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Technical Report

Analysis of Pesticides in Food Matrix using QuEChERS by Triple Quadrupole GC/MS/MS and LC/MS/MS

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Abstract:

Inspection results for 138 pesticides using GC/MS/MS and LC/MS/MS have been reported by the EURL (European Union Reference Laboratory). Of the 138 substances, it was recommended that 66 of those substances be analyzed using the triple quadrupole GC/MS/MS, and that the remaining 72 substances be analyzed by the triple quadrupole LC/MS/MS. Thus, the pesticides were analyzed using the GCMS-TQ8030 and LCMS-8040 as recommended. The combined use of GC/MS/MS and LC/MS/MS permits high-sensitivity comprehensive analysis of residual pesticides in foods.

Keywords: Positive List, Residual Pesticide, QuEChERS Method, GC/MS/MS, LC/MS/MS

1. Introduction

The heightened concern for food safety in Japan led to the introduction of a positive list system on May 29, 2006, which now includes residue limits and analytical methods for 800 types of pesticides and veterinary drugs. However, due to the differing maximum residue limits (MRLs) and regulated components among countries, implementation of a more rapid global response has become imperative with the increasing import and export of food products. In the EU, the QuEChERS method (Quick, Easy, Cheap, Effective, Rugged, Safe) is used as a pretreatment method for easy residual pesticide analysis by GC/MS/MS and LC/MS/MS.

The evaluation results by LC/MS/MS and GC/MS/MS for the main 138 pesticides used in Europe have been reported[1)] by the EURL (European Union Reference Laboratory), and these have become one indicator associated with development of measurement methods in each country. Of the 138 substances, the triple quadrupole GC/MS/MS was recommended for analysis of 66 substances, and the triple quadrupole LC/MS/MS was recommended for 72 substances. This study presents the results of analysis using the LCMS-8040 and GCMS-TQ8030 for the recommended components.

2. Method

The QuEChERS method is a pretreatment method for pesticide residue analysis that was reported by Anastassiades *et al*. in 2003. It is a fast, easy cleanup method in which acetonitrile extraction, salting-out and degassing are first conducted in a polypropylene centrifuge tube, and the extract solution obtained following centrifugation is mixed directly with solid phase extraction packing to carry out dispersive SPE. The simplicity and ease with which extraction can be achieved with this method have led to its widespread use throughout the world. For this study leeks and paprika were prepared, and the sample extracts obtained following QuEChERS cleanup of the vegetables (Fig. 1) were used for measurement.

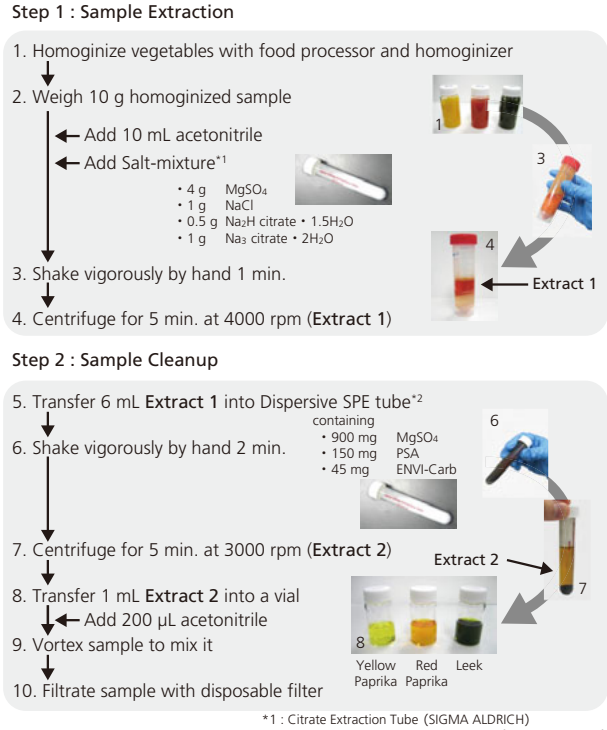

Fig. 1 Preparation of Actual Sample (QuEChERS Method Used in EU)

1) "Multiresidue Method using QuEChERS following by GC-QqQ/MS/MS and LC-QqQ/MS/MS for Fruits and Vegetables," EURL

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3. MRM Measurement by GC/MS/MS

The list of pesticides recommended for analysis by GC/MS/MS by the EURL is shown in Fig. 2. Analysis of these 66 components was conducted using the GCMS-TQ8030 with the analytical conditions shown in Table 1. These conditions and the *m/z* values to be monitored were determined based on the EURL report.

The mass chromatograms of MRM transitions for six of the 66 components are shown in Fig. 3. High-sensitivity analysis is clearly achieved even at <10 ppb. Moreover, it is clear that even residues present at the 1 ppb trace level can be detected with a high S/N ratio. We also used the GCMS-TQ8030 SIM mode and MRM mode to conduct analysis of a paprika extract solution spiked with pesticides at 10 ppb. In SIM mode, the same conditions shown in Table 1 were used, except for the CID Ar gas, which was not used. The respective analytical results are compared in Fig. 4. In SIM mode, detection of the target was sometimes difficult due the matrix effect even when monitoring a specific ion. In MRM mode, however, the matrix effect was largely eliminated by the employment of two highly selective mass analyzers, permitting analysis of the target with high sensitivity.

The use of GC/MS/MS makes it possible to analyze trace components with high sensitivity; however, analytical precision is also necessary. We therefore conducted repeat analysis (n=6) of the paprika extract solution spiked with pesticides at 10 ppb. Excellent repeatability of area values was obtained for all components.

The results of analysis using the GCMS-TQ8030 indicated that all 66 components recommended for analysis by GC/MS/MS could be analyzed reliably and with high sensitivity.

Pesticides Recommended for GC/MS/MS Analysis (66 Compounds)

Acrinathrin	Endosulfan	Myclobutanil	Tebufenpyrad
Bifenthrin	Ethion	Oxadixyl	Tefluthrin
Bromopropylate	Etofenprox	Parathion	Tetraconazole
Bupirimate	Etoprofos	Parathion-methyl	Tetradifon
Buprofezin	Fenarimol	Pendimethalin	Tolclofos-methyl
Captan	Fenazaquin	Permethrin	Tolyfluanid
Chlorfenvinphos	Fenitrothion	Phenthoate	Triazophos
Chlorotalonil	Fenpropathrin	Phosalone	Trifluralin
Chlorpropham	Fenthion	Pirimicarb	Vinclozolin
Chlorpyriphos	Fenvalerate	Pirimiphos-methyl	
Clorpyriphos-methyl	Fipronil	Procymidone	
Cyfluthrin	Flusilazole	Profenofos	
Cypermethrin	Folpet	Propiconazole	
Cyprodinil	Iprodione	Propyzamide	
Deltamethrin	λ-Cyhalothrin	Pyridaben	
Diazinon	Malathion	Pyrimethanyl	
Dichlofluanid	Mepanipyrim	Pyriproxyfen	
Dichloran	Metalaxyl	Taufluvalinate	
Diphenylamine	Methidathion	Tebuconazole	

Fig. 2 Compounds Recommended for GC/MS/MS

Table 1 GC/MS/MS Analytical Conditions

GC/MS/MS	: GCMS-TQ8030
Column	: Rxi-5Sil MS (30 m × 0.25 mmI.D., df = 0.25 µm, Restek)
GC condition	
Column Temp.	: 70 °C (2 min) – (25 °C/min) – 150 °C - (3 °C/min) – 200 °C - (8 °C/min) – 280 °C (10 min)
Carrier Gas	: He (Constant Linear Velocity Mode)
Carrier Gas Velocity	: 58 cm/sec
Injection Mode	: Splitless
Sample Injection Volume	: 1 µL
MS condition	
Interface Temp.	: 250 °C
Ion Source Temp.	: 230 °C
Data Acq. Mode	: SIM, MRM (For MRM, the EURL-recommended transitions were used.)
Loop Time	: 0.3 sec

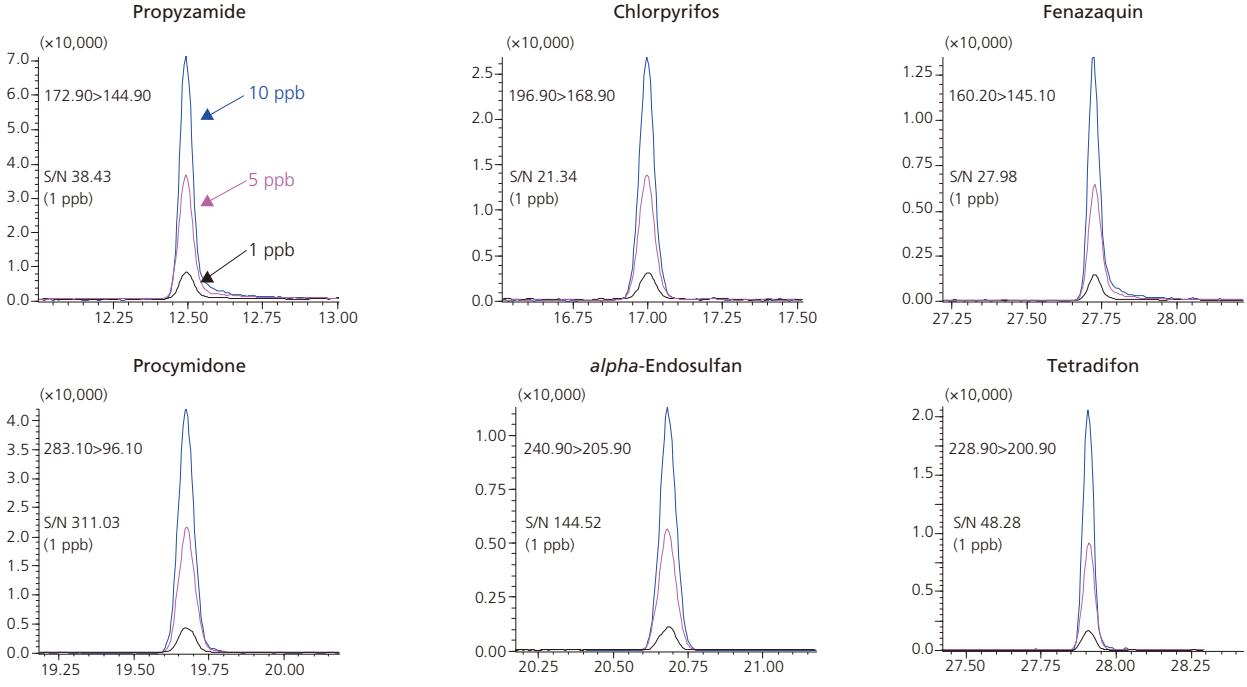


Fig. 3 MRM Mass Chromatograms of Six Typical Pesticides

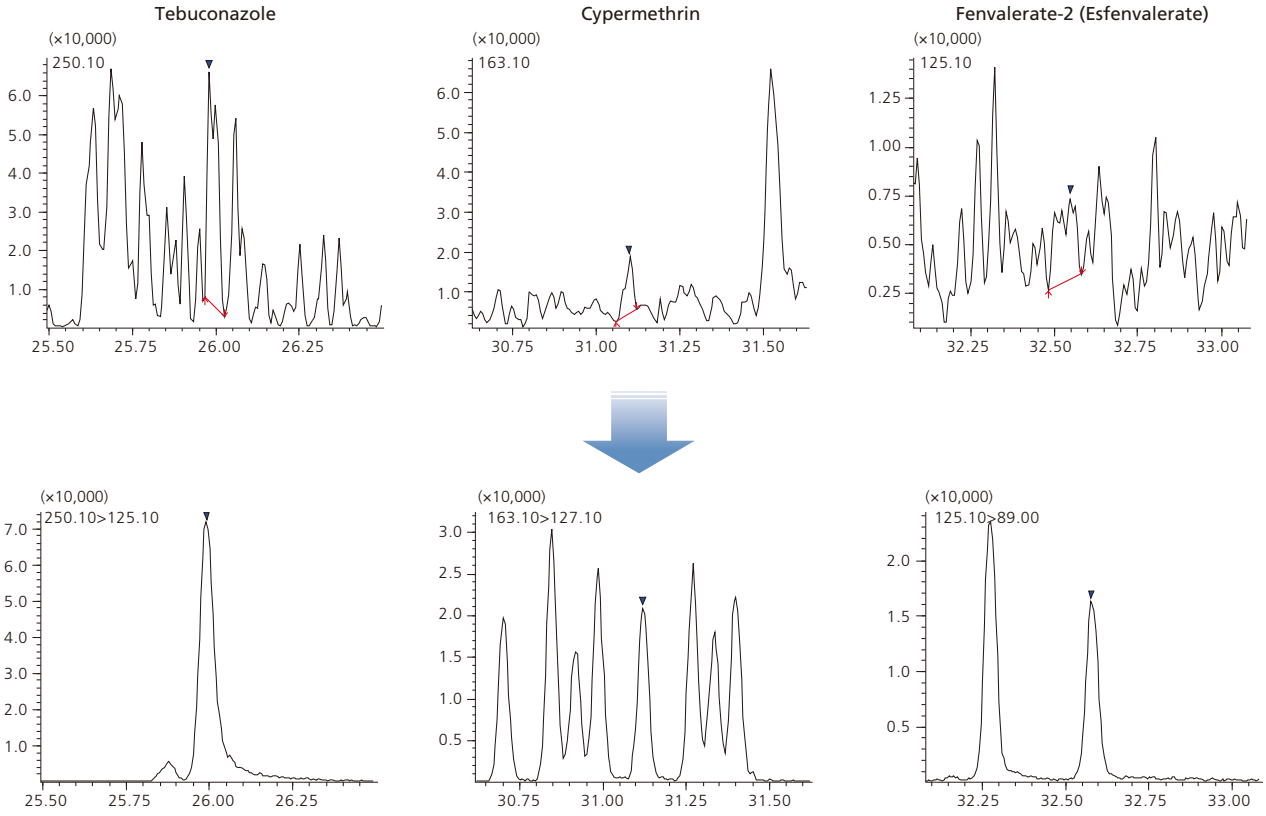


Fig. 4 Comparison of SIM and MRM Mass Chromatograms for Three Pesticides Analyzed in the Study

Table 2 Repeatability (n=6) of Area Values of Pesticides (10 ppb) in Spiked Paprika Extract

Compound Name	%RSD
Diphenylamine	4.99
Ethoprophos	4.95
Chlorpropham	6.26
Trifluralin	5.33
Dicloran	6.49
Propyzamide	5.52
Chlorothalonil	4.46
Diazinon	5.45
Pyrimethanil	3.18
Tefluthrin	5.13
Pirimicarb	5.00
Chlorpyrifos-methyl	5.27
Vinclozolin	6.33
Parathion-methyl	5.81
Tolclofos-methyl	4.89
Metalaxyl (Mefenoxam)	5.43
Fenitrothion	5.10
Pirimiphos-methyl	5.35
Dichlofluanid	4.04
Malathion	6.31

Compound Name	%RSD
Chlorpyrifos	5.23
Fenthion	5.75
Parathion	6.93
Tetraconazole	6.96
Pendimethalin	6.29
Cyprodinil	5.21
(E)-Chlorfenvinphos	5.35
Tolylfluanid	4.81
Fipronil	6.76
Captan	5.74
(Z)-Chlorfenvinphos	5.52
Phenthoate	6.40
Folpet	6.56
Procymidone	6.40
Methidathion	6.17
alpha-Endosulfan	6.27
Mepanipyrim	6.41
Profenofos	5.92
Myclobutanil	5.46
Flusilazole	5.63

Compound Name	%RSD
Buprofezin	4.92
Bupirimate	5.47
beta-Endosulfan	6.29
Oxadixyl	5.74
Ethion	6.18
Triazophos	3.45
Endosulfansulfate	4.26
Propiconazole-1	6.02
Propiconazole-2	5.56
Tebuconazole	7.59
Iprodione	1.72
Bromopropylate	5.71
Bifenthrin	5.29
Fenpropathrin	4.00
Fenazaquin	4.84
Tebufenpyrad	5.62
Tetradifon	6.09
Phosalone	5.90
Pyriproxyfen	5.16
lamda-Cyhalothrin	5.38

Compound Name	%RSD
Fenarimol	5.16
Acrinathrin	2.03
Permethrin-1	6.34
Pyridaben	7.11
Permethrin-2	6.24
Cyfluthrin-1	4.44
Cyfluthrin-2	3.77
Cyfluthrin-3	7.35
Cyfluthrin-4	8.19
Cypermethrin-1	8.58
Cypermethrin-2	3.71
Cypermethrin-3	8.08
Cypermethrin-4	2.48
Ethofenprox	5.03
Fenvalerate-1	4.20
tau-Fluvarlinate-1	2.16
Fenvalerate-2	5.65
tau-Fluvarlinate-2	2.14
Deltamethrin-1	7.58
Deltamethrin-2	7.32

4. MRM Measurement by LC/MS/MS

Similarly, we conducted simultaneous MRM analysis of the 72 components recommended for LC/MS/MS analysis, as listed in Fig. 5. The analytical conditions are shown in Table 3. Simultaneous positive and negative ionization measurement was conducted using electrospray ionization (ESI).

Fig. 6 shows the calibration curves and MRM chromatograms of two representative pesticides. Good linearity was obtained over the concentration range of 1–1000 ppb. We also analyzed leek and paprika extract solutions that were prepared via QuEChERS, and spiked at 5 ppb with the 72 pesticides specified for LC/MS/MS analysis. We then calculated the recoveries. Fig. 7 shows the repeatability and recovery for all of the pesticides in each of the solutions. Since the QuEChERS method is a simple pretreatment method, the matrix is likely to include many contaminants. Despite this, however, good pesticide recoveries of 80–90 percent were obtained from both matrices. It should be noted that a few of the pesticides added to the matrix were detected at recovery rates exceeding 120 %, but these were also detected in a matrix blank. However, all of these pesticides, as listed in Table 4, showed quantitation values that were below the Maximum Residue Levels (MRLs).

Thus, it was confirmed that by using the LCMS-8040, analysis of the LC/MS/MS-recommended substances can be conducted using simultaneous positive and negative ionization with high sensitivity and at high rates of recovery.

In addition, we also used the LCMS-8040 for analysis of all 138 pesticides, including those recommended for GC/MS/MS analysis. Details regarding this analysis are available in our IMSC poster[2].

Pesticides Recommended for LC/MS/MS Analysis (72 Compounds)

Acephate	Epoxiconazole	Linuron	Spinosad
Acetamiprid	Fenbutatin oxide	Lufenuron	Spiroxamine
Aldicarb	Fenbuconazole	Metconazole	Tebufenozide
Amitraz	Fenhexamid	Methamidophos	Teflubenzuron
DMF, DMPF	Fenoxycarb	Methiocarb	Thiabendazole
Azinphos-methyl	Fenpropimorph	Methomyl	Thamethoxam
Azoxystrobin	Fenthion sulfoxide	Methoxyfenozide	Thiacloprid
Bitertanol	Fludioxonyl	Monocrotophos	Thiphanate-m
Boscarid	Flufenoxuron	Oxamyl	ethyl
Cadusafos	Fluquinconazole	Oxydemeton-methyl	Triadimenol
Carbaryl	Flutriafol	Paclobutrazole	Trichlorfon
Carbendazim	Formetanate	Pencycuron	Trifloxystrobin
Carbofuran	Fosthiazate	Penconazole	Triflumuron
Clorfentezin	Hexaconazole	Phosmet	Triticonazole
Clothianidin	Hexythiazox	Pyraclostrobin	Zoxamide
Cyproconazole	Imazalil	Prochloraz	
Dichlorvos	Imidacloprid	Propamocarb	
Difenoconazole	Indoxacarb	Propargite	
Dimethoate	Iprovalicarb	Prothioconazole	
Dimethomorph	Kresoxim-methyl	Quinoxyfen	

Fig. 5 Compounds Recommended for LC/MS/MS

Table 3 LC/MS/MS Analytical Conditions

HPLC: Nexera UHPLC system	
Column	: Shimpack-XR ODS II (75 mm × 2 mm.I.D., 2.2 µm)
Mobile phase A	: 2 mM ammonium formate containing 0.1% formic acid - water
Mobile phase B	: Methanol
Gradient program	: 5% B (0–2.5 min.) → 55% B (2.51–6 min.) → 80% B (6.01–12 min.) → 100% B (12–15 min.) → 5% B (15.01–20 min.)
Flow rate	: 0.2 mL / min.
Oven temperature	: 40 °C
LC/MS/MS: LCMS-8040	
Ionization	: ESI (Positive / Negative)
Ion spray voltage	: +4.5 kV / –3.5 kV
MRM	: 276 MRM transitions (2 MRMs / compound) Dwell time 5 msec. / Pause time 1 msec.

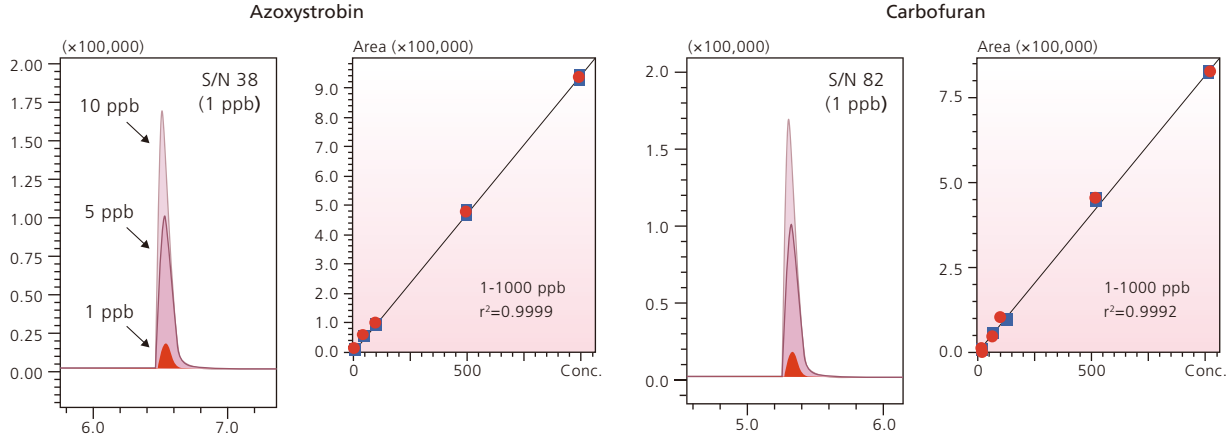


Fig. 6 MRM Mass Chromatograms and Calibration Curves of Two Typical Pesticides

2) IMSC 2012 Poster No. PO-CON1216E
"Multi-class pesticides analysis in challenging vegetable matrices using fast 5 msec MRM with 15 msec polarity switching"
http://www.shimadzu.com/an/literature/lcms/jpo112166.html

Compounds	Leek		Paprika		Compounds	Leek		Paprika	
	%RSD	Recovery (%)	%RSD	Recovery (%)		%RSD	Recovery (%)	%RSD	Recovery (%)
Acephate	7.00	87	10.89	81	Indoxacarb	6.45	98	4.93	84
Acetamiprid	2.87	83	2.29	88	Iprovalicarb	2.20	95	3.15	90
Aldicarb	4.63	76	3.59	90	Kresoxim-methyl	5.97	171	8.83	95
Amitraz	1.62	80	2.10	74	Linuron	7.66	59	4.87	91
Azinphos-methyl	1.62	73	2.89	94	Lufenuron	13.51	103	11.99	87
Azoxystrobin	2.90	162	2.21	106	Methamidophos	5.55	102	3.17	104
Bitertanol	16.03	104	6.59	108	Methiocarb	3.92	71	3.30	87
Boscalid	13.79	77	10.08	111	Methomyl	3.30	97	2.05	90
Cadusafos	2.29	92	1.75	87	Methoxyfenozide	2.75	98	3.41	91
Carbaryl (NAC)	2.20	83	3.93	79	Monocrotophos	4.96	91	5.69	93
Carbendazim	1.13	90	1.05	87	Oxamyl	1.20	95	2.30	90
Carbofuran	1.85	92	1.12	91	Oxydemeton methyl	1.93	100	18.96	82
Clorfentezin	4.07	80	2.95	83	Pacrobutrazole	3.92	83	8.29	87
Clothianidin	3.47	622	6.12	116	Penconazole	3.66	89	3.97	88
Cyproconazole	7.98	98	5.41	105	Pencycuron	3.77	92	2.66	89
Dichlorvos	16.13	37	5.16	42	Prochloraz	4.90	78	3.23	87
Difenoconazole	9.23	99	7.45	98	Propamocarb	2.74	86	2.62	83
Dimethoate	3.79	83	2.94	90	Propargite	1.59	85	1.62	91
Dimetomorph	4.81	94	3.22	92	Pyraclostrobin	6.46	101	4.90	118
Epoxiconazole	2.27	88	0.70	88	Quinoxyfen	11.08	73	11.21	90
Fenbutatin oxide	10.76	77	6.31	100	Spinosad A	1.89	104	3.56	93
Fenhexamid	7.71	88	6.99	100	Spiroxamine	1.86	82	1.74	86
Fenoxycarb	2.20	87	2.57	84	Tebufenozide	1.41	100	1.39	97
Fenpropimorph	4.27	92	3.16	94	Teflubenzuron	8.02	110	5.85	89
Fenthion sulfoxide	2.90	82	1.87	91	Thiabendazole	7.85	80	5.24	81
Fludioxonil	1.33	11439	10.27	82	Thiacloprid	3.35	50	2.00	90
Flufenoxuron	4.34	64	5.55	76	Thiamethoxam	7.89	88	4.36	121
Fluquinconazole	8.28	71	6.13	85	Thiphanate-methyl	5.80	103	2.37	123
Flutriafol	5.00	38	2.25	80	Triadimenol	4.13	118	9.26	99
Formetanate	2.55	92	0.71	93	Trichlorfon	11.22	72	9.69	110
Fosthiazate	1.85	91	2.50	95	Trifloxystrobin	3.49	98	3.47	91
Hexaconazole	5.83	81	4.02	91	Triflumuron	6.31	72	5.89	77
Hexythiazox	1.33	72	2.44	79	Triticonazole	10.01	85	15.46	92
Imazalil	4.65	94	5.40	92	Zoxamide	4.77	88	1.63	80
Imidacloprid	7.69	76	5.44	105					

Fig. 7 Peak Area Repeatability (n=6) and Recoveries of Vegetable Extract Solutions (Paprika and Leek) Spiked with Pesticides (5 ppb)

Table 4 Quantitation Results for Four Pesticides (Sample: Leek)

	Azoxystrobin	Clothianidin	Fludioxonil	Kresoxim-methyl
5ppb standards spiked	8.19 ppb	25.78 ppb	556.58 ppb	9.63 ppb
Matrix blank	3.77 ppb	21.39 ppb	550.92 ppb	2.94 ppb
MRL (Japan)	5 ppm	15 ppm	10 ppm	30 ppm

5. Conclusion

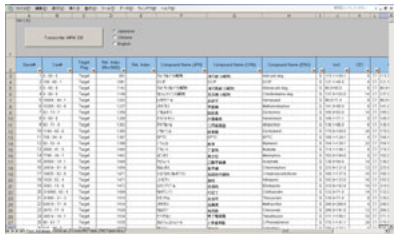
According to the EURL report regarding 138 pesticides, it was found that high-sensitivity analysis of all the pesticides is possible using the GCMS-TQ8030 and LCMS-8040, as specifically recommended. The QuEChERS method is a pretreatment method applicable to both LC/MS /MS and GC/MS /MS, and because it permits extraction of a wide range of pesticides, we consider it useful for analysis using a combination of these analytical systems.

GCMS-TQ8030

—Speed Beyond Comparison

Excellent, User-Friendly Operation

ALL transitions of GC/MS/MS used in this report are assembled as EURL database. GCMS-TQ8030 users can obtain this database and create method file for free.

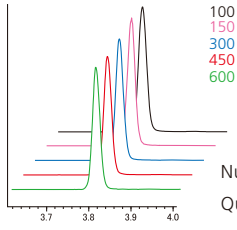


High Sensitivity and High Selectivity

MRM measurement by GC/MS/MS is effective for samples in complex matrices. As mass separation is accomplished in two steps, even components that could not be separated using the conventional scan mode and SIM mode can now be easily separated and detected.

High Speed

UFsweeper® is a unique Shimadzu technology that achieves high CID efficiency and high-speed ion transport while keeping the length of the collision cell to the absolute minimum. This effectively prevents the occurrence of cross-talk and diminished signal intensity even during high-speed measurement.



MRM/sec	%RSD
100	1.06%
150	1.13%
300	0.60%
450	0.62%
600	1.32%

Number of MRM Transitions and Repeatability

Quetiapine-TMS *m/z* 321 > 211

LCMS-8040

—Enhanced Sensitivity

User-Friendly Operation

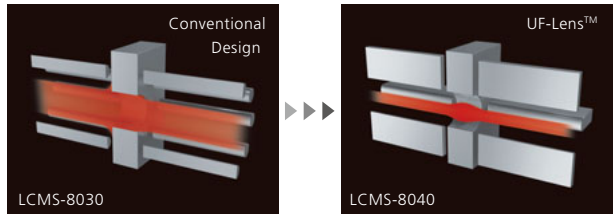
All the conditions included in the various method packages released with the LCMS-8030 base package can be used as is with the LCMS-8040. All consumables as well, including the desolvation line (DL) and ESI capillary, etc., are common to both models. And, users who have experience with LabSolutions will feel perfectly at ease using the LCMS-8040.

High Speed

The LCMS-8040 delivers incomparable analytical capabilities, with a maximum 555 ch/sec ultra-high-speed MRM measurement (1 msec minimum dwell time, 1 msec minimum pause time), 15 msec ultra-high-speed positive/negative polarity switching, and a maximum 15000 u/sec ultra-high-speed scan measurement.

High Sensitivity

Multiple Reaction Monitoring (MRM) sensitivity is increased five-fold (based on the S/N ratio of reserpine analysis) with the combination of the UF-Lens™ and UFsweeper® II collision cell. Higher sensitivity is also achieved in scan mode analysis, making the LCMS-8040 suitable for a wide range of laboratory applications.



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