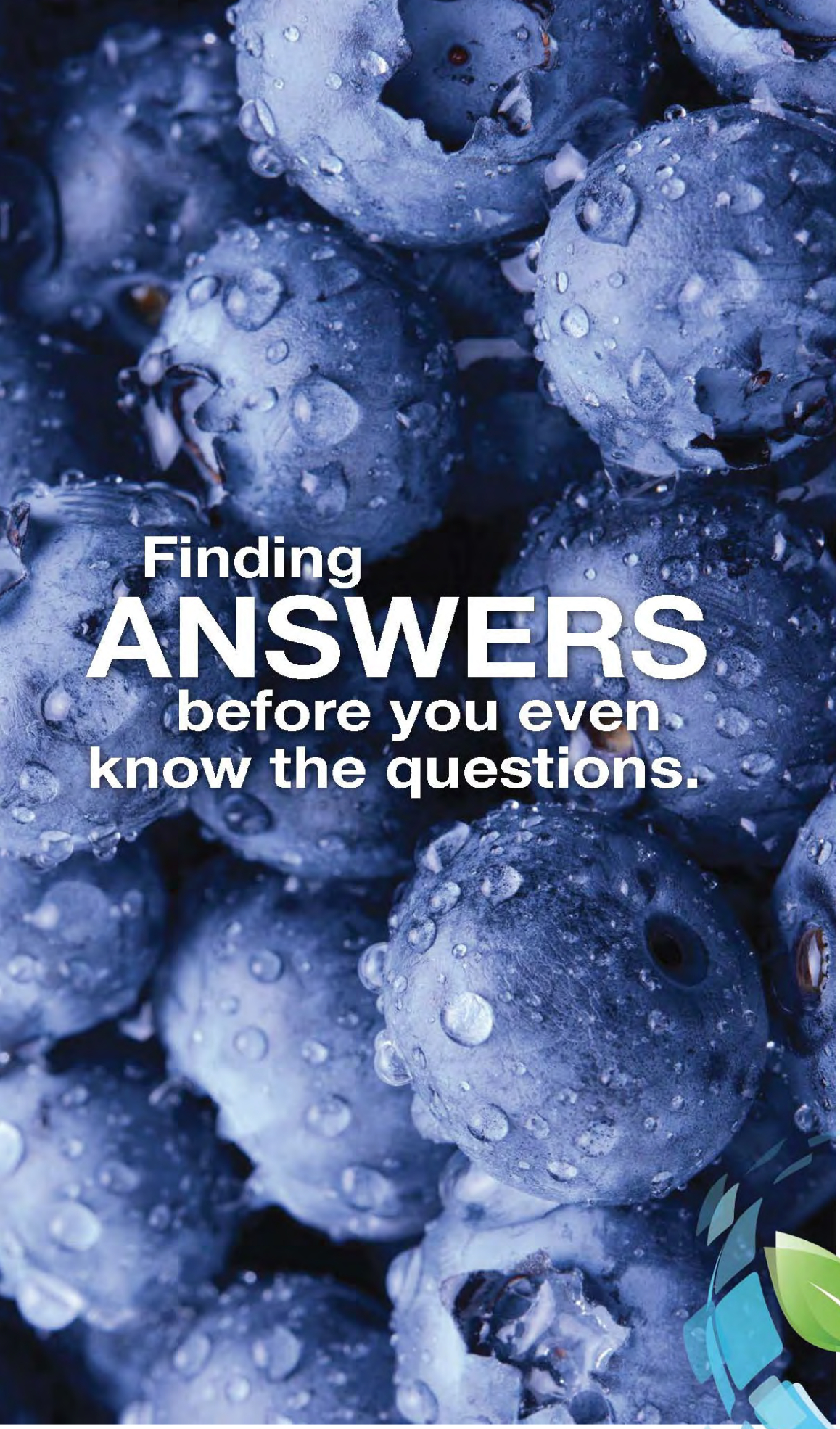

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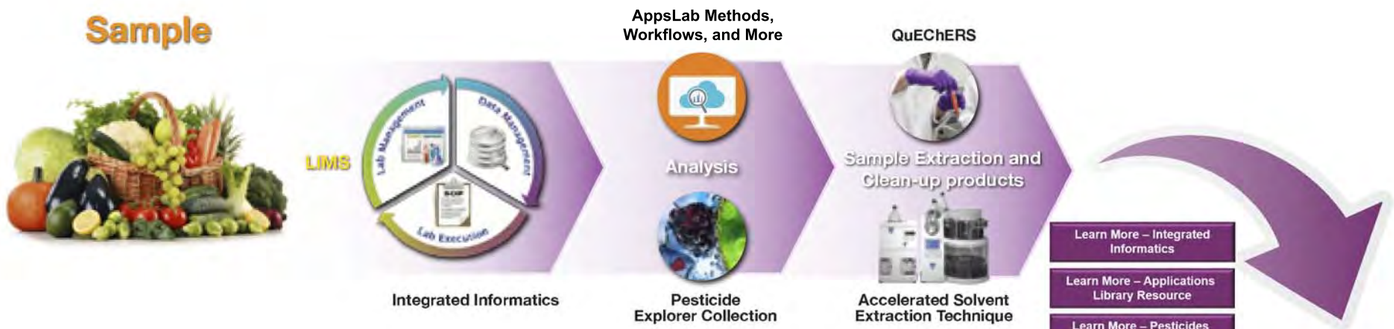

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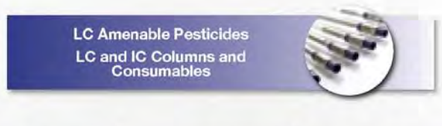

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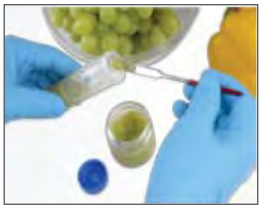

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Sample Preparation Application Notes

- Extraction of Organochlorine Pesticides from Oyster Tissue Using Accelerated Solvent Extraction
- Multi-Residue Pesticide Analysis in Herbal Products Using Accelerated Solvent Extraction with a Triple Quadrupole GC-MS/MS System
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Extraction of Organochlorine Pesticides from Oyster Tissue Using Accelerated Solvent Extraction

Pranathi Perati and SM Rahmat Ullah
Thermo Fisher Scientific, Sunnyvale, CA, USA

Application Brief 152

Key Words
Persistent organic pollutants, moisture absorbing polymer, wet samples, accelerated solvent extraction, sample preparation

Introduction

Organochlorine pesticides (OCPs) are a class of chemicals that were used to control insect pests since the 1940s. The use of OCPs was banned in the later part of the last century due to their longevity, a trait that made them effective for long term pest control, but also increased concerns of potential health outcomes such as cancer in humans and ecosystem disruption. Pesticides are regulated in the U.S. by the Environmental Protection Agency (EPA) under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). Some states also regulate pesticides under FIFRA, in a more restrictive manner than the EPA. In the European Union, water intended for human consumption must meet a maximum level of 0.1 µg/L for each pesticide and a maximum of 0.5 µg/L for total pesticides, except for aldrin, dieldrin, heptachlor, and heptachlor epoxide, which are each limited to maximum levels of 0.03 µg/L. Maximum contaminant levels have been established for OCPs by the United States Environmental Protection Agency ranging from 0.2 µg/L for Lindane to 2 µg/L for Endrin.

Many OCPs are endocrine disrupting chemicals, meaning they have subtle toxic effects on the body's hormonal systems. Endocrine disrupting chemicals often mimic the body's natural hormones, disrupting normal functions contributing to adverse health effects. OCPs are persistent organic pollutants (POPs), a class of chemicals that are ubiquitous environmental contaminants because they break down very slowly in the environment and accumulate in lipid rich tissue such as body fat. According to the Centers for Disease Control and Prevention (CDC), most people have OCPs present in their bodies. Exposure to low concentrations of organochlorine chemicals over a long period may eventually lead to a substantial body burden of toxic chemicals. Organochlorine compounds have long been recognized as the most deleterious contaminants to biota in the world's marine and estuarine waters. Various biomonitoring strategies have therefore been developed to monitor and evaluate the adverse impact of these compounds on the marine ecosystems. Analyses of OCPs are becoming increasingly important, and often with the need to isolate and analyze trace levels of compounds from a variety of matrices such as soil, sediment, animal tissue, fruits, and vegetables. Sample pretreatment constitutes an important step prior to analysis. The purpose of the sample pretreatment step is to selectively isolate the analytes of interest from matrix components and present a sample suited for routine analysis by an established analytical techniques such as gas chromatography or high-pressure liquid chromatography. Accelerated solvent extraction is an established technique for extracting analytes of interest from a solid, semisolid or an adsorbed liquid sample using an organic solvent at an elevated temperature and pressure. The elevated pressure elevates the boiling temperature of the solvent thereby allowing faster extractions to be conducted at relatively high temperatures. Thus the extraction process is significantly faster than traditional methods such as Soxhlet extraction.

This Application Brief discusses the use of Thermo Scientific™ Dionex™ ASE Prep MAP, a proprietary polymer designed to remove moisture and increase extraction efficiencies from wet samples including soils, tissues and food products. This polymer is useful for in-cell extraction of trace level organics from a variety of moisture containing samples with no additional pre or post extraction steps. The Dionex ASE Prep MAP polymer has a high-capacity for water removal and does not suffer from some of the limitations of clumping or precipitation observed in some of the traditional drying methods.

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Equipment

- Thermo Scientific™ Dionex™ ASE™ 350 Accelerated Solvent Extractor system, equipped with 34 mL Stainless Steel Extraction Cell Kit, (P/N 060071)
- Filters, Glass Fiber Cell (P/N 056781)
- 250 mL Clear Collection Bottles (P/N 056284)
- Analytical Balance (read to the nearest 0.001 g or better)
- Mortar and Pestle (Fisher Scientific or equivalent)
- Gas Chromatograph (GC) with Electron-Capture Detector (ECD)

Consumables, Regents and Standards

- Dionex ASE Prep Map, Moisture Absorbing Polymer (P/N 083475)
- Thermo Scientific Dionex ASE Prep DE (diatomaceous earth) Dispersant, 1 kg Bottle (P/N 062819)
- Sodium Sulfate
- Acetone
- Hexane
- Heptachlor
- Lindane
- Aldrin
- Dieldrin
- Endrin
- Dichlorodiphenyltrichloroethane (DDT)

All solvents are optima-grade or equivalent and are available at Fisher Scientific.

Sample Preparation and Experimental Conditions

Sample Preparation Using Sodium Sulfate as the Drying Agent

The Oyster samples were prepared by blending or chopping to produce a unifrom homogenate. 2.5 g of the spiked oyster sample was treated with 9 g of sodium sulfate as the drying agent prior to in-cell extraction in the Dionex ASE 350 system. The extraction was pursued at 100 °C using hexane:acetone (1:1) as solvents. The extracts were analyzed by GC-ECD.

Sample Preparation Using Dionex ASE Prep MAP as the Drying Agent

A 5 g portion of the homogenate was accurately weighed and mixed with 1.7 g of Dionex ASE Prep DE and 1.7 g of Dionex ASE Prep MAP. Carefully transfer the samples to the extraction cells, ensuring that the sample is completely removed from the container. Load the extraction cells and collection vials into the Dionex ASE 350 system and perform the extraction according to the conditions listed. In the case of spiked samples the spikes were added to the sample prior to extraction.

Accelerated Solvent Extraction Conditions	
Oven Temperature:	100 °C
Pressure:	1500 psi
Static Time:	5 min
Static Cycles:	3
Rinse Volume:	60%
Solvent:	Hexane/Acetone (1:1, v/v)
Total Extraction Time:	22–25 min
Pure Time:	120 sec

Results and Discussion

Sample preparation is challenging for a wet animal tissue sample such as an oyster sample. The presence of water in such a sample can result in poor recoveries of the analyte of interest. A drying step is therefore needed before the extraction. Mixtures of six OCPs at concentrations of 500 ng/g each were spiked on to the wet oyster samples. The spiked oyster samples were mixed with Dionex ASE Prep MAP and Dionex ASE Prep DE (1:1) or mixed with sodium sulfate as the drying agent prior to in-cell extraction in the Dionex ASE system. The extraction was pursued at 100 °C using hexane: acetone (1:1) as solvents. The extracts were analyzed by GC-ECD. The results in Table 1 show recoveries ranging from 91% for Lindane to 114% for DDT when the extractions are done using Dionex ASE Prep MAP and Dionex ASE Prep DE. The recoveries for extractions done with sodium sulfate are considerably lower ranging from 69% for DDT to 81% for Lindane. The data shows that Dionex ASE Prep DE and Dionex ASE Prep MAP were an effective drying agent for wet oyster samples with excellent recoveries for the six OCPs. In contrast the sodium sulfate treated sample showed poorer recoveries.

Table 1. In-cell moisture removal of oyster sample using Dionex ASE Prep MAP and Dionex ASE Prep DE, in comparison to sodium sulfate.

Compound	% Recovery Oyster dried with Dionex ASE Prep MAP and Dionex ASE Prep DE* (n = 3)	% Recovery Oyster dried with sodium sulfate** (n = 3)
Lindane	91	81
Heptachlor	93	64
Aldrin	94	66
Dieldrin	105	75
Endrin	106	70
DDT	114	69
Total	101	71

* Data is courtesy of Dr. Todd Anderson from the Department of Toxicology, Texas Tech University, Lubbock

** In-cell drying with sodium sulfate is not recommended using accelerated solvent extraction

Conclusion

This Application Brief describes a simple and reliable method to extract OCPs from oyster tissue. This method also demonstrates the use of Dionex ASE Prep DE and Dionex ASE Prep MAP for in-cell extractions without any pre and post extraction steps to remove moisture and increase extraction efficiencies in wet samples. The method is ideal for routine extractions of OCPs from wet samples.

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Acknowledgment

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Multi-Residue Pesticide Analysis in Herbal Products Using Accelerated Solvent Extraction with a Triple Quadrupole GC-MS/MS System

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Nicole Rueckert, Johann Kirchner, Elmar Häfner, Phytolab GmbH & Co KG, Vestenbergsgreuth, Germany

Application Note 52291

Key Words
Pesticides, Tea, Herbal products, ASE, SRM, MRM, Multi-residue analysis, TSQ 8000 GC-MS/MS

Introduction

The residue analysis of pesticides has developed in recent years into a comprehensive methodology for the detection of many hundreds of potential contaminating compounds. A multi-residue method for herbal products and teas is faced with additional challenges from the worldwide origin of the products and the complex matrix of the dried materials. In the due quality control of raw materials, the unknown or undeclared local plant protection treatments must be taken into account with a wide variety of potential pesticide contaminations.

Dried leaves, fruits or seeds and other herbal products of medical use deliver highly complex extracts from the sample preparation due to the rich content of active ingredients, essential oils and the typical high boiling natural polymer compounds from broken cells, leaves or fruit skins. A thorough clean up of the extracted sample can lead to losses of critical analytes of interest. A complete characterization of pesticide, and other residue, contamination is done by both LC and GC-MS/MS to cover the complete range of functional groups.

This application report describes the methodology used for the multi-residue pesticide analysis of herbal products using accelerated solvent extraction (ASE) and gel permeation chromatography (GPC) sample preparation with detection and quantitation by the Thermo Scientific TSQ 8000 GC-MS/MS system.

A routine screening method for more than 200 pesticide compounds was applied to a wide variety of different sample types, ranging from regular black tea or sage leaves, to seeds like fennel and herbs of medical and fragrance use like thyme and chamomile. The data processing and reporting was achieved by using the Thermo Scientific TraceFinder quantitation software suite.

The sensitivity requirement for this analysis was determined by the regulatory background. The analysis of pesticide residues in tea and herbal products follows the regulations of the European Directorate General for Health and Consumer Affairs (SANCO) for "Method Validation and Quality Control Procedures for Pesticide Residue Analysis in Food and Feed" [1]. The sensitivity requirements for these products as referenced in the Codex Alimentarius [2] result in maximum residue levels of 0.01 mg/kg for most of the pesticide compounds.

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Sample Preparation

Herbal and tea samples were extracted with an accelerated solvent extraction method using the Thermo Scientific Dionex ASE 350 Accelerated Solvent Extractor. The ASE method used is described in an official pesticide standard method [3]. The collected extracts were concentrated using a rotary evaporator (Rotavap) and further cleaned up via gel permeation chromatography (GPC). The GPC step used a polystyrene gel (Bio-Beads® S-X3) with an ethylacetate/cyclohexane mobile phase. After additional concentration by the Rotavap, the extracts were ready for GC injection using ethylacetate as the main solvent.

Method Setup

The analytical method comprised sample handling and injection using the Thermo Scientific TriPlus RSH liquid autosampler, TRACE GC 1310 gas chromatograph equipped with an instant connect, temperature programmable PTV injection system, and the TSQ™ 8000 triple quadrupole GC-MS/MS detection system. The MRM detection method was taken from a routinely employed Thermo Scientific TSQ Quantum XLS GC-MS/MS method without any further optimization on the TSQ 8000 GC-MS/MS system [4]. The TSQ 8000 system automatically optimized acquisition windows and optimized instrument duty cycle using timed-SRM (t-SRM) for maximum sensitivity. This enabled the avoidance of lengthy manual set-ups usually required when adopting new instrumentation (Figure 1).

ASE™ 350 Accelerated Solvent Extraction

Sample weight	10 g
Extraction solvent	Ethylacetate/cyclo-Hexane 1:1, same as GPC solvent
Temperature	120 °C
Pressure	100 bar
Extraction time	5 min, 1 cycle
Flushing with solvent	60% of cell volume
Flushing with nitrogen	100 s

TriPlus™ RSH Autosampler

Syringe	10 µL
Injection volume	1 µL
Injection type	Fast liquid band injection, 100 ms injection time
Washing cycles	3 x 10 µL, solvent ethylacetate

TRACE™ 1310 Gas Chromatograph

Injector PTV	Splitless mode
Base temperature	50 °C
Transfer	10 °C/s to 250 °C, until end of run
Flow	Constant flow, 1.2 mL/min, helium
Analytical column	40 m, ID 0.18 mm, 0.18 µm film, 5%-phenyl phase (5MS type)
Pre-column	5 m, ID 0.18 mm, empty deactivated, no backflush
Column oven	Temperature programmed
Start	70 °C, for 1.50 min
Ramp 1	15 °C/min to 190 °C
Ramp 2	7 °C/min to 290 °C, 12 min
Transfer line	250 °C

TSQ 8000 Mass Spectrometer

Ion source temperature	220 °C
MRM Detection	Timed SRM mode (see Appendix)

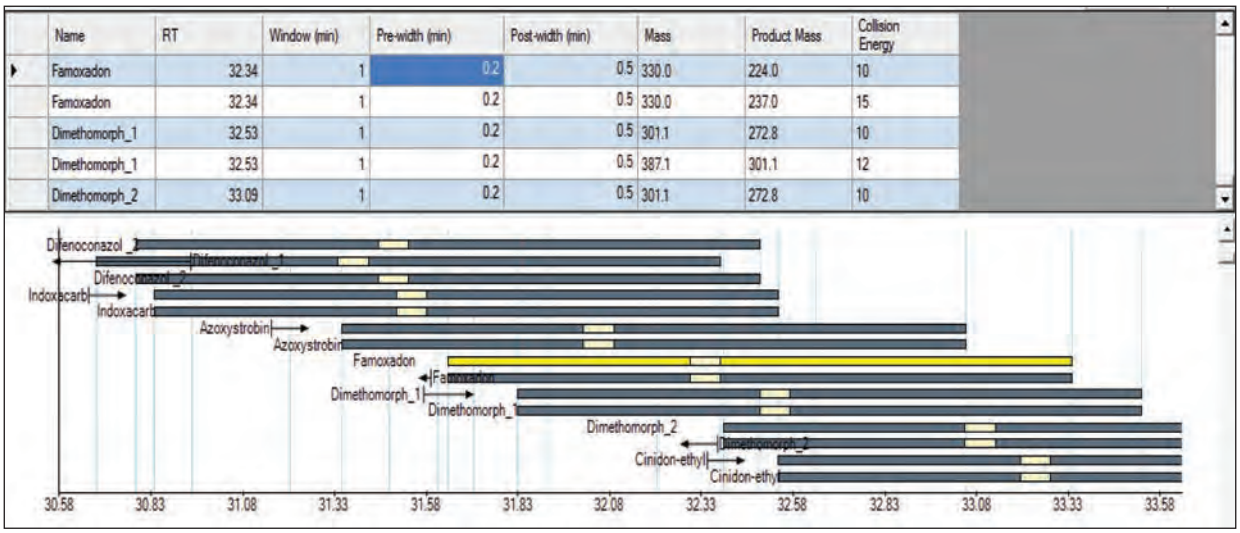

Figure 1. Screenshot of a section of the analytical run showing the "acquisition map" automatically created by the TSQ 8000 system using t-SRM. This mode ensures the instrument only monitors for compounds when they elute to optimize sensitivity.

Calibration and Linearity

The quantitative calibration and linearity check for the method was performed by using six calibration points in the range of 0.004 µg/mL to 1.0 µg/mL. This range represents an analyte concentration of 0.01 to 2.5 mg/kg in the samples (10 – 2500 ppb).

For setting up the calibration solutions, a stock solution containing target pesticide compounds in herbal products was used. The calibration solution was prepared in a standard matrix with a matrix load equivalent to the typical herbal extracts used. The standard matrix blank consisted of lemon peel extracted using the standard procedure. The pesticide blank level was tested before applying as a blank standard matrix. Standard solutions were prepared containing lemon peel extract dissolved 1:1 with ethyl acetate. The correlation coefficients, R^2, achieved during method calibration exceeded 0.99 for all compounds (Figure 2).

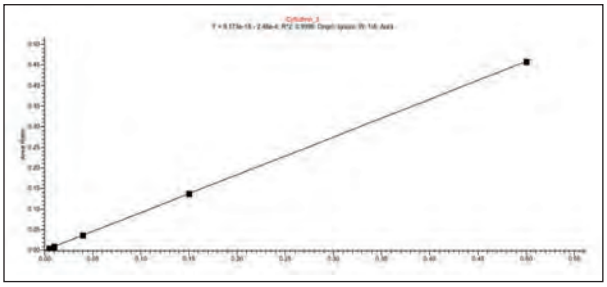

Figure 2. Calibration curve for Cyfluthrin, $R^2 = 0.9996$

Results and Discussion

Sensitivity (LOD)

Using the standard pool of pesticides, the method detection limits in the standard lemon peel were estimated. Using the 4 ppb (pg/µL) matrix standard level, S/N values were used to estimate the limits of detection (LOD). The S/N values in matrix are given in Table 1 for a selection of critical compounds taken at retention times that are affected most from the eluting matrix. Although the compounds are eluting in heavily impacted matrix regions of the chromatogram, the high selectivity of the TSQ 8000 GC-MS/MS for the target pesticides at low level against an intense matrix load is demonstrated in Figure 3 and Figure 4.

Table 1. Detection limit S/N for selected pesticide compounds in matrix

Pesticide	RT [min]	S/N @ 4 ppb
Terbacil	13:83	24
Alachlor	14:78	12
Tolylfluanid	16:75	44
Pyridaben	24:17	83

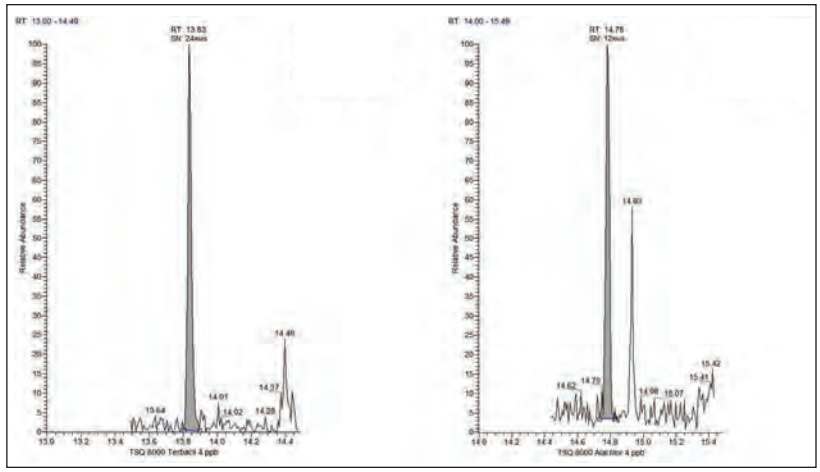

Figure 3. SRM peaks at 4 ppb from Terbacil (left, 161.1 › 88.0, CE 15 V) and Alachlor (right, 188.1 › 130.1, CE 25 V). SRM transitions were taken from the Pesticide Method Reference, 2nd ed. 2011. [4]

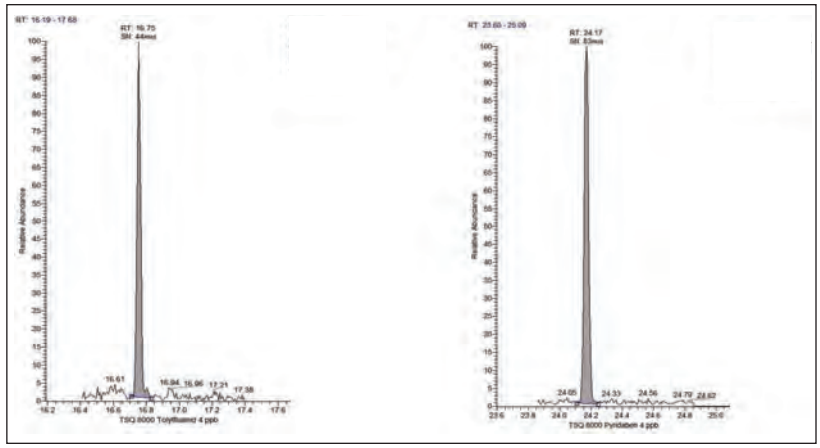

Figure 4. SRM peaks at 4 ppb from Tolylfluanid (left, 238.1 › 137.1, CE 15 V) and Pyridaben (right, 309.1 › 147.1, CE 15 V). SRM transitions were taken from the Pesticide Method Reference, 2nd ed. 2011. [4]

Robustness and Maintenance

Routine preventative maintenance on the GC was performed using routine standard operating procedures. The calibration chromatograms seen in Figures 3 and 4 have been acquired after a persistent matrix load to the system through routine analysis of more than 500 matrix samples.

This level of robustness meant that even with persistent and very high matrix load, it was not necessary to clean the removable ion source short term.

The innovative instant connect modularity of the injectors and detectors of the TRACE 1310 GC, used here as the front-end to the mass spectrometer, allows the user quick accessibility to any injector part for rapid cleaning. Furthermore the unique ability to replace the entire injector module within minutes represents an excellent way of postponing routine maintenance to when the laboratory schedule allows while keeping the GC-MS/MS system operational.

Analytical Precision

Within a routine series of 50 commercial samples, the quality control samples were measured with replicate injections. The results for a range of compounds is given in Table 2. The relative effects on known problematic pesticide compounds can be seen, while coefficients of variation (CV%) for unaffected compounds all stay well below 10% even within this long series of matrix injections.

Table 2. Coefficients of variation for lemon peel matrix spiked QC samples for a set of 60 pesticides under investigation (avg. 7.4%, 24 injections)

Diflubenzofuron	10.0%	Penconazol	7.5%	Diniconazol	2.9%
Biphenyl-d10	7.5%	Allethrin	8.4%	Aclonifen	9.0%
Biphenly	9.5%	Pyrifenox	5.5%	Trifloxystrobin	6.0%
o-Phenylphenol	8.2%	Procymidon	5.7%	Propiconazol	3.1%
Fenobucarb	6.0%	Triadimenol	11.5%	Propargit	6.0%
Diphenylamin	5.7%	Picoxystrobin	7.0%	Tebuconazol	4.3%
Terbutylazin	4.4%	Flutriafol	6.3%	Nitralin	9.2%
Propyzamid	3.1%	Hexaconazol	9.2%	Piperonyl butoxid	8.3%
Terbazil	5.8%	Isoprothiolan	9.7%	Brompropylat	5.8%
Fipronil-desulfinyl	6.9%	Uniconazol	7.0%	Fenoxycarb	9.1%
Alachlor	6.7%	Kresoxim-methyl	9.9%	Etoxazol	8.8%
Prometryn	8.3%	Myclobutanil	9.2%	Fenazaquin	3.3%
Ethofumesat	7.4%	Flusilazol	4.4%	Metconazol	5.3%
Bromacil	8.3%	Cinerin 1	8.1%	Pyriproxyfen	8.5%
Chlorpyrifos	6.9%	Buprofezin	7.4%	Fenamirol	8.5%
Tetraconazol	6.2%	Diclobutrazol	2.6%	Fluquinconazol	4.9%
Triadimefon	11.7%	Cyproconazol	2.6%	Pyridaben	5.2%
Dicapton	10.7%	Chlorbenzilat	3.3%	Etofenprox	10.2%
Butralin	6.6%	Etoconazol	4.4%	Silafluofen	10.2%
Fipronil	5.5%	Iprodion	11.1%	Indoxacarb	8.5%

Results from Real Life Samples

The above method was used for the analysis of a wide variety of herbs, teas and dried fruit known as one of the most challenging analytical task for controlling the pesticide maximum residue levels due to the heavy matrix impact. Table 3 gives a representative overview of positive results from different samples with the indication of the pesticide compound and concentration found. All compounds were detected by using at least two SRM traces and were subsequently confirmed by checking the calibrated ion ratios. The concentration ranges covered were from close to the MRL level of 10 mg/kg to high levels of up to 50 times above the regulated maximum. Figure 5 provides an example of confirmed residue detection in a thyme sample.

Table 3. Positive results above MRL level found in samples of various matrices

Sample Matrix	Pesticide Residues Found	Concentration (mg/kg)
Dried Herbs	o-Phenylphenol	0.017
Dried Herbs	Tebuconazol	0.023
Dried Fruit	Diflubenzuron	0.049
Dried Fruit	Myclobutanil	0.023
Dried Fruit	Propargit	0.479
Dried Fruit	Tebuconazol	0.081
Dried Fruit	Difenconazol	0.013
Dried Herbs	Picoxystrobin	0.228
Dried Herbs	Picoxystrobin	0.233
Dried Herbs	o-Phenylphenol	0.011
Herbal Tea	o-Phenylphenol	0.014
Herbal Tea	o-Phenylphenol	0.011
Herbal Tea	Terbutylazin	0.016

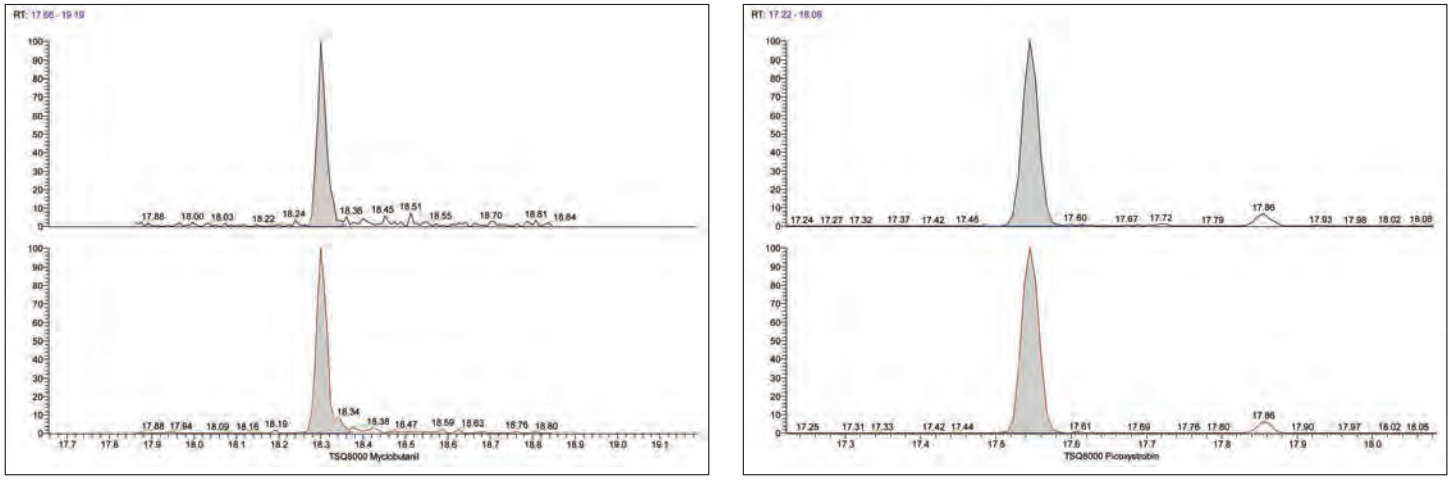

Figure 5. Positive results for Myclobutanil in green apple (0.023 mg/kg, left) and Picoxystrobin in thyme (0.228 mg/kg, right), both detected on two SRM traces

Data Analysis and Reporting

The data processing was performed using TraceFinder™ quantitation software. TraceFinder software contains a compound data store containing a large number of pesticide compound entries from which required compounds for the method had been selected. For each pesticide, the necessary parameters for MRM acquisition and compound identification, such as SRM transition, retention time, and ion ratios, as well as quantitation details like quantitation mass and recovery requirement, are stored.

The analytical sequence setup, data acquisition and result processing was done from one software platform integrating the complete analytical process. In Figure 6, the analytical sequence is shown in the upper part of the screen, with the compounds included in the method to the right. The actual chromatograms for the selected pesticide compounds are displayed in the bottom part for review by the operator.

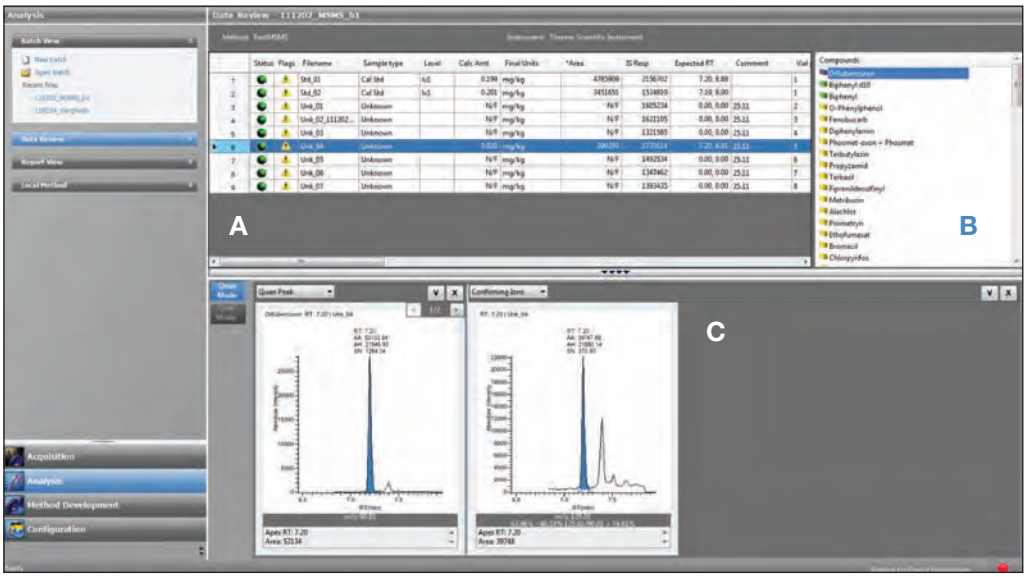

Figure 6. TraceFinder software analysis view:

A. Acquisition sequence table for calibration, QC and sample runs

B. Compound list with status flags

C. Compound chromatogram windows with integrated quantitation and confirmation peaks

Expanded Productivity

The total cycle time of the analytical runs was 30 minutes, which allowed the throughput of two samples per hour and resulted in a load of up to 48 samples, including QC checks during the day for the control of more than 200 pesticide compounds in each run.

This expanded productivity was a combined result of the TSQ 8000 triple quadrupole GC-MS/MS system with its enhanced analyte selectivity in matrix samples, the high method and system robustness, and the advanced data processing using TraceFinder software. Pesticide peaks were typically baseline-separated with a high signal-to-noise ratio allowing for an accurate automated area integration with significantly reduced manual control required. A number of quality control parameters within TraceFinder software immediately provided visible flagging for compounds that may need manual attention. Automatic ion ratio checks provided a fast and solid confirmation in the case of positive findings. The high processing speed of TraceFinder software provided for multi-residue analysis and quick and comprehensive reporting for each sample.

Conclusion

The TSQ 8000 GC-MS/MS delivered high sensitivity and matrix selectivity for routine pesticide analysis even in difficult matrix samples. The data acquisition using the unique timed-SRM allowed for the detection of a virtually unlimited number of pesticide compounds in one run without sacrificing the high sensitivity for individual compounds. Quantitative calibrations were performed in a standard matrix and showed excellent linearity and precision over the relevant concentration range to control the regulated MRL levels.

The high matrix selectivity of the TSQ 8000 system allowed for reduced sample preparation, providing high recoveries for a wide range of chemically diverse pesticide compounds. The very high matrix selectivity delivered low chemical matrix background with well-defined pesticide peaks that were safe and easy to integrate, thus eliminating the need for time-consuming manual baseline corrections.

Positive pesticide compound signals were confirmed by TraceFinder software checking the calibrated ion ration of the two monitored SRM transitions.

The TSQ 8000 GC-MS/MS system is well prepared for routine analysis and provides high robustness of the chromatographic system and ion source, thus reducing the need for frequent maintenance and avoiding system downtime for high sample throughput and productivity. The system is easy to use, durable, and robust even with the most challenging sample types and is fully automated in sampling capabilities to found and not-found report generation.

References

1. SANCO Document N° SANCO/12495/2011, Method Validation and Quality Control Procedures for Pesticide Residue Analysis in Food and Feed, Implemented by 01/01/2012.
2. Codex Alimentarius (www.codexalimentarius.net/mrls/pesticides/jsp/pest-q-e.jsp)
3. Pesticide determination according to § 64 LFGB L 00.00-34 (German legislation) Modul E9 (ASE); GPC
4. Pesticide Method Reference, 2nd Edition, 2011 Thermo Fisher Scientific, p/n 120390.

Appendix: List of pesticides with MRM transitions used (from [4])

Pesticide Name	RT (min)	Precursor Mass (*m/z*)	Product Mass (*m/z*)	Collision Energy (V)
Difluorobenzamid Degradation (Isocyanat)	6.93	152.93	90.01	20
Difluorobenzamid Degradation (Isocyanat)	6.93	152.93	125.01	20
Carbofuran 1	8.80	149.06	121.05	10
Carbofuran 1	8.80	164.08	149.07	10
Difluorobenzamid Degradation	8.62	141.00	63.11	25
Difluorobenzamid Degradation	8.62	141.00	113.09	15
Biphenyl-d10_ISTD	9.24	160.00	160.16	10
Biphenyl	9.28	154.08	153.08	15
Biphenyl	9.28	153.08	152.08	15
Carbofuran-3-hydroxy 1	10.43	137.05	81.01	18
Carbofuran-3-hydroxy 1	10.43	180.05	137.01	15
Tetrahydrophthalimid	10.84	151.04	79.01	25
Tetrahydrophthalimid	10.84	151.04	122.09	10
O-Phenylphenol	11.00	170.07	141.06	20
O-Phenylphenol	11.00	170.07	115.05	20
Molinate	11.10	187.10	126.07	10
Molinate	11.10	126.07	98.05	5
Chlorfenprop methyl	11.59	196.00	165.00	10
Chlorfenprop methyl	11.59	165.00	137.00	10
Fenobucarb	11.20	121.07	77.05	15
Fenobucarb	11.20	150.09	121.07	10
Propachlor	11.76	176.06	120.04	10
Propachlor	11.76	120.04	92.03	10
Propachlor	11.76	169.06	120.04	10
Propachlor	11.76	196.07	120.04	10
Cycloate	11.98	154.10	83.05	10
Cycloate	11.98	215.13	154.10	5
Diphenylamin	11.49	169.01	168.09	20
Diphenylamin	11.49	169.01	167.09	20
Chloropropham	12.26	213.06	127.03	15
Chloropropham	12.26	213.06	171.04	10
Phosmet-oxon	12.09	160.00	132.96	15
Phosmet-oxon	12.09	104.00	75.88	10
Phosmet-oxon	12.09	160.00	76.96	20
Prometon	13.10	225.16	183.13	10
Prometon	13.10	225.16	210.15	10
Carbofuran 2	13.13	149.06	121.05	10
Carbofuran 2	13.13	164.08	149.07	10
Profluralin	13.22	318.10	199.06	15
Profluralin	13.22	330.23	252.45	25
Swep	13.46	187.05	123.95	18
Swep	13.46	219.11	174.02	15
Trietazine	13.48	229.14	200.14	15
Trietazine	13.48	214.14	186.10	15
Dimethipin	13.53	117.98	57.97	10
Dimethipin	13.53	210.10	76.02	10
Terbutylazin	12.97	214.10	132.06	10
Terbutylazin	12.97	214.10	104.05	10
Propyzamid	13.04	173.01	145.01	15
Propyzamid	13.04	173.01	109.01	18
Propyzamid	13.04	175.02	147.01	15
Propyzamid	13.04	254.02	226.02	15
Isocarbamide	13.67	142.03	70.01	15
Isocarbamide	13.67	142.03	113.01	10
Dinoseb	13.92	211.13	116.99	15
Dinoseb	13.92	211.13	163.11	10
Terbazil	13.42	161.05	88.03	15
Terbazil	13.42	160.05	76.02	15
Bromocylen	14.37	358.79	242.85	15
Bromocylen	14.37	356.93	241.24	15
Dimethenamid	14.60	230.06	154.04	10
Dimethenamid	14.60	232.06	154.04	10
Dimethachlor	14.61	197.08	148.06	10
Dimethachlor	14.61	199.08	148.06	10
Acetochlor	14.65	174.11	146.15	15
Acetochlor	14.65	223.19	147.17	10
Desmetryn	14.68	213.11	171.08	10
Desmetryn	14.68	213.11	198.10	10
Flurprimidol	14.77	269.12	106.98	20
Flurprimidol	14.77	270.18	107.04	20
Alachlor	14.26	188.10	160.07	10
Alachlor	14.26	188.10	130.12	25
Alachlor	14.26	237.14	160.15	10
Metribuzin	14.14	198.08	82.03	20
Metribuzin	14.14	198.08	89.04	16
Propanil	15.00	217.01	161.00	10
Propanil	15.00	219.01	163.00	10
Fipronildesulfinyl	14.15	333.00	231.20	20
Fipronildesulfinyl	14.15	333.00	281.30	20
Carbofuran-3-hydroxy 2	15.02	137.05	81.01	18
Carbofuran-3-hydroxy 2	15.02	180.05	137.01	15
Prometryn	14.49	241.14	184.10	15
Prometryn	14.49	226.13	184.10	12
Tridiphan	15.18	186.94	158.94	15
Tridiphan	15.18	219.09	184.09	20
Ethofumesat	14.80	206.82	160.86	10
Ethofumesat	14.80	285.75	206.82	12
Pentanochlor	15.73	141.05	106.05	15
Pentanochlor	15.73	239.05	141.05	15
Chlorpyrifos	15.78	257.97	165.98	20
Chlorpyrifos	15.78	314.05	258.18	15
Bromacil	15.03	205.01	188.01	15
Bromacil	15.03	207.01	190.01	15

Pesticide Name	RT (min)	Precursor Mass (*m/z*)	Product Mass (*m/z*)	Collision Energy (V)
Anthrachinon	15.44	207.97	151.99	20
Anthrachinon	15.44	180.04	152.05	15
Anthrachinon	15.44	207.97	180.10	10
Nithrothal isopropyl	16.09	236.08	194.07	10
Nithrothal isopropyl	16.09	236.08	148.05	20
Triadimefon	15.41	208.07	181.06	10
Triadimefon	15.41	210.07	183.06	10
Tiocarbazil	16.15	156.08	100.05	8
Tiocarbazil	16.15	279.10	156.07	6
Tetraconazol	15.39	336.02	218.01	20
Tetraconazol	15.39	338.02	220.01	20
Butralin	15.54	266.14	220.11	15
Butralin	15.54	266.14	190.10	15
Dicapthon	15.44	262.00	262.00	9
Dicapthon	15.44	262.00	216.00	13
Crufomat	16.30	256.20	226.15	25
Crufomat	16.30	276.20	182.09	10
Allethrin	16.17	123.07	80.98	10
Allethrin	16.17	136.04	92.98	10
Dinobuton	16.89	163.06	116.04	15
Dinobuton	16.89	211.07	117.04	18
Penconazol	16.89	248.06	157.04	25
Penconazol	16.89	248.06	192.04	15
Pyrifenox 1	16.17	262.03	192.02	20
Pyrifenox 1	16.17	262.03	200.02	20
Pyrifenox 2	16.81	262.03	192.02	20
Pyrifenox 2	16.81	262.03	200.02	20
Tolylfluanid	16.92	238.09	137.05	15
Tolylfluanid	16.92	240.09	137.05	15
Fipronil	17.01	368.95	214.97	30
Fipronil	17.01	366.95	254.96	25
Triflumizol	17.20	206.05	179.04	15
Triflumizol	17.20	179.04	144.04	15
Procymidon	17.22	283.05	95.93	10
Procymidon	17.22	285.05	95.97	10
Procymidon	17.22	285.05	257.30	10
Triadimenol 1	16.45	168.11	69.99	15
Triadimenol 1	16.45	128.05	100.04	10
Triadimenol 2	16.64	168.11	69.99	15
Triadimenol 2	16.64	128.05	100.04	10
Butachlor	17.54	237.13	160.09	10
Butachlor	17.54	176.09	146.08	10
Chlorbenside	17.57	124.97	88.98	20
Chlorbenside	17.57	124.97	63.02	30
Fenothiocarb	17.68	160.07	72.01	15
Fenothiocarb	17.68	160.07	106.00	10
Picoxystrobin	17.69	335.09	303.09	10
Picoxystrobin	17.69	303.09	157.04	20
Paclobutrazole	17.75	236.10	125.06	15

Pesticide Name	RT (min)	Precursor Mass (*m/z*)	Product Mass (*m/z*)	Collision Energy (V)
Paclobutrazole	17.75	238.11	127.06	15
Chinomethionat	17.78	206.06	147.98	15
Chinomethionat	17.78	234.08	206.06	10
Napropamid	18.07	271.16	128.07	5
Napropamid	18.07	128.07	72.04	10
Flutriafol	18.11	219.07	123.04	15
Flutriafol	18.11	123.04	75.03	15
Flurodifen	18.14	190.02	126.01	10
Flurodifen	18.14	190.02	146.01	5
Bisphenol A	18.17	213.14	119.06	15
Bisphenol A	18.17	213.14	164.99	20
Bisphenol A	18.17	228.15	213.07	10
Chlorfenson_ISTD	18.20	302.00	110.90	20
Hexaconazol	18.22	214.08	159.07	20
Hexaconazol	18.22	214.08	151.98	25
Imazalil	18.24	172.96	144.96	15
Imazalil	18.24	172.96	108.95	25
Isoprothiolan	18.24	203.99	117.95	7
Isoprothiolan	18.24	203.99	84.90	25
Isoprothiolan	18.24	290.06	118.03	15
Flamprop-methyl	18.39	230.05	170.04	10
Flamprop-methyl	18.39	276.06	105.02	10
Kresoximmethyl	18.48	206.10	131.09	15
Kresoximmethyl	18.48	206.10	116.01	10
Buprofezin	18.51	175.08	116.96	20
Buprofezin	18.51	175.08	131.99	15
Buprofezin	18.51	249.16	105.93	20
Buprofezin	18.51	249.16	193.20	10
Uniconazol	18.57	234.12	136.99	15
Uniconazol	18.57	234.12	101.95	25
Uniconazol	18.57	234.12	165.08	10
Cinerin 1	18.60	123.08	95.06	10
Cinerin 1	18.60	123.08	81.05	10
Cinerin 1	18.60	150.10	108.09	10
Flusilazol	18.60	233.16	165.13	25
Flusilazol	18.60	233.16	152.06	20
Myclobutanil	18.65	179.00	125.00	15
Myclobutanil	18.65	179.00	89.95	25
Methoprotryne	18.66	256.14	212.11	15
Methoprotryne	18.66	256.14	200.11	15
Diclobutrazol	18.75	270.07	159.04	15
Diclobutrazol	18.75	272.08	161.04	15
Azaconazole	18.78	217.02	173.01	15
Azaconazole	18.78	219.02	175.01	15
Perthane	18.95	223.15	179.10	18
Perthane	18.95	223.15	167.06	18
Cyproconazol	19.14	222.09	125.05	20
Cyproconazol	19.14	224.09	127.05	20
Flamprop-isopropyl	19.14	276.08	105.03	15

Pesticide Name	RT (min)	Precursor Mass (*m/z*)	Product Mass (*m/z*)	Collision Energy (V)
Flamprop-isopropyl	19.14	278.17	104.99	20
Chloropropylat	19.16	251.02	139.01	20
Chloropropylat	19.16	251.02	111.01	20
Ancymidol	19.18	228.15	121.02	15
Ancymidol	19.18	215.15	107.02	15
Chlorbenzilat	19.22	251.02	139.01	20
Chlorbenzilat	19.22	251.02	111.01	20
Cyprofuram	19.36	211.12	132.02	10
Cyprofuram	19.36	211.12	166.05	10
Etaconazol 1	19.38	245.04	173.03	15
Etaconazol 1	19.38	245.04	191.03	10
Etaconazol 2	19.38	245.04	173.03	15
Etaconazol 2	19.38	245.04	191.03	10
Diniconazol	19.47	268.06	232.05	15
Diniconazol	19.47	270.06	234.05	15
Jasmolin 1	19.58	123.08	81.05	10
Jasmolin 1	19.58	123.08	95.06	10
Jasmolin 1	19.58	164.16	109.15	10
Aclonifen	19.70	212.02	182.02	10
Aclonifen	19.70	264.03	194.02	15
Tetrasul	19.85	251.92	216.93	20
Tetrasul	19.85	253.92	218.93	20
Carfentrazone ethyl	19.95	340.03	312.03	10
Carfentrazone ethyl	19.95	312.15	150.99	20
Benodanil	19.99	322.98	230.99	15
Benodanil	19.99	322.98	195.99	5
Trifloxystrobin	20.02	222.13	162.14	10
Trifloxystrobin	20.02	115.99	88.95	15
Trifloxystrobin	20.02	222.13	130.02	15
Chlordecone	20.06	271.91	237.16	15
Chlordecone	20.06	273.91	239.15	20
Famophos (Famphur)	20.16	218.07	108.94	15
Famophos (Famphur)	20.16	218.07	126.95	20
Iprodion Degradation	18.63	186.87	123.99	20
Iprodion Degradation	18.63	186.87	159.02	15
Iprodion Degradation	18.63	243.94	187.02	10
Iprodion	20.57	314.06	245.25	15
Iprodion	20.57	186.99	123.87	20
Iprodion	20.57	316.00	247.35	15
Iprodion	20.57	316.00	273.11	10
Propiconazol 1	19.38	259.02	173.02	20
Propiconazol 1	19.38	172.94	144.91	15
Propiconazol 2	19.54	259.02	173.02	20
Propiconazol 2	19.54	172.94	144.91	15
Pyraflufen-ethyl	20.30	412.02	349.02	15
Pyraflufen-ethyl	20.30	349.02	307.02	15
Clodinafop-propargyl	20.36	349.05	266.04	15
Clodinafop-propargyl	20.36	349.05	238.04	15
Lenacil	20.70	153.05	136.06	15

Pesticide Name	RT (min)	Precursor Mass (*m/z*)	Product Mass (*m/z*)	Collision Energy (V)
Lenacil	20.70	153.05	135.15	15
Diclofop methyl	20.77	253.02	162.01	15
Diclofop methyl	20.77	340.04	253.02	15
Propargit	20.79	173.08	135.04	15
Propargit	20.79	173.08	106.93	20
Propargit	20.79	350.21	173.10	15
Diflufenican	20.83	394.07	266.05	10
Diflufenican	20.83	266.05	246.05	10
Piperonylbutoxid	20.87	176.11	131.08	15
Piperonylbutoxid	20.87	176.11	103.06	10
Piperonylbutoxid	20.87	176.11	145.09	15
Tebuconazol	20.97	250.12	125.06	20
Tebuconazol	20.97	252.12	127.06	20
Nitralin	21.09	316.02	274.15	10
Nitralin	21.09	273.99	216.07	10
Benzoylpropethyl	21.22	292.05	105.02	15
Benzoylpropethyl	21.22	172.03	145.02	14
Captafol	21.22	311.06	78.94	20
Captafol	21.22	311.06	276.21	10
Epoxyconazol	21.29	192.04	138.03	10
Epoxyconazol	21.29	192.04	111.02	10
Bromuconazol 1	21.73	294.96	174.98	15
Bromuconazol 1	21.73	292.96	172.98	15
Brompropylat	21.76	340.93	183.05	20
Brompropylat	21.76	340.93	185.04	20
Etoxazol	21.83	300.14	270.38	20
Etoxazol	21.83	330.17	300.44	25
Fenoxycarb	21.85	186.08	109.05	15
Fenoxycarb	21.85	255.11	186.08	10
Phosmet	20.79	160.00	133.00	15
Phosmet	20.78	160.00	104.00	20
Phosmet	20.78	316.99	160.00	5
Fenpiclonil	21.94	235.99	200.99	15
Fenpiclonil	21.94	237.99	200.99	15
Fenazaquin	22.22	160.09	145.08	10
Fenazaquin	22.22	145.05	116.99	15
Fenazaquin	22.22	160.09	117.08	20
Phenothrin 1	22.27	183.10	153.08	18
Phenothrin 1	22.27	183.10	165.09	10
Phenothrin 2	22.42	183.10	153.08	18
Phenothrin 2	22.42	183.10	165.09	10
Bromuconazol 2	22.35	294.97	174.97	15
Bromuconazol 2	22.35	292.97	172.97	15
Metconazol	22.41	125.00	88.93	20
Metconazol	22.41	250.20	124.88	25
Triticonazole	22.80	235.10	217.09	10
Triticonazole	22.80	235.10	182.07	10
Pyriproxyfen	22.82	226.15	186.22	15
Pyriproxyfen	22.82	136.00	95.95	15

Pesticide Name	RT (min)	Precursor Mass (*m/z*)	Product Mass (*m/z*)	Collision Energy (V)
Azinphosmethyl	22.95	160.00	132.00	10
Azinphosmethyl	22.95	160.00	104.64	10
Pyriproxyfen	23.06	136.00	77.92	20
Fenamirol	23.55	251.02	139.01	15
Fenamirol	23.55	330.03	139.01	10
Pyridaben	24.50	364.14	309.12	5
Pyridaben	24.50	309.12	147.06	15
Fluquinconazol	24.59	340.01	298.01	22
Fluquinconazol	24.59	342.01	300.01	22
Etofenprox	26.05	163.09	107.06	16
Etofenprox	26.05	163.09	135.07	10
Etofenprox	26.05	376.14	135.02	30
Etofenprox	26.05	376.14	163.09	10
Silafluofen	26.25	179.00	151.00	7
Silafluofen	26.25	286.13	258.12	15
Difenconazol 1	26.91	323.05	265.04	15
Difenconazol 1	26.91	325.05	267.04	20
Difenconazol 2	27.05	323.05	265.04	15
Difenconazol 2	27.05	325.05	267.04	20
Indoxacarb	28.55	264.02	176.14	10
Indoxacarb	28.55	264.02	148.03	20
Indoxacarb	28.55	321.05	289.34	10

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Analysis of Pesticide Residues in Lettuce Using a Modified QuEChERS Extraction Technique and Single Quadrupole GC/MS

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Key Words

- DSQ II GC/MS
- QuanLab Forms
- Food Safety
- QuEChERS
- Pesticide Residue Analysis

Introduction

The determination of pesticides in fruits and vegetables has been simplified by a new sample preparation method, QuEChERS (**Qu**ick, **E**asy, **Ch**eap, **E**ffective, **R**ugged and **S**afe), and published recently as AOAC Method 2007.01.[1] The sample preparation is shortened by using a single step buffered acetonitrile (MeCN) extraction and liquid-liquid partitioning from water in the sample by salting out with sodium acetate and magnesium sulfate ($MgSO_4$).[1] This technical note describes the application of the QuEChERS sample preparation procedure to analysis of pesticide residues in a lettuce matrix using gas chromatography/mass spectrometry (GC/MS) on the Thermo Scientific TRACE GC Ultra™ and Thermo Scientific DSQ™ II single quadrupole mass spectrometer. Thermo Scientific QuanLab Forms 2.5 software was used for data review and reporting. The MeCN extract is solvent exchanged to hexane/acetone for splitless injection with detection by electron ionization and selected ion monitoring (SIM).[2] A calibration curve was constructed in iceberg lettuce and then the precision and accuracy of the analytical method were tested by preparing matrix spikes at 5 ng/g and 50 ng/g.

Experimental Conditions

During the method validation, several experiments were performed to determine the effect of minor modifications to the QuEChERS method which may impact the performance of the analysis in the laboratory. The recommended consumables required for sample preparation and analysis were rigorously tested (Table 1). A list of the pesticides to be studied was created that would address various functional groups of most pesticides. A surge splitless injection was made into a Thermo Scientific TRACE™ TR-Pesticide capillary column (5% diphenyl/95% dimethyl polysiloxane column, (0.25 mm x 30 m, and a film thickness of 0.25 µm) with a guard column (0.25 mm x 5 m). The closed exit ion volume was used on the DSQ II. In order to test the implementation of the QuEChERS method, each facet of the method was evaluated to determine if any error may arise from slight modifications of the method. Since there are so many steps from sample preparation to actual detection on the MS, each portion of the method was studied separately. The following sections were evaluated:

- Sample Extraction and Clean Up
- Solvent Exchange
- Injection
- Separation
- Detection

Item Descriptions
TRACE TR-Pesticide (0.25 mm x 30 m, 0.25 µm with 5 m guard column)
5 mm ID liner, 105 mm long (pk of 5)
10 µL syringe
Septa (pk of 50)
Liner graphite seal (pk of 10)
Closed Exit Ion Volume and ion volume holder for DSQ II
Graphite ferrule 0.1-0.25 (pk of 10)
Ferrule, 0.4 mm ID 1/16 G/V
Blank vespel ferrule for MS Interface
2 mL amber glass vial, silanized glass, with write-on patch (pk of 100)
Blue cap with ivory PTFE/red rubber seal (pk of 100)
Acetonitrile analytical grade (4L)
Hexane GC Resolv* Grade (4L)
Acetone GC Resolv* Grade (4L)
Organic bottle top dispenser
HPLC grade glacial acetic acid
50 ml FEP centrifuge tubes (pk of 2)
Clean up tube: 15 mL tubes ENVIRO 900 mg $MgSO_4$, 300 mg PSA 150 mg C18 (pk of 50)
50 mL PP tubes 6 g $MgSO_4$, 1.5 g $CH_3CHOONa$ (anhydrous) (pk of 250)
Clean up tube: 2 mL tubes 150 mg $MgSO_4$, 50 mg PSA (pk of 100)

Table 1: Consumables for QuEChERS Sample Prep and Analysis

Sample Extraction and Clean Up

The QuEChERS sample prep procedure consists of the steps shown in Figure 1. There are three main parts: the extraction, clean up, and solvent exchange from acetonitrile (MeCN) to a solvent mixture of hexane and acetone (9:1). The solvent exchange provides a more amenable solvent for the splitless injection. Care must be taken to adequately homogenize the sample to the consistency of baby food or purée.

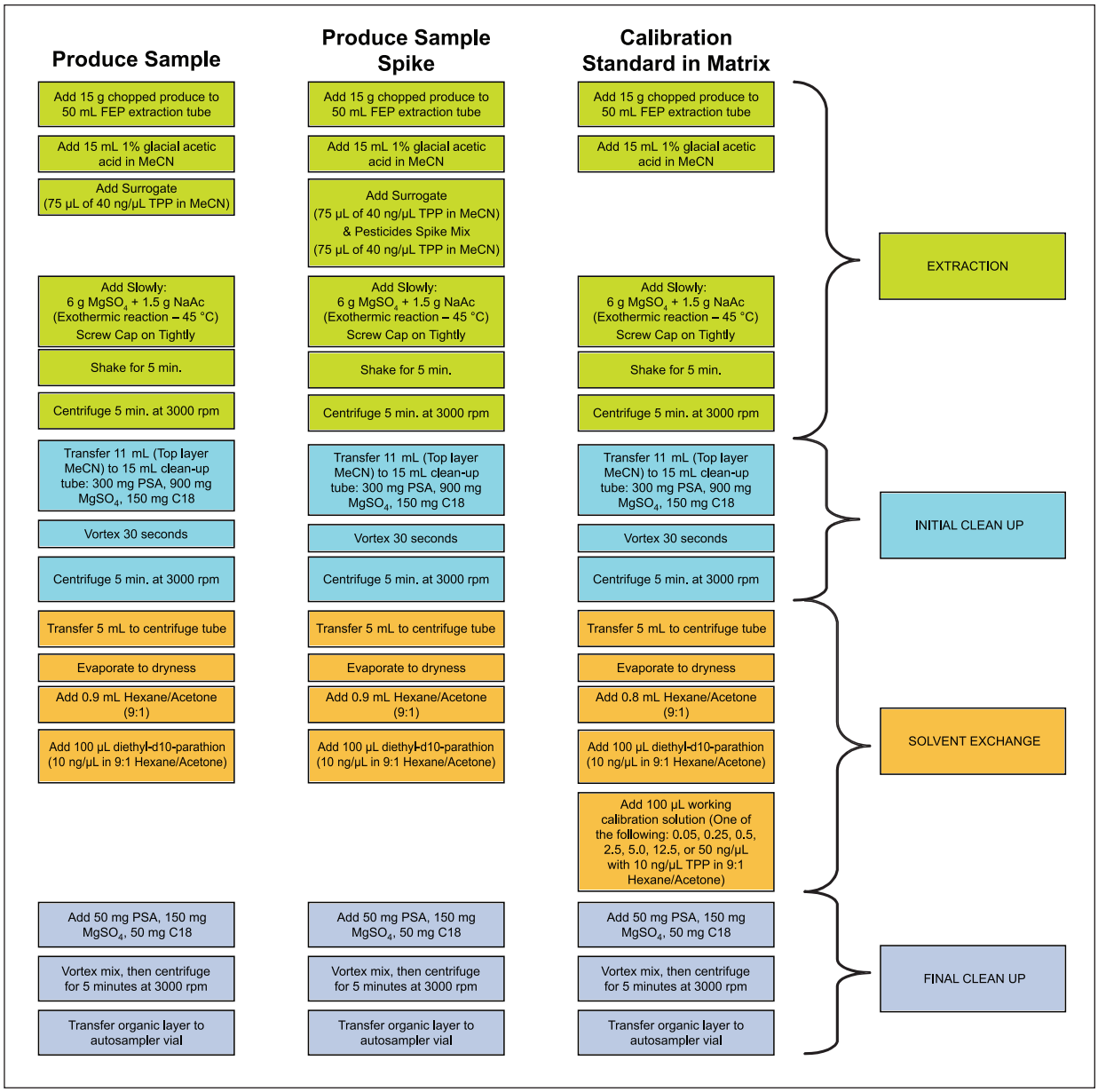

Figure 1: Flow Diagram of Modified QuEChERS Sample Prep

During the extraction phase of the sample preparation, an observation was made that if the MeCN extract was poured into the $MgSO_4$, poor spike recoveries were observed. This is due to the exothermic reaction of the water in the sample and $MgSO_4$. Although most vendors offer the pre-measured powder reagents in a separate capped extraction tube; these tubes should not be used, only the reagent in them. A change was implemented to add an empty 50 mL FEP extraction tube to the list of consumables for the sample preparation (Table 1). A well-homogenized 15 g sample of iceberg lettuce was weighed into this extraction tube. Then 15 mL of 1% glacial acetic acid:MeCN extraction solvent were poured into the tube on top of the sample. The surrogate was spiked into this MeCN layer along with the pesticide solution for the determination of the Method Validation Detection (MVD) and Limit of Detection (LOD). Then the tube was capped and vortex for 30 seconds.

The cap was removed and the powder reagents were poured slowly into the MeCN layer. The cap was tightened securely on the 50 mL extraction tube, and then it was vortexed for 30 seconds until all of the powder reagents were mixed with the liquid layers. The tubes were placed on a mechanical shaker for 5 minutes. Then the tubes were centrifuged for 5 minutes at 3000 rpm. Next 11 mL of the top MeCN layer were removed and transferred to a 15 mL clean up tube. This tube was capped and vortexed for 30 seconds and then centrifuged for 5 minutes at 3000 rpm. Then 5 mL of the top layer were transferred into a clean test tube for solvent exchange.

Solvent Exchange

The 5 mL aliquot of cleaned up extract was blown down to dryness with a gentle stream of nitrogen at 40 °C in about one hour. Care was taken to not allow the tube to remain dry for more than a few minutes. 900 µL of hexane/acetone (9:1) were added and then 100 µL of the internal standard solution, d10-parathion, were spiked into the organic solution. The individual calibration levels were spiked in at this point for preparation of the calibration curve in matrix (Figure 1). The tube was capped and vortexed for 15 seconds. Then the 1 mL of extract was transferred to a 1 mL clean up tube, capped tightly, and vortexed for 30 seconds. After centrifuging for 5 minutes at 3000 rpm, 200 µL of the light green clear extract was transferred to an autosampler vial with a small glass insert for injection onto the GC/MS.

Injection

The injection must be optimized to inject the high and low molecular weight pesticides. The inlet temperature was set to 250 °C. This temperature was adequate to vaporize all of the pesticides studied. The 5 mm i.d. splitless liner with a volume of 1.6 mL was selected for the surged pressure injection. The inlet was set at an elevated pressure of 250 kPa for the 0.5 minute injection time. The vapor cloud is actually reduced for the 2 µL injection from 0.49 to 0.19 mL using this surge pressure injection mode. Then at an elevated injection flow rate of 4.7 mL/min, the liner is swept 1.5 times during the injection time. The target compounds move through the inlet so rapidly (10 seconds) that they do not have time to interact with the inside walls of the liner. The result is reduced breakdown of the more fragile pesticides. A Performance Solution was run at the beginning of each shift to test the endrin breakdown. This test proved that no maintenance was required. The results were < 5% endrin breakdown on a daily basis. This is determined by adding up the response for the two breakdown products – endrin aldehyde and endrin ketone – and dividing by the total response for the breakdown products and endrin in percent. Usually the liner is changed when the breakdown reaches > 20%. The injection port liner tested showed very good results, with minimal breakdown (Figure 2).

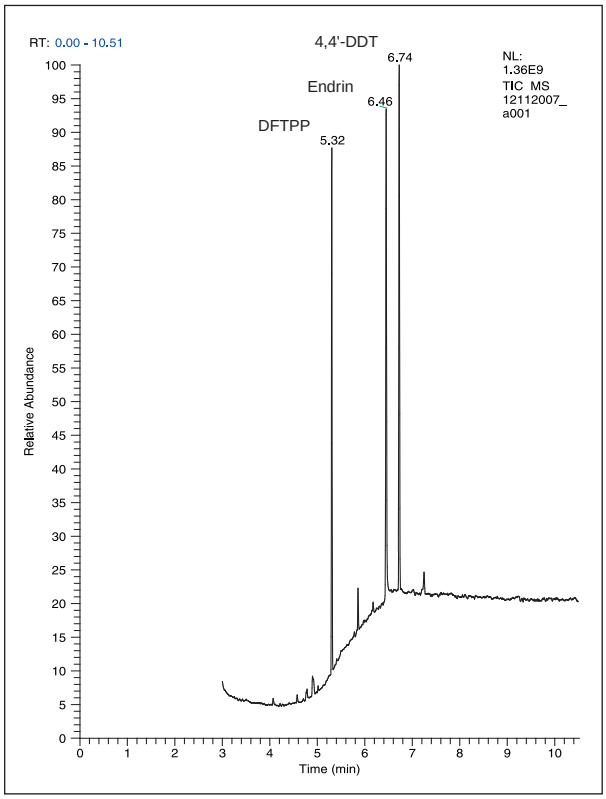

Figure 2: Total ion chromatogram of endrin breakdown QC test, demonstrating low system activity

AS 3000 Autosampler

Sample Volume	2 µL
Plunger Strokes	10
Viscous Sample	no
Sampling Depth in Vial	bottom
Injection Depth	standard
Pre-inj Dwell Time	0
Post-inject Dwell Time	0
Pre-inject Solvent Wash Vial Position	A
Pre-inject Solvent Wash Cycles	0
Sample Rinses	0
Post-inject Solvent	A
Post-inject Solvent Cycles	10

TRACE GC Ultra Gas Chromatograph

Column	TRACE TR-Pesticide 0.25 mm x 30 m, 0.25 µm with Integra-Guard Column (0.25 mm x 5 m)
Column Constant Flow	1 mL/min.
Oven Program	40°, 1.5 min., 25°/min.; 150°, 0.0 min., 7°/min., 225°, 0 min.; 25°/min., 290°, 10 min.
S/SL Temperature	250°
S/SL Mode	Splitless with Surge Pressure
Surge Pressure	250 kPa
Inject Time	0.5 min.
Split Flow	50 mL/min.
Transferline Temperature	290°

DSQ II Mass Spectrometer

Source Temperature	250°
Ion Volume	CEI
Emission Current	50 µA
Detector Gain	3 (1674V)
Lens 1	-25V
Lens 2	-5.4V
Lens 3	-25V
Prefilter Offset	-5.5
Electron Lens	15V
Electron Energy	-70V
Resolution Factors	Start Mass 1: 1.0, Ion Offset 1: 3.6, Res Factor 1: 1.89; Start Mass 2: 1050, Ion Offset 2: 3.6, Res Factor 2: 2.1
Tuning Factors	NA
Filament Delay Time	5.5 min.
End of Run Filament Off	25 min.
Tune	Autotune
Scan Parameters	(see Table 3)

Table 2: Selected instrument parameters for DSQ II, TRACE GC Ultra and Thermo Scientific AS 3000 autosampler

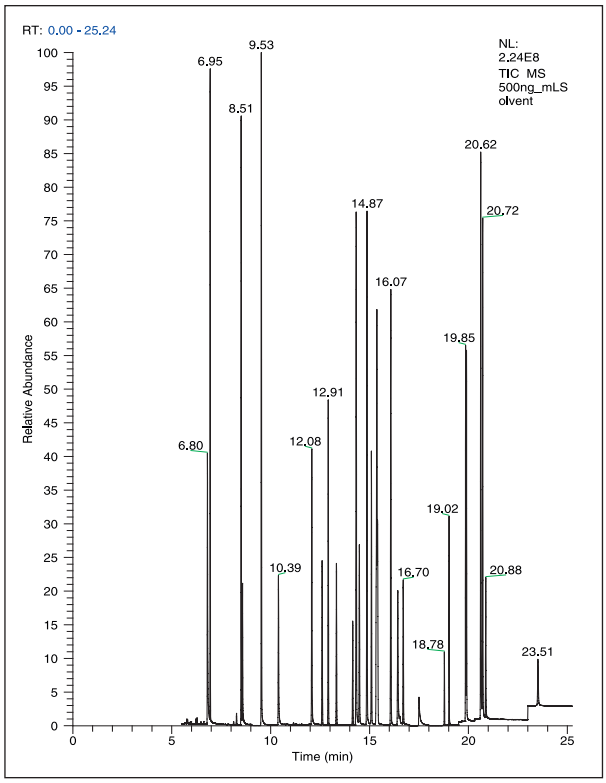

Figure 3: Pesticide Standard in Solvent at 500 ng/g (TIC of SIM)

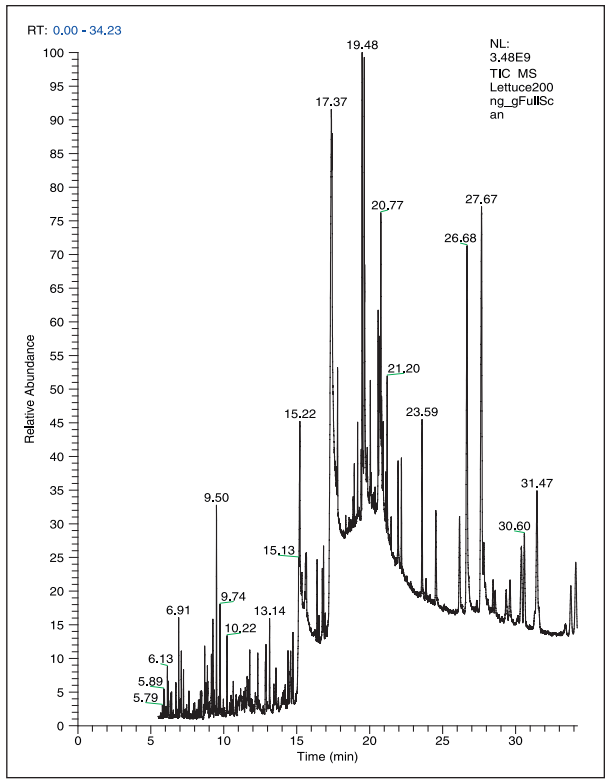

Figure 4: Iceberg Lettuce Matrix Spike at 200 ng/g in Full Scan

Separation

The separation was achieved by using a 5% diphenyl/ 95% dimethyl polysiloxane column, (0.25 mm x 30 m, and a film thickness of 0.25 µm) with a guard column (0.25 mm x 5 m). It is a non-polar phase and works quite well for heavily chlorinated pesticides. Some interactions within the stationary phase showed a loss of some pesticides at concentrations below 100 pg. These losses may be overcome by the addition of protectants.[5] The matrix-spiked calibration curve gave better linear fits than observed with the pesticide standards made in solvent only. This was due to the interaction of the matrix with the stationary phase, tying up active sites during the elution of the pesticide. The inlet was set at 250 °C and the MS source at 250 °C. The oven was programmed: 40 °C, 1.5 min., 15 °C/min., 150 °C; 7 °C/min., 225 °C; 25 °C/min., 290 °C, 15 min with a constant column flow rate of 1 mL/min.

The remaining instrument parameters are listed in Table 2. Separation of the pesticides studied was sufficient to set up the SIM ion windows for the analysis (Table 3). Deterioration of the peak shape that was observed for some pesticides when injected in solvent only was not observed when co-injected with matrix. A probable explanation is some activity in the flow path through the column. A total ion chromatogram (TIC) of the standard in solvent at 500 ng/mL is shown in Figure 3. An injection of the matrix extract in Full Scan was used to set the final hold temperature for the oven program (Figure 4). The filament was turned off after elution of the last pesticide in the final SIM method to help keep the mass spectrometer clean.

Compound	Retention Time	Segment #	Start Time (min.)	Quan Ion m/z	Quan Ion %	Qualifier Ions m/z	Qualifier Ions %	Qualifier Ions m/z	Qualifier Ions %	Width (amu)	Dwell Time (ms)
mevinphos	8.7	2	8.00	127	100	192	28	109	31	0.5	10
dimethoate	12.36			125	42	87	100	93	57	0.5	10
gamma BHC	12.86			219	49	181	100	217	40	0.5	10
diazinone	13.16	5	12.95	179	100	137	99	152	59	0.5	10
vinclozolin	14.42	6	14.00	285	41	178	99	212	100	0.5	10
metalaxyl	14.76			206	100	160	87	220	44	0.5	10
methiocarb	15.15	7	14.90	168	100	109	32	153	67	0.5	10
dichlofluanid	15.38			123	100	167	50	224	23	0.5	10
d10-parathion	15.61			301	40	99	100			0.5	10
cyprodinil	16.38	8	15.90	224	100	210	12	226	8	0.5	10
imazalil	17.72	9	17.20	215	100	173	86	217	63	0.5	100
endosulfan sulfate	18.95	10	18.50	272	100	274	75	229	71	0.5	50
TPP	19.17			326	100	325				0.5	50

Table 3: DSQ II SIM parameters for pesticides, surrogate and internal standard

Detection

The mass spectrometer scan speed was adjusted to accurately detect co-eluting pesticides. Ion ratios were monitored to prevent false positives from matrix interferences. The identification of the pesticides was performed by selected ion monitoring (SIM) by setting up discrete retention time windows and scanning events for prominent ions present in the pesticide (Table 3). Some overlays of ion ratio tests are shown in Figure 5. The closed exit ion volume was used on the DSQ II with an emission current of 50 µA.

Figure 5: Overlay of Ion Ratios for chlorothalonil (5 ng/g)

Results and Discussion

A calibration curve was prepared in lettuce matrix and analyzed using Thermo Scientific QuanLab™ Forms reporting software, which measured the Pass/Fail of multiple Quality Control (QC) criteria specified in both AOAC Method 2007.01 and the European mass spectrometry identification criteria for SIM.[1,3] The internal standard used in the method was parathion-d10, and triphenylphosphate (TPP) served as the surrogate. Quantitation was based on linear least squares calibration with a correlation coefficient of $R^2 > 0.99$ for most pesticides. The average Limit of Detection (LOD) was 1.1 ng/g, well below most Method Regulatory Limits (MRLs) specified in CODEX.[4] The average Limit of Quantitation (LOQ) was 3.6 ng/g. The Method Validation study of four replicate analyses of 50 ng/g showed an average relative percent standard deviation of 10.5% and percent recoveries ranged from 68-102%, with an average percent recovery of 88%.

Linearity

The method specifies preparation of the calibration curve in matrix. They were prepared as shown in Figure 1. The average R^2 was 0.997. The results of the linearity study are shown in Table 4. Some typical calibration curve plots are shown for dimethoate and vinclozolin in Figures 6 and 7, respectively.

Figure 6: QuanLab Forms Data Review showing Dimethoate at 1 ng/g, with linearity from 1 ng/g to 75 ng/g

Figure 7: QuanLab Forms Data Review showing vinclozolin at 1 ng/g, with linearity from 1 ng/g to 75 ng/g

Component in Lettuce Matrix	Linearity (R^2)
mevinphos	0.9942
gamma BHC	0.9964
diazinone	0.9972
vinclozolin	0.9962
metalaxyl	0.9988
methiocarb	0.9956
dichlofluanid	0.9975
cyprodinil	0.9982
imazalil	0.9971
endosulfan sulfate	0.9972
Average	**0.9968**

Table 4: Pesticide calibration curve results, using linear least squares fit

MVDs

The replicate analyses of four matrix spikes at 50 ng/g provide information on the accuracy and precision of the method. In Table 5, the average calculated amount for the 50 ng/g spike in matrix was 44 ng/g. The percent recovery ranged from 68 to 102% with an average recovery of 88%. The precision of the MVD study was 10.5%RSD.

LOQs and LODs

The actual Limit of Quantitation (LOQ) was determined by preparing matrix spikes at a level near the expected detection limit. A concentration of 5 ng/g was analyzed in eight matrix samples and the LOD and LOQ were calculated from these results by multiplying the standard deviation by 3 and 10 respectively. The average calculated concentration of the spike was 5.4 ng/g. The average precision was 7.0%RSD and the average LOD was 1.1 ng/g with an average LOQ of 3.6 ng/g. The Method Regulatory Limits (MRLs) for the pesticides and the results of this study are shown in Table 6.

Component in Lettuce Matrix	Average Concentration (ng/g)	Theoretical Concentration (ng/g)	% RSD	% Recovery
mevinphos	42.5	50	11.0	85
gamma BHC	49.5	50	6.4	99
diazinone	51.1	50	6.1	102
vinclozolin	51.0	50	12.4	102
metalaxyl	44.9	50	4.8	90
methiocarb	38.9	50	14.8	78
dichlofluanid	41.4	50	13.4	83
cyprodinil	47.6	50	7.7	95
imazalil	34.1	50	12.0	68
endosulfan sulfate	39.3	50	16.3	79
Average	44.01		10.51	88.03

Table 5: Method Validation Results for pesticides in lettuce matrix

						WHO	Japan	EU	EU	US-EPA
Component	Ave. Conc. (ng/g)	Std. Dev.	% RSD	LOD	LOQ (ng/g)	MRL[1] (ng/g)	MRL[2] (ng/g)	MRL[3] (ng/g)	LOD_3	MRL[4] (ng/g)
mevinphos	4.21	0.61	14.5	1.83	6.10		400	500		
gamma BHC	5.26	0.368	7.0	1.10	3.68		2000	10	10	3000
diazinone	5.26	0.32	6.1	0.96	3.20	500	100			700
vinclozolin	5.97	0.205	3.4	0.62	2.05	5000	5000			
metalaxyl	5.12	0.24	4.7	0.72	2.40	2000	2000	1000	50	5000
methiocarb	5.47	0.21	3.8	0.63	2.10	50	100			
dichlofluanid	5.80	0.42	7.3	1.26	4.20	10,000	10,000			
cyprodinil	6.12	0.251	4.1	0.75	2.51	10,000	1000			
imazalil	4.70	0.574	12.2	1.72	5.74		20	20	20	
endosulfan sulfate	5.99	0.408	6.8	1.22	4.08	1000	1000	50	50	2000
Average	**5.39**		**6.99**	**1.08**	**3.61**					

1. CODEX alimentarius (www.codexalimentarius.net/mrls/pesticides/jsp/pest-q-e.jsp)
2. Japanese Food Chemical Research Foundation (www.m5.ws001.squarestart.ne.jp/foundation/search.html)
3. Informal coordination of MRLs established in Directives 76/895/EEC, 86/362/EEC, 86/363/EEC, and 90/642/EEC (5058/VI/98)
4. 40CFR180 (www.access.gpo.gov/nara/cfr/waisidx_02/40cfr180_02.html)
Values are listed in ng/g (ppb); converted to mg/kg (ppm) by dividing by 1000

Table 6: Comparison of limits of detection and quantitation to maximum residue limits (MRLs) from various agencies

Conclusion

AOAC Method 2007.01 was validated using the Thermo Scientific DSQ II operating in EI SIM. The DSQ II system is able to reliably meet detection limits and quality control requirements for determination of pesticide residues in lettuce using a modified QuEChERS sample preparation. The QuEChERS sample prep was modified to include a solvent exchange to hexane/acetone. The calibration curves for the pesticides studied met a linear least squares calibration with a correlation coefficient of $R^2 > 0.997$ for most compounds. The Method Validation Study generated an average %RSD of 10.5% for four replicate analyses at a 50 ng/g and a calculated average LOD of 1 ng/g in iceberg lettuce based on 8 replicate analyses of a 5ng/g with an average LOQ of 3.6 ng/g. The injector showed endrin breakdown at below 5% on a daily basis. The surged splitless injection with detection by three ion SIM met the criteria for the AOAC Method in iceberg lettuce matrix.

Reference

1. AOAC Official Method 2007.01 Pesticide Residues in Foods by Acetonitrile Extraction and Partitioning with Magnesium Sulfate, S. Lehotay, Journal of AOAC International Vol. 90, No. 2, (2007) 485-520
2. Rapid Method for the Determination of 180 Pesticide Residues in Foods by Gas Chromatography/Mass Spectrometry and Flame Photometric Detection, M. Okihashi, Journal Pesticide Science, 304 (4), (2005) 368-377
3. Commission Decision of August 12, 2002 Implementing Council Directive 96/23/EC Concerning the Performance of Analytical Methods and the Interpretation of Results, Official Journal of European Communities, 17.8.2002
4. MRLs for lettuce as listed at http://www.codexalimentarius.net/mrls/pestdes/jsp/pest_q-e.jsp
5. Combination of Analyte Protectants to Overcome Matrix Effects in Routine GC Analysis of Pesticide Residues in Food Matrixes, K. Mastovska, S. Lehotay, M. Anastassiades, Analytical Chemistry (77) (2005), 8129-8137

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Application Note 332

Accelerated Solvent Extraction of Pesticide Residues in Food Products

Introduction

Residue analysis in crops and food products is routinely performed in regulatory and industrial laboratories around the world. Many of the traditional procedures used to perform these extractions are time-consuming and solvent-intensive. Accelerated solvent extraction is an extraction technique that speeds the extraction process and reduces the total amount of solvent used. The system uses conventional liquid solvents at elevated temperatures and pressures, which results in increased extraction kinetics. Extraction of samples ranging from 1 to 30 g typically requires 12–17 min and 15–50 mL of solvent.

In the environmental industry, accelerated solvent extraction has been compared extensively to traditional preparation techniques, and has been found to generate similar extracts in a more efficient manner. Accelerated solvent extraction is now widely used in environmental applications to replace time- and solvent-intensive techniques such as Soxhlet and sonication. The principles of accelerated solvent extraction technology are based on conventional liquid extraction theory, so the transfer of existing solvent-based extraction processes to accelerated solvent extraction is simple. In addition, the ability to extract up to 24 samples unattended can result in a dramatic increase in laboratory efficiency.

Equipment

Thermo Scientific Dionex ASE 200 accelerated solvent extraction system* equipped with 11, 22, or 33 mL cells

Thermo Scientific Dionex vials for collection of extracts (40 mL, P/N 049465; 60 mL, P/N 049466)

Cellulose filter disks (P/N 049458)

*Thermo Scientific Dionex ASE 150 and 350 accelerated solvent extraction systems can be used for equivalent results.

Reagents

Fisher Scientific Acetone, Optima grade

Fisher Scientific Acetonitrile, Optima grade

Fisher Scientific Hexane, Optima grade

Thermo Scientific Dionex ASE Prep DE (P/N 062819)

Fisher Scientific sodium sulfate, anhydrous added after extraction

Extraction Conditions

Temperature:	100 °C
Pressure:	1500 psi*
Heatup Time:	5 min
Static Time:	5 min
Flush Volume:	60%
Purge Time:	100 s
Static Cycles:	1–2
Total Extraction Time:	14–18 min per sample
Total Solvent Used:	15–45 mL per sample

*Pressure studies show that 1500 psi is the optimum extraction pressure for all accelerated solvent extraction applications.

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Sample Preparation

Weigh dry samples (1–20 g) and add directly to extraction cells containing a cellulose extraction filter. Grind wet samples (1–10 g) and mix with 6 g of Dionex ASE™ Prep DE (diatomaceous earth) using a mortar and pestle. Rinse the mortar and pestle with 2–3 mL of the extraction solvent. Add this volume to the sample in the extraction cell.

Extraction

Perform the sample extractions according to the outlined conditions. Following extraction, add 5 g of anhydrous sodium sulfate to the collection vial to absorb coextracted water. Shake the vial for 15 s and decant the water-free extract into a clean 60-mL vial. Rinse the original vial with 5 mL of the extraction solvent and decant this volume into a second vial. Concentrate the combined volume to approximately 10 mL under nitrogen.

Analytical

Analyze organochlorine pesticides using a gas chromatograph with a 30 m × 0.25 mm i.d. RTX-5 capillary column (Restek Corporation, Belefonte, USA). Set up a 1-µL splitless injection volume with the injector at 275 °C and the electron capture detector (ECD) maintained at 300 °C with a nitrogen atmosphere. Program the run from 140 °C (3 min) to 265 °C at 10 °C/min. Quantify results using endosulfan I or endrin aldehyde as the internal standard. Pass pesticide extracts through carbon or C18 cleanup cartridges prior to analysis. Quantify results by GC analysis with ECD detection (U.S. EPA Method 8151) or GC with MS detection (U.S. EPA Method 8270).

Results and Discussion

Samples (10 g) of raw potato and banana were spiked with 100 µL of a standard solution in hexane containing 12 organochlorine pesticides. Hexane with 10% acetone was chosen as the extraction solvent because it delivered good recoveries of the analytes with fewer interferences (co-extractables) than a 1:1 mixture. Resulting extracts were clear (after sodium sulfate treatment) upon concentration and suitable for GC/ECD analysis. The necessity of the drying step limits the amount of raw sample that can be extracted to 10 g. Results are presented in Tables 1 and 2. These results represent three extractions with duplicate GC injections of each extract.

Table 1. Recovery of Organochlorine Pesticides Spiked onto Raw Banana at the 100 ppm Level*

Compound	Av. Recovery (%)	SD (µg/kg)	RSD (%)
α-BHC	100.3	2.3	2.3
β-BHC	102.2	2.3	2.3
Υ-BHC	98.9	3.2	3.2
Heptachlor	89.2	7.6	8.5
Aldrin	89.4	2.2	2.5
Heptachlor Epoxide	93.5	2.1	2.2
Dieldrin	93.7	1.6	1.7
4,4'-DDE	92.1	1.8	1.9
2,4'-DDD	95.4	2.5	2.6
Endrin	94.4	2.7	3.0
4,4'-DDD	88.0	2.7	3.0
4,4'-DDT	89.6	5.8	6.4

* n = 3.

Table 2. Recovery of Organochlorine Pesticides Spiked onto Raw Potato at the 100 ppm Level*

Compound	Av. Recovery (%)	SD (µg/kg)	RSD (%)
α-BHC	96.3	6.3	6.6
β-BHC	108.6	2.3	2.1
Υ-BHC	97.4	6.6	6.8
Heptachlor	93.9	3.5	3.7
Aldrin	95.9	3.3	3.4
Heptachlor Epoxide	95.2	2.4	2.6
Dieldrin	97.1	0.55	0.57
4,4'-DDE	95.4	0.67	0.70
2,4'-DDD	95.7	0.85	0.89
Endrin	97.8	1.8	1.9
4,4'-DDD	93.7	1.8	1.9
4,4'-DDT	93.0	4.5	4.8

* n = 3.

Table 3. Recovery of Spiked Pesticides from Wheat by Accelerated Solvent Extraction

Compound	Spike Level (µg/kg)	Spike Level (µg/kg)
o-Methoate	74	85.4
Trifluralin	44	99.6
Dichlorvos	18	60.5
Phorate	18	92.8
Demeton	38	96.7
Dimethoate	58	87.8
Carbofuran	22	96.6
Atrazine	14	92.8
Diazinon	26	96.9
Disulfoton	22	87.9
Triallate	68	87.8
Parathion-methyl	40	115.7
Chlorpyrifos-methyl	8	115.4
Carbaryl	92	54.1
Linuron	102	83.6
Malathion	22	104.5
Phorate-sulfone	32	105.7
Parathion	84	101.2
Endosulfan-alpha	56	94.1
Disulfoton-sulfone	98	77.1
Imazalil	40	108.8
Endosulfan-beta	68	93.3
Endosulfan sulfate	20	77.0
Methoxychlor-*o,p*	48	89.9
Diclofop-methyl	36	81.8
Methoxychlor-*p,p'*	50	114.9
Azinphos-methyl	56	94.2

A 5-g sample of ground wheat grain was spiked with 100 µL of a standard solution containing 29 pesticides and herbicides at levels ranging from 8–102 ppb (see Table 3) and extracted at 100 °C with acetonitrile. Spike levels and recovery results are shown in Table 3. Recoveries ranged from 54.1–115.7%. The average recovery was 95.3% if the two outliers, dichlorvos and carbaryl, are excluded. Following the spike studies, 12 naturally incurred grain samples were extracted by the traditional wrist shaker extraction with acetonitrile, using post-extraction solid phase extraction (SPE) cleanup, and by accelerated solvent extraction using either acetone or acetonitrile as the extraction solvent. The accelerated solvent extraction took 12 min per sample and required 12–15 mL of solvent, while the shaker extraction took approximately 1 h per sample (including post-extraction SPE cleanup on carbon or C18) and used 130 mL of acetonitrile per sample. The accelerated solvent extraction extracts did not require post-extraction processing.

Extraction results for two compounds identified in these extracts, methyl chlorpyrifos and malathion, are shown in Table 4. The detected amounts compared well between the two techniques, with the accelerated solvent extraction values generally 10–20% higher. In all cases, samples with nondetectable levels (ND) were identified as such by both techniques. Acetonitrile and acetone appear to be good solvent choices for this application.

Table 4. Extraction of Incurred Pesticides in Wheat by accelerated solvent extraction and Conventional Wrist Shaker Extraction

Sample No.	Solvent	Sample Weight (g)	Methyl Chlorpyrifos (µg/kg)		Malathion (µg/kg)	
			Shaker	Accelerated Solvent Extraction	Wrist Shaker	Accelerated Solvent Extraction
1	Acetone	20.31	70	90	40	50
2	Acetone	19.78	80	100	40	50
3	Acetone	20.91	50	60	60	70
4	Acetone	10.13	ND	ND	ND	ND
5	Acetone	10.24	30	70	40	100
6	Acetone	9.93	ND	ND	ND	ND
7	Acetone	5.32	ND	ND	ND	ND
8	Acetone	5.39	ND	ND	ND	ND
9	Acetonitrile	19.85	60	80	60	80
10	Acetonitrile	20.4	70	90	60	70
11	Acetonitrile	5.30	ND	ND	ND	ND

ND = not detected.

Conclusion

Using accelerated solvent extraction, pesticide residue analysis laboratories can increase sample throughput while reducing overall solvent usage. The simplicity of the accelerated solvent extraction technique, combined with results showing excellent correlation to existing methods, have resulted in the rapid acceptance of accelerated solvent extraction for environmental analysis. The promulgation of U.S. EPA Method 3545 now provides a means for environmental test laboratories to take full advantage of accelerated solvent extraction technology. In addition to the wide range of target analytes covered under Method 3545 for organic pollutants in solid waste, accelerated solvent extraction has been applied successfully to the extraction of total petroleum hydrocarbons (TPH), dioxins, and furans from a variety of matrices. accelerated solvent extraction has also been applied to the extraction of explosives from soil, PCBs from fish and other marine tissues, and polyurethane foam (PUF) air sampling cartridges.

Suppliers

Fisher Scientific, 2000 Park Lane, Pittsburgh, PA 15275-1126 USA, Tel: 800-766-7000, www.fishersci.com.

Restek Corporation, 110 Benner Cir., Bellefonte, Pennsylvania, 16823 USA, Tel.: 814-353-1300, www.restekcorp.com.

"Extraction of TCL/PPL (Target Compound List/Priority Pollutant List) BNAs and Pesticides Using Accelerated Solvent Extraction with Analytical Validation by GC/MS and GC/ECD" Document 116064.A, Dionex Corporation (now part of Thermo Fisher Scientific), June 16, 1994.

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- High Efficiency, Broad Scope Screening of Pesticides Using Gas Chromatography High Resolution Orbitrap Mass Spectrometry
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- High Mass Resolution is Essential for Confident Compound Detection
- Three-fold Increase in Productivity for Pesticide Residue Analysis in Baby Food Using Fast Triple Quadrupole GC-MS/MS
- GC-MS/MS Analysis of Pesticide Residue in Green Tea Extracted by QuEChERS with Acetonitrile as Final Solvent
- Broad Scope Pesticide Screening in Food Using Triple Quadrupole GC-MS
- Validation of the Method for Determination of Pesticide Residues by Gas Chromatography – Triple-Stage Quadrupole Mass Spectrometry
- Comparing LC and GC Triple Quadrupole MS for the Screening of 500 Pesticides in Matrix
- Simplifying Complex Multi-Residue Pesticide Methodology in GC-MS/MS
- Analysis of Multi-Residue Pesticides Present in Ayurvedic Churna by GC-MS/MS
- Multi-Residue Pesticide Analysis in Herbal Products Using Accelerated Solvent Extraction with a Triple Quadrupole GC-MS/MS System
- Analysis of Dithiocarbamate Pesticides by GC-MS
- Determination of Organochlorine Pesticides Using GC-MS with a Helium-conserving Injector
- Analysis of Organophosphorus Pesticides by GC
- Trace Determination of Organo-Phosphorous Pesticides in Olive Oil by GC Analysis

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High Efficiency, Broad Scope Screening of Pesticides Using Gas Chromatography High Resolution Orbitrap Mass Spectrometry

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Application Note 10448

Keywords
Accurate Mass, Complex Matrices, GC Orbitrap Mass Spectrometry, Pesticide Analysis, QuEChERS, Screening, TraceFinder Software

Introduction

Pesticides are used globally to improve the production and yields of agricultural crops and their use is essential to ensure a sufficient global food supply. However, this widespread use of pesticides and the potential for them to remain in the final product is of significant concern to consumers and to governments whose responsibility it is to ensure a safe food supply. Consequently, legislation exists to protect consumers from exposure to contaminated foods. This legislation requires that foods are monitored for both the type and quantity of the pesticide present, with each pesticide given a maximum residue limit (MRL) in a particular sample commodity. The list of compound and sample combinations is extensive, creating a challenge for accurate and reliable routine monitoring.

Laboratories are under ever-increasing pressure to screen samples for pesticides in a single analysis, with a fast turnaround time and at a competitive cost. Most existing laboratories rely on targeted analytical approaches using both gas chromatography and liquid chromatography coupled to mass spectrometry instrumentation. These techniques cover the wide range of chemical classes that need to be monitored and at the required levels of sensitivity and selectivity. However, they are limited to only those compounds in the target list, which are usually selected based on the residue definition and legislation requirements to demonstrate that the food is fit for consumption. These techniques require careful optimization of acquisition parameters for each compound and the monitoring of acquisition time windows to ensure detection of the analyte.

To increase the scope of the analysis, chemical screening methods using high-resolution, full-scan mass spectrometry have received significant attention in recent years. These methods use non-targeted acquisition, in which a generic full scan acquisition is run, followed by targeted data processing of a list of compounds within a database.

Although data interrogation is performed against a list of target compounds, retrospective data analysis is possible in order to identify new compounds that were not screened for at the time of acquisition. For this approach to be used in routine analysis, screening data processing software needs to be fast and accurate enough to detect residues at low concentrations with an acceptably low level of false negative results, as described in the European Union guidelines.[1] There is no recommendation for the number of false positives, but it is necessary for routine laboratories to keep this number as low as possible to minimize the time required for additional investigation. The majority of samples that pass through a laboratory are compliant with the legislation. Therefore, it is efficient to quickly screen compliant samples from those that are suspected to be contaminated. Following an initial screen, the suspect positive samples are reanalyzed using a second confirmatory method (e.g., GC-MS/MS) to confirm suspect positives and to accurately determine the concentration of the pesticide present. The confirmatory analysis contains a complete calibration series in an appropriate matrix that is not included in the screening analysis.

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In this study, we evaluate the performance of the Thermo Scientific™ Q Exactive™ GC hybrid quadrupole-Orbitrap mass spectrometer (MS) for the accurate screening of GC-amenable pesticides. The Q Exactive GC Orbitrap MS provides high mass resolving power up to 120,000 (*m/z* 200) full width half maxima (FWHM) to facilitate highly accurate mass measurements and to enable confident discrimination of co-eluting and isobaric compounds in complex samples. Fast scan speeds and a high intrascan dynamic range (>5000) facilitate the detection of trace compounds in the presence of high matrix components.

Experimental Conditions

Sample Preparation

Food and feed samples were extracted following an acetate buffered QuEChERS-based approach. Briefly, 10 mL of acidified (1% acetic acid) acetonitrile was added to 5 g (cereals/feed) or 10 g (fruit/vegetables) of homogenized sample. A mixture of salts was added and the centrifuge tube shaken and spun. The final acetonitrile extracts (0.5 or 1 g/mL in acetonitrile) were fortified with a mixture of 55 pesticides at concentrations corresponding to 0.5–100 ng/g (ppb). A variety of difficult sample matrices were studied including wheat, leek, and horse feed.

Instrument and Method Setup

In all experiments, a Q Exactive GC hybrid quadrupole-Orbitrap mass spectrometer was used. Sample introduction was performed using a Thermo Scientific™ TriPlus™ RSH autosampler and chromatographic separation was obtained using a Thermo Scientific™ TRACE™ 1310 gas chromatograph (GC) and a Thermo Scientific™ TraceGOLD TG-5SilMS™ 15 m × 0.25 mm I.D. × 0.25 µm film capillary column (P/N: 26096-1301).

Additional details of instrument parameters are displayed.

GC and Injector Conditions	
TRACE 1310 GC Parameters	
Injection Volume (µL):	1
Liner:	Asymmetric baffled (P/N: 45352062)
Inlet (°C):	75
Inlet Module and Mode:	PTV, cold splitless
PTV Transfer delay (min):	1
Injection time (min):	0.1
Transfer rate (°C/sec):	2.5
Transfer temperature (°C):	300
PTV Transfer time (min)	3
Cleaning rate (°C/sec):	330
Carrier Gas, (mL/min):	He, 1.2
Oven Temperature Program	
Temperature 1 (°C):	40
Hold Time (min):	1.5
Temperature 2 (°C):	180
Rate (°C/min)	25
Temperature 3 (°C):	300
Rate (°C/min)	100
Hold Time (min):	3

Mass Spectrometer Conditions	
Q Exactive Mass Spectrometer Parameters	
Transfer line (°C):	280
Ionization type:	EI
Ion source(°C):	230
Electron energy (eV):	70
Acquisition Mode:	Full scan
Mass range (Da):	50–500
Resolving power (FWHM):	60,000
Lockmass (*m/z*):	207.03235

The Q Exactive GC system was operated in EI full scan mode using 60,000 (FWHM *m/z* 200) resolving power. Additional experiments were run at different resolution modes of 15K, 30K, and 120K. Chromatographic data was acquired with a minimum of 11 points/peak to ensure consistent peak integration.

Data Processing

Data was acquired and processed using the Thermo Scientific™ TraceFinder™ software. This single software package integrates instrument control, method development functionality, and qualitative-screening and quantitation-focused workflows.

Results and Discussion

The objective of this study was to screen for a wide range of pesticides in different sample matrices with the highest level of confidence. The aim of the analysis was to determine if a pesticide is present in a sample above the lowest MRL, which is typically 10 ppb. This assessment was made by screening fortified wheat, horse feed, and leek extracts spiked at different concentrations to determine their limits of detection for screening under the conditions described. These matrices were selected because they are known to be highly complex and challenging matrices for pesticide analysis, as is shown in the total ion chromatograms in Figure 1.

The sample extraction techniques used in routine pesticide analysis are very generic (e.g., QuEChERS) and produce highly complex and variable solutions. The lack of selectivity in sample preparation stages has to be made up for by selectivity in the instrumental analysis. This selectivity can be achieved using high mass resolving power and high mass accuracy. As sample types increase in complexity, the resolving power of the mass spectrometer becomes a key factor in reliable pesticide detection. This resolving power has already been demonstrated for the analysis of LC-amenable pesticides.[2] Furthermore, high-resolution, full-scan analysis increases the scope of the analysis without the need for optimization of the acquisition parameters.

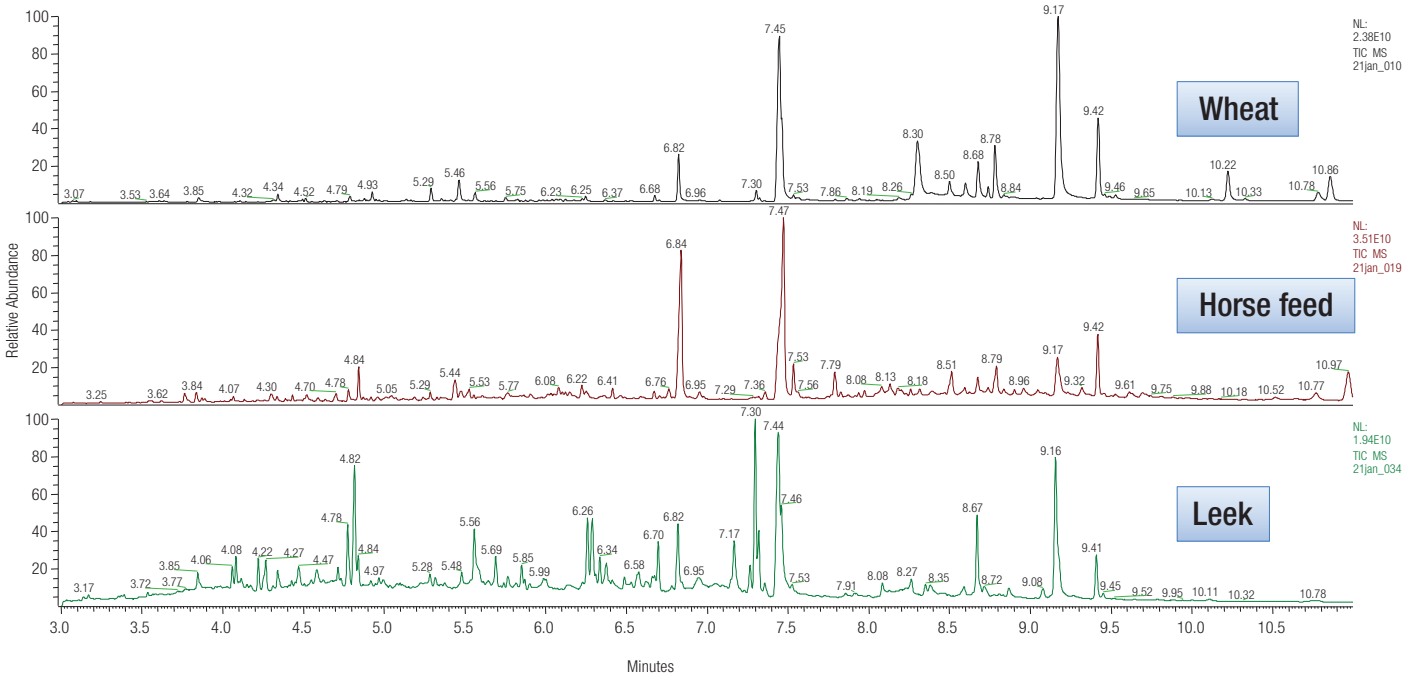

Figure 1. Full scan total ion chromatogram (TIC) for 55 pesticides spiked into wheat, horse feed, and leek extracts showing the complexity of the samples

Sample Throughput

Sample throughput is a key consideration in pesticide analysis. As such, a fast chromatographic method was used to test the system under typical conditions. This method resulted in a complete analysis within 17 minutes (injection to injection), enabling up to 84 analyses to be performed within a 24 hour period. Although this is a fast GC method, the scan speed of the mass spectrometer provided a minimum of at least 11 points/peak. Figure 2 shows the peak for diazinon with 11 points across the 1.8 second peak.

Screening

Following full scan analysis at a mass resolution of 60,000, TraceFinder software was used to process the data. An in-house database of 183 pesticides, containing information for formula, accurate mass, retention time, isotopic pattern (via formula of diagnostic ion), and fragments was used to screen the samples. Although all parameters can be used for identification, the criterion used by the software for a positive identification was that a peak must be observed in the extracted ion chromatogram (XIC) of the main diagnostic ion at the expected retention time within ± 20 seconds, and the exact mass of the ion should be within ± 2 ppm of the theoretical value.

Pesticide detection can be confirmed by assessing the retention time and mass accuracy of the fragment ions as well as the isotopic pattern fit. The inclusion of these parameters increases the confidence in the detection and reduces the number of false positives.

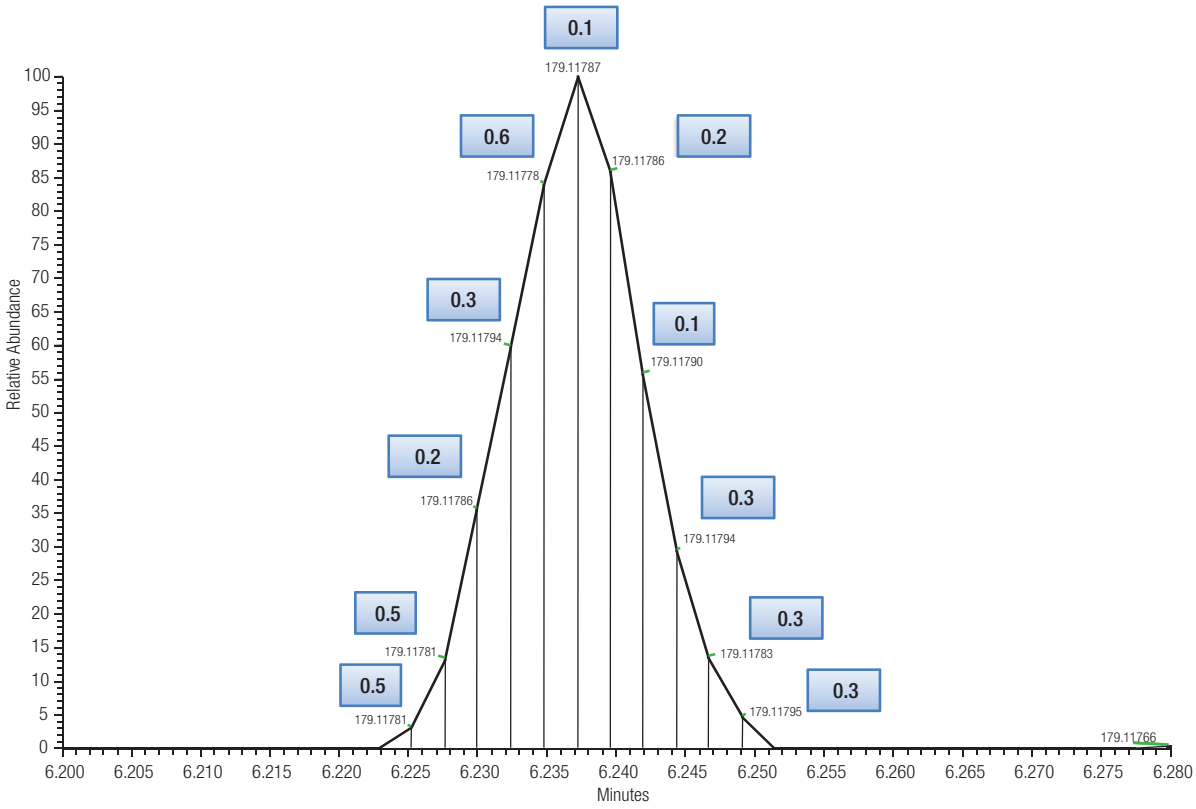

Figure 2. Extracted ion chromatogram (XIC) of diazinon (m/z 179.11789 ± 5 ppm mass window) in wheat spiked at 10 ng/mL showing ~11 scans/peak (peak width 1.8 sec). Data acquired in full scan at 60,000 FWHM at m/z 200 resolving power. Excellent accurate mass is shown for each individual scan as well as mass difference (in ppm). Average mass difference of 0.3 ppm across the peak.

Screening Software

The processing software is critical to the successful implementation of routine screening. TraceFinder software was used to quickly screen the data for the presence of the target pesticides. A target compound database was used
to detect and report the pesticides found and to indicate which criteria were satisfied. Figure 3 shows an example TraceFinder browser window for some of the detected pesticides in wheat spiked with 10 ng/mL. The pesticide p,p'-DDT, which has been detected and confirmed based on retention time, accurate mass (0.21 ppm), fragment, and isotopic match is highlighted in the summary window.

The data is displayed to the user in a traffic light system that enables quick review of the data. More detailed information is available in the summary columns and in the window panes, showing in this example the XIC and the measured and theoretical isotopic pattern for p,p'-DDT. The exceptional accurate mass provided by this system, even in complex matrices, enables compounds to be detected with a high degree of confidence. All pesticides are screened at < 2ppm and, as shown in Figure 3, the accurate mass is typically sub ppm. This specificity of accurate mass for both the main diagnostic ions and fragments enables the false detects to be screened out

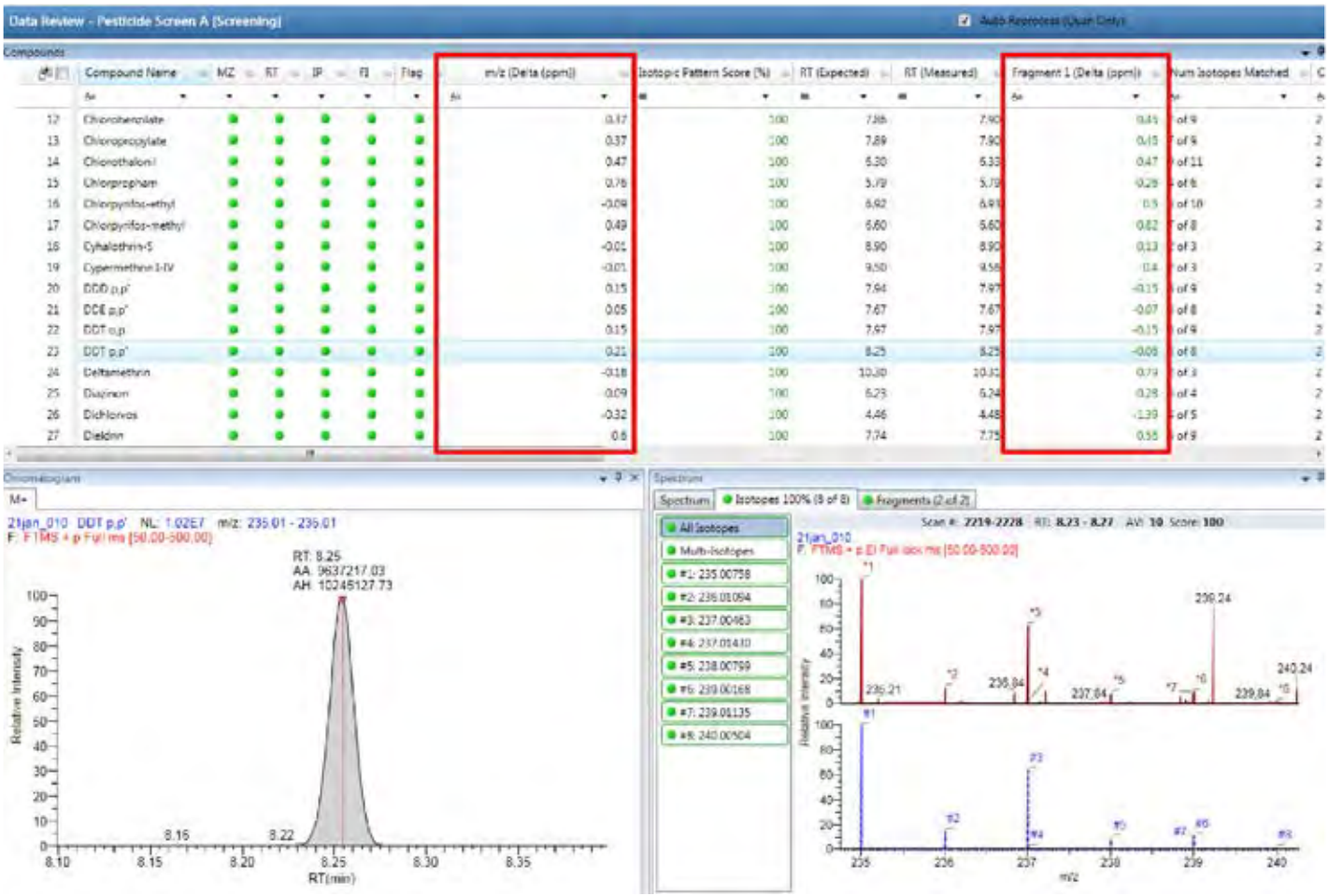

Figure 3. TraceFinder screening browser showing positively identified pesticides (p,p'-DDT as an example) in wheat spiked at 10 ng/mL, based on accurate mass confirmation (± 2 ppm mass window), retention time (RT), isotopic pattern (IP), fragment ions (FI). Sub-ppm mass accuracy for both main and confirmatory ions is highlighted in red boxes.

automatically or quickly assessed by the user.

Screening Below MRL

In this study, all 55 pesticides were detected in the wheat, horse feed, and leek samples when spiked with 10 ng/mL. The majority of pesticides were detected at much lower concentrations. As shown in Figures 4 and 5, 53 pesticides were detected at a concentration of < 2.5 ng/mL in wheat matrix with 47 detected in the 0.5 ng/mL spiked extract. This excellent sensitivity in complex matrices makes confident screening at, or even below, the MRL a unique feature

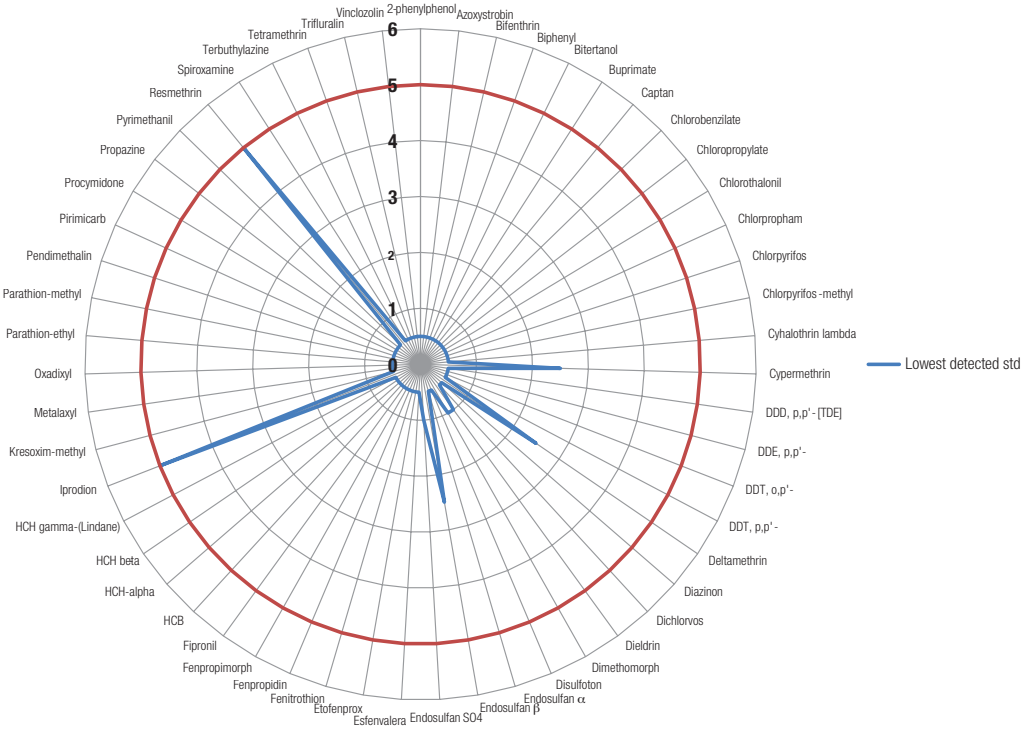

Figure 4. Graph showing the lowest detected standard for 55 pesticides in wheat. Identification based on accurate mass < 2ppm and retention time ± 20 seconds. 5 ng/mL level displayed.

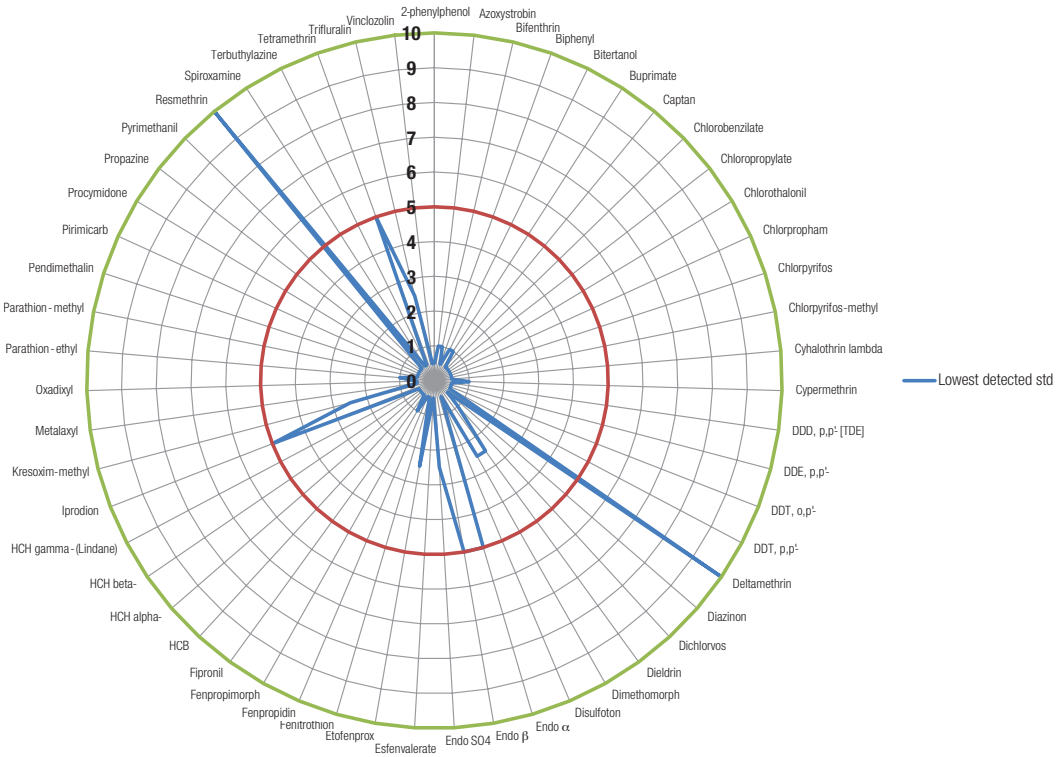

Figure 5. Graph showing the lowest detected standard for 55 pesticides in horse feed. Identification based on accurate mass < 2ppm and retention time ± 20 seconds. 5 ng/mL and 10 ng/mL levels highlighted.

of the Q Exactive GC system.

Avoiding False Negatives Using Resolving Power

The use of a narrow mass accuracy tolerance is possible only when the resolving power is sufficient to isolate target compounds from matrix interferences or other target compounds. When two mass profiles overlap, the measured mass profile is the sum of the two individual profiles. This summed profile results in the incorrect assignment of the mass of the target compound. This phenomenon is demonstrated in Figure 6, where the leek sample was analyzed four times at resolving powers of 15K, 30K, 60K, and 120K. The mass spectra show a diagnostic ion of chlorpropham and a background matrix ion at a similar mass, resulting in interference. The expected mass accuracy was achieved at 60K and 120K with near baseline resolution. However, at 15K and 30K, chlorpropham was not resolved from the interference, resulting in poorer mass accuracy. At 15K, the mass accuracy is significantly affected with a value of 18.4 ppm mass difference. Under the screening criteria used in this study, and even under a wider tolerance of 10 ppm, this peak would have resulted in a false negative for chlorpropham. This result shows that a minimum resolving power is needed. The required minimum resolving power depends on the complexity of the sample being analyzed and the concentration of both target analytes and interferences.

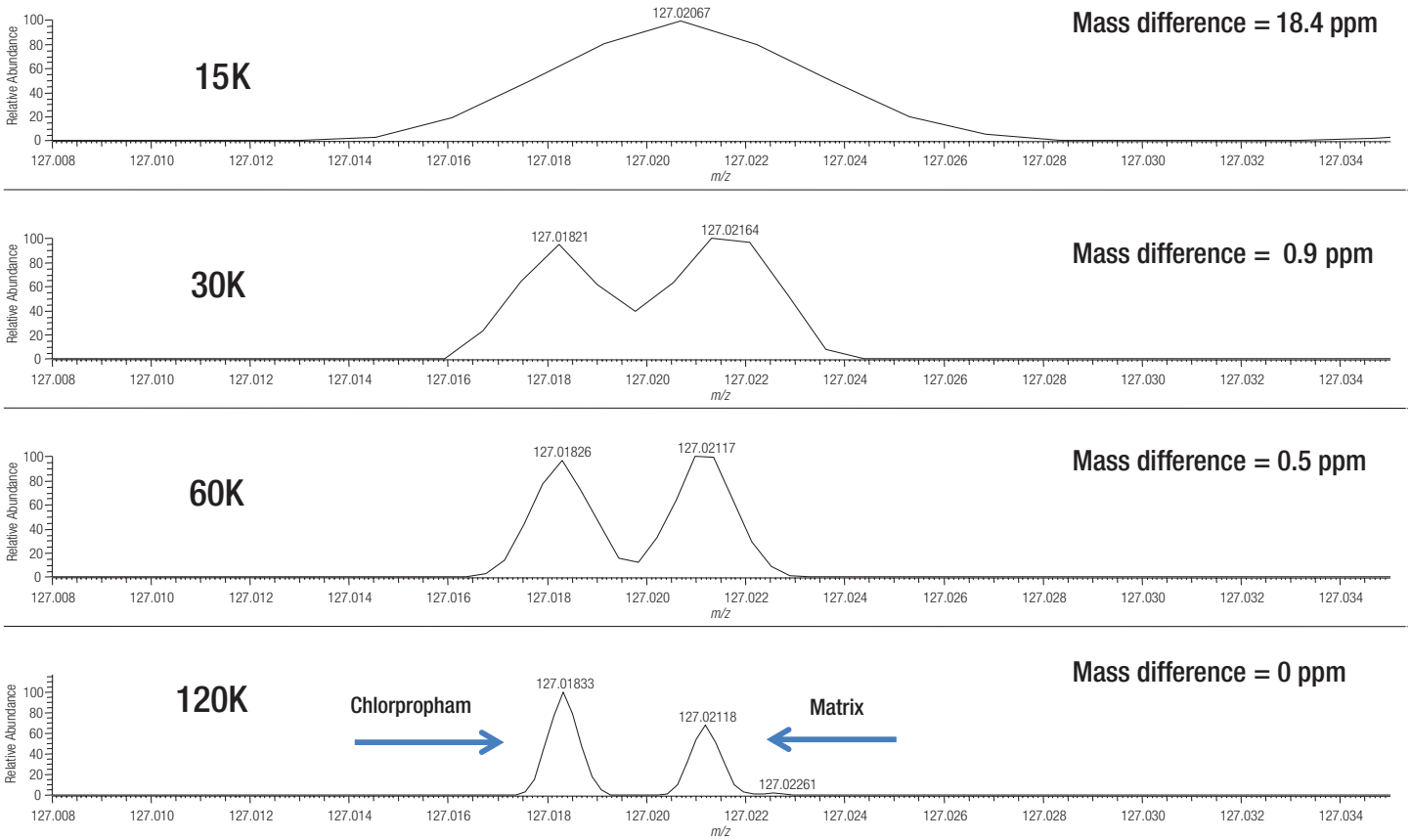

Figure 6. Effect of resolving power on mass accuracy of an analyte in matrix. Mass profiles of a diagnostic ion of chlorpropham at 10 ng/mL in leek, acquired at different resolutions of 15K, 30K, 60K, and 120K. At 15K and 30K the chlorpropham ion is not resolved from matrix interference resulting in poorer mass accuracy. At 15K, under screening criteria applied in this study, this pesticide would have been missed (false negative).

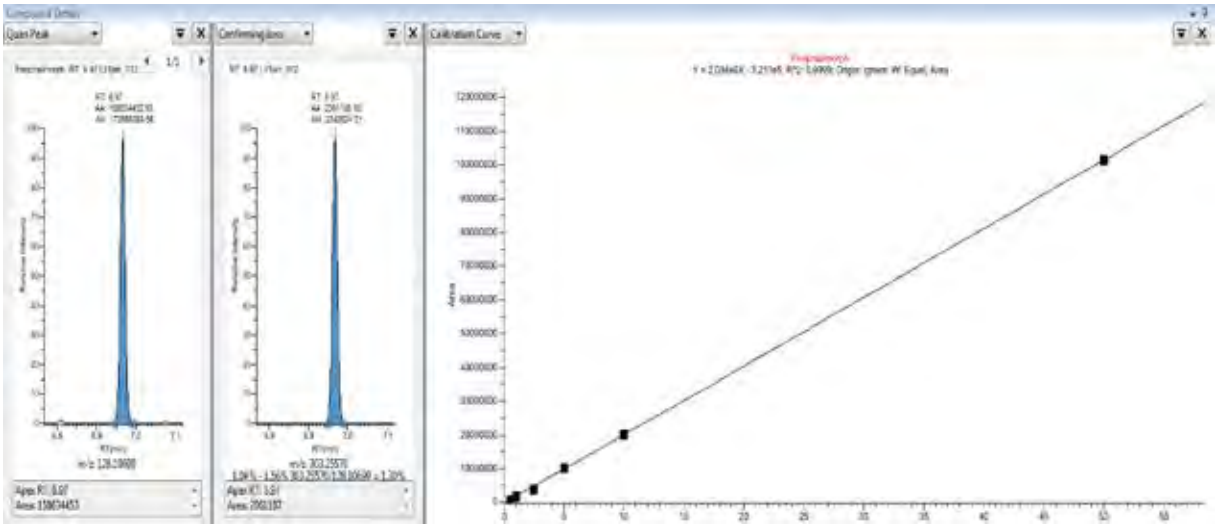

Figure 7. TraceFinder software view of the extracted ion chromatograms and calibration curve for fenpropimorph in leek. Triplicate injections of the calibration series were performed with good linearity.

Quantitative Pesticide Performance

The next step in routine analysis is to determine the concentration of the pesticide detected in the sample. Pesticide linearity was assessed across a concentration range of 0.5–50 ng/mL using matrix-matched standards and using triplicate injections of each calibration standard. In all cases, the coefficient of determination (R^2) was >0.99 with an average value of $R^2 = 0.997$ and with residual values from the regression line of <25%. An example of compound linearity for fenpropimorph is shown in Figure 7. Full quantitation of detected compounds was not in the scope of this study, but is reported in more detail for pesticides in Thermo Scientific Application Note 10449.[3]

Conclusions

The results of this evaluation demonstrate that the Thermo Scientific Q Exactive GC hybrid quadrupole-Orbitrap mass spectrometer, in combination with TraceFinder software, is an extremely effective tool for the routine screening of pesticides in food and feed samples. The Orbitrap mass spectrometer delivers excellent resolving power, mass accuracy, and sensitivity.

- Screening using full-scan, high-resolution mass spectrometry is an effective way to increase the scope of an analysis. This technology allows for more compounds to be analyzed from a single injection without prior optimization of the acquisition parameters.
- Fast GC analysis and acquisition speeds allow for increased laboratory productivity and sample throughput. The outstanding mass accuracy, in combination with excellent sensitivity, makes confident routine pesticide screening possible.
- Routine resolving power of 60,000 FWHM eliminates matrix interferences, increasing confidence in results when screening pesticides in complex matrices. Consistent sub-ppm mass accuracy achieved for all compounds ensures confident compound identification.

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Fast Screening, Identification, and Quantification of Pesticide Residues in Baby Food Using GC Orbitrap MS Technology

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Application Note 10449

Keywords
Baby Food, Exact Mass, Screening, Food Safety, GC Orbitrap, High Resolution GC-MS, Pesticide Analysis, Quantification, TraceFinder

Introduction

Pesticides are chemicals widely used to control a variety of pests, such as insects, plant pathogens, weeds, etc. The use of pesticides may result in residues in crops, therefore, strict regulations are in place to control the use of these chemicals and to ensure that concentrations do not exceed statutory maximum residue levels (MRLs).[1]

Pesticides are measured almost exclusively by liquid chromatography (LC) and gas chromatography (GC) analytical methodologies. GC coupled to a mass spectrometer (MS) as a detector is widely used in many pesticide residue laboratories, because many pesticides are not amenable to LC-MS or ionize poorly under soft ionization techniques. GC offers good separation efficiency and a choice of MS detectors, including single or triple quadrupoles. Quadrupole mass analyzers are selective, sensitive, and cost-effective instruments that operate at nominal mass resolution. When using quadrupole MS, the selectivity required to separate target pesticides from chemical background is achieved by by the use of either selected ion monitoring (SIM) or selected reaction monitoring (SRM). Both SIM and SRM are used in targeted experiments in which the mass spectrometer is pre-programmed using a list of preselected pesticides. However, targeting specific compounds during acquisition limits the scope of analysis and can result in false negative results (non-detection) for both unknown and untargeted compounds, which may be of concern with respect to food safety.

This limitation has led to increased interest in developing methods using MS analyzers that can operate in full scan with a higher mass resolving power than triple quadrupoles, but provide similar levels of selectivity and quantitative performance. Until now, high-resolution, accurate-mass GC-MS instruments have not gained wide acceptance due to their limited ability to provide full scan selectivity and quantitative performance comparable to triple quadrupole instruments operated in SRM.

In this work, we demonstrate the use of GC coupled with Orbitrap™ MS technology for fast, high throughput pesticide residues analysis in baby food samples, with an almost unlimited scope in the analysis through full scan acquisition. Quantitative performance comparable to triple quadrupoles and compliance with SANCO® guidelines[2] will also be demonstrated.

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Sample Preparation

Baby food samples were extracted using the a citrate buffered QuEChERS protocol, described previously.[4] The final extracts (1 g/mL in acetonitrile) were spiked with a mixture of 132 pesticides at concentrations corresponding to 0.5–100 ng/g (ppb) for the majority of analytes and 1.0–200 for some analytes.

Instrument and Method Setup

In all experiments, a Thermo Scientific™ Q Exactive™ GC hybrid quadrupole-Orbitrap mass spectrometer was used. Sample introduction was performed using a Thermo Scientific™ TriPlus™ RSH Autosampler, and chromatographic separation was obtained with a Thermo Scientific™ TRACE™ 1310 GC and a Thermo Scientific™ TraceGOLD™ TG-5SilMS 15 m × 0.25 mm I.D. × 0.25 µm film capillary column (P/N: 26096-1301). Additional details of instrument parameters are shown.

GC and Injector Conditions

TRACE 1310 GC Parameters	
Injection Volume (µL):	1.0
Liner:	asymmetric baffled (P/N: 45352062)
Inlet (°C):	75
Inlet Module and Mode:	PTV, cold splitless
Injection time (min):	0.1
Transfer rate (°C/sec):	2.5
Transfer temperature (°C):	300
Transfer time (min):	3
Cleaning rate (°C/sec):	330
Carrier Gas, (mL/min):	He, 1.2
Oven Temperature Program	
Temperature 1 (°C):	40
Hold Time (min):	1.5
Temperature 2 (°C):	180
Rate (°C/min)	25
Temperature 3 (°C):	300
Rate (°C/min)	100
Hold Time (min):	3

The Q Exactive GC system was tuned and calibrated using peaks of known mass from a calibration solution (FC 43, CAS 311-89-7) to achieve mass accuracy of < 0.5 ppm RMS. The system was operated in electron ionization mode (EI) using full scan and 60,000 mass resolution (Full Width at Half Maxima, measured at *m/z* 200), meeting the recommended SANCO resolution criteria[2] for high resolution analytical instrumentation. Chromatographic data was acquired with a minimum of 12 points/peak to ensure consistent peak integration.

Mass Spectrometer Conditions

Q Exactive GC Mass Spectrometer Parameters	
Transfer line (°C):	280
Ionization type:	EI
Ion source (°C):	230
Electron energy (eV):	70
Acquisition Mode:	Full scan
Mass range (*m/z*):	50–500
Mass resolution (FWHM at *m/z* 200):	60,000
Lockmass (*m/z*):	207.03235

Data Processing

Data was acquired and processed using Thermo Scientific™ TraceFinder™ software. TraceFinder software allows the analyst to build acquisition and processing methods for high throughput screening and quantitative analysis and incorporates library searching capabilities as well as easy data reviewing and data reporting.

Results and Discussion

The objective of this study was to evaluate the utility of Orbitrap-based GC-MS technology for fast pesticides screening and quantification to increase sample throughput and laboratory productivity. Various analytical parameters were assessed and the results of these experiments are described.

Chromatography

Good chromatographic separation was obtained using the GC conditions described. An example of chromatography for the matrix-matched standard (corresponding to 100/200 ng/g) is given in Figure 1. The total ion chromatogram, as well as the extracted ion chromatograms (XIC, ±2 ppm extraction mass window) of the first (dichlorvos, *m/z* 184.97650, RT = 4.46 min) and last (deltamethrin, *m/z* 252.90451, RT = 10.33 min) eluting pesticides, are shown. The fast separation allowed for a high sample throughput as described elsewhere.[4]

MS Acquisition Speed

When using short GC run times, the analyte chromatographic peak widths are narrow, typically 2.5 seconds. This narrow peak width necessitates fast MS acquisition rates in order to obtain enough scans/chromatographic peak. When the number of points per peak is not sufficient to define a Gaussian shape, the peaks of interest can be integrated inaccurately, which in turn affects the reproducibility, peak integration, and ultimately, the accuracy of target compound quantification. An example of typical number of scans acquired using the Q Exactive GC system operated at 60,000 resolution for EPTC in baby food is shown in Figure 2. Aside from producing an adequate number of scans/peak (17), excellent mass accuracy (0.5 ppm RMS) was obtained for every scan across the peak.

Pesticides Targeted Screening

A simple, targeted screening experiment was set up as a first test to screen for pesticides that were spiked into the baby food matrix. This was performed using the TraceFinder software against an in-house compound database containing 183 pesticides. The database contains the compound name, theoretical exact masses for at least three fragment ions, and expected retention time information for the GC conditions used for the sample analysis.

Compound detection and identification was based on retention time (±0.1 min window), accurate mass information (±2 ppm window), isotopic pattern similarity (measured versus theoretical), and library search hit (NIST14). Using these criteria, all 132 pesticides were positively detected and confirmed in the 10/20 ng/g baby food sample.

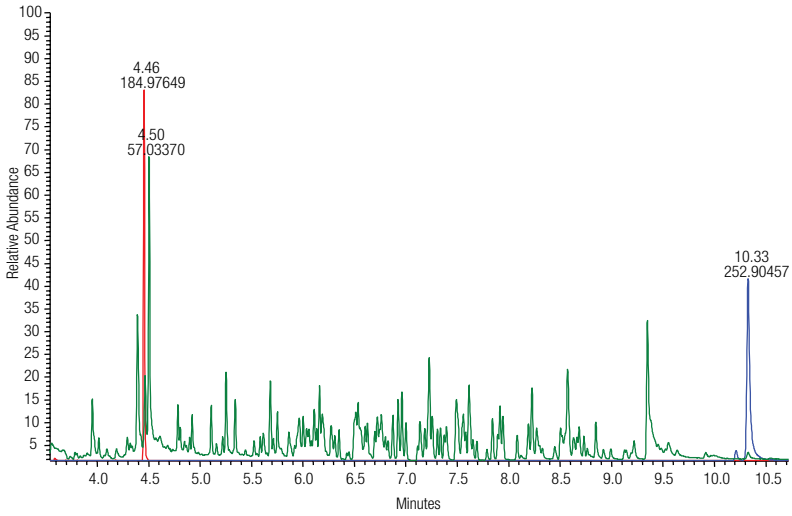

Figure 1. Overlay of the total ion chromatogram (EI full scan) and the extracted ion chromatograms (XIC) of the first (dichlorvos, RT = 4.46 min) and the last (deltamethrin, RT = 10.33 min) eluting pesticides. Relative abundance (Y axis) adjusted to emphasize XIC for dichlorvos and deltamethrin.

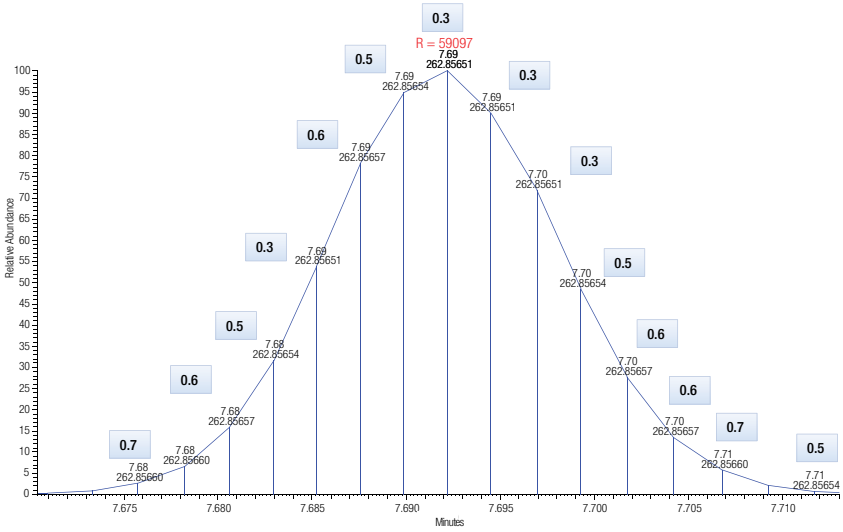

Figure 2. Extracted ion chromatogram (XIC) of dieldrin (*m/z* 262.85642, ±2 ppm mass window) showing 17 scans/peak (peak width 2.4 sec). Data acquired in full scan at 60,000 FWHM resolution (the exact resolution used is annotated in red). Measured accurate mass for each scan is shown as well as mass difference (ppm).

Figure 3. TraceFinder software screening result browser showing positively identified pesticides in the 10 ng/g sample. Compound identification and confirmation (aldrin showed as an example) was based on accurate mass identification (±2 ppm mass window), retention time (RT), isotopic pattern (IP), and fragment ions (FI). Measured and theoretical isotopic clusters are shown.

An example of the compound detection and identification workflow for aldrin is shown in Figure 3. Data acquired in full scan is deconvoluted and retention time and accurate mass information are then used to identify the compound. Aldrin was identified based on the RT, and the presence of an accurate mass quantification ion (<0.5 ppm mass error) and the characteristic fragment ions. Moreover, the elemental composition of the quantification ion ($C_7C_{15}H_2$) was used to check the isotopic pattern fit against the measured isotopic pattern. As shown in Figure 3, a 100% isotopic fit was obtained for aldrin, adding to the confidence in compound identification.

Pesticide Residue Quantification

The quantitative performance of the Q Exactive GC system for compound quantification was tested for all 132 pesticides. To assess quantitative performance, a matrix-match calibration curve was constructed over a concentration range of 0.5–100 ng/g (or 1.0–200 ng/g). System sensitivity, linearity, and peak area reproducibility were evaluated. Additionally, mass accuracy of the target pesticides was assessed across the concentration levels.

Sensitivity

Almost all pesticides (95%) were detected in the lowest calibration matrix-matched standard 0.5 (or 1.0) ng/g. Examples of chromatography at this concentration level are shown in Figure 4. At the 5 ng/g level, all of the compounds detected had ion ratios valued within a 15% limit of the average ion ratio values derived from the calibration curve across all concentrations.

Estimation of Instrument Detection Limit (IDL) and Peak Area Repeatability

System sensitivity was assessed by calculating the IDL for each pesticide. The IDL of the target pesticides represents the smallest signal above background noise that an instrument can consistently and reliably detect. This signal was determined empirically by repeatedly injecting (n=10) the 5 ng/g (and 10 ng/g) matrix-matched standard and taking into account the Student's-*t* critical values for the corresponding degrees of freedom (99% confidence). The results of this experiment showed an average %RSD for the peak area reproducibility of 6 % (Figure 5).

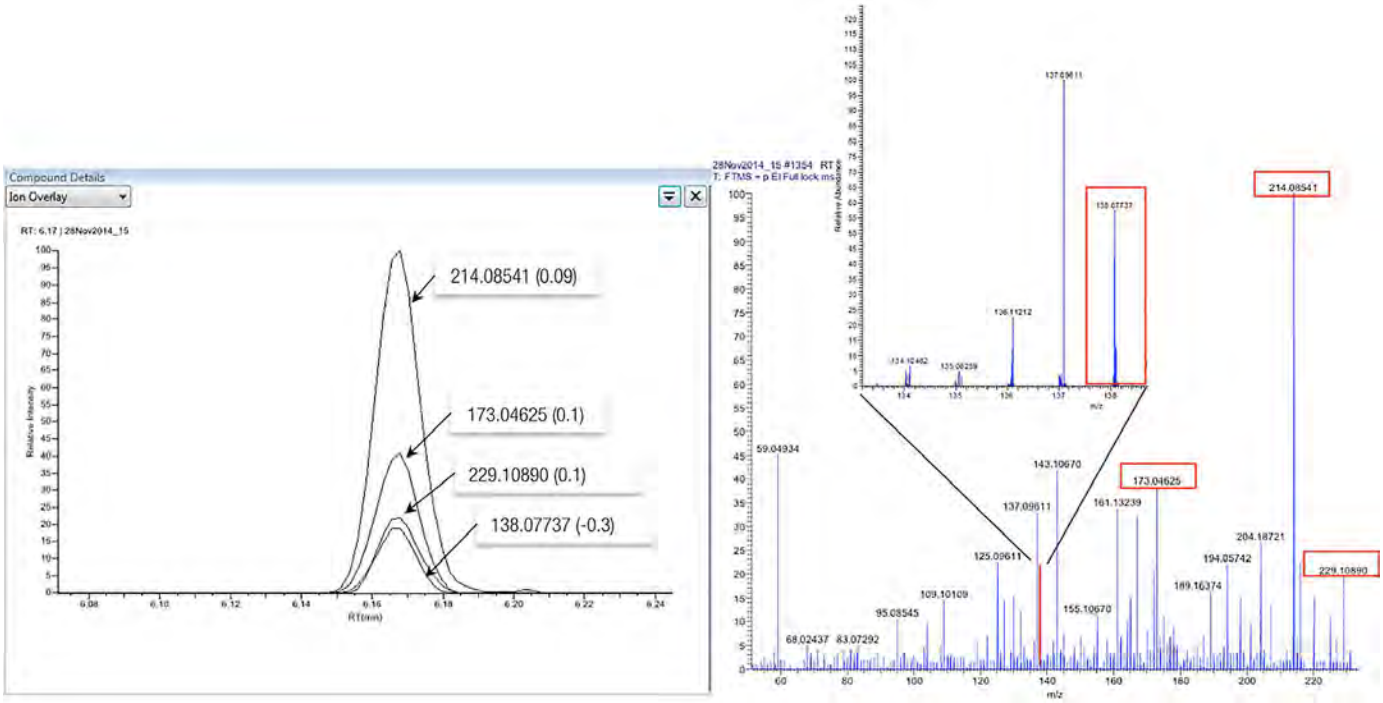

Figure 4. Terbuthylazine at 0.5 pg (on column concentration) showing an XIC overlay for the quantification ion and three additional confirmation fragment ions (left). The measured mass for each ion and mass error (in ppm) are annotated. Mass spectrum (right) highlighting the ions used for quantification and confirmation; the zoomed area shows the least intense fragment (*m/z* 138.07737) measured with a mass accuracy of 0.3 ppm.

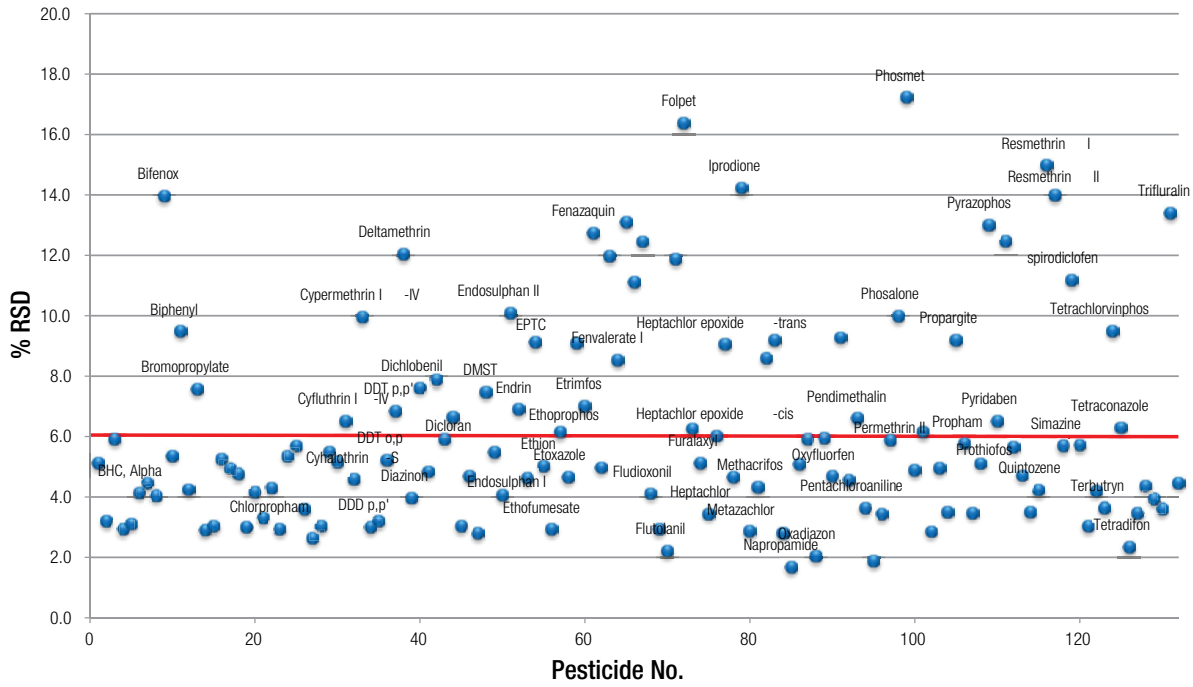

Figure 5. Absolute peak area repeatability (% RSD, n=10) at 5 or 10 pg injected on column for all 132 pesticides measured. The average %RSD value (solid line) is shown.

All the IDLs derived from the Q Exactive GC system data were lower than the typical MRLs established by the European Union for baby food samples. For most pesticides, these MRLs are currently set at <0.01 mg/kg (10 ng/g).[3] Calculated IDLs were compared to the IDL values obtained for the same pesticides using the Thermo Scientific™ TSQ™ 8000 Evo Triple Quadrupole GC-MS/MS system.[4] The results of this experiment demonstrated that the sensitivity of the Q Exactive GC system is comparable to that of the TSQ 8000 Evo GC-MS/MS system, with 91% of pesticides having an IDL < 2 ng/g (Figure 6).

Mass Accuracy

Obtaining accurate mass information in a consistent manner is critical for determining the identity of a pesticide as well as maintaining a high degree of discrimination through the resolving power of the instrument, against matrix interference.[5] The mass accuracy for all 132 pesticides was assessed at the 5 ng/g (or 10 ng/g, depending on compound) level from a series of n = 10 repeat injections. The mass deviation values did not exceed 1 ppm for any of the analytes and the overall mass accuracy average value was 0.4 ppm, providing the highest confidence in accurate and selective detection (Figure 7).

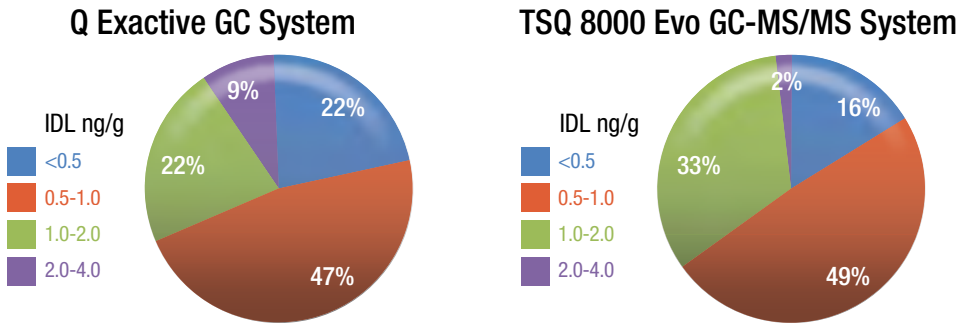

Figure 6. Comparison of the IDL99 (ng/g) calculated for 132 pesticides from a 5 ng/g matrix-matched standard from the Q Exactive GC System (left) and TSQ 8000 Evo GC-MS/MS system (right). The percentage of pesticides and corresponding IDL interval, relative to the total number of target compounds (132), is indicated.

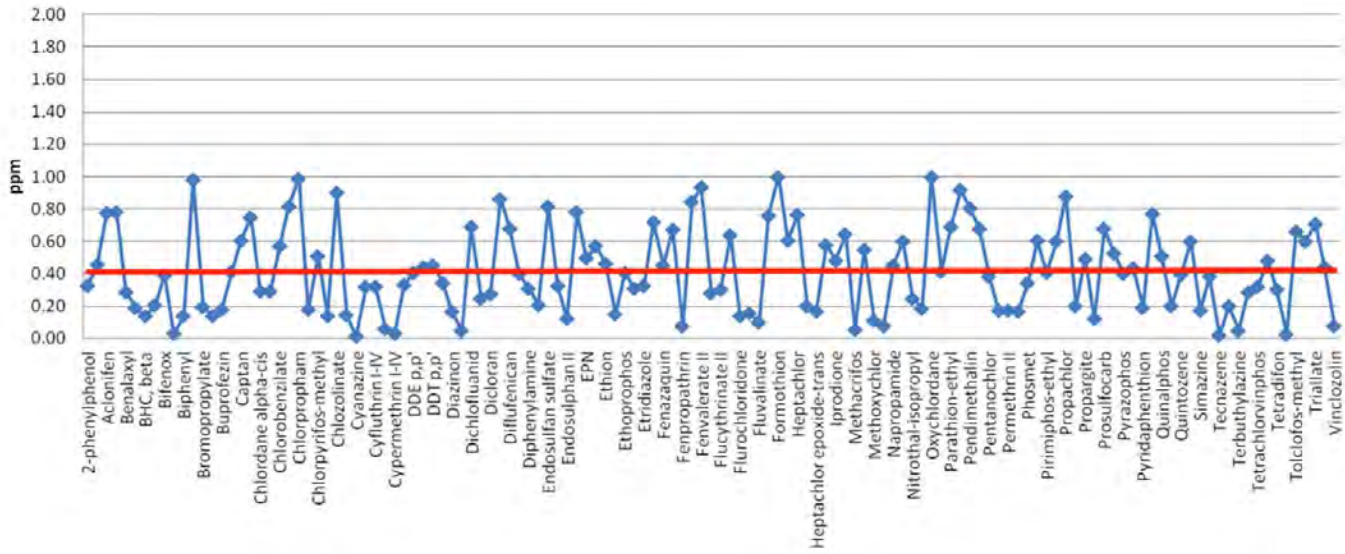

Figure 7. Accurate mass measurements (average value of n = 10) for the pesticides identified in the baby food sample at the 5 (or 10) ng/g level.

Linearity of Response

Quantitative linearity was assessed across a concentration range of 0.5–100 ng/g (or 1–200 ng/g for some analytes) using matrix-matched calibration standards injected in triplicate at each level. In all cases, the coefficient of determination (R^2) was >0.99 with an average value of R^2 = 0.997 and with residual values from the regression line of <25%. Examples of compound linearity are shown in Figure 8.

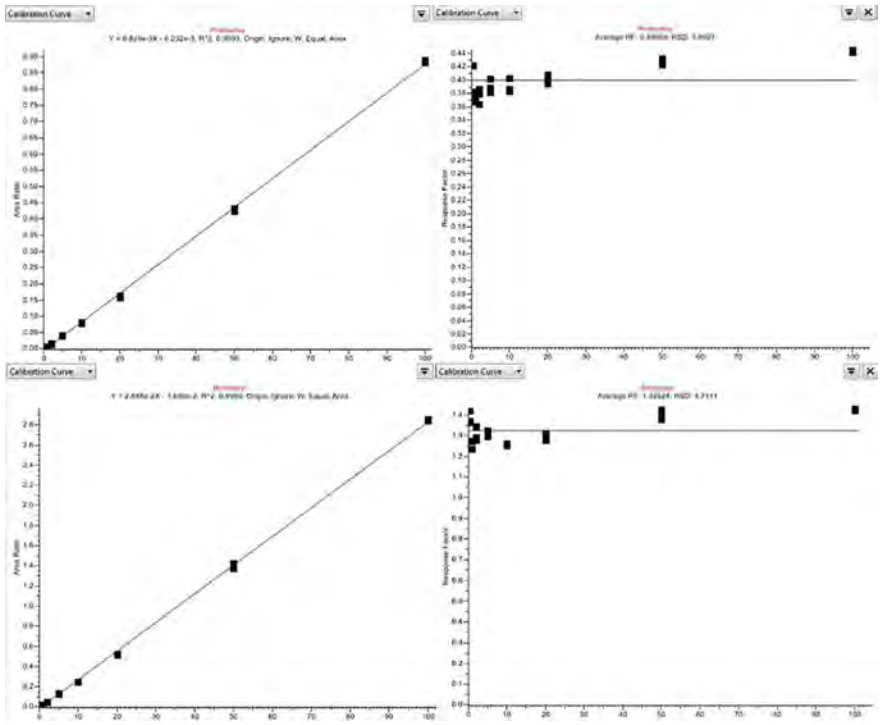

Figure 8. Coefficient of determination (left) and residuals values (%RSD) for prothiofos and benalaxyl calculated for a linear range of 0.5–100 ng/g.

Conclusions

- The Q Exactive GC system provides high performance quantitative analysis in full scan for broad-scope pesticide residue testing, even with fast GC separations.
- The fast scan speed, high resolution, and outstanding mass accuracy, together with full scan sensitivity allow reproducible and accurate pesticide quantification at very low levels.
- Acquisition with a routine mass resolution of 60,000 FWHM at *m/z* 200 eliminates isobaric interferences, increasing confidence in results when screening pesticides in complex matrices. The consistent sub-ppm mass accuracy achieved for all compounds ensures confident compound identification.
- The Q Exactive GC system provides quantitative performance that is highly comparable to that of GC triple quadrupole MS instruments.
- Thermo Scientific TraceFinder software enables analysts to develop high throughput screening and quantitative analyses quickly and accurately.

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Technical Note 10455

High Mass Resolution is Essential for Confident Compound Detection

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Keywords
Accurate Mass, Complex Matrices, GC Orbitrap Mass Spectrometry, High Resolution, Screening

Introduction

Analytical laboratories are under ever-increasing pressure to deliver fast results, while maintaining the highest levels of accuracy and confidence. The majority of these laboratories rely on targeted analytical approaches, using both gas chromatography and liquid chromatography coupled to triple quadrupole mass spectrometry (MS) instrumentation. These techniques cover the wide range of chemical classes to be monitored at the required levels of sensitivity and selectivity. However, they are limited to those compounds in the target list and they require careful optimization of acquisition parameters for each compound. High resolution, full scan mass spectrometry using Orbitrap technology provides a solution to:

- the demand for detection and quantification of a growing number of compounds.
- retrospective analysis of samples long after data acquisition.
- identification and elucidation of the chemical composition and structure of unknown compounds.

Until now, high resolution Orbitrap mass spectrometry has been available only with liquid chromatography and has proven to be a highly valuable analytical technique.[1] Orbitrap mass spectrometry technology has now been coupled to gas chromatography (GC) in the Thermo Scientific™ Q Exactive™ GC hybrid quadrupole-Orbitrap mass spectrometer system. This novel configuration of a benchtop hybrid quadrupole-Orbitrap mass spectrometer opens up new possibilities for GC-amenable compounds. The following examples highlight the benefits of high resolution MS coupled to GC.

Q Exactive GC Orbitrap GC-MS/MS system

The Impact of Mass Resolution on Selectivity for Targeted Analysis

High-resolution, accurate-mass (HR/AM) experiments typically provide a full scan analysis of a sample and, for small molecule analyses, the scan range is typically 50–1000 Da. Orbitrap technology provides the required selectivity to resolve the target compound from other compounds or from matrix ions of similar mass. For targeted compound analysis, the accurate mass of the diagnostic ion is extracted with a narrow mass extraction window (typically <5 ppm). This narrow window is possible only when the instrument provides sufficient mass accuracy, for which high mass resolving power is essential. However, when two mass profiles overlap, the measured mass profile is the sum of the two individual profiles. This overlap results in the incorrect assignment of the mass of the target compound. The problem is demonstrated in Figure 1, where a QuEChERS leek extract in acetonitrile was analyzed four times at resolving powers of 15K, 30K, 60K, and 120K (*m/z* 200).

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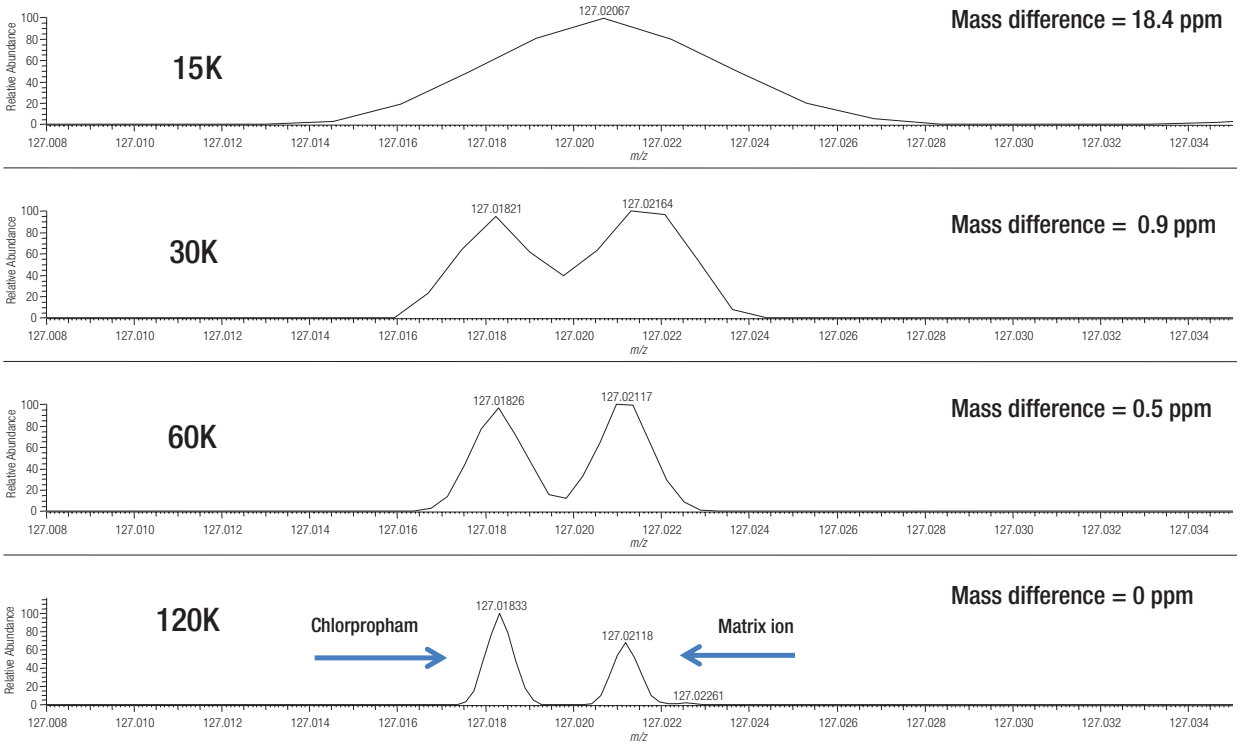

Figure 1. Effect of resolving power on mass accuracy of an analyte in matrix. Mass profiles of chlorpropham 10 ng/g in leek acquired at resolutions of 15K, 30K, 60K, and 120K. Matrix interference at 15K and 30K prevents separation of the pesticide from the interference and higher-than-expected mass difference. Chlorpropham is resolved at 60K and 120K with improvements in mass accuracy. Under normal screening criteria this pesticide would have been missed (false negative).

The mass spectra show the pesticide chlorpropham (*m/z* 127.01833) and a background matrix ion at a similar mass creating interference. Excellent mass accuracy was achieved for chlorpropham at 60K and 120K, with near baseline resolution. However, at 15K and 30K, chlorpropham was not sufficiently resolved from the interference, resulting in a poorer mass accuracy assignment. At 15K, the mass accuracy was significantly affected with a value of 18.4 ppm mass difference. Under typical screening criteria of <5ppm, and even under a wider tolerance of 10 ppm, this mass difference would have resulted in a false negative (non detection) for this pesticide. This example clearly shows that a minimum resolving power is needed. The required resolving power depends on the complexity of the sample being analyzed and the concentration of both target analytes and interferences.

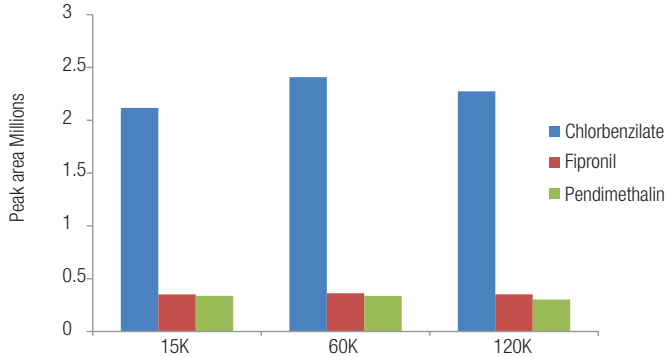

Figure 2. Chlorbenzilate, fipronil, and pendimethalin in a QuEChERS carrot extract at a concentration of 10 ng/g showing peak area responses obtained at 15, 60, and 120K FWHM resolution (*m/z* 200). Sensitivity is maintained across the resolution modes for both high and low responding analytes. A minimum of 12 scans/peak was maintained.

Maintaining Sensitivity at High Resolution

With other types of GC-MS technology, increasing mass resolution results in a decrease in ion transmission. Consequently, the precision of the measurement can be affected. For low-level targeted compound screening and quantification in complex matrices, it is essential to maintain instrument sensitivity while operating at high resolving power. In Figure 1, the need for high resolution was demonstrated. While resolution is extremely important, it is also essential to maintain sensitivity at the higher resolution modes of 60K and 120K. The Q Exactive GC system does not lose signal intensity as significantly with increasing resolution as other types of mass spectrometers. Figure 2 shows an example of three pesticides (chlorbenzilate, fipronil, and pendimethalin) and the corresponding peak area responses at a concentration of 10 ng/g in carrot QuEChERS acetonitrile extracts. These extracts were analyzed at resolution modes of 15, 60, and 120K in full scan. Absolute peak areas are maintained across the resolution modes. This consistency provides the superior mass resolution required for excellent mass accuracy, without sacrificing sensitivity.

High Resolution for Unknown Compound Identification

One of the advantages of having full scan, accurate mass capabilities is that data can be mined retrospectively and unknown peaks can potentially be identified. The mass accuracy of an ion allows elemental compositions to be proposed based on the measured accurate mass and isotopic pattern. The number of possible chemical formulae proposed is based on the elements used in the calculator and the quality of the spectral data. High resolution measurements that consistently provide sub-1-ppm mass accuracy accelerate the identification process by reducing the number of proposed formulae to a manageable number. This process is illustrated in Figure 3, where an ion at *m/z* 304.10058 was submitted to the elemental formula calculator and hits were reported using the following elements Carbon 1-50, Hydrogen 1-50, Oxygen 1-20, Nitrogen 1-20, Phosphorus 1-10, and Sulphur 1-10.

Different ppm mass tolerances from 0.5 to 10 ppm were used to suggest possible formulae. The number of hits is reported in Figure 3. As expected, the wider the tolerance, the greater the number of suggestions. At 10 ppm, 60 possible hits are proposed. Even at a relatively low value of 3 ppm, 20 elemental formulae fit the criteria. However, with the sub-ppm mass accuracy expected from the Q Exactive GC system, the number is limited to two formulae at 0.5 ppm. The top suggestion for this mass is $C_{12}H_{21}N_2O_3PS$, with a mass accuracy of 0.3 ppm and, when submitted to the ChemSpider online database, the top hit returned is the pesticide diazinon. This identification can be further confirmed by investigation of the fragment ions, matching with spectral libraries as data are acquired using electron impact (EI).

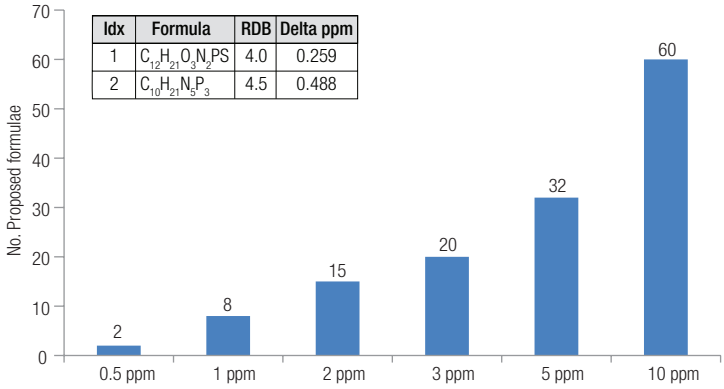

Figure 3. Number of suggested elemental compositions for *m/z* 304.10058 with different mass tolerances applied. Inset shows the top two hits at 0.5 ppm.

Conclusion

- With unmatched routine high resolving power and consistent sub-ppm mass accuracy, the Thermo Scientific Q Exactive GC mass spectrometer is a unique laboratory tool suitable for compound discovery, screening, quantitation, compound identification, and structural elucidation applications.
- Routine mass resolution of at least 60,000 FWHM (at *m/z* 200) was required to resolve chlorpropham from background interfering ions of a similar accurate mass. This resolution is essential for the confident detection of compounds.
- The Q Exactive GC system provides high sensitivity in complex matrices and importantly, the sensitivity is maintained across all resolution modes used (15–120K FWHM at *m/z* 200).
- Excellent sub-ppm mass accuracy accelerates the identification of unknown peaks by allowing the use of narrow mass tolerances.

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Three-Fold Increase in Productivity for Pesticide Residue Analysis in Baby Food Using Fast Triple Quadrupole GC-MS/MS

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Application Note 10432

Key Words
Pesticide Analysis, Baby Food, GC-MS/MS, TraceFinder, Food Safety

Goal

To assess the performance and productivity of the Thermo Scientific™ TSQ™ 8000 Evo GC-MS/MS for pesticide residues analysis.

Introduction

Pesticides include more than 1000 different substances used to control or eradicate pests. Strict regulatory controls are in place to ensure that these chemicals are used safely and effectively without harmful effects to humans, wildlife, and the environment. Maximum residue levels (MRLs) of pesticides in food and feed have been set by many international bodies including the EU.[1] Detection, quantification, and correct identification of pesticide residues at trace levels requires sensitive, selective, and robust analytical instrumentation. With ever-increasing pressure to analyze a greater number of samples of perishable commodities with shorter turnaround times, high throughput laboratories seek continuous improvements in analytical productivity. In recent times, substantial productivity gains have been achieved using the QuEChERS (quick, easy, cheap, effective, robust and safe) sample extraction approach in combination with gas or liquid chromatography (GC or LC) mass spectrometry (MS). Here, we report the possibility of further productivity gains using advanced, rapid GC-MS/MS technology in combination with new software developments to reduce the time needed to acquire and process the data.

Acetonitrile is commonly used as the extraction solvent for QuEChERS. Direct analysis of pesticide residues in acetonitrile is preferred to avoid the need for solvent exchange, which is time consuming and, hence, costly. However, the polar nature of acetonitrile results in poor focusing of chromatographic peaks and the high expansion coefficient limits the injection volume that can be used.

In this study, a fast, easy, and robust workflow was used to analyze pesticide residues in baby food. Accurate and sensitive detection, quantification, and identification of pesticides in baby foods is of particular importance because babies are more vulnerable to adverse health effects from these chemicals.

This work shows that laboratory productivity can be accelerated by direct injection of low sample volumes of QuEChERS acetonitrile extracts, in combination with fast temperature ramps to shorten GC run times. This is made possible using the innovative EvoCell collision chamber technology combined with the efficient selected reaction monitoring (SRM) scheduling of timed-SRM software in the TSQ 8000 Evo triple quadrupole GC-MS/MS.

A thorough assessment of the robustness of this fast GC analysis using acetonitrile was conducted following the SANCO guidelines.[2]

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Instrument and Method Setup

A TSQ 8000 Evo triple quadrupole GC-MS/MS instrument coupled with a Thermo Scientific™ TRACE™ 1310 GC was used. Sample introduction was performed a Thermo Scientific™ TriPlus™ RSH autosampler, and chromatographic separation using a Thermo Scientific™ TraceGOLD TG-5SilMS 15 m × 0.25 mm I.D. × 0.25 µm film capillary column (P/N: 26096-1300). Additional details of instrument parameters are displayed in tables below.

GC and Injector Conditions	
TRACE 1310 GC	
Injection Volume (µL): 1.0	
Liner:	SSL single taper (P/N: 453A2342)
Inlet (°C):	240
Inlet Module and Mode:	Splitless
Carrier Gas, (mL/min):	He, 1.2
Oven Temperature Program	
Temperature 1 (°C):	60
Hold Time (min):	1
Temperature 2 (°C):	180
Rate (°C/min)	50
Temperature 3 (°C):	320
Rate (°C/min)	35
Hold Time (min):	4
Mass Spectrometer Conditions	
TSQ 8000 Evo Mass Spectrometer	
Transfer Line (°C):	280
Ionization Type:	EI
Ion Source (°C):	320
Electron Energy (eV):	70
Acquisition Mode:	t-SRM
Q2 Gas Pressure(argon)(psi):	60
Q1 Peak Width (Da):	0.7
Q3 Peak Width (Da):	0.7

The TSQ 8000 Evo triple quadrupole mass spectrometer was operated in MS/MS mode using electron ionization (EI+). For each pesticide, two SRM transitions were chosen—one for quantification and one for identification purposes. A total of 264 SRM transitions were acquired with dwell times varying from 1 ms to 52 ms, depending on the number of SRM transitions monitored simultaneously. Chromatographic data was acquired data using timed-selected reaction monitoring (t-SRM) with a minimum of 12 points/peak.

Sample Preparation

Baby food samples were extracted using the citrate buffered QuEChERS protocol. The homogenized sample was extracted (10 g) with acetonitrile (10 mL) followed by the addition of MgSO4 (4 g), NaCl (1.0 g), disodium hydrogen citrate sesquihydrate (0.5 g), and trisodium citrate dihydrate (1.0 g). Dispersive solid phase extraction [MgSO4 (150 mg), C18 (50 mg), PSA (50 mg) and carbon (7.5 mg) per mL of extract] was used for sample clean-up. Final extracts (1 g/mL in acetonitrile) were spiked with a mixture of 132 pesticides at concentrations corresponding to 0.5–100 ng/g (ppb) and 1.0–200 ng/g (ppb) for some analytes.

Data Processing

Data were acquired and processed using the Thermo Scientific™ TraceFinder™ version 3.2 software, a single software package that integrates instrument control, method development functionality, and quantitation-focused workflows. For each compound, one SRM transition was used for quantitation and the second one for positive identification of the pesticide.

Results and Discussion

This study describes the methodology used for multi-residue pesticides analysis in baby food using fast GC for increasing laboratory productivity. The results described below were obtained with acetonitrile as the final extract solvent from the QuEChERS extraction and low-volume, hot splitless injection. The performance of the TSQ 8000 Evo GC-MS/MS system was evaluated by assessing the chromatography, sensitivity, linearity, and reproducibility of the target pesticides analyzed in the extracts of baby food samples.

Three-Fold Increase in Sample Throughput

Typically, a GC analysis of 132 target pesticides has a run time of around 42 minutes in order to obtain a sufficient number of scans per chromatographic peak (Figure 1), especially in time windows containing many co-eluting peaks. At least 10–12 scans across a chromatographic peak are needed in order to accurately integrate the peaks of interest.

Previously, fast scan speeds compromised instrument sensitivity, especially when several SRM transitions were monitored simultaneously. Using the fast GC conditions described above, the GC run time was decreased to ~11 min with no compromise in the number of data points acquired for each chromatographic peak (Figures 2 and 3). This advance is possible because the fast EvoCell technology allows fast clearance of ions from the collision cell and hence faster data acquisition, without adversely affecting instrument sensitivity. Fast data acquisition enables more information to be collected in a shorter time, ultimately resulting in faster GC runs. Using this fast methodology, sample productivity is improved by approximately three-fold, as around three times as many injections of sample/standard extracts can be carried out in an overnight sequence.

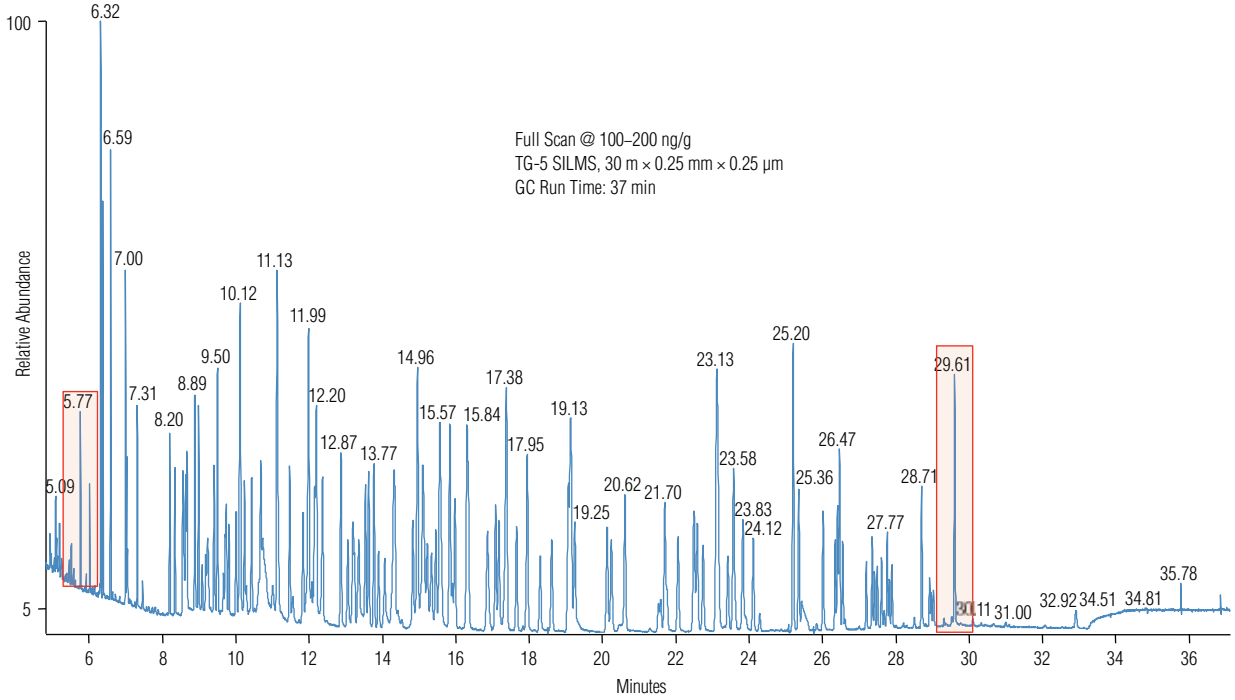

Figure 1. Total ion chromatogram (TIC, full scan) for a typical GC-MS chromatographic run of 132 pesticides at 100–200 ng/g with a total run time of approximately 40 minutes. The first (dichlorvos, RT = 5.77 min) and the last (deltamethrin, RT = 29.61 min) eluting pesticides are highlighted.

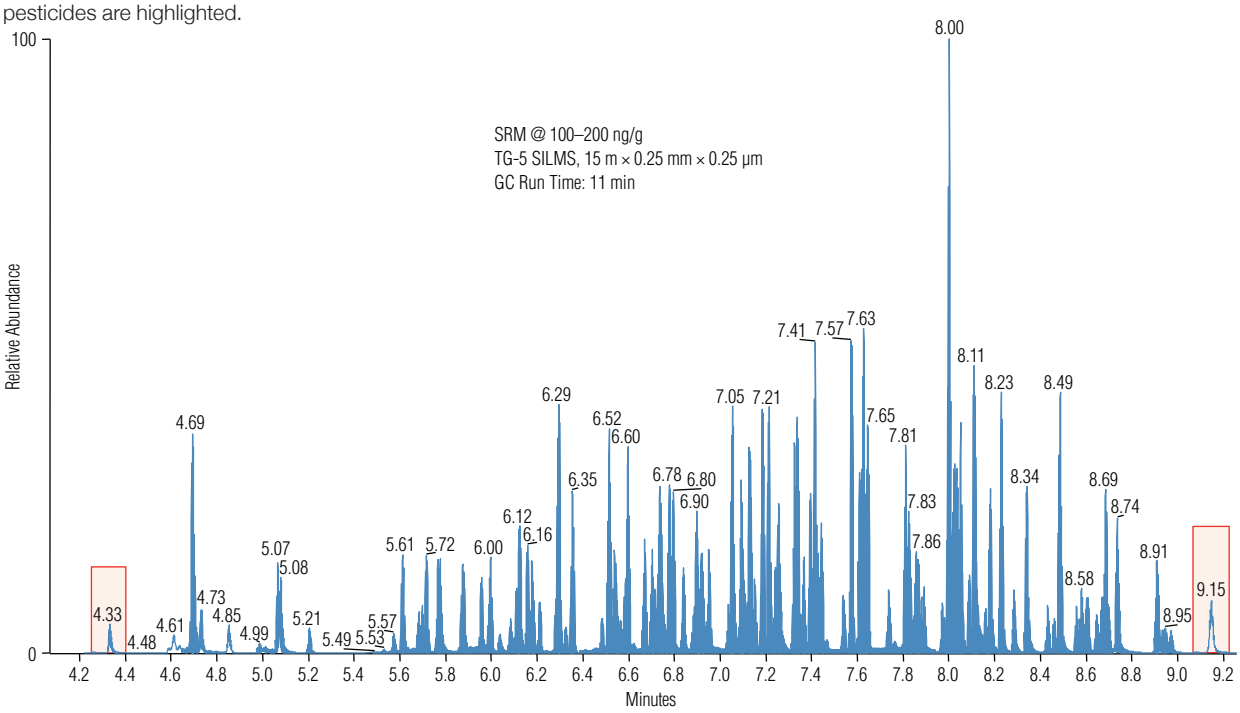

Figure 2. SRM chromatogram for a fast GC-MS chromatographic run of 132 pesticides at 100–200 ng/g with a total run time of 11 minutes. The first (dichlorvos, RT = 4.33 min) and the last (deltamethrin, RT = 9.15 min) eluting pesticides are highlighted.

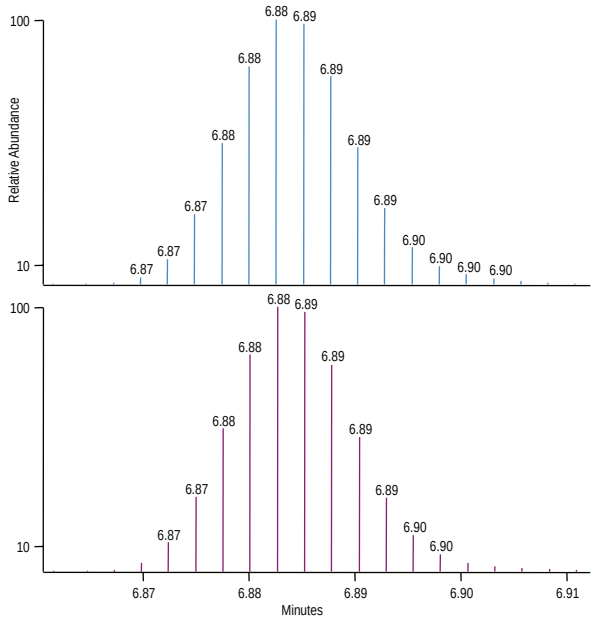

Figure 3. SRM chromatogram for parathion ethyl eluting at RT = 6.89 min showing 13 scans/peak (peak width 1.8 sec, dwell time of 1.7 ms).

Sensitivity

Almost all pesticides (97%) were detected at a concentration of 0.5 or 1.0 ng/g (ng/mL) and calibration curves were linear over the range 0. 5–100 ng/g (or 1.0–200 ng/g). Examples of chromatography at this low concentration and calibration curves are shown in Figure 4. At the lowest calibration concentration of 5–10 ng/g (0.5–1 × default MRL), all compounds were comfortably detected with all the ion ratios for compound identification within 15% of the average ion ratio values derived from the calibration curve across all concentrations.

Estimation of Instrument Detection Limit (IDL) and Peak Area Repeatability

The IDL of the target pesticides was determined empirically by repeatedly injecting (n=20) the 5 ng/g (and 10 ng/g) matrix-matched standard and taking into account the Student's-t critical values for the corresponding degrees of freedom (99% confidence).

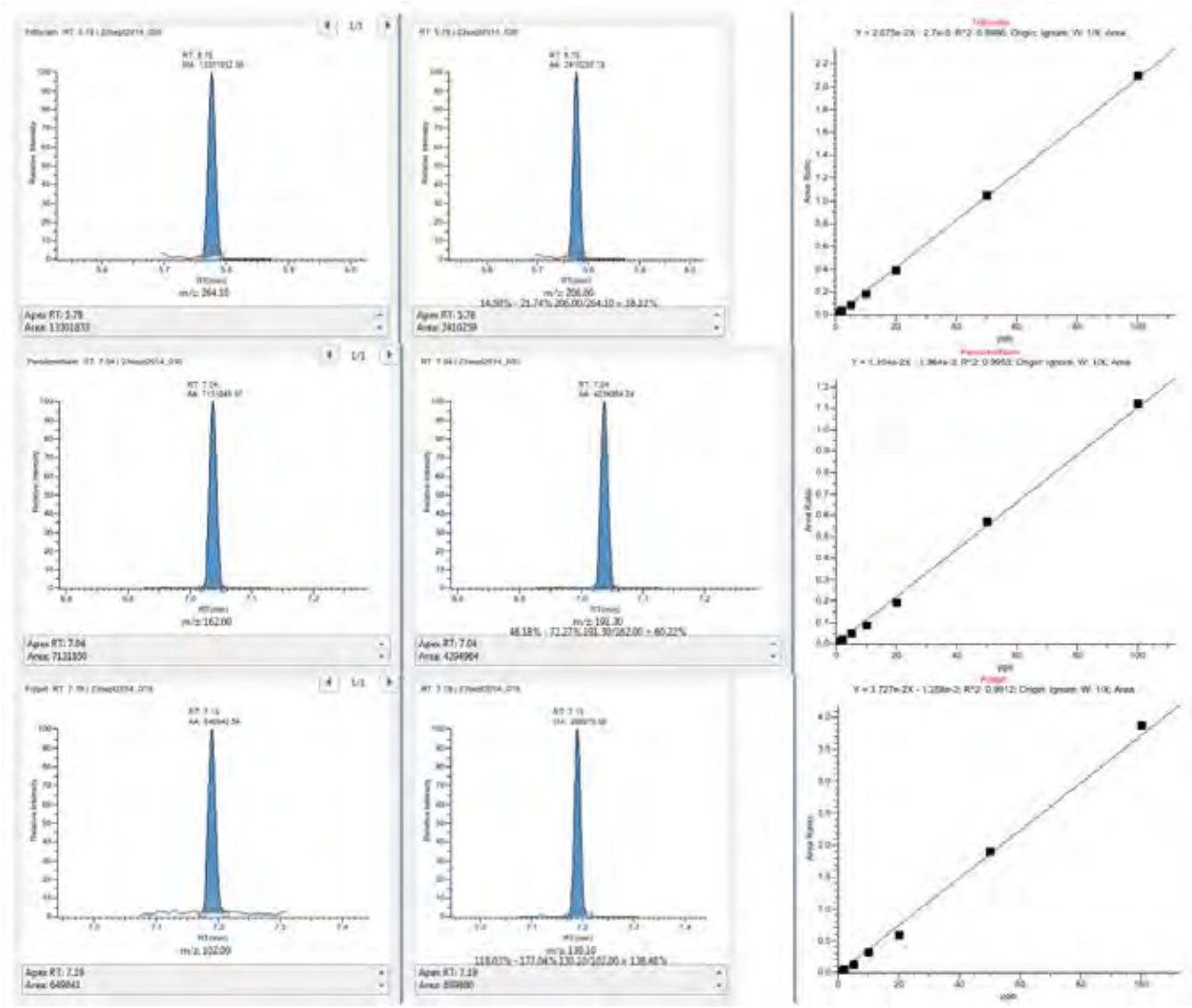

Figure 4. Examples of chromatography (0.5 pg on column) and linearity (no internal standard correction) for trifluralin, pendimethalin, and folpet.

The results of this experiment showed an average %RSD for the peak area reproducibility of 7.3 % and IDL values varying from 0.2 ng/g for dimethamid to 3.7 ng/g for captan (Table 3). By using internal standard correction to compensate for the injection errors both %RSD for peak area repeatability values can be improved even further.

Table 1. Peak area reproducibility (% RSD, n=20) at 5 or 10 pg absolute amount on column and calculated instrument IDL99 (in ng/g).

No	Compound	RT (min)	pg on Column	% RSD	IDL
1	Acetochlor	6.53	5	4.5	0.6
2	Aclonifen	7.64	10	8.3	2.1
3	Aldrin	6.90	5	5.9	0.7
4	Azinphos-ethyl	8.34	10	10.0	2.5
5	Benalaxyl	7.74	5	4.4	0.6
6	BHC, Alpha	5.96	5	5.3	0.7
7	BHC, Beta	6.11	5	8.7	1.1
8	BHC, gamma	6.18	5	6.6	0.8
9	Bifenox	8.09	10	10.0	2.6
10	Bifenthrin	8.00	5	4.3	0.5
11	Biphenyl	4.85	5	10.0	1.3
12	Bromophos-ethyl	7.21	5	6.8	0.9
13	Bromopropylate	8.04	5	5.7	0.7
14	Bupirimate	7.43	5	3.0	0.4
15	Buprofezin	7.45	5	4.9	0.6
16	Cadusafos	5.88	5	5.1	0.6
17	Captan	7.16	10	14.0	3.7
18	Carbetamide	6.90	10	9.9	2.5
19	Chlorbufam	6.09	10	7.6	1.9
20	Chlordane alpha-cis	7.25	5	6.4	0.8
21	Chlordane gamma-trans	7.32	5	5.7	0.7
22	Chlorothalonil	6.29	5	5.7	0.7
23	Chlorpropham	5.77	10	4.6	1.2
24	Chlorpyrifos-ethyl	6.85	5	4.3	0.5
25	Chlorpyrifos-methyl	6.55	5	4.2	0.5
26	Chlozolinate	7.07	5	7.9	1.0
27	Clomazone	6.12	5	4.1	0.5
28	Coumaphos	8.48	5	14.0	1.8
29	Cyanazine	6.85	5	17.0	2.2
30	Cycloate	5.72	5	8.8	1.1
31	Cyfluthrin peaks I-IV	8.59	10	11.0	2.8
32	Cyhalothrin-S	8.23	10	6.8	1.7
33	Cypermethrin peaks I-IV	8.68	10	10.0	2.6
34	DDD p,p	7.63	5	5.6	0.7
35	DDE p, p	7.42	5	4.0	0.5
36	DDT o,p	7.65	5	8.2	1.0
37	DDT p,p	7.80	5	12.0	1.6
38	Deltamethrin	9.15	10	9.8	2.5
39	Diazinon	6.21	5	4.9	0.6
40	Dichlobenil	4.69	10	8.3	2.1
41	Dichlofluanid	6.80	5	3.7	0.5
42	Dichloran	6.04	5	13.0	1.7
43	Dichlorvos	4.33	5	12.0	1.5
44	Dicrotophos	5.79	5	6.8	0.9
45	Dieldrin	7.47	5	8.0	1.0
46	Diflufenican	7.86	5	5.0	0.6
47	Dimethenamid	6.52	5	1.9	0.2
48	Diphenylamine	5.68	10	6.8	1.7
49	Endosulfan I	7.36	5	5.6	0.7
50	Endosulfan II	7.62	5	4.3	0.5
51	Endosulfan sulfate	7.81	5	4.1	0.5
52	Endrin	7.58	5	13.0	1.6
53	EPN	8.03	5	6.0	0.8
54	EPTC	4.73	10	10.0	2.6
55	Ethion	7.61	5	4.2	0.5
56	Ethofumesate	6.74	5	8.6	1.1
57	Ethoprop (Ethoprophos)	5.70	5	7.1	0.9
58	Etoxazole	8.04	10	7.5	1.9
59	Etridiazole	5.07	10	1.0	2.6
60	Etrimfos	6.33	5	4.4	0.6
61	Fenazaquin	8.11	5	4.5	0.6
62	Fenitrothion	6.74	5	4.1	0.5
63	Fenpropathrin	8.05	10	8.9	2.3
64	Fenvalerate I	8.91	5	7.4	0.9
65	Fenvalerate II	8.98	5	7.6	1.0
66	Flucythrinate I	8.69	10	8.9	2.3
67	Flucythrinate II	8.74	5	7.5	1.0
68	Flurochloridone	6.92	5	4.9	0.6
69	Flutolanil	7.33	5	10.0	1.2
70	Fluvalinate	8.93	5	11.0	1.4
71	Folpet	7.19	5	9.2	1.2
72	Furalaxyl	7.13	5	3.6	0.5
73	Heptachlor	6.67	5	3.7	0.5
74	Heptachlor epoxide-cis	7.12	5	6.3	0.8
75	Heptachlor epoxide-trans	7.14	5	9.4	1.2
76	Hexachlorobenzene	6.00	5	9.6	1.2
77	Hexazinone	7.83	5	6.9	0.9
78	Iprodione	7.99	10	12.0	3.1
79	Malaoxon	6.55	5	4.1	0.5
80	Mephosfolan	7.11	5	9.2	1.2
81	Metazachlor	7.06	10	6.4	1.6
82	Methacrifos	5.21	5	9.6	1.2
83	Methidathion	7.21	5	4.7	0.6
84	Methoxychlor	8.05	5	8.6	1.1
85	Metribuzin	6.54	10	3.8	1.0
86	Napropamide	7.34	10	6.2	1.6
87	Nitrofen	7.54	5	7.0	0.9
88	Nitrothal-isopropyl	6.92	5	4.3	0.5
89	Oxadiazon	7.39	5	4.7	0.6
90	Oxychlordane	7.12	5	5.9	0.7
91	Oxyfluorfen	7.42	10	7.7	2.0
92	Paraoxon-methyl	6.30	5	10.0	1.3
93	Parathion (ethyl)	6.88	5	6.5	0.8
94	Parathion-methyl	6.59	5	4.4	0.6
95	Pendimethalin	7.04	5	6.7	0.9
96	Pentachloroaniline	6.48	5	5.4	0.7
97	Pentanochlor (Solan)	6.78	5	5.0	0.6
98	Permethrin I	8.43	5	10.0	1.3
99	Permethrin II	8.46	5	9.7	1.2
100	Phosalone	8.18	5	8.5	1.1
101	Phosmet	8.02	5	6.8	0.9
102	Pirimiphos methyl	6.72	5	4.0	0.5
103	Pirimiphos-ethyl	6.95	5	4.0	0.5
104	Procymidone	7.15	5	5.7	0.7
105	Propachlor	5.61	5	6.9	0.9
106	Propanil	6.52	10	13.0	3.2
107	Propargite	7.86	5	10.0	1.3
108	Propetamphos	6.16	5	4.3	0.5
109	Propham	5.08	5	5.7	0.7
110	Prosulfocarb	6.71	10	4.2	1.1
111	Prothiofos	7.37	5	7.7	1.0
112	Pyrazophos	8.29	5	9.0	1.1
113	Pyridaben	8.49	5	10.0	1.3
114	Pyridaphenthion	7.97	5	16.0	2.0
115	Pyrifenox-E	7.11	5	11.0	1.4
116	Quinalphos	7.13	5	6.9	0.9
117	Quinomethionate	7.25	5	8.8	1.1
118	Quintozene	6.16	5	6.3	0.8
119	Resmethrin	7.89	5	9.6	1.2
120	Spirodiclofen	8.42	5	7.9	1.0
121	Tecnazene	5.57	5	9.6	1.2
122	Tefluthrin	6.30	5	4.5	0.6
123	Terbuthylazine	6.18	10	5.1	1.3
124	Terbutryn	6.73	5	5.6	0.7
125	Tetrachlorvinphos	7.24	5	8.7	1.1
126	Tetradifon	8.16	5	8.0	1.0
127	Tetramethrin	8.01	5	6.2	0.8
128	Tolclofos-methyl	6.60	5	4.6	0.6
129	Tolylfluanid	7.09	5	5.3	0.7
130	Triallate	6.36	10	5.9	1.5
131	Trifluralin	5.78	5	7.5	1.0
132	Vinclozolin	6.57	5	7.1	0.9

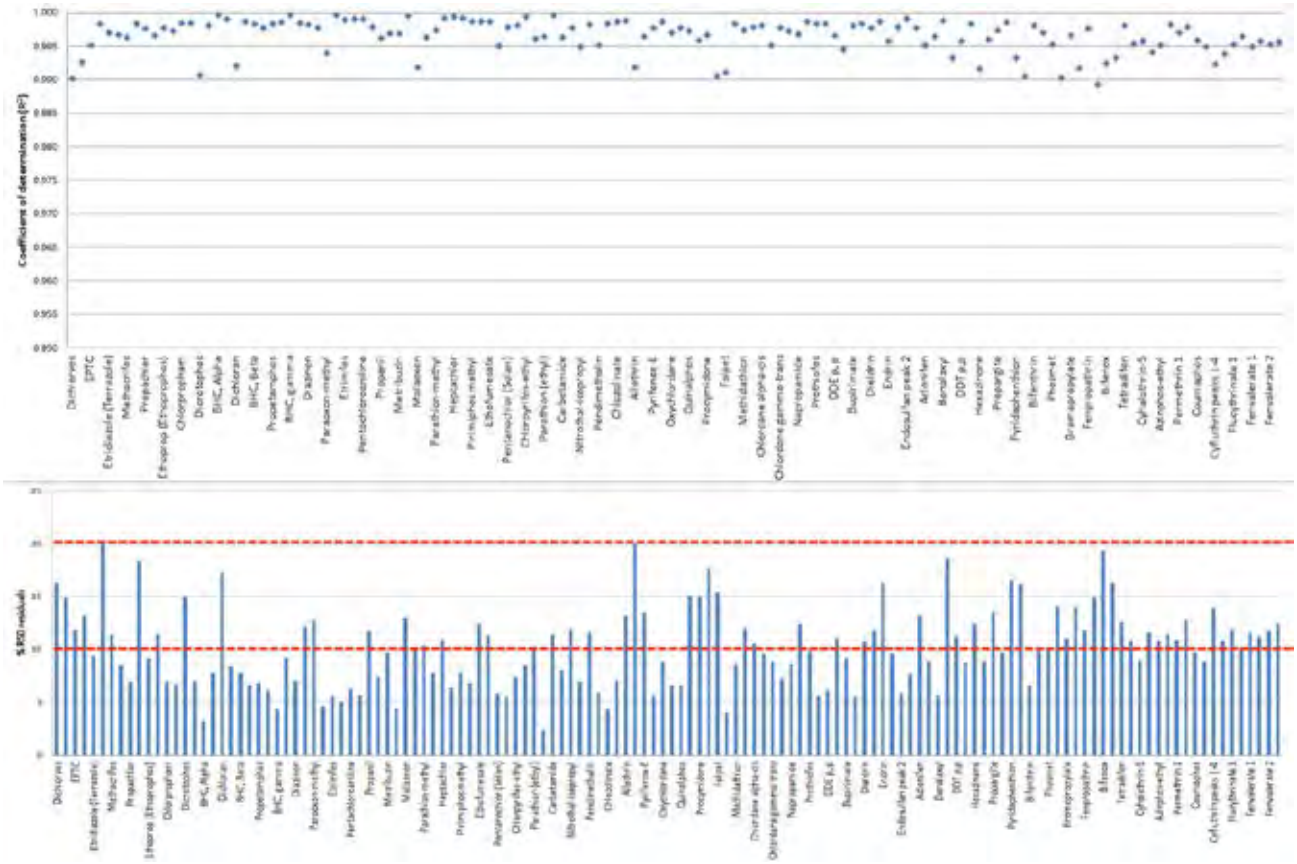

Figure 5. Coefficient of determination (R2) and residuals values (%RSD) calculated for a linear range of 0.5–100 ng/g (or 1.0–200 ng/g). Dashed lines represent the 10% and 20% RSD residual limits.

Linearity of Response

Linearity of the GC-MS/MS system was evaluated across a concentration range of 0.5–100 ng/g (or 1–200 ng/g for some analytes) using matrix-matched standards. In all cases the coefficient of determination (R^2) was higher than 0.99 with an average value of $R^2 = 0.997$. Moreover, individual residual values were <20% with an average value of 10% (Figure 5).

Comprehensive Analysis of Additional Pesticides

Targeted screening and quantification of a given number of pesticides is important, but there is increasing interest in screening samples for compounds other than those in a target list. To answer the question, "What else is in my sample?", samples have to be screened for unexpected or new pesticides or for metabolic/transformation products that could be present in the samples in addition to the targeted compounds. The capability of fast analytical instrumentation enables simultaneous acquisition of full scan and SRM/SIM data.

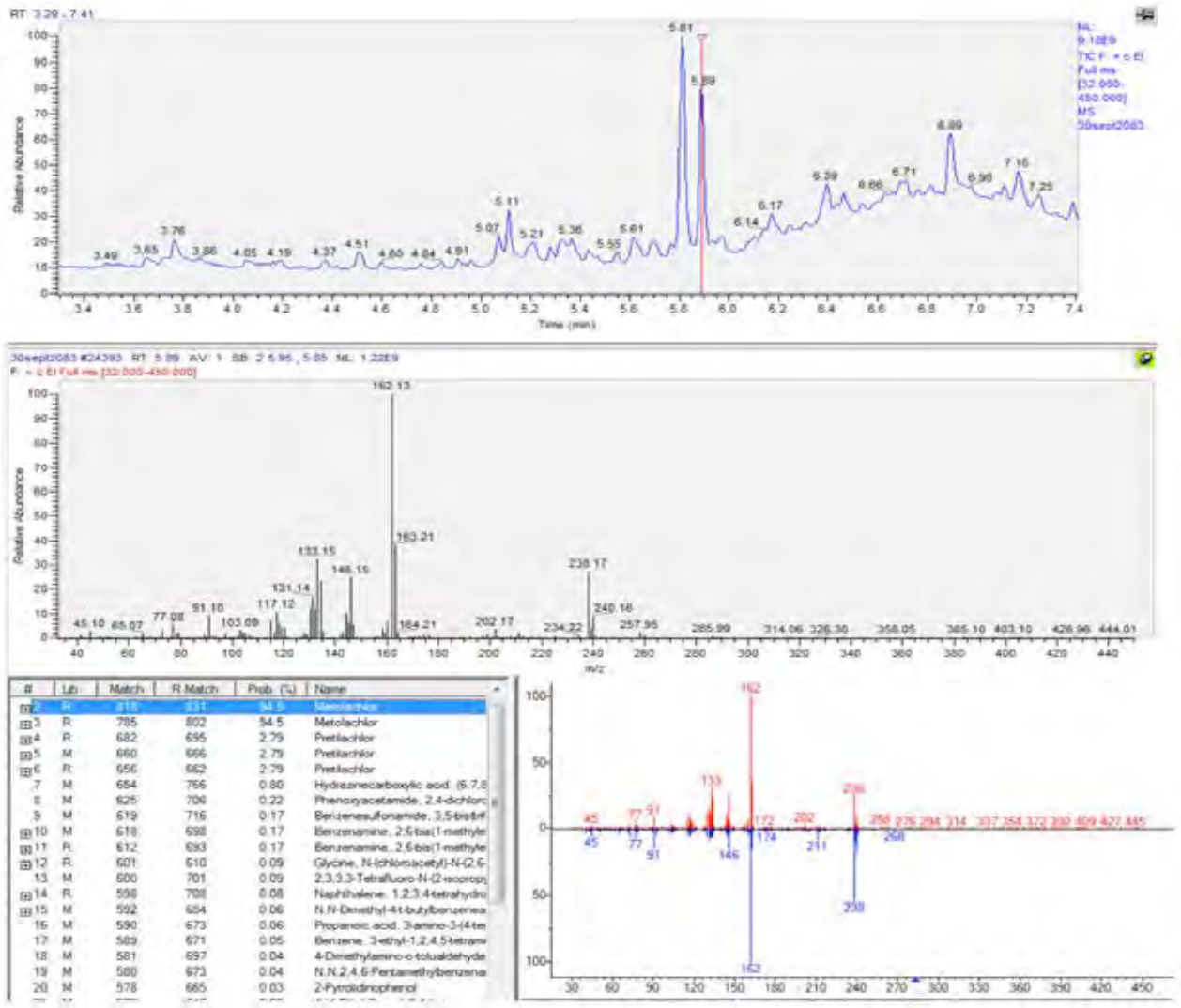

Figure 6. Comprehensive analysis of baby food contaminants using simultaneous full scan/SRM data acquisition. Compound at RT = 5.89 min identified as metolachlor (using NIST) in the full scan acquisition window.

Using the TSQ 8000 Evo GC-MS/MS system, the baby food samples were screened for additional compounds. Data was acquired in full scan and SRM modes simultaneously. An example of a full scan/SRM chromatogram is shown in Figure 6. The extracted mass spectrum of the peak eluting at RT = 5.89 min was submitted to NIST mass spectral library and identified as metolachlor (a compound not in the spiking solution or the target list) with a probability of 95%. This result shows the advantage of using such simultaneous data acquisition, which is possible only using fast instrumentation such as the TSQ 8000 Evo GC-MS/MS.

Conclusion

The results of this work show that laboratory productivity can be tripled using the Thermo Scientific TSQ 8000 Evo triple quadrupole GC-MS system. Acceleration of sample analysis is made possible by:

- direct analysis of acetonitrile extracts with no need for an additional solvent exchange step.
- shorter GC run times using fast data acquisition with the EvoCell fast collision cell technology.
- comprehensive detection of target pesticides and nontargeted pesticides using simultaneous full scan and SRM data acquisition. Additional pesticides were identified by searching the full scan data against the NIST library.

Excellent sensitivity was achieved. All pesticides were detected and identified at a concentration of 5–10 ng/g with IDL values from 0.2–3.7 ng/g.

These results demonstrate that fast GC data acquisition using the TSQ 8000 Evo GC-MS/MS system delivers excellent peak area reproducibility and compound linearity.

References

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3. EN 15662 Version 2.2, Date: 2008-04, Foods of plant origin – Determination of pesticide residues using GC-MS and/or LC-MS/MS following acetonitrile extraction/partitioning and cleanup by dispersive SPE – QuEChERS-method.

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GC-MS/MS Analysis of Pesticide Residue in Green Tea Extracted by QuEChERS with Acetonitrile as Final Solvent

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Overview

Purpose

This poster describes the analysis of several challenging pesticides from green tea samples using GC-MS/MS and acetonitrile as final extraction solvent. The compounds analysed are representatives of various classes of pesticides, such as carboxamids, OC, OP, pyrethriods, aromatic, phenylamides. QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Safe) is a well known approach used for the extraction and clean-up of pesticide residue in various matrices. Typically, the final extract ends up with the pesticides in acetonitrile. Direct injection of acetonitrile extracts is problematic in GC-MS compared to LC-MS because of poor focusing of chromatographic peaks due to the high polarity of acetonitrile, limitations on injection volumes due to the high expansion coefficient of acetonitrile and contamination of the system by matrix co-extractives [1]. Here we present a simple and robust analytical method which employs low volume splitless injections of acetonitrile sample extracts and the selectivity of the Thermo Scientific™ TSQ™ 8000 triple quadrupole GC-MS/MS instrument. With this approach, pesticide target reporting limits of <0.01 mg/kg can be easily achieved. This also overcomes the problems associated with the thermal expansion of acetonitrile and reduces the amount of matrix injected.

Methods

Green tea samples have been extracted using a typical QuEChERS protocol, and the final extracts were spiked with a mixture of 19 pesticides at levels corresponding to 0.005 to 0.5 mg/kg. The analysis was done by GC-MS/MS using a timed-SRM detection method on the TSQ 8000 instrument, employing two SRM transitions for each pesticide compound in a typical MRM method setup. Data processing and reporting is performed by using the Thermo Scientific™ TraceFinder™ software with one SRM transition used for quantitation and the second one for ion ratio confirmation of the positively identified pesticide compounds.

Results

The described method can be confidently used for the routine analysis of pesticides in complex matrices, such as teas with challenging heavy matrix impact for the control of the regulated maximum pesticide residue levels. Excellent sensitivity, linearity and reproducibility were obtained for all target compounds spiked in the green tea samples.

Introduction

QuEChERS involves an initial step when a few grams of the sample are extracted with acetonitrile followed by a clean-up step (with dispersive-SPE) used to remove, to a certain extent, unwanted matrix compound (such as pigments, sugars, organic acids). With QuEChERS, the final extract ends up with the pesticides in acetonitrile, which, being polar solvent, can be problematic in GC-MS. Poor focusing of chromatographic peaks and high expansion coefficient are issues that need to be addressed when acetonitrile is used as a solvent for GC-MS analysis. To overcome this, an additional step can be added to the QuEChERS method where acetonitrile is replaced with solvents that are more amenable to splitless injections in GC-MS.

The aim of this study was to assess the chromatography, repeatability, robustness and linearity of these compounds when using acetonitrile as extraction solvent and splitless injections.

Methods

Sample Preparation

Organically grown green tea leaves (Pure Tea Ltd., Radstock, UK) were used for the experiments described below. For the QuEChERS, 2 g of green tea was weighted and hydrated for 30 min in 10 mL deionized water. Acetonitrile (10 mL) was added followed by 4g $MgSO_4$ and 1g NaCl. After a centrifugation step (10k rpm for 5 min), 6 mL of the supernatant were transferred to a dSPE tube containing 1200 mg $MgSO_4$, 400 mg PSA, 400 mg C_{18} and 400 mg GCB. This mixture was vortexed and centrifuged and 1 mL of the upper layer was spiked with the pesticides of interest at various levels ranging from 1.0 – 100 pg/µL (corresponding to 0.005 – 0.5 mg/kg) and used for the GC-MS analysis.

TSQ 8000 GC-MS/MS Method setup

All experiments were performed using the Thermo Scientific TSQ 8000 Pesticide Analyzer (P/N TSQ8000EI-PA230) which comprises of sample handling (Thermo Scientific™ TriPlus™ RSH liquid autosampler), sample introduction and chromatographic separation (TRACE™ 1300 Series GC equipped with a SL/SSL injector), and the TSQ 8000 triple quadrupole mass analyser.

The TSQ 8000 MS was operated in SRM mode using two transitions per compound. SRM transitions are readily available from a Thermo Scientific Pesticide Compound Database (CDB) containing >600 with retention times and pre-optimized SRMs.

TriPlus RSH Autosampler

Injection vol. and type:	1.0 µL, fast liquid band injection
Washing cycles:	5 x 7 µL, solvent acetonitrile

TRACE 1310 Gas Chromatograph

Injector Split/Splitless:	splitless mode
Liner:	SSL single taper (P/N: 453A2342)
Inj. temp.:	250 °C
Flow:	const flow, 1.5 mL/min, helium
Analytical column	30 m, ID 0.25 mm, 0.25 um TraceGOLD TG-5SILMS (P/N 26096-1420)
Pre-column deactivated	5 m x 0.25 mm, empty
Column oven	temp. programmed
Start	100 °C, for 2.0 min
Ramp 1	50 °C/min to 150 °C
Ramp 2	6°C/min to 200°C
Ramp 3	16°C/min to 320°C, 6 min hold
Transfer line	280 °C

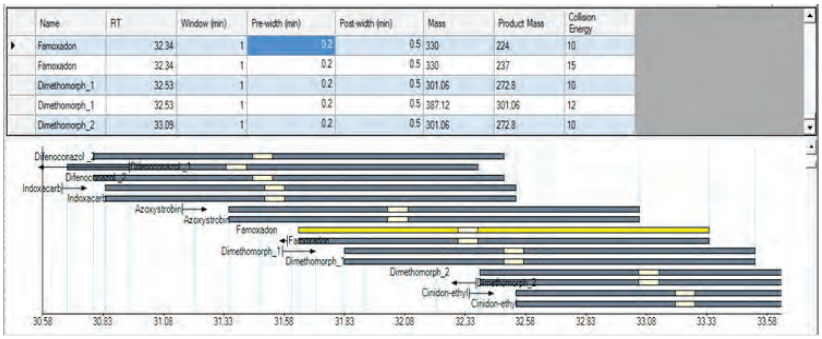
TSQ 8000 Mass Spectrometer in EI mode

Source Temp.: 300 ° C
Ionization: EI+, 70 eV
Emission Current: 50 µA
Resolution: Q1 & Q3 @ 0.7 Da
Collision Gas: Argon
MRM Detection timed SRM mode, see Figure 1

Data Acquisition/Processing

Each compound SRM transition was only monitored for a narrow time window around the established retention time (timed-SRM). This led to a fully optimized instrument duty cycle for maximum analytical performance being handled automatically by the system (Figure 1). The data processing and reporting was achieved by using TraceFinder quantitation and reporting software suite [2].

FIGURE 1. Principle of the Timed-SRM acquisition setup of the TSQ 8000 GC-MS. The white center parts show the peak width, and the gray area shows the full SRM acquisition window.



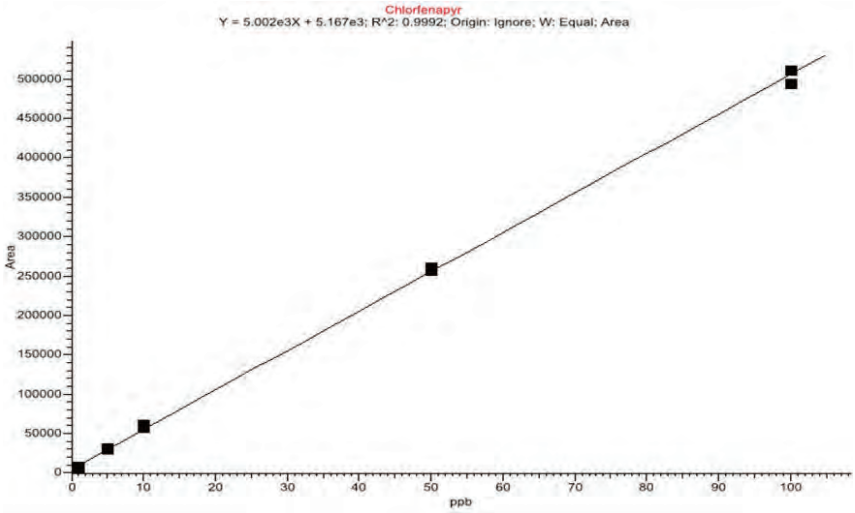
Results

This method describes the methodology used for the multi-residue pesticides analysis in green tea using acetonitrile as final extraction solvent and splitless injections of low sample volume. The performance of the TSQ 8000 GC-MS/MS system was evaluated by assessing the sensitivity, linearity and reproducibility of the targeted compounds in green tea samples.

Calibration and Linearity

The calibration solution have been prepared from green tea extracts spiked in the range of 1.0 pg/µL to 100 pg/µL (corresponding to 0.005 to 0.5 mg/kg level for each of the pesticides in the samples). Two repeat injections per calibration point were performed. The standard matrix blank consisted of green tea extracted as of the standard procedure. The pesticide blank level was tested before applying as blank standard matrix. Excellent linearity with correlation coefficients R^2 exceeding 0.996 (residual error for each calibration point <10% RSD had been achieved for all pesticides (see an example for Chlorfenapyr in Figure 2).

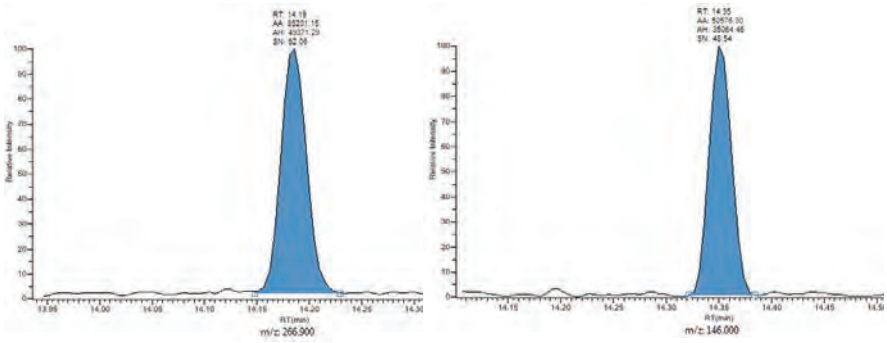
FIGURE 2. Quantitative calibration for Chlorfenapyr, range 1 ppb to 100 pg/µL, 2 injections/calibration point. No internal standard correction.



Sensitivity

All 19 pesticides were easily detected in the lowest calibration matrix-matched standard with excellent chromatography (Figure 3).

FIGURE 3. Pesticide peaks at 5 ppb (0.005 mg/kg) in green tea matrix for Profenofos (337 > 267, CE 12V) and Oxyfluorfen (252 > 146, CE 30V)

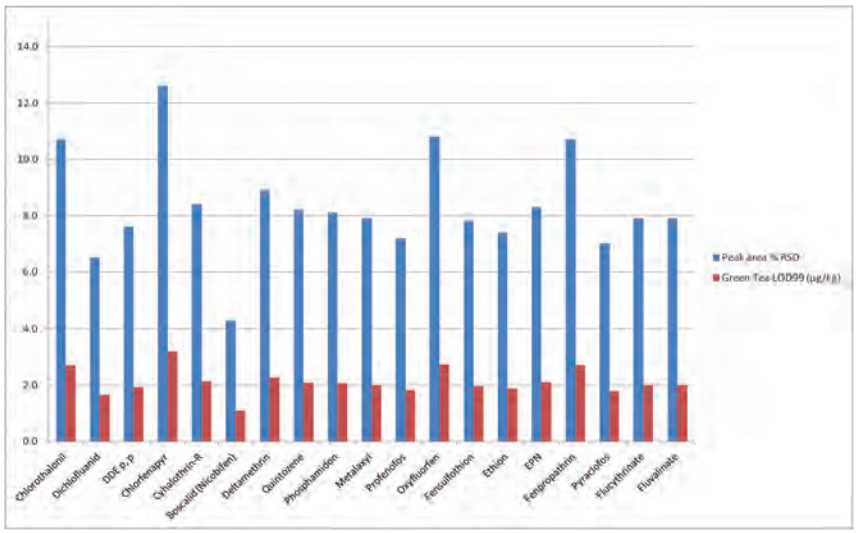


The instrument LOD was assessed by repeatedly (n = 20) injecting the 10 ppb (0.01 mg/kg) calibration standard taking into account the student's-*t* critical values for the corresponding degrees of freedom (99% confidence), the concentration of each native compound, and %RSD. The results of this test show excellent LODs for the pesticides analyzed with values between 1 ppb (200 fg on column) (Boscalid) - 3 ppb (600 fg on column) (Chlorfenapyr) (Figure 4).

Repeatability

Peak area repeatability was assessed using n = 20 replicate injections of the green tea extracts spiked at 10 ppb level (2 pg on column). The results of this experiment shows excellent coefficients of variation values (%RSD) with minimum values of 4.3% for Boscalid, maximum of 12.6 % for Chlorfenapyr and an overall average value of 8.3% (Figure 4).

FIGURE 4. Limits of Detection (LOD) and peak area repeatability (%RSD) of n=20 consecutive injections of green tea spiked at 10 ppb (0.01 mg/kg) level.



Conclusion

The QuEChERS-GC/MS/MS multi-residue method described here allows for rapid and accurate monitoring of GC amenable pesticides in green tea extracts using acetonitrile as final solvent without the need of an additional solvent exchange step.

Low volume splitless injection of the green tea sample extracts overcomes the problems associated with the thermal expansion of acetonitrile and reduces the amount of matrix injected.

The sensitivity and selectivity of the TSQ 8000 GC-MS/MS reached significantly below the regulated levels in green tea samples.

Excellent linearity, chromatography, sensitivity and peak area repeatability were reported.

Taken together, the TSQ 8000 GC-MS/MS system delivers very reliable results, reducing significantly the manual quality control reducing a typical bottleneck in trace analysis laboratories and increasing the productivity for the final sample report processing.

References

1. Rapid Analysis of Pesticides in Difficult Matrices Using GC-MS/MS, Thermo Fisher Scientific Application Note 51880, 2010.
2. Pesticide Method Reference 2nd Edition, Thermo Fisher Scientific, p/n 120390.

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Broad Scope Pesticide Screening in Food Using Triple Quadrupole GC-MS

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Thermo Fisher Scientific, Austin TX, USA

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Overview

Purpose: To demonstrate two different ways to perform targeted and non-targeted screening of pesticides in one analytical run

Methods: Screening for 600 pesticides in selected reaction monitoring (SRM) mode or a smaller subset in selected reaction monitoring/ full scan (SRM/FS) mode

Results: Either method can be used to analyze targeted and non-targeted compounds with little loss of sensitivity

Introduction

The increased accessibility of high selectivity GC-MS has enabled more generic sample preparation in pesticide testing, allowing consolidation of multiple analyte lists and matrices into one method. GC-MS/MS is well suited to multi-residue analysis in a diverse range of matrices. However, as the number of targeted compounds increases, the complexity of method optimization increases and analytical performance becomes compromised. Furthermore, there is a desire to look beyond targeted lists for other potentially harmful food contaminants. Presented here is the use of smart instrument control and data processing software applied to GC-MS/MS analysis of 600 pesticides in matrix to mitigate analytical performance degradation through MS duty cycle optimization. Also discussed is the combining of this optimized targeted quantitation with general unknown analysis through full scan/SRM.

Method 1 – Screening For 600 Pesticides

Sample Preparation

Lettuce was purchased from a local grocery store and was extracted with 1:1 ethyl acetate/cyclohexane following the QuEChERS method of extraction and clean-up, then 5 mL of solvent exchanged into 1 mL of hexane:acetone (9:1). The concentrated extract was spiked with various mixes of calibration standards.

Gas Chromatography

The Thermo Scientific™ TRACE™ 1310 GC was equipped with both an SSL and PTV inlet. A 1 µL injection was performed on the PTV inlet. The liner was a Siltek™ deactivated baffled liner (Thermo Scientific part number 453T2120). Chromatographic separation was achieved by using a 5% diphenyl/95 % dimethyl polysiloxane column (30 m x 0.25 mm 0.25 µm). See Table 1 for the parameters for the PTV and oven.

TABLE 1. PTV and Oven Parameters.

PTV	Mode	Temp	Split Flow	Splitless Time	Purge Flow
	Splitless	75	50	2.00	5.00

Flow Ramps	Rate (mL/min)	Flow (ml/min)	Hold (min)
		1.2	30
	2	3	7.2

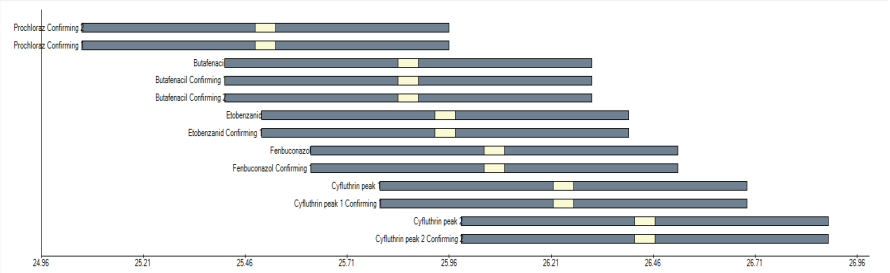
Injection phases	Pressure (kPa)	Rate (°C/sec)	Temp (°C)	Time (min)	Flow (mL/min)
Injection	70			0.1	50
Transfer	210	2.5	300	3.00	
Cleaning		14.5	330	20	75

Oven Program	Ramp	Rate (°C/min)	Temp (°C)	Hold Time (min)
	Initial		90	5
	1	25	180	0
	2	5	280	0
	3	10	300	5

Mass Spectrometry

The targeted screening using SRM of 600 compounds was performed using the Thermo Scientific™ TSQ™ 8000 triple quadrupole MS. After retention times were determined in full scan, a timed-SRM method using selected reaction monitoring (SRM) was constructed to analyze all compounds in a single injection. Over 1,300 transitions were entered into the method from the TSQ 8000 Pesticide Analyzer Compound Database. This automatically populated both the processing and instrument method through the TSQ 8000 system Method Synch. The transfer line was set to 250 C, and the ion source was at 300 C. Figure 1 demonstrates timed-SRM (t-SRM) which allows for the analysis of the 600 pesticides and provides for good sensitivity.

FIGURE 1. Small Section of Timed-SRM.



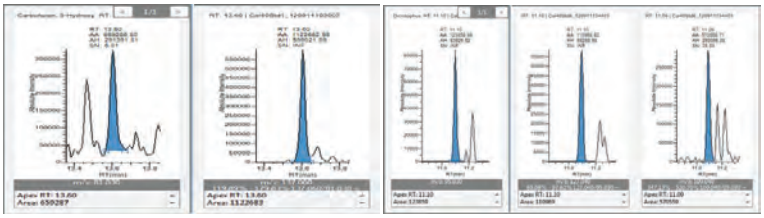
Results

Quantitative performance was determined for 52 pesticides in lettuce matrix during the screening for all 600 pesticides. The linearity for all of the compounds was $R^2 > 0.98$. Curves were generated using Thermo Scientific™ TraceFinder™ software. Ten replicates of a 40 ppb matrix spike sample were also analyzed. To test screening capability, a few additional compounds were added to the 40 ppb spike which had not been part of the calibration, but could be identified through the use of this method. The average concentration and %RSD of the 40 ppb standard are given in Table 2. Figure 5 shows the quantitation ions and confirming ions of the compounds in the 40 ppb spiked sample that were not a part of the original calibration. This demonstrates the ability of the method and the instrument to identify targeted compounds in samples for which the instrument is not calibrated.

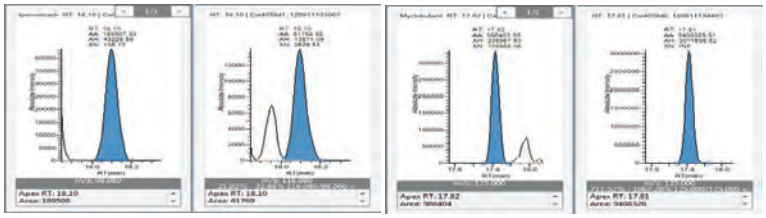
TABLE 2. 40 ppb Standard Spiked into Lettuce Matrix.

Compound Name	Avg	%RSD		Avg	%RSD
Acibenzolar-S-methyl	32.1	8.8	Flutolanil	35.1	6.0
Azinphos-methyl	48.3	4.4	Fuberidazol	45.5	9.8
Azoxystrobin	39.5	2.3	Furalaxyl	62.4	4.4
Benalaxyl	43.8	6.3	Imazalil	45.6	3.5
Bendiocarb	50.7	3.9	Indoxacarb	47.2	9.2
Bitertanol	48.4	7.1	Isoprocarb	43.9	2.3
Boscalid (Nicobifen)	44.0	3.2	Mefenacet	47.1	2.9
Buprofezin	39.6	5.5	Metalaxyl	38.8	8.3
Carbaryl	56.1	2.3	Methiocarb	58.7	4.0
Carbofuran	45.1	11.8	Mevinphos	46.2	6.0
Carboxin	44.6	4.2	Oxadixyl	41.4	4.6
Carfentrazon-ethyl	39.1	5.4	Piperonyl butoxide	42.6	2.0
Clethodim	30.6	15.4	Pirimicarb	26.6	16.5
Cyprodinil	42.5	2.9	Propargite	55.9	6.5
Diethofencarb	41.2	6.7	Propham	40.2	1.7
Difenoconazole peak 1	53.7	3.0	Propiconazole peak 1	43.7	18.5
Difenoconazole peak 2	45.5	3.6	Propiconazole peak 2	49.3	6.0
Dimethomorph-1	52.8	7.1	Propoxur	46.9	2.1
Dimethomorph-2	49.7	3.2	Pyridaben	39.0	1.4
Ethofumesate	40.9	4.3	Pyrimethanil	37.5	15.3
Fenamidone	49.8	5.0	Spiromesifen	62.8	6.0
Fenbuconazol	40.7	1.2	Spiroxamine	52.3	7.0
Fenoxycarb	44.4	3.0	Thiabendazole	49.6	9.9
Flonicamid	44.7	6.1	Triazophos	46.7	4.3
Fludioxonil	45.2	5.7	Triflumizole	48.5	14.3
Flusilazole	44.8	6.1	Zoxamide	58.6	4.3

FIGURE 2. Pesticides Identified by Ion Ratio Not in the Targeted Calibration Curve. First Peak is the Quan Peak, and the Others are for Confirmation.



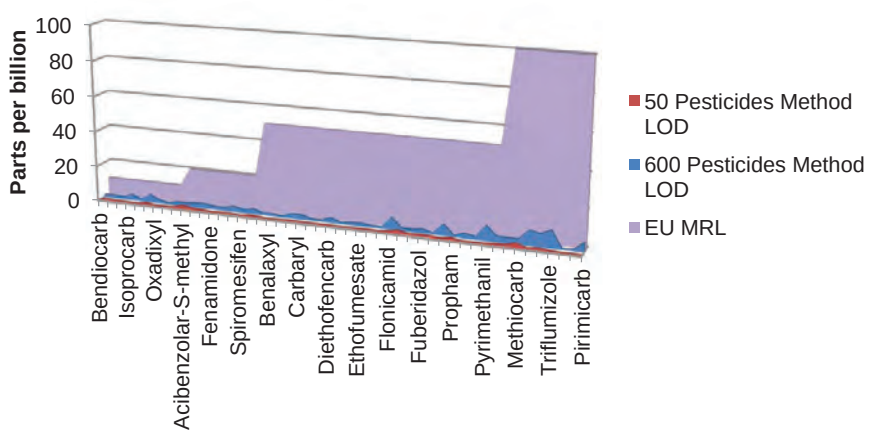
3-Hydroxycarbofuran Dicrotophos



Improvalicarb Myclobutanil

A second method was generated that targeted only the 52 compounds and contained only 104 transitions. Ten replicates of a 5 ppb and 10 ppb standard were analyzed to determine the MDLs for the two instrument methods, one with 1300+ transitions, and the other containing only 104 transitions. The results of compounds with MRLs for lettuce are shown in Figure 3. Although lower detection limits result from longer dwell times in the method with 104 transitions, the screening method that scans for 600 compounds is still capable of reaching the limits in lettuce set by the EU for the compounds requiring a targeted analysis in our list.

FIGURE 3. Comparison of MDLs: 52 Compounds vs. 600 Compounds.



Method 2 – Alternating SRM/FS

Sample Preparation and Gas Chromatography

The sample preparation and GC parameters remained the same as in the first study.

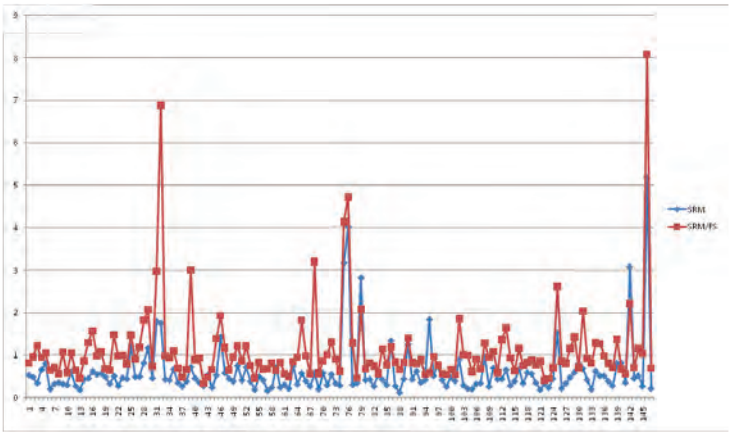
Mass Spectrometry

The scanning of 147 compounds was performed using the TSQ 8000 triple quadrupole MS. After retention times were determined in full scan, a timed-SRM method using selected reaction monitoring (SRM) was constructed to analyze all 147 compounds in a single injection. A second method was constructed, adding full scan to the analysis.

Results

A sample of fruit drink was extracted using the QuEChERS method of extraction and cleanup. The extract was concentrated 5x, then 147 pesticides were spiked into the extract to produce calibration curves from 1 ppb to 200 ppb. The calibration curves were constructed using TraceFinder software for both methods, SRM and alternating SRM/full scan for 147 pesticides. The linearity for most of the compounds was $R^2 >$ 0.98 for both methods of analysis. Ten replicates of a 1 ppb and 10 ppb standard in fruit juice extract were analyzed to determine the MDLs for the two instrument methods, SRM only and alternating SRM/full scan A comparison of the MDLs of both methods are shown in Figure 4. MDLs are slightly higher with the full scan added to the instrument method, but very comparable.

FIGURE 4. Comparison of MDLs from SRM vs. SRM/FS analysis (ppb).



Fruit drink was spiked at 100 ppb and analyzed using the SRM/FS instrument mode. This extract was also spiked with two phthalates at a 1 ppm level. The full scan chromatogram shows several peaks above the 100 ppb pesticide spike. Peaks are at retention times of 9.29, 9.73, 10.39, 10.91, and a very large saturated peak at 31.00 minutes. A close-up view of the first four compounds is shown in Figure 5. Figure 6 displays the NIST library matches for those non-targeted compounds.

FIGURE 5. Close-up View of Four Unknown Peaks in 100 ppb Spiked Fruit Drink.

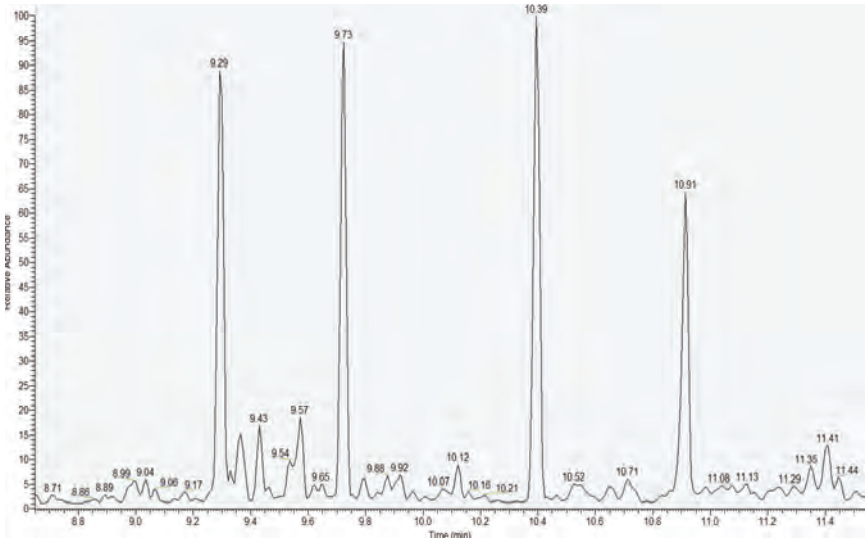
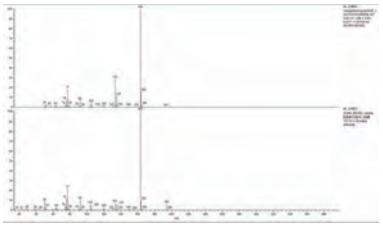
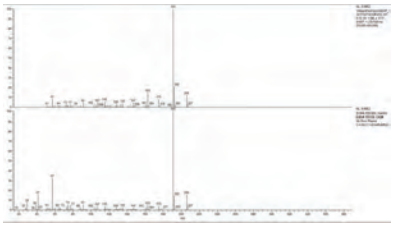


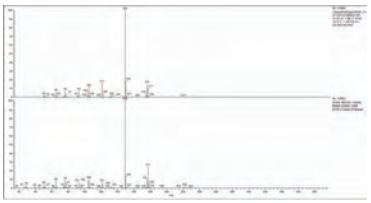
FIGURE 6. NIST Library Match for 4 Unknown Peaks.



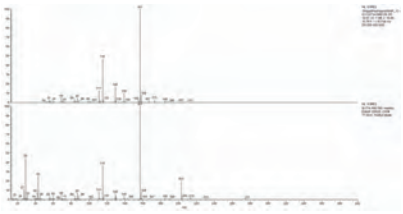
Dimethyl phthalate at 9.29 min



Phenol, 2,4-bis (1,1-dimethylethyl) at 9.72 min
used as a UV stabilizer in plastics



Diethyl phthalate at10.39 min



Triethyl citrate at 0.91 min
used as a plasticizer and food additive

Conclusion

Two different ways of analyzing targeted and non-targeted compounds have been demonstrated using the TSQ 8000 MS paired with the TRACE 1310 GC. Method 1 utilized the high SRM scan rate of the TSQ 8000 to scan for 600 pesticides in one analytical run without sacrificing sensitivity. Without having to calibrate all 600 pesticides, an analyst can still identify additional pesticides that may appear in the sample. Method 2 utilizes the ability of the TSQ 8000 to generate high quality library searchable full scan spectra at high scan speeds by operating the instrument in SRM/FS mode. This was done by selecting a number of target compounds for low level SRM analysis, while using full scan to identify unknowns of any classification, such as leachates from packaging, or nutritional compounds and preservatives added to food products.

Listed below is a summary of the two methods.

Screening for 600 Pesticides

- Screening for 600 pesticides without sacrificing sensitivity due to the high scan speed of the TSQ 8000
- 52 compounds calibrated with $R^2 > 0.98$
- Ability to identify pesticides not in the calibration through ion ratios
- Customizable compound list using AutoSRM feature to optimize new compounds

Alternating SRM/FS

- Target large number of compounds while collecting full scan data
- Quantitate targeted compounds while looking for non-targeted compounds
- Unknown identification of non-targeted compounds using the NIST library
- Calibration curves for most pesticides were $R^2 > 0.98$
- Comparable MDLs with or without full scan data collection
- Can be used for identifying contamination from packaging, nutritional components, or preservatives added to food products
- Customizable compound list using AutoSRM to optimize new compounds

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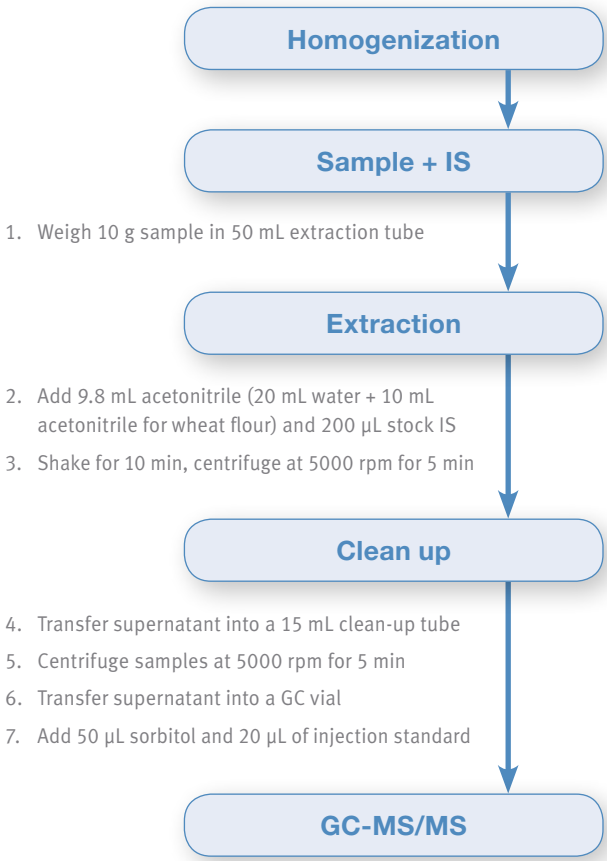
Validation of the Method for Determination of Pesticide Residues by Gas Chromatography – Triple-Stage Quadrupole Mass Spectrometry

Laszlo Hollosi, Katerina Bousova, Michal Godula
Thermo Fisher Scientific, Food Safety Response Center, Dreieich, Germany

Method 63899

Key Words
TraceFinder, TSQ, Chromatography, GC, GC-MS, Pesticide Residues, QuEChERS, Triple Quadrupole

1. Schematic of Method



2. Introduction

Pesticide residue analysis in food is one of the most important and challenging tasks in routine laboratory practice. The European legislation, which is currently the most strict legislation (European Regulation 396/2005 and Commission Directive 2006/125/EC), sets maximum residue limits (MRL) of pesticides in different products of plant and animal origin. This presents a significant analytical challenge with respect to the low limits of quantification (LOQ) required for some specified food matrices. A variety of GC and HPLC methods have been developed for multi-residue determination of pesticides employing a variety of sample preparation and cleanup techniques. In recent years the QuEChERS method has become widely adopted for preparing samples of fruit and vegetables, but the continuous need for more sensitive and accurate measurements requires new developments from the instrument producers as well.

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This method reports on in-house validation results and assessment of performance parameters of a complete multi-residue pesticide analysis method employing QuEChERS sample preparation kits, sample measurement by the newly developed Thermo Scientific™ TSQ™ 8000 Pesticide Analyzer system and rapid data analysis by Thermo Scientific™ TraceFinder™ software.

3. Scope

The objective of this validation study was to prove a complete workflow solution (delivered by Thermo Scientific chemicals, consumables and instrumentation) that can be implemented for routine multi-residue pesticide analysis (approximately 140 priority pesticides) in representative matrices (strawberry, wheat flour and leek). This was achieved in accordance with current legislation requirements, demonstrating that sensitivity of the assay conforms with the MRL values at the limits of detection (LOQ).[1-4]

4. Principle

Sub-portions of previously homogenized (for some instable compound cryogenic milling is recommended) samples were treated according to a standard QuEChERS method protocol (extraction and clean-up) prior to injection in the TSQ 8000 Triple-Stage Quadrupole GC-MS system.[5, 6]

Ready to use QuEChERS kit containing both extraction and clean-up tubes and associated protocol were used for sample preparation (Thermo Fisher Scientific, Runcorn UK). Identification of pesticide residues was based on retention time and ion-ratio confirmation using selective reaction monitoring (SRM) of characteristic transition ions, while quantification was calculated on matrix matched calibration and internal standardization. All method performance criteria were established according to the relevant guidelines.[1-4, 7]

5. Reagent List

		Part Number
5.1	Acetone, HPLC Grade	A/0606/17
5.2	Acetonitrile, LC-MS Grade	A/0638/17
5.3	Methanol, Optima LC-MS grade	A456-212
5.4	Toluene, HPLC grade	T/2200/08
5.5	Water, LC-MS grade	W/0112/17
5.6	Sorbitol, 500 g	10396733

6. Standard List

6.1 Pesticides

All individual pesticide compounds – Acephate, Acrinathrin, Amitraz, Azinphos-methyl, Azoxystrobin, Bifenthrin, Bitertanol, Boscalid, Bromopropylate, Bromuconazole, Bupirimate, Buprofezin, Cadusafos, Captan, Carbaryl, Carbofuran, Carboxin, Chlorfenapyr, Chlorfenvinphos, Chlorobenzilate, Chlorothalonil, Chlorpropham, Chlorpyrifos-ethyl, Chlorpyrifos-methyl, Cyfluthrin, Cyhalothrin, Cypermethrin, Cyproconazole, Cyprodinil, DDD, DDE,DDT, Deltamethrin, Demeton-S-methyl, Diazinon, Dichlofluanid, Dichloran, Dichlorbenzophenon, Dichlorvos, Dicofol, Difenoconazole, Dimethoate, Dimethomorph, Diphenylamine, Endosulfan, Endosulfan sulfate, EPN, Epoxiconazole, Ethion, Ethoprop (Ethoprophos), Etofenprox, Fenamiphos, Fenamiphos sulfone, Fenamiphos-sulfoxid, Fenarimol, Fenbuconazol, Fenitrothion, Fenoxycarb, Fenpropathrin, Fenpropidin, Fenpropimorph, Fenthion, Fenvalerate, Fipronil, Fludioxonil, Fluquinconazole, Flusilazole, Flutolanil, Flutriafol, Fluvalinate, Folpet, HCH alpha, HCH beta, HCH gamma Lindane, Hexaconazole, Imazalil, Iprodione, Isofenphos-methyl, Kresoxim-methyl, Linuron, Malathion, Mepanipyrim, Metalaxyl, Methacrifos, Methamidophos, Methidathion, Methiocarb, Metribuzin, Monocrotophos, Myclobutanil, Ortho-phenylphenol, Oxadiazon, Oxadixyl, Paclobutrazol, Paraoxon-methyl, Parathion (ethyl), Parathion-methyl, Pendimethalin, Permethrin, Phenthoate, Phosalone, Phosmet, Phosphamidon, Pirimicarb, Pirimicarb-p-desmetyl, Pirimiphos methyl, Prochloraz, Procymidone, Profenofos, Propargite, Propiconazole, Propyzamide, Prothiofos, Pyraclostrobin, Pyridaben, Pyrimethanil, Pyriproxyfen, Quinoxyfen, Spirodiclofen, Tebuconazole, Tebufenocide, Tebufenpyrad, Tefluthrin, Tetraconazole, Tetradifon, Tetrahydrophthalimide, Thiabendazole, Tolclofos-methyl, Tolyfluanid, Triadimefon, Triadimenol, Trifloxystrobin, Trifluralin, Triticonazole, Vinclozolin) were obtained from Sigma-Aldrich® (Germany) and Laboratory Instruments Srl (CASTELLANA GROTTE, Italy).

6.2 Internal standards

1-bromo-4-fluorobenzene (BFB), triphenylphosphate (TPP) (both from Sigma-Aldrich, Germany)

6.3 Quality Control Materials

FAPAS #19140QC (lettuce), FAPAS #19141QC (green bean) and FAPAS #19142QC (melon puree)

Note: FAPAS samples were selected primarily on content of target pesticides. However, due to limited availability, matrices are slightly different from the validated matrices.

7. Standards and Reagent Preparation

7.1 Individual Pesticide Standard Stock Solutions

Prepared gravimetrically in ~1000 mg/L concentration by weighing 10 mg from each analyte into a 20 mL amber screw cap vial on a five digit analytical balance and dissolving in 10 mL of appropriate solvent (acetone, toluene or acetonitrile depending on the individual compound). Concentrations of each individual standard stock solutions were calculated gravimetrically using weight of added compounds and solvents. All individual standard stocks were stored in a freezer at -20 °C. Validity of individual standard stock solutions was 6 months.

7.2 Intermediate Standard Stock and Working Standard Solutions

Prepared by pipetting the appropriate amount of each individual standard stock and diluting it with acetonitrile. The concentration of intermediate standard stock solutions was 5000 ng/mL. Working standards were prepared by diluting intermediate standard stock solution accordingly. Intermediate standard stock solutions were stored in a freezer at -20 °C, and the working solutions in a fridge at 4 °C. Validity of intermediate stock solutions was 3 months.

7.3 Individual Internal Standard Stock Solutions

Prepared gravimetrically in ~1000 mg/L concentration by weighing 10 mg from each analyte into a 20 mL amber screw cap vial on a five digit analytical balance and dissolving in 10 mL of acetone for TPP and 10 mL toluene for BFB. Exact concentration values were determined based on the gravimetrical values of both weighed compound and added solvent. Individual internal standard stock solutions were stored in a freezer at -20 °C. Validity of individual internal standard stock solutions was 6 months.

7.4 Working Internal Standard Stock Solutions

Prepared individually by pipetting the appropriate amount of each individual standard stock solution and diluting it with acetonitrile. The concentration of working internal standard stock solutions was 5000 ng/mL and was used for direct spiking of the samples. Validity of working stock solutions was 3 months.

7.5 1% Sorbitol Solution (Analyte Protectant)

Prepared in 70/30 v/v% ACN/H_2O and used for adding prior to injection. Protectant solution was added to the sample prior to injection in order to prevent undesired analyte interaction and consequent losses during the injection.[8]

8. Apparatus

		Part Number
8.1	Fisher precision balance	XP-1500FR
8.2	Sartorius analytical balance	ME235S
8.3	Thermo Barnstead EASYpure®II water	3125753
8.4	ULTRA-TURRAX® – G25 dispergation tool	1713300
8.5	ULTRA-TURRAX	3565000
8.6	Vortex shaker	3205025
8.7	Vortex universal cap	3205029
8.8	Horizontal shaker	1069-3391
8.9	Horizontal shaker plate	1053-0102
8.10	Thermo Heraeus Freco 17 micro centrifuge	3208590
8.11	Pesticide Analyzer (TSQ 8000 Triple Stage Quadrupole GC-MS with Thermo Scientific™ TRACE™ 1310)	

9. Consumables

		Part Number
9.1	GC vial kit	60180-599
9.2	Pipette Finnpipette 100–1000 µL	3214535
9.3	Pipette Finnpipette 10–100 µL	3166472
9.4	Pipette Finnpipette 500–5000 µL	3166473
9.5	Pipette holder	3651211
9.6	Pipette tips 0.5–250 µL, 500/box	3270399
9.7	Pipette tips 1–5 mL, 75/box	3270420
9.8	Pipette tips 100–1000 µL, 200/box	3270410
9.9	Spatula, 18/10 steel	3458179
9.10	Spatula, nylon	3047217
9.11	Centrifuge tube rack	1066-3721
9.12	QuEChERS extraction tube, 50 mL, 250 pack	60105-216
9.13	QuEChERS clean-up tube, 15 mL, 50 pack	60105-225
9.14	GC column Thermo Scientific™ TraceGOLD™ TG-5SilMS, 30 m × 0.25 × 0.25 mm	10177894
9.15	PTV Baffle Liner (Siltek), Deactivated, 2 mm ID × 2.75 mm OD × 120 mm Length	453T2120
9.16	2 mL vial rack	12211001

10. Glassware

		Part Number
10.1	Volumetric flask, 10 mL	FB50143
10.2	Volumetric flask, 25 mL	FB50147
10.3	40 mL screw cap vial	1054-1593
10.4	Caps for 40 mL screw cap vial	1009-0962
10.5	500 mL bottle	9653640
10.6	100 mL bottle	1006-8060

11. Procedure

11.1 Sample Preparation

Blank matrix samples (strawberry (SB), wheat flour (WF) and leek (LK)) used for validation experiments were purchased in local retail stores and were homogenized with an Ultra-Turrax homogenizer, extracted and cleaned-up prior to sample preparation. Matrix extracts were used as matrix blank samples and dilution solvents for matrix-matched calibration. Ready to use Thermo Scientific QuEChERS extraction kits were used for sample preparation, and contained 4 g $MgSO_4$, 1 g NaCl, 1 g trisodiumcitrate dehydrate and 0.5 g disodiumcitrate sesquihydrate for buffered extraction of target compounds. Pre-prepared clean-up tubes contained 1200 mg $MgSO_4$, 400 mg PSA and 400 mg C18 for increased clean-up efficiency for more complex matrices such as leek. The same QuEChERS protocol was applied for all of the matrices.

11.1.1 Homogenization of Matrices

11.1.1.1 Select larger amount of strawberry (~500 g) and bunch of leek matrices and put into an appropriate size beaker and label it.

11.1.1.2 Attach the G25 dispergation tool to the Ultra-Turrax homogenizer. (For better recovery for some unstable compounds cryogenic homogenization is advised).

11.1.1.3 Start homogenization at middle rotation speed (speed level 2–3) and continue to form a smooth homogenate.

11.1.2 Sample Extraction and Clean-up

11.1.2.1 Weigh 10 g sample into a 50 mL QuEChERS extraction tube containing 4 g $MgSO_4$, 1 g NaCl, 1 g trisodiumcitrate dehydrate and 0.5 g disodiumcitrate sesquihydrate.

11.1.2.2 Add 200 µL 5000 ng/mL internal standard #141 to the samples.

11.1.2.3 Add 10 mL ACN to SB and LK samples. For WF, first add 20 mL H_2O to the samples, let it completely wet the sample and then add 10 mL ACN to it.

11.1.2.4 Shake samples for 10 min on a horizontal shaker and centrifuge with 5000 rpm for 5 min. Transfer supernatant (~8 mL) into the 15 mL QuEChERS clean-up tubes containing 1200 mg $MgSO_4$, 400 mg PSA and 400 mg C18.

11.1.2.5 Vortex for 1 min and centrifuge samples with 5000 rpm for 5 min.

11.1.2.6 Collect supernatant and transfer 1 mL into a GC vial for instrumental analysis.

11.1.2.7 Add 50 µL sorbitol solution (protectant) and 20 µL 5000 ng/mL injection standard (BFB) to the GC vials prior to injection.

11.2 GC-MS/MS Analysis

Sample measurements were carried out using the TRACE 1310 gas chromatograph coupled to the TSQ 8000 Triple Stage Quadrupole Mass Spectrometer (Pesticide Analyzer). For instrument control, analysis, data review and reporting TraceFinder 3.1 software was used.

11.2.1 GC method settings

The injector settings were as follows:

Injector:	Thermo Scientific™ TriPlus RSH Autosampler with 10 µL injection syringe
Liner:	PTV Baffle Liner (Siltek), Deactivated, 2 mm ID × 2.75 mm OD × 120 mm Length (recommended to be changed after 40 injections of matrix samples)
Injection mode:	splitless PTV, basic mode
Carrier mode:	constant flow
Inlet temp:	75 °C
Split flow:	50 mL/min
Splitless time:	1 min
Injection volume:	1 µL
Plunger strokes:	3
Air filling mode:	auto
Carrier flow:	1.2 mL/min
PTV injection time:	0.1 min
PTV transfer rate:	2.5 °C/s
PTV transfer temp:	300 °C
PTV transfer time:	3 min
PTV cleaning rate:	14.5 °C
PTV cleaning temp:	330 °C
PTV cleaning time:	20 min
PTV cleaning flow:	75 mL/min
PTV cleaning phase:	post cycle temperature cool down

The GC oven settings were as follows:

Carrier gas:	1.2 mL/min Helium (constant flow)
PTV cleaning phase:	post cycle temperature cool down

Table 1. GC temperature programming

#	Rate [°C/min]	Temperature [°C]	Hold Time [min]
Initial		40	1.5
1	25	90	1.5
2	25	180	0
3	5	280	0
4	10	300	5

11.2.2 Triple Quadrupole MS Settings

Mass spectrometric detection was carried out using the TSQ 8000 triple-quadrupole mass spectrometer in timed-SRM mode. All method and SRM settings were taken from the Thermo Scientific TSQ 8000 Pesticide Analyzer system method.[6] Ion ratio values were revised and adapted for each investigated matrices.

The settings were as follows:

Scan type:	timed-SRM (details in Table 2)
Ionization:	EI +
MS transfer line temp:	250 °C
Ion source temp:	300 °C
Cycle time:	0.3 s
Minimum baseline peak width:	3 s
Desired scans per peak:	10
Minimum dwell time:	0.001 s
Q1 resolution:	normal (0.7 Da)

11.3 Calculation of Results

Internal standardization was applied for quantification of target pesticides. The relevant response factors (R_f) were defined by the equation below. Calculation of final result was performed using the following equations.

11.3.1 Equations

Calculation of the response factor:

$$R_f = \frac{A_{St} \times c_{[IS]}}{A_{[IS]} \times c_{St}}$$

R_f – the response factor

A_{St} – the area of the pesticide peak in the calibration standard

$A_{[IS]}$ – the area of the internal standard peak of the calibration standard

c_{St} – pesticide concentration of the calibration standard solution

$c_{[IS]}$ – the internal standard concentration of the calibration standard solution

Calculations of sample amount in each sample (the absolute amount of pesticide extracted from the sample):

$$X_{analyte} = \frac{A_{analyte} \times X_{IS}}{A_{IS} \times R_f}$$

$X_{analyte}$ – the absolute amount of pesticide that was extracted from the sample

$A_{analyte}$ – the area of pesticide peak in the sample

$A_{[IS]}$ – the area of the internal standard peak in the sample

$X_{[IS]}$ – the absolute amount of internal standard added to the sample

Calculations of sample amount in each sample (the absolute amount of pesticide extracted from the sample):

$$C = \frac{X_{analyte}}{m}$$

m – the weight of sample [g]

$X_{analyte}$ – absolute analyte amount [ng]

12. Method Performance Characteristics

In-house validation of the method was carried out on all matrices and target pesticides. European guidelines for single laboratory validation and pesticide residue analysis were used for establishing method performance criteria.[1, 2] All method performance parameters were compared to the relevant legislative requirements and maximum residue limit (MRLs).[2-4, 7] For compounds containing more isoforms, only one performance criteria was established.

12.1 Selectivity

Method (SRM) selectivity was assessed based on the presence of specific ion transitions (quantifier ion and two transitions for compound confirmation) at the corresponding retention time (Table 2), as well as the observed ion ratio values corresponding to those of the standards. Acceptance criteria for retention time and ion ratios were set according to current quality control criteria.[1, 3] Matrix blank samples were also inspected for the presence of interfering peaks in close vicinity of the target retention times for which (according to SANCO guideline definitions) <30% of LOQ acceptance criteria was applied.[3] Additional peaks in close vicinity of target peaks in blank samples were observed for chlorpropham (LK), demethon-s-methyl (SB), fenhexamide (WF, LK), fenitrothion (WF, LK), procymidon (WF), phosphalone (SB), permethrin (WF, LK), fenpropathrin (LK), o-phenylphenol (WF) and carbofuran (SB, WF). However, they were all clearly resolved by retention time from the target peaks (Rs>1.5) except carbofuran in SB and WF and propargite in WF and LK matrices.

12.2 Linearity, Response Factor, Matrix Effect

The calibration curves were created at six levels (matrix-matched) and injected in duplicate. R_f values for internal standardization were determined from the calibration curves for all matrices and internal standards by calculating cumulative average response factor over the whole calibration range. The linearity of calibration curves was assessed in three groups of compounds (depending on the relevant MRL values) in calibration ranges of 0–200, 0–1000 and 0–2000 ng/g, respectively, (details and results in Table 3). Calibration levels were equidistantly distributed over the calibration range. Linear function was evaluated according to Mandel's fitting test and plotting of residuals for which <20% acceptance limit was set.[3] Correlation coefficient values were additionally established for which an artificial 0.985 was set as an acceptance limit, as no legislative limits are defined for them. The set value wasn't met for fenpropathrin and dichlofluanid (LK) and propargite (WF) based on the high LOQ values related to the calibration levels. No weighted function was applied.

Matrix effects were evaluated by (Youden-) plotting of measured relative peak areas of calibration standards in solvent against the areas in the relevant matrix. No matrix effect is observed if the difference of the slope (dif%) of the fitted line is less than 20% from the ideal (y=x) curve, while matrix effects are observed when the difference is between 20–50% (minor matrix effect) or exceeds 50% (major matrix effect). Matrix effect results are listed in Table 3. For the compounds with demonstrated matrix effect application of matrix matched calibration is required.

12.3 Accuracy

Method trueness was assessed by recovery studies using blank matrices spiked at three concentration levels (L1, L2 and L3) and injected in six individually prepared replicates. (Table 4). Spiking of samples occurred prior to sample preparation. Found concentrations, recovery and relative standard deviation (% RSD) were calculated (Table 5). According to SANCO requirements recovery values are deemed acceptable if between 70–120%.[3] Values were calculated only for those cases in which spiking levels were higher than the compound LOQ in the particular matrix. Recovery values could not been established for amitraz in WF and captan, chlorthalonil and tolyfluanid in LK matrices due to the high LOQ values measured relative to the spiked levels. Strong influence of matrix on the results were observed in several cases and results could not been established at one or two spiking levels based on the measured different LOD/LOQ values in the different matrices (details in Table 4). For routine measurement these compounds in these matrices have to be measured with separate, specially optimized analytical methods. Method bias was established by means of external quality control materials obtained from FAPAS (York, UK). Available FAPAS materials were #19140QC (lettuce puree), #19141QC (green bean puree) and #19142QC (melon puree). The available Fapas samples represented only a limited number of the target compounds and different matrices from those targeted. However, measured values showed good agreement with the assigned values in all cases except carbofuran, in which the measured value was slightly below the acceptance range. This could be due to differences between the two different matrix characteristics. Details on the measured FAPAS values are listed in Table 7.

12.4 (Intermediate) Precision

Instrument injection precision was tested for both retention time and peak area for all target compounds by subsequent injections (n=6) of low concentration level (L1) standard solutions. Instrument injection precision for retention time was below 0.5% for all compounds and between 1.2–18.04% (fipronil and fenamiphos-sulfoxide) for peak area without internal standard compensation indicating reliable instrument performance. Method within-day and between-day precision values were determined for each matrix at middle spiking level (L2) and expressed as %RSD over 3 days with individually prepared samples (n=6). Mean within-day precision values were determined as an average of the 3 individual days' mean precision, while between-day precision was expressed as mean of the overall precision data. According to SANCO requirements <20% was set as acceptance criteria for the target compounds and matrices.[3] Measured values are shown in Table 5.

12.5 Limit of Detection, Limit of Quantification

Limits of detection and quantification were estimated following the IUPAC. Measured method LOD, LOQ and the relevant legislative limits (MRLs) are listed in Table 6.[7] An artificial MRL=10 ng/g was set as target value for compounds, for which no MRL values are legislatively defined. The expectation of the method was to meet MRL values at least at LOQ level which was achieved for the vast majority of target compounds. For methiocarb (WF, LK), carbofuran (SW), oxadyxil (WF) and propargite (WF, LK) the established LOQ values were below the targeted MRLs' value. However, with exchanging of quantifier and qualifier ions the target values can be reached. For fenpropathrin (WF, LK), amitraz (WF) and tebufenocid (all matrices), the target values could not be reached even when exchanging the quantifier and qualifier ions.

12.6 Robustness

A robustness study was performed by varying parameters like laboratory personnel, extraction and clean-up batches. Results were compared to the original method and significant differences were sought based on ANOVA analysis. None of the parameters which were varied led to significant differences in measured values, consequently indicating that the method was robust.

13. Conclusion

Full in-house validation of a complete method intended for routine pesticide residue measurements was carried out. The goal of the study was to obtain an objective and realistic overview of the analytical performance of a widely used and accepted sample preparation method combined with state of the art analytical instrumentation. The method performance parameters indicate that the performance for the majority of target compounds complies with current regulatory requirements. Independent, external quality control materials were additionally applied to improve confidence in the measurement results. In some cases method performance parameters could not be established or measured values fell outside of the targeted range due to individual properties of compounds or strong matrix influences on the analytical results. For those compounds (in the relevant matrix), individually optimized sample preparation (additional or special clean-up) and instrumental methods have to be applied. From a practical point of view (especially for instable or active compounds) the best performance can be achieved by replacing the liner (and septum) after 40–50 injections. Overall it can be concluded that the complete workflow solution offered by Thermo Fisher Scientific in conjunction with the newly developed TSQ 8000 GC-MS system delivers the required system performance for the target compounds especially regarding sensitivity, selectivity and recovery.

14. References

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2. European Commission 2006/125/EC
3. European Commission SANCO/12495/2011
4. European Commission 788/2012/EC
5. Fussell et al. (2007) *Food Additives & Contam*, 24:1247-1256
6. Thermo Scientific TSQ 8000 Pesticide Analyzer Brochure, https://static.thermoscientific.com/images/D22018~.pdf
7. EU Pesticides Database, http://ec.europa.eu/sanco_pesticides/public/index.cfm
8. Anastassiades et al. (2003) *J. Chromatogr A*, 1015:163-184

15. Annex

Tables and Figures

Table 2. Selectivity parameters for the target compounds

** retention times for all isomers ** internal standard compound*

Name	RT (min)	Quantifier Ion			Qualifier Ion 1			Qualifier Ion 2			Ion Ratio (for qualifier ion 1/ qualifier ion 2) [% of quant. ion]
		Precursor Mass [m/z]	Product Mass [m/z]	Collision Energy [V]	Precursor Mass [m/z]	Product Mass [m/z]	Collision Energy [V]	Precursor Mass [m/z]	Product Mass [m/z]	Collision Energy [V]	
Acephate	9.36	95.5	65.4	8	136.0	42.1	8	136.0	94.0	12	0.2 / 99
Acrinathrin	24.33	181.0	152.0	22	208.1	180.9	8	289.0	93.1	8	110 / 52
Amitraz	24.03	121.0	106.1	10	131.9	117.1	16	161.9	132.0	8	85 / 78
Azinphos-methyl	23.29	132.0	77.0	12	160.0	50.9	34	160.0	77.0	16	55 / 120
Azoxystrobin	30.33	344.1	156.0	34	344.1	171.9	36	344.1	329.0	14	100 / 250
Bifenthrin	22.08	165.1	163.6	24	181.0	165.9	10	181.0	179.0	12	3800 / 400
Bitertanol	25.25	170.0	115.1	34	170.0	141.1	20	170.0	169.1	16	140 / 40
Boscalid (Nicobifen)	27.09	112.0	76.0	12	139.9	76.0	22	139.9	112.0	10	240 / 350
Bromopropylate	22.09	184.9	75.5	30	184.9	156.9	12	340.8	185.0	14	2500 / 600
Bromuconazole	21.87/ 22.6*	172.9	74.0	38	172.9	109.0	26	172.9	144.9	16	100 / 150
Bupirimate	18.08	208.1	140.1	12	208.1	165.0	12	273.1	193.2	8	260 / 60
Buprofezin	18.08	105.1	50.9	32	105.1	77.0	18	175.0	132.1	12	275 / 75
Cadusafos	11.5	159.0	96.9	16	159.0	130.9	8	213.0	89.1	12	550 / 15
Captan	16.35	149.0	70.0	20	149.0	78.8	14	149.0	105.0	6	120 / 130
Carbaryl	14.13	115.0	89.0	16	144.0	115.1	22	144.0	116.1	10	800 / 400
Carbofuran	11.98	149.1	77.0	24	149.1	121.1	8	164.0	149.1	8	120 / 120
Carboxin	18.11	87.0	43.0	6	143.0	43.0	16	143.0	87.0	8	200 / 100
Chlorfenapyr	18.37	136.9	102.0	12	248.9	112.0	24	248.9	137.1	18	45 / 30
Chlorfenvinphos	16.13	266.9	159.0	16	266.9	203.0	10	323.0	266.9	14	25 / 80
Chlorobenzilate	18.89	111.0	75.1	14	139.0	74.9	26	139.0	111.0	12	215 / 440
Chlorothalonil	12.72	228.8	168.0	8	265.8	133.0	36	265.8	170.0	24	350 / 160
Chlorpropham	11.17	171.0	127.0	8	213.0	127.0	14	213.0	171.0	6	65 / 45
Chlorpyrifos-ethyl	14.88	196.7	107.0	36	196.7	168.9	12	313.9	257.9	12	240 / 135
Chlorpyrifos-methyl	13.67	125.0	47.0	12	125.0	79.0	6	285.9	93.0	20	110 / 55
Cyfluthrin	26.67	163.0	65.1	26	163.0	91.1	12	163.0	127.1	6	100 / 25
Cyhalothrin	23.94	180.9	151.9	22	197.0	141.1	10	208.1	180.9	8	95 / 80
Cypermethrin	27.28/ 27.53/ 27.63/ 27.72*	163.0	91.1	12	163.0	127.1	6	180.9	152.1	20	100 / 50
Cyproconazole	18.53	222.0	82.1	10	222.0	89.3	38	222.0	125.0	20	35 / 210
Cyprodinil	15.85	224.1	196.9	20	224.1	208.0	18	225.1	209.7	16	500 / 40
DDD p,p	19.16	235.0	165.1	20	235.0	199.0	14	236.8	165.0	20	21 / 48
DDE p, p	17.85	246.0	176.1	28	317.8	246.0	20	317.8	248.0	18	28 / 30
DDT p,p	20.39	235.0	165.1	22	235.0	199.5	10	236.8	165.0	22	1.5 / 48
Deltamethrin	30.04	181.0	152.1	22	252.8	92.9	16	252.8	172.0	8	40 / 35
Demeton-S-methyl	10.91	88.0	59.8	6	109.0	79.0	6	141.9	79.0	12	10.1 / 25
Diazinon	12.51	137.1	54.1	20	137.1	84.1	12	179.1	121.5	26	170 / 10

Table 2 continued

** retention times for all isomers ** internal standard compound*

Name	RT (min)	Quantifier Ion			Qualifier Ion 1			Qualifier Ion 2			Ion Ratio (for qualifier ion 1/ qualifier ion 2) [% of quant. ion]
		Precursor Mass [m/z]	Product Mass [m/z]	Collision Energy [V]	Precursor Mass [m/z]	Product Mass [m/z]	Collision Energy [V]	Precursor Mass [m/z]	Product Mass [m/z]	Collision Energy [V]	
Dichlofluanid	14.69	123.0	51.0	32	123.0	77.0	18	223.9	123.0	10	210 / 120
Dichloran	12.03	175.9	148.0	10	205.9	147.9	20	205.9	176.0	10	50 / 160
Dichlorbenzophe-non, p,p'-	16.61	139.0	110.9	15	249.9	139.0	10				0.3
Dichlorvos	8.10	109.0	79.0	6	185.0	93.0	12	186.9	93.0	12	60 / 16
Dicofol	24.18	111.0	74.9	12	139.0	111.0	12	251.0	139.0	15	460 / 160
Difenoconazole	29.51/ 29.62*	265.0	139.0	36	265.0	202.1	16	323.0	265.0	14	90 / 220
Dimethoate	11.92	87.0	42.1	10	93.0	63.0	8	125.0	79.0	8	70 / 55
Dimethomorph	30.51/ 31.00*	165.0	77.0	18	165.0	137.0	10	301.0	165.1	12	390 / 130
Diphenylamine	10.96	167.1	139.4	26	167.1	140.1	18	167.1	166.1	16	130 / 550
Endosulfan	17.19/ 19	194.7	125.0	22	194.7	159.4	8	240.6	205.9	14	140 / 120
Endosulfan sulfate	20.23	238.7	203.9	12	271.7	234.9	12	271.7	236.8	12	47 / 550
EPN	22.04	157.0	77.0	22	169.0	77.0	22	169.0	141.0	8	120 / 210
Epoxiconazole	21.34	165.0	138.0	8	192.0	111.0	22	192.0	138.0	12	150 / 300
Ethion	19.17	153.0	97.0	10	230.9	128.9	22				90
Ethoprop (Ethoprophos)	11.02	157.9	96.9	16	157.9	113.9	6	200.0	158.0	6	75 / 70
Etofenprox	27.66	163.1	77.1	32	163.1	107.1	16	163.1	135.1	10	300 / 350
Fenamiphos	17.39	154.0	139.0	10	216.9	202.0	12	303.1	195.2	8	85 / 50
Fenamiphos sulfone	21.74	320.0	213.9	14	320.0	249.1	18	320.0	292.1	8	95 / 420
Fenamiphos-sulfoxid	21.59	304.0	196.0	10	304.0	234.0	10				35
Fenarimol	24.16	139.0	74.9	26	139.0	111.0	14	219.0	107.0	10	185 / 80
Fenbuconazol	26.31	129.0	77.8	18	129.0	102.0	14	198.1	129.1	8	230 / /370
Fenitrothion	14.44	125.0	79.0	8	277.0	109.0	16	277.0	260.0	6	45 / 48
Fenoxycarb	22.19	116.0	44.1	16	116.0	88.0	8	255.1	186.1	10	460 / 60
Fenpropathrin	22.39	97.1	55.1	6	181.0	126.8	28	181.0	151.9	22	22 / 92
Fenpropidin	14.38	98.2	41.5	18	98.2	55.1	14	98.2	70.0	10	1650 / 1850
Fenpropimorph	15.06	128.1	41.7	24	128.1	70.1	12	128.1	110.1	8	400 / 300
Fenthion	14.98	245.3	125.0	12	278.0	109.0	18	278.0	169.0	14	1300 / 500
Fenvalerate	28.73	125.0	89.0	18	167.0	89.0	32	167.0	125.0	10	45 / 300
Fipronil	15.96	366.9	212.9	28	366.9	244.9	20	368.8	214.9	30	30 / 65
Fludioxonil	17.61	153.7	127.0	8	248.0	127.0	26	248.0	153.8	18	290 / 160
Fluquinconazole	25.61	340.0	108.1	36	340.0	298.0	16	340.0	313.0	14	160 / 65
Flusilazole	18.05	206.0	151.3	14	233.0	151.9	14	233.0	164.9	16	230 / 350
Flutolanil	17.47	173.0	95.0	28	173.0	145.0	14	281.0	173.0	10	350 / 56
Flutriafol	17.31	123.0	75.0	24	123.0	95.0	12	219.0	123.0	12	180 / 72

Table 2 continued

** retention times for all isomers ** internal standard compound*

Name	RT (min)	Quantifier Ion			Qualifier Ion 1			Qualifier Ion 2			Ion Ratio (for qualifier ion 1/ qualifier ion 2) [% of quant. ion]
		Precursor Mass [m/z]	Product Mass [m/z]	Collision Energy [V]	Precursor Mass [m/z]	Product Mass [m/z]	Collision Energy [V]	Precursor Mass [m/z]	Product Mass [m/z]	Collision Energy [V]	
Fluvalinate	29.03/ 29.16*	180.8	152.1	22	250.0	55.1	16	250.0	199.9	18	45 / 35
Folpet	16.54	104.0	76.0	10	130.0	102.0	12	259.9	130.1	14	92 / 62
HCH alpha	11.71	216.9	180.9	8	218.8	182.9	8				95
HCH beta	12.19	216.9	180.9	8	218.8	182.9	8				90
HCH gamma_ Lindane	12.39	216.9	180.9	8	218.8	182.9	8				100
Hexaconazole	17.54	213.9	123.5	28	213.9	159.0	18	231.0	175.0	10	950 / 1100
Imazalil	17.58	172.8	109.0	26	174.7	147.0	16	215.0	173.0	8	90 / 130
Iprodione	21.77	314.0	245.0	10	315.7	247.0	10	315.7	273.0	8	50 / 22
Isofenphos-methyl	15.65	199.0	65.0	34	199.0	121.0	10	241.1	121.1	20	395 / 70
Kresoxim-methyl	18.12	116.0	62.9	24	116.0	89.0	14	130.9	130.1	10	324 / 102
Linuron	14.63	159.8	133.0	12	187.0	124.0	20	248.0	61.1	8	70 / 120
Malathion	14.68	92.8	63.0	8	125.0	79.0	8	173.1	99.0	12	110 / 300
Mepanipyrim	17.21	222.0	206.0	26	222.0	207.1	14	223.1	207.4	24	220 / 41
Metalaxyl	14.01	131.9	117.0	12	160.1	130.0	18	160.1	144.8	10	100 / 80
Methacrifos	9.8	125.0	79.0	8	180.0	93.0	10	240.0	180.0	10	55 / 40
Methamidophos	8.03	141.0	64.0	18	141.0	79.0	20	141.0	94.8	8	420 / 520
Methidathion	16.7	145.0	58.0	14	145.0	85.0	6	302.6	284.9	14	370
Methiocarb	14.98	153.0	45.0	12	153.0	109.1	6	168.1	153.0	10	225 / 554
Metribuzin	13.67	198.0	55.0	26	198.0	82.1	16	198.0	110.0	10	300 / 100
Monocrotophos	11.4	96.9	82.0	10	127.0	95.0	16	127.0	109.0	10	105 / 350
Myclobutanil	17.98	179.0	90.0	28	179.0	125.0	14	179.0	151.7	8	320 / 60
Ortho-phenyl-phenol	10.09	141.1	115.1	14	170.1	115.0	34	170.1	141.1	22	91 / 100
Oxadiazon	17.87	174.9	76.0	28	174.9	112.0	12	174.9	147.2	6	226 / 52
Oxadixyl	19.12	131.9	117.0	16	163.1	117.0	24	163.1	132.1	8	110 / 260
Paclobutrazol	16.97	125.0	89.0	18	236.0	125.0	12	236.0	167.0	10	290 / 90
Paraoxon-methyl	12.83	95.9	65.0	12	109.0	79.0	6	230.0	105.9	16	140 / 110
Parathion (ethyl)	15.07	109.0	81.0	10	124.9	97.0	6	291.0	109.0	12	75 / 48
Parathion-methyl	13.85	124.9	47.0	12	124.9	79.0	6	263.0	109.0	12	105 / 60
Pendimethalin	15.81	252.1	161.0	14	252.1	162.0	8	252.1	191.3	8	130 / 85
Permethrin	25.38/ 25.64*	163.0	91.1	12	183.1	153.0	12	183.1	168.0	12	100 / 105
Phenthoate	16.25	121.0	77.0	22	246.0	121.0	8	274.0	121.0	10	100 / 120
Phosalone	23.15	121.1	65.0	10	182.0	74.8	30	182.0	111.0	14	105 / 190
Phosmet	21.89	160.0	50.9	38	160.0	76.9	22	160.0	133.0	10	170 / 110
Phosphamidon	13.47	127.0	94.9	16	127.0	109.0	12	264.1	127.0	12	380 / 100
Pirimicarb	13.08	166.1	55.0	18	166.1	96.0	12	238.1	166.1	10	120 / 230
Pirimicarb-p-desmetyl	13.36	152.1	42.0	25	152.1	96.0	10	224.1	152.1	10	230 / 120
Pirimiphos methyl	14.37	290.1	125.0	20	290.1	233.0	8	305.1	180.1	8	60 / 70
Prochloraz	25.74	69.9	42.0	8	180.1	138.1	12	308.0	147.1	12	160 / 10
Procymidone	16.4	95.9	53.0	16	95.9	67.1	8	283.0	96.1	8	400 / 65

Table 2 continued

** retention times for all isomers ** internal standard compound*

Name	RT (min)	Quantifier Ion			Qualifier Ion 1			Qualifier Ion 2			Ion Ratio (for qualifier ion 1/ qualifier ion 2) [% of quant. ion]
		Precursor Mass [m/z]	Product Mass [m/z]	Collision Energy [V]	Precursor Mass [m/z]	Product Mass [m/z]	Collision Energy [V]	Precursor Mass [m/z]	Product Mass [m/z]	Collision Energy [V]	
Profenofos	17.73	296.7	268.9	10	336.9	266.9	12	336.9	308.9	8	190 / 35
Propargite	20.97	135.1	77.1	26	135.1	107.1	12	150.1	135.1	8	310 / 110
Propiconazole	20.19/ 20.39*	172.9	74.0	38	172.9	109.0	26	172.9	145.0	16	110 / 155
Propyzamide	12.5	172.9	74.0	38	172.9	109.0	26	172.9	145.0	14	105 / 190
Prothiofos	17.57	266.7	220.9	18	266.7	238.9	8	308.9	239.0	14	142 / 160
Pyraclostrobin	28.89	132.0	51.1	35	132.0	77.0	20	164.0	132.1	10	230 / 220
Pyridaben	25.62	147.1	117.1	20	147.1	119.1	8	147.1	132.1	12	55 / 58
Pyrimethanil	12.66	198.1	117.9	30	198.1	157.6	18	198.1	182.9	14	10 / 120
Pyriproxyfen	23.54	136.1	78.0	20	136.1	96.0	10	226.1	186.1	12	90 / 10
Quinoxyfen	20.18	237.0	208.0	26	271.8	237.1	12	307.0	237.0	18	55 / 33
Spirodiclofen	25.09	156.9	73.0	20	156.9	86.7	32	312.2	259.0	8	60 / 105
Tebuconazole	20.85	125.0	89.0	16	125.0	99.0	16	250.0	125.0	20	50 / 110
Tebufenocide	22.58	145.1	117.0	10	160.1	145.1	12				8
Tebufenpyrad	22.58	276.1	171.0	10	318.1	131.1	14	318.1	145.1	14	43 / 31
Tefluthrin	12.79	177.0	127.0	14	177.0	137.0	16	197.0	141.1	10	34 / 40
Tetraconazole	15.18	100.9	51.0	10	159.0	123.4	16	336.0	204.0	28	8 / 100
Tetradifon	22.97	159.0	74.8	32	159.0	111.0	20	159.0	131.0	10	125 / 252
Tetrahydroph-thalimide (THPI)	9.96	151.0	77.1	30	151.0	79.9	6	151.0	122.1	8	140 / 80
Thiabendazole	16.36	174.0	103.0	18	174.0	130.1	10	201.0	174.0	14	110 / 700
Tolclofos-methyl	13.86	265.0	219.9	20	265.0	250.0	12	266.8	252.0	12	285 / 80
Tolyfluanid	16.1	137.0	65.1	28	137.0	91.1	18	238.0	137.0	10	150 / 110
Triadimefon	15.17	208.0	111.0	20	208.0	126.7	12	208.0	180.8	8	65 / 120
Triadimenol	16.39	112.0	57.6	8	128.0	65.0	18	168.2	70.0	10	
Trifloxystrobin	20.16	116.1	63.0	24	116.1	89.0	14	145.0	95.0	14	295 / 40
Trifluralin	11.17	306.1	159.7	20	306.1	206.0	10	306.1	264.1	8	150 / 900
Triphenylphos-phate (TPP)**	21.01	215.0	168.1	16	326.1	168.6	28	326.1	325.3	10	6 / 62
Triticonazole	23.17	217.0	167.0	18	235.1	181.9	12	235.1	217.1	8	92 / 120
Vinclozolin	13.73	241.1	58.1	12	241.1	184.1	10	284.9	269.9	12	160

Table 3. Linearity and matrix effect results *(see text 12.2 for details on Youden plot slope results).* – *residue plot RSD% <20%* – *residue plot RSD% >20%*

Compound	Calibration Range [ng/g]	Strawberry			Wheat Flour			Leek		
		r2	Residue Plot Deviation [%RSD]	Youden Plot Slope [diff%]	r2	Residue Plot Deviation [%RSD]	Youden Plot Slope [diff%]	r2	Residue Plot Deviation [%RSD]	Youden Plot Slope [diff%]
Acephate	0-200	0.9998		12	0.9995		9	0.9998		35
Acrinathrin	0-200	0.9976		9	0.9985		270	0.9976		61
Amitraz	0-2000	0.9884		39	n.d.	n.d.	n.d.	0.9920		38
Azinphos-methyl	0-1000	0.9885		20	0.9956		0	0.9890		52
Azoxystrobin	0-1000	0.9911		24	0.9979		130	0.9918		63
Bifenthrin	0-200	0.9997		10	0.9939		12	0.9947		24
Bitertanol	0-200	0.9993		24	0.9956		67	0.9986		18
Boscalid (Nicobifen)	0-200	0.9983		16	0.9946		61	0.9976		8
Bromopropylate	0-200	0.9986		9	0.9908		2	0.9988		19
Bromuconazole	0-200	0.9989		6	0.9965		7	0.9994		17
Bupirimate	0-1000	0.9970		5	0.9981		3	0.9995		21
Buprofezin	0-1000	0.9993		16	0.9984		13	0.9961		31
Cadusafos	0-200	1.0000		3	0.9997		14	0.9970		27
Captan	0-200	0.9963		63	0.9967		56	n.d.	n.d.	n.d.
Carbaryl	0-1000	0.9995		54	0.9991		50	0.9833		68
Carbofuran	0-200	0.9987		11	0.9907		31	0.9816		64
Carboxin	0-200	0.9989		6	0.9988		16	0.9998		18
Chlorfenapyr	0-1000	0.9991		16	0.9971		18	0.9994		35
Chlorfenvinphos	0-200	0.9996		9	0.9958		97	0.9982		10
Chlorobenzilate	0-200	0.9999		2	0.9971		5	0.9991		17
Chlorothalonil	0-200	0.9952		77	0.9991		25	n.d.	n.d.	n.d.
Chlorpropham	0-200	0.9999		1	0.9997		11	0.9971		18
Chlorpyrifos-ethyl	0-200	0.9998		11	0.9995		6	0.9994		22
Chlorpyrifos-methyl	0-200	0.9998		25	0.9995		32	0.9991		39
Cyfluthrin	0-200	0.9995		4	0.9918		130	0.9899		5
Cyhalothrin	0-200	0.9979		15	0.9972		39	0.9973		16
Cypermethrin	0-200	0.9993		10	0.9947		105	0.9900		15
Cyproconazole	0-200	0.9994		17	0.9975		29	0.9997		2
Cyprodinil	0-200	0.9594		5	0.9970		5	0.9993		10
DDD p,p	0-200	0.9984		4	0.9982		20	0.9987		7
DDE p, p	0-200	0.9999		11	0.9985		21	0.9983		9
DDT p,p	0-200	0.9974		21	0.9963		26	0.9926		18
Deltamethrin	0-200	0.9994		7	0.9935		149	0.9911		40
Demeton-S-methyl	0-1000	0.9997		0	0.9994		2	0.9995		6
Diazinon	0-200	0.9998		18	0.9996		23	0.9928		36
Dichlofluanid	0-1000	0.9962		6	0.9997		10	0.7016		99
Dichloran	0-200	0.9996		7	0.9993		21	0.9994		25
Dichlorbenzophenon, p,p'-	0-200	0.9976		24	0.9988		65	0.9904		99
Dichlorvos	0-200	0.9996		15	0.9992		37	0.9993		20
Dicofol	0-200	0.9989		2	0.9952		11	0.9991		20
Difenoconazole	0-200	0.9989		13	0.9965		225	0.9995		51
Dimethoate	0-200	0.9996		17	0.9997		4	0.9996		20

Table 3 continued

– residue plot RSD% <20% *– residue plot RSD% >20%*

Compound	Calibration Range [ng/g]	Strawberry			Wheat Flour			Leek		
		r2	Residue Plot Deviation [%RSD]	Youden Plot Slope [diff%]	r2	Residue Plot Deviation [%RSD]	Youden Plot Slope [diff%]	r2	Residue Plot Deviation [%RSD]	Youden Plot Slope [diff%]
Dimethomorph	0-200	0.9995		37	0.9996		181	0.9984		42
Diphenylamine	0-200	0.9999		12	0.9994		24	0.9969		22
Endosulfan	0-1000	0.9994		7	0.9961		4	0.9969		17
Endosulfan sulfate	0-200	0.9988		4	0.9920		2	0.9980		20
EPN	0-200	0.9960		2	0.9947		56	0.9926		0
Epoxiconazole	0-200	0.9992		5	0.9966		14	0.9994		8
Ethion	0-200	0.9977		11	0.9967		21	0.9995		3
Ethoprop (Ethoprophos)	0-200	0.9998		7	0.9997		16	0.9978		17
Etofenprox	0-200	0.9985		15	0.9939		60	0.9986		3
Fenamiphos	0-200	0.9996		2	0.9992		40	0.9999		41
Fenamiphos sulfone	0-200	0.9968		16	0.9981		74	0.9933		25
Fenamiphos-sulfoxid	0-2000	0.9907		10	0.9940		101	0.8709		44
Fenarimol	0-200	0.9979		2	0.9958		8	0.9987		22
Fenbuconazol	0-200	0.9990		7	0.9949		33	0.9991		6
Fenitrothion	0-200	0.9994		15	0.9993		15	0.9992		23
Fenoxycarb	0-200	0.9990		9	0.9970		52	0.9989		4
Fenpropathrin	0-200	0.9981		7	0.9972		45	0.9146		6
Fenpropidin	0-1000	0.9998		18	0.9997		7	0.9962		17
Fenpropimorph	0-200	0.9998		10	0.9997		5	0.9943		27
Fenthion	0-200	0.9987		17	0.9998		21	0.9997		5
Fenvalerate	0-200	0.9999		10	0.9949		84	0.9973		19
Fipronil	0-200	0.9998		8	0.9984		26	0.9991		29
Fludioxonil	0-200	0.9800		1	0.9979		11	0.9992		23
Fluquinconazole	0-200	0.9976		22	0.9990		153	0.9995		39
Flusilazole	0-200	0.9984		2	0.9953		13	0.9977		11
Flutolanil	0-200	0.9989		15	0.9996		38	0.9997		7
Flutriafol	0-200	0.9996		1	0.9991		14	0.9996		23
Fluvalinate	0-200	0.9995		20	0.9956		131	0.9938		1
Folpet	0-2000	0.9959		76	0.9984		48	n.d.	n.d.	n.d.
HCH alpha	0-200	0.9999		8	0.9951		8	0.9977		15
HCH beta	0-200	0.9999		14	0.9993		16	0.9981		29
HCH gamma_Lindane	0-200	0.9999		12	0.9945		17	0.9961		21
Hexaconazole	0-1000	0.9938		8	0.9995		11	0.9999		11
Imazalil	0-1000	0.9987		14	0.9985		14	0.9998		26
Iprodione	0-200	0.9981		5	0.9984		34	0.9917		13
Isofenphos-methyl	0-200	0.9996		6	0.9996		54	0.9992		6
Kresoxim-methyl	0-200	0.9990		15	0.9974		15	0.9992		35
Linuron	0-1000	0.9986		50	0.9967		55	0.9996		42
Malathion	0-200	0.9985		14	0.9995		11	0.9816		30
Mepanipyrim	0-200	0.9993		24	0.9928		38	0.9995		11
Metalaxyl	0-1000	0.9999		20	0.9996		30	0.9980		37
Methacrifos	0-200	0.9994		3	0.9983		16	0.9951		19

Table 3 continued

– residue plot RSD% <20% *– residue plot RSD% >20%*

Compound	Calibration Range [ng/g]	Strawberry			Wheat Flour			Leek		
		r2	Residue Plot Deviation [%RSD]	Youden Plot Slope [diff%]	r2	Residue Plot Deviation [%RSD]	Youden Plot Slope [diff%]	r2	Residue Plot Deviation [%RSD]	Youden Plot Slope [diff%]
Methamidophos	0-200	0.9995		0	0.9995		9	0.9967		33
Methidathion	0-200	0.9984		13	0.9997		14	0.9988		32
Methiocarb	0-2000	0.9988		2	0.9963		20	0.9876		33
Metribuzin	0-1000	0.9997		21	0.9996		22	0.9995		28
Monocrotophos	0-1000	0.9997		36	0.9990		11	0.9982		45
Myclobutanil	0-200	0.9994		2	0.9979		8	0.9991		20
Ortho-phenylphenol	0-200	0.9999		4	0.9995		18	0.9945		24
Oxadiazon	0-200	0.9999		8	0.9968		11	0.9956		28
Oxadixyl	0-200	0.9997		5	0.9969		4	0.9989		25
Paclobutrazol	0-200	0.9996		4	0.9997		1	0.9988		15
Paraoxon-methyl	0-1000	0.9957		40	0.9964		27	0.9875		43
Parathion (ethyl)	0-1000	0.9968		7	0.9956		4	0.9964		20
Parathion-methyl	0-200	0.9996		24	0.9985		30	0.9997		35
Pendimethalin	0-200	0.9950		15	0.9910		121	0.9937		75
Permethrin	0-200	0.9951		27	0.9961		70	0.9970		13
Phenthoate	0-1000	0.9991		18	0.9989		25	0.9996		32
Phosalone	0-200	0.9976		2	0.9921		33	0.9939		12
Phosmet	0-200	0.9972		28	0.9961		34	0.9922		61
Phosphamidon	0-200	0.9989		42	0.9997		37	0.9961		70
Pirimicarb	0-200	0.9998		16	0.9997		22	0.9990		32
Pirimicarb-p-desmetyl	0-1000	0.9999		26	0.9998		28	0.9994		36
Pirimiphos methyl	0-200	0.9987		15	0.9980		4	0.9986		25
Prochloraz	0-1000	0.9924		9	0.9974		37	0.9925		12
Procymidone	0-200	0.9999		17	0.9996		6	0.9969		26
Profenofos	0-200	0.9988		2	0.9992		>200	0.9940		34
Propargite	0-200	0.9991		9	0.8967		17	0.9997		51
Propiconazole	0-200	0.9986		13	0.9976		15	0.9877		10
Propyzamide	0-200	0.9999		9	0.9995		14	0.9946		25
Prothiofos	0-200	0.9993		20	0.9987		80	0.9986		4
Pyraclostrobin	0-200	0.9997		6	0.9954		56	0.9964		1
Pyridaben	0-200	0.9961		29	0.9967		79	0.9953		14
Pyrimethanil	0-200	0.9999		13	0.9997		13	0.9963		20
Pyriproxyfen	0-200	0.9982		1	0.9964		12	0.9996		17
Quinoxyfen	0-200	0.9977		15	0.9979		28	0.9998		2
Spirodiclofen	0-200	0.9995		7	0.9974		8	0.9950		34
Tebuconazole	0-200	0.9995		17	0.9969		22	0.9986		3
Tebufenocide	0-1000	0.9980		11	0.9975		34	0.9984		12
Tebufenpyrad	0-200	0.9987		8	0.9996		126	0.9996		4
Tefluthrin	0-200	1.0000		14	0.9994		20	0.9929		31
Tetraconazole	0-1000	0.9997		17	0.9997		13	0.9975		33
Tetradifon	0-200	0.9998		10	0.9959		11	0.9989		30
Tetrahydrophthalimide	0-200	0.9645		106	0.9638		51	0.8388		93

Table 3 continued

– residue plot RSD% <20% *– residue plot RSD% >20%*

Compound	Calibration Range [ng/g]	Strawberry			Wheat Flour			Leek		
		r2	Residue Plot Deviation [%RSD]	Youden Plot Slope [diff%]	r2	Residue Plot Deviation [%RSD]	Youden Plot Slope [diff%]	r2	Residue Plot Deviation [%RSD]	Youden Plot Slope [diff%]
Thiabendazole	0-1000	0.9987		9	0.9996		8	0.9998		28
Tolclofos-methyl	0-200	0.9998		27	0.9990		57	0.9987		6
Tolyfluanid	0-1000	0.9970		6	0.9989		47	n.d.	n.d.	n.d.
Triadimefon	0-1000	0.9987		7	0.9996		8	0.9995		22
Triadimenol	0-1000	0.9993		2	0.9991		8	0.9992		26
Trifloxystrobin	0-200	0.9985		17	0.9978		61	0.9994		3
Trifluralin	0-200	0.9913		311	0.9973		62	0.9821		30
Triticonazole	0-200	0.9977		27	0.9975		70	0.9983		20
Vinclozolin	0-200	0.9996		18	0.9983		22	0.9973		27

Table 4: Recovery values [%] at 10 ng/g (level 1), 20 ng/g (level 2) and 100 ng/g (level 3) spike levels.

** spiking levels are 50, 100 & 500 ng/g* *** spiking levels are 100, 200 & 1000 ng/g* *<LOD/LOQ – spiking value below LOD/LOQ value*

Compound	Strawberry			Wheat Flour			Leek		
	Level 1	Level 2	Level 3	Level 1	Level 2	Level 3	Level 1	Level 2	Level 3
Acephate	84	88	63	68	75	60	68	72	56
Acrinathrin	100	79	67	121	118	85	129	69	24
Amitraz**	98	79	57	n.d.	n.d.	n.d.	126	95	69
Azinphos-methyl*	127	102	79	101	128	99	126	88	68
Azoxystrobin*	101	87	67	111	123	95	78	88	82
Bifenthrin	101	104	73	94	117	76	94	108	84
Bitertanol	101	109	82	116	118	81	82	109	88
Boscalid (Nicobifen)	93	101	81	111	116	83	111	111	86
Bromopropylate	92	109	90	117	114	82	97	111	89
Bromuconazole	87	106	90	108	114	79	88	106	88
Bupirimate*	83	111	101	105	113	83	93	120	99
Buprofezin*	82	112	97	100	112	80	100	125	97
Cadusafos	78	109	88	96	111	85	68	111	95
Captan	74	42	71	42	32	66	n.d.	n.d.	n.d.
Carbaryl*	106	81	65	110	100	71	83	76	72
Carbofuran	87	99	85	106	133	107	<LOQ	54	43
Carboxin	96	107	94	99	100	80	83	107	89
Chlorfenapyr*	86	112	100	104	118	83	84	118	99
Chlorfenvinphos	101	110	89	105	119	91	84	98	79
Chlorobenzilate	87	114	94	115	123	73	85	123	97
Chlorothalonil	133	73	36	76	56	62	n.d.	n.d.	n.d.
Chlorpropham	84	113	94	87	109	86	73	118	100
Chlorpyrifos-ethyl	86	110	87	95	113	88	91	132	100
Chlorpyrifos-methyl	114	112	80	100	121	95	93	135	103
Cyfluthrin	102	103	77	127	114	73	119	98	67
Cyhalothrin	103	85	79	117	118	86	104	77	65

Table 4 continued

** spiking levels are 50, 100 & 500 ng/g ** spiking levels are 100, 200 & 1000 ng/g <LOD/LOQ – spiking value below LOD/LOQ value*

Compound	Strawberry			Wheat Flour			Leek		
	Level 1	Level 2	Level 3	Level 1	Level 2	Level 3	Level 1	Level 2	Level 3
Cypermethrin	84	86	73	181	136	84	112	112	80
Cyproconazole	83	103	88	111	112	80	73	107	89
Cyprodinil	21	30	24	106	109	81	84	120	92
DDD p,p	96	105	86	88	108	85	79	120	100
DDE p, p	76	104	85	89	100	75	80	121	96
DDT p,p	97	94	68	124	141	120	82	118	90
Deltamethrin	100	77	56	114	107	70	93	84	58
Demeton-S-methyl*	93	106	84	97	111	93	96	122	92
Diazinon	87	113	91	95	110	86	77	125	101
Dichlofluanid*	110	72	62	37	48	73	<LOD	<LOD	55
Dichloran	83	109	95	106	120	92	78	116	90
Dichlorbenzophenon, p,p'-	77	104	86	<LOD	<LOQ	84	<LOQ	105	103
Dichlorvos	89	122	92	98	118	112	98	112	85
Dicofol	86	98	85	114	114	80	83	103	85
Difenoconazole	93	104	80	101	113	90	66	87	69
Dimethoate	86	95	82	79	113	95	94	117	86
Dimethomorph	92	99	73	90	124	114	86	102	81
Diphenylamine	102	107	74	56	70	79	75	122	95
Endosulfan*	86	101	78	114	121	67	76	118	97
Endosulfan sulfate	102	109	87	126	129	86	114	122	95
EPN	121	113	84	134	123	96	122	122	85
Epoxiconazole	103	116	88	109	119	86	89	116	95
Ethion	112	110	84	116	120	86	77	116	97
Ethoprop (Ethoprophos)	91	99	73	99	111	89	72	114	97
Etofenprox	91	101	79	119	114	78	89	103	82
Fenamiphos	90	103	92	68	84	71	75	103	87
Fenamiphos sulfone	106	95	66	119	117	92	63	51	57
Fenamiphos-sulfoxid**	144	150	117	119	137	131	65	89	91
Fenarimol	95	100	79	111	115	79	85	101	83
Fenbuconazol	100	110	85	123	123	85	92	113	92
Fenitrothion	105	102	83	107	123	94	111	129	96
Fenoxycarb	98	103	85	114	120	89	97	112	91
Fenpropathrin	86	105	91	<LOD	<LOD	82	<LOD	<LOD	77
Fenpropidin*	35	36	23	43	29	26	n.d.	9	20
Fenpropimorph	59	79	65	68	79	62	40	80	73
Fenthion	87	100	108	61	84	77	108	122	102
Fenvalerate	82	93	79	111	118	85	99	109	81
Fipronil	89	110	92	119	119	96	74	104	83
Fludioxonil	<LOD	<LOD	55	104	117	68	87	117	98
Fluquinconazole	99	102	82	96	108	84	92	110	91
Flusilazole	90	119	99	123	112	85	75	99	101
Flutolanil	88	116	100	93	114	86	87	122	99
Flutriafol	85	108	91	77	114	66	81	114	92
Fluvalinate	35	97	77	121	122	91	98	101	76
Folpet**	133	34	45	66	36	29	<LOD	<LOD	<LOQ

Table 4 continued

** spiking levels are 50, 100 & 500 ng/g ** spiking levels are 100, 200 & 1000 ng/g <LOD/LOQ – spiking value below LOD/LOQ value*

Compound	Strawberry			Wheat Flour			Leek		
	Level 1	Level 2	Level 3	Level 1	Level 2	Level 3	Level 1	Level 2	Level 3
HCH alpha	79	109	87	113	121	88	84	135	108
HCH beta	85	111	90	109	110	85	87	138	109
HCH gamma_Lindane	87	110	89	115	123	88	81	132	106
Hexaconazole*	95	97	84	95	103	75	90	111	88
Imazalil*	72	97	84	87	102	67	69	96	78
Iprodione	109	111	86	120	124	94	109	102	84
Isofenphos-methyl	85	111	92	99	112	89	94	128	103
Kresoxim-methyl	86	111	96	114	119	85	86	120	101
Linuron*	126	118	95	95	100	63	126	133	98
Malathion	108	106	90	83	122	101	150	121	88
Mepanipyrim	82	111	96	123	138	72	93	121	95
Metalaxyl*	84	111	91	97	115	90	75	115	95
Methacrifos	89	108	78	82	109	96	66	130	103
Methamidophos	56	60	63	59	61	50	97	73	51
Methidathion	110	106	84	99	118	94	106	125	98
Methiocarb**	85	98	81	<LOD	<LOD	75	<LOD	<LOQ	78
Metribuzin*	87	111	98	89	117	84	94	129	99
Monocrotophos*	90	92	74	110	99	60	107	87	63
Myclobutanil	91	115	96	104	109	83	77	116	94
Ortho-phenylphenol	95	102	74	63	75	78	61	120	99
Oxadiazon	84	115	95	111	117	81	69	117	100
Oxadixyl	89	108	87	116	118	84	76	108	93
Paclobutrazol	81	106	91	95	109	85	90	111	91
Paraoxon-methyl*	102	108	109	137	146	111	132	117	73
Parathion (ethyl)*	69	98	101	54	95	95	120	132	100
Parathion-methyl	83	107	98	108	129	95	101	138	105
Pendimethalin	45	81	118	51	73	85	117	132	96
Permethrin	109	107	83	109	115	81	91	112	94
Phenthoate*	83	111	105	124	124	95	99	125	97
Phosalone	115	106	82	97	87	83	103	108	86
Phosmet	114	87	71	104	115	88	107	85	63
Phosphamidon	109	112	95	115	131	98	53	64	120
Pirimicarb	85	110	87	90	113	90	77	118	94
Pirimicarb-p-desmetyl*	79	99	81	85	106	82	82	122	89
Pirimiphos methyl	90	109	93	116	113	93	71	111	92
Prochloraz*	117	94	72	112	124	87	76	86	71
Procymidone	85	107	87	84	115	86	82	119	98
Profenofos	112	107	89	112	108	90	119	89	77
Propargite	104	104	90	<LOD	<LOD	58	62	89	88
Propiconazole	89	95	74	102	110	77	79	107	90
Propyzamide	84	110	89	100	116	88	90	133	103
Prothiofos	73	96	92	95	99	82	89	104	81
Pyraclostrobin	100	116	90	128	139	97	101	123	92
Pyridaben	111	110	86	108	114	81	92	108	87
Pyrimethanil	78	103	84	84	103	84	70	118	95

Table 4 continued ** spiking levels are 50, 100 & 500 ng/g ** spiking levels are 100, 200 & 1000 ng/g <LOD/LOQ – spiking value below LOD/LOQ value*

Compound	Strawberry			Wheat Flour			Leek		
	Level 1	Level 2	Level 3	Level 1	Level 2	Level 3	Level 1	Level 2	Level 3
Pyriproxyfen	101	107	84	106	113	81	90	112	92
Quinoxyfen	89	97	79	95	100	75	81	105	85
Spirodiclofen	91	88	78	132	113	75	<LOQ	96	71
Tebuconazole	80	95	75	100	111	79	89	110	91
Tebufenocide*	86	101	43	<LOD	<LOQ	84	<LOQ	106	87
Tebufenpyrad	80	102	89	101	104	89	70	91	78
Tefluthrin	85	109	87	86	109	86	72	126	102
Tetraconazole*	84	108	93	98	115	89	79	118	101
Tetradifon	77	119	106	104	112	78	75	116	95
Tetrahydrophthalimide (THPI)	<LOQ	<LOQ	90	<LOQ	117	115	<LOQ	111	95
Thiabendazole*	77	96	82	83	88	67	75	97	79
Tolclofos-methyl	81	108	89	102	110	84	91	119	83
Tolyfluanid*	111	71	67	87	79	77	n.d.	n.d.	n.d.
Triadimefon*	76	106	98	95	111	88	91	121	100
Triadimenol*	79	106	87	96	110	82	77	103	90
Trifloxystrobin	103	111	87	103	112	87	94	123	97
Trifluralin	121	84	59	54	39	50	77	50	87
Triticonazole	101	105	82	106	112	81	88	106	86
Vinclozolin	89	114	94	130	107	75	67	111	90

Table 5. Method precision and intermediate precision values [RSD %] at 10 ng/g (level 1), 20 ng/g (level 2) and 100 ng/g (level3). ** spiking levels are 50, 100 & 500 ** spiking levels are 100, 200 & 1000 <LOD/LOQ – spiking value below LOD/LOQ value*

Compound	Strawberry				Wheat Flour				Leek			
	Precision			Intermediate Precision	Precision			Intermediate Precision	Precision			Intermediate Precision
	Level 1	Level 2	Level 3		Level 1	Level 2	Level 3		Level 1	Level 2	Level 3	
Acephate	7	7	12	15	29	4	27	9	12	18	8	22
Acrinathrin	32	51	18	37	9	3	7	5	4	20	17	22
Amitraz**	5	11	11	14	n.d.	n.d.	n.d.	n.d.	3	7	15	27
Azinphos-methyl*	2	4	7	5	6	1	6	3	6	10	7	23
Azoxystrobin*	3	6	2	7	12	4	4	12	5	6	10	11
Bifenthrin	6	10	3	9	13	6	5	9	15	12	8	13
Bitertanol	2	3	2	4	4	2	4	2	2	6	11	10
Boscalid (Nicobifen)	3	3	2	3	3	2	3	2	6	2	11	7
Bromopropylate	7	8	4	10	10	5	6	8	5	13	7	13
Bromuconazole	2	4	2	4	7	3	4	3	2	6	9	11
Bupirimate*	6	3	3	4	3	3	3	3	6	5	9	6
Buprofezin*	6	4	3	5	4	3	3	4	2	4	8	4
Cadusafos	7	9	3	8	15	3	12	5	2	7	6	6
Captan	31	64	15	75	28	21	52	66	n.d.	n.d.	n.d.	n.d.
Carbaryl*	10	13	8	25	18	3	20	9	<LOQ	22	15	29
Carbofuran	18	5	4	16	27	5	17	11	11	40	20	50
Carboxin	7	4	2	7	6	4	3	6	4	5	7	7

Table 5 continued

** spiking levels are 50, 100 & 500 ** spiking levels are 100, 200 & 1000 <LOD/LOQ – spiking value below LOD/LOQ value*

Compound	Strawberry				Wheat Flour				Leek			
	Precision			Intermediate Precision	Precision			Intermediate Precision	Precision			Intermediate Precision
	Level 1	Level 2	Level 3		Level 1	Level 2	Level 3		Level 1	Level 2	Level 3	
Chlorfenapyr*	5	7	5	6	11	5	6	6	4	5	6	8
Chlorfenvinphos	4	5	4	7	33	5	15	37	3	4	8	10
Chlorobenzilate	3	7	4	6	5	3	38	4	3	5	8	6
Chlorothalonil	4	18	18	16	38	9	27	11	n.d.	n.d.	n.d.	n.d.
Chlorpropham	4	5	3	4	20	4	12	6	4	8	5	5
Chlorpyrifos-ethyl	5	7	2	6	11	7	10	8	13	7	7	6
Chlorpyrifos-methyl	5	3	5	6	17	3	12	6	8	5	6	5
Cyfluthrin	6	7	3	7	4	2	5	3	9	19	13	19
Cyhalothrin	8	25	9	19	3	3	3	3	3	20	15	18
Cypermethrin	6	12	5	9	11	2	3	3	17	9	12	13
Cyproconazole	5	4	3	4	4	2	2	3	5	6	7	4
Cyprodinil	3	8	3	6	7	5	8	7	8	6	6	8
DDD p,p	2	3	2	5	2	4	3	4	4	5	6	7
DDE p, p	7	7	4	6	6	4	2	5	4	6	5	16
DDT p,p	4	9	4	18	10	3	7	6	3	4	9	9
Deltamethrin	9	32	11	23	15	2	4	6	5	18	13	19
Demeton-S-methyl*	1	6	4	5	12	4	13	9	5	4	7	11
Diazinon	7	9	3	6	14	4	13	6	12	8	6	6
Dichlofluanid*	8	25	12	20	56	20	17	17	<LOD	<LOD	90	n.d.
Dichloran	11	10	4	8	11	5	16	6	10	8	5	8
Dichlorbenzophenon, p,p'-	14	18	5	14	<LOD	<LOQ	11	n.d.	<LOQ	16	7	18
Dichlorvos	5	7	8	13	28	5	19	9	8	7	10	9
Dicofol	9	4	2	5	5	1	4	4	5	2	10	8
Difenoconazole	7	4	3	10	19	5	5	14	10	5	3	12
Dimethoate	10	10	6	12	17	3	11	10	5	5	6	8
Dimethomorph	5	3	3	11	18	7	9	13	5	7	9	6
Diphenylamine	7	7	3	6	33	12	19	21	8	12	7	8
Endosulfan*	9	10	5	8	6	7	46	21	17	8	6	6
Endosulfan sulfate	9	3	4	5	4	3	4	3	4	6	9	6
EPN	4	3	3	11	8	4	4	11	4	6	9	6
Epoxiconazole	4	5	2	4	6	3	6	2	5	4	7	5
Ethion	1	3	2	3	4	2	3	2	8	7	9	5
Ethoprop (Ethoprophos)	2	7	2	5	16	4	13	12	4	6	6	5
Etofenprox	3	5	2	6	4	2	2	2	3	3	9	6
Fenamiphos	9	7	3	8	10	4	7	6	5	9	12	10
Fenamiphos sulfone	11	30	10	27	12	2	5	11	15	19	17	16
Fenamiphos-sulfoxid**	8	22	7	28	26	3	12	17	9	5	21	9
Fenarimol	3	3	1	3	7	2	3	3	3	5	9	9
Fenbuconazol	3	5	3	4	3	3	3	2	1	6	10	5
Fenitrothion	9	7	4	8	16	4	11	8	5	5	7	7
Fenoxycarb	3	3	2	3	6	5	5	7	2	7	8	8
Fenpropathrin	9	4	2	4	<LOD	<LOD	8	n.d.	<LOD	<LOD	13	n.d.
Fenpropidin*	27	26	11	21	29	12	61	15	n.d.	37	17	42

Table 5 continued

** spiking levels are 50, 100 & 500 ** spiking levels are 100, 200 & 1000 <LOD/LOQ – spiking value below LOD/LOQ value*

Compound	Strawberry				Wheat Flour				Leek			
	Precision			Intermediate Precision	Precision			Intermediate Precision	Precision			Intermediate Precision
	Level 1	Level 2	Level 3		Level 1	Level 2	Level 3		Level 1	Level 2	Level 3	
Fenpropimorph	7	11	3	7	14	6	8	8	18	10	9	9
Fenthion	16	5	6	13	30	12	9	48	12	13	5	10
Fenvalerate	5	8	3	7	7	2	3	2	3	4	12	9
Fipronil	8	5	3	7	24	4	18	17	11	4	4	17
Fludioxonil	<LOD	<LOD	6	n.d.	7	5	46	5	6	9	5	8
Fluquinconazole	7	7	5	7	13	4	4	11	3	6	10	16
Flusilazole	12	14	3	9	7	7	4	7	14	15	6	15
Flutolanil	5	2	2	5	8	4	6	5	4	8	8	8
Flutriafol	6	1	2	5	23	2	40	5	6	6	7	8
Fluvalinate	8	12	5	9	6	5	4	4	9	14	14	14
Folpet**	30	71	21	74	27	12	43	22	<LOD	<LOD	<LOQ	n.d.
HCH alpha	7	9	3	6	9	4	11	5	3	8	5	13
HCH beta	7	8	3	7	18	4	10	7	7	8	7	12
HCH gamma_Lindane	10	9	1	7	12	4	11	4	10	9	6	10
Hexaconazole*	14	12	3	9	11	6	4	7	13	4	10	8
Imazalil*	7	4	3	4	9	5	10	4	6	4	10	8
Iprodione	12	4	4	5	6	5	8	7	7	7	11	10
Isofenphos-methyl	3	3	3	3	13	4	9	6	4	4	7	7
Kresoxim-methyl	1	6	4	5	5	2	5	3	8	4	6	5
Linuron*	5	5	7	18	18	6	20	12	5	4	10	9
Malathion	3	8	4	8	14	4	14	10	10	11	10	12
Mepanipyrim	10	4	3	6	13	4	22	8	4	7	9	5
Metalaxyl*	5	5	5	5	17	5	12	6	2	7	4	6
Methacrifos	11	11	3	7	75	3	17	8	7	11	6	11
Methamidophos	15	12	14	31	23	7	25	8	7	14	9	22
Methidathion	6	7	5	7	14	3	13	5	5	4	8	6
Methiocarb**	15	15	5	17	<LOD	<LOD	11*	n.d.	<LOD	<LOQ	11	n.d.
Metribuzin*	8	7	6	9	15	5	11	6	5	7	7	7
Monocrotophos*	13	13	11	20	9	2	13	6	6	15	10	18
Myclobutanil	5	3	3	6	1	5	3	4	6	9	5	7
Ortho-phenylphenol	4	7	3	6	31	12	15	20	4	8	6	6
Oxadiazon	3	9	4	7	5	4	6	4	9	8	6	8
Oxadixyl	4	4	4	5	8	2	4	3	7	5	8	8
Paclobutrazol	3	6	5	4	16	4	10	7	4	4	8	6
Paraoxon-methyl*	7	10	12	17	12	4	17	10	4	16	16	16
Parathion (ethyl)*	8	10	3	12	25	8	11	17	4	5	7	5
Parathion-methyl	13	11	6	9	8	7	12	8	4	5	7	4
Pendimethalin	22	20	5	25	17	13	11	32	8	9	6	14
Permethrin	3	4	3	4	2	3	4	3	4	10	11	8
Phenthoate*	5	7	4	6	5	3	13	23	3	5	8	4
Phosalone	2	5	2	4	42	3	3	22	4	10	8	10
Phosmet	7	10	9	14	6	1	8	4	6	23	13	26
Phosphamidon	7	9	10	21	20	2	15	8	76	42	24	62

Table 5 continued

** spiking levels are 50, 100 & 500 ** spiking levels are 100, 200 & 1000 <LOD/LOQ – spiking value below LOD/LOQ value*

Compound	Strawberry				Wheat Flour				Leek			
	Precision			Intermediate Precision	Precision			Intermediate Precision	Precision			Intermediate Precision
	Level 1	Level 2	Level 3		Level 1	Level 2	Level 3		Level 1	Level 2	Level 3	
Pirimicarb	9	7	5	6	13	5	12	6	7	9	6	7
Pirimicarb-p-desmetyl*	12	16	8	11	13	3	12	5	4	8	9	5
Pirimiphos methyl	6	6	4	5	10	4	11	22	6	7	6	5
Prochloraz*	4	3	4	7	9	5	7	10	5	6	14	6
Procymidone	8	6	4	7	16	2	8	10	8	6	7	9
Profenofos	19	12	7	13	14	8	12	12	13	27	12	31
Propargite	5	4	4	11	<LOD	<LOD	56	n.d.	10	9	8	16
Propiconazole	2	2	3	4	8	4	11	3	3	6	8	6
Propyzamide	4	6	3	5	11	3	11	5	2	7	5	5
Prothiofos	9	3	4	6	17	5	8	17	8	10	6	11
Pyraclostrobin	5	3	3	8	2	2	3	2	2	3	11	5
Pyridaben	3	2	3	4	3	2	2	3	6	8	12	9
Pyrimethanil	4	7	2	6	13	3	8	5	3	9	7	6
Pyriproxyfen	3	4	2	4	4	1	3	1	2	4	8	6
Quinoxyfen	2	4	2	3	4	2	2	5	5	3	8	3
Spirodiclofen	10	6	5	13	14	5	10	10	<LOQ	11	12	17
Tebuconazole	3	2	1	3	6	3	7	2	3	3	9	7
Tebufenocide*	6	5	2	6	<LOD	<LOQ	6	7	<LOQ	6	9	7
Tebufenpyrad	3	6	3	5	18	7	10	16	3	8	8	6
Tefluthrin	7	7	4	5	16	5	11	6	4	8	6	6
Tetraconazole*	4	6	4	4	13	4	11	6	4	4	6	4
Tetradifon	11	7	4	5	7	2	6	4	7	6	7	7
Tetrahydrophthalimide (THPI)	<LOQ	<LOQ	8	n.d.	<LOQ	27	11	23	<LOQ	9	6	8
Thiabendazole*	5	3	4	3	16	3	14	6	4	5	9	7
Tolclofos-methyl	4	6	6	9	15	6	15	18	5	8	5	20
Tolyfluanid*	9	22	10	20	21	8	17	10	n.d.	n.d.	n.d.	n.d.
Triadimefon*	4	4	2	5	12	3	9	6	1	6	6	7
Triadimenol*	9	7	3	7	10	3	9	10	9	7	7	5
Trifloxystrobin	4	4	2	3	6	4	3	3	4	8	8	6
Trifluralin	3	16	8	17	1	12	13	20	2	12	8	32
Triticonazole	7	3	3	3	4	3	2	3	5	6	9	6
Vinclozolin	15	4	6	8	16	6	14	14	23	9	7	10

Table 6: Method LOD, LOQ and current legislative residue level values *(all values in ng/g)*.

** default value of 10 ng/g set as no MRL values defined*

Compound	Strawberry			Wheat Flour			Leek		
	LOD	LOQ	MRL	LOD	LOQ	MRL	LOD	LOQ	MRL
Acephate	0.3	1	20	0.6	2	20	1.5	5	20
Acrinathrin	6	20	200	2.7	9	50	6	20	50
Amitraz	6	20	50	300	1000	50	12	40	50
Azinphos-methyl	3	10	50	0.9	3	50	2.4	8	50
Azoxystrobin	0.9	3	1000	0.3	1	300	1.5	5	1000
Bifenthrin	6	20	500	4.8	16	500	7.5	25	50
Bitertanol	0.9	3	50	0.6	2	50	0.6	2	50
Boscalid (Nicobifen)	0.3	1	1000	0.15	0.5	500	0.6	2	5000
Bromopropylate	3	10	10	2.1	7	10	1.5	5	10
Bromuconazole	2.4	8	50	0.27	0.9	200	1.2	4	50
Bupirimate	3	10	1000	3	10	50	4.5	15	50
Buprofezin	6	20	3000	15	50	50	4.5	15	50
Cadusafos	1.5	5	10	0.3	1	10	1.5	5	10
Captan	3	10	3000	3	10	20	1000	1500	2000
Carbaryl	4.5	15	50	4.5	15	500	4.5	15	50
Carbofuran	9	30	20	3	10	20	4.5	15	20
Carboxin	1.8	6	20	6	20	20	0.6	2	20
Chlorfenapyr	4.5	15	10*	1.5	5	10*	3	10	10*
Chlorfenvinphos	1.5	5	10*	0.3	1	10*	1.2	4	10*
Chlorobenzilate	0.9	3	20	0.3	1	20	1.2	4	20
Chlorothalonil	12	40	5000	0.3	1	100	1500	2500	40000
Chlorpropham	1.5	5	50	0.6	2	20	1.2	4	50
Chlorpyrifos-ethyl	1.5	5	10*	0.3	1	10*	1.5	5	10*
Chlorpyrifos-methyl	1.5	5	500	0.3	1	3000	0.75	2.5	50
Cyfluthrin	4.5	15	20	3.6	12	20	2.4	8	20
Cyhalothrin	1.8	6	10*	0.9	3	10*	1.5	5	10*
Cypermethrin	4.5	15	70	15	50	2000	4.5	15	500
Cyproconazole	1.5	5	50	1.8	6	100	1.5	5	50
Cyprodinil	1.2	4	5000	0.3	1	500	1.5	5	50
DDD p,p	0.3	1	50	0.21	0.7	50	0.75	2.5	50
DDE p,p	0.3	1	10*	0.24	0.8	10*	1.2	4	10*
DDT o,p	0.6	2	10*	0.9	3	10*	0.6	2	10*
Deltamethrin	4.5	15	200	2.4	8	2000	7.5	25	200
Demeton-S-methyl	1.5	5	10*	1.5	5	10*	1.2	4	10*
Diazinon	0.3	1	10	0.3	1	20	0.3	1	10
Dichlofluanid	13.5	45	10*	3	10	10*	150	500	10*
Dichloran	4.5	15	300	3	10	10	2.4	8	100
Dichlorbenzophenon, p,p'-	3	10	10*	15	50	10*	4.5	15	10*
Dichlorvos	3	10	10	3	10	10	2.7	9	10
Dicofol	2.4	8	20	1.5	5	20	1.5	5	20
Difenoconazole	1.5	5	400	1.2	4	100	0.9	3	500
Dimethoate	1.2	4	20	0.6	2	50	0.6	2	20
Dimethomorph	1.5	5	10*	1.5	5	10*	0.6	2	10*

Table 6 continued

** default value of 10 ng/g set as no MRL values defined*

Compound	Strawberry			Wheat Flour			Leek		
	LOD	LOQ	MRL	LOD	LOQ	MRL	LOD	LOQ	MRL
Diphenylamine	0.3	1	50	0.3	1	50	0.6	2	50
Endosulfan	1.5	5	50	6	20	50	1.2	4	50
Endosulfan sulfate	0.6	2	10*	1.5	5	10*	0.9	3	10*
EPN	2.1	7	10*	2.1	7	10*	0.9	3	10*
Epoxiconazole	1.2	4	50	0.6	2	600	0.6	2	50
Ethion	1.2	4	10	0.9	3	10	0.6	2	10
Ethoprop (Ethoprophos)	0.3	1	20	0.6	2	20	0.9	3	20
Etofenprox	0.9	3	1000	0.9	3	500	0.6	2	10
Fenamiphos	1.2	4	20	1.2	4	20	0.9	3	20
Fenamiphos sulfone	3.6	12	10*	0.9	3	10*	1.5	5	10*
Fenamiphos-sulfoxid	0.9	3	10*	10.5	35	10*	7.5	25	10*
Fenarimol	0.9	3	300	0.3	1	20	0.3	1	20
Fenbuconazol	0.6	2	50	0.6	2	100	0.75	2.5	50
Fenitrothion	3	10	10	1.5	5	50	2.4	8	10
Fenoxycarb	0.9	3	50	1.2	4	50	0.75	2.5	50
Fenpropathrin	7.5	25	2000	30	100	10	30	100	10
Fenpropidin	4.5	15	50	12	40	500	7.8	26	50
Fenpropimorph	0.15	0.5	1000	0.3	1	500	1.2	4	1000
Fenthion	1.5	5	10	1.8	6	10	1.5	5	10
Fenvalerate	2.25	7.5	20	1.5	5	50	0.9	3	20
Fipronil	0.3	1	5	1.5	5	5	0.9	3	10
Fludioxonil	30	100	3000	1.2	4	200	1.2	4	50
Fluquinconazole	0.6	2	50	0.3	1	100	0.6	2	50
Flusilazole	4.5	15	20	2.4	8	100	1.5	5	20
Flutolanil	0.6	2	50	0.3	1	50	0.6	2	50
Flutriafol	0.3	1	500	0.9	3	500	0.45	1.5	50
Fluvalinate	6	20	10*	3.6	12	10*	4.5	15	10*
Folpet	75	250	3000	450	1500	2000	600	2000	20
HCH alpha	0.3	1	10	0.3	1	20	0.3	1	10
HCH beta	0.3	1	10	0.3	1	20	0.3	1	10
HCH gamma_Lindane	0.15	0.5	10	0.3	1	10	0.6	2	10
Hexaconazole	9	0	200	4.5	15	100	4.5	15	20
Imazalil	1.5	0	50	6	20	50	1.8	6	50
Iprodione	1.5	5	1000	1.5	5	500	1.2	4	20
Isofenphos-methyl	0.3	1	10*	0.3	1	10*	1.2	4	10*
Kresoxim-methyl	1.5	5	1000	1.8	6	50	1.5	5	5000
Linuron	3	10	50	1.8	6	50	1.5	5	50
Malathion	3	10	20	10.5	35	8000	3.6	12	20
Mepanipyrim	1.8	6	2000	2.4	8	10	1.2	4	10
Metalaxyl	9	30	500	10.5	35	50	7.5	25	200
Methacrifos	0.9	3	50	1.8	6	50	0.9	3	50
Methamidophos	0.75	2.5	10	0.9	3	10	1.5	5	10
Methidathion	0.6	2	20	0.9	3	20	1.5	5	20

Table 6 continued

** default value of 10 ng/g set as no MRL values defined*

Compound	Strawberry			Wheat Flour			Leek		
	LOD	LOQ	MRL	LOD	LOQ	MRL	LOD	LOQ	MRL
Methiocarb	150	500	1000	300	1000	100	135	450	200
Metribuzin	0.6	2	100	1.8	6	100	2.1	7	100
Monocrotophos	3	10	10*	4.5	15	10*	3	10	10*
Myclobutanil	0.3	1	1000	1.2	4	20	1.2	4	20
Ortho-phenylphenol	1.5	5	10*	1.5	5	10*	1.5	5	10*
Oxadiazon	0.3	1	50	0.9	3	50	0.6	2	50
Oxadixyl	3	10	10	5.4	18	10	3	10	70
Paclobutrazol	0.9	3	500	0.3	1	20	1.2	4	20
Paraoxon-methyl	6	20	20	6	20	20	3	10	20
Parathion (ethyl)	12	40	10*	37.5	125	10*	12	40	10*
Parathion-methyl	0.6	2	10*	1.2	4	10*	1.5	5	10*
Pendimethalin	1.5	5	50	1.2	4	50	2.1	7	50
Permethrin	2.4	8	50	1.8	6	50	4.5	15	50
Phenthoate	12	40	10*	1.8	6	10*	7.5	25	10*
Phosalone	1.8	6	50	1.2	4	50	1.5	5	50
Phosmet	0.24	0.8	50	0.3	1	50	0.6	2	50
Phosphamidon	0.3	1	10	3	10	10	3	10	10
Pirimicarb	0.9	3	3000	0.9	3	500	0.6	2	1000
Pirimicarb-p-desmetyl	0.9	3	10*	1.2	4	10*	1.5	5	10*
Pirimiphos methyl	0.27	0.9	50	0.6	2	5000	3	10	50
Prochloraz	15.6	52	50	30	100	500	15	50	50
Procymidone	3	10	20	3.9	13	20	1.8	6	20
Profenofos	3	10	50	2.1	7	50	2.1	7	50
Propargite	3	10	10	30	100	10	7.5	25	10
Propiconazole	1.8	6	50	1.2	4	50	0.6	2	100
Propyzamide	0.21	0.7	20	0.9	3	20	0.6	2	20
Prothiofos	2.4	8	10*	0.9	3	10*	1.5	5	10*
Pyraclostrobin	0.75	2.5	1000	0.3	1	100	0.3	1	500
Pyridaben	0.9	3	1000	1.8	6	50	1.5	5	50
Pyrimethanil	0.9	3	5000	1.5	5	50	1.2	4	1000
Pyriproxyfen	0.3	1	50	1.2	4	50	0.6	2	50
Quinoxyfen	0.15	0.5	300	0.24	0.8	20	0.6	2	20
Spirodiclofen	6	20	2000	6	20	20	6	20	20
Tebuconazole	1.5	5	50	0.24	0.8	200	0.3	1	1000
Tebufenocide	30	100	50	60	200	50	30	100	50
Tebufenpyrad	0.3	1	500	0.6	2	50	0.6	2	50
Tefluthrin	0.15	0.5	50	0.3	1	50	1.5	5	50
Tetraconazole	2.4	8	200	1.5	5	100	1.2	4	20
Tetradifon	1.2	4	10	1.8	6	10	0.9	3	10
Tetrahydrophthalimide (THPI)	7.5	25	10*	4.5	15	10*	4.5	15	10*
Thiabendazole	4.5	15	50	1.5	5	50	2.7	9	50
Tolclofos-methyl	0.3	1	50	0.6	2	50	2.1	7	50
Tolyfluanid	7.5	25	5000	1.8	6	50	1000	3000	10*

Table 6 continued

** default value of 10 ng/g set as no MRL values defined*

Compound	Strawberry			Wheat Flour			Leek		
	LOD	LOQ	MRL	LOD	LOQ	MRL	LOD	LOQ	MRL
Triadimefon	0.6	2	500	2.1	7	200	1.2	4	100
Triadimenol	7.5	25	500	2.1	7	200	2.7	9	100
Trifloxystrobin	1.5	5	500	1.2	4	50	1.2	4	200
Trifluralin	15	50	100	4.5	15	100	3	10	500
Triticonazole	1.5	5	10	0.6	2	10	1.5	5	10
Vinclozolin	2.4	8	50	0.9	3	50	2.7	9	50

Table 7: External quality control (FAPAS) results for the relevant compounds.

Compound	Fapas Sample Number	Assigned Value [µg/kg]	Acceptance Range [µg/kg]	Measured Value [µg/kg] (RSD%)
Carbaryl	T19142	89	49.9-128.2	51.2 (22)
beta Endosulfan	T19140	93.6	52.4-134.9	91.3 (7)
Chlorpyrifos-methyl	T19141	86.0	48.2-123.9	88.8 (8)
Cypermethrin	T19141	128.8	72.3-184.1	111.9 (8)
Cypermethrin	T19142	140.4	80.0-200.7	120.2 (17)
DDT, o,p	T19141	67.4	37.8-97.1	38.7 (16)
Dicloran	T19142	66.3	37.1-95.5	63.1 (15)
Dimethoate	T19141	69.0	38.6-99.4	62.3 (15)
Ethoprophos	T19142	29.3	16.4-42.4	25.7 (10)
Methidathion	T19141	29.0	16.3-41.8	29.1 (19)
Monocrotophos	T19141	26.4	14.8-38.0	36.8 (13)
Phosalone	T19140	70.4	39.4-101.4	68.3 (9)
Propyzamide	T19140	89.9	50.4-129.5	94.7 (4)

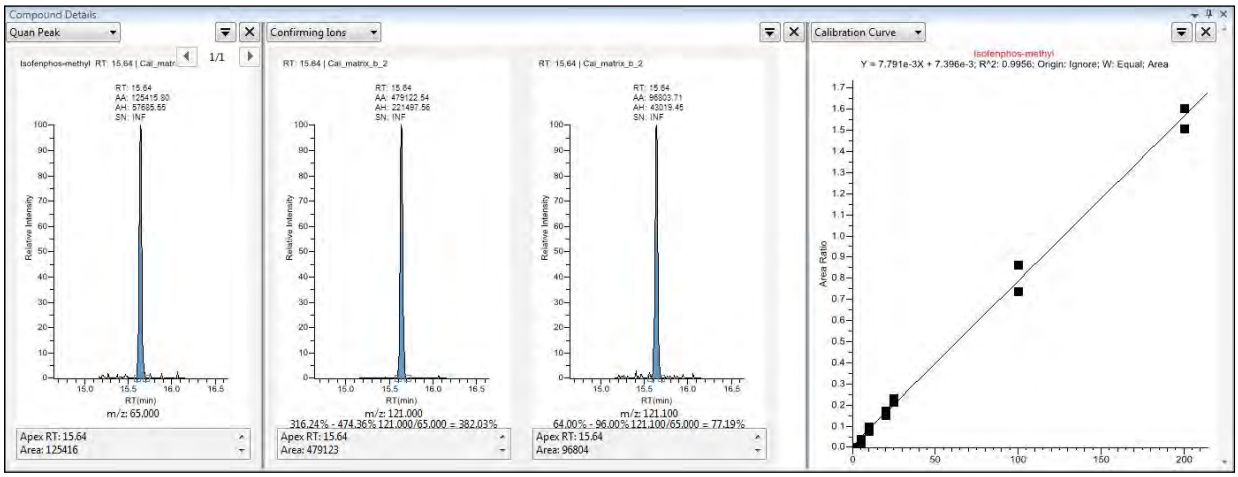

Figure 1. Chromatogram of isofenphos-methyl in leek at at calibration level 2 [5ng/g].

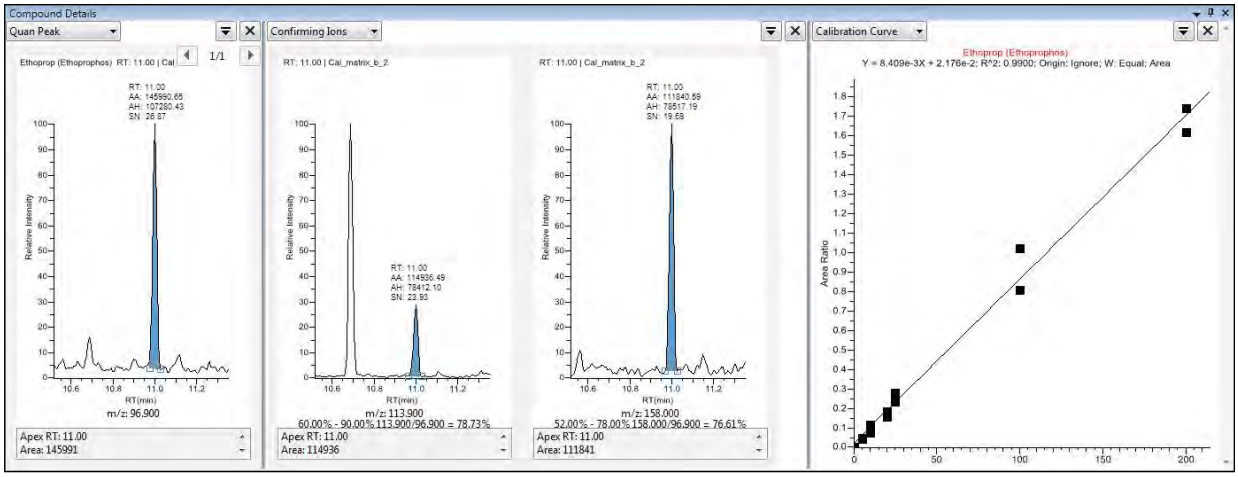

Figure 2. Chromatogram of ethoprop in leek at calibration level2 [5ng/g].

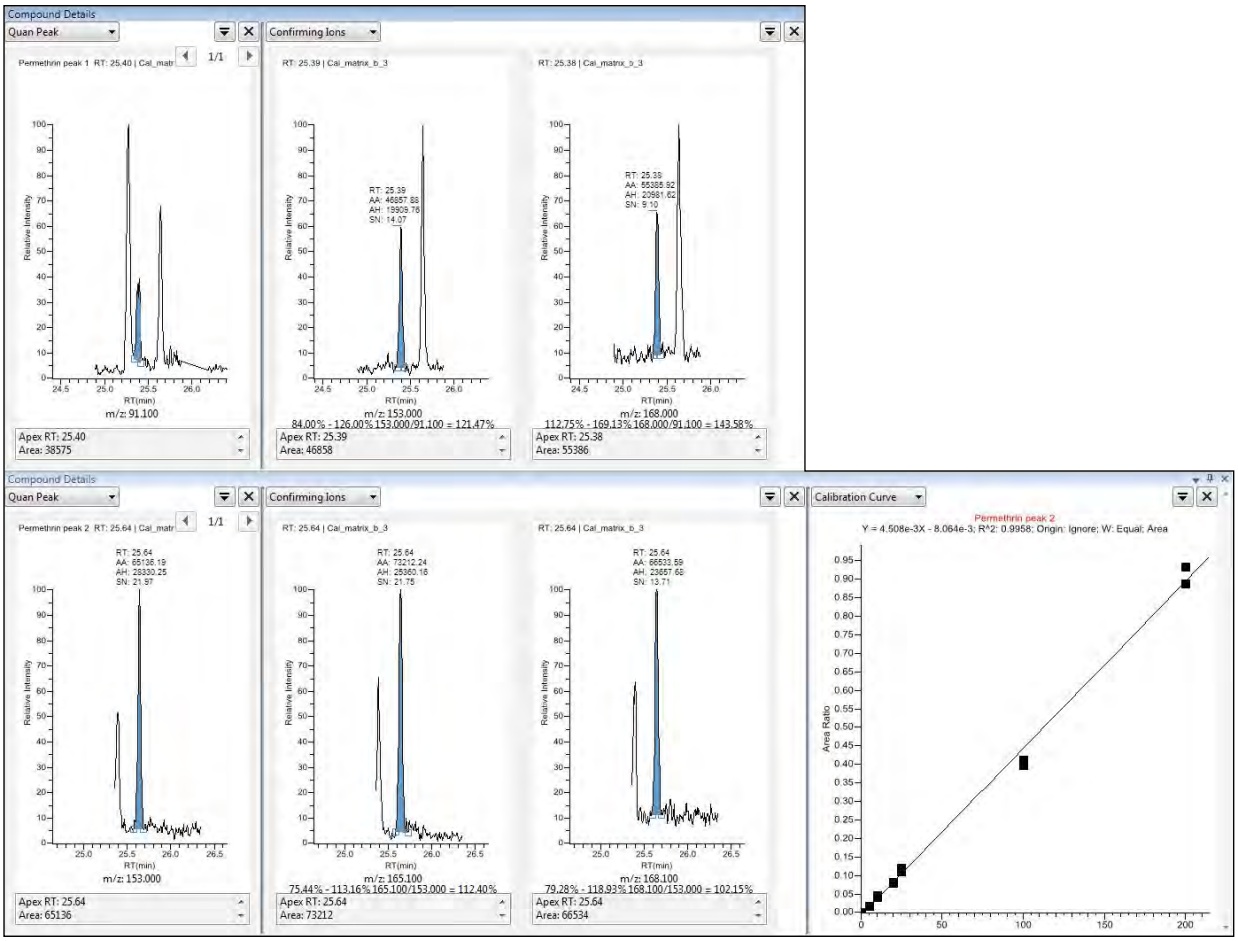

Figure 3. Chromatogram of both permethrine peaks in leek at calibration level3 [10ng/g].

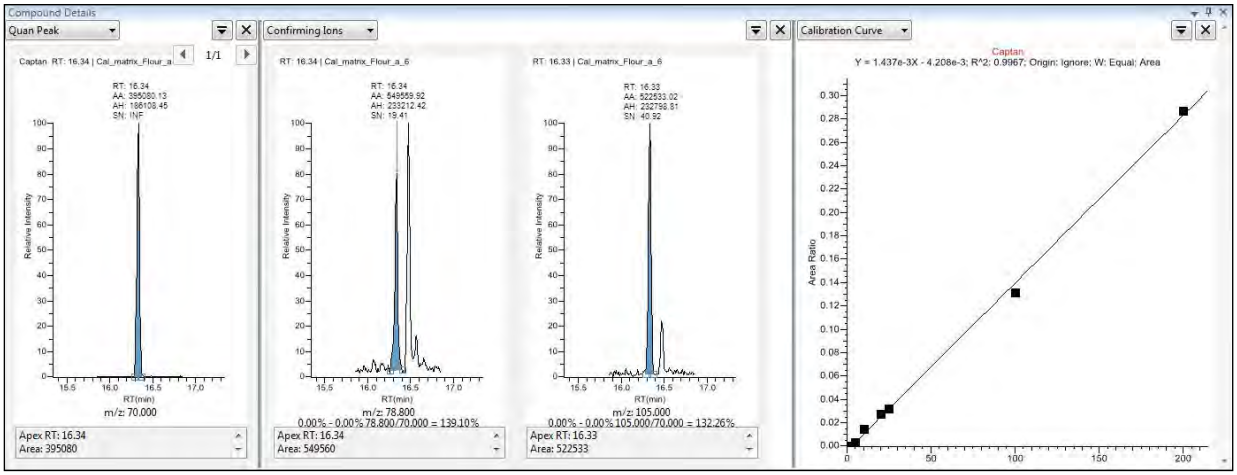

Figure 4. Chromatogram of captan in wheat flour at calibration level6 [100 ng/g].

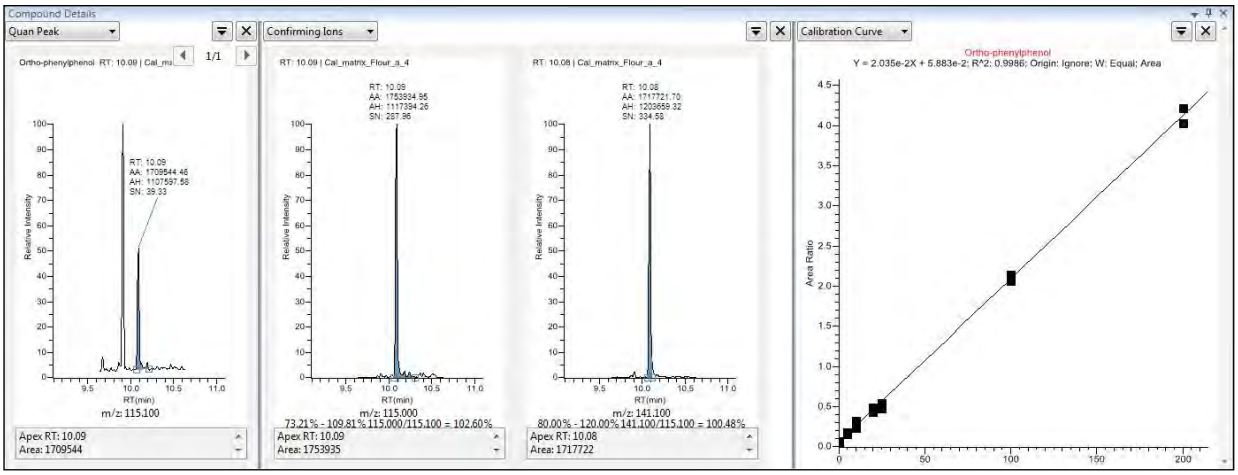

Figure 5. Chromatogram of o-phenylphenol wheat flour at calibration level4 [25ng/g].

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Comparing LC and GC Triple Quadrupole MS for the Screening of 500 Pesticides in Matrix

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Overview

Purpose: The goal of this project is to compare the screening of more than 500 pesticides in matrix by LC and GC triple quadrupole, and determine the value of a comprehensive LC and GC screening approach.

Methods: The methodology included the vegetable extraction by QuEChERS followed by GC-MS/MS and LC-MS/MS analysis of over 500 pesticides in matrix.

Results: The majority of compounds could be detected to levels acceptable by EU standards by either GC/MS or LC/MS. All but eight pesticides could be determined to acceptable levels by the combined GC/LC methodology.

Introduction

Modern pesticide analysis is extremely challenging due to the diversity of compounds required to be reported, especially in the area of food safety control. Furthermore, the pressure to report large numbers of pesticides quickly makes it attractive to use large single injection methods. Triple quadrupole mass spectrometry has emerged as a primary technique for screening large target lists of pesticides due to its high sensitivity and selectivity against matrix. However, because of the chemical diversity of pesticides, LC or GC introduction alone may not be ideal, or even sufficient for a comprehensive analysis. Presented is a comparison of both LC and GC sample introduction techniques coupled to triple quadrupole mass spectrometer for the screening of more than 500 pesticides at ppb levels.

Methods

Sample Preparation

Pesticide standards were obtained from the U.S. Food and Drug Administration (FDA). In order to determine detection limits of such a wide range of pesticides, standards were prepared at multiple levels, enabling the selection of an appropriate level to determine the detection limit of each compound.

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Vegetable matrices were prepared for analysis by using a modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method, which is a sample preparation procedure used to extract pesticides from food[1]. The QuEChERS extracts were obtained from California Department of Food and Agriculture. For the QuEChERS extraction, 15 g of homogenized sample and 15 mL of acetonitrile were used.

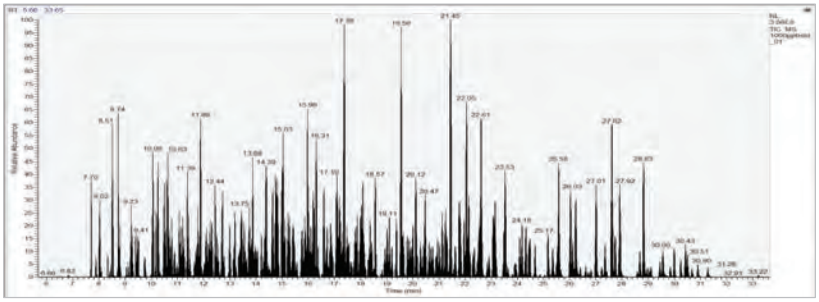
GC/MS Instrument Methodology

Gas Chromatograph Method Conditions

A method was developed for the Thermo Scientific™ TRACE™ 1310 Gas Chromatograph and Thermo Scientific™ TSQ™ 8000 Mass Spectrometer. A Programmable Temperature Vaporization (PTV) injector was used on the TRACE 1310. The ability to program a temperature ramp with this injector was utilized so that thermally labile pesticides would be transferred to the analytical column at as low a temperature possible.

Similarly, the oven on the TRACE 1310 gas chromatograph was ramped, volatilizing pesticides on the column as their boiling points were reached. A slow ramp of 5 ºC/min was employed between an oven temperature of 180 ºC and 280 ºC, which is the range in which the majority of these pesticides are volatilized, to achieve optimal separation during this most dense part of the chromatogram. Figure 1 shows the total ion chromatogram resulting from the GC/MS method, and Figure 2 lists the GC method parameters.

FIGURE 1. GC/MS Total Ion Chromatogram.



The analytical column used was a Thermo Scientific™ TraceGOLD™ TG-5SILMS, with dimensions 30 m x 0.25 mm x 0.25 µm. The liner employed was a baffled, Siltek™ deactivated inlet liner.

FIGURE 2. Gas Chromatograph Parameters.

Injection Volume	
Injection Volume (µL):	1.0
Trace 1310 GC PTV Inlet	
PTV mode:	Splitless
Inlet (°C):	75
Split flow(ml/min):	50
Splitess time (min)	1
PTV inject:	75 °C , 0.1 min to transfer step
PTV transfer:	300 °C, 2.5 °C/sec for 3 min to clean step
PTV Clean:	330 °C, 14.5 °C/sec for 20 min
Carrier Flow He (mL/min):	1.2
Oven Temperature Program	
Temperature 1 (°C):	40
Hold Time (min):	1.5
Rate (°C/min)	25
Temperature 2 (°C):	90
Hold Time (min):	1.5
Rate (°C/min)	25
Temperature 3 (°C):	180
Hold Time (min):	0
Rate (°C/min)	5
Temperature 4 (°C):	280
Hold Time (min):	0
Rate (°C/min)	10
Temperature 5 (°C):	300
Hold Time (min):	5

GC-Triple Quadrupole Method Conditions

Transitions for all pesticides were taken from the Thermo Scientific™ TSQ 8000 Pesticide Analyzer. These transitions were originally developed with the use of AutoSRM software, which provided automated SRM development with collision energies optimized to ± 1 eV. Thermo Scientific TraceFinder™ software was used for acquisition and processing of the extracted samples. Selecting the appropriate compounds from the pesticide analyzer automatically populated the SRM acquisition list in the instrument method and the compound processing parameters in the Thermo Scientific™ TraceFinder™ software processing method. One ion per compound was used for quantitation and two additional ions were used for ion ratio confirmation. Figure 3 lists additional MS parameters used.

FIGURE 3. GC-Mass Spectrometer Parameters

Mass Spec Parameters	
Transfer line (°C):	**250**
Source temperature (°C):	**300**
Mode:	**SRM**
Ionization:	**EI, 70 eV**
Collision Gas:	**Argon**
Resolution:	**Q1 normal**

LC/MS Instrument Methodology

U-HPLC Method Conditions

Chromatographic analysis was performed using the Thermo Scientific™ Accela™ 1250 UHPLC system. The autosampler was an HTC-PAL™ Autosampler (CTC Analytics, Zwingen, Switzerland). The column used was a Thermo Scientific™ Hypersil™ GOLD aQ column (100 x 2.1 mm, 1.9 µm particle size). Displayed in Figure 4 is the total ion chromatogram. The UHPLC conditions are listed in Figure 5.

FIGURE 4. LC/MS Total Ion Chromatogram

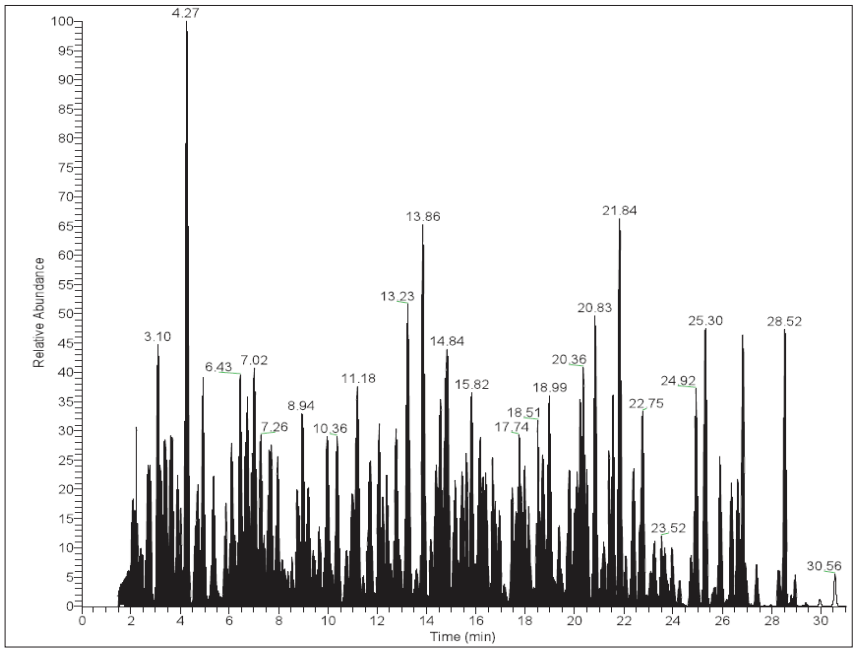


FIGURE 5. HPLC Parameters

HPLC Parameters			
Mobile Phase A:	Water with 0.1% formic acid and 4 mM ammonium formate		
Mobile Phase B:	Methanol with 0.1% formic acid and 4 mM ammonium formate		
Flow Rate:	300 µL/min		
Column Temperature:	40 °C		
Sample Injection Volume:	10 µL		
Gradient:	Gradient Time (min)	%A	%B
	0.00	98	2
	0.25	70	30
	35.00	0	100
	40.00	0	100
	40.01	98	2
	45.00	98	2

TSQ Quantum Access MAX LC-Triple Quadrupole Method Conditions

All samples were analyzed on the Thermo Scientific™ TSQ Quantum Access MAX™ triple stage quadrupole mass spectrometer with a heated electrospray ionization (HESI) source. To maximize the performance of the mass spectrometer, time-specific SRM windows were employed at the retention times of the target compounds. In addition, Quantitation-Enhanced Data-Dependent scanning, which delivers SRM-triggered MS/MS data, was used for structural confirmation. Alternating positive and negative polarity switching was utilized in the method. The MS conditions are listed in Figure 6 below.

FIGURE 6. LC-Mass Spectrometer Parameters.

Mass Spec Parameters	
Sheath Gas Flow Rate:	55 units
Aux Gas Flow Rate:	15 units
Spray Voltage:	3500 V
Capillary Temp:	280 °C
Heater Temp:	295 °C
Cycle Time:	0.2 s

Results and Discussion

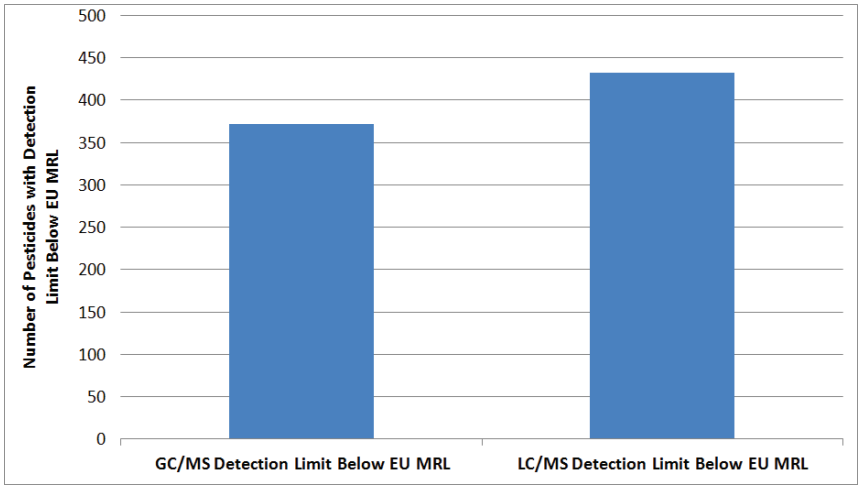
Determination of Method Detection Limit

For both GC/MS and LC/MS methods, spiked matrix samples were analyzed at several concentrations close to or below the European Union Method Reporting Limit (EU MRL). Each concentration level was injected several times and a statistical determination[2] of the method detection limit was calculated for comparison to the EU MRL for an onion matrix for each pesticide. When a required MRL was not available for the pesticide in onion, a 10 parts per billion MRL was used as stated in EU regulations.

Comparison of GC/MS to LC/MS

The majority of compounds were detected below EU MRLs by either the GC/MS or LC/MS method used (Figure 7). Out of the total 524 compounds analyzed, 372 pesticides had MDLs less than EU MRLs for the GC/MS methodology, compared with 432 pesticides with MDLs below the EU MRLs for the LC/MS methodology. Note that a10 µL injection was used in the LC/MS methodology compared with a 1 µL injection employed in the GC/MS methodology.

FIGURE 7. Number of compounds with method detection limits lower than EU MRLs for GC/MS and LC/MS methods

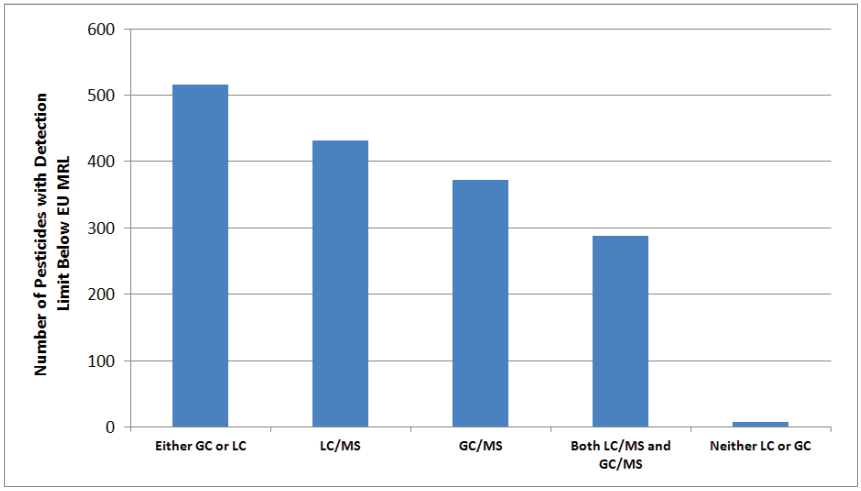


Benefits of Comprehensive GC/LC Methodology

By combining both GC and LC methodologies in a comprehensive screening methodology, 516 pesticides were detected below their MRLs for an onion matrix. This is 144 more than were detected below their MRLs for GC/MS methodology alone, and 84 more than by LC/MS alone. Only 8 pesticides had calculated detection limits for both GC/MS and LC/MS greater than their EU MRLs. On average, these 8 compounds" detection limits were four times their EU MRLs for the technique that gave them their lowest detection limit.

Furthermore, 288 compounds were able to be detected at concentrations below the EU MRL by both GC/MS and LC/MS methodology. This indicates that for a majority of these pesticides the two orthogonal techniques can be used together to increase confidence in the identification and quantitation. Figure 8 displayed below details these results.

FIGURE 8. Number of pesticides with detection limits below the EU MRL for GC/LC combined methodology compared with LC and GC methodology separately. Also displayed are numbers of pesticides detected below the MRL for both GC and LC methodology, and by neither methodology.



Conclusion

Methodology for both GC and LC/MS was developed and employed to analyze over 500 pesticides in a food matrix extracted with QuEChERS methodology. A summary of results, conclusions and possible future investigations for this project are as follow:

- 372 of 524 total pesticides were detected at levels under EU MRLs for onion samples by GC/MS
- 432 of 524 were detected at levels under EU MRLs for onion samples by LC/MS
- 516 of 524 were detected by either GC/MS, LC/MS, or by both GC/MS and LC/MS, demonstrating the power of combining these two techniques.
- For future work, a 10 µL large volume GC injection could be employed for the GC/MS methodology to better compare with the LC/MS methodology, and to try to lower the eight problematic pesticides detection limits under the EU MRL.
- Also, future work could explore techniques to selectively increase sensitivity for the eight problematic compounds, such as weighting SRM dwell time more heavily for these compounds, or decreasing resolution for these compounds, trading selectivity for sensitivity.

References

1. Steven J. Lehotay, Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERs) Approach for Determining Pesticide Residues. Methods in Biotechnology, 2006, 19, 239-261.

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Simplifying Complex Multi-Residue Pesticide Methodology in GC-MS/MS

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Technical Note 10319

Key Words
Pesticide analysis, triple quadrupole GC/MS, AutoSRM, SRM, MRM

Overview

Easing Implementation of Multi-Residue Pesticide Methodology

The task of setting up a triple quadrupole GC/MS pesticide analysis can be daunting, regardless of your starting point. Perhaps you are brand new to GC/MS pesticide analysis, and you need all the help you can get. Maybe you analyze a small set of pesticides and want to expand your target list, or you analyze a large pesticide set in multiple runs on a single quadrupole and want to combine these into a single MRM analysis. Perhaps you already have a comprehensive MRM method, but want to move this to a Thermo Scientific™ TSQ™ 8000 triple quadrupole GC-MS/MS system to take advantage of its robustness, removable ion source under vacuum, and its ease in adding new target pesticides through AutoSRM. Whatever your starting point, when adopting new technology to address complex analytical challenges, you need tools that enable you to be productive, quickly.

With your needs and requirements in mind, the Thermo Scientific TSQ 8000 Pesticide Analyzer (Figure 1) has been developed. Provided within this comprehensive package are all the tools you need to set up a complex pesticide method, regardless of your starting point.

Everyone who is new to pesticide analysis on the TSQ 8000 GC-MS/MS system will appreciate the provided list of optimized pesticide transitions. Also, with an easy to follow step-by-step description of how to develop new transitions using AutoSRM, you'll find the ease of adding new pesticides to your MRM method is now a competitive advantage for your laboratory. And for those who need more assistance, the TSQ 8000 Pesticide Analyzer contains a complete instrument method developed on an included column with provided compound retention times and MRM parameters—eliminating days, if not weeks, of method development.

Figure 1. The TSQ 8000 Pesticide Analyzer. Details of its contents can be found in the *TSQ 8000 Pesticide Analyzer Brochure (BR10318)*.

In addition to simplified method startup, another advantage of using the analyzer is that it utilizes Timed-SRM methodology, allowing for easy-to-use, high-analyte-capacity methodology. The usability and scanning efficiency of Timed-SRM are complemented by the fast-scanning capability of the TSQ 8000 instrument, making the analysis of hundreds of pesticides, with a total of over one thousand transitions, not just possible, but easy.

Finally, the TSQ 8000 Pesticide Analyzer has the ability to analyze full scan data at the same time as your targeted MRM analysis. This allows you to harness the power of existing EI full scan libraries to, for example, find potential high-level contaminants you would otherwise miss in a targeted analysis, or monitor the matrix background for possible interference.

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Using the Startup Kit

Starting Point 1: Starting from Scratch

When creating your method within Thermo Scientific™ TraceFinder™ EFS software, the instrument control and data processing software included with the TSQ 8000 Pesticide Analyzer, the use of the TraceFinder Pesticide Compound Database (CDB) will greatly simplify the method development process. Multiple transitions for each compound in the database have been optimized on the TSQ 8000 instrument with AutoSRM to within ± 1 eV of the optimum collision energy.

Simply select the compounds of interest in the CDB (Figure 2). This will create not only the TraceFinder software processing method, but also the TSQ 8000 mass spectrometer acquisition list. Since the instrument employs Timed-SRM, SRM windows for data acquisition will be centered on your retention times, so that all peaks elute far from acquisition-window breaks. The complete step-by-step procedure, including software screen captures, is detailed in the *TSQ 8000 Pesticide Analyzer Installation Guide*, which is also included with the TSQ 8000 Pesticide Analyzer.

After selecting your compounds of interest, you are now ready to acquire samples in MRM with your TSQ 8000 instrument.

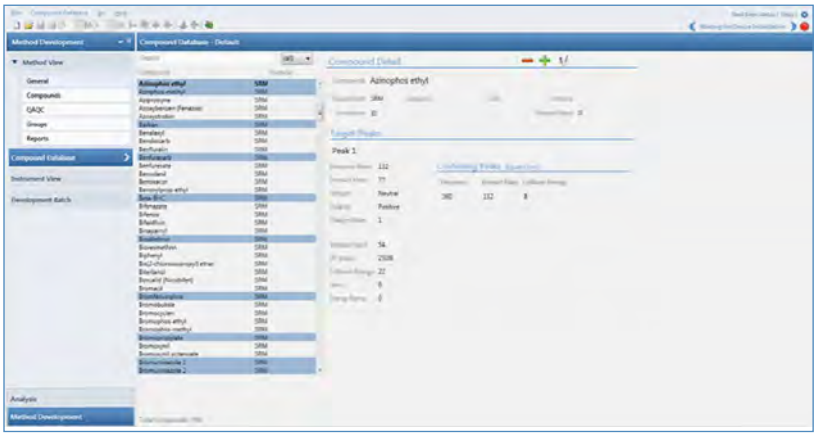

Figure 2. Selecting compounds from the TraceFinder EFS Compound Data Base. This will populate both your TraceFinder Processing Method and your acquisition list. For more information on creating TSQ 8000 methods with the TraceFinder CDB, see *AB52300: Thermo Scientific TSQ 8000 GC-MS/MS Method Sync.*

Starting Point 2: Starting from an Established GC Method

If you already have a preferred GC method, and know the retention times of your target compounds, you can update the pesticides in the CDB with the known retention times. Next, simply select the compounds you are interested in analyzing from the updated CDB, as shown in Figure 2. Again, this will create both the TraceFinder EFS processing method and the TSQ 8000 system Timed-SRM acquisition list, with acquisition windows centered on the retention times of the target peaks.

If you do not know exact retention times, you can easily widen acquisition windows while in TraceFinder EFS software for all compounds (Figure 3) to ensure your peaks fall within their acquisition window. Now update your TraceFinder EFS software method with the new retention times as you would in a normal data review, and your acquisition windows will be centered on each compound. After updating the retention times, follow the same step to reduce acquisition windows back to defaults in order to maximize dwell time for the analysis.

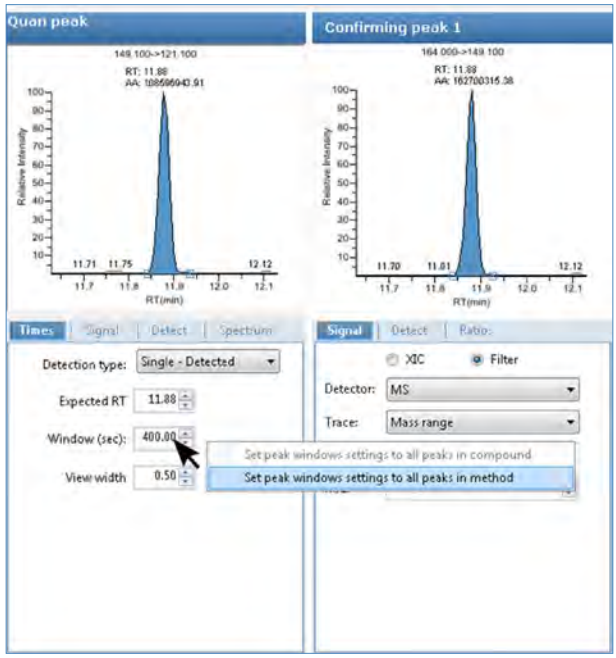

Figure 3. Widening acquisition windows in TraceFinder EFS software to find peaks with unknown retention times.

Tools to Get You Productive

The software features of the TSQ 8000 system have been designed with complex pesticide analysis in mind. These features include AutoSRM, a tool that makes the instrument the easiest for developing and adding new compounds to an existing pesticide method. Another useful feature is Timed-SRM, which enables accurate pesticide identification and quantitation, even for very dense pesticide methodologies. Finally, the ability of the TSQ 8000 instrument to perform simultaneous full scan/MRM provides the capability to identify general unknowns in conjunction with your target pesticides, filling a classic gap in targeted MRM analysis.

Addition of New Compounds

For those compounds provided in the TSQ 8000 Pesticide Analyzer CDB, the addition of new compounds to your methodology is extremely simple. If you are using the method and GC column provided with the TSQ 8000 Pesticide Analyzer, simply select additional compounds to your method from the CDB. The instrument software now adds the selected compounds to both the method acquisition list and the TraceFinder EFS software processing list with the correct retention times.

For those pesticides not yet in the TSQ 8000 Pesticide Analyzer CDB, AutoSRM can be used to quickly develop these new transitions (Figure 4). Once fully developed, the new compounds are easily imported into the CDB and added to your TraceFinder software method. A step-by-step walkthrough of this is described in detail in the *TSQ 8000 Pesticide Analyzer Installation Guide*, which is provided as part of the TSQ 8000 Pesticide Analyzer package. For more details on how AutoSRM works, see *AB52298: Introducing AutoSRM*.

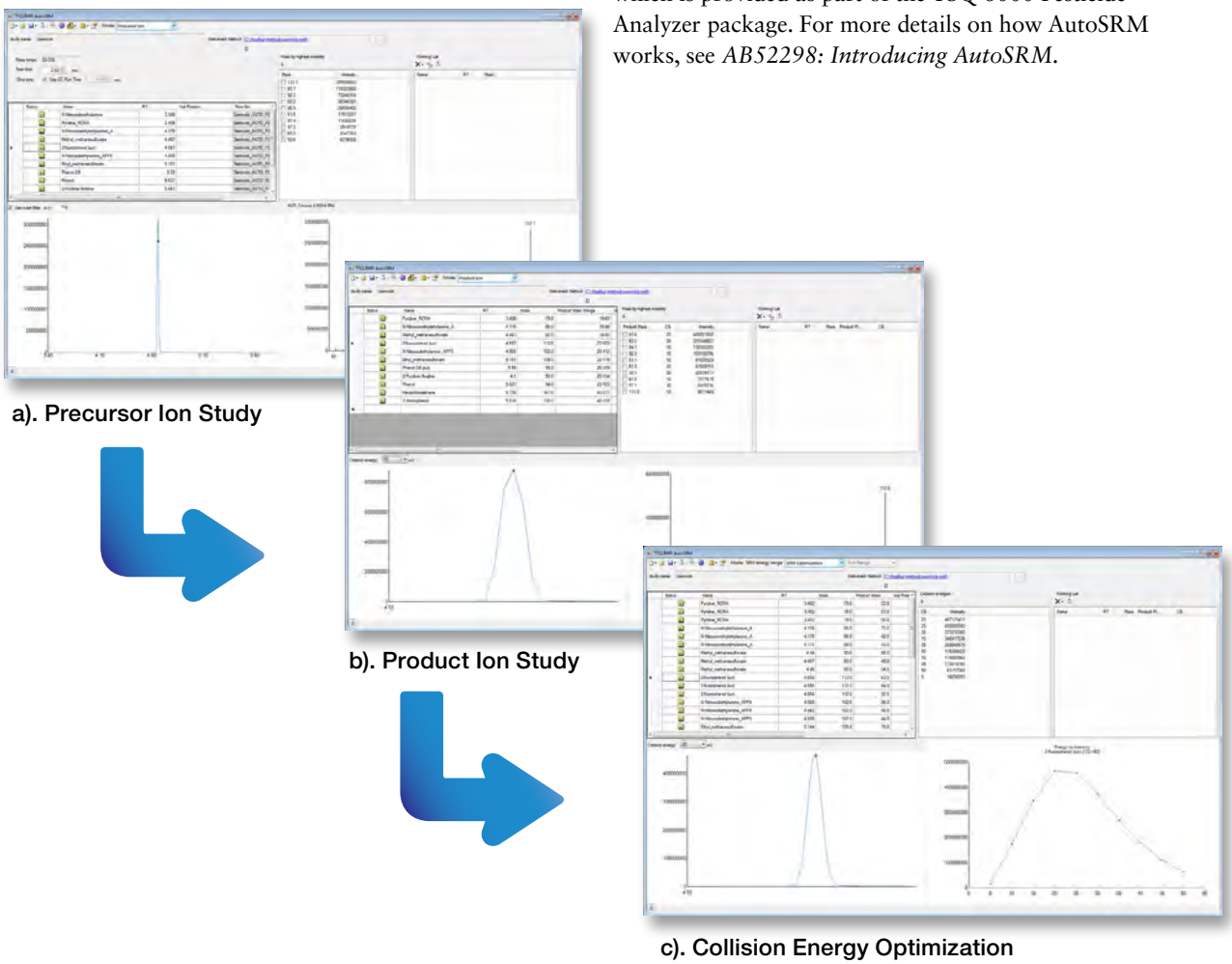

Figure 4. Screen shots showing the three-step process of AutoSRM. a.) In the first step, AutoSRM acquires full scan data for selecting precursor ions. b.) In the second step, product ions are selected from product ion scan data. c.) In the final step, collision energies are varied for each of the selected SRM's to determine the optimal collision energy.

High Compound Capacity Methods

One of the primary challenges of modern pesticide analysis is the sheer number of pesticides that need monitoring in order to meet international standards. This is particularly true in food analysis where products are transported across country borders, requiring exporters to meet the regulatory demands of many countries. Triple quadrupole instruments help meet this demand due to the high selectivity of MRM analysis, which allows for spectral separation of coeluting peaks due to unique reactions in the collision cell. This enables monitoring of more compounds in a single chromatographic run without prohibitive interference. However, due to the targeted nature of the MRM process, individual scan events must be created for each pesticide to be monitored, placing a strain on the amount of time devoted to the monitoring of each compound, and thus the sensitivity of the analysis of each compound.

With a traditional style analysis, this issue can be partially resolved by slicing up the acquisition list into discreet time segments, so that all transitions are not being monitored at the same time. However, this can quickly lead to problems when analyzing more than 50 pesticides in one run. This is because, due to the density of the peaks in the heart of the method, it is difficult to find a time for a segment break when no target peaks are eluting.

This then forces a compromise between adding many compounds per segment, reducing individual SRM dwell times and sensitivity, and adding segment breaks between closely eluting peaks, which causes the risk of false negatives due to shifts in peak retention times outside of acquisition windows because, for example, a large bit of matrix coelutes with a peak.

The TSQ 8000 system takes an approach called Timed-SRM that eliminates this compromise. Timed-SRM removes the limitations of segmented SRM by centering acquisition windows on the retention time of each peak and allowing for acquisition window overlap, so that acquisition windows for all nearby eluting compounds are not forced to start and stop at the same time (Figure 5). The user simply needs to enter the retention time of each compound, and the instrument method takes care of the rest, eliminating the need for creating segments.

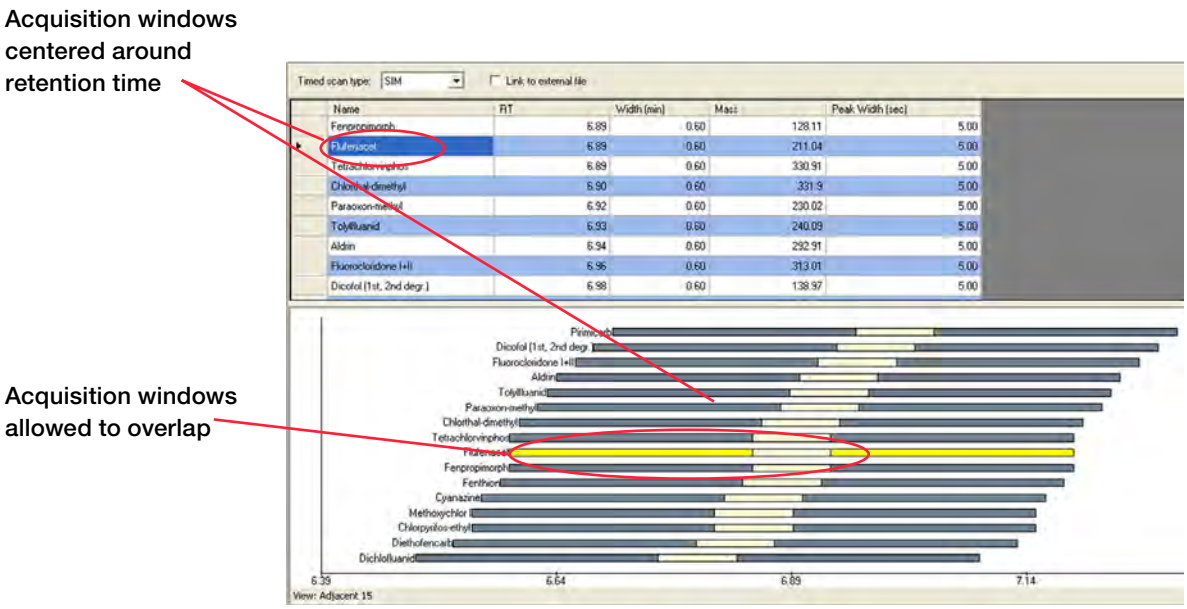

Figure 5. The TSQ 8000 system Timed-SRM Acquisition list, showing SRM acquisition windows centered on retention times and overlapping nearby transitions.

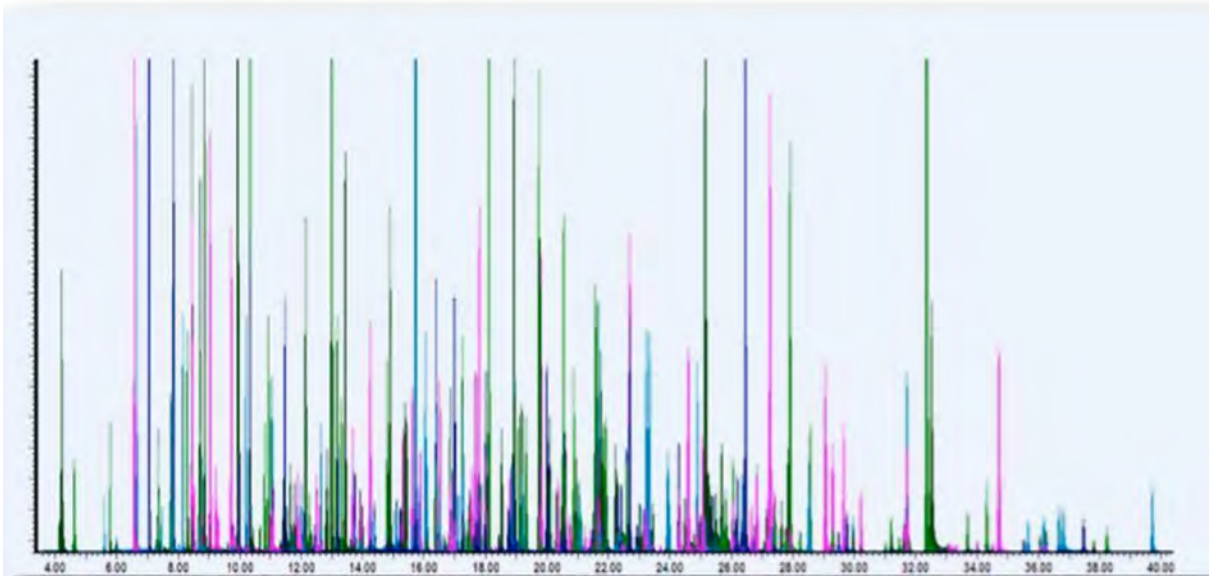

Figure 6. Real-world acquisition of over 300 pesticides in a single chromatographic run using Timed-SRM.

Figure 6 shows a real-world example of a pesticide analysis of over 300 compounds using Timed-SRM. As shown in the Table 1 comparison with Segmented-SRM, Timed-SRM increases both the sensitivity of the analysis by reducing the number of transitions being acquired simultaneously and the time between when target peaks elute and when their acquisition window begins or ends.

Table 1. Comparison of Segmented-SRM vs. Timed-SRM for method of over 300 pesticides. Timed-SRM can dramatically reduce the average number of transitions occurring simultaneously, while increasing the minimum time between an eluting peak and an acquisition window break.

	Segmented-SRM	Timed-SRM
Average number of simultaneous transtions during run	55 Transitions	15 Transitions
Shortest time interval between a compound retnetion time and an acquisiton window break	5 Seconds	15 Seconds

General Unknown Screening

Another limitation of the classic MRM approach to pesticide analysis is that, due to its targeted nature, if a compound is not part of your target list, you are not going to find it, even if it is present in large quantities in your sample. This limitation is removed with capability of the TSQ 8000 system to perform simultaneous full scan/MRM.

The TSQ 8000 system allows you to set up a full scan acquisition throughout the duration of your MRM analysis. Each acquisition will then have full scan data to identify non-target compounds, in addition to MRM data to confirm and quantitate the target list. This mode of analysis is facilitated with the TraceFinder EFS software qualitative processing view within its standard quantitative batch analysis, which automatically detects, identifies, and reports non-target compounds (Figure 7).

Figure 7. Qualitative view of TraceFinder EFS software for analyzing fruit juice with simultaneous full scan/Timed-SRM on the TSQ 8000 system. In addition to quantitating and confirming the 158 target compounds by MRM (top), TraceFinder EFS software has identified three high-level unknowns by full scan analysis (bottom): 2,4-bis(1,1-dimethylethyl)-phenol, triethyl citrate, and Vitamin E.

Conclusion

For the lab just starting up a complex pesticide analysis by triple quadrupole GC-MS, the TSQ 8000 Pesticide Analyzer offers the easiest and quickest path to success. The included methodology, consumables, and SRM transition list with accurate retention times enable the creation of your pesticide method to be as simple as selecting the compounds you want to analyze. With multiple SRM transitions per compound optimized to within ± 1 eV, the pesticide analyzer is useful for anyone who wants to take advantage of the unique features of the TSQ 8000 system designed to make complex pesticide analysis simple.

The TSQ 8000 Pesticide Analyzer fully takes advantage of these features, including the ability to do Timed-SRM, which significantly increases low-level sensitivity through a more efficient SRM scheduling. Also, the full scan/MRM capability of the TSQ 8000 mass spectrometer combines the elite quantitation capabilities of MRM analysis with classic general unknown identification through full scan quadrupole library searching. Finally, the ability to easily develop and add new pesticides to an existing pesticide method through AutoSRM makes the TSQ 8000 Pesticide Analyzer the most flexible system for expanding your pesticide target list to meet future regulatory or client demands.

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Analysis of Multi-Residue Pesticides Present in Ayurvedic Churna by GC-MS/MS

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Key Words
Traditional herbal medicine, fast liquid/liquid extraction, QuEChERS, timed-SRM, retention time synchronization, MRM, ion ratio confirmation, TraceFinder data processing

Introduction

Ayurveda is a Sanskrit term, made up of the words "ayus" and "veda. "meaning life and science; together translating to 'science of life'. A blend of several herbs and spices make up the powdered mixture known as "churna". Depending on its intended use for medicinal, beauty, or culinary purpose, the recipe varies. Avipittakara "churna" is a traditional Ayurvedic formula used widely and almost daily to control vitiated pitta dosha, remove heat in the digestive system, control indigestion, constipation, vomiting and anorexia. A major analytical challenge for these types of samples is mainly addition of multiple herbs with sugar and the natural color of herbs.[1]

The dried leaves result in highly complex extracts from the sample preparation due to the rich content of active ingredients, essential oils and the typical high boiling natural polymer compounds. Due to the use of pesticides in the fresh herbs, the "churna" may contain residual pesticides. Analysis of pesticide residues is thus important and governmentally regulated.[2] Strict quality parameters have been mented to preserve the quality and efficacy of these "churnas".

Sample Preparation

In brief, the QuEChERS sample preparation (see Figure 1) involved the extraction of 15 g of a powder sample of Avipittakara "churna" with 15 mL acetonitrile (containing 1% acetic acid) in the presence of 3 g magnesium sulfate, 1.5 g sodium acetate and 1 g NaCl.

The supernatant (1 mL) was collected after centrifugation, and dispersive cleanup was performed using 200 mg PSA and 10 mg GCB. The extract was centrifuged at 10,000 rpm for 5 min, and 3 µL of supernatant was injected via autosampler for analysis. For recovery and validation studies 15 g of the "churna" was fortified with appropriate quantities of the pesticide standard mixture.

Weigh 15 g of *Churna*

↓

15 mL acetonitrile (with 1% acetic acid) was added, shaken well.Further 3 g $MgSO_4$+1.5 g NaOAc+1 g NaCl was added and homogenized using a Vortex mixer

↓

After centrifugation (5000 rpm, 5 min), cleanup of 1 mL supernatant performed by dispersive SPE using 200 mg PSA and 10 mg GCB

↓

Centrifuge at 1000 rpm for 5 min, 3 µL of supernatant injected to GC-MS/MS

Figure 1. Sample preparation for extraction of pesticides from ayurvedic churnas.

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Calibration

Stock standard solutions of each pesticide compound were prepared by weighing 10±0.1 mg, dissolving in 10 mL acetonitrile and storing the solution in ambercolored glass vials at -20 °C. A total of ten intermediate mixtures (each containing 15-20 compounds) of 10 mg/L concentration were prepared by diluting an adequate quantity of each compound in acetonitrile. A working standard solution (1 mg/L) was prepared by mixing an adequate quantity of intermediate standard solution and dilution with acetonitrile and storing the solution at -20 °C. The calibration standards at 2.5, 5, 10, 25 and 50 µg/L were freshly prepared for measurement of the calibration curves. The calibration graphs (five points) for all the compounds were obtained by plotting the individual peak areas against the concentration of the corresponding calibration standards.

Instrument and Method Setup

The analytical method comprises the sample handling using the Thermo Scientific™ TriPlus™ RSH liquid auto sampler, the Thermo Scientific™ TRACE™ 1300 Series gas chromatograph equipped with a temperature programmable PTV injector, and the Thermo Scientific™ TSQ 8000TM triple quadrupole GC-MS/MS system. The instrument method parameters are summarized in Table 1.

Table 1. Instrument method parameters.

TRACE™ 1310 Gas Chromatograph Parameters

Carrier gas	Helium
Injector	PTV
Mode	splitless
Splitless time	3 min, split flow: 30 mL/min
PTV program	87 °C, 0.3 min (injection)
	14.5 °C/min to 285 °C (transfer)
	285 °C, 2.5 min (transfer)
	14.5 °C/min to 290 °C (cleaning)
	290 °C , 20 min (cleaning)
Column	Thermo Scientific TraceGOLD™
	TG-5 SilMS, 30 m × 0.25 mm ×
	0.25 µm (P/N 10177894)
Column flow	1.2 mL/min, constant flow
Oven program	70 °C , 2 min
	10 °C/min to 200 °C
	200 °C , 1 min
	10 °C/min to 28 °C
	285 °C , 8.5 min
Injection	3 µL by TriPlus RSH Autosampler

The Thermo Scientific™ TraceFinder™ software was used for method setup and data processing. The TraceFinder software provides a compound database of pesticides compoundsof more than 800 compounds with all required analytical details such as retention times and the optimized SRM transitions for data acquisition and processing. These software features were employed to create the processing method for the screening a large pesticides compound list.[2]

For all pesticide compounds two SRM transitions were chosen for the overall MRM acquisition method. The first transition was used for quantitation, the second transition for confirmation by checking the ion intensity ratio by the TraceFinder software during data processing. Retention times had been synchronized between data processing of standards with the acquisition method for the timed-SRM protocol (see Figure 2) in order to lock all compound retention times for robustness independent on the impact of the matrix carried by real life sample.

TSQ-8000 MS/MS Parameters

Ion source temperature	230 °C
Interface temperature	285 °C
Acquisition mode	EI, 70 eV
MRM detection	Timed SRM mode (see Figure 1)
Acquisition rate	500 ms
MRM parameter	See Table 1

The timed-SRM acquisition method used with the TSQ 8000 MS avoids the laborious and time-consuming process of segment creation and method maintenance. The scan times are automatically calculated based upon the specified cycle time so that uniform cycle times are obtained for each mass transition, thus reducing the extensive optimization process for scan times and data points across a peak. The dwell times for data acquisition are maximized independently for the number of compounds in the MRM method. Table 2 lists the MRM parameters for the compounds analyzed in this method.

The data processing and reporting was done using the quantitation and reporting suite. The software allows retention time locking by synchronization between the data processing and the acquisition setup for all compounds in the method.

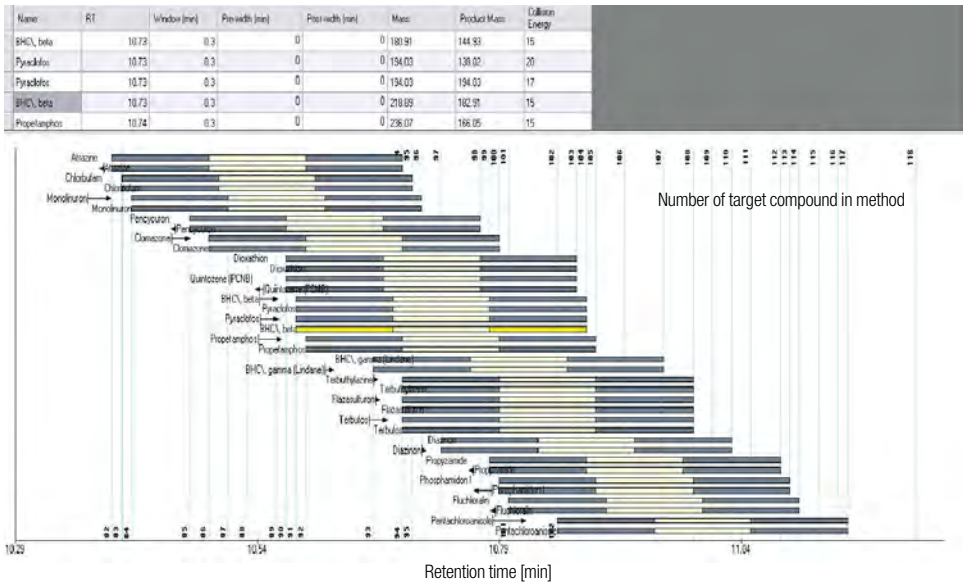

Figure 2. Principle of the timed-SRM acquisition setup of the TSQ 8000. The white center parts show the peak width centered to the compound retention time, the grey areas before and after the peak the full SRM acquisition window of 0.3 min.

Results

The multi-residue pesticide analysis of Ayurvedic churnas for routine target analytes detection and quantitationis described using liquid-liquid extraction and GC-MS/MS detection with the TSQ 8000 GC-MS/MS system. All standards and samples were processed using TraceFinder software with high speed and throughput.

All compounds included into this method had very good calibration correlation coefficients of > 0.99 for the concentration range of 2.5 to 50 ng/g, as shown Figure 3. The obtained recoveries were high within 70-120% with < 20% associated RSDs.

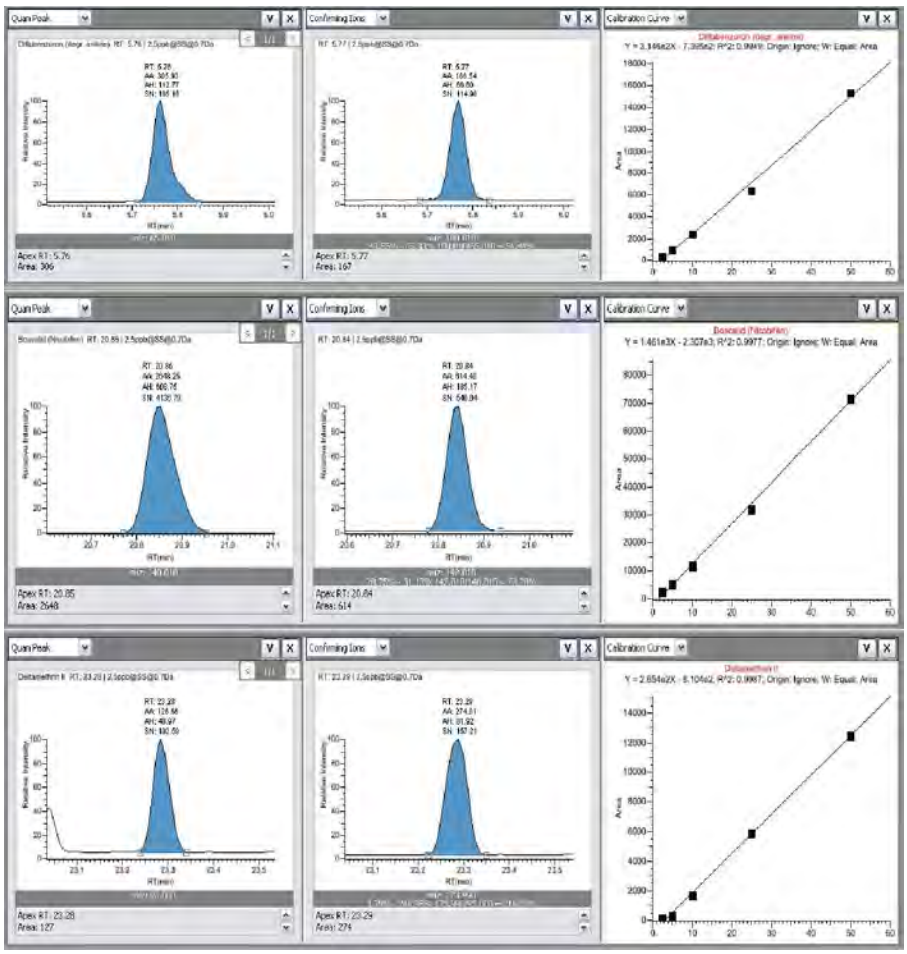

Figure 3. Selected pesticide chromatograms at 2.5 ng/g and their calibration curves.

Sample Analysis

Approximately 200 pesticide compounds were included in a routine screening method with an approximately 28 min total run time. The method setup as described above was applied for analyzing samples bought from the regional market. The results from analysis of market samples are presented in Figure 4.

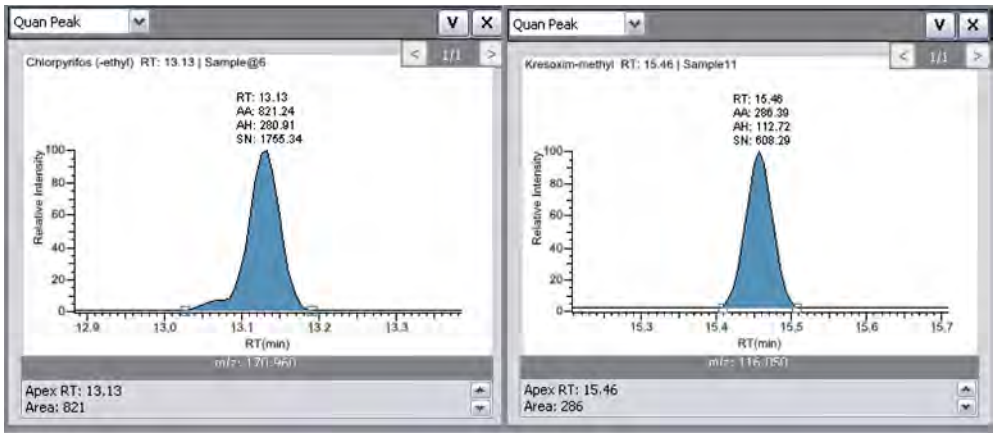

Figure 4. Traces of Chlorpyrifos ethyl and Kresoxim methyl were detected at 2.3 and 2.7 µg/kg respectively in regional market samples.

Conclusion

A rapid and sensitive quantitative method for a large number of compounds is always a major goal for analytical laboratories involved in pesticide analysis. Within 28 minutes, 200 pesticides were screened and quantitatively determined using the described pesticide analysis method. The QuEChERS sample preparation method provided high recoveries and good reproducibility. The generic TRACE TR-5MS column coupled with TRACEGuard provided good chromatographic resolution of the pesticides studied. The triple quadrupole mass analyzer TSQ 8000 GC-MS/MS system with TraceFinder™ software was used for data processing to reduce the processing time, thereby resulting in a high throughput method space missing. Linearity, specificity, recovery, and repeatability of the method were established with minimal sample preparation time. The TSQ 8000 system provided very high selectivity for the sensitive detection and reliable quantitation of the pesticides even from these samples with a high matrix load from the short QuEChERS sample preparation.

This method can be utilized for detection and confirmation of trace amounts of pesticides in difficult matrices such as herbal churnas. The method has potential to detect trace level compounds at concentration as low as 2.5 ng/g. As per the available guidelines, the concentration of the detected pesticides (0.0023 and 0.0027 mg/kg) were below the required limits of the Unani Guidelines.[3]

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Table 2. MRM parameter for the pesticide compounds analyzed.

Nr.	Compound Name	RT [min]	Quantitation *m/z*	CE [V]	Confirmation *m/z*	CE [V]	r^2
1	Diflubenzuron (degr. i-cyanat)	5.24	153.02 > 90.01	20	153.02 > 125.01	20	0.9969
2	Diflubenzuron (degr. aniline)	5.75	127.01 > 65.01	30	127.01 > 100.01	30	0.9949
3	Methamidophos	5.87	141.00 > 95.00	10	141.00 > 126.00	5	0.9930
4	Dichlorphos (DDVP)	5.94	184.95 > 92.98	17	219.95 > 184.95	10	0.9960
5	Dichlobenil	6.82	135.97 > 99.98	10	170.96 > 135.97	15	0.9960
6	Mevinphos	7.39	127.03 > 109.02	10	192.04 > 127.03	12	0.9964
7	Acephate	7.50	136.01 > 42.00	10	136.01 > 94.01	15	0.9904
8	Dichloraniline, 3,5-	7.61	160.98 > 89.99	25	160.98 > 98.99	25	0.9989
9	Molinate (Ordram)	8.58	126.07 > 55.03	10	187.10 > 126.07	10	0.9941
10	TEPP	8.60	263.06 > 179.04	15	263.06 > 235.06	5	0.9946
11	Omethoate	9.00	110.01 > 79.01	15	156.02 > 110.01	10	0.9969
12	Fenobucarb	9.11	121.07 > 77.05	15	150.09 > 121.07	10	0.9977
13	Propoxur	9.13	110.06 > 64.03	10	152.08 > 110.06	10	0.9981
14	Propachlor	9.16	176.06 > 120.04	10	196.07 > 120.04	10	0.9980
15	Ethoprophos	9.38	158.00 > 80.90	15	158.00 > 114.00	5	0.9949
16	Trifluralin	9.58	264.09 > 160.05	15	306.10 > 264.09	15	0.9944
17	Chlorpropham	9.62	213.00 > 127.00	5	213.00 > 171.00	5	0.9981
18	Benfluralin	9.63	292.10 > 160.05	21	292.10 > 264.09	10	0.9923
19	Sulfotep	9.70	322.02 > 202.01	15	322.02 > 294.02	10	0.9943
20	Bendiocarb	9.72	166.06 > 151.06	15	166.06 > 166.06	15	0.9996
21	Monocrotophos	9.80	127.03 > 95.03	20	127.03 > 109.03	10	0.9971
22	Methabenzthiazuron	9.82	164.05 > 136.04	12	164.05 > 164.05	15	0.9974
23	BHC, alpha	10.15	180.91 > 144.93	15	218.89 > 182.91	10	0.9970
24	Metamitron	10.36	202.09 > 174.07	5	202.09 > 186.08	10	0.9969
25	Atrazine	10.54	215.09 > 173.08	10	215.09 > 200.09	10	0.9945
26	Pencycuron	10.62	125.05 > 89.04	12	180.07 > 125.05	12	0.9914
27	Dioxathion	10.72	125.00 > 97.00	15	125.00 > 141.00	15	0.9936
28	BHC, beta	10.73	180.91 > 144.93	15	218.89 > 182.91	15	0.9933
29	Propetamphos	10.74	236.07 > 166.05	15	236.07 > 194.06	5	0.9918
30	BHC, gamma (Lindane)	10.81	180.91 > 144.93	15	218.89 > 180.91	5	0.9939
31	Terbuthylazine	10.84	214.10 > 132.06	10	229.11 > 173.08	10	0.9935
32	Diazinon	10.88	137.05 > 84.03	10	304.10 > 179.06	15	0.9987
33	Propyzamide	10.93	173.01 > 145.01	15	175.02 > 147.01	15	0.9939
34	Fluchloralin	10.95	264.04 > 206.03	10	306.05 > 264.04	10	0.9967
35	Pyroquilon	11.07	173.08 > 130.06	20	173.08 > 145.07	20	0.9974
36	Pyrimethanil	11.11	198.11 > 158.09	30	198.11 > 183.10	15	0.9953
37	Tefluthrin	11.16	177.02 > 127.02	20	197.03 > 141.02	15	0.9991
38	Etrimfos	11.29	292.06 > 153.03	10	292.06 > 181.04	10	0.9935
39	Pirimicarb	11.50	166.10 > 96.06	10	238.14 > 166.10	15	0.9937
40	BHC, delta	11.54	180.91 > 144.93	15	204.07 > 91.03	15	0.9949
41	Iprobenfos	11.54	204.07 > 122.04	15	218.89 > 182.91	15	0.9997
42	Formothion	11.74	126.00 > 93.00	8	172.00 > 93.00	5	0.9982
43	Phosphamidon II	11.83	227.05 > 127.03	15	264.06 > 193.04	15	0.9977
44	Dichlofenthion	11.90	222.98 > 204.98	10	278.97 > 222.98	15	0.9946
45	Dimethachlor	11.94	197.08 > 148.06	10	199.08 > 148.06	10	0.9992
46	Dimethenamid	11.95	230.06 > 154.04	10	232.06 > 154.04	10	0.9953
47	Propazine	12.02	214.09 > 172.08	12	214.09 > 214.09	10	0.9970
48	Propanil	12.06	217.01 > 161.00	10	219.01 > 163.00	10	0.9934
49	Malaoxon	12.07	127.02 > 99.02	10	127.02 > 109.02	20	0.9978
50	Chlorpyrifos-methyl	12.08	124.96 > 78.97	10	285.91 > 92.97	20	0.9945
51	Metribuzin	12.13	198.08 > 82.03	20	198.08 > 110.05	20	0.9997
52	Spiroxamine I	12.15	100.09 > 58.05	15	100.09 > 72.06	15	0.9909
53	Vinclozolin	12.16	212.00 > 172.00	15	285.00 > 212.00	15	0.9957
54	Carbofuran, 3-Hydroxy	12.21	137.06 > 81.03	18	180.08 > 137.06	15	0.9974

Nr.	Compound Name	RT [min]	Quantitation *m/z*	CE [V]	Confirmation *m/z*	CE [V]	r^2
55	Parathion-methyl	12.22	263.00 > 109.00	15	263.00 > 246.00	15	0.9966
56	Alachlor	12.23	161.07 > 146.06	12	188.08 > 160.07	10	0.9997
57	Tolclofos-methyl	12.25	264.96 > 92.99	20	264.96 > 249.96	15	0.9932
58	Propisochlor	12.31	162.08 > 144.07	10	223.11 > 147.07	10	0.9983
59	Metalaxyl	12.37	249.13 > 190.10	10	249.13 > 249.13	5	0.9911
60	Carbaryl	12.41	144.06 > 115.05	20	144.06 > 116.05	20	0.9919
61	Fuberidazol	12.41	183.80 > 156.10	10	183.80 > 183.10	20	0.9902
62	Fenchlorfos (Ronnel)	12.47	284.91 > 269.92	13	286.91 > 271.91	20	0.9994
63	Prosulfocarb	12.63	100.00 > 72.00	10	128.00 > 43.10	5	0.9938
64	Pirimiphos-methyl	12.66	290.09 > 233.07	10	305.10 > 290.09	15	0.9911
65	Spiroxamine II	12.75	100.09 > 58.05	15	100.09 > 72.06	15	0.9916
66	Ethofumesate	12.80	207.08 > 161.06	10	277.02 > 109.01	8	0.9907
67	Fenitrothion Confirming 1	12.80	277.02 > 260.02	10	286.11 > 207.08	12	0.9997
68	Methiocarb	12.84	168.06 > 109.04	15	168.06 > 153.06	15	0.9971
69	Malathion	12.92	127.01 > 99.01	10	173.02 > 127.01	10	0.9951
70	Dichlofluanid	12.95	223.97 > 122.99	15	225.97 > 122.99	15	0.9971
71	Phorate sulfone	13.01	153.00 > 125.00	5	199.00 > 143.00	20	0.9942
72	Dipropetryn	13.02	241.90 > 149.80	20	254.90 > 180.30	20	0.9906
73	Chlorpyrifos (-ethyl)	13.12	198.96 > 170.96	15	313.93 > 285.94	12	0.9995
74	Fenthionoxon	13.22	277.80 > 109.10	25	329.60 > 298.90	10	0.9927
75	Chlorthal-dimethyl (DCPA)	13.24	300.91 > 300.91	15	331.90 > 300.91	15	0.9986
76	Flufenacet	13.26	211.04 > 123.02	10	211.04 > 183.03	10	0.9959
77	Endosulfan I (alpha)	13.43	240.89 > 205.91	20	264.88 > 192.91	22	0.9942
78	Imazethapyr	13.49	201.9 > 133.00	15	252.00 > 145.90	20	0.9944
79	Butralin	13.50	266.14 > 190.10	15	266.14 > 220.11	15	0.9996
80	Pirimiphos (-ethyl)	13.54	304.12 > 168.06	15	333.13 > 318.12	15	0.9992
81	Pendimethalin	13.86	252.12 > 162.08	12	252.12 > 191.09	12	0.9912
82	Fipronil	13.87	212.97 > 177.98	16	366.95 > 212.97	25	0.9938
83	Cyprodinil	13.91	224.13 > 208.12	20	225.13 > 210.12	18	0.9959
84	Metazachlor	13.92	133.05 > 117.04	20	209.07 > 132.05	12	0.9939
85	Penconazole	14.01	248.06 > 157.04	25	248.06 > 192.04	15	0.9977
86	Tolylfluanid	14.05	137.05 > 91.03	20	238.09 > 137.05	15	0.9922
87	Chlorfenvinphos-Z	14.05	266.98 > 158.99	15	322.97 > 266.98	15	0.9904
88	Allethrin	14.06	123.08 > 81.05	10	136.08 > 93.06	10	0.9923
89	Mecarbam	14.09	226.04 > 198.03	5	329.05 > 160.03	10	0.9979
90	Phenthoate	14.18	146.01 > 118.01	10	274.03 > 246.02	10	0.9951
91	Mephosfolan	14.20	196.02 > 140.02	15	196.02 > 168.02	10	0.9973
92	Quinalphos	14.21	146.03 > 118.02	15	157.03 > 129.02	13	0.9943
93	Triflumizole	14.31	179.04 > 144.04	15	206.05 > 179.04	15	0.9925
94	Procymidone	14.31	283.02 > 96.01	15	283.02 > 255.02	10	0.9983
95	Bromophos-ethyl	14.50	358.89 > 302.91	20	358.89 > 330.90	10	0.9985
96	Methidathion	14.60	124.98 > 98.99	22	144.98 > 84.99	10	0.9945
97	Chlordane, alpha (cis)	14.62	372.81 > 265.87	18	374.81 > 267.87	15	0.9967
98	DDE, o,p	14.63	245.95 > 175.97	25	317.94 > 245.95	20	0.9946
99	Sulfallate	14.68	188.02 > 132.02	22	188.02 > 160.02	16	0.9945
100	Paclobutrazol	14.72	236.10 > 125.06	15	236.10 > 167.07	15	0.9926
101	Disulfoton sulfone	14.74	213.01 > 125.01	10	213.01 > 153.01	5	0.9912
102	Picoxystrobin	14.77	303.09 > 157.04	20	335.09 > 303.09	10	0.9937
103	Endosulfan II (beta)	14.88	271.88 > 236.89	18	338.85 > 265.88	15	0.9973
104	Mepanipyrim	14.89	222.11 > 207.10	15	223.11 > 208.10	15	0.9965
105	Chlordane, gamma (trans)	14.89	372.81 > 265.87	18	374.81 > 267.87	15	0.9991
106	Flutriafol	14.97	123.04 > 75.03	15	219.07 > 123.04	15	0.9915
107	Napropamide	15.00	128.07 > 72.04	10	271.16 > 128.07	5	0.9972
108	Flutolanil	15.03	173.06 > 145.05	15	173.06 > 173.06	15	0.9988
109	Pretilachlor	15.13	162.09 > 147.08	15	216.05 > 174.04	20	0.9935

Nr.	Compound Name	RT [min]	Quantitation *m/z*	CE [V]	Confirmation *m/z*	CE [V]	r^2
110	Hexaconazole, confirming 1	15.13	231.06 > 175.04	10	262.14 > 202.11	15	0.9962
111	Isoprothiolane	15.14	290.06 > 118.03	15	290.06 > 204.05	15	0.9961
112	Profenofos	15.21	138.98 > 96.98	8	338.94 > 268.95	20	0.9939
113	Oxadiazon	15.26	258.05 > 175.04	10	304.06 > 260.05	10	0.9927
114	DDE, p,p	15.32	245.95 > 175.97	25	317.94 > 245.95	20	0.9964
115	Myclobutanil	15.40	179.07 > 125.05	15	179.07 > 152.06	15	0.9912
116	Buprofezin	15.43	172.09 > 57.03	10	249.13 > 193.10	10	0.9906
117	Kresoxim-methyl	15.44	206.09 > 116.05	15	206.09 > 131.06	15	0.9921
118	DDT, o,p'	15.47	234.94 > 164.96	15	234.97 > 164.98	20	0.9935
119	DDT, o,p', confirming 1	15.47	236.94 > 164.96	20	236.97 > 164.98	20	0.9963
120	Aramite-1	15.48	185.06 > 63.02	15	319.10 > 185.06	15	0.9959
121	Aramite-2	15.69	185.06 > 63.02	15	319.10 > 185.06	15	0.9971
122	Carpropamid	15.78	139.00 > 103.10	10	222.00 > 125.00	18	0.9982
123	Cyproconazole	15.79	222.09 > 125.05	20	224.09 > 127.05	20	0.9989
124	Nitrofen	15.85	201.99 > 138.99	21	282.98 > 252.98	15	0.9997
125	Chlorobenzilate	15.98	251.02 > 139.01	20	253.03 > 141.01	15	0.9978
126	Oxadiargyl	15.99	149.90 > 122.90	15	285.00 > 255.00	14	0.9963
127	Fenthion sulfoxide	16.05	279.01 > 153.01	15	294.02 > 279.01	8	0.9958
128	Diniconazole	16.11	268.06 > 232.05	15	270.06 > 234.05	15	0.9949
129	Ethion	16.12	230.99 > 202.99	15	383.99 > 230.99	10	0.9973
130	Oxadixyl	16.16	132.06 > 117.05	15	163.07 > 132.06	10	0.9985
131	DDT, p,p'	16.20	234.94 > 164.96	20	234.94 > 164.96	20	0.9979
132	DDD, p,p'	16.20	234.97 > 164.98	20	236.97 > 164.98	20	0.9959
133	Chlorthiophos1	16.20	324.96 > 268.97	15	324.96 > 296.97	10	0.9969
134	Imiprothrin	16.36	123.00 > 81.00	5	324.90 > 269.20	14	0.9967
135	Mepronil	16.45	269.14 > 119.06	10	269.14 > 210.11	10	0.9945
136	Triazophos	16.46	161.03 > 134.03	10	257.05 > 162.03	10	0.9936
137	Ofurace	16.58	186.05 > 158.05	10	232.07 > 186.05	10	0.9973
138	Carfentrazone-ethyl	16.59	330.03 > 310.03	20	340.03 > 312.03	10	0.9919
139	Benalaxyl	16.63	234.12 > 174.09	10	266.14 > 148.08	10	0.9951
140	Trifloxystrobin	16.65	116.04 > 89.03	15	190.06 > 130.04	10	0.9962
141	Propiconazole, peak 1	16.77	259.02 > 69.01	20	259.02 > 173.02	20	0.9989
142	Edifenphos	16.78	173.01 > 109.01	15	310.03 > 173.01	10	0.9904
143	Quinoxyfen	16.84	272.00 > 237.00	20	307.00 > 272.00	10	0.9982
144	Endosulfan sulfate	16.85	271.88 > 236.89	15	273.88 > 238.89	15	0.9929
145	Clodinafop-propargyl	16.87	349.05 > 238.04	15	349.05 > 266.04	15	0.9991
146	Flupicolide	16.90	208.80 > 182.00	20	261.00 > 175.00	24	0.9988
147	Hexazinone	17.02	171.00 > 71.00	10	171.00 > 85.00	10	0.9998
148	Propargite	17.16	135.06 > 107.05	15	350.16 > 201.09	10	0.9991
149	Diflufenican	17.21	266.05 > 246.05	10	394.07 > 266.05	10	0.9981
150	Triphenylphosphate (TPP)	17.26	325.07 > 169.04	25	326.07 > 325.07	10	0.9995
151	Iprodione	17.65	187.02 > 124.01	20	187.02 > 159.02	40	0.9979
152	Bifenthrin	17.77	181.05 > 153.05	6	181.05 > 166.05	15	0.9922
153	Picolinafen	17.90	376.08 > 238.05	15	376.08 > 239.05	15	0.9981
154	Bromopropylate	17.91	184.98 > 156.98	20	342.96 > 184.98	20	0.9967
155	Fenoxycarb	17.93	186.08 > 186.08	10	255.11 > 186.08	20	0.9933
156	Fenpropathrin	18.01	181.09 > 152.07	23	265.13 > 210.10	15	0.9956
157	Fenamidone	18.10	238.08 > 237.08	20	268.09 > 180.06	20	0.9994
158	Tebufenpyrad	18.11	276.13 > 171.08	15	333.16 > 276.13	10	0.9997
159	Fenazaquin	18.23	145.08 > 117.07	15	160.09 > 117.07	20	0.9951
160	Imazalil	18.25	173.03 > 145.02	20	215.04 > 173.03	15	0.9954
161	Furathiocarb	18.27	163.07 > 107.04	10	325.13 > 194.08	10	0.9989
162	Flurtamone	18.38	199.06 > 157.05	20	333.10 > 120.04	15	0.9945
163	Tetradifon	18.46	226.93 > 198.94	18	353.88 > 158.95	15	0.9973
164	Phosalone	18.54	181.99 > 111.00	15	181.99 > 138.00	10	0.9985

Nr.	Compound Name	RT [min]	Quantitation *m/z*	CE [V]	Confirmation *m/z*	CE [V]	r^2
165	Triticonazole	18.57	217.09 > 182.07	10	235.10 > 217.09	10	0.9945
166	Pyriproxyfen	18.68	136.06 > 78.03	15	136.06 > 96.04	15	0.9941
167	Cyhalofop butyl	18.70	256.10 > 120.05	10	256.10 > 256.10	10	0.9969
168	Tralkoxydim	18.80	137.00 > 57.20	10	181.04 > 152.03	23	0.9995
169	Cyhalothrin, lambda	18.80	197.04 > 141.03	15	234.90 > 217.20	15	0.9997
170	Lactofen	18.83	344.04 > 223.02	15	344.04 > 300.03	15	0.9975
171	Benfuracarb	19.03	164.08 > 149.07	10	190.09 > 144.07	10	0.9975
172	Pyrazophos	19.05	221.05 > 193.04	10	232.05 > 204.05	10	0.9930
173	Fenarimol	19.15	139.01 > 111.01	15	219.02 > 107.01	15	0.9993
174	Azinphos-ethyl	19.20	132.01 > 77.01	20	160.02 > 132.01	5	0.9944
175	Fenoxaprop-P	19.41	288.03 > 260.03	10	361.04 > 288.03	10	0.9998
176	Bitertanol1	19.59	170.09 > 115.06	25	170.09 > 141.07	20	0.9993
177	Permethrin, peak 1	19.68	183.04 > 165.03	15	183.04 > 168.03	15	0.9973
178	Bitertanol2	19.71	170.09 > 115.06	25	170.09 > 141.07	20	0.9993
179	Permethrin, peak 2	19.81	183.04 > 165.03	15	183.04 > 168.03	15	0.9909
180	Prochloraz	19.88	180.01 > 138.01	15	310.03 > 268.02	10	0.9932
181	Cafenstrole	20.21	100.04 > 72.03	15	188.08 > 119.05	15	0.9991
182	Cyfluthrin, peak 1	20.26	163.02 > 91.01	12	163.02 > 127.02	10	0.9915
183	Fenbuconazole	20.34	129.04 > 102.03	15	198.07 > 129.04	10	0.9996
184	Cypermethrin I	20.65	163.03 > 127.02	10	181.03 > 152.03	25	0.9996
185	Boscalid (Nicobifen)	20.84	342.03 > 140.01	15	344.03 > 142.01	15	0.9977
186	Flucythrinate, peak 1	20.85	199.07 > 107.04	22	199.07 > 157.06	10	0.9958
187	Quizalofop-Ethyl	20.92	299.07 > 255.06	20	372.09 > 299.07	15	0.9969
188	Etofenprox	21.08	163.09 > 107.06	16	163.09 > 135.07	10	0.9987
189	Flucythrinate, peak 2	21.12	199.07 > 107.04	22	199.07 > 157.06	10	0.9989
190	Fenvalerate, peak 1	21.94	167.05 > 125.04	10	419.13 > 225.07	10	0.9978
191	Fluvalinate, peak 1	22.09	250.06 > 200.05	20	252.06 > 200.05	20	0.9973
192	Pyraclostrobin	22.17	132.03 > 77.02	15	325.08 > 132.03	20	0.9936
193	Fluvalinate, peak 2	22.20	250.06 > 200.05	20	252.06 > 200.05	20	0.9977
194	Fenvalerate, peak 2	22.28	167.05 > 125.04	10	419.13 > 225.07	10	0.9996
195	Difenoconazole, peak 1	22.76	323.05 > 265.04	15	325.05 > 267.04	20	0.9995
196	Indoxacarb	22.95	203.03 > 106.01	20	203.03 > 134.02	20	0.9995
197	Deltamethrin II	23.28	252.99 > 93.00	18	252.99 > 173.99	18	0.9987
198	Azoxystrobin	23.63	344.10 > 329.10	20	388.11 > 345.10	15	0.9991
199	Dimethomorph-1	23.91	301.10 > 165.05	10	387.12 > 301.10	12	0.9992
200	Dimethomorph-2	24.60	301.10 > 165.05	10	387.12 > 301.10	12	0.9990

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Multi-Residue Pesticide Analysis in Herbal Products Using Accelerated Solvent Extraction with a Triple Quadrupole GC-MS/MS System

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Application Note 52291

Key Words
Pesticides, Tea, Herbal products, ASE, SRM, MRM, Multi-residue analysis, TSQ 8000 GC-MS/MS

Introduction

The residue analysis of pesticides has developed in recent years into a comprehensive methodology for the detection of many hundreds of potential contaminating compounds. A multi-residue method for herbal products and teas is faced with additional challenges from the worldwide origin of the products and the complex matrix of the dried materials. In the due quality control of raw materials, the unknown or undeclared local plant protection treatments must be taken into account with a wide variety of potential pesticide contaminations.

Dried leaves, fruits or seeds and other herbal products of medical use deliver highly complex extracts from the sample preparation due to the rich content of active ingredients, essential oils and the typical high boiling natural polymer compounds from broken cells, leaves or fruit skins. A thorough clean up of the extracted sample can lead to losses of critical analytes of interest. A complete characterization of pesticide, and other residue, contamination is done by both LC and GC-MS/MS to cover the complete range of functional groups.

This application report describes the methodology used for the multi-residue pesticide analysis of herbal products using accelerated solvent extraction (ASE) and gel permeation chromatography (GPC) sample preparation with detection and quantitation by the Thermo Scientific TSQ 8000 GC-MS/MS system.

A routine screening method for more than 200 pesticide compounds was applied to a wide variety of different sample types, ranging from regular black tea or sage leaves, to seeds like fennel and herbs of medical and fragrance use like thyme and chamomile. The data processing and reporting was achieved by using the Thermo Scientific TraceFinder quantitation software suite.

The sensitivity requirement for this analysis was determined by the regulatory background. The analysis of pesticide residues in tea and herbal products follows the regulations of the European Directorate General for Health and Consumer Affairs (SANCO) for "Method Validation and Quality Control Procedures for Pesticide Residue Analysis in Food and Feed" [1]. The sensitivity requirements for these products as referenced in the Codex Alimentarius [2] result in maximum residue levels of 0.01 mg/kg for most of the pesticide compounds.

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Sample Preparation

Herbal and tea samples were extracted with an accelerated solvent extraction method using the Thermo Scientific Dionex ASE 350 Accelerated Solvent Extractor. The ASE method used is described in an official pesticide standard method [3]. The collected extracts were concentrated using a rotary evaporator (Rotavap) and further cleaned up via gel permeation chromatography (GPC). The GPC step used a polystyrene gel (Bio-Beads® S-X3) with an ethylacetate/cyclohexane mobile phase. After additional concentration by the Rotavap, the extracts were ready for GC injection using ethylacetate as the main solvent.

Method Setup

The analytical method comprised sample handling and injection using the Thermo Scientific TriPlus RSH liquid autosampler, TRACE GC 1310 gas chromatograph equipped with an instant connect, temperature programmable PTV injection system, and the TSQ™ 8000 triple quadrupole GC-MS/MS detection system. The MRM detection method was taken from a routinely employed Thermo Scientific TSQ Quantum XLS GC-MS/MS method without any further optimization on the TSQ 8000 GC-MS/MS system [4]. The TSQ 8000 system automatically optimized acquisition windows and optimized instrument duty cycle using timed-SRM (t-SRM) for maximum sensitivity. This enabled the avoidance of lengthy manual set-ups usually required when adopting new instrumentation (Figure 1).

ASE™ 350 Accelerated Solvent Extraction

Sample weight	10 g
Extraction solvent	Ethylacetate/cyclo-Hexane 1:1, same as GPC solvent
Temperature	120 °C
Pressure	100 bar
Extraction time	5 min, 1 cycle
Flushing with solvent	60% of cell volume
Flushing with nitrogen	100 s

TriPlus™ RSH Autosampler

Syringe	10 µL
Injection volume	1 µL
Injection type	Fast liquid band injection, 100 ms injection time
Washing cycles	3 x 10 µL, solvent ethylacetate

TRACE™ 1310 Gas Chromatograph

Injector PTV	Splitless mode
Base temperature	50 °C
Transfer	10 °C/s to 250 °C, until end of run
Flow	Constant flow, 1.2 mL/min, helium
Analytical column	40 m, ID 0.18 mm, 0.18 µm film, 5%-phenyl phase (5MS type)
Pre-column	5 m, ID 0.18 mm, empty deactivated, no backflush
Column oven	Temperature programmed
Start	70 °C, for 1.50 min
Ramp 1	15 °C/min to 190 °C
Ramp 2	7 °C/min to 290 °C, 12 min
Transfer line	250 °C

TSQ 8000 Mass Spectrometer

Ion source temperature	220 °C
MRM Detection	Timed SRM mode (see Appendix)

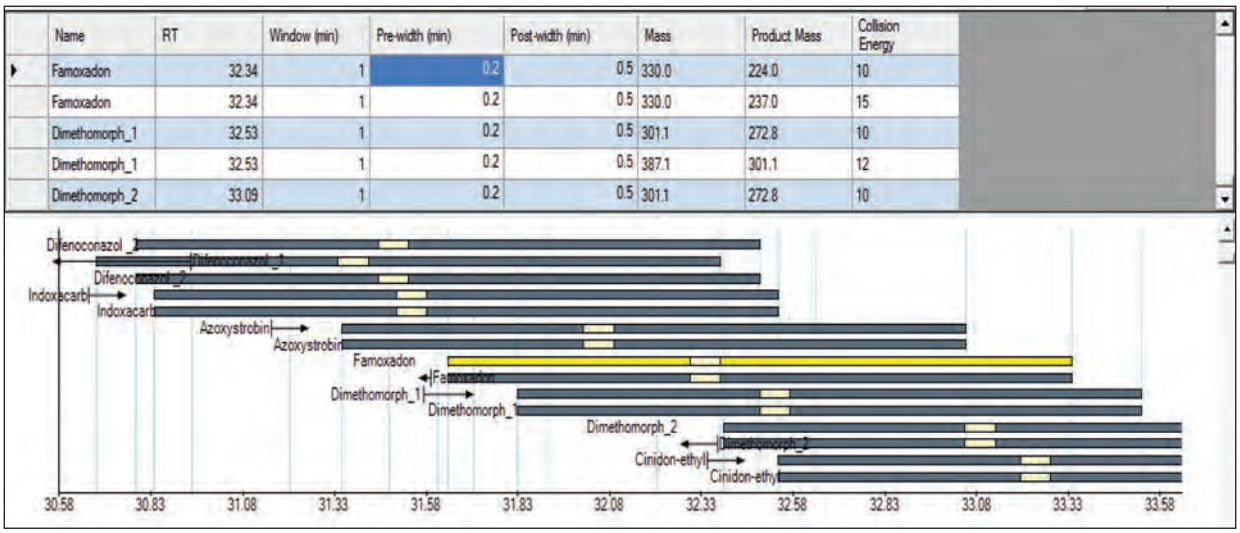

Figure 1. Screenshot of a section of the analytical run showing the "acquisition map" automatically created by the TSQ 8000 system using t-SRM. This mode ensures the instrument only monitors for compounds when they elute to optimize sensitivity.

Calibration and Linearity

The quantitative calibration and linearity check for the method was performed by using six calibration points in the range of 0.004 µg/mL to 1.0 µg/mL. This range represents an analyte concentration of 0.01 to 2.5 mg/kg in the samples (10 – 2500 ppb).

For setting up the calibration solutions, a stock solution containing target pesticide compounds in herbal products was used. The calibration solution was prepared in a standard matrix with a matrix load equivalent to the typical herbal extracts used. The standard matrix blank consisted of lemon peel extracted using the standard procedure. The pesticide blank level was tested before applying as a blank standard matrix. Standard solutions were prepared containing lemon peel extract dissolved 1:1 with ethyl acetate. The correlation coefficients, R^2, achieved during method calibration exceeded 0.99 for all compounds (Figure 2).

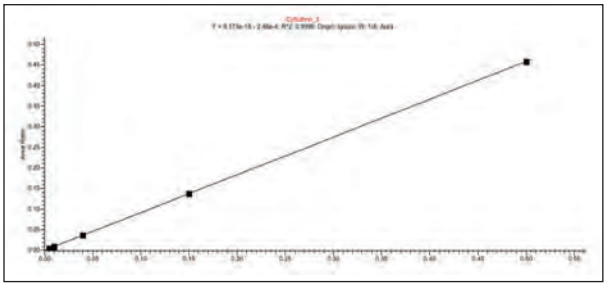

Figure 2. Calibration curve for Cyfluthrin, $R^2 = 0.9996$

Results and Discussion

Sensitivity (LOD)

Using the standard pool of pesticides, the method detection limits in the standard lemon peel were estimated. Using the 4 ppb (pg/µL) matrix standard level, S/N values were used to estimate the limits of detection (LOD). The S/N values in matrix are given in Table 1 for a selection of critical compounds taken at retention times that are affected most from the eluting matrix. Although the compounds are eluting in heavily impacted matrix regions of the chromatogram, the high selectivity of the TSQ 8000 GC-MS/MS for the target pesticides at low level against an intense matrix load is demonstrated in Figure 3 and Figure 4.

Table 1. Detection limit S/N for selected pesticide compounds in matrix

Pesticide	RT [min]	S/N @ 4 ppb
Terbacil	13:83	24
Alachlor	14:78	12
Tolylfluanid	16:75	44
Pyridaben	24:17	83

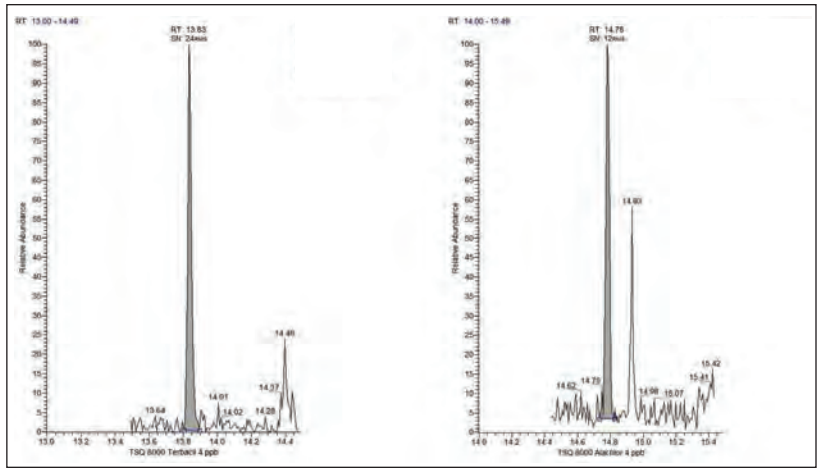

Figure 3. SRM peaks at 4 ppb from Terbacil (left, 161.1 › 88.0, CE 15 V) and Alachlor (right, 188.1 › 130.1, CE 25 V). SRM transitions were taken from the Pesticide Method Reference, 2nd ed. 2011. [4]

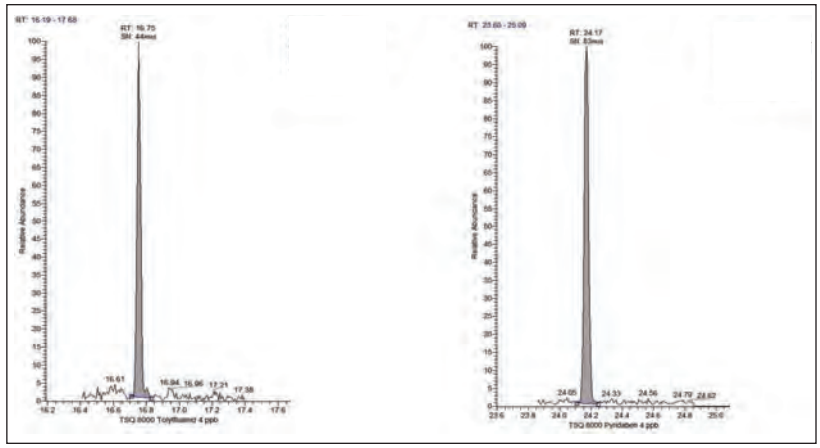

Figure 4. SRM peaks at 4 ppb from Tolylfluanid (left, 238.1 › 137.1, CE 15 V) and Pyridaben (right, 309.1 › 147.1, CE 15 V). SRM transitions were taken from the Pesticide Method Reference, 2nd ed. 2011. [4]

Robustness and Maintenance

Routine preventative maintenance on the GC was performed using routine standard operating procedures. The calibration chromatograms seen in Figures 3 and 4 have been acquired after a persistent matrix load to the system through routine analysis of more than 500 matrix samples.

This level of robustness meant that even with persistent and very high matrix load, it was not necessary to clean the removable ion source short term.

The innovative instant connect modularity of the injectors and detectors of the TRACE 1310 GC, used here as the front-end to the mass spectrometer, allows the user quick accessibility to any injector part for rapid cleaning. Furthermore the unique ability to replace the entire injector module within minutes represents an excellent way of postponing routine maintenance to when the laboratory schedule allows while keeping the GC-MS/MS system operational.

Analytical Precision

Within a routine series of 50 commercial samples, the quality control samples were measured with replicate injections. The results for a range of compounds is given in Table 2. The relative effects on known problematic pesticide compounds can be seen, while coefficients of variation (CV%) for unaffected compounds all stay well below 10% even within this long series of matrix injections.

Table 2. Coefficients of variation for lemon peel matrix spiked QC samples for a set of 60 pesticides under investigation (avg. 7.4%, 24 injections)

Diflubenzofuron	10.0%	Penconazol	7.5%	Diniconazol	2.9%
Biphenyl-d10	7.5%	Allethrin	8.4%	Aclonifen	9.0%
Biphenly	9.5%	Pyrifenox	5.5%	Trifloxystrobin	6.0%
o-Phenylphenol	8.2%	Procymidon	5.7%	Propiconazol	3.1%
Fenobucarb	6.0%	Triadimenol	11.5%	Propargit	6.0%
Diphenylamin	5.7%	Picoxystrobin	7.0%	Tebuconazol	4.3%
Terbutylazin	4.4%	Flutriafol	6.3%	Nitralin	9.2%
Propyzamid	3.1%	Hexaconazol	9.2%	Piperonyl butoxid	8.3%
Terbazil	5.8%	Isoprothiolan	9.7%	Brompropylat	5.8%
Fipronil-desulfinyl	6.9%	Uniconazol	7.0%	Fenoxycarb	9.1%
Alachlor	6.7%	Kresoxim-methyl	9.9%	Etoxazol	8.8%
Prometryn	8.3%	Myclobutanil	9.2%	Fenazaquin	3.3%
Ethofumesat	7.4%	Flusilazol	4.4%	Metconazol	5.3%
Bromacil	8.3%	Cinerin 1	8.1%	Pyriproxyfen	8.5%
Chlorpyrifos	6.9%	Buprofezin	7.4%	Fenamirol	8.5%
Tetraconazol	6.2%	Diclobutrazol	2.6%	Fluquinconazol	4.9%
Triadimefon	11.7%	Cyproconazol	2.6%	Pyridaben	5.2%
Dicapton	10.7%	Chlorbenzilat	3.3%	Etofenprox	10.2%
Butralin	6.6%	Etoconazol	4.4%	Silafluofen	10.2%
Fipronil	5.5%	Iprodion	11.1%	Indoxacarb	8.5%

Results from Real Life Samples

The above method was used for the analysis of a wide variety of herbs, teas and dried fruit known as one of the most challenging analytical task for controlling the pesticide maximum residue levels due to the heavy matrix impact. Table 3 gives a representative overview of positive results from different samples with the indication of the pesticide compound and concentration found. All compounds were detected by using at least two SRM traces and were subsequently confirmed by checking the calibrated ion ratios. The concentration ranges covered were from close to the MRL level of 10 mg/kg to high levels of up to 50 times above the regulated maximum. Figure 5 provides an example of confirmed residue detection in a thyme sample.

Table 3. Positive results above MRL level found in samples of various matrices

Sample Matrix	Pesticide Residues Found	Concentration (mg/kg)
Dried Herbs	o-Phenylphenol	0.017
Dried Herbs	Tebuconazol	0.023
Dried Fruit	Diflubenzuron	0.049
Dried Fruit	Myclobutanil	0.023
Dried Fruit	Propargit	0.479
Dried Fruit	Tebuconazol	0.081
Dried Fruit	Difenconazol	0.013
Dried Herbs	Picoxystrobin	0.228
Dried Herbs	Picoxystrobin	0.233
Dried Herbs	o-Phenylphenol	0.011
Herbal Tea	o-Phenylphenol	0.014
Herbal Tea	o-Phenylphenol	0.011
Herbal Tea	Terbutylazin	0.016

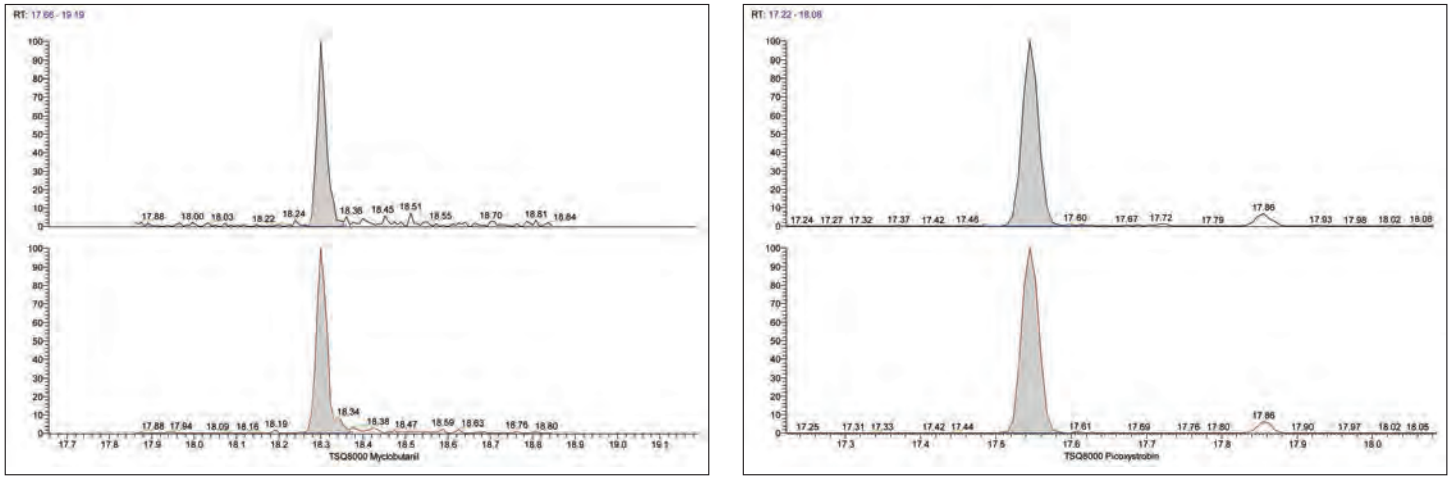

Figure 5. Positive results for Myclobutanil in green apple (0.023 mg/kg, left) and Picoxystrobin in thyme (0.228 mg/kg, right), both detected on two SRM traces

Data Analysis and Reporting

The data processing was performed using TraceFinder™ quantitation software. TraceFinder software contains a compound data store containing a large number of pesticide compound entries from which required compounds for the method had been selected. For each pesticide, the necessary parameters for MRM acquisition and compound identification, such as SRM transition, retention time, and ion ratios, as well as quantitation details like quantitation mass and recovery requirement, are stored.

The analytical sequence setup, data acquisition and result processing was done from one software platform integrating the complete analytical process. In Figure 6, the analytical sequence is shown in the upper part of the screen, with the compounds included in the method to the right. The actual chromatograms for the selected pesticide compounds are displayed in the bottom part for review by the operator.

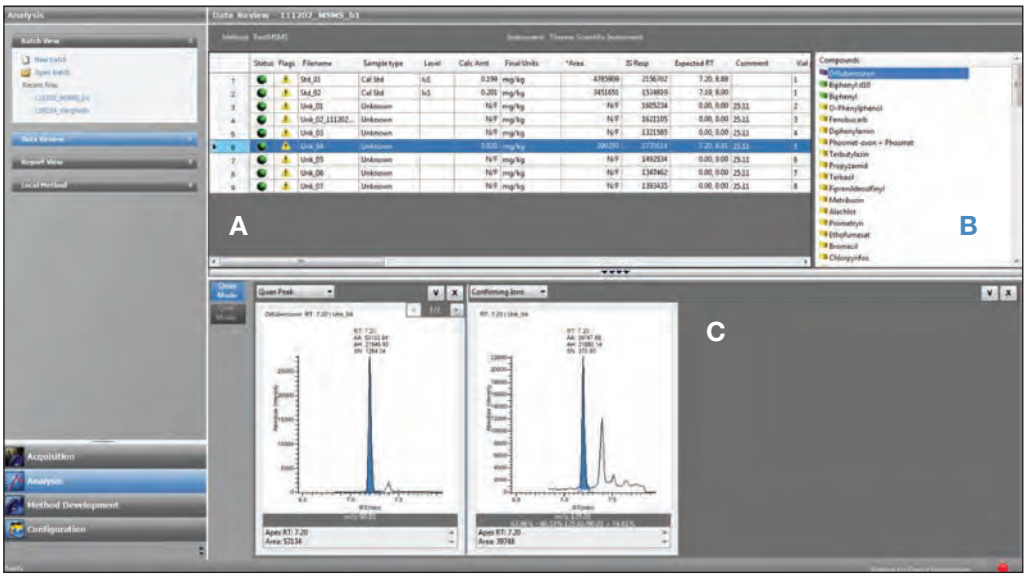

Figure 6. TraceFinder software analysis view:

A. Acquisition sequence table for calibration, QC and sample runs

B. Compound list with status flags

C. Compound chromatogram windows with integrated quantitation and confirmation peaks

Expanded Productivity

The total cycle time of the analytical runs was 30 minutes, which allowed the throughput of two samples per hour and resulted in a load of up to 48 samples, including QC checks during the day for the control of more than 200 pesticide compounds in each run.

This expanded productivity was a combined result of the TSQ 8000 triple quadrupole GC-MS/MS system with its enhanced analyte selectivity in matrix samples, the high method and system robustness, and the advanced data processing using TraceFinder software. Pesticide peaks were typically baseline-separated with a high signal-to-noise ratio allowing for an accurate automated area integration with significantly reduced manual control required. A number of quality control parameters within TraceFinder software immediately provided visible flagging for compounds that may need manual attention. Automatic ion ratio checks provided a fast and solid confirmation in the case of positive findings. The high processing speed of TraceFinder software provided for multi-residue analysis and quick and comprehensive reporting for each sample.

Conclusion

The TSQ 8000 GC-MS/MS delivered high sensitivity and matrix selectivity for routine pesticide analysis even in difficult matrix samples. The data acquisition using the unique timed-SRM allowed for the detection of a virtually unlimited number of pesticide compounds in one run without sacrificing the high sensitivity for individual compounds. Quantitative calibrations were performed in a standard matrix and showed excellent linearity and precision over the relevant concentration range to control the regulated MRL levels.

The high matrix selectivity of the TSQ 8000 system allowed for reduced sample preparation, providing high recoveries for a wide range of chemically diverse pesticide compounds. The very high matrix selectivity delivered low chemical matrix background with well-defined pesticide peaks that were safe and easy to integrate, thus eliminating the need for time-consuming manual baseline corrections.

Positive pesticide compound signals were confirmed by TraceFinder software checking the calibrated ion ration of the two monitored SRM transitions.

The TSQ 8000 GC-MS/MS system is well prepared for routine analysis and provides high robustness of the chromatographic system and ion source, thus reducing the need for frequent maintenance and avoiding system downtime for high sample throughput and productivity. The system is easy to use, durable, and robust even with the most challenging sample types and is fully automated in sampling capabilities to found and not-found report generation.

References

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2. Codex Alimentarius (www.codexalimentarius.net/mrls/pesticides/jsp/pest-q-e.jsp)
3. Pesticide determination according to § 64 LFGB L 00.00-34 (German legislation) Modul E9 (ASE); GPC
4. Pesticide Method Reference, 2nd Edition, 2011 Thermo Fisher Scientific, p/n 120390.

Appendix: List of pesticides with MRM transitions used (from [4])

Pesticide Name	RT (min)	Precursor Mass (*m/z*)	Product Mass (*m/z*)	Collision Energy (V)
Difluorobenzamid Degradation (Isocyanat)	6.93	152.93	90.01	20
Difluorobenzamid Degradation (Isocyanat)	6.93	152.93	125.01	20
Carbofuran 1	8.80	149.06	121.05	10
Carbofuran 1	8.80	164.08	149.07	10
Difluorobenzamid Degradation	8.62	141.00	63.11	25
Difluorobenzamid Degradation	8.62	141.00	113.09	15
Biphenyl-d10_ISTD	9.24	160.00	160.16	10
Biphenyl	9.28	154.08	153.08	15
Biphenyl	9.28	153.08	152.08	15
Carbofuran-3-hydroxy 1	10.43	137.05	81.01	18
Carbofuran-3-hydroxy 1	10.43	180.05	137.01	15
Tetrahydrophthalimid	10.84	151.04	79.01	25
Tetrahydrophthalimid	10.84	151.04	122.09	10
O-Phenylphenol	11.00	170.07	141.06	20
O-Phenylphenol	11.00	170.07	115.05	20
Molinate	11.10	187.10	126.07	10
Molinate	11.10	126.07	98.05	5
Chlorfenprop methyl	11.59	196.00	165.00	10
Chlorfenprop methyl	11.59	165.00	137.00	10
Fenobucarb	11.20	121.07	77.05	15
Fenobucarb	11.20	150.09	121.07	10
Propachlor	11.76	176.06	120.04	10
Propachlor	11.76	120.04	92.03	10
Propachlor	11.76	169.06	120.04	10
Propachlor	11.76	196.07	120.04	10
Cycloate	11.98	154.10	83.05	10
Cycloate	11.98	215.13	154.10	5
Diphenylamin	11.49	169.01	168.09	20
Diphenylamin	11.49	169.01	167.09	20
Chloropropham	12.26	213.06	127.03	15
Chloropropham	12.26	213.06	171.04	10
Phosmet-oxon	12.09	160.00	132.96	15
Phosmet-oxon	12.09	104.00	75.88	10
Phosmet-oxon	12.09	160.00	76.96	20
Prometon	13.10	225.16	183.13	10
Prometon	13.10	225.16	210.15	10
Carbofuran 2	13.13	149.06	121.05	10
Carbofuran 2	13.13	164.08	149.07	10
Profluralin	13.22	318.10	199.06	15
Profluralin	13.22	330.23	252.45	25
Swep	13.46	187.05	123.95	18
Swep	13.46	219.11	174.02	15
Trietazine	13.48	229.14	200.14	15
Trietazine	13.48	214.14	186.10	15
Dimethipin	13.53	117.98	57.97	10
Dimethipin	13.53	210.10	76.02	10
Terbutylazin	12.97	214.10	132.06	10
Terbutylazin	12.97	214.10	104.05	10
Propyzamid	13.04	173.01	145.01	15
Propyzamid	13.04	173.01	109.01	18
Propyzamid	13.04	175.02	147.01	15
Propyzamid	13.04	254.02	226.02	15
Isocarbamide	13.67	142.03	70.01	15
Isocarbamide	13.67	142.03	113.01	10
Dinoseb	13.92	211.13	116.99	15
Dinoseb	13.92	211.13	163.11	10
Terbazil	13.42	161.05	88.03	15
Terbazil	13.42	160.05	76.02	15
Bromocylen	14.37	358.79	242.85	15
Bromocylen	14.37	356.93	241.24	15
Dimethenamid	14.60	230.06	154.04	10
Dimethenamid	14.60	232.06	154.04	10
Dimethachlor	14.61	197.08	148.06	10
Dimethachlor	14.61	199.08	148.06	10
Acetochlor	14.65	174.11	146.15	15
Acetochlor	14.65	223.19	147.17	10
Desmetryn	14.68	213.11	171.08	10
Desmetryn	14.68	213.11	198.10	10
Flurprimidol	14.77	269.12	106.98	20
Flurprimidol	14.77	270.18	107.04	20
Alachlor	14.26	188.10	160.07	10
Alachlor	14.26	188.10	130.12	25
Alachlor	14.26	237.14	160.15	10
Metribuzin	14.14	198.08	82.03	20
Metribuzin	14.14	198.08	89.04	16
Propanil	15.00	217.01	161.00	10
Propanil	15.00	219.01	163.00	10
Fipronildesulfinyl	14.15	333.00	231.20	20
Fipronildesulfinyl	14.15	333.00	281.30	20
Carbofuran-3-hydroxy 2	15.02	137.05	81.01	18
Carbofuran-3-hydroxy 2	15.02	180.05	137.01	15
Prometryn	14.49	241.14	184.10	15
Prometryn	14.49	226.13	184.10	12
Tridiphan	15.18	186.94	158.94	15
Tridiphan	15.18	219.09	184.09	20
Ethofumesat	14.80	206.82	160.86	10
Ethofumesat	14.80	285.75	206.82	12
Pentanochlor	15.73	141.05	106.05	15
Pentanochlor	15.73	239.05	141.05	15
Chlorpyrifos	15.78	257.97	165.98	20
Chlorpyrifos	15.78	314.05	258.18	15
Bromacil	15.03	205.01	188.01	15
Bromacil	15.03	207.01	190.01	15

Pesticide Name	RT (min)	Precursor Mass (*m/z*)	Product Mass (*m/z*)	Collision Energy (V)
Anthrachinon	15.44	207.97	151.99	20
Anthrachinon	15.44	180.04	152.05	15
Anthrachinon	15.44	207.97	180.10	10
Nithrothal isopropyl	16.09	236.08	194.07	10
Nithrothal isopropyl	16.09	236.08	148.05	20
Triadimefon	15.41	208.07	181.06	10
Triadimefon	15.41	210.07	183.06	10
Tiocarbazil	16.15	156.08	100.05	8
Tiocarbazil	16.15	279.10	156.07	6
Tetraconazol	15.39	336.02	218.01	20
Tetraconazol	15.39	338.02	220.01	20
Butralin	15.54	266.14	220.11	15
Butralin	15.54	266.14	190.10	15
Dicapthon	15.44	262.00	262.00	9
Dicapthon	15.44	262.00	216.00	13
Crufomat	16.30	256.20	226.15	25
Crufomat	16.30	276.20	182.09	10
Allethrin	16.17	123.07	80.98	10
Allethrin	16.17	136.04	92.98	10
Dinobuton	16.89	163.06	116.04	15
Dinobuton	16.89	211.07	117.04	18
Penconazol	16.89	248.06	157.04	25
Penconazol	16.89	248.06	192.04	15
Pyrifenox 1	16.17	262.03	192.02	20
Pyrifenox 1	16.17	262.03	200.02	20
Pyrifenox 2	16.81	262.03	192.02	20
Pyrifenox 2	16.81	262.03	200.02	20
Tolylfluanid	16.92	238.09	137.05	15
Tolylfluanid	16.92	240.09	137.05	15
Fipronil	17.01	368.95	214.97	30
Fipronil	17.01	366.95	254.96	25
Triflumizol	17.20	206.05	179.04	15
Triflumizol	17.20	179.04	144.04	15
Procymidon	17.22	283.05	95.93	10
Procymidon	17.22	285.05	95.97	10
Procymidon	17.22	285.05	257.30	10
Triadimenol 1	16.45	168.11	69.99	15
Triadimenol 1	16.45	128.05	100.04	10
Triadimenol 2	16.64	168.11	69.99	15
Triadimenol 2	16.64	128.05	100.04	10
Butachlor	17.54	237.13	160.09	10
Butachlor	17.54	176.09	146.08	10
Chlorbenside	17.57	124.97	88.98	20
Chlorbenside	17.57	124.97	63.02	30
Fenothiocarb	17.68	160.07	72.01	15
Fenothiocarb	17.68	160.07	106.00	10
Picoxystrobin	17.69	335.09	303.09	10
Picoxystrobin	17.69	303.09	157.04	20
Paclobutrazole	17.75	236.10	125.06	15

Pesticide Name	RT (min)	Precursor Mass (*m/z*)	Product Mass (*m/z*)	Collision Energy (V)
Paclobutrazole	17.75	238.11	127.06	15
Chinomethionat	17.78	206.06	147.98	15
Chinomethionat	17.78	234.08	206.06	10
Napropamid	18.07	271.16	128.07	5
Napropamid	18.07	128.07	72.04	10
Flutriafol	18.11	219.07	123.04	15
Flutriafol	18.11	123.04	75.03	15
Flurodifen	18.14	190.02	126.01	10
Flurodifen	18.14	190.02	146.01	5
Bisphenol A	18.17	213.14	119.06	15
Bisphenol A	18.17	213.14	164.99	20
Bisphenol A	18.17	228.15	213.07	10
Chlorfenson_ISTD	18.20	302.00	110.90	20
Hexaconazol	18.22	214.08	159.07	20
Hexaconazol	18.22	214.08	151.98	25
Imazalil	18.24	172.96	144.96	15
Imazalil	18.24	172.96	108.95	25
Isoprothiolan	18.24	203.99	117.95	7
Isoprothiolan	18.24	203.99	84.90	25
Isoprothiolan	18.24	290.06	118.03	15
Flamprop-methyl	18.39	230.05	170.04	10
Flamprop-methyl	18.39	276.06	105.02	10
Kresoximmethyl	18.48	206.10	131.09	15
Kresoximmethyl	18.48	206.10	116.01	10
Buprofezin	18.51	175.08	116.96	20
Buprofezin	18.51	175.08	131.99	15
Buprofezin	18.51	249.16	105.93	20
Buprofezin	18.51	249.16	193.20	10
Uniconazol	18.57	234.12	136.99	15
Uniconazol	18.57	234.12	101.95	25
Uniconazol	18.57	234.12	165.08	10
Cinerin 1	18.60	123.08	95.06	10
Cinerin 1	18.60	123.08	81.05	10
Cinerin 1	18.60	150.10	108.09	10
Flusilazol	18.60	233.16	165.13	25
Flusilazol	18.60	233.16	152.06	20
Myclobutanil	18.65	179.00	125.00	15
Myclobutanil	18.65	179.00	89.95	25
Methoprotryne	18.66	256.14	212.11	15
Methoprotryne	18.66	256.14	200.11	15
Diclobutrazol	18.75	270.07	159.04	15
Diclobutrazol	18.75	272.08	161.04	15
Azaconazole	18.78	217.02	173.01	15
Azaconazole	18.78	219.02	175.01	15
Perthane	18.95	223.15	179.10	18
Perthane	18.95	223.15	167.06	18
Cyproconazol	19.14	222.09	125.05	20
Cyproconazol	19.14	224.09	127.05	20
Flamprop-isopropyl	19.14	276.08	105.03	15

Pesticide Name	RT (min)	Precursor Mass (*m/z*)	Product Mass (*m/z*)	Collision Energy (V)
Flamprop-isopropyl	19.14	278.17	104.99	20
Chloropropylat	19.16	251.02	139.01	20
Chloropropylat	19.16	251.02	111.01	20
Ancymidol	19.18	228.15	121.02	15
Ancymidol	19.18	215.15	107.02	15
Chlorbenzilat	19.22	251.02	139.01	20
Chlorbenzilat	19.22	251.02	111.01	20
Cyprofuram	19.36	211.12	132.02	10
Cyprofuram	19.36	211.12	166.05	10
Etaconazol 1	19.38	245.04	173.03	15
Etaconazol 1	19.38	245.04	191.03	10
Etaconazol 2	19.38	245.04	173.03	15
Etaconazol 2	19.38	245.04	191.03	10
Diniconazol	19.47	268.06	232.05	15
Diniconazol	19.47	270.06	234.05	15
Jasmolin 1	19.58	123.08	81.05	10
Jasmolin 1	19.58	123.08	95.06	10
Jasmolin 1	19.58	164.16	109.15	10
Aclonifen	19.70	212.02	182.02	10
Aclonifen	19.70	264.03	194.02	15
Tetrasul	19.85	251.92	216.93	20
Tetrasul	19.85	253.92	218.93	20
Carfentrazone ethyl	19.95	340.03	312.03	10
Carfentrazone ethyl	19.95	312.15	150.99	20
Benodanil	19.99	322.98	230.99	15
Benodanil	19.99	322.98	195.99	5
Trifloxystrobin	20.02	222.13	162.14	10
Trifloxystrobin	20.02	115.99	88.95	15
Trifloxystrobin	20.02	222.13	130.02	15
Chlordecone	20.06	271.91	237.16	15
Chlordecone	20.06	273.91	239.15	20
Famophos (Famphur)	20.16	218.07	108.94	15
Famophos (Famphur)	20.16	218.07	126.95	20
Iprodion Degradation	18.63	186.87	123.99	20
Iprodion Degradation	18.63	186.87	159.02	15
Iprodion Degradation	18.63	243.94	187.02	10
Iprodion	20.57	314.06	245.25	15
Iprodion	20.57	186.99	123.87	20
Iprodion	20.57	316.00	247.35	15
Iprodion	20.57	316.00	273.11	10
Propiconazol 1	19.38	259.02	173.02	20
Propiconazol 1	19.38	172.94	144.91	15
Propiconazol 2	19.54	259.02	173.02	20
Propiconazol 2	19.54	172.94	144.91	15
Pyraflufen-ethyl	20.30	412.02	349.02	15
Pyraflufen-ethyl	20.30	349.02	307.02	15
Clodinafop-propargyl	20.36	349.05	266.04	15
Clodinafop-propargyl	20.36	349.05	238.04	15
Lenacil	20.70	153.05	136.06	15

Pesticide Name	RT (min)	Precursor Mass (*m/z*)	Product Mass (*m/z*)	Collision Energy (V)
Lenacil	20.70	153.05	135.15	15
Diclofop methyl	20.77	253.02	162.01	15
Diclofop methyl	20.77	340.04	253.02	15
Propargit	20.79	173.08	135.04	15
Propargit	20.79	173.08	106.93	20
Propargit	20.79	350.21	173.10	15
Diflufenican	20.83	394.07	266.05	10
Diflufenican	20.83	266.05	246.05	10
Piperonylbutoxid	20.87	176.11	131.08	15
Piperonylbutoxid	20.87	176.11	103.06	10
Piperonylbutoxid	20.87	176.11	145.09	15
Tebuconazol	20.97	250.12	125.06	20
Tebuconazol	20.97	252.12	127.06	20
Nitralin	21.09	316.02	274.15	10
Nitralin	21.09	273.99	216.07	10
Benzoylpropethyl	21.22	292.05	105.02	15
Benzoylpropethyl	21.22	172.03	145.02	14
Captafol	21.22	311.06	78.94	20
Captafol	21.22	311.06	276.21	10
Epoxyconazol	21.29	192.04	138.03	10
Epoxyconazol	21.29	192.04	111.02	10
Bromuconazol 1	21.73	294.96	174.98	15
Bromuconazol 1	21.73	292.96	172.98	15
Brompropylat	21.76	340.93	183.05	20
Brompropylat	21.76	340.93	185.04	20
Etoxazol	21.83	300.14	270.38	20
Etoxazol	21.83	330.17	300.44	25
Fenoxycarb	21.85	186.08	109.05	15
Fenoxycarb	21.85	255.11	186.08	10
Phosmet	20.79	160.00	133.00	15
Phosmet	20.78	160.00	104.00	20
Phosmet	20.78	316.99	160.00	5
Fenpiclonil	21.94	235.99	200.99	15
Fenpiclonil	21.94	237.99	200.99	15
Fenazaquin	22.22	160.09	145.08	10
Fenazaquin	22.22	145.05	116.99	15
Fenazaquin	22.22	160.09	117.08	20
Phenothrin 1	22.27	183.10	153.08	18
Phenothrin 1	22.27	183.10	165.09	10
Phenothrin 2	22.42	183.10	153.08	18
Phenothrin 2	22.42	183.10	165.09	10
Bromuconazol 2	22.35	294.97	174.97	15
Bromuconazol 2	22.35	292.97	172.97	15
Metconazol	22.41	125.00	88.93	20
Metconazol	22.41	250.20	124.88	25
Triticonazole	22.80	235.10	217.09	10
Triticonazole	22.80	235.10	182.07	10
Pyriproxyfen	22.82	226.15	186.22	15
Pyriproxyfen	22.82	136.00	95.95	15

Pesticide Name	RT (min)	Precursor Mass (*m/z*)	Product Mass (*m/z*)	Collision Energy (V)
Azinphosmethyl	22.95	160.00	132.00	10
Azinphosmethyl	22.95	160.00	104.64	10
Pyriproxyfen	23.06	136.00	77.92	20
Fenamirol	23.55	251.02	139.01	15
Fenamirol	23.55	330.03	139.01	10
Pyridaben	24.50	364.14	309.12	5
Pyridaben	24.50	309.12	147.06	15
Fluquinconazol	24.59	340.01	298.01	22
Fluquinconazol	24.59	342.01	300.01	22
Etofenprox	26.05	163.09	107.06	16
Etofenprox	26.05	163.09	135.07	10
Etofenprox	26.05	376.14	135.02	30
Etofenprox	26.05	376.14	163.09	10
Silafluofen	26.25	179.00	151.00	7
Silafluofen	26.25	286.13	258.12	15
Difenconazol 1	26.91	323.05	265.04	15
Difenconazol 1	26.91	325.05	267.04	20
Difenconazol 2	27.05	323.05	265.04	15
Difenconazol 2	27.05	325.05	267.04	20
Indoxacarb	28.55	264.02	176.14	10
Indoxacarb	28.55	264.02	148.03	20
Indoxacarb	28.55	321.05	289.34	10

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Application Note 10333

Analysis of Dithiocarbamate Pesticides by GC-MS

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Keywords: Food safety, Dithiocarbamate fungicides, DTCs, Hydrolysis, Thiram, GC-MS, SIM

Introduction

The class of dithiocarbamate fungicides (DTCs) is widely used in agriculture. They are non-systemic and both the formulation and their break-down products typically remain at the site of application. DTCs are characterized by a broad spectrum of activity against various plant pathogens, low acute mammal toxicity, and low production costs [1]. The dithiocarbamate moiety is highly reactive: it readily chelates most heavy metals, reacts with sulfhydryl groups of proteins, rendering itself neurotoxic, teratogenic, and cytotoxic.

DTCs are not stable and cannot be extracted or analyzed directly. Contact with acidic plant juices degrades DTCs rapidly and they decompose into carbon disulfide (CS_2) and the respective amine [1]. It is not possible to homogenize plant samples and extract DTCs by organic solvents, as it is, for instance, with the QuEChERS standard procedure in pesticide-residue analyses. Maximum residue levels (MRLs) of DTCs are generally expressed as mg CS_2/kg food.

Dithiocarbamates can be quantitatively converted to carbon disulphide by reaction with tin(II)chloride in

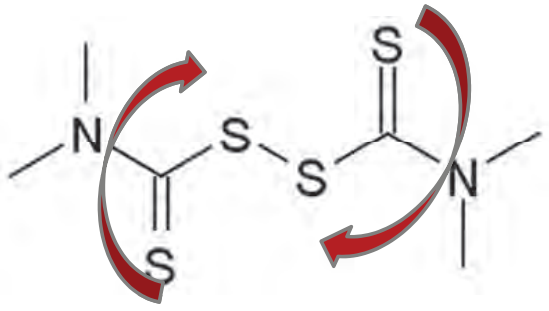

Figure 1. Thiram - 1 mole of Thiram generates 2 mole of CS_2.

aqueous HCl (1:1) in a closed bottle at 80 °C. The CS_2 gas produced is absorbed into iso-octane and measured by GC-MS. The analysis of DTCs for this application follows the acid-hydrolysis method using $SnCl_2$/HCl [2]. For method validation of the DTC pesticides, Thiram (99.5% purity) was used as representative bis (dithiocarbamate) compound considering its simple structure (1 mole of Thiram = 2 mole of CS_2 =>1 mg of Thiram theoretically generates 0.6333 mg CS_2, 1 mL of 100 ppm Thiram in 25 g of grapes = 2.5 ppm of CS_2); see Figure 1. The total DTC residues were estimated by analysing CS_2 as the DTC hydrolysis products by GC-MS. This is a non-specific DTC sum method that does not distinguish between the different species of DTCs in the sample. Interferences are known from natural precursors e.g. from crops or brassica, that can produce CS_2 as well during the hydrolysis [1, 2].

Sample Preparation

A previously reported $SnCl_2$/HCl acid-hydrolysis method was employed for sample preparation [3]. The described method follows the established methods applied in the EU reference laboratories and European commercial testing laboratories for CS_2 analysis. From the homogenized sample, 25 g are taken in a 250 mL glass bottle, 75 mL of the reaction mixture is added, followed by 25 mL iso-

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octane. The bottle is closed immediately (gas-tight) and placed in a water bath at 80 °C for 1 h with intermittent shaking and inverting the bottle every 20 min. After cooling the bottle to < 20 ˚C by ice water, a 1-2 mL aliquot of the upper isooctane layer is transferred into a micro centrifuge tube, and centrifuged at 5000 rpm for 5 min at 10 °C. The supernatant is then transferred into GC vials, and the residues of DTCs are estimated by determining the CS_2 concentration by GC-MS. The sample preparation procedure depending on the type of food used takes approx. 1-2 hrs.

Preparation of Standard Solutions and Reaction Mixture

For method validation, Thiram (99.5% purity) was used as representative DTC compound considering its simple structure (1 mole of Thiram = 2 mole of CS_2).

Carbon disulphide standard solution

A stock solution of CS_2 (2000 µg/mL) was prepared by accurately pipetting out 79.0 µL of CS_2 into a volumetric flask (certified A class, 50 mL) containing approximately 45 mL of iso-octane and made up to 50 mL with iso-octane. The CS_2 stock solution was kept in a refrigerator at -20 °C and used within two days of preparation. The CS_2 working standard solutions of 200 and 20 µg/mL concentrations (10 mL each) were prepared by serial dilution of stock solution with iso-octane.

Standard Solution of Thiram

10 mg (± 0.05) of Thiram was weighed into a 10 mL volumetric flask (certified A class) and dissolved in ethyl acetate up to the mark to get a stock solution of 1000 µg/mL. A 100 µg/mL Thiram working standard was prepared from stock solution by 10-times dilution.

Preparation of Reaction Mixture

An amount of 30 g of tin (II) chloride was accurately weighed in the 1000 mL volumetric flask (certified A class) to which 1000 mL of concentrated HCL (35%) was added. The solution was then gradually added to 1000 mL water with continuous stirring until a clear solution was obtained.

Calibration Standards

Calibration standard solutions of CS_2 at six different concentration levels (0.04, 0.08, 0.16, 0.32, 0.64, and 1.3 µg/mL) were prepared by appropriate dilutions of 20 µg/mL CS_2 working standard in iso-octane.

Matrix matched standards at the same concentrations were prepared by spiking the iso-octane extract of fresh control grapes, potato, tomato, green chili, and eggplant (all organically grown) using the following formula derived from above conversion of Thiram to CS_2:

$$\text{Spike quantity} = \frac{\text{Concentration to be achive} * \text{weight of the sample}}{0.6333 * \text{concentration of the stock solution}}$$

Before the preparation of matrix matched standards, the control samples were carefully monitored for absence of DTCs (in terms of CS_2).

Experimental Conditions

A Thermo Scientific™ TRACE GC Ultra™ gas chromatograph equipped with Thermo Scientific™ Triplus™ RSH liquid autosampler and coupled to a Thermo Scientific™ ITQ™ 900 ion trap mass spectrometer was used for analysis. See Tables 1 and 2 for instrument parameters.

Two GC columns of different polarity, stationary phase, and film thickness have been evaluated. The first column was a medium polarity cyanopropylphenyl phase (6% cyanopropylphenyl/94% dimethyl polysiloxane, 30 m x 0.32 mm ID, 1.8 µm film thickness, e.g. Thermo Scientific™ TraceGOLD™ TG-624, p/n 26085-3390) and as a second column a low polarity 5%-phenyl stationary

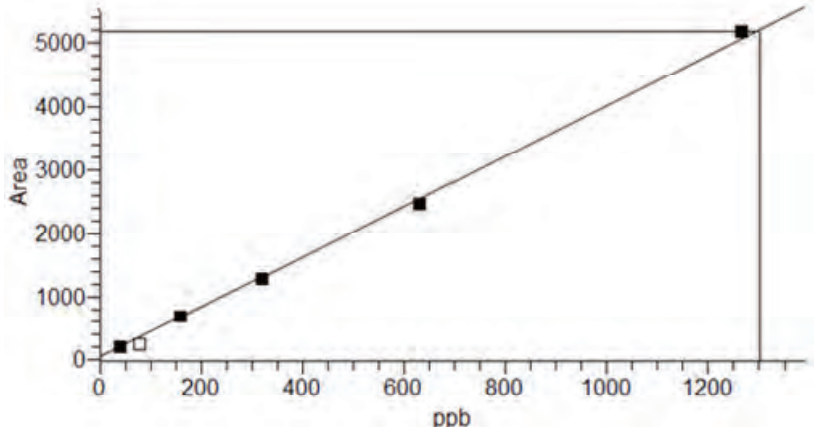

Figure 2. Calibration curve, range 0.04 - 1.300 µg/mL Thiram matrix spike, R_2 = 0.9990.

phase (5% diphenyl/95% dimethylpolysiloxane, 30 m x 0.25 mm ID, 0.25 µm film thickness, e.g. Thermo Scientific™ TraceGOLD™ TG-5MS p/n 26098-1420). The TG-624 column type is a mid-polarity column ideally suited for the analysis of volatile analytes, whereas the TG-5MS column is more commonly used especially for pesticide analysis and is commonly available in all laboratories. Both columns were thus tested for the applicability of the method. Either column can be used for the DTC analysis.

Table 1. GC Conditions

Injector, temperature prog.	PTV-LVI
	40 °C, 0.1 min (injection phase, @ 100 kPa)
	10 °C/min to 80 °C, 0.3 min (@ 200 kPa)
	10 °C/min to 110 °C (transfer phase)
	14.5 °C/min to 290 °C (cleaning phase)
Split flow	20 mL/min
Solvent vent	open until 0.17 min
	closed until 4.17 min
	open until end of run
Injection mode, volume	split, 4 µL
Carrier gas, flow	Helium, constant flow 1 mL/min
Oven program	40 °C, 5 min
	40 °C/min to 200 °C
	200 °C, 5 min
Transfer line temperature	205 °C

Table 2. MS Conditions

Ionization	EI, 70 eV
Scan mode, range	SIM, *m/z* 76, 78
Acquisition rate	2 scans/s
Ion source temperature	200 °C (optimized for CS_2 S/N ratio)

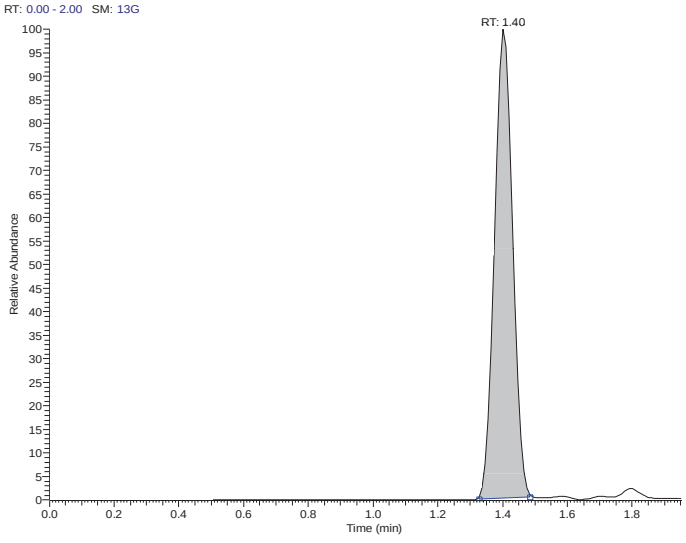

Figure 3. CS_2 chromatogram, 5 ppb matrix spike calibration.

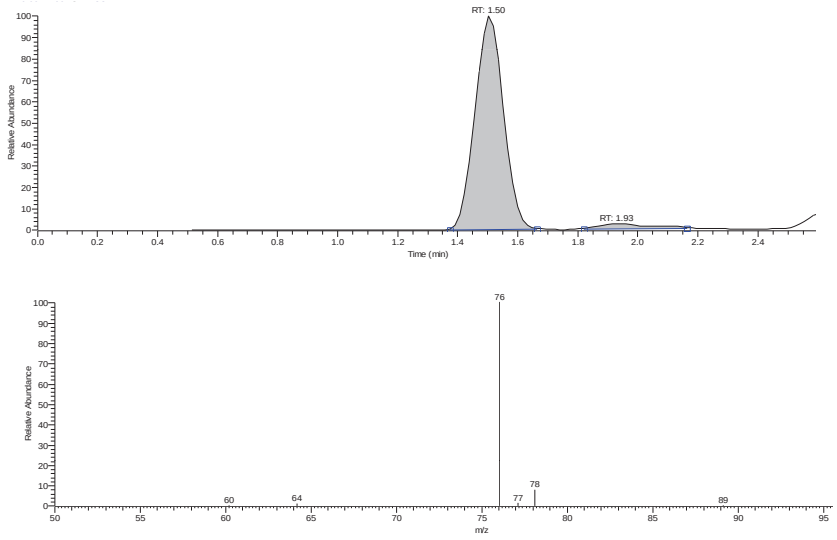

Figure 4. Chili sample analysis with confirming CS_2 ion ratio 100:10 for m/z 76:78.

Sample Measurements

A typical GC-MS batch consisted of matrix-matched calibration standards, samples, one matrix blank and one recovery sample for performance check after a set of every six samples.

The data acquisition was carried out in Full Scan mode using the compound-specific ions *m/z* 76 and 78 (the 34S isotope, ion ratio 10:1) as extracted chromatograms for a selective identification of CS_2.

Results

Sensitivity

The sensitivity of the method was evaluated in terms of the limit of detection (LOD) and limit of quantification (LOQ) which were respectively 0.005 and 0.04 µg/mL. The LOD is the concentration at which the signal to noise ratio (S/N) for the quantifier ion is > 3, whereas LOQ is the concentration for which the S/N is > 10.

Recovery

The recovery experiments were carried out on fresh untreated potato, tomato, eggplant, green chili, and grapes by fortifying 25 g of the samples with Thiram solution at 0.04, 0.16, and 1.30 µg/g levels in six replicates. The control samples of each of the tested commodities were obtained from an organic farm near Pune, India, and screened for absence of DTC residues before spiking. The spiked the samples were extracted using the sample preparation method described above. The quantitation of the residues was performed using matrix matched standards.

Table 3. Recoveries from different foods:

Spike level [ppb]	Grapes [%]	Chili [%]	Potato [%]	Egg plant [%]	Tomato [%]
1300	96 (±4)	81 (±10)	90 (±9)	90(±5)	81 (±4)
160	94 (±10)	80 (±13)	94 (±10)	92 (±8)	85 (±10)
40	104 (±15)	79 (±9)	104 (±15)	86 (±10)	96(±15)

Precision

The precision of repeatability was determined by three analysts preparing six samples each on a single day. The intermediate precision was determined by the same analysts with six samples each on six different days. The method precision was determined with 0.04 mg/kg.

General Guidelines for DTC Analysis

The analysis of cruciferous crops, including brassica samples, may not be unequivocal, because they contain naturally occurring compounds that may generate carbon disulfide.

It is necessary to avoid the use of rubber material (natural/synthetic) e.g. gloves, when performing DTC analyses as they contain dithiocarbamates, and this could lead to contamination problems. Silicone rubber and polyethylene do not contain dithiocarbamate.

Samples, other than fresh foodstuffs, will be comminuted by cryogenic milling. Fresh samples should be sub-sampled prior to extraction by removing segments from fresh samples following current Codex Alimentarius guidelines.

The samples should be analyzed within 4 weeks of cryogenic milling. If the storage of fresh produce is necessary it should be in a cool place (<-10°C) keeping condensation at minimum [4].

Conclusions

A reliable routine method for the analysis of dithiocarbamates with high precision in different vegetable and fruits has been developed. The method allows a wide calibration range of 0.04 – 1.300 µg/mL Thiram. The LOQ has been determined as 0.04 µg/mL.

The extraction uses a $SnCl_2$/HCl acid-hydrolysis with iso-octane as solvent to form CS_2 which finally gets quantified by GC-MS. The recovery from different food commodities has been shown to be very high with 79 to 104%.

The GC injection method and column separation has been optimized for the injection of 4 uL of extract, using GC columns of standard film and dimensions, typically used for other types of residue analysis as well, so that a column change to a specific column for CS_2 determination only is not required.

The mass spectrometer ion source conditions had been optimized for best sensitivity and S/N ratio. The analysis in SIM mode is preferred providing a high selectivity with easy to integrate chromatograms.

This method has been developed initially for the ITQ ion trap mass spectrometer, but the same parameter setup is suitable for the Thermo Scientific™ ISQ™ series single quadrupole or Thermo Scientific™ TSQ™ Quantum XLS Ultra or Thermo Scientific™ TSQ 8000™ triple quadrupole mass spectrometers, as well.

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Application Note 10446

Determination of Organochlorine Pesticides Using GC-MS with a Helium-conserving Injector

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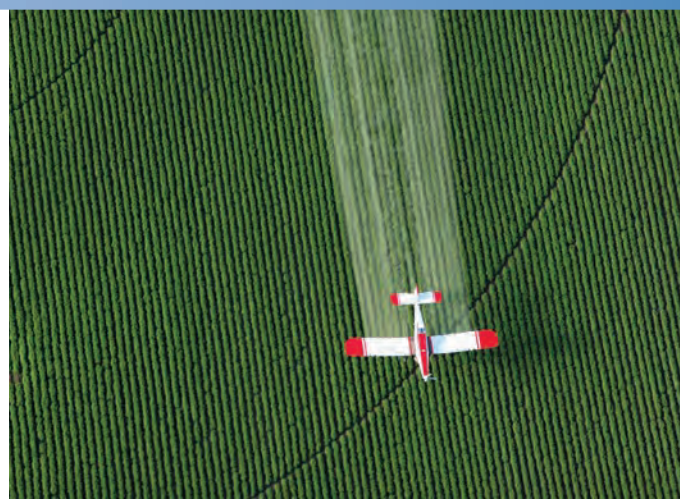
Keywords
Chromeleon CDS software, Environmental, Gas chromatography, GC-MS, Instant Connect Helium Saver injector, ISQ

Goal
To describe the performance of the Thermo Scientific™ ISQ™ LT GC-MS system equipped with an Instant Connect Helium Saver injector and Thermo Scientific Dionex™ Chromeleon™ 7.2 Chromatography Data System (CDS) software in identifying and quantifying organochlorine pesticides.



Introduction
Organochlorine pesticides are among the most toxic synthetic pesticides in the world. For this reason, their presence in the environment must be constantly and carefully monitored. Gas chromatography coupled with mass spectrometry for detection is one of the techniques of choice used to analyze these compounds.

To guarantee the accuracy and reproducibility of the analysis, the sample introduction phase is very important, the injector must provide optimal inertness to prevent compound breakdown and degradation and ensure a constant supply of carrier gas. Modern split/splitless injectors can ably handle this analysis. However, they require a large amount of helium for their operations.

To overcome this problem, Thermo Fisher Scientific introduced the innovative Instant Connect Helium Saver injector module. This injector employs a double-gas system that allows it to minimize helium consumption. Helium is used only as a carrier gas inside the column. All other operations, such as the split and purge flow feeding, use nitrogen. Here we demonstrate the performance of a GC-MS system equipped with the Instant Connect Helium Saver injector for the analysis of organochlorine pesticides.

Method Setup

GC Conditions	
TRACE 1310 GC	
Injection volume:	1 µL
Liner:	Splitless w/glass wool
Carrier gas:	Helium
Column type:	Thermo Scientific™ TraceGOLD™ TG-5 30 m, 0.25 mm, 0.25 µm
Column oven:	100 °C, hold 2 min. Ramp 15 °C/min to 160 °C, hold 5 min. Ramp 5 °C/min to 270 °C hold 2 min
SSL Injector:	225 °C; splitless mode for 2 min with a split ratio of 50:1. Helium delay 0.1 min
Column flow:	Constant flow at 1 mL/min
ISQ LT Mass Spectrometer	
Source temperature:	270 °C
Transfer line temperature:	270 °C

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Table 1. Recommended instrument parameters.

FS Conditions				
Acquisition start	Mass range	Dwell Time		
4.00	50-350	0.2		
SIM settings				
Compound	Retention time	Polarity	Window	Ion
a-Lindane	11.58	Positive	0.3	181, 219
g-Lindane	12.38	Positive	0.3	183, 219
b-Lindane	12.60	Positive	0.3	181, 219
d-Lindane	13.34	Positive	0.3	183, 219
Heptachlor	14.96	Positive	0.3	100, 272
Aldrin	16.14	Positive	0.3	66, 263
Heptachlor epoxyde	17.52	Positive	0.3	81, 263
g-Chlordane	18.35	Positive	0.3	237, 272
Endosulfan I	18.78	Positive	0.3	195, 241
a-Chlordane	18.89	Positive	0.3	237, 272
DDE	19.66	Positive	0.3	246, 318
Dieldrin	19.70	Positive	0.3	79, 263
Endrin	20.46	Positive	0.3	263, 281
Endosulfan II	20.78	Positive	0.3	159, 195
DDD	21.14	Positive	0.3	165, 235
Endrin aldehyde	21.47	Positive	0.3	67, 250
Endosulfan sulfate	22.30	Positive	0.3	229, 272
DDT	22.45	Positive	0.3	165, 235
Endrin ketone	23.93	Positive	0.3	67, 317
Methoxychlor	24.52	Positive	0.3	152, 227

Methods

The TRACE 1310 GC portion of the ISQ LT GC-MS system was equipped with one Instant Connect Helium Saver injector with a splitless w/glass wool liner (P/N 453A1925). A Thermo Scientific™ TraceGOLD™ TG-5MS 30 m, 0.25 mm 0.25 µm (P/N 26098-1420) column was used. The standards used for calibration were ordered from Restek: Organochlorine Pesticide Mix AB # 3 (P/N 32415).

The analysis was first run as a full scan to set up the single ion monitoring (SIM) conditions and then in SIM mode. The data were collected and processed using the Chromeleon 7.2 CDS software.

Results and Discussion

Figure 1 shows a chromatogram of the full scan analysis at a concentration of 1 ppm. The full scan is used to set up the time windows and target ions for the SIM for each compound. The SIM is used for quantitation and identification.

The calibration curve built comprises six points at concentrations of 1, 5, 10, 20, 50, and 100 ppb. Calibration results are reported in Table 2.

The area and retention time repeatability of the system has been assessed performing 10 consecutive runs of a sample at a concentration of 20 ppb.

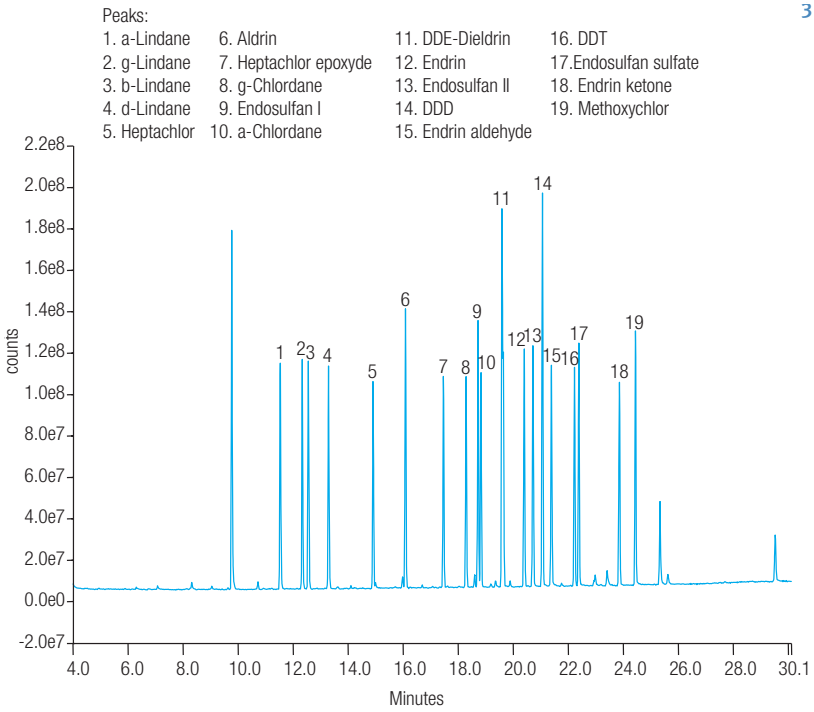

Figure 1. Analysis of organochlorine pesticides at 1 ppm in Full Scan mode.

Table 2. Calibration results.

Peak Name	Ret.Time	Cal.Type	Number of Points	Coeff. of Determination
a-BHC	11.565	Lin, WithOffset	6	0.999
g-BHC	12.362	Lin, WithOffset	6	0.998
b-BHC	12.585	Lin, WithOffset	6	0.998
d-BHC	13.322	Lin, WithOffset	6	0.997
Heptachlor	14.943	Lin, WithOffset	6	0.997
Aldrin	16.125	Lin, WithOffset	6	0.999
Heptachlor epoxyde	17.507	Lin, WithOffset	6	0.999
g-Chlordane	18.334	Lin, WithOffset	6	0.998
Endosulfan I	18.767	Lin, WithOffset	6	0.998
a-Chlordane	18.875	Lin, WithOffset	6	0.998
DDE	19.637	Lin, WithOffset	6	0.997
Dieldrin	19.684	Lin, WithOffset	6	0.997
Endrin	20.445	Lin, WithOffset	6	0.998
Endosulfan II	20.760	Lin, WithOffset	6	0.997
DDD	21.102	Lin, WithOffset	6	0.996
Endrin aldehyde	21.435	Lin, WithOffset	6	0.998
Endosulfan sulfate	22.272	Lin, WithOffset	6	0.996
DDT	22.431	Lin, WithOffset	6	0.996
Endrin ketone	23.907	Lin, WithOffset	6	0.997
Methosychlor	24.488	Lin, WithOffset	6	0.995

Table 3. Area repeatability.

Compound	RSD %
a-BHC	2.62
d-BHC	3.40
b-BHC	2.35
g-BHC	2.42
Heptachlor	2.42
Aldrin	2.56
Heptachlor epoxide	2.57
g-Chlordane	2.53
Endosulfan	2.50
a-Chlordane	2.67
DDE	2.71
Dieldrin	2.32
Endrin	2.54
Endosulfan II	2.83
DDD	2.61
Endrin aldehyde	2.24
DDT	8.32
Endosulfan sulfate	2.64
Endrin ketone	2.17
Metoxychlor	2.89

Table 4 and 5. Retention time repeatability.

	a-BHC	d-BHC	b-BHC	g-BHC	Heptachlor	Aldrin	Heptachlor epoxide	g-Chlordane	Endosulfan	a-Chlordane
	11.6007	12.4201	12.6255	13.3573	14.9839	16.1607	17.5427	18.3694	18.8037	18.9112
	11.5955	12.3966	12.62	13.3519	14.9786	16.1553	17.5373	18.3642	18.7984	18.9059
	11.6007	12.4021	12.6255	13.3573	14.9839	16.1607	17.5427	18.3694	18.8037	18.9112
	11.5906	12.39767	12.6201	13.352	14.7986	16.1556	17.35374	18.3642	18.7986	18.9061
	11.6008	12.4019	12.6252	13.357	14.9839	16.1606	17.5425	18.3693	18.8036	18.911
	11.6007	12.4043	12.6251	13.3569	14.9836	16.1655	17.5472	18.3689	18.8084	18.9108
	11.5957	12.3993	12.6252	13.3569	14.9837	16.1605	17.5423	18.3961	18.8035	18.9059
	11.5957	12.3994	12.6252	13.3572	14.984	16.1608	17.5425	18.3694	18.8037	18.9112
	11.5909	12.3946	12.6154	13.3523	14.974	16.1557	17.5377	18.3644	18.7987	18.9062
	11.591	12.3922	12.6156	13.3473	14.974	16.1508	17.5327	18.3594	18.7937	18.9012
Avg	11.60	12.40	12.62	13.35	14.96	16.16	17.52	18.37	18.80	18.91
Std.Dev	0.0043	0.0077	0.0042	0.0035	0.0578	0.0042	0.0593	0.0100	0.0042	0.0035
RSD%	0.04	0.06	0.03	0.03	0.39	0.03	0.34	0.05	0.02	0.02

	DDE	Dieldrin	Endrin	Endosulfan II	DDD	Endrin aldehyde	DDT	Endosulfan sulfate	Endrin ketone	Metoxychlor
	19.6733	19.7207	20.481	20.7985	21.1411	21.4737	22.3123	22.4647	23.9399	24.5217
	19.6679	19.7203	20.4756	20.7983	21.1358	21.4684	22.3069	22.4643	23.9394	24.5162
	19.6733	19.7207	20.481	20.7985	21.1411	21.4737	22.3123	22.4647	23.9399	24.5217
	19.668	19.72053	20.4756	20.7982	21.1409	21.4685	22.307	22.4645	23.9396	24.5164
	19.6779	19.7254	20.4805	20.8031	21.1407	21.4732	22.3167	22.4692	23.9444	24.5262
	19.6778	19.7253	20.4855	20.803	21.1456	21.4734	22.312	22.4695	23.9446	24.5214
	19.6729	19.7204	20.4806	20.8034	21.1409	21.4735	22.312	22.4695	23.9396	24.5214
	19.673	19.7205	20.4807	20.8032	21.1408	21.4735	22.3119	22.4694	23.9395	24.5214
	19.6682	19.7206	20.4759	20.7986	21.1412	21.4688	22.3073	22.4647	23.9348	24.5166
	19.6631	19.7106	20.4707	20.7884	21.131	21.4586	22.3021	22.4546	23.9298	24.5116
Avg	19.67	19.72	20.48	20.80	21.14	21.47	22.31	22.47	23.94	24.52
Std.Dev	0.0047	0.0040	0.0042	0.0045	0.0039	0.0048	0.0042	0.0045	0.0043	0.0042
RSD%	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02

Conclusions

This work highlights the excellent results that can be achieved using a GC/MS system, such as the ISQ LT GC-MS system. The analytical performance of the Instant Connect Helium Saver injector is remarkable in terms of both area and retention time reproducibility. As with other TRACE 1300 Series injectors and detectors, the Helium Saver benefits from its modularity, providing freedom from helium supply shortages, while maintaining performance identical to that of traditional SSL injectors. These unique features of the Helium Saver reduce cost without the need to change analytical methods or routine. Using a data system, such as the Chromeleon CDS software, provides a powerful tool for data acquisition and reprocessing along with unsurpassed ease of use.

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Application Note 20705

Analysis of Organophosphorus Pesticides by GC

Anila I. Khan, Thermo Fisher Scientific, Runcorn, UK

Key Words
Organophosphorus pesticides, TraceGOLD TG-5MS column, TRACE 1310, US EPA Method 8141B, quartz liner

Abstract
This application note demonstrates the use of a deactivated, splitless quartz liner with single taper and a 5% phenyl polysiloxane phase column for the separation of an organophosphorus pesticides standard mix. This was analyzed on a Thermo Scientific™ TRACE™ 1310 GC equipped with a modular split/splitless (SSL) injector and a flame ionization detector (FID).

Introduction

US EPA 8141B is one of a number of standard analytical methods used for the determination of organophosphorus pesticides (OPPs) in aqueous and solid samples by gas chromatography. OPP can easily degrade in the injector port, which can lead to poor peak profiles. This causes activity within the GC inlet port when repeated injections are made, producing matrix effects. These pesticides can then interact with the active sites and produce peak tailing and poor reproducibility of results.

Using a Thermo Scientific deactivated, packed splitless quartz liner results in a reduction of activity on the surface of the liner, giving excellent reproducibility when compared to several other liner formats. The liner is treated using a proprietary process to reduce any surface activity. These characteristics lead to highly symmetrical peak shapes. In addition, deactivated quartz wool helps in trapping the non-volatile compounds.

This analysis is performed on an ultra-low bleed 5% phenylpolysiloxane phase GC column. The OPP analysis was performed in splitless injection mode using a Thermo Scientific™ TraceGOLD™ TG-5MS 30 m × 0.25 mm × 0.25 µm GC column and a deactivated, packed splitless quartz wool liner for the TRACE 1310 GC, which is equipped with a modular plug and play split/splitless (SSL) injector and a flame ionization detector (FID). This fulfills the requirement of US EPA Method 8141B for the analysis of the OPPs listed in Table 1.

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Consumables		Part Number
Column:	TraceGOLD TG-5MS 30 m × 0.25 mm × 0.25 µm	26098-1420
Septum:	BTO coated 11 mm center guide (50/pk)	31303233
Liner:	Splitless liner with single taper 78.5 × 4 × 6.3 mm	453A1925
Column ferrules:	Graphite ferrule for 0.1–0.32 mm i.d. columns 10/pk	290GA139
Injection syringe:	10 µL syringe FN 50 mm T Gauge 26, cone tip	36500525
Vials and closures:	Thermo Scientific 9 mm Wide Opening Screw Thread Vials Convenience Kit, 2 mL Clear Glass Vial with ID Patch, Blue Closure with PTFE/Blue Silicone Septa	60180-599

Solutions
A working standard solution of 20 µg/mL of EPA 8141 was prepared in acetone. The stock solution was obtained commercially at a concentration of 1000 µg/mL.

Separation Conditions		Part Number
Instrumentation:	TRACE 1310 mainframe 230 V GC	14800302
Carrier gas:	Helium	
Split flow:	50 mL/min	
Column flow:	1.2 mL/min, constant flow	
Oven temperature:	40 °C (1 min), 12 °C/min, 280 °C (10 min)	
Injector type:	TRACE 1310 SSL Injector module	29903010
Injector mode:	Splitless	
Injection details:	Splitless (1 min)	
Injector temperature:	220 °C	
Detector details:	TRACE 1310 FID module	29903001
FID parameters:		
Temperature:	280 °C	
Air flow:	350 mL/min	
Hydrogen flow:	35 mL/min	
Nitrogen makeup flow:	30 mL/min	

Injector Conditions	
Instrumentation:	Thermo Scientific AS1300 Autosampler
Injection Volume:	1 µL
Wash solvent:	Acetone/hexane (1:1 v/v)

Data Processing	
Software:	Thermo Scientific™ Chrom-Card™ data system

Results

Figure 1 shows the TIC chromatogram for 22 OPPs at 20 µg/µL using a TraceGOLD TG-5MS column and a standard, deactivated, splitless quartz liner for the TRACE 1310 GC instrument. Table 1 shows the peak identification of the OPPs according to their retention times. Table 1 includes the reproducibility data for ten injections. The stationary phase of the TG-5MS GC column, in combination with the deactivated splitless liner, provides excellent performance due to minimal interaction of active compounds with active sites on the column, the glass wall of the liner, or the deactivated quartz wool. This minimizes peak tailing of the OPPs and gives highly symmetrical peak shapes. The combination of a TG-5MS GC column, the deactivated liner, and the TRACE 1310 GC gave excellent injection reproducibility of between 1.7% and 3.4% for the 22 OPPs tested (Table 2).

The tailing factors calculated according to the USP method for all peaks were 0.82–0.97 apart from mevinphos, which gave a tailing factor of 0.77. The resolution value between peaks 17 and 18 was 1.75 according to the USP criteria. For peaks 12 and 13, the calculated resolution was 0.90.

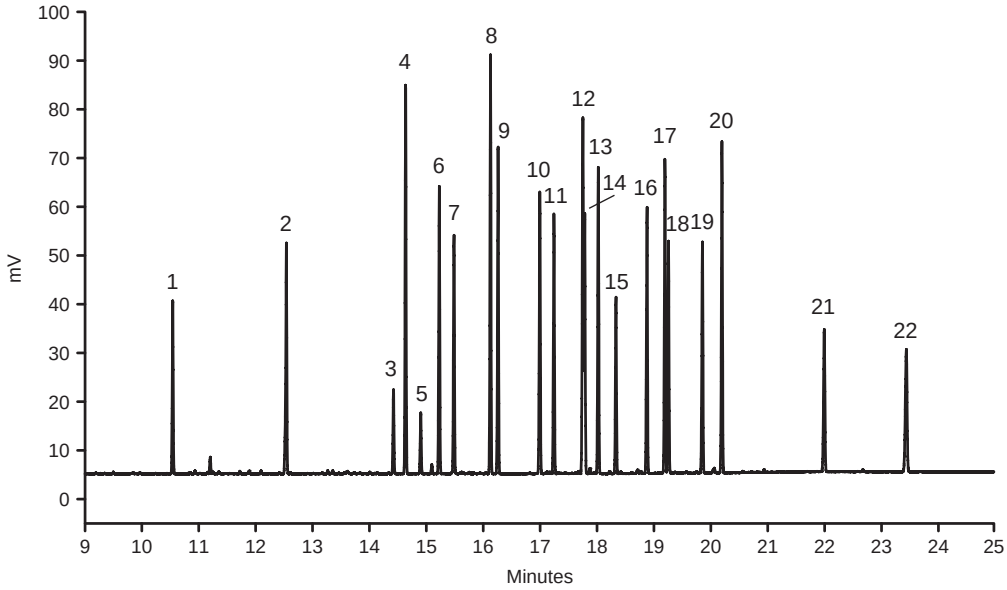

Figure 1: Chromatogram of 22 OPP standards at 20 µg/mL

Peak Number	Compound	t_R (min)	t_R %RSD (n=10)	Peak Area %RSD (n=10)
1	Dichlorvos	10.55	0.02	1.8
2	Mevinphos	12.55	0.02	2.0
3	Demeton O	14.43	0.02	2.6
4	Ethroprophos	14.65	0.01	2.0
5	Naled	14.91	0.01	2.6
6	Phorate	15.24	0.01	1.8
7	Demeton S	15.50	0.01	1.9
8	Diazinon	16.14	0.01	1.9
9	Disulfoton	16.27	0.01	1.7
10	Methyl parathion	17.01	0.01	2.2
11	Fenchlorphos	17.26	0.01	2.0
12	Fenthion	17.77	0.02	2.3
13	Chlorpyrifos	17.80	0.02	3.4
14	Trichloronate	18.04	0.01	1.9
15	Merphos	18.35	0.01	1.9
16	Stirofos	18.90	0.01	2.0
17	Tokuthion	19.21	0.02	2.1
18	Impurity	19.27	0.01	2.2
19	Fensulfothion	19.87	0.01	2.1
20	Bolstar	20.22	0.01	2.0
21	Azinphos methyl	22.02	0.01	2.3
22	Coumaphos	23.46	0.01	2.1

Table 1: List of OPPs and their retention times peak area reproducibility

Conclusion

The TraceGOLD TG-5MS column and the deactivated, splitless quartz liner with quartz wool, when used in a TRACE 1310 GC instrument, demonstrated excellent performance for the separation and analysis of organophosphorus compounds with excellent peak shape, resolution, and reproducibility.

Reference

US EPA 8141B: http://water.epa.gov/scitech/methods/cwa/index.cfm

Acknowledgements

Many thanks to Fausto Pigozzo, Silvia Gemme, Stefano Pelagatti, Magni Paolo, Franco Ponciroli, and Massimo Santoro at Thermo Fisher Scientific in Milan and Ruth Jones at Thermo Fisher Scientific in Runcorn for their help and support in this work.

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Application Note: 10049

Trace Determination of Organo-Phosphorous Pesticides in Olive Oil by GC Analysis through PTV Backflush / FPD

Thermo Fisher Scientific Inc., Milan, Italy

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Key Words

- TRACE GC Ultra
- Olive Oil
- Organo-Phosphorus Pesticides
- ppb Levels
- PTV Backflush
- FPD Selectivity

Introduction

Organo-Phosphorous Pesticides (OPP) are widely used in agriculture, due to their relatively low cost, broad spectrum of activity, and high impact on insects compared to other pesticides. However, because the OPPs are well known to cause irreversible effects on the nervous system (reduced activity of neurotransmitters), their possible presence as trace residues in food must be strictly monitored. In this respect, one critical application is the control for OPPs in olive oil.

This class of compounds can effectively be analyzed by Gas Chromatography using a Programmable Temperature Vaporizing (PTV) injector and a Flame Photometric Detector featuring extremely high sensitivity and selectivity for phosphorus containing compounds.

The PTV injector is found to be particularly suitable for samples like edible oils, characterized by the presence of heavy fractions in potentially dirty matrices. The conventional Split-Splitless injector is advantageously able to be kept at a low temperature during the sample introduction phase. This prevents any sample evaporation from the syringe needle, hence eliminating a source of discrimination of higher boiling components. On the other hand, compared to the On-column injector, it allows non-volatile sample by-products to be retained in the vaporization chamber, thus preventing any decay of the column performance in time due to by-products accumulation.

This type of analysis requires high oven temperatures and short columns with a very thin film in order to allow complete elution of the main constituents of vegetable oil, triglycerides. Additionally, the sample must also be very diluted in order to avoid overloading the column with this primary fraction (for quantity) and consequent contamination of the detector. These two factors make trace analysis of contaminants even more complex. To overcome these problems, the heavier fraction is usually completely eliminated with an extended sample preparation step prior to GC analysis.

This paper describes an alternative way to effectively and rapidly analyze OPPs in oils eliminating any interference with the heavy fraction. The use of a special accessory vents the heavier components of the sample when these are not of interest.

Back-flush Device for PTV Injector

The Thermo Scientific TRACE GC Ultra™ equipped with a PTV inlet and a reverse flow device (back-flush) is used for this application. This accessory consists basically of a 3-way solenoid valve (back-flush valve) placed in the carrier gas line, a wide-bore pre-column, a high temperature "T" connector housed in the GC oven connecting the pre-column to the column, and a calibrated flow restrictor (Figure 1).

When the back-flush valve is off (Figure 2), the carrier gas flows in its normal direction through the inlet. A very small flow provided by the restrictor is able to constantly purge the "T" connector between the pre-column, analytical column, and back-flush inlet line. The pre-column consists of a 2 m x 0.53 mm i.d. uncoated fused silica tubing, and the purge flow is about 5 % of the column flow.

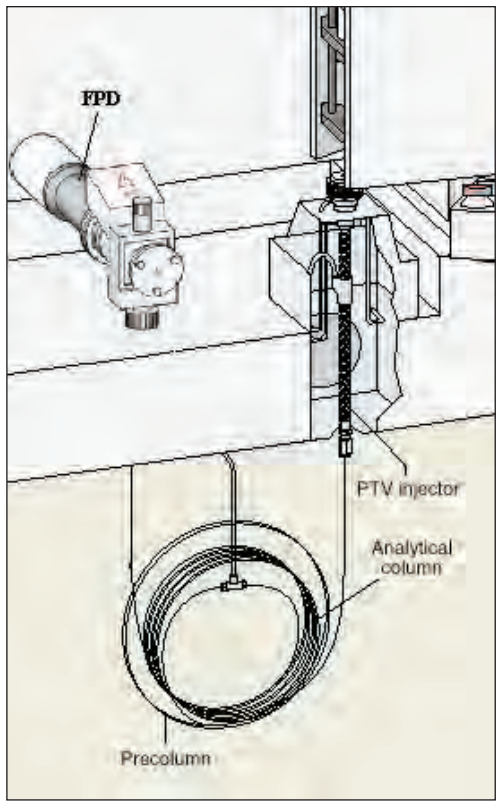

Figure 1: PTV-FPD configuration.

When the back-flush valve is switched on, the system diverts the gas directly to the "T" connection at the end of the pre-column, therefore, sweeping both the latter and the inlet in the opposite direction, with a so called "reverse flow". In this configuration, the carrier gas is able to "flush" anything still in the pre-column or in the injector directly to the vent and through the injector's split line. The small flow provided by the restrictor in the other direction will prevent the back-flushed material to flow through the inlet liner.

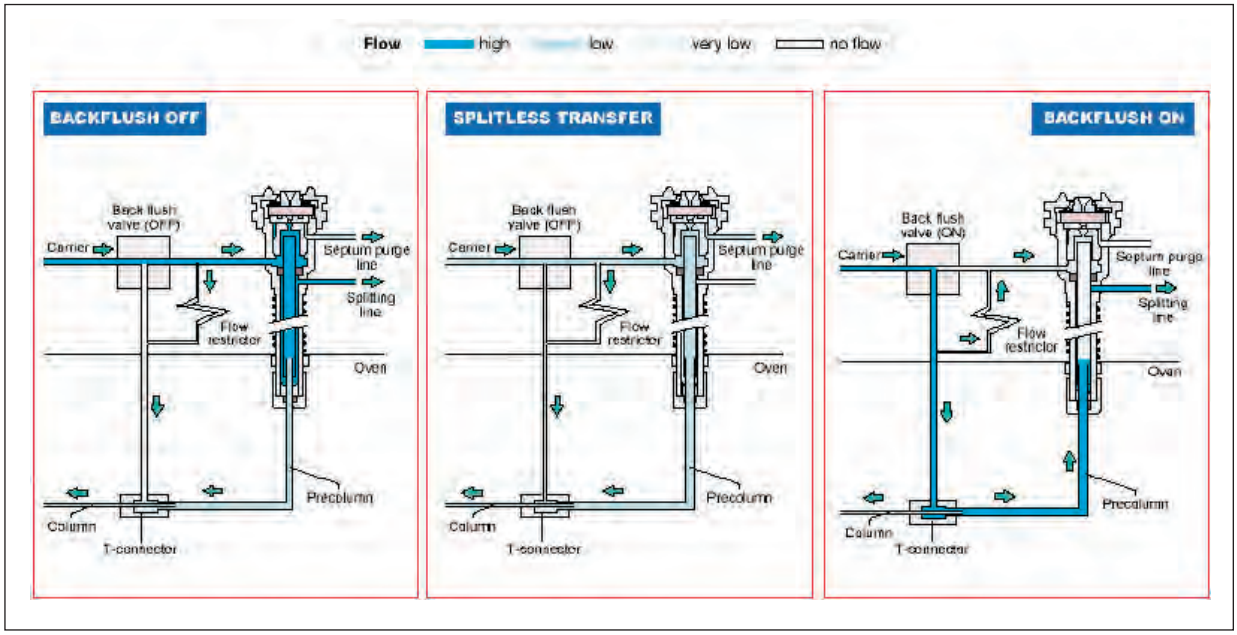

Figure 2: Reverse flow device

In order to clearly demonstrate the effect of the reverse flow device, 2 µL of virgin olive oil diluted 1:10000 in acetone are injected in a TRACE GC Ultra equipped with PTV injector and FID detector. An OV-5, 7 m long, 0.25 mm i.d., 0.25 µm f.t. column is used, together with a 2 m, 0.53 mm i.d. deactivated pre-column. The oven ramp is 60 °C (3 min) to 100 °C at 8 °C/min, then to 380 °C (10 min) at 20 °C/min. The PTV initial Temperature is 80 °C (hold 0.1 min) then ramped at 14.5 °C/sec up to 380 °C (held for all the analysis), with a splitless time of 3 minutes and a split flow of 50 mL/min. Helium is used as carrier gas at constant pressure (55 kPa). Finally the FID detector base body temperature is set at 350 °C.

The same sample is then injected in the PTV equipped with the back-flush device. Since the heavier fraction is now vented out by the reversed flow, the sample is diluted only 1:1 in acetone.

Sensitivity towards the compounds of interest is simply increased by 4 orders of magnitude, and the absence of the predominant fraction allows both to eliminate the risk of column overloading and to target separation optimization on the lighter components only.

Figure 3 shows the two chromatograms obtained with and without back-flush valve activation respectively. The complete absence of the triglycerides in the second chromatogram proves the effective reliability of the reverse flow enabled after 3 minutes. This timing is proven to be sufficient to allow transfer of the compounds of interest into the analytical column, while diverting any residual heavy fraction into the pre-column for venting.

Analysis of OPPs in Olive Oil

The same equipment is used for the determination of Organo-Phosphorous Pesticides with exception of the detection system. A highly sensitive phosphorous-selective FPD detector is used in place of the FID. Performance and repeatability tests are performed by injecting 2 µL of virgin olive oil spiked with 50/100 ppb of OPPs mixture. Also, in this case, the sample is diluted only 1:1 with acetone, and the optimum conditions for the separation of OPPs are applied. An SE54, 10 m long, 0.25 mm i.d., 0.1 µm f.t. capillary column is used, together with a 2 m, 0.53 mm deactivated pre-column. The GC oven temperature starts with an isotherm at 60 °C (1 min) and is then raised to 350 °C (10 min) at 8 °C/min. The PTV Temperature ranges between 50 °C (0.1 min) and 400 °C (held for all the analysis) at 10 °C/sec, with a splitless time of 1 minute. Helium is used as carrier gas at constant flow (1.5 mL/min), and the FPD detector is set at 300 °C. A 300 mL/min back-flush flow is enabled after 16 minutes.

Figure 4 reports the related chromatogram, together with the repeatability of retention times and peak areas based on 10 consecutive injections, showing excellent separation and sensitivity. Three different commercial olive oils were tested under the same conditions (Figure 5): only Fenthion resulted present in Oil 1 and Oil 3 in different amounts, while Oil 2 was found to be completely destitute of such pesticides. A large number of injections of oil (over 100) were performed without replacing the liner or the pre-column, and no degradation of chromatographic performance was observed.

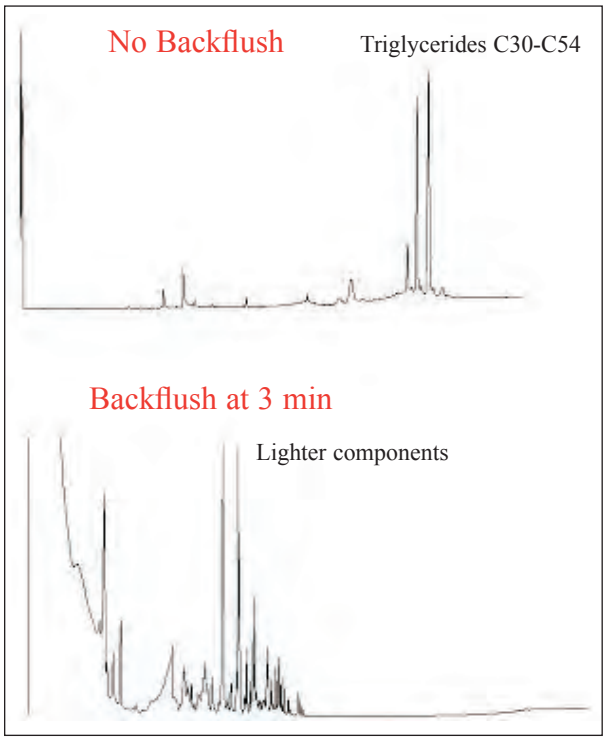

Figure 3: Olive Oil analysis with and without reverse flow; Detector: FID

PEAK NUMBER	SAMPLE COMPOUND	RETENTION TIMES AVERAGE (MIN)	RSD%	PEAK AREAS AVERAGE (COUNTS)	RSD%
1	Dimethoate	16.24	0.08	371681	3.1
2	Parathion-methyl	19.85	0.06	290948	2.6
3	Chlorphiriphos-methyl	18.97	0.05	134474	3.0
4	Malathion	20.04	0.08	174849	5.8
5	Fenthion	20.23	0.04	229989	2.5
6	Chlorphiriphos-ethyl	20.98	0.08	132520	3.7
7	Methidathion	21.89	0.04	826901	3.8

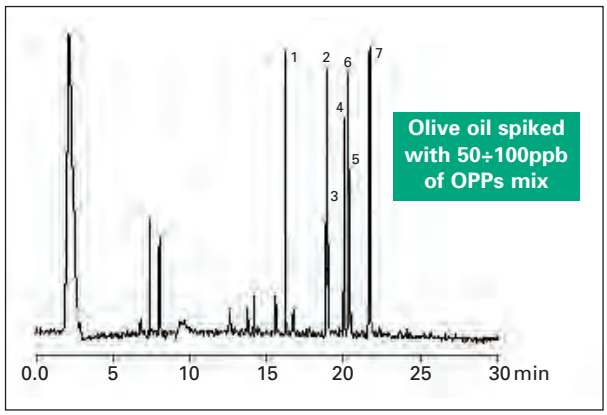

Figure 4: Repeatability Test based on 10 injections; Detector: FPD

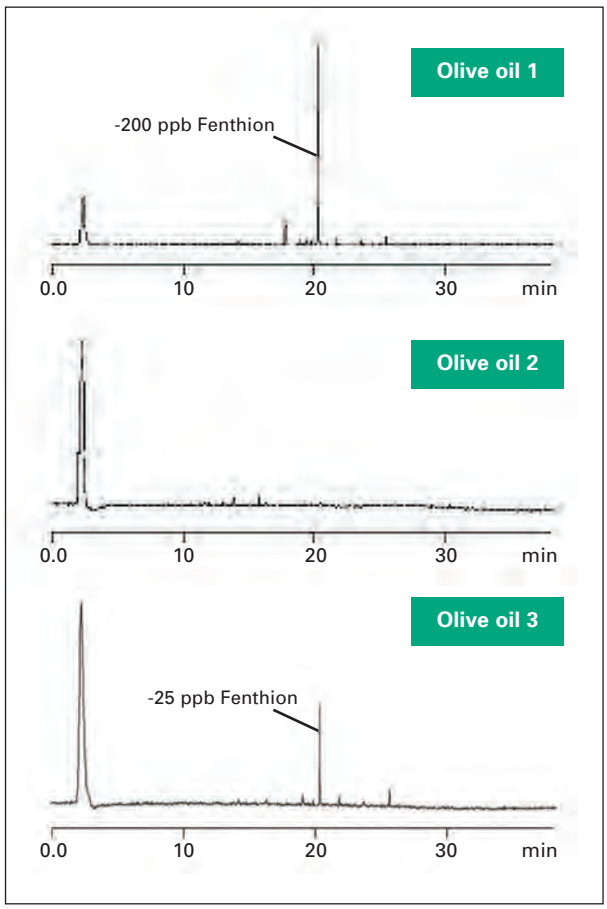

Figure 5: Detection of Fenthion in 3 commercial olive oils; Detector: FPD

Conclusions

OPPs in olive oil matrix can effectively be analyzed with PTV and FPD, provided that the triglycerides are vented out by a reverse flow device. Under these conditions, performance of the PTV injector is found to be greatly improved. The total analysis time is much shorter since no extra waiting time for complete elution of the high boiling components is now required. Sensitivity can be increased by four orders of magnitude (a few ppb) simply through the injection of a more concentrated sample.

Two additional important benefits obtained with the use of the back-flush are the highly extended column lifetime and the strongly simplified sample preparation procedure, which now only requires the dilution of the olive oil with acetone solvent.

Acknowledgement

Authors: Fausto Munari, Stefano Pelagatti, Andrea Cadoppi

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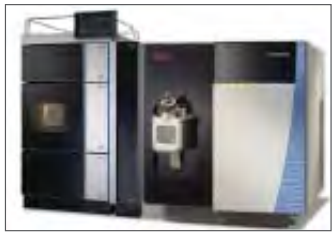

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- Increased Productivity in Pesticide Residue Analysis – Quantifying 440 Pesticides Following China GB 2763-2014: The Pesticide Explorer Collection – Standard Quantitation
- Fast and Ultrafast LC-MS/MS Methods for Robust and Reliable Analysis of Pesticides in Food Using the Vanquish UHPLC System
- Quantitative and Qualitative Confirmation of Pesticides in Beet Extract Using a Hybrid Quadrupole-Orbitrap Mass Spectrometer
- Use of UHPLC and High-Resolution MS for Quantitative Analysis of Pesticides in Onion Matrix
- Qualitative and Quantitative Analysis of Pesticides in Horse Feed Matrix Using Orbitrap MS
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- Testing LC-MS System Robustness with Automated Sample Cleanup Using Red Wine as a Matrix

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Fast Screening and Quantification of Pesticide Residues Using a Comprehensive LC-MS Solution: The Pesticide Explorer Collection – Standard Quantitation

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[1]Thermo Fisher Scientific, Special Solution Center Europe, Dreieich, Germany
[2]Thermo Fisher Scientific, San Jose, California

Application Note 643

Key Words
Pesticide Explorer Collection, European Regulation 396/2005, Commission Directive 2006/125/EC, European Commission 2002/657/EC, SANCO/12571/2013, European Commission 788/2012/EC, pesticide, food, QuEChERS, UltiMate 3000, TSQ Endura, TraceFinder

Goal
To present a fully tested LC-MS/MS workflow for rapid and robust quantification of more than 250 pesticides below maximum residue limits (MRLs) with sensitivity, accuracy, and precision that meets stringent EU guidelines.

Introduction
Pesticides are chemicals used on crops to protect them from the negative activity of pests. Inappropriate application of pesticides can have adverse effects on health; therefore, determination and quantification of pesticide residues in foods and food products is an important part of routine food control. The European Union (EU) legislation (European Regulation 396/2005 and Commission Directive 2006/125/EC) requires an extensive and comprehensive study determining pesticides in various products of plant and animal origin. The requirements for low limits of quantification (LOQ) of pesticides pose significant analytical challenges, especially for some complicated food matrices.

This study presents a multi-residue analysis method enabled by Thermo Scientific™ Pesticide Explorer Collection Standard Quantitation Solution, comprising liquid chromatography–triple-stage mass spectrometry (LC-MS/MS), for rapid and robust quantitation of more than 250 pesticides below their required maximum residue limits (MRL). This comprehensive solution includes the Thermo Scientific™ QuEChERS sample preparation kit, Dionex™ UltiMate™ 3000 LC system, TSQ Endura™ triple quadrupole mass spectrometer, TraceFinder™ software, Accucore™ aQ column, and method parameters to provide a start-to-finish workflow for pesticide analysis. The method results address the stringent EU guidelines concerning sensitivity, accuracy and precision.

Experimental
Overview
The workflow overview from sample preparation through LC-MS/MS analysis is shown in Figure 1. Samples were homogenized and extracted according to the European EN 15662 QuEChERS protocol prior to injection into the LC-MS/MS system.[1,2] The ready-to-use QuEChERS sample preparation kit containing extraction tubes and associated protocol was used for sample preparation. Identification of pesticide residues was based on retention time, the presence of a minimum of two product ions, and ion-ratio confirmation using selected reaction monitoring (SRM) of characteristic transition ions. Quantification was calculated using matrix-matched calibration. All method performance criteria were established according to the relevant EU guidelines.[3-7]

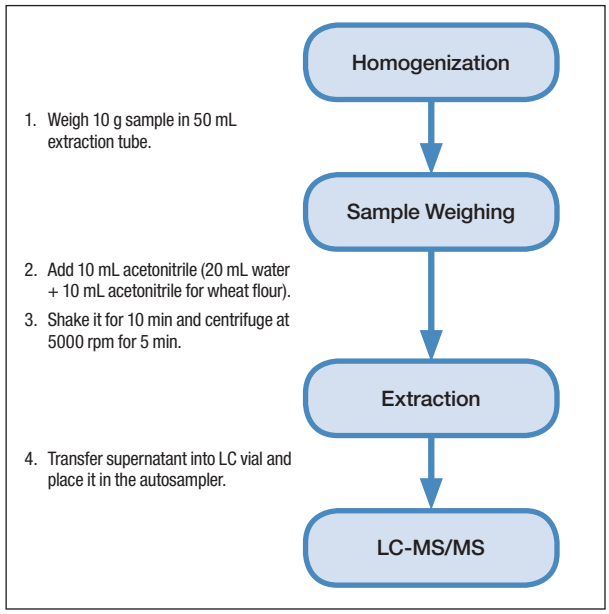

Figure 1. Workflow overview.

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Pesticide Explorer Collection:
www.thermoscientific.com/Pesticides-LCMS

Method Supplies

Table 1 lists reagents, instruments, and consumables used.

The pesticides standards were purchased from Sigma-Aldrich® (Germany) and Laboratory Instruments Srl (CASTELLANA GROTTE, Italy). Quality control materials used were FAPAS #T19140 (lettuce puree), FAPAS #19110 (lettuce puree), FAPAS #T19142 (melon puree), and FAPAS #T0983 (wheat flour). FAPAS samples were selected primarily based on their content of target pesticides. However, due to limited availability, some of the matrices are different from the matrices spiked and analyzed (i.e. lettuce and melon puree versus strawberry and leek).

Table 1. Reagents, instruments, and consumables.

Method Supplies	Fisher Scientific Part Number/ Source
Reagents	
Acetonitrile (ACN), LC/MS grade	AC61514-0025
Ammonium formate, 99%	AC40115-2500
Formic acid, Optima™ LC/MS grade	A117-50
Methanol, Optima™ LC/MS grade	A456-212
Purified water	Obtained from Thermo Scientific™ Barnstead™ Easypure™ II water system
Water, LC-MS grade	AC61515-0025
Instruments	
TSQ Endura triple quadrupole mass spectrometer	
UltiMate 3000 RSLC	
Method Supplies	**Thermo Fisher Scientific Part Number**
Consumables	
QuEChERS extraction tube, 50 mL, 250 pack	60105-216
Accucore aQ column 100 x 2.1 mm, 2.6 µm	17326-102130

Sample Preparation

Blank matrix samples [strawberry (SB), wheat flour (WF) and leek (LK)] used for validation experiments were purchased in local retail stores and were homogenized with an Ultra-Turrax homogenizer and extracted prior to fortified sample preparation. Matrix extracts were used as matrix blank samples and for preparation of matrix-matched calibration standards. Ready to use QuEChERS extraction kits were used for sample preparation, and contained 4 g $MgSO_4$, 1 g NaCl, 1 g trisodium citrate dehydrate, and 0.5 g sodium citrate for buffered extraction of target compounds. The same QuEChERS sample preparation protocol was applied to all three of the matrices analyzed, however a modification was made for flour in which water was added to wet the matrix. No cleanup was used.

Homogenization of matrices was performed using the following steps:

1. A relatively large amount of each matrix (~500 g) was placed into an appropriately sized beaker and labeled.
2. A G25 dispergation tool was attached to the Ultra-Turrax homogenizer. (Note: For better recovery for some unstable compounds cryogenic homogenization is advised.[8]).
3. Homogenization was performed at middle rotation speed (speed level 2–3) to create smooth homogenate.

Sample extraction was performed using the following steps:

1. 10 g sample was weighed into a 50 mL QuEChERS extraction tube.
2. 10 mL ACN was added to the SB and LK samples. For WF, 20 mL water was added to completely wet samples, and then 10 mL ACN was added.
3. Samples were shaken for 10 min on a horizontal shaker and centrifuged at 5000 rpm for 5 min.
4. The supernatant was collected and 1 mL was transferred into a LC vial for instrumental analysis.

LC-MS/MS Analysis

LC-MS/MS analysis was carried out using an UltiMate 3000 RSLC system coupled to a TSQ Endura triple quadrupole mass spectrometer. TraceFinder software (revision 3.2 SP2) was used for instrument control, analysis, data review, and reporting. The LC conditions and gradient are shown in Tables 2 and 3. The LC gradient was optimized to reduce analysis time to 15 minutes, while maintaining good chromatographic separation.

Table 2. LC conditions.

LC conditions	
Injection volume	1 µL
Column temperature	25 °C
Flow rate	300 µL/min
Analytical column	Accucore aQ column, 100 x 2.1 mm, 2.6 µm
Run time	15 minutes
Tray temperature	10 °C
Needle-cleaning solvent	20% Methanol in water
Sample loop	100 µL
Mobile phases	A: Water with 5 mM ammonium formate and 0.1% formic acid
	B: Methanol with 5 mM ammonium formate and 0.1% formic acid

Table 3. LC gradient.

Time (min)	Flow (mL/min)	A%	B%
0	0.300	100	0
0.5	0.300	100	0
7	0.300	30	70
9	0.300	0	100
12	0.300	0	100
12.1	0.300	100	0
15	0.300	100	0

The TSQ Endura triple quadrupole mass spectrometer was operated in timed-SRM mode. All SRM traces (parent, qualifier, quantifier ion) were individually tuned for each target analyte by direct infusion of each working standard solution. The mass spectrometer settings are provided in Table 4. For convenience and fast method implementation, the complete method including SRM settings is included with the Pesticide Explorer Collection Standard Quantitation Configuration.

Table 4. MS settings.

MS settings	
Ionization mode	Heated electrospray (HESI)
Scan type	Timed-SRM
Polarity	Positive/Negative switching
Spray voltage for Positive mode	3700 V
Spray voltage for Negative mode	2500 V
Sheath gas pressure	30 arbitrary units (Arb)
Aux gas pressure	6 Arb
Sweep gas pressure	1 Arb
Ion transfer tube temperature	325 °C
Vaporizer temperature	350 °C
CID gas pressure	2 mTorr
Cycle time	0.5 s
Q1 resolution (FWHM)	0.7
Q3 resolution (FWHM)	0.7
Chrom filter	3 s

Results and Discussion

To evaluate method performance, three matrices, strawberry, leek (the most complex), and wheat flour, were analyzed. European Union guidelines for single laboratory validation and pesticide residue analysis were used to establish method performance criteria, including linearity, matrix effect, LOD, LOQ, precision, and trueness (bias). All method performance parameters were compared to the relevant legislative requirements and MRLs. For compounds containing more than one isoform, only one performance criterion was established.

Figure 2 shows the LC-MS/MS chromatogram of the strawberry extract spiked with more than 250 pesticides at a concentration of 100 µg/kg (1 µL injection). Despite the short chromatographic run time (15 min), good separation and detection of the pesticide compounds were achieved using the timed-SRM mode. With timed-SRM, data acquisition for a particular target compound is performed in a short retention time window around the known compound retention time. Timed-SRM significantly reduces the number of SRM transitions that are monitored in parallel within a certain retention time window. A longer measurement time (dwell time) is therefore available for each transition, resulting in higher sensitivity and lower quantitation limits, improved RSDs and more data points per chromatographic peak—in this case a minimum of 10 to 12 data points.

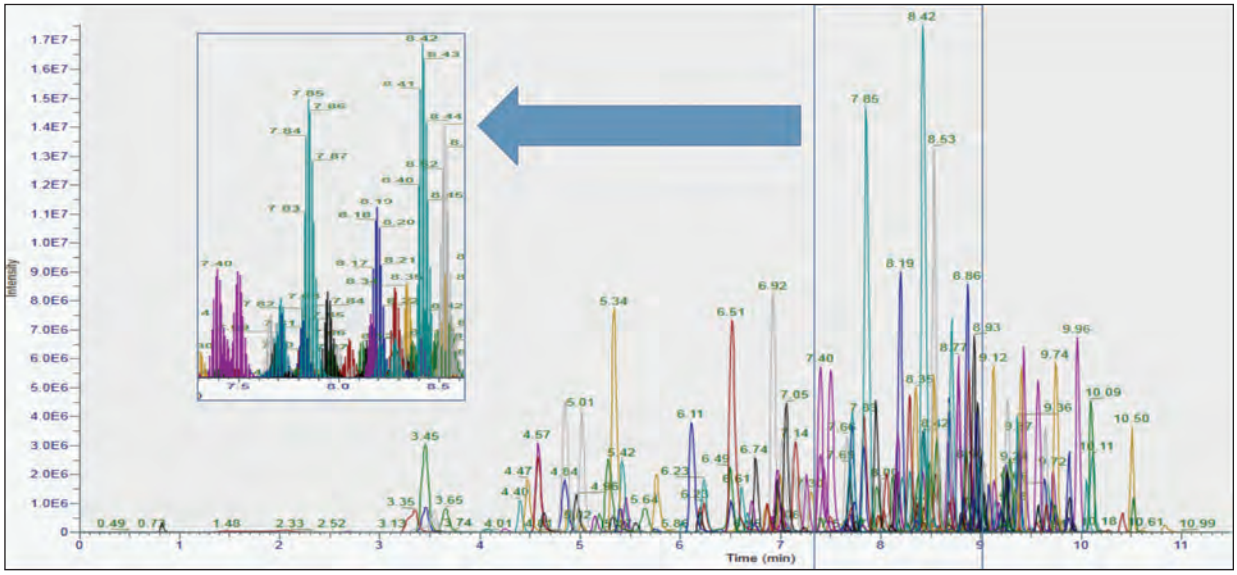

Figure 2. The LC-MS/MS chromatogram of more than 250 pesticides spiked into strawberry extract at 100 µg/kg shows good separation of compounds. Enough scans across the chromatographic peak were obtained throughout the chromatogram.

For the three matrices, including the very complex leek matrix, the LOD and LOQ values obtained demonstrated that the method enabled quantification of target pesticides below regulated MRLs. Table 5 presents the method LODs and LOQs for the target pesticides in the matrices tested. Table 6 compares the LOQ values obtained with the MRLs for selected pesticides. The pesticides selected in Table 6 represent different ionization modes and a range of retention times across the chromatogram. All compounds were detected and quantified below established MRLs.

Table 5. Method performance: LODs and LOQs (µg/kg) for target pesticides by matrix tested. LOQs were estimated taking into account reproducibility (RSDs≤ 15%) and ion ratio criteria.

	Name	Polarity	RT	Strawberry		Leek		Flour	
				LOD	LOQ	LOD	LOQ	LOD	LOQ
1	2,4-D	-	7.6	5	10	5	10	2	5
2	Abamectin b1a (NH_4)	+	10.2	0.1	0.2	0.3	1	0.3	1
3	Acephate	+	2.9	0.5	1	0.5	1	1	3
4	Acetamiprid	+	5.6	0.1	0.5	0.3	1	0.1	0.3
5	Acibenzolar-S-methyl	+	8.8	1	2	2	5	0.1	0.3
6	Alachlor	+	8.9	1	5	1	5	1	3
7	Aldicarb sulfone	+	4.8	0.5	1	0.5	1	0.3	1
8	Allethrin	+	8.7	0.3	1	0.3	1	1	3
9	Ametryn	+	7.8	0.2	0.6	0.1	0.3	0.1	0.3
10	Aminocarb	+	3.5	0.05	0.1	0.05	0.1	0.03	0.1
11	Ancymidol	+	7.1	0.5	1	0.5	1	0.1	0.3
12	Anilofos	+	9.1	0.03	0.1	0.03	0.1	0.1	0.3
13	Aramite (NH_4)	+	9.7	0.03	0.1	0.03	0.1	0.03	0.1
14	Atrazine	+	7.7	0.1	0.5	0.1	0.5	0.03	0.1
15	Azaconazole	+	8.0	0.5	1	0.1	0.5	0.2	0.6
16	Azamethiphos	+	6.7	0.05	0.1	0.3	1	0.1	0.3
17	Azinphos-ethy	+	8.8	5	10	5	10	0.3	1
18	Azinphos-methyl	+	8.2	0.5	1	1	5	1	5
19	Azoxystrobin	+	8.2	0.003	0.01	0.003	0.01	0.1	0.3
20	Bendiocarb	+	6.9	0.3	1	0.3	1	0.3	1
21	Benodanil	+	7.7	0.1	0.5	0.1	0.3	0.1	0.3
22	Benoxacor	+	8.1	0.3	1	0.3	1	0.3	1
23	Bensulfuron methyl	+	8.1	0.1	0.5	0.3	1	0.3	1
24	Bentazon	-	6.7	0.3	1	0.2	0.6	0.1	0.3
25	Benzoximate	+	9.3	0.1	0.5	0.3	1	0.3	1
26	Benzoylprop-ethyl	+	9.2	0.1	0.5	0.3	1	0.3	1
27	Bifenazate	+	8.7	0.3	1	2	5	2	5
28	Bitertanol	+	9.3	0.5	2	0.5	2	2	5
29	Boscalid	+	8.4	0.5	1	0.05	0.1	0.1	0.3
30	Brodifacoum	+	10.4	0.1	0.5	0.2	0.5	0.2	0.6
31	Bromacil	+	6.9	0.3	1	0.3	1	0.3	1
32	Bromoxynil	+	7.6	0.1	0.5	0.2	0.6	0.1	0.3
33	Bromuconazole	+	8.7	0.5	1	0.5	1	1	3
34	Bupirimate	+	8.8	0.5	1	0.5	1	0.3	1
35	Buprofezin	+	9.7	0.2	0.5	0.3	1	0.3	1
36	Butachlor	+	9.8	0.2	0.6	1	3	2	5
37	Butafenacil (NH_4)	+	8.7	0.1	0.3	0.1	0.3	0.03	0.1
38	Butocarboxim sulfoxide	+	3.5	0.5	1	1	3	1	3
39	Butoxycarboxim	+	4.8	0.1	0.3	0.1	0.3	0.3	1
40	Carbaryl	+	7.3	0.3	1	0.3	1	0.3	1
41	Carbendazim	+	4.6	0.2	0.5	0.2	0.5	0.1	0.3
42	Carbetamide	+	6.6	0.03	0.1	0.1	0.5	0.3	1
43	Carbofuran	+	6.9	0.03	0.1	0.03	0.1	0.03	0.1
44	Carbofuran-3-hydroxy	+	5.4	0.3	1	0.3	1	0.3	1
45	Carfentrazone-ethyl	+	9.0	0.3	1	0.03	0.1	0.1	0.3
46	Carpropamid	+	9.2	0.3	1	0.3	1	0.1	0.3
47	Chlorantraniliprole	+	8.0	0.3	1	0.3	1	0.3	1

	Name	Polarity	RT	Strawberry		Leek		Flour	
				LOD	LOQ	LOD	LOQ	LOD	LOQ
48	Chlorbromuron	+	8.6	0.2	0.5	0.3	1	0.3	1
49	Chlorfenvinphos	+	9.1	0.03	0.1	0.05	0.1	0.1	0.3
50	Chlorfluazuron	+	10.1	0.3	1	0.3	1	0.1	0.3
51	Chloridazon (pyrazone)	+	5.6	0.3	1	0.3	1	0.3	1
52	Chlormequat	+	0.7	0.2	0.5	0.03	0.1	0.01	0.03
53	Chlorotoluron	+	7.7	0.3	1	0.3	1	0.3	1
54	Chloroxuron	+	8.8	0.2	0.5	0.2	0.6	0.1	0.3
55	Chlorpyrifos	+	9.9	0.03	0.1	0.1	0.3	0.3	1
56	Cinosulfuron	+	6.7	0.03	0.1	0.2	0.6	0.001	0.005
57	Clethodim	+	9.5	0.3	1	2	6	2	5
58	Clomazone	+	8.2	0.03	0.1	0.03	0.1	0.1	0.3
59	Clothianidin	+	5.2	0.3	1	0.5	2	1	3
60	Coumaphos	+	9.2	0.3	1	0.3	1	0.3	1
61	Crotoxyphos (NH_4)	+	8.4	0.03	0.1	0.1	0.3	0.3	1
62	Cumyluron	+	8.7	0.03	0.1	0.3	1	0.1	0.3
63	Cyanazine	+	6.7	0.03	0.1	0.3	1	0.1	0.3
64	Cyazofamid	+	8.9	0.03	0.1	0.3	1	0.3	1
65	Cycloate	+	9.5	0.3	1	0.3	1	0.1	0.3
66	Cycluron	+	7.9	0.3	1	0.3	1	0.1	0.3
67	Cyflufenamid	+	9.2	0.3	1	0.1	0.3	0.1	0.3
68	Cyromazine	+	1.7	2	5	2	5	5	10
69	Demeton-S-methyl sulfone	+	4.4	0.03	0.1	0.1	0.3	0.1	0.3
70	Desmedipham	+	8.0	10	30	2	5	10	20
71	Desmethyl-pirimicarb	+	5.0	0.1	0.5	0.2	0.6	0.3	1
72	Desmetryn	+	7.1	0.1	0.5	0.2	0.6	0.2	0.6
73	Diclobutrazol	+	8.8	0.3	1	1	3	0.1	0.3
74	Dicrotophos	+	4.9	0.3	1	1	3	1	3
75	Diethofencarb	+	8.1	0.2	0.5	0.3	1	0.1	0.3
76	Difenacoum	+	10.1	0.03	0.1	0.2	0.6	0.1	0.3
77	Difenoconazole	+	9.4	0.2	0.5	0.03	0.1	0.03	0.1
78	Diflubenzuron	+	9.0	0.3	1	0.1	0.3	0.03	0.1
79	Dimefuron	+	8.0	0.1	0.3	0.3	1	0.3	1
80	Dimethametryn	+	8.9	0.1	0.3	0.1	0.3	0.2	0.6
81	Dimethenamid	+	8.4	0.3	1	0.1	0.3	0.1	0.3
82	Dimethoate	+	5.5	0.03	0.1	0.1	0.3	0.1	0.3
83	Dimethomorph	+	8.3	0.3	1	0.03	0.1	0.1	0.3
84	Dimoxystrobin	+	9.0	0.2	0.5	0.03	0.1	0.1	0.3
85	Diniconazole	+	9.4	0.3	1	0.3	1	0.3	1
86	Dinotefuran	+	3.7	0.3	1	0.3	1	0.3	1
87	Dithiopyr	+	9.5	0.3	1	2	5	0.3	1
88	Diuron	+	7.5	0.03	0.1	0.1	0.3	0.3	1
89	DNOC	-	7.7	0.3	1	1	3	0.3	1
90	Dodemorph	+	8.1	0.3	1	1	3	0.3	1
91	Epoxiconazole	+	8.9	0.3	1	0.03	0.1	0.1	0.3
92	Esprocarb	+	9.7	0.03	0.1	0.1	0.3	0.1	0.3
93	Etaconazol	+	8.8	0.3	1	0.3	1	0.1	0.3
94	Ethiofencarb	+	8.2	0.3	1	2	5	1	3
95	Ethiofencarb-sulfone	+	4.9	0.3	1	0.3	1	0.1	0.3

	Name	Polarity	RT	Strawberry		Leek		Flour	
				LOD	LOQ	LOD	LOQ	LOD	LOQ
96	Ethiofencarb-sulfoxide	+	5.0	0.03	0.1	0.03	0.1	0.1	0.3
97	Ethiprole	+	8.3	0.3	1	1	3	1	3
98	Ethirimol	+	6.2	0.3	1	0.1	0.5	0.1	0.3
99	Ethofumesate	+	8.3	0.3	1	5	20	5	20
100	Ethoxyquin	+	7.7	0.3	1	1	3	0.3	1
101	Etofenprox (NH_4)	+	10.5	0.03	0.1	0.3	1	1	3
102	Etoxazole	+	10.0	0.02	0.05	0.03	0.1	0.1	0.3
103	Etrimfos	+	9.1	0.3	1	0.1	0.3	1	3
104	Fenamidone	+	8.3	0.03	0.1	0.3	1	0.3	1
105	Fenamiphos	+	8.9	0.03	0.1	0.03	0.1	0.03	0.1
106	Fenarimol	+	8.8	0.3	1	0.1	0.3	0.1	0.3
107	Fenazaquin	+	10.5	0.1	0.3	1	3	0.5	1.5
108	Fenbuconazole	+	8.9	0.3	1	0.3	1	2	5
109	Fenhexamid	+	8.7	0.003	0.01	1	3	0.3	1
110	Fenobucarb	+	8.2	0.03	0.1	0.1	0.3	0.1	0.3
111	Fenoxanil	+	9.4	0.1	0.3	0.03	0.1	0.1	0.3
112	Fenoxycarb	+	9.0	0.1	0.5	0.03	0.1	0.03	0.1
113	Fenpyroximat	+	10.1	0.003	0.01	0.01	0.03	0.03	0.1
114	Fensulfothion	+	7.8	0.03	0.1	0.03	1	0.3	1
115	Fenthion	+	9.2	0.3	1	1	3	0.3	1
116	Fenthion-sulfoxide	+	7.3	0.06	0.2	0.1	0.3	0.1	0.3
117	Fenuron	+	5.3	0.1	0.3	0.1	0.3	0.03	0.1
118	Flazasulfuron	+	8.1	0.1	0.3	0.3	1	0.03	0.1
119	Florasulam	+	6.2	0.1	0.3	0.03	0.1	0.1	0.3
120	Fluazifop	+	8.3	0.3	0.6	0.3	1	1	3
121	Fluazinam	-	9.7	0.03	0.1	0.3	1	0.1	0.3
122	Flubendiamide	+	9.0	1.5	5	1.5	5	5	10
123	Flufenacet	+	8.8	0.1	0.3	0.3	1	0.1	0.3
124	Flufenoxuron	+	9.9	0.3	1	0.1	0.3	0.3	1
125	Flumetsulam	+	5.3	0.1	0.3	0.3	1	0.3	1
126	Fluometuron	+	7.5	0.3	1	0.1	0.3	0.03	0.1
127	Fluopicolide	+	8.5	0.3	1	0.1	0.3	0.1	0.3
128	Fluopyram	+	8.7	0.03	0.1	0.03	0.1	0.1	0.3
129	Fluorochloridone	+	8.7	0.3	1	0.3	1	1	3
130	Fluoxastrobin	+	8.7	0.2	0.5	0.3	1	0.1	0.3
131	Fluquinconazole	+	8.7	0.3	1	0.5	1	0.3	1
132	Flusilazole	+	9.0	0.2	0.5	0.2	0.5	0.2	0.6
133	Flutriafol	+	7.7	0.1	0.3	0.3	1	0.3	1
134	Forchlorfenuron	+	8.0	0.1	0.5	0.1	0.3	0.1	0.3
135	Formetanate hydrochloride	+	3.4	0.3	1	0.3	1	0.1	0.3
136	Formothion	+	6.6	2	5	2	5	3	10
137	Fosthiazate	+	7.4	0.1	0.3	0.1	0.3	0.01	0.03
138	Fuberidazole	+	5.4	0.2	0.6	1	3	0.3	1
139	Furathiocarb	+	9.6	0.3	1	5	10	1	3
140	Griseofulvin	+	7.7	0.3	0.6	0.3	1	0.3	1
141	Halofenozide	-	8.4	0.3	0.6	0.3	1	0.01	0.03
142	Haloxyfop	+	8.9	0.3	1	0.1	0.3	0.03	0.1
143	Haloxyfop-methyl	+	9.4	0.02	0.05	0.3	1	0.1	0.3

	Name	Polarity	RT	Strawberry		Leek		Flour	
				LOD	LOQ	LOD	LOQ	LOD	LOQ
144	Heptenophos	+	7.9	0.3	1	1	3	0.3	1
145	Hexaconazole	+	9.2	0.3	1	0.5	1.5	0.3	1
146	Hexaflumuron	-	9.5	0.3	1	0.3	1	0.1	0.3
147	Hexazinone	+	7.0	0.03	0.1	0.1	0.3	0.1	0.3
148	Hexythiazox	+	9.9	0.03	0.1	0.1	0.3	0.1	0.3
149	Imazalil	+	7.7	0.3	1	0.1	0.3	0.3	1
150	Imazaquin	+	7.0	0.2	0.6	0.3	1	0.1	0.3
151	Imazethapyr	+	6.5	0.03	0.1	0.1	0.3	0.1	0.3
152	Imibenconazole	+	9.8	0.1	0.3	0.3	1	1	3
153	Imidacloprid	+	5.1	0.1	0.3	0.1	0.3	0.5	1.5
154	Indoxacarb	+	9.4	0.3	1	1	3	0.1	0.3
155	Ioxynil	-	8.1	1	3	0.3	1	1	3
156	Iprovalicarb	+	8.7	0.3	1	1	3	1	3
157	Isocarbophos	+	7.8	0.3	1	0.1	0.3	2	5
158	Isoprocarb	+	7.7	0.2	0.6	0.3	1	0.3	1
159	Isoprothiolane	+	8.5	0.3	1	0.1	0.3	1	3
160	Isoproturon	+	7.8	0.1	0.3	0.1	0.3	0.03	0.1
161	Isoxaben	+	8.4	0.02	0.05	0.03	0.1	0.1	0.3
162	Isoxadifen-ethyl	+	9.0	0.3	1	0.3	1	0.1	0.3
163	Kresoxim-methyl	+	9.0	0.3	1	1	3	2	5
164	Lenacil	+	7.7	0.03	0.1	0.3	1	0.3	1
165	Malaoxon	+	7.0	0.1	0.3	0.5	1.5	0.1	0.3
166	Mandipropamid	+	8.4	0.3	1	0.3	1	0.3	1
167	MCPA	-	7.8	0.5	2	1	3	2	5
168	Mefenacet	+	8.7	0.03	0.1	0.03	0.1	0.03	0.1
169	Mepiquat chloride	+	0.8	0.1	0.3	0.03	0.1	0.03	0.1
170	Mepronil	+	8.6	0.1	0.3	0.1	0.3	0.03	0.1
171	Metamitron	+	5.4	0.3	1	1	3	1	3
172	Metazachlor	+	7.7	0.3	1	0.1	0.3	0.1	0.3
173	Metconazole	+	7.2	0.1	0.3	0.1	0.3	0.1	0.3
174	Methabenzthiazuron	+	8.0	0.1	0.3	0.03	0.1	0.03	0.1
175	Methamidophos	+	2.1	0.3	1	1	3	1	3
176	Methiocarb	+	8.4	0.2	0.6	0.1	0.3	0.1	0.3
177	Methiocarb-sulfone	+	5.8	0.1	0.3	0.1	0.3	0.1	0.3
178	Methiocarb-sulfoxide	+	5.3	0.03	0.1	0.1	0.3	0.1	0.3
179	Methomyl	+	4.2	0.03	0.1	0.1	0.3	0.3	1
180	Methoprotryne	+	7.9	0.1	0.3	0.1	0.3	0.3	1
181	Methoxyfenozide	+	8.6	0.1	0.3	1	3	1	3
182	Metobromuron	+	7.8	0.1	0.3	1	3	0.3	1
183	Metolachlor	+	8.9	0.3	1	0.1	0.3	0.3	1
184	Metolcarb	+	6.6	0.2	0.6	0.3	1	0.3	1
185	Metosulam	+	7.1	0.1	0.3	0.3	1	0.1	0.3
186	Metoxuron	+	6.4	0.3	1	2	5	1	3
187	Metrafenone	+	9.3	0.1	0.3	0.3	1	0.3	1
188	Metsulfuron-methyl	+	7.0	0.3	1	0.1	0.3	0.1	0.3
189	Mevinphos	+	6.0	0.03	0.1	1	3	0.1	0.3
190	Mexacarbate	+	4.8	0.03	0.1	0.1	0.3	0.3	1
191	Monocrotophos	+	4.6	0.2	0.6	0.1	0.3	0.1	0.3

	Name	Polarity	RT	Strawberry		Leek		Flour	
				LOD	LOQ	LOD	LOQ	LOD	LOQ
192	Monolinuron	+	7.5	0.03	0.1	0.1	0.3	0.1	0.3
193	Napropamide	+	8.9	0.1	0.3	0.3	1	0.03	0.1
194	Neburon	+	9.1	0.3	1	0.1	0.3	2	5
195	Nicosulfuron	+	6.9	0.3	1	0.1	0.3	0.1	0.3
196	Nuarimol	+	8.3	0.3	1	0.3	1	1	3
197	Ofurace	+	7.0	0.3	1	0.3	1	0.3	1
198	Omethoate	+	3.3	0.1	0.3	0.1	0.3	0.1	0.3
199	Oxadixyl	+	6.5	0.3	1	0.3	1	0.3	1
200	Oxamyl (NH4)	+	4.0	0.1	0.3	0.1	0.3	0.01	0.05
201	Paclobutrazol	+	8.5	0.3	1	0.3	1	1	3
202	Penconazole	+	9.1	0.1	0.3	0.1	0.3	0.2	0.6
203	Pencycuron	+	9.4	0.1	0.3	0.1	0.3	0.1	0.3
204	Phenmedipham	+	8.0	2	5	2	5	5	10
205	Phenthoate	+	9.0	0.1	0.3	0.1	0.3	0.3	1
206	Phoxim	+	9.3	0.3	1	2	5	2	5
207	Picoxystrobin	+	9.0	0.03	0.1	0.3	1	0.3	1
208	Piperonyl butoxide	+	9.8	0.003	0.01	0.1	0.3	0.03	0.1
209	Piperophos	+	9.4	0.03	0.1	0.03	0.1	0.03	0.1
210	Pirimicarb	+	6.2	0.1	0.3	0.3	1	0.3	1
211	Pirimiphos-methyl	+	9.3	0.03	0.1	0.03	0.1	0.03	0.1
212	Primisulfuron-methyl	+	8.6	0.2	0.5	0.3	1	0.1	0.3
213	Prochloraz	+	9.3	0.1	0.3	0.1	0.3	0.3	1
214	Profenophos	+	9.6	0.03	0.1	0.01	0.03	0.03	0.1
215	Promecarb	+	8.5	0.1	0.3	0.3	1	0.1	0.3
216	Prometon	+	7.4	0.03	0.1	0.1	0.3	0.3	1
217	Prometryn	+	8.4	0.2	0.6	0.2	0.6	0.2	0.6
218	Propamocarb	+	3.5	0.03	0.1	0.03	0.1	0.03	0.1
219	Propazine	+	8.3	0.1	0.3	0.3	1	0.3	1
220	Propetamphos	+	8.6	0.3	1	1	3	0.03	0.1
221	Propiconazole	+	9.2	0.1	0.3	0.1	0.3	0.1	0.3
222	Propoxur	+	6.9	0.3	1	0.3	1	0.03	0.1
223	Propyzamide	+	8.6	0.3	1	0.3	1	1	3
224	Prosulfocarb	+	9.6	0.1	0.3	0.3	1	0.1	0.3
225	Pymetrozine	+	3.5	0.1	0.3	0.1	0.3	0.1	0.3
226	Pyraclostrobin	+	9.3	0.03	0.1	0.1	0.3	0.01	0.03
227	Pyrimethanil	+	8.3	0.3	1	0.3	1	0.3	1
228	Pyroxsulam	+	7.0	0.1	0.3	0.03	0.1	0.1	0.3
229	Quinoxyfen	+	10.1	0.03	0.1	0.1	0.3	0.1	0.3
230	Quizalofop-ethyl	+	9.6	0.03	0.1	0.1	0.3	0.1	0.3
231	Quizalofop-p	+	8.9	0.3	1	2	5	2	5
232	Resmethrin	+	10.3	1	3	n	n	10	20
233	Rimsulfuron	+	7.4	0.03	0.1	0.3	1	0.3	1
234	Rotenone	+	8.9	0.3	1	0.3	1	0.3	1
235	Schradan	+	5.8	0.03	0.1	0.3	1	0.03	0.1
236	Sethoxydim	+	9.7	2	5	50	100	2	5
237	Simeconazole	+	8.8	0.3	1	5	10	5	10
238	Simetryn	+	7.1	0.3	1	0.3	1	0.3	1
239	Spinosad A	+	9.3	0.3	1	0.3	1	0.3	1

	Name	Polarity	RT	Strawberry		Leek		Flour	
				LOD	LOQ	LOD	LOQ	LOD	LOQ
240	Spiromesifen	+	9.9	0.3	1	1	3	1	3
241	Spirotetramat	+	8.7	0.03	0.1	0.1	0.3	0.3	1
242	Spiroxamine	+	8.6	0.1	0.3	0.3	1	0.3	1
243	Sulfotep	+	9.1	0.1	0.3	0.3	1	0.3	1
244	Sulprofos	+	9.9	1	3	1	3	2	5
245	Tebuconazole	+	9.1	0.3	1	0.03	0.1	0.1	0.3
246	Tebufenozide	+	9.0	0.3	1	1	3	2	5
247	Tebufenpyrad	+	9.7	0.03	0.1	0.3	1	0.1	0.3
248	Tebuthiuron	+	7.1	0.03	0.1	0.1	0.3	0.3	1
249	Teflubenzuron	+	9.4	1	3	2	5	5	10
250	Tepraloxydim	+	8.7	1	3	5	10	5	10
251	Terbumeton	+	7.5	0.1	0.3	0.1	0.3	0.1	0.3
252	Terbuthylazine	+	8.4	0.1	0.3	0.1	0.3	0.1	0.3
253	Terbutryn	+	8.5	0.1	0.3	0.3	1	0.3	1
254	Tetraconazole	+	8.8	0.3	1	0.3	1	0.1	0.3
255	Tetramethrin	+	9.7	0.3	1	2	5	1	3
256	Thiabendazole	+	5.3	0.3	1	0.3	1	0.3	1
257	Thiacloprid	+	6.1	0.03	0.1	0.03	0.1	0.03	0.1
258	Thiamethoxam	+	4.5	0.03	0.1	0.1	0.3	0.1	0.3
259	Thidiazuron	+	7.1	0.3	1	0.3	1	0.3	1
260	Thiobencarb	+	9.4	0.1	0.3	0.1	0.3	0.1	0.3
261	Thiophanate-methyl	+	6.9	0.3	1	0.3	1	0.3	1
262	Tolfenpyrad	+	9.7	0.03	0.1	0.1	0.3	0.1	0.3
263	Tralkoxydim	+	9.9	0.1	0.3	0.03	0.1	0.03	0.1
264	Triadimefon	+	8.6	1	3	5	10	1	3
265	Triadimenol	+	8.5	0.3	1	0.3	1	1	3
266	Triazophos	+	8.7	0.01	0.05	0.1	0.3	0.1	0.3
267	Trichlorfon	+	5.2	1	3	1	3	1	3
268	Tricyclazole	+	6.5	0.03	0.1	0.1	0.3	0.1	0.3
269	Tridemorph	+	9.2	0.3	1	2	5	0.1	0.3
270	Trietazine	+	8.8	0.3	1	0.1	0.3	0.1	0.3
271	Trifloxystrobin	+	9.4	0.03	0.1	0.03	0.1	0.03	0.1
272	Triflumizole	+	9.6	0.03	0.1	0.3	1	0.3	1
273	Vamidothion	+	5.4	0.01	0.03	0.03	0.1	0.1	0.3
274	Zoxamide	+	9.2	0.1	0.3	0.3	1	0.3	1

Table 6. Comparison of the method LOQ to the MRL for selected pesticides.

Analyte	MRL (µg/kg)			LOQ (µg/kg)		
	Strawberry	Leek	Flour	Strawberry	Leek	Flour
Acephate	10	10	10	1	1	1
Azoxystrobin	50000	10000	300	0.01	0.01	0.3
Carbaryl	50	10	500	1	1	1
Dimethomorph (sum of isomers)	50	1500	10	1	0.1	0.3
Diniconazole	50	10	10	1	1	1
Oxamyl	50	10	10	0.3	0.3	0.05
Pencycurone	50	50	50	0.3	0.3	0.3
Pyraclostrobin	100	700	200	0.1	0.3	0.03
Spinosad A	50	500	1000	1	1	1
Zoxamide	50	20	20	0.3	1	1

The relative standard deviation (RSD) is an important qualitative parameter that can be used instead of signal-to-noise ratio to provide a better estimate of LODs and LOQs. For pesticide residue analysis, SANCO/12571/2013 specifies repeatability criteria of 20% RSD for all compounds within the method scope. For example, as shown in Figure 3, 2.9% RSD was obtained for seven replicate injections of benzaton in the leek matrix at 10 µg/kg. The method RSDs at the MRLs were below 15%, establishing method reproducibility.

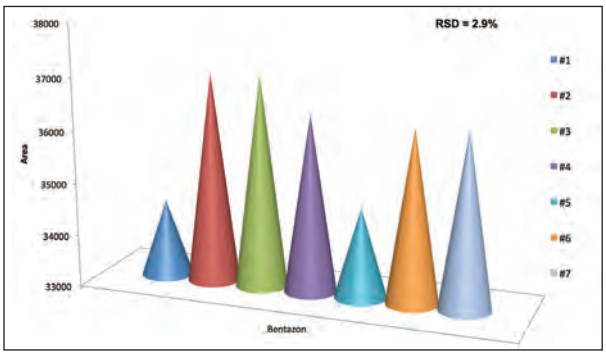

Figure 3. An RSD of 2.9% was obtained for seven replicate injections of bentazon in leek matrix at 10 µg/kg.

When using ESI, matrix effects can challenge accurate quantitation of pesticides. Though there are different strategies to compensate for these effects, the results presented in this application note are based on matrix-matched calibration. Figure 4 shows the effect of matrix on peak area. Although ion suppression is observed in the leek and wheat flour matrices, the method proved effective regardless of the matrix analyzed.

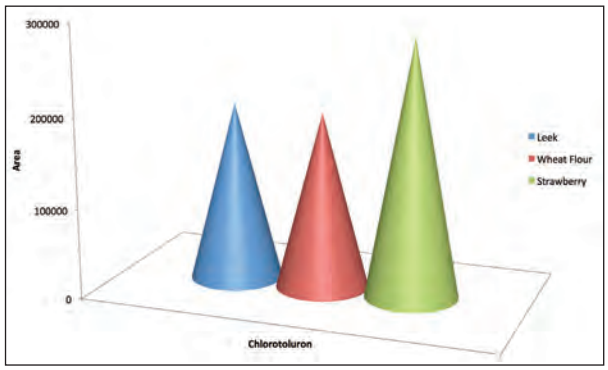

Figure 4. Matrix effects on peak area of chlorotoluron.

QuEChERS sample preparation offers a convenient and effective approach for extraction of pesticide residues in food matrices. The robust procedure has a number of compelling advantages: high recoveries, accurate results, high sample throughput, low solvent and glassware usage, reduced labor and bench space, and lower reagent costs. As shown in Figure 5, the percent recoveries achieved for selected pesticides at the 10 µg/kg level were acceptable and generally between 80 and 110% in the matrices analyzed. The pesticides selected represent results typical of all pesticides studied.

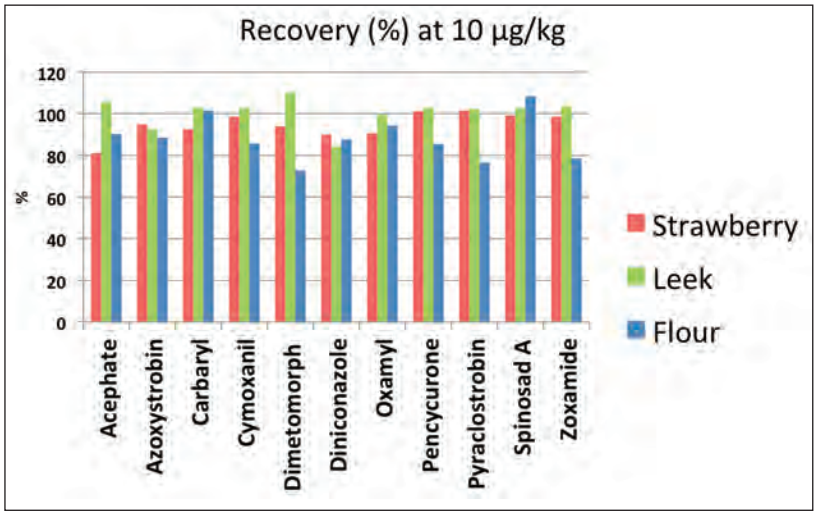

Figure 5. Recovery (%) of selected pesticides at the 10 µg/kg-level by matrix.

The quality control samples FAPAS #T19140 (lettuce puree), FAPAS #19110 (lettuce puree), FAPAS #T19142 (melon puree) and FAPAS #T0983 (wheat flour) were analyzed for their content of target pesticides to provide external quality control for method validation. As shown in Table 7, the measured target analyte values consistently fell within the acceptance range with acceptable %RSD values.

Table 7. External quality control (FAPAS) results for the relevant compounds.

Analyte	Fapas No.	Fapas Matrix	Assigned value (µg/kg)	Acceptance range (µg/kg)	Measured value (µg/kg)	RSD (%)
Carbaryl	T19142	Melon Puree	89.0	49.9–128.2	91.1	1.1
Diniconazol			52.3	29.3–75.3	59.7	9.0
Zoxamide			91.7	51.4–132.1	108.4	3.0
Pencycuron	T19140	Lettuce Puree	73.2	41.0–105.4	45.9	6.0
Thiamethoxam			48.8	27.3–70.3	36.3	9.1
Azoxystrobin	19110	Lettuce Puree	188.0	110–265	132.5	15.4
Dimetomorph (sum of isomers)			181.0	106–256	160.1	11.9
Propyzamide			197.0	116–277	195.1	16.5
Azoxystrobin	T0983	Wheat Flour	383.0	241–524	361.2	1.7
Fenhexamid			110.0	61–158	125.4	10.4
Imazalil			161.0	93–229	157.2	8.2
Thiabendazole				49.3–126.7	67.6	7.3

Conclusion

Regulations of the European Union pose some significant challenges to the analytical methods quantifying pesticide residues in complex matrices. This application note described a multi-residue LC-MS/MS method that uses the TSQ Endura triple quadrupole mass spectrometer-based Pesticide Explorer Collection Standard Quantitation solution for rapid and robust quantitation of more than 250 pesticides in fruit and vegetable matrices at their respective MRLs. For convenience and fast method implementation, the complete instrument and data processing method including SRM settings is included with the Pesticide Explorer Collection start-to-finish workflow solution.

The method results were shown to comply with the stringent guidelines set forth in SANCO/12571/2003 concerning sensitivity, accuracy, and precision. In 15 minutes, all target pesticides were detected and quantified in food matrices below established MRLs. Method RSDs at the MRLs were below 15%, establishing the method's reproducibility. Percent recoveries achieved at the 100 µg/kg-level using a standard QuEChERS sample preparation protocol were in general between 80 and 110%. The QuEChERS sample extraction procedure enabled analysis of only 1 µL sample, without need for dispersive SPE sample cleanup or sample dilution, with increased robustness and throughput.

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Increased Productivity in Pesticide Residue Analysis – Quantifying 440 Pesticides Following China GB 2763-2014: The Pesticide Explorer Collection – Standard Quantitation

Application Note 642

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Key Words
Pesticides analysis, food safety, TSQ Endura, TraceFinder, MRL, tSRM, residue analysis

Goal
Developing a robust, sensitive, high-throughput method for quantitation of 440 pesticide residues in bell pepper in a regulated environment.

Introduction
In recent years, growing concerns over food safety and the expanding world agricultural trade have led to the promulgation and enforcement of stricter pesticide regulations. In 2014, China's Ministry of Agriculture and Ministry of Health jointly issued a revised national food safety standard, GB 2763-2014 - Maximum Residue Limits for Pesticides in Food.[1] This new standard expanded the number of categories of pesticide residues and the total number of maximum residue limits (MRLs). Together with the Japanese Positive List System[2] and EU/EC Directive No. 752/2014,[3] these standards constitute some of the strictest food safety regulations globally and have fueled the need for faster and more sensitive analytical methods for cost-efficient, high-throughput screening and quantitation of multi-class pesticide residues. While it is critical to address the challenge of developing sensitive, robust analytical methods for pesticide residues, most existing solutions lack the ability to quantify multiple pesticide residues in one single experiment.

Here, a method utilizing the Thermo Scientific™ TSQ Endura™ triple quadrupole mass spectrometer and Thermo Scientific™ TraceFinder™ software is described for the simultaneous, high-throughput, quantitative analysis of 440 pesticide residues in bell pepper.

Experimental
Sample Preparation
Pesticide standards were obtained from ULTRA Scientific (North Kingstown, RI). The stock solution was prepared in acetonitrile at a concentration of 2.5 µg/mL. Calibration solutions were prepared by serial dilution of the pesticide stock solution in acetonitrile/water (40/60 v:v).

Bell pepper samples, provided by the California Department of Food and Agriculture (CDFA), were extracted using a QuEChERS method in which 5 g of homogenized bell pepper and 15 mL of acetonitrile were used. The final QuEChERS extracts were diluted with 1.5 times their volume of ultrapure water. Finally, the extracts were spiked with the pesticides standard, mixed, and vortexed thoroughly to produce a set of solutions with concentrations of 0.001 to 200 pg/µL (ppb).

Liquid Chromatography Method
Chromatographic separation was performed using the Thermo Scientific™ Dionex™ UltiMate™ 3000 ultra-high-performance liquid chromatography system, equipped with an UltiMate HPG3400-RS Rapid Separation Binary High-Pressure Gradient Pump, WPS-3000TRS Rapid Separation Well Plate Autosampler, and TCC-3000RS Rapid Separation Thermostatted Column Compartment.

The chromatographic conditions were as follows:

Column	Thermo Scientific™ Accucore™ aQ (100 x 2.1 mm, 2.6 µm), P/N 17326-102130
Mobile phases	Aqueous phase: Water + 5 mM ammonium formate + 0.1% formic acid Organic phase: Methanol + 5 mM ammonium formate + 0.1% formic acid
Flow rate	300 µL/min
Column temperature	30 °C

Gradient

Time (min)	% Aqueous	%Organic
0.0	98	2
0.5	98	2
2.0	60	40
20.0	5	95
22.0	5	95
22.1	98	2
25.0	98	2

Pesticide Explorer Collection:
www.thermoscientific.com/Pesticides-LCMS

Mass Spectrometry Method

Compounds were detected using the TSQ Endura MS equipped with a Thermo Scientific™ Easy-Max NG™ HESI III heated electrospray ionization source. Timed selected-reaction monitoring (tSRM) scan mode employing fast polarity switching was used. The tSRM times were based on the peak width differences of the pesticide residues; they were set to 1 min for the majority of compounds and up to 5 min for tridemorph, propiconazole and a small number of other substances.

The MS conditions were as follows:

Vaporizer temperature	450 °C
Ion transfer tube temperature	200 °C
Spray voltage	3500 V (ESI+); 2500 V (ESI-)
Sheath gas	60 arb
Auxiliary gas	5 arb
Sweep gas	1 arb
Q1 (FWHM)	0.7
Q3 (FWHM)	0.7
Collision cell pressure	1.5 mTorr
tSRM scan cycle time	1.2 s

Data Processing

Method development, data acquisition, and data processing were performed with TraceFinder software. TraceFinder software uses a compound database (CDB) that includes retention times and CAS numbers, plus other relevant information needed for confirmation of pesticides (Figure 1). Using the CDB, standard samples are no longer necessary for method optimization and development. Instead, relevant conditions can be imported from the database to directly conduct sample analysis.

The various tSRM conditions, including retention time, SRM fragmentations, RF lens voltage, and collision energy, were imported directly from the CDB within TraceFinder software (Figure 2). The drag-and-drop method editor accelerated method development and supported the flexible customization of various method templates.

As shown in Figure 3, TraceFinder software streamlines the laborious process of analyzing hundreds of pesticide residues simultaneously.

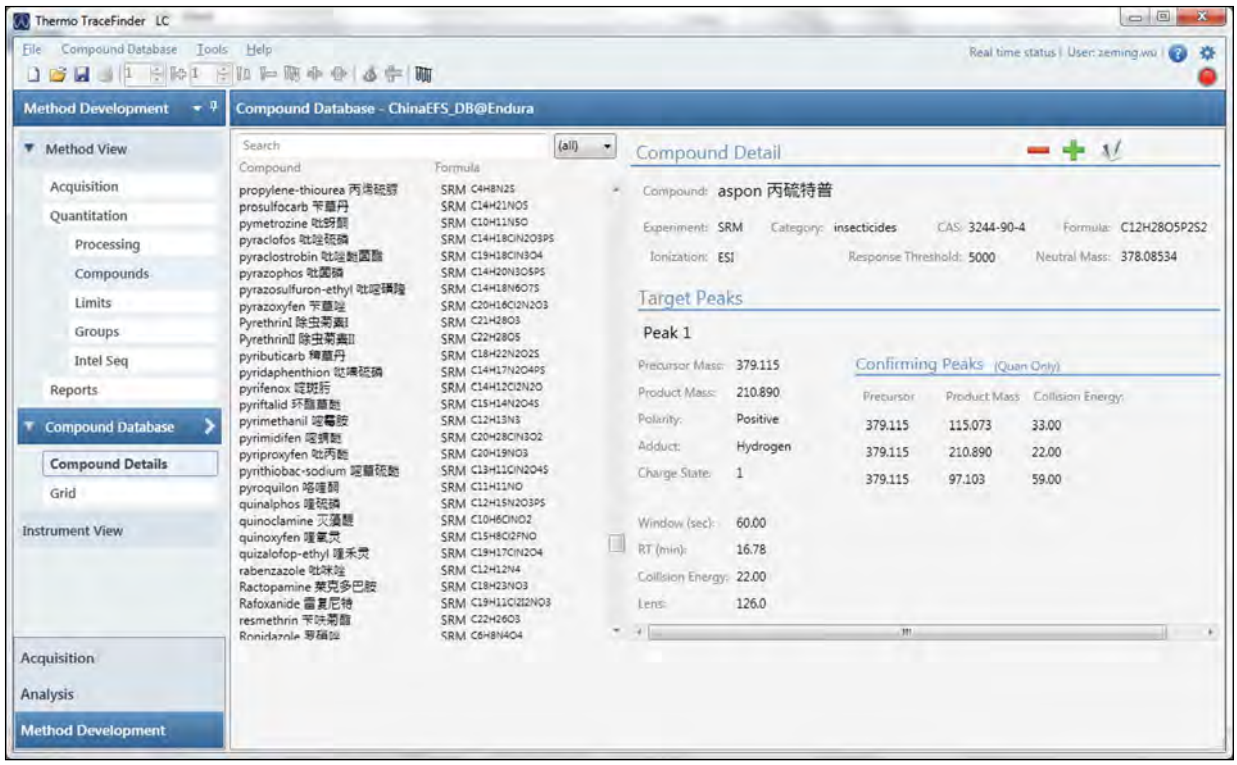

Figure 1. Pesticide detection method from TraceFinder CDB database.

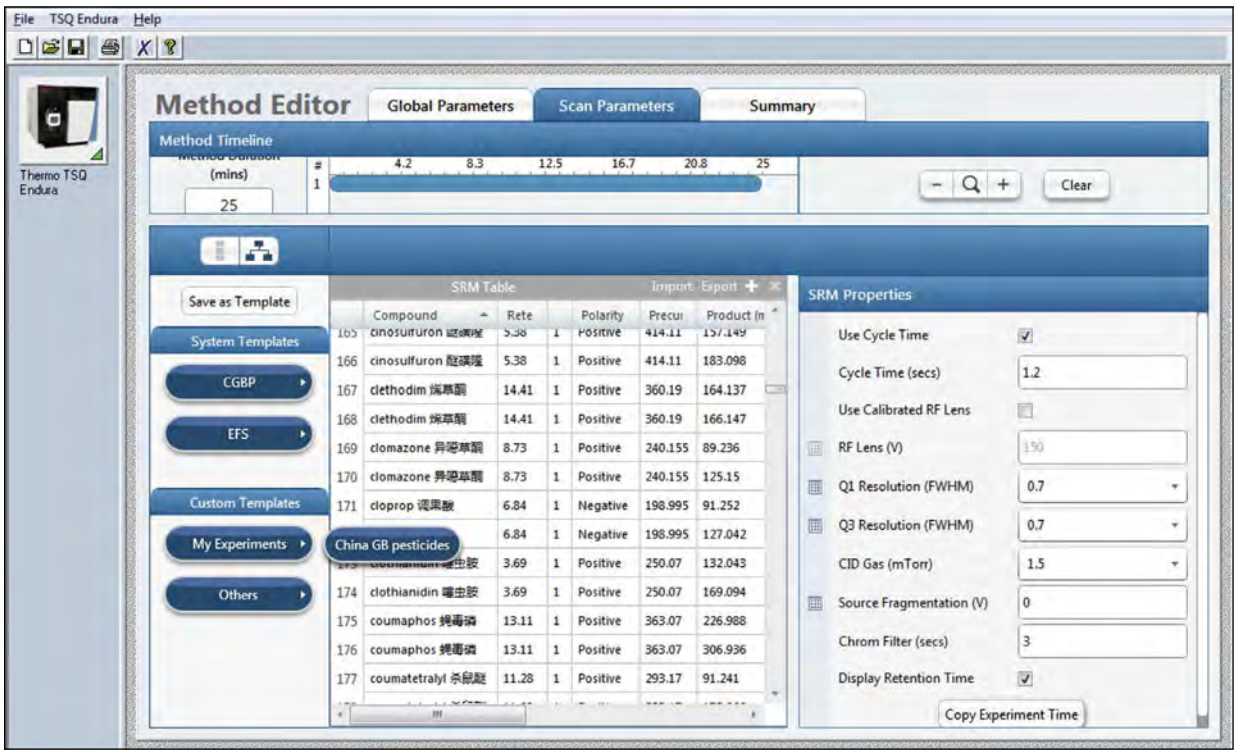

Figure 2. TraceFinder Method Editor, showing experimental conditions.

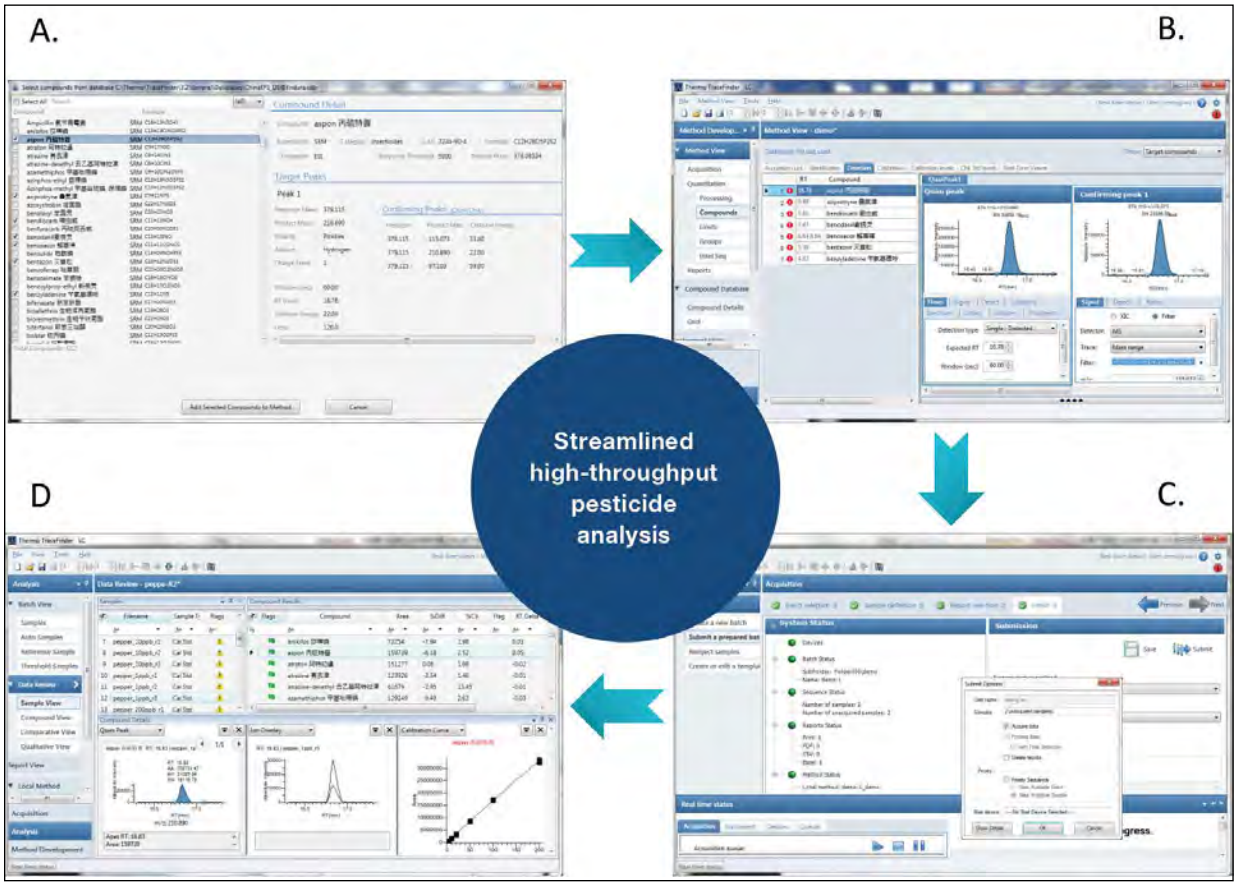

Figure 3. TraceFinder software streamlines pesticide residues analysis. (A) Choose pesticide residues needed for analysis from CDB. (B) Create instrument method and data processing method. (C) Compile analysis, operation, and data collection sequence. (D) Analyze data, browse results, and create reports.

Results and Discussion

The TSQ Endura MS, which uses simple tSRM scan functions, can quickly calculate the correct dwell time needed to run hundreds of pesticides simultaneously within a rapid gradient to achieve sensitive detection (Figure 4). Figure 5 displays the optimized chromatographic conditions needed to detect the 440 pesticide residues.

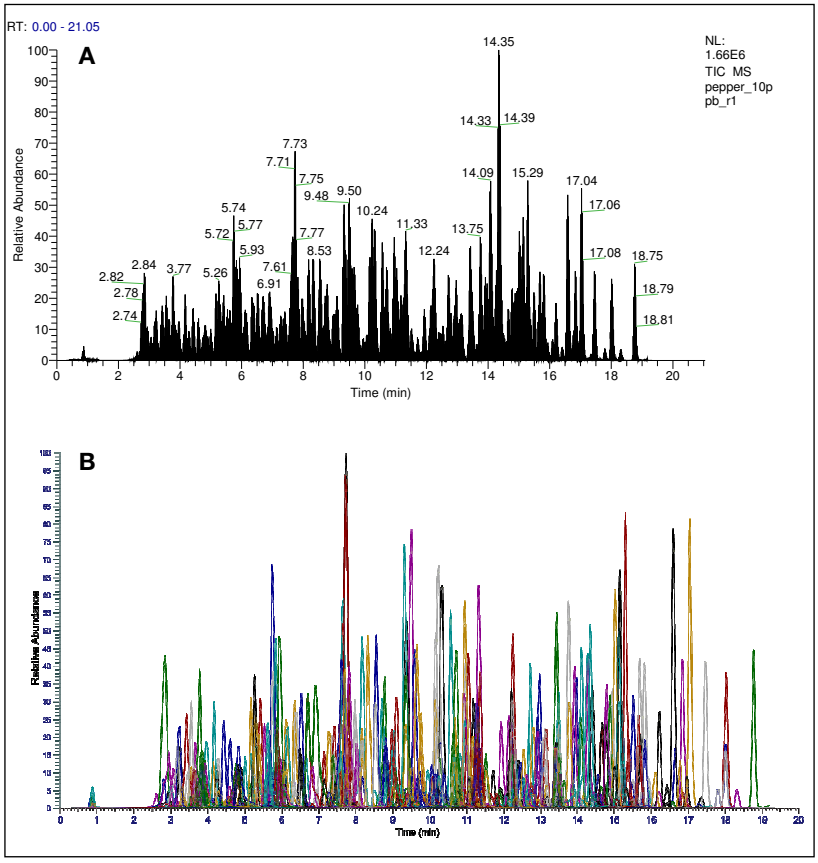

Figure 4. (A) Total ion chromatogram of 440 pesticide residues simultaneously detected in bell pepper; (B) Extracted ion chromatogram of 440 pesticide residues (10 pg/µL).

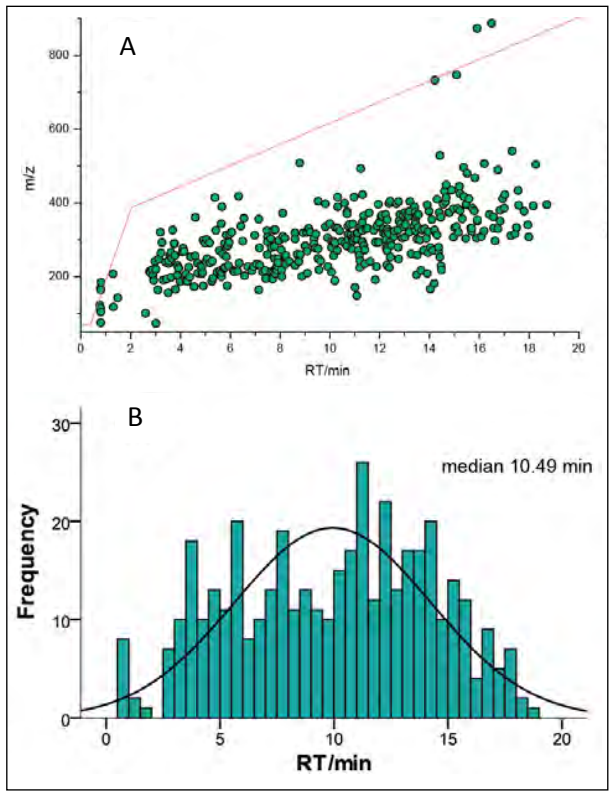

Figure 5. (A) Scatter plot of pesticide residue *m/z* vs. retention time; (B) Frequency distribution of retention times.

TraceFinder software provides a comprehensive system for high-throughput pesticide residue analysis that incorporates built-in methods for commonly found pesticides, processing methods, library searching capabilities, data review, and reporting with built-in, customizable templates. Figure 6 shows the results displayed graphically. Sample and reference mass spectra can be inspected, peak integration evaluated, different curve fits reviewed, and ion ratio values observed easily and fully interactively

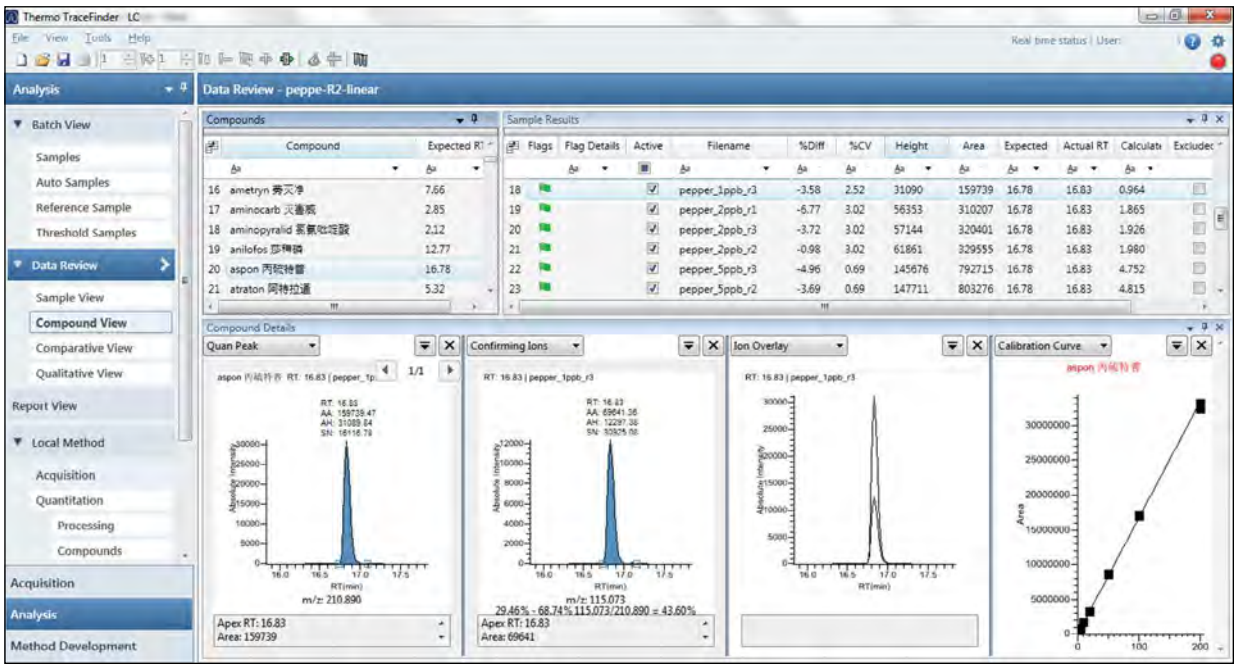

Figure 6. TraceFinder software Data Review page, showing the Compound View with the results for specific compounds.

Three factors—the coefficient of variation (CV) of the peak area, the peak shape, and the signal-to-noise ratio—were analyzed to determine the LOD and LOQ of the 440 pesticide residues in bell pepper. The CV for the reproducibility and stability of the three sample injections was less than 30% at the LOD concentrations and less than 20% at the LOQ concentrations. LOQs are represented in Figure 7.

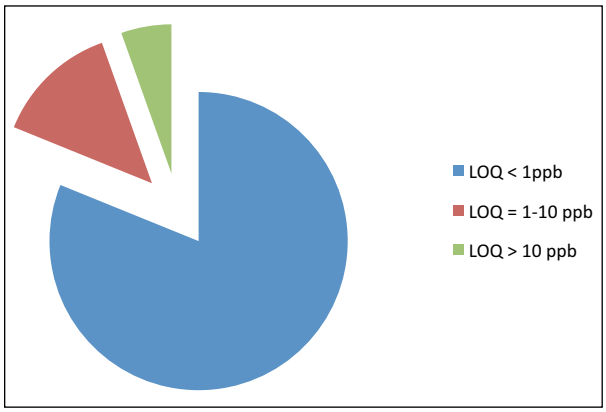

Figure 7. Representation of the LOQs detected in bell pepper matrix.

Conclusion

Addressing a critical challenge of developing a sensitive, robust, reproducible quantitative assay to quantify pesticide residues, a multi-residue method was developed for the screening and determination of 440 pesticides in a single run on the TSQ Endura triple quadrupole mass spectrometer. Data analysis was streamlined by using TraceFinder software, which is ideally suited for quantitation of large amounts of data. For this multi-pesticide residue study, a timed SRM experiment provided accurate and sensitive results for the analysis of each compound per experiment. The majority of the pesticides were detected in the spiked matrices at concentrations lower than the MRLs established by China, Japan, and the EU.

Acknowledgement

The authors wish to thank the CFDA for providing the bell pepper samples for this study.

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3. Commission Regulation (EU) No 752/2014 of 24 June 2014 replacing Annex I to Regulation (EC) No 396/2005 of the European Parliament and of the Council: http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32014R0752

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Fast and Ultrafast LC-MS/MS Methods for Robust and Reliable Analysis of Pesticides in Food Using the Vanquish UHPLC System

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Application Note 1138

Key Words
Vanquish UHPLC System, TSQ Endura Triple Quadrupole MS, Retention Time Reproducibility, Timed SRM, High-throughput, Food Safety, Chromeleon 7.2 CDS

Goal
Demonstrate the benefits of method transfer and retention time reproducibility in fast and ultra fast UHPLC separations with timed selected reaction monitoring MS detection for the analysis of pesticides in food matrices.

Introduction
Food safety is an increasing concern that has resulted in stringent pesticide regulation globally and in continuous recalls of food products. Food safety regulations require the screening and the quantitation of a large number of pesticides in food at maximum residue levels generally set in the ppb-ppm range to minimize their possible negative effects on human health. This has prompted the development of generic and reliable analytical multi-residue methods for the analysis of hundreds of pesticides simultaneously.[1] Triple stage quadrupole (TSQ) instruments operating in selected reaction monitoring (SRM) mode are widely used for this purpose and are by far the methodology of choice in routine quantitative analysis. The hyphenation of ultra high performance liquid chromatography (UHPLC) with mass spectrometry brings several advantages to the analyst because it combines high-throughput UHPLC separation with the sensitivity of the mass analyzer. With timed SRM, the compounds are monitored only in a specific time range where they are expected to elute, and not during the full chromatographic run. This brings the benefit of getting increased numbers of scans for each chromatographic peak, thus achieving maximum sensitivity and reproducibility.

Here we present a comparison between fast and ultrafast LC-MS/MS methods in timed SRM mode for the analysis of 250+ pesticides in food extracts. The two methods were compared in terms of analysis time and data quality.

Experimental
Sample Preparation
Three matrices representing soft fruit (strawberry), green vegetable (leek), and cereal grain (wheat flour) were selected for method testing. Each homogenized food sample (10 g) was weighed into a QuEChERS extraction tube (P/N 60105-216). After the addition of 10 mL of acetonitrile (+ 20 mL of water in case of wheat flour), the tube was shaken for 10 min and centrifuged at 5000 rpm for 5 min. Pesticide stock solutions were prepared in acetonitrile and matrix extracts. Working neat solutions and matrix fortified samples were obtained by dilution in the corresponding solvent or matrix to get the final concentration of 5, 10, and 100 µg/L (5–100 ppb).

Instrumentation
- Thermo Scientific™ Vanquish™ UHPLC System including:
  - System Base Vanquish (P/N VH-S01-A)
  - Binary Pump H (P/N VH-P10-A)
  - Split Sampler HT (P/N VH-A10-A)
  - Column Compartment H (P/N VH-C10-A)
- Thermo Scientific™ TSQ Endura™ Triple Quadrupole Mass Spectrometer
- Vanquish MS Connection Kit (P/N 6720.0405)

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LC Conditions	
Column	Thermo Scientific™ Accucore™ aQ 100 x 2.1 mm, 2.6 µm
Mobile Phase	A) Water/Methanol (98:2, v/v, %) with 5 mM ammonium formate and formic acid 0.1% (v/v, %) B) Water/Methanol (2:98, v/v, %) with 5 mM ammonium formate and formic acid 0.1% (v/v, %)
Temperature	25 °C
Injection Volume	1 µL
15 Min Method	
Gradient	0.00–0.82 min: 0% B; 0.82–7.32 min: 0–70% B; 7.32–9.32 min: 70–100% B; 9.32–12.32 min: 100% B; 12.32–12.42 min: 100–0% B; 12.42–15.00 min: 0% B
Flow Rate	0.300 mL/min
5 Min Method	
Gradient	0.00–0.31 min: 0% B; 0.31–2.44 min: 0–70% B; 2.44–3.11 min: 70–100% B; 3.11–4.11 min: 100% B; 4.11–4.14 min: 100–0% B; 4.14–5.00 min: 0% B
Flow Rate	0.900 mL/min

MS Conditions	
Ionization Conditions	HESI
Polarity	Positive/Negative switching
15 Min Method	
Sheath Gas Flow Rate	40 units
Aux Gas Flow Rate	6 units
Spray Voltage Positive Ion	3,700 V
Spray Voltage Negative Ion	2,500 V
Ion Transfer Tube Temp.	325 °C
Vaporizer Temp.	350 °C
CID Gas	2 mTorr
Cycle Time	0.5 s
5 Min Method	
Sheath Gas Flow Rate	58 units
Aux Gas Flow Rate	15 units
Spray Voltage Positive Ion	3,700 V
Spray Voltage Negative Ion	2,500 V
Ion Transfer Tube Temp.	350 °C
Vaporizer Temp.	400 °C
CID Gas	2 mTorr
Cycle Time	0.34 s

Data Acquisition and Processing

Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System (CDS) software, version 7.2 SR2.

Results and Discussion

Method Transfer from an UltiMate 3000 RSLC System to Vanquish UHPLC System

An already existing Thermo Scientific LC-MS method developed with the Thermo Scientific™ Dionex™ UltiMate™ 3000 RSLC system for the quantitative analysis of more than 250 pesticides in food extracts was transferred to the Vanquish UHPLC system. On the basis of the estimated differences in the gradient delay volumes of the two LC systems, the method for the Vanquish UHPLC system was corrected with the addition of an initial isocratic step of 0.32 min. This gradient delay volume between two systems was due not only to the modules but also to the specific configurations used in these applications, such as solvent mixer and capillaries. Alternatively, the Vanquish UHPLC system features various convenient fluidics adjustments to reflect the original extra-column and delay volumes. In this case the LC gradient requires no changes. Retention time shift between the two LC systems was on average less than 5 s. The negligible difference between calculated and experimentally observed shift of retention times was extremely important to avoid time consuming correction of narrow SRM scan windows of 30 s (Figure 1). Therefore, the SRM MS method was not further optimized.

Method Transfer from Fast to Ultrafast Separation with Vanquish UHPLC System

The 15 min method was shortened to 5 min with an increase of the sample throughput of 300%. The flow rate was increased from 0.3 mL/min to 0.9 mL/min, and the gradient slope was adjusted accordingly, as shown in Figure 2. The maximum system pressure was 360 bar for the 15 min method and 1010 bar for the 5 min method.

Taking benefit from the significant reduction of the peak widths in UHPLC mode with the ultrafast separation, the timed SRM scan window was decreased from 30 s to 9 s. The TSQ Endura MS has a 500 SRM/s data acquisition rate capability[2] and, a decrease of the SRM scan window gave the possibility to decrease the cycle time to 0.34 s. This allowed acquisition of 10–15 data points across the LC peak, which is optimal for accurate quantitation.

The Vanquish UHPLC system showed an outstanding retention time precision from run-to-run and from sample-to-sample that was the key factor for the development of the ultrafast UHPLC-MS method with very narrow SRM scan window (Figure 3).[3,4] The run-to-run retention time repeatability was evaluated by seven consecutive injections for 50 compounds detected in all three matrices at 5 ppb level and reveled SD below 0.30 s. The matrix-to-matrix retention time reproducibility was evaluated for the same compounds at 5 ppb level in the three matrices and revealed SD below 0.15 s (Figure 4). LC-MS analysis with ultranarrow SRM scan windows are possible only in combination with LC systems that can ensure high retention time precision because each minimal retention time shift would lead the LC peaks outside the SRM scan window compromising significantly data quality with an increased number of false negatives.

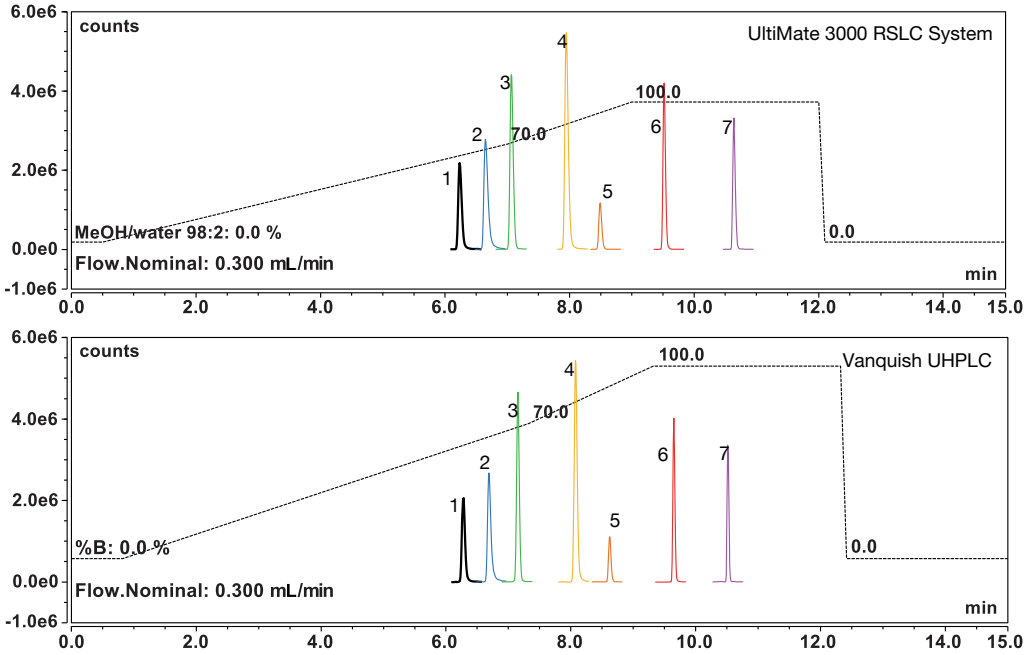
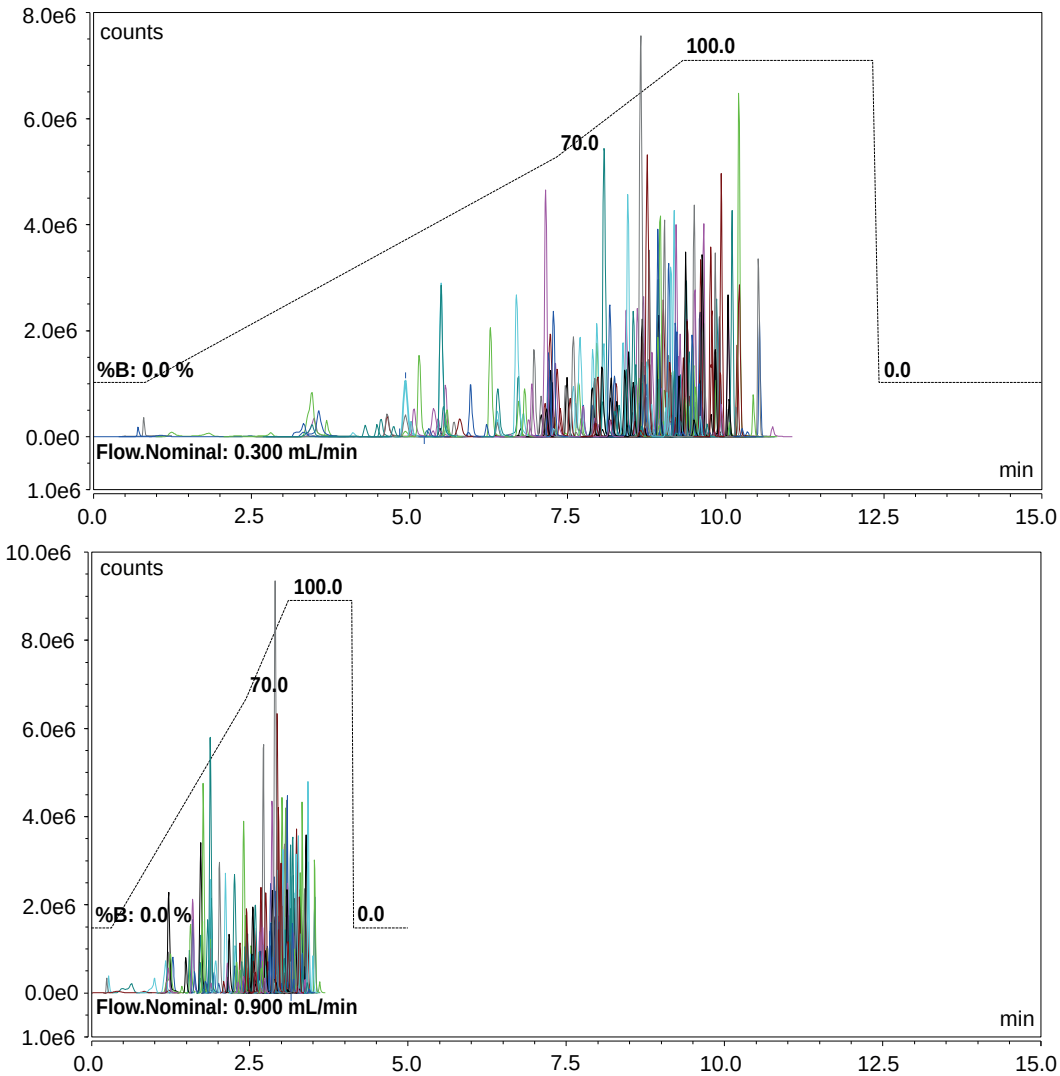


Figure 1. Comparison of extracted ion chromatograms of seven pesticides in strawberry extracts analyzed with an UltiMate 3000 RSLC system and a Vanquish UHPLC system after method transfer.



Fast Gradient
15 min run time
0.3 mL/min
360 bar
SRM-MS
Scan win. 30 s
Cycle Time 0.5

Ultrafast Gradient
5 min run time
0.9 mL/min
1010 bar
SRM-MS
Scan win. 9 s
Cycle Time 0.34

Figure 2. Extracted ion chromatograms of pesticides in strawberry matrix extract applying a gradient length of 15 and 5 min. Other conditions are described in figure.

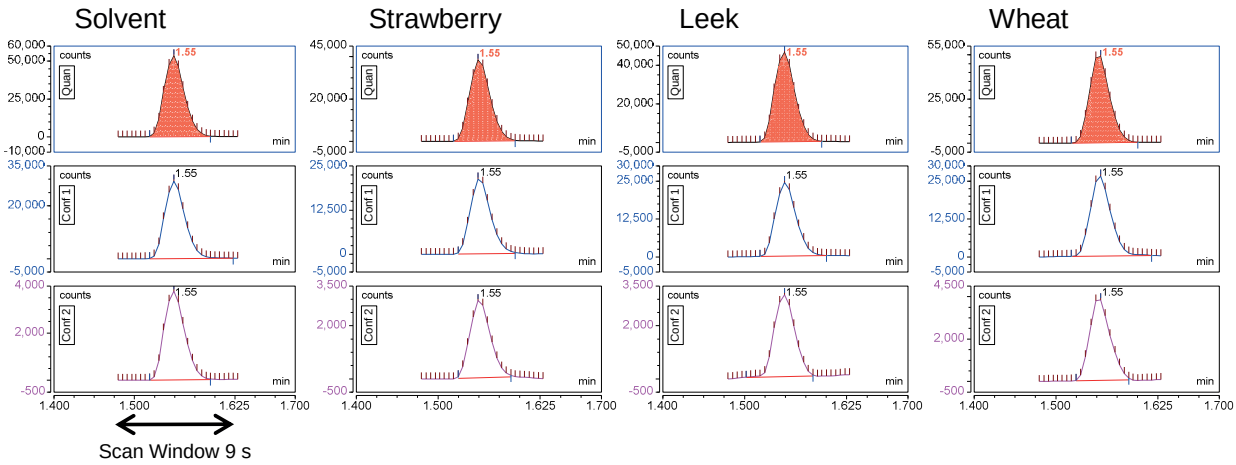


Figure 3. Demeton-S-methyl sulfone at 10 µg/kg in solvent and food matrix extracts acquired with fast cycle time and scan window of 9 s. More than 10 data points are acquired for both quantitation and confirmation ions.

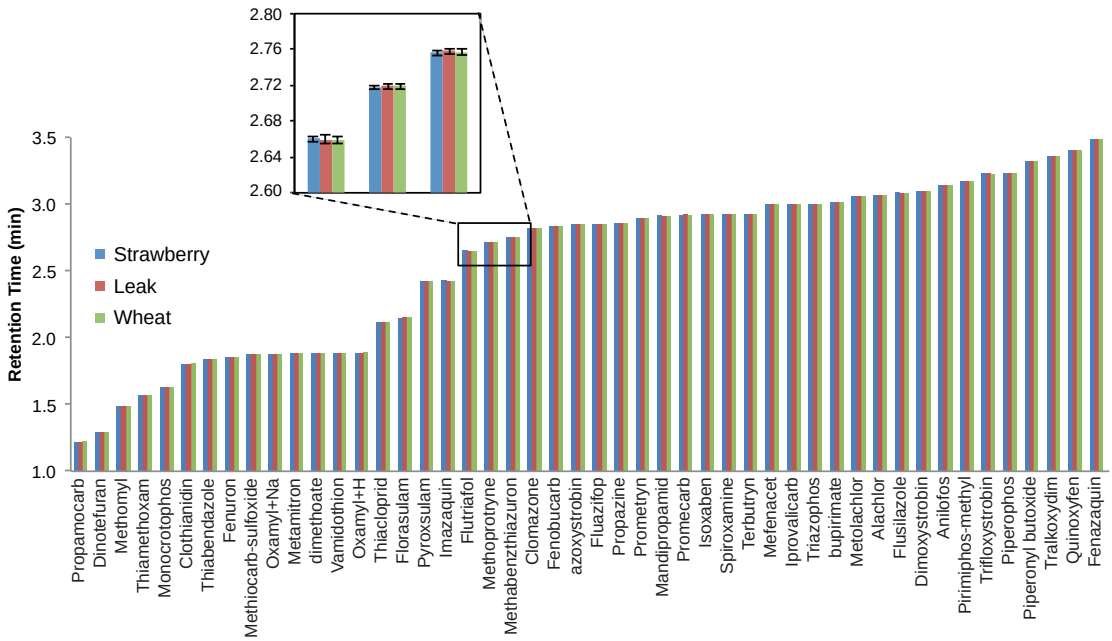


Figure 4. Retention times for 50 compounds at 5 ppb level in strawberry, leek, and wheat extracts. For each compound in each matrix is displayed the average of seven consecutive injections (±SD<0.30 s, see inset).

Method Validation

The 15 min and 5 min LC-MS methods were validated based on the following criteria: 1) accuracy, estimated at the 3 different levels in the 3 different matrices, 2) limits of quantification (LOQs), based on RSD ≤ 15% and ion ratios, 3) repeatability (%), based on RSDs%, 4) linearity measured as squared correlation coefficient.

Pesticide residues were considered reliably measured if they passed all the following evaluation criteria:

- Accuracy 80–120%
- RSDs ≤ 15%
- Ion Ratio tolerance ± 30% rel., ion co-elution 0.010 min

The results obtained with the 5 min LC-MS method in terms of accuracy, LOQs, and repeatability were compared with the 15 min method. As shown in Figure 5, the UHPLC method provided similar results saving 67% of analysis time.

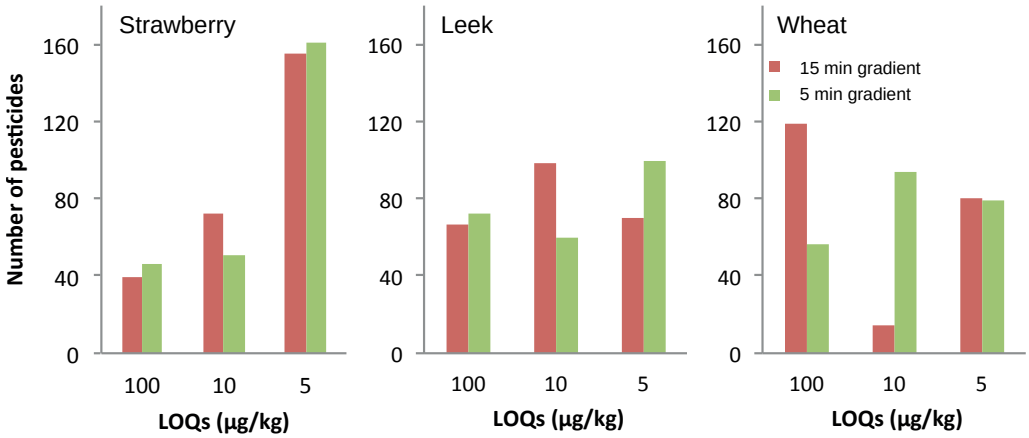


Figure 5. The 15 min and 5 min LC-MS method provide comparable data quality in all three food matrices.

Conclusion

This application note compared fast and ultrafast UHPLC-MS/MS analysis for the quantitation of 250+ pesticides in food extracts. The results showed that:

- Fast UHPLC separations in combination with ultranarrow timed SRM scan windows allowed maintaining the number of monitored transitions without compromising data quality.
- Outstanding retention time stability achieved with the Vanquish UHPLC system is the key factor for fast timed SRM MS analysis with a high number of data points across the peak.
- Ultrafast UHPLC separation resulted in saving 67% of analysis time and an increase of the sample throughput of 300% without losing information.

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Finland +358 10 3292 200
France +33 1 60 92 48 00
Germany +49 6103 408 1014
India +91 22 6742 9494
Italy +39 02 950 591
Japan +81 6 6885 1213
Korea +82 2 3420 8600
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Quantitative and Qualitative Confirmation of Pesticides in Beet Extract Using a Hybrid Quadrupole-Orbitrap Mass Spectrometer

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Olaf Scheibner, Thermo Fisher Scientific, Bremen, Germany

Application Note 617

Key Words
Q Exactive Focus, Orbitrap, pesticides, high resolution, accurate mass, quantitation, target screening, unknown screening, retrospective data analysis

Goal
To describe a method for the analysis of pesticides, showing the utility of a full-scan data-dependent MS/MS workflow to achieve regulatory levels while providing a complete targeted and screening analysis using a high-resolution, accurate mass (HRAM) spectral library for identification and confirmation.

Introduction
As world agricultural trade has expanded and concerns over food safety have grown, the enforcement of stricter pesticide regulations has become of utmost importance. In 2006, Japan introduced the Positive List System that established maximum residue levels (MRLs) for hundreds of agricultural chemicals in food, including approximately 400 pesticides, and set a uniform limit of 10 µg/kg (ppb) for chemicals for which MRLs have not been determined.[1] In 2008, the European Parliament implemented Regulation (EC) No. 396/2005, which harmonized all pesticide MRLs for European Union (EU) member states and set default limits of 10 µg/kg for all pesticide/commodity combinations for which no MRLs have been set.[2] A pesticide safety review of about 1,000 active substances on the market was mandated by EU Directive 91/414/EEC and, upon its completion in 2009, led to the approval of only about 250 substances and effectively set the permissible levels of over 700 de-listed pesticides to the default limit.[3] The EU and Japanese regulations are among the most stringent in the world and have fueled the need for faster and more sensitive analytical methods for cost-efficient, high-throughput screening and quantitation of multi-class pesticide residues.

Here, a method utilizing the Thermo Scientific™ Q Exactive™ Focus hybrid quadrupole-Orbitrap™ mass spectrometer is described. It consists of a generic chromatographic method and a full-scan data-dependent MS/MS (FS-ddMS^2) mass spectrometric method with library searching and fragment confirmation. The FS-ddMS^2 approach was used to generate calibration curves and analyze samples for targeted known compounds. In the typical acquisition setup demonstrated here, a simple full-scan data-dependent MS/MS experiment was associated with new preset confirmation settings for easier and faster method development (Figures 1 and 2).

For evaluation of the method, spiked matrix samples were analyzed by high-resolution, accurate-mass LC-MS/MS.

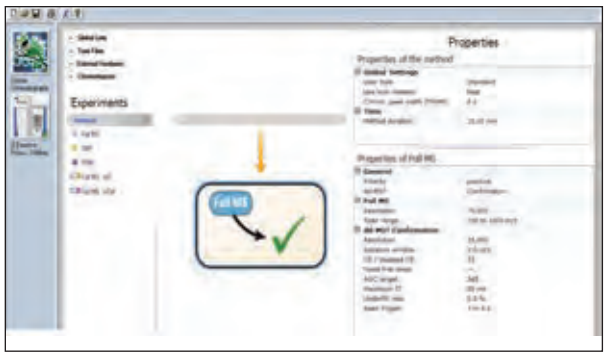

Figure 1. Instrument Setup page, showing full-scan data-dependent MS/MS.

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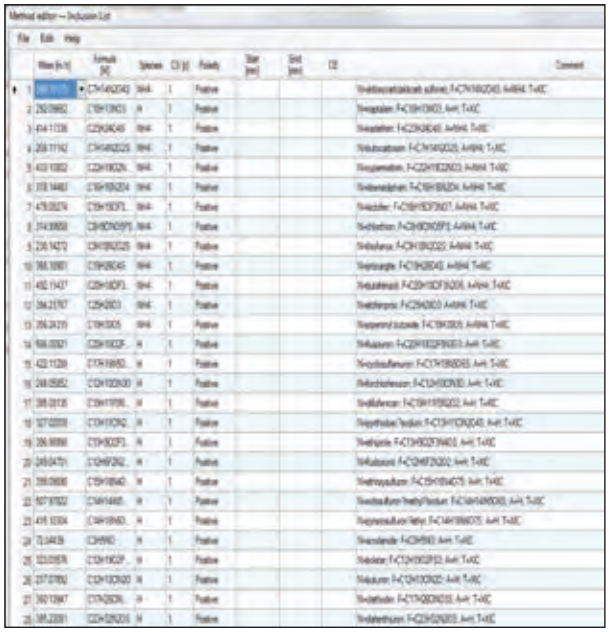

Figure 2. An example of an inclusion list that was added for targeted confirmation of known pesticides in the sample.

Sample Preparation

Beet samples, provided by the California Department of Food and Agriculture, were extracted using a modified QuEChERS method. Pesticide stock standards (ULTRA Scientific, N. Kingston, RI) were spiked into the QuEChERS extract. Then, the appropriate amount of acetonitrile was added to adjust the organic composition of the final standard solution to 50:25:25 water/matrix/acetonitrile. The concentration of the standards ranged from 0.05 to 200 µg/kg.

Liquid Chromatography Method

A generic LC method was used for all samples:

Instrumentation	Thermo Scientific™ Dionex™ UltiMate™ 3000 LC system, consisting of: · Pump: HPG-3200RS · Autosampler: WPS3000TRS · Column Warmer: TCC3000RS · Degasser: SRD3400
Column	Thermo Scientific™ Accucore™ aQ 100 x 2.1 mm, 2.6 µm particle size (p/n 17326-102130)
Column temperature	30 °C
Mobile phase A	0.1% formic acid, 5 mM ammonium formate in water
Mobile phase B	0.1% formic acid, 5 mM ammonium formate in methanol
Gradient	Refer to Figure 3
Sample injection	10 µL
Instrument run time	25 min

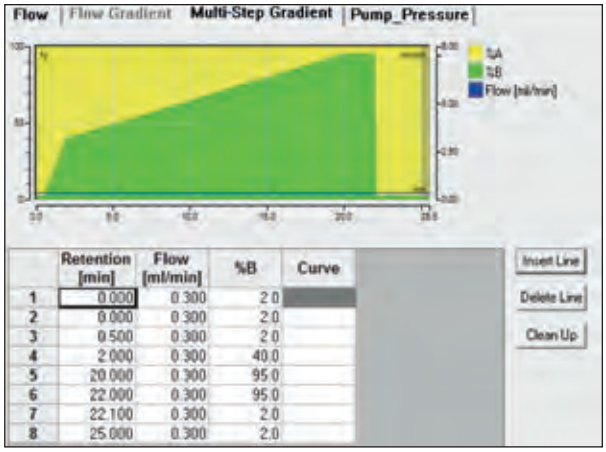

Figure 3. Flow gradient.

Mass Spectrometry Method

A generic FS-ddMS^2 method on a Q Exactive Focus MS system was used for all samples as described below:

Full Scan	
Resolution setting	70,000 (FWHM) at m/z 200
Mass range	100–1000 m/z
ddMS^2	
Resolution setting	35,000 (FWHM) at m/z 200
Isolation windows	2.0 m/z
Spray voltage	3500 V
Sheath gas	35 arb
Aux gas	10 arb
Sweep gas	1 arb
Capillary temperature	325 °C
Heater temperature	350 °C
RF-lens level	50
HCD collision energy	33 eV

Data Processing

Data processing was performed using Thermo Scientific™ TraceFinder™ software version 3.2. For generation of extracted ion chromatograms, an extraction window of 5 ppm was used. For targeted screening, a built-in compound database (>1500 compounds), consisting of compound name, precursor and fragment m/z values, and retention time, was used together with a spectral library (>7500 spectra) for confirmation of targeted residues.

Results and Discussion

Data analysis was performed within TraceFinder software with the help of green, yellow, and red flags that can quickly be sorted for review. Figure 4 demonstrates the capability of the flagging feature within TraceFinder software, which can identify issues with compounds and help the analyst make quick decisions if the sample contains that compound.

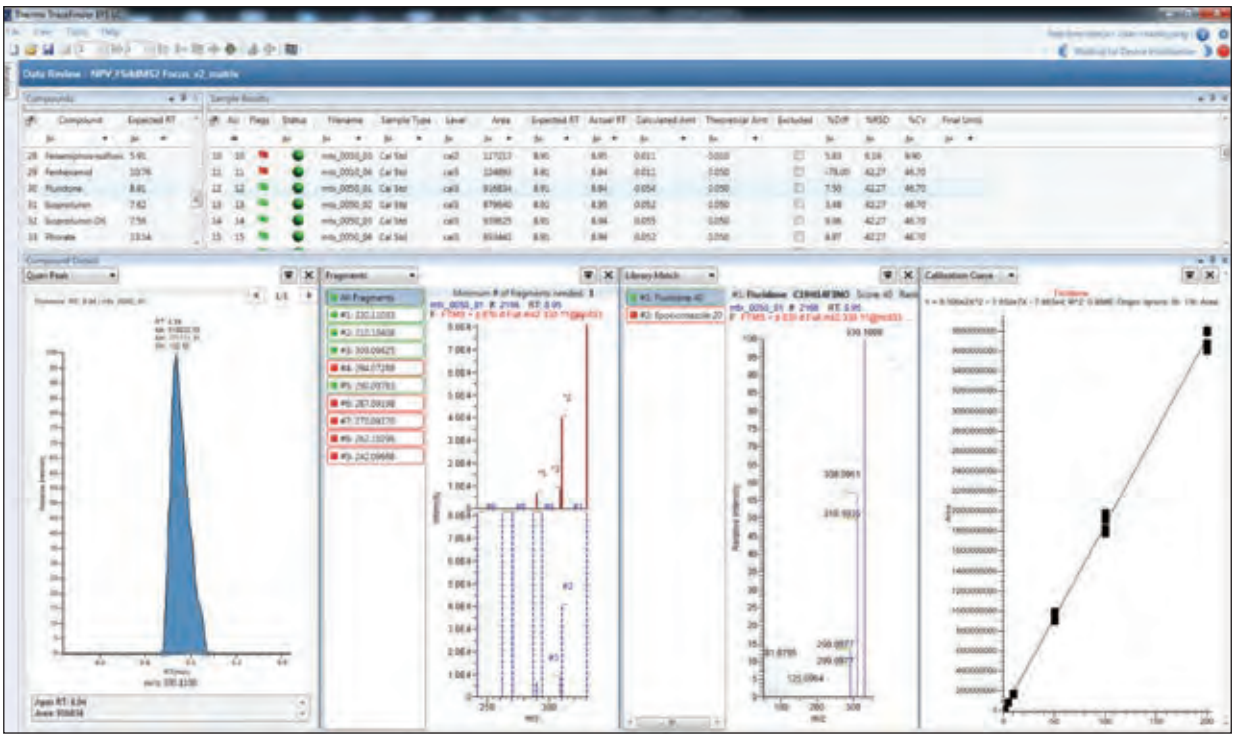

Figure 4. Flagging feature, showing that one fluridone sample has a green flag while the other sample has a red flag. A green flag indicates that all of the parameters were met and there was no issue with the calibration curve. A red flag indicates that there was a problem with the sample from the library search, fragment ion confirmation, or the calculated amount was out of range. The flagging details describe the issues with the sample.

Figure 5 demonstrates the compound details in the Quan Peak, Fragment Matching, Spectra Matching, and Calibration Curve views of TraceFinder software, which can assist the analyst in quickly looking through the data set for confirmation.

Figure 5. Tryfloxystrobin at 5 ppb, showing compound details in lower portion to highlight quick data review.

The detection results of the pesticides analyzed in the beet matrix are shown in Table 1. Detection limits varied depending on the compound. The determination of the limit of quantitation (LOQ) was based on the presence of a minimum of one fragment ion as well as reproducibility at each level as stated by the EU SANCO regulations.[4] Table 1 also shows the %RSD of n=4 at each level and available EU regulation limits for listed pesticides.[4] All RSDs were found to be well below the guidelines which require RSDs of less than 15% in order to be accepted as the LOQ.

All compounds showed good calibration curves with R^2 better than 0.99 as shown in Table 2.

Table 1. LOD/LOQ based on fragment confirmation and %RSD values compared to available EU regulation limits for pesticides.

Compound	LOD (µg/kg)	%RSD	LOQ (µg/kg)	%RSD	EU Regulation Limits (µg/kg)
Allethrin	0.91	6.25	5.13	3.27	
Atrazine	0.10	6.09	0.58	10.34	50
Azoxystrobin	0.10	3.99	0.55	11.77	15,000
Bendiocarb	0.73	6.88	0.73	6.88	
Benoxacor	0.09	6.99	0.53	8.99	
Bioresmethrin	0.77	4.44	5.24	4.98	
Boscalid	0.10	4.37	0.55	12.19	30,000
Bupirimate	0.10	3.83	0.10	3.83	50
Cadusafos	0.44	11.63	0.88	6.11	10
Carbendazim	0.53	12.39	1.04	5.58	100
Chloropyrifos	0.44	11.79	0.91	5.41	50
Coumaphos	0.10	6.00	0.60	13.19	
Cyazofamid	0.44	11.26	0.86	4.66	10
Cyproconazole	0.44	11.98	0.90	5.65	50
DEF	0.09	5.67	0.59	8.80	
Dimethenamid	0.10	2.23	0.57	11.82	10
DMST	0.09	3.46	0.58	10.71	
Fenamiphos-sulfone	0.09	5.97	0.57	12.16	20
Fluoridone	0.09	5.00	0.53	12.34	
Isoproturon	0.10	7.37	0.63	12.76	10
Phorate	0.91	5.23	4.92	2.46	10
Propetamphos	0.73	7.66	4.92	3.19	
Rotenone	0.05	42.34	0.10	4.72	10
Sulprofos	0.45	9.10	0.86	2.88	
Spirodiclofen	0.44	9.98	0.86	5.59	20
Thiobencarb	0.47	8.55	0.91	3.61	10
Triadimenol	5.12	2.93	5.12	2.93	100
Trifloxystrobin	0.10	2.91	0.10	2.91	20
Uniconcazole	0.45	13.48	0.87	4.54	

Table 2. R^2 results of 29 pesticides in beet matrix.

Compound	R^2
Allethrin	0.9989
Atrazine	0.9988
Azoxystrobin	0.9983
Bendiocarb	0.992
Benoxacor	0.9987
Bioresmethrin	0.9989
Boscalid	0.9988
Bupirimate	0.9984
Carbendazim	0.9982
Chlorpyrifos	0.9987
Coumaphos	0.9989
Cyazofamid	0.9985
Cyproconazole	0.9987
DEF	0.9988
Dimethenamid	0.9987
DMST	0.9989
Fenamiphos-sulfone	0.9988
Fluridone	0.9986
Isoproturon	0.9985
Phorate	0.9984
Propetamphos	0.9985
Rotenone	0.9986
Spirodiclofen	0.9983
Sulprofos	0.9989
Thiobencarb	0.9989
Thiodicarb	0.9986
Triadimenol	0.9977
Trifloxystrobin	0.9987
Uniconazole	0.9988

Figure 6 shows the capability of the Q Exactive Focus MS to scan quickly with polarity switching at 10 ppb. Due to the many pesticides that were spiked into the matrix, it was necessary to include internal standards to check and correct for shifts in retention times, as shown in Figures 7, 8, and 9.

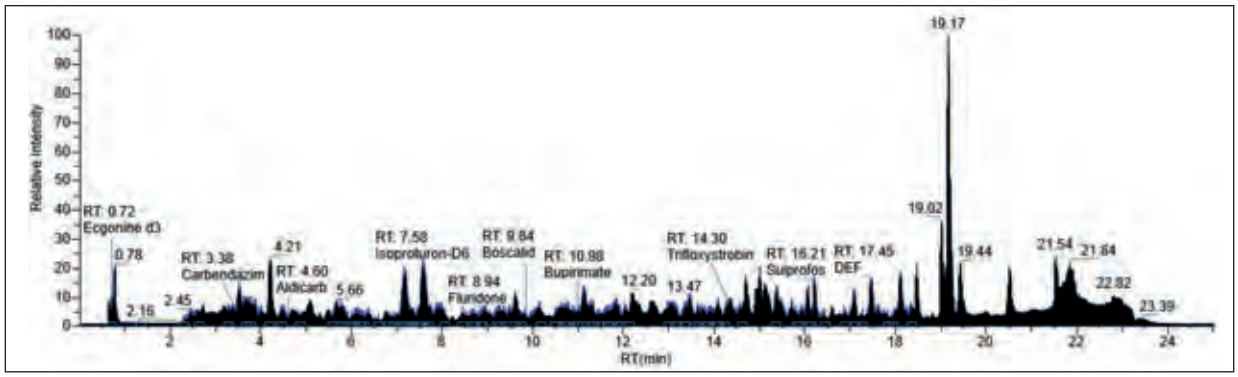

Figure 6. Total ion current (TIC) chromatogram of spiked pesticides in beets at 10 ppb.

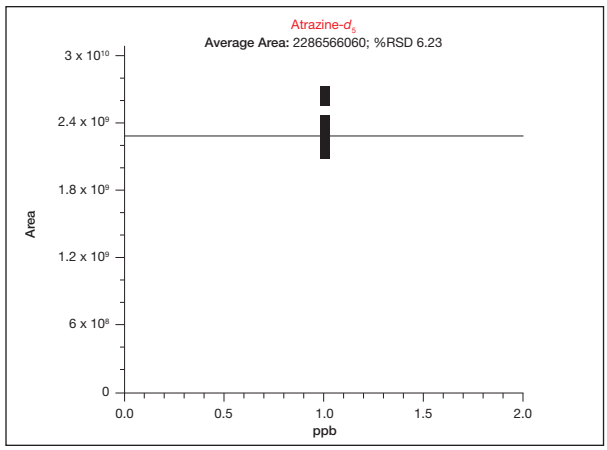

Figure 7. Atrazine-d_5, %RSD = 6.23 (beet matrix).

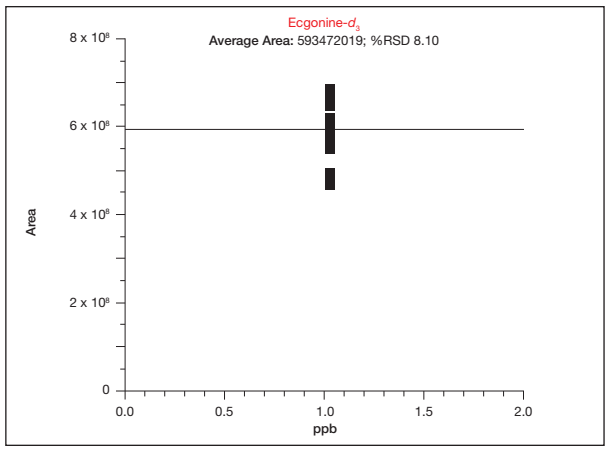

Figure 8. Ecgonine-d_3, – early eluter, %RSD = 8.10 (beet matrix).

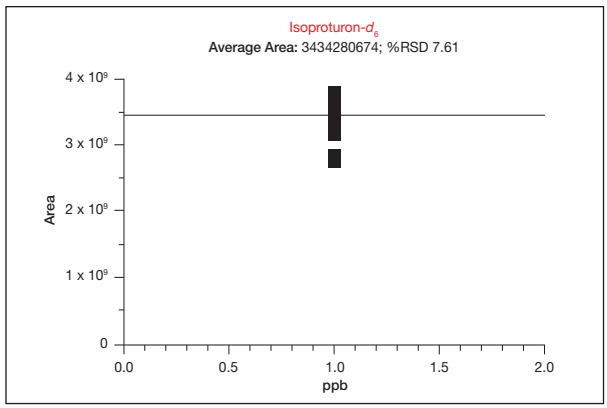

Figure 9. Isoproturon-d_6, %RSD = 7.61 (beet matrix).

Increasingly, more and more compounds are being analyzed in a single run, which can cause issues with co-elutors. A new HRAM MS/MS spectral library and compound database has been generated that is fully integrated and searchable using TraceFinder software to identify compounds with high levels of confidence. The spectral library includes more than five individual, high-resolution spectra for every compound it contains. Each compound was analyzed at multiple collision energies. Figures 10–13 showcase the matching significance of having an extensive spectral library with more than five individual spectra per compound.

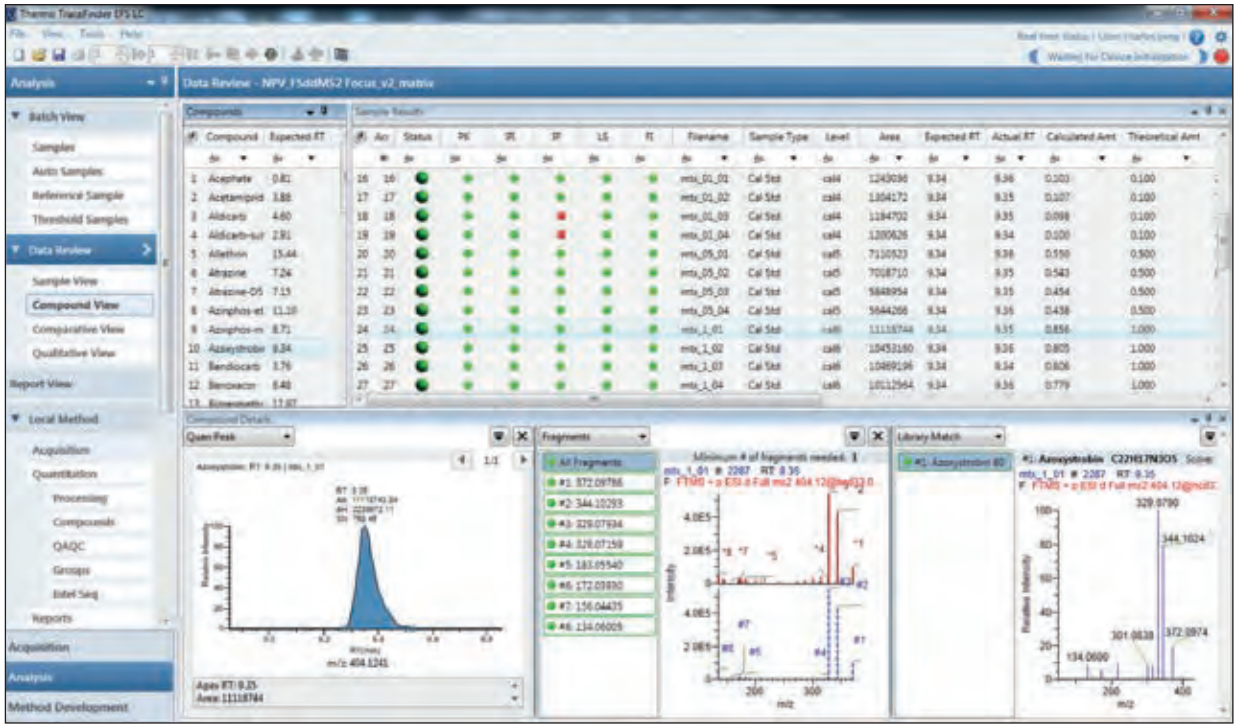

Figure 10. Azoxystrobin library match confirmation with fragmentation confirmation at 1 ppb, showing a library match score of 80% confidence in the lower right pane.

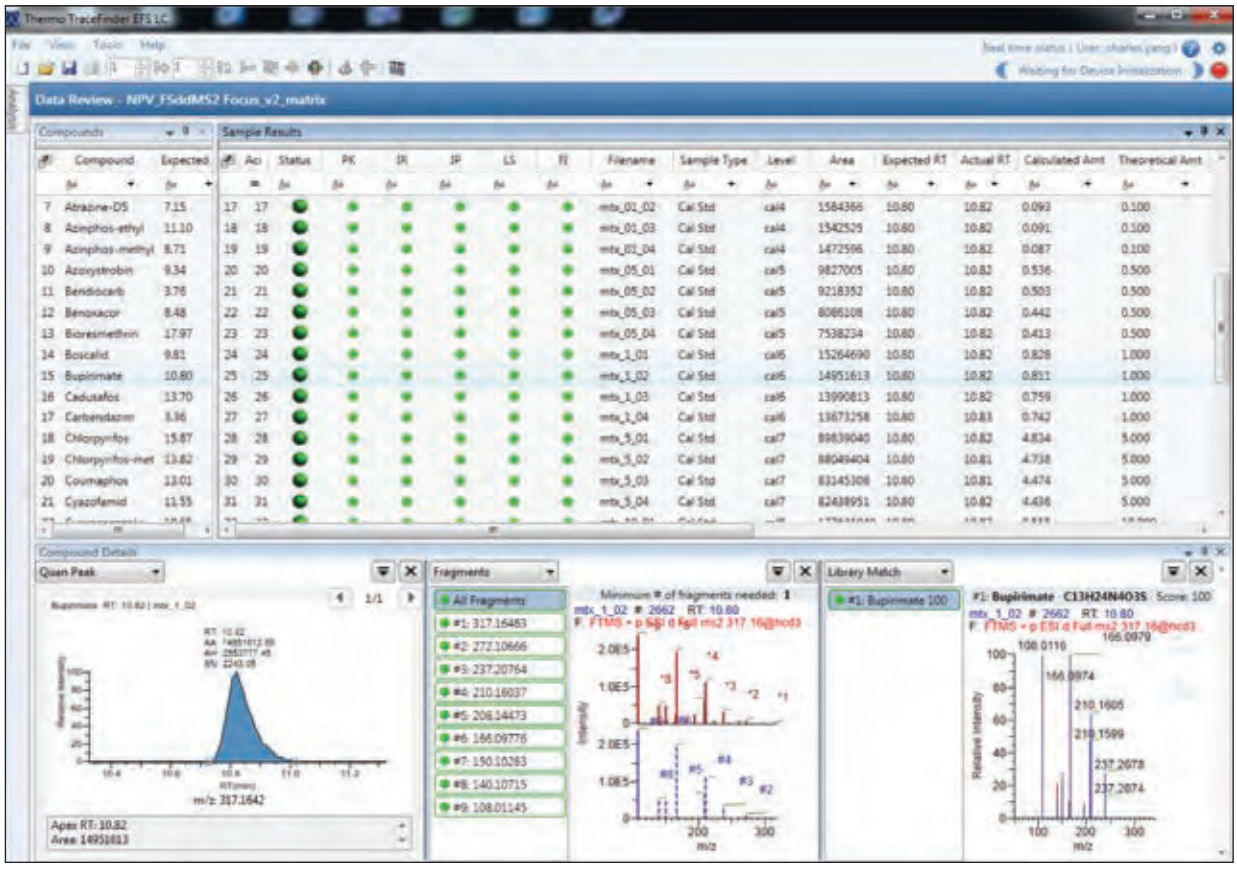

Figure 11. Bupirimate library match confirmation with fragmentation confirmation at 5 ppb, showing a library match score of 100% confidence in the lower right pane.

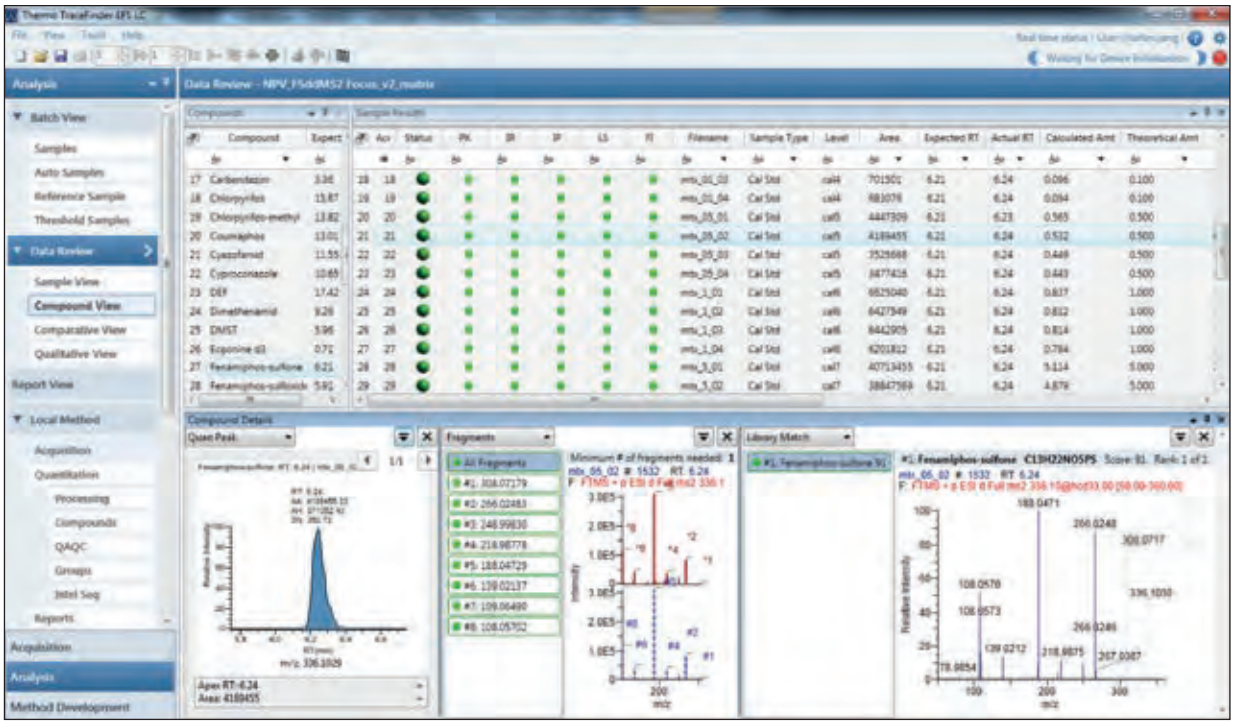

Figure 12. Fenamiphos-sulfone library match confirmation with fragmentation confirmation at 5 ppb, showing a library match score of 91% confidence in the lower right pane.

Figure 13. Rotenone library match confirmation with fragmentation confirmation at 1 ppb, showing a library match score of 93% confidence in the lower right pane.

Conclusion

The benchtop Q Exactive Focus MS provided easy access to full quantitative, confirmation, and screening data in a single injection. The high resolution and mass accuracy enabled quantification of the compounds over a wide dynamic range (0.05–200 ng/mL) with linear fit, correlation better than 0.99, and %RSD below 15%. Confirmation by the precursor-selected MS/MS gave an option to use spectral and library matching and pattern recognition within TraceFinder software. The new environmental and food safety HRAM spectral library provided more confidence in the data with its multiple, high-resolution spectra at numerous collision energies for use in any experiment.

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Application Note 579

Use of UHPLC and High-Resolution MS for Quantitative Analysis of Pesticides in Onion Matrix

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Key Words

Exactive Plus, TraceFinder, high resolution, accurate mass, food safety, UltiMate UHPLC

Goal

To demonstrate the ability of a high-resolution, accurate-mass UHPLC-MS system, combined with appropriate application-specific workflow software, to provide fast, confident, and precise screening and quantitative analysis of pesticides in onion matrix.

Introduction

Monitoring for pesticide and other chemical residues in produce is essential to maintaining a safe food supply. Monitoring data can also be used to better understand the relationship of pesticide residues to agriculture practices, enhance integrated pest management, and support the export of U.S. commodities. Monitoring is typically done by public agencies, but budget restrictions have increased pressure on these agencies to improve productivity while lowering costs.

Traditionally, triple quadrupole mass spectrometers have been used for the identification and quantitation of pesticide and chemical residues. However, MS/MS analysis with triple quadrupole mass spectrometers requires time-consuming selection of mass transitions and optimization of collision energies. The introduction of affordable benchtop, Orbitrap™-based, high-resolution, accurate-mass (HR/AM) mass spectrometers has provided an alternative method for unequivocal identification of trace contaminants without time-consuming MS/MS optimization.

A liquid chromatography/mass spectrometry methodology employing ultrahigh performance liquid chromatography (UHPLC) and HR/AM mass spectrometry makes it possible to identify, quantify, and confirm more trace-level contaminants in complex mixtures in a single analytical run. The results of this unique solution are improved sensitivity and precision, as well as unmatched throughput.

Experimental

Sample Preparation

Onion was prepared for analysis by using a modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method, which is a sample preparation procedure used to extract pesticides from food. For the QuEChERS extraction, 15 g of homogenized sample and 15 mL of acetonitrile were used. Then, 200 µL of final QuEChERS extract, 300 µL of acetonitrile, and 500 µL of water were transferred into an autosampler vial, spiked with 20 µL of the pesticides standard, and mixed thoroughly. A mixture of 120 pesticides with different starting concentrations was prepared in neat matrix (70:30 methanol/water) to make the standard calibration curve and spiked into onion matrix to determine if there was any ion suppression.

Liquid Chromatography

Chromatographic analysis was performed using a Thermo Scientific™ Dionex™ UltiMate™ 3000 RSLC UHPLC system with high-pressure mixing binary pump and 35 µL gradient mixing kit. High-purity Fisher Chemical LC/MS solvents were used.

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The chromatographic conditions were as follows:

Column:	Thermo Scientific Hypersil GOLD aQ™ column (50 x 2.1 mm, 1.9 µm)
Oven:	TCC-3300RS
Autosampler:	WPS-3000RS thermostated autosampler
Pump:	HPG3200RS binary with 35 µL gradient mixing kit, SRD-3400 solvent rack, and degasser
Mobile Phase A:	Water with 0.1% formic acid and 4 mM ammonium formate
Mobile Phase B:	Methanol with 0.1% formic acid and 4 mM ammonium formate
Flow Rate:	300 µL/min
Column Temperature:	40 °C
Sample Injection Volume:	5 µL

Gradient:	Gradient Time (min)	%A	%B
	-2.50	98	2
	0.00	98	2
	0.25	70	30
	10.00	0	100
	12.49	0	100
	12.50	98	2

Mass Spectrometry

All samples were analyzed on a Thermo Scientific Exactive™ Plus benchtop Orbitrap mass spectrometer.

The MS conditions were as follows:

Ion Source:	Heated electrospray (HESI-II)
Ion Mode:	Positive/Negative
Capillary Temperature:	280 °C
Vaporizer Temperature:	295 °C
Spray Voltage:	2200 V
Sheath Gas:	32 arbitrary units
Aux Gas:	7 arbitrary units
Scan Type:	Full MS scan
Mass Range:	*m/z* 120–1000
Mass Resolution:	70,000

Unlike triple quadrupole mass spectrometers, the high-resolution, accurate-mass Exactive Plus instrument required no optimization of mass transitions or collision energies for each analyte. Therefore, the effort for method development was significantly reduced. Table 1 lists the pesticides targeted in this analysis.

Data Analysis

Data processing was carried out with Thermo Scientific TraceFinder™ software for quantitation and targeted-screening workflows. Specificity of analysis was achieved by applying a mass extraction window of 5 ppm to the theoretical mass of the analytes.

Table 1. Targeted pesticides and their associated retention times (RT), actual and theoretical *m/z*, and calculated mass errors

Compound	RT	Formula	Theoretical *m/z*	Detected *m/z*	Delta (ppm)
Acetamiprid	2.27	$C_{10}H_{11}ClN_4$	223.0745	223.0746	0.51
Aldicarb	2.80	$C_7H_{14}N_2O_2S$	208.1114	208.1117	1.23
Aldicarb sulfone	1.49	$C_7H_{14}N_2O_4S$	240.1013	240.1013	0.14
Aldicarb sulfoxide	1.55	$C_7H_{14}N_2O_3S$	224.1063	224.1065	0.48
Atrazine	4.38	$C_8H_{14}ClN_5$	216.1010	216.1013	1.03
Azinphos methyl	5.01	$C_{10}H_{12}N_3O_3PS_2$	318.0130	318.0137	1.97
Azinphos methyl OA	2.90	$C_{10}H_{12}N_3O_4PS$	302.0359	302.0359	-0.10
Azoxystrobin	5.40	$C_{22}H_{17}N_3O_5$	404.1241	404.1245	1.03
Bendiocarb	3.52	$C_{11}H_{13}NO_4$	224.0917	224.0918	0.28
Benoxacor	4.97	$C_{11}H_{11}Cl_2NO_2$	260.0240	260.0241	0.57
Bifenazate	6.04	$C_{17}H_{20}N_2O_3$	301.1547	301.1550	0.99
Boscalid	5.61	$C_{18}H_{12}Cl_2N_2O$	343.0399	343.0403	1.07
Buprofezin	7.70	$C_{16}H_{23}N_3OS$	306.1635	306.1637	0.67
Carbaryl	3.88	$C_{12}H_{11}NO_2$	202.0863	202.0864	0.62
Carbofuran	3.52	$C_{12}H_{15}NO_3$	222.1125	222.1126	0.42
Carbofuran, 3-hydroxy	2.19	$C_{12}H_{15}NO_4$	255.1339	255.1339	-0.09
Carboxin	3.77	$C_{12}H_{13}NO_2S$	236.0740	236.0741	0.38
Carfentrazone ethyl	6.62	$C_{15}H_{14}Cl_2F_3N_3O_3$	429.0703	429.0706	0.70
Chlorpyrifos OA	6.37	$C_9H_{11}Cl_3NO_4P$	350.9830	350.9831	0.31
Clofentezine	7.27	$C_{14}H_8Cl_2N_4$	303.0199	303.0200	0.37
Clothianidin	2.03	$C_6H_8ClN_5O_2S$	250.0160	250.0162	0.82
Cymoxanil	2.53	$C_7H_{10}N_4O_3$	199.0826	199.0828	1.07
Difenoconazole	7.40	$C_{19}H_{17}Cl_2N_3O_3$	406.0720	406.0723	0.79
Diflubenzuron	6.50	$C_{14}H_9ClF_2N_2O_2$	311.0393	311.0395	0.39
Dimethomorph	5.80	$C_{21}H_{22}ClNO_4$	388.1310	388.1313	0.63
Dinotefuran	1.49	$C_7H_{14}N_4O_3$	203.1139	203.1140	0.50
Diuron	4.68	$C_9H_{10}Cl_2N_2O$	233.0243	233.0245	0.71
Famoxadone	7.04	$C_{22}H_{18}N_2O_4$	392.1605	392.1608	0.88
Fenamidone	5.46	$C_{17}H_{17}N_3OS$	312.1165	312.1167	0.51
Fenamiphos sulfone	3.77	$C_{13}H_{22}NO_4PS$	320.1080	320.1081	0.22
Fenamiphos sulfoxide	3.93	$C_{13}H_{22}NO_5PS$	336.1029	336.1029	0.09
Fenbuconazole	6.46	$C_{19}H_{17}ClN_4$	337.1215	337.1216	0.57
Fludioxonil	5.71	$C_{12}H_6F_2N_2O_2$	266.0736	266.0737	0.41
Fluridone	5.19	$C_{19}H_{14}F_3NO$	330.1100	330.1100	-0.12
Flutolanil	5.76	$C_{17}H_{16}F_3NO_2$	324.1206	324.1207	0.42
Formetanate	1.43	$C_{11}H_{15}N_3O_2$	222.1237	222.1238	0.48
Halosulfuron methyl	5.96	$C_{13}H_{15}ClN_6O_7S$	435.0484	435.0490	1.28
Hexaconazole	6.98	$C_{14}H_{17}Cl_2N_3O$	314.0821	314.0823	0.42
Hexythiazox	8.18	$C_{17}H_{21}ClN_2O_2S$	353.1085	353.1087	0.65
Imazalil	4.49	$C_{14}H_{14}Cl_2N_2O$	297.0556	297.0559	0.95
Imidacloprid	1.99	$C_9H_{10}ClN_5O_2$	256.0596	256.0596	-0.03
Indoxacarb	7.54	$C_{22}H_{17}ClF_3N_3O_7$	528.0780	528.0785	0.95
Isoprocarb	4.39	$C_{11}H_{15}NO_2$	194.1174	194.1178	1.33
Linuron	5.34	$C_9H_{10}Cl_2N_2O_2$	249.0192	249.0194	0.92
Metalaxyl	4.59	$C_{15}H_{21}NO_4$	280.1543	280.1546	0.85
Methidathion OA	2.70	$C_6H_{11}N_2O_5PS_2$	286.9920	286.9919	-0.43
Methiocarb	5.38	$C_{11}H_{15}NO_2S$	226.0896	226.0898	0.76
Methomyl	1.45	$C_5H_{10}N_2O_2S$	163.0536	163.0537	0.74

Table 1 (continued). Targeted pesticides and their associated retention times (RT), actual and theoretical *m/z*, and calculated mass errors

Compound	RT	Formula	Theoretical *m/z*	Detected *m/z*	Delta (ppm)
Methoxyfenozide	5.86	$C_{22}H_{28}N_2O_3$	369.2173	369.2176	0.79
Metribuzin	3.35	$C_8H_{14}N_4OS$	215.0961	215.0963	0.67
Monocrotophos	1.74	$C_7H_{14}NO_5P$	224.0682	224.0684	0.83
Myclobutanil	5.92	$C_{15}H_{17}ClN_4$	289.1215	289.1218	1.09
Norflurazon	4.78	$C_{12}H_9ClF_3N_3O$	304.0459	304.0461	0.80
Norflurazon desmethyl	4.27	$C_{11}H_7ClF_3N_3O$	290.0303	290.0305	0.92
Oxamyl	1.57	$C_7H_{13}N_3O_3S$	237.1016	237.1017	0.34
Oxamyl oxide	1.66	$C_5H_{10}N_2O_2S$	163.0536	163.0537	0.74
Oxydemeton methyl sulfone	4.41	$C_6H_{15}O_4PS_2$	247.0222	247.0224	0.82
Phorate sulfone	4.41	$C_7H_{17}O_4PS_3$	293.0099	293.0101	0.57
Phorate sulfoxide	4.25	$C_7H_{17}O_3PS_3$	277.0150	277.0153	0.97
Pirimicarb	2.80	$C_{11}H_{18}N_4O_2$	239.1503	239.1503	0.07
Promecarb	5.57	$C_{12}H_{17}NO_2$	208.1332	208.1335	1.26
Propamocarb	1.51	$C_9H_{20}N_2O_2$	189.1598	189.1599	0.75
Propargite	8.38	$C_{19}H_{26}O_4S$	368.1890	368.1893	0.88
Propiconazole	6.89	$C_{15}H_{17}Cl_2N_3O_2$	342.0771	342.0775	1.15
Propoxur	3.46	$C_{11}H_{15}NO_3$	210.1125	210.1125	0.23
Pyraclostrobin	7.08	$C_{19}H_{18}ClN_3O_4$	388.1059	388.1062	0.80
Pyridaben	8.90	$C_{19}H_{25}ClN_2OS$	365.1449	365.1452	0.78
Pyrimethanil	4.72	$C_{12}H_{13}N_3$	200.1182	200.1184	0.62
Pyriproxyfen	8.05	$C_{20}H_{19}NO_3$	322.1438	322.1439	0.37
Quinoxyfen	8.20	$C_{15}H_8Cl_2FNO$	308.0040	308.0042	0.67
Sethoxydim	7.72	$C_{17}H_{29}NO_3S$	328.1941	328.1942	0.28
Simazine	3.48	$C_7H_{12}ClN_5$	202.0854	202.0855	0.40
Spinosad A	7.27	$C_{41}H_{65}NO_{10}$	732.4681	732.4687	0.77
Spinosad D	7.66	$C_{42}H_{67}NO_{10}$	746.4838	746.4838	-0.01
Spiromesifen	8.36	$C_{23}H_{30}O_4$	388.2482	388.2485	0.62
Sulfentrazone	3.81	$C_{11}H_{10}Cl_2F_2N_4O_3S$	404.0157	404.0159	0.57
Tebuconazole	6.75	$C_{16}H_{22}ClN_3O$	308.1524	308.1526	0.65
Tebufenozide	6.58	$C_{22}H_{28}N_2O_2$	353.2224	353.2226	0.59
Tebuthiuron	3.62	$C_9H_{16}N_4OS$	229.1118	229.1119	0.39
Thiabendazole	1.95	$C_{10}H_7N_3S$	202.0433	202.0435	0.71
Thiabendazole, 5-hydroxy	1.65	$C_{10}H_7N_3OS$	218.0383	218.0384	0.61
Thiacloprid	2.60	$C_{10}H_9ClN_4S$	253.0309	253.0310	0.27
Thiobencarb	7.16	$C_{12}H_{16}ClNOS$	258.0714	258.0715	0.56
Triadimefon	5.82	$C_{14}H_{16}ClN_3O_2$	294.1004	294.1005	0.49
Triadimenol	5.96	$C_{14}H_{18}ClN_3O_2$	296.1160	296.1163	0.92
Trifloxystrobin	7.51	$C_{20}H_{19}F_3N_2O_4$	409.1370	409.1373	0.73
Triflumizole	7.56	$C_{15}H_{15}ClF_3N_3O$	346.0929	346.0930	0.39

Results and Discussion

The extracted ion chromatograms shown in Figure 1 illustrate the quality of the UHPLC separation at 1 ppb in onion matrix. All analytes gave very good linear response in the calibration range of 1.35–1280 ppb depending on the starting concentration in the mixture. The quantification data showed good reproducibility and recovery rates.

Table 2 shows the retention time, R^2, and LOQ for the pesticides analyzed in onion matrix. The mass accuracy of the LOQ (less than 2 ppm), as well as the retention times and curve fits, increase the confidence level for the analyst.

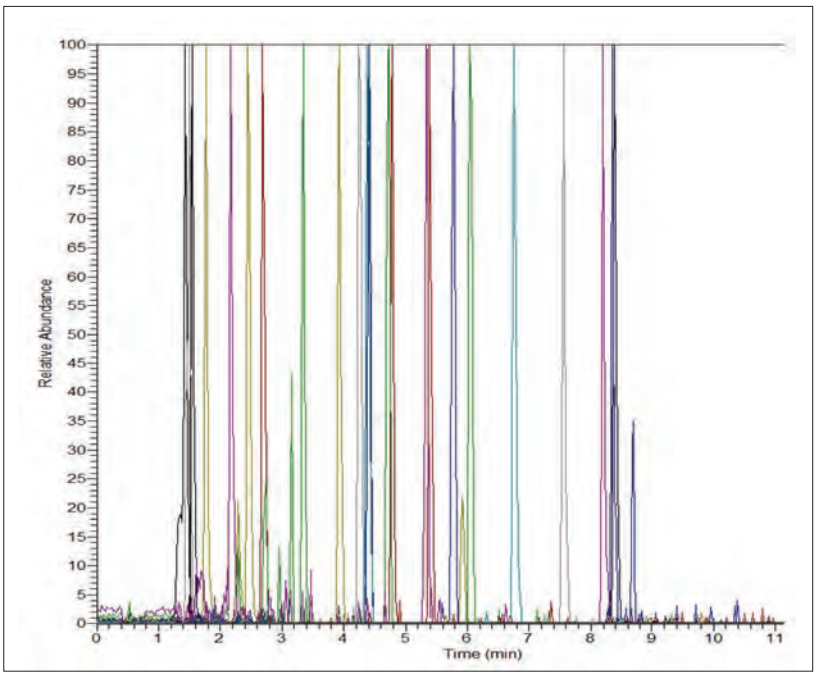

Figure 1. Extracted ion chromatograms showing peak shape and elution time at 1 ppb level in onion matrix

Table 2. Tabulated results of LOQs for each targeted compound, with retention times and curve fit R^2

Compound	RT	R^2	LOQ (ppb)
Acetamiprid	2.27	0.9990	0.125
Aldicarb	2.80	0.9956	2.000
Aldicarb sulfone	1.49	0.9986	0.500
Aldicarb sulfoxide	1.55	0.9979	0.500
Atrazine	4.38	0.9994	0.500
Azinphos methyl	5.01	0.9959	2.500
Azinphos methyl OA	2.90	0.9992	0.500
Azoxystrobin	5.40	0.9992	0.125
Bendiocarb	3.52	0.9991	0.250
Benoxacor	4.97	0.9986	0.500
Bifenazate	6.04	0.9706	0.500
Boscalid	5.61	0.9991	0.500
Buprofezin	7.70	0.9989	0.025
Carbaryl	3.88	0.9989	0.250
Carbofuran	3.52	0.9988	0.250
Carbofuran, 3-hydroxy	2.19	0.9985	0.500
Carboxin	3.77	0.9990	0.250
Carfentrazone ethyl	6.62	0.9991	0.250
Chlorpyrifos OA	6.37	0.9994	0.050
Clofentezine	7.27	0.9937	1.000
Clothianidin	2.03	0.9978	0.250
Cymoxanil	2.53	0.9029	0.500
Difenoconazole	7.40	0.9994	0.250
Diflubenzuron	6.50	0.9996	1.000
Dimethomorph	5.80	0.9993	0.250
Dinotefuran	1.49	0.9974	0.050
Diuron	4.68	0.9990	1.000
Famoxadone	7.04	0.9992	0.250
Fenamidone	5.46	0.9990	0.025
Fenamiphos sulfone	3.77	0.9992	0.250
Fenamiphos sulfoxide	3.93	0.9992	0.025
Fenbuconazole	6.46	0.9993	0.500
Fludioxonil	5.71	0.9991	0.500
Fluridone	5.19	0.9987	0.250
Flutolanil	5.76	0.9990	0.125
Formetanate	1.43	0.9983	0.050
Halosulfuron methyl	5.96	0.9866	0.500
Hexaconazole	6.98	0.9986	1.000
Hexythiazox	8.18	0.9990	0.250
Imazalil	4.49	0.9995	0.250
Imidacloprid	1.99	0.9965	0.050
Indoxacarb	7.54	0.9989	0.500
Isoprocarb	4.39	0.9971	0.500
Linuron	5.34	0.9989	0.500
Metalaxyl	4.59	0.9992	0.125

Table 2 (continued). Tabulated results of LOQs for each targeted compound, with retention times and curve fit R^2

Compound	RT	R^2	LOQ (ppb)
Methidathion OA	2.70	0.9991	2.000
Methiocarb	5.38	0.9990	0.500
Methomyl	1.45	0.9968	0.100
Methoxyfenozide	5.86	0.9996	0.250
Metribuzin	3.35	0.9992	0.250
Monocrotophos	1.74	0.9989	0.025
Myclobutanil	5.92	0.9988	0.500
Norflurazon	4.78	0.9992	0.500
Norflurazon desmethyl	4.27	0.9988	0.050
Oxamyl	1.57	0.9992	0.500
Oxamyl oxide	1.66	0.9966	1.000
Oxydemeton methyl sulfone	4.41	0.9979	0.250
Phorate sulfone	4.41	0.9984	0.025
Phorate sulfoxide	4.25	0.9990	0.050
Pirimicarb	2.80	0.9988	0.100
Promecarb	5.57	0.9990	0.250
Propamocarb	1.51	0.9981	0.500
Propargite	8.38	0.9993	0.025
Propiconazole	6.89	0.9992	0.500
Propoxur	3.46	0.9993	0.500
Pyraclostrobin	7.08	0.9990	0.125
Pyridaben	8.90	0.9991	0.125
Pyrimethanil	4.72	0.9995	0.250
Pyriproxyfen	8.05	0.9990	0.125
Quinoxyfen	8.20	0.9993	0.125
Sethoxydim	7.72	0.9964	0.250
Simazine	3.48	0.9999	0.250
Spinosad A	7.27	0.9995	0.420
Spinosad D	7.66	0.9994	0.080
Spiromesifen	8.36	0.9987	0.250
Sulfentrazone	3.81	0.9986	0.500
Tebuconazole	6.75	0.9994	0.050
Tebufenozide	6.58	0.9989	0.500
Tebuthiuron	3.62	0.9990	0.125
Thiabendazole	1.95	0.9986	0.250
Thiabendazole, 5-hydroxy	1.65	0.9993	0.250
Thiacloprid	2.60	0.9993	0.125
Thiodicarb	4.23	0.9953	20.000
Triadimefon	5.82	0.9990	0.500
Triadimenol	5.96	0.9981	1.500
Trifloxystrobin	7.51	0.9989	0.125
Triflumizole	7.56	0.9994	0.251

TraceFinder software comes with many features including user-customizable flagging. A green flag next to the name of the compound (Figure 2) indicates the compound was found in the unknown sample, whereas a yellow flag indicates the compound was not found. A red flag indicates the compound has an issue with the calibration curve and that it exceeded the flagging threshold (Figures 3 and 4). A yellow triangle caution sign indicates there is an above-threshold quantitation error with a single or multiple compounds in the sample that needs to be checked.

Figure 2. TraceFinder software displays imidacloprid calibration curve plot of matrix, R^2, list of compounds, and chromatogram. A green flag in the compound list indicates the compound was found in the unknown sample, whereas a yellow flag indicates the compound was not found.

Figure 3. TraceFinder software displays boscalid calibration curve plot of matrix, R^2, list of compounds, and chromatogram. The red flag indicates the compound has an issue with the calibration curve and that it did not meet the flagging requirement. The yellow triangle caution sign indicates there is an issue with a single or multiple compounds in the sample that needs to be checked.

Figure 4. TraceFinder software displays diuron calibration curve plot of matrix, R^2, list of compounds, and chromatogram. The highlighting of diuron in the upper right section indicates that the compound was found within the calibration curve. Therefore, there are no flags present next to the name.

Conclusion

The Exactive Plus benchtop mass spectrometer paired with TraceFinder software provided easy access to full quantitative and targeted screening data in one package. The results showed good linearity with excellent sensitivity at very low LOQs, which will assist in detecting pesticides. The Exactive Plus instrument's exceptionally high mass resolution helped resolve matrix compounds that would otherwise interfere with detection of low-level analytes. The measured mass errors showed high confidence in the data acquired with regard to mass accuracy.

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Qualitative and Quantitative Analysis of Pesticides in Horse Feed Matrix Using Orbitrap MS

Olaf Scheibner and Maciej Bromirski, Thermo Fisher Scientific, Bremen, Germany

Application Note 573

Key Words

Exactive Plus, UHPLC, high resolution, accurate mass, high throughput, Orbitrap, Data-Dependent All-Ion Fragmentation, ExactFinder

Goal

To test the ability of a high-resolution, accurate-mass benchtop Orbitrap™ mass spectrometer to achieve high sensitivity and selectivity when analyzing modern, very-short-gradient UPHLC separations of complex samples.

Introduction

Productivity of a liquid chromatograph-mass spectrometer (LC-MS) system is measured in samples per day. To achieve higher productivity, modern ultra-high-performance LC-MS (UHPLC-MS) methods use very short gradients. Chromatographic peak widths are often below 5 seconds at the base. A high-resolution, accurate-mass (HR/AM) mass spectrometer operating in full-scan mode must be able to provide a sufficient number of scans (≥10) across the chromatographic peak without compromising sensitivity and selectivity. As reported earlier, a resolving power in excess of 50,000 (FWHM at *m/z* 200) combined with a mass extraction window of 5 ppm is necessary to ensure selectivity comparable to established MS/MS techniques.[1]

The Thermo Scientific™ Exactive™ Plus Orbitrap mass spectrometer (Figure 1) is the second generation of the Exactive product family. It features two major changes over the first generation instrument. First, in the ion optics the tube-lens / skimmer assembly has been replaced by an S-Lens (Figure 2) that provides significantly higher ion transmission, increasing the instrument's sensitivity. Second, the Orbitrap mass analyzer and related electronics have been improved,[2] resulting in higher scan speed and resolution, as well as improved polarity switching. As a result, the range of resolving power is from 17,500 to 140,000 at *m/z* 200, with a maximum scan rate of 12 Hz.

In this research, the Exactive Plus instrument was used to analyze extracts of horse feed spiked with common pesticides.

Figure 1. Exactive Plus mass spectrometer with Accela 1250 UHPLC

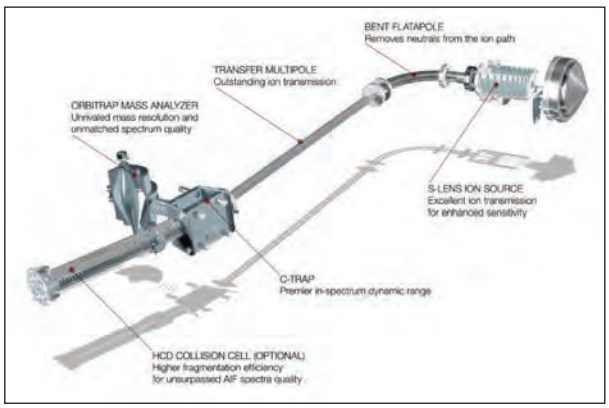

Figure 2. Exactive Plus ion optics and mass analyzer components

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Experimental

Sample Preparation

QuEChERS extracts of horse feed were spiked with 85 common pesticides (Table 1) at levels of 10 and 100 ppb, and diluted 1:1 with acetonitrile. Six calibration standards with the 85 pesticides in acetonitrile were mixed 1:1 with horse feed matrix that, through previous analysis, was proven to be free of pesticides. The final calibration levels were 5, 10, 25, 50, 100, and 150 ppb (5–150 µg/kg).

Table 1. Pesticides spiked into QuEChERS extracts

Pesticide	Chemical Formula
Acephate	$C_4H_{10}NO_3PS$
Acetamiprid	$C_{10}H_{11}ClN_4$
Aldicarb	$C_7H_{14}N_2O_2S$
Aldicarb-sulfone	$C_7H_{14}N_2O_4S$
Azinphos-ethyl	$C_{12}H_{16}N_3O_3PS_2$
Azinphos-methyl	$C_{10}H_{12}N_3O_3PS_2$
Azoxystrobin	$C_{22}H_{17}N_3O_5$
Bromacil	$C_9H_{13}BrN_2O_2$
Bromuconazole	$C_{13}H_{12}BrCl_2N_3O$
Carbaryl	$C_{12}H_{11}NO_2$
Carbendazim	$C_9H_9N_3O_2$
Carbofuran	$C_{12}H_{15}NO_3$
Carbofuran-3-hydroxy	$C_{12}H_{15}NO_4$
Chlorfluazuron	$C_{20}H_9Cl_3F_5N_3O_3$
Clofentezine	$C_{14}H_8Cl_2N_4$
Cymiazole	$C_{12}H_{14}N_2S$
Cymoxanil	$C_7H_{10}N_4O_3$
Cyproconazole	$C_{15}H_{18}ClN_3O$
Cyromazine	$C_6H_{10}N_6$
Demeton-S-methyl-sulfone	$C_6H_{15}O_5PS_2$
Dichlorvos	$C_4H_7Cl_2O_4P$
Diethofencarb	$C_{14}H_{21}NO_4$
Difenoconazole	$C_{19}H_{17}Cl_2N_3O_3$
Diflubenzuron	$C_{14}H_9ClF_2N_2O_2$
Dimethoate	$C_5H_{12}NO_3PS_2$
Disulfoton	$C_8H_{19}O_2PS_3$
Disulfoton-sulfone	$C_8H_{19}O_4PS_3$
Diuron	$C_9H_10Cl_2N_2O$
Ethiofencarb	$C_{11}H_{15}NO_2S$
Fenamiphos	$C_{13}H_{22}NO_3PS$
Fenazaquin	$C_{20}H_{22}N_2O$
Fenhexamid	$C_{14}H_{17}Cl_2NO_2$
Fenobucarb	$C_{12}H_{17}NO_2$
Fenoxycarb	$C_17H_{19}NO_4$
Fenthion	$C_{10}H_{15}O_3PS_2$
Flucycloxuron	$C_{25}H_{20}ClF_2N_3O_3$
Flufenoxuron	$C_{21}H_{11}ClF_6N_2O_3$
Formetanate	$C_{11}H_{15}N_3O_2$
Furathiocarb	$C_{18}H_{26}N_2O_5S$
Hexaflumuron	$C_{16}H_8Cl_2F_6N_2O_3$
Hexythiazox	$C_{17}H_{21}ClN_2O_2S$
Imazalil	$C_{14}H_{14}Cl_2N_2O$
Imidacloprid	$C_9H_{10}ClN_5O_2$

Pesticide	Chemical Formula
Indoxacarb	$C_{22}H_{17}ClF_3N_3O_7$
Iprovalicarb	$C_{18}H_{28}N_2O_3$
Isofenphos-methyl	$C_{14}H_{22}NO_4PS$
Isofenphos-oxon	$C_{15}H_{24}NO_5P$
Isoprothiolane	$C_{12}H_{18}O_4S_2$
Isoproturon	$C_{12}H_{18}N_2O$
Linuron	$C_9H_{10}Cl_2N_2O_2$
Mepanipyrim	$C_{14}H_{13}N_3$
Metconazole	$C_{17}H_{22}ClN_3O$
Methiocarb	$C_{11}H_{15}NO_2S$
Methiocarb-sulfone	$C_{11}H_{15}NO_4S$
Methoxyfenozide	$C_{22}H_{28}N_2O_3$
Metobromuron	$C_9H_{11}BrN_2O_2$
Monocrotophos	$C_7H_{14}NO_5P$
Napropamide	$C_{17}H_{21}NO_2$
Nitenpyram	$C_{11}H_{15}ClN_4O_2$
Omethoate	$C_5H_{12}NO_4PS$
Oxamyl	$C_7H_{13}N_3O_3S$
Pencycuron	$C_{19}H_{21}ClN_2O$
Phenmedipham	$C_{16}H_{16}N_2O_4$
Pirimicarb	$C_{11}H_{18}N_4O_2$
Prochloraz	$C_{15}H_{16}Cl_3N_3O_2$
Propamocarb	$C_9H_{20}N_2O_2$
Propoxur	$C_{11}H_{15}NO_3$
Prosulfocarb	$C_{14}H_{21}NOS$
Prosulfuron	$C_{15}H_{16}F_3N_5O_4S$
Pymetrozine	$C_{10}H_{11}N_5O$
Pyraclostrobin	$C_{19}H_{18}ClN_3O_4$
Pyridaphenthion	$C_{14}H_{17}N_2O_4PS$
Spinosyn-A	$C_{41}H_{65}NO_{10}$
Spinosyn-D	$C_{42}H_{67}NO_{10}$
Spiroxamine	$C_{18}H_{35}NO_2$
Tebufenozide	$C_{22}H_{28}N_2O_2$
Tebufenpyrad	$C_{18}H_{24}ClN_3O$
Teflubenzuron	$C_{14}H_6Cl_2F_4N_2O_2$
Tetraconazole	$C_{13}H_{11}Cl_2F_4N_3O$
Thiabendazole	$C_{10}H_7N_3S$
Thiacloprid	$C_{10}H_9ClN_4S$
Thiodicarb	$C_{10}H_{18}N_4O_4S_3$
Trichlorfon	$C_4H_8Cl_3O_4P$
Trifloxystrobin	$C_{20}H_{19}F_3N_2O_4$
Triflumuron	$C_{15}H_{10}ClF_3N_2O_3$

Liquid Chromatography

A Thermo Scientific Accela™ UHPLC system consisting of an Accela open autosampler in combination with an Accela 1250 UHPLC pump was used. A 2 minute chromatographic gradient of water and methanol, both spiked with 0.1% formic acid, was applied resulting in a total chromatographic cycle time of 5 minutes (Figure 3). Ten microliters of each sample were injected onto a Thermo Scientific Hypersil™ GOLD PFP column (50 x 2.1 mm, 1.9 µm particle size) with a flow rate of 800 µL/min. This resulted in peak widths of 3–6 seconds for the analytes of interest.

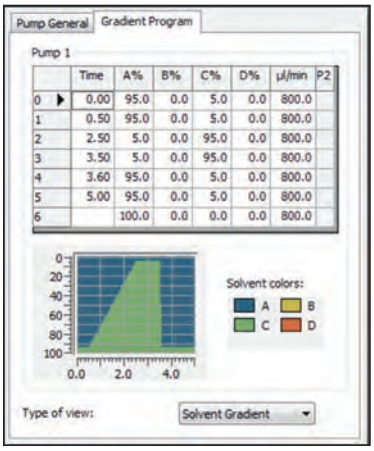

Figure 3. Chromatographic gradient

Mass Spectrometry

Given that resolution in excess of 50,000 was needed for this application, the Exactive Plus system was set to a resolving power of 70,000 at *m/z* 200, resulting in a scan rate of 3.7 Hz. As shown in Figure 4, this provided 13 scans across a 3.2 second peak.

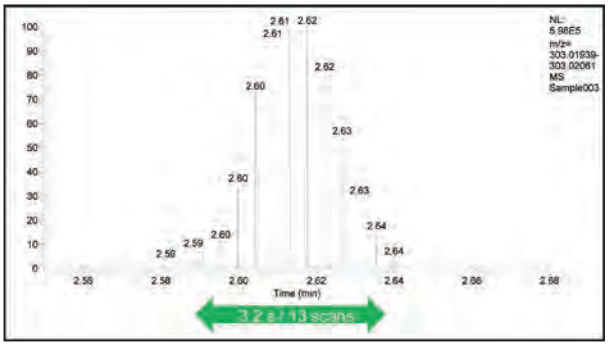

Figure 4. Scans achieved across a narrow chromatographic peak

For improved component identification, it would have been useful to have fragmentation scans on the analytes of interest. However, continual switching between full-scan and all-ion fragmentation scan modes (FS/AIF) would have required resolution to be reduced to maintain the number of scans. As an optimal solution, data-dependent AIF scans (dd-AIF) were introduced into the full scans (FS/dd-AIF) by means of a mass inclusion list containing the masses of the spiked components. One AIF scan was triggered for each target compound as soon as the abundance of the target compound crossed a given intensity threshold in a full scan. This significantly reduced the number of fragmentation scans and kept the overall data rate close to what could have been achieved in full-scan-only mode. Method details are shown in Figure 5.

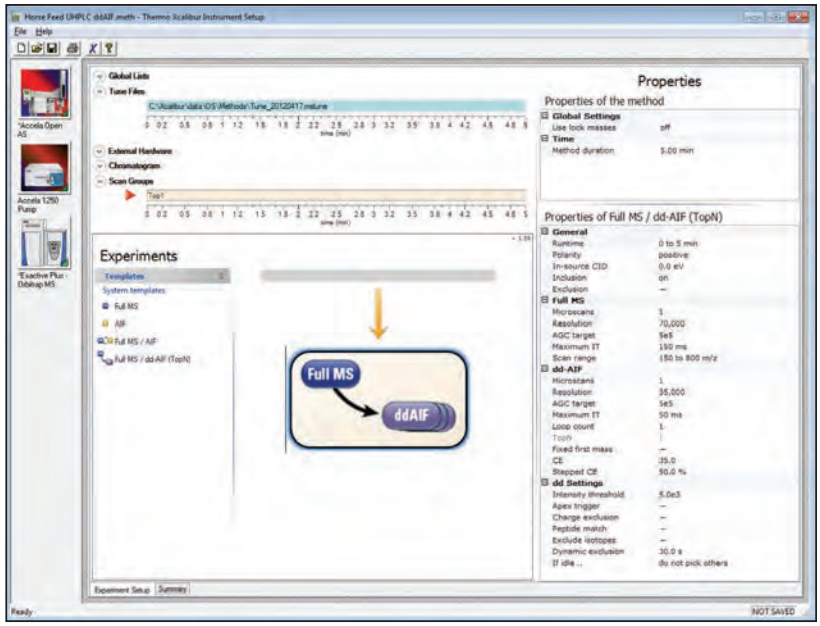

Figure 5. Exactive Plus instrument method setup

Data Analysis

The same data set was used for quantitative and qualitative data processing. Thermo Scientific ExactFinder™ software version 2.0 was used to process the data. Qualitative processing included targeted screening in combination with general unknown screening. The 85 common pesticides were selected using built-in databases from ExactFinder software. These selection could be exported directly into the mass inclusion list used by the Exactive Plus instrument method to trigger the dd-AIF scans. No further optimization of the LC-MS system was needed.

Results and Discussion

Quantitative Analysis

The six calibration standards, with spike levels ranging from 5 to 150 µg/kg, were analyzed to establish calibration curves for each of the target pesticides. The majority of pesticides eluted at between 1.3 and 3.0 minutes, so a number of target components and matrix components coeluted (Figure 6). However, the extracted ion chromatograms of most target components were free from additional peaks, demonstrating that the 5 ppm extraction window combined with the resolving power of the mass spectrometer provided very high selectivity. Linear calibration curves were achieved for nearly all target pesticides (example shown in Figure 7), confirming that the compounds could be clearly distinguished from the matrix.

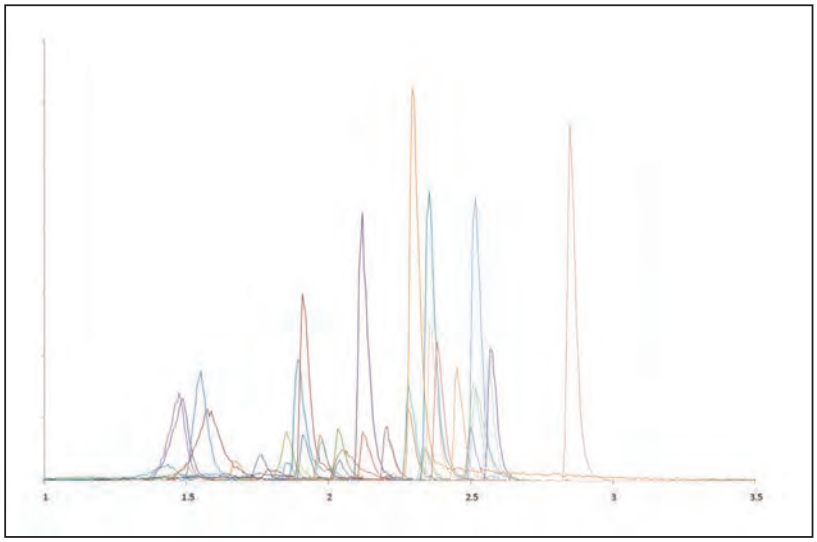

Figure 6. Extracted chromatograms demonstrate coelution of target and matrix compounds (only 20 traces compound shown)

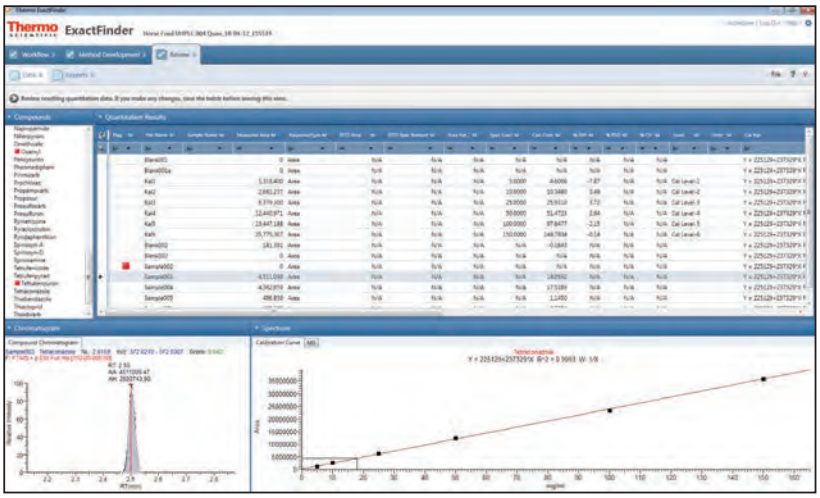

Figure 7. Example of quantitative results from one target compound (tetraconazole)

Qualitative Analysis

Qualitative analysis was carried out as a combination of targeted analysis and general unknown screening. In a first step, targeted analysis was carried out. In a second step, all peaks not identified in the targeted search were automatically forwarded for general unknown screening.

The same list of analytes used for quantitative analysis (Table 1) was applied for the targeted search. Retention time, isotopic pattern match, fragment search, and library search were used as confirmation criteria for targeted search. The fragment information for the analytes of interest and the fragmentation spectra for the library search were taken from databases included with the ExactFinder software. Even at the lower end of the concentration range, most components quantified could be easily confirmed on all four stages of confirmation (see Figure 8). With its built-in reporting capabilities, the ExactFinder software version 2.0 provided a quick, easy overview of the screening results.

Figure 8. Qualitative results as displayed by the ExactFinder software

It quickly became clear that sufficient resolution was the key to successful full-scan quantitation and screening of complex samples like the ones analyzed in this work. As shown in Figure 9, most analyte signals were surrounded by numerous matrix signals. Only sufficient resolving power ensured proper separation of analyte and matrix signals. This applies to the monoisotopic signals used for analysis as well as for the isotopic signals used for confirmation. The peaks of interest showed a resolution of close to 60,000. It was apparent that significantly lower resolving power at these masses would have led to interference and merged signals, causing significant mass shifts. The mass shifts would have led to false negatives or would have required to widening of the extraction window. Widening the extraction window would have lowered the selectivity of the analysis and resulted in false positives.

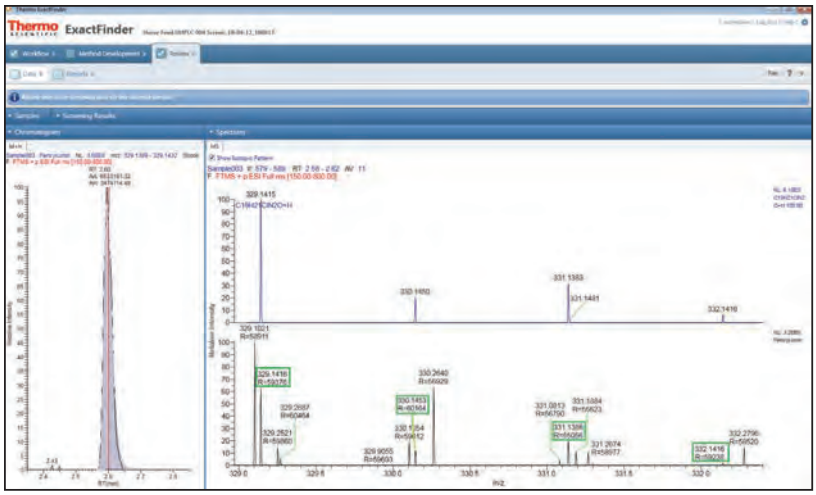

Figure 9. Isotopic pattern match of pencycuron. Green boxes mark the isotope signals surrounded by matrix signals

The general unknown screening carried out on the remaining peaks offers several options for automatic identification of the found peaks: database search, elemental composition determination based on isotopic pattern matching, spectral library search, and internet search. For the samples, roughly 15,000 components were detected; all of them went through the identification process. Database and spectral library searches were carried out using built-in resources. Internet search was carried out using a selection of databases listed in the ChemSpider® online search portal. Numerous additional contaminants could be identified, especially pesticides and a selection of aflatoxins (results not shown).

Conclusion

HR/AM analysis is a versatile method for residue analysis. It offers full quantitation capabilities in combination with unrestricted target and unknown screening options. Ultra-high resolution delivered by the Orbitrap mass analyzer in the Exactive Plus mass spectrometer provides reliability and selectivity comparable to established MS/MS techniques. The Exactive Plus mass spectrometer is compatible with UHPLC without compromising resolution or mass accuracy.

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Non-targeted Screening and Accurate Mass Confirmation of 510 Pesticides on the High Resolution Exactive Benchtop LC/MS Orbitrap Mass Spectrometer

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Key Words

- Exactive
- High Mass Accuracy
- High Resolution
- Orbitrap Technology
- Pesticide Analysis

Overview

As agricultural trade grows and food safety concerns mount, stricter pesticide regulations are being enforced around the world. Increased pesticide testing and reductions in maximum permissible residue levels have driven demand for fast, sensitive and cost-effective analytical methods for high-throughput screening of multi-class pesticides in food. Detection of 510 pesticides at low ppb levels was achieved within 12 minutes using the Thermo Scientific Exactive benchtop LC/MS system powered by Orbitrap technology. The high resolving power of the Thermo Scientific Orbitrap platform enables accurate mass confirmation of all compounds, including isobaric pesticides. Accurate, robust, easy to use and cost-efficient, the Exactive™ LC/MS is ideally suited for routine, comprehensive screening of targeted and non-targeted pesticides at or below the 0.01 mg/kg (10 ppb) default limit set by EU and Japanese legislation.

Introduction

In 2007, the United States Environmental Protection Agency (EPA) completed a ten-year reassessment of 9,721 pesticide tolerances to meet more stringent safety standards and recommended the revocation or modification of thousands of uses of pesticides in food.[1] China published national standard GB 2763-2005 in 2005, which established 478 maximum residue levels (MRLs) for 136 pesticides.[2] Japan's Positive List System, introduced in 2006, established MRLs for hundreds of agricultural chemicals, including approximately 400 pesticides, in food and set a uniform limit of 10 ppb to chemicals for which MRLs have not been determined.[3] Regulation (EC) No. 396/2005 of the European Parliament, implemented in 2008, harmonized all pesticide MRLs for European Union (EU) member states and set default limits of 0.01 mg/kg for all pesticide/commodity combinations for which no MRLs have been set.[4] A pesticide safety review of about 1,000 active substances on the market was mandated by EU Directive 91/414/EEC and, upon completion in 2009, led to the approval of only about 250 substances, effectively setting the permissible levels of over 700 de-listed pesticides to the default limit.[5] The EU and Japanese regulations are among the most stringent in the world and have fueled the need for faster and more sensitive analytical methods for cost-efficient, high-throughput screening of multi-class pesticide residues.

Pesticides in food were traditionally monitored and quantified using gas chromatography (GC) coupled with either selective detectors (e.g. electron capture) or mass spectrometry (MS). GC/MS continues to be widely used in pesticide analysis because it is highly selective, provides confirmation of multiple classes of pesticides in a single analytical run, and is relatively inexpensive and easy to operate. However, GC/MS cannot detect polar, thermally unstable or low volatility compounds without derivatization. Recent improvements in liquid chromatography (LC) throughput and MS detection capabilities have led to a surge in the use of LC/MS-based techniques for screening, confirmation and quantitation of ultra-trace levels of multi-class pesticide residues, including those that are not GC-amenable. LC-triple quadrupole tandem MS (LC/MS/MS) enables highly selective and sensitive quantification and confirmation of hundreds of target pesticides in a single run, but this approach requires extensive compound-dependent parameter optimization and cannot be used to screen for untargeted pesticides. Full scan approaches using high performance time-of-flight (TOF) or Orbitrap™ mass spectrometers coupled to ultra-high pressure LC (U-HPLC) facilitate rapid and sensitive screening and detection of LC-amenable pesticide residues present in a sample. The superior resolving power of the Orbitrap mass spectrometer (up to 100,000 FWHM) compared to TOF instruments (10,000–20,000) ensures the high mass accuracy required for complex sample analysis.[6] High resolution LC/MS instrumentation, however, can be cost-prohibitive for many routine monitoring laboratories.

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The Thermo Scientific Exactive benchtop LC/MS Orbitrap mass spectrometer was designed for accurate and reliable screening of complex samples in a wide range of demanding high-throughput applications. Built on Orbitrap mass analyzer technology, the Exactive delivers exceptional mass resolution (up to 100,000) to ensure highly accurate mass measurements and to enable confident discrimination of co-eluting, isobaric compounds in complex samples.[6,7] A wide in-scan dynamic range (3-4 orders of magnitude) facilitates the detection of trace levels of compounds in the presence of highly abundant matrix interferences. High scan speeds and polarity switching ensure full compatibility with U-HPLC and high-throughput methods. Cost-effective and easy to operate, the Exactive is an ideal tool for compliance monitoring in regulatory labs. In this note, we demonstrate rapid screening and accurate mass confirmation of 510 pesticides at low ppb levels using U-HPLC coupled to a high resolution Exactive benchtop Orbitrap mass spectrometer. Full scan U-HPLC-single stage Orbitrap MS can be used to screen a virtually limitless number of pesticides and, unlike MS/MS methods, does not require compound-dependent parameter optimization.

Materials and Methods

Sample Preparation

Pesticide standards were obtained from the U.S. Food and Drug Administration (FDA). A stock solution of a mixture of 510 pesticides was prepared at a concentration of 3 mg/L. Calibration solutions, with concentrations of 1-250 ppb, were prepared by serial dilution of the stock solution in 50:50 (v/v) acetonitrile/water.

Spiked spinach samples were prepared for analysis using a modified QuEChERS method (Figure 1). QuEChERS, an acronym for Quick, Easy, Cheap, Effective, Rugged, and Safe, is a sample preparation procedure used to extract pesticides from food.[8] Malathion D6 was used as an internal standard for calibration.

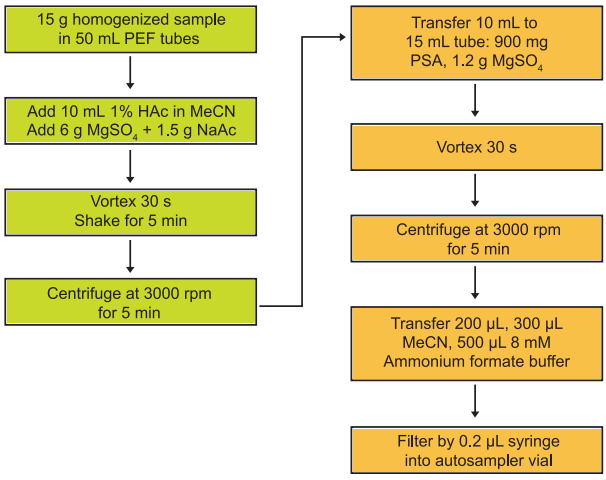


Figure 1: Schematic of the modified QuEChERS workflow used to extract pesticides from spinach matrices

Experimental Conditions

Instrumentation

LC/MS analysis was performed using a Thermo Scientific Accela U-HPLC system. With a CTC Analytics PAL autosampler coupled to an Exactive benchtop Orbitrap mass spectrometer (Figure2). Data acquisition was performed using Thermo Scientific Xcaliber software. Thermo Scientific Pathfinder software was used for data processing.

Figure 2: LC/MS analysis was performed using an Accela™ U-HPLC system coupled to an Exactive benchtop Orbitrap mass spectrometer

LC Parameters

Column:	Thermo Scientific Hypersil GOLD aQ C18 column (100 x 2.1 mm, 1.9 µm particle size)		
Mobile Phase:	A: Water with 0.1% formic acid and 4 mM ammonium formate B: Methanol with 0.1% formic acid and 4 mM ammonium formate		
Flow Rate:	300 µL/min		
Column Temperature:	ambient		
Sample Injection Volume:	10 µL		
Gradient:	Time (min)	%A	%B
	0	100	0
	1	100	0
	8	0	100
	12	0	100
	12.5	100	0
	14	100	0

MS Parameters

Full mass scan positive/negative ion mode (mass range = 100 to 1500)	
Resolution:	50,000
Automatic Gain Control (AGC) Target Value:	10e6
Heated Electrospray Ionization Source Conditions:	
Spray Voltage:	2200 V
Capillary Temperature:	280 °C
Sheath Gas:	32 au
Auxiliary Gas:	7 au
Vaporizer Temperature:	200 °C

Results and Discussion

U-HPLC improves chromatographic resolution, speed and sensitivity, and when coupled to MS, facilitates rapid, high-throughput analysis of challenging samples. Using U-HPLC-single stage Orbitrap MS, a mixture of 510 pesticides representing a broad spectrum of chemical classes was separated and detected within 12 minutes (Table 1). High resolution (50,000) and high mass accuracy (< 5 ppm without internal calibration for most compounds) enabled identification of all analytes (Table 1). Separation of isobaric pesticides was achieved only at the high resolving powers provided by Orbitrap MS, as demonstrated in Figure 3. Excellent linearity in detector response was observed over the range of 1-250 ppb, with correlation coefficients greater than 0.99 for the majority of pesticides (Table 1). Chromatograms and calibration curves for eight representative pesticides are shown in Figure 4. For the concentration range studied (1-250 ppb), limits of quantitation (LOQs) were estimated from triplicate injections (CV < 15%) of standard solutions at concentration levels corresponding to a signal-to-noise ratio of 10. As shown in Table 1, LOQs ranged from 1-50 ppb, and for 499 pesticides, LOQs were at or below 10 ppb, the MRL imposed by EU and Japanese regulations.

To evaluate the applicability of this technique to complex food samples, U-HPLC-single stage Orbitrap MS was used to screen for pesticides extracted from a spiked spinach matrix. An extraction procedure based on fast and efficient QuEChERS methodology was used to facilitate rapid high-throughput multiresidue analysis. Table 2 summarizes this and mass spectral data obtained for a representative set of extracted pesticides. Extracted ion chromatograms and calibration curves for six pesticides extracted from the spiked spinach matrix are depicted in Figure 5. The detection and quantitation capabilities of this method were assessed using the EPA method detection limit (MDL) procedure.[9] For all pesticides, limits of detection (LODs) and LOQs were lower than 1 ppb (Table 2).

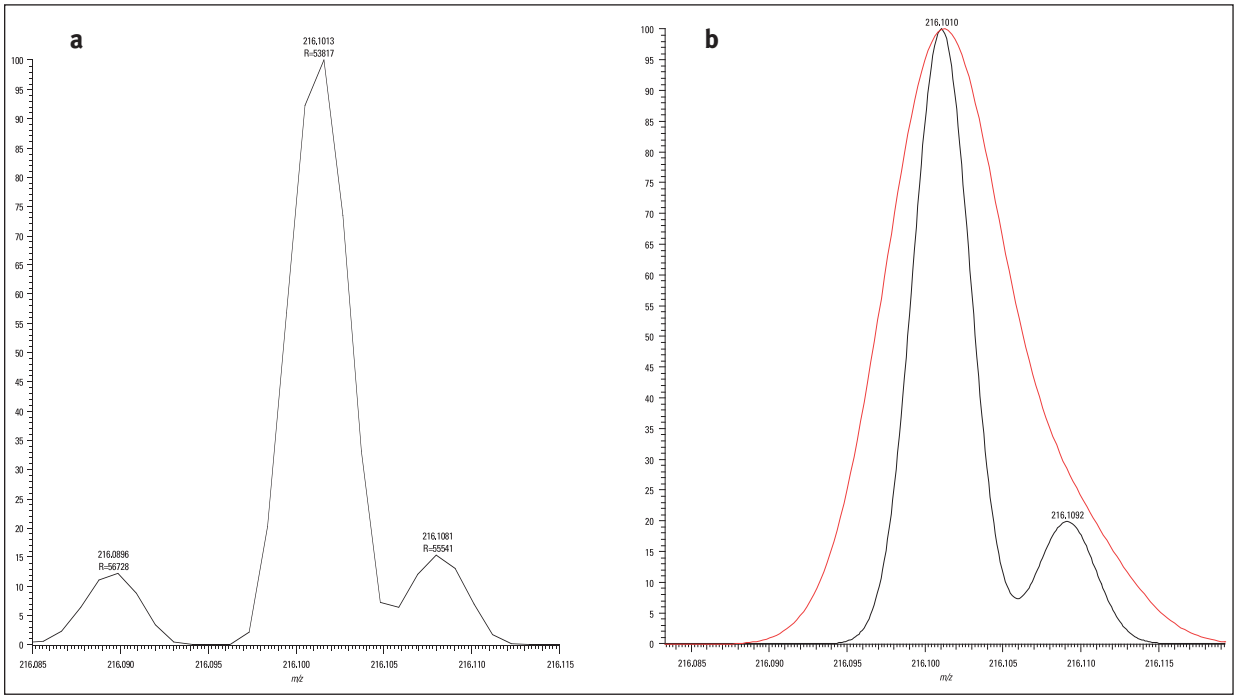


Figure 3: The high resolving power of the Exactive benchtop Orbitrap mass spectrometer enabled separation of the $[M+H]^+$ ion of atrazine (m/z = 216.1012) from the $[M+NH_4]^+$ ion of cymoxanil (m/z = 216.1088). (a) Mass spectra of the two isobaric pesticides at a resolution of 50,000. (b) Simulated mass spectra of the isobaric pesticides at resolutions of 25,000 (red line) and 50,000 (black line).

LC/MS data for Pesticide Standards *(Table 1)*

Compound	Formula	Polarity	Theoretical Mass (*m/z*)	Experimental Mass (*m/z*)	Mass Deviation (ppm)	LOQ (ppb)	R^2
Abamectin B1a	C48H72O14	+	890.526	890.5261	0	10	0.9898
Abamectin B1b	C47H70O14	+	876.5104	876.5138	3.8	10	0.9315
Acephate	C4H10NO3PS	+	184.0192	184.0193	0.8	1	0.9994
Acequinocyl	C24H32O4	+	402.2639	402.2638	0.2	1	0.9886
Acetamiprid	C10H11ClN4	+	223.0745	223.0747	0.7	1	0.9989
Acibenzolar S-methyl	C8H6N2OS2	+	210.9994	211.0004	4.4	10	0.9936
Acifluorfen	C14H7ClF3NO5	-	359.9892	359.9896	1.1	1	0.9961
Aclonifen	C12H9ClN2O3	+	282.064	282.065	3.6	25	0.9812
Acrinathrin	C26H21F6NO5	+	559.1662	559.1664	0.3	1	0.9931
Akton	C12H14Cl3O3PS	+	374.954	374.9536	1	25	0.9859
Alachlor	C14H20ClNO2	+	270.1255	270.1255	0.3	1	0.9890
Alanycarb	C17H25N3O4S2	+	400.1359	400.1369	2.5	1	0.9049
Aldicarb	C7H14N2O2S	+	208.1114	208.1116	0.6	1	0.9989
Aldicarb sulfone	C7H14N2O4S	+	223.0747	223.0747	0.3	1	0.9987
Aldicarb sulfoxide	C7H14N2O3S	+	207.0798	207.0798	0.1	1	0.9998
Allethrin	C19H26O3	+	303.1955	303.1957	0.6	1	0.9983
Allidochlor	C8H12ClNO	+	174.068	174.068	0	1	0.9936
Ametryn	C9H17N5S	+	228.1277	228.1278	0.4	1	0.9979
Amicarbazone	C10H19N5O2	+	242.1612	242.1612	0.1	1	0.9986
Aminocarb	C11H16N2O2	+	209.1285	209.1285	0.3	1	0.9997
Aminopyralid	C6H4Cl2N2O2	-	204.9577	204.9571	2.9	1	0.9629
Amitraz	C19H23N3	+	294.1965	294.1965	0.1	1	0.9725
Ancymidol	C15H16N2O2	+	257.1285	257.1284	0.4	1	0.9954
Anilazine	C9H5Cl3N4	-	272.9507	272.9572	2.4	1	0.9653
Anilofos	C13H19ClNO3PS2	+	368.0305	368.0304	0.3	1	0.9986
Anilofos	C13H19ClNO3PS2	+	368.0305	368.0304	0.3	1	0.9971
Antimycin A	C28H40N2O9	-	547.2661	547.2668	1.2	1	0.9928
Aramite	C15H23ClO4S	+	352.1344	352.1345	0.4	1	0.9946
Aspon	C12H28O5P2S2	+	379.0926	379.0927	0.1	1	0.9853
Asulam	C8H10N2O4S	+	248.07	248.07	0	1	0.9986
Atrazine	C8H14ClN5	+	216.1011	216.1012	0.8	1	0.9991
Azaconazole	C12H11Cl2N3O2	+	300.0301	300.0302	0.1	1	0.9940
Azadirachtin	C35H44O16	+	738.2968	738.2968	0	1	0.9904
Azafenidrin	C15H13Cl2N3O2	+	338.0458	338.0458	0	1	0.9932
Azamethiphos	C9H10ClN2O5PS	+	324.9809	324.981	0.2	1	0.9991
Azinphos methyl oxon	C10H12N3O4PS	+	302.0359	302.0359	0.1	1	0.9969
Azinphos-ethyl	C12H16N3O3PS2	+	346.0444	346.0443	0.1	1	0.9906
Azinphos-methyl	C10H12N3O3PS2	+	318.0131	318.0129	0.3	1	0.9957
Azoxystrobin	C22H17N3O5	+	404.1241	404.124	0.2	1	0.9948
Barban	C11H9Cl2NO2	+	275.0349	275.0355	2.4	10	0.9953
Benalaxyl	C20H23NO3	+	326.1751	326.175	0.4	1	0.9986
Benazolin	C9H6ClNO3S	+	243.983	243.9827	1.2	1	0.9863
Bendiocarb	C11H13NO4	+	224.0917	224.0919	0.8	1	0.9993
Benfluralin	C13H16F3N3O4	+	353.1431	353.143	0.2	1	0.9883
Benfuracarb	C20H30N2O5S	+	428.2214	428.2211	0.6	1	0.9956
Benodanil	C13H10INO	+	323.988	323.9879	0.4	1	0.9925
Benoxacor	C11H11Cl2NO2	+	260.024	260.024	0.1	10	0.9989
Bensulide	C14H24NO4PS3	+	415.0943	415.0944	0.1	1	0.9872
Bentazone	C10H12N2O3S	+	241.0641	241.0643	0.5	1	0.9982
Benthiavalicarb	C15H18FN3O3S	+	340.1126	340.114	4.2	1	0.9047
Benzoximate	C18H18ClNO5	+	364.0946	364.0944	0.7	1	0.9965
Bifenazate	C17H20N2O3	+	301.1547	301.1546	0.1	1	0.9892
Bifenox	C14H9Cl2NO5	+	359.0196	359.0193	0.8	10	0.9668
Bifenthrin	C23H22ClF3O2	+	423.1333	423.1322	2.6	10	0.9729
Binapacryl	C15H18N2O6	+	340.1503	340.1496	2.2	10	0.9688
Bispyribac-sodium	C19H17N4NaO8	+	453.1017	453.1018	0.1	10	0.9843
Bitertanol	C20H23N3O2	+	338.1863	338.1861	0.5	1	0.9916
Boscalid	C18H12Cl2N2O	+	343.04	343.0399	0.1	1	0.9797
Brodifacoum	C31H23BrO3	-	521.0758	521.0755	0.5	1	0.9905

Compound	Formula	Polarity	Theoretical Mass (*m/z*)	Experimental Mass (*m/z*)	Mass Deviation (ppm)	LOQ (ppb)	R^2
Bromadiolone	C30H23BrO4	-	525.0707	525.0706	0.1	1	0.9879
Bromoxynil	C7H3Br2NO	-	273.8509	273.8506	1	1	0.9990
Bromuconazole(cis-)	C13H12BrCl2N3O	+	375.9614	375.9613	0	1	0.9961
Bromuconazole(trans-)	C13H12BrCl2N3O	+	375.9614	375.9613	0	1	0.9912
Bufencarb	C13H19NO2	+	222.1489	222.149	0.6	1	0.9965
Bupirimate	C13H24N4O3S	+	317.1642	317.1641	0.2	1	0.9978
Buprofezin	C16H23N3OS	+	306.1635	306.1632	0.7	1	0.9974
Butachlor	C17H26ClNO2	+	329.199	329.1989	0.3	10	0.9928
Butafenacil	C20H18ClF3N2O6	+	492.1144	492.1144	0.1	1	0.9981
Butocarboxim	C7H14N2O2S	+	208.1114	208.1116	0.6	1	0.9971
Butoxycarboxim	C7H14N2O4S	+	223.0747	223.0747	0.3	1	0.9990
Butralin	C14H21N3O4	+	296.1605	296.1604	0.4	1	0.9983
Butylate	C11H23NOS	+	218.1573	218.1575	0.9	1	0.9993
Cadusafos	C10H23O2PS2	+	271.095	271.0948	0.8	1	0.9874
Carbaryl	C12H11NO2	+	202.0863	202.0855	3.9	1	0.9923
Carbendazim	C9H9N3O2	+	192.0768	192.0767	0.4	1	0.9986
Carbetamide	C12H16N2O3	+	237.1234	237.1235	0.6	1	0.9982
Carbofuran	C12H15NO3	+	222.1125	222.1126	0.4	1	0.9980
Carbofuran, 3OH-	C12H15NO4	+	255.1339	255.1338	0.5	1	0.9986
Carboxin	C12H13NO2S	+	236.074	236.074	0.3	1	0.9972
Carfentrazone-ethyl	C15H14Cl2F3N3O3	+	429.0703	429.0702	0.1	1	0.9957
Carpropamid	C15H18Cl3NO	+	334.0527	334.0526	0.1	1	0.9982
Chinomethionate	C10H6N2OS2	+	252.026	252.0267	2.7	10	0.9963
Chlorantraniliprole	C18H14BrCl2N5O2	+	481.9781	481.978	0.1	1	0.9718
Chlorbromuron	C9H10BrClN2O2	+	292.9687	292.9688	0.2	1	0.9958
Chlorbufam	C11H10ClNO2	+	241.0738	241.073	3.5	1	0.9864
Chlordimeform	C10H13ClN2	+	197.084	197.084	0.1	10	0.9973
Chlorfenvinphos	C12H14Cl3O4P	+	358.9768	358.9767	0.2	1	0.9976
Chlorfluazuron	C20H9Cl3F5N3O3	+	556.9968	556.9968	0.1	1	0.9963
Chloroxuron	C15H15ClN2O2	+	291.0895	291.0893	0.8	1	0.9978
Chlorpropham	C10H12ClNO2	+	214.0629	214.0632	1.3	10	0.9910
Chlorpyrifos	C9H11Cl3NO3PS	+	349.9336	349.9336	0	1	0.9951
Chlorpyrifos oxon	C9H11Cl3NO4P	+	333.9564	333.9564	0.1	1	0.9903
Chlorpyrifos-methyl	C7H7Cl3NO3PS	+	321.9023	321.9022	0.1	25	0.9763
Chlorthiamid	C7H5Cl2NS	+	222.9858	222.9852	2.8	10	0.9857
Chlorthion	C8H9ClNO5PS	+	314.9966	314.9971	1.6	25	0.9812
Chlorthiophos	C11H15Cl2O3PS2	+	360.965	360.9643	1.9	25	0.9632
Chlortoluron	C10H13ClN2O	+	213.0789	213.079	0.6	1	0.9976
Clethodim	C17H26ClNO3S	+	360.1395	360.1395	0.2	1	0.9923
Clofentezine	C14H8Cl2N4	+	320.0464	320.045	4.5	10	0.9935
Clothianidin	C6H8ClN5O2S	+	250.016	250.016	0.2	1	0.9916
Coumaphos	C14H16ClO5PS	+	363.0217	363.0217	0.1	1	0.9983
Coumaphos oxon	C14H16ClO6P	+	347.0446	347.0446	0	1	0.9951
Crotoxyphos	C14H19O6P	+	332.1258	332.1255	0.7	1	0.9982
Crufomate	C12H19ClNO3P	+	309.1129	309.112	3.1	1	0.9914
Cumyluron	C17H19ClN2O	+	303.1259	303.1258	0.2	1	0.9989
Cyanazine	C9H13ClN6	+	241.0963	241.0963	0.2	1	0.9951
Cyazofamid	C13H13ClN4O2S	+	342.0786	342.077	4.6	1	0.9895
Cyclanilide	C11H9Cl2NO3	-	271.9887	271.9891	1.8	1	0.9991
Cycloate	C11H21NOS	+	216.1417	216.1418	0.4	1	0.9913
Cyclohexamide	C15H23NO4	+	299.1965	299.1966	0.3	1	0.9977
Cycluron	C11H22N2O	+	199.1805	199.1805	0.1	1	0.9922
Cyflufenamid	C20H17F5N2O2	+	413.1283	413.1282	0.2	1	0.9977
Cyfluthrin	C22H18Cl2FNO3	+	451.0986	451.098	1.3	10	0.7124
Cyhalothrin	C23H19ClF3NO3	+	467.1344	467.1339	1	1	0.9859
Cymoxanil	C7H10N4O3	+	216.1091	216.1088	1.3	1	0.9885
Cypermethin	C22H19Cl2NO3	+	433.108	433.108	0	10	0.9859
Cyphenothrin	C24H25NO3	+	393.2173	393.2173	0	1	0.9959
Cyproconazole	C15H18ClN3O	+	292.1211	292.1211	0.2	1	0.9978

LC/MS data for Pesticide Standards *(Table 1 continued)*

Compound	Formula	Polarity	Theoretical Mass (*m/z*)	Experimental Mass (*m/z*)	Mass Deviation (ppm)	LOQ (ppb)	R^2
Cyprodinil	C14H15N3	+	226.1339	226.1339	0.3	1	0.9967
Cyprosulfamide	C18H18N2O5S	+	375.1009	375.1009	0.1	1	0.9977
Cyromazine	C6H10N6	+	167.104	167.1039	0.2	1	0.9445
Daimuron	C17H20N2O	+	269.1648	269.1646	0.7	1	0.9992
Dazomet	C5H10N2S2	+	163.0358	163.0358	0.1	1	0.9451
DEF (Tribufos)	C12H27OPS3	+	315.1034	315.1033	0.3	1	0.9840
Deltamethrin	C22H19Br2NO3	+	521.007	521.0073	0.5	1	0.9986
Demeton S-methyl	C6H15O3PS2	+	231.0273	231.0275	0.9	1	0.9966
Demeton S-sulfone	C6H15O5PS2	+	263.0171	263.0173	0.8	10	0.9914
Demeton-O	C8H19O3PS2	+	259.0586	259.0586	0.1	1	0.9960
Demeton-S (Disulfoton oxon)	C8H19O3PS2	+	259.0586	259.0586	0.1	1	0.9960
Desmedipham	C16H16N2O4	+	318.1448	318.1448	0	1	0.9975
Desmetryn	C8H15N5S	+	214.1121	214.1122	0.6	1	0.9986
Dialifor	C14H17ClNO4PS2	+	411.0363	411.0363	0.1	1	0.9984
Diallate	C10H17Cl2NOS	+	270.0481	270.0482	0.5	1	0.9636
Diamidafos (Nellite)	C8H13N2O2P	+	201.0787	201.0787	0	1	0.9986
Diazinon	C12H21N2O3PS	+	305.1083	305.1081	0.9	1	0.9983
Diazinon hydroxy	C12H21N2O4PS	+	321.1032	321.1031	0.6	1	0.9985
Diazinon oxon	C12H21N2O4P	+	289.1312	289.1311	0.2	1	0.9385
Dicapthon	C8H9ClNO5PS	+	314.9966	314.9971	1.6	25	0.9812
Dichlofluanid	C9H11Cl2FN2O2S2	+	349.9961	349.9961	0.2	1	0.9930
Dichlorfenthion	C10H13Cl2O3PS	+	314.9773	314.9768	1.5	10	0.9966
Dichlormid	C8H11Cl2NO	+	208.0291	208.0292	0.6	1	0.9923
Dichlorvos	C4H7Cl2O4P	+	220.9532	220.9533	0.4	10	0.9920
Diclobutrazol	C15H19Cl2N3O	+	328.0978	328.0978	0.1	1	0.9949
Dicrotophos	C8H16NO5P	+	238.0839	238.0839	0.2	1	0.9991
Diethofencarb	C14H21NO4	+	268.1543	268.1543	0.1	1	0.9994
Difenacoum	C31H24O3	+	445.1798	445.1798	0.1	1	0.9972
Difenoconazole	C19H17Cl2N3O3	+	406.072	406.0719	0.3	1	0.9914
Diflenoxuron	C16H18N2O3	+	287.139	287.1389	0.6	1	0.9938
Diflubenzuron	C14H9ClF2N2O2	-	309.0248	309.0246	0.6	1	0.9985
Dimepiperate	C15H21NOS	+	264.1417	264.1429	4.9	1	0.9994
Dimethachlor	C13H18ClNO2	+	256.1099	256.1098	0.3	1	0.9921
Dimethametryn	C11H21N5S	+	256.159	256.1588	0.8	1	0.9983
Dimethenamid	C12H18ClNO2S	+	276.082	276.0818	0.5	1	0.9977
Dimethoate	C5H12NO3PS2	+	230.0069	230.007	0.3	1	0.9993
Dimethomorph	C21H22ClNO4	+	388.131	388.131	0	1	0.9970
Dimethylvinphos. Z-	C10H10Cl3O4P	+	330.9455	330.9455	0.1	1	0.9950
Dimetilan	C10H16N4O3	+	241.1295	241.1295	0.1	1	0.9990
Dimoxystrobin	C19H22N2O3	+	327.1703	327.1702	0.4	1	0.9905
Diniconazole	C15H17Cl2N3O	+	326.0821	326.0821	0.2	1	0.9899
Dinotefuran	C7H14N4O3	+	203.1139	203.1139	0.1	1	0.9957
Dioxacarb	C11H13NO4	+	224.0917	224.0919	0.8	1	0.9978
Dioxathion	C12H26O6P2S4	+	474.0426	474.0426	0	1	0.9900
Diphenamid	C16H17NO	+	240.1383	240.1383	0.1	1	0.9992
Diphenylamine	C12H11N	+	170.0964	170.0965	0.3	1	0.9952
Dipropetryn	C11H21N5S	+	256.159	256.1588	0.8	1	0.9983
Disulfoton	C8H19O2PS3	+	275.0358	275.0355	0.9	1	0.9935
Ditalimfos	C12H14NO4PS	+	300.0454	300.0452	0.6	1	0.9967
Dithianon	C14H4N2O2S2	+	314.0052	314.0064	3.6	10	0.9235
Dithiopyr	C15H16F5NO2S2	+	402.0615	402.0617	0.3	10	0.9866
Diuron	C9H10Cl2N2O	+	233.0243	233.0244	0.6	1	0.9947
DNOC	C7H6N2O5	-	197.0204	197.0205	1.5	1	0.9948
Dodemorph	C18H35NO	+	282.2791	282.279	0.6	1	0.9946
Doramectin	C50H74O14	+	916.5417	916.5418	0.1	10	0.9888
Edifenphos	C14H15O2PS2	+	311.0324	311.0322	0.6	1	0.9952
EPN	C14H14NO4PS	+	341.0719	341.0721	0.3	1	0.9983
Epoxiconazole	C17H13ClFN3O	+	330.0804	330.0803	0.2	1	0.9953
Eprinomectin B1a	C50H75NO14	+	914.526	914.526	0	1	0.9852

Compound	Formula	Polarity	Theoretical Mass (*m/z*)	Experimental Mass (*m/z*)	Mass Deviation (ppm)	LOQ (ppb)	R^2
Eprinomectin B1b	C49H73NO14	+	900.5104	900.5131	3	10	0.9738
EPTC (eptam)	C9H19NOS	+	190.126	190.1261	0.2	1	0.9938
Esprocarb	C15H23NOS	+	266.1573	266.1572	0.4	1	0.9981
Etaconazol	C14H15Cl2N3O2	+	328.0614	328.0613	0.3	1	0.9980
Ethaboxam	C14H16N4OS2	+	321.0838	321.0839	0.3	1	0.9907
Ethalfluralin	C13H14F3N3O4	+	334.1009	334.0994	4.6	1	0.9845
Ethidimuron	C7H12N4O3S2	+	265.0424	265.0422	0.6	1	0.9805
Ethiofencarb	C11H15NO2S	+	226.0896	226.0898	0.8	1	0.9987
Ethiolate	C7H15NOS	+	162.0947	162.0947	0.2	1	0.9960
Ethion	C9H22O4P2S4	+	384.9949	384.9948	0.1	1	0.9914
Ethion monoxon	C9H22O5P2S3	+	369.0177	369.0177	0	1	0.9975
Ethiprole	C13H9Cl2F3N4OS	+	414.0165	414.0164	0.1	1	0.9817
Ethirimol	C11H19N3O	+	210.1601	210.1602	0.4	1	0.9984
Ethofumesate	C13H18O5S	+	304.1213	304.1213	0.1	1	0.9986
Ethoprop	C8H19O2PS2	+	243.0637	243.0637	0	1	0.9865
Ethoxyquin	C14H19NO	+	218.1539	218.1541	0.7	1	0.9967
Etobenzanid	C16H15Cl2NO3	+	340.0502	340.0502	0.1	1	0.9969
Etofenprox	C25H28O3	+	394.2377	394.2379	0.6	1	0.9928
Etoxazole	C21H23F2NO2	+	360.177	360.1769	0.1	1	0.9976
Etrimfos	C10H17N2O4PS	+	293.0719	293.0718	0.6	1	0.9982
Famoxadone	C22H18N2O4	+	392.1605	392.1603	0.4	1	0.9937
Famphur	C10H16NO5PS2	+	343.0546	343.0531	4.4	1	0.9973
Famphur oxon	C10H16NO6PS	+	327.0774	327.0775	0.2	1	0.9955
Fenamidone	C17H17N3OS	+	312.1165	312.1163	0.6	1	0.9986
Fenamiphos	C13H22NO3PS	+	304.1131	304.113	0.3	1	0.9944
Fenamiphos sulfone	C13H22NO5PS	+	336.1029	336.1029	0.1	1	0.9924
Fenamiphos sulfoxide	C13H22NO4PS	+	320.108	320.1079	0.2	1	0.9936
Fenarimol	C17H12Cl2N2O	+	331.04	331.0399	0.3	1	0.9825
Fenazaquin	C20H22N2O	+	307.1805	307.1805	0.1	1	0.9881
Fenbuconazole	C19H17ClN4	+	337.1215	337.1214	0.1	1	0.9970
Fenhexamid	C14H17Cl2NO2	+	302.0709	302.0709	0.2	1	0.9965
Fenitrothion	C9H12NO5PS	+	295.0512	295.0517	1.6	10	0.9971
Fenoxanil	C15H18Cl2N2O2	+	346.1084	346.1083	0.1	1	0.9914
Fenoxycarb	C17H19NO4	+	302.1387	302.1386	0.5	1	0.9943
Fenpiclonil	C11H6Cl2N2	+	254.0246	254.0246	0.3	1	0.9817
Fenpropathrin	C22H23NO3	+	350.1751	350.1759	2.4	1	0.9954
Fenpropimorph	C20H33NO	+	304.2635	304.2633	0.5	1	0.9919
Fenpyroximate	C24H27N3O4	+	422.2074	422.2074	0.2	1	0.9966
Fensulfothion	C11H17O4PS2	+	309.0379	309.0378	0.3	1	0.9969
Fenthion	C10H15O3PS2	+	279.0273	279.0286	4.5	1	0.9941
Fenthion oxon	C10H15O4PS	+	263.0501	263.0501	0.1	1	0.9975
Fenthion sulfone	C10H15O5PS2	+	328.0437	328.0439	0.6	1	0.9993
Fenthion sulfoxide	C10H15O4PS2	+	295.0222	295.022	0.6	1	0.9957
Fenuron	C9H12N2O	+	165.1022	165.1022	0.4	1	0.9998
Fenvalerate	C25H22ClNO3	+	437.1627	437.1629	0.7	10	0.9919
Fipronil	C12H4Cl2F6N4OS	-	434.9314	434.9316	0.4	1	0.9968
Flonicamid	C9H6F3N3O	-	228.039	228.0384	2.6	1	0.9989
Florasulam	C12H8F3N5O3S	+	360.0373	360.0374	0.2	1	0.9956
Fluazinam	C13H4Cl2F6N4O4	-	462.9441	462.945	1.9	1	0.9946
Flubendiamide	C23H22F7IN2O4S	-	681.016	681.0154	0.9	1	0.9917
Flucarbazone	C12H11F3N4O6S	+	414.069	414.069	0	1	0.9924
Fluchloralin	C12H13ClF3N3O4	+	373.0885	373.0894	2.4	10	0.9605
Flucythrinate	C26H23F2NO4	+	469.1933	469.1933	0.2	1	0.9932
Fludioxonil	C12H6F2N2O2	+	266.0736	266.0736	0.1	1	0.9749
Flufenacet	C14H13F4N3O2S	+	364.0737	364.0736	0.4	1	0.9980
Flufenoxuron	C21H11ClF6N2O3	+	489.0435	489.0436	0.1	1	0.9929
Flumetralin	C16H12ClF4N3O4	+	422.0525	422.0537	2.8	25	0.9917
Flumetsulam	C12H9F2N5O2S	+	326.0518	326.0516	0.6	1	0.9988
Flumioxazin	C19H15FN2O4	+	355.1089	355.1089	0	10	0.9677

LC/MS data for Pesticide Standards *(Table 1 continued)*

Compound	Formula	Polarity	Theoretical Mass (*m/z*)	Experimental Mass (*m/z*)	Mass Deviation (ppm)	LOQ (ppb)	R^2
Fluometuron	C10H11F3N2O	+	233.0896	233.0897	0.4	1	0.9983
Fluopicolide	C14H8Cl3F3N2O	+	382.9727	382.9728	0.2	1	0.9911
Fluorochloridone	C12H10Cl2F3NO	+	329.043	329.0431	0.4	1	0.9837
Fluorodifen	C13H7F3N2O5	+	346.0645	346.0652	2	10	0.9963
Fluoxastrobin	C21H16ClFN4O5	+	459.0866	459.0865	0.3	1	0.9983
Fluquinconazole	C16H8Cl2FN5O	+	376.0163	376.0163	0	10	0.9939
Fluroxypyr	C7H5Cl2FN2O3	-	252.9588	252.9581	2.7	10	0.9928
Flusilazole	C16H15F2N3Si	+	316.1076	316.1076	0.1	1	0.9932
Flutolanil	C17H16F3NO2	+	341.1471	341.1471	0	1	0.9948
Flutriafol	C16H13F2N3O	+	302.11	302.11	0	1	0.9942
Fluvalinate ?	C26H22ClF3N2O3	+	520.1609	520.1613	0.7	10	0.9968
Fonophos	C10H15OPS2	+	247.0375	247.0375	0.2	1	0.9165
Fonophos O-analog	C10H15O2PS	+	231.0603	231.0601	0.8	10	0.9526
Forchlorfenuron	C12H10ClN3O	+	248.0585	248.0585	0.1	1	0.9967
Formasafen	C15H10ClF3N2O6S	-	436.9827	436.9817	2.2	1	0.9972
Formetanate	C11H15N3O2	+	239.1503	239.1503	0.1	1	0.9981
Fosthiazate	C9H18NO3PS2	+	284.0539	284.0538	0.2	1	0.9958
Fuberidazole	C11H8N2O	+	185.0709	185.0708	0.9	1	0.9972
Furalaxyl	C17H19NO4	+	302.1387	302.1386	0.5	1	0.9943
Furathiocarb	C18H26N2O5S	+	383.1635	383.1635	0.1	1	0.9980
Griseofulvin	C17H17ClO6	+	353.0786	353.0787	0.2	1	0.9968
Halofenozide	C18H19ClN2O2	-	329.1062	329.1063	0.3	1	0.9984
Haloxyfop-methyl	C16H13ClF3NO4	+	376.0558	376.0556	0.4	1	0.9965
Heptenophos	C9H12ClO4P	+	251.0235	251.0235	0.2	10	0.9983
Hexaconazole	C14H17Cl2N3O	+	314.0821	314.082	0.4	1	0.9947
Hexaflumuron	C16H8Cl2F6N2O3	-	458.9743	458.9745	0.4	1	0.9834
Hexazinone	C12H20N4O2	+	253.1659	253.1658	0.5	1	0.9975
Hexythiazox	C17H21ClN2O2S	+	353.1085	353.1084	0.4	1	0.9807
Hydramethylnon	C25H24F6N4	+	495.1978	495.1976	0.3	1	0.9965
Imazalil	C14H14Cl2N2O	+	297.0556	297.0555	0.4	1	0.9960
Imazamox	C15H19N3O4	+	306.1448	306.1447	0.5	1	0.9962
Imazapyr	C13H15N3O3	+	262.1186	262.1185	0.3	1	0.9972
Imazaquin	C17H17N3O3	+	312.1343	312.1341	0.5	1	0.9970
Imibenconazole	C17H13Cl3N4S	+	410.9999	411	0.2	1	0.9909
Imidacloprid	C9H10ClN5O2	+	256.0596	256.0595	0.5	1	0.9983
Imiprothrin	C17H22N2O4	+	319.1652	319.1651	0.4	1	0.9663
Inabenifide	C19H15ClN2O2	+	339.0895	339.0895	0	1	0.9974
Indanofan	C20H17ClO3	+	341.0939	341.0938	0.4	1	0.9824
Indoxacarb	C22H17ClF3N3O7	+	528.078	528.0779	0.2	1	0.9922
Ioxynil	C7H3I2NO	-	369.8231	369.8237	0.2	1	0.9955
Ipconazole	C18H24ClN3O	+	334.1681	334.1679	0.4	1	0.9968
Iprobenfos	C13H21O3PS	+	289.1022	289.1021	0.1	1	0.9977
Iprovalicarb	C18H28N2O3	+	321.2173	321.2171	0.4	1	0.9993
Isazophos	C9H17ClN3O3PS	+	314.049	314.0489	0.3	1	0.9988
Isocarbamid	C8H15N3O2	+	186.1237	186.1237	0	1	0.9967
Isocarbophos	C11H16NO4PS	+	307.0876	307.0876	0.1	1	0.9941
Isofenfos	C15H24NO4PS	+	346.1236	346.1236	0.2	1	0.9911
Isofenfos O-analog	C15H24NO5P	+	330.1465	330.1473	2.6	10	0.9344
Isoprocarb	C11H15NO2	+	194.1176	194.1177	0.8	1	0.9978
Isopropalin	C15H23N3O4	+	310.1761	310.1761	0.2	1	0.9932
Isoprothiolane	C12H18O4S2	+	291.0719	291.0718	0.6	1	0.9961
Isoproturon	C12H18N2O	+	207.1492	207.1492	0.2	1	0.9939
Isoxaben	C18H24N2O4	+	333.1809	333.1809	0.1	1	0.9982
Isoxadifen-ethyl	C18H17NO3	+	296.1281	296.1281	0	1	0.9968
Isoxaflutole	C15H12F3NO4S	+	377.0777	377.0779	0.4	1	0.9919
Isoxathion	C13H16NO4PS	+	314.061	314.0608	0.7	1	0.9895
Ivermectin B1a	C48H74O14	+	892.5417	892.5415	0.2	10	0.9915
Ivermectin B1b	C47H72O14	+	883.4814	883.4818	0.4	50	0.9695
Kresoxim-methyl	C18H19NO4	+	314.1387	314.1386	0.2	1	0.9969

Compound	Formula	Polarity	Theoretical Mass (*m/z*)	Experimental Mass (*m/z*)	Mass Deviation (ppm)	LOQ (ppb)	R^2
Lactofen	C19H15ClF3NO7	+	479.0827	479.0828	0.1	1	0.9883
Linuron	C9H10Cl2N2O2	+	249.0192	249.0191	0.3	1	0.9977
Lufenuron	C17H8Cl2F8N2O3	+	510.9857	510.9833	4.7	1	0.9808
Malathion	C10H19O6PS2	+	348.0699	348.07	0.4	1	0.9950
Malathion O-analog	C10H19O7PS	+	315.0662	315.0661	0.2	1	0.9948
Mandipropamid	C23H22ClNO4	+	412.131	412.131	0.1	1	0.9978
Mefenacet	C16H14N2O2S	+	299.0849	299.0848	0.4	1	0.9985
Mefluidide	C11H13F3N2O3S	+	328.0937	328.0937	0.1	1	0.9987
Mepanipyrim	C14H13N3	+	224.1182	224.1184	0.6	1	0.9887
Mephospholan	C8H16NO3PS2	+	270.0382	270.038	0.6	1	0.9915
Mepronil	C17H19NO2	+	270.1489	270.1487	0.4	1	0.9938
Mesotrione	C14H13NO7S	+	340.0486	340.0502	4.9	1	0.9952
Metaflumizone	C24H16F6N4O2	-	505.1105	505.1106	0.1	1	0.9745
Metalaxyl	C15H21NO4	+	280.1543	280.1542	0.6	1	0.9988
Metazachlor	C14H16ClN3O	+	278.1055	278.1054	0.3	1	0.9984
Metconazole	C17H22ClN3O	+	320.1524	320.1523	0.4	1	0.9881
Methabenzthiazuron	C10H11N3OS	+	222.0696	222.0698	0.9	1	0.9982
Methacrifos	C7H13O5PS	+	258.056	258.0559	0.1	1	0.9958
Methamidophos	C2H8NO2PS	+	142.0086	142.0087	0.4	1	0.9990
Methidathion	C6H11N2O4PS3	+	319.9957	319.9956	0.2	1	0.9971
Methiocarb	C11H15NO2S	+	226.0896	226.0898	0.8	1	0.9987
Methomyl	C5H10N2O2S	+	163.0536	163.0534	0.9	1	0.9991
Methoprotryne	C11H21N5OS	+	272.154	272.1537	1	1	0.9978
Methoxyfenozide	C22H28N2O3	+	369.2173	369.2172	0.2	1	0.9935
Metobromuron	C9H11BrN2O2	+	259.0077	259.0077	0.2	1	0.9948
Metofluthrin	C18H20F4O3	-	359.1276	359.1277	0.2	1	0.9887
Metolachlor	C15H22ClNO2	+	284.1412	284.1411	0.1	1	0.9981
Metominostrobin(E-)	C16H16N2O3	+	285.1234	285.1232	0.7	1	0.9957
Metosulam	C14H13Cl2N5O4S	+	418.0138	418.0137	0.3	1	0.9924
Metoxuron	C10H13ClN2O2	+	229.0738	229.074	0.6	1	0.9995
Metrafenone	C19H21BrO5	+	409.0645	409.0643	0.4	1	0.9963
Metribuzin	C8H14N4OS	+	215.0961	215.0963	0.7	1	0.9969
Mevinphos	C7H13O6P	+	242.0788	242.0788	0.1	1	0.9977
Mexacarbate	C12H18N2O2	+	223.1441	223.1443	0.7	1	0.9991
Milbemectin A3	C31H44O7	+	546.3425	546.3421	0.8	10	0.9819
Milbemectin A4	C32H46O7	+	560.3582	560.3584	0.4	1	0.9905
Molinate	C9H17NOS	+	188.1104	188.1104	0.2	1	0.9881
Monocrotophos	C7H14NO5P	+	224.0682	224.0685	1	1	0.9989
Monolinuron	C9H11ClN2O2	+	215.0582	215.0583	0.7	1	0.9977
Moxidectin	C37H53NO8	+	640.3844	640.3847	0.5	1	0.9966
Myclobutanil	C15H17ClN4	+	289.1215	289.1214	0.1	1	0.9940
Naled	C4H7Br2Cl2O4P	+	395.8164	395.8164	0.1	10	0.9908
Naphthol	C10H8O	+	145.0648	145.0648	0.2	1	0.9939
Napropamide	C17H21NO2	+	272.1645	272.1644	0.5	1	0.9933
Naptalam sodium	C18H12NNaO3	+	331.1053	331.1067	4.2	1	0.9931
Neburon	C12H16Cl2N2O	+	275.0713	275.0711	0.5	1	0.9941
Nitenpyram	C11H15ClN4O2	+	271.0956	271.0948	3.2	1	0.9876
Nitralin	C13H19N3O6S	+	346.1067	346.1083	4.6	1	0.9824
Nitrothal-isopropyl	C14H17NO6	+	313.1394	313.1385	3.5	10	0.8345
Norflurazon	C12H9ClF3N3O	+	304.0459	304.0458	0.3	1	0.9858
Novaluron	C17H9ClF8N2O4	-	491.005	491.0053	0.6	1	0.9902
Noviflumuron	C17H7Cl2F9N2O3	-	526.9617	526.9613	0.7	1	0.9759
Nuarimol	C17H12ClFN2O	+	315.0695	315.0693	0.5	1	0.9907
Octhilinone (2-Octyl-4-isothiazoline-3-one)	C11H19NOS	+	214.126	214.1262	0.8	1	0.9977
Ofurace	C14H16ClNO3	+	299.1157	299.1156	0.2	1	0.9974
Omethoate (Dimethoate oxon)	C5H12NO4PS	+	214.0297	214.0298	0.4	1	0.9997
Orbencarb	C12H16ClNOS	+	258.0714	258.0712	0.6	1	0.9969

LC/MS data for Pesticide Standards *(Table 1 continued)*

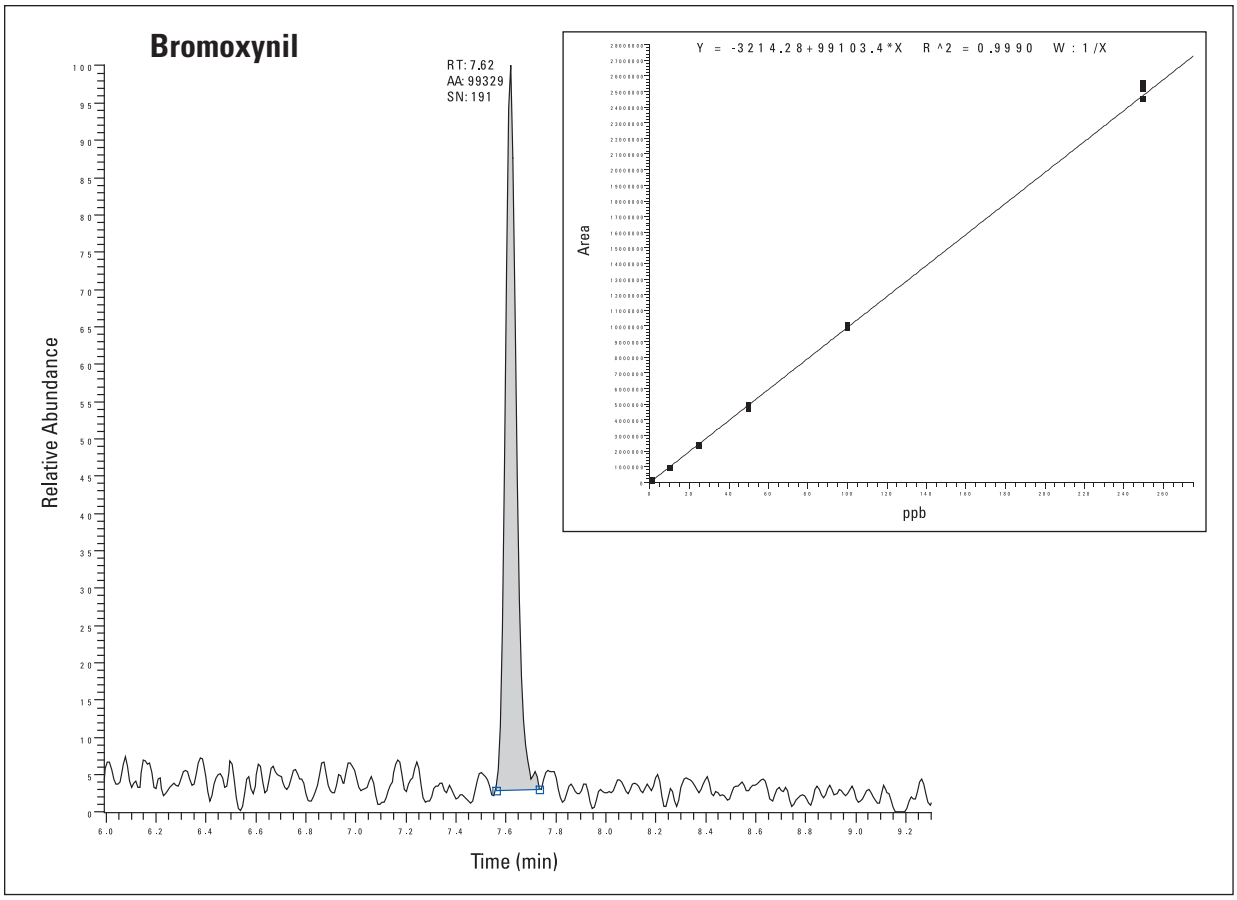
Compound	Formula	Polarity	Theoretical Mass (*m/z*)	Experimental Mass (*m/z*)	Mass Deviation (ppm)	LOQ (ppb)	R^2
Oryzalin	C12H18N4O6S	-	345.0874	345.0876	0.5	1	0.9895
Oxadiazon	C15H18Cl2N2O3	+	362.1033	362.1032	0.1	1	0.9969
Oxadixyl	C14H18N2O4	+	279.1339	279.1339	0	1	0.9994
Oxamyl	C7H13N3O3S	+	237.1016	237.1017	0.5	1	0.9997
Paclobutrazol	C15H20ClN3O	+	294.1368	294.1367	0.3	1	0.9955
Parathion	C10H14NO5PS	+	309.0669	309.0679	3.2	10	0.9645
Parathion methyl oxon	C8H10NO6P	+	265.0584	265.0585	0.5	10	0.9903
Parathion oxon	C10H14NO6P	+	293.0897	293.0896	0.3	1	0.9928
Pebulate	C10H21NOS	+	204.1417	204.1417	0.1	1	0.9929
Penconazole	C13H15Cl2N3	+	284.0716	284.0715	0.4	1	0.9931
Pencycuron	C19H21ClN2O	+	329.1415	329.1414	0.5	1	0.9986
Pendimethalin	C13H19N3O4	+	282.1448	282.1448	0.2	10	0.9949
Penoxsulam	C16H14F5N5O5S	+	484.0709	484.071	0.3	1	0.9928
Penthiopyrad	C16H20F3N3OS	+	360.1352	360.1352	0.1	1	0.9935
Permethrin(cis-)	C21H20Cl2O3	+	408.1128	408.1129	0.2	1	0.9935
Permethrin(trans-)	C21H20Cl2O3	+	408.1128	408.1129	0.2	1	0.9935
Phenmedipham	C16H16N2O4	+	318.1448	318.1448	0	1	0.9975
Phenothrin	C23H26O3	+	368.222	368.2222	0.6	1	0.9944
Phenthoate	C12H17O4PS2	+	321.0379	321.0378	0.4	1	0.9929
Phenylphenol(o-)	C12H10O	+	188.107	188.107	0.2	1	0.9854
Phorate	C7H17O2PS3	+	261.0201	261.02	0.3	10	0.9812
Phorate oxon	C7H17O3PS	+	230.0974	230.0982	3.5	1	0.9973
Phorate oxon sulfone	C7H17O5PS2	+	277.0328	277.0327	0.5	1	0.9979
Phorate oxon sulfoxide	C7H17O4PS2	+	261.0379	261.0377	0.8	1	0.9995
Phorate sulfone	C7H17O4PS3	+	310.0365	310.0363	0.6	1	0.9951
Phorate sulfoxide	C7H17O4PS2	+	261.0379	261.0377	0.8	1	0.9995
Phosalone	C12H15ClNO4PS2	+	385.0207	385.0206	0.3	1	0.9945
Phosmet	C11H12NO4PS2	+	318.0018	318.0018	0.1	1	0.9938
Phosphamidon	C10H19ClNO5P	+	317.1028	317.1026	0.4	1	0.9936
Phoxim	C12H15N2O3PS	+	299.0614	299.0613	0.4	1	0.9963
Picloram	C6H3Cl3N2O2	+	240.9333	240.9331	0.7	10	0.9594
Picoxystrobin	C18H16F3NO4	+	368.1104	368.1104	0.1	1	0.9981
Pinoxaden	C23H32N2O4	+	401.2435	401.2434	0.3	1	0.9968
Piperonyl butoxide	C19H30O5	+	356.2432	356.2433	0.3	1	0.9872
Piperophos	C14H28NO3PS2	+	354.1321	354.132	0.3	1	0.9932
Pirimicarb	C11H18N4O2	+	239.1503	239.1503	0.1	1	0.9992
Pirimiphos-ethyl	C13H24N3O3PS	+	334.1349	334.1348	0.2	1	0.9977
Pirimiphos-methyl	C11H20N3O3PS	+	306.1036	306.1034	0.7	1	0.9952
Pretilachlor	C17H26ClNO2	+	329.199	329.1989	0.3	1	0.9928
Probenazole	C10H9NO3S	+	224.0376	224.0378	0.9	1	0.9989
Prochloraz	C15H16Cl3N3O2	+	376.0381	376.0379	0.4	1	0.9933
Profenophos	C11H15BrClO3PS	+	372.9424	372.9424	0.1	1	0.9939
Prohexadione	C10H12O5	-	211.0612	211.0613	0.4	1	0.9936
Promecarb	C12H17NO2	+	208.1332	208.1333	0.4	1	0.9972
Prometon	C10H19N5O	+	226.1662	226.1664	0.7	1	0.9991
Prometryn	C10H19N5S	+	242.1434	242.1434	0.2	1	0.9985
Propachlor	C11H14ClNO	+	212.0837	212.0839	0.8	1	0.9962
Propamocarb	C9H20N2O2	+	189.1598	189.1597	0.5	1	0.9992
Propanil	C9H9Cl2NO	-	215.9988	215.9987	0.4	1	0.9855
Propargite	C19H26O4S	+	368.189	368.1891	0.1	1	0.9961
Propazine	C9H16ClN5	+	230.1167	230.1168	0.5	1	0.9976
Propetamphos	C10H20NO4PS	+	299.1189	299.1188	0.3	1	0.9929
Propham	C10H13NO2	+	180.1019	180.1019	0.1	1	0.9131
Propiconazole	C15H17Cl2N3O2	+	342.0771	342.077	0.1	1	0.9885
Propisochlor	C15H22ClNO2	+	284.1412	284.1411	0.1	1	0.9981
Propoxur	C11H15NO3	+	210.1125	210.1126	0.7	1	0.9949
Prothioconazole	C14H15Cl2N3OS	-	342.024	342.0245	1.4	1	0.9864
Prothoate	C9H20NO3PS2	+	286.0695	286.0693	0.8	1	0.9982
Pymetrozine	C10H11N5O	+	218.1036	218.1037	0.5	1	0.9985

Compound	Formula	Polarity	Theoretical Mass (*m/z*)	Experimental Mass (*m/z*)	Mass Deviation (ppm)	LOQ (ppb)	R^2
Pyracarbolid	C13H15NO2	+	218.1176	218.1177	0.6	1	0.9986
Pyraclofos	C14H18ClN2O3PS	+	361.0537	361.0537	0.1	1	0.9969
Pyraclostrobin	C19H18ClN3O4	+	388.1059	388.1057	0.5	1	0.9951
Pyraflufen-ethyl	C15H13Cl2F3N2O4	+	430.0543	430.0527	3.7	1	0.9833
Pyrasulfotole	C14H13F3N2O4S	-	361.0475	361.0476	0.2	1	0.9926
Pyrazone (Chloridazon)	C10H8ClN3O	+	239.0694	239.0687	3.1	50	0.9448
Pyrazophos	C14H20N3O5PS	+	374.0934	374.0933	0.3	1	0.9958
Pyridaben	C19H25ClN2OS	+	365.1449	365.145	0.3	1	0.9881
Pyridalyl	C18H14Cl4F3NO3	+	489.9753	489.9755	0.4	1	0.9958
Pyridaphenthion	C14H17N2O4PS	+	341.0719	341.0721	0.3	1	0.9938
Pyridate	C19H23ClN2O2S	+	379.1242	379.1242	0.2	1	0.9902
Pyrifenox	C14H12Cl2N2O	+	295.04	295.0397	0.7	1	0.9979
Pyrimethanil	C12H13N3	+	200.1182	200.1183	0.2	1	0.9977
Pyriproxyfen	C20H19NO3	+	322.1438	322.1438	0	1	0.9977
Pyroquilon	C11H11NO	+	174.0913	174.0913	0.5	1	0.9992
Pyroxsulam	C14H13F3N6O5S	+	435.0693	435.0693	0.1	1	0.9962
Quinalphos	C12H15N2O3PS	+	299.0614	299.0613	0.4	1	0.9963
Quinclamine	C10H6ClNO2	+	208.016	208.0158	1	1	0.9879
Quinoxyfen	C15H8Cl2FNO	+	308.004	308.0039	0.4	1	0.9980
Resmethrin	C22H26O3	+	339.1955	339.1955	0.1	1	0.9948
Rotenone	C23H22O6	+	395.1489	395.1489	0.1	1	0.9948
Saflufenacil	C17H17ClF4N4O5S	+	518.0883	518.0883	0	1	0.9868
Schradan	C8H24N4O3P2	+	287.1396	287.1389	2.7	1	0.9937
Secbumeton	C10H19N5O	+	226.1662	226.1664	0.7	1	0.9991
Sethoxydim	C17H29NO3S	+	328.1941	328.1939	0.5	1	0.9977
Siduron	C14H20N2O	+	233.1648	233.165	0.5	1	0.9996
Simazine	C7H12ClN5	+	202.0854	202.0855	0.3	1	0.9963
Simeconazole	C14H20FN3OSi	+	294.1432	294.1431	0.5	1	0.9949
Simetryn	C8H15N5S	+	214.1121	214.1122	0.6	1	0.9986
Spinetoram	C42H69NO10	+	748.4994	748.4992	0.3	1	0.9878
Spinetoram 1	C43H69NO10	+	760.4994	760.4995	0.1	1	0.9934
Spinosad A	C41H65NO10	+	732.4681	732.468	0.2	1	0.9960
Spinosad D	C42H67NO10	+	746.4838	746.4836	0.3	1	0.9932
Spirodiclofen	C21H24Cl2O4	+	428.139	428.1389	0.2	1	0.9991
Spiromefisen	C23H30O4	+	388.2482	388.2482	0	1	0.9934
Spirotetramat	C21H27NO5	+	374.1962	374.1963	0.3	1	0.9990
Spiroxamine	C18H35NO2	+	298.2741	298.2739	0.4	1	0.9910
Sulcotrione	C14H13ClO5S	+	346.0511	346.0519	2.6	10	0.9706
Sulfentrazone	C11H10Cl2F2N4O3S	+	386.9892	386.9906	3.8	1	0.9906
Sulfotep-ethyl	C8H20O5P2S2	+	323.03	323.03	0.1	1	0.9950
Sulfuramid	C10H6F17NO2S	-	525.9775	525.9779	0.7	1	0.9828
Sulprofos	C12H19O2PS3	+	340.0623	340.0636	3.7	1	0.9950
Tebuconazole	C16H22ClN3O	+	308.1524	308.1522	0.7	1	0.9924
Tebufenozide	C22H28N2O2	+	353.2224	353.2223	0.3	1	0.9946
Tebufenpyrad	C18H24ClN3O	+	334.1681	334.1679	0.4	1	0.9968
Tebupirimphos	C13H23N2O3PS	+	319.124	319.124	0.1	1	0.9953
Tebuthiuron	C9H16N4OS	+	229.1118	229.1119	0.5	1	0.9947
Teflubenzuron	C14H6Cl2F4N2O2	-	378.967	378.9675	1.3	1	0.9785
Tefluthrin	C17H14ClF7O2	+	419.0643	419.0635	1.9	50	0.9203
Tembotrione	C17H16ClF3O6S	+	458.0647	458.0649	0.5	10	0.9866
Temephos	C16H20O6P2S3	+	484.0236	484.0236	0.1	1	0.9953
Tepraloxydim	C17H24ClNO4	-	340.1321	340.1322	0.2	1	0.9947
Terbacil	C9H13ClN2O2	-	215.0593	215.0596	1.3	1	0.9911
Terbufos	C9H21O2PS3	+	289.0514	289.052	2	1	0.9928
Terbufos oxon sulfoxide	C9H21O4PS2	+	289.0692	289.0691	0.4	1	0.9927
Terbufos sulfone	C9H21O4PS3	+	338.0678	338.0678	0.1	1	0.9963
Terbumeton	C10H19N5O	+	226.1662	226.1664	0.7	1	0.9991
Terbuthylazine	C9H16ClN5	+	230.1167	230.1168	0.5	1	0.9976
Terbutryn	C10H19N5S	+	242.1434	242.1434	0.2	1	0.9985

LC/MS data for Pesticide Standards *(Table 1 continued)*

Compound	Formula	Polarity	Theoretical Mass (*m/z*)	Experimental Mass (*m/z*)	Mass Deviation (ppm)	LOQ (ppb)	R^2
Tetrachlorvinphos	C10H9Cl4O4P	+	381.9331	381.9331	0	1	0.9973
Tetraconazole	C13H11Cl2F4N3O	+	372.0288	372.0289	0.3	1	0.9967
Tetramethrin	C19H25NO4	+	332.1856	332.1856	0.3	1	0.9977
Thiabendazole	C10H7N3S	+	202.0433	202.0433	0.4	1	0.9967
Thiacloprid	C10H9ClN4S	+	253.0309	253.0309	0.3	1	0.9975
Thiamethoxam	C8H10ClN5O3S	+	292.0266	292.0266	0.1	1	0.9908
Thiazopyr	C16H17F5N2O2S	+	397.1004	397.1003	0.1	1	0.9972
Thidiazuron	C9H8N4OS	+	221.0492	221.0492	0.4	1	0.9922
Thiofanox	C9H18N2O2S	+	236.1427	236.1428	0.5	1	0.9923
Thiometon	C6H15O2PS3	+	264.031	264.0301	3.3	10	0.9594
Thiophanate-methyl	C12H14N4O4S2	+	343.0529	343.0531	0.4	1	0.9932
Tolclofos-methyl	C9H11Cl2O3PS	+	300.9616	300.9626	3.3	25	0.8855
Tolfenpyrad	C21H22ClN3O2	+	384.1473	384.1475	0.3	1	0.9878
Topramezone	C16H17N3O5S	+	364.0962	364.0944	5	1	0.9250
Tralkoxydim	C20H27NO3	+	330.2064	330.2063	0.2	1	0.9918
Tralomethrin	C22H19Br4NO3	+	678.8437	678.8447	1.4	10	0.9880
Triadimefon	C14H16ClN3O2	+	294.1004	294.1003	0.4	1	0.9973
Triadimenol	C14H18ClN3O2	+	296.116	296.1161	0.3	1	0.9905
Tri-allate	C10H16Cl3NOS	+	304.0091	304.009	0.3	10	0.9673
Triazophos	C12H16N3O3PS	+	314.0723	314.0721	0.7	1	0.9984
Trichlamide	C13H16Cl3NO3	+	340.0269	340.026	2.6	1	0.9986
Trichlorfon	C4H8Cl3O4P	+	256.9299	256.9298	0.1	1	0.9983
Triclopyr	C7H4Cl3NO3	-	253.9184	253.9186	0.7	1	0.9891
Tricyclazole	C9H7N3S	+	190.0433	190.0433	0.4	1	0.9996
Tridemorph	C19H39NO	+	298.3104	298.3103	0.4	1	0.9972
Trietazine	C9H16ClN5	+	230.1167	230.1168	0.5	1	0.9976
Trifloxystrobin	C20H19F3N2O4	+	409.137	409.1367	0.8	1	0.9981
Triflumizole	C15H15ClF3N3O	+	346.0929	346.0928	0.1	1	0.9957
Triflumuron	C15H10ClF3N2O3	-	357.0259	357.0251	2.2	1	0.9914
Trifluralin	C13H16F3N3O4	+	353.1431	353.143	0.2	10	0.9871
Triforine	C10H14Cl6N4O2	+	449.9586	449.9587	0.1	10	0.9851
Trinexapac-ethyl	C13H16O5	+	253.1071	253.1071	0.2	1	0.9871
Triticonazole	C17H20ClN3O	+	318.1368	318.1367	0.3	1	0.9943
Uniconazole	C15H18ClN3O	+	292.1211	292.1211	0.2	1	0.9884
Validamycin	C20H35NO13	+	498.2181	498.2172	1.9	1	0.8371
Vamidothion	C8H18NO4PS2	+	288.0488	288.0484	1.3	1	0.9984
Vamidothion sulfone	C8H18NO6PS2	+	320.0386	320.0386	0.1	1	0.9986
Vernolate	C10H21NOS	+	204.1417	204.1417	0.1	1	0.9929
Warfarin	C19H16O4	+	309.1121	309.112	0.5	1	0.9871
Zoxamide	C14H16Cl3NO2	+	336.0319	336.0318	0.4	1	0.9975

Table 1: LC/MS data for 510 pesticide standards



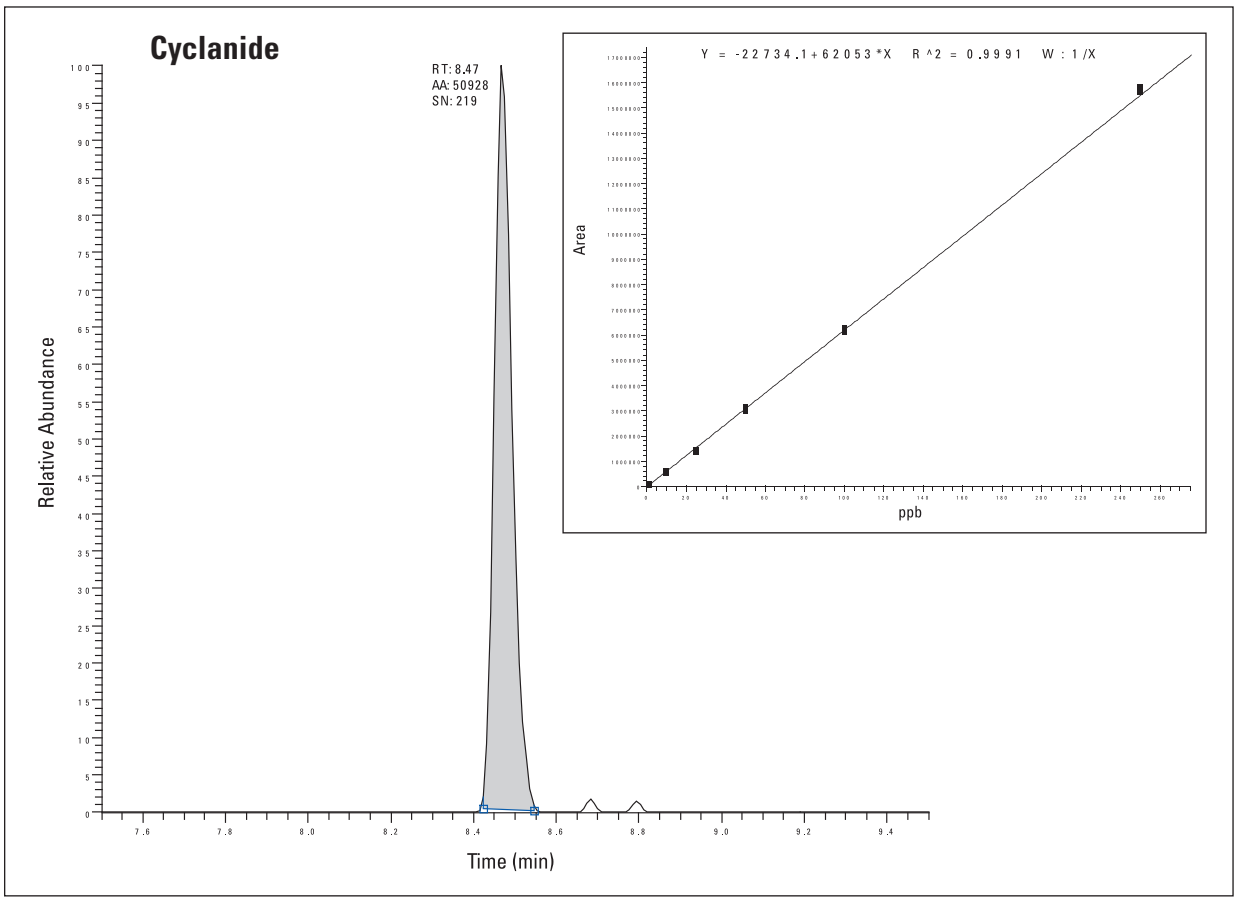
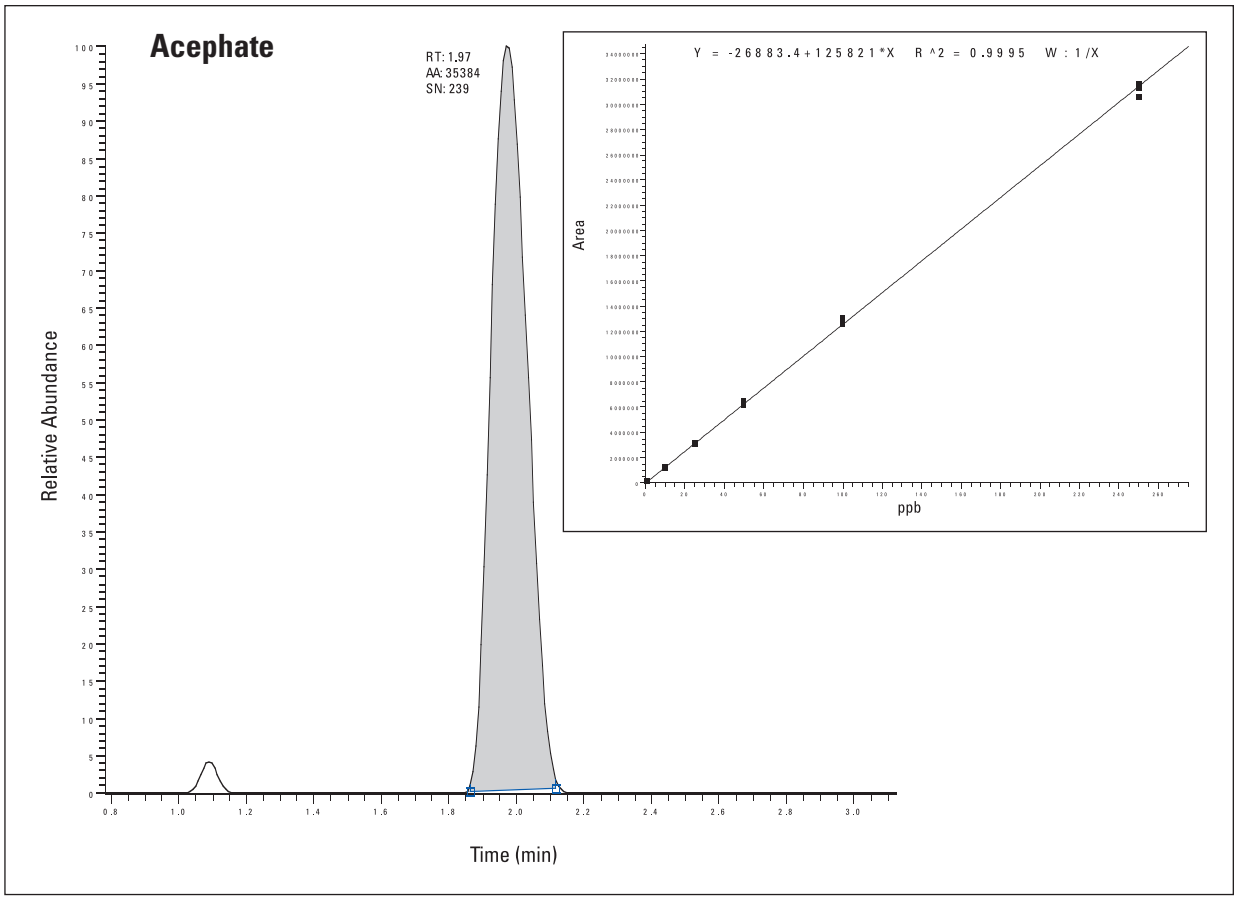


Figure 4: Extracted ion chromatograms (at 1 ppb level) and calibration curves (1-250 ppb) of eight pesticides



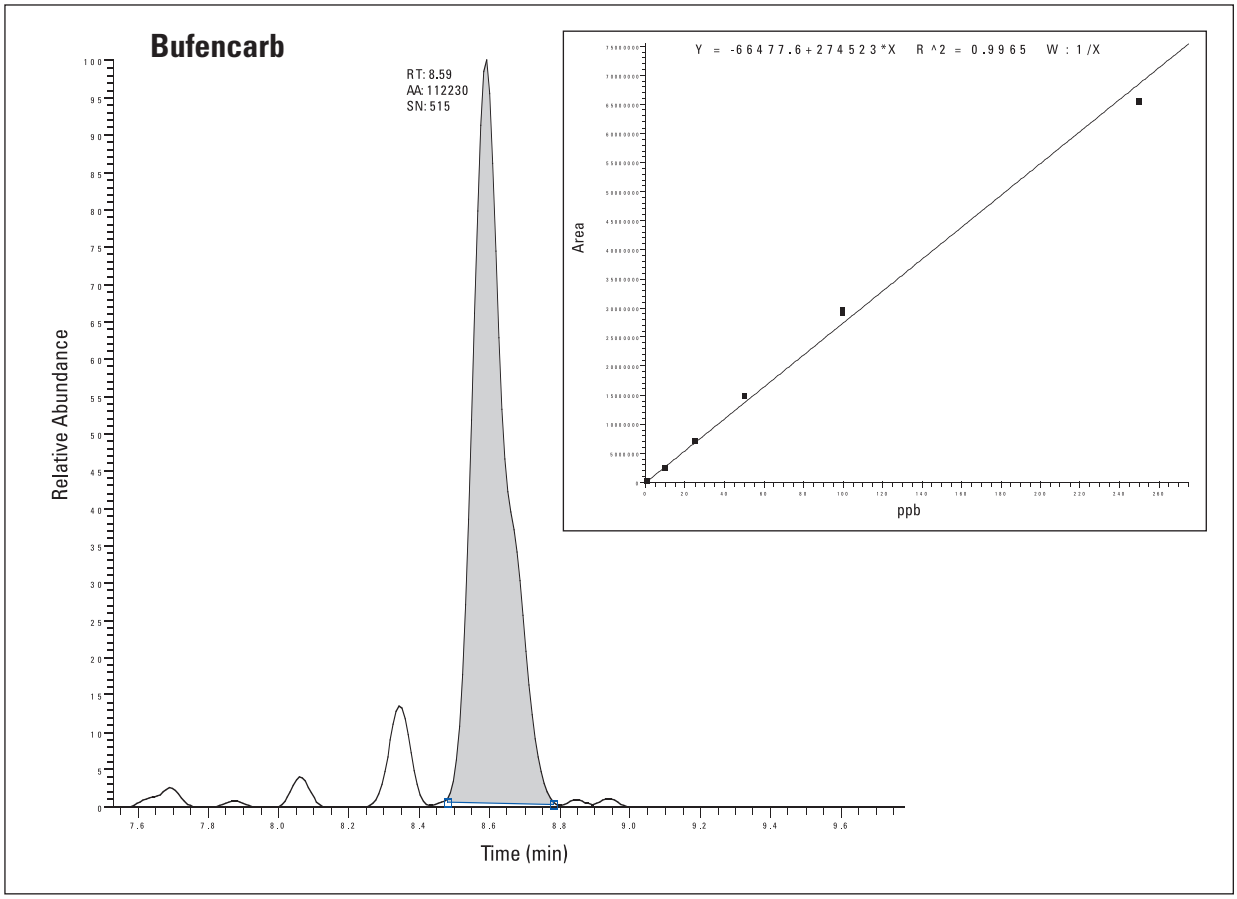
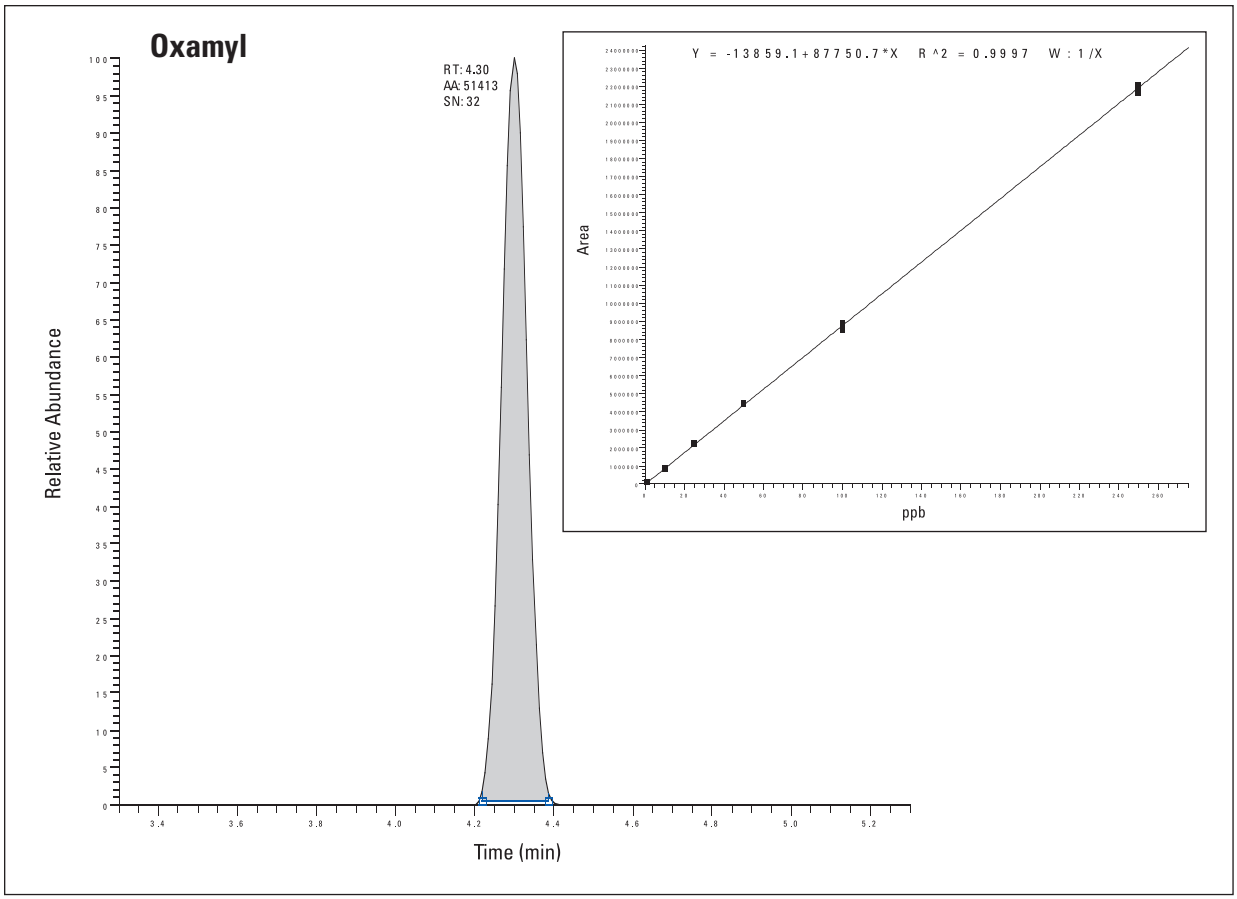


Figure 4 Continued: Extracted ion chromatograms (at 1 ppb level) and calibration curves (1-250 ppb) of eight pesticides



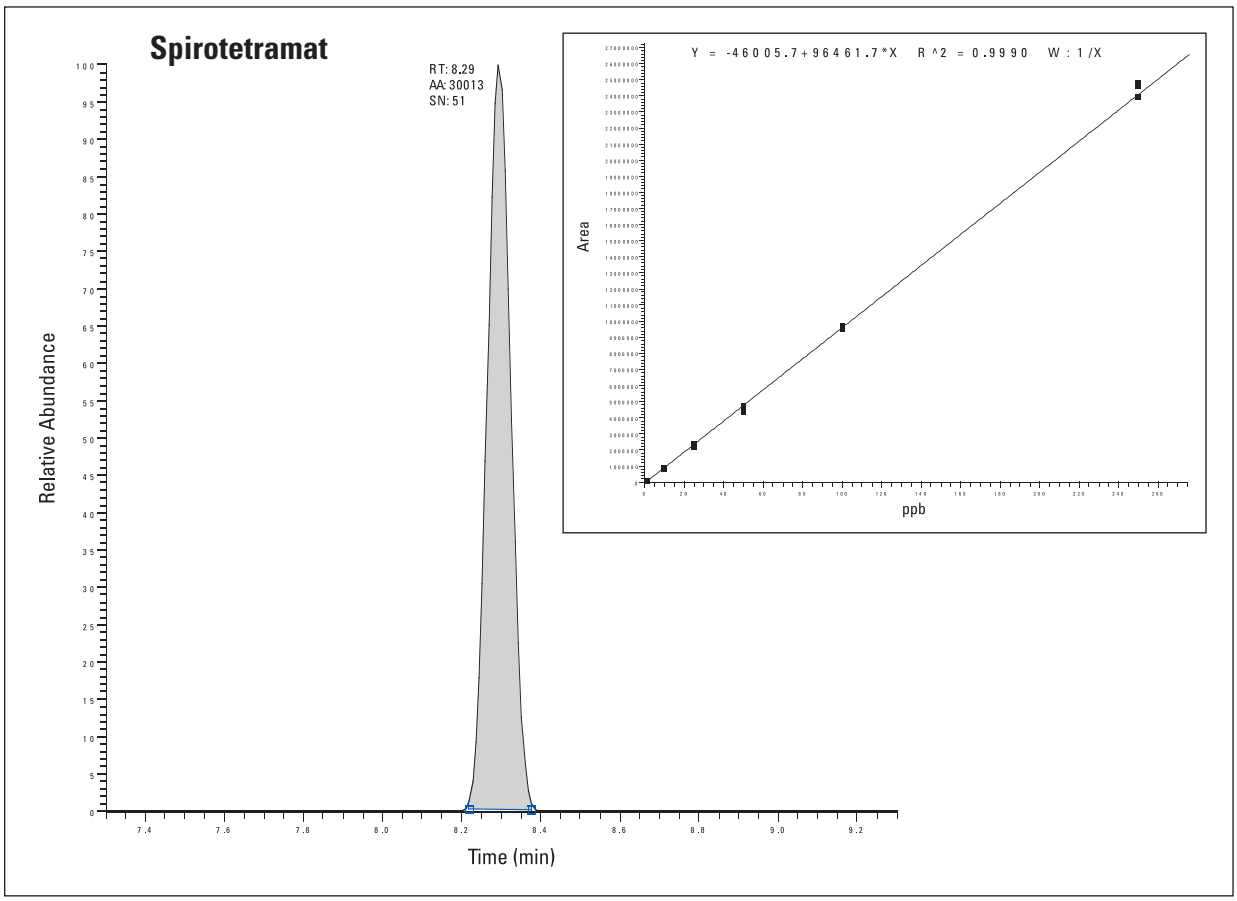
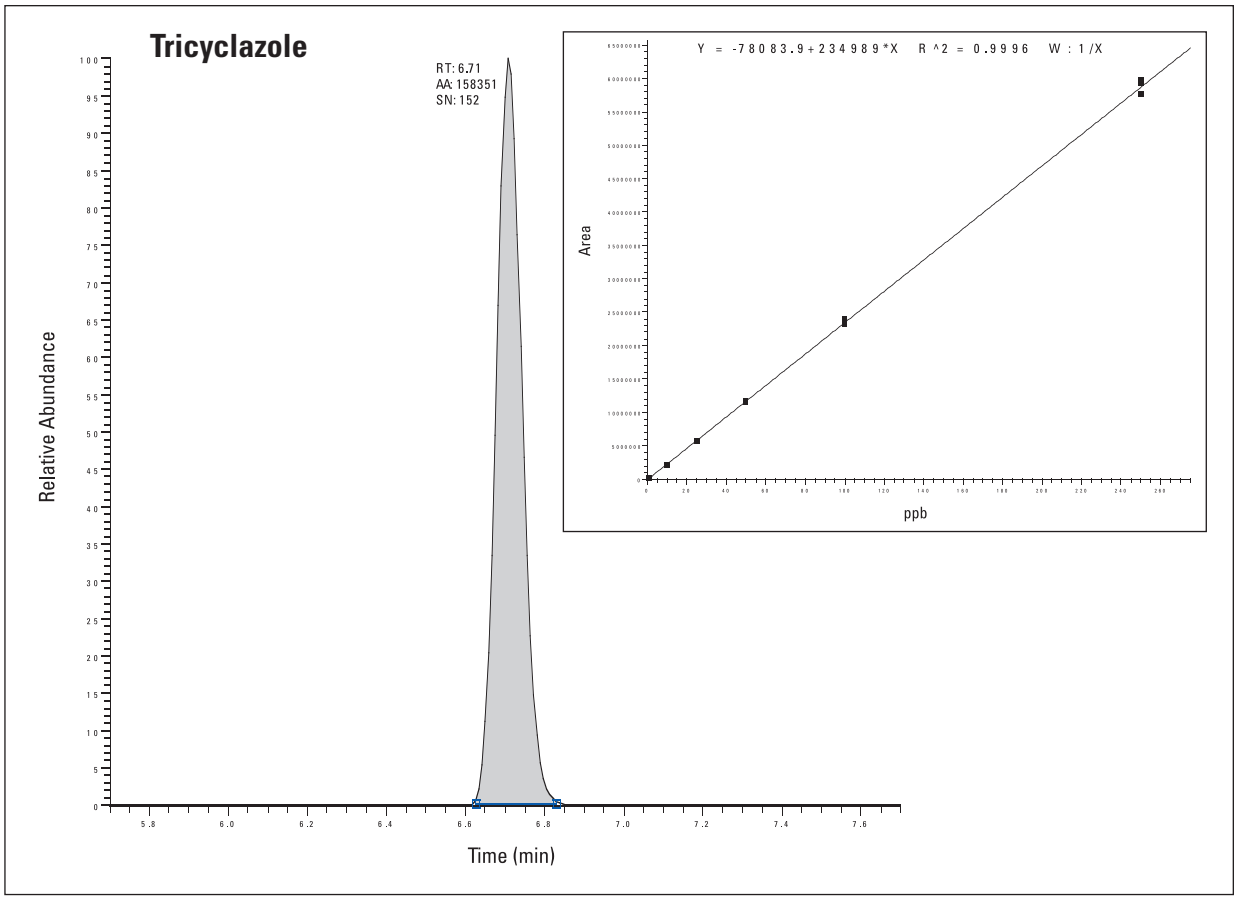


Figure 4 Continued: Extracted ion chromatograms (at 1 ppb level) and calibration curves (1-250 ppb) of eight pesticides



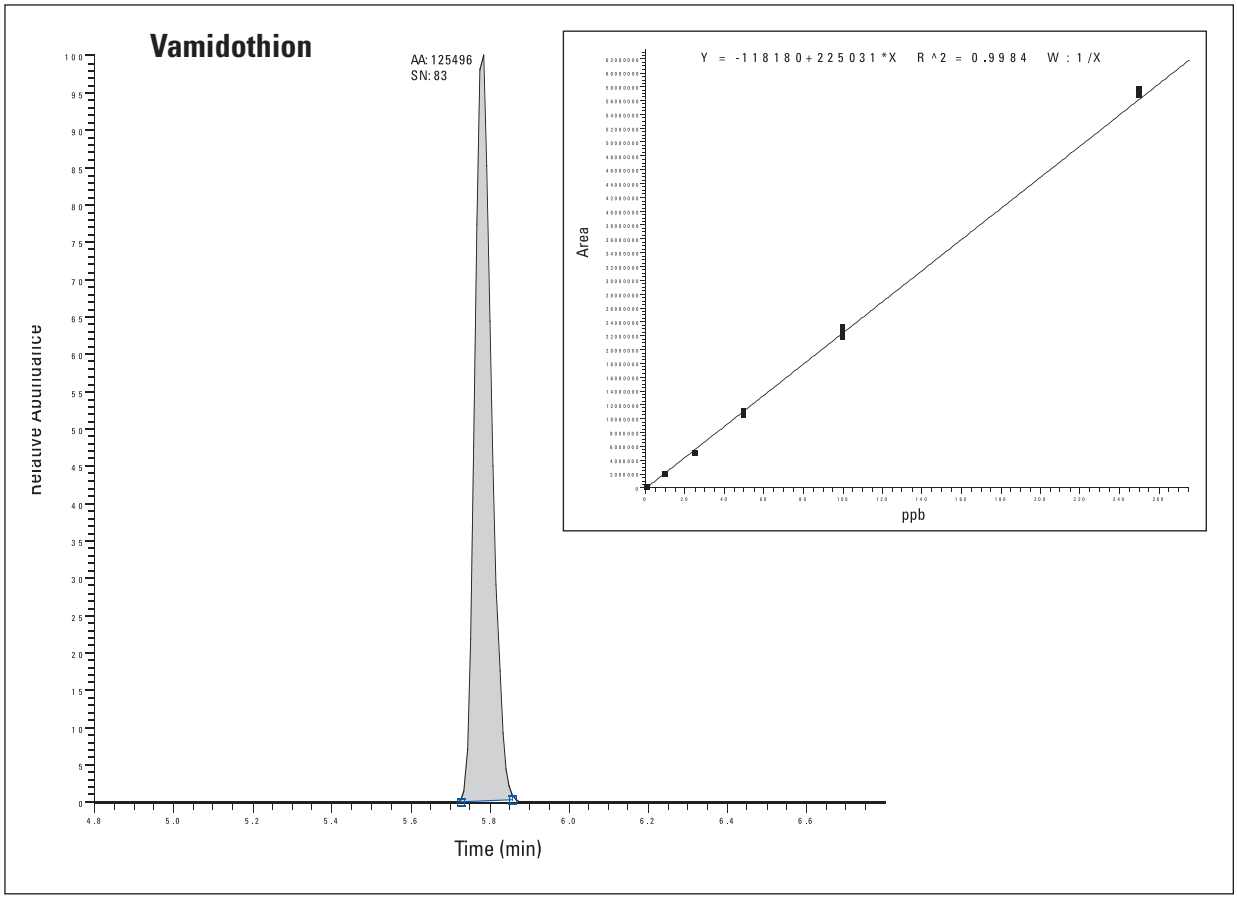


Figure 4 Continued: Extracted ion chromatograms (at 1 ppb level) and calibration curves (1-250 ppb) of eight pesticides

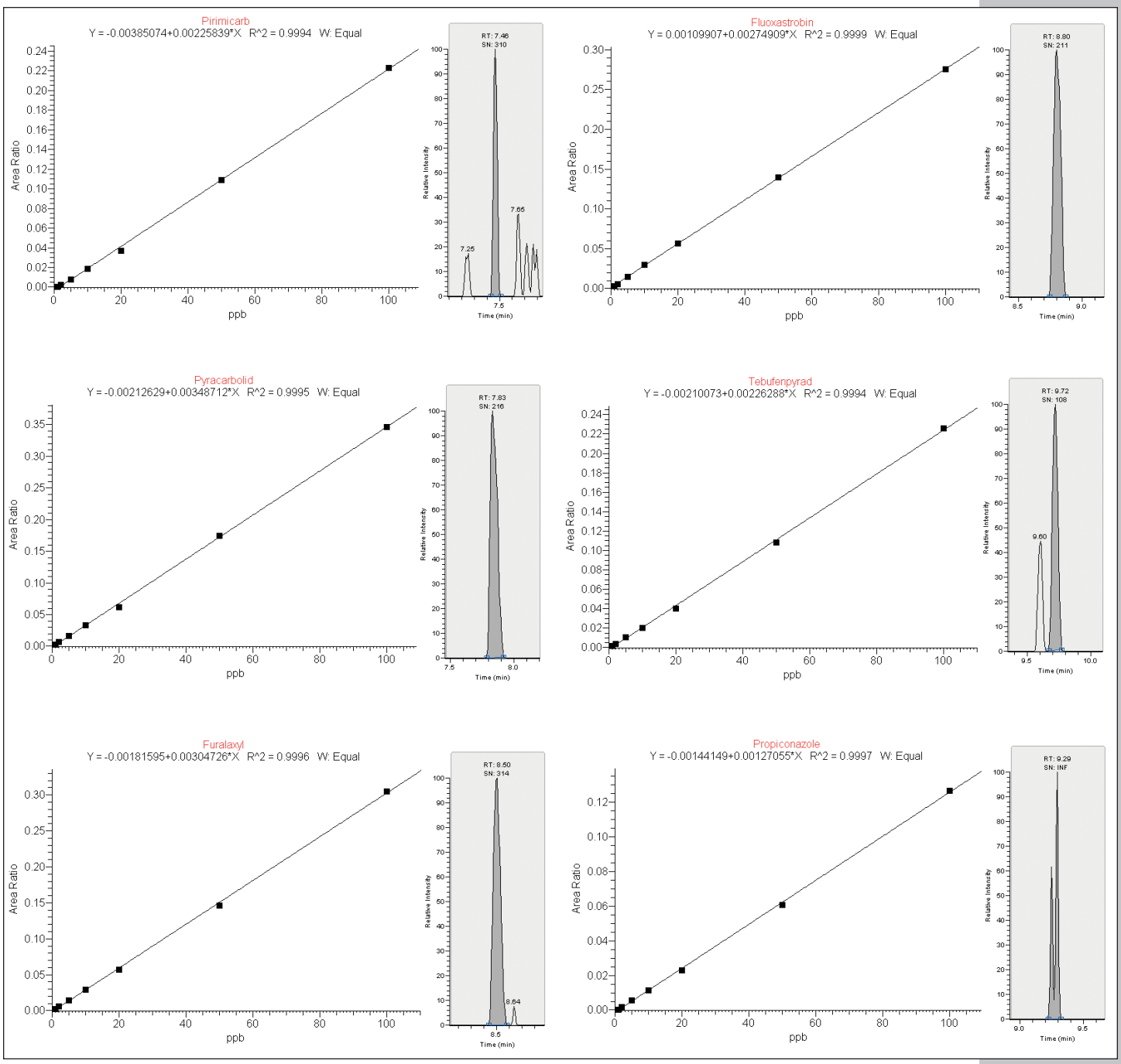


Figure 5: Extracted ion chromatograms (at 1 ppb) and calibration curves (1-250 ppb) of six pesticides extracted from spiked spinach sample

LC/MS data for representative pesticides extracted from spinach matrix *(Table 2)*

Compound	Formula	Theoretical Mass (*m/z*)	Experimental Mass (*m/z*)	Mass Deviation (ppm)	LOD (ppb)	LOQ (ppb)
Azoxystrobin	C22H17N3O5	404.1241	404.12466	1.4	0.2	0.7
Bendiocarb	C11H13NO4	224.09173	224.09169	0.2	0.2	0.7
Benthiavalicarb	C18H24FN3O3S	382.15952	382.1597	0.5	0.2	0.7
Benzoximate	C18H18ClNO5	364.09463	386.07663	0.2	0.2	0.5
Bifenazate	C17H20N2O3	301.15467	301.15457	0.3	0.3	0.9
Bupirimate	C13H24N4O3S	317.16419	317.16431	0.4	0.2	0.5
Buprofezin	C16H23N3OS	306.16346	306.16354	0.3	0.2	0.6
Butafenacil	C20H18ClF3N2	492.11437	492.11469	0.6	0.3	0.9
Carbaryl	C12H11NO2	219.1128	219.1127	0.5	0.3	0.9
Carbendazim	C9H9N3O2	192.07675	192.07684	0.5	0.2	0.7
Carbofuran	C12H15NO3	222.11247	222.11241	0.3	0.2	0.7
Carboxin	C12H13NO2S	236.07398	236.07358	1.7	0.2	0.5
Chlortoluron	C10H13ClN2O	213.07892	213.07925	1.6	0.2	0.6
Clethodim	C17H26ClNO3S	360.13947	360.13962	0.4	0.2	0.6
Clofentezine	C14H8Cl2N4	303.01988	303.01993	0.2	0.1	0.4
Cyazofamid	C13H13ClN4O2S	342.0786	342.077	4.7	0.3	0.8
Cycluron	C11H22N2O	199.18049	199.18054	0.3	0.2	0.7
Cyproconazole	C15H18ClN3O	292.12112	292.12115	0.1	0.2	0.7
Cyprodinil	C14H15N3	226.13387	226.13385	0.1	0.2	0.7
Diclobutrazol	C15H19Cl2N3O	328.09779	328.09781	0	0.2	0.5
Dicrotophos	C8H16NO5P	238.08389	238.08391	0.1	0.3	0.8
Difenoconazol	C19H17Cl2N3O3	406.07197	406.07251	1.3	0.2	0.6
Dimethoate	C5H12NO3PS2	230.0069	230.00685	0.2	0.3	0.8
Dimethomorph	C21H22ClNO4	388.13101	388.13113	0.3	0.3	0.9
Dimoxystrobin	C19H22N2O3	327.17032	327.17047	0.5	0.2	0.5
Dinotefuran	C7H14N4O3	203.11387	203.11389	0.1	0.2	0.7
Dioxacarb	C11H13NO4	203.11387	224.09169	0.2	0.2	0.7
Emamectin B1b	C49H75NO13	886.53112	886.53168	0.6	0.3	0.8
Epoxiconazole	C17H13ClFN3O	330.08039	330.08029	0.3	0.2	0.6
Etaconazole	C14H15Cl2N3O2	328.06141	328.06143	0.1	0.3	0.9
Ethiofencarb	C11H15NO2S	226.08963	226.08969	0.3	0.3	0.9
Etoxazole	C21H23F2NO2	360.17696	360.17715	0.5	0.1	0.4
Famoxadone	C22H18N2O4	392.16048	397.11591	0.1	0.2	0.7
Fenamidone	C17H17N3OS	312.11651	312.11652	0	0.2	0.6
Fenazaquin	C20H22N2O	307.18049	307.18039	0.3	0.3	0.8
Fenbuconazole	C19H17ClN4	337.12145	337.12128	0.5	0.2	0.6
Fenoxycarb	C17H19NO4	302.13868	324.12073	0.3	0.1	0.4
Fenpropimorph	C20H33NO	304.26349	304.26349	0	0.1	0.3
Fenpyroximate	C24H27N3O4	422.20743	422.20789	1.1	0.3	0.9
Fenuron	C9H12N2O	165.10224	165.10239	0.9	0.3	0.9
Flufenacet	C14H13F4N3O	364.07374	364.07401	0.7	0.2	0.6
Fluometuron	C10H11F3N2O	233.08962	233.08958	0.2	0.2	0.7
Fluoxastrobin	C21H16ClFN4O5	459.0866	459.08704	0.9	0.3	0.8
Flusiazole	C16H15F2N3Si	316.10761	316.10776	0.5	0.2	0.7
Flutolanil	C17H16F3NO2	324.12059	324.12073	0.4	0.3	0.9
Flutriafol	C16H13F2N3O	302.10995	302.10999	0.1	0.1	0.3
Forchlorfenuron	C12H10ClN3O	248.05852	248.05832	0.8	0.2	0.6
Formetanate	C11H15N3O2	239.15025	239.15018	0.3	0.2	0.5
Fuberidazole	C11H8N2O	185.07094	185.07108	0.7	0.3	0.9
Furalaxyl	C17H19NO4	302.13868	324.12073	0.3	0.1	0.4
Hexaconazole	C14H17Cl2N3O	314.08214	314.08206	0.3	0.2	0.7

Compound	Formula	Theoretical Mass (*m/z*)	Experimental Mass (*m/z*)	Mass Deviation (ppm)	LOD (ppb)	LOQ (ppb)
Hydramethylnon	C25H24F6N4	495.19779	495.19824	0.9	0.2	0.6
Imazalil	C14H14Cl2N2O	297.0556	297.05566	0.2	0.2	0.6
Iprovalicarb	C18H28N2O3	321.21727	321.21744	0.5	0.1	0.4
Isoproturon	C12H18N2O	207.14919	207.14932	0.6	0.2	0.5
Mefenacet	C16H14N2O2S	299.08487	299.08484	0.1	0.2	0.7
Mepanipyrim	C14H13N3	224.11822	224.11821	0.1	0.2	0.7
Mepronil	C17H19NO2	270.14886	270.14886	0	0.1	0.1
Metalaxyl	C15H21NO4	280.15433	280.15445	0.4	0.2	0.5
Methabenzhiazuron	C10H11N3OS	222.06956	222.06952	0.2	0.1	0.4
Methamidophos	C2H8NO2PS	142.00861	142.00865	0.3	0.2	0.5
Methiocarb	C11H15NO2S	226.08963	226.08969	0.3	0.3	0.9
Methomyl	C5H10N2O2S	163.05357	163.05357	0	0.2	0.6
Methoprotryne	C11H21N5OS	272.15396	272.15393	0.1	0.2	0.6
Methoxyfenozide	C22H28N2O3	369.21727	369.21738	0.3	0.1	0.2
Neburon	C12H16Cl2N2O	275.07125	275.07126	0	0.3	0.8
Oxadixyl	C14H18N2O4	279.13393	279.13397	0.1	0.1	0.4
Penconazole	C13H15Cl2N3	284.07158	284.07153	0.2	0.3	0.8
Pinoxaden	C23H32N2O4	401.24348	401.24393	1.1	0.1	0.1
Pirimicarb	C11H18N4O2	239.15025	239.15018	0.3	0.2	0.5
Promecarb	C12H17NO2	208.13321	208.13329	0.4	0.2	0.5
Prometon	C10H19N5O	226.16624	226.16623	0	0.2	0.5
Prometryn	C10H19N5S	242.14339	242.14348	0.4	0.2	0.5
Propamocarb	C9H20N2O2	189.15975	189.15988	0.7	0.1	0.4
Propargite	C19H26O4S	189.15975	368.18933	0.9	0.2	0.6
Propiconazole	C15H17Cl2N3O2	342.07706	342.077	0.2	0.3	0.9
Pyrimethanil	C12H13N3	200.11822	200.11826	0.2	0.2	0.6
Pyriproxyfen	C20H19NO3	322.14377	322.14392	0.5	0.2	0.6
Quinoxyfen	C15H8Cl2FNO	308.00397	308.00394	0.1	0.2	0.6
Rotenone	C23H22O6	395.14891	395.14923	0.8	0.2	0.6
Siduron	C14H20N2O	233.16484	233.16492	0.3	0.3	0.9
Simetryn	C8H15N5S	214.11209	214.11174	1.6	0.2	0.4
Spiroxamine	C18H35NO2	298.27406	298.27417	0.4	0.2	0.5
Tebuconazole	C16H22ClN3O	308.15242	308.15234	0.2	0.2	0.5
Tebufenozide	C22H28N2O2	353.22235	353.22247	0.3	0.1	0.2
Tebufenpyrad	C18H24ClN3O	334.16807	334.16821	0.4	0.2	0.7
Terbumeton	C10H19N5O	226.16624	226.16623	0	0.2	0.5
Terbutryn	C10H19N5S	242.14339	242.14348	0.4	0.2	0.5
Tetraconazole	C13H11Cl2F4N	372.02881	372.02902	0.6	0.3	0.8
Thiabendazole	C10H7N3S	202.04334	202.04344	0.5	0.2	0.6
Thiamethoxam	C8H10ClN5O3S	292.02656	292.02655	0	0.3	1
Thiobencarb	C12H16ClNOS	258.07139	280.05246	3.1	0.3	0.8
Triadimefon	C14H16ClN3O2	294.10038	294.10031	0.2	0.3	0.8
Tricyclazole	C9H7N3S	190.04334	190.04356	1.2	0.1	0.4
Trifloxystrobin	C20H19F3N2O4	409.13697	409.13745	1.2	0.2	0.6
Triflumizole	C15H15ClF3N3O	346.09285	346.09302	0.5	0.1	0.2
Triticonazole	C17H20ClN3O	318.13677	318.13687	0.3	0.3	0.8
Uniconazole	C15H18ClN3O	292.12112	292.12115	0.1	0.2	0.6
Vamidothion	C8H18NO4PS2	288.04876	288.04883	0.2	0.2	0.5
Zoxamide	C14H16Cl3NO2	336.03194	336.03189	0.1	0.3	0.9

Table 2: LC/MS data for representative pesticides extracted from spiked spinach matrix. All MS data reported below was obtained with Orbitrap MS operating in positive ion mode. LODs and LOQs were assessed using the EPA method detection limit (MDL) procedure.[9]

Conclusion

A rapid and robust U-HPLC Exactive Orbitrap MS method for multiresidue pesticide screening was developed and validated. Screening of 510 pesticides at low ppb levels was achieved within 12 minutes, and the high mass resolution and accuracy of the Exactive mass spectrometer enabled identification of all compounds. LOQs for the majority of pesticides in a standard mixture and in spiked matrix were lower than MRLs established by the EU and Japan. The Exactive LC/MS platform is ideally suited for the routine monitoring of targeted and non-targeted pesticides by regulatory laboratories.

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Acknowledgment

The authors wish to thank Dr. Jon Wong at the FDA for providing the pesticide standards used in this study.

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Analysis of Early Eluting Pesticides in a C18-Type Column Using a Divert Valve and LC-MS/MS

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Application Note 572

Key Words
TSQ Quantum Access MAX, Divert Valve, Split Peaks, Reversed-Phase Liquid Chromatography, Pesticides

Goal
To demonstrate the ability to override the solvent effects from a sample extract using gradient solvents with liquid chromatography. Additionally, to increase injection volume without overloading the column.

Introduction
Many pesticide analyses are based on the QuEChERS extraction method, which uses acetonitrile (ACN) in the final extraction step. However, injecting a solvent stronger than the HPLC mobile phase can cause peak shape problems, such as peak splitting or broadening, especially for the early eluting analytes (low capacity factor, k). The common practice is to exchange the solvent of the final extraction step for one similar to the mobile phase, for example methanol / water, but this procedure is laborious and can lead to analyte losses.

There are several possible causes of peak splitting or broadening. This study presents the peak shape differences between acetonitrile and methanol / water [1:1 v/v] solutions due to the interaction of gradient and sample solvent, as indicated in Figure 1. The lowest detection limit is achieved when an analyte is in as compact a band as possible within the flow stream of mobile phase and with larger injection volumes. However, this is limited by maximum loop volume and column capacity.

Mobile phase composition and the use of a divert valve have been evaluated for the analysis of seven selected pesticides in acetonitrile solutions (Table 1). The sample solutions were chosen to represent both low and high analyte levels for compounds that elute either early or middle-early from a C18 column. Performance was evaluated in terms of linearity (injection volume range 1–8 µL), robustness (RSD), and sensitivity as measured by signal-to-noise ratio (S/N) and peak area reproducibility.

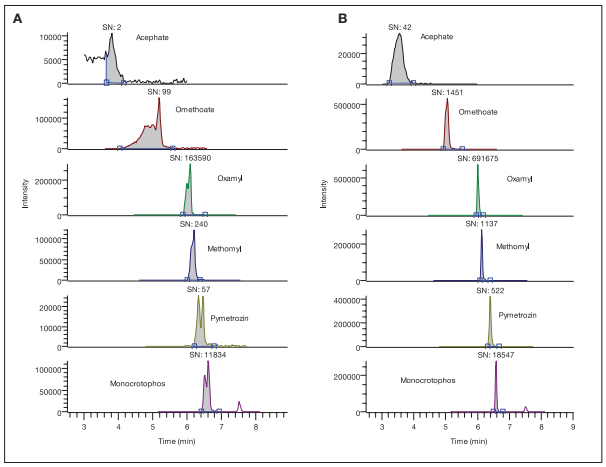

Figure 1. Chromatograms of 5 µL injections of acephate, omethoate, oxamyl, methomyl, pymetrozin, and monocrotophos in 50 µg/L acetonitrile (A) and methanol / water [1:1 v/v] solution (B), with no divert valve used

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Table 1. List of studied pesticides and their physicochemical properties

Name	Pesticide Class	Chemical Formula	Water Solubility [mg/L] / pKow	Vapor Pressure [Pa]	Molecular Weight [g/mol]
Acephate	Organophosphorous	$C_4H_{10}NO_3PS$	790,000 / -0.85	2.26 x 10^{-4} (24 °C)	183.165862
Aldicarb sulfone	Oxime carbamate	$C_7H_{14}N_2O_4S$	10,000 (25 °C) / -0.57 (calculated)	0.012 (25 °C)	222.26206
Metamitron	Triazinone	$C_{10}H_{10}N_4O$	1770 (25 °C; pH 5) / 0.85 (21 °C, not pH dependent)	7.44 x 10^{-7} (25 °C)	202.2126
Methomyl	Oxime carbamate	$C_5H_{10}N_2O_2S$	55,000 (25 °C, pH 7) / 0.09 (25 °C, pH 4-10)	7.2 x 10^{-4} (25 °C)	162.210100
Monocrotophos	Organophosphorous	$C_7H_{14}NO_5P$	water miscible	2.9 x 10^{-4} (20 °C)	223.163522
Omethoate	Organophosphorous	$C_5H_{12}NO_4PS$	water-miscible / -0.74 (20 °C)	3.3 x 10^{-3} (20 °C)	213.191842
Oxamyl	Oxime carbamate	$C_7H_{13}N_3O_3S$	148,100 (20 °C, pH 5) / -0.44 (25 °C, pH 5)	5.12 x 10^{-5} (25 °C)	219.26142

Experimental Conditions

Sample Preparation

Individual stock solutions of pesticides were prepared at concentrations that were sufficient to evaluate the linearity of peak area versus injection volume at the same concentration e.g. 10 µg/L, but different injection volumes (e.g. 1, 2, 3, 4, 5, 6, 7 µL, etc.). Additional solutions with different concentrations (5, 10, 25, 50, 70, 100, 200 µg/L) were prepared to study the linearity of peak area versus compound concentration. Finally, solutions with different solvents (acetonitrile or methanol / water [1:1 v/v]) were prepared to study the solvent effect on the methanol / water gradient mobile phase during the injection.

HPLC

HPLC analysis was performed using a Thermo Scientific Accela UHPLC system. The chromatographic conditions were as follows:

HPLC Column	Thermo Scientific Hypersil GOLD, 100 mm x 2.1 mm, 1.9 µm particle size
Trap Column	Hypersil™ GOLD, 10 mm x 2.1 mm, 5 µm particle size
Column Temperature	40 °C
Mobile Phase A	Water with ammonium formate (5 mM) and formic acid (2 mM)
Mobile Phase B	Methanol with ammonium formate (5 mM) and formic acid (2 mM)

The trap column was used to trap the analytes, while the divert valve was switched to the waste position. A tee union between the trap column and the analytical column was connected to the divert valve. The two positions of the divert valve are shown in Figure 2.

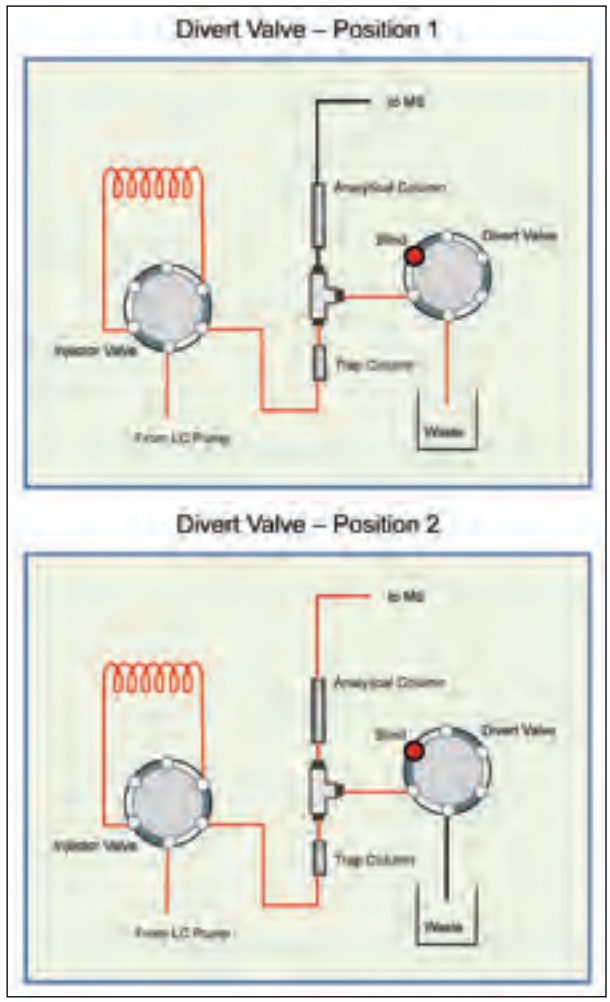

Figure 2. Divert valve positions

The gradient used is detailed in Table 2. The duration of the gradient was 21 minutes and the column equilibration time was 10 minutes. The flow rate increased at 21.10 min and decreased at 25.10 min to increase the speed of column equilibration for the next run (larger column volumes in less time). The maximum backpressure was 9,500 psi.

Table 2. HPLC Gradient. Mobile phase A is water with ammonium formate (5 mM) and formic acid (2 mM), and mobile phase B is methanol with ammonium formate (5 mM) and formic acid (2 mM).

No.	Time	A%	B%	µL/min
0	0.00	90.0	10.0	450.0
1	2.40	90.0	10.0	450.0
2	7.00	40.0	60.0	450.0
3	14.00	10.0	90.0	450.0
4	21.00	10.0	90.0	450.0
5	21.10	90.0	10.0	560.0
6	25.00	90.0	10.0	560.0
7	25.10	90.0	10.0	450.0
8	31.00	90.0	10.0	450.0

Mass Spectrometry

MS analysis was carried out on a Thermo Scientific TSQ Quantum Access MAX triple stage quadrupole mass spectrometer with an electrospray ionization (ESI) probe. The MS conditions were as follows:

Ion polarity	Positive
Q1 Resolution	0.7 Da
Spray Voltage	4000 V
Sheath/Auxiliary Gas	Nitrogen
Sheath Gas Pressure	40 (arbitrary units)
Auxiliary Gas Pressure	25 (arbitrary units)
Ion Transfer Tube Temperature	325 °C
Scan Type	Selected-Reaction Monitoring (SRM)
Collision Gas	Argon
Collision Gas Pressure	1.5 mTorr
Divert Valve	Rheodyne® model 7750E-185

The divert valve was connected to the front of the TSQ Quantum Access MAX™ and was fully controlled from the data system software.

Results and Discussion

The comparison of peak shapes between the acetonitrile and methanol / water sample solutions demonstrated that only early eluting analytes were altered by the mobile phase composition (Figure 3). Without the divert valve, the peak shape of omethoate, which elutes earlier than methomyl, was unacceptable in acetonitrile solution; whereas the peak shape of methomyl was better but not optimum (Figure 3a). The peak shape of metamitron, which elutes later than methomyl, was good in both acetonitrile and methanol / water sample solutions (Figures 3a, 3b). With the divert valve switched to the waste position for 1.30 minutes in the beginning of the run, the peak shapes of both omethoate and methomyl resembled those in the methanol / water sample solutions (Figure 3c).

The amount of time the valve was in the waste position affected the combination of peak shape and S/N ratio. As shown in Figure 4, the optimum combination of peak shape and RMS S/N ratio was achieved with a divert valve time of 1.30 minutes. Longer duration times were avoided, since the column equilibration was disturbed.

Figure 5 shows the range of injection volumes used. To assess the dependence between each compound peak area and the corresponding injection volume, eight injection volumes (1–8 µL) at a level of 10 µg/L were run three times each. The linear correlation coefficients (R^2 values) of the curve plots for all analytes studied were >0.99, and relative standard deviations were <20% (range 1%–14%). A S/N ratio greater than 10 for acephate and omethoate could not be achieved for injection volumes of 1 µL and 2 µL.

Figure 6 shows the curve of each compound's peak area versus concentration for a 5 µL injection volume. Seven different concentration levels (5, 10, 25, 50, 70, 100, 200 µg/L) with 5 µL injection volumes were run three times. The linear correlation coefficients (R^2 values) of the curve plots for all analytes studied were >0.99 and relative standard deviations were <20% (range 2%–16%). Using 5 µL injections of 5 µg/L acetonitrile solutions, RMS S/N ranged between 75 and 263,000.

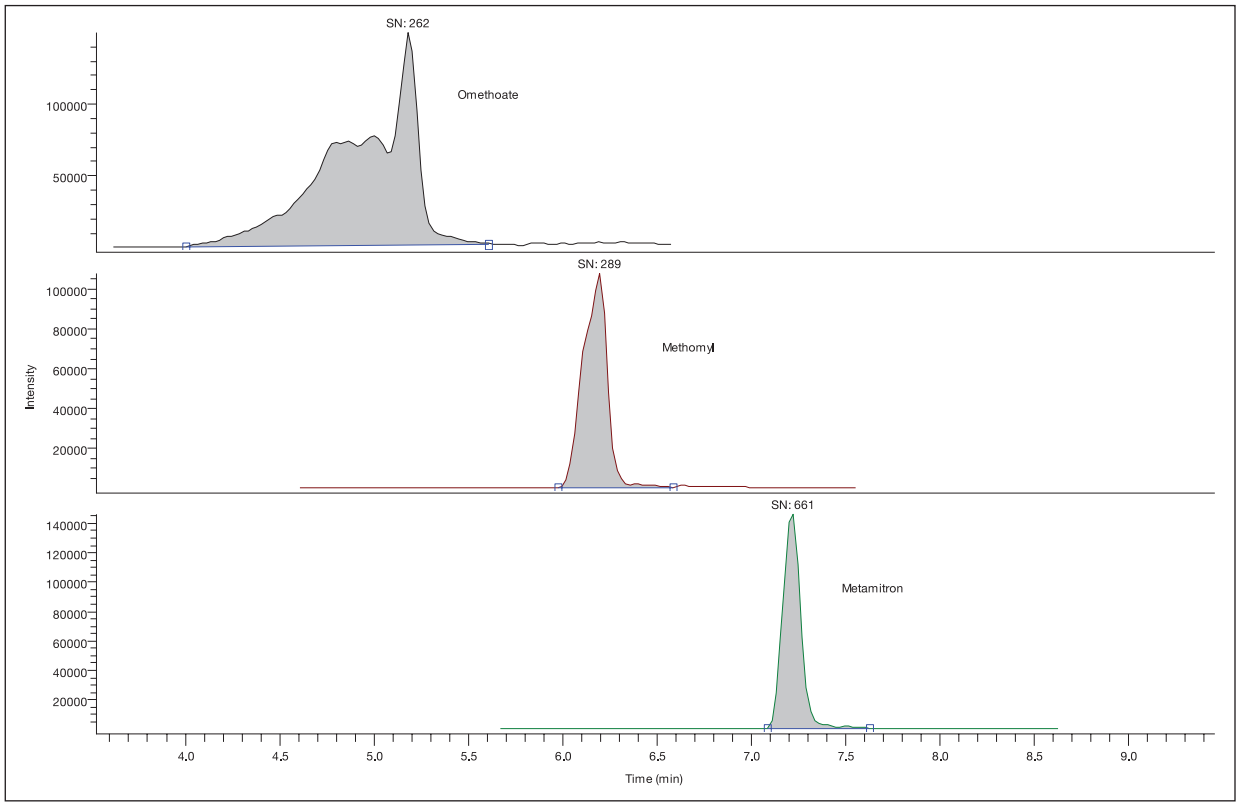

Figure 3a. Extracted chromatograms of 50 µg/L omethoate, methomyl, and metamitron in acetonitrile solution with no divert valve

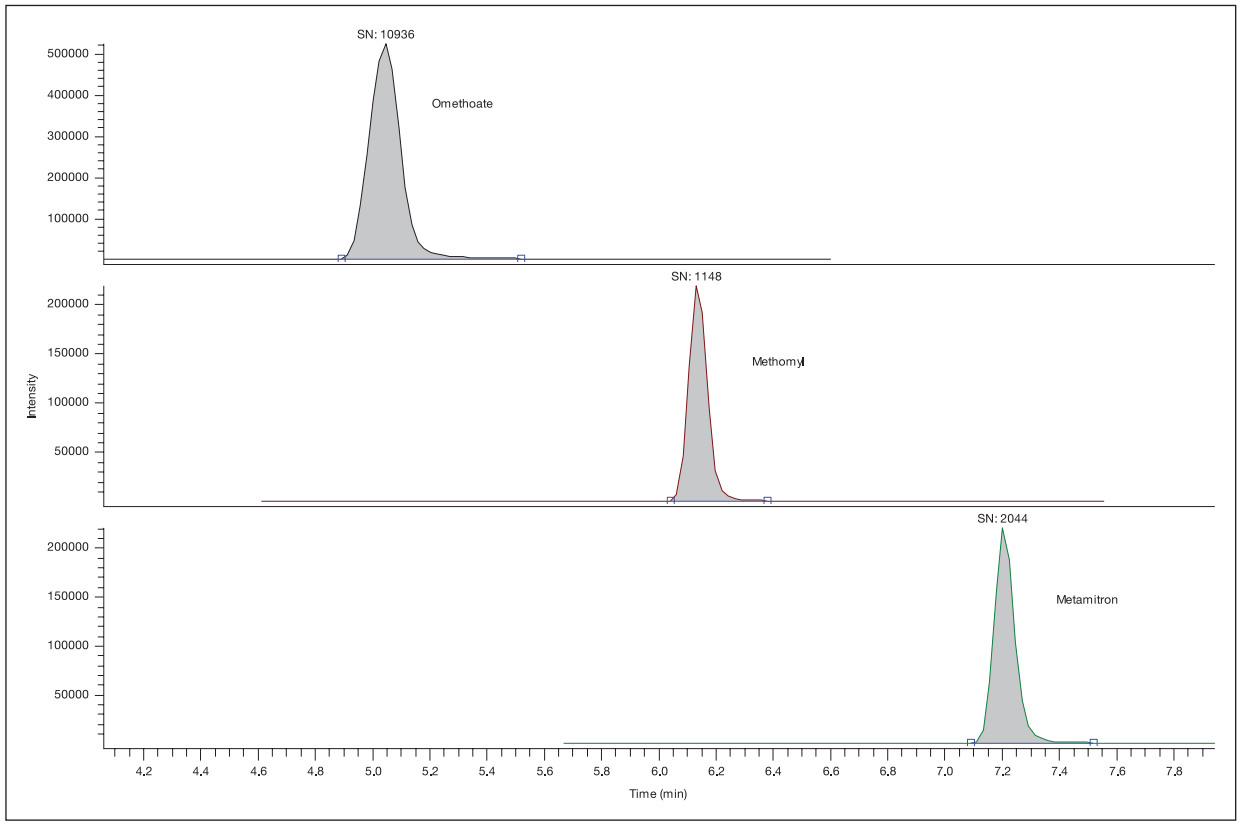

Figure 3b. Extracted chromatograms of 50 µg/L omethoate, methomyl, and metamitron in methanol / water [1:1 v/v] solution with no divert valve

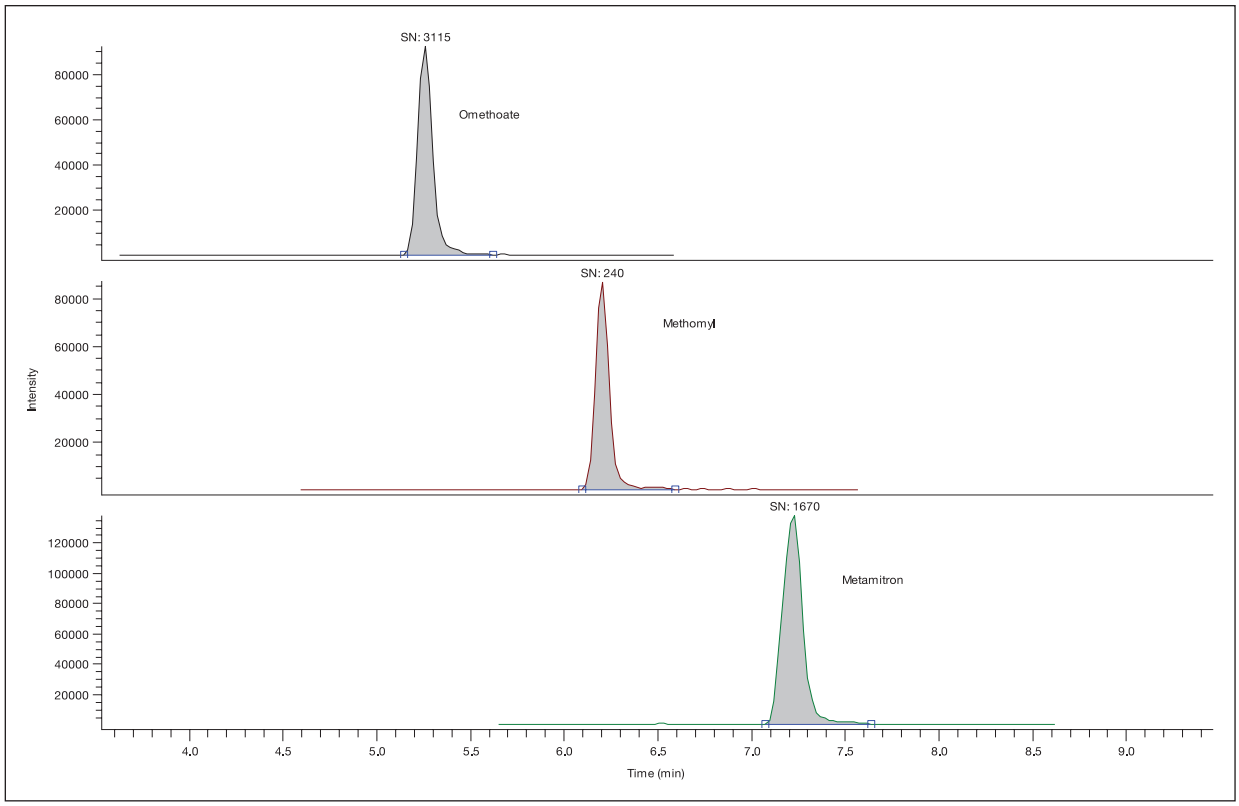

Figure 3c. Extracted chromatograms of 50 µg/L omethoate, methomyl, and metamitron in acetonitrile solution with divert valve open for 1.30 minutes

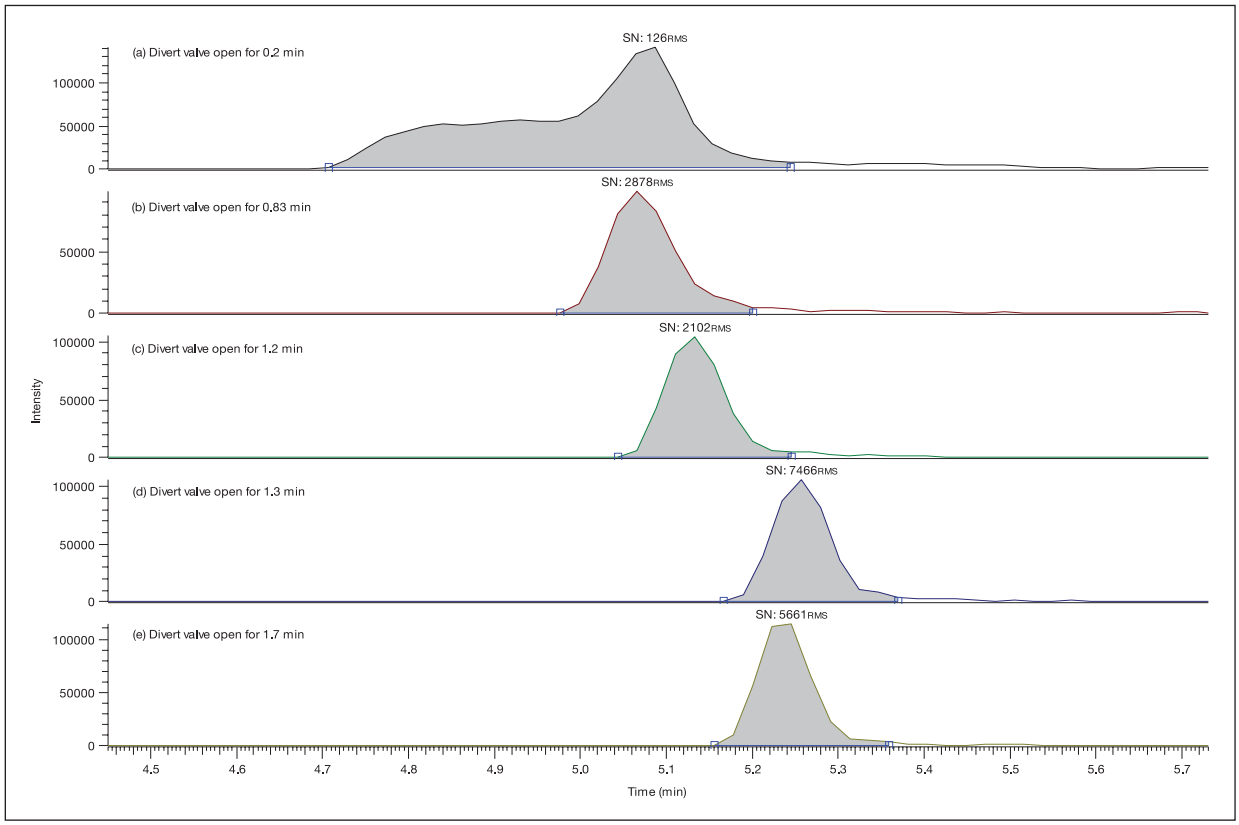

Figure 4. Extracted chromatograms of 5 µL injections of omethoate in 50 µg/L acetonitrile solution with various divert valve duration times used

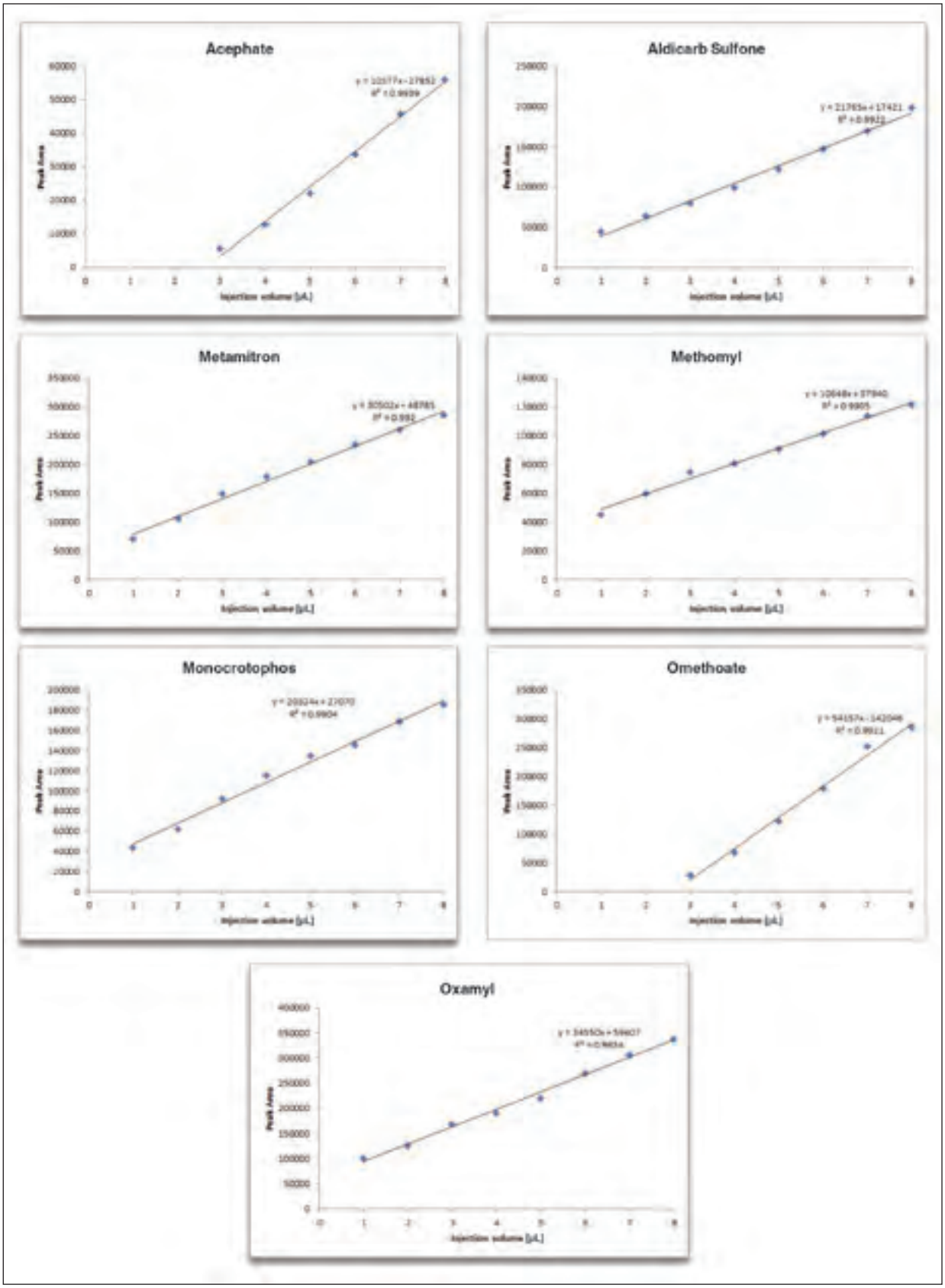

Figure 5. Curves for analyte peak area versus injection volumes 1-8 µL in 10 µg/L acetonitrile solution

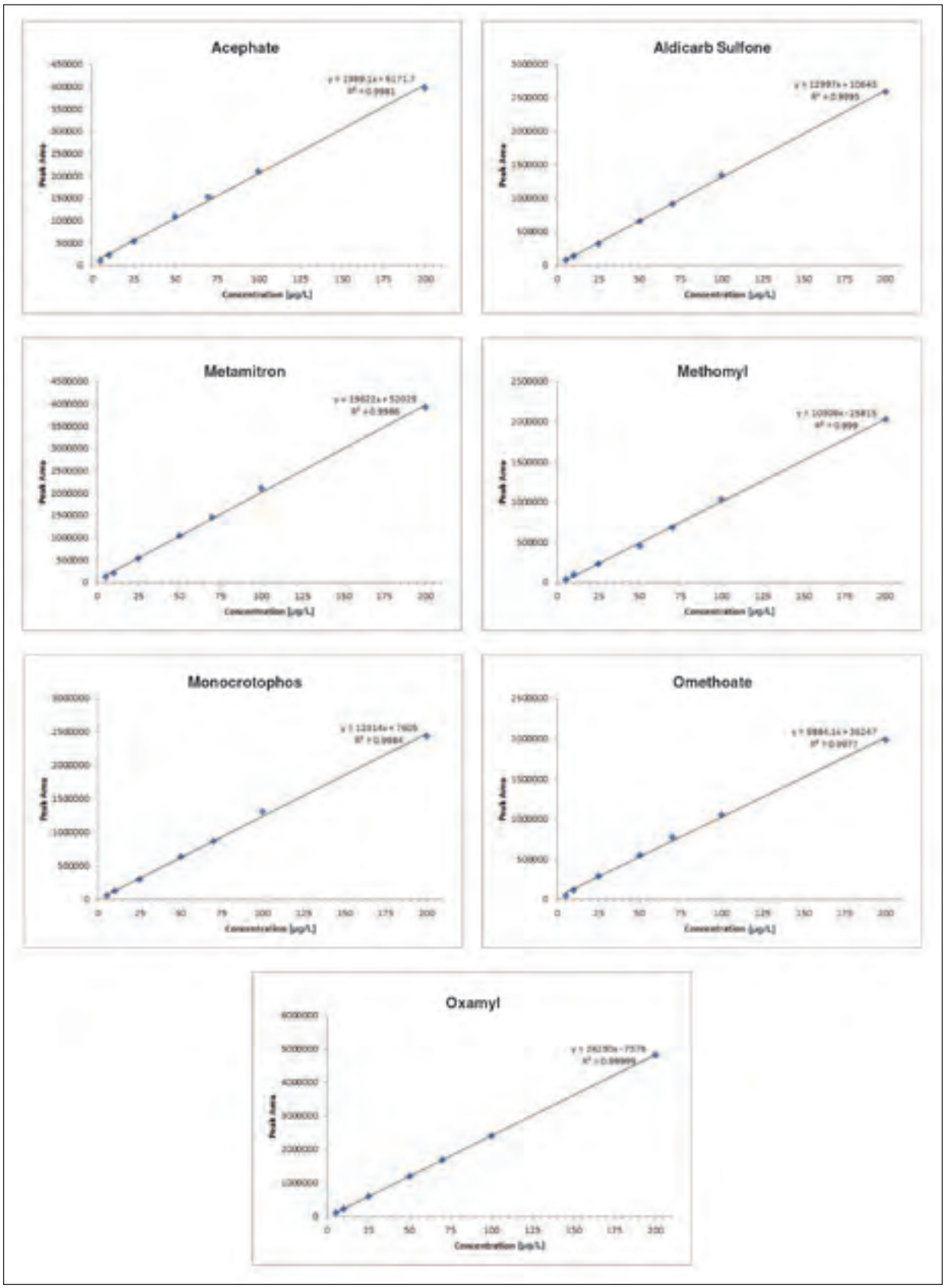

Figure 6. Curves for analyte peak area versus concentration 5-200 µg/L acetonitrile solution with 5 µL injection volume

Conclusion

The use of a divert valve proved suitable for the analysis of early eluting pesticides in acetonitrile solutions. Good peak shapes and S/N ratios were achieved and chromatographic problems, such as peak splitting or broadening, were overcome. In addition, the injection volume was increased up to 8 µL, reaching low detection limits with good linearity and repeatability, even for a sample concentration of 5 µg/L. It may be possible to increase the injection volume to 10 µL, and in some cases up to 15 µL, but with a larger loop volume. After the initial experiments, we concluded that a 5 µL injection volume is sufficient to achieve RMS S/N ratio greater than 10.

This technique resolves chromatographic issues involving interactions of gradient and sample solvent in a simple way and offers an increased laboratory sample capacity by avoiding solvent exchange in the final extract.

Reference

1. Jake L. Rafferty, J. Ilja Siepmanna, Mark R. Schure *Journal of Chromatography A*, **2011**, *1218*, 2203–2213.

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Method: 52213

Determination of Pesticides in Grapes, Baby Food and Wheat Flour by Automated Online Sample Preparation LC-MS/MS

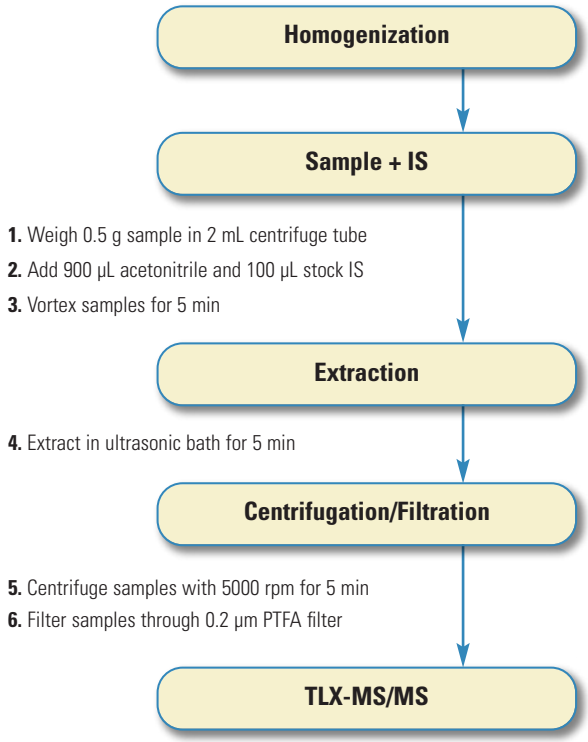
Laszlo Hollosi, Klaus Mittendorf, Thermo Fisher Scientific, Dreieich, Germany

DOWNLOAD

Key Words

- Transcend TLX
- TSQ Quantum Access MAX
- TurboFlow Technology
- Food Safety

1. Schematic of Method



2. Introduction

European Regulation 396/2005 sets maximum residue levels of pesticides in different products of plant and animal origin. These regulations present a significant analytical challenge with respect to the low limits of quantification which are required for some specified food matrices such as baby food. Many gas chromatography (GC) and high pressure liquid chromatography (HPLC) methods have been developed for multi-residue determination of pesticides and are in widespread use – employing a variety of sample preparation and cleanup techniques. In recent years the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method has become widely adopted for handling fruit and vegetables. However, QuEChERS requires many manual sample manipulation steps, making it labor-intensive when large numbers of samples have to be analyzed. It is therefore beneficial to consider options for automation of multi-residue methods, which can be cost-effective and can offer a high degree of reliability in recovery and repeatability. While the preliminary stages of homogenization and solvent extraction of food matrices inevitably require manual intervention, once a crude extract has been obtained, the procedure is fully automated thereafter. This automated procedure is included in the method, which utilizes turbulent flow chromatography with online liquid chromatography-mass spectrometry (LC-MS/MS).

Thermo Scientific Transcend TLX system coupled with the TSQ Quantum Access Max triple quadrupole mass spectrometer

Thermo SCIENTIFIC

3. Scope

This multi-residue pesticide method can be applied to fruits, cereals and composite baby foods at limits of detection (LODs) in the range of 0.8–10.3 µg/kg which are below respective EU maximum residue limits (MRLs). The method has been validated for 48 pesticides from different classes, but can be readily extended to a larger number of residues.

4. Principle

This method describes a novel sample preparation technique as a possible alternative to the QuEChERS method for high throughput pesticide analysis. Sample concentration, cleanup and analytical separation are carried out in a single run using an online coupled turbulent flow chromatography – reversed phase chromatography system (Thermo Scientific Transcend TLX system powered by Thermo Scientific TurboFlow technology). TurboFlow™ technology enables very effective separation of matrix and target compounds – resulting in relatively clean sample extracts. Macromolecules are removed from the sample extract with high efficiency, while target analytes are retained on the column based on different chemical interactions. After application of a wash step, the trapped compounds are transferred onto the analytical LC column and separated conventionally. The complete method involves internal standardization, solvent extraction of the homogenized food sample, centrifugation and injection into an automated cleanup system. Cleanup using TurboFlow technology has been optimized for maximum recovery of pesticide residues and minimal injection of co-extractives into the MS/MS. Identification of residues is based on ion-ratios using multiple reaction monitoring (MRM) of characteristic transition ions, and quantification using matrix-matched standards of one of the selected MRM ions.

5. Reagent List

		Part Number
5.1	Acetone, HPLC grade	A/0606/17
5.2	Acetonitrile, LC/MS grade	A/0638/17
5.3	Ammonium formate, for HPLC	A/5080/53
5.4	Methanol, Optima LC/MS grade	A456-212
5.5	Formic acid, extra pure for HPLC	F/1850/PB08
5.6	Isopropanol, HPLC grade	P/7507/17
5.7	Water, LC/MS grade	W/0112/17

6. Standard List

6.1 Pesticides: all standards from Sigma-Aldrich

Abamectin, ametryn, azinphos-me, azoxystrobin, bifenazate, carbaryl, carbendazim, carfentrazone-ethyl, chlormequate, clofentezin, cymoxanil, cypermethrin, dazomet, diazinon, dimethoate, dimethomorph A, dimethomorph B, ediphenfos, fenazaquin, fluazifop P, fluzilazol, hexithaizox, imazalil, imidacloprid, isoproturon, isoxaben, lactofen, malathion, metalaxyl, methomyl, metribuzin, myclobutanyl, omethoate, oxadyxil, oxamyl, pethoxamid, profenofos, promecarb, propoxur, pymetrozin, pyperonil-butoxide, pyrimethanyl, quinoxifen, spirodiclofen, tebuconazol, thiacloprid, triadimefon, trifloxistrobin.

6.2 Internal Standards

d_4-imidacloprid-, d_6-isoproturon, d_6-primicarb, d_{10}-parathion-ethyl (Sigma)

6.3 Quality Control Materials

FAPAS #963 (pasta matrix), FAPAS #966 (maize flour matrix), FAPAS #19110 (lettuce puree matrix)
(Note: FAPAS samples were selected primarily on content of target pesticides, however, matrices are different from the validated matrices with the exception of flour.)

7. Standards and Reagent Preparation

7.1 Concentration of mixed **pesticide working stock solution** (2 µg/mL and 1 µg/mL) in methanol. Prepare 2 µg/mL working stock standard solution by 10× dilution of intermediate stock standard solution in a 10 mL volumetric flask with methanol. Prepare 1 µg/mL working stock standard mix, by diluting intermediate stock standard solution by 20× in a 10 mL volumetric flask.

7.2 To prepare **individual stock standard solutions**, weigh 10 mg from each analyte into a 20 mL amber screw cap vial on the five digit analytical balance. Add 10 mL methanol from a calibrated pipette and note the weight of both analyte and solvent. If undissolved crystals are seen, put the vial in an ultrasonic bath until complete dissolution.

7.3 To prepare **intermediate stock standard solution**, pipette 200 µL from each individual stock standard into a 10 mL volumetric flask and fill up to the mark with methanol.

7.4 Concentration of **stock internal standard** (for sample spiking for internal standardization) is 100–100 ng/mL for d_4-imidacloprid and d_6-isoproturon, 10000 ng/mL for d_6-primicarb and 700 µg/mL d_{10}-parathion-ethyl in methanol. Prepare stock internal standard mixture by pipetting 7 mL of d_{10}-parathion-ethyl individual stock solution and 1 mL of intermediate stock internal standard mixture into a 10 mL volumetric flask and fill up to the mark with methanol.

7.5 To prepare **individual stock internal standard** solutions, weigh 10 mg of each analyte into a 20 mL amber screw cap vial on the five digit analytical balance. Add 10 mL methanol from a calibrated pipette and note the weight of both analyte and solvent.

7.6 To prepare **intermediate stock internal standard** mixture, pipette 1000 µL d_6-primicarb individual solution and 100–100 µL d_4-imidacloprid and d_6-isoproturon individual solutions into a 10 mL volumetric flask and fill to the mark with methanol.

8. Apparatus

		Part Number
8.1	Fisher precision balance	XP-1500FR
8.2	Sartorius analytical balance	ME235S
8.3	Thermo Scientific Barnstead EASYpure II water	3125753
8.4	Ultrasonic bath Elmasonic S40H	1002006
8.5	ULTRA-TURRAX® – G25 dispergation tool	1713300
8.6	ULTRA-TURRAX	3565000
8.7	Vortex shaker	3205025
8.8	Vortex universal cap	3205029
8.9	Accu-Jet pipettor	3140246
8.10	Thermo Scientific Heraeus Fresco 17 micro centrifuge	3208590
8.11	Transcend™ TLX-1 system	
8.12	Thermo Scientific TSQ Quantum Access MAX triple stage quadrupole mass spectrometer	

9. Consumables

		Part Number
9.1	LC vials	24014019
9.2	Pipette Finnpipette 100–1000 µL	3214535
9.3	Pipette Finnpipette 10–100 µL	3166472
9.4	Pipette Finnpipette 500–5000 µL	3166473
9.5	Pipette holder	3651211
9.6	Pipette tips 0.5–250 µL, 500/box	3270399
9.7	Pipette tips 1–5 mL, 75/box	3270420
9.8	Pipette tips 100–1000 µL, 200/box	3270410
9.9	Spatula, 18/10 steel	3458179
9.10	Spatula, nylon	3047217
9.11	Tube holder	3204844
9.12	Wash bottle, PTFE	3149330
9.13	2 mL vial rack	12211001
9.14	0.2 µm PTFA syringe filter	F2513-4
9.15	1 mL disposable plastic syringe	S7510-1
9.16	1.7 mL centrifuge plastic tube	3150968
9.17	TurboFlow Cyclone MCX-2 (50 × 0.5 mm) column	CH-953457
9.18	Thermo Scientific Hypersil GOLD 150 × 4.6 mm, 5 µm column	25005-154630
9.19	UNIGUARD holder	850-00
9.20	Hypersil GOLD™ 10 × 4 mm, 5 µm guard column	25005-014001

10. Glassware

		Part Number
10.1	Volumetric flask, 10 mL	FB50143
10.2	Volumetric flask, 25 mL	FB50147
10.3	1 mL glass pipette	FB50211
10.4	1 L bottle	9653650
10.5	500 mL bottle	9653640

11. Procedure

11.1 Sample Preparation

Solid Samples

Extract solid samples prior to injection into the Transcend system coupled to the TSQ Quantum Access MAX™ mass spectrometer. If samples are table grapes, these are treated as semisolid samples and need to be homogenized prior to extraction. Baby food and flour samples are treated as fine and homogenous solid matrices, so intensive manual mixing with a spatula is satisfactory.

11.2 Homogenization of Semisolid Samples

11.2.1 Select approximately 10–15 individual grapes randomly from the bunch and put into an appropriate size (depending on grape type and size ~100 mL) beaker and label it.

11.2.2 Attach the G25 dispergation tool to the ULTRA-TURRAX homogenizer

11.2.3 Start homogenization at middle rotation speed (speed level 2-3) and continue it to form a smooth puree

11.3 Extraction

11.3.1 Weigh 0.5 g sample into a 1.7 ml centrifuge tube

11.3.2 Add 900 µL acetonitrile stock IS

11.3.3 Vortex the sample for 5 min (to wet all the solid samples throughout)

11.3.4 Put the well-mixed samples into the Ultrasonic bath for 5 min.

11.3.5 Centrifuge in the micro centrifuge at 5000 rpm for 5 min.

11.3.6 Remove supernatant and filter it through 0.2 µm PTFE syringe filter directly into the LC vial

12. Analysis

Sample concentration, cleanup and analytical separation are carried out in a single run using an automated online sample preparation system, which includes the Transcend system and Thermo Scientific Aria operating software. TurboFlow technology with the Transcend system enables very effective separation of matrix and target compounds due to its special size exclusion and reversed phase chemistry. Macromolecules are removed from the sample extract with high efficiency, while target analytes are retained on the column based on different chemical interactions. After application of a wash step, the trapped compounds are transferred onto the analytical LC column and separated conventionally. Consequently the method was optimized for both TurboFlow technology and analytical chromatography.

		TurboFlow Technology								LC					
Step	**Duration [s]**	**Flow**	**Grad**	**A%**	**B%**	**C%**	**D%**	**Tee**	**Loop**	**Flow**	**Grad**	**A%**	**B%**	**C%**	**D%**
1	60	1.50	step		100			–	out	0.50	step		100		
2	60	1.50	step		95		5	–	out	0.50	step		100		
3	80	0.16	step		100			Tee	in	1.44	step		100		
4	60	1.00	step			100		–	in	1.60	ramp		55		45
5	60	1.00	step	10			90	–	in	1.60	ramp		40		60
6	220	0.20	step		100			–	out	1.60	ramp				100
7	60	0.20	step		100			–	out	1.60	step				100
8	180	0.20	step		100			–	out	1.00	step		100		
		Mobile phases for the TurboFlow method: A: water pH=3 B: water C: 40% acetonitrile 40% isopropanol and 20% acetone D: 5 mM ammonium-formiate in methanol + 0.1% formic acid								Solvent channels for LC: A: acetonitrile B: 5 mM ammonium-formiate in water + 0.1% formic acid C: water D: 5 mM ammonium-formiate in methanol + 0.1% formic acid *Note: LC channel C can be used for column wash purposes*					

Table 1: Gradient program table for Aria™ control software

12.1 LC Conditions for Transcend TLX System

Operation was carried out in focus mode setup (Figure 1) with 1:1 splitting before the TSQ Quantum Access MAX mass spectrometer entrance using a divert valve connection. The TurboFlow Cyclone MCX-2 column was installed as the TurboFlow column (9.17). The Hypersil GOLD column equipped with a guard column was used as the analytical LC column (9.18–9.20). Installed loop volume was 200 µL.

Sample load (Step 1) was applied with 1.5 mL/min flow rate, whereby matrix components were eluted in the waste, and target pesticides were trapped on the TurboFlow column. After washing the TurboFlow column with 5% organic/aqueous mixture (Step 2), the trapped pesticides were eluted and transferred (Step 3) after 2 min from the TurboFlow column to the analytical LC column. Simultaneous dilution of the eluate occurs enabling pre-concentration of pesticides at the beginning of the analytical column. The analytical LC column was equilibrated and conditioned during loading and washing steps. After transfer of the pesticides, the analytical separation started with gradient elution (Steps 4–7), while the TurboFlow column was washed and conditioned, and the loop was filled with the eluent. After the gradient run, the Hypersil GOLD column was washed in acetonitrile and conditioned for the next run. The total run time of the method with automated online sample preparation and analytical separation was 13 min. Table 1 gives details of the method program. In order to minimize sample carry-over and cross-contamination, the injection needle as well as the injection valve was washed 4 times with both cleaning solvents.

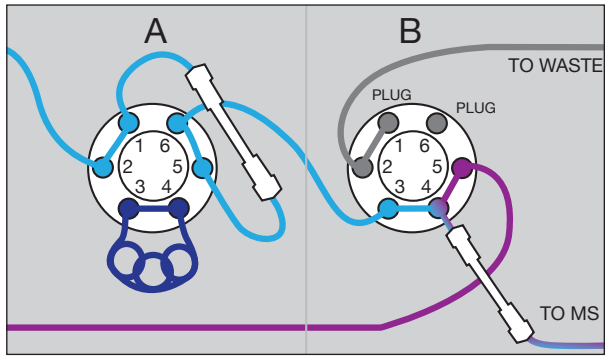


Figure 1: Focus mode system set up and method setting in Aria control software on the Transcend TLX system

12.1.1 Injector settings

Injector: Transcend TLX autosampler with 100 µL injection syringe volume

Sample holder temperature: 10 °C

Cleaning solvents: Solvent channel 1–80%MeOH/acetone
Solvent channel 2–50%MeOH/H_2O

Injector settings:

- Pre Clean with solvent 1 [steps]: 2
- Pre Clean with solvent 2 [steps]: 2
- Pre Clean with sample [steps]: 1
- Filling speed [µL/s]: 50
- Filling strokes [steps]: 2
- Injection port: LC Vlv1 (TurboFlow method channel)
- Pre inject delay [ms]: 500
- Post inject delay [ms]: 500
- Post clean with solvent 1 [steps]: 4
- Post clean with solvent 2 [steps]: 4
- Valve clean with solvent 1 [steps]: 4
- Valve clean with solvent 2 [steps]: 4
- Injection volume: 10 µL

12.1.2 Mass Spec conditions

Mass spectrometric detection was carried out by TSQ Quantum Access MAX triple stage quadrupole mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) in selected reaction monitoring (SRM) mode. All SRM traces were individually tuned for each target pesticide. MS programming was set in Thermo Scientific Xcalibur software in Eazy set up mode.

Settings were:

- Scan type: SRM (details in table below)
- Cycle time [s]: 0.3
- Peak width: 0.7 Da FWHM
- Collision gas pressure [mTorr]: 1.0
- Capillary Temperature [°C]: 290
- Vaporizer Temperature [°C]: 290
- Sheath gas pressure [arb]: 40
- Aux gas pressure [arb]: 10
- Ion sweep pressure [arb]: 0
- Spray voltage [V]: 3200
- Polarity: positive for all compounds
- Trigger: 1.00e5

12.2 Calculation of Results

Calibration by the internal standardization is applied for the determination of pesticides. This quantification method requires determination of response factors R_f defined by the equation below. Calculation of final results is performed using the following equations.

Calculation of the response factor:

$$R_f = \frac{A_{St} \times c_{[IS]}}{A_{[IS]} \times c_{St}}$$

$\mathbf{R_f}$ – the response factor

$\mathbf{A_{St}}$ – the area of the pesticide peak in the calibration standard

$\mathbf{A_{[IS]}}$ – the area of the internal standard peak of the calibration standard

$\mathbf{c_{St}}$ – pesticide concentration of the calibration standard solution

$\mathbf{c_{[IS]}}$ – the internal standard concentration of the calibration standard solution

Calculations for each sample of the absolute amount of pesticide that was extracted from the sample:

$$X_{analyte} = \frac{A_{analyte} \times X_{[IS]}}{A_{[IS]} \times R_f}$$

$\mathbf{X_{analyte}}$ – the absolute amount of pesticide that was extracted from the sample

$\mathbf{A_{analyte}}$ – the area of pesticide peak in the sample

$\mathbf{A_{[IS]}}$ – the area of the internal standard peak in the sample

$\mathbf{X_{[IS]}}$ – the absolute amount of internal standard added to the sample

The concentration of pesticide in the sample [ng/g]:

$$c = \frac{X_{analyte}}{m}$$

m – the weight of sample [g]

$\mathbf{X_{analyte}}$ – absolute analyte amount [ng]

13. Method Performance Characteristics

In-house validation of the method was carried out on all matrices and target pesticides. International Union of Pure and Applied Chemistry (IUPAC)/Association of Official Analytical Chemists (AOAC) guideline for single laboratory validation[1,2] was used as a template and it was also demonstrated that method performance characteristics fulfilled the legislative criteria set for pesticide residue methods.[3]

13.1 Selectivity

Method (SRM) selectivity was confirmed based on the presence of specific ion transitions at the corresponding retention time (Table 2), as well as the observed ion ratio values corresponding to those of the standards. Acceptance criteria for retention time and ion ratios were set according to Reference 1.

13.2 Linearity, Response Factor

The linearity of calibration curves was assessed over the range from 10–500 ng/g. In all cases, the correlation coefficients of linear functions were better than 0.985. The calibration curves were created at five levels (matrix-matched) and injected in duplicate. R_f values for internal standardization were determined from the calibration curves for each matrix, and internal standards by calculating cumulative average response factors over the whole calibration range.

13.3 Accuracy

Method accuracy and precision was assessed by recovery studies using blank matrices spiked at three concentration levels injected in six individually prepared replicates. Samples were spiked at 10, 100 and 250 ng/g concentration levels. Found concentrations, recovery and relative standard deviation (%RSD) were calculated (Table 3). Recovery values are deemed acceptable if between 70–125%. Additional accuracy was established for selected target analytes by analysing FAPAS #963, 966 and 19110 proficiency test materials. All measured concentrations of the relevant compounds (diazinon, tebuconazole, trifloxistrobin, malathion, azoxystrobin and dimethomorph) were within the acceptable satisfactory ranges.

13.4 Precision

Method within-day and between-day precision values were determined for each matrix at middle spiking level (100 ng/g) each in 6 replicates and expressed as %RSD over 3 days with individually prepared samples. Mean within-day precision values were determined as average of the 3 individual days' mean precision, while between-day precision was expressed as mean of the overall precision data. Measured values are shown in Table 4.

13.5 Limits of Detection (LODs) and Quantification (LOQs)

LODs and LOQs were estimated following the IUPAC approach which consisted of analyzing the blank sample to establish noise levels and then testing experimentally estimated LODs and LOQs for signal/noise, 3 and 10 respectively. The method LOD values are listed in Table 5. The expectation of the method was to meet MRL values at least at LOD level. The lowest MRL values were defined for baby food matrices (10 ng/g), which were achieved in all cases.

13.6 Robustness

A robustness study was performed by varying parameters like extraction time, centrifugation speed, time by 20%, shaker (horizontal shaker, vortex) and extraction mode (ultrasonic bath, vortex shaking). Results were compared to the original method and significant differences were sought based on ANOVA analysis. None of the parameters which were varied led to significant differences in measured values, consequently indicating that the method was robust.

14. Conclusion

The method described here enables convenient, fast and cost-effective automated determination of selected pesticides, from polar to non-polar compound chemistry, in different matrix types. Based on the short total run time and Transcend system with TurboFlow technology, 100 samples per day can be analyzed under controlled sample preparation conditions. Method performance characteristics were established by in-house validation for baby food, grapes and wheat flour matrices. The method performance indicates it is suitable for routine use for regulatory purposes and can be readily extended to a larger and wider range of pesticide residues.

15. References

1. http://www.aoac.org/Official_Methods/slv_guidelines.pdf
2. http://www.scribd.com/doc/4922271/Harmonized-Guidelines-for-Single-Laboratory-Validation-of-Methods-Of
3. http://ec.europa.eu/sanco_pesticides/public/index.cfm

16. Annex

16.1 Tables and Chromatograms

Analyte	Precursor Ion	Product Ion (CE)	Product Ion2 (CE)	Retention Time [min]
Abamectin	890.2	305.1 (25)	567.4 (12)	10.1
Ametryn	228.1	96.1 (25)	116.1 (26)	7.76
Azinphos methyl	339.8	132.1 (19)	160.2 (12)	7.87
Azoxystrobin	404.1	344.1 (25)	372.1 (14)	7.99
Bifenazate	301.1	198.1 (7)	170.1 (19)	8.36
Carbaryl	219.1	202.1 (5)	127.1 (32)	7.31
Carbendazim	191.8	160.1 (18)	132.1 (29)	5.96
Carfentrazone-ethyl	429.1	412.2 (12)	384.2 (18)	8.71
Chlormequate	122.1	58.5 (31)	63.3 (21)	4.06
Clofentezin	304.7	138.1 (26)	102.1 (38)	9.07
Cymoxanil	199.3	83.9 (20)	111.1 (20)	6.71
Cypermethrin	433.1	416.3 (5)	191.2 (15)	8.72
Dazomet	163.1	120.1 (11)	90.2 (9)	5.83
Diazinon	304.9	169.1 (21)	153.1 (21)	8.90
Dimethoate	230.2	125.3 (21)	170.7 (13)	6.43
Dimethomorph A&B	388.1	300.9 (21)	164.9 (31)	8.12/8.34
Ediphenfos	310.8	283.1 (11)	111.2 (19)	8.80
Fenazaquin	307.2	161.2 (16)	57.2 (21)	10.18
Fluazifop P	384.3	282.1 (18)	254.2 (27)	9.29
Fluzilazol	316.1	165.1 (27)	247.1 (18)	8.66
Hexithaizox	353.1	228.1 (14)	167.8 (24)	9.66
Imazalil	296.9	159.1 (23)	176.2 (20)	7.50
Imidacloprid	256.1	209.2 (15)	175.2 (17)	6.16
d4-Imidacloprid	259.9	213.1 (17)	179.1 (20)	6.24
Isoproturon	207.1	72.1 (18)	165.3 (14)	7.73
d6-Isoproturon	213.2	78.3 (19)	171.1 (14)	7.71
Isoxaben	333.1	165.1 (20)	149.9 (38)	8.15
Lactofen	479.1	462.1 (5)	344.2 (15)	9.35
Malathion	347.9	330.7 (5)	99.4 (29)	8.22
Metalaxyl	279.9	220.2 (13)	192.1 (18)	7.63
Methomyl	163.1	106.1 (10)	88.1 (8)	5.95
Metribuzin	215.2	187.1 (16)	74.1 (34)	7.21
Myclobutanyl	289.1	70.3 (18)	124.9 (30)	8.38
Omethoate	214.2	125.1 (22)	155.2 (15)	5.58
Oxadyxil	296.2	279.2 (5)	219.3 (15)	6.80
Oxamyl	236.9	72.2 (14)	90.3 (5)	5.75
d10-Parathion-ethyl	302.1	238.1 (17)	270.1 (11)	8.83
Pethoxamid	296.1	131.1 (20)	250.2 (12)	8.48
d6-Primicarb	245.2	185.1 (16)	78.3 (28)	6.86
Profenofos	374.8	304.9 (17)	222.8 (31)	9.37
Promecarb	225.2	207.9 (7)	151.2 (6)	8.29
Propoxur	210.1	111.1 (14)	168.2 (7)	7.12
Pymetrozin	218.0	105.2 (23)	78.3 (37)	5.53
Pyperonil-butoxide	356.0	177.1 (13)	147.1 (29)	9.49
Pyrimethanyl	200.1	181.2 (35)	168.1 (28)	8.00
Quinoxifen	307.9	196.8 (31)	214.1 (33)	9.68
Spirodiclofen	410.9	313.1 (9)	71.1 (12)	9.83
Tebuconazol	308.2	70.2 (22)	124.9 (33)	8.88
Thiacloprid	253.1	126.1 (19)	90.1 (33)	6.55
Triadimefon	294.1	197.1 (15)	69.4 (20)	8.32
Trifloxistrobin	409.5	186.3 (17)	206.4 (13)	9.24

Table 2: Ion transitions for SRM setting

	Grape [Rec %] (%RSD)			Baby Food [Rec %] (%RSD)			Wheat Flour [Rec %] (%RSD)		
Analyte	**10 ng/g**	**100 ng/g**	**250 ng/g**	**10 ng/g**	**100 ng/g**	**250 ng/g**	**10 ng/g**	**100 ng/g**	**250 ng/g**
Abamectin	66 (17)	64 (18)	71 (11)	68 (19)	76 (5)	76 (4)	89 (17)	99 (5)	101 (7)
Ametryn	111 (16)	99 (18)	118 (9)	111 (8)	115 (5)	125 (5)	108 (16)	111 (4)	109 (7)
Azinphos-me	111 (9)	121 (19)	110 (11)	105 (5)	100 (4)	112 (5)	85 (13)	92 (6)	124 (4)
Azoxystrobin	105 (15)	69 (8)	104 (9)	86 (4)	90 (5)	88 (2)	87 (5)	118 (3)	117 (2)
Bifenazate	90 (14)	88 (5)	96 (9)	101 (5)	106 (5)	113 (4)	121 (5)	112 (4)	108 (3)
Carbaryl	69 (8)	86 (8)	90 (8)	98 (5)	111 (6)	120 (4)	110 (4)	110 (3)	107 (3)
Carbendazim	93 (14)	108 (5)	104 (8)	122 (7)	89 (5)	97 (3)	73 (14)	123 (6)	116 (3)
Carfentrazone-ethyl	85 (14)	74 (11)	84 (11)	92 (6)	102 (5)	104 (3)	112 (7)	119 (4)	114 (2)
Chlormequate	LOD	90 (12)	77 (17)	74 (16)*	90 (10)	89 (10)	LOD	106 (7)	100 (7)
Clofentezin	78 (18)*	71 (9)	84 (6)	71 (18)	73 (12)	82 (10)	123 (10)*	110 (7)	94 (13)
Cymoxanil	110 (13)	93 (14)	114 (13)	96 (19)	80 (17)	78 (7)	89 (19)	101 (15)	83 (12)
Cypermethrin	121(13)*	84 (17)	74 (11)	122 (12)	79 (12)	87 (9)	123 (13)*	115 (9)	114 (11)
Dazomet	106 (19)	107 (18)	117 (9)	80 (17)	114 (5)	118 (5)	84 (7)	102 (5)	99 (5)
Diazinon	80 (15)	75 (5)	87 (10)	87 (9)	99 (6)	103 (4)	122 (3)	108 (2)	105 (3)
Dimethoate	90 (4)	88 (10)	95 (4)	106 (3)	114 (4)	117 (3)	73 (7)	118 (4)	112 (4)
Dimethomorph A	70 (15)	84 (8)	74 (8)	81 (5)	85 (4)	86 (4)	112 (4)	98 (3)	98 (2)
Dimethomorph B	89 (11)	71 (4)	77 (4)	86 (4)	91 (4)	89 (4)	110 (8)	114 (5)	118 (4)
Ediphenfos	94 (14)	72 (7)	90 (8)	109 (6)	110 (5)	114 (4)	105 (11)	111 (8)	110 (6)
Fenazaquin	101 (6)	88 (12)	78 (4)	78 (4)	83 (7)	85 (8)	104 (10)	81 (12)	73 (16)
Fluazifop P	101 (17)	72 (16)	86 (13)	101 (8)	100 (7)	103 (6)	116 (5)	107 (4)	106 (4)
Fluzilazol	87 (12)	69 (9)	89 (9)	91 (9)	102 (6)	107 (5)	122 (5)	110 (3)	106 (5)
Hexithaizox	75 (17)	82 (15)	93 (15)	93 (15)	119 (8)	120 (12)	102 (5)*	94 (11)	91 (14)
Imazalil	79 (8)	82 (11)	85 (8)	88 (5)	95 (8)	102 (6)	85 (19)	81 (5)	77 (12)
Imidacloprid	86 (8)	93 (6)	97 (5)	111 (4)	117 (3)	124 (2)	107 (3)	112 (3)	110 (3)
Isoproturon	95 (8)	74 (10)	86 (7)	104 (5)	109 (4)	101 (4)	123 (18)	109 (4)	114 (3)
Isoxaben	84 (14)	74 (5)	87 (7)	95 (4)	103 (4)	103 (3)	115 (5)	121 (3)	114 (2)
Lactofen	91 (17)	70 (15)	81 (12)	104 (7)	108 (5)	116 (9)	131 (7)	111 (6)	109 (7)
Malathion	117 (9)	83 (13)	75 (10)	103 (6)	91 (4)	88 (5)	104 (9)	94 (5)	112 (4)
Metalaxyl	79 (9)	76 (9)	80 (5)	88 (5)	98 (5)	97 (5)	74 (8)	123 (4)	115 (3)
Methomyl	75 (9)	68 (8)	81 (10)	73 (12)	81 (4)	87 (5)	99 (10)	96 (10)	89 (10)
Metribuzin	89 (11)	73 (6)	87 (4)	106 (10)	112 (5)	113 (7)	103 (13)	112 (4)	107 (3)
Myclobutanyl	90 (17)	75 (11)	90 (10)	102 (8)	104 (5)	110 (4)	105 (3)	119 (4)	117 (3)
Omethoate	70 (20)*	72 (8)	76 (9)	76 (18)	78 (7)	81 (11)	71 (16)*	75 (14)	70 (6)
Oxadyxil	71 (9)	72 (7)	87 (5)	84 (4)	101 (4)	100 (4)	87 (6)	123 (4)	117 (2)
Oxamyl	69 (9)	71 (9)	69 (7)	74 (8)	78 (5)	79 (6)	96 (11)	95 (10)	88 (7)
Pethoxamid	74 (10)*	70 (6)	77 (8)	89 (5)	88 (8)	91 (6)	123 (3)	115 (3)	108 (2)
Profenofos	112 (17)	72 (12)	95 (11)	109 (6)	115 (4)	120 (4)	115 (8)	106 (3)	105 (2)
Promecarb	90 (10)	86 (5)	94 (5)	104 (6)	114 (3)	115 (4)	128 (4)	122 (3)	112 (2)
Propoxur	84 (6)	87 (6)	84 (7)	98 (6)	106 (4)	108 (4)	91 (6)	115 (4)	110 (4)
Pymetrozin	101 (8)	94 (4)	121 (14)	101 (4)	112 (5)	113 (3)	89 (3)	117 (3)	110 (2)
Pyperonil-butoxide	78 (17)	93 (9)	86 (9)	95 (4)	102 (4)	109 (4)	115 (10)	113 (6)	111 (3)
Pyrimethanyl	120 (13)	121 (7)	108 (13)	80 (14)	114 (5)	101 (4)	94 (10)	106 (5)	110 (6)
Quinoxifen	90 (19)	78 (20)	104 (6)	87 (10)	99 (8)	105 (7)	98 (12)	90 (7)	86 (9)
Spirodiclofen	83 (11)	79 (17)	78 (17)	89 (16)	102 (6)	103 (7)	83 (4)	98 (5)	96 (5)
Tebuconazol	83 (15)	79 (8)	83 (6)	94 (4)	93 (6)	98 (4)	121 (7)	115 (4)	117 (3)
Thiacloprid	95 (8)	80 (10)	89 (8)	109 (5)	113 (5)	109 (3)	69 (8)	124 (6)	116 (4)
Triadimefon	69 (12)	68 (8)	83 (5)	96 (8)	104 (6)	109 (4)	118 (8)	115 (3)	114 (3)
Trifloxistrobin	82 (5)	76 (8)	81 (11)	99 (6)	97 (6)	104 (4)	109 (4)	98 (5)	92 (4)

Table 3: Average method recovery [%] and %RSD [%] values at 3 different spike levels in the investigated matrices (n=6)

*LOD: spike level at or below LOD, * spike level at or below LOQ*

		Grape		Baby Food		Wheat Flour	
Analyte	**Spike Level [ng/g]**	**Mean within day precision [%RSD]**	**Between day precision [%RSD]**	**Mean within day precision [%RSD]**	**Between day precision [%RSD]**	**Mean within day precision [%RSD]**	**Between day precision [%RSD]**
Abamectin	100	11	14	6	11	10	11
Ametryn	100	11	19	9	12	8	16
Azinphos-me	100	12	15	5	6	9	11
Azoxystrobin	100	14	22	7	10	6	6
Bifenazate	100	10	17	7	9	6	9
Carbaryl	100	16	19	7	16	8	17
Carbendazim	100	8	11	7	12	7	9
Carfentrazone-ethyl	100	9	17	10	15	8	10
Chlormequate	100	12	15	10	15	8	10
Clofentezin	100	14	21	11	15	9	11
Cymoxanil	100	16	19	14	21	11	15
Cypermethrin	100	12	16	12	16	10	12
Dazomet	100	15	20	13	20	15	21
Diazinon	100	9	17	6	16	8	12
Dimethoate	100	12	17	9	17	10	13
Dimethomorph A	100	11	17	7	16	8	10
Dimethomorph B	100	6	10	7	11	7	14
Ediphenfos	100	10	11	7	7	6	6
Fenazaquin	100	12	21	9	13	13	13
Fluazifop P	100	9	14	8	8	11	10
Fluzilazol	100	9	19	6	10	5	8
Hexithaizox	100	8	19	9	18	15	19
Imazalil	100	10	18	10	15	10	17
Imidacloprid	100	7	8	5	6	14	16
Isoproturon	100	15	21	6	12	7	12
Isoxaben	100	12	17	7	9	7	7
Lactofen	100	12	17	7	20	12	15
Malathion	100	7	19	8	17	5	17
Metalaxyl	100	12	19	6	11	8	8
Methomyl	100	12	18	7	14	10	20
Metribuzin	100	8	16	7	8	8	9
Myclobutanyl	100	10	14	8	10	8	14
Omethoate	100	18	19	14	16	13	14
Oxadyxil	100	12	18	4	10	6	13
Oxamyl	100	10	19	7	15	9	15
Pethoxamid	100	8	19	8	16	5	10
Profenofos	100	8	19	5	19	11	11
Promecarb	100	10	20	4	5	12	14
Propoxur	100	7	19	6	8	8	9
Pymetrozin	100	11	16	6	10	9	10
Pyperonil-butoxide	100	6	19	6	15	6	15
Pyrimethanyl	100	14	20	6	8	9	11
Quinoxifen	100	9	18	9	10	10	13
Spirodiclofen	100	9	18	8	18	10	13
Tebuconazol	100	8	13	9	10	6	6
Thiacloprid	100	16	17	9	13	9	13
Triadimefon	100	9	19	8	11	7	8
Trifloxistrobin	100	13	18	8	11	10	13

Table 4: Method (intermediate) precision values for all matrices

	Baby Food		Grape		Wheat Flour	
Compound	**LOD [ng/g]**	**LOQ [ng/g]**	**LOD [ng/g]**	**LOQ [ng/g]**	**LOD [ng/g]**	**LOQ [ng/g]**
Abamectin	2.4	7.2	2.0	6.0	3.1	9.3
Ametryn	2.5	7.5	2.5	7.5	1.4	4.2
Azinphos-Me	1.1	3.3	1.1	3.3	1.2	3.6
Azoxystrobin	0.9	2.7	0.9	2.7	0.9	2.7
Bifenazate	2.8	8.4	2.7	8.1	2.9	8.7
Carbaryl	1.5	4.5	1.6	4.8	1.2	3.6
Carbendazim	1.3	3.9	1.4	4.2	2.6	7.8
Carfentrazone-ethyl	1.5	4.5	1.5	4.5	2.1	6.4
Chlormequate	6.0	18.0	10.3	31.0	9.2	27.7
Clofentezin	3.2	9.6	4.1	12.3	4.5	13.5
Cymoxanil	3.3	9.9	3.1	9.3	3.2	9.6
Cypermethrin	3.0	9.0	5.0	15.0	4.5	13.5
Dazomet	1.4	4.3	1.3	4.0	1.2	3.6
Diazinon	1.1	3.3	1.0	3.0	1.3	3.9
Dimethoate	1.2	3.6	1.2	3.6	1.2	3.6
Dimethomorph	1.0	3.0	1.0	3.0	2.0	6.0
Edifenphos	1.2	3.6	1.1	3.3	1.2	3.6
Fenazaquin	2.0	6.0	2.5	7.5	2.2	6.6
Fluazifop P	1.0	3.0	1.2	3.6	1.8	5.4
Fluzilazol	1.0	3.0	1.0	3.0	1.5	4.5
Hexithiazox	3.0	9.1	3.4	10.2	4.0	12.0
Imazalil	1.2	3.6	1.4	4.2	1.5	4.5
Imidacloprid	1.1	3.3	1.2	3.6	1.2	3.6
Isoproturon	1.7	5.1	1.8	5.4	1.3	4.0
Isoxaben	1.0	3.0	1.0	3.0	1.1	3.3
Lactofen	1.4	4.2	1.9	5.7	2.5	7.5
Malathion	3.0	9.0	1.8	5.4	1.6	4.8
Metalaxyl	0.9	2.7	0.9	2.7	2.1	6.3
Methamyl	1.6	4.8	1.4	4.2	1.7	5.1
Metribuzin	1.5	4.5	1.6	4.8	1.9	5.7
Myclobutanyl	2.0	6.0	1.4	4.2	1.5	4.5
Omethoate	3.0	9.0	3.5	10.5	3.6	10.8
Oxadyxil	1.8	5.4	1.7	5.1	2.5	7.5
Oxamyl	2.5	7.5	3.3	9.9	2.9	8.7
Pethoxamid	2.7	8.1	3.5	10.5	2.9	8.7
Profenofos	1.9	5.7	1.9	5.7	2.5	7.5
Promecarb	1.8	5.4	1.7	5.1	1.9	5.7
Propoxur	1.6	4.8	1.5	4.5	1.2	3.6
Pymetrozin	1.1	3.3	1.4	4.2	1.1	3.3
Pyperonil-butoxide	0.8	2.4	0.8	2.4	0.8	2.4
Pyrimethanil	1.9	5.7	2.3	6.9	3.1	9.2
Quinoxifen	1.5	4.5	1.8	5.4	2.0	6.0
Spirodiclofen	2.5	7.5	2.6	7.8	3.2	9.6
Tebuconazol	1.3	3.9	1.8	5.4	2.2	6.6
Thiacloprid	1.0	3.0	1.0	3.0	1.4	4.2
Triadimefon	1.4	4.2	1.5	4.5	2.9	8.7
Trifloxistrobin	1.2	3.6	1.6	4.8	1.6	4.8

Table 5: Limits of detection and limits of quantification (LODs and LOQs) of the method for different matrices

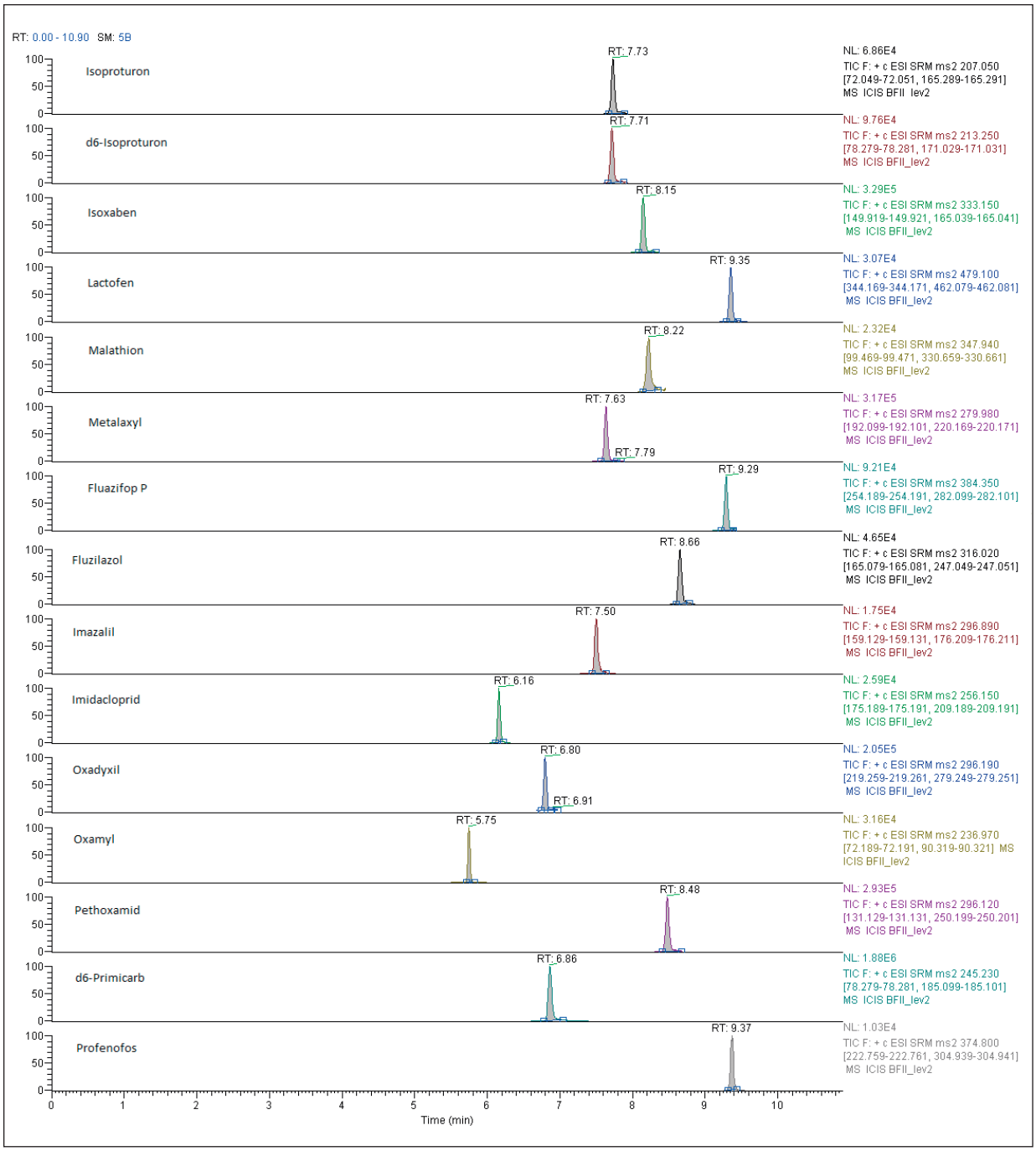


Figure 2: Illustration of selected target substance peaks and internal standards in baby food matrix spiked at legislation limit 10 ng/g

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TG52213_E 02/12M

Application Note: 525

Streamlined Analysis of 400+ Pesticides in a Single Run Using the TSQ Quantum Access MAX Mass Spectrometer and TraceFinder Software

DOWNLOAD

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Key Words

- TSQ Quantum Access MAX
- TraceFinder software
- T-SRM
- Pesticide analysis
- Food safety

Introduction

Growing concerns over food safety and the expanding world agricultural trade have lead to the enforcement of stricter pesticide regulations. In 2006, Japan introduced the Positive List System that established maximum residue levels (MRLs) for hundreds of agricultural chemicals in food, including approximately 400 pesticides, and set a uniform limit of 10 µg/kg (ppb) for chemicals for which MRLs have not been determined.[1] In 2008, the European Parliament implemented Regulation (EC) No. 396/2005, which harmonized all pesticide MRLs for European Union (EU) member states and set default limits of 10 µg/kg for all pesticide/commodity combinations for which no MRLs have been set.[2] A pesticide safety review of about 1,000 active substances on the market was mandated by EU Directive 91/414/EEC and, upon its completion in 2009, led to the approval of only about 250 substances and effectively set the permissible levels of over 700 de-listed pesticides to the default limit.[3] The EU and Japanese regulations are among the most stringent in the world and have fueled the need for faster and more sensitive analytical methods for cost-efficient, high-throughput screening and quantitation of multi-class pesticide residues.

Liquid chromatography-triple quadrupole tandem mass spectrometry (LC/MS/MS) enables highly selective, targeted, and sensitive quantitation and confirmation of hundreds of target pesticides in a single run. A multi-residue method was developed for screening and quantitation of 437 pesticides in one 45-minute run using Thermo Scientific TraceFinder software and a Thermo Scientific TSQ Series LC-MS/MS system. At least one, and often two or three, ion ratios were used to confirm each analyte. In addition, the use of the Quantitation-Enhanced Data-Dependent scan mode (QED-MS/MS) provided MS/MS mass spectra that was used for structural confirmation.

Goal

To analyze large numbers of pesticides in a single run on a triple quadrupole mass spectrometer using TraceFinder™ software with built-in workflows for streamlining method development and routine analysis.

Experimental Conditions

Sample Preparation

Pesticide standards were obtained from the U.S. Food and Drug Administration (FDA). The stock solution was prepared at a concentration of 3 mg/L. Calibration solutions, with concentrations of 0.1-250 µg/L (ppb), were prepared by serial dilution of the stock solution in 50:50 (v/v) acetonitrile/water.

Apple, orange, and asparagus matrices were prepared for analysis by using a modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method, which is a sample preparation procedure used to extract pesticides from food.[4] The QuEChERS extracts were obtained from California Department of Food and Agriculture. For the QuEChERS extraction, 15 g of homogenized sample and 15 mL of acetonitrile were used. Then, 200 µL of final QuEChERS extract, 300 µL of acetonitrile, and 500 µL of water were transferred into an autosampler vial, spiked with 20 µL of the pesticides standard, and mixed well.

HPLC

Chromatographic analysis was performed using the Thermo Scientific Accela 1250 U-HPLC system. The autosampler was an HTC-PAL Autosampler (CTC Analytics, Zwingen, Switzerland). The chromatographic conditions were as follows:

Column:	Thermo Scientific Hypersil GOLD aQ column (100 x 2.1 mm, 1.9 µm particle size)		
Mobile Phase A:	Water with 0.1% formic acid and 4 mM ammonium formate		
Mobile Phase B:	Methanol with 0.1% formic acid and 4 mM ammonium formate		
Flow Rate:	300 µL/min		
Column Temperature:	40 °C		
Sample Injection Volume:	10 µL		
Gradient:	Gradient Time (min)	%A	%B
	0.00	98	2
	0.25	70	30
	35.00	0	100
	40.00	0	100
	40.01	98	2
	45.00	98	2

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MS

All samples were analyzed on the Thermo Scientific TSQ Quantum Access MAX triple stage quadrupole mass spectrometer with a heated electrospray ionization (HESI) source. To maximize the performance of the mass spectrometer, time-specific SRM windows were employed at the retention times of the target compounds. In addition, Quantitation-Enhanced Data-Dependent scanning, which delivers SRM-triggered MS/MS data, was used for structural confirmation. Alternating positive and negative polarity switching was utilized in the method. The MS conditions were as follows:

Sheath Gas Flow Rate:	55 units
Aux Gas Flow Rate:	15 units
Spray Voltage:	3500 V
Capillary Temp:	280 °C
Heater Temp:	295 °C
Cycle Time:	0.2 s

Software

Method development, data acquisition, and data processing were performed with TraceFinder software. TraceFinder software streamlines method development, acquisition, and data review. It provides a comprehensive system incorporating processing methods, library searching capabilities, data review, reporting, and built-in methods for commonly found contaminants. The Compound Data Store (CDS) in TraceFinder software includes selective reaction monitoring (SRM) transitions and collision energies for several hundred pesticides, herbicides, personal care products, and pharmaceutical compounds (Figure 1).

In this experiment, the appropriate SRM transitions of the pesticides were chosen from the CDS and inserted into the method for detection. No compound optimization was necessary for compounds that were included in the Compound Data Store.

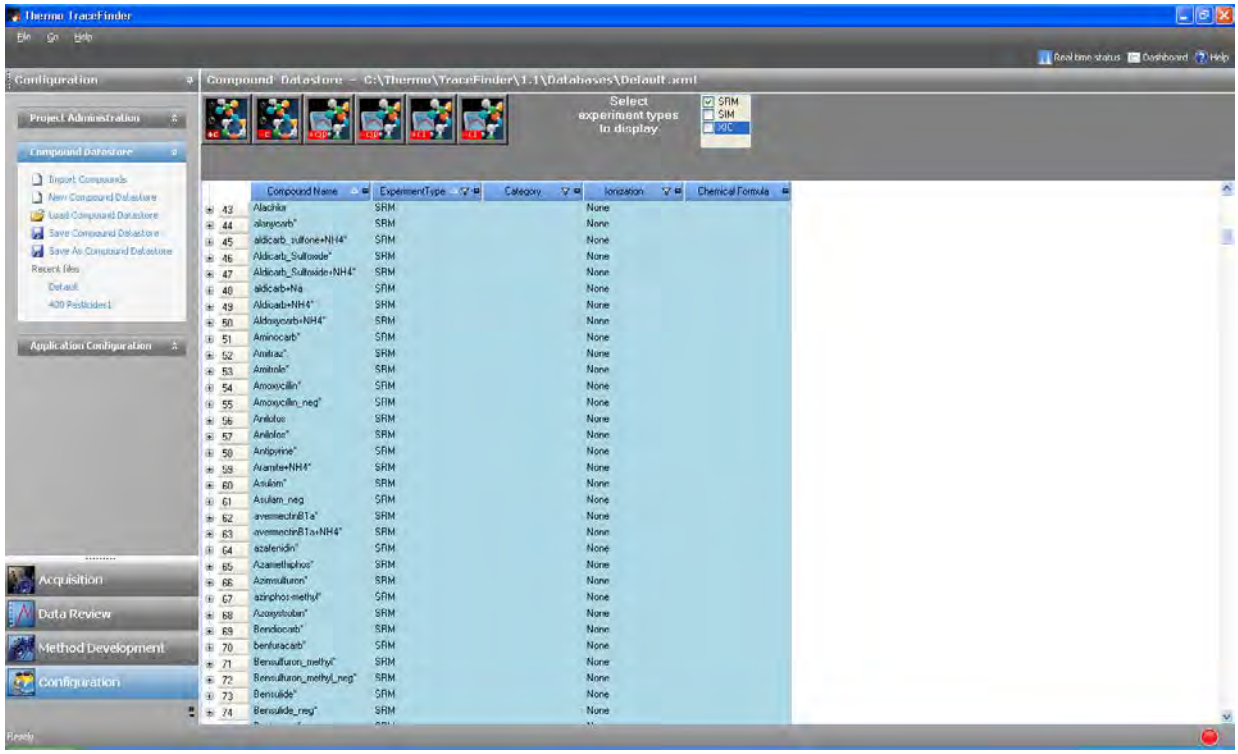


Figure 1. TraceFinder software Compound Data Store (CDS)

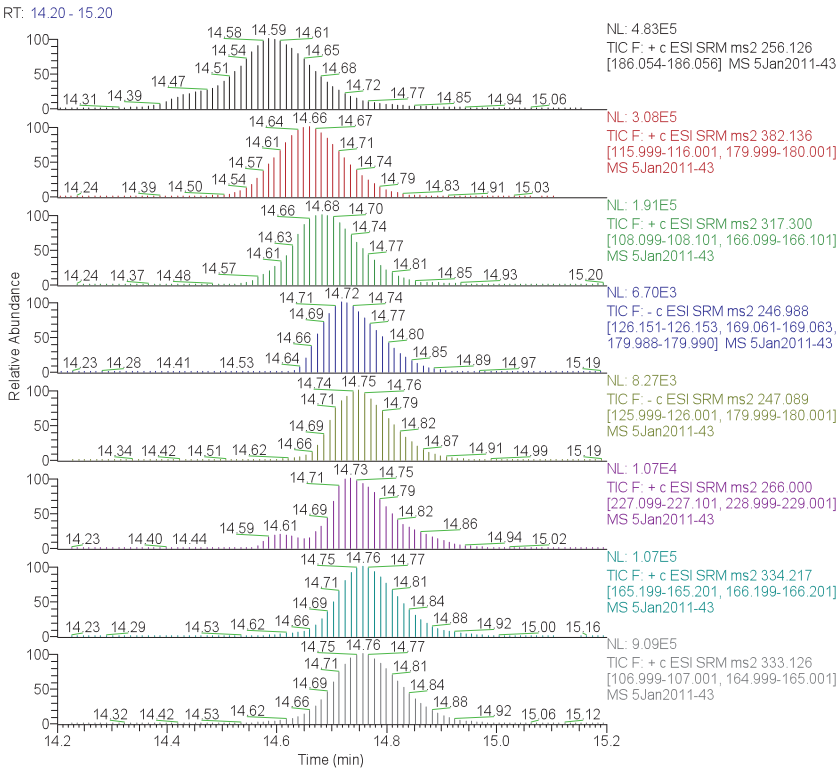


Figure 2. Eight extracted ion chromatograms showing number of scans with positive and negative switching

Results and Discussion

Multi-residue screening studies can generate very large SRM transition lists in a single experiment. T-SRM can be a useful tool to enhance qualitative and quantitative analyses. In a T-SRM experiment, using prior knowledge of the pesticide retention times, the method is set to look for specific transitions only during the expected retention time window. This increases the number of SRM transitions that can be monitored effectively per experiment. T-SRM also increases the scan time and duty cycle for monitoring individual compounds per experiment, providing more accurate and sensitive quantitation. In this screening, after retention times were determined by standard SRM run, a T-SRM method containing a total of 933 T-SRMs was constructed to analyze the compounds in one single mix. For most compounds, the time window was 60 s. Figure 2 shows that by using T-SRM, enough scans were obtained for closely and overlapping peaks with positive and negative polarity switching. T-SRM enabled the efficient detection of a large list of SRM transitions without compromising the scan time for each SRM.

A mixture of 437 pesticides representing a broad spectrum of chemical classes was separated and detected within 45 minutes (Table 1). For the concentration range studied (0.1-250 µg/L), limits of detection (LOD) were estimated from standard solutions. The LOD ranged from 0.1 to 50 µg/L, depending on the analytes.

Table 1. LC-MS/MS data for 437 pesticide standards

Compound	Precursor Ion	Quantitation Ion	CE	Confirming Ion 1	CE	Confirming Ion 2	CE	RT (min)	Polarity
Acephate	184.08	143.05	10	95.20	25			2.11	+
Acetamiprid	223.10	126.10	22	90.20	36			3.87	+
Acibenzolar-S-methyl	211.09	136.00	32	140.00	24			13.17	+
Acifluorfen	360.00	316.00	10					15.26	-
Acrinathrin+NH_4	559.00	208.00	16	181.00	33	317.00	12	27.78	+
Akton	374.80	304.90	20	97.10	40			22.05	+
Alachlor	270.10	162.00	19					15.86	+
Aldicarb sulfone+NH_4	240.12	86.20	22	148.05	12			2.38	+
Aldicarb sulfoxide	207.00	132.00	10	89.00	16			2.3	+
Aldicarb sulfoxide +NH_4	224.20	89.00	19	131.70	15			2.3	+
Aldicarb+NH_4	208.10	116.10	10	89.20	17			4.97	+
Allethrin	303.16	135.05	13	123.11	18	91.16	33	23.14	+

Table 1. LC-MS/MS data for 437 pesticide standards (continued)

Compound	Precursor Ion	Quantitation Ion	CE	Confirming Ion 1	CE	Confirming Ion 2	CE	RT (min)	Polarity
Allidochlor	174.09	98.23	12	41.44	23	39.44	45	5.24	+
Ametryn	228.20	185.90	19	96.00	26			8.94	+
Amicarbazone	242.18	143.10	12	85.20	32			6.21	+
Aminocarb	209.12	137.10	25	152.10	15			2.2	+
Amitraz	294.08	122.19	33					2.77	+
Ancymidol	257.11	135.05	26	81.21	26	77.20	45	7.44	+
Anilofos	368.00	199.00	16	171.00	23	125.00	34	18.96	+
Aramite+NH_4	352.00	191.00	12	255.00	10			23.2	+
Aspon	378.90	210.90	21	115.10	33			25.28	+
Asulam	231.00	156.00	12	92.00	25			2.29	+
Atrazine	216.00	174.00	16					9.32	+
Avermectin B1a +NH_4	890.45	305.28	22	307.00	29	567.41	11	27.65	+
Avermectin B1a+Na	895.39	751.50	45	183.08	50			27.65	+
Avermectin B1b +NH_4	876.45	291.00	30	553.40	15	145.00	35	26.77	+
Azaconazole	300.00	158.93	27	230.92	17	122.99	51	11.07	+
Azafenidrin	338.11	264.03	30	302.10	17	298.98	20	10.94	+
Azamethiphos	324.98	182.91	17	112.04	36	138.96	23	6.41	+
Azinphos-ethyl	345.96	132.10	16	160.10	7			16.14	+
Azinphos-methyl	317.93	260.98	8	125.03	19			11.9	+
Azoxystrobin	404.12	372.14	14	329.11	32			13.86	+
Benalaxyl	326.18	148.00	22	208.00	15			18.7	+
Bendiocarb	224.16	167.06	10	109.10	20			6.94	+
Benodanil	324.01	241.98	25	261.96	18	132.03	19	14.84	+
Benoxacor	260.03	148.69	17	133.98	13			11.31	+
Bensulide	398.00	314.00	12	158.00	25	218.00	18	18.34	+
Bentazone	239.07	132.00	28	197.00	22			6.51	-
Benthiavalicarb	382.14	180.00	33	116.00	23			14.65	+
Benzoximate	364.35	199.20	11	105.20	33			20.06	+
Bifenazate	301.23	170.00	20	152.00	40			16.02	+
Bifenox	342.00	310.00	15					18.99	+
Bifenthrin+NH_4	440.00	181.00	14	166.00	42			29.35	+
Bispyribac-sodium	453.14	296.96	19					13.92	+
Bitertanol	338.08	269.00	10	99.00	16			20.15	+
Boscalid	343.24	307.00	19	271.00	34			14.21	+
Brodifacoum	522.88	335.00	23	178.20	35			28.91	+
Bromadiolone	525.07	249.96	37	263.27	40	218.93	50	23.67	-
Bromoxynil	276.07	81.00	36	79.00	36			8.86	+
Bromuconazole1	377.92	158.92	28	160.88	28	123.02	35	15.16	+
Bromuconazole2	377.92	158.92	28	160.88	28	123.02	35	17.83	+
Bufencarb	222.11	95.20	34	77.20	43			17.19	+
Bupirimate	317.30	166.10	25	108.10	27			14.68	+
Buprofezin	306.21	201.00	12	116.00	18			20.78	+
Butachlor	312.20	238.00	11					22.92	+
Butafenacil+NH_4	492.31	331.00	26	180.00	46			16.26	+
Butocarboxin	208.10	109.20	15	91.40	39			13.82	+
Butoxycarboxin	223.11	106.10	10	86.20	20			2.35	+
Butoxycarboxin+NH_4	240.11	86.20	18	106.10	25			2.36	+
Butralin	296.14	240.03	14	222.03	22	208.00	28	24.95	+
Butylate	218.20	156.00	11					21.14	+
Cadusafos	270.97	158.90	16	97.00	36			20.21	+
Carbaryl	202.08	145.00	12	127.00	30			8.13	+
Carbendazim	192.10	160.06	20	132.10	33			2.75	+

Table 1. LC-MS/MS data for 437 pesticide standards (continued)

Compound	Precursor Ion	Quantitation Ion	CE	Confirming Ion 1	CE	Confirming Ion 2	CE	RT (min)	Polarity
Carbetamide	237.12	192.05	10	118.10	15			5.83	+
Carbofuran	222.14	165.06	14	123.10	25			6.91	+
Carbofuran-3-hydroxy	238.08	220.08	9	181.08	11			3.64	+
Carboxin	235.95	142.97	17	86.98	24			7.6	+
Carfentrazone-ethyl	412.19	384.00	15	366.20	19			17.94	+
Carpropamid	334.00	139.00	22	196.00	14	103.00	38	18.84	+
Chlorantraniliprole	482.13	450.89	21	283.81	19			11.79	+
Chlorbromuron	292.91	203.88	20	181.95	19	124.94	33	13.73	+
Chlordimeform	197.02	117.20	29	89.00	50			3.15	+
Chlorfenvinphos	358.81	155.20	14	99.10	33			19.16	+
Chlorfluazuron	541.90	385.00	25					26.79	+
Chlorfluazuron	539.70	383.00	20					26.79	+
Chloroxuron	291.11	72.20	20	46.20	19			15.97	+
Chlorpropham	214.00	172.00	12	154.00	19			9.96	+
Chlorpyrifos	350.00	198.00	18	97.00	35			23.81	+
Chlortoluron	213.08	140.00	22	168.00	20			9.18	+
Clethodim	360.19	164.00	20	268.00	14			21.38	+
Clofentezine	303.07	138.00	18	102.00	36			20.42	+
Clothianidin	250.12	169.06	14	132.10	18			3.38	+
Coumaphos	363.02	226.90	25					19.38	+
Coumaphos oxon	347.02	290.92	18	210.92	28	318.93	14	12.72	+
Crotoxyphos	332.07	126.99	23	99.04	27			14.36	+
Dumyluron	303.00	185.00	14	125.00	34	119.00	22	15.44	+
Cyanazine	241.10	214.00	17					6.18	+
Cyazofamid	325.22	108.00	15	261.00	10			17.23	+
Dycloate	216.00	154.00	12	134.00	14	83.00	18	19.81	+
Cyclohexamide	299.18	264.16	14	246.12	19	159.16	30	5.5	+
Cycluron	199.11	89.10	16	72.20	24			10.42	+
Cyflufenamid	413.00	295.00	16	241.00	25	203.00	42	20.34	+
Cyfluthrin	434.10	191.00	17					26.68	+
Cyhalothrin+NH_4	467.00	225.00	18	450.00	10			26.79	+
Cymoxanil	199.06	128.10	10	111.10	20			4.07	+
Cyphenothrin	393.08	315.89	23	376.00	10			20.84	+
Cyproconazole	292.13	125.00	32					15.58	+
Cyromazine	167.09	85.17	19	68.23	28	81.21	26	1.97	+
Daimuron	269.00	151.00	14	91.00	45	119.00	25	14.55	+
DEF	315.02	169.00	17	259.09	13			26.36	+
Deltamethrin	506.10	281.00	11					26.9	+
Demeton S-methyl	231.01	89.16	10	61.26	32			7.06	+
Demeton-O	259.00	89.10	11	61.21	29			11.72	+
Demeton-S	259.00	89.25	12	61.20	47			11.72	+
Desmedipham+NH_4	318.16	182.00	15	136.00	28			11.72	+
Desmetyrn	214.11	172.07	18	82.21	30	57.34	33	6.63	+
Di-allate	269.99	86.15	17	109.04	30	143.03	20	20.67	+
Diamidafos (Nellite)	201.10	107.20	28					3.96	+
Diazinon	305.03	169.10	25	153.13	23			18.51	+
Diazinon Oxon	289.00	233.00	20					16.12	+
Dichlorfenthion	314.98	258.82	16					26.36	+
Dichlormid	208.04	81.26	13	98.18	13	41.47	20	6.85	+
Dichlorvos	221.00	109.00	18	145.00	14	127.00	10	6.72	+
Dichlorvos+NH_4	238.00	109.00	24	221.00	18	127.00	24	6.72	+
Diclobutrazol	328.14	159.00	35	70.20	25			16.24	+

Table 1. LC-MS/MS data for 437 pesticide standards (continued)

Compound	Precursor Ion	Quantitation Ion	CE	Confirming Ion 1	CE	Confirming Ion 2	CE	RT (min)	Polarity
Dicrotophos	238.10	193.10	10	112.10	14			3.04	+
Diethofencarb	268.21	226.00	13	180.10	18			12.43	+
Difenacoum	445.13	179.00	30	256.97	21	177.83	59	26.63	+
Difenoconazole	406.17	251.00	25	111.00	55			21.12	+
Difenoxuron	287.09	123.04	22	72.22	26	95.15	30	11.19	+
Dimepiperate	264.12	146.08	11	119.13	17	91.15	35	20.36	+
Dimethametryn	256.13	186.05	22					14.59	+
Dimethenamid	276.00	243.97	14	168.02	23	111.15	33	12.76	+
Dimethoate	230.11	199.10	12	125.10	23			3.68	+
Dimethomorph	388.14	301.00	22	165.00	34			15.25	+
Dimethylvinphos1	331.00	127.04	13	99.06	26			14.47	+
Dimethylvinphos2	331.00	127.04	13	99.06	26			15.55	+
Dimetilan	241.10	72.20	21					4	+
Dimoxystrobin	327.13	205.00	12	116.00	25			17.73	+
Diniconazole	326.17	148.20	27	70.20	35			18.7	+
Dinotefuran	203.02	129.00	10	114.00	15			2.31	+
Dioxacarb	224.08	167.06	10	123.10	18			6.94	+
Dioxathion	473.99	271.09	10	153.04	28			22.8	+
Diphenamid	240.12	134.13	21	167.09	24	165.09	48	11.18	+
Diphenylamine	170.09	114.09	17	100.13	22	69.21	26	7.91	+
Dipropetryn	256.15	214.06	19	144.06	29	172.03	21	14.46	+
Disulfoton	274.94	89.27	5	61.28	34			19.59	+
Ditalimfos	300.10	145.30	22	144.20	21			14.47	+
Dithiopyr	402.10	354.00	20	272.30	32			21.84	+
Diuron	233.11	72.00	20	46.30	35			8.81	+
DNOC	199.14	117.10	28	89.00	53			3.15	+
Dodemorph	282.23	116.16	20	98.22	25	69.29	31	11.66	+
Doramectin	916.40	331.40	35	593.50	25			28.79	+
Edifenphos	310.98	283.00	12	109.11	35			18.62	+
Emamectin	886.70	158.00	33	302.00	20			24.99	+
Emamectin B1b	872.40	158.20	33	302.30	20			24.02	+
Epoxiconazole	330.20	121.00	21	123.00	20			16.84	+
Eprinomectin B1a	936.53	490.22	52	352.13	57			27.15	+
EPTC	190.07	128.20	13	86.20	14			16.67	+
Esprocarb	266.20	91.00	24	71.10	17			22.34	+
Etaconazole	328.19	159.00	32	123.00	58			16.62	+
Ethaboxam	321.00	183.10	24	200.10	28			8.89	+
Ethalfluralin	334.22	166.20	21	165.20	20			14.76	+
Ethidimuron	265.09	208.20	16	114.20	20			3.32	+
Ethiofencarb	226.09	107.00	16					13.16	+
Ethiolate	162.10	132.16	23	147.16	15	117.14	30	22.92	+
Ethion	384.92	142.97	29	97.09	49			23.56	+
Ethion monoxon	368.85	199.20	13	142.90	27			17.7	+
Ethiprole	397.12	351.00	20	255.00	34			14.03	+
Ethirimol	210.20	140.10	23	98.10	28			4.82	+
Ethofumesate	286.96	258.90	11	120.90	20			12.86	+
Ethoprophos	243.07	97.10	30	131.10	40			15.93	+
Ethoxyquin	218.00	174.00	34	160.00	34			8.81	+
Etobenzanid	340.13	179.10	20	121.00	33			19.13	+
Etofenprox	394.15	177.07	14	107.11	38	135.03	28	28.5	+
Etoxazole	360.21	177.10	22					19.06	+
Etrimfos	293.10	265.00	17					17.81	+

Table 1. LC-MS/MS data for 437 pesticide standards (continued)

Compound	Precursor Ion	Quantitation Ion	CE	Confirming Ion 1	CE	Confirming Ion 2	CE	RT (min)	Polarity
Famoxadone+NH_4	392.11	331.22	8	238.03	18			20.08	+
Famphur	325.96	217.03	21	280.98	13			10.36	+
Famphur oxon	327.14	201.00	26	265.00	19	186.01	35	4.91	+
Fenamidone	312.20	236.20	16	264.20	12			13.57	+
Fenamiphos	304.03	217.01	24	234.03	8			17.47	+
Fenamiphos sulfone	336.09	279.87	17	199.98	28			16.95	+
Fenarimol	331.12	268.00	23	81.00	34			16.32	+
Fenazaquin	307.20	57.20	23	160.90	18			20.77	+
Fenbuconazole	337.04	125.14	35	70.41	27			17.8	+
Fenhexamid	302.09	97.00	26	55.00	36			15.84	+
Fenitrothion	277.95	245.95	17	125.10	21			12.76	+
Fenoxycarb	302.17	116.00	13	88.00	20			18.07	+
Fenpiclonil	254.07	172.01	17					7	+
Fenpropathrin	350.20	97.00	34	125.00	16			23.82	+
Fenpropathrin+NH_4	367.20	125.00	18	97.00	34			25.65	+
Fenpropimorph	304.40	147.10	31	130.10	26			13.16	+
Fenpyroximate	422.21	366.00	15	214.00	34			25.9	+
Fensulfothion	309.18	251.00	21	163.00	18			14.17	+
Fenthion	278.95	247.01	13	169.06	20			12.76	+
Fenthion sulfone	328.09	311.04	9	109.12	37			9.11	+
Fenthion sulfoxide	294.90	108.90	32	114.90	27			8.39	+
Fenuron	165.03	72.10	17	46.30	18			3.53	+
Flonicamid	230.12	174.10	18					13.18	+
Florasulam	360.00	129.00	26	192.00	18			4.98	+
Florasulam+NH_4	377.00	129.00	30					4.98	+
Fluazinam	463.19	416.00	20	398.00	17			23.95	-
Flubendiamide	681.00	253.94	29	273.93	19	271.89	19	19.03	+
Flucarbazone	397.13	129.90	21	115.00	48			5.01	+
Fludioxinil	266.00	229.00	17	227.10	10			14.74	+
Fludioxonil	246.99	179.99	34	169.06	32	126.15	34	14.74	-
Flufenacet	364.23	194.00	12	152.00	20			16.23	+
Flufenoxuron	487.16	304.00	20	156.00	16			25.95	-
Flumetsulam	326.00	109.00	53					3.46	+
Flumioxazin	355.06	170.81	24	212.82	17	142.87	29	20.84	+
Fluometuron	233.08	72.10	18	46.30	17			8.81	+
Fluopicolide	383.01	172.94	23	144.95	47	365.01	17	14.44	+
Fluorochloridone	329.11	302.04	12	188.98	20			17.61	+
Fluoxastrobin	459.20	427.10	18	188.00	37			16.67	+
Fluquinconazole	376.17	349.20	21	307.00	20			15.8	+
Flusiazole	316.18	247.10	19	165.00	34			18.02	+
Flutolanil	324.21	242.00	26	262.00	18			14.84	+
Flutriafol	302.16	70.10	19	123.00	33			10.18	+
Fluvalinate	503.00	181.00	34	208.00	12			28.24	+
Fonophos	246.98	109.10	23	137.10	12			18.44	+
Forchlorfenuron	248.14	129.00	18	93.00	26			10.77	+
Formetanate	222.10	165.00	30					10.01	+
Fosthiazate	284.00	228.00	12	104.00	23			8.77	+
Fuberidazole	185.05	157.05	23	156.03	29	130.18	23	3.41	+
Furalaxyl	302.11	242.10	17	95.00	35			13.23	+
Furathiocarb	383.19	195.00	20	252.00	14			22.38	+
Griseofulvin	353.10	215.00	19	285.06	18	165.03	19	10.97	+
Halofenozide	329.10	121.14	22	77.33	37	155.15	29	13.57	+

Table 1. LC-MS/MS data for 437 pesticide standards (continued)

Compound	Precursor Ion	Quantitation Ion	CE	Confirming Ion 1	CE	Confirming Ion 2	CE	RT (min)	Polarity
Haloxyfop-methyl	376.03	315.96	17	287.98	25	91.13	31	20.84	+
Hexaconazole	314.14	70.20	20	159.00	20			19.39	+
Hexaflumuron	458.92	439.00	12	175.00	39			22.79	-
Hexazinone	253.09	171.05	17	85.19	29	71.27	30	7	+
Hexythiazax	353.24	228.20	18	168.10	25			24.03	+
Hydramethylnon	495.27	323.00	35	150.90	55			23.22	+
Imazalil	297.18	159.00	24	201.00	18			10.18	+
Imazamox	306.09	261.10	23	193.10	27			4.05	+
Imazapyr	262.06	216.98	19	201.97	27			9.64	+
Imazaquin	312.00	267.00	22	199.00	30	252.00	27	7.29	+
Imibenconazole	411.00	125.00	36	171.00	21			23.76	+
Imidacloprid	256.12	209.10	18	175.10	20			3.29	+
Inabenifide	339.26	80.20	38	78.90	55			13.09	+
Indanofan	341.00	187.00	14	175.00	17			16.23	+
Indoxacarb	528.30	203.00	40	293.00	15			21.9	+
Ipconazole	334.13	70.20	22	125.00	42			21.54	+
Iprobenfos	289.02	204.96	11	91.23	24			17.82	+
Iprovalicarb	321.16	119.00	20	203.00	10			15.55	+
Isocarbamid	186.08	145.05	22					21.54	+
Isocarbophos	307.12	230.93	17	171.12	22			10.71	+
Isofenfos	346.04	216.94	23	244.99	12			19.79	+
Isofenfos O-analog	330.15	121.10	43					16.84	+
Isoprocarb	194.09	95.00	16	137.00	11			9.5	+
Isopropalin	310.15	225.94	20	222.07	20	210.01	19	26.19	+
Isoprothiolane	291.00	189.00	22	231.00	12			14.5	+
Isoproturon	207.10	72.00	19	165.15	14			10.09	+
Isoxaben	333.13	165.00	20	107.00	61			14.76	+
Isoxaflutole	360.25	220.00	42					19.04	+
Isoxathion	314.00	286.00	10	105.00	18	258.00	12	20.09	+
Isozophos	314.03	162.01	16	97.03	34	120.02	28	15.81	+
Ivermectin B1a +NH_4	892.50	307.00	28	569.00	17			29.92	+
Kresoxim-methyl	314.07	267.14	8	222.13	15			17.77	+
Lactofen+NH_4	479.00	344.00	15	223.00	36			23.62	+
Linuron	249.10	182.00	18	160.00	17			12.87	+
Loxynil	369.86	242.95	28		28			11.26	-
Lufenuron	509.21	326.00	18	175.00	37			24.97	-
Malathion	330.97	126.99	13	99.02	25	124.98	32	14.48	+
Mandipropamid	412.10	327.90	15	355.90	11			15.16	+
Matoxuron	229.02	72.22	25	156.03	24			5.25	+
Mefenacet	299.17	148.00	14	120.10	31			15.4	+
Mefluidide	328.09	311.04	14	135.12	30	121.10	41	7.58	+
Mepanipyrim	224.14	106.00	27	77.00	40			15.48	+
Mephospholan	270.03	139.98	25	196.02	14	167.96	17	6.7	+
Mepronil	270.15	228.00	16	119.00	21			14.37	+
Mesotrione	340.16	227.95	16					4.72	+
Metaflumizone	505.15	302.04	22	285.10	52	117.15	34	24.67	-
Metalaxyl	280.11	220.10	16	192.10	16			10.36	+
Metazachlor	278.02	134.07	24	105.11	41			9.96	+
Metconazole	320.20	70.10	22	124.90	41			19.62	+
Methabenzhiazuron	222.13	165.00	17					6.91	+
Methacrifos	258.05	209.01	12	125.04	25	79.21	32	11.44	+
Methamidophos	142.00	94.00	20	125.00	10			1.95	+

Table 1. LC-MS/MS data for 437 pesticide standards (continued)

Compound	Precursor Ion	Quantitation Ion	CE	Confirming Ion 1	CE	Confirming Ion 2	CE	RT (min)	Polarity
Methidathion	302.90	85.20	23	144.92	5			10.92	+
Methiocarb	226.09	169.00	10					8.28	+
Methomyl	163.05	106.10	10	88.10	10			2.63	+
Methoprotryne	272.20	240.00	10	198.00	15			9.55	+
Metobromuron	259.10	170.00	20	148.00	25			9.34	+
Metolachlor	284.14	252.10	17	148.20	24			16.14	+
Metominostrobin	285.08	193.96	17	166.02	28	139.95	41	11.15	+
Metosulam	418.00	174.88	27	139.96	45	189.68	24	8.21	+
Metrafenone	409.03	209.10	16	227.10	20			20.13	+
Metribuzin	215.09	187.07	17	130.97	17			6.23	+
Mevinphos1	225.09	127.10	15	192.80	8			3.63	+
Mevinphos2	225.09	127.10	15	192.80	8			4.57	+
Mexacarbate	223.15	151.00	26	166.00	16			3.07	+
Milbemycin A3	511.40	493.20	10	475.20	10			26.77	+
Milbemycin A4+NH_4	560.40	525.20	10	507.20	12			27.95	+
Milbemycin A4-H_2O	525.40	507.20	10	489.20	10			27.96	+
Molinate	188.06	126.20	16	83.10	20			13.75	+
Monocrotophos	224.08	127.05	28	193.10	19			2.83	+
Monolinuron	215.08	126.00	17	99.00	36			8.31	+
Moxidectin	640.20	528.50	15	498.50	20			29.19	+
Myclobutanil	289.13	125.00	31	70.20	19			15.58	+
Naled	396.12	324.13	20	308.15	22			16.22	+
Naphthol	145.11	115.10	18	102.12	22			18.1	+
Napropamide	272.14	171.07	20	129.15	16	114.17	22	16.4	+
Naptalam sodium	331.14	105.16	18	139.04	19			13.57	+
Neburon	275.10	57.20	35	88.00	30			17.82	+
Nitenpyram	271.22	225.00	12	237.00	20			2.53	+
Nitralin	346.12	303.98	15	241.87	17	196.00	36	17.44	+
Nitrothal-isopropyl	313.03	148.95	15	91.14	41			15.23	+
Norflurazon	304.07	284.00	25	88.00	39			11.01	+
Novaluron	493.26	158.00	18	141.00	42			23.17	+
Novaluron	491.23	471.00	15	305.00	19			23.18	-
Noviflumuron	527.00	344.00	15	193.00	35			25.7	+
Nuarimol	315.11	251.90	26	81.00	36			13.33	+
Octhilinone	214.14	102.12	16	57.36	17			16.78	+
Ofurace	299.09	254.05	17	236.04	21	160.09	28	7.25	+
Omethoate	214.07	183.00	13	155.00	18			2.23	+
Orbencarb	258.06	125.05	28	100.15	13	89.13	43	19.38	+
Oryzalin	345.00	281.00	19	147.00	30	78.00	38	16.82	-
Oxadiazon	362.06	302.93	18	219.69	25	184.89	35	23.09	+
Oxadixyl	279.00	219.00	15	132.00	25			5.92	+
Oxamyl+NH_4	237.10	72.08	15	90.09	10			2.44	+
Paclobutrazole	294.10	70.00	20	125.00	35			14.26	+
Parathion	292.00	236.00	15	97.00	30			17.68	+
Parathion-methyl	263.94	232.07	18	109.13	20	124.90	25	12.11	+
Penconazole	284.12	159.00	35	70.10	17			18.43	+
Pencycuron	329.00	125.00	30	218.00	16			20.49	+
Pendimethalin	282.09	212.00	11	194.11	18	119.07	25	24.1	+
Penoxsulam	484.06	195.20	29	194.70	36			9.33	+
Permethrin+NH_4	408.00	183.00	22	355.00	10			28.45	+
Phenmediphame	301.17	136.00	22	168.00	10			12.23	+
Phenothrin	368.20	183.00	24	237.04	12	165.03	42	28.24	+

Table 1. LC-MS/MS data for 437 pesticide standards (continued)

Compound	Precursor Ion	Quantitation Ion	CE	Confirming Ion 1	CE	Confirming Ion 2	CE	RT (min)	Polarity
Phenthoate	320.93	247.02	11	79.26	46			18.01	+
Phorate	260.97	75.08	14	142.94	19			19.08	+
Phorate oxon sulfone	276.98	142.92	22	97.00	36	152.97	16	9.2	+
Phorate sulfone	276.05	94.15	35	173.97	21			9.85	+
Phosalone	368.00	182.00	17					20	+
Phosmet	317.91	160.05	15	133.15	39			12.18	+
Phosphamiden	317.08	300.01	10	127.04	25	226.93	19	6.07	+
Phoxim	299.00	129.00	10	77.00	20			19.81	+
Phropham	180.00	138.00	10	120.00	15	92.00	26	9.11	+
Picloram	241.00	195.00	24					2.67	+
Picoxystrobin	368.20	145.00	23	205.10	11			18.1	+
Pinoxaden	401.19	317.00	23	57.10	34			20.09	+
Piperonyl butoxide	356.19	177.00	13	119.00	33			22.74	+
Piperophos	354.09	170.85	22	212.83	16	142.90	32	20.84	+
Pirimicarb	239.09	182.00	16	72.00	21			4.59	+
Pirimiphos ethyl	334.07	198.11	24	182.14	26			21.8	+
Pirimiphos-methyl	306.01	164.12	24	108.18	34			18.17	+
Pretilachlor	312.20	252.00	17					22.53	+
Prochloraz	376.21	308.00	14	266.00	18			18.99	+
Profenophos	372.90	302.80	19	143.86	36	127.97	40	22.05	+
Prohexadione	211.07	167.19	17	123.24	17	111.18	23	4.83	+
Promecarb	208.09	151.00	10	109.00	17			13.82	+
Prometon	226.21	184.00	21	141.90	24			7.65	+
Prometryn	242.21	157.90	24	199.90	20			11.65	+
Propachlor	212.06	169.99	15	94.13	25	77.18	41	9.95	+
Propamocarb	189.05	102.10	19	144.05	14			2.32	+
Propanil	215.99	160.02	21					12.9	+
Propargite	368.18	231.00	11	174.90	18			24.9	+
Propazine	230.00	124.00	17					15.09	+
Propetamphos	282.04	138.08	18	156.00	10			15.22	+
Propiconazole	342.20	159.00	29	69.20	21			18.91	+
Propoxur	210.07	111.10	16	168.06	10			6.62	+
Prothioconazole	341.98	306.00	16	100.00	30			19.09	+
Prothoate	286.04	97.02	35					10.73	+
Pymetrozine	218.00	105.00	25	79.00	30			2.18	+
Pyracarbolid	218.20	124.90	21	96.90	31			7.03	+
Pyraclofos	361.10	257.00	23					20	+
Pyraclostrobin	388.22	194.00	14	163.00	26			20.01	+
Pyraflufen-ethyl	413.10	339.00	19					19.46	+
Pyrasulfotole	361.06	159.08	46	64.35	61	79.25	18	24.99	+
Pyrazophos	374.04	222.10	22	194.04	36			19.73	+
Pyridaben	365.20	309.10	13	147.00	23			26.81	+
Pyridalyl	489.95	109.00	29	163.90	38			30.53	+
Pyridaphenthion	340.94	189.09	23	205.04	22			15.62	+
Pyridate	379.20	207.00	19					28.28	+
Pyrifenox	294.97	93.12	26	92.07	52	67.19	50	12.88	+
Pyrimethanil	200.07	107.00	26	82.00	30			9.74	+
Pyriproxyfen	322.22	96.00	16	185.30	27			23.49	+
Pyroquilon	174.10	132.13	23	117.15	31	130.13	38	6.77	+
Pyrosulam	434.95	195.20	28	194.10	39			7.42	+
Quinalphos	299.05	163.01	23	147.06	24	38.00		17.63	+
Quinoxyfen	307.88	196.80	33	161.90	47			23.92	+

Table 1. LC-MS/MS data for 437 pesticide standards (continued)

Compound	Precursor Ion	Quantitation Ion	CE	Confirming Ion 1	CE	Confirming Ion 2	CE	RT (min)	Polarity
Resmethrin	356.16	171.01	15	143.01	26	128.03	43	27.36	+
Rotenone	395.30	213.20	23	192.10	26			17.69	+
Salflufenacil	518.19	348.94	30	459.00	16			12.36	+
Schradan	287.12	242.02	14	135.08	26	92.15	40	4.25	+
Secbumeton	226.21	169.90	19	99.90	33			7.91	+
Sethoxydim	328.00	178.00	20					7.58	+
Siduron	233.12	137.00	20	94.00	38			12.55	+
Simazine	202.10	132.00	20	104.00	27			6.7	+
Simetryne	214.10	124.00	20	96.00	26			6.56	+
Spinetoram1	748.32	141.92	30	98.03	37			22.65	+
Spinetoram2	760.2	141.88	31					24.11	+
Spinosyn A	732.50	142.00	35	98.00	47			21.19	+
Spinosyn D	746.50	142.00	34	98.00	47			22.6	+
Spirodiclofen	411.00	313.10	15	213.10	25			25.6	+
Spiromefesin	371.30	273.30	15	255.30	25			24.73	+
Spirotetramat	374.20	330.20	17	302.20	19			16.21	+
Spiroxamine	298.22	144.00	21	100.00	35			14.74	+
Sulfentrazone	404.00	387.00	10	307.00	15			7.9	+
Sulfotep-ethyl	323.19	219.00	16	247.10	15			24.39	+
Sulfuramid	525.99	219.02	26	168.94	27	269.07	23	25.97	-
Sulprofos	322.93	218.95	17	246.95	12			24.39	+
Tebuconazole	308.22	70.20	21	125.00	34			18.57	+
Tebufenozide	353.12	133.00	19	297.00	10			17.95	+
Tebufenpyrad	334.21	145.20	28	117.00	36			22.77	+
Tebupirimfos	319.10	210.20	22					14.7	+
Tebuthiuron	229.16	172.06	18	116.10	28			7.29	+
Teflubenzuron	379.16	339.00	13	196.00	22			23.82	-
Tefluthrin	419.03	174.85	27	140.72	47			8.21	+
Temephos	466.95	419.13	20	405.08	14			24.23	+
Tepraloxydim	340.00	220.00	34	248.00	18			8.38	-
Terbufos	288.97	103.10	12	57.50	21			22.23	+
Terbufos sulfone	338.08	171.00	16	115.01	31	97.06	42	12.39	+
Terbumeton	226.22	169.90	20	113.90	25			7.66	+
Terbutryn	242.22	185.90	20	91.00	28			12.03	+
Tetrachlorvinphos-a	365.00	204.00	40	127.00	16			17.79	+
Tetrachlorvinphos-a+NH_4	382.00	127.00	20					17.79	+
Tetrachlorvinphos-b	366.87	127.03	16	205.96	37	240.74	23	17.79	+
Tetrachlorvinphos-b+NH_4	383.88	126.95	19	205.81	49	240.88	24	17.79	+
Tetraconazole	372.19	159.00	39	70.00	24			17.13	+
Tetramethrin	332.10	127.04	28	174.03	19	226.92	18	14.29	+
Thiabendazole	202.04	175.05	28	131.05	35			3.2	+
Thiacloprid	253.13	126.10	22	90.20	37			4.68	+
Thiamethoxam	292.15	211.10	14	132.05	24			2.76	+
Thiazopyr	397.05	377.04	22	335.00	26			18.67	+
Thidiazuron	221.13	102.10	16	94.20	14			7.13	+
Thiobencarb	258.07	125.00	18	100.20	15			19.38	+
Thiofanox+NH_4	236.09	57.20	16	76.10	12			8.52	+
Thiometon+Na	268.88	89.10	25	61.10	36			14.52	+
Thiophanate-methyl	343.21	151.06	24	311.20	12			6.78	+
Tolclofos-methyl	301.00	175.00	22					6.16	+
Tolfenpyrad	384.08	196.95	29	181.69	30			23.59	+
Tralkoxydim	330.00	284.00	13	138.00	22			16.13	+

Table 1. LC-MS/MS data for 437 pesticide standards (continued)

Compound	Precursor Ion	Quantitation Ion	CE	Confirming Ion 1	CE	Confirming Ion 2	CE	RT (min)	Polarity
Tralomethrin+NH_4	682.80	440.60	18	665.80	10	412.60	22	27.59	+
Triadimefon	294.17	197.10	16	225.10	16			14.86	+
Triadimenol	296.10	70.00	15					14.26	+
Triazophos	313.99	162.10	21	119.17	36			15.82	+
Trichlamide	340.00	121.00	22					19.14	+
Trichlorfon	256.90	127.00	19	109.10	19			4.57	+
Tricyclazole	190.07	163.06	24	136.10	30			5.33	+
Tridemorph	298.00	130.00	28	98.00	32			19.42	+
Trifloxystrobin	409.30	186.00	21	206.10	16			21.54	+
Triflumizole	346.16	278.10	12	73.00	18			21.4	+
Triflumuron	359.10	156.20	17	139.00	31			20.24	+
Triforine-a	434.90	390.00	12					12.45	+
Triforine-b	432.90	388.00	12					12.46	+
Triforine-c	436.90	392.00	12					12.45	+
Trinexapac-ethyl	253.11	207.02	11	69.27	20	165.02	17	10.28	+
Triconazole	318.12	70.00	25	125.00	30			16.16	+
Uniconazole	292.13	70.20	25	125.00	32			17.32	+
Vamidothion	288.07	146.05	14	118.10	27			3.6	+
Vernolate	204.15	128.21	11	86.22	13	43.47	19	19.47	+
Warfarin	307.03	160.94	20					26.95	+
Zoxamide	336.22	187.00	23	159.00	38			18.7	+

Excellent linearity in detector response was observed over the calibration range. The correlation coefficients of 319 analytes were greater than 0.99, and those of 52 analytes were greater than 0.98. The total ion chromatogram is shown in Figure 3.

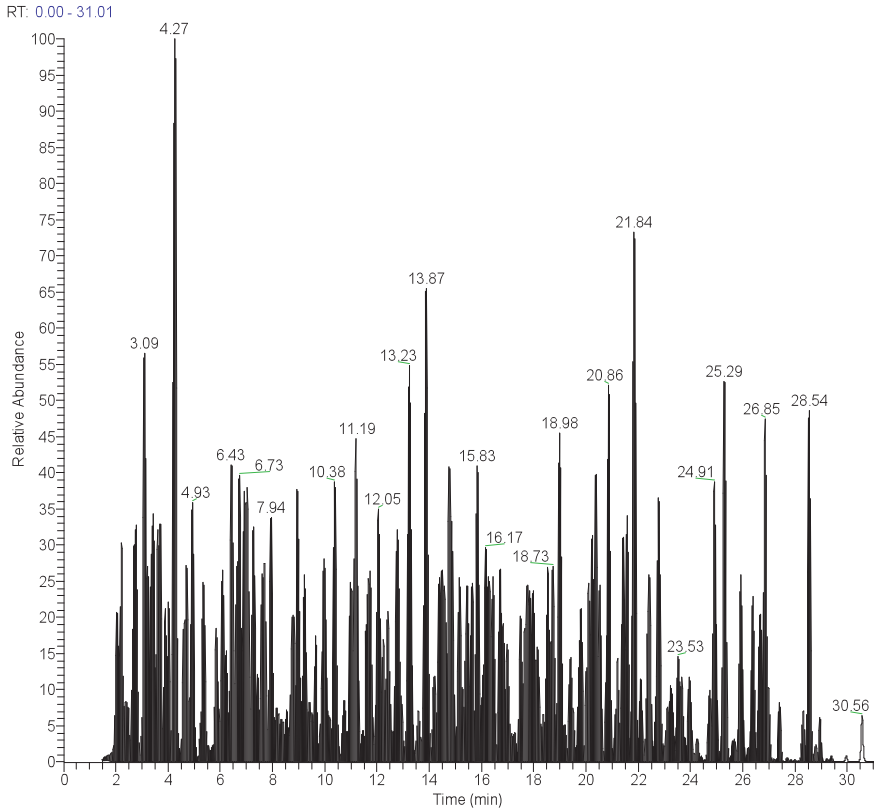
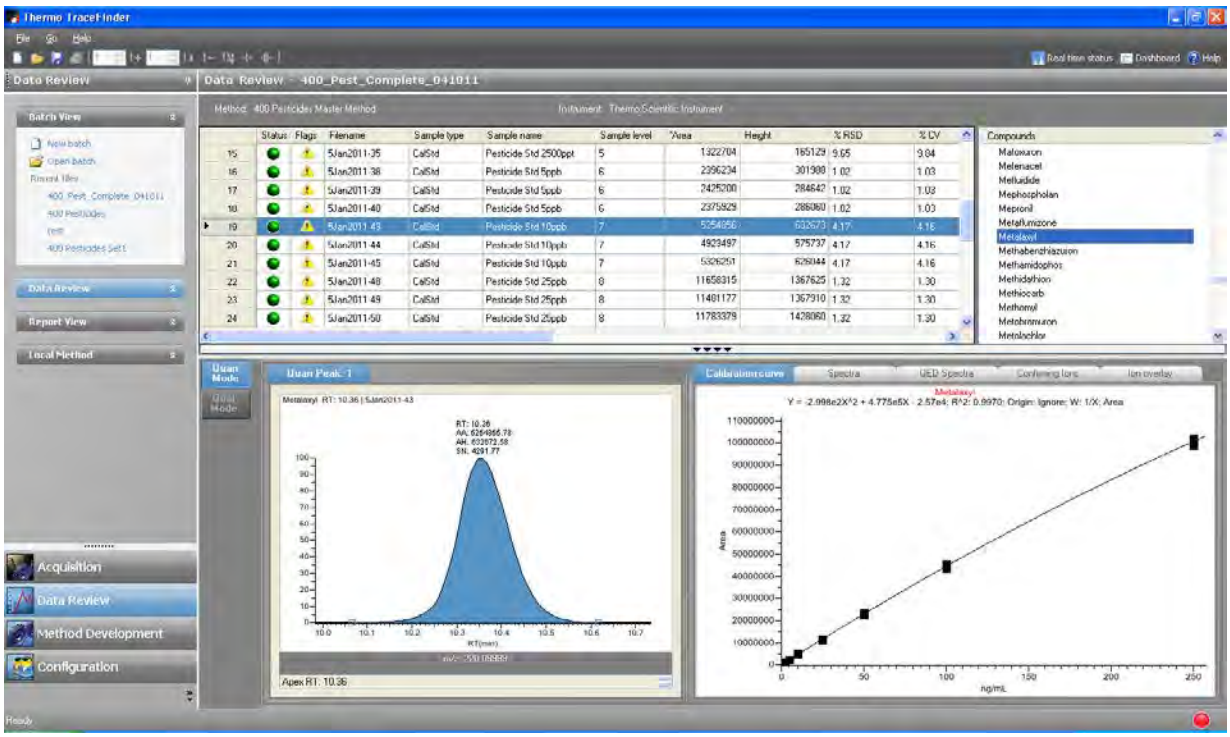


Figure 3. Chromatogram of 437 pesticides (10 µg/L standard solution)

The analysis of the pesticides was reviewed in the Data Review section of TraceFinder software (Figure 4). In this section, calibration curves, ion ratios, peak integration, and MS spectra can be monitored, and samples that meet user-set criteria can be flagged. In addition, user adjustments, such as peak re-integration, are permitted. The effects of the changes on the results are instantly updated in the results grid and standard reports. The extracted ion chromatogram and solvent standard calibration curve for two example pesticides, metalaxyl and pyridaben, are shown in Figure 4. Three replicates of each calibration standard were injected at each level.

(A) Metalaxyl



(B) Pyridaben

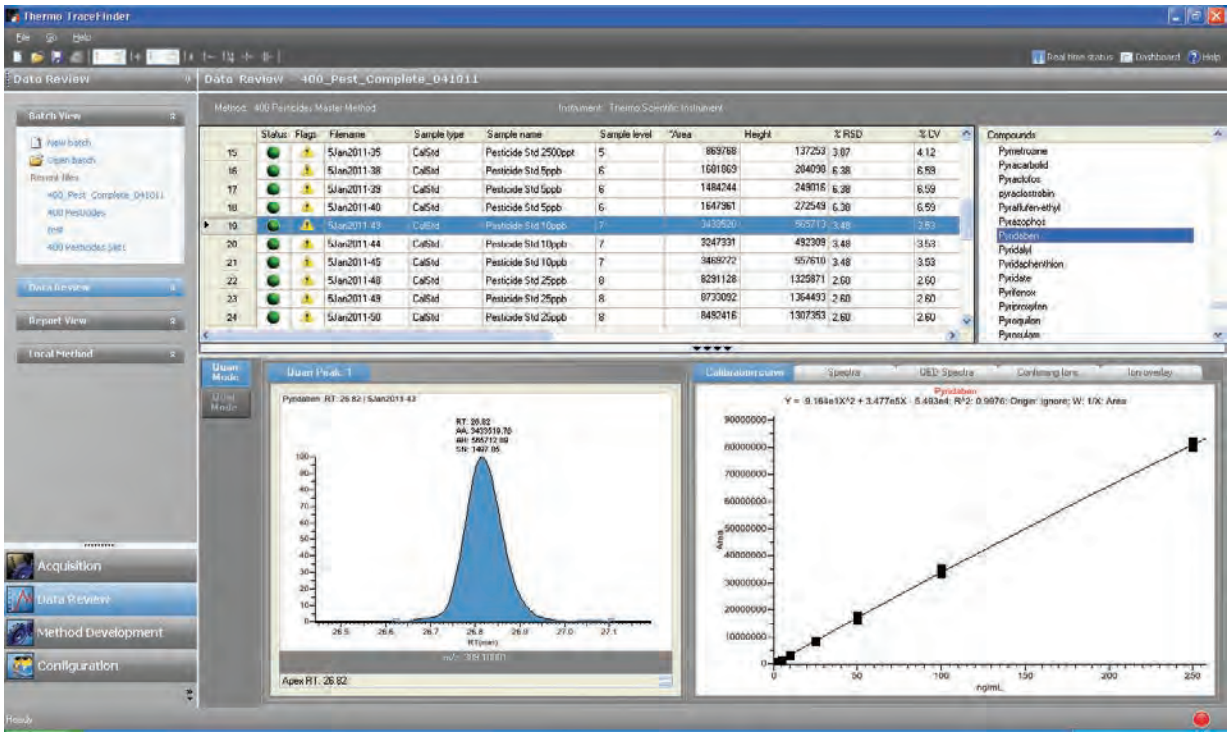


Figure 4. TraceFinder software view of extracted ion chromatogram and solvent standard calibration curve [metalaxyl (A) and pyridaben (B), 10 µg/L, 3 replicates, quadratic curve fit]

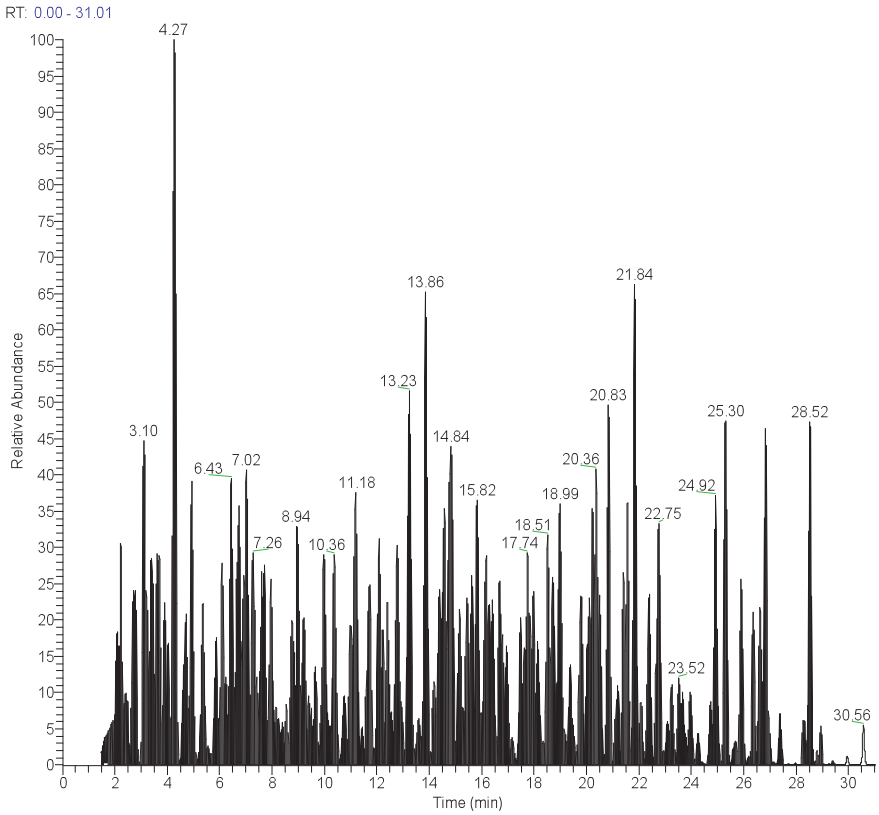
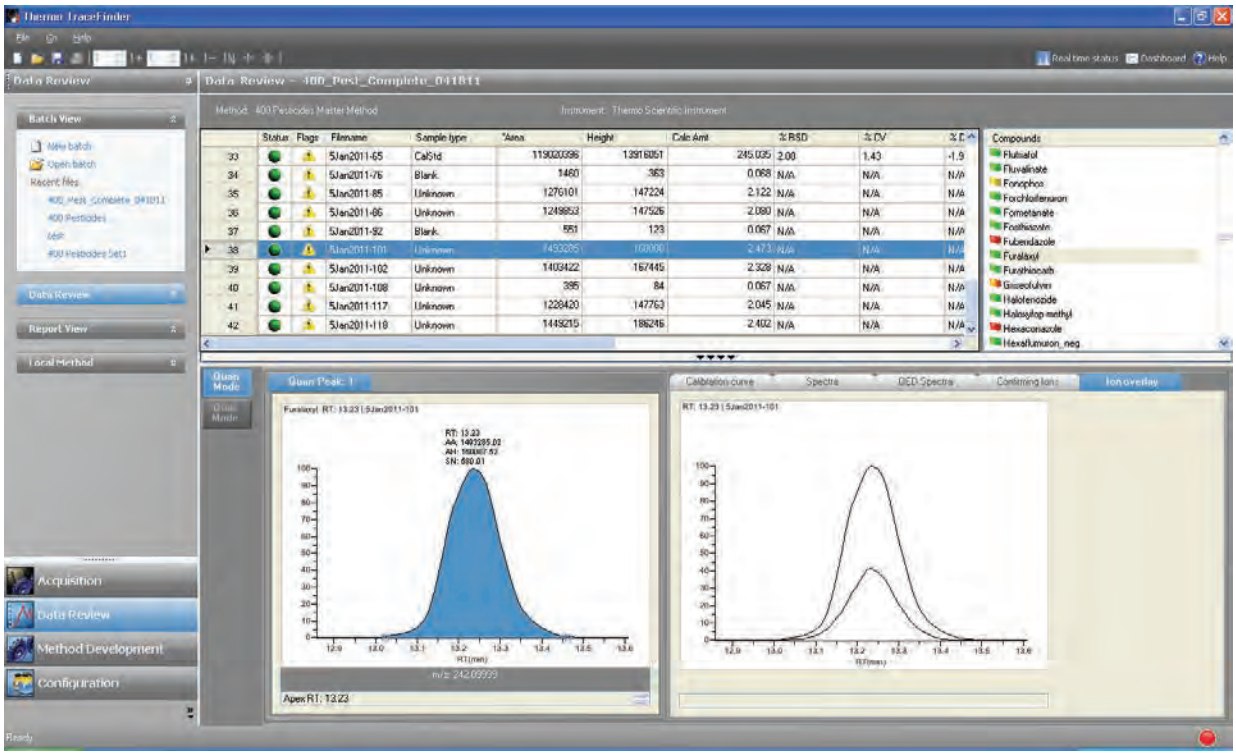


Figure 5. Chromatogram of 437 pesticides in orange extract at 2 µg/kg



Green flag – Compound found, above limit of reporting (LOR), all criteria passed.
Orange flag – Compound close to limit of detection (LOD) or LOR. User may want to double check results.
Yellow flag – Compound not found.
Red flag – Error, such as ion ratio, linearity, carryover, etc.

Figure 6. TraceFinder software view of quantitation peak and ion overlay of confirmation by secondary SRM (furalaxyl in orange extract 2 µg/kg)

To evaluate the applicability of this technique to complex food samples, the pesticide mixture was spiked into apple, orange, and asparagus matrices and analyzed. Figure 5 shows the chromatogram of 437 pesticides at 2 µg/kg in the orange matrix. The majority of the pesticides were detected at 2 µg/kg. The confirmation of target analytes was achieved by the second or third SRM. In Figure 6, the quantification ion and two qualification ions for furalaxyl are displayed in the Data Review section of TraceFinder software. The acceptance criteria percentage can be set for the ion ratio confirmation. If the ion ratio fails, the Confirmation Ion box is flagged in red by the software.

QED-MS/MS experiments were also applied to pesticide analysis in orange, asparagus, and apple extract to confirm the existence of compounds while they were being quantified. A full-scan MS/MS mass spectrum was obtained by data dependent scanning for confirmatory analysis during the SRM experiment. After a particular SRM transition reached the specified intensity threshold, the instrument automatically triggered the QED-MS/MS scan using the Reverse Energy Ramp (RER) scan function. The collision energy was linearly ramped from a high to a low value while Q3 was scanned from low *m/z* to high *m/z*. A highly sensitive, fragment-rich spectrum that was used to positively confirm the existence of a compound was collected. An example of a QED-MS/MS full scan spectrum is shown in Figure 7 for the compound fenamiphos. This QED-MS/MS scan function fragmented the precursor ion *m/z* 304 for fenamiphos over a reversed energy ramp of 10 to 50 eV.

TraceFinder software includes a large number of report templates. Reports can be created in PDF format, printed directly to the printer, or saved in XML format, which is useful for LIMS systems. Figure 8 shows the onscreen preview function of a report generated by TraceFinder software. The chromatogram shown is an apple sample spiked with 437 pesticides at 2 µg/kg. The top of the page contains a sample summary, and the quantitated results follow beneath the chromatogram. TraceFinder software can generate results for the entire batch with the click of a button, or the user can choose to view reports individually and print only those of interest.

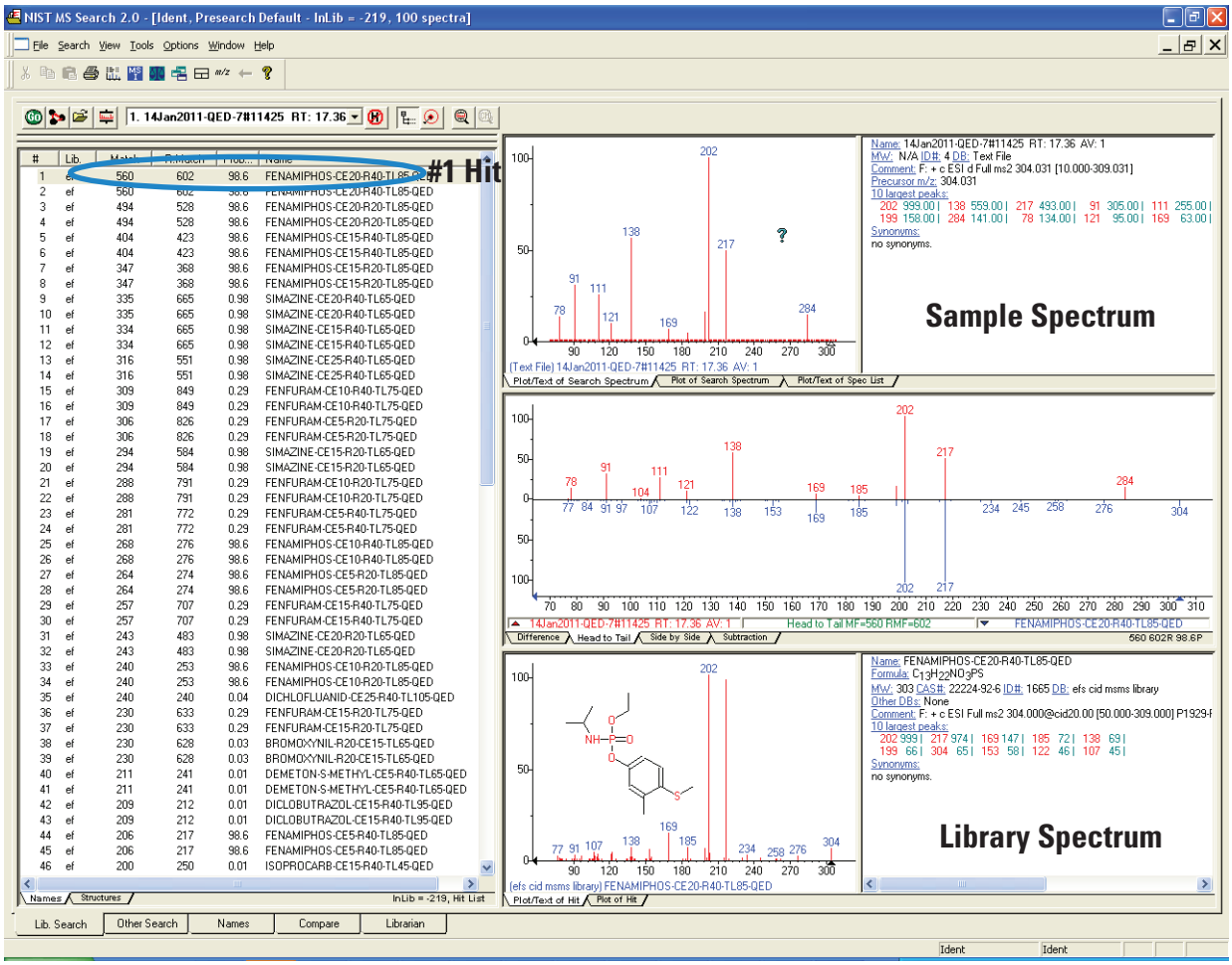


Figure 7. QED spectrum of fenamiphos at 2 µg/kg in asparagus. Searching against the standard library available on the TSQ Quantum Access MAX™ instrument platform yields a positive confirmation.

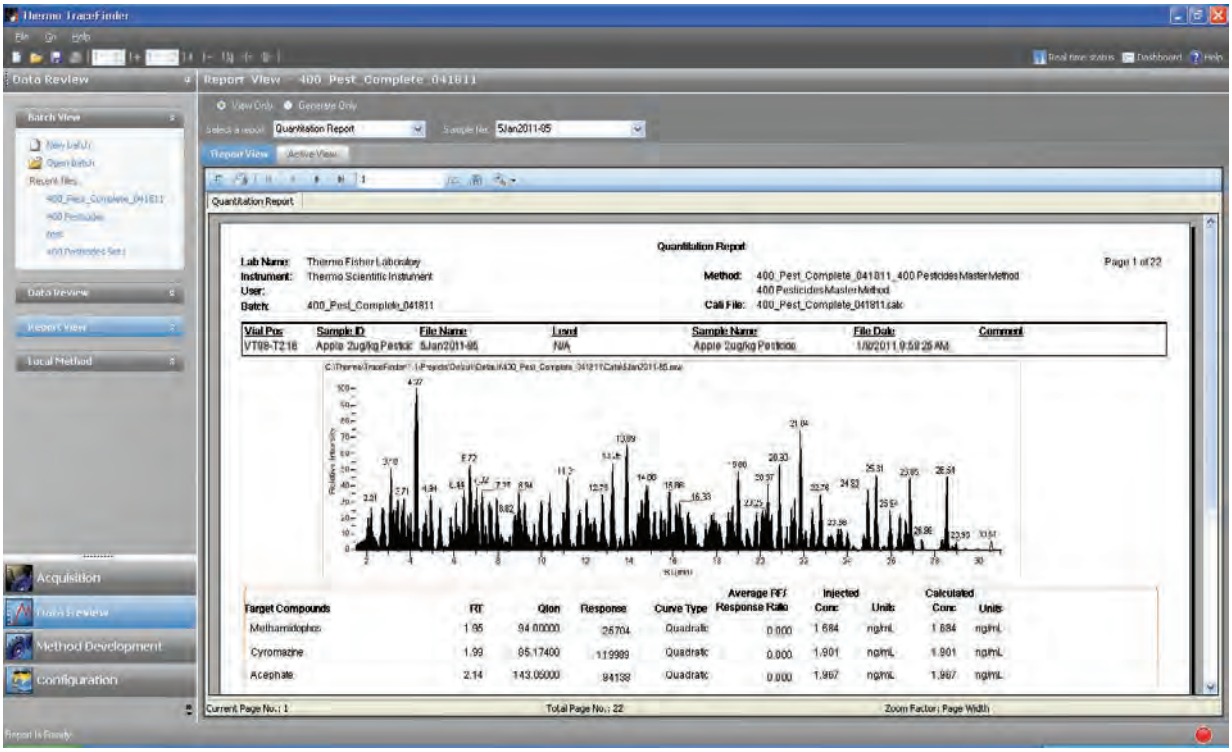


Figure 8. Report View section of TraceFinder software showing quantitative results

Conclusion

A multi-residue method was developed for the screening and determination of 437 pesticides in 45 minutes in a single run on a triple quadrupole mass spectrometer. Data analysis was streamlined by using TraceFinder software, which is ideally suited for quantitation of large amounts of data. For this large-scale multi-pesticide residue study, a timed SRM experiment provided accurate and sensitive analysis, without compromising the dwell time (and duty cycle) for detecting each compound per experiment. Quantitation-Enhanced Data-Dependent scanning provided confirmatory data following quantitative analysis. The majority of the pesticides were detected in the spiked matrices at concentrations lower than the MRLs established by EU and Japan.

Acknowledgment

The authors wish to thank Dr. Jon Wong at the FDA for providing the pesticide standards used in this study. The authors are also grateful to Roland Carlson at CDFA for providing the QuEChERS extracts for this study.

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AN63420_E 05/11S

Application Note: 514

Screening Method for 30 Pesticides in Green Tea Extract Using Automated Online Sample Preparation with LC-MS/MS

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Key Words

- Transcend TLX-1 System
- TurboFlow Technology
- TSQ Access MAX
- Food Safety

Introduction

Analysis of pesticide residues has been one of the most important tasks of food safety laboratories. Mass spectrometers (MS), with liquid chromatography coupled to triple stage quadrupole mass spectrometers (LC-MSMS), have been the main tools used in pesticide residue analysis. There is a consensus that sample preparation is becoming the bottleneck to the entire workflow. Traditional sample preparation methods, usually involving liquid-liquid extraction (LLE) or solid phase extraction (SPE), can be time-consuming and labor-intensive. In addition, low recovery, matrix interference and poor reproducibility are among other major concerns. In recent years, a rapid processing method, QuEChERs, has gained popularity. The QuEChERs method makes it easier and less expensive for analytical chemists to examine pesticide residues in various food matrices[1]. However, some reports show matrix interference tends to be severe after QuEChERs, and the mass spectrometer is more vulnerable to contamination by highly complex food matrices[2].

In this study, we describe an easy, comprehensive, online screening LC method using a Thermo Scientific Transcend TLX-1 system powered by Thermo Scientific TurboFlow technology to analyze multiple pesticide residues in green tea extract. Figure 1 illustrates a typical Transcend™ TLX-1 system with the Thermo Scientific TSQ Access MAX triple stage quadrupole mass spectrometer.

Goal

Develop a rapid and sensitive automated online sample preparation LC-MS/MS method to screen for multiple pesticides in green tea extract.

Experimental

The matrix standard curve

One gram of Chinese green tea was extracted using 10 mL HPLC grade acetonitrile followed by 15 minutes of ultra-sonication. The extract was then filtered through a 0.45 µm membrane filter. The resultant solution was used to prepare the matrix calibrators and QC samples. The matrix calibrant concentrations are 6.25 µg/L, 12.5 µg/L, 25 µg/L, 50 µg/L and 100 µg/L, respectively. The matrix QC sample concentration is 10 µg/L.

TurboFlow™ Method Parameters

System:	Transcend TLX-1 system controlled by Thermo Scientific Aria OS 1.6.3 software
Column:	TurboFlow Cyclone 0.5 x 50 mm
Injection Volume:	10 µL
Loading Solvent:	0.1% formic acid in water
Loading Flow Rate:	1.5 mL/min
Eluting Solvent:	0.1% formic acid in methanol

Figure 1. Typical layout of a Transcend TLX-1 system with a TSQ Access MAX™ triple stage quadrupole mass spectrometer.

Thermo SCIENTIFIC

HPLC Method Parameters

Analytical Column:	Thermo Scientific Hypersil GOLD 2.1 x 100 mm, 3 µm
Solvent A:	0.1% formic acid in water
Solvent B:	0.1% formic acid in methanol

Mass Spectrometer Parameters

MS:	TSQ Quantum Access MAX
MS Ionization Source:	Heated Electrospray Ionization (H-ESI)
Ion Polarity:	Positive ion mode
Spray Voltage:	2 KV
Sheath Gas Pressure (N_2):	30 arbitrary units
Auxiliary Gas Pressure (N_2):	15 arbitrary units
Vaporizer Temperature:	300 °C
Capillary Temperature:	300 °C
Collision Gas Pressure:	1.5 mTorr

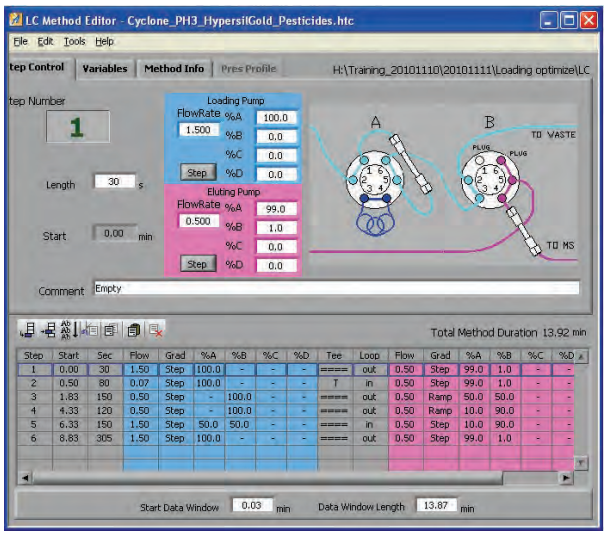

Figure 2. TurboFlow method schematic as viewed in the Aria OS software.

Results and Discussion

The Extraction and Separation of 30 Pesticide Residues

In 2006, Japan released the most stringent pesticide-related regulation in history entitled "Positive List System for Agricultural Chemical Residues in Foods"[3]. Since Japan is China's major tea importer, the limits discussed in the current study follow this regulation. As described in the Experimental section, the tea matrix standard samples are 6.25 µg/L, 12.5 µg/L, 25 µg/L, 50 µg/L and 100 µg/L, respectively. The matrix QC samples are 10 µg/L. Figure 3 shows the representative chromatograms at 6.25 µg/L, which has been determined as the lower limit of quantitation (LLOQ). The data demonstrate that 30 pesticides were well separated with good peak shape. The peaks' signal to noise ratios are far greater than the required 10:1 at the LLOQ. Table 1 shows the linear curve for these 30 analytes. All R^2 values are between 0.993-0.999. The relative standard deviation (RSD) for 6 consecutive injections of 6.25 µg/L calibrator was in the range of 2.85% -7.48%.

Background Reduction Effects using TurboFlow Technology

By using the Transcend TLX system with TurboFlow technology, the background noise and interference peaks are reduced significantly. Figure 4 compares chromatograms of Clomazone at 6.25 µg/L in tea extract using standard HPLC (top) and the TurboFlow method (bottom). The left panel (A-1 and B-1) shows the primary transition of *m/z* 240 > 125. The right panel (A-2 and B-2) shows the secondary transition of *m/z* 240 > 89. It clearly shows the effectiveness of background reduction using TurboFlow technology while the signal to noise ratio increased by 3 and 4 times for *m/z* 125 and 89 transitions, respectively. The area responses of both peaks also increase by more than 50% due to the minimization of ion suppression incurred by matrix. We also noticed the mass spectrometry response become more stable across the entire tested concentration range, thus improving the method reliability.

A Simple Method Optimization Process

During TurboFlow method development, the sample loading condition, elution solvents and many other parameters may need to be optimized. Aria™ OS 1.6.3 operation software for Transcend systems offers a method variable function. By utilizing this unique tool, different parameters can be easily tried using the same method in a single batch. For example, in this study, one of the critical steps was to find the optimal solvent content in the transfer loop to elute the target analytes completely from the TurboFlow column without introducing unnecessarily high organic solvent into the analytical column. We compared 5 different concentration ratios of 0.1% formic acid in acetonitrile to 0.1% formic acid in water (10:90, 30:70, 50:50, 70:30 and 90:10). The results indicated that with the increase of organic content, the target compounds were more completely washed off from TurboFlow column. However, once the organic concentration reached 50%, the elution strength was approaching a balance. Therefore, we chose 50:50 as the optimal elution ratio of organic to aqueous solvent in the transfer loop. Another example of method optimization appears in Figure 5, showing the effects of the loading flow rate on Dimethametryn's elution peak shape. All these tests were done in just one sample batch without writing multiple methods, which simplified the method development process and improved method reliability.

The Comparison of TurboFlow Technology with Two of the Most Popular Pesticides Sample Preparation Methods

As shown in Figure 6, we compared a TurboFlow method and two currently popular methods for pesticide residue sample preparation, SPE and QuEChERs. A typical SPE method involves equilibrating the cartridge, loading, washing and eluting analytes. It usually takes about 1 week to process 100 samples. Although QuEChERs was designed to simplify sample preparation, it still requires two-step centrifugation and concentration. A few days are typically required to prepare 100 samples with QuEChERs. TurboFlow technology minimizes preparation of 100 samples to less than 3 hours, dramatically improving the efficiency and throughput of this routine lab test.

Table 1: Standard curve linearity and QC results for the 30 pesticides in tea extract.

Compound	RT (min)	Parent ion *(m/z)*	Product ion *(m/z)*	Collision Energy (V)	Linear Curve	R^2	CV% (n = 6) QC = 10 µg/L
Prometon	4.82	226.0	184.0 142.1	20 27	Y=167343+396533X	0.999	2.99%
Ametryn	5.07	228.0	186.0 96.0	26 34	Y=83264.1+194461X	0.999	2.85%
Dimethametryn	5.68	256.1	186.1 158.1	21 27	Y=166875+605055X	0.999	3.29%
Mefenoxam	5.79	280.0	220.0 192.0	17 20	Y=460109+272420X	0.998	4.23%
Monolinuron	5.85	215.0	126.0 99.0	17 36	Y=-10985.6+18335.3X	0.998	6.51%
Isoprocarb	5.94	194.0	95.0 137.0	16 11	Y=-18662+12428.2X	0.999	6.43%
Dimethachlor	6.01	256.0	224.0 148.0	15 28	Y=-23531.9+96341.3X	0.997	5.53%
Clomazone	6.05	240.0	125.0 89.0	20 37	Y=-43447.5+42181.6X	0.998	6.37%
Furalaxyl	6.21	302.0	242.0 270.0	15 10	Y=358101+267257X	0.998	4.85%
Azoxystrobin	6.33	404.0	372.0 329.0	15 33	Y=538988+377945X	0.997	4.00%
Triadimefon	6.39	294.0	197.0 225.0	19 19	Y=-20167.4+16685.8X	0.997	7.31%
Ethoprophos	6.41	243.0	131.0 97.0	21 33	Y=-13814+14313.8X	0.997	7.48%
Iprobenfos	6.52	289.0	205.0 91.0	12 23	Y=53008.6+137376X	0.999	6.28%
Isoprothiolane	6.57	291.0	189.0 231.0	22 12	Y=123106+87284X	0.998	6.00%
Flutolanil	6.60	324.0	242.0 262.0	26 18	Y=6077+47866X	0.998	6.56%
Propiconazole	6.65	342.0	159.0 69.0	30 31	Y=-17113.2+36428.7X	0.997	6.74%
Benalaxyl	6.78	326.0	148.0 208.0	25 20	Y=172291+126493X	0.997	5.92%
Pirimiphos-methyl	6.81	306.0	164.0 108.0	22 33	Y=227752+204491X	0.994	4.73%
Picoxystrobin	6.82	368.0	145.1 205.0	22 7	Y=320093+78661.3X	0.993	4.03%
Diazinon	6.90	305.0	169.0 153.0	24 26	Y=182248+386247X	0.998	4.96%
Thiazopyr	6.95	397.0	335.0 275.0	30 40	Y=-5052.12+18434.8X	0.997	6.47%
Piperophos	7.09	354.0	171.0 143.0	25 33	Y=142671+143459X	0.996	4.68%
Trifloxystrobin	7.13	409.0	186.0 206.0	21 16	Y=-18755.2+43150.6X	0.998	6.22%
Tebufenpyrad	7.16	334.0	145.0 117.0	28 36	Y=-3267.09+9390.51X	0.998	7.01%
Piperonyl butoxide	7.25	356.0	177.0 119.0	13 33	Y=-300922+175066X	0.996	4.16%
Pyriproxyfen	7.34	322.0	96.0 185.2	16 27	Y=-19160.9+56881.6X	0.999	4.73%
Tralkoxydim	7.39	330.0	284.0 138.0	15 20	Y=-8119.47+46536.4X	0.997	5.01%
Fenazaquin	7.55	307.0	161.0 57.0	18 23	Y=-56587.3+97365.3X	0.998	2.76%
Butralin	7.58	296.0	240.0 222.0	15 20	Y=-2485.92+25777.9X	0.998	4.72%
DEF	7.85	315.0	169.0 113.0	15 25	Y=-8658.91+7992.12X	0.998	3.89%

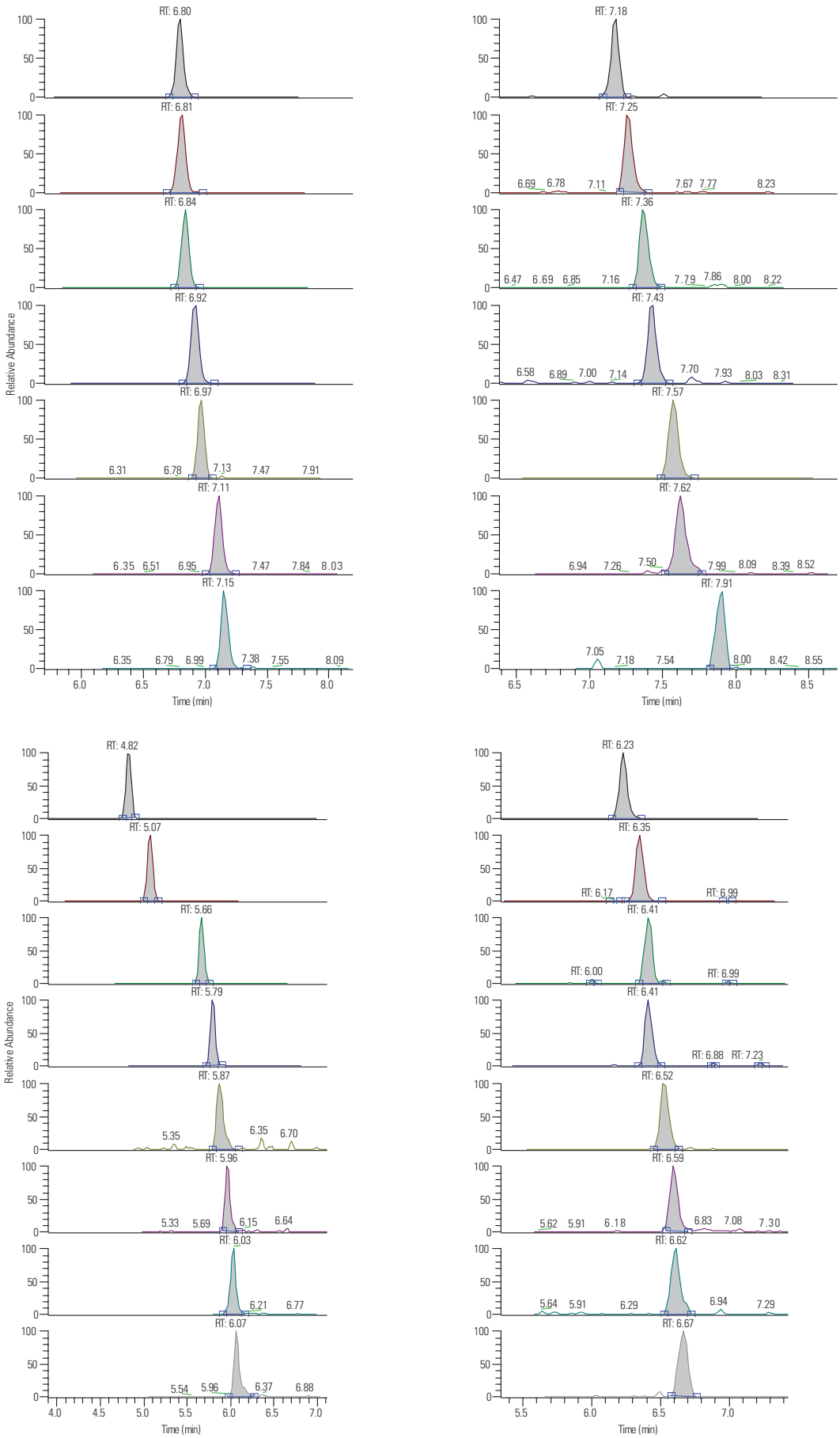

Figure 3. Selected ion chromatograms at LLOQ of 6.25 µg/L for all 30 analytes (same as the order in Table 1).

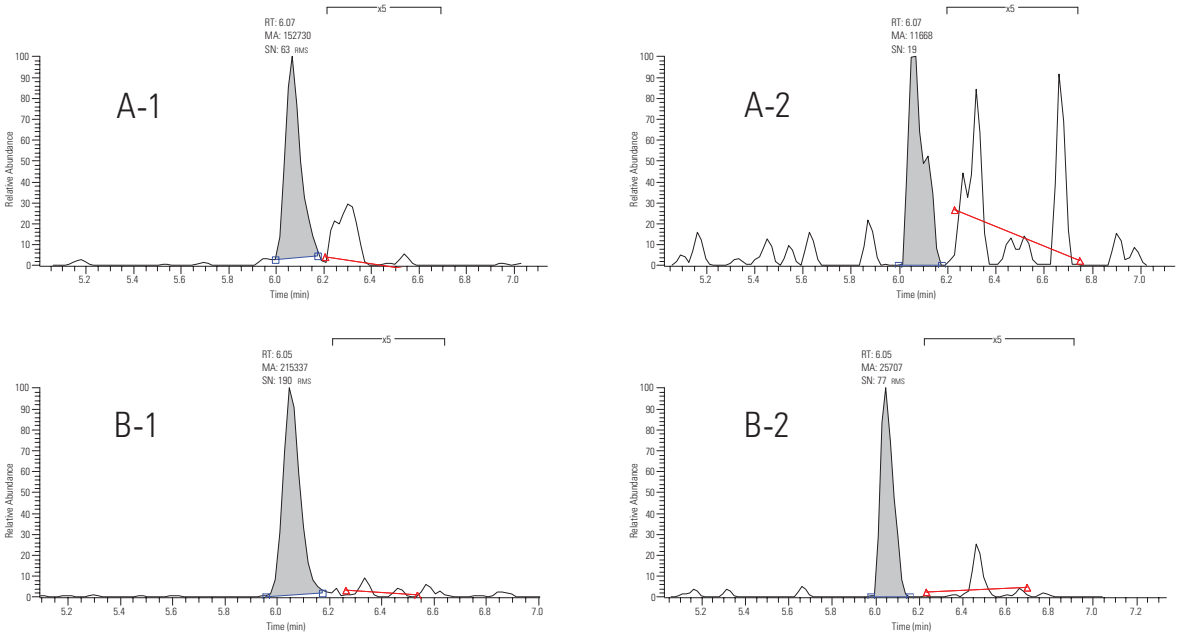

Figure 4: Comparison of chromatograms of Clomazone at 6.25 µg/L in tea extract using standard HPLC (top) and the TurboFlow method (bottom).

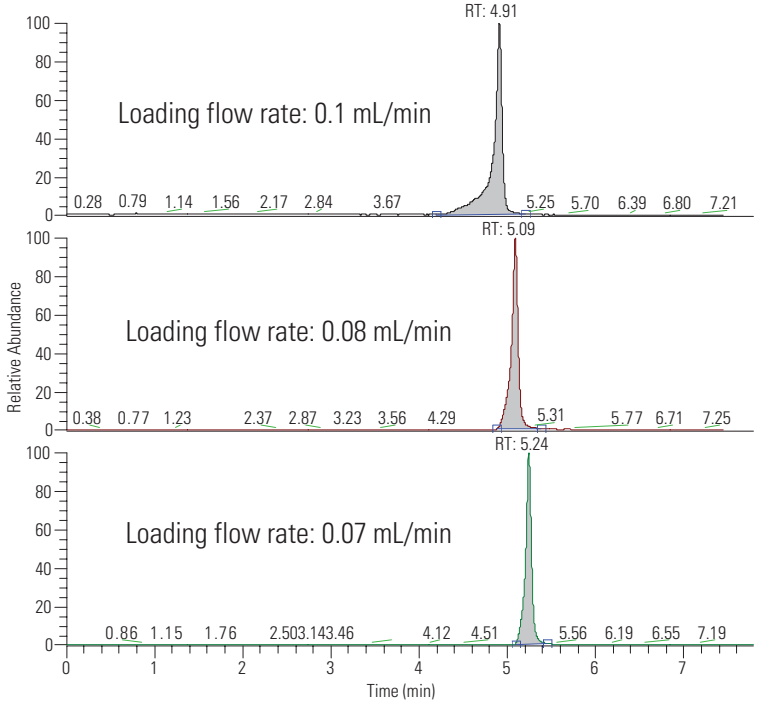

Figure 5. Effect of the loading flow rate on Dimethametryn's elution peak shape.

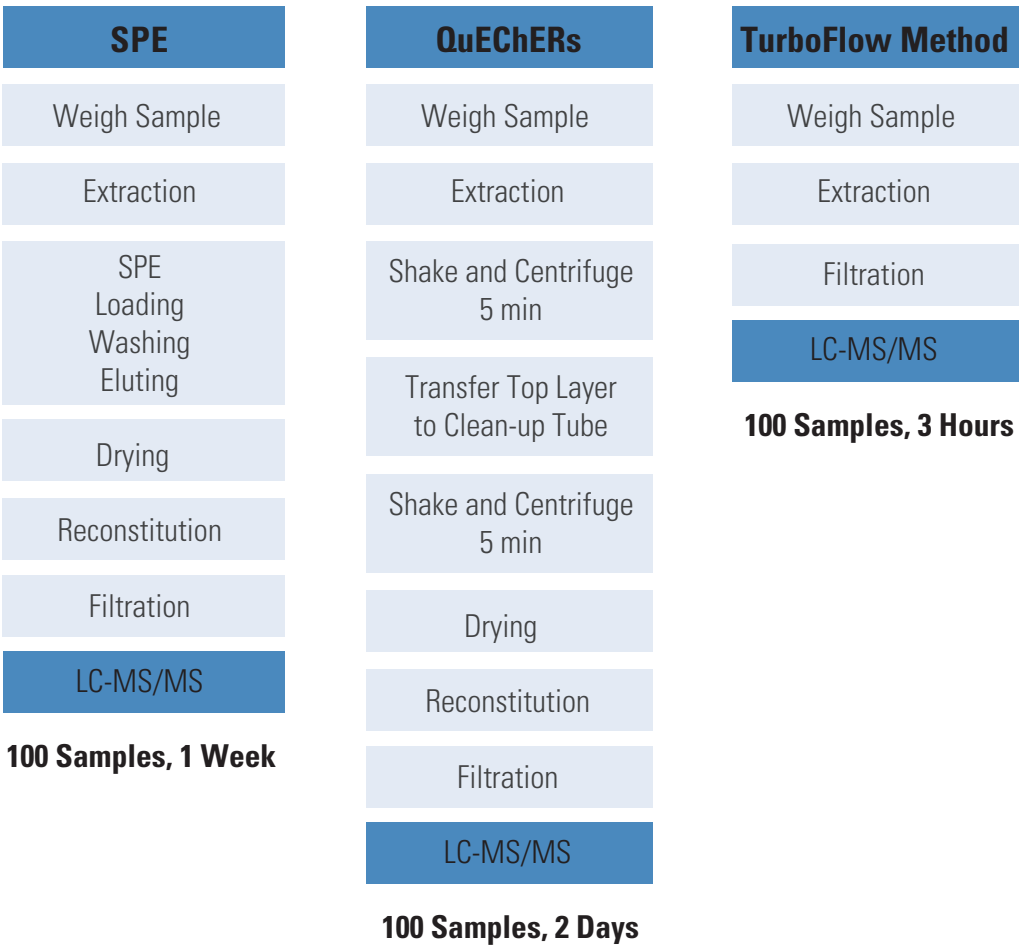

Figure 6. Comparison of the TurboFlow method to SPE and QuEChERs.

Conclusion

A quick, automated online sample preparation LC-MS/MS method has been developed that is sensitive enough to screen the tested pesticides in tea extracts. The method detection and quantitation limits are significantly lower than the strictest limits set by the Japanese government. TurboFlow technology eliminates the need for time-consuming sample preparation procedures such as SPE and QuEChERs. By using Aria OS software, the method development and optimization process is greatly simplified.

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AN63386_E 01/11S

Method: 52292

Determination of Carbendazim and Benomyl Residues in Oranges and Orange Juice by Automated Online Sample Preparation Using TLX-LC-MS/MS

Laszlo Hollosi, Katerina Bousova, Ebru Ates, Klaus Mittendorf, Thermo Fisher Scientific Food Safety Response Center, Dreieich, Germany

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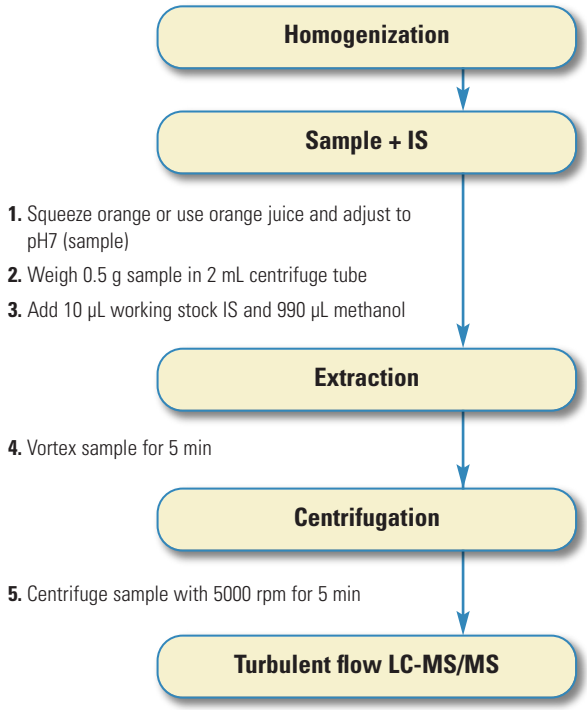
Key Words

- Transcend™ TLX
- TSQ Quantum Access MAX™
- Triple Quadrupole Mass Spec
- TurboFlow™ Technology
- Food Safety
- Fungicides

1. Schematic of Method



2. Introduction

Methyl 2-benzimidazole carbamate, most commonly known as carbendazim, is a widely used broad-spectrum benzimidazole fungicide and a decomposition product of benomyl. Carbendazim is used to control plant diseases in cereals and fruit, including citrus, bananas, strawberries, pineapples, and pome fruits. Although not permitted for use to treat citrus fruit in the USA and Australia, it is permitted in the EU and European Regulation 559/2011 sets a limit for carbendazim and benomyl (sum of carbendazim and benomyl expressed as carbendazim) of 0.2 mg/kg in oranges. Incidences of MRL exceedance have been common in the EU, with 23 Rapid Alert Notifications in 2011 for levels of carbendazim as high at 4 mg/kg in fruit, vegetables and herbs from Africa, S. America and Asia.[1] The most common occurrence was in yams and no instances of carbendazim in oranges or orange juice were reported. Orange juice from Brazil imported into the USA has been found to contain carbendazim and an action limit of 0.01 m/kg has been applied by the FDA.[2]

Many methods in widespread use for monitoring carbendazim have been developed for multi-residue determination of fungicides and employ a variety of sample preparation and cleanup techniques. In recent years the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method has become widely adopted for handling

Thermo Scientific Transcend TLX system coupled with the TSQ Quantum Access MAX triple quadrupole mass spectrometer

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fruit such as oranges. However, despite its undoubted advantages, it requires many manual sample manipulation steps, making it labor-intensive, especially when large numbers of samples have to be analyzed. Therefore, it is beneficial to consider options for automation of multi-residue methods, which can be both cost-effective as well as offer a high degree of reliability in recovery and repeatability. While the preliminary stages of homogenization and solvent extraction of food matrices inevitably require manual intervention, once a crude extract has been obtained there is scope for a fully automated procedure thereafter. The method described in this document is an adaptation of an existing online, multi-residue pesticide method (Thermo Scientific Method 52213[3]) proven and verified specially for the actual carbendazim contamination issue of orange juices in the US.

3. Scope

This method can be applied to oranges and orange juice at a limit of quantification (LOQ) below 0.01 mg/kg, the action limit used by the FDA for monitoring purposes. The method has been validated for carbendazim and the sum of benomyl + carbendazim in oranges and orange juice, but can be readily extended to a larger number of residues.

4. Principle

This method is the adaptation of carbendazim and extension for benomyl of an online sample preparation technique based on an existing in-house validated method (Thermo Scientific Method 52213[3]) for the determination of 50 pesticides in grape, baby food and wheat flour matrices. The method uses TurboFlow technology as a possible alternative to the QuEChERS method since TurboFlow is more suitable for high-throughput fungicide analysis. Sample pre-concentration, cleanup and analytical separation is carried out in a single run, using an online coupled TurboFlow method (Thermo Scientific Transcend TLX). TurboFlow technology serves as a novel sample preparation technique due to its special flow profile, size exclusion, reversed phase column chemistry and very effective separation of matrix and target compounds, resulting in relatively clean sample extracts. Macromolecules such as sugars, fats and proteins are removed from the sample extract with high efficiency, while target analytes are retained on the column based on different chemical interactions. After application of a wash step, the trapped compounds are transferred onto the analytical LC column and separated conventionally. The complete method involves internal standardization, solvent extraction of the homogenized orange juice, solvent extraction, centrifugation and injection into an automated cleanup system. Cleanup using Transcend TLX system has been optimized for maximum recovery of carbendazim or benomyl and minimal injection of co-extractives into the MS/MS. Identification of carbendazim and benomyl is based on retention time, ion-ratios using selected reaction monitoring (SRM) of characteristic transition ions, and quantification using matrix-matched standards of one of the selected SRM ions.

5. Reagent List

		Part Number
5.1	Acetone, HPLC Grade	A/0606/17
5.2	Acetonitrile, LC-MS Grade	A/0638/17
5.3	Ammonium formate, for HPLC	A/5080/53
5.4	Methanol, Optima LC/MS grade	A456-212
5.5	Formic acid, extra pure for HPLC	F/1850/PB08
5.6	Isopropanol, HPLC grade	P/7507/17
5.7	Water, LC-MS grade	W/0112/17
5.8	Ammonia (35% solution)	10508610

6. Calibration Standards

6.1 Standards

6.1.1 Carbendazim (analytical standard) from Sigma-Aldrich®

6.1.2 Benomyl (analytical standard) from Sigma-Aldrich

6.2 Internal standards:

6.2.1 Imidaclorprid-4,4,5,5-d_4 (analytical standard) from Sigma-Aldrich

7. Standards and Reagent Preparation

7.1 **Stock solution:** Weigh 10.00 mg of the compounds (recalculate the amount regarding actual purity of the standard) into a volumetric flask, dissolve in methanol and dilute to 100 mL. The final concentration of the two fungicides is 100 µg/mL. The solution of carbendazim can be used for 3 months when stored refrigerated, however benomyl stock solution remains stable only for 0.5 days.

7.2 **Individual working mixture:** Transfer 50 µL of stock solution of either carbendazim or benomyl (100 µg/mL), respectively, to a 50 mL volumetric flasks and dilute to the mark with methanol. The solution should be prepared fresh every time before using. Final concentration of each standard is 0.1 µg/mL.

7.3 **Stock standard solution of internal standard:** Weigh 10.00 mg of Imidacloprid-d_4 (recalculate the amount regarding actual purity of the standard) into volumetric flask, dissolve in methanol and dilute to 100 mL. The solution can be stored at 4 °C for at least 3 months. Final concentration is 100 µg/mL.

7.4 **Working standard solution of internal standard:** Transfer 100 µL of stock solution of imidacloprid-d4 (100 µg/mL) to a 10 mL volumetric flask and dilute to marked volume with methanol. The solution should be prepared fresh every time before using. The final concentration of imidacloprid-d_4 is 1 µg/mL.

7.5 **5 M Ammonia solution:** Weigh 24.3 g of ammonia (35% solution) to 100 mL volumetric flask and dilute to marked volume with deionized water.

8. Apparatus

		Part Number
8.1	Sartorius analytical balance	ME235S
8.2	Thermo Scientific Barnstead EASYpure II water	3125753
8.3	Vortex shaker	3205025
8.4	Vortex universal cap	3205029
8.5	Accu-Jet pipettor	3140246
8.6	Orion™ 2 Star, pH meter	10539752
8.7	Thermo Scientific Heraeus Fresco 17 micro centrifuge	208590
8.8	Transcend TLX-1 system with TSQ Quantum Access MAX MS/MS	40500

9. Consumables

		Part Number
9.1	LC vials	3205111
9.2	LC caps	3151266
9.3	Thermo Scientific Pipette Finnpipette 100–1000 µL	321453
9.4	Pipette Finnpipette™ 10–100 µL	3166472
9.5	Pipette Finnpipette 500–5000 µL	3166473
9.6	Pipette holder	3651211
9.7	Pipette tips 0.5–250 µL, 500/box	3270399
9.8	Pipette tips 1–5 mL, 75/box	3270420
9.9	Pipette tips 100–1000 µL, 200/box	3270410
9.10	Spatula, 18/10 steel	3458179
9.11	Spatula, nylon	3047217
9.12	Tube holder	3204844
9.13	Wash bottle, PTFE	3149330
9.14	Vial rack (2 mL)	12211001
9.15	Centrifuge plastic tube (2 mL)	3150968
9.16	TurboFlow Cyclone MCX-2 (50 × 0.5 mm) column	CH-953457
9.17	Thermo Scientific Hypersil GOLD 150 × 4.6 mm, 5 µm column	25005-154630
9.18	UNIGUARD holder	850-00
9.19	Hypersil GOLD™ 10 × 4 mm, 5 µm guard column	25005-014001

10. Glassware

		Part Number
10.1	Volumetric flask, 10 mL	FB50143
10.2	Volumetric flask, 25 mL	FB50147
10.3	1 mL glass pipette	FB50211
10.4	1 L bottle	9653650
10.5	500 mL bottle	9653640
10.6	100 mL volumetric flask	FB50151

11. Procedure

11.1 Sample Preparation

11.1.1 **Orange samples:** Prepare orange samples prior to injection into TLX-MS/MS system: Collect at least 10 representative oranges (min 1 kg) and cut into two halves.[4] Squeeze them on a kitchen squeezer and collect the pressed juice. Adjust the pH of the juice to 7 by adding 5 M ammonia solution.

11.1.2 **Orange juice samples:** Orange juice can be used directly after vigorous shaking and adjusting the pH to 7 with 5 M ammonia solution.

11.2 Sample Extraction

11.2.1 Weigh 0.5 g sample on an analytical balance into a 2 mL centrifuge tube

11.2.2 Add 990 µL methanol and 10 µL working IS solution

11.2.3 Vortex the sample for 5 min

11.2.4 Centrifuge in the centrifuge at 5000 rpm for 5 min

11.2.5 Transfer the supernatant into the LC vial for TLX-LC-MS/MS clean up and determination

		TurboFlow								Analytical					
Step	Duration [s]	Flow mL/min	Grad	A%	B%	C%	D%	Tee	Loop	Flow mL/min	Grad	A%	B%	C%	D%
1	60	1.50	step		100			–	out	0.50	step		100		
2	60	1.50	step		95		5	–	out	0.50	step		100		
3	80	0.16	step		100			Tee	in	1.44	step		100		
4	60	1.00	step			100		–	in	1.60	ramp		55		45
5	60	1.00	step	10			90	–	in	1.60	ramp		40		60
6	220	0.20	step		100			–	out	1.60	ramp				100
7	60	0.20	step		100			–	out	1.60	step				100
8	180	0.20	step		100			–	out	1.00	step		100		
		Mobile phases for the TurboFlow: A: water pH=3 B: water C: 40% acetonitrile 40% isopropanol and 20% acetone D: 5 mM ammonium-formiate in methanol + 0.1% formic acid								Solvent channels for analytical: A: not in use B: 5 mM ammonium-formiate in water + 0.1% formic acid C: not in use D: 5 mM ammonium-formiate in methanol + 0.1% formic acid					

Table 1: Gradient program table for Thermo Scientific Aria control software

12. Analysis

12.1 LC Operating Conditions

The TLX system was optimized for both TurboFlow methods and analytical separation.

12.1.1 LC conditions for TurboFlow and analytical columns

Operation was carried out in focus mode setup (Figure 1) with 1:0.75 splitting before MS/MS entrance using a divert valve connection. A TurboFlow Cyclone MCX-2 column was installed (9.17) and a Hypersil Gold column equipped with guard column was used (9.18–9.20). Installed loop volume was 200 µL.

Table 1 gives details of the method program. Sample load (Step 1) was applied with 1.5 mL/min flow rate in turbulent flow, whereby matrix components were eluted in the waste and target fungicides were trapped on the TurboFlow column. After washing the TurboFlow column with a 5% organic/aqueous mixture (Step 2), the trapped fungicides were eluted and transferred (Step 3) after 2 minutes from the TurboFlow to the analytical column with simultaneous dilution of the eluate enabling pre-concentration of fungicides at the beginning of the analytical column. The analytical column was equilibrated and conditioned during loading and washing steps. After transfer of the fungicides, the analytical separation started with gradient elution (Step 4–7), while the TurboFlow column was washed and conditioned and the loop was filled with the TurboFlow eluent. After the gradient run, analytical column was washed in acetonitrile and conditioned for the next run. The total run time of the method with TurboFlow sample preparation and analytical separation, with preparation for the next run, is 13 minutes to keep method capable for multi-fungicide residue analysis. In order to minimize sample carry-over and cross-contamination, the injection needle and valve were washed with both strong and weak wash solvents 4 times (conditions in 12.1.2).

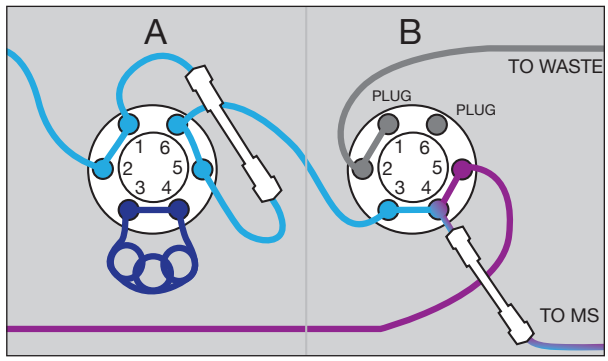

Figure 1: Focus mode system set up and method setting in Aria control software on the Transcend TLX system

12.1.2 Injector set up

Injector: Thermo Scientific Pal injector with 100 µL injection syringe volume

Sample holder temperature: 10 °C

Cleaning solvents: Solvent channel 1 – 80:20 methanol/acetone
Solvent channel 2 – acetonitrile

Injector settings:

- Pre clean with solvent 1 [steps]: 2
- Pre clean with solvent 2 [steps]: 2
- Pre clean with sample [steps]: 1
- Filling speed [µL/s]: 50
- Filling strokes [steps]: 2
- Injection port: LC Vlv1 (TX channel)
- Pre inject delay [ms]: 500
- Post inject delay [ms]: 500
- Post clean with solvent 1 [steps]: 4
- Post clean with solvent 2 [steps]: 4
- Valve clean with solvent 1 [steps]: 4
- Valve clean with solvent 2 [steps]: 4
- Injection volume: 20 µL

12.2 Mass Spectrometric Conditions

Mass spectrometric detection was carried out using a TSQ Quantum Access MAX triple quadrupole mass spectrometer in SRM mode. All SRM traces were individually tuned for the target fungicides (Table 2). MS software programming was set in Thermo Scientific Xcalibur Eazy mode set up.

MS settings:

- Scan type: SRM (details in Table 2)
- Cycle time [s]: 0.3
- Peak width: 0.7 Da FWHM
- Collision gas pressure [mTorr]: 1.5
- Capillary temperature [°C]: 290 °C
- Vaporizer temperature [°C]: 290 °C
- Sheath gas pressure [arb]: 40
- Aux gas pressure [arb]: 10
- Ion sweep pressure [arb]: 0
- Spray voltage [V]: 3200
- Skimmer offset [V]: 2
- Polarity: positive for all compounds
- Trigger: 1.00e5

13. Calculation of Results

Calibration by internal standardization is applied for the determination of carbendazim and benomyl. This quantification method requires determination of response factors R_f defined by the equation below. Calculation of final result is performed using the following equations.

Calculation of the response factor:

$$R_f = \frac{A_{St} \times c_{[IS]}}{A_{[IS]} \times c_{St}}$$

$\mathbf{R_f}$ – the response factor

$\mathbf{A_{St}}$ – the area of the fungicide peak in the calibration standard

$\mathbf{A_{[IS]}}$ – the area of the internal standard peak of the calibration standard

$\mathbf{c_{St}}$ – fungicide concentration of the calibration standard solution

$\mathbf{c_{[IS]}}$ – the internal standard concentration of the calibration standard solution

Calculations for each sample of the absolute amount of fungicide that was extracted from the sample:

$$X_{analyte} = \frac{A_{analyte} \times X_{[IS]}}{A_{[IS]} \times R_f}$$

$\mathbf{X_{analyte}}$ – the absolute amount of fungicide that was extracted from the sample

$\mathbf{A_{analyte}}$ – the area of fungicide peak in the sample

$\mathbf{A_{[IS]}}$ – the area of the internal standard peak in the sample

$\mathbf{X_{[IS]}}$ – the absolute amount of internal standard added to the sample

The concentration of fungicide in the sample [ng/g]:

$$c = \frac{X_{analyte}}{m}$$

$\mathbf{m}$ – the weight of sample [g]

$\mathbf{X_{analyte}}$ – absolute analyte amount [ng]

14. Method Performance Characteristics

In-house validation of the method was carried out according to IUPAC and AOAC guidelines for single laboratory validation and it was also demonstrated that method performance characteristics fulfilled the legislative criteria set for pesticide residue methods.[5-8]

Samples used for the determination of method performance characteristic parameters were prepared by spiking of appropriate amount of working standard solution and work solution of internal standard into the 0.5 g sample and total volume was adjusted to 1 mL with methanol (equivalent total volume according to 10.2.).

With reference to the low stability and fast transformation of benomyl into carbendazim, the validation study was carried out with samples spiked only with carbendazim to establish the method performance parameters.[6] After establishing validation parameters, samples were run additionally with spiked carbendazim and benomyl, in order to check degradation and contribution of benomyl to the carbendazim peak area (Figure 2). In order to keep control on benomyl degradation, all these samples were analyzed within 2 hours after preparation.

14.1 Selectivity

Method (SRM) selectivity was confirmed based on presence of specific ion transitions at the corresponding retention time (Table 2), as well as the observed ion ratio values corresponding to those of the standards. Acceptance criteria for retention time and ion ratios were set according to Reference 4.

14.2 Linearity, Response Factor

The linearity of calibration curves was assessed by internal standardization over the range from 0–0.1 mg/kg. The matrix-matched calibration curves were created at seven levels (and blank) and injected in duplicate. Calibration levels were 0, 0.005, 0.010, 0.015, 0.025, 0.035, 0.050 and 0.100 mg/kg. R_f values for internal standardization were determined from the calibration curves by calculating cumulative average response factor over the whole calibration range and resulted R_f = 3.2, which was used for quantitative analysis. The details on calibration are shown in Table 3.

14.3 Accuracy

Method accuracy and precision was assessed by recovery studies using blank matrices spiked at three concentration levels injected in six individually prepared replicates. Samples were spiked at 0.005, 0.010 and 0.050 mg/kg concentration levels. Found concentrations, recovery and relative standard deviation (% RSD) were calculated (Table 3). Recovery values were in the range 96–115% and were deemed to be acceptable (criteria 70–120%).

14.4 Repeatability and Intermediate Precision

Method within-day (repeatability) and between-day precision (intermediate precision) values ranged from 6.8–9.8% (Table 4) and were deemed acceptable (below 20%).

14.5 Limits of Detection (LODs) and Quantification (LOQs)

Limits of detection and quantification were estimated following the IUPAC approach which consisted of analyzing the blank sample to establish noise levels and then testing experimentally estimated LODs and LOQs for signal/noise, 3 and 10 respectively. The method LOD and LOQ values resulted as 0.00015 mg/kg and 0.0005 mg/kg (Figures 3 and 4). The expectation of the method was to meet the US rejection limit for orange juices set by the FDA at 0.010 mg/kg as well as the European MRL value (0.2 mg/kg) at LOQ level. Method LOQ fulfilled both legislation criteria.

14.6 Matrix Effect

Matrix effect was investigated by comparison of calibration results in solvent and in matrix. Youden plot of both calibration series was applied. Slope of fitted linear resulted y=0.8497× which represents less than 20 % deviation from the idealistic y=× value indicating no matrix effect for the investigated matrix (Figure 5).

14.7 Survey Samples

The method was applied to 6 different orange juice samples (n=3) and oranges (n=3) purchased from local stores. Survey samples were of organic origin from Spain and Germany. No carbendazim was found above 0.01 mg/kg in any of survey samples (Table 5).

15. Conclusion

This method enables convenient, fast and cost-effective automated determination of carbendazim and benomyl in oranges and orange juice. Based on the short total run time and a simple online sample preparation technique, 100 samples per day can be analyzed at a level of 0.01 mg/kg, with faster and more precise analysis compared to the QuEChERS technique. Method performance characteristics were established by in-house validation for oranges and orange juice. Based on its method performance parameters, the developed TLX system is suitable for routine use for regulatory purposes and possesses potential as alternative to the widely used QuEChERS method. The TLX system can readily be extended to a larger and wider range of fungicide residues, and has previously been demonstrated as being applicable to other matrices such as cereals, grapes and baby food.[1]

16. References

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17. Annex

17.1 Tables, Chromatograms and Matrix Study

Analyte	Retention time [min]	Precursor Ion	Product Ion (EcoII)	Product Ion2 (EcoII)	Ion Ratio	Tube Lens
Carbendazim	6.01	191.8	160.1 (18)	132.1 (29)	0.25	100
d4-Imidacloprid	6.21	259.9	213.1 (17)	179.1 (20)	0.82	97
Benomyl	9.03	291.1	192.1 (12)	160.1 (27)	0.85	101

Table 2: Ion transitions of target compounds for SRM setting

	Linearity			Recovery [%] (RSD%)		
Compound	**Slope**	**Intercept**	**R^2**	**0.005 mg/kg**	**0.010 mg/kg**	**0.050 mg/kg**
Carbendazim	0.1501	0.1787	0.9981	99 (5.5)	101 (6.8)	108 (4.0)
Carbendazim + Benomyl	0.3377	0.1840	0.9891	115 (14.6)	96 (9.4)	104 (3.6)

Table 3: Linearity (n=2) and recovery (n=6) of target compounds

	Precision [%]			
	Identification (tr)		Quantification (Peak Area)	
Compound	**Repeatability**	**Intermediate Precision**	**Repeatability**	**Intermediate Precision**
Carbendazim	0.1	0.1	6.8	9.5
Carbendazim + Benomyl	–	–	7.5	9.8

Table 4: Repeatability and intermediate precision of target compounds

Sample #	Type of Sample	Carbendazim [mg/kg]
1	juice	0.001
2	juice	0.002
3	juice	0.005
4	orange	0.001
5	orange	<LOD
6	orange	<LOD

Table 5: Survey sample results

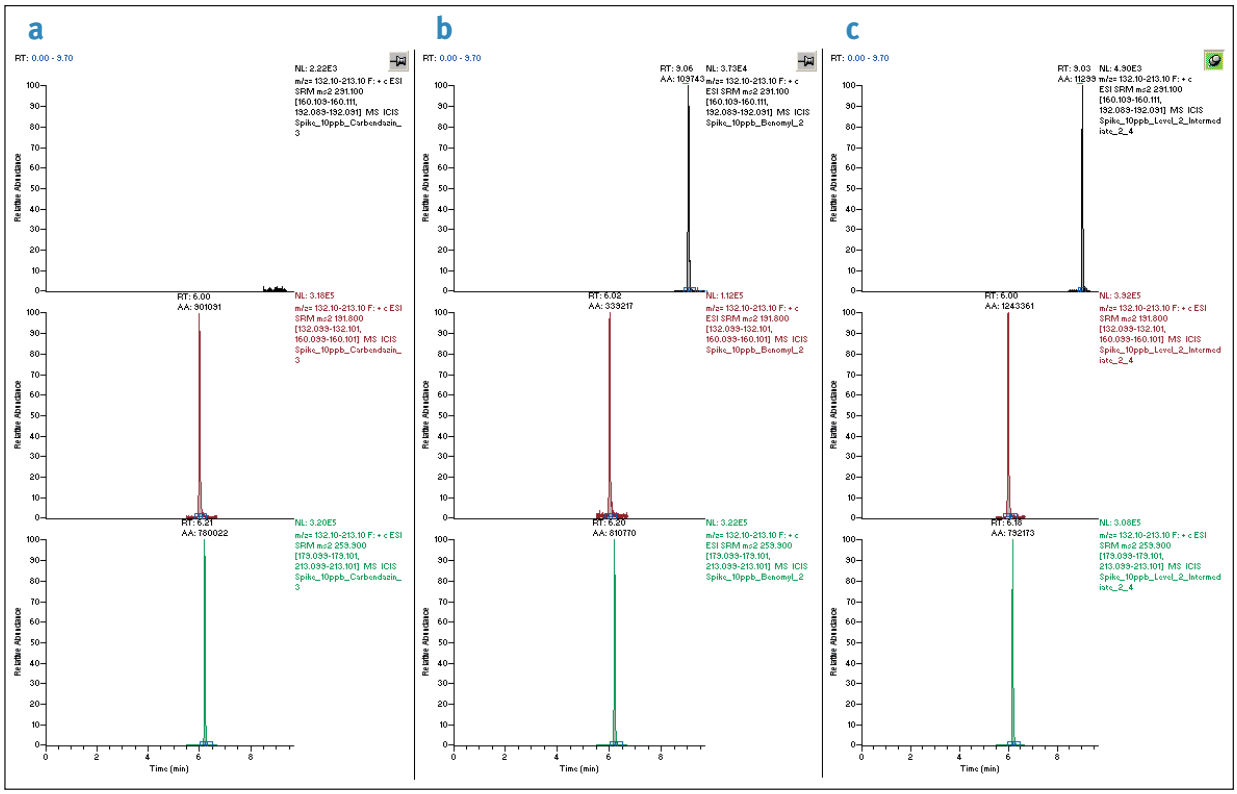

Figure 2: Demonstration of transformation of benomyl into carbendazim. Traces from top: benomyl, carbendazim and d4-imidacloprid (IS). Chromatograms showing a) 10 ng/mL carbendazim solution, b) 10 ng/mL benomyl solution after 2 hrs of preparation, c) chromatogram of solution containing 10 ng/mL carbendazim and benomyl after 2 hrs of preparation. Significant amount of benomyl transforms into carbendazim.

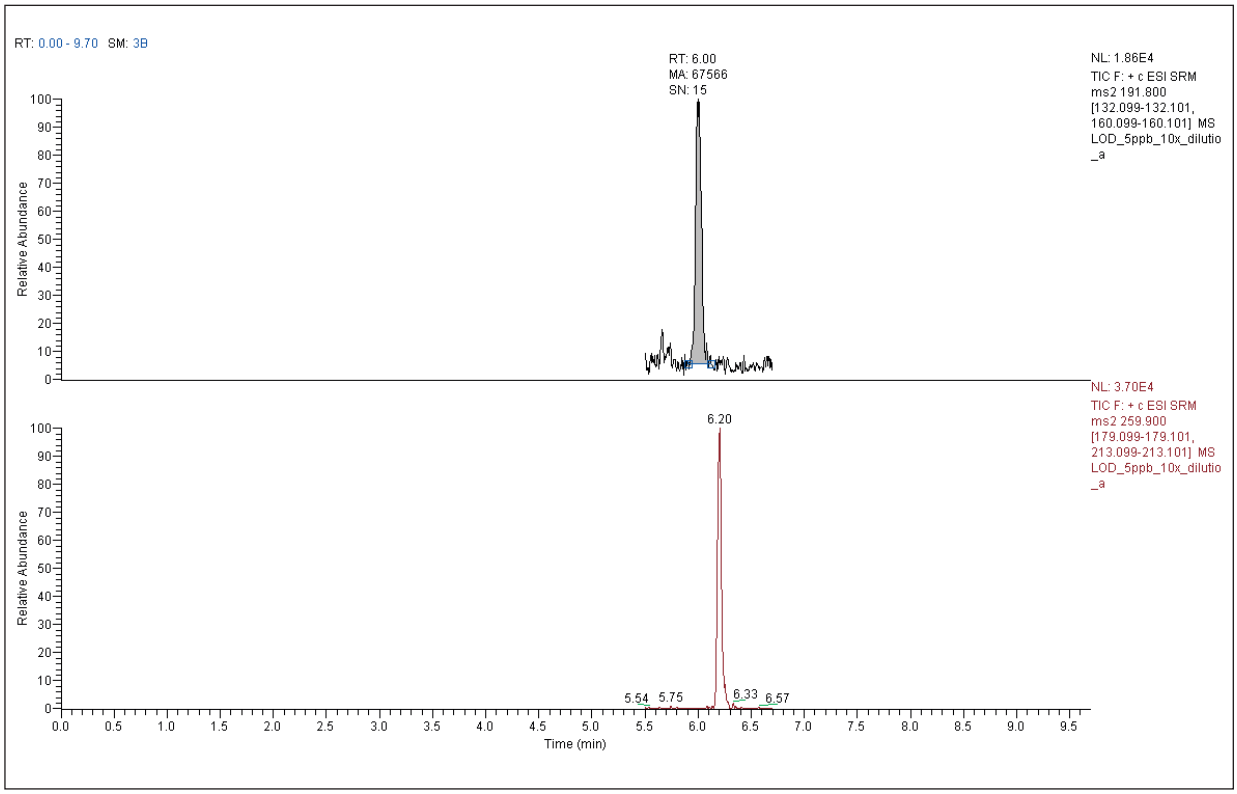

Figure 3: Chromatogram of 0.0005 mg/kg carbendazim in orange juice representing signal intensity at LOQ level.
On top: carbendazim, below: d4-imidacloprid (IS).

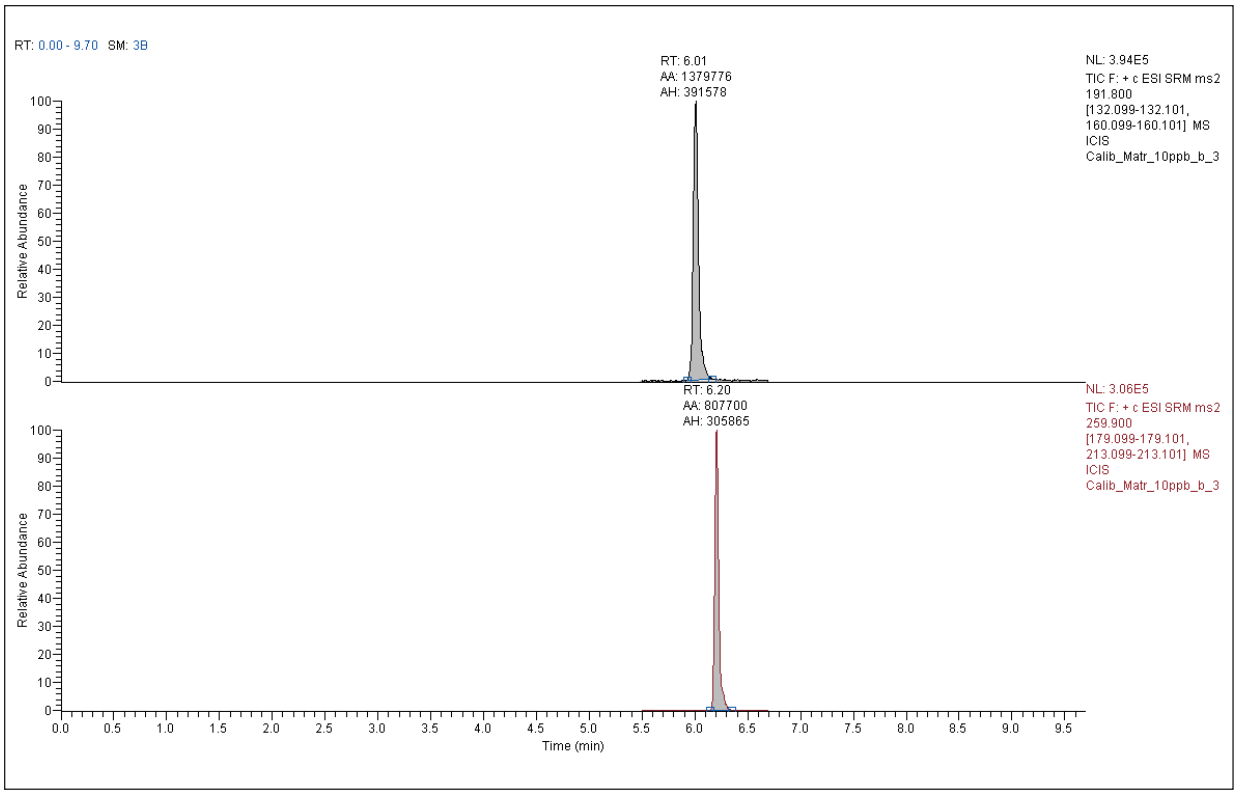

Figure 4: Chromatogram of 0.01 mg/kg carbendazim matrix (orange juice) matched calibration standard representing peak intensity at current US (FDA) rejection level. On top: carbendazim, below: d4-imidacloprid (IS).

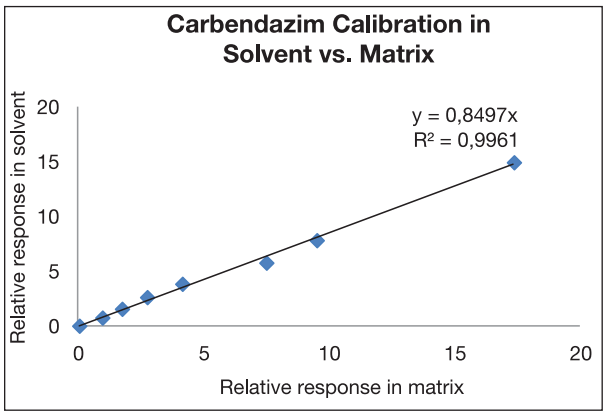

Figure 5: Matrix effect study. Plot of relative responses of calibration levels in solvent vs in orange juice.

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TG52292_E 01/12M

Application Note: 373

Testing LC-MS System Robustness with Automated Sample Cleanup Using Red Wine as a Matrix

Jonathan Beck, Thermo Fisher Scientific, San Jose, CA, USA

DOWNLOAD

Key Words

- TSQ Quantum Ultra™
- EQuan™ System
- Hypersil GOLD™ Columns
- Pesticides

Introduction

Achieving low limits of detection (LODs) of pesticides, antibiotics and veterinary residues in food residues and drinking water is of paramount importance in order to monitor the regulatory levels as stated by the US, Japanese and EU directives. These substances pose a significant health threat and therefore, need to be accurately detected at the lowest levels, typically at part per trillion (ppt). Traditionally, LC-MS/MS has been used by the environmental and food industries for the identification and quantitation of these residues. However, this methodology typically requires extensive offline sample preparation, which can be time consuming and expensive.

The Thermo Scientific EQuan environmental quantitation system consists of a Thermo Scientific TSQ Quantum™ series mass spectrometer, two Thermo Scientific Surveyor™ HPLC pumps with a preconcentration column, an analytical column, and a CTC autosampler. The unique capabilities of EQuan for online preconcentration and cleanup of samples result in improved sensitivity and precision, as well as unmatched throughput.

In previous experiments, using the EQuan system for online sample preconcentration and detection of pesticides in ground water yielded lower limits of detection compared to standard injection techniques. See Table 1.

Typically, when red wine is analyzed using LC-MS/MS, some form of sample preparation and/or extraction is necessary prior to injection. In this application note, the EQuan system was tested for robustness using a matrix of neat red wine spiked with a mixture of pesticides using large volume (1000 µL) injections.

Goal

To test the robustness of an LC-MS system for an automated online preconcentration system using a dirty matrix.

Experimental Conditions

Sample Preparation

Red Burgundy wine was spiked with a mixture of nine herbicides and six fungicides at a level of 500 pg/mL (500 ppt). The following herbicides were analyzed: atrazine, cyanazine, simazine, propazine, trietazine, metazachlor, propachlor, pendimethalin, and propyzamide. The following fungicides were analyzed: flutriafol, triadimefon, epoxiconazole, flusilazole, tebuconazole, and propiconazole. No other sample treatment was performed prior to injection.

HPLC

HPLC analysis was performed using an HTC PAL™ Autosampler with two LC quaternary pumps and two LC columns, the first for preconcentration of the sample and the second for the analytical analysis. A sample of 1000 µL of the spiked neat wine was injected directly onto the Thermo Scientific Hypersil GOLD 20 × 2.1 mm, 12 µm loading column in a high aqueous mobile phase (see Figure 1a). After 1 minute, a six-port valve on the mass spectrometer was switched by LCQUAN™ 2.5 instrument control software. This enabled the load column to be back flushed onto the analytical column (Thermo Scientific Hypersil GOLD 50 × 2.1 mm, 3 µm), where the

	1 mL Injection Area	100 µL Injection Area	Gain Factor	1 mL Injection Area	100 µL Injection Area	Gain Factor	1 mL Injection Area	100 µL Injection Area	Gain Factor	1 mL Injection Area	100 µL Injection Area	Gain Factor
	Propham			Isoproturon			Diuron			Linuron		
1 ppt				5.53E+04	NA		1.97E+04	1.73E+03	11			
5 ppt	2.17E+04			3.35E+05	3.17E+04	11	4.15RE+04	5.65E+03	7	6.96E+03		
10 ppt	2.71E+04			6.68E+05	4.90E+04	14	8.25E+04	1.18E+04	7	1.99E+04		
50 ppt	5.09E+04			3.33E+06	2.82E+05	12	4.47E+05	3.72E+04	12	5.91E+04	7.98E+03	7
100 ppt	6.51E+04			6.54E+06	5.24E+05	12	8.83E+05	7.60E+04	12	1.34E+05	2.50E+04	5
500 ppt	2.47E+05	3.00E+04	8	3.11E+07	2.60E+06	12	4.65E+06	3.80E+05	12	7.36E+05	1.28E+05	6
1000 ppt	5.29E+05	5.69E+04	9	5.81E+07	5.23E+06	11	9.39E+06	7.63E+05	12	1.43E+06	2.47E+05	6
5000 ppt	2.59E+06	2.82E+05	9	2.58E+08	2.44E+07	11	4.95E+07	3.68E+06	13	9.49E+06	1.25E+06	8

Table 1: Calculations demonstrating the gain in peak areas due to larger injection volumes in ground water samples

Thermo SCIENTIFIC

compounds were separated prior to introduction into the mass spectrometer (see Figure 1b). After all of the compounds were eluted from the analytical column, the 6-port valve was switched back to the starting position, and the loading and analytical columns were cleaned with a high organic phase before being re-equilibrated to their starting conditions. The total run time for each analysis was 22 minutes. The mobile phases for the analysis were water and methanol, both with 0.1% formic acid.

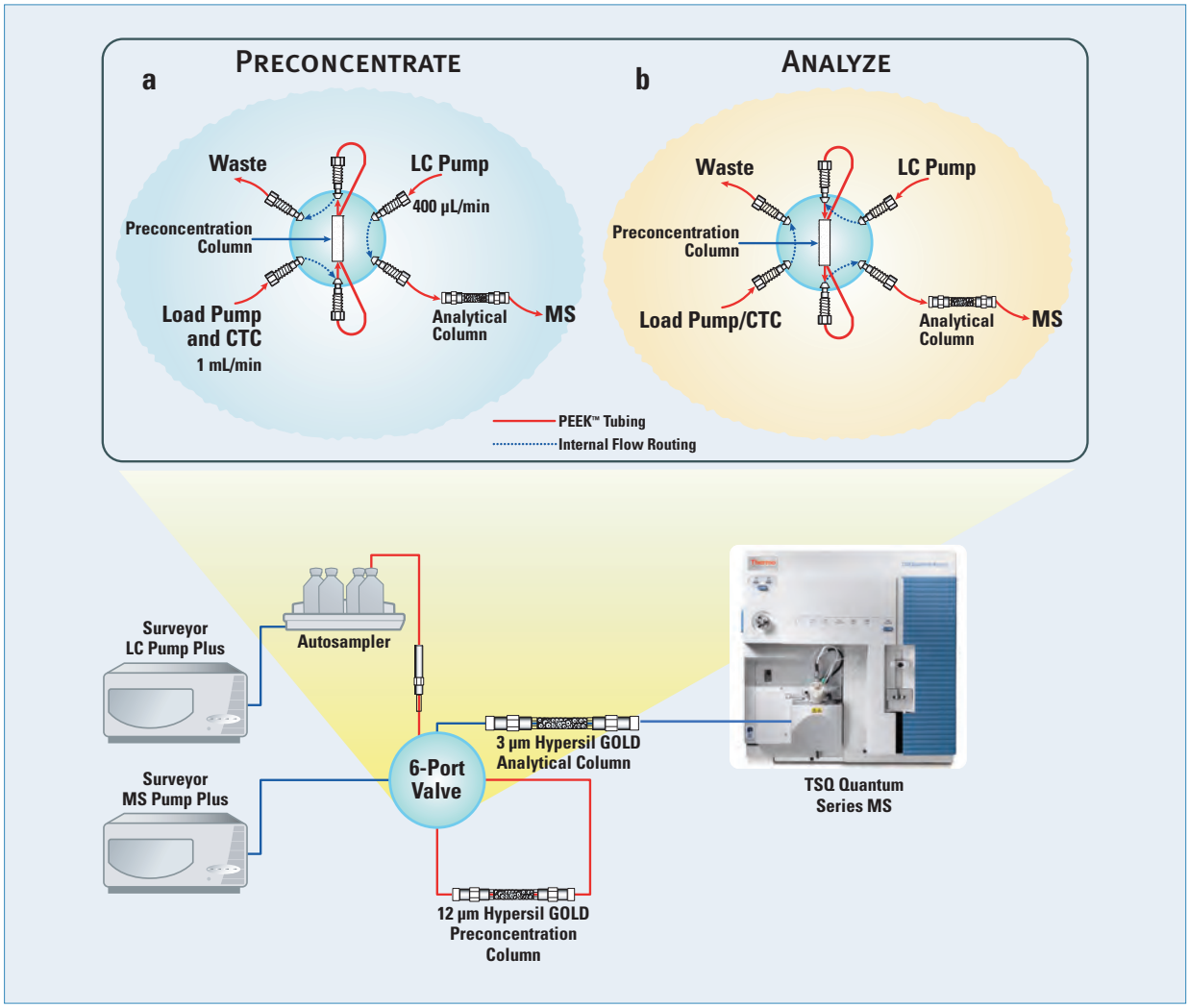

Figure 1: The schematic of the EQuan system used for this assay

Figure 2: Ion sweep cap after several hundred injections, showing contamination from red wine

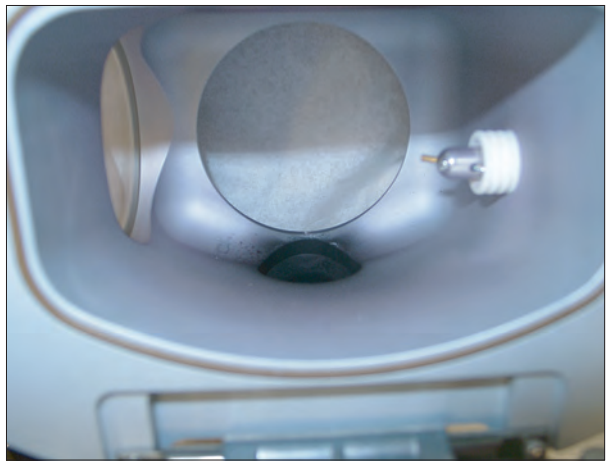

Figure 3: Electrospray ionization source with the electrospray probe removed, showing the main spray pattern directed towards the drain

MS

MS analysis was carried out on a Thermo Scientific TSQ Quantum Ultra triple quadrupole mass spectrometer with an electrospray ionization source. The MS conditions were as follows:

Electrospray ionization: Positive
Spray voltage: 3.0 kV
Ion transfer tube temperature: 350 °C
Sheath gas pressure: 45 arbitrary units
Auxiliary gas pressure: 5 arbitrary units
Ion sweep gas pressure: 3 arbitrary units
Collision gas (Ar): 1.0 mTorr
Q1/Q3 peak resolution: 0.7 Da
Scan width: 0.002 Da

The source of the mass spectrometer was adjusted so that the ESI probe was off axis to prevent contamination of the ion transfer tube. The position of the probe was set so that the main spray pattern of the electrospray hit the Ion Sweep™ cone below the center line and off to the left by about 0.5 cm. The probe depth was set to position "C" on the electrospray probe. An ion sweep gas of three arbitrary units was set to prevent any large droplets from entering the ion transfer tube of the mass spectrometer.

Results and Discussion

The back pressure of the loading column and the analytical column were monitored over the course of the wine injections to determine if the columns were becoming clogged with any particulates from the wine. Over 600 injections, the back pressure on the 12 µm loading column remained at approximately 20 bar under the starting conditions of the analytical run, while the back pressure on the 3 µm analytical column remained at approximately 72 bar.

The resulting spray pattern of the electrospray can be seen in Figure 2. A thick deposit of red wine residue is clearly visible from just below the center of the sweep cone to the outside radius. The red wine spray can also be seen on the inside of the electrospray housing in Figure 3. In the picture, the drain is dark purple in color, illustrating that the main excess spray of the red wine was directed to the bottom of the ion source and away from the main orifice of the mass spectrometer. Additionally, the ESI probe can be adjusted to be closer to the ion transfer tube, which increases robustness by allowing less side scatter from the electrospray beam, thus focusing the main spray pattern lower on the ion sweep cap.

The reproducibility of the method is shown in Figure 4. The graph plots the peak area for metazachlor for 164 injections of red wine. The first four injections were excluded from the %RSD calculation. Because the loading column was new at the beginning of the runs, several injections were required to condition the column before a stable peak area was achieved. A representative chromatogram is shown in Figure 5.

As shown in Figure 6, after several hundred injections of the spiked red wine matrix, no degradation in column performance or source robustness was observed. In total, over 600 injections were made on the system with no loss in column performance.

Conclusion

This application note demonstrates the robustness of the TSQ Quantum Ultra triple quadrupole mass spectrometer and an online extraction and preconcentration method. The described sample cleanup technique improves signal-to-noise ratios by a factor of 10 to 100 (based on injection volume) for low concentration samples in red wine matrices. Preliminary results using onion and tobacco matrices have yielded similar results in terms of column performance and mass spectrometer robustness. Further studies will be conducted in other matrices, as well as with other pesticides, herbicides, and insecticides.

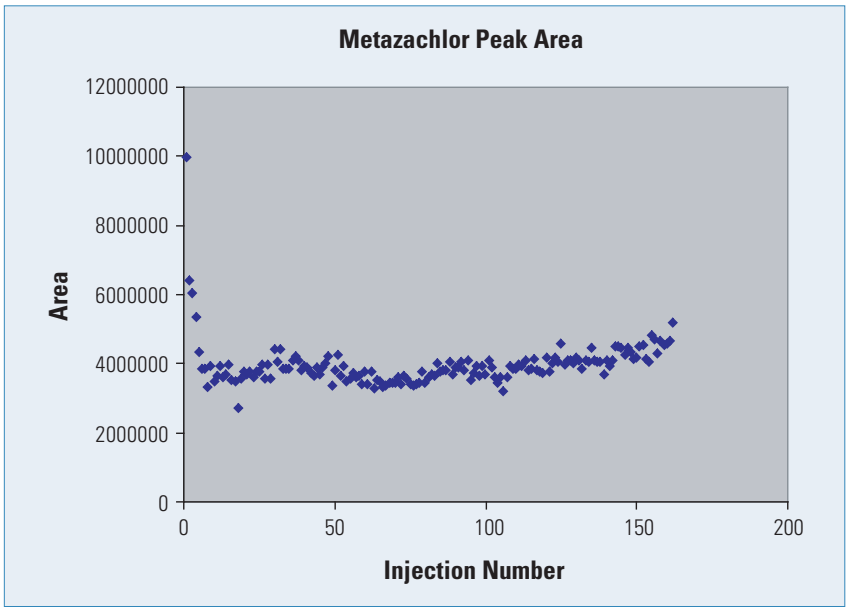

Figure 4: Scatter plot of the peak area for 164 injections (1000 µL) of metazachlor spiked in red wine. The %RSD is 9% when the first four points are excluded.

References

[1] Wang, Y.; Catana, F.; Yang, Y.; Roderick, R.; van Breemen, R.B. "An LC-MS Method for Analyzing Total Resveratrol in Grape Juice, Cranberry Juice, and in Wine"; *J Agric. Food Chem.* **2002**, 50(3), 431-435.

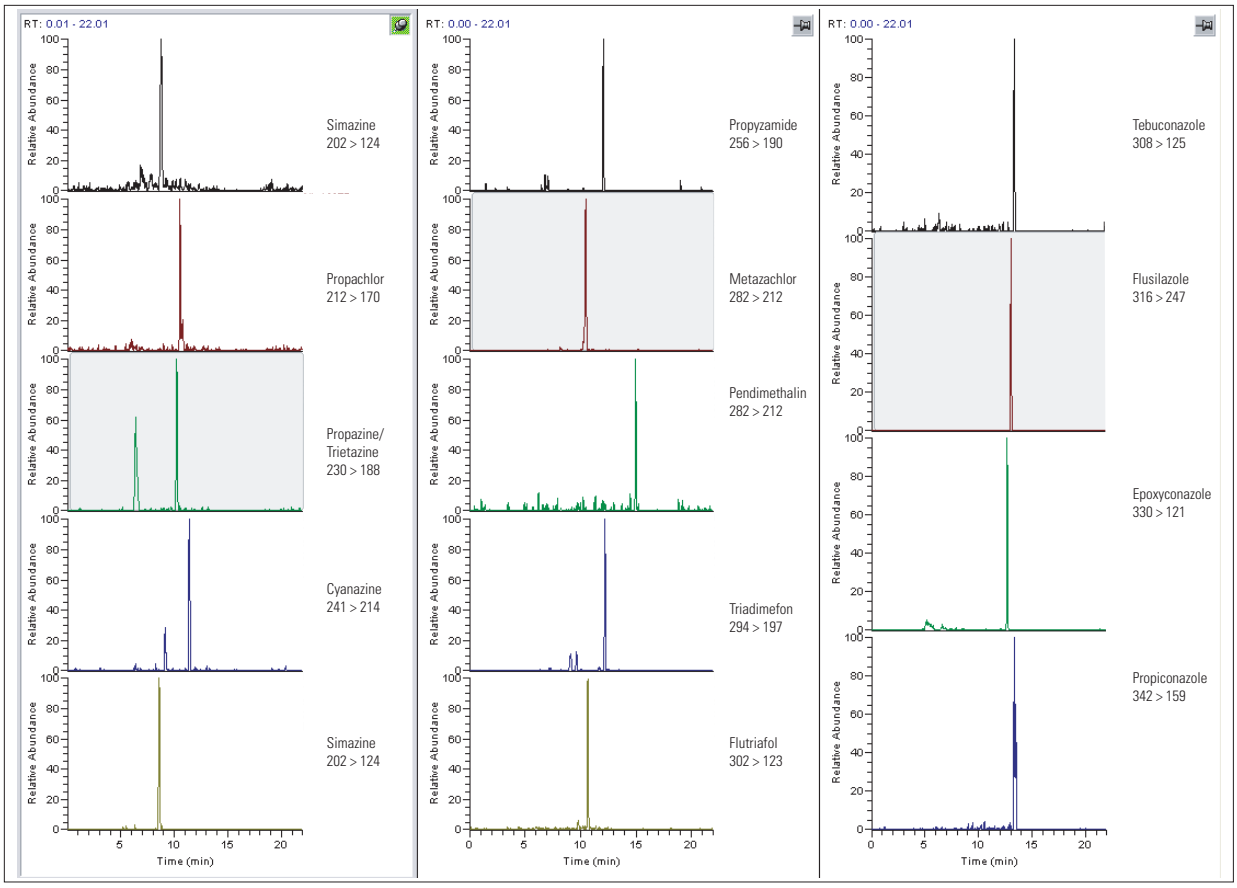

Figure 5: Example chromatograms for a 1000 µL injection of spiked red wine

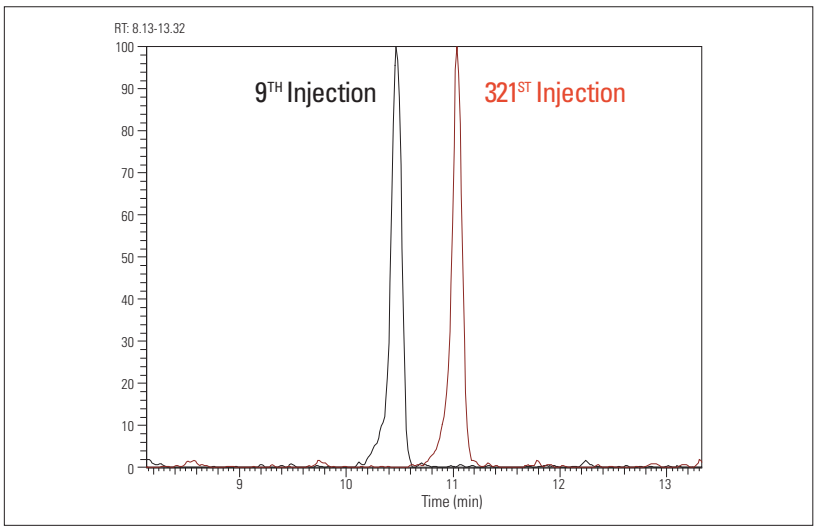

Figure 6: Different injections of metazachlor (retention times have been offset for greater visibility)

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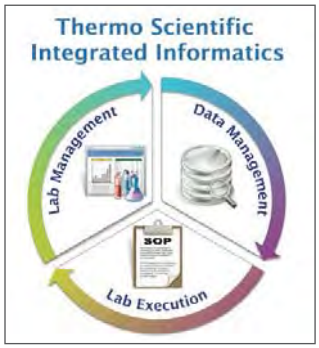

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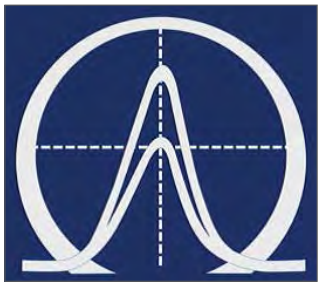

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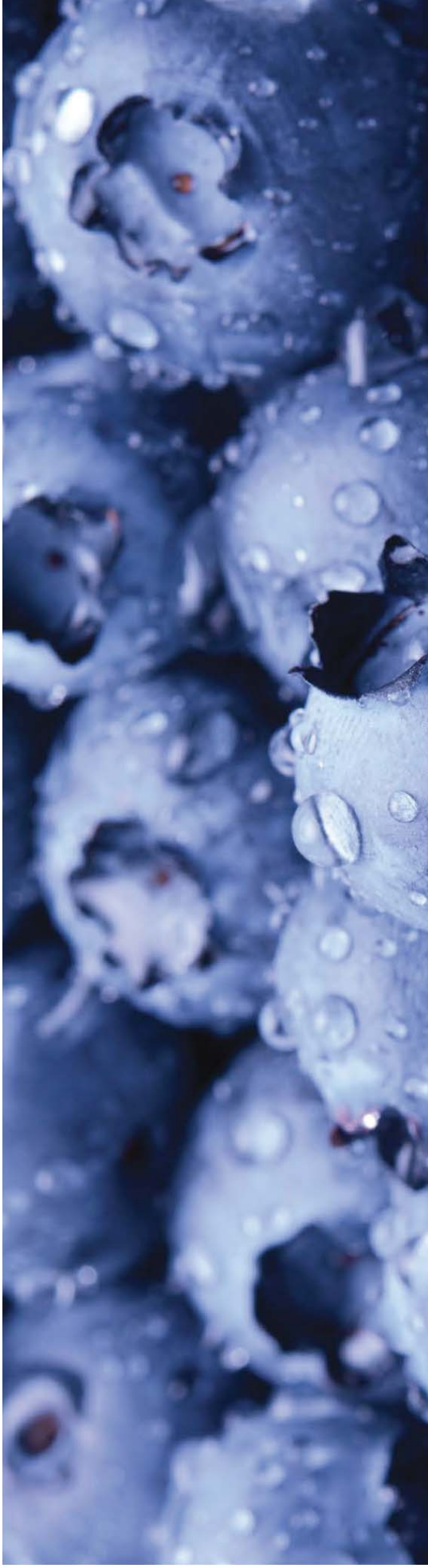

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