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Excellence in Science

# Food Safety Booklet

Complete compilation of applications for food analysis

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- Total Solutions for Food Analysis
- Applications
- Advanced Technologies & Techniques
- Additional Resources
- Approach Shimadzu for Dedicated Solutions

# Overview

Food and agricultural industries play a major role in safeguarding global food security, through advanced scientific research and technological developments. Due to these developments, issues such as GMO validation, food authenticity and food contaminants are a major concern in the industry. To ensure public health and address food safety quality and traceability issues, food compositional analysis, regulations and good practices have been established.

However, food analysis is often complicated due to the variation of the food matrices and the diverse groups of contaminants (pesticides, drugs, toxins, etc.), which differs from one another in chemical and biological properties. With the increasingly stringent and rigorous requirements in food safety standards and regulations, food industries and regulators require trace-level detection and quantification of compounds in food. Hence, there is an increasing demand for more sophisticated instruments that give higher sensitivity, precision, speed and ease of implementation.

Shimadzu is a leading analytical instrument manufacturer that provides a complete solution and supports your food safety analysis with a wide range of analytical instruments and food-related applications. These applications are incorporated with our unique UFMS technologies and advanced techniques which increases confidence in your analyses. This food safety booklet compiles and offers all necessary information for global food manufacturing industries, food safety regulators and testing laboratories.
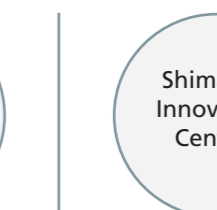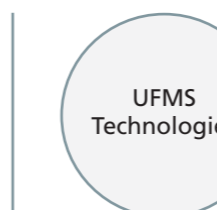


Identification and quality of food authenticity

Identification and quantification of chemical and biochemical food contaminants

Relevant issues in food analysis by modern techniques

Determination of changes in genetically modified foods

Safety, quality and traceability of global food

- Hot Topics
- Vegetables, Fruits & Spices
- Grains & Edible Oil
- Dairy Products
- Meat & Seafood
- Fertilizers
- Others
- UFMS Technologies
- Specific Techniques
- Shimadzu Innovation Centers
- Find your local contact

# HARDWARE SOLUTIONS
## ROBUST & RELIABLE INSTRUMENTS FOR ALL ANALYSES

### MASS SPECTROMETERS
**PERFORM ANALYSIS WITH UNPARALLELED THROUGHPUT**

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- Increase specificity using the Library Screening MRM Spectrum mode
- Reduce instrument maintenance with excellent robustness
- Versatile configurations to fit your specific analysis
- Suitable for various agricultural and food contaminants analyses

LCMS
GCMS
ICPMS
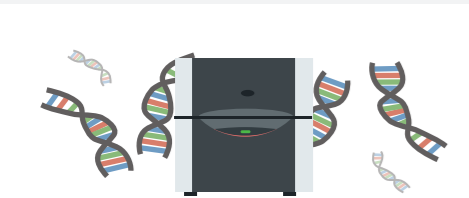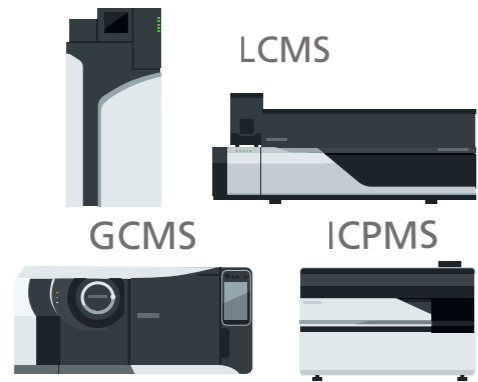
### MultiNA
**Microchip Electrophoresis System**

- Automated microfluidic device
- Wide range of amplicon detection (food allergens, pathogens, etc.)
- Higher sample throughput compared to conventional gel electrophoresis
- Compatible with 96-well PCR plates

### CHROMATOGRAPHS
**LIQUID CHROMATOGRAPHS**
**SIMPLIFY YOUR STARTUP**

- Quick method setup for your specific applications
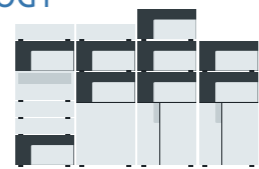- Reduce method development cost

**GAS CHROMATOGRAPHS**
**FLEXIBLE CONFIGURATION**

- [Carrier gas save] mode reduces operation costs
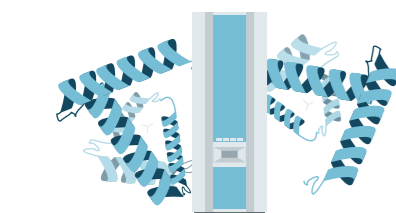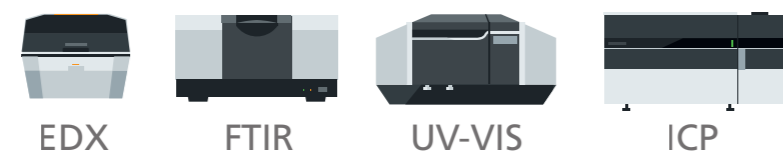- Tool-free routine maintenance

**NEXERA UC**
**SUPERCRITICAL FLUID BASED TECHNOLOGY**

- Suitable for both polar and non-polar samples
- Fully automated online sample preparation and analysis

### SPECTROSCOPIES

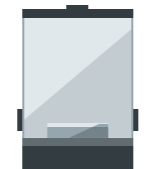- Routine toxic metal and foreign substance analysis

EDX    FTIR    UV-VIS    ICP

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- Automated microbial identification with high accuracy

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**SHORT RESPONSE TIME**

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### Understand your solutions

**Analytical Network Data System**
## LabSolutions

LIMS

## Connectivity

**Increase**
Productivity
Accuracy
Robustness

### Identify your challenges

**1 Method Development**
Tedious method development resulting in wasted resources and time
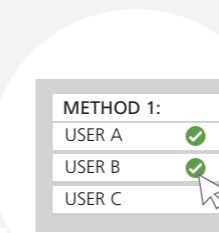
**2 Data Processing**
Inefficient data processing causing massive accumulation of unprocessed data

**3 Data Security**
Lack of effective data management system causing higher risk of data security and integrity

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# SOFTWARE SOLUTIONS
## SIMPLE WORKFLOW

**Fast & easy** analysis setup
Easy-to-use databases, method package and user access configuration

METHOD 1:
USER A
USER B
USER C

**High throughput, swift and accurate** analysis
Enabled with Shimadzu's UFMS™ technologies

**Oversee** your analysis with ease
Monitor instruments' operation status via multiple workstations and devices

**Different brand? No problem!**
LabSolutions DB/CS can also control instruments from other vendors
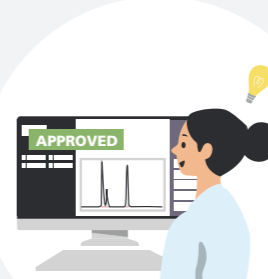
**Reduce time** spent on data processing
Peak integration algorithm resolves problematic analyses

**Go paperless,** go green
Sign off with electronic signatures, no more manual transcriptions

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All data and reports summarized in a single PDF
Create multi-data reports to combine data from various instruments

**No tampering** of results
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Post-run analysis automatically assigned with data revision numbers

**Automatic and secure** data backup

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# Find the application that suits your needs

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- 🥛 Dairy Products
- 🌾 Grains & Edible Oil
- 🟣 Fertilizers
- 🍖 Meat & Seafood
- 🥦 Vegetables, Fruits & Spices
- ⚗️ Others

| Click to navigate | GC | GC-MS | LC | LC-MS | ICP / ICP-MS | FTIR / FTIR Imaging / EDX | MALDI TOF-MS | Microchip Electrophoresis (DNA/RNA) | UV-VIS | TOC |
|---|---|---|---|---|---|---|---|---|---|---|
| Hot Topics | | ⭐ Dioxins ⭐ Mineral Oil Residues ⭐ Mineral Oil Residues | | ⭐ Allergens ⭐ Allergens ⭐ Fipronil ⭐ Aminoglycosides ⭐ Fipronil | | | | ⭐ Allergens | | |
| Pesticides | ⚗️ | 🌾 🥦 ⚗️ | 🥦 🥦 | 🥦 🥦 🥦 | | | | | | |
| Veterinary Drugs | | | 🍖 🍖 | 🍖 🍖 🍖 | | | | | | |
| Mycotoxin & Biotoxins | | | 🥛 🥦 | 🥛 🥦 🥦 | | | | | | |
| Food Additives | | | 🥛 ⚗️ | 🥦 🥦 | | | | | | |
| Heavy Metals | | | | | 🍖 🥛 🥛 | | | | | |
| Foreign Substances | | | | | | 🌾 ⚗️ ⚗️ | | | | |
| Halal Authentication | | | | 🍖 | | | | 🍖 | | |
| Microbial Identification | | | | | | | ⚗️ | | | |
| Food Adulteration | | | | | | 🌾 | 🌾 🥛 | 🌾 🍖 | | |
| GMOs | | | | | | | | 🌾 | | |
| Carbon Dioxide | ⚗️ | | | | | | | | | ⚗️ |
| Other Analyses | | 🥦 PCB / 🌾 MCPD / 🍖 POPs | 🟣 Melamine / 🟣 Nitrous Acid | 🥛 Melamine / 🍖 Synthetic Dyes / 🟣 Antibiotics | | | | | 🌾 Olive Oil Classification | |

# Hot Topics

1. Sensitive and Fast Measurement of Aminoglycoside Antibiotics in Milk, Meat or Eggs by HILIC-MS/MS and Identification using MRM Spectrum Mode

2. Determination of Polychlorinated Dibenzo-p-dioxins and Dibenzofurans (PCDD/Fs) in Foodstuffs and Animal Feed Using a Triple Quadrupole GCMS-TQ8040 System with Smart MRM Transforms Laboratory Analysis

3. Analysis of Dioxins in Feed and Food Using GC-MS/MS as Confirmatory Method in Complying with EU Regulation

4. Sensitive Method for the Determination of Fipronil in Egg using UHPLC-MS/MS [LCMS-8060]

5. Detection of Allergenic Substances Using MCE-202 MultiNA Microchip Electrophoresis System

6. High Sensitivity Analysis of Peanut Allergen in Cumin and Spice Mix [LCMS-8060]

7. Simultaneous Analysis of Major Allergens in Food Matrices by High Sensitive Mass Spectrometer

8. Sensitive Method for the Determination of Fipronil and its Metabolite Fipronil Sulfone in Egg using QuEChERS Sample Pretreatment and LC-MS/MS Detection [LCMS-8060]

9. Ultra-Sensitive and Rapid Assay of Neonicotinoids, Fipronil and Some Metabolites in Honey by UHPLC-MS/MS [LCMS-8060]

10. Determination of MOSH Contamination in Baby Foods by Using LC–GC and LC–GC×GC–MS

11. Mineral Oil Residues in Food Part 1- Dry Food (Flour, Noodles and Rice)

# Sensitive and Fast Measurement of Aminoglycoside Antibiotics in Milk, Meat or Eggs by HILIC-MS/MS and Identification using MRM Spectrum Mode

## ASMS 2017 TP-184

Mikaël LEVI[1], Hisashi KATO[2], David BAKER[3], Ichiro HIRANO[1]
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2 BIOTAGE Japan, Tokyo, Japan; 3 SHIMADZU MS Business Unit Overseas, Manchester, UK.

# Introduction

Aminoglycoside are an antibiotic family widely used for the treatment of bacterial infections in cattle, sheep, pigs and poultry. Due to their high affinity for tissues, the consumption of meat, milk or eggs containing aminoglycosides (AGs) can be potentially hazardous for human health. Regulatory agencies have set maximum residue limits (MRL) for these compounds with veterinary use. Depending on the countries, the animal species, the commodity or the AG, these MRL are different. For food safety laboratories testing large numbers of samples, a method capable to cover as many compounds, matrices and regulated range as possible would be of great help. In addition, some AGs are strictly banned for some commodities (e.g. spectinomycin in eggs) or have low MRL. So in the case of a positive sample, a strict identification of the compound is necessary to confidently report potential fraud.

AGs are very polar compounds poorly retained by reversed-phase liquid chromatography and ion-pairing reagents are not desirable when users share several methods on a single system.

Here we present a method using hydrophilic interaction liquid chromatography (HILiC) with high sensitivity mass spectrometer to reach limits of quantification at femtogram level on column, combined with MRM Spectrum Mode for formal identification.

# Methods and Materials

## Sample Preparation

Frozen meat samples were homogenized using a knife mill (Grindomix GM200, Retsch). 5 g of homogenized sample or mixed eggs or 10 mL of milk were placed in a polypropylene tube. After addition of 20 mL of extraction solution (10 mM NH4OAc, 0.4mM EDTA, 0.5% NaCl, 2% trichloroacetic acid in water), sample were vortex mixed and shaked for 10 min. After centrifugation for 10 min at 4000 rpm, the supernatant was transferred to a clean PP tube. Extraction was repeated and supernatant combined. Extract pH was then adjusted to pH 6.5 +/- 0.25.

Further purification was then perfomed by Solid Phase Extraction (SPE) using mixed-mode sorbent (WCX Express 96-well plate 30 mg, Biotage) and Extrahera automate (Biotage). 1 mL of extract was loaded without prior conditioning. Then the sample was washed with 1 mL of ammonium acetate buffer 50mM pH7. After sorbent drying, target compounds were eluted with 250 µL of aqueous formic acid 10% (v/v). Purified extracts were then diluted 25 times with aqueous formic acid 1% (v/v) prior to transfer into a polypropylene vial and analysis.

## Analytical Conditions

Two methods were used. First method for fast quantitative screening is using a fast gradient with two MRM by compound. The second method for positive sample confirmation, used same mobile phases and column but with a longer gradient and 15 MRM per compound. Parameters are described parameters in Table 1 and 2.

# Sensitive and Fast Measurement of Aminoglycoside Antibiotics in Milk, Meat or Eggs by HILIC-MS/MS and Identification using MRM Spectrum Mode

Table 1  HILiC conditions

| | |
|---|---|
| System | : Nexera X2 |
| Column | : GL Sciences Inertsil Amide 3μm 100x2.1mm |
| Temperature | : 50°C |
| Mobile Phases | : A: Water + 250 mM ammonium formate  + 1% formic acid  B: Acetonitrile |
| Flow Rate | : 800 μL/min |
| Injection Volume (Quant screening) | : 5 μL |
| Gradient | : 80 % B (0.1min) to 30%B in 1 min. 30%B (0.5 min). 30%B to 80%B in 0.1 min |
| Total Run Time (ID Confirmation) | : 3.5 min |
| Gradient | : 70 % B (0.1min) to 60%B in 6 min. 60%B to 50 (3min). 50%B to 70%B in 0.1 min |
| Total Run Time | : 11 min |

Table 2  MS/MS conditions for fast quantitative screening

| | |
|---|---|
| System | : LCMS-8060 |
| Ionization | : Heated ESI |
| Probe Voltage | : +1.5 kV (positive ionization) |
| Temperature | : Interface: 400°C  Desolvation Line: 150°C  Heater Block: 300°C |
| Gas Flow | : Nebulizing Gas: 3 L/min  Heating Gas: 20 L/Min  Drying Gas: 3 L/min |
| Dwell Time / Pause time | : 6 ms / 1.5 ms |

| MRM : Compound | MRM Quant | MRM Qual |
|---|---|---|
| Spectinomycin | 351.1 > 207.0 | 351.1 > 98.2 |
| Apramycin | 540.3 > 217.1 | 540.3 > 378.3 |
| Dihydrostreptomycin | 584.3 > 263.2 | 584.3 > 246.0 |
| Gentamicin C1a | 450.2 > 322.2 | 450.2 > 163.0 |
| Gentamicin C1 | 478.3 > 322.3 | 478.3 > 157.1 |
| Gentamicin C2 | 464.3 > 322.1 | 464.3 > 160.0 |
| Hygromycin B | 528.3 > 177.1 | 528.3 > 352.1 |
| Kanamycin | 485.3 > 163.0 | 485.3 > 324.2 |
| Neomycin B | 615.3 > 161.1 | 615.3 > 163.1 |
| Streptomycin | 582.3 > 263.2 | 582.3 > 246.12 |
| Amikacin | 586.3 > 425.2 | 586.3 > 163.3 |
| Netilmicin | 476.3 > 299.2 | 476.3 > 191.2 |
| Paromomycin | 616.3 > 163.1 | 616.3 > 293.2 |
| Sisomycin | 448.3 > 254.1 | 448.3 > 271.2 |
| Tobramycin | 468.3 > 324.0 | 468.3 > 163.0 |

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Sensitive and Fast Measurement of Aminoglycoside
Antibiotics in Milk, Meat or Eggs by HILIC-MS/MS and
Identification using MRM Spectrum Mode

# Results

## Calibration

As the method should fit any type of sample, calibration standards were prepared in aqueous 1% formic acid. The calibration range was set up by combining all MRL from Europe, Japan and USA for all target compounds and all commodities. For each compound, the lowest MRL divided by 10, or the practical achievable concentration with S/N > 10 was considered as the target limit of quantification. The highest MRL +50% was used to

define the highest calibration level. When no MRL was defined, the Japan rule using 10 ng/g as default MRL was employed for the highest level. Finally, taking into account the sample preparation protocol, volumetric concentration were established. The table 3 summarize the calibration ranges used. Some typical chromatograms at LOQ are provided in figure 1.

Table 3  Calibration ranges

| Compound | Calibration range (pg/mL) | |
|---|---|---|
| | lowest (Low MRL/10) | highest (high MRL + 50%) |
| Amikacin | 20 | 150 |
| Apramycin | 30 | 30000 |
| Dihydrostreptomycin | 5 | 30000 |
| Gentamicin(*) | 80 | 75000 |
| Hygromycin | 10 | 150 |
| Kanamycin | 20 | 37500 |
| Neomycin (+) | 100 | 150000 |
| Netilmicin | 10 | 150 |
| Paromomycin | 40 | 22500 |
| Sisomycin | 10 | 150 |
| Spectinomycin | 10 | 75000 |
| Streptomycin | 5 | 30000 |
| Tobramycin | 10 | 150 |

(*) sum of C1, C1a and C2 congeners
(+) using Neomycin B as marker

# Sensitive and Fast Measurement of Aminoglycoside Antibiotics in Milk, Meat or Eggs by HILIC-MS/MS and Identification using MRM Spectrum Mode
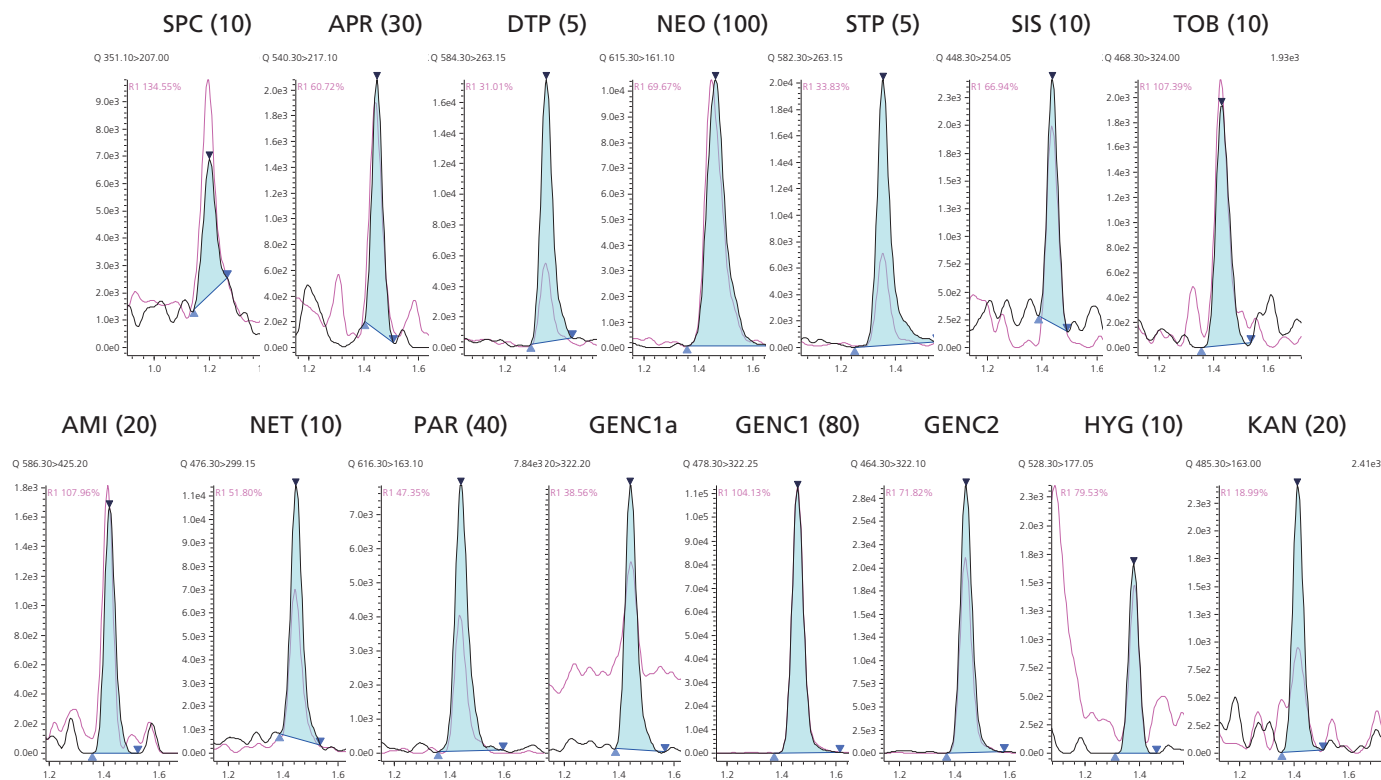


Figure 1  Chromatograms at the LOQ (concentrations in brackets, in pg/mL)

## Recovery

Several meat samples, eggs and milk samples were purchased from the local supermarket. All samples were processed as described in 2.1. Blank samples and samples spiked at 100 ng/g before extraction were analysed. No compound were found in blank samples. Peak areas were compared to an aqueous standard at the same concentration. Results are presented in table 4.

The mean recoveries for each compound were superior to 80% and moreover, were homogenous within the type of samples tested. This illustrates the good extraction recoveries and low matrix effect obtained.

## Identification using MRM Spectrum Mode

In MRM Spectrum Mode, 15 MRM transitions were acquired per compound. Signals were merged by the software to create a spectrum with optimized sensitivity for each fragment. By comparing this spectrum to a predefined library, identification becomes unambiguous.

Thanks to ultrafast MRM features of the mass spectrometer used, there is no significant difference in sensitivity when acquiring 2 or 15 MRM per compound. Figure 2 illustrates this approach.

# Sensitive and Fast Measurement of Aminoglycoside Antibiotics in Milk, Meat or Eggs by HILIC-MS/MS and Identification using MRM Spectrum Mode

Table 4  Total recovery in real samples

|  | SPC | APR | DTP | GEN C1a | GEN C1 | GEN C2 | HYG | KAN |
|---|---|---|---|---|---|---|---|---|
| Eggs | 93% | 83% | 86% | 83% | 86% | 96% | 89% | 85% |
| Fat Milk | 94% | 81% | 109% | 88% | 86% | 88% | 84% | 82% |
| Low Fat Milk | 88% | 90% | 106% | 85% | 89% | 82% | 88% | 78% |
| Low Fat Beef | 99% | 88% | 99% | 78% | 90% | 87% | 89% | 101% |
| Fat Beef | 93% | 82% | 102% | 82% | 80% | 89% | 76% | 73% |
| Chicken Leg | 94% | 100% | 97% | 109% | 93% | 106% | 77% | 103% |
| Chicken Liver | 83% | 86% | 90% | 90% | 81% | 87% | 88% | 89% |
| Chicken Breast | 93% | 83% | 96% | 89% | 90% | 81% | 84% | 81% |
| Pork Cutlet Muscle | 86% | 87% | 102% | 84% | 83% | 88% | 86% | 86% |
| Pork Cutlet Fat | 94% | 83% | 92% | 82% | 91% | 91% | 88% | 90% |
| Pork Bacon | 92% | 79% | 98% | 96% | 93% | 96% | 89% | 96% |
| Mean | 92% | 86% | 98% | 88% | 87% | 90% | 85% | 88% |
| %RSD | 4.9% | 6.9% | 6.9% | 9.7% | 5.2% | 7.9% | 5.6% | 10.6% |

|  | NEO | STP | AMI | NET | PAR | SIS | TOB |
|---|---|---|---|---|---|---|---|
| Eggs | 79% | 89% | 84% | 84% | 85% | 89% | 81% |
| Fat Milk | 80% | 101% | 83% | 83% | 90% | 73% | 87% |
| Low Fat Milk | 81% | 105% | 75% | 85% | 73% | 85% | 87% |
| Low Fat Beef | 80% | 100% | 98% | 85% | 82% | 84% | 87% |
| Fat Beef | 89% | 103% | 83% | 83% | 73% | 83% | 59% |
| Chicken Leg | 98% | 99% | 108% | 108% | 106% | 105% | 109% |
| Chicken Liver | 82% | 95% | 84% | 85% | 87% | 86% | 95% |
| Chicken Breast | 89% | 93% | 78% | 90% | 91% | 87% | 90% |
| Pork Cutlet Muscle | 79% | 103% | 82% | 74% | 88% | 92% | 94% |
| Pork Cutlet Fat | 75% | 92% | 88% | 88% | 84% | 86% | 86% |
| Pork Bacon | 89% | 93% | 102% | 99% | 97% | 96% | 85% |
| Mean | 84% | 98% | 88% | 88% | 87% | 88% | 87% |
| %RSD | 8.0% | 5.5% | 11.8% | 10.3% | 11.0% | 9.3% | 13.6% |
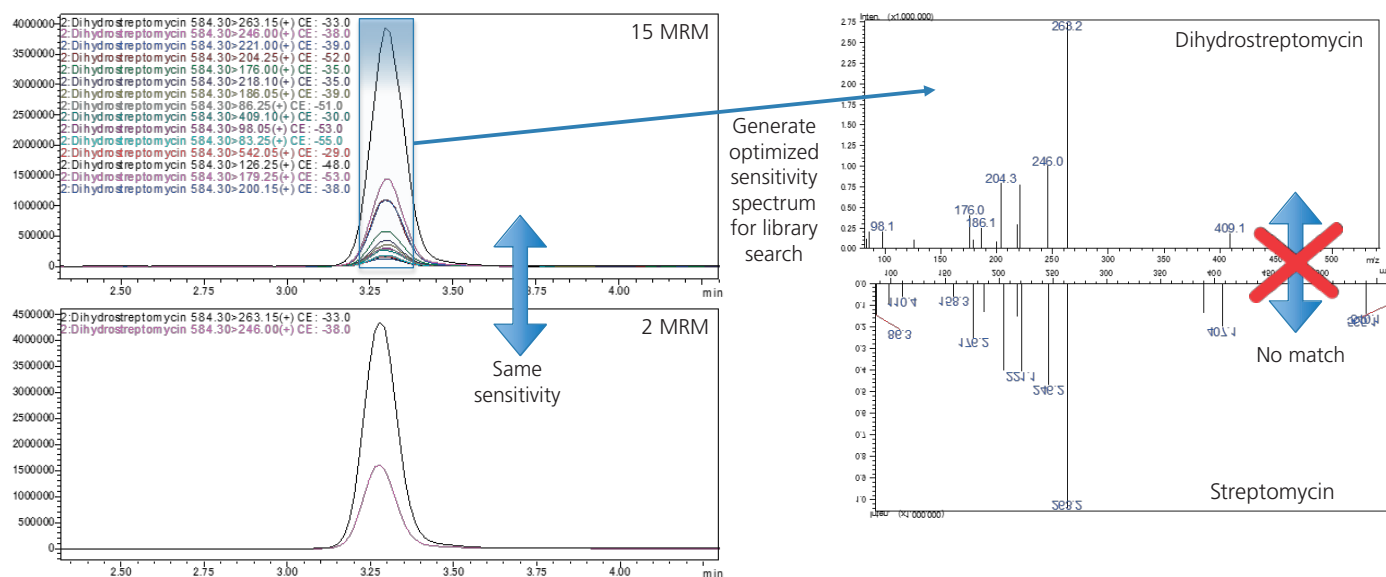
Figure 2  MRM Spectrum Mode ID confirmation

# Conclusions

A very sensitive HILiC-MS/MS method was developed to detect a large panel of aminoglycoside antibiotics without ion-pairing.
One method can be used for all kind of animal species or commodities, covering major food safety regulations.
The complete workflow, including sample preparation has been optimized to provide  high-throughput. The good recoveries obtained across the tested matrices eliminate the use of matrix-matched calibration standards.
In addition to the fast quantitative screening method, a ID confirmation method using MRM Spectrum Mode can be performed with same mobile phases and column.

## SHIMADZU

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# Technical Report

# Determination of Polychlorinated Dibenzo-*p*-dioxins and Dibenzofurans (PCDD/Fs) in Foodstuffs and Animal Feed Using a Triple Quadrupole GCMS-TQ8040 System with Smart MRM Transforms Laboratory Analysis

Pu Wang[1], Huizhong Sun[1], Qinghua Zhang[1], Feifei Tian[2], Lei Cao[2]

## Abstract:

A method was developed on a high selectively triple quadrupole GCMS-TQ8040 system for screening trace levels of polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) in foodstuffs and animal feed. Smart MRM technology was employed for method development and GC-MS/MS analysis. The results showed good sensitivity and repeatability for PCDD/Fs at low levels, as well as a good linear response over the required concentration range. The performance on real sample analysis indicated the feasibility of Shimadzu GCMS-TQ8040 system for PCDD/Fs measurement in food and feed, as required by European Union legislation.

Keywords: GC-MS/MS, MRM, PCDDs, PCDFs, dioxins, food, feed

## 1. Introduction

Polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) are of special concern, due to their harmful health effects. They have neurotoxic potential and are linked to causing cancer, endocrine disruption, and reproductive disorders. PCDD/Fs were never produced intentionally as marketable products, while they are widespread around the world. They can also bioaccumulate and biomagnify through the food chain, and finally pose a threat to human body. Most dioxin exposure to human derives from food (>90%), 90 % of which is of animal origin [1]. Consequently, incidents of dioxin contamination involving food and feed have generally raised great public concern in the world. For example, Germany's dioxin-tainted food scandal in 2011 led to a shutdown of more than 4700 farms and tremendous economic losses in Germany.

European regulations specifying official sampling and analysis methods for controlling PCDD/F levels and polychlorinated biphenyls (PCBs) in food and feed were initially issued in the early 2000s, followed by several amendments thereafter. The latest amendment was Commission Regulation (EU) Nos. 589/2014 [2] and 709/2014 [3], where a major update is that gas chromatography–triple quadrupole mass spectrometry (GC-MS/MS) was recognized as an appropriate confirmatory method for checking compliance with the maximum levels of PCDD/Fs and PCBs in food and feed control. This means GC-MS/MS can be used to provide full or complementary information enabling the PCDD/Fs and dioxin-like PCBs to be identified and quantified unequivocally at the maximum or in case of need at the action threshold.

This Technical Report describes a highly sensitive and reproducible method for determining PCDD/Fs (Table 1) in food and feed using a triple quadrupole GCMS-TQ8040 system with Smart MRM, which transforms laboratory analysis process. The results show performance comparable to using high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS) for quantitation of PCDD/Fs in food and feed.

Table 1　PCDD/Fs Specified in EU Legislation and TEF Values Established by WHO

| Congener | WHO$_{1998}$-TEF | WHO$_{2005}$-TEF | Congener | WHO$_{1998}$-TEF | WHO$_{2005}$-TEF |
|---|---|---|---|---|---|
| 2,3,7,8-TCDD | 1 | 1 | 2,3,7,8-TCDF | 0.1 | 0.1 |
| 1,2,3,7,8-PeCDD | 1 | 1 | 1,2,3,7,8-PeCDF | 0.05 | 0.03 |
| 1,2,3,4,7,8-HxCDD | 0.1 | 0.1 | 2,3,4,7,8-PeCDF | 0.5 | 0.3 |
| 1,2,3,6,7,8-HxCDD | 0.1 | 0.1 | 1,2,3,4,7,8-HxCDF | 0.1 | 0.1 |
| 1,2,3,7,8,9-HxCDD | 0.1 | 0.1 | 1,2,3,6,7,8-HxCDF | 0.1 | 0.1 |
| 1,2,3,4,6,7,8-HpCDD | 0.01 | 0.01 | 2,3,4,6,7,8-HxCDF | 0.1 | 0.1 |
| OCDD | 0.0001 | 0.0003 | 1,2,3,7,8,9-HxCDF | 0.1 | 0.1 |
| | | | 1,2,3,4,6,7,8-HpCDF | 0.01 | 0.01 |
| | | | 1,2,3,4,7,8,9-HpCDF | 0.01 | 0.01 |
| | | | OCDF | 0.0001 | 0.0003 |

1 Dioxin Lab, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, China
2 Analytical Applications Center, Shimadzu (China) Co., Ltd., Beijing, China

# 2. Experimental

## 2-1. Sample Preparation and Instrumental Analysis

The sample preparation of food and feed was mainly followed US EPA method 1613B [4] with proper modification [5]. The samples were extracted using an accelerated solvent extraction (ASE) system, followed by purification steps (including acidic/basic silica gel, alumina, and carbon columns). Samples were spiked with $^{13}$C-isotope labeled surrogate standards (1613-LCS) and internal standards (1613-IS) prior to extraction and instrumental analysis, respectively.

Samples were analyzed in a Shimadzu GCMS-TQ8040 system with Smart MRM, which transforms laboratory analysis process. The GC unit was equipped with a split/splitless injection port. The capillary column was a 60 m DB-5MS fused silica capillary column (J&W, Scientific, 0.25 µm film thickness, 0.25 mm I.D.). Table 2 showed the selected instrumental conditions for PCDD/Fs detection. Measurements were performed in EI-MS/MS Multiple Reaction Monitoring (MRM) mode. The MRM method for PCDD/Fs was developed using the Shimadzu

GCMS-TQ8040 MRM optimization tool, which automatically determines the optimum transitions and collision energies in a single sequence, and then seamlessly incorporates them into the Smart Database series for full method development (Fig. 1). At that point, the two most intense ions of the molecular chlorine isotope cluster of each congener and internal standard were chosen. Using flexible MS event, the Smart MRM function routinely determines the best dwell and loop times for the two transitions in a single method, providing optimum precision and sensitivity. In order to improve the peak shape for 2,3,7,8-TCDD/F at very low concentration levels (10 fg/µL), the event times were manually increased to 0.35 seconds for the native 2,3,7,8-TCDD/F and decreased to 0.20 seconds for the $^{13}$C-labeled 2,3,7,8-TCDD/F to maintain the same loop time as other congeners. Information on the MRM settings and analyte retention times are given in Table 3.

### Table 2   Conditions for Gas Chromatograph and Mass Spectrometer

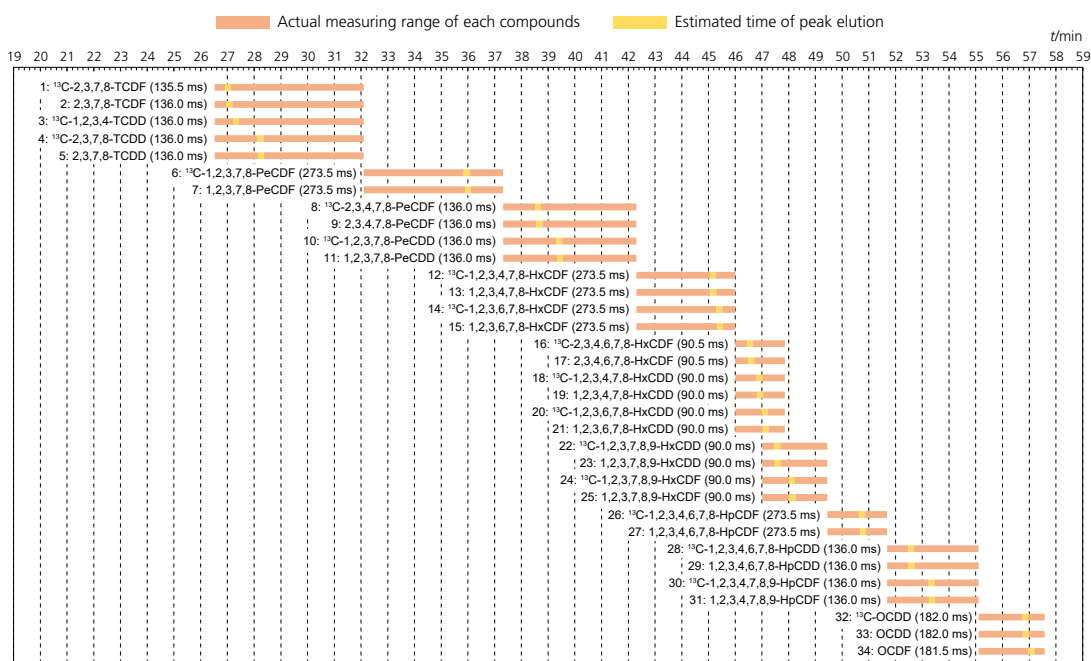| GC Conditions | | MS Conditions | |
|---|---|---|---|
| Column | : DB-5MS (60 m × 0.25 mm × 0.25 µm) | Ionization Mode | : EI |
| Injection Mode | : Splitless | Ion Source Temperature | : 250 °C |
| Injection Volume | : 2 µL | Interface Temperature | : 270 °C |
| High Pressure Injection | : 300 kPa (2 min) | Acquisition Mode | : MRM Mode |
| Sampling Time | : 1 min | Collision Gas | : Argon |
| Injection Temperature | : 290 °C | Emission Current | : 250 µA |
| Column Oven Temperature | : 150 °C (3 min) → (20 °C/min) → 230 °C (18 min) → (5 °C/min) → 235 °C (10 min) → (4 °C/min) → 320 °C (1 min) | Loop Time | : 1.1 sec |
| | | Pressure of CID Gas | : 150 kPa |
| | | Detector Voltage | : 1.8 kV |
| Flow Control Mode | : Linear Velocity | | |
| Column Flow | : 1.03 mL/min | | |



Fig. 1   Optimized MS Event Times for PCDD/F Congeners with Smart MRM Function

# 3. Results and Discussion

## 3-1. Chromatography

The chromatographic separation of the 17 native 2,3,7,8-substituted PCDD/F congeners is shown in Fig. 2 (EPA 1613 CS3). The sample was completely separated in a total run time of 60 minutes.
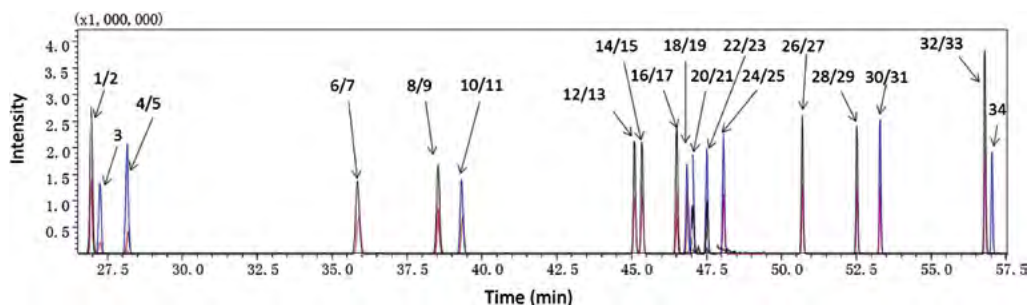


Fig. 2   Mass Chromatogram of PCDD/Fs in EPA 1613 CS3 (10 – 100 pg/µL) (Peak numbers refer to analytes listed in Table 3.)

Table 3   MS/MS Settings for Native PCDD/F Congeners and ^{13}C-Internal Standards

| Peak No. | Analyte | RT (min) | Precursor > Product | CE (V) | Precursor > Product | CE (V) |
|---|---|---|---|---|---|---|
| 1 | ^{13}C-2,3,7,8-TCDF | 27.024 | 315.90 > 251.90 | 31 | 317.90 > 253.90 | 31 |
| 2 | 2,3,7,8-TCDF | 27.076 | 303.90 > 240.90 | 31 | 305.90 > 242.90 | 31 |
| 3 | ^{13}C-1,2,3,4-TCDD | 27.323 | 331.90 > 267.90 | 25 | 333.90 > 269.90 | 25 |
| 4 | ^{13}C-2,3,7,8-TCDD | 28.235 | 331.90 > 267.90 | 25 | 333.90 > 269.90 | 25 |
| 5 | 2,3,7,8-TCDD | 28.253 | 319.90 > 256.90 | 25 | 321.90 > 258.90 | 25 |
| 6 | ^{13}C-1,2,3,7,8-PeCDF | 35.953 | 351.90 > 287.90 | 34 | 349.90 > 285.90 | 34 |
| 7 | 1,2,3,7,8-PeCDF | 36.01 | 339.90 > 276.90 | 34 | 337.90 > 274.90 | 34 |
| 8 | ^{13}C-2,3,4,7,8-PeCDF | 38.625 | 351.90 > 287.90 | 37 | 349.90 > 285.90 | 37 |
| 9 | 2,3,4,7,8-PeCDF | 38.673 | 339.90 > 276.90 | 37 | 337.90 > 274.90 | 37 |
| 10 | ^{13}C-1,2,3,7,8-PeCDD | 39.419 | 367.90 > 303.90 | 25 | 365.90 > 301.90 | 25 |
| 11 | 1,2,3,7,8-PeCDD | 39.441 | 355.90 > 292.90 | 25 | 353.90 > 290.90 | 25 |
| 12 | ^{13}C-1,2,3,4,7,8-HxCDF | 45.168 | 385.80 > 321.90 | 37 | 387.80 > 323.90 | 37 |
| 13 | 1,2,3,4,7,8-HxCDF | 45.183 | 373.80 > 310.90 | 37 | 375.80 > 312.90 | 37 |
| 14 | ^{13}C-1,2,3,6,7,8-HxCDF | 45.413 | 385.80 > 321.90 | 37 | 387.80 > 323.90 | 37 |
| 15 | 1,2,3,6,7,8-HxCDF | 45.438 | 373.80 > 310.90 | 37 | 375.80 > 312.90 | 37 |
| 16 | ^{13}C-2,3,4,6,7,8-HxCDF | 46.568 | 385.80 > 321.90 | 37 | 387.80 > 323.90 | 37 |
| 17 | 2,3,4,6,7,8-HxCDF | 46.604 | 373.80 > 310.90 | 37 | 375.80 > 312.90 | 37 |
| 18 | ^{13}C-1,2,3,4,7,8-HxCDD | 46.911 | 401.80 > 337.90 | 25 | 403.80 > 339.80 | 25 |
| 19 | 1,2,3,4,7,8-HxCDD | 46.935 | 389.80 > 326.90 | 25 | 391.80 > 328.80 | 25 |
| 20 | ^{13}C-1,2,3,6,7,8-HxCDD | 47.1 | 401.80 > 337.90 | 25 | 403.80 > 339.80 | 25 |
| 21 | 1,2,3,6,7,8-HxCDD | 47.139 | 389.80 > 326.90 | 25 | 391.80 > 328.80 | 25 |
| 22 | ^{13}C-1,2,3,7,8,9-HxCDD | 47.577 | 401.80 > 337.90 | 25 | 403.80 > 339.80 | 25 |
| 23 | 1,2,3,7,8,9-HxCDD | 47.591 | 389.80 > 326.90 | 25 | 391.80 > 328.80 | 25 |
| 24 | ^{13}C-1,2,3,7,8,9-HxCDF | 48.126 | 385.80 > 321.90 | 34 | 387.80 > 323.90 | 34 |
| 25 | 1,2,3,7,8,9-HxCDF | 48.145 | 373.80 > 310.90 | 34 | 375.80 > 312.90 | 34 |
| 26 | ^{13}C-1,2,3,4,6,7,8-HpCDF | 50.755 | 419.80 > 355.80 | 37 | 421.80 > 357.80 | 37 |
| 27 | 1,2,3,4,6,7,8-HpCDF | 50.788 | 407.80 > 344.80 | 37 | 409.80 > 346.80 | 37 |
| 28 | ^{13}C-1,2,3,4,6,7,8-HpCDD | 52.584 | 435.80 > 371.80 | 25 | 437.80 > 373.80 | 25 |
| 29 | 1,2,3,4,6,7,8-HpCDD | 52.596 | 423.80 > 360.80 | 25 | 425.80 > 362.80 | 25 |
| 30 | ^{13}C-1,2,3,4,7,8,9-HpCDF | 53.348 | 419.80 > 355.80 | 37 | 421.80 > 357.80 | 37 |
| 31 | 1,2,3,4,7,8,9-HpCDF | 53.357 | 407.80 > 344.80 | 37 | 409.80 > 346.80 | 37 |
| 32 | ^{13}C-OCDD | 56.85 | 469.70 > 405.80 | 25 | 471.70 > 407.80 | 25 |
| 33 | OCDD | 56.867 | 457.70 > 394.80 | 25 | 459.70 > 396.80 | 25 |
| 34 | OCDF | 57.081 | 441.70 > 378.80 | 34 | 443.70 > 380.80 | 34 |

## 3-2. Sensitivity

To verify the performance of the GC-MS/MS system in the low concentration range, 1:50 dilutions of the calibration standard EPA-1613CS1 were also measured (10 fg/µL 2,3,7,8-TCDD). Based on the 2 µL injection volume, the dilutions of the calibra-tion standard EPA-1613CS1 indicate an absolute amount of 20 fg 2,3,7,8-TCDD in the column. The MRM chromatograms for native PCDD/Fs are shown in Fig. 3.
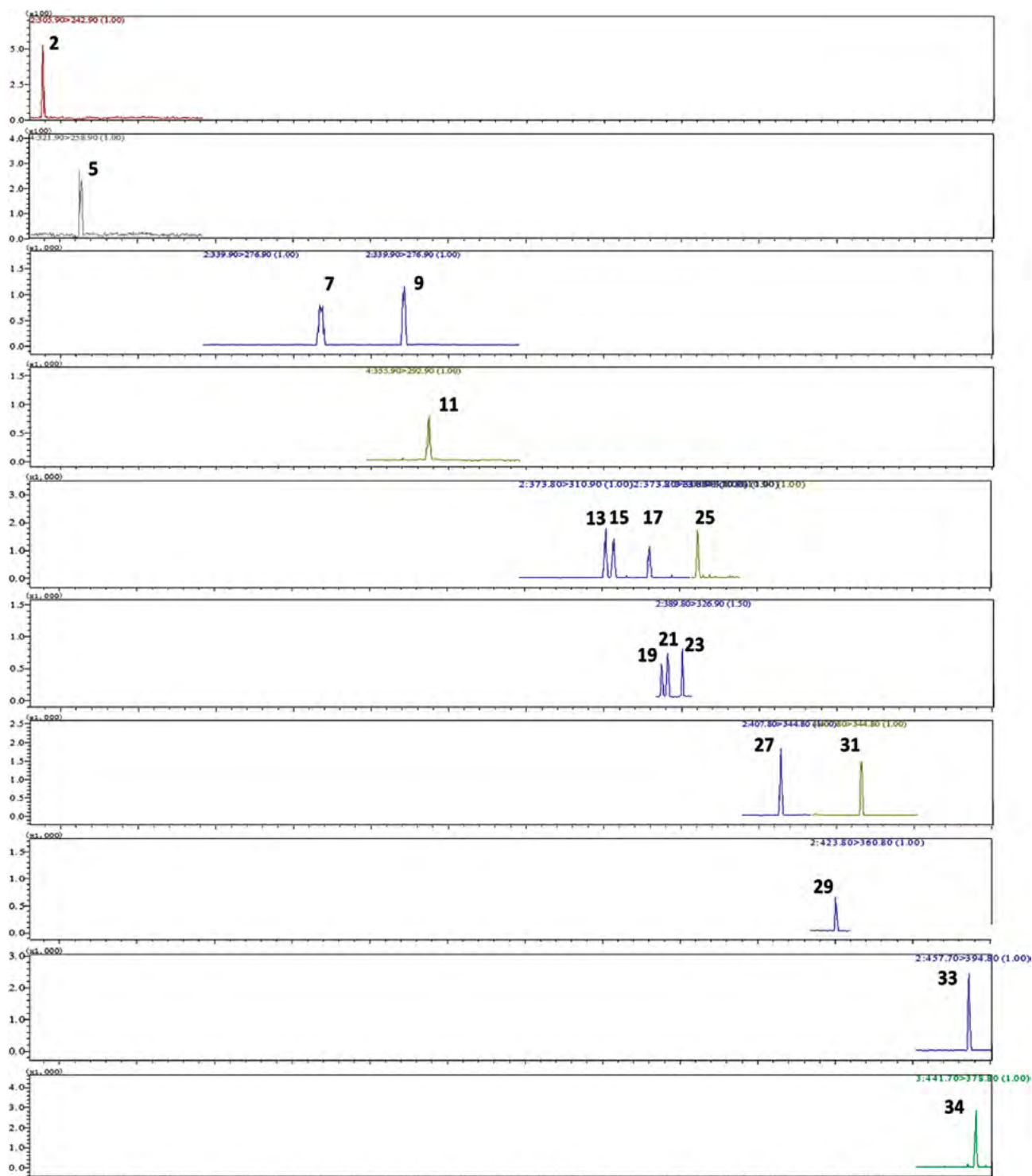
3

Fig. 3  MRM Chromatograms of Native PCDD/F Congeners (10 – 100 fg/µL, 2 µL injection volume) (Peak numbers refer to analytes listed in Table 3.)

## 3-3.  Linearity of Response

A seven-point calibration curve was prepared based on US EPA method 1613, defined for calibration verification solutions (CS1-CS5), together with EPA-1613CSL and EPA-1613CS0.5. Excellent linearity was obtained for the calibration standards over the concentration range from 0.1 to 200 ng/mL (TCDD), with $R^2$ values >0.999 for an injection volume of 1 µL (Table 4). The mean response factor for each congener is also given in Table 4. The calibration curves for 2,3,7,8-TCDD, 2,3,7,8-TCDF and 1,2,3,7,8-PeCDD are shown in Fig. 4 to 6.

Table 4   Linear Regression for Seven-Point Calibration Curves over the Range 0.1 to 200 ng/mL (TCDD) and the Mean Response Factor for Each Congener

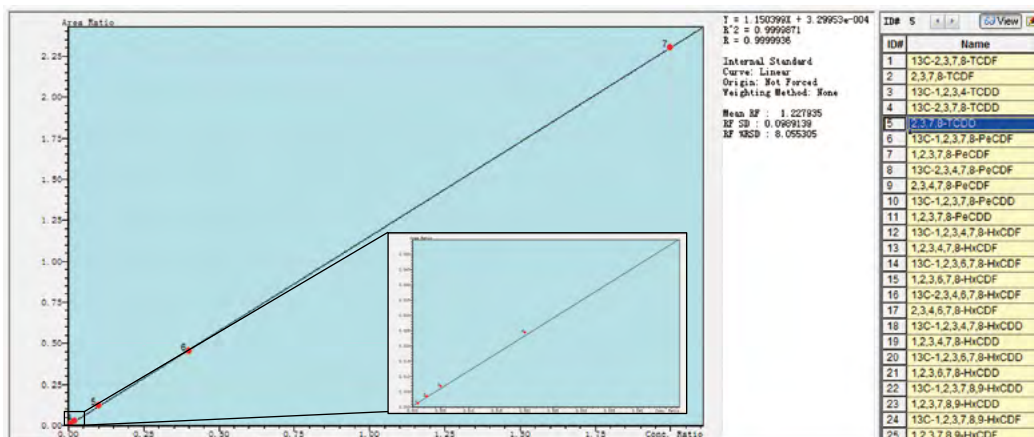| Congener | Regression line equation | $R^2$ | Mean RF | RF %RSD |
|---|---|---|---|---|
| 2,3,7,8-TCDD | Y = 1.150399X + 3.29953e-004 | 0.99999 | 1.22794 | 8.06 |
| 1,2,3,7,8-PeCDD | Y = 1.014733X + 3.009239e-003 | 1.00000 | 1.03887 | 1.96 |
| 1,2,3,4,7,8-HxCDD | Y = 1.079761X − 5.260601e-004 | 0.99997 | 1.09358 | 3.65 |
| 1,2,3,6,7,8-HxCDD | Y = 0.9705907X + 5.362575e-002 | 0.99915 | 1.08710 | 5.37 |
| 1,2,3,7,8,9-HxCDD | Y = 1.024768X + 3.682249e-002 | 0.99967 | 1.00394 | 14.48 |
| 1,2,3,4,6,7,8-HpCDD | Y = 0.9429045X + 1.331675e-002 | 0.99998 | 1.02985 | 6.92 |
| OCDD | Y = 1.242978X − 6.145206e-002 | 0.99929 | 1.14683 | 6.10 |
| 2,3,7,8-TCDF | Y = 1.15754X + 9.032785e-004 | 0.99996 | 1.18104 | 3.57 |
| 1,2,3,7,8-PeCDF | Y = 1.015266X − 5.771587e-003 | 0.99997 | 1.07846 | 7.83 |
| 2,3,4,7,8-PeCDF | Y = 1.045151X − 6.304552e-003 | 0.99998 | 1.04210 | 4.49 |
| 1,2,3,4,7,8-HxCDF | Y = 1.006328X + 2.605984e-002 | 0.99988 | 1.09930 | 6.04 |
| 1,2,3,6,7,8-HxCDF | Y = 0.9307018X + 3.432044e-002 | 0.99971 | 1.06611 | 8.06 |
| 2,3,4,6,7,8-HxCDF | Y = 0.9080292X + 3.053454e-002 | 0.99983 | 1.00464 | 6.34 |
| 1,2,3,7,8,9-HxCDF | Y = 0.960272X + 2.450491e-002 | 0.99993 | 1.03403 | 9.10 |
| 1,2,3,4,6,7,8-HpCDF | Y = 0.9732686X + 4.031919e-002 | 0.99958 | 1.08255 | 4.88 |
| 1,2,3,4,7,8,9-HpCDF | Y = 0.9562794X + 3.622056e-002 | 0.99969 | 1.06788 | 5.48 |
| OCDF | Y = 1.424071X + 3.271179e-003 | 0.99999 | 1.50245 | 8.94 |



Fig. 4   Seven-Point Calibration Curve for 2,3,7,8-TCDD with Both Linear Fit and Mean Response Factors
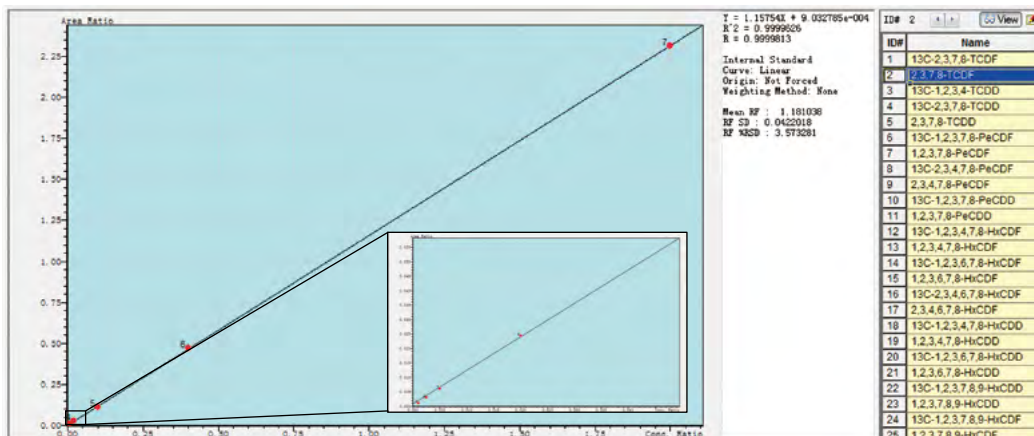


Fig. 5   Seven-Point Calibration Curve for 2,3,7,8-TCDF with Both Linear Fit and Mean Response Factors
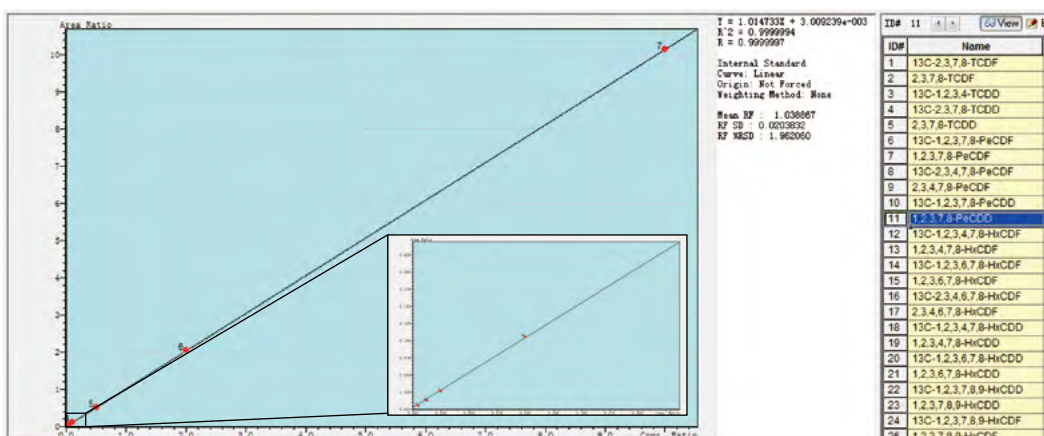
Fig. 6 Seven-Point Calibration Curve for 1,2,3,7,8-PeCDD with Both Linear Fit and Mean Response Factors

## 3-4. Repeatability

The repeatability was confirmed for within the same day and over different days. Total 12 injections (four 2 µL injections per day for three days) of 1:50 diluted EPA-1613CS1 (10 fg/µL 2,3,7,8-TCDD) were performed. Results exhibited good repeatability for the peak areas of each congener, with a relative standard deviations (RSD) less than 15 % (Fig. 7).
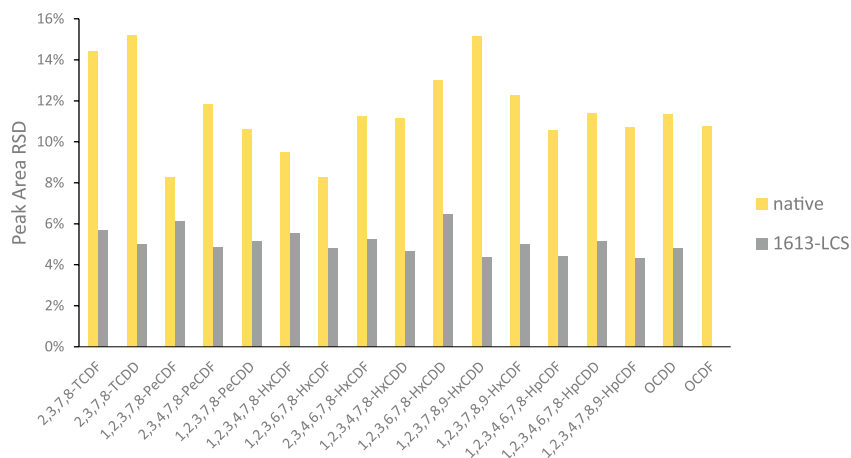


Fig. 7 Repeatability of Peak Areas for Native PCDD/Fs and 1613-LCS (n = 12)
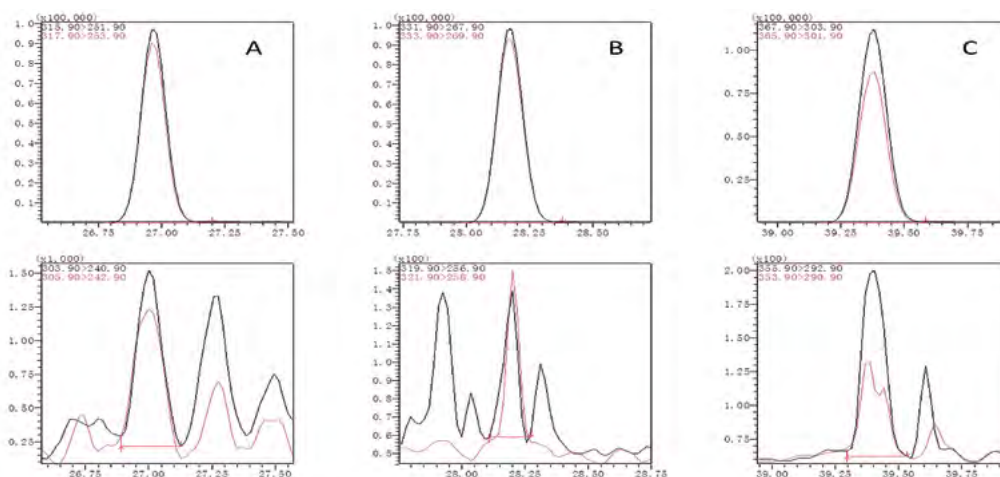


Fig. 8 MRM Chromatograms for ¹³C-Labeled and Native 2,3,7,8-TCDF (A), 2,3,7,8-TCDD (B), and 1,2,3,7,8-PeCDD (C) in Fish Oil

6

## 3-5. Sample Analysis

For comparison, each fish oil and milk sample was analyzed in both GC-MS/MS and HRGC/HRMS systems, using the same GC conditions for the HRGC/HRMS system. The MRM chromatograms for 2,3,7,8-TCDF, 2,3,7,8-TCDD, and 1,2,3,7,8-PeCDD in the fish oil sample are shown in Fig. 8. The congener profiles in both samples are exhibited in Fig. 9 and 10. GC-MS/MS results showed good consistency with HRGC/HRMS results. The toxic equivalents (TEQ) of PCDD/Fs were 29.5 pg WHO-TEQ$_{2005}$/g fat and 1.38 pg WHO-TEQ$_{2005}$/g fat (upper bound values) in the fish oil and milk samples, respectively, which were comparable to the results of 24.9 and 1.37 pg WHO-TEQ$_{2005}$/g fat obtained from the HRGC/HRMS system.
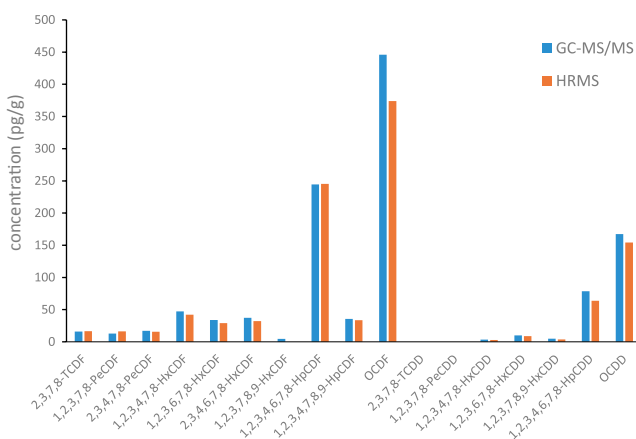


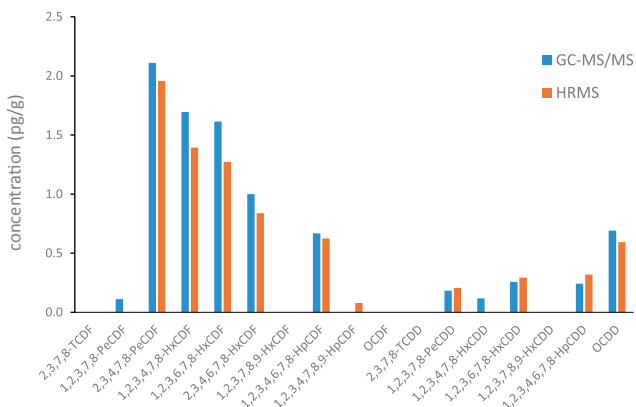Fig. 9  Congener Profiles of PCDD/Fs in Fish Oil



Fig. 10  Congener Profiles of PCDD/Fs in Milk

## 4. Conclusion

The Shimadzu GCMS-TQ8040 system facilitates the screening and quantitation of low concentration PCDD/Fs in different food-stuffs and animal feed samples. The method showed good linearity, sensitivity, and repeatability. The analytical results from real samples also indicated good precision using this method, when compared with HRGC/HRMS results. This suggests that the Shimadzu GCMS-TQ8040 system provides a substitute solution for routine screening and quantitation of PCDD/Fs in food and feed, as required by European Union legislation.

### References

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2. Commission Regulation (EU) No. 589/2014 of 2 June 2014 laying down methods of sampling and analysis for the control of levels of dioxins, dioxin-like PCBs and non-dioxin-like PCBs in certain foodstuffs and repealing Regulation (EU) No. 252/2012, *Offic. J. Eur. Commun.* 2014, L164/18–40.
3. Commission Regulation (EU) No. 709/2014 of 20 June 2014 amending Regulation (EC) No. 152/2009 as regards the determination of the levels of dioxins and polychlorinated biphenyls, *Offic. J. Eur. Commun.* 2014, L188/1–18.
4. US EPA method 1613B Tetra- through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS. 1997.
5. Wang P, Zhang Q, Wang Y, Wang T, Li X, Ding L, Jiang G. Evaluation of Soxhlet extraction, accelerated solvent extraction and microwave-assisted extraction for the determination of polychlorinated biphenyls and polybrominated diphenyl ethers in soil and fish samples. *Anal. Chim. Acta.* 2010, 663(1):43–48.

**Triple Quadrupole Gas Chromatograph Mass Spectrometer**

# GCMS-TQ8040

### Finally, a triple quadrupole GCMS *Smart* enough for everyday use in your laboratory.

The Shimadzu GCMS-TQ8040 is the first triple quadrupole with Smart Productivity for high efficiency sample throughput, Smart Operation for quick and easy method development, and Smart Performance for low detection limits and Scan/MRM. These three smart technologies contribute to Smart MRM, and provide the most accurate, cost-effective, and easy-to-use triple quadrupole GCMS you have ever imagined.

## Smart Productivity

- Retention time synchronized MRM provides simultaneous sensitivity and precision for hundreds of compounds in one run
- Smart MRM optimizes analysis of 400+ compounds in a single acquisition with maximum sensitivity
- Reduced downtimes with the Twin Line configuration MS system

## Smart Operation

- MRM Optimization Tool to optimize MRM transitions automatically
- Smart Database Series with fully optimized transitions for hundreds of compounds
- Smart MRM for automatic method creation in a single step

## Smart Performance

- The patented ion source design and uniform temperature prevent active spots and boost sensitivity
- The OFF-AXIS ion optics eliminate chemical noise and lower detection limits
- High-sensitivity analysis even in single GC-MS mode

## Smart Environmental Database
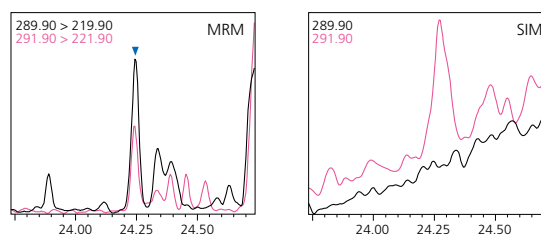
### Create MRM Methods for GC-MS/MS

The Smart Environmental Database contains all the information necessary to create MRM methods for over 500 environmental pollutants, including PCBs, BFRs, dioxins, PAHs, organochlorine pesticides (OCPs), and stable isotopically labeled compounds that are commonly used as Internal and Surrogate Standards.
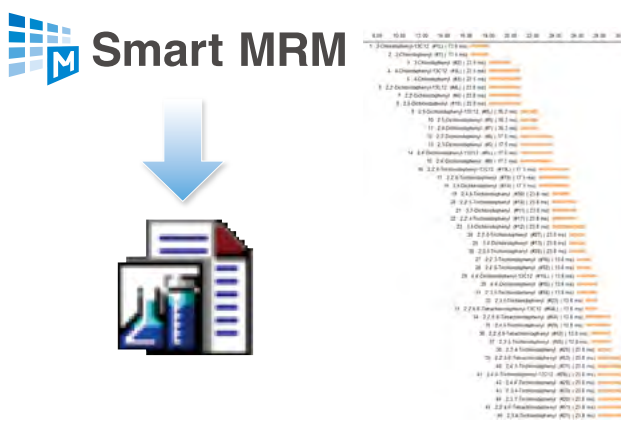
### Smart MRM Optimizes Methods Automatically

The Smart MRM feature allows the user to create fully optimized MRM and Scan/MRM methods automatically. GC-MS/MS Dwell, Event, and Loop times can be difficult to optimize when dozens, or even hundreds of compounds are to be analyzed simultaneously. The Smart MRM feature automatically determines the optimum Dwell, Event, and Loop settings using flexible MRM events, and creates MRM and Scan/MRM methods that provide the best sensitivity for all compounds in a single method.

Analysis of PCB in River Water
(2,2',5,5'-Tetrachlorobiphenyl (#52) concentration in water of 0.080 ng/L)



| Number of Registered Compounds | Number of Registered Native Compounds | Number of Registered Compounds Labeled with Stable Isotopes |
|---|---|---|
| Polychlorinated biphenyls | 209 | 45 |
| Brominated flame retardants | 55 | 28 |
| Dioxins | 32 | 26 |
| Polycyclic aromatic hydrocarbons | 38 | 37 |
| Organochlorine pesticides | 32 | 25 |

### Smart MRM

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# Technical Report

# Analysis of Dioxins in Foods and Feeds Using GC-MS/MS

Masato Takakura[1], Thomas Lehardy[2], Philippe Marchand[2]

## Abstract:

Until recently, the analysis of dioxins in foods and feeds was performed using magnetic sector GC-MS (GC-HRMS), which provided highly accurate quantitation. In recent years, the quantitative accuracy of GC-MS/MS has improved significantly. Accordingly, this method has become officially recognized in the EU as can be used for analyzing dioxins (EU589/2014, 644/2017). In this investigation, dioxins were analyzed in 44 types and 201 samples of foods and feeds using the GCMS-TQ8050 and the GC-MS/MS method package for dioxins in foods, which is compliant with EU regulations. Additionally, the GC-MS/MS analysis results were compared with the analysis results from GC-HRMS, to compare the quantitative capabilities of both methods. For the comparison, the TEQ ratio was calculated for various samples. From the comparison of the results, for samples with a higher TEQ than 0.060 pg/uL (TEQ when any of the compounds was detected at a higher concentration than LOQ), GC-MS/MS and GC-HRMS provided similar TEQ values in at least 98 % of the samples. Accordingly, it was evident that analysis with GCMS-TQ8050 and method package provides a quantitative capability equivalent to that from GC-HRMS for samples at the concentration levels required for analysis.

Keywords: GC-MS/MS, foods, feeds, dioxins

## 1. Introduction

Residual organic compounds (persistent organic pollutants or POPs) in foods and feeds are analyzed using a variety of methods. In particular, dioxins are particularly toxic even for POPs, so quantitative analysis is required down to low concentrations.

Until recently, the analysis of dioxins was performed using magnetic sector (double focusing) GC-MS (hereinafter "GC-HRMS"), which provides highly accurate quantitation. However, triple quadrupole GC-MS (hereinafter "GC-MS/MS") is less expensive and easier to handle than GC-HRMS, so its use is being increasingly investigated. In recent years, the quantitative accuracy of GC-MS/MS has improved significantly. Accordingly, the use of this analysis method has become officially recognized in the EU (EU589/2014, 644/2017).

However, in order to change from GC-HRMS to GC-MS/MS, it is first necessary to compare their respective quantitative capabilities.

The Shimadzu GCMS-TQ8050 combines a high-sensitivity detector, capable of detection at femtogram order concentrations, with noise-reduction technology, enabling the analysis of dioxins in foods and feeds. Additionally, the "EU Regulation Compliant GC-MS/MS Method Package for Dioxins in Foods" consists of method files registered with the optimal conditions for the analysis of dioxins, as well as a report creation tool, which can output the items required by EU regulations. As a result, analysis can start without spending time on investigating conditions.

In this technical report, dioxins (polychlorinated dibenzo-p-dioxin (PCDD) and polychlorinated dibenzofuran (PCDF) only) were analyzed in 44 types and 201 samples of foods and feeds using the GCMS-TQ8050 in combination with the method package. Additionally, the GC-MS/MS analysis results were compared with the analysis results from GC-HRMS, in order to evaluate the quantitative capabilities of both techniques.

## 2. EU Regulation Compliant GC-MS/MS Method Package for Dioxins in Foods

The features of the "EU Regulation Compliant GC-MS/MS Method Package for Dioxins in Foods" are shown below.

### Method Files Registered with the Optimal Conditions for the Analysis of Dioxins

To perform an analysis with TQ, the transition and collision energy (CE) of each compound must be optimized.

Additionally, when creating method files, it is necessary to calculate the retention times of all the target compounds and then to set up a complicated time program based on those results.

Optimized analytical conditions (including transition and CE) are pre-registered in the method files in this product. Additionally, the files are registered with retention times and retention indices, and the retention times can be adjusted automatically using the retention time adjustment function (AART: Automatic Adjustment of Retention Time), allowing analysis to start immediately.

The retention times and time programs can be adjusted automatically, even if the retention times for the measured compounds change, such as when conducting maintenance of the column tip.

### Report Creation Tool, Capable of Outputting Items Required by EU Regulations

Complicated calculations are required in analysis reports for dioxins in foods. A report creation tool is included in this product. It can automatically create reports showing items required by EU regulations.

In the analysis of dioxins, a single sample is fractionated into a dioxin (DXN) analysis sample and a polychlorinated biphenyl (PCB) analysis sample. However, depending on the pretreatment method, some of the PCBs can be eluted in the fraction for DXNs, so the analysis results for PCBs are sometimes divided into two parts (the analysis sample for both DXNs and PCBs, and the analysis sample for PCBs only).

With the report creation tool in this product, even if the analysis results for PCBs are divided into two parts, they can be combined, enabling support for a variety of samples and pretreatment methods.

## 3. Experiment

For the various food samples, pretreatment was performed using an automatic pretreatment unit (extraction: SpeedExtractor (BUCHI); purification: GO-xHT (Miura Co., Ltd.)). Nonane was used as the final solvent for the sample, and the amount of final solvent for the samples was 10 uL. For the STD, a mixture of DF-ST and DF-LCS from Wellington Laboratories was used.

In terms of the analytical conditions for GC-MS/MS, the conditions registered in the method package were used. The analytical conditions in detail are shown in Table 1. Additionally, the transition and collision energies for the compounds measured in this investigation are shown in Table 2.

1 Shimadzu Corporation
2 Laboratoire d'Etude des Résidus et Contaminants dans les Aliments

## Table 1   GC-MS/MS Analytical Conditions

**System Configuration**

| | |
|---|---|
| Pretreatment Unit (Extraction) | : SpeedExtractor (BUCHI) |
| Pretreatment Unit (Purification) | : GO-xHT (Miura Co., Ltd.) |
| Autosampler | : AOC-20i/s |
| GC-MS/MS | : GCMS-TQ8050 |
| Software | : GCMSsolution™ Ver. 4.45 SP1 |
| | LabSolutions Insight™ Ver. 3.2 SP1 |
| | GC-MS/MS method package for dioxins in foods |

**Analytical Conditions (AOC-20i/s)**

| | |
|---|---|
| # of Rinses with Solvent (Pre-run) | : 3 |
| # of Rinses with Solvent (Post-run) | : 3 |
| # of Rinses with Sample | : 0 |
| Plunger Speed (Suction) | : Low |
| Viscosity Comp. Time | : 0.2 sec. |
| Plunger Speed (Injection) | : High |
| Syringe Insertion Speed | : High |
| Pumping Times | : 5 |
| Inj. Port Dwell Time | : 0.3 sec. |
| Terminal Air Gap | : No |
| Plunger Washing Speed | : High |
| Washing Volume | : 6 uL |
| Injection Volume | : 2 uL |

**Analytical Conditions (GC)**

| | |
|---|---|
| Insert Liner | : Topaz® single gooseneck liner, with wool (Restek Corp., P/N: 23336) |
| Column | : SH-Rxi™-5Sil MS (60 m, 0.25 mm I.D., 0.25 µm), (SHIMADZU, P/N: 227-36036-02) |
| Injection Mode | : Splitless |
| Sampling Time | : 1.00 min. |
| Injection Temp. | : 280 °C |
| Column Oven Temp. | : 150 °C (1min.) → (20 °C/min.) → 220 °C → (2 °C /min.) → 260 °C (3 min.) → (5 °C /min.) → 320 °C (3.5 min.) |
| High Pressure Injection | : 450 kPa (1.5 min.) |
| Flow Control Mode | : Linear Velocity (45.6 cm/sec.) |
| Purge Flow | : 20 mL/min. |
| Carrier Gas | : Helium |

**Analytical Conditions (MS)**

| | |
|---|---|
| Ion Source Temp. | : 230 °C |
| Interface Temp. | : 300 °C |
| Detector Voltage | : 1.8 kV (Absolute) |
| Loop Time | : 0.8 sec. (for native compounds) 0.2 sec. (for labeled compounds) |
| Transitions | : Refer to Table 2 |

## Table 2   Transition and Collision Energies for the Measured Compounds

| I.D. | Compound Name | Retention Index | Quantitative Ion | CE | Reference Ion | CE |
|---|---|---|---|---|---|---|
| 1 | 2,3,7,8-Tetrachlorodibenzo-p-dioxin | 2383 | 319.9>256.9 | 20 | 321.9>258.9 | 20 |
| 2 | 1,2,3,7,8-Pentachlorodibenzo-p-dioxin | 2567 | 355.9>292.9 | 20 | 353.9>290.9 | 20 |
| 3 | 1,2,3,4,7,8-Hexachlorodibenzo-p-dioxin | 2742 | 389.8>326.9 | 22 | 391.8>328.9 | 22 |
| 4 | 1,2,3,6,7,8-Hexachlorodibenzo-p-dioxin | 2748 | 389.8>326.9 | 22 | 391.8>328.9 | 22 |
| 5 | 1,2,3,7,8,9-Hexachlorodibenzo-p-dioxin | 2762 | 389.8>326.9 | 22 | 391.8>328.9 | 22 |
| 6 | 1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin | 2936 | 423.8>360.8 | 22 | 425.8>362.8 | 22 |
| 7 | Octachlorodibenzo-p-dioxin | 3128 | 457.7>394.7 | 22 | 459.7>396.7 | 22 |
| 8 | 2,3,7,8-Tetrachlorodibenzofuran | 2357 | 303.9>240.9 | 28 | 305.9>242.9 | 28 |
| 9 | 1,2,3,7,8-Pentachlorodibenzofuran | 2513 | 339.9>276.9 | 30 | 337.9>274.9 | 30 |
| 10 | 2,3,4,7,8-Pentachlorodibenzofuran | 2553 | 339.9>276.9 | 30 | 337.9>274.9 | 30 |
| 11 | 1,2,3,4,7,8-Hexachlorodibenzofuran | 2694 | 373.8>310.9 | 30 | 375.8>312.9 | 30 |
| 12 | 1,2,3,6,7,8-Hexachlorodibenzofuran | 2701 | 373.8>310.9 | 30 | 375.8>312.9 | 30 |
| 13 | 2,3,4,6,7,8-Hexachlorodibenzofuran | 2732 | 373.8>310.9 | 30 | 375.8>312.9 | 30 |
| 14 | 1,2,3,7,8,9-Hexachlorodibenzofuran | 2778 | 373.8>310.9 | 30 | 375.8>312.9 | 30 |
| 15 | 1,2,3,4,6,7,8-Heptachlorodibenzofuran | 2867 | 407.8>344.8 | 30 | 409.8>346.8 | 30 |
| 16 | 1,2,3,4,7,8,9-Heptachlorodibenzofuran | 2965 | 407.8>344.8 | 30 | 409.8>346.8 | 30 |
| 17 | Octachlorodibenzofuran | 3137 | 441.8>378.8 | 30 | 443.8>380.8 | 30 |
| 18 | 1,2,3,4-Tetrachlorodibenzo-p-dioxin-13C12 | 2287 | 331.9>268.0 | 20 | 333.9>270.0 | 20 |
| 19 | 2,3,7,8-Tetrachlorodibenzo-p-dioxin-13C12 | 2382 | 331.9>268.0 | 20 | 333.9>270.0 | 20 |
| 20 | 1,2,3,7,8-Pentachlorodibenzo-p-dioxin-13C12 | 2567 | 367.9>303.9 | 20 | 365.9>301.9 | 20 |
| 21 | 1,2,3,4,7,8-Hexachlorodibenzo-p-dioxin-13C12 | 2742 | 401.8>337.9 | 22 | 399.9>335.9 | 22 |
| 22 | 1,2,3,6,7,8-Hexachlorodibenzo-p-dioxin-13C12 | 2747 | 401.8>337.9 | 22 | 399.9>335.9 | 22 |
| 23 | 1,2,3,7,8,9-Hexachlorodibenzo-p-dioxin-13C12 | 2762 | 401.8>337.9 | 22 | 399.9>335.9 | 22 |
| 24 | 1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin-13C12 | 2935 | 435.8>371.8 | 22 | 437.8>373.8 | 22 |
| 25 | Octachlorodibenzo-p-dioxin-13C12 | 3127 | 469.8>405.8 | 22 | 471.8>407.8 | 22 |
| 26 | 2,3,7,8-Tetrachlorodibenzofuran-13C12 | 2357 | 315.9>251.9 | 28 | 317.9>253.9 | 28 |
| 27 | 1,2,3,7,8-Pentachlorodibenzofuran-13C12 | 2513 | 351.9>287.9 | 30 | 349.9>285.9 | 30 |
| 28 | 2,3,4,7,8-Pentachlorodibenzofuran-13C12 | 2553 | 351.9>287.9 | 30 | 349.9>285.9 | 30 |
| 29 | 1,2,3,4,7,8-Hexachlorodibenzofuran-13C12 | 2694 | 385.8>321.9 | 30 | 387.8>323.9 | 30 |
| 30 | 1,2,3,6,7,8-Hexachlorodibenzofuran-13C12 | 2701 | 385.8>321.9 | 30 | 387.8>323.9 | 30 |
| 31 | 2,3,4,6,7,8-Hexachlorodibenzofuran-13C12 | 2732 | 385.8>321.9 | 30 | 387.8>323.9 | 30 |
| 32 | 1,2,3,7,8,9-Hexachlorodibenzofuran-13C12 | 2778 | 385.8>321.9 | 30 | 387.8>323.9 | 30 |
| 33 | 1,2,3,4,6,7,8-Heptachlorodibenzofuran-13C12 | 2867 | 419.8>355.9 | 30 | 421.8>357.9 | 30 |
| 34 | 1,2,3,4,7,8,9-Heptachlorodibenzofuran-13C12 | 2965 | 419.8>355.9 | 30 | 421.8>357.9 | 30 |
| 35 | Octachlorodibenzofuran-13C12 | 3137 | 453.8>389.8 | 30 | 455.8>391.8 | 30 |

# 4. Analysis Results

## 4-1. Analysis Results for the STD

In the analysis of dioxins in foods, the maximum permitted concentrations (Maximum Levels, hereinafter "ML") are prescribed for each food and feed. With the food and feed samples in this investigation, the ML for pig's fat and pig's meat were the lowest at 1 pg/g of fat (sum of dioxins (WHO-PCDD/F-TEQ)). Additionally, the limit of quantitation (hereinafter "LOQ") required for each compound in the analysis depends on the food or feed sample's ML, the pretreatment method, and the TEF (toxic equivalence factor) of each compound. The compounds 2,3,7,8-Tetrachlorodibenzo-p-dioxin and 1,2,3,7,8-Pentachlorodibenzo-p-dioxin have the highest TEF (TEF=1), so their LOQ are lower than for other compounds. In this investigation, the LOQ for 2,3,7,8-Tetrachlorodibenzo-p-dioxin and 1,2,3,7,8-Pentachlorodibenzo-p-dioxin in pig's fat and pig's meat was 0.060 pg/uL at the concentration in the final vial.

In the EU regulations, for each compound, at least one of the criteria shown below (a partial excerpt from EU589/2014 and 644/2017) must be satisfied at the LOQ.

### 1. S/N Ratio (Hereinafter "Method 1")

The concentration of an analyte in the extract of a sample which produces an instrumental response at two different ions to be monitored with a S/N (signal/noise) ratio of 3:1 for the less intensive raw data signal.

### 2. Lowest Concentration Point on the Calibration Curve (Method 2)

The lowest concentration point on a calibration curve that gives an acceptable ($\leq$ 30 %) and consistent (measured at least at the start and at the end of an analytical series of samples) deviation to the average relative response factor calculated for all points on the calibration curve in each series of samples.

In this technical report, for the purposes of confirmation, an evaluation was performed using both criteria.

As noted above, for 2,3,7,8-Tetrachlorodibenzo-p-dioxin, it is necessary to set the LOQ to 0.060 pg/uL or less. Accordingly, before analysis, the STD was prepared so that the concentration of each compound was 0.050 pg/uL. (The concentration was double however for Octachlorodibenzo-p-dioxin and Octachlorodibenzofuran.) From the results of the analysis, it was evident that the criteria for Method 1 were satisfied for all compounds. The S/N ratios for each compound are shown in Fig. 1.

Additionally, with Method 2, a calibration curve was created with all six points used, including the two at concentrations less than 0.060 pg/uL (0.025 pg/uL and 0.050 pg/uL). The concentrations for each compound at each calibration curve point (level) are shown in Table 3. For each compound, when the level 1 RRF and average RRF were compared, it was found that all compounds satisfied the criteria for Method 2. The RRF deviations for each compound are shown in Table 3.

From the above-mentioned results, it was evident that at the LOQ, the criteria were satisfied for all compounds.

Table 3  Each Calibration Point Concentration and RRF for the Measured Compounds

| I.D. | Compound Name | TEF | Calibration Point Concentration | | | | | | Average RRF | RRF (level 1) | RRFDev (%) (Level 1) |
|------|---------------|-----|---------|---------|---------|---------|---------|---------|-------------|--------------|---------------------|
| | | | Level 1 | Level 2 | Level 3 | Level 4 | Level 5 | Level 6 | | | |
| 1 | 2,3,7,8-Tetrachlorodibenzo-p-dioxin | 1 | 0.025 | 0.050 | 0.100 | 0.250 | 0.500 | 1.000 | 1.07 | 1.15 | 8.10 |
| 2 | 1,2,3,7,8-Pentachlorodibenzo-p-dioxin | 1 | 0.025 | 0.050 | 0.100 | 0.250 | 0.500 | 1.000 | 1.09 | 0.97 | 10.56 |
| 3 | 1,2,3,4,7,8-Hexachlorodibenzo-p-dioxin | 0.1 | 0.025 | 0.050 | 0.100 | 0.250 | 0.500 | 1.000 | 1.14 | 1.39 | 22.26 |
| 4 | 1,2,3,6,7,8-Hexachlorodibenzo-p-dioxin | 0.1 | 0.025 | 0.050 | 0.100 | 0.250 | 0.500 | 1.000 | 0.95 | 0.92 | 2.72 |
| 5 | 1,2,3,7,8,9-Hexachlorodibenzo-p-dioxin | 0.1 | 0.025 | 0.050 | 0.100 | 0.250 | 0.500 | 1.000 | 1.03 | 1.25 | 21.44 |
| 6 | 1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin | 0.01 | 0.025 | 0.050 | 0.100 | 0.250 | 0.500 | 1.000 | 0.92 | 0.82 | 11.46 |
| 7 | Octachlorodibenzo-p-dioxin | 0.0003 | 0.050 | 0.100 | 0.200 | 0.500 | 1.000 | 2.000 | 1.19 | 1.04 | 12.21 |
| 8 | 2,3,7,8-Tetrachlorodibenzofuran | 0.1 | 0.025 | 0.050 | 0.100 | 0.250 | 0.500 | 1.000 | 1.10 | 1.05 | 4.66 |
| 9 | 1,2,3,7,8-Pentachlorodibenzofuran | 0.03 | 0.025 | 0.050 | 0.100 | 0.250 | 0.500 | 1.000 | 1.04 | 1.00 | 3.23 |
| 10 | 2,3,4,7,8-Pentachlorodibenzofuran | 0.3 | 0.025 | 0.050 | 0.100 | 0.250 | 0.500 | 1.000 | 0.97 | 0.89 | 7.59 |
| 11 | 1,2,3,4,7,8-Hexachlorodibenzofuran | 0.1 | 0.025 | 0.050 | 0.100 | 0.250 | 0.500 | 1.000 | 1.03 | 0.82 | 20.72 |
| 12 | 1,2,3,6,7,8-Hexachlorodibenzofuran | 0.1 | 0.025 | 0.050 | 0.100 | 0.250 | 0.500 | 1.000 | 1.09 | 1.36 | 24.62 |
| 13 | 2,3,4,6,7,8-Hexachlorodibenzofuran | 0.1 | 0.025 | 0.050 | 0.100 | 0.250 | 0.500 | 1.000 | 1.09 | 1.39 | 27.83 |
| 14 | 1,2,3,7,8,9-Hexachlorodibenzofuran | 0.1 | 0.025 | 0.050 | 0.100 | 0.250 | 0.500 | 1.000 | 1.06 | 1.23 | 16.10 |
| 15 | 1,2,3,4,6,7,8-Heptachlorodibenzofuran | 0.01 | 0.025 | 0.050 | 0.100 | 0.250 | 0.500 | 1.000 | 1.17 | 1.05 | 10.37 |
| 16 | 1,2,3,4,7,8,9-Heptachlorodibenzofuran | 0.01 | 0.025 | 0.050 | 0.100 | 0.250 | 0.500 | 1.000 | 1.02 | 0.97 | 4.97 |
| 17 | Octachlorodibenzofuran | 0.0003 | 0.050 | 0.100 | 0.200 | 0.500 | 1.000 | 2.000 | 1.00 | 0.84 | 15.80 |

2,3,7,8-Tetrachlorodibenzo-p-dioxin
S/N:285

1,2,3,7,8-Pentachlorodibenzo-p-dioxin
S/N:1658

1,2,3,4,7,8-Hexachlorodibenzo-p-dioxin
S/N:396

Octachlorodibenzo-p-dioxin
S/N:2518

2,3,7,8-Tetrachlorodibenzofuran
S/N:2117

1,2,3,7,8-Pentachlorodibenzofuran
S/N:1882

1,2,3,7,8,9-Hexachlorodibenzofuran
S/N:546

1,2,3,4,6,7,8-Heptachlorodibenzofuran
S/N:1784

Octachlorodibenzofuran
S/N:4282
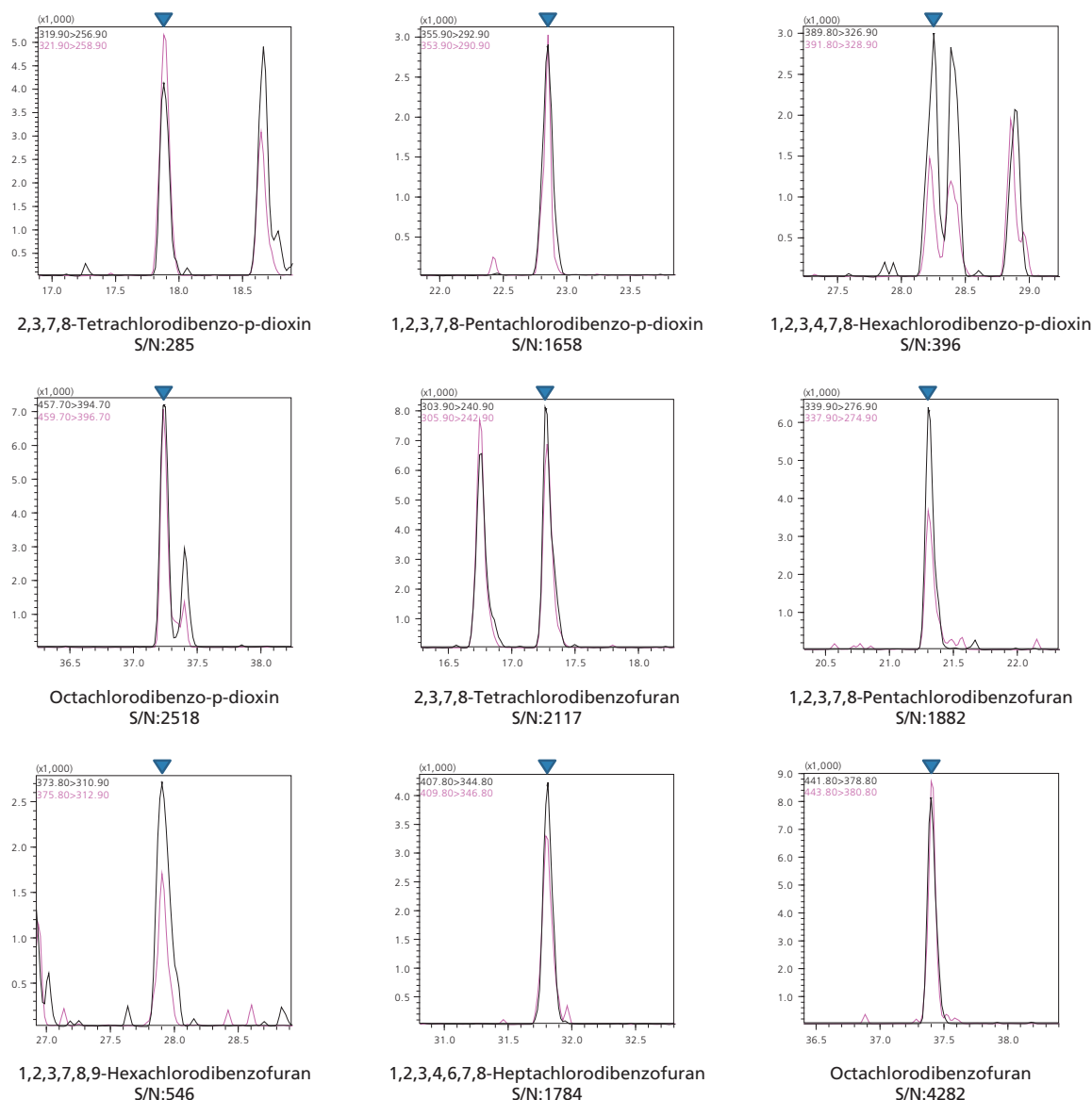
Fig. 1   Chromatograms for a Concentration of 0.050 pg/uL

## 4-2.  Analysis Results for the Test Samples

As previously noted, the strength of toxicity differs for each dioxin compound. The TEF, which is calculated for each compound by taking the toxicity of 2,3,7,8-Tetrachlorodibenzo-p-dioxin as 1, is used as an index of strength. Note that the TEF values for each compound are as shown in Table 3.

The ML for the dioxins in foods and feeds are prescribed by their toxic equivalents (TEQ). The TEQ is calculated by multiplying the concentration of each compound by the TEF, and then calculating the total TEQ for all compounds.

In this investigation, 44 types and 201 samples of foods were analyzed using GC-MS/MS. Additionally, the same samples were analyzed with GC-HRMS, and the results were compared with the GC-MS/MS analysis results. For this comparison, the TEQ was calculated by multiplying the concentration in the final vial for each compound by the TEF, and then calculating a total TEQ for all com-

pounds. The results were tallied separately for each food and feed.

The results for typical foods and feeds are shown in Fig. 2. The results for all foods and feeds are shown in Fig. 3. Additionally, typical chromatograms for each compound are shown in Fig. 4.

Fig. 2 shows a comparison of the TEQ values for GC-MS/MS and GC-HRMS by food and feed. The sample is indicated on the horizontal axis, and the TEQ for each sample is indicated on the vertical axis.

Fig. 3 shows the GC-HRMS TEQ on the horizontal axis, and the GC-MS/MS TEQ on the vertical axis. If they were correlated, the values would approach a straight line with a slope of 1 (the blue dashed line in the figure).

A TEQ of 0.060 pg/uL and a TEQ of 0.025 pg/uL are marked as indicators for the samples.

TEQ 0.060 pg/uL: If even one compound is detected at a concentration higher than the LOQ, the total TEQ value will be higher than 0.060 pg/uL. Accordingly, a straight line (red dashed line in the figure) is drawn on the vertical axis in Fig. 2, and on the horizontal axis and vertical axis in Fig. 3 to mark 0.060 pg/uL.

TEQ 0.025 pg/uL: If a compound with the highest TEF (2,3,7,8-Tetrachlorodibenzo-p-dioxin or 1,2,3,7,8-Pentachlorodibenzo-p-dioxin) is detected at a higher concentration than the lowest point in the calibration curve, the total TEQ value will be higher than 0.025 pg/uL. Accordingly, a straight line (green dashed line in the figure) is drawn on the vertical axis in Fig. 2, and on the horizontal axis and vertical axis in Fig. 3 to mark 0.025 pg/uL.

In order to check the correlation between GC-MS/MS and GC-HRMS, a regression line was calculated with respect to Fig. 3, and a t-test was performed for the slope and intercept. The calculated results are shown in Table 4. The 95 % confidence limits for the intercept and slope are extremely close to 0 and 1, respectively.

Next, the distribution of the TEQ ratios for GC-MS/MS and GC-HRMS was calculated and checked in detail.

For samples with a TEQ of at least 0.060 pg/uL, when the TEQ ratio (%) was calculated, for at least 98 % of the samples, the ratio was between 50 % and 200 %, indicating a similar TEQ value for both systems. (Table 5)

In contrast, for samples with a TEQ less than 0.060 pg/uL, 79 % of the samples had a ratio between 50 % and 200 %, indicating a significant difference for 21 % of the samples. For many of the samples, the lower the TEQ, the greater the difference. 92 % of the samples with a ratio less than 50 % or more than 200 % had a TEQ less than 0.025 pg/uL. (Table 6)

From the above-mentioned results, it was evident that GC-MS/MS and GC-HRMS provide similar TEQ values for samples with a TEQ higher than 0.060 pg/uL. Additionally, it was evident that the lower the TEQ, the greater the number of samples with a significant difference in TEQ values.

Table 4   Results of the t-Tests for the Intercept and Slope

| | Coefficient | Standard Error | t | 95 % Confidence Interval | |
| --- | --- | --- | --- | --- | --- |
| | | | | Lower Limit | Upper Limit |
| Intercept | -0.005 | 0.001 | -3.235 | -0.008 | -0.002 |
| Slope | 1.049 | 0.001 | 741.500 | 1.046 | 1.051 |

Table 5   Distribution of the Ratio of Total TEQ Values
for Samples with a TEQ of at Least 0.060 pg/uL

| | TEQ Ratio (TQ/Sector) (%) | | |
| --- | --- | --- | --- |
| | <50 | 50 - 200 | 200< |
| Number of Samples (pc) | 2 | 87 | 0 |
| Distribution (%) | 2 | 98 | 0.00 |

Table 6   Distribution of the Ratio of Total TEQ Values
for Samples with a TEQ Less Than 0.060 pg/uL

| | TEQ Ratio (TQ/Sector) (%) | | |
| --- | --- | --- | --- |
| | <50 | 50 - 200 | 200< |
| Number of Samples (pc) | 21* | 92 | 4 |
| Distribution (%) | 18 | 79 | 3 |

* 19 of the 21 samples with a ratio under 50 % had a TEQ less than 0.025 pg/uL. It was evident that the lower the total TEQ value, the greater the tendency for a difference to arise.
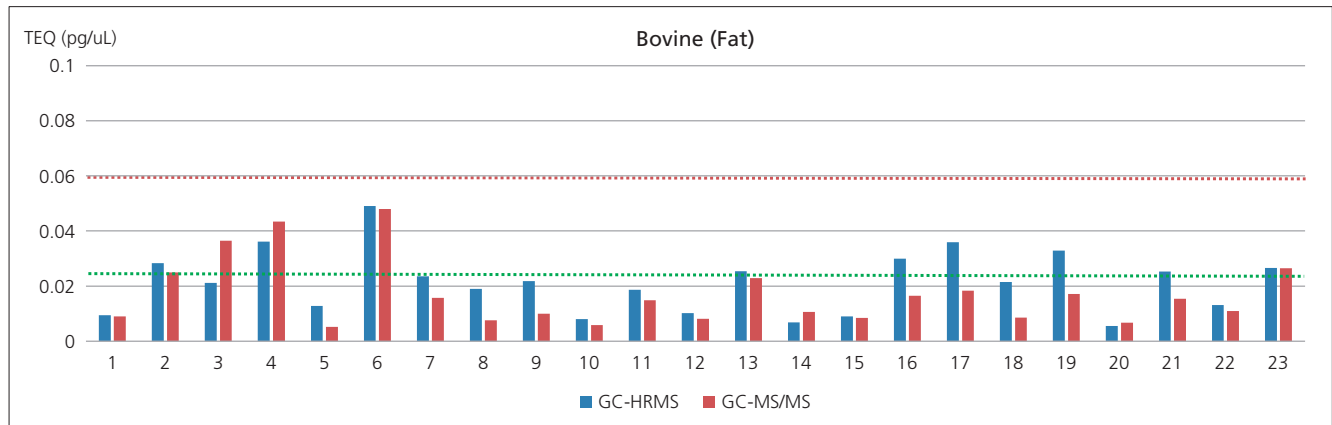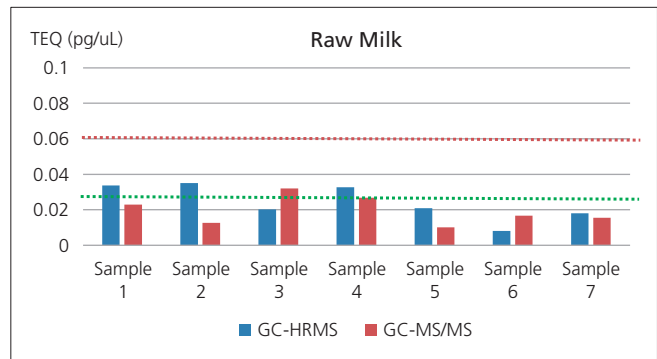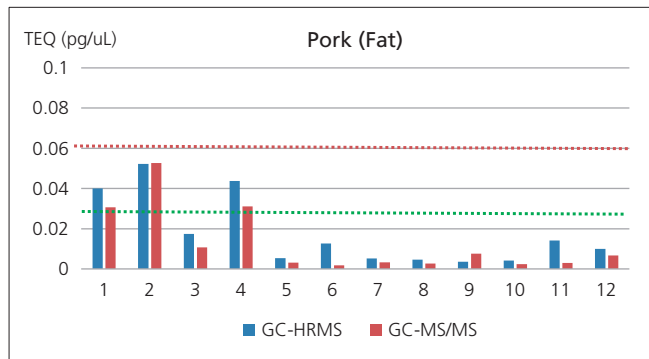


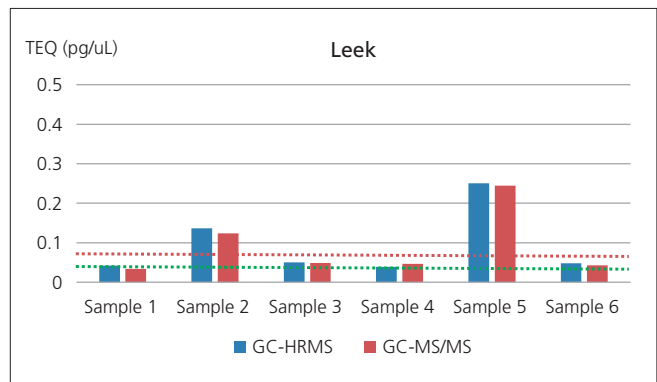Fig. 2   Comparison of the TEQ Results for Each Food and Feed

Fig. 2   Comparison of the TEQ Results for Each Food and Feed

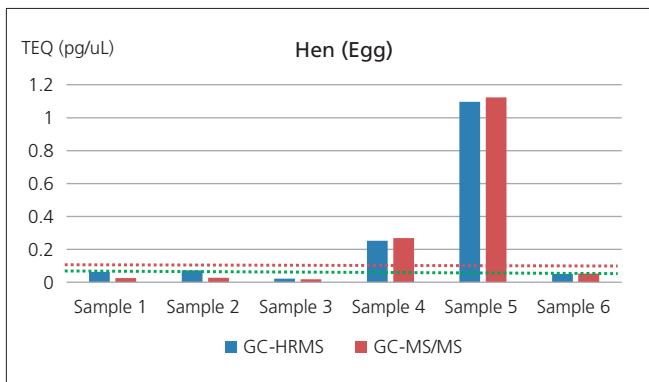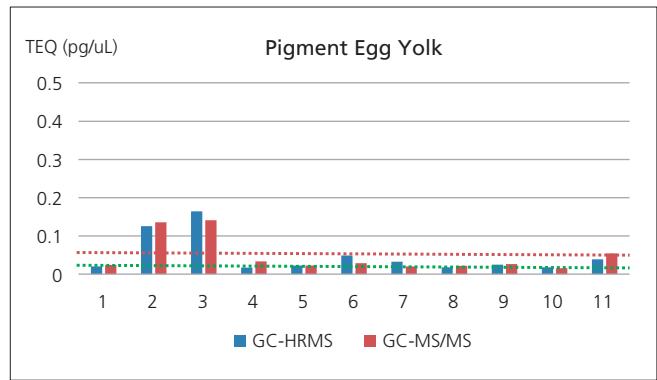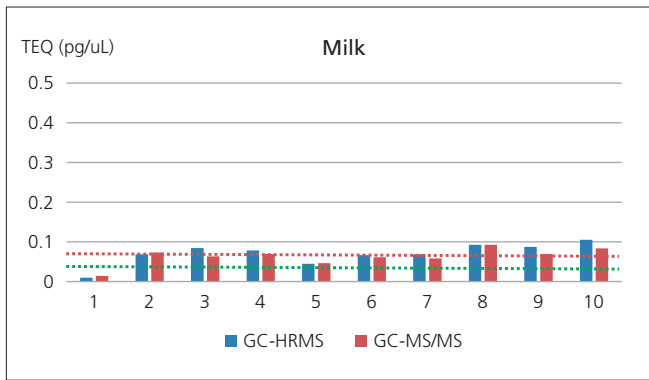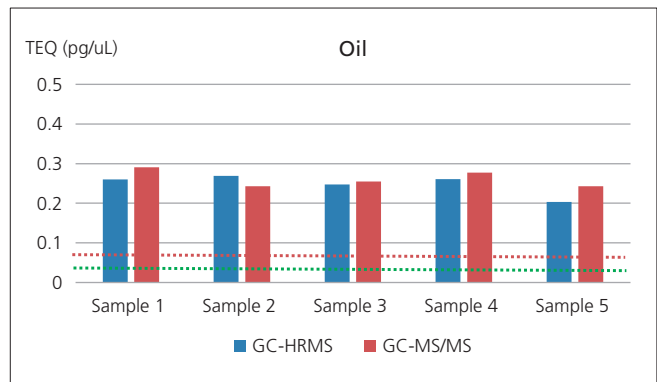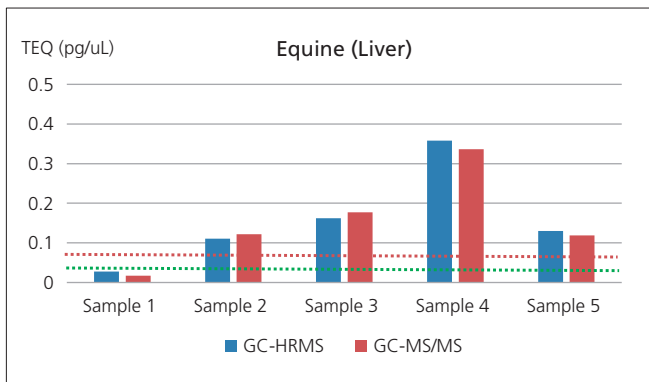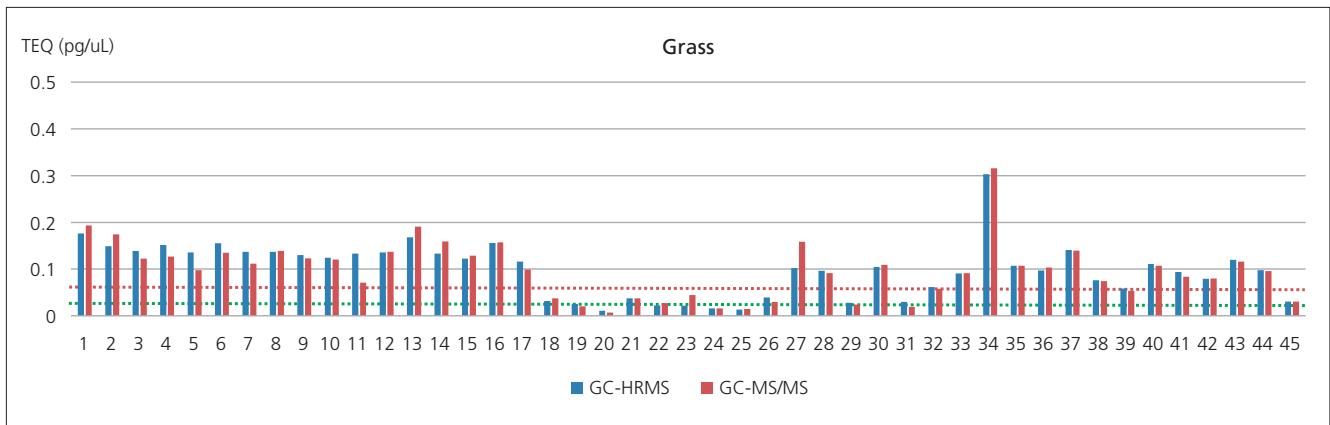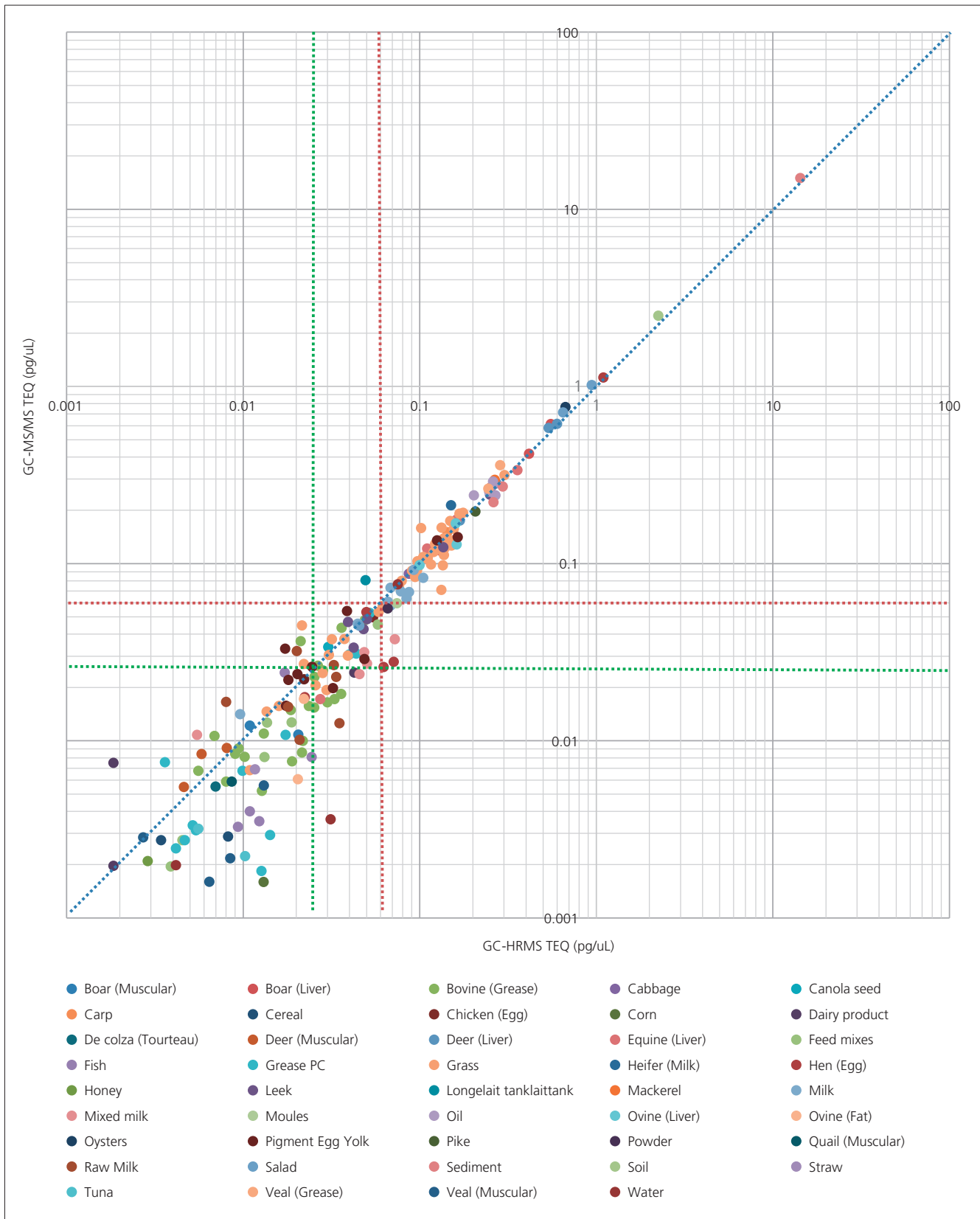Fig. 3   Comparison of the TEQ Results for Each Food and Feed

2,3,7,8-Tetrachlorodibenzo-p-dioxin
(Concentration 0.030 pg/uL)

1,2,3,7,8-Pentachlorodibenzo-p-dioxin
(Concentration 0.055 pg/uL)

1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin
(Concentration 0.030 pg/uL)

2,3,4,7,8-Pentachlorodibenzofuran
(Concentration 0.066 pg/uL)

1,2,3,4,7,8,9-Heptachlorodibenzofuran
(Concentration 0.018 pg/uL)

Octachlorodibenzofuran
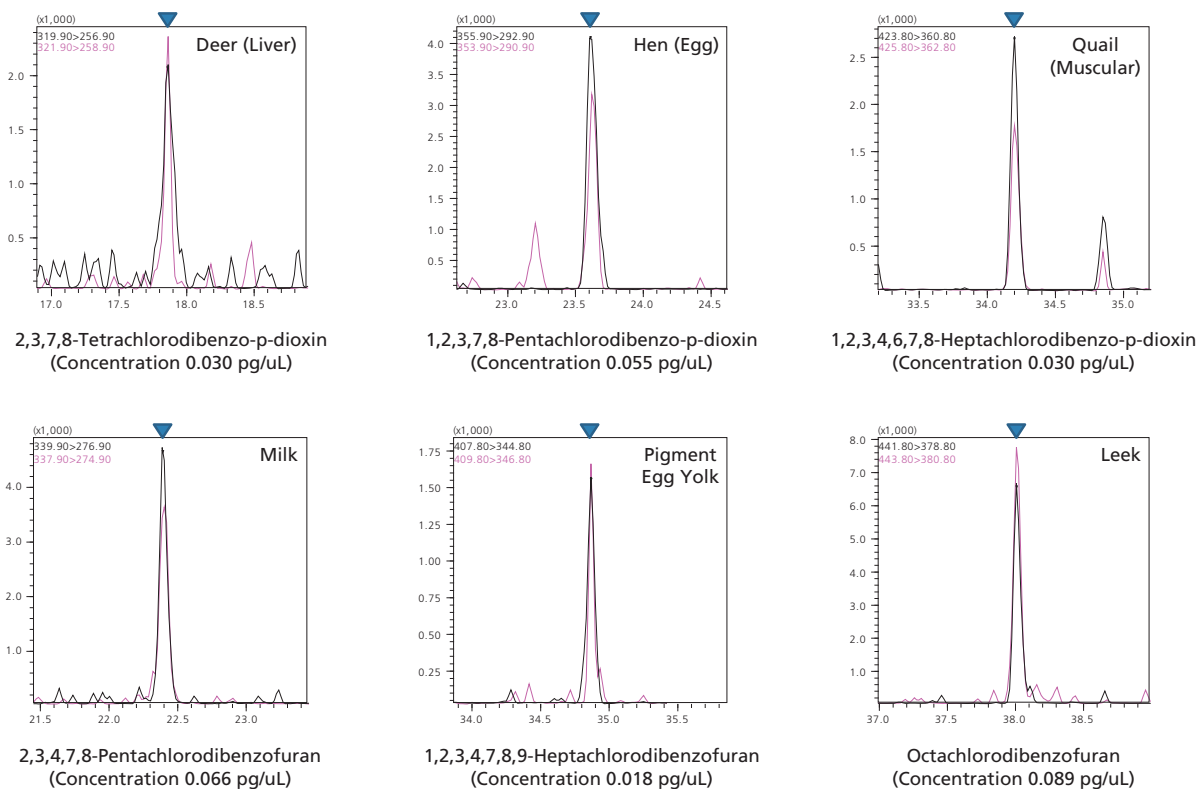(Concentration 0.089 pg/uL)

Fig. 4   Chromatograms of Dioxins in Various Samples

# 5. Conclusion

In this technical report, dioxins were analyzed in 44 types and at least 201 samples of foods and feeds using the GCMS-TQ8050 and the "EU Regulation Compliant GC-MS/MS Method Package for Dioxins in Foods". Additionally, the GC-MS/MS analysis results were compared with the analysis results from GC-HRMS in order to assess the quantitative capabilities of both methods.

Firstly, before analyzing the foods and feeds, a STD was analyzed using GC-MS/MS, and it was confirmed that the criteria were satisfied at the LOQ.

Next, the foods and feeds were analyzed, and the results were compared with those from magnetic sector GC-MS. For the comparison, the TEQ ratio was calculated for GC-MS/MS and GC-HRMS. For samples with a higher TEQ than 0.060 pg/uL (TEQ when any of the compounds was detected at a higher concentration than the LOQ), GC-MS/MS and GC-HRMS provided similar TEQ values in at least 98 % of the samples. Additionally, it was evident that the number of samples with a significant difference in TEQ increases as the TEQ value decreases.

From the above-mentioned results, it is evident that analysis with GCMS-TQ8050 and method package provides a quantitative capability equivalent to that from GC-HRMS for samples at the concentration levels required for analysis. However, at concentrations below the required level, differences in quantitative capability could arise. For this reason, it is necessary to be aware of the system status by confirming quantitative capability at the LOQ, and evaluating whether there has been a decrease in sensitivity.

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## Application News

Liquid Chromatography Mass Spectrometry

### Sensitive method for the determination of Fipronil in egg using UHPLC-MS/MS [LCMS-8060]

**No.** SCA_210_038

### ▪ Introduction

The broad-spectrum insecticide Fipronil from the group of phenylpyrazoles is used in many countries as a biocide and plant protection product against fleas, lice, ticks, cockroaches, mites and other insects. The use as plant protection product is restricted to seed treatment in the European Union since 2007. It is also an active compound in veterinary products fighting tick and flea infestations in dogs and cats. But its use in food-producing animals is not permitted. However, due to the illegal use as addition to the cleaning supplies used in poultry farm the eggs, egg products and meat were found to be contaminated in summer 2017 in 15 European countries.
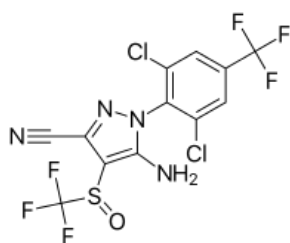
**Fipronil**
MF $C_{12}H_4Cl_2F_6N_4OS$
MW 437,1 g/mol

### ▪ Sample preparation

Compound extraction was performed using a simplyfied QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method.
5 g of egg (egg white and egg yolk) were weighted into a 50 mL polypropylene tube and spiked with a respective amount of Fipronil (and other pesticides using dilutions of RESTEK LC Multiresidue Pesticide Standard #5, Cat. 31976). 5 mL of acetonitrile was added and the samples were mixed vigorously. After that 2 g of $MgSO_4$ and 0.5 g of NaCl were added, samples were mixed again and centrifuged at 3000 rpm for 5 minutes. The supernatant was transferred into a glass vial.

### ▪ Materials and methods

Extracts were analyzed using a method set up with Shimadzus LC/MS/MS Method Package for Residual Pesticides Version 2 and a Nexera X2 UHPLC system coupled to a LCMS-8060 mass spectrometer. Analysis was carried out using MRM (Multi Reaction Monitoring) mode.

| LC system | Nexera X2 (Shimadzu, Japan) |
|---|---|
| Analytical column | Raptor Biphenyl™ 100 x 2.1 mm, 2.7 µm (RESTEK) |
| Column oven temperature | 35 °C |
| Injection volume | 2 µl (using POISe*) |
| Mobile Phase A | 2 mM ammonium formate + 0.002% formic acid - Water |
| Mobile Phase B | 2 mM ammonium formate + 0.002% formic acid - Methanol |
| **Mass spectrometer** | **LCMS-8060 (Shimadzu, Japan)** |
| Interface voltage | -3 kV |
| Q1 resolution | Unit (0.7 Da FWHM) |
| Q3 resolution | Unit (0.7 Da FWHM) |
| Nebulizing gas flow | 3 L/min |
| Drying gas flow | 10 L/min |
| Heating gas flow | 10 L/min |
| DL temperature | 150 °C |
| Heat block temperature | 300 °C |
| Interface Temperature | 350 °C |

*Performance Optimising Injection Sequence

### ▪ Calibration

The matrix matched calibration curve (Figure 1) was prepared according to the method described before ranging from 0.025 mg/kg to 2 mg/kg.

Control samples at 0.05 mg/kg and 0.5 mg/kg correspond to the calibration curve. Figure 2 shows a typical chromatogram of the lowest calibration point (0.025 mg/kg)
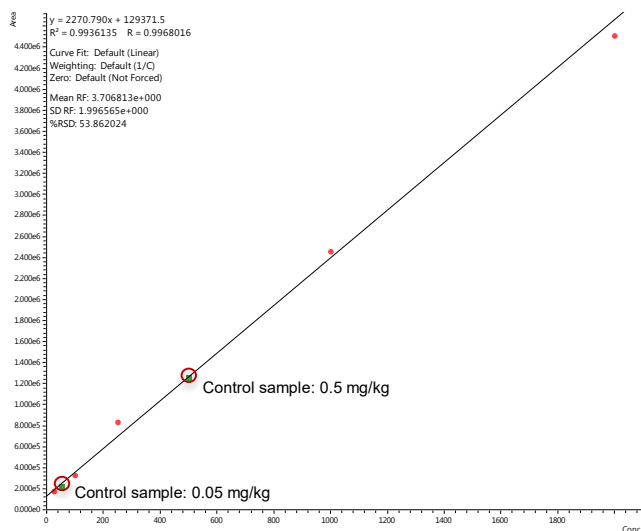


Figure 1: Calibration curve of Fipronil in egg ranging from 0.025 mg/kg to 2 mg/kg
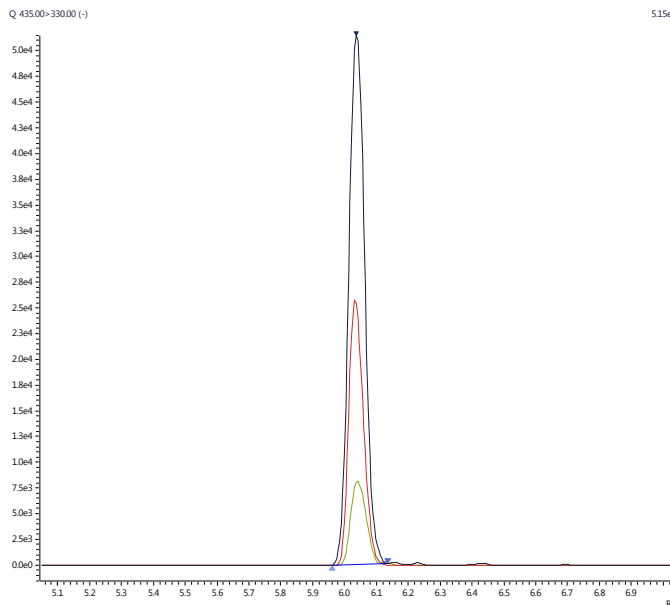


Figure 2: Chromatogram of Fipronil in egg at a concentration of 0.025 mg/kg

### ▪ Conclusion

By using the LC/MS/MS method package for residual pesticides V2 and a simplyfied QuEChERS sample preparation a method for the determination of Fipronil in eggs could be set up rapidly without further method development covering the calibration range from 0.025 mg/kg to 2 mg/kg.

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# *Application Note*

## Application Note No.4 (Lifescience)

**Lifescience**

## Food Allergen Test
## – Application of MultiNA –

Yuji Sogabe

## 1. What are Allergies?

We have immune functions that protect our bodies by creating antibodies to substances entering the body (antigens) that are foreign and hostile to the body. This is known as the "antigen-antibody reaction" or "immune response." When the same antigen subsequently enters the body, the memorized antibody activates and binds to the antigen to render it harmless.

However, if the immune functions go out of control, excess antibodies can be created or harmful antibodies produced in the body. This imbalance causes allergic diseases. Typical allergic diseases include atopic dermatitis, allergic rhinitis (hay fever, etc.), allergic conjunctivitis, allergic gastroenteritis, asthma, childhood asthma, food allergy, drug allergy, and hives.

Immunity was originally intended to protect the body from harmful substances. However, for people with a certain disposition, the immune function can activate in response to foods, pollen, dust and other substances which are usually harmless. People predisposed to such symptoms are said to have an "allergic predisposition."

When specific substances enter the body of a person with an allergic predisposition, the antibodies act abnormally to cause specific symptoms.

## 2-1 What are Food Allergies?

A hypersensitive immune reaction resulting from eating specific foods is called a "food allergy." Food allergies can cause a diverse range of symptoms, including skin symptoms such as hives and eczemas; gastrointestinal symptoms such as diarrhea, vomiting, and stomachache; and respiratory symptoms such as coughing and breathing difficulties. In severe cases, food allergies can lead to systemic symptoms, such as anaphylactic shock.

The substances causing food allergies and the amounts required differ from person to person. The reaction also differs according to the person's physical condition. For children, in particular, food allergies are often caused by the so-called "three major allergens": eggs, milk, and wheat. Of these, chicken eggs are the major cause of food allergies. Other causes are fish (in particular, blue-backed fish), meat (in particular, pork), shellfish, shrimp, crab, soybeans, cereals, and buckwheat.

Food allergies are mainly caused by proteins derived from the ingredients in the food. The three major allergens – eggs, milk, and wheat – are all foods with a high protein content. Normally, the proteins in foods are broken down in the stomach and intestines and absorbed as amino acids and peptides (several amino acids linked together). These small molecules do not normally cause allergies.

However, when the digestive tract and its functions are immature during infancy, inadequately digested proteins (oligopeptides) are often absorbed and are said to cause many food allergies. When the digestive functions are suppressed during illness, in particular, more undigested substances than normal pass through the digestive tract. As a result, undigested substances are more frequently absorbed and the incidence of allergies also increases.

The 2005 Ministry of Health, Labour and Welfare Science Research Report (Fig. 1) of Japan lists the following incidences of food allergies:

1. Eggs (38 % of total)
2. Dairy products (16 % of total)
3. Wheat (8 % of total)
4. Fruit (6 % of total)
5. Buckwheat (5 % of total)
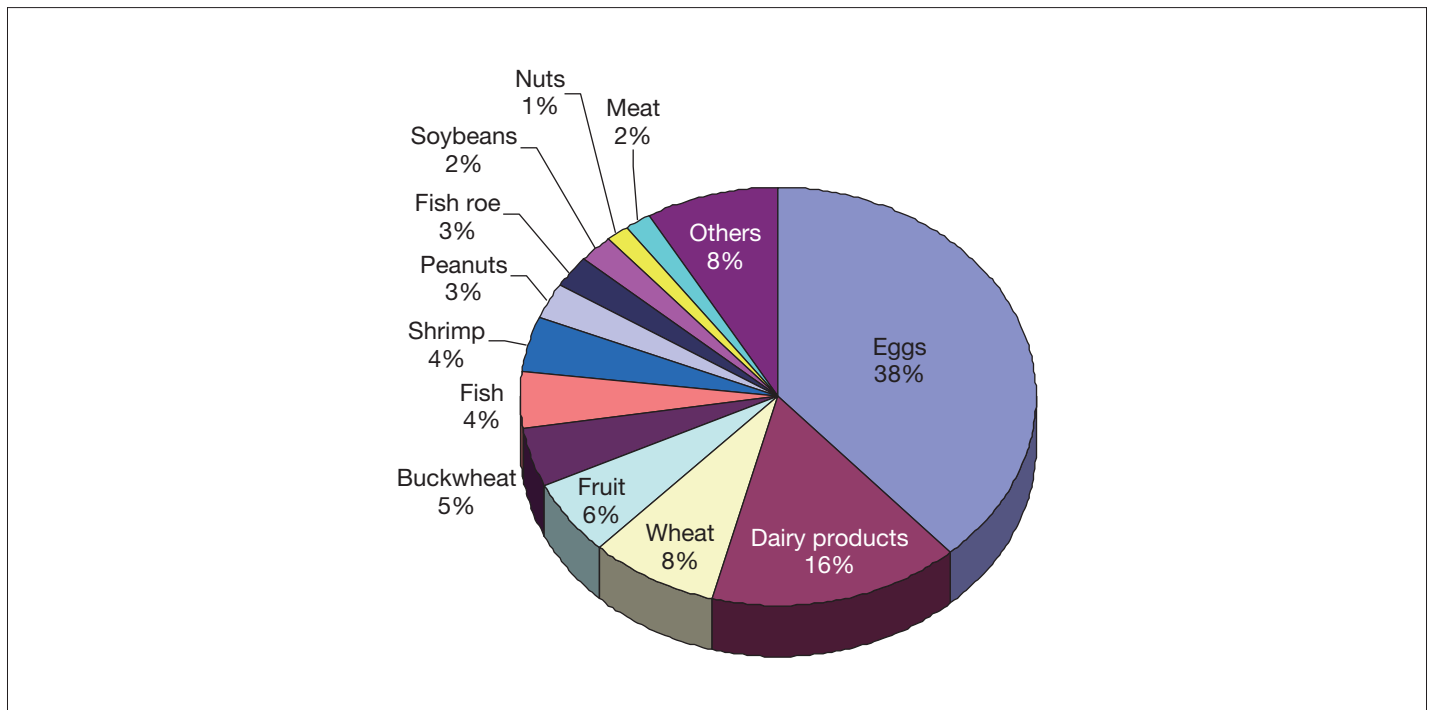6. Shrimp (4 % of total)
7. Peanuts (3 % of total)



**Fig. 1 Proportions of Foods Causing Allergies (source: 2005 Ministry of Health, Labour and Welfare Science Research Report of Japan)**

### 3. Food Labeling

Japan was the world's earliest adopter of a labeling system for foods containing allergens (see Fig. 2).

The labeling of foods containing allergens is categorized into "Mandatory" (7 specified ingredients) and "Recommended" (18 specified ingredients).

Of the foods discovered to cause allergies in recent investigations, the five items with a high incidence or severity – eggs, milk, wheat, buckwheat, and peanuts – were prescribed as "specified ingredients" under the Japanese Ordinance for Enforcement of the Food Sanitation Act. Foods containing these ingredients were subject to mandatory labeling from April 2002. Two more items were added from June 2008: shrimp and crab. Labeling is required for foods containing 10 μg/g or higher of these seven specified ingredients, even if they are impurities mixed in during the manufacturing process. Labeling the possibility that the items could be included, such as "May contain xxx," is not permitted.

Eighteen other items for which labeling is recommended (items corresponding to specified ingredients) have been notified: abalone, squid, salmon roe, orange, kiwi fruit, beef, walnuts, salmon, mackerel, soybeans, chicken, banana, pork, matsutake mushroom, peach, yam, apple, and gelatin (Table 1). Labeling is intended to provide information to consumers to avoid health hazards due to allergies. Consequently, labeling must inform of even trace levels of specified ingredients contained in or mixed in a food product.

(＊However, mandatory labeling of manufactured, processed, or imported food products was deferred to 3 June 2010.)

Recently, more and more food companies are producing products free of egg, milk, wheat, buckwheat, peanuts and other allergens. They implement strict product development, ingredient selection, production line cleaning (and subsequent checks), and inspections of individual production lots (according to the official method prescribed by the Japanese Ministry of Health, Labour and Welfare).
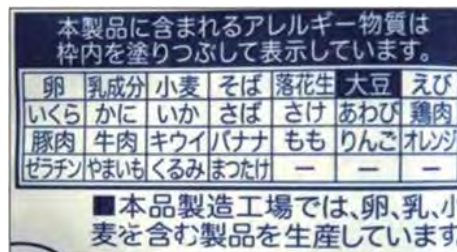


This product contains the allergens in the highlighted frames below.

| Egg | Milk | Wheat | Buckwheat | Peanuts | Soybeans | Shrimp |
|---|---|---|---|---|---|---|
| Salmon roe | Crab | Squid | Mackerel | Salmon | Abalone | Chicken |
| Pork | Beef | Kiwi fruit | Banana | Peach | Apple | Orange |
| Gelatin | Yam | Walnuts | Matsutake mushroom | – | – | – |

■ The factory that produced this product manufactures products containing egg, milk, and wheat.

**Fig. 2 Example of Labeling**

**Table 1 Items Labeled as Allergens**
**(Source: March 2009 revision of "Handbook for Labeling of Processed Foods Containing Allergens," Japanese Ministry of Health, Labour and Welfare)**

| Labelling | Term | Name |
|---|---|---|
| Mandatory | Specified ingredients (7 items) | Egg, milk, wheat, buckwheat, peanuts, shrimp, crab |
| Recommended | Items pursuant to specified ingredients (18 items) | Abalone, squid, salmon roe, orange, kiwi fruit, beef, walnuts, salmon, mackerel, soybeans, chicken, banana, pork, matsutake mushroom, peach, yam, apple, and gelatin |

*The scope of the specified ingredients is basically the range designated by the numbers in the Japan Standard Commodity Classification (JSCC). (For more details, see the March 2009 revision of "Handbook for Labeling of Processed Foods Containing Allergens," Japanese Ministry of Health, Labour and Welfare.)

## 4. Analysis of Allergenic Substances

Test methods have been established for 20 items: egg, milk, wheat, buckwheat, peanuts, shrimp, crab, abalone, squid, kiwi fruit, beef, walnuts, salmon, mackerel, soybeans, chicken, pork, yam, apple, and banana.

These test methods are included in the Japanese Ministry of Health, Labour and Welfare Notification "Regarding the testing method for foods containing allergenic substances," No. 0622003 issued by the Dept. of Food Safety, June 22, 2006.

Test methods for proteins derived from specified ingredients in foods include the ELISA method (*1) based on antigen-antibody reactions for quantitative analysis, Western blotting method (*2) for qualitative analysis, and PCR method (*3) (Table 2).

The ELISA quantitative test method is used for the screening of the seven specified ingredients – egg, milk, wheat, buckwheat, peanuts, shrimp, crab – as well as soybeans, which are listed as items corresponding to specified ingredients.

Western blotting method is generally used for the qualitative analysis of egg and milk.

The polymerase chain reaction (PCR) method uses specific sequences for the confirmation testing of the specified ingredients wheat, buckwheat, peanuts, shrimp, and crab (excluding egg and milk) and for soybeans, beef, pork, chicken, salmon, mackerel, abalone, squid, kiwi fruit, walnuts, yam, apple, and banana that are items corresponding to specified ingredients.

Table 3 summarizes the test methods applicable for each item.

### Table 3 Test Methods for Each Item

|  | Item | Test Method |
|---|---|---|
| Specified Ingredients<br><br>Mandatory Labeling | Egg | ELISA, Western blotting |
|  | Milk | ELISA, Western blotting |
|  | Wheat | ELISA, PCR |
|  | Buckwheat | ELISA, PCR |
|  | Peanuts | ELISA, PCR |
|  | Shrimp | ELISA, PCR |
|  | Crab | ELISA, PCR |
| Items Corresponding to Specified Ingredients<br><br>Recommended Labeling | Soybeans | ELISA, PCR |
|  | Beef | PCR |
|  | Pork | PCR |
|  | Chicken | PCR |
|  | Salmon | PCR |
|  | Mackerel | PCR |
|  | Abalone | PCR |
|  | Squid | PCR |
|  | Kiwi fruit | PCR |
|  | Walnuts | PCR |
|  | Yam | PCR |
|  | Apple | PCR |
|  | Banana | PCR |

### Table 2 Test Methods of Allergen

| Test Method | Application |
|---|---|
| ELISA | Screening (quantitative) |
| Western blotting | Confirmation testing (qualitative) |
| PCR | Confirmation testing (qualitative) |

(*1) ELISA (Enzyme-Linked ImmunoSorbent Assay) Method
The Enzyme-Linked ImmunoSorbent Assay is an analysis method that combines an immunoreaction (antigen-antibody reaction) and an enzyme-substrate reaction. This method is used to detect and quantify the concentration of antibodies and antigens contained in the sample. This method is known as ELISA.

(*2) Western Blotting Method
After separating a sample by electrophoresis, it is transferred and bound to a membrane. It is reacted with an antibody (primary antibody) for the protein of interest. A secondary enzyme-marked antibody is reacted with the primary antibody and the target substance is detected through luminescence or fluorescence.

(*3) Polymerase Chain Reaction (PCR) Method
This method selectively amplifies part of the DNA, using the sample DNA as a template. Cycle reactions (separation of double-stranded DNA → primer binding → DNA synthesis) are performed using a primer (short sequence-specific single-stranded DNA with each end of the region to be amplified) and DNA polymerase to amplify the required DNA region. In principle, even a single DNA molecule can be amplified in multiples of the number of reaction cycles. The presence of the substance of interest can be evaluated from whether the regions straddling the primer are amplified.

## 5. Analysis by PCR

**1) Extracting and Purifying DNA from Food Samples**
The extraction and purification of DNA can be performed by the cetyltrimethylammonium bromide surfactant (CTAB) method or methods using a silica gel membrane or ion-exchange resin. Each method has its own characteristics. The CTAB method makes it difficult for PCR inhibitors to remain in the food. Commercial kits are available for extraction and purification methods using a silica gel membrane or ion-exchange resin, making them relatively simple to perform.
The CTAB method is applicable to test samples with a low degree of processing, such as wheat flour or buckwheat flour. Methods using a silica gel membrane or ion-exchange resin are applicable to test samples subjected to a high degree of processing, including sweetening, oil treatment, hot mixing, or fermentation.

**2) Confirming DNA Purification and Quantitation**
The extracted and purified DNA sample solution is diluted ten times and the absorbance measured at 230 nm, 260 nm, and 280 nm. In principle, the DNA sample solution is prepared at 20 ng/µL concentration.

**3) PCR**
The base sequence region of interest contained in the extracted and purified DNA is amplified by performing polymerase chain reaction (PCR) using the appropriate organism-specific primer (Table 4). These amplification products are separated and detected by electrophoresis to determine the absence or presence of the specified ingredient in the inspected sample. Fig. 3 shows the detection procedure.
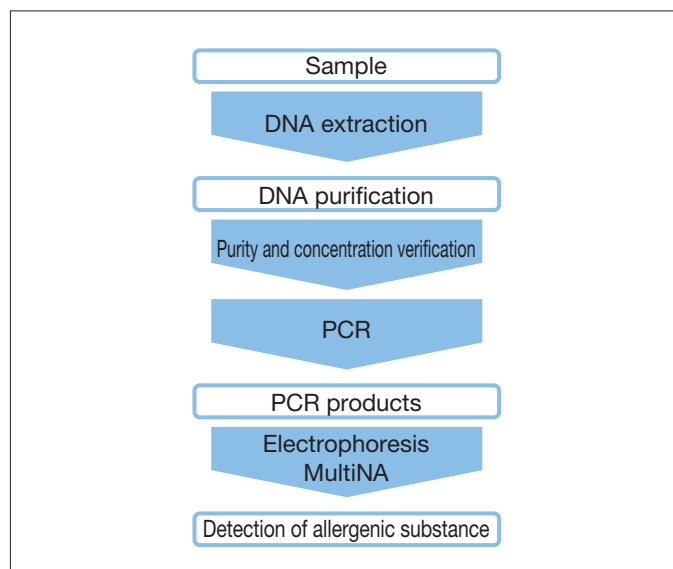


**Fig. 3 Experimental Procedure for Detection of Allergenic Substances**

**Table 4 Primers for Enzyme Detection**
**Japanese Ministry of Health, Labour and Welfare Notification "Regarding the testing method for foods containing allergenic substances," No. 0724, Publication No. 1 issued by the Dept. of Food Safety, July 24, 2009 (See Note)**

| | Plant DNA | Animal DNA | Wheat | Buckwheat | Peanuts | Shrimp | Crab |
|---|---|---|---|---|---|---|---|
| PCR Amplification Product Size (bp) | 124 | 370-470 | 141 | 127 | 95 | 187 | 62 |

| | F-Primer | R-Prime |
|---|---|---|
| Plant DNA | CP03-5' : 5'-CGG ACG AGA ATA AAG ATA GAG T-3' | CP03-3' : 5'-TTT TGG GGA TAG AGG GAC TTG A-3' |
| Animal DNA | AN1-5': 5'-TGA CCG TGC GAA GGT AGC-3'<br>AN2-5': 5'-TAA CTG TGC TAA GGT AGC-3'<br>Use a 1:1 mixture of AN1-5' and AN2-5'. | AN-3' : 5'-CTT AAT TCA ACA TCG AGG TC-3' |
| Wheat | Wtr01-5' : 5'-CAT CAC AAT CAA CTT ATG GTG G-3' | Wtr10-3' : 5'-TTT GGG AGT TGA GAC GGG TTA-3' |
| Buckwheat | FAG19-5' : 5'-AAC GCC ATA ACC AGC CCG ATT-3' | FAG22-3' : 5'-CCT CCT GCC TCC CAT TCT TC-3' |
| Peanuts | agg04-5' : 5'-CGA AGG AAA CCC CGC AAT AAA T-3 | agg05-3' : 5'-CGA CGC TAT TTA CCT TGT TGA G-3' |
| Shrimp | ShH12-05' : 5'-TTA TAT AAA GTC TRG CCT GCC-3'<br>ShH12-05' is synthesized as A and G mixed bases (R) to the 8th base from the 3' terminal. | ShH13-03'-1: 5'-GTC CCT CTA GAA CAT TTA AGC CTT TTC-3'<br>ShH13-03'-2: 5'-GTC CCT TTA TAC TAT TTA AGC CTT TTC-3'<br>ShH13-03'-3: 5'-GTC CCC CCA AAT TAT TTA AGC CTT TTC-3<br>Use a 1:1:1 mixture of ShH13-03'-1, ShH13-03'-2, and ShH13-03'-3. |
| Crab | CrH16-05'-1: 5'-GCG TTA TTT TTT TTG AGA GTT CWT ATC GTA-3'<br>CrH16-05'-2: 5'-GCG TAA TTT TTT CTG AGA GTT CTT ATC ATA-3'<br>CrH16-05'-3: 5'-GCG TTA TTT TTT TTA AGA GTA CWT ATC GTA-3'<br>CrH16-05'-4: 5'-GCG TTA TTT CTT TTG AGA GCT CAT ATC GTA-3'<br>CrH16-05'-1 and CrH16-05'-3 are synthesized as A and T mixed bases (W) to the 8th base from the 3' terminal.<br>Use a 10:1:6:3 mixture of CrH16-05'-1, CrH16-05'-2, CrH16-05'-3, and CrH16-05'-4. | CrH11-03' : 5'-TTT AAT TCA ACA TCG AGG TCG CAA AGT-3' |

* The amplification products of Shanghai hairy crab, Dungeness crab, giant spider crab, red queen crab, deep sea red crab, or swimming crab may be detected by PCR for shrimp. If it is unknown whether the amplification products obtained are derived from shrimp or crab, they can be identified by performing restriction enzyme digestion on the PCR products. For details, see the Japanese Ministry of Health, Labour and Welfare Notification "Regarding the testing method for foods containing allergenic substances."
Note) See rear cover for details.

## 6. MCE-202 MultiNA Microchip Electrophoresis System

The long series of operations required for agarose gel electrophoresis – reagent preparation, gel preparation, electrophoresis, acquiring result images, and clean-up – requires a lot of time and effort. Moreover, the data obtained is objectively poor in terms of sensitivity, separation, reproducibility, and quantitativeness.

The MCE-202 MultiNA Microchip Electrophoresis System overcomes the problems with agarose gel electrophoresis.



**Fig. 4 MultiNA Microchip**



**Fig. 5 MultiNA Regent Kit**

### Features of MultiNA

- Microchip electrophoresis by MultiNA offers superior sensitivity, separation, reproducibility, and quantitativeness to agarose gel electrophoresis.

- Simply load the samples and reagents for automated, unmanned analysis of up to 120 samples. Pretreatment and electrophoresis proceed in parallel to achieve an analysis time of just 80 s (*) per sample.

- MultiNA offers extremely easy analysis operation. Once the analysis schedule is created, simply load the samples and reagents and click the Start button.

- Reusable high-performance microchip achieves running costs equal to or lower than agarose gel electrophoresis.

(*) DNA standard analysis (DNA-100 kit/Pre-Mix mode) using four microchips.
However, this time does not include the times for initial and subsequent rinsing or the time for initial analysis.
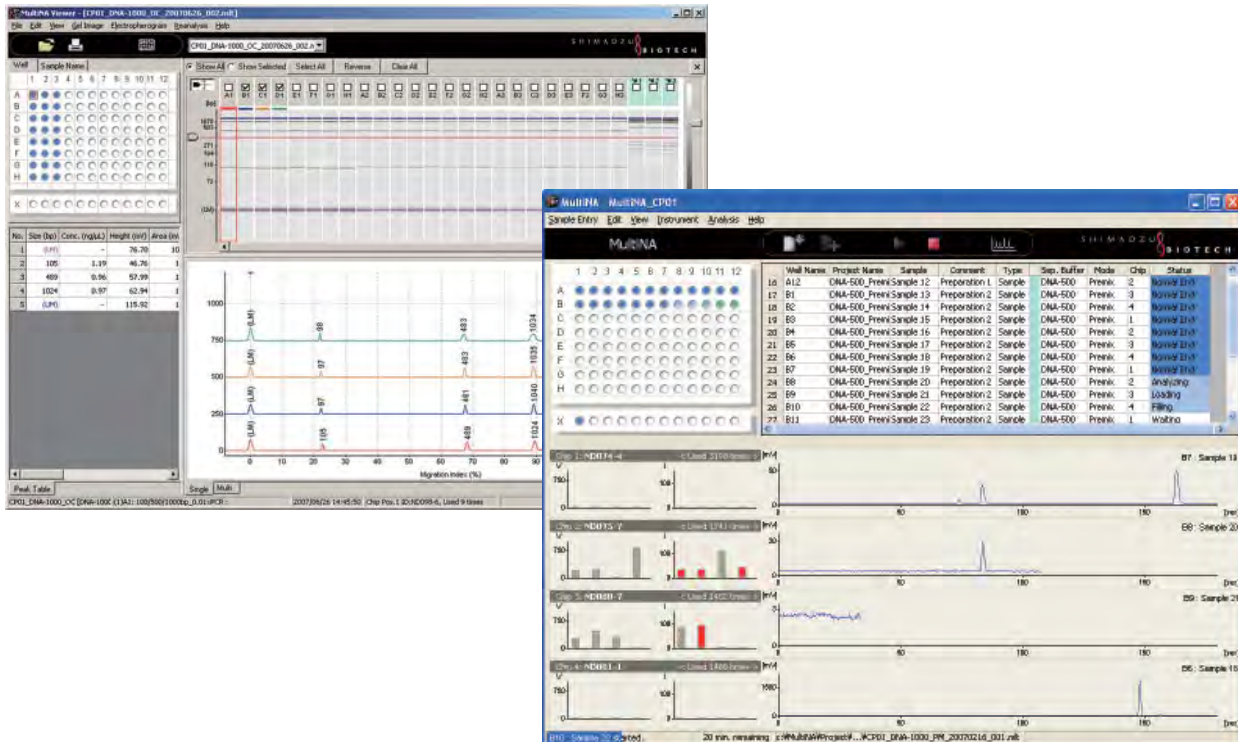


**Fig. 6 MultiNA Operation Screen**

## 7. Detection of Allergenic Substances Using MCE-202 MultiNA Microchip Electrophoresis System

7. Detection of Allergenic Substances Using MCE-202 MultiNA Microchip Electrophoresis System
The results of analysis of the PCR amplification products of DNA derived from wheat, buckwheat, peanuts, shrimp and crab, respectively, using the MultiNA are shown in Fig. 7. The PCR amplification products derived from the wheat, buckwheat, peanuts, shrimp and crab substances were all clearly detected using the MultiNA. (The estimated sizes shown in the figure were obtained in this experiment.)
The results of analysis by agarose gel electrophoresis of the same PCR amplification products are shown in Fig.8 [Reference]. The sizes of the PCR amplification products are imprecise, resulting in the lack of objectivity in interpreting the gel electrophoresis. However, the results obtained using the MultiNA consist of an electropherogram (Fig. 7-b) in addition to a gel image (Fig. 7-a), ensuring a high level of accuracy.  Despite the proximity of the wheat and buckwheat amplification products, they could be separated.  Compared to agarose gel electrophoresis, the MultiNA's excellent resolution and sensitivity allow these to be clearly detected.
Fig. 9 shows a photograph of the MCE-202 MultiNA Microchip Electrophoresis System.



(a) Gel Image          (b) Electropherogram
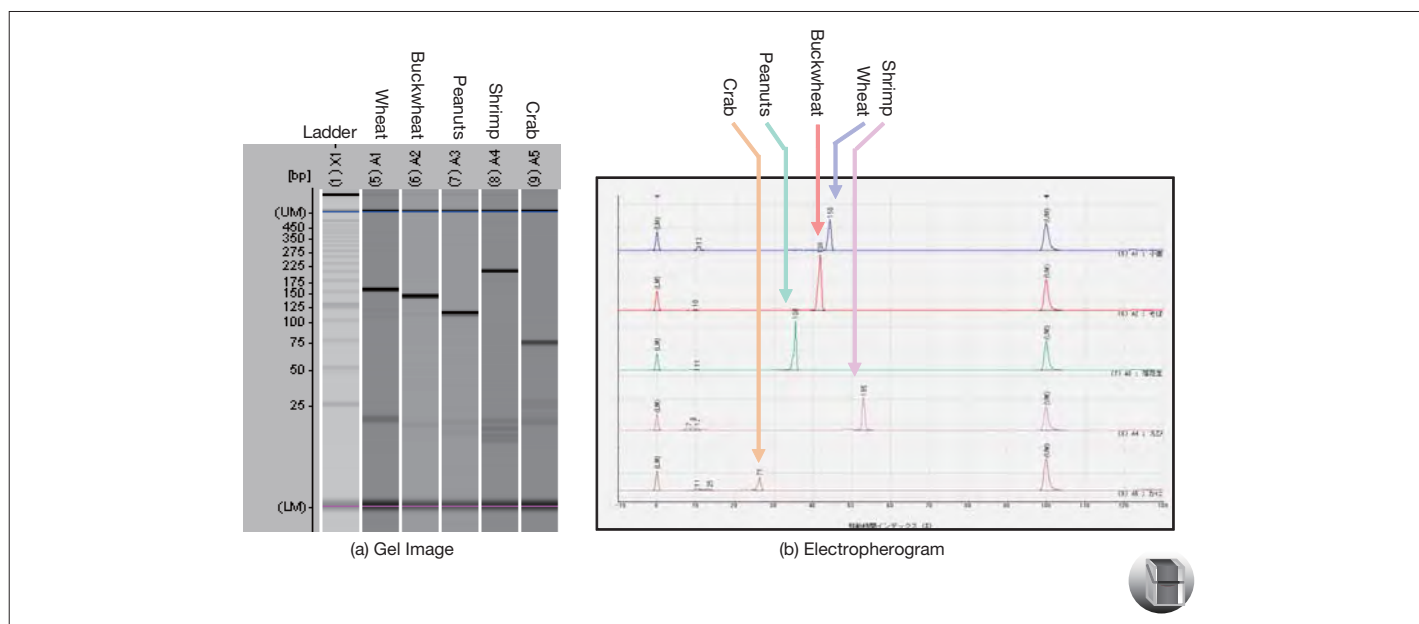
**Fig. 7 Analytical Results for PCR Products from Allergenic Substances**
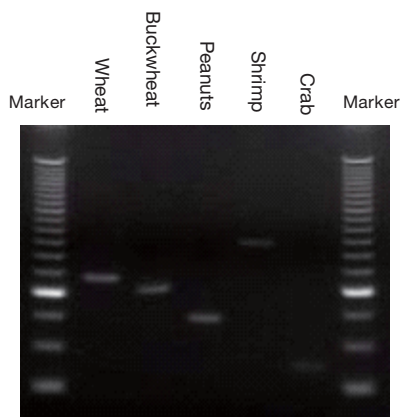


**Fig. 8 [Reference] Agarose Gel Electrophoresis of PCR Products from Allergenic Substances**



**Fig. 9 MCE-202 MultiNA Microchip Electrophoresis System**

References:
"Regarding the testing method for foods containing allergenic substances," No. 0724, Publication No. 1, the Dept. of Food Safety, Ministry of Health, Labour and Welfare of Japan, July 24, 2009
"Handbook for Labeling of Processed Foods Containing Allergens," Japanese Ministry of Health, Labour and Welfare, March 2009 revision
"What You Need to Know About Food Labeling," Japanese Ministry of Health, Labour and Welfare, Japanese Ministry of Agriculture, Forestry and Fisheries, Japan Fair Trade Commission, March 2009

Note)
Separate arrangements are required for contract testing using the primers described above for commercial applications on behalf of analytical laboratories, with the exception of public institutions.
Contact the appropriate company below. The synthesis and application of these primers for research applications is unrestricted.

• Animal: Nissin Food Products Co., Ltd.
• Wheat, buckwheat, soybeans: Nisshin Seifun Group Inc.
• Shrimp, crab: House Foods Corporation

* MCE®-202 MultiNA is not available in the United States.

* This document is based on information valid at the time of publication. It may be changed without notice.

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# Application News

**Liquid Chromatography Mass Spectrometry**

## High Sensitivity Analysis of Peanut Allergen in Cumin and Spice Mix [LCMS-8060]

# No.C141

Food allergens are a major public health concern. Among them, peanut allergy is one of the common food allergies. To avoid unexpected contact with food allergens, food labels are strictly used to indicate the presence of specific allergens. With the increasing awareness of food allergies, the presence of undeclared peanut in cumin lead to huge recalls in recent years. Although ELISA is the most commonly used technique to detect allergens, its false-positive rate is a major concern due to its cross-reactivity. We developed a method with high specificity and sensitivity to overcome this issue by using a high sensitivity triple quadrupole mass spectrometer to detect peanut allergen Ara h1 (Fig.1) in commercially available spices and seasonings.
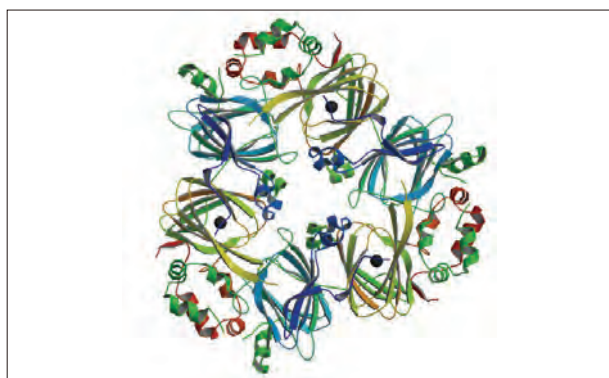


**Fig. 1  Structure of Ara h1 [3S7I] (68kDa) Vicilin Like Protein**

### ■ Sample Preparation

Commercially available defatted peanut flour was purchased and used for the initial development work. The test samples were ground and protein content was enriched by liquid-liquid extraction. Extracted proteins were denatured, reduced and alkylated before subjecting to tryptic digestion to obtain peptides that were quantitated as proxies of original protein abundance.
Cinnamon, cumin, chilli pepper, ginger, garlic, mustard seed, nutmeg, oregano, rosemary, sage, turmeric and thyme were selected as test food samples for evaluating cross-reactivity and sensitivity of the developed method. Food samples were pretreated as above with or without 2 ppm peanut powder.

### ■ Selection of MRM Transitions Using Skyline

Ara h1 is known as is known as the sensitizing allergen in 95 % of peanut allergy. Tryptic digest of protein extracted from peanuts were analyzed by monitoring theoretically calculated transitions of peptides based on amino acid sequences of two clones P17 and P41B of Ara h1.

MRM transitions for each clone was determined by using Skyline (MacCoss Lab Software). The transition list, which contained more than ten peptides for each clone, was reviewed by removing several peptides that could be susceptible by post translational modification and Maillard reaction during food processing.
Finally, nine peptides including three common peptides to both clones were selected based on sensitivity. Three transitions were set for each peptide.
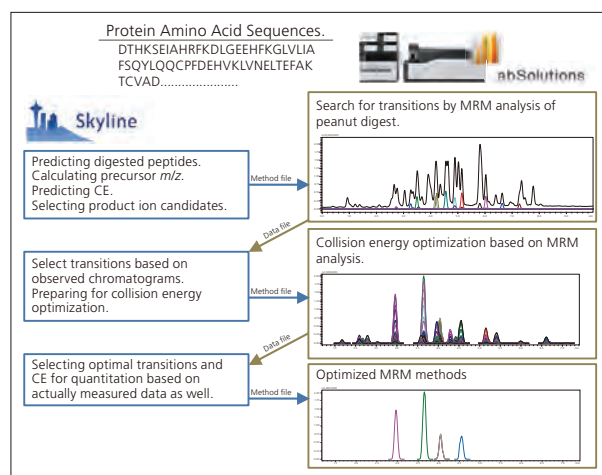


**Fig. 2  Workflow of MRM Transition Optimization Using Skyline**
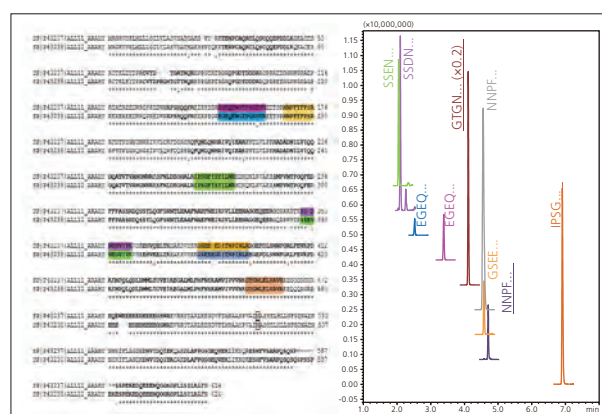


**Fig. 3  AA Sequences of P17/P41B and Nine MRM Chromatograms**

**Table 1  Analytical Conditions**

| | | | | |
|---|---|---|---|---|
| System | : Nexera X2 | | System | : LCMS-8060 |
| Column | : Shim-pack XR-ODS Ⅱ | | Ionization | : Heated ESI |
| | (50 mm L. × 2 mm I.D., 1.6 µm) | | Probe Voltage | : +1 kV (positive ionization) |
| Column Temperature | : 40 °C | | Temperature | : Interface: 250 °C |
| Mobile Phases | : A: Water + 0.1 % formic acid | | | Desolvation Line: 150 °C |
| | B : Acetonitrile | | | Heater Block: 200 °C |
| Flowrate | : 500 µL/min | | Gas Flow | : Nebulizing Gas: 3 L/min |
| Gradient | : 2 %B (0.00 min) > 25 %B (7.00 min) > | | | Heating Gas: 20 L/min |
| | 95 %B (7.10-8.00 min) > 2 %B (8.10-10.00 min) | | | Drying Gas: 5 L/min |
| Injection Volume | : 10 µL | | | |

**Table 2  MS/MS Acquisition Parameters**

| MRM Transitions | Name | Polarity | Quan | Qual1 | Qual2 |
|---|---|---|---|---|---|
| | EGEQEWGTPGSEVR | + | 780.85 > 802.40 | 780.85 > 644.35 | 780.85 > 316.10 |
| | NNPFYFPSR | + | 571.25 > 669.35 | 571.25 > 506.25 | 571.25 > 229.10 |
| | IPSGFISYILNR | + | 690.40 > 765.45 | 690.40 > 211.15 | 690.40 > 502.25 |
| | SSDNEGVIVK | + | 524.25 > 515.35 | 524.25 > 359.25 | 524.25 > 175.05 |
| | GSEEEDITNPINLR | + | 793.90 > 726.45 | 793.90 > 612.40 | 793.90 > 402.25 |
| | GTGNLELVAVR | + | 564.80 > 686.40 | 564.80 > 557.40 | 564.80 > 444.30 |
| | EGEQEWGTPGSHVR | + | 784.85 > 652.35 | 784.85 > 555.30 | 784.85 > 316.10 |
| | SSENNEGVIVK | + | 588.30 > 515.35 | 588.30 > 359.25 | 588.30 > 246.20 |
| | GSEEEGDITNPINLR | + | 822.40 > 726.45 | 822.40 > 612.40 | 822.40 > 402.25 |

| | |
|---|---|
| Dwell Time | : 41 to 130 msec depending upon the number of concomitant transitions to ensure to have at least 15 points per peak (max total loop time 400 msec). |
| Pause Time | : 3 msec |
| CID Pressure | : 300 kPa |
| Quadrupole Resolution | : Q1: Unit   Q3: Unit |

■ **Interface Optimization**

Ionization parameters optimization was performed using companion software ISSS (Interface Setting Support Software, Shimadzu Corp.). As a result, sensitivity was improved more than twofold compared to default values.

■ **Effect of Surfactant During Digestion**

A higher intensity of peptides by addition of a surfactant during tryptic digestion was expected due to improved digestion efficiency. However, the intensity of peptides were relatively worse by adding surfactant. Thus, no surfactant was used for tryptic digestion.
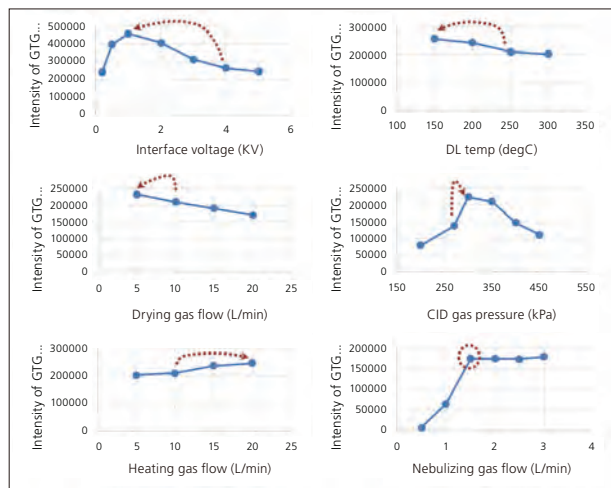


**Fig. 4  Interface Optimization Results**
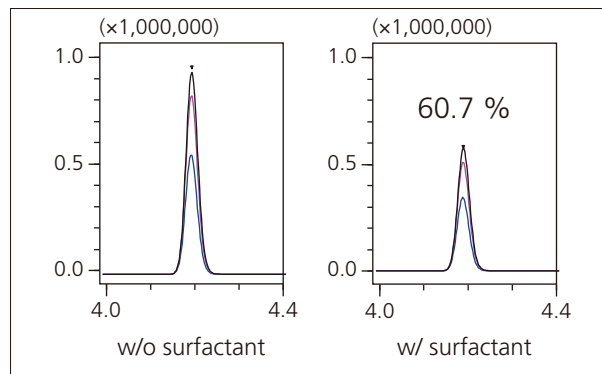


**Fig. 5  Difference of the Chromatograms of Peptide GTG… by Addition of Surfactant**

## ■ Peanut Allergen in Other Nuts

Walnuts, cashew nuts, and almonds were analyzed to test specificity. These nuts were spiked with 2 ppm (2 mg/kg) of peanut before sample preparation. The spiked peanut peptides were successfully detected and any obvious peak was detected in blank samples.
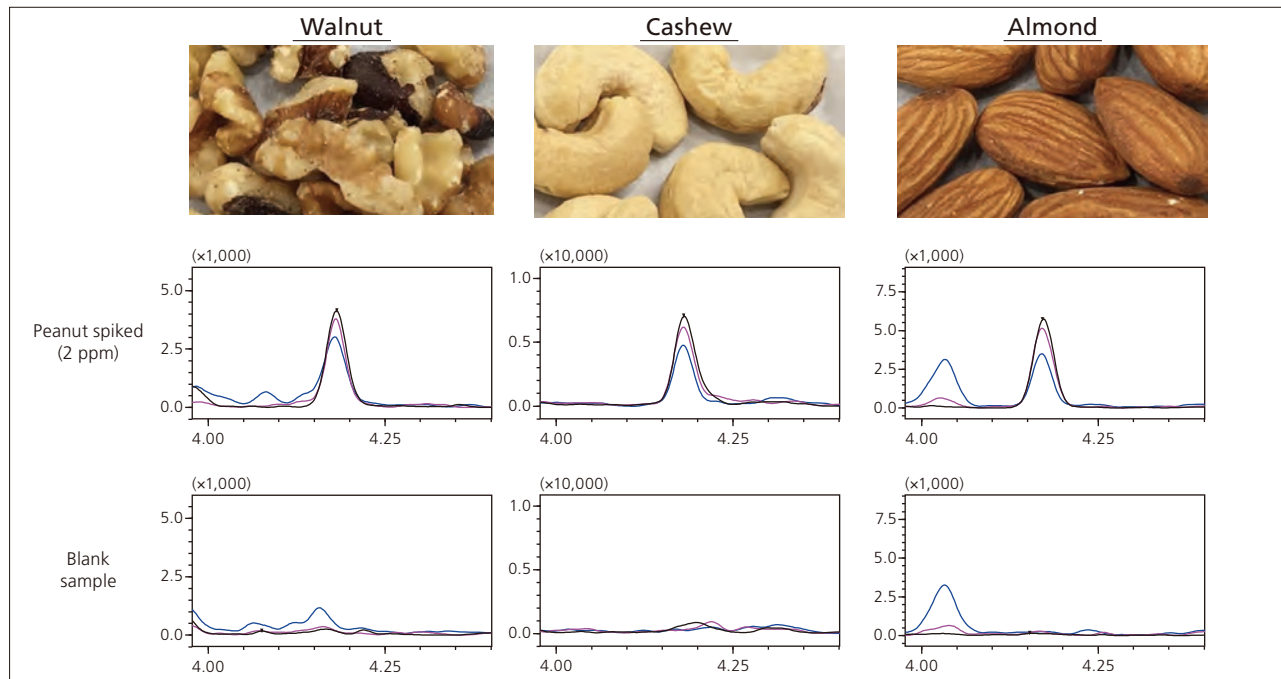


Fig. 6  Chromatograms of Peptide GTG... in Other Kind of Nuts With or Without Spiking with Peanuts

## ■ Detection of ARA h1 in Spice Mixes and Seasonings

Several spice mixes and seasonings were analyzed using sample preparation and analytical conditions described here. Peaks of tryptic peptides of Ara h1 from samples without spiking of peanut peptides were detected.
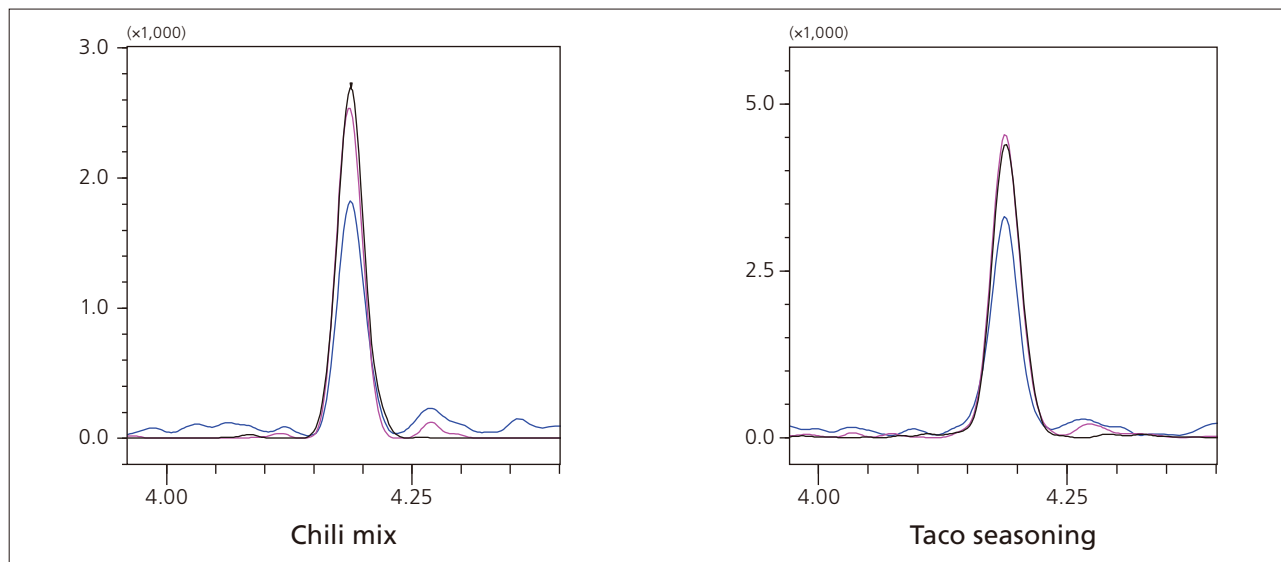


Fig. 7  Detected Peaks of Peptide GTG... in Chili Mix and Seasoning

## ■ Peanut Allergen in Spices

Contaminated spice samples were prepared and analyzed to confirm that the low amount of peanuts added into the various spices can be detected. Peptides of Ara h1 were successfully observed from the spice samples spiked with 2 ppm of peanuts. It was also confirmed that there are no obvious false-positive peaks from the blank samples.
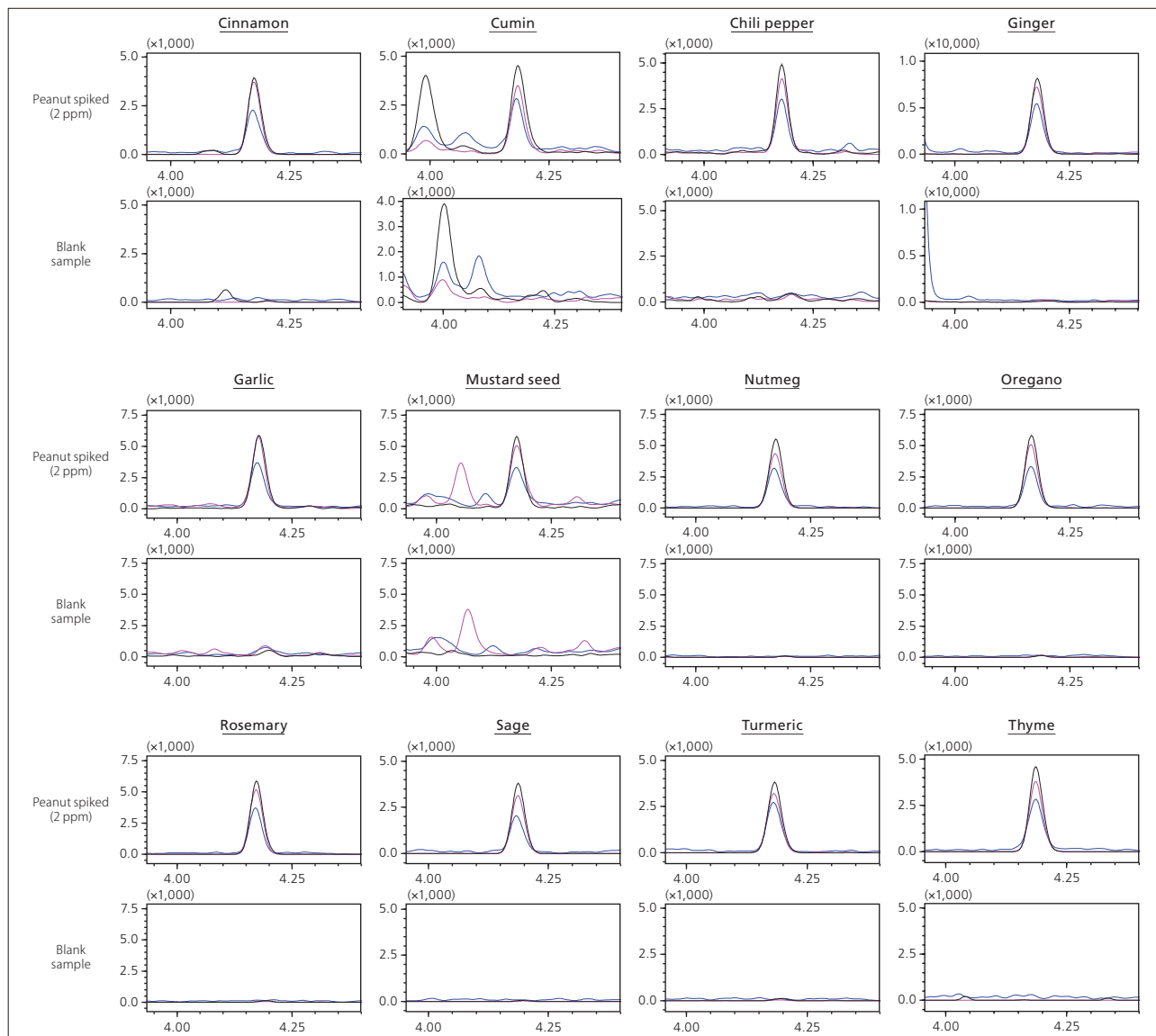


**Fig. 8  Chromatograms of Peptide GTG… in Spices With or Without Spiking with Peanuts**

## ■ Conclusion

A method for the analysis of Ara h1 in spices and seasonings was successfully developed.
The combination of the developed method and a high sensitivity triple quadrupole mass spectrometer enabled the detection of 2 ppm or lower of peanut allergen Ara h1 in spices and seasonings.

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Excellence in Science

# Simultaneous analysis of major allergens in food matrices by high sensitive mass spectrometer

## ASMS 2017  TP 202

Tairo Ogura[1]
Shimadzu Scientific Instruments, Inc. Columbia, MD, USA

# Introduction

Food allergy is an abnormal overreaction of immune system to a particular protein in food. It is becoming a major concern for public health and food industries. Typical food allergens are proteins and peptides. The signs and symptoms may range widely from itching, red skin, swelling, anaphylaxis etc. There is no cure for food allergy at present, so people with allergy must avoid food triggers. To avoid unexpected contact with food allergens, food labels are strictly used to indicate presence of specific allergens. The Food Allergen Labeling and Consumer Protection Act (FALCPA) identified eight foods or food groups as major allergens which include milk, eggs, fish (e.g., bass, flounder, cod), crustacean shellfish (e.g., crab, lobster, shrimp), tree nuts (e.g., almonds, walnuts, pecans), peanuts, wheat and soybeans and FALCPA mandates that the labels of foods containing eight major food allergens declare the presence of allergens. ELISA (enzyme-linked immunosorbent assay) and PCR (Polymerase chain reaction) are most commonly used technique to detect allergenic foods due to relatively simple handling. Even so,

cross-reactivity of ELISA can raise a the risk of false positive results. Additionally, ELISA requires separated analysis for each target. Since PCR assay is based on detection of DNAs rather than allergenic proteins, milk cannot be distinguished from beef and will be difficult to detect food contains egg white. Therefore, it is important to determine allergens in food by using more reliable detection method. Recently, liquid chromatography mass spectrometry becomes an alternative technique to detect allergenic proteins with high selectivity, sensitivity, and capability to analyze multiple allergens simultaneously. We developed a method to detect 31 peptides derived from eight allergens. We analyzed commercially available samples such as bread and gluten free bread etc to evaluate this method. We did not detected any peptides derived from gluten in gluten free bread and gluten free cracker. And we could detect peptides of 20 ppm wheat fortified to gluten free bread. We could detect other allergens shown on the label from commercial available food matrices.

# Materials and methods

## Sample preparation

Commercially available allergenic food materials were purchased at local grocery store and used for development of analytical methods. The samples were ground in fine powders by GM-200 (Retsch). 0.5 - 1 g of each ground samples was transferred into 50 mL tube. Hexane was used for removal of oils and fats from samples. Proteins were extracted by using the extraction

buffer containing 50 mM Tris-HCl (pH8.0) 2M Urea and protease inhibitors. Aliquot of extract containing 100 – 250 µg of proteins were denatured, alkylated, and digested into peptides by traditional in-solution protein digestion technique. Digested peptides were desalted by SPE, lyophilized, and stored until analysis.

## LC/MS analytical conditions

LC/MS analysis was conducted by using Shimadzu Nexera X2 UHPLC coupled to triple quadrupole mass spectrometer LCMS-8050. 0.1 % formic acid in water (A) and acetonitrile (B) were used for mobile phase at a flow rate of 0.5 mL/min. Shim-pack XR-ODS III (2.0 mmID x 75 mmL., 1.6 µm) was used as analytical column. The high pressure gradient elution was set as follows: 2%B (0.0 min), 15%B (4 min), 40%B (7 min), 95%B (7.10-8.00

min), 2%B (9.10-10.00 min). Peptides were detected by MRM acquisition. Other parameters for mass spectrometer were set as follows: positive mode electrospray ionization, nebulizing gas flow of 3 L/min, heating gas flow of 20 L/min, drying gas flow of 5 L/min, interface temperature of 250 °C, DL temperature of 150 °C, heat block temperature of 200 °C.

# Simultaneous analysis of major allergens in food matrices by high sensitive mass spectrometer



Calculate peptides, charge state, modification, and product ion

Select detected transitions, calculate RT, and set CE in steps for optimization

Select peptides, MRM transitions, and optimized CE

Transition screening
Acquires MRM data based on calculated peptides information
(For example, 600 channels)

CE optimization
Acquires MRM data for selected peptides
(For example, 1500 channels)

Sample analysis
Acquire data using optimized method
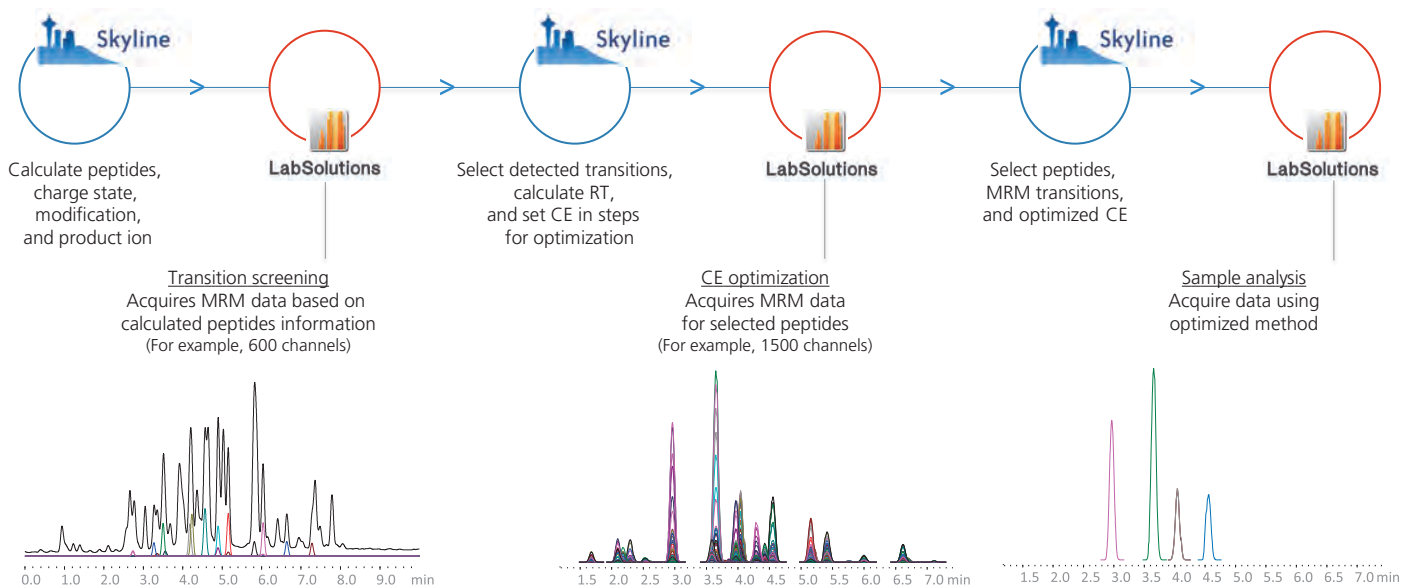
Figure 1   Work flow of MRM transition optimization using Skyline.

# Result

## Detection of allergenic proteins by LC-MS/MS

To establish analytical method, we selected MRM transitions of signature peptides by using Skyline (Figure 1) based on their peak intensity, peak shape, and similarity to other peptides of target proteins. As a result of method development, we finally selected 150 MRM transitions for monitoring 33 peptides derived from 13 proteins as allergenic proteins of eight foods or food groups. As Figure 2 shows, all of peptides were eluted within 6.5 min with good separation. Figure 2 also shows the linearity of peptides.



SVAVSQVAR (1 – 50 ppm)
Y=151.944x + 231.865
R2 > 0.999188
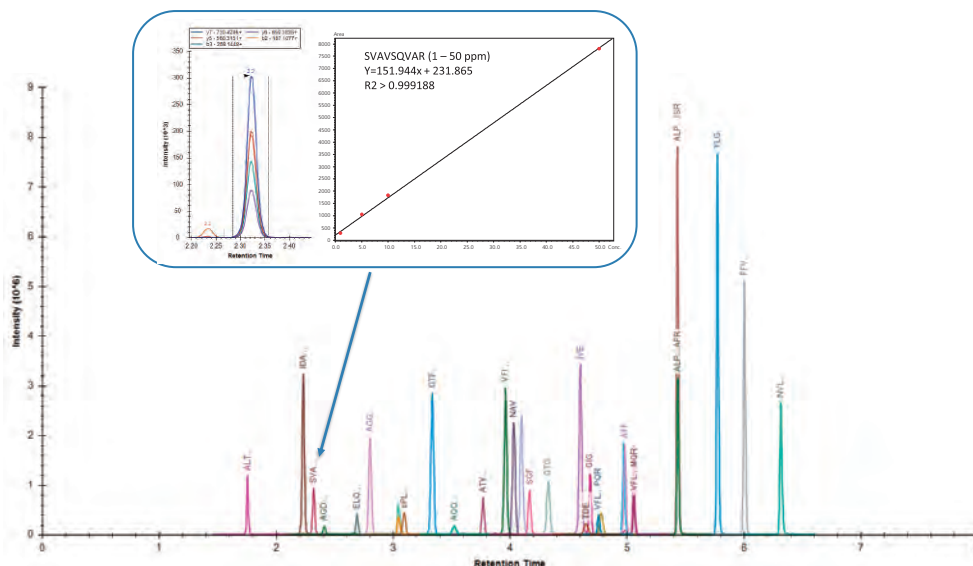
Figure 2   Chromatogram of peptides mixture derived from eight food allergens, and magnified view of five MRM transitions for wheat peptides and its calibration curve.

3

# Simultaneous analysis of major allergens
# in food matrices by high sensitive mass spectrometer

Table 1  The target food matrices, protein name, peptides, and UniProt ID found in same food.

| Food (Binomial name) | | Protein name (IUIS name) | Peptides | Uniprot ID |
|---|---|---|---|---|
| Milk (Bos taurus) | | Caseins (Bos d 8) | FFVAPFPEVFGK | P02662, B5B3R8 |
| | | | YLGYLEQLLR | P02662, B5B3R8 |
| | | | NAVPITPTLNR | P02662, B5B3R8 |
| | | | FALPQYLK | P02663 |
| | | Beta-lactoglobulin (Bos d 5) | IDALNENK | P02754, G5E5H7, B5B0D4 |
| Egg (Gallus gallus) | | Ovalbumin (Gal d 2) | NVLQPSSVDSQTAMVLVNAIVFK | P01012 |
| | | Ovotransferrin (Gal d 3) | ATYLDCIK | P02789, Q4ADJ7, Q4ADJ6, E1BQC2, Q4ADG4, A0A1D5P4L7 |
| | | | TDERPASYFAVAVAR | P02789, Q4ADJ7, Q4ADJ6, E1BQC2, Q4ADG4, A0A1D5P4L7, A0A1L1RSU6 |
| Fish | Atrantic cod (Gadus morhua) | Beta-parvalbumin (Gad m 1) | ALTDAETK | P02622, A5I873, Q90YL0 |
| | | | AFFVIDQDK | Q90YL0, A5I873 |
| | | | SGFIEEDELK | Q90YL0, A5I873 |
| Crustacean shellfish | Whiteleg shrimp (Litopenaeus vannamei) | Tropomyosin (Lit v 1) | IQLLEEDLER | B4YAH6 |
| | | | IVELEEELR | B4YAH6 |
| | | Myosin, light chain 2 (Lit v 3) | EGFQLMDR | B7SNI3 |
| | | | GTFDEIGR | B7SNI3 |
| | | Sarcoplasmic calcium-binding protein (Lit v 4) | VFIANQFK | C7A639 |
| | | | AGGLTLER | C7A639 |
| Tree nuts | Almonds (Prunus dulcis) | Amandin, 11S globulin legumin-like protein (Pru du 6) | ALPDEVLANAYQISR | E3SH28, Q43607 |
| | | | ALPDEVLQNAFR | E3SH29 |
| Peanuts (Arachis hypogaea) | | Cupin Vicillin-type, 7S globulin (Ara h 1) | NNPFYFPSR | P43237, P43238, E5G076, B3IXL2, N1NG13, Q6PSU3 |
| | | | GTGNLELVAVR | P43237, P43238, B3IXL2, Q6PSU6, Q6PSU3, N1NG13, Q6PSU5, E5G076, Q6PSU4 |
| Wheat (Triticum aestivum) | | High molecular weight glutenin (Tri a 26) | ELQELQER | P10388, P08489 and 22 others in wheat |
| | | | SVAVSQVAR | P10387, P08488, and 21 others in wheat |
| | | | AQQPATQLPTVCR | P10387, P08488, and 21 others in wheat |
| | | Low molecular weight glutenin GluB3-23 (Tri a 36) | VFLQQQCIPVAMQR | P10385 and 71 others in wheat |
| | | | VFLQQQCSPVAMPQR | P10386, P04729, P04730 and 114 others in wheat |
| Soybeans (Glycine max) | | Trypsin inhibitor (Gly m TI) | CPLTVVQSR | P01070, P01071, P25272 and 13 others |
| | | | NKPLVVQFQK | P01070, P01071, P25272 and 8 others |
| | | | NKPLVVEFQK | P25273 |

# Simultaneous analysis of major allergens in food matrices by high sensitive mass spectrometer

## Allergens in cooked food matrices

Chromatograms of commercially available food matrices were shown below. A mixture of eight allergenic food and seven cooked food were analyzed. As summarized in Table 3, even we missed soybeans from several food, these data shows that we could detect expected allergens from actual samples overall.
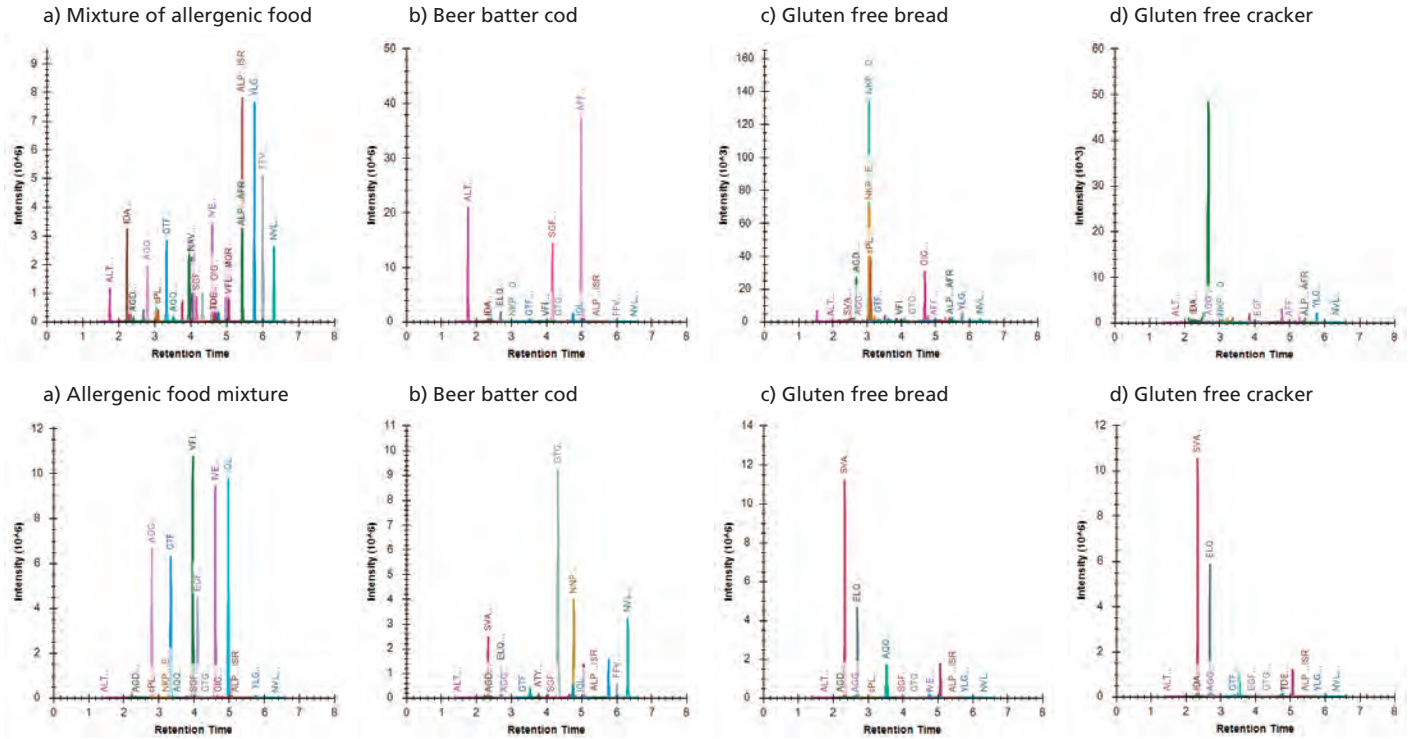


a) Mixture of allergenic food    b) Beer batter cod    c) Gluten free bread    d) Gluten free cracker

a) Allergenic food mixture    b) Beer batter cod    c) Gluten free bread    d) Gluten free cracker

Figure 3    Chromatograms of seven cooked food matrices and mixture of allergenic food as positive control.

# Simultaneous analysis of major allergens in food matrices by high sensitive mass spectrometer

Table 2  The results of seven cooked food samples.

| Food | Allergens | Gluten free bread | | Gluten free cracker | | Bread | | Cracker | |
|---|---|---|---|---|---|---|---|---|---|
| | | Label | Detect | Label | Detect | Label | Detect | Label | Detect |
| Milk | Caseins (Bos d 8) | | | x | x | | | (x) | |
| | Beta-lactoglobulin (Bos d 5) | | | | x | | | | |
| Egg | Ovalbumin (Gal d 2) | | | x | x | x | x | (x) | |
| | Ovotransferrin (Gal d 3) | | | | x | | x | | |
| Atrantic cod | Beta-parvalbumin (Gad m 1) | | | | | | | | |
| Whiteleg shrimp | Tropomyosin (Lit v 1) | | | | | | | | |
| | Myosin, light chain 2 (Lit v 3) | | | | | | | | |
| | Sarcoplasmic CBP (Lit v 4) | | | | | | | | |
| Almonds | Amandin (Pru du 6) | | | | | | | (x) | |
| Peanuts | Cupin, vicillin-type, 7S globulin (Ara h 1) | | | x | | | | (x) | |
| Wheat | High molecular weight glutenin (Tri a 26) | | | | | x | x | x | x |
| | Low molecular weight glutenin (Tri a 36) | | | | | | x | | x |
| Soybeans | Trypsin inhibitor (Gly m TI) | x | x | x | | | | x | |

| Food | Allergens | Peanuts cookies | | Frozen fish "fried cod" | | Frozen pasta "garlic shrimp" | |
|---|---|---|---|---|---|---|---|
| | | Label | Detect | Label | Detect | Label | Detect |
| Milk | Caseins (Bos d 8) | - | x | x | x | x | x |
| | Beta-lactoglobulin (Bos d 5) | | x | | x | | x |
| Egg | Ovalbumin (Gal d 2) | - | x | | | | |
| | Ovotransferrin (Gal d 3) | | x | | | | |
| Atrantic cod | Beta-parvalbumin (Gad m 1) | - | | x | x | | |
| Whiteleg shrimp | Tropomyosin (Lit v 1) | - | | | | x' | x |
| | Myosin, light chain 2 (Lit v 3) | | | | | | x |
| | Sarcoplasmic CBP (Lit v 4) | | | | | | x |
| Almonds | Amandin (Pru du 6) | - | | | | | |
| Peanuts | Cupin, vicillin-type, 7S globulin (Ara h 1) | - | x | | | | |
| Wheat | High molecular weight glutenin (Tri a 26) | - | x | x | x | x | x |
| | Low molecular weight glutenin (Tri a 36) | - | x | | x | | x |
| Soybeans | Trypsin inhibitor (Gly m TI) | - | | | | x | |

'Labeled as "Crustacean shellfish (Shrimp)"

## "Gluten-free" food samples

As a part of evaluation of the method, we analyzed bread containing gluten and gluten-free bread. In US, as one of the criteria for using the claim "gluten-free", FDA set a gluten limit of less than 20 ppm in foods that carry this label. Then, we also analyzed gluten free-bread spiked with wheat extract at 10 ppm. As shown in Figure 4, those level of glutens was detected successfully.
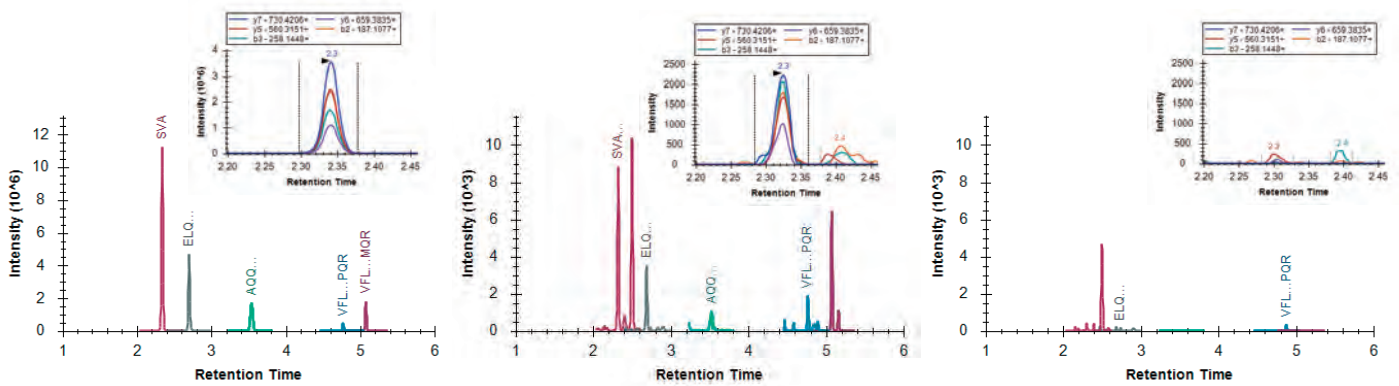


Figure 3   Chromatograms of seven cooked food matrices and mixture of allergenic food as positive control.

## Similarity to other food ingredients

In method development, we performed peptides search for amino acid sequences of theoretically calculated peptides by using UniProt database. Since gluten is a major protein in grains, those peptides sequences are commonly preserved in other edible grains as well (Table 3). To avoid miss identification of food ingredients, we selected the sequences not found in Barley or Rye as significant peptides. On the other hand, these peptides are also found in some sort of goat grass.



Common peptides to D3XQB8 in Rye

Wheat
*(Triticum aestivum)*
Tri a 26
Glutenin, high molecular
weight subunit DX10
P10387
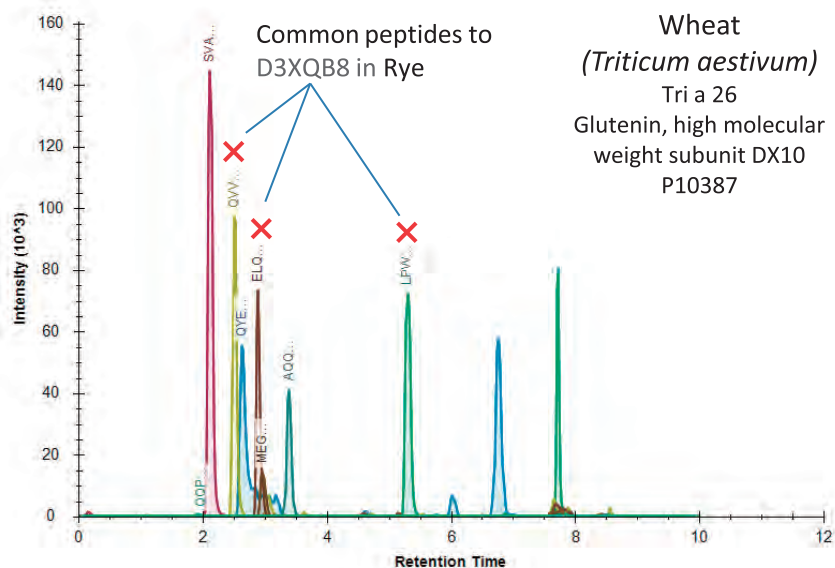
Figure 3   Work flow of MRM transition optimization using Skyline.

Table 1   Peptides search result of predicted peptides

| Analyzed wheat peptides (P10387) | Positions | Barley | Rye |
|---|---|---|---|
| AQQPATQLPTVCR | 624-636 | | |
| ELQESSLEACR | 33-43 | x | x |
| LPWSTGLQMR | 54-63 | x | x |
| MEGGDALSASQ | 637-647 | | x |
| QGSYYPGQASPQQPGQGQQPGK | 135-156 | | x |
| QQPGQGQHPEQGK | 469-481 | | x |
| QVVDQQLAGR | 44-53 | | x |
| QYEQTVVPPK | 86-95 | | |
| SVAVSQVAR | 75-85 | | |

x: found, blank: not found

# Conclusion

- Major food allergens were successfully detected by LC-MS/MS method.
- The method contains 150 MRMs for 31 peptides of 13 allergenic proteins identified in 8 foods.
- The presence of allergenic ingredients in cooked meal could be detected.

LCMS-8050 triple quadrupole mass spectrometer

The product and application are Research Use Only. Not for use in human clinical diagnostics or in vitro diagnostic procedures.

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## Application News

Liquid Chromatography Mass Spectrometry

## Sensitive method for the determination of Fipronil and its metabolite Fipronil Sulfone in egg using QuEChERS sample pretreatment and LC-MS/MS detection [LCMS-8060]

### ▪ Introduction

Fipronil concerns a broad-spectrum insecticide from the group of phenylpyrazoles used in many countries as a biocide and plant protection product against fleas, lice, ticks, cockroaches, mites and other insects. Fipronil is an active compound in veterinary products fighting tick and flea infestations in dogs and cats. The use as plant protection product is restricted to seed treatment in the European Union since 2007. However, due to the illegal use as addition to the cleaning supplies used in chicken coops the eggs and meat might get contaminated as well.

The MRL (maximum residue levels) for Fipronil and and its metabolite Fipronil sulfone (which is classified as having similar toxicity) in eggs is set to 0.005 mg/kg by the EU (by definition the sum of fipronil and fipronil-sulfone expressed as fipronil) [1], so that there is an actual requirement for the determination of both compounds in egg matrix at a relatively low level.

This application news presents a simple method using a standard QuEChERS extraction protocol followed by LC-MS/MS detection.
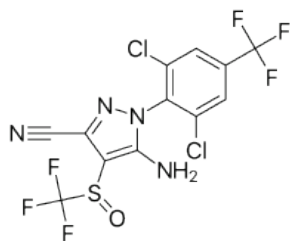
**Fipronil**
MF      $C_{12}H_4Cl_2F_6N_4OS$
MW      437,1 g/mol

### ▪ Sample preparation

Compound extraction was performed using a simple QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method.

5 g of egg (egg white and egg yolk) were weighted into a 50 mL polypropylene tube, diluted with 5 mL of water and spiked with a respective amount of Fipronil, Fipronil sulfone and in addition Fipronil-desulfinyl and Fipronil-sulfide (neochema, Germany).

10 mL of acetonitrile was added and the samples were mixed vigorously. After that ready to use QuEChERS extraction salts (Q-sep$^{TM}$ Q110, Pouch and tubes – cat. #26235, Restek) were added for sample drying and buffering. Samples were mixed again and centrifuged at 4500 rpm for 5 minutes. 1 mL of the supernatant was transferred into a dSPE tube (Q-sep$^{TM}$ QuEChERS dSPE – cat. #26217, Restek), shaken for 2 minutes, centrifuged, the supernatant was transferred into a glass vial and the pH was adjusted with 5% formic acid solution in acetonitrile (10 µL/mL super-natant).

### ▪ Materials and methods

Extracts were analyzed using a method set up with Shimadzu's LC/MS/MS Method Package for Residual Pesticides Version 2 and a Nexera X2 UHPLC system coupled to a LCMS-8060 mass spectrometer. Analysis was carried out using MRM (Multi Reaction Monitoring) mode.

| LC system | Nexera X2 (Shimadzu, Japan) |
|---|---|
| Analytical column | Raptor Biphenyl™ 100 x 2.1 mm, 2.7 µm (RESTEK) |
| Column oven temperature | 35 °C |
| Injection volume | 2 µl |
| Mobile Phase A | 2 mM ammonium formate + 0.002% formic acid - Water |
| Mobile Phase B | 2 mM ammonium formate + 0.002% formic acid - Methanol |

| Mass spectrometer | LCMS-8060 (Shimadzu, Japan) |
|---|---|
| Interface voltage | -3 kV |
| Q1 resolution | Unit (0.7 Da FWHM) |
| Q3 resolution | Unit (0.7 Da FWHM) |
| Nebulizing gas flow | 3 L/min |
| Drying gas flow | 10 L/min |
| Heating gas flow | 10 L/min |
| DL temperature | 150 °C |
| Heat block temperature | 300 °C |
| Interface Temperature | 350 °C |

In addition, the so-called "MRM spectrum mode" was used for analysis. Here, not only the fragments of the quantifier and the qualifiers are determined, but also a higher number (typically 6-10) of MRM fragment ions. Using this MRM spectrum mode, conventional MRM quantification is combined with a high-quality MRM product ion spectrum, which can be used in a library search routine, thus increasing the specificity and verification of results (Figures 1 and 2).
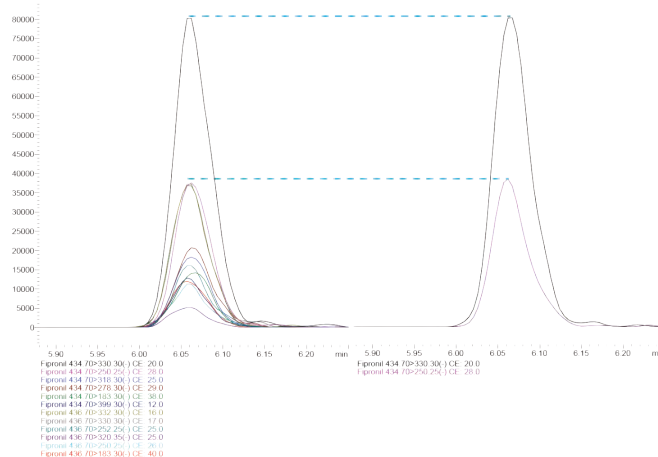


Figure 2: The figure shows MRM chromatograms for Fipronil, one recorded with the usual 2 fragment ions, and compared with a method with higher number (12) of fragment ions which, despite this fact, have the same sensitivity.
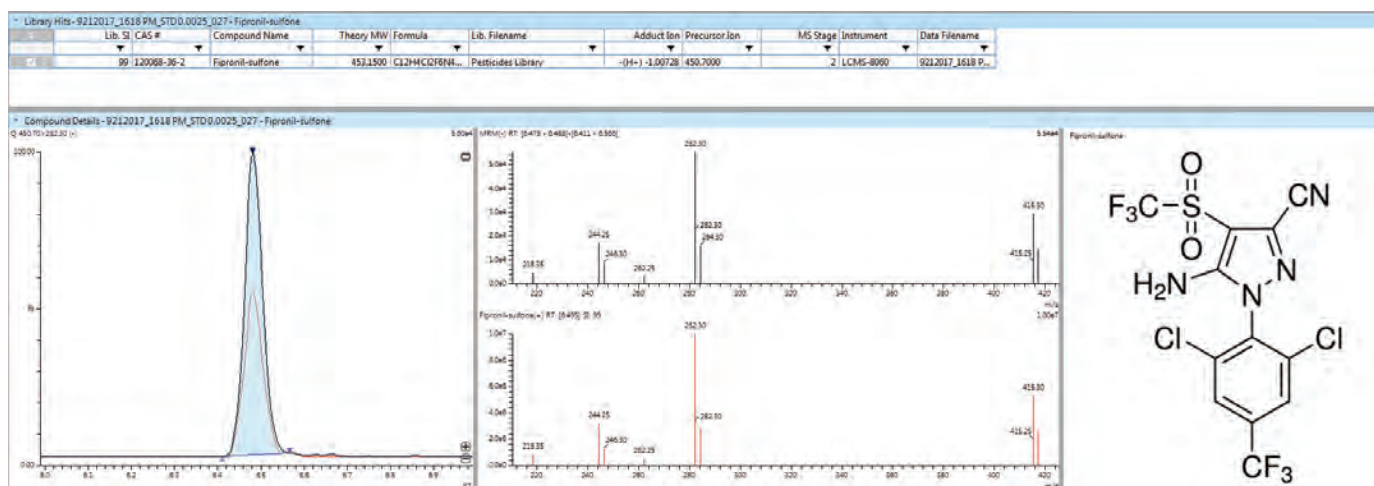


Figure 1: Result of the library search, presented with LabSolutions Insight Screening software

▪ **Calibration**

The matrix matched calibration curve (Figure 3-6) was prepared according to the method described before ranging from 0.0005 mg/kg to 0.05 mg/kg.

Control samples at 0.001 mg/kg and 0.01 mg/kg correspond to the calibration curve.
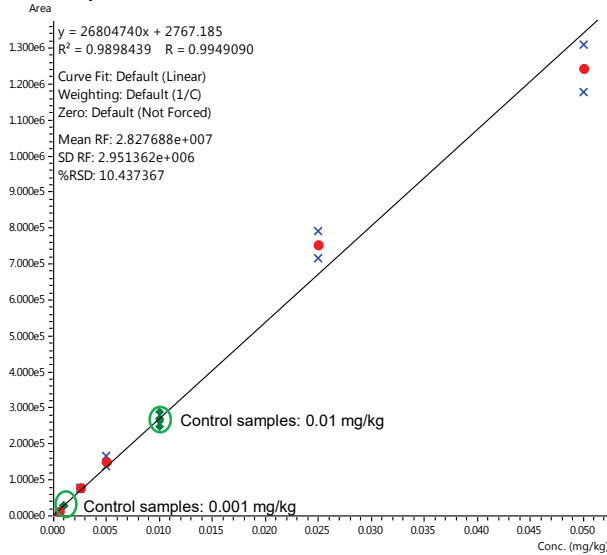
y = 26804740x + 2767.185
R² = 0.9898439   R = 0.9949090

Curve Fit: Default (Linear)
Weighting: Default (1/C)
Zero: Default (Not Forced)

Mean RF: 2.827688e+007
SD RF: 2.951362e+006
%RSD: 10.437367

Control samples: 0.01 mg/kg

Control samples: 0.001 mg/kg

Figure 3:  Calibration curve of Fipronil in egg ranging from 0.0005 mg/kg to 0.05 mg/kg

y = 63084160x + 1031.500
R² = 0.9992798   R = 0.9996398

Curve Fit: Default (Linear)
Weighting: Default (1/C)
Zero: Default (Not Forced)

Mean RF: 6.363292e+007
SD RF: 2.641896e+006
%RSD: 4.151775

Control samples: 0.01 mg/kg

Control samples: 0.001 mg/kg
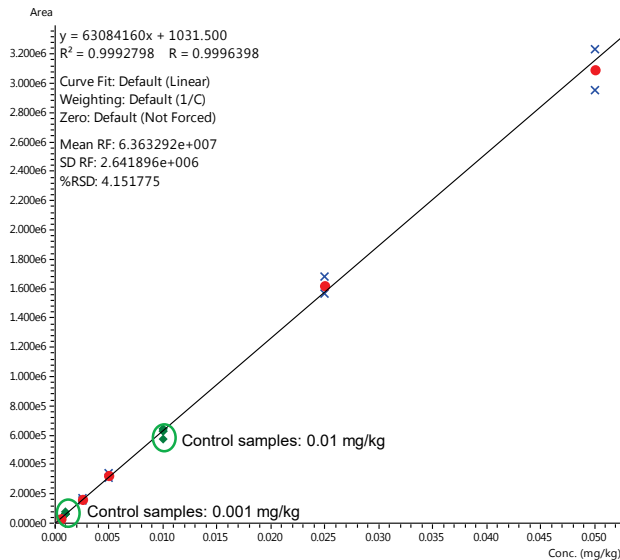
Figure 4:  Calibration curve of Fipronil-sulfide in egg ranging from 0.0005 mg/kg to 0.05 mg/kg

[1] EU Comission Regulation No 1127/2014 of 20 October 2014 Amending Annexes II and III to Regulation (EC) No 396/2005 of the European Parliament and of the Council in regards to maximum residual levels for amitrole, dinocap, fipronil, flufenacet, pendimethalin, propyzamide and pyridate in or on certain products.

y = 67657490x + 6167.372
R² = 0.9989034   R = 0.9994516

Curve Fit: Default (Linear)
Weighting: Default (1/C)
Zero: Default (Not Forced)

Mean RF: 7.093854e+007
SD RF: 3.424479e+006
%RSD: 4.827389

Control samples: 0.01 mg/kg

Control samples: 0.001 mg/kg
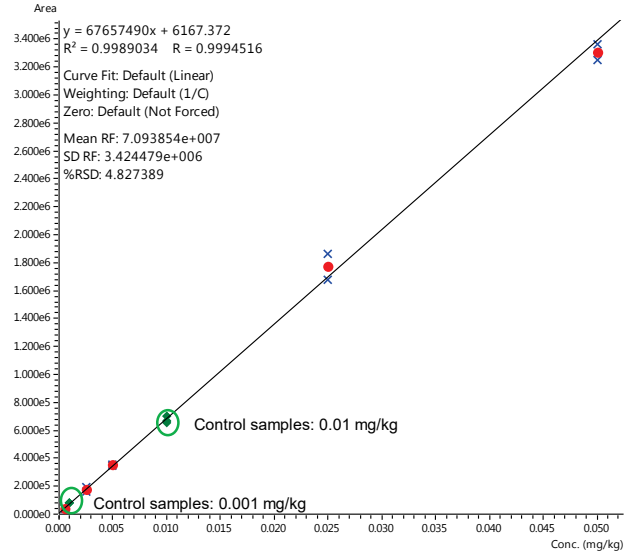
Figure 5:  Calibration curve of Fipronil-sulfone in egg ranging from 0.0005 mg/kg to 0.05 mg/kg

y = 50916160x + 5542.805
R² = 0.9986046   R = 0.9993021

Curve Fit: Default (Linear)
Weighting: Default (1/C)
Zero: Default (Not Forced)

Mean RF: 5.386494e+007
SD RF: 2.93148e+006
%RSD: 5.442279

Control samples: 0.01 mg/kg

Control samples: 0.001 mg/kg
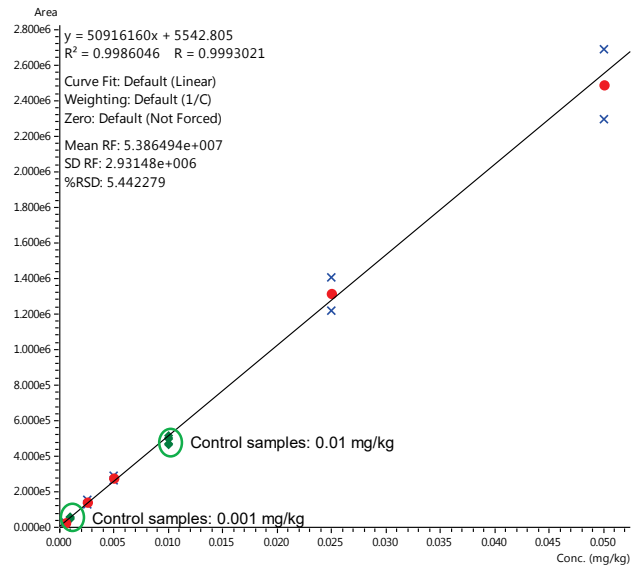
Figure 6:  Calibration curve of Fipronil-desulfinyl in egg ranging from 0.0005 mg/kg to 0.05 mg/kg

▪ **Conclusion**

By using the LC/MS/MS method package for residual pesticides V2 and a QuEChERS sample preparation a method for the determination of Fipronil and Fipronil-sulfone in eggs below the requested MRL of 0.005 mg/kg could be set up rapidly without further method development.

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# SHIMADZU
## Excellence in Science

# Application News

**Liquid Chromatography Mass Spectrometry**

## No.C140

## Ultra-Sensitive and Rapid Assay of Neonicotinoids, Fipronil and Some Metabolites in Honey by UHPLC-MS/MS [LCMS-8060]

Neonicotinoids are a class of insecticides widely used to protect fields as well as fruits and vegetables.

Recently the use of these compounds became very controversial as they were pointed as one cause of the honeybees colony collapse disorder. Since pollination is essential for agriculture, extensive studies have been conducted to evaluate the impact of neonicotinoids on bee health. Following this the European Food Security Authoritiy (EFSA) limited the use of thiamethoxam, clothianidin and imidacloprid. Fipronil, a pesticide from a different chemical class, has been also banned by EFSA for maize seed treatment due to its high risk for honeybee health.

In order to better understand the effect of these compounds on bees and their contamination in pollen and honey, a highly sensitive assay method was necessary. A method was set up using Nexera X2 with LCMS-8060.

### ■ Sample Preparation

Thiamethoxam-d3, imidacloprid-d4 and chlothianidin-d3 were used as internal standards.

Compound extraction was performed using a QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method with an additional dispersive Solid Phase Extraction (dSPE) step.

5 g of honey (±1 %) were weighted in a 50 mL polypropylene tube. 5 µL of internal standard solution at 5 µg/mL of each compound in acetonitrile was added on honey and let dry for 10 minutes. 10 mL of ultra pure water were added and the samples were homogenized by vortex mixing for 1 minute. 10 mL of acetonitrile were then added followed by vortex mixing for 1 minute.

After incubation at room temperature for one hour with gentle shaking, a commercially available salt mix from Biotage (4 g MgSO4, 1 g Sodium Citrate, 0.5 g Sodium Citrate sesquihydrate, 1g NaCl) was added. After manual shaking, samples were centrifuged at 3000 g for 5 minutes at 10 °C.

Supernatant (6 mL) was transferred into a 15 mL tube containing 1200 mg of MgSO4, 400 mg PSA and 400 mg C18 from Biotage. After centrifugation at 3000 g and 10 °C for 5 minutes the supernatant was transferred into a LCMS certified inert glass vial for analysis (Shimadzu LabTotal 227-34001-01).

### ■ Recovery

An "all-flowers" honey from the local supermarket was extracted with or without spike at 50 ppt. A blank extract (no honey) was prepared to evaluate losses or non specific interactions. Results are presented in Table 1.

Calculated recoveries are within acceptance values 70-120 % from EU SANTE/11945/2015.

**Table 1  Measured Recoveries in Honey**

| Compound | Recovery | Compound | Recovery |
|---|---|---|---|
| Acetamiprid | 78.8 % | Fipronil sulfone | 74.2 % |
| Acetamiprid-N-desmethyl | 93.4 % | Imidaclorpid | 83.2 % |
| Chlothianidin | 70.6 % | Nitenpyram | 87.0 % |
| Dinotefuran | 76.5 % | Thiacloprid | 82.2 % |
| Fipronil | 78.1 % | Thiamethoxam | 75.6 % |
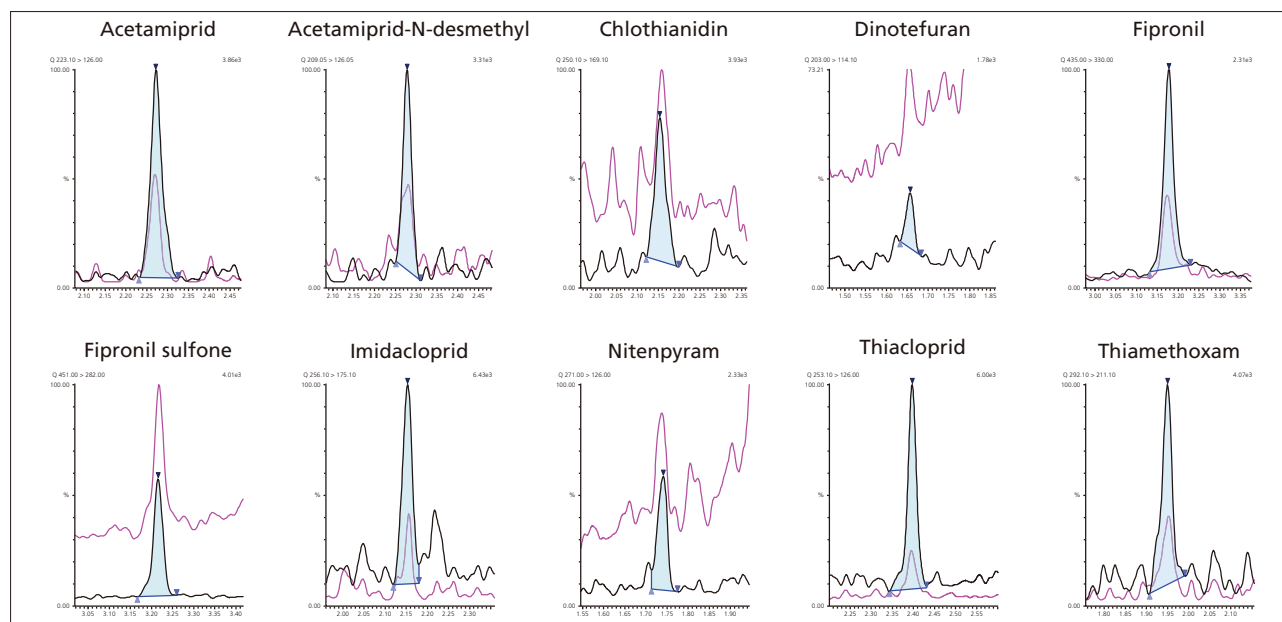


**Fig. 1  Chromatogram of the Target Compounds at Their Lower Limit of Quantification**

**Table 2  Analytical Conditions**

| | | | |
|---|---|---|---|
| System | : Nexera X2 | System | : LCMS-8060 |
| Column | : ACE SuperC18 (100 mm L. × 2.1 mm I.D., 2 µm) | Ionization | : Heated ESI |
| Column Temperature | : 30 °C | Probe Voltage | : +1 kV (positive ionization) / -1.5 kV (negative ionization) |
| Mobile Phases | : A: Water = 0.05 % ammonia | | |
| | B: Methanol + 0.05 % ammonia | Temperature | : Interface: 400 °C |
| Flowrate | : 600 µL/min | | Desolvation Line: 200 °C |
| Gradient | : 5 %B to 100 %B in 3 min | | Heater Block: 400 °C |
| | 100 %B to 5 %B in 0.1 min | Gas Flow | : Nebulizing Gas: 3 L/min |
| Total Run Time | : 4 min | | Heating Gas: 10 L/min |
| Injection Volume | : 2 µL (POISe mode with 10 µL of water) | | Drying Gas: 5 L/min |

**Table 3  MS/MS Acquisition Parameters**

| MRM Transitions | Name | Polarity | MRM Quan | MRM Qual | ISTD |
|---|---|---|---|---|---|
| | Acetamiprid | + | 223.1 > 126.0 | 223.1 > 56.1 | 2 |
| | Acetamiprid-N-desmethyl | + | 209.1 > 126.0 | 211.1 > 128.0 | 2 |
| | Clothianidin | + | 250.1 > 169.1 | 250.1 > 132.0 | 3 |
| | Dinotefuran | + | 203.0 > 114.0 | 203.0 > 87.0 | 1 |
| | Fipronil | - | 435.0 > 330.0 | 435.0 > 250.0 | 3 |
| | Fipronil sulfone | - | 451.0 > 415.0 | 451.0 > 282.0 | 3 |
| | Imidacloprid | + | 256.1 > 175.1 | 258.1 > 211.1 | 2 |
| | Nitenpyram | + | 271.0 > 126.0 | 271.0 > 225.0 | 3 |
| | Thiacloprid | + | 253.1 > 126 | 253.1 > 90.1 | 1 |
| | Thiamethoxam | + | 292.1 > 211.1 | 292.1 > 181.1 | 1 |
| | | | | | |
| | Thiamethoxam-D3 | + | 295.1 > 214.05 | --- | 1 |
| | Imidacloprid-D4 | + | 260.1 > 179.1 | --- | 2 |
| | Clothianidin-D3 | + | 253.1 > 132.05 | --- | 3 |

| | |
|---|---|
| Dwell Time | : 3 to 34 msec depending upon the number of concomitant transitions to ensure to have at least 30 points per peak (max total loop time 140 msec). |
| Pause Time | : 1 msec |
| Quadrupole Resolution | : Q1: Unit   Q3: Unit |

### ■ Calibration

Calibration curves were prepared in acetonitrile to obtain final concentrations ranging from 0.5 pg/mL (1 fg on column) to 5 ng/mL. These concentrations corresponds to 1 ng/kg and 10 µg/kg in honey, respectively.

For each compound, the lower limit of quantification was selected to give an accuracy between 80-120 % (see table 4).

A typical calibration curve is shown in Fig. 2.

**Table 4  Limits of Quantification in Honey**

| Compound | LOQ (µg/kg) | Compound | LOQ (µg/kg) |
|---|---|---|---|
| Acetamiprid | 0.005 | Fipronil sulfone | 0.001 |
| Acetamiprid-N-desmethyl | 0.005 | Imidacloprid | 0.020 |
| Chlothianidin | 0.020 | Nitenpyram | 0.020 |
| Dinotefuran | 0.010 | Thiacloprid | 0.005 |
| Fipronil | 0.001 | Thiamethoxam | 0.005 |



$y = 0.0x^2 + 0.008386x + 0.040184$
$R^2 = 0.9988971$  $R = 0.9994484$

Curve Fit: Quadratic
Weighting: 1/x
Zero: Default (Not Forced)

Mean RF: 6.529647
SD RF: 12.56448
%RSD: 192.4221

n = 1

| Standard (µg/kg) | Accuracy (%) |
|---|---|
| 0.005 | 106 |
| 0.010 | 97.2 |
| 0.020 | 95.6 |
| 0.100 | 107 |
| 0.200 | 98.4 |
| 0.500 | 91.5 |
| 1.000 | 104 |
| 5.000 | 99.9 |
| 10.000 | 100 |
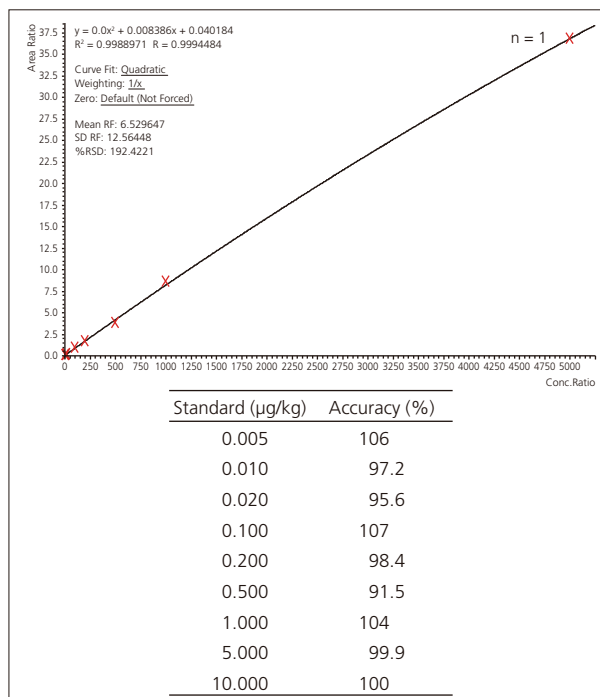
**Fig. 2  Calibration Curve of Acetamiprid**

■ **Real Samples Analysis**

Nine honey samples purchased at the local supermarket or used as raw materials in cosmetics (orange tree honey) were assayed as unknowns.

All tested honeys showed concentrations far below the authorized maximum residue limit. But thanks to the very high sensitivity reached, even low concentrations of neonicotinoids were quantified. Results are presented in table 5.

A representative chromatogram of a sample honey is shown in Fig. 3.

**Table 5  Honey Samples Results (concentrations in µg/kg)**

| Honey | Acetamiprid | Clothianidin | Imidacloprid | Thiacloprid | Thiamethoxam |
|---|---|---|---|---|---|
| 1. Provence creamy | --- | --- | 0.20 | --- | 0.010 |
| 2. Italy creamy | 0.15 | --- | 0.17 | --- | --- |
| 3. Pyrenees liquid | 0.38 | --- | 0.043 | 0.020 | --- |
| 4. French-Spanish creamy | 0.27 | --- | 0.047 | 0.020 | --- |
| 5. Thyme liquid | --- | --- | --- | --- | --- |
| 6. Lemon tree creamy | 1.7 | --- | 0.15 | 0.033 | --- |
| 7. Orange tree liquid | 1.2 | --- | 0.62 | --- | --- |
| 8. Flowers creamy | 0.14 | --- | 0.055 | 0.39 | --- |
| 9. Flowers liquid | 0.34 | --- | 0.11 | 0.010 | --- |

| Honey | Dinotefuran | Nitenpyram | Acetamiprid-N-desmethyl | Fipronil | Fipronil sulfone |
|---|---|---|---|---|---|
| 1. Provence creamy | --- | 0.052 | 0.005 | --- | --- |
| 2. Italy creamy | --- | 0.040 | --- | --- | --- |
| 3. Pyrenees liquid | --- | --- | 0.015 | 0.004 | --- |
| 4. French-Spanish creamy | --- | 0.032 | --- | --- | --- |
| 5. Thyme liquid | --- | --- | --- | --- | --- |
| 6. Lemon tree creamy | --- | --- | 0.020 | --- | --- |
| 7. Orange tree liquid | --- | 0.024 | 0.018 | --- | --- |
| 8. Flowers creamy | --- | --- | 0.016 | --- | --- |
| 9. Flowers liquid | --- | --- | 0.006 | --- | --- |
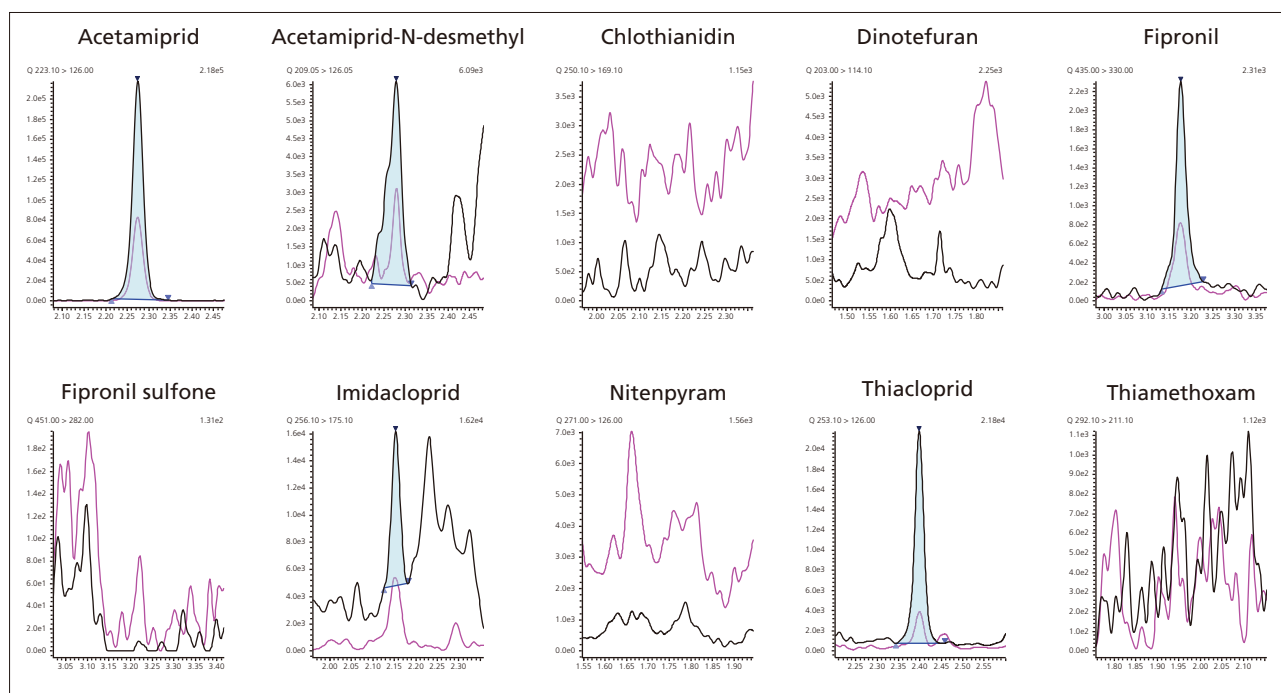


**Fig. 3  Chromatogram of a Sample Honey (Pyrenees)**

### ■ Stability

The thyme honey sample with no detectable target compound was spiked at 50 ng/kg with all compounds prior to extraction. The extract obtained was then consecutively injected 150 times in the system.

The results presented in Fig. 4 show excellent stability of the signal even at these low concentrations. This demonstrates that the excellent sensitivity can be maintained over long series of real sample analysis thanks to the ion source ruggedness.



Acetamiprid RSD = 3.2 %
Acetamiprid-N-desmethyl RSD = 4.8 %
Clothianidin RSD = 7.3 %
Dinotefuran RSD = 17.2 %
Fipronil RSD = 1.2 %
Fipronil sulfone RSD = 1.1 %
Imidacloprid RSD = 6.1 %
Nitenpyram RSD = 3.5 %
Thiacloprid RSD = 10.8 %
Thiamtehoxam RSD = 7.2 %
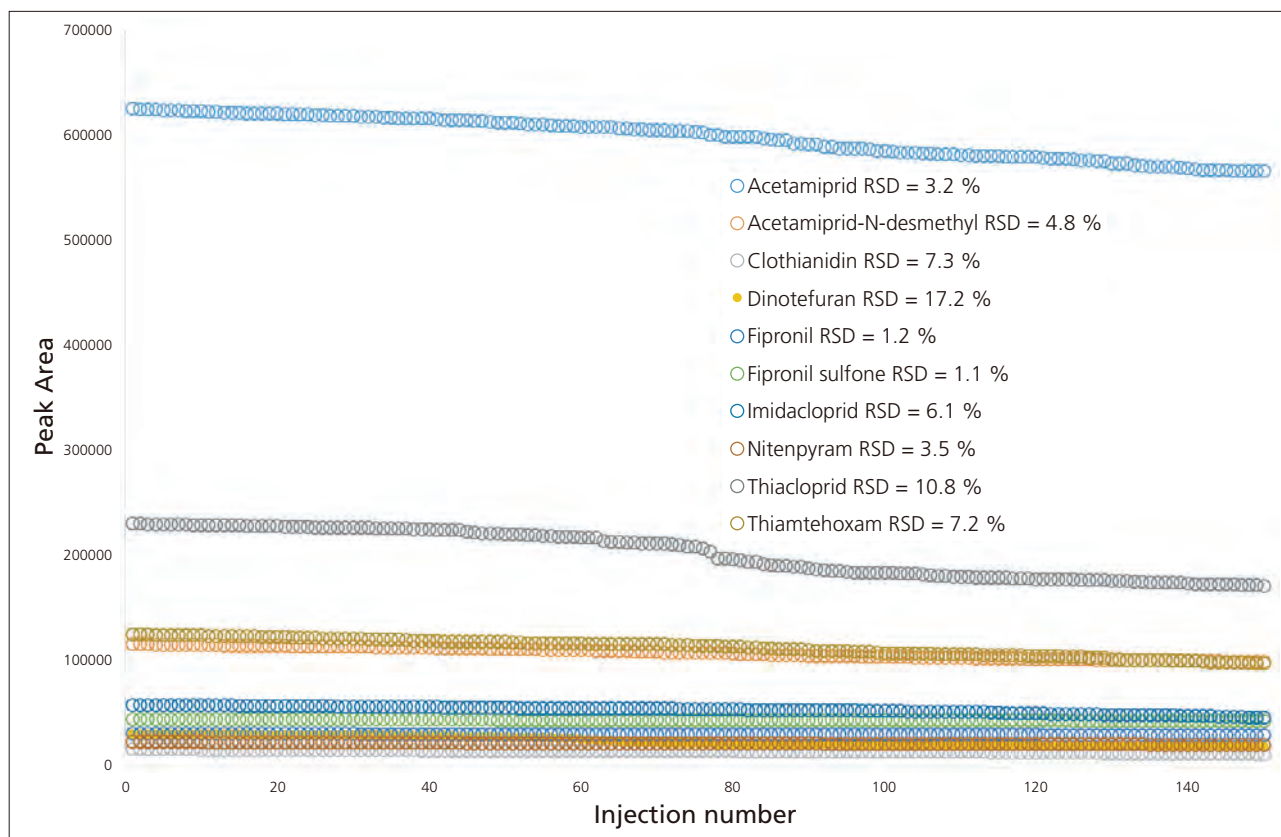
**Fig. 4  Stability of Peak Areas in Real Honey Samples**

### ■ Conclusion

A method for ultra sensitive assay of neonicotinoids in honey was set up. The sample preparation was simple but provided excellent recoveries. The injection mode used prevented the use of tedious evaporation/reconstitution or dilution steps.

Thanks to the high sensitivity obtained enabled assay in real samples at very low levels far under the regulated residue levels. Furthermore, even at low measured concentrations, the system demonstrated its stability after long analytical series of real samples.

This method can be a very efficient support tool to better understand the impact of neonicotinoids on honey bee colonies and could be easily transposed to pollen or bee samples.

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# Technical Report

# Determination of MOSH Contamination in Baby Foods by Using LC–GC and LC–GC×GC–MS
## Advances in food contaminant analysis

Mariosimone Zoccali[1], Peter Q. Tranchida[1], Paola Dugo[1, 2], Luigi Mondello[1, 2]

## Abstract:

The present research is based on an investigation directed towards the use of a rapid heart-cutting multidimensional LC–GC–FID method for the analysis of mineral oil saturated hydrocarbons (MOSH), contained in different types of homogenized solid baby food. Sixteen commercial baby food samples were subjected to analysis. The results were confirmed, in qualitative terms, by collecting the LC fractions, relative to some of the food samples, and subjecting them to comprehensive two-dimensional GC-quadrupole mass spectrometry.

Keywords: multidimensional liquid-gas chromatography, comprehensive 2D gas chromatography, baby food

## 1. Introduction

Mineral oil is derived from crude oil and consists, mainly, of saturated and aromatic hydrocarbons. The former class of compounds, generically defined mineral oil saturated hydrocarbons (MOSH), is formed of straight and branched alkanes, as well as cyclic constituents (naphthenes). The contamination of foods with MOSH is a common occurrence, and many works have been published. For example, Grob et al. in 1991 found a series of contamination sources, namely lubricating oils and release agents exploited in the food industry, or mineral batching oil used for the production of jute [1, 2]. In a later study, it was found that mineral oil can be transferred to dry baby food products via ink printed on cardboard containers [3].

At present, there are no legal limits related to the amounts of MOSH contained in foods; a single exception (50 mg/kg) is that related to Ukrainian sunflower oil, following a severe case of contamination [4]. A limit of 0.6 mg/kg for MOSH (from $C_{10}$ to $C_{25}$) contamination in foods, as a consequence of migration from cardboard packaging, has been recently proposed by the German Federal Ministry of Food, Agriculture and Consumer Protection (BMELV).

Such a limit was derived from a calculation made considering an adult of 60 kg, consuming 1 kg of contaminated food per day.

Heart-cutting LC–GC, with a flame ionization detector (FID), is a prime choice for the quantification of MOSH. The first LC dimension is always exploited to separate the hydrocarbons from the lipid matrix.

The present investigation is based on the development and validation of an LC–GC–FID method for the analysis of mineral oil saturated hydrocarbons in baby food. The main novelties of the research, are related to the high analysis speed and to the use of a novel comprehensive LC–GC (LC×GC) interface, capable of working in both heart-cutting or "comprehensive" (LC×GC) conditions. In the latter configuration, continuous and sequential LC fractions are transferred to the GC column.

The MOSH fraction is transferred and analyzed in the GC dimension, where it appears typically as a "hump". Moreover, the use of LC–GC×GC–qMS appears to be an interesting option to "see and identify" what is beneath anonymous LC–GC–FID hydrocarbon humps.



Fig. 1   LC–GC–FID chromatogram for the salmon (I) baby food sample.
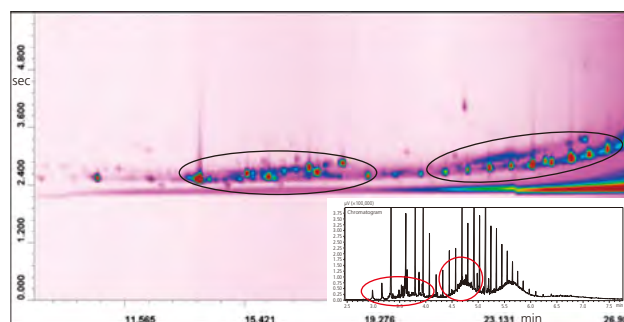


Fig. 2   TIC GC×GC–MS chromatogram relative to the MOSH fraction derived from the salmon (I) baby food.

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## 2. Experimental

## 2-1. Sample Preparation

All the samples (500 mg) were extracted for three times with hexane (1 mL each, for 15 min), and then each time filtered. After, the filtrates were added together and dried under a gentle nitrogen stream. The extract was weighed (to derive the % of extract) and then diluted to a final concentration of 25% *v/v* in hexane. Quantification was achieved through a calibration curve, constructed through external standardization. The MOSH hump was integrated through a manual software function, performing the subtraction of the linear alkanes.

## 2-2. LC–GC–FID Analyses

All sixteen samples were analyzed by using an LC×GC system (Shimadzu) consisting of:

(1) A Shimadzu Prominence LC-20A system, equipped with a CBM-20A communication bus module, two LC-20AD dual-plunger parallel-flow pumps, a DGU-20A degassing unit, an SPD-M20A photodiode array detector, a CTO-20A column oven, and an SIL-20AC autosampler. Data were acquired by the LCsolution software (Shimadzu). LC conditions: a 100 mm L. × 3 mm I.D. × 5 µm $d_p$ silica column (SUPELCOSIL LC-Si, Supelco, Milan, Italy) was operated under isocratic conditions, using hexane as mobile phase (0.35 mL/min). Injection volume: 20 µL. At the end of the heart cut, the column was backflushed using $CH_2Cl_2$.

(2) A Shimadzu AOC-5000 autoinjector equipped with a dedicated dual side-port syringe, employed as a transfer device. Chromatography band transfer is achieved, in the stop-flow mode, through a modified 25-µL syringe. The lower part of the syringe is connected, *via* two transfer lines, to the LC detector exit and to waste. A Teflon plug is located at the end of the syringe plunger; the latter is characterized by a lower OD with respect to the barrel ID, thus enabling mobile phase flow inside the syringe. In the waste mode, the syringe plug is located below both lines and the effluent is directed to waste. In the cut position, the syringe plug is located between the upper and lower line and the effluent flows to the GC. From 0 to 1.5 min the LC effluent was directed to waste, while from 1.5 to 2.0 min the LC effluent was directed to the GC.

(3) A Shimadzu GC-2010 Plus, equipped with an Optic-3 PTV injector (GL Sciences B.V., Eindhoven, The Netherlands). The Optic-3 injector was temperature-programmed as follows: from 75 °C (1 min) to 360 °C at 250 °C/min. Injection mode: split, at a ratio of 200:1 for 1 min during sample introduction and solvent vent, then splitless for 1 min during sample desorption and transfer, then 50:1 for the remaining analysis time.
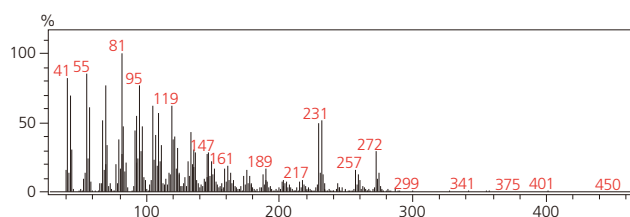


Fig. 3   Mass spectrum extracted from the first "cycloalkane zone".

Data were acquired by the GCsolution software. GC conditions: an SLB-5 ms [silphenylene polymer, virtually equivalent in polarity to poly (5% diphenyl/95% methylsiloxane)] 15 m L. × 0.10 mm I.D. × 0.10 µm $d_f$ column (Supelco) was heated from 50 °C (1 min) to 360 °C (4 min) at 70 °C/min. Carrier gas, hydrogen, was supplied at an initial pressure of 529 kPa (constant linear velocity: 100 cm/s). FID (360 °C) sampling frequency was 50 Hz.

A dedicated LC×GC software enabled the control of each instrument through the respective native software. The LC×GC software controlled the transfer process. All parameters, including the LC and GC methods, can be saved with the LC×GC method.

## 2-3. GC×GC–MS Analyses

All GC×GC applications were carried out on a Shimadzu GC×GC–MS system, consisting of a GC-2010 gas chromatograph, and a GCMS-QP2010 Plus quadrupole mass spectrometer. The primary column, an SLB-5 ms 30 m L. × 0.25 mm I.D. × 0.25 µm $d_f$ column, was connected to an uncoated capillary segment (1.0 m L. × 0.25 mm I.D., used to create a double-loop), by using an SGE SilTite mini-union (SGE, Ringwood, Victoria, Australia). The uncoated capillary was then connected to a segment of BPX50 (50% phenyl polysilphenylene–siloxane) 1.5 m L. × 0.10 mm I.D. × 0.10 µm $d_f$ column (SGE), by using another union. Modulation was carried out every 6 s, by using a loop-type modulator (under license from Zoex Corporation, Houston, TX, USA). The duration of the hot pulse (350 °C) was 375 ms.

Table 1   Samples analyzed, total levels of contamination, and contamination considering an upper boundary defined by $C_{25}$.

| Baby food | MOSH (mg/kg) | ≤ $C_{25}$ alkanes (mg/kg) |
|---|---|---|
| 1 - Salmon I | 13.8 | 2.0 |
| 2 - Plaice | 3.5 | 0.9 |
| 3 - Chicken | 3.0 | 1.4 |
| 4 - Beef I | 1.9 | 0.8 |
| 5 - Beef II | 5.6 | 2.2 |
| 6 - Beef-Ham | 4.9 | 2.0 |
| 7 - Turkey | 3.2 | 1.1 |
| 8 - Sea bass | 1.4 | 0.9 |
| 9 - Calf | 2.5 | 0.7 |
| 10 - Rabbit | 3.5 | 1.0 |
| 11 - Ostrich | 1.6 | — |
| 12 - Salmon II | 1.1 | — |
| 13 - Prune I | 0.6 | — |
| 14 - Pear | 3.6 | 0.6 |
| 15 - Fruit mix | 0.3 | — |
| 16 - Prune II | 10.5 | 1.8 |

MS parameters: the samples were analyzed in the full scan mode, using a mass range of 40–460 *m/z*; spectra generation frequency: 25 Hz; interface and ion source temperatures were 300 °C and 200 °C, respectively. MS ionization mode: electron ionization. GC oven temperature program: 80–360 °C at 10 °C/min. Carrier gas, helium, was supplied at an initial pressure of 250 kPa (constant linear velocity). Injection temperature: 350 °C. Injection mode and volume: split (1:10), 4 µL. Data were collected by the GCMSsolution software; bi-dimensional visualization was carried out by using the Chrom-Square v. 2.0 software.
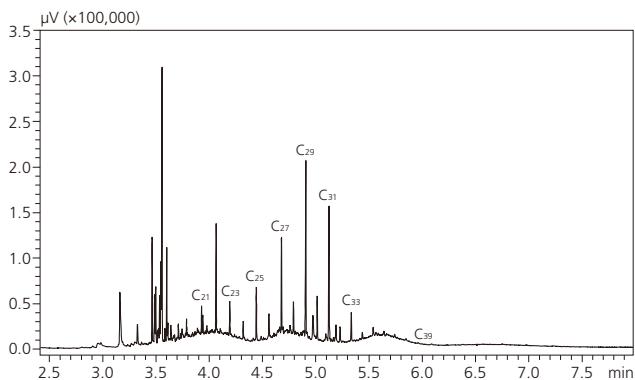


Fig. 4  LC–GC–FID chromatogram for the beef (II) baby food sample.

## 3. Method Validation

A seven-point calibration curve was constructed using solutions of mineral oil saturated hydrocarbons in hexane at the 1, 5, 10, 20, 50, 100, and 200 ppm levels (n = 3). The area of each MOSH hump was integrated by using the GC–FID "manual integration" software function; the *n*-alkane areas on top of the hump were subtracted from the final result. The derived calibration curve was linear in the range of concentrations considered and was characterized by a regression coefficient ($R^2$) of 0.9994. Intra-day precision was calculated by analyzing one sample, six times consecutively. The resulting standard deviation equaled ± 1.5 mg/kg (average value: 35.2 mg/kg), while the coefficient of variation was 4.1%. Inter-day precision was calculated by analyzing sample no. 9 three times, on five consecutive days. The resulting standard deviation equaled ± 0.34 mg/kg (average value: 14.5 mg/kg), while the coefficient of variation was 2.4%. Limits of detection and quantification were calculated by multiplying the standard deviation of the area of the "blank" olive oil sample (n = 3) three and ten times, respectively, and then dividing the result by the slope of the calibration curve. The values extrapolated, namely 0.15 and 0.5 mg/kg, gave final LOD and LOQ values of 0.6 and 2 ppm, respectively. Such LOD and LOQ values can be considered as acceptable.

## 4. Results and Discussion

Initially, the scope of the present research was to apply an LC–GC method to baby foods, prepared using vegetable oil. Twelve homogenized solid baby foods, containing either meat or fish (and vegetable oil), were subjected to analysis (Table 1). Three products were fish-based (2 salmon, sea bass, plaice), while all the others contained meat as the main ingredient (chicken, 2 beef, calf, beef-ham, turkey, rabbit, ostrich). Hexane baby food extraction was performed three times; the fourth extraction gave a negligible analytical response. The vegetable oil used as an ingredient was, in all twelve cases, sunflower oil and on the basis of its position in the ingredient list it was certainly present in low amounts (most probably less than 5%). A hexane blank was analyzed periodically throughout the period of research, by drying a 3 mL volume of hexane (the volume used for sample extraction), by adding a 50 µL volume of hexane to the residue, and then subjecting a 20 µL quantity to LC–GC analysis. The resulting chromatograms were always characterized by a negligible response. As can be observed in Table 1, the meat and fish baby foods were all contaminated (the results are the average of two applications), to various degrees. Also reported in the table are (approximate) MOSH concentrations considering the upper alkane boundary on the basis of the BMELV proposal (MOSH up to $C_{25}$). The highest MOSH level, namely 13.8 mg/kg, was found in the salmon I sample.
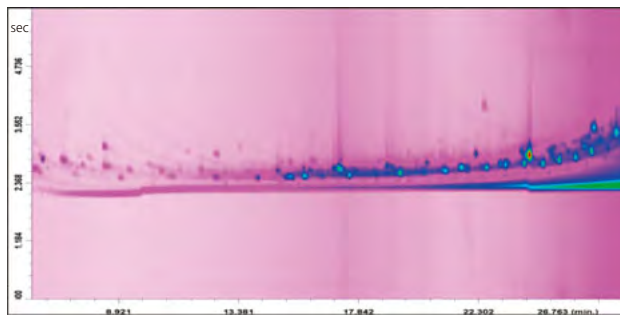


Fig. 5  TIC GC×GC–MS chromatogram relative to the MOSH fraction derived from the beef (II) baby food sample.

Considering contamination up to the $C_{25}$ point, the MOSH concentration was approximately 2.0 mg/kg, well over the proposed maximum limit (0.6 mg/kg). The GC chromatogram, relative to the salmon I baby food, is reported in Fig. 1. The chromatogram is characterized by two predominant humps (and two external smaller ones) centered roughly at *n*-alkane $C_{27}$ and $C_{36}$. After quantification, the MOSH "humps" were subjected to a qualitative investigation. A four-dimension off-line LC–GC×GC–qMS experiment was carried out as follows: the 175 µL MOSH fraction was collected, dried, and the residue was solubilised in 10 µL of hexane. Four microliters were injected into the GC×GC–qMS system (split ratio: 1:10), leading to an injected MOSH quantity about 25 times lower than that analyzed by using LC–GC–FID. However, the enhanced sensitivity of cryogenic modulation was something that was counted on. The LC–apolarGC×polarGC–qMS result, which is shown in Fig. 2, was attained in a relatively short time (<30 min), but obviously could not match the speed of the LC–GC second dimension. The LC–GC–FID result, for the salmon I sample, is also included in the same figure to make a direct comparison easier.

A mass spectrum derived from the "cycloalkane zone" is shown in Fig. 3. The "cyclics" were identified on the basis of (I) bidimensional chromatogram location, and (II) information reported in the literature, related to the fragmentation of cycloalkanes [5]: the molecular ion intensity is more abundant in cycloalkanes than in straight-chain alkanes, as is also the tendency to lose even-numbered fragment ions, producing a greater number of even-numbered mass ions. Compounds containing cyclohexyl rings tend to produce ions at *m/z* 83, 82 and 81 corresponding to ring fragmentation, and loss of one and two hydrogen atoms, respectively. A further example of MOSH contamination can be observed in Fig. 4 and 5, which illustrates the LC–GC–FID result for beef sample II. The MOSH levels in the beef sample were approximately one third (5.6 ppm) of that found in the salmon. However, the contamination type is different because three MOSH humps are present, with the second and third spanning the $C_{25}$–$C_{39}$ range, and the first starting approximately at $C_{19}$ and ending at $C_{24}$. Considering the $C_{25}$ boundary, the MOSH concentration was 2.2 mg/kg. With regards to the other samples reported in Table 1, it can be affirmed as follows: the third most contaminated sample, namely beef-ham (4.9 ppm), generated a GC chromatogram characterized by two humps, in the $C_{19}$–$C_{24}$ (≈40%) and $C_{25}$–$C_{42}$ range. Such a contamination level and type is comparable to that observed in beef sample II. Five other samples were characterized by MOSH levels around the 3 ppm mark (plaice, chicken, turkey, calf, rabbit), while the remaining four baby foods, namely beef (sample I), ostrich, sea bass, and salmon II were the less contaminated ones, with values under 2 ppm. It must be emphasized that only two baby foods, namely ostrich and salmon II, were characterized by a MOSH contamination lower than the "$C_{25}$" limit. At this point, it was decided to extend the study to fruit-based baby foods, namely products containing no vegetable oil. Four samples (2 prunes, 1 pear, 1 fruit mix) were subjected to analysis, and found to contain MOSH concentrations in the range 0.3–10.5 mg/kg (Table 1). Two baby foods, namely prune I and fruit mix, were characterized by a MOSH presence within the "$C_{25}$" limit, while the pear sample was right at the limit; the prune II sample was, on the other hand, the second most contaminated, considering all the baby foods subjected to analysis (Table 1). The results attained from the fruit products clearly indicated that the presence of MOSH was not only due to that of the vegetable oil.

## 5. Conclusions

All the baby foods analyzed in the present research were found to be MOSH contaminated, with considerable levels being observed (average value: 3.8 mg/kg). In fact, if one refers to the 0.6 mg/kg limit proposed by the BMELV, the contamination levels must be evaluated as high, especially considering the age and body weight of the consumers. In fact, taking into account the $C_{25}$ boundary, the average MOSH concentration was approx. 1 mg/kg. The presence of MOSH has been found in human milk [6], and on the basis of the present findings, there is a high probability that MOSH intake continues as soon as the infant passes on to solid food. It is clear that the origin of the MOSH contamination observed, in all samples, cannot be exactly pinpointed, though the vegetable oil probably plays a role. The hydrocarbon molecular-weight range also varied, meaning that the contamination most certainly came from different sources.

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Food – MOSH/MOAH

## Mineral Oil Residues in Food
## Part 1- Dry Food (Flour, Noodles and Rice)

### ■ Introduction

Mineral oil (MO) residues in food raised public concern due to some elevated concentrations up to several thousand milligrams per kilogram food [1]. Due to the chemical structures two groups of MOs can be differentiated. Mineral oil saturated hydrocarbons (MOSH) consist of linear and branched alkanes, and alkyl-substituted cyclo-alkanes, whilst mineral oil aromatic hydrocarbons (MOAH) include mainly alkyl-substituted polyaromatic hydrocarbons. Technical grades of mineral oil contain aromatic hydrocarbons in a concentration range from 15-35%. Food grade mineral oils (white oils) can have lower MOAH concentrations. Rice and pasta and other grain based food products including flour from different grain varieties are consumed in high amounts all over the world. The annual rice consumption per capita is over 54 kg per capita [2]. The annual pasta consumption in Italy is 26 kg and approximately 8 kg in Germany [3].

At the moment there are no legal limits in Europe for MOSH/MOAH, but MOSH concentrations up to 2 mg/kg and MOAH levels below 0,5 mg/kg are considered to be acceptable.

### ■ System Setup

For the determination of the two mineral oil fractions an online LC-GC –FID system was used. The LC is directly connected to two high temperature GC columns with retention gaps which are installed in one GC oven. MOSH and MOAH fractions are separated on a silica gel column using a n-hexane /dichloromethane gradient. The interface between LC and GC is controlled by Axel Semrau LC-GC Chronect interface. After transferring the MOSH fraction on column 1 and MOAH on column 2 the temperature programme is started and both fractions are separated simultaneously and detected by FID. Figure 1 shows a typical LC-Chromatogram with UV-signal in black, pump pressure in green, $CH_2Cl_2$ concentration in blue and total flow in purple. Figure 2 shows the LC-GC-FID system.

**LC Parameters:**
Shimadzu LC-20AD solvent delivery pump
Column: Allure Silica 5 µm (250 × 2.1 mm)
Gradient: Start with 100 % n-Hexane (flow 0.3 ml/min), $CH_2Cl_2$ raised to 35 % within 2 min (hold for 4.20 min), column was flushed at 6.30 min with 100 % $CH_2Cl_2$ (flow 0.5 ml/min; hold for 9 min) and reconditioned to 100 % n-Hexane (flow 0.5 ml/min; hold for 10 min). Flow was decreased afterwards to 0.3 ml/min until next injection.
UV-Detector: $D_2$-lamp; 230 nm, 40 °C cell temperature
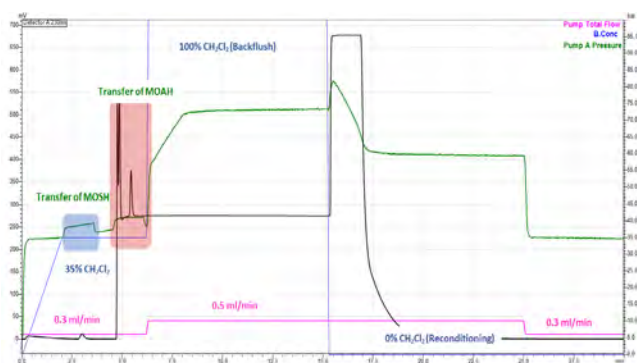
Fig. 1: LC-Chromatogram

**GC Parameters:**
Shimadzu GC-2010 Plus dual FID
Guard Columns: Restek MXT Siltek (10 m × 0.53 mm id)
Columns: Restek MTX ®-1 (15 m × 0.25 mm id × 0.1 µm df)
Carrier gas: Hydrogen (150 kPa analysis pressure; evaporation pressure: 87 kPA MOSH, 85 kPA MOAH
Temperature program:
60 °C (6 min) @ 20 °C/min to 100 °C (0 min) and followed by 35 °C/min to 370 °C (9.29 min)



Fig. 2: LC-GC-FID System

■ **Experimental Work**

Sample preparation
Depending on the expected mineral oil concentration 1-10 g of homogenized and finely ground samples were used. The sample was extracted in Hexane/Ethanol 1/1 after the addition of an internal standard mixture (Restek MOSH/MOAH standard Cat.#: 31070 containing 9 internal standards) at room temperature for 2 hours under occasionally shaking the flask. After the extraction water was added and centrifuged. The washing step was repeated a second time. Afterwards the organic layer was dried over anhydrous sodium sulphate and the volume was adjusted to 1 mL in an automated solvent concentrator. The extract was transferred into a 2 mL autosampler vial and put in the autosampler rack of the LC-GC system. Aliquots of 50 µl were injected into the LC. Here the separation into the MOSH- and MOAH fraction is performed. Each fraction has a size of 450 µL and is transferred to the respective channel of the GC for parallel MOSH/MOAH determination.

**Quantification**

For the quantification of the MOSH fraction the internal standard Bicyclohexyl (CyCy) is used. For the MOAH fraction 2-Methyl-naphtahlene (2-MN) is used. All other internal standards are used to ensure no losses of analytes and a good separation between the MOSH and MOAH fraction.
According to a proposed method published by the German Bundesinstitut für Risikobewertung (BfR) quantification is done by integration of the hump for different molecular weight regions. They propose for food contact materials three ranges for the MOSH fraction ($C_{10}$-$C_{16}$, $C_{16}$-$C_{25}$ and $C_{25}$-$C_{35}$) and two ranges for the MOAH fraction ($C_{10}$-$C_{25}$, $C_{25}$-$C_{35}$). For dry food only the ranges up to $C_{25}$ are used [4]. Figure 3 shows the MOSH (black trace) and MOAH (purple trace) of a spaghetti sample with a MOSH concentration of 12.7 mg/kg from $C_{16}$-$C_{35}$ and the marked regions of $C_{16}$-$C_{25}$ (blue) and $C_{25}$-$C_{35}$ (red). The internal standards are marked with symbols (black squares internal standards MOSH: $C_{11}$, Bicyclohexyl, $C_{13}$ and Cholestane eluting with the MOSH hump, purple stars internal standards MOAH: Pentylbenzene, 1 & 2-Methylnaphthalene, Tri-tert-butylbenzene and Perylene) .The rice sample (Figure 3) shows also additional peak in the rear part of the chromatogram. These are naturally occurring odd-numbered alkanes with a chain length of $C_{21}$ to $C_{35}$.
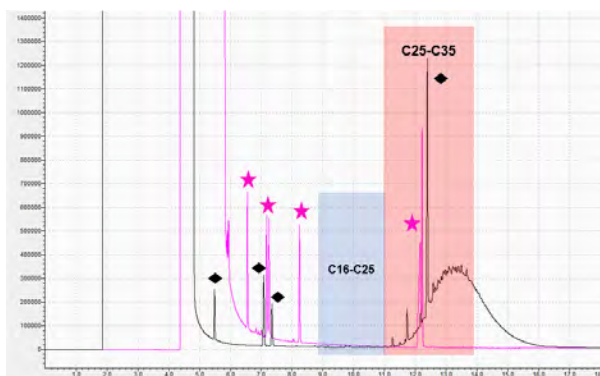
Fig. 3: Spaghetti sample
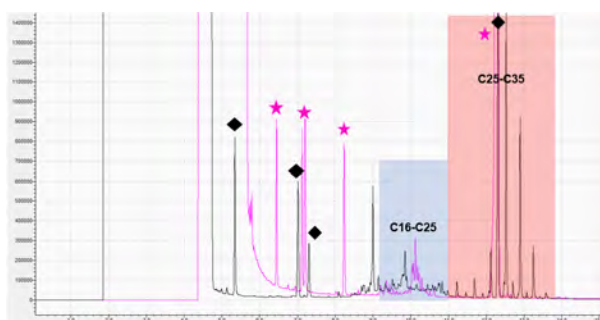


Fig. 4: Rice sample
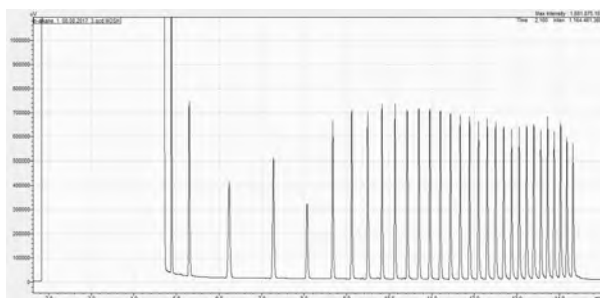


Fig. 5: n-Alkane standard C10-C40 as retention time marker

## ■ Conclusion

The new LC-GC-FID provides a tool for fast and reliable routine analysis of MOSH and MOAH in dry non fatty food. After the extraction step the samples are analysed fully automated with a high sample through put.

## ■ References

1) EFSA Panel on Contaminants in the Food Chain (CONTAM) Scientific Opinion on Mineral Oil Hydrocarbons in Food DOI: 10.2903/j.efsa.2012.2704

2) Link: https://www.statista.com/statistics/256002/global-per-capita-rice-use-since-2000/

3) Link: http://www.internationalpasta.org/index.aspx?idsub=118

4) Link: http://www.bfr.bund.de/cm/343/bestimmung-von-kohlenwasserstoffen-aus-mineraloel-oder-kunststoffen.pdf

# Dairy Products

1. A High Sensitivity LC/MS/MS Method with QuEChERS Sample Pre-treatment for Analysis of Aatoxins in Milk Powder Samples

2. Assay of Aflatoxin M1 in Milk Based on Notification Test Methodology, Using Prominence-i and the RF-20AXS Fluorescence Detector

3. Determination of Polychlorinated Dibenzo-p-dioxins and Dibenzofurans (PCDD/Fs) in Foodstuffs and Animal Feed Using a Triple Quadrupole GCMS-TQ8040 System with Smart MRM Transforms Laboratory Analysis

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# A High Sensitivity LC/MS/MS Method with QuEChERS Sample Pre-treatment for Analysis of Aflatoxins in Milk Powder Samples

## ASMS 2016  WP230

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# Introduction

Aflatoxins (B1, B2, G1 and G2) are metabolites produced by fungi (Aspergillus favus and Aspergillus parasiticus) in crops, animal feed and dairy products. Aflatoxins are highly toxic contaminants in food and feed and their amounts increase under bad storage conditions favourite for fungal growth. Aflatoxin M1 is a hydroxylated metabolite of aflatoxin B1 found in milk of cow fed with a diet contaminated with aflatoxin B1[1]. Aflatoxin B1 is known the most carcinogenic among all the aflatoxins, and hence its metabolite aflatoxin M1 is given critical attention. Strict regulations for aflatoxin M1 in milk and dairy products have been set. For example, European Union (EU) limits the level of aflatoxin M1 to no more than 0.05 µg/kg in milk and dairy products and 0.025 µg/kg in infant food. We present a high sensitivity LC/MS/MS method for quantitative analysis of the five aflatoxins (B1, B2, G2, G2 and M1) in milk powder incorporating QuEChERS sample pre-treatment procedure, which is more cost effective as compared to the traditional procedure using immunoaffinity column (IAC)[2]. High sensitivity and good recoveries were achieved using this LC/MS/MS method.

# Experimental

A mixed standard of aflatoxin B1, B2, G1 and G2 was obtained from Supelco. Aflatoxin M1 was obtained from Romer Labs. A stock solution of the mixture of 5 aflatoxins was prepared using methanol as the diluent, from which calibrant series and spiked samples were prepared. The QuEChERS kits were purchased from RESTEK. Two grams of milk powder was first extracted with the extraction kits followed by cleaning up using dSPE tubes. A LCMS-8060 triple quadrupole LC/MS/MS (Shimadzu Corporation, Japan) was used in this work. A C18 column (Kinetex, 2.1 x 100mm, 1.7um) was used for fast separation of aflatoxins using a gradient elution program. Method performance evaluation were carried out using spiked aflatoxins in milk powder samples. Table 1 shows the analytical conditions on LCMS-8060.



Shimadzu LCMS-8060, an UFMS triple quadrupole system with a heated ESI interface

Table 1: LC/MS/MS analytical conditions of aflatoxins on LCMS-8060

| | |
|---|---|
| Column | : Kinetex C18 (2.1mmI.D x 100mmL., 1.7 μm) |
| Flow rate | : 0.5 mL/min |
| Mobile phase | : A: 5 mM ammonium acetate in water with 0.1% FA |
| | B: 5 mM ammonium acetate in MeOH |
| Oven temp. | : 40ºC |
| Injection vol. | : 5 μL |
| Elution mode | : Gradent elution, B%: 5% (0-5 min) 50% (4- 5.5 min) |
| | 85% (6-7.5 min) 5% (8.1-10 min) |
| Interface | : ESI (Heated) |
| MS mode | : Positive, MRM, 2 transitions each compound |
| Interface temp. | : 350ºC |
| Block temp. | : 400ºC |
| DL temp. | : 250ºC |
| CID gas | : Ar (350 kPa) |
| Nebulizing gas flow | : 3.0 L/min |
| Drying gas flow | : 10.0 L/min |
| Heating gas flow | : 10.0 L/min |

# Results and Discussion

## QuEChERS sample pre-treatment

Hexane was used in the procedure to remove fats, oils and non-polar components from the milk powder samples. The extraction step was completed using Q-sep QuEChERS extraction salt packet (4 g MgSO4, 1 g NaCl, 1 g trisodium citrate dehydrate, 0.5 g disodium hydrogen citrate). Dispersive SPE tube containing MgSO4, PSA and C18 was used in the clean-up process to remove remaining water, organic acid and non-polar components respectively. The process of the sample preparation is illustrated in Figure 1.

## Method Development

Automated MRM optimisation of the five aflatoxins was carried out using the LabSolutions workstation. Two MRM transitions for every aflatoxin were chosen as quantifier and confirmation ion (Table 2).

A milk powder matrix free from aflatoxins was used as a "blank" and matrix for the preparation of post-spiked calibrants to build calibration curves. The blank and every post-spiked calibrant was injected thrice and the average area was calculated to obtain reliable results.

# A High Sensitivity LC/MS/MS Method with QuEChERS Sample Pre-treatment for Analysis of Aflatoxins in Milk Powder Samples
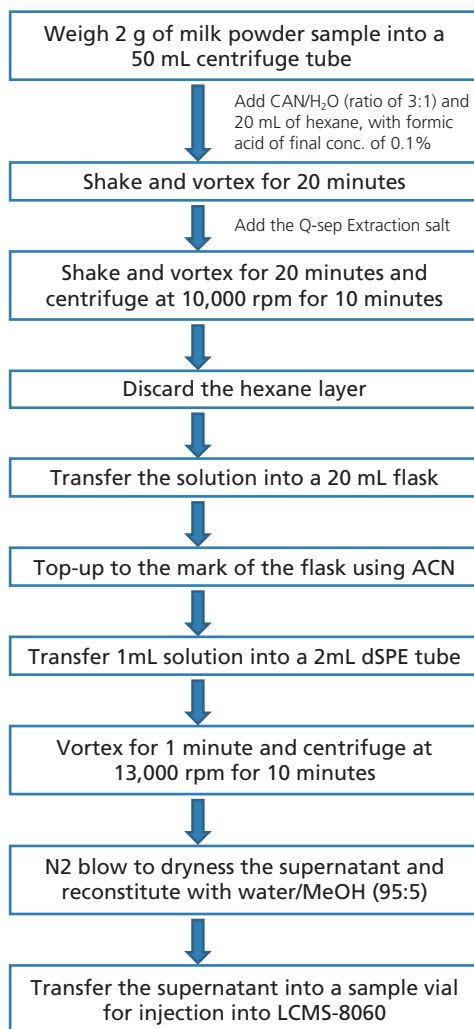


Figure 1: Flowchart of sample pre-treatment for aflatoxins in milk powders by modified QuEChERS method.

Table 2: LC/MS/MS analytical conditions of LCMS-8050 for aflatoxins

| Compound | MRM (m/z) | CID Voltage (V) | | |
|---|---|---|---|---|
| | | Q1 | CE | Q3 |
| Aflatoxin B1 | 313.1>241.0* | -12 | -40 | -17 |
| | 313.1>213.0 | -21 | -44 | -15 |
| Aflatoxin B2 | 315.1>287.0* | -22 | -27 | -20 |
| | 315.1>259.1 | -11 | -30 | -18 |
| Aflatoxin G1 | 329.1>243.0* | -12 | -28 | -17 |
| | 329.1>200.0 | -12 | -40 | -22 |
| Aflatoxin G2 | 331.1>189.0* | -24 | -43 | -19 |
| | 331.1>245.0 | -12 | -31 | -18 |
| Aflatoxin M1 | 329.0>273.0* | -12 | -23 | -18 |
| | 329.0>259.0 | -23 | -24 | -29 |

* MRM transitions used as quantifiers.

# A High Sensitivity LC/MS/MS Method with QuEChERS Sample Pre-treatment for Analysis of Aflatoxins in Milk Powder Samples

A chromatogram of spiked sample is shown in Figure 2. Linear calibration curves were obtained for all five aflatoxin compounds with good linearity (r² >0.999). The calibration curves of aflatoxins spiked in milk powder matrix are shown in Figure 4.

## Method Performance Evaluation

The LOD and LOQ of aflatoxins in milk powder matrix are lower than 0.83 pg/mL and 2.50 pg/mL respectively (Table 3). The repeatability of the method was evaluated using spiked samples at two concentrations. The peak area %RSD of aflatoxins were found to be lower than 7.46%.
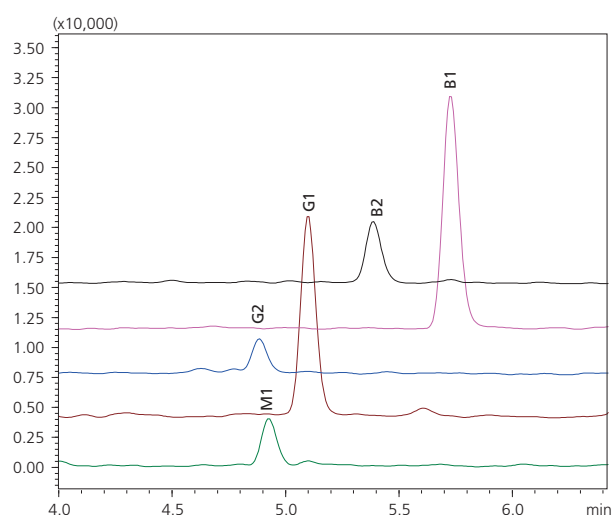


Figure 2: Total ion chromatogram of 5 aflatoxins (Concentrations of B1,G1 and M1 at 10 pg/mL; B2 and G2 at 3 pg/mL)
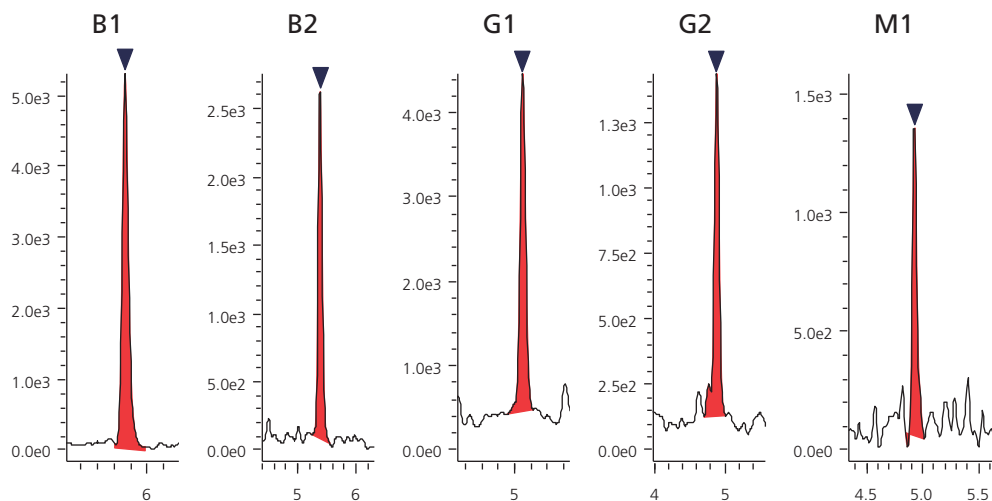


Figure 3: Single LOQ MRM chromatograms of 5 aflatoxins
(Concentrations of B1, G1 and M1 (5 pg/mL); B2 and G2 (3 pg/mL))

5

# A High Sensitivity LC/MS/MS Method with QuEChERS Sample Pre-treatment for Analysis of Aflatoxins in Milk Powder Samples
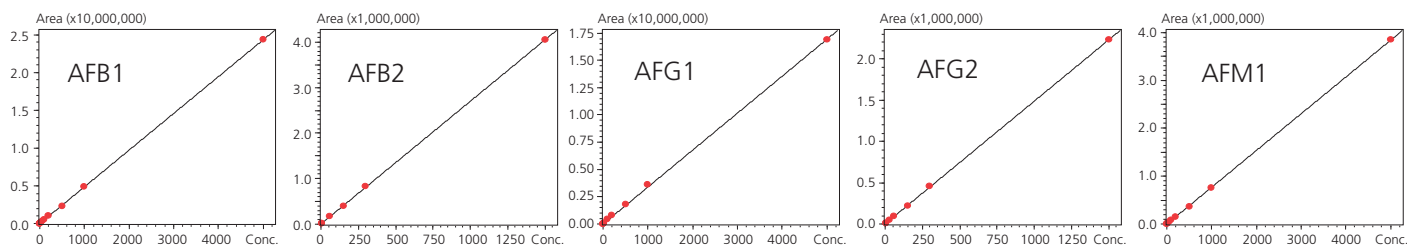


Figure 4: Calibration curves of aflatoxins B1, B2, G1, G2 and M1 in milk powder matrix.

Table 3: LOD, LOQ and repeatability of aflatoxin spiked samples at different concentrations

| Aflatoxin | Range (pg/mL) | Linearity | LOD (pg/mL) | LOQ (pg/mL) | %RSD (n=6) | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | 5 pg/mL | 6 pg/mL | 30 pg/mL | 50 pg/mL |
| B1 | 1-5000 | 0.9999 | 0.14 | 0.44 | 3.1 | | | 2.3 |
| B2 | 3-1500 | 0.9999 | 0.36 | 1.09 | | 6.4 | 2.4 | |
| G1 | 3-5000 | 0.9998 | 0.71 | 2.16 | 4.0 | | | 2.44 |
| G2 | 3-1500 | 0.9999 | 0.41 | 1.22 | | 5.8 | 3.5 | |
| M1 | 3-5000 | 0.9999 | 0.83 | 2.50 | 7.5 | | | 2.7 |

Both the matrix effect and recoveries of aflatoxins were evaluated by using a duplicate set of samples at different concentrations. Each duplicate was obtained from the average of three injections. The results are shown in Table 4 and Table 5.

Table 4: Matrix effects of the MRM method for aflatoxins in spiked milk powder samples

| Concentration (pg/mL) | Matrix effect (%) | | | Concentration (pg/mL) | Matrix effect (%) | |
|---|---|---|---|---|---|---|
| | B1 | G1 | M1 | | B2 | G2 |
| 5.0 | 105.1 | 116.0 | 99.4 | 6.0 | 105.8 | 116.3 |
| 50.0 | 105.3 | 107.9 | 105.4 | 30.0 | 110.2 | 109.3 |

Table 5: Recoveries of aflatoxins in spiked milk powder samples

| Concentration (pg/mL) | Recovery (%) | | | Concentration (pg/mL) | Recovery (%) | |
|---|---|---|---|---|---|---|
| | B1 | G1 | M1 | | B2 | G2 |
| 5.0 | 76.6 | 87.3 | 83.8 | 6.0 | 71.6 | 70.8 |
| 50.0 | 73.8 | 76.5 | 75.6 | 30.0 | 73.9 | 75.6 |

## Analysis of aflatoxins in actual milk powder samples

Three milk powder samples from local supermarket were analysed using the established MRM method. The results showed that no aflatoxin was detected in all three samples.

# A High Sensitivity LC/MS/MS Method with QuEChERS Sample Pre-treatment for Analysis of Aflatoxins in Milk Powder Samples
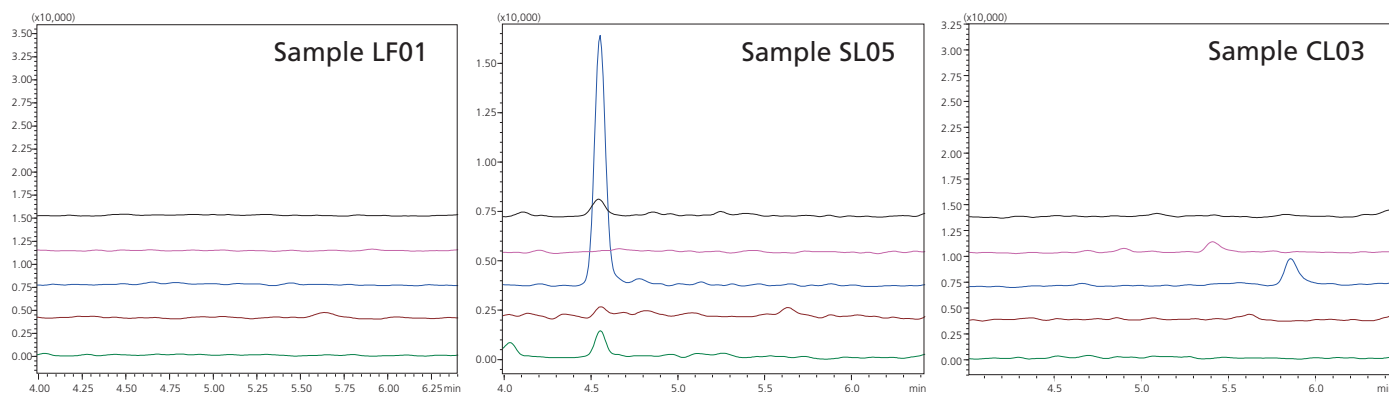


Figure 5:  MRM Chromatograms for Aflatoxin B2, B1, G2, G1 and M1 (top to bottom) of three milk powder samples from local supermarket. Targets were not detected in all samples.

# Conclusions

A high sensitivity LC/MS/MS method with QuEChERS for sample pre-treatment was established using Shimadzu LCMS-8060 system. The QuEChERS sample preparation method was proven effective and easy to operate. The method performance including sensitivity, linearity, repeatability, matrix effect and recovery were carried out and the results confirm that the method is feasible and reliable for determination of aflatoxins in milk powder samples.

# References

1. Iqbal, S. Z.; Jinap, S.; Pirouz, A. A.; Ahmad Faizal, A.R. *Trends in Food Science & Technology* 2015, *46*, 110-119.
2. Cherkani-Hassani, A.; Mojemmi, B.; Mouane, N. *Trends in Food Science & Technology* 2016, *50*, 56-69.

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# SHIMADZU
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# Application News

## No.L506

# Assay of Aflatoxin M₁ in Milk Based on Notification Test Methodology, Using Prominence-i and the RF-20Axs Fluorescence Detector

Aflatoxin $M_1$ (AFM₁) is a mycotoxin suspected of carcinogenicity in humans that is detected in the milk of mammals that eat food contaminated with aflatoxin $B_1$. The notification "Handling of Aflatoxin $M_1$ in Milk" issued on July 23, 2015 (Notification No. 0723-[1] of the Department of Food Safety, PFSB, MHLW) [1] sets a regulatory level for AFM₁ in milk of 0.5 µg/kg, and came into force on January 23, 2016.

The assay methodology for AFM₁ in milk was included in "Test Methodology for Aflatoxin $M_1$ in Milk" (Notification No. 0723-[5] of the Department of Food Safety, PFSB, MHLW) [2], which was announced on the same day and describes two test methodologies.

(1) Test method consisting of quantitation by HPLC with attached fluorescence detector and confirmation by LC/MS or LC/MS/MS.

(2) Screening method using an assay kit.

We describe an analysis of commercially available milk that is compliant with test method (1). We analyzed for AFM₁ in bovine milk using the Prominence-i integrated HPLC and the RF-20Axs fluorescence detector. Under these conditions we were able to measure AFM₁ at a concentration of 1/10th Japan's regulatory level for AFM₁ in milk.

## ■ Analysis of Standard Aflatoxin M₁ Solutions

Chromatograms obtained after analysis of standard AFM₁ solutions (0.1 µg/L, equivalent to 1/100th the regulatory concentration) are shown in Fig. 1, and the analytical conditions used are shown in Table 1. The relative standard deviation (%RSD) of peak areas after repeating analysis six times was 3.4 %. Fig. 2 shows the calibration curve for 0.1 to 20 µg/L. Good linearity was achieved with a contribution ratio $R^2$ of $\geq 0.9999$ within the concentration range. These results show the RF-20Axs fluorescence detector can be used to analyze trace quantities of AFM₁ with high sensitivity and high precision.

When the standard AFM₁ solution of 0.1 µg/L is processed according to the pretreatment procedure shown in Fig. 3, which follows the notification methodology, it produces a sample equivalent to 1/100th the regulatory level for AFM₁ in milk (0.005 µg/kg).
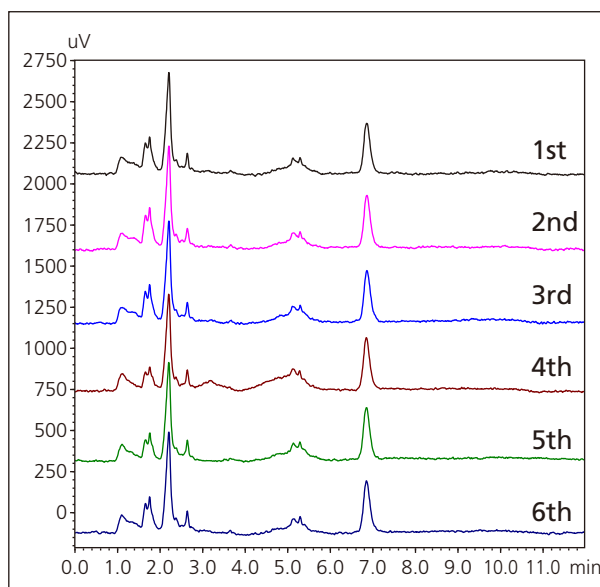


**Fig. 1  Chromatograms for Standard AFM₁ Solution Equivalent to 1/100th the Regulatory Concentration (0.1 µg/L, Test Repeated 6 Times)**
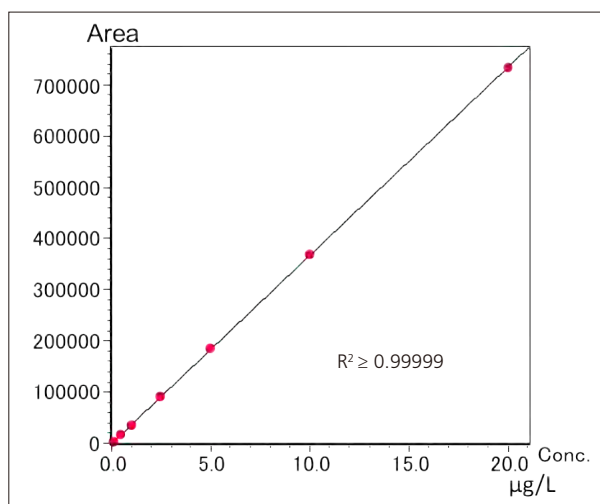


**Fig. 2  AFM₁ Calibration Curve (0.1-20 µg/L)**

### Table 1  HPLC Analytical Conditions

| | |
|---|---|
| System | : Prominence-i |
| Column | : Shim-pack VP-ODS (150 mm L. × 4.6 mm I.D., 5 µm) |
| Mobile Phase | : Water/Acetonitrile = 3/1(v/v) |
| Flowrate | : 1.0 mL/min |
| Column Temp. | : 40 ℃ |
| Detection | : RF-20Axs, Ex. at 365 nm, Em. at 435 nm |
| RF Cell. | : Conventional Cell |
| Cell Temp. | : 25 ℃ |
| Injection Volume | : 100 µL |

## ■ Analysis of Aflatoxin M₁ in Milk

We analyzed commercially available milk and milk with added AFM₁. AFM₁ was added to make up a concentration of 0.05 µg/kg in milk (1/10th the regulatory level), and pretreatment was performed according to the notification methodology.[2] The pretreatment procedure is shown in Fig. 3. Refer to the notification methodology [2] for further details.

An AflaStar™ R* immunoaffinity column from Romer Labs was used to remove contaminant constituents. The chromatograms obtained after analysis of these samples are shown in Fig. 4. (A) is the chromatogram for milk with added AFM₁, and (B) is the chromatogram for milk with no added AFM₁.

The analytical conditions were the same as those used in Fig. 1, which are shown in Table 1.

\* "AflaStar" is a registered trademark of Romer Labs.
　The AflaStar™ R can be purchased from Shimadzu GLC Ltd.

The percentage recovery calculated according to Eqn. 1 shown below was 98 %. We found that using the RF-20A$_{XS}$ fluorescence detector allows for analysis at concentrations 1/10th the regulatory level with high sensitivity and good precision.

A small peak was observed at the AFM₁ elution position when milk with no added AFM₁ was analyzed. Using LC/MS/MS to analyze the milk with no added AFM₁ suggested this peak was derived from AFM₁, and the concentration of the substance present was below 1/100th Japan's regulatory level.

**Fig. 3  Milk Pretreatment Procedure**

Sample (homogenized milk*1)
↓ ← (Aflatoxin M₁ standard solution (100 µg/L) 10 µL)
Heating up to 37 °C
↓
Filtration with Glass filter
↓
20.0 g
↓
Cleaning-up by immunoaffinity column*2 "AflaStar™R"
↓
Elution by Acetonitrile (3 mL)
↓
Drying-up *3 by N₂ gas
↓ ← 1.0 mL Water/Acetonitrile = 4/1(v/v)
Vortex
↓
HPLC *4

*1 A pretreatment centrifugation step is needed for raw milk and other milks that are not homogenized. Refer to reference [2] for details.
*2 Refer to the annotations in reference [2] for detailed information on use of the immunoaffinity column.
*3 AFM₁ can adhere to the container during drying, so it is recommended that silane-treated containers be washed with 20 % to 30 % aqueous acetonitrile then dried before use.
*4 AFM₁ can adhere to glass containers used to hold samples for HPLC even when these containers have been treated with silane, so it is recommended that plastic containers be used.

$$\text{Recovery rate (\%)} = \frac{(\text{Peak area of milk with added standard AFM}_1) - (\text{Peak area of milk with no added standard AFM}_1)}{\text{Peak area of standard AFM}_1 \text{ sample}} \times 100$$

**Eqn. 1  Percentage Recovery Equation**
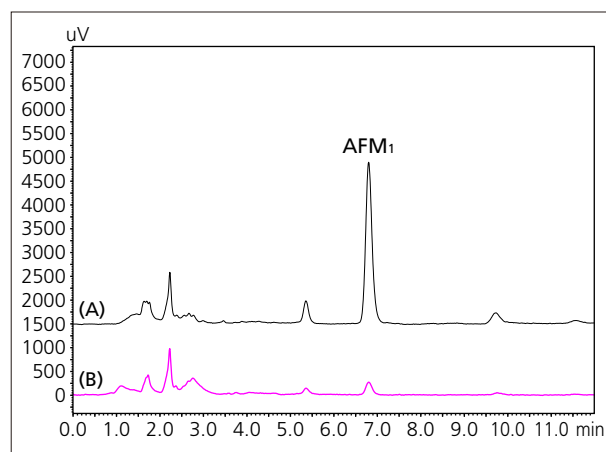
**Fig. 4  HPLC Chromatograms for Commercially Available Milk (A) With added standard AFM₁, (B) with no added standard AFM₁**

References
1) "Handling of Aflatoxin M₁ in Milk" (July 23, 2015, Notification No. 0723-[1] of the Department of Food Safety, PFSB, MHLW)
2) "Test Methodology for Aflatoxin M₁ in Milk" (Notification No. 0723-[5] of the Department of Food Safety, PFSB, MHLW)

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## Technical Report

# Determination of Polychlorinated Dibenzo-*p*-dioxins and Dibenzofurans (PCDD/Fs) in Foodstuffs and Animal Feed Using a Triple Quadrupole GCMS-TQ8040 System with Smart MRM Transforms Laboratory Analysis

Pu Wang[1], Huizhong Sun[1], Qinghua Zhang[1], Feifei Tian[2], Lei Cao[2]

## Abstract:

A method was developed on a high selectively triple quadrupole GCMS-TQ8040 system for screening trace levels of polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) in foodstuffs and animal feed. Smart MRM technology was employed for method development and GC-MS/MS analysis. The results showed good sensitivity and repeatability for PCDD/Fs at low levels, as well as a good linear response over the required concentration range. The performance on real sample analysis indicated the feasibility of Shimadzu GCMS-TQ8040 system for PCDD/Fs measurement in food and feed, as required by European Union legislation.

Keywords: GC-MS/MS, MRM, PCDDs, PCDFs, dioxins, food, feed

## 1. Introduction

Polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) are of special concern, due to their harmful health effects. They have neuro-toxic potential and are linked to causing cancer, endocrine disruption, and reproductive disorders. PCDD/Fs were never produced intentionally as marketable products, while they are widespread around the world. They can also bioaccumulate and biomagnify through the food chain, and finally pose a threat to human body. Most dioxin exposure to human derives from food (>90%), 90 % of which is of animal origin [1]. Consequently, incidents of dioxin contamination involving food and feed have generally raised great public concern in the world. For example, Germany's dioxin-tainted food scandal in 2011 led to a shutdown of more than 4700 farms and tremendous economic losses in Germany.

European regulations specifying official sampling and analysis methods for controlling PCDD/F levels and polychlorinated biphenyls (PCBs) in food and feed were initially issued in the early 2000s, followed by several amendments thereafter. The latest amendment was Commission Regulation (EU) Nos. 589/2014 [2] and 709/2014 [3], where a major update is that gas chromatography–triple quadrupole mass spectrometry (GC-MS/MS) was recognized as an appropriate confirmatory method for checking compliance with the maximum levels of PCDD/Fs and PCBs in food and feed control. This means GC-MS/MS can be used to provide full or complementary information enabling the PCDD/Fs and dioxin-like PCBs to be identified and quantified unequivocally at the maximum or in case of need at the action threshold.

This Technical Report describes a highly sensitive and reproducible method for determining PCDD/Fs (Table 1) in food and feed using a triple quadrupole GCMS-TQ8040 system with Smart MRM, which transforms laboratory analysis process. The results show performance comparable to using high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS) for quantitation of PCDD/Fs in food and feed.

Table 1   PCDD/Fs Specified in EU Legislation and TEF Values Established by WHO

| Congener | WHO$_{1998}$-TEF | WHO$_{2005}$-TEF | Congener | WHO$_{1998}$-TEF | WHO$_{2005}$-TEF |
|---|---|---|---|---|---|
| 2,3,7,8-TCDD | 1 | 1 | 2,3,7,8-TCDF | 0.1 | 0.1 |
| 1,2,3,7,8-PeCDD | 1 | 1 | 1,2,3,7,8-PeCDF | 0.05 | 0.03 |
| 1,2,3,4,7,8-HxCDD | 0.1 | 0.1 | 2,3,4,7,8-PeCDF | 0.5 | 0.3 |
| 1,2,3,6,7,8-HxCDD | 0.1 | 0.1 | 1,2,3,4,7,8-HxCDF | 0.1 | 0.1 |
| 1,2,3,7,8,9-HxCDD | 0.1 | 0.1 | 1,2,3,6,7,8-HxCDF | 0.1 | 0.1 |
| 1,2,3,4,6,7,8-HpCDD | 0.01 | 0.01 | 2,3,4,6,7,8-HxCDF | 0.1 | 0.1 |
| OCDD | 0.0001 | 0.0003 | 1,2,3,7,8,9-HxCDF | 0.1 | 0.1 |
| | | | 1,2,3,4,6,7,8-HpCDF | 0.01 | 0.01 |
| | | | 1,2,3,4,7,8,9-HpCDF | 0.01 | 0.01 |
| | | | OCDF | 0.0001 | 0.0003 |

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2 Analytical Applications Center, Shimadzu (China) Co., Ltd., Beijing, China

## 2. Experimental

## 2-1. Sample Preparation and Instrumental Analysis

The sample preparation of food and feed was mainly followed US EPA method 1613B [4] with proper modification [5]. The samples were extracted using an accelerated solvent extraction (ASE) system, followed by purification steps (including acidic/basic silica gel, alumina, and carbon columns). Samples were spiked with $^{13}$C-isotope labeled surrogate standards (1613-LCS) and internal standards (1613-IS) prior to extraction and instrumental analysis, respectively.

Samples were analyzed in a Shimadzu GCMS-TQ8040 system with Smart MRM, which transforms laboratory analysis process. The GC unit was equipped with a split/splitless injection port. The capillary column was a 60 m DB-5MS fused silica capillary column (J&W, Scientific, 0.25 μm film thickness, 0.25 mm I.D.). Table 2 showed the selected instrumental conditions for PCDD/Fs detection. Measurements were performed in EI-MS/MS Multiple Reaction Monitoring (MRM) mode. The MRM method for PCDD/Fs was developed using the Shimadzu

GCMS-TQ8040 MRM optimization tool, which automatically determines the optimum transitions and collision energies in a single sequence, and then seamlessly incorporates them into the Smart Database series for full method development (Fig. 1). At that point, the two most intense ions of the molecular chlorine isotope cluster of each congener and internal standard were chosen. Using flexible MS event, the Smart MRM function routinely determines the best dwell and loop times for the two transitions in a single method, providing optimum precision and sensitivity. In order to improve the peak shape for 2,3,7,8-TCDD/F at very low concentration levels (10 fg/μL), the event times were manually increased to 0.35 seconds for the native 2,3,7,8-TCDD/F and decreased to 0.20 seconds for the $^{13}$C-labeled 2,3,7,8-TCDD/F to maintain the same loop time as other congeners. Information on the MRM settings and analyte retention times are given in Table 3.

Table 2   Conditions for Gas Chromatograph and Mass Spectrometer

| GC Conditions | | MS Conditions | |
|---|---|---|---|
| Column | : DB-5MS (60 m × 0.25 mm × 0.25 μm) | Ionization Mode | : EI |
| Injection Mode | : Splitless | Ion Source Temperature | : 250 °C |
| Injection Volume | : 2 μL | Interface Temperature | : 270 °C |
| High Pressure Injection | : 300 kPa (2 min) | Acquisition Mode | : MRM Mode |
| Sampling Time | : 1 min | Collision Gas | : Argon |
| Injection Temperature | : 290 °C | Emission Current | : 250 μA |
| Column Oven Temperature | : 150 °C (3 min) → (20 °C/min) → 230 °C (18 min) | Loop Time | : 1.1 sec |
| | → (5 °C/min) → 235 °C (10 min) → (4 °C/min) | Pressure of CID Gas | : 150 kPa |
| | → 320 °C (1 min) | Detector Voltage | : 1.8 kV |
| Flow Control Mode | : Linear Velocity | | |
| Column Flow | : 1.03 mL/min | | |



Fig. 1   Optimized MS Event Times for PCDD/F Congeners with Smart MRM Function

# 3. Results and Discussion

## 3-1. Chromatography

The chromatographic separation of the 17 native 2,3,7,8-substituted PCDD/F congeners is shown in Fig. 2 (EPA 1613 CS3). The sample was completely separated in a total run time of 60 minutes.



Fig. 2   Mass Chromatogram of PCDD/Fs in EPA 1613 CS3 (10 – 100 pg/µL) (Peak numbers refer to analytes listed in Table 3.)

Table 3   MS/MS Settings for Native PCDD/F Congeners and ^{13}C-Internal Standards

| Peak No. | Analyte | RT (min) | Precursor > Product | CE (V) | Precursor > Product | CE (V) |
|---|---|---|---|---|---|---|
| 1 | ^{13}C-2,3,7,8-TCDF | 27.024 | 315.90 > 251.90 | 31 | 317.90 > 253.90 | 31 |
| 2 | 2,3,7,8-TCDF | 27.076 | 303.90 > 240.90 | 31 | 305.90 > 242.90 | 31 |
| 3 | ^{13}C-1,2,3,4-TCDD | 27.323 | 331.90 > 267.90 | 25 | 333.90 > 269.90 | 25 |
| 4 | ^{13}C-2,3,7,8-TCDD | 28.235 | 331.90 > 267.90 | 25 | 333.90 > 269.90 | 25 |
| 5 | 2,3,7,8-TCDD | 28.253 | 319.90 > 256.90 | 25 | 321.90 > 258.90 | 25 |
| 6 | ^{13}C-1,2,3,7,8-PeCDF | 35.953 | 351.90 > 287.90 | 34 | 349.90 > 285.90 | 34 |
| 7 | 1,2,3,7,8-PeCDF | 36.01 | 339.90 > 276.90 | 34 | 337.90 > 274.90 | 34 |
| 8 | ^{13}C-2,3,4,7,8-PeCDF | 38.625 | 351.90 > 287.90 | 37 | 349.90 > 285.90 | 37 |
| 9 | 2,3,4,7,8-PeCDF | 38.673 | 339.90 > 276.90 | 37 | 337.90 > 274.90 | 37 |
| 10 | ^{13}C-1,2,3,7,8-PeCDD | 39.419 | 367.90 > 303.90 | 25 | 365.90 > 301.90 | 25 |
| 11 | 1,2,3,7,8-PeCDD | 39.441 | 355.90 > 292.90 | 25 | 353.90 > 290.90 | 25 |
| 12 | ^{13}C-1,2,3,4,7,8-HxCDF | 45.168 | 385.80 > 321.90 | 37 | 387.80 > 323.90 | 37 |
| 13 | 1,2,3,4,7,8-HxCDF | 45.183 | 373.80 > 310.90 | 37 | 375.80 > 312.90 | 37 |
| 14 | ^{13}C-1,2,3,6,7,8-HxCDF | 45.413 | 385.80 > 321.90 | 37 | 387.80 > 323.90 | 37 |
| 15 | 1,2,3,6,7,8-HxCDF | 45.438 | 373.80 > 310.90 | 37 | 375.80 > 312.90 | 37 |
| 16 | ^{13}C-2,3,4,6,7,8-HxCDF | 46.568 | 385.80 > 321.90 | 37 | 387.80 > 323.90 | 37 |
| 17 | 2,3,4,6,7,8-HxCDF | 46.604 | 373.80 > 310.90 | 37 | 375.80 > 312.90 | 37 |
| 18 | ^{13}C-1,2,3,4,7,8-HxCDD | 46.911 | 401.80 > 337.90 | 25 | 403.80 > 339.80 | 25 |
| 19 | 1,2,3,4,7,8-HxCDD | 46.935 | 389.80 > 326.90 | 25 | 391.80 > 328.80 | 25 |
| 20 | ^{13}C-1,2,3,6,7,8-HxCDD | 47.1 | 401.80 > 337.90 | 25 | 403.80 > 339.80 | 25 |
| 21 | 1,2,3,6,7,8-HxCDD | 47.139 | 389.80 > 326.90 | 25 | 391.80 > 328.80 | 25 |
| 22 | ^{13}C-1,2,3,7,8,9-HxCDD | 47.577 | 401.80 > 337.90 | 25 | 403.80 > 339.80 | 25 |
| 23 | 1,2,3,7,8,9-HxCDD | 47.591 | 389.80 > 326.90 | 25 | 391.80 > 328.80 | 25 |
| 24 | ^{13}C-1,2,3,7,8,9-HxCDF | 48.126 | 385.80 > 321.90 | 34 | 387.80 > 323.90 | 34 |
| 25 | 1,2,3,7,8,9-HxCDF | 48.145 | 373.80 > 310.90 | 34 | 375.80 > 312.90 | 34 |
| 26 | ^{13}C-1,2,3,4,6,7,8-HpCDF | 50.755 | 419.80 > 355.80 | 37 | 421.80 > 357.80 | 37 |
| 27 | 1,2,3,4,6,7,8-HpCDF | 50.788 | 407.80 > 344.80 | 37 | 409.80 > 346.80 | 37 |
| 28 | ^{13}C-1,2,3,4,6,7,8-HpCDD | 52.584 | 435.80 > 371.80 | 25 | 437.80 > 373.80 | 25 |
| 29 | 1,2,3,4,6,7,8-HpCDD | 52.596 | 423.80 > 360.80 | 25 | 425.80 > 362.80 | 25 |
| 30 | ^{13}C-1,2,3,4,7,8,9-HpCDF | 53.348 | 419.80 > 355.80 | 37 | 421.80 > 357.80 | 37 |
| 31 | 1,2,3,4,7,8,9-HpCDF | 53.357 | 407.80 > 344.80 | 37 | 409.80 > 346.80 | 37 |
| 32 | ^{13}C-OCDD | 56.85 | 469.70 > 405.80 | 25 | 471.70 > 407.80 | 25 |
| 33 | OCDD | 56.867 | 457.70 > 394.80 | 25 | 459.70 > 396.80 | 25 |
| 34 | OCDF | 57.081 | 441.70 > 378.80 | 34 | 443.70 > 380.80 | 34 |

## 3-2. Sensitivity

To verify the performance of the GC-MS/MS system in the low concentration range, 1:50 dilutions of the calibration standard EPA-1613CS1 were also measured (10 fg/µL 2,3,7,8-TCDD). Based on the 2 µL injection volume, the dilutions of the calibration standard EPA-1613CS1 indicate an absolute amount of 20 fg 2,3,7,8-TCDD in the column. The MRM chromatograms for native PCDD/Fs are shown in Fig. 3.

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Fig. 3 MRM Chromatograms of Native PCDD/F Congeners (10 – 100 fg/μL, 2 μL injection volume) (Peak numbers refer to analytes listed in Table 3.)

## 3-3. Linearity of Response

A seven-point calibration curve was prepared based on US EPA method 1613, defined for calibration verification solutions (CS1-CS5), together with EPA-1613CSL and EPA-1613CS0.5. Excellent linearity was obtained for the calibration standards over the concentration range from 0.1 to 200 ng/mL (TCDD), with $R^2$ values >0.999 for an injection volume of 1 μL (Table 4). The mean response factor for each congener is also given in Table 4. The calibration curves for 2,3,7,8-TCDD, 2,3,7,8-TCDF and 1,2,3,7,8-PeCDD are shown in Fig. 4 to 6.

Table 4  Linear Regression for Seven-Point Calibration Curves over the Range 0.1 to 200 ng/mL (TCDD) and the Mean Response Factor for Each Congener

| Congener | Regression line equation | $R^2$ | Mean RF | RF %RSD |
|---|---|---|---|---|
| 2,3,7,8-TCDD | Y = 1.150399X + 3.29953e-004 | 0.99999 | 1.22794 | 8.06 |
| 1,2,3,7,8-PeCDD | Y = 1.014733X + 3.009239e-003 | 1.00000 | 1.03887 | 1.96 |
| 1,2,3,4,7,8-HxCDD | Y = 1.079761X − 5.260601e-004 | 0.99997 | 1.09358 | 3.65 |
| 1,2,3,6,7,8-HxCDD | Y = 0.9705907X + 5.362575e-002 | 0.99915 | 1.08710 | 5.37 |
| 1,2,3,7,8,9-HxCDD | Y = 1.024768X + 3.682249e-002 | 0.99967 | 1.00394 | 14.48 |
| 1,2,3,4,6,7,8-HpCDD | Y = 0.9429045X + 1.331675e-002 | 0.99998 | 1.02985 | 6.92 |
| OCDD | Y = 1.242978X − 6.145206e-002 | 0.99929 | 1.14683 | 6.10 |
| 2,3,7,8-TCDF | Y = 1.15754X + 9.032785e-004 | 0.99996 | 1.18104 | 3.57 |
| 1,2,3,7,8-PeCDF | Y = 1.015266X − 5.771587e-003 | 0.99997 | 1.07846 | 7.83 |
| 2,3,4,7,8-PeCDF | Y = 1.045151X − 6.304552e-003 | 0.99998 | 1.04210 | 4.49 |
| 1,2,3,4,7,8-HxCDF | Y = 1.006328X + 2.605984e-002 | 0.99988 | 1.09930 | 6.04 |
| 1,2,3,6,7,8-HxCDF | Y = 0.9307018X + 3.432044e-002 | 0.99971 | 1.06611 | 8.06 |
| 2,3,4,6,7,8-HxCDF | Y = 0.9080292X + 3.053454e-002 | 0.99983 | 1.00464 | 6.34 |
| 1,2,3,7,8,9-HxCDF | Y = 0.960272X + 2.450491e-002 | 0.99993 | 1.03403 | 9.10 |
| 1,2,3,4,6,7,8-HpCDF | Y = 0.9732686X + 4.031919e-002 | 0.99958 | 1.08255 | 4.88 |
| 1,2,3,4,7,8,9-HpCDF | Y = 0.9562794X + 3.622056e-002 | 0.99969 | 1.06788 | 5.48 |
| OCDF | Y = 1.424071X + 3.271179e-003 | 0.99999 | 1.50245 | 8.94 |



Fig. 4  Seven-Point Calibration Curve for 2,3,7,8-TCDD with Both Linear Fit and Mean Response Factors



Fig. 5  Seven-Point Calibration Curve for 2,3,7,8-TCDF with Both Linear Fit and Mean Response Factors

Fig. 6   Seven-Point Calibration Curve for 1,2,3,7,8-PeCDD with Both Linear Fit and Mean Response Factors

## 3-4. Repeatability

The repeatability was confirmed for within the same day and over different days. Total 12 injections (four 2 µL injections per day for three days) of 1:50 diluted EPA-1613CS1 (10 fg/µL 2,3,7,8-TCDD) were performed. Results exhibited good repeatability for the peak areas of each congener, with a relative standard deviations (RSD) less than 15 % (Fig. 7).



Fig. 7   Repeatability of Peak Areas for Native PCDD/Fs and 1613-LCS (n = 12)



Fig. 8   MRM Chromatograms for $^{13}$C-Labeled and Native 2,3,7,8-TCDF (A), 2,3,7,8-TCDD (B), and 1,2,3,7,8-PeCDD (C) in Fish Oil

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## 3-5. Sample Analysis

For comparison, each fish oil and milk sample was analyzed in both GC-MS/MS and HRGC/HRMS systems, using the same GC conditions for the HRGC/HRMS system. The MRM chromatograms for 2,3,7,8-TCDF, 2,3,7,8-TCDD, and 1,2,3,7,8-PeCDD in the fish oil sample are shown in Fig. 8. The congener profiles in both samples are exhibited in Fig. 9 and 10. GC-MS/MS results showed good consistency with HRGC/HRMS results. The toxic equivalents (TEQ) of PCDD/Fs were 29.5 pg WHO-TEQ$_{2005}$/g fat and 1.38 pg WHO-TEQ$_{2005}$/g fat (upper bound values) in the fish oil and milk samples, respectively, which were comparable to the results of 24.9 and 1.37 pg WHO-TEQ$_{2005}$/g fat obtained from the HRGC/HRMS system.



Fig. 9   Congener Profiles of PCDD/Fs in Fish Oil



Fig. 10   Congener Profiles of PCDD/Fs in Milk

## 4. Conclusion

The Shimadzu GCMS-TQ8040 system facilitates the screening and quantitation of low concentration PCDD/Fs in different food-stuffs and animal feed samples. The method showed good linearity, sensitivity, and repeatability. The analytical results from real samples also indicated good precision using this method, when compared with HRGC/HRMS results. This suggests that the Shimadzu GCMS-TQ8040 system provides a substitute solution for routine screening and quantitation of PCDD/Fs in food and feed, as required by European Union legislation.

### References

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**Triple Quadrupole Gas Chromatograph Mass Spectrometer**

# GCMS-TQ8040

## Finally, a triple quadrupole GCMS *Smart* enough for everyday use in your laboratory.

The Shimadzu GCMS-TQ8040 is the first triple quadrupole with Smart Productivity for high efficiency sample throughput, Smart Operation for quick and easy method development, and Smart Performance for low detection limits and Scan/MRM. These three smart technologies contribute to Smart MRM, and provide the most accurate, cost-effective, and easy-to-use triple quadrupole GCMS you have ever imagined.

## Smart Productivity

- Retention time synchronized MRM provides simultaneous sensitivity and precision for hundreds of compounds in one run
- Smart MRM optimizes analysis of 400+ compounds in a single acquisition with maximum sensitivity
- Reduced downtimes with the Twin Line configuration MS system

## Smart Operation

- MRM Optimization Tool to optimize MRM transitions automatically
- Smart Database Series with fully optimized transitions for hundreds of compounds
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- High-sensitivity analysis even in single GC-MS mode

# Smart Environmental Database

## Create MRM Methods for GC-MS/MS

The Smart Environmental Database contains all the information necessary to create MRM methods for over 500 environmental pollutants, including PCBs, BFRs, dioxins, PAHs, organochlorine pesticides (OCPs), and stable isotopically labeled compounds that are commonly used as Internal and Surrogate Standards.

## Smart MRM Optimizes Methods Automatically

The Smart MRM feature allows the user to create fully optimized MRM and Scan/MRM methods automatically. GC-MS/MS Dwell, Event, and Loop times can be difficult to optimize when dozens, or even hundreds of compounds are to be analyzed simultaneously. The Smart MRM feature automatically determines the optimum Dwell, Event, and Loop settings using flexible MRM events, and creates MRM and Scan/MRM methods that provide the best sensitivity for all compounds in a single method.

Analysis of PCB in River Water
(2,2',5,5'-Tetrachlorobiphenyl (#52) concentration in water of 0.080 ng/L)



| Number of Registered Compounds | Number of Registered Native Compounds | Number of Registered Compounds Labeled with Stable Isotopes |
|---|---|---|
| Polychlorinated biphenyls | 209 | 45 |
| Brominated flame retardants | 55 | 28 |
| Dioxins | 32 | 26 |
| Polycyclic aromatic hydrocarbons | 38 | 37 |
| Organochlorine pesticides | 32 | 25 |

**Smart MRM**

# SHIMADZU

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# SHIMADZU
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# Application News

## No.J120

**Inductively Coupled Plasma Mass Spectrometry**

## Analysis of Minerals and Harmful Elements in Formula Milk Powder Using ICPMS-2030

### ■ Introduction

Formula milk powder is formulated to contain a good balance of minerals necessary for infant growth. Japan's Health Promotion Law includes stipulations on the essential mineral content, including calcium (Ca), iron (Fe), and copper (Cu), of food for special dietary use (e.g., formulated milk powder for infants) and requires the labeling of their content. [1]

Harmful elements like lead (Pb) have a negative effect on infant development, and require strict safety controls from raw materials to the finished product.

We describe using Shimadzu's ICPMS-2030 inductively coupled plasma mass spectrometer to perform a simultaneous analysis of elements present in formula milk powder (National Metrology Institute of Japan certified reference material [NMIJ CRM]). In addition to being highly sensitive, the ICPMS-2030 uses a helium gas collision system that greatly reduces the spectral interference caused by argon and chlorine.

[References]
1) Permission standards for labeling and component composition of breast milk and formulated milk powder for infants (Permission for labeling of food for special dietary use, Food Labeling Division, Consumer Affairs Agency, No. 221 of March 31, 2016)

### ■ Sample

NMIJ CRM milk powder (for trace element analysis)
(NMIJ CRM 7512-a: No. MI-040)

### ■ Sample Preparation

Compared to the normal wet digestion method, the microwave sample decomposition method has the advantages of completing decomposition in a shorter period of time and within a closed system, which results in little volatilization loss of As and other analytes. We used the ETHOS One (Milestone General K.K., Japan) to perform sample decomposition.

After placing a sample 0.25 g, hydrochloric acid 0.5 mL, and nitric acid 6.5 mL in a quartz decomposition vessel, decomposition was performed using a microwave sample pretreatment system.

After decomposition, pure water was added to make up 250 mL of solution for analysis (1000-fold dilution). At this point, Sc, Co, Ga, Y, In, and Ti internal standard elements were added (to an analytical solution concentration of 10 µg/L).

Table 1 shows the decomposition conditions.

**Table 1  Decomposition Conditions for Microwave Digestion System**

| STEP | Temperature (°C) | Time (min) | Power (W) |
|------|------------------|------------|-----------|
| 1 | 50 | 2 | 1000 |
| 2 | 30 | 3 | 0 |
| 3 | 180 | 25 | 1000 |
| 4 | 150 | 1 | 0 |
| 5 | 180 | 4 | 1000 |
| 6 | 180 | 15 | 1000 |

### ■ Instrument and Analytical Conditions

Shimadzu's ICPMS-2030 inductively coupled plasma mass spectrometer was used for analysis. Analytical conditions are shown in Table 2.

In addition to being highly sensitive, the ICPMS-2030 uses a helium gas collision system that greatly reduces the spectral interference caused by argon and chlorine.

**Table 2  Analytical Conditions**

| | |
|---|---|
| Instrument | : ICPMS-2030 |
| High-frequency output | : 1.2 kW |
| Plasma gas flowrate | : 8.0 L/min |
| Auxiliary gas flowrate | : 1.10 L/min |
| Carrier gas flowrate | : 0.60 L/min |
| Sample introduction | : Nebulizer 10 |
| Chamber | : Cyclone chamber (electronic cooling) |
| Plasma torch | : Mini-torch |
| Collision gas | : He |

## ■ Analysis

Minerals and harmful elements present in formula milk powder were measured simultaneously using a calibration curve method.

To confirm the validity of measured values, a spike and recovery test sample was prepared by adding a standard solution of analyte elements (Cd, Cr, Pb, As) after sample decomposition treatment.

## ■ Analytical Results

Table 3 shows the analytical results. The results for mineral elements were within the range of certified levels, and good spike and recovery was obtained for trace amounts of harmful elements.

## ■ Conclusions

We successfully used the ICPMS-2030 to simultaneously measure mineral constituents present in high concentrations in formula milk powder along with trace amounts of harmful elements.

**Table 3  Analytical Results for Certified Reference Material in Formula Milk Powder (NMIJ CRM 7512-a)**

|  | Unit | Measured Value (in Powder) | NMIJ Certified Value | Expanded Uncertainty | Spike and Recovery (%) |
|---|---|---|---|---|---|
| Ca | g/kg | 8.5 | 8.65 | 0.38 | - |
| Fe | | 0.102 | 0.104 | 0.007 | - |
| K | | 8.3 | 8.41 | 0.33 | - |
| Mg | | 0.82 | 0.819 | 0.024 | - |
| Na | | 1.81 | 1.87 | 0.09 | - |
| P | | 5.4 | 5.62 | 0.23 | - |
| Mn | mg/kg | 0.91 | 0.931 | 0.032 | - |
| Mo | | 0.230 | 0.223 | 0.012 | - |
| Sr | | 5.7 | 5.88 | 0.20 | - |
| Zn | | 41 | 41.3 | 1.4 | - |
| Cd | | <0.005 | - | - | 100 |
| Cr | | <0.06 | - | - | 101 |
| Pb | | <0.03 | - | - | 100 |
| As | | <0.03 | - | - | 108 |

Spike and recovery (%) = { (Spike and recovery test sample analysis result - Measured value) / Spiked concentration} × 100

# SHIMADZU

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## Application News

iD*plus*

## No.MO398 | Milk and Dairy Product Profiling Using iD*plus*

- Foodstuff authenticity screening
- Effective detection of adulteration in dairy products
- Molecular profiling with minimal sample preparation
- Patented SuperSpectrum™ concept
- High degree of flexibility: customizable open database
- Visualization, clustering and dendrogramming tools for simple interpretation of results

### Introduction

iD*plus*™ is an established MALDI-TOF MS based platform in microbial identification but it is far from limited to this application. The flexibility of the open database associated with iD*plus* allows the use of the platform for molecular profiling experiments and differentiation of related samples based on the unique features in their profile. New custom sample-specific entries (SuperSpectra) can be added to the existing microbial database to create a sub-database relevant to a particular area of research. This has been reported in areas as diverse as cell line identification, entomology, zooplankton research, fish speciation and the study of food-borne bacteria (ref 1-7).

Adulteration of dairy products is a significant problem in the food industry. Methods for the detection of fraudulent addition of cows' milk to other more expensive types of milk, such as goat or sheep, is important to eradicate economically motivated milk adulteration. This illegal practice, however, is not limited solely to milk production: other areas of the dairy industry such as cheese manufacture have also been targeted.

For example: the European protected designation of origin (PDO) legislation protects highly sought-after buffalo mozzarella from the Campania region of Italy (Mozzarella di Bufala Campana). While mozzarella can be made from cows' milk, it would not receive PDO certification and would be a significantly cheaper product. This has led to widespread fraudulent attempts to misrepresent cow mozzarella as buffalo mozzarella, a practice highlighted in 2010 when checks of PDO-protected Mozzarella di Bufala Campana by the ministry of agriculture in Italy found that at least 25% contained cows' milk.

This work demonstrates the effectiveness of the iD*plus* platform for rapid differentiation of dairy products and identification of fraudulent practices. Proof of principle of this technique is demonstrated using milk profiling from several different species. The established method is then applied to foodstuff authentication using mozzarella cheese as a model product.

## The SuperSpectrum Concept

SuperSpectra are database entries within the iD$^{plus}$ database (SARAMIS™) that represent a typical population of a species or sample. They are computed from multiple mass spectra (Reference Spectra) acquired from a given sample that are combined into a consensus spectrum weighted by specificity. The weighting algorithm favors sample-specific peaks and devalues common-feature peaks, further increasing confidence when using database matching. The diagram in figure 1 illustrates the process used to create a SuperSpectrum.



multiple mass profiles of different samples

similarity dendrogram of mass profiles

consensus spectrum with average signal intensity

SuperSpectrum with signals weighted by specificity
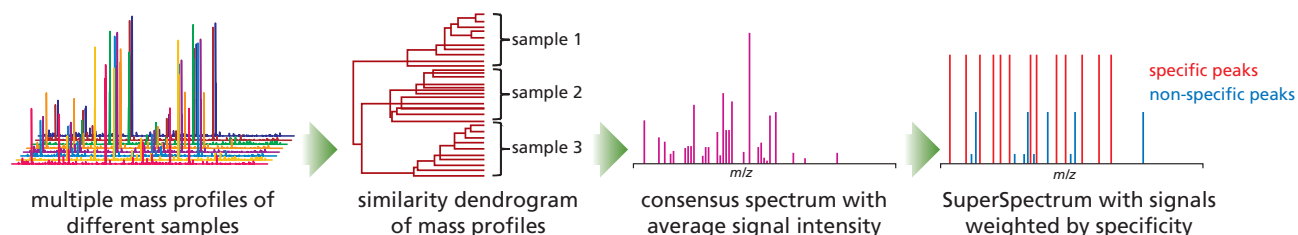
**Figure 1: Creation of a SuperSpectrum**

## Experimental

Three types of milk (cow, buffalo and goat) and two types of mozzarella cheese (cow and buffalo) were obtained from several sources (table 1). Multiple reference spectra were acquired and combined into a characteristic SuperSpectrum for each milk product to populate a custom dairy-specific database.

Briefly, each of the milk samples was diluted 10-fold into 0.1% aqueous TFA. 1 µL of this solution was then deposited onto the target plate and left until almost dry before adding 1 µL of CHCA matrix. For the mozzarella samples, a small amount of the cheese was smeared directly onto the FlexiMass-DS™ target surface using an inoculation loop before adding 1 µL of CHCA matrix. Spectra were acquired across the m/z range 2000 to 20 000.

| Product Name | Product Type | Species |
|---|---|---|
| Tesco Semi-skimmed | Milk | Cow |
| Tesco Pure Filtered Semi-skimmed | Milk | Cow |
| Tesco Jersey & Guernsey Cow | Milk | Cow |
| Delamere Sterilized Whole | Milk | Cow |
| Laverstock Park Semi-skimmed | Milk | Buffalo |
| Laverstock Park Whole | Milk | Buffalo |
| St Helen's Farm Semi-skimmed | Milk | Goat |
| Galbani | Mozzarella | Cow |
| Waitrose Italian | Mozzarella | Cow |
| Cantile | Mozzarella | Buffalo |
| Laverstock Park | Mozzarella | Buffalo |
| Garofalo | Mozzarella | Buffalo |

**Table 1: Summary of the milk and mozzarella samples**

## Results

### Proof of Principle

The different types of milk analyzed (cow, goat and buffalo) generated highly taxon-specific mass profiles exhibiting many species-specific masses. Figure 2 highlights the differences observed between these profiles.
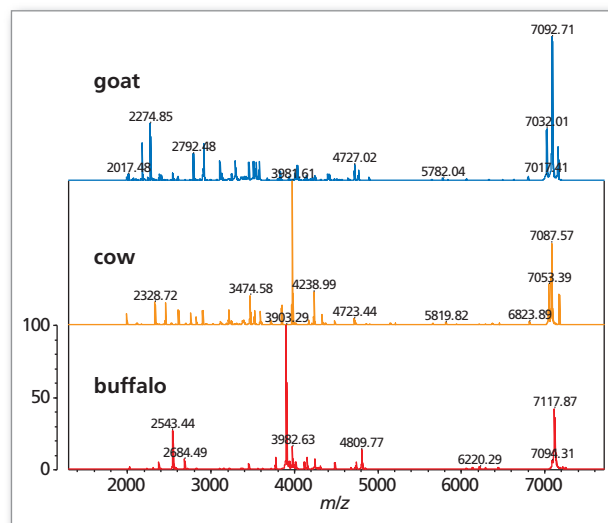


**Figure 2: iD$^{plus}$ mass profiles for goat, cow and buffalo milk**

Additional samples of cow, buffalo and goat milk were analyzed and processed for cluster analysis. The dendrogram results obtained confirmed effective differentiation of the milk samples (figure 3).
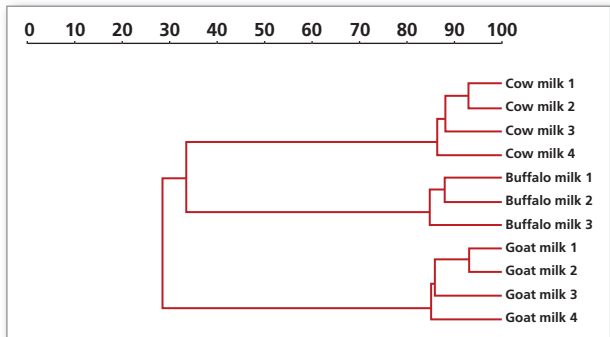


**Figure 3: Cluster analysis of milk originating from cow, buffalo and goat**

### Food Adulteration Detection

Similarly to milk, the different types of mozzarella cheese generated distinguishable mass profiles exhibiting many product specific masses (figure 4).
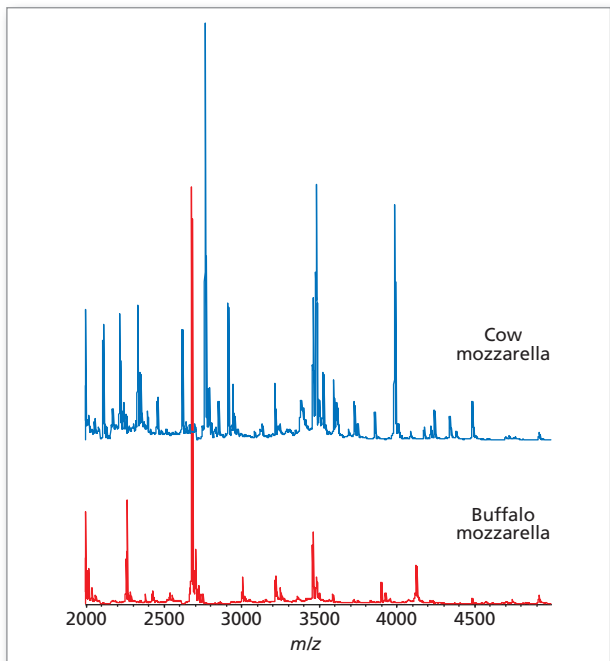


**Figure 4: iD*plus* mass profiles for cow and buffalo mozzarella cheese**

To simulate adulteration of a mozzarella sample, Mozzarella di Bufala Campana cheese was mixed with cow mozzarella and prepared in the same manner as previously. The mass profile obtained for the adulterated sample is shown in figure 5 and clearly exhibits mass signals from both taxa.
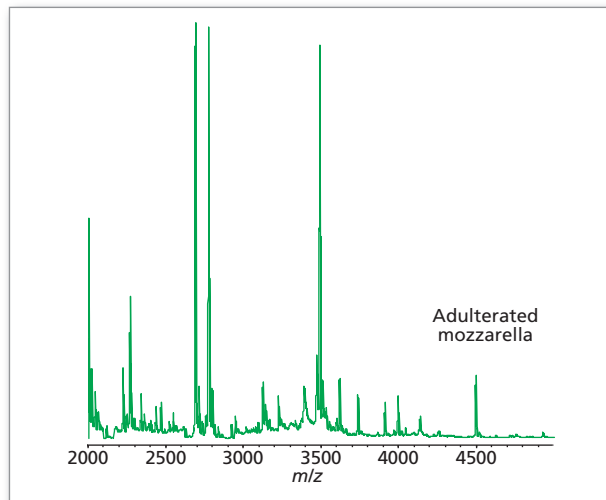


**Figure 5: iD*plus* mass profiles for adulterated mozzarella cheese**

When the adulterated mozzarella sample results were submitted to search the custom dairy database an unambiguous identification was not achieved (result flagged in red in figure 6). Further inspection of the detailed result showed that the sample had matched both cow and buffalo mozzarella in the database, a clear indication of an adulterated sample (figure 6).
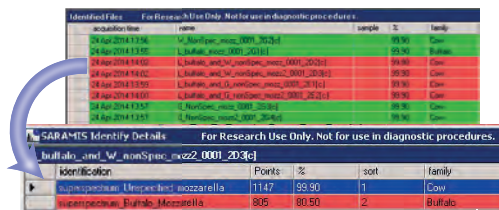


**Figure 6: Search result confirming mozzarella adulteration**

Further adulterated mozzarella samples were analyzed. Cluster analysis of the results clearly displayed three distinct groups: buffalo mozzarella, cow mozzarella and adulterated mozzarella (cow and buffalo mix) confirming that the iD$^{plus}$ system can be applied to dairy foodstuff adulteration detection (figure 7).
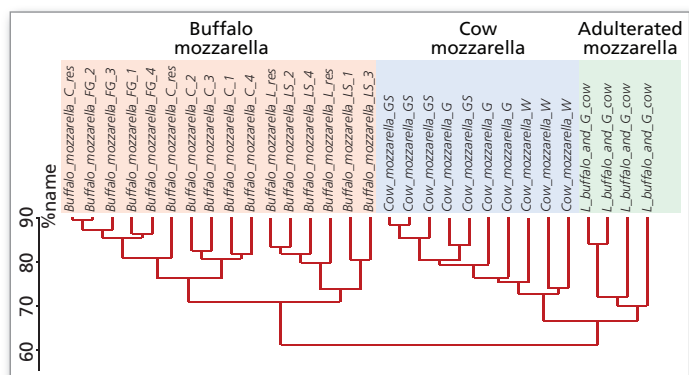


**Figure 7: Cluster analysis of buffalo, cow and adulterated mozzarella**

## Conclusion

Foodstuff adulteration is a prevalent problem within the industry. It comprises both misrepresentation of products and deliberate contamination with lesser ingredients. The results shown here demonstrate effective detection of adulteration in dairy products. Construction of custom databases is straightforward and provides a very high level of confidence due to the use of the patented SuperSpectrum concept and the cluster analysis tool provides a simple graphical representation of the results obtained. The iD$^{plus}$ is an ideal platform for simple and efficient foodstuff adulteration detection and authenticity screening.

References
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| **Application News** | *Food Safety Analysis / Nexera X2* |
|---|---|
| **No. AD-0096** | **Development of An UHPLC Method for Simultaneous Determination of Thirteen Bisphenols in Milk Samples** |

## ❑ Introduction

Bisphenol A (BPA) and Bisphenol F (BPF) are the monomers to make polycarbonate plastics and epoxy resins. Their diglycidyl esters, i.e., BADGE and BFDGE (Figure 1), are also present in the polymeric products. These materials are made into a variety of consumer products or used as inner coatings for baby milk bottles and reusable food containers which are allowed to use in refrigerator and microwave for food storage and heating. It has been reported that polycarbonate plastics and epoxy-based coatings can release BPA, BPF BADGE and BFDGE as well as their reaction products as illustrated in Figure 1 [1]. These leached chemicals can migrate into food and become contaminants consumed by consumers eventually. Although researches indicate that the migration of these chemicals is normally extremely low [2], the specific migration limits (SMLs) of bisphenols were listed in the EU legislation No 1895/2005 on the restriction of use of certain epoxy derivatives in materials contacted with food. BPA has estrogenic effect and can disrupt normal hormone levels and development in fetuses and babies. In U.S., FDA has published food additive regulations prohibiting the use of BPA-based epoxy resins as inner coatings of containers for infant formula packaging [3]. We describe in this Application News a new UHPLC method for simultaneous determination of thirteen concerned bisphenols including BPA, BPF, BADGE, BFDGE and some structural analogues. An UHPLC system (Nexera X2, Shimadzu Corporation) with a high sensitivity fluorescence detector [4] was adopted to develop a fast and high sensitivity method to meet the requirements of regulations.
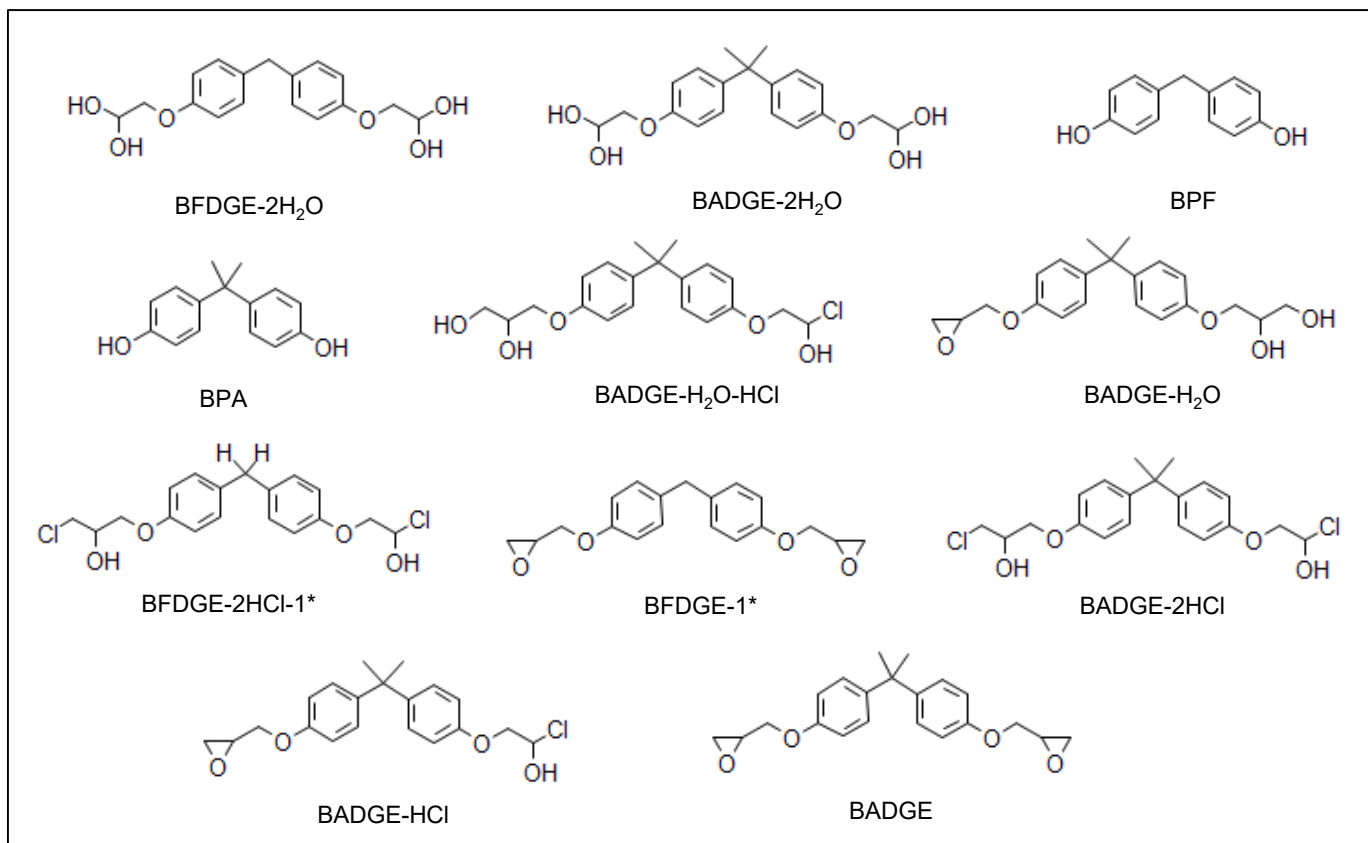


**Figure 1** Chemical structures and abbreviation names of bisphenol A, bisphenol F, their diglycidyl esters and derivatives [1].
*Note: positional isomers of BFDGE-2 and BFDGE-2HCl-2 are not shown.

## ❑ Experimental

### Instrumental and analytical conditions

An UHPLC system - Nexera X2 (Shimadzu Corporation) equipped with a fluorescence detector (RF-20Axs) was employed in this work. Separation of bisphenol A (BPA), bisphenol F (BPF) and other 11 derivatives are performed using a Shim-pack HR-ODS column (250 x 3.0mm, 3µm) with an optimized gradient elution program. Pure water and acetonitrile (ACN) were used as UHPLC mobile phases without any additive. The detailed analytical conditions of the UHPLC method are shown in Table 1.

### Standards and spiked milk samples

A mixed standard stock solution of thirteen bisphenols (Refer to Table 2) containing BPA, BPF and other derivative compounds were prepared in ACN/$H_2O$ (30:70). A serial of calibration standards of concentrations from 5 µg/L to 2,000 µg/L were prepared from the stock to set up multi-point calibration curves. Two blank milk matrix spiked with known concentrations of standards (100 and 1,000 µg/L) obtained from a third party laboratory were used for evaluation of the method performance.

**Table 1:** UHPLC conditions of Bisphenols and derivatives.

| Column | Shim-pack HR-ODS (250 x3.0 mm, 3µm) |
|---|---|
| Mobile phase | A: Water B: Acetonitrile |
| Elution program | 0.1min, 30% B; 13min, 45% B; 37min, 70% B; 38-43min, 85% B; 43.1min, 30% B. |
| Flow rate | 0.40 mL/min |
| Detection | Ex 235 nm, Em 317nm |
| Oven temp. | 30 ºC |
| Injection | 10 µL |

## ❑ Results and Discussion

### Development of fast UHPLC method

For well separation of the thirteen bisphenols studied, a reference HPLC method has a long running time of 95 mins. As shown in Figure 3, the current UHPLC method was optimized to achieve fast elution for every compounds with sufficient separation resolution, especially for the separation of BADGE-$H_2O$ and BADGE-$H_2O$-HCl at 19.4 and 19.9 mins. Due to the similarity in compound structure and chemical properties, separation of these two peaks was a main obstacle to achieve fast analysis speed. The results obtained show clearly the advantages of an UHPLC column with small particle size (3 µm) of the C18 stationary phase.

The main targets BPA (18.1 min) and BPF (13.4 min), BADGE (34.3 min) are separated completely without any inference. BFDGE has two positional isomers, which appeared as a pair at 28.8 min and 30.0 min, respectively. Noted that, another pair of positional isomers BFDGE-2HCl appeared just before the BFDGE peaks at 26.9 min and 27.9 min, respectively.
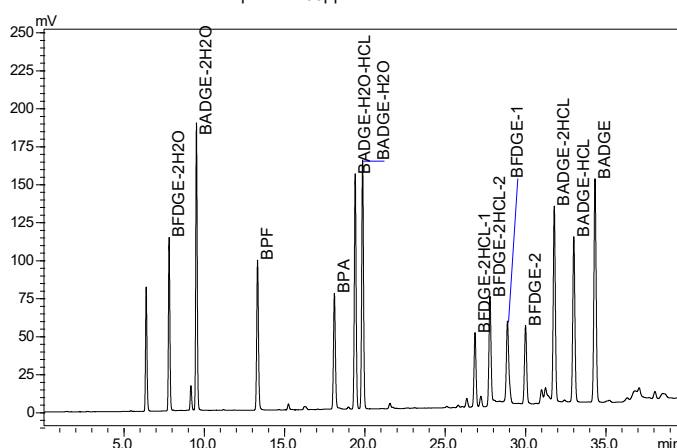


**Figure 2:** UHPLC-RF chromatogram of mixed standards of thirteen bisphenols at concentration of 100 µg/L each.

**Table 2:** Summary of UHPLC method and performance evaluation results for analysis of thirteen bisphenols

| ID # | Name | Ret Time (min) | Calibration range: 5-2000 µg/L | | RSD (%), n=6 | | Sensitivity [2] | |
|---|---|---|---|---|---|---|---|---|
| | | | $R^2$ | Accuracy (%) [1] | 5µg/L | 100µg/L | LOD (µg/L) | LOQ (µg/L) |
| 1 | BFDGE-2$H_2O$ | 7.9 | 0.9998 | 100.9 | 1.0 | 0.9 | 0.7 | 2.0 |
| 2 | BADGE-2$H_2O$ | 9.5 | 0.9991 | 98.7 | 0.4 | 0.4 | 0.4 | 1.2 |
| 3 | BPF | 13.4 | 0.9997 | 101.7 | 0.7 | 0.3 | 0.8 | 2.5 |
| 4 | BPA | 18.1 | 0.9997 | 101.2 | 0.8 | 0.3 | 1.0 | 3.1 |
| 5 | BADGE-$H_2O$-HCL | 19.4 | 0.9998 | 101.5 | 0.5 | 0.3 | 0.5 | 1.5 |
| 6 | BADGE-$H_2O$ | 19.9 | 0.9997 | 103.2 | 0.3 | 0.3 | 0.5 | 1.5 |
| 7 | BFDGE-2HCL-1 | 26.9 | 0.9996 | 98.8 | 0.6 | 0.1 | 1.5 | 4.6 |
| 8 | BFDGE-2HCL-2 | 27.8 | 0.9997 | 100.8 | 2.3 | 0.2 | 1.2 | 3.6 |
| 9 | BFDGE-1 | 28.9 | 0.9997 | 99.3 | 0.9 | 0.3 | 1.4 | 4.2 |
| 10 | BFDGE-2 | 30.0 | 0.9997 | 101.3 | 1.2 | 0.4 | 1.6 | 4.7 |
| 11 | BADGE-2HCL | 31.8 | 0.9997 | 98.9 | 1.8 | 0.3 | 0.6 | 1.7 |
| 12 | BADGE-HCL | 33.0 | 0.9997 | 101.7 | 0.5 | 0.4 | 0.7 | 2.2 |
| 13 | BADGE | 34.3 | 0.9997 | 100.7 | 1.0 | 0.5 | 0.5 | 1.6 |

Notes: 1. Average of 12 concentration levels 5-2000 µg/L
2. Estimated using 5 ug/L mixed stds data based on S/N=3 for LOD and S/N=10 for LOQ

## Calibration curves, range and linearity

Linear calibration curves of the thirteen bisphenols are established using mixed standards samples for concentrations ranging from 5 µg/L to 2000 µg/L as shown in Figure 3. A total of 12 concentration levels were used with each compound in the mixture being 5, 10, 20, 50. 100, 200, 400, 600, 800, 1200, 1500 and 2000 µg/L. All of the thirteen bisphenols peaks give excellent linearity with $R^2$ greater than 0.999 as tabulated in Table 2.
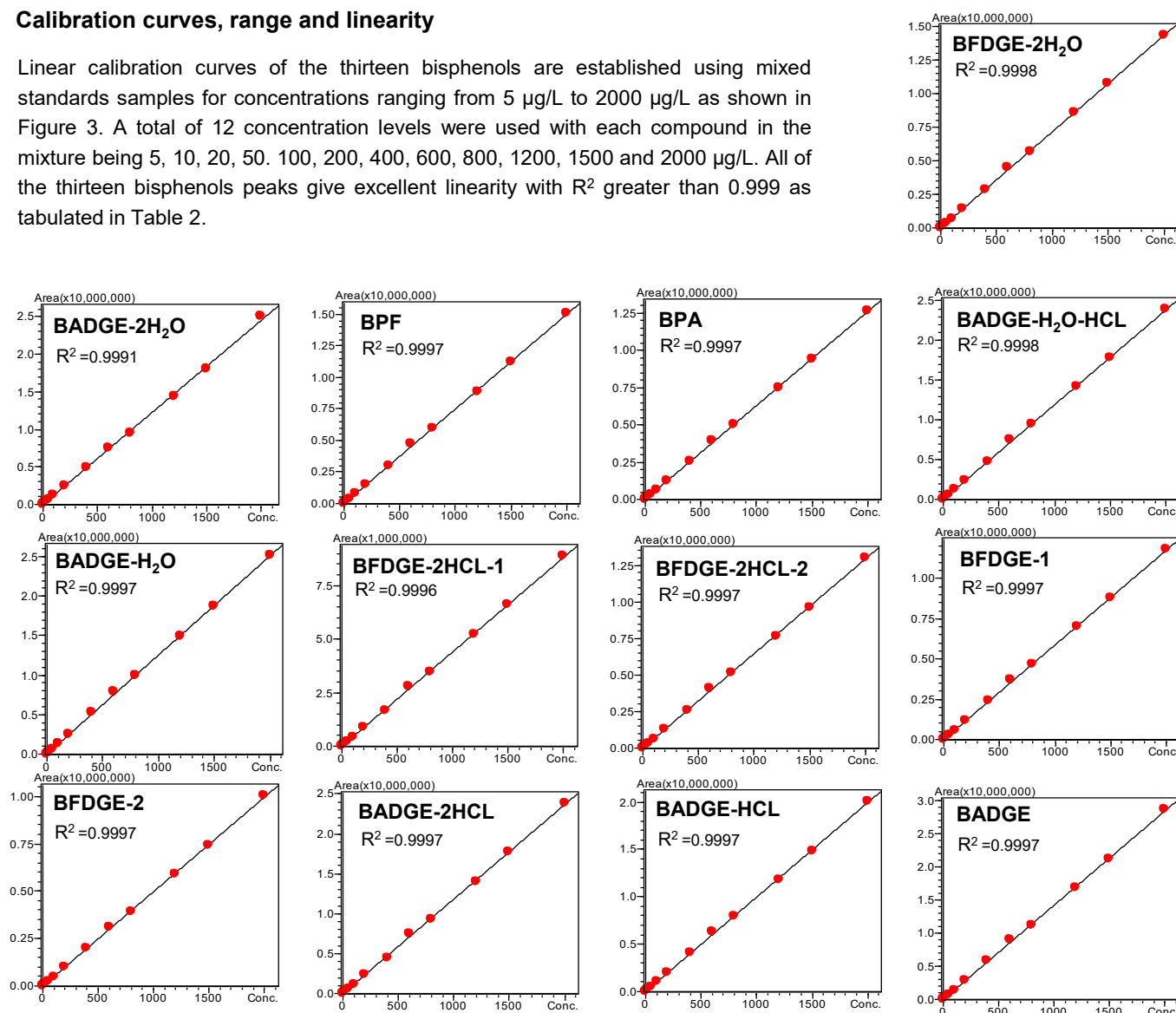
**Figure 3:** Calibration curves of the thirteen bisphenols with concentration range from 5 µg/L to 2,000 µg/L.

## Evaluation of method performance

The accuracy of the method at every calibration levels were calculated and the average accuracy values for every compounds are presented in Table 2. To evaluate repeatability of the method, six consecutive runs of the lowest concentration mixed standard sample (5 µg/L) and a mixed standard sample of 100 µg/L were performed. The RSD values for the 5µg/L mixed standards are less than 2.3%, while RSD values for 100µg/L concentration level are less than 1%, as can be seen in Table 2. The limit of detection (LODs) and limit of quantification (LOQs) were determined from the chromatogram of the lowest concentration mixed standards (5µg/L) as shown in Figure 4, following the rule of S/N=3 for LOD and S/N=10 for LOQ. The obtained LODs and LOQs are at 0.4~1.6 µg/L, and 1.5~4.7 µg/L for the thirteen bisphenols (Table 2).

Dataf ile Name:HSA-18Aug 007.lcd
Sample Name:HSA mix std
Sample ID:5ppb
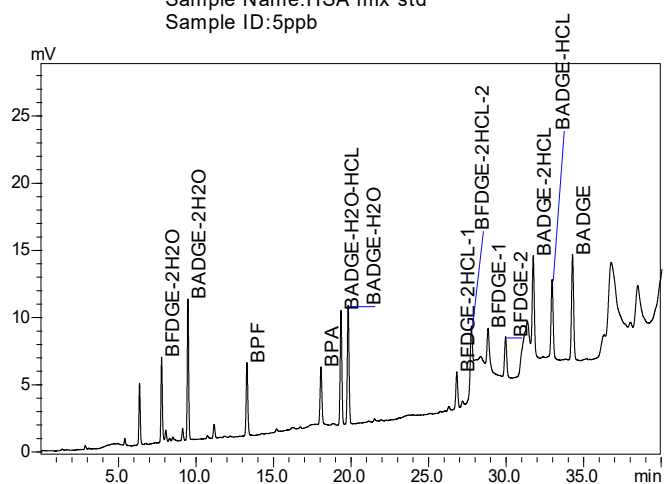
**Figure 4:** UHPLC-RF chromatogram of mixed standards of thirteen bisphenols, 5 µg/L each compound.

## Spiked milk samples and recovery

A blank milk sample and two spiked samples of 13 mixed standards in the same blank were obtained from an analytical laboratory, labeled as Blank, S1 (spiked 100 µg/L) and S2 (spiked 1,000 µg/L). The blank milk matrix was analyzed first and the result showed no any detection of the 13 bisphenols studied. The chromatogram of spiked sample S1 is shown in Figure 5. The quantitative results and recovery data of the 13 bisphenols in both samples are tabulated in Table 3. In both samples, the measured concentrations of BADGE-H2O-HCl are higher than the expected levels with a recovery around 130%. On the other hand, three compounds with longer retentions (peaks 11~13) exhibit much lower concentrations as expected and low recovery of about 40% and 60%.

Datafile Name:HSA-18Aug 022.lcd
Sample Name:HSA sample 1 low conc
Sample ID:HSA sample 1 low conc
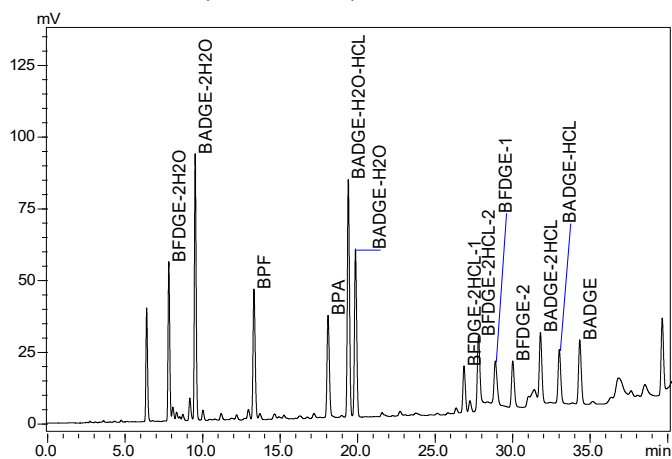


**Figure 5:** UHPLC-RF chromatogram of spiked milk sample S1 (100 µg/L). The sample was diluted with water for two time prior to injection (10 uL)

**Table 3:** Analysis results of spiked milk samples for 13 bisphenols determined by the UHPLC-RF method established

| ID # | Name | Ret Time (min) | Spiked S1 | | Spiked S2 | |
|------|------|----------------|-----------|----------|-----------|----------|
| | | | Conc. (µg/L) | Recovery % | Conc. (µg/L) | Recovery % |
| 1 | BFDGE-2H$_2$O | 7.9 | 115.0 | 115 | 1140.8 | 114.1 |
| 2 | BADGE-2H$_2$O | 9.5 | 114.5 | 114.5 | 1123.6 | 112.4 |
| 3 | BPF | 13.4 | 114.9 | 114.9 | 1152.4 | 115.2 |
| 4 | BPA | 18.1 | 114.1 | 114.1 | 1162.9 | 116.3 |
| 5 | BADGE-H$_2$O-HCL | 19.4 | 131.3 | 131.3 | 1284.9 | 128.5 |
| 6 | BADGE-H$_2$O | 19.9 | 91.1 | 91.1 | 983.1 | 98.3 |
| 7 | BFDGE-2HCL-1 | 26.9 | 79.3 | 79.3 | 947.6 | 94.8 |
| 8 | BFDGE-2HCL-2 | 27.8 | 90.5 | 90.5 | 983.0 | 98.3 |
| 9 | BFDGE-1 | 28.9 | 68.6 | 68.6 | 823.8 | 82.4 |
| 10 | BFDGE-2 | 30.0 | 77.2 | 77.2 | 824.2 | 82.4 |
| 11 | BADGE-2HCL | 31.8 | 47.3 | 47.3 | 668.9 | 66.9 |
| 12 | BADGE-HCL | 33.0 | 43.3 | 43.3 | 635.7 | 63.6 |
| 13 | BADGE | 34.3 | 37.4 | 37.4 | 563.7 | 56.4 |

## ❑ Conclusions

An UHPLC method with using a high sensitivity fluorescence detector was developed for fast, well-separation and high sensitivity analysis of thirteen bisphenols, including the most concerned BPA, BPF, BADGE and BFDGE, in milk samples. This new method shows high sensitivity to low µg/L levels, high accuracy and excellent repeatability. The method was applied to spiked milk samples and the results indicated the good feasibility, high sensitivity and reliability in simultaneous determination of thirteen bisphenols in milk samples.

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## SHIMADZU
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# Application News

## No. C164

**Liquid Chromatograph Mass Spectrometry**

# Ultra-High-Speed Analysis of Melamine in Powdered Milk Using LDTD-MS/MS

The deliberate contamination of powdered milk and pet food with melamine has become a serious social issue. If melamine is contained in food at high concentrations together with cyanuric acid, which is produced in the manufacturing process of melamine, contamination can lead to kidney stones and even kidney failure. In many cases, melamine is added for producing adulterated products, and when added, is done so at very high concentrations. In order to stop these sorts of adulterated products at the border, high-speed screening analysis that can be performed together with easy sample preparation is required. A widely reported analysis technique for melamine in powdered milk involves using LCMS and GCMS after performing pretreatment to remove impurities. This article describes an ultra-high-speed analysis of melamine in powdered milk without column separation by using a laser diode thermal desorption (LDTD) ion source together with the LCMS-8060.

An ion source for ultra-high-speed screening analysis developed by Phytronix Technologies Inc. (https://phytronix.com/) in Canada was employed as the LDTD ion source. Mass spectrometry can be completed within a few seconds by sample vaporization using laser irradiation and subsequent APCI ionization. By applying samples to 96-well plates, up to 10 plates can undergo consecutive analysis. When using the LDTD ion source together with a Shimadzu LCMS-8060, each instrument can be utilized as necessary, such as for direct analysis using LDTD or for LC/MS analysis with column separation, simply by loading a method file with no need to disconnect the LDTD ion source from the LCMS-8060 (Fig. 1). This allows for MRM optimization of the compound for analysis on the LCMS-8060 and then ultra-high-speed analysis with LDTD using the determined MRM transitions. Conversely, polyspecimen analysis screening using ultra-high-speed analysis with LDTD can be performed first, and then using the results, LC/MS analysis can be performed with respect to a particular sample. In this way the combination of the LDTD ion source and LCMS-8060 can be used to switch between two completely different analysis methods according to the purpose of analysis.

In this research, we connected an LDTD ion source, performed MRM optimization of melamine using DUIS (dual ion sources of ESI and APCI), and then used the obtained MRM transitions in ultra-high-speed analysis by LDTD-MS. In performing ultra-high-speed analysis by LDTD-MS, we used a mass spectrometry system comprising an LDTD ion source and the LCMS-8060 and used samples prepared by adding melamine to powdered milk and collecting the melamine using liquid-liquid extraction. The following introduces an example of analyzing melamine in powdered milk by switching between the two analysis systems of LCMS and LDTD-MS.

## ■ MRM Optimization Using LC-MS with an LDTD System Connected

First, MRM optimization was performed in DUIS mode using a standard sample of melamine. The LC conditions used in optimization were the MRM optimization conditions used for general flow injection analysis (FIA). Fig. 2 shows the MS/MS spectrum (CE: −25 V) obtained when optimizing melamine in DUIS mode. Of the MRM transitions ($m/z$ 126 > 85, 127 > 68, and 127 > 43) identified under these conditions, the MRM transition ($m/z$ 127 > 68) with low background noise in LDTD-MS analysis was used to perform the analysis of melamine in powdered milk with LDTD-MS.

T. Nakanishi
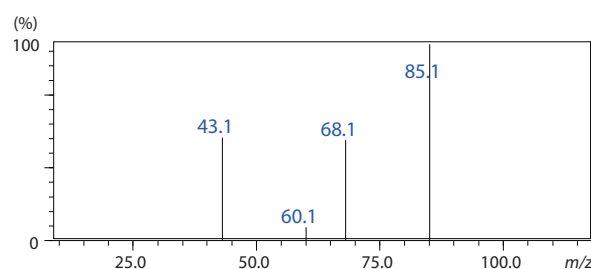


**Fig. 2 MS/MS Spectrum of Melamine Using DUIS Mode**
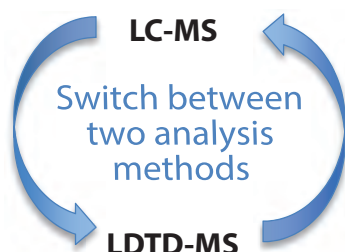


While independently utilizing the three ionization methods of ESI, APCI, and DUIS, MRM optimization of target components can be performed to ensure a smooth start to ultra-high-speed analysis using LDTD, and in cases of complex analysis samples, detailed analysis by LC/MS can be performed following the LDTD analysis.

Easy application of samples to 96-well plates for LDTD-MS allows ultra-high-speed analysis (four second ionization) of multiple components by LDTD-MS.

**Fig. 1 Two Methods of Analysis Using LC-MS and LDTD-MS**

## ■ Extraction of Melamine Added to Powdered Milk

Commercially-available powdered milk was weighed out (125 mg portions) and transferred to 1.5 mL Eppendorf tubes. Next, 0.5 mL of ultra pure water and 0.5 mL of acetonitrile were added and the mixtures were thoroughly agitated for one minute. Then, 12.5 µL of 0, 5, 10, 25, 50, 100, 500, and 1000 µg/mL melamine solutions prepared in advance were added to each powdered milk suspension. These correspond to the concentrations of 0, 0.5, 1, 2.5, 5, 10, 50, and 100 ppm in the powdered milk. Further agitation was performed for another minute to ensure that the added melamine was sufficiently mixed into each solution. Powdered milk components were precipitated by centrifugal separation (14,000 g, room temperature, 5 min) and 200 µL of supernatant containing melamine was collected and transferred to new tubes. Next, 200 µL of sodium carbonate buffer solution (saturated NaCl, pH 10) was added and thoroughly agitated, and then 1 mL of ethyl acetate was added and sufficiently agitated. Since this separates into an aqueous layer and organic layer, centrifugal separation was performed. From the organic layer which contains melamine, 4 µL was taken and dispensed into a LazWell plate (96 well) and then dried. The LazWell plate was set into the LDTD ion source and batch analysis was performed on each sample.

**Table 1  LDTD-MS Analysis Conditions**

| LDTD Analysis Conditions | |
|---|---|
| Laser pattern | : 65 % laser power, 2 seconds |
| Gas flow rate | : 3.0 L/min |

| MS Analysis Conditions | |
|---|---|
| Mode | : MRM (pos) |
| Interface | : APCI |
| DL temperature | : 250 °C |
| Heat block temperature | : 400 °C |

## ■ LDTD-MS Analysis of Melamine Added to Powdered Milk

Table 1 summarizes the LDTD-MS analysis conditions. Fig. 3 shows MRM chromatograms of melamine added to powdered milk (corresponding to 0.5, 5, and 50 ppm concentrations in the powdered milk). It is apparent that the LDTD ion source ionized the melamine within just six seconds (within 0.1 minute). Also, analysis at n = 3 of the samples with melamine added at each concentration resulted in favorable repeatability as shown in Fig. 3. These results indicate that ultra-high-speed analysis by LDTD-MS has unparalleled throughput and is capable of quantitative analysis with high repeatability that is comparable to LCMS analysis. Next, the peak area for each additive concentration of melamine was graphed based on the analysis results of each sample concentration (Fig. 4). A linearity of $R^2 = 0.998$ was verified from these analysis results. From these results we can see that LDTD-MS enables ultra-high-speed analysis with both high repeatability and linearity, even for samples that contain many impurities, such as melamine in powdered milk.

Melamine 126.90 > 68.10(+) CE: -28.0 V



| Area: 387 | Area: 3,541 | Area: 31,563 |
| RSD: 8.2 % | RSD: 4.1 % | RSD: 0.7 % |
| 0.5 µg/mL | 5 µg/mL | 50 µg/mL |
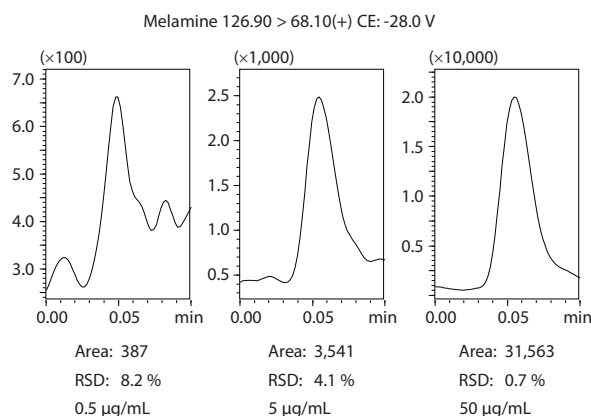
**Fig. 3  MRM Chromatograms of Melamine Added to Powdered Milk**
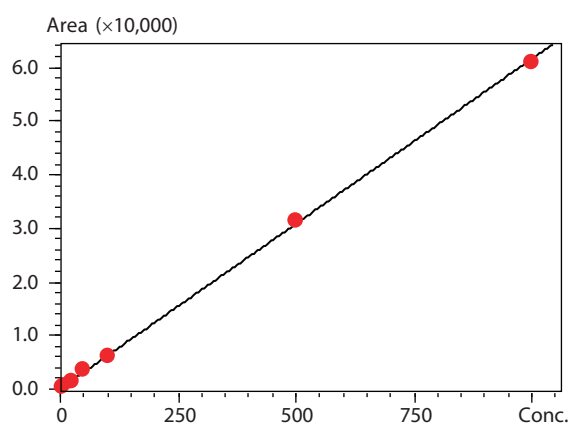


**Fig. 4  Linearity of Melamine Added to Powdered Milk**

In this research, we performed MRM optimization in DUIS mode on the LCMS-8060 followed by ultra-high-speed analysis using LDTD-MS with respect to melamine added to powered milk, and verified the level of repeatability and linearity. As demonstrated, the combination of the LCMS-8060 with an LDTD ion source allows easy switching of the analysis system according to the purpose of analysis, thereby allowing multicomponent optimization by LCMS, or LCMS analysis of complex analysis samples as necessary based on the results of simple ultra-high-speed screening analysis by LDTD. These two characteristic analysis methods can be utilized as necessary.

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# Determination of 5 Kinds of Estrogens in Milk using LCMS-8045

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## Abstract

A method for determination of three kinds of natural estrogen (estrone, 17β-estradiol, and estriol) and two kinds of synthetic estrogen (hexestrol and diethylstilbestrol) in milk was developed using Shimadzu's ultra-high performance liquid chromatograph (UHPLC) LC-30A coupled with the triple quadrupole mass spectrometer LCMS-8045. Proteins in milk samples were precipitated with acetonitrile and extracted by sonication. Without any further pretreatment and derivatization, the samples were analyzed by the ESI -MS/MS in negative ion mode. The samples were quantified by an external standard method. The linearity of the calibration curve was good and all linear correlation coefficients were at or above 0.9993. Samples of low, medium and high concentrations were tested in 6 replicates. The relative standard deviations of retention time and peak area were 0.05 to 0.12% and 0.42 to 8.26%, respectively. The method's limit of quantitation was 0.006 to 0.033 ng/mL. This method, characterized by simple sample preparation, high sensitivity and good repeatability, can be used for determination of estrogen content in milk products.

Estrogen is a type of steroid hormones and is widely used in dairy farming to increase milk yield in dairy cows. While the use of estrogen can increase economic efficiency, it may also give rise to estrogen residues in milk. In recent years, a large number of studies have demonstrated that estrogen can enter the human body through the food chain and may induce cancers of the breast, uterus, testis, bone, kidney, and other tissues. Therefore, the analysis and detection of estrogen in milk are of practical significance.

In this application news, esterone (E1), 17β-estradiol (E2), estriol (E3), hexestrol (HEX) and diethylstilbestrol (DES) were used as target substances to establish a method for detection of estrogen content in milk using Shimadzu LCMS-8045. First, proteins in milk samples were precipitated with acetonitrile and extracted by sonication. Subsequently, without further pretreatment and derivatization, the samples were ionized by ESI negative ion mode and data was analyzed and collected under MRM mode. The samples were then quantitated by the external standard method. This method, characterized by simple sample preparation, high sensitivity and good repeatability, can be used for determination of estrogen content in milk products.
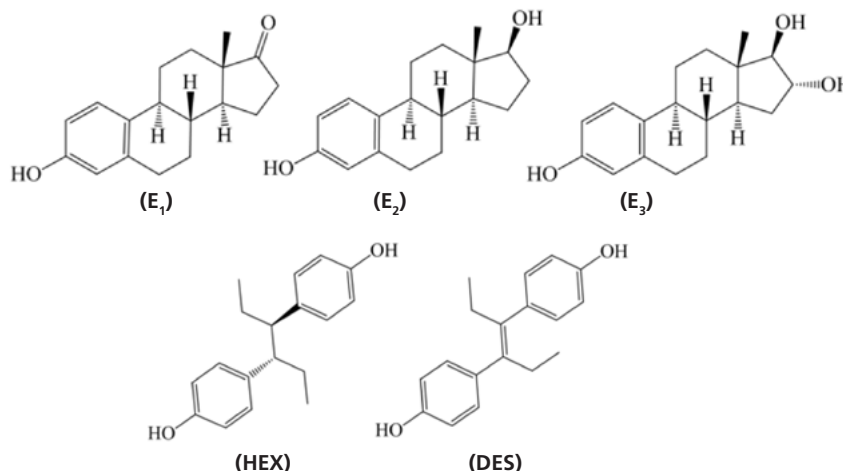


Figure 1  Structural formulas of five estrogen molecules

## EXPERIMENTAL
### Instrumentation

The experiment employed Shimadzu's UHPLC LC-30A and triple quadrupole mass spectrometer LCMS-8045. The configurations are two LC-30AD pumps, DGU-20A5 online degassing unit, SIL-30AC autosampler, CTO-30A column oven, CBM-20A system controller, LCMS-8045 triple quadrupole mass spectrometer and LabSolutions Ver. 5.86 chromatography workstation.

### Analytical Conditions
#### LC Chromatography (LC) Conditions

| | | |
|---|---|---|
| Column | : | Shim-pack GISS Column (2.1 mm I.D.×100 mm L., 1.9 μm C18) |
| Mobile phase | : | Mobile phase A - 0.03% ammonia in water Mobile phase B-acetonitrile |
| Flow rate | : | 0.4 mL/min |
| Column Temp. | : | 40 °C |
| Injection volume | : | 5 μL |
| Elution method | : | Gradient elution with initial concentration of mobile phase B at 20%. Refer to Table 1 for detailed elution program. |

### Standard Solution Preparation

Preparation of standard solution: mixed standard stock solution at a concentration of 100 mg/L was serially diluted with an aqueous solution of 50% methanol to obtain standard solutions at concentrations of 0.1, 0.2, 0.5, 1.0, 5.0, 10.0, 50.0 and 100.0 ng/mL.

### Sample Preparation Method

200 μL of milk sample were added

**Table 1 Time program**

| Time (min) | Module | Command | Value (%) |
|---|---|---|---|
| 0.30 | Pumps | Pump B Conc. | 20 |
| 0.50 | Pumps | Pump B Conc. | 50 |
| 3.50 | Pumps | Pump B Conc. | 50 |
| 3.70 | Pumps | Pump B Conc. | 95 |
| 4.50 | Pumps | Pump B Conc. | 95 |
| 4.60 | Pumps | Pump B Conc. | 20 |
| 7.00 | Controller | Stop | |

### Mass Spectrometry (MS) Conditions

| | | |
|---|---|---|
| Ion source | : | ESI (-) |
| Heated air | : | Air 12.0 L/min |
| Nebulizing gas | : | Nitrogen 12.0 L/min |
| Drying gas | : | Nitrogen 8.0 L/min |
| Collision gas | : | Argon |
| Interface temperature | : | 350 °C |
| DL temperature | : | 150 °C |
| Block heater temperature | : | 350 °C |
| Mode | : | Multiple reaction monitoring (MRM) |
| Dwell time | : | 100 ms |
| Pause time | : | 3 ms |
| MRM transitions | : | Refer to Table 2 |

to 800 μL of acetonitrile and vortexed for 1 min. Subsequently, the sample was subjected to 20 min of sonication extraction and centrifuged at 10,000 rpm for 3 min. Finally, the supernatant was collected for testing.

**Table 2 MRM transition**

| No. | Compound | CAS No. | Precursor Ion | Product Ion | Q1 Pre Bias (V) | CE (V) | Q3 Pre Bias (V) |
|---|---|---|---|---|---|---|---|
| 1 | Estriol (E3) | 50-27-1 | 287.20 | 171.10* | 20.0 | 40.0 | 27.0 |
| | | | | 145.10 | 20.0 | 47.0 | 22.0 |
| 2 | 17 β-Estradiol | 50-28-2 | 271.20 | 145.10* | 13.0 | 44.0 | 21.0 |
| | | | | 183.20 | 28.0 | 45.0 | 30.0 |
| 3 | Estrone (E1) | 53-16-7 | 269.20 | 145.10* | 30.0 | 41.0 | 25.0 |
| | | | | 143.00 | 13.0 | 66.0 | 12.0 |
| 4 | Hexestrol (HEX) | 84-16-2 | 269.20 | 119.05* | 19.0 | 43.0 | 18.0 |
| | | | | 134.10 | 19.0 | 16.0 | 11.0 |
| 5 | Diethylstilbestrol (DES) | 6898-97-1 | 267.20 | 251.10* | 18.0 | 27.0 | 23.0 |
| | | | | 237.10 | 18.0 | 31.0 | 22.0 |

Note: * indicates quantification ion

## RESULTS AND DISCUSSION
### Chromatogram of Standard Mixture
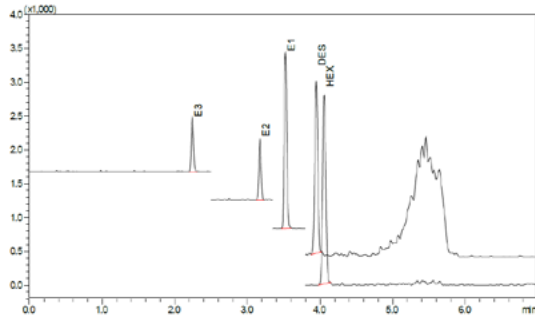


**Figure 2 MRM chromatograms of standard mixture (0.2 ng/mL)**

### Calibration and Linearity

Standard calibration solutions of the four target compounds at concentrations of 0.1, 0.2, 0.5, 1.0, 5.0, 10.0, 50.0 and 100.0 ng/mL were determined based on conditions listed in the experimental section. An example of the MRM chromatograms of a standard sample (0.2ng/mL) is shown in Figure 2. Calibration curves were established using the external standard method and shown in Figure 3. The linear equation, linear range, and correlation coefficients are tabulated in Table 3.

### Precision Test

Mixed standard samples containing 0.2, 5.0, and 100 ng/mL of target substances were prepared and tested in 6 replicates to determine the repeatability. The retention time and peak area are shown in Table 4 and it demonstrates good repeatability.
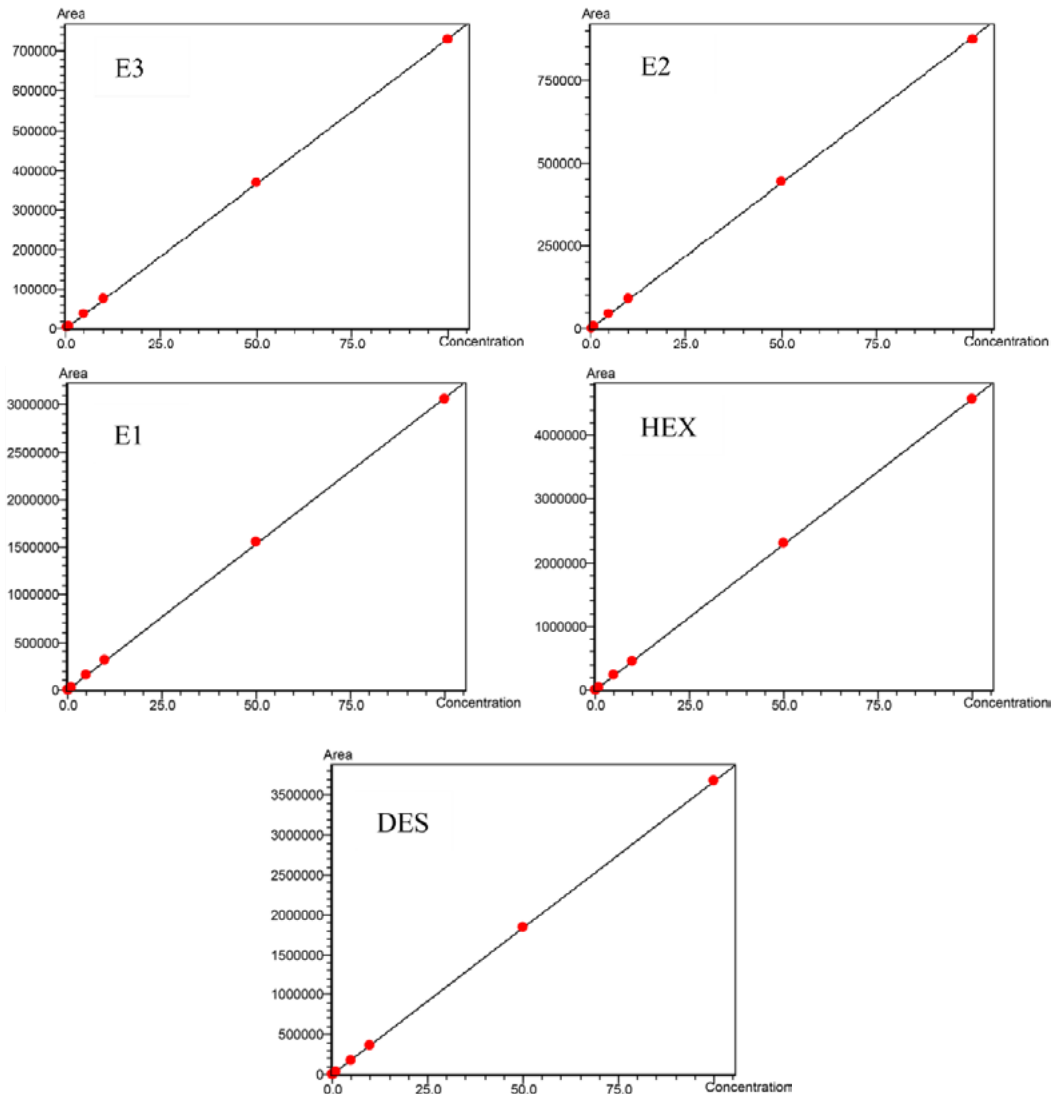


**Figure 3 Calibration curve**

**Table 3  Parameters of calibration curve**

| Compound | Calibration Curve | Linear Range (ng/mL) | Correlation Coefficient (r) | Accuracy (%) |
|---|---|---|---|---|
| E3 | Y = (7335.5) X +150.426 | 0.2-100 | 0.9999 | 96.01-103.4% |
| E2 | Y = (8932.6) X -2.7160 | 0.2-100 | 0.9999 | 95.5-104.7% |
| E1 | Y = (30778.2) X -18.0396 | 0.1-100 | 0.9998 | 94.7-104.9% |
| HEX | Y = (45714.2) X-376.316 | 0.1-100 | 0.9999 | 96.5-106.1% |
| DES | Y = (36057.1) X -196.210 | 0.1-100 | 0.9997 | 93.4-105.1% |

**Table 4 Repeatability results of retention time and peak area (n=6)**

| Compound | RSD% (0.2 ng/mL) | | RSD% (5.0 ng/mL) | | RSD% (100 ng/mL) | |
|---|---|---|---|---|---|---|
| | R.T. | Area | R.T. | Area | R.T. | Area |
| E3 | 0.12 | 3.99 | 0.11 | 1.33 | 0.08 | 0.42 |
| E2 | 0.12 | 5.34 | 0.09 | 1.48 | 0.06 | 0.54 |
| E1 | 0.10 | 2.20 | 0.09 | 0.62 | 0.06 | 0.51 |
| HEX | 0.11 | 3.35 | 0.09 | 0.84 | 0.05 | 0.49 |
| DES | 0.08 | 8.26 | 0.09 | 1.62 | 0.05 | 0.39 |

### Sensitivity Test

Standard solutions containing 0.20 ng/mL of target compounds were injected and analyzed. The limit of detection (ILOD, S/N=3) and limit of quantitation (ILOQ, S/N=10) for each target component were calculated based on the signal to noise ratios (S/N). The results are shown in Table 5.

**Table 5 Limit of detection and limit of quantitation**

| Compound | Limit of Detection (ng/L) | Limit of Quantitation (ng/L) |
|---|---|---|
| E3 | 0.008 | 0.025 |
| E2 | 0.007 | 0.021 |
| E1 | 0.002 | 0.006 |
| HEX | 0.007 | 0.020 |
| DES | 0.010 | 0.033 |

### Recovery Test

A certain brand of milk purchased from a supermarket was processed, injected and analyzed according to the method listed in the experimental section. The results showed that none of the five target compounds were detected, as shown in Figure 4. The five target compounds were added into the milk to determine the spike recovery. The results are shown in Table 6.

### CONCLUSION

This application news describes a method for determination of three kinds of natural estrogen and two kinds of synthetic estrogen in milk using Shimadzu's ultra-high performance liquid chromatograph (UHPLC) LC-30A and triple quadrupole mass spectrometer LCMS-8045. Proteins in milk samples were precipitated with acetonitrile and extracted by sonication.

**Table 6 Spike Recovery Test**

| Compound | Spiked Concentration (ng/mL) | Recovery (%) | Spiking Concentration (ng/mL) | Recovery (%) | Spiking Concentration (ng/mL) | Recovery (%) |
|---|---|---|---|---|---|---|
| E3 | 0.2 | 103.3 | 4.0 | 86.5 | 80 | 92.0 |
| E2 | 0.2 | 87.8 | 4.0 | 103.3 | 80 | 106.0 |
| E1 | 0.2 | 86.8 | 4.0 | 102.4 | 80 | 106.3 |
| HEX | 0.2 | 81.0 | 4.0 | 90.8 | 80 | 95.9 |
| DES | 0.2 | 83.3 | 4.0 | 102.9 | 80 | 109.4 |

Without any further pretreatment or derivatization, the samples were ionized by the ESI negative ion mode and the data was obtained under MRM mode. The samples were then quantified by an external standard method. The linearity of the calibration curve was good and all linear correlation coefficients were at or above 0.9993. Samples of low, medium and high concentrations were tested in 6 replicates. The relative standard deviations of retention time and peak area were 0.05 to 0.12% and 0.42 to 8.26%, respectively. The limit of quantitation was 0.006 to 0.033 ng/mL. This method, characterized by simple sample preparation, high sensitivity and good repeatability, can be used for determination of estrogen in milk products.
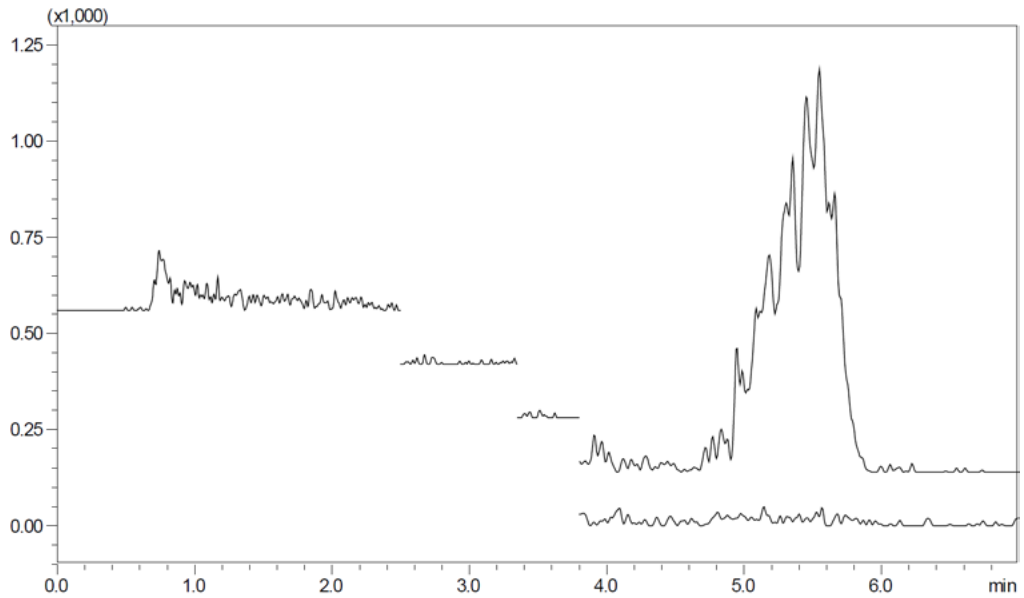


**Figure 4 Chromatogram of milk sample found to not contain any targeted estrogen**
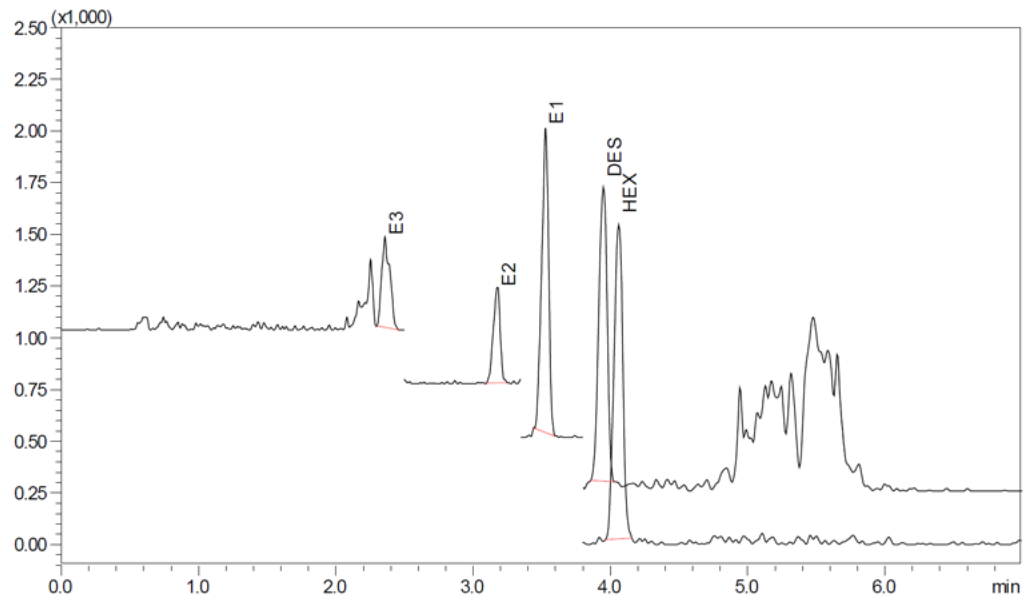


**Figure 5  Chromatogram of blank sample matrix spiked with 0.2 ng/mL estrogens**

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# Determination of Imidocarb Residues in Milk by Ultra-High Performance Liquid Chromatograph Coupled with Triple Quadrupole Mass Spectrometer

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## Abstract

In this application news, Shimadzu's Ultra-High Performance Liquid Chromatograph (UHPLC) LC-30A was coupled with Triple Quadrupole Mass Spectrometer LCMS-8045 for the determination of imidocarb residues in milk. This method is made in reference to the "Inspection and Quarantine Industry Standard, SN/T 4252-2015: Method for Detection of Imidocarb Residues in Animal-Derived Food for Export". The linearity was excellent over the range of 0.02 – 50 ng/mL and the correlation coefficient of the method was 0.9999. In the precision experiment, the RSD% of the retention time and peak area of imidocarb samples were 0.1 – 0.2% and 1.5 – 5.4% respectively, indicating good precision. In the spike recovery test, the spike recovery of the matrix at different concentrations ranged from 86.0 to 98.6%.

Imidocarb is a derivative of 1,3-Diphenylurea and is commonly used as a novel antiprotozoal chemical drug (veterinary medicine) used in animals. In clinical applications, imidocarb preparations are usually used in forms of dipropionate salt or bis-hydrochloride salt. Through intramuscular or subcutaneous injection, imidocarb is used in the treatment and prevention of various parasitic infections such as babesiosis, piroplasmosis, trypanosomiasis, eperythrozoonosis, anaplasmosis and theileriosis. Imidocarb is concentrated in kidneys and resorbed in its original forms. The detoxification (metabolism) of imidocarb occurs in the liver. Therefore, the amount of imidocarb use is small and its efficacy is long-lasting, thus inducing minimal stimulation. As a veterinary medicine, imidocarb is characterized by small dosage, convenient administration, short course of treatment and low drug resistance.

Studies have shown that imidocarb has a long metabolic period and high residue levels in animals. High concentrations of imidocarb residues may pose a threat to human health. CODEX, EU, Japan, and Australia have all established standards regarding the Maximum Residue Level (MRL) of imidocarb in foods. In particular, the MRL of imidocarb in both milk and beef fat is 50 µg/Kg.

Milk contains a high nutritional content and is the most ideal natural food for humans. Therefore, the detection of imidocarb residues in milk is of great significance. In this experiment, in reference to the "Inspection and Quarantine Industry Standard, SN/T 4252-2015: Method for Detection of Imidocarb Residues in Animal-Derived Food for Export", Shimadzu's UHPLC LC-30A was used together with the Triple Quadrupole Mass Spectrometer LCMS-8045 to establish a method for determination of imidocarb residues in milk. UHPLC-tandem mass spectrometry is characterized by high selectivity, high sensitivity, strong qualitative and quantitative capabilities, and high accuracy. Therefore, in the inspection and quarantine industry, UHPLC-tandem mass spectrometry is ideal for the analysis of veterinary drug residues in animal-derived foods.

## EXPERIMENTAL

### Instrumentation

The experiment employed Shimadzu's UHPLC LC-30A and Triple Quadrupole Mass Spectrometer LCMS-8045. The specific configurations are two LC-30AD pumps, DGU-20A$_{5R}$ online degassing unit, SIL-30AC autosampler,

CTO-20AC column oven, CBM-20A system controller, LCMS-8045 triple quadrupole mass spectrometer and LabSolutions LCMS DB Ver. 6.80 Chromatographic Workstation.

## Analytical Conditions
## Liquid Chromatography (LC) Conditions

| | | |
|---|---|---|
| Column | : | Shim-pack GISS, 2.1 mm I.D. × 100 mm L., 1.9 μm |
| Mobile Phase | : | Mobile Phase A-0.1% formic acid in water<br>Mobile Phase B-methanol |
| Flow rate | : | 0.40 mL/min |
| Column temp. | : | 40 °C |
| Injection volume | : | 1 μL |
| Type of elution | : | Gradient elution with the initial concentration of Mobile Phase B at 25%. Refer to Table 1 for elution program. |

**Table 1 Gradient elution time program**

| Time (min) | Module | Command | Value (%) |
|---|---|---|---|
| 0.50 | pumps | B Conc. | 25 |
| 1.00 | pumps | B Conc. | 50 |
| 2.00 | pumps | B Conc. | 50 |
| 2.10 | pumps | B Conc. | 90 |
| 3.00 | pumps | B Conc. | 90 |
| 3.10 | pumps | B Conc. | 25 |
| 5.00 | controller | Stop | |

## Mass Spectrometry (MS) Conditions

| | | |
|---|---|---|
| Analytical Instrument | : | LCMS-8045 |
| Ion source | : | ESI (+) |
| Heating gas | : | Air 10.0 L/min |
| Nebulizing gas | : | Nitrogen 3.0 L/min |
| Drying gas | : | Nitrogen 10.0 L/min |
| Collision gas | : | Argon |
| Interface temp. | : | 300 °C |
| DL temp. | : | 250 °C |
| Heating block temp. | : | 400 °C |
| Scanning mode | : | Multiple reaction monitoring (MRM) |
| Interface voltage | : | 0.2 kV |
| MRM parameters | : | See Table 2 |

## Standard Solution Preparation

An appropriate amount of imidocarb standard was weighed and dissolved in methanol to prepare a standard stock solution at a concentration of 1.0 μg/mL. The standard stock solution was stored at 4 °C. The standard stock solution was diluted with the mobile phase at its initial ratio (0.1% formic acid in water: methanol = 75:25, v/v) to obtain standard working solutions at concentrations of 0.02 ng/mL, 0.1 ng/mL, 1 ng/mL, 10 ng/mL, and 50 ng/mL.

## Sample Preparation

Sample preparation was performed according to the milk extraction and purification method in the "Inspection and Quarantine Industry Standard, SN/T 4252-2015: Method for Detection of Imidocarb Residues in Animal-Derived Food for Export". 5.00 g of the sample (to the nearest 0.01 g) was weighed and placed

**Table 2 MRM optimized parameters**

| Compound Name | CAS No. | Precursor Ion | Product Ion | Q1 Pre Bias (V) | CE (V) | Q3 Pre Bias (V) |
|---|---|---|---|---|---|---|
| Imidocarb | 27885-92-3 | 349.1 | 188.2* | -17 | -30 | -18 |
| | | | 162.2 | -17 | -25 | -15 |

Note: * indicates quantification ion

into a 50 mL centrifuge tube. After adding the extraction solution (acetonitrile: water = 8:2, v/v) to a final volume of 25 mL, the solution was vortexed and mixed for 2 min. Subsequently, the solution was centrifuged at 4500 r/min for 8 min, and 5 mL of the supernatant was transferred into another centrifuge tube. After adding sodium hydroxide solution into the supernatant (sample solution) to adjust the pH to approximately 8.0, the solution was centrifuged at 4000 r/min for 5 min. The sample solution was added to a WCX solid-phase extraction (SPE) column, followed by washing with 2 mL of water and 3 mL of methanol. The entire wash solution was discarded and the column was eluted with 5 mL of methanol. The eluate was collected and completely dried with nitrogen. The residues were dissolved in 1 mL of solution (0.1% formic acid in water : methanol = 75:25, v/v) and filtered through a 0.22 µm membrane filter to obtain the final sample for analysis

## RESULTS AND DISCUSSION

### Q1 MS Scan and Product Ion Scan of Imidocarb Standard

The Q1 MS scan and product ion scan of the imidocarb standard are shown in Figures 1 and 2.

### MRM Chromatogram of 0.02 ng/mL Imidocarb Standard Solution

The MRM chromatogram is shown in Figure 3.
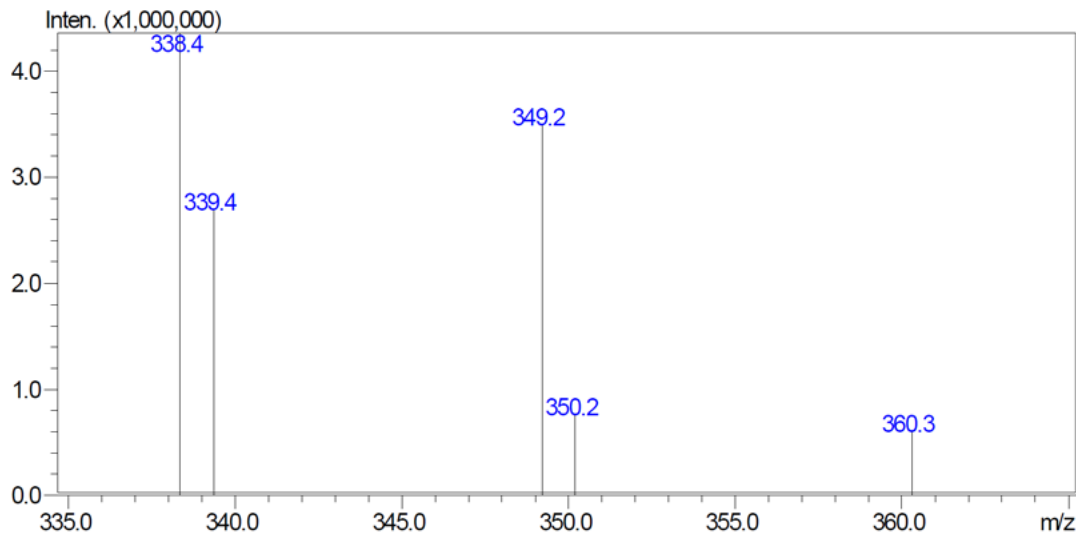


Figure 1  Q1 MS scan of imidocarb
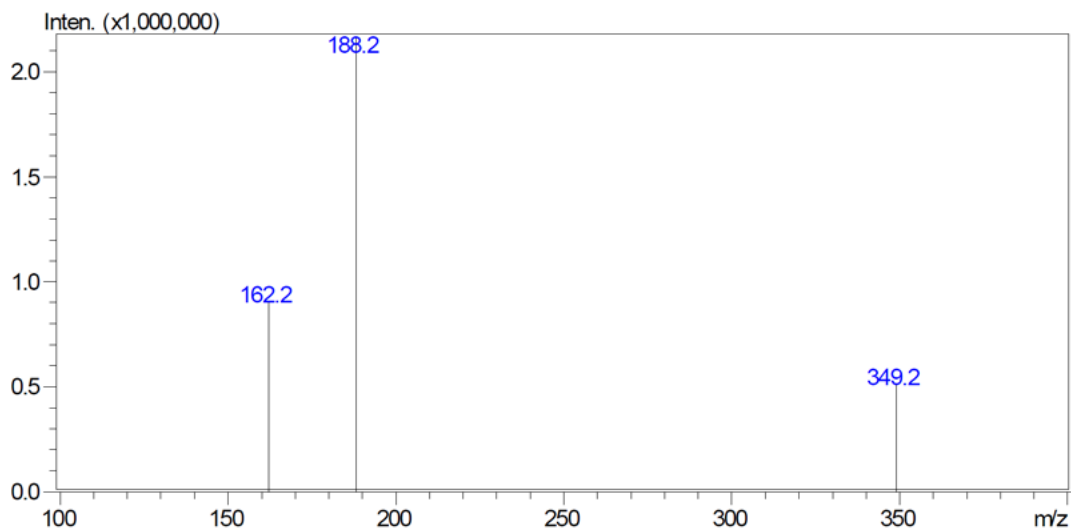


Figure 2  Product ion scan of imidocarb (the CE value was -25 V)
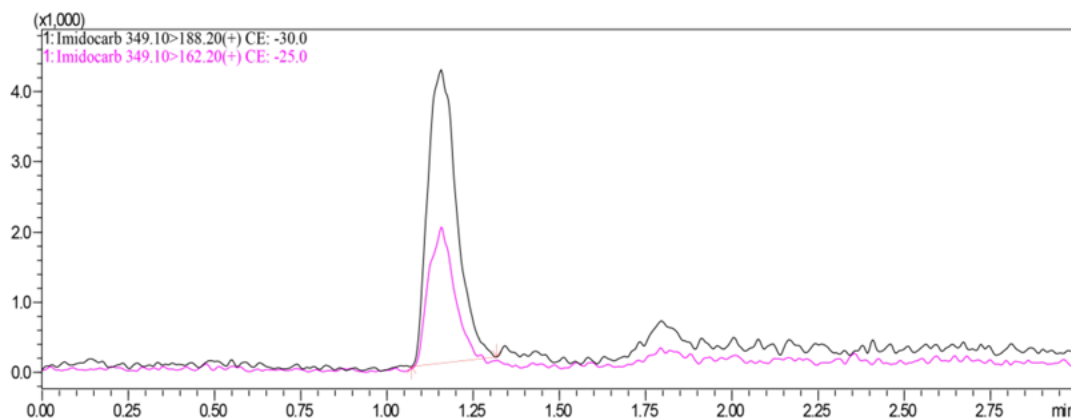
**Figure 3 MRM chromatogram of 0.02 ng/mL imidocarb standard solution**

### Calibration Curve and Linear Range

After the measurement was performed according to the analytical conditions specified in the experimental section, a standard curve was established by the external standard method. The results are shown in Figure 4. In the linear concentration range of 0.02-50 ng/mL, the correlation coefficients of imidocarb were all greater than 0.9999 and the linear correlation of the results was excellent. The detailed results are shown in Table 3.
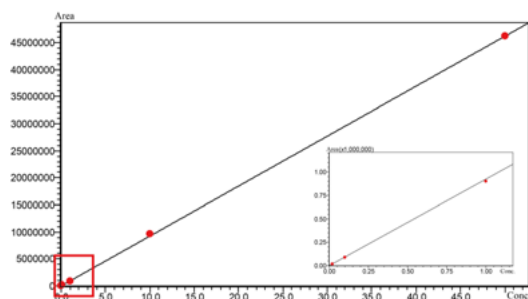


**Figure 4  The standard curve of imidocarb**

### Precision Test

According to the analytical conditions specified in previously, the standard solutions of imidocarb at concentrations of 0.02 ng/mL, 1 ng/mL, and 50 ng/mL were injected consecutively and tested in 6 replicates. The relative standard deviation (RSD) for the retention time of imidocarb was in the range of 0.1 – 0.2%. The RSD for the peak area was in the range of 1.5 – 5.4% (see Table 4 for detailed results). The results showed that the precision was adequate.

### Sensitivity Test

In order to examine the sensitivity, the imidocarb standard solution at a low concentration of 0.02 ng/mL was measured under the analytical conditions specified in the experimental section. The signal-to-noise ratio, limit of detection, and limit of quantitation were calculated using the LabSolutions LCMS DB Ver. 6.80 software. The results are shown in Table 5.

**Table 3  Parameters of the calibration curve**

| Analyte | Linear Equation | Correlation Coefficient | Concentration Point of the Curve (ng/mL) | Accuracy (%) |
|---------|-----------------|-------------------------|------------------------------------------|--------------|
|         |                 |                         | 0.02                                     | 92.8         |
|         |                 |                         | 0.1                                      | 94.6         |
| Imidocarb | Y = 923549X   | 0.9999                  | 1                                        | 99.4         |
|         |                 |                         | 10                                       | 103.3        |
|         |                 |                         | 50                                       | 99.9         |

**Table 4  Precision results (n=6)**

| Analyte | 0.02 ng/mL | | 1 ng/mL | | 50 ng/mL | |
|---|---|---|---|---|---|---|
| | Retention Time (Min) | Peak Area | Retention Time (Min) | Peak Area | Retention Time (Min) | Peak Area |
| | 1.158 | 22343 | 1.147 | 688961 | 1.143 | 40201400 |
| | 1.162 | 21251 | 1.148 | 697204 | 1.141 | 40360042 |
| Imidocarb | 1.155 | 24061 | 1.152 | 690845 | 1.141 | 40507473 |
| | 1.156 | 24457 | 1.152 | 718771 | 1.143 | 40860853 |
| | 1.156 | 22054 | 1.147 | 711057 | 1.142 | 41454168 |
| | 1.160 | 22913 | 1.147 | 718038 | 1.145 | 41716697 |
| Average | 1.157 | 22846 | 1.149 | 704146 | 1.143 | 40850106 |
| RSD% | 0.2 | 5.4 | 0.2 | 1.9 | 0.1 | 1.5 |

**Table 5 Signal-to-noise ratio(S/N), limit of detection and limit of quantitation**

| Analyte | Conc. (ng/mL) | S/N | LOD (ng/mL) | LOQ (ng/mL) |
|---|---|---|---|---|
| Imidocarb | 0.02 | 19.2 | 0.003 | 0.01 |

**Spike Recovery**

The milk sample solutions, prepared as mentioned in experimental section, were injected and analyzed to obtain the chromatogram shown in Figure 5. The imidocarb standard solution was added into 5.00 g of milk sample and the matrix spike sample was prepared according to the pretreatment method specified in experimental section. The final volume of the sample was 1.0 mL.

As specified in SN/T 4252-2015, the lower limit of detection for imidocarb is 25 µg/kg. No imidocarb was detected in the sample. The samples were spiked to obtain the final spiked concentrations of 0.02 ng/mL (0.02 µg/kg), 1 ng/mL (1 µg/kg) and 50 ng/mL (50 µg/kg). Each spiked sample was continuously injected 6 times. The chromatograms of the spiked samples and the spike recovery rates are shown in Figure 6 and Table 6 respectively. From



**Figure 5 Chromatogram of milk samples**



**Figure 6 Chromatogram of milk samples using 0.02 µg/kg of imidocarb standard addition**

**Table 6  Spike recovery (n=6)**

| Analyte/Spiked Amount | Detected Concentration | Spike Recovery (%) | | |
|---|---|---|---|---|
| | µg/kg | 0.02 µg/kg | 1 µg/kg | 50 µg/kg |
| | | 92.0 | 89.0 | 99.0 |
| | | 83.5 | 90.9 | 97.7 |
| Imidocarb | N.D. | 82.0 | 87.5 | 99.8 |
| | | 85.5 | 86.8 | 97.9 |
| | | 89.5 | 87.9 | 99.5 |
| | | 85.5 | 88.7 | 97.7 |
| Average Recovery (%) | | 86.0 | 88.5 | 98.6 |
| RSD% | | 5.0 | 1.6 | 0.9 |

(N.D.: Not Detected)

Table 6, it is observed that when the level of imidocarb spiked in the matrix was 0.02 µg/kg, the recovery was in the range of 82.0-92.0%; at the level of 1 µg/kg, the recovery was in the range of 86.8–90.9%; at the level of 50 µg/kg, the recovery was in the range of 97.7–99.5%. The spiked samples showed an adequate response above the limit of quantitation. Therefore, the method can meet the requirements for the quantitative analysis of the samples.

**CONCLUSION**

This application news established a method for determination of imidocarb residues in milk using Shimadzu's UHPLC LC-30A coupled with Triple Quadrupole Mass Spectrometer LCMS-8045. The linearity was excellent over the concentration range of 0.02–50 ng/mL and the correlation coefficient was 0.9999. The precision was evaluated and the RSD% of the retention time and peak area of imidocarb samples were 0.1 – 0.2% and 1.5 – 5.4% respectively, indicating the adequate precision. In the spike recovery test, the spike recovery of the matrix at different concentrations ranged from 86.0 to 98.6%. This method, characterized by fast analysis, high sensitivity and excellent reproducibility, is suitable for the detection and analysis of imidocarb residues by the inspection/quarantine department and related industries.

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# Analysis of toxic elements in processed milk products using ICP-MS

## ASMS 2017   TP 195

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# Introduction

Milk is considered as complete food as it provides all essential macro and micronutrients. Macronutrients consist of protein, carbohydrate and lipids, whereas micronutrients consist of elements, vitamins and enzymes[1][2]. Milk as an excretion of the mammary gland can carry numerous xenobiotic substances that constitute a technological risk factor for milk and milk products.

Toxic elements like Arsenic (As), Cadmium (Cd), Lead (Pb) and Mercury (Hg) have caused adverse effects on human health. These can be transferred from contaminated soil to plants and grass, causing accumulation of these toxic metals in cattle, but also in humans consuming milk. Milk processing may also cause contamination of milk products with toxic elements.

The objective of this study is to develop a sensitive, selective, accurate and reliable method using Shimadzu ICPMS-2030 to determine the risk of toxic heavy metals in milk product.



Figure 1   Milk and processed milk products

# Methods and Materials

Commercially available milk products e.g. toned milk and cheese (Figure 1) were used for the extraction of toxic heavy metals in this study. Recovery studies were established by spiking milk product samples with standard solution of elements. The extracts obtained were analysed on Shimadzu ICPMS-2030.

## Sample Preparation:

The samples were digested using microwave digestion system (Anton Paar).

| Transfer 1g of milk/ cheese in microwave digester PTFE vessel |
|:---:|

⬇

| Add 5mL nitric acid, 1mL perchloric acid, 1mL hydrogen peroxide |
|:---:|

⬇

| Sample digestion in microwave digester as per programme (Table 1) & volume make up to 20mL |
|:---:|
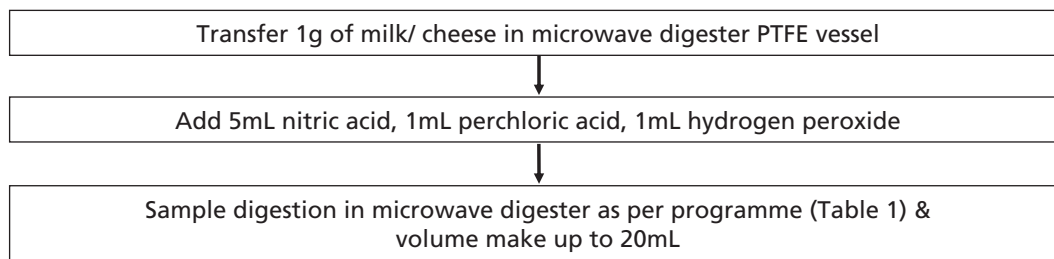
Table 1: Microwave digester programme

| Steps | Ramp (min) | Temp (°C) | Hold time (min) |
|-------|------------|-----------|-----------------|
| 1 | 10 | 100 | 05 |
| 2 | 10 | 150 | 10 |
| 3 | 10 | 180 | 10 |
| 4 | 10 | 200 | 10 |

The extracts obtained were analysed on Shimadzu ICPMS-2030. Analysis was done using plasma generated by specially designed mini torch as an ionization source. ICPMS LabSolutions software and special features like Profile integration time and Total mass measurement were used for identification, detection and quantitation. NIST traceable standards were used for quantification of elements at low level in QuantBase mode (used for quantitation purpose).

## Key features of ICPMS-2030

Inductively coupled plasma mass spectrometer ICPMS-2030 by Shimadzu (shown in Figure 3), sets a new benchmark in ICP-MS technology with minimum consumption of Argon gas. This system ensures highest quality of data, with very high degree of reliability. The newly developed collision cell (shown in Figure 3) based on UFsweeper technology uses high purity Helium gas for removal of polyatomic interferences. The mini-torch design allows the operation of ICPMS at low RF power without compromising the sensitivity.



Figure 2   Shimadzu ICPMS-2030 Inductively coupled plasma mass spectrometer



Figure 3   Newly developed Collision Cell

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# Analysis of toxic elements in processed milk products using ICP-MS

| Analytical Conditions (ICPMS-2030) | |
|---|---|
| Torch | : Mini torch |
| Radiofrequency | : 1.2 kW |
| Sampling depth | : 5 mm |
| Plasma gas (L/min) | : 8.0 |
| Auxiliary gas (L/min) | : 1.1 |
| Carrier gas (L/min) | : 0.7 |
| Cell voltage | : -21 V |
| Cell gas (mL/min) | : 6.0 |
| Energy Filter | : 7V |
| Chamber temp. | : 5 °C |
| Number of scans | : 10 |
| Integration time | : 2sec |
| Peristaltic pump speed | : 60 r.p.m.- High |
|  | 20 r.p.m.- Low |
| Isotopes monitored | = $^{75}$As, $^{111}$Cd, $^{208}$Pb, $^{202}$Hg |
| Internal standard | = $^{89}$Y |

Linearity data for elemental standards in the concentration range of 0.1 µg/L to 10 µg/L for toned milk and cheese is shown in Figure 4 and Figure 5.
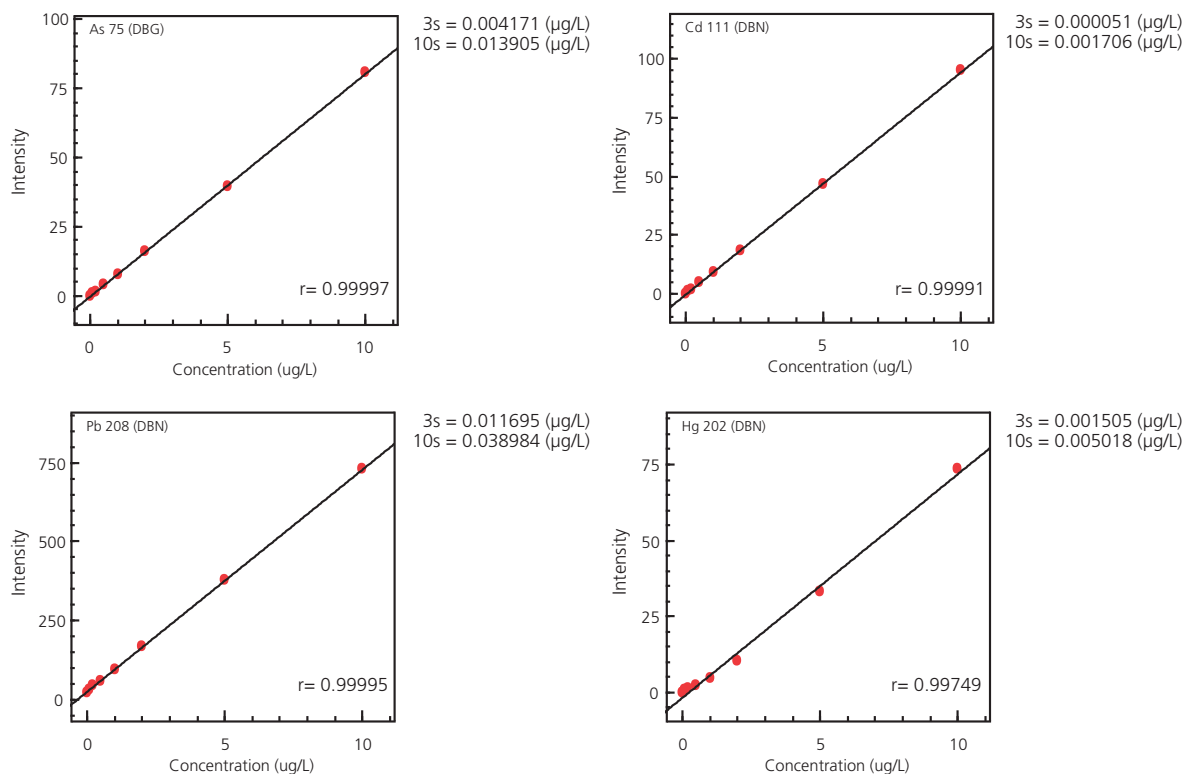


Figure 4   Standard linearity curves for 0.1-10µg/L for Milk

# Analysis of toxic elements in processed milk products using ICP-MS
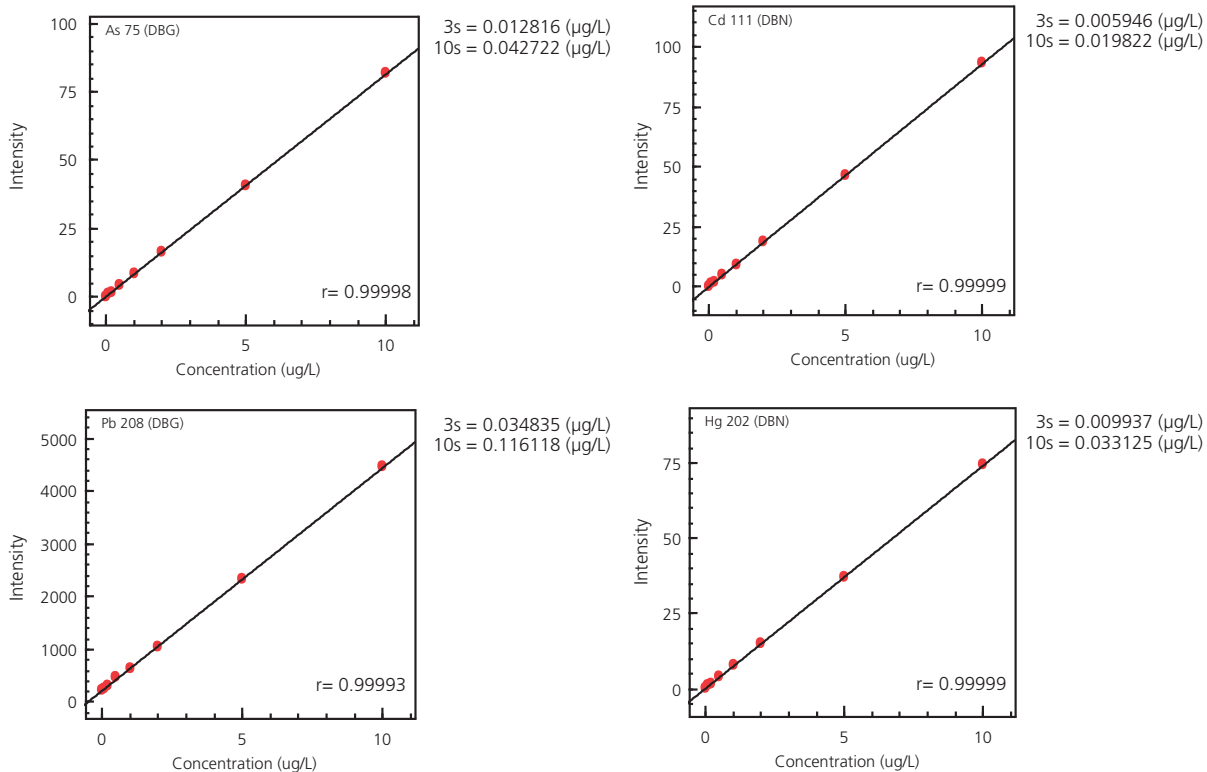


Figure 5   Standard linearity curves for 0.1-10µg/L for Cheese

# Results

Extracts were analysed on ICPMS-2030 in QuanBase mode. The elements were further quantified using calibration curves plotted from analytical standards (Merck). Measurements were made using an off axis Collison cell system which helped to remove neutrals and photon interferences.
The results obtained were evaluated for statistical parameters like accuracy and linearity. Accuracy in terms of recovery was found to be between 70 to 120% for pre-spiked samples. The results showed good linear response with correlation coefficient $\geq 0.995..$ The results obtained for toned milk and cheese are given in Table 1. RSD and % recoveries for toned milk and cheese are shown in Table 2 and Table 3 respectively. The RSD of six replicates was within 7%, showing good precision of the method.

Table 2  Average elemental results obtained for processed milk products (n = 6 replicates)

| Elements | Toned milk (µg/L) | Cheese (µg/L) |
|---|---|---|
| As | Not detected | 5.0 |
| Cd | Not detected | Not detected |
| Pb | 5.6 | Not detected |
| Hg | 1.6 | 1.2 |

Table 3  Average accuracy results at 0.25 µg/L of for toned milk & cheese sample (n = 6 replicates)

| Elements | Toned milk | | Cheese | |
|---|---|---|---|---|
| | %Recovery (Accuracy) | %RSD | %Recovery (Accuracy) | %RSD |
| As | 95-108 | 5.4 | 96-117 | 6.8 |
| Cd | 87-98 | 1.6 | 92-97 | 2.2 |
| Pb | 75-106 | 2.1 | 99 -115 | 6.3 |
| Hg | 100-110 | 0.9 | 101-107 | 2.4 |

# Conclusion

ICPMS-2030 was found to be best technique for the determination of toxic heavy metals at very low concentration. The above methodology proved to be selective, sensitive, accurate and reliable.

Disclaimer: Shimadzu ICPMS-2030 and application in this poster are intended for Research Use Only (RUO). Not for use in diagnostic procedures. Not available in the USA, Canada and China.

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# Application News

## No.J116

Inductively Coupled Plasma Atomic Emission Spectrometry

# Analysis of Nutritional and Harmful Elements in Powdered Milk by ICPE-9820 / HVG-1

## ■ Introduction

Minerals required for infant growth are well balanced in powdered infant formula. According to Japan's Health Promotion Law, a specific formulation of essential minerals, including calcium (Ca), iron (Fe) and copper (Cu), etc., has been established for infant formula as a special-use food, and is required to be displayed on the product label.[1]

However, the potential adverse effects of certain harmful elements such as arsenic (As) on infant growth and development is of utmost concern, accentuating the importance of strict safety management in the production of infant formula from the raw material stage to the finished product.

Here, using the Shimadzu ICPE-9820 simultaneous ICP atomic emission spectrometer, we conducted simultaneous analysis of elements in powdered milk (NMIJ-certified reference material). In addition, high-sensitivity analysis using an HVG-1 hydride vapor generator was conducted for the detection and quantification of the minute levels of arsenic in the sample.

The ICPE-9820 permits analysis using both high-sensitivity axial (AX) direction observation and high-concentration radial (RD) observation, thereby allowing simultaneous analysis of elements present at concentrations ranging from very low to high levels. As for detection of arsenic, the HVG-1 permits detection of As at the several tens ng/L trace level.

## ■ Sample

NMIJ-certified reference material; Trace Elements in Milk Powder (NMIJ CRM 7512-a: No. MI-040)

## ■ Sample Preparation

(1) Acid Digestion
Sample decomposition was conducted using a microwave sample digestion system. Each sample was weighed out to approximately 0.5 g, and digestion was conducted by adding 5 mL nitric acid, 2 mL hydrochloric acid and 1 mL hydrogen peroxide. Table 1 shows the sample digestion conditions. Following sample digestion, 0.5 mL of perchloric acid was added, and after conducting digestion again using the same digestion conditions, the total volume was adjusted to 20 mL using purified water. At this time, yttrium (Y) and indium (In) were added as internal standard elements to the measurement solution to obtain concentrations of 0.5 mg/L for Y, and 5.0 mg/L for In.

**Table 1 Digestion Conditions Using Microwave Digestion System**

| STEP | Temperature (°C) | Time (Minutes) | Power (W) |
|------|------------------|----------------|-----------|
| 1 | 50 | 2 | 1000 |
| 2 | 30 | 3 | 0 |
| 3 | 180 | 25 | 1000 |
| 4 | 150 | 1 | 0 |
| 5 | 180 | 4 | 1000 |
| 6 | 180 | 15 | 1000 |

(2) Pretreatment for High-Sensitivity Analysis of As Using HVG-1
After conducting digestion as described in step (1), the sample was heated (180 °C) to near-dryness on a hot plate. Then, 3 mL hydrochloric acid, 2 mL potassium iodide (200 g/L) and 0.4 mL ascorbic acid (100 g/L)

were added, and the mixture was left standing for 60 minutes. The total volume was then adjusted to 20 mL using purified water.
In addition, as reagents for operation of the HVG-1, 6 M hydrochloric acid solution and sodium borohydride solution were prepared.

(3) Validation
For validation of the analytical values, a spike and recovery test sample spiked with the standard solution containing the trace-level analyte elements (As, Cd, Cr, Pb) was prepared prior to digestion.

## ■ Instrument and Analytical Conditions

Measurement was conducted using the Shimadzu ICPE-9820 simultaneous ICP atomic emission spectrometer and the HVG-1 hydride vapor generator. The typical measurement conditions are shown in Table 2, and the measurement conditions using the HVG-1 are shown in Table 3.

Constituents present at high and trace level concentrations were measured using radial (RD) and high-sensitivity axial (AX) observation, respectively. This all-at-once analysis of both high-concentration components and trace components is possible due to the automatic switching between the radial and axial observation directions featured in the ICPE-9820.

The HVG-1 permits analysis of As with sensitivity that is several hundred times higher than that possible using typical measurement. Moreover, the proprietary design of the gas-liquid separator permits acquisition with stable analytical results over an extended period of time.

**Table 2 Analytical Conditions**

| | |
|---|---|
| Instrument | : ICPE-9820 |
| Radio Frequency Power | : 1.20 kW |
| Plasma Gas Flowrate | : 10.0 L/min |
| Auxiliary Gas Flowrate | : 0.60 L/min |
| Carrier Gas Flowrate | : 0.70 L/min |
| Sample Introduction | : Nebulizer 10 |
| Misting Chamber | : Cyclone chamber |
| Plasma Torch | : Mini torch |
| Observation | : Axial (AX) / Radial (RD) |

**Table 3 Analytical Conditions (HVG-1)**

| | |
|---|---|
| Instrument | : ICPE-9820, HVG-1 |
| Radio Frequency Power | : 1.20 kW |
| Plasma Gas Flowrate | : 10.0 L/min |
| Auxiliary Gas Flowrate | : 0.60 L/min |
| Carrier Gas Flowrate | : 0.80 L/min |
| Plasma Torch | : Mini torch |

## ■ Analysis

The calibration curve method (internal standard method) was used to conduct simultaneous analysis of the minerals and harmful elements in powdered milk.

[Reference]
1) Allowable Standard for Component Composition and Display of Breast Milk and Infant Formula (published by Japan's Ministry of Health, Labour and Welfare)

■ **Analytical Results**

The analytical results are shown in Table 4. The results for the mineral elements were within the certification range, and good spike and recovery test results were obtained for the trace level toxic elements.

Table 5 shows the analysis result and spike and recovery test result for As using the HVG-1. The As detection limit in aqueous solution was 0.04 µg/L, and in powder, 2 µg/L. As for the spike and recovery test, excellent result of 99 % was obtained.

Fig. 1 shows the results of continuous analysis of a standard solution of As over a 4-hour period. The relative standard deviation (RSD) was 1.6 %, demonstrating stable results over an extended period.

■ **Conclusion**

These results demonstrate that the ICPE-9820 can be used for simultaneous analysis of the elements in powdered milk, from the minerals present at high concentrations to the toxic substances present at trace levels. Further, in combination with the HVG-1, measurement of As at trace levels is also possible.

**Table 4  Analytical Results for Powdered Milk (NMIJ CRM 7512-a)**

| Element | Unit | Analytical Value in Powder | NMIJ-Certified Value | Expanded Uncertainty | Detection Limit in Powder (DL: 3$\sigma$) | Analytical Value in Measurement Solution mg/L | Spike Concentration mg/L | Spike and Recovery % | Detection Limit in Measurement Solution (DL: 3$\sigma$) mg/L |
|---|---|---|---|---|---|---|---|---|---|
| Ca | g/kg | 8.63 | 8.65 | 0.38 | 0.0000002 | 211 | - | - | 0.000005 |
| Fe | | 0.100 | 0.104 | 0.007 | 0.000006 | 2.45 | - | - | 0.0001 |
| K | | 8.66 | 8.41 | 0.33 | 0.00002 | 215 | - | - | 0.0004 |
| Mg | | 0.838 | 0.819 | 0.024 | 0.0000002 | 20.5 | - | - | 0.000005 |
| Na | | 1.78 | 1.87 | 0.09 | 0.00001 | 50.2 | - | - | 0.0003 |
| P | | 5.52 | 5.62 | 0.23 | 0.0002 | 135 | - | - | 0.005 |
| Cu | mg/kg | 4.70 | 4.66 | 0.23 | 0.02 | 0.115 | - | - | 0.0005 |
| Mn | | 0.957 | 0.931 | 0.032 | 0.002 | 0.023 | - | - | 0.00005 |
| Mo | | 0.229 | 0.223 | 0.012 | 0.02 | 0.006 | - | - | 0.0006 |
| Sr | | 5.89 | 5.88 | 0.20 | 0.0008 | 0.144 | - | - | 0.00002 |
| Zn | | 40.9 | 41.3 | 1.4 | 0.01 | 1.00 | - | - | 0.0003 |
| Cd | µg/kg | <DL | - | - | 10 | <DL | 0.5 | 98 | 0.0002 |
| Cr | | <DL | - | - | 15 | <DL | 0.5 | 100 | 0.0004 |
| Pb | | <DL | - | - | 97 | <DL | 0.5 | 100 | 0.002 |

<DL: Below detection limit (3$\sigma$) (concentration in measurement solution)

Spike and Recover Rate (%) = (Analytical value of spike-and-recovery test solution – Analytical Value) / Spike Concentration × 100

**Table 5  Results of Analysis of As in Powdered Milk Using HVG-1 and Results of Spike and Recovery Test**

| Element | Unit | Analytical Value in Powder | NMIJ Reference Value | Detection Limit in Powder (DL: 3$\sigma$) | Analytical Value in Measurement Solution µg/L | Spike Concentration µg/L | Spike and Recovery % | Detection Limit in Measurement Solution (DL: 3$\sigma$) µg/L |
|---|---|---|---|---|---|---|---|---|
| As | µg/kg | (2.5) | 2.1 | 2 | (0.06) | 4 | 99 | 0.04 |

Reference values in (  ) indicate value greater than detection limit, and value less than the lower limit of quantitation.
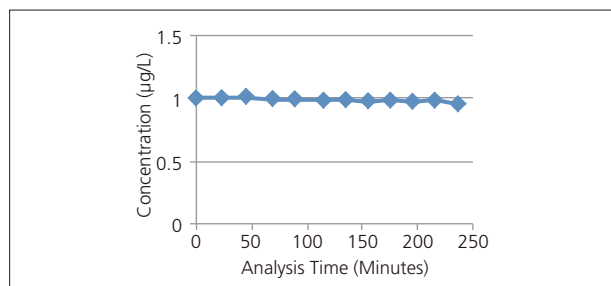


**Fig. 1  Long Term Stability of As Value Using HVG-1**

Continuous measurement of As 1 µg/L solution (10 % HCl-based) measured continuously every 20 minutes for four hours.
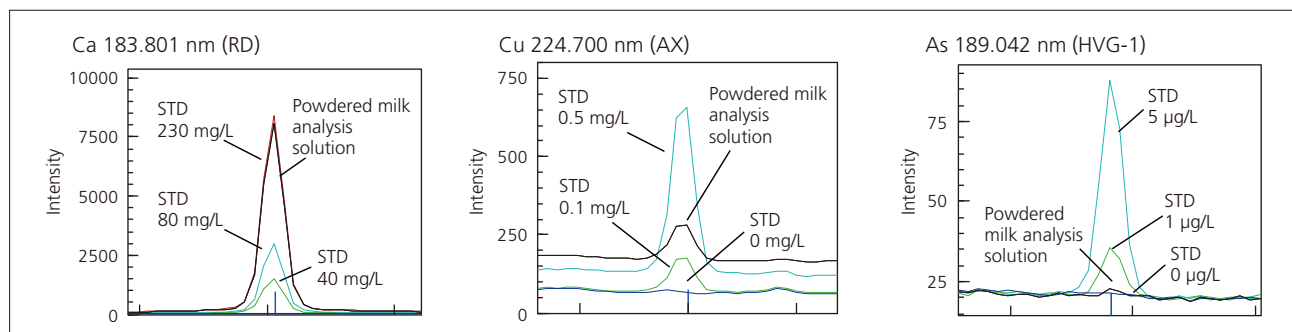


**Fig. 2  Spectral Profiles of Ca , Cu, and As**

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# Grain and Edible Oil

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# Determination of Fatty Acid Esters of 2- and 3-Monochloro-1,2-propanediol (MCPD) and Glycidol in Edible Oil Using GC/Triple Quadrupole MS

**ASMS 2017**

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Determination of Fatty Acid Esters of 2- and
3-Monochloro-1,2-propanediol (MCPD) and Glycidol
in Edible Oil Using GC/Triple Quadrupole MS

# Introduction

Esterified and free forms of 2-MCPD, 3-MCPD and glycidol (Figure 1) are heat-induced contaminants found in various types of processed food.[1] Following ingestion, esterified forms of these compounds are metabolised to the respective free forms which have been associated with a range of toxicities such as carcinogenicity and nephrotoxicity.

Various studies have investigated the levels of these contaminants in oils/fats from different sources and mitigation strategies are currently under development. Well-validated analytical methods are critical for these studies and currently, the American Oil Chemists' Society (AOCS) has adopted three methods for the analysis of

these contaminants in edible oils and fats using gas chromatography-mass spectrometry.[2]
However, to support future studies where higher sensitivity and selectivity are necessary, analytical solutions based on triple-quadrupole mass spectrometer will be required. In the present study, a novel gas chromatography-triple quadrupole mass spectrometry (GC/TQMS) method was developed and validated for the simultaneous analysis of 2-MCPD, 3-MCPD and glycidyl fatty acid esters in edible oil. Subsequently, this method was applied for the quantitation of these contaminants in commercial edible oil samples.
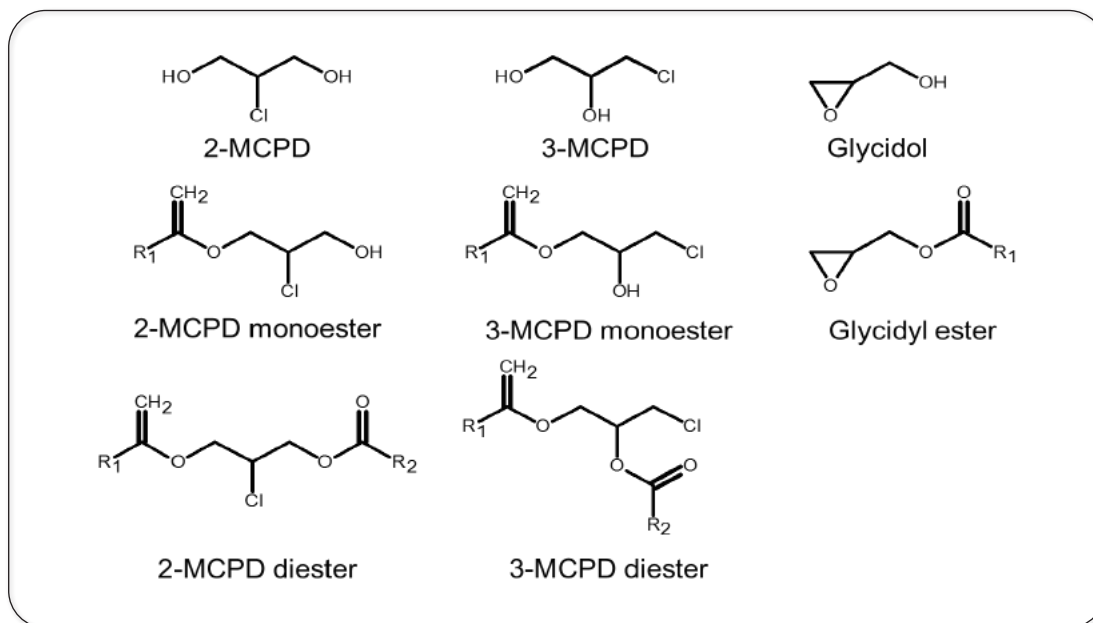


Figure 1. Structures of free and bound forms of 2-MCPD, 3-MCPD and glycidol.

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# Methods and Materials

Fatty acid esters of 2-MCPD, 3-MCPD and glycidol in oil were extracted and derivatised using phenylboronic acid according to the validated AOCS Official Method Cd 29a-13.[2] The analyte standards used were 1,3-dipalmitoyl-2-chloropropanediol (PP-2-MCPD), 1,2-dipalmitoyl-3-chloropropanediol (PP-3-MCPD) and glycidyl palmitate (GlyP) (Toronto Research Chemical, Ontario, Canada).
The internal standards were 1,2-dipalmitoyl-3-chloropropanediol-d5 (PP-2-MCPD-d5) (Santa Cruz Biotechnology, and glycidyl palmitate-d5 (GlyP-d5) (Toronto Research Chemical, Ontario, Canada).

Cold-pressed extra virgin olive oil was used as blank matrix. For simplicity, the target analytes in this analysis are named as 2-MCPD, 3-MCPD and glycidol although the actual analytes are the derivatives.
The extracts were analysed on a GC/TQMS system (GCMS-TQ8040, Shimadzu Corporation, Japan). Separation was performed using a 30 m × 0.25 mm × 1.0 µm capillary column (SH-Rxi-1MS, Shimadzu Corporation, Japan). Detailed instrumental conditions are presented in Table 1 and MRM parameters for the different analytes are shown in Table 2.

Table 1. Instrumental conditions used for analysis

| Parameter | Setting |
|---|---|
| **GC conditions** | |
| Injection mode/volume | : Splitless/0.5 µL |
| Injector temperature | : 250 °C |
| Flow control mode | : Pressure |
| Pressure | : 49.7 kPa |
| Oven temperature programme | : 80 °C (1 min) → 10 °C/min to 170 °C (5 min) → 3 °C/min to 200 °C → 15 °C/min to 300 °C → 300 °C (15 min) |
| **MS conditions** | |
| Interface temperature | : 300 °C |
| Ion source temperature | : 230 °C |

Table 2. MRM parameters used in analysis

| ISP | Start Time (min) | End Time (min) | Mode | Event Time (s) | Ch1 m/z | Ch1 CE | Ch2 m/z | Ch2 CE | Ch3 m/z | Ch3 CE | Ch4 m/z | Ch4 CE | Ch5 m/z | Ch5 CE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3-MCPD-d5 | 17.0 | 19.2 | MRM | 0.167 | 150.00>93.10 | 15 | 201.00>150.20 | 10 | 201.00>93.20 | 30 | 203.00>150.10 | 10 | 203.00>93.20 | 25 |
| 3-MCPD | 17.0 | 19.2 | MRM | 0.133 | 147.00>91.10 | 15 | 196.05>91.20 | 25 | 147.00>65.10 | 25 | 198.10>147.20 | 15 | - | - |
| 2-MCPD | 19.2 | 21.2 | MRM | 0.300 | 196.00>104.10 | 20 | 198.00>104.10 | 20 | 196.00>91.20 | 10 | 196.00>62.00 | 25 | 198.00>91.10 | 10 |
| Glycidol-d5 | 21.2 | 23.0 | MRM | 0.167 | 150.00>93.10 | 15 | 245.00>150.10 | 10 | 247.00>150.10 | 10 | 245.00>93.10 | 25 | 247.00>93.10 | 25 |
| Glycidol | 21.2 | 23.0 | MRM | 0.133 | 242.00>147.10 | 10 | 240.00>147.10 | 15 | 240.00>91.20 | 30 | 242.00>91.10 | 25 | - | - |

# Results

MRM chromatograms of the analytes (using target MRM transitions) showed good peak shapes (Figure 2). The retention times of 3-MCPD-d5, 3-MCPD, 2-MCPD, glycidol-d5 and glycidol were approximately 18.4 min, 18.6 min, 19.6 min, 21.6 min and 21.7 min respectively.
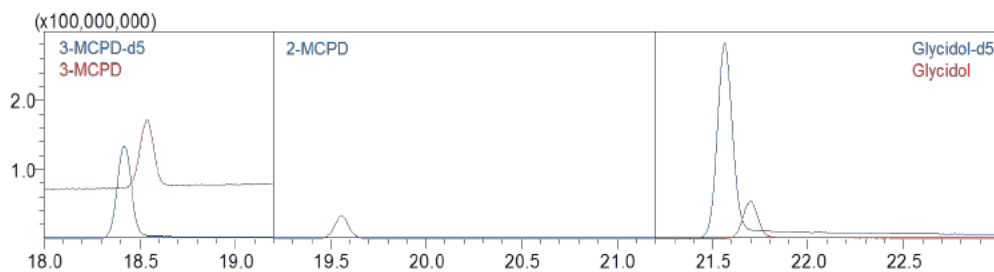


Figure 2. Representative MRM chromatogram where 3-MCPD-d5, 3-MCPD, 2-MCPD, glycidol-d5 and glycidol were eluted at 18.4 min, 18.6 min, 19.6 min, 21.6 min and 21.7 min, respectively.

Method validation was carried out to assess parameters such as sensitivity, accuracy, precision, linearity and repeatability using calibration standards or quality control (QC) sample. Subsequently, this method was applied for the quantitation of the different analytes in commercially available edible oil samples

Sensitivity
The sensitivity of the assay was examined by measuring the signal to noise (S/N) ratio of the analyte peaks. The was defined as S/N ratio of at least 5. The LOD (limit of detection) for this method was 0.003 µg of 2-MCPD and 3-MCPD and 0.006 µg of glycidol. The limit of quantitation (LOQ) was defined as S/N ratio of at least 10. The LOQ of this method was 0.01 µg of 2-MCPD and 3-MCPD and 0.024 µg of glycidol.

Linearity
Eight calibration standards ranging from 2-MCPD and 3-MCPD: 0.010 µg, glycidol: 0.024 µg to 2-MCPD and 3-MCPD: 0.930 µg, glycidol: 2.130 µg were used to construct the calibration curves. The calibration curves (Figure 3) for all analytes showed excellent linearity ($R^2 > 0.998$) (n = 6).

Repeatability
Excellent repeatability of the peak areas of consecutive injections were achieved for all analytes (< 3%) (n = 6).

Accuracy and Precision
QC samples at three concentrations (QC1, QC2 and QC3) were used to investigate the accuracy and precision of the method. High accuracy and precision were demonstrated for this method as the accuracy of the QC samples were all within 100 ± 7 % and the %RSD were all < 10% (n = 6).

Application of method for quantitation of analytes in commercially available edible oil samples
The validated method was applied for the quantitation of esters of 2-MCPD, 3-MCPD and glycidol in edible oil samples from different sources (Table 3, Figure 4). Generally, samples containing palm oil were found to contain the highest levels of the contaminants.

# Determination of Fatty Acid Esters of 2- and 3-Monochloro-1,2-propanediol (MCPD) and Glycidol in Edible Oil Using GC/Triple Quadrupole MS



**2-MCPD**     **3-MCPD**     **Glycidol**
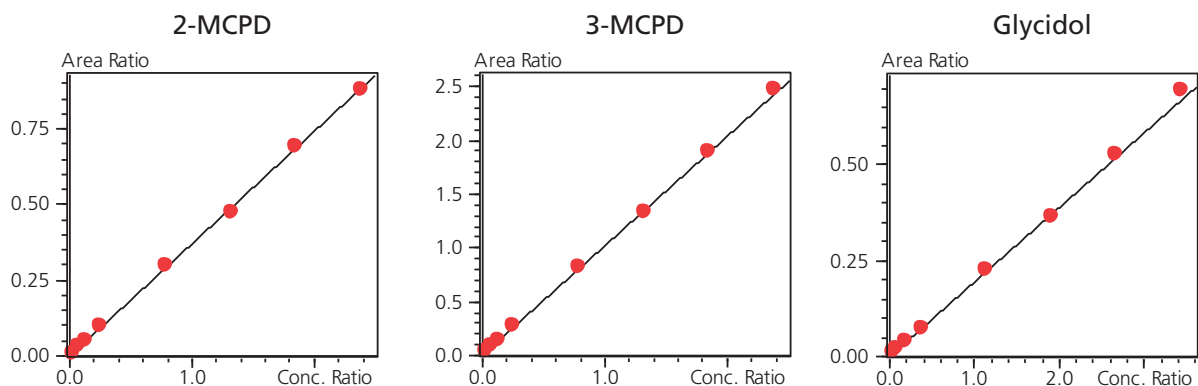
Figure 3. Calibration curves for the analytes 2-MCPD, 3-MCPD and glycidol where the x axis is the concentration ratio and y axis is the peak area ratio between analyte and IS peak areas.

Table 3. Concentration of contaminants in commercially available edible oil samples

| Sample | Analyte Concentration (ppm) (n=3) | | |
|---|---|---|---|
| | 2-MCPD | 3-MCPD | Glycidol |
| Kenaf seed oil | 0.173 ± 0.007 | 0.354 ± 0.008 | 0.367 ± 0.003 |
| Mustard oil | < LOQ | < LOQ | < LOQ |
| Olive oil | < LOQ | < LOQ | < LOQ |
| Palm oil A | 2.820 ± 0.062 | 5.313 ± 0.032 | 8.813 ± 0.131 |
| Palm oil B | 0.653 ± 0.032 | 1.145 ± 0.023 | 5.891 ± 0.032 |
| Peanut oil | 0.491 ± 0.008 | 0.898 ± 0050 | 0.589 ± 0.016 |
| Vegetable cooking oil* | 1.346 ± 0.0182 | 2.804 ± 0.061 | 4.273 ± 0.046 |

*Vegetable cooking oil contains palm olein and soyabean oil
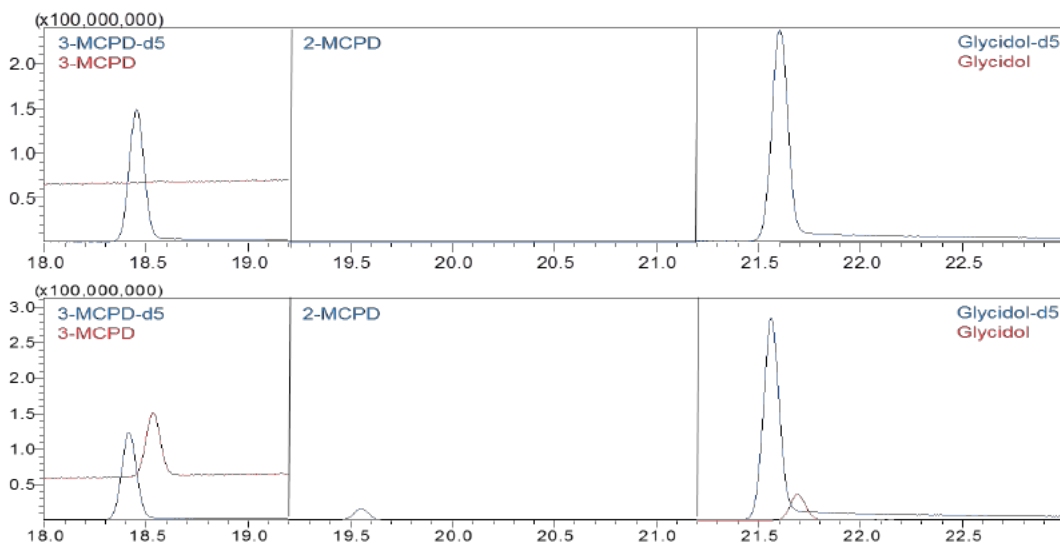


Figure 4. Representative MRM chromatograms of olive oil (top) and kenaf seed oil (bottom) shown below.

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Determination of Fatty Acid Esters of 2- and
3-Monochloro-1,2-propanediol (MCPD) and Glycidol
in Edible Oil Using GC/Triple Quadrupole MS

# Conclusions

In summary, a novel GC/TQMS method was developed and validated for the simultaneous analysis of 2- and 3-MCPD and glycidol fatty acid esters in edible oil. This method showed more than 3-fold improvement in sensitivity compared to the official method currently available and demonstrated excellent linearity, repeatability, accuracy and precision. Application of this method to the analyses of commercially available edible oil samples confirmed that samples containing palm oil show higher levels of contaminants.

# References

[1] Federation for European Oil and Proteinmeal Industry. 2016. FEDIOL Q&A on 2- and 3-MCPD and Their Esters and Glycidyl Esters
[2] The American Oil Chemists' Society. 2013. 2- and 3-MCPD Fatty Acid Esters and Glycidol Fatty Acid Esters in Edible Oils and Fats by Acid Transesterification. AOCS Official Method Cd29a-13, Cd29b-13 and Cd29c-13

Disclaimer: The products and applications in this poster are intended for Research Use Only (RUO). Not for use in diagnostic procedures.

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# SHIMADZU
## Excellence in Science

**Application Data Sheet**

**No.106**

## GC-MS
Gas Chromatograph Mass Spectrometer

# Simultaneous Analysis of 477 Residual Pesticides in Agricultural Crops Using GC-MS/MS - Part 1

GC-MS/MS systems can measure more than 400 residual pesticides in foods. However, analyzing more than 400 pesticides simultaneously requires a short dwell time (data loading time) during MRM measurements, which results in problems with inadequate sensitivity and the tedious process of creating MRM measurement programs. Consequently, several different methods are used for target pesticides and the same sample is measured multiple times to analyze all components. That can decrease productivity, due to the time required for analyzing all the components involved in the large number of pesticides being inspected. This Application Data Sheet describes a solution to these problems with the creation of a method for simultaneously analyzing 477 components and evaluating the resulting sensitivity and accuracy.

## Experiment

Matrix solutions were prepared by processing soy bean, orange, brown rice, and spinach samples according to a pretreatment procedure for residual pesticide analysis, and then purifying them using the GPC Cleanup System (from Shimadzu Corporation).[1] Measurement sample solutions (1 g/mL sample concentration) were then prepared by spiking the prepared matrix solutions with 477 components (including internal standard substances) to a concentration of 5 ppb (or 200 ppb for the internal standard substances). 19 kinds[*1] of surrogate pesticides were used as the internal standard substances.

The GCMS-TQ8040 combined with the Twin Line MS System was used to measure samples based on the analytical conditions listed in Table 1. Two transitions were specified for each component, one for quantitation and the other for confirmation, and Smart MRM was used to automatically create a measurement program.

Table 1: Analysis Conditions

| | |
|---|---|
| GC-MS: | GCMS-TQ8040 (Twin Line MS System) |
| Column 1: | SH-Rxi-5Sil MS (30m L., 0.25 mm I.D., df=0.25 μm) (Shimadzu, P/N: 221-75954-30) |
| Column 2: | SH-Rtx-200 MS (30m L., 0.25 mm I.D., df=0.25 μm) (Shimadzu, P/N: 221-75811-30) |
| Glass Insert : | Sky Liner, Splitless Single Taper Gooseneck w/Wool (Restek, P/N: 567366) |

**[GC]**

| | |
|---|---|
| Injection Temp.: | 250 °C |
| Column Oven Temp.: | 60 °C (1 min) → (25 °C /min) → 160 °C → (4 °C /min) → 240 °C → (10 °C /min) → 290 °C (11 min) |
| Injection Mode: | Splitless |
| High Pressure Injection: | 250 kPa (1.5 min) |
| Carrier Gas Control: | Linear Velocity (40.0 cm/sec) |
| Injection Volume: | 2 μL |

**[MS]**

| | |
|---|---|
| Interface Temp.: | 300 °C |
| Ion Source Temp.: | 200 °C |
| Measurement Mode: | MRM |
| Loop Time: | 0.4 sec |
| Processing Time Required: | 0.3 min |

## Analysis Results

The relationship between the dwell time and retention time in the measurement program created using Smart MRM is shown in Fig. 1. The average dwell time for all components was 12.3 msec, with over 6.5 msec provided even for retention time bands where a high number of pesticides were eluted. Consequently, compared to conventional measurement methods that divide analysis into segments, Smart MRM provides, on average, 2.5 time longer dwell times and makes it easy to create optimal MRM measurement programs.
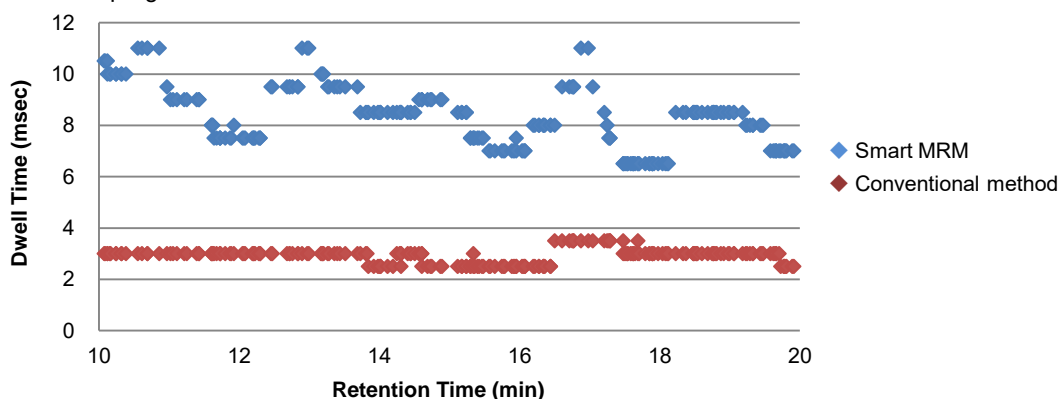


Fig. 1 Relationship Between Retention Time and Dwell Time (for retention times from 10 to 20 minutes)

1) E. Ueno, et al., *J. AOAC INT.* **87**, (2004) 1003-1015

*1 Dichlorvos-$d_6$, acephate-$d_6$, diazinon-$d_{10}$, iprobenfos-$d_7$, carbaryl-$d_7$, fenitrothion-$d_6$, linuron-$d_6$, metolachlor-$d_6$, chlorpyrifos-$d_{10}$, diethofencarb-$d_7$, fosthiazate-$d_5$, pendimethalin-$d_5$, thiabendazole-$^{13}C_6$, imazalil-$d_5$, isoprothiolane-$d_4$, isoxathion-$d_{10}$, EPN-$d_5$, etofenprox-$d_5$, and esfenvalerate-$d_7$

The repeatability for each matrix was used to evaluate whether the measurement program created using Smart MRM provided adequate sensitivity. The %RSD distribution obtained for each matrix is shown in Fig. 2 and the %RSD values for 100 of the 477 components are tabulated in Table 2. These results show that %RSD (n = 5) was 10 % or less for 88 % of targets (1618 of the 1832 components in four types of matrix), which indicates that high analytical accuracy was achieved when analyzing as many as 477 components simultaneously. By eliminating the need to split the analysis using multiple methods, the number of injections is reduced and productivity increased. This also allows maintenance frequency and costs to be minimized.

Though matrix interference was identified for a few components, high-accuracy detection was possible by using the Twin Line MS system, which uses two columns with different separation characteristics. For information regarding the Twin Line MS System, refer to Application Data Sheet 107.
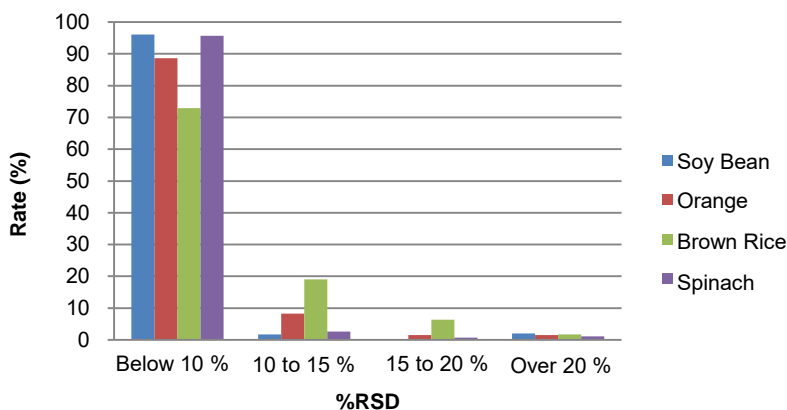


Fig. 2 %RSD Distribution for Each Matrix

Table 2: %RSD (n = 5) of Samples Spiked with Pesticides (5 ppb)

| Name of Compound | Soy Bean | Orange | Brown Rice | Spinach | Name of Compound | Soy Beas | Orange | Brown Rice | Spinach | Name of Compound | Soy Bean | Orange | Brown Rice | Spinach |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Methamidophos | 4.82 | 7.66 | 8.84 | 1.86 | Fthalide | 6.92 | 5.01 | 12.09 | 4.79 | Trifloxystrobin | 6.80 | 8.83 | 8.84 | 5.89 |
| Acephate | 4.72 | 4.29 | 4.85 | 6.89 | Fosthiazate-1 | 9.53 | 4.33 | 14.06 | 3.16 | Tebuconazole | 6.63 | 4.46 | 9.34 | 5.18 |
| Propham | 4.55 | 3.84 | 15.43 | 3.13 | Fosthiazate-2 | 7.90 | 3.44 | 15.15 | 8.60 | Piperonyl butoxide | 4.41 | 3.91 | 9.63 | 2.78 |
| Clothianidin | 3.66 | 4.84 | 7.53 | 2.92 | Pendimethalin | 9.80 | 5.24 | 9.81 | 6.47 | Acetamiprid | 7.56 | 7.09 | 8.10 | 3.44 |
| Chloroneb | 4.35 | 2.74 | 12.91 | 5.54 | Fipronil | 9.68 | 11.76 | 9.33 | 9.72 | Iprodione | 8.26 | 3.90 | 9.09 | 3.65 |
| Fenobcarb | 2.95 | 3.02 | 7.72 | 2.14 | Heptachlor-exo-epoxide | 5.92 | 13.72 | 8.89 | 7.93 | EPN | 7.68 | 9.03 | 5.85 | 7.96 |
| Phorate | 6.20 | 4.87 | 10.62 | 4.72 | Thiabendazole | 4.22 | 3.90 | 9.34 | 5.03 | Bromopropylate | 4.58 | 3.65 | 9.49 | 3.71 |
| Dimethoate | 7.03 | 5.21 | 8.75 | 6.91 | Captan | 14.99 | 3.41 | 5.66 | 10.28 | Bifenthrin | 4.52 | 2.87 | 8.77 | 2.67 |
| gamma-BHC | 9.73 | 3.38 | 9.13 | 7.18 | Phenthoate | 8.96 | 1.92 | 9.77 | 5.60 | Bifenazate | 9.05 | 7.67 | 9.58 | 6.96 |
| Cyanophos | 5.89 | 3.82 | 8.64 | 4.01 | Quinalphos | 6.63 | 5.12 | 8.38 | 7.46 | Fenpropathrin | 8.53 | 4.96 | 9.79 | 9.01 |
| Terbufos | 2.89 | 4.37 | 7.94 | 5.04 | Procymidone | 4.31 | 5.49 | 12.58 | 5.87 | Tebufenpyrad | 3.66 | 4.18 | 9.73 | 2.88 |
| Diazinon | 8.13 | 4.68 | 9.35 | 7.42 | Triflumizole | 7.71 | 7.73 | 7.93 | 8.74 | Tetradifon | 8.47 | 4.02 | 8.83 | 7.17 |
| Pyrimethanil | 2.80 | 3.38 | 8.13 | 5.52 | Chinomethionat | 7.98 | 4.48 | 11.60 | 1.82 | Azinphos-methyl | 7.95 | 8.06 | 8.35 | 5.72 |
| Iprobenfos | 3.77 | 3.83 | 12.89 | 3.25 | Trichlamide | 7.78 | 3.23 | 9.93 | 5.82 | Pyriproxyfen | 4.25 | 6.00 | 5.30 | 3.39 |
| Benoxacor | 7.31 | 1.86 | 8.91 | 4.25 | Butachlor | 9.05 | 5.75 | 8.79 | 5.25 | Fenarimol | 1.45 | 4.13 | 9.64 | 2.72 |
| Acetochlor | 6.74 | 6.94 | 8.74 | 3.01 | Alpha-endosulfan | 8.92 | 3.48 | 9.39 | 3.12 | Acrinathrin | 5.27 | 9.02 | 8.37 | 8.17 |
| Parathion methyl | 7.86 | 4.91 | 7.77 | 3.41 | Mepanipyrim | 4.63 | 3.89 | 9.55 | 3.77 | Coumaphos | 5.15 | 6.18 | 7.79 | 4.18 |
| Tolclofos-methyl | 8.51 | 7.87 | 8.79 | 1.95 | Hexaconazole | 5.49 | 8.17 | 8.81 | 5.20 | Pyridaben | 6.42 | 3.16 | 7.25 | 1.59 |
| Carbaryl | 4.44 | 8.21 | 8.83 | 6.73 | Imazalil | 8.84 | 5.09 | 8.01 | 4.24 | Cypermethrin-1 | 8.23 | 8.70 | 7.71 | 1.42 |
| Heptachlor | 7.92 | 3.29 | 8.59 | 4.05 | Flutolanil | 4.88 | 3.61 | 9.69 | 1.93 | Boscalid | 5.29 | 14.34 | 9.02 | 3.51 |
| Metalaxyl | 2.88 | 6.82 | 14.92 | 5.22 | Prothiofos | 9.31 | 4.77 | 10.21 | 4.80 | Cypermethrin-2 | 8.68 | 5.80 | 8.49 | 7.71 |
| Prometryn | 4.48 | 5.90 | 8.83 | 7.87 | Isoprothiolane | 3.65 | 4.46 | 8.04 | 5.86 | Cypermethrin-3 | 9.28 | 5.31 | 8.79 | 5.44 |
| Pirimiphos-methyl | 7.24 | 9.41 | 9.11 | 6.64 | Dieldrin | 9.55 | 8.16 | 9.39 | 6.59 | Cypermethrin-4 | 4.59 | 12.36 | 2.67 | 7.80 |
| Fenitrothion | 9.87 | 6.55 | 5.77 | 7.20 | Myclobutanil | 4.80 | 5.72 | 9.55 | 2.11 | Ethofenprox | 4.72 | 7.17 | 7.04 | 3.51 |
| Linuron | 7.87 | 6.27 | 13.16 | 4.65 | o,p'-DDD | 5.51 | 3.71 | 11.30 | 3.02 | Silafluofen | 3.09 | 10.17 | 8.81 | 2.84 |
| Malathion | 9.97 | 7.47 | 7.37 | 2.98 | Flusilazole | 7.51 | 7.35 | 8.85 | 5.56 | Fenvalerate-1 | 8.28 | 14.86 | 9.21 | 6.41 |
| Metolachlor | 3.77 | 3.78 | 12.20 | 4.78 | Kresoxim-methyl | 6.77 | 6.34 | 13.15 | 3.58 | Fenvalerate-2 | 8.60 | 16.74 | 8.23 | 4.30 |
| Chlorpyrifos | 7.22 | 3.28 | 9.78 | 6.08 | Chlorfenapyr | 10.54 | 7.30 | 5.37 | 7.93 | Difenoconazole-1 | 1.52 | 9.27 | 7.86 | 2.83 |
| Thiobencarb | 7.77 | 2.08 | 9.59 | 4.36 | Isoxathion | 9.10 | 7.85 | 12.21 | 9.12 | Difenoconazole-2 | 5.84 | 9.25 | 7.16 | 7.27 |
| Diethofencarb | 5.44 | 4.17 | 12.25 | 6.75 | Beta-endosulfan | 8.66 | 8.25 | 12.65 | 4.06 | Azoxystrobin | 5.01 | 4.22 | 4.54 | 5.80 |
| Fenthion | 4.11 | 5.06 | 9.33 | 5.36 | Ethion | 5.81 | 4.81 | 9.01 | 4.22 | | | | | |
| Parathion | 7.43 | 8.93 | 9.05 | 5.05 | Triazophos | 6.42 | 4.64 | 8.64 | 2.63 | **Among Total of 458 Components*2** | | | | |
| Triadimefon | 5.66 | 7.30 | 9.52 | 9.15 | Edifenphos | 7.40 | 6.61 | 9.89 | 7.70 | Number of Components with 10 % or Lower | 440 | 406 | 334 | 438 |
| Tetraconazole | 9.01 | 8.56 | 9.70 | 6.59 | Endosulfan sulfate | 8.23 | 4.19 | 7.14 | 5.54 | Average %RSD (excluding N.D.) | 6.62 | 6.46 | 9.90 | 5.55 |
| Dicofol degradation products | 4.91 | 3.99 | 11.87 | 7.33 | Quinoxyfen | 4.23 | 8.59 | 12.91 | 2.30 | | | | | |

Items determined to have 20 % or more overlap (area values) between pesticide-spiked and blank samples are underlined (reference data).
*2   Excludes the 19 internal standard substances.

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# SHIMADZU
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**Application Data Sheet**

**No. 107**

## GC-MS
Gas Chromatograph Mass Spectrometer

# Simultaneous Analysis of 477 Residual Pesticides in Agricultural Crops Using GC-MS/MS - Part 2

Application Data Sheet No. 106 showed that it is possible to simultaneously analyze 477 components with high sensitivity and high accuracy by using a measurement program created using Smart MRM. However, there were still cases where matrix interference was unavoidable even when using highly selective MRM analysis. Therefore, this Application Data Sheet presents results from analysis using two columns with different separation characteristics: a general-purpose 5 % phenyl / 95 % methylpolysiloxane column and a trifluoropropyl methyl polysiloxane column.

By using the Twin Line MS System, both of these columns can be installed in the same GC-MS/MS system at the same time for continuous analysis without having to release the vacuum or replace columns.

## Experiment

Matrix solutions were prepared by processing soy bean, orange, brown rice, and spinach samples according to a pretreatment procedure for residual pesticide analysis, and then purifying them using the GPC Cleanup System (from Shimadzu Corporation).[1] Measurement sample solutions (1 g/mL sample concentration) were then prepared by spiking the prepared matrix solutions with 477 components (including internal standard substances) to a concentration of 5 ppb (or 200 ppb for the internal standard substances). 19 kinds[*1] of surrogate pesticides were used as the internal standard substances.

The GCMS-TQ8040 combined with the Twin Line MS System was used to measure samples based on the analytical conditions listed in Table 1. Two transitions were specified for each component, one for quantitation and the other for confirmation, and Smart MRM was used to automatically create a measurement program.

Table 1: Analysis Conditions

| | |
|---|---|
| GC-MS: | GCMS-TQ8040 (Twin Line MS System) |
| Column 1: | SH-Rxi-5Sil MS (30 m L., 0.25 mm I.D., df=0.25 μm) (Shimadzu, P/N: 221-75954-30) |
| Column 2: | SH-Rtx-200 MS (30 m L., 0.25 mm I.D., df=0.25 μm) (Shimadzu, P/N: 221-75811-30) |
| Glass Insert : | Sky Liner, Splitless Single Taper Gooseneck w/Wool (Restek, P/N: 567366) |

**[GC]**

| | |
|---|---|
| Injection Temp.: | 250 °C |
| Column Oven Temp.: | 60 °C (1 min) → (25 °C /min) → 160 °C → (4 °C /min) → 240 °C → (10 °C /min) → 290 °C (11 min) |
| Injection Mode: | Splitless |
| High Pressure Injection: | 250 kPa (1.5 min) |
| Carrier Gas Control: | Linear Velocity (40.0 cm/sec) |
| Injection Volume: | 2 μL |

**[MS]**

| | |
|---|---|
| Interface Temp.: | 300 °C |
| Ion Source Temp.: | 200 °C |
| Measurement Mode: | MRM |
| Loop Time: | 0.4 sec |
| Processing Time Required: | 0.3 min |



Fig. 1: GCMS-TQ8040 with Twin Line MS System

## Analysis Results

Results from analysis using columns 1 and 2 are shown in Figs. 2 and 3. Due to matrix interference, some pesticide peaks cannot be detected properly with column 1, but using column 2 allows separation of the matrix and results in accurate detection. Furthermore, high-precision analytical results can be obtained even when using column 2.

If a peak is detected in data from column 1, then the data from column 2 can be used to confirm that the peak is from a pesticide.

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1) E. Ueno, et al., *J. AOAC INT.* **87**, (2004) 1003-1015
*1 Dichlorvos-$d_6$, acephate-$d_6$, diazinon-$d_{10}$, iprobenfos-$d_7$, carbaryl-$d_7$, fenitrothion-$d_6$, linuron-$d_6$, metolachlor-$d_6$, chlorpyrifos-$d_{10}$, diethofencarb-$d_7$, fosthiazate-$d_5$, pendimethalin-$d_5$, thiabendazole-$^{13}C_6$, imazalil-$d_5$, isoprothiolane-$d_4$, isoxathion-$d_{10}$, EPN-$d_5$, etofenprox-$d_5$, and esfenvalerate-$d_7$

Column 1 (SH-Rxi-5Sil MS)

Column 2 (SH-Rtx-200 MS)

(×1,000,000)

1.00

0.75

0.50

0.25

206.00 > 191.00
206.00 > 141.00

Matrix Blank

7.75　　8.00　　8.25　　8.50

(×10,000)

2.5

2.0

1.5

1.0

0.5

0.0

206.00 > 191.00
206.00 > 141.00

Matrix Blank

7.00　　7.25　　7.50　　7.75

(×100,000)

6.0

5.0

4.0

3.0

2.0

1.0

0.0

206.00 > 191.00
206.00 > 141.00

Spiked sample

7.75　　8.00　　8.25　　8.50

(×10,000)

2.5

2.0

1.5

1.0

0.5

0.0

206.00 > 191.00
206.00 > 141.00

Spiked sample

%RSD
= 5.30 %

7.00　　7.25　　7.50　　7.75
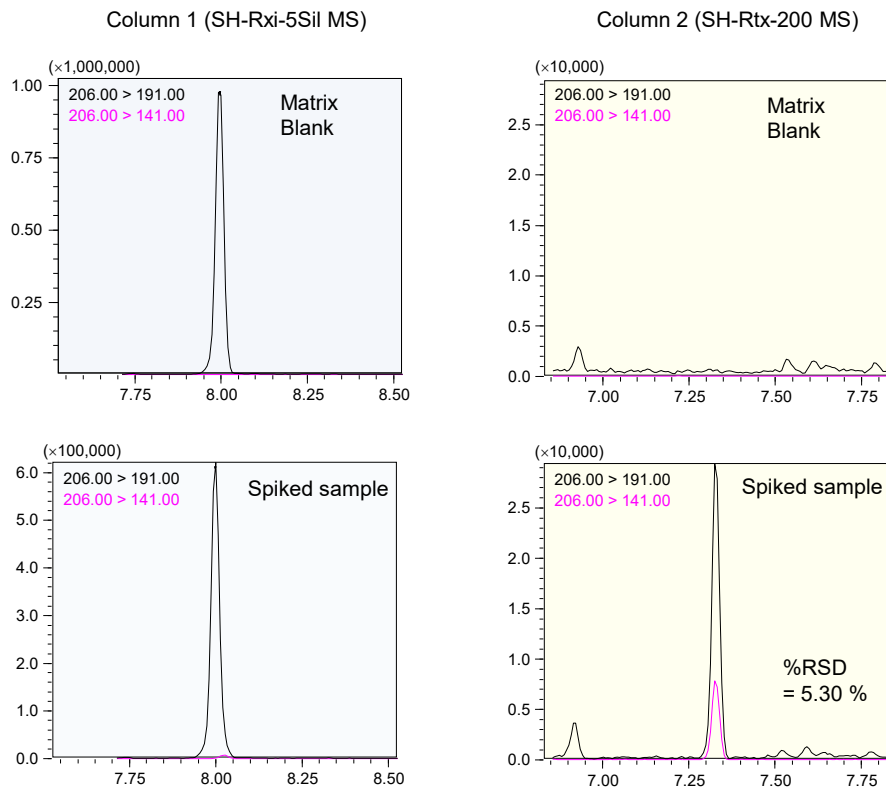
Fig. 2: MRM Chromatograms of Chloroneb in a Soy Bean Sample Using Column 1 (left) and Column 2 (right)

Column 1 (SH-Rxi-5Sil MS)

Column 2 (SH-Rtx-200 MS)

(×10,000)

2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25

300.10 > 258.10
300.10 > 199.10

Matrix Blank

25.50　　25.75　　26.00　　26.25

(×1,000)

2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00

300.10 > 258.10
300.10 > 199.10

Matrix Blank

23.50　　23.75　　24.00　　24.25

(×10,000)

1.50

1.25

1.00

0.75

0.50

0.25

300.10 > 258.10
300.10 > 199.10

Spiked sample

25.50　　25.75　　26.00　　26.25

(×1,000)

2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00

300.10 > 258.10
300.10 > 199.10

Spiked sample

%RSD
= 8.44 %

23.50　　23.75　　24.00　　24.25

Fig. 3: MRM Chromatograms of Bifenazate in a Brown Rice Sample Using Column 1 (left) and Column 2 (right)

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# Application News

## No.J114A

**Inductively Coupled Plasma Atomic Emission Spectrometry**

## Analysis of Additive Elements, Wear Metals, and Contaminants in Used Lubricating Oil According to ASTM D5185: ICPE-9820

### ◼ Introduction

Analysis of lubricants added to engine oils such as those used in automobiles and ships is an effective as well as important way to diagnose the state of the engine and other equipment.

According to ASTM International Standard D5185 [1], inductively coupled plasma (ICP) atomic emission spectrometry with organic solvent dilution is specified for measurement of additive elements, wear metals and contaminants present in used lubricants. Also, the Japan Petroleum Institute standard JPI-5S-44-2011 stipulates the use of ICP atomic emission spectrometry in Japan for analysis of Fe, Cu, Al, Pb, Cr and Sn in used lubricating oil. [2]

Here, using the Shimadzu ICPE-9820 multi-type ICP atomic emission spectrometer, we conducted analysis of 22 elements specified according to ASTM D5185 in samples consisting of a used lubricant (commercially available automotive lubricating oil) and, as a reference, the same, but unused lubricating oil, both of which were diluted with organic solvent. The ICPE-9820, which adopts a vertically-oriented plasma torch which reduces the possibility of carbon precipitation, provides stable analytical results for organic solvent samples without requiring the flow of oxygen through the system.

### ◼ Samples

- Used lubricating oil (commercially available automotive lubricant, used for approximately 4000 km)
- Same lubricating oil as above, but in unused state

### ◼ Sample Preparation

Approximately 10 g of each sample was weighed and then diluted with 100 mL of kerosene. The standard solutions were prepared by appropriately diluting with kerosene the SPEX oil-based 21-element mixed standard solution (500 µg/g), the Conostan® and SPEX oil-based single-element standard solution (5000 µg/g), and the Tokyo Kasei Kogyo Co., Ltd. heavy oil sulfur content standard sample (1.05 % by weight).

For validation of the measurement values, the above standard solution was added to the used lubricating oil to prepare a 5 mg/L solution to serve as a low-concentration element spike-and-recovery test sample. In addition, for high-concentration elements, the used lubricant was diluted 50-fold with kerosene to prepare a diluted test sample.

Finally, the Conostan® oil-based Y (yttrium) single-element standard solution (5000 µg/g) was diluted with kerosene and added to all the samples as the internal standard element so as to occupy a fixed concentration in all the samples.

### ◼ Instrument and Analytical Condition

Measurement was conducted using the Shimadzu ICPE-9820 multi-type ICP atomic emission spectrometer. The measurement conditions are shown in Table 1.

When conducting analysis of organic solvent samples with most conventional ICP instruments, oxygen must typically be introduced into the plasma torch to suppress carbon deposition at the tip of the torch. With the Shimadzu ICPE-9820, however, the vertical orientation of the plasma torch and adoption of a plasma torch that suppresses carbon deposition has nearly completely eliminated the deposition of carbon originating from the sample. Therefore, even in analysis of organic solvent samples such as kerosene, xylene and MIBK, the ICPE-9820 eliminates the need to introduce oxygen to suppress the precipitation of carbon.

Also, since the Shimadzu ICPE-9820 adopts a vacuum spectrometer, elements such as S with a wavelength in the vacuum ultraviolet region can be analyzed at a low running cost without the need for costly high-purity gas, typically required with a purge-type spectrometer.

**Table 1 Analytical Conditions**

| Instrument | : ICPE-9820 |
|---|---|
| Radio Frequency Power | : 1.40 kW |
| Plasma Gas Flowrate | : 16.0 L/min |
| Auxiliary Gas Flowrate | : 1.40 L/min |
| Carrier Gas Flowrate | : 0.70 L/min |
| Sample Introduction | : Nebulizer, 10UES |
| Misting Chamber | : Organic solvent chamber |
| Plasma Torch | : Torch |
| Observation | : Radial (RD) |

### ◼ Analysis

The calibration curve method – internal standard method was used to conduct analysis of 22 elements (Al, Ba, B, Ca, Cr, Cu, Fe, Pb, Mg, Mn, Mo, Ni, P, K, Si, Ag, Na, S, Sn, Ti, V, Zn) specified according to the ASTM standard.

### ◼ Analytical Results

Table 2 shows the analytical results. Excellent results near 100 % were obtained in the dilution test for the high-concentration elements and the spike-and-recovery test for the low-concentration elements, both with respect to the used lubricating oil. In addition, the analytical results obtained in analysis of the unused lubricating oil are also listed for reference.

The spectral line profiles for Fe and P are shown in Fig. 1. The calibration curves for Fe, Mg and S are shown in Fig. 2.

### ◼ Conclusion

Using the ICPE-9820, dissolved elements in used lubricating oil can be analyzed stably without the introduction of oxygen.

### ◼ References

1) ASTM International Standard D5185
   Standard Test Method for Determination of Additive Elements, Wear Metals, and Contaminants in Used Lubricating Oils and Determination of Selected Elements in Base Oils by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES)

2) The Japan Petroleum Institute Standard JPI-5S-44-2011 Method for Analyzing Fe, Cu, Al, Pb, Cr and Sn Contents in Used Lubricating Oil Using Solvent Dilution - Inductively Coupled Plasma Atomic Emission Spectrometry

**Table 2 Analytical Results of Lubricating Oil**

| Element | Used lubricant (µg/g) | Used lubricant spike recovery rate (%) | Used lubricant dilution test (%) | Unused lubricant (µg/g) | Detection limit (µg/g) |
|---|---|---|---|---|---|
| Ag | < | 100 | - | < | 0.02 |
| Al | 10 | 101 | - | 6.51 | 0.3 |
| B | 65.9 | - | 98 | 121 | - |
| Ba | 0.123 | 101 | - | < | 0.02 |
| Ca | 3970 | - | 98 | 2250 | - |
| Cr | 1.03 | 101 | - | < | 0.01 |
| Cu | 0.65 | 100 | - | < | 0.02 |
| Fe | 10.8 | 101 | - | 0.43 | 0.01 |
| K | 22.1 | 99 | - | < | 0.6 |
| Mg | 10.4 | 100 | - | 5.48 | 0.02 |
| Mn | 0.618 | 101 | - | 0.139 | 0.002 |
| Mo | 184 | - | 98 | 183 | - |
| Na | 2.5 | 100 | - | < | 0.4 |
| Ni | < | 102 | - | < | 0.05 |
| P | 756 | - | 99 | 731 | - |
| Pb | < | 100 | - | < | 0.5 |
| S | 3980 | - | 100 | 3810 | - |
| Si | 8.96 | 103 | - | 5.07 | 0.03 |
| Sn | < | 100 | - | < | 0.5 |
| Ti | < | 100 | - | < | 0.01 |
| V | < | 103 | - | < | 0.02 |
| Zn | 872 | - | 97 | 882 | - |

Spike recovery rate (%) = (C1-C2)/B×100 (C1: Spiked sample quantitative value; C2: Non-spiked sample quantitative value; B: Spike concentration)

Dilution test (%) = I/S ×100 (I: Quantitative value of sample before dilution; S: Quantitative value of 5-fold diluted sample ×5)

Detection limit: DL = 3×$\sigma_{BL}$× $\kappa$  ($\sigma_{BL}$: Standard deviation of background intensity; $\kappa$: Concentration/intensity)

<: Less than the detection limit
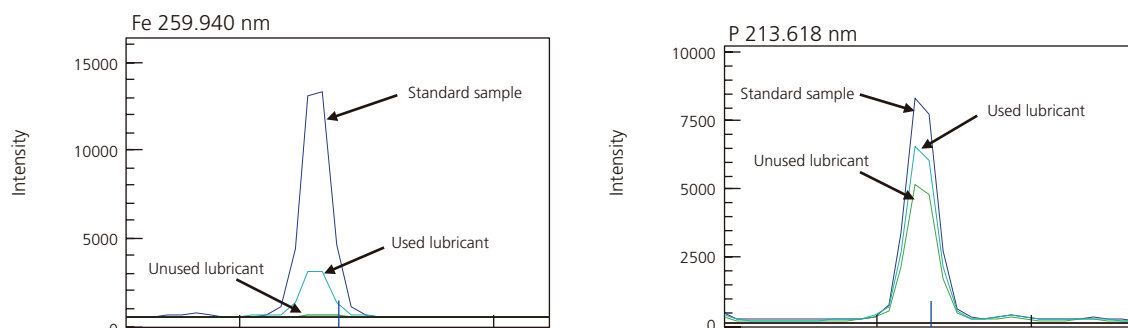


**Fig. 1 Spectral Profiles of Fe and P**
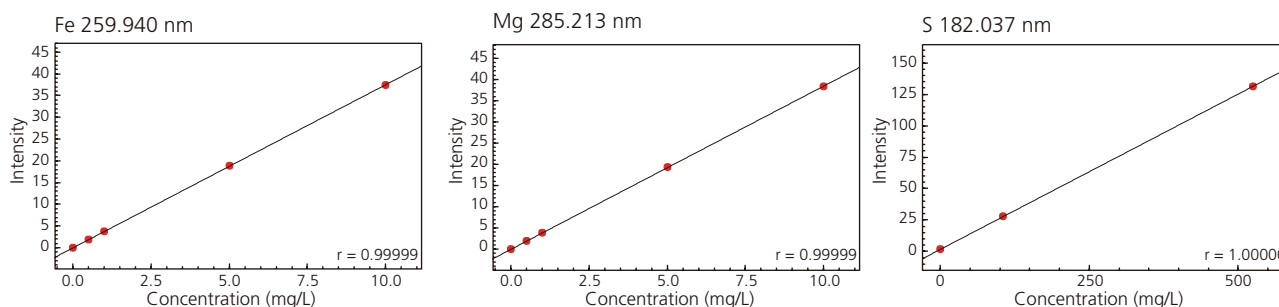


**Fig. 2 Calibration Curves of Fe, Mg and S**

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## Application News

### No.A524

**Spectrophotometric Analysis**

## Differentiating Olive Oils Using UV-VIS Spectrophotometer and Spectrofluorophotometer

### ■ Introduction

There is growing interest in food safety and organic foods, with an increasing number of shops specializing in a variety of food products and increasing circulation of high price import goods. One of these foods is olive oil, which is supposed to have both health and aesthetic benefits. The most expensive form of olive oil is extra virgin olive oil, which is regulated by the International Olive Council. Only olive oil that is chemically unprocessed, produced by squeezing and filtering olive fruit, and with an acidity of no more than 0.8 % qualifies as extra virgin olive oil. Another olive oil called pure olive oil is created by purification and high-temperature treatment. Differentiating between extra virgin olive oil and pure olive oil based on appearance alone is difficult. This article describes an attempt to differentiate between these two olive oil types by spectrum measurement using Shimadzu UV-2700 UV-VIS spectrophotometer and RF-6000 spectrofluorophotometer, then performing multivariate analysis.

### ■ Absorbance Measurement of Olive Oils

Fig. 1 shows Shimadzu UV-2700 that was used to measure absorbance. Fig. 2 shows some of the olive oils tested. They each differ in terms of color, odor, and place of origin. Ten different extra virgin olive oils were prepared from a total of 6 producers. Samples were named in the format "○ × E", where "○" was replaced by letters A through F to refer to each producer, and "×" was replaced by each producer's consecutive numbers in order of increasing olive oil price. Pure olive oil was also prepared from producers A and B. These samples were named in the format "○ × P". Each olive oil was placed in a quartz cell, then its absorption spectrum was measured. Measurement conditions are shown in Table 1, and three spectra representative of the spectra obtained are shown in Fig. 3. Absorption peak wavelengths are almost identical between spectra, though with obvious differences in their degree of absorption. Results confirmed the extra virgin olive oils tended to exhibit higher absorbance than the pure olive oils.



**Fig. 1  UV-2700 UV-VIS Spectrophotometer**
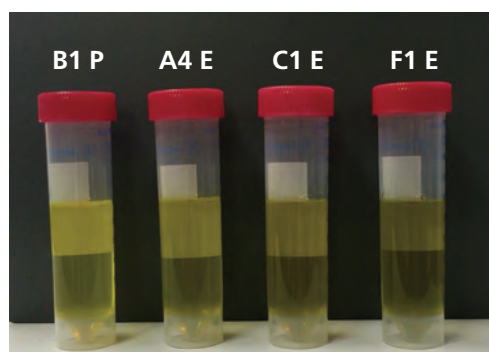


**Fig. 2  Various Olive Oils**
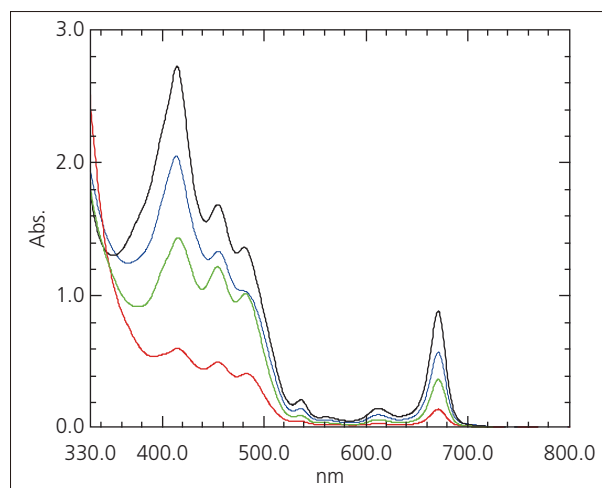


**Fig. 3  Olive Oil Absorption Spectra**
**Red: B1 P, Green: A4 E, Blue: C1 E, Black: F1 E**

**Table 1  UV-2700 Measurement Conditions**

| | |
|---|---|
| Spectrum Type | : Absorption spectrum |
| Measurement Wavelength Range | : 330 nm to 800 nm |
| Scanning Speed | : Intermediate |
| Sampling Pitch | : 0.5 nm |
| Light Source Switching Wavelength | : 323 nm |

## ■ Multivariate Analysis Using Absorption Spectra

We attempted to differentiate between pure olive oil and extra virgin olive oil by performing a multivariate analysis of the results obtained by absorbance measurement. The Unscrambler®X[1] multivariate analysis software was used to perform difference analysis on absorbance at 7 peak wavelengths.

Principal component analysis (PCA) and cluster analysis were used to differentiate between olive oil types. With PCA, scores are calculated to allow visual differentiation by the analyst (score plot). A loading plot is also used to determine characteristic factors in each grouping that have a strong influence on the score plot. Cluster analysis differentiates samples based on a tree diagram. The shorter the horizontal line that connects each sample, the more similar those samples.

The score plot obtained by PCA is shown in Fig. 4. Pure olive oils are clustered in the negative direction along the dominant PC-1 axis, while extra virgin olive oils are clustered in the positive direction along the dominant PC-1 axis. This shows successful differentiation between olive oil types. The loading plot in Fig. 5 shows a characteristic of the extra virgin olive oils tested is strong signals at short wavelengths, such as 415 nm and 454.5 nm.
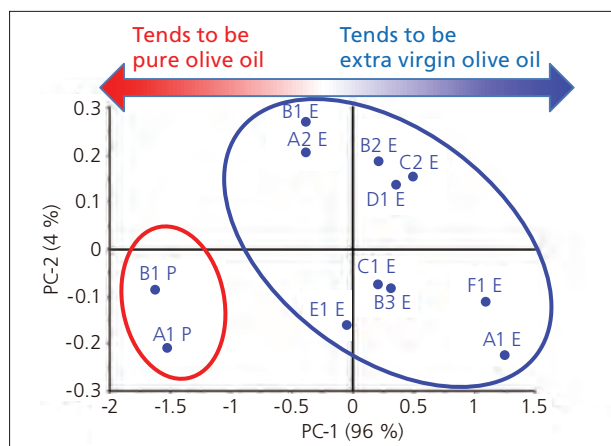


**Fig. 4 Score Plot Based on Olive Oil Absorbance**
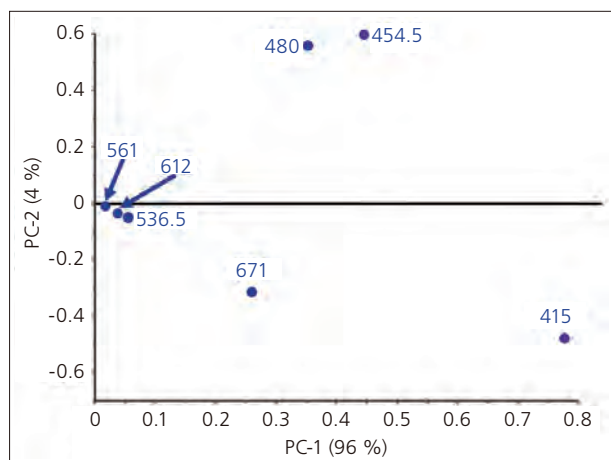


**Fig. 5 Loading Plot Based on Olive Oil Absorbance**

Cluster analysis results are shown in Fig. 6. At a glance, the tree diagram shows the olive oils separated into 2 groups and the degree of similarity between the samples. This result shows we successfully differentiated between pure olive oils and extra virgin olive oils.
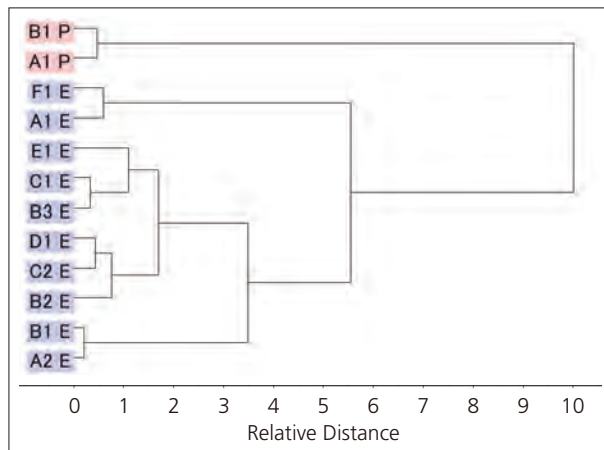


**Fig. 6 Cluster Analysis Result Based on Olive Oil Absorbance**

## ■ Three-Dimensional Spectra Measurements of Olive Oils

The three-dimensional emission spectra of olive oils were measured using Shimadzu RF-6000 spectrofluorophotometer. Fig. 7 shows the instrument used. Due to the high absorbance exhibited by the samples, a solid sample holder (Fig. 8) was used to compensate for self-absorption effects. Self-absorption is the phenomenon of light emitted by the sample being absorbed by the sample itself. When the absorption spectrum of a highly absorbing sample is measured in a normal cell holder, the amount of emission light that enters the detector can be reduced due to emission light being absorbed by the sample itself. A solid sample holder was used to direct excitation light towards the corner of the quartz cell as shown in Fig. 9. This reduces the amount of sample through which emission light travels, and so reduces the effects of self-absorption.
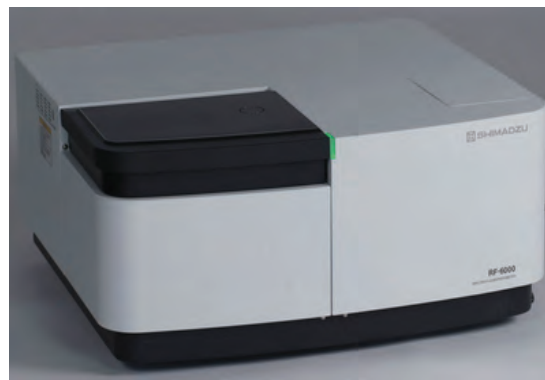


**Fig. 7 RF-6000 Spectrofluorophotometer**
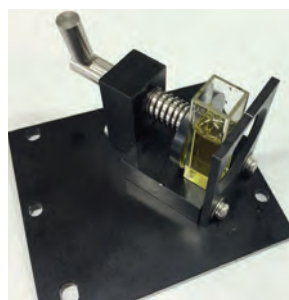


**Fig. 8 Solid Sample Holder**     **Fig. 9 Measurement Method**
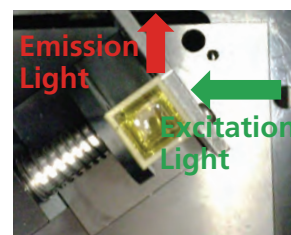
Table 2 shows the measurement conditions used. A filter (IHU310) that blocks light below 310 nm was placed in the path of emission light to prevent high-order excitation light reaching the detector. Fig. 10 shows two of the three-dimensional spectral diagrams obtained. Emission light predicted to be derived from chlorophyll was confirmed at Em 680 nm in both samples. Emission light at this wavelength was detected more strongly from extra virgin olive oil samples compared to pure olive oil samples. Also, strong emission light in the vicinity of Em 400 nm was mainly detected from pure olive oil samples. This 400 nm emission light is predicted to be derived from vitamins. Fig. 11 compares a pure olive oil and an extra virgin olive oil showing emission spectra obtained at an excitation wavelength of 300 nm. The excitation light region shown in Fig. 11 is indicated by the white dotted lines in Fig. 10. The emission spectra show different peak strengths and peak tail shapes between 300 nm and 500 nm.



**Fig. 10  Three-Dimensional Spectra of Olive Oils**
**Top: A1 P, Bottom: F1 E**

### ■ Multivariate Analysis Based on Three-Dimensional Spectra

Similar to absorbance analysis, a multivariate analysis was performed on the three-dimensional spectra to differentiate between pure olive oils and extra virgin olive oils. Emission light intensity at the 10 points shown in Fig. 12 (A through J) was used for this analysis.

**Table 2  RF-6000 Measurement Conditions**

| | |
|---|---|
| Optional Accessory | : Solid sample holder, IHU310 |
| Spectrum Type | : 3D spectrum |
| Measurement Wavelength Range | : Ex 200 nm to 800 nm |
| | Em 250 nm to 800 nm |
| Scanning Speed | : 6000 nm/min |
| Wavelength Interval | : Ex 5.0 nm, Em 1.0 nm |
| Bandwidth | : Ex 5.0 nm, Em 5.0 nm |
| Sensitivity | : Low |
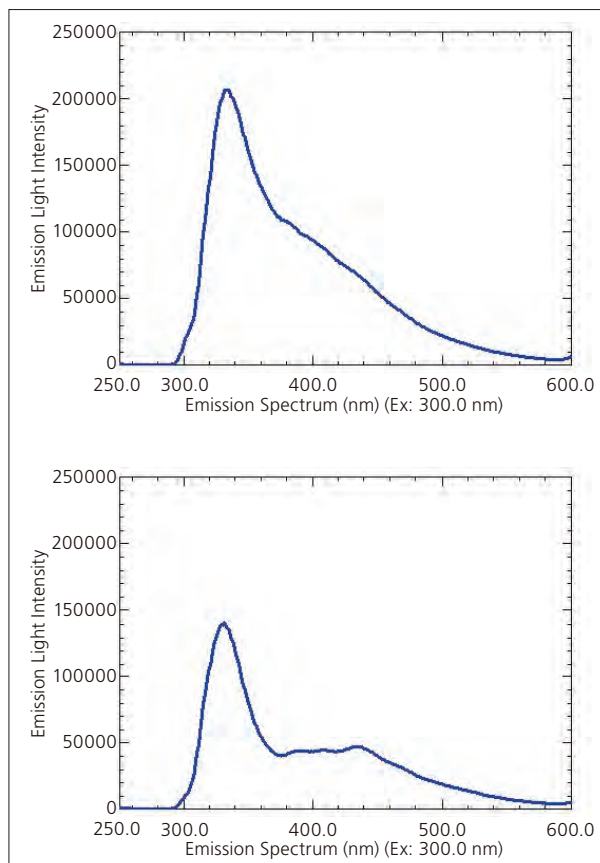


**Fig. 11  Emission Spectrum of Olive Oils Excited at 300 nm**
**Top: A1 P, Bottom: F1 E**
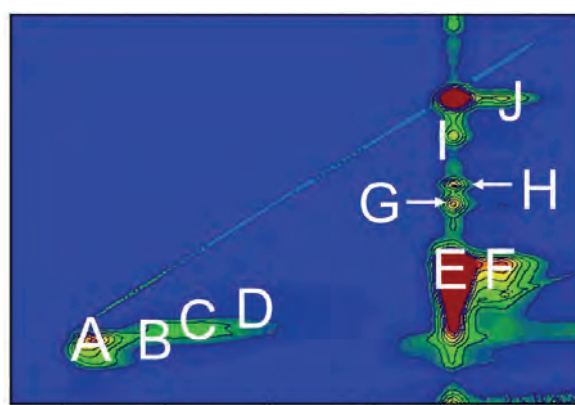


**Fig. 12  Analysis Points for Multivariate Analysis**

The score plot obtained by PCA is shown in Fig. 13. Pure olive oils are clustered in the positive direction along the dominant PC-1 axis and extra virgin olive oils are clustered in the negative direction along the dominant PC-1 axis. This shows the olive oil types have been separated.

Compared to the score plot created based on absorbance, the A2 E and B1 E samples are positioned closer to the pure olive oil samples. While A2 E and B1 E are both extra virgin olive oils, we can predict they are similar to pure olive oils. The loading plot is shown in Fig. 14. It shows a characteristic of the pure olive oils is for strong signals at emission light analysis points A through D, which are points predicted to be derived from vitamins.
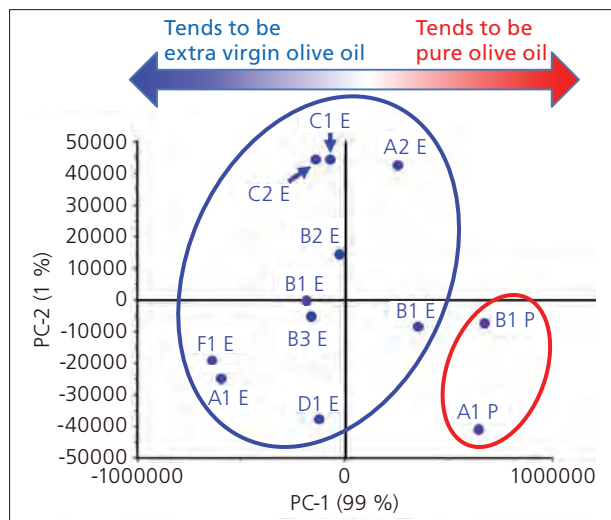
**Fig. 13　Score Plot Based on Olive Oil Absorbance**
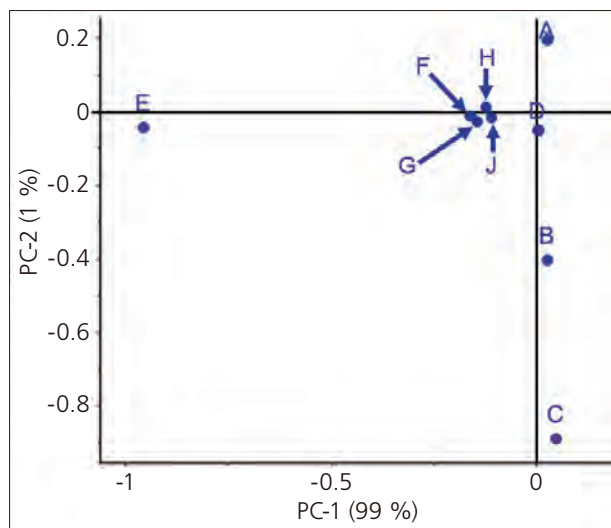
**Fig. 14　Loading Plot Based on Olive Oil Absorbance**

Results of the cluster analysis are shown in Fig. 15. Compared to the results obtained based on absorbance, the A2 E and B1 E samples are included in the pure olive oil group. This result also shows that while these samples are extra virgin olive oils, they tend to be closer to pure olive oils. Using emission light spectra, we could predict which extra virgin olive oils are likely to contain more vitamins.
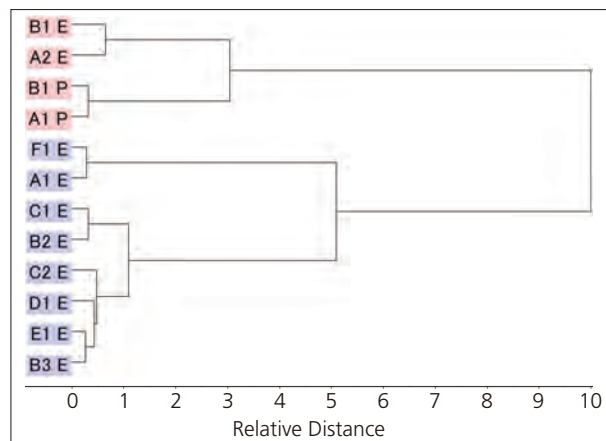
**Fig. 15　Cluster Analysis Result Based on Olive Oil Absorbance**

■ **Conclusion**

Shimadzu UV-2700 UV-VIS spectrophotometer was used to measure absorption spectra. Results showed the extra virgin olive oils have a higher absorbance than the pure olive oils. Next, the three-dimensional emission spectra of olive oils were measured using Shimadzu RF-6000 spectrofluorophotometer. Differences in emission light intensity and peak width were confirmed in the three-dimensional emission spectra results. Multivariate analysis was then performed based on each spectrum. This allowed successful differentiation between the extra virgin olive oils and pure olive oils, while results obtained from the RF-6000 allowed identification of extra virgin olive oils particularly similar to pure olive oils.

1) The Unscrambler®X is a trademark or registered trademark of CAMO Software.

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# SHIMADZU
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# Application News

## No. A543

## Analysis of Food Contaminants Using KBr Cuttings: KBr Plates for KBr Pellet Formation

The KBr pellet method is a technique mainly used to measure solid samples. This method exploits the plasticity of alkali halides that form a transparent plate when subjected to pressure. While potassium bromide (KBr) is the most common alkali halide used in pellet formation, potassium chloride (KCl) and cesium iodine (CsI) may also be used. Conventionally, pellets were formed by pulverizing KBr and the measurement sample each with an agate mortar, mixing the two to an appropriate concentration, and then applying pressure. However, compared to its crystallized state, crushed KBr readily absorbs moisture and there is also a risk of contamination from the mortar. Furthermore, press-forming work was a burden to analysts and preparing concentrations also took time.

By using KBr Cuttings, the onerous tasks of pulverizing KBr and mixing it with samples using an agate mortar are no longer required. KBr Cuttings are plates of cut KBr crystals. Good quality KBr disks can be produced by simply setting the sample for measurement between two KBr plates, placing the combination into a pelletizer, and applying pressure. When using KBr Cuttings, FTIR measurement is done using the transmittance mode. In this mode, the detector receives a greater amount of light compared to that with the reflectance mode and the ATR method, and therefore features measurement with good sensitivity.

In Application News No. A536, we introduced the procedure for using KBr Cuttings and an example analysis of pharmaceutical identification testing.[1]
In this article we introduce an example analysis of food contaminants using KBr Cuttings.

R. Fuji

### ■ KBr Cuttings Used

Material: KBr
Shape: 3 × 3 × 0.75 mm

**Fig. 1 KBr Cuttings**

Fig. 1 shows a photo of KBr Cuttings. The shape of KBr Cuttings is either 3 × 3 × 0.75 mm or 5 × 5 × 1 mm.

### ■ Analysis of Food Contaminants

Using KBr Cuttings, we measured a black contaminant which was caught in a mesh from filtering inspection, a quality inspection process. Fig. 2 shows the stereo microscope image of the contaminant. The IRTracer-100 Fourier transform infrared spectrophotometer and AIM-9000 infrared microscope, indicated in Fig. 3, were used for measurement. In measurement with an infrared microscope, usage of KBr Cuttings suppresses baseline distortions which occur due to scattering of light at the sample surface as well as interference fringes which may occur when measuring thin samples with a flat and smooth surface. Fig. 4 shows the KBr disk set for measurement. Since the formed KBr disk is the same size as the hole on the sample stage of AIM-9000, the disk can be fixed in place just simply by placing it. Table 1 lists the analysis conditions and Fig. 5 shows the measurement result.

100 μm
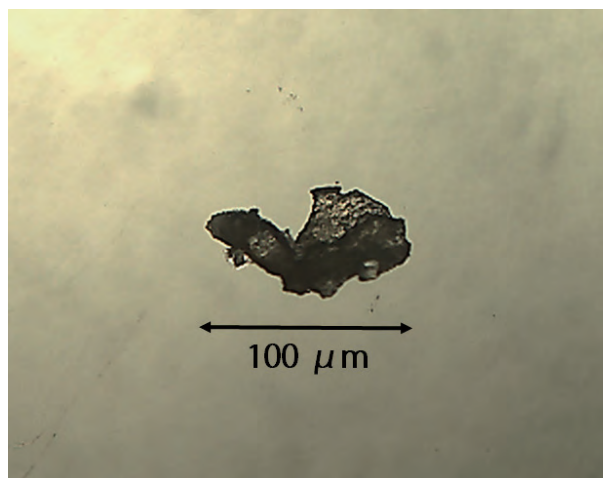
**Fig. 2 Stereo Microscope Image of Contaminant**

**Fig. 3 IRTracer-100 Fourier Transform Infrared Spectrophotometer and AIM-9000 Infrared Microscope**
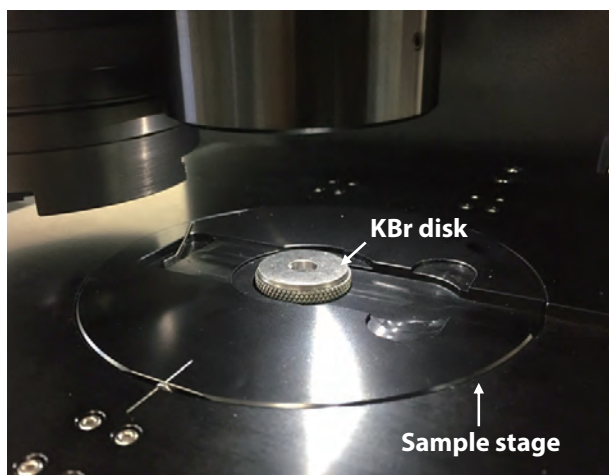
**Fig. 4 KBr Disk Set for Measurement**

**Table 1 Measurement Conditions**

| | |
|---|---|
| Instrument | : IRTracer-100, AIM-9000 |
| Resolution | : 8 cm$^{-1}$ |
| Accumulation | : 40 times |
| Apodization function | : Happ-Genzel |
| Detector | : MCT |
| Aperture size | : 100 μm × 100 μm |
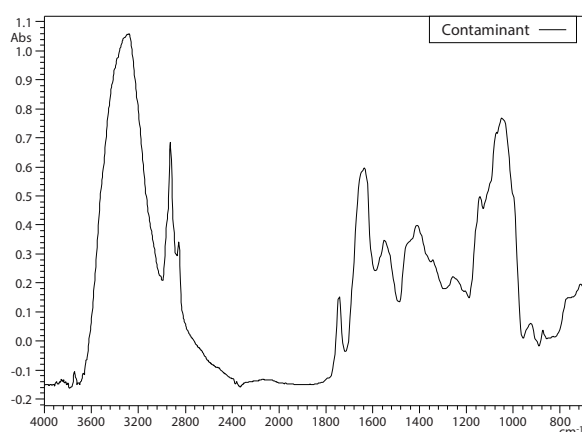


**Fig. 5 Measurement Result**

The measurement result shown in Fig. 5 indicates a large peak in the vicinity of 3200 cm$^{-1}$ caused by O-H bonds originating from water. Other detected peaks include a peak from C-H bonds near 2800 cm$^{-1}$, a peak from C=O bonds which are frequently found in foods containing oil near 1750 cm$^{-1}$, and a peak from amide bonds originating from protein in the range of 1650 to 1550 cm$^{-1}$.

## ■ Analysis of Measurement Result

Contaminant analysis was done using the standard library which contains 12,000 entries. Fig. 6 shows the analysis results.

Based on the library search, the contaminant was found to be a mixture of oil, protein and starch. Since all components are generally included in foods, we can presume that the contaminant is a part of a food.

In analysis of food contaminants, it is often the case that the found contaminant is a part of the food, or food components are attached to the contaminant.
In the latter case, there are times when measurement and analysis are required again after measuring the contaminant and then dissolving the food components such as with water. (The first measurement must be done since the contaminant may also dissolve through pretreatments.) In order to obtain more accurate qualitative results in contaminant analysis, information such as where the contaminant was found and the appearance of the contaminant observed with an optical microscope are also important.
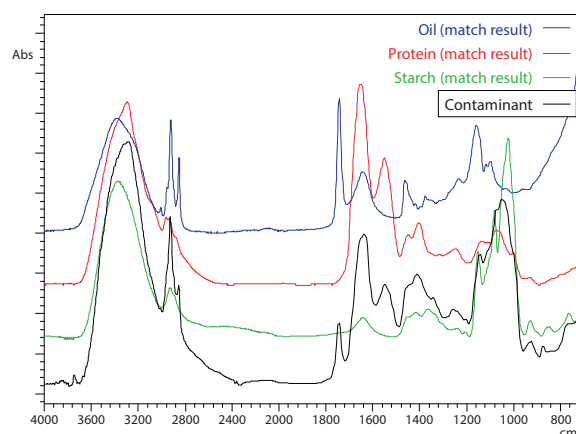


**Fig. 6 Analysis Results**

## ■ Conclusion

We introduced an example analysis of food contaminants using KBr Cuttings. Unlike the conventional KBr pellet method, measurement can be done easily and simply. Also, in addition to measurement with an infrared microscope, KBr disks can be used for the transmittance mode on FTIR instruments. We hope this method will be useful in measurements for contaminant analysis.

References:
*1   Application News No.A536
     "Introduction to KBr Cuttings: Convenient KBr Plates for KBr Pellet Formation"

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# Sensitive multi-mycotoxins analysis with a single sample preparation by LC-MS/MS

## ASMS 2018   MP 266

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2 Yokohama University Pharmacy, Kanagawa, Japan.

# Introduction

There are various substances that can threaten the food safety, such as pesticides, mycotoxins. LC-MS/MS analysis is a prevailing technique for the detection of these substances in food. Mycotoxins are especially frequent contaminants of agricultural products, and brewers are concerned that they can give serious damages to consumers, for example liver cancer, nephritis, pulmonary edema and so on. This is the reason why most countries have adopted regulations to limit exposure to mycotoxins, while the regulated mycotoxins and value differ with countries. The toxicity and potential health hazards induced by mycotoxins demand the need for sensitive, robust analytical methodologies. This research provides a LC-MS/MS system for quantitative screening of mycotoxins and includes a multi-mycotoxin sample preparation column to cover worldwide regulations. Although LC-MS/MS is a highly sensitive analytical technique, the problem of carryover occurs frequently. Metal-free column and multi-rinse mode were performed for reduction of carryover.



Fig 1. LC-MS/MS system (Nexera X2+LCMS-8060, Shimadzu Corporation.)

# Methods and Pretreatment

19 mycotoxins (Nivalenol, Patulin, Doxynivalenol-3-Glucoside, Deoxynivalenol, Fusarenon-X, Neosoraniol, 3-Acetyl-Deoxynivalenol, 15-Acetyl-Deoxynivalenol, Aflatoxin B1, B2, G1, G2, Diacetoxyscirpenol, Fumonisin B1, B2, B3, T-2 toxin, Ochratoxin A, Zearalenone) were used for evaluation of matrix effect and recovery rates in wheat. These mycotoxins were diluted with ACN at 5 ng/mL. Ground wheat flour samples were mixed with water/acetonitrile. After filteration, extracts were diluted with aqueous acetic acid solution and mixed with mycotoxins at 5 ng/mL. The solution were loaded to into the spin purification column (MycoSpin™400, Romer Lab) and analyzed using a triple quadrupole mass spectrometer (LCMS-8060, Shimadzu Corp.).

# Sensitive multi-mycotoxins analysis with a single sample preparation by LC-MS/MS

Table 1. LC and MS conditions

**[LC] Nexera™ X2 System**

| | |
|---|---|
| Analytical Column | : Mastro™ PFP2 (Shimadzu GLC Ltd) 2.1 mmI.D.×150 mmL., 3 µm |
| Solvent A | : 10 mmol/L ammonium acetate |
| Solvent B | : 2% acetic acid in methanol |
| Gradient Program | : |

| Time (min) | %B |
|---|---|
| 1.00 | 40 |
| 1.50 | 40 |
| 1.51 | 50 |
| 5.50 | 50 |
| 5.51 | 65 |
| 9.50 | 70 |
| 9.51 | 95 |
| 13.00 | 95 |
| 13.01 | 20 |
| 15.00 | STOP |

| | |
|---|---|
| Flow Rate | : 0.4 mL/min |
| Column Temp | : 40 °C |

**[MS] LCMS-8060**

| | |
|---|---|
| Ionization | : ESI (Positive/Negative) |
| Nebulizer Gas | : 2 L/min |
| Interface temperature | : 300 °C |
| Desolvation Line | : 250 °C |
| Heat Block temperature | : 500 °C |
| Heating Gas | : 10 L/min |
| Drying Gas | : 10 L/min |



< Rinse Program >

| R0 | 10 mmol/L ammonium acetate |
|---|---|
| R1 | 10 mmol/L sodium citrate |
| R2, R3 | 1% Formic acid + water/MeOH/ACN/IPA= 1/1/1/1 (v/v) |

# Sensitive multi-mycotoxins analysis with a single sample preparation by LC-MS/MS

Table 2. MRM transitions for mycotoxins        :Positive        :Negative

| No. | Mycotoxins | RT (min) | Polarity | transition | CE |
|-----|-----------|----------|----------|------------|-----|
| 1 | NIV | 1.88 | - | 371.10>281.10 | 16 |
| 2 | PAT | 2.22 | - | 153.00>109.00 | 11 |
| 3 | D3G | 2.30 | - | 517.20>427.20 | 21 |
| 4 | DON | 2.40 | - | 355.10>265.10 | 15 |
| 5 | FUX | 2.94 | - | 413.10>353.10 | 9 |
| 6 | NEO | 3.05 | + | 400.20>305.10 | -12 |
| 7 | 15-ADON | 3.74 | + | 339.10>261.10 | -11 |
| 8 | 3-ADON | 3.86 | + | 339.10>231.10 | -14 |
| 9 | AF G2 | 4.87 | + | 331.10>245.10 | -31 |
| 10 | AF G1 | 5.55 | + | 329.10>243.10 | -30 |
| 11 | DAS | 5.78 | + | 384.20>307.10 | -13 |
| 12 | AF B2 | 6.22 | + | 315.10>259.10 | -30 |
| 13 | AF B1 | 6.96 | + | 313.10>241.10 | -39 |
| 14 | FB1 | 7.37 | + | 722.40>334.10 | -43 |
| 15 | FB3 | 8.08 | + | 706.40>336.10 | -38 |
| 16 | T-2 | 8.71 | + | 484.30>185.10 | -20 |
| 17 | FB2 | 8.97 | + | 706.40>336.10 | -39 |
| 18 | OTA | 9.73 | + | 404.10>239.10 | -24 |
| 19 | ZEN | 10.8 | - | 317.10>130.10 | 35 |

## Details of sample preparation

1. Mix a ground wheat flour sample (50.0 g) with 100.0 mL of water/acetonitrile (15/85), and shake for 30 minutes

2. Filter the supernatant using glass-fiber filter paper (pore size < 0.7 um)
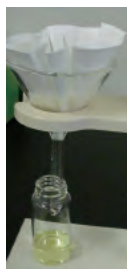
3. Add 500.0 µL of acetic acid to the filtrate (10.0 mL): Solution A

4. Load 1.0 mL of Solution A into the spin purification column and mix using vortex mixer for 1 minute while capped

5. Remove the bottom tip of the column and centrifugation for 2 minutes at 10,000 rpm

6. Transfer the supernatant into a vial then serve to the sample

# Sensitive multi-mycotoxins analysis with a single sample preparation by LC-MS/MS

# Results

Typical MS chromatogram for mycotoxins in ACN are shown in Fig. 2. An LC-MS/MS method was developed that achieved good separation and sensitivity for the detection of all mycotoxins without separating method for its polarity. Autosampler rinsing capabilities and metal free column were used to minimize the carryover of the fumonisins. Matrix effect was calculated by the peak area of mycotoxins (5 ng/mL) in ACN and post spiked samples.

Recovery rate was calculated by the peak area of post spiked samples (5 ng/mL) and pre spiked samples (5 ng/mL) which is shown in Table 3. NIV, DON, AF B1, T-2, ZEN were influenced wheat extractions which dramatically decrease the ionization efficiency of the mycotoxins. Recovery rate of the NIV, D3G, DON, T-2, ZEN were also insufficient. Therefore, internal standards are required for achieving accurate quantitative results.
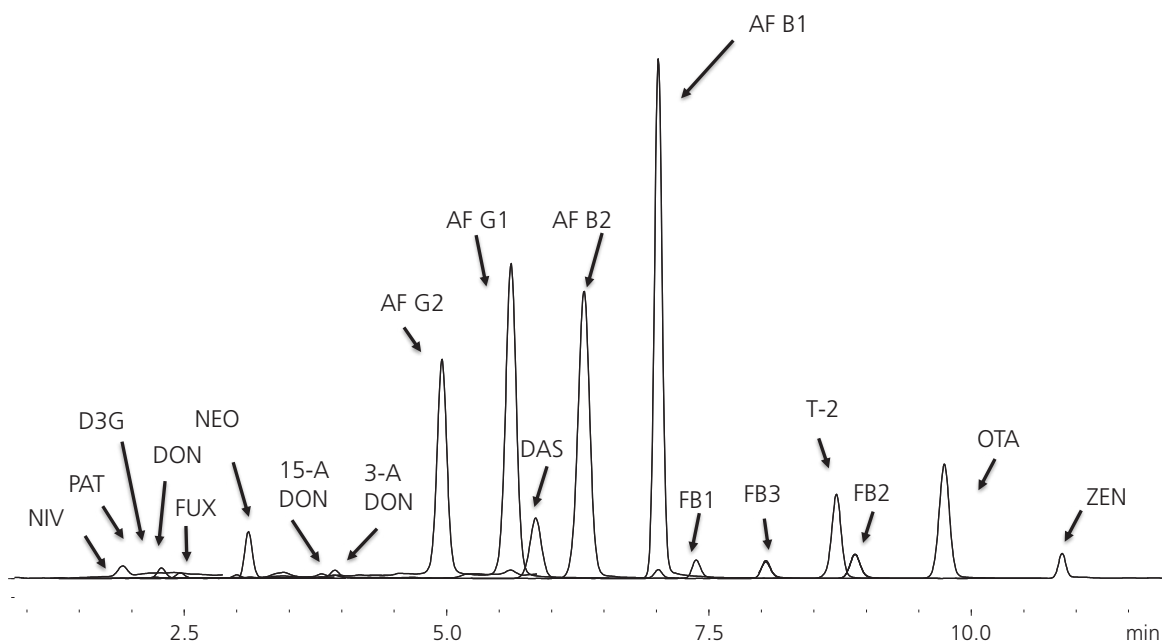


Fig 2. Typical MS chromatogram for mycotoxins mixture (50 ppb)

Table 3. Matrix effect and recovery rate of the mycotoxins in wheat matrix (5 ppb)

| No. | Mycotoxins | Matrix Effect (%) | Recovery Rate (%) | No. | Mycotoxins | Matrix Effect (%) | Recovery Rate (%) |
|---|---|---|---|---|---|---|---|
| 1 | NIV | 35.0 | 156.6 | 11 | DAS | 84.6 | 76.4 |
| 2 | PAT | 71.6 | 115.8 | 12 | AF B2 | 80.6 | 75.0 |
| 3 | D3G | 34.4 | 166.8 | 13 | AF B1 | 33.8 | 65.3 |
| 4 | DON | 47.3 | 143.9 | 14 | FB1 | 73.6 | 128.6 |
| 5 | FUX | 81.8 | 99.3 | 15 | FB3 | 71.5 | 120.1 |
| 6 | NEO | 74.2 | 95.1 | 16 | T-2 | 51.8 | 52.0 |
| 7 | 15-ADON | 72.6 | 87.7 | 17 | FB2 | 68.6 | 122.2 |
| 8 | 3-ADON | 87.6 | 78.6 | 18 | OTA | 42.3 | 111.5 |
| 9 | AF G2 | 78.3 | 70.7 | 19 | ZEN | 40.4 | 28.0 |
| 10 | AF G1 | 85.6 | 65.5 | | | | |

# Sensitive multi-mycotoxins analysis with a single sample preparation by LC-MS/MS



Fig 3. MS chromatograms of NIV, D3G, DON, T-2, ZEN which are pre-spiked in and post-spiked in wheat extraction at 5 ng/mL

# Conclusion

- This LC-MS/MS method and one step sample preparation measured various types of mycotoxins which spiked in wheat matrix.
- Sensitivity of some mycotoxins were decreased because of matrix effect.
- For accurate quantitative measurement, internal standard is necessary

# Reference

1) Masayoshi TAMURA, Keiko MATSUMOTO, Jun WATANABE, Naoki MOCHIZUKI, et al., *Journal of separation science*, 2014, **37**, 1552-1560

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# Use of MALDI-TOF mass spectrometry and machine learning to detect the adulteration of extra virgin olive oils

ASMS 2018    MP 521

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CLOVER
BioSoft

## Use of MALDI-TOF mass spectrometry and machine learning to detect the adulteration of extra virgin olive oils

# Introduction

Extra virgin olive oil (EVOO) is known for its nutraceutical properties, which associate it with several health benefits and a high economic value. For these reasons, EVOO is often a target of adulteration with cheaper, lower-grade vegetable oils, typically, sunflower, corn and soybean. Within the quality control process of EVOO products, it is fundamental to develop rapid, simple and robust analytical methods to detect any fraud. Here, we present a simple approach based on the profiling of triacylglycerols (TAGs) using MALDI-TOF mass spectrometry and an evolved neural network based on a logistic regression machine learning algorithm to reveal the adulteration of extra virgin olive oils by seed oils.

**Shimadzu MALDI-8020**

**1. Sample preparation**
- Preparation of oil solutions containing an internal standard

**2. Analyses**
- LDI (matrix-free) analysis for TAGs profiling

**3. Classification**
- Distinguish among pure and adulterated oils using a neural network machine learning software
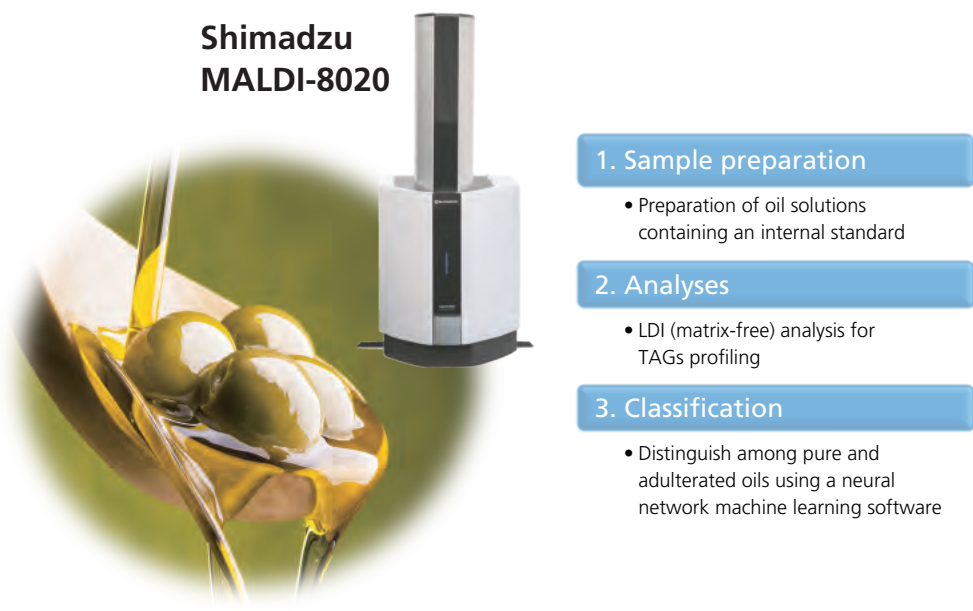
Figure 1. Left: MALDI-8020 Benchtop Linear MALDI-TOF mass spectrometer. Right: sample analysis workflow. (Picture taken from: https://www.iobenessereblog.it/olio-extravergine-di-oliva-benefici/26429).

# Methods and Materials

EVOO and sunflower oils were purchased from local stores. Sample preparation involved dissolution of oil aliquots in chloroform. To simulate the adulteration, mixtures of EVOOs containing 5%, 10% and 20% of sunflower oil were prepared. Tricaprin was used as internal standard for mass alignment and the semi-quantitative analyses. LDI (matrix-free) analyses were conducted on a MALDI-8020 benchtop linear MALDI-TOF mass spectrometer (Shimadzu, Manchester, UK; Figure 1), by spotting the oil sample solutions directly onto the MALDI target which was previously pre-coated with NaTFA. Data were acquired in quadruplicates for each scenario and processed using Clover MS software (Clover Bioanalytical Software, Granada, Spain) for peak area calculation and classification with neural networks.

# Use of MALDI-TOF mass spectrometry and machine learning to detect the adulteration of extra virgin olive oils

# Results and Discussion

## MALDI analyses

Figure 2a shows a comparison between the TAG profiles of an EVOO and a sunflower oil. It can be seen how, in EVOO (red trace), naturally rich in palmitic (P) and oleic (O) acids, the TAGs at *m/z* 881 and 907, i.e. most likely OPO and OOO, are predominant. In sunflower oil (blue trace), highly rich in linoleic acid (L), the most representative TAGs are those at m/z 901, 903 and 905, i.e. most likely LLL, OLL

and OLO, respectively. In the oil mixture scenario (Figure 2b), the alteration of the natural TAG ratios in EVOO, e.g. *m/z* 877/907, 881/907, 903/907 and 905/907, can be observed. Interestingly, the TAG at *m/z* 901 (LLL), characteristic of sunflower oil but not normally present in EVOO, is revealed in the EVOO/sunflower mixtures even at the smallest adulteration level.
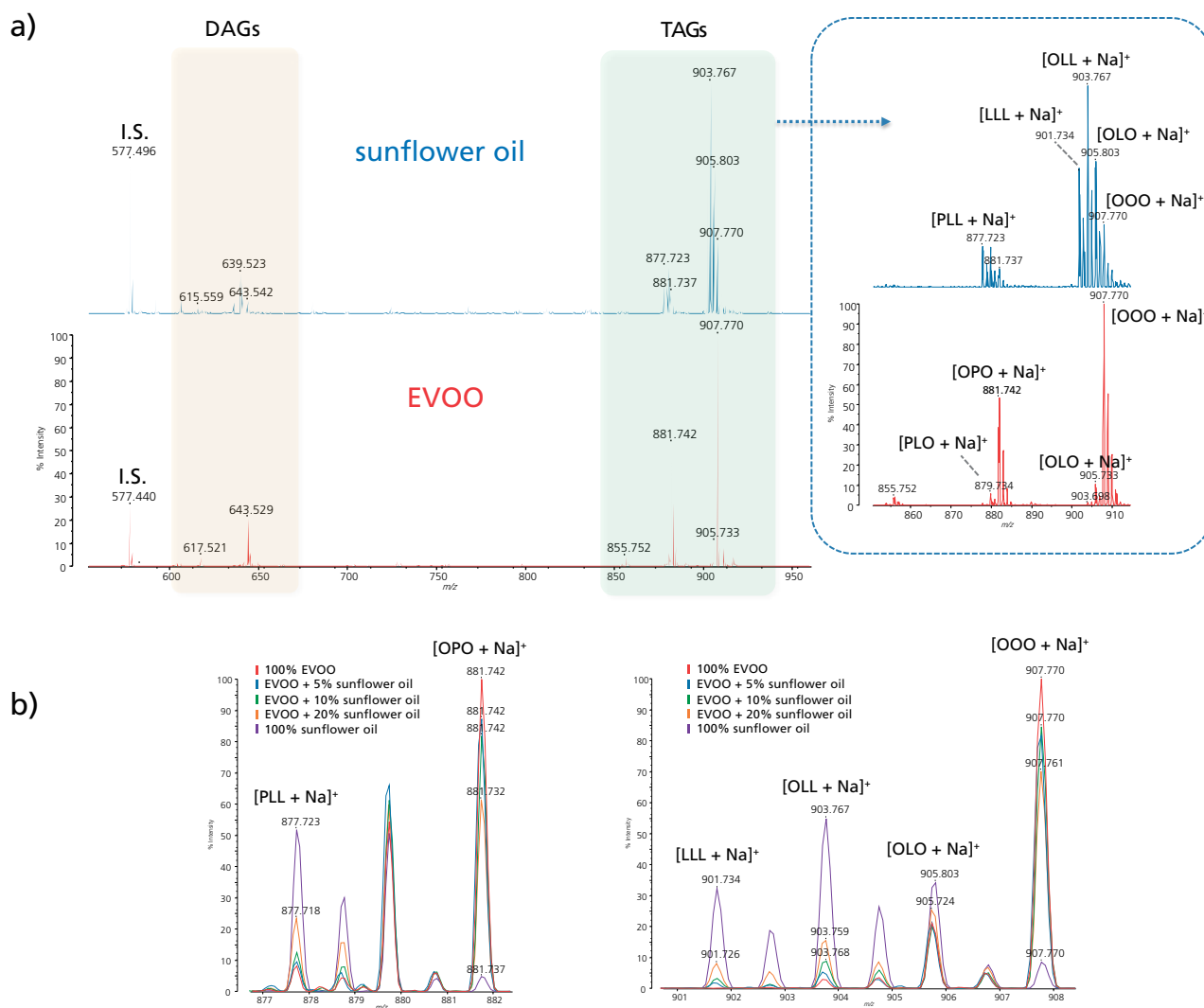


Figure 2. a) LDI MS spectra of a pure EVOO (red trace) and sunflower oil (blue trace). Right panel: expansion of the mass spectra showing the region of representative TAGs of EVOO (red trace) and sunflower oil (blue trace). b) Expansions of the overlaid mass spectra of EVOO (red trace), EVOO + 5% sunflower oil (blue trace), EVOO + 10% sunflower oil (green trace), EVOO + 20% sunflower oil (orange trace), sunflower oil (purple trace), showing the variation of TAGs and their ratios. P = palmitic acid; O = oleic acid; L = linoleic acid. I.S. = internal standard.

## Semi-quantitative analyses

Figure 3 shows the plots of the ratios of EVOO's TAG markers and the TAG at *m/z* 907 (the most abundant and representative in EVOO), versus the different levels of adulterant oil (from 0%, i.e. pure EVOO, to 20% sunflower oil). All TAGs were normalised against the internal standard using the area of the peaks from quadruplicate analyses. A good linearity has been achieved along with good coefficients of determination ($R^2$).



**877/907**

$y = 809x - 9.3124$
$R^2 = 0.9965$

**901/907**

$y = 460.56x + 0.1249$
$R^2 = 0.9998$

**903/907**

$y = 203.59x - 3.9913$
$R^2 = 0.9999$

**905/907**

$y = 303.19x - 44.684$
$R^2 = 0.9964$
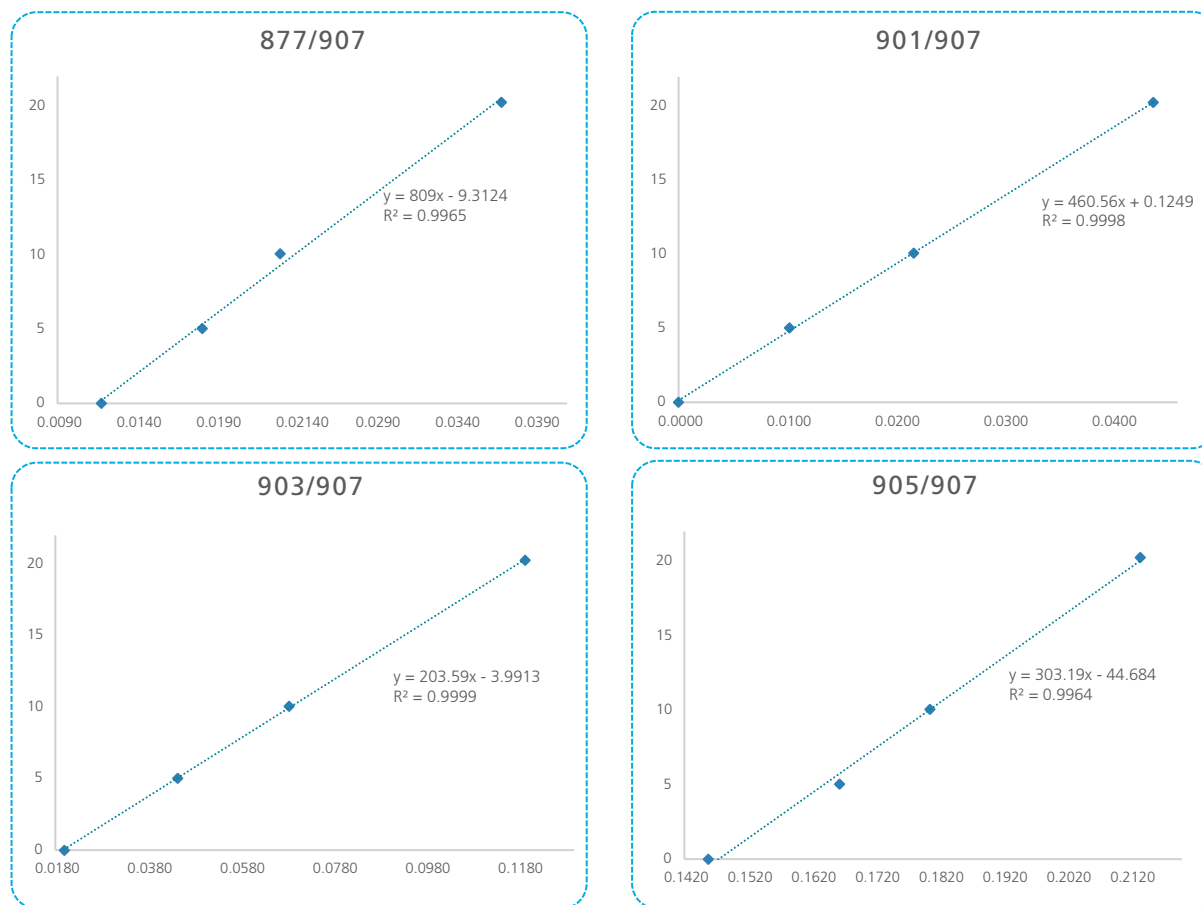
Figure 3.  Plots of *m/z* 877/907, 901/907, 903/907 and 905/907 ratios (*x*-axis) versus percentage
of EVOO adulteration (from 0%, i.e. pure EVOO, to 20% sunflower oil; *y*-axis).

## Neural Network Training and Classification

Artificial Neural Networks (ANNs) are one of the well-known cutting edge technologies used for classification problems given the huge amount of data available nowadays. They are able to learn specific features from a given dataset. On the other hand, logistic regression models have been typically used for binary classification on linearly separable datasets. We show that the use of ANNs with a logistic regression model seems to be a fast and efficient combination to detect different types of oil samples including the adulterated ones. We have created a three layers neural network able to classify between the EVOO, adulterated EVOO and sunflower oil categories (Figure 4). Prior to the classification, all spectra were aligned and normalised by the 903 Da mass. A total of 267 spectra were used to train and validate the neural network. Thirty single-blinded spectra were used to test the model accuracy (Figure 5).

# Use of MALDI-TOF mass spectrometry and machine learning to detect the adulteration of extra virgin olive oils

**NN Parameters Definition:**
- Input Data: (1188, 222)
- Validation Data: (1188, 45)
- Categories: 3
- Epochs: 75
- Batch Size: 10
- Nodes Hidden I: 50
- Nodes Hidden II: 25
- Learning Rate: 0.00025

**NN Accuracy and Testing:**
- Validation Accuracy: 97.78%
- 30 single-blinded spectra: 100%
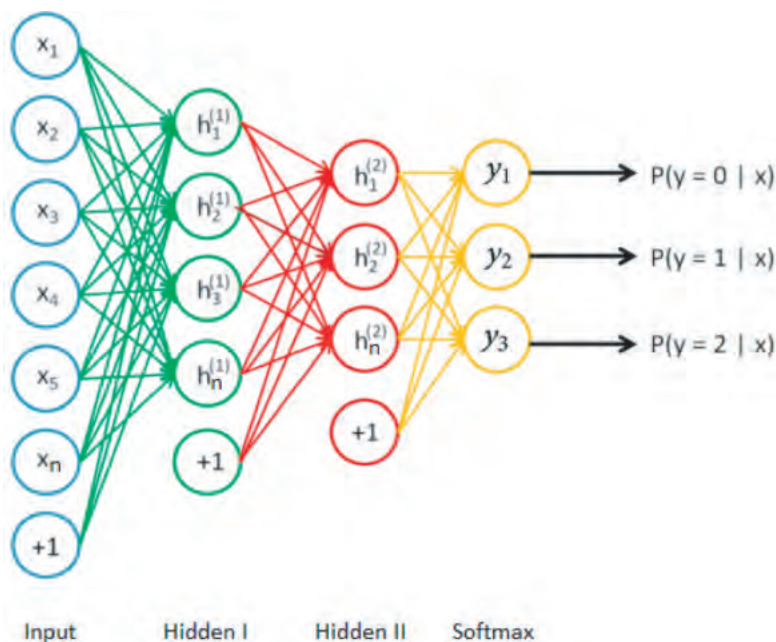


Figure 4. Representation of the neural network defined.
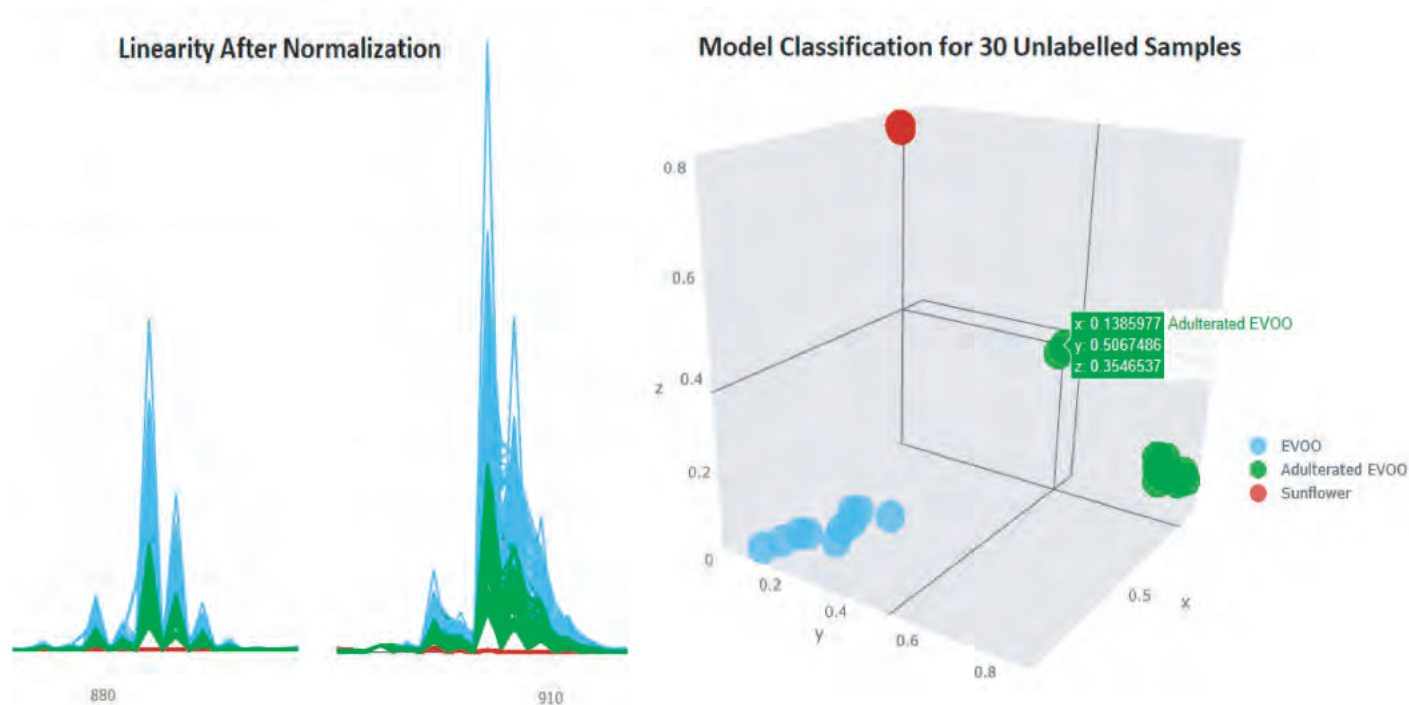


Figure 5. The linearity shown in the spectrum (left) after alignment and normalization of the input data using the Clover MS Software. The trained model classification results (left) over the 30 single-blinded samples.

# Conclusions

The combination of MALDI-TOF MS and the use of a cutting edge machine learning technique has been proven to be suitable for the detection of adulterated EVOO. The efficiency and simplicity of the methodology proposed is the key point of this research. The promising results achieved, and the expansion of the dataset and categories to be detected will determine the future viability of the system and its introduction into the oil industry.

Disclaimer: The products and applications in this presentation are intended for Research Use Only (RUO). Not for use in diagnostic procedures.

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# A rapid screening method of mycotoxins in grains by liquid chromatograph tandem mass spectrometry

**ASMS 2018**   MP-290

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# A rapid screening method of mycotoxins in grains by liquid chromatograph tandem mass spectrometry

## Overview

Development of a rapid simple screening method by LC/MS/MS for mycotoxins containing fumonisins.

## Introduction

Mycotoxin is a chemical substance produced by mold. In terms of harmful substances to health of human and livestock, regulatory limitation is defined in each country. In recent years, the risk management of mycotoxins has been gaining wider acceptance all over the world. In this report, we demonstrate a simultaneous screening analysis for 18 kinds of mycotoxins in grains by LC/MS/MS. For the purification of grain extract, Multitoxin Spin Column (Romer Labs) was utilized in order to perform a simple and rapid clean-up treatment for mycotoxins, which has individual chemical properties, such as aflatoxins, ochratoxin A, trichothecenes, and fumonisins.

## Methods and Materials

### Analytical conditions

Analysis was performed by a LCMS-8050 which was equipped with a Nexera™ X2 UHPLC. Pentafluorophenyl (PFP) bounded column was used to separate the regioisomeric pair (3-AcDON / 15-AcDON, FB2 / FB3) by gradient elution with a series of mobile phases containing ammonium acetate, acetic acid and methanol. Quantitative limits had been deemed to be less than or equivalent to the minimum values specified in EC/1886/2006.
The developed method achieved the simultaneous determination of mycotoxins such as aflatoxins (B1, B2, G1, G2), fumonisins (B1, B2, B3), ochratoxin A (OTA), trichothecenes [(3-acetyldeoxynivalenol(3-AcDON), 15-acetyldeoxynivalenol (15- AcDON), deoxynivalenol (DON), HT-2, nivalenol (NIV), T-2, zearalenone (ZEN)), Fusarenon-X (FUX) , Diacetoxy- scirpenol (DAS)] and patulin (PAT) in 15 minutes analytical cycle. This analytical method was developed by the modified LC/MS/MS method package for mycotoxin (Shimadzu Corporation, Japan).



sample

air

Sample     Air     Water     Inject
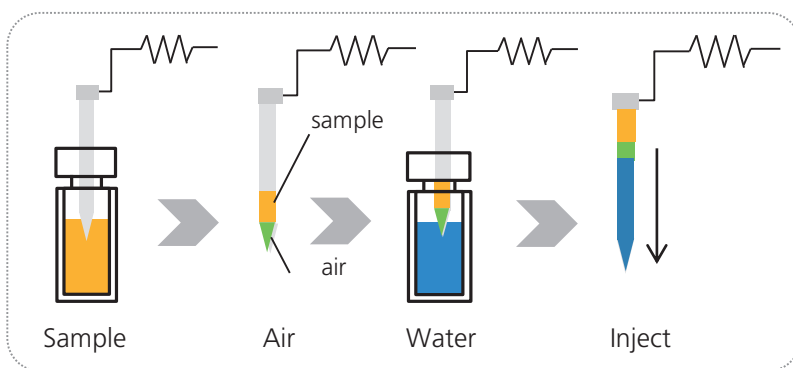
Inject method

Improving peak shape of NIV solved in more than 50% of acetonitrile aqueous solution, the sample solutions should be injected with additional water. SIL-30AC autosampler has this useful function shown as above.

Nexera is a trademark of Shimadzu Corporation.
MycoSpin is a trademark of Romer Labs Division Holding GmbH.

# A rapid screening method of mycotoxins in grains by liquid chromatograph tandem mass spectrometry



High Speed Mass Spectrometer
 Ultra Fast Polarity Switching -5 msec
 Ultra Fast MRM -Max.555 transition/sec

Figure 1    LCMS-8050 triple quadrupole mass spectrometer

**UHPLC conditions (Nexera™ X2 system)**

| | |
|---|---|
| Column | : Mastro PFP 2 (150 mm×2.1 mm, 3 µm) |
| Mobile phase  A | : 10 mmo/L Ammonium acetate-water |
| Mobile phase  B | : Methanol including 2% acetic acid |
| Flow rate | : 0.4 mL/min |
| Time program | : B conc.15%(0 min) -35%(1.51 min) – 45%(5.50 min) - 60%(5.51 min) – 95%(9.50-12.00 min) - 15%(12.01-15.00 min) |
| Column temp. | : 40 ℃ |
| Injection vol. | : 2.5 µL with 50 µL Water |
| Rince R0 | : Mobile phase A |
| Rince R1 | : 10 mmol/L Sodium citrate aqueous solution |
| Rince R2 | : Water/ Methanol / Acetonitrile/ IPA = 1/1/1/1 including 1% formic acid |
| Needle rinse program | : inside: R1 → R0 → R2 → R0, outside: R3(1 sec) → R0 |

**MS conditions (LCMS-8050)**

| | |
|---|---|
| Ionization | : ESI, Positive/Negative MRM mode |
| DL temp. | : 150 ℃ |
| Interface temp. | : 200 ℃ |
| Heat block temp. | :  400 ℃ |
| Nebulizer gas | : 2.5 L /min |
| Heating gas | : 15 L/min |
| Drying gas | : 5 L/min |

Nexera is a trademark of Shimadzu Corporation.

# A rapid screening method of mycotoxins in grains by liquid chromatograph tandem mass spectrometry

Table 1   MRM transitions of Mycotoxins

| No. | Mycotoxin | Retention Time (min) | Polarity | Precursor Ion (*m/z*) | Product Ion (*m/z*) | CE (V) |
|-----|-----------|----------------------|----------|-----------------------|---------------------|--------|
| 1 | Nivalenol (NIV) | 2.261 | - | 371.10 | 281.10 | 15 |
| 2 | Patulin (PAT) | 2.569 | - | 153.00 | 109.10 | 13 |
| 3 | Deoxynivalenol (DON) | 2.998 | - | 355.10 | 295.10 | 10 |
| 4 | Fusarenon-X (FUX) | 3.827 | - | 413.10 | 353.10 | 9 |
| 5 | 15-AcetylDeoxynivarenol (15-AcDON) | 5.582 | + | 339.10 | 261.10 | -12 |
| 6 | 3-AcetylDeoxynivarenol (3-AcDON) | 5.751 | + | 339.10 | 231.10 | -14 |
| 7 | Aflatoxin G2 (AF G2) | 7.197 | + | 331.10 | 245.10 | -30 |
| 8 | Diacetoxy- scirpenol (DAS) | 7.480 | + | 384.20 | 307.10 | -13 |
| 9 | Aflatoxin G1 (AF G1) | 7.433 | + | 329.10 | 243.10 | -27 |
| 10 | Aflatoxin B2 (AF B2) | 7.669 | + | 315.10 | 259.10 | -30 |
| 11 | Fumonisin B1 (FB1) | 7.804 | + | 722.40 | 334.10 | -42 |
| 12 | Aflatoxin B1 (AF B1) | 7.904 | + | 313.10 | 241.10 | -39 |
| 13 | HT-2 toxin (HT-2) | 8.060 | + | 442.20 | 263.10 | -13 |
| 14 | Fumonisin B3 (FB3) | 8.107 | + | 706.40 | 336.10 | -35 |
| 15 | Fumonisin B2 (FB2) | 8.475 | + | 706.40 | 336.10 | -39 |
| 16 | T2-toxin (T-2) | 8.705 | + | 484.30 | 185.10 | -23 |
| 17 | Ochratoxin A (OTA) | 8.987 | + | 404.10 | 239.10 | -24 |
| 18 | Zearalenone (ZEN) | 9.532 | - | 317.10 | 131.10 | 30 |

## Sample preparation

Analytical samples were prepared through the extraction protocol of MycoSpin™400 (Romer Labs), which is a very convenient method without evaporator nor nitrogen purge procedures. The operation of MycoSpin™400 was completed within 5 minutes.

MycoSpin is a trademark of Romer Labs Division Holding GmbH.

# A rapid screening method of mycotoxins in grains by liquid chromatograph tandem mass spectrometry

2.5 g Wheat / Corn (add 100 ng each standard)

Add 10 mL 50% Acetonitrile Solution
(or 85% Acetonitrile Solution)

Shake for 90 minutes

Centrifuge at 3200 rpm., 10 minutes

1 mL of the supernatant, add 50 μL acetic acid

Transfer 750 μL of the solution
to the MycoSpin™ column

Cap MycoSpin™ column and vortex for 1 minute

Break bottom tip off of MycoSpin™

Centrifuge at 10000 rpm., 30 seconds

Obtain 350 μL of purified extract

Each 45 μL of purified extract with 5 μL standard solution
(0, 100 ,200, 500 ng/mL)

Inject onto the LC/MS/MS system

5 min

Figure 2   Protocol of sample preparation

# Result

## Analysis of Standard Solution

Figure 3 shows MRM chromatograms of the 18 mycotoxin standards (each 10 ng/mL).
At first, we evaluated the solvent for better recovery of the mycotoxin from MycoSpin™ column.

In comparison, with 85% acetonitrile aqueous solution, better recovery of fumonisins was obtained with 50% acetonitrile aqueous solution (Fig.4). Thus, we decided to use 50% acetonitrile aqueous solution for the extraction solvent.



14:NIV 371.10>281.10(-) CE: 15.0(50.00)
15:PAT 153.00>109.10(-) CE: 13.0(100.00)
16:DON 355.10>295.10(-) CE: 10.0(20.00)
17:FUX 413.10>353.10(-) CE: 9.0(20.00)
1:15-ADON 339.10>261.10(+) CE: -12.0(20.00)
2:3-ADON 339.10>231.10(+) CE: -14.0(10.00)
3:AF G2 331.10>245.10(+) CE: -30.0
4:AF G1 329.10>243.10(+) CE: -27.0
5:DAS 384.20>307.10(+) CE: -13.0
6:AF B2 315.10>259.10(+) CE: -30.0
7:FB1 722.40>334.10(+) CE: -42.0(10.00)
8:AF B1 313.10>241.10(+) CE: -39.0
9:HT-2 442.20>263.10(+) CE: -13.0
10:FB3 706.40>336.10(+) CE: -35.0(20.00)
12:T-2 484.30>185.10(+) CE: -23.0
13:OTA 404.10>239.10(+) CE: -24.0
18:ZEN 317.10>131.10(-) CE: 30.0(10.00)

Figure 3   MRM chromatograms of the 18 mycotoxin standards (each 10 ng/mL).



Figure 4   Recovery (%) of the mycotoxin standard from MycoSpin™ column.
(Each 10 ng/mL standard mixture was applied on the column.)

## Evaluation of the matrix effect

Figure 5 shows recovery (%) of the mycotoxin standards in the four kinds of extraction as wheat, corn powder, peanut powder, and almond powder. MycoSpin™ protocol was convenient in short timescale. However, even after the clean-up, many matrix compounds remained and was

affected.(Fig. 5). Although under this situation, it usually requires each labeled internal standard for target compound, we tried to investigate the quantify by the standard additive method.

# A rapid screening method of mycotoxins in grains by liquid chromatograph tandem mass spectrometry
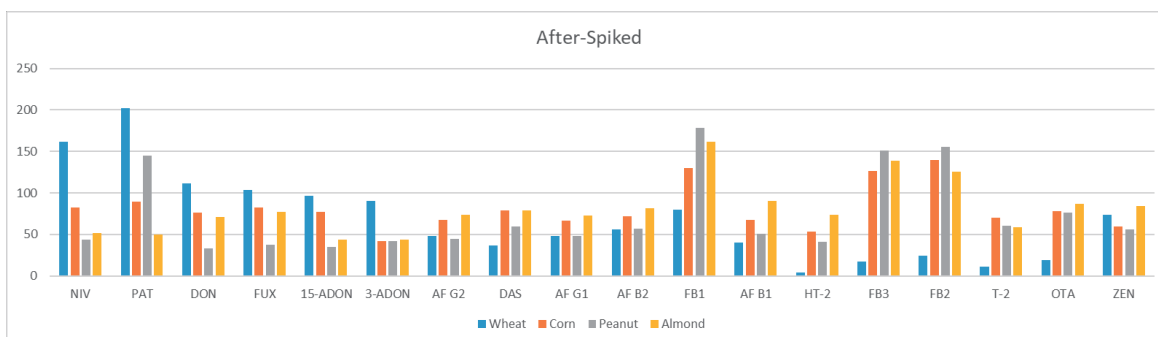


Figure 5   Recovery (%) of the mycotoxins under the four kinds of extractions.
The each standard was spiked after clean-up procedure (final each 10 ng/mL).

## Quantitative analysis

The results of quantitative analysis from wheat and corn powder using the sample preparation protocol (2.2) is summarized in table 2 through using the standard additive method instead of the internal standard method. The results indicates that the standard addition calibration method could help correct and improve the recovery rate even under the influence of the matrix effect.
Using this method, only a small amount of mycotoxins were detected in corn powder below the regulation value.

Table 2   The results of wheat and corn powder

|  | Wheat | | | Corn | | |
|---|---|---|---|---|---|---|
|  | Result (mg/Kg) | Recovery (%) | %RSD (n=2) | Result (mg/Kg) | Recovery (%) | %RSD (n=2) |
| NIV | N.D. | 93 | 0.86 | N.D. | 134 | 2.00 |
| PAT | 0.0350 | 94 | 5.36 | N.D. | 79 | 15.60 |
| DON | 0.0148 | 128 | 6.34 | 0.1376 | 114 | 10.66 |
| FUX | N.D. | 101 | 0.30 | N.D. | 149 | 0.02 |
| 15-AcDON | N.D. | 119 | 10.73 | 0.0213 | 114 | 14.30 |
| 3-AcDON | N.D. | 106 | 7.64 | N.D. | 117 | 8.26 |
| AF G2 | N.D. | 104 | 4.27 | N.D. | 115 | 1.80 |
| DAS | N.D. | 118 | 4.24 | N.D. | 137 | 2.25 |
| AF G1 | N.D. | 101 | 3.45 | N.D. | 119 | 1.11 |
| AF B2 | N.D. | 110 | 0.07 | N.D. | 125 | 5.95 |
| FB1 | N.D. | 60 | 18.33 | 0.0407 | 94 | 15.16 |
| AF B1 | N.D. | 104 | 2.06 | 0.0011 | 124 | 2.32 |
| HT-2 | N.D. | 107 | 9.21 | 0.0008 | 132 | 3.91 |
| FB3 | N.D. | 70 | 19.66 | 0.0078 | 83 | 7.19 |
| FB2 | N.D. | 66 | 6.23 | 0.0094 | 79 | 5.80 |
| T-2 | N.D. | 105 | 4.79 | 0.0005 | 119 | 1.80 |
| OTA | N.D. | 97 | 12.29 | N.D. | 109 | 4.38 |
| ZEN | N.D. | 97 | 0.90 | 0.0120 | 103 | 3.37 |

# A rapid screening method of mycotoxins in grains by liquid chromatograph tandem mass spectrometry
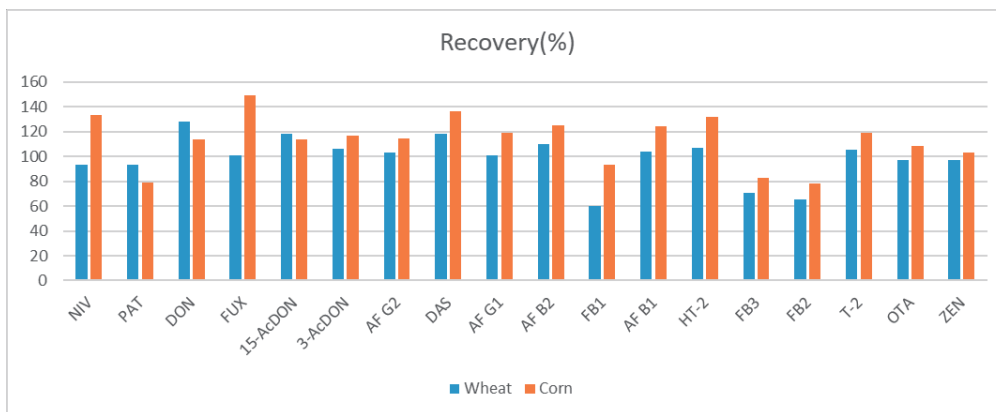


Figure 6   Recovery (%) of the mycotoxin standards from wheat and corn powder
(Each 100 ng standard was spiked)
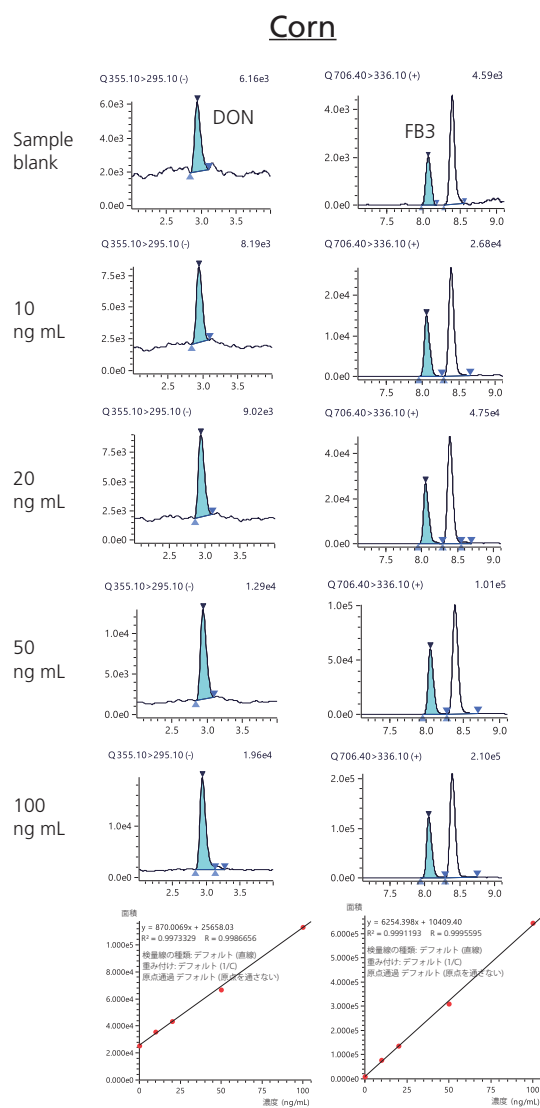
## Corn



Figure 7   Chromatograms and calibration curves of the corn extraction (DON and FB3).

# Conclusions

- A rapid screening method for mycotoxins had been established.
- The LC/MS/MS method package for mycotoxin (Shimadzu Corporation, Japan) is useful tools for this type of analysis.
- The extraction solvent to improve the recovery rate of fumonisins was optimized.
- We investigated the standard addition method in order to compensate for the effect of the matrix.
- We plan to continue to evaluate multi-function columns or ion exchange columns for further improvement of the clean-up.

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# SHIMADZU
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# Application News

## MultiNA

# Qualitative Determination of Edible Corn Oil Using the MultiNA and the PCR Method

Lixiao Li

Analytical Instruments Dept. Analytical Application Center Shimadzu (China) Co., LTD

■ **Abstract:**

Currently, edible oil adulteration is a common practice. This not only affects the physical health of consumers, but also lowers their confidence and has a serious impact on their interests. This paper proposes the design of a PCR primer for the specific gene of the species to be identified by DNA-based molecular biological means because different species have different DNA sequences, and determines the existence and chain length of the PCR amplified product using the MultiNA, thereby establishing a MultiNA-based method for edible oil identification. The specific gene PCR extracted from corn oil was amplified, and the size of its amplified product as determined by the MultiNA was 196 bp, largely consistent with the size of the PCR target product of the corn gene (190 bp). The experimental results indicate that this method can realize the qualitative determination of edible oils.

■ **Introduction**

Together with rice and flour, edible oils are foods essential to people's daily lives. In order to reduce costs and earn extra profits, some illegal traders sell lower-cost edible oils in place of higher-cost types, or substantially blend lower-cost edible oils with higher-cost types. This not only affects the physical health of consumers, but also lowers their confidence and has a serious impact on their interests.Edible oil identification and adulteration detection using molecular biological techniques feature high sensitivity and reliability. A PCR primer is designed to identify the specific gene of the species because different species have different DNA sequences, and the existence and chain ength of the PCR amplified product are determined to realize substance identification.This paper establishes a MultiNA-based method for edible oil identification. A modified reagent kit method was used to extract the corn genome from corn oil. A primer was designed for the corn endogenous gliadin gene, and subject to PCR amplification using PCR instrument. The size of its amplified product based on the MultiNA was 196 bp, largely consistent with 190 bp of the expected product amplified from the primer. The experimental results indicate that through edible oil gene extraction using this method, PCR amplification, and MultiNA determination, edible oil identification can be realized.

■ **1. Experimental Materials and Methods**

**1.1 Instruments**
MCE-202 MultiNA, PCR instrument

## 1.2 Reagents
Vegetable oil gene extraction reagent kit
 (Beijing Kwinbon Biotechnology Co., Ltd.) FZ-002
SYBR ® Premix Ex TaqTM II (Takara Bio Inc.) RR820A
Primers:   5'-TGAACCCATGCATGCAGT-3'
            5'-GGCAAGACCATTGGTGA-3
            ' (primer synthesized by Sangon Biotech)
DNA-500 Reagent Kit for MultiNA
      (Shimadzu Corporation) 292-27910-91
SYBR ® Gold Nucleic Acid Gel Stain (Invitrogen) S-11494
1×TE Buffer
25 bp DNA Ladder (Invitrogen) 10597-011
DNA-500 Reagent Kit for MultiNA
      (Shimadzu Corporation) 292-27910-91
Sample: commercially available edible pure corn oil

## 1.3 Analysis Conditions
DNA-500 on chip mode

## 1.4 Analysis Procedure
**1.4.1 DNA Extraction and Purification in Sample**
**1.4.1.1** Mix the sample oil with the 1x extract in the extraction reagent kit at a ratio of 2:1, and strongly stir the mixture with a magnetic stirrer for 30 minutes. Add the 1x extract obtained after centrifugation to the new sample oil again, and repeat this step. The total amount of sample oil used is 4,000 mL.
**1.4.1.2** Centrifuge the thoroughly mixed mixture at high speeds (at 12,000 rpm for 10 min), then remove the upper oil phase completely, and put the aqueous phase extract in a rotary evaporator for dry concentration at 65 ℃.
**1.4.1.3** Dissolve the freeze-dried matter with 1 mL of 1x extract, extract 0.375 mL of the dissolved matter and place in a 1.5 mL centrifuge tube, add 0.375 mL of extract A, mix, and put in a 65 ℃ water bath for 1 hour.
**1.4.1.4** After bathing, add 0.75 mL of extract B: extract C=1:1 mixture to the tube, mix for 30 seconds, and then centrifuge at 12,000 rpm for 5 minutes.
**1.4.1.5** Pipette the upper aqueous phase into a new 1.5 mL centrifuge tube, add double volume of the pre-cooled anhydrous alcohol (4 ℃), 10 % volume of settling agent 1 and 1.5 mL of settling agent 2, mix, and allow to settle at -20 ℃ for 1 hour.
**1.4.1.6** After settlement, centrifuge at 12,000 rpm for 15 minutes, and pour away the supernatant carefully. At this point, white sediment can be seen at the bottom of the EP tube. This sediment is the extracted DNA.
**1.4.1.7** Add 1 mL of pre-cooled washing solution (4 ℃), flip the EP tube to mix, centrifuge at 12,000 rpm for 5 minutes, then discard the supernatant, and invert the EP tube onto a filter paper for drying.

**1.4.1.8** Add 30 mL of dissolving solution to the dried EP tube for sediment dissolution, and maintain the resultant solution at -20 ℃. Preheat the resultant solution at 65 ℃ for improved dissolution. The resultant solution can be used directly for subsequent PCR determination.

**1.4.2 PCR reaction system**
See Tables 1 and 2 for the PCR reaction reagents and conditions.

**1.4.3 MultiNA Determination**
After PCR amplification, MultiNA determination begins. When the MultiNA is used for high-accuracy DNA fragment length analysis, a ladder analysis should be performed first to prepare a standard curve. When an unknown sample is analyzed, fragment length can be determined from the standard curve as long as its migration time is measured.

Table 1 PCR Reaction Reagents

|  | Amount | Final concentration |
|---|---|---|
| SYBR® Premix Ex Taq II（Tli RNaseH Plus）（2×） | 10.0 µl | 1× |
| PCR Forward Primer（10 mM） | 0.8 µl | 0.4 µM |
| PCR Reverse Primer（10 mM） | 0.8 µl | 0.4 µM |
| DNA template | 2.0 µl | <100 ng |
| dH2O (sterile purified water) | 6.4 µl | |
| Total volume | 20.0 µl | |

Table 2 PCR Reaction Parameters

| Impact | Time/s | Temperature/℃ |
|---|---|---|
| Active DNA enzyme and initial denaturation | 30 | 95 |
| PCR（45 cycles） | | |
| Denaturation | 30 | 95 |
| Annealing | 30 | 55 |
| Extending | 60 | 72 |
| Holding after cycles | 180 | 72 |

## ■ Discussion of Results

Fig. 1 and Fig. 2 are the gel diagram and electropherogram obtained by analyzing the ladder, DNA amplified product extracted from corn oil, positive control (used the corn gene template during PCR) and negative control (did not use the corn gene template during PCR), using the MultiNA, respectively. A remarkable 197 bp band was obtained from the positive control. Since the chain length of the PCR target product was 190 bp, and no corresponding band near this area was obtained from the negative control, the PCR procedure has been executed successfully. The DNA amplified product extracted from corn oil was subject to a MultiNA analysis, and the results indicate that a 196 bp band was detected, showing that this corn oil contains a corn endogenous gene. The fragment length determined using the MultiNA differs slightly from that of the PCR target product. This result is deemed rational in consideration of the instrument error of 5 %.
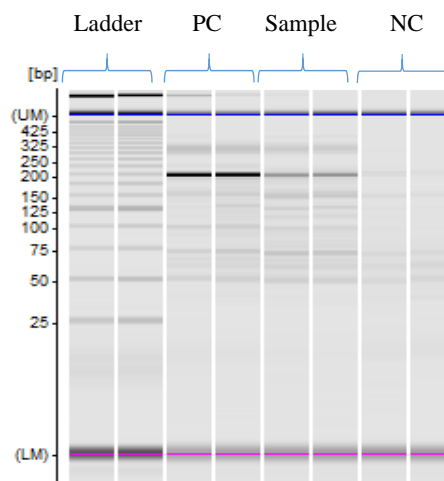


Fig. 1 Gel Analysis Results of Edible Corn Oil
(PC: positive control; NC: negative control)
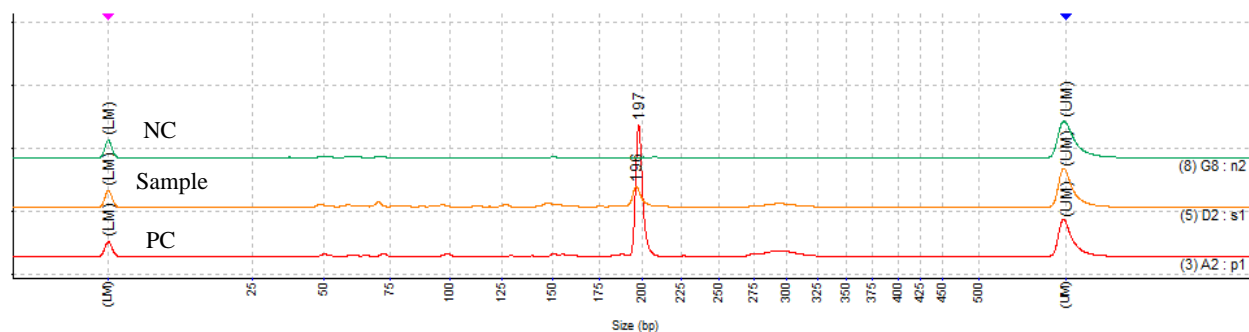


Fig. 2 Electrophoresis Results of Edible Corn Oil  (PC: positive control; NC: negative control)

## ■ Conclusion

This paper establishes a method for the qualitative determination of edible vegetable oils using the Shimadzu MCE-202 MultiNA based on molecular biological technology. For the species identification of edible oils, this method is reliable and easy to operate, and can be applied for the qualitative and quantitative measurement of edible oils in complex systems, thus allowing full exposure of edible oil adulteration.

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# Cost-effective SPE and LC/MS/MS for Analysis of 18 Mycotoxins

<u>Background:</u> Authorities such as European Union (EU) and US Food and Drug Administration (FDA) impose strict regulations on mycotoxins in agriculture products due to their extreme toxicity. There are many mycotoxins that are regulated under the European Union EC/1886/2006. Mycotoxins are extracted and cleaned up by immunoaffinity column or solid phase extraction. Due to their different polarities, different immunoaffinity cartridges or different SPE tubes and conditions must be used for recovering multiple mycotoxins, which is time-consuming and expensive. A cost-effective and reliable approach was developed using Biotage ISOLUTE Myco SPE tubes and LCMS-8060 with a Mastro PFP column. A total of 18 polar and less polar mycotoxins regulated by EU regulation could be effectively extracted and cleaned up using the same SPE tube under two conditions and analysed using the same LC/MS/MS method on LCMS-8060.

<u>Technical approach & solution:</u> the 18 targeted mycotoxins are extracted and cleaned up using ISOLUTE Myco SPE tubes under two conditions for polar (6) and less polar compounds (12) for different crop matrices. The operation of the two conditions is similar and easy as illustrated below. The obtained eluents can be injected to LCMS directly and analysed using a same MRM based method.

### SPE Condition 1 – For polar mycotoxins (6)

1. Powered sample extracted with acidified water. Vortex and centrifuge.
2. Dilution with water.
3. SPE clean-up
   - Add ACN
   - Add water
   - Loading
   - Wash with water
   - Elute with ACN

### SPE Condition 2 – For less polar mycotoxins (12)

1. Powered sample extracted with acidified water and ACN. Vortex and centrifuge.
2. Dilution with water.
3. SPE clean-up
   - Add ACN
   - Add water
   - Loading
   - Wash with water; followed by 10% ACN
   - Elute with acidified ACN and MeOH

Workflow of ISOLUTE Myco SPE for extraction and clean-up for 6 polar and 12 less polar mycotoxins under different conditions

LC conditions for quantitative screening of 18 mycotoxins on LCMS-8060

| | |
|---|---|
| Column: | Shimadzu Mastro PFP (100mmL x 2.1mmI.D, 3 µm) |
| Flow rate: | 0.4 mL/min |
| Mobile phase: | A: 0.15 mM Ammonium fluoride in water<br>B: 0.15 mM Ammonium fluoride in MeOH with 2% acetic acid |
| Oven Temp: | 40°C |
| Injection vol: | 10 µL |
| LC program: | Gradient elution, 12.5 minutes<br>B% : 15% (0.0 to 1.0 min) → 25% (1.0 min) → 40% (2.0 min) → 41% (4.5 min) → 100% (7.5 to 10.0 min) → 15% (10.1 to 12.5 min) |

**Biotage**

**ISOLUTE® Myco**

- One product for multiple mycotoxins analysis
- Quick and easy to use
- No special storage requirements
- Cost-effective

MRM Chromatograms of 18 mycotoxins spiked in barley sample (NIV, DON, FUS-X, NEO, 15-AcDON, and 3-AcDON at 2.5 ng/mL; AFB1 and AFG1 at 0.5 ng/mL; AFB2 and AFG2 at 0.15 ng/mL; DAS, FB1, FB2, FB3, HT-2, T-2, OA and ZON at 25 ng/mL).
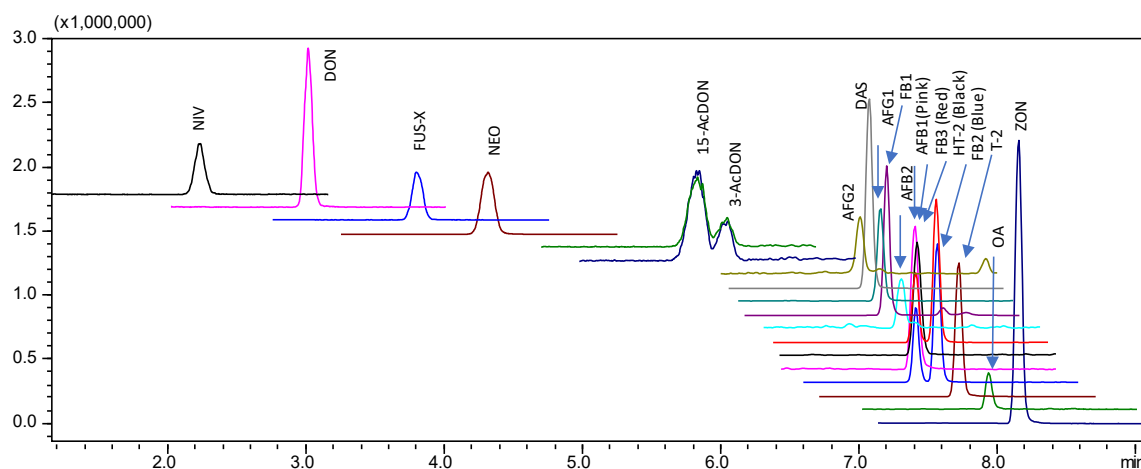


MRM transitions and LOQ and LOD (ng/mL) of 18 mycotoxins spiked in different matrices, dilution factor (DF) = 40 for No. 1~6 and DF = 20 for No. 7~18.

| No | Compound | MRM transition 1 | MRM transition 2 | Barley | | Rice | | Corn | | Wheat Flour | | Cashew | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | LOQ | LOD | LOQ | LOD | LOQ | LOD | LOQ | LOD | LOQ | LOD |
| 1 | NIV | 371.1 > 281.2 | 371.1 > 311.2 | 0.15 | 0.05 | 0.22 | 0.07 | 2.50 | 0.80 | 0.50 | 0.17 | 0.50 | 0.17 |
| 2 | DON | 297.2 > 249.2 | 297.2 > 279.2 | 0.25 | 0.08 | 0.05 | 0.02 | 0.50 | 0.17 | 0.25 | 0.08 | 2.50 | 0.8 |
| 3 | FUS-X | 355.2 > 247.2 | 355.2 > 277.2 | 0.06 | 0.02 | 0.10 | 0.03 | 0.09 | 0.03 | 0.25 | 0.08 | 1.57 | 0.52 |
| 4 | NEO | 400.2 > 305.2 | 400.2 > 215.2 | 0.01 | <0.01 | 0.03 | 0.01 | 0.01 | <0.01 | 0.01 | <0.01 | 0.01 | <0.01 |
| 5 | 15-AcDON | 339.3 > 261.2 | 339.3 > 297.2 | 0.5 | 0.17 | 0.25 | 0.08 | 0.51 | 0.17 | 0.24 | 0.08 | 0.99 | 0.33 |
| 6 | 3-AcDON | 339.2 > 261.2 | 339.2 > 297.2 | 0.44 | 0.15 | 0.50 | 0.17 | 0.63 | 0.21 | 0.27 | 0.09 | 2.50 | 0.8 |
| 7 | AFG2 | 331.2 > 245.1 | 331.2 > 285.1 | 0.15 | 0.05 | 0.02 | 0.01 | 0.08 | 0.02 | 0.03 | 0.01 | 0.10 | 0.03 |
| 8 | DAS | 384.2 > 307.2 | 384.2 > 229.2 | 0.10 | 0.03 | 0.05 | 0.02 | 0.10 | 0.03 | 0.05 | 0.02 | 0.04 | 0.02 |
| 9 | AFG1 | 329.1 > 243.1 | 329.1 > 200.1 | 0.01 | <0.01 | 0.01 | <0.01 | 0.04 | 0.01 | 0.01 | <0.01 | 0.02 | 0.01 |
| 10 | FB1 | 722.4 > 352.4 | 722.4 > 334.3 | 0.24 | 0.08 | 0.35 | 0.11 | 0.2 | 0.06 | 0.26 | 0.09 | 0.50 | 0.17 |
| 11 | AFB2 | 315.2 > 287.1 | 315.2 > 259.1 | 0.03 | 0.01 | 0.01 | <0.01 | 0.03 | 0.01 | 0.03 | 0.01 | 0.03 | 0.01 |
| 12 | AFB1 | 313.1 > 285.1 | 313.1 > 241.1 | 0.01 | <0.01 | 0.01 | <0.01 | 0.02 | 0.07 | 0.02 | 0.01 | 0.03 | 0.01 |
| 13 | HT-2 | 706.4 > 318.3 | 706.40 > 354.4 | 1.00 | 0.33 | 0.04 | 0.01 | 2.13 | 0.70 | 4.85 | 1.60 | 2.50 | 0.8 |
| 14 | FB3 | 447.3 > 345.2 | 447.3 > 285.1 | 0.50 | 0.17 | 0.50 | 0.17 | 0.27 | 0.09 | 0.50 | 0.17 | 0.50 | 0.17 |
| 15 | FB2 | 706.4 > 318.3 | 706.4 > 354.4 | 0.50 | 0.17 | 0.50 | 0.17 | 0.85 | 0.28 | 0.08 | 0.03 | 0.50 | 0.17 |
| 16 | T-2 | 484.30 > 215.2 | 484.3 > 185.1 | 0.29 | 0.09 | 0.03 | 0.01 | 0.20 | 0.06 | 0.05 | 0.02 | 0.45 | 0.15 |
| 17 | OA | 404.2 > 221.0 | 404.2 > 239.1 | 0.05 | 0.02 | 0.08 | 0.03 | 0.31 | 0.10 | 0.05 | 0.02 | 0.05 | 0.02 |
| 18 | ZON | 317.1 > 175.1 | 317.1 > 131.2 | 0.10 | 0.03 | 0.05 | 0.02 | 0.22 | 0.07 | 0.10 | 0.03 | 0.10 | 0.03 |
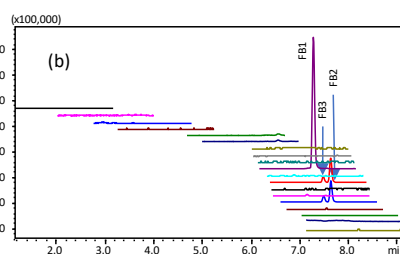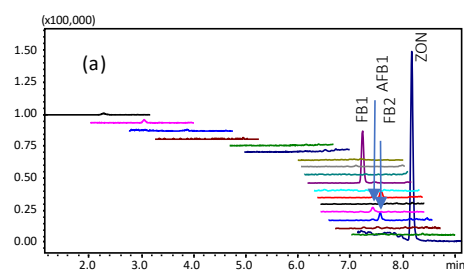


MRM chromatograms of real samples; (a) Barley J1 and (b) Corn G9. The quantitative results of few mycotoxins detected are below the EU limits.

Conclusions: a cost-effective method using ISOLUTE Myco SPE and LC/MS/MS was developed for quantitative screening for 18 mycotoxins regulated by EU regulation. The method performance was evaluated with 5 different crop matrices. The sensitivity of the method is sufficient for detection of the 18 targeted mycotoxins to the limits set by EU regulation.

Reference:

[1] Validation of A Low-Cost and Highly-Sensitive Method for Determination of Eighteen Mycotoxins in Food Matrixes Using SPE and LC/MS/MS, ASMS-2018 poster accepted

# SHIMADZU
## Excellence in Science

**Application Note**

## Testing and Analysis of Genetically Modified Food – Application of MultiNA –

Yoshiyuki Harada

**No.15**



(Photo Above: Natural product, unrelated to text contents)

*LifeScience*

## 1. Introduction

Genetically modified organism (GMO) has burgeoned over the years in order to satiate the global appetite or to add value to natural agriculture products. Technology to increase crop yields has been a constant demand, and the introduction and success of increased agricultural yield by using gene recombinant technology has indeed increased productivity in crop yields. On the other hand, the question arises as to whether these genetically modified food sources safe to eat, and nutritionally beneficial compared to natural products? The necessity to protect consumers while assisting agricultural food producers is a challenge for regulatory agencies globally, requiring that they keep up with quickly evolving technology and increasing genetically modified foods. In this environment, various regulations are conducted in many countries. For example, in Japan, only genetically modified foods that have received approval through safety assessment are permitted to be circulated in the domestic food market.

Domestic consumers tend to avoid such genetically modified foods though many GMO food have been approved by the safety assessment, the cultivation and circulation of foods developed using gene recombinant technology are currently rare in Japan. However, genetically modified organisms are actively cultivated globally, and large quantities of genetically modified organisms and their processed foods spread all over the world. Thus, genetically modified may penetrate gradually into the Japanese market in future.

## 2. Genetically Modified Organism (GMO)

Genetically modified organisms are farm products that breed improvement is introduced by gene recombinant technology. Comparing to conventional methods of hybridization and artificial mutation, the gene recombinant technology transforms plants artificially and enables the introduction of genes from other species and a wider variety of breed improvements in a much shorter period of time.

Various characteristic forms including insect resistance, virus resistance and herbicide tolerance for the improvement of cultivation, high lysine for the increment of nutritive value and high oleic for health promotion are introduced to genetically modified organisms distributed globally.

According to ISAAA[1] 2009 statistics, the cultivation area for genetically modified organisms has reached as much as 134 million hectares. The proportion of genetically modified organisms to whole organisms has reached 77 % for soybeans, 26 % for corn, 21 % for rapeseed, and 49 % for cotton respectively. Genetically modified organisms and their processed food are referred to as genetically modified food. According to the Food Sanitation Act in Japan, the safety assessment of genetically modified foods is mandatory and only foods approved in the assessment are permitted to be imported and circulated.

As of July 2010, genetically modified foods corresponding to 126 varieties of genetically modified organisms have been approved through safety assessments. These include 8 varieties of potatoes, 7 varieties of soybeans, 3 varieties of sugar beets, 70 varieties of corn, 15 varieties of rapeseed, 20 varieties of cotton, and 3 varieties of alfalfa[2].

## 3. Labeling of Genetically Modified Foods in Japan

According to Food Sanitation Act and Japanese Agricultural Standard (JAS) Law[2, 3] (Law Concerning Standardization and Proper Labeling of Agricultural and Forestry Products ~ Law No. 175, 1950), the genetically modified foods which are specified to be labeled as such are shown in Table 1[2, 3]. The items for which food labeling is obligatory include the 7 types of agricultural products along with the 32 types of processed foods containing those products listed in (1) of 1 of Table 1, as well as high oleic soybean and high lysine corn of category 2 in Table 1. The labeling of processed foods (corresponding to (2) of 1 in Table 1) in which genetically modified DNA or resulting protein does not remain after processing, is voluntary. The main raw ingredients of processed foods (those among the top 3 ingredients in terms of weight ratio of all ingredients, and where the weight ratio is at least 5 %) must be included in the label. The labeling of genetically modified foods is summarized in Table 2[2, 3].

The segregation of genetically modified and non-genetically modified agricultural products (foods) is extremely important for labeling of genetically modified products . Whether or not identity preserved handling (IP) has been conducted is specified on the label.

Identity preserved handling refers to the management system in which genetically modified and non-genetically modified foods are segregated through every stage including production, distribution (truck, silo, container ship, etc.), and processing (at processing companies) under the greatest care. Further, its strict management should be confirmed by documents.

The implementation of Identity preserved handling cannot necessarily prevent the mixing of genetically modified foods into non-genetically modified foods.

If Identity preserved handling for soybean or corn is conducted and its mixing rate of genetically modified organism into non-genetically modified organism (GMO content) is less than 5 %, this Identity preserved handling is regarded as proper. The subject of the GMO content is described further on page 7.

[References]
1) ISAAA (International Service for the Acquisition of Agri-biotech Applications), http://www.isaaa.org
2) Department of Food Safety, The Ministry of Health, Labour and Welfare, Japan, http://www.mhlw.go.jp/english/topics/foodsafety/dna/index.html
3) Ministry of Agriculture, Forestry and Fisheries, Japan, "Food Labeling for Processed Foods", http://www.maff.go.jp/e/jas/labeling/modified.html

**Table 1 Foods to Be Labeled as Genetically Modified Food in Japan**

| 1 | Produce whose composition and nutritional value are similar to that of conventional produce | | |
|---|---|---|---|
| | (1) Agricultural products and processed foods containing genetically modified DNA or protein even after processing | | |
| | Agricultural Products | Soybean (including green soybeans and soybean sprouts), Corn, Potato, Rapeseed, Cottonseed, Alfalfa, and Sugar beet | |
| | Processed Foods | Items subject to labeling | Ingredient to be labeled |
| | | 1. *Tofu* (soybean curd) and fried *tofu* | Soybean |
| | | 2. Dried soybean curd, soybean refuse, *yuba* | |
| | | 3. *Natto* (fermented soy beans) | |
| | | 4. *To-nyu* (Soy milk) | |
| | | 5. *Miso* (soybean paste) | |
| | | 6. Cooked soy bean | |
| | | 7. Canned or bottled soybean | |
| | | 8. *Kinako* (roasted soybean fluor) | |
| | | 9. Roasted soybean | |
| | | 10. Item containing food of items 1 to 9 as a main ingredient | |
| | | 11. Item containing soybeans (for cooking) as a main ingredient | |
| | | 12. Item containing soybean flour as a main ingredient | |
| | | 13. Item containing soybean protein as a main ingredient | |
| | | 14. Item containing *edamame* (green soybean) as a main ingredient | *Edamame* |
| | | 15. Item containing soybean sprouts as a main ingredient | Soybean sprouts |
| | | 16. Corn snacks | Corn |
| | | 17. Corn starch | |
| | | 18. Popcorn | |
| | | 19. Frozen corn | |
| | | 20. Canned corn or bottled corn | |
| | | 21. Item containing corn flour as a main ingredient | |
| | | 22. Item containing corn grits as a main ingredient (except corn flakes) | |
| | | 23. Item containing corn (for cooking) as a main ingredient | |
| | | 24. Item containing food of items 16 to 20 as a main ingredient | |
| | | 25. Frozen potato | Potato |
| | | 26. Dried potato | |
| | | 27. Potato starch | |
| | | 28. Potato snacks | |
| | | 29. Item containing food of items 25 to 28 as a main ingredient | |
| | | 30. Item containing potatoes (for cooking) as a main ingredient | |
| | | 31. Item containing alfalfa as a main ingredient | Alfalfa |
| | | 32. Item containing sugar beet as a main ingredient | Sugar beet |
| | (2) Processed foods in which genetically modified DNA or resulting protein does not remain after processing ( e.g., soybean oil, soy source, corn oil, isomerized liquid sugar) | | |
| 2 | Produce whose composition or nutritional value is markedly different from that of conventional produce (high oleic acid soybeans, high lysine corn) | | |

Table 2  Labeling of Genetically Modified Foods in Japan

| | Classification | | Labeling Example | Labeling |
|---|---|---|---|---|
| 1 | Produce whose composition and nutritional value are similar to that of conventional produce | | | |
| | (1) Agricultural products and processed foods containing genetically modified DNA or resulting protein even after processing (corresponding to 7 agricultural products and 32 processed food categories in Table 1) | GM agricultural products under the identity preserved handling or processed foods made form those | "GMO segregated from non-GMO", "GMO" | Mandatory |
| | | Agricultural products, not segregated GM products and non-GM products, or processed foods made from those the identity preserved handling2 or processed foods made form those | "Not segregated from GMO" | Mandatory |
| | | non-GM agricultural products under the identity preserved handling or processed foods made from those | "Non-GMO segregated from GMO", "Non-GMO" | Voluntary |
| | (2) Processed foods in which genetically modified DNA or resulting protein does not remain after processing (e.g., soybean oil, soy source, corn oil, isomerized liquid sugar) | | "Non-GMO segregated from GMO", "Non-GMO" | Voluntary |
| 2 | Produce whose composition or nutritional value is markedly different from that of conventional produce(high oleic acid soybeans, high lysine corn) | | "soybeans (high oleic, genetically modified)" | Mandatory |

# 4. Testing and Analysis of Genetically Modified Foods in Japan

The standard tests and methods used for analysis of genetically modified foods are specified in "Testing for Foods Produced by Recombinant DNA Techniques"[1], notifications concerning inspection and guidance of imported foods[2] – [5] by The Ministry of Health, Labour and Welfare, and "JAS analytical test handbook"[6] by Food and Agricultural Materials Inspection Center.
Table 3 shows the genetically modified foods that are subject to testing, and test methods to be used. Both approved and unapproved genetically modified foods based on safety assessment are subject to the testing.
As of July, 2010, papaya (55-1), corn (CBH351), corn (Bt10), corn (DAS59132), rice (LLRICE601), rice (Bt), and rapeseed (RT73 B.rapa) have not been unapproved by the safety assessment.
The tests can be classified to qualitative testing to determine the presence or absence of genetically modified organisms (GMO) and quantitative testing to determine the ratio of genetically modified organisms to non-genetically modified organisms (GMO content). The methods adopted for qualitative testing include lateral flow immunoassay, qualitative PCR, and the GUS gene test, while quantitative PCR and ELISA (enzyme-linked immunosorbent assay) are adopted for quantitative testing.

Table 3  Testing Methods for Genetically Modified Foods in Japan

| Food Product | Genetically Modified Gene | Test Type | Test Method | References |
|---|---|---|---|---|
| Papaya (raw or processed) | Papaya (55-1) | Qualitative test | Qualitative PCR, GUS gene test | 1) |
| Corn (grain) | Corn (CBH351) | | Lateral flow immunoassay | |
| Corn (partially processed) | | | Lateral flow immunoassay, Qualitative PCR | |
| Corn (processed) | | | Qualitative PCR | |
| Corn (grain) | Corn (Bt10) | | | |
| Corn (partially processed) | | | | |
| Corn (grain) | Corn (DAS59132) | | | |
| Corn | Corn (GA21) | Quantitative test or Qualitative test / Quantitative test | Quantitative PCR Qualitative PCR / Quantitative PCR | 1) 6) |
| | Corn (Event176) | | | |
| | Corn (Bt11) | | | |
| | Corn (T25) | | | |
| | Corn (Mon810) | | | |
| Soybean | Soybean (Roundup Ready Soybean) | Quantitative test or Qualitative test / Quantitative test | Quantitative PCR Qualitative PCR / Quantitative PCR | 1) 6) |
| Soybean | CP4EPSPS protein | Quantitative test | ELISA | 1) |
| Rice | Rice (LLRICE601) | Qualitative test | Qualitative PCR | 3) |
| Rice | Rice (Bt) | | | 4) |
| Rapeseed | Rapeseed (RT73 B.rapa) | | | 5) |
| Potato | Potato (New Leaf) | | | 6) |
| | Potato (New Leaf Plus) | | | |

[References]
1) The Ministry of Health, Labour and Welfare, Japan, "Testing for Foods Produced by Recombinant DNA Techniques",
   http://www.mhlw.go.jp/english/topics/food/sec05-1a.html
2) http://www.mhlw.go.jp/english/topics/importedfoods/index.html
3) Department of Food Safety, The Ministry of Health, Labour and Welfare, Japan, Notification No. 0915002, September 15, 2006.
4) Department of Food Safety, The Ministry of Health, Labour and Welfare, Japan, Notification No. 0220002, February 20, 2007.
5) Department of Food Safety, The Ministry of Health, Labour and Welfare, Japan Notification No. 0914 -5, September 14, 2009.
6) "Japanese Agricultural Standard (JAS) analytical test handbook:
   genetically modified food quality, labeling analysis manual for individual products, 2nd revision (2002)", Food and Agricultural Materials Inspection Center, Japan.

Table 4 presents an overview of analysis methods for testing genetically modified foods. Since the ELISA and lateral flow immunoassay methods are based on antigen-antibody reactions, they are not applicable to testing for processed foods because antigenicity is lost due to protein denaturation during heat processing, etc. DNA exhibits superior stability to protein because DNA has better thermal stability and is more tolerant to decomposition and denaturation upon heating or other processes.

On the other hand, qualitative PCR is applicable for testing of both agricultural products and processed foods due to the high possibility of target gene amplification by PCR. However, quantitative PCR cannot be applied to determination of recombinant gene content in processed foods, as discussed later in this document.

Table 4  Analysis Methods Used for Testing Genetically Modified Foods

| Analysis Method | Overview |
|---|---|
| ELISA (Enzyme-Linked Immunosorbent Assay) | It is used for quantitative analysis or qualitative analysis (detection) of antigens and antibodies in a sample. It utilizes the high specificity of the antigen-antibody reaction and the high sensitivity of enzymatic reactions. |
| Lateral Flow Immunoassay | This is one type of immunochromatography that uses the antigen-antibody reaction as in the ELISA method. Here, a drop of sample is applied to a test strip, and as it migrates along the strip by capillary action, the presence or absence of an antigen in the sample is determined based on comparison of the color patterns in the test line and a control line. When the a sample including the target antigen passes through a zone including dyed antigen-specific antibodies, complex between antigen in the sample and dyed antibody (antigen- dyed antibody complex) is formed. Antigen-specific antibody is fixed in the test line zone, and it binds to the antigen- dyed antibody complex. The control line includes an antibody that binds dyed-antibody. If antigen is present in the sample, both the test line and control line are colored. If there are no antigens present in the sample, only the control line is colored. |
| GUS Gene Test | A β-glucuronidase (GUS) gene might be introduced along with an exogenous gene for use as an indicator (reporter) of genetic recombination. In this type of gene recombination, the GUS gene is expressed along with the recombinant gene, making it possible to confirm the success of recombination process based on the presence or absence of GUS activity. In the GUS gene test, the reagent containing the substrate 5-bromo-4-chloro-3-indolyl-β-D-glucuronide (X-Gluc) is added, and GUS activity is confirmed by the appearance of blue coloring. |
| Qualitative PCR | PCR (Polymerase Chain Reaction) is a technique in which a specific region of a DNA from template DNA is selectively amplified. In PCR, two single chain DNA fragments (primer pair) which are complimentary to both ends of the specific region to be amplified are used. An enzyme for DNA synthesis (DNA polymerase) is also added to reaction tube and a cycle reaction (dissociation of double-strand DNA to single-strand DNA ( Denaturation) $\rightarrow$ primers binding to each strand (Annealing) $\rightarrow$ DNA synthesis (Elongation ) is repeated to amplify the specific gene region selectively.<br>Thus, in principle, the specific gene region is amplified by a factor of 2 during each reaction cycle. In qualitative PCR, PCR is conducted to detect a target gene region included in a DNA template extracted from the sample, and the obtained amplification product (PCR product) is subjected to electrophoretic analysis. If the target gene region is included in the extracted DNA, the PCR product corresponding to the target gene region will be detected. |
| Quantitative PCR | In quantitative PCR, PCR is conducted to amplify the specific gene region using a template DNA extracted from a sample and its amplification process is monitored just-timely.<br>A fluorescent compound (intercalator) which can bind with double-stranded DNA, or a fluorescent marker probe to enable recognition a specific part of the amplification region is added to allow monitoring of the amplification products at each cycle. Analysis of the obtained amplification curve allows determination of the quantity (number of copies) of the target genes. |

Fig. 1 shows an example of the procedure used for analysis of genetically modified food using qualitative PCR. The sample is pulverized, and the DNA is extracted using an extraction kit. The DNA concentration of the extracted DNA is determined using the BioSpec-nano Ultraviolet-Visible spectrophotometer for life sciences, and PCR is conducted using a specified quantity of extracted DNA as the template. Electrophoretic analysis of the obtained PCR products is conducted using the 'MCE-202' MultiNA microchip electrophoresis system, and the presence or absence of PCR products corresponding to the target region is confirmed.

Due to extremely high analysis sensitivity, qualitative PCR can detect even minute levels of modified genes in extracted DNA.When Identity preserved handling is implemented, the permissible genetically modified organism (GMO) content to non-GMO is 5 %.
However, recombinant gene is often detected even though the GMO content is below 5 %. When recombinant DNA is detected by qualitative PCR, quantitative PCR testing will be conducted to determine the GMO content. DNA extracted from the sample is used as the template in quantitative PCR, and PCR is conducted using a primer to detect recombinant and endogenous genes.
The number of copies of recombinant and endogenous genes in the extracted DNA can be determined by analysis of the quantitative PCR amplification curve. The GMO content and internal standard ratio are defined according to Equation 1 and 2, respectively. When the content of genetically modified species exceeds 5 % in identity preserved handling foods labeled as "non-GM (non-Genetically Modified)" or "Non-GM segregated from GM", a close inspection of the identity preserved handling is necessary. It should be mentioned that since the degradation rates of recombinant genes and endogenous genes are not necessarily same in processed food, obtaining the GMO content accurately by quantitative PCR method is impossible.

Sample

DNA extraction

Extracted DNA

Nucleic acid quantitation using BioSpec-nano
UV-VIS spectrophotometer

PCR

PCR products

Electrophoretic analysis using
MultiNA

Detection of recombinant genes

Fig. 1  Example of Procedure for Analysis of Genetically Modified Food by
Qualitative PCR

GMO content (%) = {(number of copies of the recombinant gene) / (number of copies of internal standard genes)} × (1 / Internal standard ratio) × 100     (Equation 1)
Internal standard ratio = (Number of recombinant gene in pure genetically modified agriculture product) / (Number of internal gene in pure genetically modified agriculture product)     (Equation 2)

[Reference]
"Japanese Agricultural Standard (JAS) analytical test handbook:
genetically modified food quality, labeling analysis manual for individual products, 2nd revision(2002)", Food and Agricultural Materials Inspection Center, Japan.

## 5. Introduction of Tools for Optimizing Inspection and Analysis of Foods by Qualitative PCR

### MCE-202 MultiNA Microchip Electrophoresis System

Agarose gel electrophoretic analysis requires a great deal of time and effort to conduct a series of operations including the mixing of reagents, preparing the gel, conducting electrophoresis, acquiring an image of the results, and post-cleanup. In addition, its data has a tendency to show inferior sensitivity, resolution and quantitation performance.

The MCE-202 MultiNA microchip electrophoresis system solves these problems of agarose gel electrophoresis all at once since the system is based on brand-new, superior platform and fully automated.



Fig. 2  MultiNA

**MultiNA Features**

**High Analysis Performance**

Compared with agarose gel electrophoresis, the microchip electrophoretic analysis with the MultiNA delivers excellent sensitivity, separation, repeatability and quantitation performance.

**Automated Operation for up to 120 Analyses**

Simply set up the samples and the separation buffer for automated analysis of up to 120 analyses. The parallel processing for analysis pretreatment and electrophoresis permits a processing speed of just 80 seconds per analysis.[1]

**Maximum Ease of Use**

Analysis operation with the MultiNA is extremely simple. Just set up the analysis schedule, and then simply load the reagents and samples and click the [Start] button.

**Reduce Analysis Costs**

The reusable, high-performance microchip achieves lower running costs per analysis than agarose gel electrophoresis.

[1] When 4 microchips are used in DNA standard analysis (for example, DNA-1000 Kit / Premix mode), this does not include the time required for the initial rinse and final rinse, and the initial analysis.

### BioSpec-nano UV-VIS Spectrophotometer for Life Sciences

To successfully guide the PCR reaction to completion, confirming the DNA concentration of the extracted DNA and checking of DNA purity using OD ratio (OD260/280) are indispensable. Conducting analysis with UV-VIS spectrophotometers which use conventional cuvettes is both labor intensive and time consuming due to the required rinsing and drying of the cuvettes.

The BioSpec-nano, which incorporates a cuvette-free optical system, an innovative, automatic mounting mechanism, and an automatic wiping mechanism, offers simple fast and simple nucleic acid analysis of 1 to 2 µL samples. High throughput analysis is achieved, requiring a mere 15 seconds to complete one analysis.



Fig. 3  BioSpec-nano

**BioSpec-nano Features**

**Drop and Click Analysis**

DNA concentration and purity can be checked by just dropping the sample on the target, and clicking the button. Measurement and wiping are both handled automatically by the instrument.

**Nucleic Acid Quantitation of 1 to 2 µL Samples**

Sample volumes of 1 µL (0.2 mm optical path length) and 2 µL (0.7 mm optical path length) can be measured.

**Simple and Quick Analysis**

Blank measurement, sample measurement, report output in PDF or CSV format, and other basic operations are conducted simply and quickly just by clicking a button.

**Support for Wide Range of Analyses**

Nucleic acid quantitation, quantitation of nucleic acid labeled for micro-array, protein quantitation by OD280, and labeled protein quantitation are all supported.

# 6. Examples of Qualitative PCR Analysis of Genetically Modified Foods Using the MultiNA
## 6.1 Analysis of Genetically Modified Corn (MON810)

Here we introduce an analysis of genetically modified corn (MON810) as an example of genetically modified food analysis. After extracting DNA from 3 powdered samples consisting of genetically modified corn (MON810) having GMO content of 0 %, 1 % and 5 %, respectively, the extracted DNA from each of the samples was used as a template. PCR was then conducted using a primer for endogenous gene *SSIIb*-3 detection[2] and a primer for the genetically modified MON810 detection[3]. The electrophoretic analysis of PCR product using the MultiNA are shown in Fig. 4. In analysis of the PCR products using the primer for the endogenous gene *SSIIb*-3 detection, the PCR product (114 bp) corresponding to *SSIIb*-3 was detected in all of the samples except for the negative control. The endogenous gene *SSIIb*-3 is a gene that is specific to corn, and detection of the endogenous gene in a sample means PCR testing of the recombinant gene in that sample is effective. On the other hand, in analysis of the PCR products using the primer for the genetically modified MON810 detection, the PCR product (113 bp) corresponding to MON810 was detected in the 1 % and 5 % GMO content samples, as well as in the positive control sample.

Gel Image



L : 25 bp DNA Ladder
1-3 : MON810 0 % GMO content sample
4-6 : MON810 1 % GMO content sample
7-9 : MON810 5 % GMO content sample
10 : Negative control (without template DNA)
11 : Negative control (without primer)
12 : Positive control plasmid
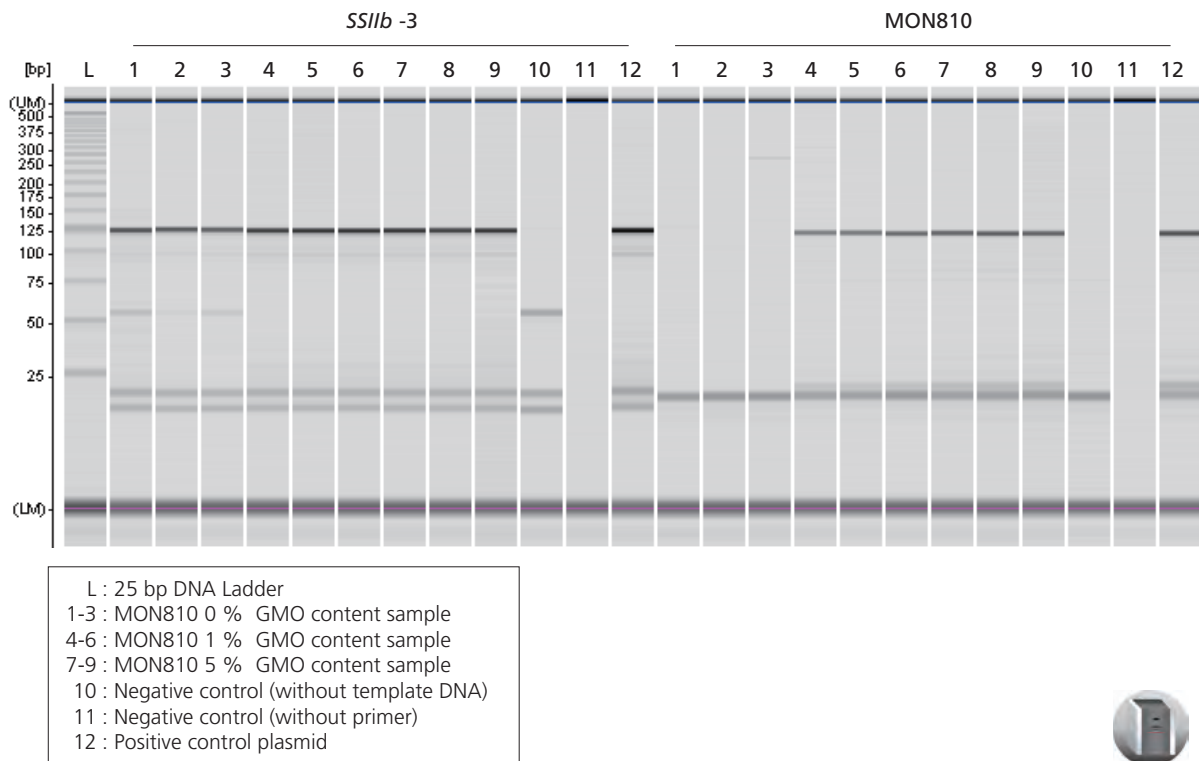
Fig. 4 Analysis of Genetically Modified Corn (MON810) Using MultiNA

[References]
1) Shimadzu Application News No. B29, Qualitative Analysis of Genetically Modified Corn by Standard Method with MCE-202 "MultiNA"
2) The Ministry of Health, Labour and Welfare, Japan, "Testing for Foods Produced by Recombinant DNA Techniques",
   http://www.mhlw.go.jp/english/topics/food/sec05-1a.html
3) "Japanese Agricultural Standard (JAS) analytical test handbook:
   genetically modified food quality, labeling analysis manual for individual products, 2nd revision (2002)", Food and Agricultural Materials Inspection Center, Japan.

## 6.2 Detection of Genetically Modified DNA (GA21) in Processed Corn

Here we introduce an example of analysis of genetically modified DNA in processed corn using qualitative PCR. DNA was extracted from 4 types of processed corn products (2 types of canned corn, 1 type of popcorn, 1 type of corn starch), and the DNA extracted from each of the samples was used as a template. PCR was conducted using a primer for the endogenous gene *SSIIb* detection and a primer for the genetically modified GA21 detection. Next, the obtained PCR products were analyzed using the MultiNA. The analysis results are shown in Fig. 5. In PCR using the primer for the endogenous gene *SSIIb* detection, the PCR product (151 bp) corresponding to *SSIIb* was detected in all of the processed food samples and in the positive control plasmid. If damage to DNA derived from processed food is considerable due to heating during processing,the endogenous genes will not be detected. In samples where the endogenous gene is not detected, qualitative testing for genetically modified genes in these samples is regarded as invalid. On the other hand, in analysis of the PCR products using the primer for the genetically modified GA21 detection, the PCR product (133 bp) corresponding to GA21 was detected only in the positive control plasmid, and was not detected in the 4 types of processed food samples, which were labeled as " Non-GMO"

As shown in the electropherograms of the negative and positive controls, the *SSIIb* gene (151 bp) and GA21 gene (133 bp) are clearly detected using only 20 copies of the positive control plasmid. Thus, high-sensitivity qualitative PCR analysis of genetically modified food is clearly achieved using the MultiNA.
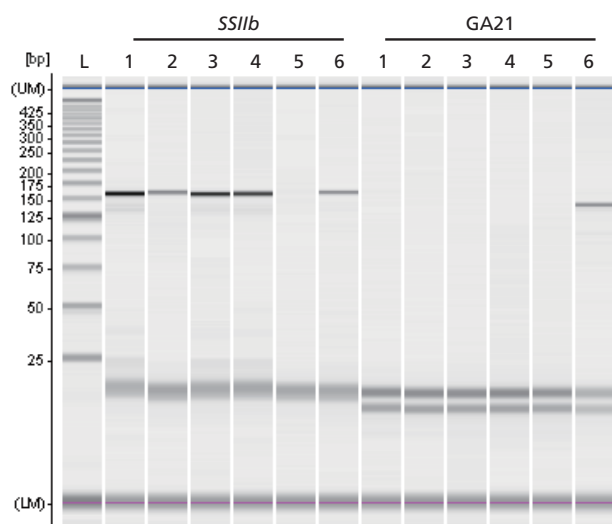


**Gel Image**

L : 25 bp DNA Ladder
1-4 : 4 types of processed corn food samples
5 : Negative control (without template DNA)
6 : Positive control plasmid (20 copies)
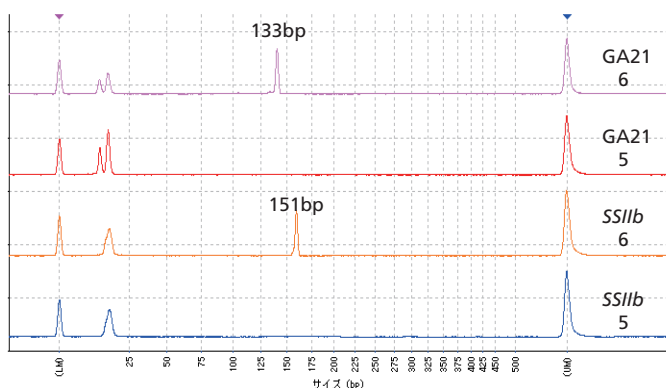
**Electropherograms**

5 : Negative control (without template DNA)
6 : Positive control plasmid (20 copies)

**Fig. 5  Analysis of Genetically Modified Gene (GA21) in Processed Corn Food Products Using MultiNA**

[Reference]
"Japanese Agricultural Standard (JAS) analytical test handbook:
genetically modified food quality, labeling analysis manual for individual products, 2nd revision (2002)", Food and Agricultural Materials Inspection Center, Japan.

## 6.3 Analysis of Genetically Modified Foods Unapproved by Safety  Assessment in Japan

Some genetically modified foods that are unapproved by Japanese safety assessment, are permitted to be circulated in global market.

These particular foods are subject to qualitative inspection in Japan (Table 3). Positive control plasmids and primers according to official inspection methods are commercially available, making it possible to conduct analysis for modified DNA in these foods by the qualitative PCR method.

Examples of analysis of 3 types of modified DNA that were unapproved by the safety inspection as of July, 2010, including corn (CBH351)[1], papaya (55-1)[1], and rice (Bt)[2], are shown below. PCR was conducted using the respective positive control plasmids as templates, and the primers specified in the respective test methods for detection and identification. The obtained PCR products were analyzed using the MultiNA, and the results shown in Fig. 6 were obtained. The PCR products corresponding to the respective target genes and the primers can be verified.

Gel Image



L : 25 bp DNA Ladder
1 : Corn (CBH351) positive control plasmid,
    detection primer, target PCR product (170 bp)
2 : Corn (CBH351) positive control plasmid,
    identification primer, target PCR product (171 bp)
3 : Papaya (55-1) positive control plasmid,
    detection primer, target PCR product (207 bp)
4 : Papaya (55-1) positive control plasmid,
    identification primer, target PCR product (250 bp)
5 : Rice (Bt) positive control plasmid,
    detection primer, target PCR product (147 bp)
6 : Rice (Bt) positive control plasmid,
    identification primer, target PCR product (120 bp)

Fig. 6  Analysis of Genetically Modified Corn (CBH351), Papaya (55-1), Rice (Bt) using MultiNA

[References]
1) The Ministry of Health, Labour and Welfare, Japan, "Testing for Foods Produced by Recombinant DNA Techniques",
   http://www.mhlw.go.jp/english/topics/food/sec05-1a.html
2) Department of Food Safety, The Ministry of Health, Labour and Welfare, Japan, Notification No. 0220002 of February 20, 2007.

MCE®-202 MultiNA is not available in the United States.
*This document is based on information valid at the time of publication. It may be changed without notice.

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# Application News

# No. AD-0162

*Halal Authentication Analysis / IRTracer-100*

## Quantitative Determination of Lard Adulteration by FTIR Spectroscopy with Chemometrics Method - Vegetable Palm Oil

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## ❑ Introduction

Food adulteration is a persistent problem which could occur either accidentally or intentionally. Among the food products, edible oil is the most prone to adulteration [1] and this poses a major concern in terms of economical and religious point of view. For example, the Islamic law prohibits Muslims from consuming pork in any form, including lard in food products [2]. Therefore, it is necessary to develop analytical techniques to identify and quantify lard adulterated edible oil.

Fourier Transform Infrared (FTIR) spectroscopy is an effective technique to differentiate fats and oils as different compounds have unique fingerprint region in the infrared (IR) region. FTIR spectroscopy in combination with chemometrics data analysis such as multi-linear regression (MLR) or partial least squares (PLS) regression is a fast and simultaneous quantitative analysis of multi-component. In this application news, we introduce a method for quantitative determination of lard adulterated vegetable palm oil using FTIR spectroscopy and PLS quantitative calibration model.

## ❑ Experimental

Pork lard and commercially available palm oil were purchased from local markets. The lard was extracted based on the procedure by Rohman and Che Man [3]. Adipose tissue of pork was cut into small pieces and heated at 90-100°C for 2 hours. The melted fat was strained through sieve cloth and dried by addition of anhydrous $Na_2SO_4$. The extracted fat was centrifuged at 3000 rpm and for 20 minutes at 30°C. The fat layer was decanted and centrifuged again, followed by filtering through filter paper to remove solid residue.

A set of 13 standards containing 1-90% (w/w) lard in palm oil was prepared. A PLS calibration model for lard was established with 11 of these standards using LabSolutions IR workstation with Chemometrics PLS function. The remaining 2 standards were used as samples for quantitative determination.

The sample was measured with horizontal type attenuated total reflection attachment (HATR) with zinc selenide (ZnSe) prism. Each sample was measured 3 times. The IR spectra were acquired in the wavenumber range from 4000 cm$^{-1}$ to 650 cm$^{-1}$. The measurement conditions are shown in Table 1.

Table 1: Instruments and Analytical Conditions

| | |
|---|---|
| Instruments | : IRTracer-100, ATR-8200H (ZnSe) |
| Resolution | : 4.0 cm$^{-1}$ |
| Accumulation | : 45 |
| Apodization | : Happ-Genzel |
| Detector | : DLATGS |

## ❑ Results and Discussion

Figure 1 shows the IR spectra of palm oil, lard and 50% w/w lard in palm oil. The IR spectra for palm oil and lard were quite similar. This is due to the similar chemical compositions between palm oil and lard. A closer examination of the IR region in the range of 1500-1000 cm$^{-1}$ revealed slight differences in terms of peak intensities ratio at around 1160 cm$^{-1}$, 1117 cm$^{-1}$ and 1097 cm$^{-1}$ as marked by arrows in the overlay spectrum of palm oil and lard (Figure 2).
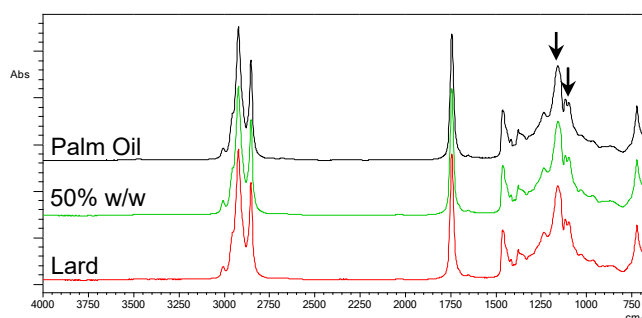


Figure 1: IR spectra of palm oil, lard and 50% w/w lard in palm oil.
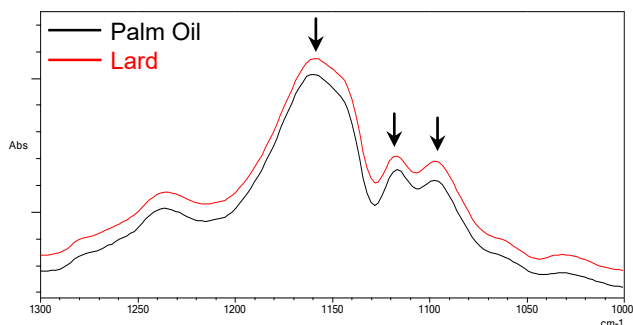
Figure 2: Overlay spectrum of lard and palm oil

Second derivative spectra were used in the PLS data analysis for better resolution of overlapping and shoulder peaks. Table 2 and Figure 3 show the PLS calibration parameter and result of lard in palm oil. A good square correlation coefficients of more than 0.999 was obtained for the PLS calibration modelling with low Mean Squared Error of Prediction (MSEP) and Standard Error of Prediction (SEP) as shown in Table 2.

Table 2: PLS calibration parameters of lard in palm oil

| Calibration Table | |
|---|---|
| Algorithm | PLS I |
| Number of references | 33 (three measurement per sample) |
| Range (cm$^{-1}$) | 1000 – 1490 |
| Pre-process | Derivative, Order = 2, Points = 15 |
| Scale | Autoscale |
| Number of factors | 5 |
| Square of correlation coefficient (R$^2$) | 0.9993 |
| MSEP | 0.0007 |
| SEP | 0.0258 |



Actual versus predicted for lard r$^2$ = 0.9993
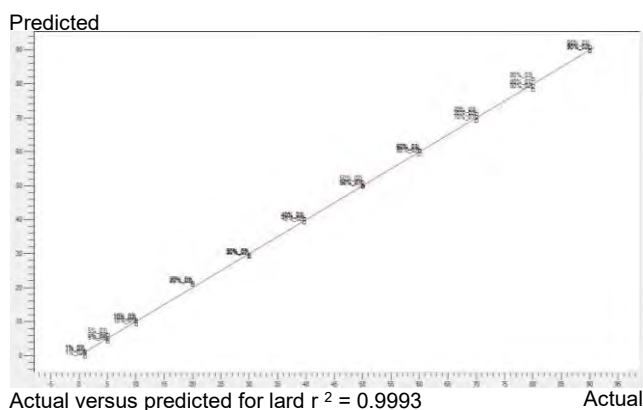
Figure 3: PLS calibration for lard predicted versus actual values.

Table 3 shows the quantitation results of lard in various types of edible oils by PLS method. The measurement results are within ±10% of the expected values for lard in palm oil. For lard in olive oil and palm soy oil, the mean predicted values differed greatly from the expected values. This is due to the difference in sample matrix of the edible oils.

Table 3: Predicted values of lard in different edible oils

| Edible Oil | | Brand A Palm Oil | | Brand B Palm Oil | | Olive Oil | Palm Soy Oil |
|---|---|---|---|---|---|---|---|
| Expected Value (% w/w) | | 8.0 | 25.0 | 8.0 | 25.0 | 25.0 | 25.0 |
| Predicted Value (% w/w) | 1 | 8.90 | 24.08 | 9.06 | 26.1 | 64.41 | 32.32 |
| | 2 | 8.24 | 23.54 | 7.60 | 25.82 | 64.56 | 32.13 |
| | 3 | 8.60 | 24.43 | 8.36 | 26.18 | 62.95 | 33.66 |
| | Mean | 8.58 | 24.02 | 8.34 | 26.03 | 63.97 | 32.70 |
| Recovery (%) | | 107.2 | 96.1 | 104.2 | 104.1 | 255.9 | 130.8 |

❑ **Conclusions**

FTIR spectroscopy in combination with PLS data analysis is a rapid technique which has potential in determination of lard adulteration in palm oil without excessive sample pre-treatment. In this application news, a percentage of verification sample within ±10 % of the expected value was obtained. For lard adulteration in other types of edible oils, respective PLS calibration models have to be prepared to account for the difference in sample matrix.

❑ **References**

1. Johnson, R. (2014). Food Fraud and economically motivated adulteration of food and food ingredients. Congressional Research Services R43358, 1–40.
2. Regenstein, J. M., Chaudry, M. M. and Regenstein, C.E. (2003). The kosher and halal food laws. Comprehensive Reviews in Food Science Food Safety 2: 111–127.
3. Rohman, A. and Che Man, Y. B. (2009). Analysis of cod-liver oil adulteration using FTIR spectroscopy. Journal of the American Oil Chemists' Society 86: 1149–1153.

# An Ultrafast LC/MS/MS Method for Characterization and Quantitation of Triton X-100 Extracted From Palm Oil

## ASMS 2017  TP 712

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# Introduction

Triton X-100, oligomers of octylphenol ethoxylate (OPEOn), is predominantly used as detergent in laboratory, industry and household [1]. The chain lengths of ethoxy units (n) of the oligomers ranged from 0 to 20 (Figure 1). Analysis of octylphenol ethoxylates by GC, HPLC and LC/MS methods were reported previously for monitoring the residues in river and wastewater due to their potential toxicity towards aquatic ecosystem [2-4]. In the last decade, oil-bearing crops has gained much attention for use as a raw material for biofuel. Palm oil (*Elais guineensis*) is one of the commercialized and most profitable oil-bearing crops to date. It has been widely used as a traditional cooking oil in Southeast Asia and Africa and commonly found in processed food, makeup, toothpaste and cleaning products. There is inquiry recently if Triton X-100 is present in edible palm oil and food products due to its involvement in the extraction process for palm oil production. We describe here an ultrafast LC/MS/MS method for sensitive analysis of Triton X-100 in palm oil samples.
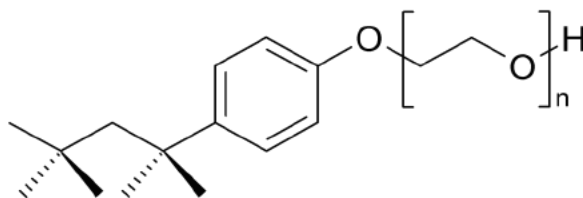


Figure 1.  Chemical structure of the octylphenol ethoxylates (OPEOn) investigated in this study.

# Experimental

Triton X-100 standard was acquired from Sigma Aldrich. An edible palm oil was obtained from local supermarket and utilized as matrix for method development. A customized liquid-liquid extraction (LLE) using acetonitrile-saturated hexane (1:1, v/v) was employed for the extraction of Triton X-100. The sample pretreatment procedure and LC/MS/MS method are described in Figure 2 and Table 1, respectively.
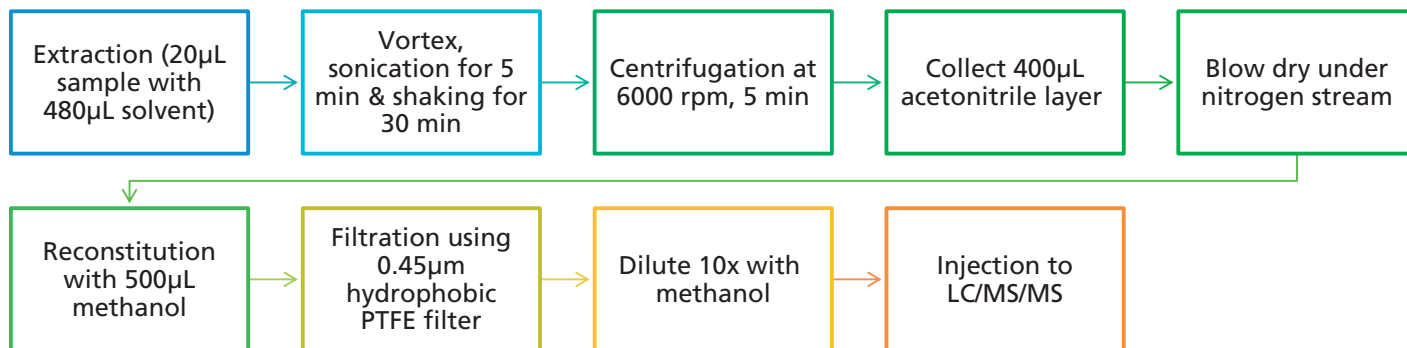


Figure 2.  Flow chart of sample pretreatment.

# An Ultrafast LC/MS/MS Method for Characterization and Quantitation of Triton X-100 Extracted From Palm Oil

Table 1.  Analytical conditions of Triton X-100 on LCMS-8060.

| | |
|---|---|
| Column | : Kinetex C18 100Å (50 x 2.1 mm, 1.7 μm) |
| Flow rate | : 0.3 mL/min |
| Mobile phase | : A: Water with 2mM ammonium acetate |
| | B: Acetonitrile with 2mM ammonium acetate |
| Elution mode | : Gradient elution, 0min (50% B) → 0.01-2.5mins (100% B) |
| | → 2.51-3.00mins (100% B) → 3.01-3.50mins (50% B) |
| | → 3.51-5.00 mins (50% B) |
| Oven temp. | : 30 °C |
| Injection vol. | : 5.0 μL |
| Interface & temp. | : ESI, 300 °C |
| MS mode | : Positive, MRM |
| Block temp. | : 400 °C |
| DL temp. | : 250 °C |
| CID gas | : Ar (270 kPa) |
| Nebulizing gas flow | : $N_2$, 2 L/min |
| Drying gas flow | : $N_2$, 15 L/min |
| Heating gas flow | : 0 air, 10 L/min |

# Results and Discussion

## Development of LC/MS/MS method

A MRM-based method for quantitation of Triton X-100 was developed. The OPEOn species forms ammonia adduct ion $[M+NH_4]^+$ under the mobile phase and ESI conditions. Using an automated MRM optimization program on LCMS-8060, each OPEOn adduct ion was subjected to optimize for highest intensity for every MRM transition. It was observed that the most intensive MRM transition of OPEOn species (n = 3~20) is not the transition losing ammonia. Two MRM transitions were selected for each OPEOn species, with the higher intensity one as the quantifier (Table 2) and the other for confirmation. A total of eighteen species of OPEOn with n = 3~20 were analysed. A characteristic pattern of peak distribution with OPEO8 peak highest is observed. The RT of the species decreases with the number of ethoxy units (*n*). The total MRM chromatograms (sum of two MRMs for each species) of the 18 OPEOn are displayed in Figure 3.
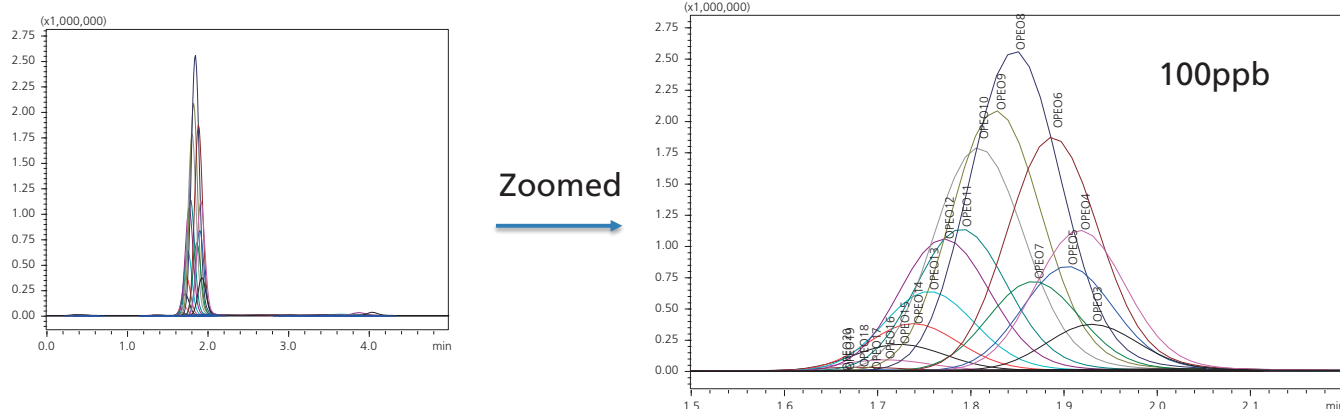


Figure 3.  Total MRM chromatograms (each is the sum of two individual MRMs of the OPEOn) of 18 species of Triton X-100 standard (n = 3~20) at 100 ppb.

## Quantitation performance with edible palm oil spiked sample

The MRM-based calibration curves (Figure 4) was established with palm oil spiked samples. Effective extraction of the OPEOn from palm oil matrix was found challenging in the study. Various extraction procedures including LLE, solid phase extraction (SPE) and dispersive SPE (dSPE) were applied and compared to extract Triton X-100 from palm oil. The extraction efficiency was determined with spiked samples. Triton X-100 standard dissolved in methanol was spiked into palm oil at low, mid, and high concentrations (8, 40 and 80 ppb). A LLE with acetonitrile-saturated with hexane (1:1, v/v) produced the best recovery ranged from 74.4 to 127.8% and without severe matrix effect (75.5 to 136.6%).
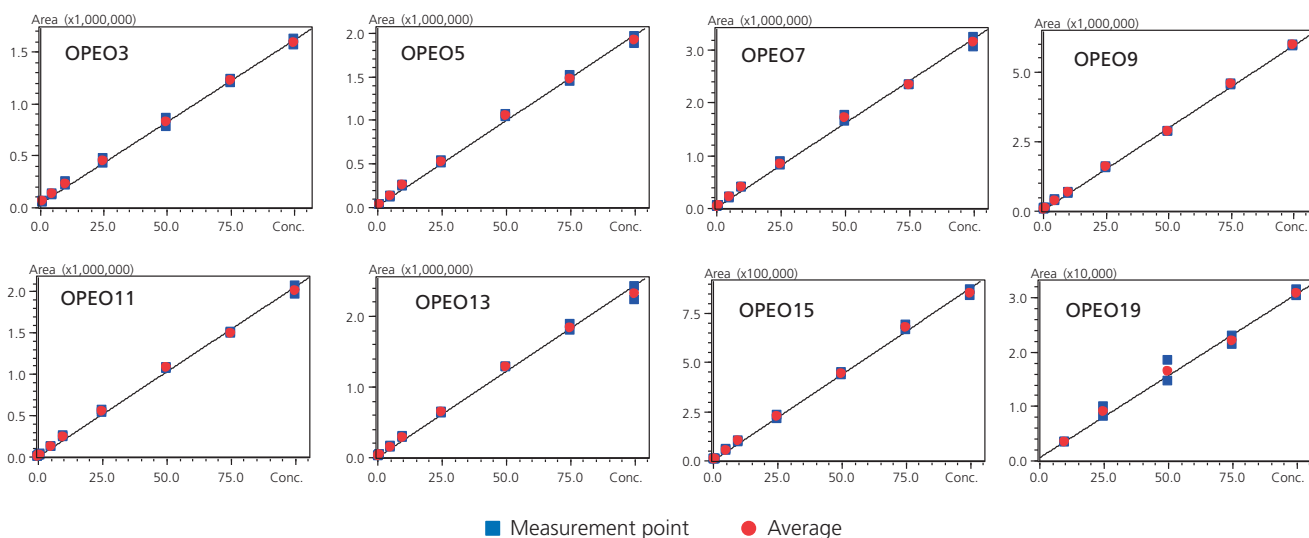


Figure 4.  Representative calibration curves of OPEO species in oil matrix (two replications, $n$=2).

The calibration curves were built using palm oil matrix spiked with Triton X-100 standard. Linear relationship with $R^2 \geq 0.994$ were obtained for all OPEO species across minimum five quantitation points from 0.5 or 1 ppb to 100 ppb for most OPEO species except for the species of n = 17, 19 and 20 (Table 2).

Most of the OPEO species exhibited a detection limit (LOD) based upon S/N > 3 in the range of 0.02~0.8 ng/mL level and a quantitation limit (LOQ) method based upon S/N > 10 of approximately lower than 3 ng/mL, except for OPEO17, OPEO19, and OPEO20. The reduced sensitivity for OPEO with a higher number of ethoxy units is probably because of poorer ionization and fragmentation efficiency of the molecules. The repeatability of the method was evaluated at 50 ppb for all OPEO species. The RSD of most species were under 8% across six repetitive injections, except for three OPEO species (OPEO17, 19, &20). The results are summarized in Table 2.

# An Ultrafast LC/MS/MS Method for Characterization and Quantitation of Triton X-100 Extracted From Palm Oil

Table 2. Calibration results of Triton X-100 standard in edible palm oil matrix (two replications, *n*=2).

| No | Triton X-100 species | MRM transition for quantitation | RT (min) | LOQ (ppb) | LOD (ppb) | Range (ppb) | Linearity ($R^2$) | Repeatability at 50 ppb* | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Area RSD (%) | Conc. RSD (%) |
| 1 | OPEO3 | 365.25>227.15 | 1.93 | 0.21 | 0.07 | 1-100 | 0.998 | 6.09 | 6.35 |
| 2 | OPEO4 | 400.25>271.10 | 1.92 | 1.25 | 0.41 | 1-100 | 0.999 | 3.97 | 4.04 |
| 3 | OPEO5 | 444.25>133.05 | 1.91 | 1.29 | 0.43 | 1-100 | 0.997 | 3.63 | 3.68 |
| 4 | OPEO6 | 488.25>471.25 | 1.89 | 0.65 | 0.22 | 1-100 | 0.997 | 1.79 | 1.81 |
| 5 | OPEO7 | 532.35>277.20 | 1.87 | 0.81 | 0.53 | 0.5-100 | 0.997 | 4.75 | 4.79 |
| 6 | OPEO8 | 576.35>559.35 | 1.85 | 0.85 | 0.28 | 0.5-100 | 0.998 | 2.93 | 2.95 |
| 7 | OPEO9 | 620.40>603.40 | 1.83 | 0.59 | 0.2 | 0.5-100 | 0.998 | 4.66 | 4.7 |
| 8 | OPEO10 | 664.40>133.10 | 1.81 | 0.07 | 0.035 | 0.5-100 | 0.998 | 5.52 | 5.55 |
| 9 | OPEO11 | 708.40>177.10 | 1.79 | 0.06 | 0.02 | 0.1-100 | 0.997 | 3.07 | 3.08 |
| 10 | OPEO12 | 752.45>133.05 | 1.78 | 0.09 | 0.03 | 0.5-100 | 0.997 | 3.72 | 3.75 |
| 11 | OPEO13 | 796.50>133.00 | 1.76 | 0.67 | 0.22 | 0.5-100 | 0.997 | 4.3 | 4.3 |
| 12 | OPEO14 | 840.50>133.10 | 1.74 | 0.64 | 0.21 | 0.5-100 | 0.997 | 3.38 | 3.4 |
| 13 | OPEO15 | 884.55>133.05 | 1.75 | 2.17 | 0.72 | 0.5-100 | 0.998 | 4.39 | 4.4 |
| 14 | OPEO16 | 928.60>88.95 | 1.71 | 1.15 | 0.38 | 0.5-100 | 0.995 | 6.43 | 6.43 |
| 15 | OPEO17 | 972.60>146.95 | 1.70 | 24.9 | 8.21 | 5-100 | 0.997 | 8.19 | 8.2 |
| 16 | OPEO18 | 1016.70>89.00 | 1.68 | 2.3 | 0.76 | 1-100 | 0.997 | 4.58 | 4.6 |
| 17 | OPEO19 | 1060.80>133.25 | 1.67 | 15.9 | 5.25 | 10-100 | 0.994 | 17.4 | 17.9 |
| 18 | OPEO20 | 1104.80>133.20 | 1.66 | 69.9 | 23.1 | 50-250 | 0.999 | 11.16 | 11.46 |

*6 replications, n=6

# LC/MS/MS analysis of crude palm oil

The above method was applied to a crude palm oil sample obtained from a research lab to detect the presence of Triton X-100 residues, which might be used in the extraction process of production. The matrix of crude palm oil is more complex compared to that of edible palm oil sold in supermarket. Thus, in addition to the blank crude palm oil sample, spiked samples with Triton X-100 at two concentrations (5 ppb and 50 ppb) were also  analyzed to compare the baselines, interference and LC/MS/MS analysis results. The same sample extraction and purification procedure shown in Figure 2 was applied for the crude palm oil sample.

# An Ultrafast LC/MS/MS Method for Characterization and Quantitation of Triton X-100 Extracted From Palm Oil

Table 3. Results of crude palm oil analysis (blank)

| No | Triton X-100 species | Crude palm oil blank sample (non spiked)* | |
|---|---|---|---|
| | | Area | Conc. (ppb) |
| 1 | OPEO3 | 24931 | ND** |
| 2 | OPEO4 | 22300 | ND** |
| 3 | OPEO5 | 30932 | 0.68 |
| 4 | OPEO6 | 17996 | ND** |
| 5 | OPEO7 | 7761 | ND** |
| 6 | OPEO8 | 14118 | ND** |
| 7 | OPEO9 | 7176 | ND** |
| 8 | OPEO10 | 7152 | ND** |
| 9 | OPEO11 | 1399 | ND** |
| 10 | OPEO12 | 2278 | ND** |
| 11 | OPEO13 | - | ND |
| 12 | OPEO14 | - | ND |
| 13 | OPEO15 | - | ND |
| 14 | OPEO16 | - | ND |
| 15 | OPEO17 | - | ND |
| 16 | OPEO18 | - | ND |
| 17 | OPEO19 | - | ND |
| 18 | OPEO20 | - | ND |

*three replications, $n$=3
**under detection limit (S/N < 3)
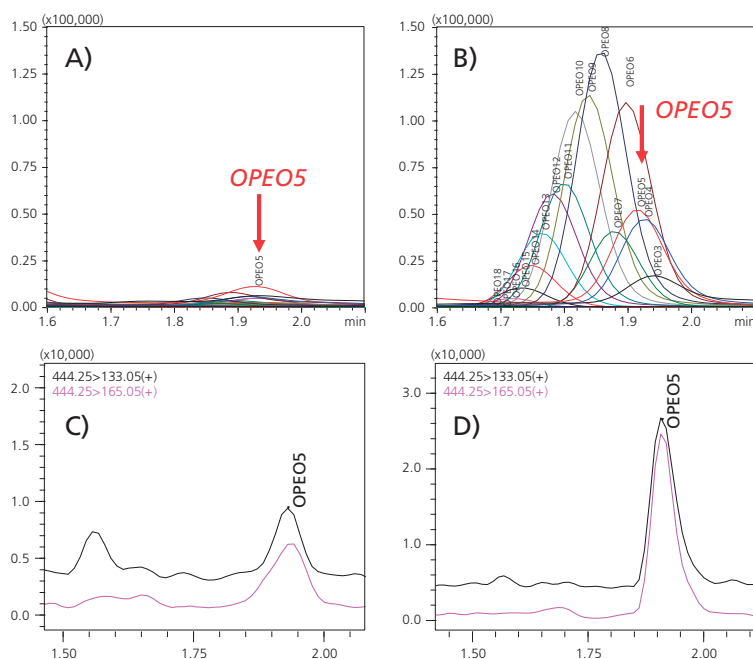


Figure 5.  Total MRMs of crude palm oil (blank) (A) and spiked with 5ppb standard (B).
Individual MRMs of OPEO5 of crude palm oil (blank) (C) and spiked with 5ppb standard (D).

The quantitative results indicate the presence of OPEO5 at very low content (~0.68 ppb) in the crude palm oil sample (Table 3 & Figure 5). However, this level is lower than the quantitation limit of the method. Despite shown with various peak area values, other 9 Triton X-100 species are considered not detected because the levels are below the detection limit (S/N < 3). The characteristic of distribution pattern of Triton X-100 species are also not observed thus the targeted Triton X-100 is unlikely presence in the sample.

## Conclusions

A MRM-based method for characterization and quantitation of Triton X-100 has been established using a Kinetex C18 100Å column on LCMS-8060. A total of 18 Triton X-100 species (n = 3~20) was analysed within 5 minute running time using gradient elution program. With remarkable system sensitivity at the level of 3 ppb or less for most of Triton species, this ultrafast LC/MS/MS method is applicable to screen and quantitate the residue of Triton X-100 in crude palm oil.

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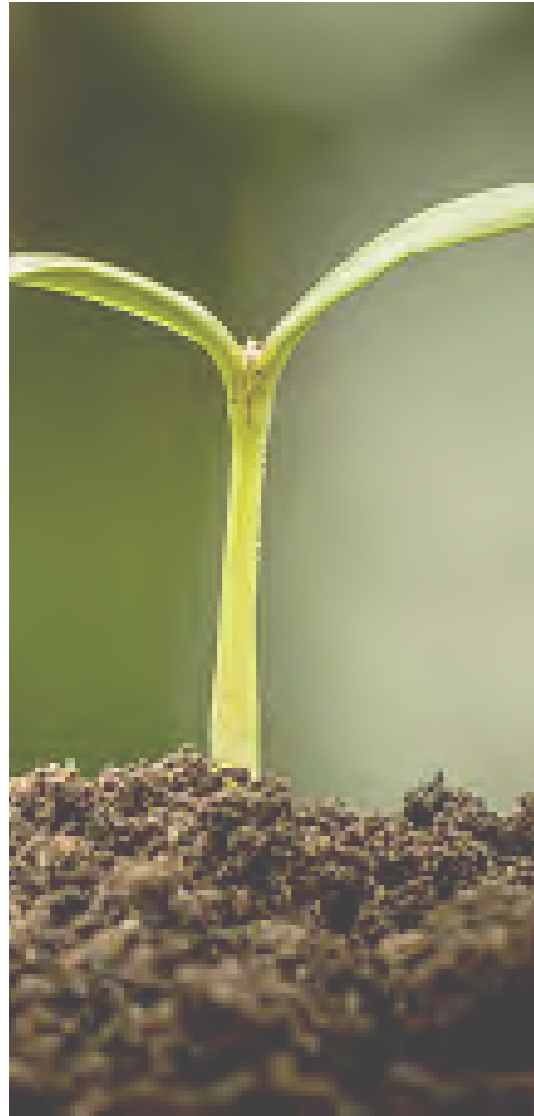
# Fertilizers

1. Analysis of Melamine and its Related Substances in Fertilizers

2. Analysis of Nitrous Acid and Ammonium Thiocyanate in Fertilizers

3. Multi-Residue Analysis of Antibiotics in Organic Fertilizers using the Ultra-High Performance Liquid Chromatography coupled with Triple Quadrupole Mass Spectrometry

4. Analysis of Sulfamic Acid in Fertilizers using LC/MS (LCMS-2020)

# SHIMADZU
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# Application News

### No.L514

**High Performance Liquid Chromatography**

## Analysis of Melamine and Its Related Substances in Fertilizers

Calcium cyanamide is effective as a fertilizer, pesticide, soil amendment, and for many other uses, and it is essential compound for producing high quality vegetables. Recently, high levels of melamine were discovered as a byproduct in some calcium cyanamide hydrate products, pelletized by adding water to calcium cyanamide. Due to the risk of agricultural products absorbing the melamine from the soil, it has been identified as a potential public health risk. For example, if both melamine and its related substance cyanuric acid are ingested at the same time, they can form crystals that can impede kidney function.[1]

As a result, the Food Safety and Consumer Affairs Bureau in the Ministry of Agriculture, Forestry and Fisheries in Japan issued a notice specifying a 0.4 % provisional maximum allowable concentration of melamine in calcium cyanamide.[2]

This article describes an example of pretreating and analyzing melamine and its related substances, namely ammeline, ammelide, and cyanuric acid, in fertilizer, in accordance with the testing methods supervised by the Food and Agricultural Materials Inspection Center (FAMIC) in Japan for fertilizers and other substances (2016, 8.1.c). [3], [4], [5]

### ■ Analysis of Standard Solution

The structure of melamine and its related substances is shown in Fig. 1. The analytical conditions are indicated in Table 1. The chromatogram of the standard mixture solution of melamine and its related substances (1 mg/L each) is shown in Fig. 2. For more details regarding the procedures used to prepare the standard solution and mobile phase, refer to the applicable test methods. Calibration curves for melamine and its related substances are shown in Fig. 3. Calibration curves were prepared for a concentration range of 0.05 to 5 mg/L. The results indicated good linearity, with a contribution rate ($R^2$) over 0.9999.

| Substance | $R_1$ | $R_2$ | $R_3$ |
|-----------|-------|-------|-------|
| Cyanuric acid | OH | OH | OH |
| Ammelide | OH | OH | $NH_2$ |
| Ammeline | OH | $NH_2$ | $NH_2$ |
| Melamine | $NH_2$ | $NH_2$ | $NH_2$ |
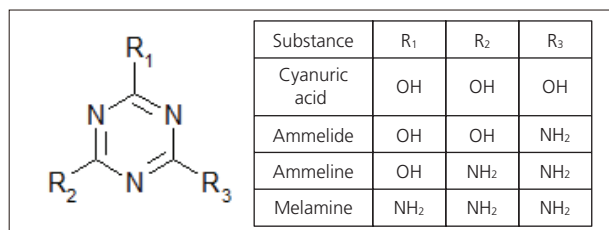
**Fig. 1  Chemical Structure of Melamine and Its Related Substances**

**Table 1  Analytical Conditions**

| | |
|---|---|
| System | : Prominence |
| Column | : TOSOH, TSKgel Amide-80 (250 mm L. × 4.6 mm I.D., 5 µm) |
| Guard Column | : TOSOH, TSKgel guardgel Amide-80 (15 mm L. × 3.2 mm I.D.) |
| Mobile Phase | : (Sodium) phosphate buffer pH 6.7±0.2 / Acetonitrile = 1/4  (v/v) |
| Flowrate | : 1.0 mL/min |
| Column Temp. | : 40 °C |
| Injection Vol. | : 10 µL |
| Detection | : UV-VIS detector (SPD-20A) at 214 nm |
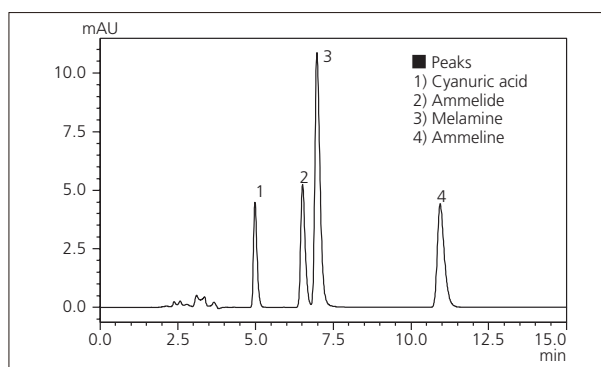
**Fig. 2  Chromatogram of Standard Mixture (1 mg/L each)**

Peaks
1) Cyanuric acid
2) Ammelide
3) Melamine
4) Ammeline
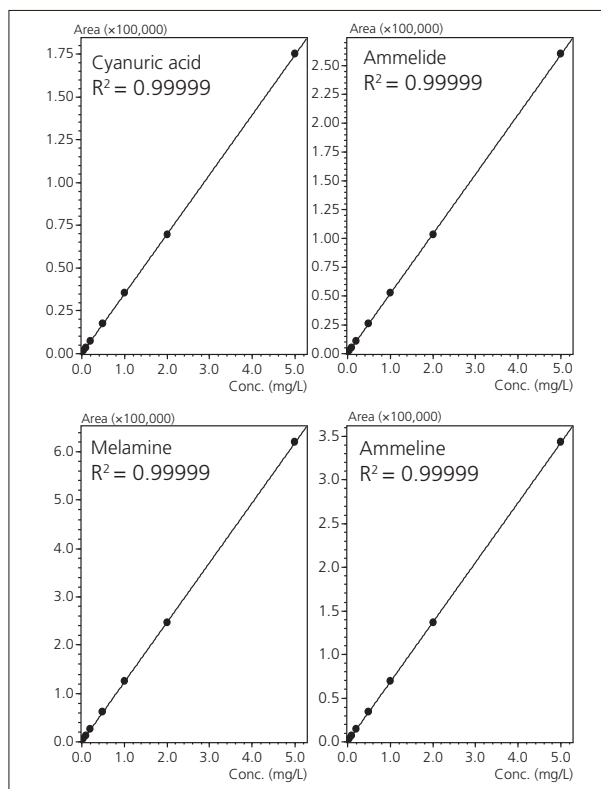
**Fig. 3  Linearity (0.05 to 5 mg/L)**

### ■ Repeatability

The relative standard deviation (%RSD) results for peak area after analyzing the standard solution (0.1 mg/L) six consecutive times were very good, with 0.41 % for cyanuric acid, 0.42 % for ammelide, 0.52 % for melamine, and 0.56 % for ammeline. When pretreated as indicated in Fig. 4, the 0.1 mg/L concentration of the standard solution is equivalent to a 0.02 % concentration of melamine and other related substances in fertilizer.

## Analysis of Melamine and its Related Substances in Fertilizer

The analytical sample (fertilizer) was pretreated in accordance with the test method by adding a standard quantity of melamine and its related substances. The pretreatment procedure is indicated in Fig. 4 and the analytical results are shown in Fig. 5. In this example, five types of samples were tested, including two types of commercially available nitrolime, a synthetic fertilizer that contains calcium cyanamide, a synthetic fertilizer, and ammonium sulfate. The quantities of the substances added to the analytical samples, as a percentage of mass, were equivalent to about 0.035 to 2.8 % melamine, about 0.035 to 1.6 % ammeline, about 0.035 to 1.1 % ammelide, and about 0.037 to 1.2 % cyanuric acid. These results demonstrate that the Prominence system provides more than adequate performance for measuring the provisional 0.4 % melamine limit issued by the Food Safety and Consumer Affairs Bureau, Ministry of Agriculture, Forestry and Fisheries for calcium cyanamide and fertilizers that contain calcium cyanamide as an ingredient.
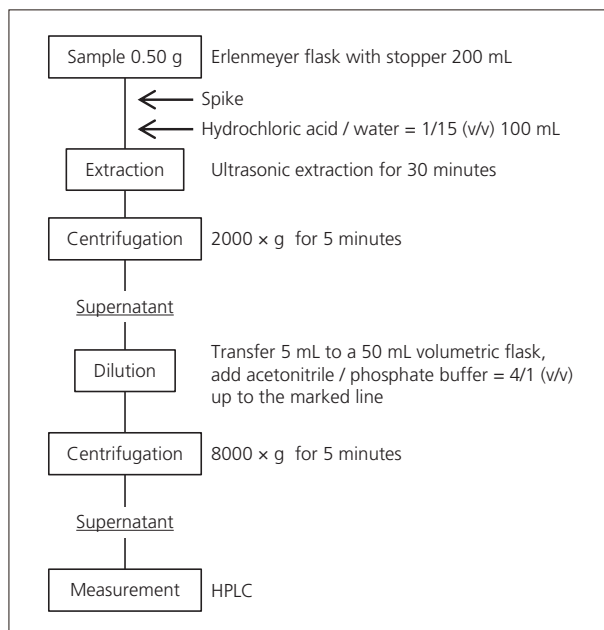


**Fig. 4  Pretreatment Procedure**
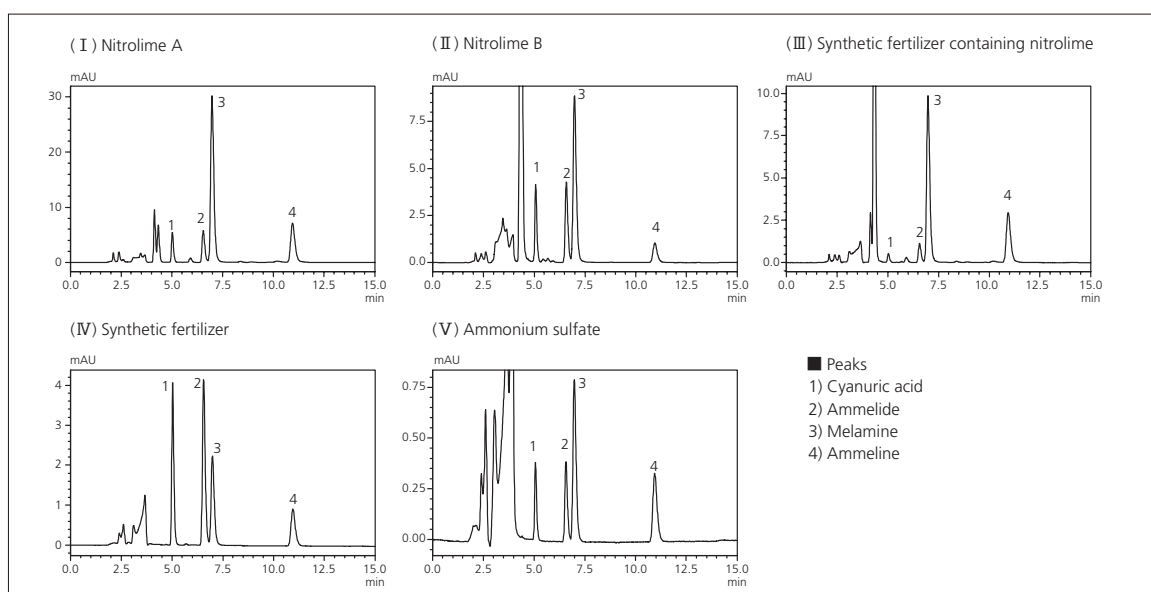


**Fig. 5  Chromatograms for (Ⅰ) Nitrolime A, (Ⅱ) Nitrolime B, (Ⅲ) Synthetic Fertilizer Containing Calcium Cyanamide, (Ⅳ) Synthetic Fertilizer, and (Ⅴ) Ammonium Sulfate**

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3) Testing Methods for Fertilizers (2016), Food and Agricultural Materials Inspection Center (FAMIC): http://www.famic.go.jp/ffis/fert/obj/shikenho_2016.pdf (in Japanese)
4) Etsuko Bando and Yuji Shirai: Validation of High Performance Liquid Chromatography (HPLC) for Determination of Melamine and Its Related Substances in Fertilizer, Research Report of Fertilizer Vol. 6, pp. 27 to 35 (2013)
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# SHIMADZU
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# Application News

**High Performance Liquid Chromatography**

## Analysis of Nitrous Acid and Ammonium Thiocyanate in Fertilizers

# No.L513

After it is spread onto agricultural land, nitrogen fertilizer converts to nitrate-nitrogen or nitrite-nitrogen and leaches into subsoil and river water polluting the groundwater. Drinking water with a high nitrate-nitrogen or nitrite-nitrogen content is a potential public health risk. It causes methemoglobinemia that inhibits the oxygen-carrying capacity of blood and has caused deaths in infants outside Japan.

Fertilizers with high concentrations of nitrous acid and ammonium thiocyanate have a negative effect on plant growth, therefore maximum content levels (permitted content levels) for toxic substances are prescribed in official specifications for commercial fertilizers according to the Fertilizer Control Law[1].

An example of simultaneous analysis of the nitrous acid and ammonium thiocyanate content of fertilizer by HPLC is described. Analysis was performed in conformance with the test method that appears in Testing Methods for Fertilizers (5.8.b and 5.9.a, 2016)[2], published by the Food and Agricultural Materials Inspection Center (FAMIC).

### ■ Analysis of a Standard Mixture

Analytical conditions are shown in Table 1. A chromatogram of a standard mixture of nitrous acid and ammonium thiocyanate (20 mg/L each) is also shown in Fig. 1. Please refer to the test method[2] for details on mobile phase preparation. Standard solution was prepared by dissolution and dilution with water. An NH2 column was used for analysis.

**Table 1  Analytical Conditions**

| | |
|---|---|
| System | : Prominence |
| Column | : Shodex Asahipak NH2P-50 4E (250 mm L. × 4.6 mm I.D., 5 µm) |
| Guard Column | : Shodex Asahipak NH2P-50G 4A (10 mm L. × 4.0 mm I.D., 5 µm) |
| Mobile Phase | : (Sodium) phosphate buffer containing sodium perchlorate |
| Flowrate | : 1.0 mL/min |
| Column Temp. | : 40 °C |
| Detection | : UV-VIS detector (SPD-20AV) at 210 nm |
| Injection Vol. | : 10 µL |
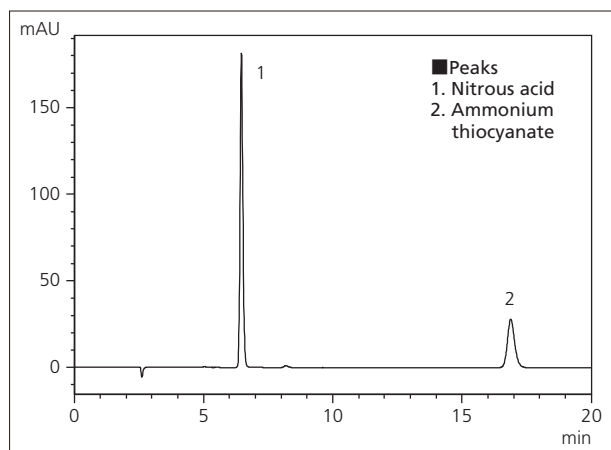


**Fig. 1  Chromatogram of Standard Mixture (20 mg/L each)**

### ■ Linearity of Calibration Curves

Fig. 2 shows calibration curves for nitrous acid and ammonium thiocyanate analyzed under the conditions shown in Table 1. The range used for calibration curves was 1 to 20 mg/L. Good linearity was obtained for both compounds with contribution rates ($R^2$) of 0.9999 or higher.
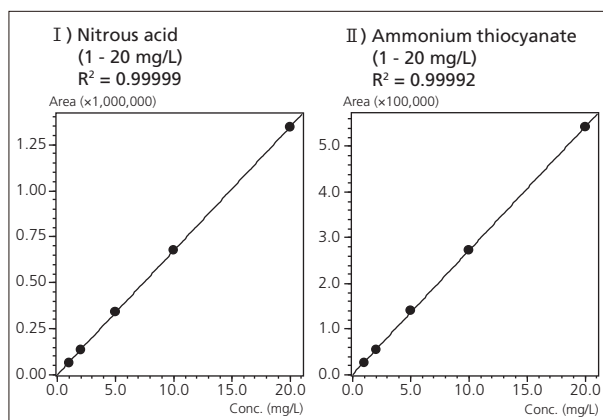


**Fig. 2  Linearity of Calibration Curves**
**Ⅰ) Nitrous acid (1 - 20 mg/L),**
**Ⅱ) Ammonium thiocyanate (1 - 20 mg/L)**

### ■ Repeatability

The relative standard deviation (%RSD) of retention times and peak areas obtained from an analysis of each compound at 0.1 mg/L repeated six times is shown in Table 2 and 3. The concentration analyzed (0.1 mg/L) was equivalent to 1/10 the lowest concentration on the calibration curve of either compound. Good repeatability was obtained for the retention times and peak areas of both compounds.

**Table 2  Repeatability of Retention Time and Peak Area for Nitrous Acid Analysis**

| | R.T. (min) | Area |
|---|---|---|
| 1st | 6.452 | 6,752 |
| 2nd | 6.452 | 6,801 |
| 3rd | 6.450 | 6,722 |
| 4th | 6.452 | 6,794 |
| 5th | 6.452 | 6,727 |
| 6th | 6.451 | 6,823 |
| Ave. | 6.452 | 6,770 |
| %RSD | 0.012 | 0.62 |

**Table 3  Repeatability of Retention Time and Peak Area for Ammonium Thiocyanate Analysis**

| | R.T. (min) | Area |
|---|---|---|
| 1st | 16.868 | 2,551 |
| 2nd | 16.870 | 2,534 |
| 3rd | 16.882 | 2,524 |
| 4th | 16.881 | 2,519 |
| 5th | 16.885 | 2,546 |
| 6th | 16.868 | 2,553 |
| Ave. | 16.876 | 2,538 |
| %RSD | 0.046 | 0.57 |

## ■ Analysis of Nitrous Acid and Ammonium Thiocyanate in Fertilizers

The method used to prepare fertilizer samples is shown in Fig. 3. The method of pretreatment differed depending on whether the sample was a powder or liquid, and on extraction liquid pH. Standard additions of nitrous acid and ammonium thiocyanate were made to the samples for analysis (fertilizer) before being further prepared according to the procedure shown in Fig. 3[3), 4)]. The results of analysis are shown in Fig. 4. Testing Methods for Fertilizers (2016) describes a method that uses ion chromatography for the analysis of ammonium thiocyanate (5.8.a). However, because this method produces a complex eluent, it takes some time for the baseline to stabilize. Another problem with the method is that it has resulted in multiple system peaks and peaks close to the elution position of ammonium thiocyanate. Nevertheless, on this occasion, the results show that good separation was achieved, including for contaminating constituents in the fertilizer.
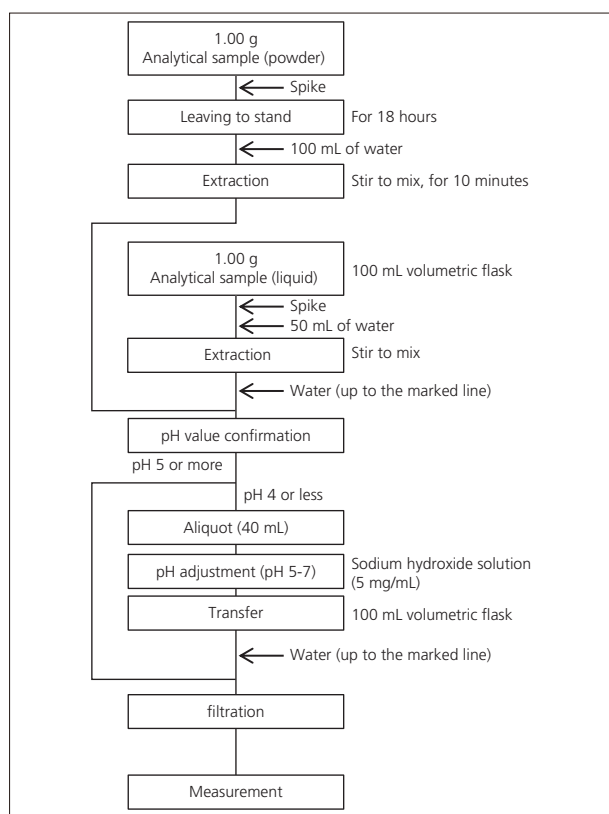


**Fig. 3  Pretreatment**
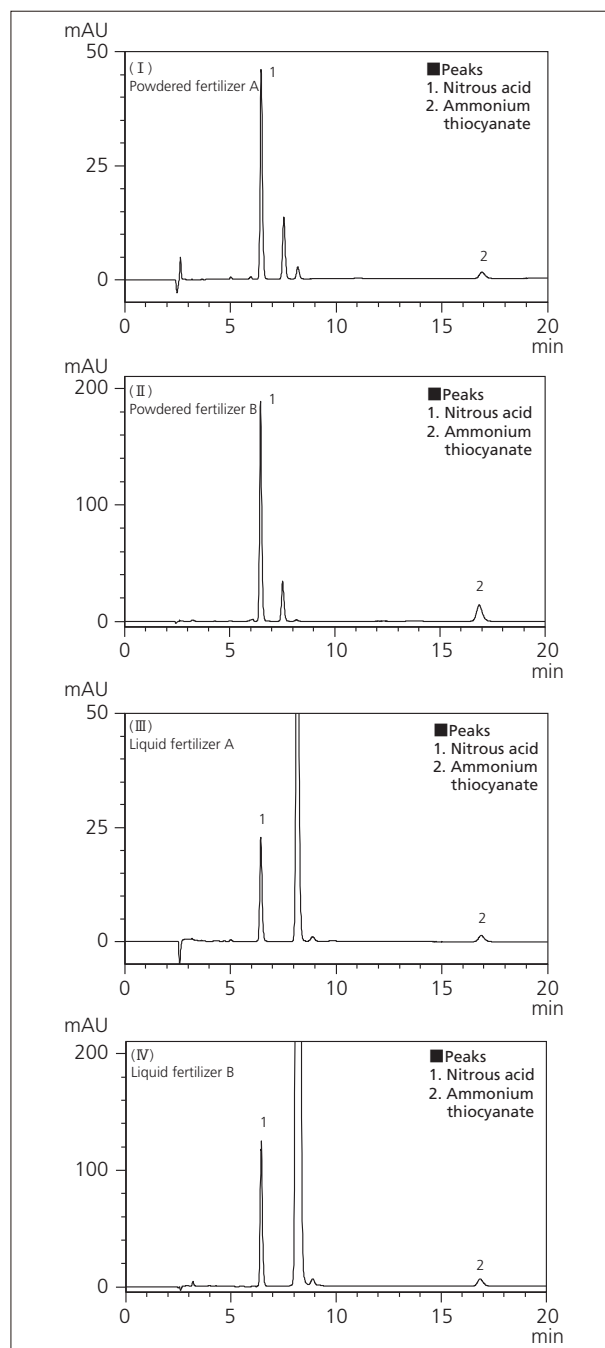


**Fig. 4  Chromatograms of Fertilizer Sample Solutions**
**( Ⅰ ) Powdered fertilizer A, ( Ⅱ ) Powdered fertilizer B,**
**( Ⅲ ) Liquid fertilizer A, ( Ⅳ ) Liquid fertilizer B**

References
1) Ministry of Agriculture, Forestry and Fisheries notification: Establishment of official specifications for commercial fertilizers according to the Fertilizer Control Law, dated February 22, 1986. Ministry of Agriculture, Forestry and Fisheries notification no. 284: Final revision, dated March 30, 2016. Ministry of Agriculture, Forestry and Fisheries notification no. 884: Enactment, dated April 1, 2016. [In Japanese]
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# Multi-Residue Analysis of Antibiotics in Organic Fertilizers using the Ultra-High Performance Liquid Chromatograph coupled with Triple Quadrupole Mass Spectrometer

Liu Zhao
Shimadzu (China), Shanghai Analysis Center

## Abstract

This report describes a method for the determination of 15 antibiotics, such as tetracyclines, penicillin, quinolones and sulfonamides, in organic fertilizer using the Shimadzu Ultra-High Performance Liquid Chromatograph LC-30A and Triple Quadrupole Mass Spectrometer LCMS-8045. In this method, 15 antibiotics are analyzed within 12 minutes, and the correlation coefficients of the calibration curve are all above 0.995 with good linearity. Six parallel tests of the mixed standard solutions with low, medium and high concentrations were performed, and the relative standard deviations of retention time and peak area of the 15 antibiotics were 0.01–0.46% and 0.29–7.09% respectively. These results show good precision. The sensitivity test result indicates that the quantitation limit of the 15 antibiotics is at 0.004–0.135 ng/mL; the samples were also tested with the standard addition concentrations of 0.5 ng/mL and 5 ng/mL, and the recovery rates were measured to be in the range 82.2–104.8%. Characterized by fast analysis speed, good repeatability and high sensitivity, the described method can determine antibiotic residues in organic fertilizers and at the same time meet requirements specified in the new national standard (GB/T32951-2016).

With the rapid growth and developments in the livestock and poultry industry, antibiotics have been widely used as a feed additive to prevent and treat diseases and promote growth. Research shows that the amount of antibiotics discharged in an animal's urine or feces in the form of matrix or metabolites accounts for 40%–90% of the antibiotic dose. Organic fertilizer, consisting of mainly livestock and poultry feces, is widely used in agricultural production because of its rich organic compounds and nitrogen and phosphorus contents. However, due to limitations in the treatment process, antibiotics cannot be effectively degraded and removed during the production of organic fertilizer. The use of organic fertilizer containing antibiotics directly cause soil pollution, especially when livestock manure containing adsorbable tetracycline antibiotics is applied to farmland. These antibiotics combines with soil particles forming stable compounds with long lifetime, polluting the soil, as well as inducing and spreading various antibiotic-resistant bacteria. This poses a major threat to human health and our environment.

The current organic fertilizer standards in China are referenced mainly from the "Organic Fertilizer" (NY525-2012) and "Bio-organic Fertilizer" (NY884-2012) standards. These standards stipulate the contents of organic material, total nutrients and heavy metal, but the amount of residual antibiotics is not included. In view of the lack of detection methods and standards for antibiotic content in organic fertilizers in China, the State General Administration of Quality Supervision, Inspection and Quarantine and the State Standardization Administration Committee approved and issued the standard GB/T32951-2016 "High Performance Liquid Chromatography for Determination of Oxytetracycline, Tetracycline, Chlortetracycline and Doxycycline Content in Organic Fertilizer" in August 2016, and implemented it on March 1st, 2017. This standard has not only provided an important method for determining tetracycline antibiotic residues in organic fertilizer products, but has also provided technical support for the analysis

and optimization of treatment processes in livestock and poultry manure processing enterprises.

With reference to the sample pretreatment method specified in GB/T32951-2016 "High Performance Liquid Chromatography for Determination of Oxytetracycline, Tetracycline, Chlortetracycline and Doxycycline Content in Organic Fertilizer", this paper demonstrates a method that uses the Shimadzu Ultra-High Performance Liquid Chromatograph LC-30A and the Triple Quadrupole Mass Spectrometer LCMS-8045 to determine the amount of 15 antibiotic residues in organic fertilizer.

### EXPERIMENTAL
**Instrumentation**

A system with a Shimadzu Ultra-High Performance Liquid Chromatograph (UHPLC) LC-30A and Triple Quadrupole Mass Spectrometer LCMS-8045 is used in this experiment. The specific configurations are two LC-30AD pumps, DGU-20A$_{5R}$ online degassing unit, SIL-30AC automatic sampler, CTO-30AC column oven, CBM-20A system controller, LCMS-8045 triple quadrupole mass spectrometer and LabSolutions Ver. 5.86 Chromatographic Workstation.

### Analytical Conditions
#### LC Chromatography (LC) Conditions

| | | |
|---|---|---|
| Column | : | Shim-pack XR-ODS III, 2.0 mm I.D.× 75 mm L., 1.6 μm |
| Mobile Phase | : | Phase A - 0.1% formic acid solution<br>Phase B - acetonitrile |
| Flow rate | : | 0.30 mL/min |
| Column temp. | : | 40 °C |
| Injection volume | : | 10 μL |
| Type of elution | : | Gradient elution with the initial concentration of Mobile Phase B at 10%. Refer to Table 1 for elution program. |

**Table 1 Gradient elution time program**

| Time (min) | Module | Command | Value (%) |
|---|---|---|---|
| 0.50 | Pumps | Pump B Conc. | 10 |
| 3.00 | Pumps | Pump B Conc. | 35 |
| 5.50 | Pumps | Pump B Conc. | 90 |
| 8.00 | Pumps | Pump B Conc. | 90 |
| 8.10 | Pumps | Pump B Conc. | 10 |
| 12.00 | Controller | Stop | |

### Mass Spectrometry (MS) Conditions

| | | |
|---|---|---|
| Analytical Instrument | : | LCMS-8045 |
| Ion sources | : | ESI |
| Nebulizer gas flow rate | : | 3.0 L/min |
| Drying gas flow rate | : | 10.0 L/min |
| Interface temp. | : | 300 °C |
| DL temp. | : | 250 °C |
| Heating module temp. | : | 400 °C |
| Heating gas flow rate | : | 10.0 L/min |
| Scanning mode | : | Multiple reaction monitoring (MRM) |
| Dwell time | : | 8 ms |
| MRM Parameters | : | Refer to Table 2 |

### Preparation of Standard Solutions

An appropriate amount of each of the 15 antibiotic standards was weighed, combined and dissolved in methanol to prepare a mixed standard stock solution containing 15 antibiotics, each of concentration 10μg/mL. It is further diluted with blank matrix to obtain mixed standard solutions of concentrations 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50 ng/mL (taking oxytetracycline calibration curve concentration as an example).

The sample pretreatment method was conducted in reference to the sample extraction and clean-up method specified in GB/T 32951-2016 "Determination of Oxytetracycline, Tetracycline, Chlortetracycline and Doxycycline in Organic Fertilizers".

**Table 2 MRM optimized parameters**

| Compound Name | CAS No. | Precursor Ion | Product Ion | Q1 Pre Bias (V) | CE (V) | Q3 Pre Bias (V) |
|---|---|---|---|---|---|---|
| Amoxicillin | 26787-78-0 | 366.2 | 114.00* | -26.0 | -22.0 | -20.0 |
| | | | 349.20 | -26.0 | -10.0 | -17.0 |
| Lincomycin | 154-21-2 | 407.3 | 126.10* | -20.0 | -40.0 | -17.0 |
| | | | 359.30 | -20.0 | -20.0 | -27.0 |
| Sulfadiazine | 68-35-9 | 251.0 | 156.00* | -30.0 | -15.0 | -29.0 |
| | | | 92.10 | -30.0 | -25.0 | -17.0 |
| Ampicillin | 69-53-4 | 350.2 | 106.10* | -17.0 | -23.0 | -11.0 |
| | | | 114.10 | -17.0 | -30.0 | -19.0 |
| Sulfathiazole | 72-14-0 | 256.0 | 156.00* | -30.0 | -14.0 | -30.0 |
| | | | 92.10 | -30.0 | -28.0 | -17.0 |
| Ciprofloxacin | 85721-33-1 | 332.2 | 314.10* | -13.0 | -16.0 | -24.0 |
| | | | 231.00 | -13.0 | -44.0 | -18.0 |
| Levofloxacin | 100986-85-4 | 362.1 | 318.10* | -11.0 | -20.0 | -21.0 |
| | | | 261.10 | -11.0 | -28.0 | -17.0 |
| Sulfapyridine | 144-83-2 | 250.0 | 156.00* | -30.0 | -16.0 | -29.0 |
| | | | 92.10 | -30.0 | -27.0 | -17.0 |
| Oxytetracyclin | 79-57-2 | 461.2 | 426.00* | -14.0 | -18.0 | -23.0 |
| | | | 443.10 | -14.0 | -14.0 | -24.0 |
| Tetracycline | 60-54-8 | 445.2 | 410.20* | -11.0 | -22.0 | -30.0 |
| | | | 427.00 | -11.0 | -16.0 | -23.0 |
| Aureomycin | 57-62-5 | 479.2 | 444.10* | -12.0 | -24.0 | -23.0 |
| | | | 154.10 | -12.0 | -28.0 | -12.0 |
| Doxycycline | 564-25-0 | 445.1 | 428.05* | -16.0 | -18.0 | -30.0 |
| | | | 154.05 | -11.0 | -32.0 | -30.0 |
| Sulfamethazine | 57-68-1 | 279.0 | 186.00* | -30.0 | -17.0 | -20.0 |
| | | | 92.10 | -30.0 | -31.0 | -17.0 |
| Chloramphenicol | 56-75-7 | 321.00 | 152.10* | 22.0 | 17.0 | 27.0 |
| | | | 257.10 | 22.0 | 11.0 | 26.0 |
| Oxacillin | 66-79-5 | 400.1 | 259.10* | 29.0 | 13.0 | 19.0 |
| | | | 356.25 | 30.0 | 8.0 | 14.0 |

Note: * indicates quantification ion

## RESULTS AND DISCUSSION
### MRM Chromatograms of Standard Samples
The MRM chromatograms for the mixed standard samples are displayed in Figure 1.

### Calibration curve and linearity
The samples were first extracted and purified to obtain blank matrix extract solutions. The stock solution was added to the blank matrix solution to prepare standard working solutions at different concentrations. LC-MS/MS analysis was conducted as specified in previously. A calibration curve was obtained using the external standard method. Refer to Table 3 for linear equations, linear range and the corresponding correlation coefficient.

### Precision experiment
Mixed standard solutions were prepared at different concentrations with blank matrix and injected for 6 consecutive times (taking oxytetracycline calibration curve concentration as an example) to check the precision. As shown in Table 4, the relative standard deviation (RSD%) of retention time and peak area are at 0.01–0.46% and 0.29–7.09% respectively, showing good precision.
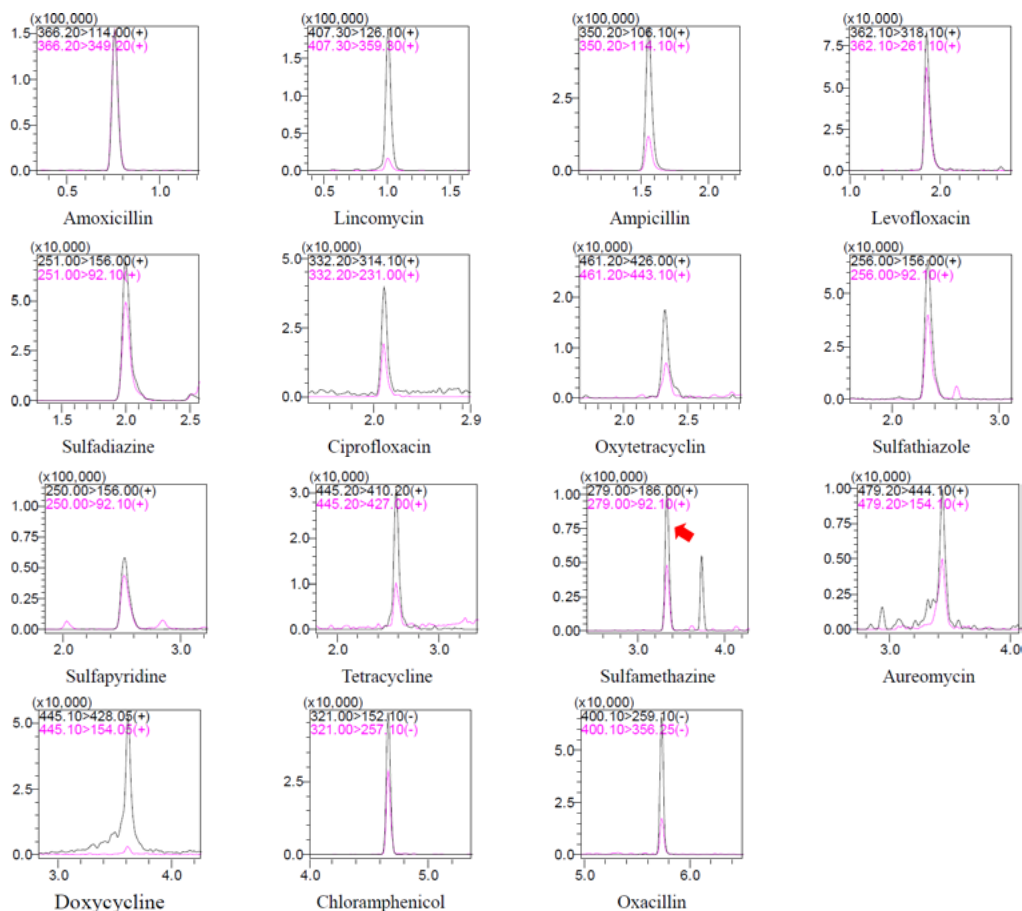
**Figure 1  MRM chromatograms of standard samples with concentration of 1 ng/mL
(taking the calibration curve concentration of Oxytetracyclin as an example)**

**Table 3  Parameters for the calibration curve (linear regression, the weight coefficient was 1/C²)**

| No. | Compound | Calibration curve | Linear Range (ng/mL) | Accuracy (%) | Correlation Coefficient (r) |
|---|---|---|---|---|---|
| 1 | Amoxicillin | Y = (31256.8)X + (5907.03) | 1~200 | 93.8~113.7 | 0.9967 |
| 2 | Lincomycin | Y = (373554)X + (186159) | 0.1~50 | 94.0~110.4 | 0.9976 |
| 3 | Ampicilin | Y = (151576)X + (-9683.21) | 1~500 | 91.2~107.0 | 0.9984 |
| 4 | Levofloxacin | Y = (107361)X + (215994) | 0.2~50 | 90.4~111.7 | 0.9963 |
| 5 | Sulfadiazine | Y = (263530)X + (26112.5) | 0.1~50 | 88.6~110.1 | 0.9965 |
| 6 | Ciprofloxacin | Y = (45160.1)X + (95808.1) | 0.2~50 | 91.3~109.4 | 0.9972 |
| 7 | Oxytetracyclin | Y = (41615.1)X + (16467.2) | 0.1~50 | 92.1~105.4 | 0.9989 |
| 8 | Sulfathiazole | Y = (276746)X + (27313.8) | 0.1~20 | 93.1~104.6 | 0.9988 |
| 9 | Sulfapyridine | Y = (237308)X + (30237.9) | 0.1~20 | 90.5~107.7 | 0.9979 |
| 10 | Tetracycline | Y = (97960.6)X + (4577.06) | 0.1~50 | 95.6~107.4 | 0.9987 |
| 11 | Sulfamethazine | Y = (314461)X + (10821.7) | 0.1~50 | 94.3~109.6 | 0.9984 |
| 12 | Aureomycin | Y = (24763.0)X + (9419.09) | 0.1~50 | 86.7~111.0 | 0.9955 |
| 13 | Doxycycline | Y = (114771)X + (30987.1) | 0.2~50 | 91.5~109.1 | 0.9976 |
| 14 | Chloramphenicol | Y = (11132.6)X + (1469.11) | 1~100 | 92.2~108.0 | 0.9971 |
| 15 | Oxacillin | Y = (15531.9)X + (-1257.09) | 1~500 | 95.5~106.2 | 0.9993 |

Table 4  Repeatability results of retention time and peak area (n=6)

| No. | Compound Name | RSD% (0.5 ng/mL) | | RSD% (2 ng/mL) | | RSD% (10 ng/mL) | |
|---|---|---|---|---|---|---|---|
| | | R.T. | Area | R.T. | Area | R.T. | Area |
| 1 | Amoxicillin | 0.09 | 3.62 | 0.08 | 2.65 | 0.03 | 1.61 |
| 2 | Lincomycin | 0.26 | 3.56 | 0.25 | 1.73 | 0.10 | 1.41 |
| 3 | Ampicilin | 0.37 | 2.02 | 0.39 | 0.89 | 0.13 | 0.29 |
| 4 | Levofloxacin | 0.45 | 3.51 | 0.46 | 1.34 | 0.16 | 0.79 |
| 5 | Sulfadiazine | 0.17 | 4.13 | 0.26 | 2.82 | 0.08 | 0.88 |
| 6 | Ciprofloxacin | 0.39 | 6.47 | 0.40 | 3.08 | 0.18 | 3.02 |
| 7 | Oxytetracyclin | 0.33 | 4.53 | 0.34 | 5.43 | 0.16 | 2.29 |
| 8 | Sulfathiazole | 0.22 | 5.11 | 0.21 | 2.73 | 0.09 | 1.41 |
| 9 | Sulfapyridine | 0.13 | 3.35 | 0.21 | 1.98 | 0.09 | 1.96 |
| 10 | Tetracycline | 0.29 | 6.96 | 0.23 | 3.68 | 0.09 | 1.08 |
| 11 | Sulfamethazine | 0.06 | 4.92 | 0.12 | 2.14 | 0.05 | 0.53 |
| 12 | Aureomycin | 0.13 | 5.45 | 0.14 | 5.70 | 0.06 | 5.40 |
| 13 | Doxycycline | 0.07 | 5.93 | 0.10 | 4.01 | 0.06 | 4.39 |
| 14 | Chloramphenicol | 0.04 | 3.75 | 0.05 | 2.77 | 0.01 | 1.12 |
| 15 | Oxacillin | 0.01 | 7.09 | 0.02 | 2.53 | 0.01 | 2.27 |

**Limit of detection and limit of quantitation**

The organic fertilizer samples are prepared according to the treatment method described in the experimental section. A matrix spike sample of concentration 0.2 ng/mL was prepared (taking the standard curve concentration of oxytetracycline as an example), and injected for analysis. The lowest detection limit (S/N=3) and the lowest quantitative limit (S/N=10) of the 15 antibiotics were calculated by software and the results are shown in Table 5.

**Matrix Spike and Recovery Experiment**

The organic fertilizer sample was prepared according to the treatment method described in experimental section to obtain a blank matrix., Matrix spike samples at concentrations of 0.5 ng/mL and 5 ng/mL are prepared and injected to determine the recovery rate. The results in Table 6 show that the spike recovery rates are in the range of 82.2–104.8%.

Table 5  Detection Limit and Quantitation Limit of 15 Antibiotics

| No. | Compound Name | Spiked Sample Concentration (0.5 ng/mL) | | Spiked Sample Concentration (5 ng/mL) | |
|---|---|---|---|---|---|
| | | Average (ng/mL) | Recovery (%) | Average (ng/mL) | Recovery (%) |
| 1 | Amoxicillin | 4.72 | 94.4 | 46.66 | 93.3 |
| 2 | Lincomycin | 0.51 | 101.8 | 4.13 | 82.6 |
| 3 | Ampicilin | 4.67 | 93.5 | 47.25 | 94.5 |
| 4 | Levofloxacin | 0.42 | 83.9 | 4.34 | 86.9 |
| 5 | Sulfadiazine | 0.48 | 96.6 | 4.11 | 82.2 |
| 6 | Ciprofloxacin | 0.41 | 82.8 | 4.31 | 86.2 |
| 7 | Oxytetracyclin | 0.49 | 98.4 | 4.98 | 99.5 |
| 8 | Sulfathiazole | 0.49 | 97.8 | 4.52 | 90.3 |
| 9 | Sulfapyridine | 0.48 | 96.5 | 4.34 | 86.7 |
| 10 | Tetracycline | 0.47 | 93.0 | 4.39 | 87.8 |
| 11 | Sulfamethazine | 0..48 | 95.4 | 4.58 | 91.6 |
| 12 | Aureomycin | 0.45 | 89.7 | 5.24 | 104.8 |
| 13 | Doxycycline | 0.43 | 86.3 | 4.52 | 90.4 |
| 14 | Chloramphenicol | 4.85 | 96.9 | 46.43 | 92.9 |
| 15 | Oxacillin | 4.49 | 89.9 | 53.89 | 107.8 |

## CONCLUSION

This paper establishes a method for the rapid determination of 15 antibiotics in organic fertilizer using the Shimadzu UHPLC LC-30A and Triple Quadrupole Mass Spectrometer LCMS-8045. With this method, 15 antibiotics are analyzed within 12 minutes, and the correlation coefficients of the calibration curve are all above 0.995 with good linearity. Six parallel tests of the mixed standard solutions with low, medium and high concentrations were performed, and the relative standard deviations of retention time and peak area of the 15 antibiotics are at 0.01–0.46% and 0.29–7.09% respectively, showing good precision. Prepared samples with concentrations of 0.5 ng/mL and 5 ng/mL had a recovery rate of 82.2–104.8%, indicating characteristics of fast analysis speed, good repeatability and high sensitivity. This method has broadened the scope of antibiotic analysis and demonstrated the multi-residue analysis of antibiotics at the same time meet requirements specified in the new national standard (GB/T32951-2016).

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# Application News

**Liquid Chromatography Mass Spectrometry**

## Analysis of Sulfamic Acid in Fertilizers Using LC/MS (LCMS-2020)

# No.C105

Sulfamic acid, due to its plant growth inhibiting effects, is subject to maximum limits in fertilizers as specified in the official standard[1] for ordinary fertilizers according to the Japanese Fertilizers Regulation Act. According to the Testing Methods for Fertilizers[2] supervised by Japan's Food and Agricultural Materials Inspection Center (FAMIC), the ion chromatography (IC) method is specified as the test method for sulfamic acid in ammonium sulfate. It has been reported, however, that when applying this IC method with byproduct compound fertilizer (fertilizer produced by concentrating and drying liquid byproducts obtained from fermentation plants involved in amino acid production, etc.) samples that contain large amounts of organic matter, it is difficult to separate the sulfamic acid peaks from contaminant peaks generated from sample matrix.[3]

In this application, we investigated the analytical conditions for LC/MS that would permit acquisition of mass information and provide high selectivity in order to eliminate the effects of contaminating components. The LCMS-2020 single quadrupole mass spectrometer was used for the analysis.

Good quantitative results were obtained, confirming the applicability of this method using byproduct compound fertilizer as the actual sample.

### ■ Analysis of Standard Solution

Table 1 shows the analytical conditions, and Fig. 1 shows chromatogram obtained using a standard solution (0.1 mg/L aqueous solution) of sulfamic acid.

As retention of a zwitterionic compound such as sulfamic acid is difficult using reversed phase conditions, we adopted conditions using a HILIC column. Isocratic analysis was conducted using a mobile phase consisting of acetonitrile / ammonium formate + formic acid (pH 3.2).

Applying the LC/MS method (ESI-Negative), we conducted selected ion monitoring (SIM) analysis using the deprotonated molecule at $m/z$ 95.9. Fig. 2 shows the calibration curve. Excellent linearity was obtained over the entire concentration range of 0.001 to 0.1 mg/L, with a correlation coefficient greater than 0.999.
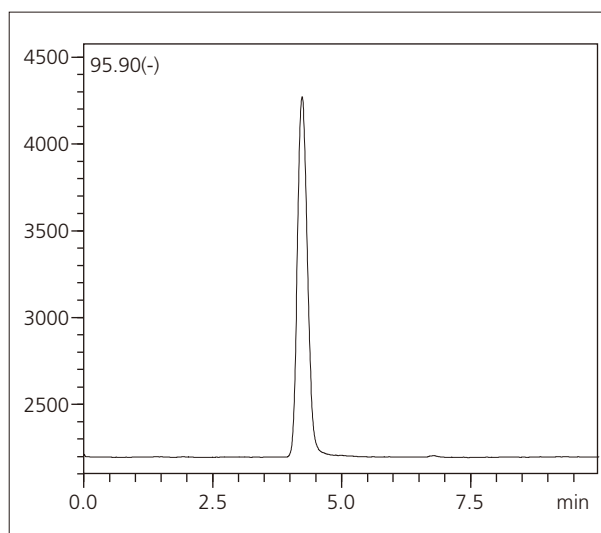


**Fig. 1  Mass Chromatogram (SIM) of Sulfamic Acid (0.1 mg/L)**



R  = 0.9991732
$R^2$ = 0.9983472

Area % RSD (0.001 mg/L, n=5) 2.72 %
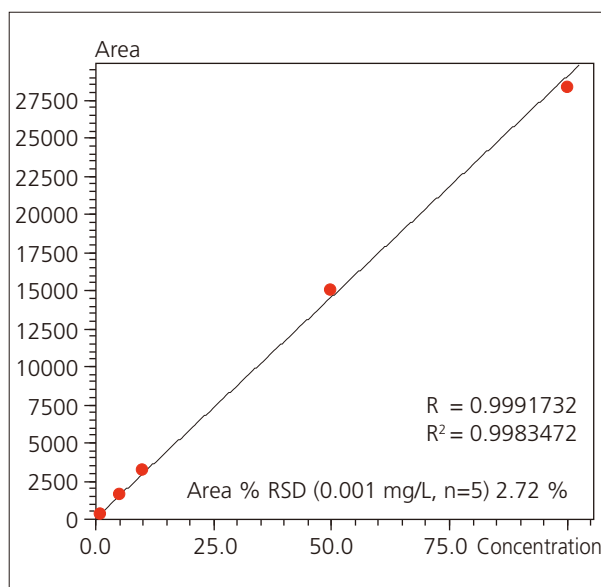
**Fig. 2  Calibration Curve (0.001 – 0.1 mg/L)**

**Table 1  Analytical Conditions**

| | |
|---|---|
| Column | : Phenomenex Luna HILIC 20A (100 mm L. × 2.0 mm I.D., 5 μm) |
| Mobile Phases | : Acetonitrile/100 mmol/L Ammonium Formate+ Formic Acid (pH 3.2) = 90:10, v/v |
| Flowrate | : 0.2 mL/min |
| Column Temperature | : 40 ℃ |
| Injection Volume | : 1 μL |
| Probe Voltage | : -3.5 kV (ESI-negative mode) |
| DL Temperature | : 250 ℃ |
| Block Heater Temperature | : 400 ℃ |
| Nebulizing Gas Flow | : 1.5 L/min |
| Drying Gas Flow | : 15 L/min |
| Monitoring Ion (SIM) | : $m/z$ 95.9 |

## ■ Analysis of Sulfamic Acid in Fertilizers

We verified the applicability of the LC/MS method using a byproduct compound fertilizer as an actual sample. The permissible content level of sulfamic acid is set based on the total amount of the principal component in each type of fertilizer. Here, taking the lower limit of quantitation of sulfamic acid in fertilizer as 1/5 the value of the minimum concentration permissible (sulfamic acid concentration 0.005 % per principal component 1 %), we conducted spike and recovery testing using a spike quantity equivalent to the lower limit of quantitation.

Fig. 3 shows the sample pretreatment procedure. The extraction method conforms to the Testing Methods for Fertilizers (2013) supervised by FAMIC. After weighing out 1 g of byproduct compound fertilizer, extraction was conducted using 100 mL of water, and after further diluting this 100 to 1 with water, the mixture was filtered to complete preparation of the fertilizer measurement solution.

As the total quantity of the principal component represented 5 % of the fertilizer content, the concentration of sulfamic acid corresponding to the lower limit of quantitation is calculated as 50 mg/kg of fertilizer. In the spike and recovery test, 0.5 mL of 100 mg/L standard sample was added to the fertilizer, and after letting the mixture stand for 30 minutes, a measurement solution was prepared using the same procedure. The concentration of sulfamic acid in the measurement solution is therefore 0.005 mg/L.

Representative chromatograms are shown in Fig. 4 including chromatograms of the standard sample (0.005 mg/L), the sample spiked with sulfamic acid, and the byproduct compound fertilizer measurement solution. Table 2 shows the analytical results. Sulfamic acid was not detected in the byproduct compound fertilizer, nor were there any noticeable peaks associated with contaminant components.

In the spike and recovery test, excellent results were obtained in continuous analysis (n=5), with an average recovery rate of 101 %. The LC/MS method investigated here in the analysis of highly contaminated byproduct compound fertilizer was demonstrated to permit quantitation by simply adding a dilution step following extraction, as opposed to the IC method which requires tedious processing to address the issue of high-contaminant content.
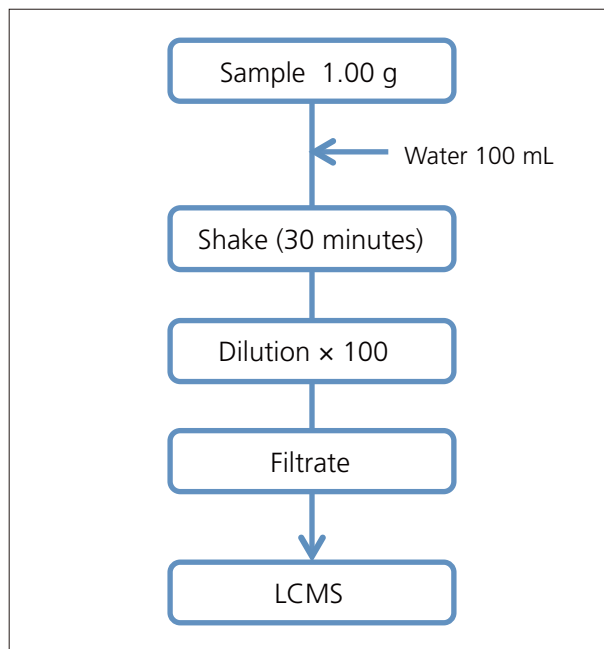


**Fig. 3  Preparation Flow**
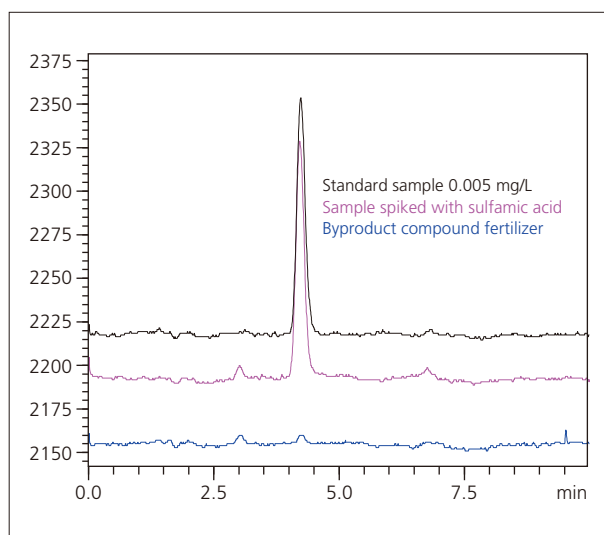


**Fig. 4  SIM Chromatograms of STD and Fertilizer Sample**

**Table 2  Repeatability of Peak Area and Retention Time in Spike and Recovery Test**

|       | R.t (min) | Peak Area | Recovery (%) |
|-------|-----------|-----------|--------------|
| 1st   | 4.217     | 1564      | 103          |
| 2nd   | 4.252     | 1561      | 102          |
| 3rd   | 4.229     | 1508      | 99           |
| 4th   | 4.224     | 1511      | 99           |
| 5th   | 4.219     | 1534      | 100          |
| Ave   | 4.228     | 1535      | 101          |
| %RSD  | 0.336     | 1.735     |              |

[References]
1) Notification Regarding Determination of the Official Standard for Ordinary Fertilizer Based on the Fertilizers Regulation Act, February 22, 1986, the Japan's Ministry of Agriculture, Forestry and Fisheries Notification No. 284, Final Revision December 5, 2013 the Ministry of Agriculture, Forestry and Fisheries Notification No. 2939 (2013)
2) Food and Agricultural Materials Inspection Center (FAMIC): Testing Methods for Fertilizers
   < http://www.famic.go.jp/ffis/fert/sub9.html >
3) Hiroi T., Shirai Y.: Simultaneous Determination of Sulfamic Acid and Ammonium Thiocyanate in Ammonium Sulfate by Nonsuppressed Ion Chromatography, Research Report of Fertilizer, 5, 1 – 12 (2012)

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# Meat and Seafood

1. Analysis of Trace Elements in Certified Fish Sample with ICP-MS

2. Analysis of Trace Elements in Fish Sample with ICP-AES (ICP-OES) on ICPE-9800

3. Multi-residual Quantitative Analytical Method for Antibiotics in Seafood by LC/MS/MS

4. Analysis of Residual Antimicrobials in Meat with Antimicrobial Screening System (Part 1)

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6. Microchip Electrophoresis-Based Quantitative Determination of Composition of Blended Meat

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12. Sensitive Detection of Pork DNA in Processed Meat Products on PCR-MultiNA Platform

13. Quantitative Analysis of Veterinary Drugs Using the Shimadzu LCMS-8050 Triple Quadrupole Mass Spectrometer

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17. Highly Sensitive and Rapid Analysis of Synthetic Dyes in Seafood by LC/MS/MS

# Application News

# No. AD-0169

*Food Analysis / ICPMS-2030*

## Analysis of Trace Elements in Certified Fish Sample with ICP-MS

❑ **Introduction**

Fish is one of the most important food resource that is widely consumed in many parts of the world due to its high protein content, low saturated fats and omega-3 fatty acid. However, because of industrial discharges from human activities, fish may have trace levels of heavy metals such as arsenic, cadmium, lead, mercury and nickel which are absorbed from the surrounding waters and the foods they eat. Not only do these heavy metals accumulate in organisms and circulate in food chain, they also remain in the sediments of the ecosystem in the long term [1]. The toxicity of these metals poses a concern to human health through the consumption of fish. Fish are the single largest sources of mercury and arsenic for man. Mercury is a known human toxicant and the primary sources of mercury contamination in man are through eating fish [2]. Minamata disease is a methylmercury poisoning associated with the daily consumption of large quantities of fish and shellfish heavily contaminated with the toxic chemical [3]. Here, Shimadzu ICPMS-2030, a inductively coupled plasma mass spectrometer (ICP-MS) was used to conduct a simultaneous analysis of elements in fish. In addition to being highly sensitive, the ICPMS-2030 uses a helium gas collision system that greatly reduces the spectral interference caused by argon and chlorine.

❑ **Experimental**

The sample used in this analysis was Fish Protein Certified Reference Material for Trace Metals (DORM-4) from National Research Council Canada. There were two sets of preparation. The sample, 0.5 g, was placed in a digestion vessel followed by addition of 5.0 mL of concentrated nitric acid, 2.0 mL of hydrogen peroxide and 1.0 mL of water. The mixture was digested using microwave-assisted digestion system based on AOAC 999.10 procedure. After the digestion process, deionized water was added to the digested sample to a final total volume of 200.0 mL.

The calibration standards were prepared from 1000 ppm copper (Cu), iron (Fe) and zinc (Zn) standards, and 100 ppm ICP multi-element standard solution IX, which contains arsenic (As), cadmium (Cd), chromium (Cr), mercury (Hg), nickel (Ni), lead (Pb) and selenium (Se). The internal standards were prepared from 1000 ppm bismuth (Bi), germanium (Ge), indium (In) and scandium (Sc) standards and diluted to a concentration of 10 ppb with pure water. The standard solutions were purchased from Merck Millipore, Germany. Type E-1 ultra pure water with resistivity of 18 ΩW was used. Four different calibration ranges were prepared to cover the range of the elements based on the certified values. The calibration standards were acid matched to the digested samples.

Measurement was conducted using the Shimadzu ICPMS-2030 ICP-MS spectrometer, equipped with the mini-torch and helium (He) gas collision system. The mini-torch reduces running cost associated with argon gas usage. The helium gas collision system reduces spectral interference caused by polyatomic ions, for example, $^{40}Ar^{16}O$ that interferes with measurement of $^{56}Fe$, $^{40}Ar^{35}Cl$ that interferes with measurement of $^{75}As$, and $^{40}Ar^{38}Ar$ that interferes with measurement of $^{78}Se$. The typical measurement conditions are shown in Table 1. The elements, mass, and calibration ranges used are shown in Table 2.

Table 1. Instrument and measurement conditions

| Instrument | : ICPMS-2030 | |
|---|---|---|
| Radio Frequency Power | : 1.20 | (kW) |
| Sampling Depth | : 6 | (mm) |
| Plasma Gas Flow Rate | : 8.0 | (L/min) |
| Auxiliary Gas Flow Rate | : 1.10 | (L/min) |
| Nebulizer Gas Flow Rate | : 0.60 | (L/min) |
| Nebulizer | : Coaxial Nebulizer | |
| Spray Chamber | : Cyclone Chamber (electronic cooling) | |
| Plasma Torch | : Mini-torch | |
| Collision gas | : He | |

Table 2. Elements, mass and calibration ranges

| Element | Mass | Calibration Range |
|---------|------|-------------------|
| As | 75 | |
| Cd | 111 | |
| Cr | 52 | |
| Hg | 202 | 0 to 20 ppb |
| Ni | 60 | |
| Pb | 208 | |
| Se | 78 | |
| Cu | 65 | 0 to 100 ppb |
| Fe | 56 | 0 to 2000 ppb |
| Zn | 66 | 0 to 200 ppb |

Table 4. Detection Limits

| Element | IDL (ppb) | LOQ (ppb) |
|---------|-----------|-----------|
| As | 0.003 | 0.0097 |
| Cd | 0.001 | 0.0044 |
| Cr | 0.005 | 0.018 |
| Cu | 0.02 | 0.065 |
| Fe | 0.06 | 0.20 |
| Hg | 0.003 | 0.011 |
| Ni | 0.01 | 0.040 |
| Pb | 0.004 | 0.014 |
| Se | 0.01 | 0.045 |
| Zn | 0.03 | 0.091 |

## ❑  Results and Discussion

The quantitative results for the trace elements were within the certification range of Fish Protein CRM DORM-4 as shown in Table 3.

The instrument detection limits (IDL) and the limits of quantitation (LOQ) are shown in Table 4. The IDL is calculated as three times the standard deviation of 10 replicate measurements of a calibration blank, and the LOQ is calculated as ten times the standard deviation of 10 replicate measurements of a calibration blank.

Table 3. Quantitation results of Fish CRM DORM-4

| Element | Fish CRM DORM-4 | | |
|---------|-----------------|---|---|
| | Measured Value (mg/kg) | (Duplicate) Measured Value (mg/kg) | Certified Value (mg/kg) |
| As | 6.59 | 6.69 | 6.80 ± 0.64 |
| Cd | 0.299 | 0.298 | 0.306 ± 0.015 |
| Cr | 1.88 | 1.89 | 1.87 ± 0.16 |
| Cu | 15.6 | 15.5 | 15.9 ± 0.9 |
| Fe | 326 | 328 | 341 ± 27 |
| Hg | 0.415 | 0.360 | 0.410 ± 0.055 |
| Ni | 1.31 | 1.29 | 1.36 ± 0.22 |
| Pb | 0.388 | 0.389 | 0.416 ± 0.053 |
| Se | 3.44 | 3.51 | 3.56 ± 0.34 |
| Zn | 55.0 | 53.3 | 52.2 ± 3.2 |

## ❑  Conclusions

The ICPMS-2030 can provide a sensitive multi-element analysis of the trace elements in fish accurately, even for low concentration of Cd, Hg and Pb.

## ❑  References

1. Sreenivasa Rao. J., Vasudeva Rao. Y., Devindra. S and Longvah. T. (2014). Analysis of heavy metal concentrations in Indian marine fish using ICP-MS after closed vessel micro wave digestion method. International Journal of Analytical and Bioanalytical Chemistry 4(3): 67-73.

2. F. Emami Khansari, M. Ghazi-Khansari, M. Abdollahi (2005). Heavy metals content of canned tuna fish. Food Chemistry 93: 293-296.

3. Noriyuki Hachiya (2006). The history and the present of Minamata disease. Japan Medical Association Journal  49 (3): 112-118.

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# Analysis of Trace Elements in Fish Sample with ICP-AES (ICP-OES) on ICPE-9800

## ❑ Introduction

Fish is one of the most important food resource that is widely consumed in many parts of the world due to its high protein content, low saturated fats and omega-3 fatty acid. It is also rich in calcium and phosphorus. However, because of industrial discharges from human activities, fish may have trace levels of toxic elements such as arsenic, cadmium and lead which are absorbed from the environmental water and the foods they eat. The toxicity of these metals poses a concern to human health through the consumption of fish. Here, Shimadzu ICPE-9820, a simultaneous inductively coupled plasma atomic emission spectrometer (ICP-AES) was used to conduct a simultaneous analysis of elements in fish. The ICPE-9820, with its mini-torch plasma and spectrometer permitting all elements / all wavelengths simultaneous analysis, provides high sensitivity and throughput measurement while reducing running cost.

## ❑ Experimental

The sample used in this analysis was Fish Protein Certified Reference Material for Trace Metals (DORM-4), from National Research Council Canada. There were two sets of preparation. The sample, 0.5 g, was placed in a digestion vessel followed by addition of 5.0 mL of concentrated nitric acid, 2.0 mL of hydrogen peroxide and 1.0 mL of water. The mixture was digested using microwave-assisted digestion system based on AOAC 999.10 procedure. After the digestion process, deionized water was added to the digested sample to a final total volume of 20.0 mL.

The calibration standards were prepared from 1000 ppm copper (Cu), iron (Fe) and zinc (Zn) standards, and 100 ppm ICP multi-element standard solution IX, which contains arsenic (As), cadmium (Cd), chromium (Cr), nickel (Ni), lead (Pb) and selenium (Se). The standard solutions were purchased from Merck Millipore, Germany. Type E-1 ultra pure water with resistivity of 18 ΩW was used. Three different calibration ranges were prepared to cover the range of the elements based on the certified values. The calibration standards were acid matched to the digested samples.

Measurement was conducted using the Shimadzu ICPE-9820 simultaneous ICP-AES spectrometer, equipped with the mini-torch.

The typical measurement conditions are shown in Table 1. The elements, wavelength and calibration ranges used are shown in Table 2.

Table 1. Instrument and measurement conditions

| | |
|---|---|
| Instrument | : ICPE-9820 |
| Radio Frequency Power | : 1.20 (kW) |
| Plasma Gas Flow Rate | : 10.0 (L/min) |
| Auxiliary Gas Flow Rate | : 0.60 (L/min) |
| Nebulizer Gas Flow Rate | : 0.70 (L/min) |
| Nebulizer | : Coaxial Nebulizer |
| Spray Chamber | : Cyclone Chamber |
| Plasma Torch | : Mini-torch |
| Observation | : Axial |

Table 2. Elements, wavelength and calibration ranges

| Element | Wavelength | Concentration Range |
|---|---|---|
| As | 193.759 | 0 to 0.2 ppm |
| Cd | 214.438 | |
| Cr | 205.552 | |
| Ni | 231.604 | |
| Pb | 220.353 | |
| Se | 203.985 | |
| Cu | 224.700 | 0 to 2 ppm |
| Zn | 206.200 | |
| Fe | 239.147 | 0 to 20 ppm |

## ❑ Results and Discussion

The quantitative results for the trace elements were within the certification range of Fish Protein CRM DORM-4 as shown in Table 3. The spectral profiles of standards and samples are displayed in Figure 1.

## ❑ Conclusions

The ICPE-9800 can provide simultaneous analysis of trace elements in fish CRM sample with high accuracy, which include low concentrations of Cd and Pb.

Table 3. Quantitation results of Fish CRM DORM-4 on ICPE-9800

| Element | Fish CRM DORM-4 | | | IDL (ppm)* |
|---|---|---|---|---|
| | Measured Value (mg/kg) | (Duplicate) Measured Value (mg/kg) | Certified Value (mg/kg) | |
| As | 6.7 | 6.6 | 6.80 ± 0.64 | 0.008 |
| Cd | 0.298 | 0.303 | 0.306 ± 0.015 | 0.0002 |
| Cr | 1.85 | 1.76 | 1.87 ± 0.16 | 0.0007 |
| Cu | 15.1 | 15 | 15.9 ± 0.9 | 0.002 |
| Fe | 336 | 323 | 341 ± 27 | 0.03 |
| Ni | 1.24 | 1.29 | 1.36 ± 0.22 | 0.0004 |
| Pb | 0.43 | 0.45 | 0.416 ± 0.053 | 0.002 |
| Se | 3.7 | 3.8 | 3.56 ± 0.34 | 0.01 |
| Zn | 51.7 | 49.4 | 52.2 ± 3.2 | 0.0003 |

*Instrument Detection limit (IDL) is calculated as three times the standard deviation of 10 replicate measurements of a calibration blank*
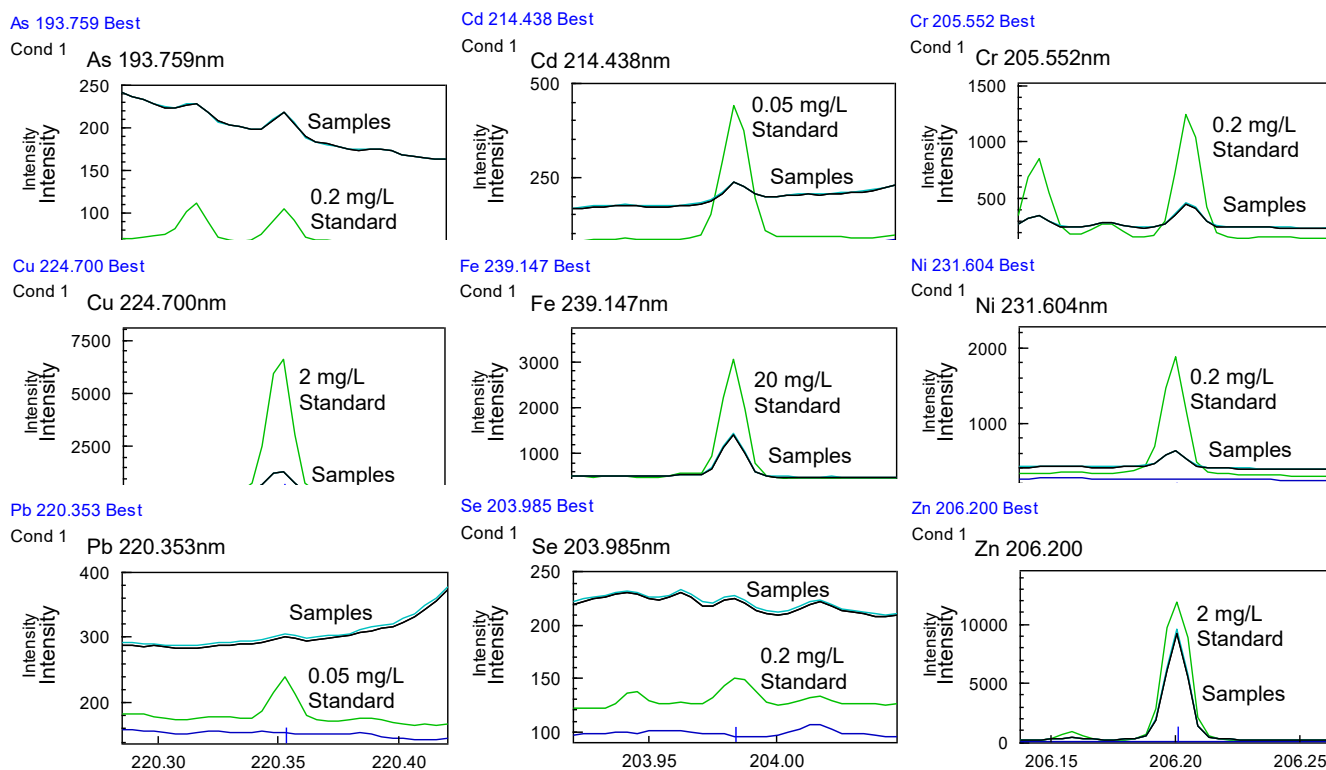
# Multi-residual quantitative analytical method for antibiotics in sea food by LC/MS/MS

**ASMS 2017**  TP 198

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# Multi-residual quantitative analytical method for antibiotics in sea food by LC/MS/MS

# Introduction

Antibiotics are widely used in agriculture as growth enhancers, disease treatment and control in animal feeding operations. Concerns for increased antibiotic resistance of microorganisms have prompted research into the environmental occurrence of these compounds. Assessment of the environmental occurrence of antibiotics depends on development of sensitive and selective analytical methods based on new instrumental technologies.

LC/MS/MS method has been developed for quantitation of multi-residual antibiotics (Table 1) from sea food sample using LCMS-8040, a triple quadrupole mass spectrometer from Shimadzu Corporation, Japan. Simultaneous analysis of multi-residual antibiotics often exhibit peak shape distortion owing to their different chemical nature. To overcome this, autosampler pre-treatment feature was used [1].

Table 1. List of antibiotics

| Sr.No. | Name of group | Name of compound | Number of compounds |
|--------|---------------|------------------|---------------------|
| 1 | Fluoroquinolones | Flumequine, Oxolinic Acid, Ciprofloxacin, Danofloxacin, Difloxacin.HCl, Enrofloxacin, Sarafloxacin HCl Trihydrate, Naldixic Acid | 8 |
| 2 | Sulfonamides | Sulfadimethoxine, Sulfadoxine, Sulfachlorpyridazine, Sulfamethoxypyridazine, Sulfadimidine, Sulfamethizole, Sulfamerazine, Sulfathiazole, Sulfamethizole, Sulfadiazine, Sulfapyridine | 11 |
| 3 | Dyes | Crystal Violet , Leucocrystal violet, Malachite green, Leucomalachite green | 4 |
| 4 | Antihelminthics | Albendazole, Albendazole Sulfone, Albendazole Sulfoxide, Albendazole-2-aminosulfone , Fenbendazole, Flubendazole | 6 |
| 5 | Nitroimidazoles | Ronidazole, Metronidazole, Dimetronidazole | 3 |
| 6 | Phenylbutazone | Phenylbutazone | 1 |
| 7 | Macrolides | Erythromycin, Spiramycin, Tilmicosin, Tylosin Tartarate, Trimethoprim | 5 |

# Methods and Materials

## Sample preparation

The antibiotic standards procured from Sigma-Aldrich were used for the analysis. All individual standards stock were prepared in the methanol. Further mixture of all antibiotics were prepared in methanol. This stock was serially diluted to prepare calibration levels ranging from 0.5 ppb to 50 ppb in methanol for solvent standard and in matrix for matrix matched standard calibration.
Commercially available shrimp sample were used for analysis. The shrimp was finely crushed by using a sample crushing mixer. Crushed sample was transferred to 50 ml centrifuge tube. To this 10 mL of acidified acetonitrile was used for extraction of anti-biotics from the shrimp sample, because some of the antibiotics require acidic condition for extraction. Solution was then centrifuged at 4°C,8000 rpm for 5 mins. Further dSPE clean-up was given to the supernatant and dSPE cleanup extract was filtered through 0.2 micron filter and injected on LCMS-8040.

# Multi-residual quantitative analytical method for antibiotics in sea food by LC/MS/MS
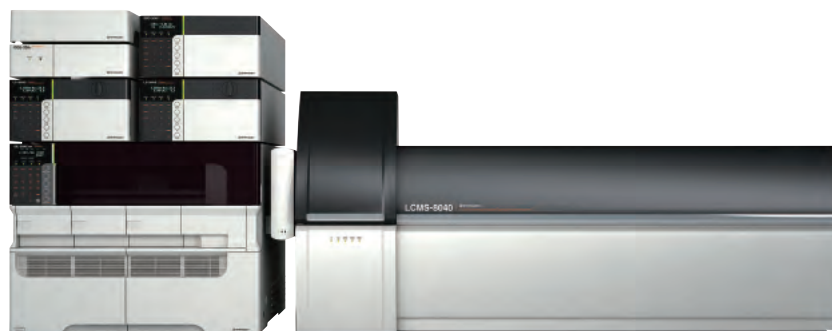


Figure 1.  LCMS-8040 triple quadrupole mass spectrometer by Shimadzu

LCMS-8040 triple quadrupole mass spectrometer by Shimadzu, sets a new benchmark in triple quadrupole technology with an unsurpassed sensitivity (UFsensitivity), ultra fast scanning speed of 15,000 u/sec (UFscanning) and polarity switching speed of 15 msec (UFswitching). This system ensures highest quality of data, with very high degree of reliability.

## LC/MS/MS analysis

All antibiotics i.e. 11 Sulfonamides, 8 Fluoroquinolones, 4 Dyes, 6 Antihelminthics, 3 Nitroimidazole,5 Macrolides and Phenylbutazone were simultaneously analyzed using Ultra High Performance Liquid Chromatography (UHPLC) Nexera coupled with LCMS-8040 triple quadrupole system (Shimadzu Corporation, Japan). The details of analytical conditions are given in Table 2.

Table 2. Optimized LC/MS/MS conditions for antibiotic analysis

| | |
|---|---|
| Column | : Shim-pack GIST Phenyl (75mm L X 3.0mm I.D, 2 µm) |
| Mobile phase | : A- 2mM ammonium formate + 0.002 % formic acid in water |
| | : B- 2mM ammonium formate + 0.002 % formic acid in methanol |
| Flow rate | : 0.4 mL/min |
| Gradient program (B %) | : 0.01-1 min → 10 (%); 1-5 min → 10-70 (%); 5-9 min → 70-95 (%) ; 9-11 min → 95 (%); 11-11.5 min → 95-10 (%); 11.5-15 min → 10 (%) |
| Injection vol. | : 5 µL |
| Column temperature | : 40°C |
| MS interface | : Electro Spray Ionization (ESI) |
| Nitrogen gas flow | : Nebulizing gas 2L/min; Drying gas 10L/min |
| MS temperature | : Desolvation line 250°C; Heating block 400°C |

Table 3. Details of MRM transitions

| Sr.No. | Name of Compound | Quantifier ions |
|---|---|---|
| 1 | Sulfamethoxypyridazine | 281.10>156.05 |
| 2 | Sulfamethizole | 271.10>156.00 |
| 3 | Sulfamerazine | 265.15>92.10 |
| 4 | Sulfadiazine | 251.10>156.00 |
| 5 | Sulfapyridine | 250.10>92.10 |
| 6 | Sulfamethazin | 279.15>186.00 |
| 7 | Sulfamethoxazole | 254.10>155.95 |
| 8 | Sulfadimethoxine | 311.10>156.00 |
| 9 | Sulfadoxine | 311.10>156.10 |
| 10 | Sulfachlorpyridazine | 285.10>155.95 |
| 11 | Sulfathiazole | 256.10>156.05 |
| 12 | Albendazole | 266.10>234.00 |
| 13 | Albendazole sulfone | 298.10>159.00 |
| 14 | Albendazole sulfoxide | 282.20>240.00 |
| 15 | Albendazole 2 aminosulfone | 240.10>133.05 |
| 16 | Fendendazole | 300.10>268.00 |
| 17 | Flubendazole | 314.15>282.15 |
| 18 | Morantel | 221.05>111.10 |
| 19 | Flumequine | 262.15>201.95 |
| 20 | Oxolinic acid | 262.15>160.05 |
| 21 | Ciprofloxacin | 332.20>314.20 |
| 22 | Difloxacin | 400.10>382.15 |
| 23 | Enrofloxacin | 360.20>342.25 |
| 24 | Sarafloxacin | 386.15>368.05 |
| 25 | Nalidixic Acid | 233.15>187.05 |
| 26 | Danofloxacin | 358.20>340.20 |
| 27 | Ronidazole | 201.10>140.00 |
| 28 | Metronidazole | 172.20>127.90 |
| 29 | Dimetronidazole | 142.20>96.10 |
| 30 | Leucocrystal violet | 374.20>358.15 |
| 31 | Malachite green | 329.20>313.15 |
| 32 | Leucomalachite green | 331.20>239.10 |
| 33 | Crystal violet | 372.20>356.20 |
| 34 | Spiramycin II | 422.20>101.15 |
| 35 | Tilmicosin I | 435.20>98.90 |
| 36 | Tylosin tartarate I | 916.30>174.20 |
| 37 | Trimethoprim | 291.25>230.20 |
| 38 | Neo spiramycin II | 366.20>174.10 |
| 39 | Phenylbutazone | 309.15>77.10 |
| 40 | Erythromycin | 734.30>576.30 |

# Results

Analysis was performed using aqueous as well as matrix matched standards. The MRM transitions used for these analysis are given in Table 3. Auto MRM optimization feature was used for optimization for MRM transitions. Linearity studies were carried out using external calibration method and linearity results are tabulated in Table 4.

The matrix matched calibration levels were prepared and injected in segmented MRM mode. The calibration curve of all antibiotics are shown in Figure 4 to Figure 6 and the correlation coefficient >0.99 was obtained for all compounds.
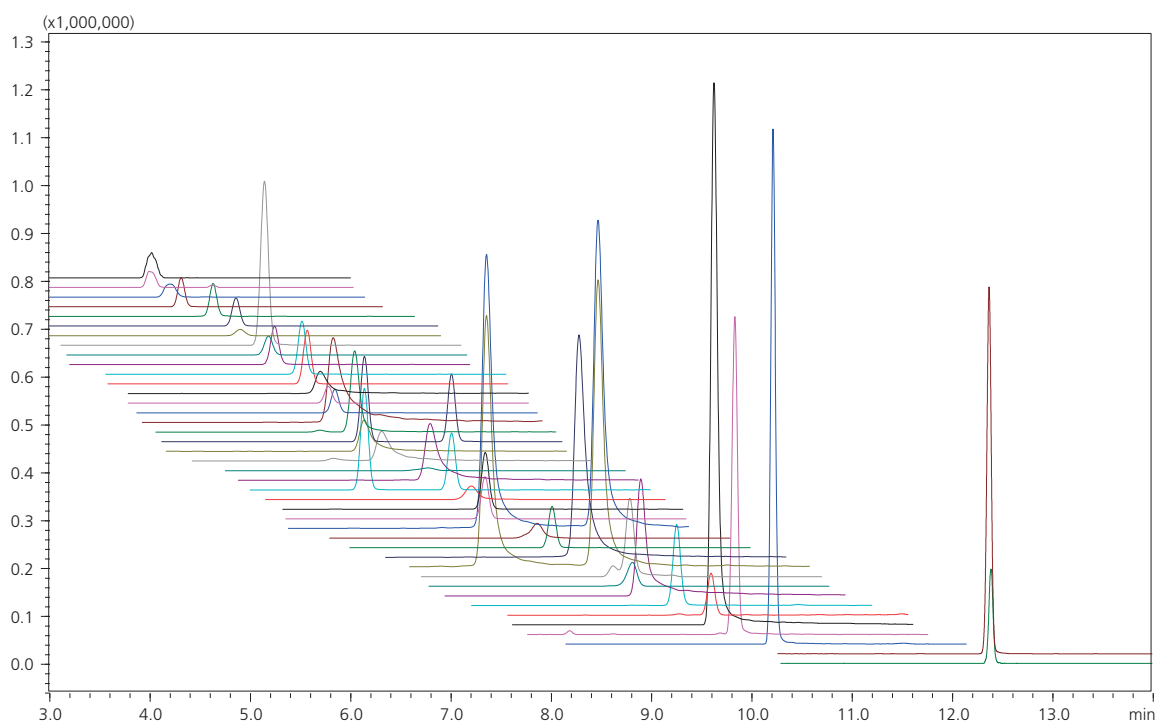


Figure 2. Chromatogram of matrix match standards of 2 ppb

# Multi-residual quantitative analytical method for antibiotics in sea food by LC/MS/MS
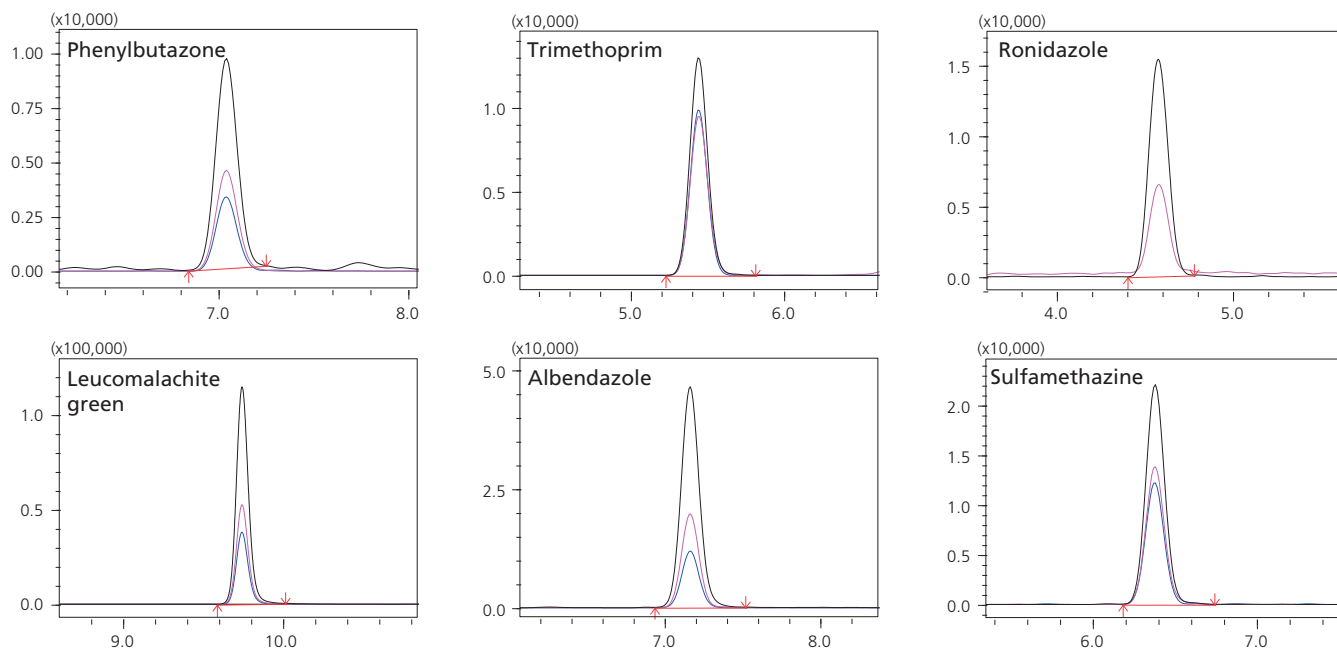


Figure 3.  Extracted ion chromatogram of representative antibiotics
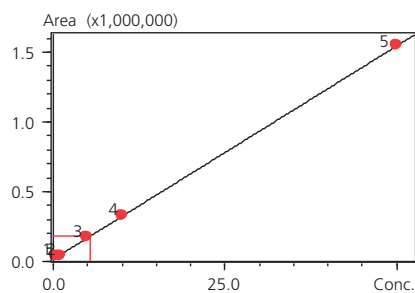


Figure 4.  Sulfamethazine
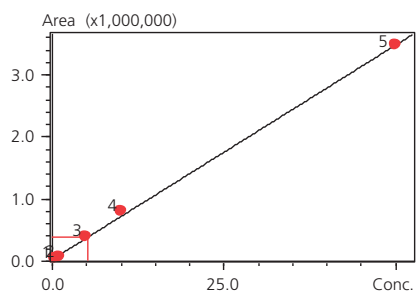
Figure 5.  Albendazole
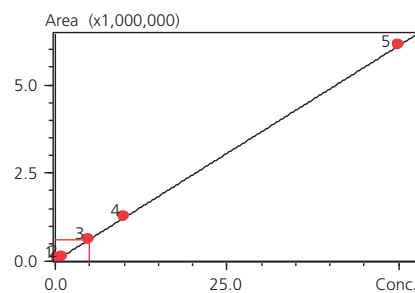
Figure 6.  Leucomalachite green

# Multi-residual quantitative analytical method for antibiotics in sea food by LC/MS/MS

Table 4. Result table for matrix matched standard calibration and recovery at different level

| Sr.No. | Class / category | Name of Compound | MRPL in ppb | LOQ achived in ppb | Linearity range in ppb | Recovery | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | 0.5 ppb | 1 ppb | 2 ppb |
| 1 | Fluoroquinolones | Flumequine | 600 | 0.5 | 0.5-50 | 106.8 | 88.76 | 87.5 |
| 2 | | Oxolinic Acid | 100 | 0.5 | 0.5-50 | 89.32 | 97.7 | 97.03 |
| 3 | | Ciprofloxacin | 100 | 2 | 0.5-50 | NA | NA | 105.36 |
| 4 | | Difloxacin.HCL | 300 | 2 | 0.5-50 | NA | NA | 84.06 |
| 5 | | Enrofloxacin | 100 | 2 | 0.5-50 | NA | NA | 88.52 |
| 6 | | Sarafloxacin | 30 | 1 | 0.5-50 | NA | 117.42 | 86.52 |
| 7 | | Naldixic Acid | Reporting>LOQ | 0.5 | 0.5-50 | 100.82 | 105.52 | 92.6 |
| 8 | Sulfonamides | Sulfadimethoxine | 100 | 0.5 | 0.5-50 | 107.38 | 104.55 | 87.9 |
| 9 | | Sulfadoxine | | 0.5 | 0.5-50 | 102.88 | 89.41 | 89.54 |
| 10 | | Sulfachlorpyridazine | | 0.5 | 0.5-50 | 80.9 | 98.12 | 86.14 |
| 11 | | Sulfamethoxypyridazine | | 0.5 | 0.5-50 | 116.08 | 108.14 | 88.95 |
| 12 | | Sulfamethizole | | 0.5 | 0.5-50 | 82.56 | 87.7 | 89.78 |
| 13 | | Sulfamerazine | | 0.5 | 0.5-50 | 113.78 | 78.61 | 89.88 |
| 14 | | Sulfathiazole | | 0.5 | 0.5-50 | 79.96 | 83.28 | 89.46 |
| 15 | | Sulfamethazin | | 0.5 | 0.5-50 | 90.8 | 90.06 | 90.47 |
| 16 | | Sulfadiazine | | 0.5 | 0.5-50 | 103.66 | 91.11 | 93.93 |
| 17 | | Sulfapyridine | | 0.5 | 0.5-50 | 93.72 | 84.48 | 101.36 |
| 18 | | Sulfamethoxazole | | 0.5 | 0.5-50 | 112.68 | 111.82 | 96.3 |
| 19 | Dyes | Leucocrystal violet | Nil | 0.5 | 0.5-50 | 56.5 | 62.45 | 57.75 |
| 20 | | Crystal violet | | 0.5 | 0.5-50 | 94.56 | 117.22 | 103.44 |
| 21 | | Leucomalachite green | 2 | 0.5 | 0.5-50 | 104.94 | 99.64 | 88.48 |
| 22 | | Malachite green | | 0.5 | 0.5-50 | 103.88 | 73.97 | 64.352 |
| 23 | Anthelminthics | Albendazole | 50 | 0.5 | 0.5-50 | 79.12 | 87.79 | 81.65 |
| 24 | | Albendazole Sulfone | 50 | 0.5 | 0.5-50 | 84.74 | 97.29 | 98.49 |
| 25 | | Albendazole Sulfoxide | 50 | 0.5 | 0.5-50 | 99.56 | 77.13 | 84.98 |
| 26 | | Albendazole-2-aminosulfone | 50 | 1 | 0.5-50 | NA | 79.08 | 75.13 |
| 27 | | Fenbendazole | 50 | 0.5 | 0.5-50 | 111.62 | 88.91 | 88.99 |
| 28 | | Flubendazole | 50 | 0.5 | 0.5-50 | 108.34 | 100.81 | 97.33 |
| 29 | | Morantel | 50 | 0.5 | 0.5-50 | 87.52 | 95.31 | 100.6 |
| 30 | | Fenbendazole sulfone | 50 | 0.5 | 0.5-50 | 78.44 | 84.16 | 93.13 |
| 31 | Nitroimidazoles | Ronidazole | 3 | 0.5 | 0.5-50 | 110.82 | 95.99 | 91.68 |
| 32 | | Metronidazole | 3 | 0.5 | 0.5-50 | 108.16 | 96.71 | 82.89 |
| 33 | | Dimetronidazole | 3 | 2 | 0.5-50 | NA | NA | 89.9 |
| 34 | Phenylbutazone | Phenylbutazone | 5 | 1 | 0.5-50 | NA | 107.11 | 96.11 |
| 35 | Macrolides | Spiramycin | NA | 1 | 0.5-50 | NA | 128.75 | 87.41 |
| 36 | | Tilmicosin | NA | 0.5 | 0.5-50 | 114.14 | 89.45 | 84.68 |
| 37 | | Tylosin Tartarate | NA | 0.5 | 0.5-50 | 119.98 | 94.15 | 105.11 |
| 38 | | Trimethoprim | NA | 0.5 | 0.5-50 | 89.62 | 85.55 | 78.88 |
| 39 | | Neo Spiramycin | NA | 5 | 0.5-50 | 70.34 | 75.9 | 79.23 |

# Conclusion

- The recovery obtained for most of the compounds are well within the acceptance range of 70-120%.
- This method will improve the overall turn around time of sample analysis, along with reduction in per sample cost.

# Reference

[1] Defoirdt, T.; Sorgeloos, P.; Bossier, P. Alternatives to Antibiotics for the Control of Bacterial Disease in Aquaculture. Curr. Opin. Microbiol. 2011, 14, 251–258.

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# SHIMADZU
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# Application News

## No.L509

**High Performance Liquid Chromatography**

## Analysis of Residual Antimicrobials in Meat with Antimicrobial Screening System (Part 1)

In May 2006, the positive list system took effect in Japan that, in principle, prohibited the sale of food products with residual levels of pesticides, animal feed additives, and veterinary drugs (collectively referred to as agricultural chemicals, etc.) above the level determined by the Minister of Health, Labour and Welfare.[1]
Antimicrobials are a type of veterinary drug and animal feed additive, and used for the treatment and prevention of disease in livestock and marine products. Quinolones and sulfonamides are two common groups of synthetic antimicrobials.
Shimadzu's quick and simple antimicrobial screening system is capable of screening 24 antimicrobials compounds. An example screening analysis targeting 12 widely used quinolones (old quinolones, new quinolones) is described here. Application News No.L510 also describes an example screening analysis targeting 12 antimicrobials including sulfonamides (also including antifolates).

### ■ Antimicrobial Screening System

Shimadzu's antimicrobial screening system is able to determine whether levels of antimicrobials subject to regulation in various countries are above a maximum residue limit (MRL). Table 1 shows MRLs for the target quinolones.
The system uses an i-Series integrated HPLC instrument and RF-20Axs high-sensitivity fluorescence detector, and comes with a sample pretreatment method, analytical column, analytical method files, and a UV spectral library that allow for immediate operation after installation. When the analysis method capable of simultaneous component analysis is used, the system can be used for simultaneous screening of multiple components. The determination of whether MRL have been exceeded can be viewed immediately after the system completes analysis. The photodiode array (PDA) detector built into the i-Series instrument supports highly accurate screening with compound identification based on retention times as well as UV spectra.

**Table 1  Maximum Residue Limits and Sample Solution Concentration of Screening Target Compounds**

| | Compound | MRL (mg/kg) | Sample Solution Concentration (mg/L) |
|---|---|---|---|
| 1 | Marbofloxacin | 0.01 | 0.025 |
| 2 | Ofloxacin | 0.01 | 0.025 |
| 3 | Ciprofloxacin | 0.01 | 0.025 |
| 4 | Danofloxacin | 0.01 | 0.025 |
| 5 | Enrofloxacin | 0.01 | 0.025 |
| 6 | Orbifloxacin | 0.01 | 0.025 |
| 7 | Sarafloxacin | 0.01 | 0.025 |
| 8 | Difloxacin | 0.01 | 0.025 |
| 9 | Oxolinic acid | 0.01 | 0.025 |
| 10 | Nalidixic acid | 0.01 | 0.025 |
| 11 | Flumequine | 0.01 | 0.025 |
| 12 | Piromidic acid | 0.01 | 0.025 |

### ■ Sample Pretreatment

Sample pretreatment was performed based on Simultaneous Analysis Method I for Veterinary Drugs by HPLC (Livestock and Marine Products).[2,3] After acetonitrile extraction and removing fat by acetonitrile/hexane partitioning, sample solution was prepared by evaporation then redissolution. Fig. 1 shows the sample pretreatment protocol, and Table 1 shows sample solution concentrations after pretreatment. Refer to the instruction manual of the system for the details of the sample pretreatment procedure.

Sample (homogenized) 1 g
    Extraction
Supernatant
Hexane Partitioning
    Fat Removal
Acetonitrile Layer          Hexane Layer
    Evaporation
Residue
    Redissolution
Acetonitrile Layer - Aqueous Layer
HPLC
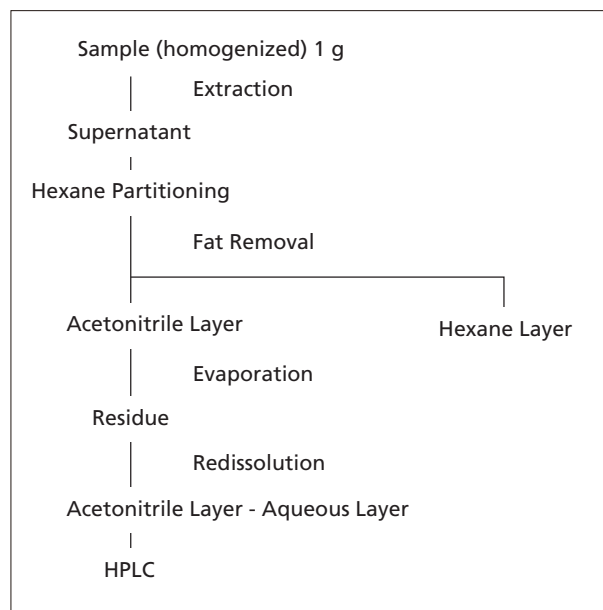
**Fig. 1  Sample Pretreatment Protocol**

**Table 2  Analytical Conditions**

| | |
|---|---|
| System | : LC-2040C 3D, RF-20Axs |
| Column | : Shim-pack FC-ODS (150 mm L. × 4.6 mm I.D., 3 µm) |
| Mobile Phase | : A) 20 mM (Sodium) Phosphate Buffer Containing 0.1 M Sodium Perchlorate |
| | B) Acetonitrile/Methanol=90/10 |
| Time Program | : Gradient Elution |
| Flowrate | : 1.0 mL/min |
| Column Temp. | : 40 °C |
| Injection Volume | : 5 µL |
| Detection | : <LC-2040C 3D> |
| | 280 nm |
| | <RF-20Axs> |
| | Ex at 290 nm, Em at 495 nm |
| | Ex at 325 nm, Em at 365 nm |
| Cell Temp. | : 40 °C (PDA), 30 °C (RF) |

## ■ Analysis of Quinolones in Meat

Chicken and pork were used as samples. Chromatograms of the pretreated matrix solutions (blue line), matrix solutions spiked with standard solution to create matrix standard solutions (red line), and neat standard solution (black line) are shown in Fig. 2. Standard solution was added to matrix solutions to create matrix standard solutions with quinolone concentrations of 0.01 mg/kg. The analytical conditions are shown in Table 2. Analysis was performed with the fluorescence detector in dual wavelength mode. New quinolones (compounds 1 to 8 in Table 1) were detected at an excitation wavelength of 290 nm and fluorescence wavelength of 495 nm, and old quinolones (compounds 9 to 11 in Table 1) were detected at an excitation wavelength of 325 nm and fluorescence wavelength of 365 nm. Piromidic acid (compound 12 in Table 1) differs from other quinolones in exhibiting no fluorescence characteristics, and was detected using the PDA detector. Employing the analytical conditions shown, all 12 compounds were separated and eluted in approximately 22 minutes.

## ■ Similarity Calculation Using UV Spectral Library

The PDA-detected compound (piromidic acid) can be analyzed qualitatively based on UV spectra as well as retention times. Its spectrum can be checked for similarity against the library spectra. Fig. 3 shows a UV spectrum of piromidic acid in pork matrix spiked with a standard solution of piromidic acid at threshold concentration. The degree of similarity with the library spectrum was 0.998.
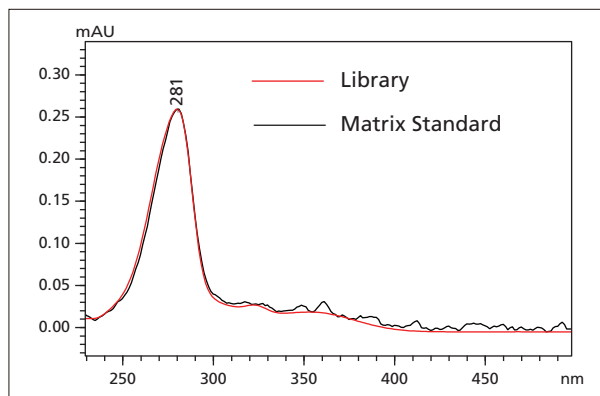


**Fig. 3 Spectra of Piromidic Acid**
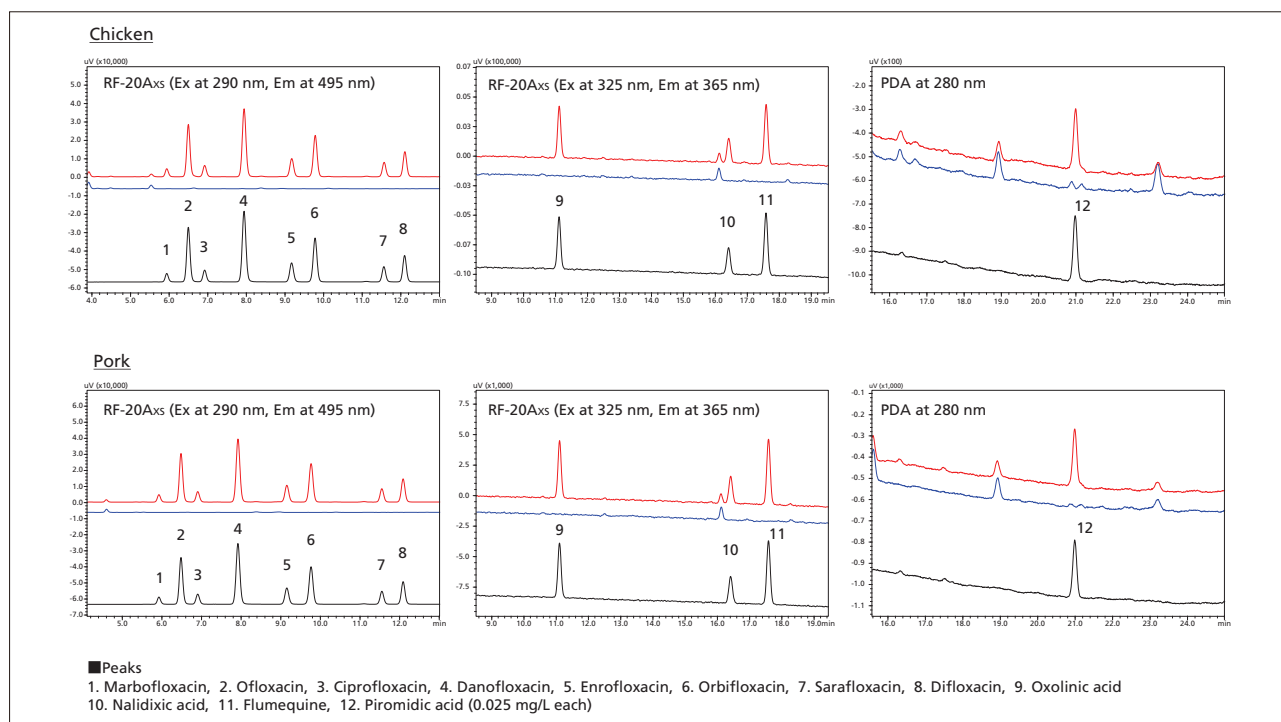


**Fig. 2 Chromatograms of Chicken and Pork:**
**Matrix Standard Solution (Red Line), Matrix Solution (Blue Line), Neat Standard Solution (Black Line)**

<References>
1) The Japanese Positive List System for Agricultural Chemical Residues in Foods, Japan's Ministry of Health, Labour and Welfare
2) Multiresidue Method I for Veterinary Drugs, Etc. by HPLC (Animal and Fishery products)
   Director Notice about Analytical Methods for Residual Compositional Substances of Agricultural Chemicals, Feed Additives, and Veterinary Drugs in Food (Syoku-An No. 0124001, January 24, 2005. Final amendments were made on May 26, 2006.), Japan's Ministry of Health, Labour and Welfare
3) "Standard methods of analysis in food safety regulation (for veterinary drugs and animal feed additives)" p.26-43, Japan Food Hygiene Association (2003), edited under the supervision of the Japan's Ministry of Health, Labour and Welfare

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# Application News

## No.L510

**High Performance Liquid Chromatography**

## Analysis of Residual Antimicrobials in Meat with Antimicrobial Screening System (Part 2)

Antimicrobials are a type of veterinary drug and animal feed additive, and are used for the treatment and prevention of disease in livestock and marine products. Residual antimicrobials are often found in livestock and marine products, so threshold levels for antimicrobials are set by regulation to ensure the safety of the consumer based on amounts that do not harm human health.

Due to ongoing reports of recent cases of regulatory violations in various countries and the large number of compounds targeted for testing, there is a demand for quick and simple antimicrobial screening.

While Application News No.L509 described an example of using the antimicrobial screening system for screening 12 quinolone compounds, this Application News describes an example screening analysis of 12 antimicrobial target compounds including sulfanomides.

### ■ Sample Pretreatment

Sample pretreatment for analysis of residual antimicrobials in meat usually employs liquid-liquid extraction (and sometimes solid phase extraction), but this process takes time and effort. In this article, we employed a QuEChERS method designed to be more efficient and reduce pretreatment times. The QuEChERS method is used to pretreat vegetables and fruits for residual pesticide analysis.

After using the QuEChERS method to perform extraction and fat removal, sample solutions were prepared by evaporation and redissolution steps. Table 1 shows the maximum residue limits (MRLs) of target compounds and sample solution concentrations after sample pretreatment, and Fig. 1 shows the sample pretreatment protocol. Refer to the instruction manual of the system for the details of the sample pretreatment procedure.

**Table 1  Maximum Residue Limits and Sample Solution Concentration of Screening Target Compounds**

| | Compound | MRL (mg/kg) | Sample Solution Concentration (mg/L) |
|---|---|---|---|
| 1 | Sulfadiazine | 0.01 | 0.025 |
| 2 | Sulfamerazine | 0.01 | 0.025 |
| 3 | Sulfadimidine | 0.01 | 0.025 |
| 4 | Sulfamonomethoxine | 0.01 | 0.025 |
| 5 | Trimethoprim | 0.01 | 0.025 |
| 6 | Sulfamethoxazole | 0.01 | 0.025 |
| 7 | Ormetoprim | 0.01 | 0.025 |
| 8 | Sulfadimethoxine | 0.01 | 0.025 |
| 9 | Sulfaquinoxaline | 0.01 | 0.025 |
| 10 | Pyrimethamine | 0.01 | 0.025 |
| 11 | Difurazon | 0.01 | 0.025 |
| 12 | Nicarbazin*1 | 0.01 | 0.025 |

*1: Concentration of N, N'-Bis(4-nitrophenyl)urea, the main constituent of nicarbazin.



**Fig. 1  Sample Pretreatment Protocol**

Sample (homogenized) 10 g
— Extraction
Supernatant
— Fat Removal
Acetonitrile Layer
— Evaporation
Residue
— Redissolution
Acetonitrile Layer - Aqueous Layer
—
HPLC
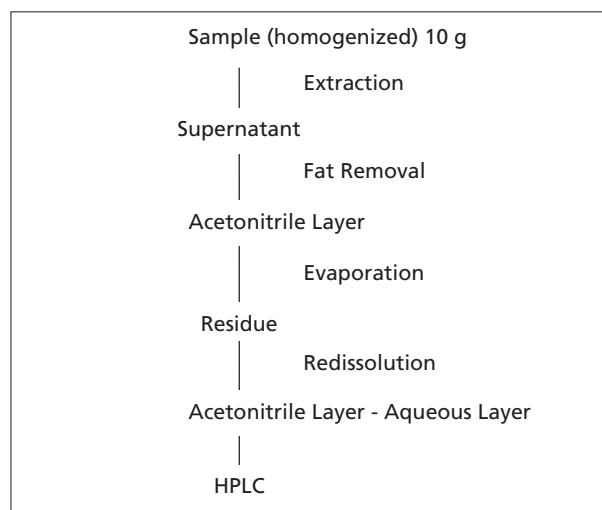
### ■ Analysis of Antimicrobials Including Sulfonamides in Meat

Chicken and beef were used as samples. The analytical conditions are shown in Table 2. Chromatograms of the pretreated matrix solutions (blue line), matrix solutions spiked with standard solution to create matrix standard solutions (red line), and neat standard solution (black line) are shown in Fig. 2.

Standard solution was added to matrix solutions to make up antimicrobial concentrations, including sulfonamide concentrations, of 0.01 mg/kg in matrix standard solutions. Standard solutions were prepared to the sample solution concentrations listed in Table 1.

The photodiode array (PDA) detector (six-wavelength) built in the i-Series instrument was used for detecting all target compounds. Employing the analytical conditions shown, all 12 compounds were separated and eluted in approximately 25 minutes.

**Table 2  Analytical Conditions**

| | |
|---|---|
| System | : LC-2040C 3D |
| Column | : Shim-pack FC-ODS (150 mm L. × 4.6 mm I.D., 3 µm) |
| Mobile Phase | : A) 20 mM (Sodium) Phosphate Buffer Containing 0.1 M Sodium Perchlorate |
| | B) Acetonitrile/Methanol=80/20 |
| Time Program | : Gradient Elution |
| Flowrate | : 1.0 mL/min |
| Column Temp. | : 50 ℃ |
| Injection Volume | : 20 µL |
| Detection | : 240 nm |
| | 270 nm |
| | 280 nm |
| | 285 nm |
| | 350 nm |
| | 380 nm |
| Cell Temp. | : 40 ℃ |

### ■ Similarity Calculation Using UV Spectral Library

All target compounds in this Application News can be analyzed qualitatively based on UV spectra as well as retention times. Sample spectra can be also checked for similarity against library spectra. Fig. 3 shows a UV spectrum of sulfaquinoxaline in a beef matrix spiked with a standard solution of sulfaquinoxaline at threshold concentration. Degree of similarity with the library spectrum was 0.997.
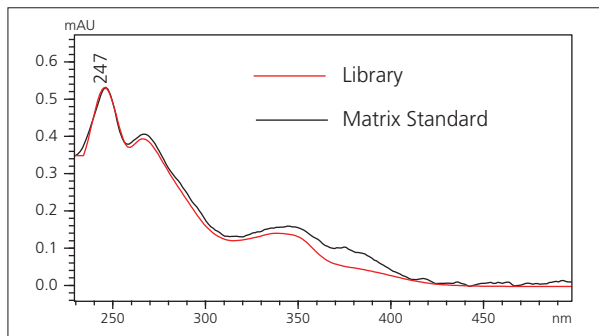


**Fig. 3  Spectra of Sulfaquinoxaline**



■Peaks
1. Sulfadiazine,  2. Sulfamerazine,  3. Sulfadimidine,  4. Sulfamonomethoxine,  5. Trimethoprim,  6. Sulfamethoxazole,  7. Ormetoprim,  8. Sulfadimethoxine,
9. Sulfaquinoxaline,  10. Pyrimethamine,  11. Difurazon,  12. Nicarbazin (0.025 mg/L each)
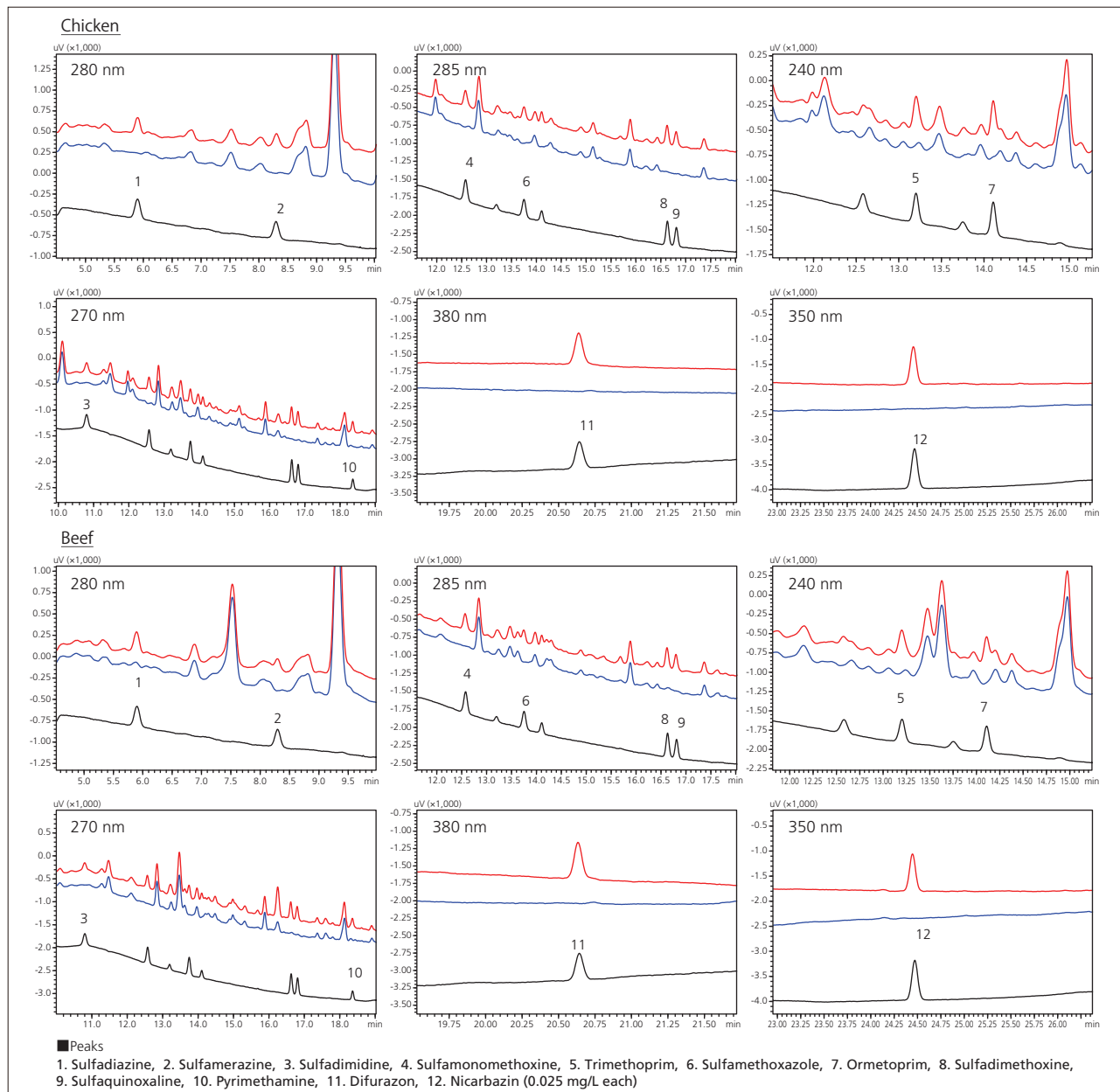
**Fig. 2  Chromatograms of Chicken and Beef:
Matrix Standard Solution (Red Line), Matrix Solution (Blue Line), Neat Standard Solution (Black Line)**

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# SHIMADZU
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# Application News

MultiNA

# No. MultiNA-013

## Microchip Electrophoresis-Based Quantitative Determination of Composition of Blended Meat

LI Lixiao

Analytical Instruments Dept. Analytical Application Center Shimadzu (China) Co., LTD

### ■ Introduction

Adulterations of meat which jeopardize customer's heath, benefit and confidence were disclosed recently in the world. For example, fake beef were made from horse and pork which are cheaper materials. It is difficult to distinguish meat species with vision and smell. To detect meat species, molecular biological approach is used based on specific DNA profiles of different varieties. With PCR primer designing towards to variety's specific DNA sequence, qualitative determination is achieved by evaluating PCR product size with electrophoresis. However, due to the strong sensitive of PCR approach, there is a risk of false positive with only qualitative determination. Therefore, quantitative determination is strongly required to obtain correct results for meat detection. In this report, a method for quantitative determination of composition of blended meat was developed based on microchip electrophoresis. With this method, blended meat of beef and pork were successfully determined quantitatively.

### ■ Experimental

#### Principle

For PCR, the relationship between the amount of PCR product of target gene and initial template DNA is shown in the following:

$$X_n = X_0(1+E)^n$$

$X_n$ is the amount of PCR product of target gene with n cycles, n is PCR cycle number, $X_0$ is the amount of initial template DNA. E is efficiency of PCR which is affected by temperature, primer and polyase. If the effect of E term is negligible with the same temperature, primer and polyase, $X_n$ has the linear relationship with $X_0$.

On the other hand, Shimadzu microchip electrophoresis MCE 202 MultiNA with quartz microchip using a standard DNA sample as internal standard can measure the amount of PCR product. In this study, sample was pretreated with Shimadzu Ampdirect kit which can eliminate PCR inhibitor. Due to the same pretreated and extracted method for all samples, obtained DNA amount is lineally dependent on the amount of sample. Thereby, quantitative determination of the amount of sample was realized by MultiNA.

#### Instrument

MCE-202 MultiNA

#### Reagent

1mol/l-Tris-HCl Buffer Solution (pH 8.0), 1L nacalai tesque, Code:35435-11

0.5mol/l-EDTA Solution (pH 8.0), 1L nacalai tesque, Code:14347-21

5mol/l-Sodium Chloride Solution, 1L nacalai tesque, Code:31334-51

10%-SDS Solution, 100 mL nacalai tesque, Code:30562-04

Proteinase K（powder）100 mg SIGMA, Code:P6556（100mg）

Ampdirect Plus (For International) WAKO pure chem. Code: 604-21469

Shimadzu corp.,Code: S241-08800-99

IMMOLASE™ DNA Polymerase BIOLINE, Code: BIO-21046

DNA-500 KIT (1000 analyses) Shimadzu corp.,

Code: 292-27910-91

SYBR Gold Life Technologies,

Code: S-11494

25bp DNA Ladder Life Technologies,

Code: 10597-011

TE buffer (pH 8.0) nacalai tesque, Code: 32739-31

#### Sample

The composition of pork, beef and blended meat of pork and beef are shown in Table 1.

**Table 1**. The composition of sample

| No. | Beef /g | Prok /g |
|-----|---------|---------|
| 1 | 0.50 | 0 |
| 2 | 0.40 | 0.10 |
| 3 | 0.30 | 0.20 |
| 4 | 0.20 | 0.30 |
| 5 | 0.10 | 0.40 |
| 6 | 0 | 0.50 |

#### Pretreatment and PCR condition

Sample preparation and reaction conditions are shown in Figure 1.
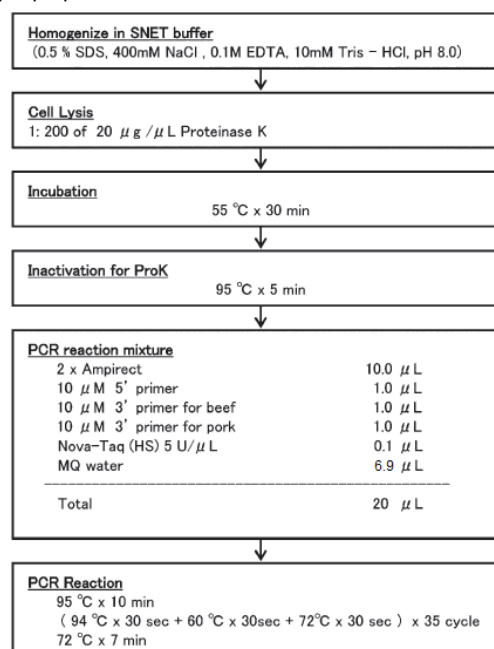


**Figure 1**. Sample preparation and reaction conditions

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# Application News

MultiNA

## No. MultiNA-013

# Microchip Electrophoresis-Based Quantitative Determination of Composition of Blended Meat

Lixiao Li

Analytical Instruments Dept., Analytical Application Center, Shimadzu (China) Co., LTD

## ■ Result and discussion

Gel diagram and electropherogram of determination of beef and pork with different composition by MultiNA are shown in Figures 2 and 3. The result suggested that the specific fragment of beef and pork were successfully amplified, in addition, size of these fragments were detected by MultiNA and agreement with expected value. Furthermore, the relationship between the concentration of pork PCR product and pork weigh is shown in Figure 4. It is indicated that the concentration of pork PCR product has the linear relationship with pork weigh. Moreover, the concentration of beef PCR product has the linear relationship with beef weigh as well shown in Figure 4. It is showed that experimental result is agreement with theoretical equation, and quantification determination of meat was achieved by this method.
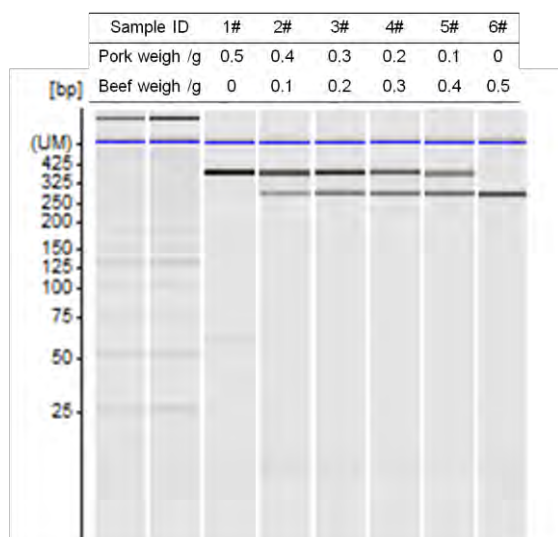


**Figure 2.** Gel diagram of determination of beef and pork with different composition by MultiNA.
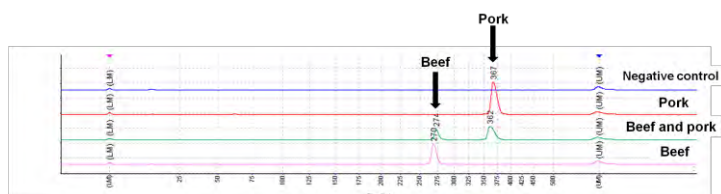


**Figure 3**. Electropherogram of determination of beef and pork with different composition by MultiNA.
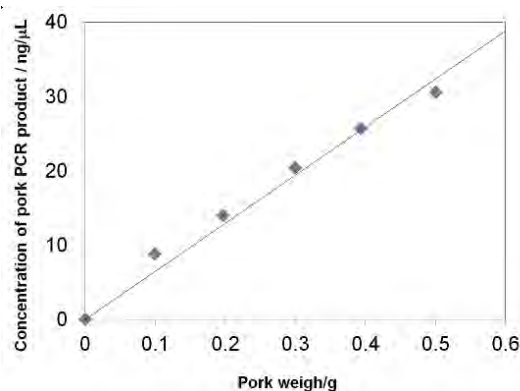


**Figure 4.** The relationship between concentration of pork PCR product and pork weigh.
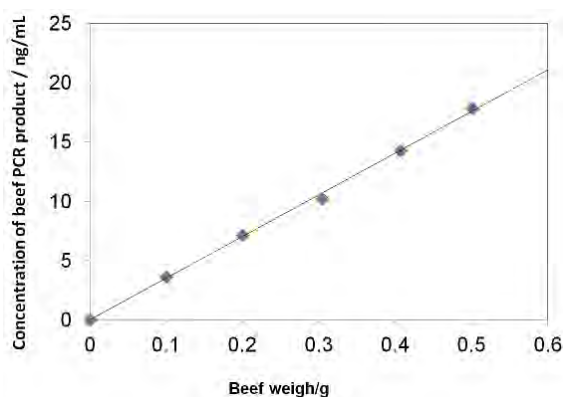


**Figure 5**. The relationship between concentration of beef PCR product and beef weigh.

## ■ Conclusion

In conclusion, a method for quantitatively determining blended meat was successfully developed. This method can be applied to further confirm positive samples of quality determination for meat, and it is a powerful approach to detect adulterations with different species.

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Shimadzu Corporation

# Determination of chemical contaminants in marine fish by GCMS/MS using QuEChERS as an extraction method

**ASMS 2015** TP 338

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# Introduction

As Persistent Organic Pollutants (POPs) are environmental contaminants with a strong potential for bioaccumulation, these chemicals are expected to be present in farm animals and food products of animal origin.[1] In recent years, the occurrence of various POPs, in the marine environment has received much attention with regards to their effect on human health. Especially in India, marine water is contaminated due to uncontrolled disposal from industries and domestic waste. Hence the fish obtained from it has high risk of contamination. Evidently, it becomes necessary to have sensitive, accurate, reliable, reproducible and fast analytical method to quantify these POPs in marine fish at ppb levels.
Fish is a complex matrix and hence requires selective extraction and extensive cleanup such as QuEChERS (Quick Easy Cheap Effective Rugged Safe) to ensure trace level detection with adequate precision and accuracy. In this study, Shimadzu GCMS-TQ8040 was used in Multiple Reaction Monitoring (MRM) mode to analyze POPs like



Figure 1.  Prawns

Organo-Chlorine Pesticides (OCP), Polycyclic Aromatic Hydrocarbons (PAH) and Poly-Chlorinated Biphenyls (PCB).
Prawns (Figure 1) sample purchased from local market was extracted, spiked and analyzed for obtaining LOD, LOQ, precision and recovery.

# Method of analysis

## Extraction of pesticides from prawns

Extraction of pesticides was done using modified AOAC QuEChERS method, as given below[2]

# Determination of chemical contaminants in marine fish by GCMS/MS using QuEChERS as an extraction method

Remove the shell of prawns. Take 10 g of finely grounded prawns sample. Add 10 mL of water. Homogenize the sample and Keep it for 30 min.

Add 10 mL of acetonitrile containing 0.5 % acetic acid. Immediately, add QuEChERS salt (Restek Cat # 26238 : 6g $MgSO_4$ + 1.5g NaAcetate)

Shake and vortex for 1 min.

Centrifuge at 5000 rpm for 5 min.

Allow to stand for 1 min for phase separation

Draw 6 mL from upper extract of acetonitrile for further cleanup

Transfer the extract to dSPE tube containing cleanup mixture [Restek Cat # 26221 : 1200mg anhydrous $MgSO_4$, 400mg PSA (Primary secondary amines) and 400mg C18]. Vortex for 2 min.

Centrifuge the mixture at 5000 rpm for 5 min.

Collect the supernatant and filter through a 0.2 μm PTFE membrane filter

Inject 2.0 μL of the clean extract into GCMS-TQ8040 (Figure 2)

## MRM method development

Individual mixtures of OCP, PAH and PCB standards were procured from Restek®. For OCP, the MRM transitions existing in Smart Database were used. For PAH, they were obtained from existing application data and imported in Smart Database. In case of PCB, MRMs were optimized. For optimization, about 1 ppm standard mixtures of PCB was analyzed using scan mode. Retention times of individual components were identified and precursor ions were selected. Using selected precursor ion, product ion scan was performed with different Collision Energies (CE). For each component of PCB, MRM transitions with appropriate CEs were determined (Refer Figure 3). All the above steps were simplified with the help of Smart MRM optimization tool. These MRM transitions for PCB were then registered to Smart Database containing OCP and PAH. From this, the final method with optimum segments (Refer Figure 4) and minimum three MRM transitions per compound was generated. The mixture of OCP, PAH and PCB was analyzed using the created method as shown in Table 2 and the MRM chromatogram for the same is shown in Figure 5.

Figure 2.  GCMS-TQ8040 Triple quadrupole system by Shimadzu

## Key Features of GCMS-TQ8040

1.  **Smart Productivity :** Analysis of 400 pesticides that used to require 2 or 3 methods, can now be accomplished in a single acquisition method by the new firmware protocol.
2.  **Smart Operation :** Smart MRM technology creates optimal MRM methods automatically. The "MRM Optimization Tool" automates best MRM transitions for new compounds.
3.  **Smart Performance :** ASSP achieves high sensitivity at scan speeds of 20,000 u/second. Fastest  MRM 800 trans/sec. Single GC/MS mode with the maximum possible sensitivity and repeatability.

## GCMS/MS Analytical Conditions

The analysis was carried out on Shimadzu GCMS-TQ8040 as per the conditions given in Table 1.

Table 1.  Analytical conditions

| Chromatographic parameters | |
|---|---|
| Column | : Rxi-5Sil MS (30 m L x 0.25 mm I.D. x 0.25 µm) |
| Injection Mode | : Splitless |
| Sampling Time | : 2.00 min |
| Split Ratio | : 5.0 |
| Carrier Gas | : Helium |
| Flow Control Mode | : Linear Velocity |
| Linear Velocity | : 40.2 cm/sec |
| Column Flow | : 1.2 mL/min |
| Injection Volume | : 1.0 µL |
| Injector Type | : High Pressure Injection |
| Total Program Time | : 45.87 min |

Column Temp. Program :

| Rate (°C /min) | Temperature (°C) | Hold time (min) |
|---|---|---|
|  | 70.0 | 2.00 |
| 25.00 | 150.0 | 0.00 |
| 3.00 | 200.0 | 0.00 |
| 8.00 | 280.0 | 10.00 |

| Mass Spectrometry parameters | |
| --- | --- |
| Ion Source Temp. | : 230.0 ℃ |
| Interface Temp. | : 280.0 ℃ |
| Ionization Mode | : EI (Electron Ionization) |
| Acquisition Mode | : MRM |

# Results

Prawns sample was extracted to prepare matrix blank, which was spiked with various concentration levels of POPs to prepare matrix match or post-extraction spike linearity. Using this linearity parameters like LOD, LOQ, precision were studied. Against this linearity, pre-extraction spike was analyzed to study the recoveries.

Table 2.  Method creation using Smart MRM feature of GCMS-TQ8040

| Type of compounds | Condition | Intermediate requirement | Step.1 | Step.2 | Step.3 |
| --- | --- | --- | --- | --- | --- |
| PCB | No information about transitions | Measure in Scan mode and Determine Pre-cursor ion | **MRM Optimization Tool** Create Batch sequence and Method file of several Collision Energy automatically. | **MRM Optimization Tool** Analyze acquired data files, and select the best transitions and collision energy automatically. And the result can be exported to Smart MRM database on a mouse click. | Method creation using Smart Database |
| PAH | Known MRM transitions but, Collision energies are not optimized | | | | |
| OCP | Present in Smart Pesticide Database | | | | |
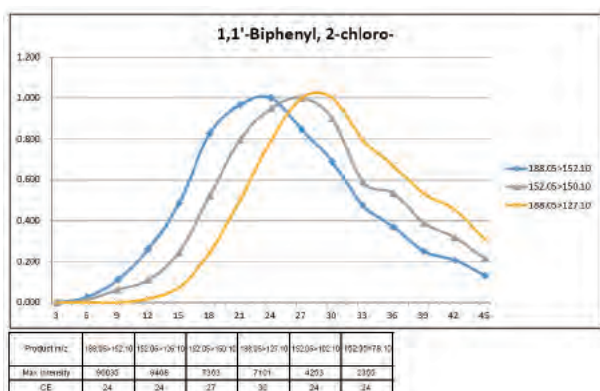


Figure 3.  CE Optimization using Smart MRM optimization tool
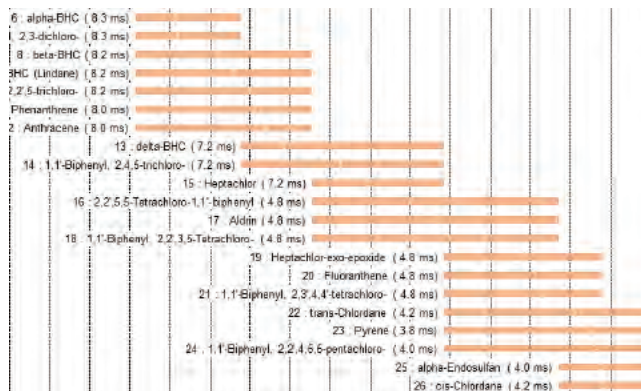


Figure 4.  Optimum segmented method created using Smart Database

Relative Standard Deviation (% RSD) for 5 ppb standard solution (n=5) was less than 15 % for all components. Calibration plot of matrix match standards ranging from 1 ppb to 50 ppb concentration level showed linear response with r2 more than 0.995. Recoveries for the prawns sample spiked with 5 ppb standard mixture were in the range of 70 to 130 %. On the basis of statistical data obtained as shown in Table 3, the method was proved to be highly selective, sensitive and accurate.
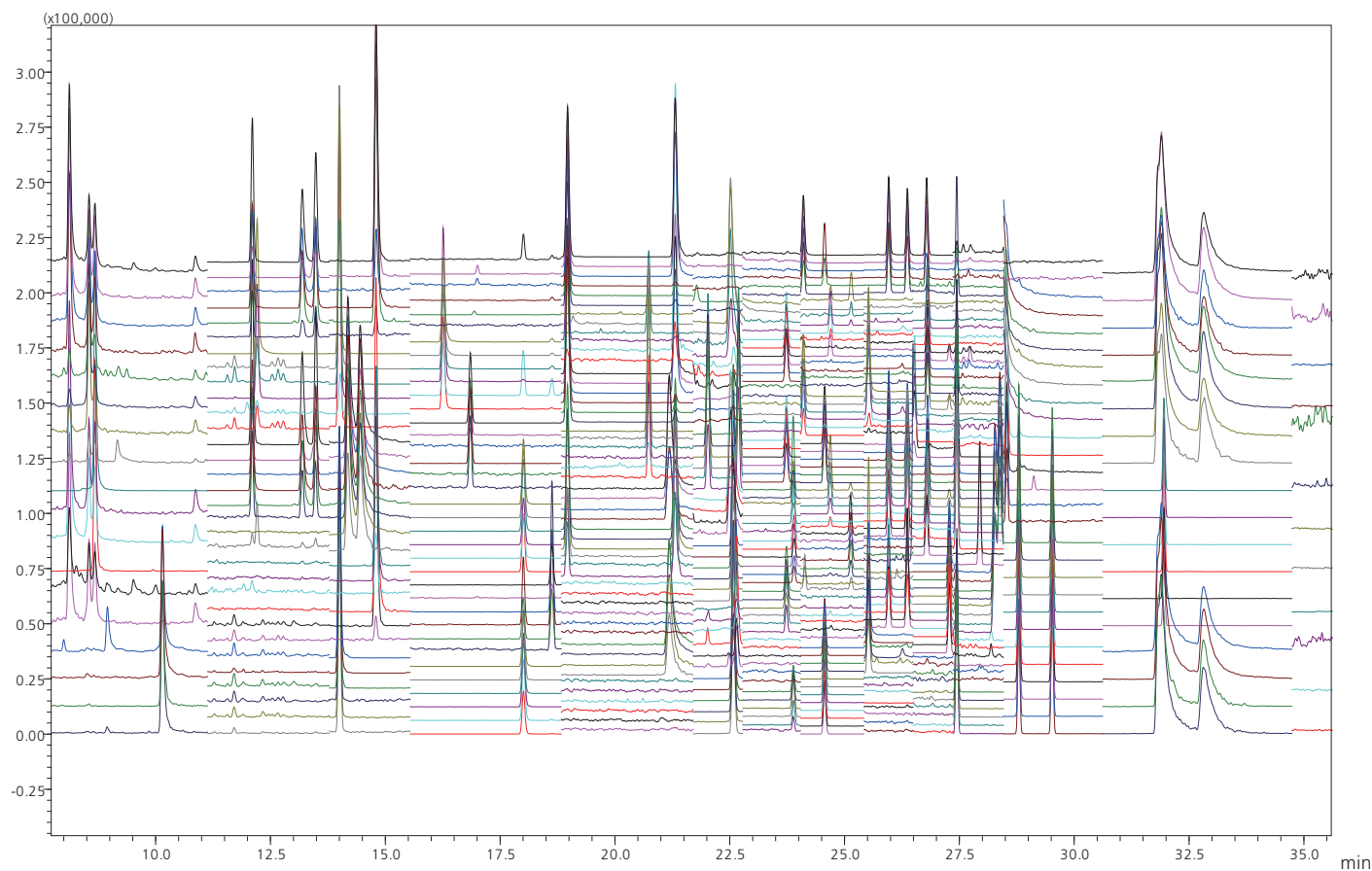


Figure 5.  MRM Chromatogram for 10 ppb POPs mixture in matrix

# Determination of chemical contaminants in marine fish by GCMS/MS using QuEChERS as an extraction method

Table 3.  Quantitation results

| ID | POPs | Retention time (min) | Target MRM (m/z) | $r^2$ | S/N at 5 ppb LOQ level | % RSD at LOQ level (n=5) | % Recovery at LOQ |
|---|---|---|---|---|---|---|---|
| 1 | Naphthalene | 5.41 | 128.10>128.10 | 0.9993 | 2261.7 | 3.97 | 117 |
| 2 | Acenaphthylene | 8.09 | 152.10>152.10 | 0.9994 | 349.37 | 5.46 | 94 |
| 3 | Acenaphthene | 8.53 | 153.10>153.10 | 0.9999 | 16.05 | 1.74 | 104 |
| 4 | Fluorene | 10.11 | 166.10>166.10 | 0.9996 | 98.46 | 8.96 | 96 |
| 5 | Phenanthrene | 14.12 | 178.10>178.10 | 1.0000 | 22.5 | 3.00 | 111 |
| 6 | Anthracene | 14.39 | 178.10>178.10 | 1.0000 | 31.09 | 5.13 | 95 |
| 7 | Fluoranthene | 21.23 | 202.10>202.10 | 1.0000 | 112.84 | 5.22 | 107 |
| 8 | Pyrene | 22.59 | 202.10>202.10 | 1.0000 | 55.95 | 7.10 | 102 |
| 9 | Benz[a]anthracene | 28.44 | 228.10>228.10 | 0.9950 | 17.2 | 4.25 | 124 |
| 10 | Chrysene | 28.44 | 228.10>228.10 | 0.9948 | 21.66 | 4.47 | 131 |
| 11 | Benzo[k]fluoranthene | 32.00 | 252.10>252.10 | 0.9999 | 999.8 | 4.80 | 85 |
| 12 | Benzo[b]fluoranthene | 32.01 | 252.10>252.10 | 0.9998 | 303.42 | 7.99 | 91 |
| 13 | Benzo[a]pyrene | 32.97 | 252.10>252.10 | 0.9993 | 88.39 | 9.18 | 79 |
| 14 | Indeno[1,2,3-cd]pyrene | 37.37 | 276.10>276.00 | 0.9996 | 22.22 | 7.19 | 70 |
| 15 | Dibenz(a,h)anthracene | 37.45 | 278.10>278.10 | 0.9948 | 36.15 | 12.28 | 94 |
| 16 | Benzo[g,h,i]Perylene | 38.42 | 276.10>276.10 | 0.9996 | 918.4 | 9.98 | 70 |
| 17 | 1,1'-Biphenyl, 2-chloro- | 8.67 | 188.05>152.10 | 0.9998 | 3181.75 | 3.25 | 95 |
| 18 | 1,1'-Biphenyl, 2,3-dichloro- | 12.19 | 222.00>152.10 | 0.9999 | 691.78 | 1.48 | 92 |
| 19 | 1,1'-Biphenyl, 2,2',5-trichloro- | 13.99 | 255.95>186.00 | 0.9999 | 1516.47 | 1.01 | 93 |
| 20 | 1,1'-Biphenyl, 2,4,5-trichloro- | 16.25 | 255.95>186.00 | 0.9999 | 582.39 | 2.17 | 85 |
| 21 | 2,2',5,5-Tetrachloro-1,1'-biphenyl | 18.00 | 289.90>220.00 | 0.9999 | 1326.79 | 2.16 | 86 |
| 22 | 1,1'-Biphenyl, 2,2',3,5-Tetrachloro- | 18.96 | 289.90>220.00 | 0.9998 | 597.52 | 4.12 | 87 |
| 23 | 1,1'-Biphenyl, 2,3',4,4'-tetrachloro- | 21.29 | 289.90>219.90 | 1.0000 | 117.87 | 2.57 | 80 |
| 24 | 1,1'-Biphenyl, 2,2',4,5,5-pentachloro- | 22.57 | 325.90>255.90 | 0.9999 | 1487.66 | 5.86 | 73 |
| 25 | 1,1'-Biphenyl, 2,2',3,4,5-pentachloro- | 23.73 | 325.90>255.90 | 0.9998 | 263.7 | 6.44 | 75 |
| 26 | 2,3,3',4,6'-Pentachloro-1,1'-biphenyl | 24.09 | 325.90>255.80 | 0.9997 | 303.94 | 1.99 | 80 |
| 27 | 2,2',3,5,5',6'-Hexachloro-1,1'-biphenyl | 24.56 | 359.85>289.80 | 0.9999 | 635.77 | 4.58 | 70 |
| 28 | alpha-BHC | 12.15 | 180.90>144.90 | 0.9999 | 740.26 | 4.36 | 106 |
| 29 | beta-BHC | 13.23 | 180.90>144.90 | 0.9999 | 424.32 | 1.78 | 113 |
| 30 | gamma-BHC (Lindane) | 13.57 | 180.90>144.90 | 0.9999 | 484.21 | 3.37 | 106 |
| 31 | delta-BHC | 14.83 | 180.90>144.90 | 0.9997 | 132.15 | 4.84 | 100 |
| 32 | Heptachlor | 16.92 | 271.80>236.90 | 1.0000 | 376.67 | 7.29 | 98 |
| 33 | Aldrin | 18.71 | 262.90>193.00 | 0.9997 | 62.23 | 3.17 | 78 |
| 34 | Heptachlor-exo-epoxide | 20.82 | 352.80>262.90 | 0.9998 | 132.2 | 6.57 | 114 |
| 35 | trans-Chlordane | 22.10 | 372.80>263.90 | 0.9999 | 424.26 | 7.84 | 90 |
| 36 | cis-Chlordane | 22.69 | 372.80>263.90 | 1.0000 | 388 | 7.73 | 82 |
| 37 | alpha-Endosulfan | 22.74 | 194.90>160.00 | 0.9999 | 11.86 | 11.71 | 97 |
| 38 | Dieldrin* | 23.95 | 262.90>193.00 | 0.9997 | 162.75 | 10.58 | 78 |
| 39 | p,p'-DDE | 23.99 | 246.00>176.00 | 1.0000 | 205.29 | 3.00 | 86 |
| 40 | Endrin* | 24.78 | 262.90>191.00 | 1.0000 | 184.28 | 8.87 | 107 |
| 41 | beta-Endosulfan* | 25.22 | 194.90>160.00 | 0.9988 | 24.94 | 11.52 | 107 |
| 42 | p,p'-DDD | 25.63 | 235.00>165.00 | 1.0000 | 365.71 | 4.30 | 109 |
| 43 | Endosulfan sulfate | 26.61 | 271.80>236.90 | 0.9988 | 183.35 | 11.75 | 95 |
| 44 | p,p'-DDT | 26.86 | 235.00>165.00 | 1.0000 | 101.05 | 5.24 | 103 |

Note * : For these compounds LOQ was 10 ppb        Key : | RAH | PCB | OCP |

# Conclusion

- Shimadzu GCMS-TQ8040 with Smart MRM feature was able to optimize MRM transitions with ease.
- New Smart Database tool creates method with optimum segments leading to increased dwell-time, which resulted in achieving high sensitivity for trace level quantitation of POPs in complex matrix like prawns.
- The MRM method developed for POPs can be used for screening of pesticides in various marine fish products. For 90 % of the POPs, LOQ of 5 ppb was achieved.

# References

[1]  Weiss, J., Paepke, O. and Bergman, A. 2005. A wordwide survey of polychlorinated dibenzo-p-dioxins, dibenzofurans, and related contaminants in butter. Ambio 34(8):22-30.
[2]  Pesticide Residue in Foods by Acetonitrile Extraction and Partitioning with Magnesium Sulfate (AOAC Official Method 2007.01), (2007), 06.

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# Application News

## No.C104

**Liquid Chromatography Mass Spectrometry**

# Analysis of Diarrhetic Shellfish Toxin Using Triple Quadrupole LC/MS/MS (LCMS-8050)

The Japanese Ministry of Health, Labour and Welfare (JMHLW) specified in July, 1980 that the mouse bioassy (MBA) be used as the official method for diarrhetic shellfish toxin, and that the permissible exposure limit be 0.05 MU per gram of edible shellfish*[)]. Shellfish in which the toxin exceeds this limit are prohibited from being sold at market according to the Japanese Food Sanitation Law Article 6, Item 2.

Due to significant technological advances since 1980, the sensitivity and accuracy obtained using the MBA method are significantly inferior compared to the high-precision, high-sensitivity possible using liquid chromatography mass spectrometry analytical instrumentation, which is currently used for this application. A complete transition to instrumental analysis for lipophilic marine biotoxins is scheduled to be implemented by January 2015 throughout the EU.

Based on this international trend, the JMHLW is currently considering migration to an instrumental analysis assay and setting new reference values to be used with instrumental analysis, in addition to the introduction of the Codex standard for okadaic acids (OA, Reference 1).

**Table 1  CODEX Standard 292-2008**

|  | Reference Value |
|---|---|
| OA Acids (OA and DTX group) | Permissible ingestion limit of 0.16 mg OA per kg of edible shellfish |

Fig. 1 shows examples of LC/MS/MS high-sensitivity analysis of okadaic acid (OA), dinophysistoxin 1 (DTX1) and pectenotoxins (PTX1, 2, 6) and yessotoxin 1 (YTX1). Thus, it is possible to conduct high-sensitivity, high-separation analysis of each component.

Fig. 2 and Fig. 3 show MRM chromatograms of standard samples of OA and DTX1, respectively.

* The amount of toxin resulting in the death of two out of three mice following intraperitoneal administration of the equivalent of 20 g per edible shellfish.



PTX6 906.50 > 835.40(+)
OA 803.50 > 255.20(-) (4.00)
YTX1 1141.50 > 1061.30(-) (9.00)
DTX1 817.50 > 255.20(-) (5.00)
PTX1 892.60 > 821.40(+)
PTX2 876.50 > 805.40(+)
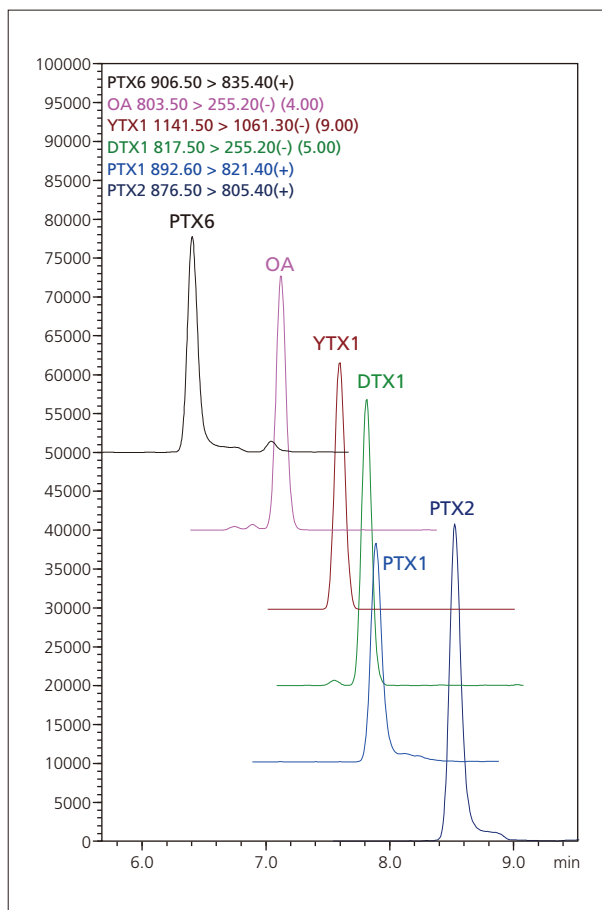
**Fig. 1  MRM Chromatograms of Diarrhetic Shellfish Toxin (1 ng/mL)**



5:803.50 > 255.20(-)

0.025     0.05     0.1     0.5     1     5     10     50 ng/mL
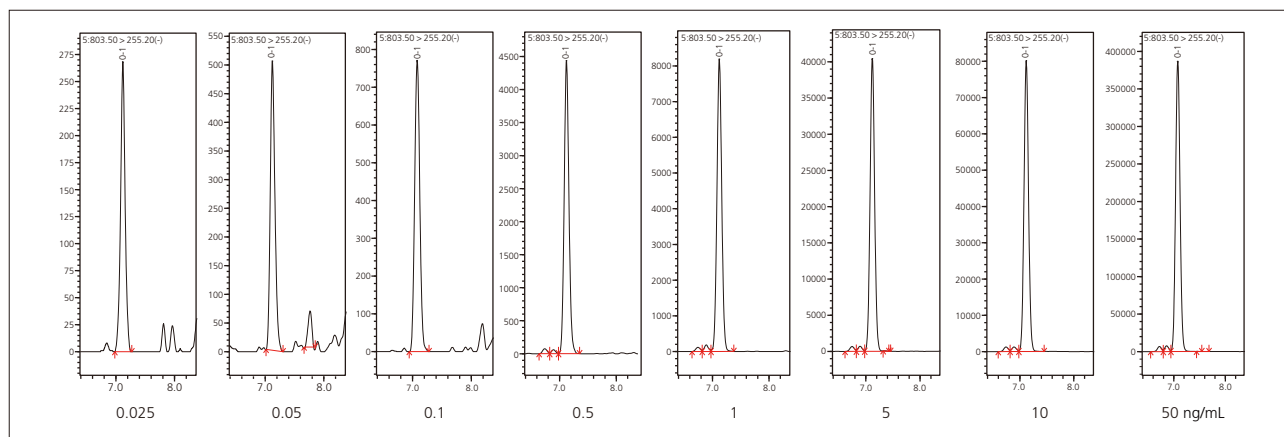
**Fig. 2  MRM Chromatograms of Okadaic Acid (OA)**
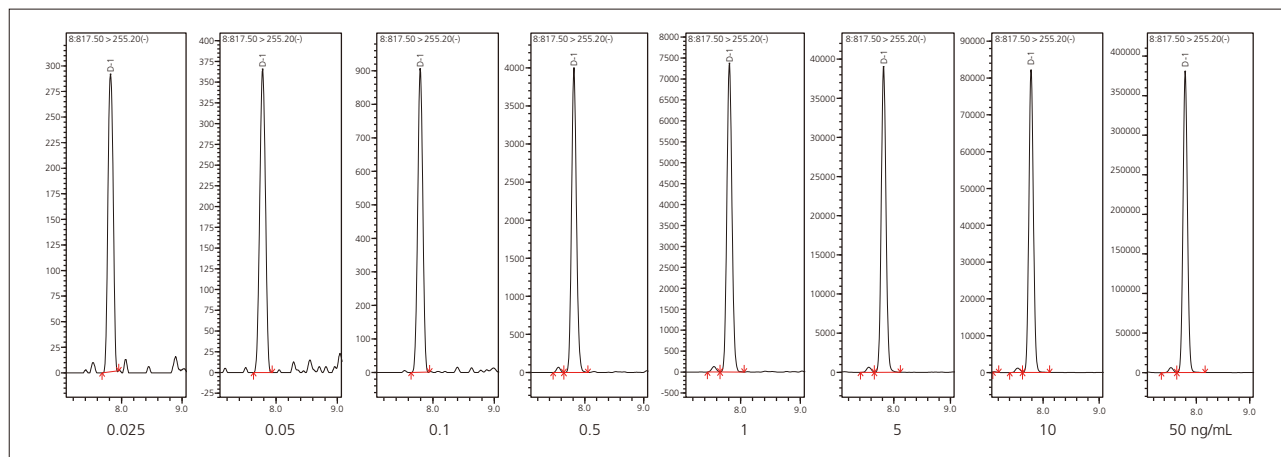
**Fig. 3  MRM Chromatograms of Dinophysistoxin 1 (DTX1)**

In addition, the calibration curves of OA and DTX1 are shown in Fig. 4. In both cases, the coefficient of determination R² was greater than 0.9999, indicating excellent linearity. Comparable linearity was also obtained for the other four substances.

Thus, instrumental analysis of shellfish by LC/MS/MS offers high sensitivity and accuracy, making it a highly effective analytical method. For this reason it is attracting attention as an alternative to the traditional MBA method.
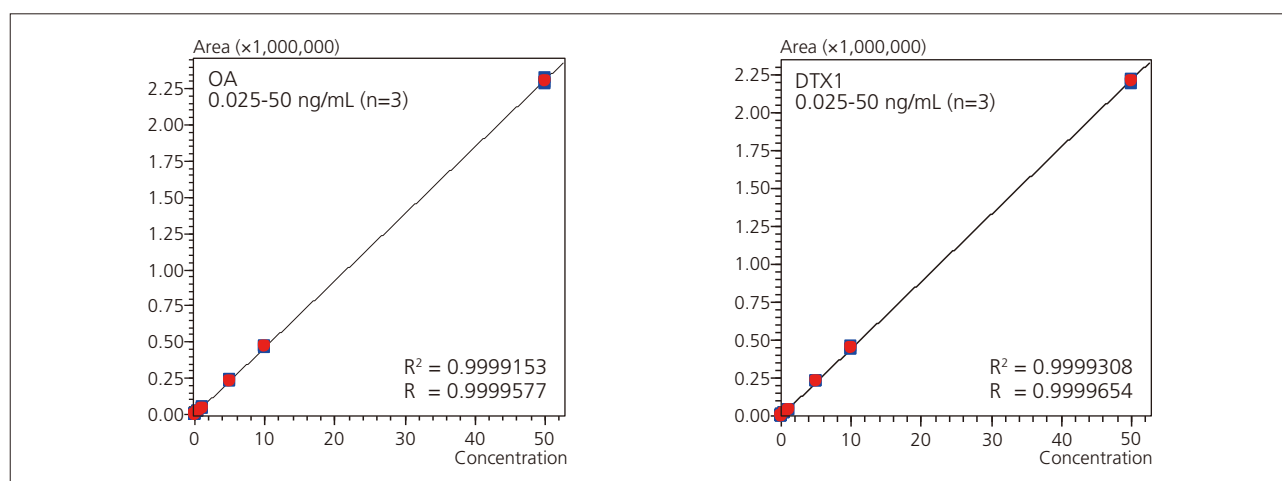


**Fig. 4  Calibration Curves of OA and DTX1**

**Table 2  Analytical Conditions**

| | |
|---|---|
| Column | : InertSustain C8 (50 mm L. × 2.1 mm I.D., 3 μm) |
| Mobile Phases | : A 2 mmol/L Ammonium Formate – Water (pH adjusted to 8.5 with ammonia water) |
| | : B Methanol |
| Time Program | : 20 %B (0 min) – 100 %B (10 min) – 20 %B (10.01 min) – STOP (15 min) |
| Flowrate | : 0.2 mL/min |
| Column Temperature | : 40 ˚C |
| Injection Volume | : 10 μL |
| Probe Voltage | : +4.0 kV/-3.0 kV (ESI-positive / negative mode) |
| DL Temperature | : 200 ˚C |
| Block Heater Temperature | : 400 ˚C |
| Interface Temperature | : 350 ˚C |
| Nebulizing Gas Flow | : 3 L/min |
| Drying Gas Flow | : 10 L/min |
| Heating Gas Flow | : 10 L/min |
| MRM Transition | : (+) PTX6 906.50 > 835.40, PTX1 892.60 > 821.40, PTX2 876.50 > 805.40 |
| | : (-) OA 803.50 > 255.20, YTX1 1141.50 > 1061.30, DTX1 817.50 > 255.20 |

The diarrhetic shellfish toxin standards were provided courtesy of Dr. Toshiyuki Suzuki of the Japanese National Research Institute of Fisheries Science.

Reference 1: July, 2014, Food Safety Commission of Japan "Natural Poison Evaluation Report – Okadaic Acid Group Among Bivalves"
http://www.fsc.go.jp/fsciis/evaluationDocument/list?itemCategory=009

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# Application News

# No. AD-0153

*Halal Authentication Analysis / LCMS-8060*

## Highly-Sensitive Detection of Multiple Porcine-Specific Peptides in Processed Foods by LC/MS/MS Method

**Udi Jumhawan, Jie Xing & Zhaoqi Zhan**
*Application Development & Support Centre, Shimadzu (Asia Pacific) Pte Ltd, Singapore*

## ❑ Introduction

In recent years, unauthorized blending of undeclared materials into food products has been a major concern among consumers. Such action violates customer rights based upon economical and safety values and it is also a critical problem for communities with ethical and religious beliefs [1,2]. Both accidental and intentional adulteration of pork meat into food products are significant issue affecting Muslims and Jews as they have dietary restrictions on foods containing pork and its-by-product, such as gelatin. Particularly for Muslims, those restrictions are part of Islamic law concerning Halal (permissible) and Haram (non-permissible) foods. With an estimated 1.6 billion Muslims worldwide, development of sensitive method for detection of porcine materials in food products is indispensable. Various approaches and targets have been utilized to trace porcine materials in processed foods including pork DNA by qPCR [3] and PCR-MCE [4]. However, DNA is prone to thermal degradation thus its viability remains dubious after food processing (cooking). In recent years, porcine-specific peptide markers were discovered and utilized for detection of pork in food [5-8]. In this Application News, a highly sensitive LC/MS/MS method is described for ensuring halal food integrity by targeting more heat-stable porcine-specific peptide markers in processed foods.

## ❑ Experimental

**Preparation of meat and processed food samples**

The experimental procedure is modified based upon previous reports [5-8] and consists of four steps as follows: protein extraction, trypsin digestion, SPE clean-up and analysis of porcine-specific peptide markers on LC/MS/MS. The workflow of protein extraction, reduction, alkylation and digestion is displayed in **Figure 1**. Raw meats of pork, chicken and beef were used in method development to confirm the porcine-specific peptides and obtain optimized MRMs and retentions. The targeted samples are processed foods. Nine processed food products

were obtained from local supermarket. It is to note that the supplementary addition of 20 µL dithiothreitol is to consume the unreacted iodoacetamide. The obtained tryptic digestion solution is cleaned up using SPE (solid phase extraction) approach prior to LC/MS/MS analysis. The details of the procedure are shown in **Figure 2**.



**Figure 2.** Workflow of SPE clean up procedure of trypsin digested proteins extracted from processed food or raw meat

**LC/MS/MS analytical conditions**

A high sensitive triple quadrupole system, LCMS-8060, coupled with a Nexera X2 UHPLC system was utilized to establish detection and semi-quantitation of porcine-specific peptides in processed food. A reversed phase Phenomenex column which has been described suitable for peptide mapping was used. The details of the UHPLC and MS/MS parameters are summarized in **Table 1**.



**Figure 1.** Workflow of protein extraction ①, reduction, alkylation, and digestion ②

**Table 1**. Analytical conditions for detection and semi quantitation of porcine-specific peptides on LCMS-8060

| Column | Aeris Peptide 1.7 µm XB-C18 100Å (150 mm x 2.1 mm I.D.) |
|---|---|
| Mobile Phase | A: Water with 0.1% formic acid<br>B: Acetonitrile with 0.1% formic acid |
| Elution Program | Gradient elution, 3%B (0min) ➔ 45%B (12min)➔70%B (12.01 −14min) ➔ 3%B (14.50 − 18min) |
| Flow Rate | 0.3 mL/min |
| Oven Temp. | 50 ºC |
| Injection | 5 µL |

| Interface | Heated ESI |
|---|---|
| MS Mode | MRM, Positive mode |
| Block Temp. | 400 ºC |
| DL Temp. | 250 ºC |
| Interface Temp. | 300 ºC |
| Nebulizing gas | $N_2$, 3 L/min |
| Drying gas | $N_2$, 10 L/min |
| Heating Gas | Zero air, 10 L/min |

## ❑ Results and discussion

### MRM-based method for detection of porcine-specific peptides

A number of porcine-specific peptides has been discovered by several researchers in past years [5-8]. The peptide markers for pork as well as other meat species were adopted in establishment of MRM based method on LC/MS/MS to achieve high sensitivity for inspection of blending pork or concerned meat species in Halal food. To enhance the detection reliability, this study incorporates all the known peptide markers reported previously [5-8] into a single LC/MS/MS method. A total of seven peptide markers was targeted and utilized.

Due to the unavailability of authentic peptide standards, development of MRM method was conducted according to the strategy shown in **Figure 3**. The targeted peptide sequences in FASTA format was retrieved from UniProt protein database and then submitted into Skyline software to obtain predicted MRM transitions and collision energies (CE). This simplified and speed it up greatly method development on LC/MS/MS. The predicted MRM parameters were imported into the LabSolutions, where MRM optimization were performed using the auto MRM optimization program. **Table 2** and **Figure 4** show the seven porcine-specific peptides and MRM method established on LCMS-8060. The peptide markers were found with good sensitivity using 5µL injection volume compared to that of higher volumes applied in previous reports (40-50 µL) [5-6].
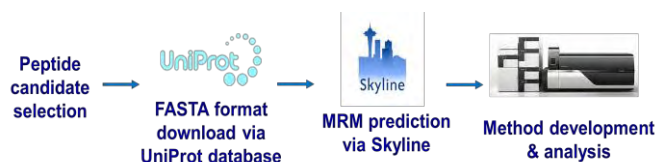


**Figure 3.** Schematic procedure of MRM method development of peptides from digested proteins with assistance of Skyline program.

**Table 2**. Porcine-specific peptides and MRM based method on LCMS-8060

| Protein | UniProt accession No. | Peptide marker (short sequence) | Precursor ion & charges | Number of MRM | RT (min) |
|---|---|---|---|---|---|
| Troponin T | Q75NG7 | YDII | 453.8++ | 5 | 6.52 |
| Myosin-1 & Myosin-4 | Q9TV61/62 | SALA | 376.1+++ | 6 | 3.25 |
| Myosin-4 | Q9TV62 | TLAF | 534.3++ | 6 | 8.14 |
| L-lactate dehydrogenase A chain | P00339 | LVVI | 450.3++ | 4 | 5.44 |
| Serum albumin | P08835 | EVTE | 412.2++ | 4 | 3.99 |
| | | FVIE | 388.8++ | 5 | 6.68 |
| | | TVLG | 647.9++ | 3 | 8.01 |
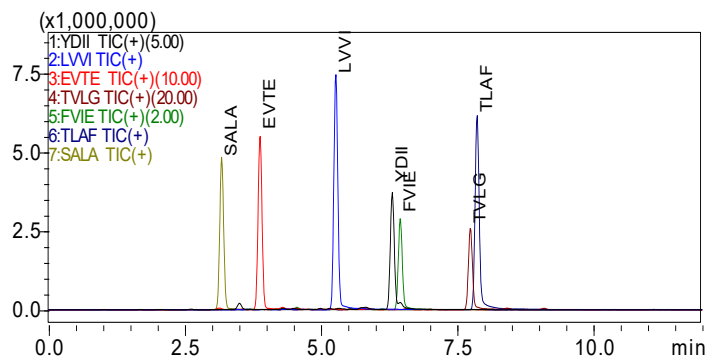


**Figure 4.** Total MRM chromatograms of seven porcine-specific marker peptides detected in trypsin-digested extract of raw pork meat.

### Specificity of porcine-specific peptide markers

The specificity of the seven porcine-specific peptides were verified with beef and chicken meats following the exact same sample preparation and analysis conditions. The criteria included at least three MRM transitions, their ratios and RT. All the seven peptide markers were not detected in beef sample. However, as shown in **Figure 5**, one peptide peak in chicken meat was overlapped with porcine peptide which identified as LVVITAGAR. This particular peptide from L-lactate dehydrogenase protein in pork has identical amino acid composition to that of the same peptide in chicken (LVIVTAGAR) as shown with the same ion mass (*m/z*) [7]. However, based on Skyline MRM prediction, the difference in amino acid sequence among these peptides resulted in one unique product ion: 588.35 for pork and 574.35 for chicken.
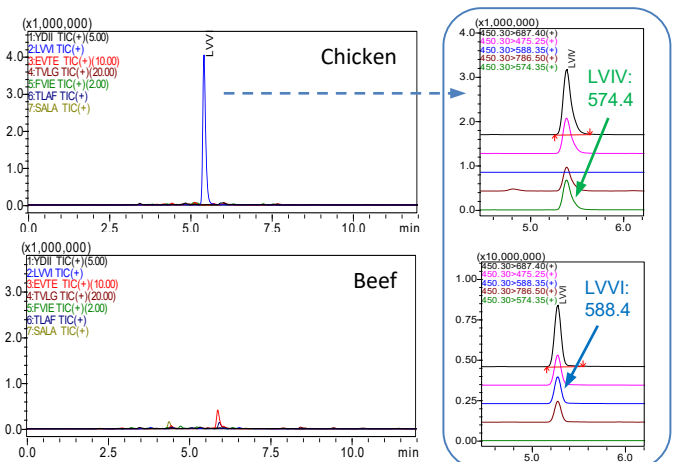


**Figure 5.** Specificity of porcine peptide markers in chicken and pork (Left). The sequence of LVVI marker peptide (LVIVTAGAR) in chicken is actually different from pork (LVVITAGAR), which could be differentiated from MRM (right)

The prediction was confirmed (**Figure 5 right**) and thereby verified method selectivity to pinpoint species-specific peptide marker.

## Thermolability test of porcine-specific peptide markers

To investigate viability of the seven peptide markers after food processing (cooking), raw pork meat was subjected to heat treatment at 200°C for 30 minutes before sample pre-treatment. The results indicated that these porcine-specific peptide markers exhibited certain depleted levels upon heat treatment but all of the peptide markers could be detected firmly. Furthermore, processed food products were also treated at 200°C for 30 minutes and the results showed that the levels of the peptide markers remained in the samples were feasible for detection of pork by the established LC/MS/MS method (**Table 3**).

## Screening analysis of porcine-specific peptides in processed foods

**Table 3** shows the results of screening analysis of five non-halal and four halal-certified processed food products using the established method aiming for halal testing application. All the samples were pre-treated at 200°C for 30 minutes before extraction, digestion and purification. This is to verify the feasibility of the method for cooked and processed food.

**Table 3**. Results of screening analysis of porcine-specific peptides in processed food products (Note: the peptide markers are indicated with first four letters of amino acid composition)

| Halal label | | Processed food | Porcine-specific peptide markers (*n*=2) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | YDII | LVVI | EVTE | TVLG | FVIE | TLAF | SALA |
| H A L A L | 1 | Chicken-Beef sausage | ND | ND | ND | ND | ND | ND | ND |
| | 2 | Lamb-Chicken sausage | ND | ND | ND | ND | ND | ND | ND |
| | 3 | Canned corned beef | ND | ND | ND | ND | ND | ND | ND |
| | 4 | Canned mutton curry | ND | ND | ND | ND | ND | ND | ND |
| N O N H A L A L | 5 | Chicken sausage | + | + | + | ND | + | ND | + |
| | 6 | Pork sausage | + | + | + | + | + | + | + |
| | 7 | Canned corned pork | + | + | + | + | + | + | + |
| | 8 | Pork meatball | + | + | + | + | + | + | + |
| | 9 | Noodle seasoning | + | + | + | + | + | + | + |

*(+) detected based on 3 MRM transitions RT matching; ND, not detected.*

All the seven porcine-specific peptide markers were detected in all the processed samples with pork material (samples No. 5-9). None of the seven porcine-specific peptide markers was detected in the Halal certified samples (sample No. 1-4). However, five peptide markers were detected in chicken sausage (sample No. 5), which was not a Halal certified product. Following the confirmation criteria (RT matching with positive control, signal-to-ratio (S/N)>3, and at least 3xMRM transitions) the detection of the

five porcine-specific peptides were confirmed. This may be resulted from cross-contamination due to non-halal sample handling during product manufacturing.

## Detection limit of pork in processed foods

To evaluate the sensitivity of the method for screening analysis, it is important to know the detection limit in terms of content level of pork materials in processed food. To estimate the detection limit and confirmation reliability of the method, cooked pork meat (200°C, 30 minutes) was pre-spiked into halal-certified mutton curry (sample No. 4) in four spiking percentages from 0.1% to 5.0% (wt) before sample preparation. The results of the spiked samples are summarized in **Table 4**.

Three porcine-specific peptide markers YDII, LVVI and SALA were detected for all spiking levels including the 0.1% (wt) with S/N >/= 3. The results were reproducible among four separate sample preparations and analyses in different days (inter-day). Peptide FVIE and EVTE had detection limits of about 0.5% and 1%, respectively. Peptide EVTE could be detected at only 5% spiking level whereas peptide TVLG could not be detected. This suggests that the detection sensitivity of the seven porcine-specific peptide markers are very different in processed food. It was found in thermolability tests that peptide YDII is most thermostable while the other six peptides are less stable at 200°C.

**Table 4**. Detection limit of porcine-specific peptides in spiked Halal food sample

| Peptide marker | Spiking percentage of cooked pork (%, wt) (*n*=4) | | | |
|---|---|---|---|---|
| | 0.1 | 0.5 | 1.0 | 5.0 |
| YDII | + | + | + | + |
| LVVI | + | + | + | + |
| EVTE | ND | ND | ND | + |
| TVLG | ND | ND | ND | ND |
| FVIE | ND | + | + | + |
| TLAF | ND | ND | + | + |
| SALA | + | + | + | + |
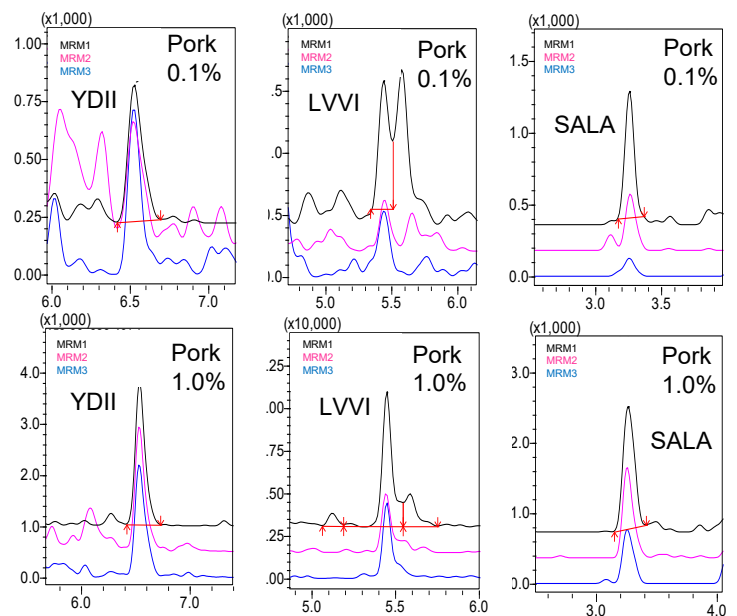


**Figure 6**. Detection of porcine-specific peptide markers YDII, LVVI and SALA in certified Halal food matrix spiked with cooked (200°C) pork at 0.1% and 1% (wt).

The detection sensitivities of the seven peptide markers are different depending on the food processing and cooking conditions. The additional heating treatment of all raw meats and processed foods at 200°C under dry air in this study is considerably a severe condition which lead to degradation of proteins. The detection results under this experiment condition indicate the method is applicable and feasible for halal testing of various processed and cooked foods.

### Semi-quantitative screening of pork blended in processed food

The four porcine-specific peptides YDII, LVVI, SALA and FVIE, which are more sensitive for detection of pork in processed food, are further evaluated for semi-quantitative screening. As shown in **Figure 7** and **Table 5,** linear relationship exists between peak area and content level of spiked pork (cooked at 200°C) in processed canned mutton curry (Halal certified). The linearity ($R^2$) is equal to or greater than 0.995 for the four peptide markers in the testing range. The quantitative results also exhibit acceptable repeatability with RSD of both peak area and concentration (spiking percentage) within acceptable value which is under 16%.

**Table 5**. Calibration curves of four peptide markers in spiked samples

| Peptide marker | Range (%) | Linearity ($R^2$) | Repeatability at 1% ($n$=6) Area RSD (%) | Repeatability at 1% ($n$=6) Conc. RSD (%) |
|---|---|---|---|---|
| YDII | 0.1-5 | 0.997 | 11.3 | 13.6 |
| LVVI | 0.1-5 | 0.998 | 9.3 | 11.0 |
| FVIE | 0.5-5 | 0.995 | 14.4 | 15.3 |
| SALA | 0.1-5 | 0.998 | 8.3 | 10.1 |

## ❑ Conclusions

Despite its tedious sample preparation procedure, porcine-specific peptides are far more prominent targets for developing halal testing method, since peptides are more heat-stable than DNA, the common target for detection of pork in foods. With remarkable sensitivity and selectivity, this LC/MS/MS method provides a more robust approach to detect as low as 0.1% (wt) of porcine material blended in processed food.



**Figure 7.** Linear relationship between peak area of four porcine-specific peptides and percentage (%) of cooked pork spiked in canned mutton curry. (A) YDII, (B) LVVI, (C) FVIE, (D) SALA. The blue and red dots represent repeated and average values, respectively.

In summary, the established MRM-based method on a LC/MS/MS platform offers a highly sensitive and selective approach to detect and monitor the presence of porcine-specific markers for semi-quantitation of the amount of pork in processed foods. The established method from sample preparation to LC/MS/MS analysis exhibits excellent reliable sensitivity, which is potentially able to support halal testing in processed food products. Analysis of various food matrices will be performed in the future.

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# Development of Sensitive and Selective Methods for Identification of Marine Toxins by Liquid Chromatography Tandem Mass Spectrometry

## ASMS 2017 TP-211

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Development of Sensitive and Selective Methods for
Identification of Marine Toxins by Liquid Chromatography
Tandem Mass Spectrometry

# Introduction

Over many decades, marine toxins have been monitored by the mouse bioassay (MBA) for the food safety purpose in many countries. In place of MBA, liquid chromatography (LC) with mass spectrometry (MS) is expected to use for the analysis of the marine toxins, deemed superior to the MBA in the point of sensitivity and accuracy.
 Our purpose is establishment of the analytical conditions using Liquid Chromatography Tandem Mass Spectrometry for three groups of marine toxins of which each structure and property is different.

 The target three groups of marine toxins are diarrhetic shellfish poisoning (DSP) toxins as okadaic acid (OA) and dinophysistoxins (DTX1 and DTX2) , ciguatera fish poisoning (CFP) as ciguatoxin 3C (CTX3C) , and globefish poisoning as tetrodotoxin (TTX).
 Since globefish poisoning, tetrodotoxin (TTX) have been reported to be detected from the bivalves in a certain sea area near New Zealand and European coast, it's argued internationally to add TTX to the shellfish poisoning toxin.

| Paralytic Shellfish Poisoning (PSP) | Diarrheic Shellfish Poisoning (DSP) | Ciguatera Fish Poisoning (CFP) |
|---|---|---|
| Serious effects. Fatal toxic symptoms. | Diarrhea and/or vomiting. Not so serious conditions. | Fatal toxic symptoms (in the limited area) |
| LC-MS/MS in Japan &EU | MBA in Japan Fluorescence HPLC method in addition to MBA in EU and the USA (AOAC 2005.06 & 2011.02) | Review of regulatory frameworks |
| OA:0.16 mg OA eq/ kg *[1]. | 4 MU/g as MBA STX 0.8 mg STX eq /kg *[1] (as 2 HCl) | |

*1 CODEX STAN 292-2008.
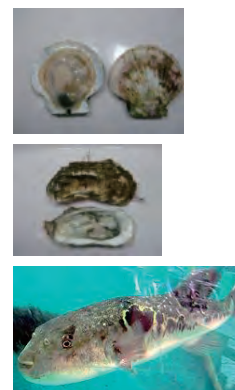
# Experimental

## Standard Solutions

Standard solutions of OA, DTX1 and DTX2 were purchased from NRC (Canada).
      CRM-OA-c (Lot #20070328), CRM-DTX1 (Lot #20071024), CRM-DTX2 (Lot #20150819)
Standard of CTX3C and TTX were purchased from Wako Chemical Industry (JAPAN).
      Ciguatoxin CTX-3C 100 ng, Wako Chemical # 030-21581
      Tetrodotoxin TTX 1 mg, Wako Chemical # 206-11071
Methanol including 0.1% formic acid was used for dilution of standard mixture from above.
Each structure of marine toxins compound is shown in Figure 1 as below.

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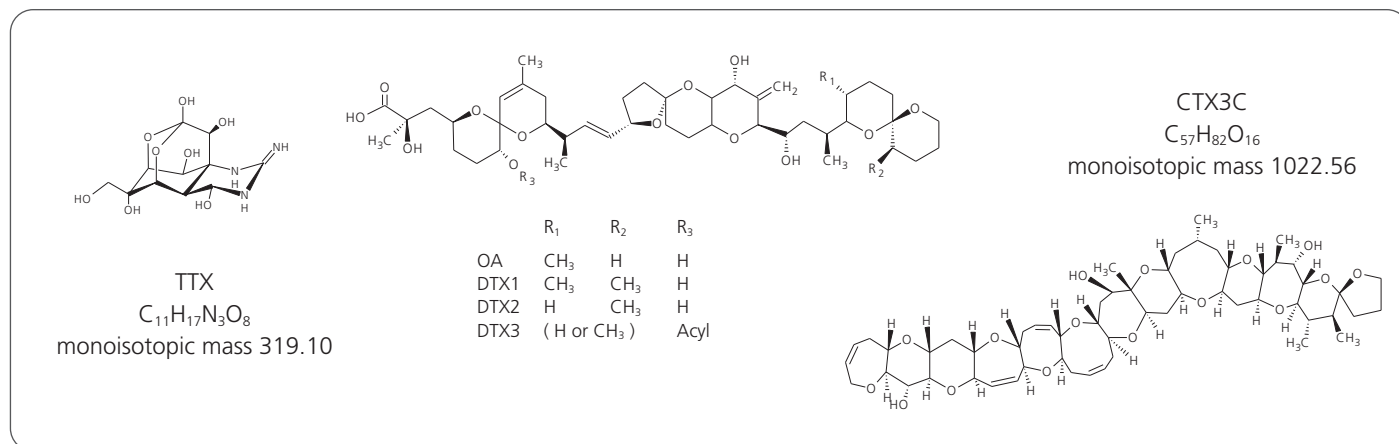


Figure 1. Structure of marine toxins



LCMS-8050 Triple quadrupole mass spectrometer

## LC/MS/MS analysis

With the shift from the MBA toward to the instrumental method, the simultaneous analytical method of DSP and PSP has been eager to be utilized; however DSP is generally hydrophobic, while PSP mostly hydrophilic. It is relatively hard to analyze simultaneously both DSP and PSP with reversed phase mode.

Our purpose in this study is evaluation of potential analytical condition such as,

1) The simultaneous analytical method for DSP and PSP with a multi mode ODS column.
2) Reversed phase condition for DSP (acidic and neutral conditions)
3) Specified method for PSP, especially TTX with a HILIC mode column.

# Development of Sensitive and Selective Methods for Identification of Marine Toxins by Liquid Chromatography Tandem Mass Spectrometry

Table 1  Analytical Conditions

| | Condition | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| **HPLC** | Instrument | UHPLC Nexera X2 (Shimadzu) | | | |
| | Target compounds | TTX<br>OA, DTX1, DTX2, CTX3C | OA, DTX1, DTX2, CTX3C | OA, DTX1, DTX2, CTX3C | TTX |
| | Total run time (min) | 40 | 17.5 | 17.5 | 15 |
| | Column | Scherzo SM-C18<br>(150×2 mm, 3 µm)<br>Imtakt | L-column2 ODS<br>(75×2.1 mm, 2 µm)<br>CERI | L-column2 ODS<br>(75×2.1 mm, 2 µm)<br>CERI | InertSutain Amide PEEK<br>(150×2.1 mm, 3 µm)<br>GL Sciences |
| | Mobile phase A | 0.05% formic acid water | 2 mM ammonium formate with 50 mM formic acid | 2 mM ammonium formate | 0.1% formic acid water |
| | Mobile phase B | Acetonitrile<br>with 0.05% formic acid | Acetonitrile / Water :<br>95 / 5 (v/v)<br>including 2 mM ammonium formate with 50 mM formic acid | Acetonitrile / Water :<br>95 / 5 (v/v)<br>including 2 mM ammonium formate | Acetonitrile<br>with 0.1% formic acid |
| | Time program | B conc. 0% (0-2 min) →<br>100% (30-35min) →<br>0% (35.01 – 40 min) | B conc. 40% (0-2.5 min) →<br>100% (7.5-12.5 min) →<br>40% (12.51 – 17.5 min) | B conc. 40% (0-2.5 min) →<br>100% (7.5-12.5 min) →<br>40% (12.51 – 17.5 min) | B conc. 100% (0-3 min) →<br>5% (10 min) →<br>100% (10.01 – 15 min) |
| | Flow rate (mL/min) | 0.2 | 0.2 | 0.2 | 0.4 |
| | Column Temp. (°C) | 25 | 30 | 30 | 30 |
| | Injection Volume | 5 µL | | | |
| **MS** | Instrument | LCMS-8050 (Shimadzu) | | | |
| | Ionization | Heated ESI (+/-) | | | Heated ESI (+) |
| | Mode | MRM | | | |
| | CID gas pressure | 330 kPa | | | |
| | Temperatures | HESI:350°C / Desolvation line:200°C / Heat block:400°C | | | |
| | Gas flow | Nebulizing gas (N$_2$) : 2.5 L/min<br>Heating gas (Air) : 15 L/min<br>Drying gas (N$_2$) : 5 L/min | | | |

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# Result and discussion

## Mass Spectra of CTX3C

With electro spray ionization (ESI) on LC-MS/MS, since DSP and CFP are lipophilic toxins, various ions represented by sodium adduct and dehydrated ion are observed at positive mode (as $[M+Na]^+$ , $[M+K]^+$ , $[M+H-H_2O]^+$). While, OA and DTX1, DTX2 are monitored as simple mass peak at negative mode. Under this observation, precursor ion of OA and DTX1, DTX2 were selected as deprotonated molecule at negative mode.
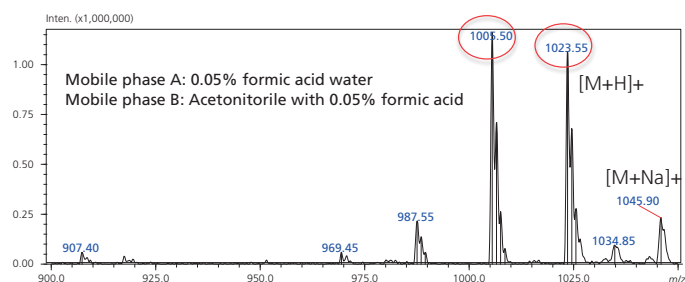


Since deprotonated molecule of CTX are weak signal at negative mode, its precursor ion were evaluated as protonated molecule or sodium adducted ion depending on the mobile phase constitutions at positive mode. It was observed that sodium adducted ion decreased with acetonitrile rather than methanol as mobile phase solvent.
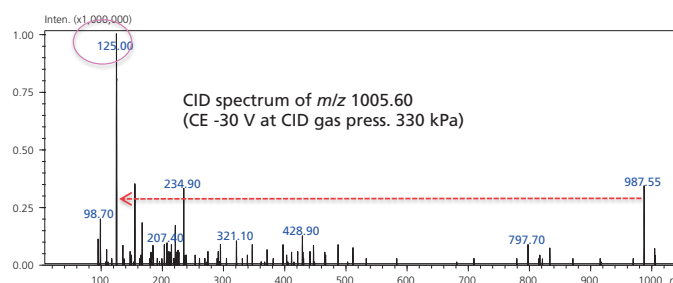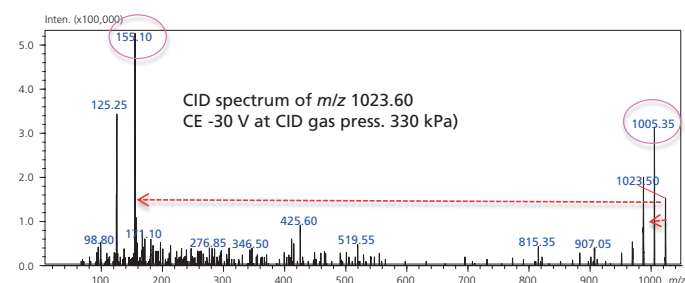
Figure 2. Mass spectra of CTX3C

## MRM chromatograms of standard solution

With multi mode ODS column, both of the separation and sensitivity of 5 compounds (TTX, OA, DTX1, DTX2 and CTX) was successfully optimized.
TTX is hard to retain in reversed phase mode due to its hydrophilicity. Thus, hydrophilic interaction (HILIC) mode is alternative choice in comparison with another condition in the point of separation and sensitivity. As a result of evaluation using several types of columns, we found the InertSustain Amide column gave the better result of TTX analysis than other columns.

# Development of Sensitive and Selective Methods for Identification of Marine Toxins by Liquid Chromatography Tandem Mass Spectrometry
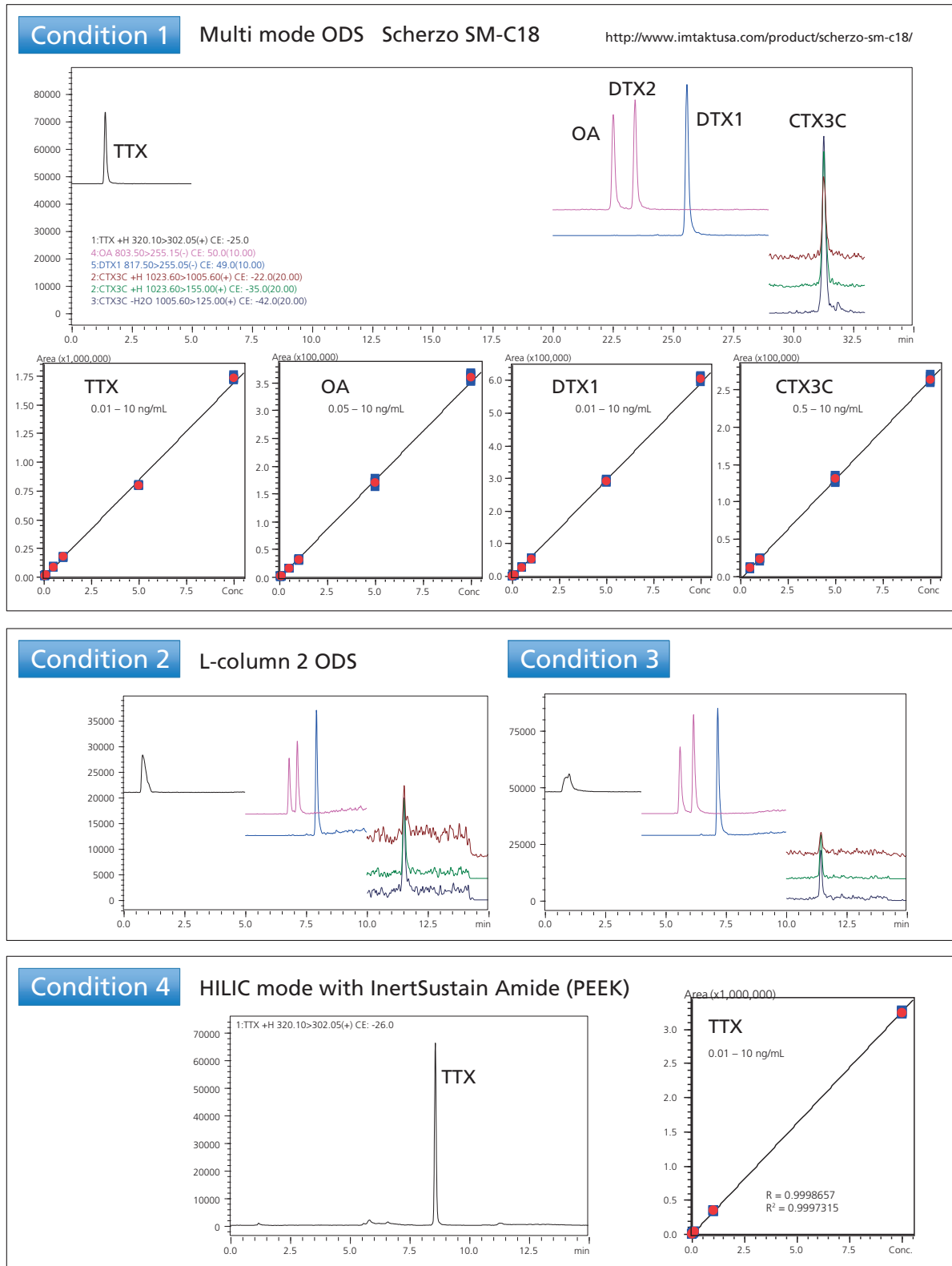


Figure 3.  MRM Chromatograms (each.1 ng/mL) and Calibration curves

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# Evaluation of SPE pretreatment (Collaborated with Biotage®)

Preliminary evaluation of recovery with two different type of SPE cartridges were performed for sample preparation using two major toxic compounds (OA,DTX1).
Schematic pretreatment protocol for each SPE is llustrated in figure 4.

The recovery of representative two compound above is shown in Table 2.
The 66 ~ 83% of recovery was achieved with OA and DTX1 in the fraction of Elution 1 to 2, respectively using ISOLUTE® C18(EC) and EVOLUTE® EXPRESS ABN SPE.

**STEP 1**      **STEP 2**

10 ppb Mixture 200 µL
(OA, DTX1)

25 µL
10% NaOH

50 µL
10% HCl

325 µL
Water

Solution 600 µL

C18(EC)          EXPRESS ABN
200 µL              200 µL

### ISOLUTE® C18 (EC)

Mixture 200 µL

25 mg SPE

40% MeOH          Sample 1
(400 µL x 2)
                        Sample 2,3

1 mL Water
                        Wash 1

1 mL
40% MeOH
                        Wash 2

1 mL
90% MeOH

**Elution 1 - 5 (200 µL x 5)**
OA, DTX1

### EVOLUTE® EXPRESS ABN

Mixture 200 µL

10 mg SPE

40% MeOH          Sample 1
(400 µL x 2)
                        Sample 2,3

500 µL Water
                        Wash 1

500 µL
40% MeOH
                        Wash 2

1 mL
90% MeOH

**Elution 1 - 5 (200 µL x 5)**
OA, DTX1

Biotage®

Figure 4.  Protocol of SPE

Table 2  Recovery (%) with each SPE cartridge

|        | C18 (EC) | EXPRESS ABN |
|--------|----------|-------------|
| OA     | 66       | 74          |
| DTX1   | 72       | 83          |

# Summary and Conclusion

- Survey of analytical conditions indicates that the Multi mode ODS column gives better result of separation for 5 representative toxins (TTX, OA, DTX1, DTX2 and CTX3C) than other mode. The Summary is shown in Table 3.
- HILIC mode is alternative selection for TTX due to its good peak shape and retention.
- These results suggest Multi mode or HILIC are ways to achieve general condition including additional toxin, especially PSP.
- Pre-treatment with Biotage® SPEs were evaluated and good recovery was obtained.
- Development of sample clean up protocol as well as the evaluation of matrix effect has been continuously investigated with Biotage®.

Table  3LOD (ppb) of each toxin with four condition

|  | +/- | Transition (m/z) | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|---|
| TTX | + | 320.10>302.05 | 0.01 | - | - | 0.01 |
| OA | - | 803.50>255.15 | 0.05 | 0.05 | 0.05 | - |
| DTX2 | - | 803.50>255.15 | 0.05 | 0.05 | 0.05 | - |
| DTX1 | - | 817.50>255.05 | 0.01 | 0.05 | 0.01 | - |
| CTX3C | + | 1023.60>1005.60 | 0.5 | 1 | 0.5 | - |
| | + | 1023.60>155.00 | 0.5 | 0.5 | 0.5 | - |
| | + | 1005.60>125.00 | 0.5 | 0.5 | 0.5 | - |

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# Application News

### No. C161

**Liquid Chromatograph Mass Spectrometry**

## Multi-Residue Veterinary Drug Analysis of >200 Compounds using MRM Spectrum Mode by LC-MS/MS

Veterinary drugs are used for therapeutic, metaphylactic, prophylactic and growth promotion purposes. To provide an assurance that food from animals is safe with regards to residues of veterinary medicines, regulatory authorities have established Maximum Residue Limits (MRL's) for certain drugs in target tissues and animal species. Some pharmacologically active compounds identified by regulatory authorities have been prohibited and their hazardousness at all levels are being considered (EU regulation EC 37/2010; Commission Decision 2003/181/EC; 21CFR Part 556 Tolerances for Residues of New Animal Drugs in Food). In this article, we describe how a triple quadrupole mass spectrometer, which is both highly sensitive and selective, contributes to reducing false positive and false negative reporting when using a measurement mode called MRM Spectrum mode. MRM Spectrum mode acquires a high number of fragment ion transitions for each target compound and generates fragmentation spectra that can be used in routine library searching and compound verification using reference library match scores.

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[*2] : Phytocontrol, Nimes, France

## ■ Samples and Analysis Conditions

Samples of beef, egg, honey, milk and salmon were extracted and spiked with veterinary drugs in the calibration range of 0.001 to 0.1 mg/kg. Repeatability was assessed at low and high concentrations. Samples were measured using Shimadzu's Nexera X2 UHPLC and LCMS-8060 triple quadrupole mass spectrometer (Table 1 and 2). Over 200 veterinary drugs were targeted and over 2,000 MRM transitions in both ESI +/- were monitored during a gradient elution time of 12 minutes.

### Table 1  UHPLC Conditions

| Liquid chromatography | | | | |
|---|---|---|---|---|
| UHPLC | Nexera LC system | | | |
| Analytical column | Restek Biphenyl (100 × 2.1, 2.7 µm) | | | |
| Column temperature | 40 °C | | | |
| Flow rate | 0.4 mL/minute | | | |
| Solvent A | 0.1 % formic acid 0.5 mM ammonium formate solution | | | |
| Solvent B | 0.1 % formic acid in methanol | | | |
| Binary Gradient | Time (mins) | %B | Time (mins) | %B |
| | 0.00 | 2 | 14.60 | 2 |
| | 12.50 | 100 | 17.50 | Stop |
| | 14.50 | 100 | | |

### Table 2  MS/MS Acquisition Parameters

| Mass spectrometry | |
|---|---|
| Mass spectrometer | Shimadzu LCMS-8060 |
| Pause time/dwell time | 1 msec/3 msec |
| Polarity switching time | Pos/neg switching time set to 5 msec |
| Scope | 218 drugs in positive ion mode (including internal standards) |
| | 11 drugs in negative ion mode |
| | Structure Analytics (in house development tool) |
| Source temperatures (interface; heat block; DL) | 350 °C; 300 °C; 150 °C |
| Gas flows (nebulising; heating; drying) | 3 L/min; 10 L/min; 10 L/min |

## ■ Advantages of MRM Spectrum Mode

The measurement method can be easily set using the MRM optimization tool and measurement window (MRM Synchronization) settings of LabSolutions LCMS. The method achieves high data densities and a high data sampling rate across each elution peak. This approach generates a consistent loop time and sampling rate producing reliable quantitation and peak integration. It also provides great operator-friendliness in routine simultaneous analysis of veterinary drugs by enhancing flexibility in qualifier and quantifier ion selection. The number of fragment ion transitions generated from a single precursor ion is limited only by the chemical structure of the veterinary drug.

## ■ Results

MRM Spectrum mode was used to acquire a high number of fragment ion transitions for each veterinary drug target. For chlortetracycline, 11 precursor-fragment ion transitions were acquired using optimized collision energies (Fig. 1). Acquiring a high number of fragment ion transitions enables generation of fragmentation spectra which can be used in library searching and compound verification for each veterinary drug. (Chlortetratcycline is a tetracycline class of antimicrobials. According to the Sixth ESVAC report published in 2016, of the overall sales of antimicrobials in the 29 EU countries in 2014, the largest amount, expressed as a proportion of mg/PCU, was accounted for by tetracyclines (33.4 %). This is followed by penicillins (25.5 %) and sulfonamides (11.0 %). Chlortetracycline was selected as a representative target).
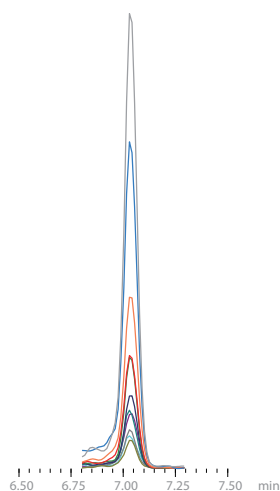
Compound name Chlortetracycline
Accurate mass 479.1216 [M+H]+
Formula C22H23ClN2O8
CAS 57-62-5
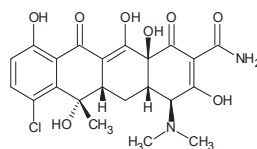
**MRM Spectrum Mode**
11 MRM's acquired for
chlortetracycline at 10pg/uL in egg.

| | | | |
|---|---|---|---|
| 1:479.10>444.00 (+) | CE: -23V | 7:479.10>300.80(+) | CE: -45V |
| 2:479.10>461.95 (+) | CE: -35V | 8:479.10>287.90(+) | CE: -53V |
| 3:479.10>154.00 (+) | CE: -34V | 9:479.10>274.95(+) | CE: -44V |
| 4:479.10>98.05(+) | CE: -45V | 10:479.10>370.95(+) | CE: -31V |
| 5:479.10>260.05(+) | CE: -60V | 11:479.10>285.85(+) | CE: -56V |
| 6:479.10>303.05(+) | CE: -37V | | |

**MRM Spectrum mode**
**Higher specificity**
**Higher reporting confidence**
**Library searchable fragment data.**
The number of precursor-fragment ion transitions monitored is limited only by the structural chemistry of the molecule.
Typically more than 10 precursor-fragment ion transitions were monitored for each veterinary drug.

**Fig. 1 Utilization of MRM Spectrum Mode (Chlortetracycline)**

Fig. 2 shows the MRM reference spectrum for chlortetracycline with assigned fragment structures. The MRM Spectrum mode is a measurement mode which combines MRM with the generation of a product ion spectrum. The product ion spectrum can be used for compound identification by searching a library.

As the collision energy was optimized for each fragment ion to generate a product ion spectrum, the library spectrum is highly specific and selective.

m/z 154.00
[Accurate mass 154.05042]
Formula C7H8NO3 (+4H)

m/z 274.95
[Accurate mass 275.04749]
Formula C15H12O3Cl (+4H)

m/z 444.00
[Accurate mass 444.08501]
Formula C22H19NO7Cl (+2H)

m/z 461.95
[Accurate mass 462.09557]
Formula C22H21NO8Cl

m/z 98.05
[Accurate mass 98.06059]
Formula C5H8NO (+4H)

m/z 303.05
[Accurate mass 303.04241]
Formula C16H12O4Cl +(4H)

m/z 260.05
[Accurate mass 260.02402]
Formula C14H9O3Cl• (+4H)

**Fig. 2 MRM Reference Spectrum with Assigned Fragment Structures (Chlortetracycline)**

## ■ Library Identification using MRM Spectrum Mode



Chlortetracycline MRM Spectrum
Similarity Score 98
Matrix **Salmon**

Chlortetracycline Library MRM Spectrum

Compound name Chlortetracycline
Precursor 479.10 [M+H]+
Formula C22H23ClN2O8
CAS 57-62-5

Chlortetracycline
Similarity Score 99
Matrix **Beef**

Chlortetracycline
Similarity Score 98
Matrix **Milk**

Chlortetracycline
Similarity Score 99
Matrix **Egg**
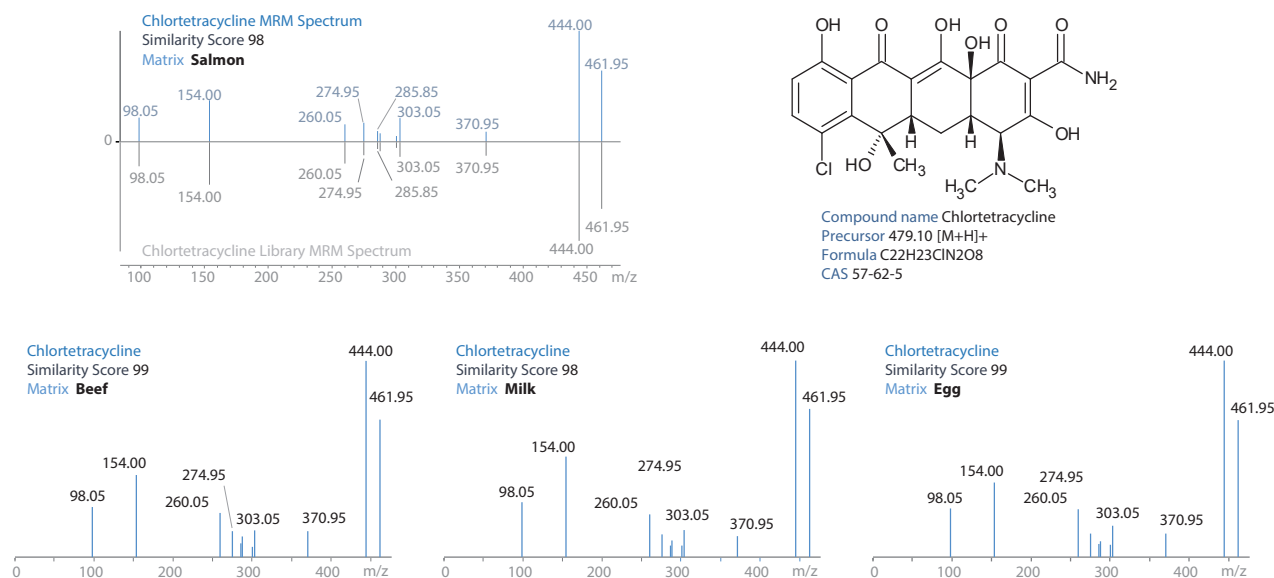
**Fig. 3 Library Searchable MRM Spectra in Different Matrices Spiked at 10 pg/μL (Chlortetracycline)**

Fig. 4 shows the MRM spectra and the n=10 measurement results of four compounds for salmon extract spiked with virginiamycin S1 at a concentration of 10 pg/μL. The library match score was above 99 in all injections (MRM spectra of injections 1, 5 and 10 are indicated). Also, the %RSD for oxytetracycline, sulfadimethoxine, ormetoprim, and virginiamycin spiked into salmon extract (n=10; 10 pg/uL) acquired using a conventional 2-MRM method was compared with that of the MRM spectrum method.



Virginiamycin S1 MRM Spectrum

m/z 205.05
[Accurate mass 205.06077]
Formula C10H9N2O3 (+4H)

m/z 290.05
[Accurate mass 290.11353]
Formula C14H16N3O4 (+4H)

Virginiamycin S1 Library MRM Spectrum

Compound name Virginiamycin S1
Precursor 824.35 [M+H]+
Formula C43H49N7O10
CAS 23152-29-6

Virginiamycin S1
Similarity Score 99
Matrix **Salmon**
**Injection1**

Virginiamycin S1
Similarity Score 99
Matrix **Salmon**
**Injection 5**

Virginiamycin S1
Similarity Score 100
Matrix **Salmon**
**Injection 10**

| Compound name | Oxytetracycline | | Sulfadimethoxine | | Ormetoprim | | Virginiamycin | |
|---|---|---|---|---|---|---|---|---|
| Number of MRM's | 2MRM's | 8MRMs | 2MRM's | 11MRMs | 2MRM's | 11MRMs | 2MRM's | 11MRMs |
| Mean peak area | | | | | | | | |
| Quantitation ion | 1890170 | 1729171 | 7809989 | 7227748 | 8291171 | 8160952 | 2232967 | 1956045 |
| **%RSD** | **3.74** | **3.04** | **1.49** | **1.46** | **1.54** | **1.18** | **0.91** | **1.65** |

**Fig. 4 MRM Spectra and n=10 Results of Salmon Extract Spiked with Virginiamycin S1 at 10 pg/μL**

## ■ Quantitation Results using MRM Spectrum Mode

To assess the robustness of the MRM Spectrum mode, the same sample was repeatedly injected. The method used complies with the identification criteria set out in the EU guidelines SANTE/11945/2015 that require the retention time and the ion ratio from at least 2 MRM ion ratios to be within acceptable tolerance limits. The absolute response and signal variability were compared to those of the MRM Spectrum mode (Fig. 4). Both methods resulted in a variance of less than 4 %RSD (n=10 for each method; 10 pg/uL spiked into salmon matrix). Fig. 5 indicates MRM spectra and the calibration curve obtained for sulfamerazine as an example of quantitation results.



Compound name Sulfamerazine
Precursor 265.10 [M+H]+
Formula C11H12N4O2S
CAS 127-79-7

**MRM Spectrum Mode**
11 MRM's acquired for sulfamerazine in beef.

| | | |
|---|---|---|
| 1: | 265.10>156.05 (+) | CE: -17V |
| 2: | 265.10>92.05 (+) | CE: -30V |
| 3: | 265.10>172.05 (+) | CE: -15V |
| 4: | 265.10>108.05 (+) | CE: -26V |
| 5: | 265.10>65.00 (+) | CE: -11V |
| 6: | 265.10>110.05 (+) | CE: -22V |
| 7: | 265.10>80.05 (+) | CE: -15V |
| 8: | 265.10>38.95 (+) | CE: -14V |
| 9: | 265.10>199.10 (+) | CE: -21V |
| 10: | 265.10>93.10 (+) | CE: -30V |
| 11: | 265.10>66.05 (+) | CE: -24V |
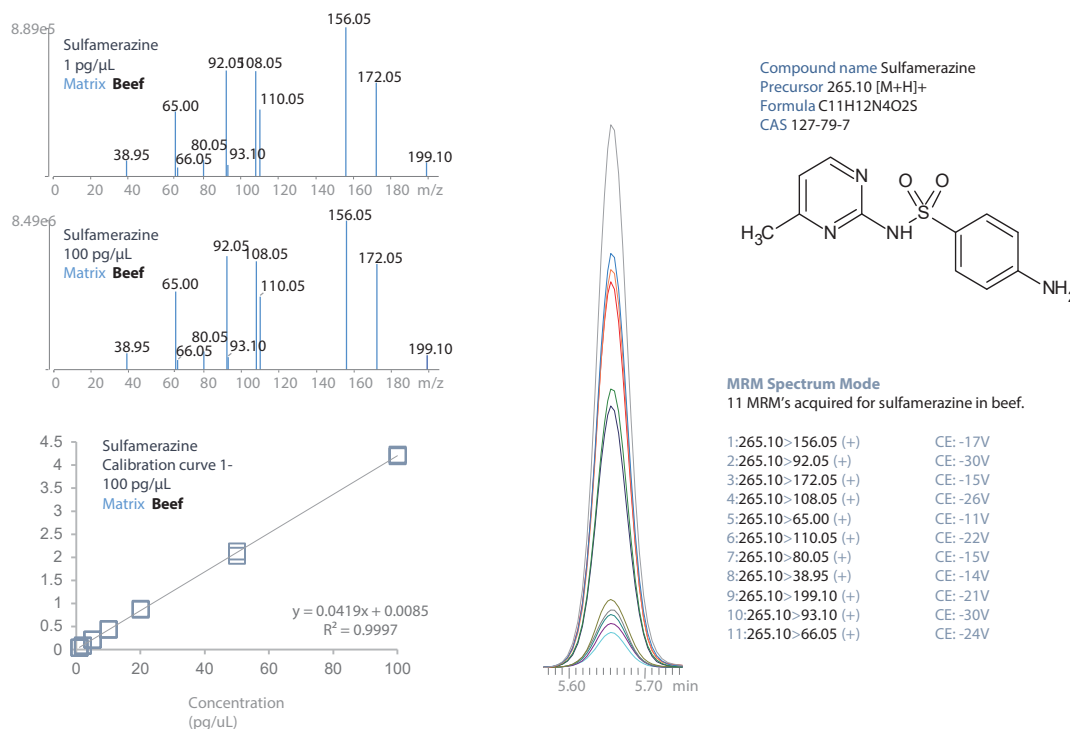
**Fig. 5 MRM Spectra and Calibration Curve of Sulfamerazine (1 pg/µL to 100 pg/µL)**

## ■ Conclusion

The level of confidence in compound identification and verification was increased by using a higher number of MRM transitions for each veterinary drug target and thereby reducing false negative and false positive reporting. Although the number of transitions for each target is dependent upon the chemical structure of the target, typically more than 10 transitions can be monitored for each compound. MRM Spectrum mode combines conventional quantitation with the generation of a high quality product ion spectrum which can be used to achieve highly reliable compound identification and verification by library searching. In this research, use of the MRM Spectrum mode was examined by quantifying and identifying 212 veterinary drugs (the method included 2,009 MRM transitions). Limits of detection, linearity or repeatability were not compromised compared to a conventional 2-MRM method.

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# Application News

# No. AD-0120

*Halal Authentication Analysis / MultiNA*

## Sensitive Detection of Pork DNA in Processed Meat products on PCR-MultiNA Platform

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## Introduction

The authentication of species content in processed meat and food products is implemented in many countries for various reasons such as economic and cultural beliefs [1, 2]. Such identification is of importance in various religious communities where consumption of a particular species of meat is prohibited. Based on Islamic Shari'ah (law), the term Halal is often used in reference to food and drinks, where they are permissible for Muslims to consume. The most common example of non-halal (Haraam) food is pork. In this Application News, a highly sensitive method is described for detection of pig DNA in processed food such as sausages. It is based on DNA extraction and species-specific PCR amplification of pork specific, followed by microchip electrophoresis detection on MultiNA [3]. This PCR-MultiNA method is highly sensitive in detecting the extracted pork DNA fragment, with housekeeping fragments as confirmation.

## Experimental

### Reagents / Kits
- DNeasy mericon™ Food Kit (50) from QIAGEN
- PCR Mastermix Pod: Pork from NeoGen Corporation
- DNA-500 Reagent Kit for MultiNA from Shimadzu
- SYBR Gold nucleic acid gel stain from *life* Technologies
- 25 base-pair ladder from Invitrogen

### Analytical Conditions for PCR Products
- Instrument       : MCE-202 MultiNA
- Analysis Mode   : DNA 500 On-Chip Mode

### Procedure for detection of Pork DNA

The process workflow is described in Figure 1. It consists of three steps: (1) extraction of DNA from sausage sample, (2) species-specific PCR amplification of the extracted DNA and (3) detection of the pork DNA fragment by microchip electrophoresis on MultiNA.

DNA extraction from sausage sample was carried out using DNeasy mericon Food Kit from QIAGEN in accordance with the protocol of the kit [4]. The extracted DNA sample was amplified via PCR using BioKits PCR Mastermix Pod, pork specific obtained from Neogen Corporation [5]. DNA amplification was carried out in a 25 µL volume reaction mixture, which contained 19.9 µL of Pork PCR mastermix, 0.1 µL of NovaTaq™ Hot Start DNA polymerase from Novagen, 4 µL of TE buffer and 1 µL of the DNA extract. The amplification was run using the following program: 94°C for 10 minutes for activation of Hot Start Taq, followed by 30 cycles at 94°C for 15 seconds, 64°C for 15 seconds, and 72°C for 15 seconds, and ended by the extension step at 72°C for 3 minutes. The PCR products were held at 4°C until subjecting to microchip electrophoresis on MultiNA. The amplified PCR products were then analysed using Shimadzu MCE-202 MultiNA.

Step (1)
DNA Extraction of sample (sausage)

Step (2)
PCR of Purified DNA

Step (3)
Electrophoresis MultiNA of PCR Product
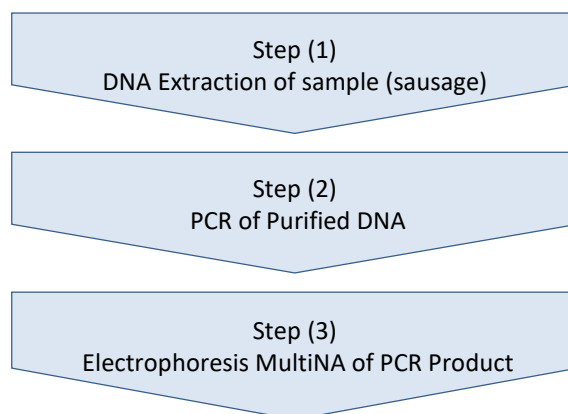
**Figure 1**: Experimental procedure for detection of Pork DNA on MultiNA

## Results and Discussion

**PCR-MultiNA procedure:** The BioKits PCR Mastermix Pod is used normally with agarose gel electrophoresis for detection of the targeted species DNA [5]. Instead of the agarose gel electrophoresis, microchip electrophoresis (MCE) was used to detect the DNA fragment on MultiNA in this work [3]. The advantages of the MCE method are high detection sensitivity, easy quantitation, fast and fully-automated operation. The PCR tubes after PCR process of food samples can be loaded onto the MultiNA directly. The subsequent analysis for detection of targeted species DNA is carried out in a fully-automated manner from adding reagents, system checking, ladder calibration to batch sample measurement on the MultiNA. The results are displayed in electropherogram (Figure 2). The precise area of the

electropherogram peak of pork DNA (314 bp) can be obtained, which is proportional to the amount of the DNA molecules in the sample.

Figure 2 shows that the PCR Mastermix, pork specific, amplified the pork DNA fragment 313 bp (target size: 314 bp) and the housekeeping fragment 395 bp (target size: 380-420 bp) in pork sausage sample selectively. In the DNA extract of chicken sausage, pork DNA fragment was not detected. The only observed fragment was the housekeeping DNA at 418 bp. A negative control of PCR mixture without any template DNA was included as blank.

The accuracy and reproducibility of the PCR-MultiNA method are summarized in Table 1. The targeted pork DNA fragment is 314 bp. The measured size is at 314-324 bp, which is within the accuracy specification of MultiNA (±5%). The reproducibility of the method is at 0.2%~1.0%.

**Table 1**: Accuracy and reproducibility of PCR-MultiNA procedure for detection of pork DNA using Neogen Biokits PCR Mastermix

| Pork Content | Repeat No. (n) | Measured Ave (bp) | Accuracy, Ave (%) | RSD (%) |
|---|---|---|---|---|
| 100% | 4 | 317.7 | 101.2 | 0.74 |
| 3% & 5% | 3 | 316.3 | 100.7 | 1.02 |
| 1% | 3 | 316.7 | 100.8 | 0.18 |
| 0.5% | 4 | 320.3 | 102.0 | 0.95 |
| 0.1% | 5 | 322.0 | 102.5 | 0.54 |

**Sensitivity:** Pork sausage was spiked into halal certified chicken sausage to prepare spiked samples with pork content in 0.1%~5%(wt) for testing the detection sensitivity and reliability of the method. The results (Figure 3, Table 1) show that the PCR-MultiNA method established is able to detect pork DNA fragment (314 bp) in chicken sausage spiked with pork sausage at level as low as 0.1 % firmly. Furthermore, the peak areas of five measurements of 0.1% pork-spiked chicken sausage samples prepared in different days are considerably consistent with a CV (Coefficient of Variation) of 40.6%. For 0.5% pork-spiked chicken samples, the CV of four measurements are 20.7%. A correlation between peak area and content of pork in the sample is plotted in Figure 4, which reveals that the peak area increases proportionally with the pork content at a low range (0.1%~1%). Based on this finding, it is possible to establish at least a semi-quantitation method to detect the presence and content level of pork in processed food.

## Conclusions

Using Neogen Biokits PCR Mastermix Pod, pork specific (314 bp), a highly sensitive procedure for detection of pork in processed meat and food products was established on a PCR-MultiNA platform. The microchip electropherosis and fluorescence detection of MultiNA exhibits the advantages of high sensitivity, reliability and easiness in operation. The method can detect as low as 0.1% (wt) of pork sausage spiked in halal certified chicken sausage sample.
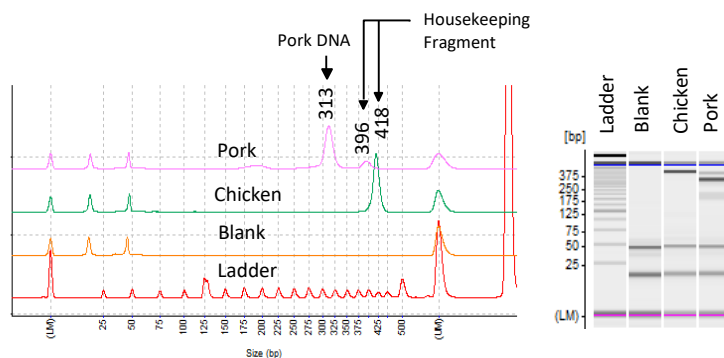


**Figure 2**: Electropherograms (EC) of PCR product of food samples (left), Detected peaks are pork DNA fragment (~313bp), housekeeping fragments (pork~395bp, chicken~418bp) and DNA ladders . A gel imaging display converted from EC (right).
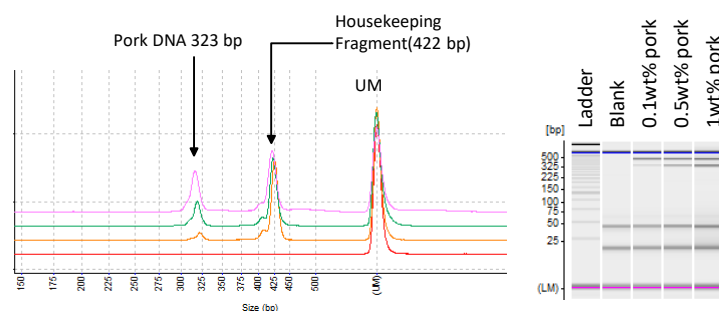


**Figure 3**: Detection of pig DNA fragment (~313bp) in chicken sausage samples spiked with 0% (blank), 0.1%, 0.5% and 1% of pork sausage (from bottom to top).
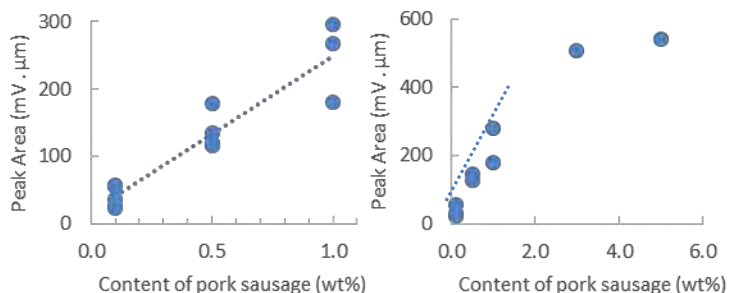


**Figure 4**: Correlation of area of electropherogram peak with content of pork sausage spiked in chicken sausage sample on PCR-MultiNA using Biokits PCR Mastermix Pod.

## References:

1. S. Edris, M.H.Z. Mutwakil, O.A., Abuzinadah, H.E. Mohammed, A. Ramadan, N.O. Hadalla, A.M. Shokry, S.M.Hassan, R.M. Shoaib, F.M. El-D0omyati and A. Bahieldin, Life Sci. Journal, 2012; 9(4), 5831-5837.
2. Fajardo, V., González, I., Rojas, M., García, T. & Martín, R. 2010, A review of current trends in Food Science & Technology, 2010. 21(8): p. 408-421.
3. Application Notes 11, Rapid Identification of Meat Species with MCE-202 "MultiNA", Shimadzu.
4. DNeasy® mericonTM Food Handbook, QIAGEN Sample & Assay Technologies. 2010.
5. Biokits Animal Speciation Selection Module, Neogen® Corporation 2013, 8 – 10.

*Note: The Application and Shimadzu Instruments are for research use only, not for use in diagnostic procedures.*

# SHIMADZU
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## Application News

### No.C99

**Liquid Chromatography Mass Spectrometry**

## Quantitative Analysis of Veterinary Drugs Using the Shimadzu LCMS-8050 Triple Quadrupole Mass Spectrometer

Foods in which chemical residues, like pesticides, feed additives, and veterinary drugs found in excess of maximum residue levels have been banned from sale in many countries around the world. Compounds that are subject to residue standards vary widely and the list is expected to grow. Because of this, there is a need for a highly sensitive and rapid analytical technique to analyze as many of these compounds as possible in a single run. This Application News introduces an example of the high-sensitivity analysis of 89 veterinary drugs in a crude extract of livestock and fishery products.

### ■ Sample Preparation

The typical samples used in the analysis of veterinary drugs contain large amounts of lipids because they are commonly meat and fish samples. Sample preparation is extremely important to ensure excellent sensitivity and repeatability. To avoid the typical time-consuming and laborious solid phase extraction sample preparation procedure, the QuEChERS method, which is typically used for the preparation of vegetables, was selected to simplify sample preparation.

The QuEChERS method normally consists of two steps, the first is an acetonitrile extraction and the second a cleanup step, but this time only the acetonitrile extraction step was used.

\* QuEChERS Extraction Salts kit: Restek Q-sep™ AOAC2007.01

(1) Homogenize 100 g sample (chicken, pork, salmon, shrimp) in food processor

(2) Weigh out 10 g homogenized sample, transfer to 50 mL test tube

(3) Add 5 mL water, shake gently by hand

(4) Add acetonitrile containing 1 % acetic acid and QuEChERS salts\*, shake by hand (1 min)

(5) Centrifuge separation (3 min)

(6) Collect acetonitrile layer and filter

(7) LC/MS/MS analysis

**Fig. 1  Sample Preparation Procedure**

### ■ Improved Peak Shape Using Sample / Water Co-Injection

When conducting reversed phase chromatography, the peaks of polar compounds may split or collapse depending on the relationship between the sample solvent and mobile phase. In cases where the sample solvent is rich in organic solvent, the elution strength must be lowered (by substitution or dilution) with the addition of water. As the pretreated sample solvent in this analysis consists of 100 % acetonitrile, injection in that state into the LC/MS will result in split peaks for some of the substances (Fig. 2 left).

To eliminate as much of the time and effort typically associated with sample preparation, the pretreatment features of the autosampler (SIL-30A) were utilized to conduct co-injection of sample and water, which resulted in improved peak shapes.



Draw Sample → Draw Air → Draw Water → Inject

Diaveridine

7:261.15>123.10 (+)

7:261.15>123.10 (+)

Difloxacin

26:400.10>356.20 (+)

26:400.10>356.20 (+)

Sample 2 µL

Sample 2 µL
+ Water 10 µL

Note: Sample solvent: 100 % Acetonitrile

**Fig. 2  Comparison of Peak Shape**

## ■ MRM Analysis of Matrix Standards

Fig. 3 shows the MRM chromatogram of the matrix standard solution consisting of the sample solution with added standard solution (data obtained using pork extract solution). Table 1 shows the lower limits of quantitation for the standard solution without added matrix and with added matrix, respectively. In a crude extract obtained by acetonitrile extraction alone, sensitivity was comparable to that obtained for most of the compounds using only standard solution. Although there were several compounds for which the lower limit of quantitation was different in the standard solution than the matrix-added solution, rather than attributing this to matrix effects, it is thought to be caused by elevated background due to ions derived from contaminating components (Refer to Fig. 5).



Fig. 3 MRM Chromatograms of 89 Veterinary Drugs (10 µg/L pork extract solution with added standard solution)

Table 1 LOQs of Veterinary Drugs in Neat Standards and Matrix Standards and Calibration Range of Veterinary Drugs in Matrix Standards

| | Std. Solution | Matrix-Added Std. Solution | |
|---|---|---|---|
| | Min. Conc. | Min. Conc. | Max. Conc. |
| Gentamicin | 0.5 | 1 | 50 |
| Sulfanilamide | 1 | 1 | 50 |
| Levamisole | 0.05 | 0.05 | 50 |
| Lincomycin | 0.01 | 0.01 | 10 |
| 5-Propylsulfonyl-1-benzimidazole-2-amine | 0.05 | 0.05 | 10 |
| Diaveridine | 0.01 | 0.01 | 10 |
| Trimethoprim | 0.02 | 0.02 | 20 |
| Marbofloxacin | 0.01 | 0.01 | 50 |
| Sulfisomidine | 0.02 | 0.02 | 20 |
| Norfloxacin | 0.5 | 0.5 | 50 |
| Ormetoprim | 0.02 | 0.02 | 10 |
| Thiabendazole | 0.01 | 0.01 | 10 |
| Ciprofloxacin | 0.05 | 0.5 | 10 |
| Neospiramycin I | 0.01 | 0.05 | 10 |
| Danofloxacin | 0.1 | 0.1 | 10 |
| Enrofloxacin | 0.05 | 0.1 | 50 |
| Oxytetracycline | 0.01 | 0.1 | 50 |
| Xylazine | 0.01 | 0.01 | 10 |
| Orbifloxacin | 0.05 | 0.05 | 50 |
| Sulfacetamide | 1 | 1 | 50 |
| Clenbuterol | 0.01 | 0.01 | 10 |
| Tetracycline | 0.05 | 0.01 | 50 |
| Spiramycin I | 0.01 | 0.01 | 50 |
| Sarafloxacin | 0.5 | 0.5 | 50 |
| Difloxacin | 0.05 | 0.1 | 50 |
| Sulfadiazine | 0.02 | 0.1 | 20 |
| Sulfathiazole | 0.02 | 0.1 | 20 |
| Sulfapyridine | 0.02 | 0.1 | 20 |
| Carbadox | 0.05 | 0.05 | 10 |
| Pyrimethamine | 0.02 | 0.02 | 20 |
| Sulfamerazine | 0.02 | 0.02 | 20 |
| Chlortetracycline | 0.1 | 0.1 | 50 |
| Tilmicosin | 0.1 | 0.1 | 50 |
| Thiamphenicol | 1 | 1 | 50 |
| Sulfadimidine | 0.02 | 0.02 | 20 |
| Sulfametoxydiazine | 0.01 | 0.02 | 10 |
| Sulfamethoxypyridazine | 0.02 | 0.02 | 20 |
| Sulfisozole | 0.01 | 0.01 | 50 |
| Trichlorfon (DEP) | 0.05 | 0.05 | 50 |
| Sulfamonomethoxine | 0.02 | 0.02 | 20 |
| Furazolidone | 1 | 1 | 50 |
| Difurazone | 0.05 | 0.05 | 50 |
| Erythromycin A | 0.01 | 0.01 | 50 |
| Cefazolin | 0.5 | 0.5 | 50 |

| | Std. Solution | Matrix-Added Std. Solution | |
|---|---|---|---|
| | Min. Conc. | Min. Conc. | Max. Conc. |
| Sulfachloropyridazine | 0.02 | 0.02 | 20 |
| Sulfadimethoxine | 0.02 | 0.02 | 10 |
| Tylosin | 0.05 | 0.05 | 50 |
| Sulfamethoxazole | 0.02 | 0.1 | 10 |
| Sulfaethoxypyridazine | 0.02 | 0.02 | 10 |
| Tiamulin | 0.01 | 0.01 | 50 |
| Florfenicol | 0.5 | 10 | 50 |
| 2Acetylamino 5nitrothiazole | 0.05 | 0.05 | 50 |
| Sulfatroxazole | 0.01 | 0.01 | 5 |
| Leucomycin | 0.01 | 0.01 | 50 |
| Sulfisoxazole | 0.01 | 0.05 | 50 |
| Oxolinic acid | 0.01 | 0.1 | 50 |
| Chloramphenicol | 0.5 | 1 | 50 |
| Clorsulon | 0.5 | 1 | 50 |
| Sulfabenzamide | 0.01 | 0.01 | 10 |
| Ethopabate | 0.01 | 0.01 | 10 |
| Sulfadoxine | 0.02 | 0.02 | 20 |
| Sulfaquinoxaline | 0.02 | 0.02 | 10 |
| Prednisolone | 0.1 | 0.05 | 20 |
| Ofloxacin | 0.5 | 0.5 | 50 |
| Flubendazole | 0.01 | 0.01 | 50 |
| Methylprednisolone | 0.5 | 0.5 | 50 |
| Nalidixic acid | 0.01 | 0.01 | 50 |
| Dexamethasone | 0.5 | 0.5 | 50 |
| Flumequine | 0.01 | 0.01 | 50 |
| Benzylpenicillin | 0.5 | 0.5 | 50 |
| Sulfanitran | 0.2 | 0.2 | 50 |
| Sulfabromomethazine | 0.01 | 0.01 | 50 |
| betaTrenbolone | 0.02 | 0.1 | 50 |
| Emamectin B1a | 0.01 | 0.01 | 50 |
| alphaTrenbolone | 0.02 | 0.1 | 50 |
| Piromidic acid | 0.01 | 0.05 | 50 |
| Zeranol | 1 | 0.1 | 50 |
| Ketoprofen | 0.01 | 0.05 | 50 |
| Testosterone | 0.01 | 0.05 | 10 |
| Famphur | 0.05 | 0.05 | 50 |
| Fenobucarb (BPMC) | 0.01 | 0.01 | 50 |
| Clostebol | 0.05 | 0.05 | 50 |
| Dichlofenac | 0.01 | 0.01 | 50 |
| Melengestrol Acetate | 0.05 | 0.05 | 50 |
| Temephos (Abate) | 0.01 | 0.5 | 50 |
| Allethrin | 0.1 | 1 | 50 |
| Closantel | 0.01 | 0.01 | 10 |
| Monensin | 0.01 | 0.01 | 10 |

(Unit: µg/L)

**Fig. 4  MRM Chromatograms in the Vicinity of the LOQ and Calibration Curves of Typical Compounds**

■ **Recoveries of Veterinary Drugs in Crude Extracts from Livestock and Fishery Products (Matrix Effect Verification)**

We examined whether or not the matrix affected measurement of actual samples. This time, four types of food product samples were used, including shrimp, chicken meat, pork, and salmon. Standard solution was added to the acetonitrile extraction solution of each of these to obtain a final concentration of 10 μg/L, after which the rates of recovery were determined. The results indicated that 90 % of the compounds were recovered at rates of 70 to 120 % and measurement was accomplished without any adverse matrix effects even though the crude extract solution was subjected only to acetonitrile extraction.



**Fig. 5  Recoveries of Veterinary Drugs in Each of the Matrices**

## ■ Acetonitrile Extraction Efficiency Using QuEChERS Method

To check the efficiency of acetonitrile extraction by the QuEChERS method, standard solution was added at stage (2) of Fig. 1 to obtain a concentration of 10 µg/L, and the recoveries were determined. Good recoveries of approximately 80 % were obtained in cases both with and without the addition of matrix. However, relatively poor recoveries were seen for highly polar compounds such as tetracycline and quinolone. For these compounds, it is necessary to examine the use of a separate extraction solvent and extraction reagent.

**Table 2  Recoveries (Pre-Spike)**

| Recovery | Without Matrix | With Matrix (Pork) | Compounds with Poor Recovery |
|---|---|---|---|
| < 50 % | 17 (19 %) | 13 (15 %) | Tetracyclines Quinolones |
| 50 % - 70 % | 1 (1 %) | 8 (9 %) | |
| > 70 % | 71 (80 %) | 68 (76 %) | |

## ■ Robustness

We checked the long-term stability of the instrument using a solution of pork crude extract (spiked with 10 µg/L standard solution). Even after continuous measurement of an extremely complex matrix over a period of 3 days, we were able to obtain stable data.



| Compounds | %RSD (%) (n=220) |
|---|---|
| Sulfaquinoxaline | 1.5 |
| Sulfamethoxazole | 2.8 |
| Ketoprofen | 2.3 |
| $\beta$ - trenbolone | 3.2 |
| Teststerone | 3.5 |

**Fig. 6  Area Plot and %RSD of Typical Compounds with Continuous Analysis**

**Table 3  Analytical Conditions**

| | |
|---|---|
| Column | : Shim-pack XR-ODS Ⅱ (75 mm × 2.0 mm I.D., 2.2 µm) |
| Mobile Phase A | : 0.1 % Formic Acid - Water |
| Mobile Phase B | : Acetonitrile |
| Time Program | : 1 %B (0 min) → 15 %B (1 min) → 40 %B (6 min) → 100 %B (10-13 min) → 1 %B (13.01-16 min) |
| Flowrate | : 0.2 mL/min. |
| Injection Volume | : 2 µL (2 µL sample solution + 10 µL water) |
| Oven Temperature | : 40 °C |
| Ionization Mode | : ESI (Positive / Negative) |
| Probe Voltage | : +2.0 kV / -1.0 kV |
| Neburizing Gas Flow | : 3.0 L/min. |
| Drying Gas Flow | : 10.0 L/min. |
| Heating Gas Flow | : 10.0 L/min. |
| Interface Temperature | : 400 °C |
| DL Temperature | : 200 °C |
| Block Heater Temperature | : 400 °C |

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# Determination of Nitrofuran Metabolites in Fish Matrix by LCMS-8045

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## Abstract

In this article, a method for the determination of nitrofuran metabolite residues in fish matrix was established using Shimadzu's Ultra-High Performance Liquid Chromatograph (UHPLC) LC-30A in conjunction with its Triple Quadrupole Mass Spectrometer LCMS-8045. After sample preparation, they were separated by UHPLC LC-30A in 3.0 minutes and then quantitatively analyzed using the Triple Quadrupole LCMS-8045. 3-amino-2-oxazolone (AOZ), 3-amino-5-morpholinomethyl-2-oxazolidinone (AMOZ), 1-amino-hydantoin (AHD), and semicarbazide (SEM) showed good linearity over the range of 0.05-20 ng/mL and their correlation coefficients were all greater than 0.9970. The precision experiments were performed with mixed standard solutions of concentrations 0.05 and 2.0 ng/mL. The results showed that the relative standard deviations for the retention times and peak areas obtained from 6 consecutive injections were 0.09 - 0.18% and 0.85 - 1.99% respectively, indicating good precision.

Nitrofurans are a class of synthetic antimicrobials drugs which inhibits the activity of acetyl-coenzyme A and interfere with the metabolism of sugar in microbes. Nitrofurans are very unstable and can easily be converted into metabolites. In the body of animals, nitrofuran drugs are rapidly decomposed into metabolites, which are subsequently bound to the membrane proteins. As the metabolites of nitrofurans are relatively stable and are associated with carcinogenic effects, the level of nitrofuran metabolites are often monitored in food safety tests. There are four common derivatives of nitrofuran metabolites, including: 3-amino-2-oxazolone (AOZ), 3-amino-5-morpholinomethyl-2-oxazolidinone (AMOZ), 1-amino-hydantoin (AHD) and semicarbazide (SEM).

In this article, a high-sensitivity detection method using Shimadzu UHPLC coupled with Triple Quadrupole LCMS-8045 is established for the assay of nitrofuran metabolite residues AOZ, AMOZ, AHD and SEM in fish matrix. This method was developed with reference to the standard method GB/T 21311-2007 and is aimed to serve as a reference method for regulatory testing.

## EXPERIMENTAL
### Instrumentation

This experiment was performed on a Shimadzu UHPLC LC-30A in conjunction with Triple Quadrupole Mass Spectrometer LCMS-8045. The specific configurations includes LC-30AD×2 solvent delivery pumps, DGU-20A$_{5R}$ online degassing unit, SIL-30AC autosampler, CTO-30AC column oven, CBM-20A system controller, LCMS-8045 triple quadrupole mass spectrometer and LabSolutions Ver. 5.86 chromatography workstation.

### Analytical Conditions
#### LC Chromatography (LC) Conditions

| | | |
|---|---|---|
| Column | : | Shim-pack GISS C18 Column (2.1 mm I.D.×100 mm L., 1.9 μm) |
| Mobile phase | : | Solvent A: 0.01% formic acid in water<br>Solvent B: 0.01% formic acid in acetonitrile |
| Flow rate | : | 0.50 mL/min |
| Column Temp. | : | 40 °C |
| Injection volume | : | 10 μL |
| Elution method | : | Gradient elution with an initial ratio of 10% B |

**Table 1 General gradient elution program**

| Time (min) | Module | Command | Value (%) |
|---|---|---|---|
| 1.50 | Pumps | Pump B Conc. | 80 |
| 1.51 | Pumps | Pump B Conc. | 10 |
| 3.00 | Controller | Stop | |

## Mass Spectrometry (MS) Conditions

| | | |
|---|---|---|
| Analytical Instrument | : | LCMS-8045 |
| Ion Source | : | ESI+ |
| Nebulizar gas flowrate | : | 3.0 L/min |
| Heating gas flow rate | : | 15.0 L/min |
| Interface Temp. | : | 400 °C |
| DL Temp. | : | 250 °C |
| Heating block Temp. | : | 450 °C |
| Needle offset distance | : | +3.0 mm |
| Drying gas flow rate | : | 5.0 L/min |
| Dwell Time | : | 22 ms |
| Scan mode | : | Multiple Reaction Monitoring (MRM) with parameters shown in **Table 2** |

## Standards and Sample Preparation

Preparation of standard solutions: an appropriate amount of nitrofuran derivatives, i.e., AOZ, AMOZ, AHD and SEM, were individually weighed and dissolved in a mixture of acetone/methanol (1:1) to prepare a single-standard stock solution at a concentration of 0.5 mg/mL. Subsequently, the stock solution was diluted with methanol to 100 ng/mL and further diluted with water to prepare a series of mixed standard working solutions at concentrations of 0.050, 0.10, 0.20, 0.50, 1.0 and 2.0 ng/mL.

Sample was prepared with reference to "GB/T 21311-2007: Determination of residues of nitrofuran metabolites in foodstuffs of animal origin HPLC-MS/MS method".

## RESULTS AND DISCUSSION

### MRM Chromatogram of Standard Samples

The MRM chromatogram of standard sample is shown in Figure 1.

**Table 2 Compound information and MRM parameters**

| Number | Analyte | CAS. | Percursor Ion | Product ion | Q1 Pre Bias (V) | CE (V) | Q3 Pre Bias (V) |
|---|---|---|---|---|---|---|---|
| 1 | SEM | 57-56-7 | 209.05 | 166.10 | -14.0 | -9.0 | -17.0 |
| | | | | 192.05 | -15.0 | -13.0 | -21.0 |
| 2 | AOZ | 80-65-9 | 236.10 | 134.00 | -11.0 | -11.0 | -23.0 |
| | | | | 104.10 | -17.0 | -22.0 | -11.0 |
| 3 | AHD | 2827-56-7 | 249.00 | 134.05 | -26.0 | -12.0 | -25.0 |
| | | | | 104.15 | -17.0 | -23.0 | -21.0 |
| 4 | AMOZ | 43056-63-9 | 335.15 | 291.10 | -16.0 | -12.0 | -30.0 |
| | | | | 262.10 | -24.0 | -18.0 | -30.0 |
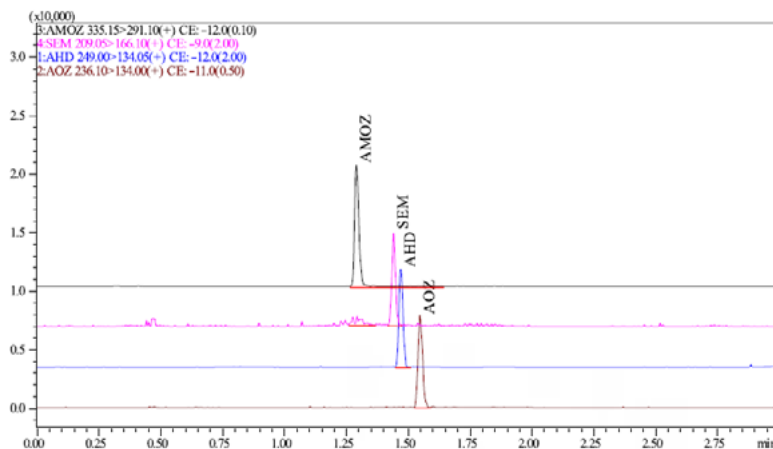


**Figure 1 MRM chromatogram of 0.5 ng/mL standard sample**

### Calibration curve and linearity

The mixed standard working solutions at concentrations of 0.050, 0.10, 0.20, 0.50, 1.0, 2.0, 5.0, 10.0, and 20 ng/mL were prepared and determined according to the analytical conditions described previously. A calibration curve was created using the external standard method and shown in Figure 2. The linearity of the method was good in the range of 0.005-20 ng/mL. The linear equations, the corresponding linear range and correlation coefficient are shown in Table 3.

### Limit of detection and limit of quantitation

For the injection and analysis of the mixed standard solution of concentration 0.05 ng/mL, the results for the limit of detection (S/N=3, LOD) and the limit of quantitation (S/N=10, LOQ) are shown in Table 4.

### Precision experiment

The mixed standard solutions at two concentrations were consecutively injected for 6 times to test the precision. The repeatability results of retention time and peak area are shown in Table 5. The relative standard deviations of the retention time and peak area for standards at two concentrations were 0.09-0.18% and 0.85-1.99%, respectively, indicating good precision.

**Table 3 Parameters of the calibration curve**

| Number | Analyte | Calibration curve | Linear range (ng/mL) | Accuracy (%) | Correlation coefficient (r) |
|--------|---------|-------------------|----------------------|--------------|------------------------------|
| 1 | SEM | Y = (8892.23)X + (-316.210) | 0.050-20 | 93.2-111.6 | 0.9970 |
| 2 | AOZ | Y = (42020.9)X + (65.5028) | 0.050-20 | 94.0-108.4 | 0.9986 |
| 3 | AHD | Y = (10603.3)X + (-260.028) | 0.050-20 | 95.5-102.1 | 0.9996 |
| 4 | AMOZ | Y = (242918)X + (-322.397) | 0.050-20 | 94.5-103.8 | 0.9996 |
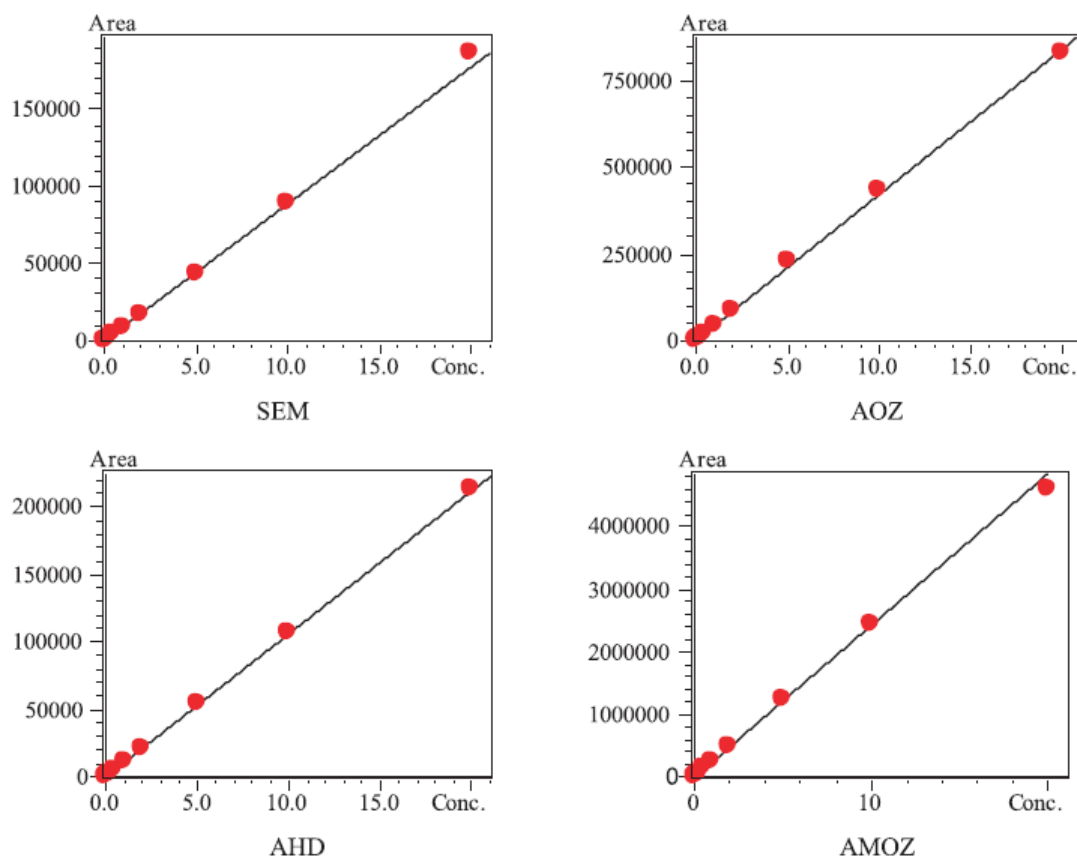


Figure 2 Standard calibration cure

**Table 4 Limit of detection and limit of quantification**

| No. | Analyte | S/N ratio | Limit of detection (ng/mL) | Limit of quantification (ng/mL) | Correlation coefficient (r) |
|-----|---------|-----------|---------------------------|--------------------------------|------------------------------|
| 1 | SEM | 10 | 0.015 | 0.05 | 0.9970 |
| 2 | AOZ | 156 | 0.00096 | 0.0032 | 0.9986 |
| 3 | AHD | 150 | 0.0010 | 0.0033 | 0.9996 |
| 4 | AMOZ | 633 | 0.00024 | 0.00079 | 0.9996 |
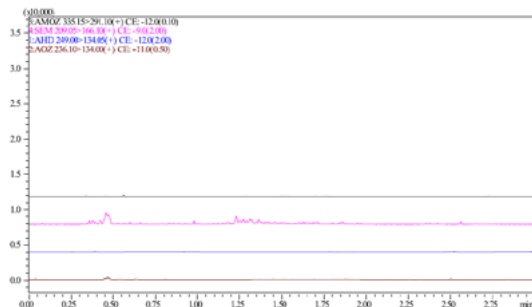


**Figure 3 Chromatogram of blank matrix**
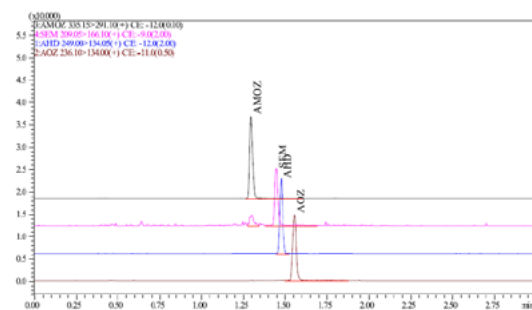


**Figure 4 Chromatogram of matrix spike samples (1.0 ng/mL)**

**Table 5  Repeatability results of retention time and peak area (n=6)**

| Analyte | RSD% (0.5 ng/mL) | | RSD% (2 ng/mL) | |
|---------|------|------|------|------|
| | R.T. | Area | R.T. | Area |
| SEM | 0.14 | 1.99 | 0.09 | 1.86 |
| AOZ | 0.13 | 1.94 | 0.12 | 1.65 |
| AHD | 0.18 | 1.97 | 0.14 | 1.61 |
| AMOZ | 0.18 | 1.97 | 0.09 | 0.85 |

**Recovery tests**

Blank fish sample was extracted according to the sample preparation method. The mixed standard solution was added with a spiked content of 1.0 µg/kg to give a final injection concentration of 1.0ng/mL. The chromatogram of blank matrix was shown in Figure 3 and the chromatogram of matrix spike samples was shown in Figure 4.

**CONCLUSION**

The nitrofuran metabolites in the fish matrix were determined using a Shimadzu UHPLC LC-30A in conjunction with a triple quadrupole LCMS-8045. In reference to "GB/T 21311-2007: Determination of residues of nitrofuran metabolites in foodstuffs of animal origin HPLC-MS/MS method", the measurement of AOZ, AMOZ, AHD and SEM showed good linearity in the range of 0.05-20 ng/mL, and their correlation coefficients were all greater than 0.9970. This method is fast and simple, with high sensitivity and selectivity, which meets the current requirements for the determination of nitrofuran metabolite residues in foodstuffs of animal origin.

# Determination of Sulfonamide Residues in Pork Using LCMS-8045

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## Abstract

This application news demonstrates a method for determination of sulfonamide residues in pork using Shimadzu's ultra-high performance liquid chromatograph (UHPLC) LC-30A together with the triple quadrupole mass spectrometer LCMS-8045. The linearity of the 11 sulfonamides was excellent and their correlation coefficients were all greater than 0.999. The limit of detection was 0.002 to 0.026 µg/L, and its limit of quantitation was 0.006 to 0.080 µg/L. The matrix spike recovery rate was between 86.6 and 119.8%. As this method meets the requirements in terms of lower limit of detection of 0.5 µg/kg as specified in the Department of Agriculture's announcement No. 1025-23-2008, it can be used to quickly and accurately determine sulfonamide residues in pork.

Sulfonamides (SAs) refer to synthetic antibiotics with sulfanilamide structures and it can be used to suppress most gram-positive bacteria and some gram-negative bacteria. When used in combination with antibacterial synergists, such as trimethoprim, SAs can enhance the antibacterial effect and expand the scope of treatment. Due to their advantages of having wide antibacterial spectra, strong curative effect and low cost, SAs are widely used in the prevention and treatment of diseases. However, one of its main drawbacks is that it can easily bring about side effects such as allergies and hematopoietic disorders, thereby causing a gradual reduction in clinical applications and usage. Instead, SAs are widely used in livestock breeding and aquaculture. Most of these drugs cannot be fully metabolized in animals and these SA residues can enter human body through the food chain, thus causing harm to human health. At present, the European Union, the United States, and Japan all list SAs as drugs with a restricted use in animal husbandry, with the maximum amount of SA residues generally limited to 50–100 µg/kg. China has also established relevant standards for detection of SA residues in animal-derived food, such as GB 29694-2013 "Determination of 13 Types of SA Residues in Animal-Derived Food Using High Performance Liquid Chromatography", GB 21316-2007 "Determination of SA Residues in Animal-Derived Food Using Liquid Chromatography-Mass Spectrometry/Mass Spectrometry", and SN/T 4057-2014 "Determination of SA Residues in Animal-Derived Food for Export Using Immunoaffinity Column Chromatography-HPLC and LC-MS/MS Method".

In reference to the sample preparation method No. 1025-23-2008 listed by the Department of Agriculture "Detection of SA Residues in Animal-Derived Food Using Liquid Chromatography-Tandem Mass Spectrometry", this application news demonstrates the use of Shimadzu's Ultra-High Performance Liquid Chromatograph (UHPLC) LC-30A together with the triple quadrupole mass spectrometer LCMS-8045 to determine SA residues in pork.

## EXPERIMENTAL

### Instrumentation

The experiment employed Shimadzu's UHPLC LC-30A and triple quadrupole mass spectrometer LCMS-8045. The configurations are two LC-30AD pumps, DGU-20A$_{5R}$ online degassing unit, SIL-30AC autosampler, CTO-30AC column oven, CBM-20A system controller, LCMS-8045 triple quadrupole mass spectrometer and LabSolutions Ver. 5.86 chromatographic workstation.

## Analytical Conditions
### Liquid Chromatography (LC) Conditions

| | | |
|---|---|---|
| Column | : | Shim-pack XR-ODS III (2.0 mm I.D.×50 mm L., 1.6 μm) |
| Mobile phase | : | Mobile phase A-0.1% formic acid in water, Mobile phase B-methanol |
| Flow rate | : | 0.3 mL/min |
| Column Temp. | : | 40 °C |
| Injection volume | : | 5 μL |
| Elution method | : | Gradient elution program with the initial concentration of Mobile Phase B at 10%. Refer to Table 1 for detailed gradient elution program. |

**Table 1 Time program**

| Time (min) | Module | Command | Value (%) |
|---|---|---|---|
| 0.20 | Pumps | Pump B Conc. | 10 |
| 1.00 | Pumps | Pump B Conc. | 30 |
| 2.00 | Pumps | Pump B Conc. | 30 |
| 4.00 | Pumps | Pump B Conc. | 90 |
| 5.00 | Pumps | Pump B Conc. | 90 |
| 5.01 | Pumps | Pump B Conc. | 10 |
| 8.00 | Controller | Stop | |

### Mass Spectrometry (MS) Conditions

| | | |
|---|---|---|
| Ion sources | : | ESI |
| Interface temperature | : | 300 °C |
| DL temperature | : | 250 °C |
| Mode | : | Multiple reaction monitoring (MRM) |
| Nebulizing gas flow rate | : | 3.0 L/min |
| Drying gas flow rate | : | 10.0 L/min |
| Heated gas flow rate | : | 10.0 L/min |
| MRM transition | : | Refer to Table 2 |

## Sample Preparation

There were 11 SA substances in total, including sulfathiazole, sulfapyridine, sulfamethiazole, sulfamethazine/ sulfadimidine, sulfameter/ sulfamethoxydiazine, sulfamethoxypyridazine, sulfachloropyridazine, sulfamethoxazole, sulfisoxazole, sulfamethoxine, and sulfaquinoxaline were prepared.

Preparation of standard solutions: mixed standard stock solutions at a concentration of 10 mg/L were prepared using acetonitrile. The mixed standard stock solutions were subsequently diluted with a methanol/water solution (V/V, 10:90) to obtain mixed standard working solutions at concentrations of 0.1, 0.5, 1, 5, 10, and 50 μg/L.

Preparation of sample: samples were prepared and injected for analysis according to the sample extraction and clean-up method listed in the Department of Agriculture's No. 1025-23-2008 "Detection of SA Residues in Animal-Derived Food Using Liquid Chromatography-Tandem Mass Spectrometry".

## RESULTS AND DISCUSSION
### MRM Chromatograms of Standard Samples

MRM chromatograms of mixed standard samples are shown in Figure 1.

### Calibration and Linearity

The mixed standard calibration solutions at concentrations of 0.1, 0.5, 1, 5, 10, and 50 μg/L were prepared and determined according to analytical conditions described previously. Linearity was good over the concentration range of 0.1-10 μg/L and 0.1-50 μg/L where the external standard method was used to generate the calibration curve. Linear equation, linear range, and coefficients of the determination are shown in Table 3.
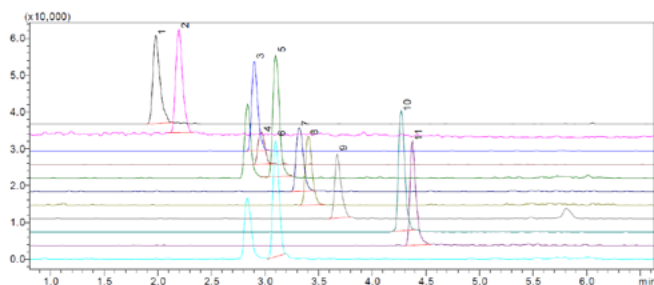
**Figure 1  MRM chromatogram of mixed standard sample (0.5 μg/L)**
(1. Sulfathiazole; 2. Sulfapyridine; 3. Sulfamethiazole; 4. Sulfamethazine/sulfadimidine; 5. Sulfameter/sulfamethoxydiazine; 6. Sulfamethoxypyridazine; 7. Sulfachloropyridazine; 8. Sulfamethoxazole; 9. Sulfisoxazole; 10. Sulfamethoxine; 11. Sulfaquinoxaline)

**Table 2  MRM transition**

| No. | Analyte | Precursor Ion | Product Ion | Q1 Pre Bias (V) | CE (V) | Q3 Pre Bias (V) |
|-----|---------|---------------|-------------|-----------------|--------|-----------------|
| 1 | Sulfathiazole | 256.10 | 156.05* | -28.0 | -16.0 | -29.0 |
|   |   |   | 108.10 | -18.0 | -25.0 | -21.0 |
| 2 | Sulfapyridine | 250.15 | 156.05* | -28.0 | -17.0 | -29.0 |
|   |   |   | 184.00 | -12.0 | -17.0 | -19.0 |
| 3 | Sulfamethiazole | 271.05 | 156.05* | -13.0 | -14.0 | -29.0 |
|   |   |   | 108.10 | -13.0 | -27.0 | -21.0 |
| 4 | Sulfamethazine/sulfadimidine | 279.20 | 186.10* | -14.0 | -18.0 | -19.0 |
|   |   |   | 156.00 | -30.0 | -20.0 | -28.0 |
| 5 | Sulfameter/sulfamethoxydiazine | 281.15 | 156.05* | -30.0 | -18.0 | -30.0 |
|   |   |   | 108.15 | -20.0 | -28.0 | -20.0 |
| 6 | Sulfamethoxypyridazine | 281.15 | 156.10* | -30.0 | -18.0 | -30.0 |
|   |   |   | 108.10 | -20.0 | -27.0 | -20.0 |
| 7 | Sulfachloropyridazine | 285.05 | 156.10* | -14.0 | -14.0 | -29.0 |
|   |   |   | 108.00 | -14.0 | -27.0 | -21.0 |
| 8 | Sulfamethoxazole | 254.10 | 156.05* | -29.0 | -16.0 | -30.0 |
|   |   |   | 108.10 | -12.0 | -24.0 | -21.0 |
| 9 | Sulfisoxazole | 268.10 | 156.10* | -29.0 | -14.0 | -28.0 |
|   |   |   | 113.15 | -13.0 | -15.0 | -22.0 |
| 10 | Sulfamethoxine | 311.15 | 156.10* | -15.0 | -23.0 | -30.0 |
|   |   |   | 108.05 | -15.0 | -30.0 | -22.0 |
| 11 | Sulfaquinoxaline | 301.15 | 156.10* | -15.0 | -17.0 | -29.0 |
|   |   |   |   | -15.0 | -27.0 | -19.0 |

Note: * indicates quantification ion

**Table 3  Parameters for calibration curve (linear regression, the weight coefficient was 1/C)**

| No. | Compound | Calibration Curve | Linear Range (ng/mL) | Accuracy (%) | Correlation Coefficient (r) |
|-----|----------|-------------------|----------------------|--------------|------------------------------|
| 1 | Sulfathiazole | Y = (208213) X + (3090.87) | 0.1~50 | 81.9~106.9 | 0.9994 |
| 2 | Sulfapyridine | Y = (244596) X + (-547.080) | 0.1~10 | 91.9~104.1 | 0.9998 |
| 3 | Sulfamethiazole | Y = (183474) X + (-83.6554) | 0.1~10 | 95.6~104.9 | 0.9999 |
| 4 | Sulfamethazine/sulfadimidine | Y = (163835) X + (1324.90) | 0.1~10 | 89.2~108.3 | 0.9997 |
| 5 | Sulfameter/sulfamethoxydiazine | Y = (250149) X + (5313.05) | 0.1~10 | 88.5~108.1 | 0.9997 |
| 6 | Sulfamethoxypyridazine | Y = (242793) X + (4103.03) | 0.1~10 | 95.2~103.3 | 0.9996 |
| 7 | Sulfachloropyridazine | Y = (157837) X + (175.633) | 0.1~10 | 95.0~104.5 | 0.9998 |
| 8 | Sulfamethoxazole | Y = (154848) X + (4259.61) | 0.1~50 | 82.0~109.0 | 0.9991 |
| 9 | Sulfisoxazole | Y = (145057) X + (1448.37) | 0.1~50 | 94.9~106.6 | 0.9999 |
| 10 | Sulfamethoxine | Y = (245919) X + (11612.2) | 0.1~50 | 97.3~103.3 | 0.9999 |
| 11 | Sulfaquinoxaline | Y = (200233) X + (6393.09) | 0.1~50 | 88.1~104.2 | 0.9997 |

**Limit of Detection and Limit of Quantitation**

Pork samples were treated according to the method specified in the previous section to obtain spiked samples at a concentration of 0.5 µg/L. After injection and analysis, the lower limit of detection (LOD, S/N=3) and the lower limit of quantitation (LOQ, S/N=10) for 11 SAs were calculated as shown in Table 4.

**Precision Test**

Mixed standard solutions at various concentrations were injected consecutively 6 times to determine precision. Repeatability results of retention time and peak area are shown in Table 5. The relative standard deviations of retention time and peak area were within ranges of 0.02 to 0.13% and 0.66 to 5.15%, respectively, indicating good precision.

**Matrix Spike Samples Test**

Pork samples were treated according to the method specified in the previous section to obtain a blank matrix, which was then used to prepare spiked samples at a concentration of 0.5 µg/L for injection and analysis. The spike recovery rate of samples ranged from 86.6 to 119.8%. The chromatograms of the blank matrix are shown in Figure 2, while the chromatograms of the spiked samples are shown in Figure 3.

**Table 4 Limit of detection and limit of quantification for 11 SAs**

| No. | Compound Name | Limit of Detection (µg/L) | Limit of Quantitation (µg/L) |
|---|---|---|---|
| 1 | Sulfathiazole | 0.002 | 0.006 |
| 2 | Sulfapyridine | 0.010 | 0.032 |
| 3 | Sulfamethiazole | 0.002 | 0.006 |
| 4 | Sulfamethazine/sulfadimidine | 0.006 | 0.017 |
| 5 | Sulfameter/sulfamethoxydiazine | 0.025 | 0.076 |
| 6 | Sulfamethoxypyridazine | 0.026 | 0.080 |
| 7 | Sulfachloropyridazine | 0.003 | 0.011 |
| 8 | Sulfamethoxazole | 0.021 | 0.062 |
| 9 | Sulfisoxazole | 0.004 | 0.014 |
| 10 | Sulfamethoxine | 0.002 | 0.008 |
| 11 | Sulfaquinoxaline | 0.009 | 0.028 |

**Table 5 Repeatability results of retention time and peak area (n=6)**

| No. | Compound Name | RSD% (1 µg/L) | | RSD% (10 µg/L) | |
|---|---|---|---|---|---|
| | | R.T. | Area | R.T. | Area |
| 1 | Sulfacetamide | 0.13 | 2.62 | 0.07 | 0.92 |
| 2 | Sulfathiazole | 0.08 | 2.47 | 0.05 | 1.28 |
| 3 | Sulfapyridine | 0.13 | 2.39 | 0.05 | 1.00 |
| 4 | Sulfamethiazole | 0.03 | 1.86 | 0.04 | 0.83 |
| 5 | Sulfamethazine/sulfadimidine | 0.09 | 4.95 | 0.05 | 0.66 |
| 6 | Sulfameter/sulfamethoxydiazine | 0.05 | 1.33 | 0.03 | 1.56 |
| 7 | Sulfamethoxypyridazine | 0.04 | 5.15 | 0.03 | 1.72 |
| 8 | Sulfachloropyridazine | 0.07 | 2.11 | 0.03 | 1.04 |
| 9 | Sulfamethoxazole | 0.03 | 3.44 | 0.04 | 0.91 |
| 10 | Sulfisoxazole | 0.03 | 2.46 | 0.03 | 1.31 |
| 11 | Sulfamethoxine | 0.01 | 1.29 | 0.03 | 1.41 |
| 12 | Sulfaquinoxaline | 0.06 | 1.91 | 0.02 | 1.26 |
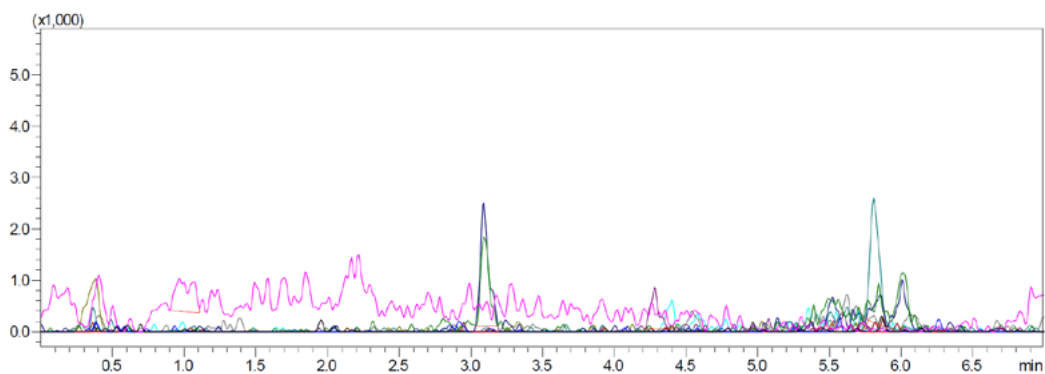
**Figure 2  Chromatogram of blank matrix**
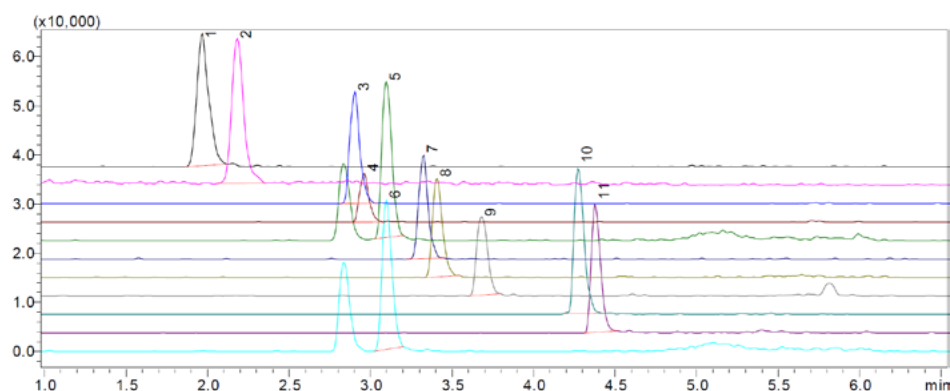


**Figure 3  Chromatogram of spike recovery test**

(1. Sulfathiazole; 2. Sulfapyridine; 3. Sulfamethiazole; 4. Sulfamethazine/sulfadimidine; 5. Sulfameter/sulfamethoxydiazine; 6. Sulfamethoxypyridazine; 7. Sulfachloropyridazine; 8. Sulfamethoxazole; 9. Sulfisoxazole; 10. Sulfamethoxine; 11. Sulfaquinoxaline)

**Table 6  Results of spiked sample recovery**

| No. | Compound Name | Measured Concentration (µg/L) | Recovery (%) |
|---|---|---|---|
| 1 | Sulfathiazole | 0.599 | 119.8 |
| 2 | Sulfapyridine | 0.553 | 110.6 |
| 3 | Sulfamethiazole | 0.509 | 101.8 |
| 4 | Sulfamethazine/sulfadimidine | 0.468 | 93.6 |
| 5 | Sulfameter/sulfamethoxydiazine | 0.494 | 98.8 |
| 6 | Sulfamethoxypyridazine | 0.508 | 101.6 |
| 7 | Sulfachloropyridazine | 0.479 | 95.8 |
| 8 | Sulfamethoxazole | 0.513 | 102.6 |
| 9 | Sulfisoxazole | 0.515 | 103.0 |
| 10 | Sulfamethoxine | 0.433 | 86.6 |
| 11 | Sulfaquinoxaline | 0.478 | 95.6 |

## CONCLUSION

In the determination of SA residues in pork using Shimadzu's UHPLC LC-30A coupled with the triple quadrupole mass spectrometer LCMS-8045, the linearity of each of these 11 SAs was good, and their correlation coefficients were all greater than 0.999. The limit of detection was 0.002 to 0.026 µg/L, and its limit of quantification was 0.006 to 0.080 µg/L. The matrix spike recovery rate was between 86.6 and 119.8%. As this method meets the requirements of lower limit of detection of 0.5 µg/kg as specified in the Department of Agriculture's No. 1025-23-2008 "Detection of SA Residues in Animal-Derived Food Using Liquid Chromatography-Tandem Mass Spectrometry", it can be used to detect SA residues in pork.

# SHIMADZU
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# Determination of Quinolone Antibiotic Residues in Chicken by Ultra-High Performance Liquid Chromatograph Coupled with Triple Quadrupole Mass Spectrometer

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## Abstract

A method was developed for the determination of 12 quinolone antibiotics in chicken using Shimadzu's ultra-high performance liquid chromatograph LC-30A coupled with triple quadrupole mass spectrometer LCMS-8045. The analysis of 12 antibiotics was completed within 9 min and the correlation coefficients of the calibration curves were all above 0.997. The mixed standard solutions with various concentrations of antibiotics were tested in 6 replicates. The relative standard deviations of retention time and peak area of the 12 target compounds were 0.03 - 0.27% and 1.13 - 4.93%, respectively, and the precision was good. The range of matrix spike recovery was 91.90 - 108.60% at different concentrations. The method can be applied to the simultaneous detection of 12 quinolone antibiotic residues in chicken.

Quinolones (QNs) are synthetic drugs with broad-spectrum bactericidal effect. Due to their strong antibacterial activity and wide spectrum range, they are widely used in the prevention and treatment of various infectious diseases in human beings, poultry and livestock. However, drug overdose or improper use will lead to a high level of QNs residues in animals, especially for food-producing animals. In addition to the immediate and direct toxic effects of QNs on human body, the long-term consumption of animal-derived food containing QNs can readily induce drug resistance, thus affecting the clinical efficacy of QNs on human body. Therefore, the issue of QN residues has raised more and more concerns. The U.S., Japan, E.U. and China have regulated the maximum residue limit of QNs in food and it varies according to the different classification, properties and characteristics of QNs and is in the range of 10-6000 µg/kg.

Chicken is a meat widely consumed in China, so the determination of QNs residues in chicken is of great significance. High performance liquid chromatography-tandem mass spectrometry is a rapid developing analytical technology in recent years. With its ability to perform highly sensitive and selective quantitative and qualitative analyses as well as provide high accuracy for antibiotic compounds in complex matrices, it is the preferred technique for ultra-trace residue analysis. A method was established in this application news for determination of 12 QNs antibiotics in chicken using a Shimadzu UHPLC LC-30A coupled with Triple Quadrupole Mass Spectrometer LCMS-8045.

## EXPERIMENTAL

### Instrumentation

The experiment employed Shimadzu's UHPLC LC-30A and triple quadrupole mass spectrometer LCMS-8045. The specific configurations are two LC-30AD pumps, DGU-20A5 online degassing unit, SIL-30AC autosampler, CTO-30A column oven, CBM-20A system controller, LCMS-8045 triple quadrupole mass spectrometer and LabSolutions Ver. 5.86 chromatographic workstation.

## Analytical Conditions

### Liquid Chromatography (LC) Conditions

| | | |
|---|---|---|
| Column | : | Shim-pack GISS (2.1 mm I.D.× 100 mm L, 1.9 µm) |
| Mobile phase | : | Phase A - 0.2% formic acid in water<br>Phase B - acetonitrile/methanol (6:4) |
| Flow rate | : | 0.40 mL/min |
| Column Temp. | : | 40 °C |
| Injection volume | : | 10 µL |
| Elution method | : | Gradient elution with the initial concentration of Mobile Phase B at 10%. Refer to Table 1 for elution program. |

## Table 1 Time program

| Time (min) | Module | Command | Value (%) |
|---|---|---|---|
| 4.50 | Pumps | Pump B Conc. | 40 |
| 4.60 | Pumps | Pump B Conc. | 95 |
| 5.50 | Pumps | Pump B Conc. | 95 |
| 5.60 | Pumps | Pump B Conc. | 10 |
| 9.00 | Controller | Stop | |

## Mass Spectrometry (MS) Conditions

| | | |
|---|---|---|
| Mass Spectrometer | : | LCMS-8045 |
| Ion sources | : | ESI (+) |
| Heating gas | : | Air 10.0 L/min |
| Nebulizing gas | : | Nitrogen 3.0 L/min |
| Drying gas | : | Nitrogen 10.0 L/min |
| Collision gas | : | Argon |
| Interface temp. | : | 300 °C |
| DL temp. | : | 250 °C |
| Heater temp. | : | 400 °C |
| Mode | : | Multiple reaction monitoring (MRM) |
| Dwell time | : | 15 ms |
| Delay time | : | 3 ms |
| MRM parameters | : | Refer to Table 2 |

## Table 2 MRM optimized parameters

| No. | Analyte | CAS No. | Precursor Ion | Product Ion | Q1 Pre Bias (V) | CE (V) | Q3 Pre Bias (V) |
|---|---|---|---|---|---|---|---|
| 1 | Pipemidic Acid | 51940-44-4 | 304.1 | 286.1* | -16 | -21 | -20 |
| | | | | 215.1 | -30 | -38 | -24 |
| 2 | Enoxacin | 84294-96-2 | 321.1 | 303.1* | -13 | -22 | -11 |
| | | | | 232.1 | -13 | -35 | -16 |
| 3 | Ofloxacin | 82419-36-1 | 362.1 | 318.2* | -12 | -21 | -22 |
| | | | | 261.1 | -12 | -29 | -18 |
| 4 | Norfloxacin | 70458-96-7 | 320.0 | 302.1* | -11 | -22 | -21 |
| | | | | 231.1 | -11 | -39 | -24 |
| 5 | Pefloxacin | 149676-40-4 | 334.1 | 316.1* | -28 | -23 | -22 |
| | | | | 290.1 | -13 | -18 | -14 |
| 6 | Ciprofloxacin | 93107-08-5 | 332.0 | 314.1* | -11 | -16 | -24 |
| | | | | 231.0 | -11 | -37 | -25 |
| 7 | Lomefloxacin | 98079-52-8 | 352.1 | 265.1* | -16 | -25 | 28 |
| | | | | 308.1 | -16 | -17 | -21 |
| 8 | Danofloxacin | 119478-55-6 | 358.1 | 340.1* | -14 | -24 | -12 |
| | | | | 255.1 | -14 | -40 | -17 |
| 9 | Enrofloxacin | 93106-60-6 | 360.1 | 316.2* | -12 | -20 | -11 |
| | | | | 342.1 | -12 | -20 | -11 |
| 10 | Cinoxacin | 28657-80-9 | 263.0 | 245.0* | -28 | -15 | -26 |
| | | | | 217.0 | -28 | -23 | -22 |
| 11 | Oxolinic Acid | 14698-29-4 | 262.1 | 244.0* | -17 | -18 | -26 |
| | | | | 216.1 | -30 | -28 | -23 |
| 12 | Flumequine | 42835-25-6 | 262.1 | 244.1* | -16 | -16 | -17 |
| | | | | 202.0 | -16 | -36 | -22 |

Note: * indicates quantification ion

### Standard Solution Preparation

Quinolone standards were weighed and dissolved in methanol to prepare mixed standard stock solutions of 1.0mg/ml. The mixed standard solutions were stored at -18 °C. Accurate volumes of mixed standard stock solution were added to blank chicken extract solutions to prepare mixed standard working solutions with concentrations of 0.2, 0.5, 1, 5, 10, 20 and 50 ng/mL.

### Sample Preparation Method

Chicken samples were prepared with reference to the national standard GB/T 21312-2007 "Analysis of 14 Quinolone in Food of Animal Origin by High Performance Liquid Chromatography Tandem Mass Spectrometry".

5.0g (accurate to 0.1g) of homogeneous chicken sample was weighed in a 50mL polypropylene centrifuge tube. 20mL of 0.1m/L EDTA-Mcllvaine buffer solution was added. The mixture was vortexed, ultrasonically extracted for 10 mins and centrifuged at 10,000 rpm for 5 mins. The extraction was repeated 3 times in total and the supernatant was combined.

SPE clean-up was performed using HLB SPE cartridges (200mg, 6mL). The SPE was first activated with 6mL of methanol and 6mL of water before use. 6mL of extracted supernatant was added to the SPE column and rinse with 2mL of 5% methanol solution. The filtrate was discarded and the SPE column drained. Elution of SPE was carried out with 6mL of methanol. The eluate was collected and completely dried with nitrogen. The dried extract was reconstituted with 1ml of mobile phase and vortexed. The reconstituted extracts were filtered through a 0.22μm filter membrane and injected into LC-MS/MS for analysis.

### RESULTS AND DISCUSSION

### MS Scan and Product Ion Scan of Standard Samples

The Q1 MS scan and product ion scan of the quinolone drugs are shown in Figures 1-12.
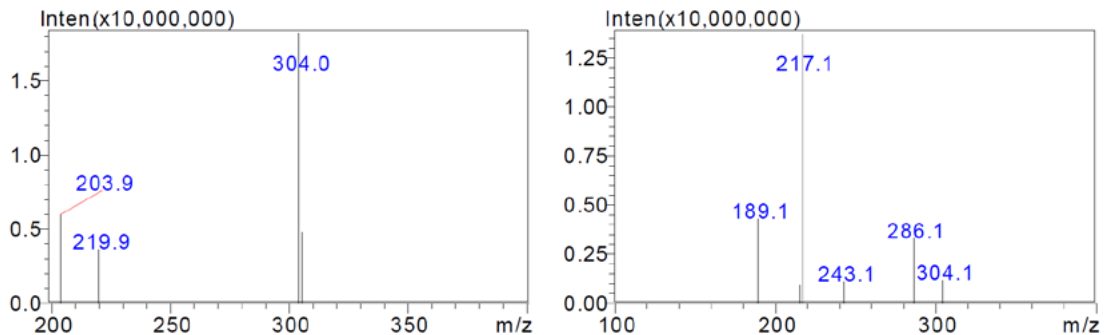


**Figure 1 Q1 MS Scan (left) and product ion scan (CE value-20 V, right) of pipemidic acid**
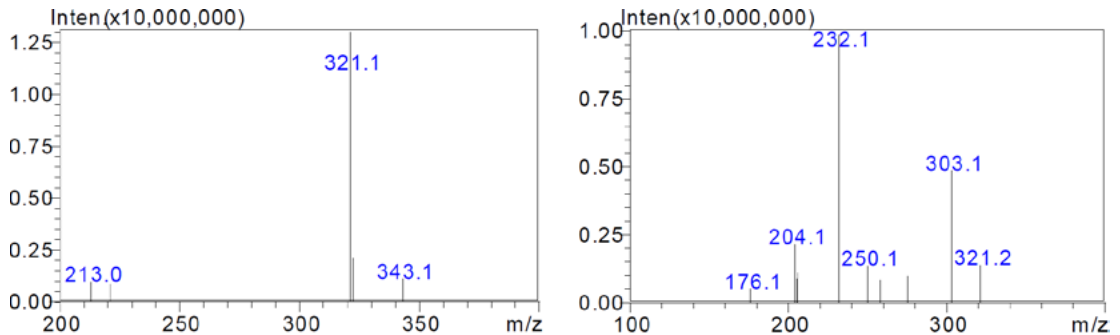


**Figure 2 Q1 MS Scan (left) and product ion scan (CE value-30 V, right) of enoxacin**
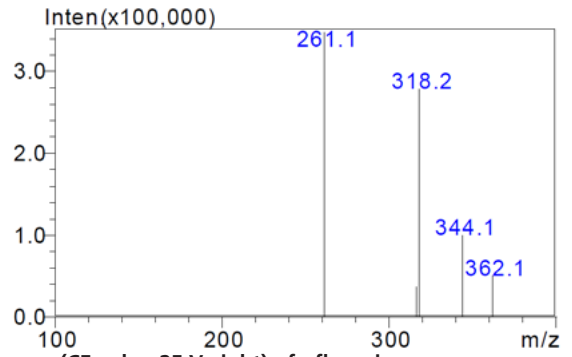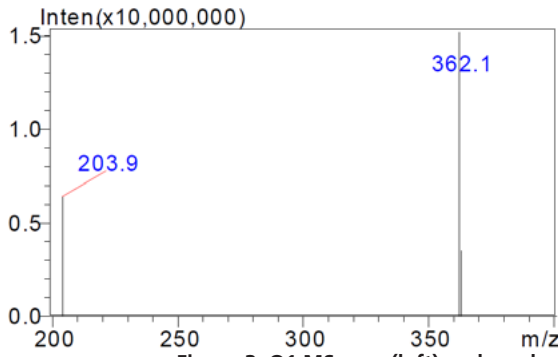
**Figure 3  Q1 MS scan (left) and product ion scan (CE value-25 V, right) of ofloxacin**
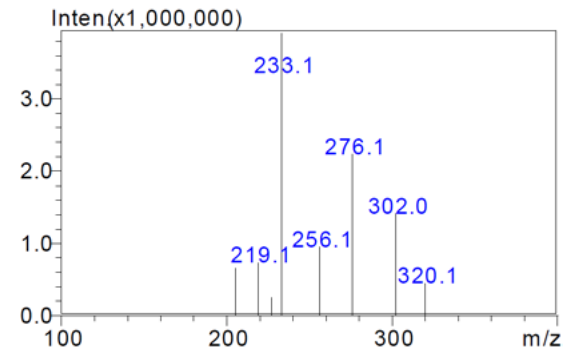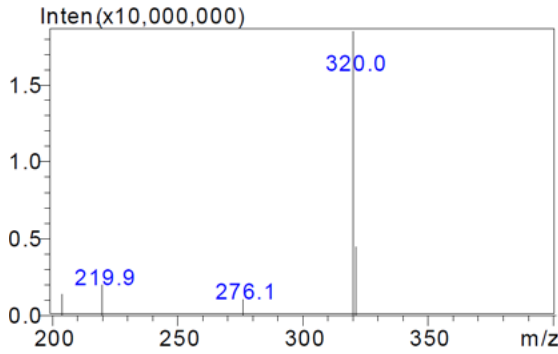


**Figure 4  Q1 MS scan (left) and product ion scan (CE value-25 V, right) of norfloxacin**
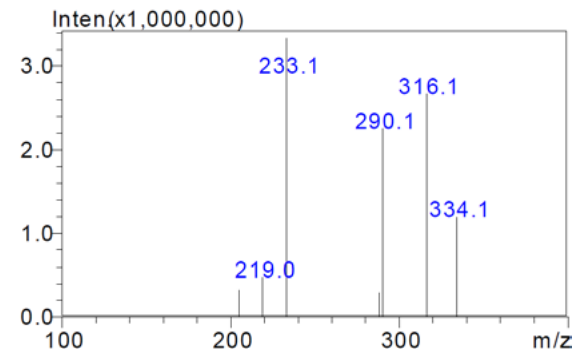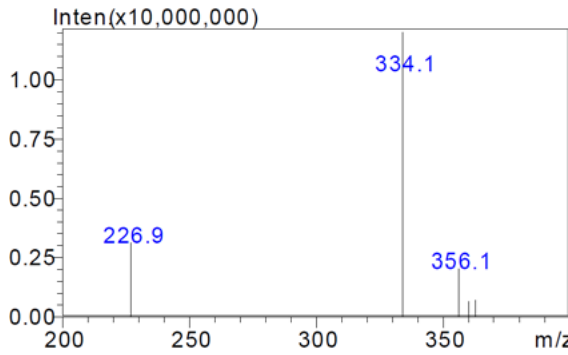


**Figure 5  Q1 MS scan (left) and product ion scan(CE value-25 V, right) of pefloxacin**
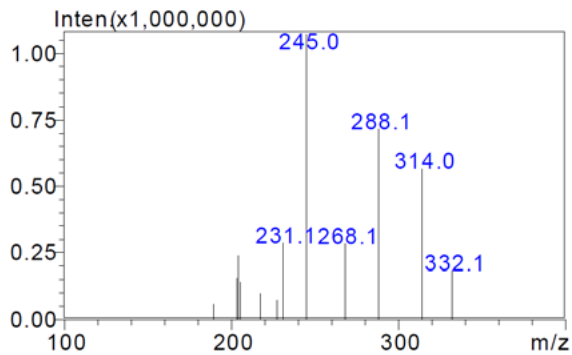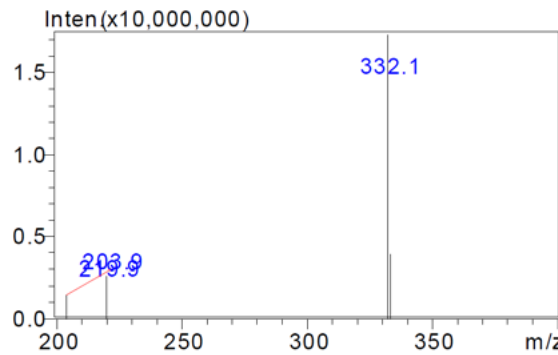


**Figure 6  Q1 MS scan (left) and product ion scan (CE value-25 V, right) of ciprofloxacin**
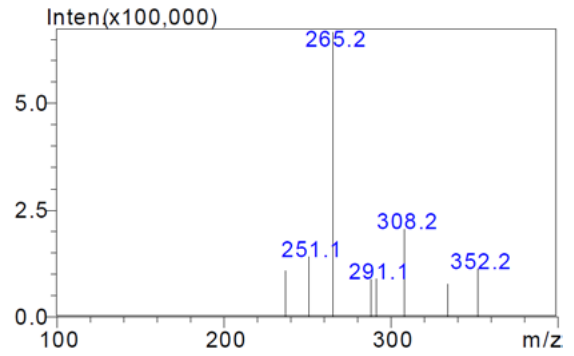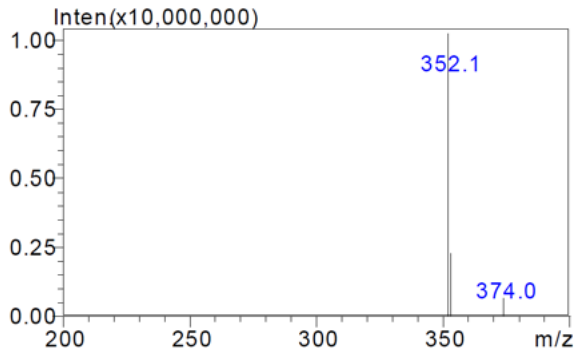
**Figure 7  Q1 MS scan (left) and product ion scan CE value-25 V, right) of lomefloxacin**
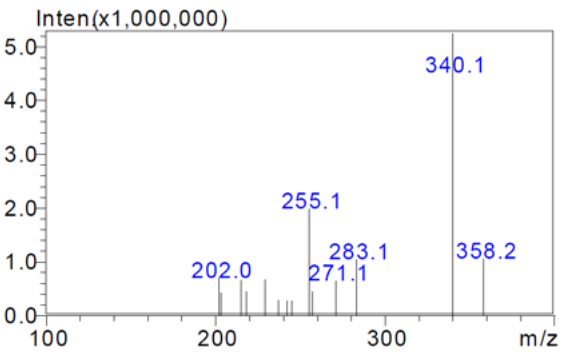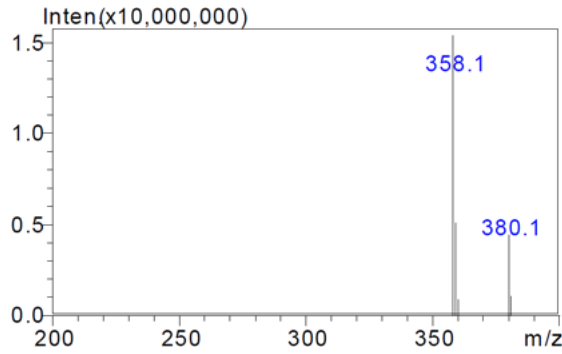


**Figure 8  Q1 MS scan (left) and product ion scan (CE value-25 V, right) of danofloxacin**
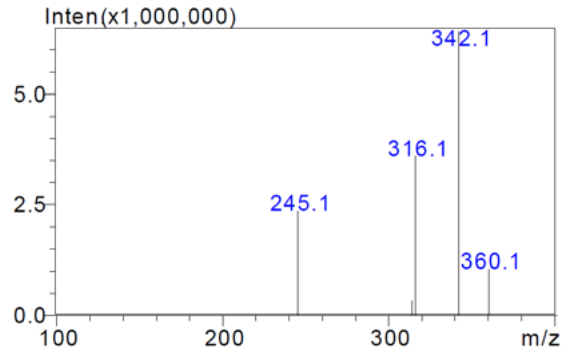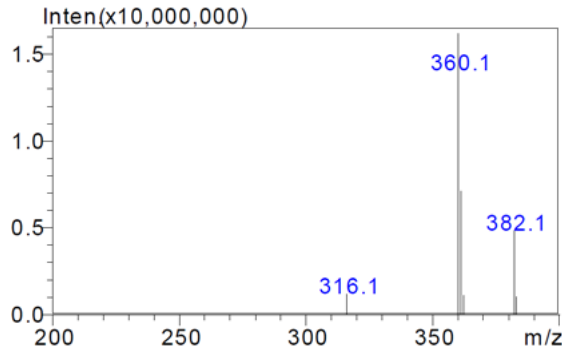


**Figure 9  Q1 MS scan (left) and product ion scan (CE value-25 V, right) of enrofloxacin**
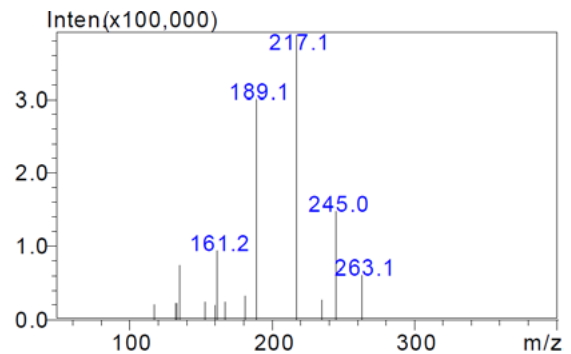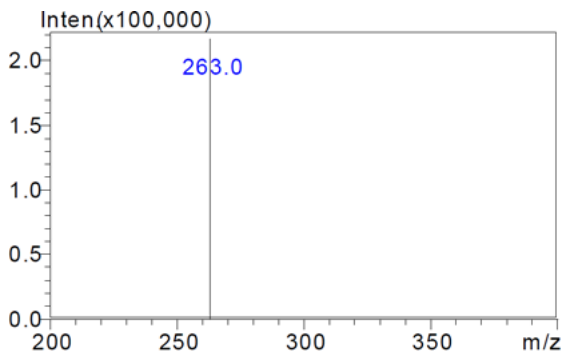


**Figure 10  Q1 MS scan (left) and product ion scan (CE value-30 V, right) of cinoxacin**
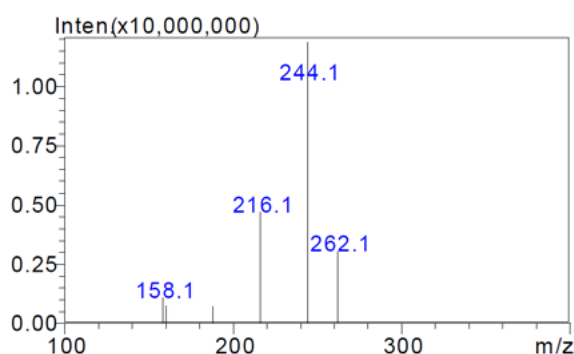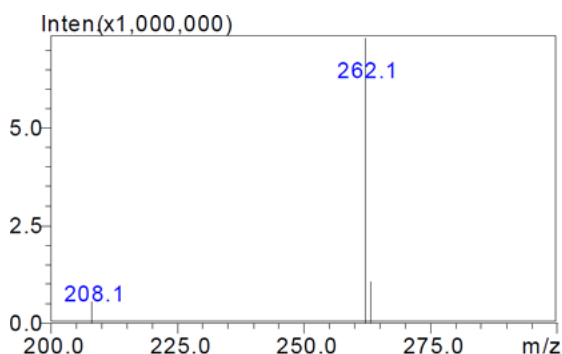
**Figure 11 Q1 MS scan (left) and product ion scan (CE value-25 V, right) of oxolinic acid**
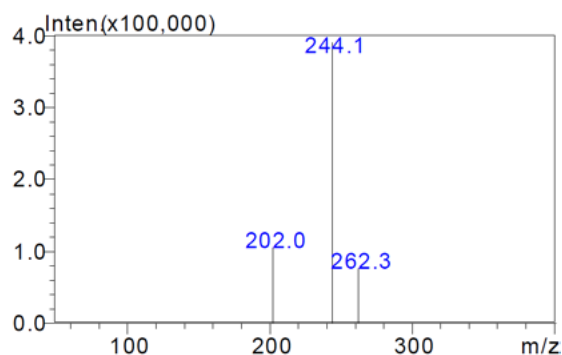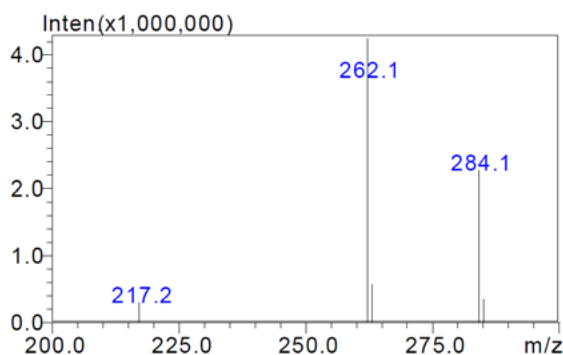


**Figure 12 Q1 MS scan (left) and product ion scan (CE value-35 V, right) of flumequine**

**MRM chromatograms of 12 quinolone standard solutions (1 ng/mL)**

The MRM chromatograms of the quinolone drugs are shown in Figure 13.

**Calibration and linearity**

About 60mL of combined liquid extract was collected after the extraction of 5.0g chicken sample with EDTA-McIlvaine buffer solution. The combined liquid extract went through similar SPE clean-up as described previously and was reconstituted to give 10mL of blank chicken extraction solution. The mixed standard calibration solutions of concentrations 0.2, 0.5, 1, 5, 10, 20 and 50 ng/mL were prepared by diluting appropriate amounts of the standard stock solution (1mg/mL) with the blank chicken extract solution. A calibration curve was plotted showing concentration of working solution against peak area (see Figure 13). The linearity was good and the linear equation and correlation coefficient are shown in Table 3.

**Precision test**

The retention times and peak areas of the mixed standard working solutions at different concentrations (6 replicates for each concentration) were determined to evaluate the precision. The repeatability results of retention time and peak area are shown in Table 4. The results indicate that the relative standard deviations of retention time and peak area of standard samples at different concentrations are 0.03 - 0.27 % and 1.13 - 4.93% respectively, showing good precision.

**Sensitivity test**

To determine the sensitivity, a low concentration (0.2ng/mL) mixed standard antibiotics solution was prepared and analyzed. The signal-to-noise (S/N) ratio, limit of detection (LOD) and limit of quantitation (LOQ) was determined with the use of RMS calculation method and LabSolutions Ver. 5.86. The S/N ratio, LOD and LOQ of the 12 compounds are shown in Table 5.
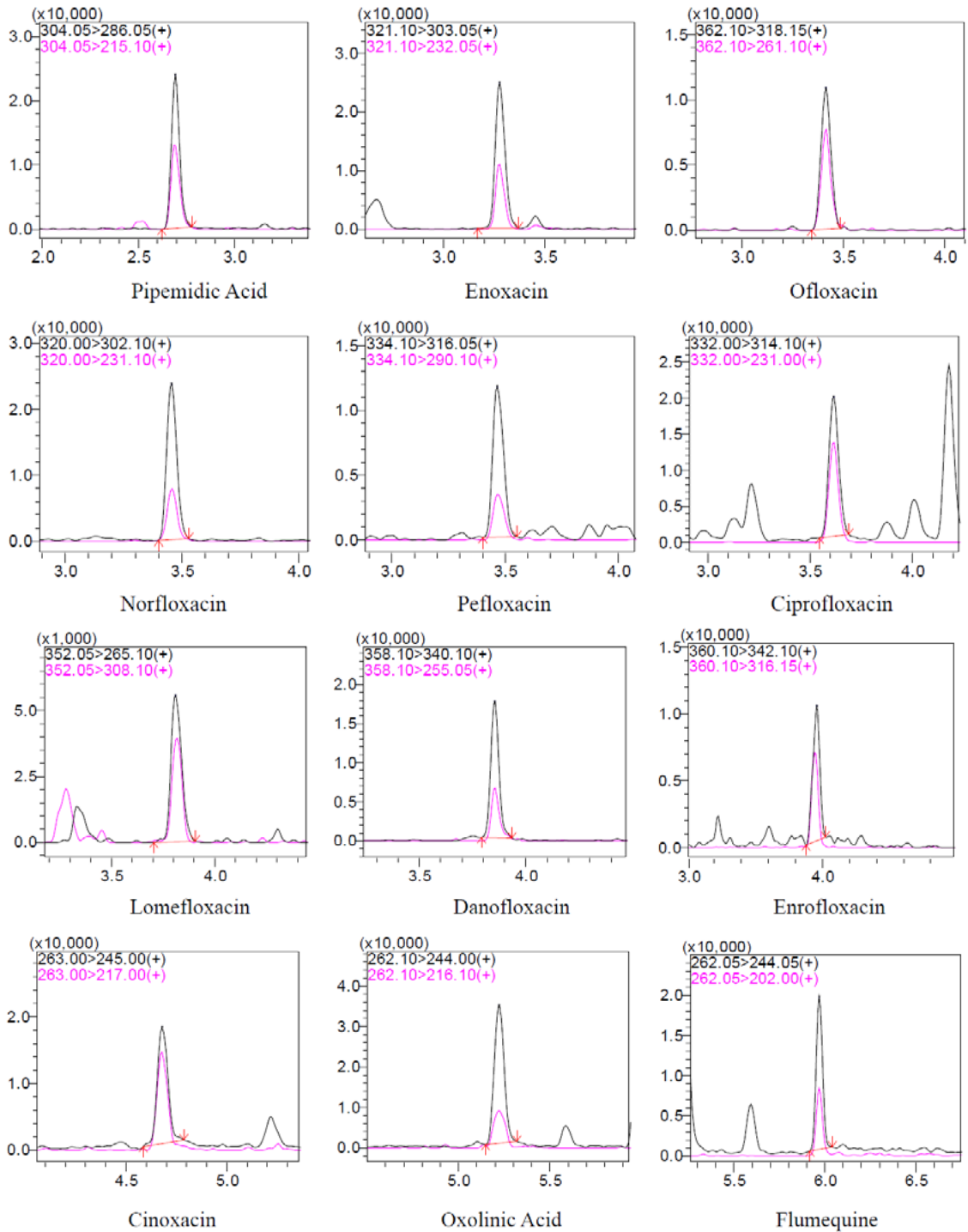
**Figure 13 Standard curves of 12 quinolones**

**Table 3  Calibration curve**

|  | Analyte | Calibration Curve | Linear Range (ng/mL) | Correlation Coefficient (r) | Accuracy (%) |
|---|---|---|---|---|---|
| 1 | Pipemidic Acid | Y = (76616.3) X + (-2517.68) | 0.2-50 | 0.9998 | 96.1-104.1 |
| 2 | Enoxacin | Y = (75235.4) X + (832.686) | 0.2-50 | 0.9996 | 87.9-109.4 |
| 3 | Ofloxacin | Y = (30271.5) X + (-849.705) | 0.2-50 | 0.9998 | 90.9-106.6 |
| 4 | Norfloxacin | Y = (64434.1) X + (1680.13) | 0.2-50 | 0.9988 | 85.7-112.4 |
| 5 | Pefloxacin | Y = (30801.7) X + (579.667) | 0.2-50 | 0.9990 | 89.5-109.8 |
| 6 | Ciprofloxacin | Y = (58040.6) X + (4785.98) | 0.2-50 | 0.9990 | 90.7-114.2 |
| 7 | Lomefloxacin | Y = (19629.0) X + (-749.499) | 0.2-50 | 0.9997 | 89.8-107.5 |
| 8 | Danofloxacin | Y = (44434.3) X + (911.073) | 0.2-50 | 0.9995 | 91.1-110.6 |
| 9 | Enrofloxacin | Y = (23178.6) X + (76.5354) | 0.2-50 | 0.9975 | 87.2-111.3 |
| 10 | Cinoxacin | Y = (69528.6) X + (284.320) | 0.2-50 | 0.9995 | 92.1-111.6 |
| 11 | Oxolinic acid | Y = (124807) X + (4369.16) | 0.2-50 | 0.9999 | 87.5-113.0 |
| 12 | Flumequine | Y = (18436.0) X + (814.851) | 0.2-50 | 0.9987 | 91.2-108.2 |

**Table 4  Repeatability results of retention time and peak area (n=6)**

| Compound Name | RSD% (0.2 ng/mL) | | RSD% (10 ng/mL) | | RSD% (50 ng/mL) | |
|---|---|---|---|---|---|---|
| | R.T. | Area | R.T. | Area | R.T. | Area |
| Pipemidic Acid | 0.23 | 4.93 | 0.21 | 2.54 | 0.24 | 1.85 |
| Enoxacin | 0.16 | 3.73 | 0.18 | 1.21 | 0.19 | 1.79 |
| Ofloxacin | 0.27 | 4.93 | 0.17 | 1.59 | 0.18 | 1.66 |
| Norfloxacin | 0.17 | 4.91 | 0.16 | 1.92 | 0.19 | 1.13 |
| Pefloxacin | 0.16 | 4.77 | 0.17 | 2.06 | 0.19 | 2.01 |
| Ciprofloxacin | 0.07 | 3.88 | 0.15 | 1.34 | 0.18 | 2.29 |
| Lomefloxacin | 0.15 | 3.37 | 0.15 | 1.70 | 0.18 | 1.45 |
| Danofloxacin | 0.17 | 3.23 | 0.15 | 2.61 | 0.16 | 3.74 |
| Enrofloxacin | 0.23 | 4.88 | 0.13 | 3.16 | 0.15 | 1.81 |
| Cinoxacin | 0.10 | 3.32 | 0.11 | 1.40 | 0.10 | 1.34 |
| Oxolinic acid | 0.09 | 2.15 | 0.09 | 1.69 | 0.07 | 1.44 |
| Flumequine | 0.04 | 3.43 | 0.03 | 3.37 | 0.03 | 2.00 |

**Table 5 Signal-to-noise ratio (S/N), LOD and LOQ**

| Compound | Concentration Level (ng/mL) | S/N | Limit of Detection (ng/L) | Limit of Quantitation (ng/L) |
|---|---|---|---|---|
| Pipemidic Acid | 0.20 | 15.43 | 0.04 | 0.13 |
| Enoxacin | 0.20 | 31.11 | 0.02 | 0.07 |
| Ofloxacin | 0.20 | 21.89 | 0.03 | 0.10 |
| Norfloxacin | 0.20 | 39.04 | 0.02 | 0.06 |
| Pefloxacin | 0.20 | 19.21 | 0.04 | 0.12 |
| Ciprofloxacin | 0.20 | 56.02 | 0.01 | 0.04 |
| Lomefloxacin | 0.20 | 39.68 | 0.02 | 0.06 |
| Danofloxacin | 0.20 | 29.48 | 0.02 | 0.07 |
| Enrofloxacin | 0.20 | 13.34 | 0.05 | 0.16 |
| Cinoxacin | 0.20 | 29.57 | 0.07 | 0.21 |
| Oxolinic acid | 0.20 | 44.84 | 0.02 | 0.05 |
| Flumequine | 0.20 | 18.66 | 0.03 | 0.09 |

**Matrix spike test**

A matrix spike using the blank chicken sample was prepared accordingly to give spiked sample at concentrations of 0.5 ng/mL, 10 ng/mL and 40 ng/mL. 3 replicates were tested and the average results are shown in Table 6. The test results indicate that the spike recovery of the 12 samples of antibiotics were 91.90–108.60%.

**CONCLUSION**

A method was established for the determination of quinolone antibiotics in chicken using Shimadzu UHPLC LC-30A coupled with triple quadrupole mass spectrometer LCMS-8045. This method analyzed 12 antibiotics within 9 min, and the correlation coefficients of the calibration curve are all above 0.997. The mixed standard antibiotics solutions at concentrations of 0.2 ng/mL, 10 ng/mL and 50 ng/mL were tested in 6 replicates. The relative standard deviations of retention time and peak area of the 12 target compounds are 0.03-0.27 % and 1.13-4.93% respectively, showing good precision. The chicken sample matrix at spiked concentrations of 0.5 ng/mL, 10 ng/mL and 40 ng/mL were tested in 3 replicates, and the spike recovery was 91.90-108.60%. The described method is fast and ensures high sensitivity and excellent reproducibility. It can be used for the determination of various antibiotic residues in chicken.

Table 6  Results of spike-recovery test

| No. | Compound Name | Spiked Sample Concentration (0.5 ng/mL) | | Spiked Sample Concentration (10 ng/mL) | | Spiked Sample Concentration (40 ng/mL) | |
| --- | --- | --- | --- | --- | --- | --- | --- |
| | | Average (ng/mL) | Recovery (%) | Average (ng/mL) | Recovery (%) | Average (ng/mL) | Recovery (%) |
| 1 | Pipemidic Acid | 0.54 | 108.60 | 10.22 | 102.20 | 39.02 | 97.55 |
| 2 | Enoxacin | 0.50 | 100.23 | 9.72 | 97.23 | 37.34 | 93.38 |
| 3 | Ofloxacin | 0.51 | 101.93 | 9.59 | 95.90 | 38.84 | 97.08 |
| 4 | Norfloxacin | 0.48 | 96.35 | 9.82 | 98.20 | 37.16 | 92.90 |
| 5 | Pefloxacin | 0.47 | 94.08 | 10.01 | 100.08 | 37.37 | 93.43 |
| 6 | Ciprofloxacin | 0.47 | 94.23 | 9.90 | 99.03 | 37.12 | 92.83 |
| 7 | Lomefloxacin | 0.46 | 93.70 | 9.33 | 93.40 | 38.51 | 96.30 |
| 8 | Danofloxacin | 0.47 | 93.05 | 9.72 | 97.20 | 36.77 | 91.90 |
| 9 | Enrofloxacin | 0.48 | 95.85 | 9.86 | 98.63 | 39.70 | 99.25 |
| 10 | Cinoxacin | 0.48 | 95.78 | 9.82 | 98.25 | 37.71 | 94.28 |
| 11 | Oxolinic acid | 0.50 | 100.65 | 10.24 | 102.45 | 39.36 | 98.43 |
| 12 | Flumequine | 0.50 | 100.50 | 10.05 | 100.60 | 39.40 | 98.50 |

# Highly sensitive and rapid analysis of synthetic dyes in sea food by LC/MS/MS

## ASMS 2017   MP 189

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# Introduction

Synthetic dyes like malachite green, crystal violet are used for wide range of industrial applications. However, they are also used in aquaculture due to their anti-bacterial and anti-fungal properties. They are cheap, very effective and readily available, but due to their toxic effects to humans, these dyes are banned in the EU and US regions with zero tolerance policy. [1] [2]

Here, LC/MS/MS method has been developed for quantitation of malachite green, leucomalachite green, crystal violet and leucocrystal violet from sea food sample using LCMS-8045, a triple quadrupole mass spectrometer from Shimadzu Corporation, Japan.
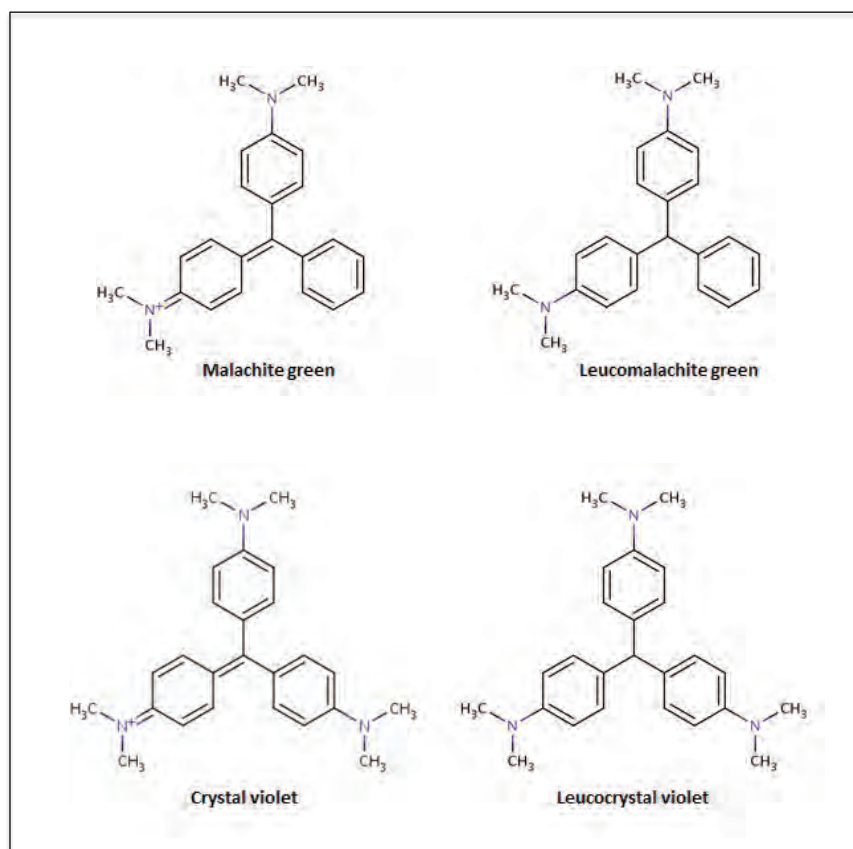


Figure 1.  Structure of synthetic dyes

# Methods and materials

## Sample preparation

Synthetic dye (Figure 1) standards procured from Sigma-Aldrich were used for the analysis. All individual standard stocks were prepared in the acetonitrile. Further mixture of all dyes were prepared in acetonitrile. This stock was serially diluted to prepare calibration levels ranging from 0.05 ppb to 10 ppb in acetonitrile for solvent standard and in matrix for matrix matched standard calibration.

Analysis of these dyes is difficult due to their instability. These synthetic dyes readily undergo oxidation-reduction reaction hence to stabilize them ascorbic acid as an anti-oxidant was added prior to the sample extraction. [3]

For sample analysis, commercially available shrimp sample was purchased and used for analysis. Sample was crushed and transferred to 15 mL centrifuge tube to which 10 mL of acidified acetonitrile and 1 mL of 1M solution of ascorbic acid was added. It was kept on mechanical shaker for 15 min for proper extraction of dyes. Sample were centrifuged for 10 min at 3000 rpm at 4 °C. Then the supernatant layer was transferred to 50 mL tube containing 20 mL McIlvaine's buffer. Phenomenex Strata SCX SPE cartridges were used for sample clean up. Methanol containing 1% triethylamine and 0.5% formic acid was used as an elution solvent for SPE. After clean up, sample were injected on LCMS-8045.



Figure 2. Nexera with LCMS-8045
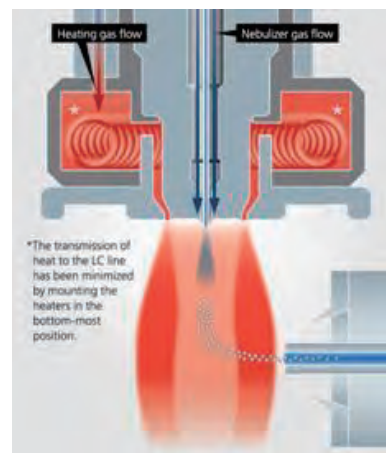


Figure 3. Heated ESI probe

LCMS-8045 triple quadrupole mass spectrometer by Shimadzu (shown in Figure 2), sets a new benchmark in triple quadrupole technology with an unsurpassed sensitivity (UFsensitivity), ultra fast scanning speed of 30,000 u/sec (UFscanning) and polarity switching speed of 5 msec (UFswitching). This system ensures highest quality of data, with very high degree of reliability.

In order to improve ionization efficiency, the newly developed heated ESI probe (shown in Figure 3) combines high-temperature gas with the nebulizer spray, assisting in the desolvation of large droplets and enhancing ionization. This development allows high-sensitivity analysis of a wide range of target compounds with considerable reduction in background.

# LC/MS/MS analysis

Malachite green, leucomalachite green, crystal violet and leucocrystal violet were simultaneously analyzed using Ultra High Performance Liquid Chromatography (UHPLC) Nexera coupled with LCMS-8045 triple quadrupole system (Shimadzu Corporation, Japan). The details of analytical conditions are given in Table 1.

Table 1. Optimized LC/MS/MS conditions for dyes analysis

| | | |
|---|---|---|
| Column | : | Shim-pack GISS (75mm L X 3.0mm I.D, 3 μm) |
| Mobile phase A | : | 2 mM ammonium formate + 0.002 % formic acid in water. |
| | : | B: 2 mM ammonium formate + 0.002 % formic acid in methanol |
| Flow rate | : | 0.4 mL/min |
| Gradient program (B %) | : | 0-0.5 min → 30 (%); 0.5-3 min → 30-95 (%); 3-4 min → 95 (%); 4-4.2 min → 30 (%); 4.2-6.5 min → 30 (%) |
| Injection volume | : | 10 μL |
| Column temperature | : | 40 °C |
| MS interface | : | Electro Spray Ionization (ESI) |
| Nitrogen gas flow | : | Nebulizing gas 3 L/min; Drying gas 10 L/min. |
| Zero air flow | : | Heating gas 10 L/min. |
| MS temperature | : | Desolvation line 150 °C; Heating block 400 °C; Interface 300 °C |

Table 2. MRM transition of synthetic dyes

| Sr.No. | Name of compound | Precursor m/z | Product 1 m/z | Product 2 m/z |
|---|---|---|---|---|
| 1 | Malachite green | 329.25 | 313.15 | 208.10 |
| 2 | Leucomalachite green | 331.20 | 239.10 | 316.20 |
| 3 | Crystal violet | 372.35 | 356.20 | 340.15 |
| 4 | Leucocrystal violet | 374.30 | 358.25 | 239.15 |

# Results

The LC/MS/MS method was developed for trace level quantitation of malachite green, leucomalachite green, crystal violet and leucocrystal violet in marine product. All MRM transitions were optimized with the help of auto optimization feature of LabSolutions.

Analysis was performed using aqueous as well as matrix matched standards. The MRM transition used for these analysis are given in Table 2. Linearity solutions were prepared from 0.05 to 0.5 ppb and acquired using external calibration method and result of linearity are tabulated in

Table 3. Overlay of 0.05 ppb standards are shown in Figure 4. Matrix matched calibration levels were prepared and injected in the same concentration range. A control shrimp sample was extracted as per the same procedure. The Figure 5 shows overlay of chromatograms of blank, control sample and 0.05 ppb matrix matched standard clearly indicates that there no matrix interference. The calibration curve of for all four dyes are shown in Figure 6 and the correlation coefficient >0.99 was obtained for all compounds.

# Highly sensitive and rapid analysis of synthetic dyes in sea food by LC/MS/MS
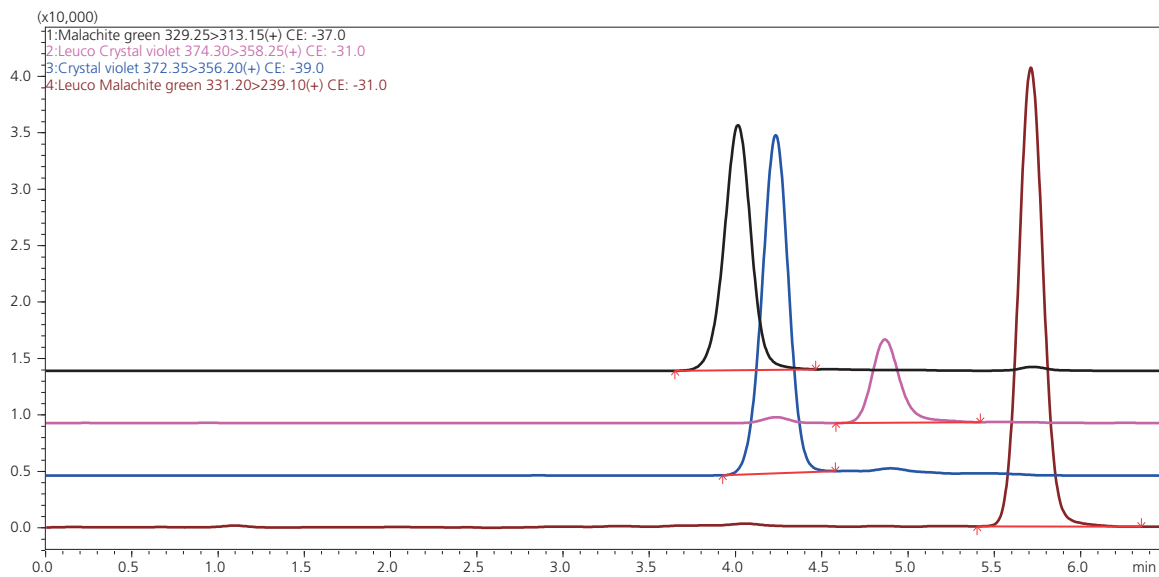


Figure 4.  Chromatogram of solvent standard of 0.05 ppb
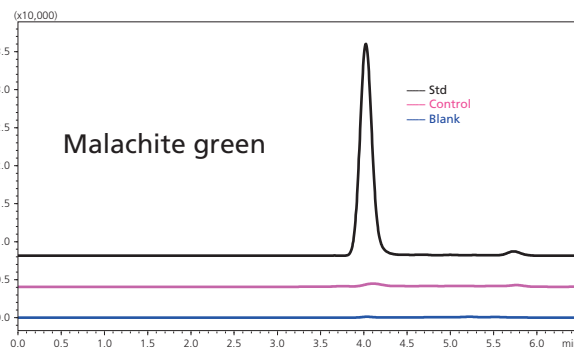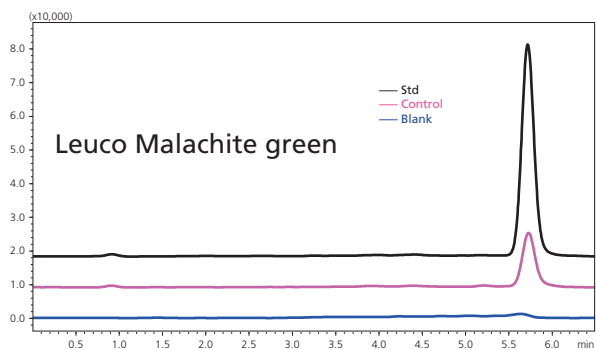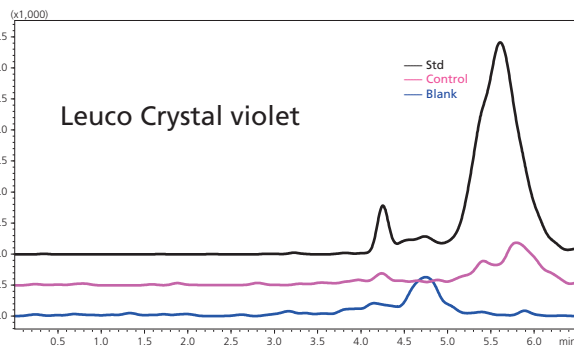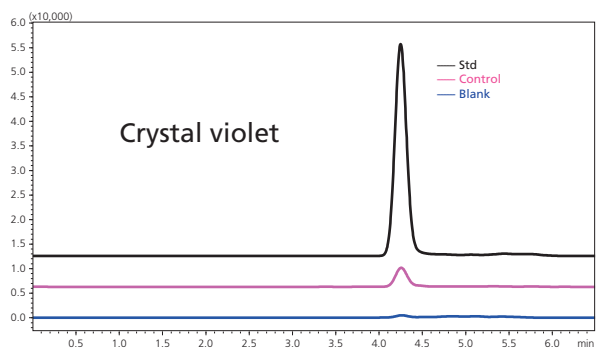


Figure 5.  Overlay of chromatograms of blank, control and 0.05 ppb matrix standard

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# Highly sensitive and rapid analysis of synthetic dyes in sea food by LC/MS/MS

In earlier study it was observed that leuco malachite green and leuco crystal violet metabolite were not stable for long period of time in water or methanol even at -20 °C and oxidizes into non leuco form, hence aprotic solvent is used to prepare standards and at the same time ascorbic acid was used as an antioxidant.

SPE cartridge was used for extraction has strong cation exchange sorbent, since most of the dyes carry positive charge and due to this their retention was better which helps in reduction of matrix interference.
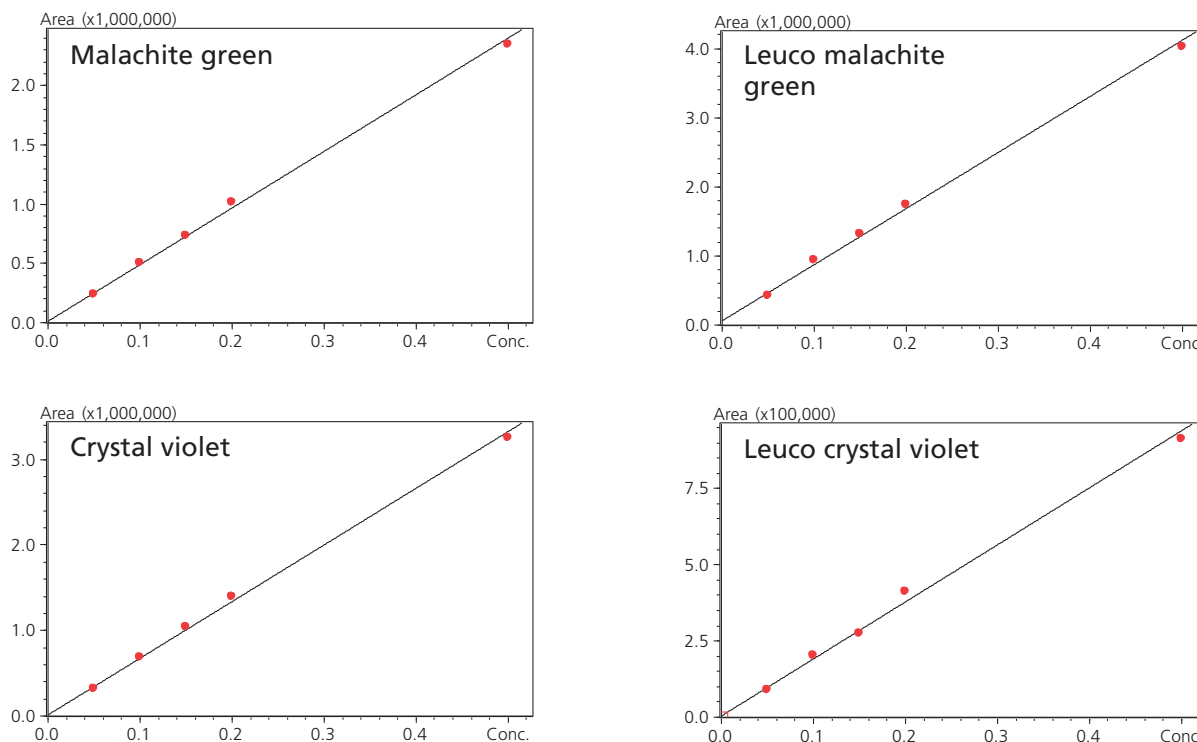


Figure 6.  Calibration curves of dyes

Table 3. Linearity and recovery of dyes

| Sr.No. | Name of compound | Linearity range (ppb) | Correlation coefficient ($r^2$) | % Recovery at 0.05 ppb |
|---|---|---|---|---|
| 1 | Malachite green | 0.05-0.5 | 0.9974 | 80.21 |
| 2 | Leucomalachite green | 0.05-0.5 | 0.9960 | 112.00 |
| 3 | Crystal violet | 0.05-0.5 | 0.9977 | 95.00 |
| 4 | Leucocrystal violet | 0.05-0.5 | 0.9930 | 81.00 |

# Conclusion

- A highly sensitive and rapid method for analysis of crystal violet, leuco crystal violet, malachite green and leuco malachite green was developed.
- Simple SPE method for the determination of malachite green, crystal violet and other synthetic dyes in seafood coupled with LC/MS/MS detection.
- Less matrix interference and sensitive LC/MS/MS instrument enabled lower detection limits and better recovery.

# Reference

[1] A A Bergwerff, P Scherpenisse. J Chromatogr B: Anal Technol Biomed Life Sci. 788: 351-359
[2] Lopez-Gutierrez et al. Anal Methods. 5: 3434–3449, 2013.
[3] J C Hashimoto et al. J AOAC Int. 95: 913–922, 2012.

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# SHIMADZU
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# Application News

No.**C99**

**Liquid Chromatography Mass Spectrometry**

## Quantitative Analysis of Veterinary Drugs Using the Shimadzu LCMS-8050 Triple Quadrupole Mass Spectrometer

Foods in which chemical residues, like pesticides, feed additives, and veterinary drugs found in excess of maximum residue levels have been banned from sale in many countries around the world. Compounds that are subject to residue standards vary widely and the list is expected to grow. Because of this, there is a need for a

highly sensitive and rapid analytical technique to analyze as many of these compounds as possible in a single run. This Application News introduces an example of the high-sensitivity analysis of 89 veterinary drugs in a crude extract of livestock and fishery products.

### ■ Sample Preparation

The typical samples used in the analysis of veterinary drugs contain large amounts of lipids because they are commonly meat and fish samples. Sample preparation is extremely important to ensure excellent sensitivity and repeatability. To avoid the typical time-consuming and laborious solid phase extraction sample preparation procedure, the QuEChERS method, which is typically used for the preparation of vegetables, was selected to simplify sample preparation.

The QuEChERS method normally consists of two steps, the first is an acetonitrile extraction and the second a cleanup step, but this time only the acetonitrile extraction step was used.

\* QuEChERS Extraction Salts kit: Restek Q-sep™ AOAC2007.01

(1) Homogenize 100 g sample (chicken, pork, salmon, shrimp) in food processor

(2) Weigh out 10 g homogenized sample, transfer to 50 mL test tube

(3) Add 5 mL water, shake gently by hand

(4) Add acetonitrile containing 1 % acetic acid and QuEChERS salts\*, shake by hand (1 min)

(5) Centrifuge separation (3 min)

(6) Collect acetonitrile layer and filter

(7) LC/MS/MS analysis

**Fig. 1  Sample Preparation Procedure**

### ■ Improved Peak Shape Using Sample / Water Co-Injection

When conducting reversed phase chromatography, the peaks of polar compounds may split or collapse depending on the relationship between the sample solvent and mobile phase. In cases where the sample solvent is rich in organic solvent, the elution strength must be lowered (by substitution or dilution) with the addition of water. As the pretreated sample solvent in this analysis consists of 100 % acetonitrile, injection in that state into the LC/MS will result in split peaks for some of the substances (Fig. 2 left).

To eliminate as much of the time and effort typically associated with sample preparation, the pretreatment features of the autosampler (SIL-30A) were utilized to conduct co-injection of sample and water, which resulted in improved peak shapes.

Draw Sample    Draw Air    Draw Water    Inject

Diaveridine

7:261.15>123.10 (+)    7:261.15>123.10 (+)

Difloxacin

26:400.10>356.20 (+)    26:400.10>356.20 (+)

Sample 2 µL

Sample 2 µL
+ Water 10 µL

Note: Sample solvent: 100 % Acetonitrile

**Fig. 2  Comparison of Peak Shape**

### ■ MRM Analysis of Matrix Standards

Fig. 3 shows the MRM chromatogram of the matrix standard solution consisting of the sample solution with added standard solution (data obtained using pork extract solution). Table 1 shows the lower limits of quantitation for the standard solution without added matrix and with added matrix, respectively. In a crude extract obtained by acetonitrile extraction alone, sensitivity was comparable to that obtained for most of the compounds using only standard solution. Although there were several compounds for which the lower limit of quantitation was different in the standard solution than the matrix-added solution, rather than attributing this to matrix effects, it is thought to be caused by elevated background due to ions derived from contaminating components (Refer to Fig. 5).



**Fig. 3 MRM Chromatograms of 89 Veterinary Drugs (10 μg/L pork extract solution with added standard solution)**

**Table 1 LOQs of Veterinary Drugs in Neat Standards and Matrix Standards and Calibration Range of Veterinary Drugs in Matrix Standards**

| | Std. Solution | Matrix-Added Std. Solution | | | Std. Solution | Matrix-Added Std. Solution | |
|---|---|---|---|---|---|---|---|
| | Min. Conc. | Min. Conc. | Max. Conc. | | Min. Conc. | Min. Conc. | Max. Conc. |
| Gentamicin | 0.5 | 1 | 50 | Sulfachloropyridazine | 0.02 | 0.02 | 20 |
| Sulfanilamide | 1 | 1 | 50 | Sulfadimethoxine | 0.02 | 0.02 | 10 |
| Levamisole | 0.05 | 0.05 | 50 | Tylosin | 0.05 | 0.05 | 50 |
| Lincomycin | 0.01 | 0.01 | 10 | Sulfamethoxazole | 0.02 | 0.1 | 10 |
| 5-Propylsulfonyl-1-benzimidazole-2-amine | 0.05 | 0.05 | 10 | Sulfaethoxypyridazine | 0.02 | 0.02 | 10 |
| | | | | Tiamulin | 0.01 | 0.01 | 50 |
| Diaveridine | 0.01 | 0.01 | 10 | Florfenicol | 0.5 | 10 | 50 |
| Trimethoprim | 0.02 | 0.02 | 20 | 2Acetylamino 5nitrothiazole | 0.05 | 0.05 | 50 |
| Marbofloxacin | 0.01 | 0.01 | 50 | Sulfatroxazole | 0.01 | 0.01 | 5 |
| Sulfisomidine | 0.02 | 0.02 | 20 | Leucomycin | 0.01 | 0.01 | 50 |
| Norfloxacin | 0.5 | 0.5 | 50 | Sulfisoxazole | 0.01 | 0.05 | 50 |
| Ormetoprim | 0.02 | 0.02 | 10 | Oxolinic acid | 0.01 | 0.1 | 50 |
| Thiabendazole | 0.01 | 0.01 | 10 | Chloramphenicol | 0.5 | 1 | 50 |
| Ciprofloxacin | 0.05 | 0.5 | 10 | Clorsulon | 0.5 | 1 | 50 |
| Neospiramycin I | 0.01 | 0.05 | 10 | Sulfabenzamide | 0.01 | 0.01 | 10 |
| Danofloxacin | 0.1 | 0.1 | 10 | Ethopabate | 0.01 | 0.01 | 10 |
| Enrofloxacin | 0.05 | 0.1 | 50 | Sulfadoxine | 0.02 | 0.02 | 20 |
| Oxytetracycline | 0.01 | 0.1 | 50 | Sulfaquinoxaline | 0.02 | 0.02 | 10 |
| Xylazine | 0.01 | 0.01 | 10 | Prednisolone | 0.1 | 0.05 | 20 |
| Orbifloxacin | 0.05 | 0.05 | 50 | Ofloxacin | 0.5 | 0.5 | 50 |
| Sulfacetamide | 1 | 1 | 50 | Flubendazole | 0.01 | 0.01 | 50 |
| Clenbuterol | 0.01 | 0.01 | 10 | Methylprednisolone | 0.5 | 0.5 | 50 |
| Tetracycline | 0.05 | 0.01 | 50 | Nalidixic acid | 0.01 | 0.01 | 50 |
| Spiramycin I | 0.01 | 0.01 | 50 | Dexamethasone | 0.5 | 0.5 | 50 |
| Sarafloxacin | 0.5 | 0.5 | 50 | Flumequine | 0.01 | 0.01 | 50 |
| Difloxacin | 0.05 | 0.1 | 50 | Benzylpenicillin | 0.5 | 0.5 | 50 |
| Sulfadiazine | 0.02 | 0.1 | 20 | Sulfanitran | 0.2 | 0.2 | 50 |
| Sulfathiazole | 0.02 | 0.1 | 20 | Sulfabromomethazine | 0.01 | 0.01 | 50 |
| Sulfapyridine | 0.02 | 0.1 | 20 | betaTrenbolone | 0.02 | 0.1 | 50 |
| Carbadox | 0.05 | 0.05 | 10 | Emamectin B1a | 0.01 | 0.01 | 50 |
| Pyrimethamine | 0.02 | 0.02 | 20 | alphaTrenbolone | 0.02 | 0.1 | 50 |
| Sulfamerazine | 0.02 | 0.02 | 20 | Piromidic acid | 0.01 | 0.05 | 50 |
| Chlortetracycline | 0.1 | 0.1 | 50 | Zeranol | 1 | 0.1 | 50 |
| Tilmicosin | 0.1 | 0.1 | 50 | Ketoprofen | 0.01 | 0.05 | 50 |
| Thiamphenicol | 1 | 1 | 50 | Testosterone | 0.01 | 0.05 | 10 |
| Sulfadimidine | 0.02 | 0.02 | 20 | Famphur | 0.05 | 0.05 | 50 |
| Sulfametoxydiazine | 0.01 | 0.02 | 10 | Fenobucarb (BPMC) | 0.01 | 0.01 | 50 |
| Sulfamethoxypyridazine | 0.02 | 0.02 | 20 | Clostebol | 0.05 | 0.05 | 50 |
| Sulfisozole | 0.01 | 0.01 | 50 | Dichlofenac | 0.01 | 0.01 | 50 |
| Trichlorfon (DEP) | 0.05 | 0.05 | 50 | Melengestrol Acetate | 0.05 | 0.05 | 50 |
| Sulfamonomethoxine | 0.02 | 0.02 | 20 | Temephos (Abate) | 0.01 | 0.5 | 50 |
| Furazolidone | 1 | 1 | 50 | Allethrin | 0.1 | 1 | 50 |
| Difurazone | 0.05 | 0.05 | 50 | Closantel | 0.01 | 0.01 | 10 |
| Erythromycin A | 0.01 | 0.01 | 50 | Monensin | 0.01 | 0.01 | 10 |
| Cefazolin | 0.5 | 0.5 | 50 | | | | |

(Unit: μg/L)

Fig. 4  MRM Chromatograms in the Vicinity of the LOQ and Calibration Curves of Typical Compounds

■ **Recoveries of Veterinary Drugs in Crude Extracts from Livestock and Fishery Products (Matrix Effect Verification)**

We examined whether or not the matrix affected measurement of actual samples. This time, four types of food product samples were used, including shrimp, chicken meat, pork, and salmon. Standard solution was added to the acetonitrile extraction solution of each of these to obtain a final concentration of 10 μg/L, after which the rates of recovery were determined. The results indicated that 90 % of the compounds were recovered at rates of 70 to 120 % and measurement was accomplished without any adverse matrix effects even though the crude extract solution was subjected only to acetonitrile extraction.



Fig. 5  Recoveries of Veterinary Drugs in Each of the Matrices

## ■ Acetonitrile Extraction Efficiency Using QuEChERS Method

To check the efficiency of acetonitrile extraction by the QuEChERS method, standard solution was added at stage (2) of Fig. 1 to obtain a concentration of 10 µg/L, and the recoveries were determined. Good recoveries of approximately 80 % were obtained in cases both with and without the addition of matrix. However, relatively poor recoveries were seen for highly polar compounds such as tetracycline and quinolone. For these compounds, it is necessary to examine the use of a separate extraction solvent and extraction reagent.

### Table 2  Recoveries (Pre-Spike)

| Recovery | Without Matrix | With Matrix (Pork) | Compounds with Poor Recovery |
|---|---|---|---|
| < 50 % | 17 (19 %) | 13 (15 %) | Tetracyclines Quinolones |
| 50 % - 70 % | 1 (1 %) | 8 (9 %) | |
| > 70 % | 71 (80 %) | 68 (76 %) | |

## ■ Robustness

We checked the long-term stability of the instrument using a solution of pork crude extract (spiked with 10 µg/L standard solution). Even after continuous measurement of an extremely complex matrix over a period of 3 days, we were able to obtain stable data.



| Compounds | %RSD (%) (n=220) |
|---|---|
| Sulfaquinoxaline | 1.5 |
| Sulfamethoxazole | 2.8 |
| Ketoprofen | 2.3 |
| $\beta$ - trenbolone | 3.2 |
| Teststerone | 3.5 |

**Fig. 6  Area Plot and %RSD of Typical Compounds with Continuous Analysis**

### Table 3  Analytical Conditions

| | |
|---|---|
| Column | : Shim-pack XR-ODS II (75 mm × 2.0 mm I.D., 2.2 µm) |
| Mobile Phase A | : 0.1 % Formic Acid - Water |
| Mobile Phase B | : Acetonitrile |
| Time Program | : 1 %B (0 min) → 15 %B (1 min) → 40 %B (6 min) → 100 %B (10-13 min) → 1 %B (13.01-16 min) |
| Flowrate | : 0.2 mL/min. |
| Injection Volume | : 2 µL (2 µL sample solution + 10 µL water) |
| Oven Temperature | : 40 °C |
| Ionization Mode | : ESI (Positive / Negative) |
| Probe Voltage | : +2.0 kV / -1.0 kV |
| Neburizing Gas Flow | : 3.0 L/min. |
| Drying Gas Flow | : 10.0 L/min. |
| Heating Gas Flow | : 10.0 L/min. |
| Interface Temperature | : 400 °C |
| DL Temperature | : 200 °C |
| Block Heater Temperature | : 400 °C |

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# Vegetables, Fruits and Spices

1. A High Sensitivity LC/MS/MS Method with QuEChERS Sample Pre-treatment for Analysis of Aflatoxins in Peanut Butter Samples

2. Determination of Phthalates in Vegetables by GPC–GCMS

3. Taking Advantage of the Contaminant Library

4. Efficient Analysis of Residual Pesticides in Foods Using High-Sensitivity GC-MS/MS

5. Quantitative Analysis of Highly Polar Pesticides in Food Using SFC/MS

6. Improved Detection of Pesticide Residues in Botanicals by LCMS

7. Fast GC-MS/MS Analysis Of Multicomponent Pesticide Residues (360) In Food Matrix

8. Multi-Residue Analysis of 18 Regulated Mycotoxins by LC/MS/MS

9. Multi-residue Analysis of 18 Regulated Mycotoxins by LC-MS/MS (2)

10. Multi-Residue Analysis of 210 Pesticides in Food Samples by Triple Quadrupole UHPLC-MS/MS

11. Expanding Capabilities in Multi-Residue Pesticide Analysis Using The LCMS-8060

12. Analysis of Nivalenol, Deoxynivalenol, 3-Acetyldeoxynivalenol and 15-Acetyldeoxynivalenol Using Triple Quadrupole LC/MS/MS (LCMS-8050)

13. Rapid analysis of polychlorinated biphenyls (PCBs) in vegetables by QuEChERS-based extraction and GPC–GCMS

14. Using the Nexera UC Online SFE-SFC-MS System to Analyze Residual Pesticides in Agricultural Products

15. Applying 'MRM Spectrum Mode' and Library Searching for Enhanced Reporting Confidence in Routine Pesticide Residue Analysis

16. Residual Pesticides Analysis of Botanical Ingredients Using Gas Chromatography Triple Quadrupole Mass Spectrometry

17. Expanding Capabilities in Multi-Residue Pesticide Analysis Using The LCMS-8060

18. Highly Polar Pesticide Multi-Residue Analysis in Food Safety by LC-MS/MS

19. Simultaneous Analysis of 16 Sweeteners Using Triple Quadrupole LC/MS/MS [LCMS-8050]

20. Simultaneous Analysis of Nine Sweeteners Using Triple Quadrupole LC/MS/MS (LCMS-8040)

21. Determination of Avermectin Drug Residues in Vinegar Using LCMS-8045

22. A Sensitive and Repeatable Method for Characterization of Sulfonamides and Trimethoprim in Honey using QuEChERS Extracts with LC-MS/MS

# A High Sensitivity LC/MS/MS Method with QuEChERS Sample Pre-treatment for Analysis of Aflatoxins in Peanut Butter Samples

## ASMS 2015 WP074

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A High Sensitivity LC/MS/MS Method with QuEChERS
Sample  Pre-treatment for Analysis of Aflatoxins in
Peanut Butter Samples

# Introduction

Aflatoxins are metabolites produced by fungi (Aspergillus flavus and Aspergillus parasiticus) in high humidity environment in crops such as maize nuts and processed food. Aflatoxin contamination in food is monitored with strict regulations worldwide due to high toxicity of the compounds [1]. Recently, several media reports revealed the exceed levels of aflatoxins found in peanut butters in the USA and Taiwan. Aflatoxins in food have been

analyzed by LC/MS/MS using various sample pre-treatment methods. We describe a high sensitivity LC/MS/MS method for quantitative analysis of aflatoxins in peanut butters using QuEChERS sample pre-treatment procedure [2], as opposed to the use of immunoaffinity column or other methods for sample pre-treatment which are more expensive and tedious. High sensitivity and good recoveries were achieved using this LC/MS/MS method.

# Experimental

A mixed standard of aflatoxin B1, B2, G2 and G2 was obtained from Supelco. A stock solution was prepared using methanol as the diluent, from which calibrant series and spiked samples were prepared. The QuEChERS kits were purchased from RESTEK. Two grams of peanut butter was first extracted with the extraction kits followed by cleaning up using dSPE tubes. The procedure was adjusted and optimized to obtain highest recovery. A

LCMS-8050 triple quadrupole LC/MS/MS (Shimadzu Corporation, Japan) was used in this work. A C18 column (Kinetex, 2.1 x 100mm, 1.7u) was used for fast separation of aflatoxins using a gradient elution program. The method development and performance evaluation were carried out using spiked aflatoxins in peanut butter samples. Table 1 shows the analytical conditions on LCMS-8050.

Table 1:  LC/MS/MS analytical conditions of aflatoxins on LCMS-8050

| | |
|---|---|
| Column | Kinetex C18 (2.1mmI.D x 100mmI.D, 1.7µm) |
| Flow rate | 0.5 mL/min |
| Mobile phase | A: 5mM ammonium acetate in water with 0.1% FA |
| | B: 5mM ammonium acetate in MeOH |
| Oven temp. | 40 °C |
| Injection vol. | 5 µL |
| Elution mode | Gradient elution, B%: 5% (0 to 0.5 min) $\rightarrow$ 50% (4 to 5.5 min) |
| | $\rightarrow$ 85% (6 to 7.5 min) $\rightarrow$ 5% (8.1 to 10 min) |
| Interface | ESI |
| MS mode | Positive, MRM, 2 transitions for each compound |
| Interface temp. | 350 °C |
| Block temp. | 400 °C |
| DL temp. | 250 °C |
| CID gas | Ar (350 kPa) |
| Nebulizing gas flow | 3.0 mL/min |
| Drying gas flow | 10.0 L/min |
| Heating gas flow | 10.0 L/min |

# A High Sensitivity LC/MS/MS Method with QuEChERS Sample Pre-treatment for Analysis of Aflatoxins in Peanut Butter Samples

# Results and Discussion

## QuEChERS sample pre-treatment

Hexane was used in the procedure to remove fats, oils and non-polar components from the peanut butter samples. The extraction step was completed using Q-sep QuEChERS extraction salt packet (4 g MgSO4, 1 g NaCl, 1 g trisodium citrate dehydrate, 0.5 g disodium hydrogen citrate). Dispersive SPE tube containing MgSO4, PSA and C18 was used in the clean-up process to remove remaining water, organic acid and non-polar components respectively. The process of the sample preparation is illustrated in Figure 1.

## Method Development

Automated MRM optimisation of aflatoxins was carried out using the LabSolutions workstation. The precursors of aflatoxins B1, B2, G1 and G2 were their protonated ions (m/z313.1, m/z315.1, m/z329.1 and m/z331.1, respectively). Two MRM transitions of every aflatoxin were chosen as quantifier and confirmation ion (Table 2). A peanut butter matrix free from aflatoxins was used as a "blank" and matrix for the preparation of post-spiked calibrants to build calibration curves. The blank and every post-spiked calibrant was injected thrice and the average area was calculated to obtain reliable results. A chromatogram of spiked sample is shown in Figure 2. Linear calibration curves were obtained for all four aflatoxin compounds. Good linearity with correlation coefficient ($r2$) greater than 0.999 across the range of 10 pg/mL - 10 ng/mL was obtained. The calibration curves of aflatoxins spiked in peanut butter matrix are shown in Figure 3.

Weigh 2 g of peanut butter sample into a 50 mL centrifuge tube

Add 15 mL of hexane, ACN:$H_2O$ at ration of 86:14 with total FA concentration of 0.1%

Shake and vortex for 20 minutes

Add the Q-sep Extraction salt

Shake and vortex for 20 minutes and centrifuge at 6000 rpm for 40 minutes

Discard the hexane layer

Transfer the solution into a 20 mL flask

Add 4 mL of ACN into centrifuge tube to wash the extraction salt, vortex and centrifuge

Combine the washing solution and the solution in the volumetric flask

Filter the solution using a PTFE filter with pore size of 0.2 μm

Transfer 1mL solution into a 2mL dSPE tube

Vortex for 1 minute and centrifuge at 13000 rpm for 15 minutes

Transfer the supernatant into a vial for injection into LCMS-8050

Figure 1: Flow chart of sample pretreatment for aflatoxins in peanut butter by modified QuEChERS method.

Table 2: LC/MS/MS analytical conditions of LCMS-8050 for aflatoxins

| Compound | MRM (m/z) | CID Voltage (V) | | |
|---|---|---|---|---|
| | | Q1 | CE | Q3 |
| Aflatoxin B1 | 313.1>241.0* | -15 | -37 | -26 |
| | 313.1>213.0 | -15 | -46 | -22 |
| Aflatoxin B2 | 315.1>259.1* | -15 | -30 | -28 |
| | 315.1>287.0 | -15 | -27 | -20 |
| Aflatoxin G1 | 329.1>243.0* | -30 | -27 | -27 |
| | 329.1>200.0 | -30 | -42 | -21 |
| Aflatoxin G2 | 331.1>245.0* | -16 | -33 | -25 |
| | 331.1>189.0 | -16 | -42 | -19 |

*MRM transitions used as quantifiers.

# A High Sensitivity LC/MS/MS Method with QuEChERS Sample Pre-treatment for Analysis of Aflatoxins in Peanut Butter Samples
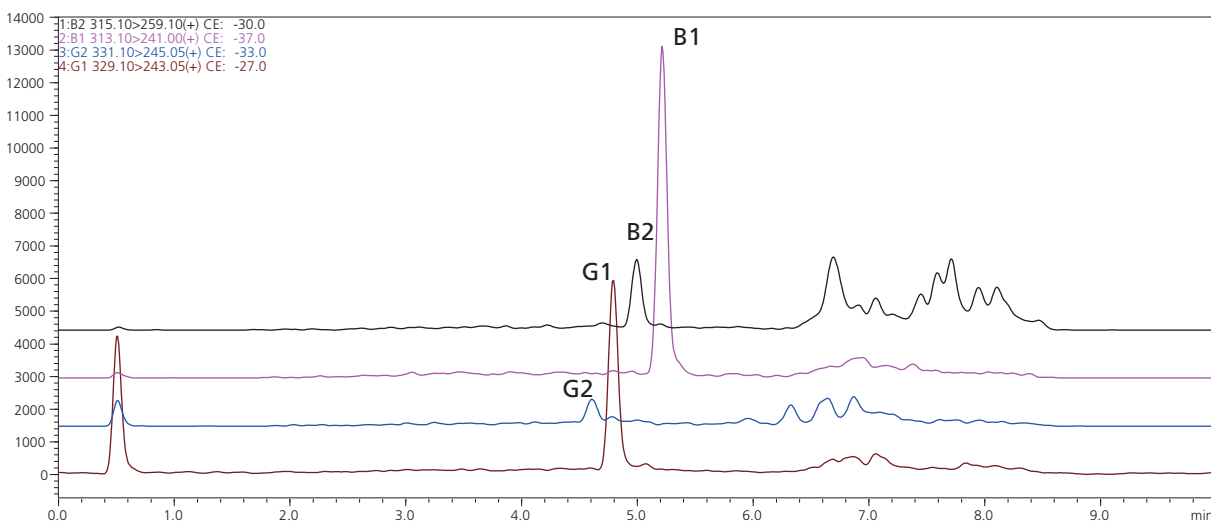


Figure 2:  MRM chromatograms of aflatoxins spiked in peanut butter sample
(B1 and G1 at concentration of 100 pg/mL; B2 and G2 at concentration of 30 pg/mL).
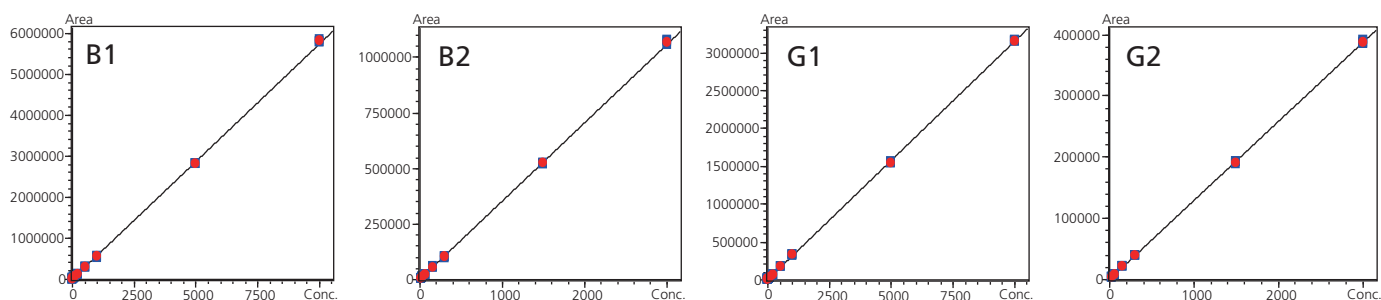


Figure 3:  Calibration curves of aflatoxins B1, B2, G1 and G2 in peanut butter matrix.

Table 3:  LOD, LOQ and repeatability of aflatoxin spiked samples at different concentrations

| Compound | Concentration range (pg/mL) | %RSD (n=6) | | | | | | LOQ | LOD | r2 |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 15 ppt | 30 ppt | 50 ppt | 60 ppt | 100 ppt | 200 ppt | | | |
| B1 | 10 - 10000 | | | 2.71 | | 2.22 | 1.20 | 4.6 | 1.5 | 0.9995 |
| B2 | 11 - 3000 | 7.73 | 7.51 | | 2.96 | | | 8.7 | 2.9 | 0.9997 |
| G1 | 10 - 10000 | | | 2.21 | | 1.82 | 1.21 | 4.2 | 1.4 | 0.9995 |
| G2 | 30 - 3000 | | 12.12 | | 9.10 | | | 22.4 | 7.4 | 0.9995 |

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A High Sensitivity LC/MS/MS Method with QuEChERS
Sample  Pre-treatment for Analysis of Aflatoxins in
Peanut Butter Samples

## Method Performance Evaluation

As shown in Table 4, the LOD and LOQ of aflatoxins in peanut butter matrix are lower than 7.4 pg/mL and 22.4 pg/mL respectively. The repeatability of the method was evaluated using using spiked samples at lower concentrations. The peak area %RSD of aflatoxins were found to be lower than 7.5% except for G2 with %RSD of 12.1%.

The matrix effect was evaluated by using the average of spiked samples injected thrice at different concentrations. The recoveries of aflatoxins were determined by using a duplicate set of samples at different concentrations. Each duplicate was obtained from the average of three injections. The results are shown in Table 4.

Table 4:  Matrix effects of the MRM method for aflatoxins in spiked peanut butter samples

| Concentration (pg/mL) | Matrix effect (%) | | Concentration (pg/mL) | Matrix effect (%) | |
|---|---|---|---|---|---|
| | B1 | G1 | | B2 | G2 |
| 50 | 78.35 | 80.63 | 60 | 72.31 | 70.14 |
| 100 | 71.88 | 71.24 | 150 | 73.81 | 74.72 |

Table 5:  Recoveries of aflatoxins in spiked peanut butter samples

| Compound | Recovery (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 30 pg/mL | | 50 pg/mL | | 60 pg/mL | | 200 pg/mL | |
| | Dup 1 | Dup 2 | Dup 1 | Dup 2 | Dup 1 | Dup 2 | Dup 1 | Dup 2 |
| B1 | | | 70.24 | 74.00 | | | 81.52 | 80.49 |
| B2 | 65.22 | 71.20 | | | 87.40 | 85.12 | | |
| G1 | | | 90.92 | 95.11 | | | 77.47 | 79.38 |
| G2 | 79.97 | 79.58 | | | 87.76 | 86.52 | | |

## Analysis of aflatoxins in actual samples

Three peanut butter samples from local supermarket were analysed using the established MRM method. The results showed that aflatoxins were found in two of the samples (Table 6). While the aflatoxins in sample M is within the

EU regulatory limits (sum of aflatoxins below 4 µg/kg), the aflatoxin B1 amount in sample UL exceeds the allowed concentration (aflatoxin B1 below 2 µg/kg).
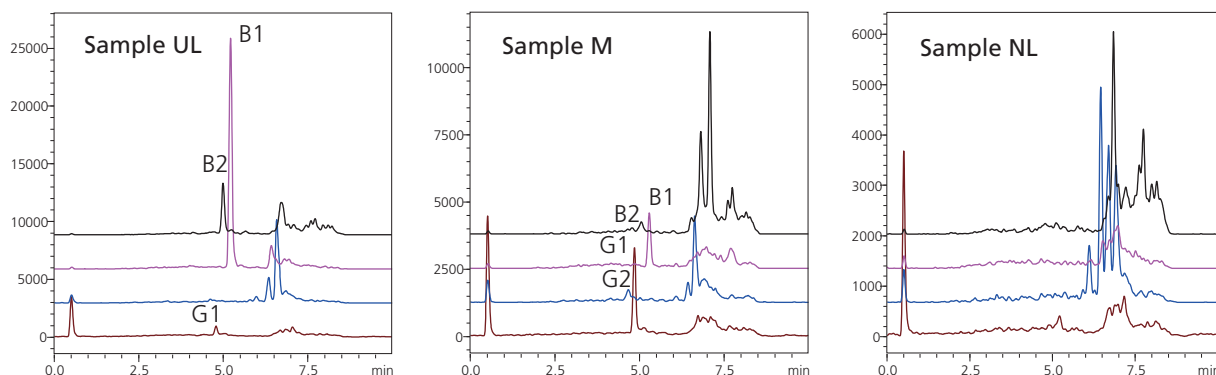


Figure 4:  Chromatograms of peanut butter samples.

5

Table 6:  The amount of aflatoxins found in peanut butter samples from supermarket

| Sample | Concentration (ng/g) | | | |
|---|---|---|---|---|
| | B1 | B1 | G1 | G2 |
| UL | 2.09 | 0.79 | 0.03 | Not detected |
| M | 0.16 | 0.06 | 0.50 | 0.18 |
| NL | Not detected | Not detected | Not detected | Not detected |

# Conclusions

A high sensitivity LC/MS/MS method with QuEChERS for sample pre-treatment was established using Shimadzu LCMS-8050 system. The QuEChERS sample preparation method was proven effective and easy to operate. The method performance including sensitivity, linearity, repeatability, matrix effect and recovery were carried out and the results confirm that the method is feasible and reliable for determination of aflatoxins in peanut butter samples.

# References

(1) Pereira, V.; Fernandes, J.; Cunha, S. *Trends in Food Science & Technology* 2014, 36, 96-136.
(2) Liu, Y.; Han, S.; Lu, M.;Wang, P.; Han, J.;Wang, J. *Journal of Chromatography B* 2014, 970, 68-76.

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# Determination of Phthalates in vegetables by GPC–GCMS

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## Determination of Phthalates in vegetables by GPC–GCMS

# Introduction

Phthalates (PAEs) are a class of compounds which can be added to plastics to increase its flexibility, transparency, durability and longevity. They can be used in electronics industry, agriculture adjuvant, building materials, toys, food packaging materials and textiles etc. Because of its medium viscosity, high stability, low volatility, easily accessible, low cost and other features, they are currently the most widely used plasticizer.

In 2011 PAEs events broke out in Taiwan drinks, and in 2012 the same thing happened to a certain brand of liquor. And recently it was reported that "vegetables wrapped in tape" in the supermarkets may contain PAEs. And this caused more and more consumers pay great attention to the PAEs.

PAEs were classified as one kind of suspected environmental hormone. Their toxicity is mainly estrogen and anti-androgen activity which can cause endocrine disorder and reproductive function hinder in the organism. Therefore, PAEs had been restricted used in the relevant national standards such as drinking water, toys, packaging materials and food etc.

In this report a method was developed using Shimadzu's GPC-GCMS to determine 22 kinds of PAEs in vegetables. This method is sensitive, easy to operate and can be applied to quickly detect PAEs in vegetables.

# Experimental

**Instrument:**

Shimadzu GPC-GCMS



Figure 1   Shimadzu GPC-GCMS

# Determination of Phthalates in vegetables by GPC–GCMS

## Experimental conditions:

### GPC conditions:

| | |
|---|---|
| Chromatographic column | : Shodex CLNpak EV-200 (2.1 mm×150 mm) |
| Mobile phase | : acetone/cyclohexane (3/7, V/V) |
| Flow rate | : 0.1 mL/min |
| Column temperature | : 40ºC |
| Sample size | : 20 µL |

### GCMS conditions:

| | |
|---|---|
| Chromatographic column | : inert quartz tube: 5 m×0.53 mm |
| Precolumn | : WondaCap WAX, 5m×0.25 mm×0.25 µm |
| Analytical column | : WondaCap WAX, 25m×0.25 mm×0.25 µm |
| Column oven temperature | : 82ºC(5min)_8ºC/min_150ºC(0min)_25ºC/min_240ºC(5min) |
| Injection temperature | : 120ºC(5min)_100ºC/min_280ºC(15.8min) |
| Pressure | : 120kPa(0min)_100kPa/min_180kPa(4.4min)_(-49.8 kPa/min)_120kPa (15.9min) |
| Purge flow | : 5.0mL/min_(-10mL/min)_0 mL/min(6min)_10mL/min_5mL/min (15.1min) |
| Sampling time | : 7min |
| Solvent cut time | : 9.7min |
| Interface temperature | : 250ºC |
| Ion source temperature | : 200ºC |
| Acquisition mode | : SIM, acquisition conditions are shown in Table 1 |

## Sample pretreatment:

Weigh accurately 1.0 g grinded vegetables into 25 mL centrifuge tube, add 5 mL Water, mix and exact for 30 min. Then add 2 mL Hexane, mix and vortex for 3 min, then take the supernatant fluid for sample analysis.
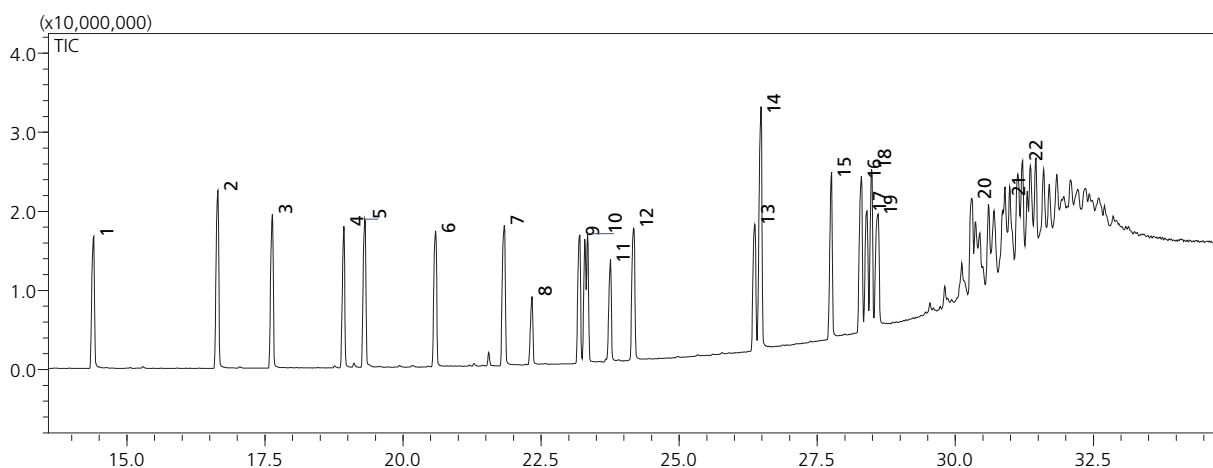


Figure 2   Total Ion Chromatogram of standard sample (1.0 mg/L)

Table 1 Characteristic fragment ions of PAEs(m/z)

| No. | Compound name | CAS | R.T. | Target Ion | Ref. Ion 1 | Ref. Ion 2 |
|-----|---------------|-----|------|-----------|-----------|-----------|
| 1 | DMP | 131-11-3 | 14.400 | 163 | 133 | 194 |
| 2 | DEP | 84-66-2 | 16.642 | 149 | 177 | 176 |
| 3 | DIPRP | 605-45-8 | 17.633 | 149 | 209 | 150 |
| 4 | DAP | 131-17-9 | 18.925 | 149 | 104 | 189 |
| 5 | DPRP | 131-16-8 | 19.308 | 149 | 209 | 191 |
| 6 | DIBP | 84-69-5 | 20.583 | 149 | 167 | 205 |
| 7 | DBP | 84-74-2 | 21.833 | 149 | 205 | 223 |
| 8 | DMEP | 117-82-8 | 22.333 | 149 | 104 | 176 |
| 9 | DIPP | 605-50-5 | 23.192 | 149 | 219 | 237 |
| 10 | BMPP | 146-50-9 | 23.342 | 167 | 149 | 251 |
| 11 | DEEP | 605-54-9 | 23.750 | 149 | 104 | 176 |
| 12 | DPP | 131-18-0 | 24.175 | 149 | 219 | 237 |
| 13 | DHXP | 84-75-3 | 26.367 | 149 | 233 | 251 |
| 14 | BBP | 85-68-7 | 26.475 | 149 | 91 | 206 |
| 15 | DBEP | 117-83-9 | 27.750 | 149 | 101 | 193 |
| 16 | DCHP | 84-61-7 | 28.292 | 149 | 167 | 249 |
| 17 | DHP | 3648-21-3 | 28.392 | 265 | 149 | 247 |
| 18 | DEHP | 117-81-7 | 28.483 | 279 | 149 | 167 |
| 19 | DPHP | 84-62-8 | 28.592 | 225 | 104 | 153 |
| 20 | DNOP | 117-84-0 | 30.292 | 149 | 261 | 279 |
| 21 | DINP | 68515-48-0 | 30.908 | 293 | 149 | 127 |
| 22 | DIDP | 26761-40-0 | 31.217 | 307 | 149 | 141 |

# Result

## Calibration curve & Repeatability

Dilute the standard stock solution into 0.005, 0.05, 0.1, 0.5, 1.0 µg/mL. Some of the calibration curves obtained are shown in Figure 2. The correlation coefficients are >0.999, the RSD% of 7 consecutive tests of 0.05 µg/mL standard samples are less than 5 % and the detection limit calculated according to the data of 0.005 µg/mL standard sample (3 S/N) are below 3 µg/mL.

## Sample & recovery results

Add PAEs standard (0.1, 0.2, and 0.4 mg/kg) into sample (lettuce, canola and celery) before sample pretreatment in accordance with the processing steps and calculate recovery rate. The results were between 60 % ~ 130 %.
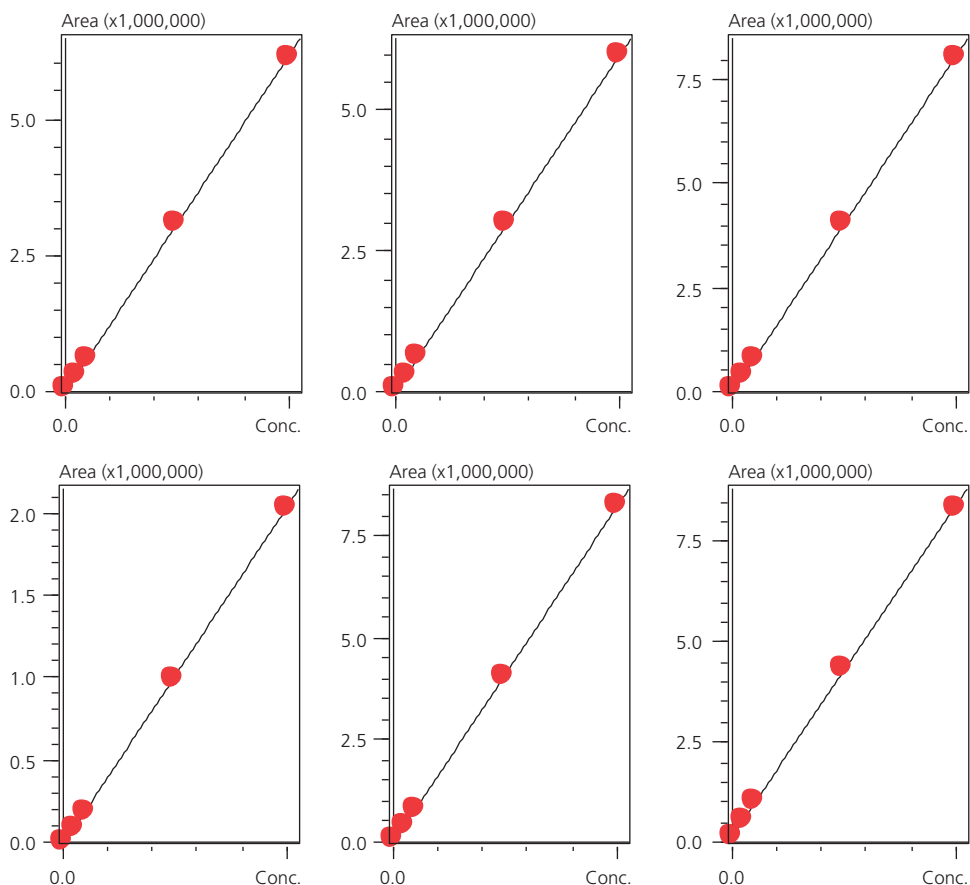
Figure 3   Calibration curve of some compounds

# Conclusions

A quick, easy and reliable method for determination PAEs in vegetables by Shimadzu's GPC-GCMS is developed. This method is sensitive, easy to operate and can be applied to quickly detect PAEs in vegetables.

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# Application News

## No. A535

**Spectrophotometric Analysis**

# Taking Advantage of the Contaminant Library

Accuracy and speed are necessary when determining the cause of contaminants. There are many causes of contamination that lead to complaints, such as contamination originating from a company's own production line to consumers inadvertently introducing contaminants themselves, and therefore contaminant analysis requires considerable knowledge and experience.

This article introduces an example of contaminant analysis using a contaminant library that consolidates Shimadzu's experience and analysis know-how.

S. Iwasaki

## ■ Contaminant Library Overview

The contaminant library can be used in the same way as the standard library by adding it as an option to the LabSolutions IR software, which performs instrument control and data analysis on Shimadzu's FTIR instruments. Unlike commercially-available libraries that only contain data on single components, this contaminant library contains as many as 485 entries including mixtures such as actually collected contaminants (provided by water supply and food companies) and gaskets, and therefore achieves a remarkably improved search accuracy. Furthermore, while conventional contaminant libraries have only provided notation of component names, which made identifying the source of contaminants difficult, this library employs notation that allows inference of contamination sources.

In addition to infrared spectra obtained through measurement using a single reflection ATR attachment, the contaminant library contains detailed information including sample images, major elements, color, shape, hardness, and metallic luster. Since all included samples have also undergone EDX analysis, the corresponding EDX profile database can be viewed as a PDF file for samples that appear in the contaminant library search results. This contains the qualitative profile and quantitative analysis results.

## ■ Example of Contaminant Analysis

FTIR analysis was performed on contaminant 1 detected on a production line. Table 1 lists the instruments and analysis conditions. Fig. 1 shows the measurement result and search result from the contaminant library. With the top search result being bone particle, the contaminant was considered a mixture of calcium phosphate and protein. The peak around 1750 cm$^{-1}$ (C=O group derivative) present in contaminant 1 can be considered to be due to the influence of cooking oil.

Fig. 2 shows the EDX profile database of bone particle, which was indicated in the library search result. Performing EDX analysis enabled verification of the presence of Ca and P, which are the main components of bone. Since this corroborates the FTIR analysis results, this example demonstrates the benefits of analysis using both FTIR and EDX instruments.

**Table 1  Instruments and Analysis Conditions**

| | |
|---|---|
| Instruments | : IRAffinity-1S, MIRacle10 (Diamond prism) |
| Resolution | : 4 cm$^{-1}$ |
| Accumulation | : 40 |
| Apodization | : Happ-Genzel |
| Detector | : DLATGS |
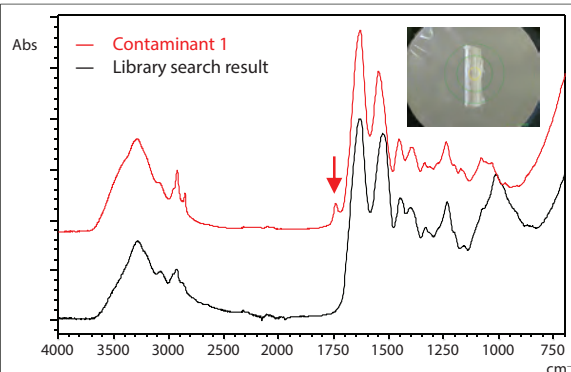


Library information:  Bone particle_white    Materials; Bone particle (Calcium phosphate, Protein)    Major elements; Ca, P, S    Color; White    Shape; Stick    Hardness; Hard    Metallic luster; No    Technique; ATR (Diamond)

**Fig. 1  Measurement Result and Search Result of Contaminant 1**
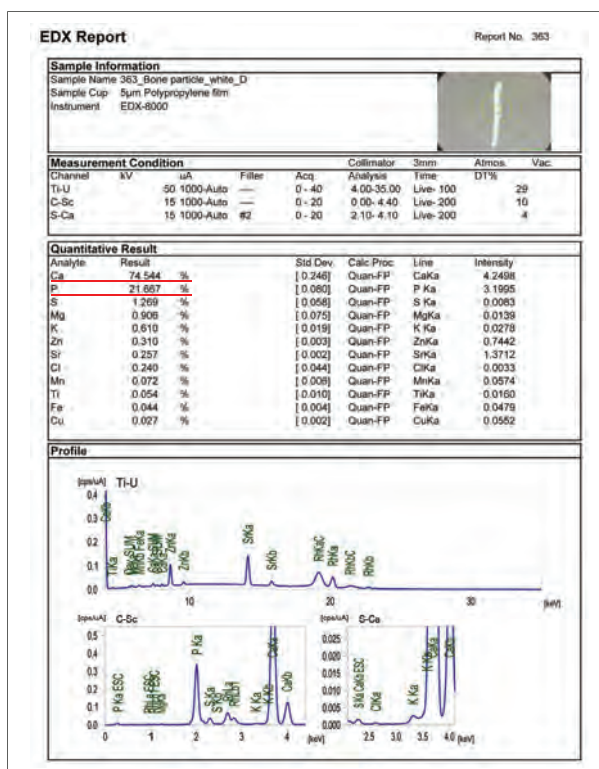


**Fig. 2  EDX Profile Database of "Bone Particle"**

Next, FTIR analysis was performed on contaminant 2 that was detected on a food production line. Fig. 3 shows the measurement result and the search result from the contaminant library. The top search result was a cluster of starch. While this contains starch, cooking oil, and protein, since the peak around 1750 cm$^{-1}$ (C=O group derivative) is hardly visible in contaminant 2 we can deduce that even if cooking oil was present in the contaminant, it would be minute.

Fig. 4 shows the EDX profile database of the cluster of starch that was indicated in the library search result. While salt (NaCl) was detected in the database, presumably from Na and Cl contained in food, almost no metallic elements were detected in contaminant 2.

This leads to the possibility that contaminant 2 is an ingredient whose main components are starch and protein.

Lastly, Table 2 lists an example of data in the contaminant library.



Library information: Cluster of starch    Materials; Starch, Cooking oil (Triacylglycerol), Protein    Major elements; Cl, Na    Color; Brown    Shape; Cluster    Hardness; Hard    Metallic luster; No    Technique; ATR (Ge)
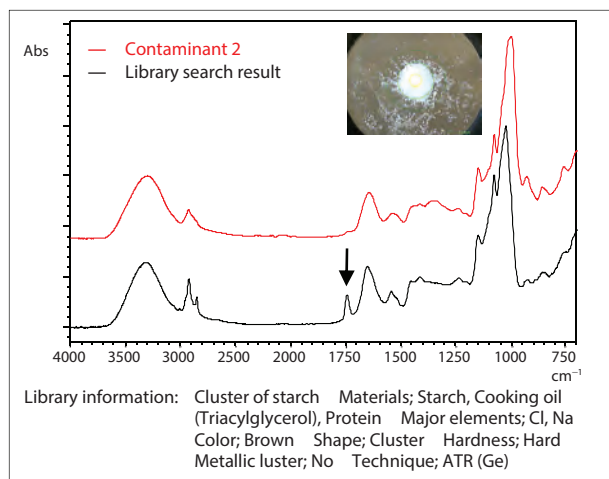
**Fig. 3** Measurement Result and Search Result of Contaminant 2



**Fig. 4** EDX Profile Database of "Cluster of starch"

## ■ Conclusion

While the data included in this library is useful for contaminant analysis since it contains the analysis results of actual contaminants, even higher accuracy in analysis can be achieved through the accumulation of samples detected as contaminants in a custom user library.

The EDX-FTIR contaminant finder/material inspector software, EDXIR-Analysis, which was released in 2016, contains data files of both FTIR and EDX analyses enabling integrated analysis using both data types. As demonstrated in the example of contaminant 1, in some cases performing EDX analysis may provide useful information for contaminant samples that prove difficult to identify with FTIR analysis alone. In contaminant analysis, a multifaceted approach which covers both organic and inorganic contaminants is very effective. For details, refer to Application News No. A522A.

**Table 2  Example of Data in the Contaminant Library**

| | Name | Comment |
|---|---|---|
| Water Supply-Related Contaminants | Pipe slip packing | Pipe slip packing    Materials; Polyethylene (PE)    Major elements; below 1%    Color; Black    Shape; Resin/Ring    Hardness; Hard    Metallic luster; No    Technique; ATR (Ge) |
| | Sealing tape | Sealing tape    Materials; Polytetrafluoroethylene (PTFE)    Major elements; F    Color; White    Shape; Film    Hardness; Soft    Metallic luster; No    Technique; ATR (Ge) |
| | Coating of inner wall_1 | Coating of inner wall_1    Materials; Polystyrene (PS), Acrylic resin    Major elements; Cl    Color; Brown    Shape; Fragment    Hardness; Soft    Metallic luster; No    Technique; ATR (Ge) |
| | Mold | Mold    Materials; Protein, Silicate    Major elements; below 1%    Color; Brown    Shape; Mold    Hardness; Soft    Metallic luster; No    Technique; ATR (Ge) |
| | Coating in pump_white | Coating in pump_white    Materials; Polyamide (Nylon 11), Titanium dioxide ($TiO_2$)    Major elements; Ti, Na    Color; White    Shape; Scraping    Hardness; Brittle    Metallic luster; No    Technique; ATR (Ge) |
| Food-Related Contaminants | Piece of plant material | Piece of plant material    Materials; Plant epidermis (Cellulose), Vegetable fat (Triacylglycerol)    Major elements; Cl, Na    Color; Brown    Shape; Cluster    Hardness; Soft    Metallic luster; No    Technique; ATR (Ge) |
| | White hair | White hair    Materials; Human hair (Protein)    Major elements; S    Color; White    Shape; Fiber    Hardness; Soft    Metallic luster; No    Technique; ATR (Ge) |
| | Nail | Nail    Materials; Nail (Keratin)    Major elements; S    Color; White    Shape; Fragment    Hardness; Hard    Metallic luster; No    Technique; ATR (Ge) |
| | Bone particle_brown | Bone particle_brown    Materials; Bone particle (Calcium phosphate, Protein)    Major elements; Ca, P, Mg    Color; Brown    Shape; Stick    Hardness; Hard    Metallic luster; No    Technique; ATR (Ge) |
| | Stapler1 | Stapler1    Materials; Zinc stearate (Adsorbate on metal surface)    Major elements; Fe, P    Color; Black    Shape; Stick    Hardness; Hard    Metallic luster; Yes    Technique; ATR (Ge) |
| | Glass fragment | Glass fragment    Materials; Glass ($SiO_2$)    Major elements; Pb, Si, K, Na, Zn    Color; Transparency    Shape; Cluster    Hardness; Hard    Metallic luster; No    Technique; ATR (Ge) |
| | Stainless steel_1 | Stainless steel_1    Materials; Unidentified (Adsorbate on metal surface)    Major elements; Fe, Cr, Ni, Mn    Color; Silver    Shape; Metal    Hardness; Hard    Metallic luster; Yes    Technique; ATR (Ge) |

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# SHIMADZU
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**Application Data Sheet**

**No.138**

### GC-MS
Gas Chromatograph Mass Spectrometer

# Efficient Analysis of Residual Pesticides in Foods Using High-Sensitivity GC-MS/MS

In the analysis of residual pesticides in foods, periodic maintenance is required. This includes the replacement of insert liners and column cutting due to the impact of impurities originating in the foods. However, because of issues such as the time for maintenance procedures and the cost of consumables, there is a need to reduce the frequency of maintenance as much as possible.

The GCMS-TQ8050, a high-sensitivity GC-MS/MS, can detect trace ions with high sensitivity, so high quantitative accuracy is obtained even for trace components. Using the high-sensitivity TQ8050, existing lower limits of quantitation can be obtained with small injection volumes. Further, reducing the injection volume lessens the analysis burden on parts such as insert liners, columns, and the ion source, which, in turn, reduces the frequency of maintenance. This Application Data Sheet presents the analysis results of pesticide standard samples and actual tomato samples, under conditions in which the injection volume is reduced to 1/4 the conventional amount, using the GCMS-TQ8050.



Fig. 1: GCMS-TQ8050,
a High-Sensitivity GC-MS/MS

**Cleaning the ion source**

**Replacing consumables**

**Conventional 2 μL injection**

**TQ8050 0.5 μL injection**

Injection volume 1/4

Cleaning frequency 1/4

Cost of consumables 1/4
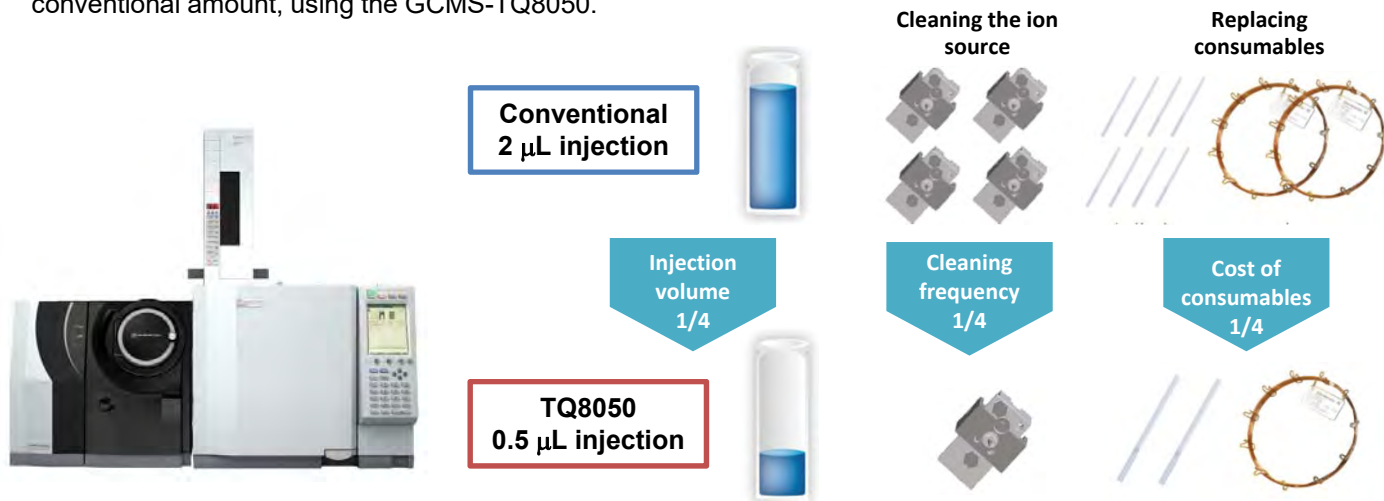
Fig. 2: Reduced Maintenance Frequency Due to Reduced Injection Volume

## Experiment

As the standard samples, pesticide standard mixed solutions (Hayashi Pure Chemical Ind. Ltd. PL2005 Pesticides GC/MS Mix I, II, III, IV, V, VI, and 7) were prepared to achieve final concentrations between 5 ng/mL and 100 ng/mL. At this point, as a virtual matrix, polyethylene glycol 300 was added to ensure a concentration of 200 μg/mL. In addition, as an actual sample for the spike recovery test, the pesticide standard mixed solution was spiked with a tomato extract solution to ensure a concentration of 10 ng/mL.

Smart Pesticides Database Ver. 2 was used to create the analytical method. In this experiment, in order to reduce the injection volume, the analysis was performed with a 0.5 μL injection using a 5 μL syringe.

Table 1 Analytical Conditions

| | |
|---|---|
| GC-MS: | GCMS-TQ8050 |
| Column: | SH-Rxi®-5Sil MS (30 m long, 0.25 mm I.D., df = 0.25 μm) (Shimadzu GLC, P/N 221-75954-30) |
| Glass Insert: | Topaz single taper with wool (Shimadzu GLC, P/N 23336) |
| Syringe for Injection: | AOC-20i 5 μL syringe (Shimadzu GLC, P/N: 221-75173) |

| GC | |
|---|---|
| Injection Port Temp.: | 250 °C |
| Column Oven Temp.: | 50 °C (1 min) → (25 °C /min) → 125 °C → (10 °C /min) → 300 °C (15 min) |
| Injection Mode: | Splitless |
| High-Pressure Injection: | 250 kPa (1.5 min) |
| Injection Volume: | 0.5 μL |
| Carrier Gas Control: | Linear velocity (47.2 cm/sec) |

| MS | |
|---|---|
| Interface Temp.: | 250 °C |
| Ion Source Temp.: | 230 °C |
| Ionization Method: | EI |
| Measurement Mode: | MRM |

## Analysis Results

The injection volume was reduced to 1/4 the conventional amount, and a 5 ng/mL standard sample was analyzed repeatedly (N = 5). Both sensitivity and repeated analysis accuracy were confirmed. Fig. 3 shows the distribution of area repeatability for a total of 387 components. Favorable results were obtained, with a %RSD of 10 % or less for 95 % of the components, and 20 % or less for 98 % of the components.

In addition, pesticides were spiked to the actual tomato sample, and the quantified values in the actual sample were confirmed. Fig. 4 shows the distribution of the recovery rates calculated from these quantitative results, and Fig. 5 shows typical pesticide chromatograms. For approximately 80 % of the components, a favorable recovery rate between 70 % and 120 % was obtained.

From these results, it is evident that using the high-sensitivity GC-MS/MS, sufficient quantitative accuracy can be obtained even at low concentrations, even when the injection volume is reduced. Reducing the injection volume reduces the frequency of instrument maintenance, enabling more efficient operation.
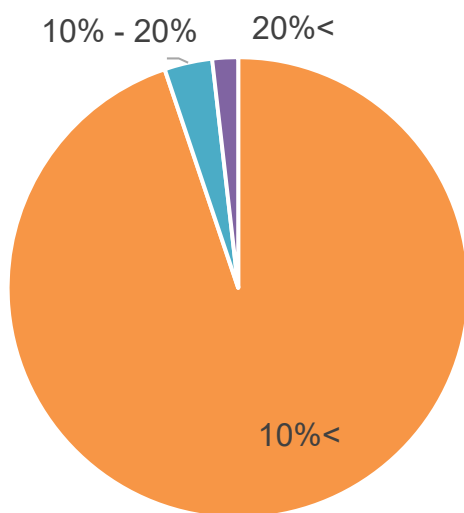
Fig. 3: Distribution of Area Repeatability (%RSD) for a 5 ppb Pesticide Standard Sample
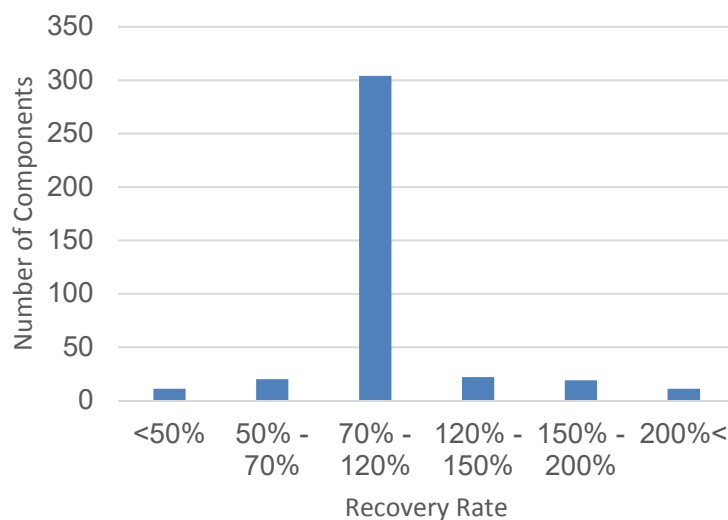
Fig. 4: Distribution of Spike Recovery Rates for an Actual Tomato Sample
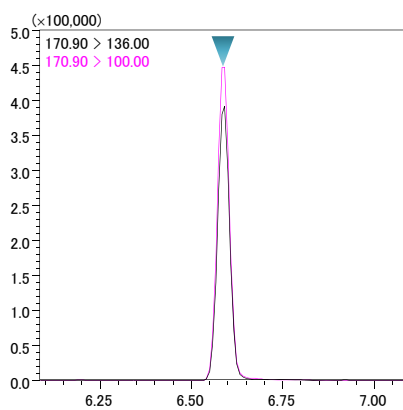
Dichlobenil
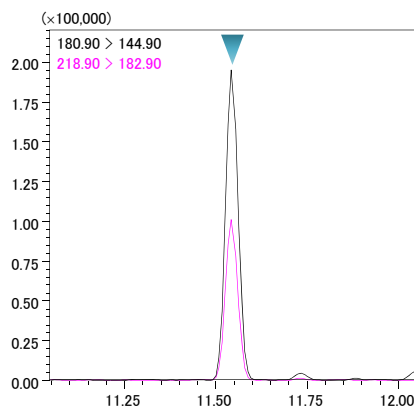
delta-BHC
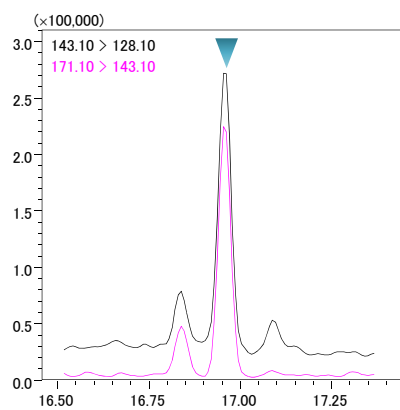
Resmethrin-1, 2

Fig. 5: Typical Chromatograms for Pesticides in an Actual Tomato Sample

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# Application News

## No. C162

**Liquid Chromatograph Mass Spectrometry**

# Quantitative Analysis of Highly Polar Pesticides in Food Using SFC/MS

Since achieving sufficient retention and favorable separation in normal batch analysis of highly polar pesticides has proved difficult due to their chemical characteristics, a number of individual analysis methods are employed for LC/MS/MS analysis. To rectify this situation, EURL-SRM (Stuttgart, Germany), an EU Reference Laboratories member in charge of individual analysis method development, is developing a batch analysis method called "QuPPe (Quick Polar Pesticides)" for highly polar pesticides that are difficult to analyze using pretreatment with the QuEChERS method as well as normal batch analysis methods. This method proposes multiple methods to suit each sample and target chemical compound (M. Anastassiades et al; QuPPe of EURL-SRM (Version 9.1; 2016)).

Until now, analysis of highly polar pesticides using LC/MS/MS has used a variety of separation methods including HILIC mode, mixed mode, normal phase, and reversed phase. However, all of these methods have restrictions on the chemical compounds that can be analyzed together and this remains a problem. On the contrary, supercritical fluid chromatography (SFC) has the advantage of being able to separate a wide array of chemical compounds at once due to the characteristics of the mobile phase that is used. In addition, since the separation behavior with SFC differs from that with LC even when using a column of the same separation mode, SFC may be effective for the analyses of chemical compounds for which retention and separation are difficult in LC. This article introduces an example of batch analysis of highly polar pesticides using SFC.

Y.Fujito, D. Baker, A. Barnes, C. Titman, J. Horner, N. Loftus
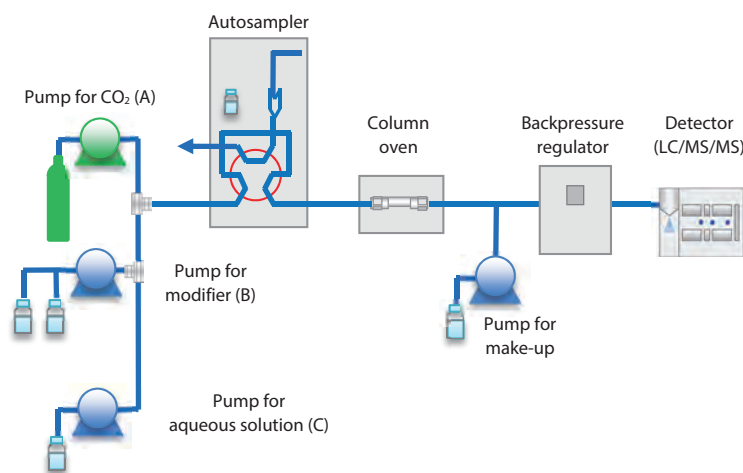


**Fig. 1** SFC/MS System Configuration Diagram

In this experiment, an examination of adding a small amount of water to a modifier was performed for the purpose of eluting and separating highly polar pesticides.
In order to simplify this examination, a low-pressure gradient pump (LPGE) was used as pump B and the modifier was automatically prepared by mobile phase blending.

**Table 1  SFC/MS Analysis Conditions**

| Supercritical fluid chromatography | | Mass spectrometry | |
|---|---|---|---|
| **SFC** | Nexera UC system | **LC-MS/MS** | LCMS-8060 |
| **Analytical column** | Restek Ultra Silica (150 × 2.1 mm 3 μm) | **Ionisation mode** | Heated ESI |
| **Column temperature** | 50 °C | **Scan speed** | 15,000 u/sec |
| **Flow rate** | 0.8 mL/min (0.6 mL/min 13-22 min) | **MRM Dwell time** | 3 msec |
| **Pump A** | $CO_2$ | **Pause time** | 1 msec |
| **Pump B (modifier solvent)** | Acetonitrile + 0.5 % formic acid + 10 mM ammonium formate | **Interface temp.** | 300 °C |
| **Pump C (modifier solvent)** | Water + 0.5 % formic acid + 10 mM ammonium formate | **Heating block** | 350 °C |
| **Pump D (make up solvent)** | Methanol | **Desolvation line** | 250 °C |
| **Makeup solvent flow rate** | 0.2 mL/min | | |

## ■ Examination of SFC Separation Conditions

Normally, SFC performs gradient separation using supercritical carbon dioxide and an organic solvent (such as methanol and acetonitrile), which is referred to as a modifier. However, some highly polar chemical compounds exhibit strong retention in columns resulting in cases where separation and elution is insufficient even with 100 % organic solvent. In this experiment, since a number of highly polar pesticides could not be eluted with 100 % organic solvent, separation was examined by adding a small amount of water to the modifier.

Supercritical carbon dioxide has low polarity and low miscibility with water. This means that only a limited amount of water can be added to the modifier (normally about 0.1 to 10 %). We therefore examined separation behavior by adding water by the amount equivalent to 0.2, 4, 6, 8, and 10 % to the modifier. Through examination based on the peak profiles and separation patterns of the eluted components, we adopted a water content of 6 %. However, there were chemical compounds that could not be eluted even with this condition.



\* Aqueous Solution: 0.5 % formic acid + 1mM ammonium formate
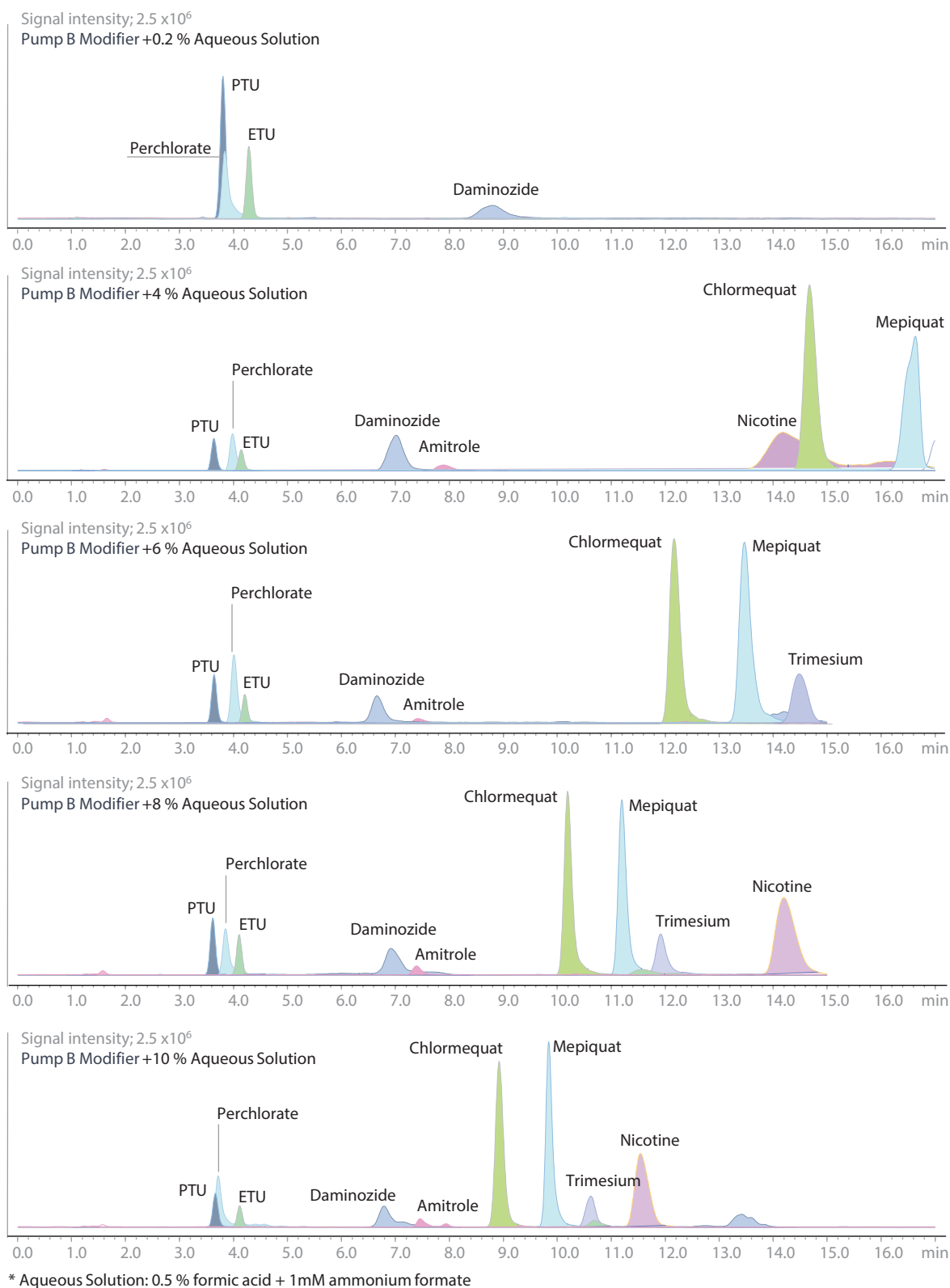
**Fig. 2** **Effect of Water on Separation Behavior of Highly Polar Pesticides in SFC/MS**

## ■ Optimization of SFC Separation Conditions

When we examined addition of water to the modifier, we were able to confirm elution of most chemical compounds with the 6 % aqueous solution. However, nicotine and kasugamycine, which both exhibit strong retention, could not be eluted. Any further addition of aqueous solution in the presence of carbon dioxide adversely affects gradient accuracy and may impair the stability of the analysis method. For this reason, aqueous solution was added using a separate pump (pump C) after the modifier reached 100 % (Fig. 4).

This allowed elution of the remaining highly polar pesticides and enabled batch separation of the highly polar pesticides from logP-3.47 to 1.96.
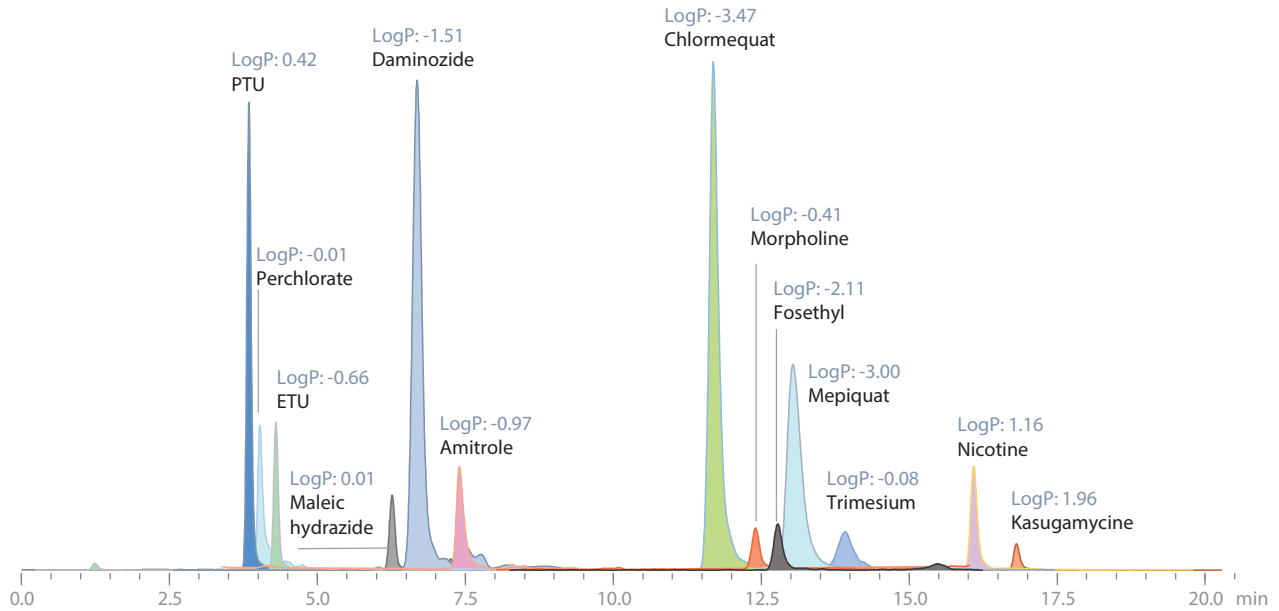


**Fig. 3** MRM Chromatogram of Highly Polar Pesticides Using SFC-MS
(Addition of 200 ppb Pesticide Standard Solution into Flaxseed Extract Using QuPPe)
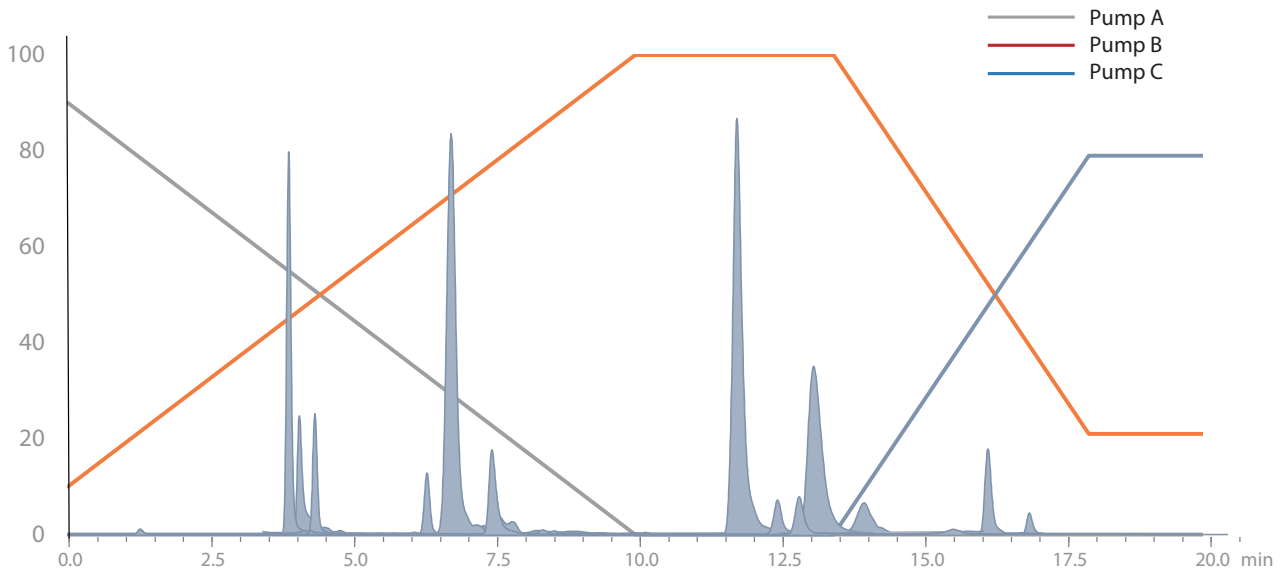


The initial SFC/MS conditions;

Pump A 90 % : Carbon Dioxide

Pump B 10 % : 6 % Water in Acetonitrile containing 0.5 % formic acid and 10 mM ammonium formate

Pump C 0 % : Aqueous solution containing 0.5 % formic acid + 10 mM ammonium formate

**Fig. 4  Ternary Gradient Program**

## ■ Sample Preparation and Analysis

Flaxseed and lemon were used as food samples and extraction was performed using a method compliant with QuPPe. (The extracts were provided by Concept Life Sciences, a contract analytical laboratory located in the U.K.) Standard solution of highly polar pesticides was added to these matrix solutions, which were then directly injected into the SFC-MS/MS.

## ■ Quantitative Analysis of Highly Polar Pesticides

In order to verify the quantitative performance of the developed SFC/MS analysis method, matrix calibration curves were created using each food extract to which standard solution of the highly polar pesticides was added. The calibration curve range was 10 to 200 ppb and accuracy was verified using the internal standard method regarding components for which an internal standard substance labeled with a stable isotope was obtained.

The calibration curve created for each sample showed favorable linearity for all chemical compounds regardless of the sample matrix.

ETU Calibration curve 10-200 ppb
Matrix comparison Lemon | Flaxseed
Peak area Ratio | ETU/($^2$H$_4$)ETU | RT 4.36 mins

Nicotine Calibration curve 10-200 ppb
Matrix comparison Lemon | Flaxseed
Peak area Ratio | Nicotine/($^2$H$_3$)Nicotine | RT 16.04 mins



ETU Lemon
Slope 0.0101
Intercept - 0.0159
$R^2$ = 0.9992

ETU Flaxseed
Slope 0.0097
Intercept - 0.0081
$R^2$ = 0.9999

Nicotine Flaxseed
Slope 0.0125
Intercept 0.0199
$R^2$ = 0.999

Nicotine Lemon
Slope 0.0117
Intercept - 0.0069
$R^2$ = 0.9999

**Fig. 5 Matrix Calibration Curves of Representative Highly Polar Pesticides**
**(ETU: fast eluting compound, Nicotine: slow eluting compound, Samples: lemon, flaxseed)**

**Table 2 Calibration Curve Linearity and Repeatability at 100 ppb of Eight Highly Polar Pesticide Components**

| Compound | RT (min) | Internal Standard | IS RT (min) | Quan MRM | %RSD 100ppb | $R^2$ |
|---|---|---|---|---|---|---|
| Perchlorate | 3.95 | $^{18}$O$_4$ Perchlorate | 3.91 | 99.00 > 82.90 | 4.98 | 0.968 |
| ETU | 4.36 | $^2$H$_4$ ETU | 4.26 | 103.10 > 44.05 | 4.84 | 0.999 |
| Maleic hydrazide | 6.28 | $^2$H$_2$ Maleic hydrazide | 6.28 | 113.00 > 67.10 | 6.81 | 0.997 |
| Chlormequat | 11.58 | $^2$H$_4$ Chlormequat | 11.54 | 121.90 > 58.10 | 1.75 | 1.000 |
| Fosethyl | 12.50 | $^2$H$_{15}$ Fosethyl | 12.50 | 109.00 > 80.95 | 6.78 | 0.999 |
| Morpholine | 12.19 | $^2$H$_8$ Morpholine | 12.23 | 87.90 > 70.05 | 10.74 | 0.996 |
| Mepiquat | 12.72 | $^2$H$_3$ Mepiquat | 12.69 | 114.30 > 98.10 | 7.66 | 0.998 |
| Nicotine | 16.06 | $^2$H$_3$ Nicotine | 16.03 | 163.00 > 130.00 | 2.31 | 0.999 |

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# Application News

**High Performance Liquid Chromatography**

## Analysis of Mycotoxins in Grain Using Mycotoxin Screening System

# No.L512

Mycotoxins are chemical products produced by organisms in the fungus kingdom and are toxic to humans, animals, and crops. As an example, aflatoxins are a type of mycotoxin that are some of the most carcinogenic naturally occurring substances in the world. They are classified as Group 1 carcinogens (carcinogenic to humans) by the WHO International Agency for Research on Cancer (IARC), and subject to strict regulations in many countries and regions of the world.

This Application News describes the screening analysis for mycotoxins in grain products (soft wheat flour and rice flour) using the i-Series Solution Package mycotoxin screening system.

### ■ i-Series Solution Package Mycotoxin Screening System

The screening system comprises a compact and easy to use integrated i-Series HPLC system together with analysis methods including sample pretreatment methods. The system comes ready to use and capable of data acquisition and analysis, including columns and method files designed for mycotoxin analysis, an instruction manual with analysis methods, and report templates. For screening applications, the system can determine whether mycotoxin levels in food are in excess of reference levels.



Nexera-i 3D, RF-20A$_{XS}$

Column
CD-ROM
Mixer
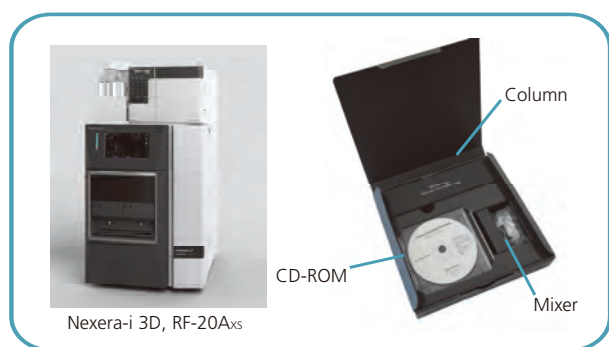
**Fig. 1  Mycotoxin Screening System**

Currently, HPLC and LC/MS are the most common techniques used to identify aflatoxins in food. With HPLC, fluorescent derivatization is often performed to improve sensitivity, though disadvantages of derivatization procedures are the time required and their complexity. Meanwhile, though LC/MS is more selective in terms of sensitivity, major financial investment into system is required.

The i-Series Solution Package comes with a built-in PDA detector, and can be further enhanced with an RF-20A$_{XS}$ fluorescence detector that offers world-class sensitivity. The package can also detect aflatoxins directly without derivatization.

### ■ Analysis of a Standard Solution

Mycotoxin targets of the screening system are shown in Table 1, chemical structures of some of these targets are shown in Fig. 2, and analytical conditions are shown in Table 2. The package includes analysis method files that eliminate the need to configure analytical conditions. An RF-20A$_{XS}$ was used to perform analysis with on-time excitation wavelength/emission wavelength switching.

**Table 1  Screening Target Compounds**

|  | Mycotoxin | Abbreviation | Matrix |
|---|---|---|---|
| 1 | Aflatoxin M$_1$ | AFM$_1$ | Milk |
| 2 | Aflatoxin G$_2$ | AFG$_2$ | Grain |
| 3 | Aflatoxin G$_1$ | AFG$_1$ | |
| 4 | Aflatoxin B$_2$ | AFB$_2$ | |
| 5 | Aflatoxin B$_1$ | AFB$_1$ | |
| 6 | Zearalenone | ZON | |
| 7 | Ochratoxin A | OTA | |
| 8 | Nivalenol | NIV | |
| 9 | Deoxynivalenol | DON | |
| 10 | Patulin | PAT | Apple |



AFB$_1$   AFB$_2$
AFG$_1$   AFG$_2$
AFM$_1$   ZON
OTA   PAT
NIV   DON
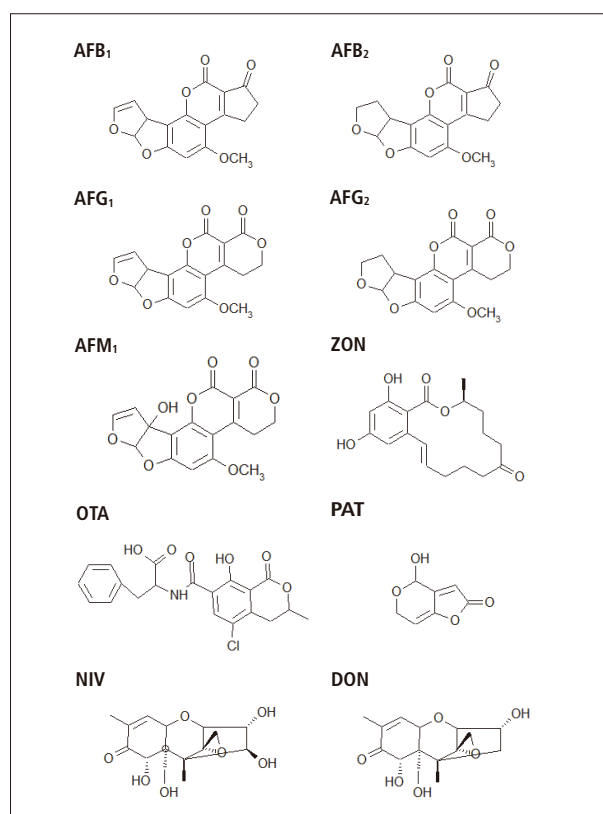
**Fig. 2  Target Mycotoxin Structures**

**Table 2  Analytical Conditions**

| System | : Nexera-i 3D, RF-20A$_{XS}$ |
|---|---|
| Column | : Shim-pack GIST C18 (75 mm L. × 3.0 mm I.D., 2 μm) |
| Mobile Phase | : A) 20 mmol/L (Sodium) phosphate buffer (pH 2.5) |
| | B) Acetonitrile |
| | C) Methanol |
| | (Gradient elution) |
| Flowrate | : 1.0 mL/min |
| Column Temp. | : 55 °C |
| Injection Vol. | : 10 μL |
| Detection (RF-20A$_{XS}$) | : AFB$_1$, AFB$_2$, AFG$_1$, AFG$_2$, AFM$_1$ : Ex 365 nm, Em 450 nm |
| | OTA, ZON : Ex 320 nm, Em 465 nm |
| Detection (Nexera-i 3D) | : NIV, DON : 220 nm (ch 1) |
| | PAT : 276 nm (ch 2) |

Although regulatory limits for mycotoxin levels in food can vary by country and region, the screening system is compatible with the strictest regulatory limits that are found in the EU (excluding regulatory limits in baby food). Chromatograms of a standard mixture with mycotoxin levels equivalent to EU reference levels[1] is shown in Fig. 3.



Peaks
1) AFM$_1$ 1 μg/L
2) AFG$_2$ 0.25 μg/L
3) AFG$_1$ 0.25 μg/L
4) AFB$_2$ 0.25 μg/L
5) AFB$_1$ 0.25 μg/L
6) ZON 50 μg/L
7) OTA 5 μg/L

Peaks
8) NIV 1000 μg/L
9) DON 1000 μg/L
10) PAT 25 μg/L
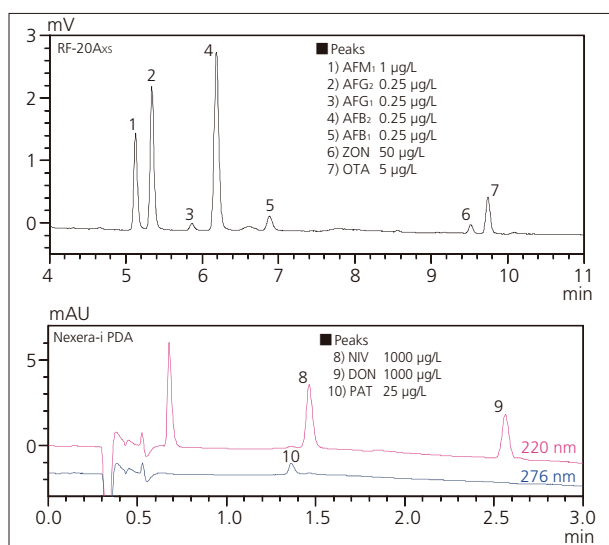
**Fig. 3  Chromatograms of a Standard Mixture**

■ **Analysis of Mycotoxins in Grain**

This section describes an analysis of milled grains after pretreatment. Fig. 4 shows an overview of the sample pretreatment method. Further details can be found in the mycotoxin screening system instruction manual. Chromatograms of pretreated samples of soft wheat flour and rice flour and of pretreated samples of soft wheat flour and rice flour spiked with a standard mixture of mycotoxins that are produced in grains (shown among the screening target compounds listed in Table 1) are shown in Fig. 5 and Fig. 6.

Comparing the area of each peak in the standard mixture that contains mycotoxins at EU reference levels and each peak in the flour samples allows identification of whether the mycotoxins present in flour samples are in excess and violation of reference levels. The system makes this determination without the need for complex analysis of results by the user, allowing for easy screening of target compounds.
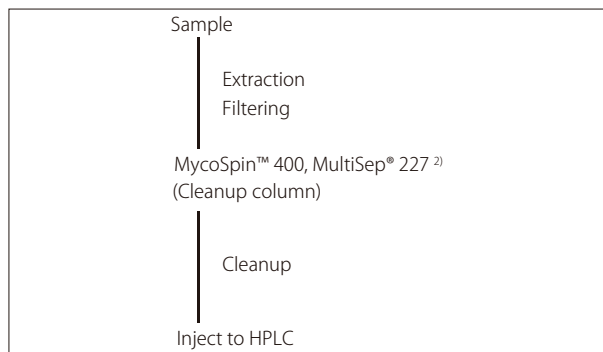


**Fig. 4  Sample Pretreatment**
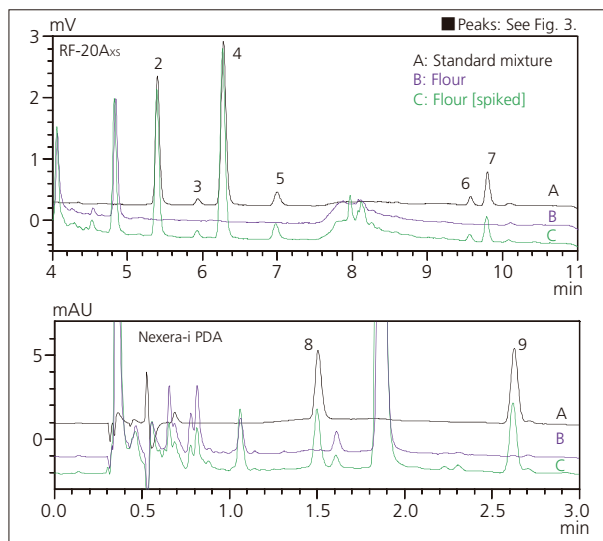


**Fig. 5  Chromatograms of Soft Wheat Flour**
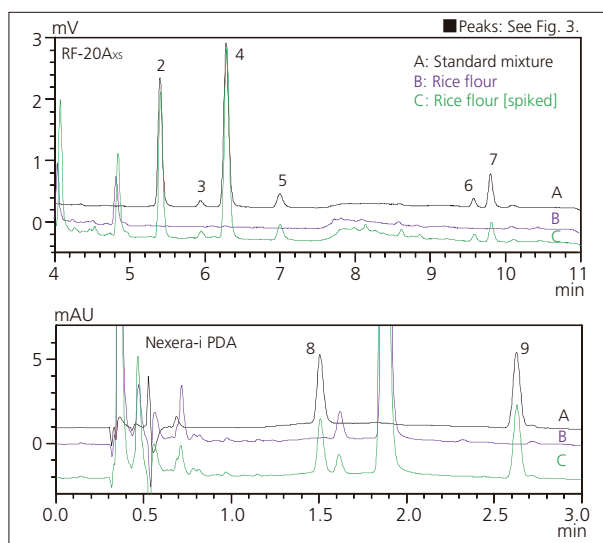


**Fig. 6  Chromatograms of Rice Flour**

Footnotes
1) Converted concentrations in the standard mixture were obtained according to a pretreatment method described in the i-Series Solution Package Mycotoxin Screening System instruction manual.
2) MycoSpin™ 400 and MultiSep® 227 are registered trademarks of Romer Labs.

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# Improved Detection of Pesticide Residues in Botanicals by LCMS

# ASMS 2018

Jeffrey H. Dahl and Tairo Ogura
Shimadzu Scientific Instruments, Columbia Maryland

# Introduction

Pesticides and other chemicals are used in the production of many agricultural products, including botanicals for use as dietary supplements. Supplements are widely used but their raw materials, often sourced from remote locations, are subjected to fewer regulatory controls than staple foods. To ensure quality the US FDA requires identity and quality testing, but most botanicals do not have specific regulations. To analyze complex botanicals for residual chemicals such as pesticides, LC-MS-MS is needed for high sensitivity, high confidence results. We developed an LCMS method with improved detection sensitivity for chemical residues in botanicals.

Photo credits:  Echinacea, Giancarlo Dessì; Cayenne, H. Zell; Valerian, Lairich Rig; Ginseng, National Institute of Korean Language; Tumeric, Simon A. Eugster; Passionflower, Bob Peterson; St Johns Wort, Glyn Baker. All photos obtained through wikimedia commons under creative commons attribution-share alike 2.0 or higher



*Echinacea* E. purpurea



*Cayenne* Capsicum annuum



*Valerian* Valeriana officinalis



*Passionflower (tea)* Passiflora sp.

*Tumeric* Curcuma Longa





*Korean Ginseng* Panax ginseng



*St. Johns Wort* Hypericum perforatum

Figure 1   A selection of popular dietary supplements tested. Various parts of the above-pictured plants may be used in actual dietary supplement formulations.

# Method

Representative samples of powdered botanicals were removed from their gelatin capsules, homogenized and extracted with acetonitrile accompanied by shaking and sonication. Samples were additionally cleaned up using dispersive solid phase extraction to remove unwanted matrix components. Analysis was carried out by LC-MS-MS using a triple quadrupole mass spectrometer. The mass spectrometer interface parameters were carefully adjusted to improve the signal for the majority of the analytes. Spiking experiments were used to determine recovery and matrix-matched standards were used to prepare calibration curves.

Table 1  Instrument parameters used for analysis

| | |
|---|---|
| LC Column | : Raptor ARC18 (2.1×150 mm, 2.7 µm) |
| Mobile Phase A | : 0.1% Formic Acid with 5 mM Am. Formate |
| Mobile Phase B | : Methanol |
| Flow Rate | : 0.5 mL/min |
| Probe Voltage | : +0.5 kV or –0.5kV |
| Interface Temp | : 100 ˚C |
| Nebulizing Gas | : 3 L/min |
| Drying Gas | : 10 L/min |
| DL Temp | : 100 ˚C |
| Heat Block Temp | : 100 ˚C |

Empty contents of capsules and homogenize. 0.5 gram sample size

Extract with 5 mL ACN, sonication and vortexing 15 minutes

Cleanup with dSPE 6 mL per tube with PSA, C18 and GCB
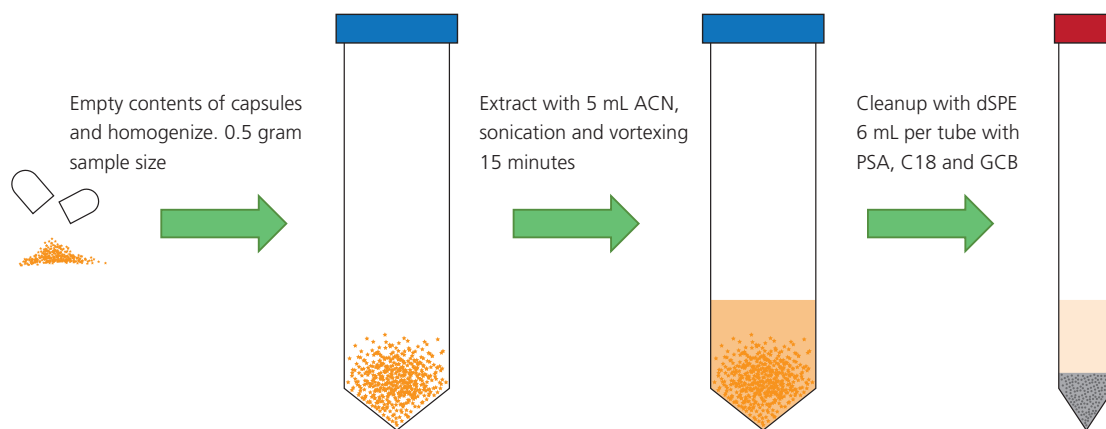
Figure 2   Sample preparation of botanicals for LC-MS analysis

# Results and Discussion

Eight popular botanical supplements were selected for testing, including Cayenne, Valerian, Passionflower tea, Korean Ginseng, St. John's Wort, Tumeric and two varieties of Echinacea. The Tea and Echinacea variety 1 were labeled as organic, while the other supplements were not labeled as organic. For each sample, a single-point standard addition sample at 500 ng/g dried material was prepared in addition to check matrix-specific effects. Compared with a conventional method, we found significant improvement in instrument response for many

analytes by careful adjustment of interface temperature and spray voltage. For quantitation, matrix matched calibration curves were linear within the quantitation limits established for each compound, which was compound dependent. Detection limits and quantitation limits were required to have 3:1 and 10:1 signal to noise respectively, and quantitation limits were required to have less than 20% RSD in triplicate injections. Using our newly developed method, we are able to characterize the extent of residual pesticides t in popular botanicals.
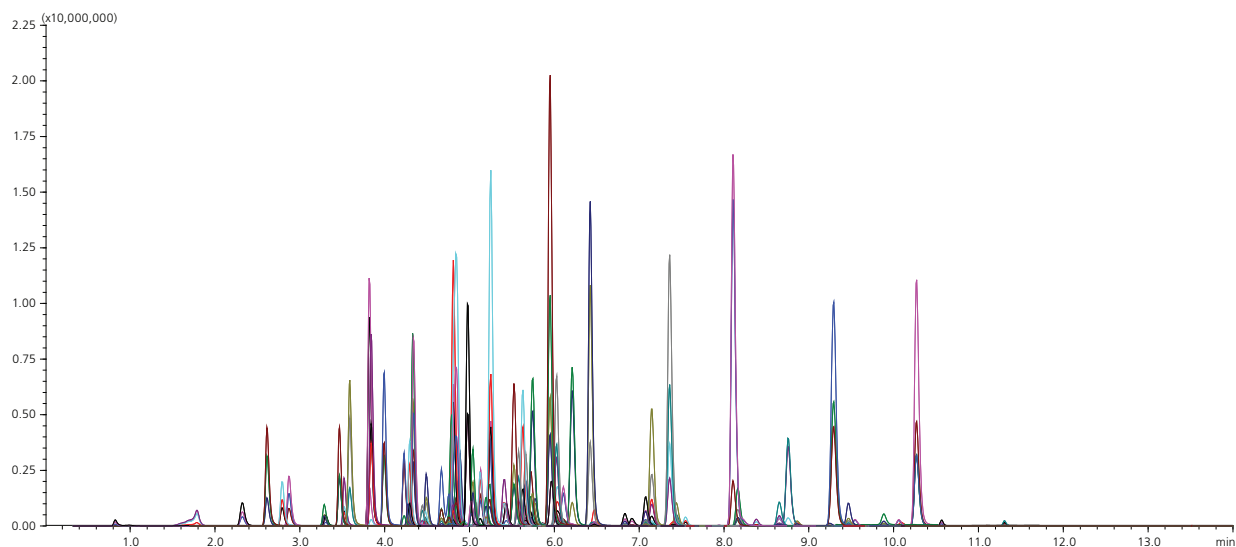


Figure 3   Representative chromatogram of pesticides spiked into a sample of Korean Ginseng at the 500 ng/g of dried material level.
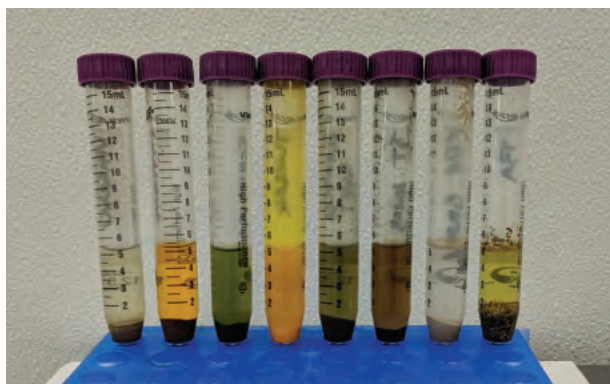


Figure 4   Acetonitrile extracts (before dSPE cleanup) of various botanicals. From left: Valerian, Cayenne, Echinacea-1, Tumeric, Echinacea-2, St. John's Wort, Korean Ginseng, and Passionflower Tea.

# Improved Detection of Pesticide Residues in Botanicals by LCMS

Table 2  Chemical residues detected in botanical extracts. Values reported in ng/g dry material.

| | Cayenne | Echinacea-1 | Echinacea-2 | Korean Ginseng | St. John's Wort | Passionflower tea | Tumeric | Valerian root |
|---|---|---|---|---|---|---|---|---|
| Azoxystrobin | 15 | ND | ND | 7 | ND | ND | ND | ND |
| Carbaryl | ND | ND | ND | ND | ND | 35 | ND | ND |
| Carbofuran | 3.7 | ND | ND | ND | ND | ND | ND | ND |
| Chlorpyrifos | ND | ND | ND | ND | ND | ND | 9.4 | ND |
| Cypermethrin | 140 | ND | ND | ND | ND | ND | ND | ND |
| Dimethomorph | ND | ND | ND | 36 | ND | ND | ND | ND |
| Imidacloprid | 11 | ND | ND | ND | ND | ND | ND | ND |
| Metalaxyl | ND | ND | ND | ND | ND | ND | 8.3 | ND |
| Methoprene | 196 | ND | ND | ND | ND | ND | ND | ND |
| Novaluron | 24 | ND | ND | ND | ND | ND | ND | ND |
| Propiconazole | ND | ND | ND | 24 | ND | ND | ND | ND |
| Pyraclastrobin | 6.5 | ND | ND | ND | ND | ND | ND | ND |
| Tebuconazole | 59 | ND | ND | 4.8 | ND | ND | ND | ND |
| Trifloxystrobin | 10 | ND | ND | ND | ND | ND | ND | ND |

Table 3  List of compounds measured and limits of quantitation in ng/g.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Abamectin | 30 | Daminozide | 15 | Hexythiazox | 15 | Propiconazole | 60 |
| Acephate | 5 | Deltamethrin | 30 | Imazalil | 10 | Propoxur | 2 |
| Acequinocyl | 60 | Diazinon | <2 | Imidacloprid | 4 | Pyraclostrobin | 10 |
| Acetamiprid | <2 | Dichlorvos | 15 | Kresoxim-methyl | 4 | Pyrethrin I | 100 |
| Aldicarb | <2 | Dimethoate | <2 | Malathion | 2 | Pyridaben | 2 |
| Allethrin | 50 | Dimethomorph | 5 | Metalaxyl | 2 | Resmethrin | 35 |
| Azoxystrobin | 4 | Dinotefuran | 2 | Methiocarb | 4 | Spinetoram | 2 |
| Bifenazate | 2 | Dodemorph | 4 | Methomyl | <2 | Spinosad | <2 |
| Bifenthrin | 4 | Endosulfan-sulfate | 4 | Methoprene | 50 | Spirodiclofen | 10 |
| Boscalid | 4 | Ethoprophos | 2 | Mevinphos | 4 | Spiromesifen | 20 |
| Buprofezin | <2 | Etofenprox | 4 | MGK-264 | 500 | Spirotetramat | 2 |
| Carbaryl | 10 | Etoxazole | <2 | Myclobutanil | 10 | Spiroxamine | 2 |
| Carbofuran | <2 | Fenhexamid | 20 | Naled | 2 | Tebuconazole | 2 |
| Chlorantraniliprole | 2 | Fenoxycarb | 2 | Novaluron | 15 | Tebufenozide | 5 |
| Chlorpyrifos | 10 | Fenpyroximate | 10 | Oxamyl | 2 | Teflubenzuron | 15 |
| Clofentazine | 4 | Fensulfothion | 5 | Paclobutrazol | 2 | Tetrachlorvinphos | 4 |
| Clothianidin | 4 | Fenthion | 10 | Permethrin | 10 | Tetramethrin | 4 |
| Coumaphos | 4 | Fenvalerate | 100 | Phenothrin | 10 | Thiacloprid | <2 |
| Cyantraniliprole | 2 | Fipronil | 2 | Phosmet | 10 | Thiamethoxam | <2 |
| Cyfluthrin | 500 | Flonicamid | 25 | Piperonyl butoxide | 5 | Thiophanate-methyl | 5 |
| Cypermethrin | 60 | Fludioxonil | 2 | Pirimicarb | 2 | Trifloxystrobin | <2 |
| Cyprodinil | 10 | Fluopyram | 2 | Prallethrin | 10 | | |
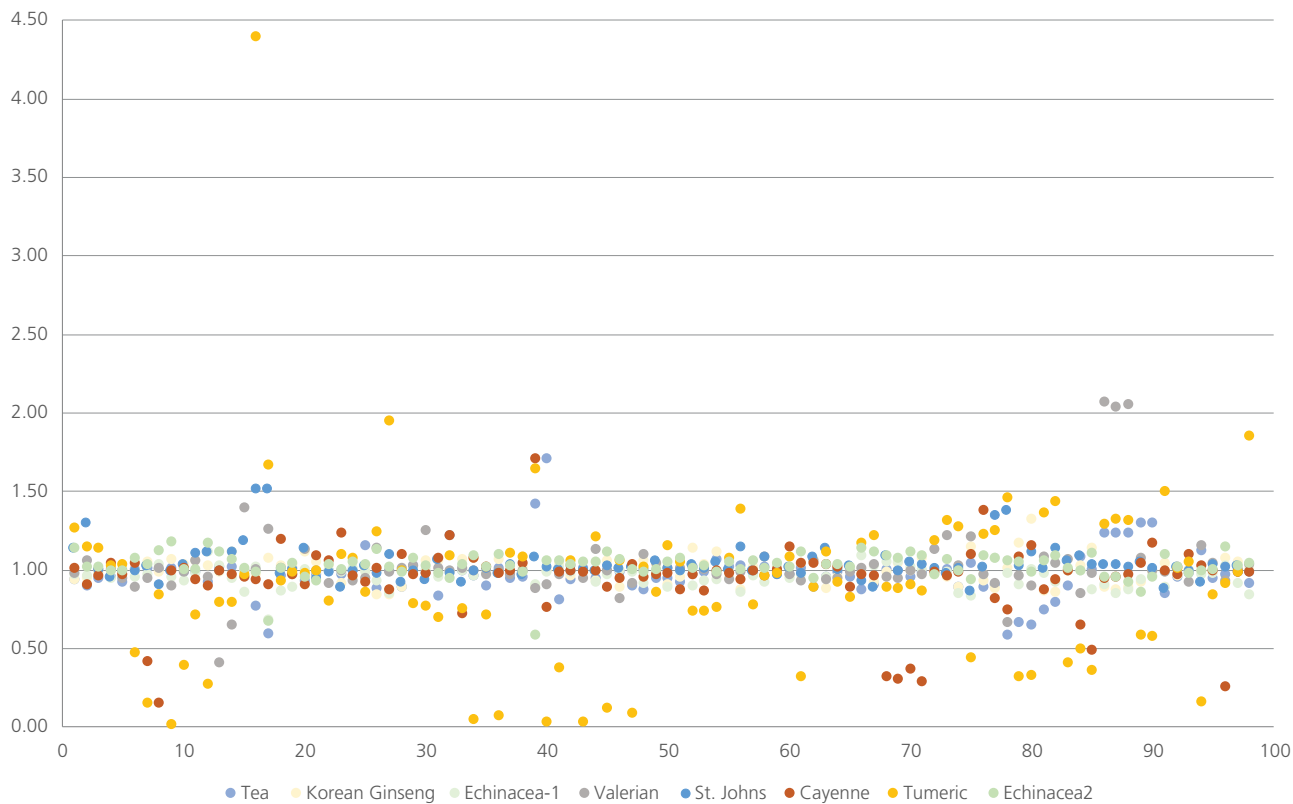
Figure 5   Recovery for each analyte in a 500 ng/g spike of each sample. Tumeric and Cayenne had the greatest number of analytes with low recovery, due to signal suppression.
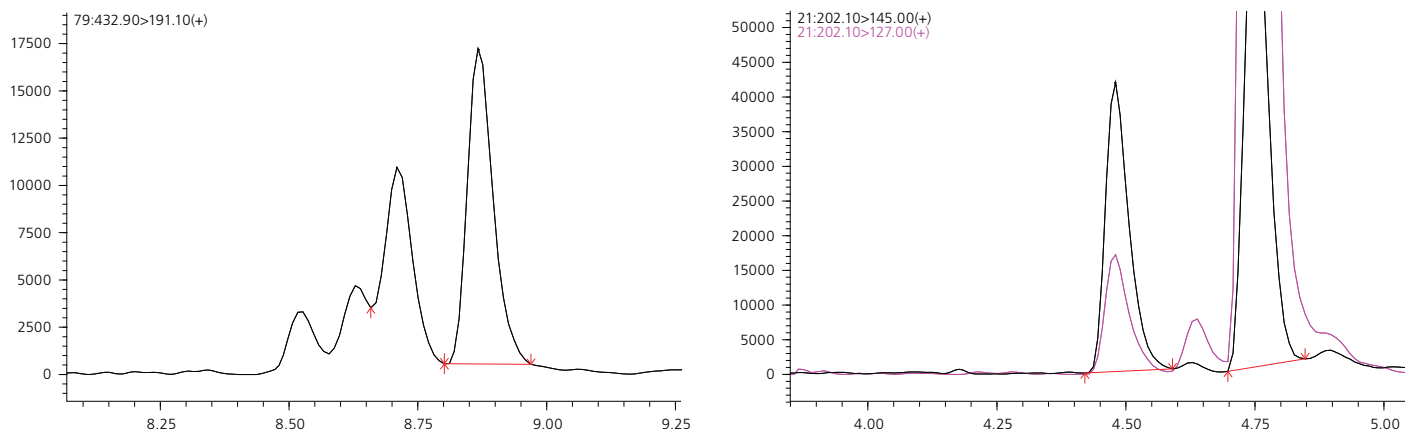


Figure 6   (Left) Cypermethrin (four isomers) detected in Cayenne sample.
(Right) Carbaryl detected in organic passionflower tea sample.

# Conclusion

We developed a high performance method for sensitive detection of pesticides in popular botanical supplements with a simple sample preparation and applied the method to measure pesticides in selected botanical products offered for retail sale. We found matrix effects to be minimal with the exception of Tumeric and Cayenne. For these matrices, additional sample cleanup may be useful to minimize signal suppression by the matrix.

Several pesticides were detected in some of the dietary supplements. Cayenne had the greatest number of detections and with the highest levels approaching 200 ng/g. Significantly, the Passionflower tea, which was labeled as organic, was found to contain 35 ng/g carbaryl. Our rapid, sensitive, and selective method is well-suited to high throughput detection of pesticide residues in popular botanical products.

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# Fast GC-MS/MS Analysis Of Multicomponent Pesticide Residues (360) In Food Matrix

## ASMS 2015   WP 053

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# Introduction

Contamination of food products with pesticides is a growing concern because of recognized adverse health effects, increasing world-wide usage of pesticides, and increasing imports of raw foodstuffs from foreign sources. Consequently, the number of samples as well as monitored pesticides became significantly higher in the last decade. To handle this high sample load, a Quick, Easy and Cheap cleanup procedure called QuEChERS was established[1]. Unfortunately, samples prepared by this method contain large matrix signals which can complicate accurate pesticide quantification by MS alone. As a consequence of matrix effects in MS, tandem MS/MS instruments using multiple reaction mechanisms (MRM), have more frequently been adopted in recent years, as it increases selectivity and sensitivity. Besides matrix interference, short analysis times are more frequently needed when handling large sample numbers in routine work. The use of narrow bore capillary columns has been shown to be a powerful tool to dramatically reduce the analysis time while maintaining chromatographic resolution in different GCMS applications[2]. In fast GC experiments typically peak width at half height (FWHM) was reduced to ~1 sec therefore requiring ultra fast scanning and polarity switching. As a result of the ultra fast GC and selectivity of tandem MS increases in laboratory efficiency can be gained in addition to reduced working costs.

In this work ultra fast GC-MS/MS analysis was tested by analyzing 360 pesticides in apple QuEChERS extract in less than 10 minutes.

# Methods and Materials

## Sample preparation

Apple extract was used as test sample matrix. The sample matrix was extracted and subjected to cleanup using the well-established QuEChERS procedure. The calibration curve had 6-points (0.5 ppb to 100 ppb) by spiking the blank sample matrix using internal standard technique. The spiked solution contained overall 360 different pesticides and TPP as internal standard.

Table 1: GC Analytical Conditions

| | |
|---|---|
| Instrument | : GCMS-TQ8040 (Shimadzu, Japan) |
| Software | : GCMSSolution 4.2 with SmartMRM and MRM Optimization Tool |
| Injector | : Optic-4, IP deactivated liner with glass insert |
| PTV Programme | : 70 °C, 15 °C/s to 280 °C, 1.2 min, 15 °C/s to 320 °C, 6 min |
| Split | : Splitless Injection (1.3 min) |
| Injection Volume | : 1 µL |
| Column | : 5 MS 20m, 0.18 mm, 0.18 µm |
| GC Oven | : 80 °C, 1 min, 35 °C/min to 210 °C, 25 °C/min to 320 °C,  2 min |

Table 2: MS Analytical Conditions (GCMS-TQ8040)

| | |
|---|---|
| Transfer Line | : 300 ºC |
| Ion Source | : 200 ºC |
| Emission Current | : 100 µA |
| Ionization Mode | : EI, 70 eV |
| Mass Resolution | : Q1 0.8 Da, Q3 at  3.0 Da (FWHM) |
| CID Gas | : Argon (200 kPa) |
| Loop Time | : 0.18 s |
| Acquisition Mode | : MRM |
| Min Dwell time per MRM | : 3 ms |
| Processing Window | : ±0.1 min |

## Sample measurement

The Shimadzu GCMS-TQ8040 equipped with the GLScience multi-mode inlet Optic-4 and an AOC-5000 Plus was used for sample measurement. MRMs and collision energies (CE) were taken from Shimadzu´s SmartDB for pesticides. MRMs and CEs for pesticides missing in the database were determined by the fully automatic MRM Optimization Tool available in the latest version of GCMSSolution (version 4.3). SmartMRM was utilized for the measurement time optimization. The algorithm guaranteed a processing time window not less than 12 seconds for each compound and a dwell time per MRM of at least 3 msec. All compounds were measured with one quantifier and one qualifier. Tables 1 & 2 provide detailed summary of analytical conditions.

# Results

Figure 1 shows the full chromatogram of the measured 360 pesticides in which all compounds elute in less than 10 minutes. Moreover, a strong tendency for co-elution is evident with an accelerated GC gradient. To enable meaningful data analysis in such a highly compressed time window, the use of tandem MS triple quadrupole technology is essential.
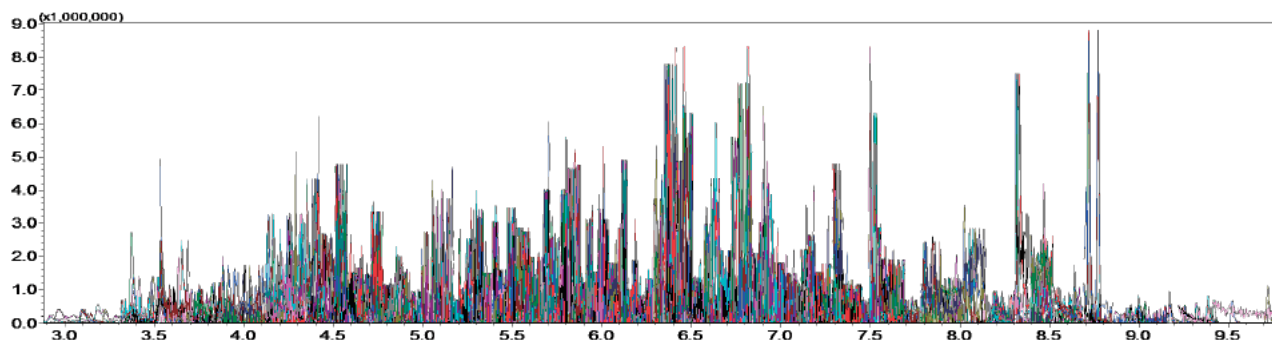


Figure 1:  Chromatogram 360 Pesticides In Apple Matrix

# Fast GC-MS/MS Analysis Of Multicomponent Pesticide Residues (360) In Food Matrix
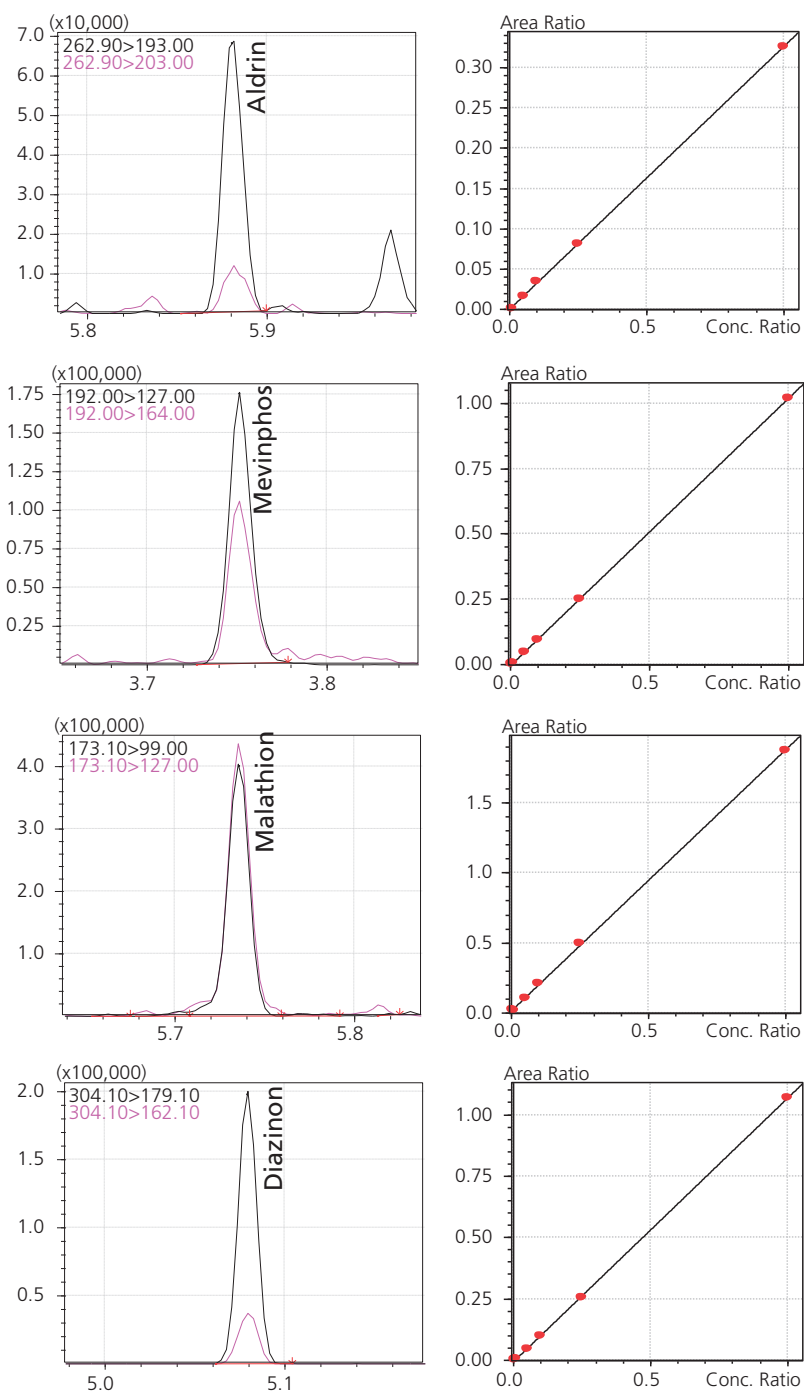


Figure 2:  Calibration Curve (0.5 ppb – 100 ppb) and Peak Profile at 5 ppb (Aldrin, Malathion, Mevinphos and Diazinon)

Results shown in figure 1 were obtained using a 5ms 20 m, 0.18 mm, 0.18 μm fast GC column. It is noteworthy that there are columns available, which have lower dimensions and offer even faster chromatographic results. Using fast GC columns, two main properties need to be taken into account when choosing ideal separation conditions. On the one hand the lower inner diameter and higher possible heating rates enable sharpened peaks and consequently higher S/N ratios. On the other hand the peak capacity decreases by lowering the column dimensions, which results in lower absolute sample amounts and reduced sensitivity[3]. Therefore, in this work an intermediate column was used which provided decreased analysis time whilst maintaining high sensitivity.

Calibration curve results were therefore determined with the aforementioned intermediate column. Matrix calibration curves (0.5 ppb – 100 ppb) were measured for all 360 pesticides. The linear correlation factor was higher than 0.9980 for every compound. Nearly all components were detectable at the lowest concentration of 0.5 ppb. Figure 2 shows peak profiles and calibration curves for some typical pesticides. As already indicated by the correlation factor, linearity was very good for all compounds.



Trifloxystrobin, %RSD = 7.6   Isazofos, %RSD = 6.4   Diazinon, %RSD = 4.6
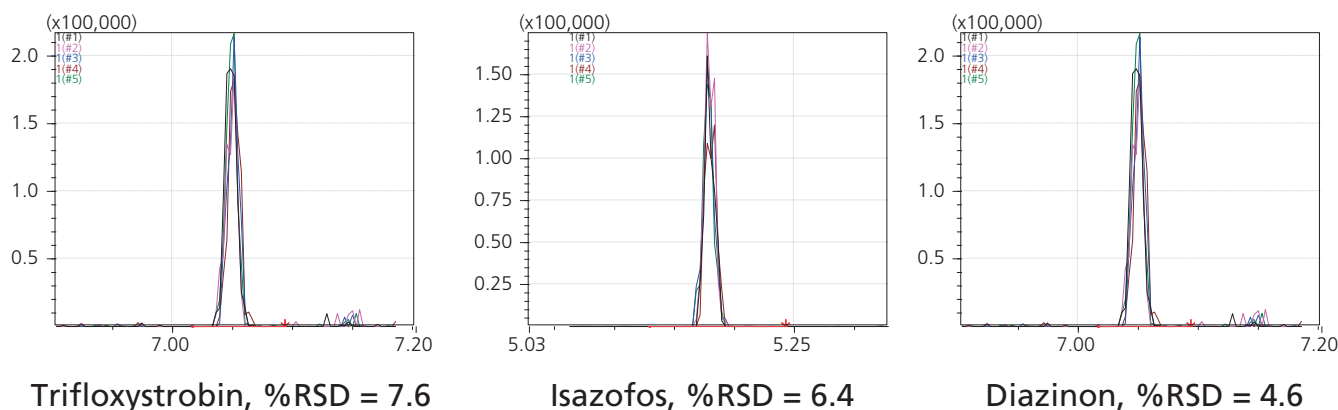
Figure 3:  Superimposition of 5 unsmoothed peaks and RSD% of 3 compounds measured at 3 ms dwell time

Peak widths at half maximum (FHMW) were typically less than 1 sec using fast GC separation compared to standard GC. Furthermore, it was known that for good reproducibility, at least 10 data points per peak are needed[4]. To enable this number of data points a loop time of 0.18 s was chosen. In some parts of the chromatogram up to 30 compounds eluted in the same processing window and for each compound two transitions (1 Quantifier and 1 Quantifier) were needed. Consequently, dwell time per MRM was in some cases reduced to as short as 3 msec. Figure 3 shows superimposed chromatograms and %RSD of three different peaks measured with a dwell time of 3 msec. It was therefore evident that %RSDs for these peaks are within acceptable limits even with such short dwell times. This degree of high precision was found for most of the compounds, and for all compounds %RSD values were below 15%. It is thought that compounds exhibiting worse precision was caused by active sites in liner or column. Therefore further optimization of the sample introduction by improved liner deactivation will help to improve %RSD for these few compounds.

# Conclusions

This study shows the successful combination of fast GC and tandem mass spectrometry. It was possible to determine 360 pesticides spiked in a QuEChERS apple extract with excellent calibration curve linearity and good reproducibility in less than 10 minutes. The shown application can help to increase routine laboratory efficiency.

# Literature

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# Application News

## No.C138

**Liquid Chromatography Mass Spectrometry**

## Multi-Residue Analysis of 18 Regulated Mycotoxins by LC/MS/MS

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Mycotoxins are one of the most important contaminants in food and feed due to their widespread distribution in the environment and toxic effects on humans and animals.[1] Structurally, mycotoxins are a very diverse group with a wide range of physicochemical properties and low molecular weights.[2] They are produced by fungi (mould) frequently found on agricultural produce, and are often not visible to the naked eye.[3] Some of the most commonly contaminated food stuffs include wheat, oats, rye, corn, barley, rice, nuts and milk.[4]

Due to the risks posed by mycotoxins in food they are regulated globally, including, the EU, US, China, Singapore and Brazil.[5] In the EU, reporting limits are harmonised in Regulation (EC) No 1886/2006 (amended by (EC) No 1126/2007) and sampling and analysis in Regulation (EC) No 401/2006.

LC/MS/MS is the technique most commonly employed for mycotoxin quantitation in order to achieve the necessary low reporting limits in complex food and feed matrices.

### ■ Experimental

Solvent extracts were provided by Scientific Analysis Laboratories (SAL, UK) following validated extraction protocols. Samples were analysed using the Nexera UHPLC and the LCMS-8060 triple quadrupole detector (Table 1). Calibration was performed using $^{13}$C internal standards spiked during sample extraction. All MRM transitions and associated internal standards for each compound are listed in Table 2. All solvents used during analysis were LCMS quality from Sigma-Aldrich.

Due to the wide range of physical and chemical properties of mycotoxins, different LC/MS/MS methods are typically developed for small groups of compounds with similar properties.

In this application paper a single LC/MS/MS method has been developed for the determination of 18 mycotoxins in food safety. Limits of quantification were at or below the maximum levels set in the EC/1886/2006 document. The scope of the method included Aflatoxins (B1, B2, G1, G2), Fumonisins (B1, B2, B3), Ochratoxin A (OTA) and Trichothecenes (3-acetyldeoxynivalenol (3AcDON), 15-acetyldeoxynivalenol (15AcDON), Deoxynivalenol (DON), Diaceteoxyscripanol (DAS), Fusarenon-X (FUS X), HT-2, Neosolaninol (NEO), Nivalenol (NIV), T2, Zearalenone (ZON)) with an analysis cycle time of 12.5 minutes.

**Table 1 Analytical Conditions**

| | |
|---|---|
| UHPLC | : Nexera LC System |
| Mobile Phase | : A; Water with additives |
| | B; Methanol with additives |
| Column | : Reversed phase column (100 mm L.× 2.1 mm I.D.) |
| Column Temperature | : 40 ˚C |
| Flowrate | : 0.4 mL/minute |
| Gradient | : B. Conc 15 % (0 min) → 25 % (1 min) |
| | → 40 % (2 min) → 41 % (4.5 min) |
| | → 100 % (7.5 - 10.0 min) → 15 % (10.10 min) |
| | → Stop (12.5 min) |
| LC-MS/MS | : LCMS-8060 |
| Dwell Time | : 10 to 40 msec. |
| Pause Time | : 1 msec. |
| Ionisation Mode | : ESI +/- |
| Polarity Switching | : 5 msec. |
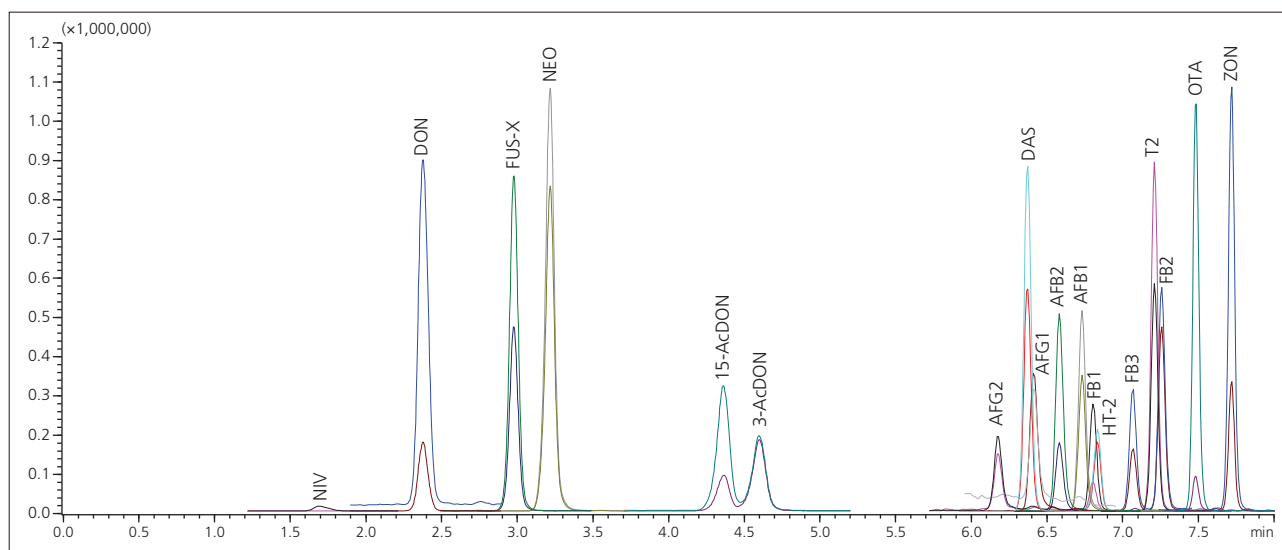


**Fig. 1 MRM Chromatograms of 18 Mycotoxins**

AFB1 (aflatoxin B1; 1 µg/kg), AFB2 (aflatoxin B2; 1 µg/kg), AFG1 (aflatoxin G1; 1 µg/kg), AFG2 (aflatoxin G2; 1 µg/kg), OTA (ochratoxin A; 4 µg/kg), FB1 (fumonisin B1; 100 µg/kg), FB2 (fumonisin B2; 100 µg/kg), FB3 (fumonisin B3; 100 µg/kg), 15-AcDON (15-acetyldeoxynivalenol; 100 µg/kg), 3-AcDON (3-acetyldeoxynivalenol; 100 µg/kg), DON (deoxynivalenol; 100 µg/kg), DAS (diaceteoxyscripanol; 100 µg/kg), FUS-X (fusarenon-X; 100 µg/kg), HT-2 (100 µg/kg), T-2 (100 µg/kg), NEO (neosolaninol; 100 µg/kg), NIV (nivalenol; 100 µg/kg), ZON (zearalenone; 100 µg/kg).
For clarity only 2 MRM transitions are displayed per compound and the following MRM chromatograms were changed; neosolaniol (x0.3), T2 (x0.3), aflatoxins (x3), fumonisins (x2).

**Table 2  All MRM's Measured in the Mycotoxin Method and Corresponding Calibration Range and R² Result**

| | Compound name | Parent ion | Ret. Time (mins) | MRM 1 | MRM 2 | MRM 3 | ISTD | Calibration range µg/kg | $R^2$ |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Aflatoxin B1 | $[M+H]^+$ | 6.773 | 313 > 241 | 313 > 285 | 313 > 269 | ¹³C Aflatoxin B1 | 0.1 - 10 | 0.9988 |
| 2 | Aflatoxin B2 | $[M+H]^+$ | 6.621 | 315 > 259 | 315 > 287 | 315 > 243 | ¹³C Aflatoxin B2 | 0.1 - 10 | 0.9995 |
| 3 | Aflatoxin G1 | $[M+H]^+$ | 6.453 | 329 > 243 | 329 > 200 | | ¹³C Aflatoxin G1 | 0.1 - 10 | 0.9998 |
| 4 | Aflatoxin G2 | $[M+H]^+$ | 6.219 | 331 > 245 | 331 > 285 | | ¹³C Aflatoxin G2 | 0.1 - 10 | 0.9965 |
| 5 | Ochratoxin A | $[M+H]^+$ | 7.509 | 404 > 239 | 404 > 221 | 404 > 358 | ¹³C Ochratoxin A | 0.4 - 40 | 0.9969 |
| 6 | Fumonisin B1 | $[M+H]^+$ | 6.811 | 722 > 352 | 722 > 334 | 722 > 704 | ¹³C Aflatoxin B2 | 10 - 1000 | 0.9937 |
| 7 | Fumonisin B2 | $[M+H]^+$ | 7.260 | 706 > 318 | 706 > 354 | 706 > 688 | ¹³C Aflatoxin B2 | 10 - 1000 | 0.9998 |
| 8 | Fumonisin B3 | $[M+H]^+$ | 7.073 | 706 > 318 | 706 > 354 | 706 > 688 | ¹³C Aflatoxin B2 | 10 - 1000 | 0.9991 |
| 9 | Deoxynivalenol | $[M+H]^+$ | 2.372 | 297 > 279 | 297 > 249 | | ¹³C Deoxynivalenol | 10 - 1000 | 0.9992 |
| 10 | Diacetoxyscirpenol | $[M+NH_4]^+$ | 6.349 | 384 > 229 | 384 > 307 | 384 > 247 | ¹³C T2 Toxin | 10 - 1000 | 0.9994 |
| 11 | T2 | $[M+NH_4]^+$ | 7.206 | 484 > 185 | 484 > 215 | 484 > 245 | ¹³C T2 Toxin | 10 - 1000 | 0.9989 |
| 12 | HT-2 | $[M+Na]^+$ | 6.822 | 447 > 345 | 447 > 285 | | ¹³C T2 Toxin | 10 - 1000 | 1.0000 |
| 13 | Nivalenol | $[M-CH_3COO]^-$ | 1.684 | 371 > 281 | 371 > 311 | | ¹³C HT-2 | 10 - 1000 | 0.9991 |
| 14 | Neosolaniol | $[M+NH_4]^+$ | 3.227 | 400 > 215 | 400 > 305 | 400 > 185 | ¹³C Deoxynivalenol | 10 - 1000 | 0.9995 |
| 15 | Fusarenon X | $[M+H]^+$ | 2.986 | 355 > 247 | 355 > 277 | | ¹³C Deoxynivalenol | 10 - 1000 | 0.9987 |
| 16 | Zearalenone | $[M-H]^-$ | 7.711 | 317 > 175 | 317 > 131 | 317 > 273 | ¹³C T2 Toxin | 10 - 1000 | 0.9985 |
| 17 | 15-Acetyldeoxynivalenol | $[M+H]^+$ | 4.406 | 339 > 261 | 339 > 297 | | ¹³C Deoxynivalenol | 10 - 1000 | 1.0000 |
| 18 | 3-Acetyldeoxynivalenol | $[M+H]^+$ | 4.618 | 339 > 261 | 339 > 297 | | ¹³C Deoxynivalenol | 10 - 1000 | 0.9986 |
| 19 | ¹³C HT-2 | $[M+NH_4]^+$ | 6.844 | 464 > 278 | | | | | |
| 20 | ¹³C T2 | $[M+NH_4]^+$ | 7.228 | 508 > 322 | | | | | |
| 21 | ¹³C Aflatoxin B1 | $[M+H]^+$ | 6.754 | 330 > 301 | | | | | |
| 22 | ¹³C Aflatoxin B2 | $[M+H]^+$ | 6.614 | 332 > 303 | | | | | |
| 23 | ¹³C Aflatoxin G1 | $[M+H]^+$ | 6.435 | 346 > 212 | | | | | |
| 24 | ¹³C Aflatoxin G2 | $[M+H]^+$ | 6.219 | 348 > 259 | | | | | |
| 25 | ¹³C Ochratoxin A | $[M+H]^+$ | 7.516 | 424 > 250 | | | | | |
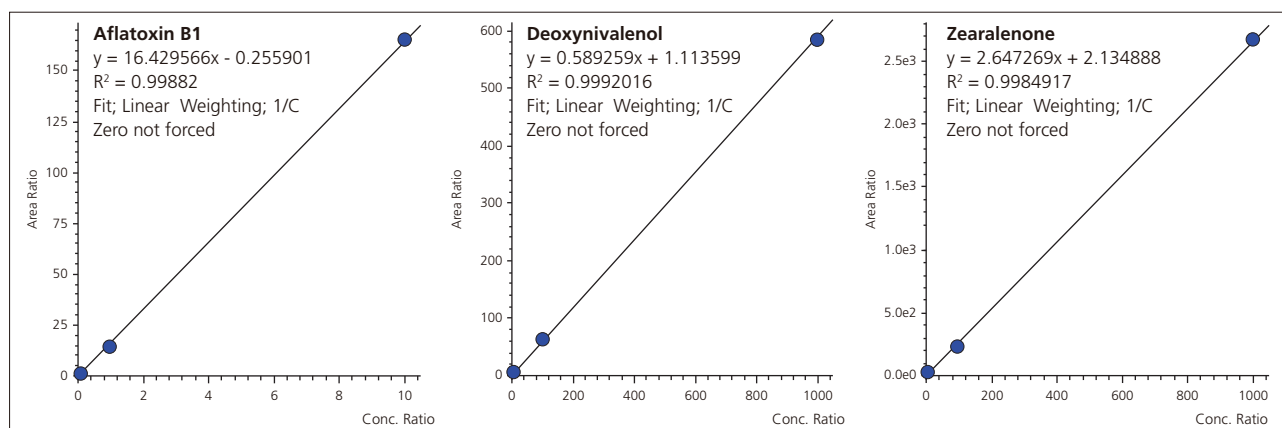


**Fig. 2  Calibration Curves for Selected Compounds**
Calibration Curves for Aflatoxin (0.1 – 10 µg/kg), Deoxynivalenol (10 – 1000 µg/kg), and Zearalenone (10 – 1000 µg/kg).

### ■ Conclusions

In this study a single method has been developed for the analysis of 18 regulated mycotoxins with an injection to injection cycle time of 12.5 minutes. This method achieves the required EU reporting limits (between 0.1 -10 µg/kg) with linear regression coefficients $R^2$ typically greater than 0.998 (Fig. 2 and Table 1). The LC mobile phase, column and gradient were all optimised and provided chromatographic resolution of 15-acetyldeoxynivalenol and 3-acetyldeoxynivalenol.

### ■ References

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# SHIMADZU
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# Application News

## No. C165

## Multi-residue analysis of 18 regulated mycotoxins by LC-MS/MS (2)

Fusarium mycotoxins are a structurally diverse group of secondary metabolites known to contaminate a diverse array of food and feed resulting in a risk for human and animal health. European guidance legislation has set maximum levels for mycotoxins in food and feed to minimize the impact to human and animal health. The most toxicologically important Fusarium mycotoxins are trichothecenes (including deoxynivalenol (DON) and T-2 toxin (T-2)), zearalenone (ZON) and fumonisin B1 (FB1).

In this work, a single LC-MS/MS method has been developed for the determination of 18 mycotoxins in food safety. Limits of quantification were at or below the maximum levels set in the EC/1886/2006 document. The scope of the method included aflatoxins (B1, B2, G1, G2), fumonisins (B1, B2, B3), ochratoxin A (OTA) and trichothecenes (3-acetyldeoxynivalenol (3-AcDON), 15-acetyldeoxynivalenol (15-AcDON), deoxynivalenol (DON), diasteoxyscripanol (DAS), fusarenon-X (FUS X), HT-2, neosolaninol (NEO), nivalenol (NIV), T2, zeareleonone (ZON)) with an analysis cycle time of 12.5 minutes.

### ■ Materials and Methods

Solvent extracts were provided by Concept Life Sciences following validated extraction protocols. Samples were measured using a Nexera UHPLC and the LCMS-8060 triple quadrupole detector (Table 1). To separate out the three pairs of regioisomers (3-AcDON/15-AcDON, FB2/FB3, and FA2/FA3) a pentafluorophenyl (PFP) column was used and compared against a C18 material. To enhance signal response a series of mobile phase additives were considered including ammonium acetate, ammonium fluoride, ammonium formate and acetic acid solutions.

In this work, ammonium fluoride solution and ammonium fluoride with acetic acid solution was the preferred solvent system as it resulted in a considerable enhancement of signal intensity in positive ion mode for all mycotoxins. Calibration was performed using ^{13}C internal standards spiked during sample extraction. All solvents used during analysis were LCMS quality from Sigma-Aldrich.

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[*1] : Shimadzu, Manchester, UK
[*2] : Concept Life Sciences, Cambridge, UK
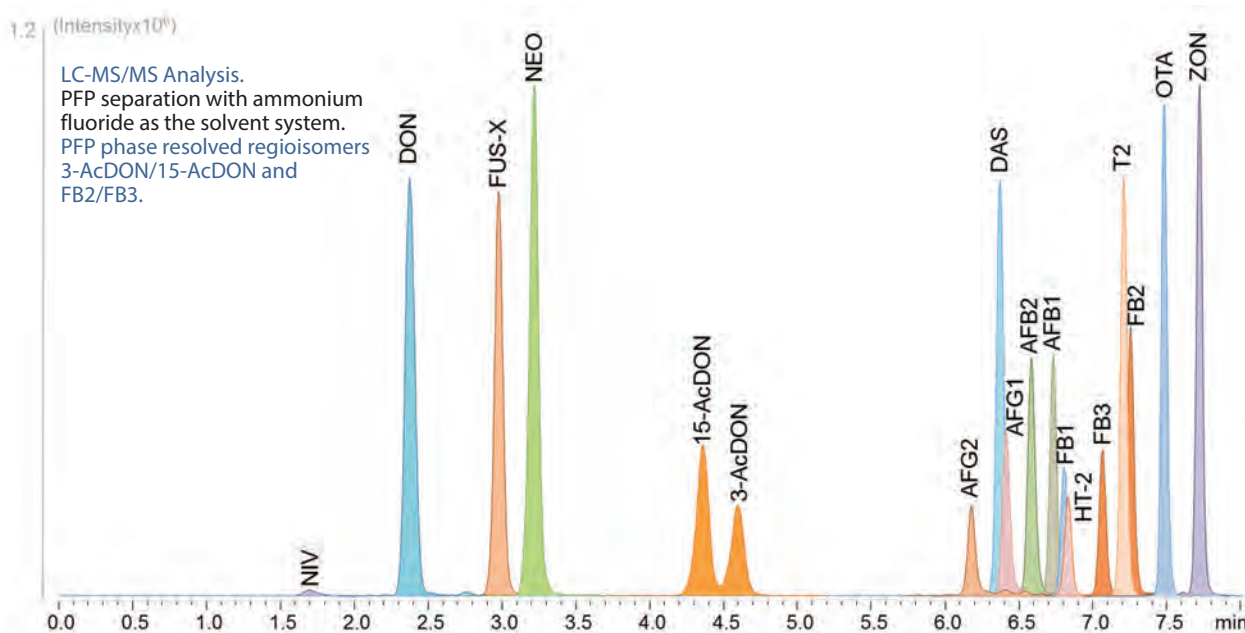


**Fig. 1  MRM chromatograms of 18 mycotoxins using a PFP bonded phase.**
AFB1 (aflatoxin B1; 1 µg/kg; rescaled x3), AFB2 (aflatoxin B2; 1 µg/kg; rescaled x3), AFG1 (aflatoxin G1; 1 µg/kg; rescaled x3),
AFG2 (aflatoxin G2; 1 µg/kg; rescaled x3), OTA (ochratoxin A; 4 µg/kg), FB1 (fumonisin B1; 100 µg/kg; rescaled x2),
FB2 (fumonisin B2; 100 µg/kg; rescaled x2), FB3 (fumonisin B3; 100 µg/kg; rescaled x2), 15-AcDON (15-acetyldeoxynivalenol; 100 µg/kg),
3-AcDON (3-acetyldeoxynivalenol; 100 µg/kg), DON (deoxynivalenol; 100 µg/kg), DAS (diasteoxyscripanol; 100 µg/kg),
FUS-X (fusarenon-X; 100 µg/kg), HT-2 (100 µg/kg), T-2 (100 µg/kg; rescaled x0.3), NEO (neosolaniol; 100 µg/kg; rescaled x0.3),
NIV (nivalenol; 100 µg/kg), ZON (zearalenone; 100 µg/kg)

## ◼ Influence of ammonium fluoride on ion signal intensity

Ammonium fluoride solution has a high gas-phase basicity and known to be effective in improving sensitivity for small molecules in negative mode LC-MS. However, ammonium fluoride has also been shown to enhance sensitivity in positive ion mode. Compared to standard mobile phases used for mycotoxin analysis the addition of ammonium fluoride has a positive impact on ion signal intensity.

Fig. 2 indicates that ammonium fluoride markedly increases ion signal intensity compared to other solvent systems. All chromatograms are normalized to the same signal intensity. Ammonium fluoride delivered higher ion signal response for mycotoxins in positive ion mode compared to other mobile phase solvent system (Fig. 2a).

**Table 1  Analytical Conditions**

| | |
|---|---|
| UHPLC | Nexera X2 LC system |
| Analytical column | Mastro PFP (100 mmL. × 2.1 mm I.D., 3 µm) |
| Column temperature | 40 °C |
| Flow rate | 0.4 mL/min |
| Solvent A | 0.15 mmol/L ammonium fluoride aqueous solution |
| Solvent B | 0.15 mM ammonium fluoride methanol solution with 2 % acetic acid |
| Binary Gradient | B conc. 15 % (0 min) - 25 % (1 min) - 40 % (2 min) - 41 % (4.5 min) - 100 % (7.5 - 10 min) - 15 % (10.1 min) – Stop (12.5 min) |
| | |
| Mass spectrometer | Shimadzu LCMS-8060 |
| Pause time/Dwell time | 1 msec/10-40 msec |
| Polarity switching time | Pos/neg switching time set to 5 msec |
| Source temperatures (interface; heat block; DL) | 300 °C; 400 °C; 250 °C |
| Gas flows (nebulising; heating; drying) | 3 L/min; 10 L/min; 10 L/min |
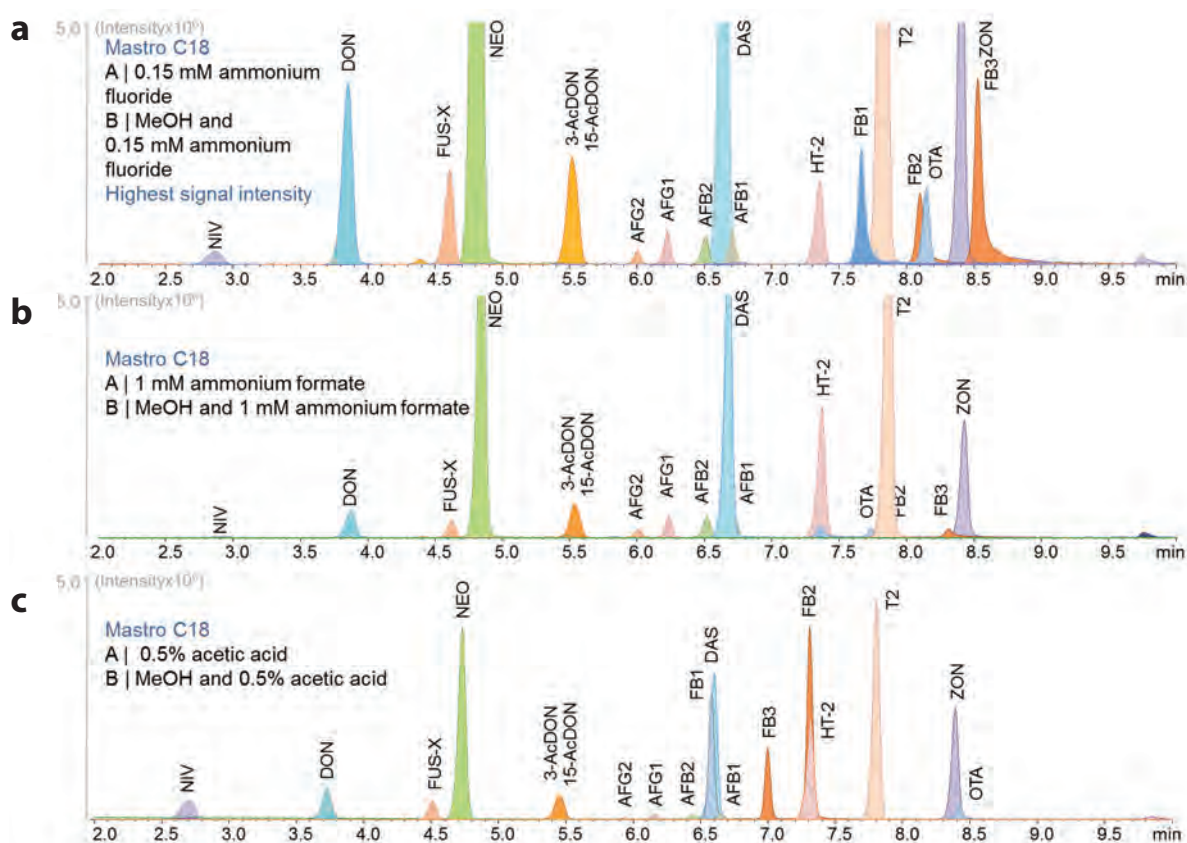


**Fig. 2  Comparison of MRM Chromatograms of 18 Mycotoxins under the Different Mobile Phase Conditions (Mastro C18 Column)**
a: Mobile Phase A = 0.15 mM Ammonium Fluoride Aqueous Solution, Mobile Phase B = 0.15 mM Ammonium Fluoride Methanol Solution
b: Mobile Phase A = 1 mM Ammonium Formate Aqueous Solution, Mobile Phase B = 1 mM Ammonium Formate Methanol Solution
c: Mobile Phase A = 0.5 % Acetic Acid Aqueous Solution, Mobile Phase B = 0.5 % Acetic Acid Methanol Solution

Fig. 3 shows 18 mycotoxins separated on a PFP phase compared to a C18 bonded material using ammonium fluoride as the mobile phase. PFP phases delivered near baseline resolution of 3- and 15-acetyldeoxynivalenol which is not possible on a C18 phase (C18 material can still be used due to preferential ionisation of 3-AcDON in negative ion mode).
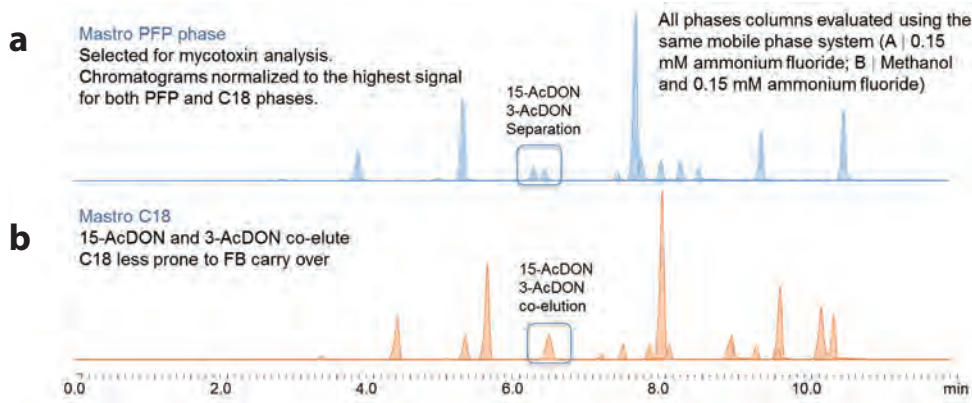


**Fig. 3** Comparison of MRM Chromatograms of 18 Mycotoxins Using Different Columns
Mobile Phase A = 0.15 mM Ammonium Fluoride Aqueous Solution,
Mobile Phase B = 0.15 mM Ammonium Fluoride Methanol Solution (for Both Columns)
a: Mastro PFP Column, b: Mastro C18 Column

## ■ Analysis of sample matrices

To separate the regioisomers 3-AcDON/15-AcDON and FB2/FB3 several PFP phases were evaluated including Mastro PFP, Kinetix PFP, Discovery HS F5 PFP and ACE PFP. Compared to a C18 bonded phase, the PFP phases delivered near baseline resolution of the regioisomers 3-AcDON/15-AcDON and FB2/FB3 but required a modification of the mobile phase to reduce FB carry over (2 % acetic acid was added to the mobile phase to negate the effects of FB's carry over).

Fig. 4 shows the analysis of a mixed spice extract and a pepper extract spiked with Aflatoxins B1, B2, G1, G2 (2.5 µg/kg) and Ochratoxin A (10 µg/kg) using ammonium fluoride solution in the mobile phase. Repeatedly injecting the extracts resulted in a %RSD typically below 10 % (n=12) for Aflatoxins B1, B2, G1, G2 (2.5 µg/kg) and Ochratoxin A (10 µg/kg).
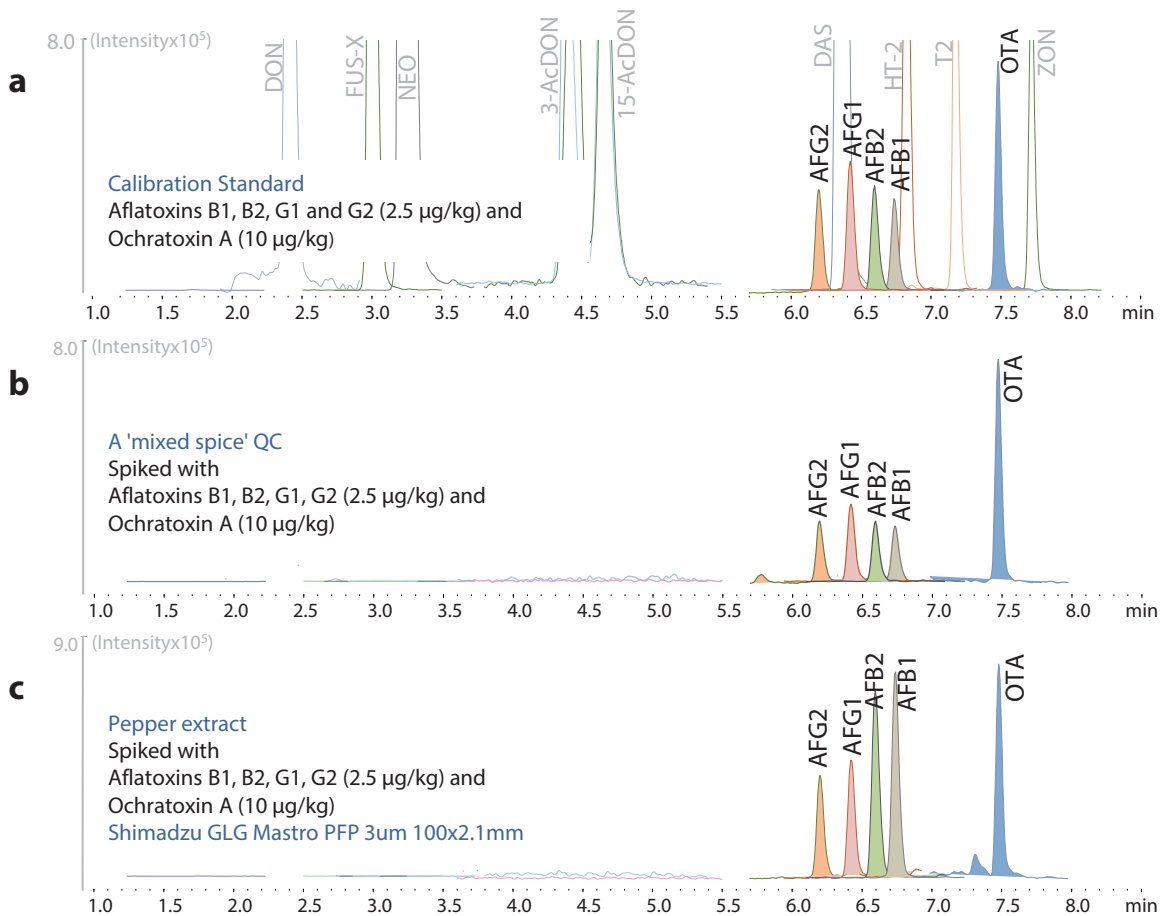


**Fig. 4** Chromatograms of the Mycotoxin Standard Solution, Mixed Spice Extract, and Pepper Extract
Spiked with Aflatoxins B1, B2, G1, G2 (2.5 µg/kg) and Ochratoxin A (10 µg/kg)
a: Mycotoxin Standard Solution, b: Mixed Spice Extract, c: Pepper Extract

**Table 2  MRM's of mycotoxins in positive and negative mode ionisation.**

| Compound name | Parent ion | RT | MRM 1 | MRM 2 | Internal Standard | Calibration range (µg/kg) | R² |
|---|---|---|---|---|---|---|---|
| Aflatoxin B1 | [M+H]⁺ | 6.773 | 313 > 241 | 313 > 285 | ¹³C Aflatoxin B1 | 0.1 - 10 | 0.9988 |
| Aflatoxin B2 | [M+H]⁺ | 6.621 | 315 > 259 | 315 > 287 | ¹³C Aflatoxin B2 | 0.1 - 10 | 0.9995 |
| Aflatoxin G1 | [M+H]⁺ | 6.453 | 329 > 243 | 329 > 200 | ¹³C Aflatoxin G1 | 0.1 - 10 | 0.9998 |
| Aflatoxin G2 | [M+H]⁺ | 6.219 | 331 > 245 | 331 > 285 | ¹³C Aflatoxin G2 | 0.1 - 10 | 0.9965 |
| Ochratoxin A | [M+H]⁺ | 7.509 | 404 > 239 | 404 > 221 | ¹³C Ochratoxin A | 0.4 - 40 | 0.9969 |
| Fumonisin B1 | [M+H]⁺ | 6.811 | 722 > 352 | 722 > 334 | ¹³C Aflatoxin B2 | 10 - 1000 | 0.9937 |
| Fumonisin B2 | [M+H]⁺ | 7.26 | 706 > 318 | 706 > 354 | ¹³C Aflatoxin B2 | 10 - 1000 | 0.9998 |
| Fumonisin B3 | [M+H]⁺ | 7.073 | 706 > 318 | 706 > 354 | ¹³C Aflatoxin B2 | 10 - 1000 | 0.9991 |
| Deoxynivalenol | [M+H]⁺ | 2.372 | 297 > 279 | 297 > 249 | ¹³C Deoxynivalenol | 10 - 1000 | 0.9992 |
| Diacetoxyscirpenol | [M+NH₄]⁺ | 6.349 | 384 > 229 | 384 > 307 | ¹³C T-2 Toxin | 10 - 1000 | 0.9994 |
| T-2 | [M+NH₄]⁺ | 7.206 | 484 > 185 | 484 > 215 | ¹³C T-2 Toxin | 10 - 1000 | 0.9989 |
| HT-2 | [M+Na]⁺ | 6.822 | 447 > 345 | 447 > 285 | ¹³C T-2 Toxin | 10 - 1000 | 1.0000 |
| Nivalenol | [M+CH₃COO]⁻ | 1.684 | 371 > 281 | 371 > 311 | ¹³C HT-2 | 10 - 1000 | 0.9991 |
| Neosolaniol | [M+NH₄]⁺ | 3.227 | 400 > 215 | 400 > 305 | ¹³C Deoxynivalenol | 10 - 1000 | 0.9995 |
| Fusarenon X | [M+H]⁺ | 2.986 | 355 > 247 | 355 > 277 | ¹³C Deoxynivalenol | 10 - 1000 | 0.9987 |
| Zearalenone | [M-H]⁻ | 7.711 | 317 > 175 | 317 > 131 | ¹³C T2 Toxin | 10 - 1000 | 0.9985 |
| 15-Acetyldeoxynivalenol | [M+H]⁺ | 4.406 | 339 > 261 | 339 > 297 | ¹³C Deoxynivalenol | 10 - 1000 | 1.0000 |
| 3-Acetyldeoxynivalenol | [M+H]⁺ | 4.618 | 339 > 261 | 339 > 297 | ¹³C Deoxynivalenol | 10 - 1000 | 0.9986 |
| ¹³C HT-2 | [M+NH₄]⁺ | 6.844 | 464 > 278 | | | | |
| ¹³C T-2 | [M+NH₄]⁺ | 7.228 | 508 > 322 | | | | |
| ¹³C Aflatoxin B1 | [M+H]⁺ | 6.754 | 330 > 301 | | | | |
| ¹³C Aflatoxin B2 | [M+H]⁺ | 6.614 | 332 > 303 | | | | |
| ¹³C Aflatoxin G1 | [M+H]⁺ | 6.435 | 346 > 212 | | | | |
| ¹³C Aflatoxin G2 | [M+H]⁺ | 6.219 | 348 > 259 | | | | |
| ¹³C Ochratoxin A | [M+H]⁺ | 7.516 | 424 > 250 | | | | |

■ **Conclusions**

Ammonium fluoride as a solvent system results in a higher signal response for mycotoxins in positive ion detection.

To negate any possible carry over effects with fumonisin's 2 % acetic acid was added to the mobile phase.

PFP bonded phases deliver a separation of mycotoxin regioisomers which can be applied routinely.

This method results in higher sensitivity for mycotoxins and can be applied to both PFP and C18 phases in routine quantitation with a cycle time of 12.5 minutes.

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## Technical Report

# Multi-Residue Analysis of 210 Pesticides in Food Samples by Triple Quadrupole UHPLC-MS/MS

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## Abstract

Pesticides and their metabolites are of great concern to society as they are harmful to human health, pollute natural resources and disturb the equilibrium of the ecosystem. Consequently, stricter food safety regulations are being enforced around the world, placing pesticide analysis laboratories under increasing pressure to expand the list of targeted pesticides, detect analytes at lower levels and with greater precision, reduce analysis turnaround times, and all the while maintaining or reducing costs. In this study a method was successfully developed for the quantitation of 210 commonly analysed pesticides in food samples using the Nexera UHPLC and LCMS-8040. Initial validation was performed to demonstrate instrument capabilities. Limits of detection (LOD) for 90 % of compounds were less than 0.001 mg kg$^{-1}$ (1 ppb) and all compounds were less than 0.01 mg kg$^{-1}$ (10 ppb) for both the quantifying and qualifying transitions using only a 2 µL injection. Repeatability at the 0.01 mg kg$^{-1}$ reporting level was typically less than 5 %RSD for compounds and correlation coefficients were typically greater than 0.997 in a variety of studied food extracts. Consequently, the LCMS-8040 is ideally suited for routine monitoring of pesticides below the 0.01 mg kg$^{-1}$ default level set by EU and Japanese legislation.

**Keywords**: Pesticides; Multi-residue analysis; LCMS-8040; Food safety; Fruit; Vegetables

## 1. Introduction

Pesticide residues in food continue to be the target of studies due to the uncertainty concerning adverse effects that those residues may have on human health after a lengthy exposure at low levels. More than 1000 active ingredients have been utilised and are formulated in thousands of different commercial products. They include a variety of compounds, mainly insecticides, herbicides and fungicides, as well as their metabolites, with very different physico-chemical characteristics and large differences in polarity, volatility and persistence.[1] Consequently, in order to ensure food safety for consumers and to facilitate international trade, regulatory bodies around the world have established maximum residue levels (MRLs) for pesticide residues in food commodities; that is, the maximum amount of pesticide residue and its toxic metabolites that might be expected on a commodity if good agricultural practice was adhered to during the use of the pesticide.[2]

In the European Union regulation 396/2005/EC was implemented in 2008 harmonising pesticide MRLS in all member states for 435 pesticide active substances in 378 commodities.[3] This EU regulation covers pesticides both currently and formerly used in agriculture in or outside the EU. For pesticide and food commodity combinations not listed in the regulation a default MRL of 0.01 mg kg$^{-1}$ applies (Art 18(1b) of European Union Regulation No 396/2005).[3] In general, MRLs in the European Food regulation are in the range 0.01 - 10 mg kg$^{-1}$ depending on the pesticide-commodity combination, with the lowest levels set for banned pesticides. For vegetables, fruits and cereals intended for the production of baby foods, Directive 2006/141/EC requires that baby food contains no detectable levels of pesticide residues defined as < 0.01 mg kg$^{-1}$ and prohibits the use of certain very toxic

pesticides in the production of infant foods and establishes even lower MRLs for a few other very toxic pesticides.[4] Regulatory bodies around the world, as in the EU, have produced similar guidelines. In the US, tolerances for more than 450 pesticides and other ingredients are stated in the electronic Code of Federal Regulations (US Environmental Protection Agency Office of Pesticide Programs) and are enforced by the US FDA.[5] Japan's positive list system for agricultural chemical residues in foods, introduced in 2006, contains MRLs for over 400 pesticides in various commodities.[6] China published national standard GB 2763-2005 in 2005 and more recently GB 28260-2011 which was introduced in 2012 and specifies 181 MRLS for 85 pesticides in food.[7,8]

Consequently, pesticide analysis laboratories are under increasing pressure to expand the list of targeted pesticides, detect analytes at lower levels and with greater precision, reduce analysis turnaround times and reduce usage of hazardous solvents while maintaining or reducing costs. Pesticide residues were traditionally analysed mainly by GC-based multi-residue methods often with MS detection. However, many modern

(semi)polar compounds and/or ionic compounds could not be analysed in this way due to poor thermal stability or volatility without the need for derivatisation.[9] Recent improvements in liquid chromatography - tandem mass spectrometry, combined with the discussed pitfalls of GCMS, have meant LCMSMS has become a vital technique. LC-tripe quadruple mass spectrometry enables highly selective and sensitive analysis and is well suited to the multi-class analysis of large numbers of pesticides at trace levels.

In this work, we discuss the development of a multi-residue pesticide method for 210 pesticides using the Nexera UHPLC and LCMS-8040 triple quadruple. Pesticides were matrix-matched in food matrix (lettuce, pear and dried fruit) following QuEChERS sample preparation. The method was evaluated in matrix to ensure that the necessary reporting limits were obtained according to the various regulatory guidelines around the world with acceptable precision, in addition to ensuring chromatographic resolution of pesticide isomers with identical SRM transitions.

## 2. Experimental

A stock of pesticides was obtained from the Food and Environment Agency, UK, at a concentration of 0.01 mg kg^{-1} (for each pesticide) in acetone:acetonitrile 1:1. Linearity was investigated over a nine-point calibration with samples ranging from 0.5 µg kg^{-1} - 0.2 mg kg^{-1} (0.5 – 200 ppb) analysed in duplicate; calibration samples were injected once in increasing order and once in decreasing order. Linearity was assessed with four calibration curves prepared by serial dilution of: (1) acetonitrile, (2) dried fruit extract, (3) lettuce extract and, (4) pear extract. Instrumental area repeatability was determined by replicate (n=6) injection of pear matrix at 0.01 mg kg^{-1}. LC-MS mobile phase solvents and additives were all of LC–MS quality and purchased from Sigma–Aldrich.

Food extracts were supplied by the Food and Environment Agency, UK, following established QuEChERS protocols. QuEChERS is acronym for Quick Easy Cheap Effective Rugged Safe and is a widely used sample preparation technique for the extraction of pesticides from food. Food samples included dried fruit, lettuce and pear, with the final extracts prepared in 100% acetonitrile.

**LC Parameters**

| UHPLC: | Nexera UHPLC system | | |
|---|---|---|---|
| Column: | Shim-pack XR-ODS III (150 x 2 mm, 2.2 µm particle size) | | |
| Column temp.: | 40 °C | | |
| Mobile phase: | A = Water with 5 mM ammonium formate and 0.01 % formic acid<br>B = Methanol with 5 mM ammonium formate and 0.01 % formic acid | | |
| Gradient: | Time (min) | A% | B% |
| | 0 | 5 | 95 |
| | 16 | 0 | 100 |
| | 18 | 0 | 100 |
| | 18.1 | 5 | 95 |
| | 20 | 5 | 95 |
| Flow rate: | 0.4 mL min^{-1} | | |
| Injection volume: | 32 µL (stacked injection: 2µL sample + 30µL water) | | |
| Needle wash: | 1000 µL Methanol | | |

**MS Parameters**

| MS: | LCMS-8040 triple quadrupole mass spectrometer |
|---|---|
| Ionisation: | ESI - Positive and negative (15 msec. polarity switch) |
| SRM: | Dwell time 5 msec.<br>Pause time 1 msec. |
| Desolvation line: | 250 °C |
| Heating block: | 400 °C |
| Drying gas: | 15 L min^{-1} |
| Nebulising gas: | 2 L min^{-1} |
| SRM optimisation: | 1:1 water:methanol with 10mM ammonium acetate<br>Flow rate: 0.5mL min^{-1}<br>Flow injection analysis (No column fitted)<br>0.2 µL (0.01 mg kg^{-1} pesticide standard solution) |
| Mobile phase screening: | Carrier 1:1 water:methanol<br>Flow rate: 0.3mL min^{-1}<br>Flow injection analysis (No column fitted)<br>5µL injection (0.01 mg kg^{-1} pesticide standard solution)<br>1µL air gap<br>(see text for mobile phase compositions) |

Pesticide limits of detection were calculated based on the method described by the US-EPA in Title 40 Code of Federal Regulation Part 136,[10] using a standard deviation of 7 replicates in pear matrix at a concentration value that corresponds to an instrument signal to noise ratio in the range of 2.5 to 5 and a Student's t 99% confidence interval:

$$MDL = St(n-1, 1-\alpha = 0.99) \; x \; s.d.$$

Where, t(n-1,1-α=0.99) = Student's t value for the 99% confidence level with n-1 degrees of freedom (t = 3.14 for 7 replicates), n = number of replicates, and s.d. = standard deviation of the replicate analyses.

## 3. Results and discussion

### 3.1 SRM optimisation

Target precursor and product ions were selected based on recommendations from the Food and Environment Agency, UK, and data from the EURL DataPool.[11] Typically the protonated or deprotonated molecule was used for the precursor ion. In order to try to prevent interference of SRM transitions from matrix, product ions greater than m/z 100 were selected where possible as they are typically more diagnostic.[12] Analyte specific MS parameters (Q1 pre-bias (V), Q3 pre-bias (V) and collision energy) were optimised using automated flow injection analysis. Briefly, this involves placing pesticide standards into the auto-sampler, from where they are then rapidly injected into the MS with a different parameter optimised on each injection. Each compound was optimised in only a few minutes using the automated software provided in LabSolutions. This allowed large numbers of compounds to be optimised overnight; this is in stark contrast to traditional time-consuming infusion in order to optimise parameters. The compounds studied and their associated transitions are shown in Table 1.

**Table 1** - *Studied compounds and their chemical formulas, CAS numbers, SRMs, retention times, limits of detection and $R^2$*

| Compound | Formula | CAS | Transition 1 | Transition 2 | Pear extract | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | RT (min.) | Transition 1 LOD (ppb) | Transition 2 LOD (ppb) | %RSD (10ppb) | $R^2$ |
| Avermectin B1a | C48H72O14 | 71751-41-2 | 891 > 305 | 891 > 567 | 16.4 | 0.35 | 0.56 | 5.0 | 0.9975 |
| Acephate | C4H10NO3PS | 30560-19-1 | 184 > 143 | 184 > 49 | 3.0 | 0.17 | 0.31 | 1.0 | 0.9999 |
| Acetamiprid | C10H11ClN4 | 135410-20-7 | 223 > 126 | 223 > 99 | 7.2 | 0.50 | 1.00 | 1.1 | 0.9979 |
| Acrinathrin | C26H21F6NO5 | 101007-06-1 | 559 > 208 | 559 > 181 | 16.1 | 1.32 | 2.36 | 4.4 | 0.9990 |
| Alachlor | C14H20ClNO2 | 15972-60-8 | 270 > 238 | 270 > 162 | 13.4 | 0.09 | 0.26 | 1.5 | 0.9995 |
| Aldicarb | C7H14N2O2S | 116-06-3 | 208 > 116 | 208 > 89 | 8.5 | 0.05 | 0.10 | 1.7 | 0.9998 |
| Aldicarb sulfone | C7H14N2O4S | 1646-88-4 | 240 > 223 | 240 > 86 | 4.3 | 0.17 | 0.13 | 1.8 | 0.9999 |
| Aldicarb sulfoxide | C7H14N2O3S | 1646-87-3 | 207 > 89 | 207 > 132 | 3.9 | 0.22 | 0.36 | 2.3 | 1.0000 |
| Amidosulfuron | C9H15N5O7S2 | 120923-37-7 | 370 > 261 | 370 > 139 | 9.3 | 0.14 | 0.22 | 2.8 | 0.9984 |
| Asulam | C8H10N2O4S | 3337-71-1 | 231 > 156 | 231 > 92 | 3.4 | 0.72 | 2.03 | 3.8 | 0.9979 |
| Atrazine | C8H14ClN5 | 1912-24-9 | 216 > 174 | 216 > 104 | 11.1 | 0.10 | 0.22 | 2.4 | 0.9989 |
| Azinphos-methyl | C10H12N3O3PS2 | 86-50-0 | 318 > 132 | 318 > 77 | 11.8 | 0.50 | 0.50 | 2.7 | 0.9903 |
| Azoxystrobin | C22H17N3O5 | 131860-33-8 | 404 > 372 | 404 > 344 | 12.1 | 0.03 | 0.30 | 2.1 | 0.9989 |
| Bendiocarb | C11H13NO4 | 22781-23-3 | 224 > 109 | 224 > 167 | 9.8 | 0.10 | 0.09 | 1.5 | 0.9996 |
| Benthiavalicarb-isopropyl | C18H24FN3O3S | 177406-68-7 | 382 > 180 | 382 > 116 | 12.7 | 0.12 | 0.41 | 0.9 | 0.9997 |
| Bispyribac sodium | C19H17N4NaO8 | 125401-92-5 | 453 > 297 | 453 > 179 | 12.1 | 1.41 | 5.43 | 7.4 | 0.9954 |
| Boscalid | C18H12Cl2N2O | 188425-85-6 | 343 > 307 | 343 > 140 | 12.5 | 0.81 | 1.19 | 4.6 | 0.9968 |
| Bromoxynil* | C7H3Br2NO | 1689-84-5 | 274 > 79 | 276 > 81 | 9.9 | 2.24 | 2.61 | 4.5 | 0.9968 |
| Bromuconazole | C13H12BrCl2N3O | 116255-48-2 | 376 > 159 | 376 > 70 | 13.0 | 0.72 | 1.79 | 2.9 | 0.9994 |
| Butachlor | C17H26ClNO2 | 23184-66-9 | 312 > 238 | 312 > 57 | 15.3 | 0.29 | 0.39 | 1.6 | 0.9998 |
| Butocarboxim | C7H14N2O2 S | 34681-10-2 | 208 > 75 | 208 > 191 | 8.4 | 0.13 | 0.87 | 3.1 | 0.9999 |
| Butocarboxim sulfone | C7H14N2O4S | 34681-23-7 | 223 > 106 | 223 > 166 | 4.1 | 2.63 | 3.23 | 9.7 | 0.9949 |
| Butocarboxim sulfoxide | C7H14N2O3S | 34681-24-8 | 207 > 88 | 207 > 75 | 3.7 | 0.22 | 0.21 | 1.9 | 0.9999 |
| Carbaryl | C12H11NO2 | 63-25-2 | 202 > 145 | 202 > 127 | 10.3 | 0.13 | 0.22 | 2.4 | 0.9988 |
| Carbendazim | C9H9N3O2 | 10605-21-7 | 192 > 160 | 192 > 132 | 7.1 | 0.50 | 1.00 | 1.1 | 0.9996 |
| Carbofuran | C12H15NO3 | 1563-66-2 | 222 > 165 | 222 > 123 | 11.1 | 0.12 | 0.18 | 0.7 | 0.9993 |
| Carboxin | C12H13NO2S | 5234-68-4 | 236 > 143 | 236 > 87 | 10.2 | 0.09 | 0.25 | 0.9 | 0.9991 |
| Chlorantraniliprole* | C18H14BrCl2N5O2 | 500008-45-7 | 482 > 284 | 482 > 177 | 11.8 | 0.50 | 1.00 | 2.3 | 0.9979 |
| Chlorfenvinfos | C12H14Cl3O4P | 470-90-6 | 361 > 155 | 361 > 99 | 14.0 | 0.28 | 0.49 | 2.3 | 0.9966 |
| Chloridazon | C10H8ClN3O | 1698-60-8 | 222 > 92 | 222 > 104 | 7.2 | 0.20 | 0.18 | 3.2 | 0.9990 |
| Chlorotoluron | C10H13ClN2O | 15545-48-9 | 213 > 72 | 213 > 46 | 10.8 | 0.05 | 0.13 | 1.3 | 0.9967 |
| Chromafenozide | C24H30N2O3 | 143807-66-3 | 395 > 175 | 395 > 91 | 13.0 | 0.05 | 0.60 | 1.0 | 0.9977 |
| Clethodim | C17H26ClNO3S | 99129-21-2 | 360 > 164 | 360 > 268 | 14.7 | 0.08 | 0.45 | 0.7 | 0.9970 |
| Clofentezine | C14H8Cl2N4 | 74115-24-5 | 303 > 138 | 303 > 102 | 14.4 | 4.03 | 5.76 | 9.5 | 0.9967 |
| Clothianidin | C6H8ClN5O2S | 210880-92-5 | 250 > 132 | 250 > 169 | 6.5 | 0.25 | 0.12 | 1.6 | 0.9978 |
| Cyazofamid | C13H13ClN4O2S | 120116-88-3 | 325 > 108 | 325 > 261 | 13.3 | 0.39 | 3.74 | 2.4 | 0.9964 |
| Cycloxydim | C17H27NO3S | 101205-02-1 | 326 > 280 | 326 > 180 | 14.8 | 0.33 | 0.73 | 1.0 | 0.9989 |
| Cyflufenamid | C20H17F5N2O2 | 180409-60-3 | 413 > 295 | 413 > 241 | 14.2 | 0.27 | 0.29 | 2.9 | 0.9982 |
| Cymoxanil | C7H10N4O3 | 57966-95-7 | 199 > 128 | 199 > 111 | 7.7 | 2.99 | 3.52 | 5.5 | 0.9960 |
| Cyproconazole | C15H18ClN3O | 113096-99-4 | 292 > 70 | 292 > 125 | 12.8 | 0.41 | 0.60 | 3.5 | 0.9988 |
| Cyprodinil | C14H15N3 | 121552-61-2 | 226 > 93 | 226 > 108 | 13.9 | 0.89 | 0.91 | 1.3 | 0.9990 |
| Cyromazine | C6H10N6 | 66215-27-8 | 167 > 85 | 167 > 125 | 2.2 | 2.57 | 4.79 | 7.4 | 0.9994 |
| Demeton-S-methyl sulfoxide | C6H15O4PS2 | 301-12-2 | 247 > 169 | 247 > 109 | 5.0 | 0.01 | 0.03 | 1.2 | 0.9999 |
| Demeton-S-methyl sulfone | C6H15O5PS2 | 17040-19-6 | 263 > 169 | 263 > 109 | 5.3 | 0.03 | 0.10 | 3.1 | 0.9999 |
| Desmedipham | C16H16N2O4 | 13684-56-5 | 318 > 182 | 318 > 136 | 11.6 | 0.08 | 0.33 | 0.5 | 0.9971 |
| Diclobutrazol | C15H19Cl2N3O | 75736-33-3 | 328 > 70 | 330 > 70 | 13.8 | 0.17 | 0.20 | 2.7 | 0.9988 |

*Table 1* – Continued...

| Compound | Formula | CAS | Transition 1 | Transition 2 | Pear extract | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | RT (min.) | Transition 1 LOD (ppb) | Transition 2 LOD (ppb) | %RSD (10ppb) | R² |
| Diethofencarb | C14H21NO4 | 87130-20-9 | 268 > 226 | 268 > 124 | 12.2 | 0.06 | 0.12 | 2.2 | 0.9996 |
| Difenoconazole | C19H17Cl2N3O3 | 119446-68-3 | 406 > 251 | 406 > 188 | 14.5 | 0.18 | 0.53 | 2.6 | 0.9994 |
| Diflubenzuron | C14H9ClF2N2O2 | 35367-38-5 | 311 > 158 | 311 > 141 | 13.5 | 2.21 | 7.48 | 9.2 | 0.9936 |
| Dimethoate | C5H12NO3PS2 | 60-51-5 | 230 > 125 | 230 > 199 | 7.0 | 0.05 | 0.07 | 1.6 | 0.9997 |
| Dimethomorph | C21H22ClNO4 | 110488-70-5 | 388 > 301 | 388 > 165 | 12.7 | 0.29 | 0.41 | 2.5 | 0.9991 |
| Dimoxystrobin | C19H22N2O3 | 149961-52-4 | 327 > 205 | 327 > 116 | 13.7 | 0.12 | 0.14 | 0.5 | 0.9997 |
| Dinotefuran | C7H14N4O3 | 165252-70-0 | 203 > 129 | 203 > 157 | 3.9 | 0.10 | 0.22 | 2.9 | 0.9994 |
| Disulfoton sulfoxide | C8H19O3PS3 | 2497-07-6 | 291 > 213 | 291 > 97 | 10.8 | 0.05 | 0.15 | 2.6 | 0.9980 |
| Diuron | C9H10Cl2N2O | 330-54-1 | 233 > 72 | 235 > 72 | 11.4 | 0.09 | 0.26 | 0.6 | 0.9971 |
| DMPF | C10H14N2 | 33089-74-6 | 163 > 107 | 163 > 122 | 4.8 | 1.00 | 2.00 | 2.5 | 0.9910 |
| Dodine | C15H33N3O2 | 2439-10-3 | 228 > 71 | 228 > 60 | 13.5 | 0.30 | 0.54 | 1.7 | 0.9946 |
| Epoxiconazole | C17H13ClFN3O | 135319-73-2 | 330 > 121 | 330 > 101 | 13.3 | 0.12 | 0.37 | 2.5 | 0.9998 |
| Ethiofencarb | C11H15NO2S | 29973-13-5 | 226 > 107 | 226 > 169 | 10.6 | 0.18 | 0.59 | 0.7 | 0.9994 |
| Ethiofencarb sulfone | C11H15NO2S2 | 53380-23-7 | 275 > 107 | 275 > 201 | 6.2 | 0.02 | 0.16 | 0.9 | 0.9999 |
| Ethiofencarb sulfoxide | C11H15NO3S | 53380-22-6 | 242 > 107 | 242 > 185 | 6.5 | 0.02 | 0.02 | 0.9 | 0.9999 |
| Ethirimol | C11H19N3O | 23947-60-6 | 210 > 140 | 210 > 98 | 10.8 | 0.14 | 0.24 | 1.8 | 0.9977 |
| Etofenprox | C25H28O3 | 80844-07-1 | 394 > 177 | 394 > 359 | 16.9 | 0.03 | 0.06 | 3.1 | 0.9983 |
| Fenamidone | C17H17N3OS | 161326-34-7 | 312 > 92 | 312 > 236 | 12.4 | 0.06 | 0.18 | 1.9 | 0.9988 |
| Fenamiphos | C13H22NO3PS | 22224-92-6 | 304 > 217 | 304 > 202 | 13.5 | 0.05 | 0.28 | 1.9 | 0.9970 |
| Fenamiphos sulfone | C13H22NO5PS | 31972-44-8 | 336 > 266 | 336 > 188 | 10.2 | 0.31 | 0.25 | 4.3 | 0.9961 |
| Fenamiphos sulfoxide | C13H22NO4PS | 31972-43-7 | 320 > 108 | 320 > 171 | 10.0 | 0.18 | 0.52 | 3.3 | 0.9976 |
| Fenbuconazole | C19H17ClN4 | 114369-43-6 | 337 > 125 | 337 > 70 | 13.4 | 0.23 | 0.40 | 5.0 | 0.9964 |
| Fenhexamid | C14H17Cl2NO2 | 126833-17-8 | 302 > 97 | 302 > 55 | 13.1 | 0.75 | 0.95 | 0.9 | 0.9944 |
| Fenoxycarb | C17H19NO4 | 79127-80-3 | 302 > 88 | 302 > 116 | 13.6 | 0.10 | 0.20 | 2.4 | 0.9989 |
| Fenpropimorph | C20H33NO | 67564-91-4 | 304 > 147 | 304 > 117 | 14.1 | 0.05 | 0.13 | 1.6 | 0.9995 |
| Fenpyroximate | C24H27N3O4 | 111812-58-9 | 422 > 366 | 422 > 215 | 15.9 | 0.02 | 0.17 | 1.2 | 0.9997 |
| Fenthion sulfoxide | C10H15O4PS2 | 3761-41-9 | 295 > 109 | 295 > 280 | 10.1 | 0.18 | 0.27 | 1.5 | 0.9985 |
| Fenthion sulfone | C10H15O5PS2 | 3761-42-0 | 311 > 109 | 311 > 125 | 10.4 | 3.75 | 3.61 | 9.8 | 0.9974 |
| Fipronil* | C12H4Cl2F6N4OS | 120068-37-3 | 435 > 330 | 435 > 250 | 13.5 | 0.11 | 0.35 | 4.1 | 0.9998 |
| Fluazifop acid* | C15H12F3NO4 | 69335-91-7 | 328 > 282 | 328 > 91 | 11.8 | 0.55 | 3.61 | 7.1 | 0.9983 |
| Fluazinam* | C13H4Cl2F6N4O4 | 79622-59-6 | 463 > 416 | 463 > 398 | 15.2 | 0.20 | 0.27 | 2.7 | 0.9994 |
| Fludioxonil* | C12H6F2N2O2 | 131341-86-1 | 247 > 126 | 247 > 180 | 12.4 | 1.00 | 1.00 | 4.2 | 0.9974 |
| Flufenacet | C14H13F4N3O2S | 142459-58-3 | 364 > 152 | 364 > 194 | 13.2 | 0.04 | 0.06 | 1.6 | 0.9986 |
| Flufenoxuron | C21H11ClF6N2O3 | 101463-69-8 | 489 > 158 | 489 > 141 | 15.7 | 0.24 | 0.63 | 8.2 | 0.9989 |
| Fluometuron | C10H11F3N2O | 2164-17-2 | 233 > 72 | 233 > 46 | 10.6 | 0.12 | 0.14 | 1.3 | 0.9996 |
| Fluopicolide | C14H8Cl3F3N2O | 239110-15-7 | 383 > 173 | 383 > 145 | 12.7 | 0.05 | 0.17 | 2.1 | 0.9967 |
| Fluoxastrobin | C21H16ClFN4O5 | 361377-29-9 | 459 > 427 | 459 > 188 | 13.1 | 0.19 | 0.22 | 1.7 | 0.9987 |
| Fluroxypyr* | C7H5Cl2FN2O3 | 69377-81-7 | 253 > 195 | 255 > 197 | 7.8 | 1.13 | 1.75 | 5.7 | 0.9993 |
| Flutriafol | C16H13F2N3O | 76674-21-0 | 302 > 70 | 302 > 123 | 11.1 | 0.29 | 0.43 | 3.2 | 0.9984 |
| Fosthiazate | C9H18NO3PS2 | 98886-44-3 | 284 > 104 | 284 > 228 | 10.7 | 0.05 | 0.12 | 2.7 | 0.9985 |
| Furathiocarb | C18H26N2O5S | 65907-30-4 | 383 > 195 | 383 > 252 | 15.1 | 0.07 | 0.13 | 1.8 | 1.0000 |
| Halofenozide | C18H19ClN2O2 | 112226-61-6 | 331 > 105 | 331 > 275 | 12.3 | 0.05 | 0.05 | 1.7 | 0.9947 |
| Halosulfuron-methyl* | C13H15ClN6O7S | 100784-20-1 | 435 > 182 | 437 > 182 | 11.5 | 0.30 | 0.96 | 3.1 | 0.9968 |
| Haloxyfop acid* | C15H11ClF3NO4 | 69806-34-4 | 360 > 288 | 362 > 290 | 13.3 | 6.20 | 6.86 | 13.4 | 0.9999 |
| Heptenophos | C9H12ClO4P | 23560-59-0 | 251 > 127 | 251 > 89 | 11.4 | 0.15 | 1.36 | 4.7 | 0.9982 |
| Hexythiazox | C17H21ClN2O2S | 78587-05-0 | 353 > 228 | 353 > 168 | 15.6 | 2.25 | 1.02 | 4.5 | 0.9956 |

*Table 1* – Continued...

| Compound | Formula | CAS | Transition 1 | Transition 2 | Pear extract | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | RT (min.) | Transition 1 LOD (ppb) | Transition 2 LOD (ppb) | %RSD (10ppb) | R² |
| Imazalil | C14H14Cl2N2O | 35554-44-0 | 297 > 159 | 297 > 69 | 11.8 | 0.30 | 0.48 | 3.5 | 0.9988 |
| Imidacloprid | C9H10ClN5O2 | 138261-41-3 | 256 > 209 | 256 > 175 | 6.4 | 0.50 | 0.50 | 1.9 | 0.9966 |
| Indoxacarb | C22H17ClF3N3O7 | 144171-61-9 | 528 > 203 | 528 > 150 | 14.5 | 0.40 | 0.37 | 3.9 | 0.9964 |
| Ioxynil* | C7H3I2NO | 1689-83-4 | 370 > 127 | 370 > 215 | 11.0 | 0.12 | 1.00 | 3.6 | 0.9961 |
| Iprovalicarb | C18H28N2O3 | 140923-17-7 | 321 > 119 | 321 > 203 | 13.1 | 0.06 | 0.23 | 2.5 | 0.9981 |
| Isazofos | C9H17ClN3O3PS | 42509-80-8 | 314 > 120 | 314 > 162 | 12.9 | 0.04 | 0.13 | 2.2 | 0.9994 |
| Isocarbofos | C11H16NO4PS | 24353-61-5 | 307 > 231 | 307 > 121 | 11.4 | 0.07 | 0.12 | 2.7 | 0.9991 |
| Isofenphos | C15H24NO4PS | 25311-71-1 | 346 > 245 | 346 > 217 | 14.3 | 0.17 | 0.13 | 1.7 | 0.9991 |
| Isofenphos-methyl | C14H22NO4PS | 99675-03-3 | 332 > 231 | 332 > 273 | 13.8 | 0.03 | 0.13 | 1.2 | 0.9996 |
| Isoprocarb | C11H15NO2 | 2631-40-5 | 194 > 95 | 194 > 137 | 11.1 | 0.20 | 0.49 | 1.9 | 0.9990 |
| Isoprothiolane | C12H18O4S2 | 50512-35-1 | 291 > 189 | 291 > 231 | 12.6 | 0.10 | 0.09 | 0.9 | 0.9994 |
| Isoproturon | C12H18N2O | 34123-59-6 | 207 > 72 | 207 > 46 | 11.3 | 0.10 | 0.11 | 1.7 | 0.9996 |
| Isoxaben | C18H24N2O4 | 82558-50-7 | 333 > 165 | 333 > 150 | 12.6 | 0.02 | 0.06 | 0.9 | 0.9989 |
| Kresoxim-methyl | C18H19NO4 | 143390-89-0 | 314 > 116 | 314 > 206 | 13.8 | 0.15 | 0.18 | 3.3 | 0.9991 |
| Lenacil | C13H18N2O2 | 2164-08-1 | 235 > 153 | 235 > 136 | 11.2 | 0.18 | 0.64 | 2.2 | 0.9987 |
| Linuron | C9H10Cl2N2O2 | 330-55-2 | 249 > 160 | 249 > 182 | 12.2 | 3.15 | 3.20 | 3.7 | 0.9979 |
| Lufenuron* | C17H8Cl2F8N2O3 | 103055-07-8 | 509 > 339 | 509 > 175 | 15.2 | 0.35 | 2.39 | 3.8 | 0.9918 |
| Malathion | C10H19O6PS2 | 121-75-5 | 348 > 127 | 348 > 331.2 | 12.6 | 0.04 | 0.31 | 1.0 | 0.9989 |
| Mandipropamid | C23H22ClNO4 | 374726-62-2 | 412 > 328 | 412 > 356 | 12.5 | 0.11 | 0.45 | 4.2 | 0.9991 |
| Mecarbam | C10H20NO5PS2 | 2595-54-2 | 330 > 227 | 330 > 97 | 13.2 | 0.15 | 0.30 | 2.0 | 0.9992 |
| Mepanipyrim | C14H13N3 | 110235-47-7 | 224 > 106 | 224 > 77 | 13.1 | 0.19 | 0.39 | 3.6 | 0.9993 |
| Mepronil | C17H19NO2 | 55814-41-0 | 270 > 119 | 270 > 91 | 12.7 | 0.05 | 0.07 | 1.1 | 0.9972 |
| Mesosulfuron-methyl | C17H21N5O9S2 | 208465-21-8 | 504 > 182 | 504 > 83 | 10.9 | 0.27 | 0.96 | 3.4 | 0.9996 |
| Metaflumizone | C24H16F6N4O2 | 139968-49-3 | 507 > 178 | 507 > 287 | 15.1 | 2.63 | 3.42 | 6.6 | 0.9986 |
| Metalaxyl | C15H21NO4 | 57837-19-1 | 280 > 220 | 280 > 192 | 11.3 | 0.04 | 0.06 | 1.9 | 0.9998 |
| Metamitron | C10H10N4O | 41394-05-2 | 203 > 175 | 203 > 104 | 7.0 | 0.21 | 0.44 | 2.3 | 0.9990 |
| Metconazole | C17H22ClN3O | 125116-23-6 | 320 > 70 | 322 > 125 | 14.2 | 0.10 | 0.30 | 3.6 | 0.9976 |
| Methabenzthiazuron | C10H11N3OS | 18691-97-9 | 222 > 165 | 222 > 150 | 11.1 | 0.11 | 0.19 | 0.9 | 0.9989 |
| Methamidophos | C2H8NO2PS | 10265-92-6 | 142 > 94 | 142 > 125 | 2.3 | 0.06 | 0.69 | 1.3 | 0.9991 |
| Methiocarb | C11H15NO2S | 2032-65-7 | 226 > 121 | 226 > 169 | 12.3 | 0.10 | 0.28 | 2.9 | 0.9948 |
| Methiocarb sulfoxide | C11H15NO3S | 2635-10-1 | 242 > 122 | 242 > 170 | 6.9 | 0.04 | 0.15 | 1.5 | 0.9996 |
| Methomyl | C5H10N2O2S | 16752-77-5 | 163 > 88 | 163 > 106 | 5.0 | 0.10 | 0.10 | 0.8 | 0.9996 |
| Methoxyfenozide | C22H28N2O3 | 161050-58-4 | 369 > 149 | 369 > 313 | 12.7 | 0.50 | 1.00 | 1.7 | 0.9980 |
| Metobromuron | C9H11BrN2O2 | 3060-89-7 | 259 > 148 | 259 > 91 | 10.9 | 0.35 | 0.63 | 3.2 | 0.9987 |
| Metolachlor | C15H22ClNO2 | 51218-45-2 | 284 > 252 | 284 > 176 | 13.4 | 0.06 | 0.31 | 1.5 | 0.9962 |
| Metolcarb | C9H11NO2 | 1129-41-5 | 166 > 109 | 166 > 94 | 9.1 | 0.12 | 0.29 | 2.4 | 0.9996 |
| Metosulam | C14H13Cl2N5O4S | 139528-85-1 | 418 > 175 | 418 > 140 | 10.1 | 0.24 | 0.23 | 2.2 | 0.9968 |
| Metoxuron | C10H13ClN2O2 | 19937-59-8 | 229 > 72 | 229 > 156 | 8.7 | 0.04 | 0.30 | 1.4 | 0.9997 |
| Metrafenone | C19H21BrO5 | 220899-03-6 | 409 > 209 | 409 > 227 | 14.4 | 0.09 | 0.10 | 1.3 | 0.9993 |
| Metsulfuron-methyl | C14H15N5O6S | 74223-64-6 | 382 > 167 | 382 > 77 | 9.2 | 0.19 | 0.97 | 1.2 | 0.9982 |
| Mevinphos | C7H13O6P | 7786-34-7 | 225 > 127 | 225 > 193 | 7.1 | 0.05 | 0.16 | 2.5 | 0.9998 |
| Molinate | C9H17NOS | 2212-67-1 | 188 > 126 | 188 > 55 | 12.9 | 2.08 | 1.25 | 3.1 | 0.9956 |
| Monocrotophos | C7H14NO5P | 6923-22-4 | 224 > 193 | 224 > 127 | 5.6 | 0.72 | 1.35 | 4.8 | 0.9991 |
| Monuron | C9H11ClN2O | 150-68-5 | 199 > 72 | 199 > 46 | 9.4 | 0.13 | 0.21 | 1.6 | 0.9995 |
| Myclobutanil | C15H17ClN4 | 88671-89-0 | 289 > 70 | 289 > 125 | 12.8 | 0.23 | 0.44 | 2.6 | 0.9990 |
| Neoquassin | C22H30O6 | 76-77-7 | 391 > 373 | 391 > 207 | 10.2 | 0.29 | 1.63 | 2.3 | 0.9970 |

*Table 1* – *Continued...*

| Compound | Formula | CAS | Transition 1 | Transition 2 | Pear extract | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | RT (min.) | Transition 1 LOD (ppb) | Transition 2 LOD (ppb) | %RSD (10ppb) | R² |
| Nitenpyram | C11H15ClN4O2 | 120738-89-8 | 271 > 126 | 271 > 225 | 4.7 | 0.15 | 0.29 | 2.6 | 1.0000 |
| Nuarimol | C17H12ClFN2O | 63284-71-9 | 315 > 252 | 315 > 81 | 12.2 | 0.75 | 2.66 | 2.8 | 0.9990 |
| Omethoate | C5H12NO4PS | 1113-02-6 | 214 > 125 | 214 > 183 | 3.6 | 0.16 | 0.18 | 1.6 | 0.9998 |
| Oxadixyl | C14H18N2O4 | 77732-09-3 | 296 > 279 | 296 > 219 | 9.0 | 0.25 | 0.26 | 1.7 | 0.9999 |
| Oxamyl | C7H13N3O3S | 23135-22-0 | 237 > 72 | 237 > 90 | 4.6 | 0.03 | 0.10 | 1.5 | 0.9999 |
| Paclobutrazol | C30H40Cl2N6O2 | 76738-62-0 | 294 > 70 | 294 > 125 | 12.6 | 0.18 | 2.74 | 2.4 | 0.9982 |
| Penconazole | C13H15Cl2N3 | 66246-88-6 | 284 > 70 | 284 > 159 | 13.9 | 0.17 | 0.20 | 2.6 | 0.9992 |
| Pencycuron | C19H21ClN2O | 66063-05-6 | 329 > 125 | 329 > 218 | 14.4 | 0.03 | 0.39 | 1.5 | 0.9992 |
| Phenmedipham | C16H16N2O4 | 13684-63-4 | 318 > 168 | 318 > 136 | 11.8 | 0.36 | 0.32 | 1.0 | 0.9949 |
| Phenthoate | C12H17O4PS2 | 2597-03-7 | 321 > 79 | 321 > 247 | 13.7 | 0.32 | 0.55 | 2.3 | 0.9993 |
| Phorate sulfone | C7H17O5PS2 | 2588-04-7 | 293 > 171 | 293 > 97 | 11.0 | 0.51 | 0.26 | 3.4 | 0.9964 |
| Phorate sulfoxide | C7H17O4PS2 | 2588-05-8 | 277 > 97 | 277 > 199 | 10.8 | 0.26 | 0.13 | 0.9 | 0.9979 |
| Phosphamidon | C10H19ClNO5P | 297-99-4 | 300 > 174 | 300 > 127 | 9.3 | 0.10 | 0.19 | 1.0 | 0.9998 |
| Phoxim | C12H15N2O3PS | 14816-18-3 | 299 > 77 | 299 > 129 | 14.1 | 0.25 | 0.30 | 2.0 | 0.9992 |
| Picolinafen | C19H12F4N2O2 | 137641-05-5 | 377 > 238 | 377 > 145 | 15.2 | 0.26 | 1.38 | 5.4 | 0.9999 |
| Picoxystrobin | C18H16F3NO4 | 117428-22-5 | 368 > 145 | 368 > 205 | 13.5 | 0.12 | 0.17 | 1.3 | 0.9994 |
| Pirimicarb | C11H18N4O2 | 23103-98-2 | 239 > 72 | 239 > 182 | 10.8 | 0.05 | 0.10 | 2.1 | 0.9996 |
| Pirimicarb-desmethyl | C10H16N4O2 | 152-16-9 | 225 > 72 | 225 > 168 | 8.5 | 0.04 | 0.04 | 1.7 | 0.9996 |
| Prochloraz | C15H16Cl3N3O2 | 67747-09-5 | 376 > 308 | 376 > 70 | 14.3 | 0.10 | 0.19 | 2.8 | 0.9987 |
| Profenofos | C11H15BrClO3PS | 41198-08-7 | 375 > 305 | 375 > 347 | 15.0 | 0.30 | 0.38 | 2.6 | 0.9997 |
| Promecarb | C12H17NO2 | 2631-37-0 | 208 > 109 | 208 > 151 | 12.5 | 0.44 | 0.42 | 3.1 | 0.9993 |
| Prometryn | C10H19N5S | 7287-19-6 | 242 > 158 | 242 > 200 | 13.1 | 0.07 | 0.08 | 1.6 | 0.9998 |
| Propamocarb free base | C9H20N2O2 | 24579-73-5 | 189 > 102 | 189 > 74 | 3.1 | 0.23 | 0.22 | 1.4 | 0.9984 |
| Propaquizafop | C22H22ClN3O5 | 111479-05-1 | 444 > 100 | 44 > 371 | 15.2 | 0.15 | 0.85 | 1.2 | 0.9990 |
| Propiconazole | C15H17Cl2N3O2 | 60207-90-1 | 342 > 159 | 342 > 69 | 14.0 | 0.23 | 0.60 | 3.6 | 0.9998 |
| Propoxur | C11H15NO3 | 114-26-1 | 210 > 111 | 210 > 168 | 9.7 | 0.07 | 0.08 | 2.6 | 0.9998 |
| Propyzamide | C12H11Cl2NO | 23950-58-5 | 256 > 190 | 258 > 192 | 12.7 | 1.83 | 1.94 | 6.0 | 0.9915 |
| Prosulfuron | C15H16F3N5O4S | 94125-34-5 | 420 > 141 | 420 > 167 | 11.7 | 0.43 | 0.82 | 2.0 | 0.9940 |
| Prothioconazole | C14H15Cl2N3OS | 178928-70-6 | 312 > 70 | 314 > 70 | 13.4 | 0.16 | 0.50 | 2.3 | 0.9952 |
| Pymetrozine | C10H11N5O | 123312-89-0 | 218 > 105 | 218 > 79 | 5.0 | 0.05 | 0.39 | 2.9 | 0.9994 |
| Pyraclostrobin | C19H18ClN3O4 | 175013-18-0 | 388 > 194 | 388 > 163 | 14.2 | 0.50 | 1.00 | 1.9 | 0.9996 |
| Pyrethrin I | C21H28O3 | 121-21-1 | 329 > 161 | 329 > 105 | 15.9 | 0.25 | 1.20 | 2.3 | 0.9998 |
| Pyrethrin II | C22H28O5 | 121-29-9 | 373 > 161 | 373 > 133 | 14.6 | 0.70 | 2.27 | 4.2 | 0.9992 |
| Pyrimethanil | C12H13N3 | 53112-28-0 | 200 > 107 | 200 > 82 | 12.3 | 0.10 | 0.50 | 0.9 | 0.9999 |
| Pyriproxyfen | C20H19NO3 | 95737-68-1 | 322 > 96 | 322 > 185 | 15.5 | 0.07 | 0.10 | 0.6 | 0.9999 |
| Quassia | C22H28O6 | 76-78-8 | 389 > 223 | 389 > 163 | 9.1 | 0.57 | 0.80 | 2.7 | 0.9968 |
| Quinmerac | C11H8ClNO2 | 90717-03-6 | 222 > 204 | 222 > 141 | 6.8 | 0.09 | 0.45 | 1.8 | 0.9966 |
| Quinoxyfen | C15H8Cl2FNO | 124495-18-7 | 308 > 197 | 308 > 162 | 15.6 | 0.18 | 0.23 | 3.2 | 0.9998 |
| Rimsulfuron | C14H17N5O7S2 | 122931-48-0 | 432 > 182 | 432 > 325 | 10.0 | 0.31 | 0.64 | 2.8 | 0.9989 |
| Rotenone | C23H22O6 | 83-79-4 | 395 > 213 | 395 > 192 | 13.5 | 0.44 | 0.52 | 3.5 | 0.9976 |
| Spinosyn A | C41H65NO10 | 131929-60-7 | 733 > 142 | 733 > 98 | 14.1 | 0.03 | 0.19 | 1.6 | 0.9997 |
| Spinosyn D | C42H67NO10 | 131929-63-0 | 747 > 142 | 747 > 98 | 14.6 | 0.20 | 0.97 | 3.3 | 1.0000 |
| Spiromesifen | C23H30O4 | 283594-90-1 | 388 > 273 | 388 > 371 | 15.6 | 0.05 | 0.34 | 2.3 | 0.9998 |
| Spiroxamine | C18H35NO2 | 118134-30-8 | 298 > 144 | 298 > 100 | 11.7 | 0.08 | 0.18 | 2.1 | 0.9999 |
| Sulcotrione | C14H13ClO5S | 99105-77-8 | 329 > 139 | 329 > 69 | 7.5 | 0.70 | 5.00 | 4.3 | 0.9969 |
| Tebuconazole | C16H22ClN3O | 107534-96-3 | 308 > 70 | 310 > 70 | 13.9 | 0.10 | 0.34 | 2.1 | 0.9993 |

*Table 1* – *Continued...*

| Compound | Formula | CAS | Transition 1 | Transition 2 | Pear extract | | | | |
|----------|---------|-----|--------------|--------------|---------|---------------------------|---------------------------|------------------|-------|
| | | | | | RT (min.) | Transition 1 LOD (ppb) | Transition 2 LOD (ppb) | %RSD (10ppb) | $R^2$ |
| Tebufenozide | C22H28N2O2 | 112410-23-8 | 353 > 133 | 353 > 297 | 13.5 | 0.04 | 0.10 | 1.5 | 0.9980 |
| Tebufenpyrad | C18H24ClN3O | 119168-77-3 | 334 > 117 | 334 > 147 | 15.2 | 0.30 | 0.28 | 0.9 | 0.9998 |
| Teflubenzuron* | C14H6Cl2F4N2O2 | 83121-18-0 | 379 > 339 | 379 > 359 | 15.3 | 0.29 | 0.40 | 3.6 | 0.9973 |
| Terbufos sulfone | C9H21O4PS3 | 56070-16-7 | 321 > 97 | 321 > 171 | 12.1 | 0.55 | 0.52 | 3.8 | 0.9956 |
| Terbufos sulfoxide | C9H21O3PS3 | 10548-10-4 | 305 > 187 | 305 > 97 | 12.1 | 0.09 | 0.09 | 1.3 | 0.9989 |
| Tetraconazole | C13H11Cl2F4N3O | 112281-77-3 | 372 > 159 | 372 > 70 | 13.2 | 0.29 | 0.55 | 2.6 | 0.9950 |
| Thiabendazole | C10H7N3S | 148-79-8 | 202 > 175 | 202 > 131 | 8.2 | 2.50 | 2.50 | 1.5 | 0.9987 |
| Thiacloprid | C10H9ClN4S | 111988-49-9 | 253 > 126 | 253 > 90 | 7.9 | 0.10 | 0.50 | 1.0 | 0.9991 |
| Thiamethoxam | C8H10ClN5O3S | 153719-23-4 | 292 > 211 | 292 > 181 | 5.3 | 0.04 | 0.08 | 2.4 | 0.9995 |
| Thiodicarb | C10H18N4O4S3 | 59669-26-0 | 355 > 88 | 355 > 108 | 10.6 | 0.08 | 0.18 | 1.1 | 0.9991 |
| Thiophanate-methyl | C12H14N4O4S2 | 23564-05-8 | 343 > 151 | 343 > 311 | 9.7 | 0.25 | 0.62 | 1.1 | 0.9967 |
| Tolfenpyrad | C21H22ClN3O2 | 129558-76-5 | 384 > 197 | 384 > 91 | 15.3 | 0.28 | 0.73 | 3.0 | 0.9983 |
| Triadimefon | C14H16ClN3O2 | 43121-43-3 | 294 > 69 | 294 > 197 | 12.8 | 0.24 | 0.31 | 2.6 | 0.9985 |
| Triadimenol | C14H18ClN3O2 | 55219-65-3 | 296 > 70 | 298 > 70 | 13.1 | 0.24 | 0.54 | 3.7 | 0.9982 |
| Triasulfuron | C14H16ClN5O5S | 82097-50-5 | 402 > 141 | 402 > 167 | 9.6 | 0.42 | 0.36 | 1.5 | 0.9993 |
| Triazamate acid* | C11H18N4O3S | 112143-82-5 | 287 > 198 | 287 > 170 | 10.1 | 0.09 | 0.26 | 4.4 | 0.9996 |
| Triazophos | C12H16N3O3PS | 24017-47-8 | 314 > 162 | 314 > 119 | 12.9 | 0.02 | 0.12 | 1.5 | 0.9992 |
| Triclopyr* | C7H4Cl3NO3 | 55336-06-3 | 256 > 198 | 254 > 196 | 11.1 | 1.95 | 1.81 | 8.9 | 0.9969 |
| Tricyclazole | C9H7N3S | 41814-78-2 | 190 > 136 | 190 > 163 | 8.3 | 0.10 | 0.20 | 2.3 | 0.9993 |
| Trifloxystrobin | C20H19F3N2O4 | 141517-21-7 | 409 > 186 | 409 > 145 | 14.6 | 0.02 | 0.05 | 1.2 | 0.9994 |
| Triflumizole | C15H15ClF3N3O | 68694-11-1 | 346 > 278 | 346 > 43 | 14.8 | 0.09 | 0.09 | 1.3 | 0.9996 |
| Triflumuron* | C15H10ClF3N2O3 | 64628-44-0 | 357 > 154 | 357 > 176 | 14.2 | 1.76 | 3.12 | 4.6 | 0.9991 |
| Triforine | C10H14Cl6N4O2 | 26644-46-2 | 435 > 390 | 437 > 392 | 11.7 | 0.92 | 3.53 | 4.8 | 0.9963 |
| Triticonazole | C17H20ClN3O | 131983-72-7 | 318 > 70 | 320 > 70 | 13.2 | 0.40 | 0.41 | 1.9 | 0.9993 |
| Zoxamide | C14H16Cl3NO2 | 156052-68-5 | 336 > 187 | 336 > 159 | 14.0 | 0.09 | 0.29 | 1.3 | 0.9951 |
| 2,4-D* | C8H6Cl2O3 | 94-75-7 | 219 > 161 | 219 > 125 | 10.3 | 1.09 | 5.00 | 9.7 | 0.9980 |

* Negative electrospray ionisation

## 3.2 Rapid screening of different mobile phase compositions on signal response

The signal intensity in LCMS can be strongly influenced by the mobile phase composition. In order to optimise the signal intensity, pesticides were added into vials containing different mobile phase compositions and injected into the interface with no column installed. The Nexera auto-sampler was setup to inject an air gap both before and after the injected sample in order to prevent the sample mixing with carrier mobile phase. This approach enables a large number of potential mobile phase compositions to be screened in a short automated period of time and without the need to manually change mobile phases. Ten different mobile phase compositions were tested, including: ammonium acetate, ammonium formate, formic acid, acetic acid, and ammonium formate with formic acid in water:methanol or acetonitrile 1:1. A total of 23 different pesticides were assessed, selected to include a range of different polarities and both positively and negatively ionised compounds. The different mobile phases tested and their peak area response, relative to the highest peak area response obtained for that compound, are shown in Table 2.

As expected with multi residue methods, there was not one optimum mobile phase for all pesticides. Overall, the lowest signal was achieved for mobile phases containing water:methanol only, and the mobile phase containing water:acetonitrile 10 mM ammonium acetate. Negatively ionised compounds (fludioxinil and ioxynil) provided superior responses in water:methanol 10mM ammonium acetate, while the addition of either formic acid or acetic acid decreased response. The highest signals were typically found in 10 mM ammonium formate, 10mM ammonium acetate, and 10 mM ammonium formate with 0.1 % formic acid. The effect of methanol and acetonitrile in the mobile phase was also investigated. Comparison of 10mM ammonium formate in methanol and acetonitrile showed that intensities were typically lower with the use of acetonitrile. Similarly the use of ammonium acetate in methanol and acetonitrile presented the same trend. The same observation with regards to methanol and acetonitrile for pesticide analysis have been reported by others.[13]

**Table 2** - *Results of rapid mobile phase screening using flow injection analysis for 23 pesticides. All peaks areas were normalised against the maximum peak area achieved for that compound. Accordingly, 100 % indicates the highest peak area achieved and is highlighted.*

| Compound | H2O:MeOH | H2O:MeOH 0.05% Formic acid | H2O:MeOH 0.1% Formic acid | H2O:MeOH 0.2% Formic acid | H2O:MeOH 5mM Ammonium acetate | H2O:MeOH 10mM Ammonium acetate | H2O:MeOH 20mM Ammonium acetate | H2O:MeOH 50mM Ammonium acetate | H2O:MeCN 10mM Ammonium acetate | H2O:MeOH 10mM Ammonium formate | H2O:MeCN 10mM Ammonium formate | H2O:MeOH 0.1% Acetic acid | H2O:MeOH 0.1% Formic acid 50mM ammonium formate |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Atrazine | 52 | **100** | 99 | 88 | 52 | 71 | 66 | 62 | 48 | 80 | 50 | 52 | 87 |
| Azinphos-methyl | 14 | 32 | 32 | 27 | 75 | 98 | 87 | 59 | 26 | **100** | 26 | 30 | 96 |
| Azoxystrobin | 27 | 30 | 29 | 25 | 69 | 87 | 77 | 58 | 65 | **100** | 82 | 29 | 99 |
| Carbendazim | 66 | **100** | 91 | 92 | 37 | 42 | 38 | 32 | 26 | 71 | 36 | 64 | 81 |
| Chlorantraniliprole | **100** | 46 | 52 | 41 | 69 | 81 | 92 | 69 | 27 | 91 | 60 | 94 | 56 |
| Cyprodinil | 66 | 94 | 88 | 86 | 55 | 63 | 57 | 41 | 51 | **100** | 82 | 67 | 78 |
| Difenoconazole | 27 | 85 | 90 | 72 | 70 | **100** | 92 | 73 | 59 | 99 | 62 | 61 | 90 |
| Fludioxinil | 69 | 42 | 38 | 37 | 74 | **100** | 95 | 84 | 60 | 94 | 81 | 55 | 76 |
| Imazalil | 85 | 69 | 62 | 63 | 66 | 78 | 73 | 62 | 51 | **100** | 68 | 58 | 74 |
| Ioxynil | **100** | 47 | 41 | 43 | 41 | 60 | 60 | 51 | 34 | 62 | 53 | 55 | 53 |
| Isoproturon | 28 | 34 | 34 | 30 | 74 | 93 | 84 | 75 | 78 | **100** | 90 | 30 | 98 |
| Metalaxyl | 30 | 31 | 31 | 25 | 68 | 92 | 81 | 76 | 79 | **100** | 87 | 31 | 92 |
| Myclobutanil | 15 | 71 | 75 | 57 | 65 | **100** | 91 | 73 | 23 | 86 | 25 | 58 | 84 |
| Pirimicarb | 82 | 85 | 76 | 78 | 66 | 90 | 80 | 68 | 68 | **100** | 80 | 66 | 78 |
| Pirimicarb-desmethyl | 72 | 90 | 81 | 83 | 64 | 85 | 74 | 67 | 64 | **100** | 82 | 70 | 86 |
| Prochloraz | 38 | **100** | 94 | 89 | 47 | 65 | 56 | 45 | 45 | 61 | 46 | 64 | 64 |
| Pyraclostrobin | 33 | 32 | 30 | 27 | 62 | 78 | 70 | 55 | 61 | **100** | 82 | 26 | 93 |
| Pyrimethanil | 54 | **100** | 92 | 91 | 54 | 65 | 54 | 31 | 48 | 92 | 74 | 62 | 76 |
| Tebufenozide | 28 | 40 | 40 | 36 | 70 | 88 | 78 | 65 | 73 | 96 | 84 | 33 | **100** |
| Thiabendazole | 96 | **100** | 91 | 89 | 58 | 69 | 61 | 48 | 37 | 99 | 60 | 67 | 84 |
| Thiacloprid | 16 | 28 | 28 | 25 | 53 | 59 | 45 | 32 | 34 | 86 | 49 | 18 | **100** |
| Thiophanate methyl | 24 | 21 | 24 | 17 | 62 | 77 | 62 | 44 | 34 | 98 | 43 | 31 | **100** |
| Triadimenol | 17 | 96 | **100** | 81 | 56 | 88 | 86 | 74 | 44 | 79 | 46 | 66 | 74 |
| Minimum | 14 | 21 | 24 | 17 | 37 | 42 | 38 | 31 | 23 | 61 | 25 | 18 | 53 |
| Maximum | 100 | 100 | 100 | 92 | 75 | 100 | 95 | 84 | 79 | 100 | 90 | 94 | 100 |
| **Average** | **50** | **64** | **62** | **57** | **61** | **80** | **72** | **58** | **49** | **91** | **63** | **52** | **83** |

## 3.3 Performance Optimising Injection Sequence (POISe)

In reversed phase UHPLC, early eluting compounds typically display the greatest peak distortion. Peak distortion is a particular problem is pesticide analysis as samples are typically extracted by QuEChERS, with samples diluted in 100% acetonitrile (a strong eluting solvent). To solve this issue, laboratories may decide to dilute the acetonitrile extracts in water before LCMS injection. However, doing so adds an additional sample preparation step and dilution in water can also negatively affect the stability of some analytes.[14]

To minimise peak dispersion with the injection of acetonitrile extracts, one potential solution is the use of a band compression technique.[15] Band compression is achieved by injecting a band of weak eluting solvent onto the column after the analytes. As the analyte and the weak eluting solvent bands travel towards the column, minute mixing occurs. Therefore, the analytes are dissolved in a weak eluting solvent when they reach the column leading to isocratic band compression.

The performance optimising injection sequence (POISe) was evaluated by injecting between 5 – 40 µL of water following a 3 µL injection of pear extract in 100% acetonitrile. This was achieved using the Nexera auto-sampler (SIL-30AC) pre-treatment program to perform this function.

Figure 1 shows the injection of pear extract *with* and *without* the performance optimising injection sequence. Using POISe, band dispersion was minimised considerably for early eluting pesticides, with peak widths reduced by 5-69%. The optimum amount of water to inject following the sample was found to be 30 µL. Increasing this volume to 40 µL did not provide any significant improvements. Early eluting compounds are affected by the injection of a weak eluting solvent band to a much larger extent in comparison to analytes with higher retention factors. This improvement is due to the reduction in the sample solvent elution strength, which has a large impact on the early eluting compounds. Whereas, analytes with higher retention factors will experience some degree of band compression in the mobile phase already. Table 3 lists the peak width for 11 early eluting compounds. Compounds are arranged in retention time order to show the improvement using the POISe on early eluting analytes.

**(A) 3 µL pear extract injection *without* the POISe**
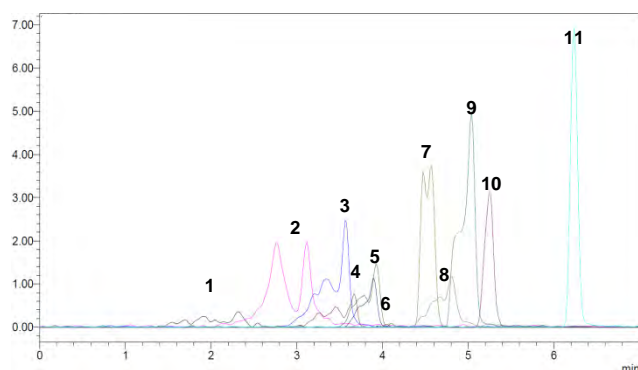
**(B) 3 µL pear extract injection *with* the POISe (30 µL water)**
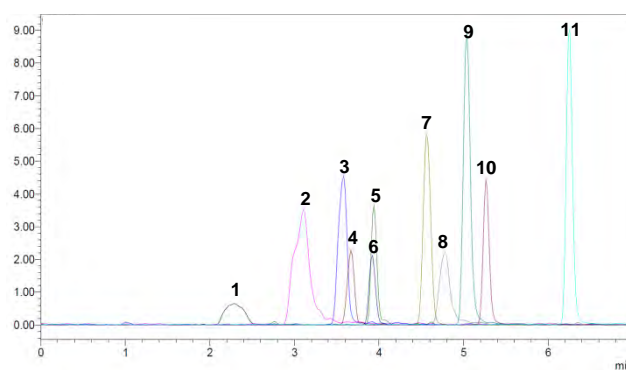


*Figure 1* – Pear extract (0.050 mg kg$^{-1}$) injected without (A) and with (B) the performance optimising injection sequence

*Table 3* – Peak widths obtained with and without the performance optimising injection sequence

| No. | Compound | Peak width (min.) | | Peak width change (%) |
|---|---|---|---|---|
| | | Without POISe | With POISe | |
| 1 | Methamidophos | 1.193 | 0.466 | **-60.9** |
| 2 | Propamocarb | 0.937 | 0.473 | **-49.5** |
| 3 | Omethoate | 0.773 | 0.247 | **-68.0** |
| 4 | Butocarboxim sulfoxide | 0.664 | 0.205 | **-69.1** |
| 5 | Aldicarb sulfoxide | 0.545 | 0.195 | **-64.2** |
| 6 | Dinotefuran | 0.460 | 0.247 | **-46.3** |
| 7 | Oxamyl | 0.317 | 0.248 | **-21.8** |
| 8 | DMPF | 0.309 | 0.254 | **-17.8** |
| 9 | Demeton-S-methyl sulfoxide | 0.418 | 0.271 | **-35.2** |
| 10 | Demeton-S-methyl sulphone | 0.277 | 0.248 | **-10.5** |
| 11 | Ethiofencarb sulphone | 0.233 | 0.220 | **-5.6** |

### 3.4 UHPLC gradient optimisation

Based on the results of the mobile phase screening investigation (section 3.2) the three superior compositions were tested: 1) 10 mM ammonium formate, 2) 10 mM ammonium acetate and 3) 10 mM ammonium formate with 0.1 % formic acid. Separation was achieved using a Shim-Pack XR-ODS III, 2.0 x 150 mm, 2.2 μm particle size. Ammonium formate was found to be the most effective compromise for all 210 compounds in terms of signal to noise ratios and peak shapes.

However two problems with ammonium formate were observed; early elution of asulam and poor peak shape of propamocarb. Consequently, 0.01 % formic acid was tested and found to increase the retention of asulam, and improve the peak shape of propamocarb. The addition of acid was found to shorten the retention time of cyromazine (RT 2.2 min.), yet this retention time was still in excess of 2 column volumes as required in quality control procedures for pesticide residues analysis in food and feed.[13]

A number of pesticide isomers have identical transitions and consequently must be separated chromatographically. Employing a 16 minute gradient resulted in resolution greater than 1 between all necessary pesticides including: butocarboxim sulphoxide / aldicarb sulphoxide, ethiofencarb sulphone / methiocarb sulphone, diuron / fluometronsulam and desmedipham / phenmedipham. Figure 2 highlights the excellent peak shapes achieved on the Nexera UHPLC.

### 3.5 Final method performance

In order to assess the performance of the LCMS-8040 for real samples, limits of detection, linearity and repeatability were determined in food extracts. Linearity was assessed from 0.5 – 200 ppb in four types of sample: (1) acetonitrile, (2) dried fruit extract, (3) lettuce extract and, (4) pear extract. All 210 pesticides achieved excellent correlation coefficients greater than 0.99 in all four types of matrix with typical values greater than 0.997. Correlation coefficients are listed in Table 1 for all pesticides in pear extract, and the calibration curves of eight selected pesticides shown in Figure 3.

Pesticide limits of detection were calculated based on the method described by the US-EPA (see experimental section). Limits of detection were assessed for both the quantifying transition and the qualifying transition and are listed in Table 1. All of the studied pesticides presented LODs less than the 0.01 mg kg$^{-1}$ reporting level for both transition 1 and 2.

A limit of detection less than 0.001 mg kg$^{-1}$ (1ppb) was achieved for the quantifying transition and less than 0.002 mg kg$^{-1}$ (2 ppb) for the qualifying transition for 90 % of compounds: thereby highlighting the excellent sensitivity of the LCMS-8040 for pesticide analysis. Furthermore, these limits of detection were achieved with an injection volume of only 2 μL. Therefore, detection limits could be reduced even further with larger injection volumes. An injection volume of 2 μL was used in the study to allow the injection of 100 % acetonitrile extracts without detriment to early eluting peak shapes.

Repeatability was assessed at the 0.01 mg kg$^{-1}$ reporting level as peak area %RSD for six replicate injections in pear extracts. Repeatability less than 5 %RSD was achieved for 92 % of the 210 pesticides studied. All of the studied compounds presented repeatability less than 10 %RSD, with exception of haloxyfop acid (13.4 %).
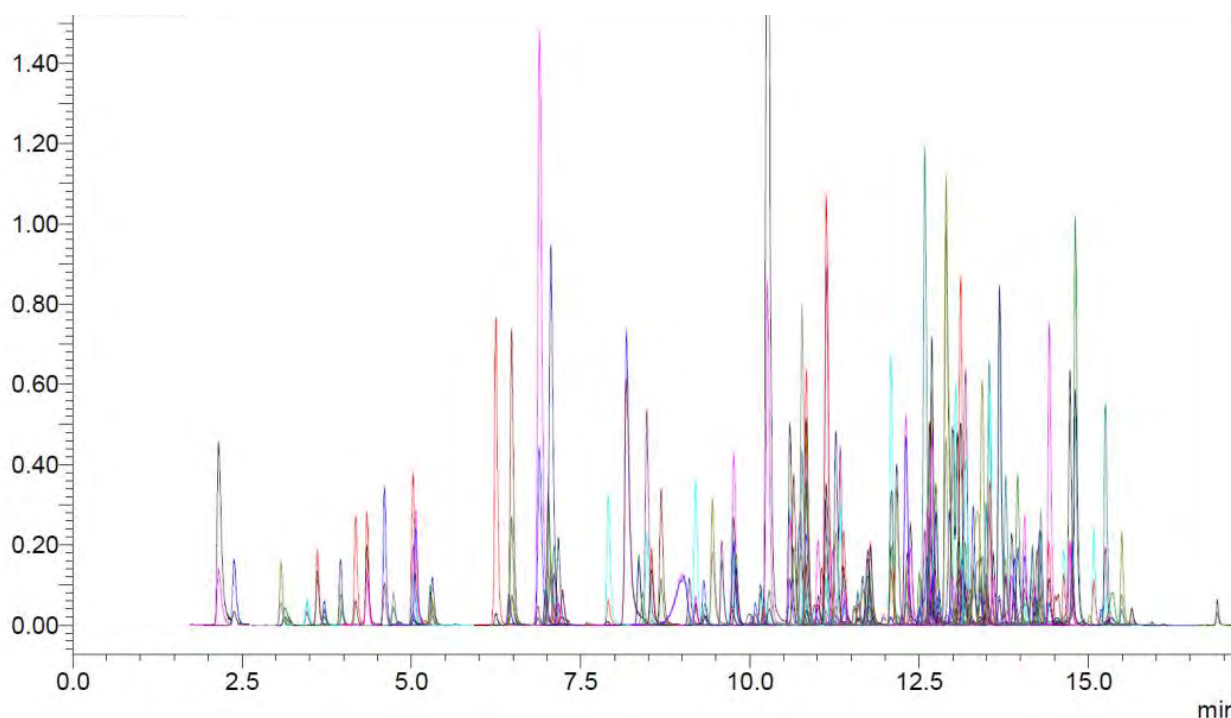


***Figure 2*** *– Extracted ion chromatogram of 210 pesticides using the Shimadzu Nexera UHPLC and the Shimadzu LCMS-8040; 2 μL injection of a 0.05 mg kg$^{-1}$ standard solution.*
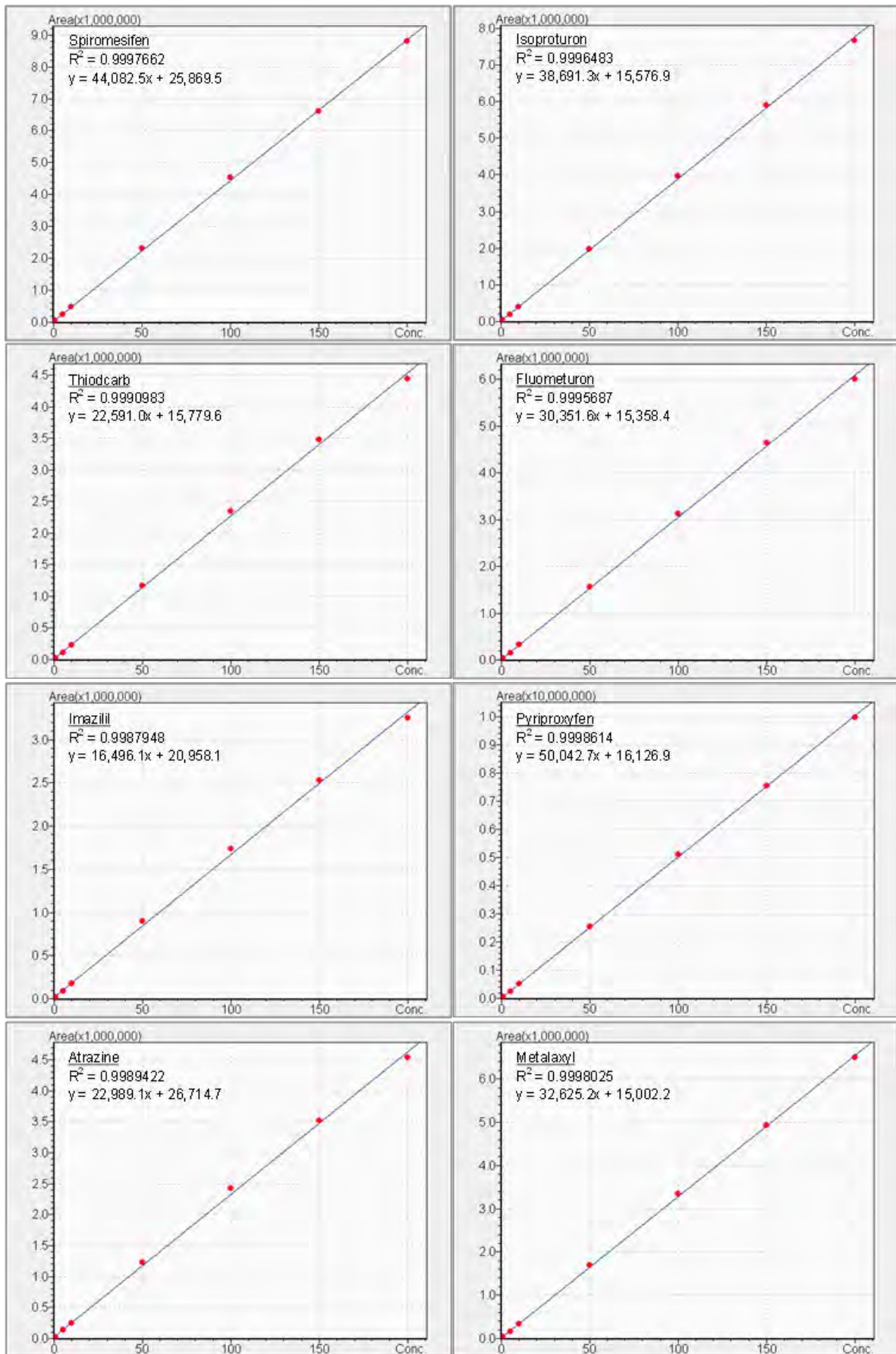
**Figure 3** – *Calibration curves,* 0.5 µg kg$^{-1}$ - 0.2 mg kg$^{-1}$ (0.5 – 200 ppb), *of eight pesticides in pear matrix*

## 4. Conclusion

The results of the developed methodology show that the Shimadzu LCMS-8040 triple quadrupole can achieve excellent sensitivity, linearity and repeatability in food extracts for over 200 commonly analysed pesticides. Limits of detection were less than 0.01 mg kg$^{-1}$ (10 ppb) for both the quantifying and qualifying transitions for all compounds studied, while for 90% of compounds was less than 0.001 mg kg$^{-1}$ (1ppb) (quantifying transition) and 0.002 mg kg$^{-1}$ (2 ppb) (qualifying transition); therefore providing excellent response, especially given that the injection volume was only 2μL. The sensitivity of the LCMS-8040 was able to meet the 0.01 mg kg$^{-1}$ (10 ppb) requirements of regulatory guidelines such as those established by the EU and Japan. Repeatability at the 0.01 mg kg$^{-1}$ reporting level was less than 5% for nearly all compounds and correlation coefficients greater than 0.99 for all compounds in a variety of food samples. Consequently the LCMS-8040 is ideally suited for routine monitoring of pesticides in regulatory laboratories.

## Acknowledgements

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# Application News

## No.C136

**Liquid Chromatography Mass Spectrometry**

## Expanding Capabilities in Multi-Residue Pesticide Analysis Using The LCMS-8060

**David R. Baker[1], Laëtitia Fages[2], Eric Capodanno[2], Neil Loftus[1]**
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### ■ Abstract

With an increasing global population, food security is increasingly under threat and there is a growing challenge for agriculture to produce more food, safely and more sustainably. The use of herbicides, insecticides, and fungicides reduce crop losses both before and after harvest, and increase crop yields. However, pesticide residues resulting from the use of plant protection products on crops may pose a risk to human health and require a legislative framework to monitor pesticide residues in food.

National programs for pesticide monitoring in the US, Europe and Japan have set Maximum Residue Levels (MRL's) or tolerance information (EPA) for pesticides in food products. A default value of 0.01 mg/kg is applied for MRL enforcement, which therefore requires highly sensitive and specific analytical technologies to monitor an increasing number of pesticides.

This application note describes the expanded capability of the LCMS-8060 to help accelerate method development workflows and support increased pesticide monitoring programs. Using the Shimadzu Pesticide MRM Library (the Library includes information on 766 certified reference materials) a single multi-residue LC/MS/MS method was developed for 646 pesticides (3 MRM transitions for over 99 % targeted pesticides resulting in 1,919 transitions in total, with a polarity switching time of 5 msec).

Keywords: Pesticides; food safety; LCMS-8060; Pesticide MRM Library, 776 compound library

### ■ Introduction

There are more than 1,000 pesticides used globally on soil and crops. With the ever increasing international trade of the food industry, regulatory bodies around the world have increased the number of regulated pesticides and the maximum residue levels (MRLs) allowed in food commodities. In the EU, regulation 396/2005/EC and its annexes set MRLs for over 500 pesticides in 370 food products.[1] In the US, tolerances for more than 450 pesticides and other ingredients are established by the US EPA[2] and Japan's positive list system for agricultural chemical residues in foods contains MRLs for over 400 pesticides in various commodities.[3]

National pesticide monitoring programs create new challenges for food safety laboratories as the number of pesticides required for analysis is increasing together with an expanded range of food products.

In this application paper we present the development of a LC-MS/MS method for screening and quantifying over 646 pesticides in a single method. The method was quickly and efficiently set up using the Shimadzu Pesticide MRM Library. For each target pesticide analysis, up to 3 MRMs (Multiple Reaction Monitoring) transitions were imported from the library. 3 MRMs transitions provided additional data confidence in reporting results in comparison to the conventional 2 transitions used in most methods. As the LCMS-8060 has a high data acquisition speed 1,919 transitions were acquired using a polarity switching speed of 5 msec over a 10.5 minutes gradient elution.

To evaluate the method QuEChERS extracts of mint, tomato and apple were provided by a commercial laboratory as raw acetonitrile extracts and spiked with 646 pesticides (data is presented on the mint extract as it is the more complex sample matrix). The method was evaluated in matrix to ensure that the reporting limits were in agreement with recognised MRL's.

### ■ Experiment

Food extracts of mint, tomato and apple were supplied by Phytocontrol, France, following established QuEChERS protocols. Final extracts were prepared in acetonitrile without any dilution. Certified reference materials for the Shimadzu Pesticide MRM Library were obtained from ACSD, France as stock solutions. All solvents were of LCMS quality purchased from Sigma-Aldrich.

A six point calibration curve from 0.002 - 0.1 mg/kg (2 - 100 pg/µL) were generated using internal standard method. Two internal standards (Atrazine-d5 and Diuron-d6) were spiked in during the auto-sampler sequence for quantitation.

The robustness of the LCMS-8060 was assessed by peak area response for 646 pesticides spiked into mint, tomato and apple matrix extracts at 0.05 mg/kg.

### ■ LC/MS/MS method development

The Shimadzu Pesticide MRM Library has 766 pesticides in its database (Application News No. C135). For each pesticide several MRM's are included in the database and in this analysis the default value used was 3 MRM's. For this method, 1,919 transitions were selected in both positive and negative ionisation mode using a switching time of 5 msec (1,819 MRM transitions were in positive mode and 100 MRM transitions in negative mode).

To optimize ion source conditions (for example, DL temperature, interface temperature, heating block temperature, heating gas flow, drying gas flow and nebulizer gas flow) the interface setting software was used. This tool provides an optimized response for all compounds.

**Table 1  LC and MS/MS Acquisition Parameters**

| Liquid chromatography | | Mass spectrometry | |
|---|---|---|---|
| UHPLC | Nexera LC system | LC/MS/MS | LCMS-8060 |
| Analytical column | Restek Raptor Biphenyl (2.1 mm I.D. × 100 mm L., 2.7 µm) | Ionisation mode | Heated electrospray |
| | | Polarity switching time | 5 msec |
| Column temperature | 35 ℃ | Pause time | 1 msec |
| Flow rate | 0.4 mL/min | Total MRM transitions | 1,919 (1,819 positive; 100 negative) |
| Solvent A | 2 mmol/L ammonium formate + 0.002 % formic acid - Water | MRM Dwell | 4 msec (target ion); 1 msec (reference ion) |
| Solvent B | 2 mmol/L ammonium formate + 0.002 % formic acid - Methanol | Interface temperature | 350 ℃ |
| | | Heating block | 300 ℃ |
| Binary Gradient B.Conc. | 3 % (0 min) - 10 % (1.00 min) - 55 % (3.00 min) - 100 % (10.50 - 12.00 min) - 3 % (12.01 - 15.00 min) | Desolvation line | 150 ℃ |
| | | Heating gas | 10 L/min |
| | | Drying gas | 10 L/min |
| Injection volume | 2 µL sample (plus 40 µL water) | Nebulizer gas | 3 L/min |



**Fig. 1  MRM chromatograms of 646 pesticides spiked into a mint extract at 0.01 mg/kg
(Up to 3 MRMs per compound and 5 msec polarity switching time).**



**Fig. 2  MRM chromatograms for pesticides most commonly detected in plant products listed in the 2015 European Food Safety Journal. In this report, residues exceeding the legal limits were related to 58 different pesticides. Compounds such as boscalid, chlorpyriphos, cyprodinil, fenhexamid, fludioxonil, pyraclostrobin and tebuconazole (highlighted in the MRM chromatogram) are some of the most frequently detected compounds present in more than 4 % of the samples analyzed.**

The MRM chromatograms show the response to each pesticide spiked into a food matrix at the default MRL of 0.01 mg/kg.

■ **Results and Discussion**

**Shimadzu Pesticide MRM Library**
(Application News No. C135 )

**A flexible tool for expanding capabilities in pesticide monitoring programs**

The Pesticide MRM Library has been created using 766 certified reference materials and is designed to help accelerate method development and compound management.

The library contains an average of 8 optimized MRM transitions for each compound (including positive and negative ion modes). In total, more than 6,000 MRM transitions are held within the 766 compound library. The library itself documents CAS#, formula, activity, mono-isotopic mass and adduct masses, rank of MRM transitions, synonyms, InChI, InChIKey, compound names translation (Japanese and Chinese) and links to websites offering further information (for example; alanwood.net, PAN pesticide database, Chemical Book, ChemSpider).

The library also serves as a powerful data repository for reporting and checking pesticide data sources.

**Creating flexible pesticide monitoring methods Building a new LC/MS/MS method**

To create new pesticide LC/MS/MS methods the user simply needs to select the target compounds from the library, identify the required number of MRMs for each compound and confirm the analytical column for the analysis. (The new method can be used to expand current capabilities or to create focused methods with a limited number of pesticides). The new method is simply imported into LabSolutions.

As the LCMS-8060 has a high data acquisition speed of 30,000 u/sec, high sensitivity and a polarity switching speed of 5 msec, the capabilities of the library can be expanded to meet the future needs of any laboratory.

**Expanded capability of the LCMS-8060**

The LCMS-8060 has a data acquisition speed of 30,000 u/sec which creates new opportunities for expanding compound lists.

As one example, between 6.45 and 6.60 minutes 25 pesticide compounds elute (Fig. 3). Even with high data density acquisitions the average variation in peak area response was less than 3 %RSD (varying between 1.1 - 5.9 %RSD).



**Fig. 3  The LCMS-8060 can acquire MRM data at a high speeds and enables precise quantitation even with high data density. Between 6.45 and 6.60 minutes 25 compounds were monitored (Table 2).**

**Table 2  Peak area variation (%RSD; n=6) for 25 pesticides eluting over a nine-second time window (6.45 - 6.60 minutes) spiked into a mint matrix extract at the reporting limit of 0.01 mg/kg.**

| Compound Name | CAS number | Formula | M | Polarity | MRM Quantitation Ion | RT | Average Peak Area | %RSD (n=6) |
|---|---|---|---|---|---|---|---|---|
| Trinexapac-ethyl | 95266-40-3 | C13H16O5 | 252.0998 | + | 252.90 > 69.05 | 6.45 | 1,780,015 | 3.1 |
| Iprovalicarb | 140923-17-7 | C18H28N2O3 | 320.2100 | + | 321.20 > 119.15 | 6.46 | 1,442,486 | 2.8 |
| Dodemorph | 1593-77-7 | C18H35NO | 281.2719 | + | 282.30 > 116.15 | 6.47 | 658,920 | 4.2 |
| Fluopyram | 658066-35-4 | C16H11ClF6N2O | 396.0464 | + | 397.00 > 145.00 | 6.47 | 2,439,146 | 1.9 |
| Flutolanil | 66332-96-5 | C17H16F3NO2 | 323.1133 | + | 324.10 > 242.00 | 6.48 | 3,372,285 | 2.7 |
| Trifloxysulfuron | 145099-21-4 | C14H14F3N5O6S | 437.0617 | + | 438.00 > 182.15 | 6.48 | 1,822,340 | 2.5 |
| Azaconazole | 60207-31-0 | C12H11Cl2N3O2 | 299.0228 | + | 300.00 > 159.00 | 6.50 | 1,580,445 | 2.0 |
| Terbutryn | 886-50-0 | C10H19N5S | 241.1361 | + | 242.10 > 157.95 | 6.50 | 755,446 | 3.4 |
| Prometryn | 7287-19-6 | C10H19N5S | 241.1361 | + | 242.10 > 158.00 | 6.50 | 1,300,193 | 2.6 |
| Azimsulfuron | 120162-55-2 | C13H16N10O5S | 424.1026 | + | 425.10 > 182.10 | 6.50 | 2,498,050 | 1.8 |
| Metominostrobin | 133408-50-1 | C16H16N2O3 | 284.1161 | + | 285.10 > 193.95 | 6.51 | 2,929,500 | 1.7 |
| Thifluzamide | 130000-40-7 | C13H6Br2F6N2O2S | 525.8421 | + | 528.60 > 148.05 | 6.51 | 193,982 | 5.9 |
| Nicarbazin | 330-95-0 | C13H10N4O5 | 302.0651 | - | 301.10 > 137.15 | 6.52 | 973,101 | 2.6 |
| Bromobutide | 74712-19-9 | C15H22BrNO | 311.0885 | + | 312.10 > 194.10 | 6.53 | 1,829,781 | 2.1 |
| Saflufenacil | 372137-35-4 | C17H17ClF4N4O5S | 500.0544 | + | 501.00 > 198.00 | 6.53 | 465,224 | 2.3 |
| Cyproconazole | 94361-06-5 | C15H18ClN3O | 291.1138 | + | 292.10 > 70.05 | 6.54 | 1,174,967 | 1.7 |
| Clomazone | 81777-89-1 | C12H14ClNO2 | 239.0713 | + | 239.90 > 125.00 | 6.54 | 3,409,656 | 1.7 |
| Fensulfothion | 115-90-2 | C11H17O4PS2 | 308.0306 | + | 309.00 > 281.00 | 6.54 | 4,267,514 | 1.4 |
| Oxasulfuron | 144651-06-9 | C17H18N4O6S | 406.0947 | + | 407.10 > 150.15 | 6.54 | 2,911,533 | 1.1 |
| Rimsulfuron | 122931-48-0 | C14H17N5O7S2 | 431.0569 | + | 432.00 > 182.00 | 6.55 | 4,722,065 | 1.8 |
| Fenthion-oxon | 6552-12-1 | C10H15O4PS | 262.0429 | + | 263.10 > 231.00 | 6.55 | 3,075,195 | 1.4 |
| Nitrothal-isopropyl | 10552-74-6 | C14H16NO6Na | 317.0875 | + | 295.10 > 230.95 | 6.56 | 2,199,581 | 3.0 |
| Chlorantraniliprole | 500008-45-7 | C18H14BrCl2N5O2 | 480.9708 | + | 483.90 > 452.90 | 6.57 | 2,407,025 | 2.7 |
| Fipronil-sulfone | 120068-36-2 | C12H4Cl2F6N4O2S | 451.9336 | - | 451.00 > 414.90 | 6.57 | 2,843,708 | 2.0 |
| Valifenalate | 283159-90-0 | C19H27ClN2O5 | 398.1608 | + | 399.20 > 155.00 | 6.59 | 3,845,335 | 1.9 |

**Final method performance for 646 pesticides**
In order to test the performance of the developed method, linearity, repeatability and longer term robustness were assessed for all 646 pesticides.

**Linearity**
Linearity was assessed over a six point calibration curve from 0.002 - 0.1 mg/kg (2 - 100 pg/µL). All 646 pesticides achieved excellent $R^2$ values greater than 0.99 in both tomato and mint spiked extracts with typical values greater than 0.996. Calibration curves were generated using a linear curve fit type and 1/C weighting. Typical calibration curve data is presented below in Fig. 4.



**Fig. 4** Calibration curves for selected pesticides spiked into a mint matrix extract in the range 0.002 - 0.1 mg/kg. The quantitation MRM chromatogram is shown in black (qualifier ion MRM chromatograms are shown in red and blue).

**Repeatability**

To assess the robustness of the system and the developed method during routine analysis, repeat injections of a mint matrix sample spiked with 646 pesticides at 0.05 mg/kg, were analyzed over a 24 hour period.

The results for selected compounds are displayed below in Fig. 5.

Compounds were selected throughout the run at equidistant points (closest elution points to 3, 4, 5, 6, 7, 8, 9 and 10 minutes), including positive and negative ion detection, (Table 3).

The peak area variance was less than 5.7 % for all pesticides measured.



**Fig. 5  Peak area response for several pesticides following 100 repeat injections of a 0.05 mg/kg spiked into mint matrix extract.**

**Table 3  Peak area variance for selected following the repeated injection of a 0.05 mg/kg spiked into mint matrix extract (number of sample replicates was 100; the analysis sequence was 24 hours).**

| Compound Name | CAS Number | Formula | M | Polarity | MRM Quantitation Ion | RT (mins) | Average Peak Area | %RSD (n=100) |
|---|---|---|---|---|---|---|---|---|
| Butocarboxim-sulfoxide | 34681-24-8 | C7H14N2O3S | 206.0725 | + | 207.10 > 75.10 | 3.042 | 1,220,391 | 2.6 |
| Thiofanox-sulfone | 39184-59-3 | C9H18N2O4S | 250.0987 | + | 268.10 > 57.00 | 4.001 | 442,724 | 5.7 |
| Monolinuron | 1746-81-2 | C9H11ClN2O2 | 214.0509 | + | 215.10 > 99.10 | 4.985 | 2,904,116 | 3.7 |
| Probenazole | 27605-76-1 | C10H9NO3S | 223.0303 | + | 224.00 > 41.05 | 5.995 | 1,145,189 | 3.5 |
| Dipropetryn | 4147-51-7 | C11H21N5S | 255.1518 | + | 256.20 > 144.05 | 6.999 | 3,289,597 | 3.4 |
| Pyraflufen-ethyl | 129630-19-9 | C15H13Cl2F3N2O4 | 412.0204 | + | 413.00 > 339.00 | 8.004 | 3,653,333 | 3.5 |
| Emamectin B1a | 138511-97-4 | C56H81NO15 | 1007.5606 | + | 886.40 > 158.20 | 9.008 | 3,109,562 | 4.5 |
| Pyridalyl | 179101-81-6 | C18H14Cl4F3NO3 | 488.9680 | - | 491.90 > 109.05 | 10.171 | 1,579,422 | 5.0 |

**Response to differing matrices**

One of the major challenges in the quantitative LC/MS/MS analysis for pesticides in food is that compound and matrix-dependent response suppression or enhancement may occur. Although matrix effects can affect the peak area response between different food types following a QuEChERS extraction protocol, the peak area variance should be minimized within a single matrix.

Food extracts of apple, mint and tomato following QuEChERS extraction were spiked with 646 pesticides at 0.05 mg/kg and were repeatedly injected on the LCMS-8060 (n=100 repeat injections for each matrix; 300 injections in the same batch sequence). Fig. 6 shows the response for 3 selected pesticides analyzed in a single batch sequence corresponding to a 72 hour analysis sequence. Within a matrix, variance was less than 5.9 %RSD for all compounds.

Although the absolute peak area changes with different food matrices, the response between injection 1 and injection 100 for 2 pesticides (probenazole and dipropetryn) within a single matrix has a variance less than 5.7 %RSD.



**Fig. 6  Peak area response for three pesticides spiked into apple, mint and tomato matrix extracts at 0.05 mg/kg over 72 hours. As in Fig. 5, compounds were selected to reflect peak area response throughout the chromatographic run (Table 3).**



**Fig. 7  MRM chromatograms for probenazole (RT 5.995 minutes) and dipropetryn (RT 6.999 minutes) for injection 1 and injection 100 spiked into apple, mint and tomato matrix extracts. The extracts were spiked at 0.05 mg/kg and analyzed over 72 hours.**

## Reducing matrix effects by extensively diluting the sample

The need to test for more pesticides in a wider range of samples at high sensitivity is very challenging as matrix effects from the sample extraction will influence both ion suppression and enhancement. Ion suppression can lead to errors in the detection capability, accuracy and precision of the method.

To reduce the effect of interfering compounds in the quantitation of complex samples extensive sample dilution is now widely used in routine analysis. It is an approach which is simple to build into multi-residue extraction methods and is cost effective.

This approach leads to greater robustness as a consequence of a reduced sample injection in the LC/MS/MS, higher data quality and increased instrument uptime.

Fig. 8 shows the results of diluting a matrix sample spiked at 0.005 mg/kg with dilution factors of 1:5, 1:10, 1:20, 1:50 and 1:100.

As matrix effects can be both significant and variable for different compounds Table 4 shows recovery data for a series of pesticides diluted from 0 to a dilution factor of 1:100.

Matrix suppression was reduced for most compounds when the sample was diluted 1:10 with recoveries in the range of 70 - 120 % with an associated repeatability RSDr ≤ 20 %. Relative standard deviations in relation to the mean values were typically less than 10 %.

Diluting the sample by a factor of 20 or 50 resulted in acceptable signal suppression from the matrix.



Fig. 8 MRM chromatograms for 3 selected compounds spiked into a mint extract at 0.005 mg/kg and diluted 1:5, 1:10, 1:20, 1:50 and 1:100 with water.

Table 4 Diluting a sample matrix extract spiked with 0.005 mg/kg with water reduced matrix ion suppression.

| Compound | CAS | Formula | M | Dilution series | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 0 | 1:5 | 1:10 | 1:20 | 1:50 | 1:100 |
| | | | | Recovery | | | | | |
| Bentazone | 25057-89-0 | C10H12N2O3S | 240.0569 | 32.1 | 44.6 | 65.5 | 72.7 | 91.7 | 98.1 |
| Demeton-S-methyl-sulfone | 17040-19-6 | C6H15O5PS2 | 262.0099 | 51.1 | 78.5 | 89.6 | 91.1 | 114.2 | 116.8 |
| Dimethoate | 60-51-5 | C5H12NO3PS2 | 228.9996 | 36.2 | 65.3 | 88.5 | 92.2 | 92.4 | 94.2 |
| Isocarbamid | 30979-48-7 | C8H15N3O2 | 185.1164 | 28.8 | 57.1 | 81.8 | 98.7 | 102.5 | 96.4 |
| Vamidothion | 2275-23-2 | C8H18NO4PS2 | 287.0415 | 53.6 | 76.3 | 98.2 | 98.5 | 101.5 | 114.1 |
| Thiazafluron | 25366-23-8 | C6H7F3N4OS | 240.0293 | 32.8 | 62.9 | 80.5 | 84.2 | 87.1 | 97.4 |
| Demeton-S-methyl | 919-86-8 | C6H15O3PS2 | 230.0200 | 57.8 | 82.1 | 93.1 | 87.6 | 108.5 | 102.4 |
| Sebuthylazine | 7286-69-3 | C9H16ClN5 | 229.1094 | 28.7 | 53.3 | 69.8 | 79.8 | 88.5 | 95.8 |
| Flutriafol | 76674-21-0 | C16H13F2N3O | 301.1027 | 27.3 | 46.1 | 71.4 | 76.1 | 81.8 | 87.3 |
| Furametpyr | 123572-88-3 | C17H20ClN3O2 | 333.1244 | 48.3 | 69.8 | 86.9 | 86.2 | 97.6 | 101.9 |
| Fenobucarb | 3766-81-2 | C12H17NO2 | 207.1259 | 60.9 | 79.2 | 100.7 | 96.1 | 102.8 | 103.9 |
| Benodanil | 15310-01-7 | C13H10INO | 322.9807 | 50.9 | 69.8 | 86.3 | 96.5 | 102.4 | 94.8 |
| Terbuthylazine | 5915-41-3 | C9H16ClN5 | 229.1094 | 50.4 | 66.6 | 83.2 | 87.2 | 89.8 | 91.0 |
| Dimethachlor | 50563-36-5 | C13H18ClNO2 | 255.1026 | 75.1 | 86.1 | 106.0 | 107.1 | 106.2 | 108.0 |
| Dimethenamid | 87674-68-8 | C12H18ClNO2S | 275.0747 | 72.6 | 84.9 | 102.9 | 100.0 | 103.6 | 97.3 |
| Furalaxyl | 57646-30-7 | C17H19NO4 | 301.1314 | 82.2 | 89.1 | 106.6 | 108.6 | 106.2 | 102.4 |
| Bixafen | 581809-46-3 | C18H12Cl2F3N3O | 413.0310 | 66.8 | 79.3 | 99.0 | 95.6 | 103.7 | 97.1 |
| Triflumuron | 64628-44-0 | C15H10ClF3N2O3 | 358.0332 | 54.2 | 71.8 | 95.5 | 84.9 | 95.3 | 101.7 |
| Epoxiconazole | 133855-98-8 | C17H13ClFN3O | 329.0731 | 61.6 | 77.2 | 98.8 | 95.3 | 90.0 | 101.2 |
| Teflubenzuron | 83121-18-0 | C14H6Cl2F4N2O2 | 379.9742 | 41.8 | 50.9 | 80.1 | 86.8 | 100.0 | 97.7 |

# ■ Conclusion

A fast, selective and highly sensitive method has been developed for the quantitation of 646 pesticides using a single method with 1,919 transitions (corresponding to up to 3 MRM transitions per compound) and a LC gradient time of only 10.5 minutes.

As the LCMS-8060 has a rapid polarity switching time of 5 msec, the single multi-residue LC/MS/MS method supported the analysis of 34 pesticides in negative ion mode and 612 compounds in positive ion mode.

The enhanced performance and higher sensitivity of the LCMS-8060 has created new opportunities in sample dilution to reduce ion signal suppression and matrix effects. For most compounds a dilution factor of 1:20 or 1:50 was sufficient to provide recoveries in the range 70 - 120 %.



# ■ References

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# Application News

## No.C103

**Liquid Chromatography Mass Spectrometry**

## Analysis of Nivalenol, Deoxynivalenol, 3-Acetyldeoxynivalenol and 15-Acetyldeoxynivalenol Using Triple Quadrupole LC/MS/MS (LCMS-8050)

Nivalenol and deoxynivalenol are mycotoxins which are produced by the fusarium fungi. A provisional reference value of 1.1 ppm was established in Japan for deoxynivalenol (Notification No. 0521001 issued by the Pharmaceutical and Food Safety Bureau, Japanese Ministry of Health, Labour and Welfare on May 21, 2002). The test methods specified for deoxynivalenol are HPLC for both qualitative and quantitative analysis, and LC/MS for verification testing (Notification No. 0717001 issued by the Dept. of Food Safety, Pharmaceutical and Food Safety Bureau, Japanese Ministry of Health, Labour and Welfare on July 17, 2003).

This paper describes an LC-MS/MS method for high-sensitivity simultaneous analysis of the four compounds, nivalenol, deoxynivalenol and the deoxynivalenol metabolites, 3-acetyl-deoxynivalenol and 15-acetyl-deoxynivalenol.

### ■ Analysis of a Standard Mixture

Fig. 1 shows the chromatograms obtained using a 2 μL injection of the four-component standard mixture (each 10 ppb), and Table 1 shows repeatability of retention time and peak areas for the four substances, respectively, using six repeat measurements.

Nivalenols are detected using the heated electrospray ionization (hESI) method in negative mode. Although water and acetonitrile alone can be used as the LC eluent for LC/MS analysis, higher sensitivity was obtained for each compound by adding low-concentration ammonium acetate (in this case, 0.5 mmol/L) to eluent A. Fig. 1 shows the mass chromatograms for the highest sensitivity MRM transitions for each compound. The analytical conditions are shown in Table 2.

Next, six repeat analyses of a 10 ppb standard solution were conducted, corresponding to approximately 1/100 the concentration of the provisional reference value. The relative standard deviations (%RSD) for the measured retention times and peak areas are shown in Table 1. Good repeatability was obtained for both retention time and peak area.

**Table 1  Repeatability (10 ppb, n=6)**

|  | R.T. %RSD | Area %RSD |
|---|---|---|
| Nivalenol | 0.04 | 2.57 |
| Deoxynivalenol | 0.04 | 6.52 |
| 15-Acetyldeoxynivalenol | 0.06 | 4.09 |
| 3-Acetyldeoxynivalenol | 0.05 | 2.58 |



**■ Peaks**
1 : Nivalenol
2 : Deoxynivalenol
3 : 15-Acetyldeoxynivalenol
4 : 3-Acetyldeoxynivalenol

371.05 > 281.20
355.10 > 59.00
397.10 > 59.00
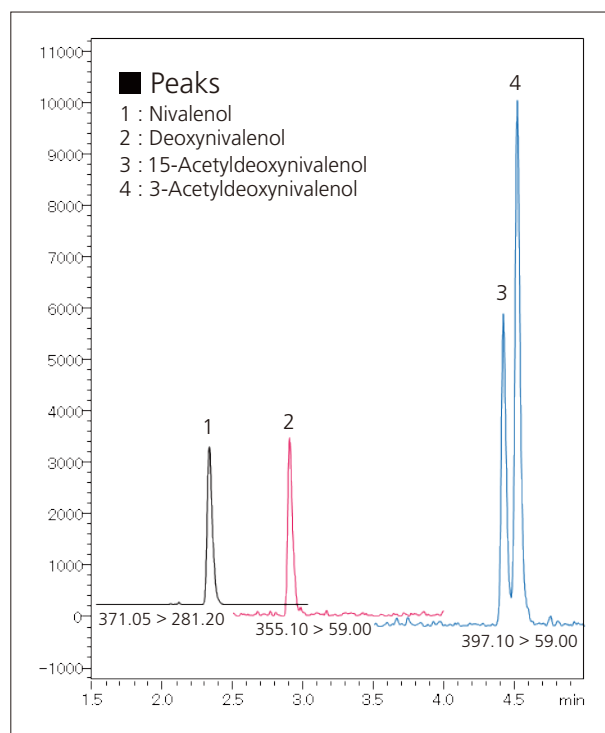
**Fig. 1  MRM Chromatograms of a Standard Mixture (10 ppb each)**

### ■ Linearity of Calibration Curves

Fig. 2 shows the calibration curves generated using the analytical conditions of Table 2. Excellent linearity with a coefficient of determination greater than $R^2 = 0.999$ was obtained for calibration curves using a concentration range from 1 to 250 ppb for each component.
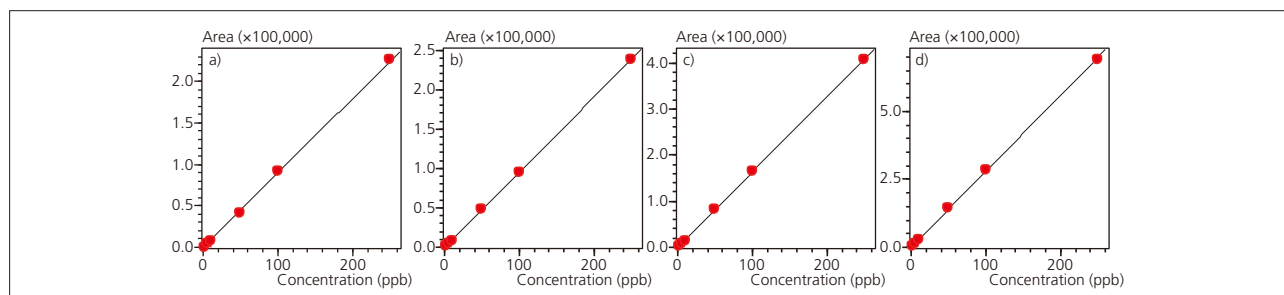


**Fig. 2  Linearity of Calibration Curves: a) Nivalenol  b) Deoxynivalenol  c)15-Acetyldeoxynivalenol  d) 3-Acetyldeoxynivalenol**

### ■ Analysis of Wheat

Fig. 3 describes the sample pretreatment procedure for wheat. The wheat extract solution was purified using either the MultiSep #227 multi-function column (Romer Labs) or the Autoprep MF-T column (Showa Denko K.K.). The chromatograms generated using the samples prepared using the MultiSep #227 (unspiked samples) and the standard-spiked samples, respectively, are shown in Fig. 4. The standard mixture was added to obtain a final concentration of 25 ppb for the four components (about 1/40 of the provisional reference value), respectively. No large contaminant peaks were detected in the chromatograms of the pretreated samples. Furthermore, although deoxynivalenol was detected, it was at a level below that of the provisional reference value. The spike-and-recovery rates for the four components were excellent, from 101 to 107 %, without any particular matrix effects. Even in samples pretreated using Autoprep MF-T, comparable spike-and-recovery test results were obtained.



**Fig. 3  Pretreatment**

Wheat
↓
Grind to less than 1000 μm
↓
50.0 g
↓ ← 200 mL Water/Acetonitrile=15/85(v/v)
Shake for 30 min
↓
Vacuum Filtration by Whatman GF/B
↓
15 mL
↓
Clean-up by multi function column
" MultiSep#227" or "Autoprep MF-T"
↓ ← Discard the first 3 mL of solution.
        Extract the next 5 mL of solution.
4 mL
↓
Evaporate by N₂ gas below 45 °C
↓ ← 1 mL Water/Acetonitrile/Methanol
        =90/5/5(v/v/v)
Filtration
↓
Sample solution
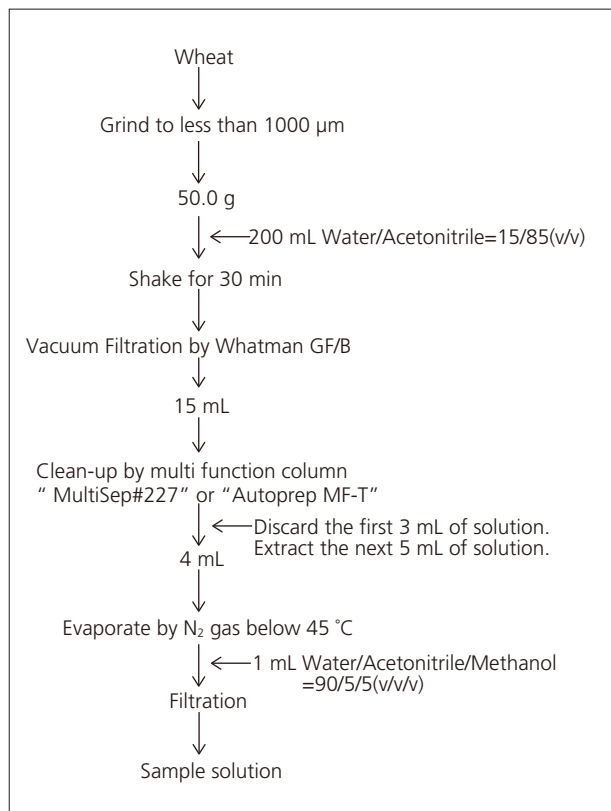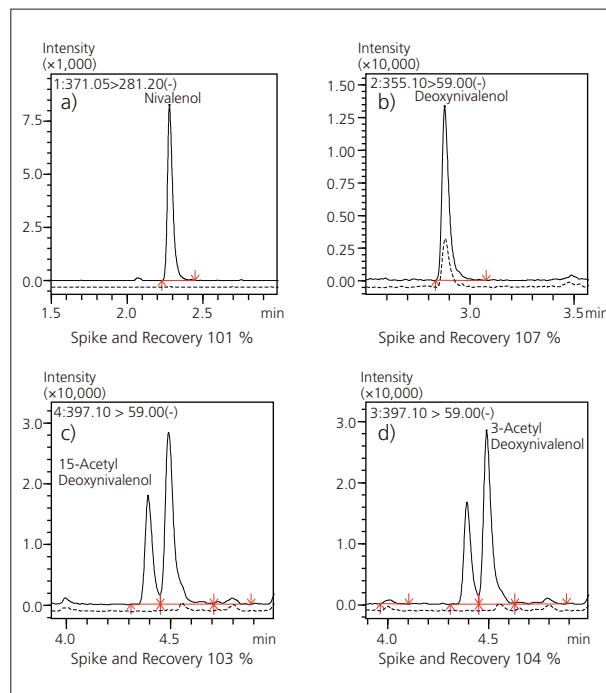


**Fig. 4  Chromatograms of Wheat**
**(Dotted line: Unspiked Sample, Solid line: Spiked Sample, Spiked at 25 ppb each)**
**a) Nivalenol  b) Deoxynivalenol**
**c) 15-Acetyldeoxynivalenol  d) 3-Acetyldeoxynivalenol**

**Table 2  Analytical Conditions**

| | |
|---|---|
| Column | : Shim-pack XR-ODS Ⅲ (150 mm L. × 2.0 mm I.D., 2.2 μm) |
| Mobile Phases | : A 0.5 mmol/L Ammonium Acetate - Water |
| | : B Acetonitrile |
| Time Program | : 5 %B (0 min) → 45 %B (5.0 min) → 95 %B (5.01-7.0 min) → 5 %B (7.01 min) → STOP (12 min) |
| Flowrate | : 0.3 mL/min |
| Column Temperature | : 40 °C |
| Injection Volume | : 2 μL |
| | |
| Probe Voltage | : -3.0 kV (ESI-negative mode) |
| DL Temperature | : 100 °C |
| Block Heater Temperature | : 200 °C |
| Interface Temperature | : 200 °C |
| Nebulizing Gas Flow | : 2 L/min |
| Drying Gas Flow | : 10 L/min |
| Heating Gas Flow | : 10 L/min |
| MRM Transition | : Nivalenol                      371.05 > 281.20         CE: 16.0 V |
| | : Deoxynivalenol              355.10 > 59.00           CE: 22.0 V |
| | : 15-Acetyldeoxynivalenol   397.10 > 59.00           CE: 22.0 V |
| | : 3-Acetyldeoxynivalenol     397.10 > 59.00           CE: 26.0 V |

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# Rapid analysis of polychlorinated biphenyls (PCBs) in vegetables by QuEChERS-based extraction and GPC–GCMS

## ASMS 2015 ThP 041

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# Overview

7 kinds of PCBs in two complicated matrix–carrot and ginger were analyzed by gel permeation chromatography coupled with gas chromatography-mass spectrometry (GPC–GCMS), which could remove majority of macromolecules such as oil and pigment.

# Introduction

Polychlorinated biphenyls (PCBs), one of the most famous "dirtydozen" persistent organic pollutants (POPs) with carcinogenicity, teratogenicity and mutagenicity, are used to be produced and commercially used as mixtures. Because of their specific properties such as good stability, low volatility, insulativity and non-flammability, PCBs have been applied in a series of industrial applications such as coating, links, flame retardants, paints, electronic appliances, heat-transfer systems and hydraulic fluids.

Even PCBs were banned by most countries as early as 1970s, they can still be detected in air, soil, water, sediment and biota at a global scale, even in remote areas such as the polar regions, deep seas and high mountains. In this research, carrot and ginger were selected as representative samples analyzed by on-line GPC-GCMS system characterized by remove macromolecules for further purification.

# Methods

## Sample Preparation

The purification procedure was referenced to the QuEChERS method: 10g crushed sample, vortex in 10 mL acetonitrile, add 4 g of $MgSO_4$ and 1 g of NaCl; vortex mixed for 1 min and centrifuged for 10 min at 3000 rpm;

2 mL of the upper layer was transferred into a 5 mL centrifuge tube containing 150 mg of $MgSO_4$ and 25 mg of PSA, after vortex mixing for 1 min, centrifuged for 10 min at 3000 rpm and was ready for injection.

## GPC-GCMS Analysis

| Instrument | : GPC-GCMS (Shimadzu Corporation, Japan) |
|---|---|
| **GPC system condition** | |
| Column | : Shodex CLNpak EV-200 (2.1mm x 150mm) |
| Mobile phase | : acetone/ cyclohexane (3/7, v/v) |
| Flow rate | : 0.1 mL/min |
| Sample volume | : 10 µL |

| GCMS system | |
| --- | --- |
| Retention gap | : 5 m x 0.53 mm |
| Pre-column | : Rtx-5 MS, 5 m x 0.25 mm x 0.25 μm |
| Analytical column | : Rtx-5 MS, 25m x 0.25mm x 0.25μm |
| Temp. program | : 82 °C (5 min)_8 °C/min_300 °C (7.75 min) |
| PTV injection temperature program | : 120 °C (5 min)_100 °C/min_250 °C (33.7min) |
| Injection pressure program | : 120 kPa (0 min)_100 kPa/min _180 kPa (4.4 min)_ (-49.8 kPa/min)_120 kPa (33.8 min) |
| Purge program | : 5.0 mL/min_(-10 mL/min)_ 0 mL/min (6 min)_ 10 mL/min_5 mL/min (5 min) |
| Sampling time | : 7 min |
| Solvent cut time | : 9.7 min |
| Interface temperature | : 300 °C |
| Ion source temperature | : 200 °C |
| Acquisition mode | : SIM |

Table 1  GCMS parameters for PCBs

| No. | Compound | CAS | Retention time | Quantitation ions | Qualification ions 1 | Qualification ions 2 |
| --- | --- | --- | --- | --- | --- | --- |
| 1 | PCB28 (2,4,4'-Trichlorobiphenyl) | 7012-37-5 | 20.74 | 256 | 258 | 186 |
| 2 | PCB52 (2,2',5,5'-Tetrachlorobiphenyl) | 35693-99-3 | 21.65 | 292 | 220 | 290 |
| 3 | PCB101 (2,2',4,5,5'-Pentachlorobipenyl) | 37680-73-2 | 23.88 | 326 | 254 | 328 |
| 4 | PCB118 (2,3',4,4',5-Pentachlorobiphenyl) | 31508-00-6 | 25.42 | 326 | 328 | 324 |
| 5 | PCB138 (2,2',3,4,4',5'-Hexachlorobiphenyl) | 35065-28-2 | 25.96 | 360 | 362 | 290 |
| 6 | PCB153 (2,2',4,4',5,5'-Hexachlorobiphenyl) | 35065-27-1 | 26.63 | 360 | 362 | 290 |
| 7 | PCB180 (2,2',3,4,4',5,5'-Heptachlorobiphenyl) | 35065-29-3 | 28.23 | 396 | 394 | 324 |

# Results

The results of this research indicated that the relative coefficients of the 7 kinds of PCBs ranged from 1 to 500 μg/L were above 0.998. Precision (n=6) of this method was measured by analyzing the sample at 1 μg/L. The overall RSDs of analysis were below 5%. The limit of detection (LOD; S/N=3) of most compounds were below 0.05 μg/L. Commercially available carrot and ginger were used for recovery test, spiked concentration was 10 μg/kg and the recoveries of carrot were between 97% and 125% and those of ginger were between 85% and104%. The developed method in this study was proved to be reliable and accurate, and permits rapid determination of PCBs can be easily applied for quality control of vegetables.

# Rapid analysis of polychlorinated biphenyls (PCBs) in vegetables by QuEChERS-based extraction and GPC–GCMS
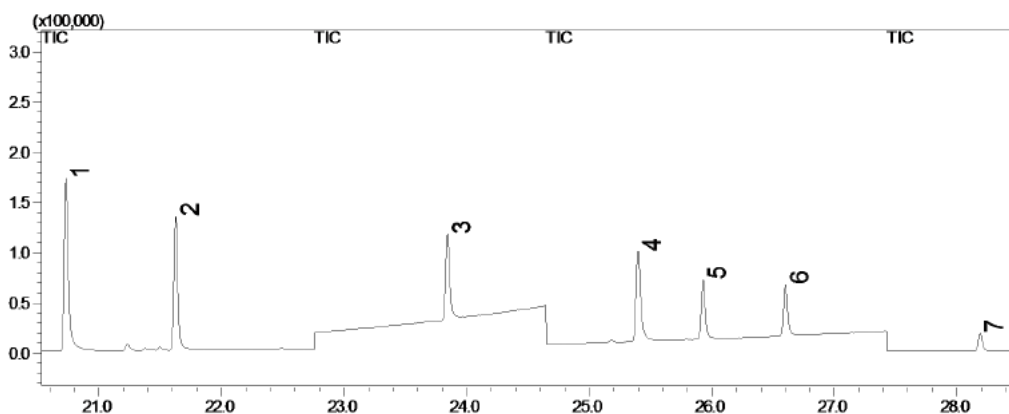


Figure 1  SIM chromatograms of PCBs (10 ng/mL)
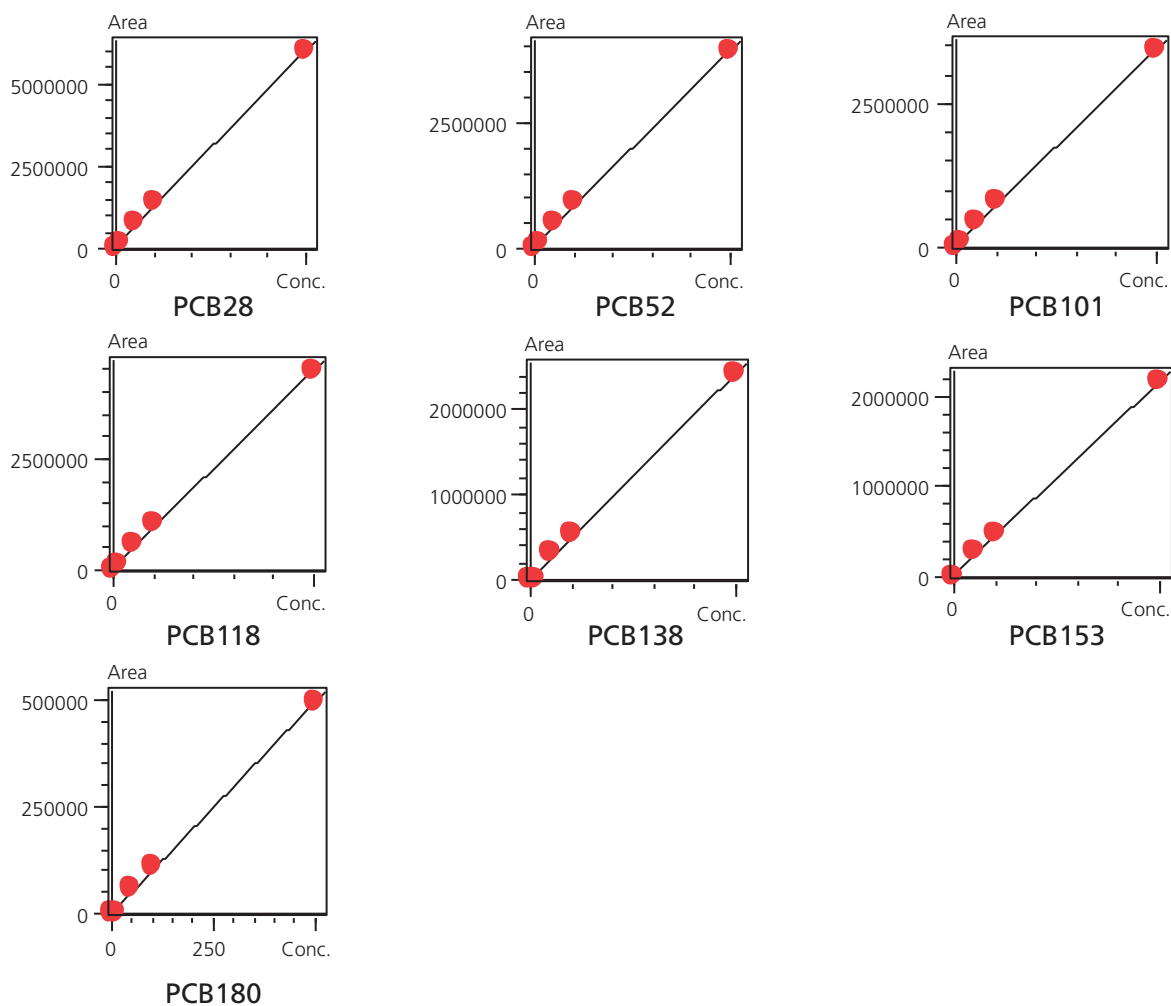


Figure 2  Calibration curve

# Rapid analysis of polychlorinated biphenyls (PCBs) in vegetables by QuEChERS-based extraction and GPC–GCMS

Table 2  Relative coefficients, limit of detection (LOD, S/N=3) and recovery of  the 7 kinds of PCBs

| No. | Compound | Relative coefficients | LOD (µg/L) | Recovery | |
|-----|----------|-----------------------|------------|----------|--------|
| | | | | Carrot | Ginger |
| 1 | PCB28 | 0.9990 | 0.02 | 100.54 | 104.39 |
| 2 | PCB52 | 0.9991 | 0.01 | 97.76 | 101.63 |
| 3 | PCB101 | 0.9991 | 0.02 | 98.01 | 84.13 |
| 4 | PCB118 | 0.9991 | 0.05 | 99.22 | 95.70 |
| 5 | PCB138 | 0.9989 | 0.02 | 108.86 | 87.79 |
| 6 | PCB153 | 0.9996 | 0.02 | 116.63 | 92.21 |
| 7 | PCB180 | 0.9994 | 0.07 | 125.67 | 84.94 |

Table 3  Precision of PCBs (1 ng/mL each, n=6)

| No. | Compound | Area | | | | | | RSD (%) |
|-----|----------|------|------|------|------|------|------|---------|
| | | 1 | 2 | 3 | 4 | 5 | 6 | |
| 1 | PCB28 | 20882 | 21137 | 21076 | 21016 | 20311 | 21474 | 1.83 |
| 2 | PCB52 | 13494 | 13944 | 13702 | 13926 | 13942 | 13706 | 1.33 |
| 3 | PCB101 | 11428 | 11678 | 11653 | 11397 | 11349 | 11558 | 1.21 |
| 4 | PCB118 | 13891 | 14251 | 13501 | 13602 | 14160 | 14300 | 2.45 |
| 5 | PCB138 | 5965 | 5993 | 5557 | 5777 | 5800 | 5861 | 2.70 |
| 6 | PCB153 | 5469 | 5470 | 5063 | 5148 | 5364 | 5091 | 3.58 |
| 7 | PCB180 | 1141 | 1082 | 1044 | 1061 | 1109 | 1100 | 3.20 |

Table 4 Sample test result

| No. | Compound | Carrot | Ginger |
|-----|----------|--------|--------|
| 1 | PCB28 | N.D | N.D |
| 2 | PCB52 | N.D | N.D |
| 3 | PCB101 | N.D | N.D |
| 4 | PCB118 | N.D | N.D |
| 5 | PCB138 | N.D | N.D |
| 6 | PCB153 | N.D | N.D |
| 7 | PCB180 | N.D | N.D |

N.D.: Not detected

# Conclusions

Using the online GPC-GC/MS to analyse 7 kinds of PCBs in carrot and ginger, the method has the advantages of simple operation, high sensitivity and excellent precision.

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# Application News

## No.L497

Supercritical Fluid Extraction / Chromatography

## Using the Nexera UC Online SFE-SFC-MS System to Analyze Residual Pesticides in Agricultural Products

The Nexera UC online SFE-SFC-MS system combines supercritical fluid extraction (SFE) and supercritical fluid chromatography (SFC) in one online system, so that the entire process from extraction of target components to acquisition of data can be performed completely automatically. Furthermore, the system can add polar organic solvents (modifiers) to the supercritical carbon dioxide fluid during SFE and SFC, so that the system can be used to extract and analyze components with a wide range of polarities.

Meanwhile, ever since the positive list system was enacted in 2006 in Japan for residual pesticides in foods, which applies to more than 800 types of pesticides, there has been increasing demand for a system able to simultaneously analyze multiple pesticides with a wide range of properties, including pretreating samples.

This article describes an example of using the Nexera UC online SFE-SFC-MS system to analyze residual pesticides in agricultural products.

### ■ Online SFE-SFC-MS System

The operating principle of the Nexera UC online SFE-SFC-MS system is shown in Fig. 1. The extraction vessel filled with the sample is placed in the SFE unit and heated to an internal temperature of 40 °C (Fig. 1A). Then supercritical carbon dioxide fluid is pumped into the extraction vessel. After filling the vessel, the flow is stopped to allow static extraction of target components (Fig. 1B). After static extraction, the fluid is pumped through the extraction vessel for dynamic extraction (Fig. 1C). During dynamic extraction, extracted substances flow from the extraction vessel and into the analytical column. However, due to the high level of contaminant components in agricultural products, passing all the extract substances through the analytical column or mass spectrometer could damage the column or contaminate the mass spectrometer. Therefore, the Nexera UC online SFE-SFC-MS system splits the flow to send only a portion of the substances extracted from dynamic extraction through the analytical column. After dynamic extraction, fluid is only sent through the analytical flow line, where the analytical column is used for gradient separation and the mass spectrometer for detecting the target components (Fig. 1D).

### ■ Sample Preparation

The QuEChERS is a well-known method that prioritizes simplicity and speed and is commonly used to pretreate agricultural products for residual pesticide analysis. However, the method involves many steps, such as adding reagents, solvent extraction, purification by dispersive solid phase extraction, and centrifugal separation. In contrast, the online SFE-SFC-MS system requires only mixing 1 g of agricultural product crushed with a mixer with 1 g of a dehydrating agent* and placing the mixture in the extraction vessel, as shown in Fig. 2. Consequently, the system improves analytical productivity, reduces the environmental impact, and also avoids human errors involved in the pretreatment steps. Using a dedicated rack changer, the system can continuously extract and analyze up to 48 samples at a time.

\* "Miyazaki Hydro-Protect" Patent No. 3645552
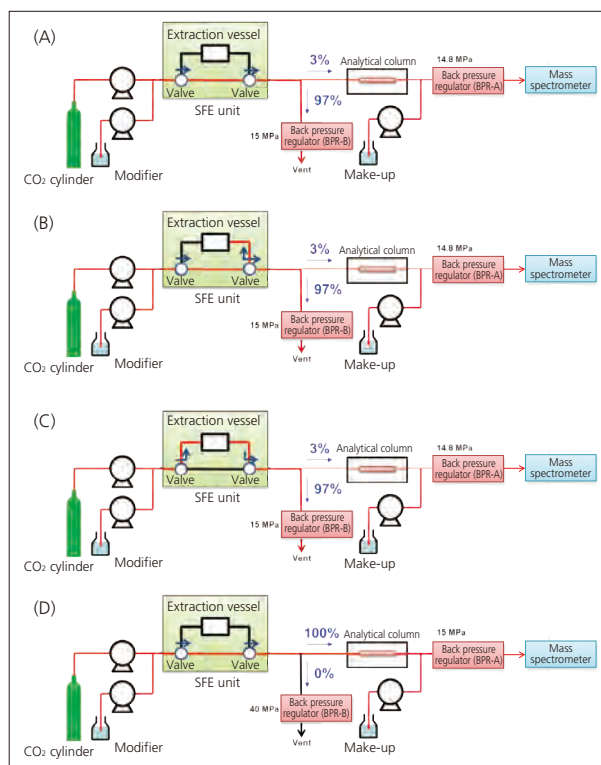


Fig. 1  Analysis Flow by Online SFE-SFC-MS
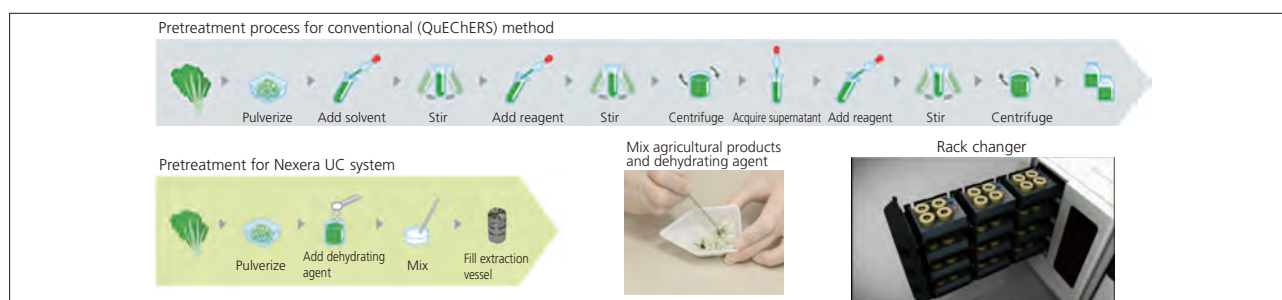


Fig. 2  Sample Preparation

**Table 1  Analytical Conditions**

| [SFE] | | [SFC] | |
|---|---|---|---|
| Solvent | : A) Super critical fluid of $CO_2$ <br> B) 0.1 % Ammmonium formate in methanol | Column | : Shim-pack UC-RP (250 mm L. × 4.6 mm I.D., 5 μm) |
| Flowrate | : 5 mL/min | Mobile Phase | : A) Super critical fluid of $CO_2$ <br> B) 0.1 % Ammmonium formate in methanol |
| Extraction | : 0-3 min. Static mode (B.Conc. 5 %) <br> 3-6 min. Dynamic mode (B.Conc. 5 %) | Time Program | : B.Conc. 0 % (0 min.) → 10 % (11 min.) → 30% (14 min.) → 40 % (14.01-17 min.) |
| Extraction Vessel Temp. | : 40 °C | Flowrate | : 3 mL/min |
| BPR Pressure | : A) 14.8 MPa, B) 15 MPa (split rate: 3 %) | Make-up | : 0.1 % Ammmonium formate in methanol (0.1 mL/min.) |
| Make-up | : 0.1 % Ammmonium formate in methanol (0.4 mL/min.) | Column Temp. | : 40 °C |
| | | BPR Pressure | : A) 15 MPa, B) 40 MPa |
| | | Detector | : LCMS-8050 MRM mode |

## ■ Analysis of Standard Mixture of Pesticides

The standard mixture sample of 510 pesticide components were mixed with a dehydrating agent and analyzed using the analytical conditions indicated in Table 1. Fig. 3 shows the results. Using the system, we were able to accomplish the entire process, from extraction to data acquisition, in about 45 minutes per analysis. For 327 components, we obtained good repeatability for the concentration range from 1 to 100 ng/g (less than 30 %RSD for relative standard deviation for peak area at respective concentrations) and good linearity (contribution ratio of at least $R^2$ = 0.99). Table 2 also shows how pesticides with a wide range of polarities were analyzed with good repeatability and linearity.
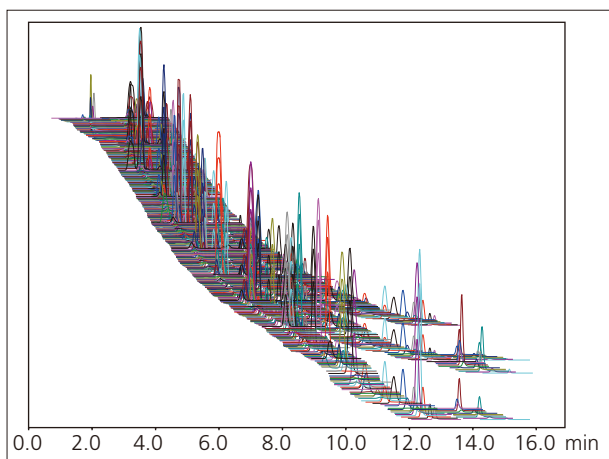


**Fig. 3  Mass Chromatogram of Standard Pesticide Mixture Solution**

**Table 2  Repeatability and Linearity for Representative Pesticides**

| Compounds | LogPow | Repeatability (%RSD, n=5) | Range (ng/g) | $R^2$ |
|---|---|---|---|---|
| Ethofenprox | 6.9 | 6.1 | 1-100 | 0.9991 |
| Hexaflumuron | 5.68 | 6.8 | 1-100 | 0.9992 |
| Benzofenap | 4.69 | 1.4 | 2-200 | 0.9990 |
| Mepronil | 3.66 | 4.6 | 1-100 | 0.9993 |
| Prometryn | 3.34 | 2.7 | 1-100 | 0.9994 |
| Fenamidone | 2.8 | 3.0 | 2-200 | 0.9991 |
| Ethylchlozate | 2.5 | 3.0 | 1-100 | 0.9996 |
| Imazosulfuron | 1.6 | 6.2 | 1-100 | 0.9998 |
| Bensulfuron methyl | 0.79 | 8.1 | 1-100 | 0.9996 |
| Primisulfuron methyl | 0.2 | 5.5 | 1-100 | 0.9994 |
| Halosulfuron methyl | -0.02 | 5.5 | 1-100 | 0.9996 |
| Azimsulfuron | -1.4 | 4.2 | 1-100 | 0.9998 |
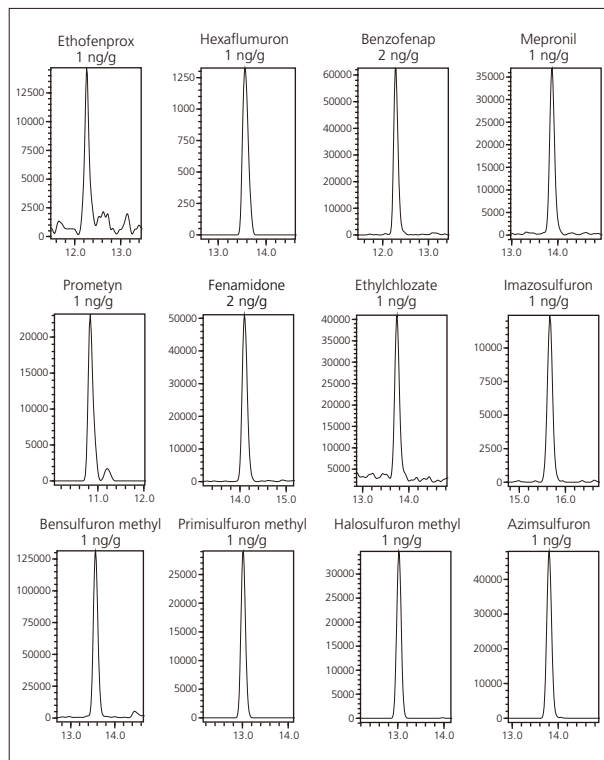


**Fig. 4  MRM Chromatograms of Representative Pesticides**

## ■ Analysis of a Tomato

Analysis of 10 ng/g of 510 pesticide components added to a tomato resulted in good repeatability (less than 20 %RSD for the relative standard deviation of the peak area) and a good recovery rate (70 to 120 %) for 248 components. Plots of LogPow and recovery rate results are shown in Fig. 5. It shows that pesticides with a wide range of polarities were analyzed with good recovery.
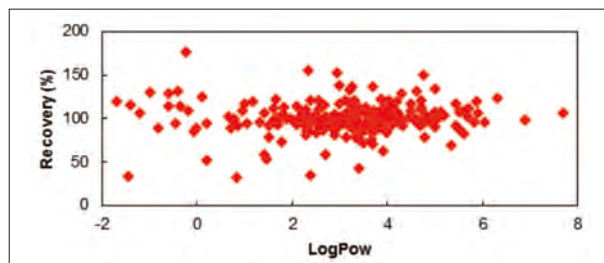


**Fig. 5  LogPow vs. Recovery Rate for Tomato Analysis**

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# Application News

# No. C154

**Liquid Chromatography Mass Spectrometry**

## Applying 'MRM Spectrum Mode' and Library Searching for Enhanced Reporting Confidence in Routine Pesticide Residue Analysis

David R. Baker[1], Chris Titman[1], Jonathan Horner[2], Neil Loftus[1]
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### ■ Abstract

To help reduce the incidence of false positive and false negative reporting in pesticide residue monitoring routine multiple-reaction monitoring (MRM) methods have been enhanced to monitor a higher number of fragment ion transitions to increase specificity and reporting confidence. In this workflow, typically 6-10 fragment ion transitions were monitored for each target pesticide as opposed to a conventional approach using 2-3 fragment ions. By acquiring a high number of fragment ion transitions, each target pesticide had a corresponding fragmentation spectra which could be used in routine library searching and compound verification using reference library match scores. This 'MRM Spectrum Mode' was applied to quantify and identify 193 pesticides using 1,291 MRM transitions without compromising limits of detection, linearity or repeatability.



Compound Name Linuron
Formula C9H10Cl2N2O2
CAS 330-55-2

**Conventional approach**
2 MRM's
1:248.80>160.00
2:248.80>182.10

**MRM Spectrum Mode**
9 MRM's
1:248.80>160.00
2:248.80>182.10
3:250.80>162.00
4:248.80>133.10
5:250.80>135.00
6:248.80>161.00
7:250.80>184.10
8:248.80>125.00
9:248.80>153.00

**Higher specificity
Higher reporting confidence
Library searchable fragment data**

7.75   8.00 min    7.75   8.00 min

**Fig. 1  Using a higher number of fragment ions in MRM data acquisition increases the specificity of detection and reduces false negative and false positive reporting. In the case of linuron, 9 precursor-fragment ion transitions were used to increase confidence in assay specificity. There is no compromise in data quality between methods despite a higher number of fragment ions monitored. Signal intensity, linearity, reproducibility are in good agreement between both methods.**

### ■ Introduction

Multiple Reaction Monitoring (MRM) based LC-MS/MS techniques are widely used on triple quadrupole platforms for targeted quantitation as a result of high selectivity, sensitivity and robustness. In a regulated environment such as food safety there is a growing need to enhance the capability in routine monitoring programs by increasing the number of pesticides measured in a single analysis and at the same time delivering the highest confidence in compound identification to reduce false detect reporting. For pesticide analysis in the EU, identification criteria in SANTE/11945/2015 requires the retention time and the ion ratio from at least 2 MRM transitions to be within acceptable tolerance limits.[*1] However, even applying this criteria it is well reported that false positives can occur in certain pesticide/commodity combinations.[*2-*4]

To reduce false negative and false positive reporting a higher number of MRM transitions were used for each target pesticide to increase the level of confidence in assay specificity. The number of fragment ion transitions monitored for each target pesticide was dependent upon the chemical structure with typically between 6-10 fragment ions for each compound. MRM Spectrum mode combines conventional MRM quantitation with the generation of a high quality MRM product ion spectrum which can be used in routine library searching and compound verification and identification.

In this application paper we present the development of a method for 193 pesticides, with 1,291 MRM transitions, and a 15 minute cycle time. In order to acquire this number of MRM transitions using a short run time a 3 msec dwell time was applied to each MRM transition and a 5 msec polarity switch was used. On average 7 MRM transitions were applied to each compound. The method was quickly set up using the Shimadzu Pesticide Method Package, a data base with more than 750 pesticides and over 6,000 MRM transitions designed to accelerate method set-up and help compound verification. MRM Spectrum mode was also compared to a conventional pesticide monitoring method with 2 MRMs per compound (386 MRMs in total) in order to assess the effect on data quality when adding additional MRM transitions to the method. Several different food commodities were analysed with varying complexity (turmeric, plum, peppermint, parsnip, cherry, lime, pumpkin, tomato, potato). Data was processed using LabSolutions Insight software which provides automated library searching of target MRM spectrum.

## ■ Experimental

Pesticide spiked samples, extracted using established QuEChERS based methods, were provided by Scientific Analysis Laboratories, UK. In order to test the performance of the MRM Spectrum Mode database and library searching a number of matrices were tested including turmeric, plum, peppermint, parsnip, cherry, lime, pumpkin, tomato and potato. Final extracts were prepared in acetonitrile without any dilution and directly injected into the LC-MS/MS. A water co-injection method, performed automatically in the auto-sampler, was used to improve early eluting peak shapes in addition to a sub 2 micron particle size column to improve peak capacity (Table 1) .

Calibration curves were prepared in the range 0.01 to 0.2 mg/kg. Repeatability of the method was tested using avocado matrix at 0.1 mg/kg. In the final method samples were analysed in ESI +/- using a polarity switching time of 5 msec.

On average 7 MRM transitions were applied to each compound, with more than 10 MRM transitions applied to 34 compounds. All MRM transitions were acquired throughout the MRM window without the need for triggering thresholds. The method includes a total of 1,291 MRM transitions for 193 pesticides in a run time of only 15 minutes. A dwell time of 3 msecs was applied to every MRM transition. In order to evaluate the data quality from the MRM Spectrum Mode method, the same method was set up with 2 MRMs applied to each compound (386 MRMs in total) using the same acquisition method (Table 2).

LabSolutions software was used to automatically optimize the fragmentation for all pesticides and generate a MRM Spectrum mode method. The MRM Spectrum Mode method for library searching and compound verification could be simply and quickly set up using the Shimadzu pesticide database. This database contains more than 6,000 MRM transitions for over 750 pesticides.

LabSolutions Insight v3.0 software was used to review quantitative data and MRM Spectrum mode library searching with advanced filtering tools to review by exception and to reduce false detect reporting.

#### Table 1  LC acquisition parameters

| Liquid chromatography | | |
|---|---|---|
| **UHPLC** | Nexera LC system | |
| **Analytical column** | HSS T3 (100 × 2.1, 1.7 μm) | |
| **Column temperature** | 40 °C | |
| **Flow rate** | 0.4 mL/minute | |
| **Solvent A** | 5 mmol/L ammonium formate and 0.004 % formic acid | |
| **Solvent B** | 5 mmol/L ammonium formate and 0.004 % formic acid in methanol | |
| **Binary Gradient** | **Time (mins)** | **%B** |
| | 1.50 | 35 |
| | 11.50 | 100 |
| | 13.00 | 100 |
| | 13.01 | 3 |
| | 15.00 | Stop |
| **Injection volume** | 0.1 μL (plus 30 μL water) | |

**Table 2  MS/MS methods used to acquire data in MRM Spectrum Mode and a conventional MRM method with 2 MRM transitions per compound. As part of the comparative study, the same LC conditions were used for both methods.**

| LC-MS/MS Mass spectrometry | MRM Spectrum Mode: generating library searchable spectra | 2 MRM method |
|---|---|---|
| **Target number of compounds** | 193 | 193 |
| **Total number of MRM transitions** | 1,291 transitions (1,229 in ESI+ and 62 in ESI-) | 386 (374 in ESI+ and 12 in ESI-) |
| **Pause time/dwell time** | 1 msec./3 msec. | 1 msec./3 msec. |
| **Ionisation mode** | ESI +/- | ESI +/- |
| **Polarity switching time** | 5 msec | 5 msec |
| **Interface temperature** | 350 °C | 350 °C |
| **Heat bl°Ck temperature** | 300 °C | 300 °C |
| **Desolvation line temperature** | 150 °C | 150 °C |
| **Nebulising gas** | 3 L/min | 3 L/min |
| **Heating gas** | 10 L/min | 10 L/min |
| **Drying gas** | 10 L/min | 10 L/min |

## ■ Results and Discussion

In developing monitoring programs for chemical contamination methods are designed to determine a list of known analytes with a focus on delivering a rapid, cost-effective analysis that generates no false-negative or false-positive results. Guidelines for compound identification have been published by the EU in directive SANTE/11945/2015 . This identification criteria requires at least two MRM transitions with an ion ratio and retention time within defined tolerance limits.

To help reduce false detect reporting in pesticide monitoring programs, a MRM method was developed with a higher number of MRM transitions for each target pesticide to increase the level of confidence in assay specificity. By combining multiple MRM transitions for a compound into a product ion spectrum, pesticide identification can be verified and confirmed against a MS/MS reference spectral library. Using MRM Spectrum mode can help markedly reduce false detect reporting without affecting the data quality for optimized quantitation or identification.

Fig. 2, shows the MRM chromatogram for all 193 pesticides spiked at 0.010 mg/kg measured with MRM Spectrum mode. Using this mode 1,291 MRM transitions were measured for 193 pesticides. Despite the high data density acquired with MRM Spectrum Mode (for example, 151 MRM transitions were registered in the same time window during the analysis, see Fig. 3) sensitivity was not affected by the high data acquisition rate.

## ■ Method performance
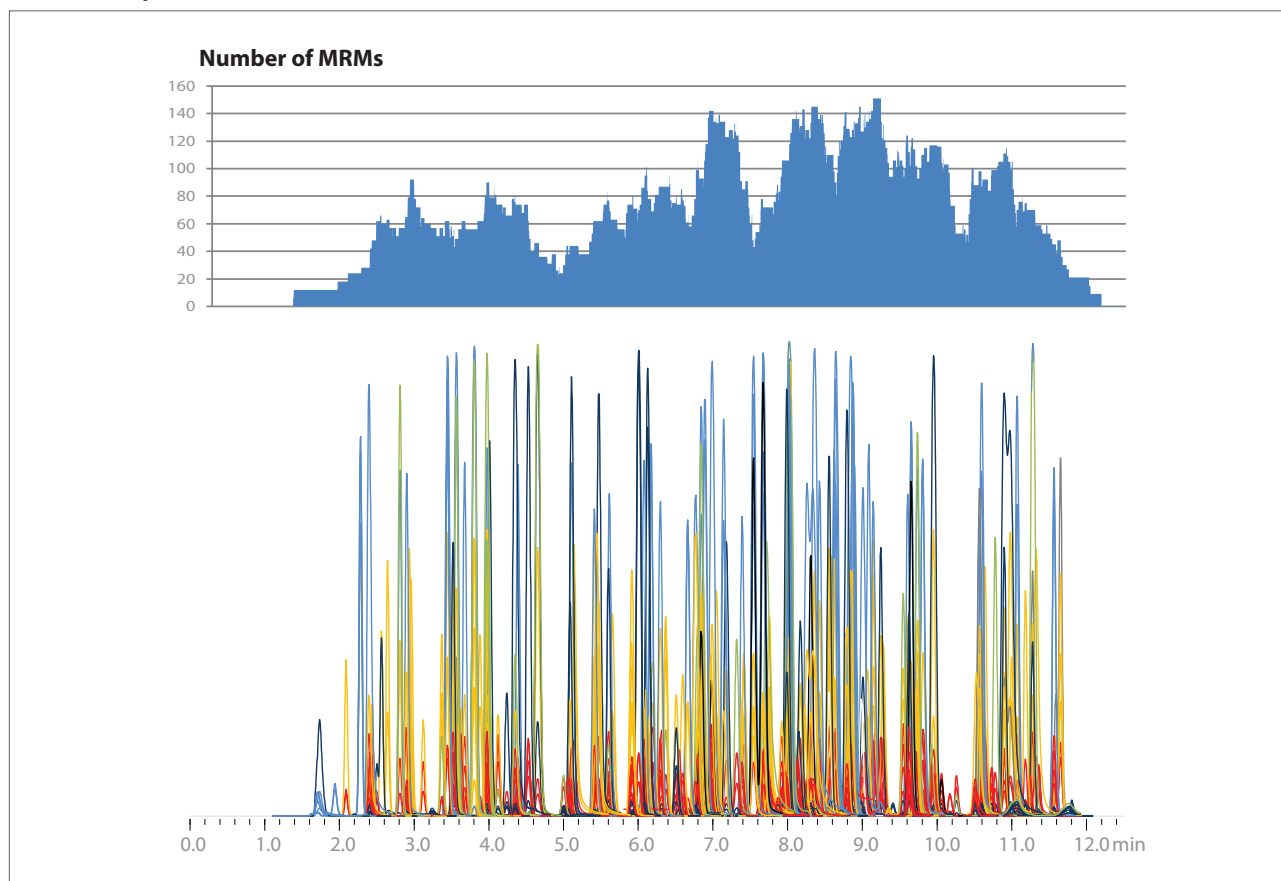
**Number of MRMs**



**Fig. 2** Histogram showing the number of MRM transitions monitored at each time point and chromatogram showing all 193 target compounds. The highest number of overlapping MRM's acquired was 151. Even at such a high data sampling rate the response was in agreement with a conventional 2 MRM method with peak area variation less than 5.2% (n=5). This data is displayed below in more detail, Fig. 3.

**Table 3** Between 8.80 mins and 9.30 mins 151 MRM transitions in both positive and negative ion were monitored. Peak area repeatability for the 22 compounds eluting in this time period is shown below.

| | Ret. Time | # MRMs | Polarity | Peak Area %RSD (n=5) |
|---|---|---|---|---|
| Dichlofluanid | 8.80 | 6 | ESI+ | 2.2 |
| Dichlofluanid 2 | 8.80 | 6 | ESI+ | 3.4 |
| Dichlofluanid 1 | 8.80 | 5 | ESI+ | 2.6 |
| Fluoxastrobin | 8.82 | 12 | ESI+ | 2.0 |
| Fenhexamid | 8.83 | 11 | ESI+ | 2.2 |
| Iprovalicarb | 8.88 | 6 | ESI+ | 2.3 |
| Spirotetramat | 8.89 | 6 | ESI+ | 2.6 |
| Azinphos-ethyl | 8.90 | 5 | ESI+ | 3.1 |
| Chromafenozide | 8.91 | 5 | ESI+ | 3.2 |
| Triticonazole | 8.93 | 5 | ESI+ | 2.1 |
| Cyazofamid | 9.01 | 5 | ESI+ | 2.1 |
| Prothioconazole desthio | 9.07 | 10 | ESI+ | 1.9 |
| Diflubenzuron | 9.09 | 4 | ESI+ | 2.0 |
| Pyrifenox | 9.11 | 8 | ESI+ | 2.0 |
| Dodemorph | 9.17 | 6 | ESI+ | 2.1 |
| Fenoxycarb | 9.17 | 6 | ESI+ | 2.0 |
| Rotenone | 9.17 | 6 | ESI+ | 2.4 |
| Fipronil | 9.20 | 10 | ESI- | 5.2 |
| Bixafen | 9.25 | 8 | ESI- | 2.8 |
| Tebufenozide | 9.27 | 6 | ESI+ | 3.9 |
| Bensulide | 9.27 | 6 | ESI+ | 2.6 |
| Neburon | 9.30 | 9 | ESI+ | 1.7 |
| | | Total MRM's 151 | | Average 2.6 %RSD |
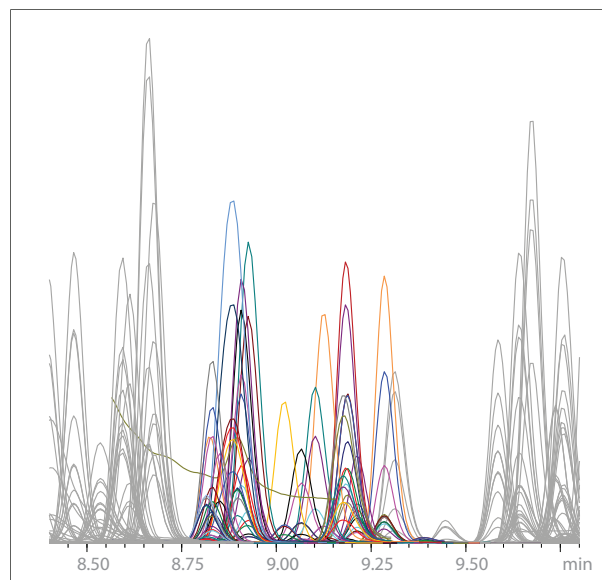


**Fig. 3** Between 8.80 mins and 9.30 mins 151 MRM transitions in both positive and negative ion were monitored. During this time period 22 target pesticides eluted with a peak area variation less than 5.2 % RSD. Data was acquired in an avocado sample matrix at a concentration of 0.1 mg/kg.

## ■ Method performance



Compound Name Ethirimol
Formula C11H19N3O
CAS 23947-60-6
RT 7.02mins

**Conventional approach**
2 MRM's
ESI+
1:210.20>140.20
2:210.20>98.20

**MRM Spectrum Mode**
12 MRM's
ESI+
1:210.20>140.20
2:210.20>98.20
3:210.20>182.20
4:210.20>193.05
5:210.20>70.20
6:210.20>165.20
7:210.20>71.20
8:210.20>138.10
9:210.20>150.20
10:210.20>95.15
11:210.20>107.25
12:210.20>167.20

Higher specificity
Higher reporting confidence
Library searchable fragment data

Compound Name Lufenuron
Formula C17H8Cl2F8N2O3
CAS 103055-07-8
RT 10.75mins

**Conventional approach**
2 MRM's
ESI-
1:508.90>339.00
2:508.90>326.00

**MRM Spectrum mode**
10 MRM's
ESI-
1:508.90>339.00
2:508.90>326.00
3:508.90>175.10
4:508.90>488.80
5:508.90>202.10
6:510.90>328.00
7:510.90>340.90
8:510.90>177.10
9:510.90>490.80
10:510.90>204.00

Higher specificity
Higher reporting confidence
Library searchable fragment data

**Fig. 4** **MRM chromatograms for ethirimol (positive ion) and lufenuron (negative ion) acquired using a conventional 2 fragment ion MRM method and compared to a method with a higher number of precursor-fragment ions to increase confidence in assay specificity and reporting.**

**Despite acquiring a higher number of MRM transitions the library searchable MRM approach (acquiring 1,291 transitions in a single method) results in the same signal intensity compared to a conventional 2 fragment ion MRM method (acquiring 386 MRM transitions in a single method). The repeatability for each MRM method was evaluated by repeatedly injecting (n=5) an avocado extract corresponding to a concentration of 0.1 mg/kg. In each MRM method the %RSD was less than 3.5% for both compounds.**

To minimize the possibility of false positive and false negative reporting LC-MS/MS methods were developed with a high number of MRM transitions for each pesticide. The performance of this approach was compared with a conventional MRM method monitoring 2 transitions for each pesticide.

In Fig. 4, the MRM chromatograms for 2 compounds, ethirimol and lufenuron, are shown for the same sample extract acquired using different MRM methods (the sample is avocado spiked at 0.1 mg/kg). The MRM chromatograms show un-smoothed data and are scaled to the same signal intensity for each compound. Ethirimol and lufenuron elute at 7.02 and 10.75 mins corresponding to time windows of high data density with more than one hundred MRM transations monitored in the same time segment. However, regardless of the high number of fragment ions monitored, the absolute signal intensity for both approach's is near identical in positive and negative ion mode.

Fig. 5 shows the correlation between the peak areas for all pesticides measured using 2 different MRM methods. The linear regression curve shows a good agreement between the peak areas measured for all pesticides spiked into sample matrix with a slope value near unity and an intercept near zero.



$$y = 1.0048x - 4928.6$$
$$R^2 = 0.9989$$
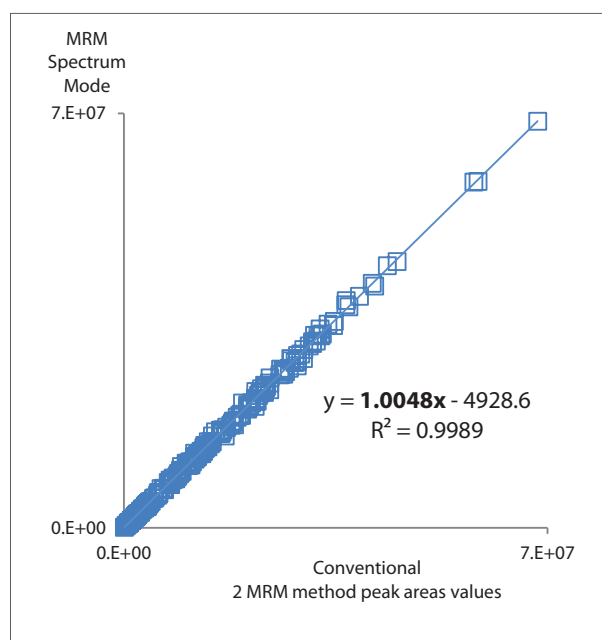
**Fig. 5** **Absolute peak area response for all 193 pesticides acquired using a conventional MRM method with 386 transitions compared to a MRM method with 1,291 transitions designed for library searchable verification. Both approaches result in near identical peak areas regardless of the number of fragment ions used to verify and identify each pesticide.**

## ■ Spectrum based identification

In this study, the number of qualifier fragment ion transitions was increased for each pesticide and the combined transitions were used to create a MRM product ion spectrum. This product ion spectrum derived from MRM acquisitions was used in conventional library matching routines comparing against a reference spectrum to generate a similarity score.

In Fig. 6, demeton-S-methyl sulphone was to highlight library matching in different matrices including cumin, potato, mucuna pruriens powder, tomato, black pepper, peppermint tea and turmeric. Even in the presence of complex spice matrices the library matching approach identified demeton-S-methyl sulphone with a high similarity score and a high degree of confidence for data reporting.



Compound Name Demeton-S-methyl sulphone
Formula C6H15O3PS2
CAS 919-86-8
RT 2.94mins

MRM spectrum
Precursor-fragment ions
11 MRM's
1:263.00>109.10 CE: -30V
2:263.00>169.10 CE: -22V
3:263.00>125.05 CE: -25V
4:263.00>121.15 CE: -16V
5:263.00>230.90 CE: -14V
6:263.00>93.10 CE: -21V
7:263.00>78.85 CE: -46V
8:263.00>143.15 CE: -16V
9:263.00>110.85 CE: -29V
10:263.00>77.05 CE: -30V
11:263.00>65.00 CE: -51V
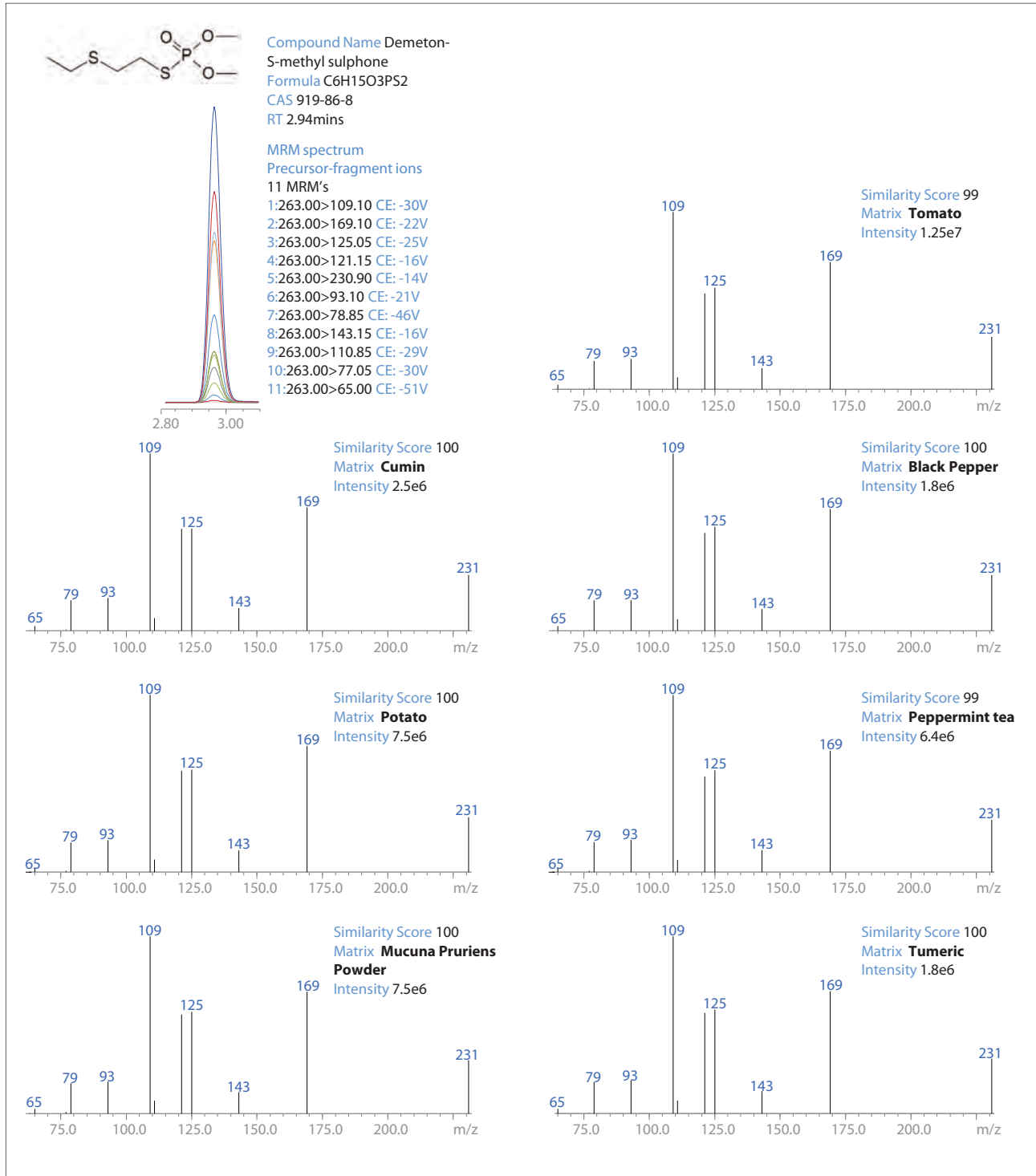
**Fig. 6 MRM spectrum identification in different matrices for demeton-S-methyl sulphone**

## ■ Spectrum based identification

To increase the confidence in reporting results the number of qualifier transitions was increased for each pesticide and the combined MRM transitions were used to create a product ion spectrum. This MRM product ion spectrum can then be automatically compared against a reference spectrum to generate a product ion spectrum match score using conventional library matching.

Fig. 7 highlights the advantage of using a library searchable fragment ion spectrum in identifying and quantifying desmedipham and phenmedipham. Both desmedipham and phenmedipham share several common fragment ions and have similar retention times. Using MRM Spectrum Mode and comparing to a library searchable spectra, both desmedipham and phenmedipham are positively identified (fragment ions at *m/z* 154 and 182 are absent in product ion spectrum for phenmedipham).
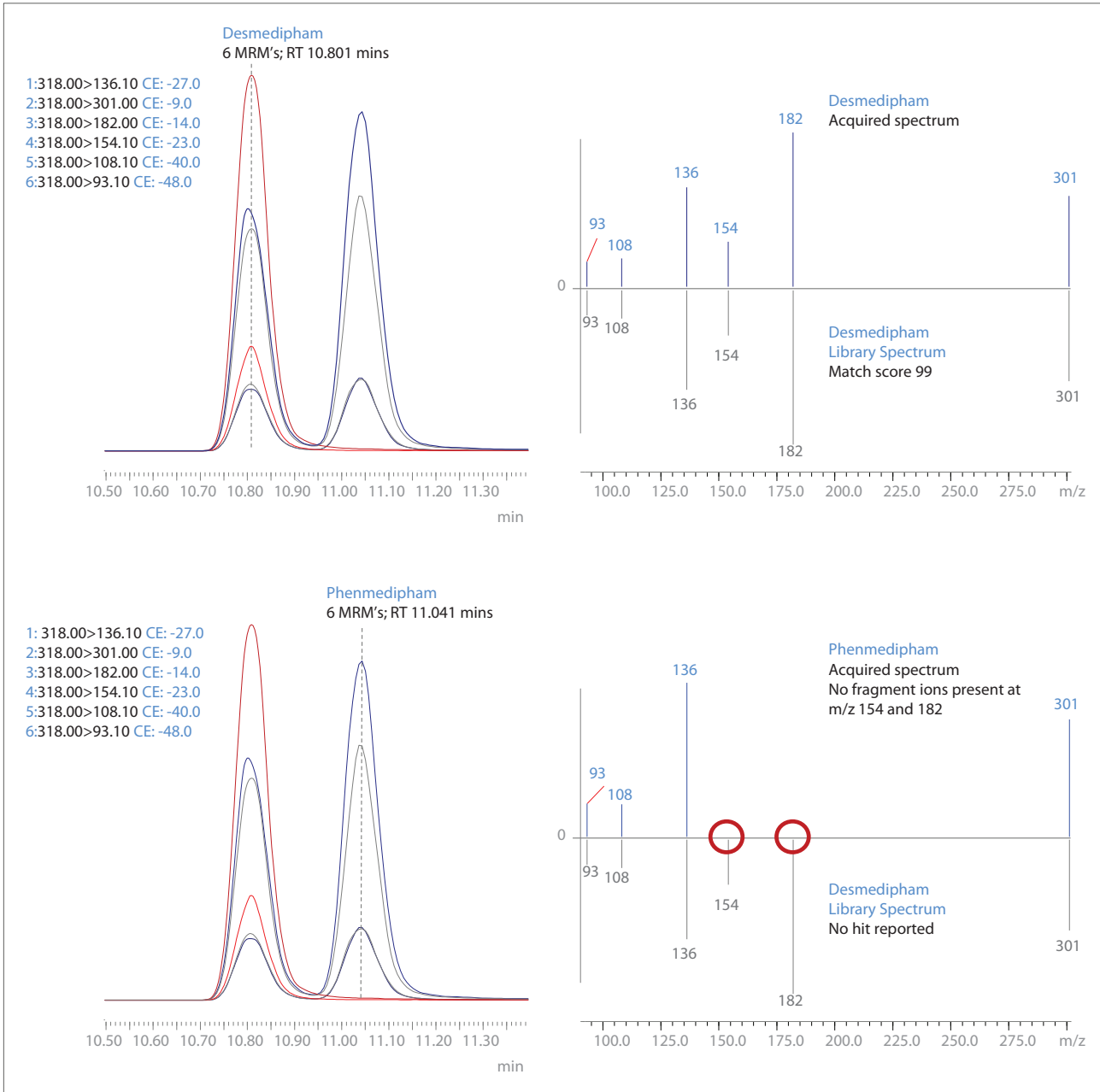


**Fig. 7** MRM chromatogram for desmedipham and phenmedipham spiked into a cumin extract at 0.1 mg/kg. As phenmedipham shares common transitions and elutes at a similar retention time as desmedipham the MRM spectrum can be used to distinguish between both pesticides to avoid false positive reporting.

## ■ Quantitation

As one example, carbendazim was spiked into a matrix at three different concentration levels. In Fig. 8, all MRM transitions were detected even at the reporting level of 0.010mg/kg with a signal to noise for all fragment ion transitions greater than 9. The response was linear for all transitions throughout the calibration range (0.010-0.200mg/kg) as shown Fig. 9.

The limit on the number of MRM transations used to generate a product ion spectrum is dependent on the chemical structure of the pesticide molecule. In the case of carbendazim, several bonds could be broken using collision energies between 10-60V resulting in a product ion spectrum of 12 fragment ions. The product ion spectrum can then be used for library search and analyte confirmation as shown in Fig. 10. For each calibration level ranging from 0.010-0.200mg/kg the library similarity score was greater than 99 confidently confirming the target analyte. The advantage of this technique is that library searchable product ion spectrum data is used in target compound identification without compromising sensitivity, accuracy and robustness in quantitative data reporting.



Compound Name Carbendazim
Formula C9H9N3O2
CAS 10605-21-71
RT 4.42mins

MRM spectrum Mode
Precursor-fragment ions
12 MRM's
1:192.10>159.95 CE: -34V
2:192.10>132.10 CE: -32V
3:192.10>105.15 CE: -41V
4:192.10>65.10 CE: -48V
5:192.10>90.15 CE: -42V
6:192.10>92.15 CE: -36V
7:192.10>117.15 CE: -33V
8:192.10>78.15 CE: -55V
9:192.10>133.10 CE: -32V
10:192.10>51.10 CE: -60V
11:192.10>106.20 CE: -42V
12:192.10>78.90 CE: -50V
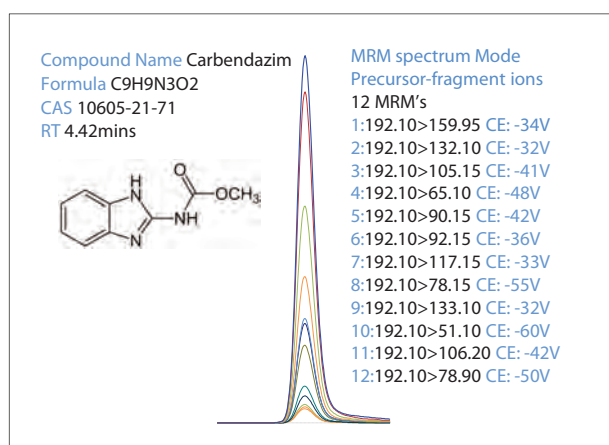
**Fig. 8 By applying a range of collision energies to carbendazim 12 precursor-fragment ions are generated. MRM 192.10>159.95 was used in generating sensitive and robust quantitation whilst the product ion spectrum using all 12 fragment ions was used in confirming peak identification.**



Peak Area

Compound Name Carbendazim
Linear regression curve No weighting
Regression  $R^2 = 0.9993576$

Concentration (mg/kg)

**Fig. 9 Calibration curve for carbendazim using the optimized quantitation ion transition (MRM 192.10>159.95). The response was linear for all calibration and QC samples. All 12 fragment ions were above a signal to noise ratio of 10 even at the reporting level of 0.010mg/kg.**



**Fig. 10 MRM Product ion spectrum data for carbendazim in 3 calibration levels (0.010-0.200mg/kg) spiked into a food matrix was compared with an authentic library spectrum of carbendazim. In all library searches the similarity score was greater than 99 indicating a very high confidence in compound verification and reporting.**

### Data Reporting

Automated reference library matching and quantitation results can be simply viewed using LabSolutions Insight software (Fig 11).

LabSolutions Insight software helps to review by exception and to reduce false positive reporting by verifying compound identification using library matching scores and retention time variation from a calibration standard.
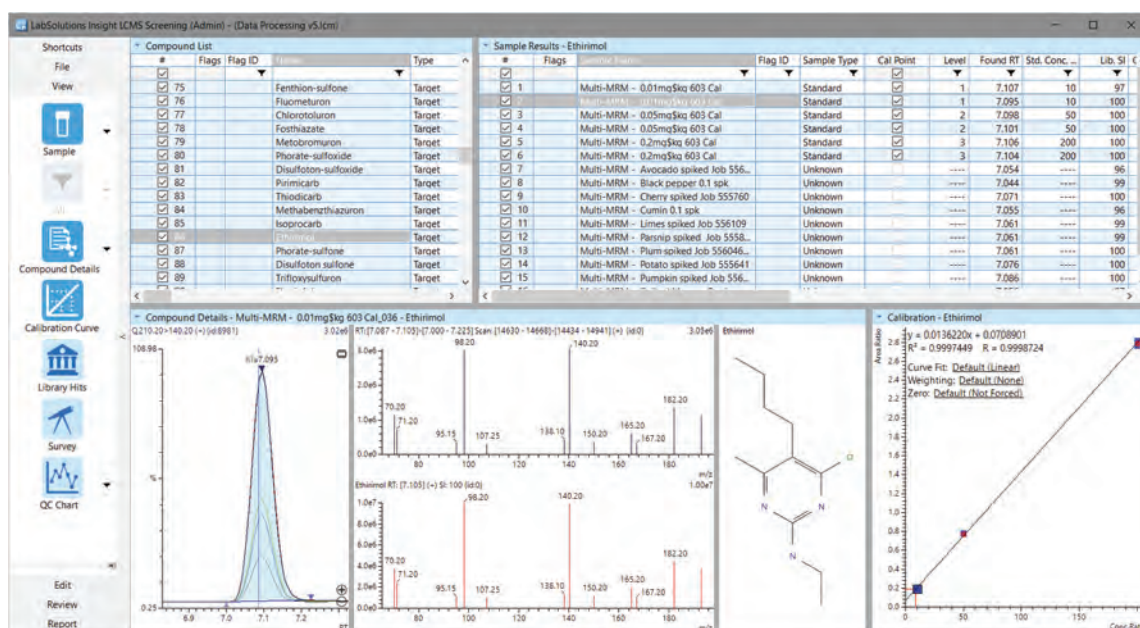


**Fig. 11 LabSolutions Insight software helps to review quantitative and reference library matching results quickly and easily. Flexible filtering and sorting tools can be used to help reduce reporting false detects, especially in high throughput laboratories by filtering results based upon a similarity score with a reference library product ion spectrum.**

### Conclusions

False positive results are a major issue for all pesticide residue monitoring laboratories. EU regulations require that retention time and the ion ratio between 2 MRM transitions are within a set threshold. However, even applying this criteria false positives may occur for certain pesticide/commodity combinations.

In this application paper, we have applied MRM Spectrum Mode to identify and quantify 193 target pesticides in a number of different sample matrices. The library score is used as an additional identification criterion in order to improve identification confidence.

Acquisition of the MRM Spectrum mode method (1,291 MRM transitions) did not compromise data quality when compared to a conventional 2 MRM per compound method (386 MRM transitions) with consistent signal response and repeatability in both methods. The MRM product ion spectrums were demonstrated to be consistent across the linear range and between different matrices. The method acquired data in both positive and negative ion modes with a polarity switching time of 5 msec enabling fast cycle times and a high data collection rate.

All 1,291 MRM transitions were acquired throughout the MRM window. No 'triggering' of MRM transitions was necessary due to the short dwell times that were applied using the LCMS-8060. Therefore, MRM transitions can be swapped between qualifier and qualifier if needed and the peak shape of the additional MRM transitions can be assessed.

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## Technical Report

# Residual Pesticides Analysis of Botanical Ingredients Using Gas Chromatography Triple Quadrupole Mass Spectrometry

Riki Kitano[1], Tairo Ogura[1], Nicole Lock[1], Robert Clifford[1], Julie Kowalski[2], Jack Cochran[2], Dan Li[2]

## Abstract:

Dietary supplements, which are consumed worldwide, are made from various botanical ingredients. To be safe from pesticides exposure, residual pesticides must be monitored by chromatographic instrumentation. Issues arise however, due to the fact these botanical samples are dried and cause large interferences in the chromatography. This study shows that the modified QuEChERS method combined with GC-MS/MS achieves consistent pesticides monitoring in botanical ingredients.

**Keywords: botanical, QuEChERS, pesticides, GC-MS/MS**

## 1. Introduction

The use of dietary supplements is increasing in the United States. These dietary supplements are made from various dried botanicals and residual pesticides and because of that, they have to be monitored to ensure their quality and prevent exposure. The ingredients are dried which requires the standard QuEChERS methods[1]) to be modified to overcome this difficulty. Gas chromatography is best technique to separates multi components including coextracted interferences, and because the triple quadruple mass spectrometry (GC-MS/MS) is highly sensitive this allows analysis of trace level contamination. In this study, we analyzed over 200 compounds simultaneously using a triple quadrupole gas chromatograph mass spectrometer with the modified QuEChERS method.

## 2. Materials and Methods

Pesticides standards, internal standards, quality control standards and QuEChERS kit were obtained from Restek:
· GC Multiresidue Pesticide Kit (Cat.#: 32562)
· GCMS Internal Standard Mix (Cat.#: 33267)
· SV Internal Standard Mix (Cat.#: 31206)
· Q-sep QuEChERS Extraction Kit (Original) (Cat.#: 23991)

The total number of targets was 232 compounds (220 pesticides, 6 internal standards, and 6 quality control standards).Ginseng, which can be purchased in any store, was used as a matrix. Using this ginseng, matrix-matched calibration standards (1 to 200 ng/mL) and fortified samples (each two 10 and 50 ng/g) were prepared. Calibration curves were generated by internal standard method, weighted 1/C and the internal standard was PCB52.

A MRM analytical method was created using the Smart Pesticides Database (Shimadzu). This database has retention indices for all registered compounds, and retention times can be predicted by running an n-alkane sample mixture (AART: Automatic Adjustment of Retention Time). According to estimated retention times, Smart MRM® creates an optimum data acquisition time program (Fig. 1).

## 2-1. Extraction and Clean-up Procedure

▶ Weigh 1.0 ± 0.05 g ground ginseng powder into 50 mL polypropylene centrifuge tube.
▶ Add 10 mL HPLC-grade water and vortex the tube vigorously.
▶ Add 10 mL of the ACN/IS Extraction Solvent.
▶ Allow the tube to sit for 15 min.
▶ Add 4 g anhydrous $MgSO_4$ and 1 g NaCl.
▶ Shake the tube vigorously on a mechanical shaker for 30 min.
▶ Centrifuge the 50 mL tubes at 3000–4500 rpm × 5 min.
▶ Condition the GCB/PSA (0.25 g/0.5 g) SPE columns with ~250 mg anhydrous $Na2SO_4$ on top using 3 column volumes of acetone.
▶ Insert a collection rack consisting of 15 mL disposable glass centrifuge tube on a SPE vacuum manifold.
▶ Add 1.25 mL of the ACN extract.
▶ Rinse with 1 mL acetone.
▶ Elute with 12 mL of 3:1 v/v acetone:toluene.
▶ Evaporate (50 °C) the eluent to ~100 µL gently.
▶ Add 500 µL of toluene to the Blank/fortified samples, calibration standard solutions to matrix matched calibration standards.
▶ Add 20 µL quality control standards (12.5 µg/mL) and ~50 mg of anhydrous $MgSO_4$ to all samples.
▶ Vortex for 5 sec.
▶ Centrifuge the tubes at 3000 g × 5 min.
▶ Transfer the toluene extract using a Pasteur pipette to ALS GC vials.

## 2-2. Analytical Condition

**System Configuration**

| | |
|---|---|
| GC-MS/MS | : GCMS-TQ8040 (Shimadzu) |
| Auto Injector | : AOC-20i + 20s (Shimadzu) |
| Column | : SH-Rxi-5MS 30 m × 0.25 mm I.D., df = 0.25 µm (Shimadzu, P/N: 221-75940-30) with Rxi guard column 5 m × 0.25 mm I.D. (Restek, Cat.#: 10029) |
| Glass Liner | : Sky Liner, Splitless Single Taper Gooseneck w/Wool (Restek, Cat.#: 23336.5) |
| Software | : GCMSsolution Ver. 4.42 |

**GC**

| | |
|---|---|
| Injection Temp. | : 250 °C |
| Oven Temp. | : 90 °C (1 min), 30 °C/min to 130 °C, 10 °C/min to 330 °C (2 min) |
| Total GC Time | : 24.33 min |
| Carrier Gas Control | : Linear Velocity (55 cm/sec) |
| Injection Mode | : Splitless with high pressure injection (250 kPa, 1.5 min) |
| Injection Volume | : 2 µL |

**MS**

| | |
|---|---|
| Interface Temp. | : 290 °C |
| Ion Source Temp. | : 230 °C |
| Ionization Mode | : EI (Ionization voltage: 70 eV) |
| Acquisition Mode | : MRM (2 transitions for each compound) |
| Resolution | : Unit (Q1) – Low (Q3) |
| Loop Time | : 0.4 sec |



Fig. 1 Data Acquisition Time Program Created by Smart MRM

# 3. Result and Discussion

## 3-1. Matrix Matched Calibration

The chromatogram in Fig. 2 shows a 10 ng/mL matrix matched calibration standard. Of the 232 compounds, 230 could be detected in ±0.1 min of estimated retention time by AART. The remaining two compounds, 1,4-Dichlorobenzene-d4 and Naphthalene-d8 of six quality control standards, had eluted before 4 min. Although retention times were shifted, they were with identified within about ±0.2 min of estimated.

Calibration curves were generated from matrix matched calibration standards, then back calculation and linearity were evaluated.

Back calculation was performed by calculating the concentration of each calibration point, and if the concentration exceeds ±20% of theoretical value, the calibration point would be interpolated with the nearest two points. Over 93% of the compounds with concentration of 1 ng/mL were within ±20% of theoretical calculations and all compounds of concentration 20 to 200 ng/mL were within ±20% (Fig. 3).

This modified QuEChERS method contains dilution steps, and samples will be diluted by quarter. To quantify 10 ng/g concentration, 2 ng/g or lower calibration point are required. Even at low concentrations, the calibration curves show good linearity (Fig. 4) and all coefficients of determination (220 pesticides) were greater than 0.99.
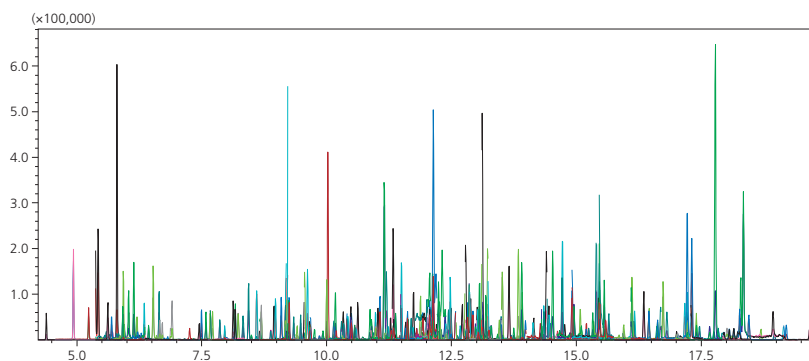


Fig. 2 MRM Chromatogram of 10 ng/mL Matrix Matched Calibration Standard (Internal standards and quality control standards are not displayed.)
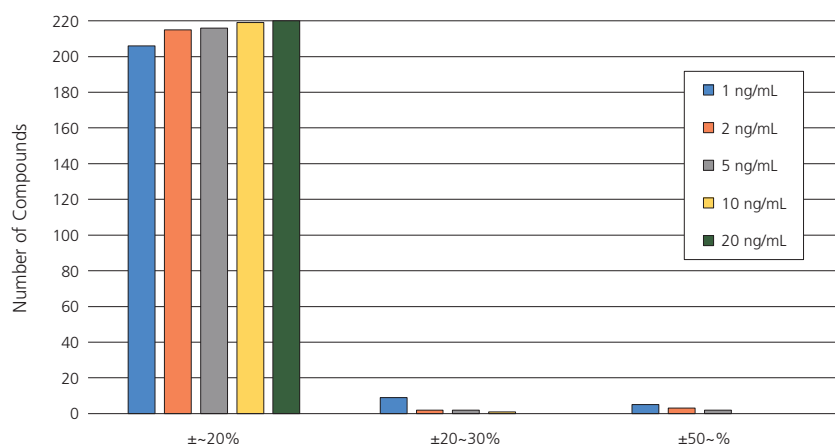
Fig. 3   Difference Between Back Calculation and Theoretical Concentration
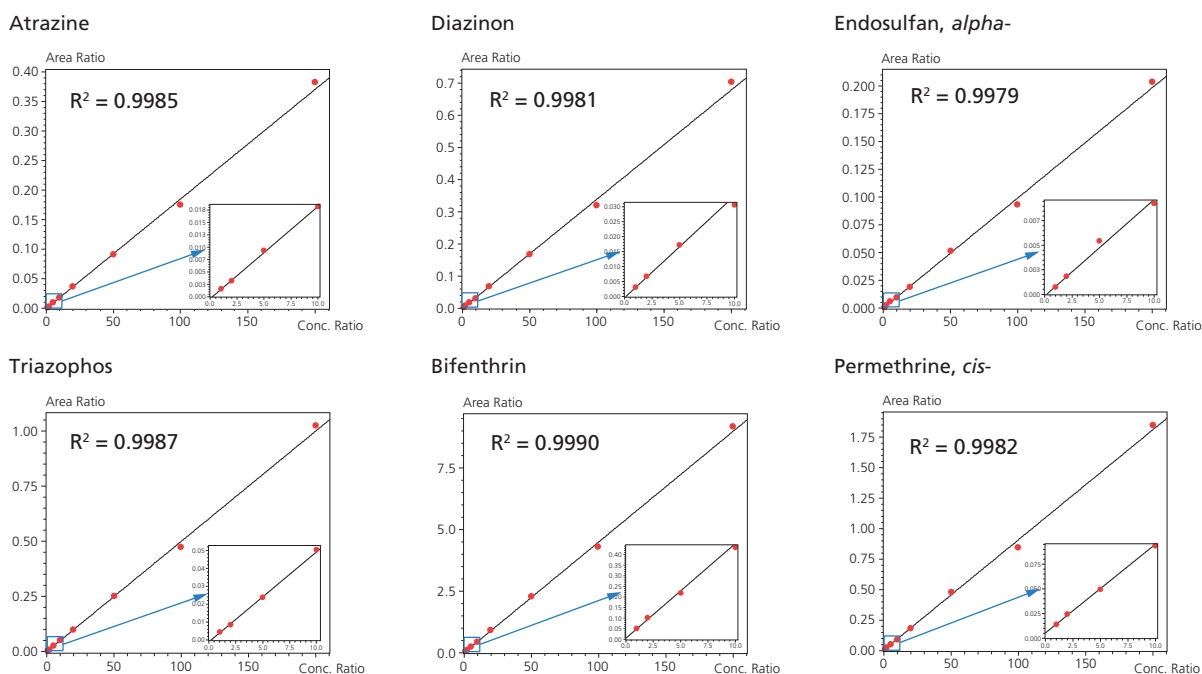


Fig. 4   Calibration Curves of Representative Six Compounds

## 3-2. Recovery of Fortified Sample

Each two 10 ng/g and 50 ng/g fortified samples were prepared (10 ng/g-1, 10 ng/g-2, 50 ng/g-1, 50 ng/g-2) and these recovery rates were evaluated from the average of three successive data points for each samples.

Of the compounds, 85% showed good recovery within the range of 70 to 120% on 10 ng/g-1 and 50 ng/g-2. As mentioned previously, fortified samples were diluted to 2.5 ng/mL and 12.5 ng/mL. Since calibration curves showed good linearity at low concentration and modified QuEChERS method suppressed interference, good recovery result were achieved. (Some compounds were not quantified correctly because the matrix for the calibration standards originally contained them. Y-intercept were lifted up and this shift might cause incorrect quantification, especially at low concentration. From the standard addition method, 16 pesticides were detected with more than 10 ng/g in matrix.)

In this study, recovery results were rechecked and combined with qualitative information, the relative ion ratio. Ion ratios between the target and reference were compared to that of the 100 ng/g standard and evaluated according to SANCO/12571/2013[2]. This mentions that the use of relative ratio ±30% as a criteria is recommended.

Table 1 shows recovery and relative ion ratio for all compounds and Fig. 5 shows the combination map of recovery and relative ion ratio, which was generated from this table. Of the compounds, 76% in the 10 ng/g-1 were within ±30% on relative ion ratio with good recovery of between 70 to 120%. For 50 ng/g-2, 83% of the compounds were within a ±30% relative ion ratio. And here, compounds which showed poor recovery and/or over ±30% relative ion ratio were examined (Page 7).

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Table 1　Recovery and Relative Ion Ratio; Relative ion ratios were calculated by those of 100 ng/mL standard solution.

| ID | Compound Name | Transitions | | | | Recovery (Average of n = 3) | | | | Relative Ion Ratio (Average of n = 3) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Target | CE | Reference | CE | 10 ng/g-1 | 10 ng/g-2 | 50 ng/g-1 | 50 ng/g-2 | 10 ng/g-1 | 10 ng/g-2 | 50 ng/g-1 | 50 ng/g-2 |
| 1 | 2,3,5,6-Tetrachloroaniline | 228.9 > 158.0 | 18 | 230.9 > 158.0 | 22 | 72.5 | 67.0 | 61.4 | 68.8 | 93.6 | 97.2 | 98.9 | 101.1 |
| 2 | 2,4'-Methoxychlor | 227.1 > 121.1 | 16 | 228.1 > 122.1 | 16 | 83.0 | 101.1 | 86.5 | 80.2 | 94.7 | 105.0 | 104.6 | 106.7 |
| 3 | 2-Phenylphenol | 170.1 > 141.1 | 24 | 170.1 > 115.1 | 28 | 72.7 | 72.3 | 63.2 | 69.0 | 113.1 | 112.9 | 99.1 | 102.1 |
| 4 | 3,4-Dichloroaniline | 161.0 > 99.0 | 22 | 161.0 > 126.0 | 14 | 74.5 | 66.2 | 56.4 | 64.4 | 103.8 | 105.5 | 105.8 | 106.0 |
| 5 | 4,4'-Dichlorobenzophenone | 139.0 > 111.0 | 14 | 139.0 > 75.0 | 26 | 77.4 | 83.2 | 73.5 | 73.8 | 97.5 | 104.7 | 102.0 | 100.3 |
| 6 | 4,4'-methoxychlor olefin | 308.0 > 238.1 | 16 | 310.0 > 238.1 | 20 | 84.4 | 94.8 | 85.7 | 83.0 | 99.4 | 101.0 | 100.0 | 98.4 |
| 7 | Acequinocyl deg. | 342.2 > 188.1 | 14 | 342.2 > 160.1 | 22 | 105.3 | 269.8 | 201.1 | 115.2 | 61.2 | 62.8 | 90.7 | 64.0 |
| 8 | Acetochlor | 223.1 > 132.1 | 22 | 223.1 > 147.1 | 10 | 77.5 | 88.3 | 77.4 | 77.1 | 109.0 | 104.1 | 102.8 | 106.2 |
| 9 | Acrinathrin | 289.1 > 93.0 | 14 | 181.1 > 152.1 | 26 | 102.5 | 121.4 | 91.0 | 82.4 | 87.0 | 94.7 | 92.3 | 95.1 |
| 10 | Alachlor | 188.1 > 160.1 | 10 | 188.1 > 132.1 | 18 | 74.1 | 87.2 | 77.8 | 74.1 | 114.7 | 107.2 | 97.3 | 103.5 |
| 11 | Aldrin | 262.9 > 191.0 | 34 | 262.9 > 193.0 | 28 | 86.0 | 66.9 | 65.2 | 74.3 | 79.8 | 101.1 | 104.6 | 93.2 |
| 12 | Allidochlor | 132.1 > 56.0 | 8 | 132.1 > 49.0 | 24 | 71.8 | 64.5 | 59.0 | 65.9 | 123.7 | 118.4 | 113.4 | 117.2 |
| 13 | Anthraquinone | 208.1 > 180.1 | 10 | 208.1 > 152.1 | 22 | 0.0 | 126.7 | 47.4 | 48.0 | 97.0 | 83.6 | 90.4 | 94.0 |
| 14 | Atrazine | 200.1 > 104.1 | 18 | 200.1 > 122.1 | 8 | 77.8 | 94.0 | 83.3 | 78.6 | 105.3 | 110.1 | 92.5 | 100.6 |
| 15 | Azinphos-ethyl | 160.1 > 132.1 | 4 | 160.1 > 77.0 | 18 | 91.4 | 115.5 | 94.5 | 86.5 | 102.1 | 94.8 | 100.0 | 98.7 |
| 16 | Azinphos-methyl | 160.1 > 132.1 | 6 | 160.1 > 77.0 | 20 | 84.1 | 124.7 | 93.0 | 83.3 | 89.6 | 103.3 | 98.2 | 100.0 |
| 17 | Benfluralin | 292.1 > 264.0 | 8 | 292.1 > 160.0 | 22 | 78.4 | 77.0 | 64.5 | 70.7 | 97.7 | 89.1 | 96.8 | 95.3 |
| 18 | BHC, *alpha-* | 180.9 > 144.9 | 16 | 218.9 > 182.9 | 8 | 62.5 | 50.4 | 58.2 | 66.1 | 99.0 | 104.0 | 99.2 | 104.3 |
| 19 | BHC, *beta-* | 180.9 > 144.9 | 16 | 218.9 > 182.9 | 8 | 69.6 | 87.0 | 76.8 | 71.8 | 102.2 | 102.9 | 98.9 | 104.1 |
| 20 | BHC, *delta-* | 180.9 > 144.9 | 16 | 218.9 > 182.9 | 8 | 28.6 | 87.6 | 69.0 | 67.8 | 102.5 | 104.3 | 106.4 | 104.2 |
| 21 | BHC, *gamma-* | 180.9 > 144.9 | 16 | 218.9 > 182.9 | 8 | 64.1 | 73.3 | 62.0 | 69.8 | 94.8 | 93.9 | 103.1 | 102.8 |
| 22 | Bifenthrin | 181.1 > 166.1 | 12 | 181.1 > 179.1 | 12 | 84.6 | 99.2 | 89.3 | 81.4 | 97.0 | 104.4 | 116.7 | 105.9 |
| 23 | Bioallethrin | 123.1 > 81.1 | 10 | 136.1 > 93.1 | 14 | 81.7 | 100.7 | 83.6 | 71.9 | 463.4 | 563.8 | 200.4 | 228.1 |
| 24 | Biphenyl | 154.1 > 128.1 | 22 | 154.1 > 115.1 | 24 | 90.0 | 67.9 | 56.2 | 65.1 | 105.5 | 105.1 | 106.3 | 106.0 |
| 25 | Bromfenvinfos-methyl | 294.9 > 109.0 | 16 | 296.9 > 109.0 | 16 | 85.5 | 98.5 | 84.2 | 77.6 | 103.5 | 92.2 | 100.9 | 102.7 |
| 26 | Bromfenvinphos | 266.9 > 159.0 | 14 | 268.9 > 161.0 | 16 | 78.4 | 97.8 | 88.2 | 79.2 | 102.1 | 94.7 | 100.9 | 102.3 |
| 27 | Bromophos | 330.9 > 315.9 | 14 | 328.9 > 313.9 | 18 | 70.9 | 86.2 | 77.2 | 76.4 | 104.6 | 98.6 | 97.2 | 98.6 |
| 28 | Bromophos-ethyl | 358.9 > 302.9 | 16 | 302.9 > 284.9 | 18 | 78.0 | 86.9 | 76.5 | 75.8 | 91.9 | 89.6 | 98.5 | 98.4 |
| 29 | Bromopropylate | 340.9 > 182.9 | 18 | 340.9 > 184.9 | 20 | 85.8 | 104.5 | 94.3 | 85.6 | 101.4 | 100.9 | 101.0 | 99.2 |
| 30 | Bupirimate | 273.1 > 108.1 | 16 | 273.1 > 193.1 | 8 | 94.4 | 102.8 | 92.2 | 87.0 | 82.0 | 98.3 | 98.1 | 92.8 |
| 31 | Captafol | 79.0 > 77.0 | 14 | 79.0 > 51.0 | 20 | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. |
| 32 | Captan | 149.1 > 105.1 | 4 | 149.1 > 79.1 | 14 | 90.7 | 95.3 | 83.4 | 75.3 | 232.6 | 240.2 | 118.7 | 135.5 |
| 33 | Carbophenothion | 341.9 > 157.0 | 14 | 341.9 > 199.0 | 8 | 81.9 | 97.6 | 84.2 | 79.2 | 58.6 | 66.3 | 80.4 | 91.6 |
| 34 | Carfentrazone-ethyl | 340.1 > 312.1 | 14 | 312.1 > 151.1 | 24 | 85.3 | 100.6 | 94.0 | 87.3 | 81.5 | 81.8 | 101.9 | 94.7 |
| 35 | Chlorbenside | 125.0 > 99.0 | 18 | 127.0 > 89.0 | 18 | 80.3 | 83.4 | 73.5 | 71.8 | 86.9 | 93.5 | 96.8 | 99.3 |
| 36 | Chlordane, *cis-* | 374.8 > 265.9 | 26 | 372.8 > 265.9 | 22 | 76.4 | 84.2 | 76.6 | 77.1 | 94.8 | 99.2 | 105.3 | 101.1 |
| 37 | Chlordane, *trans-* | 374.8 > 265.9 | 26 | 372.8 > 265.9 | 22 | 76.6 | 77.3 | 75.9 | 72.9 | 96.3 | 106.5 | 103.2 | 102.7 |
| 38 | Chlorfenapyr | 247.1 > 227.0 | 16 | 247.1 > 200.0 | 24 | 84.3 | 114.9 | 87.1 | 85.0 | 51.3 | 71.3 | 112.2 | 98.6 |
| 39 | Chlorfenson | 175.0 > 111.0 | 12 | 301.9 > 175.0 | 8 | 77.0 | 89.1 | 81.4 | 76.4 | 99.5 | 100.6 | 100.5 | 98.0 |
| 40 | Chlorfenvinphos, (E)- | 323.0 > 267.0 | 16 | 267.0 > 159.0 | 18 | 110.3 | 114.8 | 74.7 | 72.9 | 270.4 | 484.8 | 103.9 | 99.9 |
| 41 | Chlorfenvinphos, (Z)- | 323.0 > 267.0 | 16 | 267.0 > 159.0 | 18 | 85.5 | 96.6 | 82.7 | 78.5 | 105.8 | 101.5 | 104.8 | 101.2 |
| 42 | Chlorobenzilate | 251.0 > 139.0 | 14 | 139.0 > 75.0 | 26 | 87.5 | 106.9 | 88.9 | 80.3 | 98.3 | 92.0 | 100.1 | 101.1 |
| 43 | Chloroneb | 206.0 > 141.0 | 20 | 193.0 > 113.0 | 18 | 65.4 | 59.8 | 59.8 | 68.6 | 111.1 | 110.8 | 107.9 | 105.9 |
| 44 | Chlorothalonil | 263.9 > 168.0 | 24 | 263.9 > 228.8 | 18 | N.D. | N.D. | 5.2 | N.D. | N.D. | N.D. | 82.3 | N.D. |
| 45 | Chlorpropham | 213.1 > 171.1 | 6 | 127.1 > 92.0 | 18 | 77.7 | 81.7 | 72.5 | 73.3 | 99.6 | 101.7 | 99.0 | 101.4 |
| 46 | Chlorpyrifos | 313.9 > 257.9 | 14 | 313.9 > 285.9 | 8 | 71.2 | 77.8 | 74.9 | 74.2 | 100.5 | 93.8 | 93.6 | 95.7 |
| 47 | Chlorpyrifos-methyl | 285.9 > 93.0 | 22 | 287.9 > 93.0 | 22 | 77.0 | 89.3 | 73.1 | 75.3 | 92.9 | 91.7 | 102.6 | 98.0 |
| 48 | Chlorthal-dimethyl | 298.9 > 220.9 | 24 | 300.9 > 222.9 | 26 | 78.8 | 81.9 | 77.3 | 73.7 | 90.7 | 95.7 | 96.1 | 99.9 |
| 49 | Chlorthiophos-1 | 256.9 > 239.0 | 14 | 256.9 > 193.0 | 22 | 91.4 | 108.5 | 84.2 | 83.5 | 8.4 | 49.4 | 77.2 | 68.6 |
| 50 | Chlorthiophos-2 | 324.9 > 268.9 | 14 | 268.9 > 205.0 | 18 | 76.1 | 96.8 | 84.6 | 80.0 | 73.8 | 79.3 | 95.8 | 101.5 |
| 51 | Chlorthiophos-3 | 324.9 > 268.9 | 14 | 268.9 > 205.0 | 18 | 78.4 | 100.2 | 86.8 | 77.8 | 92.6 | 85.9 | 100.8 | 97.8 |
| 52 | Chlozolinate | 258.9 > 188.0 | 14 | 330.9 > 258.9 | 6 | 75.6 | 82.2 | 75.9 | 76.1 | 91.7 | 98.3 | 103.8 | 104.3 |
| 53 | Clomazone | 204.1 > 107.0 | 20 | 204.1 > 78.0 | 26 | 72.7 | 72.2 | 70.9 | 74.9 | 100.7 | 99.6 | 101.8 | 98.6 |
| 54 | Coumaphos | 362.0 > 109.0 | 16 | 362.0 > 226.0 | 14 | 90.1 | 110.4 | 98.2 | 89.7 | 84.4 | 84.7 | 94.5 | 94.9 |
| 55 | Cycloate | 154.2 > 72.0 | 6 | 215.1 > 154.2 | 4 | 69.8 | 66.7 | 61.2 | 69.5 | 89.3 | 89.9 | 93.3 | 94.0 |
| 56 | Cyfluthrin-1 | 226.1 > 206.1 | 14 | 163.1 > 127.1 | 6 | 92.8 | 111.7 | 98.2 | 92.1 | 105.2 | 118.8 | 100.9 | 96.1 |
| 57 | Cyfluthrin-2 | 226.1 > 206.1 | 14 | 163.1 > 127.1 | 6 | 92.5 | 113.1 | 91.9 | 90.2 | 124.6 | 121.5 | 105.1 | 97.6 |
| 58 | Cyfluthrin-3 | 226.1 > 206.1 | 14 | 163.1 > 127.1 | 6 | 81.4 | 96.8 | 92.5 | 81.7 | 117.8 | 160.7 | 124.5 | 128.0 |
| 59 | Cyfluthrin-4 | 226.1 > 206.1 | 14 | 163.1 > 127.1 | 6 | 76.5 | 95.7 | 106.8 | 88.7 | 167.1 | 159.4 | 121.8 | 126.4 |
| 60 | Cyhalothrin, *lambda-* | 208.1 > 181.1 | 8 | 197.1 > 141.0 | 12 | 88.4 | 108.0 | 92.1 | 85.5 | 99.2 | 113.2 | 99.5 | 96.9 |
| 61 | Cypermethrin-1 | 163.1 > 127.1 | 6 | 163.1 > 109.1 | 22 | 96.0 | 113.8 | 94.6 | 89.9 | 108.8 | 93.7 | 117.4 | 111.3 |
| 62 | Cypermethrin-2 | 163.1 > 127.1 | 6 | 163.1 > 109.1 | 22 | 89.6 | 119.7 | 98.0 | 88.7 | 99.2 | 113.1 | 94.8 | 100.1 |
| 63 | Cypermethrin-3 | 163.1 > 127.1 | 6 | 163.1 > 109.1 | 22 | 75.9 | 124.9 | 103.1 | 96.9 | 126.1 | 114.1 | 96.6 | 104.1 |
| 64 | Cypermethrin-4 | 163.1 > 127.1 | 6 | 163.1 > 109.1 | 22 | 76.6 | 100.1 | 84.6 | 81.5 | 115.3 | 119.9 | 124.9 | 120.1 |
| 65 | Cyprodinil | 224.1 > 197.1 | 22 | 224.1 > 131.1 | 14 | 82.0 | 88.9 | 80.0 | 71.2 | 138.1 | 117.5 | 100.5 | 112.6 |
| 66 | DDD, *o,p'-* | 235.0 > 165.0 | 24 | 235.0 > 199.0 | 16 | 83.6 | 96.0 | 79.8 | 75.3 | 87.0 | 86.6 | 100.1 | 99.1 |
| 67 | DDD, *p,p'-* | 235.0 > 165.0 | 24 | 235.0 > 199.0 | 16 | 80.2 | 94.6 | 83.6 | 78.2 | 104.4 | 98.8 | 102.6 | 104.3 |
| 68 | DDE, *o,p'-* | 246.0 > 176.0 | 30 | 248.0 > 176.0 | 28 | 73.7 | 83.1 | 75.7 | 71.7 | 101.7 | 97.9 | 99.0 | 100.3 |
| 69 | DDE, *p,p'-* | 246.0 > 176.0 | 30 | 317.9 > 248.0 | 24 | 76.9 | 99.7 | 76.2 | 73.5 | 101.1 | 93.8 | 98.8 | 98.3 |
| 70 | DDT, *o,p'-* | 235.0 > 165.0 | 24 | 235.0 > 199.0 | 16 | 79.0 | 89.1 | 78.0 | 74.3 | 93.9 | 97.6 | 100.1 | 101.2 |
| 71 | DDT, *p,p'-* | 235.0 > 165.0 | 24 | 235.0 > 199.0 | 16 | 75.2 | 95.1 | 80.7 | 75.8 | 96.8 | 95.0 | 102.9 | 97.7 |
| 72 | Deltamethrin | 252.9 > 93.0 | 20 | 252.9 > 171.9 | 8 | 83.8 | 109.4 | 92.2 | 85.3 | 99.9 | 99.3 | 102.5 | 99.7 |
| 73 | Di-allate-1 | 234.1 > 150.0 | 20 | 234.1 > 192.1 | 14 | 75.3 | 66.5 | 63.3 | 70.7 | 93.8 | 93.0 | 100.9 | 103.3 |
| 74 | Di-allate-2 | 234.1 > 150.0 | 20 | 234.1 > 192.1 | 14 | 72.4 | 65.1 | 63.0 | 70.5 | 88.9 | 107.7 | 100.0 | 94.1 |
| 75 | Diazinon | 304.1 > 179.1 | 10 | 304.1 > 162.1 | 8 | 76.4 | 70.3 | 69.5 | 74.4 | 62.3 | 80.6 | 97.9 | 86.4 |
| 76 | Dichlobenil | 170.9 > 100.0 | 24 | 170.9 > 136.0 | 14 | 71.2 | 65.3 | 58.2 | 65.4 | 96.5 | 97.4 | 96.5 | 98.2 |
| 77 | Dichlofluanid | 223.9 > 123.1 | 8 | 223.9 > 77.0 | 28 | 65.0 | 71.1 | 57.8 | 56.9 | 75.4 | 87.4 | 94.4 | 101.7 |
| 78 | Dicloran | 206.0 > 176.0 | 10 | 206.0 > 124.0 | 24 | 78.1 | 76.6 | 69.7 | 77.0 | 79.0 | 87.7 | 100.6 | 91.0 |

4

| ID | Compound Name | Transitions | | | | Recovery (Average of n = 3) | | | | Relative Ion Ratio (Average of n = 3) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Target | CE | Reference | CE | 10 ng/g-1 | 10 ng/g-2 | 50 ng/g-1 | 50 ng/g-2 | 10 ng/g-1 | 10 ng/g-2 | 50 ng/g-1 | 50 ng/g-2 |
| 79 | Dieldrin | 276.9 > 241.0 | 8 | 262.9 > 193.0 | 34 | 80.2 | 80.3 | 89.0 | 85.0 | 154.2 | 185.9 | 102.7 | 91.4 |
| 80 | Dimethachlor | 197.1 > 148.1 | 10 | 199.1 > 148.1 | 10 | 78.4 | 90.6 | 77.9 | 76.8 | 99.6 | 89.1 | 100.2 | 101.6 |
| 81 | Diphenamid | 239.1 > 167.1 | 8 | 239.1 > 72.0 | 16 | 94.7 | 96.0 | 88.3 | 79.8 | 126.1 | 123.6 | 112.2 | 115.5 |
| 82 | Diphenylamine | 169.1 > 66.0 | 24 | 169.1 > 77.0 | 28 | 78.2 | 74.6 | 65.6 | 71.1 | 85.3 | 99.9 | 98.3 | 100.6 |
| 83 | Disulfoton | 186.0 > 153.0 | 6 | 186.0 > 97.0 | 16 | 71.4 | 66.2 | 64.6 | 78.9 | 142.4 | 124.1 | 107.9 | 85.8 |
| 84 | Edifenphos | 173.0 > 109.0 | 10 | 310.0 > 173.0 | 14 | 82.6 | 103.7 | 91.0 | 83.6 | 97.9 | 94.6 | 100.3 | 98.2 |
| 85 | Endosulfan ether | 240.9 > 205.9 | 16 | 238.9 > 203.9 | 16 | 62.7 | 64.4 | 67.2 | 69.5 | 126.7 | 95.3 | 104.8 | 101.2 |
| 86 | Endosulfan sulfate | 271.8 > 236.9 | 18 | 386.8 > 252.9 | 16 | 83.8 | 88.4 | 87.0 | 85.1 | 42.5 | 55.6 | 83.3 | 85.8 |
| 87 | Endosulfan, *alpha-* | 194.9 > 160.0 | 8 | 194.9 > 125.0 | 24 | 74.0 | 80.0 | 75.6 | 80.7 | 77.6 | 76.9 | 92.3 | 83.4 |
| 88 | Endosulfan, *beta-* | 194.9 > 160.0 | 8 | 194.9 > 125.0 | 24 | 83.1 | 99.4 | 81.6 | 81.7 | 102.1 | 67.3 | 93.2 | 100.2 |
| 89 | Endrin | 262.9 > 193.0 | 28 | 262.9 > 228.0 | 22 | 84.1 | 89.9 | 77.2 | 75.3 | 50.2 | 49.4 | 85.3 | 88.2 |
| 90 | Endrin aldehyde | 249.8 > 214.9 | 26 | 344.9 > 244.9 | 16 | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. |
| 91 | Endrin ketone | 316.9 > 244.9 | 20 | 314.9 > 242.9 | 18 | 73.4 | 91.5 | 84.1 | 88.0 | 108.9 | 95.4 | 107.6 | 101.9 |
| 92 | EPN | 169.1 > 140.9 | 8 | 169.1 > 77.0 | 22 | 90.3 | 107.4 | 89.2 | 82.3 | 94.3 | 102.9 | 100.2 | 98.8 |
| 93 | Ethalfluralin | 276.0 > 202.0 | 18 | 316.1 > 276.0 | 10 | 73.3 | 67.7 | 63.0 | 67.1 | 95.2 | 96.4 | 100.9 | 100.6 |
| 94 | Ethion | 230.9 > 129.0 | 24 | 230.9 > 174.9 | 14 | 81.4 | 97.1 | 84.2 | 77.9 | 118.3 | 108.6 | 101.8 | 102.6 |
| 95 | Etofenprox | 163.1 > 135.1 | 10 | 163.1 > 107.1 | 18 | 87.1 | 101.1 | 95.6 | 87.3 | 97.4 | 103.1 | 98.8 | 97.8 |
| 96 | Etridiazole | 210.9 > 182.9 | 10 | 210.9 > 139.9 | 22 | 70.0 | 60.1 | 58.0 | 66.9 | 88.2 | 102.0 | 102.0 | 100.4 |
| 97 | Fenamiphos | 303.1 > 195.1 | 8 | 288.1 > 260.1 | 6 | 88.7 | 104.6 | 99.3 | 87.1 | 120.9 | 106.0 | 96.8 | 100.1 |
| 98 | Fenarimol | 251.0 > 139.0 | 14 | 330.0 > 139.0 | 18 | 83.9 | 106.7 | 96.5 | 89.4 | 97.4 | 92.6 | 99.3 | 96.0 |
| 99 | Fenchlorphos | 284.9 > 269.9 | 16 | 286.9 > 271.9 | 18 | 80.5 | 81.4 | 71.5 | 74.8 | 89.8 | 83.8 | 99.1 | 97.2 |
| 100 | Fenitrothion | 277.0 > 260.0 | 6 | 260.0 > 125.1 | 12 | 78.0 | 89.3 | 79.3 | 75.8 | 104.9 | 99.2 | 111.4 | 109.6 |
| 101 | Fenpropathrin | 265.1 > 210.1 | 12 | 265.1 > 89.0 | 28 | 90.3 | 102.8 | 96.8 | 88.2 | 68.9 | 75.9 | 90.9 | 94.4 |
| 102 | Fenson | 141.0 > 77.0 | 16 | 267.9 > 141.0 | 6 | 78.3 | 88.0 | 77.9 | 74.5 | 91.3 | 92.7 | 100.8 | 96.8 |
| 103 | Fenthion | 278.0 > 169.0 | 14 | 278.0 > 125.0 | 20 | 80.4 | 81.9 | 78.2 | 76.0 | 93.9 | 96.0 | 102.2 | 104.1 |
| 104 | Fenvalerate-1 | 225.1 > 147.1 | 10 | 419.1 > 225.1 | 6 | 82.2 | 100.7 | 94.6 | 88.9 | 95.5 | 92.4 | 103.2 | 99.3 |
| 105 | Fenvalerate-2 | 225.1 > 147.1 | 10 | 419.1 > 225.1 | 6 | 75.7 | 106.7 | 93.1 | 86.5 | 73.5 | 70.0 | 86.2 | 89.7 |
| 106 | Fipronil | 366.9 > 212.9 | 30 | 368.9 > 214.9 | 30 | 92.8 | 113.2 | 99.5 | 82.8 | 93.8 | 96.2 | 88.0 | 96.7 |
| 107 | Fluazifop-P-butyl | 282.1 > 91.0 | 18 | 383.1 > 282.1 | 14 | 77.4 | 94.0 | 89.0 | 80.6 | 101.9 | 96.4 | 99.6 | 100.5 |
| 108 | Fluchloralin | 306.0 > 264.0 | 8 | 326.0 > 63.0 | 16 | 76.4 | 78.4 | 76.9 | 75.1 | 95.8 | 96.0 | 90.4 | 96.5 |
| 109 | Flucythrinate-1 | 157.1 > 107.1 | 12 | 199.1 > 107.1 | 22 | 86.9 | 111.9 | 95.6 | 87.3 | 119.8 | 120.4 | 107.1 | 105.2 |
| 110 | Flucythrinate-2 | 157.1 > 107.1 | 12 | 199.1 > 107.1 | 22 | 89.4 | 111.0 | 96.0 | 86.6 | 105.1 | 108.4 | 102.6 | 104.6 |
| 111 | Fludioxonil | 248.0 > 127.0 | 26 | 248.0 > 154.0 | 20 | 94.1 | 110.3 | 96.5 | 86.1 | 91.9 | 101.1 | 98.1 | 96.0 |
| 112 | Fluquinconazole | 340.0 > 298.0 | 20 | 340.0 > 313.0 | 14 | 85.2 | 107.0 | 98.9 | 88.9 | 97.7 | 98.5 | 101.4 | 101.1 |
| 113 | Fluridone | 328.1 > 259.0 | 24 | 328.1 > 127.0 | 24 | 91.5 | 117.7 | 100.3 | 91.4 | 80.1 | 77.3 | 93.4 | 95.7 |
| 114 | Flusilazole | 233.1 > 165.1 | 14 | 233.1 > 152.1 | 14 | 78.1 | 93.3 | 84.9 | 83.2 | 105.9 | 91.5 | 94.8 | 95.4 |
| 115 | Flutolanil | 173.0 > 95.0 | 26 | 281.1 > 173.0 | 12 | 84.1 | 108.0 | 93.4 | 84.7 | 104.1 | 101.1 | 100.0 | 99.8 |
| 116 | Flutriafol | 219.1 > 123.1 | 14 | 219.1 > 95.0 | 28 | 82.9 | 109.6 | 89.8 | 84.2 | 120.1 | 115.2 | 103.9 | 103.9 |
| 117 | Fluvalinate-1, *tau-* | 250.1 > 55.0 | 18 | 250.1 > 200.1 | 16 | 86.7 | 112.8 | 91.9 | 85.5 | 79.4 | 79.9 | 90.8 | 90.4 |
| 118 | Fluvalinate-2, *tau-* | 250.1 > 55.0 | 18 | 250.1 > 200.1 | 16 | 90.4 | 112.0 | 89.1 | 83.9 | 89.6 | 95.5 | 102.5 | 104.7 |
| 119 | Folpet | 259.9 > 130.0 | 14 | 261.9 > 130.0 | 18 | 65.2 | 82.9 | 72.8 | 67.0 | 97.2 | 89.1 | 99.1 | 98.3 |
| 120 | Fonofos | 246.0 > 137.1 | 6 | 246.0 > 109.1 | 18 | 76.6 | 74.7 | 65.8 | 71.4 | 106.8 | 97.6 | 102.1 | 101.2 |
| 121 | Heptachlor | 271.8 > 236.9 | 20 | 273.8 > 238.9 | 16 | 68.6 | 65.4 | 65.0 | 68.6 | 92.3 | 102.0 | 104.0 | 101.2 |
| 122 | Heptachlor-exo-epoxide | 352.8 > 262.9 | 14 | 352.8 > 316.9 | 10 | 81.1 | 80.4 | 71.8 | 79.1 | 33.7 | 39.0 | 88.6 | 71.5 |
| 123 | Hexachlorobenzene | 283.8 > 248.8 | 24 | 283.8 > 213.8 | 28 | 45.7 | 28.8 | 48.1 | 62.3 | 101.4 | 100.7 | 102.7 | 99.3 |
| 124 | Hexazinone | 171.1 > 71.0 | 16 | 171.1 > 85.0 | 16 | 93.2 | 112.9 | 96.1 | 88.2 | 105.3 | 101.3 | 104.0 | 105.2 |
| 125 | Iodofenphos | 376.9 > 361.8 | 22 | 376.9 > 331.8 | 32 | 80.0 | 86.8 | 75.8 | 74.2 | 83.8 | 102.3 | 97.3 | 97.1 |
| 126 | Iprodione | 314.0 > 245.0 | 12 | 314.0 > 56.0 | 22 | 110.5 | 156.0 | 102.0 | 89.9 | 91.0 | 99.9 | 102.2 | 107.0 |
| 127 | Isazofos | 257.0 > 162.0 | 8 | 257.0 > 119.0 | 18 | 80.0 | 87.4 | 74.4 | 74.7 | 109.5 | 107.1 | 96.0 | 102.6 |
| 128 | Isodrin | 192.9 > 157.0 | 20 | 262.9 > 192.9 | 28 | 76.4 | 73.7 | 67.4 | 71.2 | 88.2 | 99.0 | 110.1 | 98.6 |
| 129 | Isopropalin | 280.1 > 238.1 | 8 | 280.1 > 133.1 | 18 | 76.5 | 88.1 | 73.6 | 71.4 | 89.8 | 82.7 | 97.9 | 106.1 |
| 130 | Lenacil | 153.1 > 136.1 | 14 | 153.1 > 82.1 | 16 | 85.1 | 103.2 | 97.5 | 85.7 | 109.0 | 108.9 | 124.1 | 111.8 |
| 131 | Leptophos | 376.9 > 361.9 | 24 | 374.9 > 359.9 | 24 | 86.8 | 102.6 | 89.0 | 82.6 | 95.4 | 99.8 | 101.9 | 103.0 |
| 132 | Linuron | 248.0 > 61.0 | 16 | 250.0 > 61.0 | 16 | 68.3 | 84.7 | 77.7 | 77.9 | 98.3 | 83.1 | 105.8 | 108.6 |
| 133 | Malathion | 173.1 > 99.0 | 14 | 158.1 > 125.0 | 10 | 79.9 | 87.3 | 77.6 | 75.3 | 95.2 | 97.4 | 101.3 | 100.9 |
| 134 | Metalaxyl | 249.2 > 190.1 | 8 | 249.2 > 146.1 | 22 | 83.5 | 88.7 | 89.5 | 81.1 | 103.0 | 122.4 | 104.0 | 100.7 |
| 135 | Metazachlor | 209.1 > 132.1 | 18 | 211.1 > 132.1 | 20 | 83.3 | 93.2 | 83.8 | 80.1 | 97.8 | 100.1 | 101.6 | 104.8 |
| 136 | Methacrifos | 208.0 > 180.0 | 8 | 240.0 > 208.0 | 4 | 75.6 | 68.7 | 62.9 | 67.2 | 93.3 | 89.0 | 95.3 | 101.8 |
| 137 | Methoxychlor | 227.1 > 169.1 | 24 | 227.1 > 212.1 | 14 | 82.8 | 101.8 | 90.8 | 83.5 | 109.0 | 108.5 | 103.5 | 103.2 |
| 138 | Metolachlor | 238.1 > 162.1 | 12 | 238.1 > 133.1 | 26 | 77.0 | 89.0 | 77.9 | 75.4 | 104.8 | 96.6 | 101.4 | 102.3 |
| 139 | Mevinphos-1 | 192.0 > 127.0 | 12 | 127.0 > 95.0 | 18 | 75.7 | 70.7 | 64.1 | 71.4 | 95.8 | 104.8 | 103.6 | 102.2 |
| 140 | MGK 264-1 | 164.1 > 93.0 | 10 | 164.1 > 80.0 | 24 | 112.8 | 102.9 | 86.2 | 81.3 | 92.5 | 104.4 | 108.1 | 104.7 |
| 141 | MGK 264-2 | 164.1 > 98.0 | 12 | 164.1 > 67.0 | 8 | 74.8 | 89.7 | 79.3 | 78.2 | 116.0 | 100.8 | 93.7 | 96.0 |
| 142 | Mirex | 271.8 > 236.8 | 18 | 273.8 > 238.8 | 18 | 71.4 | 80.4 | 73.6 | 71.2 | 99.9 | 97.3 | 100.7 | 99.6 |
| 143 | Myclobutanil | 179.1 > 125.0 | 14 | 179.1 > 152.0 | 8 | 83.7 | 104.4 | 90.8 | 84.1 | 122.4 | 127.5 | 112.5 | 108.8 |
| 144 | N-(2,4-dimethylphenyl) formamide | 149.1 > 106.1 | 16 | 149.1 > 121.1 | 6 | 86.6 | 88.5 | 73.0 | 71.4 | 334.2 | 367.6 | 157.5 | 169.6 |
| 145 | Nitralin | 316.1 > 274.0 | 8 | 274.0 > 169.0 | 12 | 101.1 | 116.0 | 95.4 | 87.2 | 98.7 | 104.0 | 106.2 | 97.4 |
| 146 | Nitrofen | 202.0 > 139.0 | 24 | 282.9 > 253.0 | 12 | 83.4 | 91.9 | 85.9 | 77.0 | 89.0 | 95.2 | 99.6 | 104.3 |
| 147 | Nonachlor, *cis-* | 406.8 > 299.9 | 24 | 406.8 > 334.9 | 16 | 80.0 | 91.3 | 79.8 | 77.0 | 59.7 | 48.5 | 84.3 | 87.0 |
| 148 | Nonachlor, *trans-* | 406.8 > 299.9 | 24 | 406.8 > 334.9 | 16 | 74.8 | 86.0 | 80.8 | 78.2 | 58.4 | 72.4 | 82.3 | 84.6 |
| 149 | Norflurazon | 303.0 > 145.0 | 22 | 145.0 > 95.0 | 18 | 94.7 | 111.0 | 98.7 | 85.0 | 92.7 | 95.4 | 99.2 | 100.8 |
| 150 | Oxadiazon | 258.0 > 175.0 | 8 | 302.0 > 175.0 | 14 | 78.8 | 88.7 | 84.7 | 76.9 | 95.1 | 101.4 | 100.4 | 103.2 |
| 151 | Oxyfluorfen | 361.0 > 300.0 | 14 | 361.0 > 317.0 | 6 | 101.4 | 106.2 | 90.9 | 80.4 | 91.2 | 97.7 | 101.4 | 102.1 |
| 152 | Paclobutrazol | 236.1 > 125.0 | 14 | 236.1 > 167.0 | 10 | 92.2 | 116.7 | 92.8 | 86.1 | 98.0 | 86.2 | 100.9 | 100.1 |
| 153 | Parathion | 291.1 > 137.0 | 6 | 291.1 > 81.0 | 24 | 99.8 | 96.8 | 80.7 | 76.0 | 100.1 | 115.2 | 104.9 | 108.1 |
| 154 | Parathion-methyl | 263.0 > 109.0 | 14 | 263.0 > 246.0 | 6 | 85.5 | 91.6 | 74.2 | 77.4 | 71.7 | 69.8 | 96.2 | 93.7 |
| 155 | Pebulate | 161.1 > 128.1 | 6 | 128.1 > 57.0 | 6 | 64.1 | 59.1 | 56.9 | 66.7 | 115.3 | 111.1 | 101.3 | 101.7 |
| 156 | Penconazole | 248.1 > 157.1 | 26 | 159.1 > 123.1 | 22 | 90.9 | 92.6 | 75.3 | 75.4 | 90.0 | 94.4 | 97.6 | 97.5 |

| ID | Compound Name | Transitions | | | | Recovery (Average of n = 3) | | | | Relative Ion Ratio (Average of n = 3) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Target | CE | Reference | CE | 10 ng/g-1 | 10 ng/g-2 | 50 ng/g-1 | 50 ng/g-2 | 10 ng/g-1 | 10 ng/g-2 | 50 ng/g-1 | 50 ng/g-2 |
| 157 | Pendimethalin | 252.1 > 162.1 | 10 | 252.1 > 191.1 | 8 | 84.0 | 84.2 | 73.9 | 73.8 | 89.5 | 98.9 | 102.2 | 104.0 |
| 158 | Pentachloroaniline | 262.9 > 191.9 | 22 | 264.9 > 193.9 | 18 | 23.6 | 83.6 | 52.0 | 72.4 | 99.4 | 99.0 | 101.1 | 98.8 |
| 159 | Pentachloroanisole | 279.9 > 236.8 | 26 | 279.9 > 264.8 | 12 | 69.0 | 65.8 | 60.2 | 67.9 | 102.8 | 93.3 | 101.7 | 101.4 |
| 160 | Pentachlorobenzene | 249.9 > 214.9 | 18 | 249.9 > 176.9 | 26 | 59.9 | 27.0 | 47.6 | 62.0 | 104.3 | 113.2 | 108.4 | 104.3 |
| 161 | Pentachlorobenzonitrile | 274.8 > 239.8 | 18 | 272.8 > 202.9 | 30 | 69.3 | 65.6 | 63.2 | 68.7 | 90.1 | 88.2 | 96.3 | 95.7 |
| 162 | Pentachlorothioanisole | 295.8 > 262.9 | 14 | 295.8 > 245.8 | 30 | 55.8 | 75.7 | 62.7 | 69.7 | 92.4 | 96.3 | 94.9 | 93.5 |
| 163 | Permethrine, cis- | 183.1 > 153.1 | 14 | 183.1 > 168.1 | 14 | 86.6 | 112.1 | 96.8 | 87.5 | 101.7 | 108.3 | 99.5 | 100.2 |
| 164 | Permethrine, trans- | 183.1 > 153.1 | 14 | 183.1 > 168.1 | 14 | 96.0 | 131.5 | 97.0 | 88.4 | 100.4 | 98.3 | 103.6 | 102.4 |
| 165 | Perthane | 223.2 > 167.1 | 14 | 223.2 > 193.1 | 28 | 85.3 | 92.7 | 81.9 | 78.1 | 97.1 | 106.8 | 102.3 | 100.3 |
| 166 | Phenothrin-1 | 183.1 > 153.1 | 14 | 183.1 > 168.1 | 14 | N.D. | N.D. | N.D. | 84.8 | N.D. | N.D. | 111.5 | 92.4 |
| 167 | Phenothrin-2 | 183.1 > 153.1 | 14 | 183.1 > 168.1 | 14 | 100.7 | 113.7 | 95.7 | 86.7 | 101.4 | 112.1 | 105.1 | 100.1 |
| 168 | Phorate | 260.0 > 75.0 | 8 | 231.0 > 129.0 | 24 | 74.3 | 63.8 | 61.9 | 69.7 | 92.7 | 116.3 | 99.8 | 101.6 |
| 169 | Phosalone | 182.0 > 102.0 | 14 | 182.0 > 111.0 | 14 | 86.3 | 101.6 | 93.9 | 84.4 | 117.0 | 123.8 | 101.2 | 104.5 |
| 170 | Phosmet | 160.0 > 77.0 | 24 | 160.0 > 105.0 | 18 | 86.7 | 105.4 | 92.6 | 83.0 | 100.8 | 100.1 | 103.5 | 101.2 |
| 171 | Piperonyl butoxide | 176.1 > 131.1 | 12 | 176.1 > 117.1 | 20 | 84.1 | 112.8 | 92.1 | 85.9 | 104.9 | 118.6 | 102.2 | 102.3 |
| 172 | Pirimiphos ethyl | 304.1 > 168.1 | 12 | 318.1 > 166.1 | 12 | 78.1 | 94.3 | 79.1 | 73.8 | 83.5 | 84.4 | 97.4 | 105.8 |
| 173 | Pirimiphos-methyl | 290.1 > 125.0 | 22 | 290.1 > 233.1 | 12 | 80.4 | 88.9 | 78.5 | 76.1 | 93.3 | 92.5 | 101.2 | 99.4 |
| 174 | Pretilachlor | 262.1 > 202.1 | 10 | 238.1 > 162.1 | 10 | 77.0 | 95.2 | 86.0 | 79.7 | 109.6 | 79.5 | 93.4 | 102.5 |
| 175 | Prochloraz | 180.1 > 138.1 | 12 | 180.1 > 69.0 | 20 | 68.1 | 110.1 | 85.4 | 81.5 | 106.2 | 102.1 | 91.1 | 91.2 |
| 176 | Procymidone | 283.0 > 96.0 | 10 | 285.0 > 96.0 | 10 | 12.4 | 140.1 | 78.8 | 79.4 | 103.0 | 101.7 | 104.7 | 101.8 |
| 177 | Prodiamine | 321.1 > 279.1 | 6 | 321.1 > 203.1 | 10 | 86.1 | 94.7 | 81.6 | 78.3 | 88.5 | 88.3 | 93.6 | 99.0 |
| 178 | Profenofos | 338.9 > 268.9 | 18 | 336.9 > 266.9 | 14 | 87.1 | 93.5 | 90.0 | 85.6 | 107.6 | 107.6 | 93.3 | 91.1 |
| 179 | Profluralin | 318.1 > 199.1 | 16 | 318.1 > 55.0 | 22 | 66.3 | 64.9 | 65.7 | 74.8 | 105.2 | 95.9 | 97.6 | 91.7 |
| 180 | Propachlor | 176.1 > 57.0 | 8 | 176.1 > 77.0 | 24 | 74.9 | 76.4 | 67.3 | 71.4 | 114.9 | 106.5 | 103.8 | 102.7 |
| 181 | Propanil | 217.0 > 161.0 | 10 | 160.9 > 126.0 | 18 | 93.5 | 105.3 | 91.4 | 78.7 | 100.7 | 106.9 | 95.8 | 104.6 |
| 182 | Propargite | 173.1 > 135.1 | 16 | 173.1 > 107.1 | 24 | 88.0 | 98.7 | 90.8 | 85.5 | 120.0 | 122.8 | 74.1 | 66.1 |
| 183 | Propisochlor | 223.1 > 132.1 | 20 | 223.1 > 147.1 | 8 | 83.7 | 90.8 | 79.4 | 77.9 | 91.0 | 100.1 | 98.4 | 101.0 |
| 184 | Propyzamide | 172.9 > 109.0 | 26 | 172.9 > 74.0 | 28 | 83.0 | 91.4 | 77.1 | 76.5 | 98.7 | 100.0 | 108.7 | 106.3 |
| 185 | Prothiofos | 266.9 > 238.9 | 10 | 309.0 > 238.9 | 14 | 74.3 | 88.9 | 78.0 | 73.9 | 102.4 | 94.4 | 102.7 | 101.2 |
| 186 | Pyraclofos | 194.0 > 138.0 | 22 | 360.1 > 194.0 | 14 | 91.6 | 111.4 | 97.0 | 87.0 | 84.1 | 89.9 | 99.3 | 100.1 |
| 187 | Pyrazophos | 221.1 > 193.1 | 12 | 221.1 > 149.1 | 14 | 88.7 | 111.9 | 97.3 | 86.1 | 101.7 | 96.0 | 99.9 | 103.7 |
| 188 | Pyridaben | 147.1 > 117.1 | 22 | 147.1 > 132.1 | 14 | 87.5 | 106.4 | 92.5 | 84.1 | 99.2 | 105.1 | 102.0 | 102.5 |
| 189 | Pyridaphenthion | 340.0 > 199.1 | 8 | 199.1 > 92.0 | 16 | 100.2 | 120.0 | 95.0 | 89.3 | 114.1 | 129.2 | 109.5 | 107.5 |
| 190 | Pyrimethanil | 198.1 > 118.1 | 28 | 198.1 > 158.1 | 18 | 74.4 | 82.5 | 75.3 | 73.2 | 95.2 | 99.1 | 96.4 | 98.5 |
| 191 | Pyriproxyfen | 136.1 > 96.0 | 14 | 226.1 > 186.1 | 14 | 81.3 | 91.9 | 92.8 | 84.4 | 59.7 | 78.1 | 88.1 | 84.8 |
| 192 | Quinalphos | 146.1 > 118.0 | 10 | 146.1 > 91.0 | 24 | 71.7 | 86.6 | 79.6 | 72.9 | 204.6 | 155.7 | 122.4 | 117.8 |
| 193 | Quintozene | 294.8 > 236.8 | 16 | 264.8 > 236.8 | 10 | 61.7 | 0.0 | 18.5 | 71.1 | 100.1 | 100.7 | 101.5 | 100.3 |
| 194 | Resmethrin-1 | 171.1 > 128.1 | 12 | 171.1 > 143.1 | 6 | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. |
| 195 | Resmethrin-2 | 171.1 > 143.1 | 6 | 171.1 > 128.1 | 14 | 85.8 | 96.4 | 85.8 | 78.1 | 86.0 | 97.9 | 95.6 | 100.0 |
| 196 | Sulfotep | 322.0 > 294.0 | 4 | 322.0 > 202.0 | 10 | 79.1 | 71.8 | 65.5 | 71.8 | 94.6 | 98.9 | 98.0 | 95.0 |
| 197 | Sulprofos | 322.0 > 156.0 | 8 | 156.0 > 108.0 | 28 | 80.7 | 97.3 | 86.0 | 83.2 | 101.1 | 95.9 | 97.3 | 95.1 |
| 198 | Tebuconazole | 250.1 > 125.1 | 22 | 250.1 > 153.1 | 12 | 76.8 | 82.9 | 68.1 | 71.6 | 112.5 | 96.6 | 98.9 | 98.1 |
| 199 | Tebufenpyrad | 333.1 > 171.1 | 20 | 333.1 > 276.1 | 8 | 87.7 | 105.2 | 96.7 | 84.7 | 97.6 | 97.3 | 94.6 | 95.6 |
| 200 | Tecnazene | 260.9 > 202.9 | 14 | 202.9 > 85.0 | 24 | 78.8 | 59.9 | 59.7 | 72.4 | 93.9 | 99.1 | 96.8 | 95.8 |
| 201 | Tefluthrin | 177.0 > 127.1 | 16 | 177.0 > 137.1 | 16 | 79.0 | 81.2 | 67.5 | 72.5 | 92.6 | 94.9 | 101.0 | 98.3 |
| 202 | Terbacil | 161.0 > 88.0 | 20 | 117.0 > 76.0 | 8 | 97.5 | 111.6 | 86.5 | 82.0 | 81.8 | 77.9 | 92.5 | 96.1 |
| 203 | Terbufos | 231.0 > 128.9 | 26 | 231.0 > 174.9 | 14 | 78.8 | 80.6 | 65.2 | 70.3 | 111.6 | 100.1 | 102.8 | 104.7 |
| 204 | Terbuthylazine | 229.1 > 173.1 | 6 | 214.1 > 71.0 | 16 | 94.9 | 91.0 | 77.6 | 77.1 | 82.6 | 91.6 | 99.7 | 103.3 |
| 205 | Tetrachlorvinphos | 328.9 > 109.0 | 20 | 330.9 > 109.0 | 22 | 87.4 | 102.5 | 87.3 | 81.0 | 96.0 | 93.6 | 99.9 | 96.6 |
| 206 | Tetradifon | 355.9 > 159.0 | 18 | 355.9 > 228.9 | 12 | 84.1 | 96.6 | 93.6 | 88.5 | 87.9 | 103.1 | 102.9 | 96.7 |
| 207 | Tetramethrin-1 | 164.1 > 107.1 | 14 | 164.1 > 77.0 | 22 | 66.8 | N.D. | 100.5 | 94.8 | N.D. | N.D. | 106.5 | 114.1 |
| 208 | Tetramethrin-2 | 164.1 > 107.1 | 14 | 164.1 > 77.0 | 22 | 103.2 | 129.6 | 98.4 | 88.4 | 115.2 | 117.0 | 107.9 | 113.8 |
| 209 | THPI | 151.1 > 79.0 | 18 | 151.1 > 77.0 | 28 | 79.4 | 85.0 | 81.5 | 78.2 | 104.7 | 104.1 | 103.6 | 108.0 |
| 210 | Tolclofos-methyl | 264.9 > 93.0 | 24 | 264.9 > 219.9 | 22 | 72.8 | 78.6 | 72.6 | 73.8 | 98.8 | 100.9 | 102.3 | 103.6 |
| 211 | Tolylfluanid | 238.0 > 137.1 | 14 | 181.1 > 138.1 | 10 | 66.8 | 79.8 | 65.9 | 64.1 | 118.7 | 117.3 | 114.3 | 107.4 |
| 212 | Transfluthrin | 163.1 > 127.1 | 6 | 163.1 > 143.1 | 16 | 84.2 | 85.7 | 77.1 | 77.6 | 106.3 | 115.0 | 97.7 | 99.0 |
| 213 | Triadimefon | 208.1 > 111.0 | 22 | 208.1 > 127.0 | 14 | 88.4 | 98.2 | 86.7 | 80.5 | 99.5 | 103.9 | 97.9 | 104.7 |
| 214 | Triadimenol | 168.1 > 70.0 | 10 | 128.1 > 65.0 | 22 | N.D. | N.D. | 101.2 | 94.2 | N.D. | N.D. | 80.9 | 85.6 |
| 215 | Tri-allate | 268.1 > 184.0 | 20 | 270.1 > 186.0 | 20 | 80.0 | 75.6 | 68.6 | 74.6 | 102.4 | 85.9 | 94.6 | 98.1 |
| 216 | Triazophos | 257.0 > 162.0 | 12 | 257.0 > 134.0 | 22 | 89.7 | 112.4 | 94.8 | 86.4 | 95.9 | 80.8 | 90.3 | 88.2 |
| 217 | Tricyclazole | 189.0 > 161.9 | 12 | 189.0 > 135.0 | 18 | 91.5 | 95.7 | 84.5 | 81.8 | 105.9 | 118.7 | 105.2 | 97.3 |
| 218 | Triflumizole | 278.1 > 73.0 | 6 | 206.1 > 186.1 | 8 | 87.2 | 88.2 | 71.5 | 76.8 | 83.2 | 110.9 | 97.0 | 99.2 |
| 219 | Trifluralin | 306.1 > 264.1 | 8 | 306.1 > 160.1 | 22 | 79.9 | 73.6 | 64.4 | 72.5 | 92.7 | 100.3 | 100.8 | 101.1 |
| 220 | Vinclozolin | 285.0 > 212.0 | 12 | 212.0 > 172.0 | 16 | 86.3 | 92.8 | 79.9 | 80.7 | 84.3 | 96.4 | 104.2 | 103.3 |
| QC-1 | 1,4-Dichlorobenzene-d4 | 150.0 > 78.0 | 24 | 115.1 > 78.0 | 12 | — | — | — | — | — | — | — | — |
| QC-2 | Acenaphthene-d10 | 164.0 > 160.0 | 30 | 164.0 > 134.0 | 38 | — | — | — | — | — | — | — | — |
| QC-3 | Chrysene-d12 | 240.0 > 236.0 | 30 | 240.0 > 212.0 | 24 | — | — | — | — | — | — | — | — |
| QC-4 | Naphthalene-d8 | 136.0 > 84.0 | 22 | 136.0 > 82.0 | 28 | — | — | — | — | — | — | — | — |
| QC-5 | Perylene-d12 | 264.0 > 263.0 | 34 | 264.0 > 262.0 | 24 | — | — | — | — | — | — | — | — |
| QC-6 | Phenanthrene-d10 | 188.0 > 160.0 | 24 | 187.0 > 159.0 | 18 | — | — | — | — | — | — | — | — |
| IS-1 | 2,2',5-Trichlorobiphenyl | 255.9 > 186.0 | 26 | 257.9 > 186.0 | 26 | — | — | — | — | — | — | — | — |
| IS-2 | 2,4,4'-Trichlorobiphenyl | 255.9 > 186.0 | 26 | 257.9 > 186.0 | 26 | — | — | — | — | — | — | — | — |
| IS-3 | 2,2',5,5'-Tetrachlorobiphenyl | 257.0 > 222.0 | 26 | 292.0 > 220.0 | 26 | — | — | — | — | — | — | — | — |
| IS-4 | Triphenylmethane | 244.1 > 167.1 | 16 | 244.1 > 165.1 | 26 | — | — | — | — | — | — | — | — |
| IS-5 | Triphenylphosphate | 215.1 > 168.1 | 16 | 325.1 > 169.1 | 20 | — | — | — | — | — | — | — | — |
| IS-6 | Tris(1,3-dichloroisopropyl)phosphate | 379.0 > 159.0 | 12 | 381.0 > 159.0 | 12 | — | — | — | — | — | — | — | — |

Compounds outside the red box (Fig. 5) were classified to four groups.

Group A showed low recovery; this group consisted mainly of compounds which have low boiling point. They may have been lost in the evaporation step. Group B showed high relative ion ratios and this was caused by interference from matrix. Group C showed high recovery; this group consisted of 10 ng/g fortified sample. Some of these were in matrix originally and quantified incorrectly. Others caused by their transitions which had low response and low stability. Group D showed low relative ion ratio. It was necessary to set higher response transitions. By modifying some procedures and parameters, positions of these compounds may improve.
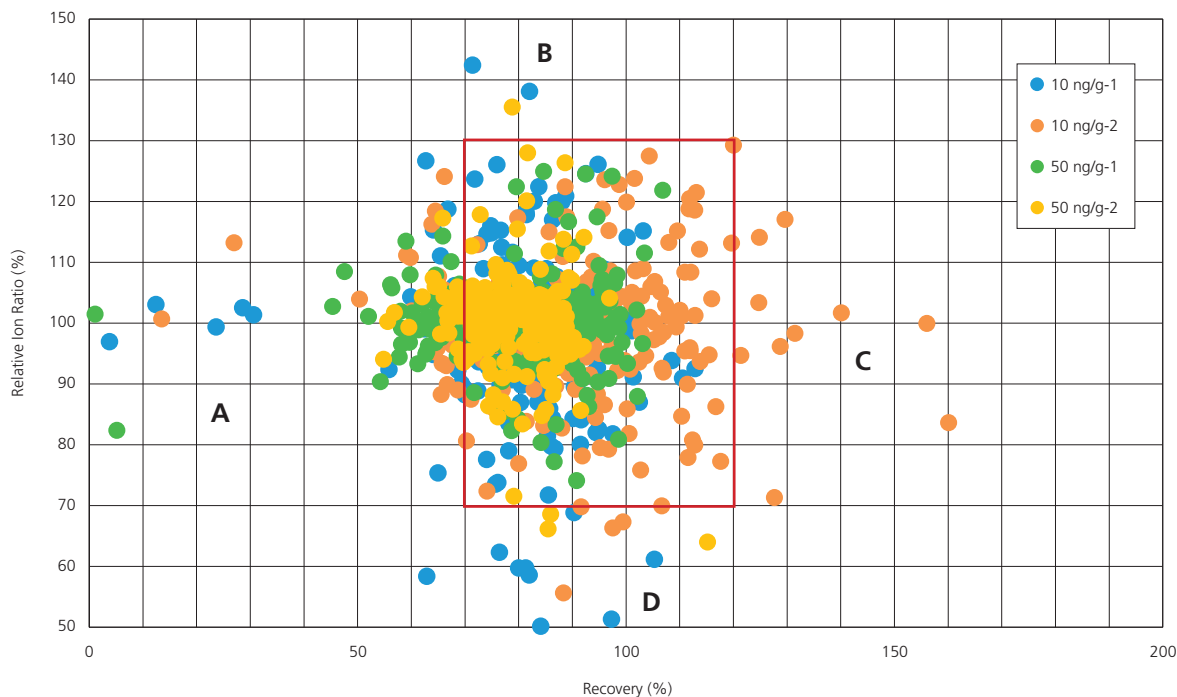


Fig. 5   Combination Map Between Recovery and Ion Ratio (Average of n = 3 for Each Fortified Samples)
Red box shows the area of 70–120% recovery and ±30% relative ion ratio.

# 4. Conclusion

This study shows that the modified QuEChERS method combined with GC-MS/MS achieved consistent pesticides monitoring in botanical ingredients.

Although dried sample could make a heavy and difficult matrix, the modified QuEChERS method, SPE column cleanup, and toluene dilution steps suppressed interference from matrix. The GC-MS/MS detected very low amount of pesticides even though the sample was diluted. This analytical method takes only 30 minutes in total run time and covers over 200 pesticides. It provides a high throughput solution in laboratories doing this type of analysis.

### Reference

1)   M. Anastassiades, S. J. Lehotay, D. Štajnbaher, F. J. Schenck, Fast and Easy Multiresidue Method Employing Acetonitrile Extraction/Partitioning and "Dispersive Solid-Phase Extraction" for the Determination of Pesticide Residues in Produce, *J. AOAC Int.*, **86** (2003) 412–431

2)   European Commission, Health & Consumer Protection Directorate-General, Guidance document on analytical quality control and validation procedures for pesticide residues analysis in food and feed, SANCO/12571/2013

**Triple Quadrupole Gas Chromatograph Mass Spectrometer**

# GCMS-TQ8040

## Smart Performance That Boosts Routine Analytical Work

GC-MS/MS is useful for measuring trace quantities of various chemical substances present in a variety of sample types. However, specifying several parameter settings and employing suitable methods are required when using this technique.

Nevertheless, GCMS-TQ8040 can dramatically increase the productivity by automating tedious method creation processes and simultaneously analyzing multiple components with high sensitivity.

### Smart Productivity

- Includes a new firmware protocol.
- Simultaneously analyzes a wide range of compounds with high sensitivity and high accuracy.
- Twin Line MS system minimizes the replacement of columns.

### Smart Operation

- Smart MRM automatically creates optimized methods.
- Automatically searches for optimal transitions.
- AART function automatically adjusts retention times.

### Smart Performance

- Patented high-sensitivity ion source technology offers even higher sensitivity.
- OFF-AXIS Ion Optics reduces noise.
- Capable of performing high-sensitivity analysis even as a single GC-MS system.

**GC/MS Residual Pesticides Database**

# Smart Pesticides Database Ver. 2

## Supports the Simultaneous Analysis of 530 Residual Pesticides in Foods via GC-MS(/MS)

Smart Pesticides Database contains the retention indices and transitions for 530 pesticides. Ver. 2 additionally contains measurement ions for SIM mode, so it can be applied to both SIM and MRM analyses. Thanks to the retention indices contained in the database and the AART function, retention times can be revised automatically without the use of pesticide standards.

Furthermore, the Smart MRM/SIM function allows automatic creation of the optimal measurement programs for multicomponent simultaneous analysis using MRM and SIM modes. Lastly, the database can be customized to the GC conditions and the addition of new components.

| Compound Name (E) | Ret. Index 1 | Ret. Index 2 | Ret. Index 3 | Ion1 Type | Ion1 m/z | Ion1 CE | Ion1 Ratio | Ion2 Type | Ion2 m/z | Ion2 CE | Ion2 Ratio | Ion1 Type | Ion1 m/z | Ion1 Ratio | Ion2 Type | Ion2 m/z | Ion2 Ratio |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Hymexazol | 1201 | 1193 | 1191 | T | 99.0>71.0 | 8 | 100.00 | Ref.1 | 99.0>54.0 | 26 | 6.13 | T | 99.0 | 100.00 | Ref.1 | 71.0 | 18.40 |
| Methamidophos | 1240 | 1231 | 1229 | T | 141.0>95.0 | 8 | 100.00 | Ref.1 | 141.0>79.0 | 22 | 31.28 | T | 141.0 | 100.00 | Ref.1 | 94.0 | 337.84 |
| Dichlorvos | 1248 | 1244 | 1243 | T | 109.0>79.0 | 8 | 100.00 | Ref.1 | 185.0>93.0 | 14 | 56.91 | T | 185.0 | 100.00 | Ref.1 | 109.0 | 403.23 |
| Nereistoxin | 1285 | 1284 | 1274 | T | 149.1>71.1 | 8 | 100.00 | Ref.1 | 149.1>102.1 | 6 | 67.94 | T | 70.0 | 100.00 | Ref.1 | 149.0 | 51.20 |
| Allidochlor | 1290 | 1288 | 1283 | T | 132.1>56.0 | 8 | 100.00 | Ref.1 | 138.1>96.0 | 6 | 26.15 | T | 138.0 | 100.00 | Ref.1 | 173.0 | 6.98 |
| Dichlobenil | 1348 | 1345 | 1335 | T | 170.9>136.0 | 14 | 100.00 | Ref.1 | 170.9>100.0 | 24 | 98.06 | T | 171.0 | 100.00 | Ref.1 | 173.0 | 76.00 |
| EPTC | 1359 | 1358 | 1353 | T | 189.1>128.1 | 4 | 100.00 | Ref.1 | 189.1>86.0 | 12 | 22.96 | T | 128.0 | 100.00 | Ref.1 | 189.0 | 25.60 |
| Biphenyl | 1393 | 1391 | 1380 | T | 154.1>128.1 | 22 | 100.00 | Ref.1 | 154.1>115.1 | 24 | 74.03 | T | 154.0 | 100.00 | Ref.1 | 153.0 | 37.60 |
| Propamocarb | 1394 | 1393 | 1390 | T | 188.2>72.0 | 4 | 100.00 | Ref.1 | 188.2>173.2 | 4 | 72.45 | T | 58.0 | 100.00 | Ref.1 | 129.0 | 2.40 |
| Mevinphos-1 | 1420 | 1420 | 1419 | T | 127.0>109.0 | 12 | 100.00 | Ref.1 | 192.0>127.0 | 12 | 61.12 | T | 127.0 | 100.00 | Ref.1 | 192.0 | 31.60 |

## Verifying the Effectiveness of Recovery Tests of Health Foods

In conducting recovery tests in health foods for 220 pesticides, using the database, recommended pretreatment kit, pretreatment protocol, and certified standard substances, we succeeded in obtaining excellent ratios in over 80 percent of components.

Health foods contain many contaminants. Because it includes transitions for the separation of contaminants, the database minimizes their impact.

Additionally, the database can separate contaminants by allowing analysis with a different column, even if peaks of target pesticides and contaminants overlap. If the database is used in combination with the Twin Line MS system, analysis with different columns can be performed smoothly, without compromising the MS vacuum.

(the number of components)
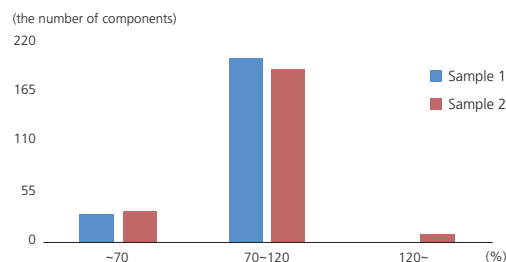


Distribution of rates for two health food samples which were spiked with a pesticide standard sample so as to obtain a final concentration of 2.5 ng/mL each.

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## Excellence in Science

| | |
|---|---|
| **Application News** | **Liquid Chromatography Mass Spectrometry** |
| **No.C136** | **Expanding Capabilities in Multi-Residue Pesticide Analysis Using The LCMS-8060** |

David R. Baker[1], Laëtitia Fages[2], Eric Capodanno[2], Neil Loftus[1]
[1]Shimadzu Corporation, UK; [2]Phytocontrol, France

## ■ Abstract

With an increasing global population, food security is increasingly under threat and there is a growing challenge for agriculture to produce more food, safely and more sustainably. The use of herbicides, insecticides, and fungicides reduce crop losses both before and after harvest, and increase crop yields. However, pesticide residues resulting from the use of plant protection products on crops may pose a risk to human health and require a legislative framework to monitor pesticide residues in food.

National programs for pesticide monitoring in the US, Europe and Japan have set Maximum Residue Levels (MRL's) or tolerance information (EPA) for pesticides in food products. A default value of 0.01 mg/kg is applied for MRL enforcement, which therefore requires highly sensitive and specific analytical technologies to monitor an increasing number of pesticides.

This application note describes the expanded capability of the LCMS-8060 to help accelerate method development workflows and support increased pesticide monitoring programs. Using the Shimadzu Pesticide MRM Library (the Library includes information on 766 certified reference materials) a single multi-residue LC/MS/MS method was developed for 646 pesticides (3 MRM transitions for over 99 % targeted pesticides resulting in 1,919 transitions in total, with a polarity switching time of 5 msec).

Keywords: Pesticides; food safety; LCMS-8060; Pesticide MRM Library, 776 compound library

## ■ Introduction

There are more than 1,000 pesticides used globally on soil and crops. With the ever increasing international trade of the food industry, regulatory bodies around the world have increased the number of regulated pesticides and the maximum residue levels (MRLs) allowed in food commodities. In the EU, regulation 396/2005/EC and its annexes set MRLs for over 500 pesticides in 370 food products.[1] In the US, tolerances for more than 450 pesticides and other ingredients are established by the US EPA[2] and Japan's positive list system for agricultural chemical residues in foods contains MRLs for over 400 pesticides in various commodities.[3]

National pesticide monitoring programs create new challenges for food safety laboratories as the number of pesticides required for analysis is increasing together with an expanded range of food products.

In this application paper we present the development of a LC-MS/MS method for screening and quantifying over 646 pesticides in a single method. The method was quickly and efficiently set up using the Shimadzu Pesticide MRM Library. For each target pesticide analysis, up to 3 MRMs (Multiple Reaction Monitoring) transitions were imported from the library. 3 MRMs transitions provided additional data confidence in reporting results in comparison to the conventional 2 transitions used in most methods. As the LCMS-8060 has a high data acquisition speed 1,919 transitions were acquired using a polarity switching speed of 5 msec over a 10.5 minutes gradient elution.

To evaluate the method QuEChERS extracts of mint, tomato and apple were provided by a commercial laboratory as raw acetonitrile extracts and spiked with 646 pesticides (data is presented on the mint extract as it is the more complex sample matrix). The method was evaluated in matrix to ensure that the reporting limits were in agreement with recognised MRL's.

## ■ Experiment

Food extracts of mint, tomato and apple were supplied by Phytocontrol, France, following established QuEChERS protocols. Final extracts were prepared in acetonitrile without any dilution. Certified reference materials for the Shimadzu Pesticide MRM Library were obtained from ACSD, France as stock solutions. All solvents were of LCMS quality purchased from Sigma-Aldrich.

A six point calibration curve from 0.002 - 0.1 mg/kg (2 - 100 pg/μL) were generated using internal standard method. Two internal standards (Atrazine-d5 and Diuron-d6) were spiked in during the auto-sampler sequence for quantitation.

The robustness of the LCMS-8060 was assessed by peak area response for 646 pesticides spiked into mint, tomato and apple matrix extracts at 0.05 mg/kg.

## ■ LC/MS/MS method development

The Shimadzu Pesticide MRM Library has 766 pesticides in its database (Application News No. C135). For each pesticide several MRM's are included in the database and in this analysis the default value used was 3 MRM's. For this method, 1,919 transitions were selected in both positive and negative ionisation mode using a switching time of 5 msec (1,819 MRM transitions were in positive mode and 100 MRM transitions in negative mode).

To optimize ion source conditions (for example, DL temperature, interface temperature, heating block temperature, heating gas flow, drying gas flow and nebulizer gas flow) the interface setting software was used. This tool provides an optimized response for all compounds.

**Table 1  LC and MS/MS Acquisition Parameters**

| Liquid chromatography | | Mass spectrometry | |
|---|---|---|---|
| UHPLC | Nexera LC system | LC/MS/MS | LCMS-8060 |
| Analytical column | Restek Raptor Biphenyl (2.1 mm I.D. × 100 mm L., 2.7 µm) | Ionisation mode | Heated electrospray |
| | | Polarity switching time | 5 msec |
| Column temperature | 35 °C | Pause time | 1 msec |
| Flow rate | 0.4 mL/min | Total MRM transitions | 1,919 (1,819 positive; 100 negative) |
| Solvent A | 2 mmol/L ammonium formate + 0.002 % formic acid - Water | MRM Dwell | 4 msec (target ion); 1 msec (reference ion) |
| Solvent B | 2 mmol/L ammonium formate + 0.002 % formic acid - Methanol | Interface temperature | 350 °C |
| | | Heating block | 300 °C |
| Binary Gradient B.Conc. | 3 % (0 min) - 10 % (1.00 min) - 55 % (3.00 min) - 100 % (10.50 - 12.00 min) - 3 % (12.01 - 15.00 min) | Desolvation line | 150 °C |
| | | Heating gas | 10 L/min |
| | | Drying gas | 10 L/min |
| Injection volume | 2 µL sample (plus 40 µL water) | Nebulizer gas | 3 L/min |



**Fig. 1  MRM chromatograms of 646 pesticides spiked into a mint extract at 0.01 mg/kg (Up to 3 MRMs per compound and 5 msec polarity switching time).**



**Fig. 2  MRM chromatograms for pesticides most commonly detected in plant products listed in the 2015 European Food Safety Journal. In this report, residues exceeding the legal limits were related to 58 different pesticides. Compounds such as boscalid, chlorpyriphos, cyprodinil, fenhexamid, fludioxonil, pyraclostrobin and tebuconazole (highlighted in the MRM chromatogram) are some of the most frequently detected compounds present in more than 4 % of the samples analyzed.**

The MRM chromatograms show the response to each pesticide spiked into a food matrix at the default MRL of 0.01 mg/kg.

■ **Results and Discussion**
**Shimadzu Pesticide MRM Library**
(Application News No. C135 )

**A flexible tool for expanding capabilities in pesticide monitoring programs**
The Pesticide MRM Library has been created using 766 certified reference materials and is designed to help accelerate method development and compound management.
The library contains an average of 8 optimized MRM transitions for each compound (including positive and negative ion modes). In total, more than 6,000 MRM transitions are held within the 766 compound library. The library itself documents CAS#, formula, activity, mono-isotopic mass and adduct masses, rank of MRM transitions, synonyms, InChI, InChIKey, compound names translation (Japanese and Chinese) and links to websites offering further information (for example; alanwood.net, PAN pesticide database, Chemical Book, ChemSpider).
The library also serves as a powerful data repository for reporting and checking pesticide data sources.

**Creating flexible pesticide monitoring methods**
**Building a new LC/MS/MS method**
To create new pesticide LC/MS/MS methods the user simply needs to select the target compounds from the library, identify the required number of MRMs for each compound and confirm the analytical column for the analysis. (The new method can be used to expand current capabilities or to create focused methods with a limited number of pesticides). The new method is simply imported into LabSolutions.
As the LCMS-8060 has a high data acquisition speed of 30,000 u/sec, high sensitivity and a polarity switching speed of 5 msec, the capabilities of the library can be expanded to meet the future needs of any laboratory.

**Expanded capability of the LCMS-8060**
The LCMS-8060 has a data acquisition speed of 30,000 u/sec which creates new opportunities for expanding compound lists.
As one example, between 6.45 and 6.60 minutes 25 pesticide compounds elute (Fig. 3). Even with high data density acquisitions the average variation in peak area response was less than 3 %RSD (varying between 1.1 - 5.9 %RSD).



**Fig. 3  The LCMS-8060 can acquire MRM data at a high speeds and enables precise quantitation even with high data density. Between 6.45 and 6.60 minutes 25 compounds were monitored (Table 2).**

**Table 2  Peak area variation (%RSD; n=6) for 25 pesticides eluting over a nine-second time window (6.45 - 6.60 minutes) spiked into a mint matrix extract at the reporting limit of 0.01 mg/kg.**

| Compound Name | CAS number | Formula | M | Polarity | MRM Quantitation Ion | RT | Average Peak Area | %RSD (n=6) |
|---|---|---|---|---|---|---|---|---|
| Trinexapac-ethyl | 95266-40-3 | C13H16O5 | 252.0998 | + | 252.90 > 69.05 | 6.45 | 1,780,015 | 3.1 |
| Iprovalicarb | 140923-17-7 | C18H28N2O3 | 320.2100 | + | 321.20 > 119.15 | 6.46 | 1,442,486 | 2.8 |
| Dodemorph | 1593-77-7 | C18H35NO | 281.2719 | + | 282.30 > 116.15 | 6.47 | 658,920 | 4.2 |
| Fluopyram | 658066-35-4 | C16H11ClF6N2O | 396.0464 | + | 397.00 > 145.00 | 6.47 | 2,439,146 | 1.9 |
| Flutolanil | 66332-96-5 | C17H16F3NO2 | 323.1133 | + | 324.10 > 242.00 | 6.48 | 3,372,285 | 2.7 |
| Trifloxysulfuron | 145099-21-4 | C14H14F3N5O6S | 437.0617 | + | 438.00 > 182.15 | 6.48 | 1,822,340 | 2.5 |
| Azaconazole | 60207-31-0 | C12H11Cl2N3O2 | 299.0228 | + | 300.00 > 159.00 | 6.50 | 1,580,445 | 2.0 |
| Terbutryn | 886-50-0 | C10H19N5S | 241.1361 | + | 242.10 > 157.95 | 6.50 | 755,446 | 3.4 |
| Prometryn | 7287-19-6 | C10H19N5S | 241.1361 | + | 242.10 > 158.00 | 6.50 | 1,300,193 | 2.6 |
| Azimsulfuron | 120162-55-2 | C13H16N10O5S | 424.1026 | + | 425.10 > 182.10 | 6.50 | 2,498,050 | 1.8 |
| Metominostrobin | 133408-50-1 | C16H16N2O3 | 284.1161 | + | 285.10 > 193.95 | 6.51 | 2,929,500 | 1.7 |
| Thifluzamide | 130000-40-7 | C13H6Br2F6N2O2S | 525.8421 | + | 528.60 > 148.05 | 6.51 | 193,982 | 5.9 |
| Nicarbazin | 330-95-0 | C13H10N4O5 | 302.0651 | - | 301.10 > 137.15 | 6.52 | 973,101 | 2.6 |
| Bromobutide | 74712-19-9 | C15H22BrNO | 311.0885 | + | 312.10 > 194.10 | 6.53 | 1,829,781 | 2.1 |
| Saflufenacil | 372137-35-4 | C17H17ClF4N4O5S | 500.0544 | + | 501.00 > 198.00 | 6.53 | 465,224 | 2.3 |
| Cyproconazole | 94361-06-5 | C15H18ClN3O | 291.1138 | + | 292.10 > 70.05 | 6.54 | 1,174,967 | 1.7 |
| Clomazone | 81777-89-1 | C12H14ClNO2 | 239.0713 | + | 239.90 > 125.00 | 6.54 | 3,409,656 | 1.7 |
| Fensulfothion | 115-90-2 | C11H17O4PS2 | 308.0306 | + | 309.00 > 281.00 | 6.54 | 4,267,514 | 1.4 |
| Oxasulfuron | 144651-06-9 | C17H18N4O6S | 406.0947 | + | 407.10 > 150.15 | 6.54 | 2,911,533 | 1.1 |
| Rimsulfuron | 122931-48-0 | C14H17N5O7S2 | 431.0569 | + | 432.00 > 182.00 | 6.55 | 4,722,065 | 1.8 |
| Fenthion-oxon | 6552-12-1 | C10H15O4PS | 262.0429 | + | 263.10 > 231.00 | 6.55 | 3,075,195 | 1.4 |
| Nitrothal-isopropyl | 10552-74-6 | C14H16NO6Na | 317.0875 | + | 295.10 > 230.95 | 6.56 | 2,199,581 | 3.0 |
| Chlorantraniliprole | 500008-45-7 | C18H14BrCl2N5O2 | 480.9708 | + | 483.90 > 452.90 | 6.57 | 2,407,025 | 2.7 |
| Fipronil-sulfone | 120068-36-2 | C12H4Cl2F6N4O2S | 451.9336 | - | 451.00 > 414.90 | 6.57 | 2,843,708 | 2.0 |
| Valifenalate | 283159-90-0 | C19H27ClN2O5 | 398.1608 | + | 399.20 > 155.00 | 6.59 | 3,845,335 | 1.9 |

## Final method performance for 646 pesticides

In order to test the performance of the developed method, linearity, repeatability and longer term robustness were assessed for all 646 pesticides.

## Linearity

Linearity was assessed over a six point calibration curve from 0.002 - 0.1 mg/kg (2 - 100 pg/μL). All 646 pesticides achieved excellent $R^2$ values greater than 0.99 in both tomato and mint spiked extracts with typical values greater than 0.996. Calibration curves were generated using a linear curve fit type and 1/C weighting. Typical calibration curve data is presented below in Fig. 4.



**Fig. 4  Calibration curves for selected pesticides spiked into a mint matrix extract in the range 0.002 - 0.1 mg/kg. The quantitation MRM chromatogram is shown in black (qualifier ion MRM chromatograms are shown in red and blue).**

## Repeatability

To assess the robustness of the system and the developed method during routine analysis, repeat injections of a mint matrix sample spiked with 646 pesticides at 0.05 mg/kg, were analyzed over a 24 hour period.

The results for selected compounds are displayed below in Fig. 5.

Compounds were selected throughout the run at equi-distant points (closest elution points to 3, 4, 5, 6, 7, 8, 9 and 10 minutes), including positive and negative ion detection, (Table 3).

The peak area variance was less than 5.7 % for all pesticides measured.



**Fig. 5  Peak area response for several pesticides following 100 repeat injections of a 0.05 mg/kg spiked into mint matrix extract.**

**Table 3  Peak area variance for selected following the repeated injection of a 0.05 mg/kg spiked into mint matrix extract (number of sample replicates was 100; the analysis sequence was 24 hours).**

| Compound Name | CAS Number | Formula | M | Polarity | MRM Quantitation Ion | RT (mins) | Average Peak Area | %RSD (n=100) |
|---|---|---|---|---|---|---|---|---|
| Butocarboxim-sulfoxide | 34681-24-8 | C7H14N2O3S | 206.0725 | + | 207.10 > 75.10 | 3.042 | 1,220,391 | 2.6 |
| Thiofanox-sulfone | 39184-59-3 | C9H18N2O4S | 250.0987 | + | 268.10 > 57.00 | 4.001 | 442,724 | 5.7 |
| Monolinuron | 1746-81-2 | C9H11ClN2O2 | 214.0509 | + | 215.10 > 99.10 | 4.985 | 2,904,116 | 3.7 |
| Probenazole | 27605-76-1 | C10H9NO3S | 223.0303 | + | 224.00 > 41.05 | 5.995 | 1,145,189 | 3.5 |
| Dipropetryn | 4147-51-7 | C11H21N5S | 255.1518 | + | 256.20 > 144.05 | 6.999 | 3,289,597 | 3.4 |
| Pyraflufen-ethyl | 129630-19-9 | C15H13Cl2F3N2O4 | 412.0204 | + | 413.00 > 339.00 | 8.004 | 3,653,333 | 3.5 |
| Emamectin B1a | 138511-97-4 | C56H81NO15 | 1007.5606 | + | 886.40 > 158.20 | 9.008 | 3,109,562 | 4.5 |
| Pyridalyl | 179101-81-6 | C18H14Cl4F3NO3 | 488.9680 | - | 491.90 > 109.05 | 10.171 | 1,579,422 | 5.0 |

### Response to differing matrices

One of the major challenges in the quantitative LC/MS/MS analysis for pesticides in food is that compound and matrix-dependent response suppression or enhancement may occur. Although matrix effects can affect the peak area response between different food types following a QuEChERS extraction protocol, the peak area variance should be minimized within a single matrix.

Food extracts of apple, mint and tomato following QuEChERS extraction were spiked with 646 pesticides at 0.05 mg/kg and were repeatedly injected on the LCMS-8060 (n=100 repeat injections for each matrix; 300 injections in the same batch sequence). Fig. 6 shows the response for 3 selected pesticides analyzed in a single batch sequence corresponding to a 72 hour analysis sequence. Within a matrix, variance was less than 5.9 %RSD for all compounds.

Although the absolute peak area changes with different food matrices, the response between injection 1 and injection 100 for 2 pesticides (probenazole and dipropetryn) within a single matrix has a variance less than 5.7 %RSD.



**Fig. 6  Peak area response for three pesticides spiked into apple, mint and tomato matrix extracts at 0.05 mg/kg over 72 hours. As in Fig. 5, compounds were selected to reflect peak area response throughout the chromatographic run (Table 3).**



**Fig. 7  MRM chromatograms for probenazole (RT 5.995 minutes) and dipropetryn (RT 6.999 minutes) for injection 1 and injection 100 spiked into apple, mint and tomato matrix extracts. The extracts were spiked at 0.05 mg/kg and analyzed over 72 hours.**

## Reducing matrix effects by extensively diluting the sample

The need to test for more pesticides in a wider range of samples at high sensitivity is very challenging as matrix effects from the sample extraction will influence both ion suppression and enhancement. Ion suppression can lead to errors in the detection capability, accuracy and precision of the method.

To reduce the effect of interfering compounds in the quantitation of complex samples extensive sample dilution is now widely used in routine analysis. It is an approach which is simple to build into multi-residue extraction methods and is cost effective.

This approach leads to greater robustness as a consequence of a reduced sample injection in the LC/MS/MS, higher data quality and increased instrument uptime.

Fig. 8 shows the results of diluting a matrix sample spiked at 0.005 mg/kg with dilution factors of 1:5, 1:10, 1:20, 1:50 and 1:100.

As matrix effects can be both significant and variable for different compounds Table 4 shows recovery data for a series of pesticides diluted from 0 to a dilution factor of 1:100.

Matrix suppression was reduced for most compounds when the sample was diluted 1:10 with recoveries in the range of 70 - 120 % with an associated repeatability RSDr ≤ 20 %. Relative standard deviations in relation to the mean values were typically less than 10 %.

Diluting the sample by a factor of 20 or 50 resulted in acceptable signal suppression from the matrix.



**Fig. 8  MRM chromatograms for 3 selected compounds spiked into a mint extract at 0.005 mg/kg and diluted 1:5, 1:10, 1:20, 1:50 and 1:100 with water.**

**Table 4  Diluting a sample matrix extract spiked with 0.005 mg/kg with water reduced matrix ion suppression.**

| Compound | CAS | Formula | M | Dilution series | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 0 | 1:5 | 1:10 | 1:20 | 1:50 | 1:100 |
| | | | | Recovery | | | | | |
| Bentazone | 25057-89-0 | C10H12N2O3S | 240.0569 | 32.1 | 44.6 | 65.5 | 72.7 | 91.7 | 98.1 |
| Demeton-S-methyl-sulfone | 17040-19-6 | C6H15O5PS2 | 262.0099 | 51.1 | 78.5 | 89.6 | 91.1 | 114.2 | 116.8 |
| Dimethoate | 60-51-5 | C5H12NO3PS2 | 228.9996 | 36.2 | 65.3 | 88.5 | 92.2 | 92.4 | 94.2 |
| Isocarbamid | 30979-48-7 | C8H15N3O2 | 185.1164 | 28.8 | 57.1 | 81.8 | 98.7 | 102.5 | 96.4 |
| Vamidothion | 2275-23-2 | C8H18NO4PS2 | 287.0415 | 53.6 | 76.3 | 98.2 | 98.5 | 101.5 | 114.1 |
| Thiazafluron | 25366-23-8 | C6H7F3N4OS | 240.0293 | 32.8 | 62.9 | 80.5 | 84.2 | 87.1 | 97.4 |
| Demeton-S-methyl | 919-86-8 | C6H15O3PS2 | 230.0200 | 57.8 | 82.1 | 93.1 | 87.6 | 108.5 | 102.4 |
| Sebuthylazine | 7286-69-3 | C9H16ClN5 | 229.1094 | 28.7 | 53.3 | 69.8 | 79.8 | 88.5 | 95.8 |
| Flutriafol | 76674-21-0 | C16H13F2N3O | 301.1027 | 27.3 | 46.1 | 71.4 | 76.1 | 81.8 | 87.3 |
| Furametpyr | 123572-88-3 | C17H20ClN3O2 | 333.1244 | 48.3 | 69.8 | 86.9 | 86.2 | 97.6 | 101.9 |
| Fenobucarb | 3766-81-2 | C12H17NO2 | 207.1259 | 60.9 | 79.2 | 100.7 | 96.1 | 102.8 | 103.9 |
| Benodanil | 15310-01-7 | C13H10INO | 322.9807 | 50.9 | 69.8 | 86.3 | 96.5 | 102.4 | 94.8 |
| Terbuthylazine | 5915-41-3 | C9H16ClN5 | 229.1094 | 50.4 | 66.6 | 83.2 | 87.2 | 89.8 | 91.0 |
| Dimethachlor | 50563-36-5 | C13H18ClNO2 | 255.1026 | 75.1 | 86.1 | 106.0 | 107.1 | 106.2 | 108.0 |
| Dimethenamid | 87674-68-8 | C12H18ClNO2S | 275.0747 | 72.6 | 84.9 | 102.9 | 100.0 | 103.6 | 97.3 |
| Furalaxyl | 57646-30-7 | C17H19NO4 | 301.1314 | 82.2 | 89.1 | 106.6 | 108.6 | 106.2 | 102.4 |
| Bixafen | 581809-46-3 | C18H12Cl2F3N3O | 413.0310 | 66.8 | 79.3 | 99.0 | 95.6 | 103.7 | 97.1 |
| Triflumuron | 64628-44-0 | C15H10ClF3N2O3 | 358.0332 | 54.2 | 71.8 | 95.5 | 84.9 | 95.3 | 101.7 |
| Epoxiconazole | 133855-98-8 | C17H13ClFN3O | 329.0731 | 61.6 | 77.2 | 98.8 | 95.3 | 90.0 | 101.2 |
| Teflubenzuron | 83121-18-0 | C14H6Cl2F4N2O2 | 379.9742 | 41.8 | 50.9 | 80.1 | 86.8 | 100.0 | 97.7 |

■ **Conclusion**

A fast, selective and highly sensitive method has been developed for the quantitation of 646 pesticides using a single method with 1,919 transitions (corresponding to up to 3 MRM transitions per compound) and a LC gradient time of only 10.5 minutes.

As the LCMS-8060 has a rapid polarity switching time of 5 msec, the single multi-residue LC/MS/MS method supported the analysis of 34 pesticides in negative ion mode and 612 compounds in positive ion mode.

The enhanced performance and higher sensitivity of the LCMS-8060 has created new opportunities in sample dilution to reduce ion signal suppression and matrix effects. For most compounds a dilution factor of 1:20 or 1:50 was sufficient to provide recoveries in the range 70 - 120 %.



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# Application News

LCMS

## No.C118

**Liquid Chromatography Mass Spectrometry**

# Highly Polar Pesticide Multi-Residue Analysis in Food Safety by LC-MS/MS

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■ **Abstract**

The analysis of highly polar pesticides by a single LC-MS/MS method is extremely challenging as a consequence of diverse separation and detection behaviour. Conventional approaches in highly polar pesticide analysis often use single residue methods or small group specific methods which are time consuming and limit throughput. In this study, the panel of target analytes selected for analysis included a series of compounds that are typically addressed by multiple methods and workflows; glufosinate, glyphosate, ethephon, fosethyl aluminium, maleic hydrazide, perchlorate, ETU, PTU, nicotine, amitrole, chlormequat, daminozide, diquat, kasugamycine, mepiquat, paraquat and trimesium.

To accelerate turnaround times and increase sample sizes for more complete testing programs two LC-MS/MS methods were developed for the measurement of a range of highly polar pesticides in their underivitised state using the LCMS-8050 triple quadrupole mass spectrometer. All target compounds were quantified at 0.01 mg/kg which is below the European Union maximum residue limit for all studied compounds delivering a measurable impact on sample cycle time and productivity.

**Keywords: Highly polar pesticides, LCMS-8050, food safety, glyphosate, diquat, paraquat, perchlorate**

■ **Introduction**

The use of pesticides in the environment is constantly under review and in recent years regulatory bodies have adopted a hazards-based approach to pesticide regulation leading to an increased use of highly polar pesticides which present lower persistence and toxicity. Enforcing pesticide limits within regulatory limits defined as the maximum residue levels (MRL's; the maximum concentration of pesticide residues permitted in food and feed) requires methods that provide results quickly and accurately for a broad spectrum of chemical structures in a diverse range of food samples.

Pesticide residue monitoring laboratories utilise multi-residue LC-MS/MS methods for the quantification of an ever increasing list of target pesticides. However, the measurement of highly polar pesticides by a single LC-MS/MS method is extremely challenging as a consequence of diverse separation and detection behaviour. For this reason, single residue methods or small group specific methods are often utilised to analyse these compounds, in some cases including the use of pre- or post-column derivatisation. Therefore, there is a clear need to reduce the number of separation methods applied to the analysis of highly polar pesticides to help accelerate sample throughput, reduce the cost platform, simplify analytical workflows and enhance data quality for regulatory reporting limits.
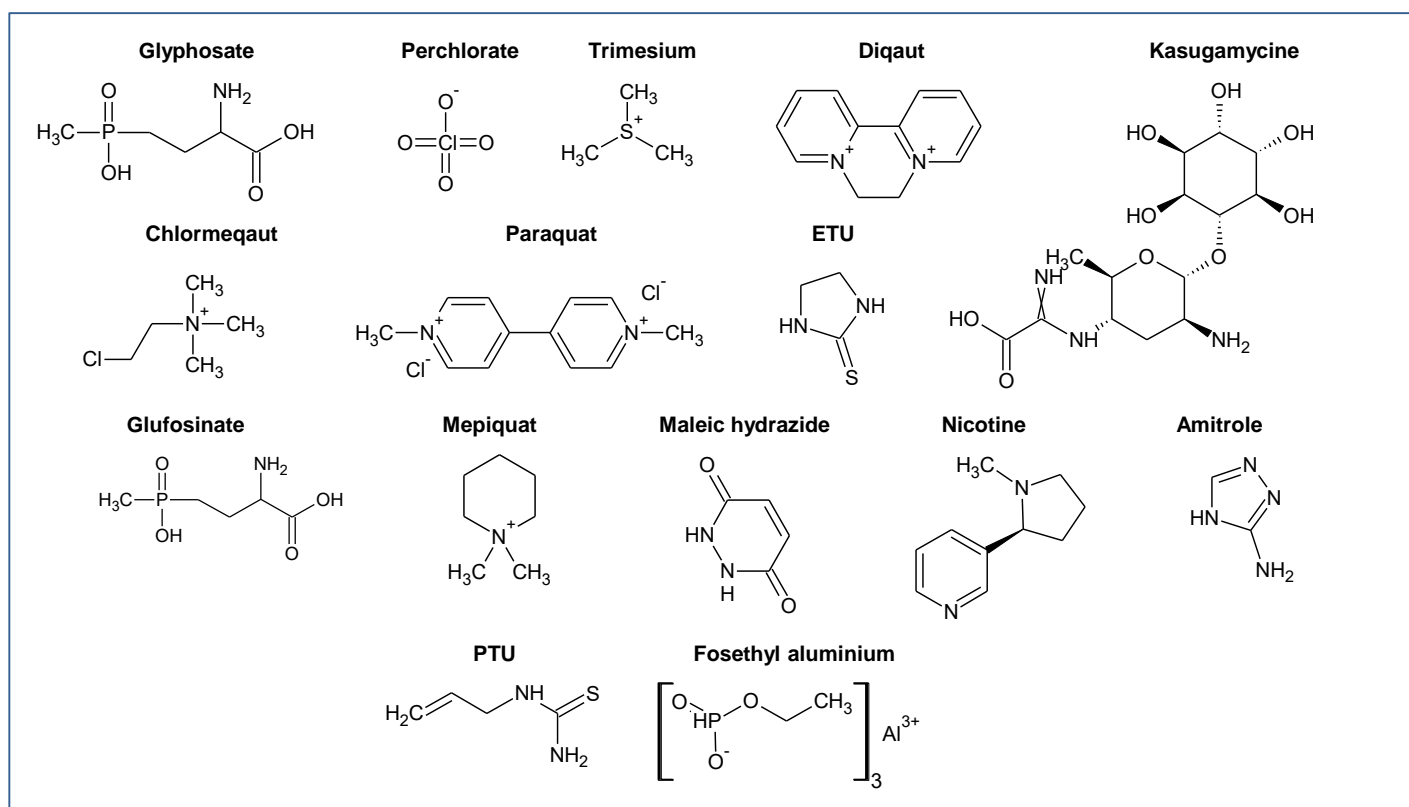


**Figure 1. Target analyte structures**

The highly polar pesticides targeted in this study included glufosinate, glyphosate, ethephon, fosethyl aluminium, maleic hydrazide, perchlorate, ETU, PTU, nicotine, amitrole, chlormequat, daminozide, diquat, kasugamycin, mepiquat, paraquat and trimesium. Structures for these compounds are displayed in Figure 1. All of the compounds included in this study were polar, characterised with LogKow < 1. The most polar compounds being the cationic quaternary ammonium herbicides diquat (LogKow -4.6) and paraquat (LogKow -4.5). Several of the compounds also have a low molecular mass, for example trimesium (77 g/mol), amitrole (84 g/mol) and ETU (102 g/mol).

The analysis of highly polar pesticides is extensively reported in literature but the methods have been limited to a small number of specific target compounds and not to a group with such a diverse chemical space. For example, a common approach for the analysis of one of the world's biggest selling herbicides glyphosate is typically achieved by FMOC derivatization. This derivatization step is specific for glyphosate, glufosinate and AMPA residues in water and food samples but it is relatively complex, limits throughput and repeatability and reproducibility can suffer due to the derivatisation step.

The aim of this study was to develop a fast, sensitive and simple methodology for a range of challenging highly polar pesticides that require single-residue methods, by as few multi-residue LC-MS/MS runs as possible and without the need for derivatisation. Several different analytical columns and mobile phases were evaluated in this study, in addition to assessing the MS/MS parameters. Isotopically labelled standards were used to compensate for matrix effects. Initial data was collected in food matrix using a triple quadrupole mass spectrometer in MRM mode.

■ **Experimental**

Individual reference standards for each compound were provided by Phytocontrol in methanol at a concentration of 10 ng/µL. Mobile phase solvents and additives were all LC–MS quality and purchased from Sigma–Aldrich. Apple extracts were provided by Phytocontrol and extracted according to the EURL-SRM QuPPe methodology.[1] Briefly, apple samples (10 g) were prepared by chopping up the sample, freezing, homogenizing with dry ice, adding 1% formic acid in methanol solution (10 mL) and centrifuging (4000 RPM). Linearity was evaluated by spiking sample extracts at the following levels:0.005, 0.01, 0.02, 0.05, 0.1 and 0.2 mg/kg. Deuterated internal standards were used for calibration. All calibration points were analysed in duplicate. Plastic vials were used for analysis to prevent interaction of certain pesticides (e.g. paraquat, diquat and glyphosate) with glass surfaces.[1]

SRM transitions and analyte specific MS parameters (Q1 pre-bias (V), Q3 pre-bias (V) and collision energy) were optimised automatically using the SRM optimisation feature available in LabSolutions software. SRM transitions are listed in Table 2 and Table 3.

Preliminary investigations involved the testing of several different analytical columns: SIELC Obselisc R (150 x 2.1mm, 5µm); Hypercarb PGC (100 x 2.1mm, 5µm); SeQuant ZiC-HILIC (100 x 2.1mm, 3.5µm), SeQuant ZIC-cHILIC (100 x 2.1mm, 3.5µm), Scherzo SM-C18 (50 x 2, 3 µm), Scherzo SW-C18 (50 x 2, 3 µm), Fortis Phenyl (100 x 2.1mm, 5µm), Luna Phenyl-Hexyl (100 x 2.1mm, 3µm), and Restek IBD (150 x 2.1mm, 3µm). These columns were tested with several different mobile phase additives including acetic acid, formic acid, ammonium formate, ammonium acetate and ammonium hydroxide (depending on appropriate conditions for each column and the progression of results). Reversed phase, HILIC, and mixed mode chromatography were tested depending on the column suitability for each mode. The final LCMS/MS method conditions are listed in Table 1.

**Table 1. LC/MS/MS parameters for Method 1 and Method 2**

| Liquid chromatography | | | | |
|---|---|---|---|---|
| | **Method 1** | | **Method 2** | |
| **UHPLC** | Nexera UHPLC system | | Nexera UHPLC system | |
| **Analytical column** | ZIC-HILIC (100 x 2.1mm, 3.5µm) | | Hypercarb PGC (100mm x 2.1mm, 5µm) | |
| **Mobile phase** | A = Water 20mM ammonium formate and 0.3% formic acid | | A = Water 1% acetic acid | |
| | B = Acetonitrile | | B = Methanol 1% acetic acid | |
| **Gradient** | **Time (mins)** | **%B** | **Time (mins)** | **%B** |
| | 0 | 97 | 0 | 0 |
| | 5.8 | 68 | 10 | 30 |
| | 9 | 15 | 15 | 35 |
| | 10 | 15 | 17.5 | 68 |
| | 10 | 97 | 18 | 100 |
| | 16 | Stop | 22 | 100 |
| | | | 22.1 | 0 |
| | | | 33 | Stop |
| **Column temp.** | 35$^o$C | | 35$^o$C | |
| **Injection volume** | 6µL (40µL acetonitrile co-injected) | | 5µL | |
| **Flow rate** | 0.4mL/min | | 0.3mL/min | |
| **Mass spectrometry** | | | | |
| **LC/MS/MS** | LCMS-8050 | | | |
| **Ionisation mode** | Heated electrospray | | | |
| **Polarity switching time** | 5 ms | | | |
| **Pause time** | 1 ms | | | |
| **Dwell times** | 5-50ms | | | |
| **Interface temperature** | 350$^o$C | | | |
| **Heating block** | 300$^0$C | | | |
| **Desolvation line** | 200$^o$C | | | |
| **Gas** | Heating gas 10 L/min; drying gas 10 L/min; Nebulising gas 3 L/min | | | |

**Table 2. Method 1 MS acquisition parameters, retention time and internal standard**

| Compound | Ret. time (min) | Polarity | SRM transitions | Q1 (V) | CE | Q3 (V) | ISTD | MS1 Res. | MS2 Res. |
|---|---|---|---|---|---|---|---|---|---|
| Amitrole | 3.1 | Positive | 85 > 43 | -14 | -25 | -14 | Paraquat d8 | Unit | Unit |
| | | | 85 > 57 | -14 | -20 | -20 | | Unit | Unit |
| | | | 85 > 58 | -14 | -23 | -22 | | Unit | Unit |
| Chlormequat | 4.1 | Positive | 122 > 58 | -28 | -27 | -21 | Chlormequat d4 | Unit | Unit |
| | | | 122 > 59 | -28 | -23 | -21 | | Unit | Unit |
| | | | 122 > 63 | -28 | -22 | -23 | | Unit | Unit |
| Daminozide | 2.2 | Positive | 161 > 143 | -16 | -14 | -25 | Chlormequat d4 | Unit | Unit |
| | | | 161 > 44 | -16 | -22 | -16 | | Unit | Unit |
| | | | 161 > 45 | -16 | -23 | -16 | | Unit | Unit |
| Diquat | 4.0 | Positive | 183 > 157 | -12 | -21 | -27 | Paraquat d8 | Unit | Unit |
| | | | 183 > 78 | -12 | -39 | -12 | | Unit | Unit |
| | | | 183 > 130 | -12 | -34 | -22 | | Unit | Unit |
| Kasugamycine | 7.8 | Positive | 380 > 112 | -18 | -20 | -18 | Chlormequat d4 | Unit | Unit |
| | | | 380 > 200 | -18 | -13 | -20 | | Unit | Unit |
| Mepiquat | 4.5 | Positive | 114 > 98 | -22 | -29 | -15 | Mepiquat d3 | Unit | Unit |
| | | | 114 > 58 | -22 | -26 | -21 | | Unit | Unit |
| | | | 114 > 42 | -22 | -45 | -14 | | Unit | Unit |
| Paraquat | 9.0 | Positive | 186 > 171 | -12 | -20 | -30 | Paraquat d8 | Unit | Unit |
| | | | 186 > 77 | -12 | -45 | -27 | | Unit | Unit |
| | | | 186 > 169 | -12 | -35 | -29 | | Unit | Unit |
| Trimesium | 5.1 | Positive | 77 > 62 | -13 | -21 | -22 | Paraquat d8 | Unit | Unit |
| | | | 77 > 47 | -13 | -27 | -17 | | Unit | Unit |
| | | | 77 > 45 | -13 | -45 | -16 | | Unit | Unit |
| Chlormequat d4 | 4.1 | Positive | 126 > 58 | -21 | -29 | -21 | | Unit | Unit |
| Mepiquat d3 | 4.5 | Positive | 117 > 101 | -20 | -29 | -18 | | Unit | Unit |
| Paraquat d8 | 9.0 | Positive | 193 > 178 | -13 | -21 | -30 | | Unit | Unit |

**Table 3. Method 2 MS acquisition parameters, retention time and internal standard**

| Compound | Ret. time (min) | Polarity | SRM transitions | Q1 (V) | CE | Q3 (V) | ISTD | MS1 Res. | MS2 Res. |
|---|---|---|---|---|---|---|---|---|---|
| Glyphosate | 3.7 | Positive | 170 > 88 | -17 | -9 | -18 | Glyphosate C13 | Unit | Unit |
| | | | 170 > 42 | -17 | -26 | -17 | | Unit | Unit |
| | | | 170 > 60 | -17 | -16 | -24 | | Unit | Unit |
| Gluphosinate | 2.9 | Positive | 182 > 136 | -12 | -11 | -26 | Maleic hydrazide d2 | Unit | Unit |
| | | | 182 > 56 | -12 | -24 | -23 | | Unit | Unit |
| | | | 182 > 119 | -12 | -19 | -23 | | Unit | Unit |
| ETU | 3.1 | Positive | 103 > 44 | -19 | -18 | -15 | ETU d4 | Unit | Unit |
| | | | 103 > 60 | -19 | -28 | -23 | | Unit | Unit |
| | | | 103 > 86 | -19 | -21 | -28 | | Unit | Unit |
| Fosethyl | 9.9 | Negative | 109 > 81 | 23 | 13 | 29 | Fosethyl d15 | Unit | Unit |
| | | | 109 > 63 | 23 | 25 | 23 | | Unit | Unit |
| | | | 109 > 79 | 23 | 24 | 28 | | Unit | Unit |
| Maleic hydrazide | 13.7 | Positive | 113 > 40 | -11 | -27 | -16 | Maleic hydrazide d2 | Unit | Unit |
| | | | 113 > 67 | -11 | -19 | -27 | | Unit | Unit |
| | | | 113 > 85 | -11 | -17 | -17 | | Unit | Unit |
| Nicotine | 2.0 | Positive | 163 > 130 | -16 | -21 | -22 | Nicotine d3 | Unit | Unit |
| | | | 163 > 117 | -16 | -25 | -20 | | Unit | Unit |
| | | | 163 > 132 | -16 | -17 | -23 | | Unit | Unit |
| Perchlorate | 30.1 | Negative | 99 > 83 | 22 | 26 | 30 | Perchlorate 18O4 | Unit | Unit |
| | | | 99 > 67 | 22 | 37 | 23 | | Unit | Unit |
| | | | 101 > 85 | 22 | 26 | 30 | | Unit | Unit |
| PTU | 3.1 | Positive | 117 > 58 | -20 | -16 | -19 | ETU d4 | Unit | Unit |
| | | | 117 > 60 | -20 | -29 | -20 | | Unit | Unit |
| | | | 117 > 72 | -12 | -22 | -26 | | Unit | Unit |
| ETU d4 | 3.0 | Positive | 107 > 48 | -18 | -19 | -16 | | Unit | Unit |
| Fosethyl d5 | 9.6 | Negative | 114 > 82 | 24 | 15 | 30 | | Unit | Unit |
| Maleic hydrazide d2 | 13.6 | Positive | 115 > 42 | -11 | -20 | -17 | | Unit | Unit |
| Glyphosate 13C2 15N | 3.6 | Positive | 173 > 91 | -11 | -8 | -19 | | Unit | Unit |
| Nicotine d3 | 1.7 | Positive | 166 > 130 | -30 | -22 | -21 | | Unit | Unit |
| Perchlorate 18O4 | 30.1 | Negative | 107 > 89 | 23 | 27 | 30 | | Unit | Unit |

## ■ Results and Discussion

Following evaluation of several different analytical columns, mobile phases and mass spectrometer settings, two methods were developed for a range of highly polar pesticides that typically require single residue methods to analyse. A ZIC-HILIC column, a zwitterionic stationary phase covalently attached to porous silica, was used in method 1 to analyse the following; amitrole, chlormequat, daminozide, diquat, kasugamycine, mepiquat, paraquat and trimesium. While a Hypercarb PGC (porous graphitic carbon), which behaves as a strongly retentive alkyl-bonded silica gel, was used in method 2 to analyse the following; glufosinate, glyphosate, ethephon, fosethyl aluminium, maleic hydrazide, perchlorate, ETU, PTU, and nicotine.

Three MRM transitions were acquired for each analyte, with exception of two transitions for kasugamycine. Linearity was evaluated for all compounds in the range 0.005 mg/kg – 0.2 mg/kg (5 – 200 ppb) in apple matrix. The concentration of each calibration level is listed in the experimental section. All seven target compounds achieved excellent correlation coefficients greater than $R^2>0.9975$, using internal standards for quantitation, linear fit and 1/C weighting. Calibration curves for several compounds are displayed in Figure 3 (using LC method 1) and Figure 5 (using LC method 2). The linearity results for all target compounds is listed in Table 4.
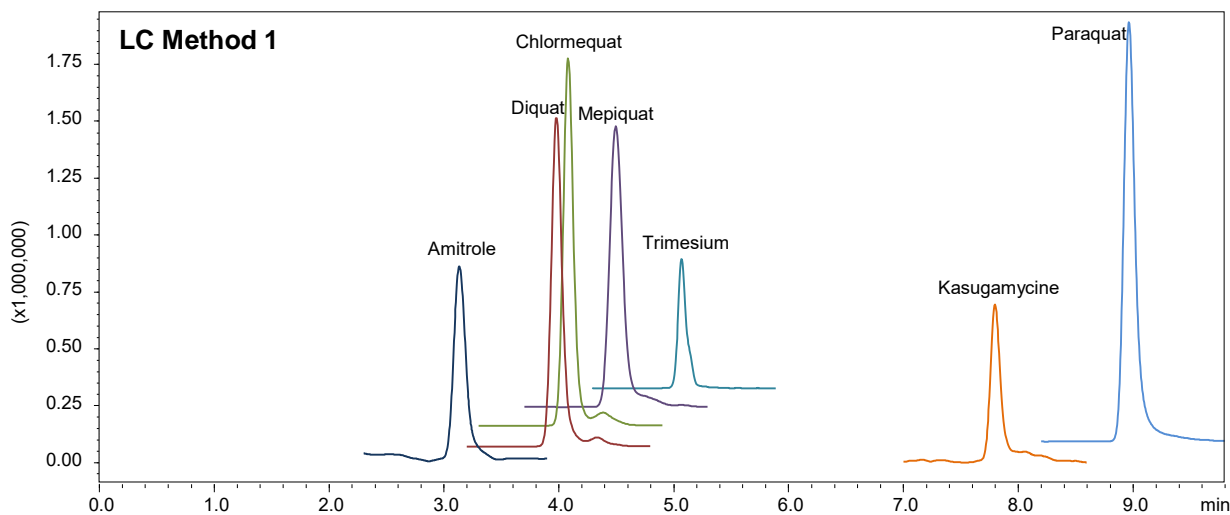


**Figure 2 . Target analytes at 0.05mg/kg in apple matrix using a ZIC-HILIC based separation (LC Method 1)**



**Mepiquat**
y = 0.0247140x + 0.000651611
R² = 0.99994
Fit; linear, weighting 1/C, zero not forced

**Diquat**
y = 0.0379254x + 0.0483990
R² = 0.9986
Fit; linear, weighting 1/C, zero not forced

**Trimesium**
y = 0.0137501x - 0.0197801
R² = 0.9982
Fit; linear, weighting 1/C, zero not forced

**Paraquat**
y = 0.106951x - 0.0114163
R² = 0.9996
Fit; linear, weighting 1/C, zero not forced
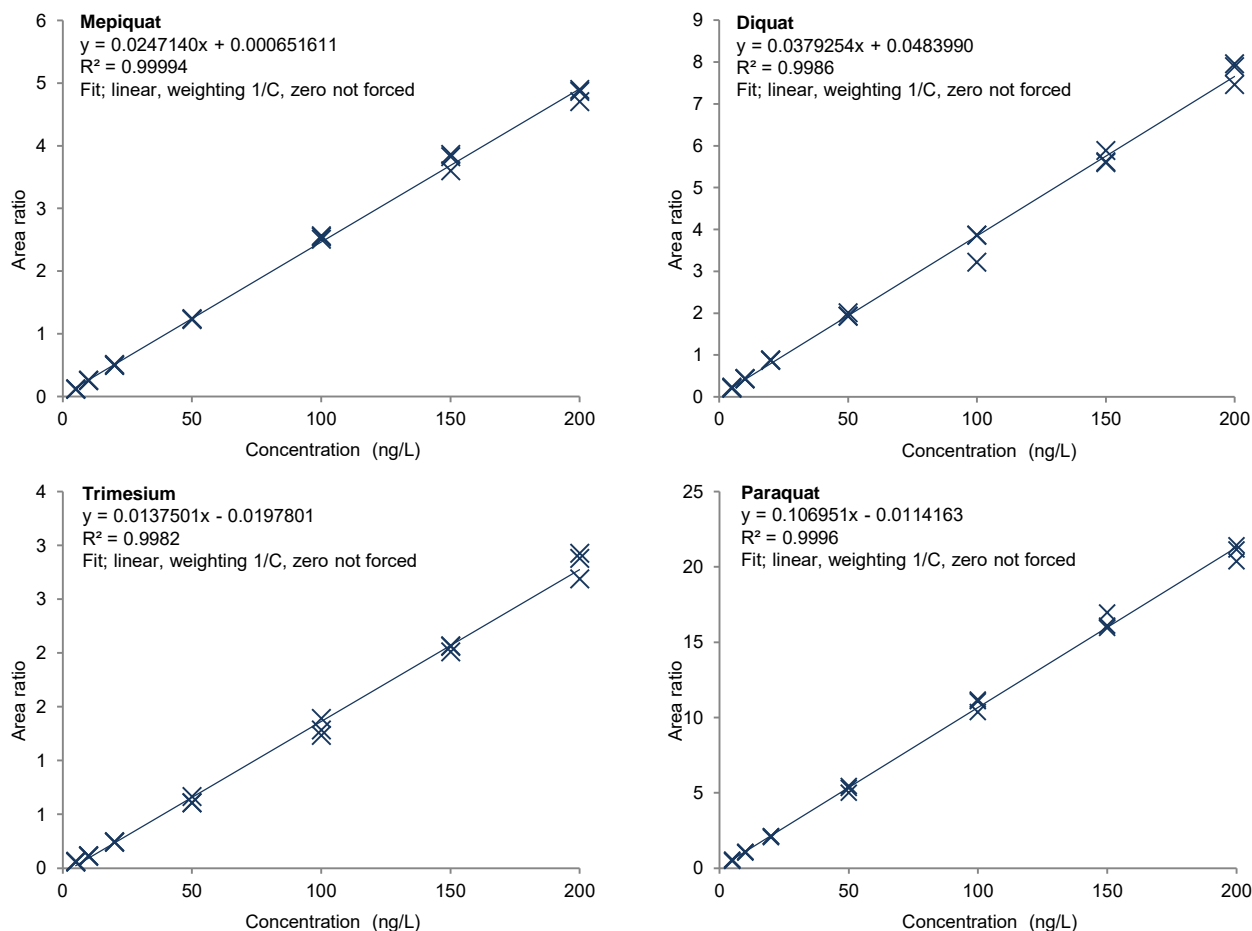
**Figure 3. Calibration curves for paraquat, mepiquat, trimesium and diquat using a ZIC-HILIC based separation (LC Method 1)**

Figure 2 displays a chromatogram of each compound at 0.05 mg/kg using a ZIC-HILIC based separation (LC method 1) and Figure 4 display a chromatogram using a Hypercarb PGC based separation (LC method 2). All target analytes were identified at 0.01 mg/kg. This concentration is below the European Union (EU) maximum residue limit (MRL) for all of the target analytes in this study. For example, the EU MRL for the following compounds in the majority of commodities is; glyphosate 0.1 mg/kg, glufosinate 0.1 mg/kg, chlormequat 0.05 mg/kg, paraquat 0.02, mepiquat 0.05 mg/kg, daminozide 0.02 mg/kg and ethephon 0.05 mg/kg.[2] Consequently, the sensitivity achieved in these methods is far below what is required and therefore dilution of sample extracts is possible in order to reduce matrix effects.
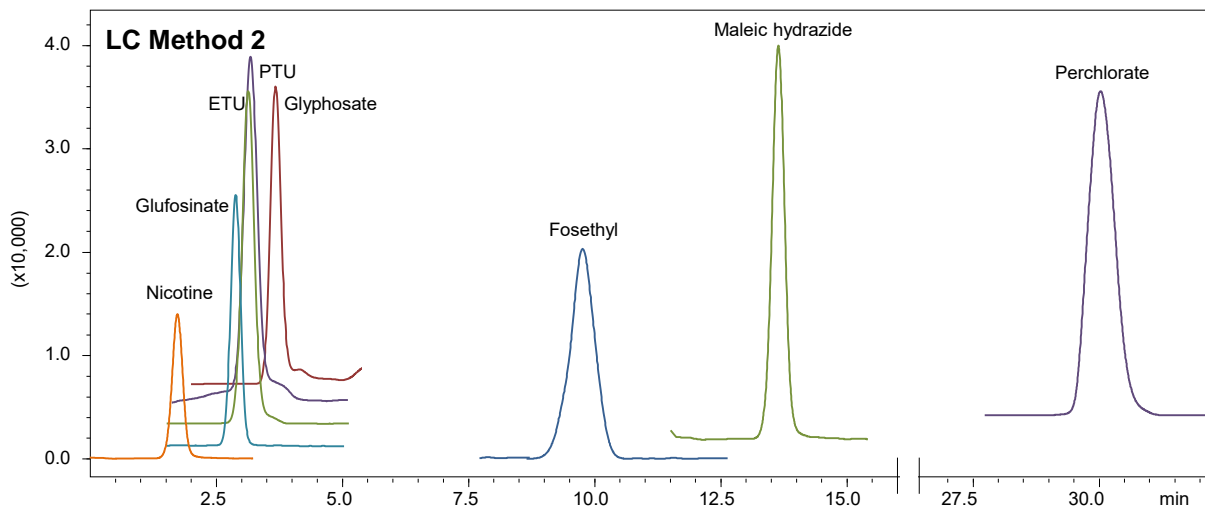


**Figure 4. Target analytes at 0.05mg/kg in apple matrix using a Hypercarb PGC based separation (LC Method 2).**



**Glyphosate**
y = 0.0119135x + 0.00216382
R² = 0.99830
Fit; linear, weighting 1/C, zero not forced

**Glufosinate**
y = 0.175785x + 0.0177350
R² = 0.99935
Fit; linear, weighting 1/C, zero not forced

**Maleic hydrazide**
y = 0.0126499x + 0.00914840
R² = 0.99823
Fit; linear, weighting 1/C, zero not forced

**Fosethyl**
y = 0.0236968x + 0.0488563
R² = 0.99752
Fit; linear, weighting 1/C, zero not forced
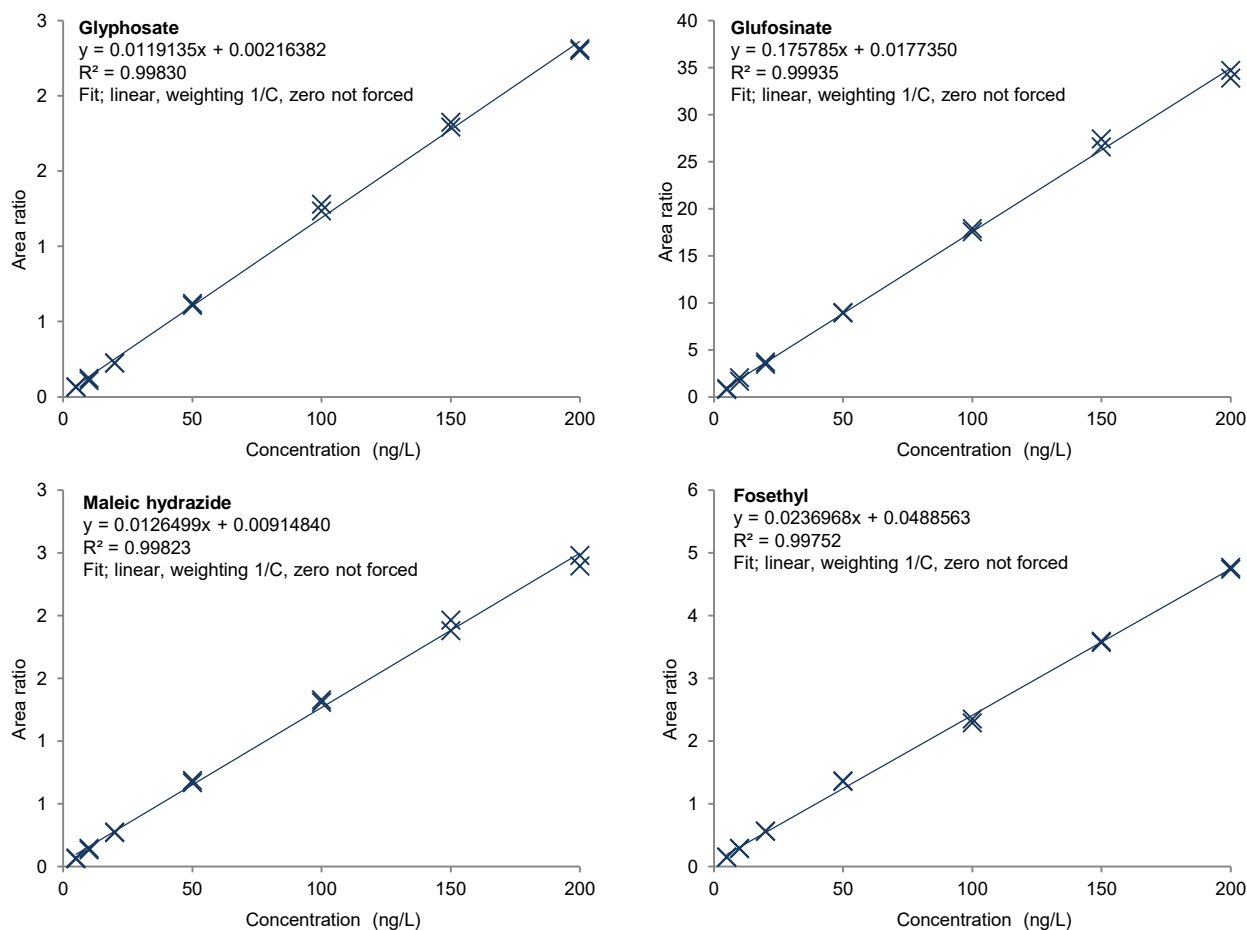
**Figure 5. Calibration curves for glyphosate, gluphosinate, maleic hydrazide and fosethyl using Hypercarb PGC based separation (LC Method 2)**

## ■ Conclusion

Two LC-MS/MS methods were developed for the measurement of a range of highly polar pesticides in their underivitised state using the LCMS-8050 triple quadrupole mass spectrometer. The developed multi-residue methods offer significant time savings in comparison to single residue methods typically used for analyse of these analytes. All compounds were quantified in the range 0.005 – 0.2 mg/kg with correlation coefficients greater than 0.997. The excellent sensitivity achieved, which is most cases is far below the EU MRL, offers the opportunity to dilute sample extracts prior to LC-MS/MS injection in order to reduce matrix effects.

## ■ References

1. Reference Laboratory for pesticides requiring Single Residue Methods (EURL-SRM). Quick Method for the Analysis of Residues of numerous Highly Polar Pesticides in Foods of Plant Origin involving Simultaneous Extraction with Methanol and LC-MS/MS Determination (QuPPe-Method). 2012. Version 7

2. Commission Regulation (EC). 2005. No 396/2005 of the European Parliament and of the Council, maximum residue levels of pesticides in or on food and feed of plant and animal origin. Official Journal of the European Union, L 70: 1-16. http://ec.europa.eu/sanco_pesticides/public/index.cfm?event=homepage&language=EN

**Table 4. Target analytes linearity results using LC method 1 and LC method 2**

| Compound | $R^2$ | Fit type | Weight | Method |
|---|---|---|---|---|
| Diquat | 0.9986 | Linear | 1/C | Method 1 |
| Chlormequat | 0.9988 | Linear | 1/C | Method 1 |
| Amitrole | 0.9981 | Quadratic | 1/C | Method 1 |
| Kasugamycine | 0.9992 | Linear | 1/C | Method 1 |
| Daminozide | 0.9995 | Quadratic | 1/C | Method 1 |
| Mepiquat | 0.9993 | Linear | 1/C | Method 1 |
| Paraquat | 0.9995 | Linear | 1/C | Method 1 |
| Trimesium | 0.9981 | Linear | 1/C | Method 1 |
| ETU | 0.9998 | Linear | 1/C | Method 2 |
| Fosethyl | 0.9975 | Linear | 1/C | Method 2 |
| Gluphosinate | 0.9993 | Linear | 1/C | Method 2 |
| Glyphosate | 0.9983 | Linear | 1/C | Method 2 |
| Maleic hydrazide | 0.9982 | Linear | 1/C | Method 2 |
| Nicotine | 0.9984 | Linear | 1/C | Method 2 |
| Perchlorate | 0.9998 | Linear | 1/C | Method 2 |
| PTU | 0.9991 | Linear | 1/C | Method 2 |

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# SHIMADZU
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# Application News

## No.C133

**Liquid Chromatography Mass Spectrometry**

## Simultaneous Analysis of 16 Sweeteners Using Triple Quadrupole LC/MS/MS [LCMS-8050]

Artificial sweeteners such as aspartame, sucralose, and acesulfame potassium fall under the category of designated additives according to Japan's Food Sanitation Act, and prescribed standards are in place for their use in some foods and quantities.

Cyclamate and other artificial sweeteners used in some regions outside Japan are included among undesignated additives in Japan, and inspection is required in specific imported foods.

Consequently, quantitation for large numbers of sweeteners, including not only permitted in Japan but also undesignated, are needed.

Application News C121 described the simultaneous analysis of nine artificial sweeteners including both designated and undesignated additives using an LCMS-8040 triple quadrupole LC/MS/MS system. In this article, we introduce an example of simultaneous analysis of 16 sweeteners using an LCMS-8050.

### ■ Standard Mixture Analysis

MRM analysis was performed on 16 sweeteners using the analytical conditions shown in Table 1. Chromatograms of each compound near their lower limit of quantitation are shown in Fig. 1, with calibration curve ranges and correlation coefficients shown in Table 2. Results that met an accuracy of 100 % ±20 % and area repeatability (%RSD) of within 20 % were used for calibration point. Good linearity was obtained for all compounds, with correlation coefficients of 0.997 or higher.

**Table 1  Analytical Conditions**

| | | | |
|---|---|---|---|
| Column | : Unison UK-C18 (150 mm L. × 3.0 mm I.D., 3.0 µm) | Injection Volume | : 1 µL |
| Mobile Phases | : A 5 mmol/L Ammonium formate - Water | Probe Voltage | : + 4.0 kV (ESI-positive mode) / -3.0 kV (ESI-negative mode) |
| | : B 5 mmol/L Ammonium formate - Methanol | Nebulizing Gas Flow | : 3 L/min |
| Gradient | : B.Conc. 0 % (0.0-2.0 min) | Heating Gas Flow | : 10 L/min |
| | → 70 % (4.5 min) → 90 % (8.0-12.0 min) | Interface Temperature | : 300 °C |
| | → 0 % (12.01-15.0 min) | DL Temperature | : 150 °C |
| Flowrate | : 0.4 mL/min | Block Heater Temperature | : 250 °C |
| Column Temperature | : 40 °C | Drying Gas Flow | : 10 L/min |



**Fig. 1-1  Chromatograms of 16 Sweeteners**
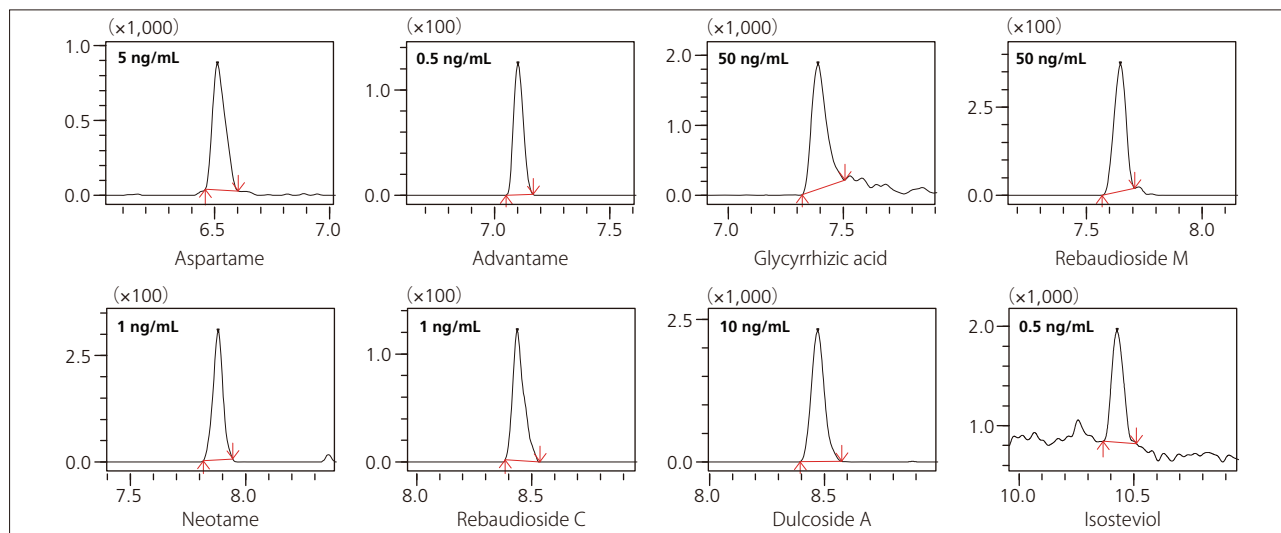
Fig. 1-2  Chromatograms of 16 Sweeteners (continued)

Table 2  Linearity of 16 Sweeteners

| Compound Name | Polarity | Transition | Holding Time (min) | Calibration Curve Range (ng/mL) | | | Correlation Coefficient |
|---|---|---|---|---|---|---|---|
| Sucralose | + | 414.00>199.10 | 6.36 | 0.5 | - | 100 | 0.999 |
| Dulcin | + | 181.20>108.10 | 6.70 | 0.05 | - | 10 | 0.999 |
| Alitame | + | 332.20>129.00 | 6.92 | 0.5 | - | 100 | 0.999 |
| Rebaudioside A | + | 984.50>325.10 | 8.21 | 0.5 | - | 100 | 0.999 |
| Stevioside | + | 822.00>319.30 | 8.23 | 0.5 | - | 100 | 0.999 |
| Acesulfame potassium | − | 161.90>82.00 | 5.23 | 0.1 | - | 10 | 0.999 |
| Saccharin | − | 181.90>42.00 | 5.58 | 0.5 | - | 50 | 0.997 |
| Cyclamate | − | 178.00>80.00 | 6.08 | 1 | - | 100 | 0.999 |
| Aspartame | − | 293.40>261.10 | 6.53 | 5 | - | 100 | 0.999 |
| Advantame | − | 457.30>200.30 | 7.12 | 0.5 | - | 100 | 0.999 |
| Glycyrrhizic acid | − | 821.20>351.10 | 7.41 | 50 | - | 1000 | 0.999 |
| Rebaudioside M | − | 1289.60>802.90 | 7.66 | 50 | - | 1000 | 0.999 |
| Neotame | − | 377.30>200.00 | 7.90 | 1 | - | 100 | 0.999 |
| Rebaudioside C | − | 949.50>787.20 | 8.46 | 1 | - | 100 | 0.999 |
| Dulcoside A | − | 787.50>625.20 | 8.50 | 10 | - | 1000 | 0.999 |
| Isosteviol | − | 317.30>317.30 | 10.46 | 0.5 | - | 1000 | 0.999 |

### ■ Recovery from Real World Samples

Sweeteners were added to sample solutions prepared according to the procedure shown in Fig. 2, and recovery of these additives was verified by measuring the samples after 100-fold or 1000-fold dilution. The results are shown in Table 3.

Dialysis and solid phase extraction are common methods used in sample pretreatment for sweetener analysis, but these operations have the drawback of being complex, time-consuming, and laborious. Pretreatment by solvent extraction requires no special equipment, and can be performed quickly and simply.

Table 3  Recovery

| Compound Name | Additive Concentration | Real World Sample | Dilution Ratio | Recovery (%) |
|---|---|---|---|---|
| Glycyrrhizic acid | 100 µg/mL | Soy sauce | 100 | 85.20 |
| Acesulfame potassium | 10 µg/mL | Powdered soft drink (café au lait) | 1000 | 81.21 |
| Aspartame | | | | 104.2 |
| Neotame | 10 µg/mL | Ketchup | 100 | 108.5 |
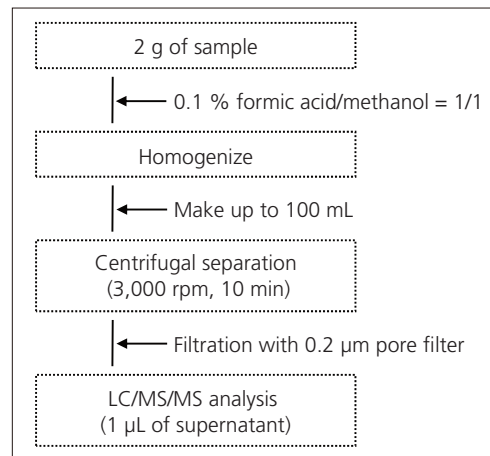


Fig. 2  Pretreatment Workflow

This Application News was prepared with the cooperation of Japan Food Research Laboratories, who provided samples and guidance.

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# Application News

## No.C121

**Liquid Chromatography Mass Spectrometry**

## Simultaneous Analysis of Nine Sweeteners Using Triple Quadrupole LC/MS/MS (LCMS-8040)

Artificial sweeteners such as saccharin sodium, aspartame, sucralose and acesulfame potassium fall under the category of specified additives in Japan's Food Sanitation Act, for which each specified criteria exist for their use in terms of eligible foods and amounts used.

Cyclamate, an artificial sweetener used in some regions of the world outside Japan, is an unspecified additive within Japan, for which inspection is required on specific imported foods.

In light of these situations, there is a demand for analyses of various different sweeteners, not only the quantitative testing of permitted sweeteners but also the testing of unspecified sweetener additives.

This article presents a simultaneous analysis of nine sweeteners including both specified additives and unspecified additives, using the LCMS-8040 high-performance liquid chromatograph-triple quadrupole mass spectrometer.

### ■ Analysis of a Standard Mixture

Fig. 1 shows chromatograms measured from a 5 µL injected sample of a 10 ng/mL standard mixture of nine sweeteners, analyzed with the analytical conditions shown in Table 1. Chromatograms at around the lower limit of quantitation (LLOQ) are shown in Fig. 2. The retention time, calibration curve range, and correlation coefficient for each compound are shown in Table 2.

A calibration point accuracy of within 100 ± 20 % and a percentage of area repeatability (%RSD) of within 20 % were employed. Good linearity was obtained for all compounds with a correlation coefficient of 0.997 or higher.
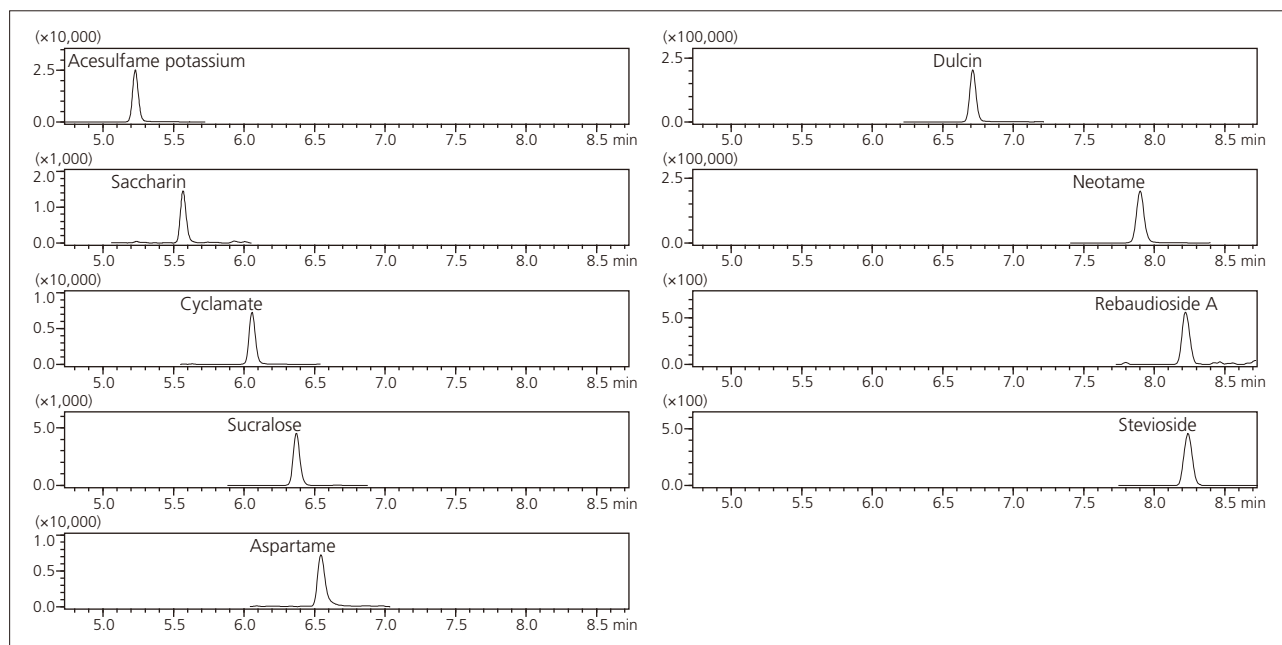


**Fig. 1  Chromatograms from a 10 ng/mL Standard Mixture of Nine Sweeteners**

**Table 1  Analytical Conditions**

| | |
|---|---|
| Column | : Unison UK-C18 (150 mm L. × 3.0 mm I.D., 3.0 µm) |
| Mobile Phases | : A 5 mmol/L Ammonium Formate - Water |
| | : B Methanol |
| Gradient | : B Conc. 0 % (0.0 - 2.0 min) → 70 % (4.5 min) → 90 % (8.0 - 12.0 min) → 0 % (12.01-15.0 min) |
| Flowrate | : 0.2 mL/min |
| Column Temperature | : 40 ℃ |
| Injection Volume | : 5 µL |
| Probe Voltage | : ＋ 4.5 kV (ESI-positive mode) / -3.5 kV (ESI-negative mode) |
| DL Temperature | : 300 ℃ |
| Block Heater Temperature | : 500 ℃ |
| Nebulizing Gas Flow | : 3 L/min |
| Drying Gas Flow | : 15 L/min |

**Fig. 2  Chromatograms of Nine Sweeteners at Around LLOQ**

**Table 2  Linearity of Nine Sweeteners**

| Compound Name | Polarity | Transition | Retention Time (min) | Calibration Curve Range (ng/mL) | | | Correlation Coefficient |
|---|---|---|---|---|---|---|---|
| Acesulfame potassium | – | 162.00 > 82.10 | 5.228 | 0.05 | – | 100 | 0.997 |
| Saccharin | – | 182.00 > 42.00 | 5.561 | 1 | – | 100 | 0.999 |
| Cyclamate | – | 178.00 > 80.00 | 6.057 | 1 | – | 100 | 0.998 |
| Sucralose | + | 413.90 > 199.00 | 6.370 | 0.5 | – | 500 | 0.999 |
| Aspartame | – | 293.10 > 261.10 | 6.543 | 0.5 | – | 1000 | 0.999 |
| Dulcin | + | 181.20 > 108.10 | 6.712 | 0.05 | – | 10 | 0.999 |
| Neotame | + | 379.10 > 172.20 | 7.898 | 0.05 | – | 1000 | 0.999 |
| Rebaudioside A | – | 965.30 > 803.40 | 8.220 | 5 | – | 1000 | 0.999 |
| Stevioside | + | 822.30 > 319.20 | 8.238 | 5 | – | 1000 | 0.999 |

■ **Recovery from Actual Samples**

Seven sweeteners were added to foods (curry paste, rice cake flavored with mugwort, and sponge cake) pretreated by dialysis (Fig. 3), and the matrix effect was evaluated. The recovery of each added sweetener is shown in Table 3. Dulcin was the only sweetener for which the recovery was calculated based on a 1000-fold dilution of the solution after dialysis treatment, while the recovery of all other sweetener samples was calculated based on 100-fold dilution. The recovery was good with all samples, ranging from 85 to 125 %.

**Table 3  Recovery of Seven Added Sweeteners**

| Compound Name | Added Concentration | Recovery (%) | | |
|---|---|---|---|---|
| | | Curry Paste | Rice Cake Flavored with Mugwort | Chocolate Sponge Cake |
| Acesulfame potassium | | 100.8 | 94.2 | 93.7 |
| Saccharin | | 97.0 | 87.7 | 88.3 |
| Cyclamate | | 99.6 | 89.3 | 92.0 |
| Sucralose | 5 µg/mL | 96.2 | 89.6 | 82.6 |
| Aspartame | | 94.0 | 89.4 | 87.2 |
| Dulcin | | 110.2 | 99.5 | 99.5 |
| Neotame | | 122.5 | 106.9 | 110.0 |



```
┌─────────────────────────┐
│  20 g sample            │
└─────────────────────────┘
         ↓
┌─────────────────────────┐
│  Dialysis (24 hours)    │
└─────────────────────────┘
         ↓
┌─────────────────────────┐
│  Solution after dialysis│
└─────────────────────────┘
         ↓ 100-fold or 1000-fold dilution
┌─────────────────────────┐
│  LC/MS/MS analysis      │
└─────────────────────────┘
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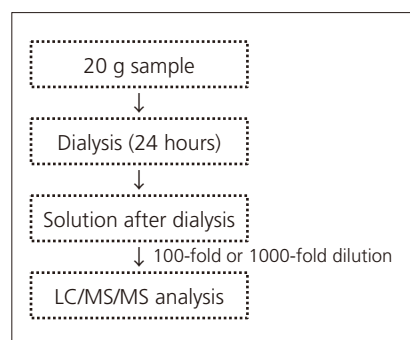
**Fig. 3  Workflow of Pretreatment**

This Application News was prepared with the cooperation of Tokyo Food Sanitation Association, who provided samples and guidance.

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# Determination of Avermectin Drug Residues in Vinegar Using LCMS-8045

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Shimadzu (China), Shanghai Analysis Center

## Abstract

This application news describes a method developed for determination of avermectin drug residues in vinegar using Shimadzu's ultra-high performance liquid chromatograph (UHPLC) LC-30A coupled with triple quadrupole mass spectrometer LCMS-8045. The analysis was completed within 6 minutes and the external standard quantification showed good linearity with a correlation coefficient above 0.997. Samples of low, medium and high concentrations were tested in 6 replicates. The relative standard deviations of retention time and peak area were 0.02 to 0.09% and 0.66 to 4.57%, respectively, showing good precision. The limit of detection and limit of quantitation of four avermectin drugs ranged from 0.22 to 0.25 ng/mL and 0.75 to 0.83 ng/mL respectively.

The main varieties of avermectin drugs include avermectin, eprinomectin, doramectin, and ivermectin. Due to their excellent insect repellent properties, these drugs are the widely used as anti-parasitic drugs. Although avermectin drugs are pesticides derived from microorganisms, their $LD_{50}$ in rats is 10 mg/kg, which is similar to that of thiophos pesticides. Therefore, the World Health Organization lists avermectin drugs as highly toxic compounds. The main methods currently used for detection of avermectin drugs include liquid chromatography-ultraviolet detection, liquid chromatography-fluorescence detection, and enzyme-linked immunosorbent assay (ELISA).

In recent years, many reports have used high performance liquid chromatography-tandem mass pectrometry assays in detection of avermectin drug residues. Both ESI and APCI ion sources can be used for LC-MS/MS analysis of avermectin drugs. The limit of detection of four avermectin drugs, avermectin, eprinomectin, doramectin, and ivermectin, specified in China's national standard GB/T 21320-2008 "Determination of Avermectin Residues in Animal-Derived Food Using Liquid Chromatography-Tandem Mass Spectrometry" is 1.5 µg/kg with an ESI source. In the People's Republic of China's entry and exit inspection and quarantine industry standard, SN/T 1973-2007 "Detection of Avermectin Residues in Import and Export Food Using High Performance Liquid Chromatography-Mass Spectrometry/Mass Spectrometry", the limit of detection of avermectin is 5 µg/kg with an APCI source. When the positive ion scanning mode of ESI is used for analysis, precursor ions of avermectin drugs are more likely to be detected in the form of $[M+Na]^+$ ions. However, if the sodium content in extraction matrix is low or if only a trace amount of sodium ions is present in the extraction liquid due to the selected extraction method, a poor linear relationship tends to be observed when the detection is carried out in the form of $[M+Na]^+$ ions. For this reason, some literature has suggested using the negative ion mode of APCI to detect the precursor ion $[M-H]^-$ to acquire a better linear relationship. Shimadzu China has already published an application report (report No.: AP_News_LCMSMS-050) for LC-MS/MS analysis of avermectin drugs using the ESI source.

In this article, in reference to SN/T 1973-2007 and GB/T 21320-2008, Shimadzu's UHPLC LC-30A coupled with the Triple Quadrupole Mass Spectrometer LCMS-8045 and an APCI source was used to establish a highly sensitive and rapid method for detection of avermectin drug residues in vinegar. The results were superior to the requirements of the above standards and can be used as a reference method by relevant personnel.

## EXPERIMENTAL

### Instrumentation

The experiment employed Shimadzu's ultra-high performance liquid chromatograph (UHPLC) LC-30A and triple quadrupole mass spectrometer LCMS-8045. The configurations are two LC-30AD pumps, DGU-20A$_5$ online degassing unit, SIL-30AC autosampler, CTO-30A column oven, CBM-20A system controller, LCMS-8045 triple quadrupole mass spectrometer and LabSolutions Ver. 5.86 chromatography workstation.

### Analytical Conditions

#### Liquid Chromatography (LC) Conditions

| | | |
|---|---|---|
| Column | : | Shim-pack XR-ODS (3.0 mm I.D.×75 mm L., 2.2 µm) |
| Mobile phase | : | Solvent A - 5 mM ammonium acetate in water Solvent B - methanol |
| Flow rate | : | 1.20 mL/min |
| Column Temp. | : | 40 °C |
| Injection volume | : | 20 µL |
| Elution method | : | Gradient elution with initial concentration of mobile phase B at 75%. Refer to Table 1 for time program. |

**Table 1 Time program**

| Time (min) | Module | Command | Value (%) |
|---|---|---|---|
| 3.00 | Pumps | Pump B Conc. | 100 |
| 4.40 | Pumps | Pump B Conc. | 100 |
| 4.50 | Pumps | Pump B Conc. | 75 |
| 6.00 | Controller | Stop | |

### Mass Spectrometry (MS) Conditions

| | | |
|---|---|---|
| Analytical Instrument | : | LCMS-8045 |
| Ionization mode | : | APCI(-) |
| Ionization Voltage | : | -4.5 kV |
| Nebulizer gas | : | Nitrogen 3.0 L/min |
| Drying gas | : | Nitrogen 6.0 L/min |
| Collision gas | : | Argon |
| Probe temp. | : | 350 °C |
| DL temp. | : | 100 °C |
| Block Heater temp. | : | 200 °C |
| Mode | : | Multiple reaction monitoring (MRM) |
| Dwell time | : | 40 ms |
| Pause time | : | 3 ms |
| MRM transitions | : | Refer to Table 2 |

### Standard Solution Preparation

Mixed standard stock solution at a concentration of 1 mg/mL was prepared using acetonitrile and was subsequently diluted with acetonitrile to make a series of standard working solutions at concentrations of 1, 2, 5, 10, 20, 50, 100, 200, and 400 ng/mL.

### Sample Preparation Method

Sample was prepared in accordance to the industry standard SN/T 1973-2007 "Detection of Avermectin Residues in Import and Export Food Using High Performance Liquid Chromatography-Mass Spectrometry/Mass Spectrometry".

**Table 2 MRM transition**

| No. | Analyte | Precursor Ion | Product Ion | Q1 Pre Bias (V) | CE (V) | Q3 Pre Bias (V) |
|---|---|---|---|---|---|---|
| 1 | Avermectin | 871.35 | 565.25* | 32.0 | 21.0 | 18.0 |
| | | | 229.20 | 46.0 | 40.0 | 50.0 |
| 2 | Eprinomectin | 912.35 | 565.35* | 20.0 | 29.0 | 18.0 |
| | | | 270.20 | 46.0 | 42.0 | 50.0 |
| 3 | Doramectin | 897.35 | 591.35* | 16.0 | 28.0 | 18.0 |
| | | | 229.20 | 44.0 | 43.0 | 46.0 |
| 4 | Ivermectin | 873.35 | 567.30* | 16.0 | 28.0 | 10.0 |
| | | | 229.15 | 44.0 | 40.0 | 50.0 |

## RESULTS AND DISCUSSION

**Q1 MS scan and Product Ion Scan of Standard Sample**

The Q1 MS scan and product ion scan of the avermectin drugs are shown in Figures 1-4.

**MRM Chromatogram of Standard Mixture**

The MRM chromatograms of standard samples of the four avermectin drugs are shown in Figure 5.
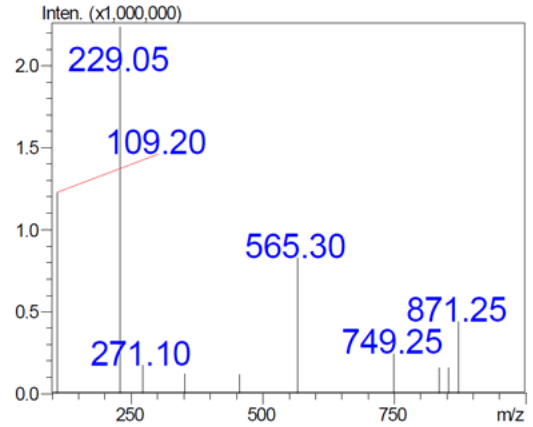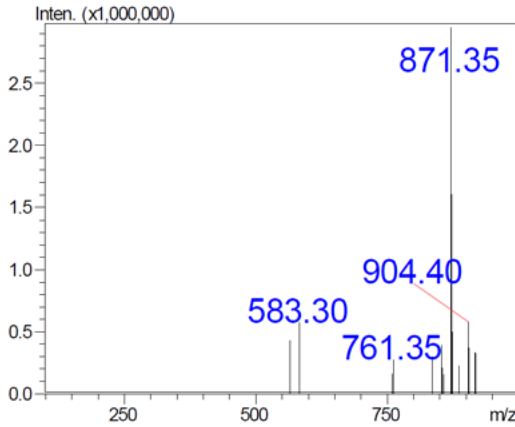


Figure 1 Q1 MS scan (left) and product ion scan (right, CE value is 40V) of avermectin
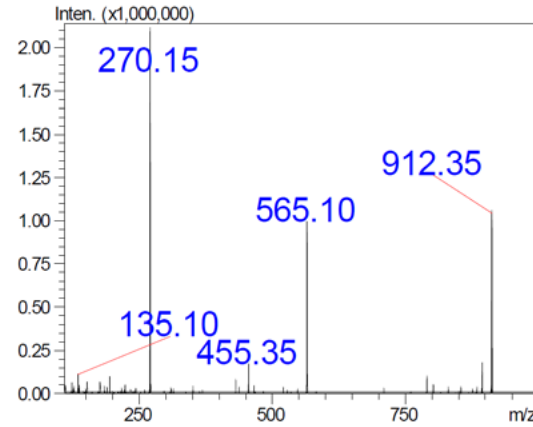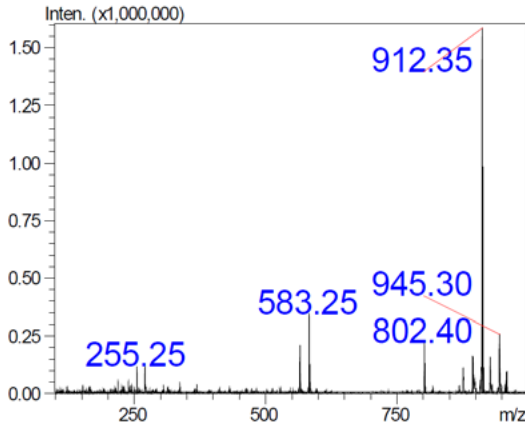


Figure 2 Q1 MS scan (left) and product ion scan (right, CE value is 40V) of eprinomectin
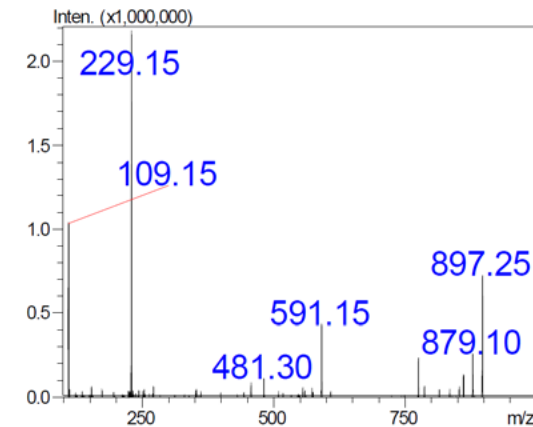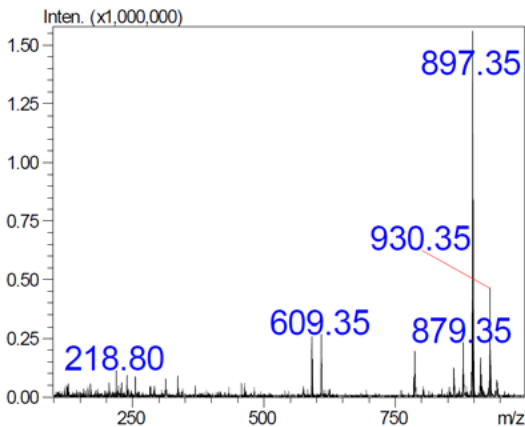


Figure 3 Q1 MS scan (left) and product ion scan (right, CE value is 40V) of doramectin
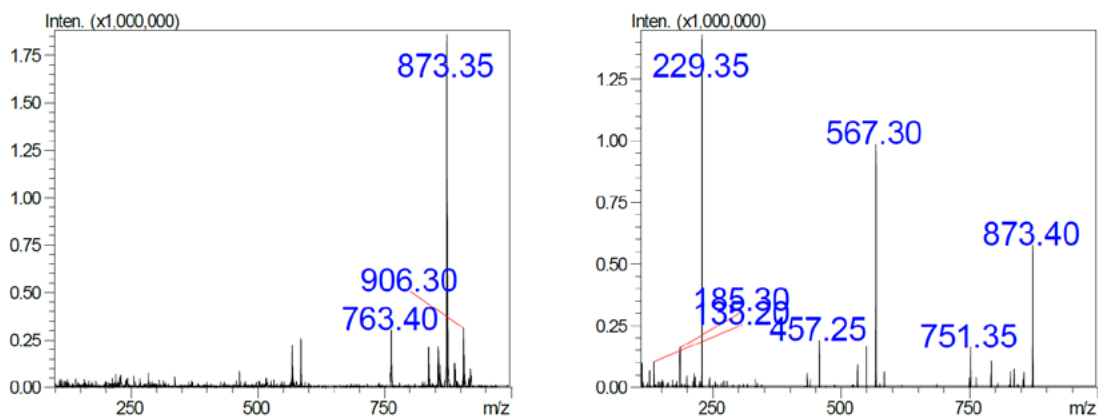
**Figure 4  Q1 MS scan (left) and product ion scan (right, CE value is 40V) of ivermectin**
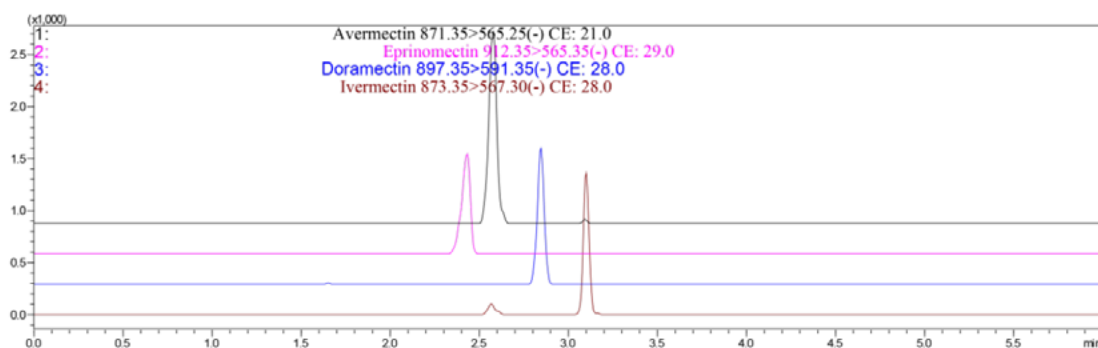


**Figure 5  MRM chromatograms of 1 ng/mL standard samples of the four avermectin drugs**

## Calibration and Linear Range

A series of standard solutions at concentrations of 1, 2, 5, 10, 20, 50, 100, 200, and 400 ng/mL were injected and analyzed based on conditions listed in the previous section. Calibration curves were established by the external standard method and the results are shown in Figures 6-9. The four avermectin drugs showed good linearity within the range of 1 to 400 ng/mL. Linear equation, linear range, and correlation coefficients are shown in Table 3.

## Limit of Detection and Limit of Quantitation

Sample solutions at a concentration of 1.0 ng/mL were injected and analyzed. A value equivalent to 3 times the noise was used as the lower limit of detection (i.e., S/N = 3, LOD), while the lower limit of quantitation was 10 times the noise (i.e., S/N=10, LOQ). Lower LOD and lower LOQ for the four avermectin drugs are shown in Table 4.

**Table 4  Limit of detection and limit of quantitation**

| Analyte | Limit of Detection (ng/mL) | Limit of Quantification (ng/mL) |
|---|---|---|
| Avermectin | 0.23 | 0.77 |
| Eprinomectin | 0.25 | 0.83 |
| Doramectin | 0.23 | 0.76 |
| Ivermectin | 0.22 | 0.77 |

**Table 3  Parameters for calibration curves of 4 avermectin drugs (weight coefficient: $1/C^2$)**

| Compound | Calibration Curve | Linear Range (ng/mL) | Accuracy (%) | Correlation Coefficient (r) |
|---|---|---|---|---|
| Avermectin | Y = (6631.81) X + (-448.237) | 1.00~400.00 | 0.9977 | 90.9~107.4 |
| Eprinomectin | Y = (4314.94) X + (-2460.15) | 1.00~400.00 | 0.9974 | 92.0~111.9 |
| Doramectin | Y = (4740.90) X + (-850.905) | 1.00~400.00 | 0.9986 | 91.5~105.4 |
| Ivermectin | Y = (5160.71) X + (-1877.62) | 1.00~400.00 | 0.9975 | 87.1~109.0 |
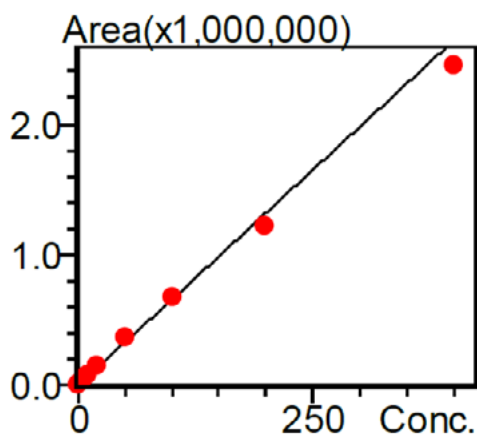
**Figure 6  The standard calibration curve of avermectin**
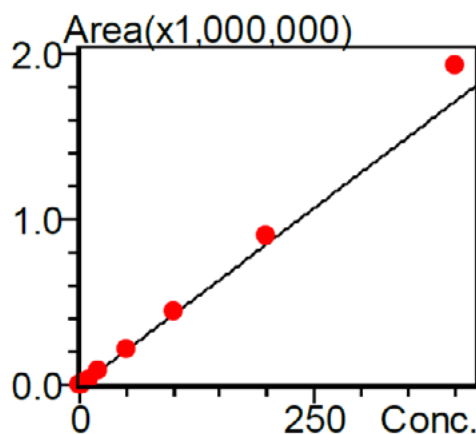


**Figure 7  The standard calibration curve of eprinomectin**
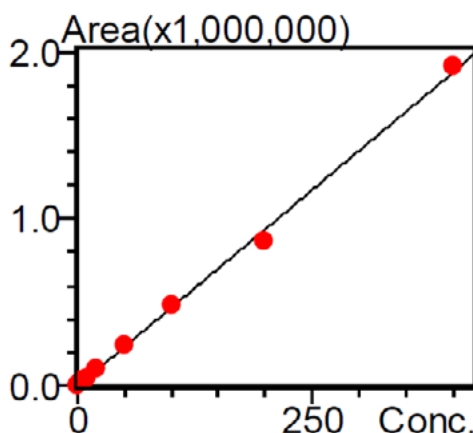


**Figure 8  The standard calibration curve of doramectin**
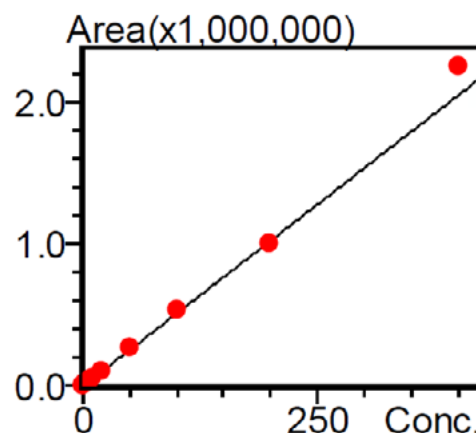


**Figure 9  The standard calibration curve of ivermectin**

**Precision Test**

After the mixed standard solutions at concentrations of 10 ng/mL, 100 ng/mL, and 200 ng/mL were analyzed by six consecutive injections, the relative standard deviations of retention time and peak area for the standards at three concentrations were 0.02 to 0.09% and 0.66 to 4.57%, respectively, indicating good precision.

**Matrix Effects**

Standard mixtures and spiked sample solution were prepared at low, medium and high concentrations. The matrix effects were assessed by comparing the peak area of the spiked sample solution with that of standard solution at the same concentration. If the obtained results of matrix effects are between 80% and 120%, the matrix effects showed minimal interference on detection of target substances. The experimental results are shown in Table 6. As seen from Table 6, the matrix effects showed minimal interference on detection of target substances.

**Table 5  Repeatability results of retention time and peak area (n=6)**

| Analyte | RSD% (10 ng/mL) | | RSD% (100 ng/mL) | | RSD% (200 ng/mL) | |
|---|---|---|---|---|---|---|
| | R.T. | Area | R.T. | Area | R.T. | Area |
| Avermectin | 0.03 | 2.79 | 0.02 | 3.18 | 0.02 | 0.66 |
| Eprinomectin | 0.09 | 4.57 | 0.02 | 1.31 | 0.05 | 1.30 |
| Doramectin | 0.05 | 3.76 | 0.02 | 2.68 | 0.02 | 1.30 |
| Ivermectin | 0.03 | 2.55 | 0.02 | 2.92 | 0.02 | 0.92 |
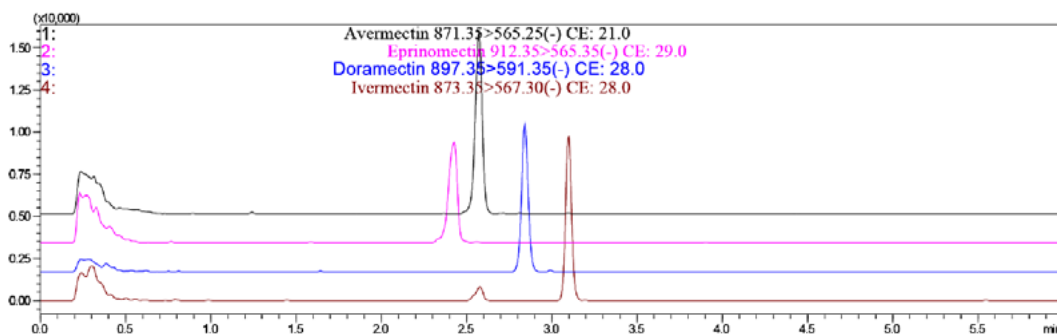
**Figure 10  MRM chromatogram of blank vinegar matrix**
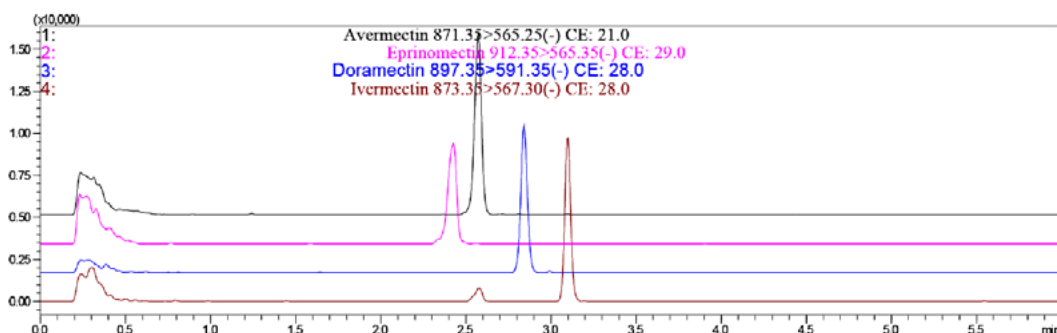


**Figure 11  MRM chromatogram of vinegar matrix spike solution (5 ng/mL)**

**Table 6 Matrix effects**

| Spiking Concentration (ng/mL) | Avermectin (%) | Eprinomectin (%) | Doramectin (%) | Ivermectin (%) |
|---|---|---|---|---|
| 10 | 103.8 | 105.7 | 117.1 | 109.7 |
| 100 | 84.5 | 91.7 | 101.1 | 90.6 |
| 200 | 94.7 | 82.0 | 118.4 | 100.4 |

**Matrix Recovery Test**

Mixed standards were first added to the blank vinegar samples. The pre-spiked samples were treated according to the method described previously, to give concentrations of pre-spiked samples of 10, 100, and 200 ng/mL.

For the preparation of the post-spiked samples, the blank vinegar sample matrix was first prepared according to the method described in the previous section. Mixed standards were then subsequently added into the blank vinegar matrix, so that the concentrations of post-spiked samples were 10, 100, and 200 ng/mL.

Both pre-spiked and post-spiked samples were injected and analyzed. The recovery was then calculated as the ratio between the peak area of pre-spiked samples and the peak area of post-spiked samples. The recovery rates obtained at various concentrations are shown in Table 7.

**Table 7  Recovery rate**

| Theoretical Concentration of Sample (ng/mL) | Avermectin (%) | Eprinomectin (%) | Doramectin (%) | Ivermectin (%) |
|---|---|---|---|---|
| 10 | 77.5 | 74.3 | 78.0 | 71.9 |
| 100 | 81.2 | 72.8 | 80.4 | 75.3 |
| 200 | 88.0 | 76.4 | 78.0 | 77.4 |

## CONCLUSION

This application news demonstrates a method for determination of avermectin drug residues in vinegar using Shimadzu's UHPLC LC-30A coupled with triple quadrupole mass spectrometer LCMS-8045. When quantified by the external standard method, the calibration curves of the method showed adequate linearity with correlation coefficients all above 0.997. Samples of low, medium and high concentrations were tested in 6 replicates. The relative standard deviations of retention time and peak area were 0.02 to 0.09% and 0.66 to 4.57% respectively, showing good precision. The limit of detection and limit of quantitation ranged from 0.22 to 0.25 ng/mL and 0.75 to 0.83 ng/mL respectively, thus complying with the current requirements for detection of avermectin drug residues in food. This method can provide a reference for relevant personnel in carrying out the detection of avermectin drug residues in food.

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# A sensitive and repeatable method for characterization of sulfonamides and trimethoprim in honey using QuEChERS extracts with Liquid-Chromatography-Tandem Mass Spectrometry

## ASMS 2015   WP 057

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**A sensitive and repeatable method for characterization of sulfonamides and trimethoprim in honey using QuEChERS extracts with Liquid-Chromatography-Tandem Mass Spectrometry**

# Introduction

The antibacterial sulfonamides (SA) and trimethoprim are widely used in veterinary and human medicine. Diverse foods from animals potentially contain residues of these drugs posing possible threats to people by triggering allergic reactions and undesirable increasing of microorganism's drug resistance. Various countries have defined their own maximum residue limits (MRLs) for sulfonamides accepted in honey, There are no MRL's for sulfonamides in honey in the UE but in 2002 a minimum required performance level (MRPL) was set for analytical methods at a level of 10 µg/kg. HPLC-MS/MS is an effective strategy to characterize and accurately measure those antibiotics considering MRLs and MRPLs in food products from animal origin tend to be continually reduced to preserve human health safety. A selective, fast and sensitive HPLC-MS-MS method has been developed for 15 sulfonamides and trimethoprim.

# Materials and Method

## Sample preparation

5 grams of honey, spiked with 17 SAs and trimethoprim (Table 1A), were extracted using QuEChERS method following manufacturer's procedure with a final 1:5 extract dilution using methanol. A multiple reaction monitoring MRM method was optimized for quantitation for each sulfonamide compound using a Shimadzu Nexera UHPLC with an LCMS-8050 fast-scanning triple quadrupole mass spectrometer model equipped with software Labsolution LCMS version 5.65 and electrospray ionization ESI.

Stock standard solutions of each sulfonamide were prepared dissolving appropriate amounts in DMSO and methanol, diluting to 100 ppm and 1 ppm at the end with mobile phase A:B 50:50. Table 1B shows the concentrations at each level used to build calibration curves for external calibration method.
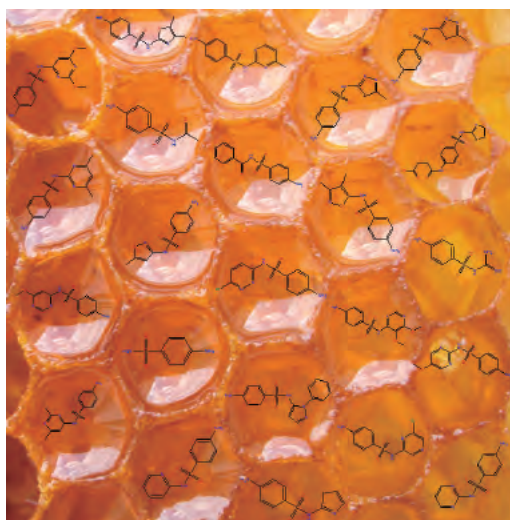


## LC conditions

A Kinetex 2.6µ PFP 100 Å column (100 × 2.1 mm) was used at 40 °C, flow rate of 0.5 mL/min, and 10 µL injection volume using QuEChERS extraction method. A binary gradient of 10% methanol (mobile phase A) and methanol, 0.3% formic Acid (mobile phase B) was used with the gradient program described in Table 1C.

A sensitive and repeatable method for characterization of sulfonamides and trimethoprim in honey using QuEChERS extracts with Liquid-Chromatography-Tandem Mass Spectrometry

## Mass Spectrometry:

Electrospray ionization was used in positive mode, spray voltage was 4.5 kV, desolvation line temperature was 250 °C, nebulization gas was 2.0 L/min, heater block was 400 °C, and drying gas 15 L/min.

Table 1.  A. Sulfonamide compounds used in this study; B. Concentration levels to define calibration curves, and C. HPLC gradient used.

### A. Sulfonamides used

| # | SULFONAMIDE | # | SULFONAMIDE |
|---|---|---|---|
| 1 | Sulfaguanidine | 10 | Sulfamethoxypyridizine |
| 2 | Sulfacetamide | 11 | Succinylsulfathiazole |
| 3 | Sulfadiazine | 12 | Sulfamethoxazole |
| 4 | Sulfathiazole | 13 | Trimethoprim |
| 5 | Sulfapyridine | 14 | Sulfamonomethoxine |
| 6 | Sulfamerazine | 15 | Sulfisoxazole |
| 7 | Sulfamethazine | 16 | Sulfabenzamide |
| 8 | Sulfameter | 17 | Sulfaclozine |
| 9 | Sulfamethizole | 18 | Sulfadimethoxine |

### B. Calibration Curve

| Level | Conc. (ng/ml) |
|---|---|
| 1 | 1000 |
| 2 | 500 |
| 3 | 250 |
| 4 | 125 |
| 5 | 62.5 |
| 6 | 31.3 |
| 7 | 15.6 |
| 8 | 7.8 |
| 9 | 3.9 |
| 10 | 2 |
| 11 | 1 |

### C. LC Gradient

| Time (min) | %B |
|---|---|
| 0 | 5 |
| 1 | 15 |
| 4.5 | 35 |
| 5 | 60 |
| 5.01 | 95 |
| 5.5 | 95 |
| 5.51 | 5 |
| 7 | 5 |

To implement sulfonamide quantitation, MRM transitions were optimized using a 0.5 µg mixture of SAs, 1 µL injections at 400 µL/min. Three transitions from parent ions and fragments were selected using the optimization tool software.

# Results
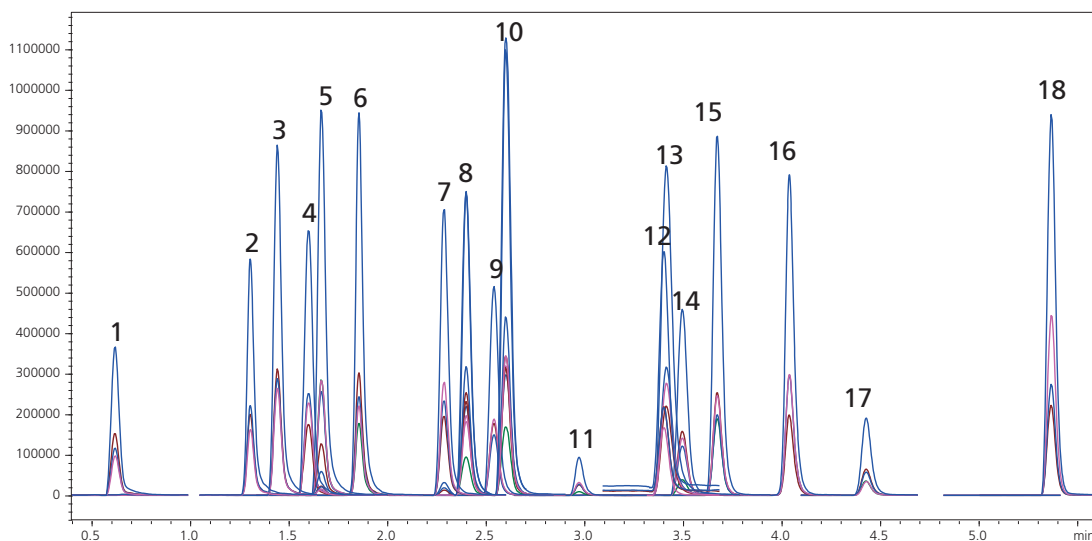


Figure 1.  Representative chromatogram of sulfonamide drugs. Standard mixture at 125 pg on-column for each standard. Peak numbers follow the order described for SA compounds in table 1A.

# A sensitive and repeatable method for characterization of sulfonamides and trimethoprim in honey using QuEChERS extracts with Liquid-Chromatography-Tandem Mass Spectrometry
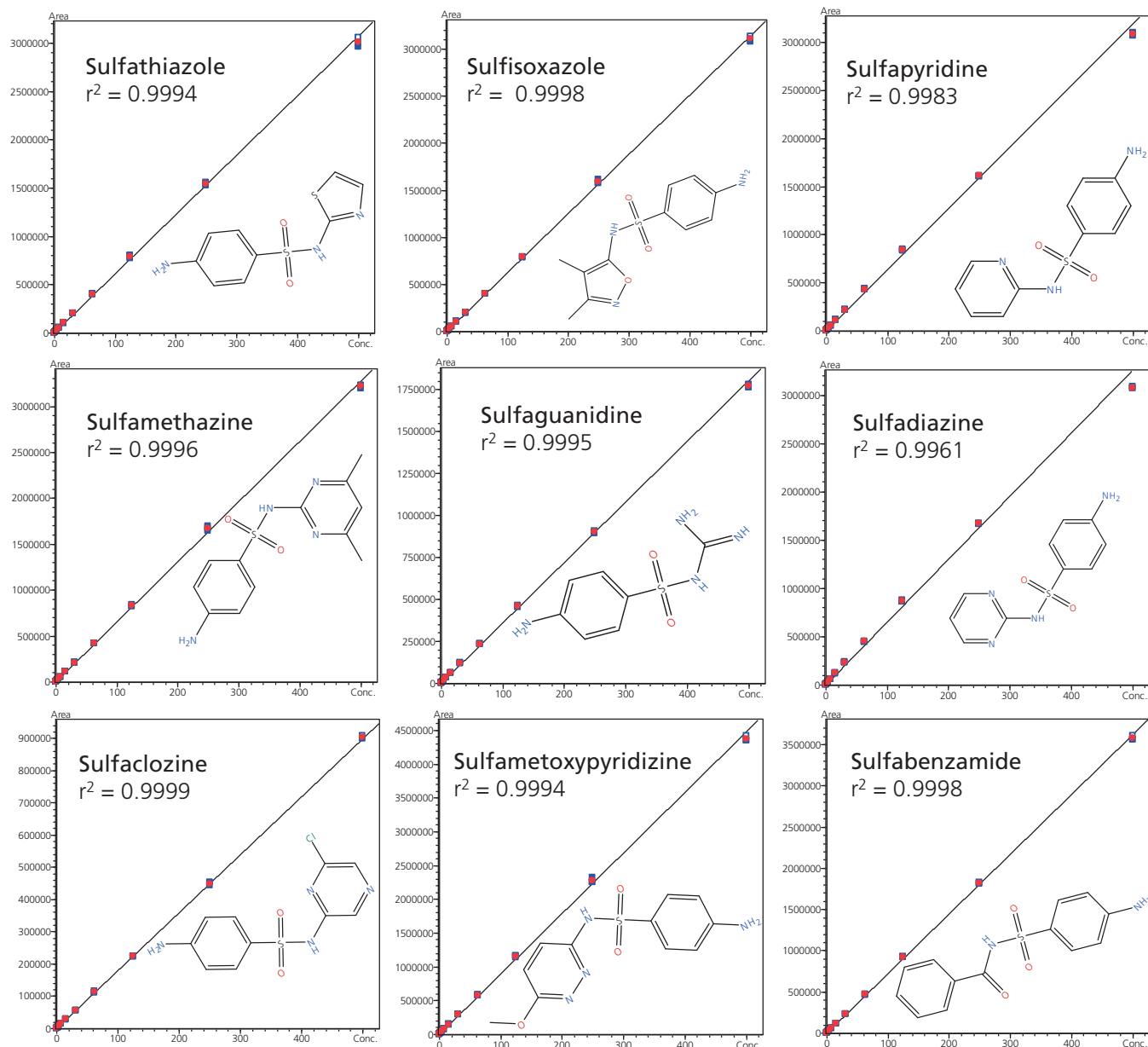


Figure 2. High degree of linearity was observed over the concentration range 0.5–500 pg on column, with values of $r^2 \geq 0.990$ for all analytes.

Authentic SAs standards were fully characterized by HPLC and MS/MS with an MRM optimized assay. The calibration curves of standards in 50% methanol matrix were linear with with $r^2 > 0.990$ (Figure 2) in the tested range of 1 to 1000 µg/Kg (0.5 to 500pg on column). The limits of quantification were 1 µg/Kg (0.5pg on column) for all compounds except succinylsulfathiazole and sulfacetamide, which were 2 µg/Kg (1pg on column). The recovery ranged from 53.9 to 91.4% for all but two compounds measured using drug residue-free organic honey.

Succinylsulfathiazole and sulfaguanidine exhibited recovery below 20% using the QuEChERS method for extraction.

# A sensitive and repeatable method for characterization of sulfonamides and trimethoprim in honey using QuEChERS extracts with Liquid-Chromatography-Tandem Mass Spectrometry



| Level | Sulfathiazole Cal point %RSD | Sulfathiazole Accuracy % | Sulfisoxazole Cal point %RSD | Sulfisoxazole Accuracy % | Sulfapyridine Cal point %RSD | Sulfapyridine Accuracy % | Sulfamethazine Cal point %RSD | Sulfamethazine Accuracy % | Sulfaguanidine Cal point %RSD | Sulfaguanidine Accuracy % | Sulfadiazine Cal point %RSD | Sulfadiazine Accuracy % | Sulfaclozine Cal point %RSD | Sulfaclozine Accuracy % | Sulfamethoxypyridizine Cal point %RSD | Sulfamethoxypyridizine Accuracy % | Sulfabenzamide Cal point %RSD | Sulfabenzamide Accuracy % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1.7 | 97.8 | 1.0 | 98.9 | 0.5 | 96.5 | 0.6 | 98.1 | 0.5 | 98.3 | 0.4 | 94.3 | 0.5 | 100.4 | 1.0 | 97.5 | 0.8 | 98.8 |
| 2 | 0.9 | 100.4 | 1.4 | 101.2 | 0.3 | 100.6 | 1.5 | 101.8 | 0.7 | 99.8 | 0.2 | 102.3 | 1.1 | 99.9 | 1.9 | 101.5 | 0.3 | 100.3 |
| 3 | 2.1 | 102.6 | 0.4 | 100.3 | 0.9 | 105.0 | 1.1 | 101.0 | 0.9 | 101.2 | 1.0 | 106.0 | 0.8 | 99.3 | 1.1 | 102.8 | 0.7 | 101.6 |
| 4 | 1.7 | 103.6 | 0.3 | 102.0 | 1.3 | 107.7 | 0.8 | 102.5 | 0.6 | 103.6 | 1.5 | 109.7 | 1.7 | 100.0 | 0.9 | 102.7 | 1.2 | 102.8 |
| 5 | 0.4 | 106.3 | 2.0 | 101.0 | 1.4 | 107.8 | 1.0 | 102.1 | 1.7 | 105.5 | 1.3 | 114.0 | 1.0 | 98.4 | 1.5 | 104.3 | 1.4 | 101.7 |
| 6 | 1.6 | 106.1 | 3.3 | 102.6 | 0.5 | 110.8 | 0.9 | 106.3 | 1.8 | 108.3 | 0.6 | 116.8 | 1.5 | 99.3 | 3.2 | 104.0 | 0.4 | 104.2 |
| 7 | 3.8 | 109.4 | 1.5 | 101.1 | 6.3 | 103.1 | 0.7 | 105.8 | 3.0 | 113.9 | 2.8 | 115.4 | 3.4 | 100.9 | 1.2 | 108.1 | 0.6 | 103.6 |
| 8 | 4.4 | 108.0 | 1.2 | 104.1 | 8.2 | 102.2 | 3.3 | 103.7 | 2.3 | 114.6 | 1.6 | 111.9 | 5.0 | 98.5 | 6.3 | 105.9 | 3.3 | 102.6 |
| 9 | 4.4 | 115.7 | 1.5 | 100.2 | 4.0 | 104.4 | 2.0 | 106.4 | 6.4 | 114.3 | 6.5 | 110.8 | 8.1 | 87.9 | 2.7 | 112.3 | 2.6 | 104.7 |
| 10 | 3.9 | 107.4 | 5.0 | 88.0 | 9.2 | 103.7 | 15.2 | 90.6 | 8.0 | 114.1 | 9.1 | 118.5 | 8.8 | 90.9 | 5.7 | 121.4 | 2.7 | 97.6 |
| 11 | 12.7 | 114.7 | 10.5 | 100.3 | 6.2 | 109.9 | 5.9 | 100.9 | 9.9 | 126.9 | 16.5 | 116.5 | 6.9 | 95.5 | 7.3 | 105.9 | 6.4 | 91.3 |

Figure 3. Representative chromatograms of sulfonamide drugs at lowest concentration showing limit of quantitation and statistics for diverse concentration levels.

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A sensitive and repeatable method for characterization of
sulfonamides and trimethoprim in honey using QuEChERS extracts with
Liquid-Chromatography-Tandem Mass Spectrometry

# Conclusions

LC-MS/MS with QuEChERS as extraction method provides a fast, simple, sensitive and accurately measuring for sulfonamide drugs and trimethoprim in honey with an acceptable recovery range. Matrix matched calibration and use of internal standards can be tested to improve performance.

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# Others

1.  Analysis of Organophosphorus Pesticides Using Nexis GC-2030

2.  Combined Analysis of a Contaminant Using a Compact FTIR and EDX

3.  Contaminant Analysis in Food Manufacturing Process by EDX and FTIR

4.  Serotype-Level Bacterial Discrimination Using Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry

5.  Simultaneous Analysis of 418 Pesticides Utilizing Smart SIM

6.  Fully Automated Derivatization and Quantication of Glyphosate and AMPA in Beer Using a Standard UHPLC-MS/MS System

7.  Determination of Pyrrolizidine Alkaloids in Plant Material Using on-line SPE Coupled to UHPLC-MS/MS

8.  Fast and High Sensitivity Analysis of Six Preservatives in Beverages by UHPLC with Photodiode Array Detection

9.  Shimadzu Pesticide MRM Library Support for LC/MS/MS

10. Determining Gamma-Hydroxybutyric Acid and its Precursors Gamma-Butyrolactone and 1,4-Butanediol in Suspected Drug-spiked Beverages using LCMS-8045

11. Carbon Dioxide Determination in Beer

# SHIMADZU
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# Application News

## No. G294

**Gas Chromatograph**

# Analysis of Organophosphorus Pesticides Using Nexis GC-2030

Cases have been reported of health problems due to foods contaminated with pesticides, and there is currently heightened interest in food safety countermeasures. Using a detector with high selectivity for specific components, or a mass spectrometer highly capable of qualitative analysis are effective when analyzing trace components in foods and other samples in which there are many impurities.

The FPD-2030 flame photometric detector, which is installed in Nexis GC-2030 gas chromatograph, has the world's highest level of sensitivity* thanks to the optimized nozzle shape and the advanced dual focus system.

In the analysis of pesticides in foods, this detector provides high sensitivity and high stability.

In this Application News, we introduce an analysis of organophosphorus pesticides using Nexis GC-2030 gas chromatograph, which is equipped with the FPD-2030.

*As of February 2017

E. Kobayashi, T. Murata

## ■ Analysis Results

A mixture standard solution of 54 organophosphorus pesticides* (20 mg/L) was introduced via split injection, and the elution positions of each pesticide were confirmed.

### Table 1  Analytical Conditions

| | |
|---|---|
| Model | : Nexis GC-2030 |
| Detector | : FPD-2030 (P-mode) |
| Column | : SH-Rtx-1701 (0.25 mm I.D. × 30 m, d.f. = 0.25 μm) |
| Column Temperature | : 60 ℃ (2 min) - 25 ℃/min - 150 ℃ (0 min) - 5 ℃/min - 200 ℃ (12 min) - 5 ℃/min - 280 ℃ (7 min)    Total 50.6 min |
| Injection Mode | : Split 1 : 20 |
| Carrier Gas Controller | : Constant Linear Velocity (He) |
| Linear Velocity | : 30 cm/sec |
| Injection Temperature | : 250 ℃ |
| Detector Temperature | : 275 ℃ |
| Injection Volume | : 1 μL |



| | | | |
|---|---|---|---|
| 1: Ethoprophos | 7: Dimethoate | 13: Isofenphos | 19: Fensulfothion |
| 2: Phorate | 8: Tolclofos-methyl | 14: PAP (Phenthoate) | 20: EPN |
| 3: Thiometon | 9: Chlorpyrifos | 15: Prothiofos | 21: PMP (Phosmet) |
| 4: Terbufos | 10: Formothion | 16: DMTP (Mathidathion) | 22: Pyraclofos |
| 5: Etrimfos | 11: MPP (Fenthion) | 17: Butamifos | |
| 6: ECP (Dichlofenthion) | 12: MEP (Fenitrothion) | 18: Sulprofos | |
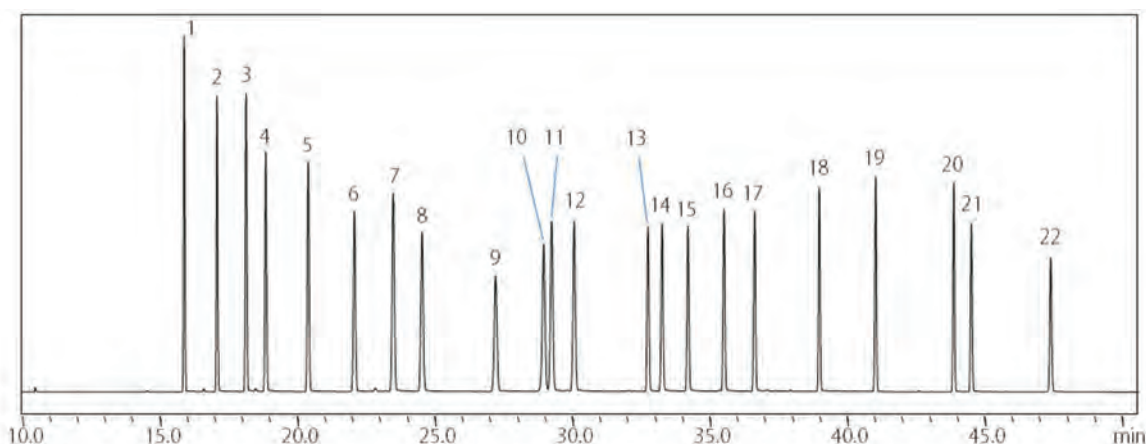
**Fig. 1** **Chromatogram of 20 mg/L Organophosphorus Pesticides**

## ■ Trace Level Analysis

Table 2 and Fig. 2 show the analysis conditions and the chromatogram respectively for a trace level analysis of 5 µg/L organophosphorus pesticides via high-pressure splitless injection.

**Table 2  Analysis Conditions for Low-Concentration Organophosphorus Pesticides**

| | |
|---|---|
| Model | : Nexis GC-2030 |
| Detector | : FPD-2030 (P-mode) |
| Column | : SH-Rtx-1701 (0.25 mm I.D. × 30 m, d.f. = 0.25 µm) |
| Column Temperature | : 60 °C (1 min) - 20 °C/min - 180 °C (0 min) - 5 °C/min - 200 °C (10 min) - 7 °C/min - 280 °C (5 min)    Total 37.4 min |
| Injection Mode | : High Pressure Splitless (300 kPa, 1 min) |
| Carrier Gas Controller | : Constan Linear Velocity (He) |
| Linear Velocity | : 46.8 cm/sec |
| Injection Temperature | : 260 °C |
| Detector Temperature | : 300 °C |
| Injection Volume | : 2 µL |



| | S/N | | S/N |
|---|---|---|---|
| 1: Ethoprophos | : 42 | 12: MEP (Fenitrothion) | : 17 |
| 2: Phorate | : 39 | 13: Isofenphos | : 15 |
| 3: Thiometon | : 42 | 14: PAP (Phenthoate) | : 16 |
| 4: Terbufos | : 30 | 15: Prothiofos | : 18 |
| 5: Etrimfos | : 33 | 16: DMTP (Mathidathion) | : 21 |
| 6: ECP (Dichlofenthion) | : 24 | 17: Butamifos | : 19 |
| 7: Dimethoate | : 26 | 18: Sulprofos | : 25 |
| 8: Tolclofos-methyl | : 23 | 19: Fensulfothion | : 25 |
| 9: Chlorpyrifos | : 16 | 20: EPN | : 25 |
| 10: Formothion | : 5 | 21: PMP (Phosmet) | : 22 |
| 11: MPP (Fenthion) | : 18 | 22: Pyraclofos | : 15 |
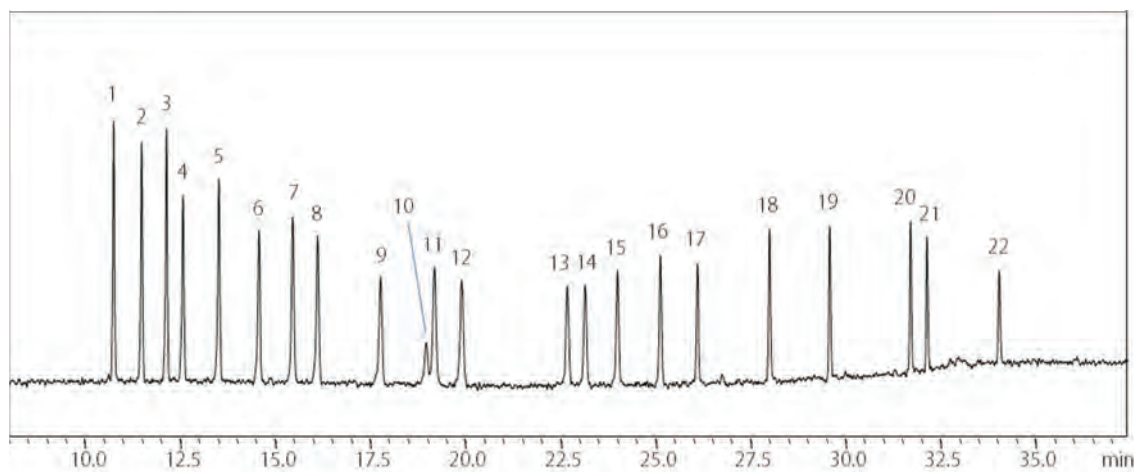
**Fig. 2  Chromatogram of Low-Concentration (5 µg/L) Organophosphorus Pesticides**

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# SHIMADZU
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**Application News**

**No. A567**

Spectrophotometric Analysis

## Combined Analysis of a Contaminant Using a Compact FTIR and EDX

Demands regarding the analysis of contaminants that are mixed in or adhered to products are increasing for food and chemical manufacturers and inspection agencies which are consigned inspections.

This increase in demands has drawn attention to energy dispersive X-ray fluorescence spectrometers (EDX) which are suited to analyzing inorganic elements such as metals and to Fourier transform infrared spectrophotometers (FTIR) which are optimal for the analysis of organic substances such as polymeric compounds. Cases where one sample is analyzed using both instruments are increasing as well. However, data obtained with each instrument requires respective analysis procedures and results are sometimes influenced by the operator's knowledge and experience. It is in light of such situations that Shimadzu developed the EDX-FTIR contaminant finder/material inspector, EDXIR-Analysis™ software. The first in the industry, this software is capable of combining and analyzing data acquired from a Shimadzu EDX and FTIR. Details of the software are introduced in Application News Nos. A522A [1] and A527 [2]. This article introduces an example analysis of a contaminant using the EDX-FTIR combined analysis system shown in Fig. 1.

R. Fuji, T. Nakao



**Fig. 1 EDX-FTIR Combined Analysis System**

### Measurement Sample

A contaminant found in a food production process (Fig. 2) was used as the measurement sample. About 4 mm in size and white on the surface, the sample is hard when handled with tweezers. The sample was measured by fixing it in place using the EDXIR-Holder™ shown in Fig. 3, which is a sample holder/stocker for contaminant measurement and effective for streamlining analysis processes for EDX and FTIR. Details of the EDXIR-Holder are introduced in Application News No. A537 [3].



**Fig. 2 Photograph of Contaminant Found in a Food Production Process**

Polypropylene film surface for X-ray fluorescence spectrometer analysis

Adhesive layer of film
Adhere sample

Without sample          With sample
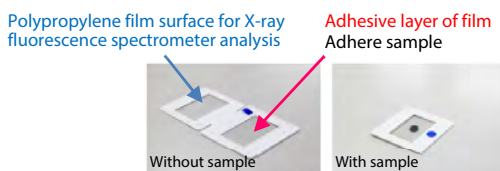
**Fig. 3 EDXIR-Holder: Sample Holder/Stocker for Contaminant Measurement**

### Measurement Using FTIR

IRSpirit™, a compact FTIR, was used for measurement with the QATR™-S single-reflection ATR accessory, which is designed especially for the IRSpirit series, with a diamond prism installed (Fig. 4). Fig. 5 shows the sample set on the instrument and Table 1 lists the measurement conditions that were used. In using the IRSpirit, the dedicated IR Pilot program was used to facilitate measurement. IR Pilot allows operators with minimal FTIR experience to analyze samples by simply selecting the analysis purpose and the accessory. The measured spectrum and the search result from the standards library with the highest similarity are drawn superimposed in Fig. 6. In this case, protein was identified as the best match.



**Fig. 4 IRSpirit with QATR-S Single-Reflection ATR Accessory (Diamond prism)**
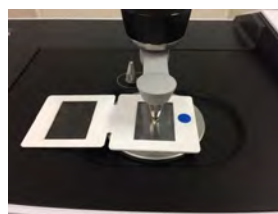


**Fig. 5 Sample Set on Instrument**

**Table 1 Measurement Conditions**

| | |
|---|---|
| Instrument | : IRSpirit-T (KRS-5 window) QATR-S |
| Resolution | : 4 cm⁻¹ |
| Accumulation Times | : 20 |
| Apodization Function | : SqrTriangle |
| Detector | : DLATGS |
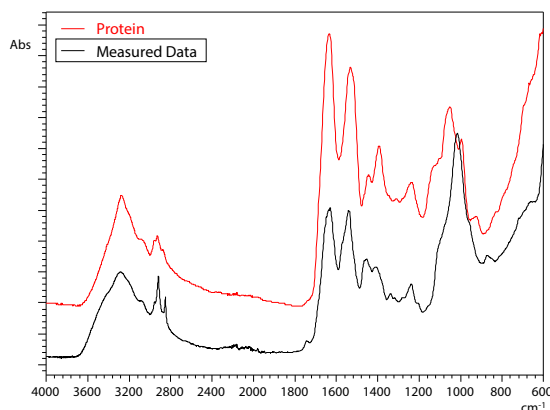


**Fig. 6 Infrared Spectra of Measured Data and the Search Result**

## ■ Measurement Using EDX

Measurement was done using the EDX-7000 energy dispersive X-ray fluorescence spectrometer (Fig. 7) according to the measurement conditions listed in Table 2. The sample was set on the instrument as shown in Fig. 8. For measurement using EDX, the EDXIR-Holder is closed and placed so that the side with polypropylene film is facing the X-ray beam source (bottom). The EDXIR-Holder enables easy setting of samples between EDX and FTIR instruments and contributes to alleviating and improving the efficiency of analysis tasks.



**Fig. 7  EDX-7000 Energy Dispersive X-Ray Fluorescence Spectrometer**
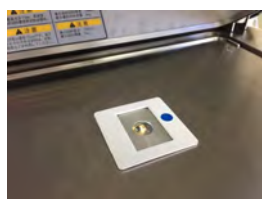


**Fig. 8  Sample Set on Instrument**

**Table 2  Measurement Conditions**

| | |
|---|---|
| Instrument | : EDX-7000 |
| X-Ray Tube Target | : Rh |
| Voltage / Current | : 50 kV (Al-U) / Auto |
| Atmosphere | : Vacuum |
| Analysis Diameter | : 1 mm$\phi$ |
| Filter | : None |
| Integration Time | : 100 s |

Fig. 9 shows the qualitative and quantitative analysis results which indicate that $_{20}$Ca and $_{15}$P are the primary elements of the contaminant. Conventionally, the identification of a contaminant requires the analysis of each of the EDX and FTIR measurement results. In this instance however, the EDXIR-Analysis software was used to read and analyze the data acquired from the instruments.



| Elements | Ca | P | Mg | K | S | Sr | Zn | Fe |
|---|---|---|---|---|---|---|---|---|
| Quantitative Value [wt%] | 68.1 | 28.3 | 1.8 | 0.95 | 0.66 | 0.09 | 0.09 | 0.04 |
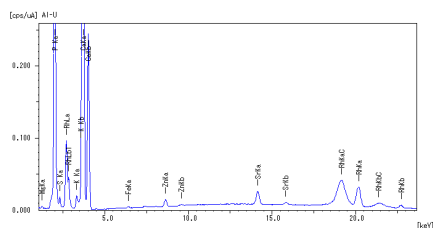
**Fig. 9  Qualitative and Quantitative Analysis Results**

EDXIR-Analysis, IRTracer, IRAffinity, EDXIR-Holder, IRSpirit and QATR are trademarks of Shimadzu Corporation.

## ■ Analysis Using EDXIR-Analysis Software

Analysis was done using the EDXIR-Analysis software. The contaminant library used in analysis was developed by measuring and accumulating data of contaminants provided by water supply organizations and food manufacturers using Shimadzu's EDX and FTIR. Comprising a total of 485 entries, various contaminants such as tap water contaminants and food contaminants are registered.

The hit list shown in Fig. 10 indicates that with a similarity of 0.9160, the most probable match is white bone particle (a mixture of calcium phosphate and protein). Similarity values are within the range from 0 to 1 and larger values indicate that the analyzed data (acquired data) and the hit data (data in the library) are more similar. By comparing the element content and X-ray fluorescence profile of the analyzed data and hit data in Fig. 11 and the infrared spectra in Fig. 12, we can see that the two are highly similar. In addition, images of the sample can be compared as shown in Fig. 13, allowing evaluation of similarity with candidate substances in terms of color, shape, and texture. Based on these various aspects it was concluded that the contaminant is bone.



**Fig. 10  Hit List**



**Fig. 11  Element Content and X-Ray Fluorescence Profiles of Analyzed Data and Hit Data**
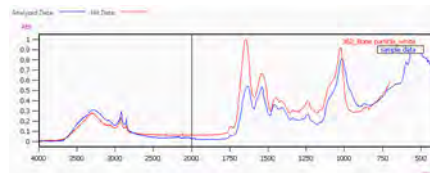


**Fig. 12  Infrared Spectra of Analyzed Data and Hit Data**



| Analyzed data | Hit data |
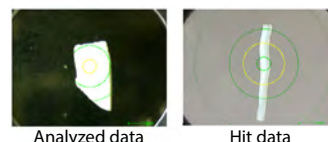
**Fig. 13  Sample Images of Analyzed Data and Hit Data**

The EDXIR-Analysis software enabled easy and swift obtaining of analysis results that combine the inorganic element information acquired using EDX and the organic compound information acquired using FTIR.

References
(1) Application News No. A522A "Contaminant Analysis Using EDXIR-Analysis Software for Combined EDX-FTIR Analysis"
(2) Application News No. A527 "Quantifying "Silent Change" Using EDXIR-Analysis Software: EDX-FTIR Contaminant Finder/Material Inspector"
(3) Application News No. A537 "Introducing the EDXIR-Holder: Sample Holder/Stocker for Contaminant Measurement"

First Edition: Apr. 2018

# SHIMADZU
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# Application News

### X-ray Analysis

# Contaminant Analysis in Food Manufacturing Process by EDX and FTIR

## No.X261

EDX and FTIR are widely used for analysis of foreign contaminant matter, but recently, these instruments are increasingly being utilized in tandem to conduct contaminant analysis[1]. While identification using any of these instruments and analytical methods independently is limited to some degree, using them in conjunction with one another permits a more detailed elucidation of the contaminant characteristics, thereby enhancing the validity of the respective results.
The analytical method and sample pretreatment method to be used depend on the degree to which a contaminant is to be characterized, whether or not the substance is altered or destroyed due to pretreatment, and the speed that is required to complete the analysis. Introduced here is an example of actual analysis of various types of foreign matter entered during the food manufacturing process.

### ■ Samples
Foreign matter that entered during the food manufacturing process Five types of samples: Sample 1, 2, 3, 4, 5

### ■ Pretreatment and Analysis Procedures
First, EDX measurement was conducted without conducting any sample pretreatment, and then FTIR measurement was conducted similarly without pretreatment. Next, the foreign matter was removed by rinsing, and then analyzed. This preparation procedure is outlined in the flowchart of Fig. 1.
Depending on the sample, there may be cases in which detailed analysis by ATR measurement using the FTIR main unit will be difficult due to such factors as small sample size relative to the prism, which could result in the sample

being crushed, such as in the current situation, or samples consisting of a mixture, etc. It was therefore decided to conduct microscopic ATR measurement with close contact of the prism at the measurement site.

[EDX]
Samples 1 to 5
Sandwiched in 4 μm thick polypropylene film, and measured → (1), (2)

[Analysis Results]

[FTIR]
Samples 3, 4, 5
Measured without pretreatment → (3), (4), (5)

[FTIR]
Sample 4
Rinsed with acetone, then measured → (4)

[EDX⇒FTIR]
Sample 5
7 % hydrochloric acid-14 % nitric acid, rinsed in purified water, then measured → (5)
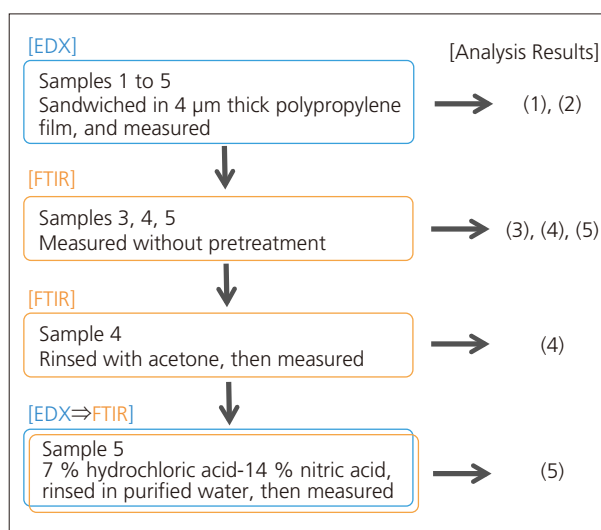
**Fig. 1  Pretreatment and Analysis Procedures**

### ■ Analysis Result
Fig. 2 to Fig. 9 and Table 1 to Table 5 show the analysis results for each sample using EDX and FTIR, in addition to the inferred and specific attributions according to those results.

## (1) Sample 1     Characteristics: Metallic luster, hard, silvery white



| Analysis Target | Analysis Result |
|---|---|
| Fe | 96.417 % |
| Sn | 3.087 % |
| Mn | 0.347 % |
| Cr | 0.149 % |

X-ray irradiation range 1 mm dia.

Sample Image
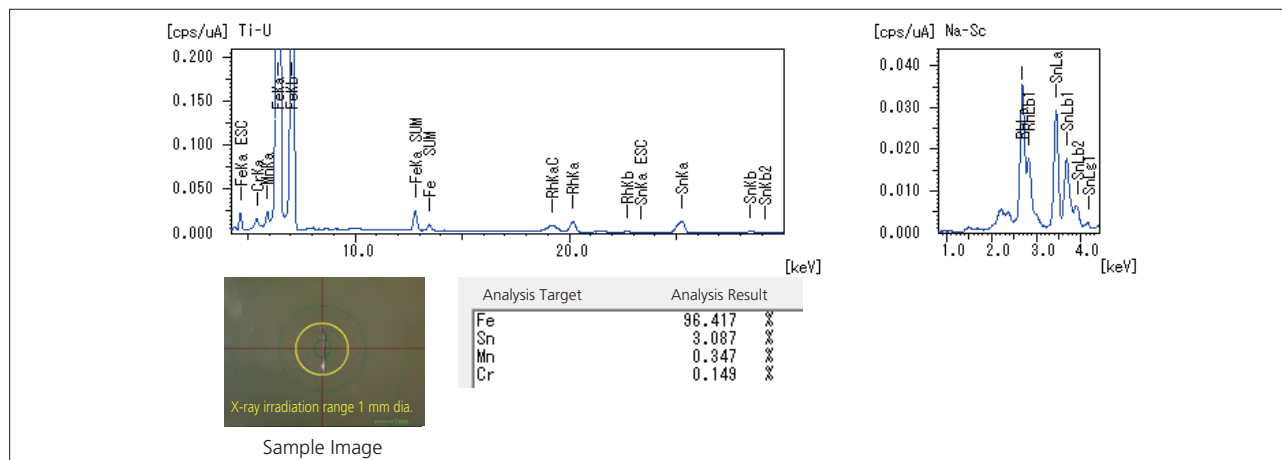
**Fig. 2  Sample 1 Qualitative-Quantitative Results by EDX**

**Table 1  Analysis Results for Sample 1**

| | Measurement Result | Possible Source | Total Findings Found by EDX and FTIR |
|---|---|---|---|
| EDX | Principal component is $_{26}$Fe, next prevalent is $_{50}$Sn. | Tin-plated steel sheet, fragment of tin can | Tin-plated steel sheet, fragment of tin can (Clearly metallic according to EDX measurement only) |
| FTIR | Omitted (Significant peak not detected) | Possibly a metal or inorganic compound | |

## (2) Sample 2　　Characteristics: Metallic luster, hard, silver color



Sample Image
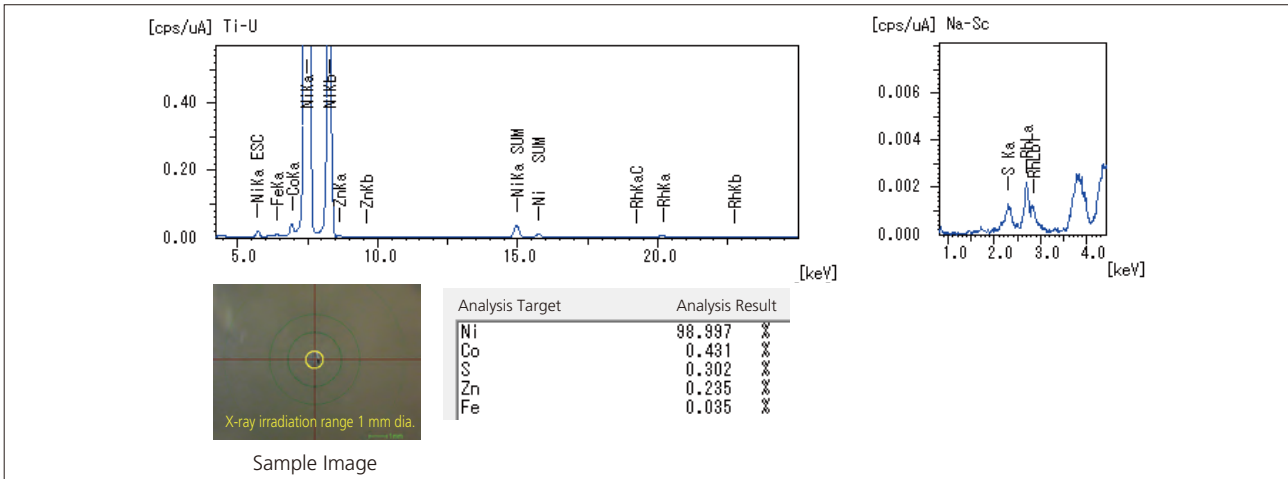
**Fig. 3　Sample 2 Qualitative-Quantitative Results by EDX**

**Table 2　Analysis Results for Sample 2**

| | Measurement Result | Possible Source | Total Findings Found by EDX and FTIR |
|---|---|---|---|
| EDX | Principal component is $_{28}$Ni, other components are in small quantity. | Nickel, peeling of the nickel plating | Nickel, peeling of the nickel plating (Clearly metallic according to EDX measurement only) |
| FTIR | Omitted (Significant peak not detected) | Possibly a metal or inorganic compound | |

## (3) Sample 3　　Characteristics: No metallic luster, brittle, brownish red



Sample Image
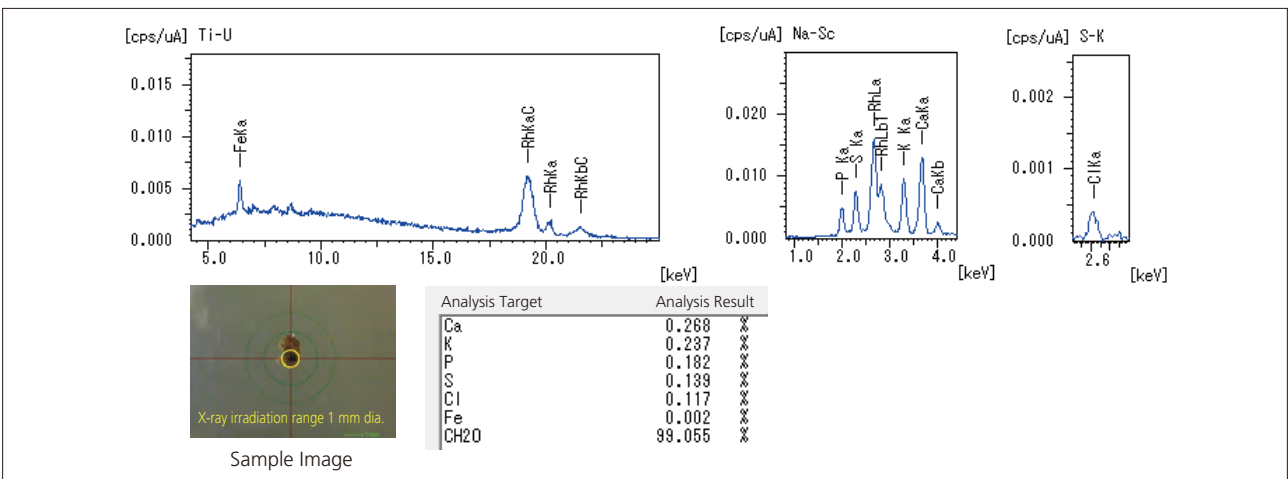
**Fig. 4　Sample 3 Qualitative-Quantitative Results by EDX**
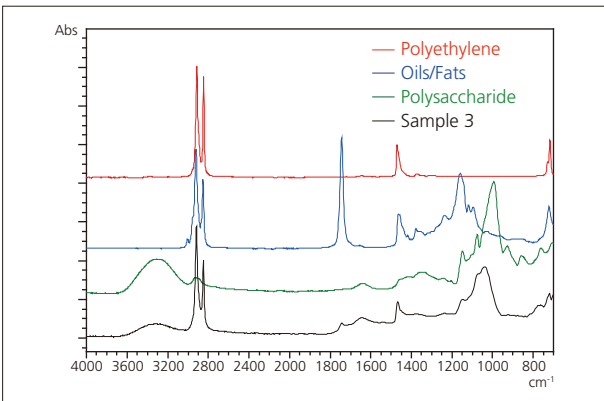


**Fig. 5　Infrared Spectrum and Search Results for Sample 3 by FTIR**

**Table 3　Analysis Results for Sample 3**

| | Measurement Result | Possible Source | Total Findings Found by EDX and FTIR |
|---|---|---|---|
| EDX | Detected $_{19}$K, $_{20}$Ca, and other food components. Principal component is $_9$F and below. (RhK$\alpha$C is big.[2)]) | Food clump | Polyethylene with attached food components |
| FTIR | Polyethylene, oils and fats, polysaccharides | Polyethylene with attached oils/fats and polysaccharides | |

### (4) Sample 4     Characteristics: Non-metallic luster, hard, black



Sample Image

| Analysis Target | Analysis Result | |
|---|---|---|
| Ca | 1.379 | % |
| Al | 0.053 | % |
| Si | 0.050 | % |
| S | 0.037 | % |
| Fe | 0.002 | % |
| CH2O | 98.480 | % |
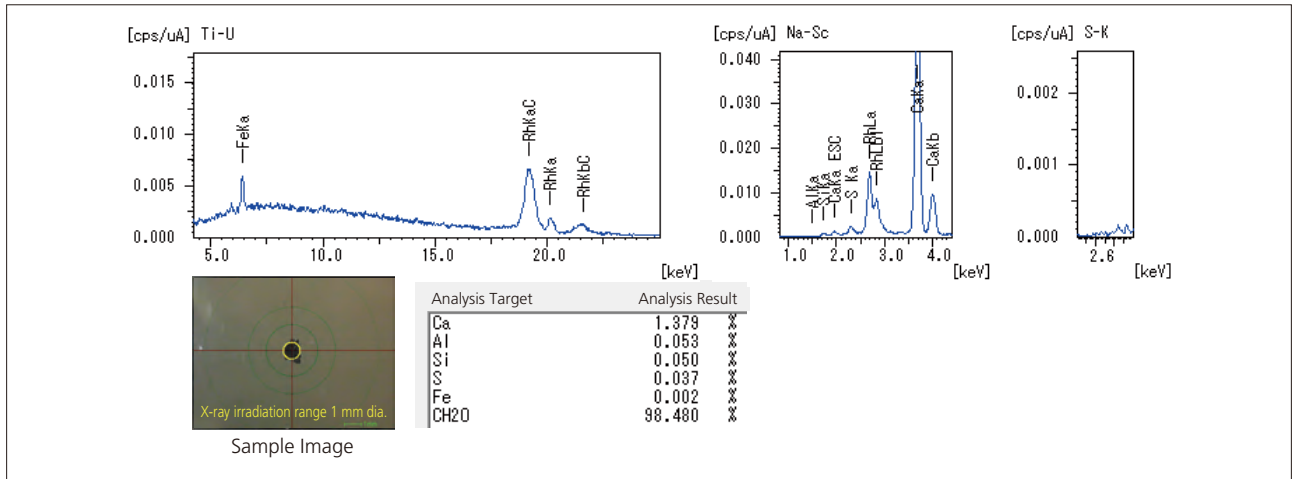
**Fig. 6  Sample 4 Qualitative-Quantitative Results by EDX**



<Before Rinsing>

<After Rinsing (Acetone) >
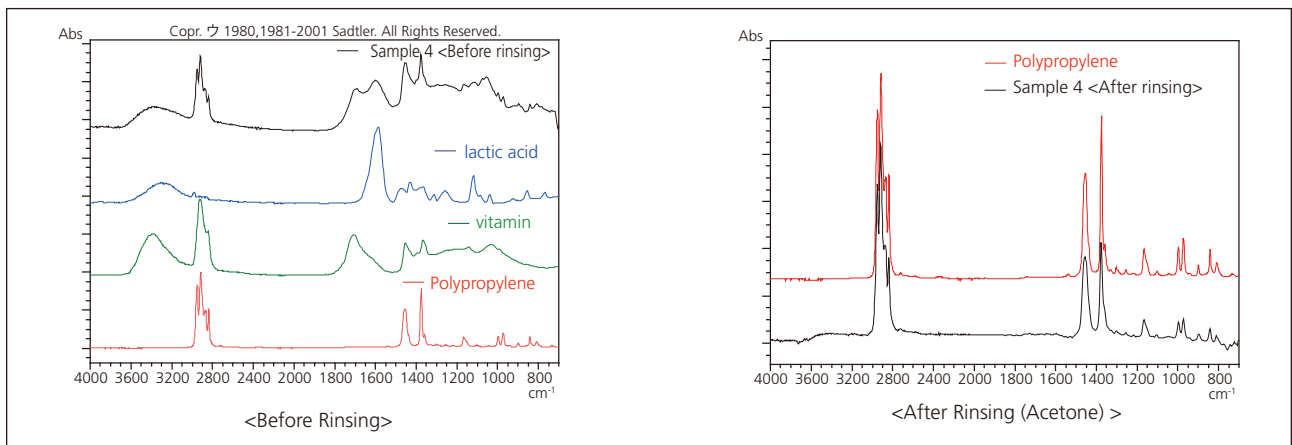
**Fig. 7  Sample 4 Infrared Spectra and Search Results by FTIR**

**Table 4  Analysis Results for Sample 4**

| | | Measurement Result | Possible Source | Total Findings Found by EDX and FTIR |
|---|---|---|---|---|
| EDX | | Detected $_{20}$Ca and other food components. Principal component is $_9$F and below. | Food clump, resins, etc. | Polyethylene with attached food components |
| FTIR | Before rinsing | Polypropylene, lactic acid, vitamins | Food components (lactic acid, vitamins, etc.) adhering to polypropylene | |
| | After rinsing | Polypropylene | | |

### (5) Sample 5     Characteristics: Some metallic luster, hard, black silver color



Sample Image

| Analysis Target | Analysis Result | |
|---|---|---|
| Cu | 0.214 | % |
| Cl | 0.081 | % |
| Zn | 0.057 | % |
| S | 0.026 | % |
| Se | 0.020 | % |
| CH2O | 99.601 | % |

<Before rinsing>

| Analysis Target | Analysis Result | |
|---|---|---|
| Cl | 1.245 | % |
| Se | 0.006 | % |
| Cu | 0.001 | % |
| CH2O | 98.748 | % |

<After rinsing>
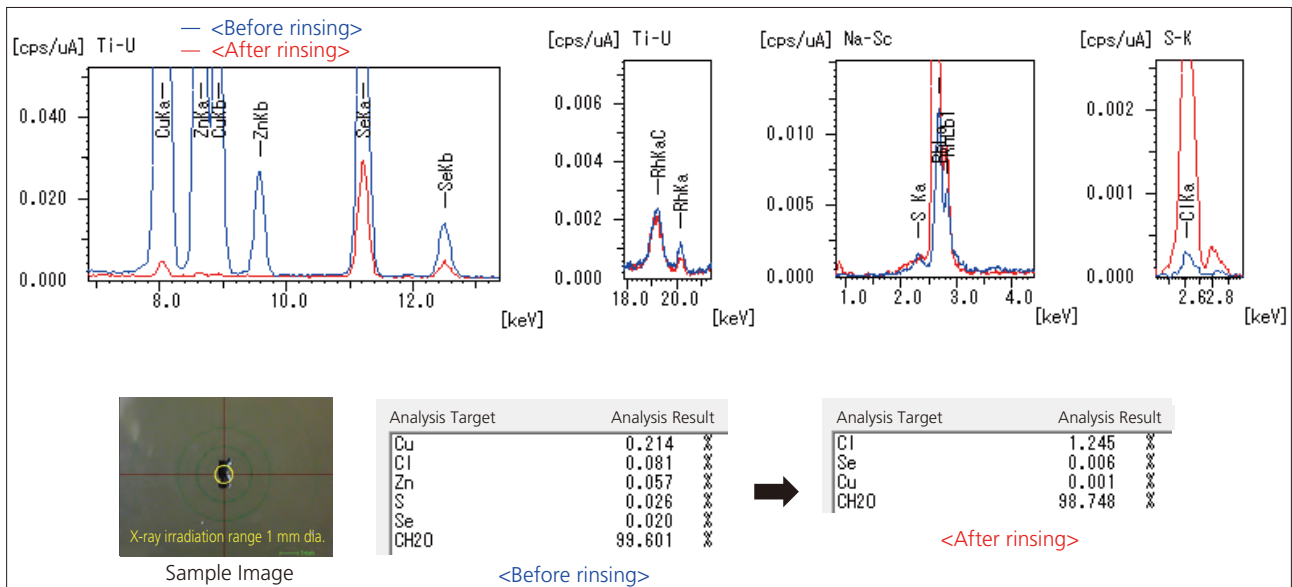
**Fig. 8  Sample 5 Qualitative-Quantitative Result by EDX**
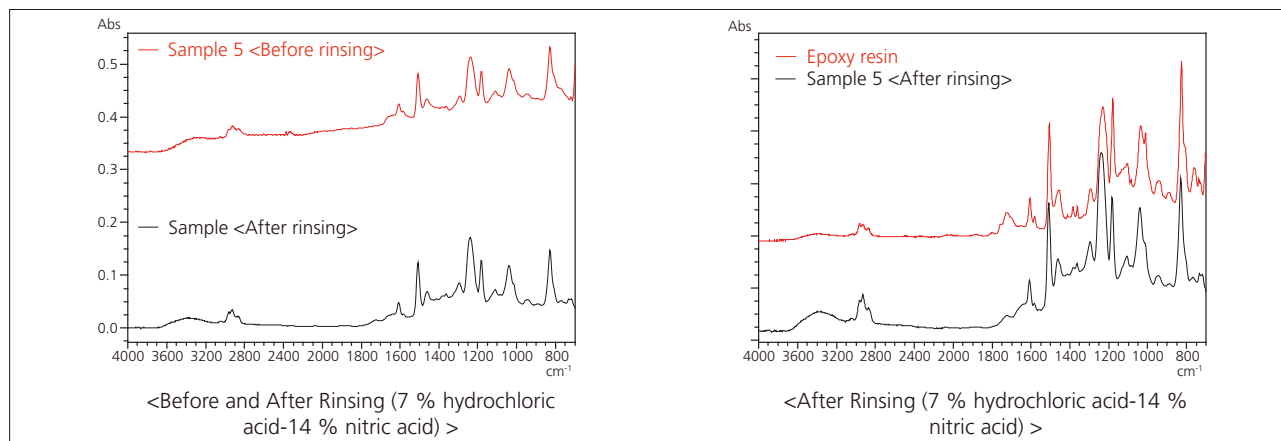
**Fig. 9  Sample 5 Infrared Spectra and Search Result by FTIR**

**Table 5  Analysis Results for Sample 5**

| | | Measurement Result | Possible Source | Total Findings Found by EDX and FTIR |
|---|---|---|---|---|
| EDX | Before rinsing | Principal components are $_9$F and below, large amounts of $_{29}$Cu, $_{30}$Zn, $_{34}$Se. | Copper alloy, resin composite material, zinc, selenium additives | Zinc and selenium food additives adhering to epoxy resin coated on copper thin film |
| | After rinsing | Principal components are $_9$F and below, with $_{29}$Cu, $_{30}$Zn nearly absent due to rinsing, and a small amount of residual $_{34}$Se. | Film | |
| FTIR | Before rinsing | Epoxy resin (with the presence of metals, etc. suggested due to rising of the infrared baseline) | Composite material consisting of epoxy resin and metal | |
| | After rinsing | Epoxy resin (no rise in the baseline in infrared spectrum) | Epoxy resin | |

- Regarding the EDX quantitative analysis results
  · Organic material is represented by $CH_2O$, and was balanced.
  · Abundant, small quantity, etc. are relative reference values.
    (In order to collectively set plating, film and deposits, etc.)

■ **Conclusion**

The analysis results by both EDX and FTIR permitted approximate identification of metals, resins, and their compounds or complex materials associated with contaminants introduced during the food product manufacturing process without the need for pretreatment. Further, by conducting relatively simple pretreatment of samples, detailed identification is also possible depending on the sample. In terms of speed and ease, these analytical techniques are quite effective.

[References]
1) Shimadzu Application News No. A452
2) Izumi Nakai (Editor), A Practical Guide for X-ray Fluorescence Analysis, Asakura Publishing, 90 (2006)

**Analytical Conditions [EDX]**

| | |
|---|---|
| Instrument | : EDX-7000 |
| Elements | : Na-U |
| Analytical Group | : Qualitative-quantitative |
| Detector | : SDD |
| X-Ray Tube | : Rh target |
| Tube Voltage [kV] | : 15, 50 |
| Current [μA] | : Auto |
| Collimator [mm $\phi$] | : 1 or 3 |
| Primary Filter | : Non, #2 |
| Atmosphere | : Vacuum |
| Integration Time [sec] | : 50 /ch |
| Dead Time [%] | : Max. 30 |

**Analytical Conditions [FTIR]**

| | |
|---|---|
| Instruments | : IRTracer-100, AIM-8800 |
| Resolution | : 8 cm$^{-1}$ |
| Accumulation | : 40 |
| Apodization | : Sqr-Triangle |
| Detector | : MCT |

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## Technical Report

# Serotype-Level Bacterial Discrimination Using Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry
### Practical Application of the Highly-Accurate Bacterial Discrimination Software "Strain Solution Ver. 2" with the iD$^{plus}$

Teruyo Ojima-Kato[1], Hiroto Tamura[1, 2], Naomi Yamamoto[2], Keisuke Shima[3]

## Abstract:

We successfully achieved serotype-level discrimination of enterohemorrhagic *Escherichia coli* using a proteotyping method (the *S10*-GERMS method) with markers consisting of ribosomal proteins detected during bacterial measurements using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS). In the future, this technique could be utilized as a routine method for the discrimination of bacteria at levels finer than the species level, which has thus far not been possible with conventional MALDI-TOF MS fingerprinting-based microbial identification methods.

Keywords: food-poisoning pathogens, MALDI-TOF MS, proteotyping, *S10*-GERMS

## 1. Introduction

Fast and accurate microbial identification is needed on a routine basis during the manufacturing of food products and pharmaceuticals, as well as in clinical microbiological testing. In particular, the rapid identification of pathogenic microorganisms and microbial contaminants (opportunistic bacteria) has become increasingly important. Conventional microbial identification methods generally include physiological and biochemical tests as well as DNA nucleotide sequence analyses targeting 16S rRNA gene sequences. However, these techniques are associated with the following challenges: 1) they lack rapidity; 2) they are labor-intensive and require expertise; 3) for some bacterial species, detailed identification is impossible beyond the genus or species level; and, importantly, 4) the discrimination of pathogenic microorganisms requires the use of costly reagents (e.g., antisera/antibodies). Thus, methods based on matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) have received wide attention as new techniques for the rapid identification of microorganisms. MALDI-TOF MS is attractive owing to its simplicity and convenience, and the fact that it allows for the rapid processing of multiple samples. Basically, analyses can be carried out by simply mixing microbial samples from a single colony with an extremely small amount of matrix solution (ionization adjuvant). In addition, the required analysis time of this method is less than 1 minute per sample, which allows for processing multiple samples in a short amount of time. Microbial identification methods using MALDI-TOF MS have been spreading rapidly since the late 2000s, mainly in the field of clinical microbiological testing, and were approved by the United States Food and Drug Administration in 2013. The procedure of microbial identification using MALDI-TOF MS consists of acquiring the mass spectrum of a microbial sample and matching it with the mass spectra of various bacterial species that are previously recorded in a database (fingerprinting method). Although the fingerprinting method allows for identification up to the genus and species levels, it does not allow for detailed discrimination of the serotypes of important food poisoning-related pathogens such as *Escherichia coli* and *Salmonella*. Therefore, we developed a method for discrimination of the serotypes of food poisoning-causing bacteria using the proteotyping method[*1] *S10-spc-alpha* operon Gene Encoded Ribosomal protein Mass Spectrum (*S10*-GERMS method) [1], which enables discrimination of microorganisms with high accuracy, surpassing that of the fingerprinting method. In addition, a highly-accurate bacterial discrimination software named Strain Solution Ver. 2 was developed, which is compatible with the iD$^{plus}$ (Fig. 1). In this communication, the use of MALDI-TOF MS and Strain Solution Ver. 2 for the discrimination of microorganisms to the level of serotypes is introduced, and an example of the application of the method for enterohemorrhagic *Escherichia coli*[*2] is described [2].

*1 proteotyping method:
In this method, protein components detected by MALDI-TOF MS are used as biomarkers for the discrimination of microorganisms. The biomarkers used for proteotyping are specified in advance on the basis of the nucleotide sequence data of the target gene. Compared to typing methods based on genetic techniques such as the conventional DNA sequencing method, proteotyping using MALDI-TOF MS offers the advantages of being faster, simpler, and easier to use. If the genome sequence of the targeted bacterial species has not yet been decoded, proteotyping can still be carried out by decoding the base sequence of the *S10-spc-alpha* operon, which encodes approximately half of all ribosomal proteins. The *S10*-GERMS method was developed by the Department of Environmental Bioscience, Faculty of Agriculture, Meijo University, and by the Environmental Measurement Technology Group, National Institute of Advanced Industrial Science and Technology.

*2 Enterohemorrhagic *Escherichia coli*:
Nearly 200 different serotypes of *Escherichia coli* (type O antigen) have been reported thus far, but the Shiga toxin-producing serotypes O157, O26, and O111 are the major pathogens responsible for food poisoning, and are therefore the most problematic. However, bacterial identification methods using conventional fingerprinting techniques are limited to the species level, which does not allow for determination of serotypes.

## 2. The *S10*-GERMS Method and Strain Solution Ver. 2

The *S10*-GERMS method identifies the genes encoding the proteins (biomarkers) detected as mass peaks by MALDI-TOF MS that are specific to serotypes or strains of microorganisms. As a result, a database of the theoretical masses of mass peaks that could potentially be used as biomarkers for the distinction between serotypes or strains can be constructed on the basis of the DNA sequence information of the gene as well as on the basis of the actual measured values. Strain Solution Ver. 2 matches the list of the mass peaks obtained from the sample with the database of theoretical values.

Unlike fingerprinting methods, the components of the biomarkers used in this method are well-defined, and therefore discrimination can be achieved even if the difference consists of only a single amino acid mutation resulting from a single-base mutation. For this reason, this method is also useful for the molecular phylogenetic analysis that follows microbial identification, as well as for the analysis of mixed samples (which will be described later).

1 Research Institute, Meijo University
2 Department of Environmental Bioscience, Faculty of Agriculture, Meijo University
3 Analytical & Measuring Instruments Division, Shimadzu Corporation
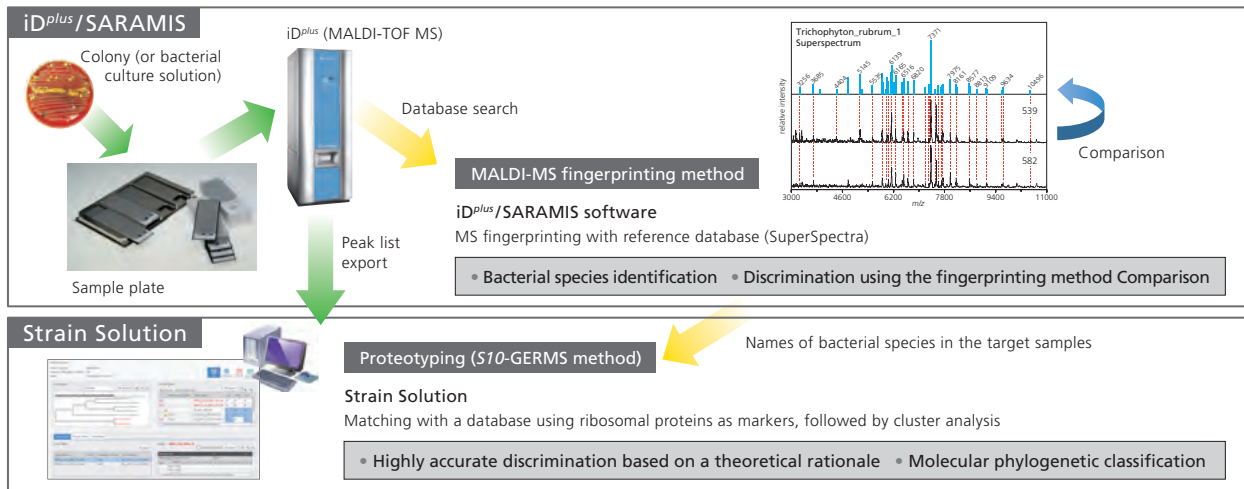
Fig. 1  iD$^{plus}$ and Strain Solution

# 3. Application of the Method

Here, we introduce the development of a database aimed at distinguishing among the major serotypes of enterohemorrhagic *Escherichia coli*, namely O157, O26, and O111, and provide an example of the analysis using Strain Solution Ver. 2.

## 3-1. Development of a Database Designed for Use with Strain Solution Ver. 2

First, 50 strains of *Escherichia coli* with various types of O antigens were obtained from culture collections; measurements were carried out with MALDI-TOF MS (iD$^{plus}$), and biomarker proteins that could potentially be used as indicators for serotype discrimination were selected. Genomic information or experimentally analyzed DNA sequence data were matched with data from actual measurements using MALDI-TOF MS, and a table of the theoretical masses of the selected biomarkers was created (Fig. 2). As a result, we found a number of biomarker candidates that showed mass numbers characteristic to each *Escherichia coli* serotype, and we were able to distinguish 12 groups (groups A–L) among the 50 strains of *Escherichia coli*.

Among these groups, we found that the presence or absence of the acid-stress protein HdeB (*m/z* 9066.2), the ribosomal protein subunits S15 (*m/z* 10166.6 or 10138.6) and L25 (*m/z* 10676.4 or 10694.4), as well as the DNA-binding protein H-NS (*m/z* 15409.4 or 15425.4) were characteristic of serotypes O157, O26, and O111, the major serotypes of enterohemorrhagic *Escherichia coli*. These biomarkers were also useful for distinguishing between the aforementioned serotypes and other *Escherichia coli* strains and serotypes.

## 3-2. Verification Using Wild-Type Strains

We carried out a verification of the practicality of the developed database using 45 wild-type strains of *Escherichia coli* of various serotypes, which were isolated from food poisoning patients as well as from food samples (Table 1) [3]. The strains were cultured in tryptone soy agar medium and common *Escherichia coli* selective media (desoxycholate agar medium, CT-SMAC medium, Chromagar X-gal medium, and VRBL medium). Specifically, the following steps were carried out for the verification (Fig. 1).

(1) On the basis of the theoretical mass list shown in Fig. 2, the marker peaks of HdeB, S15, L25, and H-NS of *Escherichia coli* strains O157, O26, O111, and K12 were recorded in the Strain Solution Ver. 2 database.

(2) Single colonies (approximately 10$^7$ cells) of the wild-type strains used in the verification experiments were cultured on agar medium and smeared on metal plates for analysis, and then mixed with 1 µL of matrix solution (20 mg/mL sinapic acid, 50% acetonitrile, 1% trifluoroacetic acid).

(3) Analyses were carried out using the iD$^{plus}$, and mass spectra were obtained.

(4) Using Strain Solution Ver. 2, the list of mass peaks in the resulting mass spectrum was matched with the marker peaks registered in the database.

Table 1  *Escherichia coli* Strains Used in the Verification Experiments [3]

| ID | Serotype | Gene | | | Source |
|---|---|---|---|---|---|
| | | stx1 | stx2 | eae | |
| KB0137 | O157 | + | + | N. D. | Patient (Japan) |
| KB0139 | O157 | + | + | N. D. | Patient (Japan) |
| KB0150 | O157 | − | − | N. D. | Patient (USA) |
| KB0152 | O157 | + | + | N. D. | Patient (Japan) |
| KB0155 | O157 | − | + | N. D. | Patient (Kenya) |
| KB0156 | O157 | + | + | N. D. | Patient (Japan) |
| KB0340 | O157 | − | + | N. D. | Beef liver |
| KB0341-1 | O157 | − | + | + | Beef omasum |
| KB0341-2 | O157 | − | − | − | Beef omasum |
| KB0514 | O157 | − | + | + | Patient (Japan) |
| KB0521 | O111 | + | − | + | Patient (Japan) |
| KB0522 | O157 | + | + | + | Patient (Japan) |
| KB0548 | O159 | + | + | N. D. | Patient (Kenya) |
| KB0549 | UT | − | + | N. D. | Patient (Kenya) |
| KB0617 | O26 | + | + | N. D. | Patient (unknown) |
| KB0618 | O26 | + | + | N. D. | Patient (unknown) |
| KB0619 | O26 | + | + | N. D. | Patient (unknown) |
| KB0620 | O26 | + | + | N. D. | Patient (unknown) |
| KB0621 | O26 | + | + | N. D. | Patient (unknown) |
| KB0622 | O26 | + | + | N. D. | Patient (unknown) |
| KB0623 | O26 | + | + | N. D. | Patient (unknown) |
| KB0624 | O26 | + | + | N. D. | Patient (unknown) |
| KB0625 | O26 | + | + | N. D. | Patient (unknown) |
| KB0626 | O26 | + | + | N. D. | Patient (unknown) |
| KB0627 | O111 | + | − | N. D. | Patient (Japan) |
| KB0628 | O111 | + | + | N. D. | Patient (Japan) |
| KB0732 | O121 | − | + | + | Patient (Japan) |
| KB0733 | UT | + | + | − | Minced meat |
| KB0734 | O145 | + | − | + | Patient (Japan) |
| KB0735 | O91 | + | − | − | Patient (Japan) |
| KB0738 | UT | − | + | + | Patient (Japan) |
| KB0739 | O121 | − | + | + | Patient (Japan) |
| KB0740 | O91 | + | + | − | Patient (Japan) |
| KB0741 | O145 | + | − | + | Patient (Japan) |
| KB0742 | O128 | + | + | − | Patient (Japan) |
| KB0743 | O91 | + | + | − | Patient (Japan) |
| KB0744 | UT | − | + | − | Patient (Japan) |
| KB0745 | O157 | + | + | + | Patient (Japan) |
| KB0746 | O115 | + | − | − | Patient (Japan) |
| KB0747 | O121 | − | + | + | Patient (Japan) |
| KB0748 | O145 | − | + | + | Patient (Japan) |
| KB0749 | O103 | + | − | + | Patient (Japan) |
| KB0750 | O103 | + | − | + | Patient (Japan) |
| KB0751 | O145 | + | − | + | Patient (Japan) |
| KB0752 | O145 | − | + | + | Patient (Japan) |

Serotypes were determined by performing antisera agglutination tests and PCR.
UT, untyped.
N. D., not determined.

2

## Theoretical mass database

| | | Group of mass pattern | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A | B | C | | D | | | | E | F | G | | H | | I | J | K | L |
| Number of strains | | 14 | 2 | 2 | 9 | 3 | 1 | 1 | 4 | 1 | 1 | 1 | 2 | 5 | 1 | 1 | | | 1 |
| Protein | Coded operon | O157 | O157 | O111 | O26 | O121 | O128 | O152 | - | O115 | O119 | O63 | - | K12 | - | - | - | - | O150 |
| L23 | S10 | 11200.1 | 11200.1 | 11200.1 | 11200.1 | 11200.1 | 11200.1 | 11200.1 | 11200.1 | 11147.1 | 11200.1 | 11200.1 | 11200.1 | 11200.1 | 11200.1 | 11200.1 | 11200.1 | 11200.1 | 11200.1 |
| L24 | spc | 11186.0 | 11186.0 | 11186.0 | 11186.0 | 11186.0 | 11186.0 | 11186.0 | 11186.0 | 11186.0 | 11186.0 | 11216.0 | 11216.1 | 11186.0 | 11186.0 | 11186.0 | 11186.0 | 11186.0 | 11216.1 |
| S14 | spc | 11450.3 | 11450.3 | 11450.3 | 11450.3 | 11450.3 | 11450.3 | 11450.3 | 11450.3 | 11450.3 | 11450.3 | 11450.3 | 11450.3 | 11450.3 | 11450.3 | 11464.3 | 11450.3 | 11450.3 | 11450.3 |
| L15 | spc | 14967.4 | 14967.4 | 14967.4 | 14967.4 | 14967.4 | 14967.4 | 14967.4 | 14967.4 | 14981.4 | 14945.0 | 14967.4 | 14967.4 | 14981.4 | 14981.4 | 14981.4 | 14967.4 | 14967.4 | 14967.4 |
| S11+Me | alpha | 13728.8 | 13728.8 | 13728.8 | 13728.8 | 13728.8 | 13728.8 | 13728.8 | 13728.8 | 13728.8 | 13728.8 | 13728.8 | 13728.8 | 13728.8 | 13728.8 | 13728.8 | 13728.8 | 13728.8 | 13756.8 |
| S15 | | 10166.6 | 10138.6 | 10138.6 | 10138.6 | 10138.6 | 10138.6 | 10138.6 | 10138.6 | 10138.6 | 10138.6 | 10138.6 | 10138.6 | 10138.6 | 10138.6 | 10137.6 | 10138.6 | 10138.6 | 10138.6 |
| L25 | | 10676.4 | 10694.4 | 10694.4 | 10694.4 | 10694.4 | 10694.4 | 10694.4 | 10694.4 | 10694.4 | 10694.4 | 10693.5 | 10693.5 | 10694.4 | 10694.4 | 10694.4 | 10694.4 | 10693.5 | 10693.5 |
| HdeB | | - | - | 9066.2 | 9066.2 | 9066.2 | 9066.2 | 9066.2 | 9066.2 | 9066.2 | 9066.2 | 9066.2 | 9066.2 | 9066.2 | 9066.2 | 9066.2 | 9066.2 | 9066.2 | 9066.2 |
| H-NS | | 15409.4 | 15409.4 | 15425.4 | 15425.4 | 15409.4 | 15409.4 | 15409.4 | 15409.4 | 15409.4 | 15409.4 | 15409.4 | 15409.4 | 15409.4 | 15409.4 | 15882.0 | 15409.4 | 15409.4 | 15409.4 |

### Biomarker peaks allowing for discrimination among *Escherichia coli* O157, O26, and O111

HdeB — Non-O157 / O157 (m/z: 8900, 9000, 9100, 9200)
S15 — Non-O157 / O157 (m/z: 10000, 10100, 10200)
L25 — Non-O157 / O157 (m/z: 10600, 10700, 10800)
H-NS — O26 / O111 / Others (m/z: 15200, 15300, 15400, 15500)

Intensity (%)
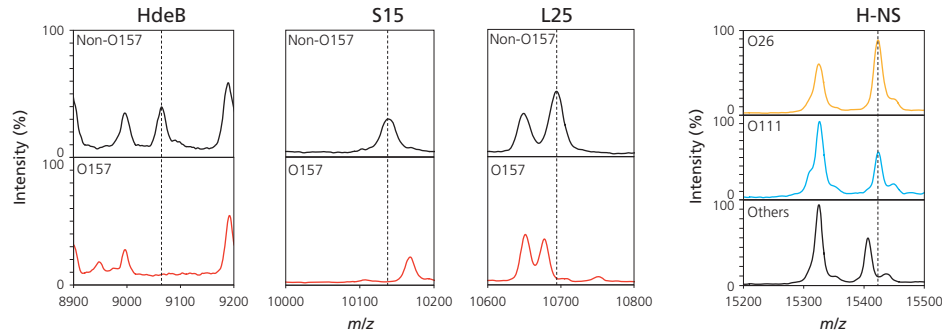
Fig. 2   Construction of the Database Used for Distinguishing Between Serotypes of Enterohemorrhagic *Escherichia coli* on the Basis of the *S10*-GERMS Method.

The results of discrimination using the cluster analysis function of Strain Solution Ver. 2 are shown in Fig. 3. The strains were broadly classified into the following four clusters: serotype O157 (cluster A), other serotypes (cluster B), serotypes O26 and O111 (cluster C), and serotype O157 (cluster D). The percentage rates of correct discrimination of the serotypes O157 (12 strains) and O26/O111 (13 strains) were 92% (11/12 strains) and 100% (13/13 strains), respectively. These findings showed a high degree of concordance with the results of the determination of serotypes using conventional methods. However, the 11 strains of the Shiga toxin-producing serotype O157 were classified with a 100% discrimination rate in the cluster of O157 (D), revealing that *Escherichia coli* O157 KB0341-2 (a non-shigatoxin producing strain), which was classified as "others" (cluster B), was different from the common O157 strains. In addition, O121 KB0747 had no HdeB peak; therefore, it was classified in the same group (group A) as GTC14550, a rare strain within the O157 serotype.

Fig. 3 shows the results of measurements conducted on colonies that were grown on tryptone soy agar medium, but similar results were also obtained from strains grown in various types of selective media. Of course, it is necessary to carry out verifications on a number of other samples as well; however, these results showed that our database and serotype discrimination by proteotyping seems to be effective for most wild-type strains. Another advantage of this method is that it allows for analyses to be conducted for colonies grown on selective culture media that are used in official methods as well as for those used in independent tests. As a result, this method can be applied to a wide variety of samples without having to change the conventional microbial testing process. The database described in this study is available as an option in the software Strain Solution Ver. 2. Further original databases can also be created depending on the purpose.

**O157 (A)**
E coli O157 GTC14550-15
KB0747 O121.txt

**Others (B)**
KB0746 O115.txt
KB0748 O145.txt
KB0744 UT.txt
KB0743 O91.txt
KB0742 O128.txt
KB0741 O145.txt
KB0740 O91.txt
KB0739 O121.txt
KB0749 O103.txt
KB0735 O91.txt
KB0734 O145.txt
KB0733 UT.txt
KB0732 O121.txt
KB0750 O103.txt
KB0549 UT.txt
KB0548 O159.txt
KB0341-2 O157.txt
E coli K12_W3110
KB0751 O145.txt
KB0752 O145.txt
KB0738 UT.txt

**O26 & O111 (C)**
KB0626 O26.txt
KB0625 O26.txt
KB0624 O26.txt
KB0623 O26.txt
KB0622 O26.txt
KB0621 O26.txt
KB0620 O26.txt
KB0619 O26.txt
KB0618 O26.txt
KB0617 O26.txt
KB0521 O111.txt
E coli O26 GTC14538-18
E coli O111 GTC14507-2
KB0627 O111.txt
KB0628 O111.txt

**O157 (D)**
KB0514 O157.txt
KB0341-1 O157.txt
KB0340 O157.txt
KB0156 O157.txt
KB0155 O157.txt
KB0152 O157.txt
KB0150 O157.txt
KB0139 O157.txt
KB0137 O157.txt
E coli O157 GTC14546-13
KB0522 O157.txt
KB0745 O157.txt

Fig. 3   Cluster Analysis Results
Strains registered in the database are shown in black, and wild-type strains used in the verification experiments are shown in red.

## 3-3. Mixed Samples

Here, *Escherichia coli* strain K12, which is a common strain used in experiments, was mixed at various ratios with the enterohemorrhagic *Escherichia coli* strain O157, and analyses were carried out using MALDI-TOF MS to verify whether Strain Solution Ver. 2 could identify the mixtures with three biomarkers as indicators. The results showed that when O157 was mixed at ratios of 10% to 80%, the software was able to correctly determine that the samples were mixtures of O157 and other *Escherichia coli* (K12 strains). When the samples were mixed at ratios of 40% to 70%, all three biomarkers were recognized as two mass peaks (Fig. 4) [3].

### Biomarker mass peaks



Mixing ratio between K12:O157
- 0:100
- 10:90
- 20:80
- 30:70
- 40:60
- 50:50
- 60:40
- 70:30
- 80:20
- 90:10
- 100:0
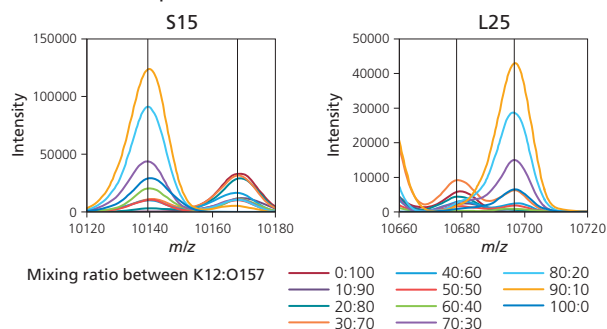
### Results from the Strain Solution

| Mixing ratio K12:O157 | Hit biomarker S15 | L25 | H-NS | Results of identifications using the Strain Solution |
|---|---|---|---|---|
| 100:0 | C | C | C | Other serotypes |
| 90:10 | A, C | C | A, C | O157 and other serotypes |
| 80:20 | A, C | C | A, C | O157 and other serotypes |
| 70:30 | A, C | C | A, C | O157 and other serotypes |
| 60:40 | A, C | A, C | A, C | O157 and other serotypes |
| 50:50 | A, C | A, C | A, C | O157 and other serotypes |
| 40:60 | A, C | A, C | A, C | O157 and other serotypes |
| 30:70 | A, C | A, C | A, C | O157 and other serotypes |
| 20:80 | A, C | A | A, C | O157 and other serotypes |
| 10:90 | A | A | A | O157 |
| 0:100 | A | A | A | O157 |

A: Mass corresponding to the biomarker of the O157 type (*Escherichia coli*).
C: Mass corresponding to the biomarker of other *Escherichia coli*

**Fig. 4   Discrimination of Mixed Bacteria**

Similar to conventional microbial identification methods, bacteria normally need to be isolated prior to analysis with MALDI-TOF MS; however, if closely related bacterial strains are mixed together, they cannot be distinguished from each other through the standard fingerprinting method. Therefore, such functions of Strain Solution Ver. 2 are useful for monitoring human error when dealing with several closely related bacterial strains. This is beneficial for the management of the quality of starter bacterial strains used in the food industry, as well as culture collections for the management of their microbial collections.

## 4. Conclusion and Future Prospects

Due to the rapidity and simplicity of MALDI-TOF MS, its use and applications are rapidly expanding in various fields, including for microbial control such as in clinical settings, the pharmaceutical industry, and the food industry. Several studies have reported the use of MALDI-TOF MS for direct measurements of bacteria from blood culture media and food samples in recent years, and simpler analyses with higher accuracy are needed. The proteotyping technique using Strain Solution Ver. 2 based on the *S10*-GERMS method allows for distinguishing the small changes in the mass of selected biomarkers with high sensitivity, which has thus far not been considered important. Therefore, this method can potentially be utilized in a wide range of applications, not only in the discrimination of serotypes or strains of single microorganisms but also in the detailed analysis and diagnosis of the intestinal flora or bacterial mixtures that do not allow for single-strain isolation.

## Acknowledgments

### References

[1]  Yudai Hotta, Kanae Teramoto, Hiroaki Sato, Hiromichi Yoshikawa, Akifumi Hosoda, and Hiroto Tamura: Classification of Genus Pseudomonas by MALDI-TOF MS Based on Ribosomal Protein Coding in *S10-spc-alpha* Operon at Strain Level. *J. Proteome Res.*, 2010, 9, 6722–6728.

[2]  Teruyo Ojima-Kato, Naomi Yamamoto, Mayumi Suzuki, Tomohiro Fukunaga, and Hiroto Tamura: Discrimination of *Escherichia coli* O157, O26 and O111 from other serovars by MALDI-TOF MS based on the *S10*-GERMS method. *PLoS ONE*, 2014, 9: e113458.

[3]  Teruyo Ojima-Kato, Naomi Yamamoto, Yoshio Iijima, and Hiroto Tamura: Assessing the performance of novel software Strain Solution on automated discrimination of *Escherichia coli* serotypes and their mixtures using matrix-assisted laser desorption ionization-time of flight mass spectrometry. *J. Microbiol. Methods*, 2015, 119, 233–238.

Strain Solution software was created on the basis of the results obtained from the Food Safety & Security Technology Development Project (group leader: Professor Hiroto Tamura of the Laboratory of Environmental Microbiology, Faculty of Agriculture, Meijo University), a "Knowledge Hub Aichi" core research project of Aichi Prefecture, which is in-turn based on ideas from the Meijo University and the National Institute of Advanced Industrial Science and Technology.

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**Application Data Sheet**

**No.127A**

## GC-MS
Gas Chromatograph Mass Spectrometer

## Simultaneous Analysis of 418 Pesticides Utilizing Smart SIM

The selected ion monitoring (SIM) method is capable of high-sensitivity measurements, so it is utilized for the analysis of trace components, such as residual pesticides in foods. With existing SIM conditions settings, however, the number of components is limited to a few hundred, and configuring the settings is difficult. In addition, when many ions are monitored simultaneously, the sensitivity is reduced. Smart SIM was developed to solve these problems. This article provides an example of its application to the simultaneous analysis of 418 pesticides.

### Smart SIM
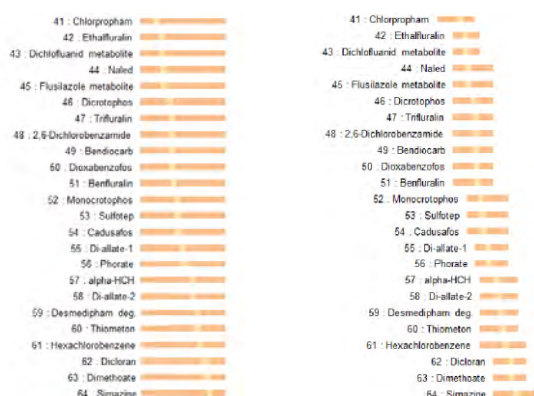


Fig. 1: SIM Measurement Time Program
(Left: Group Measurement Method;
Right: Measurement Method Using Smart SIM)
By using Smart SIM, the optimal time program synchronized to the retention times for each compound can be created.

As shown in Fig. 1, with the existing SIM method, a time program must be configured in order to collect data on designated ions only for designated time periods. If the number of target compounds exceeds 100, this time program becomes very complex. With the existing group measurement method (Fig. 1, left), configuring the settings is difficult, and sufficient sensitivity is not obtained because many ions are measured simultaneously.

In this example, a method was created utilizing Smart SIM, which automatically creates the optimal time program synchronized to the retention times for each compound (Fig. 1, right), and the sensitivity and accuracy were then evaluated.

In order to create a method with Smart SIM, the first step is to prepare the Smart Database (Fig. 2), which can be created simply by importing an existing method file if there is one. The retention index information is also registered; therefore, by utilizing the AART function with Smart Database, retention times can be estimated and adjusted without analyzing standard samples. Here, a method was created utilizing retention times adjusted with the AART function. The analytical conditions are indicated in Table 1.

(For details on retention time adjustments utilizing retention indices, check the Shimadzu website. http://www.an.shimadzu.co.jp/gcms/gcmssol/sol1.htm)

| Serial# | Type | Acq. Mode | Compound Name (E) | Ret. Index 1 | Ret. Time | Ion1 Type | Ion1 m/z | Ion1 Rati | Ion2 Type | Ion2 m/z | Ion2 Rati | Ion3 Type | Ion3 m/z | Ion3 Rati |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 41 | Target | SIM | Chlorpropham | 1666 | 10.228 | T | 213.0 | 100.00 | Ref.1 | 127.0 | 219.30 | | 154.0 | 56.14 |
| 42 | Target | SIM | Ethalfluralin | 1675 | 10.324 | T | 276.0 | 100.00 | Ref.1 | 316.0 | 78.70 | | 333.0 | 23.15 |
| 43 | Target | SIM | Dichlofluanid metabolite | 1676 | 10.334 | T | 200.0 | 100.00 | Ref.1 | 92.0 | 126.89 | | 108.0 | 22.13 |
| 44 | Target | SIM | Naled | 1678 | 10.355 | T | 185.0 | 100.00 | Ref.1 | 109.0 | 595.24 | | 145.0 | 152.38 |
| 45 | Target | SIM | Flusilazole metabolite | 1678 | 10.355 | T | 235.0 | 100.00 | Ref.1 | 250.0 | 14.80 | | 155.0 | 9.60 |
| 46 | Target | SIM | Dicrotophos | 1688 | 10.461 | T | 127.0 | 100.00 | Ref.1 | 193.0 | 9.60 | | 237.0 | 6.80 |
| 47 | Target | SIM | Trifluralin | 1691 | 10.493 | T | 306.0 | 100.00 | Ref.1 | 264.0 | 88.68 | | 335.0 | 8.18 |
| 48 | Target | SIM | 2,6-Dichlorobenzamide | 1691 | 10.493 | T | 173.0 | 100.00 | Ref.1 | 175.0 | 63.60 | | 189.0 | 44.00 |
| 49 | Target | SIM | Dioxabenzofos (Salithion) | 1692 | 10.504 | T | 216.0 | 100.00 | Ref.1 | 183.0 | 45.60 | | 201.0 | 26.80 |
| 50 | Target | SIM | Benfluralin | 1695 | 10.535 | T | 292.0 | 100.00 | Ref.1 | 264.0 | 24.40 | | 293.0 | 12.40 |
| 51 | Target | SIM | Monocrotophos | 1700 | 10.588 | T | 127.0 | 100.00 | Ref.1 | 97.0 | 18.00 | | 192.0 | 10.00 |
| 52 | Target | SIM | Sulfotep | 1702 | 10.609 | T | 322.0 | 100.00 | Ref.1 | 238.0 | 39.60 | | 266.0 | 39.20 |
| 53 | Target | SIM | Cadusafos | 1703 | 10.619 | T | 159.0 | 100.00 | Ref.1 | 158.0 | 69.20 | | 270.0 | 5.20 |
| 54 | Target | SIM | Di-allate-1 | 1711 | 10.702 | T | 234.0 | 100.00 | Ref.1 | 236.0 | 39.73 | | 86.0 | 254.79 |
| 55 | Target | SIM | Phorate | 1713 | 10.723 | T | 260.0 | 100.00 | Ref.1 | 231.0 | 54.29 | | 75.0 | 714.29 |

Fig. 2: Smart Database Screen

### Table 1: Analytical Conditions

GC-MS:          GCMS-QP2020
Column:         SH-Rtx-5MS (30 m long, 0.25 mm I.D., df = 0.25 μm) (Shimadzu GLC, P/N: 221-75855-30)
Glass Insert:   Sky Single Taper Inlet Liner w/ Wool (Shimadzu GLC, P/N: 23336.5)

**GC**
Injection Port Temperature:  250 °C
Column Oven Temperature:     50 °C (1.0 min) → (25 °C /min) → 125 °C → (10 °C/min) → 300 °C (15 min)
Injection Mode:              Splitless
High-Voltage Injection:      250 kPa (1.5 min)
Injection Volume:            2 μL
Carrier Gas Control:         Linear velocity (47.2 cm/sec)

**MS**
Interface Temperature:    250 °C
Ion Source Temperature:   200 °C
Ionization Mode:          EI
Measurement mode:         SIM
Loop Time:                0.5 sec

## Results

To create the sample, polyethylene glycol 300 was added as a pseudo matrix to a pesticides standard mixed solution to reach a concentration of 200 $\mu$g/mL. Fig. 3 shows the SIM chromatogram for the 100 ng/ml standard solution, and Fig. 4 shows the SIM chromatograms for each pesticide, as well as the %RSD and S/N ratios. Fig. 5 shows the results for the same sample, measured with a 244 component simultaneous analysis method via the existing group measurement method.
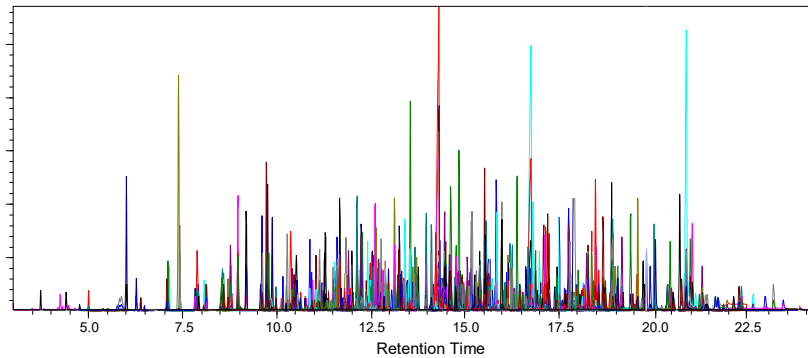


Fig. 3: SIM Chromatogram for a 100 ng/mL Pesticides Standard Mixed Solution



Folpet — %RSD = 8.3 % — S/N = 35.6

Zoxamide — %RSD = 5.9 % — S/N = 66.2

Flucythrinate-1,2 — %RSD = 6.0, 4.8 % — S/N = 34.2, 25.8

Fig. 4: SIM Chromatograms, %RSD and Average S/N Ratios (n=6) for 2 ng/mL Sample



%RSD = 15.2 % — S/N = 16.0

%RSD = 24.0 % — S/N = 21.0

%RSD = 9.1, 11.3 % — S/N = 16.1, 11.3
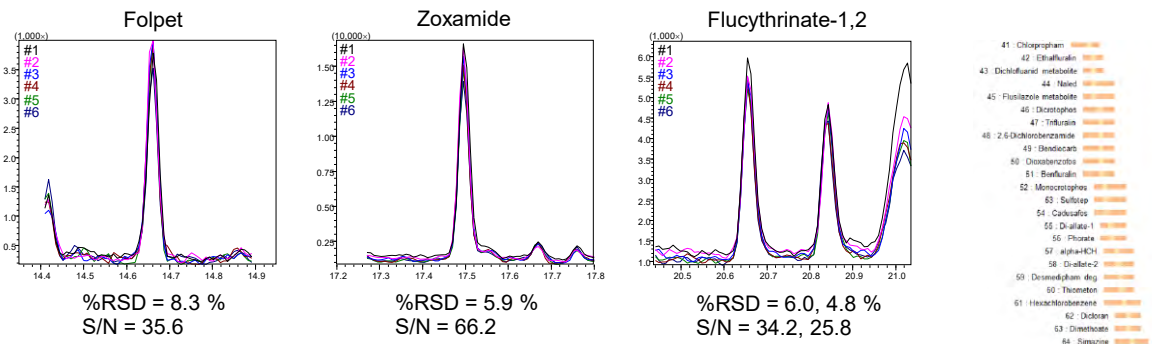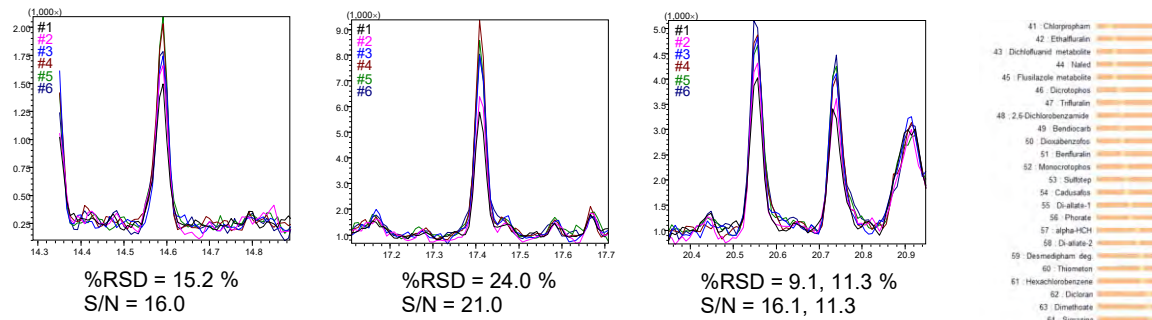
Fig. 5: SIM Chromatograms, %RSD and Average S/N Ratios (n=6) for 2 ng/mL Sample with the Group Measurement Method

Using Smart SIM, a method was easily created that enables the simultaneous analysis of more than 400 components. In addition, it was evident that the analysis could be performed with higher sensitivity and higher accuracy in comparison to a method configured with the existing group measurement method. Further, as shown in Fig. 6, for more than 90 % of the 2 ng/mL components, the %RSD was 10 % max., indicating a highly accurate analysis.

Furthermore, with the existing method, to ensure sensitivity and accuracy, when the number of components exceeds 200, multiple measurement cycles are performed by dividing the method into several sections. In contract, Smart SIM allows obtaining the results with a single measurement. This can significantly reduce the analysis time. In addition, since the number of analyses per sample is reduced, so too are the frequency of maintenance and the cost, which will significantly improve laboratory productivity.



- < 10% : 93.0
- 10 - 20% : 5.0
- > 20% : 1.9
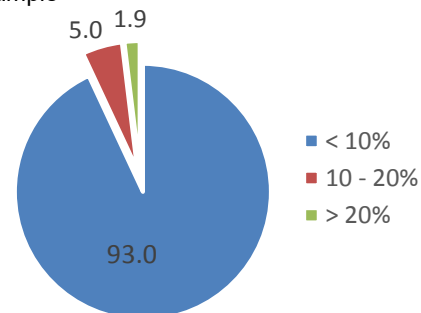
Fig. 6: %RSD Distribution for 2 ng/mL Sample
(Excluding oxpoconazole-formyl deg., which were not detected.)

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# Fully automated derivatization and quantification of Glyphosate and AMPA in beer using a standard UHPLC-MS/MS system

## ASMS 2017 MP205

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**Fully automated derivatization and quantification of Glyphosate and AMPA in beer using a standard UHPLC-MS/MS system**

# Introduction

Glyphosate is currently one of the most common pesticides used worldwide. In spite of its approval by regulatory bodies all over the world, the concern about its harm to humans and the environment persists. Therefore, the strict control of Glyphosate and its metabolite Aminomethylphosphonic acid (AMPA) in food and environment is mandatory.
The chromatography of glyphosate is challenging due to its high polarity. In order to overcome this, there exists a well-established method including a derivatization step with 9-fluorenylmethyl chloroformate (FMOC) followed by LCMS analysis.
Here we report a fully automated derivatization followed by LC-MS/MS analysis of beer samples. The instrumental set-up does not require any additional hardware for sample pretreatment but uses the built-in pretreatment function of the autosampler.



**FMOC-Cl**

**Glyphosat**
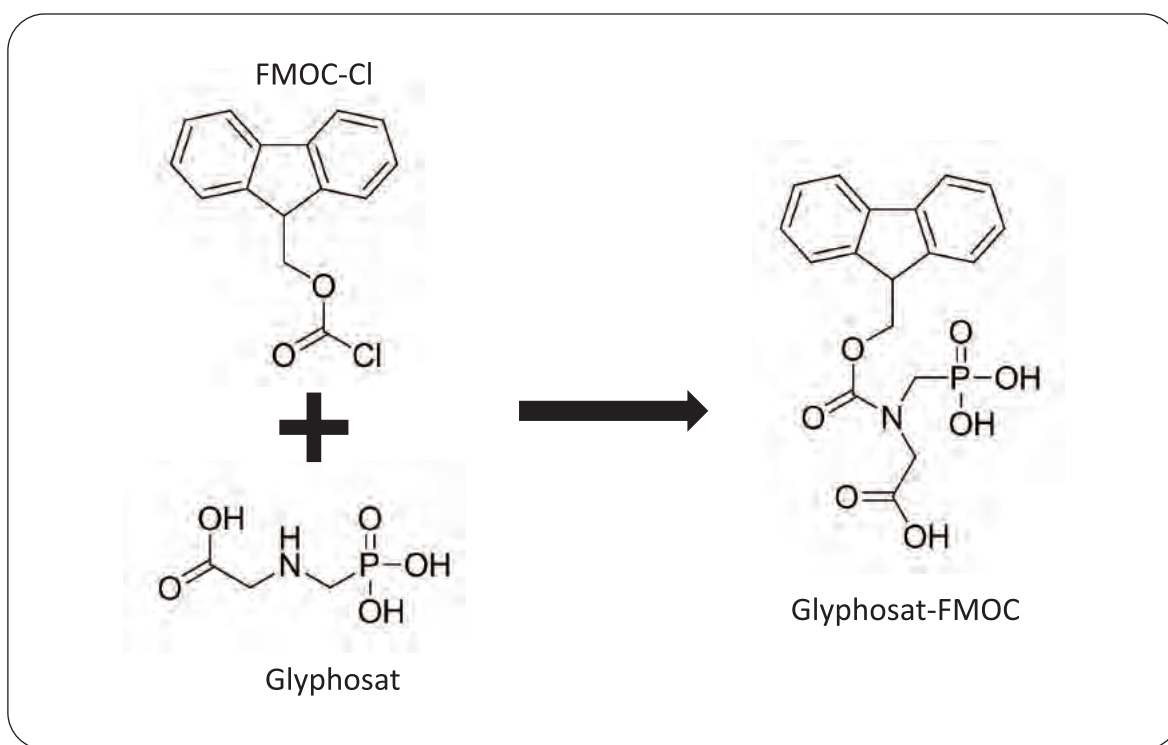
**Glyphosat-FMOC**

Figure 1   Derivatization of Glyphosat with FMOC

# Methods and Materials

## Sample Preparation

After precipitation with methanol (50:50) and centrifugation the beer samples were set into the autosampler.

## UHPLC method

| | | |
|---|---|---|
| Instrument | : | Nexera UHPLC, Shimadzu |
| Column | : | Gemini 5 µm C18, 150 x 2 mm |
| Mobile phase A | : | 2 mm $NH_4HCO_3$, pH 9.5 |
| B | : | acetonitrile |
| Flow rate | : | 0.4 mL/min |
| Time program | : | B conc. 5%(0 min) -50%(7 min) - 95%(7.01-12min) – 5% (12.01 min – 15 min) |
| Injection vol. | : | 50 µL |
| Column temperature | : | 35 °C |

## MS conditions

| | | |
|---|---|---|
| Instrument | : | LCMS-8060, Shimadzu |
| Ionization | : | pos/neg ESI |
| Nebulizing gas | : | 3 L/min |
| Heating gas | : | 15 L/min |
| Drying gas | : | 5 L/min |
| Interface temperature | : | 325 °C |
| DL temperature | : | 150 °C |
| Heat block temperature | : | 400 °C |
| CID gas | : | 270 kPa |
| Interface voltage | : | 4 kV/ -3 kV |

# Results

## Method development for automatization of derivatization

The addition of internal standards as well as the derivatization of Glyphosate and AMPA with FMOC was done fully automated by the autosampler SIL-30AC within 15 minutes. After derivatization the sample was injected directly to the LC-MS/MS and analyzed accordingly.

# Fully automated derivatization and quantification of Glyphosate and AMPA in beer using a standard UHPLC-MS/MS system



**Protein precipitation**
- Mix 1mL beer with 1 mL MeOH
- Vortex thoroughly
- Centrifuge 15 min, 12,000 RPM, RT

**Internal standard**
- Transfer 75 µL sample to a new vial
- Add 3 µL internal standard

**derivatization**
- Add 5 µL EDTA-Borate
- Add 15 µL FMOC

**Stop reaction**
- Add 5 µL formic acid

Done by SIL-30AC autosampler within 15 minutes including incubation

**Automated injection to LCMS/MS**
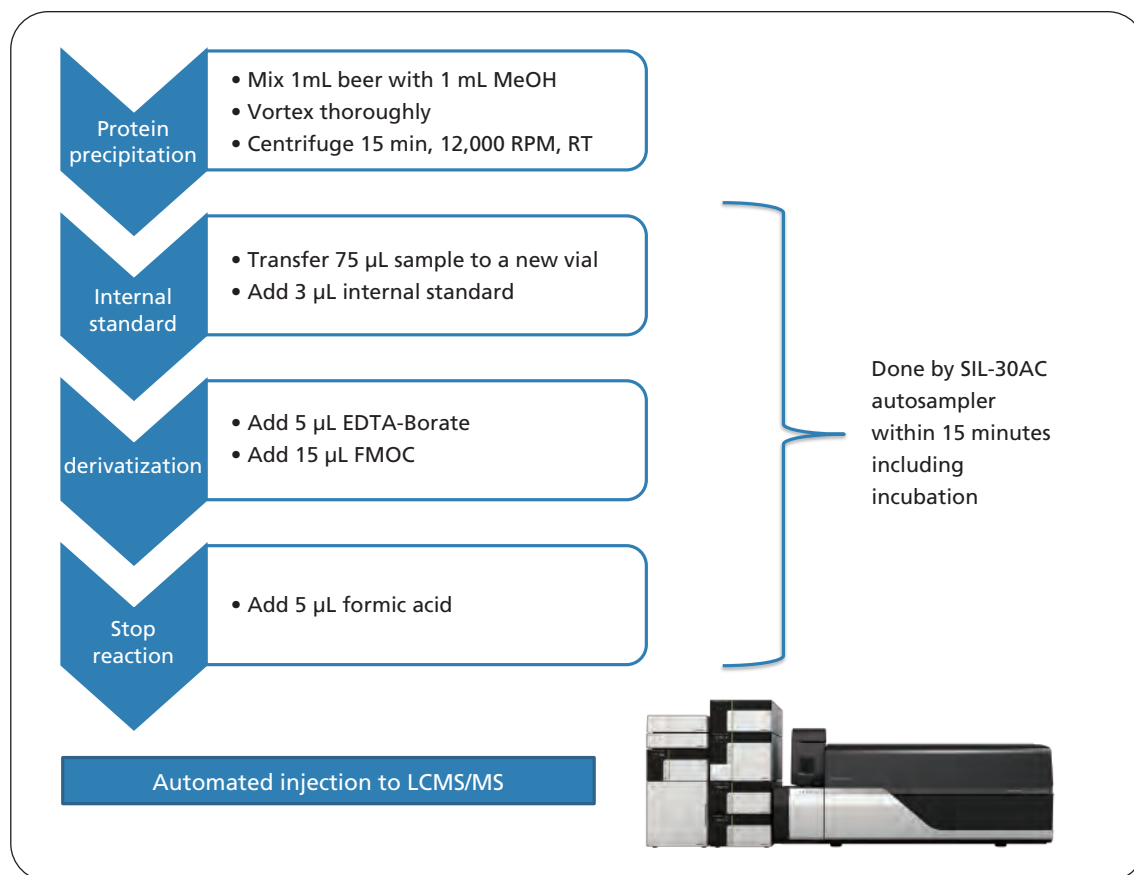
Figure 2: Workflow of sample pretreatment. Addition of internal standard as well as derivatization is done by the autosampler.

Due to overlapping sample pretreatment functionality, the next sample was already pretreated during the on-going analysis in order to maximize sample throughput. Except for the first and the last sample, the total time per sample for automated pretreatment and analysis can be reduced to 15 minutes.



injection     injection     injection

15 min     15 min     15 min     15 min

LCMS analysis     LCMS analysis     LCMS analysis

derivatization     derivatization     derivatization
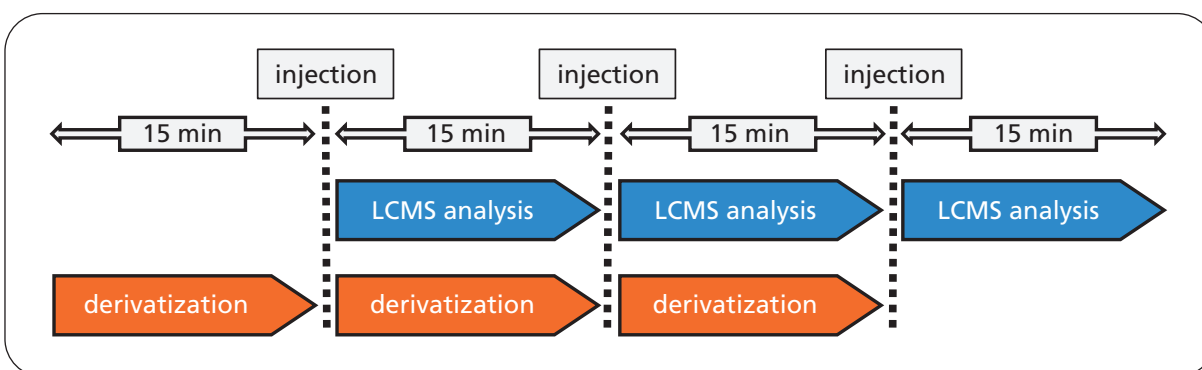
Figure 3: Overlapping sample pretreatment and analysis done by SIL-30AC. Total time per sample is reduced to 15 minutes.

# Fully automated derivatization and quantification of Glyphosate and AMPA in beer using a standard UHPLC-MS/MS system

Table 1: QC sample results

| Batch | Glyphosate-FMOC | | | | | | AMPA-FMOC | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | QC 3 ng/mL | | QC 15 ng/mL | | QC 75 ng/mL | | QC 3 ng/mL | | QC 15 ng/mL | | QC 75 ng/mL | |
| | Conc. | Acc.% | Conc. | Acc.% | Conc. | Acc.% | Conc. | Acc.% | Conc. | Acc.% | Conc. | Acc.% |
| A | 2,60 | 86,5 | 14,89 | 99,3 | 74,14 | 98,9 | 4,76 | 158,5 | 15,66 | 104,4 | 80,80 | 107,7 |
| A | 2,87 | 95,7 | 14,96 | 99,7 | 81,22 | 108,3 | 2,71 | 90,3 | 16,16 | 107,7 | 85,65 | 114,2 |
| A | 3,41 | 113,5 | 15,14 | 100,9 | 77,94 | 103,9 | 3,15 | 105,0 | 15,99 | 106,6 | 81,38 | 108,5 |
| B | 2,81 | 93,7 | 16,00 | 106,7 | 79,18 | 105,6 | 4,11 | 137,0 | 15,33 | 102,2 | 78,40 | 104,5 |
| B | 3,20 | 106,7 | 16,08 | 107,2 | 76,19 | 101,6 | 3,49 | 116,2 | 15,20 | 101,3 | 82,23 | 109,6 |
| B | 3,46 | 115,3 | 15,42 | 102,8 | 83,74 | 111,6 | 3,02 | 100,8 | 15,66 | 104,4 | 84,15 | 112,2 |
| C | 2,82 | 93,9 | 14,94 | 99,6 | 67,88 | 90,5 | 3,48 | 115,9 | 15,48 | 103,2 | 83,97 | 112,0 |
| C | 2,73 | 91,1 | 15,67 | 104,5 | 76,89 | 102,5 | 3,25 | 108,3 | 16,55 | 110,3 | 79,72 | 106,3 |
| C | 3,27 | 109,0 | 15,87 | 105,8 | 84,87 | 113,2 | 3,38 | 112,6 | 16,87 | 112,5 | 82,65 | 110,2 |
| D | 3,19 | 106,2 | 16,42 | 109,5 | 82,82 | 110,4 | 2,73 | 90,9 | 16,85 | 112,3 | 75,46 | 100,6 |
| D | 3,33 | 110,9 | 16,00 | 106,7 | 85,29 | 113,7 | 3,31 | 110,4 | 14,35 | 95,7 | 72,06 | 96,1 |
| D | 3,23 | 107,6 | 17,14 | 114,3 | 84,74 | 113,0 | 3,55 | 118,3 | 15,50 | 103,3 | 75,97 | 101,3 |
| Mean | 3,08 | | 15,71 | | 79,57 | | 3,41 | | 15,80 | | 80,20 | |
| SD | 0,2915 | | 0,6816 | | 5,2735 | | 0,5676 | | 0,7306 | | 4,0615 | |
| RSD (%) | 9,5 | | 4,3 | | 6,6 | | 16,6 | | 4,6 | | 5,1 | |
| | | | | | | | | extrapolated | | | | |
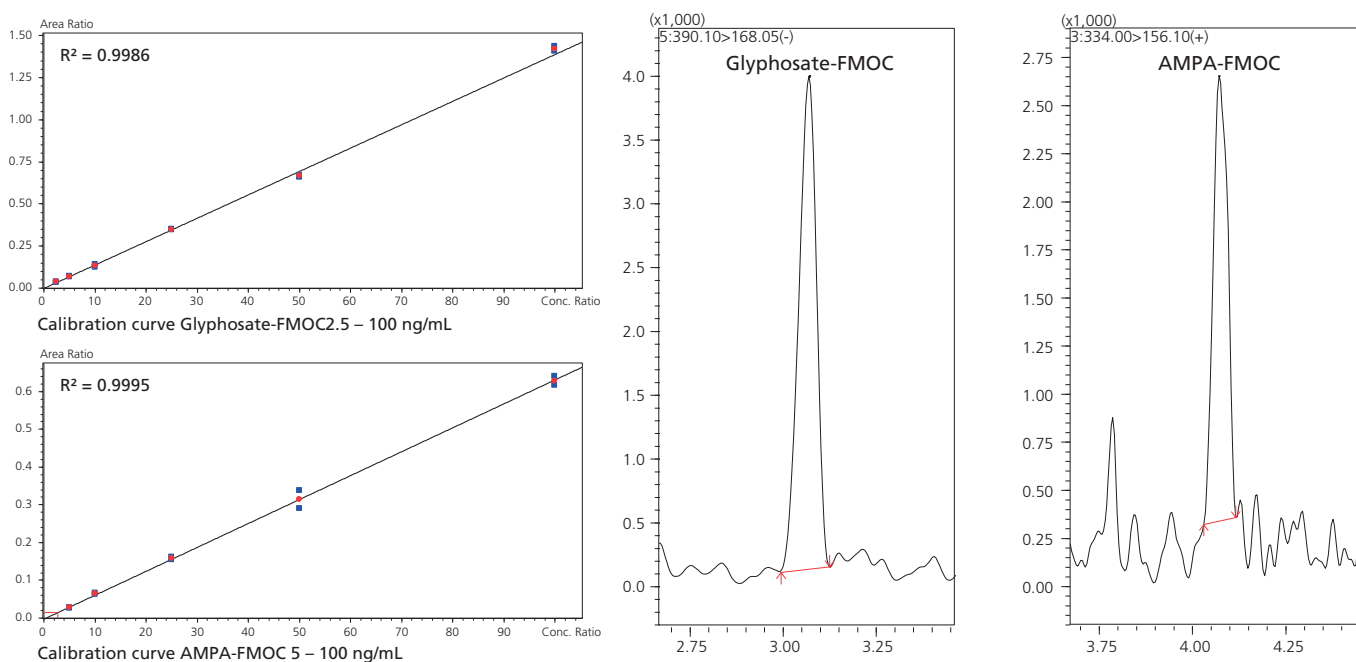


Figure 4:  Chromatogram of Glyphosate-FMOC (2.5 ng/mL) and AMPA-FMOC (5 ng/mL) at their respective LOQs and calibration curves.

## Quantitative Analysis of 40 beer samples

A total of 40 commercially available beer samples were analysed. Among these samples there were 21 samples of beer brewed according to Pilsener style, 3 samples of organic beer, 10 samples of other types of beer and 6 samples of alcohol-free beers or non alcoholic beer mix drinks. All samples were analysed in duplicate in two consecutive runs. While Glyphosate was detected in 60 % of all samples its metabolite AMPA was below LOQ in all samples.

Table 2: Analysis of beer samples

| | Glyphosat-FMOC | | | | | AMPA-FMOC | |
| --- | --- | --- | --- | --- | --- | --- | --- |
| | Conc. ng/mL | Conc. ng/mL | Mean | SD | % RSD | Conc. ng/mL | Conc. ng/mL |
| Pils | | | | | | | |
| Sample 1 | <LOQ | <LOQ | | | | <LOQ | <LOQ |
| Sample 2 | 8,37 | 8,95 | 8,7 | 0,4087 | 4,7 | <LOQ | <LOQ |
| Sample 3 | 20,85 | 20,28 | 20,6 | 0,4038 | 2,0 | <LOQ | <LOQ |
| Sample 4 | <LOQ | <LOQ | | | | <LOQ | <LOQ |
| Sample 5 | 6,78 | 6,57 | 6,7 | 0,1549 | 2,3 | <LOQ | <LOQ |
| Sample 6 | 11,34 | 12,08 | 11,7 | 0,5240 | 4,5 | <LOQ | <LOQ |
| Sample 7 | <LOQ | <LOQ | | | | <LOQ | <LOQ |
| Sample 8 | 8,61 | 9,41 | 9,0 | 0,5706 | 6,3 | <LOQ | <LOQ |
| Sample 9 | 4,74 | 4,63 | 4,7 | 0,0834 | 1,8 | <LOQ | <LOQ |
| Sample 10 | <LOQ | <LOQ | | | | <LOQ | <LOQ |
| Sample 11 | 10,81 | 12,03 | 11,4 | 0,8627 | 7,6 | <LOQ | <LOQ |
| Sample 12 | 13,95 | 14,65 | 14,3 | 0,4943 | 3,5 | <LOQ | <LOQ |
| Sample 13 | 33,06 | 27,61 | 30,3 | 3,8509 | 12,7 | <LOQ | <LOQ |
| Sample 14 | 20,29 | 18,68 | 19,5 | 1,1377 | 5,8 | <LOQ | <LOQ |
| Sample 15 | 25,28 | 22,09 | 23,7 | 2,2578 | 9,5 | <LOQ | <LOQ |
| Sample 16 | 3,23 | 2,93 | 3,1 | 0,2171 | 7,1 | <LOQ | <LOQ |
| Sample 17 | 3,66 | 3,48 | 3,6 | 0,1308 | 3,7 | <LOQ | <LOQ |
| Sample 18 | 5,25 | 5,65 | 5,4 | 0,2807 | 5,2 | <LOQ | <LOQ |
| Sample 19 | 2,67 | 2,93 | 2,8 | 0,1881 | 6,7 | <LOQ | <LOQ |
| Sample 20 | 3,87 | 4,39 | 4,1 | 0,3698 | 9,0 | <LOQ | <LOQ |
| Sample 21 | <LOQ | <LOQ | | | | <LOQ | <LOQ |
| Organic Beer | | | | | | | |
| Sample 22 | <LOQ | <LOQ | | | | <LOQ | <LOQ |
| Sample 23 | <LOQ | <LOQ | | | | <LOQ | <LOQ |
| Sample 24 | <LOQ | <LOQ | | | | <LOQ | <LOQ |
| Others | | | | | | | |
| Sample 25 | 2,79 | 3,26 | 3,0 | 0,3323 | 11,0 | <LOQ | <LOQ |
| Sample 26 | 4,61 | 4,15 | 4,4 | 0,3260 | 7,4 | <LOQ | <LOQ |
| Sample 27 | <LOQ | <LOQ | | | | <LOQ | <LOQ |
| Sample 28 | <LOQ | <LOQ | | | | <LOQ | <LOQ |
| Sample 29 | 2,52 | <LOQ | | | | <LOQ | <LOQ |
| Sample 30 | <LOQ | <LOQ | | | | <LOQ | <LOQ |
| Sample 31 | <LOQ | <LOQ | | | | <LOQ | <LOQ |
| Sample 32 | 8,06 | 7,27 | 7,7 | 0,5621 | 7,3 | <LOQ | <LOQ |
| Sample 33 | 11,19 | 11,57 | 11,4 | 0,2737 | 2,4 | <LOQ | <LOQ |
| Sample 34 | <LOQ | <LOQ | | | | <LOQ | <LOQ |
| Non alcoholic | | | | | | | |
| Sample 35 | 4,75 | 4,47 | 4,6 | 0,1952 | 4,2 | <LOQ | <LOQ |
| Sample 36 | 16,05 | 15,71 | 15,9 | 0,2454 | 1,5 | <LOQ | <LOQ |
| Sample 37 | <LOQ | <LOQ | | | | <LOQ | <LOQ |
| Sample 38 | <LOQ | <LOQ | | | | <LOQ | <LOQ |
| Sample 39 | <LOQ | <LOQ | | | | <LOQ | <LOQ |
| Sample 40 | 2,50 | 2,85 | 2,7 | 0,2482 | 9,3 | <LOQ | <LOQ |

# Conclusions

- Fully automated FMOC-derivatization of Glyphosate and AMPA within 15 minutes.
- No additional hardware required
- Sample derivatization and internal standard addition done by autosampler SIL-30AC
- Maximized sample throughput due to overlapping sample pretreatment functionality
- Robust and reliable method for Glyphosat and AMPA even in a complex matrix like beer

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# Determination of pyrrolizidine alkaloids in plant material using on-line SPE coupled to UHPLC-MS/MS

## ASMS 2018 WP-249

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# Introduction

Pyrrolizidine alkaloids (PAs) are secondary plant metabolites that are supposed to be carcinogenic and genotoxic. They occur mainly in plants of the Boraginaceae, Asteraceae and Fabaceae families. They contain a pyrrolizidine core and make up a large group of heterocyclic alkaloids mainly derived from the 4 Necin bases platynecine, retronecine, heliotridin and ontonecin. PAs are hepatotoxic if they carry a 1,2-double bond as well as an esterified side chain which is a structural prerequisite for their hepatic activation. Plant food and beverage, phytopharmaceuticals or even animal feed can easily be contaminated with PAs and enter the food chain. Currently there are discussions on possible regulatory measures caused by the presence of PAs in honey, tea, herbal infusions and food supplements. Existing methods include laborious sample preparation, e.g. solid-liquid extraction followed by solid phase extraction for clean-up. Here we report an on-line SPE UHPLC-MS/MS method, which overcomes the difficulties of combining low pressure online SPE with high pressure analytical UHPLC.
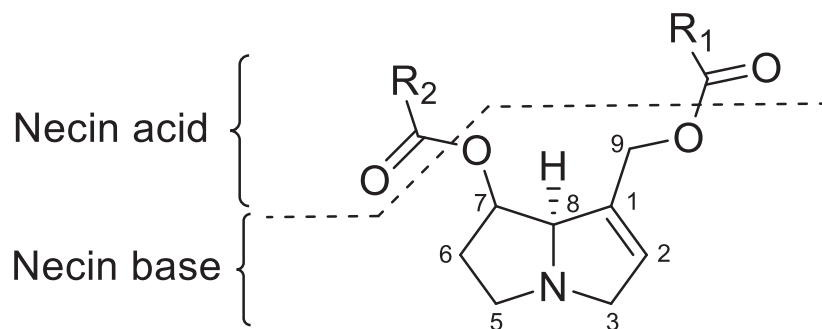


Figure 1　General structure of pyrrolizidine alkaloids

# Methods and Materials

## Sample Preparation

Tea samples were extracted twice with 0.05M sulfuric acid by sonication. Before centrifugation the pH of the combined extracts was adjusted with ammonium hydroxide.

## UHPLC method

| | | |
|---|---|---|
| Instrument | : | Nexera UHPLC, Shimadzu |
| Column | : | Shim-pack XR-ODS III, 150 mm x 2.0 mm, 2.2 µm, Shimadzu |
| Mobile phase A | : | 5 mM ammonium formate + 0.1% formic acid |
| B | : | methanol + 5 mM ammonium formate + 0.1% formic acid |
| Flow rate | : | 0.4 mL/min |
| Time program | : | B conc. 1% (0-1.6 min) -50% (14.6 min) – 71.5% (18.1 min) |
| | | – 95% (18.2 min – 20.2 min) -1% (20.3 min – 25 min) |
| Column temperature | : | 30 °C |

## Online SPE method

| | | |
|---|---|---|
| Column | : | EVOLUTE® EXPRESS ABN, 30 x 2.1 mm, Biotage |
| Mobile phase | : | 5 mM ammonium formate + 0.1% formic acid for sample loading |
| | | methanol / H2O + 5 mM ammonium formate + 0.1% formic acid |
| | | methanol, isopropanol for washing of SPE column |
| Flow rate | : | 0.2 / 2 mL/min |
| Injection vol. | : | 50 µL |
| Column temperature | : | RT |

## MS conditions

| | | |
|---|---|---|
| Instrument | : | LCMS-8060, Shimadzu |
| Ionization | : | pos ESI |
| Nebulizing gas | : | 3 L/min |
| Heating gas | : | 15 L/min |
| Drying gas | : | 5 L/min |
| Interface temperature | : | 400 °C |
| DL temperature | : | 300 °C |
| Heat block temperature | : | 400 °C |
| CID gas | : | 270 kPa |
| Interface voltage | : | 1 kV |

# Result

## Method development of the online SPE

The neutralized and centrifuged tea extract samples were put into the autosampler and transferred to the on-line SPE column using an aqueous solution. After washing the sample was eluted with only 10 µL solvent and trapped into a loop. By switching the loop the eluted sample was transferred to the analytical column. A binary gradient separated the PAs for quantification. Due to this hardware set-up UHPLC with high backpressure and on-line SPE which is pressure limited were successfully combined. By careful fine-tuning of the SPE elution and the chromatographic conditions the separation of critical peak pairs could be maintained.
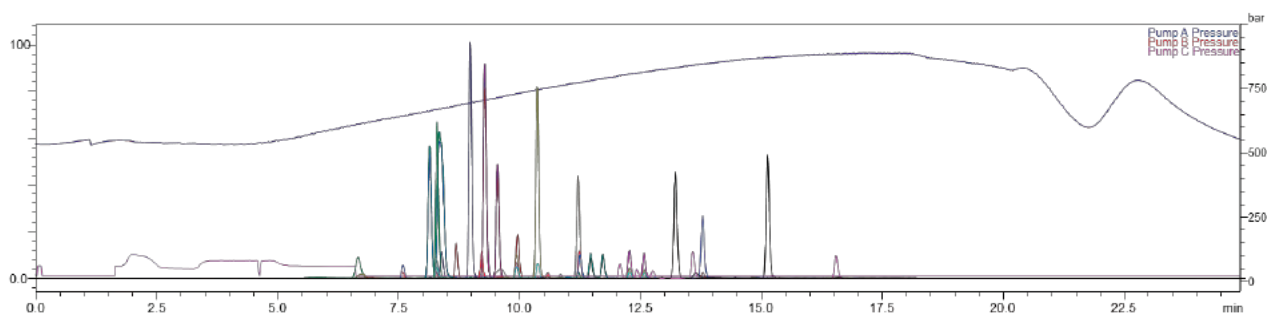


Figure 2   Typical chromatogram of pyrrolizidine alkaloids in tea matrix including the separated pressure curves
of the analytical column (Pump A and B pressure) and the online SPE column (Pump C pressure)

# Determination of pyrrolizidine alkaloids in plant material using on-line SPE coupled to UHPLC-MS/MS
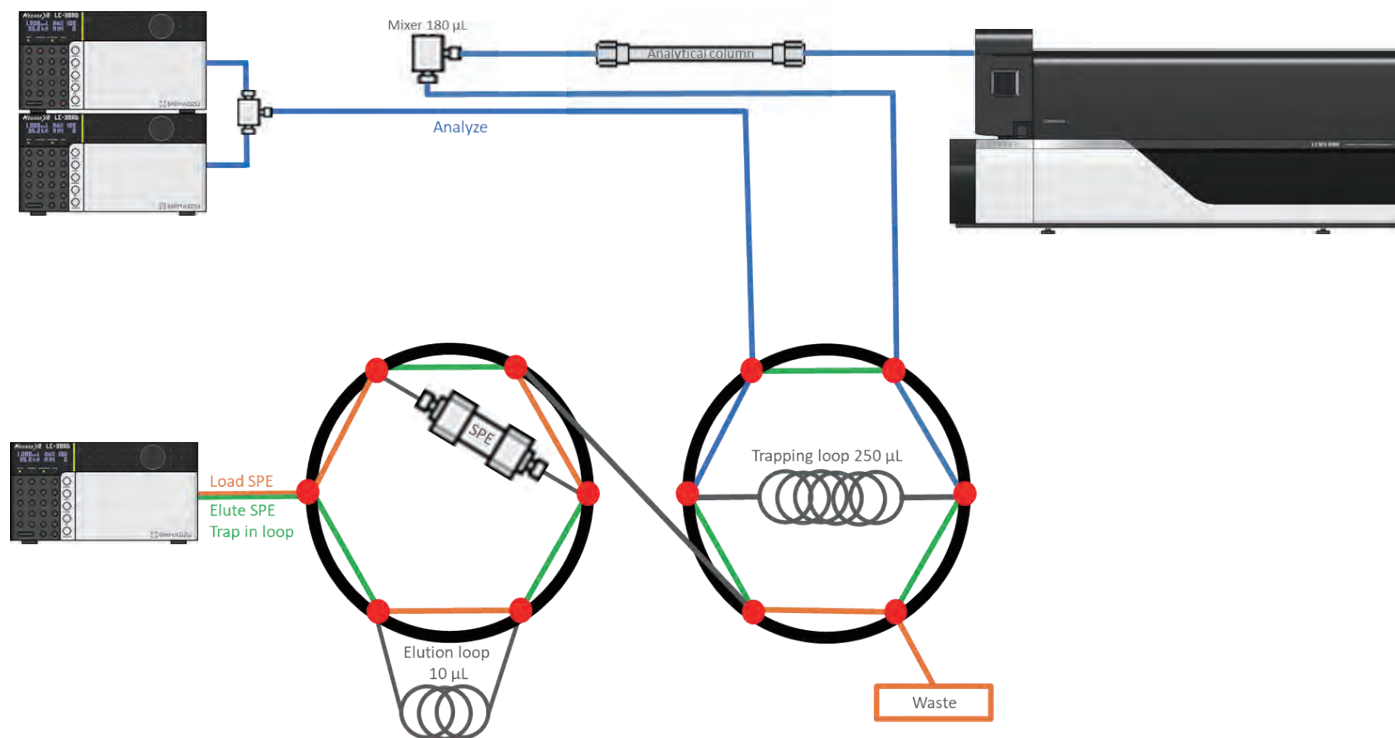


Figure 3   Setup of the on-line SPE analytical system

## Quantitative Analysis of tea samples

By using the reported instrument set-up, analysis and thus the quantification of 16 PAs and 14 of their related N-Oxides could be performed. Calibration curves in different tea matrices (black tea, green tea and herbal tea) determined in duplicate showed good precision and accuracy and even in a complex matrix like tea we were able to easily quantify the PAs in at least the range of 10 to 400 µg/kg. This is comparable to the established methods using manual sample preparation. For all analytes, weighted regression resulting in r² 0.99 could be achieved, with S/N >10 for LLOQ levels.

Exemplary calibration curves obtained for the 30 compounds are shown in Figure  4, Chromatograms of exemplary LLOQs are shown in Figure 5, the LLOQs which could be achieved in the different tea matrices are shown in Table 1.

# Determination of pyrrolizidine alkaloids in plant material using on-line SPE coupled to UHPLC-MS/MS



Calibration Curve Echimidine in herbal tea
0.05 - 20 ng/mL (1 – 400 µg/kg)

Calibration Curve Lycopsamine–N-oxide in green tea
0.05 - 20 ng/mL (1 – 400 µg/kg)

Calibration Curve Europine in black tea
0.05 - 20 ng/mL (1 – 400 µg/kg)

Calibration Curve Jacobine in herbal tea
0.5 - 20 ng/mL (10 – 400 µg/kg)

Calibration Curve Senecyphilline in green tea
0.2 - 20 ng/mL (4 – 400 µg/kg)

Calibration Curve Retrosine-N-oxide in black tea
0.2 - 20 ng/mL (4 – 400 µg/kg)
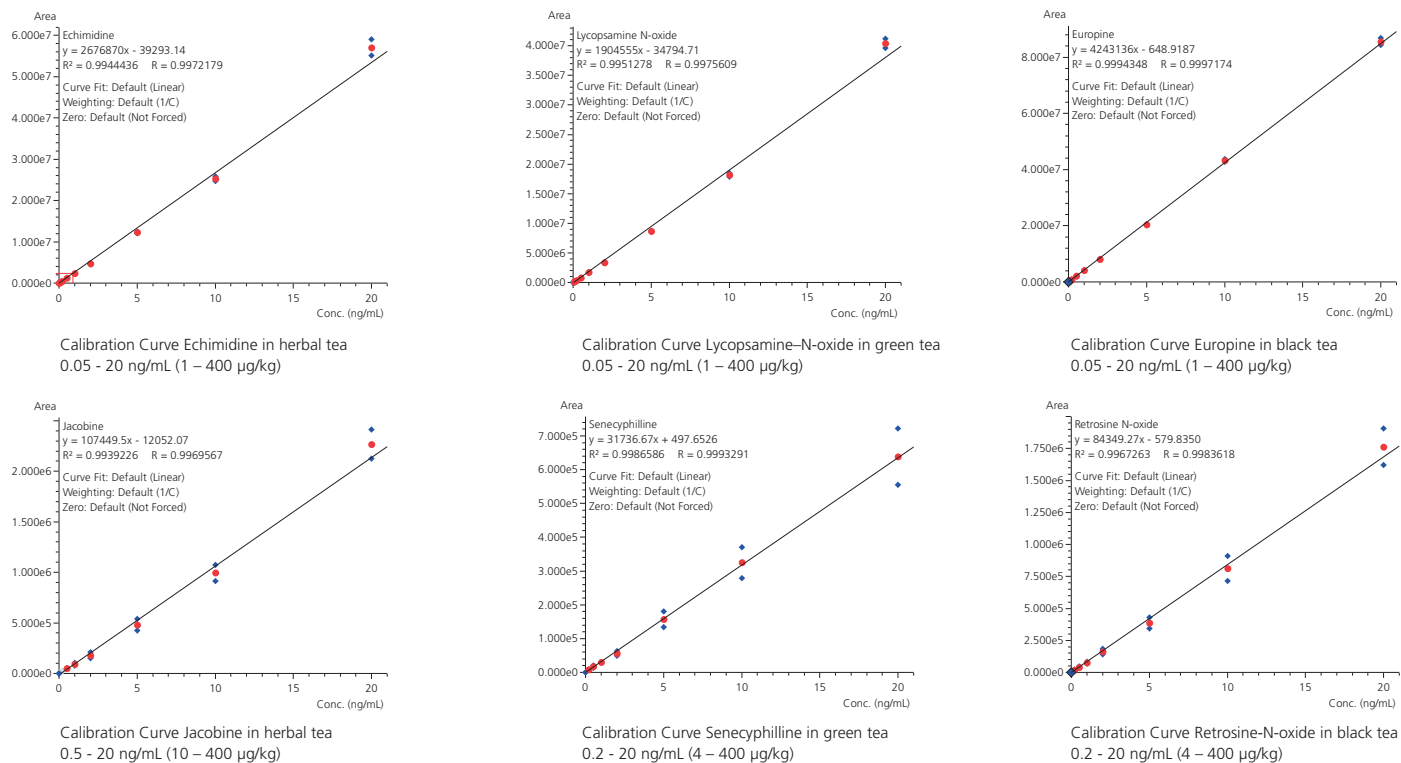
Figure 4   Exemplary calibration curves in different tea matrices



LLOQ of Echimidine in herbal tea / 1.0 µg/kg

LLOQ of Lycopsamine-N-oxide in green tea / 1.0 µg/kg

LLOQ of Europine in black tea / 1.0 µg/kg

LLOQ of Jacobine in herbal tea / 10 µg/kg

LLOQ of Senecyphilline in green tea / 4.0 µg/kg

LLOQ of Retrosine-N-oxide in black tea / 10µg/kg
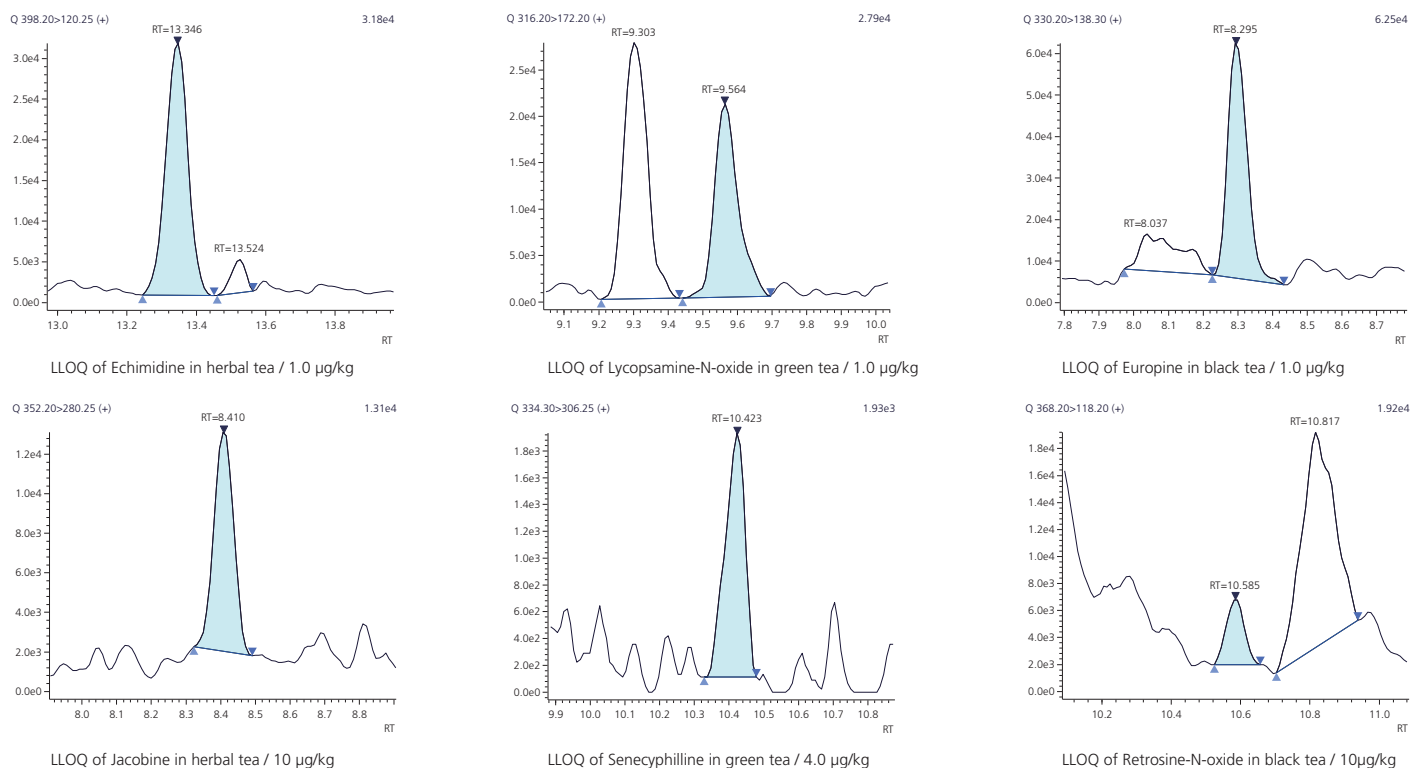
Figure 5   Exemplary chromatograms of LLOQs different tea matrices

# Determination of pyrrolizidine alkaloids in plant material using on-line SPE coupled to UHPLC-MS/MS

Table 1  LLOQs of the pyrrolizidine alkaloids in different tea matices

|  | Black tea LLOQ | | Green tea LLOQ | | Herbal tea LLOQ | |
|---|---|---|---|---|---|---|
|  | ng/mL | µg/kg | ng/mL | µg/kg | ng/mL | µg/kg |
| Echimidine | 0.05 | 1.0 | 0.05 | 1.0 | 0.05 | 1.0 |
| Echimidine-N-oxide | 0.05 | 1.0 | 0.05 | 1.0 | 0.05 | 1.0 |
| Erucifoline | 0.20 | 4.0 | 0.20 | 4.0 | 0.50 | 10.0 |
| Erucifoline-N-Oxide | 0.10 | 2.0 | 0.20 | 4.0 | 0.10 | 2.0 |
| Europine | 0.05 | 1.0 | 0.05 | 1.0 | 0.05 | 1.0 |
| Europine -N-Oxide | 0.05 | 1.0 | 0.05 | 1.0 | 0.05 | 1.0 |
| Heliotrine | 0.05 | 1.0 | 0.05 | 1.0 | 0.05 | 1.0 |
| Heliotrine N-oxide | 0.05 | 1.0 | 0.05 | 1.0 | 0.05 | 1.0 |
| Intermedine | 0.05 | 1.0 | 0.05 | 1.0 | 0.05 | 1.0 |
| Intermedine N-oxide / Indicine N-oxide | N.A | N.A | 0.05 | 1.0 | 0.05 | 1.0 |
| Jacobine | 0.20 | 4.0 | 0.50 | 10.0 | 0.50 | 10.0 |
| Jacobine N-oxide | 0.05 | 1.0 | 0.10 | 2.0 | 0.10 | 2.0 |
| Lasiocarpine | 0.05 | 1.0 | 0.10 | 2.0 | 0.05 | 1.0 |
| Lasiocarpine N-oxide | 0.10 | 2.0 | 0.10 | 2.0 | 0.05 | 1.0 |
| Lycopsamine / Indicine | N.A | N.A | 0.05 | 1.0 | 0.05 | 1.0 |
| Lycopsamine N-oxid | 0.05 | 1.0 | 0.05 | 1.0 | 0.05 | 1.0 |
| Monocrotaline | 0.50 | 10.0 | 0.20 | 4.0 | 0.50 | 10.0 |
| Monocrotaline-N-oxide | 0.10 | 2.0 | 0.10 | 2.0 | 0.10 | 2.0 |
| Retrosine | 0.10 | 2.0 | 0.05 | 1.0 | 0.10 | 2.0 |
| Retrosine N-oxide | 0.20 | 4.0 | 0.50 | 10.0 | 0.50 | 10.0 |
| Senecionine | 0.05 | 1.0 | 0.50 | 10.0 | 0.50 | 10.0 |
| Senecionine N-oxide | 0.05 | 1.0 | 0.20 | 4.0 | 0.50 | 10.0 |
| Senecyphilline | 0.10 | 2.0 | 0.20 | 4.0 | 0.50 | 10.0 |
| Senecyphilline N-oxide | 0.05 | 1.0 | 0.20 | 4.0 | 0.10 | 2.0 |
| Senecivernine | 0.20 | 4.0 | 0.50 | 10.0 | 0.20 | 4.0 |
| Senecivernine N-oxide | 0.05 | 1.0 | 0.20 | 4.0 | 0.10 | 2.0 |
| Senkirkine | 0.05 | 1.0 | 0.05 | 1.0 | 0.05 | 1.0 |
| Trichodesmine | 0.05 | 1.0 | 0.10 | 2.0 | 0.20 | 4.0 |

A total of 29 commercially available tea samples were analyzed. Among these samples there were 6 samples of green tea, 10 samples of black tea and 13 samples of herbal tea. In 59% of all analyzed tea samples one or more of the pyrrolizidine alkaloids could be detected above their LLOQ. 3 out of 6 green tea samples, 5 out of 10 black tea samples and 9 out of 13 herbal tea samples where contaminated with pyrollizidine alkaloids.

# Conclusions

An on-line SPE method for high-sensitivity analysis was successfully developed for PA analysis in plant material. The manual sample preparation could be reduced to a minimum as the set up of on-line SPE followed by UHPLC-MS/MS saves additional clean-up steps without compromising the performance of the assay.

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# Application News

## No. AD-0095

*Food Additives / Nexera X2*

# Fast and High Sensitivity Analysis of Six Preservatives in Beverages by UHPLC with Photodiode Array Detection

## ❑ Introduction

Food preservatives are additives to inhibit, retard or prevent mould, acidification or other deterioration of foodstuffs caused by microbial contamination. The most commonly used preservatives in beverages are benzoic acid, sorbic acid and four para-hydroxybenzoic acid esters (parabens). However, excess amounts of these additives can be harmful to consumer health. In this regard, the minimum permissible concentrations of preservatives are regulated in most countries to ensure safety for consumer [1]. Therefore, quantitative analysis of these preservatives in food is not only required for food quality assurance but also important for consumer interest and protection. High performance liquid chromatography (HPLC) has been used for analysis of the preservatives in beverage [2-4]. In this Application News, a new rapid and high sensitivity UHPLC method for simultaneous determination of the six preservatives in beverages is described. A gradient elution was optimized for separation and quantitation of the six preservatives with a photodiode array detector. A capillary flow cell with extra long optical path of 85 mm was employed to achieve high sensitivity for a very small injection amount of sample (1 μL) which was not cleaned up except filtration.

## ❑ Experimental

### Preparation of standards and samples

Benzoic acid, sorbic acid and parabens were obtained from chemicals suppliers. A mixed stock solution of 1.0 g/L of benzoic acid, sorbic acid and methyl, ethyl, propyl, butyl parabens were prepared with ethanol/water (70/30) solvent as the diluent. A set of nine working standards was prepared from the stock solution using the same diluent at the concentrations shown in Table 1. Soft drink, mango juice and cocoa drink were purchased at the local supermarket. The soft drink and mango juice were diluted 20 times and 2 times with diluent respectively while cocoa drink was not diluted. All the samples were filtered through a 0.45 μm syringe filter prior to injection to UHPLC.

**Table 1:** Concentrations of working standards of six preservatives for setting calibration curves

| No. | Working Standard | Benzoic acid (mg/L) | Sorbic acid (mg/L) | Parabens (mg/L) |
|-----|-----|-----|-----|-----|
| 1 | S1 | 0.2 | 0.008 | 0.01 |
| 2 | S2 | 2.0 | 0.08 | 0.1 |
| 3 | S3 | 4.0 | 0.16 | 0.2 |
| 4 | S4 | 20.0 | 0.8 | 1.0 |
| 5 | S5 | 60.0 | 2.4 | 3.0 |
| 6 | S6 | 80.0 | 3.2 | 4.0 |
| 7 | S7 | 100.0 | 4.0 | 5.0 |
| 8 | S8 | 150.0 | 6.0 | 7.5 |
| 9 | S9 | 200.0 | 8.0 | 10.0 |

### Instrumental and analytical conditions

A Nexera X2 UHPLC system (Shimadzu Corporation, Japan) was used in this work. The system is consisted of a high pressure binary gradient solvent delivery unit (LC-30AD pumps) and an UHPLC autosampler (SIL-30AC) coupled to a photodiode array detector (SPD-M30A) with a high sensitivity capillary flow cell (85mm optical path length) featured as total reflection and low dispersion. A YMC Triart C18 column of 1.9μm particle size (150mmL. x 2.0mm I.D.) was used for the separation of preservatives (benzoic acid, sorbic acid and methyl, ethyl, propyl, butyl parabens) with an optimized linear gradient program developed. The details of the LC conditions are shown in Table 2.

**Table 2:** Analytical conditions of preservatives in beverages on Nexera X2 UHPLC

| | |
|-----|-----|
| Column | YMC Triart C18 1.9 μm 150 x 2.0mm I.D. |
| Flow Rate | 0.45 mL/min |
| Mobile Phase | A: 1.5% acetic acid+1.5% ammonium acetate in H2O<br>B: 1.5% ammonium acetate in MeOH |
| Elution Mode | Gradient elution: 40% B (0.01 to 4.0 min) ➔ 80% B (4.01 to 5.5 min) ➔ 40% B (5.51 to 8.5min) |
| Oven Temp. | 45°C |
| Injection Volume | 1 μL |
| Detection (PDA) | Wavelength 240~600nm; Ref: 720nm Quant, 240nm for benzoic acid, 260nm for other compounds |

## ❑ Results and Discussion

### Method Development

The six preservatives were well-separated as sharp peaks between 1.7 min and 5.1 min as shown in Figure 1. The total run time of the UHPLC method is 8.5 mins, which is several times faster than the HPLC method reported [2-4]. It is worth to note that two wavelengths were selected for quantitative data processing, i.e., 240 nm for benzonic acid and 260 nm for the rest five compounds [4].
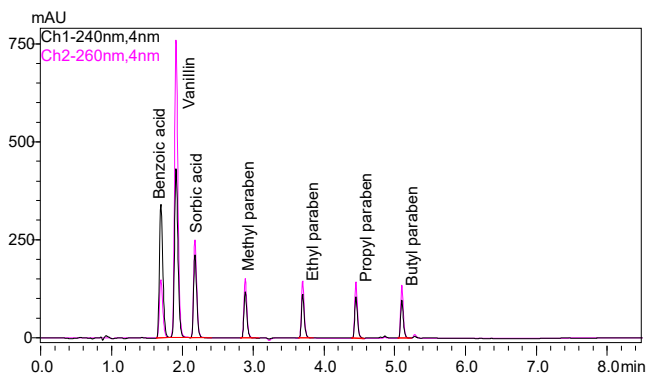


**Figure 1:** Chromatograms of mixed standard (S3) with 1μL injection volume on Nexera X2.
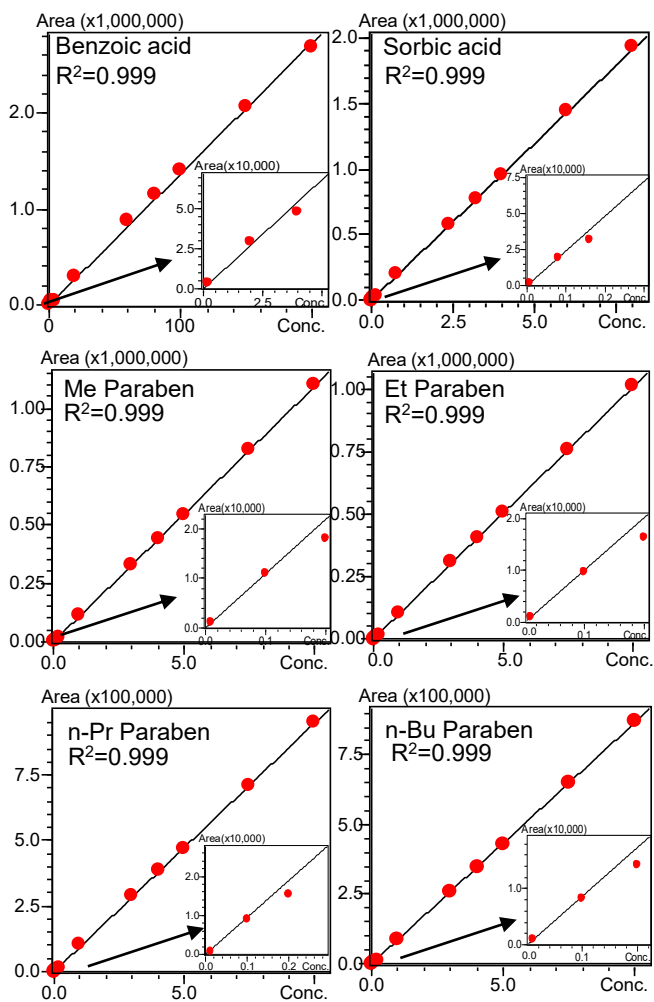


**Figure 2:** Calibration curves of six preservatives at concentration S1~S9 (see Table 1)

Figure 2 shows the calibration curves of the six compounds established with 1μL injection volume. The linearity with correlation coefficient ($R^2$) greater than 0.999 across the wide calibration range of 0.008~200 mg/L was obtained for the six compounds.

The repeatability of the method was evaluated at the levels S2 and S5. The peak area %RSD for the six compounds were lower than 5.1% and 0.3% respectively (Table 3).

**Table 3:** Results of repeatability evaluation using working standard S2 and S5 (n=6, 1μL injection)

| Compound | Conc. (mg/L) | RSD% | Conc. (mg/L) | RSD% |
|---|---|---|---|---|
| Benzoic acid | 2.0 | 1.1 | 60.0 | 0.2 |
| Sorbic acid | 0.08 | 1.5 | 60.0 | 0.2 |
| Methyl paraben | 0.1 | 1.2 | 2.4 | 0.2 |
| Ethyl paraben | 0.1 | 3.8 | 3.0 | 0.2 |
| Propyl paraben | 0.1 | 3.2 | 3.0 | 0.2 |
| Butyl paraben | 0.1 | 5.1 | 3.0 | 0.3 |

The LOD and LOQ of the method, and peak identification criteria (RT & $\lambda_{Max}$) are summarized in Table 4. The results were obtained from the mixed standard S1 (Figure 3). The high sensitivity achieved, i.e., LOQs ranging at 8~10 μg/L of the compounds except benzoic acid (280 μg/L), is attributed partially to the use of a high sensitivity SPD-M30A detector with using a capillary cell of 85mm optical path.

**Table 4:** LOD (S/N=3), LOQ (S/N=10) and peak identification criteria of UHPLC method obtained from S1 chromatogram

| Compound | Conc. (μg/L) | RT | $\lambda_{Max}$ | LOD (μg/L) | LOQ (μg/L) |
|---|---|---|---|---|---|
| Benzoic acid | 200 | 1.702 | 238 | 90 | 280 |
| Sorbic acid | 8 | 2.183 | 257 | 2.7 | 8 |
| Methyl paraben | 10 | 2.866 | 258 | 3 | 10 |
| Ethyl paraben | 10 | 3.687 | 257 | 3 | 10 |
| Propyl paraben | 10 | 4.445 | 258 | 3 | 10 |
| Butyl paraben | 10 | 5.091 | 256 | 3 | 10 |
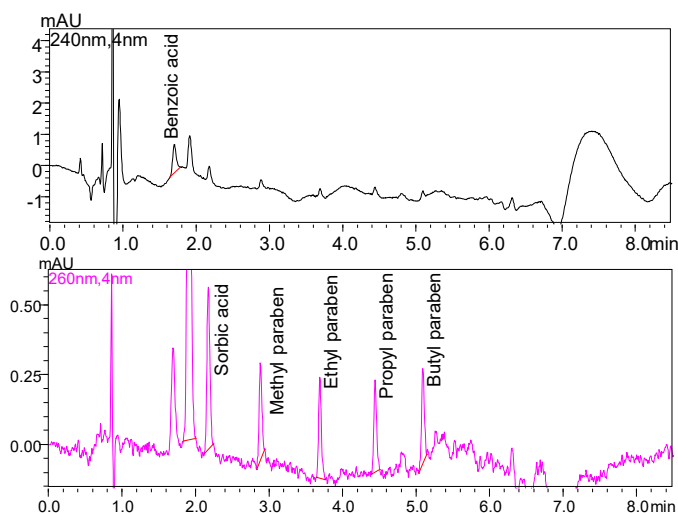


**Figure 3:** Chromatogram of mixed standard S1 (1μL).

### Analysis of beverage samples

The UHPLC method established was applied for quantitation of preservatives in three kinds of beverages: soft drink B1, fruit juice B2 and cocoa drink B3. The chromatograms of the samples are shown in Figure 4 and the results are summarized into Table 5. No preservatives was detected in cocoa drink. Benzoic acid and sorbic acid were detected in the soft drink and fruit juice. The identification of both benzoic acid and sorbic acid peaks were confirmed by UV spectra.
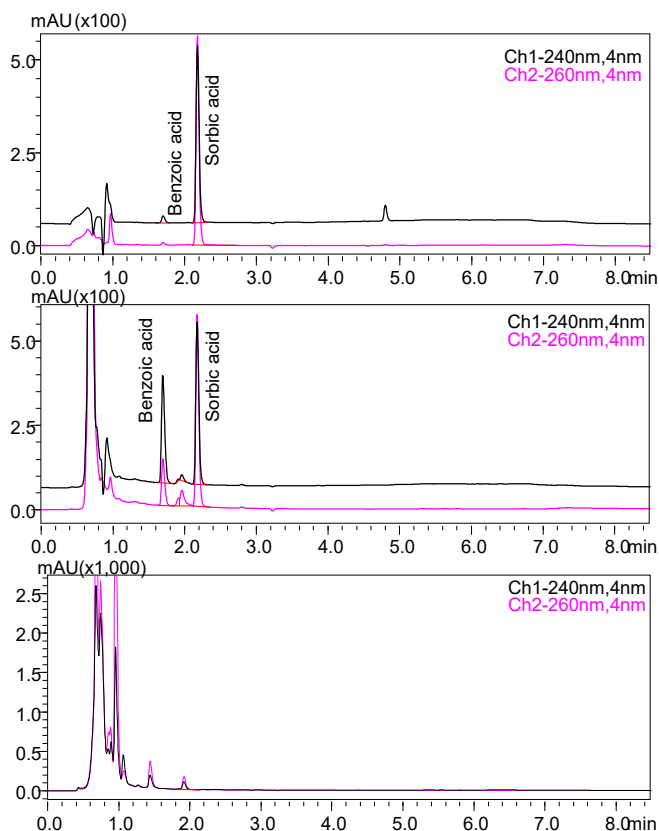


**Figure 4:** Chromatogram of beverage samples (injection: 1$\mu$L): Soft drink with 20 times dilution (top); Fruit drink with 2 times dilutions; Cocoa drink without dilution (bottom).

Table 5: Quantitative results of six preservatives in three beverages, each with duplicate injections

| Sample Name | Benzoic acid | | Sorbic acid | | Parabens | |
|---|---|---|---|---|---|---|
| | RT (min) | Conc (mg/L) | RT (min) | Conc (mg/L) | RT (min) | Conc (mg/L) |
| B1 | 1.70 | 82.4 | 2.18 | 137.4 | | |
| B2 | 1.70 | 142.4 | 2.18 | 13.8 | ND | |
| B3 | ND | | | | | |

### ☐ Conclusions

A rapid and high sensitivity UHPLC method for quantitation of six preservatives, benzoic acid, sorbic acid and four para-hydroxybenzoic acid esters (parabens), in beverages was established using a reversed phase UHPLC column (1.9$\mu$m particle size). A capillary flow cell with extra long optical path of 85 mm was employed in the photodiode array detector. The method achieves LOQs ranging 8-10 $\mu$g/L for the compounds except benzoic acid (280 $\mu$g/L), with 1$\mu$L injection volume. The very small injection volume minimizes the contamination of beverage samples to the column and system, as such suitable for direct analysis of beverage samples without need for clean up procedure.

### ☐ References

1. Singapore Agri-Food and Veterinary Services website: Food Regulations – AVA (2014)
2. United States Department of Agriculture Food Safety and Inspection Service, Office of Public Health Science: *Determination of Benzoic acid, Sorbic acid and Methyl, Ethyl, Propyl and Butyl Parabens by HPLC* (2004)
3. Tzu-Yun Chu, Chine-Lin Chen and Hsueh-Fang Wang, *A Rapid Method for the Simultaneous Determination of Preservatives in Soy Sauce,* Journal of Food and Drug Analysis, Vol. 11, No. 3, Pages 246-250 (2003)
4. Shu-Xiang Sun, Shu-Bai Li and Pei Yao, *Simultaneous Determination of Six Preservatives in Beverage by HPLC,* 3rd International Conference on Material, Mechanical and Manufacturing Engineering (2015)

# SHIMADZU
## Excellence in Science

## Application News

No.**C135**

**Liquid Chromatography Mass Spectrometry**

## Shimadzu Pesticide MRM Library Support for LC/MS/MS

**David R. Baker, Alan Barnes, Neil Loftus**
**Shimadzu Corporation, UK**

### ■ Abstract

To help expand capabilities in LC/MS/MS pesticide monitoring programs we have created the Shimadzu Pesticide MRM Library. The Library has been created with 766 certified reference standards and has been verified for use with Shimadzu LCMS-8050 and 8060 systems.

The Library contains information that can be used to accelerate method development in LC/MS/MS pesticide analysis including;

An average of 8 MRM transitions for each reference standard (with optimized collision energies) are registered in the database including positive and negative ionization mode. In total, more than 6,000 MRM transitions are part of the Library.

Meta-data for each library entry such as CAS#, formula, activity, mono-isotopic mass and adduct masses, rank of MRM transitions, synonyms, InChI, InChIKey, compound names translation (Japanese and Chinese) and links to websites offering further information (alanwood.net, PAN pesticide database, Chemical Book, ChemSpider). The meta-data is intended not only to set up new methods quickly but to help search for compound properties.

Key words; Pesticide MRM Library,
766 compound library

### ■ Using the Shimadzu Pesticide MRM Library

Expanding pesticide monitoring programmes (or creating focused methods) can be quickly set up using the Library data base (Table 1) and create fully optimized MRM methods for LC/MS/MS analysis.

Users select the target pesticides and corresponding transitions from the Library and simply copy the list into a Shimadzu LabSolutions analytical method. The method will include optimized MRM transitions. Once the acquisition method is created users can start to acquire data for screening or quantitative LC/MS/MS analysis.

**Table 1  The Shimadzu Pesticide MRM Library supports a list of over 766 compounds. Designed to build extended LC/MS/MS methods quickly and to review pesticide information easily.**

| Library entries | |
| --- | --- |
| Compound information | Compound Name<br>Synonyms<br>Japanese name<br>Chinese name<br>CAS<br>Chemical Formula<br>Mono-isotopic mass<br>Theoretical $m/z$ ([M+H]+,<br>[M+Na]+, [M+K]+, [M+NH$_4$]+,<br>[M-H]-)<br>Activity<br>InChI<br>InChIKey |
| MS/MS parameters | Ionization mode<br>Q1 ($m/z$)<br>Q3 ($m/z$)<br>Q1 Pre Bias<br>CE<br>Q3 Pre Bias |
| Web links | Alanwood.net<br>PAN Pesticide Database<br>Chemical Book<br>ChemSpider |

| | Compound | CAS | Formula | M | [M+H]+ | [M-H]- | Ionisation Mode | MRM Transitions |
|---|---|---|---|---|---|---|---|---|
| 1 | (E)-Fenpyroximate | 134098-61-6 | C24H27N3O4 | 421.2002 | 422.2075 | 420.1929 | ESI+ | 6 |
| 2 | (E)-Ferimzone | 89269-64-7 | C15H18N4 | 254.1531 | 255.1604 | 253.1458 | ESI+ | 2 |
| 3 | (Z)-Fenpyroximate | 149054-53-5 | C24H27N3O4 | 421.2002 | 422.2075 | 420.1929 | ESI+ | 2 |
| 4 | (Z)-Ferimzone | 89269-64-7 | C15H18N4 | 254.1531 | 255.1604 | 253.1458 | ESI+ | 6 |
| 5 | 1-(3, 4-Dichlorophenyl)-3-methylurea | 3567-62-2 | C8H8Cl2N2O | 218.0014 | 219.0087 | 216.9941 | ESI+ | 19 |
| 6 | 1-(3, 4-Dichlorophenyl)urea | 2327-02-8 | C7H6Cl2N2O | 203.9857 | 204.9930 | 202.9784 | ESI+ | 17 |
| 7 | 1-(4-Isopropylphenyl)-3-methylurea | 34123-57-4 | C11H16N2O | 192.1263 | 193.1336 | 191.1190 | ESI+ | 6 |
| 8 | 1-(4-Isopropylphenyl)urea | 56046-17-4 | C10H14N2O | 178.1106 | 179.1179 | 177.1033 | ESI+ | 6 |
| 9 | 1-naphthaleneacetamide | 86-86-2 | C12H11NO | 185.0841 | 186.0914 | 184.0768 | ESI+ | 4 |
| 10 | 1-Naphthaleneacetic Acid | 86-87-3 | C12H10O2 | 186.0681 | 187.0754 | 185.0608 | ESI- | 1 |
| 11 | 2, 4, 5-T | 93-76-5 | C8H5Cl3O3 | 253.9304 | 254.9377 | 252.9231 | ESI- | 7 |
| 12 | 2, 4, 6-Tribromophenol | 118-79-6 | C6H3Br3O | 327.7734 | 328.7807 | 326.7661 | ESI- | 10 |
| 13 | 2, 4, 6-Trichlorophenol | 88-06-2 | C6H3Cl3O | 195.9249 | 196.9322 | 194.9176 | ESI- | 3 |
| 14 | 2, 4-D (2, 4-PA) | 94-75-7 | C8H6Cl2O3 | 219.9694 | 220.9767 | 218.9621 | ESI- | 7 |
| 15 | 2, 4-DB | 94-82-6 | C10H10Cl2O3 | 248.0007 | 249.0080 | 246.9934 | ESI- | 5 |
| 16 | 2, 4-dimethylaniline | 95-68-1 | C8H11N | 121.0891 | 122.0964 | 120.0818 | ESI+ | 5 |
| 17 | 2, 6-Dichlorobenzamide | 2008-58-4 | C7H5Cl2NO | 188.9748 | 189.9821 | 187.9675 | ESI+ | 13 |
| 18 | 2-Naphthoxy acetic acid | 120-23-0 | C12H10O3 | 202.0630 | 203.0703 | 201.0557 | ESI- | 2 |
| 19 | 2-Phenylphenol | 90-43-7 | C12H10O | 170.0732 | 171.0805 | 169.0659 | ESI- | 2 |
| 20 | 3-(3-Indolyl)-propionic acid | 830-96-6 | C11H11NO2 | 189.0790 | 190.0863 | 188.0717 | ESI+ | 6 |
| 21 | 3, 4, 5-Trimethacarb | 2686-99-9 | C11H15NO2 | 193.1103 | 194.1176 | 192.1030 | ESI+ | 12 |
| 22 | 3-Indolyl-acetic acid | 87-51-4 | C10H9NO2 | 175.0633 | 176.0706 | 174.0560 | ESI+ | 12 |
| 23 | 3-Methylphosphinicopropionic acid | 15090-23-0 | C4H9O4P | 152.0238 | 153.0311 | 151.0165 | ESI+ | 12 |
| 24 | 4-(3-Indolyl)-butyric acid | 133-32-4 | C12H13NO2 | 203.0946 | 204.1019 | 202.0873 | ESI+ | 14 |
| 25 | 4-Chlorophenoxyacetic acid | 122-88-3 | C8H7ClO3 | 186.0084 | 187.0157 | 185.0011 | ESI- | 4 |
| 26 | 6-chloro-3-phenylpyridazin-4-ol | 40020-01-7 | C10H7ClN2O | 206.0247 | 207.0320 | 205.0174 | ESI+ | 6 |
| 27 | 6-Furfurylaminopurine | 525-79-1 | C10H9N5O | 215.0807 | 216.0880 | 214.0734 | ESI+ | 9 |
| 28 | Acephate | 30560-19-1 | C4H10NO3PS | 183.0119 | 184.0192 | 182.0046 | ESI+ | 6 |
| 29 | Acequinocyl | 57960-19-7 | C24H32O4 | 384.2301 | 385.2374 | 383.2228 | ESI+ | 6 |
| 30 | Acetamiprid | 135410-20-7 | C10H11ClN4 | 222.0672 | 223.0745 | 221.0599 | ESI+ | 10 |
| 31 | Acibenzolar-S-methyl | 135158-54-2 | C8H6N2OS2 | 209.9922 | 210.9995 | 208.9849 | ESI+ | 6 |
| 32 | Acifluorfen | 50594-66-6 | C14H7ClF3NO5 | 360.9965 | 362.0038 | 359.9892 | ESI- | 12 |
| 33 | Aclonifen | 74070-46-5 | C12H9ClN2O3 | 264.0302 | 265.0375 | 263.0229 | ESI+ | 2 |
| 34 | Acrinathrin | 101007-06-1 | C26H21F6NO5 | 541.1324 | 542.1397 | 540.1251 | ESI+ | 12 |
| 35 | Alachlor | 15972-60-8 | C14H20ClNO2 | 269.1183 | 270.1256 | 268.1110 | ESI+ | 12 |
| 36 | Alanycarb | 83130-01-2 | C17H25N3O4S2 | 399.1286 | 400.1359 | 398.1213 | ESI+ | 6 |
| 37 | Aldicarb | 116-06-3 | C7H14N2O2S | 190.0776 | 191.0849 | 189.0703 | ESI+ | 5 |
| 38 | Aldicarb-sulfone (Aldoxycarb) | 1646-88-4 | C7H14N2O4S | 222.0674 | 223.0747 | 221.0601 | ESI+ | 5 |
| 39 | Aldicarb-sulfoxide | 1646-87-3 | C7H14N2O3S | 206.0725 | 207.0798 | 205.0652 | ESI+ | 8 |
| 40 | Allethrin | 584-79-2 | C19H26O3 | 302.1882 | 303.1955 | 301.1809 | ESI+ | 12 |
| 41 | Allidochlor | 93-71-0 | C8H12ClNO | 173.0607 | 174.0680 | 172.0534 | ESI+ | 12 |
| 42 | Ametoctradin | 865318-97-4 | C15H25N5 | 275.2110 | 276.2183 | 274.2037 | ESI+ | 6 |
| 43 | Ametryn | 834-12-8 | C9H17N5S | 227.1205 | 228.1278 | 226.1132 | ESI+ | 6 |
| 44 | Amidosulfuron | 120923-37-7 | C9H15N5O7S2 | 369.0413 | 370.0486 | 368.0340 | ESI+ | 8 |
| 45 | Aminocarb | 2032-59-9 | C11H16N2O2 | 208.1212 | 209.1285 | 207.1139 | ESI+ | 6 |
| 46 | Aminopyralid | 150114-71-9 | C6H4Cl2N2O2 | 205.9650 | 206.9723 | 204.9577 | ESI+ | 7 |
| 47 | Amisulbrom | 348635-87-0 | C13H13BrFN5O4S2 | 464.9576 | 465.9649 | 463.9503 | ESI+ | 10 |
| 48 | Amitraz | 33089-61-1 | C19H23N3 | 293.1892 | 294.1965 | 292.1819 | ESI+ | 2 |
| 49 | Amitrole | 61-82-5 | C2H4N4 | 84.0436 | 85.0509 | 83.0363 | ESI+ | 5 |
| 50 | AMPA | 1066-51-9 | CH6NO3P | 111.0085 | 112.0158 | 110.0012 | ESI- | 3 |
| 51 | Ancymidol | 12771-68-5 | C15H16N2O2 | 256.1212 | 257.1285 | 255.1139 | ESI+ | 6 |
| 52 | Anilazine | 101-05-3 | C9H5Cl3N4 | 273.9580 | 274.9653 | 272.9507 | ESI+ | 12 |
| 53 | Anilofos | 64249-01-0 | C13H19ClNO3PS2 | 367.0232 | 368.0305 | 366.0159 | ESI+ | 12 |
| 54 | Aramite | 140-57-8 | C15H23ClO4S | 334.1006 | 335.1079 | 333.0933 | ESI+ | 12 |
| 55 | Asulam | 3337-71-1 | C8H10N2O4S | 230.0361 | 231.0434 | 229.0288 | ESI+ | 9 |
| 56 | Atraton | 1610-17-9 | C9H17N5O | 211.1433 | 212.1506 | 210.1360 | ESI+ | 6 |
| 57 | Atrazine | 1912-24-9 | C8H14ClN5 | 215.0938 | 216.1011 | 214.0865 | ESI+ | 8 |
| 58 | Atrazine-2-hydroxy | 2163-68-0 | C8H15N5O | 197.1277 | 198.1350 | 196.1204 | ESI+ | 6 |
| 59 | Atrazine-desethyl | 6190-65-4 | C6H10ClN5 | 187.0625 | 188.0698 | 186.0552 | ESI+ | 9 |
| 60 | Atrazine-desethyl-2-hydroxy | 19988-24-0 | C6H11N5O | 169.0964 | 170.1037 | 168.0891 | ESI+ | 5 |
| 61 | Atrazine-desisopropyl | 1007-28-9 | C5H8ClN5 | 173.0468 | 174.0541 | 172.0395 | ESI+ | 10 |
| 62 | Avermectin B1a | 65195-55-3 | C48H72O14 | 872.4922 | 873.4995 | 871.4849 | ESI+ | 4 |
| 63 | Avermectin B1b | 65195-56-4 | C47H70O14 | 858.4766 | 859.4839 | 857.4693 | ESI+ | 3 |
| 64 | Azaconazole | 60207-31-0 | C12H11Cl2N3O2 | 299.0228 | 300.0301 | 298.0155 | ESI+ | 8 |
| 65 | Azadirachtin | 11141-17-6 | C35H44O16 | 720.2629 | 721.2702 | 719.2556 | ESI+ | 8 |
| 66 | Azamethiphos | 35575-96-3 | C9H10ClN2O5PS | 323.9737 | 324.9810 | 322.9664 | ESI+ | 11 |
| 67 | Azimsulfuron | 120162-55-2 | C13H16N10O5S | 424.1026 | 425.1099 | 423.0953 | ESI+ | 5 |
| 68 | Azinphos-ethyl | 2642-71-9 | C12H16N3O3PS2 | 345.0371 | 346.0444 | 344.0298 | ESI+ | 5 |
| 69 | Azinphos-methyl | 86-50-0 | C10H12N3O3PS2 | 317.0058 | 318.0131 | 315.9985 | ESI+ | 6 |
| 70 | Aziprotryne | 4658-28-0 | C7H11N7S | 225.0797 | 226.0870 | 224.0724 | ESI+ | 4 |
| 71 | Azobenzene | 103-33-3 | C12H10N2 | 182.0844 | 183.0917 | 181.0771 | ESI+ | 2 |
| 72 | Azoxystrobin | 131860-33-8 | C22H17N3O5 | 403.1168 | 404.1241 | 402.1095 | ESI+ | 5 |
| 73 | Barban | 101-27-9 | C11H9Cl2NO2 | 257.0010 | 258.0083 | 255.9937 | ESI+ | 11 |
| 74 | Beflubutamid | 113614-08-7 | C18H17F4NO2 | 355.1195 | 356.1268 | 354.1122 | ESI+ | 10 |
| 75 | Benalaxyl | 71626-11-4 | C20H23NO3 | 325.1678 | 326.1751 | 324.1605 | ESI+ | 6 |

| | Compound | CAS | Formula | M | [M+H]+ | [M-H]- | Ionisation Mode | MRM Transitions |
|---|---|---|---|---|---|---|---|---|
| 76 | Benazolin | 3813-05-6 | C9H6ClNO3S | 242.9757 | 243.9830 | 241.9684 | ESI+ | 6 |
| 77 | Benazolin-ethyl | 25059-80-7 | C11H10ClNO3S | 271.0070 | 272.0143 | 269.9997 | ESI+ | 18 |
| 78 | Bendiocarb | 22781-23-3 | C11H13NO4 | 223.0845 | 224.0918 | 222.0772 | ESI+ | 6 |
| 79 | Benfuracarb | 82560-54-1 | C20H30N2O5S | 410.1875 | 411.1948 | 409.1802 | ESI+ | 5 |
| 80 | Benfuresate | 68505-69-1 | C12H16O4S | 256.0769 | 257.0842 | 255.0696 | ESI+ | 2 |
| 81 | Benodanil | 15310-01-7 | C13H10INO | 322.9807 | 323.9880 | 321.9734 | ESI+ | 6 |
| 82 | Benoxacor | 98730-04-2 | C11H11Cl2NO2 | 259.0167 | 260.0240 | 258.0094 | ESI+ | 17 |
| 83 | Bensulfuron-methyl | 83055-99-6 | C16H18N4O7S | 410.0896 | 411.0969 | 409.0823 | ESI+ | 6 |
| 84 | Bensulide | 741-58-2 | C14H24NO4PS3 | 397.0605 | 398.0678 | 396.0532 | ESI+ | 9 |
| 85 | Bentazone | 25057-89-0 | C10H12N2O3S | 240.0569 | 241.0642 | 239.0496 | ESI- | 5 |
| 86 | Benthiavalicarb-isopropyl | 177406-68-7 | C18H24FN3O3S | 381.1522 | 382.1595 | 380.1449 | ESI+ | 5 |
| 87 | Benthiazole | 21564-17-0 | C9H6N2S3 | 237.9693 | 238.9766 | 236.9620 | ESI+ | 6 |
| 88 | Benzanilide | 93-98-1 | C13H11NO | 197.0841 | 198.0914 | 196.0768 | ESI+ | 4 |
| 89 | Benzofenap | 82692-44-2 | C22H20Cl2N2O3 | 430.0851 | 431.0924 | 429.0778 | ESI+ | 2 |
| 90 | Benzoximate | 29104-30-1 | C18H18ClNO5 | 363.0874 | 364.0947 | 362.0801 | ESI+ | 12 |
| 91 | Benzoylprop-ethyl | 22212-55-1 | C18H17Cl2NO3 | 365.0585 | 366.0658 | 364.0512 | ESI+ | 6 |
| 92 | Benzthiazuron | 1929-88-0 | C9H9N3OS | 207.0466 | 208.0539 | 206.0393 | ESI+ | 9 |
| 93 | Benzyldimethyldodecylammonium | 139-07-1 | C21H37N | 303.2926 | 304.2999 | 302.2853 | ESI+ | 4 |
| 94 | Benzyldimethylhexadecylammonium | 122-18-9 | C25H45N | 359.3552 | 360.3625 | 358.3479 | ESI+ | 3 |
| 95 | Benzyldimethyltetradecylammonium | 139-08-2 | C23H41N | 331.3239 | 332.3312 | 330.3166 | ESI+ | 3 |
| 96 | Bifenazate | 149877-41-8 | C17H20N2O3 | 300.1474 | 301.1547 | 299.1401 | ESI+ | 6 |
| 97 | Bifenox | 42576-02-3 | C14H9Cl2NO5 | 340.9858 | 341.9931 | 339.9785 | ESI+ | 8 |
| 98 | Bifenthrin | 82657-04-3 | C23H22ClF3O2 | 422.1260 | 423.1333 | 421.1187 | ESI+ | 5 |
| 99 | Bioresmethrin | 28434-01-7 | C22H26O3 | 338.1882 | 339.1955 | 337.1809 | ESI+ | 6 |
| 100 | Bispyribac-sodium | 125401-92-5 | C19H17N4NaO8 | 452.0944 | 453.1017 | 451.0871 | ESI+ | 8 |
| 101 | Bitertanol | 55179-31-2 | C20H23N3O2 | 337.1790 | 338.1863 | 336.1717 | ESI+ | 6 |
| 102 | Bixafen | 581809-46-3 | C18H12Cl2F3N3O | 413.0310 | 414.0383 | 412.0237 | ESI+ | 12 |
| 103 | Boscalid | 188425-85-6 | C18H12Cl2N2O | 342.0327 | 343.0400 | 341.0254 | ESI+ | 12 |
| 104 | Brodifacoum | 56073-10-0 | C31H23BrO3 | 522.0831 | 523.0904 | 521.0758 | ESI+ | 12 |
| 105 | Bromacil | 314-40-9 | C9H13BrN2O2 | 260.0160 | 261.0233 | 259.0087 | ESI+ | 9 |
| 106 | Bromadiolone | 28772-56-7 | C30H23BrO4 | 526.0780 | 527.0853 | 525.0707 | ESI- | 12 |
| 107 | Bromfenvinfos | 33399-00-7 | C12H14BrCl2O4P | 401.9190 | 402.9263 | 400.9117 | ESI+ | 17 |
| 108 | Bromobutide | 74712-19-9 | C15H22BrNO | 311.0885 | 312.0958 | 310.0812 | ESI+ | 10 |
| 109 | Bromophos-ethyl | 4824-78-6 | C10H12BrCl2O3PS | 391.8805 | 392.8878 | 390.8732 | ESI+ | 3 |
| 110 | Bromophos-methyl | 2104-96-3 | C8H8BrCl2O3PS | 363.8492 | 364.8565 | 362.8419 | ESI+ | 6 |
| 111 | Bromoxynil | 1689-84-5 | C7H3Br2NO | 274.8581 | 275.8654 | 273.8508 | ESI- | 11 |
| 112 | Bromuconazole | 116255-48-2 | C13H12BrCl2N3O | 374.9541 | 375.9614 | 373.9468 | ESI+ | 11 |
| 113 | Bupirimate | 41483-43-6 | C13H24N4O3S | 316.1569 | 317.1642 | 315.1496 | ESI+ | 6 |
| 114 | Buprofezin | 69327-76-0 | C16H23N3OS | 305.1562 | 306.1635 | 304.1489 | ESI+ | 6 |
| 115 | Butachlor | 23184-66-9 | C17H26ClNO2 | 311.1652 | 312.1725 | 310.1579 | ESI+ | 12 |
| 116 | Butafenacil | 134605-64-4 | C20H18ClF3N2O6 | 474.0805 | 475.0878 | 473.0732 | ESI+ | 10 |
| 117 | Butamifos | 36335-67-8 | C13H21N2O4PS | 332.0960 | 333.1033 | 331.0887 | ESI+ | 12 |
| 118 | Butocarboxim | 34681-10-2 | C7H14N2O2S | 190.0776 | 191.0849 | 189.0703 | ESI+ | 3 |
| 119 | Butocarboxim-sulfone | 34681-23-7 | C7H14N2O4S | 222.0674 | 223.0747 | 221.0601 | ESI+ | 14 |
| 120 | Butocarboxim-sulfoxide | 34681-24-8 | C7H14N2O3S | 206.0725 | 207.0798 | 205.0652 | ESI+ | 6 |
| 121 | Butralin | 33629-47-9 | C14H21N3O4 | 295.1532 | 296.1605 | 294.1459 | ESI+ | 6 |
| 122 | Buturon | 3766-60-7 | C12H13ClN2O | 236.0716 | 237.0789 | 235.0643 | ESI+ | 9 |
| 123 | Butylate | 2008-41-5 | C11H23NOS | 217.1500 | 218.1573 | 216.1427 | ESI+ | 3 |
| 124 | Cadusafos | 95465-99-9 | C10H23O2PS2 | 270.0877 | 271.0950 | 269.0804 | ESI+ | 5 |
| 125 | Cafenstrole | 125306-83-4 | C16H22N4O3S | 350.1413 | 351.1486 | 349.1340 | ESI+ | 3 |
| 126 | Captafol | 2425-06-1 | C10H9Cl4NO2S | 346.9108 | 347.9181 | 345.9035 | ESI+ | 1 |
| 127 | Carbaryl (NAC) | 63-25-2 | C12H11NO2 | 201.0790 | 202.0863 | 200.0717 | ESI+ | 6 |
| 128 | Carbendazim | 10605-21-7 | C9H9N3O2 | 191.0695 | 192.0768 | 190.0622 | ESI+ | 5 |
| 129 | Carbetamide | 16118-49-3 | C12H16N2O3 | 236.1161 | 237.1234 | 235.1088 | ESI+ | 6 |
| 130 | Carbofuran | 1563-66-2 | C12H15NO3 | 221.1052 | 222.1125 | 220.0979 | ESI+ | 6 |
| 131 | Carbofuran-3-hydroxy (3-Hydroxycarbofuran) | 16655-82-6 | C12H15NO4 | 237.1001 | 238.1074 | 236.0928 | ESI+ | 12 |
| 132 | Carbofuran-3-keto | 16709-30-1 | C12H13NO4 | 235.0845 | 236.0918 | 234.0772 | ESI+ | 12 |
| 133 | Carbophenothion | 786-19-6 | C11H16ClO2PS3 | 341.9739 | 342.9812 | 340.9666 | ESI+ | 9 |
| 134 | Carbosulfan | 55285-14-8 | C20H32N2O3S | 380.2134 | 381.2207 | 379.2061 | ESI+ | 6 |
| 135 | Carboxin | 5234-68-4 | C12H13NO2S | 235.0667 | 236.0740 | 234.0594 | ESI+ | 6 |
| 136 | Carfentrazone-ethyl | 128639-02-1 | C15H14Cl2F3N3O3 | 411.0364 | 412.0437 | 410.0291 | ESI+ | 5 |
| 137 | Carpropamid | 104030-54-8 | C15H18Cl3NO | 333.0454 | 334.0527 | 332.0381 | ESI+ | 18 |
| 138 | Cartap | 15263-53-3 | C7H15N3O2S2 | 237.0606 | 238.0679 | 236.0533 | ESI+ | 3 |
| 139 | Chinomethionat | 2439-01-2 | C10H6N2OS2 | 233.9922 | 234.9995 | 232.9849 | ESI+ | 6 |
| 140 | Chloramphenicol | 56-75-7 | C11H12Cl2N2O5 | 322.0123 | 323.0196 | 321.0050 | ESI- | 17 |
| 141 | Chlorantraniliprole | 500008-45-7 | C18H14BrCl2N5O2 | 480.9708 | 481.9781 | 479.9635 | ESI+ | 28 |
| 142 | Chlorbromuron | 13360-45-7 | C9H10BrClN2O2 | 291.9614 | 292.9687 | 290.9541 | ESI+ | 12 |
| 143 | Chlorbufam | 1967-16-4 | C11H10ClNO2 | 223.0400 | 224.0473 | 222.0327 | ESI+ | 4 |
| 144 | Chlordimeform | 6164-98-3 | C10H13ClN2 | 196.0767 | 197.0840 | 195.0694 | ESI+ | 12 |
| 145 | Chlorfenvinphos | 470-90-6 | C12H14Cl3O4P | 357.9695 | 358.9768 | 356.9622 | ESI+ | 12 |
| 146 | Chlorfluazuron | 71422-67-8 | C20H9Cl3F5N3O3 | 538.9630 | 539.9703 | 537.9557 | ESI+ | 17 |
| 147 | Chloridazon | 1698-60-8 | C10H8ClN3O | 221.0356 | 222.0429 | 220.0283 | ESI+ | 11 |
| 148 | Chlorimuron-ethyl | 90982-32-4 | C15H15ClN4O6S | 414.0401 | 415.0474 | 413.0328 | ESI+ | 12 |
| 149 | Chlormequat-chloride | 999-81-5 | C5H13Cl2N | 157.0425 | 158.0498 | 156.0352 | ESI+ | 6 |
| 150 | Chlorophacinone | 3691-35-8 | C23H15ClO3 | 374.0710 | 375.0783 | 373.0637 | ESI- | 15 |

| | Compound | CAS | Formula | M | [M+H]+ | [M-H]- | Ionisation Mode | MRM Transitions |
|---|---|---|---|---|---|---|---|---|
| 151 | Chlorotoluron | 15545-48-9 | C10H13ClN2O | 212.0716 | 213.0789 | 211.0643 | ESI+ | 8 |
| 152 | Chloroxuron | 1982-47-4 | C15H15ClN2O2 | 290.0822 | 291.0895 | 289.0749 | ESI+ | 12 |
| 153 | Chloroxynil | 1891-95-8 | C7H3Cl2NO | 186.9592 | 187.9665 | 185.9519 | ESI- | 6 |
| 154 | Chlorpropham | 101-21-3 | C10H12ClNO2 | 213.0557 | 214.0630 | 212.0484 | ESI+ | 2 |
| 155 | Chlorpyrifos | 2921-88-2 | C9H11Cl3NO3PS | 348.9263 | 349.9336 | 347.9190 | ESI+ | 16 |
| 156 | Chlorpyrifos-methyl | 5598-13-0 | C7H7Cl3NO3PS | 320.8950 | 321.9023 | 319.8877 | ESI+ | 12 |
| 157 | Chlorpyrifos-oxon | 5598-15-2 | C9H11Cl3NO4P | 332.9491 | 333.9564 | 331.9418 | ESI+ | 24 |
| 158 | Chlorsulfuron | 64902-72-3 | C12H12ClN5O4S | 357.0299 | 358.0372 | 356.0226 | ESI+ | 18 |
| 159 | Chlorthiamid | 1918-13-4 | C7H5Cl2NS | 204.9520 | 205.9593 | 203.9447 | ESI+ | 16 |
| 160 | Chromafenozide | 143807-66-3 | C24H30N2O3 | 394.2256 | 395.2329 | 393.2183 | ESI+ | 6 |
| 161 | Cinidon-ethyl | 142891-20-1 | C19H17Cl2NO4 | 393.0535 | 394.0608 | 392.0462 | ESI+ | 24 |
| 162 | Cinosulfuron | 94593-91-6 | C15H19N5O7S | 413.1005 | 414.1078 | 412.0932 | ESI+ | 6 |
| 163 | Clethodim | 99129-21-2 | C17H26ClNO3S | 359.1322 | 360.1395 | 358.1249 | ESI+ | 10 |
| 164 | Climbazole | 38083-17-9 | C15H17ClN2O2 | 292.0979 | 293.1052 | 291.0906 | ESI+ | 9 |
| 165 | Clodinafop (free acid) | 114420-56-3 | C14H11ClFNO4 | 311.0361 | 312.0434 | 310.0288 | ESI+ | 8 |
| 166 | Clodinafop-propargyl | 105512-06-9 | C17H13ClFNO4 | 349.0517 | 350.0590 | 348.0444 | ESI+ | 12 |
| 167 | Clofentezine | 74115-24-5 | C14H8Cl2N4 | 302.0126 | 303.0199 | 301.0053 | ESI+ | 10 |
| 168 | Clomazone | 81777-89-1 | C12H14ClNO2 | 239.0713 | 240.0786 | 238.0640 | ESI+ | 8 |
| 169 | Clomeprop | 84496-56-0 | C16H15Cl2NO2 | 323.0480 | 324.0553 | 322.0407 | ESI+ | 21 |
| 170 | Cloprop | 101-10-0 | C9H9ClO3 | 200.0240 | 201.0313 | 199.0167 | ESI- | 2 |
| 171 | Clopyralid | 1702-17-6 | C6H3Cl2NO2 | 190.9541 | 191.9614 | 189.9468 | ESI- | 2 |
| 172 | Cloquintocet-mexyl | 99607-70-2 | C18H22ClNO3 | 335.1288 | 336.1361 | 334.1215 | ESI+ | 9 |
| 173 | Cloransulam-methyl | 147150-35-4 | C15H13ClFN5O5S | 429.0310 | 430.0383 | 428.0237 | ESI+ | 12 |
| 174 | Clothianidin | 210880-92-5 | C6H8ClN5O2S | 249.0087 | 250.0160 | 248.0014 | ESI+ | 7 |
| 175 | Coumachlor | 81-82-3 | C19H15ClO4 | 342.0659 | 343.0732 | 341.0586 | ESI+ | 18 |
| 176 | Coumaphos | 56-72-4 | C14H16ClO5PS | 362.0145 | 363.0218 | 361.0072 | ESI+ | 12 |
| 177 | Coumatetralyl | 5836-29-3 | C19H16O3 | 292.1099 | 293.1172 | 291.1026 | ESI+ | 6 |
| 178 | Crimidine | 535-89-7 | C7H10ClN3 | 171.0563 | 172.0636 | 170.0490 | ESI+ | 12 |
| 179 | Crotoxyphos | 7700-17-6 | C14H19O6P | 314.0919 | 315.0992 | 313.0846 | ESI+ | 6 |
| 180 | Crufomate | 299-86-5 | C12H19ClNO3P | 291.0791 | 292.0864 | 290.0718 | ESI+ | 12 |
| 181 | Cumyluron | 99485-76-4 | C17H19ClN2O | 302.1186 | 303.1259 | 301.1113 | ESI+ | 2 |
| 182 | Cyanazine | 21725-46-2 | C9H13ClN6 | 240.0890 | 241.0963 | 239.0817 | ESI+ | 6 |
| 183 | Cyanofenphos | 13067-93-1 | C15H14NO2PS | 303.0483 | 304.0556 | 302.0410 | ESI+ | 6 |
| 184 | Cyazofamid | 120116-88-3 | C13H13ClN4O2S | 324.0448 | 325.0521 | 323.0375 | ESI+ | 5 |
| 185 | Cyclanilide | 113136-77-9 | C11H9Cl2NO3 | 272.9959 | 274.0032 | 271.9886 | ESI- | 20 |
| 186 | Cycloate | 1134-23-2 | C11H21NOS | 215.1344 | 216.1417 | 214.1271 | ESI+ | 5 |
| 187 | Cycloheximide | 66-81-9 | C15H23NO4 | 281.1627 | 282.1700 | 280.1554 | ESI+ | 12 |
| 188 | Cycloprothrin | 63935-38-6 | C26H21Cl2NO4 | 481.0848 | 482.0921 | 480.0775 | ESI+ | 2 |
| 189 | Cyclosulfamuron | 136849-15-5 | C17H19N5O6S | 421.1056 | 422.1129 | 420.0983 | ESI+ | 6 |
| 190 | Cycloxydim | 101205-02-1 | C17H27NO3S | 325.1712 | 326.1785 | 324.1639 | ESI+ | 10 |
| 191 | Cycluron | 2163-69-1 | C11H22N2O | 198.1732 | 199.1805 | 197.1659 | ESI+ | 5 |
| 192 | Cyflufenamid | 180409-60-3 | C20H17F5N2O2 | 412.1210 | 413.1283 | 411.1137 | ESI+ | 6 |
| 193 | Cyflumetofen | 400882-07-7 | C24H24F3NO4 | 447.1657 | 448.1730 | 446.1584 | ESI+ | 8 |
| 194 | Cyhalofop-butyl | 122008-85-9 | C20H20FNO4 | 357.1376 | 358.1449 | 356.1303 | ESI+ | 3 |
| 195 | Cymiazole | 61676-87-7 | C12H14N2S | 218.0878 | 219.0951 | 217.0805 | ESI+ | 6 |
| 196 | Cymoxanil | 57966-95-7 | C7H10N4O3 | 198.0753 | 199.0826 | 197.0680 | ESI+ | 4 |
| 197 | Cypermethrin | 52315-07-8 | C22H19Cl2NO3 | 415.0742 | 416.0815 | 414.0669 | ESI+ | 10 |
| 198 | Cyphenothrin | 39515-40-7 | C24H25NO3 | 375.1834 | 376.1907 | 374.1761 | ESI+ | 12 |
| 199 | Cyproconazole | 94361-06-5 | C15H18ClN3O | 291.1138 | 292.1211 | 290.1065 | ESI+ | 10 |
| 200 | Cyprodinil | 121552-61-2 | C14H15N3 | 225.1266 | 226.1339 | 224.1193 | ESI+ | 6 |
| 201 | Cyromazine | 66215-27-8 | C6H10N6 | 166.0967 | 167.1040 | 165.0894 | ESI+ | 6 |
| 202 | Daimuron (Dymron) | 42609-52-9 | C17H20N2O | 268.1576 | 269.1649 | 267.1503 | ESI+ | 6 |
| 203 | Dalapon | 75-99-0 | C3H4Cl2O2 | 141.9588 | 142.9661 | 140.9515 | ESI- | 10 |
| 204 | Daminozide | 1596-84-5 | C6H12N2O3 | 160.0848 | 161.0921 | 159.0775 | ESI+ | 6 |
| 205 | Dazomet | 533-74-4 | C5H10N2S2 | 162.0285 | 163.0358 | 161.0212 | ESI+ | 6 |
| 206 | Deet | 134-62-3 | C12H17NO | 191.1310 | 192.1383 | 190.1237 | ESI+ | 2 |
| 207 | Deltamethrin | 52918-63-5 | C22H19Br2NO3 | 502.9732 | 503.9805 | 501.9659 | ESI+ | 12 |
| 208 | Demeton-O | 298-03-3 | C8H19O3PS2 | 258.0513 | 259.0586 | 257.0440 | ESI+ | 2 |
| 209 | Demeton-S | 126-75-0 | C8H19O3PS2 | 258.0513 | 259.0586 | 257.0440 | ESI+ | 3 |
| 210 | Demeton-S-methyl | 919-86-8 | C6H15O3PS2 | 230.0200 | 231.0273 | 229.0127 | ESI+ | 2 |
| 211 | Demeton-S-methyl-sulfone | 17040-19-6 | C6H15O5PS2 | 262.0099 | 263.0172 | 261.0026 | ESI+ | 6 |
| 212 | Desmedipham | 13684-56-5 | C16H16N2O4 | 300.1110 | 301.1183 | 299.1037 | ESI+ | 6 |
| 213 | Desmetryn | 1014-69-3 | C8H15N5S | 213.1048 | 214.1121 | 212.0975 | ESI+ | 4 |
| 214 | Diafenthiuron | 80060-09-9 | C23H32N2OS | 384.2235 | 385.2308 | 383.2162 | ESI+ | 12 |
| 215 | Dialifos | 10311-84-9 | C14H17ClNO4PS2 | 393.0025 | 394.0098 | 391.9952 | ESI+ | 12 |
| 216 | Diallate | 2303-16-4 | C10H17Cl2NOS | 269.0408 | 270.0481 | 268.0335 | ESI+ | 12 |
| 217 | Diazinon | 333-41-5 | C12H21N2O3PS | 304.1010 | 305.1083 | 303.0937 | ESI+ | 6 |
| 218 | Dicamba | 1918-00-9 | C8H6Cl2O3 | 219.9694 | 220.9767 | 218.9621 | ESI- | 2 |
| 219 | Dichlofenthion | 97-17-6 | C10H13Cl2O3PS | 313.9700 | 314.9773 | 312.9627 | ESI+ | 8 |
| 220 | Dichlofluanid | 1085-98-9 | C9H11Cl2FN2O2S2 | 331.9623 | 332.9696 | 330.9550 | ESI+ | 11 |
| 221 | Dichlormid | 37764-25-3 | C8H11Cl2NO | 207.0218 | 208.0291 | 206.0145 | ESI+ | 19 |
| 222 | Dichlorprop | 120-36-5 | C9H8Cl2O3 | 233.9850 | 234.9923 | 232.9777 | ESI- | 8 |
| 223 | Dichlorvos | 62-73-7 | C4H7Cl2O4P | 219.9459 | 220.9532 | 218.9386 | ESI+ | 17 |
| 224 | Diclobutrazol | 75736-33-3 | C15H19Cl2N3O | 327.0905 | 328.0978 | 326.0832 | ESI+ | 4 |
| 225 | Diclofop | 40843-25-2 | C15H12Cl2O4 | 326.0113 | 327.0186 | 325.0040 | ESI- | 4 |

| | Compound | CAS | Formula | M | [M+H]+ | [M-H]- | Ionisation Mode | MRM Transitions |
|---|---|---|---|---|---|---|---|---|
| 226 | Diclofop-methyl | 51338-27-3 | C16H14Cl2O4 | 340.0269 | 341.0342 | 339.0196 | ESI+ | 12 |
| 227 | Dicloran | 99-30-9 | C6H4Cl2N2O2 | 205.9650 | 206.9723 | 204.9577 | ESI+ | 4 |
| 228 | Diclosulam | 145701-21-9 | C13H10Cl2FN5O3S | 404.9865 | 405.9938 | 403.9792 | ESI+ | 9 |
| 229 | Dicrotophos | 141-66-2 | C8H16NO5P | 237.0766 | 238.0839 | 236.0693 | ESI+ | 6 |
| 230 | Dicyclanil | 112636-83-6 | C8H10N6 | 190.0967 | 191.1040 | 189.0894 | ESI+ | 6 |
| 231 | Didecyldimethylammonium | 7173-51-5 | C22H47N | 325.3709 | 326.3782 | 324.3636 | ESI+ | 6 |
| 232 | Diethanolamine | 111-42-2 | C4H11NO2 | 105.0790 | 106.0863 | 104.0717 | ESI+ | 6 |
| 233 | Diethofencarb | 87130-20-9 | C14H21NO4 | 267.1471 | 268.1544 | 266.1398 | ESI+ | 6 |
| 234 | Difenacoum | 56073-07-5 | C31H24O3 | 444.1725 | 445.1798 | 443.1652 | ESI+ | 12 |
| 235 | Difenoconazole | 119446-68-3 | C19H17Cl2N3O3 | 405.0647 | 406.0720 | 404.0574 | ESI+ | 12 |
| 236 | Difenoxuron | 14214-32-5 | C16H18N2O3 | 286.1317 | 287.1390 | 285.1244 | ESI+ | 6 |
| 237 | Difenzoquat-methyl-sulfate | 43222-48-6 | C17H16N2 | 248.1313 | 249.1386 | 247.1240 | ESI+ | 6 |
| 238 | Diflubenzuron | 35367-38-5 | C14H9ClF2N2O2 | 310.0321 | 311.0394 | 309.0248 | ESI+ | 9 |
| 239 | Diflufenican | 83164-33-4 | C19H11F5N2O2 | 394.0741 | 395.0814 | 393.0668 | ESI+ | 12 |
| 240 | Dimefuron | 34205-21-5 | C15H19ClN4O3 | 338.1146 | 339.1219 | 337.1073 | ESI+ | 5 |
| 241 | Dimepiperate | 61432-55-1 | C15H21NOS | 263.1344 | 264.1417 | 262.1271 | ESI+ | 6 |
| 242 | Dimethachlon | 24096-53-5 | C10H7Cl2NO2 | 242.9854 | 243.9927 | 241.9781 | ESI- | 2 |
| 243 | Dimethachlor | 50563-36-5 | C13H18ClNO2 | 255.1026 | 256.1099 | 254.0953 | ESI+ | 12 |
| 244 | Dimethametryn | 22936-75-0 | C11H21N5S | 255.1518 | 256.1591 | 254.1445 | ESI+ | 6 |
| 245 | Dimethenamid | 87674-68-8 | C12H18ClNO2S | 275.0747 | 276.0820 | 274.0674 | ESI+ | 12 |
| 246 | Dimethirimol | 5221-53-4 | C11H19N3O | 209.1528 | 210.1601 | 208.1455 | ESI+ | 3 |
| 247 | Dimethoate | 60-51-5 | C5H12NO3PS2 | 228.9996 | 230.0069 | 227.9923 | ESI+ | 6 |
| 248 | Dimethomorph | 110488-70-5 | C21H22ClNO4 | 387.1237 | 388.1310 | 386.1164 | ESI+ | 12 |
| 249 | Dimetilan | 644-64-4 | C10H16N4O3 | 240.1222 | 241.1295 | 239.1149 | ESI+ | 6 |
| 250 | Dimoxystrobin | 149961-52-4 | C19H22N2O3 | 326.1630 | 327.1703 | 325.1557 | ESI+ | 6 |
| 251 | Diniconazole | 83657-24-3 | C15H17Cl2N3O | 325.0749 | 326.0822 | 324.0676 | ESI+ | 7 |
| 252 | Dinocap | 39300-45-3 | C18H24N2O6 | 364.1634 | 365.1707 | 363.1561 | ESI+ | 6 |
| 253 | Dinoseb | 88-85-7 | C10H12N2O5 | 240.0746 | 241.0819 | 239.0673 | ESI- | 4 |
| 254 | Dinotefuran | 165252-70-0 | C7H14N4O3 | 202.1066 | 203.1139 | 201.0993 | ESI+ | 6 |
| 255 | Dinoterb | 1420-07-1 | C10H12N2O5 | 240.0746 | 241.0819 | 239.0673 | ESI- | 4 |
| 256 | Dioxacarb | 6988-21-2 | C11H13NO4 | 223.0845 | 224.0918 | 222.0772 | ESI+ | 6 |
| 257 | Dioxathion | 78-34-2 | C12H26O6P2S4 | 456.0087 | 457.0160 | 455.0014 | ESI+ | 6 |
| 258 | Diphenamid | 957-51-7 | C16H17NO | 239.1310 | 240.1383 | 238.1237 | ESI+ | 6 |
| 259 | Diphenylamine | 122-39-4 | C12H11N | 169.0891 | 170.0964 | 168.0818 | ESI+ | 4 |
| 260 | Dipropetryn | 4147-51-7 | C11H21N5S | 255.1518 | 256.1591 | 254.1445 | ESI+ | 6 |
| 261 | Diquat | 6385-62-2 | C12H12N2 | 184.1000 | 185.1073 | 183.0927 | ESI+ | 3 |
| 262 | Disulfoton | 298-04-4 | C8H19O2PS3 | 274.0285 | 275.0358 | 273.0212 | ESI+ | 3 |
| 263 | Disulfoton-sulfone | 2497-06-5 | C8H19O4PS3 | 306.0183 | 307.0256 | 305.0110 | ESI+ | 6 |
| 264 | Disulfoton-sulfoxide | 2497-07-6 | C8H19O3PS3 | 290.0234 | 291.0307 | 289.0161 | ESI+ | 6 |
| 265 | Ditalimfos | 5131-24-8 | C12H14NO4PS | 299.0381 | 300.0454 | 298.0308 | ESI+ | 6 |
| 266 | Dithianon | 3347-22-6 | C14H4N2O2S2 | 295.9714 | 296.9787 | 294.9641 | ESI- | 4 |
| 267 | Dithiopyr | 97886-45-8 | C15H16F5NO2S2 | 401.0543 | 402.0616 | 400.0470 | ESI+ | 6 |
| 268 | Diuron (DCMU) | 330-54-1 | C9H10Cl2N2O | 232.0170 | 233.0243 | 231.0097 | ESI+ | 7 |
| 269 | DMST | 66840-71-9 | C9H14N2O2S | 214.0776 | 215.0849 | 213.0703 | ESI+ | 4 |
| 270 | DNOC | 534-52-1 | C7H6N2O5 | 198.0277 | 199.0350 | 197.0204 | ESI- | 6 |
| 271 | Dodemorph | 1593-77-7 | C18H35NO | 281.2719 | 282.2792 | 280.2646 | ESI+ | 6 |
| 272 | Dodine | 2439-10-3 | C15H33N3O2 | 287.2573 | 288.2646 | 286.2500 | ESI+ | 6 |
| 273 | Doramectin | 117704-25-3 | C50H74O14 | 898.5079 | 899.5152 | 897.5006 | ESI+ | 10 |
| 274 | Edifenphos | 17109-49-8 | C14H15O2PS2 | 310.0251 | 311.0324 | 309.0178 | ESI+ | 6 |
| 275 | Emamectin B1a | 119791-41-2 | C49H75NO13 | 885.5238 | 886.5311 | 884.5165 | ESI+ | 5 |
| 276 | Emamectin B1b | 137335-79-6 | C55H79NO15 | 871.5082 | 872.5155 | 870.5009 | ESI+ | 3 |
| 277 | Endosulfan-sulfate | 1031-07-8 | C9H6Cl6O4S | 419.8118 | 420.8191 | 418.8045 | ESI- | 3 |
| 278 | EPN | 2104-64-5 | C14H14NO4PS | 323.0381 | 324.0454 | 322.0308 | ESI+ | 6 |
| 279 | Epoxiconazole | 133855-98-8 | C17H13ClFN3O | 329.0731 | 330.0804 | 328.0658 | ESI+ | 9 |
| 280 | EPTC | 759-94-4 | C9H19NOS | 189.1187 | 190.1260 | 188.1114 | ESI+ | 5 |
| 281 | Esfenvalerate | 66230-04-4 | C25H22ClNO3 | 419.1288 | 420.1361 | 418.1215 | ESI+ | 2 |
| 282 | Esprocarb | 85785-20-2 | C15H23NOS | 265.1500 | 266.1573 | 264.1427 | ESI+ | 5 |
| 283 | Etaconazole | 60207-93-4 | C14H15Cl2N3O2 | 327.0541 | 328.0614 | 326.0468 | ESI+ | 12 |
| 284 | Ethametsulfuron-methyl | 97780-06-8 | C15H18N6O6S | 410.1009 | 411.1082 | 409.0936 | ESI+ | 6 |
| 285 | Ethephon | 16672-87-0 | C2H6ClO3P | 143.9743 | 144.9816 | 142.9670 | ESI- | 6 |
| 286 | Ethidimuron | 30043-49-3 | C7H12N4O3S2 | 264.0351 | 265.0424 | 263.0278 | ESI+ | 11 |
| 287 | Ethiofencarb | 29973-13-5 | C11H15NO2S | 225.0823 | 226.0896 | 224.0750 | ESI+ | 10 |
| 288 | Ethiofencarb-sulfone | 53380-23-7 | C11H15NO4S | 257.0722 | 258.0795 | 256.0649 | ESI+ | 8 |
| 289 | Ethiofencarb-sulfoxide | 53380-22-6 | C11H15NO3S | 241.0773 | 242.0846 | 240.0700 | ESI+ | 4 |
| 290 | Ethion | 563-12-2 | C9H22O4P2S4 | 383.9876 | 384.9949 | 382.9803 | ESI+ | 6 |
| 291 | Ethiprole | 181587-01-9 | C13H9Cl2F3N4OS | 395.9826 | 396.9899 | 394.9753 | ESI+ | 30 |
| 292 | Ethirimol | 23947-60-6 | C11H19N3O | 209.1528 | 210.1601 | 208.1455 | ESI+ | 6 |
| 293 | Ethofumesate | 26225-79-6 | C13H18O5S | 286.0875 | 287.0948 | 285.0802 | ESI+ | 11 |
| 294 | Ethoprophos | 13194-48-4 | C8H19O2PS2 | 242.0564 | 243.0637 | 241.0491 | ESI+ | 6 |
| 295 | Ethoxyquin | 91-53-2 | C14H19NO | 217.1467 | 218.1540 | 216.1394 | ESI+ | 4 |
| 296 | Ethoxysulfuron | 126801-58-9 | C15H18N4O7S | 398.0896 | 399.0969 | 397.0823 | ESI+ | 6 |
| 297 | Ethylenethiourea | 96-45-7 | C3H6N2S | 102.0252 | 103.0325 | 101.0179 | ESI+ | 6 |
| 298 | Etofenprox | 80844-07-1 | C25H28O3 | 376.2038 | 377.2111 | 375.1965 | ESI+ | 6 |
| 299 | Etoxazole | 153233-91-1 | C21H23F2NO2 | 359.1697 | 360.1770 | 358.1624 | ESI+ | 6 |
| 300 | Etrimfos | 38260-54-7 | C10H17N2O4PS | 292.0647 | 293.0720 | 291.0574 | ESI+ | 6 |

| | Compound | CAS | Formula | M | [M+H]+ | [M-H]- | Ionisation Mode | MRM Transitions |
|---|---|---|---|---|---|---|---|---|
| 301 | Famoxadone | 131807-57-3 | C22H18N2O4 | 374.1267 | 375.1340 | 373.1194 | ESI+ | 6 |
| 302 | Famphur | 52-85-7 | C10H16NO5PS2 | 325.0208 | 326.0281 | 324.0135 | ESI+ | 12 |
| 303 | Fenamidone | 161326-34-7 | C17H17N3OS | 311.1092 | 312.1165 | 310.1019 | ESI+ | 6 |
| 304 | Fenaminosulf | 140-56-7 | C8H10N3NaO3S | 251.0341 | 252.0414 | 250.0268 | ESI+ | 2 |
| 305 | Fenamiphos | 22224-92-6 | C13H22NO3PS | 303.1058 | 304.1131 | 302.0985 | ESI+ | 6 |
| 306 | Fenamiphos-sulfone | 31972-44-8 | C13H22NO5PS | 335.0956 | 336.1029 | 334.0883 | ESI+ | 6 |
| 307 | Fenamiphos-sulfoxide | 31972-43-7 | C13H22NO4PS | 319.1007 | 320.1080 | 318.0934 | ESI+ | 6 |
| 308 | Fenarimol | 60168-88-9 | C17H12Cl2N2O | 330.0327 | 331.0400 | 329.0254 | ESI+ | 12 |
| 309 | Fenazaquin | 120928-09-8 | C20H22N2O | 306.1732 | 307.1805 | 305.1659 | ESI+ | 6 |
| 310 | Fenazox | 495-48-7 | C12H10N2O | 198.0793 | 199.0866 | 197.0720 | ESI+ | 6 |
| 311 | Fenbuconazole | 114369-43-6 | C19H17ClN4 | 336.1142 | 337.1215 | 335.1069 | ESI+ | 8 |
| 312 | Fenbutatin-oxide | 13356-08-6 | C60H78OSn2 | 1054.4121 | 1055.4194 | 1053.4048 | ESI+ | 11 |
| 313 | Fenchlorazol-ethyl | 103112-35-2 | C12H8Cl5N3O2 | 400.9059 | 401.9132 | 399.8986 | ESI+ | 118 |
| 314 | Fenfuram | 24691-80-3 | C12H11NO2 | 201.0790 | 202.0863 | 200.0717 | ESI+ | 6 |
| 315 | Fenhexamid | 126833-17-8 | C14H17Cl2NO2 | 301.0636 | 302.0709 | 300.0563 | ESI+ | 23 |
| 316 | Fenitrothion | 122-14-5 | C9H12NO5PS | 277.0174 | 278.0247 | 276.0101 | ESI+ | 2 |
| 317 | Fenobucarb | 3766-81-2 | C12H17NO2 | 207.1259 | 208.1332 | 206.1186 | ESI+ | 6 |
| 318 | Fenoprop | 93-72-1 | C9H7Cl3O3 | 267.9461 | 268.9534 | 266.9388 | ESI- | 8 |
| 319 | Fenothiocarb | 62850-32-2 | C13H19NO2S | 253.1136 | 254.1209 | 252.1063 | ESI+ | 4 |
| 320 | Fenoxanil | 115852-48-7 | C15H18Cl2N2O2 | 328.0745 | 329.0818 | 327.0672 | ESI+ | 29 |
| 321 | Fenoxaprop | 95617-09-7 | C16H12ClNO5 | 333.0404 | 334.0477 | 332.0331 | ESI+ | 23 |
| 322 | Fenoxaprop-ethyl | 66441-23-4 | C18H16ClNO5 | 361.0717 | 362.0790 | 360.0644 | ESI+ | 12 |
| 323 | Fenoxaprop-P-ethyl | 71283-80-2 | C18H16ClNO5 | 361.0717 | 362.0790 | 360.0644 | ESI+ | 12 |
| 324 | Fenoxycarb | 79127-80-3 | C17H19NO4 | 301.1314 | 302.1387 | 300.1241 | ESI+ | 6 |
| 325 | Fenpropathrin | 64257-84-7 | C22H23NO3 | 349.1678 | 350.1751 | 348.1605 | ESI+ | 11 |
| 326 | Fenpropidin | 67306-00-7 | C19H31N | 273.2457 | 274.2530 | 272.2384 | ESI+ | 6 |
| 327 | Fenpropimorph | 67564-91-4 | C20H33NO | 303.2562 | 304.2635 | 302.2489 | ESI+ | 6 |
| 328 | Fensulfothion | 115-90-2 | C11H17O4PS2 | 308.0306 | 309.0379 | 307.0233 | ESI+ | 6 |
| 329 | Fensulfothion-oxon | 6552-21-2 | C11H17O5PS | 292.0534 | 293.0607 | 291.0461 | ESI+ | 6 |
| 330 | Fensulfothion-oxon-sulfone | 6132-17-8 | C11H17O6PS | 308.0483 | 309.0556 | 307.0410 | ESI+ | 4 |
| 331 | Fensulfothion-sulfone | 14255-72-2 | C11H17O5PS2 | 324.0255 | 325.0328 | 323.0182 | ESI+ | 6 |
| 332 | Fenthion | 55-38-9 | C10H15O3PS2 | 278.0200 | 279.0273 | 277.0127 | ESI+ | 6 |
| 333 | Fenthion-oxon | 6552-12-1 | C10H15O4PS | 262.0429 | 263.0502 | 261.0356 | ESI+ | 6 |
| 334 | Fenthion-oxon-sulfone | 14086-35-2 | C10H15O6PS | 294.0327 | 295.0400 | 293.0254 | ESI+ | 12 |
| 335 | Fenthion-oxon-sulfoxide | 6552-13-2 | C10H15O5PS | 278.0378 | 279.0451 | 277.0305 | ESI+ | 3 |
| 336 | Fenthion-sulfone | 3761-42-0 | C10H15O5PS2 | 310.0099 | 311.0172 | 309.0026 | ESI+ | 4 |
| 337 | Fenthion-sulfoxide | 3761-41-9 | C10H15O4PS2 | 294.0149 | 295.0222 | 293.0076 | ESI+ | 6 |
| 338 | Fenuron | 101-42-8 | C9H12N2O | 164.0950 | 165.1023 | 163.0877 | ESI+ | 6 |
| 339 | Fenvalerate | 51630-58-1 | C25H22ClNO3 | 419.1288 | 420.1361 | 418.1215 | ESI+ | 6 |
| 340 | Fipronil | 120068-37-3 | C12H4Cl2F6N4OS | 435.9387 | 436.9460 | 434.9314 | ESI- | 12 |
| 341 | Fipronil-desulfinyl | 205650-65-3 | C12H4Cl2F6N4 | 387.9717 | 388.9790 | 386.9644 | ESI- | 12 |
| 342 | Fipronil-sulfide | 120067-83-6 | C12H4Cl2F6N4S | 419.9438 | 420.9511 | 418.9365 | ESI- | 12 |
| 343 | Fipronil-sulfone | 120068-36-2 | C12H4Cl2F6N4O2S | 451.9336 | 452.9409 | 450.9263 | ESI- | 12 |
| 344 | Flamprop-isopropyl | 52756-22-6 | C19H19ClFNO3 | 363.1037 | 364.1110 | 362.0964 | ESI+ | 10 |
| 345 | Flamprop-methyl | 52756-25-9 | C17H15ClFNO3 | 335.0724 | 336.0797 | 334.0651 | ESI+ | 4 |
| 346 | Flamprop-M-isopropyl | 63782-90-1 | C19H19ClFNO3 | 363.1037 | 364.1110 | 362.0964 | ESI+ | 10 |
| 347 | Flazasulfuron | 104040-78-0 | C13H12F3N5O5S | 407.0511 | 408.0584 | 406.0438 | ESI+ | 6 |
| 348 | Flocoumafen | 90035-08-8 | C33H25F3O4 | 542.1705 | 543.1778 | 541.1632 | ESI+ | 12 |
| 349 | Flonicamid | 158062-67-0 | C9H6F3N3O | 229.0463 | 230.0536 | 228.0390 | ESI+ | 8 |
| 350 | Florasulam | 145701-23-1 | C12H8F3N5O3S | 359.0300 | 360.0373 | 358.0227 | ESI+ | 2 |
| 351 | Fluacrypyrim | 229977-93-9 | C20H21F3N2O5 | 426.1403 | 427.1476 | 425.1330 | ESI+ | 6 |
| 352 | Fluazifop | 69335-91-7 | C15H12F3NO4 | 327.0718 | 328.0791 | 326.0645 | ESI+ | 12 |
| 353 | Fluazifop-butyl | 69806-50-4 | C19H20F3NO4 | 383.1344 | 384.1417 | 382.1271 | ESI+ | 6 |
| 354 | Fluazifop-P (free acid) | 83066-88-0 | C15H12F3NO4 | 327.0718 | 328.0791 | 326.0645 | ESI+ | 12 |
| 355 | Fluazifop-P-butyl | 79241-46-6 | C19H20F3NO4 | 383.1344 | 384.1417 | 382.1271 | ESI+ | 6 |
| 356 | Fluazinam | 79622-59-6 | C13H4Cl2F6N4O4 | 463.9514 | 464.9587 | 462.9441 | ESI- | 12 |
| 357 | Fluazuron | 86811-58-7 | C20H10Cl2F5N3O3 | 505.0019 | 506.0092 | 503.9946 | ESI+ | 17 |
| 358 | Flubendiamide | 272451-65-7 | C23H22F7IN2O4S | 682.0233 | 683.0306 | 681.0160 | ESI+ | 5 |
| 359 | Flucycloxuron | 94050-52-9 | C25H20ClF2N3O3 | 483.1161 | 484.1234 | 482.1088 | ESI+ | 10 |
| 360 | Flucythrinate | 70124-77-5 | C26H23F2NO4 | 451.1595 | 452.1668 | 450.1522 | ESI+ | 4 |
| 361 | Fludioxonil | 131341-86-1 | C12H6F2N2O2 | 248.0397 | 249.0470 | 247.0324 | ESI- | 6 |
| 362 | Flufenacet | 142459-58-3 | C14H13F4N3O2S | 363.0665 | 364.0738 | 362.0592 | ESI+ | 6 |
| 363 | Flufenoxuron | 101463-69-8 | C21H11ClF6N2O3 | 488.0362 | 489.0435 | 487.0289 | ESI+ | 8 |
| 364 | Flumetralin | 62924-70-3 | C16H12ClF4N3O4 | 421.0452 | 422.0525 | 420.0379 | ESI+ | 3 |
| 365 | Flumetsulam | 98967-40-9 | C12H9F2N5O2S | 325.0445 | 326.0518 | 324.0372 | ESI+ | 2 |
| 366 | Flumioxazin | 103361-09-7 | C19H15FN2O4 | 354.1016 | 355.1089 | 353.0943 | ESI+ | 2 |
| 367 | Fluometuron | 2164-17-2 | C10H11F3N2O | 232.0823 | 233.0896 | 231.0750 | ESI+ | 4 |
| 368 | Fluopicolide | 239110-15-7 | C14H8Cl3F3N2O | 381.9654 | 382.9727 | 380.9581 | ESI+ | 11 |
| 369 | Fluopyram | 658066-35-4 | C16H11ClF6N2O | 396.0464 | 397.0537 | 395.0391 | ESI+ | 12 |
| 370 | Fluoroglycofen-ethyl | 77501-90-7 | C18H13ClF3NO7 | 447.0333 | 448.0406 | 446.0260 | ESI+ | 12 |
| 371 | Fluoxastrobin | 361377-29-9 | C21H16ClFN4O5 | 458.0793 | 459.0866 | 457.0720 | ESI+ | 12 |
| 372 | Flupyrsulfuron-methyl | 144740-54-5 | C15H14F3N5O7S | 465.0566 | 466.0639 | 464.0493 | ESI+ | 12 |
| 373 | Fluquinconazole | 136426-54-5 | C16H8Cl2FN5O | 375.0090 | 376.0163 | 374.0017 | ESI+ | 10 |
| 374 | Fluridone | 59756-60-4 | C19H14F3NO | 329.1027 | 330.1100 | 328.0954 | ESI+ | 4 |
| 375 | Flurochloridone | 61213-25-0 | C12H10Cl2F3NO | 311.0092 | 312.0165 | 310.0019 | ESI+ | 18 |

| | Compound | CAS | Formula | M | [M+H]+ | [M-H]- | Ionisation Mode | MRM Transitions |
|---|---|---|---|---|---|---|---|---|
| 376 | Fluroxypyr | 69377-81-7 | C7H5Cl2FN2O3 | 253.9661 | 254.9734 | 252.9588 | ESI+ | 15 |
| 377 | Fluroxypyr-1-methylheptylester | 81406-37-3 | C15H21Cl2FN2O3 | 366.0913 | 367.0986 | 365.0840 | ESI+ | 12 |
| 378 | Flurprimidol | 56425-91-3 | C15H15F3N2O2 | 312.1086 | 313.1159 | 311.1013 | ESI+ | 6 |
| 379 | Flurtamone | 96525-23-4 | C18H14F3NO2 | 333.0977 | 334.1050 | 332.0904 | ESI+ | 6 |
| 380 | Flusilazole | 85509-19-9 | C16H15F2N3Si | 315.1003 | 316.1076 | 314.0930 | ESI+ | 6 |
| 381 | Fluthiacet-methyl | 117337-19-6 | C15H15ClFN3O3S2 | 403.0227 | 404.0300 | 402.0154 | ESI+ | 9 |
| 382 | Flutolanil | 66332-96-5 | C17H16F3NO2 | 323.1133 | 324.1206 | 322.1060 | ESI+ | 12 |
| 383 | Flutriafol | 76674-21-0 | C16H13F2N3O | 301.1027 | 302.1100 | 300.0954 | ESI+ | 5 |
| 384 | Fluxapyroxad | 907204-31-3 | C18H12F5N3O | 381.0901 | 382.0974 | 380.0828 | ESI+ | 11 |
| 385 | Fomesafen | 72178-02-0 | C15H10ClF3N2O6S | 437.9900 | 438.9973 | 436.9827 | ESI+ | 21 |
| 386 | Fonofos | 944-22-9 | C10H15OPS2 | 246.0302 | 247.0375 | 245.0229 | ESI+ | 5 |
| 387 | Foramsulfuron | 173159-57-4 | C17H20N6O7S | 452.1114 | 453.1187 | 451.1041 | ESI+ | 6 |
| 388 | Forchlorfenuron | 68157-60-8 | C12H10ClN3O | 247.0512 | 248.0585 | 246.0439 | ESI+ | 12 |
| 389 | Fosetyl-aluminium | 39148-24-8 | C2H7O3P | 110.0133 | 111.0206 | 109.0060 | ESI- | 3 |
| 390 | Fosthiazate | 98886-44-3 | C9H18NO3PS2 | 283.0466 | 284.0539 | 282.0393 | ESI+ | 6 |
| 391 | Fuberidazole | 3878-19-1 | C11H8N2O | 184.0637 | 185.0710 | 183.0564 | ESI+ | 6 |
| 392 | Furalaxyl | 57646-30-7 | C17H19NO4 | 301.1314 | 302.1387 | 300.1241 | ESI+ | 3 |
| 393 | Furametpyr | 123572-88-3 | C17H20ClN3O2 | 333.1244 | 334.1317 | 332.1171 | ESI+ | 12 |
| 394 | Furathiocarb | 65907-30-4 | C18H26N2O5S | 382.1562 | 383.1635 | 381.1489 | ESI+ | 6 |
| 395 | Furmecyclox | 60568-05-0 | C14H21NO3 | 251.1521 | 252.1594 | 250.1448 | ESI+ | 6 |
| 396 | Gibberellic acid (Gibberellin) | 77-06-5 | C19H22O6 | 346.1416 | 347.1489 | 345.1343 | ESI- | 11 |
| 397 | Gluphosinate | 77182-82-2 | C5H12NO4P | 181.0504 | 182.0577 | 180.0431 | ESI+ | 10 |
| 398 | Glyphosate | 1071-83-6 | C3H8NO5P | 169.0140 | 170.0213 | 168.0067 | ESI+ | 8 |
| 399 | Halofenozide | 112226-61-6 | C18H19ClN2O2 | 330.1135 | 331.1208 | 329.1062 | ESI+ | 12 |
| 400 | Halosulfuron-methyl | 100784-20-1 | C13H15ClN6O7S | 434.0411 | 435.0484 | 433.0338 | ESI+ | 11 |
| 401 | Haloxyfop | 69806-34-4 | C15H11ClF3NO4 | 361.0329 | 362.0402 | 360.0256 | ESI+ | 9 |
| 402 | Haloxyfop-2-ethoxyethyl | 87237-48-7 | C19H19ClF3NO5 | 433.0904 | 434.0977 | 432.0831 | ESI+ | 12 |
| 403 | Haloxyfop-methyl | 69806-40-2 | C16H13ClF3NO4 | 375.0485 | 376.0558 | 374.0412 | ESI+ | 12 |
| 404 | Haloxyfop-R-methyl | 72619-32-0 | C16H13ClF3NO4 | 375.0485 | 376.0558 | 374.0412 | ESI+ | 12 |
| 405 | Heptenophos | 23560-59-0 | C9H12ClO4P | 250.0162 | 251.0235 | 249.0089 | ESI+ | 9 |
| 406 | Hexaconazole | 79983-71-4 | C14H17Cl2N3O | 313.0749 | 314.0822 | 312.0676 | ESI+ | 10 |
| 407 | Hexaflumuron | 86479-06-3 | C16H8Cl2F6N2O3 | 459.9816 | 460.9889 | 458.9743 | ESI- | 12 |
| 408 | Hexazinone | 51235-04-2 | C12H20N4O2 | 252.1586 | 253.1659 | 251.1513 | ESI+ | 3 |
| 409 | Hexythiazox | 78587-05-0 | C17H21ClN2O2S | 352.1012 | 353.1085 | 351.0939 | ESI+ | 11 |
| 410 | Hydramethylnon | 67485-29-4 | C25H24F6N4 | 494.1905 | 495.1978 | 493.1832 | ESI+ | 12 |
| 411 | Hymexazol | 10004-44-1 | C4H5NO2 | 99.0320 | 100.0393 | 98.0247 | ESI+ | 3 |
| 412 | Imazalil | 35554-44-0 | C14H14Cl2N2O | 296.0483 | 297.0556 | 295.0410 | ESI+ | 12 |
| 413 | Imazamethabenz-methyl | 81405-85-8 | C16H20N2O3 | 288.1474 | 289.1547 | 287.1401 | ESI+ | 12 |
| 414 | Imazamox | 114311-32-9 | C15H19N3O4 | 305.1376 | 306.1449 | 304.1303 | ESI+ | 10 |
| 415 | Imazapic | 104098-48-8 | C14H17N3O3 | 275.1270 | 276.1343 | 274.1197 | ESI+ | 11 |
| 416 | Imazapyr | 81334-34-1 | C13H15N3O3 | 261.1113 | 262.1186 | 260.1040 | ESI+ | 11 |
| 417 | Imazaquin | 81335-37-7 | C17H17N3O3 | 311.1270 | 312.1343 | 310.1197 | ESI+ | 6 |
| 418 | Imazethapyr | 81335-77-5 | C15H19N3O3 | 289.1426 | 290.1499 | 288.1353 | ESI+ | 12 |
| 419 | Imazosulfuron | 122548-33-8 | C14H13ClN6O5S | 412.0357 | 413.0430 | 411.0284 | ESI+ | 13 |
| 420 | Imibenconazole | 86598-92-7 | C17H13Cl3N4S | 409.9927 | 411.0000 | 408.9854 | ESI+ | 22 |
| 421 | Imidacloprid | 138261-41-3 | C9H10ClN5O2 | 255.0523 | 256.0596 | 254.0450 | ESI+ | 8 |
| 422 | Indanofan | 133220-30-1 | C20H17ClO3 | 340.0866 | 341.0939 | 339.0793 | ESI+ | 6 |
| 423 | Indoxacarb | 173584-44-6 | C22H17ClF3N3O7 | 527.0707 | 528.0780 | 526.0634 | ESI+ | 12 |
| 424 | Iodosulfuron-methyl | 144550-36-7 | C14H14IN5O6S | 506.9710 | 507.9783 | 505.9637 | ESI+ | 8 |
| 425 | Ioxynil | 1689-83-4 | C7H3I2NO | 370.8304 | 371.8377 | 369.8231 | ESI- | 4 |
| 426 | Ipconazole | 125225-28-7 | C18H24ClN3O | 333.1608 | 334.1681 | 332.1535 | ESI+ | 5 |
| 427 | Iprobenfos | 26087-47-8 | C13H21O3PS | 288.0949 | 289.1022 | 287.0876 | ESI+ | 3 |
| 428 | Iprodione | 36734-19-7 | C13H13Cl2N3O3 | 329.0334 | 330.0407 | 328.0261 | ESI+ | 4 |
| 429 | Iprovalicarb | 140923-17-7 | C18H28N2O3 | 320.2100 | 321.2173 | 319.2027 | ESI+ | 6 |
| 430 | Irgarol 1051 | 28159-98-0 | C11H19N5S | 253.1361 | 254.1434 | 252.1288 | ESI+ | 6 |
| 431 | Isazofos | 42509-80-8 | C9H17ClN3O3PS | 313.0417 | 314.0490 | 312.0344 | ESI+ | 12 |
| 432 | Isocarbamid | 30979-48-7 | C8H15N3O2 | 185.1164 | 186.1237 | 184.1091 | ESI+ | 6 |
| 433 | Isocarbofos | 24353-61-5 | C11H16NO4PS | 289.0538 | 290.0611 | 288.0465 | ESI+ | 6 |
| 434 | Isofenphos | 25311-71-1 | C15H24NO4PS | 345.1164 | 346.1237 | 344.1091 | ESI+ | 6 |
| 435 | Isofenphos-methyl | 99675-03-3 | C14H22NO4PS | 331.1007 | 332.1080 | 330.0934 | ESI+ | 6 |
| 436 | Isofenphos-oxon | 31120-85-1 | C15H24NO5P | 329.1392 | 330.1465 | 328.1319 | ESI+ | 3 |
| 437 | Isomethiozin | 57052-04-7 | C12H20N4OS | 268.1358 | 269.1431 | 267.1285 | ESI+ | 6 |
| 438 | Isonoruron | 28805-78-9 | C13H22N2O | 222.1732 | 223.1805 | 221.1659 | ESI+ | 6 |
| 439 | Isoprocarb | 2631-40-5 | C11H15NO2 | 193.1103 | 194.1176 | 192.1030 | ESI+ | 3 |
| 440 | Isopropalin | 33820-53-0 | C15H23N3O4 | 309.1689 | 310.1762 | 308.1616 | ESI+ | 6 |
| 441 | Isoprothiolane | 50512-35-1 | C12H18O4S2 | 290.0647 | 291.0720 | 289.0574 | ESI+ | 6 |
| 442 | Isoproturon | 34123-59-6 | C12H18N2O | 206.1419 | 207.1492 | 205.1346 | ESI+ | 6 |
| 443 | Isopyrazam | 881685-58-1 | C20H23F2N3O | 359.1809 | 360.1882 | 358.1736 | ESI+ | 9 |
| 444 | Isoxaben | 82558-50-7 | C18H24N2O4 | 332.1736 | 333.1809 | 331.1663 | ESI+ | 6 |
| 445 | Isoxadifen-ethyl | 163520-33-0 | C18H17NO3 | 295.1208 | 296.1281 | 294.1135 | ESI+ | 12 |
| 446 | Isoxaflutole | 141112-29-0 | C15H12F3NO4S | 359.0439 | 360.0512 | 358.0366 | ESI+ | 5 |
| 447 | Isoxathion | 18854-01-8 | C13H16NO4PS | 313.0538 | 314.0611 | 312.0465 | ESI+ | 6 |
| 448 | Ivermectine | 70288-86-7 | C48H74O14 | 874.5079 | 875.5152 | 873.5006 | ESI+ | 6 |
| 449 | Karbutilate | 4849-32-5 | C14H21N3O3 | 279.1583 | 280.1656 | 278.1510 | ESI+ | 16 |
| 450 | Kasugamycin | 6980-18-3 | C14H25N3O9 | 379.1591 | 380.1664 | 378.1518 | ESI+ | 3 |

| | Compound | CAS | Formula | M | [M+H]+ | [M-H]- | Ionisation Mode | MRM Transitions |
|---|---|---|---|---|---|---|---|---|
| 451 | Kresoxim-methyl | 143390-89-0 | C18H19NO4 | 313.1314 | 314.1387 | 312.1241 | ESI+ | 6 |
| 452 | Lactofen | 77501-63-4 | C19H15ClF3NO7 | 461.0489 | 462.0562 | 460.0416 | ESI+ | 12 |
| 453 | Lambda-Cyhalothrin | 91465-08-6 | C23H19ClF3NO3 | 449.1006 | 450.1079 | 448.0933 | ESI+ | 4 |
| 454 | Lenacil | 2164-08-1 | C13H18N2O2 | 234.1368 | 235.1441 | 233.1295 | ESI+ | 6 |
| 455 | Linuron | 330-55-2 | C9H10Cl2N2O2 | 248.0119 | 249.0192 | 247.0046 | ESI+ | 12 |
| 456 | Lufenuron | 103055-07-8 | C17H8Cl2F8N2O3 | 509.9784 | 510.9857 | 508.9711 | ESI- | 10 |
| 457 | Malaoxon | 1634-78-2 | C10H19O7PS | 314.0589 | 315.0662 | 313.0516 | ESI+ | 6 |
| 458 | Malathion | 121-75-5 | C10H19O6PS2 | 330.0361 | 331.0434 | 329.0288 | ESI+ | 12 |
| 459 | Maleic-hydrazide | 123-33-1 | C4H4N2O2 | 112.0273 | 113.0346 | 111.0200 | ESI+ | 3 |
| 460 | Mandipropamid | 374726-62-2 | C23H22ClNO4 | 411.1237 | 412.1310 | 410.1164 | ESI+ | 12 |
| 461 | MCPA (MCP) | 94-74-6 | C9H9ClO3 | 200.0240 | 201.0313 | 199.0167 | ESI- | 3 |
| 462 | MCPA-butoxyethyl ester | 19480-43-4 | C15H21ClO4 | 300.1128 | 301.1201 | 299.1055 | ESI+ | 12 |
| 463 | MCPB | 94-81-5 | C11H13ClO3 | 228.0553 | 229.0626 | 227.0480 | ESI- | 3 |
| 464 | Mecarbam | 2595-54-2 | C10H20NO5PS2 | 329.0521 | 330.0594 | 328.0448 | ESI+ | 6 |
| 465 | Mecoprop (MCPP) | 93-65-2 | C10H11ClO3 | 214.0397 | 215.0470 | 213.0324 | ESI- | 2 |
| 466 | Mecoprop-P | 16484-77-8 | C10H11ClO3 | 214.0397 | 215.0470 | 213.0324 | ESI- | 4 |
| 467 | Mefenacet | 73250-68-7 | C16H14N2O2S | 298.0776 | 299.0849 | 297.0703 | ESI+ | 6 |
| 468 | Mefenpyr-diethyl | 135590-91-9 | C16H18Cl2N2O4 | 372.0644 | 373.0717 | 371.0571 | ESI+ | 24 |
| 469 | Mefluidide | 53780-34-0 | C11H13F3N2O3S | 310.0599 | 311.0672 | 309.0526 | ESI+ | 10 |
| 470 | Mepanipyrim | 110235-47-7 | C14H13N3 | 223.1109 | 224.1182 | 222.1036 | ESI+ | 6 |
| 471 | Mephosfolan | 950-10-7 | C8H16NO3PS2 | 269.0309 | 270.0382 | 268.0236 | ESI+ | 6 |
| 472 | Mepiquat | 24307-26-4 | C7H16N | 114.1283 | 115.1356 | 113.1210 | ESI+ | 6 |
| 473 | Mepronil | 55814-41-0 | C17H19NO2 | 269.1416 | 270.1489 | 268.1343 | ESI+ | 5 |
| 474 | Meptyldinocap | 6119-92-2 | C18H24N2O6 | 364.1634 | 365.1707 | 363.1561 | ESI- | 6 |
| 475 | Mesosulfuron-methyl | 208465-21-8 | C17H21N5O9S2 | 503.0781 | 504.0854 | 502.0708 | ESI+ | 6 |
| 476 | Mesotrione | 104206-82-8 | C14H13NO7S | 339.0413 | 340.0486 | 338.0340 | ESI+ | 6 |
| 477 | Metaflumizone | 139968-49-3 | C24H16F6N4O2 | 506.1177 | 507.1250 | 505.1104 | ESI+ | 6 |
| 478 | Metalaxyl | 57837-19-1 | C15H21NO4 | 279.1471 | 280.1544 | 278.1398 | ESI+ | 6 |
| 479 | Metalaxyl-M | 70630-17-0 | C15H21NO4 | 279.1471 | 280.1544 | 278.1398 | ESI+ | 6 |
| 480 | Metamitron | 41394-05-2 | C10H10N4O | 202.0855 | 203.0928 | 201.0782 | ESI+ | 3 |
| 481 | Metazachlor | 67129-08-2 | C14H16ClN3O | 277.0982 | 278.1055 | 276.0909 | ESI+ | 6 |
| 482 | Metconazole | 125116-23-6 | C17H22ClN3O | 319.1451 | 320.1524 | 318.1378 | ESI+ | 4 |
| 483 | Methabenzthiazuron | 18691-97-9 | C10H11N3OS | 221.0623 | 222.0696 | 220.0550 | ESI+ | 6 |
| 484 | Methacrifos | 62610-77-9 | C7H13O5PS | 240.0221 | 241.0294 | 239.0148 | ESI+ | 12 |
| 485 | Methamidophos | 10265-92-6 | C2H8NO2PS | 141.0013 | 142.0086 | 139.9940 | ESI+ | 6 |
| 486 | Methfuroxam | 28730-17-8 | C14H15NO2 | 229.1103 | 230.1176 | 228.1030 | ESI+ | 4 |
| 487 | Methidathion | 950-37-8 | C6H11N2O4PS3 | 301.9619 | 302.9692 | 300.9546 | ESI+ | 7 |
| 488 | Methiocarb | 2032-65-7 | C11H15NO2S | 225.0823 | 226.0896 | 224.0750 | ESI+ | 6 |
| 489 | Methiocarb-sulfone | 2179-25-1 | C11H15NO4S | 257.0722 | 258.0795 | 256.0649 | ESI+ | 9 |
| 490 | Methiocarb-sulfoxide | 2635-10-1 | C11H15NO3S | 241.0773 | 242.0846 | 240.0700 | ESI+ | 6 |
| 491 | Methomyl | 16752-77-5 | C5H10N2O2S | 162.0463 | 163.0536 | 161.0390 | ESI+ | 6 |
| 492 | Methoprene | 40596-69-8 | C19H34O3 | 310.2508 | 311.2581 | 309.2435 | ESI+ | 12 |
| 493 | Methoprotryne | 841-06-5 | C11H21N5OS | 271.1467 | 272.1540 | 270.1394 | ESI+ | 6 |
| 494 | Methoxyfenozide | 161050-58-4 | C22H28N2O3 | 368.2100 | 369.2173 | 367.2027 | ESI+ | 6 |
| 495 | Metobromuron | 3060-89-7 | C9H11BrN2O2 | 258.0004 | 259.0077 | 256.9931 | ESI+ | 12 |
| 496 | Metolachlor | 51218-45-2 | C15H22ClNO2 | 283.1339 | 284.1412 | 282.1266 | ESI+ | 12 |
| 497 | Metolcarb | 1129-41-5 | C9H11NO2 | 165.0790 | 166.0863 | 164.0717 | ESI+ | 6 |
| 498 | Metominostrobin | 133408-50-1 | C16H16N2O3 | 284.1161 | 285.1234 | 283.1088 | ESI+ | 6 |
| 499 | Metosulam | 139528-85-1 | C14H13Cl2N5O4S | 417.0065 | 418.0138 | 415.9992 | ESI+ | 24 |
| 500 | Metoxuron | 19937-59-8 | C10H13ClN2O2 | 228.0666 | 229.0739 | 227.0593 | ESI+ | 5 |
| 501 | Metrafenone | 220899-03-6 | C19H21BrO5 | 408.0572 | 409.0645 | 407.0499 | ESI+ | 12 |
| 502 | Metribuzin | 21087-64-9 | C8H14N4OS | 214.0888 | 215.0961 | 213.0815 | ESI+ | 5 |
| 503 | Metsulfuron-methyl | 74223-64-6 | C14H15N5O6S | 381.0743 | 382.0816 | 380.0670 | ESI+ | 6 |
| 504 | Mevinphos | 7786-34-7 | C7H13O6P | 224.0450 | 225.0523 | 223.0377 | ESI+ | 5 |
| 505 | Mexacarbate | 315-18-4 | C12H18N2O2 | 222.1368 | 223.1441 | 221.1295 | ESI+ | 6 |
| 506 | Molinate | 2212-67-1 | C9H17NOS | 187.1031 | 188.1104 | 186.0958 | ESI+ | 6 |
| 507 | Monalide | 7287-36-7 | C13H18ClNO | 239.1077 | 240.1150 | 238.1004 | ESI+ | 20 |
| 508 | Monocrotophos | 6923-22-4 | C7H14NO5P | 223.0610 | 224.0683 | 222.0537 | ESI+ | 12 |
| 509 | Monolinuron | 1746-81-2 | C9H11ClN2O2 | 214.0509 | 215.0582 | 213.0436 | ESI+ | 10 |
| 510 | Monuron | 150-68-5 | C9H11ClN2O | 198.0560 | 199.0633 | 197.0487 | ESI+ | 10 |
| 511 | Morpholine | 110-91-8 | C4H9NO | 87.0684 | 88.0757 | 86.0611 | ESI+ | 6 |
| 512 | Moxidectin | 113507-06-5 | C37H53NO8 | 639.3771 | 640.3844 | 638.3698 | ESI+ | 12 |
| 513 | Myclobutanil | 88671-89-0 | C15H17ClN4 | 288.1142 | 289.1215 | 287.1069 | ESI+ | 8 |
| 514 | N-(2, 4-Dimethylphenyl) formamide | 60397-77-5 | C9H11NO | 149.0841 | 150.0914 | 148.0768 | ESI+ | 6 |
| 515 | N-(2, 4-Dimethylphenyl) -N'-methylformamidine | 33089-74-6 | C10H14N2 | 162.1157 | 163.1230 | 161.1084 | ESI+ | 6 |
| 516 | N, N'-Diphenylurea | 102-07-8 | C13H12N2O | 212.0950 | 213.1023 | 211.0877 | ESI+ | 4 |
| 517 | Naled | 300-76-5 | C4H7Br2Cl2O4P | 377.7826 | 378.7899 | 376.7753 | ESI+ | 6 |
| 518 | Naproanilide | 52570-16-8 | C19H17NO2 | 291.1259 | 292.1332 | 290.1186 | ESI+ | 2 |
| 519 | Napropamide | 15299-99-7 | C17H21NO2 | 271.1572 | 272.1645 | 270.1499 | ESI+ | 6 |
| 520 | Naptalam | 132-66-1 | C18H13NO3 | 291.0895 | 292.0968 | 290.0822 | ESI+ | 6 |
| 521 | Neburon | 555-37-3 | C12H16Cl2N2O | 274.0640 | 275.0713 | 273.0567 | ESI+ | 9 |
| 522 | Nicarbazin | 330-95-0 | C19H18N6O6 | 426.1288 | 427.1361 | 425.1215 | ESI- | 3 |
| 523 | Nicosulfuron | 111991-09-4 | C15H18N6O6S | 410.1009 | 411.1082 | 409.0936 | ESI+ | 6 |
| 524 | Nicotine | 54-11-5 | C10H14N2 | 162.1157 | 163.1230 | 161.1084 | ESI+ | 6 |
| 525 | Nitenpyram | 150824-47-8 | C11H15ClN4O2 | 270.0884 | 271.0957 | 269.0811 | ESI+ | 6 |

| | Compound | CAS | Formula | M | [M+H]+ | [M-H]- | Ionisation Mode | MRM Transitions |
|---|---|---|---|---|---|---|---|---|
| 526 | Nitralin | 4726-14-1 | C13H19N3O6S | 345.0995 | 346.1068 | 344.0922 | ESI+ | 12 |
| 527 | Nitrothal-isopropyl | 10552-74-6 | C14H17NO6 | 295.1056 | 296.1129 | 294.0983 | ESI+ | 6 |
| 528 | Norflurazon | 27314-13-2 | C12H9ClF3N3O | 303.0386 | 304.0459 | 302.0313 | ESI+ | 16 |
| 529 | Norflurazon-desmethyl | 23576-24-1 | C11H7ClF3N3O | 289.0230 | 290.0303 | 288.0157 | ESI+ | 12 |
| 530 | Novaluron | 116714-46-6 | C17H9ClF8N2O4 | 492.0123 | 493.0196 | 491.0050 | ESI+ | 18 |
| 531 | Noviflumuron | 121451-02-3 | C17H7Cl2F9N2O3 | 527.9690 | 528.9763 | 526.9617 | ESI- | 6 |
| 532 | Nuarimol | 63284-71-9 | C17H12ClFN2O | 314.0622 | 315.0695 | 313.0549 | ESI+ | 11 |
| 533 | Ofurace | 58810-48-3 | C14H16ClNO3 | 281.0819 | 282.0892 | 280.0746 | ESI+ | 17 |
| 534 | Omethoate | 1113-02-6 | C5H12NO4PS | 213.0225 | 214.0298 | 212.0152 | ESI+ | 4 |
| 535 | Orbencarb | 34622-58-7 | C12H16ClNOS | 257.0641 | 258.0714 | 256.0568 | ESI+ | 12 |
| 536 | Orthosulfamuron | 213464-77-8 | C16H20N6O6S | 424.1165 | 425.1238 | 423.1092 | ESI+ | 6 |
| 537 | Oryzalin | 19044-88-3 | C12H18N4O6S | 346.0947 | 347.1020 | 345.0874 | ESI+ | 5 |
| 538 | Oxabetrinil | 94593-79-0 | C12H12N2O3 | 232.0848 | 233.0921 | 231.0775 | ESI+ | 2 |
| 539 | Oxadiargyl | 39807-15-3 | C15H14Cl2N2O3 | 340.0381 | 341.0454 | 339.0308 | ESI+ | 14 |
| 540 | Oxadiazon | 19666-30-9 | C15H18Cl2N2O3 | 344.0694 | 345.0767 | 343.0621 | ESI+ | 6 |
| 541 | Oxadixyl | 77732-09-3 | C14H18N2O4 | 278.1267 | 279.1340 | 277.1194 | ESI+ | 12 |
| 542 | Oxamyl | 23135-22-0 | C7H13N3O3S | 219.0678 | 220.0751 | 218.0605 | ESI+ | 3 |
| 543 | Oxasulfuron | 144651-06-9 | C17H18N4O6S | 406.0947 | 407.1020 | 405.0874 | ESI+ | 6 |
| 544 | Oxaziclomefone | 153197-14-9 | C20H19Cl2NO2 | 375.0793 | 376.0866 | 374.0720 | ESI+ | 2 |
| 545 | Oxycarboxin | 5259-88-1 | C12H13NO4S | 267.0565 | 268.0638 | 266.0492 | ESI+ | 3 |
| 546 | Oxydemeton-methyl | 301-12-2 | C6H15O4PS2 | 246.0149 | 247.0222 | 245.0076 | ESI+ | 6 |
| 547 | Paclobutrazol | 76738-62-0 | C15H20ClN3O | 293.1295 | 294.1368 | 292.1222 | ESI+ | 8 |
| 548 | Paraoxon-ethyl | 311-45-5 | C10H14NO6P | 275.0559 | 276.0632 | 274.0486 | ESI+ | 6 |
| 549 | Paraoxon-methyl | 950-35-6 | C8H10NO6P | 247.0246 | 248.0319 | 246.0173 | ESI+ | 3 |
| 550 | Paraquat | 1910-42-5 | C12H14Cl2N2 | 256.0534 | 257.0607 | 255.0461 | ESI+ | 5 |
| 551 | Parathion | 56-38-2 | C10H14NO5PS | 291.0330 | 292.0403 | 290.0257 | ESI+ | 3 |
| 552 | Pebulate | 1114-71-2 | C10H21NOS | 203.1344 | 204.1417 | 202.1271 | ESI+ | 6 |
| 553 | Penconazole | 66246-88-6 | C13H15Cl2N3 | 283.0643 | 284.0716 | 282.0570 | ESI+ | 12 |
| 554 | Pencycuron | 66063-05-6 | C19H21ClN2O | 328.1342 | 329.1415 | 327.1269 | ESI+ | 10 |
| 555 | Pendimethalin | 40487-42-1 | C13H19N3O4 | 281.1376 | 282.1449 | 280.1303 | ESI+ | 6 |
| 556 | Penoxsulam | 219714-96-2 | C16H14F5N5O5S | 483.0636 | 484.0709 | 482.0563 | ESI+ | 6 |
| 557 | Pentachlorophenol | 87-86-5 | C6HCl5O | 263.8470 | 264.8543 | 262.8397 | ESI- | 3 |
| 558 | Pentoxazone | 110956-75-7 | C17H17ClFNO4 | 353.0830 | 354.0903 | 352.0757 | ESI+ | 2 |
| 559 | Permethrin | 52645-53-1 | C21H20Cl2O3 | 390.0790 | 391.0863 | 389.0717 | ESI+ | 12 |
| 560 | Pethoxamid | 106700-29-2 | C16H22ClNO2 | 295.1339 | 296.1412 | 294.1266 | ESI+ | 7 |
| 561 | Phenmedipham | 13684-63-4 | C16H16N2O4 | 300.1110 | 301.1183 | 299.1037 | ESI+ | 6 |
| 562 | Phenothrin | 26002-80-2 | C23H26O3 | 350.1882 | 351.1955 | 349.1809 | ESI+ | 9 |
| 563 | Phenthoate | 2597-03-7 | C12H17O4PS2 | 320.0306 | 321.0379 | 319.0233 | ESI+ | 12 |
| 564 | Phorate | 298-02-2 | C7H17O2PS3 | 260.0128 | 261.0201 | 259.0055 | ESI+ | 6 |
| 565 | Phorate-oxon | 2600-69-3 | C7H17O3PS2 | 244.0357 | 245.0430 | 243.0284 | ESI+ | 6 |
| 566 | Phorate-sulfone | 2588-04-7 | C7H17O4PS3 | 292.0027 | 293.0100 | 290.9954 | ESI+ | 6 |
| 567 | Phorate-sulfoxide | 2588-03-6 | C7H17O3PS3 | 276.0077 | 277.0150 | 275.0004 | ESI+ | 6 |
| 568 | Phosalone | 2310-17-0 | C12H15ClNO4PS2 | 366.9869 | 367.9942 | 365.9796 | ESI+ | 12 |
| 569 | Phosfolan | 947-02-4 | C7H14NO3PS2 | 255.0153 | 256.0226 | 254.0080 | ESI+ | 6 |
| 570 | Phosmet | 732-11-6 | C11H12NO4PS2 | 316.9945 | 318.0018 | 315.9872 | ESI+ | 12 |
| 571 | Phosphamidon | 13171-21-6 | C10H19ClNO5P | 299.0689 | 300.0762 | 298.0616 | ESI+ | 12 |
| 572 | Phoxim | 14816-18-3 | C12H15N2O3PS | 298.0541 | 299.0614 | 297.0468 | ESI+ | 6 |
| 573 | Picloram | 1918-02-1 | C6H3Cl3N2O2 | 239.9260 | 240.9333 | 238.9187 | ESI+ | 9 |
| 574 | Picolinafen | 137641-05-5 | C19H12F4N2O2 | 376.0835 | 377.0908 | 375.0762 | ESI+ | 6 |
| 575 | Picoxystrobin | 117428-22-5 | C18H16F3NO4 | 367.1031 | 368.1104 | 366.0958 | ESI+ | 6 |
| 576 | Pinoxaden | 243973-20-8 | C23H32N2O4 | 400.2362 | 401.2435 | 399.2289 | ESI+ | 6 |
| 577 | Piperonyl-butoxide | 51-03-6 | C19H30O5 | 338.2093 | 339.2166 | 337.2020 | ESI+ | 12 |
| 578 | Piperophos | 24151-93-7 | C14H28NO3PS2 | 353.1248 | 354.1321 | 352.1175 | ESI+ | 6 |
| 579 | Pirimicarb | 23103-98-2 | C11H18N4O2 | 238.1430 | 239.1503 | 237.1357 | ESI+ | 3 |
| 580 | Pirimicarb-desmethyl | 30614-22-3 | C10H16N4O2 | 224.1273 | 225.1346 | 223.1200 | ESI+ | 6 |
| 581 | Pirimicarb-desmethyl-formamido | 27218-04-8 | C11H16N4O3 | 252.1222 | 253.1295 | 251.1149 | ESI+ | 2 |
| 582 | Pirimiphos-ethyl | 23505-41-1 | C13H24N3O3PS | 333.1276 | 334.1349 | 332.1203 | ESI+ | 6 |
| 583 | Pirimiphos-methyl | 29232-93-7 | C11H20N3O3PS | 305.0963 | 306.1036 | 304.0890 | ESI+ | 6 |
| 584 | Prallethrin | 23031-36-9 | C19H24O3 | 300.1725 | 301.1798 | 299.1652 | ESI+ | 6 |
| 585 | Pretilachlor | 51218-49-6 | C17H26ClNO2 | 311.1652 | 312.1725 | 310.1579 | ESI+ | 6 |
| 586 | Primisulfuron-methyl | 86209-51-0 | C15H12F4N4O7S | 468.0363 | 469.0436 | 467.0290 | ESI+ | 9 |
| 587 | Probenazole | 27605-76-1 | C10H9NO3S | 223.0303 | 224.0376 | 222.0230 | ESI+ | 4 |
| 588 | Prochloraz | 67747-09-5 | C15H16Cl3N3O2 | 375.0308 | 376.0381 | 374.0235 | ESI+ | 15 |
| 589 | Profenofos | 41198-08-7 | C11H15BrClO3PS | 371.9351 | 372.9424 | 370.9278 | ESI+ | 12 |
| 590 | Profoxydim | 139001-49-3 | C24H32ClNO4S | 465.1741 | 466.1814 | 464.1668 | ESI+ | 24 |
| 591 | Promecarb | 2631-37-0 | C12H17NO2 | 207.1259 | 208.1332 | 206.1186 | ESI+ | 6 |
| 592 | Prometon | 1610-18-0 | C10H19N5O | 225.1590 | 226.1663 | 224.1517 | ESI+ | 6 |
| 593 | Prometryn | 7287-19-6 | C10H19N5S | 241.1361 | 242.1434 | 240.1288 | ESI+ | 6 |
| 594 | Propachlor | 1918-16-7 | C11H14ClNO | 211.0764 | 212.0837 | 210.0691 | ESI+ | 6 |
| 595 | Propamocarb | 24579-73-5 | C9H20N2O2 | 188.1525 | 189.1598 | 187.1452 | ESI+ | 6 |
| 596 | Propanil | 709-98-8 | C9H9Cl2NO | 217.0061 | 218.0134 | 215.9988 | ESI+ | 9 |
| 597 | Propaphos | 7292-16-2 | C13H21O4PS | 304.0898 | 305.0971 | 303.0825 | ESI+ | 10 |
| 598 | Propaquizafop | 111479-05-1 | C22H22ClN3O5 | 443.1248 | 444.1321 | 442.1175 | ESI+ | 12 |
| 599 | Propargite | 2312-35-8 | C19H26O4S | 350.1552 | 351.1625 | 349.1479 | ESI+ | 6 |
| 600 | Propazine | 139-40-2 | C9H16ClN5 | 229.1094 | 230.1167 | 228.1021 | ESI+ | 6 |

| | Compound | CAS | Formula | M | [M+H]+ | [M-H]- | Ionisation Mode | MRM Transitions |
|---|---|---|---|---|---|---|---|---|
| 601 | Propetamphos | 31218-83-4 | C10H20NO4PS | 281.0851 | 282.0924 | 280.0778 | ESI+ | 18 |
| 602 | Propham | 122-42-9 | C10H13NO2 | 179.0946 | 180.1019 | 178.0873 | ESI+ | 6 |
| 603 | Propiconazole | 60207-90-1 | C15H17Cl2N3O2 | 341.0698 | 342.0771 | 340.0625 | ESI+ | 12 |
| 604 | Propisochlor | 86763-47-5 | C15H22ClNO2 | 283.1339 | 284.1412 | 282.1266 | ESI+ | 12 |
| 605 | Propoxur | 114-26-1 | C11H15NO3 | 209.1052 | 210.1125 | 208.0979 | ESI+ | 6 |
| 606 | Propoxycarbazone | 181274-15-7 | C15H18N4O7S | 398.0896 | 399.0969 | 397.0823 | ESI+ | 20 |
| 607 | Propylene-thiourea | 2122-19-2 | C4H8N2S | 116.0408 | 117.0481 | 115.0335 | ESI+ | 6 |
| 608 | Propyzamide | 23950-58-5 | C12H11Cl2NO | 255.0218 | 256.0291 | 254.0145 | ESI+ | 10 |
| 609 | Proquinazid | 189278-12-4 | C14H17IN2O2 | 372.0335 | 373.0408 | 371.0262 | ESI+ | 6 |
| 610 | Prosulfocarb | 52888-80-9 | C14H21NOS | 251.1344 | 252.1417 | 250.1271 | ESI+ | 4 |
| 611 | Prosulfuron | 94125-34-5 | C15H16F3N5O4S | 419.0875 | 420.0948 | 418.0802 | ESI+ | 7 |
| 612 | Prothioconazole | 178928-70-6 | C14H15Cl2N3OS | 343.0313 | 344.0386 | 342.0240 | ESI+ | 10 |
| 613 | Prothioconazole-desthio | 120983-64-4 | C14H15Cl2N3O | 311.0592 | 312.0665 | 310.0519 | ESI+ | 10 |
| 614 | Prothiophos | 34643-46-4 | C11H15Cl2O2PS2 | 343.9628 | 344.9701 | 342.9555 | ESI+ | 12 |
| 615 | Prothoate | 2275-18-5 | C9H20NO3PS2 | 285.0622 | 286.0695 | 284.0549 | ESI+ | 6 |
| 616 | Pymetrozine | 123312-89-0 | C10H11N5O | 217.0964 | 218.1037 | 216.0891 | ESI+ | 4 |
| 617 | Pyracarbolid | 24691-76-7 | C13H15NO2 | 217.1103 | 218.1176 | 216.1030 | ESI+ | 3 |
| 618 | Pyraclofos | 89784-60-1 | C14H18ClN2O3PS | 360.0464 | 361.0537 | 359.0391 | ESI+ | 12 |
| 619 | Pyraclostrobin | 175013-18-0 | C19H18ClN3O4 | 387.0986 | 388.1059 | 386.0913 | ESI+ | 11 |
| 620 | Pyraflufen-ethyl | 129630-19-9 | C15H13Cl2F3N2O4 | 412.0204 | 413.0277 | 411.0131 | ESI+ | 12 |
| 621 | Pyrasulfotole | 365400-11-9 | C14H13F3N2O4S | 362.0548 | 363.0621 | 361.0475 | ESI+ | 9 |
| 622 | Pyrazolynate | 58011-68-0 | C19H16Cl2N2O4S | 438.0208 | 439.0281 | 437.0135 | ESI+ | 2 |
| 623 | Pyrazophos | 13457-18-6 | C14H20N3O5PS | 373.0861 | 374.0934 | 372.0788 | ESI+ | 12 |
| 624 | Pyrazosulfuron-ethyl | 93697-74-6 | C14H18N6O7S | 414.0958 | 415.1031 | 413.0885 | ESI+ | 6 |
| 625 | Pyrazoxyfen | 71561-11-0 | C20H16Cl2N2O3 | 402.0538 | 403.0611 | 401.0465 | ESI+ | 12 |
| 626 | Pyributicarb | 88678-67-5 | C18H22N2O2S | 330.1402 | 331.1475 | 329.1329 | ESI+ | 6 |
| 627 | Pyridaben | 96489-71-3 | C19H25ClN2OS | 364.1376 | 365.1449 | 363.1303 | ESI+ | 12 |
| 628 | Pyridalyl | 179101-81-6 | C18H14Cl4F3NO3 | 488.9680 | 489.9753 | 487.9607 | ESI+ | 18 |
| 629 | Pyridaphenthion | 119-12-0 | C14H17N2O4PS | 340.0647 | 341.0720 | 339.0574 | ESI+ | 6 |
| 630 | Pyridate | 55512-33-9 | C19H23N2O2S | 378.1169 | 379.1242 | 377.1096 | ESI+ | 12 |
| 631 | Pyrifenox | 88283-41-4 | C14H12Cl2N2O | 294.0327 | 295.0400 | 293.0254 | ESI+ | 8 |
| 632 | Pyriftalid | 135186-78-6 | C15H14N2O4S | 318.0674 | 319.0747 | 317.0601 | ESI+ | 2 |
| 633 | Pyrimethanil | 53112-28-0 | C12H13N3 | 199.1109 | 200.1182 | 198.1036 | ESI+ | 6 |
| 634 | Pyrimidifen | 105779-78-0 | C20H28ClN3O2 | 377.1870 | 378.1943 | 376.1797 | ESI+ | 12 |
| 635 | Pyriminobac-methyl (E) | 136191-64-5 | C17H19N3O6 | 361.1274 | 362.1347 | 360.1201 | ESI+ | 6 |
| 636 | Pyriproxyfen | 95737-68-1 | C20H19NO3 | 321.1365 | 322.1438 | 320.1292 | ESI+ | 6 |
| 637 | Pyroquilon | 57369-32-1 | C11H11NO | 173.0841 | 174.0914 | 172.0768 | ESI+ | 6 |
| 638 | Pyroxsulam | 422556-08-9 | C14H13F3N6O5S | 434.0620 | 435.0693 | 433.0547 | ESI+ | 6 |
| 639 | Quinalphos | 13593-03-8 | C12H15N2O3PS | 298.0541 | 299.0614 | 297.0468 | ESI+ | 6 |
| 640 | Quinclorac | 84087-01-4 | C10H5Cl2NO2 | 240.9697 | 241.9770 | 239.9624 | ESI+ | 11 |
| 641 | Quinmerac | 90717-03-6 | C11H8ClNO2 | 221.0244 | 222.0317 | 220.0171 | ESI+ | 12 |
| 642 | Quinoclamine | 2797-51-5 | C10H6ClNO2 | 207.0087 | 208.0160 | 206.0014 | ESI+ | 19 |
| 643 | Quinoxyfen | 124495-18-7 | C15H8Cl2FNO | 306.9967 | 308.0040 | 305.9894 | ESI+ | 12 |
| 644 | Quizalofop (free acid) | 76578-12-6 | C17H13ClN2O4 | 344.0564 | 345.0637 | 343.0491 | ESI+ | 24 |
| 645 | Quizalofop-ethyl | 76578-14-8 | C19H17ClN2O4 | 372.0877 | 373.0950 | 371.0804 | ESI+ | 12 |
| 646 | Quizalofop-methyl | 76578-13-7 | C18H15ClN2O4 | 358.0720 | 359.0793 | 357.0647 | ESI+ | 12 |
| 647 | Quizalofop-P | 94051-08-8 | C17H13ClN2O4 | 344.0564 | 345.0637 | 343.0491 | ESI+ | 9 |
| 648 | Quizalofop-P-ethyl | 100646-51-3 | C19H17ClN2O4 | 372.0877 | 373.0950 | 371.0804 | ESI+ | 12 |
| 649 | Rabenzazole | 40341-04-6 | C12H12N4 | 212.1062 | 213.1135 | 211.0989 | ESI+ | 12 |
| 650 | Resmethrin | 10453-86-8 | C22H26O3 | 338.1882 | 339.1955 | 337.1809 | ESI+ | 6 |
| 651 | Rimsulfuron | 122931-48-0 | C14H17N5O7S2 | 431.0569 | 432.0642 | 430.0496 | ESI+ | 9 |
| 652 | Rotenone | 83-79-4 | C23H22O6 | 394.1416 | 395.1489 | 393.1343 | ESI+ | 6 |
| 653 | Saflufenacil | 372137-35-4 | C17H17ClF4N4O5S | 500.0544 | 501.0617 | 499.0471 | ESI+ | 8 |
| 654 | Sebuthylazine | 7286-69-3 | C9H16ClN5 | 229.1094 | 230.1167 | 228.1021 | ESI+ | 6 |
| 655 | Sebuthylazine-desethyl | 37019-18-4 | C7H12ClN5 | 201.0781 | 202.0854 | 200.0708 | ESI+ | 12 |
| 656 | Secbumeton | 26259-45-0 | C10H19N5O | 225.1590 | 226.1663 | 224.1517 | ESI+ | 4 |
| 657 | Sethoxydim | 74051-80-2 | C17H29NO3S | 327.1868 | 328.1941 | 326.1795 | ESI+ | 12 |
| 658 | Siduron | 1982-49-6 | C14H20N2O | 232.1576 | 233.1649 | 231.1503 | ESI+ | 5 |
| 659 | Silafluofen | 105024-66-6 | C25H29FO2Si | 408.1921 | 409.1994 | 407.1848 | ESI+ | 2 |
| 660 | Silthiofam | 175217-20-6 | C13H21NOSSi | 267.1113 | 268.1186 | 266.1040 | ESI+ | 5 |
| 661 | Simazine | 122-34-9 | C7H12ClN5 | 201.0781 | 202.0854 | 200.0708 | ESI+ | 12 |
| 662 | Simazine-2-hydroxy | 2599-11-3 | C7H13N5O | 183.1120 | 184.1193 | 182.1047 | ESI+ | 5 |
| 663 | Simeconazole | 149508-90-7 | C14H20FN3OSi | 293.1360 | 294.1433 | 292.1287 | ESI+ | 6 |
| 664 | Simetryn | 1014-70-6 | C8H15N5S | 213.1048 | 214.1121 | 212.0975 | ESI+ | 4 |
| 665 | Spinetoram A | 187166-40-1 | C42H69NO10 | 747.4921 | 748.4994 | 746.4848 | ESI+ | 2 |
| 666 | Spinetoram B | 187166-15-0 | C43H69NO10 | 759.4921 | 760.4994 | 758.4848 | ESI+ | 3 |
| 667 | Spinosyn A | 131929-60-7 | C41H65NO10 | 731.4608 | 732.4681 | 730.4535 | ESI+ | 4 |
| 668 | Spinosyn D | 131929-63-0 | C42H67NO10 | 745.4765 | 746.4838 | 744.4692 | ESI+ | 4 |
| 669 | Spirodiclofen | 148477-71-8 | C21H24Cl2O4 | 410.1052 | 411.1125 | 409.0979 | ESI+ | 11 |
| 670 | Spiromesifen | 283594-90-1 | C23H30O4 | 370.2144 | 371.2217 | 369.2071 | ESI+ | 4 |
| 671 | Spirotetramat | 203313-25-1 | C21H27NO5 | 373.1889 | 374.1962 | 372.1816 | ESI+ | 6 |
| 672 | Spiroxamine | 118134-30-8 | C18H35NO2 | 297.2668 | 298.2741 | 296.2595 | ESI+ | 6 |
| 673 | Sulcotrione | 99105-77-8 | C14H13ClO5S | 328.0172 | 329.0245 | 327.0099 | ESI+ | 2 |
| 674 | Sulfallate | 95-06-7 | C8H14ClNS2 | 223.0256 | 224.0329 | 222.0183 | ESI+ | 9 |
| 675 | Sulfaquinoxaline | 59-40-5 | C14H12N4O2S | 300.0681 | 301.0754 | 299.0608 | ESI+ | 6 |

| | Compound | CAS | Formula | M | [M+H]+ | [M-H]- | Ionisation Mode | MRM Transitions |
|---|---|---|---|---|---|---|---|---|
| 676 | Sulfometuron-methyl | 74222-97-2 | C15H16N4O5S | 364.0841 | 365.0914 | 363.0768 | ESI+ | 6 |
| 677 | Sulfosulfuron | 141776-32-1 | C16H18N6O7S2 | 470.0678 | 471.0751 | 469.0605 | ESI+ | 6 |
| 678 | Sulfotep | 3689-24-5 | C8H20O5P2S2 | 322.0227 | 323.0300 | 321.0154 | ESI+ | 6 |
| 679 | Sulprofos | 35400-43-2 | C12H19O2PS3 | 322.0285 | 323.0358 | 321.0212 | ESI+ | 6 |
| 680 | Tau-Fluvalinate | 102851-06-9 | C26H22ClF3N2O3 | 502.1271 | 503.1344 | 501.1198 | ESI+ | 15 |
| 681 | Tebuconazole | 107534-96-3 | C16H22ClN3O | 307.1451 | 308.1524 | 306.1378 | ESI+ | 10 |
| 682 | Tebufenozide | 112410-23-8 | C22H28N2O2 | 352.2151 | 353.2224 | 351.2078 | ESI+ | 6 |
| 683 | Tebufenpyrad | 119168-77-3 | C18H24ClN3O | 333.1608 | 334.1681 | 332.1535 | ESI+ | 12 |
| 684 | Tebupirimfos | 96182-53-5 | C13H23N2O3PS | 318.1167 | 319.1240 | 317.1094 | ESI+ | 6 |
| 685 | Tebutam | 35256-85-0 | C15H23NO | 233.1780 | 234.1853 | 232.1707 | ESI+ | 6 |
| 686 | Tebuthiuron | 34014-18-1 | C9H16N4OS | 228.1045 | 229.1118 | 227.0972 | ESI+ | 6 |
| 687 | Teflubenzuron | 83121-18-0 | C14H6Cl2F4N2O2 | 379.9742 | 380.9815 | 378.9669 | ESI- | 12 |
| 688 | Tembotrione | 335104-84-2 | C17H16ClF3O6S | 440.0308 | 441.0381 | 439.0235 | ESI+ | 12 |
| 689 | Temephos | 3383-96-8 | C16H20O6P2S3 | 465.9897 | 466.9970 | 464.9824 | ESI+ | 6 |
| 690 | Tepraloxydim | 149979-41-9 | C17H24ClNO4 | 341.1394 | 342.1467 | 340.1321 | ESI+ | 8 |
| 691 | Terbacil | 5902-51-2 | C9H13ClN2O2 | 216.0666 | 217.0739 | 215.0593 | ESI- | 10 |
| 692 | Terbucarb | 1918-11-2 | C17H27NO2 | 277.2042 | 278.2115 | 276.1969 | ESI+ | 12 |
| 693 | Terbufos | 13071-79-9 | C9H21O2PS3 | 288.0441 | 289.0514 | 287.0368 | ESI+ | 5 |
| 694 | Terbufos-sulfone | 56070-16-7 | C9H21O4PS3 | 320.0340 | 321.0413 | 319.0267 | ESI+ | 6 |
| 695 | Terbufos-sulfoxide | 10548-10-4 | C9H21O3PS3 | 304.0390 | 305.0463 | 303.0317 | ESI+ | 6 |
| 696 | Terbumeton | 33693-04-8 | C10H19N5O | 225.1590 | 226.1663 | 224.1517 | ESI+ | 6 |
| 697 | Terbumeton-desethyl | 30125-64-5 | C8H15N5O | 197.1277 | 198.1350 | 196.1204 | ESI+ | 3 |
| 698 | Terbuthylazine | 5915-41-3 | C9H16ClN5 | 229.1094 | 230.1167 | 228.1021 | ESI+ | 10 |
| 699 | Terbuthylazine-2-hydroxy | 66753-07-9 | C9H17N5O | 211.1433 | 212.1506 | 210.1360 | ESI+ | 6 |
| 700 | Terbuthylazine-desethyl | 30125-63-4 | C7H12ClN5 | 201.0781 | 202.0854 | 200.0708 | ESI+ | 12 |
| 701 | Terbutryn | 886-50-0 | C10H19N5S | 241.1361 | 242.1434 | 240.1288 | ESI+ | 6 |
| 702 | Tetrachlorvinphos (CVMP) | 22248-79-9 | C10H9Cl4O4P | 363.8993 | 364.9066 | 362.8920 | ESI+ | 16 |
| 703 | Tetraconazole | 112281-77-3 | C13H11Cl2F4N3O | 371.0215 | 372.0288 | 370.0142 | ESI+ | 7 |
| 704 | Tetraethylpyrophosphate | 107-49-3 | C8H20O7P2 | 290.0684 | 291.0757 | 289.0611 | ESI+ | 6 |
| 705 | Tetramethrin | 7696-12-0 | C19H25NO4 | 331.1784 | 332.1857 | 330.1711 | ESI+ | 12 |
| 706 | Thenylchlor | 96491-05-3 | C16H18ClNO2S | 323.0747 | 324.0820 | 322.0674 | ESI+ | 12 |
| 707 | Thiabendazole | 148-79-8 | C10H7N3S | 201.0361 | 202.0434 | 200.0288 | ESI+ | 6 |
| 708 | Thiacloprid | 111988-49-9 | C10H9ClN4S | 252.0236 | 253.0309 | 251.0163 | ESI+ | 6 |
| 709 | Thiamethoxam | 153719-23-4 | C8H10ClN5O3S | 291.0193 | 292.0266 | 290.0120 | ESI+ | 12 |
| 710 | Thiazafluron | 25366-23-8 | C6H7F3N4OS | 240.0293 | 241.0366 | 239.0220 | ESI+ | 6 |
| 711 | Thiazopyr | 117718-60-2 | C16H17F5N2O2S | 396.0931 | 397.1004 | 395.0858 | ESI+ | 6 |
| 712 | Thidiazuron | 51707-55-2 | C9H8N4OS | 220.0419 | 221.0492 | 219.0346 | ESI+ | 6 |
| 713 | Thiencarbazone-methyl | 317815-83-1 | C12H14N4O7S2 | 390.0304 | 391.0377 | 389.0231 | ESI+ | 3 |
| 714 | Thifensulfuron-methyl | 79277-27-3 | C12H13N5O6S2 | 387.0307 | 388.0380 | 386.0234 | ESI+ | 6 |
| 715 | Thifluzamide | 130000-40-7 | C13H6Br2F6N2O2S | 525.8421 | 526.8494 | 524.8348 | ESI+ | 29 |
| 716 | Thiobencarb | 28249-77-6 | C12H16ClNOS | 257.0641 | 258.0714 | 256.0568 | ESI+ | 11 |
| 717 | Thiodicarb | 59669-26-0 | C10H18N4O4S3 | 354.0490 | 355.0563 | 353.0417 | ESI+ | 6 |
| 718 | Thiofanox | 39196-18-4 | C9H18N2O2S | 218.1089 | 219.1162 | 217.1016 | ESI+ | 2 |
| 719 | Thiofanox-sulfone | 39184-59-3 | C9H18N2O4S | 250.0987 | 251.1060 | 249.0914 | ESI+ | 9 |
| 720 | Thiofanox-sulfoxide | 39184-27-5 | C9H18N2O3S | 234.1038 | 235.1111 | 233.0965 | ESI+ | 12 |
| 721 | Thiometon | 640-15-3 | C6H15O2PS3 | 245.9972 | 247.0045 | 244.9899 | ESI+ | 2 |
| 722 | Thionazin | 297-97-2 | C8H13N2O3PS | 248.0384 | 249.0457 | 247.0311 | ESI+ | 6 |
| 723 | Thiophanate-ethyl | 23564-06-9 | C14H18N4O4S2 | 370.0769 | 371.0842 | 369.0696 | ESI+ | 6 |
| 724 | Thiophanate-methyl | 23564-05-8 | C12H14N4O4S2 | 342.0456 | 343.0529 | 341.0383 | ESI+ | 6 |
| 725 | Thiram | 137-26-8 | C6H12N2S4 | 239.9883 | 240.9956 | 238.9810 | ESI+ | 6 |
| 726 | Tolclofos-methyl | 57018-04-9 | C9H11Cl2O3PS | 299.9544 | 300.9617 | 298.9471 | ESI+ | 12 |
| 727 | Tolylfluanid | 731-27-1 | C10H13Cl2FN2O2S2 | 345.9780 | 346.9853 | 344.9707 | ESI+ | 22 |
| 728 | Topramezone | 210631-68-8 | C16H17N3O5S | 363.0889 | 364.0962 | 362.0816 | ESI+ | 12 |
| 729 | Tralkoxydim | 87820-88-0 | C20H27NO3 | 329.1991 | 330.2064 | 328.1918 | ESI+ | 6 |
| 730 | Tralomethrin | 66841-25-6 | C22H19Br4NO3 | 660.8098 | 661.8171 | 659.8025 | ESI+ | 7 |
| 731 | Triadimefon | 43121-43-3 | C14H16ClN3O2 | 293.0931 | 294.1004 | 292.0858 | ESI+ | 12 |
| 732 | Triadimenol | 55219-65-3 | C14H18ClN3O2 | 295.1088 | 296.1161 | 294.1015 | ESI+ | 7 |
| 733 | Tri-allate | 2303-17-5 | C10H16Cl3NOS | 303.0018 | 304.0091 | 301.9945 | ESI+ | 16 |
| 734 | Triapenthenol | 76608-88-3 | C15H25N3O | 263.1998 | 264.2071 | 262.1925 | ESI+ | 12 |
| 735 | Triasulfuron | 82097-50-5 | C14H16ClN5O5S | 401.0561 | 402.0634 | 400.0488 | ESI+ | 12 |
| 736 | Triazamate | 112143-82-5 | C13H22N4O3S | 314.1413 | 315.1486 | 313.1340 | ESI+ | 4 |
| 737 | Triazophos | 24017-47-8 | C12H16N3O3PS | 313.0650 | 314.0723 | 312.0577 | ESI+ | 6 |
| 738 | Triazoxide | 72459-58-6 | C10H6ClN5O | 247.0261 | 248.0334 | 246.0188 | ESI+ | 11 |
| 739 | Tribenuron-methyl | 101200-48-0 | C15H17N5O6S | 395.0900 | 396.0973 | 394.0827 | ESI+ | 5 |
| 740 | Trichlorfon | 52-68-6 | C4H8Cl3O4P | 255.9226 | 256.9299 | 254.9153 | ESI+ | 10 |
| 741 | Triclopyr | 55335-06-3 | C7H4Cl3NO3 | 254.9257 | 255.9330 | 253.9184 | ESI- | 2 |
| 742 | Tricyclazole | 41814-78-2 | C9H7N3S | 189.0361 | 190.0434 | 188.0288 | ESI+ | 6 |
| 743 | Tridemorph | 81412-43-3 | C19H39NO | 297.3032 | 298.3105 | 296.2959 | ESI+ | 6 |
| 744 | Trietazine | 1912-26-1 | C9H16ClN5 | 229.1094 | 230.1167 | 228.1021 | ESI+ | 6 |
| 745 | Triethanolamine | 102-71-6 | C6H15NO3 | 149.1052 | 150.1125 | 148.0979 | ESI+ | 6 |
| 746 | Trifloxystrobin | 141517-21-7 | C20H19F3N2O4 | 408.1297 | 409.1370 | 407.1224 | ESI+ | 6 |
| 747 | Trifloxysulfuron | 145099-21-4 | C14H14F3N5O6S | 437.0617 | 438.0690 | 436.0544 | ESI+ | 9 |
| 748 | Triflumizole | 68694-11-1 | C15H15ClF3N3O | 345.0856 | 346.0929 | 344.0783 | ESI+ | 9 |
| 749 | Triflumizole Metabolite | 131549-75-2 | C12H14ClF3N2O | 294.0747 | 295.0820 | 293.0674 | ESI+ | 2 |
| 750 | Triflumuron | 64628-44-0 | C15H10ClF3N2O3 | 358.0332 | 359.0405 | 357.0259 | ESI+ | 8 |

| | Compound | CAS | Formula | M | [M+H]+ | [M-H]- | Ionisation Mode | MRM Transitions |
|---|---|---|---|---|---|---|---|---|
| 751 | Triflusulfuron-methyl | 126535-15-7 | C17H19F3N6O6S | 492.1039 | 493.1112 | 491.0966 | ESI+ | 8 |
| 752 | Triforine | 26644-46-2 | C10H14Cl6N4O2 | 431.9248 | 432.9321 | 430.9175 | ESI+ | 7 |
| 753 | Trinexapac-ethyl | 95266-40-3 | C13H16O5 | 252.0998 | 253.1071 | 251.0925 | ESI+ | 6 |
| 754 | Triphenyl phosphate | 115-86-6 | C18H15O4P | 326.0708 | 327.0781 | 325.0635 | ESI+ | 6 |
| 755 | Tris (2-chloro-1-(chloromethyl)ethyl) phosphate | 13674-87-8 | C9H15Cl6O4P | 427.8839 | 428.8912 | 426.8766 | ESI+ | 26 |
| 756 | Triticonazole | 131983-72-7 | C17H20ClN3O | 317.1295 | 318.1368 | 316.1222 | ESI+ | 9 |
| 757 | Tritosulfuron | 142469-14-5 | C13H9F6N5O4S | 445.0279 | 446.0352 | 444.0206 | ESI+ | 4 |
| 758 | Valifenalate | 283159-90-0 | C19H27ClN2O5 | 398.1608 | 399.1681 | 397.1535 | ESI+ | 16 |
| 759 | Vamidothion | 2275-23-2 | C8H18NO4PS2 | 287.0415 | 288.0488 | 286.0342 | ESI+ | 6 |
| 760 | Vamidothion-sulfone | 70898-34-9 | C8H18NO6PS2 | 319.0313 | 320.0386 | 318.0240 | ESI+ | 6 |
| 761 | Vamidothion-sulfoxide | 20300-00-9 | C8H18NO5PS2 | 303.0364 | 304.0437 | 302.0291 | ESI+ | 6 |
| 762 | Vernolate | 1929-77-7 | C10H21NOS | 203.1344 | 204.1417 | 202.1271 | ESI+ | 5 |
| 763 | Warfarin | 81-81-2 | C19H16O4 | 308.1049 | 309.1122 | 307.0976 | ESI+ | 6 |
| 764 | XMC (3, 5-xylyl methylcarbamate) | 2655-14-3 | C10H13NO2 | 179.0946 | 180.1019 | 178.0873 | ESI+ | 12 |
| 765 | Ziram | 137-30-4 | C6H12N2S4Zn | 303.9175 | 304.9248 | 302.9102 | ESI+ | 2 |
| 766 | Zoxamide | 156052-68-5 | C14H16Cl3NO2 | 335.0247 | 336.0320 | 334.0174 | ESI+ | 18 |

■ **Further Information**

Application News No.C136 describes the analysis of 646 pesticides in a single multi-residue method built using the Shimadzu Pesticide Library.

■ **Scope and Legal Disclaimers**

Whilst every effort has been made to ensure the accuracy of the Library, the method will need to be verified in a laboratory as conditions may differ marginally. The influence of sample matrices, extraction protocols, LC behaviour and technical experience may affect the performance of the LC/MS/MS analysis.

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# Determining Gamma-Hydroxybutyric Acid and its Precursors Gamma-Butyrolactone and 1,4-Butanediol in Suspected Drug-spiked Beverages using LCMS-8045

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## Abstract

This paper established a method to determine the quantity of gamma-hydroxybutyric acid and its precursors gamma-butyrolactone and 1,4-butanediol in suspected drug-spiked beverages using Shimadzu's Ultra-High Performance Liquid Chromatograph LC-30A and Triple Quadrupole Mass Spectrometer LCMS-8045. Analysis was completed within 2 min with good linearity. The standard samples at various concentrations were tested in 6 replicates, and the relative standard deviations of retention time and peak area were 0.26 – 0.48% and 0.70 – 2.31% respectively, showing good precision. The spike recovery of the sample was 92.6 – 104.3%. Given the fast analysis and accurate results acquired, this method can be used for the determination of gamma-hydroxybutyric acid and its precursors gamma-butyrolactone and 1,4-butanediol in beverages.

Media recently reported that energy drinks are being used as alcohol-substitute beverages in karaoke establishments (KTVs) and other entertainment venues. These beverages have been detected to contain high concentrations of gamma-hydroxybutyric acid (GHB) and gamma-butyrolactone (GBL). GHB is listed as a psychotropic drug strictly controlled in China. Substance abuse or drug misuse can cause temporary memory loss, nausea, vomiting, headache, loss of reflex, immediate loss of consciousness, coma and even death. In addition, the consumption of GHB with alcoholic beverage further increase these risks. Both GBL and 1,4-butanediol (1,4-BD) are precursors of GHB and they are rapidly metabolized to GHB inside the human body.

For all these reasons, it is necessary to accurately detect GHB and its precursors in beverages. Traditional detection methods mainly involve the use of GCMS, which requires derivatization of target substances before sample analysis. In this paper, a rapid and accurate method using Shimadzu's LCMS-8045 is established to determine the quantity of GHB and its precursors in beverages. The method preparation is simple, which doesn't call for derivatization. Sample preparation consists of dilution and filtration of samples. The analysis is fast and ensures good precision, which is ideal for quantitative analysis of such drug substances in beverages.

### EXPERIMENTAL

#### Instrumentation

The experiment used Shimadzu's UHPLC LC-30A and Triple Quadrupole Mass Spectrometer LCMS-8045. The specific configurations are LC-30AD×2 pumps, DGU-20A5 online degassing unit, SIL-20AC autosampler, CTO-20AC column oven, CBM-20A system controller, LCMS-8045 triple quadrupole mass spectrometer and LabSolutions Ver. 5.89 chromatographic workstation.
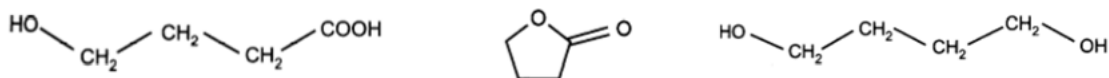


**Figure 1  Structures of the target drug substances (left: GHB, middle: GBL, right: 1,4-BD)**

## Analytical Conditions

### LC Chromatograpy (LC) Conditions

| | | |
|---|---|---|
| Column | : | Shim-pack XR-ODS III (150 mm. L×2.0 mm I.D., 2.2 µm) |
| Mobile phase | : | Phase A - 0.1% acetic acid in water<br>Phase B - acetonitrile |
| Flow rate | : | 0.30 mL/min |
| Column Temp. | : | 25 °C |
| Injection volume | : | 10 µL |
| Elution method | : | Isocratic elution, where the concentration of Phase B is 5% |

### Mass Spectrometry (MS) Conditions

| | | |
|---|---|---|
| Analytical Instrument | : | LCMS-8045 |
| Ion Source | : | ESI, simultaneous scanning of positive and negative ions |
| Heating gas | : | Air 10.0 L/min |
| Nebulizer gas | : | Nitrogen 3.0  L/min |
| Drying gas | : | Nitrogen 10.0 L/min |
| Scan mode | : | Multiple reaction monitoring (MRM) |
| Dwell time | : | 50 ms |
| DL temp. | : | 200 °C |
| Heating block temp. | : | 400 °C |
| Scan mode | : | Multiple reaction monitoring (MRM) |
| Dwell time | : | 50 ms |
| Collisions gas | : | Argon |
| Interface temp. | : | 300 °C |
| DL temp. | : | 200 °C |
| Heat block temp. | : | 400 °C |
| Delay time | : | 3 ms |
| MRM Parameters | : | Refer to Table 1 |

## Standard solution preparation

Preparation of Standard Working Solutions: The drug standards were weighed and dissolved in methanol or ultra-pure water to give a standard stock solution at concentration of 1000 µg/mL. The stock solution were further diluted with ultra-pure water to obtain standard working solutions of the following concentrations: 1.0, 2.0, 5.0, 10.0, 20.0, 50.0, 100.0, 200.0, 500.0 ng/mL.

## Sample Preparation Method

1mL of the beverage sample were collected and diluted with ultra-pure water. The solution was filtered with a 0.22 µm filter membrane prior to analysis.

## RESULTS AND DISCUSSION

### MRM Chromatogram of Standard Drug Samples

The MRM chromatogram is shown in Figure 2.

### Calibration Curve and Linear Range

A series of standard solutions with concentrations of 1.0, 2.0, 5.0, 10.0, 20.0, 50.0, 100.0, 200.0 and 500.0 ng/ml were prepared and injected for analysis according to the conditions in the experimental section and quantified with external standard calibration method. A calibration curve, shown in Figure 3, was plotted showing peak area against concentration,. The linear equation, linear range, and correlation coefficients are shown in Table 2.

### Table 1  MRM optimized parameters

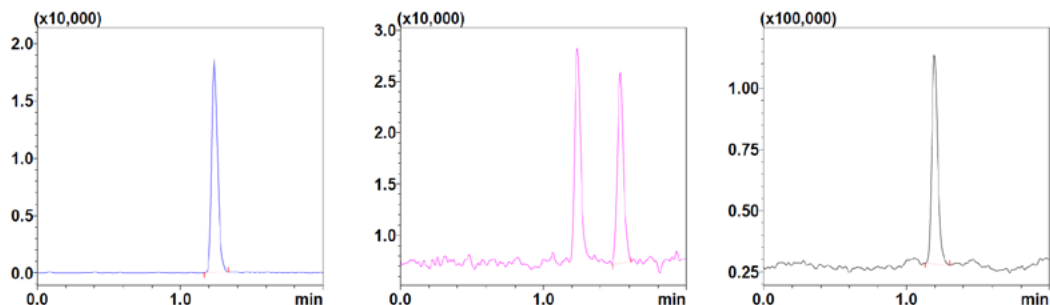| Compound | Ionization Mode | Precursor Ion | Product Ion | Q1 Pre Bias (V) | CE (V) | Q3 Pre Bias (V) |
|---|---|---|---|---|---|---|
| Gamma-Hydroxybutyric Acid (GHB) | ESI (-) | 103.1 | 85.1* | 10.0 | 15.0 | 11.0 |
| | | | 57.1 | 10.0 | 12.0 | 16.0 |
| Gamma-Butyrolactone (GBL) | ESI (+) | 87.2 | 45.1* | -13.0 | -25.0 | -18.0 |
| | | | 43.1 | -15.0 | -25.0 | -20.0 |
| 1,4-Butanediol (1,4-DB) | ESI (+) | 91.2 | 55.1* | -15.0 | -12.0 | -22.0 |
| | | | 73.1 | -15.0 | -10.0 | -13.0 |

Note: * indicates quantification ion

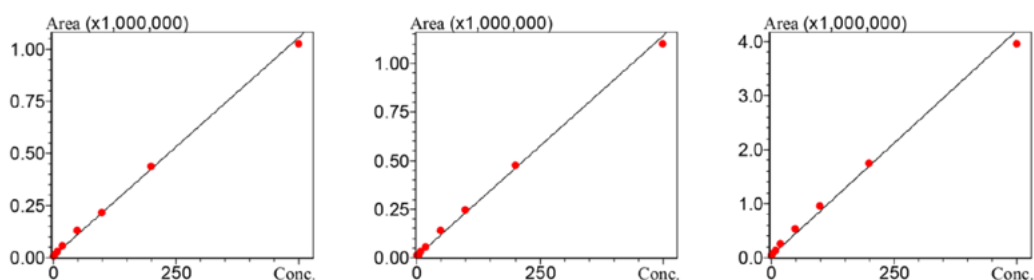**Figure 2  Chromatogram of 20 ng/ml standard samples (left: GHB, middle: GBL, right: 1,4-BD)**



**Figure 3  Calibration curve (left: GHB, middle: GBL, right: 1,4-BD)**

**Table 2  Parameters of the calibration curve**

| Compound Name | Calibration curve | Linear range (ng/mL) | Correlation coefficient (R) | Accuracy (%) |
|---|---|---|---|---|
| GHB | Y = (2413.4) X + (945.6) | 1-500 | 0.9979 | 85.4-111.1 |
| GBL | Y = (2379.9) X + (3704.9) | 2-500 | 0.9982 | 88.3-114.6 |
| 1,4-BD | Y = (10000.5) X + (9547.5) | 2-500 | 0.9986 | 86.7-113.6 |

**Precision Test**

The standard samples with concentrations of 10.0 and 200 ng/ml were consecutively injected for 6 times to investigate the repeatability; and the result of retention time and peak area were shown as Table 3. It shows good repeatability.

**Table 3  Repeatability results of retention time and peak area (n=6)**

| Compound Name | RSD% (10 ng/ml) | | RSD% (200 ng/ml) | |
|---|---|---|---|---|
| | R.T. | Area | R.T. | Area |
| GHB | 0.47 | 2.31 | 0.41 | 0.81 |
| GBL | 0.30 | 2.08 | 0.26 | 0.70 |
| 1,4-BD | 0.44 | 1.87 | 0.48 | 0.97 |

**Actual Sample Test and Spike Recovery Test**

The samples were prepared and injected for analysis. The results are shown as Table 5. The spike recovery at a spiking concentration of 0.5 mg/mL was calculated.

**CONCLUSION**

A method was established in this paper to determine the quantity of gamma-hydroxybutyric acid and its precursors gamma-butyrolactone and 1,4-butanediol in suspected drug-spiked beverages using Shimadzu's UHPLC LC-30A and Triple Quadrupole Mass Spectrometer LCMS-8045. Analysis was completed within 2 min with good linearity. The standard samples at various concentrations were tested in

**Table 5  Sample detection and spike recovery test**

| Compound Name | Detected concentration (μg/ml) | Dilution Factor | Actual Sample concentration (mg/ml) | Spiking concentration (mg/ml) | Detected concentration of spiked samples (mg/ml) | Recovery (%) |
|---|---|---|---|---|---|---|
| GHB | 0.11 | | 0.44 | 0.50 | 0.91 | 93.1 |
| GBL | 0.18 | 4000 | 0.70 | 0.50 | 1.23 | 104.3 |
| 1,4-BD | N.D. | | N.D. | 0.50 | 0.46 | 92.6 |

Note: The samples were provided by the Drug Control Branch of the Wuhan Municipal Public Security Bureau

N.D. = Not detected

6 replicates, and the relative standard deviations of retention time and peak area were 0.26 – 0.48% and 0.70 – 2.31% respectively, showing good precision. The spike recovery of the sample was in the range of 92.6 – 104.3%. Given the fast analysis and accurate results acquired, this method can be used for the determination of gamma-hydroxybutyric acid and its precursors gamma-butyrolactone and 1,4-butanediol in beverages.

# Application News

Sum parameter – Total Organic Carbon

## Carbon dioxide determination in beer

**No.** SCA-130-403

Carbon dioxide is an important ingredient in many soft drinks. This is also the case for beer. It creates a sparkling and refreshing (tangy) taste and is important for the formation of foam.

The $CO_2$ content of a beer affects the threshold values for various fragrance and aroma components. In addition, bottling under $CO_2$ increases the shelf life of beer..

In the manual of the 'central- European brewery technological analysis commission)' (MEBAK) various methods for the determination of $CO_2$ are listed. These are generally based on manometric or titrimetric method, or they are methods that use specialized detectors.

Disadvantages of these methods are often the lack of selectivity for $CO_2$ (other gases or substances are also determined), high expenditure in terms of personnel and time, and the lack of possibilities for automation.

In order to develop a method that does not have these disadvantages, a TOC analyzer was used.

## ■ Innovative methods

In this method, the sample (beer) is directly placed in a 40 mL autosampler vial. 5 mL of a 32% NaOH solution was added to the autosampler vial to preserve the $CO_2$.

The sample is subsequently added directly to the autosampler and the IC (inorganic carbon) content is measured.

**Preservation step:**

$$CO_2 + OH^- \rightarrow HCO_3^-$$

$$CO_2 + 2\,OH^- \rightarrow CO_3^{2-} + H_2O$$
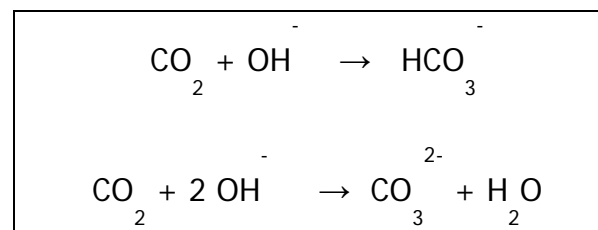
In the TOC analyzer, the sample is injected in a concentrated phosphoric acid solution (25%). The $CO_2$ is subsequently released again and is transferred via the carrier gas to a $CO_2$-selective NIDR detector where it is detected.

**Displacement reaction:** (the strong acid displaces the weak acid from its salt)

$$HCO_3^- + H^+ \rightarrow H_2O + CO_2$$

$$CO_3^{2-} + 2H^+ \rightarrow H_2O + CO_2$$
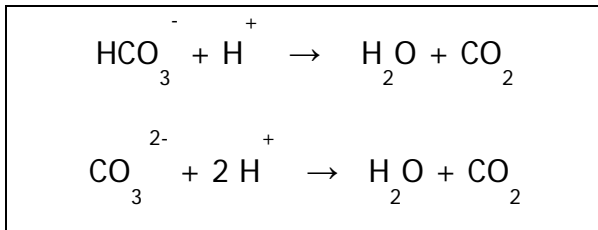
To calculate the results, the IC function of the TOC system is calibrated using a sodium hydrogen carbonate standard in the range of 100 – 1000 mg/L. The dilution of the individual calibration points is performed automatically via the dilution function of the instrument.

■ **Advantages of this method**
- can be automated to a high degree
- fast
- good reproducibility and high accuracy (precision)
- multiple determinations from one sample is possible
- effortless calibration
- simple operation
- highly specific

Using the modern TOC-L software, evaluation can be carried out automatically or can be recalculated manually. Another function enables further processing of the measurement results. This way the carbon dioxide content can be directly presented in the desired dimension. Due to the possibility for multiple injections, the evaluation contains all the important statistical quantities

Another sample preparation variant is to be carried out during the determination of carbon

dioxide in bottled or canned beer. In this step, 5 mL of a 32% solution of NaOH was directly added to the freshly opened bottle or can for preservation.

■ **Comparison of the methods**
The following graph shows the good agreement between the TOC method (blue bars) and the Corning method (green bars).
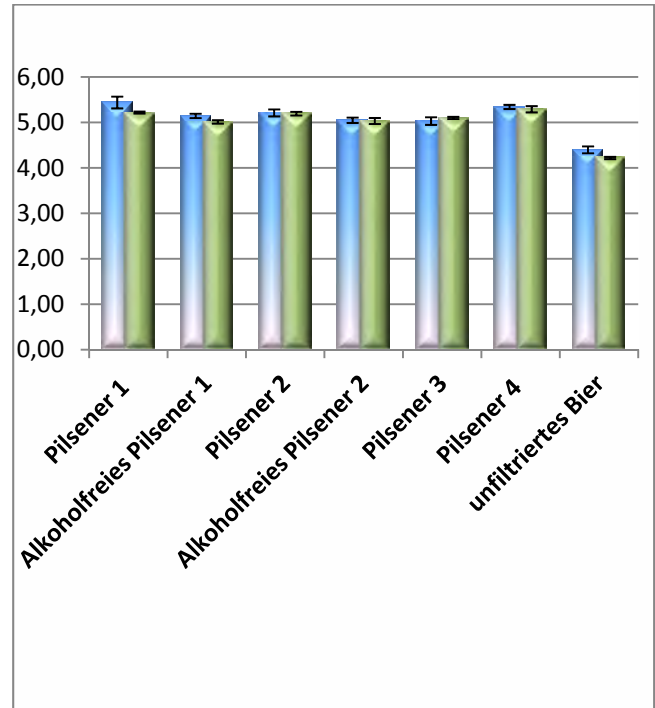




■ **Recommended Analyzer / Configuration**
TOC-L CPH
ASI-L (40ml)

**SHIMADZU**

Shimadzu Europa GmbH
www.shimadzu.eu

# Fundamental Techniques for Food Analysis

## Mass Spectrometry in Food Analysis

Single quadrupole mass spectrometry have long been acknowledged as the gold standard for food analysis and quantification. With more stringent demands in food safety standards and regulations, the use of triple quadrupole mass spectrometry (QqQ) in the food and agricultural industries have been increasingly popular. Shimadzu concentrates on these key MS features to achieve high speed, sensitivity, specificity and selectivity for your food analyses.

## Challenging Limits with Shimadzu

Shimadzu Corporation, one of the leading world-class mass spectrometry manufacturer produced the **world's first mass spectrometry** GCMS- LKB9000 in **1970**.

### UFMS
ULTRA FAST MASS SPECTROMETRY

LC-MS   GC-MS   ICP-MS   MALDI TOF-MS   MS Imaging

### UF Productivity
• UF-MRM™
• UFswitching™
• Smart MRM™
• Smart SIM™

### UF Accuracy
• MRM Library
  Screening
• LabSolutions
  Software

### UF Robustness
• Ion Optics
• Ion Source
• Inert Flow Path
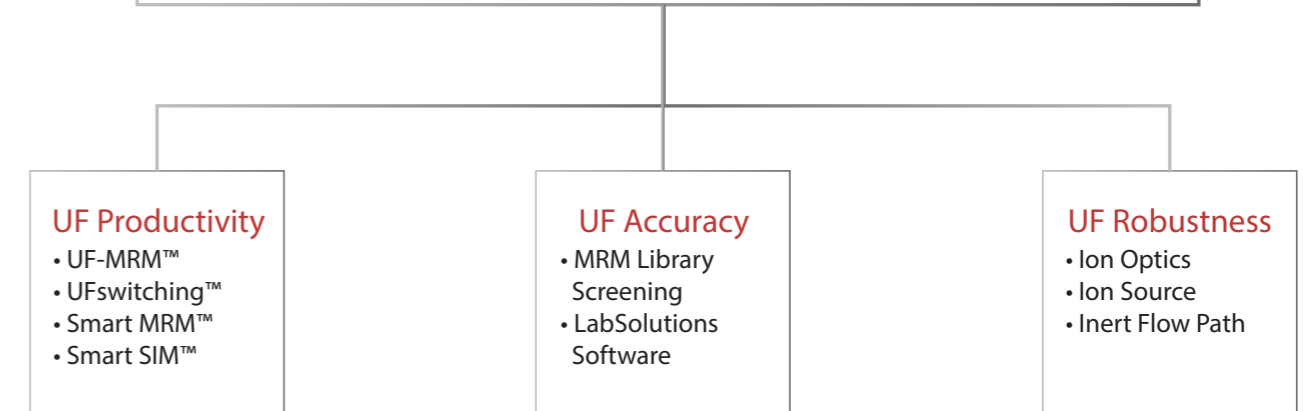
Since 2010, Shimadzu has moved quadrupole MS into a new analytical space by delivering class-leading sensitivity and a higher capability in data quality through Ultra-Fast Mass Spectrometry (UFMS). To date, Shimadzu's UFMS portfolio covers multiple instruments, including GC-MS, LC-MS, ICP-MS, MALDI TOF-MS and MS imaging, driving advancements across a broad range of applications.

These advancements have brought the food safety industry multiple unique benefits and increased confidence in food analysis. Shimadzu's total solution, from method development, workflows, LabSolutions software to data reporting, together addresses several challenges that you may face in your food analysis

# UF-MRM™ and UFswitching™

## What is Multiple Reaction Monitoring (MRM)?
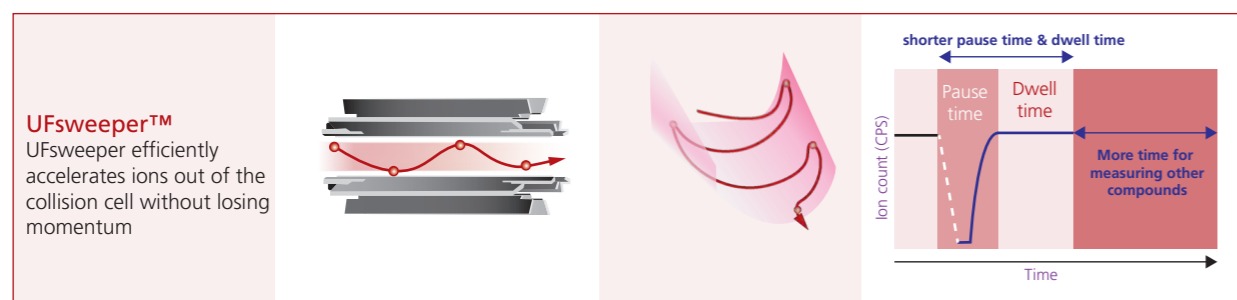


Collision Cell

MS1

MS2
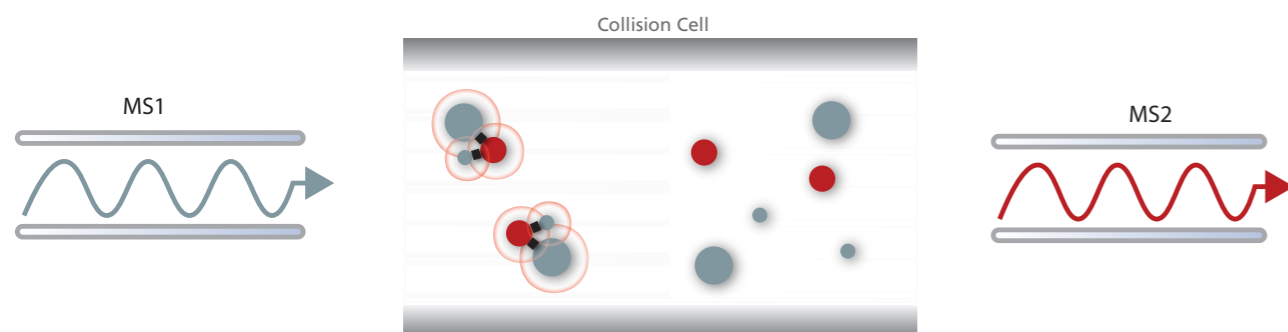
MRM is widely used on triple quadrupole platforms for targeted quantitation as a result of high selectivity, sensitivity and robustness. In a regulated environment such as food safety, along with the increasing complexity in food matrices, there is a growing need to enhance the capability in routine monitoring programs.

## ✓ Advantages of applying UF-MRM™

Conventional MRM



**Conventional design**
Ions lose momentum due to collision with gas

End of previous MRM

Pause time    Dwell time

Ion count (CPS)

Time

In UF-MRM™



**UFsweeper™**
UFsweeper efficiently accelerates ions out of the collision cell without losing momentum

shorter pause time & dwell time

Pause time    Dwell time

More time for measuring other compounds

Ion count (CPS)

Time
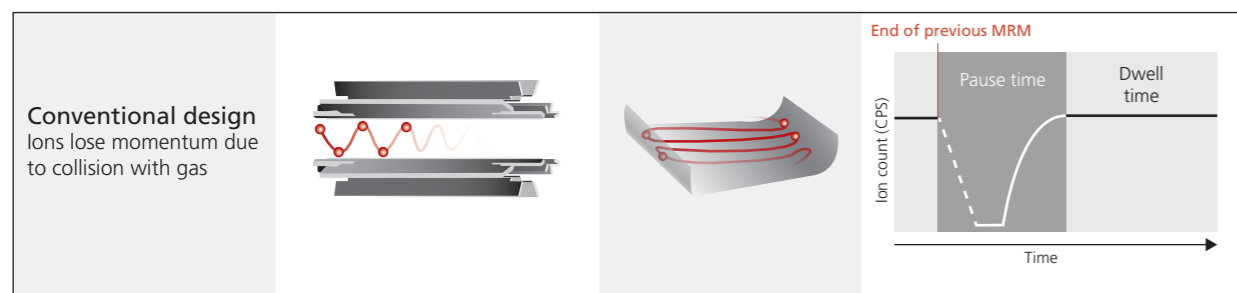
A high-sensitivity and high-throughput MRM approach requires universally ultra-fast collision cell technology. With the new pseudo potential surface, ions entering the collision cell are accelerated and maintain their momentum upon collision. Under these circumstances, the efficiency of the fragmentation or CID is improved. This technology allows quicker and more efficient ion transmission in the collision cell, maintaining signal intensity and dramatically suppressing crosstalk, even when shorter dwell and pause[1] times are used.

[1]Measurement conditions must be switched to perform simultaneous measurements of multiple compounds, (multi-component analysis). The time needed for this switching is termed as "pause-time". As data cannot be acquired during the pause time, it should be as short as possible.

## ✓ High-speed polarity switching pushes the boundaries further

High-speed polarity switching[2] has a high impact on LC-MS/MS method capability by optimizing the signal response for each target compound resulting in a single injection analysis cycle.

UFswitching™ enables high-speed positive/negative ionization switching in 5 msec, allowing you to measure simultaneous positive/negative compounds in a single analysis, whereas sensitivity and repeatability were not compromised as well.



UFswitching™

[2]Separate positive ionization and negative ionization modes may be used in LCMS. However, switching between the positive and negative ionizations during analysis is required to and the time taken to switch between these modes is known as the "polarity switching time".

## ✓ Expanding Capabilities in Multi-Residue Pesticide Analysis Using LC-MS/MS
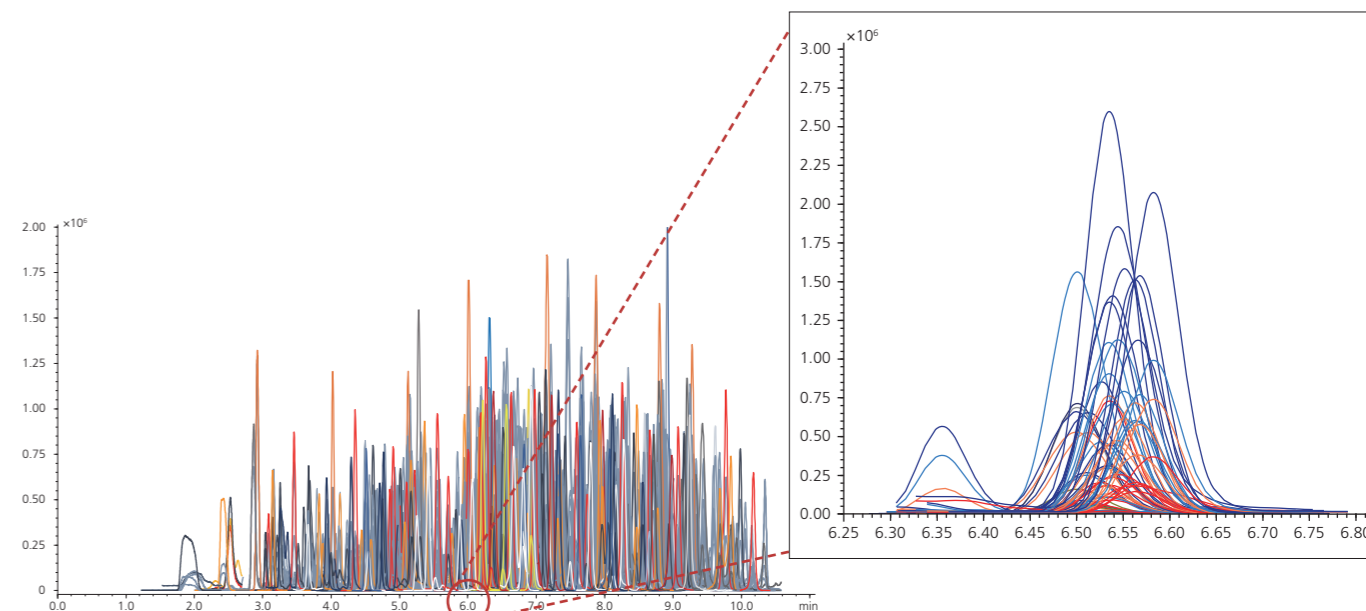


MRM chromatograms of 646 pesticides spiked into a mint extract at 0.01 mg/kg
(Up to 3 MRMs per compound and 5 msec polarity switching time).

Based on the example of the mint extract analysis (Phytocontrol, France), QuEChERS protocols were used and 25 pesticide compounds were eluted at the 6.45 - 6.60 time range. Even with high data density acquisitions, the average variation in peak area response was less than 3 %RSD (varying between 1.1 - 5.9 %RSD).



Click to read the application news in details: Expanding Capabilities in Multi-Residue Pesticide Analysis Using The LCMS-8060
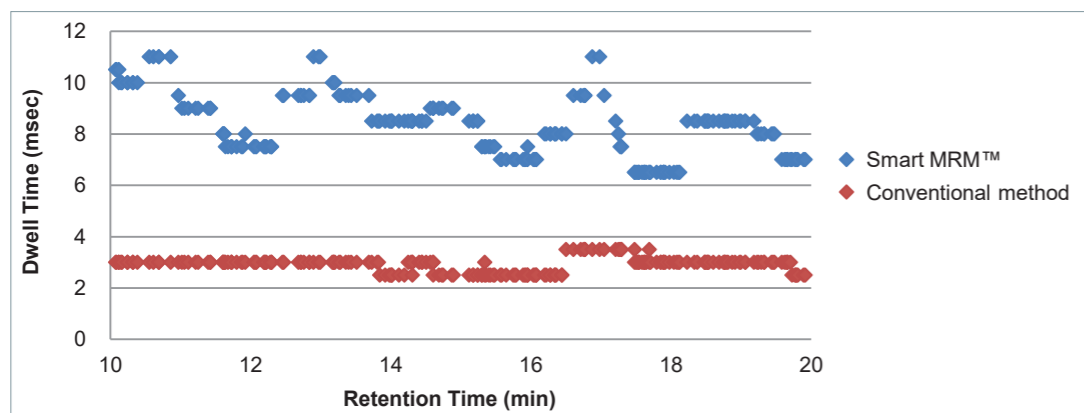
# Smart MRM™

✓ **Automatic method development with increased throughput**

Smart MRM

| Serial# | Type | Acq. Mode | Compound Name (E) | Ret. Index 1 | Ret. Time | Ion1 | | | Ion2 | | | Ion3 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Type | m/z | Rati | Type | m/z | Rati | Type | m/z | Rati |
| 41 | Target | SIM | Chlorpropham | 1666 | 10.228 | T | 213.0 | 100.00 | Ref.1 | 127.0 | 219.30 | | 154.0 | 56.14 |
| 42 | Target | SIM | Ethalfluralin | 1675 | 10.324 | T | 276.0 | 100.00 | Ref.1 | 316.0 | 78.70 | | 333.0 | 23.15 |
| 43 | Target | SIM | Dichlofluanid metabolite | 1676 | 10.334 | T | 200.0 | 100.00 | Ref.1 | 92.0 | 128.69 | | 108.0 | 22.13 |
| 44 | Target | SIM | Naled | 1678 | 10.355 | T | 185.0 | 100.00 | Ref.1 | 109.0 | 595.24 | | 145.0 | 152.38 |
| 45 | Target | SIM | Flusilazole metabolite | 1678 | 10.355 | T | 235.0 | 100.00 | Ref.1 | 250.0 | 14.80 | | 155.0 | 9.60 |
| 46 | Target | SIM | Dicrotophos | 1688 | 10.461 | T | 127.0 | 100.00 | Ref.1 | 193.0 | 9.60 | | 237.0 | 6.80 |
| 47 | Target | SIM | Trifluralin | 1691 | 10.493 | T | 306.0 | 100.00 | Ref.1 | 264.0 | 88.68 | | 335.0 | 8.18 |
| 48 | Target | SIM | 2,6-Dichlorobenzamide | 1691 | 10.493 | T | 173.0 | 100.00 | Ref.1 | 175.0 | 63.60 | | 189.0 | 44.00 |
| 49 | Target | SIM | Dioxabenzofos (Salithion) | 1692 | 10.504 | T | 216.0 | 100.00 | Ref.1 | 183.0 | 45.60 | | 201.0 | 26.80 |
| 50 | Target | SIM | Benfluralin | 1695 | 10.535 | T | 292.0 | 100.00 | Ref.1 | 264.0 | 24.40 | | 293.0 | 12.40 |
| 51 | Target | SIM | Monocrotophos | 1700 | 10.588 | T | 127.0 | 100.00 | Ref.1 | 97.0 | 18.00 | | 192.0 | 10.00 |
| 52 | Target | SIM | Sulfotep | 1702 | 10.609 | T | 322.0 | 100.00 | Ref.1 | 238.0 | 39.60 | | 266.0 | 39.20 |
| 53 | Target | SIM | Cadusafos | 1703 | 10.619 | T | 159.0 | 100.00 | Ref.1 | 158.0 | 69.20 | | 270.0 | 5.20 |
| 54 | Target | SIM | Di-allate-1 | 1711 | 10.702 | T | 234.0 | 100.00 | Ref.1 | 236.0 | 39.73 | | 86.0 | 254.79 |
| 55 | Target | SIM | Phorate | 1713 | 10.723 | T | 260.0 | 100.00 | Ref.1 | 231.0 | 54.29 | | 75.0 | 714.29 |

Smart MRM database incorporated with Smart MRM technology supports automatic creation of analysis methods.

### Dwell Time Optimization using Smart MRM



The relationship between the dwell time and retention time in the measurement program created using Smart MRM is shown.(for retention times from 10 to 20 minutes)

Smart MRM technology can automatically adjust the analytical dwell time for each transition, and acquires data during peak elution, to fully optimize sensitivity.



Simultaneous Analysis Method of 447 Pesticides
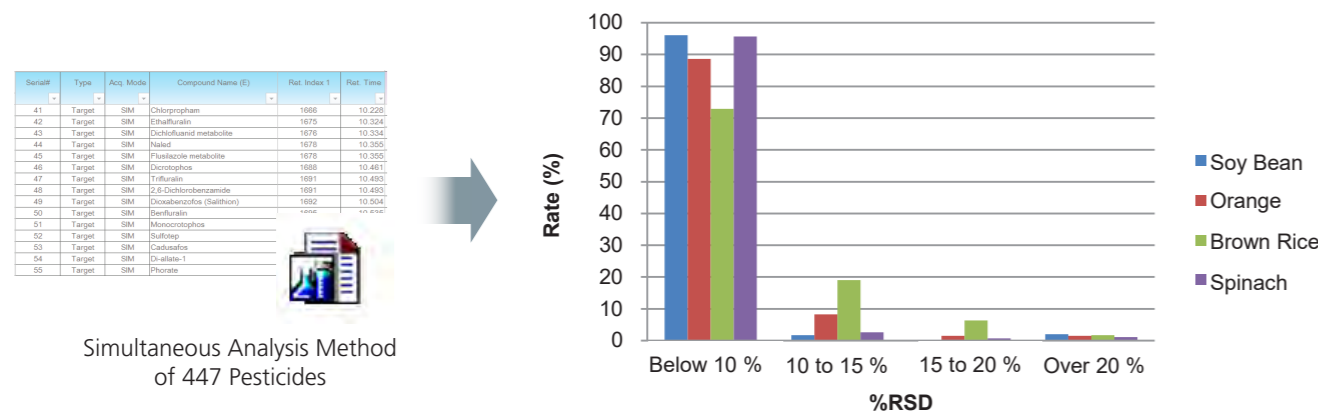
%RSD distribution for each matrix spiked with 477 pesticides at 5 ppb.

Click to read the application data sheet in detail: Simultaneous Analysis of 477 Residual Pesticides in Agricultural Crops Using GC-MS/MS

# Smart SIM™

✓ **Effortless SIM method building**

Smart SIM technology improved dramatically the efficiency of multi-component simultaneous analysis, which function is the same as Smart MRM. Moreover, highly reliable data can be obtained as the result of the optimized analytical conditions.
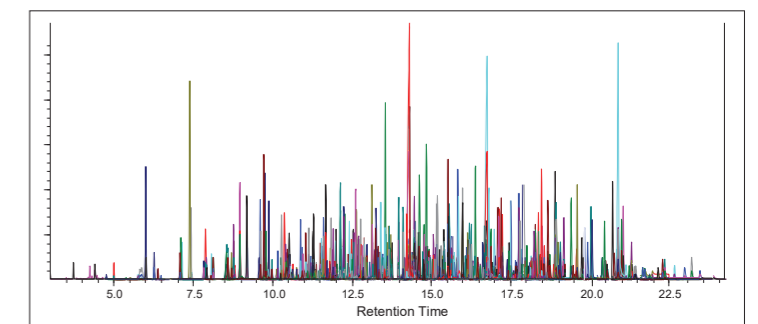
### Group Measurement Method
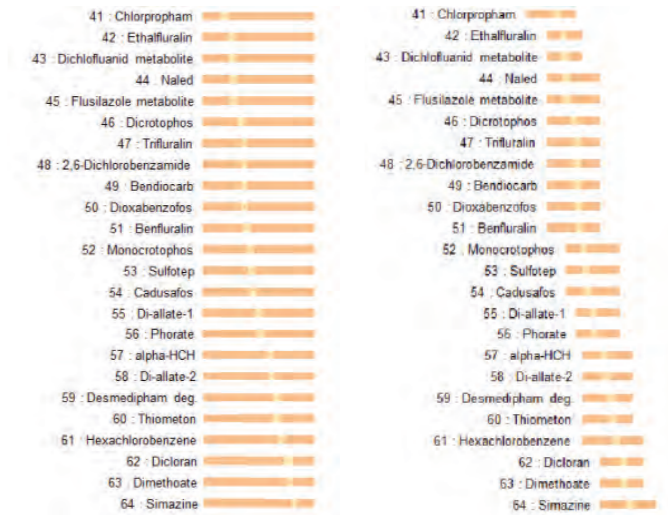


SIM measurement time program
Left: Group measurement method
Right: Measurement method using Smart SIM

Automatic Method Creation

Simultaneous Analysis Method of 418 Pesticides

SIM Chromatogram for 418 Pesticides Standard Mixed Solution at 100 ng/mL

Click to read the application data sheet in detail: Simultaneous Analysis of 418 Pesticides Utilizing Smart SIM
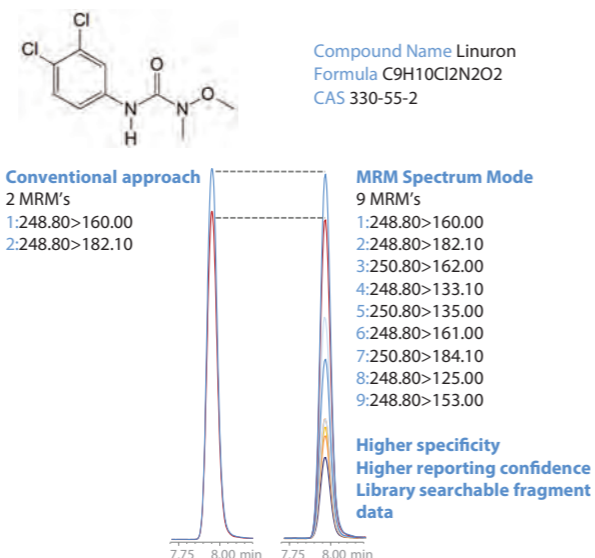
# Library Screening MRM Spectrum Mode

## ✓ Enhanced specificity and reporting confidence

The integrated UF technologies (UF-MRM™, UFSwitching™) can increase the specificity of detection and reduce false negative and false positive reporting, by acquiring multi-fragment ions in MRM data acquisition.
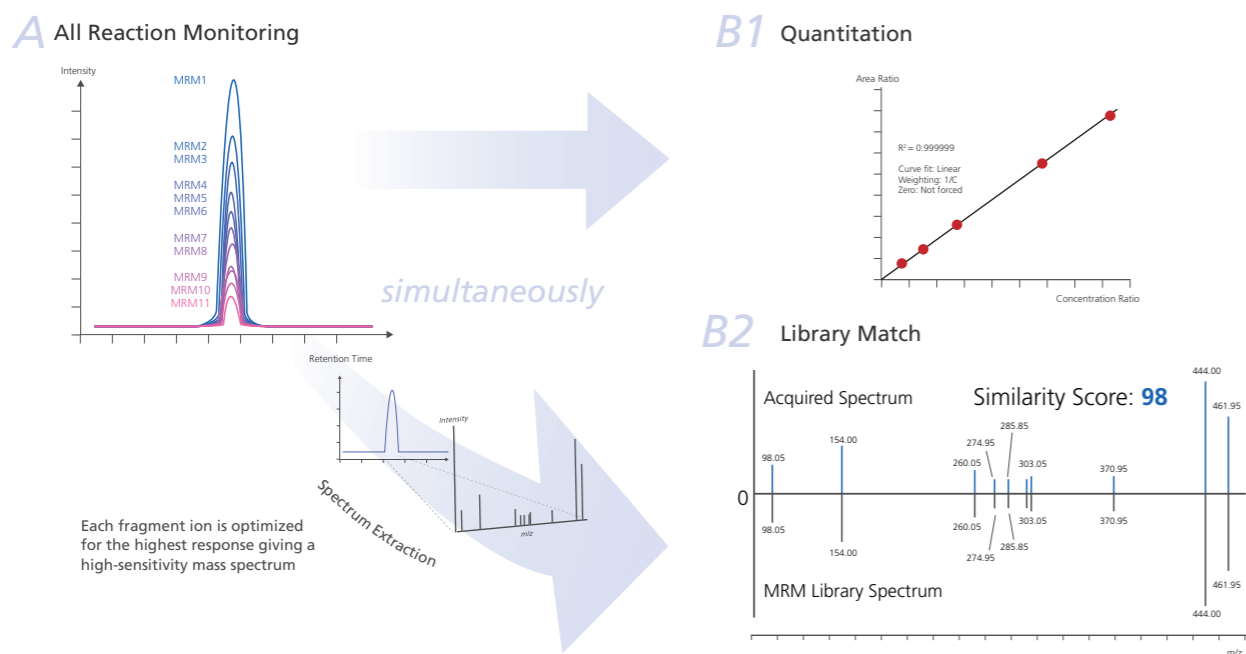
Compound Name Linuron
Formula C9H10Cl2N2O2
CAS 330-55-2

### Comparison of conventional 2-MRM method and MRM Spectrum mode

With reference to the analysis of linuron, data quality of MRM Spectrum mode was not compromised despite monitoring a larger number of fragment ions.

**Conventional approach**
2 MRM's
1: 248.80>160.00
2: 248.80>182.10

**MRM Spectrum Mode**
9 MRM's
1: 248.80>160.00
2: 248.80>182.10
3: 250.80>162.00
4: 248.80>133.10
5: 250.80>135.00
6: 248.80>161.00
7: 250.80>184.10
8: 248.80>125.00
9: 248.80>153.00

**Higher specificity
Higher reporting confidence
Library searchable fragment data**

7.75  8.00 min      7.75  8.00 min

## ✓ Improved data review efficiency

Shimadzu provides effective ease-of-use tool — Library Screening MRM Spectrum Mode, to deliver reliable data review. This serves as an additional identification criterion to enhance reporting confidence.

A **All Reaction Monitoring**

Intensity

MRM1
MRM2
MRM3
MRM4
MRM5
MRM6
MRM7
MRM8
MRM9
MRM10
MRM11

*simultaneously*

Retention Time

Spectrum Extraction

Intensity

m/z

Each fragment ion is optimized for the highest response giving a high-sensitivity mass spectrum

B1 **Quantitation**

Area Ratio

R² = 0.999999

Curve fit: Linear
Weighting: 1/C
Zero: Not forced

Concentration Ratio

B2 **Library Match**

Acquired Spectrum          Similarity Score: **98**

444.00
461.95
285.85
274.95
98.05  154.00  260.05  303.05  370.95
0
98.05
154.00    260.05  303.05  370.95
274.95  285.85
461.95
MRM Library Spectrum
444.00
m/z

Click to read in detail: LabSolutions Insight Library Screening - MRM Spectrum Mode

## ✓ Enhanced Reporting Confidence in Routine Pesticide Residue Analysis

Compound Name Demeton-S-methyl sulphone
Formula C6H15O3PS2
CAS 919-86-8
RT 2.94mins

**MRM spectrum**
Precursor-fragment ions
11 MRM's
1: 263.00>109.10 CE: -30V
2: 263.00>169.10 CE: -22V
3: 263.00>125.05 CE: -25V
4: 263.00>121.15 CE: -16V
5: 263.00>230.90 CE: -14V
6: 263.00>93.10 CE: -21V
7: 263.00>78.85 CE: -46V
8: 263.00>143.15 CE: -16V
9: 263.00>110.85 CE: -29V
10: 263.00>77.05 CE: -30V
11: 263.00>65.00 CE: -51V

2.80   3.00

Similarity Score 100
Matrix **Cumin**
Intensity 2.5e6

109
169
125
143
65  79  93
231
75.0   100.0   125.0   150.0   175.0   200.0   m/z

Similarity Score 100
Matrix **Black Pepper**
Intensity 1.8e6

109
125
169
79  93  143
65
231
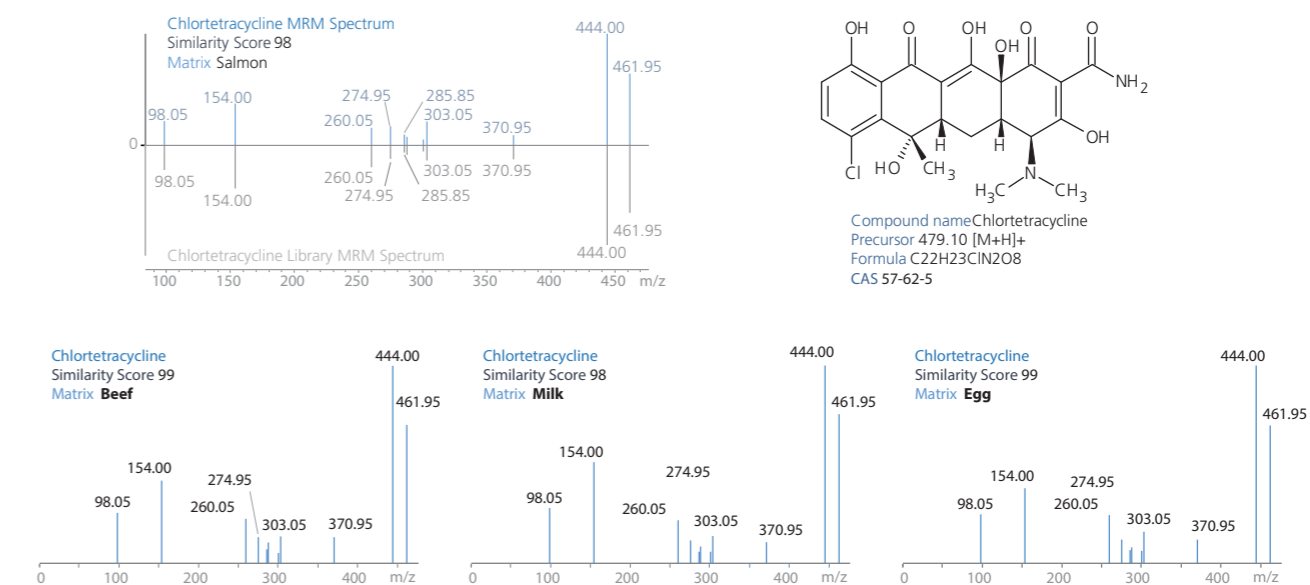75.0   100.0   125.0   150.0   175.0   200.0   m/z

Demeton-S-methyl sulphone was to highlight library matching in cumin and black pepper. Even in the presence of complex spice matrices the library matching approach identified demeton-S-methyl sulphone with a high similarity score and a high degree of confidence for data reporting.

Click to read the application news in detail: Application News Applying 'MRM Spectrum Mode' and Library Searching for Enhanced Reporting Confidence in Routine Pesticide Residue Analysis

## ✓ Identification of Residual Veterinary Drug

Chlortetracycline MRM Spectrum
Similarity Score 98
Matrix **Salmon**

444.00
461.95
154.00  274.95  285.85
260.05  303.05
98.05   370.95
0
98.05   260.05  303.05  370.95
154.00  274.95  285.85
461.95
Chlortetracycline Library MRM Spectrum
444.00
100  150  200  250  300  350  400  450  m/z

Compound name Chlortetracycline
Precursor 479.10 [M+H]+
Formula C22H23ClN2O8
CAS 57-62-5

Chlortetracycline
Similarity Score 99
Matrix **Beef**

444.00
461.95
154.00  274.95
98.05  260.05
303.05  370.95
0   100   200   300   400   m/z

Chlortetracycline
Similarity Score 98
Matrix **Milk**

444.00
461.95
154.00  274.95
98.05  260.05
303.05  370.95
0   100   200   300   400   m/z

Chlortetracycline
Similarity Score 99
Matrix **Egg**

444.00
461.95
154.00  274.95
98.05  260.05  303.05  370.95
0   100   200   300   400   m/z

Identification and verification of chlortetracycline using reference library match scores. The library match score was above 98 for salmon, beef, milk, egg extract spiked with chlortetracycline at a concentration of 10 pg/µL.

Click to read the application news in detail: Multi-Residue Veterinary Drug Analysis of >200 Compounds using MRM Spectrum Mode by LC-MS/MS

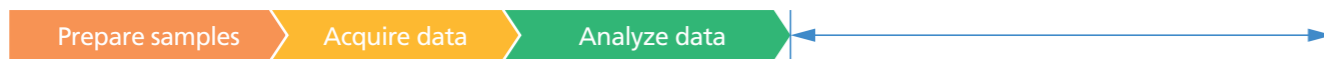# LabSolutions Software Platform

## LabSolutions Insight™

✓ Accelerates data processing productivity and quantitative data review. Library Screening MRM Spectrum Mode is a featured in LabSolutions Insight.

## Conventional data acquisition and processing

| Prepare samples | Acquire data | Analyze data |
|---|---|---|

## Data acquisition and processing using LabSolutions Insight

| Prepare samples | Acquire data | Analyze data |
|---|---|---|

✓ Intuitive Operation
An easy-to-use and configurable interface allows new users to be productive quickly

✓ Automated QA/QC Flagging and Peak Comparison
Contains multiple QA/QC flagging criteria for retention times, reference ion ratios, concentration limits, etc.

✓ Data Integrity
Compatible with LabSolutions DB, enabling traceability, audit trail, and user management capabilities.

✓ Efficient Workflow
Flag-based labeling, filtering, peak comparison functions, and centralized results files to work more efficiently.



Clean and comprehensive layout.

Peaks can be manually identified and integrated by simply dragging markers.

Peak Survey Mode can display all analytes in a single sample, or all sample results for a specified analyte.

## LabSolutions Report Plus™ Software

✓ Create user-defined reports with information freely arranged in any layout



LabSolutions / LabSolutions Report Plus
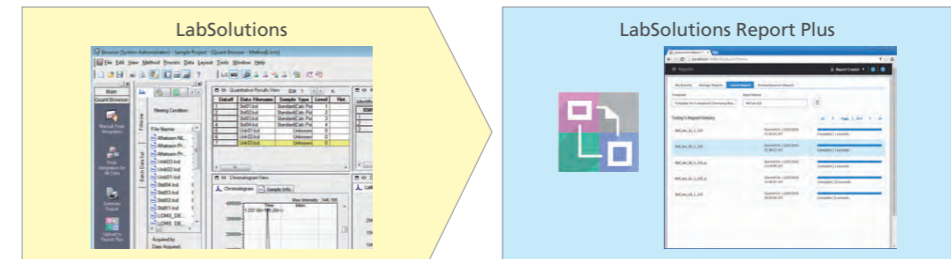
An analytical condition summary report lists the analytical conditions for each compound

A calibration curve summary report lists the calibration curve for each compound

A compound summary report summarizes quantitation results for each compound of unknown samples

Click to read the technical report in detail: Data Integrity Compliance Using the LabSolutions Report Set

## Various Database Series and Application-Specific Method Packages

✓ Simplify your workflows and quickly set up a method for targeted and suspect screening using GC-MS and LC-MS.

**Food Safety Analysis**

GC/MS
· Quick-DB Database for Residual Pesticide Analysis
· Smart Pesticides Database

LC/MS
· Method Package for Residual Pesticides
· Method Package for Veterinary Drugs

**Metabolite Component Analysis**

GC/MS
· Smart Metabolites Database

LC/MS
· MRM Library for Metabolic Enzymes (Yeasts) Library
· Method Package for Lipid Mediators
· Method Package for Primary Metabolites

**Environmental Pollutant Analysis**

GC/MS
· Compound Composer
· Smart Environmental Database

LC/MS
· Method Package for Water Analysis

**Forensic Toxicological Analysis**

GC/MS
· Smart Forensic Database
· Quick-DB GC/MS/MS Forensic Toxicological Database

LC/MS
· Rapid Toxicological Drug Screening System
· Method Package for Forensic Toxins

← Return to
main page
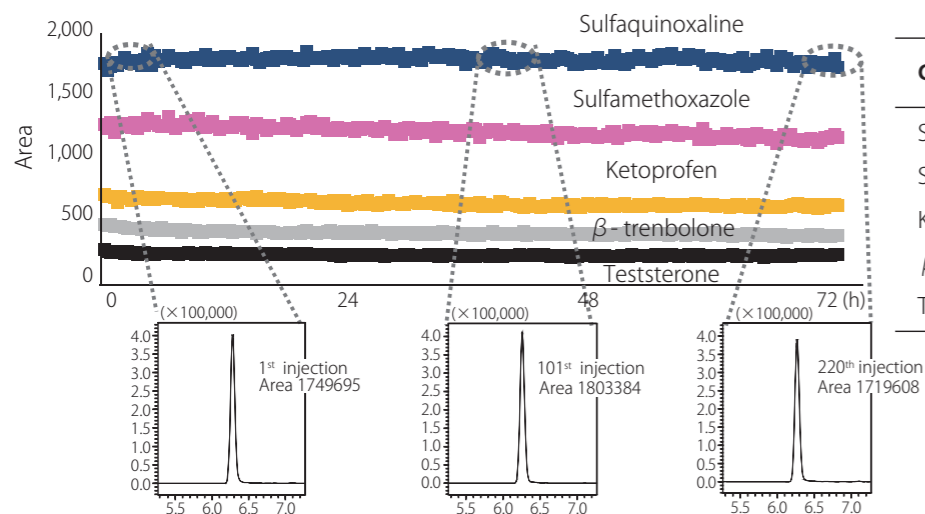
# Cutting-edge Ion Optics

## ✓ Long-term stability with minimum downtime



Shimadzu LC-MS/MS series have engineered a unique and innovative ion optics that routinely delivers robust detection at very high sensitivities even with complex matrices at trace levels.
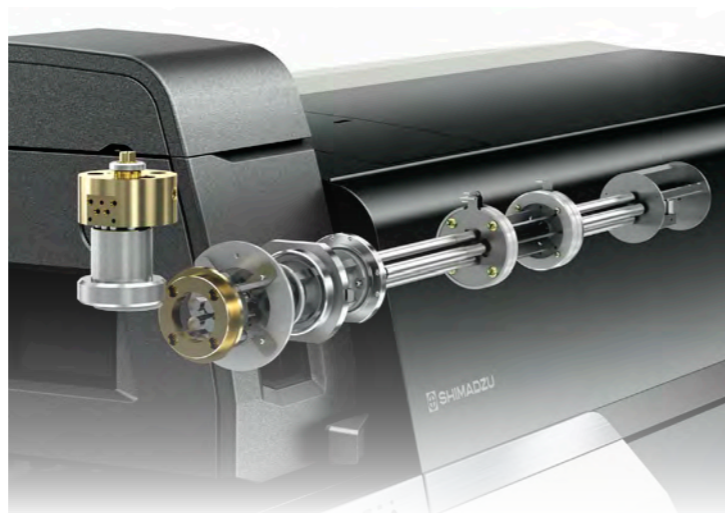
LC-MS/MS robustness was demonstrated by long-term stability of the instrument using a solution of pork crude extract (spiked with 10µg/L standard solution). Even after continuous measurement of an extremely complex matrix over a period of 3 days, Shimadzu instruments can deliver consistent, and higher quality results.

## ✓ Highly repeatable quantification of veterinary drug



| Compounds | %RSD (%) (n=220) |
|---|---|
| Sulfaquinoxaline | 1.5 |
| Sulfamethoxazole | 2.8 |
| Ketoprofen | 2.3 |
| $\beta$ - trenbolone | 3.2 |
| Teststerone | 3.5 |

Sample preparation of typical samples based on QuEChERS Extraction Salts Kit.
Area Plot and %RSD of Typical Compounds with Continuous Analysis

Click to read the application news in detail: Quantitative Analysis of Veterinary Drugs Using the Shimadzu LCMS-8050 Triple Quadrupole Mass Spectrometer

## ✓ Confident pesticide residue analysis for various matrices
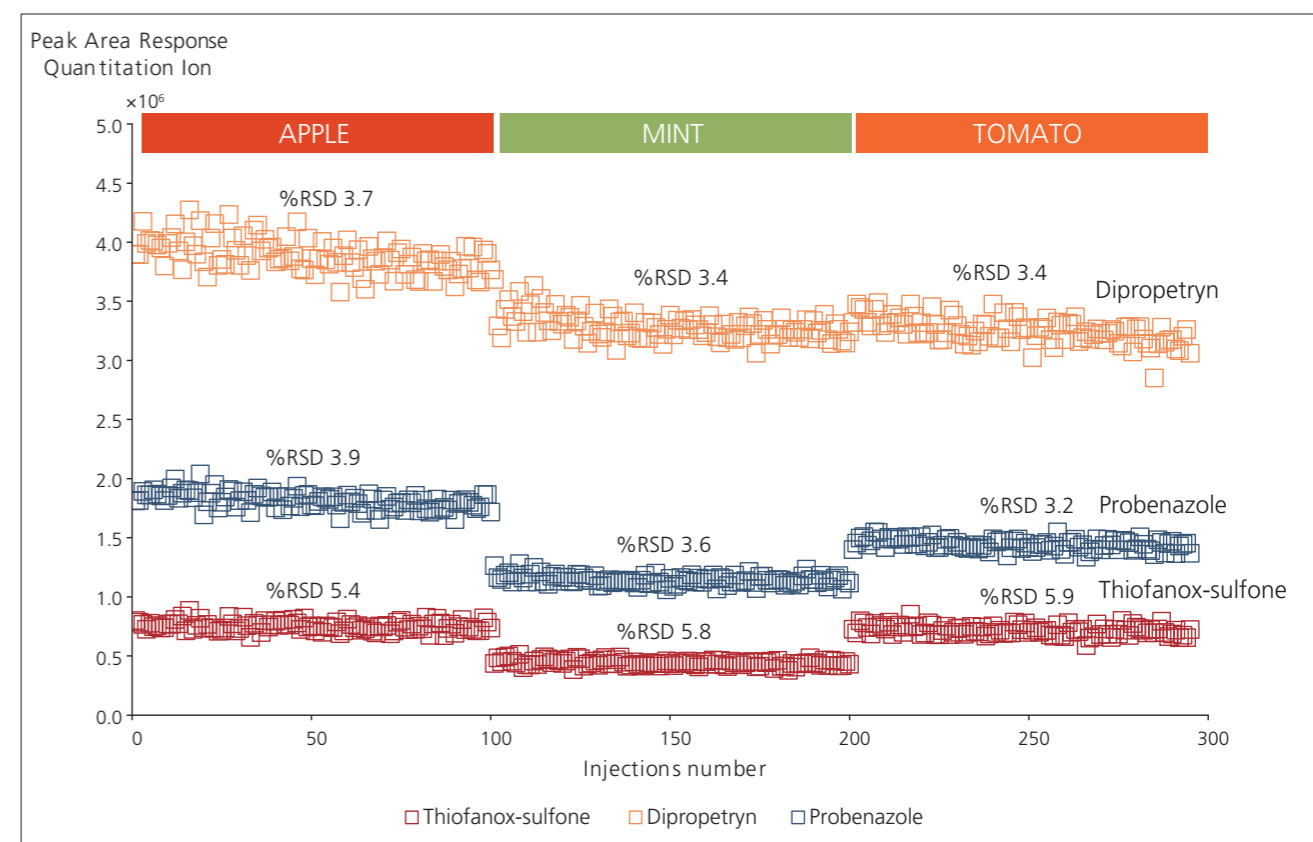


Peak area response for three pesticides spiked into apple, mint and tomato matrix extracts at 0.05 mg/kg over 72 hours.
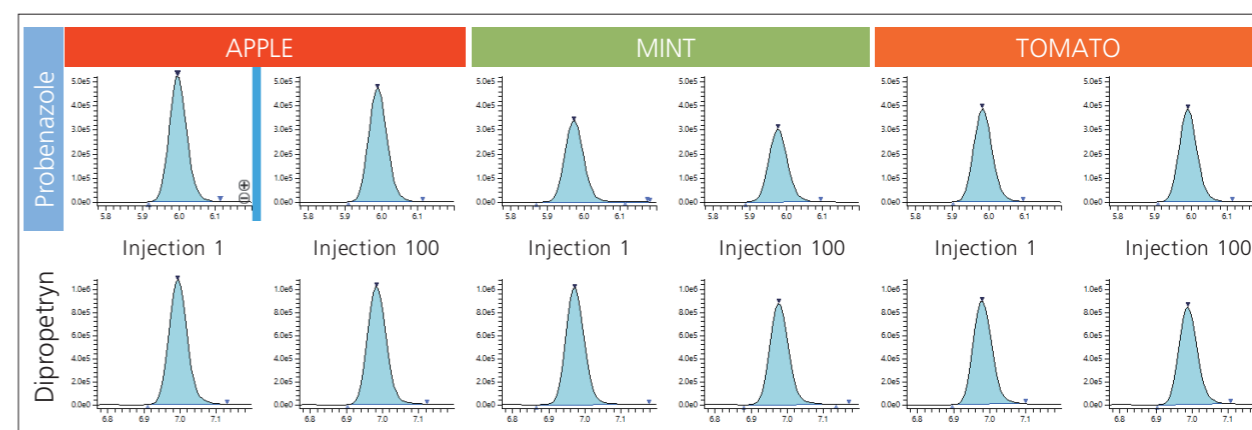


MRM chromatogram for probenazole and dipropetryn for injection 1 and injection 100.

Click to read the application news in detail: Expanding Capabilities in Multi-Residue Pesticide Analysis Using The LCMS-8060

# Patented Ion Source

✓ **Reduces maintenance frequency and long-term operational costs**

The effect of the filament's electric potential on the ion source is reduced by increasing the distance between the filament and ion source box. In addition, a shield blocks out radiant heat generated from the filament to ensure the ion source box temperature remains uniform. Since this prevents any active spots within the ion source, it provides higher sensitivity for analysis (Patent: US7939810).

Shield
Shield

Temperature
Low    High

● : Filament
···· : Electric field
→ : Heat rays
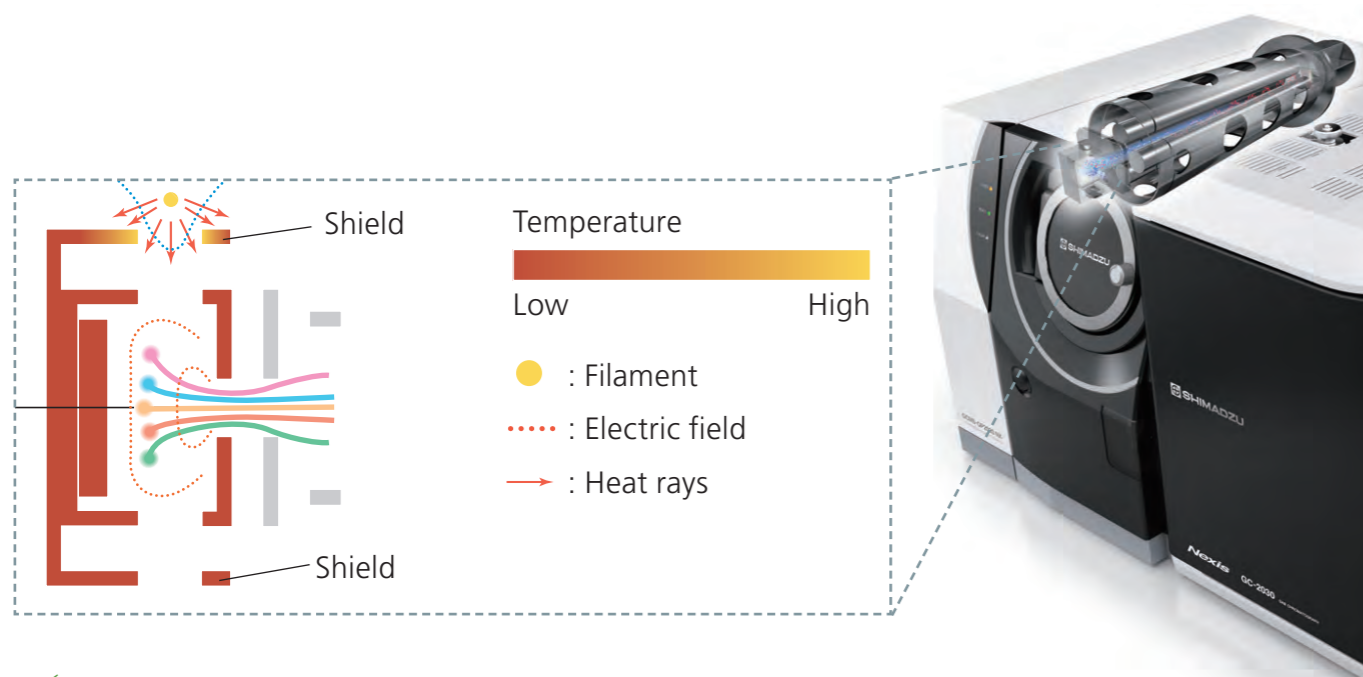
✓ **Improved robustness through ultra-sensitive design**

maintenance is performed per year
Annual cost of consumables

2 µL injection of 5ng/mL

8 inserts    2 columns

4 times per year

**4x Lesser Injection Volume**
**4x Lesser Cleaning**
**4x Lesser Consumables**

GCMS-TQ8050 NX

1 time per year
2 inserts    1 column

0.5 µL injection of 5 ng/mL
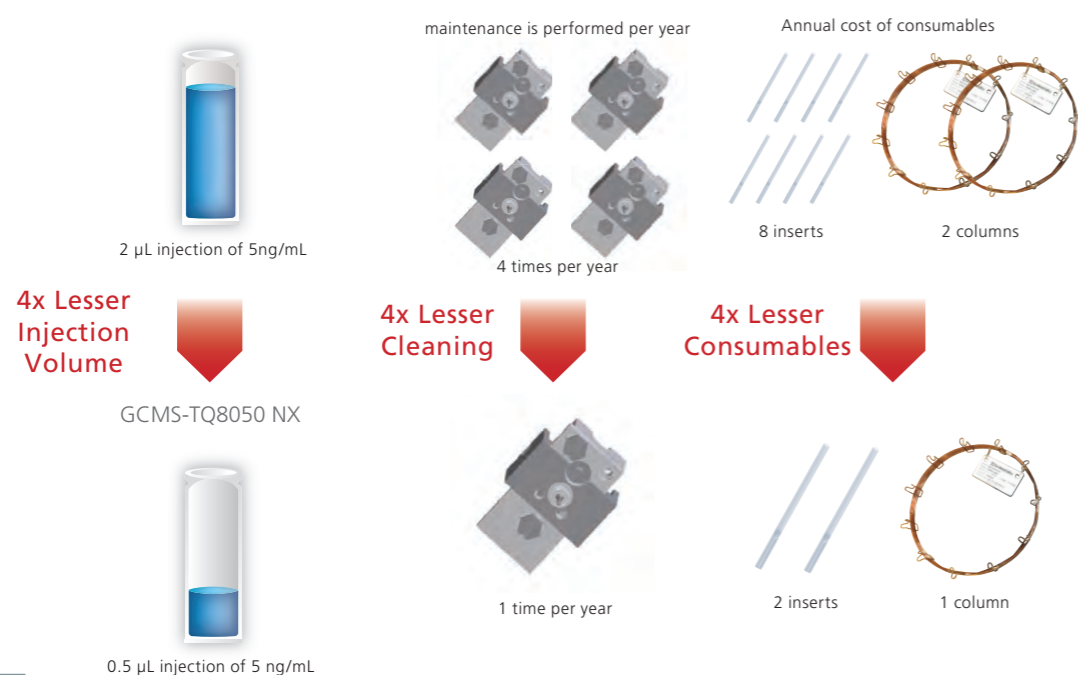
Click to read the application data sheet in detail: Efficient Analysis of Residual Pesticides in Foods Using High-Sensitivity GC-MS/MS

# Inert Flow Path

✓ **Detect trace components with high sensitivity and repeatability**

**Glass liner**
The glass liner recommended for GC/MS analysis uses a proprietary inactivation technology to dramatically suppress active sites. After packing into the insert, the wool is subjected to a complete inactivation treatment. This product is controlled throughout from production to final inspection to provide 100 % satisfaction.

TIC
Resolution (%)
Endrin: 1.34
DDT:    1.44

14.0    15.0    16.0    17.0

**Micro-syringe**
Autosampler syringes feature improved durability, clarity, and accuracy, achieving reliable injection accuracy.

**GC septum**
Our lineup now includes low bleed septa, which maintain optimal seal performance even when the injection cycles are increased, and can be used even at high temperatures. This reduces sensitivity variations due to leaks.

**Ferrules and gold gasket**
The high-quality Vespel ferrule is easily attached and designed to resist leaking. The gold gasket is inactive, and adsorption does not occur.

**Ion source**
Designed with a shield that blocks radiant heat generated by the filament, and an ion source treated with an oxide coating, active spots inside the ion source are not prone to occur, which enables high-sensitivity analysis with long-term stability.

**Capillary columns**
For the SH-Rxi™ series, a high-quality fused silica like no other is used as the raw material. Our proprietary surface inactivation technology and optimal process to mask silanol groups result in a low-bleed column with very impressive inactivation performance, even with respect to polar compounds comparable to acidic and basic substances.
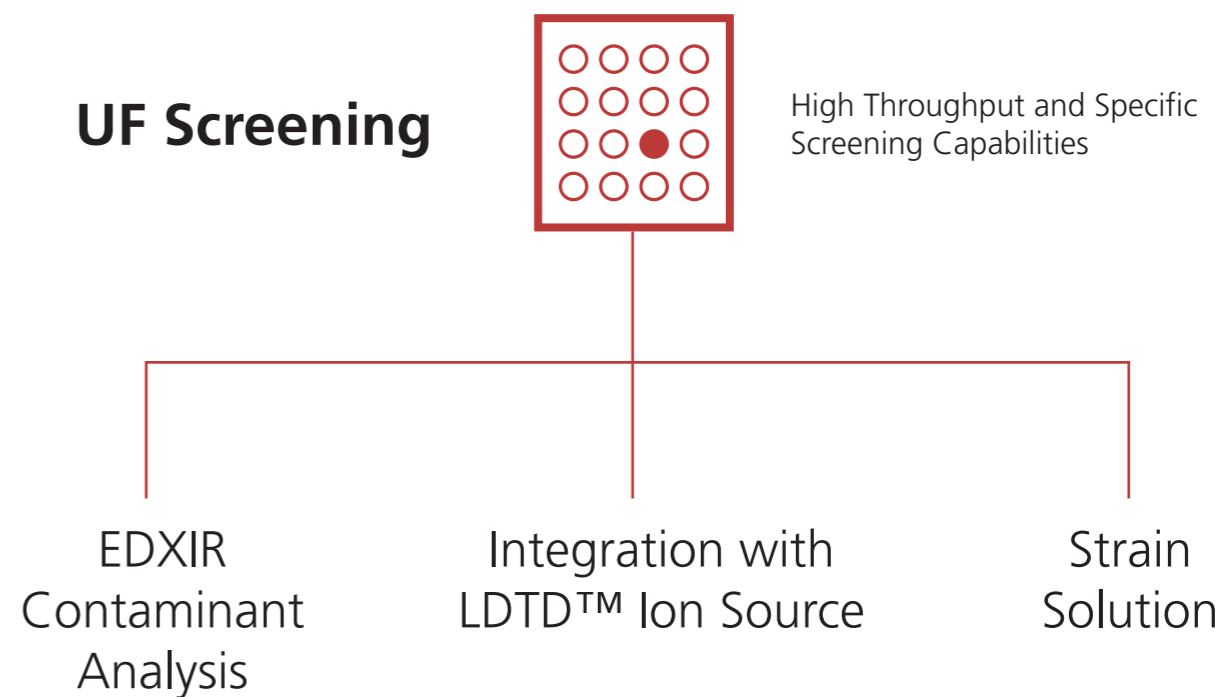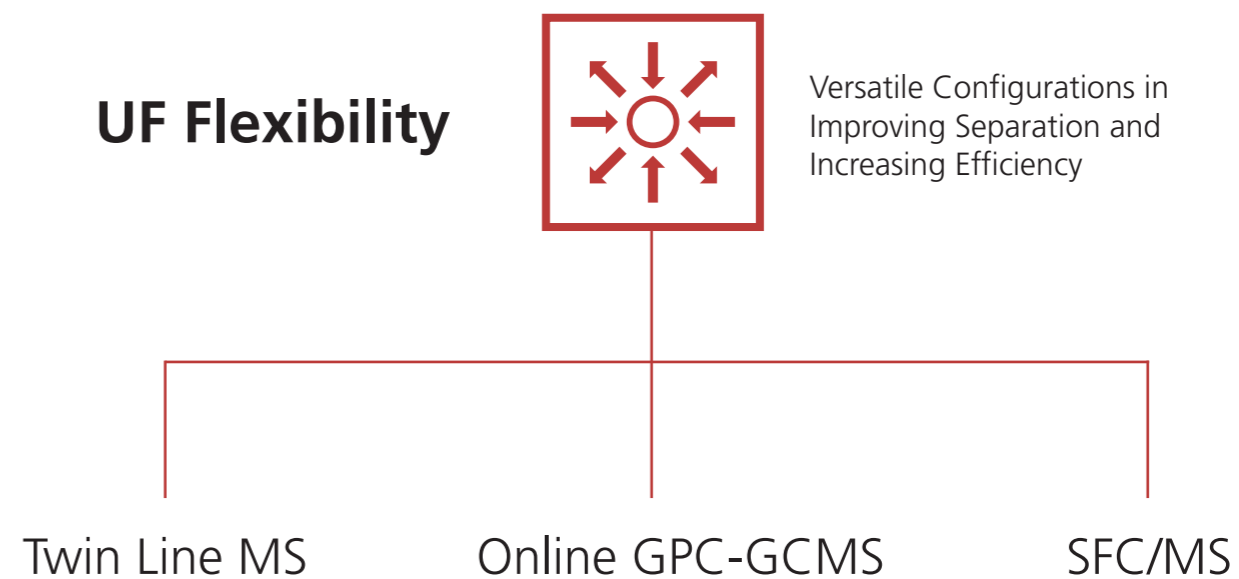
# Advanced Techniques for Food Analysis

Complex food analysis can be made simpler and more efficient by several advanced separation and screening techniques. In addition to Shimadzu UFMS technologies that addressed truly scalable performance for routine analysis, complex food analysis can be made simpler and more efficient with the incorporation of advanced separation and screening techniques.

Shimadzu has developed several instrumentation, software and configurations to simplify and expedite your analytical workflow, and improve your screening capabilities. This section describes in detail the key features and benefits of these advanced techniques.

**UF Flexibility**

Versatile Configurations in Improving Separation and Increasing Efficiency

Twin Line MS          Online GPC-GCMS          SFC/MS

**UF Screening**

High Throughput and Specific Screening Capabilities

EDXIR
Contaminant
Analysis

Integration with
LDTD™ Ion Source

Strain
Solution

# Twin Line MS System

✓ **Makes second-column confirmation on the same instrument simpler**

The highly complex nature of food samples has always been a challenging issue in sample preparation and analysis. With the use of simple sample preparation for food such as QuEChERS, the detection and identification of target compounds may not be directly achieved. In some cases, even the use of SIM or MRM mode may not obtain the required resolution, sensitivity and selectivity.

✓ **Discover the key benefit enabling quantitation with confidence**

Using a second-column allows for peak separation and identification of the target compound. Both single quadrupole MS and triple quadrupole MS are compatible with the Twin Line MS system, and can be used to reliably and accurately analyze target compounds in complex food matrices without the need to vent the MS.

## Simultaneous Analysis of 477 Residual Pesticides in Agricultural Crops Using GC-MS/MS

Column 1 (SH-Rxi-5Sil MS)         Column 2 (SH-Rtx-200 MS)



( 1,000,000)
206.00 > 191.00
206.00 > 141.00
Matrix Blank

( 10,000)
206.00 > 191.00
206.00 > 141.00
Matrix Blank

( 100,000)
206.00 > 191.00
206.00 > 141.00
Spiked sample

( 10,000)
206.00 > 191.00
206.00 > 141.00
Spiked sample
%RSD = 5.30 %
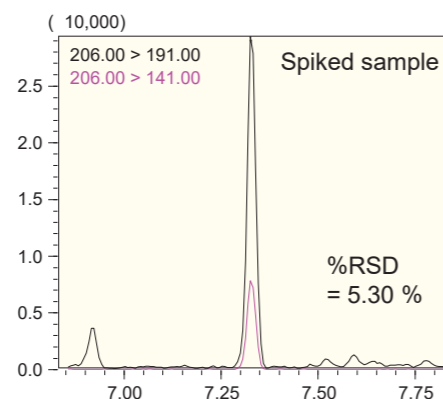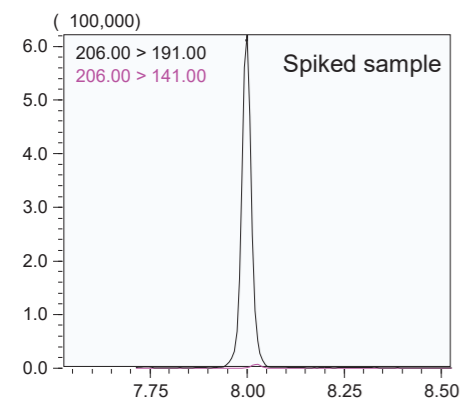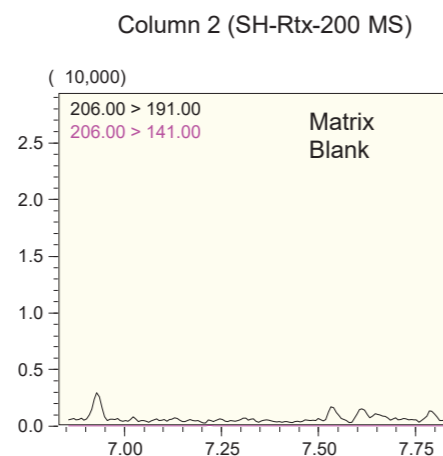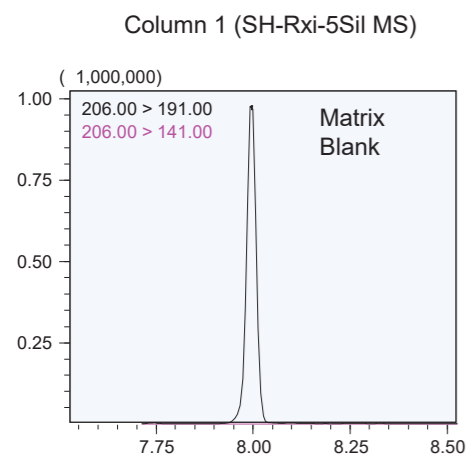
Separated from Impurities

Click to read in detail the application note on the use of Twin Line MS for second-column confirmation of pesticides on the same GC-MS without venting the MS.

# Online GPC-GCMS

✓ **Online cleanup technique specifically for residual pesticides analysis in food**

In this online system, Gel Permeation Chromatography (GPC) works as a cleanup technique, by removing unwanted compounds from complex food matrices, prior to a GC-MS analysis. This coupling of GPC to GC-MS provides a rapid streamlined workflow specifically for analysis of pesticides in foods. Also, some food contaminants are analyzed by GPC-GCMS.

**Accessible online analysis**

1. Unwanted compounds (e.g. oil, fat and dye analytes) are separated from target pesticides compounds using a GPC column.
2. Unwanted compounds are removed using a flow line switching valve.
3. Target pesticides are trapped and concentrated in the sample loop for large-volume injection into the GC-MS for simultaneous multi-component analysis.
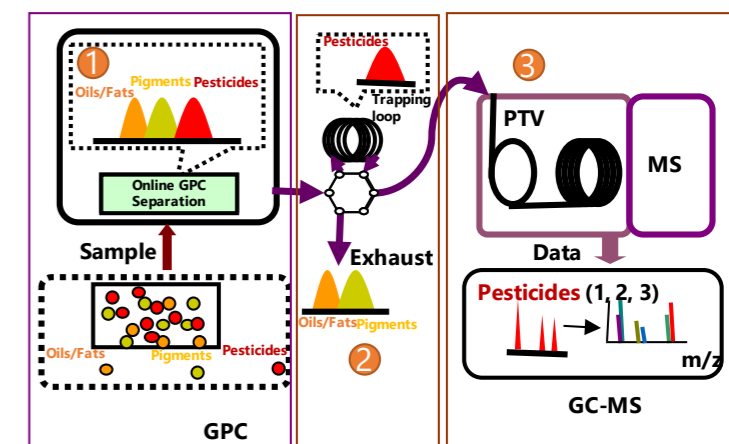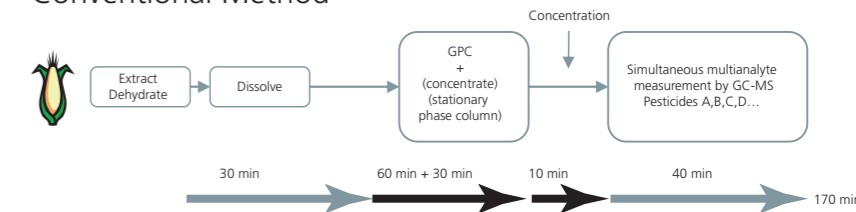


✓ Reduced Manual Sample Preparation

✓ Automated System
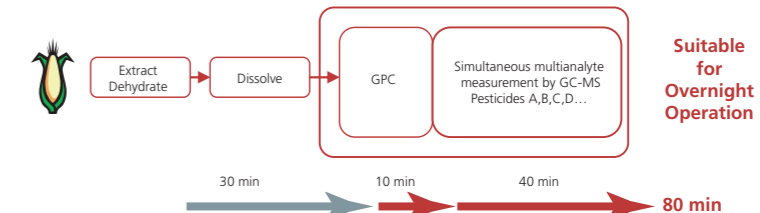
✓ GC-MS analysis + Simultaneous removal of unwanted compounds

✓ Lower Solvent Consumption

Conventional Method



Online GPC-GCMS



Click to read more on the analysis of PCBs in vegetables using GPC–GCMS

# SFC/MS System

## ✓ Increased coverage of polar pesticides

Supercritical fluid chromatography (SFC) has the advantage of being able to separate a wide array of chemical compounds at once due to the characteristics of the mobile phase that is used. In addition, since the separation behavior with SFC differs from that with LC even when using the same column chemistry, SFC can be an effective alternative for the analyses of compounds for which retention and separation are difficult in LC.

### Quantitative Analysis of Highly Polar Pesticides in Food Using SFC/MS
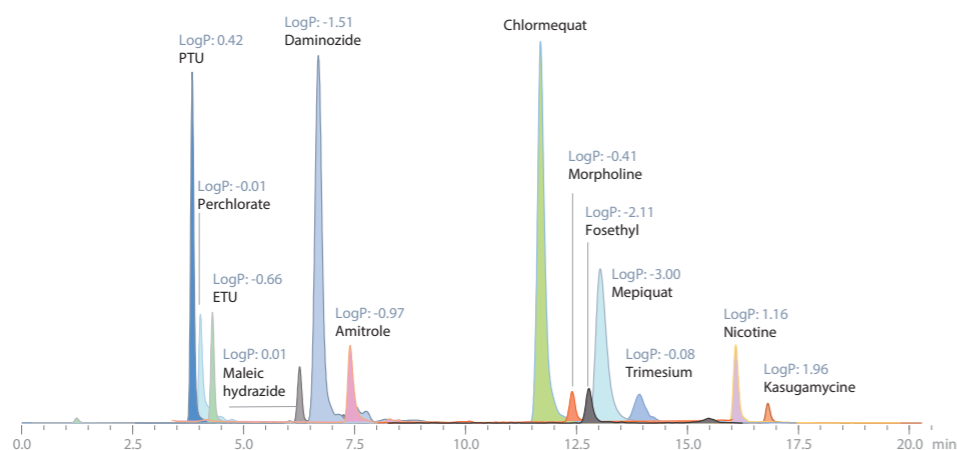


SFC/MS analysis covers the broadest range of compounds than any other existing methods

## ✓ Higher productivity

Facilitates fast chromatography through low solvent viscosity and superior diffusion characteristics.

## ✓ Higher sensitivity

Achieves excellent detection capabilities by splitless coupling of SFC eluent to MS

## ✓ Eco-friendly

Reduces cost of solvent purchase and usage

SPC/MS can be upgraded to on-line SFE-SFC-MS system*, which unites sample separation, analysis with various separation modes, and high-sensitivity detection.

* SFE (Supercritical Fluid Extraction) : An extraction method using supercritical fluid. It is available as a pretreatment method for solid sample analyses.
* SFC (Supercritical Fluid Chromatography) : The chromatographic technique using supercritical fluids as mobile phases. With its unique properties, it enables high-speed, high-resolution analyses.

Click to read more on: Quantitative Analysis of Highly Polar Pesticides in Food using SFC/MS

# EDXIR–Analysis

## ✓ EDX-FTIR Contaminant Finder/Material Inspector

EDXIR system is a comprehensive search system that provide an effective way to identify contaminants by an energy dispersive X-ray (EDX) fluorescence spectrometer and a Fourier transform infrared spectrophotometer (FTIR). Additionally, EDXIR-Analysis software is specially designed to perform qualitative analysis with library used for matching foreign substances in search results.

### Procedure for Qualitative Analysis of Contaminants
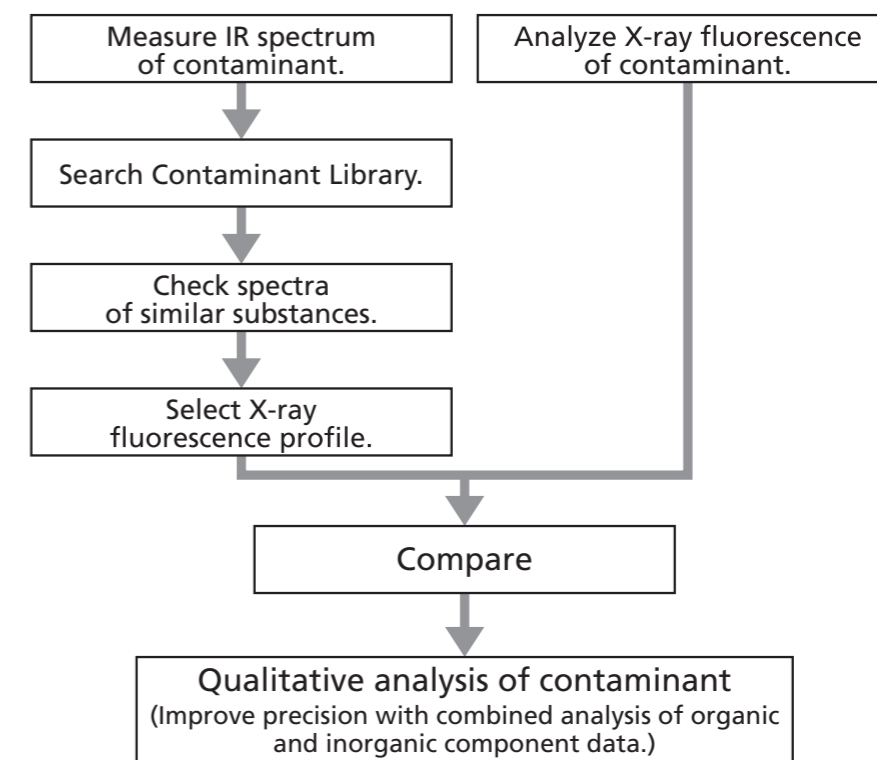


### Features

✓ Shimadzu's proprietary contaminant library was prepared with cooperation from organizations in the public water supply industry and food manufactures.

✓ Includes information from actual contaminant samples both an infrared spectral library, and X-ray fluorescence proles (in PDF file format).

### EDXIR-Analysis Library

The library used for data analysis (containing 485 data as standard) originates from Shimadzu, and was created through cooperation with water supply agencies and food manufacturers. Additional data can be registered to the library as well.

Click to read more on: Combined Analysis of a Contaminant Using a Compact FTIR and EDX

# Laser Diode Thermal Desorption (LDTD)™

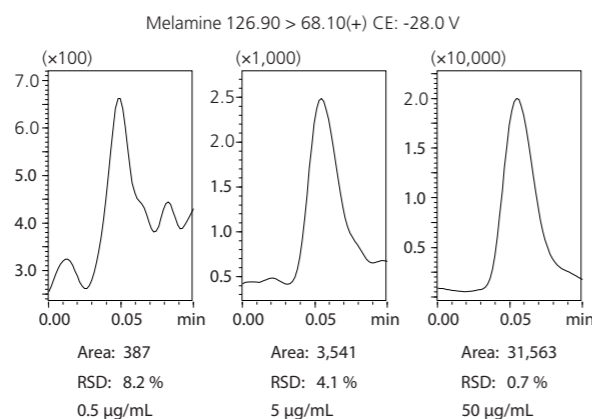✓ **Scalable performance revolutionize laboratory productivity**

The LDTD™ ion source (Phytronix Technologies Inc.) delivers solutions that complement screening and identification testing in various fields such as food safety. It is a direct ion source for mass spectrometers that provides ultra-high speed analysis in less than 4 seconds per sample.

**Features**

✓ LDTD Ion Source
- Direct sample introduction with no carryover
- Supports 10 LazWell plates (960 samples, 2 to 10 µL)

✓ Compatible with LCMS-8050/8060
- High speed performance of mass spectrometer enables high-throughput analysis.
- No need to replace LC-MS and LDTD-MS in screening and confirmation test

✓ Software Supports Serial Analysis Workflows
- Easy installation procedure
- Batch analysis with LabSolutions software

**LC-MS**

Switch between
two analysis
methods
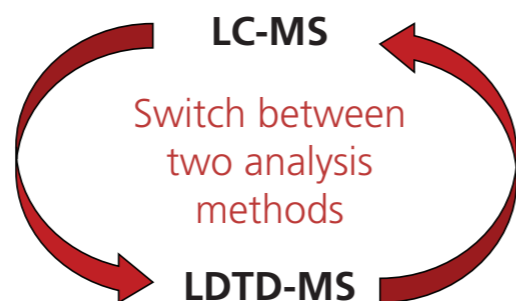
**LDTD-MS**

### Analysis of melamine added into milk powder using LDTD-MS

Melamine 126.90 > 68.10(+) CE: -28.0 V

(×100)

| | |
|---|---|
| 7.0 | |
| 6.0 | |
| 5.0 | |
| 4.0 | |
| 3.0 | |

0.00    0.05    min

Area: 387
RSD:  8.2 %
0.5 µg/mL

(×1,000)

2.5
2.0
1.5
1.0
0.5

0.00    0.05    min

Area: 3,541
RSD:  4.1 %
5 µg/mL

(×10,000)

2.0
1.5
1.0
0.5

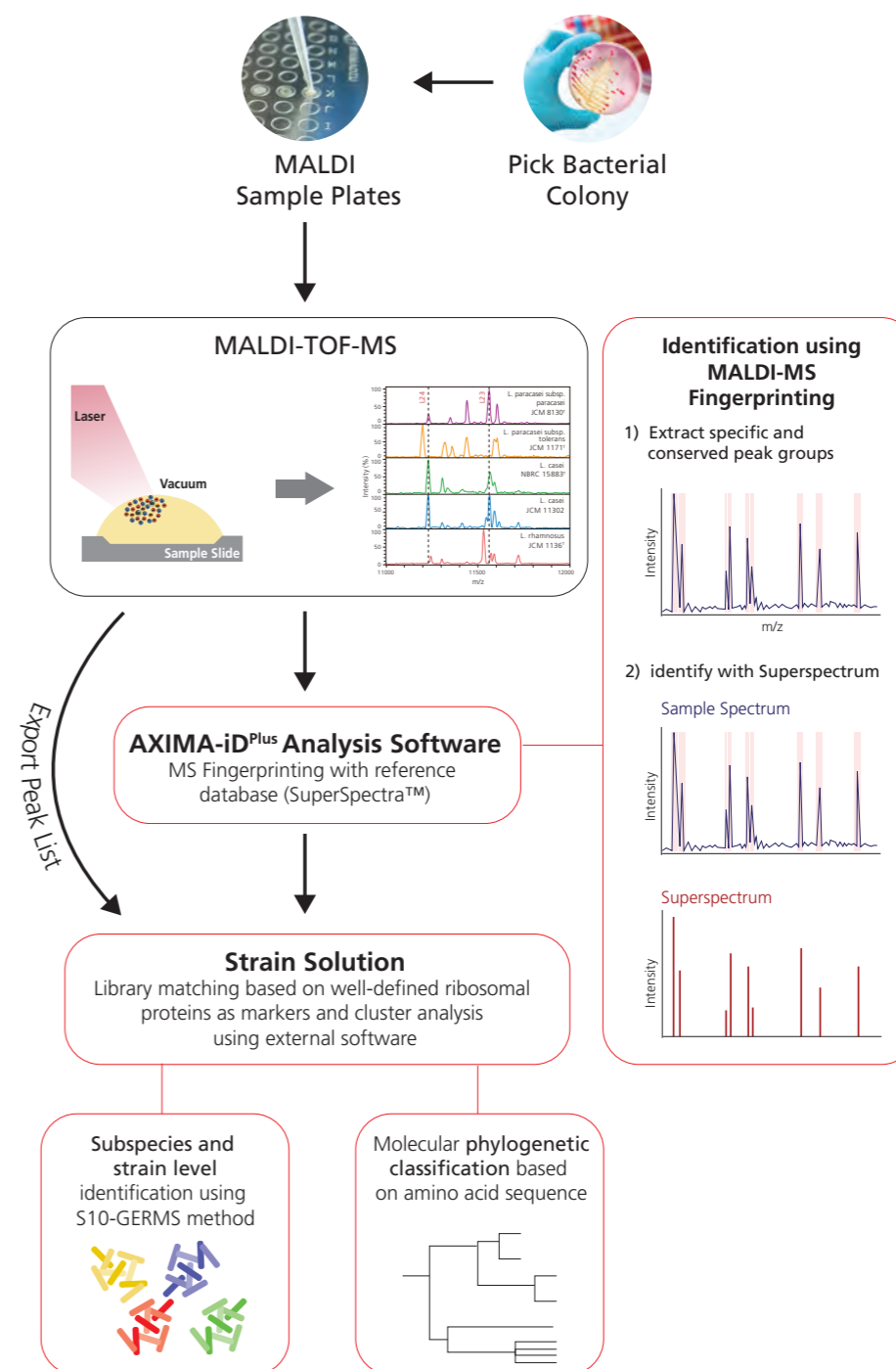0.00    0.05    min

Area: 31,563
RSD:  0.7 %
50 µg/mL

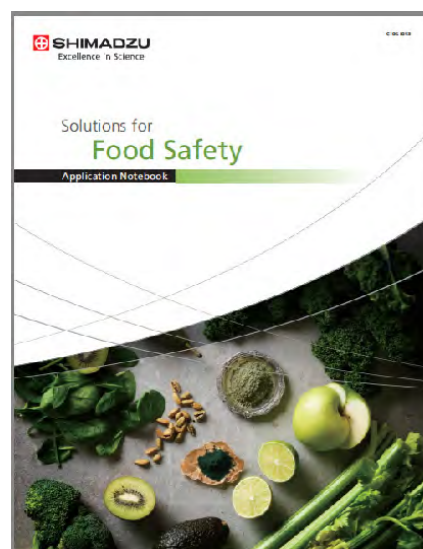Click to read more on: Ultra-high-speed Analysis of Melamine Powdered Milk using LDTD-MS/MS

# Strain Solution with AXIMA series

✓ **Strain level identification using S10-GERMS method**

Microbial identification using Shimadzu's AXIMA Series MALDI-TOF-MS allows rapid analysis with minimal sample preparation and reagents. Together with the AXIMA-ID[plus] software, consistent species-level identification can be conducted using SARAMIS microorganism identification. For detailed identification at the species and strain level, Shimadzu's Strain Solution Software facilitates proteotyping based on the S10-GERMS method, discriminating single amino acid mutations.
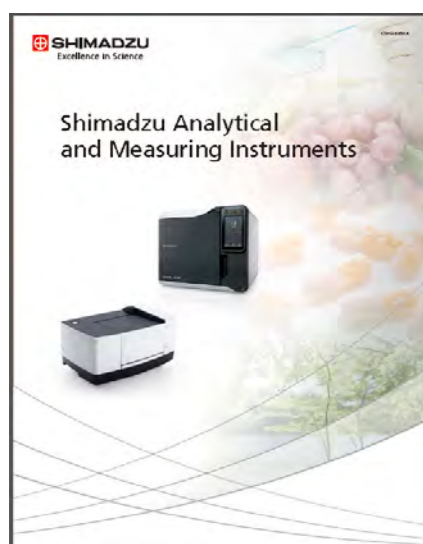
MALDI
Sample Plates

Pick Bacterial
Colony

MALDI-TOF-MS

Laser

Vacuum

Sample Slide

Sample Slide

L. paracasei subsp.
paracasei
JCM 8130[T]
L. paracasei subsp.
tolerans
JCM 1171[T]
L. casei
NBRC 15883[T]
L. casei
JCM 11302
L. rhamnosus
JCM 1136[T]

**Identification using MALDI-MS Fingerprinting**

1) Extract specific and conserved peak groups

Intensity

m/z

2) identify with Superspectrum

Sample Spectrum

Intensity

Superspectrum

Intensity

Export Peak List

**AXIMA-iD[Plus] Analysis Software**
MS Fingerprinting with reference database (SuperSpectra™)

**Strain Solution**
Library matching based on well-defined ribosomal proteins as markers and cluster analysis using external software

**Subspecies and strain level** identification using S10-GERMS method

Molecular **phylogenetic classification** based on amino acid sequence
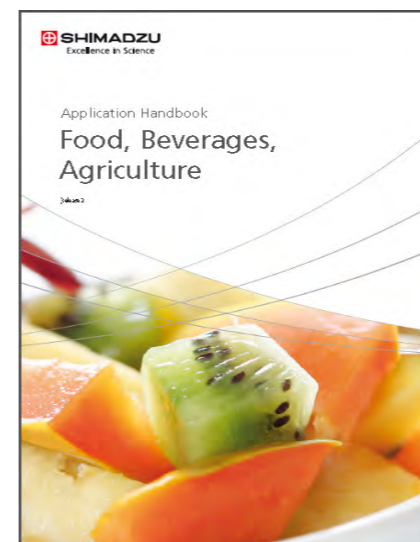
## Solutions for Food Safety

A compilation of application notes targeted for the analysis of pesticides, veterinary drugs, preservatives and more in food.
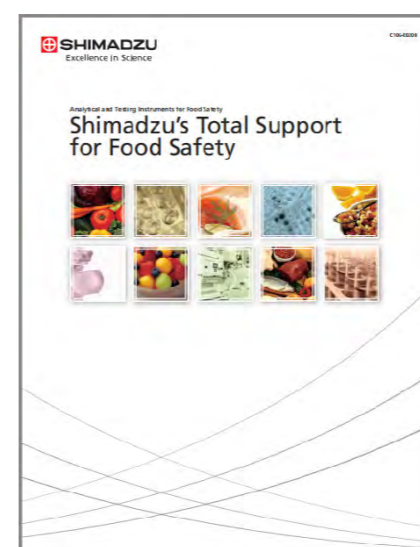
## Application Handbook
## Food, Beverage, Agriculture

A compilation of applications focusing on food safety and quality.

## Shimadzu Analytical and Measuring Instruments

A comprehensive booklet on service and support for chromatograhy, spectroscopy, X-ray and surface analysis apparatus and more.

## Shimadzu's Total Support for Food Safety

A full range of analytical instruments specially catered to accelerate productivity, enhance sensitivity and accuracy for food safety compliance.

## Shimadzu Innovation Centers and MS Centers

Strengthening our ability to listen closely to customers - this is what Shimadzu has been focusing on. Shimadzu is continuously expanding its innovation centers and MS centers worldwide to strengthen our insight into customers' needs and contribute to collaborative researches.

Healthcare    Enviromental    Chemical    Food    Composite    Environmental    Imaging
              Energy                              Material



European Innovation Center

China MS Center

Asia Pacific
Innovation Centre

US Innovation Center

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R<sup>G</sup> ResearchGate

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# SHIMADZU

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