Mycotoxins and Other Natural Toxins Testing

Application Notebook

Waters™

Introduction

EFFICIENT ANALYTICAL SOLUTIONS TO SUPPORT THE SAFETY OF FOOD, FEED, AND WATERS SUPPLIES

Welcome to Waters Natural Toxins testing application notebook. Many foods naturally contain chemicals that are toxic to a greater or lesser degree. Drinking water and surface waters can also become contaminated with algal toxins. The toxicity of these compounds has adverse effects on humans and animals, which consequently led to the generation of regulatory frameworks for control of natural toxins in food, drinking water, and in the environment. Inside this eBook you will find a compilation of our scientists' application notes, supporting your development and implementation of new testing methods and technologies for the determination of natural toxins:

- Mycotoxins
- Plant toxins
- Marine biotoxins
- Freshwater biotoxins

Suppliers of food and drinking water have always tried to balance quality and safety assurance with economy and efficiency, but in the context of an increasingly globalized food supply, climate change and rising concern about food- and waterborne illnesses, the need for natural toxin test methods that help companies do more with less has taken on a new urgency. Whether you are a manufacturer operating an internal testing laboratory, a contract testing laboratory, or a government laboratory, Waters can help you expand your capabilities to include a complete portfolio of natural toxin testing solutions.

Mycotoxins are toxic compounds that are naturally produced by different types of fungi. They enter the food chain due to infection of crops and are typically found in foods such as cereals, dried fruits, nuts, and spices. They have well established health impacts both in humans and animals that have been fed contaminated feed. The most common mycotoxins are regulated in many countries, typically including values for maximum allowed levels in various food and feed commodities. These are very low in concentration due to their severe toxicity. There are many other chemically diverse mycotoxins for which no current regulations exist and the co-occurrence of multiple mycotoxins in food and feed samples is a growing matter of concern.

In addition to risk to public health, mycotoxins generate high levels of economic loses for the food industry due to reduced crop yields, lost trade revenues due to failure to comply with regulatory limits, and livestock illnesses.

Mycotoxin contamination can occur pre-harvest when the crop plant is growing or post-harvest during processing, packaging, distribution, and storage of food products, so the timing of the analysis is vitally important and constitutes a major challenge for farmers, processors, and distributors of agricultural commodities. Consumers and companies in the retail sector might be more concerned about the contamination levels to be determined in finished products on sale.

Plant toxins can occur naturally in edible crops, while others enter the food chain due to contamination of edible crops with weeds, both of which can have an impact on human health. Some toxins occur only in specific plant genera or are even species specific, others are present in several plant families.

Some species of marine phytoplankton produce biotoxins that can accumulate within the shellfish and fish that feed on them. These groups of toxins are classified by the type of resulting food poisoning in humans:

- Paralytic Shellfish Poisoning (saxitoxin and its analogues)
- Diarrhetic Shellfish Poisoning (okadaic acid, azaspiracid, yessotoxins and pectenotoxins) – also called Lipophilic Marine Biotoxins
- Amnesic Shellfish Poisoning (domoic acid)

Tetrodotoxin is another extremely potent marine biotoxin, which has been found to accumulate in the tissues of shellfish and some fish.

Many species of cyanobacteria, often associated with bodies of freshwater, produce secondary metabolites, some of which are toxic to higher organisms and pose a risk to human health when water is ingested. Over 100 different microcystins have been reported to date, of which microcystin-LR is the most common.

CONTENTS

Mycotoxins in Food

Rapid Testing for Six Major Mycotoxins in Agricultural Commodities by Lateral Flow Device

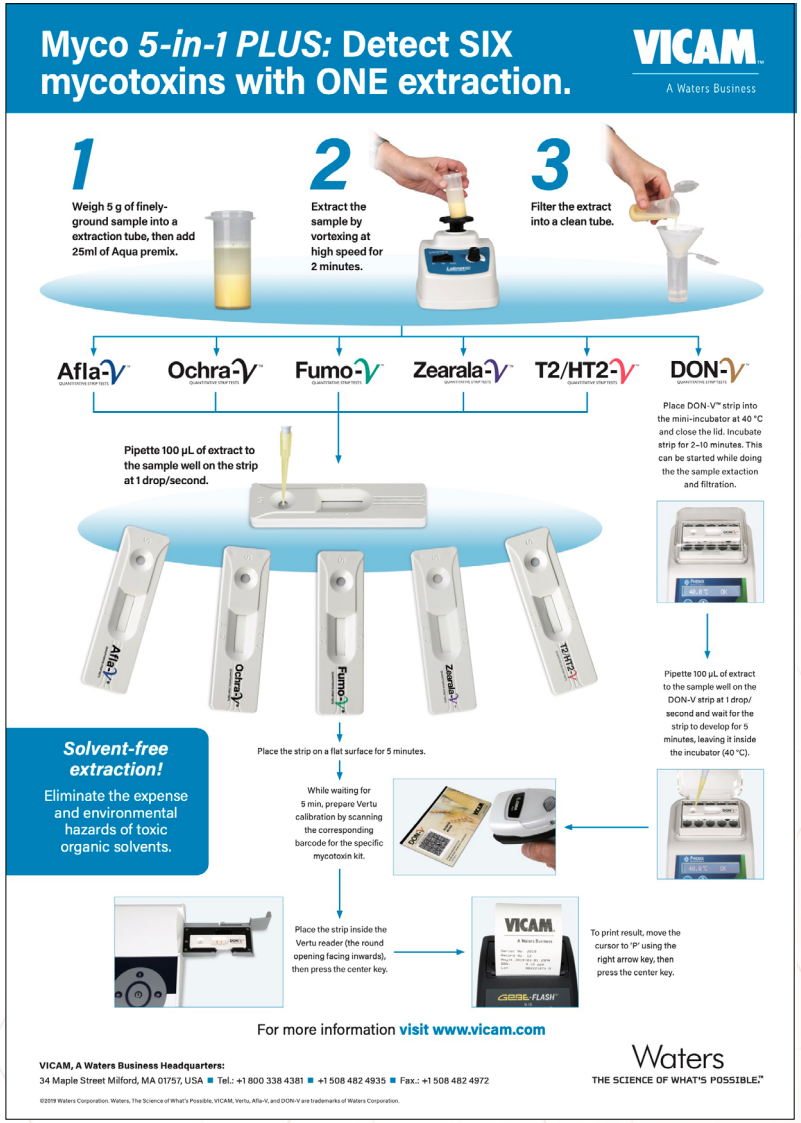
Upstream monitoring for the major mycotoxins is carried out on raw commodities, to make decisions on contamination of crops at harvest and various points along the supply chain or to support export shipment release. Lateral flow strip tests possess the advantages of easy operation and provide accurate, on-time quantitative data to help prevent the introduction of mycotoxins into raw commodity and finished product value streams. Testing needs to be accurate but fast so that large numbers of tests can be undertaken in a cost-effective manner.

The VICAM™ portfolio of quantitative strip tests, which are used in conjunction with the Vertu™ PREP and Vertu TOUCH Lateral Flow Reader, provide monitoring solutions for companies seeking to minimize the costly consequences of mycotoxin contamination. For example, grain handlers and processors need to adopt rapid quantitative testing strategies for multiple toxins in the same sample. VICAM's Myco 5-in-1 PLUS strip tests can deliver results for up to six groups of mycotoxins from a single extract. When the method was validated in corn, results for all six mycotoxin groups showed trueness between 80% and 138% and repeatability ≤22% RSD, without any false positives.

BENEFITS

- VICAM's AQUA range use water-based extraction with no or little organic solvent waste, while still providing sensitive, accurate results.
- Many of VICAM's lateral flow strip tests are approved by USDA-FGIS.
- The Myco 5-in-1 PLUS tests delivers accurate, quantitative results for up to six mycotoxins groups in just 10 minutes, with just one extraction.

Read the Whitepaper on Onsite Testing



Individual isomer peak integration of branched (yellow) and linear (black) PFHxS with the total isomer concentration calculated automatically in MS Quan to support reporting requirements.

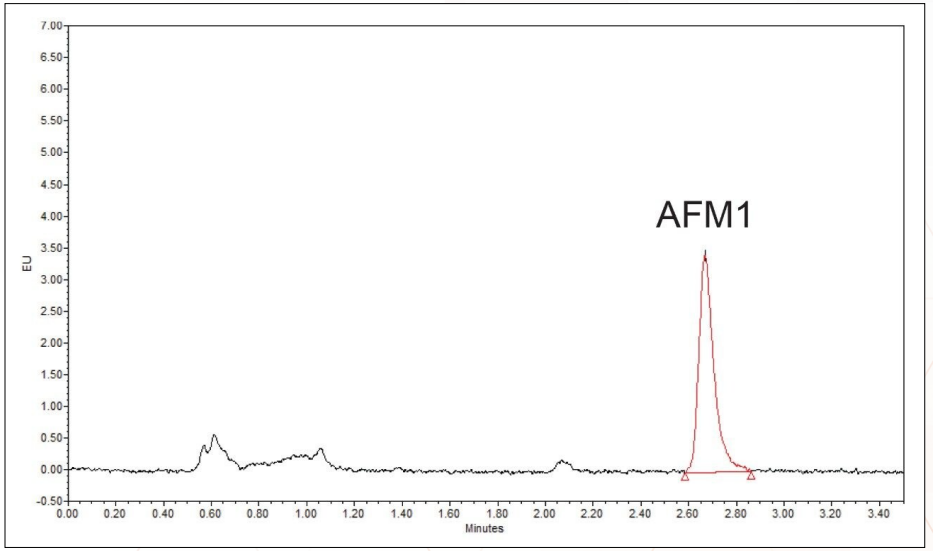
Determination of Aflatoxin M1 in Milk by UPLC With Fluorescence Detection After Immunoaffinity Chromatography Clean-Up

Aflatoxin M1 (AFM1) is a metabolite of aflatoxin B1 (AFB1), which is present in the milk of animals that ingest feed contaminated with AFB1. A method has been developed for the highly sensitive and selective determination of AFM1 in milk. Samples were prepared by centrifuging the milk, separating out and removing the fat layer. The skim portion was then applied to the VICAM Afla M1 IAC Column, which contains specific antibodies that selectively bind to AFM1. Once AFM1 is bound to the antibody on the column, the column was washed to remove matrix components and AFM1 eluted from the column. The AFM1 concentration was determined using an ACQUITY™ UPLC H-Class PLUS System with fluorescence detection. The performance of the method was evaluated through replicate analysis of spiked test portions. The limit of detection for this procedure was 0.005 µg/kg (ppb). Overall recovery was shown to be satisfactory, greater than 80%, with repeatability <10% RSD. The method was found to be specific as no interference peaks were observed for blank samples.

Read the Full Application Note

BENEFITS

- The application note describes a modification of an established AOAC official method.
- UPLC offers a quicker analysis time than with HPLC, decreasing the run time from about 10 to 3.5 minutes.
- Method is suitable for checking compliance with regulatory limits in milk in various parts of the world.



Chromatogram from analysis of AFM1 standard at 0.025 ng/mL.

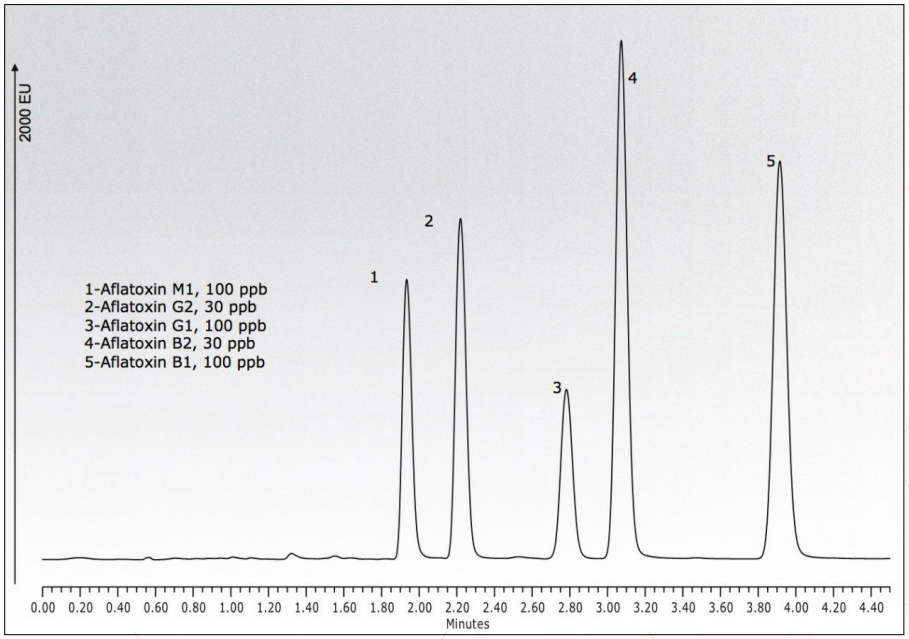
Determination of Aflatoxins in a Range of Commodities by UPLC with Fluorescence Detection After Immunoaffinity Chromatography Clean-Up

Aflatoxins are carcinogenic mycotoxins that have adverse health effects on both humans and animals consuming contaminated food and feed, respectively. A method has been developed for the highly sensitive and selective determination of regulated aflatoxins in a wide range of commodities. The extraction of aflatoxins from representative commodities of interest (nutmeg, red chili, black pepper, cocoa, roasted coffee, dog food, and a traditional Chinese medicine) was performed using liquid-liquid extraction and then immunoaffinity chromatography (IAC) clean-up on the AflaTest™ WB SR+ Column. Chromatographic separation was demonstrated using both HPLC (Alliance™) and UPLC (ACQUITY UPLC H-Class PLUS) systems, using fluorescence detection, supported with post-column derivatization and without derivatization, using a large flow cell, respectively. The performance of the method was evaluated through replicate analysis of spiked test portions of seven different matrices. Overall recovery was shown to be satisfactory, between 82% and 119%, with repeatability <8% RSD. The method was found to be specific as no interference peaks were observed for blank samples. The method has been demonstrated as suitable for monitoring compliance with regulatory limits set for aflatoxins in food commodities globally.

Read the Full Application Note

BENEFITS

- The method is suitable for analysis of a range of agricultural, botanical and food commodities for checking compliance with regulatory limits in many parts of the world.
- There is no need for the complicated post-column derivatization step when using the ACQUITY UPLC System.
- UPLC offers improved sensitivity, resolution, and speed (5 minutes compared to 12 minutes using HPLC).



UPLC chromatograms for aflatoxins without derivatization.

Determination of Aflatoxins and Ochratoxin A in Complex Commodities by LC-MS/MS After Immunoaffinity Chromatography Clean-Up

Aflatoxins and ochratoxin A can occur naturally in a variety of food products via pre- and post-harvest contamination mechanisms and are regulated worldwide. This application note describes three different methods using IAC columns and LC-MS/MS (ACQUITY UPLC I-Class and Xevo™ TQ-S cronos), for analysis of specific, challenging food commodities:

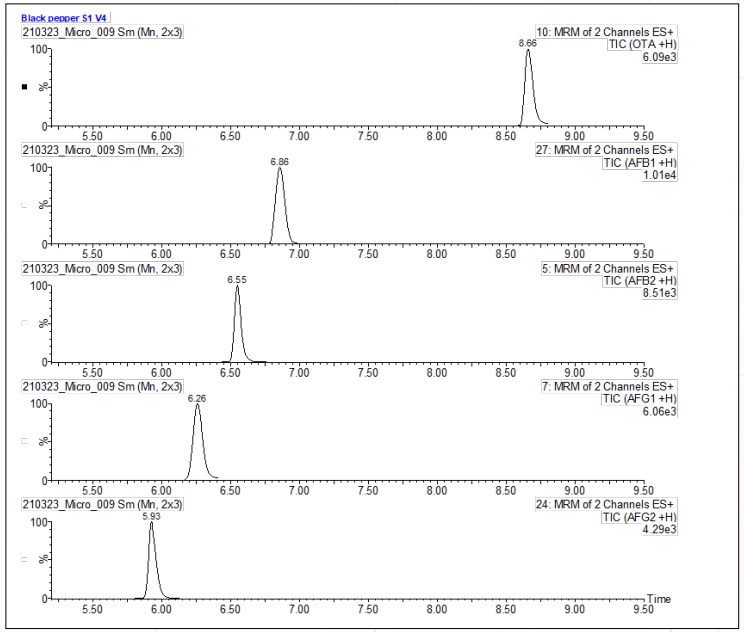
- Aflatoxins in groundnuts, pistachio, and hazelnuts using Aflatest WB IAC Column
- Ochratoxin A in roasted coffee and cocoa using OchraTest WB IAC Column
- Aflatoxins and ochratoxin A in black pepper using AflaOchra IAC Column

The performance of all three methods was evaluated using spiked samples as well as naturally contaminated reference materials, where available. The methods provide optimal performance in terms of trueness and repeatability, with method limit of quantitations (LOQs) as low as 0.050 µg/kg for aflatoxins and 0.4 µg/kg for ochratoxin A. Recoveries were within the range 71–108% (mean 90%) due to the highly specific antibody-based binding, and repeatability was between 0.5% and 5% RSD, thus making it suitable to be coupled with LC-MS/MS for analysis of challenging commodities.

Read the Full Application Note

BENEFITS

- Highly selective methods for targeted mycotoxins in complex commodities allows the use of a low-tier tandem quadrupole mass spectrometer (MS/MS).
- There is no need for internal standards at any point of the sample preparation or matrix-matched calibration standards; solvent standard can be used for a reliable quantitation.
- The performance of the method shows it is suitable for checking compliance with maximum levels for aflatoxins and ochratoxin A in the commodities tested.



Chromatogram of black pepper spiked at 0.5 µg/kg of aflatoxins (each) and 2 µg/kg of ochratoxin A.

Determination of Multiple Regulated Mycotoxins in Cereals by LC-MS/MS

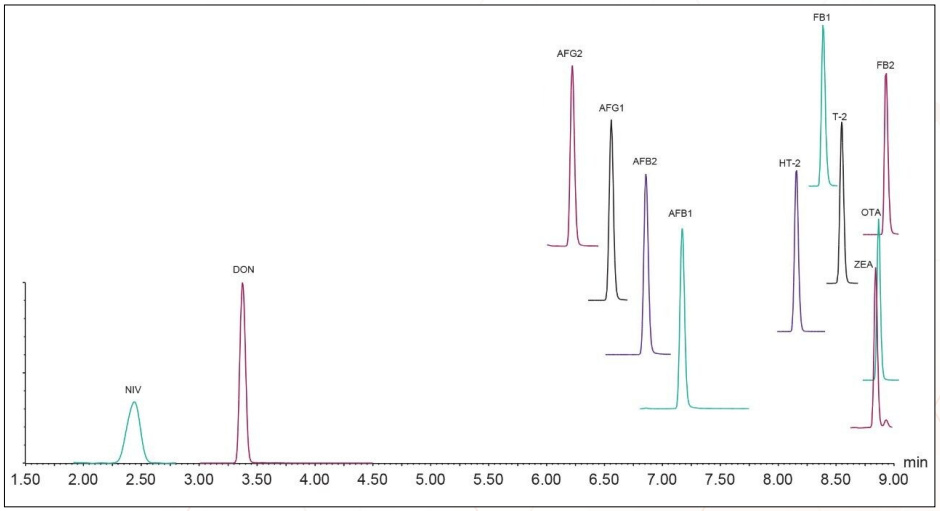
Multi-toxin methods, using LC-MS/MS, provide a more comprehensive view of the type and level of contamination of a wide range of mycotoxins and associated metabolites known as modified mycotoxins. A simple, highly sensitive "dilute and shoot" approach was developed and validated, based upon determination using ACQUITY UPLC I-Class and Xevo TQ-XS. It uses 13C-labeled analogues as internal standards to correct for both matrix effects and analyte losses during the sample extraction. The method has been be applied to the determination of 12 common mycotoxins in wheat flour, oatmeal (ground oats) and to a gluten-free mix of different flours (rice, potato, tapioca, maize, and buckwheat).

The performance of the methods was evaluated using test portions of a sample of wheat flour, spiked at three concentrations. The mean apparent recoveries were all within the range 95–105%, whereas repeatability was shown to be ≤9% RSD.

Read the Full Application Note

BENEFITS

- The application note describes a simple "dilute and shoot" approach with no need for clean-up.
- The use of labelled internal standards negates the need for matrix-matched calibration.
- The performance described shows the method is suitable for checking compliance with regulatory limits for mycotoxins in cereal grain commodities for the EU and elsewhere.



Chromatograms from analysis of matrix-matched calibrant in oat extract at 10x LOQ (which vary for each mycotoxin).

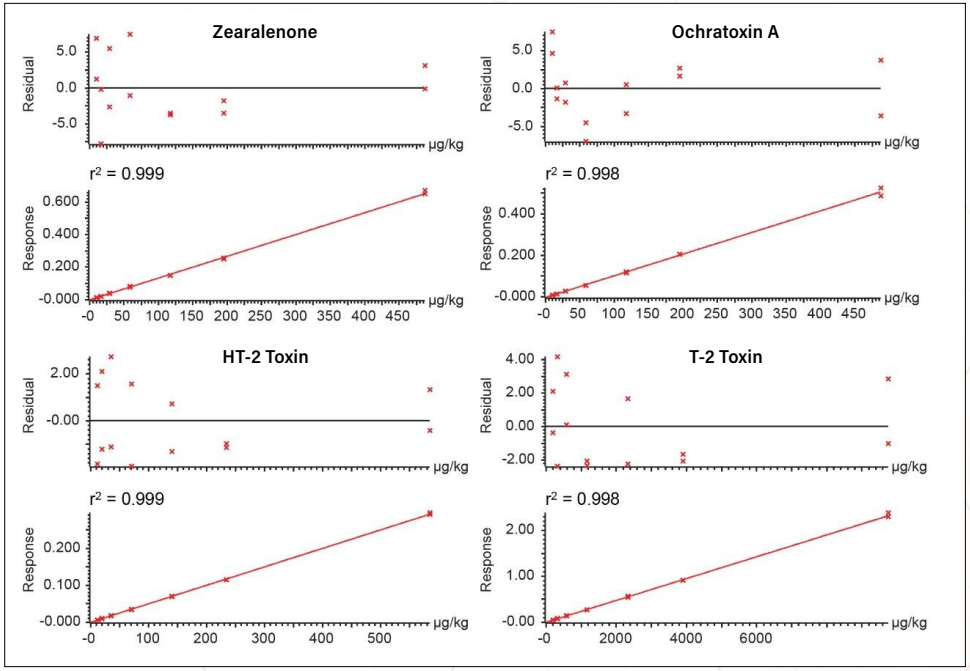
Evaluation of the Performance of a Method for Regulated Mycotoxins using an Interlaboratory Study

Waters previously reported the development and single laboratory validation of a method for the determination of the 12 mycotoxins regulated in the EU in various cereals based upon LC-MS/MS after a simple generic extraction method without any clean-up. This application brief shows the successful evaluation of the performance of this method by interlaboratory study. Two cereal FAPAS QC materials were sent to four laboratories in Europe and the USA. Each material was analyzed in triplicate by the four laboratories. The laboratories demonstrated good accuracy and precision for the determination of the 8 mycotoxins in the two FAPAS QC materials. Reported concentrations matched the assigned values provided by FAPAS. Trueness was within the range of 85% to 113%, the within-laboratory repeatability was between 3.0% to 13% and between laboratory reproducibility was between 3.1% and 23%.

Read the Full Application Note

BENEFITS

- The study shows that the method could be easily transferrable to different laboratories.
- The performance of the method, sensitivity, trueness and precision, was verified using independent reference materials.
- The results further demonstrates the method is suitable for the monitoring of mycotoxins in cereals for both official control and testing conducted by food business operators.



Typical calibration graphs for some of the 12 regulated mycotoxins.

Determination of Multiple Mycotoxins in Various Commodities by LC-MS/MS After SPE Clean-Up

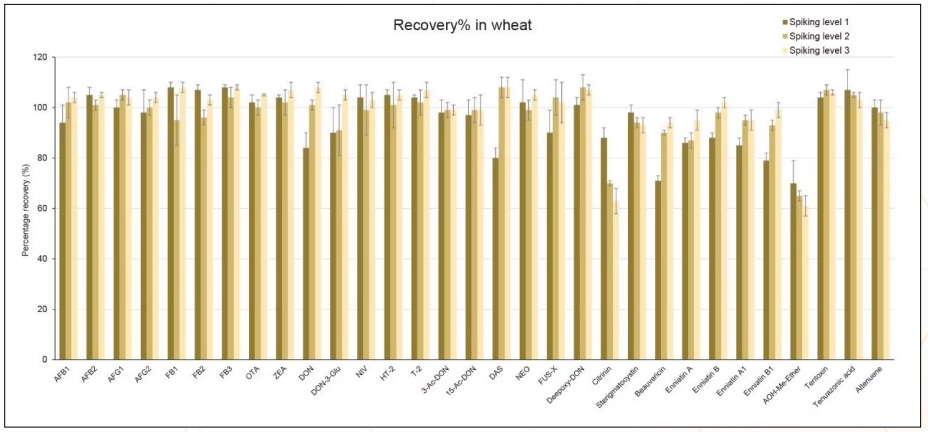
This work describes a multi-toxin method for a total of 31 regulated and emerging mycotoxins using a quick and simple pass-through SPE clean-up prior to LC-MS/MS (ACQUITY UPLC I-Class and Xevo TQ-XS). The method was applied to the analysis of cereal-based products, ground- and tree-nuts, dried figs, and animal feeds. It was developed to improve robustness, avoid the use of internal standards and hence maximize absolute recovery, whilst enhancing ease-of-use. The use of Oasis™ PRiME HLB SPE clean-up involves a simple and quick protocol, which retains the co-extractives whilst allowing the analytes to pass through.

The performance of the method has been validated using spiked samples and verified using reference materials, where available. Matrix-matched standards were used for quantification. The method is sensitive with LOQs as low as 0.25 µg/kg for aflatoxins and 1.0 µg/kg for ochratoxin A. Recoveries were in the range 60–108% and repeatability <11% RSD. Trueness and repeatability were both also successfully assessed using analysis of independent reference materials.

Read the Full Application Note

BENEFITS

- The application note describes the determination of multiple mycotoxins in a wide range of commodities using a single method.
- The performance of the method fulfils the criteria set by the European Regulation No 401/2006 and SANTE guidelines.
- A simple and quick pass-through SPE clean-up was incorporated and allows the effective removal of some major interferences thus improving method robustness.



Graph showing mean recoveries and repeatability for the 31 mycotoxins in wheat flour.

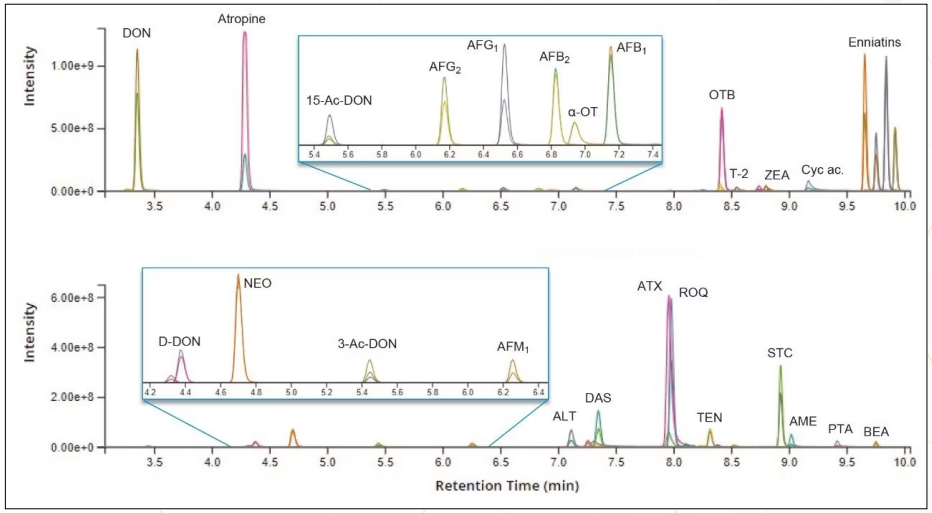
Determination of Multiple Mycotoxins and Plant Toxins in Cereals by LC-MS/MS

In this application note we describe the performance of a multi-toxin UPLC-MS/MS method for 50 regulated and emerging mycotoxins and two plant toxins, atropine and scopolamine, in cereal-based products. The high sensitivity tandem quadrupole mass spectrometer, the Xevo TQ-XS, was used in combination with an ACQUITY UPLC I-Class PLUS to reach very low limits of detection and quantification. A mixture of cereal flours was extracted using a simple "dilute-and-shoot" protocol, without any use of clean-up or internal standards. Matrix effects were calculated for all compounds and were found to be significant, illustrating the need for matrix-matched calibration. Coefficients of determination (R2) from matrix-matched calibration curves were almost all >0.99 with residuals <20%. The LOQ was for aflatoxins (0.1 µg/kg). The method fulfilled the identification criteria set out in the SANTE guidelines for mycotoxins.

Read the Full Application Note

BENEFITS

- The application note describes the simultaneous determination of more than 50 mycotoxins and plant toxins in a single LC-MS/MS method.
- The high sensitivity of the Xevo TQ-XS allows considerable dilution of the sample extract while still reaching extremely low LOQs.
- The performance of the method demonstrates it to be suitable for checking compliance with regulatory limits and to investigate the levels of other toxins for risk assessment purposes.
- The MS Quan application in waters_connect™ for quantitation software, reduces the time taken to review results by up to 50%.



Chromatograms from the analysis of a spiked cereal sample.

Plant Toxins

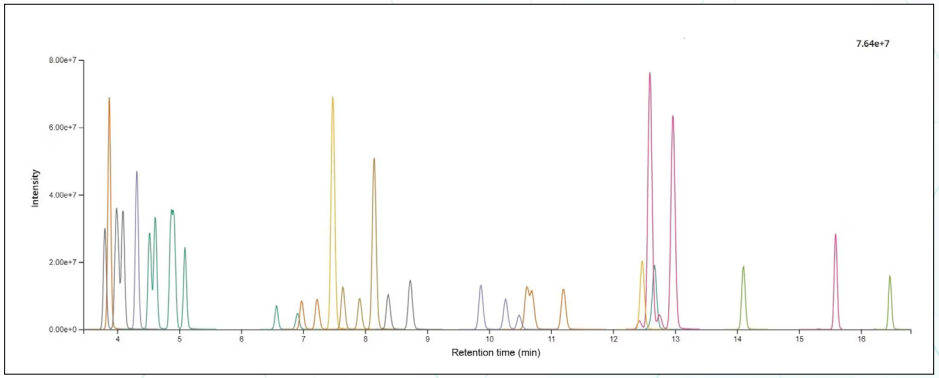
Determination of Pyrrolizidine Alkaloids in a Range of Plant-Based Foods and Honey Using LC-MS/MS

Pyrrolizidine alkaloids (PAs) are toxins exclusively biosynthesised by plants. Expressing both genotoxic and carcinogenic properties, an increasing number of reports reveal relatively high contaminations with PAs in food, herbal infusions, and teas. This application note describes an analytical method for the determination of PAs in plant-derived product (e.g. botanicals such as tea, herbs, spices, and cumin seeds) and honey. Samples were extracted with a sulfuric acid solution, cleaned up using Oasis MCX SPE cartridges, concentrated and resuspended prior to LC-MS/MS analysis (ACQUITY UPLC I-Class PLUS and Xevo TQ-S micro). The chromatographic resolution of critical pairs of isomers was addressed in this study. Good method recoveries and excellent repeatability were obtained, which complied with the acceptance criteria in the CEN standard for single laboratory validation. The LOQ for individual compounds were 0.6 µg/kg, which were shown to exceed regulatory compliance, such that the method can also be applied to food intended for infants and young children.

Read the Full Application Note

BENEFITS

- The application note describes a method for the determination of 35 PAs in plant-based foods and honey suitable for checking EU regulatory compliance.
- Removal of major co-extractives using Oasis MCX SPE clean-up decreases the amount of isobaric interference.
- RADAR™, a unique feature of Xevo tandem quadrupole mass spectrometers, can be used to simplify and accelerate the development of robust methods.
- The method recoveries and repeatability complied with the acceptance criteria in the CEN standard for single laboratory validation.

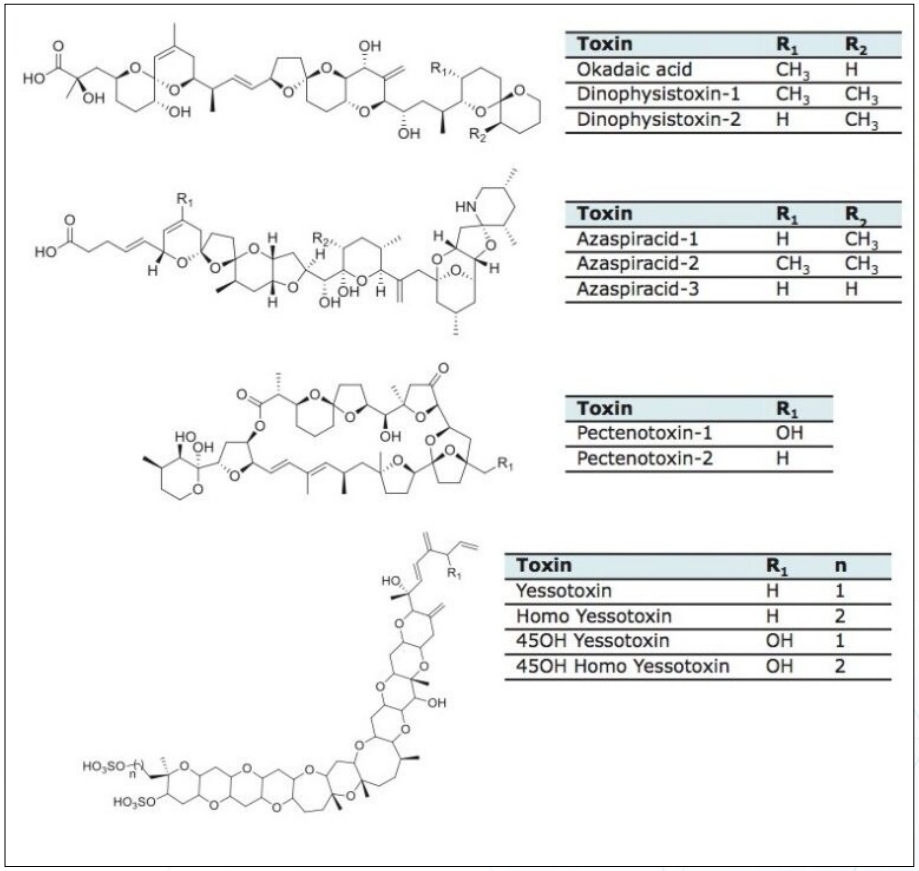


Chromatograms from the analysis of the matrix-matched standard in tea at 250 µg/kg.

Marine Biotoxins

Determination of Lipophilic Marine Biotoxins in Shellfish by LC-MS/MS

This application note describes a robust, rapid method for the analysis of both regulated and non-regulated lipophilic marine biotoxins in shellfish. Due to their lipophilic properties, Diarrhetic Shellfish Poisoning toxins are often classified as lipophilic marine biotoxins. This group of toxins comprises many compounds with a variety of physiochemical properties, such as carboxylic acids, sulfonic acids, and amino and imino functionalities. The aims of this study were to produce a much faster routine analysis than the conventional HPLC method under alkaline conditions and to include additional non-regulated compounds that are of interest for risk assessment purposes. Homogenized shellfish tissue was extracted with methanol, centrifuged and toxins determined by LC-MS/MS; ACQUITY UPLC System coupled with a Xevo TQ-S Tandem Quadrupole Mass Spectrometer. The method, which could be easily transferred to current Xevo TQ platforms, was sensitive enough to detect all the toxins, even at levels of 0.125x the various maximum levels. For all regulated toxins, the recovery was between 99% to 103%, with repeatability <10% RSD. This method is approved for use to check compliance with regulatory maximum levels for the lipophilic toxin group instead of the mouse bioassay in the EU.



Toxin	R_1	R_2
Okadaic acid	CH_3	H
Dinophysistoxin-1	CH_3	CH_3
Dinophysistoxin-2	H	CH_3

Toxin	R_1	R_2
Azaspiracid-1	H	CH_3
Azaspiracid-2	CH_3	CH_3
Azaspiracid-3	H	H

Toxin	R_1
Pectenotoxin-1	OH
Pectenotoxin-2	H

Toxin	R_1	n
Yessotoxin	H	1
Homo Yessotoxin	H	2
45OH Yessotoxin	OH	1
45OH Homo Yessotoxin	OH	2

Chemical structure of the lipophilic marine biotoxins regulated in shellfish in the EU.

BENEFITS

- The application note describes a rapid, robust method for the analysis of both regulated and non-regulated lipophilic marine biotoxins in shellfish.
- UPLC offers significant improvements in the speed of analysis compared to HPLC.
- It can be used as reference method for the detection of lipophilic toxins and used as matter of routine, both for the purposes of official controls at any stage of the food chain and own-checks by food business operators.

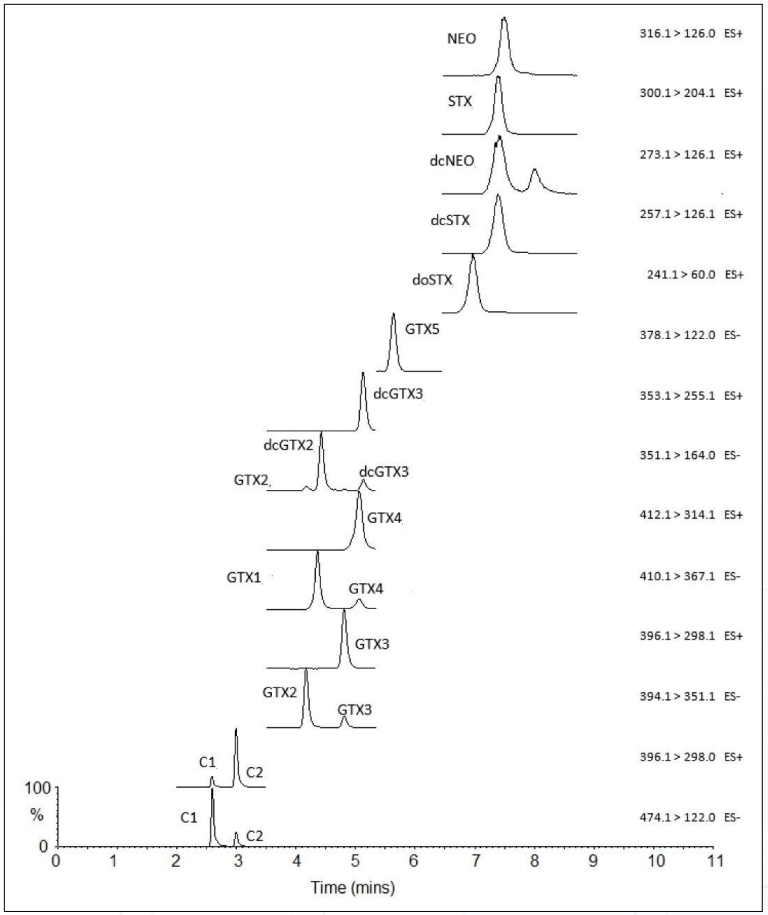
Read the Full Application Note

Determination of Paralytic Shellfish Toxins and Tetrodotoxins in Shellfish by LC-MS/MS

The ACQUITY UPLC I-Class System with the Xevo TQ-S Tandem Mass Spectrometer provided excellent sensitivity for detection, identification, and quantification of paralytic shellfish toxins (PST) and tetrodotoxin in shellfish tissues. A simple single-step extraction of shellfish tissues in weak acetic acid prior to graphitized carbon solid phase extraction provided good recoveries for the compounds of interest and effective removal of co-extractives and salts, the source of significant matrix suppression during the analysis of shellfish by LC-MS/MS. Separation is achieved using hydrophilic interaction liquid chromatography (HILIC) on the BEH™ Amide Column. The Xevo TQ-S exhibited excellent sensitivity and robustness, but this same method could easily be transferred to other Xevo Tandem Quadrupole MS/MS instruments to meet the same performance criteria or extracts diluted further prior to UPLC-MS/MS.

BENEFITS

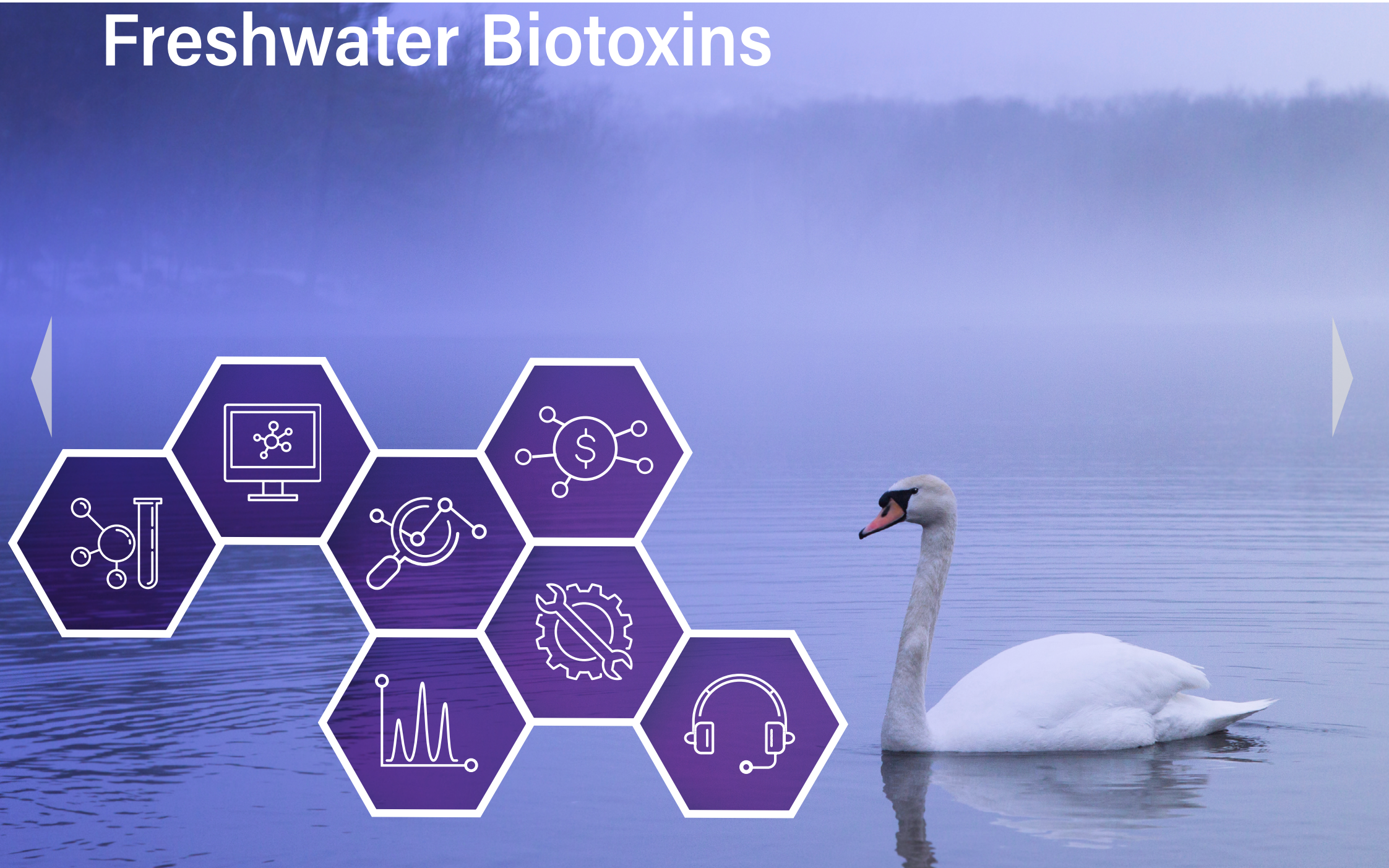
- This work represents a simple, rapid and cost-effective method for the determination of a range of hydrophilic marine biotoxins in shellfish tissue samples.
- This method is suitable for use to check compliance with regulatory maximum levels for the PST group instead of the HPLC reference method.
- It can be used for both screening and confirmation for the purpose of shellfish food safety testing for both official control and by the shellfish industry.



Chromatograms showing PSTs from the analysis of high-level calibration standard.

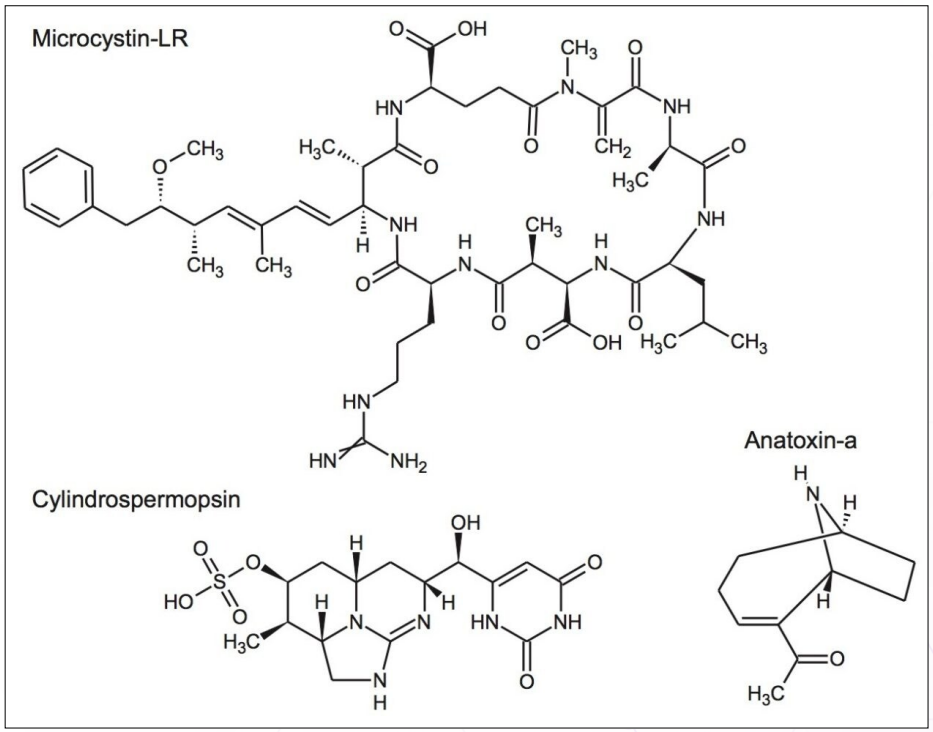
Read the Full Application Note

Freshwater Biotoxins



Determination of Cyanotoxins, Including Microcystins, in Drinking and Surface Waters by LC-MS/MS

Cyanobacteria (blue-green algae) are photosynthetic organisms found in both marine and freshwater environments, many species of which produce secondary metabolites, some of which are toxic to higher organisms. This application note describes a method for the analysis of drinking and surface waters for 10 well-known cyanotoxins; cylindrospermopsin, anatoxin-a and microcystins nodularin, microcystin-LR (MC-LR), MC-DeRR, MC-RR, MC-YR, MC-LY, MC-LW, and MC-LF. The method, using direct injection of water samples onto the ACQUITY UPLC I-Class System coupled to Xevo TQ-S Tandem Quadrupole Mass Spectrometer, was sufficiently sensitive to detect microcystins and the other cyanotoxins at concentrations well below the WHO provisional guideline of 1 µg/L for MC-LR. This method can be transferred to other current Xevo TQ platforms such as Xevo TQ-S micro.[1]



Structures of the three types of cyanotoxin determined in this study.

BENEFITS

- Direct injection negates the need for expensive and time-consuming sample extraction.
- Sensitive detection at sub-ppb levels allows the method to be used to check the compliance with any guidance or mandatory limits in both drinking and surface waters.
- The use of standard addition for quantification mitigates for any matrix effects and avoids the need for expensive internal standards.

[1] *Sensitive Analysis of Nodularin and Microcystins of Concern in Drinking Water Using Simplified Sample Preparation*

Read the Full Application Note

Targeted and Untargeted Screening for Microcystins in Lake Water Samples Using High Resolution Mass Spectrometry (HRMS)

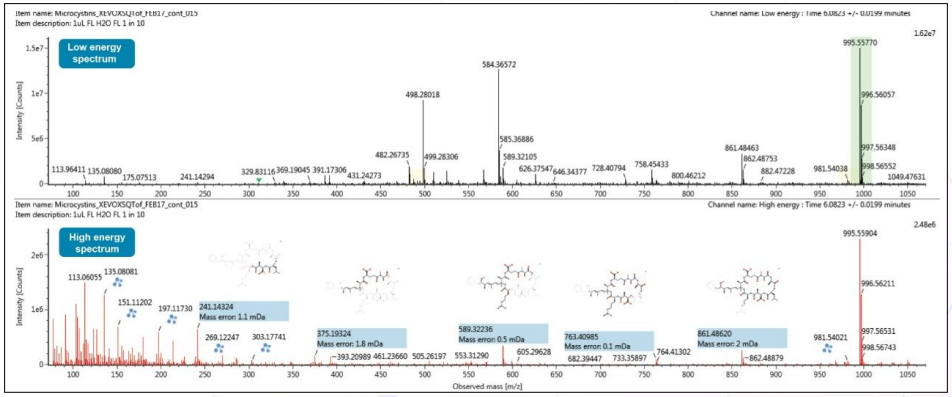
Methods based upon LC-MS/MS in MRM mode are restricted in scope to the compounds included in the acquisition method and for which reference standards are commercially available. In such cases data is available only on toxins monitored rather than toxins present so there can be considerable bias in what has been reported. Non-targeted approaches, based upon comparison of experimental data with those derived from empirical formulae and structures of likely toxins, provides an opportunity for the detection of a much greater number of cyanotoxins in water samples and algal blooms.

This application describes the use of HRMS on a Xevo G2-XS QTof Mass Spectrometer using data independent acquisition coupled with both targeted and non-targeted data analysis. A 2D-UPLC ACQUITY System, in trap and elute mode for injection of large volumes of water, improves sensitivity so that MC-LR could easily be detected at 0.001 µg/L (1 ppt) in drinking water (500 µL injection). Extending the target list to include likely ions for a further 82 microcystins resulted in detection of 44 additional microcystins. Structure elucidation of some remaining unknown microcystins was made possible by matching the experimental masses with the possible microcystin amino acid configurations within a specific mass range and fragmentation prediction software.

Read the Full Application Note

BENEFITS

- This application note describes the use of direct analysis of water by UPLC-HRMS to quantify specific targeted microcystins to check compliance with regulatory current limits.
- It also provides a non-targeted approach to analysis of toxins in water including retrospective data review and the opportunity to search for a wide range of microcystins using in-silico tools.
- Significant improvements in sensitivity and degree of automation can be achieved using the UPLC System in a 2D-UPLC configuration.



Identification of microcystin-LR detected in a lake sample from MS^E mass spectra and in silico fragmentation using the compound's structure.

Links to Other Useful Materials

[Carryover in UPLC Methods: The Case Study of Fumonisin B2]

- Comprehensive description of the types of carryover including an evaluation of different autosampler configurations and injection modes.
- Describes a stepwise protocol to locate the source of carryover and a detailed description of mitigation actions.

[Improving the Robustness of an LC-MS/MS Mycotoxins Method]

- Clean-up reduces the introduction of unwanted co-extractives into the system, mitigates the impact of matrix effects and reduces both the contamination of the quadrupoles and hence the rate at which charging effects occur and the frequency of intervention for maintenance.

[The Benefits of ACQUITY Premier UPLC for Mycotoxin Testing]

- The ACQUITY Premier System and column effectively reduce carryover of fumonisins compared to conventional UHPLC systems, negates the need for the addition of metal chelators in the mobile phase or washing solutions and improves throughput as the number of washing cycles can be reduced.

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