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TRANSFORM YOUR RESULTS FROM ACCEPTABLE TO EXCEPTIONAL

Applications Resource Handbook for
Agilent Food Analyzers, Databases, and Libraries

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MINIMIZING SETUP, MAXIMIZING RESULTS

The safety of our food supply is a paramount global concern; however, today's labs are short-staffed, under tight deadlines, and facing tough regulatory scrutiny. To succeed with any new application, the number of steps between startup and results must be reduced.

Agilent has your back with customizable and preconfigured innovations to help you protect consumers.

LC/MS and GC/MS solutions from Agilent Technologies empower your lab to solve problems faster, run more efficiently, and enable every user to employ advanced chromatographic techniques.

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Only Agilent has customized solutions to meet your unique food analysis challenges.

How this Guide works

The table of contents is conveniently organized by analyte type, so you can find the solution you're looking for quickly. Simply click the links to immediately jump to the section you want to explore.

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PESTICIDES

Identify and confirm pesticides in difficult matrixes

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CONFIDENTLY CONFIRM PESTICIDES AND ENVIRONMENTAL POLLUTANTS IN COMPLEX MATRICES

Agilent Pesticides and Environmental Pollutants Analyzer 4.0

Powered by market-leading Triple Quadrupole GC/MS technology, this factory configured, chemically tested analyzer puts you on the fast track to measuring pesticides and environmental pollutants in complex matrices

Concern about trace-level pollutants in food and the environment is driving the demand for rapid and reliable identification of chemical residues. Meeting this challenge requires technologies that can differentiate pesticides, PCBs, PAHs, PBDE, and other targets from organic interferences at low ppb concentrations.

The **Agilent Pesticides and Environmental Pollutants (P&EP) Analyzer 4.0** follows SANCO guidelines and lets you accurately confirm target pesticides while reducing the time required from start-up to results. It combines the leading-edge innovations of the Agilent 7890B GC and 7010B Series Triple Quadrupole GC/MS with pre-tested methods and a comprehensive MRM database to transform your results from acceptable to exceptional.

The Agilent P&EP Analyzer 4.0 includes the following to maximize performance and support your complete workflow:

- Choice of pre-tested, retention time-locked methods
- Dynamic MRM enhances data efficiency by automatically optimizing an analyte's dwell time
- Capillary Flow Technology and backflush-ready configurations
- Reverse sandwich injection saves bench work
- P&EP MRM database with > 1,100 compounds
- Over 7,500 matrix-optimized MRM transitions
- QuEChERS and EMR—Lipid sample prep kits
- Installation with a checkout sample for verification of chromatographic performance
- OPTIONAL: JetClean self-cleaning ion source and Method and Application Services

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MARKET-LEADING TECHNOLOGY DELIVERS SUPERIOR ANALYTICAL PERFORMANCE

Automated Retention Time Locking (Auto RTL)

Pre-tested methods provide optimal analysis

A choice of four (4) pre-tested methods to meet your individual analytical needs for performance or flexibility driven method setup. As a core method parameter, Auto RTL precisely matches RTs from column to column, instrument to instrument, and lab to lab for methods that have the same nominal parameters. The result: peaks are where you expect them in the chromatogram. Retention time locking provides the basis for effective dynamic MRM methods.

Capillary Flow Technology (CFT) Backflush

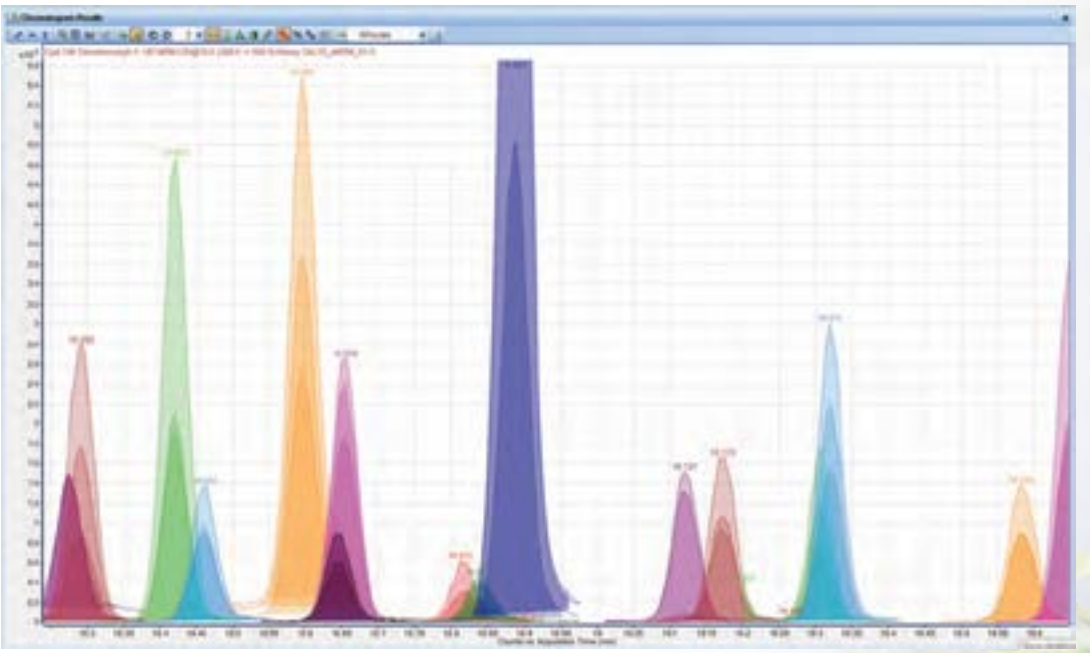
Three configurations remove heavy sample components

Injecting heavy matrix samples can retain high-boiling compounds at the head of the column, causing analyte interferences and degrading chromatographic performance. Reversing column flow using CFT backflush eliminates the need to bake columns at the end of the run to remove heavy materials, reduces system cycle time, minimizes source cleaning, and provides stable baselines and retention times for target analytes.

Dynamic Multiple Reaction Monitoring (dMRM)

Easier method maintenance and better performance

Enable larger target lists in a single run due to more efficient use of instrument data acquisition time using dMRM with retention-time scheduled MRM transitions. Convert your time segment-based methods to dMRM methods for easier method maintenance and better performance. Achieve faster maximum scan speeds in combined scan/MRM mode for simultaneous analysis of non-targets. dMRM also provides an intuitive way to build and modify acquisition methods using the P&EP MRM database.



MassHunter qualitative chromatogram of an organic honey dMRM run

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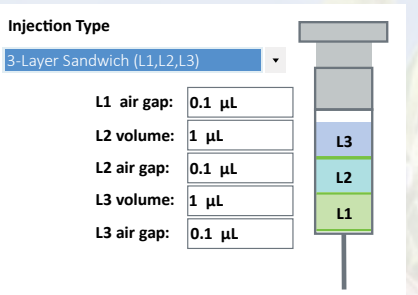
Reverse Sandwich Injection

Streamlined sample injection

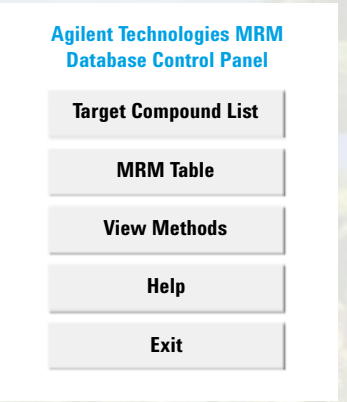
Using sandwich injections can save significant bench work otherwise needed to prepare matrix-matched calibration standards and/or addition of internal standards to the samples. Injecting matrix before the sample benefits the analysis. Updated MassHunter Data Acquisition Software simplifies the sandwich injection options.



Updated P&EP MRM Database

Simplify the creation of compound lists and analytical methods

The P&EP 4.0 Analyzer not only provides the most comprehensive MRM Database on the market, it also guarantees state-of-the-art analyses with a 3-year site subscription with free updates. It also includes over 7500 MRM transitions that have been optimized in a variety of complex matrices like spinach, jasmine rice, and black tea. Alternate MRM transitions help avoid matrix interferences and increase confidence in the identification of target compounds in difficult matrices. The database is fully compatible with Agilent GC/MS MassHunter Data Acquisition's dynamic MRM (dMRM) functionality.

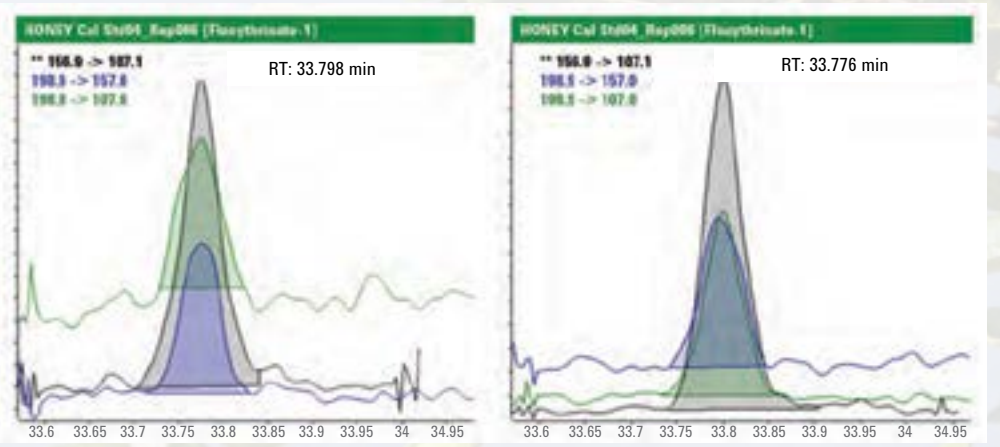


JetClean Self-Cleaning Ion Source

Reduce source maintenance and get more consistent results

The Agilent JetClean self-cleaning ion source keeps your P&EP Analyzer free of matrix deposits that would otherwise build up over time and degrade instrument performance. Using a carefully controlled hydrogen flow, JetClean technology significantly reduces the need for cleaning the ion source, thereby extending maintenance-free operation periods of the analyzer.

Flucythrinate-1 MW 451



No JetClean **0.13 mL/min (continuous) JetClean**

To review our full line of analyzers, visit **www.agilent.com/chem/food-ms-solutions**

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Agilent Method and Application Services

Achieve the best scientific outcome at each step of the workflow

Agilent application consultants provide you with comprehensive guidance for trace-level analysis of pesticides and environmental pollutants in food and environmental matrices, including optimization of the MRM acquisition method. Optional sample prep consultancy can provide you with step-by-step guidance.

Agilent QuEChERS and EMR—Lipid Startup Kits

Sample preparation made easy

Agilent offers a broad range of QuEChERS sample prep products for a variety of matrices. The P&EP 4.0 Analyzer includes a QuEChERS+EMR—Lipid Startup Kit applicable to frequently analyzed sample matrices. The innovative sorbent in the Enhanced Matrix Removal—Lipid dispersive SPE (EMR—Lipid dSPE) product selectively removes lipids in complex matrices without losing analytes of interest.

Agilent P&EP Analyzer 4.0 Ordering Information:

Choose one of the following options when you order an **Agilent 7000D/7010B Series Triple Quadrupole GC/MS** with an **Agilent 7890B GC analyzer system:**

Part No	Backflush Method	Run Time	Dimensions	Backflush
M7411AA	Flexible Constant Pressure	40 min	30 m column x 0.5 m restrictor	Post Column
M7412AA	Constant Flow (CF)	20 or 40 min	15 m column x 15 m column	Mid Column
M7414AA	Selective CF	20 min	5 m column x 15 m column	Mid Column

The industry-leading Agilent 7890B GC boosts productivity, protects our environment, and generates data with confidence through better resource management. GC system inertness provides an advantage for pesticide analysis.

The Agilent 7010B Triple Quadrupole GC/MS redraws the boundaries for GC/MS/MS workflow productivity. Its High Efficiency Source (HES) is critical for trace level analysis of pesticides.

MassHunter Software is a single powerful software solution for all Agilent MS platforms, saving training costs in multi-instrument labs.

Put your lab on the productivity *fast track*.

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Call **800-227-9770** (in the U.S. or Canada)
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5991-7418EN

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An Optimal Method for the Analysis of Pesticides in a Variety of Matrices

Application Note

Author

Jessica Westland and Joan Stevens
Agilent Technologies, Inc.

Abstract

Matrix effects have been a common complaint among MRM acquisition methods in pesticides analysis. The usefulness of a given compound's MRMs can change depending on the matrix being measured. The ability to have multiple MRMs from which to choose aids in lab productivity, improved quant method generation, and achieving optimal analysis. A total of 195 target compounds were selected for the analysis. Each compound was analyzed in each of the eight matrixes as well as in ACN (Figure 3). The top five MRM transitions for each target compound were selected based on response, ion ratios, and selectivity. From these, the top three to four MRMs were transferred to a matrix specific method for further analysis. As a result, 90% of all target compounds achieved a calibration curve with a $R^2 \geq 0.990$. All analyzed pesticides obtained a %RSD of repeated measurements of ≤30%, and 90% of the analyzed pesticides were found to have a limit of quantitation (LOQ) ≤1.5 pg/µL.

Introduction

The global agriculture industry uses over a thousand different pesticides for the production of food and foodstuffs. Producers require pesticides to meet the increasing demand for reasonably priced food. This growing demand has increased the use of pesticides and expanded poor agricultural practices, elevating risks in the food supply and the environment. Analytical laboratories are strained to evaluate and quantitate hundreds of pesticides in a wide range of matrixes. Not only are laboratories faced with time constraints, but they also face matrix interferences that degrade their ability to accurately identify and quantitate the multitude of target pesticides.

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Many laboratories focused on pesticide residue analysis in food commodities routinely use the Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) method [1,2]. This straightforward sample preparation allows for the analysis of hundreds of pesticides at low concentrations with a single extraction.

The Agilent G9250AA Rev. A.04.00 Pesticides and Environmental Pollutants (P&EP) Standard MRM Database is the most comprehensive GC MRM Database on the market. With over 1,100 compounds and up to 10 MRMs/compound, analysts have the ability to optimize their acquisition methods for their target compounds in a wide variety of matrixes. The availability of multiple MRM transitions not only helps to address matrix effects, but it also aids in accurately identifying compounds that may have several MRMs in common.

Experimental

Sample preparation

A selection of eight different matrixes were examined. These commodities included yellow onion, navel orange, organic honey, basic cucumber, jasmine rice, fresh leaf baby spinach, black loose leaf tea, and extra virgin olive oil. Each matrix was extracted with a specified QuEChERS methodology, in which various dispersive SPE (dSPE) were used for matrix cleanup (Table 1).

A 10 g sample of homogenized yellow onion; a 10 g sample of homogenized navel orange; a 3 g sample of homogenized jasmine rice with 7 mL of water; a 3 g sample of homogenized loose leaf black tea with 7 mL of water; a 10 g of homogenized baby spinach; a 10 g sample of homogenized cucumber; a 5 g sample of organic honey with 5 mL of water followed the same QuEChERS extraction procedure. Each sample was vortexed with two ceramic homogenizers. 10 mL of acetonitrile (ACN) was added, and the sample was vortexed for 2 minutes. The QuEChERS EN salts (p/n 5982-5650) were added, and capped tubes were placed on a GenoGrinder vertical shaker for 2 minutes, then centrifuged at 5,000 rpm for 5 minutes. Six milliliters of the extract was transferred to QuEChERS dSPE (p/n 5982-5256) used with fatty matrix for the onion, orange and rice extract; or 6 mL of the extract transferred to QuEChERS dSPE (p/n 5982-5256) used with pigmented matrix for tea; or 6 mL of the extract was transferred to the QuEChERS dSPE (p/n 5982-5356) for highly pigmented fruits and vegetables for baby spinach; or 6 mL of the extract was transferred to the QuEChERS dSPE (p/n 5982-5056) general fruit and vegetables for honey and cucumber extract . Then the extracts were vortexed for 2 minutes, and centrifuged at 5,000 rpm for 5 minutes.

Table 1. Matrix selection and sample preparation used for optimal MRM application.

Category	Matrix	Sample prep
High oil	Extra virgin olive oil	3 g oil/7 mL water, EN salts (5982-5650), EMR-L (5982-1010), Polish Pouch (5982-0102), Dry step
Difficult	Black loose leaf tea	3 g tea/7 mL water, EN salts, EN dSPE pigment (5982-5256)
High pigment	Fresh leaf baby spinach	10 g, EN salts, EN dSPE pigment (5982-5356)
High starch	Jasmine rice	3 g rice/7 mL water, EN salts, EN dSPE Fatty (5982-5156)
High water	Basic cucumber	10 g, EN salts, EN dSPE General (5982-5056)
High sugar	Organic honey	5 g honey/5 mL water, EN salts, EN dSPE General (5982-5056)
High acid	Navel orange	10 g, EN salts, EN dSPE Fatty (5982-5156)
Clean 15	Yellow onion (not sweet)	10 g, EN salts, EN dSPE Fatty (5982-5156)

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A 3 g sample of extra virgin olive oil and 7 mL of water were vortexed for 2 minutes with two ceramic homogenizers. Ten milliliters of ACN were added, and the sample was vortexed for 2 minutes. The QuEChERS EN salts were added, and the tubes were placed on a GenoGrinder vertical shaker for 2 minutes, then centrifuged at 5,000 rpm for 5 minutes. Five milliliters of water was added to an EMR —Lipid tube (p/n 5982-1010) containing 1 g of EMR—Lipid sorbent, and vortexed for 30 seconds. Five milliliters of the ACN extract were added to the activated EMR—Lipid, vortexed for 2 minutes, and centrifuged at 5,000 rpm for 5 minutes. The entire extract was decanted into a 50 mL centrifuge tube, and the entire contents of a Polish Pouch (p/n 5982-0102) was added. The tube was capped, vortexed aggressively, and centrifuged at 5,000 rpm for 5 minutes. Four milliliters of the extract was transferred to a 15 mL centrifuge tube along with 300 mg/mL of $MgSO_4$ from a Polish Pouch. The tube was vortexed, then centrifuged at 5,000 rpm for 5 minutes.

After the final centrifugation, all sample extracts were transferred to their own 4 mL vial, and stored at –20 °C until analysis.

Instrumentation

All analyses were run on an Agilent 7890B GC equipped with an Agilent 7693B Autosampler and the Agilent 7010A Triple Quadrupole GC/MS. Tables 2 and 3 display the GC, backflush, and MS/MS method parameters. The GC was configured with a Multimode Inlet (MMI) equipped with an 4 mm ultra inert, splitless, single taper, glass wool liner (p/n 5190-2293). From the inlet, two HP-5ms UI columns (15 m × 0.25 mm, 0.25 µm; p/n 19091S-431 UI) were coupled to each other through a purged ultimate union (PUU) for the use of midcolumn/post run backflushing (Figure 1).

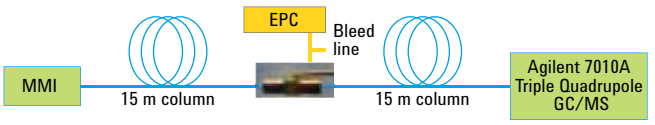

Figure 1. Column configuration for an optimal MRM application.

Table 2. Agilent 7890B GC method conditions.

Parameter	Value
MMI Injection mode	Hot-splitless
Injection volume	1 µL
Inlet temperature	280 °C
Carrier gas	He, constant flow 1.00 mL/min (column 2 = 1.20 mL/min)
Oven program	60 °C for 1 min 40 °C/min to 120 °C, 0 min 5 °C/min to 310 °C, 0 min
MS transfer line temperature	280 °C
PUU Backflush settings*	
Timing	1.5 min duration during post-run
Oven temperature	310 °C
Aux EPC pressure	~50 psi
Inlet pressure	~2 psi

* Backflush conditions optimized for application method in an Agilent Laboratory. A 1.5 minute backflush duration may be too short for other methods; recommendations can be made for a 5 minute backflush duration.

Table 3. Agilent 7010A Triple Quadrupole GC/MS parameters.

Parameter	Value
Electron energy	70 eV
Tune	atunes.eihs.tune.xmL
EM gain	10
MS1 and MS2 resolution	Wide
Collision cell	1.5 mL/min N_2 and 2.25 mL/min He
Quant/Qual transitions	Matrix optimized
Dwell times	Time segment (TS) specific*
Source temperature	300 °C**
Quad temperatures	150 °C

* All dwells in each TS were given the same value (no value under 10 was set) to attain a scan rate of ~5 scans/sec for the TS.

** The recommended source temperature is 280 °C. The source temp here was run hotter due to internal lab settings.

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Identification of Matrix Optimized MRM Transitions

Matrix effects have been a common complaint for MRM acquisitions in pesticides analysis. The usefulness of a given compound's MRMs can change depending on the matrix being analyzed. The ability to have multiple MRMs from which to choose aids in lab productivity, improved quant method generation, and achieving optimal analysis. Agilent offers the most comprehensive GC MRM Database for Pesticides and Environmental Pollutants (Figure 2). This MRM Database contains 1,100+ compounds and up to 10 MRMs/compound. The all-inclusive database provides a surplus of MRMs to aid in accurate identification, use MRMs that fall within the ion ratio confidence limits, and avoid matrix effects.

Figure 2. Screen capture of the top portion of the Target Compound List from the Agilent MassHunter P&EP MRM Standard Database (A.04.00).

Globally, there are a multitude of different applications and regulations that are followed. The P&EP MRM Database provides all of the material for users to identify the optimal MRMs for their specific analysis. To further provide guidance on identifying optimal MRMs, Agilent has analyzed 195 target compounds in a variety of matrices to analyze (as well as in ACN; Figure 3). The top five MRM transitions for each target compound were selected based on response, ion ratios, and selectivity. From these, the top three to four MRMs were transferred to a matrix-specific method for further analysis.

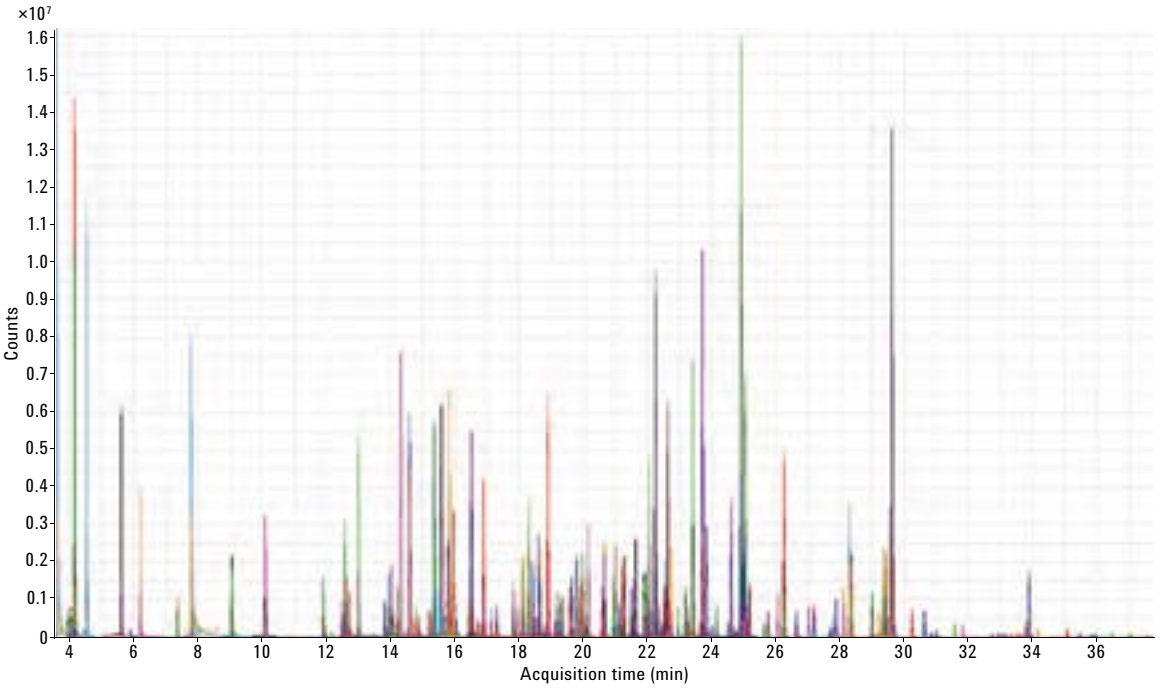

Figure 3. Chromatogram of all target compounds in acetonitrile (~200-400 ppb; compound dependent).

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Changes in quant (Q0) and qual ions (Q1, Q2, ...)

The majority of pesticides analyzed indicated that the responses of the optimal MRM transitions often change when in different matrixes. Figures 4-11 and Tables 4-11 display examples of various target compounds and their ACN solvent-based MRMs compared to specific matrix-optimized MRMs.

Matrix effects are real

Figures 12-14 and Tables 12-14 illustrate a few examples of various target compounds and their ACN solvent-based MRMs compared to specific matrix-optimized MRMs. These figures also illustrate the various matrix effects that can occur, such as ion supression, ion enhancement, RT shift, and MRM transition interferences.

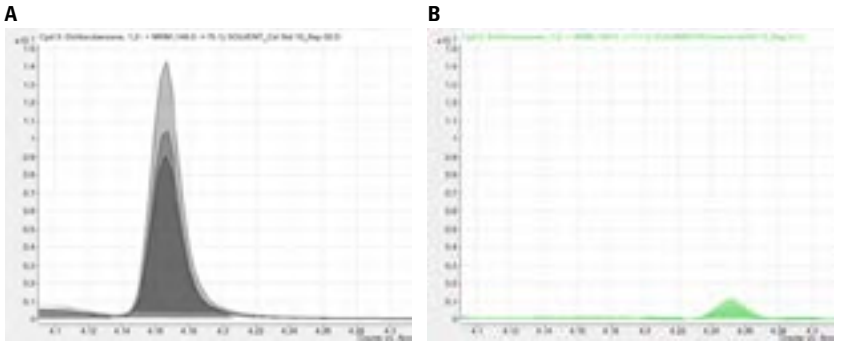

Figure 4. Chromatogram of MRM transitions of 1,2-dichlorobenzene in ACN (A) and cucumber (B).

Table 4. ACN solvent-based and matrix-optimized MRMs in cucumber for 1,2-dichlorobenzene.

	Solvent MRMs			Cucumber MRMs		
Ion	*m/z*	prod.	CE	*m/z*	prod.	CE
Q0	148	75.1	25	146	75.1	25
Q1	111	75.1	10	146	111.1	15
Q2	146	75.1	25	111	75.1	10

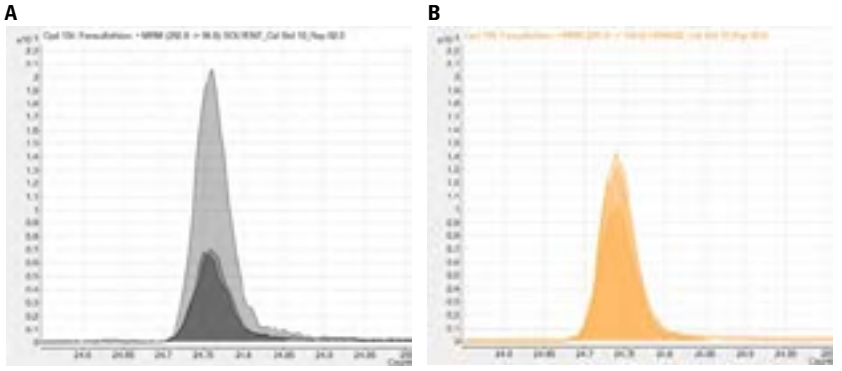

Figure 5. Chromatogram of MRM transitions of fensulfothion in ACN (A) and navel orange (B).

Table 5. ACN solvent-based and matrix-optimized MRMs in navel orange for fensulfothion.

	Solvent MRMs			Navel orange MRMs		
Ion	*m/z*	prod.	CE	*m/z*	prod.	CE
Q0	292.8	96.8	20	140	125	10
Q1	140	125	10	156	141	10
Q2	156	141	10	291.8	156	15

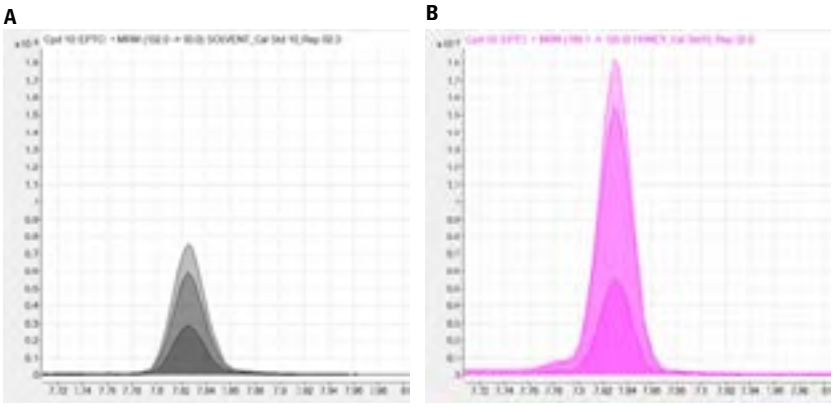

Figure 6. Chromatogram of MRM transitions of EPTC in ACN (A) and organic honey (B).

Table 6. ACN solvent-based and matrix-optimized MRMs in organic honey for EPTC.

	Solvent MRMs			Organic honey MRMs		
Ion	*m/z*	prod.	CE	*m/z*	prod.	CE
Q0	128	86	5	128	86	5
Q1	132	90	5	132	90	5
Q2	132	62	10	189.1	128	5

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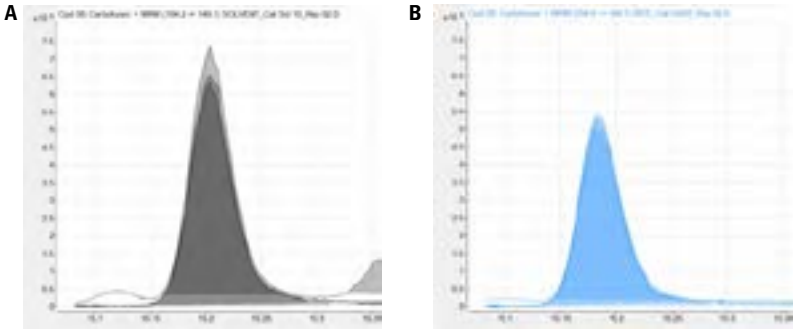

Figure 7. Chromatogram of MRM transitions of carbofuran (A) in ACN and jasmine rice (B).

Table 7. ACN solvent-based and matrix-optimized MRMs in jasmine rice for carbofuran.

	Solvent MRMs			Jasmine rice MRMs		
Ion	*m/z*	prod.	CE	*m/z*	prod.	CE
Q0	149.1	77.1	30	149.1	77.1	30
Q1	164.2	149.1	10	164.2	149.1	10
Q2	164.2	103.1	25	164.2	103.1	25

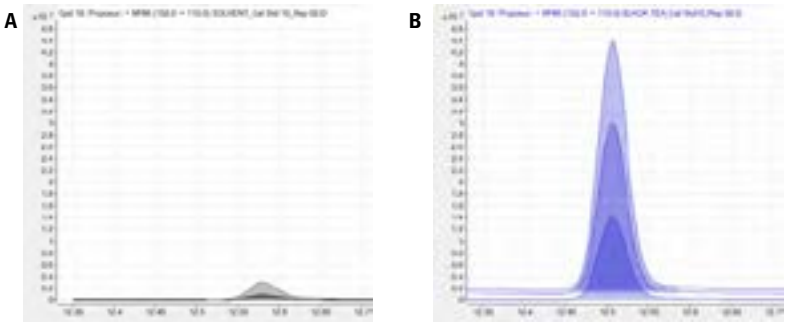

Figure 8. Chromatogram of MRM transitions of propoxur in ACN (A) and black tea (B).

Table 8. ACN solvent-based and matrix-optimized MRMs in black tea for propoxur.

	Solvent MRMs			Black tea MRMs		
Ion	*m/z*	prod.	CE	*m/z*	prod.	CE
Q0	110	63	25	110	63	25
Q1	152	110	10	110	64	15
Q2	110	92	10	152	110	10

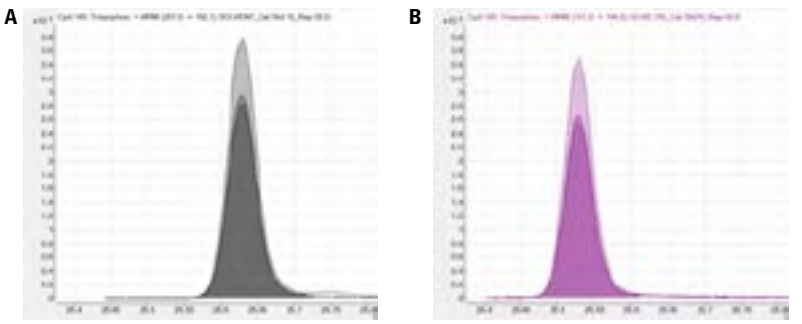

Figure 9. Chromatogram of MRM transitions of aldrin in ACN (A) and yellow onion (B).

Table 9. ACN solvent-based and matrix-optimized MRMs in yellow onion for aldrin.

	Solvent MRMs			Yellow onion MRMs		
Ion	*m/z*	prod.	CE	*m/z*	prod.	CE
Q0	262.9	192.9	35	262.9	192.9	35
Q1	254.9	220	20	262.9	190.9	35
Q2	262.9	190.9	35	254.9	220	20

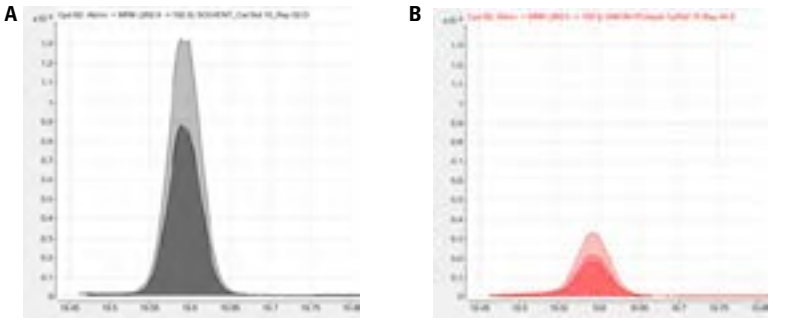

Figure 10. Chromatogram of MRM transitions of triazophos in ACN (A) and extra virgin olive oil (B).

Table 10. ACN solvent-based and matrix-optimized MRMs in extra virgin olive oil for triazophos.

	Solvent MRMs			Extra virgin olive oil MRMs		
Ion	*m/z*	prod.	CE	*m/z*	prod.	CE
Q0	161.2	134.2	5	161.2	134.2	5
Q1	161.2	106.1	10	161.2	106.1	10
Q2	257	162.1	5	161.2	91	15

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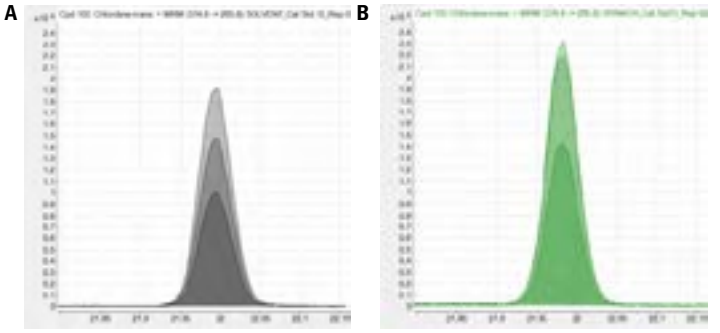

Figure 11. Chromatogram of MRM transitions of *trans*-chlordane in ACN (A) and spinach (B).

Table 11. ACN solvent-based and matrix-optimized MRMs in baby spinach for *trans*-chlordane.

	Solvent MRMs			Baby spinach MRMs		
Ion	*m/z*	prod.	CE	*m/z*	prod.	CE
Q0	271.8	236.9	15	372.8	265.9	25
Q1	372.8	265.9	25	271.7	236.9	15
Q2	374.8	265.8	15	374.8	265.8	15

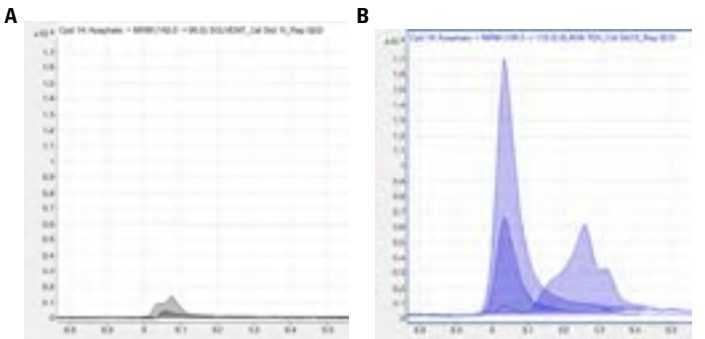

Figure 12. Chromatogram of MRM transitions of acephate in ACN (A) and in black tea (B).

Table 12. ACN solvent-based and matrix-optimized MRMs in black tea for acephate.

	Solvent MRMs			Black tea MRMs		
Ion	*m/z*	prod.	CE	*m/z*	prod.	CE
Q0	136	94	15	142	96	5
Q1	78.9	47	10	78.9	47	10
Q2	142	96	5	124.9	47	15

Table 13. ACN solvent-based and matrix-optimized MRMs in extra virgin olive oil, and matrix-optimized MRMs in jasmine rice for vamidothion.

	Solvent MRMs			Extra virgin olive oil MRMs			Jasmine rice MRMs		
Ion	*m/z*	prod.	CE	*m/z*	prod.	CE	*m/z*	prod.	CE
Q0	141.9	78.9	10	145	87	5	145	87	5
Q1	145	87	5	141.9	78.9	10	141.9	78.9	10
Q2	108.9	78.9	5	108.9	78.9	5	108.9	78.9	5

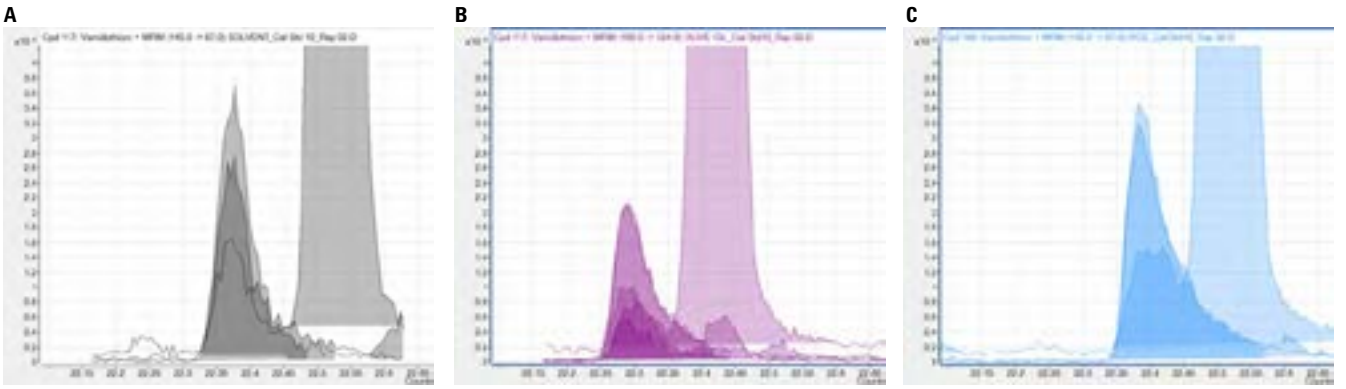

Figure 13. Chromatograms of MRM transitions of vamidothion in ACN (A), extra virgin olive oil (B), and jasmine rice (C).

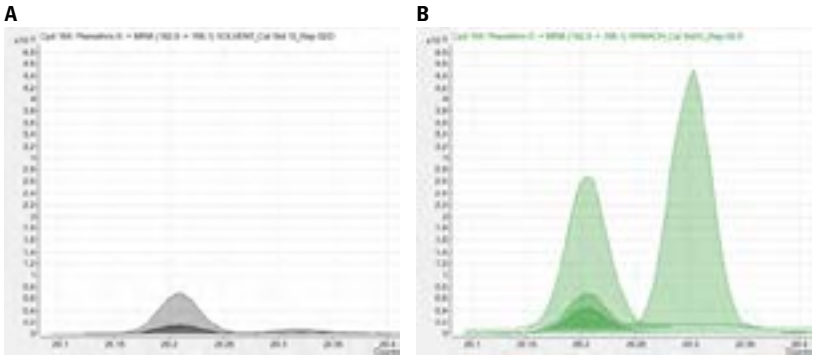

Figure 14. Chromatogram of MRM transitions of phenothrin II in ACN (A) and spinach (B).

Table 14. ACN solvent-based and matrix-optimized MRMs in baby spinach for phenothrin II.

	Solvent MRMs			Spinach MRMs		
Ion	*m/z*	prod.	CE	*m/z*	prod.	CE
Q0	122.9	81.1	5	122.9	81.1	5
Q1	182.9	168.1	10	122.9	79.1	20
Q2	182.9	153.1	15	182.9	168.1	10

Results and Discussion

The 7010A Series Triple Quadrupole GC/MS system can confirm pesticide residues at the low ppb level even in the most complex extracts. The top three to four MRMs for each target compound in each of the eight different matrixes were used for all of the analyses. The calibration standards were prepared at concentrations ranging from 0.12 pg/µL to 50 pg/µL. As a result, 90% of all target compounds achieved a calibration curve with a $R^2 \geq 0.990$. All analyzed pesticides obtained a %RSD of repeated measurements of ≤30%, and 90% of the analyzed pesticides were found to have a limit of quantitation (LOQ) ≤1.5 pg/µL. A representative selection of compounds and their calculated values are shown for organic honey and baby spinach compared to ACN solvent (Tables 15-17)

Table 15. A representative selection of compounds and their calculated values are shown for analysis in acetonitrile.

Compound	%RSD	IDL_{RSD} (pg)	MDL (pg/µL)	iLOQ (pg/µL)	%Error
Ethoprophos	11.13	0.39	0.41	1.48	5.72
BHC-*alpha*	9.38	0.33	0.34	1.24	5.51
Dazomet	11.15	0.39	0.41	1.49	6.81
BHC-*beta*	9.27	0.32	0.34	1.23	5.80
Aminocarb	19.89	0.69	0.74	2.67	6.75
Phenanthrene-D10	7.68	0.27	0.28	1.01	5.19
Diazinon	9.63	0.33	0.35	1.27	5.69
2,4-D butyl ester	15.67	0.54	0.58	2.08	6.35
Chlorpyrifos-methyl	9.96	0.35	0.36	1.32	5.37
Triadimefon	12.71	0.44	0.46	1.68	5.41
Heptachlor endo-epoxide	9.57	0.66	0.70	2.53	5.41
Flurenol-butyl	9.09	0.31	0.33	1.19	5.47
Chlordane-*cis*	8.35	0.29	0.31	1.10	5.26
DDT-*o,p´*	4.42	0.15	0.16	0.59	5.31
Hexazinone	11.71	0.41	0.43	1.56	5.67
Azinphos-ethyl	9.01	0.31	0.33	1.19	5.45
Permethrin, (1R)-*trans*-	10.89	0.38	0.40	1.43	5.05

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Table 16. A representative selection of compounds and their calculated values are shown for analysis in organic honey.

Compound	%RSD	IDL_{RSD} (pg)	MDL (pg/µL)	iLOQ (pg/µL)	%Error
Ethoprophos	8.72	0.30	0.29	1.06	3.11
BHC-*alpha*	7.83	0.27	0.26	0.94	4.01
Dazomet	4.38	0.15	0.15	0.55	0.45
BHC-*beta*	17.19	0.60	0.54	1.96	9.10
Aminocarb	8.40	0.29	0.29	1.03	2.09
Phenanthrene-D10	6.59	0.23	0.22	0.79	4.92
Diazinon	7.33	0.25	0.24	0.86	6.15
2,4-D butyl ester	8.09	0.28	0.26	0.94	7.08
Chlorpyrifos-methyl	7.76	0.27	0.25	0.91	6.42
Triadimefon	4.26	0.15	0.14	0.50	6.97
Heptachlor endo-epoxide	7.75	0.54	0.49	1.78	8.13
Flurenol-butyl	6.85	0.23	0.22	0.79	6.32
Chlordane-*cis*	13.08	0.45	0.41	1.49	9.42
DDT-*o,p'*	8.78	0.31	0.27	0.98	11.35
Hexazinone	4.91	0.17	0.16	0.57	7.85
Azinphos-ethyl	13.77	0.48	0.44	1.58	8.53
Permethrin, (1R)-*trans*-	10.25	0.35	0.34	1.21	5.57

Table 17. A representative selection of compounds and their calculated values are shown for analysis in baby spinach.

Compound	%RSD	IDL_{RSD} (pg)	MDL (pg/µL)	iLOQ (pg/µL)	%Error
Ethoprophos	8.25	0.29	0.30	1.07	3.18
BHC-*alpha*	7.94	0.28	0.28	1.00	0.16
Dazomet	9.10	0.32	0.32	1.17	2.22
BHC-*beta*	8.76	0.30	0.30	1.10	0.24
Aminocarb	9.76	0.34	0.36	1.31	6.91
Phenanthrene-D10	8.95	0.31	0.31	1.13	0.49
Diazinon	5.78	0.20	0.20	0.73	0.46
2,4-D butyl ester	19.66	0.68	0.69	2.49	1.13
Chlorpyrifos-methyl	7.04	0.24	0.24	0.88	0.20
Triadimefon	10.17	0.35	0.36	1.31	2.95
Heptachlor endo-epoxide	7.17	0.50	0.49	1.77	1.25
Flurenol-butyl	18.80	0.64	0.65	2.35	1.13
Chlordane-*cis*	21.67	0.75	0.75	2.71	0.14
DDT-*o,p'*	23.04	0.80	0.79	2.84	1.84
Hexazinone	7.40	0.26	0.26	0.95	2.10
Azinphos-ethyl	16.08	0.56	0.56	2.04	1.25
Permethrin, (1R)-*trans*-	22.36	0.77	0.79	2.87	2.56

Conclusions

The growing demand on the global agriculture industry has increased the number of targeted pesticides, and expanded to include a multitude of different matrixes. Not only are analytical laboratories faced with time constraints, but they also face matrix effects that degrade their ability to accurately identify and quantitate the multitude of target pesticides. There were 195 target compounds analyzed in eight various matrixes spanning multiple varieties.

The following observations recognized:

- Changes in Q0 and Q1, Q2, ... responses are the most common. These changes merely affect the relative abundances of the MRMs, which plays a part in method development for optimum quantitative data analysis.
- The availability of multiple MRMs per compound allows a user to discriminate among compounds with similar transitions, and to select MRMs that fulfill desired ion ratio confidence limits.
- The main challenges come from extremely large matrix effects, which are encountered more often in complex matrixes such as loose leaf black tea or spinach. The number of usable MRMs for a given target compound can be reduced, and the shift in retention time can push a target out of a time segment.

In these cases, great care must be exercised to produce accurate results for all analytes. Overall, matrix-optimized MRM transitions aid in lab productivity, improved quant method generation, and optimal analysis.

The Agilent G9250AA Rev. A.04.00 Pesticides and Environmental Pollutants (P&EP) Standard MRM Database is the most comprehensive GC MRM database on the market. With the evolving market and demand for matrix-optimized transitions, the Agilent P&EP 4.0 Analyzer includes the addition of 7,800 matrix-optimized transitions to provide customers with their optimal pesticides analysis.

References

1. Anastassiades, M.; Lehotay, S. J.; Štajnbaher, D.; Schenck, F. S. J. *AOAC Int.* **2003**, *86*, 412-431.
2. Lehotay, S. J.; Mastovská, K.; Lightfield, A. R. J. *AOAC Int.* **2005**, *88*, 615-629.

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Accurately Identify and Quantify One Hundred Pesticides in a Single GC Run

Application Note

Author

Jessica Westland
Agilent Technologies, Inc.

Abstract

A selected target compound list of 195 various pesticides was chosen for the evaluation of both the traditional time segment (TS) acquisition and the dMRM acquisition structures. Not only were the MRM acquisition setup procedures examined, but the acquired data were also evaluated. As sample complexity increases, the ability to use dMRM will provide laboratories with the capability to better tackle their large multi-analyte analysis, and to accurately quantify trace quantities of pesticides from high-throughput methods. The use of dynamic MRM (dMRM) acquisition method development provides users the ability to achieve equivalent or better quality data and results by:

- Monitoring the MRM transitions based on the compounds' retention times as they elute from GC
- Reducing the number of MRM transitions active at any given time, allowing for longer dwell times
- Optimizing the dwell times to maintain a constant MS cycle time and constant sampling rate across all peaks

Introduction

The global agriculture industry uses over a thousand different pesticides for the production of food and foodstuffs. Producers require pesticides to meet the increasing demand for reasonably priced food. Analytical laboratories are then strained to evaluate and quantitate hundreds of pesticides in a single run. Currently, GC/MS/MS MRM analyses use time segment (TS) acquisition methods. TS methods focus on specified MRM transitions within a fixed retention time (RT) window. The more transitions in a time segment, the lower the dwell time and thus the sensitivity of the data acquired. Adding new compounds to the method usually results in redoing the time segments manually, and can be very time-consuming. Using the automated process of dynamic MRM (dMRM) acquisition saves a large amount of method development time. dMRM uses retention time locking (RLT) of the GC/MS system to set the RT of concurrent MRM transitions in a RT window. This automated procedure determines the number of these transitions to group in a RT window based on dwell criteria entered by the user to determine optimal sensitivity for the instrument.

Experimental

Sample preparation

Many laboratories focused on pesticide residue analysis in food commodities routinely use the Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) method [1,2]. This straightforward sample preparation allows for the analysis of hundreds of pesticides at low concentrations with a single extraction.

A selection of eight different matrices were analyzed. These commodities included yellow onion, navel orange, organic honey, basic cucumber, jasmine rice, fresh leaf baby spinach, black loose leaf tea, and extra virgin olive oil [3]. Each matrix was extracted with a specified QuEChERS methodology, in which various dispersive SPE (dSPE) were used for matrix cleanup (Table 1).

Instrumentation

All analyses were run on an Agilent 7890B GC equipped with an Agilent 7693B Autosampler and an Agilent 7010A Triple Quadrupole GC/MS. Table 2 displays the GC and backflush parameters, and Tables 3 and 4 show the MS/MS method parameters for TS and dMRM, respectively. The GC was configured with a multimode inlet (MMI) equipped with an 4 mm ultra inert, splitless, single taper, glass wool liner (p/n 5190-2293). From the inlet, two Agilent J&W HP-5ms Ultra Inert columns (15 m × 0.25 mm, 0.25 µm; p/n 19091S-431 UI) were coupled to each other through a purged ultimate union (PUU) for the use of midcolumn/post run backflushing (Figure 1).

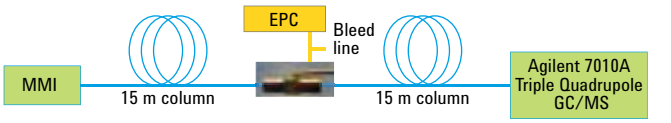

Figure 1. Column configuration for an optimal MRM application.

Table 1. Matrix Selection and Sample Preparation Used for Optimal MRM Application.

Category	Matrix	Sample Prep
High oil	Extra virgin olive oil	3 g oil/7 mL water, EN salts (5982-5650), EMR—L (5982-1010), Polish Pouch (5982-0102), Dry step
Difficult	Black loose leaf tea	3 g tea/7 mL water, EN salts, EN dSPE pigment (5982-5256)
High pigment	Fresh leaf baby spinach	10 g, EN salts, EN dSPE pigment (5982-5356)
High starch	Jasmine rice	3 g rice/7 mL water, EN salts, EN dSPE Fatty (5982-5156)
High water	Basic cucumber	10 g, EN salts, EN dSPE General (5982-5056)
High sugar	Organic honey	5 g honey/5 mL water, EN salts, EN dSPE General (5982-5056)
High acid	Navel orange	10 g, EN salts, EN dSPE Fatty (5982-5156)
Clean 15	Yellow onion (not sweet)	10 g, EN salts, EN dSPE Fatty (5982-5156)

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MS Acquisition Method Development

The Agilent MassHunter Pesticide & Environmental Pollutant MRM Database (Rev. A.04.00) and Matrix Optimized Transitions [3] were used to develop the MRM acquisition methods for the evaluation of 195 target pesticides in each matrix (Figure 2). Both the 40 minute and 20 minute constant flow methods referenced in the MRM Database were followed. The top three (highest responding) MRMs for each compound were selected for analysis.

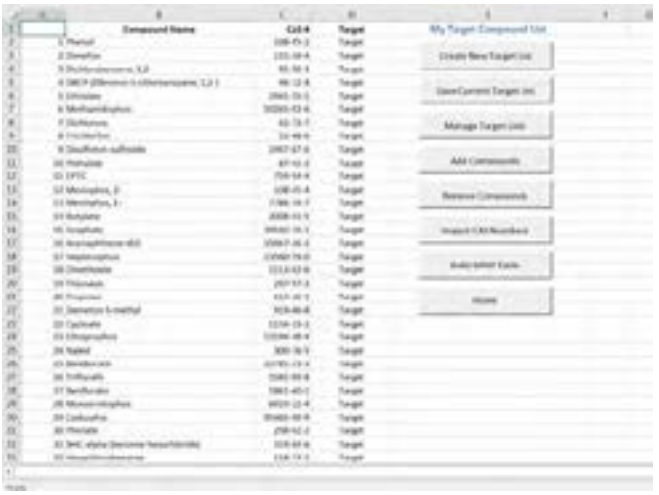

Figure 2. Screen capture of the top portion of the Target Compound List from the P&EP MRM Database (A.04.00).

Table 2. Agilent 7890B GC method conditions.

Parameter	Value
MMI Injection mode	Hot-splitless
Injection volume	1 µL
Inlet temperature	280 °C
Carrier gas	He, constant flow 1.00 mL/min (column 2 = 1.20 mL/min)
MS transfer line temperature	280 °C
Oven program (40 minute method)	60 °C for 1 min 40 °C/min to 120 °C, 0 min 5 °C/min to 310 °C, 0 min
Oven program (20 minute method)	60 °C for 1 min 40 °C/min to 170 °C, 0 min 10 °C/min to 310 °C, 3 min
PUU Backflush settings*	
Timing	1.5 min duration during post run
Oven temperature	310 °C
Aux EPC pressure	~50 psi
Inlet pressure	~2 psi

* Backflush conditions are optimized for an application method in an Agilent Laboratory. A 1.5 minute backflush duration may be too short for other methods; recommendations can be made for a 5 minute backflush duration.

Table 3. Agilent 7010A Triple Quadrupole GC/MS time segment (TS) MRM parameters.

Parameter	Value
Electron energy	70 eV
Tune	atunes.eihs.tune.xmL
EM gain	10
MS1 and MS2 resolution	Wide
Collision cell	1.5 mL/min N_2 and 2.25 mL/min He
Quant/Qual transitions	Matrix Optimized
Dwell times	Time Segment (TS) specific*
Source temperature	300 °C**
Quad temperatures	150 °C

* All dwells in each TS were given the same value (no value under 10 was set) to attain a scan rate of ~5 scans/sec for the TS.

** The recommended source temperature is 280 °C. The source temperature here was run hotter due to internal lab settings.

Table 4. Agilent 7010A Triple Quadrupole GC/MS dynamic MRM (dMRM) parameters.

Parameter	Value
Electron energy	70 eV
Tune	atunes.eihs.tune.xmL
EM gain	10
MS1 and MS2 resolution	dMRM unit
Collision cell	1.5 mL/min N_2 and 2.25 mL/min He
Quant/Qual transitions	Matrix optimized
Dwell times	Optimized by dMRM*
Source temperature	300 °C
Quad temperatures	150 °C

* All dwells in each dMRM RT window were given the same value (no value under 10 was set) to attain a scan rate of ~5 scans/sec for the TS.

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Time Segment Method Development

Time segment acquisition development was completed using the graphical user interface (GUI) in the MRM Database and the MassHunter Compound List Assistant (CLA). The Organic Honey Matrix Optimized MRM Database was used as an example for the TS method development (Figure 3). After the Target List was created, the **Build MRM Table** option was selected (Figure 4). Two selections are needed for the development of the MRM Table:

- Method selection (the 40 minute constant flow method was selected in this example)
- Quantifier and qualifier ion selections (Figure 5)

Figure 3. The GUI Homepage of the Organic Honey Matrix Optimized MRM Database, used for TS method development.

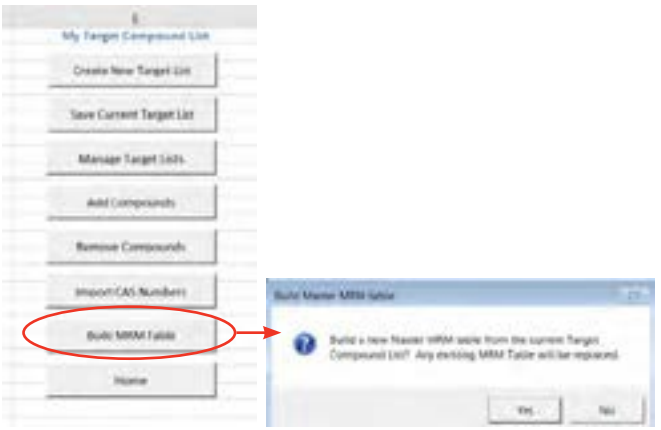

Figure 4. Selecting **Build MRM Table** from the generated Target Compound List.

A

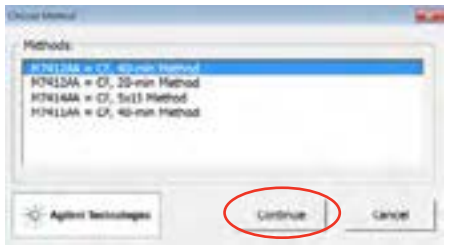

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Figure 5. Two selections for MRM Table development: A) Method Selection (the 40 minute M7412AA method option was selected here); B) Quant and Qual Ion selections.

Once the MRM Table was completed, the **Export for CLA Optimizer** option was selected, and the CLA program was launched. The Database saved this export file as a .csv file, and was then imported into the CLA (Figure 6). The optimization parameters were set to use a constant cycle time of 5 msec throughout each TS (Figure 7). The RT deltas can also be edited within the CLA. The method was saved and loaded into MassHunter GC/MS Data Acquisition (DA) B.07.05 (Figure 8).

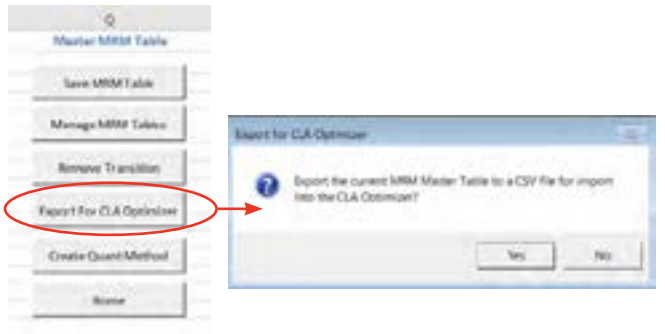

Figure 6. Exporting the MRM Table to a .csv file for the CLA.

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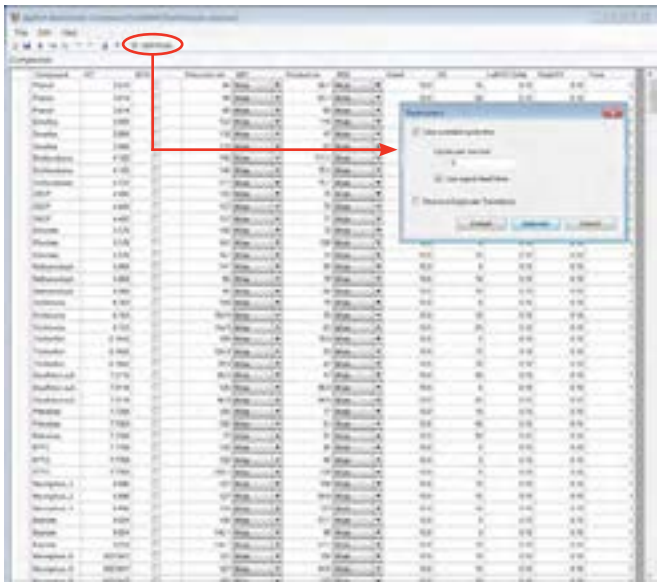

Figure 7. The acquisition optimization parameters were set to use a constant cycle time, of 5 cycles/sec, throughout each TS.

Key Elements of TS method development

- **Typical method development time**: ~ 5 minutes
- **Adding target compounds**: One-by-one selection or import CAS# list
- **Removing target compounds**: One-by-one selection
- **Adding MRM transitions**: Recreation of the MRM Table from the Target List
- **Removing MRM transitions**: One-by-one selection; must rerun CLA to re-optimize
- **Quant and Qualifier selection**: Same selection and amount for each target compound
- **Use of CLA for method optimization**: RT deltas can be set one-by-one or filled down within columns; dwell optimization by algorithm or constant cycles/sec

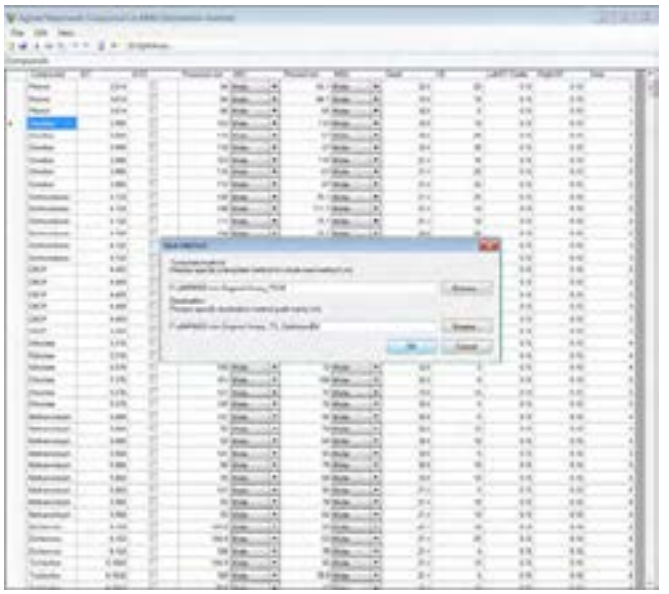

Figure 8. The TS MRM method was saved by the CLA, and made ready for Agilent MassHunter GC/MS Data Acquisition.

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dMRM Method Development

dMRM acquisition development was completed using the MS Method Editor within MassHunter Workstation GC/MS Acquisition Software. From within the MS Parameters of MassHunter GC/MS Data Acquisition (B.07.05), the Organic Honey Matrix Optimized MRM Database was imported, and the 40 minute M7412AA constant flow method was selected (Figure 9). The MRM Acquisition Method page is where all of the target compounds for the method are shown (Figure 10). The Compound Browser was used to locate target compounds and their respective MRMs (the same target list and ions were used as the TS method development). Once chosen, the MRMs are applied to the Import List (Figure 11). The Import List maintains all of the target compounds that are to be used in the method, and their respective MRMs. Once the target list is finalized, they are imported to the Method (Figure 12). The Method Acquisition page is where the RT deltas can be edited, the cycles/sec can be defined, and the dwell times are optimized (Figure 13). Figure 14 displays a view of the 20 minute dMRM acquisition method for the same Target List and respective MRMs.

Figure 9. The Organic Honey Matrix Optimized MRM Database was imported into the MS Parameters of Agilent MassHunter GC/MS Data Acquisition B.07.05.

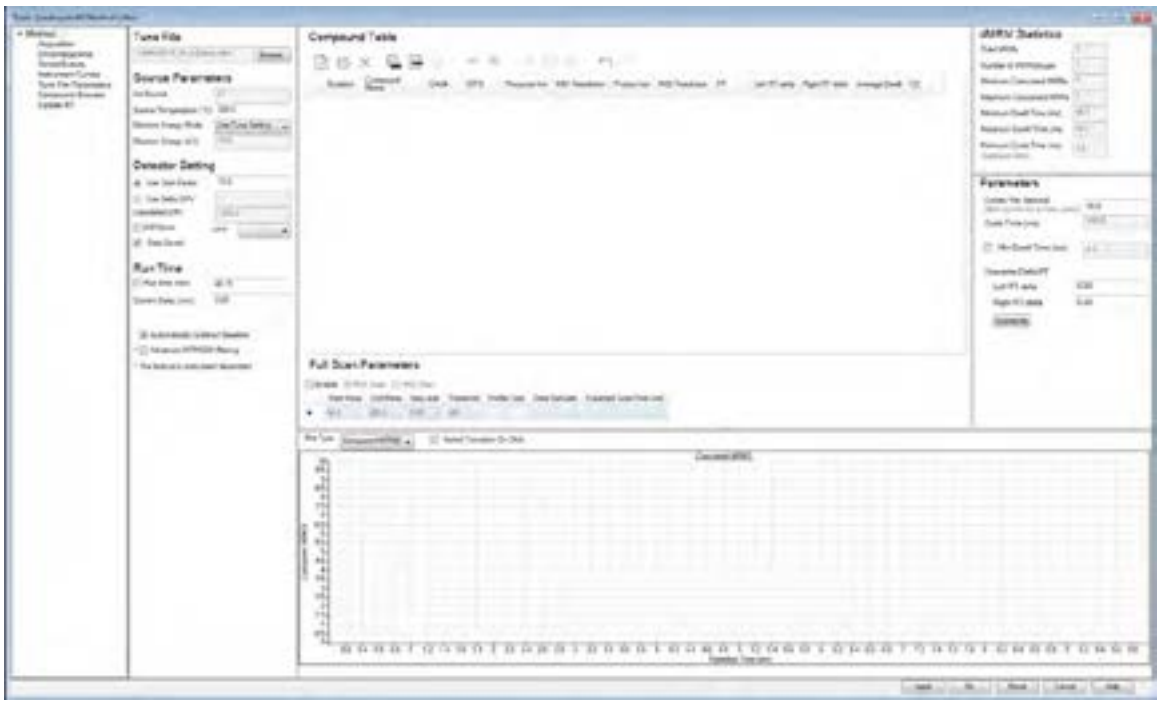

Figure 10. A blank Agilent MRM Acquisition Method page.

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Figure 11. The compound browser displays all of the compounds and respective MRMs that are within the loaded Database.

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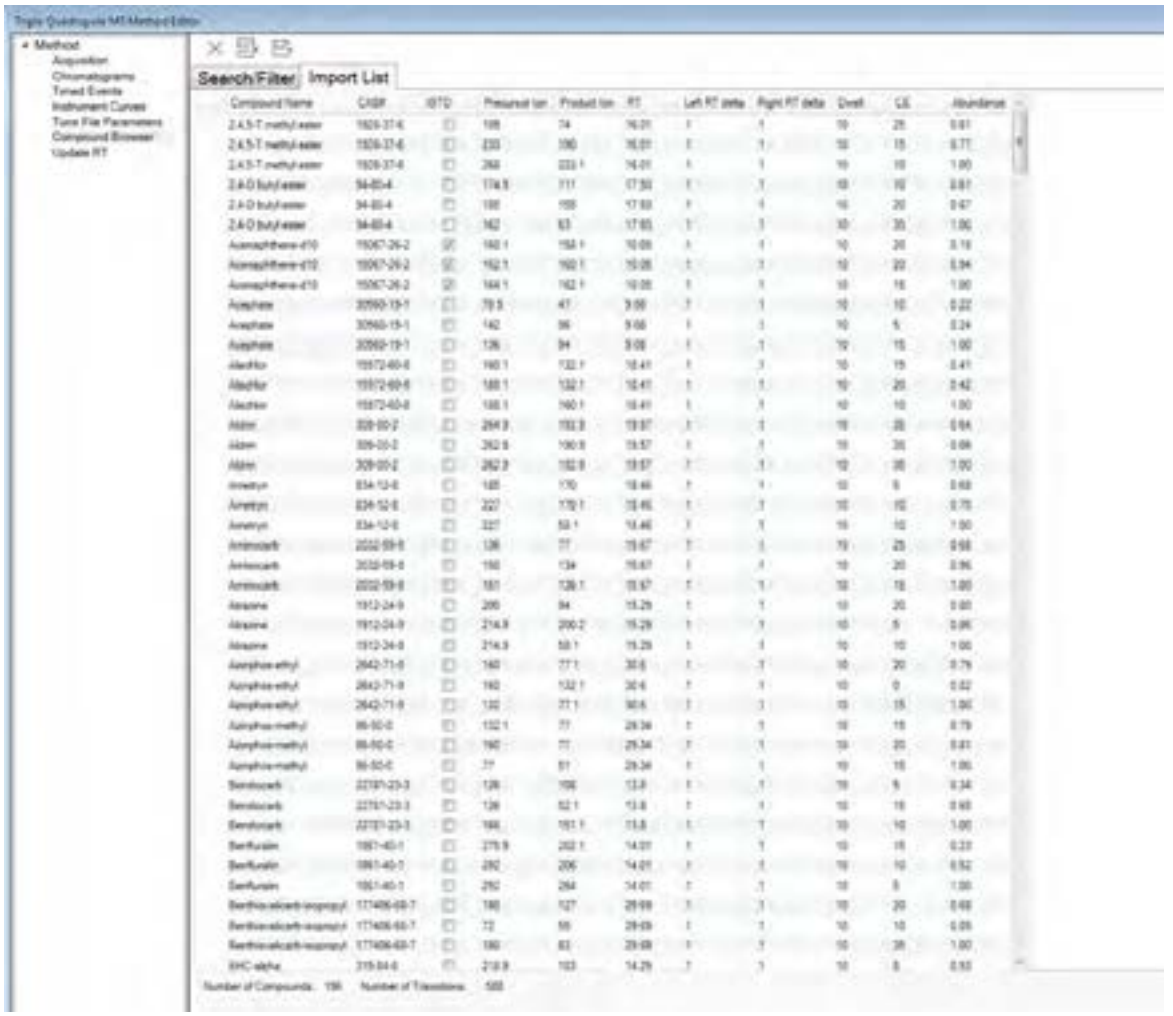

Figure 12. The Import List maintains all of the target compounds and their respective MRMs that are intended for the acquisition method.

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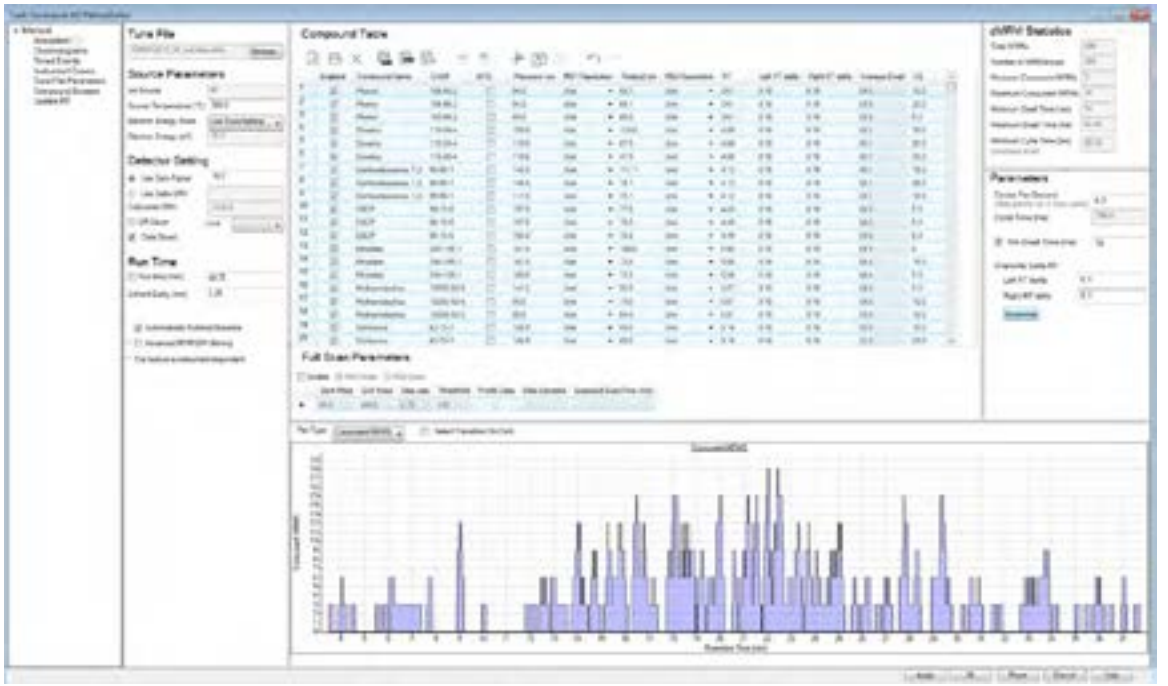

Figure 13. The Method Acquisition page shows the Target List and respective MRMs for the 40 minute method.

Figure 14. The Method Acquisition page shows the same Target List and respective MRMs for the 20 minute method.

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Key elements of dMRM method development

- **Typical method development time**: ~5-10 minutes depending on how detailed the MS method is
- **Adding target compounds**: One-by-one selection, group selection, or searching a CAS# list
- **Removing target compounds**: One-by-one or multiple selection
- **Adding MRM transitions**: One-by-one or multiple selection
- **Removing MRM transitions**: One-by-one or multiple selection removal
- **Quant and qualifier selection**: Same selection for all or choice for each target compound
- **Use of MassHunter DA for method optimization**: RT deltas can be set one-by-one or filled down within columns; dwell optimization by algorithm or user-defined settings

Evaluation

The dMRM acquisition method provides users with another way to set up their MS acquisition method parameters. Whether the user chooses to use TSs or the dMRM functionalities, they both aid in achieving optimal analysis. Figures 15-20 are various selected chromatograms that were observed and analyzed in both TS and dMRM acquisition methods.

Results and Discussion

There are two ways to view the difference in the chromatographic displays:

- MassHunter Qualitative Analysis Software (B.07.00 SP1, or later)
- MassHunter Quantitative Analysis Software (B.07.01, or later)

Figures 19-26 show a selected representation of the 195 target compounds in various matrices. The concentration shown for the various target analytes ranged between 180-380 ppb. A higher concentration was used for viewing ability; further analysis was done showing that 90% of all target compounds achieved a calibration curve with $R^2 \geq 0.990$. All analyzed pesticides obtained a %RSD of repeated measurements of ≤30%, and 90% of the analyzed pesticides were found to have a limit of quantitation (LOQ) ≤1.5 pg/µL [3].

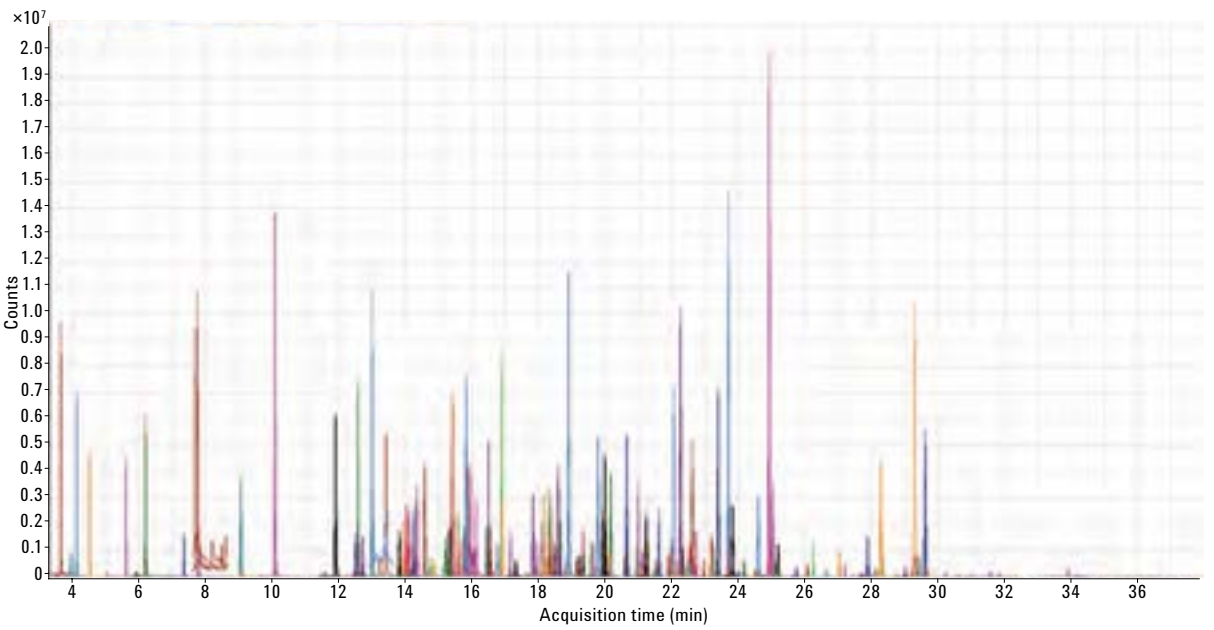

Figure 15. Organic honey 40 minute analysis chromatogram of 195 target compounds with three MRM transitions per compound using the TS MS parameters.

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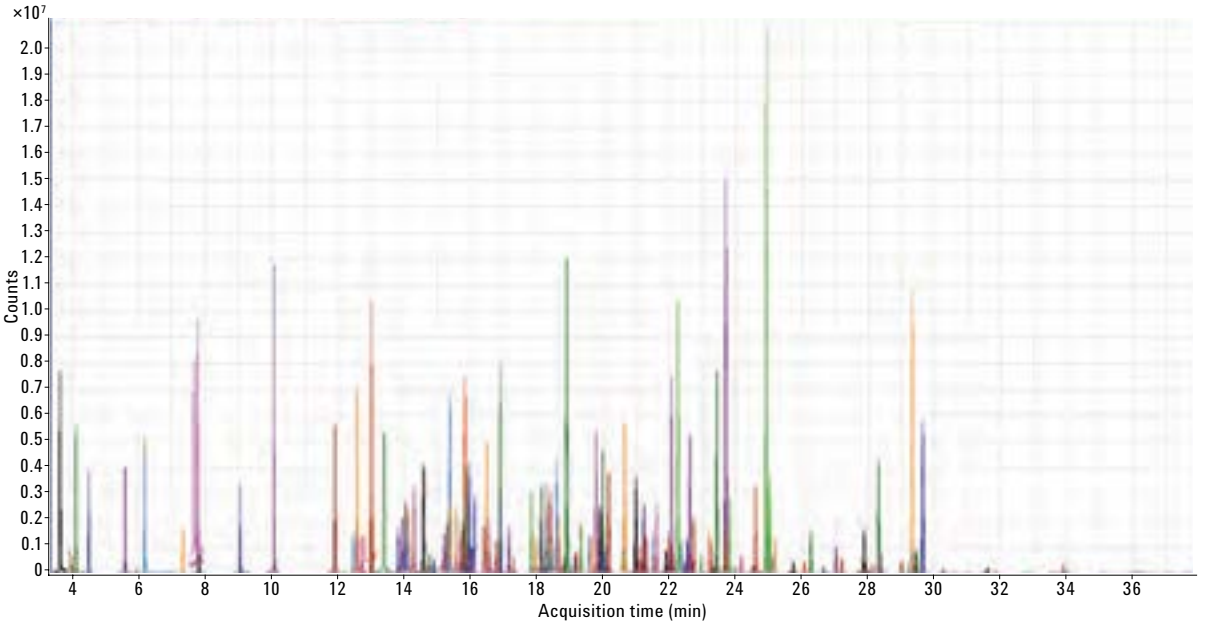

Figure 16. Organic honey 40 minute analysis chromatogram of 195 target compounds with three MRM transitions per compound using the dMRM MS parameters.

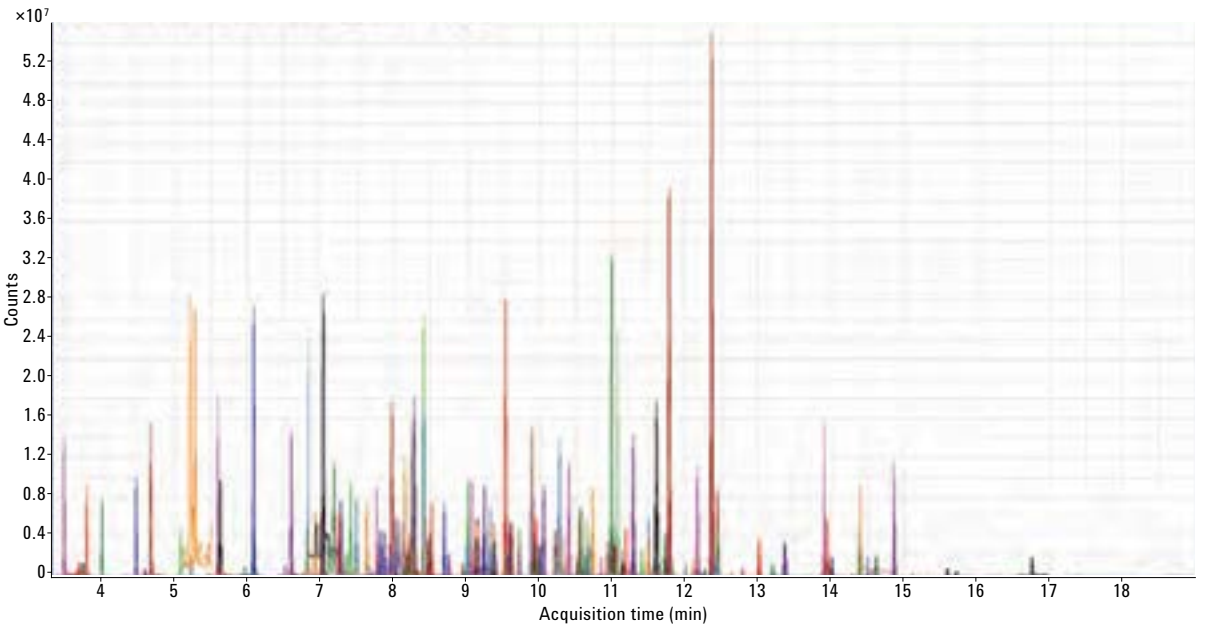

Figure 17. Organic honey 20 minute analysis chromatogram of 195 target compounds with three MRM transitions per compound using the dMRM MS parameters.

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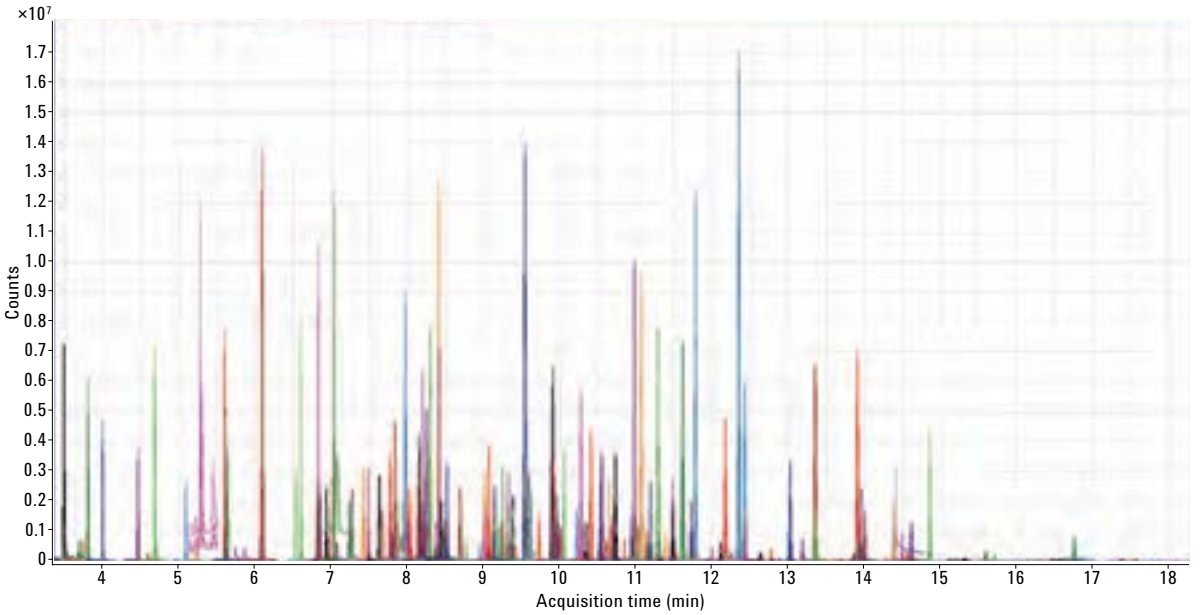

Figure 18. Extra virgin olive oil 20 minute analysis chromatogram of 195 target compounds with three MRM transitions per compound using the dMRM MS parameters.

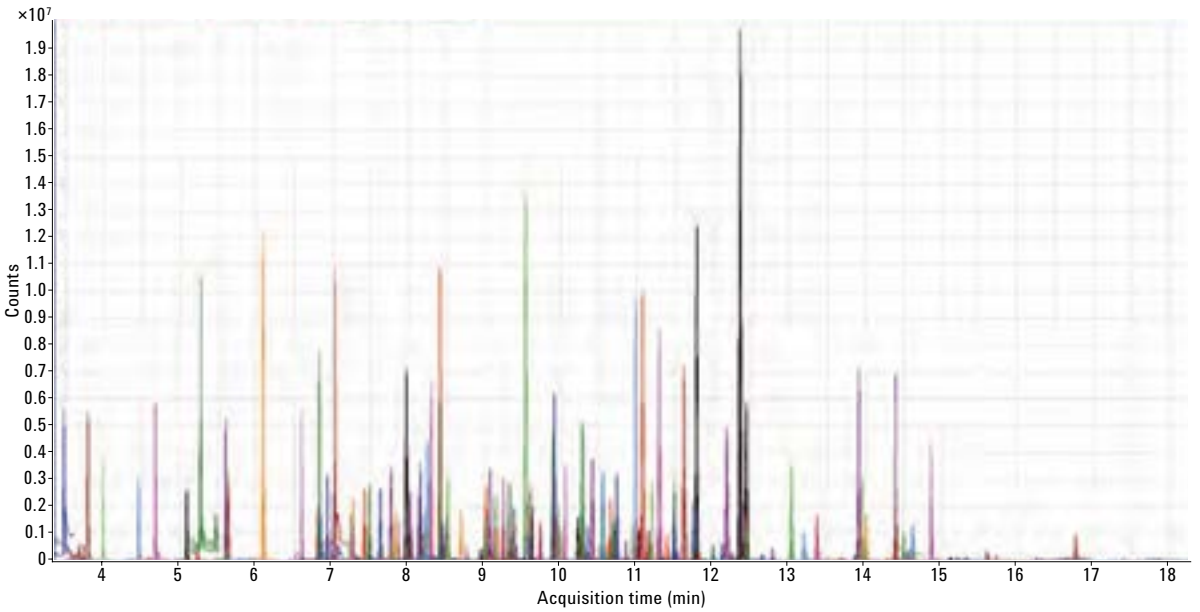

Figure 19. Navel orange 20 minute analysis chromatogram of 195 target compounds with three MRM transitions per compound using the dMRM MS parameters.

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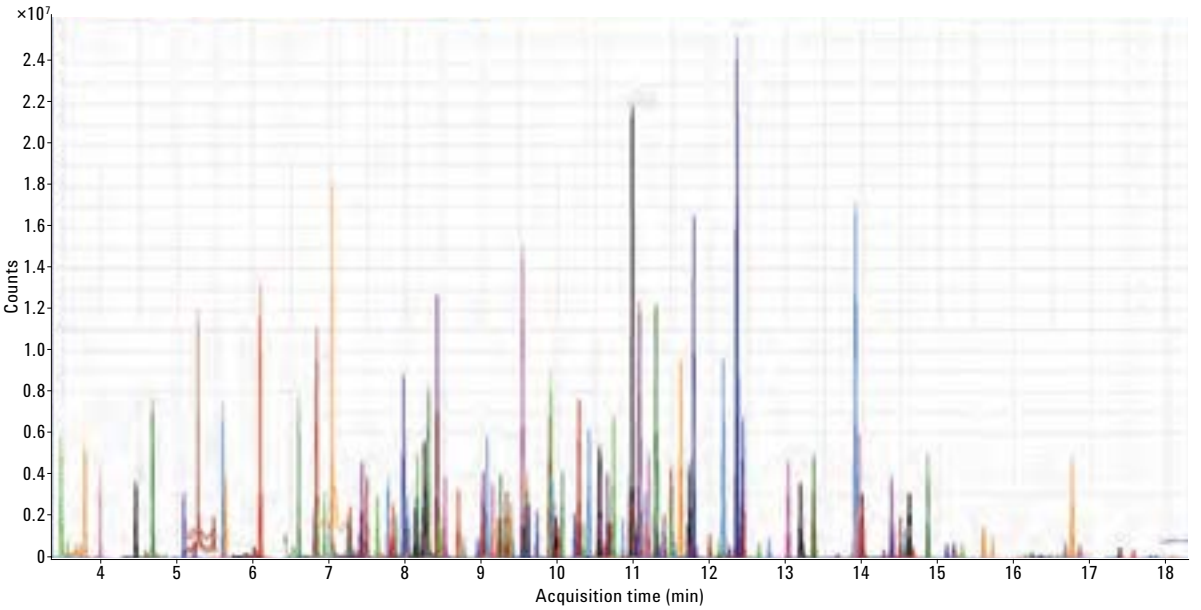

Figure 20. Fresh leaf baby spinach 20 minute analysis chromatogram of 195 target compounds with three MRM transitions per compound using the dMRM MS parameters.

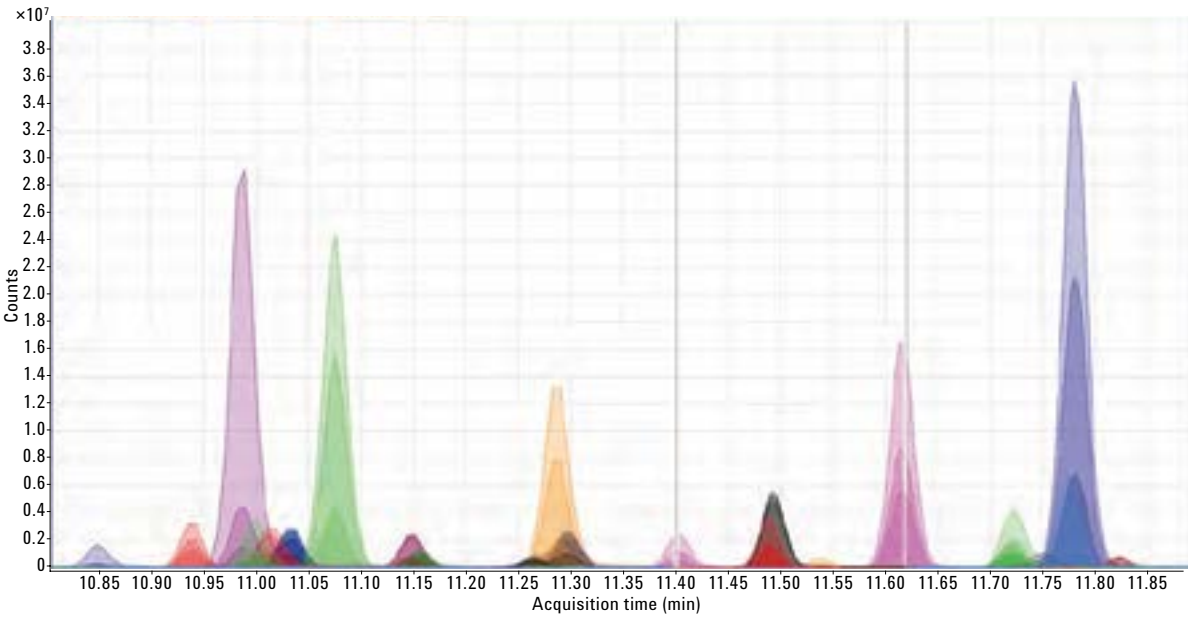

Figure 21. Organic honey TS chromatogram of RT range (40 minute method) in Agilent MassHunter Qualitative Analysis.

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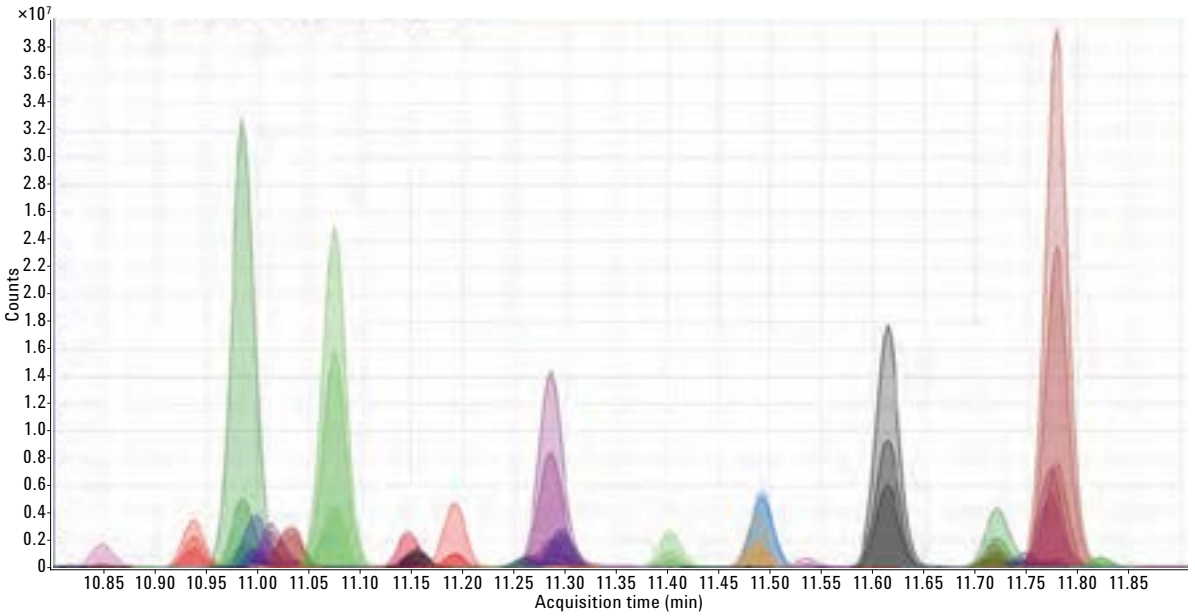

Figure 22. Organic honey dMRM chromatogram of RT range (40 minute method) in Agilent MassHunter Qualitative Analysis.

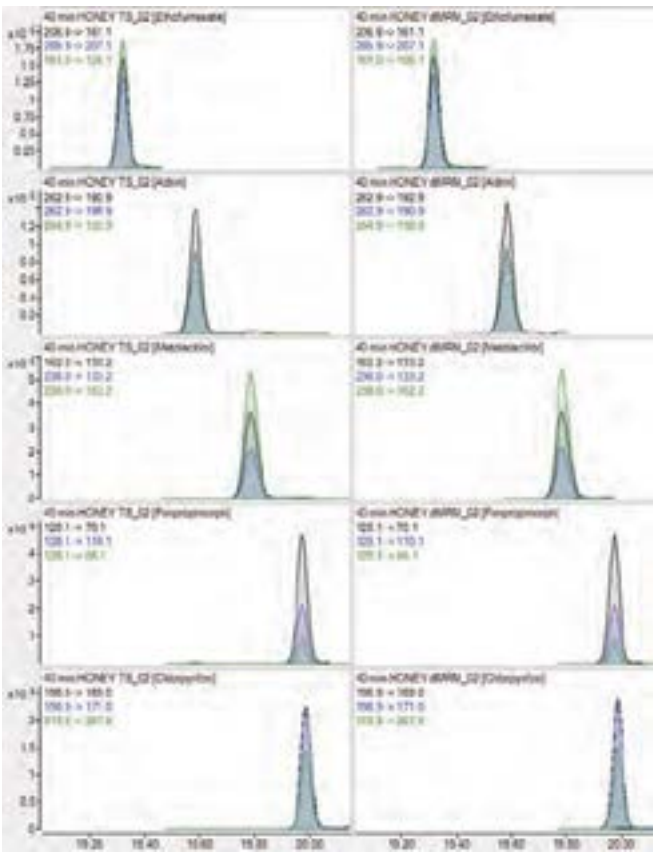

Figure 23. Organic honey TS chromatograms (left) and dMRM chromatograms (right) of selected compounds for RT range (40 minute method) in Agilent MassHunter Quantitative Analysis.

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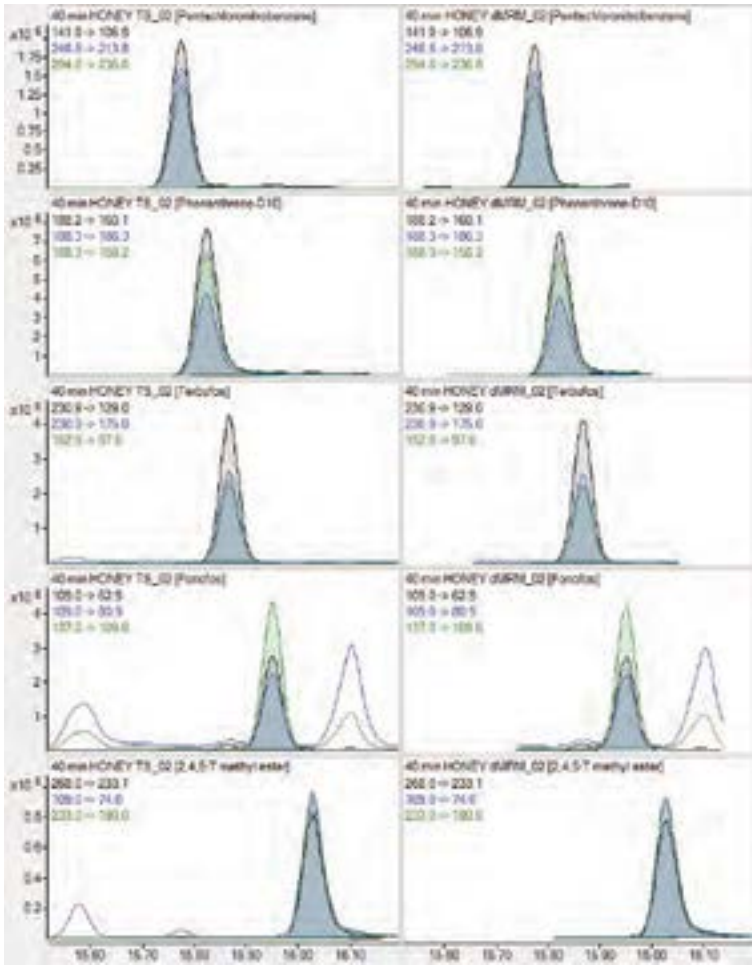

Figure 24. Organic honey TS chromatograms (left) and dMRM chromatograms (right) of selected compounds for RT range (40 minute method) in Agilent MassHunter Quantitative Analysis.

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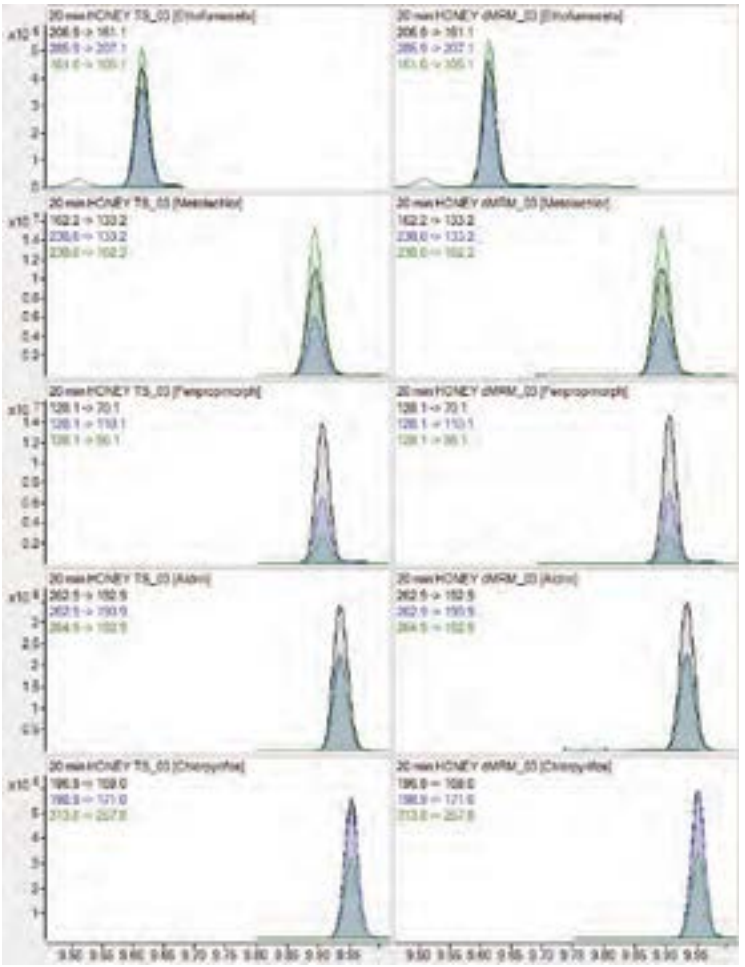

Figure 25. Organic honey TS chromatograms (left) and dMRM chromatograms (right) of selected compounds for RT range (20 minute method) in Agilent MassHunter Quantitative Analysis

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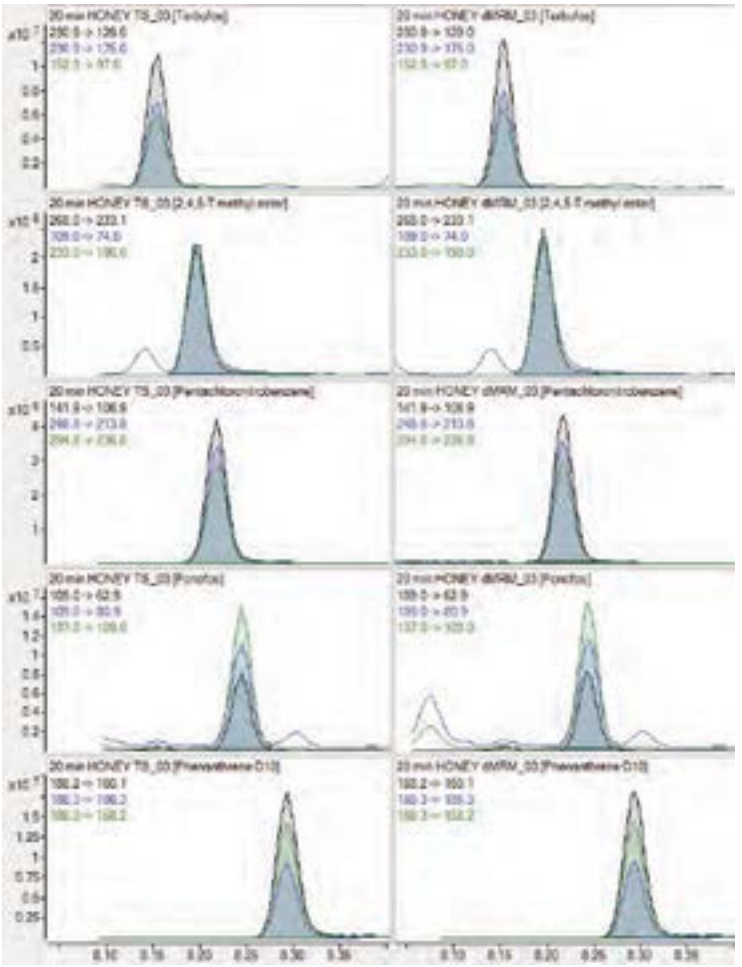

Figure 26. Organic honey TS chromatograms (left) and dMRM chromatograms (right) of selected compounds for RT range (20 minute method) in Agilent MassHunter Quantitative Analysis.

Conclusions

Standard GC/MS/MS Pesticide methods use TS acquisition methods with a gain of 10, dwell times of 10 msec, and 2-3 MRMs/compound. The Agilent MassHunter Data Acquisition's dMRM functionality for MS acquisition method development provides users the ability to achieve equivalent or better quality data and results by:

- Monitoring the MRM transitions based on the compounds' retention times as they elute from the GC
- Reducing the number of MRM transitions active at any given time allowing for longer dwell times
- Optimizing the dwell times to maintain a constant MS cycle time and constant sampling rate across all peaks

As sample complexity increases, the ability to use dMRM will provide laboratories with the capability to better tackle their large multi-analyte analysis, and to accurately quantify trace quantities of pesticides from high-throughput methods.

References

1. Anastassiades, M.; Lehotay, S. J.; Štajnbaher, D.; Schenck, F. S. J. *AOAC Int.* **2003**, *86*, 412-431.
2. Lehotay, S. J.; Mastovská, K.; Lightfield, A. R. J. *AOAC Int.* **2005**, *88*, 615-629.
3. Westland, J.; Stevens, J. *An Optimal Method for the Analysis of Pesticides in a Variety of Matrices*; Application note, Agilent Technologies, Inc. Publication number 5991-7303EN, **2016**.

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April 12, 2017
5991-7302EN

Agilent Technologies

Maintaining Sensitivity and Reproducibility with the Agilent JetClean Self-Cleaning Ion Source for Pesticides in Food and Feed

Application Note

Author

Jessica Westland
Agilent Technologies, Inc.

Abstract

Approximately 200 various pesticides were analyzed in organic honey extract on the Agilent 7010A Series Triple Quadrupole GC/MS with and without the use of the Agilent JetClean self-cleaning ion source. The chromatographic peak shape and baseline improved with the use of JetClean at 0.13 mL/min continuous H_2 flow, particularly for the later eluting, higher molecular weight (MW) analytes. The resulting R^2 values both with and without JetClean were very comparable. The MDLs were calculated from 10 replicate measurements of 2.5 ppb spiked honey extract using a 99 % confidence level. Low ppb MDLs were obtained for the majority of the analytes using JetClean, with an average of 0.170 ppb MDL without use of JetClean, and an average of 0.147 ppb with JetClean. The replicate measurements performed with and without JetClean at 2.5 ppb resulted in comparable %RSDs. All results identified that the use of a low continuous flow of H_2 into the MS source can be considered as an option for maintaining performance during pesticide analyses.

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Introduction

The global agricultural industry uses over a thousand different pesticides for food and foodstuffs cultivation. Producers are compelled to use pesticides to meet the growing demand for reasonably priced food, resulting in the need for pesticide residue monitoring in commodities worldwide. Concurrently, simple sample preparation methods, such as Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) are routinely used for the analysis of food and feed samples, often leaving a significant amount of matrix in the extracts. Analytical laboratories are challenged by these matrix residues, which negatively affect the responses of the analyzed pesticides, and eventually require source cleaning.

The use of the Agilent JetClean self-cleaning ion source (JetClean) reduces the time between manual source cleanings while still allowing for the analysis of complex samples without losing sensitivity and reproducibility [1]. The JetClean self-cleaning ion source introduces a precisely measured hydrogen gas (H_2) flow into the MS source, controlled by Agilent MassHunter Data Acquisition Software (B.07.05). The appropriate H_2 flow (µL/min) generates conditions that clean the surfaces of the source, the lenses, and other components. These actions aid in maintaining a stable detection environment and provide for response stability of the pesticides in difficult matrixes. The JetClean is equipped with two operational modes:

- Acquire and Clean (also known as On-line) mode: H_2 is running continuously during the analysis
- Clean only (also known as Off-line) mode: H_2 is introduced only post run or post sequence

Experimental

Sample preparation

Many laboratories focused on pesticide residue analysis in food commodities routinely use the QuEChERS method [2,3]. This straightforward sample preparation allows for the analysis of hundreds of pesticides at low concentrations with a single extraction. A 5-g sample of organic honey with 5 mL of water was vortexed with two ceramic homogenizers. Ten milliliters of acetonitrile (ACN) was added, and the sample was vortexed for 2 minutes. The QuEChERS EN salts (p/n 5982-5650) were added, and the capped tubes were placed on a GenoGrinder vertical shaker for 2 minutes, then centrifuged at 5,000 rpm for 5 minutes. Six milliliters of the honey extract was transferred to the QuEChERS dSPE (p/n 5982-5056) general fruit and vegetables. Then, the extract was vortexed for 2 minutes, and centrifuged at 5,000 rpm for 5 minutes [4].

Instrumentation

All analyses were run on an Agilent 7890B GC equipped with an Agilent 7693B Autosampler and the Agilent 7010A Triple Quadrupole GC/MS. Table 1 displays the GC and backflush parameters, and Table 2 shows the MS/MS method parameters. The GC was configured with a Multimode Inlet (MMI) equipped with an 4 mm ultra inert, splitless, single taper, glass wool liner (p/n 5190-2293). From the inlet, two Agilent J&W DB-5ms Ultra Inert columns (15 m × 0.25 mm, 0.25 µm; p/n 19091S-431 UI) were coupled to each other through a purged ultimate union (PUU) for the use of midcolumn/post run backflushing (Figure 1).

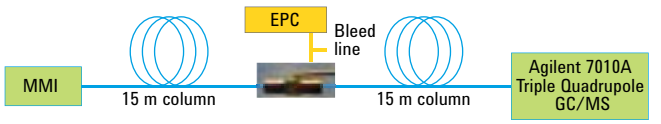

Figure 1. Column configuration for an optimal MRM application.

Table 1. Agilent 7890B GC Method Conditions

Parameter	Value
MMI Injection mode	Hot-splitless
Injection volume	1 µL
Inlet temperature	280 °C
Carrier gas	He, constant flow 1.00 mL/min (column 2 = 1.20 mL/min)
MS transfer line temperature	280 °C
Oven program (40-minute method)	60 °C for 1 minute, 40 °C/min to 120 °C, 0 minutes 5 °C/min to 310 °C, 0 minutes
PUU Backflush settings*	
Timing	1.5-minute duration during post run
Oven temperature	310 °C
Aux EPC pressure	~50 psi
Inlet pressure	~2 psi

* Backflush conditions were optimized for this application method.

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MS Acquisition Method Development

The organic honey matrix-optimized transitions of the Agilent MassHunter Pesticide & Environmental Pollutant Enhanced MRM Database (Rev. A.04.00) was used to develop the MRM method for the evaluation of 195 target pesticides (Figure 2) [5]. The top three (highest responding) MRMs for each compound were selected for analysis.

Table 2. Agilent 7010A MS/MS Parameters

Parameter	Value
Electron energy	70 eV
Tune	atunes.eihs.tune.xmL
EM gain	10
MS1 and MS2 resolution	Wide
Collision cell	1.5 mL/min N_2 and 2.25 mL/min He
Quant/Qual transitions	Matrix-optimized
Dwell times	Time segment (TS) specific*
Source temperature	300 °C
Quad temperatures	150 °C
Cleaning operation	Acquire & Clean
H_2 flow (mL/min)	0.13 mL/min**

* All dwells in each TS were given the same value (no value under 10 was set) to attain a scan rate of ~5 scans/sec for the TS

** H_2 flow (mL/min) was set at the lowest achievable flow

Agilent JetClean Operation

Previously, the introduction of the H_2 flow into the MS source was introduced through an EPC module. The next stage of Agilent innovative technology, the JetClean self-cleaning ion source, moves the control of H_2 flow to the MS (Figure 3). This application used JetClean in the Acquire and Clean operation mode for continuous on-line cleaning (Figures 4–6). The Agilent MassHunter software allowed for the simple setup and operation of the process, all controlled in the MS domain.

Figure 2. Screen capture of the top portion of the Target Compound List from the Agilent P&EP MRM Enhanced Database (A.04.00).

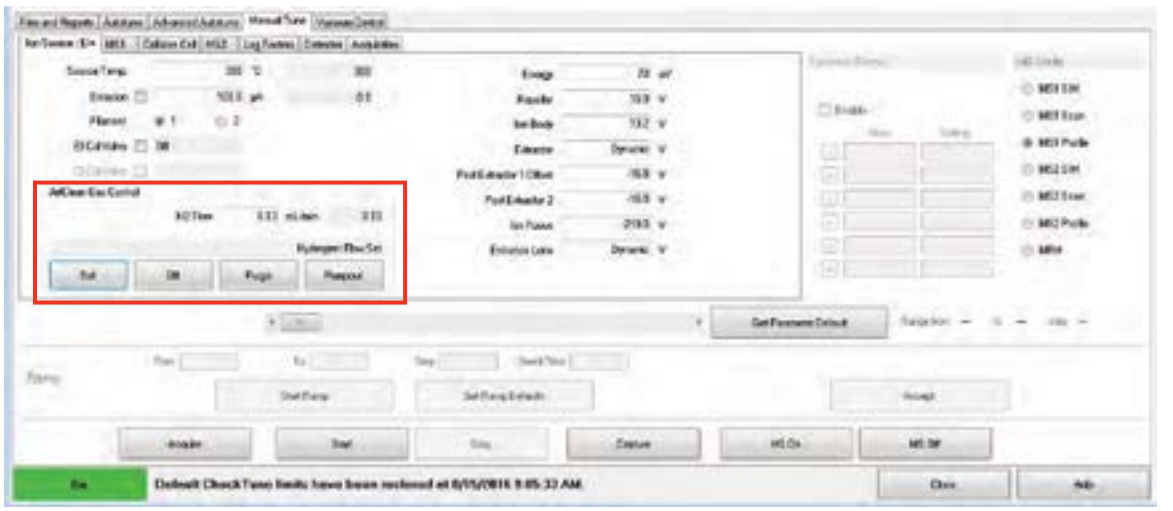

Figure 3. View of Agilent MassHunter Data Acquisition Triple Quadrupole MS Tune (B.07.05) and the Agilent JetClean Gas Control.

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Figure 4. View of Agilent MassHunter Data Acquisition Triple Quadrupole MS Method Editor Jet Clean Settings (B.07.05).

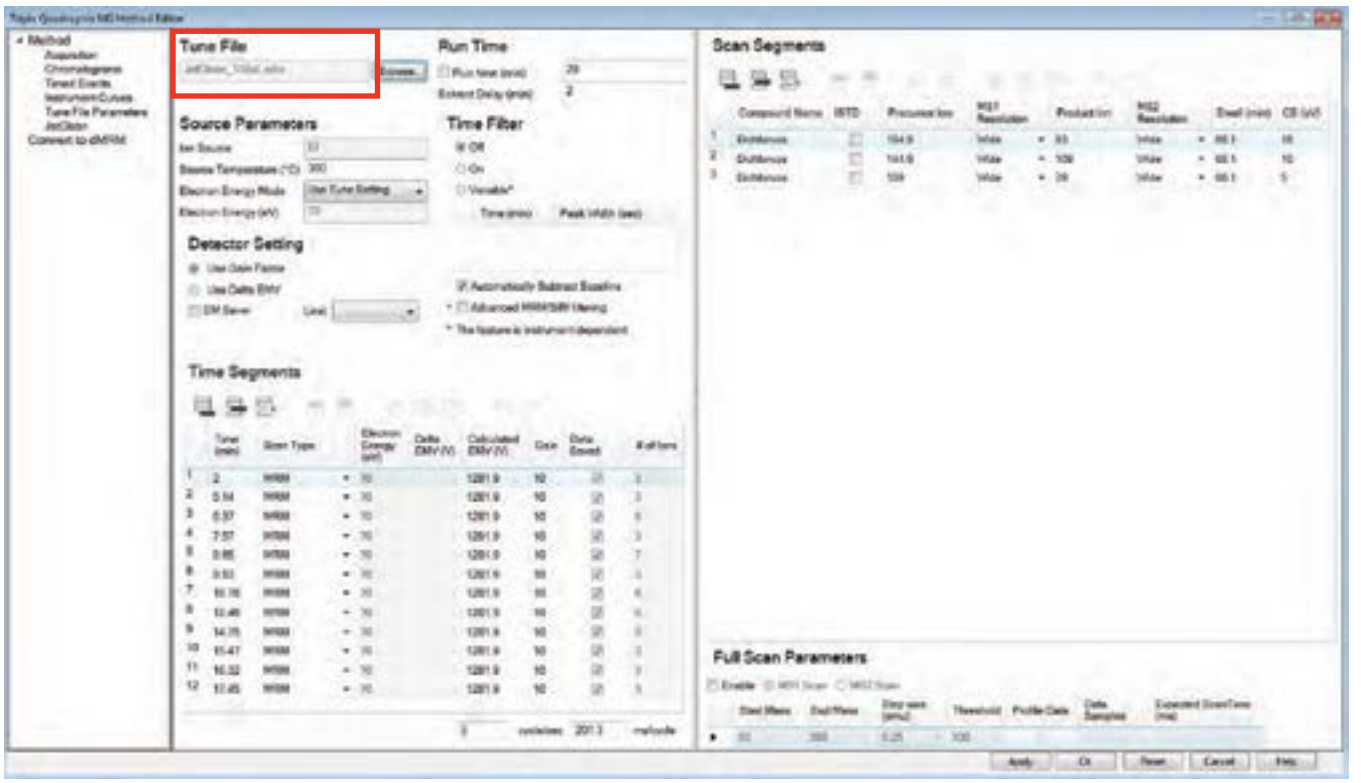

Figure 5. View of Agilent MassHunter Data Acquisition Triple Quadrupole MS Method Editor Acquisition Tab with loaded Agilent JetClean Tune File (B.07.05).

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Figure 6. View of Agilent MassHunter Data Acquisition Instrument Control (B.07.05) with JetClean monitors.

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Chromatographic Performance

The following chromatograms (Figures 7–13) show analytes eluting throughout the 40-minute chromatographic run at ~2.5 ppb in organic honey (concentration varies by compound). The chromatograms are of target compounds and their respective matrix-optimized MRM transitions with and without the use of JetClean. Chromatographically, the use of JetClean was seen to improve peak shape and the baseline on the later-eluting, higher molecular weight (MW) analytes.

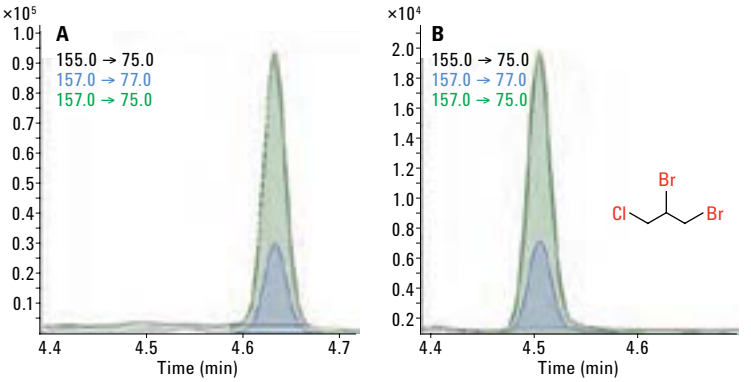

Figure 7. Example chromatograms for DBCP (in organic honey) without Agilent JetClean (A) and with Agilent JetClean Acquire and Clean at 0.13 mL/min (B).

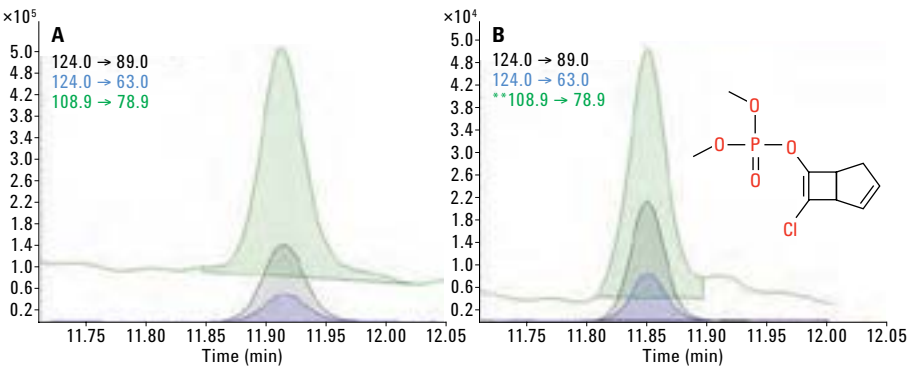

Figure 8. Example chromatograms for heptenophos (in organic honey) without Agilent JetClean (A) and with Agilent JetClean Acquire and Clean at 0.13 mL/min (B).

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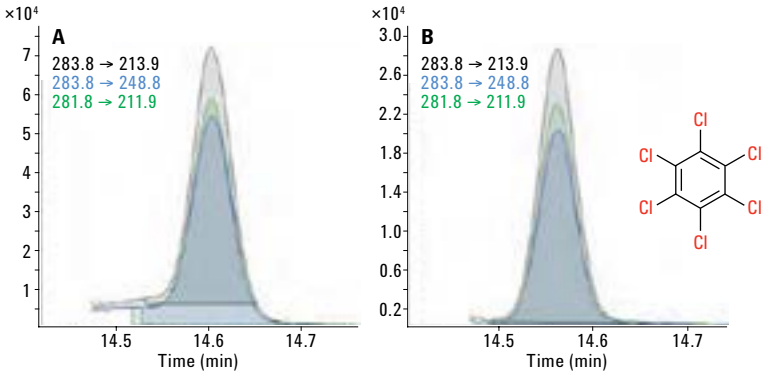

Figure 9. Example chromatograms for hexachlorobenzene (in organic honey) without Agilent JetClean (A) and with Agilent JetClean Acquire and Clean at 0.13 mL/min (B).

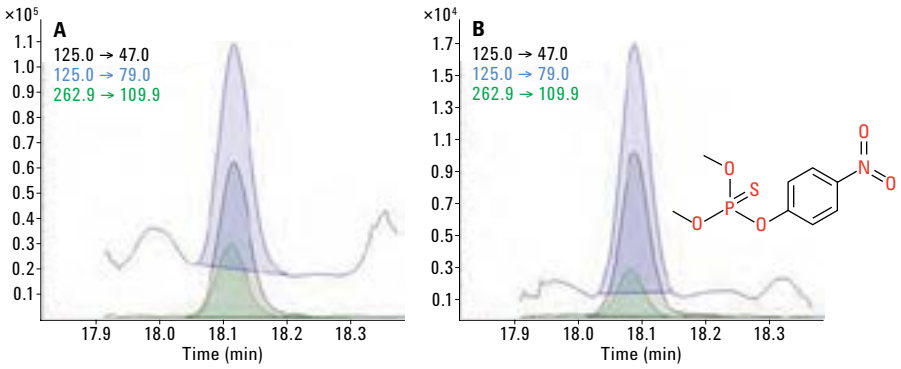

Figure 10. Example chromatograms for parathion-methyl (in organic honey) without Agilent JetClean (A) and with Agilent JetClean Acquire and Clean at 0.13 mL/min (B).

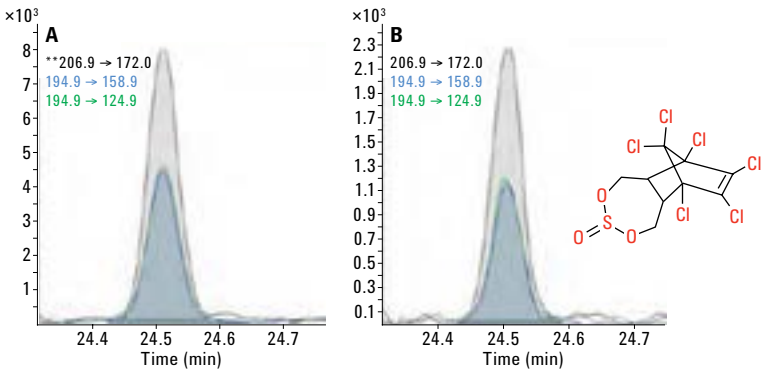

Figure 11. Example chromatograms for endosulfan-II (in organic honey) without Agilent JetClean (A) and with Agilent JetClean Acquire and Clean at 0.13 mL/min (B).

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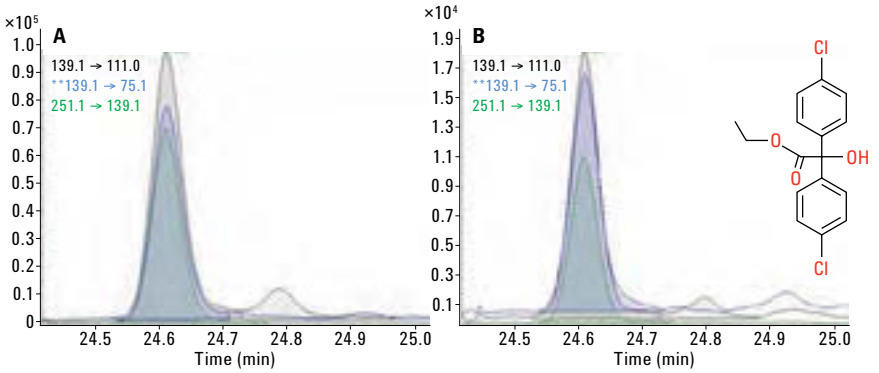

Figure 12. Example chromatograms for chlorobenzilate (in organic honey) without Agilent JetClean (A) and with Agilent JetClean Acquire and Clean at 0.13 mL/min (B).

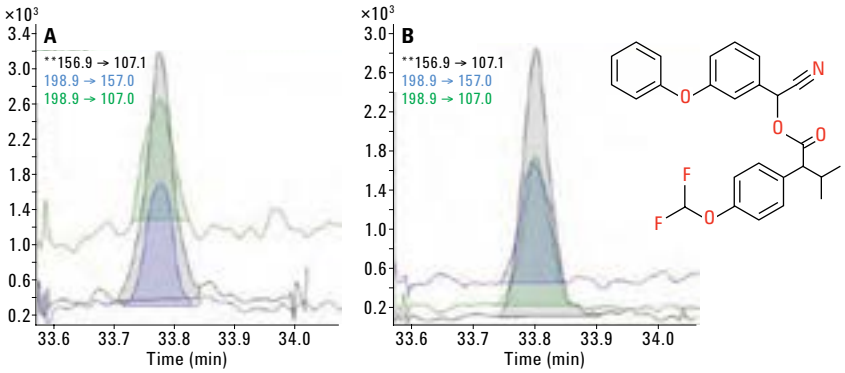

Figure 13. Example chromatograms for flucythrinate-I (in organic honey) without Agilent JetClean (A) and with Agilent JetClean Acquire and Clean at 0.13 mL/min (B).

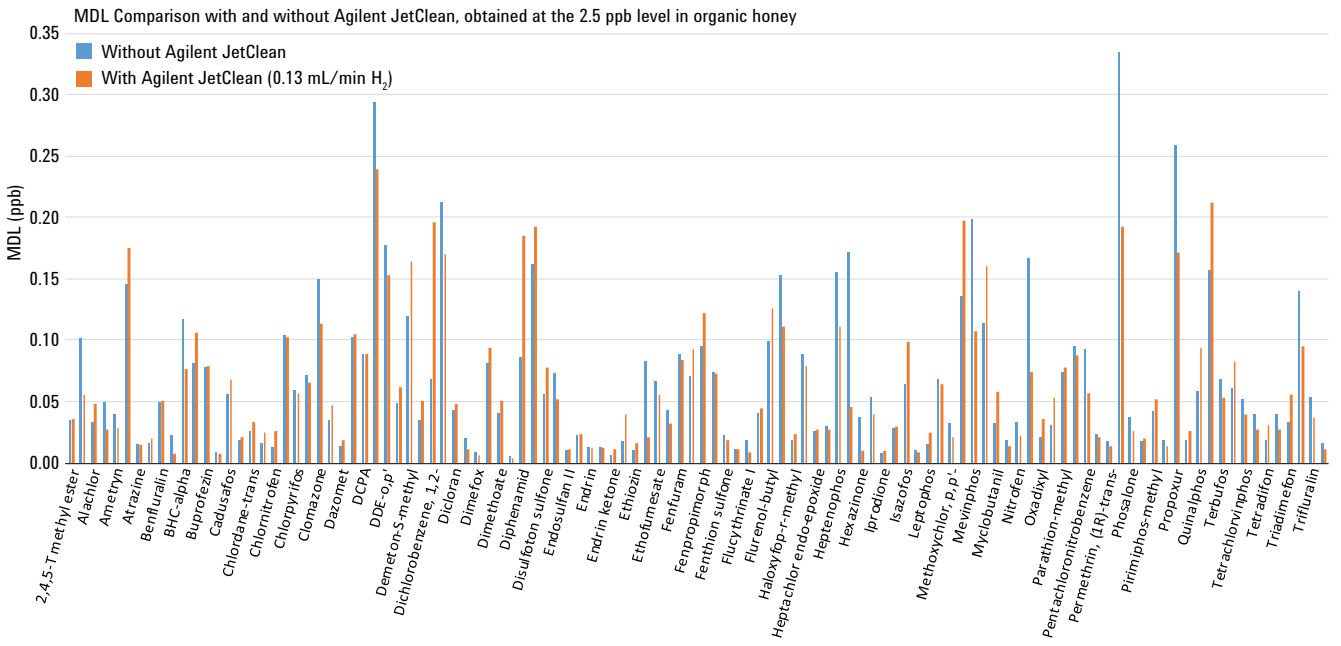

Figure 14. MDL comparison of selected target compounds with and without Agilent JetClean obtained at the 2.5 ppb level in organic honey.

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Results and Discussion

Table 3 lists the R^2 values and the statistically derived method detection limits (MDLs) for representative target analytes of the various pesticides tested. The calibration ranged from 0.12 ppb–50 ppb for the majority of the analytes, although some were not included at the lowest level. The resulting R^2 values both with and without JetClean (0.13 mL/min H_2) were very comparable. The MDLs were calculated from 10 replicate measurements of 2.5 ppb spiked honey extract using 99 % confidence level. Lower MDLs were obtained for the majority of the analytes using JetClean (0.13 mL/min H_2), with an average of 0.170 ppb MDL without the use of JetClean, and an average of 0.147 ppb with JetClean.

It was observed that the use of JetClean for pesticide analysis exhibited a decrease in overall analyte response throughout the analysis (the degree of the response reduction was compound dependent). Even though this reduction in response was observed, it did not affect the ability to confidently identify the analytes and quantitate under the required limits. The replicate measurements performed with and without JetClean at 2.5 ppb resulted in comparable %RSDs.

Table 3. R^2 values and MDLs of Selected Target Analytes at the 2.5 ppb Level in Organic Honey

	R^2		%RSD			R^2		%RSD	
Analyte	**Without JetClean**	**With JetClean**	**Without JetClean**	**With JetClean**	**Analyte**	**Without JetClean**	**With JetClean**	**Without JetClean**	**With JetClean**
Aldrin	0.998	0.997	0.05	0.03	Flucythrinate I	0.998	0.992	0.02	0.01
Atrazine	0.998	0.997	0.01	0.02	Flurenol-butyl	0.997	0.997	0.10	0.13
Azinphos-ethyl	0.997	0.995	0.02	0.02	Genite	0.999	0.997	0.15	0.11
Benfluralin	0.998	0.994	0.05	0.05	Haloxyfop-r-methyl	0.998	0.994	0.02	0.02
Butralin	0.997	0.991	0.01	0.01	Heptachlor	0.998	0.996	0.09	0.08
Cadusafos	0.998	0.996	0.06	0.07	Heptachlor endo-epoxide	0.997	0.992	0.03	0.03
Carboxin	0.997	0.994	0.02	0.02	Iprobenfos	0.997	0.993	0.05	0.04
Chlordane-*trans*	0.997	0.998	0.03	0.03	Iprodione	0.998	0.999	0.01	0.01
Chlornitrofen	0.998	0.997	0.01	0.03	Irgarol	0.998	0.997	0.03	0.03
Chlorpyrifos	0.998	0.994	0.06	0.06	Isazofos	0.997	0.993	0.06	0.10
Cloquintocet-mexyl	0.998	0.993	0.03	0.05	Methidathion	0.997	0.995	0.07	0.06
DCPA	0.997	0.994	0.09	0.09	Methoxychlor, *p,p′*-	0.998	0.996	0.03	0.02
DDD-*p,p′*	0.997	0.997	0.29	0.24	Metolachlor	0.998	0.990	0.14	0.20
DDE-*o,p′*	0.997	0.995	0.18	0.15	Mirex	0.997	0.995	0.11	0.16
DDT-*p,p′*	0.998	0.996	0.05	0.06	Myclobutanil	0.998	0.998	0.03	0.06
Dicloran	0.997	0.993	0.04	0.05	Napropamide	0.997	0.996	0.02	0.01
Dieldrin	0.997	0.994	0.02	0.01	Nitrofen	0.998	0.995	0.03	0.02
Dimethenamid-P	0.998	0.993	0.08	0.09	Oxadixyl	0.998	0.994	0.02	0.04
Dimethomorph I	0.999	0.995	0.00	0.00	Oxythioquinox	0.998	0.996	0.03	0.05
Disulfoton-sulfoxide	0.997	0.992	0.07	0.05	Parathion-methyl	0.997	0.995	0.07	0.08
Endosulfan sulfate	0.997	0.996	0.02	0.02	Pentachloronitrobenzene	0.997	0.997	0.09	0.06
Endrin	0.999	0.994	0.01	0.01	Pentoxazone	0.999	0.993	0.02	0.02
Endrin	0.999	0.994	0.01	0.01	Piperonyl butoxide	0.998	0.997	0.02	0.02
Endrin ketone	0.997	0.996	0.01	0.01	Profenofos	0.997	0.996	0.02	0.01
EPN	0.997	0.996	0.02	0.04	Pyrazophos	0.998	0.996	0.02	0.03
Ethiozin	0.997	0.992	0.01	0.02	Quinalphos	0.998	0.996	0.06	0.09
Ethofenprox	0.998	0.992	0.08	0.02	Tefluthrin	0.998	0.994	0.16	0.21
Ethofumesate	0.998	0.991	0.07	0.06	Terbufos	0.997	0.996	0.07	0.05
Fenitrothion	0.997	0.991	0.07	0.09	Terbufos sulfone	0.998	0.992	0.06	0.08
Fenpropimorph	0.997	0.995	0.10	0.12	Thionazin	0.998	0.994	0.04	0.03
Fenthion	0.998	0.995	0.07	0.07	Trifluralin	0.997	0.994	0.05	0.04
Fenthion sulfone	0.997	0.994	0.02	0.02	Triphenyl phosphate	0.997	0.993	0.02	0.01

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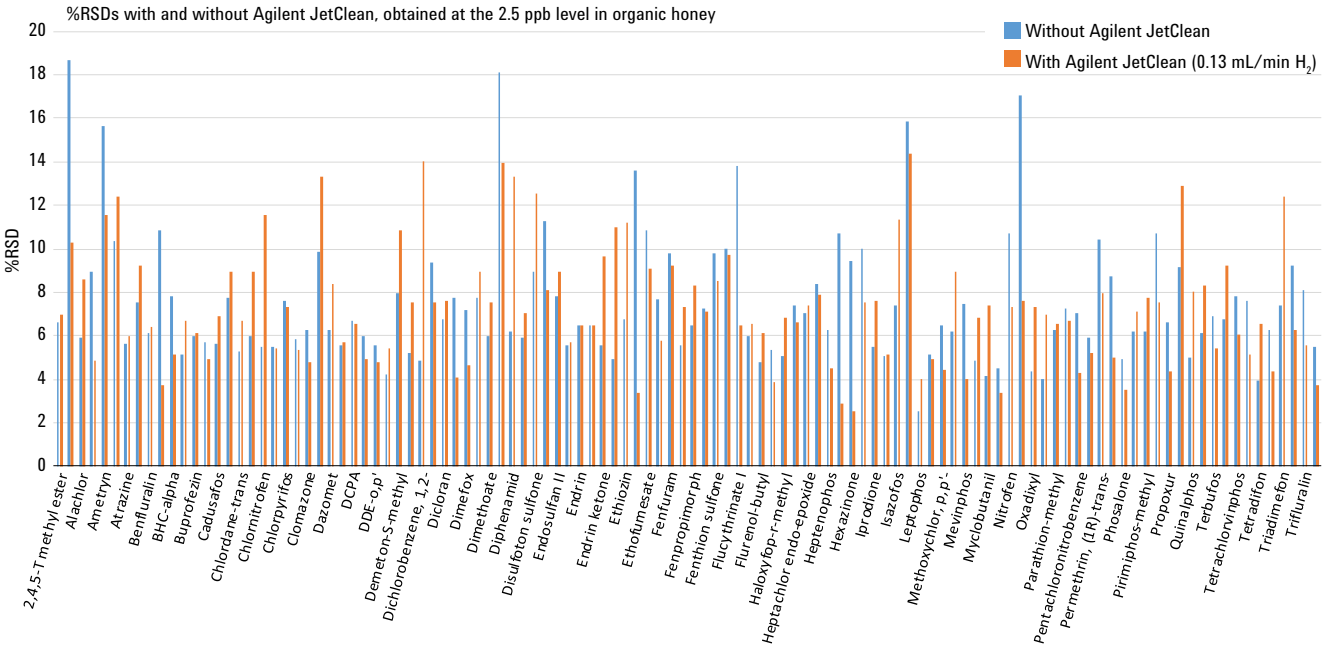

Figure 15. %RSDs of selected target compounds obtained with and without Agilent JetClean at the 2.5 ppb level in organic honey.

Conclusions

Approximately 200 pesticides were analyzed in organic honey extract on the Agilent 7010 Series Triple Quadrupole GC/MS with and without the use of the Agilent JetClean self-cleaning ion source in the Acquire and Clean mode (0.13 mL/min continuous flow). The improvements of the chromatographic peak shape and baselines (for later, higher molecular weight compounds), the comparable R^2 values, the equivalent %RSDs, as well as the low ppb MDLs; all indicate that the use of a low continuous flow of H_2 into the MS source can be considered as an option for maintaining performance during pesticide analyses.

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5. Pesticides and Environmental Pollutants (P&EP) Standard MRM Database User's Manual G9250AA Rev. A.4.00, *Agilent Technologies*, publication number G3445-90040.

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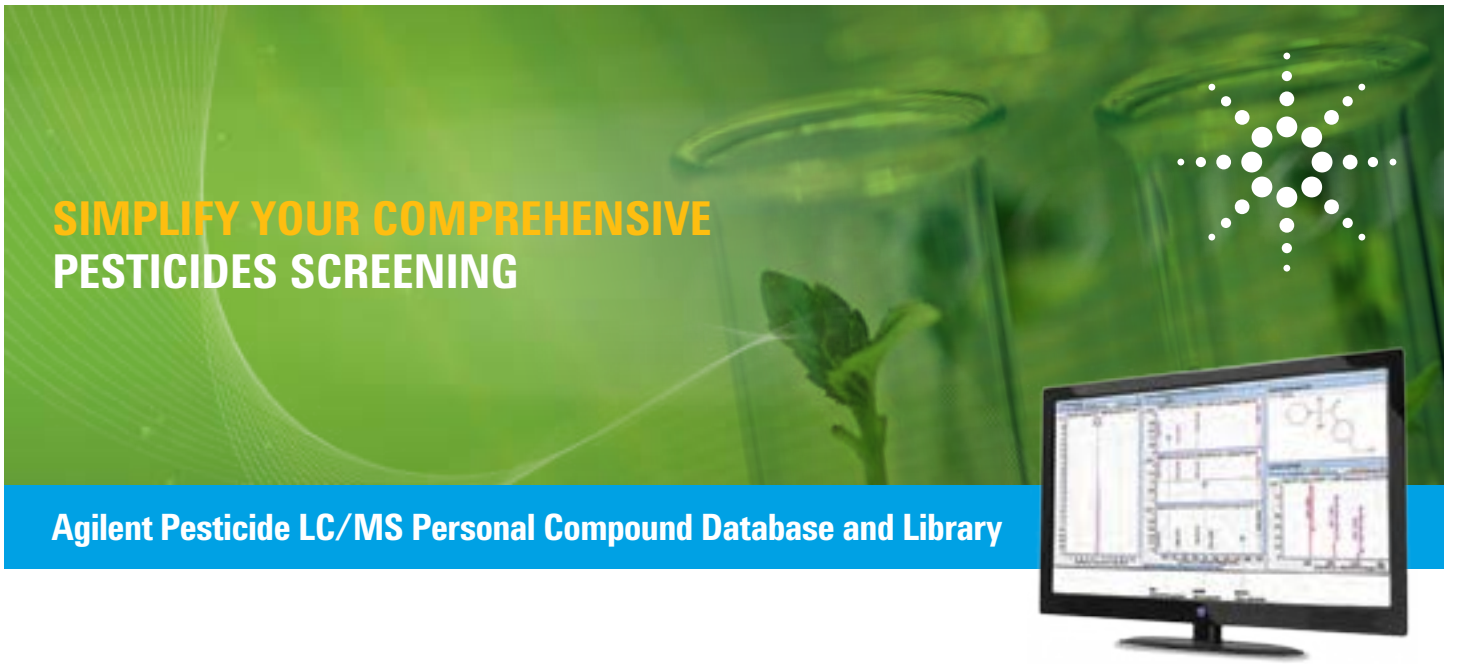

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- Quick-start guide with data examples and familiarization exercises
- Method Setup Guide that shows you how to create acquisition methods
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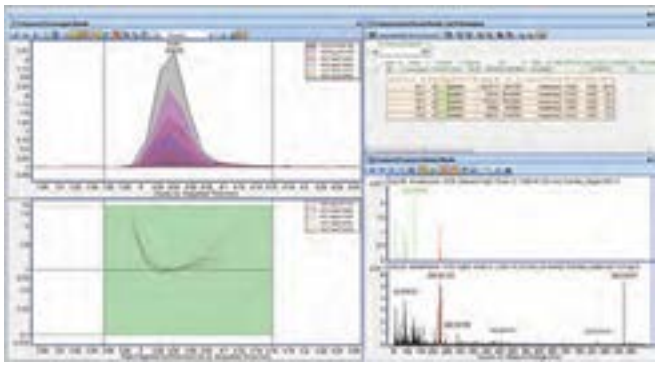
- **Perform presumptive matching** of acquired and library spectra—without the need to source standards
- **Create a custom PCDL** for more focused screening
- **Propose a suspect list** based on MS data and the "Find by Formula" algorithm
- **Confirm contaminants and eliminate false positives** with targeted MS/MS and library search
- **Mine data from Auto MS/MS experiments** using "Molecular Feature Extraction," and search for proposed compounds against the PCDL
- **Add your own compounds and library spectra** to create PCDLs specific to your needs
- **Perform retrospective data analysis** using newly added PCDL compounds—without the need to re-run samples

Simply put, the Pesticide PCDL makes compound confirmation and data mining easier, even for high-throughput labs.



Straightforward data mining and unambiguous identification using All Ions Software.

Top: Auto MS/MS spectrum

Middle: Mirror spectrum

Bottom: Library spectrum

Reliably confirm compounds by library matching using Auto MS/MS.

PCDL Manager Software makes it easy to control the database and library.

PROTECT OUR FOOD SUPPLIES AND COMPLY WITH REGULATORY STANDARDS

The Agilent Pesticide PCDL can help you meet the strict requirements established by global food regulatory agencies, including the following:

EU
EC 396/2005, EU WFD

China/Japan
GB 2763-2014, NY 235, JPL, JDWQS

U.S.
40 CFR part 180, EPA 535/1699, Draft CCL4

Available class tags
Pesticides, insecticides, herbicides, fungicides, etc.

Maximize your data quality with database and library curation

- Compound common name and IUPAC name
- Accurate mass of neutral molecule
- Molecular formula and structure
- Ion type (anion, cation, or neutral)
- CAS number/PubChem link (if existing)
- ChemSpider ID and hyperlink (if existing)
- Precursor and product ion peaks corrected to theoretical accurate mass
- Spectra acquired at 10, 20, and 40 V collision energy
- Includes adduct & loss spectra
- Spectra measured in positive and/or negative ion mode, where applicable
- Spectra filtered for signal intensity and curated for spectrum noise, chemical impurities, and incorrectly set instrument parameters

Application consulting lets you focus on what you do best

Installation and familiarization (optional):

Experienced service personnel will install the PCDL, verify all functions with an Agilent checkout sample, and familiarize you with the supporting software.

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Pesticide Personal Compound Database and Library (G3878CA)
Required but not included with the Pesticide PCDL:
Agilent 1260 or 1290 Infinity II LC
Agilent 6200 Series TOF or 6500 Series Q-TOF LC/MS
Agilent MassHunter Acquisition Software (B.05 or higher) and Windows 7 (64-Bit)
Agilent MassHunter Qualitative Analysis Software (B.07 SP1 or higher)
Agilent MassHunter Quantitative Analysis Software (B.07 or higher)
OPTIONAL: G3878CA #001 Installation and Familiarization Service
OPTIONAL: Advanced Application Consulting H2149A (Americas); R1736A (other regions)

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Development and Application of an Exact Mass LC/MS/MS Library for the Accurate Mass Screening and Quantitation of Pesticides in Fruits and Vegetables

Application Note

Food, Pesticide Analysis

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Abstract

This application note describes the creation of an exact mass library for pesticides and its application to the screening, quantitation and verification of pesticide residues in fruit and vegetables. An Agilent 1290 Infinity LC System was coupled to an Agilent 6540 Ultra High Definition QTOF LC/MS System which was operated in positive and negative electrospray using Dual Spray Agilent Jet Stream Technology. Target MS/MS acquisition was used for quantitation and verification of pesticide residues. Results of the successful validation of a fast UHPLC-QTOF-MS/MS method for three different commodity groups are shown. The method was appropriate for the analysis of pesticides in food extracts with regards to the required limits of quantitation (LOQs), linearity, and reproducibility. When applied to real-world samples from a routine monitoring program, all pesticides detected by triple quadrupole LC/MS and GC/MS methods were identified by the UHPLC-QTOF/MS method. Quantitation results were also in good agreement.

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Introduction

There is an ever increasing number of pesticides, and although the use of many of them is no longer permitted, they still occur from time to time as contaminants in food products. Accurate-Mass LC/MS screening for pesticides in food is of growing interest since it allows testing of a large number of potential contaminations. This is especially useful for commercial testing labs as a means of increasing the scope of the analysis, increasing the sample throughput, and minimizing cost-per-analysis. This increased interest is due to the recent implementation of guideline SANCO/12495/2011 [1] establishing method validation and quality control procedures for the analysis of pesticide residues in food and feed. For the first time, there are specified criteria for qualitative screening without the use of expensive standards for each pesticide in each batch of samples.

In the EU, maximum residue levels (MRLs) for pesticides are set by European Commission regulation (EC) 396/2005 and its amendments [2]. Appendix II and III specify more than 170,000 MRLs for various matrix pesticide combinations. The equivalent for the US is 40 CFR part 180 which sets tolerances and exemptions for pesticides in food. In addition, it specifies methodologies allowed for the analyses.

Modern LC/QTOF/MS instruments allow the analysis of most LC/MS amenable pesticides well below the MRLs specified by EU and US legislation. A typical workflow includes quantitation of all regulated pesticide residues considered to be a risk for a given country by using MS domain data. A list of less likely contaminants can be found and identified, when comprehensive database searches are applied, using the additional information resident in the full scan accurate mass data that comes from a time-of-flight instrument [3]. In complex samples such as QuEChERS extracts [4], the challenge is to rule out potential false positives. In accurate mass LC/TOF/MS, the retention time, mass accuracy, isotope distribution, and adduct pattern are used to verify positives. The availability of true MS/MS information gives a higher level of confidence in the identification especially if accurate mass fragment information is available.

This application note describes the creation of an exact mass LC/MS/MS library containing CID spectra for three different collision energies for over 300 LC/MS amenable pesticides listed high in the Check-your-scope ranking of the EURL for pesticides. This exact mass MS/MS library was then used as part of a LC/QTOF/MS/MS workflow for the screening and identification of pesticides in fruit and vegetable extracts. Pesticides verified by MS/MS spectral comparison were also quantified using the MS domain data. Results of the successful validation of the workflow, according to SANCO/12495/2011, for more than 50 pesticides and three representative fruit and vegetable commodities belonging to different commodity groups [5] are presented. When the workflow was applied to real world samples which were part of routine pesticide monitoring, all pesticides detected earlier by triple quadrupole LC/MS and GC/MS analysis were identified and quantitation results were also in agreement.

Experimental

Sample preparation

Fruits and vegetables were obtained from a local grocer. Samples were extracted according to the official citrate buffered QuEChERS protocol using Agilent BondElut QuEChERS kits. Ten grams homogenized fruit and vegetable (cucumber, lemon, and rucola) samples were weighed in 50 mL polypropylene tubes and extracted with 10 mL acetonitrile for 1 minute while shaking vigorously by hand. The lemon homogenate was neutralized afterwards by adding 600 µL of a 5 M sodium hydroxide solution. After adding an extraction salt packet containing 4 g anhydrous $MgSO_4$, 1 g NaCl, and 1.5 g buffering citrate salts, the mixture was again shaken for 1 minute and then centrifuged at 3,000 rpm for 5 minutes.

After phase separation, a 6-mL aliquot of the upper acetonitrile phase was transferred into an Agilent BondElut QuEChERS EN dispersive SPE tube (p/n 5982-5256) containing 150 mg primary secondary amine (PSA) and 15 mg graphitized carbon black for sample cleanup and 900 mg anhydrous $MgSO_4$ to remove water. The tubes were closed and shaken for another minute. Afterwards, the tubes were centrifuged at 3,000 rpm for 5 minutes. A 4-mL amount of the final extract was transferred into a clean polypropylene vial. To improve the stability of the target pesticides, 40 µL formic acid was added to the final extract. For use in method validation, a pesticide mixture containing 55 target compounds was spiked into an aliquot of the final extracts at four different levels corresponding to 5, 10, 50, and 100 µg/kg.

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LC/MS/MS Analyses

Separation was carried out using an Agilent 1290 Infinity UHPLC system consisting of an Agilent 1290 Infinity Binary Pump (G4220A), an Agilent 1290 Infinity High Performance Autosampler (G4226A), and an Agilent 1290 Infinity Thermostatted Column compartment (G1316C). The UHPLC system was coupled to an Agilent G6540A UHD Quadrupole Time-of-Flight LC/MS System equipped with a Dual Spray Agilent Jet Stream electrospray ionization source and operated in the 2 GHz extended dynamic range mode.

Reference mass ions were delivered using an Agilent Infinity 1260 Isocratic pump (G1310B) at a flow rate of 10 µL/min using a 1 in 100 flow splitter (G1607-60000). The importance of a reliable reference mass delivery is described in great detail in [6]. Agilent MassHunter workstation B.05.00 software was used for data acquisition, MassHunter Qualitative Analysis B.06.00 and Quantitative Analysis B.05.02 was used for data analysis. Table 1 shows the UHPLC parameters; Table 2 shows the Agilent Jet Stream parameters.

Exact mass LC/MS/MS library spectra for more than 300 pesticides were acquired at collision energies of 10, 20, and 40 eV by injecting individual standards in acetonitrile with a concentration of 1 ng/µl in flow injection analysis (FIA) into the LC/QTOF/MS/MS system operated in target MS/MS mode. The $[M+H]^+$ and the $[M-H]^-$ ions were specified as the target masses. After curation of the acquired MS/MS spectra for their exact fragment masses, the spectra were included in the pesticide PCDL which was then applied for pesticide discovery and verification.

In this step, final QuEChERS extracts were injected onto the Q-TOF system, operating with an acquisition rate of 5 scans/sec in the MS domain, and 3 scans/sec in the MS/MS domain. Data was collected in positive and negative ion mode in two consecutive analytical runs. In the TOF mode (MS domain), a mass range of *m/z* 100 to 1,100 amu was acquired. In the target MS/MS mode, a mass range of *m/z* 50 to 1,000 amu was acquired for more than 200 target masses rated high in the Check-your-scope list of the EURL for pesticides, using an acquisition window of 0.6 minutes. A collision energy ramp was applied to the target masses using an offset of 4 eV and a slope of 6 eV per 100 amu.

Table 1. UHPLC Method Parameters

UHPLC column	Agilent ZORBAX Eclipse Plus C18 RRHD 2.1 × 150 mm, 1.8 µm at 30 °C	
Mobile phase	A: 5 mM NH_4 formate + 0.1% formic acid B: 5 mM NH_4 formate + 0.1% formic acid in methanol	
Gradient program	Min	% B
	0	10
	0.5	10
	3.5	50
	17.0	100
	20.0	100
	20.1	10
Stop time	22 min	
Flow rate	0.40 mL/min	
Injection volume	3 µL	

Table 2. Agilent Jet Stream Parameters

Parameter	Value	
Gas temperature	200 °C	
Gas flow	8.0 L/min	
Nebulizer	35 psi	
Sheath gas temperature	350 °C	
Sheath gas flow	11.0 L/min	
	Value positive (V)	**Value negative (V)**
Vcap	4,000	3,000
Nozzle voltage	300	0
Fragmentor	120	120
Skimmer 1	65.0	65.0
Octopole RF peak	750	750
Reference mass correction	Enabled	
	Detection window	50 ppm
	Minimum height	500 counts
Reference mass ions	Positive	Negative
	121.050873	119.03632
	922.009798	966.000725

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Pesticide discovery was done using the Find by Formula (FBF) data mining algorithm. In the Find by Formula workflow, compounds are searched in the MS domain using the molecular formula information out of the Personal Compound Database and Library (PDCL) along with the user supplied definition of the ion species. Table 3 shows the parameters used in the FBF algorithm which represent good values for the screening of residues and contaminants in samples with high chemical background.

Results were scored based on retention time, mass accuracy, and isotope pattern matching. When a compound was identified in the MS domain, CID spectra were automatically extracted and searched against the exact-mass MS/MS library spectra included in the PCDL. When suspect compounds were verified by a MS/MS library search, pesticides were included in quantitation for an efficient batch review and to assign a concentration value.

Figure 1 illustrates the applied workflow for the screening, identification, and quantitation of pesticides in fruit and vegetable extracts. This approach was used also for real world samples. Identified pesticides as well as quantitation results were compared to results previously collected on triple quadrupole LC/MS and GC/MS systems.

Table 3. Parameter Settings for the FBF Data Mining Algorithm

Parameter	Value	
Extraction data format	Centroid for both, chromatographic and spectral extraction	
Integrator	Agile, no peak thresholds	
Spectra to include	> 10% peak height < 20% saturation (in the *m/z* ranges used in the chromatogram Background subtraction of average spectra at peak start and end No peak thresholds	
Charge state	Limited to # 1	
Isotope model	Common organic molecules; except for fenbutatinoxide (separate evaluation with unbiased)	
PCDL	G3878CA Pesticide PCDL containing 741 compounds with exact mass spectra for both polarities and up to three different collision energies	
Positive ions	$[M+H]^+$, $[M+NH_4]^+$, $[M+Na]^+$	
Negative ions	$[M-H]^-$, $[M+HCOO]^-$	
Formula matching	Match tolerance (spectra)	± 6 ppm
	Recognition window	± 0.35 minutes
	EIC extraction	± 10 ppm
	Extraction window	± 1.0 minute
	No peak thresholds	
	Extraction of MS/MS spectrum if available with ± 20 ppm precursor tolerance	
Matching criteria	Warning threshold	Score < 80
	Compound matching	Score > 50
Library search criteria	Precursor ion expansion	± 10 ppm
	Product ion expansion	± 20 ppm
	Collision energy spread	± 20 eV
	Minimum reverse score	> 50
	No peak thresholds	

Results and Discussion

Creation of an exact mass MS/MS library

The presence of pesticides in a sample detected by a qualitative screening method according to guideline SANCO/12495/2011 can be verified by the comparison of the accurate mass MS/MS spectrum with an exact mass reference library spectrum. To create a user defined accurate mass library, accurate mass spectra for more than 300 pesticides were acquired with collision energies of 10, 20, and 40 eV. In either positive or negative ionization mode, meaningful MS/MS spectra were acquired for most of the investigated pesticides. For several compounds, MS/MS library spectra were captured in both ionization modes. To eliminate mass assignment errors, fragment masses in the acquired spectra were compared to the theoretical fragment formulas and corrected to their true (empirical) masses. The corrected spectra were included in the Agilent Pesticide Personal Compound Database and Library (p/n G3878CA) which was used for the screening and verification of pesticide residues in fruit and vegetable samples. Retention time information was added to the library by analyzing comprehensive pesticide standards with the given UHPLC method. Figure 2 shows the Personal Compound Database and Library (PCDL) software along with the exact mass spectrum of Omethoate acquired with a collision energy of 10 eV.

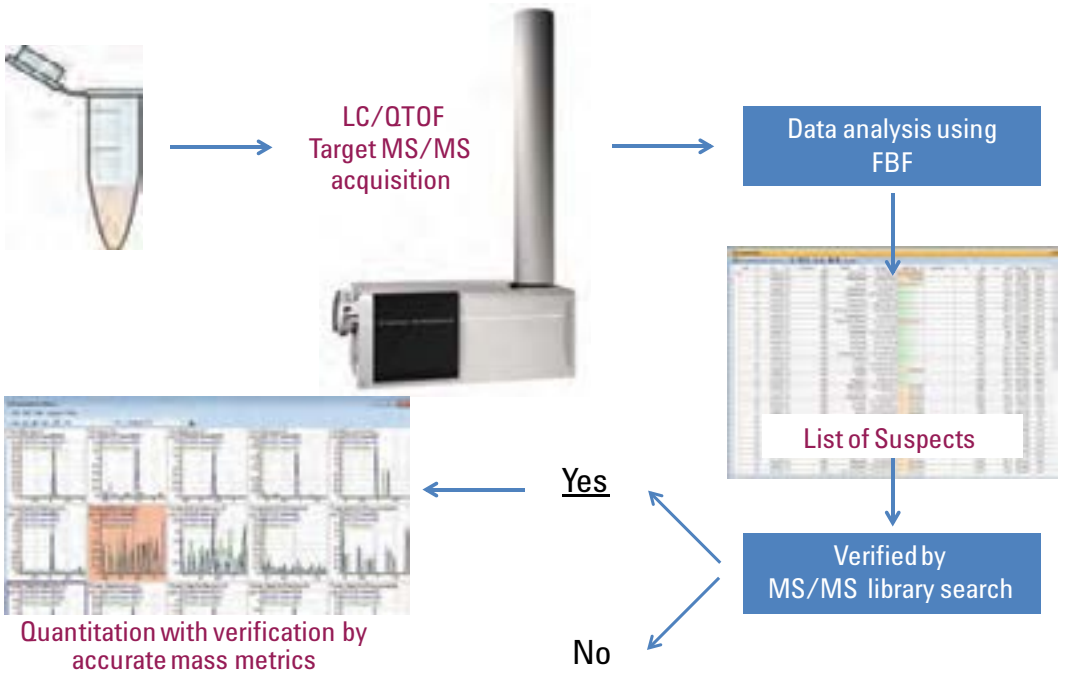

Figure 1. UHPLC-QTOF/MS/MS workflow for discovery, verification and quantitation of pesticides in food extracts.

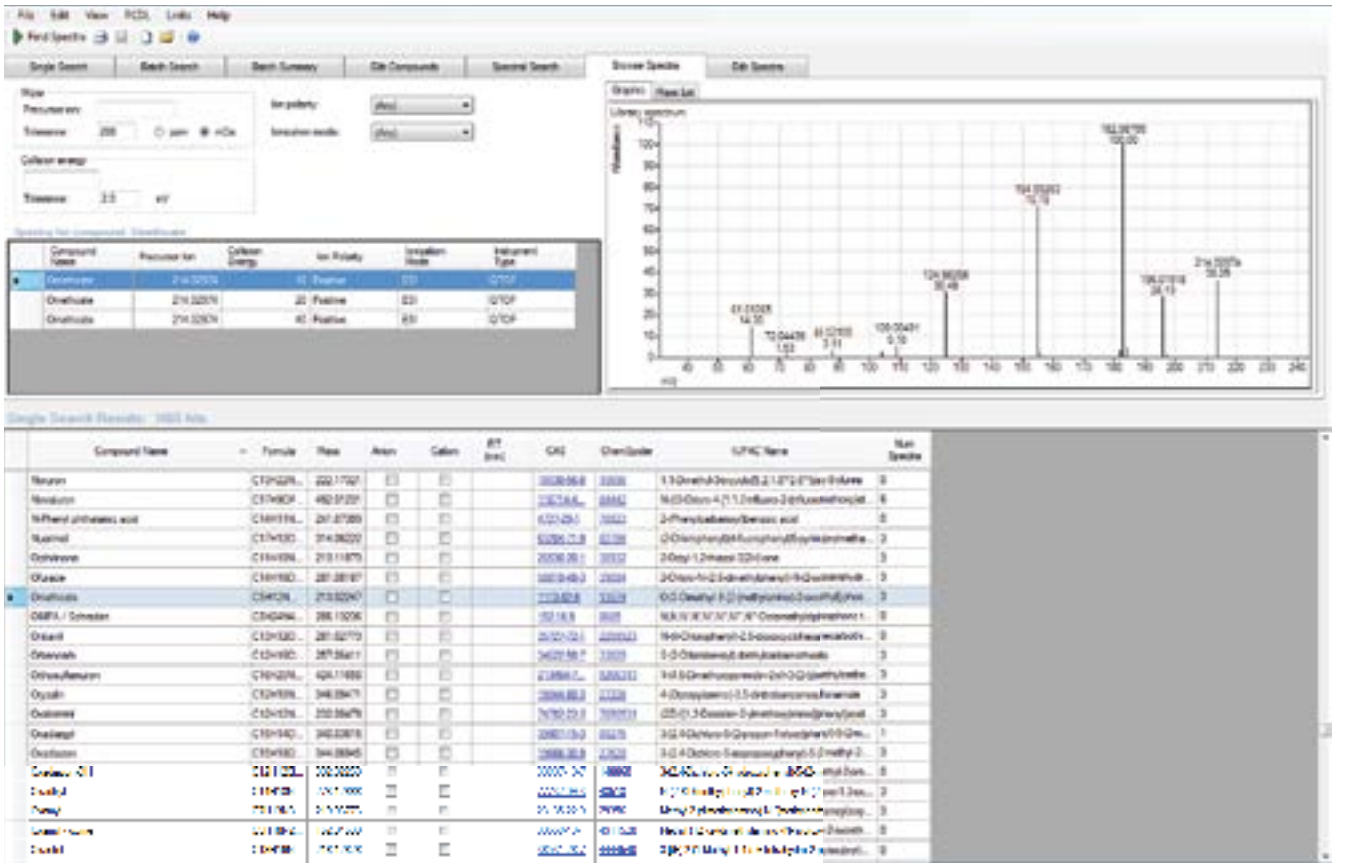

Figure 2. PCDL software showing the pesticide library and the exact mass spectrum of omethoate.

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Validation of the screening and verification workflow for the identification of pesticides in fruit and vegetable matrices

Cucumber, lemon, and rucola extracts spiked with 55 relevant pesticides were analyzed using UHPLC separation and target MS/MS acquisition. The masses of the precursor ions ($[M+H]^+$) of the spiked pesticides were included in the target list along with the precursor masses of 150 other relevant pesticides. The FBF algorithm was used for compound searching. It automatically generates an extracted ion chromatogram for the expected ion species for all target compounds in the accurate mass database. Peak spectra are extracted and the experimentally measured results are compared against the calculated results for the database entries. The results are scored depending on the agreement of the accurate monoisotopic mass, the isotope ratio, the isotope spacing, and the retention time. Figure 3 shows, as an example, the compound chromatogram and peak spectrum for methidathion spiked into a QuEChERS extract of rucola and obtained by the FBF algorithm.

The automatically generated extracted ion chromatogram (EIC) summarizes the signals for all selected adduct species of methidathion (A). Even for a low spiking concentration corresponding to 10 µg/kg and the most complex matrix, a very good signal-to-noise (S/N) ratio of 238.2 (peak-to-peak noise algorithm) was observed. Figure 3B shows the compound spectrum of methidathion (green centroided signals) in comparison to the theoretical isotope pattern (red boxes) for all detected adducts. Figure 3C shows the same picture zoomed in for the major $[M+H]^+$ species. The software could allocate 11 ions to the different adducts of methidathion including their isotope signals. In addition, the signal intensities were in good agreement with the expected isotope ratios even with coeluting background signals from the matrix with intensities of up to 3 x 10^6 counts. Consequently, a high overall score of 95.7 (out of 100) was observed for methidathion which reflects a combination of the mass accuracy score (97.6 out of 100), the isotope abundance score (93.5 out of 100), and the isotope spacing score (97.8 out of 100).

Table 4 shows the compound table for all 53 pesticides measured in positive mode and spiked in cucumber extract.

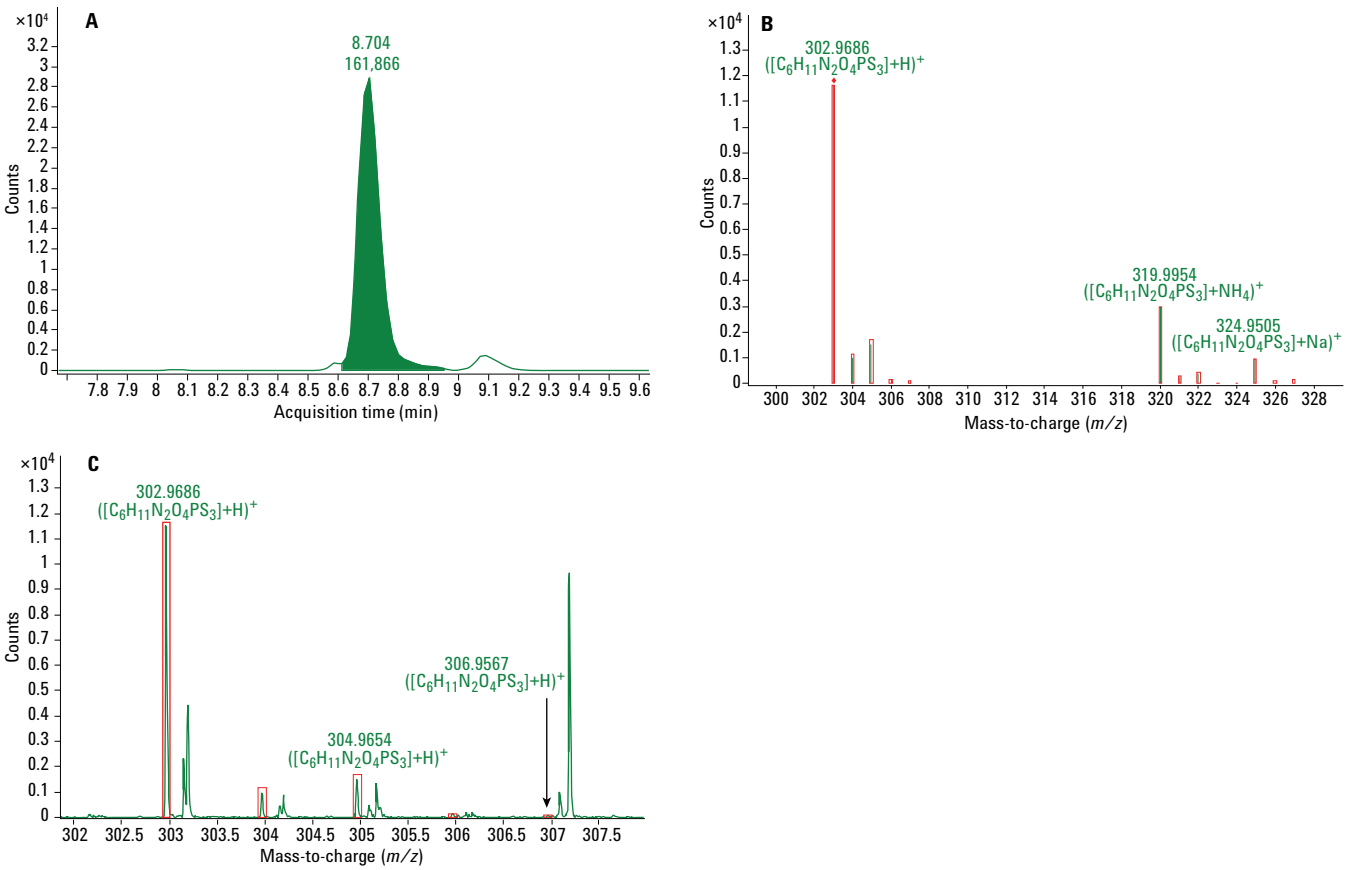

Figure 3. Compound chromatogram and peak spectrum obtained by the Find by Formula algorithm for methidathion spiked into a QuEChERS extract of rucola equivalent to a concentration of 10 µg/kg (50% of the MRL for rucola).

Table 4. Compound Table for 53 Pesticides Measured in Positive Mode and Spiked in QuEChERS Extract of Cucumber Equivalent to a Concentration of 10 µg/kg

Compound name	RT	Mass	Formula	Mass deviation (ppm)	Total score	Mass score	Isotope abundance score	Isotope spacing score
Acetamiprid	4.704	222.0672	$C_{10} H_{11} Cl N_4$	–0.12	97.88	99.94	97.37	98.85
Azoxystrobin	9.384	403.1171	$C_{22} H_{17} N_3 O_5$	0.77	97.48	99.38	99.33	99.92
Bifenazate	10.48	300.1474	$C_{17} H_{20} N_2 O_3$	–2.89	81.58	94.96	76.84	48.77
Boscalid	9.85	342.0323	$C_{18} H_{12} C_{12} N_2 O$	–1.15	95.49	98.18	82.18	97.04
Buprofezin	13.956	305.1563	$C_{16} H_{23} N_3 O S$	0.37	98.44	99.92	92.61	99.37
Carbaryl	7.012	201.0785	$C_{12} H_{11} N O_2$	–1.58	98.63	98.92	97.08	99.72
Carbendazim	4.047	191.0694	$C_9 H_9 N_3 O_2$	–0.4	93.35	99.94	88.37	73.67
Chlorpyrifos	14.529	348.9259	$C_9 H_{11} Cl_3 N O_3 P S$	–0.97	95.03	99.27	76.61	98.7
Chlorpyrifos-methyl	12.905	320.895	$C_7 H_7 Cl_3 N O_3 P S$	–0.82	83.91	95.55	78.24	67.44
Cyhexatin	14.711	360.1564	$C_{18} H_{32} Sn$	–0.66	79.62	99.67	n.a.	94.31
Cyprodinil	11.385	225.1267	$C_{14} H_{15} N_3$	0.4	99.52	99.92	99.44	97.83
DEET (Diethyltoluamide)	7.989	191.1304	$C_{12} H_{17} N O$	–0.46	87.17	99.92	97.26	49.5
Dichlorvos	6.272	219.9448	$C_4 H_7 Cl_2 O_4 P$	–2.83	94.04	96.36	79.63	95.54
Difenconazole(I)	12.935	405.0648	$C_{19} H_{17} Cl_2 N_3 O_3$	0.35	94.31	99.73	88.64	92.25
Diflubenzuron	11.404	310.0321	$C_{14} H_9 Cl F_2 N_2 O_2$	0.17	95.61	99.03	87.03	99.0
Dimethoate	4.734	228.9993	$C_5 H_{12} N O_3 P S_2$	–1.49	98.18	99.01	99.56	91.78
Dimethomorph	10.122	387.1236	$C_{21} H_{22} Cl N O_4$	–0.29	97.73	99.86	96.81	90.05
Famoxadon	12.282	374.1288	$C_{22} H_{18} N_2 O_4$	–0.89	79.47	99.4	75.86	41.24
Fenhexamid	10.798	301.0641	$C_{14} H_{17} Cl_2 N O_2$	–1.46	95.37	99	84.52	91.85
Fluazifop	9.353	327.071	$C_{15} H1_2 F_3 N O_4$	–2.56	86.13	95.34	87.34	52.34
Fludioxonil	9.771	248.0392	$C_{12} H_6 F_2 N_2 O_2$	–2.01	84.76	97.95	58.57	48.92
Fluquinconazole	10.738	375.0095	$C_{16} H_8 Cl_2 F N_5 O$	–1.36	93.7	98.5	84.06	96.44
Flutriafol	7.915	301.1025	$C_{16} H_{13} F_2 N_3 O$	0.64	93.15	99.71	76.03	96.21
Imazalil	7.5	296.0481	$C_{14} H_{14} Cl_2 N_2 O$	0.77	98.46	99.66	95.78	98.73
Imidacloprid	4.377	255.0522	$C_9 H_{10} Cl N_5 O_2$	0.41	97.53	99.99	91.34	97.02
Iprodione	11.29	329.0335	$C_{13} H_{13} Cl2 N_3 O_3$	0.95	94.0	99.06	84.76	88.52
Kresoxim-methyl	11.721	313.1312	$C_{18} H_{19} N O_4$	0.64	99.13	99.94	98.53	96.47
Linuron	9.414	248.0117	$C_9 H_{10} Cl_2 N_2 O_2$	1.02	94.33	99.41	75.86	95
Mandipropamid	9.881	411.1236	$C_{23} H_{22} Cl N O_4$	0.26	98.21	100	91.13	99.52
Metalaxyl	8.162	279.1471	$C_{15} H_{21} N O_4$	–0.17	98.03	99.89	99.21	98.3
Methidathion	8.64	301.9614	$C_6 H_{11} N_2 O_4 P S_3$	1.54	97.46	98.34	98.73	99.92
Myclobutanil	10.313	288.1147	$C_{15} H_{17} Cl N_4$	–1.9	89.78	97.46	79.86	90.79
Penconazole	11.825	283.064	$C_{13} H_{15} Cl_2 N_3$	1.15	97.95	99.22	91.67	98.87
Pendimethalin	14.675	281.1376	$C_{13} H_{19} N_3 O_4$	–2.19	75.62	97.09	26.23	50.0
Phosmet	9.032	316.9943	$C_{11} H_{12} N O_4 P S_2$	0.72	98.79	98.83	96.57	98.95
Phoxim	12.39	298.0544	$C_{12} H_{15} N_2 O_3 P S$	–0.86	99.23	99.95	98.95	96.6
Piperonyl butoxide	14.252	338.2097	$C_{19} H_{30} O_5$	–1.06	97.33	99.44	99.55	98.41
Pirimicarb	6.346	238.1429	$C_{11} H_{18} N_4 O_2$	0.36	96.46	99.96	99.04	79.31
Pirimicarb, desmethyl-	4.529	224.128	$C_{10} H_{16} N_4 O_2$	–2.13	81.3	96.34	89.45	13.11
Propamocarb	2.885	188.1521	$C_9 H_{20} N_2 O_2$	1.89	98.72	98.46	99.66	97.59
Propargite	14.94	350.1553	$C_{19} H_{26} O_4 S$	–0.47	98.41	99.52	93.17	99.3
Propiconazole(I)	12.132	341.0688	$C_{15} H_{17} Cl_2 N_3 O_2$	3.02	94.19	99.2	95.45	95.38
Prosulfocarb	13.45	251.1343	$C_{14} H_{21} N O S$	0.43	97.6	99.99	95.94	92.63
Pyraclostrobin	12.451	387.0987	$C_{19} H_{18} Cl N_3 O_4$	–0.28	97.65	99.92	99.8	98.95
Pyridaben	15.757	364.1379	$C_{19} H_{25} Cl N_2 O S$	–0.74	96.98	99.46	88.62	98.56
Teflubenzuron	14.216	379.9735	$C_{14} H_6 Cl_2 F_4 N_2 O_2$	1.9	83.77	97.16	25.64	94.31
Thiabendazole	4.565	201.0359	$C_{10} H_7 N_3 S$	1.01	97.01	99.58	96.38	95.7
Thiacloprid	5.114	252.0235	$C_{10} H_9 Cl N_4 S$	0.49	98.67	99.77	97.28	97.94
Thiamethoxam	3.818	291.0191	$C_8 H_{10} Cl N_5 O_3 S$	0.76	98.79	99.35	97.35	97.87
Thiophanate-methyl	6.519	342.0452	$C_{12} H_{14} N_4 O_4 S_2$	1.18	95.87	99.09	89.6	97.49
Triadimefon	10.199	293.093	$C_{14} H_{16} Cl N_3 O_2$	0.52	93.03	99.78	68.44	95.1
Triazophos	10.522	313.0649	$C_{12} H_{16} N_3 O_3 P S$	0.33	94.09	99.94	94.34	94.62
Trifloxystrobin	13.148	408.1298	$C_{20} H_{19} F_3 N_2 O_4$	–0.16	99.43	99.98	98.57	98.19

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The mass error of the major ion species, and the resulting scores for all compounds are given. At a concentration corresponding to 10 µg/kg, all compounds were found by the FBF algorithm with the specified settings. The mass deviations of the measured masses compared to the theoretical masses generally were below 1 ppm, and for only seven compounds, which were detected with lower peak intensities, a mass deviation between 2 and 3 ppm, was observed. Most of the pesticides got a score of 90 (out of 100) or above. The quality filter used in this data processing method requires at least two or more individual ions for the compound. In combination with the retention time, this typically is sufficient for the identification. Three compounds had an overall score below 80 and were flagged for inspection. In all cases, one of the specified adducts showed up with low abundance and, thus, had either a bigger mass deviation or missing isotope signals.

MS/MS spectra were extracted automatically over the peak window and were matched against the library spectra contained in the Agilent Pesticide Personal Compound Database and Library. Since a precursor mass dependent collision energy ramping was used in the MSMS experiments, a search filter on collision energy of ± 20 eV was applied to focus comparisons of library spectra to those library entries of similar collision energy decent. Figure 4 shows the MS/MS spectrum for methidathion acquired in the rucola extract spiked at a concentration corresponding to 10 µg/kg in comparison to the library spectrum from the Agilent Pesticide PCDL. All major fragment ions listed in the library spectrum of methidathion were found in the measured spectrum within a narrow mass extraction window and in a similar ratio to the reference spectrum for a collision energy of 20 eV. The forward search against the exact mass pesticide library resulted in a score of 95.9 out of 100 and verified the presence of methidathion in the sample. Additional signals in the acquired mass spectrum belong to matrix components with similar precursor masses.

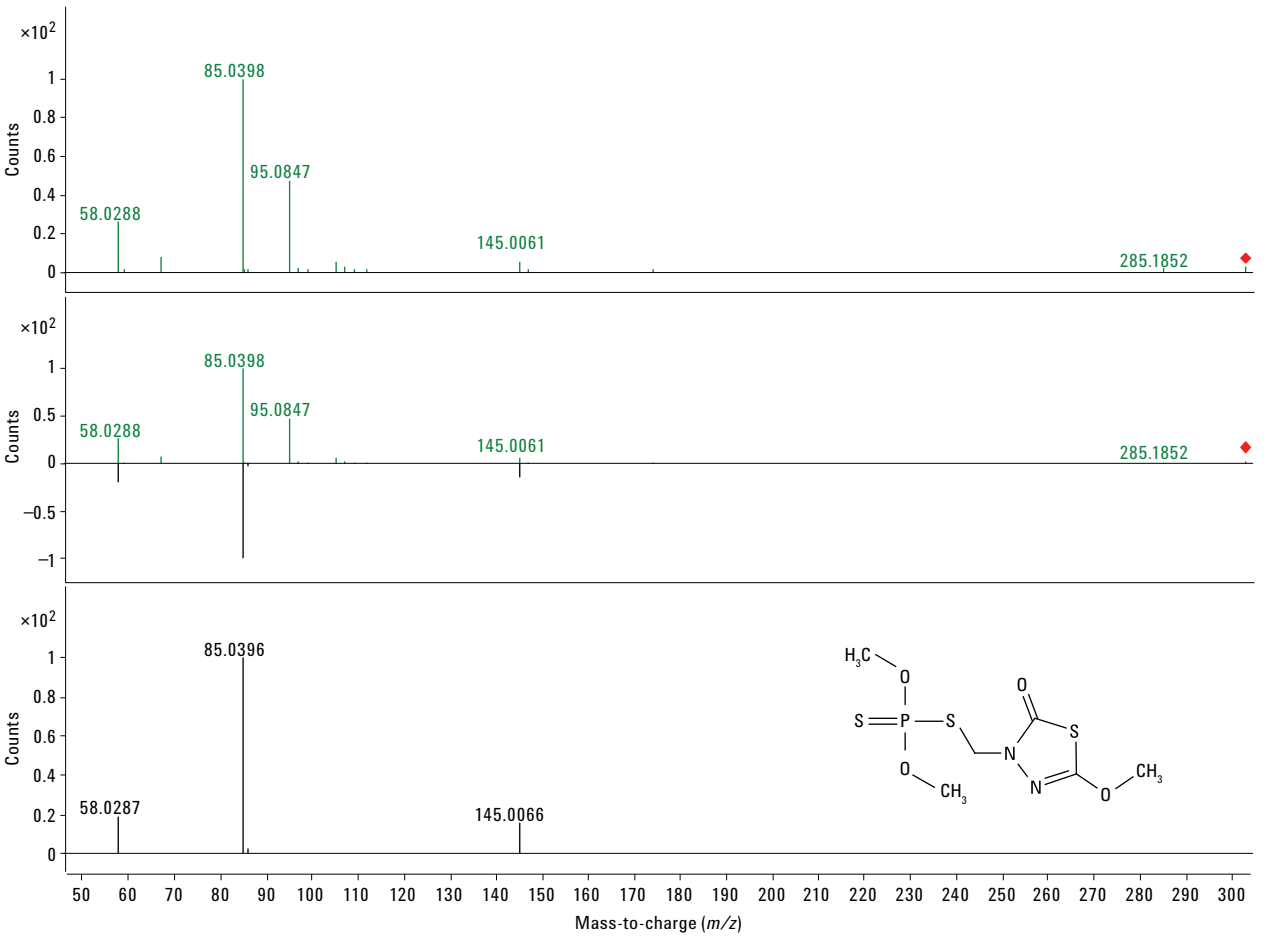

Figure 4. Comparison of the measured spectrum of methidathion in the spiked rucola extract (corresponding to a concentration of 10 µg/kg) with the reference spectra of methidathion from the Agilent Pesticide PCDL.

Accurate mass screening for pesticides, combined with confirmation of identified contaminants by MS/MS library searching, was validated for solvent standards as well as for spiked QuEChERS extracts of cucumber, lemon, and rucola. Pesticide concentrations in solvent and in the spiked QuEChERS extracts were 5, 10, 50, and 100 ng/mL. Table 5 summarizes the results which were obtained by the automatic data analysis using the Find by Formula algorithm and the parameters described in Table 3. Most of the compounds were detected in positive mode, 2,4-D and MCPA were only detected in negative mode, and several compounds were detected in both modes.

The majority of the spiked pesticides was successfully detected by the FBF algorithm even at a concentration equivalent to 5 µg/kg and in complex matrices. Moreover, for most of the pesticides meaningful MS/MS spectra were generated at this concentration which allowed the verification of the compound by library searching against the MSMS spectra in the PCDL.

Under the conditions used, the predominant adduct species for some of the compounds were not the [M+H] ion and no library spectra were available for the corresponding sodium or ammonium adduct. Those compounds are marked with an asterisk. The cucumber sample used for the spiking experiment was most probably contaminated with low levels of iprodione which is used as fungicide for the cultivation of cucumbers. All other positive findings in the blank samples represent very low concentrations and are most probably caused by a carry-over effect.

By adding more compounds to the database and library, the scope of the data analysis was extended to more than 570 of the most important pesticides. For approximately 300 of these pesticides, retention time information was available. Applying this database as formula source to the FBF algorithm, the screening of pesticides in matrix samples yielded several additional contaminant suspects which are summarized in Table 6. For the rucola extract, applying a 300 compound database with retention time information, resulted in the detection of 55 additional suspect pesticides. When applying a 570 compound database without retention time information, 166 additional suspect pesticides were detected. The precursor masses for all suspect pesticides were included as precursor masses in the target MS/MS method. None of the suspect pesticides present could be verified by comparing the target MS/MS spectra against the pesticide reference library. Similar results were obtained for the less complex matrices cucumber and lemon. In the lemon extract, 13 additional suspect pesticides were observed when using the 300 compound database, and 119 additional suspect pesticides were observed when using the 570 compound database without retention time information. In the cucumber extract, 6 and 79 suspects were detected when using the 300 compound database with, and the 570 compound database (without retention time information), respectively. For both matrices, library searching against the Agilent Pesticide PCDL successfully helped to eliminate potential false positives.

Quantitative review

The MS domain data from this method was also used to obtain (semi-)quantitative information for the spiked extracts as well as for several official control samples. The best ions to select for the quantitative method were derived from the compound results extracted from the qualitative software using the 50 ng/mL solvent standard data file. These were exported to a compound exchange file (.cef) which was used in the MassHunter Quantitative software for the automatic creation of a quantitation method. In this way, quantifier and qualifier ions were automatically selected from the observed adduct species and isotope signals, based on their relative abundance.

The limit of quantification for most of the 55 targeted pesticides in the TOF mode was below 5 ng/g in all tested matrices with a linear range of up to four orders of magnitude. Figures 6E and 6F show the linear calibration curves for pirimicarb and boscalid obtained from the MS domain data.

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*Table 5. Results Summary for the Screening and Verification of Pesticides in Fruits and Vegetables by Target MS/MS Acquisition and Library Searching. (Green: Compound Automatically Found and Presence Verified by MS/MS Library Confirmation; Yellow: Compound Automatically Found but no Qualified MS/MS Spectrum Available; *No Spectrum Available for Predominant Adduct of Compound; ***Compound Results Acquired in Negative Ion Mode*

	Cucumber					Lemon					Rucola				
	Blank	5 ppb	10 ppb	50 ppb	100 ppb	Blank	5 ppb	10 ppb	50 ppb	100 ppb	Blank	5 ppb	10 ppb	50 ppb	100 ppb
2,4-D***															
Acetamiprid															
Azoxystrobin															
Bifenazate															
Boscalid															
Buprofezin															
Carbaryl															
Carbendazim															
Chlorpyrifos															
Chlorpyrifos-methyl															
Cyhexatin dehydrate*															
Cyprodinil															
DEET															
Dichlorvos*															
Difenconazole															
Diflubenzuron															
Dimethoate															
Dimethomorph															
Famoxadon*															
Fenhexamid															
Fluazifop															
Fludioxonil***															
Fluquinconazole															
Flutriafol															
Imazalil															
Imidacloprid															
Iprodione															
Kresoxim-methyl															
Linuron															
Mandipropamid															
MCPA***															
Metalaxyl															
Methidathion															
Myclobutanil															
Penconazole															
Pendimethalin															
Phosmet															
Phoxim															
Piperonyl butoxide*															
Pirimicarb															
Pirimicarb, desmethyl-															
Propamocarb															
Propargite*															
Propiconazole															
Prosulfocarb															
Pyraclostrobin															
Pyridaben															
Teflubenzuron															
Thiabendazole															
Thiacloprid															
Thiamethoxam															
Thiophanate-methyl															
Triadimefon															
Triazophos															
Trifloxystrobin															

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Table 6. Number of Suspects Detected and Verified by Accurate Mass Library Searching in the Blank Quechers Matrices (N = 5) When Applying Large Compound Databases With and Without Retention Time Information as Formula Source for the Find-By-Formula Data Mining Algorithm

	Cucumber	Lemon	Rucola
Pesticide suspects identified with find by formula			
RT required (300 target compounds)	6 ± 2	13 ± 6	55 ± 20
Verified by MS/MS library searching	0	0	0
RT optional (570 target compounds)	73 ± 2	119 ± 6	166 ± 24
Verified by MS/MS library searching	0	0	0

Figure 5 shows a screenshot of the compounds at a glance view for the review of multiple samples (organized in rows) and multiple pesticides (organized in columns) in MassHunter Quantitative software.

As illustrated in Table 4, it is possible to compare the isotope pattern of a peak's spectrum with that which is predicted by theory, and then to assign score based on equivalency. This approach can also be implemented in MassHunter Quantitative software (pattern matching). The quantifier-qualifier concept and the accurate mass metrics, increases the confidence in the results and allows an efficient data review in batch processing. Figure 5 shows that a minimum isotope match score has been specified as outlier and has been used to flag samples (in red) for which the mass accuracy and the isotope pattern match was not sufficient for a positive identification of the compound.

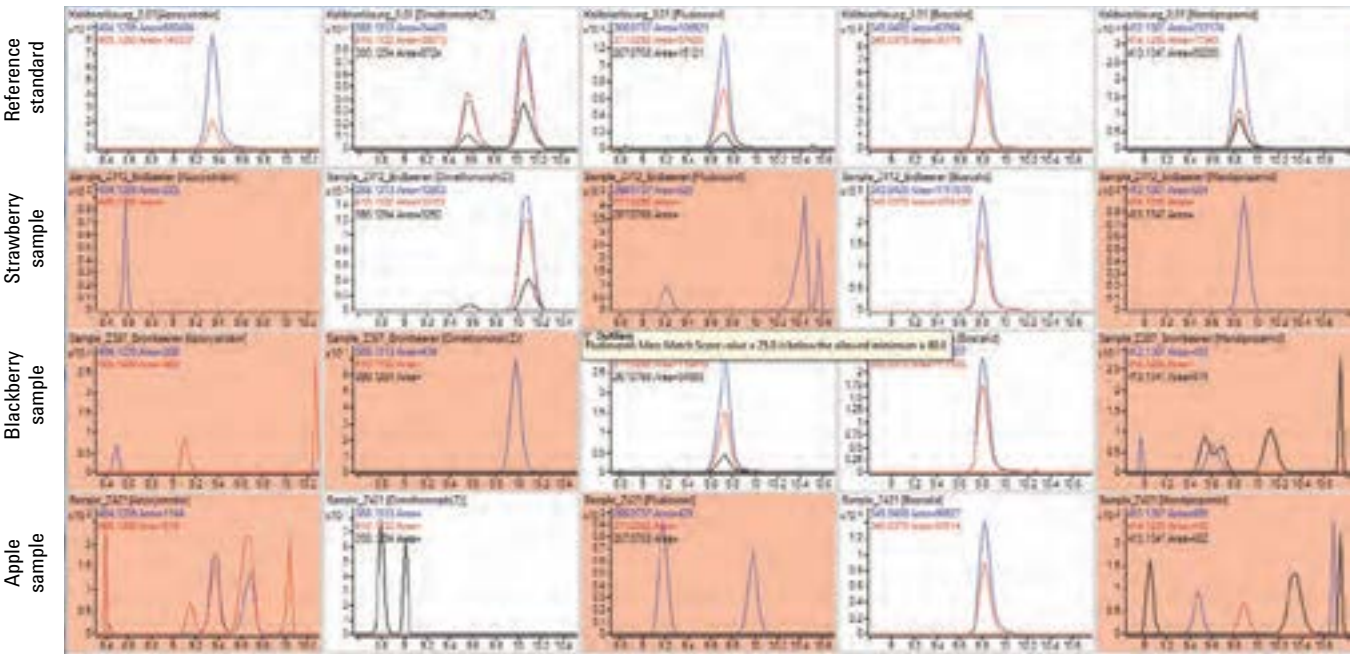

Figure 5. Screenshot of the compounds at a glance view of the MassHunter quantitative software showing multiple compounds for multiple samples. The accurate mass metrics has been used for the flagging of outliers.

Analysis of real samples

In addition to the spiked matrices used to validate the workflow, samples obtained from a routine monitoring laboratory were analyzed using the UHPLC-QTOF method. These samples were also analyzed by LC/MS/MS or GC/MS and several pesticide residues were detected.

Figures 6A and 6B show the chromatograms and spectra for pirimicarb and boscalid, which were found as residues in an apple sample. Figures 6C and 6D show the comparison of the acquired spectra with the associated library spectra (pattern matching). For pirimicarb, the mass deviation of the molecular ion was –0.54 ppm which resulted in a mass score of 99.2. The isotope pattern match score was 97.0. For boscalid, a mass deviation of –0.15 ppm was observed which led to a mass score of 97.5. The isotope pattern match score was 93.0.

Ten pesticides were found in this apple sample which gave good mass match scores and pattern match scores. These scores were consolidated with their associated retention time score which gave values of over 85 for all 10. Seven of these were further verified by target MS/MS acquisition and accurate mass library searching. The other three compounds had no MS/MS spectra in the library for comparison.

Five additional pesticides were detected in the target MS/MS data and identified by library searching with high library match scores. Concentrations of these pesticides were all below 5 µg/kg and, therefore, below the reporting limit.

Table 7 summarizes the comparison of the results of the official control measurements with the results obtained for the UHPLC-QTOF/MS/MS method for four different samples. All of the pesticides found previously with triple quadrupole LC/MS/MS or GC/MS were found by the accurate mass screening method and concentrations of the identified compounds were in good agreement with the concentrations previously determined. Only the parsley sample had concentrations of some compounds significantly lower than the results obtained during the control analysis. This is due to the fact that calibration was based on a solvent calibration, and matrix suppression in the real samples would reduce the apparent recovery of the pesticide compound. By using matrix matched calibrations or other methods to compensate for matrix effects, it can be expected that concentrations would be in better agreement with the results from the official control.

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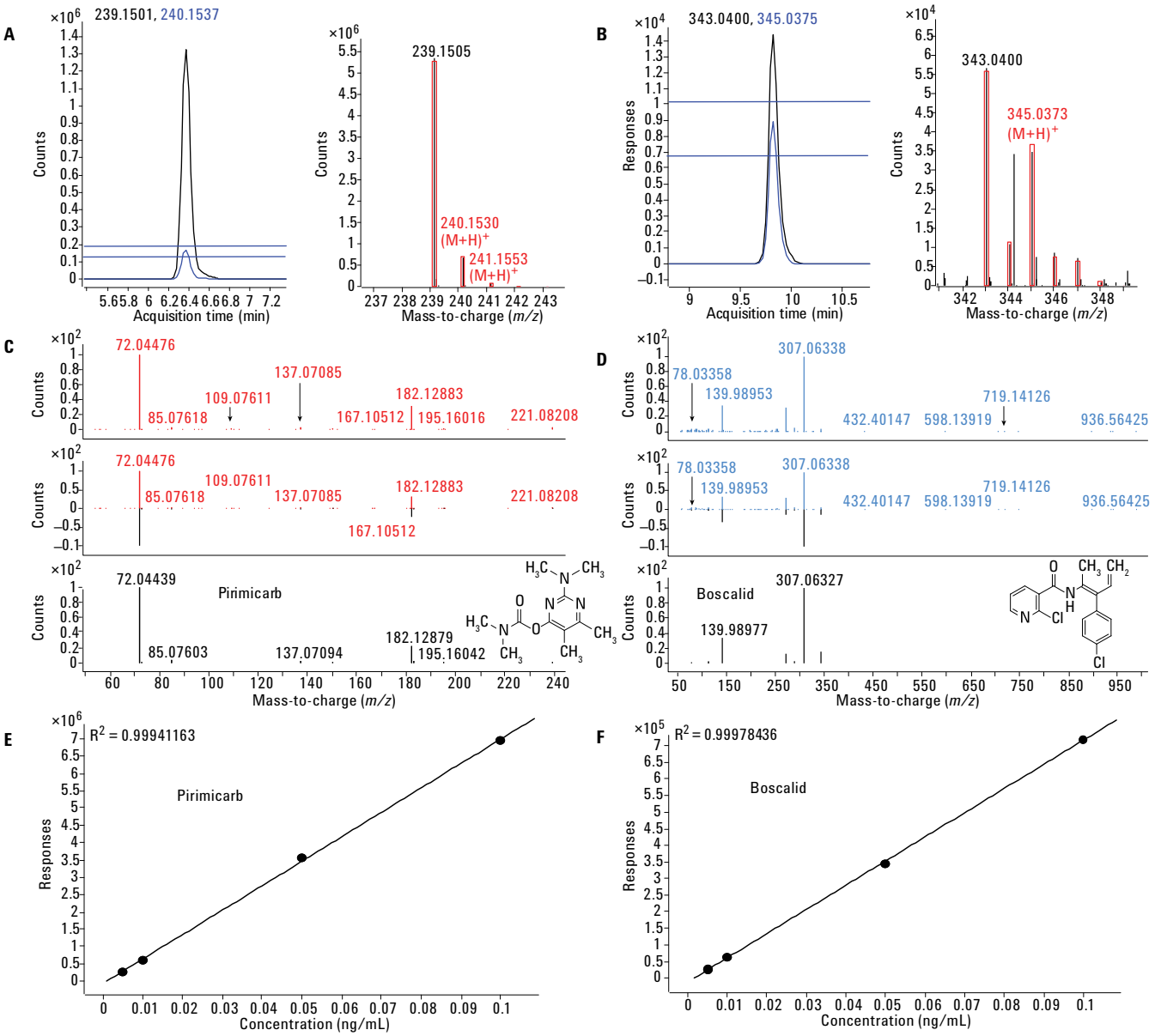

Figure 6. Results of the LC/QTOF/MS screening, confirmation and quantitation of pesticides in an apple sample obtained from a pesticide monitoring laboratory. Pirimicarb and Boscalid are shown with their associated compound chromatogram, TOF and MS/MS spectrum in comparison to the library spectrum, and their calibration curves.

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Table 7. Comparison of the Results of the Official Control Measurements (Triple Quadrupole LCMS and GCMS) With the Results Obtained for the UHPLC-QTOF/MS/MS Method for Four Different Samples

	Apple Germany			Strawberry Netherlands			Grapes Brazil			Parsley Germany		
Compounds	**QTOF**		**LC-QQQ and GCMS**	**QTOF**		**LC-QQQ and GCMS**	**QTOF**		**LC-QQQ and GCMS**	**QTOF**		**LC-QQQ and GCMS**
Acetamiprid	YES	0.01	0.01									
Azoxystrobin							YES	0.06	0.09	YES	0.15	0.34
Boscalid	YES	0.02	0.02	YES	0.24	0.02				YES	0.03	0.14
Carbendazim	YES	0.02	0.02				YES	<0.01				
Difenoconazole	YES	<0.01					YES	<0.01	0.02	YES	0.03	0.04
Dimethomorph				YES	<0.01					YES	0.38	0.74
Imidacloprid	YES	<0.01					YES	0.01	0.01			
Linuron										YES	0.01	0.01
Mandipropamid							YES	<0.01	0.01			
Penconazole	YES	<0.01		YES	0.05	0.1						
Pirimicarb	YES	0.01	0.18									
Pirimicarb-desmethyl	YES	0.01	0.01									
Prosulfocarb										YES	0.01	0.01
Pyraclostrobin	YES	<0.01	0.01	YES	0.05	0.09	YES	<0.01		YES	0.02	0.03
Thiacloprid				YES	0.03							
Thiophanate methyl	YES	<0.01					YES	<0.01	0.01			
Trifloxystrobin	YES	0.01	0.01							YES	<0.01	

Conclusions

An exact mass MS/MS library for pesticides was created and applied to the LC/QTOF/MS/MS screening of pesticides in fruit and vegetable extracts. The method was successfully validated for the screening and identification of more than 50 pesticides in three different commodity groups. When applied to real world samples obtained from a routine monitoring program, all pesticides detected previously by triple quadrupole LC/MS and GC/MS were identified. The quantitation results were in good agreement with quantitative results obtained previously.

The Agilent Pesticide PCDL mentioned in this application note is available as (p/n G3878CA) or p/n (G3878AA) which also contains a chromatography column, a comprehensive pesticide standard and application support.

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Rapid Pesticide Screening and Identification Using the High Resolution All Ions MS/MS Technique

Application Note

Food Safety

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Abstract

The All Ions MS/MS technique was used to rapidly screen, quantify, and identify pesticides in food matrices. This analytical method uses a high resolution Time-of-Flight (TOF) or Quadrupole-TOF mass spectrometer to rapidly analyze samples and generate quantitative information for target compounds.

To validate the effectiveness of this new methodology in complex matrices and at low concentrations, three different food matrices were spiked with a comprehensive pesticide standard and were analyzed using the All Ions MS/MS technique. This technique helps eliminate false positives, and has the speed and accuracy to significantly improve the productivity of pesticide screening and quantitation.

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Introduction

Consumers continue to be concerned about the public health impacts of pesticide residues in food. Especially with the expanding global trade in food products, detection of pesticides has pushed forward regulations such as European Commission (EC) Regulation 396/2005[1] and 40 CFR Part 180 in the United States[2]. However, pesticide residues continue to be a quality issue in food. For example, in 2009, there were 173 alert notifications in the European Union[3] related to pesticide residues entered in the Rapid Alert System for Food and Feed (RASFF)[4].

State-of-the-art pesticide screening requires the consideration of more than 1,000 pesticides and their metabolites. Of these, approximately 600 to 800 compounds are included in routine monitoring programs and approximately 150 are typically detected in food commodities. The analytical method used for pesticide screening needs to be validated and must comply with quality standards laid down in the SANCO 12495/2011[5] guidelines.

The most widely-used sample preparation method for pesticide screening is the Quick Easy Cheap Effective Rugged Safe (QuEChERS)[6] for sample preparation. This is widely accepted as a universal extraction procedure. The analytical method of choice is liquid chromatographic separation followed by detection using a triple quadrupole mass spectrometer. While the use of Multiple Reaction Monitoring (MRM) is the most sensitive technique for the analysis of pesticides in complex matrices, this technique has drawbacks. The MRM technique requires thorough method development, constant maintenance, need for expert knowledge, and days of tedious work to enter MRM transitions for all compounds and estimate their retention times. In addition, identification of a compound requires the acquisition of two or more product ions with a constant ratio, hence limiting the number of compounds that can be acquired in one single analytical method. Most importantly, the ability to re-interrogate data for new and unexpected residues without reacquisition is not possible.

Agilent Technologies has developed the All Ions MS/MS technique for the screening and identification of pesticides in a single analytical run. The technique uses an accurate mass LC/TOF or Q-TOF and features easy setup of the acquisition method, verification of the pesticide compounds using MS/MS spectral libraries, and chromatographic coelution of the precursor and product ions, and rapid development of a quantitative method including product ions as qualifiers. The user can quickly verify the identities of compounds with high resolution accurate mass data, and then create a quantitative method for the compounds of interest in minutes. Potential false positives can be eliminated by assessing the quality of the product ion chromatograms. With this technique, hundreds of pesticides can be quantified in a single analysis.

Experimental

Sample preparation

Tomato, avocado, and lemon samples obtained from a local grocery store were prepared according to the citrate buffered QuEChERS method, using Agilent BondElut QuEChERS kits (p/n 5982-5650). Sample extracts were cleaned up using Agilent BondElut QuEChERS EN dispersive SPE tubes (p/n 5982-5256). Before the cleanup, only the lemon extracts were neutralized by adding sodium hydroxide solution. After the cleanup, all samples were acidified using 5% formic acid in acetonitrile to improve the stability of the target pesticides. Samples were spiked at three relevant concentrations with a comprehensive pesticide standard containing more than 190 pesticides.

System configuration

Separation was carried out using an Agilent 1290 Infinity UHPLC System consisting of an Agilent 1290 Infinity Binary Pump (G4220A), an Agilent 1290 Infinity High Performance Autosampler (G4226A), and an Agilent 1290 Infinity Thermostatted Column compartment (G1316C).

An Agilent 6540 UHD Q-TOF was operated with MassHunter Acquisition Software rev. B.05.01 using 2 GHz extended dynamic range mode with an acquisition rate of three scans/s in MS and two discrete collision energies for the All Ions MS/MS method. The use of precursor scan with any collision energies and MS/MS scans with two collision energies resulted in alternating spectra with a low energy channel containing the precursor ion and two high energy channels containing the precursor and product ions.

Chromatographic conditions		
UHPLC column	Agilent ZORBAX Eclipse Plus C18 RRHD, 2.1 × 150 mm, 1.8 µm	
Column temperature	30 °C	
Mobile phase	A: 5 mM NH_4 formate + 0.1% formic acid B: 5 mM NH_4 formate + 0.1% formic acid in methanol	
Gradient program	Min	% B
	0	5
	0.2	5
	2.2	30
	10.5	100
	13.0	100
	13.5	5
Stop time	15 minutes	
Flow rate	0.50 mL/min	

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Results and Discussion

Data analysis was performed using the MassHunter Qualitative Analysis Software (B.06.00) using the Find by Formula (FbF) algorithm. FbF uses a formula and determines if a compound with that formula is present in the high resolution mass spec data. The FbF algorithm has been updated to support the All Ions MS/MS technique. The mass peaks in the low energy channel were first searched against the Pesticide Personal Compound Database and Library (PCDL) (B.04.01) for compounds that had the same *m/z* values. A set of putative identifications was then compiled. Figure 1 shows that carbendazim was confidently identified from the samples by using the FbF algorithm.

For the identified compounds, the fragment ions in the MS/MS spectra from the PCDL were compared to the ions in the high energy channel to confirm the presence of the correct fragments (Figure 2).

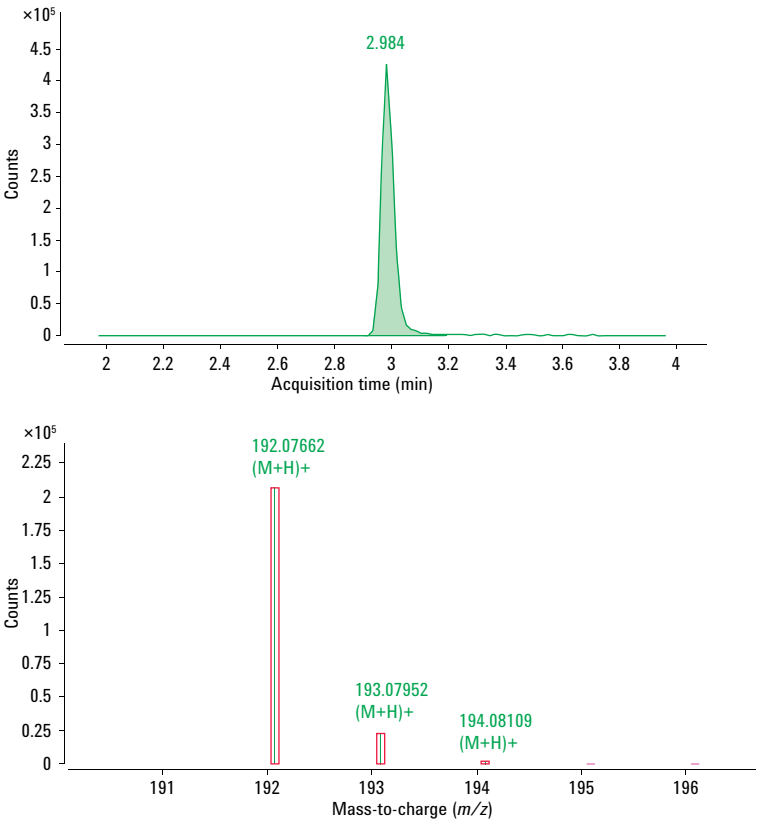

Figure 1. Identification of carbendazim using the Find by Formula (FbF) algorithm.

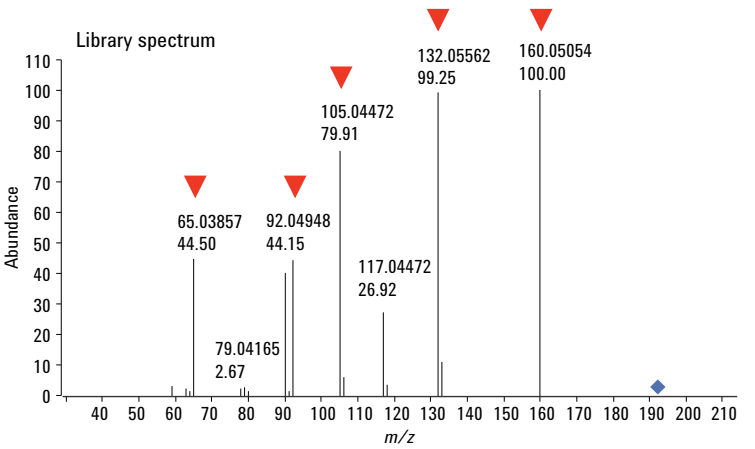

Figure 2. High energy (40 eV) spectrum of carbendazim.

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Both the precursors and product ions were extracted as chromatograms (Figure 3A) and evaluated using a coelution score. The coelution score was derived from a technique similar to UV chromatography's Peak Purity[7], where the software calculates a number that takes into account multiple factors, such as abundance, peak shape (symmetry), peak width, and retention time. The scores were plotted and were easily viewable as a coelution plot (Figure 3B).

The software analysis reported that carbendazim was found with five valid qualifier fragments from the PCDL MS/MS spectrum (Figure 4).

A parallel analysis was performed for all other putative precursor ions found in the low energy channel and were searched against the 741 compounds with MS/MS spectra in the Pesticides PCDL.

As a validation study, 190 pesticides were spiked into three different matrices (tomato, lemon, and avocado) at increasing concentration levels. Table 1 shows the results for 50 important and frequently found pesticides. Most of the compounds were found in the lowest levels of all matrices and their presence was verified by at least one additional fragment ion (as indicated by green cells). In some cases, the compounds were found by the FbF algorithm, but the fragment ions were not qualified (yellow cells).

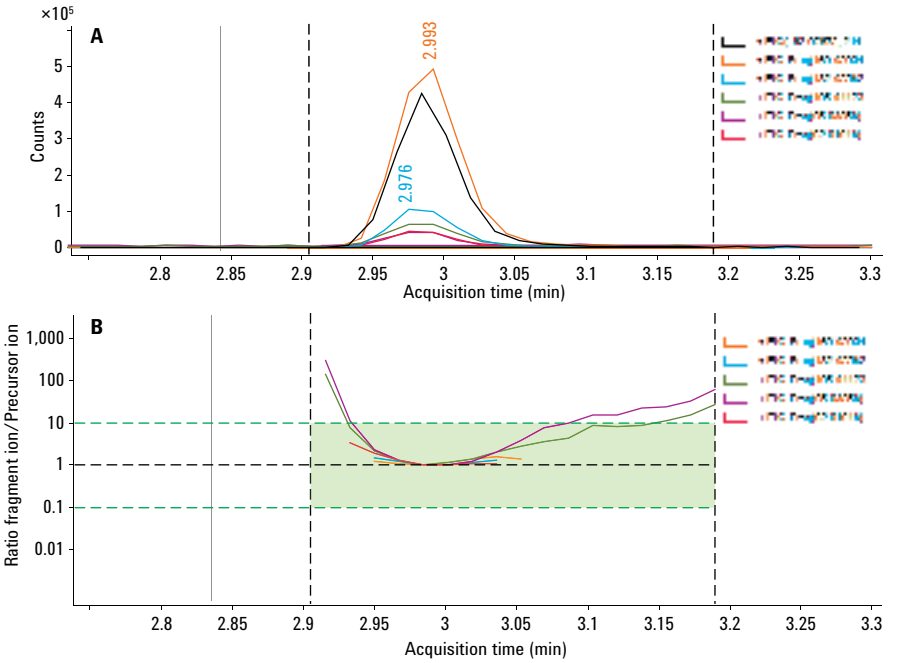

Figure 3. Overlaid ion chromatograms (A) and calculated coelution plot (B).

Figure 4. Compound identification results.

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Table 1. Compound confirmation results.

Compound	Tomato Blank	Tomato 0.005	Tomato 0.01	Tomato 0.05	Lemon Blank	Lemon 0.005	Lemon 0.01	Lemon 0.05	Avocado Blank	Avocado 0.005	Avocado 0.01	Avocado 0.05
Acetamiprid												
Aldicarb												
Azoxystrobin												
Bifenazate (D 2341)												
Buprofezin												
Carbaryl												
Carbendazim (Azole)												
Chlorfenvinphos(I)												
Chloroxuron												
Chlorpyrifos												
Chlorpyrifos-methyl												
Cyprodinil												
Difenconazole(I)												
Dimethoate												
Dimethomorph(E)												
Dimoxystrobin												
Dinotefuran												
Dioxacarb												
Ethoxyquin												
Fenamiphos												
Fenhexamid												
Fluquinconazole(I)												
Flutriafol												
Imazalil(II) (Enilconazole)												
Imidacloprid												
Metalaxyl												
Methidathion												
Myclobutanil												
Penconazole(I)												
Pendimethalin (Penoxalin)												
Phosmet (Imidan)												
Pirimicarb												
Propamocarb												
Propiconazole(II)												
Pyraclostrobin												
Pyridaben												
Quinalphos (Diethquinalphione)												
Spinosyn A												
Spiroxamine												
Sulfentrazone												
Tebuconazole (II) (Terbuconazole)												
Tebufenpyrad												
Thiabendazole												
Thiacloprid												
Thiamethoxam												
Triadimefon												
Triazophos												
Trifloxystrobin												
Uniconazole-P(I)												
Vamidothion												
Zoxamide												

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The data were then exported to MassHunter Quantitative Analysis rev. B.05.02 using a Compound Exchange Format (CEF) file. The CEF file contained information necessary to rapidly set up a quantitative processing method including compound name, retention time, precursor ion, fragment ions (to create qualifiers required by regulation), collision energy, and relative abundances. The Quantitative Analysis software automatically selected the major precursor and fragment ions with a relative abundance above 10% for each compound, saving tedious manual labor and time. Fragment ions with different collision energies were selected and used by the software (Figure 5).

The Quantitative Analysis software extracted chromatograms for the quantifier (target), qualifier ions, and isotopic cluster of the pseudo molecular ion. The isotope pattern can be confirmed by viewing an overlay with the theoretical pattern (Figure 6).

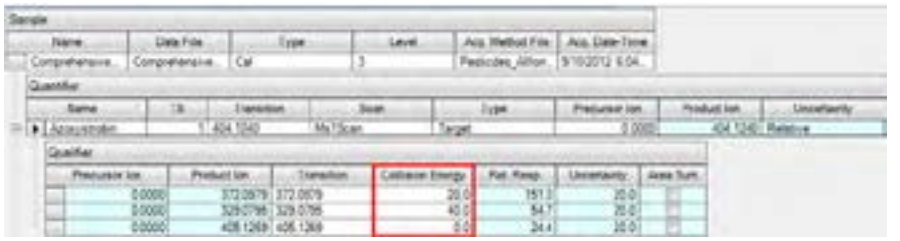

Figure 5. Quantitative method setup with multiple collision energies.

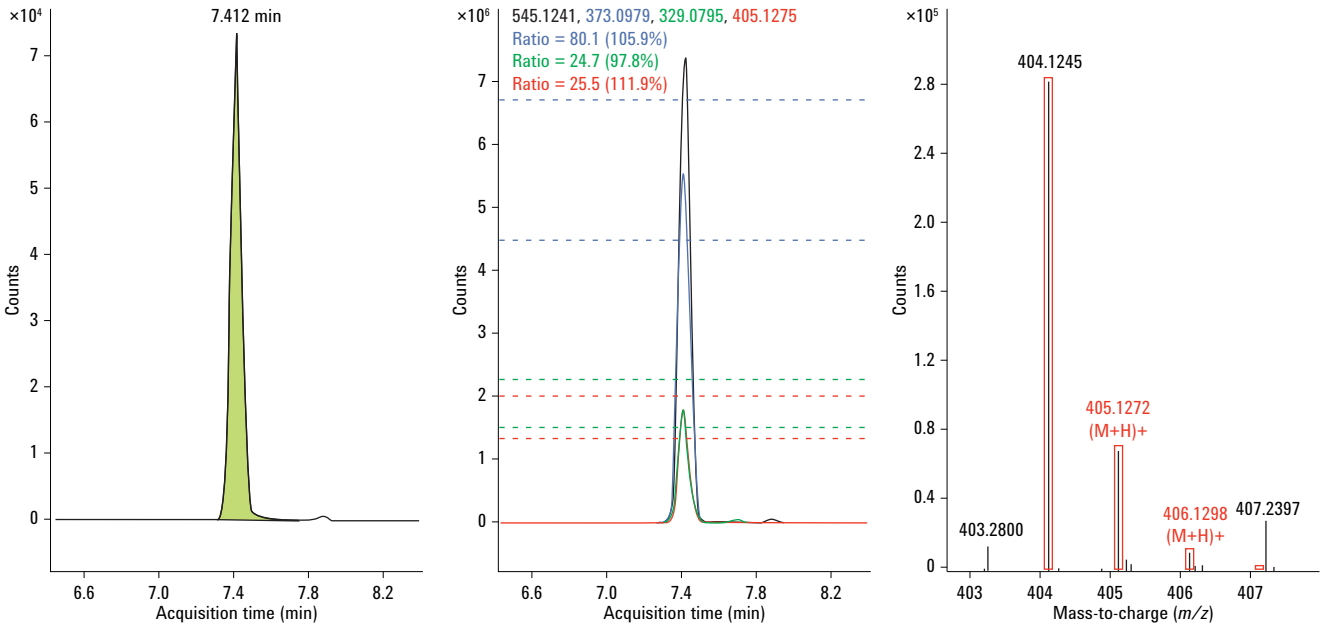

Figure 6. Compound information with quantifier, qualifiers, and isotopic cluster.

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After analyzing a large batch of samples, it is possible to easily review the results by sample and compound in the Compounds-at-a-glance module. Figure 7 shows chromatograms with compounds, annotated in red, that were outside of user defined outlier limits.

Conclusions

Samples containing pesticides spiked into food matrices were used to rapidly generate a quantitative data processing method for a Q-TOF mass spectrometer. The All Ions MS/MS technique was used to screen for the presence of compounds prior to the creation of the quantitative method. With the All Ion MS/MS technique, analysts benefit by gaining increased productivity as they do not have to enter hundreds of compound names or select specific product ions. In addition, large batches of sample results can easily be reviewed at a time. The data can also be re-interrogated at a later time by adding more compounds to the screen using an expanded PCDL. The Quantitative Analysis software provides an added level of confidence in the results by providing the interface to view quantifier and qualifier ions, including scoring the quality of identifications with accurate mass metrics.

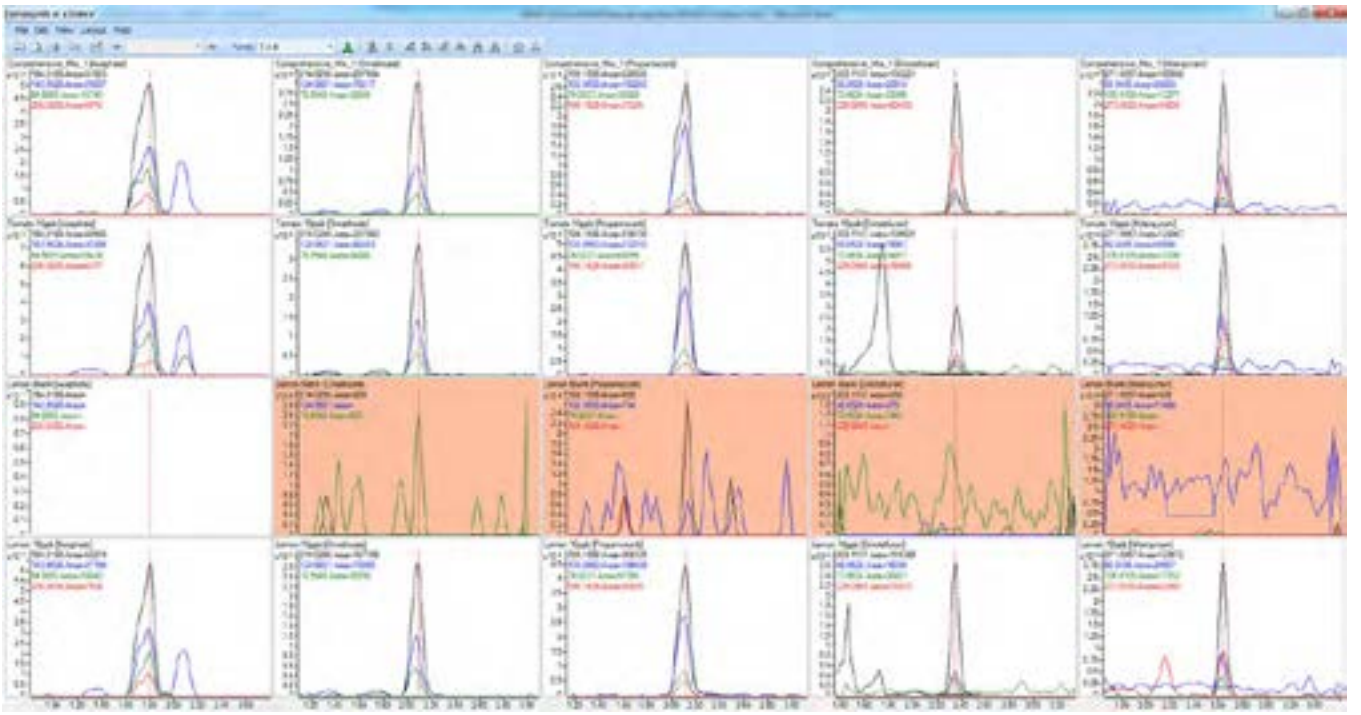

Figure 7. Compounds-at-a-glance view.

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Agilent Technologies

Screening and Quantitation of 240 Pesticides in Difficult Food Matrixes Using the Agilent 6545 Q-TOF LC/MS System

Application Note

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Abstract

This Application Note describes an UHPLC/Q-TOF/MS method for screening 240 pesticides and pesticide metabolites in difficult food matrixes. The method benefits from increased chromatographic resolution using the Agilent 1290 Infinity UHPLC System and improved ionization capabilities with an Agilent Jet Stream ionization source. More importantly, the successful screening of analytes is accomplished by the innate sensitivity improvement of the Agilent 6545 Q-TOF LC/MS System and ion transmission tuning to facilitate the optimal ion transmission of small, fragile organic molecules. Targeted MS/MS acquisition was used for analyte quantitation and confirmation. Black tea matrix was chosen for its complexity, and avocado matrix was chosen to represent food commodities with high lipid content.

Our results demonstrate that the improved detection of small organic molecules by the 6545 Q-TOF LC/MS System allows the screening and quantitation of most of the targeted pesticides below the maximum residue limits (MRLs) specified by the European Commission regulations.

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Introduction

Pesticide residue screening in food products is one of the most important and most demanding applications in food safety. There are more than 1,000 pesticides and pesticide metabolites which can be present in food. European Commission regulation (EC) 396/2005 and its annexes set maximum residue limits (MRLs) for more than 170,000 matrix-pesticide combinations[1]. Similar regulations are in place in other regions[2]. Accurate mass LC/MS in food safety applications permits the detection of a large number of analytes. This is especially important for some metabolites where chemical standards are hard to obtain, and the development of a triple quadrupole method is rendered difficult. Accurate mass LC/MS method setup is relatively easy and can be accomplished without knowing the retention time or fragmentations. This is especially important for a commercial testing lab to increase the scope of testing and throughput.

A typical workflow in accurate mass LC/MS includes the screening and quantitation of regulated pesticide residues by using MS domain data. Mass accuracy, isotopic abundance, isotopic spacing, and adduct pattern are used to verify positives. Quite often, retention time is also considered a critical factor for compound matching. Using a comprehensive personal compound database and library (PCDL) search can disclose a list of likely pesticide residues. The pesticide residues can be further confirmed by auto or targeted MS/MS with the application of appropriate collision energy for fragmentation, and searched against the PCDL. This is of critical importance to rule out potential false positives in the context of complex matrixes such as QuEChERS extracts. The MS/MS information gives a higher level of identification confidence.

Most pesticides are analyzed with multiresidue methods covering hundreds of compounds applied to various food commodities. Therefore, fast and reliable analytical methods are required for pesticide identification at low concentrations in a broad range of food matrixes. Criteria for the identification of pesticide residues and requirements for method validation and quality control procedures for quantitation are specified in guidance documents such as SANCO/12571/2013[3].

This Application Note describes the development of an UHPLC/QTOF/MS method for the screening and quantitation of hundreds of pesticides in food samples. The method was developed using the Pesticide Comprehensive Test Mix (p/n 5190-0551). An Agilent 1290 Infinity UHPLC System was coupled to the Agilent 6545 Q-TOF LC/MS System. The acquisition was carried out in positive ion mode. Several modifications associated with the 6545 Q-TOF LC/MS System have resulted in higher analytical performance. Hardware improvements include a new:

- Slicer design with the option to operate under high sensitivity or high resolution mode
- High performance high voltage power supply that improves the mass resolution for higher molecular weight entities
- Enhanced gain shifted detector that provides much longer detector lifetime

More importantly, for the first time, Particle Swarm Optimization technology is commerically used to optimize mass spectrometers, resulting in much faster (4x) and more robust tuning of the instrument. The improvements in ion transmission for small molecules also results in mass accuracy enhancement below 100 *m/z*. A substantial 4x increase in signal response compared with the previous generation of the Agilent 6540 Q-TOF LC/MS System is achieved.

Experimental

Reagents and chemicals

All reagents and solvents were HPLC or LC/MS grade. Acetonitrile and methanol were purchased from Honeywell (Morristown, NJ, USA). Ultrapure water was produced using a Milli-Q Integral system equipped with a LC-Pak Polisher and a 0.22 µm point-of-use membrane filter cartridge (EMD Millipore, Billerica, MA, USA). Formic acid was from Fluka (Sigma-Aldrich Corp., St. Louis, MO, USA) and ammonium formate solution (5 M) was from Agilent (p/n G1946-85021). Pesticides were included in the Agilent Pesticide Comprehensive Test Mix (p/n 5190-0551). A 10 ppm amount of pesticides working solution was used for spiking the QuEChERS extracts and preparing the calibration samples.

Sample preparation

Organic black tea and organic avocado were obtained from a local grocery store. Samples were extracted according to the official citrate-buffered QuEChERS protocol using Agilent BondElut QuEChERS kits (p/n 5982-5650)[4]. Ten grams of homogenized avocado and 2 g of black tea were weighed into 50-mL polypropylene tubes and extracted with 10 mL acetonitrile for 1 minute while shaking vigorously. The tea samples were wetted with 8 mL ultrapure water for 2 hours prior to extraction. Raw extracts were cleaned up by dispersive SPE with lipid removal for avocado (p/n 5982-5158) and with graphitized carbon black (GCB) (p/n 5982-5356H) for black tea. Final extracts were spiked in six relevant concentrations with the pesticides at 1 ng/g, 5 ng/g, 10 ng/g, 20 ng/g, 50 ng/g, and 100 ng/g. The matrix-matched standards were prepared before injection and were measured with five technical replicates at lower concentration levels.

Equipment and software

Separation was carried out using an Agilent 1290 Infinity UHPLC System consisting of:

- Agilent 1290 Infinity Binary Pump (G4220A)
- Agilent 1290 Infinity High Performance Autosampler (G4226A) and sample cooler (G1330B)
- Agilent 1290 Infinity Thermostatted Column compartment (G1316C)

The UHPLC system was coupled to an Agilent 6545 Q-TOF LC/MS System equipped with an Agilent Jet Stream electrospray dual ionization source. Agilent MassHunter workstation software was used for data acquisition (B.06.01, build 6.01.6145), qualitative analysis (B.07.00, build 7.0.7024.0) and quantitative analysis (B.07.00, build 7.0.457.0).

Methods

The 1290 Infinity UHPLC conditions are summarized in Table 1. Analysis was carried out with positive ion mode. Three microliters of the final extract were injected. Source parameters are optimized with a subset of 14 pesticides that represents the cohort of 240. The summary of the 6545 Q-TOF LC/MS System parameters are listed in Table 2.

Data were evaluated using the MassHunter Qualitative and Quantitative Analysis software. Calibration curves were generated using quadratic fitting, 1/x weighting, and including the origin.

Table 1. Agilent 1290 UHPLC parameters.

Parameter	Value
Column	Agilent ZORBAX Eclipse Plus C18 2.1 × 150 mm, 1.8 µm (p/n 959759-902)
Column temperature	45 °C
Injection volume	3 µL
sampler temperature	5 °C
Needle wash	10 seconds (80 % MEOH/20 % water)
Mobile phase	MPA: Water, 5 mM NH_4formate + 0.1 % formic acid MPB: MeOH, 5 mM NH_4formate + 0.1 % formic acid
Flow rate	0.4 mL/min
Gradient program	Time % B 0 5 1 5 4 50 17 100 20 100 20.1 5 Stop time 22 minutes Post time 1 minute

Table 2. Agilent 6545 Q-TOF LC/MS System parameters.

Parameter	Value
Mode	Positive; 4 GHz High Resolution
Tune	50–250 *m/z*
Drying gas temperature	150 °C
Drying gas flow	10 L/min
Sheath gas temperature	375 °C
Sheath gas flow	11 L/min
Nebulizer pressure	35 psi
Capillary voltage	3,500 V
Nozzle voltage	200 V
Fragmentor	125 V
Skimmer	45 V
Oct1 RF Vpp	750 V
Acq mass range	100–1,000 *m/z* (MS only)
Acq rate	3 spectra/s
Ref mass ions	121.050873, 922.009798

Results and Discussion

New tuning algorithm: particle swarm optimization tune (SWARM tune)

An artificial intelligent optimization algorithm that simulates social learning behavior in a bird flock was used to optimize the mass spectrometer with different criteria. Unlike classic Auto Tune, that generates one-size-fits-all optimization, the SWARM tune opens a new chapter for customizable instrument optimization based on application needs. The algorithm provides the possibility to optimize the ion transmission for fragile smaller molecules (for example, 50–250 *m/z*, and 50–750 *m/z*) depending on the user selection. In conjunction with the hardware improvement, the optimization dramatically improves signal response for small molecules. Moreover, acetonitrile-sodium adducts can be used as an additional calibrant to improve the mass accuracy in the 50–100 *m/z* range under the fragile ions tune. In addition to sensitivity and mass accuracy improvements associated with the 6545 Q-TOF LC/MS System, a substantial increase in tuning and calibration speed can be achieved. Comparing with the classic auto tune on the 6540 Q-TOF LC/MS System, the system tuning time was reduced by a factor of four. The new algorithm and methodology also let the system tune TOF and Quad simultaneously under both polarities.

Figure 1 shows a new user interface under the tune context.

Agilent 6545 Q-TOF LC/MS System performance

The changes in hardware and ion transmission tune result in a factor of ~4 signal response improvement by the 6545 Q-TOF LC/MS System compared to the 6540 Q-TOF LC/MS System when the high resolution slicer position is chosen. Figure 2 shows the overlay of 10 ppb thiabendazole chromatographs on a 6540 Q-TOF LC/MS System and a 6545 Q-TOF LC/MS System. Thiabendazole has a molecular weight of 201.3, and the optimized ion transmission of the *m/z* 50–250 range further improved the signal response. In addition to thiabendazole, the other 13 pesticides in this small study demonstrated a similar improvement trend.

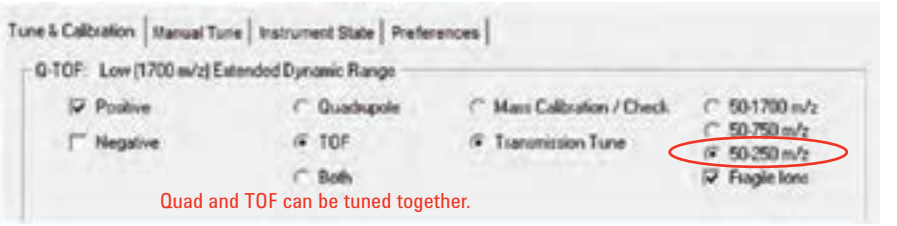

Figure 1. User interface under the tune context: the SWARM tune is implemented and defines optimal conditions for ion transmission based on application needs.

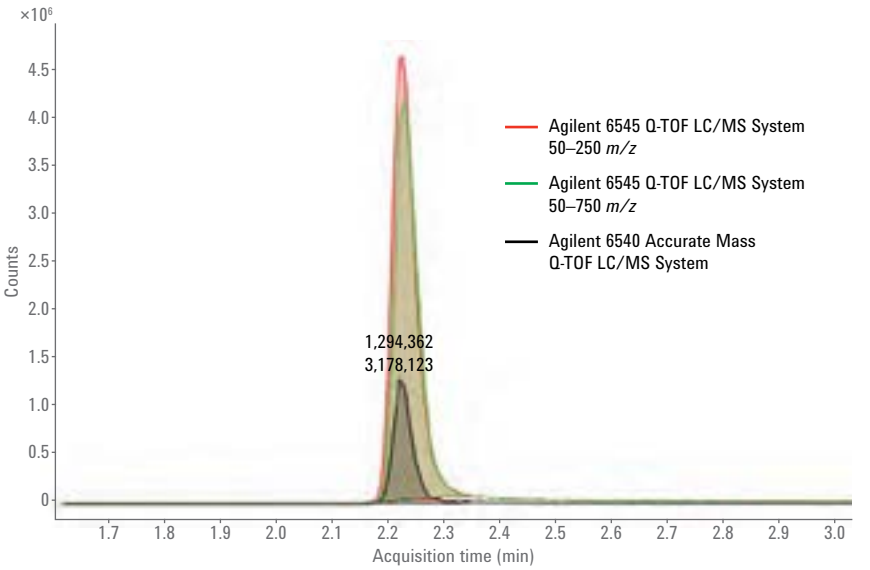

Figure 2. Exemplary chromatogram of signal response of 10 ppb thiabendazole in acetonitrile on an Agilent 6540 Q-TOF LC/MS System and an Agilent 6545 Q-TOF LC/MS System. The optimized ion transmission at 50–250 *m/z* provides further improvement compared with optimized ion transmission at 50–750 *m/z* for the analyte with *m/z* 202.3.

Similar improvement for a group of 240 pesticides has been observed in black tea and avocado matrixes at 10 ng/g spike concentration, corresponding to 2 ppb and 10 ppb respectively. For compounds that can be detected at these levels, the ratios are taken from the average of five replicates with each instrument. Histograms are shown in Figure 3. The improvement in sensitivity results in more compounds identified by the 6545 Q-TOF LC/MS System than by the 6540 Q-TOF LC/MS System. An example of 10 ng/g alanycarb spiked in black tea matrix is shown in Figure 4. The 6540 Q-TOF LC/MS System failed to detect the compound. The above results were obtained with a high resolution slicer position. It is expected that another two-fold signal response improvement can be achieved when a high sensitivity slicer position is chosen.

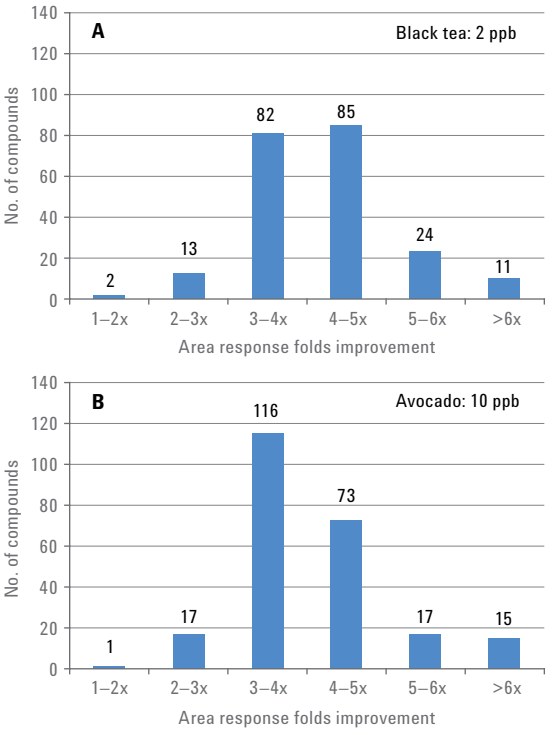

Figure 3. Signal response improvements of ~240 pesticides in black tea and avocado matrixes. The area response ratio of Agilent 6545 Q-TOF LC/MS System/Agilent 6540 Q-TOF LC/MS System was taking from the average of five replicates for each analyte. Another two-fold signal response improvement is expected when the high sensitivity slicer position is chosen.

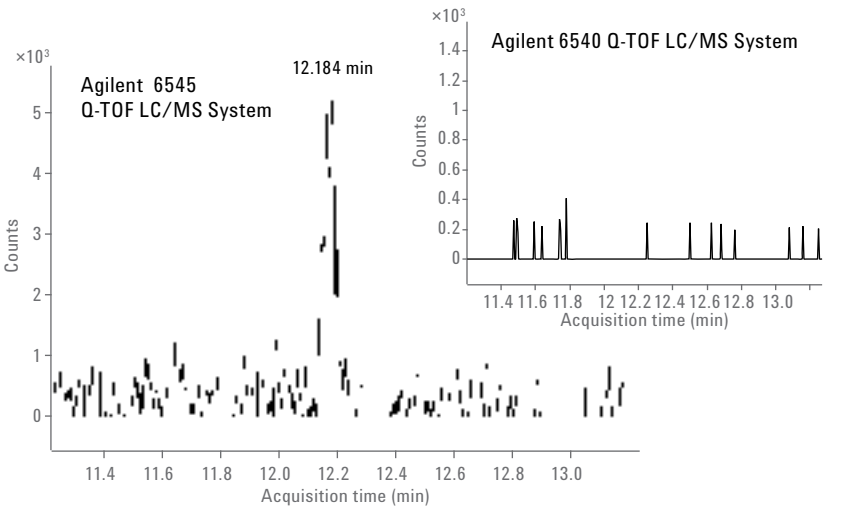

Figure 4. The Agilent 6545 Q-TOF LC/MS System sensitivity improvement benefits the analyte at the borderline of detection. Alanycarb could be detected by the Agilent 6545 Q-TOF LC/MS System, but not by the Agilent 6540 Q-TOF LC/MS System in black tea matrix at 10 ng/g spike level.

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Compounds identification using Find by Formula (FbF)

The PCDL containing all spiked pesticides was generated from Agilent Pesticide PCDL (G3878-60003 MassHunter Personal Pesticide Library). The FbF search of analytes in matrixes against the PCDL was conducted, and some parameters settings of FbF algorithm are listed in Table 3. Software automatically generated an extracted ion chromatogram for the expected ion species of the target compounds in the accurate mass database. Peak spectra were extracted and the measured results were compared with calculated results to generate a matching score based on mass accuracy, isotopic abundance, and isotopic spacing[5].

Figure 5 shows, as an example, the methidathion chromatogram and peak spectrum in black tea matrix at 10 ng/g spike level, which corresponds to 2 ppb. At such a low concentration, and in one of the most complex matrixes, outstanding signal-to-noise (S/N) ratio can be obtained. Both proton adduct and ammonium adduct give excellent matching scores, with up to 400–500x coeluting background ions from the matrix. Consequently, the overall score for H^+ adduct is 99.66 out of 100 (mass accuracy 99.81 out of 100; isotopic abundance 99.74 out of 100; isotopic spacing 99.26 out of 100), and the overall score for ammonium adduct is 97.36 out of 100 (mass accuracy 99.29 out of 100, isotopic abundance 94 out of 100, isotopic spacing 97.52 out of 100). The mass error for major ion species is generally low, resulting in a matching score for most of the analytes > 90 out of 100.

Table 3. Parameter Settings for FbF Data Mining

Parameter	Value
Extraction data file	Profile for chromatographic and spectral extraction
Charge state	1
Isotopic model	Common organic molecule
PCDL	Subset of G3878CA
Adduct	$[M+H]^+$, $[M+NH_4]^+$, $[M+Na]^+$
Mass tolerance	6 ppm
RT window	0.5 minutes
Mass accuracy weighting	100
Isotopic abundance weighting	60
Isotopic spacing weighting	50

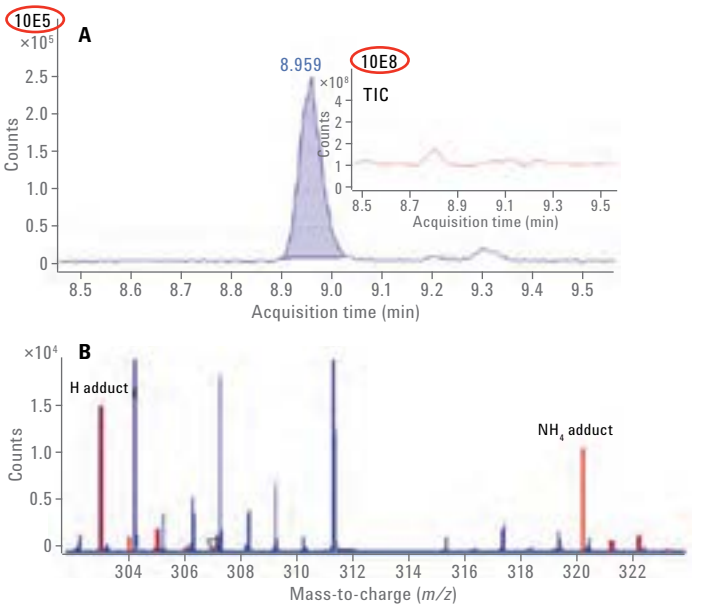

Figure 5. Compound chromatograph and peak spectrum by FbF algorithm for methidathion spiked into a QuEChERS extract of black tea at 10 ng/g. The identification of the compound with high confidence can be achieved even with 400–500x coeluting background ions.

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The MS domain data can be used to obtain quantitative information for the spiked pesticides. The best ions for the quantitative method are derived from the compound results extracted from the MassHunter Qualitative Analysis Software using 20 ppb pesticides in neat solvent. The results are exported as a compound exchange file (.cef) that is used in quantitative analysis. Quantifier and qualifier are automatically selected from the observed adduct species and isotopic signals based on their relative abundance. All pesticides could be detected at 10 ng/g spike concentration in avocado matrix except propagite, which could not be detected in neat solvent at higher concentration, possibly due to degradation. We did not detect 20 compounds at 10 ng/g in black tea matrix due to the combination of 5x lower concentration and severe matrix effect. However, the improved sensitivity of the 6545 Q-TOF LC/MS System still achieved ~70 % analyte detection at a 1 ng/g spike level in black tea matrix, corresponding to 0.2 ppb. The number of the compounds detected at different spike concentrations is shown in Figure 6.

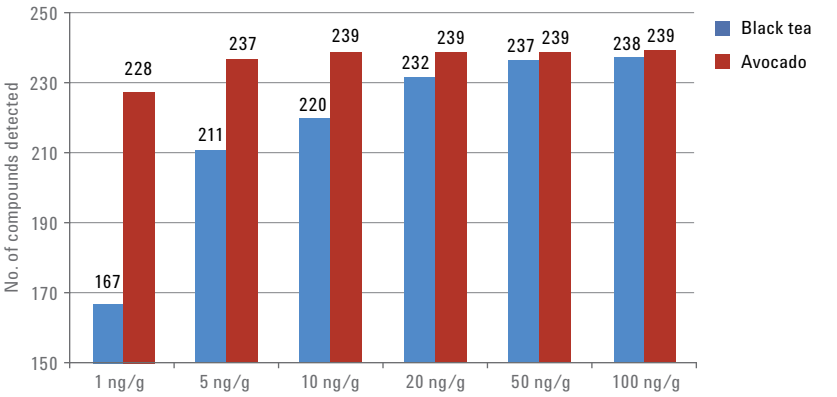

Figure 6. Number of the compound detected at each spike concentration in black tea and avocado matrixes. The improvement in sensitivity of the Agilent 6545 Q-TOF LC/MS System results in more compounds to be detected.

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Quantitation and confirmation with targeted MS/MS

Targeted MS/MS is an important feature in data acquisition for analyte quantitation as well as for structure confirmation by comparing the fragmentation pattern against the database and library. Knowledge of the retention time of a particular ion species is required to set up the acquisition method. The retention times were retrieved from MS domain data that could be extracted from Agilent MassHunter Qualitative Analysis Software. Retention time window (delta RT) was set to 1 minute. Reference ions were excluded for the MS/MS spectrum. Acquisition rates were 15 spectra/s for MS and 12 spectra/s for MS/MS. Collision energy was set as a linear regression of 4 + 6 × mass/100, depending on the molecular weight of the target ions. As a result, a search filter on collision energy of ± 20 eV was applied to focus comparison of measured spectra to those library entries of similar collision energy. Most compounds were confirmed and quantified at or below the MRL in black tea matrix with reverse matching. Most of the compounds detected on MS domain could be detected by targeted MS/MS. Figure 7 shows a calibration curve and library match at 10 ng/g spike level of metobromuron in black tea matrix (top) and dimethoate in avocado matrix (bottom). It is expected that the measure MS/MS spectrum in the presence of matrix can be noisier than the library spectrum often acquired in neat solvent at relatively higher concentration. In MassHunter Quantitative Analysis, users can either set up the reference library with PCDL, or use the standards in neat solvent under the same LC/MS conditions through the compound exchange file.

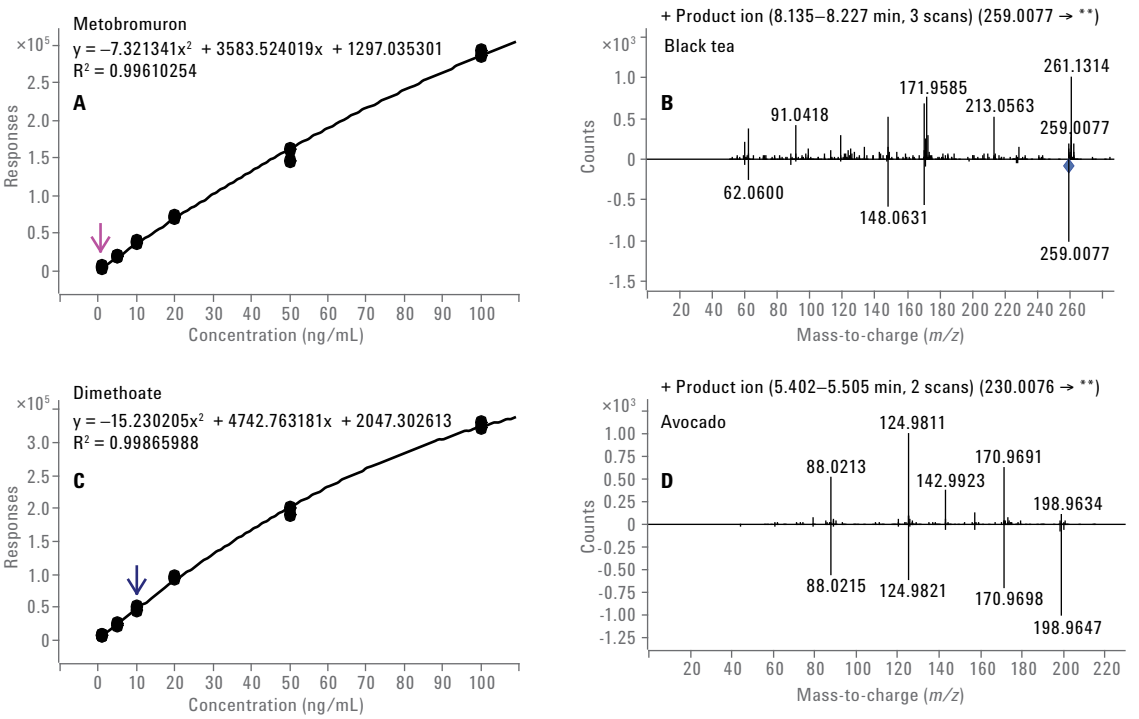

Figure 7. Targeted MS/MS can be used to quantify pesticides as well as confirm its structure by fragments comparing with PCDL spectrum. Most of the compounds detected on MS domain could be quantified by targeted MS/MS. A 10 ng/g spike of metobromuron in black tea (top) and dimethoate in avocado (bottom) are shown as examples.

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Structure confirmation with Auto MS/MS

Auto MS/MS is a feature frequently used for untargeted analysis. In pesticide screening, to eliminate false positives in complex matrixes such as black tea and avocado, further structure confirmation is needed. The confirmation is conducted by comparing the measured MS/MS spectrum to the library spectrum. Caution should be taken when seting up auto MS/MS methods. Sometimes, a preferred ion list is required to overcome matrix interference and obtain meaningful MS/MS spectra. Ion species and associated retention times can be imported from MassHunter Qualitative Analysis on MS domain data. The advantage of auto MS/MS over targeted MS/MS is that the data mining can be performed retrospectively should the library be updated with more analytes for their presence in the samples. The acquisition typically lasts only 2–3 cycles for more compound coverage. Thus, auto MS/MS is not recommended for analyte quantitation. Two examples are shown in Figure 8 on the stucture confirmation for chlorfevinphos (II) in black tea and diethofencarb in avocado at 10 ng/g spike concentration.

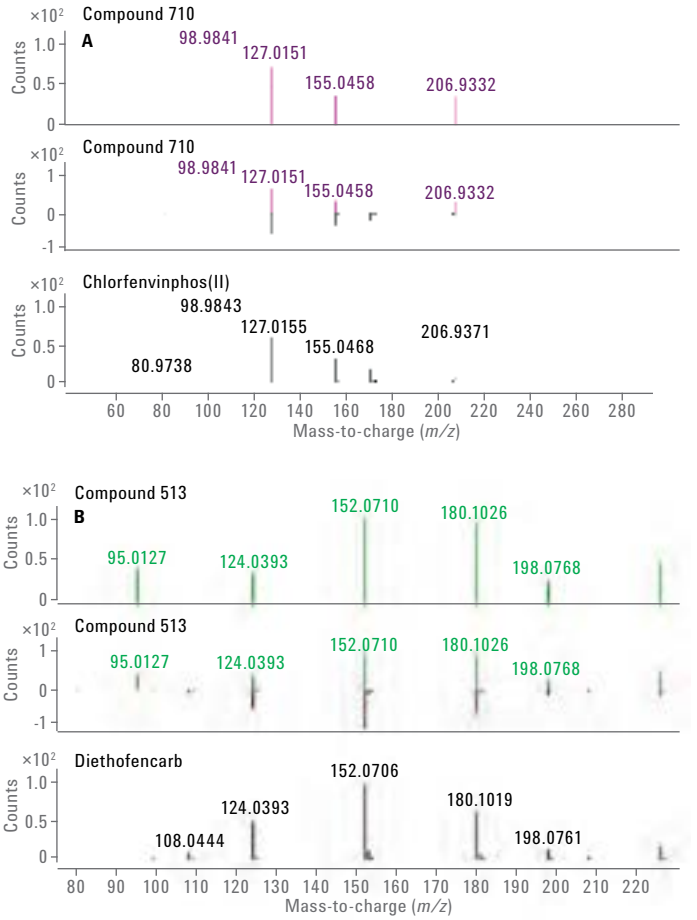

Figure 8. Auto MS/MS is useful for structure confirmation by the comparison of the measured spectrum and library spectrum. The examples show 10 ng/g chlorfevinphos (II) in black tea (A) and 10 ng/g diethofencarb in avocado (B).

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Conclusions

The Agilent 6545 Q-TOF LC/MS System is a valuable addition to the product family for the applied market with its improvement in sensitivity and ion transmission tune for fragile organic molecules. We have demonstrated that most of the pesticides and pesticide metabolites can be detected well below the MRL in complex matrixes. The method can easily be extended for more analyte screening and quantitation. This can potentially allow laboratories to increase their testing scale and throughput. The Agilent total solution, from comprehensive reagent kit, UHPLC/MS, PCDL, and MassHunter Qualitative/Quantitative Analysis Software, has allowed us to facilitate method development and validation for the end users.

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5. Kempe, G; Spitzbarth, F; Glauner, T. Development and Application of an Exact Mass LC/MS/MS Library for the Accurate Mass Screening and Quantitation of Pesticides in Fruits and Vegetables, *Agilent Technologies Application Note*, publication number 5991-2227EN, **2013**.

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- Method Setup Guide that shows you how to create MRM, dMRM, and tMRM methods
- Application notes with detailed LC/MS method information
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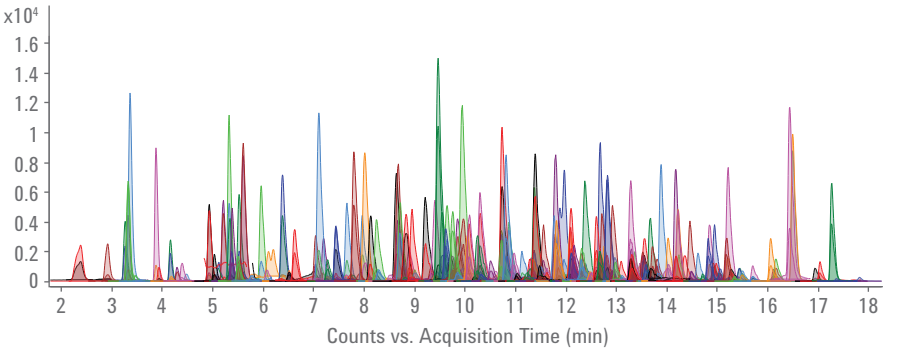
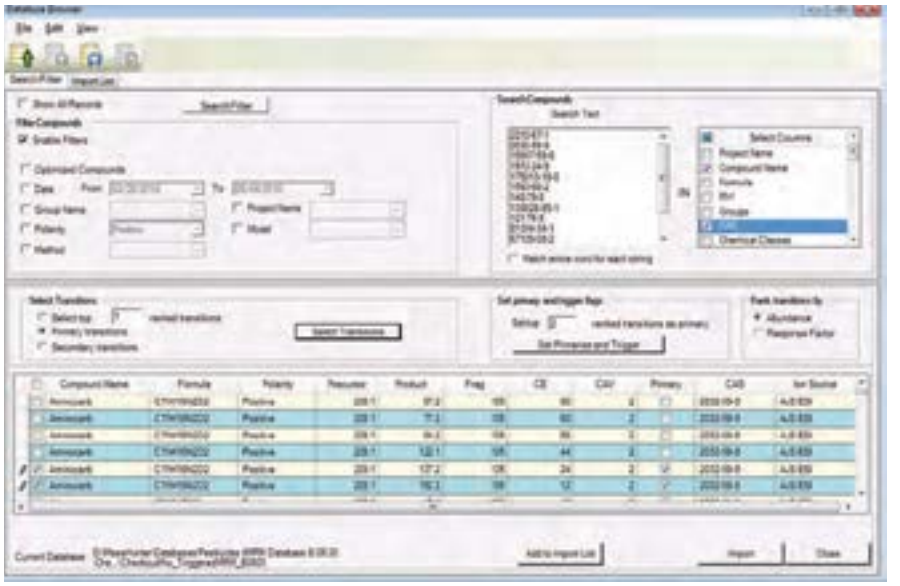
- **For additional specificity, you can create a tMRM method** by adding secondary transitions to a dMRM method. tMRM is particularly beneficial for isobaric compounds, and compounds affected by matrix interference. Additionally, tMRM is compatible with fast cycle times, so you can apply it to a subset of problematic compounds—or to all compounds in a multi-residue screen.
- **Create a reference library from your data** specific to your analysis and confirm results with a library match to confidently assess borderline results.

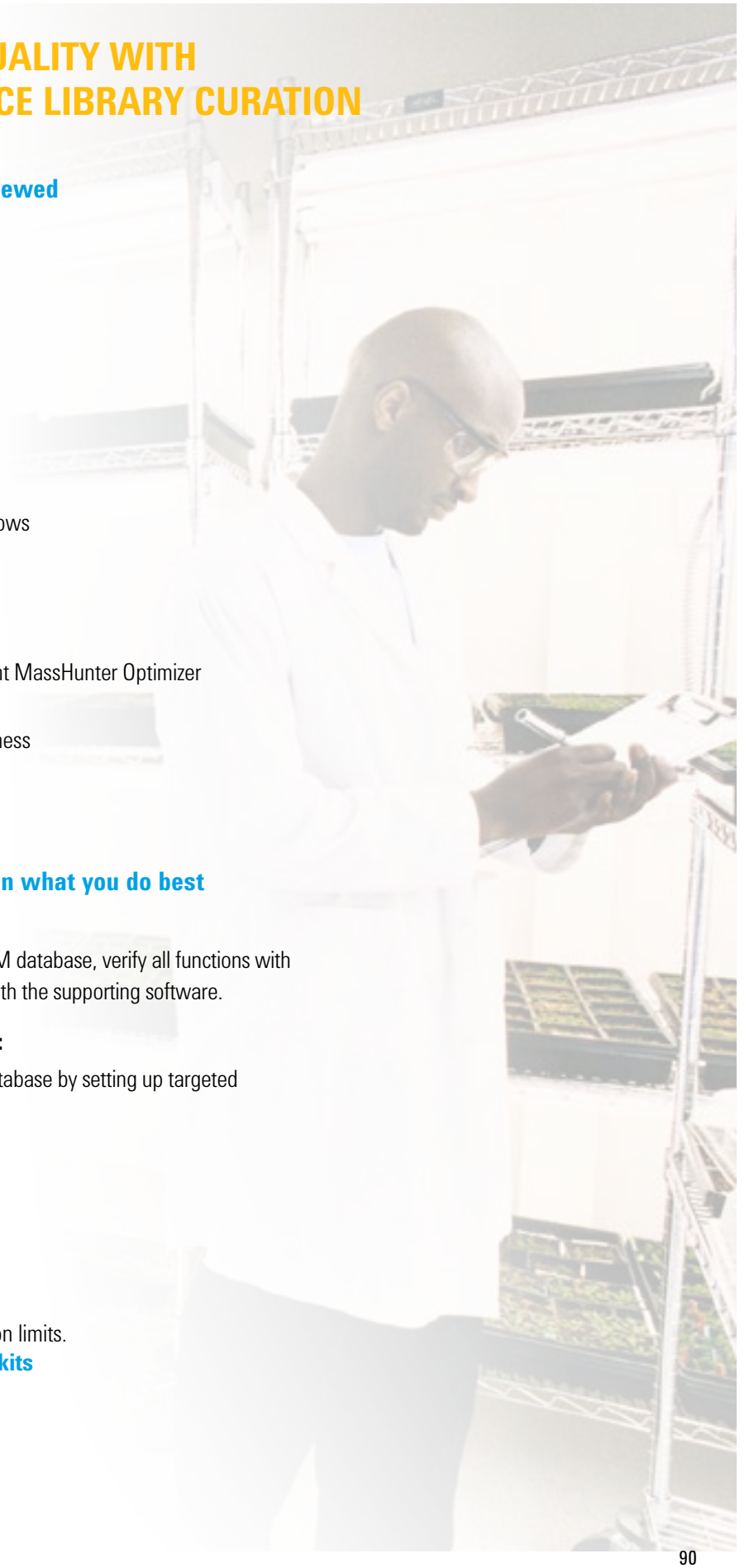


MRM chromatograms of a 20-fold dilution of more than 250 pesticides spiked into black tea at an MRL of 10 µg/kg. The sample was analyzed using an Agilent 1290 Infinity II UHPLC and 6470 Triple Quadrupole LC/MS System.



The Agilent Pesticide tMRM database ensures fast, customized method development and can be easily expanded, or have subset databases created.

MAXIMIZE YOUR DATA QUALITY WITH DATABASE AND REFERENCE LIBRARY CURATION



Curated databases—each entry is reviewed for correctness, and includes:

- Compound common name
- CAS number of the native compound
- Molecular formula
- Unit mass of the neutral molecule
- MRM transitions (precursor and product m/z)
- Fragmentor voltage
- Collision energy
- Added retention times and retention time windows
- Added trigger parameters

MRM transition curation:

- MRM transitions are optimized using the Agilent MassHunter Optimizer software for more than 600 compounds
- Optimized MRM data are reviewed for correctness
- Compounds include: Pesticides, insecticides, herbicides, fungicides

Application consulting lets you focus on what you do best

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Agilent 6400 Series Triple Quadrupole LC/MS System
Agilent MassHunter Acquisition Software (B.06 or higher) and Windows 7 (64-Bit)
Agilent MassHunter Qualitative Analysis Software (B.06 or higher)
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5991-7061EN

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Triggered MRM LC/MS/MS Method Development – Practical Considerations for MRM Optimization Using Agilent MassHunter Optimizer Software

Technical Overview

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Introduction

Optimization of multiple reaction monitoring (MRM) conditions is an essential part of any liquid chromatographic-tandem mass spectrometric (LC/MS/MS) method development. A typical MRM or dynamic MRM (dMRM) method employs two MS/MS transitions per analyte: one for quantification, and the second one as a qualifier for identification purposes. In a triggered MRM (tMRM) method using Agilent 6400 Series triple quadrupole LC/MS systems, up to 10 MS/MS transitions can be acquired for each analyte, and combined into a product ion spectrum (at optimum collision energies for each product ion), which is used for library matching, and provides increased identification confidence. Using the tMRM function, some of the transitions (primary transitions) are acquired during the entire analyte acquisition window. The acquisition of the additional transitions is triggered (and performed for a defined number of scans) when one of the primary transitions exceeds the set abundance threshold [1].

Agilent MassHunter Optimizer software is a versatile tool for automated optimization of MRM conditions, including the selection of precursor and product ions and optimization of collision energies (CE) [2,3]. Using Optimizer software significantly reduces the time needed for MRM optimizations, especially for multi-analyte tMRM methods when more than two MRMs need to be optimized for multiple analytes. Optimizer software is simple to use, and provides a high degree of flexibility but, as with any tool, it should be used appropriately. This technical overview provides practical considerations for routine optimization of compounds using MassHunter Optimizer, drawing from our experience of developing a large pesticide multiresidue tMRM LC/MS/MS method [4].

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MRM Optimization Using Agilent MassHunter Optimizer

MassHunter Optimizer automates the selection of:

- Precursor ions using MS2 Selected Ion Monitoring (SIM)
- Optimum fragmentor voltage for each precursor ion (not done in the case of Agilent 6490 and Agilent 6495 instruments)
- Product ions using product ion scan for each precursor ion
- Optimum CE for each transition using MRM acquisition mode

Compound information (name and formula or nominal mass) and location of a vial with an appropriate standard solution can be entered directly into Optimizer, or can be imported from Microsoft Excel [3]. The latter option is convenient, especially when a larger list of compounds is being automatically optimized. Using a template method, Optimizer creates all necessary MS data acquisition methods (SIM, product ion scan, and MRM), and generates a report with the list of optimized MRM conditions. These conditions can be stored in a MassHunter database, loaded into the MassHunter Acquisition software, and exported into Microsoft Excel.

Precursor Ion Selection

Optimizer provides a highly flexible selection of potential precursor ions, including both positive and negative ions, various adducts (different options can be added manually as needed), and charge states. It is possible to conduct the optimization for the most abundant precursor ion, or set the search order based on user priorities. Certain masses or low-abundance precursor ions can be excluded from consideration.

The actual precursor ion selection is based on data acquired using MS2 SIM. The monitored *m/z* values in MS2 SIM are determined based on the entered compound formula or nominal mass, using the specified adducts, neutral losses, and charge states. If the molecular formula is used, Optimizer calculates monoisotopic mass. In certain cases, such as when the analyte molecule contains bromine or chlorine atoms, a mass other than the monoisotopic mass may result in more abundant precursor ions (for example, in the case of two or more bromines or a bromine/chlorine combination). This specific mass could be entered in the nominal mass field without entering compound formula. Similarly, for identification purposes, it may be useful to optimize two masses with different isotopic composition when the compounds do not generate a sufficient number of strong product ions (see Figure 1 for an example of the pesticide diuron).

Depending on the compound structure, mobile phase composition and user preferences (preferred ionization mode, exclusion of Na^+ adducts, and so forth), the precursor ion options may be limited to one or two possibilities. This streamlines the optimization process and results/data review. For example, when using a mobile phase containing ammonium ions for small organic compounds ionizing in positive electrospray, single charge, and positive mode, one can have only H^+ and NH_4^+ adduct options selected to simplify the process.

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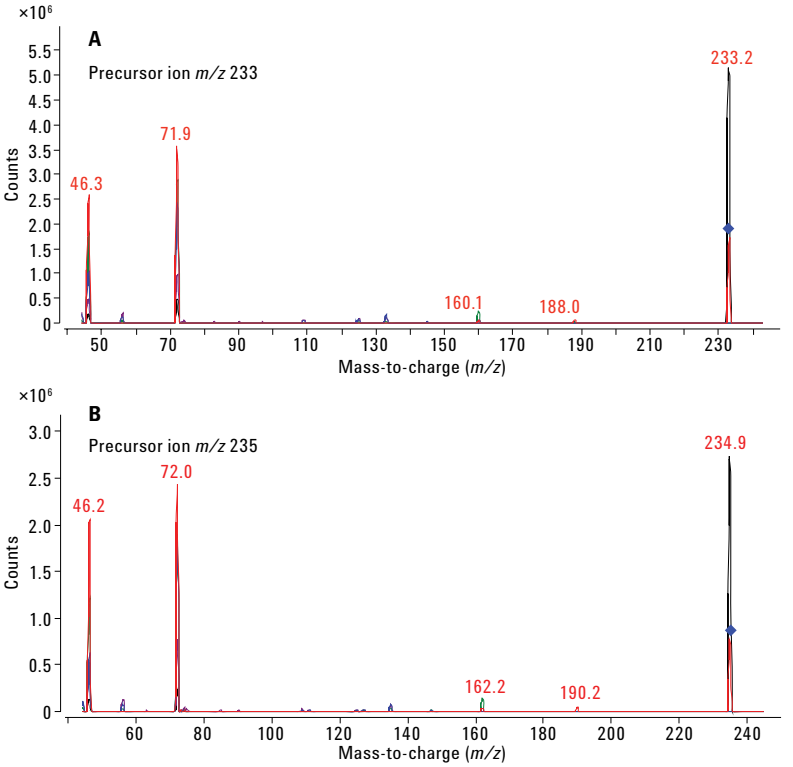

Figure 1. Overlay of product ion spectra obtained at CEs of 0, 15, 30, 45, and 60 eV for two diuron ($C_9H_{10}Cl_2N_2O$) precursor ions (m/z 233 and 235) with different chlorine isotope composition (^{35}Cl and $^{35}Cl/^{37}Cl$, respectively).

Product Ion Selection

Product ions are selected as the most abundant ions in a composite product ion scan spectrum obtained for a given precursor ion at multiple CEs. Certain ions can specifically be excluded based on their *m/z* values, minimum abundance, neutral loss (for example, a generic loss of H_2O or NH_3), and by setting a low-mass cut-off. The low-mass cut-off is an important consideration. For example, if the cut-off were set at *m/z* 100 for the diuron example in Figure 1, sensitivity (and also selectivity) of diuron determination would be severely compromised because the most abundant product ions (*m/z* 46 and 72) would not be selected for MRM optimization. Similarly, if the cut-off were set at *m/z* 50, the second most abundant product ion (*m/z* 46) would not be considered. For relatively small molecules (precursor ions less than *m/z* 300), we recommend setting the cut-off at *m/z* 45.

The product ion spectra are obtained at different CEs across the CE range specified by the user in the Optimizer settings. Product ion scans at 0, 15, 30, 45, and 60 eV were collected for CEs ranging from 0 to 60 eV within one analytical run (Figure 2). This range provides good generic conditions for optimization of a wide range of compounds, typically resulting in a sufficient selection of both lower- and higher-mass product ions. The precursor ions were selected from the composite product ion spectrum collected during the entire data acquisition time. This could lead to potential problems if isobaric precursor ion interferences are present, as demonstrated in Figure 2, and discussed in more detail below.

The maximum number of product ions that should be found at this stage depends on the intended application. For tMRM methods, it is recommended that up to 10 product ions are selected for MRM optimization.

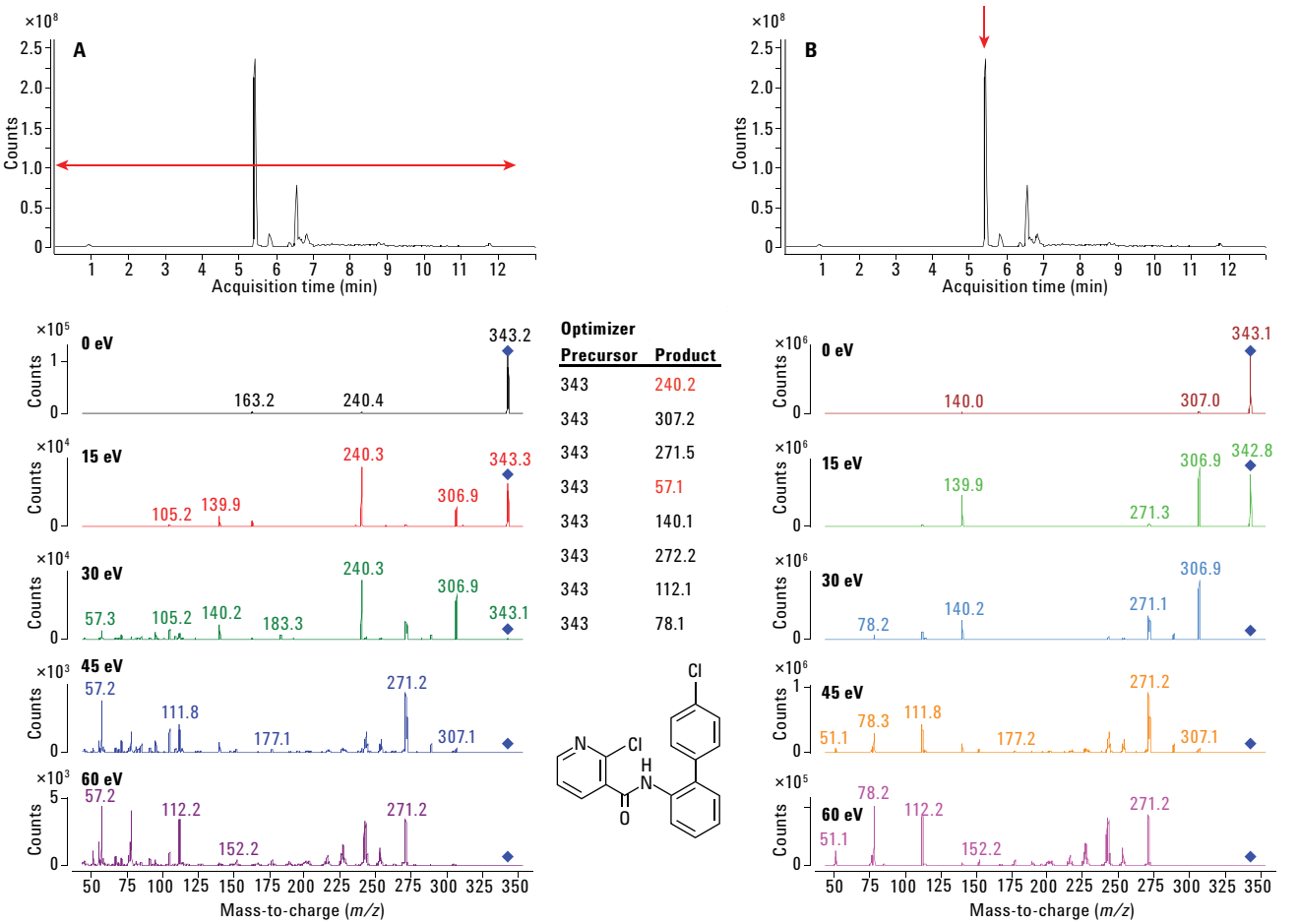

Figure 2. Product ion spectra obtained at CEs of 0, 15, 30, 45, and 60 eV during boscalid (formula: $C_{18}H_{12}Cl_2N_2O$; precursor ion: m/z 343; retention time: 5.45 minutes) optimization (A) for the entire data acquisition time (with respective Optimizer results showing product ion selection), and (B) across the boscalid peak. Product ions m/z 240 and 57 (highlighted in red) do not belong to boscalid.

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CE Optimization

The CE optimization involves the acquisition of each precursor-to-product ion transition at varying CEs. The CEs are varied across the selected CE range at a predefined step of 4 eV. When a CE range of 0 to 60 eV was used, 16 different CEs were tested for each transition within a single analytical run. This resulted in 160 different MRM settings when 10 transitions were optimized. Therefore, if a chromatographic separation is used for analyte optimization, it is important to set an appropriate dwell time in the Optimizer software to collect enough data points across the expected width of the analyte peak. For UHPLC separations, a dwell time of 5 ms or less is recommended for the above number of MRMs.

Another important consideration is the obtained analyte signal and related amount of analyte introduced into the system. For accurate CE optimization, it is important to avoid signal saturation by using an appropriate analyte concentration or injection volume. Figure 3 compares extracted ion chromatograms for three different diuron transitions obtained during the CE optimization (CE range 0 to 60 eV) in the same optimization run, and shows that if the analyte signal is saturated, it is not possible to determine the optimum CE for the given transition. The signals for the more abundant transition *m/z* 233 → 72 became saturated close to the optimum value (CE range of 12 to 32 eV), making the determination of the actual optimum CE value difficult. Whereas, the optimum CE values for the less abundant transitions *m/z* 233 → 133 and 233 → 188 were easily determined under the same conditions. Based on our experience, signal saturation might be suspected when analyte abundance exceeds approximately 5×10^6 counts. If this happens, CE optimization should be repeated using an adequately reduced analyte concentration or injection volume.

If the initial optimization gives an optimum CE value at the maximum evaluated CE (such as at 60 eV in the above mentioned example), the CE optimization should be redone with a higher CE value range.

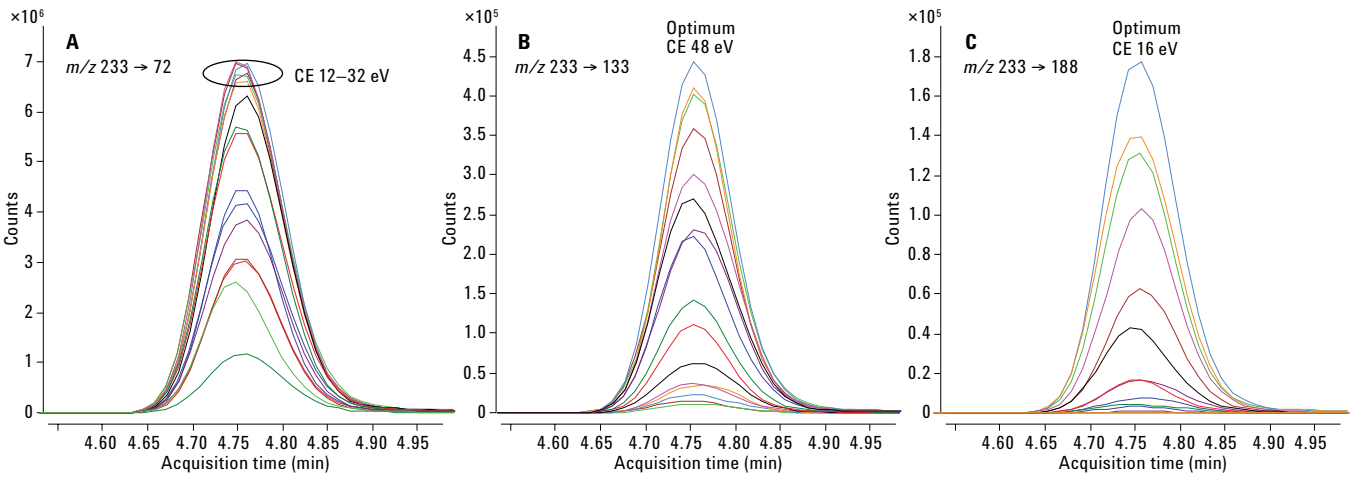

Figure 3. Overlay of extracted ion chromatograms for three different diuron transitions obtained during CE optimization (CE range 0 to 60 eV) in the same optimization run. As opposed to the less abundant transitions m/z 233 → 133 and 233 → 187, the more intense transition m/z 233 → 72 shows signal saturation without a clear CE optimum.

LC Conditions

MassHunter Optimizer is highly flexible regarding sample introduction options into the MS system. It enables manual infusion using a syringe, automatic infusion using loop injection, and sample injection with or without a LC column. We recommend optimization using LC column separation under LC conditions as close to the final method as possible. This approach requires more time, but provides increased robustness due to a better separation from potential interferences and optimization under relevant mobile phase conditions (for adduct formation). Retention time information is collected at this stage, and can be used for faster development of the final method while minimizing potential analyte misidentification. This is a crucial aspect when combining a larger number of compounds into one dMRM or tMRM method.

For a large list of compounds, the mobile phase composition and optimization gradient should be selected using multiple representative analytes evaluated in SIM mode. The LC column and mobile phase gradient should provide adequate separation from potential interferences. The use of fast gradient and short columns (such as guard columns) provides faster run times, but the probability of a coelution with interfering components is higher. Potential interferences may originate from the optimization standard solution or from LC/MS system contamination (especially mobile phase contamination). Ideally, the optimization solution should contain only one analyte. If a mixture of analytes is used, it should be selected carefully to avoid isobaric interferences from various adducts of monoisotopic precursor ions and other isotopic contributions, especially chlorinated or brominated molecules. In-source generated fragments may also interfere. However, even single-analyte solutions may contain interfering components originating from solvent, glassware, or reference standard contamination.

As noted above, Optimizer selects precursor ions, product ions, and the optimum CE based on the most abundant signal from the entire data collection window. If we use generic data acquisition conditions for automated optimization of multiple analytes, it is possible that the optimum signal for some or all optimized conditions does not originate from the analyte of interest, as demonstrated in Figure 2. Figure 2 shows that, even though the analyte of interest (boscalid) produces the largest peak in the obtained chromatogram, the most abundant product ion (*m/z* 240) reported as the first choice by Optimizer comes from a later eluting, smaller interference peak. This product ion (and a less abundant *m/z* 57 ion) is not present in the product ion spectrum obtained by spectra extraction around the boscalid peak apex. Similarly, an incorrect precursor ion could be selected in the initial SIM analysis, or optimum CE values may originate from a different peak in the chromatogram. Therefore, it is critical to review the results and raw data (chromatograms and spectra) generated by the Optimizer program and respective data acquisition methods and, if needed, re-optimize affected analytes or individual MRMs.

Review of Results and Raw Data Generated by Optimizer

A full Optimizer sequence includes the following runs, which are saved in separate data files in a subfolder entitled DataFiles in a respective Optimizer project folder (the file names are a combination of the compound and method name with suffixes _P1.d to _P4.d):

- _P1.d file: MS2 SIM for each precursor ion
- _P2.d file: Product ion scans for each precursor ion at multiple CEs
- _P3.d file: MRM (at CEs covering the defined range) for precursor-to-product ion transitions found in the previous step
- _P4.d file: Product ion scan using the optimal CEs, and a smaller scan range to determine *m/z* of the fragment ion to one decimal place. (The system assumes a good mass calibration, and Optimizer knows the *m/z* of the precursor to the first decimal place from the exact mass of the formula. Optimizer does not know the formula of the fragment ion, thus, this step is necessary to determine the optimum *m/z* for the fragment ion.)

When the optimization is complete, Optimizer generates a report, displaying the optimized MRM conditions. The export of the optimized MRM conditions into Microsoft Excel is a practical option when further re-optimization is needed because, for instance, a correction of product ion selection can easily be done in Excel, followed by import back into Optimizer.

For review of raw data, first evaluate the product ion scan results in the _P2.d file. The presence of multiple peaks in the chromatogram could lead to potential problems with product ion selection and CE optimization. For proper identification of the analyte peak, obtain as much information as possible about fragmentation pathways and precursor and product ions from reliable sources (such as peer-reviewed publications or curated spectral libraries). Known product ions can then be extracted from the chromatogram to help identify the analyte peak. If no information about potential product ions is available, then the main product ions generated for the peak or peaks in its or their product ion spectrum should be elucidated. A presence of a single peak does not guarantee that it belongs to the analyte, because it may be an interference peak, while the analyte peak may be missing due to low detection sensitivity, poor ionization efficiency, or even injection issues (interferences originating from mobile phase contamination could be present in the chromatogram even if no injection was made).

If the analyte peak could not be identified in the product ion _P2.d file, it is important to review the MS2 SIM results in the _P1.d file to evaluate the precursor ion selection. Further troubleshooting may be needed, such as new optimization at a higher concentration, or infusion in full scan to search for precursor ions and check the suitability of the used source conditions for a given analyte. In general, default source conditions are recommended for automated MRM optimization of a large number of compounds with different chemical structures and properties. For problematic analytes, source optimization may help provide better conditions for successful MRM optimization.

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Figure 4A shows the non-smoothed MRM chromatograms of napropamide spiked into lemon extract to a concentration of 1 µg/kg acquired with the triggered MRM method. Although napropamide elutes in the most crowded region of the chromatogram with 34 primary transitions and additionally 86 confirmatory ions acquired at the tip of the peak, the peak shape of the quantifier and first qualifier is not impinged. Even at a concentration 50 times below the maximum residue limit (MRL) for lemons, the observed area ratio of the two primary transitions was in good agreement with the expected ratio. The triggered MRM spectra of napropamide in lemon extract acquired for spiking concentrations of 1, 10, and 100 µg/kg are shown in Figure 4B. Across the different concentration levels the in spectrum ratio of the fragments were extremely reproducible with RSDs well below 5% for five replicate injections. Consequently, Reference Library Match Scores above 90 were observed even for the lowest spiking levels. This was verified for several other pesticides within the test suite. The high quality spectra acquired with triggered MRM even at very low concentrations are a result of an improved ion statistics due to the use of optimized collision energies for each transition and reasonably long dwell times.

For standard dynamic MRM, the average dwell time of a transition is constant for different samples. When data dependent triggering is added to a method this will result in lower dwell times for the primary transitions when the confirmatory ions are triggered. This might be different for various samples or calibration standards. It is essential that these differences in the average dwell times are not reflected in the peak areas and do not have negative effects on the quantitation and the reproducibility.

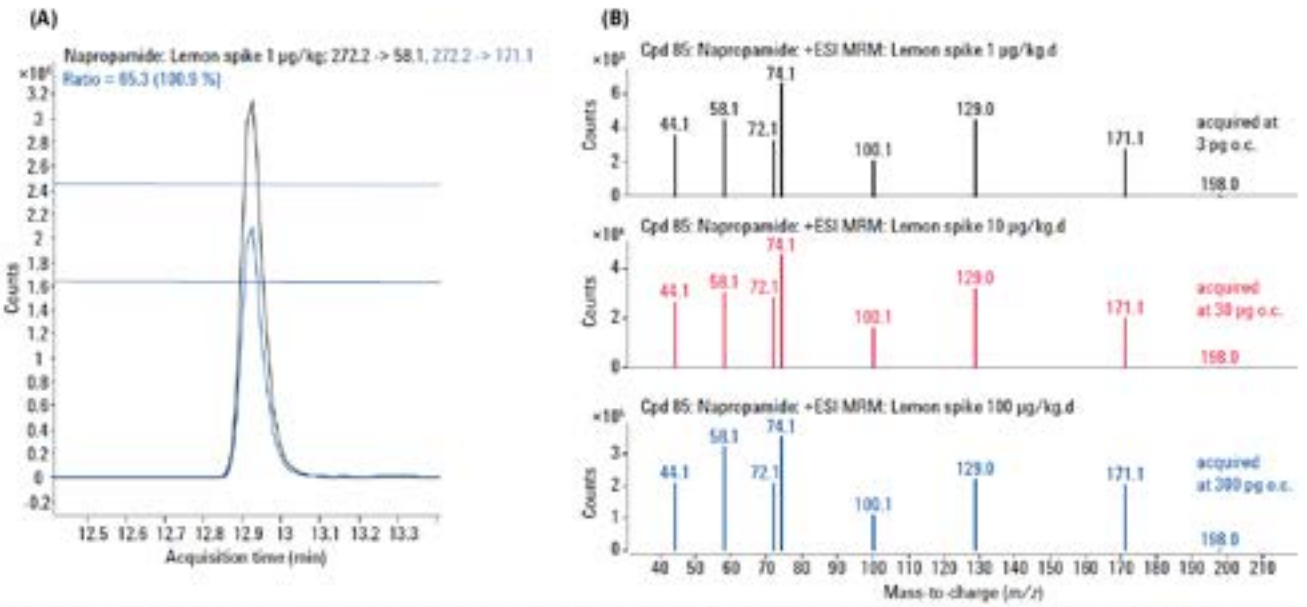

Figure 4. MRM chromatograms for the primary transitions for napropamide spiked into lemon extract at a concentration corresponding to 1 µg/kg (A) and triggered MRM spectra of napropamide spiked into lemon extract (B) at concentrations of 1 (black), 10 (red), and 100 µg/kg (blue).

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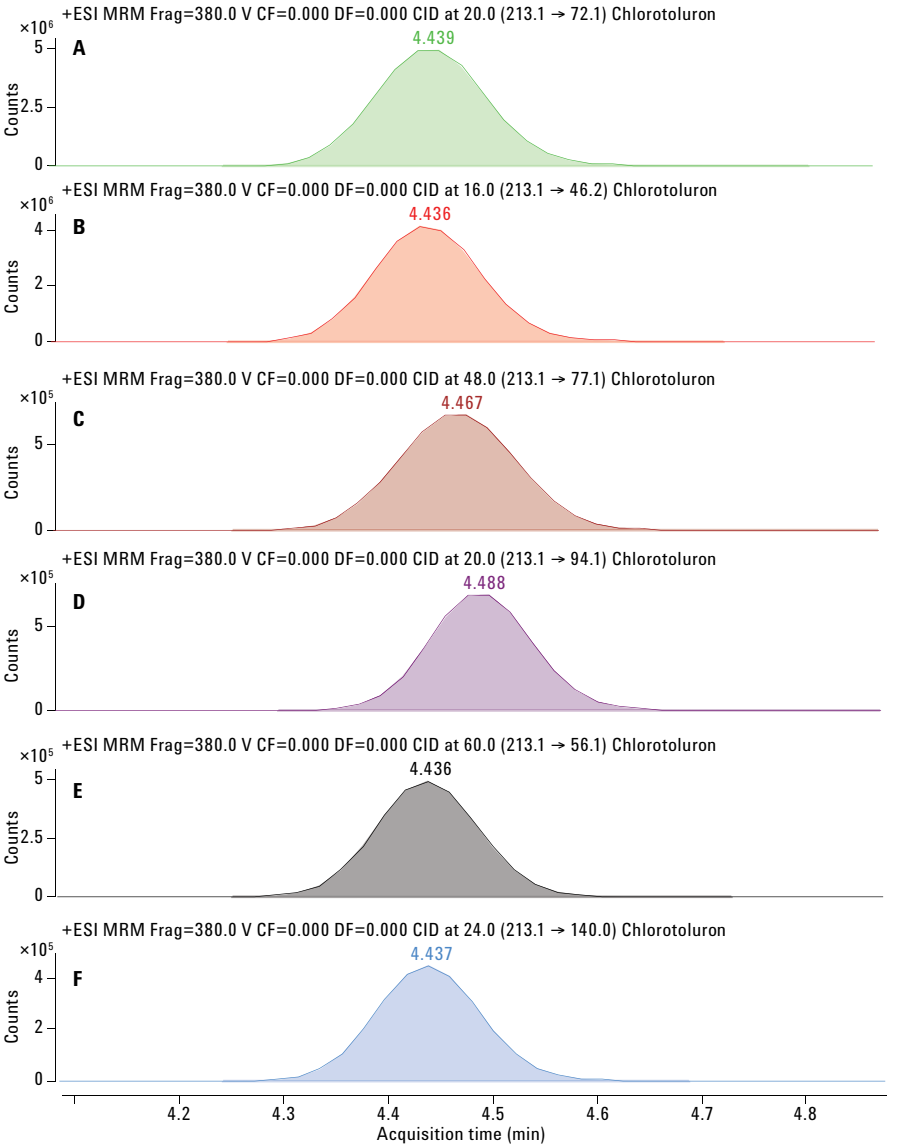

Figure 4. Extracted ion chromatograms of six MRMs (at their optimum CEs) obtained during optimization of chlorotoluron. The third and fourth most abundant transitions, m/z 213 → 77 and 213 → 94, do not belong to chlorotoluron, as indicated by their different chromatographic behavior (different retention times).

Re-Optimization Using Optimizer

If the raw data review suggests that re-optimization of certain steps is needed, new information can easily be entered into Optimizer or imported from Excel. Optimizer then creates new methods, and collects only the required data. For example, if a different precursor ion was used for product ion selection, this information could be directly entered/imported into Optimizer. As a result, the first optimization step (MS2 SIM) is skipped, and no _P1.d file is generated. Similarly, if new product ions were selected from product ion spectra in a _P2.d file, they could be directly entered/imported into Optimizer for the CE optimization step. If the CE values are left blank, Optimizer creates a method collecting data for the entire CE optimization range (for example, 0 to 60 eV). If CE values are included in the Optimizer information (for some or all transitions), only CEs around the given value (with two steps above and two below) will be optimized for the given transitions (for example, if CE = 20 eV is entered in the Optimizer, MRM data for CEs = 12, 16, 20, 24, and 28 eV will be collected). The latter option is useful for quick verification or fine-tuning around the optimum CE value. The CE range selected is not trivial, and may have to be reiterated. Too wide a range for a fragile molecule may not produce significant product ions, and too narrow a range for more intractable molecules may not produce fragments. An input CE value that produces a maximum at the beginning or end of the range indicates that the optimal value may not have been reached.

Collision Cell Accelerator Voltage Optimization

Collision cell accelerator voltage (CAV) is another MRM-dependent parameter that should be optimized in addition to CE. The CAV setting affects the speed at which a given product ion moves out of the collision cell. It can be set to values ranging from 1 to 8 V; the lower the value, the slower the speed. For methods with many MRMs and short dwell times, avoid using 1 to 2 V settings, due to a potential risk of cross talk in the collision cell.

The Optimizer process does not include optimization of this parameter (a CAV of 4 V can be used as a generic setting in the Optimizer program), but it could easily be varied in an MRM/dMRM method (values of 3 to 8 V, step = 1 V), and evaluated using MassHunter Quantification software. For this reason, name each compound transition with a unique name (for example, compoundX_01, compoundX_02, and so on), and include them in the quantification method individually to determine the optimum CAV value for each of them.

Depending on the particular MRM, CAV optimization may or may not result in a significant improvement of the MRM signal as compared to a CAV of 4 V. We observed improvements up to approximately 50 % in certain cases.

Analysis of real samples

During the validation runs several matrix-pesticide combinations were observed for which natural compounds showed high analogies to the targeted pesticides, as for example, identical precursors and fragments and similar retention times. By using a single qualifier/quantifier ratio, this could result in false detects, especially in a high-throughput environment. The key advantage of using triggered MRM is the acquisition of additional information allowing for the unequivocal verification of compounds by the comparison of a compound spectrum with spectra saved in a reference library.

Figure 8 shows the chromatograms and triggered MRM spectra of a natural compound in a QuEChERS extract of chamomile flowers (A) which has a similar retention time and qualifier/quantifier ratio as the herbicide tebuthiuron (B) in a solvent standard (10 ng/mL). The triggered MRM spectra are shown in comparison to the reference library spectrum. While the spectrum of the calibration sample (B) shows a perfect match and consequently results in a match score of 100.0, the fragment spectrum of the chamomile constituent (A) shows low abundances for the low mass fragments 57.1, 62.0, 74.0, and 89.1 (red arrows), the fragments 116.0 and 157.1 show high abundances (green arrows) compared to the quantifier transition.

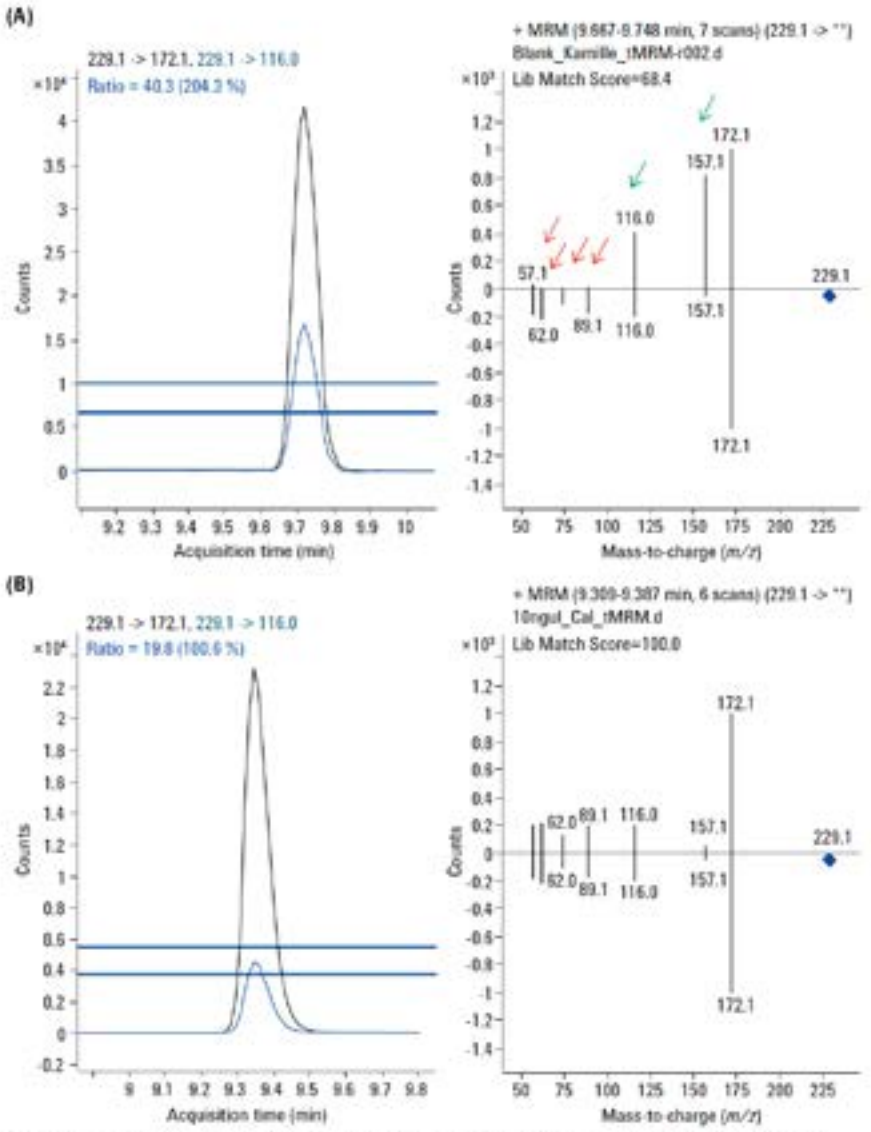

Figure 8. Chromatograms and triggered MRM spectra of a natural chamomile constituent (A) and the herbicide tebuthiuron (B). Spectra are shown in comparison to the reference library spectrum of tebuthiuron.

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Validation Results for LC/MS/MS Pesticide Multiresidue Analysis Using Triggered MRM and Online Dilution

Supplementary Information for Application Note 5991-7193EN

Technical Overview

Introduction

This technical overview presents the validation results as supplementary information for the application note entitled “Improved LC/MS/MS Pesticide Multiresidue Analysis Using Triggered MRM and Online Dilution”, publication number 5991-7193EN, including trueness (recovery) and precision (repeatability and intermediate precision) results obtained for pesticides included in the Agilent LC/MS mixes 1–8 (p/n 5190-0551) during the method validation in tomato, orange juice, spinach, and wheat flour.

This study was conducted in accordance with the document SANTE/11945/2015 entitled “Guidance document on analytical quality control and method validation procedures for pesticide residue analysis in food and feed”, which was issued by the European Commission Directorate General for Health and Food Safety, and became effective on January 1, 2016.

Results and Discussion

Trueness and precision were evaluated for each analyte using blank matrices fortified at 0.01 and 0.02 mg/kg. The fortified samples were prepared in five replicates, and each preparation was injected once. Intermediate precision was evaluated based on data generated by a second analyst repeating the trueness and precision experiments for wheat flour on a different day using different matrix-matched standard preparations. The intermediate precision results were used for the determination of measurement uncertainty (MU) at the 95 % confidence level. The obtained MU results were less than 50 % in the majority of cases; therefore, a default expanded MU of 50 % can be used for the interpretation of results based on the SANTE/11945/2015 guidelines.

Based on the SANTE/11945/2015 guidelines, mean recoveries calculated from the results of individual analysts should be between 70 and 120 %, with corresponding relative standard deviations (RSDs) ≤ 20 %. Recoveries outside of this range are acceptable in multiresidue methods (especially if low and consistent), but should be explained and documented. Tables 1 through 5, respectively summarize the trueness and precision results for tomato, orange juice, spinach, wheat flour (0.01 mg/kg), and wheat flour (0.02 mg/kg). Results outside of the acceptable criteria are highlighted in bold. Mean recoveries of the majority of analytes (at concentrations ≥ LOQ) were between 70 and 120 %, with RSDs ≤ 20 %; exceptions are discussed and explained in the following paragraphs.

Quinmerac and tribenuron-methyl gave lower recoveries (with acceptable RSDs) due to their higher polarity and reduced transfer into acetonitrile during the QuEChERS partition step.

In the spinach matrix, alanycarb, hydramethylnon, pyridate, and tolylfluanid gave slightly lower recoveries (with acceptable RSDs) due to degradation in this matrix. Benfuracarb and carbosulfan showed more pronounced degradation in spinach. These analytes are known to be prone to degradation in certain matrices. Based on the SANTE/11945/2015 guidelines, standard addition should be used when highly accurate, matrix-based quantification is required for detected pesticide residues, and no suitable blank commodity is available for the preparation of matrix-matched standards. The standard addition should be done into the sample matrix prior to the extraction, thus inherently taking into account the recovery of the analytical procedure (including potentially lower recoveries of the above discussed pesticides), and also compensating for any matrix effects.

Ethoxyquin gave higher recoveries in orange juice, and lower recoveries in wheat flour. This analyte is also prone to degradation, which depends on the matrix composition and exposure to air (ethoxyquin acts as an antioxidant). The degradation can occur in matrix-matched standards or sample extracts, leading to high or low recoveries, respectively.

Spinosad (spinosyns A and D) gave more variable results in spinach (results in all other matrices were acceptable with low RSDs).

Certain analytes, such as bifenthrin, dichlorvos, and disulfoton, included in the Agilent LC/MS mixes (p/n 5190-0551) are more suitable for GC/MS analysis. Most of these analytes were included in the LC/MS/MS method for confirmation purposes, with the exception of isocarbophos (mix 4), methacrifos (mix 8), procymidone (mix 3), and tolclofos-methyl (mix 3). Propham (mix 3), another compound with a better performance in GC/MS/MS, was included in the LC/MS/MS method, but its results are not listed in the following tables due to inadequate sensitivity at the evaluated concentration levels. Fluazinam (mix 8) would require electrospray negative mode, and was omitted from the presented LC/MS/MS method.

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Table 1. Trueness (% Recovery) and Precision (% RSD) Data Obtained for Pesticides Included in the Agilent LC/MS Mixes 1–8 in Tomato Matrix at the Fortification Levels of 0.01 and 0.02 mg/kg Evaluated in Five Replicates (A–E)

		Fortification level 0.01 mg/kg							Fortification level 0.02 mg/kg						
		Trueness (%)						RSD	Trueness (%)						RSD
Analyte	Mix	A	B	C	D	E	Mean	(%)	A	B	C	D	E	Mean	(%)
Abamectin - Avermectin B1a	7	67.2	80.8	55.8	66.0	78.2	69.6	15	80.0	67.1	78.3	80.5	94.7	80.1	12
Acephate	1	80.3	71.1	82.0	82.1	82.5	79.6	6.0	72.1	70.7	66.7	70.4	69.9	70.0	2.8
Acetamiprid	5	83.4	84.4	96.0	91.2	94.5	89.9	6.4	88.5	85.8	94.7	90.9	86.7	89.3	4.0
Alanycarb	5	78.7	74.4	79.9	83.6	84.8	80.3	5.1	85.3	86.1	65.1	69.7	72.5	75.8	13
Aldicarb	5	79.3	89.9	85.8	80.2	82.6	83.6	5.2	80.7	76.8	77.3	85.4	91.1	82.3	7.3
Amidosulfuron	4	91.6	83.6	90.2	85.0	93.8	88.8	4.9	87.9	87.4	75.3	75.5	75.6	80.3	8.3
Aminocarb	4	90.7	89.3	89.5	85.0	85.3	87.9	3.0	85.4	72.2	78.0	79.7	79.9	79.0	6.0
Azaconazole	1	83.5	81.9	87.4	85.7	83.5	84.4	2.6	87.2	82.2	89.2	96.3	86.2	88.2	5.9
Azamethiphos	2	87.8	76.0	92.6	84.3	85.7	85.3	7.1	86.1	84.4	76.7	73.7	75.8	79.3	7.0
Azinphos-ethyl	1	85.9	83.8	77.8	81.4	75.9	81.0	5.1	67.9	80.0	87.7	82.3	84.4	80.5	9.4
Azinphos-methyl	1	85.6	86.6	84.1	82.9	87.1	85.3	2.1	73.9	74.0	78.3	80.4	77.4	76.8	3.7
Azoxystrobin	5	83.1	81.0	83.3	82.3	80.8	82.1	1.4	85.6	84.5	95.6	90.0	86.8	88.5	5.1
Beflubutamid	8	81.3	75.0	83.2	83.3	80.7	80.7	4.2	73.2	76.3	81.3	81.1	75.8	77.5	4.6
Benalaxyl	2	91.8	86.6	87.2	83.7	93.1	88.5	4.4	76.2	86.1	81.8	76.9	78.9	80.0	5.1
Benfuracarb	4	72.6	69.2	71.8	82.2	75.9	74.3	6.7	79.4	82.9	72.4	75.1	77.3	77.4	5.2
Benzoximate	7	87.1	83.5	81.3	91.2	89.2	86.5	4.7	80.2	86.5	89.2	80.0	76.3	82.4	6.4
Bifenazate	8	76.1	94.6	88.9	88.7	92.6	88.2	8.2	75.6	82.0	74.8	84.3	79.3	79.2	5.1
Bifenthrin	2	81.2	81.7	86.2	86.4	98.0	86.7	7.8	81.3	90.6	82.4	80.5	88.5	84.7	5.4
Bispyribac	7	91.6	90.4	83.6	82.0	88.6	87.2	4.8	89.7	82.9	69.3	70.8	77.9	78.1	11
Bitertanol	3	89.5	89.4	85.6	77.3	92.6	86.9	6.8	77.4	81.0	83.1	92.3	80.6	82.9	6.8
Boscalid	4	76.3	86.9	89.5	89.0	84.7	85.3	6.3	73.1	83.4	85.7	91.6	84.0	83.6	8.0
Bromuconazole (2 diastereoisomers)	2	92.5	92.2	92.5	90.1	89.6	91.4	1.6	78.6	80.0	89.9	91.2	89.4	85.8	7.0
Bupirimate	2	81.6	80.7	87.8	83.6	87.1	84.2	3.8	86.5	70.7	82.4	96.0	78.3	82.8	11
Buprofezin	1	82.1	82.0	89.1	88.4	83.8	85.1	4.0	80.7	81.0	77.6	74.7	81.6	79.1	3.7
Butocarboxim	4	95.4	81.1	81.3	75.2	82.8	83.2	9.0	84.1	81.2	79.3	82.4	73.9	80.2	4.9
Carbaryl	6	79.1	79.8	82.4	80.1	85.1	81.3	3.0	75.7	77.5	90.3	84.5	88.4	83.3	7.8
Carbendazim	5	87.2	86.8	95.2	81.3	88.5	87.8	5.7	106	78.2	71.8	89.9	83.2	85.7	15
Carbofuran	8	83.0	88.1	83.4	81.4	85.8	84.3	3.1	80.9	81.9	89.4	94.0	93.3	87.9	7.1
Carbosulfan	6	82.1	78.1	84.6	87.3	84.2	83.3	4.1	73.8	74.8	82.8	82.8	76.0	78.1	5.7
Carboxin	5	82.2	78.1	86.0	80.5	85.0	82.4	3.9	82.2	81.9	79.6	84.0	79.8	81.5	2.2
Carfentrazone-ethyl	4	93.1	78.5	91.4	82.9	92.3	87.6	7.5	97.6	95.3	89.7	86.7	79.7	89.8	7.9
Chlorantraniliprole	8	81.2	81.1	92.8	74.7	91.4	84.2	9.1	80.1	85.6	86.6	78.9	72.1	80.6	7.2
Chlorfenvinphos (E- and Z-isomers)	2	89.3	82.2	86.6	90.9	90.0	87.8	4.0	86.7	84.4	81.5	83.6	81.5	83.5	2.6
Chloridazon (Pyrazon)	4	88.8	85.8	88.6	89.8	89.2	88.4	1.7	83.5	84.3	85.9	79.6	80.4	82.7	3.2
Chlorotoluron (Chlortoluron)	7	84.3	78.4	81.9	85.3	84.7	82.9	3.4	78.4	81.0	84.0	81.9	88.1	82.7	4.4
Chloroxuron	7	93.3	89.0	85.9	89.0	91.7	89.8	3.2	66.0	79.1	88.6	89.6	83.6	81.4	12
Chlorpyrifos	2	85.0	83.8	87.7	84.4	87.5	85.7	2.1	80.5	83.1	79.1	81.6	84.2	81.7	2.5
Chlorpyrifos-methyl	2	87.2	92.2	82.4	93.5	85.8	88.2	5.2	78.3	86.4	75.2	85.7	85.0	82.1	6.1
Chlorsulfuron	4	81.7	86.1	83.3	89.2	87.1	85.5	3.5	78.8	84.0	81.7	84.7	79.4	81.7	3.2

Table 1. Trueness (% Recovery) and Precision (% RSD) Data Obtained for Pesticides Included in the Agilent LC/MS Mixes 1–8 in Tomato Matrix at the Fortification Levels of 0.01 and 0.02 mg/kg Evaluated in Five Replicates (A–E) (continued)

		Fortification level 0.01 mg/kg							Fortification level 0.02 mg/kg						
		Trueness (%)						RSD	Trueness (%)						RSD
Analyte	Mix	A	B	C	D	E	Mean	(%)	A	B	C	D	E	Mean	(%)
Clethodim (E- and Z-isomers)	3	96.6	82.2	87.4	83.4	96.2	89.2	7.7	84.5	85.9	87.0	81.5	80.2	83.8	3.4
Clofentezine	4	80.6	81.0	90.5	79.0	79.9	82.2	5.7	86.3	79.8	79.5	85.4	78.0	81.8	4.6
Clomazone	8	82.0	77.6	80.0	82.1	81.6	80.6	2.4	81.9	77.4	83.6	84.1	82.3	81.9	3.3
Coumaphos	2	80.2	75.8	71.9	77.2	82.9	77.6	5.4	72.5	76.6	74.8	69.5	79.9	74.6	5.3
Cyazofamid	4	87.6	83.7	90.0	92.6	87.9	88.4	3.7	83.9	80.6	87.8	82.6	86.8	84.3	3.5
Cycloate	1	81.9	96.2	83.7	93.1	81.5	87.3	7.9	78.5	77.6	82.7	78.4	94.4	82.3	8.6
Cycluron	7	86.0	84.2	80.7	79.7	89.2	83.9	4.6	78.1	83.9	88.0	89.0	84.5	84.7	5.1
Cymiazole	1	96.9	88.4	85.1	86.6	82.7	87.9	6.2	80.5	75.5	86.2	82.2	91.4	83.2	7.2
Cymoxanil	4	94.7	82.5	91.5	79.7	103	90.3	10	89.5	94.6	97.5	87.9	88.0	91.5	4.7
Cyproconazole (2 diastereoisomers)	1	93.0	93.3	96.0	91.2	92.0	93.1	2.0	88.3	87.7	85.3	88.4	85.6	87.1	1.7
Cyprodinil	8	83.7	89.7	88.5	96.7	89.2	89.6	5.2	85.5	87.7	101	107	89.8	94.1	9.8
DEET (Diethyltoluamide)	4	84.1	87.2	85.3	94.5	87.5	87.7	4.6	74.2	83.9	83.8	84.3	92.5	83.8	7.7
Desmedipham	8	89.1	80.7	79.5	79.8	80.2	81.9	5.0	85.8	81.9	88.1	91.4	82.6	86.0	4.6
Diazinon	2	78.2	82.8	83.2	85.9	84.0	82.8	3.4	85.4	78.6	88.0	74.0	77.7	80.7	7.2
Dichlorvos	2	86.5	93.7	96.4	90.1	97.9	92.9	5.0	91.0	95.5	90.1	90.0	98.1	93.0	4.0
Diethofencarb	6	88.7	85.5	94.4	91.3	89.2	89.8	3.7	89.0	82.8	95.2	98.7	85.0	90.1	7.4
Difenoconazole (*cis*- and *trans*-)	3	86.4	79.8	87.3	84.0	88.0	85.1	3.9	90.6	85.8	86.5	86.4	80.4	86.0	4.2
Diflubenzuron	4	97.0	97.1	102	96.2	93.3	97.0	3.1	91.8	98.3	84.2	88.0	83.6	89.2	6.8
Diflufenican	1	78.1	76.6	80.4	87.6	86.8	81.9	6.1	80.8	88.2	84.6	86.2	81.4	84.2	3.7
Dimethachlor	1	88.8	82.9	89.2	86.2	86.0	86.6	2.9	78.8	82.1	83.1	85.5	82.7	82.5	2.9
Dimethoate	8	78.6	85.5	88.0	90.1	82.0	84.8	5.5	83.5	89.5	83.5	90.3	88.3	87.0	3.8
Dimethomorph (E- and Z-isomers)	5	67.9	69.5	69.9	69.9	77.0	70.8	5.0	78.2	74.6	81.7	75.7	70.3	76.1	5.6
Dimoxystrobin	1	87.1	82.5	88.3	91.7	81.7	86.3	4.8	91.4	93.0	99.6	100	83.5	93.6	7.4
Diniconazole	2	81.9	81.2	81.1	89.6	84.1	83.6	4.3	82.7	84.3	79.8	79.2	82.5	81.7	2.6
Dinotefuran	7	82.3	81.0	82.2	76.1	79.7	80.2	3.2	81.0	77.0	82.2	78.5	76.5	79.0	3.1
Dioxacarb	7	102	83.9	86.1	93.6	91.5	91.5	7.9	100	94.9	96.2	94.1	93.8	95.8	2.7
Disulfoton	1	86.3	81.2	97.8	80.0	88.2	86.7	8.2	81.1	76.4	85.6	83.3	76.9	80.7	5.0
Diuron	5	84.0	80.8	80.7	82.6	85.4	82.7	2.5	82.3	84.2	85.0	89.4	84.1	85.0	3.1
Epoxiconazole	2	89.5	90.4	86.5	82.0	92.1	88.1	4.5	81.6	86.1	86.8	85.4	75.6	83.1	5.6
Ethidimuron (Sulfadiazole)	8	69.2	81.1	84.7	87.5	78.1	80.1	8.8	101	89.5	88.5	91.4	82.4	90.6	7.5
Ethion	2	85.7	84.1	91.5	83.2	91.4	87.2	4.6	89.3	79.0	77.4	77.4	85.6	81.8	6.6
Ethirimol	4	93.3	90.8	93.8	97.2	86.6	92.3	4.3	87.4	83.5	94.2	98.4	86.8	90.0	6.7
Ethofumesate	4	76.4	82.6	85.8	82.7	80.8	81.7	4.2	85.3	83.7	94.0	81.9	84.4	85.9	5.5
Ethoprophos (Ethoprop)	2	74.6	80.3	85.1	84.1	89.2	82.6	6.7	87.5	86.4	92.2	85.2	84.7	87.2	3.5
Ethoxyquin	8	87.8	91.2	93.5	89.5	84.4	89.3	3.8	85.5	90.5	98.2	93.4	90.2	91.6	5.1
Etofenprox	3	81.0	81.0	84.1	88.3	95.8	86.0	7.2	88.7	89.9	91.5	84.8	91.2	89.2	3.0
Famoxadone	4	80.5	96.9	78.5	78.1	91.8	85.2	10	86.5	97.0	90.2	99.9	90.8	92.9	5.8
Fenamidone	5	82.6	80.5	87.2	85.2	87.5	84.6	3.5	93.7	86.0	87.7	77.9	76.1	84.3	8.6
Fenamiphos	1	87.4	81.0	83.0	89.5	84.3	85.1	4.0	66.9	78.4	88.2	90.6	79.6	80.7	12

Table 1. Trueness (% Recovery) and Precision (% RSD) Data Obtained for Pesticides Included in the Agilent LC/MS Mixes 1–8 in Tomato Matrix at the Fortification Levels of 0.01 and 0.02 mg/kg Evaluated in Five Replicates (A–E) (continued)

		Fortification level 0.01 mg/kg							Fortification level 0.02 mg/kg						
		Trueness (%)						RSD	Trueness (%)						RSD
Analyte	Mix	A	B	C	D	E	Mean	(%)	A	B	C	D	E	Mean	(%)
Fenarimol	2	89.9	90.9	86.3	89.7	90.5	89.5	2.0	77.9	84.3	80.8	81.8	86.4	82.3	4.0
Fenazaquin	5	89.0	80.6	88.9	85.7	86.6	86.2	4.0	73.2	76.0	75.8	82.0	72.5	75.9	4.9
Fenbuconazole	2	84.3	89.4	84.2	84.4	92.4	87.0	4.3	94.1	88.0	91.0	84.3	78.9	87.2	6.8
Fenhexamid	3	82.6	74.9	84.7	87.1	94.6	84.8	8.5	82.2	86.9	81.8	81.6	96.0	85.7	7.2
Fenobucarb	5	88.0	88.4	91.8	87.5	95.2	90.2	3.6	89.2	84.8	79.5	73.7	77.8	81.0	7.5
Fenoxycarb	6	82.6	82.0	95.2	72.8	88.5	84.2	9.9	75.6	83.7	86.6	81.7	94.4	84.4	8.2
Fenpropidin	5	83.0	81.7	88.9	82.8	89.1	85.1	4.2	86.4	85.1	94.2	89.1	83.0	87.6	4.9
Fenpyroximate	5	83.7	78.7	86.6	90.4	87.7	85.4	5.2	83.8	87.5	79.9	80.0	79.8	82.2	4.2
Fenuron	7	96.3	88.8	93.6	88.7	84.1	90.3	5.2	93.1	100	90.9	88.8	91.5	92.9	4.7
Fipronil	4	91.1	89.7	68.5	92.0	79.7	84.2	12	87.2	113	89.2	106	70.6	93.3	18
Flazasulfuron	4	76.4	80.3	86.4	80.7	84.5	81.7	4.8	72.4	79.2	74.7	85.4	78.1	78.0	6.4
Flonicamid	6	91.3	81.0	86.2	92.3	83.8	86.9	5.6	88.6	77.1	89.1	71.4	83.1	81.9	9.3
Flubendiamide	7	105	86.1	96.1	100	88.1	95.1	8.4	78.3	79.3	95.7	84.9	91.6	86.0	8.9
Fludioxonil	2	88.8	81.3	89.2	87.1	79.0	85.1	5.5	86.9	79.7	87.1	92.1	86.1	86.4	5.1
Flufenacet	1	92.5	84.0	86.1	89.5	80.0	86.4	5.6	77.3	80.4	85.6	81.7	76.3	80.3	4.6
Flufenoxuron	4	89.7	83.5	82.5	86.3	91.3	86.6	4.4	86.8	82.6	80.4	85.8	76.7	82.5	5.0
Flumetsulam	8	94.4	78.3	88.2	82.1	91.4	86.9	7.6	87.3	69.6	96.9	97.3	80.1	86.2	14
Flumioxazin	6	88.3	107	85.3	81.5	99.3	92.2	11	84.2	74.5	88.6	91.2	88.6	85.4	7.7
Fluometuron	8	86.4	84.4	85.2	86.9	88.5	86.3	1.9	84.3	81.3	89.6	88.1	90.7	86.8	4.5
Fluopicolide	1	87.5	83.4	84.3	85.6	80.1	84.2	3.3	93.7	94.8	95.3	91.0	93.2	93.6	1.8
Fluoxastrobin	8	89.4	72.1	83.9	89.5	84.1	83.8	8.4	89.6	78.3	96.2	102	95.5	92.2	9.6
Fluquinconazole	2	80.3	91.7	87.3	92.1	82.3	86.7	6.2	77.3	80.7	88.8	89.3	83.2	83.9	6.1
Flusilazole	2	79.0	75.4	82.6	67.3	74.5	75.8	7.5	84.5	76.5	75.7	79.4	85.3	80.3	5.5
Flutriafol	8	85.2	80.5	86.3	91.4	82.3	85.2	4.9	88.0	83.4	83.8	88.5	85.7	85.9	2.7
Foramsulfuron	3	84.4	72.5	82.5	81.4	82.1	80.6	5.8	77.8	77.9	80.9	76.3	77.3	78.0	2.2
Forchlorfenuron	7	84.6	81.5	87.3	83.9	87.3	84.9	2.9	76.3	80.9	78.7	80.7	75.8	78.5	3.0
Fosthiazate (sum of isomers)	1	81.4	88.0	83.3	86.9	81.4	84.2	3.7	75.2	82.0	83.4	79.5	84.9	81.0	4.7
Fuberidazole	4	87.4	83.3	92.9	91.3	85.8	88.1	4.5	76.8	72.4	88.3	87.0	78.1	80.5	8.5
Furalaxyl	7	80.4	77.0	78.3	76.9	77.3	78.0	1.9	81.2	73.3	86.3	84.7	81.3	81.4	6.2
Furathiocarb	6	90.5	80.7	92.9	77.2	87.0	85.7	7.7	78.1	83.3	78.4	83.7	85.8	81.9	4.2
Halofenozide	8	89.7	81.3	87.3	79.6	89.4	85.5	5.5	87.6	83.5	88.0	91.4	83.0	86.7	4.0
Halosulfuron-methyl	8	78.9	77.1	82.1	78.5	71.8	77.7	4.8	72.2	75.5	84.7	83.0	78.3	78.7	6.6
Hexaconazole	2	80.9	89.2	94.9	88.1	87.1	88.0	5.7	84.2	83.1	95.6	92.8	83.2	87.8	6.8
Hexaflumuron	7	90.0	91.8	92.1	102	86.9	92.5	6.0	87.1	80.3	86.2	78.9	79.4	82.4	4.8
Hexythiazox	4	83.6	80.9	84.9	86.3	88.0	84.7	3.2	88.3	88.1	87.3	81.0	83.2	85.6	3.8
Hydramethylnon	7	82.0	79.3	86.9	89.3	92.8	86.1	6.4	77.8	77.4	70.6	77.9	74.8	75.7	4.2
Imazalil	2	78.2	82.9	90.5	83.4	78.9	82.8	5.9	79.3	79.0	91.4	94.3	83.6	85.5	8.2
Imidacloprid	5	85.0	75.9	92.9	88.8	82.5	85.0	7.6	90.0	83.8	88.3	81.5	82.8	85.3	4.3
Indoxacarb	3	85.5	79.0	89.4	82.8	85.1	84.3	4.5	88.4	96.6	93.9	96.7	80.1	91.1	7.7

Table 1. Trueness (% Recovery) and Precision (% RSD) Data Obtained for Pesticides Included in the Agilent LC/MS Mixes 1–8 in Tomato Matrix at the Fortification Levels of 0.01 and 0.02 mg/kg Evaluated in Five Replicates (A–E) (continued)

		Fortification level 0.01 mg/kg							Fortification level 0.02 mg/kg						
		Trueness (%)						RSD	Trueness (%)						RSD
Analyte	Mix	A	B	C	D	E	Mean	(%)	A	B	C	D	E	Mean	(%)
Ipconazole	2	90.5	101	121	98.3	104	103	11	91.1	99.7	119	126	117	111	13
Iprovalicarb	5	93.7	88.1	87.8	94.6	96.1	92.1	4.2	84.0	87.2	75.9	84.5	80.0	82.3	5.4
Isofenphos-methyl	1	79.1	92.8	88.7	84.3	80.2	85.0	6.8	84.4	81.9	93.1	90.1	81.4	86.2	6.0
Isoprothiolane	1	97.7	98.9	92.9	87.6	96.0	94.6	4.8	84.0	76.5	92.3	85.3	94.9	86.6	8.4
Isoxaben	4	86.0	86.1	88.3	78.9	86.8	85.2	4.3	89.6	85.8	102	93.3	86.4	91.5	7.3
Isoxaflutole	3	88.4	86.8	88.8	91.0	85.6	88.1	2.3	84.2	85.6	87.7	90.3	91.7	87.9	3.6
Ivermectin B1a	7	89.5	86.0	88.4	89.2	83.8	87.4	2.7	75.8	82.7	77.2	86.2	79.6	80.3	5.3
Kresoxim-methyl	4	88.1	89.8	88.5	82.9	83.4	86.5	3.6	88.9	84.0	90.8	82.9	93.9	88.1	5.3
Lenacil	1	82.4	85.0	86.0	80.7	84.7	83.8	2.6	88.7	91.1	86.2	98.3	89.8	90.8	5.0
Linuron	4	73.6	77.3	88.5	81.5	90.7	82.4	8.8	82.4	82.6	86.8	81.7	77.4	82.2	4.1
Lufenuron	4	77.3	72.4	90.4	93.3	87.6	84.2	11	89.2	95.9	85.2	81.5	76.7	85.7	8.6
Malaoxon	3	81.7	83.7	82.1	88.6	95.3	86.3	6.7	79.7	80.9	88.3	91.2	94.2	86.9	7.3
Malathion	3	105	80.5	90.5	71.6	91.6	87.9	14	84.6	83.0	89.2	88.5	95.0	88.1	5.3
Mandipropamid	4	83.9	74.4	91.7	87.3	83.5	84.2	7.6	93.4	81.1	82.5	84.2	71.6	82.6	9.4
Mecarbam	3	80.5	84.8	94.4	93.2	84.4	87.5	6.9	83.7	89.8	78.6	86.1	86.7	85.0	4.9
Mepanipyrim	3	86.9	86.7	86.8	85.6	84.4	86.1	1.2	77.7	81.1	85.1	78.1	85.0	81.4	4.4
Mesosulfuron-methyl	6	86.4	82.5	87.5	91.9	85.6	86.8	3.9	83.1	81.1	101	108	94.7	93.6	12
Metaflumizone	4	96.5	99.1	78.7	90.5	89.7	90.9	8.7	88.7	87.7	73.7	74.8	76.2	80.2	9.2
Metalaxyl	3	79.5	79.0	87.9	83.1	79.4	81.8	4.7	75.4	79.8	89.4	89.8	89.3	84.8	7.9
Metamitron	4	86.6	72.2	83.4	81.4	94.3	83.6	9.6	108	94.3	76.4	58.2	68.1	81.1	25
Metazachlor	3	82.4	84.5	83.6	84.6	84.1	83.9	1.1	78.4	77.7	86.0	90.5	89.8	84.5	7.2
Metconazole	2	81.8	76.2	83.6	89.3	88.7	83.9	6.4	84.9	85.5	82.4	83.8	86.7	84.7	2.0
Methabenzthiazuron	5	85.3	80.1	86.0	81.6	89.8	84.6	4.5	80.5	83.7	90.3	86.8	88.2	85.9	4.5
Methamidophos	1	82.4	71.8	77.9	77.9	81.9	78.4	5.4	80.9	79.1	76.4	78.2	80.1	78.9	2.2
Methidathion	3	87.5	92.3	91.2	95.4	89.9	91.3	3.2	80.2	86.1	87.8	94.7	93.3	88.4	6.6
Methiocarb	7	85.6	80.3	84.1	79.4	86.5	83.2	3.8	81.9	84.4	89.7	94.3	81.9	86.5	6.3
Methomyl	5	88.9	82.9	106	96.5	80.3	90.9	11	79.4	84.8	86.0	86.3	80.6	83.4	3.9
Methoprotryne	7	86.5	86.3	91.1	90.9	91.0	89.2	2.8	81.8	86.9	88.2	86.8	93.7	87.5	4.8
Methoxyfenozide	5	79.5	90.5	88.7	87.6	92.3	87.7	5.6	78.1	74.6	107	121	93.7	94.9	21
Metobromuron	8	80.0	78.4	81.1	84.6	86.8	82.2	4.2	78.4	83.2	84.6	84.2	82.6	82.6	3.0
Metolachlor	3	88.1	97.7	92.4	87.1	81.8	89.4	6.7	78.6	82.4	122	114	104	100	19
Metrafenone	4	93.1	88.6	88.8	85.5	89.6	89.1	3.1	81.7	83.0	79.2	79.1	75.9	79.8	3.4
Metribuzin	4	88.2	80.9	90.1	81.5	92.8	86.7	6.1	84.8	89.8	81.7	85.1	80.9	84.4	4.1
Metsulfuron-methyl	4	76.9	95.4	83.6	84.9	80.5	84.2	8.2	73.0	78.0	102	107	95.7	90.9	16
Mevinphos (E- and Z-isomers)	3	91.5	91.9	94.8	90.7	89.0	91.6	2.3	86.3	85.4	82.7	80.2	82.3	83.4	2.9
Mexacarbate	7	79.6	90.3	85.5	85.3	85.6	85.2	4.4	83.6	76.4	79.0	83.2	86.1	81.6	4.8
Molinate	3	77.4	71.5	78.2	73.0	81.8	76.4	5.5	77.6	68.7	80.5	81.7	77.5	77.2	6.6
Monocrotophos	4	94.7	86.3	94.2	80.9	91.0	89.4	6.5	80.8	82.4	90.6	89.0	87.6	86.1	4.9
Moxidectin	7	93.1	87.1	93.2	97.2	98.4	93.8	4.7	95.6	85.2	87.1	76.4	84.6	85.8	8.0

Table 1. Trueness (% Recovery) and Precision (% RSD) Data Obtained for Pesticides Included in the Agilent LC/MS Mixes 1–8 in Tomato Matrix at the Fortification Levels of 0.01 and 0.02 mg/kg Evaluated in Five Replicates (A–E) (continued)

		Fortification level 0.01 mg/kg							Fortification level 0.02 mg/kg						
		Trueness (%)						RSD	Trueness (%)						RSD
Analyte	Mix	A	B	C	D	E	Mean	(%)	A	B	C	D	E	Mean	(%)
Myclobutanil	1	86.8	83.7	74.9	91.9	83.8	84.2	7.3	93.9	98.5	95.5	91.2	94.2	94.6	2.8
Nicosulfuron	4	88.7	92.3	91.0	84.0	88.4	88.9	3.6	77.7	70.2	79.9	77.1	71.9	75.4	5.4
Nitenpyram	7	91.0	95.2	85.7	86.2	92.0	90.0	4.5	79.8	82.3	81.7	81.2	87.7	82.5	3.7
Novaluron	4	88.9	91.7	94.6	91.7	96.4	92.7	3.1	83.8	89.3	89.6	86.4	82.7	86.4	3.6
Omethoate	6	88.8	69.5	77.1	83.0	71.6	78.0	10	92.2	81.3	84.9	84.1	70.5	82.6	9.5
Oxadiazon	3	86.3	80.9	98.9	91.0	85.3	88.5	7.7	103	104	87.0	86.8	88.1	93.6	9.3
Oxadixyl	3	87.8	84.8	95.1	89.4	87.3	88.9	4.3	87.7	84.6	85.8	89.1	84.0	86.2	2.5
Oxamyl	5	93.2	77.3	89.7	80.5	87.7	85.7	7.7	86.9	82.2	80.7	81.0	92.1	84.6	5.8
Oxasulfuron	4	73.9	83.9	88.8	77.8	73.2	79.5	8.4	75.7	74.6	83.9	86.0	76.8	79.4	6.5
Paclobutrazol	3	85.0	82.8	85.8	84.2	87.4	85.0	2.0	81.6	81.4	77.3	77.3	78.4	79.2	2.7
Penconazole	3	83.3	81.6	88.0	85.1	89.3	85.5	3.7	83.9	80.7	85.3	86.7	85.4	84.4	2.7
Pencycuron	6	87.3	83.4	86.9	87.5	89.9	87.0	2.7	71.1	79.3	79.0	83.6	78.6	78.3	5.8
Pendimethalin	3	91.5	73.8	88.8	84.3	81.6	84.0	8.2	90.2	84.6	81.4	80.9	79.4	83.3	5.2
Phenmedipham	4	81.6	86.9	82.9	86.2	80.9	83.7	3.2	77.4	75.8	80.3	85.8	79.3	79.7	4.8
Phenthoate	3	105	91.5	77.5	86.8	90.7	90.3	11	96.4	85.7	97.6	99.0	92.1	94.1	5.7
Phosalone	3	82.3	68.8	91.8	81.1	93.9	83.6	12	81.1	84.2	81.9	84.7	81.0	82.6	2.1
Phosmet	6	83.7	85.5	85.2	84.8	86.2	85.1	1.1	84.3	87.8	94.3	95.1	98.8	92.1	6.4
Phosphamidon (E- and Z-isomers)	3	86.9	82.1	86.3	83.4	88.7	85.5	3.1	86.6	77.5	75.2	77.4	80.2	79.4	5.6
Phoxim	4	89.3	87.0	88.6	83.4	88.6	87.4	2.8	85.5	74.8	80.7	85.1	79.1	81.0	5.5
Picolinafen	3	92.6	86.2	75.3	85.8	84.8	85.0	7.3	83.2	78.5	78.0	76.2	80.4	79.3	3.3
Picoxystrobin	5	86.7	81.9	88.5	83.3	87.3	85.5	3.3	77.7	83.4	80.3	84.7	85.8	82.4	4.1
Pirimicarb	3	85.9	82.9	86.4	87.7	90.0	86.6	3.0	78.8	75.0	82.6	82.9	79.8	79.8	4.0
Pirimiphos-methyl	3	79.1	76.3	78.3	82.7	84.9	80.2	4.3	87.1	82.2	87.6	79.6	92.8	85.9	5.9
Prochloraz	1	89.5	78.4	85.6	87.0	85.9	85.3	4.8	82.9	78.1	84.2	84.6	78.7	81.7	3.8
Profenofos	3	89.1	79.1	88.9	83.5	90.6	86.2	5.6	85.0	77.3	62.1	64.4	66.3	71.0	14
Promecarb	7	77.3	86.8	86.2	82.7	87.1	84.0	4.9	82.8	81.0	86.0	82.0	83.7	83.1	2.3
Prometon	4	85.9	81.0	88.9	85.8	89.5	86.2	3.9	80.9	88.3	91.0	87.4	85.4	86.6	4.3
Propamocarb	5	81.4	77.5	82.3	74.8	86.3	80.5	5.5	79.0	67.9	79.6	86.5	84.3	79.5	9.0
Propaquizafop	4	79.3	78.3	84.7	79.6	86.4	81.7	4.5	78.5	72.0	84.8	83.3	78.9	79.5	6.3
Propargite	4	81.9	82.4	82.7	85.0	90.3	84.5	4.1	86.9	84.1	78.4	80.1	81.8	82.3	4.1
Propetamphos	3	76.9	77.9	81.8	87.0	80.6	80.8	4.9	75.8	75.9	82.3	85.3	83.2	80.5	5.4
Propiconazole (sum of isomers)	2	79.7	88.4	83.0	85.5	81.9	83.7	4.0	85.6	81.8	86.7	92.6	88.6	87.0	4.6
Propoxur	6	100	82.3	94.4	89.9	97.2	92.8	7.5	93.7	90.7	67.9	73.1	70.8	79.3	15
Propyzamide (Pronamide)	3	86.1	79.0	87.4	83.7	74.8	82.2	6.4	78.5	78.0	91.6	90.6	91.5	86.0	8.3
Proquinazid	1	87.5	76.4	86.1	77.4	85.0	82.5	6.3	78.8	74.4	72.2	70.1	77.1	74.5	4.8
Prosulfocarb	4	73.4	77.8	76.7	82.2	81.3	78.3	4.6	76.5	73.3	70.1	62.5	69.9	70.5	7.4
Pymetrozine	7	81.8	86.9	90.2	87.3	81.6	85.5	4.4	84.0	90.5	91.1	99.0	89.9	90.9	5.9
Pyracarbolid	7	80.8	82.2	81.9	79.7	83.9	81.7	1.9	83.7	83.6	85.7	88.5	87.1	85.7	2.5
Pyraclostrobin	5	87.0	81.4	82.5	88.3	91.8	86.2	5.0	80.6	78.5	81.6	80.5	78.0	79.9	1.9

Table 1. Trueness (% Recovery) and Precision (% RSD) Data Obtained for Pesticides Included in the Agilent LC/MS Mixes 1–8 in Tomato Matrix at the Fortification Levels of 0.01 and 0.02 mg/kg Evaluated in Five Replicates (A–E) (continued)

		Fortification level 0.01 mg/kg							Fortification level 0.02 mg/kg						
		Trueness (%)						RSD	Trueness (%)						RSD
Analyte	Mix	A	B	C	D	E	Mean	(%)	A	B	C	D	E	Mean	(%)
Pyridaben	5	80.5	85.5	86.7	81.6	85.9	84.1	3.3	80.3	81.4	82.7	73.2	85.5	80.6	5.7
Pyridate	5	80.9	79.0	79.8	76.6	86.0	80.5	4.3	75.3	84.3	84.5	73.8	82.1	80.0	6.4
Pyrimethanil	6	82.9	83.0	86.8	81.1	83.4	83.4	2.5	81.2	84.3	89.3	90.0	81.9	85.3	4.8
Pyriproxyfen	5	85.2	80.7	95.0	89.7	89.9	88.1	6.1	83.9	79.9	88.8	84.2	81.8	83.7	4.0
Quinalphos	3	83.0	76.3	92.8	82.9	78.4	82.7	7.7	80.9	88.0	80.8	79.0	82.9	82.3	4.2
Quinmerac	7	63.8	55.5	71.1	59.3	56.1	61.2	11	64.9	55.5	75.6	64.5	70.4	66.2	11
Quinoclamine	4	83.5	86.9	89.0	81.1	84.2	84.9	3.6	77.8	86.5	83.7	88.4	96.2	86.5	7.8
Quinoxyfen	3	86.2	83.6	80.0	84.6	93.4	85.6	5.8	82.0	79.2	79.8	71.1	79.8	78.4	5.4
Rimsulfuron	4	76.8	73.8	82.3	77.2	83.6	78.7	5.2	71.1	79.1	81.8	77.0	77.4	77.3	5.1
Rotenone	7	91.9	86.7	79.0	87.6	88.7	86.8	5.5	82.4	85.6	82.3	75.3	82.4	81.6	4.6
Secbumeton	7	83.0	80.9	92.6	83.0	84.5	84.8	5.4	77.1	79.1	77.4	73.0	79.6	77.3	3.4
Silthiofam	4	83.4	75.7	89.5	83.1	80.2	82.4	6.1	82.6	90.1	85.9	76.7	86.8	84.4	6.0
Spinosad - Spinosyn A	7	87.9	89.6	85.2	86.6	86.5	87.2	1.9	88.4	77.5	76.2	83.6	71.2	79.4	8.4
Spinosad - Spinosyn D	7	81.5	75.4	82.6	75.6	88.2	80.7	6.6	84.8	73.6	69.8	72.5	76.2	75.4	7.6
Spirodiclofen	1	89.3	94.7	91.0	89.7	97.2	92.4	3.7	87.8	86.6	83.0	81.0	87.6	85.2	3.6
Spiromesifen	6	85.6	82.5	85.4	82.8	96.7	86.6	6.7	87.8	91.2	85.9	82.0	83.5	86.1	4.2
Spirotetramat	6	84.0	76.4	92.1	93.5	83.5	85.9	8.1	76.1	77.3	81.2	82.9	70.6	77.6	6.2
Spiroxamine (2 diastereoisomers)	1	90.0	88.6	90.0	85.0	96.0	89.9	4.4	86.8	83.3	83.6	79.3	81.6	82.9	3.3
Sulfentrazone	6	81.8	86.7	85.2	88.3	92.0	86.8	4.3	84.8	91.1	95.4	82.0	91.2	88.9	6.1
Tebuconazole	2	85.7	84.5	84.4	89.7	84.3	85.7	2.7	84.4	82.3	85.9	89.1	93.3	87.0	5.0
Tebufenozide	5	59.7	64.5	80.1	80.2	69.5	70.8	13	94.2	98.7	106	90.7	80.6	94.1	10
Tebufenpyrad	3	89.8	81.8	90.5	87.5	92.3	88.4	4.6	80.1	82.2	79.4	79.8	80.8	80.5	1.4
Tebuthiuron	7	87.4	82.5	84.0	82.1	89.5	85.1	3.8	78.2	80.7	86.9	86.3	86.6	83.7	4.8
Teflubenzuron	4	76.5	77.9	91.9	78.5	80.1	81.0	7.7	83.0	83.6	85.0	79.3	85.3	83.2	2.9
Temephos	7	85.1	81.6	81.1	92.2	84.9	85.0	5.2	82.6	80.6	78.3	70.6	67.6	75.9	8.6
Tepraloxydim (E- and Z-isomers)	3	86.4	87.0	89.2	94.5	94.3	90.3	4.3	86.7	89.6	94.6	85.7	90.6	89.4	3.9
Terbufos	3	83.0	82.6	85.9	90.9	87.7	86.0	4.0	80.9	88.0	81.5	85.1	79.0	82.9	4.3
Tetraconazole	2	84.9	87.4	83.3	86.3	89.0	86.2	2.6	83.8	85.5	88.5	87.8	82.3	85.6	3.0
Thiabendazole	5	83.1	81.7	92.5	75.3	83.6	83.2	7.4	77.0	85.7	92.8	79.4	89.7	84.9	7.9
Thiacloprid	5	94.4	81.4	79.2	78.5	81.8	83.1	7.8	72.4	90.4	79.1	84.6	83.3	82.0	8.2
Thiamethoxam	5	88.2	97.4	101	92.5	84.2	92.6	7.2	94.7	90.6	98.4	97.2	88.9	94.0	4.4
Thidiazuron	7	85.3	80.0	85.2	85.4	82.3	83.6	2.9	85.0	84.2	81.1	88.7	78.5	83.5	4.6
Thifensulfuron-methyl	4	76.3	80.3	79.3	87.4	82.4	81.2	5.1	87.7	80.4	92.5	90.7	83.8	87.0	5.7
Thiodicarb	5	70.9	87.5	97.0	93.3	74.3	84.6	14	69.1	93.4	74.2	81.4	74.9	78.6	12
Thiofanox	5	76.4	92.2	87.4	81.8	79.4	83.4	7.6	68.3	81.4	123	135	124	106	28
Tolylfluanid	3	78.7	73.3	76.3	77.8	85.1	78.2	5.6	69.9	74.2	69.6	65.6	69.5	69.8	4.4
Tralkoxydim	1	86.6	85.5	85.9	85.3	91.0	86.9	2.7	83.7	87.3	77.2	76.4	80.8	81.1	5.6
Triadimefon	3	86.3	83.1	81.0	83.9	86.9	84.2	2.9	76.0	74.5	88.7	87.9	82.8	82.0	8.0
Triadimenol	6	74.1	64.9	82.9	78.1	74.6	74.9	8.8	84.7	80.4	86.3	83.8	82.4	83.5	2.7

Table 1. Trueness (% Recovery) and Precision (% RSD) Data Obtained for Pesticides Included in the Agilent LC/MS Mixes 1–8 in Tomato Matrix at the Fortification Levels of 0.01 and 0.02 mg/kg Evaluated in Five Replicates (A–E) (continued)

		Fortification level 0.01 mg/kg							Fortification level 0.02 mg/kg						
		Trueness (%)						RSD	Trueness (%)						RSD
Analyte	Mix	A	B	C	D	E	Mean	(%)	A	B	C	D	E	Mean	(%)
Triasulfuron	4	85.9	86.2	93.7	91.1	82.1	87.8	5.2	73.4	79.1	85.2	90.7	88.7	83.4	8.5
Triazophos	3	92.9	86.5	85.4	90.2	94.1	89.8	4.3	85.6	87.4	90.1	93.7	95.4	90.4	4.6
Tribenuron-methyl	4	69.5	68.4	67.9	66.6	69.0	68.3	1.6	72.0	72.8	72.4	66.3	73.1	71.3	4.0
Trichlorfon (Metrifonate)	6	86.0	72.5	88.3	80.9	82.8	82.1	7.4	86.3	91.3	94.1	79.9	81.7	86.7	7.0
Tricyclazole	2	91.5	82.9	87.4	80.9	86.2	85.8	4.8	86.8	79.6	82.7	83.3	77.0	81.9	4.6
Trietazine	6	90.8	86.9	84.1	83.9	91.5	87.4	4.1	84.4	81.8	89.9	87.8	86.1	86.0	3.6
Trifloxystrobin	5	86.1	83.8	83.8	81.7	90.9	85.3	4.1	86.1	87.3	84.2	80.9	78.9	83.5	4.2
Triflumizole	3	85.2	81.2	78.6	80.7	90.2	83.2	5.5	87.6	85.8	84.2	84.1	82.3	84.8	2.3
Triflumuron	4	84.8	67.4	83.9	78.1	86.4	80.1	9.7	86.7	76.3	56.2	67.7	59.3	69.2	18
Trimethacarb	6	86.9	86.2	91.7	88.1	87.3	88.0	2.5	91.5	93.1	98.1	92.2	88.1	92.6	3.9
Triticonazole	2	82.3	80.1	79.5	90.5	75.6	81.6	6.8	97.5	87.2	85.0	86.6	90.5	89.3	5.6
Uniconazole	2	95.2	106	91.0	114	105	102	8.8	114	104	102	109	124	110	7.9
Vamidothion	2	82.7	83.2	91.9	86.4	88.1	86.5	4.4	82.5	79.0	92.8	81.5	83.5	83.9	6.3
Zoxamide	6	85.8	86.7	82.2	88.9	92.7	87.3	4.4	76.7	85.1	79.6	86.1	85.8	82.7	5.2

Table 2. Trueness (% Recovery) and Precision (% RSD) Data Obtained for Pesticides Included in the Agilent LC/MS Mixes 1–8 in Orange Juice Matrix at the Fortification Levels of 0.01 and 0.02 mg/kg Evaluated in Five Replicates (A–E)

		Fortification level 0.01 mg/kg							Fortification level 0.02 mg/kg						
		Trueness (%)						RSD	Trueness (%)						RSD
Analyte	Mix	A	B	C	D	E	Mean	(%)	A	B	C	D	E	Mean	(%)
Abamectin - Avermectin B1a	7	102	94.7	94.0	88.5	69.5	89.8	14	99.2	121	96.1	89.2	92.4	99.6	13
Acephate	1	95.0	94.0	91.6	91.8	86.6	91.8	3.5	80.7	94.0	89.8	86.5	84.6	87.1	5.8
Acetamiprid	5	93.9	83.4	105	102	85.2	94.0	10	84.8	100	84.5	84.9	96.6	90.3	8.5
Alanycarb	5	93.7	93.8	85.2	86.5	93.4	90.5	4.7	89.2	101	99.0	83.5	95.4	93.6	7.7
Aldicarb	5	95.2	93.6	93.5	96.1	96.8	95.0	1.5	83.5	102	97.3	83.1	81.9	89.6	11
Amidosulfuron	4	86.2	90.1	96.2	89.3	89.7	90.3	4.0	76.5	92.4	82.4	86.0	92.3	85.9	7.9
Aminocarb	4	92.4	112	76.8	89.8	79.1	89.9	15	86.4	105	93.8	95.3	100	96.1	7.2
Azaconazole	1	83.9	79.2	93.4	84.7	95.9	87.4	8.0	89.2	91.2	94.1	87.3	91.1	90.6	2.8
Azamethiphos	2	92.9	92.0	98.0	92.9	91.0	93.4	2.9	79.6	88.3	87.3	84.1	80.1	83.9	4.8
Azinphos-ethyl	1	96.9	106	91.2	102	106	100	6.4	79.7	96.9	100	97.6	97.5	94.4	8.8
Azinphos-methyl	1	82.5	83.9	80.7	86.6	84.1	83.6	2.6	91.3	95.3	85.9	82.4	87.3	88.5	5.7
Azoxystrobin	5	84.3	78.9	83.7	89.9	90.9	85.5	5.7	75.1	103	90.8	88.6	81.1	87.6	12
Beflubutamid	8	84.3	87.4	96.4	83.2	102	90.6	8.9	78.8	102	97.4	93.5	88.7	92.0	9.6
Benalaxyl	2	92.2	98.0	84.1	86.4	97.0	91.5	6.8	78.9	90.9	92.1	89.5	84.2	87.1	6.3
Benfuracarb	4	71.9	84.1	70.1	91.7	92.6	82.1	13	79.1	102	93.8	93.2	91.5	91.9	9.0
Benzoximate	7	85.3	93.8	86.6	97.1	88.8	90.3	5.5	83.0	97.0	85.2	83.1	87.8	87.2	6.7
Bifenazate	8	71.7	94.8	86.3	89.3	82.0	84.8	10	77.1	94.4	99.7	95.0	83.4	89.9	10
Bifenthrin	2	92.2	93.5	97.4	85.6	93.5	92.5	4.6	88.3	96.2	95.7	81.2	98.2	91.9	7.7
Bispyribac	7	96.4	91.7	86.9	90.6	102	93.4	6.1	84.7	97.1	101	89.1	82.6	90.9	8.6

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Table 2. Trueness (% Recovery) and Precision (% RSD) Data Obtained for Pesticides Included in the Agilent LC/MS Mixes 1–8 in Orange Juice Matrix at the Fortification Levels of 0.01 and 0.02 mg/kg Evaluated in Five Replicates (A–E) (continued)

		Fortification level 0.01 mg/kg							Fortification level 0.02 mg/kg						
		Trueness (%)						RSD	Trueness (%)						RSD
Analyte	Mix	A	B	C	D	E	Mean	(%)	A	B	C	D	E	Mean	(%)
Bitertanol	3	101	95.0	90.6	91.7	86.0	92.8	5.8	84.0	99.8	90.3	80.4	106	92.1	12
Boscalid	4	83.1	80.6	85.0	96.6	91.9	87.4	7.6	78.8	105	94.7	90.8	86.6	91.2	11
Bromuconazole (2 diastereoisomers)	2	91.7	86.5	89.3	93.8	90.4	90.3	3.0	84.4	97.7	94.8	85.9	85.8	89.7	6.8
Bupirimate	2	83.5	81.6	94.3	94.6	80.3	86.9	8.1	74.4	98.1	104	97.9	94.9	93.8	12
Buprofezin	1	97.1	83.8	87.7	92.0	86.7	89.5	5.8	82.7	108	84.1	91.1	90.9	91.3	11
Butocarboxim	4	91.2	86.8	83.4	75.5	89.4	85.2	7.3	77.7	97.4	92.5	92.1	82.1	88.3	9.2
Carbaryl	6	86.5	92.5	83.0	92.6	91.8	89.3	4.9	84.5	92.5	96.5	89.4	86.3	89.8	5.4
Carbendazim	5	73.2	76.1	61.5	71.1	71.3	70.6	7.8	73.1	81.4	71.8	74.5	97.7	79.7	13
Carbofuran	8	87.5	83.6	83.5	85.8	78.3	83.7	4.1	84.1	103	92.8	86.9	86.9	90.7	8.1
Carbosulfan	6	82.7	76.5	80.9	78.7	75.6	78.9	3.8	72.7	98.8	98.6	74.9	77.1	84.4	16
Carboxin	5	92.9	88.6	93.0	88.5	90.4	90.7	2.4	83.1	97.0	89.6	87.1	84.5	88.3	6.2
Carfentrazone-ethyl	4	81.4	88.0	74.7	79.3	94.4	83.5	9.2	80.4	93.5	88.3	78.2	88.6	85.8	7.4
Chlorantraniliprole	8	91.8	95.7	92.0	93.7	97.0	94.0	2.4	84.0	107	98.8	88.3	91.2	93.9	9.7
Chlorfenvinphos (E- and Z-isomers)	2	102	105	107	98.4	102	103	3.2	91.0	107	100	100	104	101	6.0
Chloridazon (Pyrazon)	4	78.6	83.4	79.2	89.7	82.3	82.6	5.4	76.3	91.4	81.3	86.1	81.7	83.3	6.8
Chlorotoluron (Chlortoluron)	7	91.8	91.8	90.6	94.9	96.5	93.1	2.6	81.9	100	95.4	87.7	81.1	89.3	9.4
Chloroxuron	7	93.1	95.7	94.6	87.0	92.6	92.6	3.6	83.4	99.8	100	90.7	88.7	92.5	7.8
Chlorpyrifos	2	88.6	89.8	87.4	87.0	87.3	88.0	1.3	82.1	99.3	93.9	88.3	88.1	90.3	7.2
Chlorpyrifos-methyl	2	90.5	99.4	95.4	79.8	84.5	89.9	8.8	74.9	99.8	93.8	88.9	82.1	87.9	11
Chlorsulfuron	4	94.3	102	102	89.6	100	97.6	5.5	84.3	97.7	90.8	91.8	79.6	88.8	7.9
Clethodim (E- and Z-isomers)	3	87.5	95.3	92.5	94.7	89.1	91.8	3.7	79.5	89.2	102	88.5	83.1	88.5	9.6
Clofentezine	4	88.8	87.5	79.0	84.6	90.2	86.0	5.2	85.9	89.8	98.2	80.1	77.4	86.3	9.5
Clomazone	8	84.5	93.3	89.9	90.0	87.2	89.0	3.7	83.7	94.7	89	92.5	87.4	89.5	4.8
Coumaphos	2	94.9	91.6	88.4	97.7	87.5	92.0	4.7	84.7	95.7	93.5	86.6	96.3	91.4	5.9
Cyazofamid	4	91.1	93.9	91.6	80.6	96.1	90.6	6.6	81.5	98.2	100	89.5	92.3	92.3	8.0
Cycloate	1	95.4	79.4	89.9	91.5	93.4	89.9	6.9	78.1	105	95.3	87.7	88.7	90.9	11
Cycluron	7	84.8	88.2	85.7	91.9	89.5	88.0	3.3	83.9	107	103	95.6	87.0	95.3	10
Cymiazole	1	98.5	95.5	111	95.9	104	101	6.6	93.4	98.2	106	83.1	89.7	94.1	9.3
Cymoxanil	4	94.8	98.4	91.0	96.5	90.8	94.3	3.6	77.7	87.3	85.5	85.3	88.2	84.8	4.9
Cyproconazole (2 diastereoisomers)	1	91.7	102	109	94.1	85.8	96.5	9.3	78.0	97.6	95.8	98.1	98.5	93.6	9.4
Cyprodinil	8	85.2	87.9	89.2	86.2	89.0	87.5	2.0	83.3	98.3	93.6	103	81.1	91.8	10
DEET (Diethyltoluamide)	4	83.8	95.5	92.2	97.9	95.7	93.0	6.0	77.6	90.8	90.5	93.4	85.5	87.6	7.2
Desmedipham	8	89.0	91.4	94.9	96.3	88.5	92.0	3.8	82.6	96.1	87.3	83.9	84.3	86.8	6.3
Diazinon	2	89.7	89.6	93.2	86.5	93.1	90.4	3.1	76.5	89.9	87.6	98.2	83.0	87.0	9.3
Dichlorvos	2	96.0	96.5	93.2	86.3	91.5	92.7	4.4	76.6	106	93.3	93.2	87.6	91.4	12
Diethofencarb	6	89.4	95.3	87.5	94.3	95.6	92.4	4.0	79.7	99.1	86.9	92.8	82.7	88.2	8.9
Difenoconazole (*cis*- and *trans*-)	3	82.2	94.8	81.2	91.2	86.0	87.1	6.7	75.8	94.0	89.4	98.1	87.9	89.0	9.5
Diflubenzuron	4	91.2	93.6	95.0	83.7	99.2	92.6	6.2	88.4	85.2	102	91.0	85.2	90.3	7.6
Diflufenican	1	91.0	95.2	96.4	87.5	96.4	93.3	4.2	82.6	93.8	94.3	84.7	86.9	88.4	6.0

Table 2. Trueness (% Recovery) and Precision (% RSD) Data Obtained for Pesticides Included in the Agilent LC/MS Mixes 1–8 in Orange Juice Matrix at the Fortification Levels of 0.01 and 0.02 mg/kg Evaluated in Five Replicates (A–E) (continued)

		Fortification level 0.01 mg/kg							Fortification level 0.02 mg/kg						
		Trueness (%)						RSD	Trueness (%)						RSD
Analyte	Mix	A	B	C	D	E	Mean	(%)	A	B	C	D	E	Mean	(%)
Dimethachlor	1	95.6	97.2	92.7	91.5	94.1	94.2	2.4	82.1	94.4	96.2	94.1	89.1	91.2	6.3
Dimethoate	8	78.7	82.6	100	88.3	103	90.6	12	76.1	106	96.7	110	93.3	96.2	14
Dimethomorph (E- and Z-isomers)	5	93.0	87.2	90.9	99.1	106	95.1	7.6	82.4	91.9	89.1	93.3	83.4	88.0	5.6
Dimoxystrobin	1	107	82.7	79.5	77.1	87.7	86.8	14	94.3	117	106	88.0	88.3	98.9	13
Diniconazole	2	99.2	96.6	96.3	100	95.3	97.6	2.2	81.9	97.2	89.5	92.9	84.1	89.1	7.0
Dinotefuran	7	87.1	78.4	78.8	89.5	91.2	85.0	7.1	73.2	97.0	95.3	93.7	93.1	90.5	11
Dioxacarb	7	106	95.8	81.0	108	89.3	96.0	12	90.7	103	93.8	95.7	106	97.9	6.7
Disulfoton	1	81.8	83.2	87.4	90.5	91.1	86.8	4.8	84.8	95.1	94.2	88.4	86.5	89.8	5.2
Diuron	5	88.2	89.6	87.4	83.6	92.8	88.3	3.8	76.5	87.0	87.6	91.0	85.2	85.5	6.3
Epoxiconazole	2	81.5	93.6	80.2	84.0	86.5	85.2	6.2	82.5	96.9	94.2	82.6	84.4	88.1	7.8
Ethidimuron (Sulfadiazole)	8	93.0	91.8	75.7	83.2	92.9	87.3	8.8	78.8	85.4	79.7	87.4	96.1	85.5	8.2
Ethion	2	90.3	104	96.3	101	102	98.7	5.6	76.0	100	94.4	87.2	85.6	88.6	10
Ethirimol	4	94.1	89.4	88.1	96.3	84.3	90.4	5.3	78.6	96.4	89.5	102	81.6	89.6	11
Ethofumesate	4	86.7	93.5	87.2	79.8	91.5	87.7	6.0	82.3	95.0	88.4	86.2	84.6	87.3	5.5
Ethoprophos (Ethoprop)	2	86.7	85.0	92.3	89.0	93.7	89.3	4.1	79.8	105	94.3	91.0	87.4	91.5	10
Ethoxyquin	8	171	165	169	166	174	169	2.1	129	143	138	140	135	137	3.7
Etofenprox	3	87.3	88.3	91.3	87.4	90.4	88.9	2.0	80.1	107	87.8	93.1	89.7	91.5	11
Famoxadone	4	79.0	89.1	76.6	76.4	90.7	82.4	8.5	79.6	81.8	104	86.6	72.0	84.7	14
Fenamidone	5	93.2	86.7	94.2	87.7	94.9	91.3	4.2	83.8	97.6	92.1	82.8	82.2	87.7	7.8
Fenamiphos	1	97.4	99.6	84.0	91.6	78.7	90.3	9.8	68.0	97.9	108	105	102	96.1	17
Fenarimol	2	91.4	89.2	89.7	102	98.7	94.2	6.1	77.1	98.2	91.6	91.3	80.5	87.7	9.9
Fenazaquin	5	99.4	95.3	104	111	96.8	101	6.4	86.8	97.0	96.8	82.9	95.4	91.8	7.1
Fenbuconazole	2	88.6	102	86.8	86.9	96.8	92.3	7.6	79.4	98.5	90.5	89.7	88.6	89.3	7.6
Fenhexamid	3	93.7	84.9	88.2	87.3	90.0	88.8	3.7	78.3	80.7	95.6	90.0	89.1	86.8	8.2
Fenobucarb	5	91.1	89.4	89.8	94.3	95.3	92.0	2.9	79.7	101	89.4	90.8	88.6	90.0	8.5
Fenoxycarb	6	96.1	87.0	99.2	85.9	94.9	92.7	6.3	85.7	98.6	88.4	85.4	81.1	87.9	7.5
Fenpropidin	5	96.5	95.9	95.2	95.2	99.4	96.5	1.8	82.0	93.5	87.2	89.4	87.1	87.9	4.7
Fenpyroximate	5	92.6	95.4	99.1	97.8	95.6	96.1	2.6	72.4	93.0	85.7	90.6	87.4	85.8	9.3
Fenuron	7	92.2	92.1	86.7	85.5	96.3	90.6	4.9	84.5	93.5	89.2	84.5	91.5	88.6	4.6
Fipronil	4	90.3	105	99.0	83.2	90.2	93.5	9.0	90.4	128	89	98.4	83.5	97.8	18
Flazasulfuron	4	96.6	90.6	94.5	77.0	87.7	89.3	8.6	78.2	93.0	82.5	89.6	85.3	85.7	6.8
Flonicamid	6	87.5	93.6	89.4	74.8	88.5	86.8	8.2	75.3	75.3	90.1	80.9	80.8	80.5	7.5
Flubendiamide	7	96.0	101	100	85.9	85.9	93.8	8.0	65.9	87.6	93.1	81.4	93.1	84.2	13
Fludioxonil	2	90.2	84.9	87.2	86.9	99.3	89.7	6.4	93.8	88.7	99.1	85.5	88.6	91.1	5.9
Flufenacet	1	96.7	101	110	93.5	106	101	6.6	90.7	107	110	93.7	99.2	100	8.4
Flufenoxuron	4	91.1	92.6	94.4	90.5	99.4	93.6	3.8	79.1	90.5	88	87.2	84.0	85.8	5.1
Flumetsulam	8	82.0	84.0	98.0	93.3	97.8	91.0	8.3	94.0	89.0	103	77.5	82.2	89.1	11
Flumioxazin	6	63.6	100	89.4	88.5	81.6	84.6	16	83.1	88.0	97.3	91.1	89.9	89.9	5.7
Fluometuron	8	94.6	94.9	92.7	93.8	95.3	94.3	1.1	80.7	95.0	87.2	90.2	86.2	87.9	6.0

Table 2. Trueness (% Recovery) and Precision (% RSD) Data Obtained for Pesticides Included in the Agilent LC/MS Mixes 1–8 in Orange Juice Matrix at the Fortification Levels of 0.01 and 0.02 mg/kg Evaluated in Five Replicates (A–E) (continued)

		Fortification level 0.01 mg/kg							Fortification level 0.02 mg/kg						
		Trueness (%)						RSD	Trueness (%)						RSD
Analyte	Mix	A	B	C	D	E	Mean	(%)	A	B	C	D	E	Mean	(%)
Fluopicolide	1	85.8	87.5	97.3	90.5	83.3	88.9	6.1	84.5	94.0	90.8	89.4	95.6	90.8	4.8
Fluoxastrobin	8	84.9	99.5	74.6	82.2	87.2	85.7	11	81.2	85.9	85.6	74.0	93.6	84.1	8.6
Fluquinconazole	2	90.7	90.3	100	89.8	103	94.7	6.6	75.7	101	95	106	99.6	95.5	12
Flusilazole	2	79.3	86.3	79.6	77.1	89.4	82.4	6.3	78.1	105	99.4	94.1	86.5	92.6	11
Flutriafol	8	86.3	94.2	92.8	90.4	91.9	91.1	3.3	84.4	100	91.3	94.5	90.3	92.2	6.4
Foramsulfuron	3	84.7	94.8	93.2	96.7	83.6	90.6	6.7	79.6	99.3	99	89.8	82.4	90.0	10
Forchlorfenuron	7	89.7	86.4	92.4	95.4	96.2	92.0	4.4	81.7	96.9	87.8	93.2	83.2	88.5	7.3
Fosthiazate (sum of isomers)	1	94.2	87.9	90.3	83.0	87.6	88.6	4.6	80.0	93.5	86.7	86.8	89.9	87.4	5.7
Fuberidazole	4	90.7	94.6	97.0	91.2	82.8	91.3	5.9	79.5	88.9	99.3	83.7	90.3	88.4	8.5
Furalaxyl	7	89.9	91.5	83.8	76.9	81.5	84.7	7.1	87.0	96.5	95.7	97.2	92.4	93.8	4.5
Furathiocarb	6	95.0	89.6	88.9	88.9	89.8	90.5	2.8	85.3	93.0	98.5	90.5	91.0	91.7	5.2
Halofenozide	8	95.8	97.5	93.2	92.7	90.1	93.9	3.1	74.4	94.0	90.3	86.2	98.3	88.7	10
Halosulfuron-methyl	8	100	104	93.7	99.3	104	100	4.2	80.4	100	90.4	92.0	90.3	90.7	7.8
Hexaconazole	2	97.3	94.5	88.5	92.8	94.5	93.5	3.5	84.8	103	92.2	94.5	90.9	93.0	6.9
Hexaflumuron	7	88.0	84.2	87.3	87.1	91.3	87.6	2.9	72.8	91.0	89.4	86.4	80.6	84.0	8.8
Hexythiazox	4	86.5	98.1	95.1	87.8	99.9	93.5	6.5	81.0	93.8	95.2	88.6	82.0	88.1	7.4
Hydramethylnon	7	103	102	98.3	90.6	99.3	98.6	4.9	89.5	92.5	93.1	85.0	88.7	89.8	3.6
Imazalil	2	83.4	97.1	101	89.6	98.8	94.0	7.7	84.6	97.7	94.4	94.9	83.2	90.9	7.2
Imidacloprid	5	75.2	80.2	90.7	72.3	77.1	79.1	9.0	83.8	104	85.2	80.0	87.0	88.1	11
Indoxacarb	3	87.1	90.6	90.6	91.0	89.8	89.8	1.8	79.5	86.2	81.3	91.0	84.8	84.5	5.3
Ipconazole	2	94.9	90.7	92.9	93.8	94.2	93.3	1.7	83.9	106	101	89.3	86.8	93.4	10
Iprovalicarb	5	83.6	89.5	91.1	90.3	87.7	88.4	3.4	74.9	92.2	85.2	86.3	86.6	85.0	7.4
Isofenphos-methyl	1	89.7	87.0	84.6	86.3	92.8	88.1	3.7	83.7	89.3	89.9	90.5	89.5	88.6	3.1
Isoprothiolane	1	117	139	104	102	68.8	106	24	89.9	99.5	109	103	92.6	98.8	7.8
Isoxaben	4	83.5	95.5	87.3	98.1	85.1	89.9	7.3	85.8	101	109	94.4	92.3	96.5	9.2
Isoxaflutole	3	89.6	89.6	92.8	92.0	90.1	90.8	1.6	80.3	97.2	91.6	85.9	82.5	87.5	7.9
Ivermectin B1a	7	101	97.2	105	95.2	92.1	98.0	5.0	76.4	92.0	86.6	83.6	88.2	85.3	6.9
Kresoxim-methyl	4	90.4	107	97.0	96.6	103	98.7	6.4	86.5	94.5	97.7	93.2	89.8	92.3	4.7
Lenacil	1	90.6	91.0	101	99.2	102	96.6	5.6	89.8	97.4	93.6	96.9	82.5	92.1	6.7
Linuron	4	83.8	90.8	84.4	97.8	92.2	89.8	6.5	76.2	94.1	101	94.7	91.7	91.6	10
Lufenuron	4	85.7	92.1	99.7	98.1	95.3	94.2	5.9	78.0	87.0	83.8	81.4	86.2	83.3	4.4
Malaoxon	3	103	96.8	98.5	86.4	86.1	94.1	8.0	80.6	90.6	97.2	97.4	93.8	91.9	7.5
Malathion	3	91.3	100	87.7	93.2	90.8	92.7	5.1	72.9	85.1	92.9	85.8	85.6	84.5	8.5
Mandipropamid	4	83.3	94.6	96.5	83.5	88.7	89.3	6.8	77.7	97.1	85.1	86.1	90.7	87.3	8.2
Mecarbam	3	94.4	90.5	89.5	85.2	86.9	89.3	4.0	75.3	95.6	87.8	93.9	91.5	88.8	9.1
Mepanipyrim	3	89.7	81.1	84.1	75.2	84.0	82.8	6.4	69.9	89.3	88.6	85.8	84.6	83.7	9.5
Mesosulfuron-methyl	6	96.0	90.6	86.2	86.4	96.9	91.2	5.6	75.4	92.7	76.3	87.9	77.7	82.0	9.5
Metaflumizone	4	97.1	102	106	95.8	107	102	5.1	85.9	98.9	88.2	101	91.0	93.0	7.1
Metalaxyl	3	88.7	93.4	82.8	88.7	83.9	87.5	4.9	80.0	96.0	87.2	87.7	85.8	87.4	6.6

Table 2. Trueness (% Recovery) and Precision (% RSD) Data Obtained for Pesticides Included in the Agilent LC/MS Mixes 1–8 in Orange Juice Matrix at the Fortification Levels of 0.01 and 0.02 mg/kg Evaluated in Five Replicates (A–E) (continued)

		Fortification level 0.01 mg/kg							Fortification level 0.02 mg/kg						
		Trueness (%)						RSD	Trueness (%)						RSD
Analyte	Mix	A	B	C	D	E	Mean	(%)	A	B	C	D	E	Mean	(%)
Metamitron	4	84.0	87.1	79.8	85.7	82.4	83.8	3.4	82.2	102	83.6	90.3	81.7	88.1	9.9
Metazachlor	3	94.9	86.5	89.2	85.4	90.5	89.3	4.2	81.9	94.4	88.9	86.5	88.2	88.0	5.1
Metconazole	2	98.9	98.4	98.9	98.2	94.5	97.8	1.9	83.9	103	96.7	89.0	93.9	93.3	7.9
Methabenzthiazuron	5	90.9	102	90.7	92.0	90.9	93.2	5.1	83.6	95.5	95.4	86.6	88.6	89.9	5.9
Methamidophos	1	85.8	83.9	86.0	83.1	93.9	86.5	5.0	80.1	92.1	88.1	83.6	84.4	85.7	5.4
Methidathion	3	92.7	92.4	87.9	78.0	92.1	88.6	7.1	81.9	106	93.9	84.7	94.5	92.1	10
Methiocarb	7	93.2	99.5	91.4	92.6	101	95.5	4.4	89.8	108	96.1	97.4	93.2	97.0	7.2
Methomyl	5	95.2	94.5	92.7	97.0	88.8	93.6	3.3	79.9	98.9	86.3	94.1	90.9	90.0	8.1
Methoprotryne	7	86.3	91.5	94.5	94.7	91.9	91.8	3.7	82.9	96.0	90.7	89.2	89.8	89.7	5.2
Methoxyfenozide	5	103	96.7	100	88.2	89.1	95.5	7.0	75.6	96.2	91	96.5	89.1	89.7	9.5
Metobromuron	8	91.9	88.3	91.0	94.7	94.0	92.0	2.7	83.1	94.0	95.8	89.7	85.6	89.6	6.0
Metolachlor	3	99.4	106	108	102	95.8	102	4.8	81.1	105	104	86.5	85.0	92.4	12
Metrafenone	4	95.8	89.8	92.4	94.9	100	94.6	4.1	90.7	97.9	93.6	93.3	86.9	92.5	4.4
Metribuzin	4	91.3	90.7	95.0	101	91.5	94.0	4.7	80.9	98.9	88.8	98.4	88.0	91.0	8.4
Metsulfuron-methyl	4	91.0	85.8	103	91.6	93.7	92.9	6.6	83.9	101	93.3	86.9	85.8	90.2	7.9
Mevinphos (E- and Z-isomers)	3	94.1	94.5	90.8	89.1	89.3	91.6	2.8	87.6	93.6	91.8	95.3	88.6	91.4	3.6
Mexacarbate	7	88.2	90.0	91.8	79.7	115	93.0	14	83.6	98.4	88.4	86.1	86.4	88.6	6.5
Molinate	3	91.9	94.7	77.5	99.1	93.3	91.3	9.0	83.1	99.5	85.3	103	85.0	91.2	10
Monocrotophos	4	82.5	87.3	77.4	73.0	85.1	81.1	7.2	72.8	89.9	93.8	84.4	88.6	85.9	9.4
Moxidectin	7	97.9	93.8	88.1	96.0	97.5	94.7	4.2	85.0	104	86.4	93.1	94.2	92.5	8.2
Myclobutanil	1	92.4	99.0	94.4	94.2	100	96.0	3.5	89.3	88.7	102	85.7	87.1	90.5	7.0
Nicosulfuron	4	88.4	89.1	95.3	90.8	97.9	92.3	4.5	67.9	87.9	86	85.0	75.6	80.5	11
Nitenpyram	7	106	96.3	103	91.9	88.5	97.0	7.5	79.5	89.6	86	67.0	85.1	81.4	11
Novaluron	4	90.0	85.3	96.0	87.6	97.3	91.2	5.7	78.9	98.0	86.7	87.3	95.2	89.3	8.5
Omethoate	6	97.8	80.1	104	81.2	95.2	91.7	12	92.4	98.8	105	101	95.0	98.4	5.1
Oxadiazon	3	89.7	91.4	79.1	90.9	92.8	88.8	6.2	89.0	93.5	95.8	80.9	89.1	89.6	6.4
Oxadixyl	3	90.8	87.6	93.7	90.2	91.6	90.8	2.5	73.6	91.2	88.3	90.5	87.1	86.2	8.4
Oxamyl	5	89.2	91.6	81.0	104	80.8	89.4	11	76.2	89.8	91.9	89.4	85.9	86.7	7.2
Oxasulfuron	4	89.9	97.1	104	102	95.7	97.7	5.6	76.8	98.9	94.3	88.7	89.7	89.7	9.2
Paclobutrazol	3	84.0	93.8	86.0	89.6	93.5	89.4	4.9	79.2	88.2	92.3	85.1	87.7	86.5	5.6
Penconazole	3	82.1	83.6	91.5	86.8	91.4	87.1	5.0	84.1	93.6	97.2	88.9	84.9	89.7	6.3
Pencycuron	6	78.7	79.4	82.5	74.4	92.0	81.4	8.1	90.3	99.5	95.5	90.3	99.9	95.1	4.9
Pendimethalin	3	91.9	84.9	98.0	85.9	89.1	90.0	5.9	75.5	95.6	91.6	89.5	89.0	88.2	8.6
Phenmedipham	4	99.8	93.1	91.0	97.9	94.6	95.3	3.7	83.9	95.8	92.9	89.9	90.2	90.5	4.9
Phenthoate	3	81.1	95.3	107	96.2	90.4	94.0	9.9	85.7	76.2	83.7	93.0	106	88.9	13
Phosalone	3	95.7	99.9	94.4	99.5	88.9	95.7	4.7	87.8	97.4	99.6	92.5	85.3	92.5	6.6
Phosmet	6	95.2	88.2	96.6	84.1	85.9	90.0	6.2	81.8	101	96	105	92.9	95.4	9.4
Phosphamidon (E- and Z-isomers)	3	92.2	91.7	93.9	90.2	91.3	91.8	1.5	81.0	95.0	93.7	91.3	86.6	89.5	6.4
Phoxim	4	86.8	91.9	82.0	91.1	90.2	88.4	4.6	79.0	90.9	90.6	89.7	88.8	87.8	5.7

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Table 2. Trueness (% Recovery) and Precision (% RSD) Data Obtained for Pesticides Included in the Agilent LC/MS Mixes 1–8 in Orange Juice Matrix at the Fortification Levels of 0.01 and 0.02 mg/kg Evaluated in Five Replicates (A–E) (continued)

		Fortification level 0.01 mg/kg							Fortification level 0.02 mg/kg						
		Trueness (%)						RSD	Trueness (%)						RSD
Analyte	Mix	A	B	C	D	E	Mean	(%)	A	B	C	D	E	Mean	(%)
Picolinafen	3	97.4	97.4	92.1	90.5	85.9	92.7	5.3	83.9	97.7	90.5	91.7	89.0	90.6	5.5
Picoxystrobin	5	91.4	86.7	88.4	82.3	82.5	86.3	4.5	87.6	91.4	84.6	92.5	83.3	87.9	4.6
Pirimicarb	3	93.2	97.8	95.6	94.2	94.5	95.1	1.9	87.9	98.0	97.8	89.8	91.1	92.9	5.0
Pirimiphos-methyl	3	97.8	91.8	101	100	99.3	98.0	3.7	76.5	113	103	90.2	95.3	95.6	14
Prochloraz	1	86.5	101	88.3	90.4	104	94.1	8.5	81.1	94.9	88.9	107	80.0	90.5	12
Profenofos	3	88.4	97.7	98.2	88.5	90.8	92.7	5.3	77.0	98.0	91.1	84.1	90.2	88.1	9.0
Promecarb	7	93.9	83.7	86.3	92.1	85.2	88.2	5.1	90.3	95.4	96.3	93.5	90.2	93.1	3.1
Prometon	4	92.8	93.0	96.9	95.9	91.0	93.9	2.6	77.1	101	97.8	87.8	83.4	89.4	11
Propamocarb	5	94.6	103	95.8	98.8	94.1	97.3	3.8	92.2	91.1	96.3	85.9	82.9	89.7	5.9
Propaquizafop	4	86.1	98.8	92.2	96.7	87.2	92.2	6.1	75.0	82.6	88	84.0	87.5	83.4	6.3
Propargite	4	94.8	93.5	94.6	91.0	91.8	93.1	1.8	82.1	101	92	92.1	89.9	91.4	7.2
Propetamphos	3	91.5	77.8	91.3	93.5	93.4	89.5	7.4	85.0	90.3	87.4	96.9	95.7	91.1	5.7
Propiconazole (sum of isomers)	2	84.1	83.8	94.0	85.0	87.3	86.8	4.9	82.1	101	93.2	86.9	83.8	89.3	8.6
Propoxur	6	91.3	83.0	95.6	88.5	102	92.2	8.0	89.0	96.5	91.6	91.3	88.0	91.3	3.6
Propyzamide (Pronamide)	3	88.3	92.6	93.2	95.7	98.9	93.7	4.2	87.7	104	94.1	93.1	88.1	93.5	7.2
Proquinazid	1	93.6	90.5	91.5	92.3	97.9	93.1	3.1	79.6	98.0	95.4	87.6	94.8	91.1	8.2
Prosulfocarb	4	89.9	89.4	91.1	86.7	92.4	89.9	2.4	84.3	95.0	94.2	91.5	79.0	88.8	7.8
Pymetrozine	7	89.4	86.8	98.4	93.7	100	93.7	6.1	82.6	98.7	90.3	86.9	81.7	88.0	7.8
Pyracarbolid	7	98.4	95.6	90.8	93.4	88.9	93.4	4.0	80.0	93.6	86.3	77.8	87.9	85.1	7.5
Pyraclostrobin	5	92.4	95.1	92.8	88.7	96.4	93.1	3.2	75.3	86.6	85.7	86.0	80.3	82.8	5.9
Pyridaben	5	87.2	88.1	88.7	86.7	92.8	88.7	2.7	82.9	88.6	91.8	81.0	86.1	86.1	5.0
Pyridate	5	84.7	94.5	85.9	88.7	76.6	86.1	7.5	82.9	107	92	85.2	83.6	90.2	11
Pyrimethanil	6	83.9	85.8	84.5	72.0	84.8	82.2	7.0	76.8	88.2	90.7	88.9	85.4	86.0	6.4
Pyriproxyfen	5	88.1	82.2	86.1	85.2	83.3	85.0	2.7	77.3	98.3	97.4	94.5	85.4	90.6	10
Quinalphos	3	91.6	92.2	96.4	77.8	90.7	89.7	7.8	79.3	100	87.6	89.3	87.1	88.7	8.4
Quinmerac	7	69.1	76.7	72.8	75.7	77.1	74.3	4.5	76.8	97.2	87.4	69.6	71.6	80.5	14
Quinoclamine	4	99.6	91.1	85.0	88.9	94.4	91.8	6.0	83.6	91.1	94	85.7	93.0	89.5	5.1
Quinoxyfen	3	85.4	83.8	86.8	91.5	95.2	88.5	5.3	85.4	95.5	85	94.9	85.7	89.3	6.1
Rimsulfuron	4	90.1	86.8	92.0	89.9	88.8	89.5	2.1	80.9	92.5	89.8	86.6	82.7	86.5	5.6
Rotenone	7	91.3	87.4	95.8	84.0	86.8	89.1	5.1	78.4	95.6	93.1	81.9	91.7	88.1	8.6
Secbumeton	7	91.0	91.3	94.9	94.0	100	94.3	4.0	84.6	100	90.4	89.9	85.8	90.1	6.7
Silthiofam	4	87.2	82.5	71.0	78.0	86.9	81.1	8.4	86.7	107	88.8	93.7	81.4	91.5	10
Spinosad - Spinosyn A	7	102	95.6	103	90.3	92.1	96.7	6.0	82.3	93.7	82.8	99.6	94.0	90.5	8.4
Spinosad - Spinosyn D	7	90.0	84.2	78.1	79.1	81.6	82.6	5.8	70.7	90.4	87.9	83.5	93.2	85.1	10
Spirodiclofen	1	81.0	85.1	87.7	88.7	80.3	84.6	4.5	74.3	104	95.8	87.8	87.7	89.8	12
Spiromesifen	6	90.3	95.8	93.0	84.9	87.0	90.2	4.9	81.9	93.0	94.3	89.9	83.5	88.5	6.3
Spirotetramat	6	89.0	85.3	90.4	85.5	95.7	89.2	4.8	87.6	105	91.2	82.5	96.5	92.6	9.3
Spiroxamine (2 diastereoisomers)	1	104	109	102	99.7	104	104	3.1	85.8	99.9	89.9	86.5	91.4	90.7	6.2
Sulfentrazone	6	89.9	88.9	94.9	92.9	95.3	92.4	3.1	83.1	105	92.1	87.3	89.2	91.4	9.2

Table 2. Trueness (% Recovery) and Precision (% RSD) Data Obtained for Pesticides Included in the Agilent LC/MS Mixes 1–8 in Orange Juice Matrix at the Fortification Levels of 0.01 and 0.02 mg/kg Evaluated in Five Replicates (A–E) (continued)

		Fortification level 0.01 mg/kg							Fortification level 0.02 mg/kg						
		Trueness (%)						RSD	Trueness (%)						RSD
Analyte	Mix	A	B	C	D	E	Mean	(%)	A	B	C	D	E	Mean	(%)
Tebuconazole	2	83.1	92.6	83.6	83.0	91.8	86.8	5.7	84.0	93.2	86.3	88.0	83.9	87.1	4.4
Tebufenozide	5	103	98.7	91.6	91.4	92.4	95.4	5.4	92.2	94.0	73.8	98.3	90.8	89.8	10
Tebufenpyrad	3	90.3	82.2	86.5	85.2	91.7	87.2	4.4	85.7	104	96.5	89.0	85.2	92.0	8.6
Tebuthiuron	7	96.2	95.2	91.0	96.9	97.9	95.4	2.8	72.9	86.1	82.1	83.4	76.1	80.1	6.8
Teflubenzuron	4	85.3	88.5	84.7	78.3	102	87.8	10	70.8	103	89.4	81.9	82.2	85.5	14
Temephos	7	103	112	107	99.9	102	105	4.5	85.2	117	101	109	94.7	101	12
Tepraloxydim (E- and Z-isomers)	3	87.6	81.2	86.8	84.0	78.4	83.6	4.7	80.5	92.1	86.8	92.0	91.6	88.6	5.7
Terbufos	3	93.4	93.2	93.6	95.9	91.5	93.5	1.7	84.9	101	89.3	87.7	82.6	89.1	7.9
Tetraconazole	2	89.9	93.2	95.3	86.6	87.9	90.6	4.0	79.8	92.2	94.5	95.9	92.5	91.0	7.1
Thiabendazole	5	84.4	84.2	90.3	82.4	84.0	85.1	3.6	78.6	93.8	93	87.2	92.2	89.0	7.1
Thiacloprid	5	89.7	82.8	87.5	90.4	93.1	88.7	4.3	77.5	90.4	74.5	89.8	95.9	85.6	11
Thiamethoxam	5	101	93.9	92.5	86.4	90.9	92.9	5.6	73.4	83.3	86	79.7	91.3	82.8	8.2
Thidiazuron	7	91.9	86.6	98.4	93.9	92.8	92.7	4.6	85.7	100	91.2	86.7	86.2	90.0	6.7
Thifensulfuron-methyl	4	95.5	104	97.7	91.1	92.4	96.2	5.5	80.2	101	96.8	94.8	86.4	91.8	9.1
Thiodicarb	5	87.1	88.0	86.9	84.4	101	89.5	7.4	83.3	101	102	86.8	83.7	91.5	10
Thiofanox	5	82.7	84.6	107	89.7	95.1	91.9	11	81.0	109	90.1	107	84.5	94.3	14
Tolylfluanid	3	90.3	92.7	113	91.8	90.1	95.5	10	81.0	83.6	94.2	73.3	78.9	82.2	9.4
Tralkoxydim	1	90.0	93.9	96.0	95.9	93.5	93.9	2.6	81.2	84.1	88.1	91.4	83.5	85.7	4.7
Triadimefon	3	95.3	97.9	92.1	98.4	90.2	94.8	3.8	75.5	90.2	103	90.1	81.3	88.0	12
Triadimenol	6	115	97.3	106	92.4	102	103	8.5	87.0	88.7	89.9	89.1	87.8	88.5	1.3
Triasulfuron	4	94.2	102	88.6	98.2	99.0	96.3	5.3	86.0	107	98.3	90.5	90.4	94.5	8.9
Triazophos	3	86.6	76.5	94.1	72.6	85.7	83.1	10	77.1	94.7	89.7	77.2	91.1	86.0	9.6
Tribenuron-methyl	4	74.5	81.9	73.3	78.4	78.8	77.4	4.5	73.6	87.3	86.7	81.7	74.7	80.8	8.0
Trichlorfon (Metrifonate)	6	77.5	77.2	82.7	83.0	86.7	81.4	4.9	73.9	87.1	87.8	97.2	84.2	86.0	9.7
Tricyclazole	2	95.4	99.2	95.9	93.1	92.3	95.2	2.9	83.4	94.8	93.2	89.7	89.2	90.1	4.9
Trietazine	6	90.7	98.3	99.7	95.0	103	97.4	5.0	80.6	92.8	83.9	87.9	93.5	87.8	6.4
Trifloxystrobin	5	88.7	102	94.4	86.8	96.4	93.6	6.5	74.7	95.2	86.3	83.1	83.9	84.6	8.7
Triflumizole	3	93.9	95.3	93.0	91.8	104	95.5	4.9	80.3	96.8	92.7	92.4	87.0	89.8	7.1
Triflumuron	4	97.0	96.3	96.7	91.9	91.0	94.6	3.1	82.9	85.5	86.7	95.0	95.3	89.1	6.4
Trimethacarb	6	91.9	89.5	86.7	91.1	95.1	90.8	3.4	84.3	101	89.9	91.1	86.7	90.6	7.0
Triticonazole	2	76.7	94.6	89.1	90.9	92.6	88.8	8.0	79.8	103	97.3	85.3	88.7	90.8	10
Uniconazole	2	96.4	100	93.9	85.7	94.3	94.1	5.7	85.5	109	101	82.0	98.8	95.2	12
Vamidothion	2	103	112	120	104	99.0	108	7.8	88.0	105	86.5	85.7	79.3	88.9	11
Zoxamide	6	89.5	91.9	93.3	94.4	94.3	92.7	2.2	86.6	98.1	90.6	94.3	83.3	90.6	6.5

Table 3. Trueness (% Recovery) and Precision (% RSD) Data Obtained for Pesticides Included in the Agilent LC/MS Mixes 1–8 in Spinach Matrix at the Fortification Levels of 0.01 and 0.02 mg/kg Evaluated in Five Replicates (A–E)

		Fortification level 0.01 mg/kg							Fortification level 0.02 mg/kg						
		Trueness (%)						RSD	Trueness (%)						RSD
Analyte	Mix	A	B	C	D	E	Mean	(%)	A	B	C	D	E	Mean	(%)
Abamectin - Avermectin B1a	7	99.1	91.6	69.9	60.0	108	85.7	23	79.1	103	91.2	71.6	107	90.3	17
Acephate	1	95.9	78.2	76.0	75.1	82.2	81.5	10	65.7	81.2	70.6	91.6	97.6	81.3	17
Acetamiprid	5	92.1	85.8	86.9	88.9	82.5	87.2	4.1	79.1	81.6	77.3	87.0	91.0	83.2	6.8
Alanycarb	5	82.1	58.2	62.2	59.1	54.1	63.1	17	71.9	73.8	68.0	64.7	79.5	71.6	7.9
Aldicarb	5	98.2	89.6	89.7	90.9	91.3	92.0	3.9	79.7	80.2	79.8	82.8	86.9	81.9	3.7
Amidosulfuron	4	92.1	92.1	87.8	88.2	91.4	90.3	2.3	86.1	82.0	83.3	88.7	90.4	86.1	4.1
Aminocarb	4	106	89.7	91.0	95.7	92.9	94.9	6.7	83.4	87.1	81.0	85.5	86.3	84.6	2.9
Azaconazole	1	94.5	96.4	88.7	89.8	97.3	93.3	4.1	82.4	89.2	82.9	80.5	91.9	85.4	5.7
Azamethiphos	2	99.1	78.7	94.8	91.5	86.1	90.0	8.8	95.4	84.3	81.3	87.2	95.2	88.7	7.2
Azinphos-ethyl	1	81.8	88.9	87.4	80.7	84.1	84.6	4.1	79.8	77.4	82.7	88.1	88.3	83.2	5.8
Azinphos-methyl	1	79.4	70.7	81.4	70.6	81.4	76.7	7.3	85.3	76.8	85.0	85.1	87.5	83.9	4.9
Azoxystrobin	5	97.2	98.1	92.4	102	102	98.5	4.2	92.9	97.2	89.1	85.3	95.3	92.0	5.2
Beflubutamid	8	85.1	95.3	85.4	82.9	93.7	88.5	6.4	87.1	79.9	88.9	92.1	95.9	88.8	6.7
Benalaxyl	2	90.1	87.5	85.9	95.6	89.2	89.7	4.1	82.4	91.6	75.2	87.2	85.6	84.4	7.3
Benfuracarb	4	50.6	18.3	18.0	12.5	12.0	22.3	72	40.5	43.2	26.5	47.3	67.7	45.1	33
Benzoximate	7	104	87.0	83.5	88.9	95.0	91.6	8.6	93.5	90.8	83.6	84.3	96.9	89.8	6.5
Bifenazate	8	108	103	73.7	93.7	94.9	94.8	14	78.8	83.0	116	63.2	92.5	86.7	22
Bifenthrin	2	85.0	79.3	73.0	75.4	74.8	77.5	6.2	73.4	73.5	73.3	81.5	76.0	75.5	4.7
Bispyribac	7	90.5	91.8	84.6	89.7	91.5	89.6	3.2	77.0	80.9	79.4	80.3	81.5	79.8	2.2
Bitertanol	3	95.9	73.1	92.5	80.0	82.6	84.8	11	84.4	84.4	78.0	75.7	71.6	78.8	7.1
Boscalid	4	91.9	88.5	84.4	84.1	90.5	87.9	4.0	81.8	87.5	80.7	87.8	82.4	84.0	4.0
Bromuconazole (2 diastereoisomers)	2	102	90.2	91.4	90.4	91.7	93.1	5.3	81.1	75.9	76.3	92.1	89.2	82.9	9.0
Bupirimate	2	92.0	97.6	88.1	102	104	96.7	6.9	91.6	91.4	85.4	98.0	107	94.7	8.7
Buprofezin	1	84.9	81.8	84.0	81.1	80.8	82.5	2.2	78.1	79.8	79.0	80.3	85.3	80.5	3.5
Butocarboxim	4	97.5	87.0	95.8	92.5	93.7	93.3	4.3	86.1	86.6	87.4	87.1	86.2	86.7	0.7
Carbaryl	6	100	95.7	94.6	91.3	90.5	94.4	4.0	81.8	83.0	81.5	85.0	89.3	84.1	3.8
Carbendazim	5	99.9	91.7	83.4	94.0	99.2	93.7	7.1	74.4	75.2	71.7	82.6	77.7	76.3	5.4
Carbofuran	8	97.1	94.8	95.9	91.8	100	95.9	3.2	94.6	87.6	91.9	91.5	95.3	92.2	3.3
Carbosulfan	6	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Carboxin	5	93.5	87.1	84.2	93.3	94.1	90.5	5.0	78.4	78.2	82.0	88.6	80.8	81.6	5.2
Carfentrazone-ethyl	4	88.9	96.7	89.0	85.6	86.9	89.4	4.8	92.1	90.4	83.2	88.4	91.2	89.0	4.0
Chlorantraniliprole	8	99.4	96.3	90.1	98.7	97.6	96.4	3.9	79.9	80.2	79.2	83.8	90.5	82.7	5.7
Chlorfenvinphos (E- and Z-isomers)	2	92.3	92.1	84.7	88.9	93.0	90.2	3.8	86.3	83.0	78.7	87.6	90.8	85.3	5.4
Chloridazon (Pyrazon)	4	87.2	90.4	86.6	86.7	82.8	86.8	3.1	92.6	83.3	81.6	83.2	84.9	85.1	5.1
Chlorotoluron (Chlortoluron)	7	94.0	90.9	84.9	90.0	90.5	90.0	3.7	84.5	83.4	82.7	86.3	90.3	85.4	3.5
Chloroxuron	7	103	102	105	98.6	103	102	2.3	89.4	88.4	77.4	91.2	91.4	87.6	6.7
Chlorpyrifos	2	99.3	89.4	90.7	92.5	89.9	92.4	4.4	82.5	86.7	87.9	89.8	89.8	87.3	3.5
Chlorpyrifos-methyl	2	90.5	90.2	90.2	85.3	95.9	90.4	4.2	82.6	87.9	77.5	85.5	83.7	83.5	4.6
Chlorsulfuron	4	77.8	89.7	77.4	81.7	80.7	81.4	6.1	81.9	77.8	78.3	84.3	87.6	82.0	5.1

Table 3. Trueness (% Recovery) and Precision (% RSD) Data Obtained for Pesticides Included in the Agilent LC/MS Mixes 1–8 in Spinach Matrix at the Fortification Levels of 0.01 and 0.02 mg/kg Evaluated in Five Replicates (A–E) (continued)

		Fortification level 0.01 mg/kg							Fortification level 0.02 mg/kg						
		Trueness (%)						RSD	Trueness (%)						RSD
Analyte	Mix	A	B	C	D	E	Mean	(%)	A	B	C	D	E	Mean	(%)
Clethodim (E- and Z-isomers)	3	96.9	91.0	101	92.6	102	96.6	5.0	83.2	85.9	90.2	88.9	85.1	86.7	3.3
Clofentezine	4	93.7	87.5	85.1	84.5	98.5	89.8	6.8	92.0	86.4	81.2	87.7	92.1	87.9	5.2
Clomazone	8	88.6	86.7	87.6	85.1	89.0	87.4	1.8	81.4	79.5	72.1	82.3	85.1	80.1	6.1
Coumaphos	2	93.6	93.1	90.3	92.2	88.1	91.5	2.5	84.1	90.8	87.5	87.0	93.4	88.6	4.1
Cyazofamid	4	91.4	89.1	86.3	95.4	88.9	90.2	3.8	89.9	90.7	87.3	90.5	87.1	89.1	2.0
Cycloate	1	99.0	88.0	91.4	94.5	82.7	91.1	6.8	81.8	94.1	79.5	85.6	85.7	85.4	6.5
Cycluron	7	80.5	83.4	86.1	84.8	85.9	84.2	2.7	82.6	83.6	81.2	84.5	79.4	82.2	2.4
Cymiazole	1	87.8	85.3	82.9	85.2	81.9	84.6	2.7	75.8	83.2	78.6	74.3	83.2	79.0	5.2
Cymoxanil	4	116	90.4	97.4	87.2	112	100	13	85.1	92.3	91.4	92.7	89.9	90.3	3.4
Cyproconazole (2 diastereoisomers)	1	93.6	90.5	85.3	90.8	89.1	89.9	3.4	81.3	84.7	83.6	86.2	86.7	84.5	2.6
Cyprodinil	8	81.0	81.4	86.0	86.4	90.2	85.0	4.5	83.1	84.5	82.4	87.3	80.0	83.5	3.2
DEET (Diethyltoluamide)	4	89.8	85.7	92.0	89.2	82.1	87.8	4.4	85.2	82.3	77.8	80.1	86.2	82.3	4.2
Desmedipham	8	93.1	101	86.3	89.7	91.9	92.5	6.0	93.7	80.4	85.8	91.5	90.0	88.3	5.9
Diazinon	2	94.7	91.7	85.5	90.7	86.2	89.8	4.3	91.4	79.8	83.2	79.5	76.4	82.1	7.0
Dichlorvos	2	125	123	114	117	118	120	3.9	107	101	110	108	111	108	3.7
Diethofencarb	6	94.6	88.7	91.7	89.2	86.8	90.2	3.4	82.2	91.3	87.2	89.4	89.6	87.9	4.0
Difenoconazole (*cis*- and *trans*-)	3	86.2	88.4	90.1	88.5	92.7	89.2	2.7	81.3	85.3	78.5	78.6	79.7	80.7	3.5
Diflubenzuron	4	100	73.5	94.5	89.4	95.2	90.6	11	87.0	86.7	80.9	85.9	86.5	85.4	3.0
Diflufenican	1	91.9	88.1	82.3	84.0	80.9	85.5	5.3	83.6	79.4	82.4	87.9	87.3	84.1	4.2
Dimethachlor	1	91.6	91.7	89.6	86.4	89.4	89.7	2.4	91.8	92.8	85.1	89.5	90.9	90.0	3.4
Dimethoate	8	91.5	85.0	82.3	88.5	96.3	88.7	6.2	89.7	95.0	93.2	101	93.3	94.5	4.6
Dimethomorph (E- and Z-isomers)	5	106	93.1	97.1	99.8	99.3	99.1	4.8	82.9	80.0	79.5	85.8	85.6	82.8	3.6
Dimoxystrobin	1	94.6	100	83.8	95.2	82.0	91.2	8.6	90.0	96.0	93.3	84.1	97.2	92.1	5.7
Diniconazole	2	95.1	99.2	92.0	96.9	88.7	94.4	4.3	84.8	83.7	88.5	89.9	87.7	86.9	3.0
Dinotefuran	7	101	79.4	80.5	88.6	83.4	86.5	10	81.0	85.6	84.1	80.6	103	86.8	11
Dioxacarb	7	109	98.6	118	90.2	80.9	99.3	15	98.0	87.9	85.6	90.9	95.0	91.5	5.5
Disulfoton	1	96.5	103	106	100	86.8	98.4	7.5	85.6	90.4	87.4	85.8	94.9	88.8	4.4
Diuron	5	95.5	94.3	97.8	92.9	101	96.2	3.1	82.8	82.6	78.8	86.1	88.8	83.8	4.5
Epoxiconazole	2	102	111	92.1	94.3	83.3	96.6	11	88.9	97.3	100	90.5	95.6	94.5	4.9
Ethidimuron (Sulfadiazole)	8	101	96.8	97.3	94.9	98.2	97.6	2.2	79.3	100	82.3	92.2	95.3	89.9	9.9
Ethion	2	98.2	91.9	100	92.0	99.1	96.3	4.2	81.1	85.0	78.6	89.7	88.1	84.5	5.5
Ethirimol	4	98.4	88.4	95.9	91.6	95.0	93.9	4.1	86.7	91.9	68.2	83.1	83.0	82.6	11
Ethofumesate	4	82.0	88.5	90.0	85.2	96.6	88.4	6.2	81.8	82.9	87.6	91.4	96.5	88.0	7.0
Ethoprophos (Ethoprop)	2	82.0	89.0	76.4	75.8	94.1	83.5	9.5	92.9	106	89.1	92.6	91.4	94.4	7.1
Ethoxyquin	8	94.8	86.5	94.2	97.2	93.6	93.3	4.3	73.1	73.2	73.0	68.0	81.5	73.8	6.6
Etofenprox	3	78.8	77.0	80.0	76.7	76.3	77.8	2.0	72.9	78.3	75.5	74.5	78.1	75.8	3.1
Famoxadone	4	89.4	78.2	75.9	72.1	94.4	82.0	12	89.4	81.3	83.7	99.8	94.7	89.8	8.5
Fenamidone	5	96.9	98.6	85.2	95.0	88.1	92.8	6.3	74.9	83.8	70.8	78.3	84.8	78.5	7.6
Fenamiphos	1	102	89.9	82.9	86.2	91.4	90.4	7.8	72.0	85.4	75.5	78.8	89.7	80.3	9.0

Table 3. Trueness (% Recovery) and Precision (% RSD) Data Obtained for Pesticides Included in the Agilent LC/MS Mixes 1–8 in Spinach Matrix at the Fortification Levels of 0.01 and 0.02 mg/kg Evaluated in Five Replicates (A–E) (continued)

		Fortification level 0.01 mg/kg							Fortification level 0.02 mg/kg						
		Trueness (%)						RSD	Trueness (%)						RSD
Analyte	Mix	A	B	C	D	E	Mean	(%)	A	B	C	D	E	Mean	(%)
Fenarimol	2	92.7	100	107	94.3	105	99.8	6.4	76.1	82.5	89.7	95.1	92.1	87.1	8.8
Fenazaquin	5	95.4	89.4	83.5	79.7	90.8	87.8	7.1	75.5	75.8	80.3	76.4	70.8	75.7	4.5
Fenbuconazole	2	96.5	92.2	82.6	85.2	94.8	90.3	6.7	100	94.2	87.9	96.1	84.0	92.5	7.1
Fenhexamid	3	97.2	91.7	82.6	85.3	86.1	88.6	6.6	79.6	79.2	80.1	99.2	87.3	85.1	10
Fenobucarb	5	96.0	96.6	89.6	91.2	89.1	92.5	3.9	92.5	88.2	86.3	90.1	87.6	89.0	2.7
Fenoxycarb	6	95.0	86.7	88.5	101	92.9	92.9	6.2	87.9	92.9	96.4	88.1	100	93.1	5.8
Fenpropidin	5	90.9	89.9	83.2	84.5	90.6	87.8	4.2	79.3	86.5	84.5	80.2	83.1	82.7	3.6
Fenpyroximate	5	86.5	82.0	74.2	80.2	82.6	81.1	5.6	80.8	73.1	84.8	83.6	81.8	80.8	5.7
Fenuron	7	92.4	100	97.3	87.0	99.6	95.3	5.8	91.7	94.2	85.6	97.7	91.7	92.2	4.8
Fipronil	4	93.5	93.3	111	106	105	102	7.7	101	92.9	96.1	87.6	83.7	92.3	7.5
Flazasulfuron	4	87.7	88.8	94.4	94.0	93.2	91.6	3.4	87.7	88.9	88.8	85.7	89.4	88.1	1.7
Flonicamid	6	128	83.8	87.4	87.9	78.4	93.2	21	78.6	101	81.4	88.3	102	90.2	12
Flubendiamide	7	95.6	85.2	78.3	77.9	82.9	84.0	8.6	72.3	94.2	70.9	87.6	88.9	82.8	13
Fludioxonil	2	104	90.1	90.2	82.9	88.4	91.1	8.6	83.3	80.0	76.5	81.1	94.2	83.0	8.1
Flufenacet	1	98.9	89.5	94.2	86.1	88.0	91.3	5.7	84.3	87.2	84.8	87.2	90.4	86.8	2.8
Flufenoxuron	4	85.2	88.3	84.5	83.0	89.8	86.2	3.3	86.0	87.8	81.4	86.1	89.3	86.1	3.4
Flumetsulam	8	94.0	90.0	101	81.6	89.4	91.2	7.8	81.2	83.1	91.8	78.8	87.9	84.6	6.2
Flumioxazin	6	89.7	109	80.7	75.2	72.2	85.4	17	69.5	77.0	79.0	80.4	92.6	79.7	10
Fluometuron	8	100	96.4	93.2	98.1	95.0	96.5	2.7	85.2	85.6	88.6	89.4	93.2	88.4	3.7
Fluopicolide	1	83.6	93.3	92.9	97.8	93.7	92.3	5.7	79.0	85.6	80.8	86.2	87.5	83.8	4.4
Fluoxastrobin	8	80.0	86.6	84.3	99.0	77.0	85.4	9.9	77.7	80.5	80.1	75.0	75.6	77.7	3.2
Fluquinconazole	2	89.3	100	81.3	86.4	78.2	87.0	9.7	84.0	79.6	91.7	91.7	81.4	85.7	6.7
Flusilazole	2	97.5	96.0	87.8	88.4	90.7	92.1	4.8	89.7	91.6	83.8	94.1	93.9	90.6	4.6
Flutriafol	8	88.6	83.9	81.2	84.0	87.6	85.1	3.5	77.5	84.4	79.6	81.0	77.6	80.0	3.6
Foramsulfuron	3	88.4	85.2	86.2	87.8	90.7	87.6	2.4	84.6	79.9	79.7	81.8	86.6	82.5	3.7
Forchlorfenuron	7	92.6	89.9	91.9	95.2	95.5	93.0	2.5	80.8	84.4	86.4	85.4	83.7	84.1	2.5
Fosthiazate (sum of isomers)	1	89.2	90.7	87.1	88.0	89.0	88.8	1.5	82.6	87.9	80.3	86.4	88.5	85.1	4.2
Fuberidazole	4	91.0	83.9	84.1	78.9	83.5	84.3	5.1	81.7	77.7	75.3	76.2	81.6	78.5	3.8
Furalaxyl	7	81.7	84.3	84.2	80.3	89.0	83.9	4.0	83.7	82.2	85.5	88.9	86.1	85.3	3.0
Furathiocarb	6	88.1	85.7	77.5	85.8	87.4	84.9	5.0	74.1	77.3	81.1	77.2	95.7	81.1	11
Halofenozide	8	97.0	85.3	82.7	84.7	89.9	87.9	6.5	80.8	80.7	85.6	88.9	90.1	85.2	5.2
Halosulfuron-methyl	8	99.7	104	93.0	99.7	95.0	98.3	4.4	94.1	81.9	84.8	101	93.8	91.1	8.5
Hexaconazole	2	89.5	88.7	92.2	83.9	86.2	88.1	3.6	83.0	88.5	84.5	83.4	86.8	85.2	2.8
Hexaflumuron	7	97.8	97.4	90.4	92.4	95.4	94.7	3.4	84.7	76.7	86.0	86.8	89.3	84.7	5.7
Hexythiazox	4	83.5	81.4	82.3	79.4	86.1	82.5	3.0	76.4	78.2	72.3	86.2	81.1	78.8	6.6
Hydramethylnon	7	72.4	66.7	65.1	65.3	62.1	66.3	5.7	70.7	68.2	70.2	76.8	66.7	70.5	5.5
Imazalil	2	82.0	86.5	77.5	80.9	83.6	82.1	4.0	78.4	74.8	76.5	78.7	84.5	78.6	4.7
Imidacloprid	5	103	99.9	91.9	94.2	96.0	97.1	4.7	83.5	90.9	86.3	93.6	91.2	89.1	4.6
Indoxacarb	3	93.4	99.7	91.2	92.2	95.1	94.3	3.5	86.9	92.7	83.8	89.4	95.5	89.7	5.1

Table 3. Trueness (% Recovery) and Precision (% RSD) Data Obtained for Pesticides Included in the Agilent LC/MS Mixes 1–8 in Spinach Matrix at the Fortification Levels of 0.01 and 0.02 mg/kg Evaluated in Five Replicates (A–E) (continued)

		Fortification level 0.01 mg/kg							Fortification level 0.02 mg/kg						
		Trueness (%)						RSD	Trueness (%)						RSD
Analyte	Mix	A	B	C	D	E	Mean	(%)	A	B	C	D	E	Mean	(%)
Ipconazole	2	91.5	94.0	89.9	87.9	97.4	92.1	4.0	85.0	84.9	83.5	91.9	87.6	86.6	3.8
Iprovalicarb	5	97.0	97.8	97.3	95.5	96.8	96.9	0.9	98.4	91.5	88.5	88.5	100	93.4	5.9
Isofenphos-methyl	1	97.1	103	102	100	102	101	2.1	88.0	91.0	86.1	89.6	95.5	90.0	4.0
Isoprothiolane	1	81.6	85.9	81.9	76.9	91.2	83.5	6.4	82.2	91.9	94.5	90.4	93.1	90.4	5.4
Isoxaben	4	88.1	81.3	81.4	70.2	86.4	81.5	8.6	87.5	82.0	84.3	92.2	94.4	88.1	5.9
Isoxaflutole	3	85.1	82.3	78.0	83.9	81.2	82.1	3.3	73.9	75.2	75.2	73.1	75.0	74.5	1.3
Ivermectin B1a	7	95.0	68.5	75.3	90.1	97.3	85.2	15	82.4	81.9	82.7	90.3	97.0	86.9	7.7
Kresoxim-methyl	4	96.4	81.8	94.8	82.9	96.8	90.5	8.3	81.7	86.7	82.6	93.1	90.1	86.9	5.6
Lenacil	1	90.3	89.6	86.5	82.2	92.5	88.2	4.5	78.5	74.5	78.3	85.6	80.7	79.5	5.1
Linuron	4	89.9	89.1	87.4	88.8	94.6	90.0	3.1	75.1	81.0	75.5	77.9	86.8	79.3	6.1
Lufenuron	4	82.5	82.7	74.2	78.4	89.2	81.4	6.9	74.9	79.7	72.2	78.5	79.4	76.9	4.2
Malaoxon	3	92.3	90.2	83.2	83.5	86.4	87.1	4.6	75.1	86.9	76.9	77.1	80.7	79.3	5.9
Malathion	3	86.0	86.8	83.4	79.4	88.6	84.8	4.2	92.7	103	80.3	85.9	112	94.8	14
Mandipropamid	4	84.9	89.2	89.7	81.1	82.5	85.5	4.5	84.5	89.6	80.5	82.0	80.8	83.5	4.5
Mecarbam	3	96.5	91.0	88.7	84.5	94.4	91.0	5.2	89.9	85.8	89.5	91.6	88.5	89.1	2.4
Mepanipyrim	3	91.6	92.9	91.7	82.6	89.2	89.6	4.6	81.5	83.4	82.7	84.2	83.0	83.0	1.2
Mesosulfuron-methyl	6	90.7	86.7	79.0	85.6	96.3	87.6	7.3	96.4	91.0	80.3	80.6	92.9	88.2	8.4
Metaflumizone	4	97.8	88.5	94.4	83.6	86.4	90.1	6.5	87.2	81.9	83.7	88.1	84.8	85.1	3.0
Metalaxyl	3	86.4	91.2	97.2	92.1	89.1	91.2	4.4	80.7	86.8	85.3	95.6	86.7	87.0	6.2
Metamitron	4	100	82.7	77.8	85.4	86.4	86.5	9.6	97.9	97.7	85.2	100	85.2	93.3	8.0
Metazachlor	3	90.7	89.5	90.6	88.3	95.7	91.0	3.1	81.9	88.5	80.9	85.8	87.1	84.8	3.9
Metconazole	2	98.1	91.4	94.9	91.9	93.3	93.9	2.9	83.5	88.2	87.2	92.0	88.8	87.9	3.5
Methabenzthiazuron	5	87.1	82.6	86.6	88.0	87.8	86.4	2.6	80.3	77.2	77.3	82.7	85.6	80.6	4.5
Methamidophos	1	89.8	70.4	68.7	67.4	73.8	74.0	12	71.3	73.7	69.5	84.2	77.4	75.2	7.7
Methidathion	3	82.1	82.5	88.9	91.6	88.5	86.7	4.8	81.2	78.1	85.1	90.5	88.0	84.6	5.9
Methiocarb	7	90.0	96.9	100	94.5	96.4	95.6	3.9	84.6	86.8	77.6	93.1	90.6	86.5	6.9
Methomyl	5	126	97.9	106	104	111	109	9.5	93.1	113	95.0	102	109	103	8.6
Methoprotryne	7	89.7	90.9	89.8	89.9	88.3	89.7	1.0	81.0	78.6	88.6	80.0	84.6	82.6	4.9
Methoxyfenozide	5	89.0	88.2	71.3	90.4	80.1	83.8	9.6	82.9	78.5	75.1	87.6	92.1	83.2	8.2
Metobromuron	8	98.7	95.1	90.8	98.5	98.8	96.4	3.6	83.8	89.9	79.3	89.5	87.6	86.0	5.2
Metolachlor	3	94.6	90.1	85.9	90.6	88.4	89.9	3.5	80.7	82.6	77.2	78.8	88.3	81.5	5.3
Metrafenone	4	84.2	92.5	87.8	85.5	81.2	86.2	4.9	83.4	77.0	80.0	82.0	84.7	81.4	3.7
Metribuzin	4	95.0	91.6	88.2	91.1	93.7	91.9	2.8	90.3	85.4	85.3	91.3	85.8	87.6	3.3
Metsulfuron-methyl	4	80.9	80.8	89.1	89.7	96.8	87.5	7.7	94.4	94.8	94.8	81.0	81.6	89.3	8.2
Mevinphos (E- and Z-isomers)	3	97.5	93.2	92.2	81.4	88.7	90.6	6.7	90.3	95.5	81.9	95.2	88.2	90.2	6.2
Mexacarbate	7	93.4	95.6	88.7	84.9	91.5	90.8	4.6	88.3	86.2	72.2	86.3	92.4	85.1	9.0
Molinate	3	105	96.9	90.6	94.6	97.0	96.7	5.3	85.0	84.4	79.5	97.8	89.4	87.2	7.9
Monocrotophos	4	93.1	91.9	83.4	84.0	91.6	88.8	5.3	79.0	81.8	77.8	83.6	86.9	81.8	4.4
Moxidectin	7	85.3	86.9	81.8	86.3	78.5	83.8	4.2	89.2	80.0	75.5	77.7	83.8	81.2	6.7

Table 3. Trueness (% Recovery) and Precision (% RSD) Data Obtained for Pesticides Included in the Agilent LC/MS Mixes 1–8 in Spinach Matrix at the Fortification Levels of 0.01 and 0.02 mg/kg Evaluated in Five Replicates (A–E) (continued)

		Fortification level 0.01 mg/kg							Fortification level 0.02 mg/kg						
		Trueness (%)						RSD	Trueness (%)						RSD
Analyte	Mix	A	B	C	D	E	Mean	(%)	A	B	C	D	E	Mean	(%)
Myclobutanil	1	93.3	98.3	85.9	93.3	93.9	92.9	4.8	92.5	91.6	84.4	81.1	103	90.5	9.3
Nicosulfuron	4	82.6	91.9	85.2	83.2	78.9	84.4	5.7	69.4	79.5	75.0	81.7	77.1	76.5	6.1
Nitenpyram	7	76.9	82.0	82.1	71.5	71.1	76.7	7.0	71.7	73.3	72.3	73.7	82.4	74.7	5.9
Novaluron	4	84.9	85.6	84.3	84.5	87.6	85.4	1.6	82.4	76.1	79.9	85.2	81.7	81.1	4.1
Omethoate	6	115	97.2	103	97.6	90.1	101	9.2	78.6	78.2	87.5	83.7	101	85.9	11
Oxadiazon	3	85.3	86.8	77.4	85.6	79.7	82.9	5.0	72.1	74.8	76.1	76.3	77.9	75.4	2.9
Oxadixyl	3	95.8	91.7	91.8	91.7	92.3	92.7	1.9	89.4	85.7	82.1	87.5	89.7	86.9	3.6
Oxamyl	5	107	102	94.2	91.8	85.8	96.2	8.8	76.6	83.2	78.6	84.1	110	86.6	16
Oxasulfuron	4	97.5	91.7	96.8	87.9	88.3	92.4	4.9	80.1	85.4	91.3	76.7	85.7	83.8	6.7
Paclobutrazol	3	88.4	85.7	87.0	84.8	88.0	86.8	1.8	83.8	82.4	81.1	82.7	87.3	83.5	2.8
Penconazole	3	91.8	96.6	87.4	89.1	93.6	91.7	4.0	84.0	85.1	93.5	94.3	94.4	90.3	5.8
Pencycuron	6	101	88.5	92.3	89.0	95.7	93.3	5.6	94.2	92.3	87.9	90.4	91.8	91.3	2.6
Pendimethalin	3	104	96.3	75.9	123	90.5	98.1	18	53.1	96.1	67.7	75.7	85.2	75.6	22
Phenmedipham	4	87.4	91.3	80.5	89.2	91.3	87.9	5.1	86.0	82.1	77.8	89.0	83.9	83.8	5.0
Phenthoate	3	106	87.9	89.9	92.1	95.8	94.4	7.7	95.5	90.4	100	94.6	87.5	93.6	5.2
Phosalone	3	89.7	80.9	82.1	76.0	92.6	84.3	8.0	89.3	84.1	77.6	87.4	92.7	86.2	6.7
Phosmet	6	97.1	90.5	82.6	88.2	95.7	90.8	6.5	88.6	82.0	95.1	87.1	92.0	89.0	5.6
Phosphamidon (E- and Z-isomers)	3	88.5	89.2	84.2	81.9	85.8	85.9	3.6	82.5	84.1	85.5	85.1	87.6	84.9	2.2
Phoxim	4	87.6	92.3	80.5	84.7	82.9	85.6	5.3	84.1	80.1	88.5	91.4	86.4	86.1	5.0
Picolinafen	3	86.9	84.3	89.3	91.8	92.5	89.0	3.8	90.9	89.6	86.6	88.5	84.0	87.9	3.1
Picoxystrobin	5	98.4	88.2	91.2	89.9	56.3	84.8	19	89.5	86.7	87.6	100	97.4	92.3	6.7
Pirimicarb	3	90.9	86.7	81.8	86.3	89.0	86.9	3.9	78.2	77.6	80.9	76.9	83.6	79.5	3.5
Pirimiphos-methyl	3	91.3	90.1	93.1	84.6	93.0	90.4	3.9	92.5	89.3	85.0	90.5	98.6	91.2	5.5
Prochloraz	1	93.9	88.7	91.8	98.0	107	95.8	7.3	82.7	88.0	77.9	85.4	90.2	84.8	5.6
Profenofos	3	96.1	93.8	89.7	89.1	90.1	91.7	3.3	87.9	90.5	88.9	91.3	94.6	90.6	2.8
Promecarb	7	88.3	94.0	91.2	86.2	83.8	88.7	4.5	85.0	93.6	90.2	95.5	94.1	91.7	4.6
Prometon	4	94.2	96.5	88.3	92.0	94.9	93.2	3.4	80.0	79.6	79.8	86.4	84.7	82.1	3.9
Propamocarb	5	98.3	86.1	82.6	91.7	88.9	89.5	6.6	77.1	86.2	80.5	78.3	91.7	82.7	7.4
Propaquizafop	4	92.7	97.6	89.2	92.6	95.8	93.6	3.4	84.8	75.2	78.7	81.9	83.5	80.8	4.8
Propargite	4	87.1	92.5	87.1	93.7	89.4	90.0	3.4	87.6	88.6	86.2	92.9	89.7	89.0	2.9
Propetamphos	3	78.4	85.1	91.1	94.8	91.8	88.2	7.4	84.4	86.2	83.4	83.7	91.4	85.8	3.9
Propiconazole (sum of isomers)	2	89.5	91.2	83.5	85.0	88.7	87.6	3.7	81.6	83.1	86.2	84.9	89.7	85.1	3.7
Propoxur	6	89.1	92.1	93.3	89.8	91.4	91.1	1.9	86.6	88.5	83.5	91.0	92.2	88.4	3.9
Propyzamide (Pronamide)	3	93.6	101	99.6	103	95.2	98.5	4.0	85.6	85.5	93.1	102	92.2	91.7	7.3
Proquinazid	1	78.6	80.2	73.6	84.8	82.2	79.9	5.3	75.2	84.5	74.1	84.4	81.7	80.0	6.2
Prosulfocarb	4	93.8	88.8	88.8	91.5	92.8	91.1	2.5	92.4	85.1	89.1	85.7	87.1	87.9	3.3
Pymetrozine	7	78.9	79.4	66.7	73.4	69.4	73.6	7.6	93.3	81.1	78.1	75.3	96.2	84.8	11
Pyracarbolid	7	96.9	92.2	83.8	89.9	87.4	90.1	5.5	89.4	85.4	78.9	88.1	93.1	87.0	6.1
Pyraclostrobin	5	96.6	93.5	92.3	94.7	85.7	92.5	4.5	91.3	84.8	93.5	96.4	98.9	93.0	5.8

Table 3. Trueness (% Recovery) and Precision (% RSD) Data Obtained for Pesticides Included in the Agilent LC/MS Mixes 1–8 in Spinach Matrix at the Fortification Levels of 0.01 and 0.02 mg/kg Evaluated in Five Replicates (A–E) (continued)

		Fortification level 0.01 mg/kg							Fortification level 0.02 mg/kg						
		Trueness (%)						RSD	Trueness (%)						RSD
Analyte	Mix	A	B	C	D	E	Mean	(%)	A	B	C	D	E	Mean	(%)
Pyridaben	5	96.9	91.0	89.5	89.5	90.1	91.4	3.4	82.6	74.1	69.8	82.5	84.2	78.6	8.1
Pyridate	5	72.1	63.9	62.6	68.9	69.0	67.3	5.8	64.7	62.5	69.9	75.7	64.0	67.4	8.0
Pyrimethanil	6	90.4	94.0	88.2	88.5	86.9	89.6	3.1	87.5	88.4	82.3	84.5	87.3	86.0	2.9
Pyriproxyfen	5	94.2	84.8	89.9	84.8	86.1	88.0	4.6	81.5	79.4	76.4	86.6	86.1	82.0	5.3
Quinalphos	3	97.8	93.5	82.8	85.5	95.8	91.1	7.3	84.2	85.3	87.9	101	96.1	90.8	7.9
Quinmerac	7	56.2	65.0	64.4	63.9	68.2	63.5	7.0	52.3	52.9	59.7	55.4	50.6	54.2	6.5
Quinoclamine	4	96.0	93.4	91.4	93.2	94.7	93.7	1.8	83.4	84.8	83.9	89.0	82.5	84.7	3.0
Quinoxyfen	3	83.2	82.5	80.9	87.3	97.5	86.3	7.8	80.2	75.1	79.1	88.9	85.7	81.8	6.7
Rimsulfuron	4	90.6	88.3	82.5	83.1	87.7	86.4	4.1	79.9	89.9	81.9	85.7	84.8	84.4	4.5
Rotenone	7	95.5	93.0	94.4	97.1	92.6	94.5	1.9	80.6	85.7	87.2	82.5	83.6	83.9	3.1
Secbumeton	7	86.1	84.2	88.3	83.5	85.9	85.6	2.2	84.6	82.9	79.6	83.0	79.6	81.9	2.7
Silthiofam	4	86.0	88.2	73.3	97.9	88.4	86.8	10	92.4	85.5	79.1	89.1	96.1	88.4	7.4
Spinosad - Spinosyn A	7	126	165	79.3	162	221	151	35	114	127	62.9	157	62.0	105	40
Spinosad - Spinosyn D	7	175	101	109	119	155	132	24	120	92.8	66.8	86.9	88.5	90.9	21
Spirodiclofen	1	92.5	77.3	78.1	85.6	88.2	84.3	7.8	84.0	84.9	81.3	76.2	81.4	81.5	4.1
Spiromesifen	6	93.3	94.9	88.8	83.5	86.6	89.4	5.3	88.6	88.0	85.5	90.3	88.5	88.2	2.0
Spirotetramat	6	87.5	76.3	82.1	79.0	89.7	82.9	6.8	77.7	84.1	81.4	91.9	92.1	85.5	7.5
Spiroxamine (2 diastereoisomers)	1	96.1	93.1	92.5	95.6	94.5	94.4	1.7	88.0	83.8	81.5	87.4	86.9	85.5	3.2
Sulfentrazone	6	86.7	89.8	89.9	104	94.2	92.9	7.2	78.1	91.1	83.7	93.9	87.6	86.9	7.2
Tebuconazole	2	97.3	91.3	87.8	94.3	96.0	93.4	4.1	83.0	87.3	86.8	91.0	84.7	86.5	3.5
Tebufenozide	5	90.5	88.6	94.8	84.7	100	91.7	6.4	88.2	89.8	92.8	101	105	95.4	7.8
Tebufenpyrad	3	93.6	94.7	92.5	91.6	91.2	92.7	1.5	83.2	80.2	84.6	87.1	87.1	84.4	3.4
Tebuthiuron	7	93.8	92.5	86.3	86.2	90.2	89.8	3.9	85.8	86.7	82.6	88.5	94.8	87.7	5.1
Teflubenzuron	4	75.4	88.7	87.2	89.5	87.4	85.6	6.8	82.7	78.1	87.4	86.8	78.6	82.7	5.3
Temephos	7	78.3	89.1	91.4	94.1	83.0	87.2	7.4	84.6	88.9	87.3	81.2	92.2	86.8	4.8
Tepraloxydim (E- and Z-isomers)	3	95.5	90.5	90.3	92.0	91.6	92.0	2.3	81.4	80.5	85.1	78.4	83.0	81.7	3.1
Terbufos	3	103	105	103	98.9	103	103	2.3	85.7	85.2	88.8	82.0	92.5	86.8	4.6
Tetraconazole	2	94.6	90.8	90.6	89.9	93.8	91.9	2.3	83.4	85.1	96.4	89.1	92.6	89.3	6.0
Thiabendazole	5	94.5	98.3	91.8	87.5	95.2	93.5	4.3	81.7	84.1	85.0	96.3	93.7	88.1	7.3
Thiacloprid	5	91.9	85.0	89.7	94.2	86.5	89.4	4.2	90.1	81.5	93.0	89.8	92.9	89.5	5.2
Thiamethoxam	5	83.2	111	92.8	94.0	87.6	93.6	11	78.1	80.9	79.1	93.3	94.2	85.1	9.3
Thidiazuron	7	82.7	81.4	78.5	75.7	87.6	81.2	5.5	68.3	80.3	78.9	80.9	82.4	78.2	7.2
Thifensulfuron-methyl	4	80.7	91.6	87.4	77.7	89.9	85.4	7.0	94.9	83.4	90.9	83.8	77.0	86.0	8.1
Thiodicarb	5	82.6	73.9	73.0	77.8	83.4	78.2	6.1	74.6	81.3	77.0	82.0	80.9	79.1	4.0
Thiofanox	5	80.2	75.5	92.9	79.6	88.0	83.2	8.4	70.4	80.9	69.1	77.6	79.9	75.6	7.2
Tolylfluanid	3	63.9	60.4	60.8	57.9	56.9	60.0	4.6	64.5	69.5	58.8	73.4	70.8	67.4	8.6
Tralkoxydim	1	88.2	95.9	82.9	92.5	92.8	90.4	5.6	84.0	86.7	84.7	92.7	96.0	88.8	5.9
Triadimefon	3	88.8	92.2	96.9	105	97.9	96.1	6.3	87.6	82.9	85.9	85.8	93.8	87.2	4.6
Triadimenol	6	82.8	95.6	86.8	90.1	91.4	89.3	5.4	90.4	80.6	85.8	98.4	92.2	89.5	7.5

Table 3. Trueness (% Recovery) and Precision (% RSD) Data Obtained for Pesticides Included in the Agilent LC/MS Mixes 1–8 in Spinach Matrix at the Fortification Levels of 0.01 and 0.02 mg/kg Evaluated in Five Replicates (A–E) (continued)

		Fortification level 0.01 mg/kg							Fortification level 0.02 mg/kg						
		Trueness (%)						RSD	Trueness (%)						RSD
Analyte	Mix	A	B	C	D	E	Mean	(%)	A	B	C	D	E	Mean	(%)
Triasulfuron	4	89.9	94.3	92.3	90.4	90.9	91.6	1.9	82.6	82.6	84.5	90.5	81.6	84.3	4.3
Triazophos	3	99.1	86.2	96.7	102	91.3	95.0	6.6	85.8	72.1	83.8	91.8	94.5	85.6	10
Tribenuron-methyl	4	85.3	84.9	76.8	74.3	75.0	79.3	6.8	75.3	77.6	74.2	83.8	82.5	78.7	5.5
Trichlorfon (Metrifonate)	6	98.9	102	103	98.1	99.3	100	2.1	82.6	90.7	85.9	87.4	85.1	86.4	3.5
Tricyclazole	2	89.7	89.0	91.7	92.2	92.5	91.0	1.7	83.3	83.8	82.1	86.4	89.0	84.9	3.3
Trietazine	6	86.0	86.2	89.9	74.1	79.4	83.1	7.6	84.3	85.7	77.7	80.4	83.1	82.2	3.9
Trifloxystrobin	5	92.0	88.3	92.3	85.0	87.0	88.9	3.6	88.6	85.9	80.9	87.5	81.0	84.8	4.3
Triflumizole	3	85.0	83.0	83.4	88.2	87.4	85.4	2.7	82.3	80.9	81.4	89.6	89.1	84.7	5.1
Triflumuron	4	84.1	86.5	87.1	85.0	93.3	87.2	4.1	87.8	79.5	75.4	82.4	79.5	80.9	5.7
Trimethacarb	6	85.1	79.4	91.7	82.7	87.2	85.2	5.5	84.6	87.6	93.4	83.6	85.6	87.0	4.5
Triticonazole	2	91.8	94.3	89.2	85.1	92.1	90.5	3.9	90.2	82.1	80.3	83.6	93.2	85.9	6.5
Uniconazole	2	78.5	88.7	90.3	88.1	95.1	88.1	6.9	83.8	79.5	77.3	85.9	85.6	82.4	4.7
Vamidothion	2	99.5	88.1	81.7	87.6	89.8	89.3	7.2	72.9	83.1	81.0	78.4	89.3	80.9	7.5
Zoxamide	6	102	102	106	95.0	109	103	5.2	90.2	87.8	86.2	85.7	90.5	88.1	2.5

Table 4. Trueness (% Recovery), Precision (% RSD), and Measurement Uncertainty (% MU) Data Obtained for Pesticides Included in the Agilent LC/MS Mixes 1–8 in Wheat Flour Matrix at the Fortification Level of 0.01 mg/kg Evaluated in Five Replicates (A–E) by Two Different Analysts on Two Different Days

		Fortification level 0.01 mg/kg (Analyst 1)							Fortification level 0.01 mg/kg (Analyst 2)							Overall		
		Trueness (%)						RSD	Trueness (%)						RSD	Trueness	RSD	MU
Analyte	Mix	A	B	C	D	E	Mean	(%)	A	B	C	D	E	Mean	(%)	(%)	(%)	(%)
Abamectin - Avermectin B1a	7	177	194	145	123	122	152	21	82.7	98.4	58.7	63.2	117	84.0	29	118	38	85
Acephate	1	116	106	101	103	99.4	105	6.4	86.8	83.5	87.6	89.0	87.8	86.9	2.4	96.0	11	25
Acetamiprid	5	94.4	110	103	97.7	104	102	5.8	112	112	105	100	100	106	5.6	104	5.7	13
Alanycarb	5	114	117	123	111	110	115	4.4	121	109	98.7	84.7	113	105	13	110	10	23
Aldicarb	5	116	118	107	114	110	113	3.8	92.7	93.1	86.1	94.2	80.9	89.4	6.4	101	13	30
Amidosulfuron	4	104	104	103	98.7	100	102	2.4	101	88.0	82.5	91.3	101	92.8	8.8	97.5	7.7	17
Aminocarb	4	127	115	118	112	112	117	5.2	81.9	72.9	85.6	83.9	86.7	82.2	6.7	99.6	19	43
Azaconazole	1	104	102	110	108	104	106	2.9	94.7	86.6	98.5	92.6	97.1	93.9	5.0	99.9	7.3	16
Azamethiphos	2	123	116	120	118	119	119	2.2	92.1	94.8	91.4	91.1	90.3	91.9	1.8	106	14	31
Azinphos-ethyl	1	109	110	120	109	127	115	7.1	104	90.9	107	110	98.0	102	7.5	109	9.3	21
Azinphos-methyl	1	112	115	110	101	110	110	4.7	99.4	93.5	96.0	102	89.6	96.2	5.2	103	8.4	19
Azoxystrobin	5	110	106	110	107	112	109	2.2	96.3	89.5	95.5	103	91.9	95.2	5.3	102	8.1	18
Beflubutamid	8	124	119	127	122	122	123	2.3	93.9	81.9	89.1	99.9	88.6	90.7	7.4	107	17	37
Benalaxyl	2	111	111	101	102	112	108	4.9	103	93.1	85.1	90.0	87.9	91.8	7.5	99.7	10	23
Benfuracarb	4	107	108	96.1	103	112	105	5.6	119	134	120	112	134	124	7.8	115	11	24
Benzoximate	7	122	123	123	112	118	120	3.8	101	105	106	97.6	96.9	101	4.1	110	9.5	21
Bifenazate	8	83.3	80.2	83.5	78.6	75.3	80.2	4.3	61.5	51.6	58.4	56.2	58.6	57.3	6.4	68.7	18	41
Bifenthrin	2	99.6	101	108	113	134	111	12	142	222	225	170	223	197	19	154	34	76

Table 4. Trueness (% Recovery), Precision (% RSD), and Measurement Uncertainty (% MU) Data Obtained for Pesticides Included in the Agilent LC/MS Mixes 1–8 in Wheat Flour Matrix at the Fortification Level of 0.01 mg/kg Evaluated in Five Replicates (A–E) by Two Different Analysts on Two Different Days (continued)

		Fortification level 0.01 mg/kg (Analyst 1)							Fortification level 0.01 mg/kg (Analyst 2)							Overall		
		Trueness (%)						RSD	Trueness (%)						RSD	Trueness	RSD	MU
Analyte	Mix	A	B	C	D	E	Mean	(%)	A	B	C	D	E	Mean	(%)	(%)	(%)	(%)
Bispyribac	7	106	97.7	99.2	97.4	98.6	99.7	3.4	86.7	83.1	68.4	80.2	89.8	81.6	10	90.7	12	28
Bitertanol	3	116	114	117	105	109	112	4.6	111	98.7	103	102	102	103	4.6	108	6.0	13
Boscalid	4	109	107	99.3	99.4	107	104	4.5	92.8	99.1	105	98.9	103	99.8	4.7	102	5.0	11
Bromuconazole (2 diastereoisomers)	2	128	116	120	122	108	119	6.3	91.1	99.4	87.0	86.7	96.4	92.1	6.1	105	15	32
Bupirimate	2	121	114	117	95.3	96.7	109	11	93.5	99.6	109	98.3	104	101	5.7	105	9.3	21
Buprofezin	1	108	110	110	104	107	108	2.6	90.5	93.7	95.9	95.8	95.2	94.2	2.4	101	7.5	17
Butocarboxim	4	119	121	120	117	120	119	1.2	99.2	95.5	95.3	95.3	102	97.5	3.2	108	11	24
Carbaryl	6	118	113	111	109	115	113	2.9	90.4	93.7	93.1	96.3	90.6	92.8	2.6	103	11	24
Carbendazim	5	99.1	92.3	92.8	89.1	96.4	93.9	4.1	82.9	87.3	83.2	84.2	78.8	83.3	3.7	88.6	7.3	16
Carbofuran	8	110	110	105	105	97.2	105	5.0	97.4	103	103	104	101	102	2.7	104	4.2	9.3
Carbosulfan	6	108	101	100	102	110	104	4.2	92.8	80.0	92.8	84.6	95.4	89.1	7.3	96.6	9.8	22
Carboxin	5	113	100	101	100	101	103	5.0	101	98.3	91.7	97.2	97.2	97.1	3.4	100	5.2	12
Carfentrazone-ethyl	4	105	94.8	98.4	99.1	93.3	98.0	4.4	94.4	90.3	89.1	83.2	89.8	89.4	4.5	93.7	6.4	14
Chlorantraniliprole	8	109	112	115	105	113	111	3.5	96.6	99.6	100	100	97.1	98.7	1.7	105	6.6	15
Chlorfenvinphos (E- and Z-isomers)	2	114	111	107	109	110	110	2.3	98.8	96.4	92.8	93.7	98.8	96.1	2.9	103	7.5	17
Chloridazon (Pyrazon)	4	97.5	105	100	98.1	105	101	3.6	97.1	88.0	86.8	89.6	83.4	89.0	5.7	95.0	8.0	18
Chlorotoluron (Chlortoluron)	7	121	117	121	113	117	118	3.0	91.5	90.6	94.6	88.8	97.2	92.5	3.6	105	13	29
Chloroxuron	7	113	102	106	104	107	106	4.0	100	95.9	97.0	96.6	100	98.0	2.1	102	5.4	12
Chlorpyrifos	2	107	109	106	101	98.8	104	4.2	91.6	84.8	94.7	82.4	84.4	87.6	6.1	95.9	10	23
Chlorpyrifos-methyl	2	111	122	104	114	114	113	5.7	104	119	110	123	112	114	6.6	113	5.8	13
Chlorsulfuron	4	122	122	120	110	119	119	4.2	90.9	89.8	88.4	85.5	88.7	88.6	2.3	104	16	35
Clethodim (E- and Z-isomers)	3	96.8	103	103	99.8	97.1	100	3.2	94.4	121	112	105	108	108	9.0	104	7.7	17
Clofentezine	4	119	111	120	114	112	115	3.6	89.2	84.1	100	109	100	96.6	10	106	11	25
Clomazone	8	119	112	115	107	114	114	3.8	99.1	102	101	93.1	102	99.4	3.7	107	7.9	18
Coumaphos	2	100	100	108	90.4	113	103	8.6	92.1	94.4	88.0	92.8	99.1	93.3	4.3	97.9	8.3	18
Cyazofamid	4	113	112	109	100	102	107	5.2	96.9	93.2	98.5	98.0	106	98.6	4.8	103	6.5	14
Cycloate	1	110	111	121	103	117	112	6.0	83.0	88.3	82.0	85.8	90.8	86.0	4.2	99.1	15	33
Cycluron	7	108	108	100	109	104	106	3.3	88.9	90.4	86.0	88.5	96.5	90.1	4.4	97.9	9.2	20
Cymiazole	1	112	107	115	112	102	110	4.6	100	101	100	101	95.8	99.6	2.1	105	6.2	14
Cymoxanil	4	122	121	123	116	103	117	7.1	92.3	98.5	82.9	84.7	91.4	90.0	7.0	104	15	34
Cyproconazole (2 diastereoisomers)	1	104	102	93.5	95.3	104	99.6	4.9	93.8	87.1	93.4	93.1	93.3	92.1	3.1	95.9	5.7	13
Cyprodinil	8	104	108	97.3	102	98.8	102	4.0	90.1	94.5	103	105	94.3	97.5	6.7	99.7	5.6	13
DEET (Diethyltoluamide)	4	113	114	115	104	109	111	4.1	88.6	97.6	106	105	96.7	98.7	7.1	105	8.1	18
Desmedipham	8	109	114	109	101	103	107	4.8	95.8	97.0	95.4	93.2	96.0	95.5	1.5	101	7.0	16
Diazinon	2	115	117	107	120	113	115	4.2	96.2	93.4	90.6	99.1	99.7	95.8	4.0	105	10	23
Dichlorvos	2	129	127	127	122	125	126	2.1	110	117	115	117	111	114	2.8	120	5.7	13
Diethofencarb	6	117	115	112	106	116	113	3.8	82.7	87.5	91.3	87.1	91.0	87.9	4.0	100	14	31
Difenoconazole (*cis*- and *trans*-)	3	109	105	113	105	112	109	3.4	86.7	85.9	88.7	93.9	98.3	90.7	5.8	99.8	11	24

Table 4. Trueness (% Recovery), Precision (% RSD), and Measurement Uncertainty (% MU) Data Obtained for Pesticides Included in the Agilent LC/MS Mixes 1–8 in Wheat Flour Matrix at the Fortification Level of 0.01 mg/kg Evaluated in Five Replicates (A–E) by Two Different Analysts on Two Different Days (continued)

		Fortification level 0.01 mg/kg (Analyst 1)							Fortification level 0.01 mg/kg (Analyst 2)							Overall		
		Trueness (%)						RSD	Trueness (%)						RSD	Trueness	RSD	MU
Analyte	Mix	A	B	C	D	E	Mean	(%)	A	B	C	D	E	Mean	(%)	(%)	(%)	(%)
Diflubenzuron	4	130	120	125	123	129	125	3.3	99.5	79.8	78.4	89.5	91.1	87.6	9.9	106	20	44
Diflufenican	1	97.2	102	98.7	98.5	105	100	3.0	93.9	94.1	96.1	101	93.2	95.7	3.3	97.9	3.8	8.5
Dimethachlor	1	119	114	113	112	112	114	2.4	98.9	98.8	94.1	95.9	104	98.3	3.9	106	8.3	18
Dimethoate	8	106	115	109	102	113	109	4.9	102	90.2	84.4	95.4	83.4	91.1	8.5	100	11	25
Dimethomorph (E- and Z-isomers)	5	86.8	90.7	88.1	90.7	91.7	89.6	2.3	98.7	93.3	102	116	85.7	99.2	11	94.4	9.7	22
Dimoxystrobin	1	113	122	106	107	121	114	6.4	91.2	88.9	103	105	90.8	95.8	7.9	105	11	25
Diniconazole	2	109	112	101	98.0	104	105	5.4	95.1	90.1	99.0	94.5	94.5	94.7	3.3	99.8	6.9	15
Dinotefuran	7	107	96.6	94.3	96.8	102	99.3	5.2	91.2	86.2	84.0	80.8	84.7	85.4	4.5	92.4	9.2	20
Dioxacarb	7	118	111	119	110	103	112	5.9	100	93.3	91.5	90.9	115	98.1	10	105	10	23
Disulfoton	1	104	113	114	104	109	109	4.1	42.9	61.8	64.1	61.5	44.0	54.8	19	81.8	36	80
Diuron	5	105	105	105	106	102	105	1.3	100	97.2	101	103	94.4	99.3	3.5	102	3.7	8.2
Epoxiconazole	2	108	106	111	100	103	105	3.8	94.7	95.3	95.1	95.9	99.2	96.1	1.9	101	5.7	13
Ethidimuron (Sulfadiazole)	8	108	93.2	105	86.7	96.6	97.9	8.8	110	114	130	91.0	107	111	13	104	12	28
Ethion	2	97.5	107	113	101	92.2	102	8.0	97.9	89.4	90.5	95.4	88.8	92.4	4.4	97.3	8.2	18
Ethirimol	4	113	102	110	104	112	108	4.5	93.9	99.5	95.6	107	93.1	97.8	5.8	103	7.2	16
Ethofumesate	4	108	102	114	106	113	109	4.6	104	104	105	107	105	105	1.1	107	3.6	8.1
Ethoprophos (Ethoprop)	2	119	114	108	106	113	112	4.7	103	93.5	99.3	98.3	110	101	6.1	106	7.5	17
Ethoxyquin	8	84.0	83.9	84.5	84.3	83.8	84.1	0.3	26.1	25.9	26.3	25.6	26.2	26.0	1.1	55.1	56	124
Etofenprox	3	117	113	113	109	143	119	11	87.9	124	119	77.6	122	106	20	113	16	36
Famoxadone	4	131	136	132	121	128	130	4.3	101	96.0	93.8	93.2	80.4	92.8	8.1	111	18	41
Fenamidone	5	115	105	102	98.8	116	108	7.1	104	94.3	91.6	107	92.5	98.0	7.4	103	8.4	19
Fenamiphos	1	116	115	101	97.9	102	106	7.9	96.5	101	103	91.0	99.9	98.2	4.7	102	7.5	17
Fenarimol	2	103	111	100	105	117	107	6.3	123	132	83.4	115	112	113	16	110	12	27
Fenazaquin	5	113	108	110	115	111	111	2.3	72.3	71.0	79.3	82.7	75.5	76.2	6.4	93.8	20	45
Fenbuconazole	2	115	109	109	100	108	108	5.0	99.5	91.7	88.5	97.2	97.7	94.9	4.9	102	8.4	19
Fenhexamid	3	101	108	96.3	104	98.8	102	4.6	97.2	92.0	83.6	83.8	109	93.1	11	97.4	9.2	20
Fenobucarb	5	103	106	105	94.8	94.0	101	5.7	95.5	98.3	88.4	90.2	95.1	93.5	4.4	97.0	6.2	14
Fenoxycarb	6	105	113	124	118	107	113	7.0	103	98.0	86.4	108	101	99.2	8.1	106	10	22
Fenpropidin	5	113	109	115	107	114	112	3.1	97.7	103	97.1	96.6	103	99.5	3.3	106	6.8	15
Fenpyroximate	5	108	111	114	120	107	112	4.9	82.7	76.0	80.7	80.2	77.3	79.4	3.4	95.6	18	41
Fenuron	7	115	110	118	105	112	112	4.5	101	102	88.7	98.9	106	99.5	6.6	106	8.2	18
Fipronil	4	119	119	126	87.7	95.2	109	15	97.5	64.3	79.1	69.0	80.9	78.2	16	93.8	23	52
Flazasulfuron	4	118	116	116	110	117	116	2.7	96.2	95.5	103	99.6	101	99.2	3.3	107	8.5	19
Flonicamid	6	126	123	115	101	127	118	9.0	94.2	92.0	93.5	91.2	83.0	90.8	5.0	105	16	35
Flubendiamide	7	114	112	101	102	121	110	7.6	96.4	133	164	153	103	130	23	120	19	43
Fludioxonil	2	114	96.4	98.0	101	102	102	6.9	104	103	100	84.8	105	99.5	8.5	101	7.5	17
Flufenacet	1	121	113	120	110	116	116	4.1	106	87.1	95.7	107	100	99.1	8.1	108	10	23
Flufenoxuron	4	110	116	116	109	113	113	3.0	80.8	77.8	70.9	85.9	81.5	79.4	7.0	96.1	19	42
Flumetsulam	8	107	91.3	89.5	98.4	90.8	95.5	7.8	82.7	92.7	95.0	112	81.7	92.8	13	94.2	10	23

Table 4. Trueness (% Recovery), Precision (% RSD), and Measurement Uncertainty (% MU) Data Obtained for Pesticides Included in the Agilent LC/MS Mixes 1–8 in Wheat Flour Matrix at the Fortification Level of 0.01 mg/kg Evaluated in Five Replicates (A–E) by Two Different Analysts on Two Different Days (continued)

		Fortification level 0.01 mg/kg (Analyst 1)							Fortification level 0.01 mg/kg (Analyst 2)							Overall		
		Trueness (%)						RSD	Trueness (%)						RSD	Trueness	RSD	MU
Analyte	Mix	A	B	C	D	E	Mean	(%)	A	B	C	D	E	Mean	(%)	(%)	(%)	(%)
Flumioxazin	6	113	123	125	119	127	121	4.5	87.3	100	65.3	65.7	82.4	80.2	19	101	24	53
Fluometuron	8	109	109	107	97.4	106	106	4.6	95.3	100	99.4	94.8	93.4	96.6	3.2	101	6.0	13
Fluopicolide	1	99.9	118	98.4	106	109	106	7.5	90.7	103	106	95.2	88.8	96.7	7.7	101	8.7	19
Fluoxastrobin	8	98.5	127	107	100	106	108	11	107	97.0	114	113	107	108	6.4	108	8.3	19
Fluquinconazole	2	116	118	116	111	115	115	2.2	94.2	94.5	104	75.4	91.5	92.0	11	104	14	31
Flusilazole	2	116	107	112	107	111	111	3.6	99.4	90.0	92.3	97.2	96.2	95.0	4.0	103	8.9	20
Flutriafol	8	107	109	104	99.9	103	105	3.5	95.5	97.7	99.9	93.1	96.6	96.5	2.6	101	5.2	12
Foramsulfuron	3	130	129	125	118	125	125	3.8	93.6	99.7	94.0	82.8	91.0	92.2	6.7	109	17	37
Forchlorfenuron	7	106	102	97.9	96.4	95.2	99.4	4.3	91.6	90.8	89.6	89.9	90.6	90.5	0.9	95.0	5.8	13
Fosthiazate (sum of isomers)	1	114	113	113	109	114	113	1.8	96.3	93.6	93.0	107	90.1	95.9	6.6	104	9.5	21
Fuberidazole	4	106	99.7	99.4	91.2	101	99.5	5.3	99.2	91.9	100	104	99.1	99.0	4.5	99.2	4.6	10
Furalaxyl	7	117	120	119	118	120	119	1.0	93.5	90.6	96.8	93.2	99.4	94.7	3.6	107	12	27
Furathiocarb	6	115	109	111	118	108	112	3.8	80.7	75.6	92.6	75.6	102	85.4	14	98.8	17	37
Halofenozide	8	112	116	113	111	105	112	3.5	97.1	90.5	92.5	89.0	94.5	92.7	3.5	102	10	23
Halosulfuron-methyl	8	104	98.9	103	102	109	103	3.5	94.8	97.5	93.4	93.6	98.0	95.5	2.3	99.4	5.0	11
Hexaconazole	2	107	108	107	103	99.3	105	3.5	89.4	96.4	102	89.9	91.3	93.9	5.8	99.4	7.4	16
Hexaflumuron	7	105	101	104	98.8	106	103	2.9	86.2	87.6	92.0	93.0	101	91.9	6.3	97.3	7.3	16
Hexythiazox	4	103	106	109	105	108	106	2.3	82.6	77.1	75.4	76.6	82.3	78.8	4.3	92.5	16	36
Hydramethylnon	7	122	124	112	105	106	114	7.6	103	99.5	99.0	106	104	102	3.0	108	8.1	18
Imazalil	2	108	121	113	114	116	114	4.1	99.3	102	105	103	85.6	99.1	7.9	107	9.5	21
Imidacloprid	5	106	113	92.9	88.5	88.7	97.9	11	101	89.6	97.9	88.6	91.3	93.7	5.8	95.8	9.0	20
Indoxacarb	3	115	118	115	105	114	113	4.5	96.8	97.1	104	96.5	99.2	98.7	3.0	106	8.1	18
Ipconazole	2	117	108	110	110	111	111	2.9	97.8	92.0	93.3	91.2	101	95.1	4.5	103	9.0	20
Iprovalicarb	5	120	111	113	109	108	112	4.1	97.8	96.5	95.1	98.2	105	98.5	3.9	105	7.8	17
Isofenphos-methyl	1	118	119	106	103	109	111	6.4	90.8	87.7	103	103	99.3	96.6	7.2	104	9.7	22
Isoprothiolane	1	117	104	105	92.7	109	105	8.2	94.7	96.2	98.1	96.5	94.3	96.0	1.6	101	7.7	17
Isoxaben	4	131	119	125	111	106	118	8.3	98.6	93.6	94.5	104	86.8	95.5	6.6	107	13	30
Isoxaflutole	3	114	105	112	106	109	109	3.6	92.9	92.6	101	98.2	105	98.0	5.5	104	7.2	16
Ivermectin B1a	7	118	123	129	124	134	126	5.0	120	114	106	130	113	117	7.7	121	7.2	16
Kresoxim-methyl	4	108	121	94.5	104	105	107	8.9	91.1	104	117	84.8	106	101	13	104	11	24
Lenacil	1	102	104	113	105	96.3	104	5.7	105	97.2	102	94.5	93.5	98.5	5.1	101	5.8	13
Linuron	4	120	98.4	102	105	108	107	7.5	92.6	96.0	96.1	91.4	101	95.3	3.8	101	8.3	18
Lufenuron	4	103	105	117	109	111	109	5.1	90.1	96.8	100	88.0	107	96.4	8.1	103	9.0	20
Malaoxon	3	112	112	110	107	105	109	3.0	91.8	92.8	95.7	102	97.3	95.9	4.1	103	7.6	17
Malathion	3	103	105	105	104	103	104	1.1	91.3	90.5	106	103	91.5	96.3	7.6	100	6.4	14
Mandipropamid	4	124	110	115	115	115	116	4.2	110	109	115	109	102	109	4.0	112	5.0	11
Mecarbam	3	105	103	95.4	96.8	91.8	98.4	5.5	96.4	100	97.4	97.6	84.4	95.2	6.5	96.8	5.9	13
Mepanipyrim	3	103	103	103	105	104	104	1.0	94.9	93.6	88.1	94.8	87.6	91.8	3.9	97.7	6.9	15
Mesosulfuron-methyl	6	111	108	112	119	127	115	6.5	92.9	121	112	121	113	112	10	114	8.2	18

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		Fortification level 0.01 mg/kg (Analyst 1)							Fortification level 0.01 mg/kg (Analyst 2)							Overall		
		Trueness (%)						RSD	Trueness (%)						RSD	Trueness	RSD	MU
Analyte	Mix	A	B	C	D	E	Mean	(%)	A	B	C	D	E	Mean	(%)	(%)	(%)	(%)
Metaflumizone	4	108	123	128	122	104	117	8.8	96.2	106	126	119	115	113	10	115	9.3	21
Metalaxyl	3	111	109	110	104	111	109	2.7	96.6	96.4	105	98.2	103	99.7	3.8	104	5.7	13
Metamitron	4	102	99.3	89.2	94.2	109	98.6	7.5	81.0	82.2	87.6	84.1	92.7	85.5	5.6	92.1	9.8	22
Metazachlor	3	111	108	105	97.5	106	106	4.8	91.7	94.8	100	102	95.8	96.8	4.2	101	6.2	14
Metconazole	2	114	111	106	105	114	110	3.8	96.1	87.8	90.5	95.7	103	94.6	6.2	102	9.2	20
Methabenzthiazuron	5	111	107	98.9	100	101	104	5.0	95.1	92.7	91.1	97.8	90.6	93.5	3.2	98.6	6.8	15
Methamidophos	1	95.9	97.8	94.6	92.0	101	96.3	3.6	77.5	77.7	79.5	80.7	79.4	79.0	1.7	87.6	11	24
Methidathion	3	108	109	107	105	107	107	1.6	83.5	85.2	91.6	98.1	95.2	90.7	6.9	99.0	9.8	22
Methiocarb	7	110	110	106	108	117	110	3.7	105	92.5	96.9	96.6	92.9	96.8	5.2	103	8.0	18
Methomyl	5	126	125	117	107	126	120	6.9	90.1	81.8	77.8	102	78.2	86.0	12	103	19	43
Methoprotryne	7	114	109	110	101	111	109	4.4	93.8	95.7	99.1	94.2	95.7	95.7	2.2	102	7.6	17
Methoxyfenozide	5	122	120	114	113	111	116	4.2	109	114	126	136	116	120	9.0	118	7.0	16
Metobromuron	8	103	105	104	104	102	104	1.2	96.5	89.2	102	89.9	90.4	93.6	5.9	98.5	6.5	15
Metolachlor	3	101	114	116	99.1	111	108	7.2	90.9	109	119	106	91.1	103	12	106	9.4	21
Metrafenone	4	108	109	107	101	101	105	3.6	99.9	103	96.8	102	103	101	2.6	103	3.7	8.2
Metribuzin	4	104	97.0	103	104	112	104	5.2	95.6	96.7	90.0	91.1	96.5	94.0	3.4	99.1	6.9	15
Metsulfuron-methyl	4	112	105	104	107	108	108	2.7	91.7	90.5	87.1	87.8	82.5	87.9	4.1	97.7	11	25
Mevinphos (E- and Z-isomers)	3	114	116	118	103	112	113	5.2	104	85.4	98.4	103	106	99.3	8.3	106	9.2	21
Mexacarbate	7	115	121	114	108	114	114	3.8	92.5	89.7	102	105	91.2	96.2	7.3	105	11	24
Molinate	3	107	108	99.9	106	99.2	104	4.0	96.2	84.6	104	113	107	101	11	103	7.8	17
Monocrotophos	4	132	128	122	121	113	123	5.8	96.6	88.6	91.9	93.5	92.9	92.7	3.1	108	16	35
Moxidectin	7	124	122	122	131	172	134	16	79.8	127	90.8	56.1	104	91.6	29	113	28	63
Myclobutanil	1	112	94.2	93.6	96.0	99.8	99.1	7.6	111	101	96.1	80.4	104	98.6	12	98.8	9.3	21
Nicosulfuron	4	108	101	103	99.0	119	106	7.5	77.0	83.1	91.9	85.0	83.5	84.1	6.3	95.0	14	31
Nitenpyram	7	107	109	116	97.9	104	107	6.2	96.6	77.7	89.2	86.7	87.7	87.6	7.7	97.2	12	27
Novaluron	4	104	115	104	105	103	106	4.9	94.1	83.2	95.7	92.0	92.1	91.4	5.3	98.8	9.2	21
Omethoate	6	113	103	103	92.0	101	102	7.4	84.0	82.7	78.6	93.7	79.2	83.6	7.3	93.0	13	28
Oxadiazon	3	104	98.1	101	94.4	103	100	4.0	96.3	83.7	91.3	102	96.1	93.8	7.2	97.0	6.4	14
Oxadixyl	3	111	118	113	110	106	112	3.9	92.4	85.1	93.0	93.9	93.5	91.6	4.0	102	11	25
Oxamyl	5	119	114	115	102	119	114	6.2	83.0	79.9	84.1	84.7	88.7	84.1	3.8	98.9	17	37
Oxasulfuron	4	105	107	105	101	107	105	2.2	104	109	108	110	108	108	2.0	107	2.4	5.4
Paclobutrazol	3	116	120	105	105	108	111	6.1	99.6	99.4	95.1	95.8	95.6	97.1	2.3	104	8.3	18
Penconazole	3	121	107	111	108	109	111	5.4	95.7	93.1	92.4	97.5	93.0	94.3	2.3	103	9.6	21
Pencycuron	6	102	92.1	99.9	94.6	93.0	96.4	4.6	90.9	94.1	83.4	94.8	92.0	91.0	5.0	93.7	5.5	12
Pendimethalin	3	96.9	120	115	114	106	110	8.2	85.3	87.9	116	103	106	99.6	13	105	11	25
Phenmedipham	4	126	112	113	117	111	116	5.1	104	106	104	108	98.1	104	3.6	110	7.1	16
Phenthoate	3	90.6	99.5	95.1	88.0	103	95.2	6.4	103	118	105	107	107	108	5.5	102	8.6	19
Phosalone	3	116	103	107	100	108	107	5.5	96.1	93.7	97.3	99.5	92.0	95.7	3.1	101	7.2	16

Table 4. Trueness (% Recovery), Precision (% RSD), and Measurement Uncertainty (% MU) Data Obtained for Pesticides Included in the Agilent LC/MS Mixes 1–8 in Wheat Flour Matrix at the Fortification Level of 0.01 mg/kg Evaluated in Five Replicates (A–E) by Two Different Analysts on Two Different Days (continued)

		Fortification level 0.01 mg/kg (Analyst 1)							Fortification level 0.01 mg/kg (Analyst 2)							Overall		
		Trueness (%)						RSD	Trueness (%)						RSD	Trueness	RSD	MU
Analyte	Mix	A	B	C	D	E	Mean	(%)	A	B	C	D	E	Mean	(%)	(%)	(%)	(%)
Phosmet	6	118	111	108	115	108	112	3.9	94.2	103	100	103	98.4	99.7	3.7	106	7.0	16
Phosphamidon (E- and Z-isomers)	3	100	103	104	110	116	107	6.0	95.0	89.9	93.8	80.3	96.2	91.0	7.1	98.9	10	23
Phoxim	4	109	109	115	102	103	108	4.8	102	102	98.5	92.0	97.4	98.3	4.1	103	6.4	14
Picolinafen	3	111	105	112	107	99.9	107	4.5	90.3	83.9	94.4	95.7	90.9	91.0	5.1	99.1	9.7	22
Picoxystrobin	5	122	110	123	104	115	114	7.0	110	88.7	95.6	97.1	97.4	97.8	8.0	106	11	24
Pirimicarb	3	102	101	101	103	103	102	1.0	101	90.6	93.2	94.5	98.1	95.5	4.4	98.8	4.5	10
Pirimiphos-methyl	3	115	100	98.3	104	109	105	6.5	89.6	92.8	87.3	99.8	106	95.2	8.2	100	8.7	19
Prochloraz	1	119	103	111	102	108	109	6.1	90.8	91.3	99.1	101	91.1	94.6	5.2	102	9.0	20
Profenofos	3	110	107	121	106	104	110	6.0	99.7	113	99.8	111	97.3	104	6.9	107	6.7	15
Promecarb	7	107	101	105	96.4	107	103	4.5	101	96.8	96.8	102	96.3	98.6	2.8	101	4.3	9.6
Prometon	4	106	105	97.2	110	106	105	4.5	89.2	94.5	99.1	97.0	93.2	94.6	4.0	99.7	6.8	15
Propamocarb	5	111	104	106	105	110	107	2.7	103	100	98.6	101	108	102	3.5	105	3.9	8.7
Propaquizafop	4	105	108	106	105	105	106	1.2	102	91.2	93.2	100	99.1	97.1	4.7	101	5.5	12
Propargite	4	115	118	116	118	117	117	1.1	86.2	81.6	84.9	82.4	83.0	83.6	2.2	100	18	39
Propetamphos	3	110	115	112	113	111	112	1.8	91.1	86.5	96.0	85.7	95.3	90.9	5.2	102	12	26
Propiconazole (sum of isomers)	2	111	116	111	106	116	112	3.8	98.6	85.7	91.3	89.1	89.1	90.8	5.3	101	12	26
Propoxur	6	108	106	101	103	103	104	2.9	100	87.0	90.9	92.0	95.0	93.0	5.2	98.5	7.1	16
Propyzamide (Pronamide)	3	112	110	108	113	116	112	2.5	97.0	91.3	107	101	88.4	97.0	7.7	104	9.0	20
Proquinazid	1	103	108	114	105	105	107	4.2	64.6	64.0	66.8	68.8	67.8	66.4	3.1	86.8	25	56
Prosulfocarb	4	102	105	101	105	101	103	2.2	88.8	90.1	95.5	99.6	89.4	92.7	5.1	97.7	6.5	15
Pymetrozine	7	109	83.2	102	98.7	99.7	98.5	9.6	106	75.7	88.5	78.6	82.6	86.2	14	92.4	13	29
Pyracarbolid	7	107	107	104	105	101	105	2.3	92.0	87.7	93.9	97.2	87.6	91.7	4.5	98.2	7.7	17
Pyraclostrobin	5	109	106	110	100	104	106	3.6	107	97.2	97.2	103	109	103	5.4	104	4.6	10
Pyridaben	5	125	129	127	119	118	124	4.0	69.1	69.9	71.5	73.4	76.7	72.1	4.2	97.8	28	62
Pyridate	5	119	117	112	109	109	113	3.9	55.8	49.6	52.5	50.6	56.0	52.9	5.5	83.1	39	86
Pyrimethanil	6	115	114	115	108	112	113	2.5	85.5	91.6	91.5	94.5	90.6	90.7	3.6	102	12	26
Pyriproxyfen	5	108	108	109	102	105	106	2.8	93.1	91.8	89.7	95.0	94.4	92.8	2.3	99.7	7.6	17
Quinalphos	3	120	107	112	106	104	110	5.9	81.4	99.8	96.2	99.2	87.3	92.8	8.7	101	11	25
Quinmerac	7	69.6	66.3	72.0	80.2	69.0	71.4	7.5	38.9	43.5	34.1	39.8	38.9	39.0	8.5	55.2	32	71
Quinoclamine	4	104	98.2	107	105	105	104	3.2	84.4	91.2	96.7	94.3	92.3	91.8	5.1	97.7	7.5	17
Quinoxyfen	3	115	115	111	107	108	111	3.3	93.4	96.0	93.5	96.1	103	96.4	4.0	104	8.2	18
Rimsulfuron	4	104	94.9	102	102	101	101	3.6	83.7	82.7	88.7	88.3	98.7	88.4	7.2	94.7	8.7	19
Rotenone	7	106	113	105	103	111	108	4.1	93.6	88.1	98.6	94.1	94.3	93.8	4.0	101	8.2	18
Secbumeton	7	115	114	103	106	96.7	107	7.3	97.9	88.9	97.2	104	95.5	96.7	5.6	102	8.2	18
Silthiofam	4	116	104	118	110	119	113	5.6	94.1	108	99.7	104	94.2	99.9	5.9	107	8.5	19
Spinosad - Spinosyn A	7	124	120	122	112	122	120	3.9	93.6	109	105	107	109	105	6.2	112	8.5	19
Spinosad - Spinosyn D	7	123	126	119	120	122	122	2.4	104	104	101	106	98.8	103	2.7	112	9.4	21
Spirodiclofen	1	119	87.3	112	117	112	109	12	70.9	75.2	72.1	76.5	70.4	73.0	3.7	91.2	23	52

Table 4. Trueness (% Recovery), Precision (% RSD), and Measurement Uncertainty (% MU) Data Obtained for Pesticides Included in the Agilent LC/MS Mixes 1–8 in Wheat Flour Matrix at the Fortification Level of 0.01 mg/kg Evaluated in Five Replicates (A–E) by Two Different Analysts on Two Different Days (continued)

		Fortification level 0.01 mg/kg (Analyst 1)							Fortification level 0.01 mg/kg (Analyst 2)							Overall		
		Trueness (%)						RSD	Trueness (%)						RSD	Trueness	RSD	MU
Analyte	Mix	A	B	C	D	E	Mean	(%)	A	B	C	D	E	Mean	(%)	(%)	(%)	(%)
Spiromesifen	6	119	119	114	111	113	115	3.0	72.4	65.2	68.9	70.2	77.6	70.8	6.5	92.9	25	57
Spirotetramat	6	141	128	135	119	118	128	7.6	123	97.1	102	102	103	106	9.5	117	13	29
Spiroxamine (2 diastereoisomers)	1	106	124	122	113	124	118	7.0	106	101	89.0	98.3	102	99.3	6.4	109	11	25
Sulfentrazone	6	122	122	127	111	123	121	4.9	86.6	96.2	105	90.9	93.0	94.4	7.4	108	14	32
Tebuconazole	2	104	98.3	98.7	103	102	101	2.6	104	105	97.7	103	100	102	2.7	102	2.5	5.6
Tebufenozide	5	139	115	122	98.4	105	116	14	112	101	93.8	97.7	80.1	97.0	12	107	15	34
Tebufenpyrad	3	105	103	101	104	107	104	2.0	95.9	98.9	93.2	94.1	92.9	95.0	2.6	99.4	5.2	12
Tebuthiuron	7	108	102	99.4	98.4	107	103	4.3	96.5	92.0	96.0	93.0	97.9	95.1	2.6	99.1	5.5	12
Teflubenzuron	4	110	108	113	86.2	115	106	11	94.0	84.1	85.0	80.6	102	89.1	9.8	97.8	14	30
Temephos	7	94.5	96.9	105	102	109	101	5.8	93.1	101	103	91.3	91.9	96.0	5.6	98.7	6.1	14
Tepraloxydim (E- and Z-isomers)	3	106	106	107	99.7	101	104	3.1	114	121	124	122	114	119	4.0	111	8.0	18
Terbufos	3	108	105	108	105	98.0	105	3.9	99.5	88.2	108	115	88.7	99.9	12	102	8.5	19
Tetraconazole	2	118	123	114	112	120	118	3.8	88.6	99.5	104	104	99.4	99.1	6.3	108	10	23
Thiabendazole	5	107	88.6	89.2	94.6	95.1	94.8	7.7	80.6	84.0	88.4	82.3	79.6	83.0	4.2	88.9	9.3	21
Thiacloprid	5	112	105	117	103	112	110	5.1	108	97.5	89.5	99.8	97.7	98.4	6.5	104	8.0	18
Thiamethoxam	5	86.8	109	103	98.7	111	102	9.5	92.1	101	109	92.2	86.2	96.1	9.3	98.9	9.4	21
Thidiazuron	7	99.5	102	95.8	90.7	103	98.2	5.1	86.5	87.9	78.2	88.1	89.0	85.9	5.2	92.0	8.5	19
Thifensulfuron-methyl	4	98.9	100	92.3	91.4	97.6	96.1	4.2	98.2	80.6	86.4	80.9	90.1	87.2	8.4	91.7	8.0	18
Thiodicarb	5	103	106	107	105	107	106	1.7	87.4	92.3	75.0	81.5	102	87.7	12	96.7	12	27
Thiofanox	5	130	120	114	122	118	121	4.9	88.3	107	128	123	101	109	15	115	11	25
Tolylfluanid	3	94.3	94.4	97.4	93.3	96.8	95.3	1.9	74.8	71.9	80.7	72.6	70.6	74.1	5.4	84.7	14	30
Tralkoxydim	1	106	111	109	102	103	106	3.5	80.1	81.1	83.3	82.4	81.7	81.7	1.5	93.9	14	31
Triadimefon	3	109	116	113	91.1	109	108	9.0	99.0	97.1	96.9	106	105	101	4.4	104	7.6	17
Triadimenol	6	106	97.3	103	103	102	102	3.2	106	97.4	98.3	94.9	100	99.3	4.1	101	3.8	8.5
Triasulfuron	4	106	105	105	111	114	108	3.9	94.7	95.6	87.6	89.7	97.8	93.1	4.6	100	8.7	19
Triazophos	3	124	112	123	118	109	117	5.7	102	84.3	87.3	88.8	88.1	90.2	7.8	104	15	34
Tribenuron-methyl	4	101	106	102	97.9	98.5	101	3.1	76.5	81.1	70.0	72.0	74.8	74.9	5.7	88.0	16	36
Trichlorfon (Metrifonate)	6	109	109	106	109	106	108	1.6	95.4	95.8	92.9	91.1	98.0	94.7	2.8	101	7.2	16
Tricyclazole	2	102	105	95.8	102	99.8	101	3.2	99.7	94.6	91.8	99.1	85.1	94.0	6.4	97.3	5.9	13
Trietazine	6	112	105	107	102	115	108	4.8	91.2	87.5	94.3	86.6	96.2	91.2	4.6	99.7	10	22
Trifloxystrobin	5	119	118	108	99.4	102	109	8.2	96.1	97.0	90.8	94.2	98.5	95.3	3.1	102	9.5	21
Triflumizole	3	110	116	107	103	112	110	4.3	99.5	98.4	92.5	96.9	100	97.4	3.1	104	7.2	16
Triflumuron	4	105	107	105	102	104	105	1.7	104	99.8	91.1	87.9	97.3	96.1	6.9	100	6.4	14
Trimethacarb	6	111	101	99.5	102	99.7	103	4.7	100	92.6	102	97.0	90.4	96.4	5.0	99.5	5.6	13
Triticonazole	2	106	98.4	113	109	112	108	5.4	102	93.3	104	104	88.5	98.3	7.2	103	7.6	17
Uniconazole	2	112	115	109	110	98.9	109	5.5	99.6	99.4	95.1	95.8	95.6	97.1	2.3	103	7.3	16
Vamidothion	2	92.4	106	105	102	106	102	5.5	103	95.9	110	103	98.3	102	5.1	102	5.0	11
Zoxamide	6	101	102	104	101	106	103	2.3	97.2	85.0	101	97.9	90.3	94.3	6.9	98.4	6.5	14

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Table 5. Trueness (% Recovery), Precision (% RSD), and Measurement Uncertainty (% MU) Data Obtained for Oesticides Included in the Agilent LC/MS Mixes 1–8 in Wheat Flour Matrix at the Fortification Level of 0.02 mg/kg Evaluated in Five Replicates (A–E) by Two Different Analysts on Two Different Days

		Fortification level 0.02 mg/kg (Analyst 1)							Fortification level 0.02 mg/kg (Analyst 2)							Overall		
		Trueness (%)						RSD	Trueness (%)						RSD	Trueness	RSD	MU
Analyte	Mix	A	B	C	D	E	Mean	(%)	A	B	C	D	E	Mean	(%)	(%)	(%)	(%)
Abamectin - Avermectin B1a	7	104	82.6	90.0	123	117	103	17	57.6	70.1	63.2	71.0	63.7	**65.1**	8.4	84.3	**28**	62
Acephate	1	89.4	85.5	91.2	86.9	84.3	87.5	3.2	73.2	75.7	80.9	76.1	75.4	76.3	3.7	81.9	7.9	18
Acetamiprid	5	92.1	100	115	97.0	98.6	101	8.5	118	92.9	104	110	108	106	8.7	103	8.6	19
Alanycarb	5	104	113	103	108	108	107	3.7	147	150	91	108	101	119	**23**	113	17	38
Aldicarb	5	99.7	95.7	101	92.7	98.6	97.5	3.4	106	89.8	89.9	103	88.8	95.3	8.5	96.4	6.2	14
Amidosulfuron	4	90.9	92.2	91.3	87.2	96.8	91.7	3.7	108	98.2	87.3	93.2	86.6	94.6	9.3	93.2	7.0	16
Aminocarb	4	91.8	101	97.1	101	101	98.4	4.1	89.4	74.3	98.6	95.3	89.4	89.4	10	93.9	8.8	20
Azaconazole	1	91.2	94.7	98.0	100	95.7	96.0	3.5	95.1	85.9	93.4	91.0	102	93.4	6.2	94.7	4.9	11
Azamethiphos	2	99.0	102	98.2	99.8	97.5	99.3	1.8	94.8	90.9	92.5	92.6	89.7	92.1	2.1	95.7	4.4	9.7
Azinphos-ethyl	1	110	116	113	104	100	109	5.9	97.6	92.4	104	97.0	102	98.6	4.6	104	7.2	16
Azinphos-methyl	1	90.2	90.0	110	106	110	101	10	105	90.3	92.9	94.4	80.2	92.6	9.8	97.0	11	24
Azoxystrobin	5	94.0	95.4	96.7	105	105	99.1	5.3	92.0	93.9	93.3	102	87.6	93.8	5.6	96.4	5.9	13
Beflubutamid	8	92.6	92.9	101	97.0	101	96.8	4.2	94.2	87.9	87.6	89.3	97.5	91.3	4.8	94.1	5.2	12
Benalaxyl	2	90.2	103	100	96.1	90.9	96.1	5.9	88.8	86.3	87.2	85.3	97.0	88.9	5.3	92.5	6.7	15
Benfuracarb	4	96.9	98.8	92.5	102.0	105	99.1	5.0	151	149	141	138	136	**143**	4.7	**121**	20	44
Benzoximate	7	115	108	106	100	106	107	5.2	103	92.5	98.2	103	96.5	98.6	4.4	103	6.3	14
Bifenazate	8	77.2	76.5	77.0	69.7	82.3	76.5	5.9	70.6	60.1	61.7	67.2	81.7	**68.3**	13	72.4	11	24
Bifenthrin	2	109	81.9	117	75.6	91.5	95.0	19	164	158	168	207	255	**190**	**22**	**143**	**41**	91
Bispyribac	7	92.9	88.2	96.0	96.7	96.6	94.1	3.8	89.9	81.7	72.7	81.1	71.0	79.3	9.6	86.7	11	25
Bitertanol	3	102	101	94.6	100	104	101	3.6	110	82.1	95.8	95.6	98.1	96.4	10	98.4	7.6	17
Boscalid	4	91.2	99.2	96.4	91.0	88.1	93.2	4.8	87.2	97.7	105	91.1	103	96.7	7.7	94.9	6.4	14
Bromuconazole (2 diastereoisomers)	2	94.7	105	112	95.2	106	102	7.2	86.7	86.7	89.7	97.7	90.1	90.2	5.0	96.3	9.0	20
Bupirimate	2	98.3	103	105	106	101	103	2.9	93.5	91.0	104	96.3	105	97.9	6.4	100	5.2	12
Buprofezin	1	95.7	93.9	102	99.7	96.7	97.7	3.5	87.2	95.0	96.0	91.0	91.8	92.2	3.8	94.9	4.6	10
Butocarboxim	4	90.7	104	96.7	96.8	98.9	97.4	4.8	103	101	94.8	96.2	95.0	98.1	4.0	97.7	4.2	9.3
Carbaryl	6	98.5	102	99.8	98.5	103	100	1.9	99.7	92.3	93.4	94.4	97.0	95.4	3.1	97.8	3.6	8.0
Carbendazim	5	87.7	92.1	102	93.4	91.4	93.3	5.6	77.4	68.1	61.7	65.4	66.1	**67.7**	8.7	80.5	18	40
Carbofuran	8	96.1	103	105	96.8	96.1	99.4	4.2	97.9	94.8	95.9	84.1	99.1	94.4	6.3	96.9	5.7	13
Carbosulfan	6	90.2	101.0	96.3	98.7	97.6	96.8	4.2	103	99.4	109	105	101	103	3.7	100	5.1	11
Carboxin	5	95.2	92.1	97.0	97.1	101	96.4	3.2	104	99.9	96.3	97.9	99.9	99.5	2.7	98.0	3.3	7.3
Carfentrazone-ethyl	4	89.4	92.0	86.7	88.1	87.4	88.7	2.4	88.0	94.6	84.5	81.1	103	90.3	9.8	89.5	6.8	15
Chlorantraniliprole	8	97.4	93.6	106	95.1	96.2	97.8	5.1	90.9	84.5	88.5	82.5	85.3	86.3	3.9	92.0	7.9	18
Chlorfenvinphos (E- and Z-isomers)	2	93.2	90.5	85.1	91.8	94.0	90.9	3.9	104	91.3	93.4	93.9	94.8	95.4	5.0	93.2	5.0	11
Chloridazon (Pyrazon)	4	92.7	89.3	99.6	87.4	94.0	92.6	5.1	93.2	91.3	94.5	91.1	89.5	91.9	2.1	92.3	3.7	8.3
Chlorotoluron (Chlortoluron)	7	96.9	99.6	99.5	100	105	100	2.9	97.6	87.8	90.0	92.4	94.8	92.5	4.2	96.4	5.4	12
Chloroxuron	7	97.6	102	105	93.0	99.0	99.2	4.5	89.5	92.0	94.0	94.8	94.6	93.0	2.4	96.1	4.8	11
Chlorpyrifos	2	95.1	95.1	103	101	93.5	97.5	4.2	92.6	84.0	82.0	90.9	86.9	87.3	5.1	92.4	7.3	16
Chlorpyrifos-methyl	2	104	105	100	103	105	103	1.9	85.1	96.1	108	89.5	117	99.2	13	101	9.1	20

Table 5. Trueness (% Recovery), Precision (% RSD), and Measurement Uncertainty (% MU) Data Obtained for Oesticides Included in the Agilent LC/MS Mixes 1–8 in Wheat Flour Matrix at the Fortification Level of 0.02 mg/kg Evaluated in Five Replicates (A–E) by Two Different Analysts on Two Different Days (continued)

		Fortification level 0.02 mg/kg (Analyst 1)							Fortification level 0.02 mg/kg (Analyst 2)							Overall		
		Trueness (%)						RSD	Trueness (%)						RSD	Trueness	RSD	MU
Analyte	Mix	A	B	C	D	E	Mean	(%)	A	B	C	D	E	Mean	(%)	(%)	(%)	(%)
Chlorsulfuron	4	98.2	91.0	101	98.1	100	97.7	4.0	82.3	85.1	87.3	86.6	82.3	84.7	2.8	91.2	8.2	18
Clethodim (E- and Z-isomers)	3	104	101	108	96.2	114	104	6.5	101	93.1	88.6	97.4	90.4	94.0	5.3	99.3	7.9	18
Clofentezine	4	93.4	96.0	96.9	99.6	99.7	97.1	2.7	91.7	90.8	93.9	93.7	92.3	92.5	1.4	94.8	3.3	7.4
Clomazone	8	102	101	98.0	100	98.5	100	1.8	100	96.9	89.4	95.3	105	97.3	5.8	98.7	4.3	9.5
Coumaphos	2	95.1	87.9	93.8	98.1	102	95.3	5.4	99.6	94.3	87.9	99.3	94.3	95.1	5.0	95.2	4.9	11
Cyazofamid	4	96.4	105	98.6	103	100	101	3.3	91.5	92.9	95.8	93.2	95.8	93.8	2.0	97.3	4.5	10
Cycloate	1	90.6	101	110	117	108	105	9.4	91.6	98.3	93.4	119	85.9	97.6	13	101	11	25
Cycluron	7	97.5	94.4	99.0	95.7	101	97.4	2.5	96.1	85.3	93.2	94.4	100	93.9	5.9	95.6	4.6	10
Cymiazole	1	108	93.2	112	94.2	98.5	101	8.2	101	99.9	86.2	82.3	99.0	93.6	9.3	97.3	9.1	20
Cymoxanil	4	107	106	105	94.0	95.2	101	6.2	101	91.8	95.0	82.8	91.5	92.5	7.3	97.0	8.0	18
Cyproconazole (2 diastereoisomers)	1	90.7	90.1	96.7	86.6	89.5	90.7	4.0	102	103	97.9	97.3	102	101	2.8	95.6	6.3	14
Cyprodinil	8	106	101	102	103	90.1	100	5.9	79.2	81.3	91.6	76.6	90.0	83.7	8.0	92.0	11	25
DEET (Diethyltoluamide)	4	93.4	91.0	98.2	98.3	100	96.2	3.9	82.9	89.7	98.3	97.5	95.1	92.7	6.9	94.4	5.6	13
Desmedipham	8	101	99.3	100	91.0	102	98.8	4.6	101	89.3	93.6	94.0	95.8	94.7	4.4	96.7	4.8	11
Diazinon	2	96.7	102	105	98.2	107	102	4.4	98.2	87.3	87.7	88.9	91.9	90.8	5.0	96.4	7.5	17
Dichlorvos	2	110	110	120	113	110	113	3.8	113	104	102	115	112	109	5.2	111	4.6	10
Diethofencarb	6	99.5	97.8	120	105	102	105	8.4	87.7	82.8	94.2	86.5	90.2	88.3	4.8	96.5	11	25
Difenoconazole (*cis*- and *trans*-)	3	99.2	102	94.2	98.8	98.8	98.6	2.9	90.9	95.3	93.9	84.3	89.5	90.8	4.7	94.7	5.7	13
Diflubenzuron	4	111	111	111	103	107	109	3.3	106	94.4	97.8	88.6	88.7	95.0	7.5	102	8.8	20
Diflufenican	1	85.8	96.9	101	94.2	100	95.6	6.4	100	92.1	89.9	98.5	99.6	96.1	5.0	95.9	5.4	12
Dimethachlor	1	103	99.7	111	103	113	106	5.6	100	97.7	98.6	101	92.4	98.0	3.5	102	6.0	13
Dimethoate	8	96.2	97.8	112	105	98.4	102	6.4	81.9	98.0	86.8	95.6	84.6	89.4	7.9	95.6	9.6	21
Dimethomorph (E- and Z-isomers)	5	87.0	89.9	92.5	91.9	95.9	91.4	3.6	94.1	92.2	92.5	94.0	92.5	93.1	1.0	92.2	2.6	5.9
Dimoxystrobin	1	99.9	94.2	98.6	104	100	99.3	3.4	93.9	86.6	92.5	87.2	90.1	90.1	3.6	94.7	6.1	14
Diniconazole	2	85.3	86.3	96.4	94.0	93.4	91.1	5.5	95.5	88.8	88.5	85.4	97.9	91.2	5.7	91.2	5.3	12
Dinotefuran	7	101	96.3	92.9	88.2	89.7	93.7	5.7	85.6	82.4	82.9	91.7	85.0	85.5	4.3	89.6	6.8	15
Dioxacarb	7	94.8	104	110	105	104	104	5.3	95.0	83.3	87.7	99.3	114	95.8	12	99.7	9.6	21
Disulfoton	1	89.2	92.2	104	100	99.5	97.0	6.3	90.4	79.8	88.6	73.3	106	87.6	14	92.3	11	25
Diuron	5	102	101	102	96.9	103	101	2.3	96.4	93.8	93.1	93.9	93.0	94.0	1.5	97.5	4.1	9.2
Epoxiconazole	2	114	114	98.5	105	111	108	6.2	84.1	94.9	93.8	101	91.6	93.0	6.5	101	10	22
Ethidimuron (Sulfadiazole)	8	88.9	98.1	89.8	100	94.8	94.3	5.2	113	112	111	123	121	116	4.7	105	12	26
Ethion	2	94.0	96.0	96.1	87.4	95.6	93.8	3.9	94.6	93.5	89.8	95.6	86.0	91.9	4.3	92.9	4.0	9.0
Ethirimol	4	102	94.3	93.3	90.5	93.7	94.7	4.5	89.1	82.8	85.5	84.9	87.1	85.9	2.7	90.3	6.3	14
Ethofumesate	4	93.0	95.3	109	93.7	107	99.7	7.9	102	87.6	95.8	93.0	100	95.7	6.0	97.7	7.0	16
Ethoprophos (Ethoprop)	2	104	93.6	97.5	92.3	90.3	95.5	5.6	88.2	80.7	101	87.8	94.0	90.3	8.2	92.9	7.2	16
Ethoxyquin	8	42.4	42.4	42.6	42.6	42.8	**42.6**	0.4	13.4	13.7	13.4	14.2	14.0	**13.7**	2.7	**28.1**	**54**	120
Etofenprox	3	113	94.1	113	83.9	102	101	12	97.5	87.4	82.1	134	127	106	**22**	104	17	39
Famoxadone	4	106	106	117	67.9	97.6	98.9	19	79.0	78.4	93.2	94.6	86.0	86.2	8.8	92.6	16	36

Table 5. Trueness (% Recovery), Precision (% RSD), and Measurement Uncertainty (% MU) Data Obtained for Oesticides Included in the Agilent LC/MS Mixes 1–8 in Wheat Flour Matrix at the Fortification Level of 0.02 mg/kg Evaluated in Five Replicates (A–E) by Two Different Analysts on Two Different Days (continued)

		Fortification level 0.02 mg/kg (Analyst 1)							Fortification level 0.02 mg/kg (Analyst 2)							Overall		
		Trueness (%)						RSD	Trueness (%)						RSD	Trueness	RSD	MU
Analyte	Mix	A	B	C	D	E	Mean	(%)	A	B	C	D	E	Mean	(%)	(%)	(%)	(%)
Fenamidone	5	97.5	93.3	98.5	98.1	96.8	96.9	2.1	95.7	91.5	88.1	106	93.6	95.0	7.1	95.9	5.0	11
Fenamiphos	1	84.9	89.0	85.4	95.2	108	92.4	10	84.7	84.4	93.5	99.5	90.2	90.5	7.0	91.4	8.4	19
Fenarimol	2	92.2	84.3	98.7	89.7	85.7	90.1	6.3	101	105	101	96.6	94.2	99.7	4.4	94.9	7.3	16
Fenazaquin	5	105	97.9	104	105	109	104	3.9	73.7	70.4	77.6	74.9	74.0	74.1	3.5	89.2	18	40
Fenbuconazole	2	97.2	103	99.2	104	95.0	99.7	3.9	93.1	89.6	98.4	89.9	96.8	93.6	4.2	96.7	5.1	11
Fenhexamid	3	94.1	91.0	111	85.9	103	97.1	10	101	91.2	91.1	98.7	94.0	95.3	4.8	96.2	7.8	17
Fenobucarb	5	91.1	94.6	103	94.3	96.5	95.9	4.6	98.8	86.2	81.9	90.5	92.5	90.0	7.1	92.9	6.5	15
Fenoxycarb	6	109	107	103	97.2	102	104	4.6	109	89.4	92.2	89.9	104	96.9	9.3	100	7.7	17
Fenpropidin	5	104	99.6	101	100	100	101	1.8	102	104	95.8	95.7	98.2	99.2	3.8	100	2.9	6.4
Fenpyroximate	5	114	103	108	104	107	107	4.2	71.3	69.0	67.4	64.2	70.5	68.5	4.1	87.8	**24**	52
Fenuron	7	110	96.7	103	94.2	106	102	6.4	89.3	86.7	91.0	99.7	96.2	92.6	5.7	97.3	7.7	17
Fipronil	4	93.3	98.0	91.9	90.4	107	96.2	7.1	77.0	76.5	60.3	78.5	98.9	78.2	18	87.2	16	36
Flazasulfuron	4	95.3	97.4	99.0	95.6	95.7	96.6	1.6	90.0	81.5	88.8	88.2	85.2	86.8	3.9	91.7	6.3	14
Flonicamid	6	107	105	123	99.1	135	114	13	73.9	73.3	88.8	74.3	88.1	79.7	10	96.7	**22**	49
Flubendiamide	7	89.0	75.7	109	98.8	91.9	92.9	13	108	99.1	129	104	100	108	11	100	14	31
Fludioxonil	2	98.8	103	106	96.4	102	101	3.8	106	86.6	97.6	88.5	89.0	93.5	8.7	97.4	7.5	17
Flufenacet	1	99.2	97.0	95.0	80.0	91.9	92.6	8.1	85.8	85.6	86.9	95.7	95.4	89.9	5.8	91.2	6.9	15
Flufenoxuron	4	98.0	99.0	98.7	100	101	99.5	1.4	91.2	87.8	74.9	88.3	92.7	87.0	8.1	93.2	8.7	19
Flumetsulam	8	103	110	98.6	97.4	109	104	5.5	106	90.4	78.8	104	92.8	94.5	12	99.0	9.7	22
Flumioxazin	6	113	112	123	112	122	116	4.9	98.0	108	93.7	80.9	95.6	95.2	10	106	13	28
Fluometuron	8	95.5	98.2	103	96.5	99.8	98.7	3.2	94.6	89.8	90.2	96.2	98.8	93.9	4.1	96.3	4.3	9.6
Fluopicolide	1	96.8	107	106	93.1	97.1	100	6.2	88.4	82.1	102	87.2	93.6	90.6	8.2	95.3	8.5	19
Fluoxastrobin	8	99.8	103	90.5	99.0	101	98.7	4.8	87.9	83.4	101	77.4	85.7	87.0	9.8	92.9	9.7	22
Fluquinconazole	2	94.1	96.9	107	110	102	102	6.3	91.5	94.0	100	92.8	92.2	94.2	3.8	98.0	6.5	14
Flusilazole	2	92.9	94.4	95.9	100	98.2	96.3	3.0	95.1	84.1	92.5	91.9	88.2	90.3	4.7	93.3	5.0	11
Flutriafol	8	95.7	96.9	101	100	103	99.4	3.1	94.6	97.4	91.1	92.3	96.7	94.4	2.9	96.9	3.9	8.7
Foramsulfuron	3	102	101	94.1	103	99.6	99.8	3.4	99.0	103	97.4	99.0	84.2	96.5	7.4	98.2	5.7	13
Forchlorfenuron	7	91.1	88.4	91.0	88.4	95.1	90.8	3.0	88.7	88.8	95.6	92.7	93.9	91.9	3.4	91.4	3.1	6.9
Fosthiazate (sum of isomers)	1	99.3	101	105	96.5	102	101	3.0	98.6	85.8	96.7	95.2	97.8	94.8	5.5	97.8	5.2	12
Fuberidazole	4	88.3	92.5	96.5	88.8	85.5	90.3	4.7	92.3	90.8	86.9	87.4	94.1	90.3	3.5	90.3	3.9	8.7
Furalaxyl	7	100	97.5	103	97.1	99.8	99.6	2.5	88.2	82.3	97.0	99.7	105	94.4	9.6	97.0	7.1	16
Furathiocarb	6	102	102	94.8	96.7	95.0	98.1	3.7	103	95.3	96.4	86.8	101	96.6	6.7	97.4	5.1	11
Halofenozide	8	99.7	100	104	101	104	102	2.2	101	91.6	89.8	89.0	98.3	93.9	5.6	97.8	5.8	13
Halosulfuron-methyl	8	91.7	92.7	90.4	91.7	102	93.8	5.2	90.7	90.1	89.1	87.2	88.4	89.1	1.6	91.4	4.5	10
Hexaconazole	2	97.8	94.7	96.1	86.3	90.4	93.1	5.0	96.2	86.5	84.7	89.7	90.9	89.6	4.9	91.3	5.1	11
Hexaflumuron	7	89.2	91.3	96.9	93.5	95.2	93.2	3.3	91.6	85.6	88.3	82.8	94.9	88.6	5.4	90.9	4.9	11
Hexythiazox	4	95.7	96.7	103	99.3	99.3	98.8	2.8	87.9	82.0	82.3	81.8	87.0	84.2	3.6	91.5	8.9	20
Hydramethylnon	7	101	106	110	102	103	105	3.5	110	99.9	100	96.0	92.9	99.8	6.5	102	5.4	12

Table 5. Trueness (% Recovery), Precision (% RSD), and Measurement Uncertainty (% MU) Data Obtained for Oesticides Included in the Agilent LC/MS Mixes 1–8 in Wheat Flour Matrix at the Fortification Level of 0.02 mg/kg Evaluated in Five Replicates (A–E) by Two Different Analysts on Two Different Days (continued)

		Fortification level 0.02 mg/kg (Analyst 1)							Fortification level 0.02 mg/kg (Analyst 2)							Overall		
		Trueness (%)						RSD	Trueness (%)						RSD	Trueness	RSD	MU
Analyte	Mix	A	B	C	D	E	Mean	(%)	A	B	C	D	E	Mean	(%)	(%)	(%)	(%)
Imazalil	2	96.7	101	101	101	110	102	4.7	85.3	88.7	98.3	85.6	88.3	89.3	5.9	95.6	8.6	19
Imidacloprid	5	84.2	90.4	98.6	97.0	92.5	92.5	6.2	86.2	88.3	89.0	82.5	109	91.1	12	91.8	8.8	20
Indoxacarb	3	94.0	94.5	100	105	103	99.5	5.1	97.4	99.9	92.3	92.8	103	97.2	4.9	98.4	4.9	11
Ipconazole	2	94.0	97.4	101	96.4	101	98.0	3.1	98.2	87.2	90.5	97.0	95.0	93.6	4.9	95.8	4.5	10
Iprovalicarb	5	100	96.6	94.0	94.5	100	97.0	3.0	96.6	87.9	103	98.9	97.5	96.7	5.7	96.9	4.3	9.5
Isofenphos-methyl	1	103	94.0	98.0	98.1	95.6	97.8	3.6	91.9	83.8	96.2	86.3	109	93.4	11	95.6	7.7	17
Isoprothiolane	1	89.0	82.3	89.1	86.8	93.3	88.1	4.5	94.6	98.2	97.5	92.0	110	98.4	6.9	93.3	8.1	18
Isoxaben	4	102	111	96.5	93.6	91.2	98.9	7.9	97.6	80.6	91.7	105	87.2	92.4	10	95.7	9.2	21
Isoxaflutole	3	86.5	90.3	96.4	96.8	101	94.3	6.2	89.4	83.4	89.2	89.4	98.6	90.0	6.1	92.1	6.3	14
Ivermectin B1a	7	135	97.8	124	97.4	108	112	15	118	108	136	112	106	116	10	114	12	27
Kresoxim-methyl	4	95.4	91.9	111	102	112	102	8.8	93.0	95.8	99.5	78.5	105	94.4	11	98.5	10	22
Lenacil	1	93.4	98.8	97.2	89.4	99.9	95.7	4.5	95.8	80.3	96.0	91.8	97.2	92.2	7.6	94.0	6.1	14
Linuron	4	93.6	102	106	101	104	101	4.7	95.4	88.5	91.7	93.1	93.8	92.5	2.8	96.8	6.0	13
Lufenuron	4	104	96.1	101	85.6	106	98.5	8.3	106	92.4	85.2	95.4	95.4	94.9	7.9	96.7	7.9	18
Malaoxon	3	95.6	84.7	104	97.7	100	96.5	7.6	89.2	84.2	94.4	101	97.7	93.3	7.1	94.9	7.2	16
Malathion	3	107	94.6	109	99.2	98.2	102	6.2	87.0	80.0	91.2	95.4	94.9	89.7	7.1	95.7	9.1	20
Mandipropamid	4	101	110	118	108	119	111	6.6	108	98.8	94.1	103	101	101	5.2	106	7.5	17
Mecarbam	3	96.1	105	106	106	96.0	102	5.1	105	102	92.5	93.8	95.8	97.7	5.4	99.7	5.4	12
Mepanipyrim	3	101	94.5	96.9	90.8	96.1	96.0	4.1	94.8	83.3	92.0	101	101	94.3	7.7	95.1	5.9	13
Mesosulfuron-methyl	6	105	98.2	109	104	96.1	102	5.0	85.3	79.4	112	91.3	96.7	92.9	13	97.7	11	23
Metaflumizone	4	95.2	91.4	85.0	104	89.4	93.0	7.6	110	84.3	95.7	96.8	102	97.8	9.6	95.4	8.6	19
Metalaxyl	3	98.9	97.2	105	98.1	97.7	99.4	3.2	107	99.4	95.8	98.2	97.5	99.6	4.5	99.5	3.7	8.2
Metamitron	4	95.8	96.7	95.8	88.2	95.4	94.4	3.7	92.2	81.2	87.1	94.3	84.2	87.8	6.2	91.1	6.1	14
Metazachlor	3	97.7	96.1	100	96.9	104	99.0	3.3	87.9	88.4	89.4	90.3	93.7	89.9	2.5	94.4	5.8	13
Metconazole	2	89.1	101	102	102	101	99.0	5.6	102	90.5	86.3	96.7	89.9	93.1	6.8	96.1	6.7	15
Methabenzthiazuron	5	92.6	90.9	101	92.8	98.4	95.1	4.6	91.0	87.6	93.1	95.0	97.8	92.9	4.2	94.0	4.3	9.6
Methamidophos	1	84.3	82.0	84.3	79.7	87.4	83.5	3.4	78.9	78.7	74.1	78.9	75.5	77.2	2.9	80.4	5.1	11
Methidathion	3	100	93.8	94.5	99.0	98.2	97.2	3.0	93.8	83.2	99.1	94.8	86.7	91.5	7.1	94.4	5.9	13
Methiocarb	7	97.3	95.0	103	99.4	95.7	98.1	3.3	98.4	92.5	85.7	95.6	91.8	92.8	5.1	95.4	5.0	11
Methomyl	5	97.6	103	102	106	100	102	3.0	103	93.0	104	99.4	85.5	97.0	8.0	99.3	6.1	14
Methoprotryne	7	95.3	92.5	103	104	103	99.6	5.3	92.4	88.1	95.9	100	91.4	93.7	5.0	96.6	5.9	13
Methoxyfenozide	5	95.2	102	109	99.2	99.2	101	5.2	104	88.8	140	118	125	115	17	108	14	32
Metobromuron	8	93.0	102	94.2	94.9	101	97.0	4.2	94.4	97.5	93.5	95.2	98.1	95.7	2.1	96.4	3.2	7.1
Metolachlor	3	110	108	106	109	113	109	2.2	71.9	73.9	90.6	93.9	89.5	84.0	12	96.5	16	35
Metrafenone	4	94.4	94.7	97.9	95.3	88.8	94.2	3.5	91.8	84.3	84.0	89.9	87.0	87.4	3.9	90.8	5.3	12
Metribuzin	4	107	105	109	101	110	106	3.4	96.4	86.7	93.7	92.5	87.0	91.3	4.7	98.7	8.8	20
Metsulfuron-methyl	4	91.7	88.6	89.7	94.6	92.1	91.4	2.5	87.6	81.8	97.9	93.3	101	92.4	8.4	91.9	5.9	13
Mevinphos (E- and Z-isomers)	3	95.0	90.1	95.5	92.2	92.6	93.1	2.4	124	109	97.5	110	99.1	108	10	101	11	24

Table 5. Trueness (% Recovery), Precision (% RSD), and Measurement Uncertainty (% MU) Data Obtained for Oesticides Included in the Agilent LC/MS Mixes 1–8 in Wheat Flour Matrix at the Fortification Level of 0.02 mg/kg Evaluated in Five Replicates (A–E) by Two Different Analysts on Two Different Days (continued)

		Fortification level 0.02 mg/kg (Analyst 1)							Fortification level 0.02 mg/kg (Analyst 2)							Overall		
		Trueness (%)						RSD	Trueness (%)						RSD	Trueness	RSD	MU
Analyte	Mix	A	B	C	D	E	Mean	(%)	A	B	C	D	E	Mean	(%)	(%)	(%)	(%)
Mexacarbate	7	93.8	90.4	113	85.3	96.2	95.8	11	85.6	88.5	99.8	95.1	90.5	91.9	6.1	93.8	8.8	20
Molinate	3	96.3	98.7	98.5	90.4	86.2	94.0	5.9	68.2	69.4	103	92.4	83.6	83.3	18	88.7	14	30
Monocrotophos	4	102	101	111	90.9	98.6	101	7.1	106	87.6	80.0	87.8	96.6	91.5	11	96.0	9.8	22
Moxidectin	7	119	100	138	92.6	88.4	108	19	95.0	109	119	147	130	120	17	114	18	39
Myclobutanil	1	91.1	96.3	108	99.2	94.0	97.8	6.7	89.5	107	98.0	97.9	103	99.2	6.8	98.5	6.4	14
Nicosulfuron	4	87.6	80.8	80.1	83.6	97.7	86.0	8.3	89.0	87.7	88.9	86.0	90.8	88.5	2.0	87.2	5.9	13
Nitenpyram	7	97.8	88.1	95.1	94.1	95.6	94.1	3.9	82.3	78.5	84.9	86.2	73.8	81.1	6.2	87.6	9.1	20
Novaluron	4	103	104	103	101	97.8	101	2.3	105	93.0	96.8	93.0	95.2	96.6	5.1	99.0	4.5	10
Omethoate	6	83.9	80.4	88.1	83.8	87.2	84.7	3.7	88.1	79.6	96.4	80.8	91.6	87.3	8.2	86.0	6.2	14
Oxadiazon	3	105	91.9	88.1	99.6	89.1	94.8	7.8	96.2	95.8	92.8	83.0	92.3	92.0	5.8	93.4	6.7	15
Oxadixyl	3	100	103	101	101	103	102	1.4	99.2	94.0	93.6	93.3	87.8	93.6	4.3	97.7	5.3	12
Oxamyl	5	106	96.3	105	97.5	102	101	4.2	78.6	83.0	77.6	75.4	78.6	78.6	3.5	90.0	14	31
Oxasulfuron	4	94.6	95.7	103	93.4	104	98.2	5.1	94.2	90.0	89.8	91.8	93.7	91.9	2.2	95.0	5.2	12
Paclobutrazol	3	100	97.1	104	100	98.2	99.9	2.7	107	102	99.7	97.8	99.4	101	3.6	101	3.1	6.8
Penconazole	3	98.7	106	97.7	93.4	95.3	98.3	5.1	108	91.6	87.9	88.9	90.7	93.4	8.7	95.9	7.2	16
Pencycuron	6	88.8	91.2	96.0	92.7	98.3	93.4	4.1	96.5	93.6	94.2	96.5	95.6	95.3	1.4	94.3	3.0	6.7
Pendimethalin	3	98.3	105	104	96.3	109	103	5.0	94.6	93.6	82.6	83.7	79.3	86.7	8.0	94.7	11	24
Phenmedipham	4	99.6	97.1	96.7	100	93.8	97.5	2.6	93.7	94.2	97.9	90.7	94.5	94.2	2.7	95.8	3.1	6.8
Phenthoate	3	83.9	90.9	86.2	93.9	99.2	90.8	6.7	83.3	91.5	109	83.2	110	95.4	14	93.1	11	24
Phosalone	3	99.1	98.3	110	101	109	104	5.4	85.5	93.1	83.0	84.8	101	89.5	8.5	96.5	10	22
Phosmet	6	100	99.4	101	99.7	110	102	4.5	90.4	95.5	93.4	88.7	91.0	91.8	2.9	96.9	6.6	15
Phosphamidon (E- and Z-isomers)	3	92.6	93.1	92.8	92.2	92.5	92.6	0.4	106	97.4	95.6	94.5	96.6	97.9	4.5	95.3	4.3	9.5
Phoxim	4	104	97.3	109	101	105	103	4.1	85.5	89.7	94.9	96.9	100	93.4	6.2	98.3	7.2	16
Picolinafen	3	107	97.9	106	100	102	103	3.9	100	80.4	85.9	90.0	83.9	88.1	8.7	95.4	10	22
Picoxystrobin	5	103	123	115	112	112	113	6.5	97.2	95.3	90.6	103	98.7	96.9	4.7	105	9.8	22
Pirimicarb	3	95.0	92.1	93.5	83.5	94.6	91.7	5.2	94.8	95.4	99.8	99.6	94.9	96.9	2.6	94.3	4.8	11
Pirimiphos-methyl	3	107	103	99.8	98.6	107	103	3.9	105	93.3	92.5	102	89.8	96.4	6.6	99.8	6.2	14
Prochloraz	1	106	97.8	112	102	99.0	104	5.7	97.6	86.7	87.8	87.7	89.2	89.8	4.9	96.7	9.1	20
Profenofos	3	99.1	92.5	104	94.1	101	98.2	5.0	102	97.4	87.5	90.0	96.6	94.7	6.2	96.5	5.6	13
Promecarb	7	91.4	102	103	101	94.6	98.6	5.3	99.6	96.9	97.8	94.5	90.5	95.8	3.7	97.2	4.6	10
Prometon	4	88.3	100	98.6	94.2	98.3	95.9	5.0	94.8	90.0	93.6	92.6	97.2	93.6	2.8	94.8	4.0	9.0
Propamocarb	5	88.6	87.1	86.8	93.8	85.8	88.4	3.6	95.2	85.8	88.8	85.1	90.6	89.1	4.6	88.8	3.9	8.7
Propaquizafop	4	91.4	99.6	104	99.2	92.3	97.3	5.6	99.1	87.7	95.5	93.9	89.7	93.2	4.9	95.3	5.5	12
Propargite	4	111	108	107	102	106	107	3.2	77.6	74.1	73.1	71.3	70.0	73.2	4.0	89.9	20	44
Propetamphos	3	92.7	101	94.8	95.1	97.1	96.2	3.3	95.3	91.2	89.1	93.1	102	94.1	5.1	95.1	4.2	9.4
Propiconazole (sum of isomers)	2	101	98.0	106	91.9	96.8	98.9	5.4	94.9	87.3	95.0	93.7	93.0	92.8	3.4	95.8	5.5	12
Propoxur	6	101	101	104	101	97.6	101	2.1	104	101	87.3	97.0	91.1	96.1	7.1	98.4	5.5	12
Propyzamide (Pronamide)	3	96.3	105	101	101	96.0	99.8	3.8	101	89.1	97.8	105	94.8	97.6	6.2	98.7	5.0	11

Table 5. Trueness (% Recovery), Precision (% RSD), and Measurement Uncertainty (% MU) Data Obtained for Oesticides Included in the Agilent LC/MS Mixes 1–8 in Wheat Flour Matrix at the Fortification Level of 0.02 mg/kg Evaluated in Five Replicates (A–E) by Two Different Analysts on Two Different Days (continued)

		Fortification level 0.02 mg/kg (Analyst 1)							Fortification level 0.02 mg/kg (Analyst 2)							Overall		
		Trueness (%)						RSD	Trueness (%)						RSD	Trueness	RSD	MU
Analyte	Mix	A	B	C	D	E	Mean	(%)	A	B	C	D	E	Mean	(%)	(%)	(%)	(%)
Proquinazid	1	93.7	107	97.7	96.0	93.7	97.6	5.4	71.0	62.9	63.3	64.8	60.6	**64.5**	6.1	81.0	**22**	49
Prosulfocarb	4	97.7	93.8	103	98.0	97.6	97.9	3.2	104	94.2	89.5	98.8	96.9	96.6	5.5	97.3	4.3	9.5
Pymetrozine	7	90.1	84.0	89.9	83.5	94.2	88.3	5.1	81.0	73.8	87.3	82.9	82.1	81.4	6.0	84.9	6.8	15
Pyracarbolid	7	96.8	94.7	103	97.4	94.6	97.3	3.6	96.7	94.5	93.3	94.9	95.3	94.9	1.3	96.1	2.9	6.4
Pyraclostrobin	5	105	98.9	102	94.4	103	101	4.1	89.5	86.3	91.3	83.1	104	90.8	8.8	95.8	8.3	18
Pyridaben	5	115	109	101	106	105	107	4.8	79.9	70.0	71.0	67.6	73.5	72.4	6.5	89.8	**21**	47
Pyridate	5	91.0	99.3	94.1	89.5	93.5	93.5	4.0	60.1	52.9	55.1	53.2	55.6	**55.4**	5.2	74.4	**27**	**61**
Pyrimethanil	6	99.1	90.3	103	96.8	95.9	97.0	4.7	80.2	88.5	82.1	89.9	93.5	86.8	6.4	91.9	7.8	17
Pyriproxyfen	5	112	93.7	96.3	98.8	99.4	100	7.0	95.2	87.1	86.7	92.7	83.2	89.0	5.5	94.5	8.6	19
Quinalphos	3	99.4	97.2	109	100	97.7	101	4.6	103	95.5	91.0	93.5	86.1	93.9	6.7	97.2	6.5	14
Quinmerac	7	53.4	51.0	48.0	53.8	51.3	**51.5**	4.5	37.2	33.3	37.8	39.2	35.1	**36.5**	6.4	**44.0**	19	41
Quinoclamine	4	98.5	94.4	98.6	91.9	98.5	96.4	3.2	92.6	88.8	91.3	88.4	88.8	90.0	2.1	93.2	4.4	9.9
Quinoxyfen	3	97.2	99.8	99.4	97.0	105	99.6	3.1	90.6	87.0	85.7	86.7	90.8	88.2	2.7	93.9	7.0	16
Rimsulfuron	4	82.4	88.2	95.4	92.4	89.4	89.6	5.5	96.1	88.7	80.9	86.8	89.6	88.4	6.2	89.0	5.5	12
Rotenone	7	95.5	94.7	95.6	96.8	98.2	96.2	1.4	102	99.2	96.6	96.6	91.4	97.1	4.0	96.7	2.9	6.4
Secbumeton	7	90.1	99.4	103	95.3	93.5	96.3	5.3	93.6	89.3	94.4	95.6	94.6	93.5	2.6	94.9	4.3	9.6
Silthiofam	4	96.3	110	107	99.1	117	106	8.0	108	106	92.0	106	96.5	102	6.9	104	7.4	16
Spinosad - Spinosyn A	7	97.7	95.4	105	96.8	105	100	4.7	97.5	95.6	96.9	96.7	103	97.9	2.9	98.9	3.8	8.6
Spinosad - Spinosyn D	7	107	113	112	97.9	95.9	105	7.5	103	105	98.3	103	98.0	101	3.1	103	5.8	13
Spirodiclofen	1	111	114	113	106	100	109	5.0	77.3	75.6	77.1	75.0	74.5	75.9	1.6	92.2	19	43
Spiromesifen	6	112	117	114	108	110	112	2.9	73.0	71.4	70.2	70.4	67.9	70.6	2.6	91.5	**24**	54
Spirotetramat	6	109	99.5	123	108	128	113	10	80.1	87.5	97.7	92.6	79.3	87.4	9.1	100	17	37
Spiroxamine (2 diastereoisomers)	1	89.4	92.7	94.9	94.0	97.2	93.6	3.1	105	97.2	96.5	101	94.2	98.8	4.3	96.2	4.5	10
Sulfentrazone	6	106	103	98.9	108	101	103	3.5	89.4	88.2	93.0	86.9	94.1	90.3	3.4	96.9	7.8	17
Tebuconazole	2	95.1	96.3	95.6	95.9	105	97.5	4.2	90.7	94.8	91.7	95.0	92.7	93.0	2.0	95.3	4.0	9.0
Tebufenozide	5	96.6	140	109	112	115	114	14	94.2	87.2	94.3	89.9	91.1	91.3	3.3	103	16	35
Tebufenpyrad	3	95.4	101	100	95.0	98.7	98.0	2.8	94.0	93.3	98.0	91.6	93.7	94.1	2.5	96.1	3.3	7.3
Tebuthiuron	7	96.0	97.7	100	98.9	94.0	97.3	2.5	92.6	92.2	94.7	101	96.1	95.3	3.7	96.3	3.1	7.0
Teflubenzuron	4	96.5	105	117	91.8	107	103	9.5	106	90.9	92.6	83.6	85.6	91.7	9.6	97.6	11	24
Temephos	7	98.8	95.2	107	101	101	101	4.4	96.5	87.9	99.0	89.4	81.2	90.8	7.8	95.7	7.9	18
Tepraloxydim (E- and Z-isomers)	3	97.4	98.4	101	98.5	107	100	3.7	121	107	108	122	122	116	6.5	108	9.2	21
Terbufos	3	100	101	94.2	94.9	87.4	95.5	5.6	94.7	81.1	92.4	87.4	91.3	89.4	6.0	92.4	6.5	14
Tetraconazole	2	101	96.2	102	104	102	101	2.8	106	99.5	96.1	98.8	101	100	3.7	101	3.1	6.9
Thiabendazole	5	95.4	94.5	105	95.0	106	99.3	6.0	84.2	78.5	84.7	90.1	88.2	85.2	5.2	92.2	9.7	22
Thiacloprid	5	96.7	102	98.4	97.5	99.9	98.9	2.1	93.4	92.1	97.3	97.3	93.8	94.8	2.5	96.8	3.1	6.9
Thiamethoxam	5	95.1	87.2	99.5	107	110	99.8	9.2	105	91.8	99.4	110	93.6	100	7.6	99.9	8.0	18
Thidiazuron	7	87.5	88.3	88.8	93.9	101	91.8	6.0	95.9	83.1	86.5	92.7	92.9	90.2	5.8	91.0	5.6	13
Thifensulfuron-methyl	4	80.3	87.7	84.6	82.7	79.3	82.9	4.1	78.7	86.8	90.0	89.4	92.8	87.5	6.1	85.2	5.7	13

Table 5. Trueness (% Recovery), Precision (% RSD), and Measurement Uncertainty (% MU) Data Obtained for Oesticides Included in the Agilent LC/MS Mixes 1–8 in Wheat Flour Matrix at the Fortification Level of 0.02 mg/kg Evaluated in Five Replicates (A–E) by Two Different Analysts on Two Different Days (continued)

		Fortification level 0.02 mg/kg (Analyst 1)							Fortification level 0.02 mg/kg (Analyst 2)							Overall		
		Trueness (%)						RSD	Trueness (%)						RSD	Trueness	RSD	MU
Analyte	Mix	A	B	C	D	E	Mean	(%)	A	B	C	D	E	Mean	(%)	(%)	(%)	(%)
Thiodicarb	5	98.5	92.9	99.6	92.7	104	97.6	5.0	85.3	79.9	83.3	77.9	96.3	84.5	8.5	91.1	9.9	22
Thiofanox	5	99.1	101	84.2	104	113	100	10	82.1	73.3	98.1	90.9	105	89.9	14	95.1	13	29
Tolylfluanid	3	85.3	80.5	83.7	80.4	76.9	81.3	4.0	64.6	70.9	67.7	62.3	66.9	**66.5**	4.9	73.9	11	25
Tralkoxydim	1	99.4	95.9	104	94.4	97.6	98.2	3.8	82.6	75.8	75.3	82.0	87.7	80.7	6.4	89.5	11	25
Triadimefon	3	97.3	93.2	97.1	99.2	95.9	96.5	2.3	100	86.3	104	101	112	101	9.1	98.6	6.8	15
Triadimenol	6	97.1	96.5	95.7	98.7	83.7	94.3	6.4	84.2	110	100	101	89.4	96.8	10	95.6	8.3	19
Triasulfuron	4	97.7	90.5	95.0	108	89.7	96.1	7.6	94.8	88.9	91.3	96.7	94.2	93.2	3.3	94.7	5.8	13
Triazophos	3	108	109	113	97.8	120	109	7.3	106	88.6	95.7	87.0	97.5	95.0	8.1	102	10	23
Tribenuron-methyl	4	86.0	92.7	83.5	90.5	82.2	87.0	5.2	68.0	66.2	66.7	71.0	72.3	68.9	3.9	77.9	13	29
Trichlorfon (Metrifonate)	6	94.2	93.8	93.1	93.7	93.7	93.7	0.4	98.6	90.8	84.3	93.5	85.1	90.4	6.6	92.1	4.7	11
Tricyclazole	2	90.9	90.5	91.7	87.3	91.1	90.3	1.9	89.3	95.1	88.5	87.0	84.3	88.9	4.5	89.6	3.3	7.4
Trietazine	6	94.3	102	109	97.2	102	101	5.5	102	85.8	101	92.6	99.8	96.2	7.1	98.5	6.5	14
Trifloxystrobin	5	95.8	95.2	97.9	91.1	101	96.2	3.8	92.4	92.8	94.4	97.0	95.4	94.4	2.0	95.3	3.0	6.7
Triflumizole	3	94.3	99.8	105	102	103	101	4.2	106	92.7	99.0	99.5	98.4	99.2	4.9	100	4.4	9.8
Triflumuron	4	101	95.5	113	99.0	103	102	6.4	117	105	91.4	98.8	102	103	9.2	102	7.5	17
Trimethacarb	6	95.9	97.2	98.8	95.4	98.1	97.1	1.5	88.4	99.1	96.6	91.5	103	95.6	6.0	96.4	4.1	9.2
Triticonazole	2	101	83.6	109	93.5	99.5	97.4	9.7	91.9	95.5	94.5	95.6	97.8	95.0	2.2	96.2	6.9	15
Uniconazole	2	91.6	96.4	98.0	80.1	87.6	90.8	8.0	107	102	99.7	97.8	99.4	101	3.6	96.0	8.1	18
Vamidothion	2	113	91.6	106	99.6	96.2	101	8.3	90.6	87.7	87.6	89.2	95.3	90.1	3.5	95.7	8.8	20
Zoxamide	6	92.6	93.7	95.7	101	111	98.9	7.8	108	96.6	81.9	82.3	94.7	92.7	12	95.8	10	22

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Improved LC/MS/MS Pesticide Multiresidue Analysis Using Triggered MRM and Online Dilution

Application Note

Food Safety

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Abstract

This application note describes the development and validation of a large pesticide multiresidue LC/MS/MS method using Agilent 1290 Infinity II LC systems coupled to Agilent 6490 triple quadrupole LC/MS instruments. The method enables the analysis of about 450 globally important pesticides in a short analysis time (analyte elution in less than 10 minutes). The MS/MS acquisition method uses triggered multiple reaction monitoring (tMRM), which provides increased confidence in analyte identification through triggered acquisition of additional MRMs when one of the primary MRMs exceeds a set abundance threshold. The mobile phase gradient was optimized to spread the analytes evenly throughout the elution window, with special attention paid to the separation of critical pairs. The LC system uses an online dilution setup, ensuring excellent peak shapes of early eluting (more polar) analytes. As a result, acetonitrile extracts (prepared using a QuEChERS-based extraction) are injected directly without a need for dilution with an aqueous buffer/solution prior to the injection. The method was validated in three different routine laboratories in multiple food commodity types/matrices, with 0.01 mg/kg method validated limit of quantitation (LOQ) achieved for the majority analyte-matrix combinations.

Introduction

Multiresidue methods, capable of simultaneous analysis of a larger number of analytes, provide the most practical approach to routine pesticide residue monitoring in food, feed, dietary supplements, and similar sample types. In addition to the economic benefits (cost, time, and labor efficiency), large multiresidue methods and careful selection of the included analytes can also address challenges associated with global trade (global sample origin due to global sourcing of raw materials and global distribution of products) and different regulatory issues in different countries when it comes to pesticide use and misuse, regulatory limits, or pesticide residue definitions. Modern multiresidue methods typically employ tandem mass spectrometry using triple quadrupole instruments coupled to both gas chromatography and liquid chromatography (GC/MS/MS and LC/MS/MS) to cover a wide range of both GC- and LC/MS/MS amenable pesticides. High-end triple quadrupole instruments provide sensitivity, selectivity, and speed for the determination of a large number of compounds at low concentration levels, even in highly complex matrices.

When a pesticide residue is detected in a sample, the first step is identification. Using MS/MS, two overlapping precursor-to-product ion transitions (multiple reaction monitoring, MRM) within a certain ion ratio and retention time tolerance are typically required for analyte identification. The widely accepted SANTE guidelines (SANTE/11945/2015) for analytical quality control and method validation procedures for pesticide residue analysis in food and feed [1] recommend the following identification criteria for GC/MS/MS and LC/MS/MS methods: retention time within ± 0.1 minutes, ≥ 2 product ions, and ± 30 % maximum relative tolerance for ion ratios (as compared to the retention times and ion ratios obtained for the given analyte in concurrently analyzed standards).

In routine practice, especially when analyzing highly complex samples, the minimum identification criteria may not be enough to prevent potentially false positive or false negative results. Therefore, additional information is beneficial for improved identification confidence and also fast decision making on whether to accept/reject the given result. For compounds amenable to both GC/MS/MS and LC/MS/MS analysis, it is helpful to include these analytes on both analytical platforms, and take advantage of orthogonal selectivity of GC/MS/MS and LC/MS/MS techniques for a high degree of identification confidence. There are compounds, however, that can be analyzed only on one platform, or for which the second platform provides inferior sensitivity or other poorer performance characteristics. This is the case for many modern pesticides that are more polar and thermally labile, and thus more suitable for LC/MS/MS analysis. To obtain additional MS/MS information without compromising the number of analytes that could be included in the LC/MS/MS method, the Agilent 6400 Series triple quadrupole LC/MS instruments offer so-called triggered MRM (tMRM) functions.

In tMRM, up to 10 MRMs can be acquired for each analyte, and combined into a product ion spectrum (at optimum collision energies for each product ion), which is used for library matching of selected pesticides, as shown in Figure 1. Using the tMRM function, some of the transitions (primary transitions) are acquired during the entire analyte acquisition window. The acquisition of the additional transitions is triggered (and performed for a defined number of scans) if one of the primary transitions exceeds the set abundance threshold [2,3].

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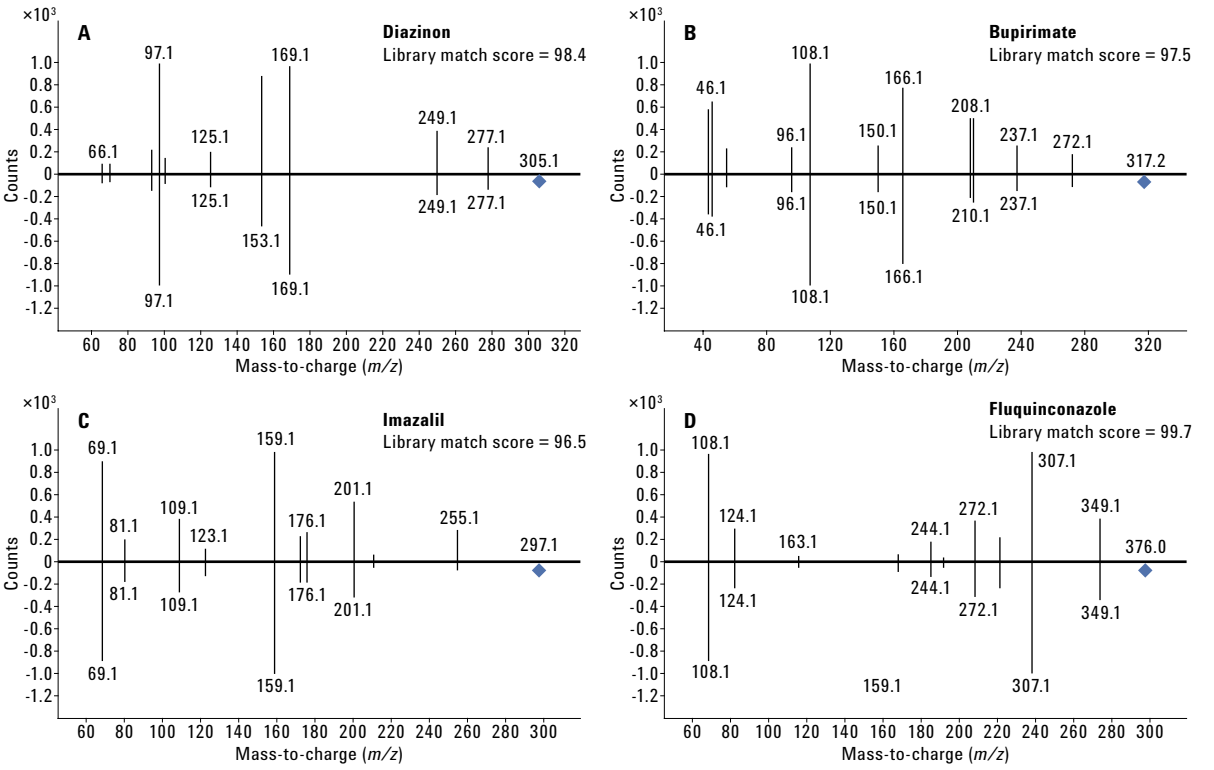


Figure 1. Examples of triggered MRM spectra and their matching against a reference library obtained using 10 MRMs for selected pesticides.

This application note describes the development and validation of a pesticide multiresidue LC/MS/MS method for the analysis of approximately 450 globally relevant pesticides using Agilent 1290 Infinity II LC systems coupled to Agilent 6490 triple quadrupole LC/MS instruments. The method is completely compatible with the Agilent 1290 Infinity II LC and an Agilent 6495 triple quadrupole LC/MS. The analytes are compounds amenable to a QuEChERS-based extraction and LC/MS/MS analysis, and represent priority pesticides included in regulatory monitoring testing programs in the US, Canada, EU, and Asia. Special attention was paid to the inclusion of pesticides specifically listed in certain regulations or guidelines, such as in the EU infant formula directive 141/2006/EC, the USDA National Organic Program, or US and European Pharmacopoeia pesticide monographs.

This method enables the analysis of a large number of analytes using a relatively short separation time (analyte elution in less than 10 minutes) and uses tMRM for increased identification confidence, using two to three primary MRMs, and typically four or more total MRMs, resulting in > 2,000 MRMs in the method. In addition, it improves retention and peak shape of early eluting, more polar analytes, which are notorious for having poor peak shapes when injected in extracts with a higher content of organic solvents, such as in QuEChERS acetonitrile extracts. This was achieved by using a special online dilution setup (a serial combination of two high-pressure mixers), enabling effective mixing of the injected sample with the initial highly aqueous mobile phase before reaching the column.

The method development was carried out at multiple laboratory sites. The final method was assembled at one location, and then transferred onto multiple instruments in three pesticide testing laboratories in the US, EU, and Asia, followed by method validation in multiple matrices using the SANTE method validation guidelines and criteria [1].

Experimental

Pesticide standards

A composite standard solution, containing all the analytes listed in Table 1 (pages 12–22) , was prepared at 1 µg/mL in acetonitrile containing 1 % acetic acid. To prepare this composite solution, the Agilent LC/MS standard mixes 1 to 8 at 100 µg/mL in acetonitrile (p/n 5190-0551) were combined with several custom mixes. The composite solution was then used for the preparation of solvent-based and matrix-matched standards. All standard solutions were stored at –20 °C.

Sample preparation

Sample preparation was based on the AOAC Int. Official method 2007.01 [4] using the acetate buffer QuEChERS extraction and partition steps but without any cleanup. High-moisture (10 g), low-moisture/low-fat (5 g), and low-moisture/high-fat and complex samples (1 g) were extracted using 10 mL of acetonitrile with 1 % acetic acid (10 mL of water was added to low-moisture samples) by shaking for 30 minutes. An internal standard mixture (100 µL of 1 µg/mL of the internal standards listed in Table 1) was added to the samples prior to extraction. After the initial shaking, 4 g of anhydrous magnesium sulfate and 1 g of sodium acetate were added to the sample tubes, followed by immediate shaking/vortexing for 1 minute. After centrifugation at >1,500 rcf for 5 minutes, an aliquot (400 µL) of the upper acetonitrile layer was placed in an autosampler vial together with 40 µL of a quality control (QC) standard containing 0.1 µg/mL triphenyl phosphate (TPP) in acetonitrile with 1 % acetic acid.

Matrix-matched standards (typically at concentrations corresponding to 0.001 to 0.050 µg/mL in the extract) were prepared by extracting blank matrices, and adding 40 µL of an appropriate standard solution to the 400 µL blank extract aliquot instead of the QC solution.

For trueness and precision (recovery and relative standard deviation, RSD) evaluation, blank matrix samples were spiked at 0.01, 0.02, or 0.05 mg/kg in five replicates during the method validation.

LC/MS/MS conditions

LC/MS/MS analyses were conducted using 1290 Infinity II LC systems (1,200 bar) coupled to 6490 triple quadrupole LC/MS instruments in three different laboratories. All systems used the same LC and MS conditions, listed in Table 2. Agilent MassHunter software was used for data acquisition and processing.

Table 2. Instrument Conditions

UHPLC parameters

Parameter	Value					
Analytical column	Agilent ZORBAX Eclipse Plus C18, Rapid Resolution HD, 2.1 × 100 mm, 1.8 µm					
Guard column	Agilent ZORBAX Eclipse Plus C18, 2.1 × 5 mm, 1.8 µm					
Online dilution setup	see Figure 2					
Column temperature	40 °C (G1316C TCC)					
Mobile phase A	10 mM ammonium formate in water-methanol (98:2, v/v) + 0.1 % formic acid					
Mobile phase B	10 mM ammonium formate in methanol-water (99:1, v/v) + 0.1 % formic acid					
Injection volume	2 µL (G4226A autosampler)					
Binary pump (G4220A) gradient and flow	Time (min)	%A	%B			Flow (mL/min)
	0.00	100	0			0.100
	0.20	100	0			0.100
	0.21	100	0			0.500
	0.50	50	50			0.500
	2.50	45	55			0.500
	5.50	25	75			0.500
	7.50	15	85			0.500
	8.30	0	100			0.500
	12.00	0	100			0.500
	12.10	100	0			0.500
	14.80	100	0			0.500
	14.90	100	0			0.100
Quaternary pump (G4204A) gradient and flow	Time (min)	%A	%B	%C	%D	Flow (mL/min)
	0.00	100	0	0	0	0.500
	0.20	100	0	0	0	0.500
	0.40	100	0	0	0	0.000
	14.80	100	0	0	0	0.000
	14.90	100	0	0	0	0.500

MS/MS parameters

Parameter	Value
Ionization mode	Positive ESI with Agilent Jet Stream (AJS)
Scan type	Triggered MRM (with three repeats)
Cycle time	650 ms
Stop time	15 minutes
Divert valve program	At 0 minutes to waste, at 1 minute to MS, at 10 minutes to waste
MS1/MS2 resolution	Unit
Gas temperature	180 °C
Gas flow	20 L/min
Nebulizer	40 psi
Sheath gas temperature	225 °C
Sheath gas flow	11 L/min
Capillary voltage	4,500 V
Nozzle voltage	0 V
iFunnel RF high/low	150/60

Results and Discussion

Optimization of MS/MS conditions

The MS/MS method development involved optimization and selection of MS/MS transitions (typically, 10 MRMs per analyte) using Agilent MassHunter Optimizer software, followed by a detailed review of the collected information. MassHunter Optimizer is a versatile tool for automated optimization of MRM conditions, including selection of precursor and product ions, and optimization of collision energies (CE) [5]. Practical considerations for routine optimization of pesticides and other compounds using MassHunter Optimizer are discussed in detail in a separate document [6].

Optimization of UHPLC conditions and online dilution

The aim of the UHPLC optimization was to achieve optimum analyte separation and detection within the relatively short separation time of less than 10 minutes. In addition, we wanted to improve retention and peak shape of early eluting, more polar analytes (such as cyromazine, methamidophos, acephate, and so forth), which are notorious for having poor peak shapes when injected in extracts with a higher content of organic solvents. Our ultimate goal was to be able to inject QuEChERS acetonitrile extracts directly, without any pre-injection dilution, while having sharp and well-focused peaks of the early eluting pesticides.

Figure 2 shows a typical situation that can be observed for early eluting pesticides when injected in acetonitrile in multiresidue methods. The early eluting peaks exhibit peak splitting and broadening. The most polar analytes usually show an unretained portion eluting at the dead time. This is caused by a breakthrough of molecules surrounded by the strong injection solvent. A lower injection volume can improve the situation, but usually not solve it completely. This option provides lower sensitivity due to the decreased sample volume introduced into the system. Another option is to dilute the sample extract and calibration standards before the injection using water or an aqueous buffer. This is a common practice but, unfortunately, the typically recommended dilution factors, such as 1:2 extract dilution, do not fully solve the peak splitting/retention problem. Higher dilution factors would be needed, but they can lead to stability and solubility issues. Moreover, the pre-injection dilution requires an additional step in the sample preparation method.

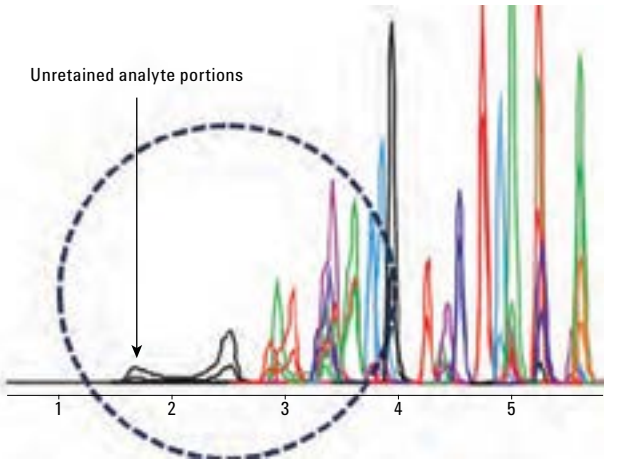
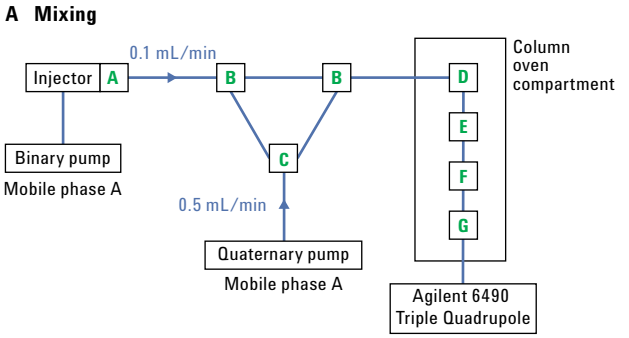


Figure 2. Illustration of problematic peak shape and retention of early eluting, more polar pesticides when injected in acetonitrile in other multiresidue LC/MS/MS methods [3].

We decided to use online dilution and mixing to improve the chromatography of early eluting compounds. Figure 3 provides the online dilution setup (a serial combination of two high-pressure mixers) used in the final method. This setup enables highly effective mixing of the injected sample with the initial highly aqueous mobile phase A before reaching the column, resulting in excellent peak shapes and retention of early eluting analytes (Figure 4). During the mixing stage, the method uses only mobile phase A. The sample is introduced at a lower flow rate of 0.1 mL/min using a binary pump, while the quaternary pump (a second high-pressure pump) flow rate is at 0.5 mL/min for more effective mixing with the aqueous mobile phase. After the mixing step, the quaternary pump flow is stopped, and the binary pump gradient starts. This online dilution design proved to be robust and easily transferable onto multiple systems in multiple locations. However, it requires the use of a second high-pressure pump (a quaternary pump in our case). To eliminate the second pump, it is possible to use a 6-port high-pressure valve and a T-piece to split the binary pump flow between the injector and the online dilution (2-mixer) system (Figure 5). This setup requires more precise timing and tubing consideration as compared to the two-pump option.



Components

- **A** 2 µm in-line direct connect SS filter (Analytical Sales & Services, p/n 48812) threaded directly to the injector valve
- **B** 25 µL high-pressure static mixer (Resolution Systems, p/n 402-0025HP)
- **C** Valco SS mixing tee 1/16 inch 0.25 mm bore (Resolution Systems, PN ZT1C or Sigma-Aldrich, p/n 58626)
- **D** Agilent 0.3 µm in-line filter (p/n 5067-4638)
- **E** Heat exchanger (p/n G1316-80002)
- **F** UHPLC (1,200 bar) guard column Agilent ZORBAX Eclipse Plus C18, 2.1 × 5 mm, 1.8 µm (p/n 821725-901)
- **G** UHPLC (1,200 bar) column Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 100 mm, 1.8 µm (p/n 959758-902)

Figure 3. Online dilution setup used in the LC/MS/MS method during (A) mixing of the injected sample with mobile phase A (initial 0.2 minutes), and (B) post-mixing when the binary pump gradient starts.

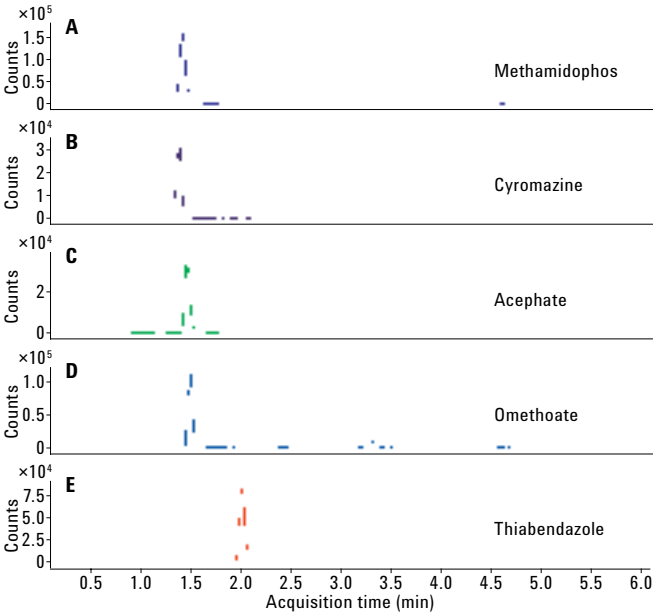
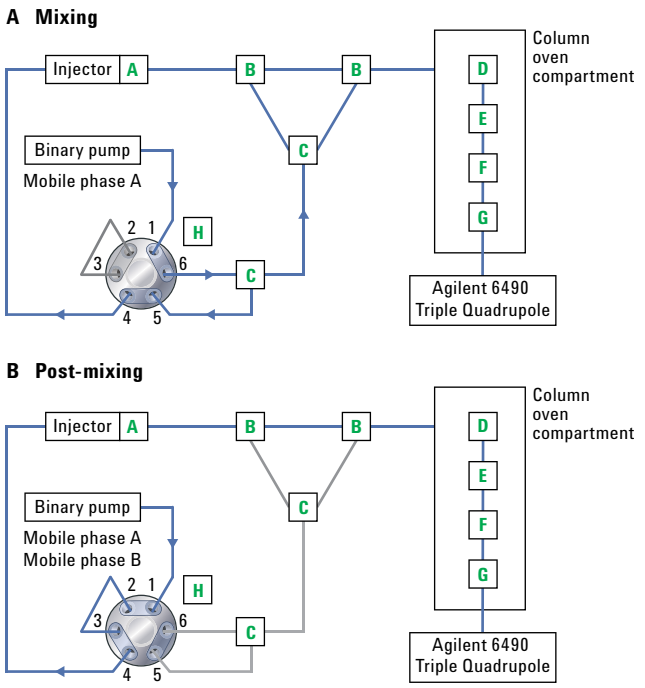


Figure 4. Peak shape of early eluting pesticides methamidophos, cyromazine, acephate, and omethoate injected in 3 µL of acetonitrile using the online dilution system depicted in Figure 2 (Note: thiabendazole was added to the picture to show the peak shape of this not as early eluting but often tailing analyte).



Components

A 2 µm in-line direct connect SS filter (Analytical Sales & Services, p/n 48812) threaded directly to the injector valve

B 25 µL high-pressure static mixer (Resolution Systems, p/n 402-0025HP)

C Valco SS mixing tee 1/16 inch 0.25 mm bore (Resolution Systems, PN ZT1C or Sigma-Aldrich, p/n 58626)

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E Heat exchanger (p/n G1316-80002)

F UHPLC (1,200 bar) guard column Agilent ZORBAX Eclipse Plus C18, 2.1 × 5 mm, 1.8 µm (p/n 821725-901)

G UHPLC (1,200 bar) column Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 100 mm, 1.8 µm (p/n 959758-902)

H 6-port, 2-position valve (1,200 bar)

Figure 5. Alternative on-line dilution setup without the use of the second high-pressure pump. Arrows indicate the mobile phase flow direction during (A) mixing of the injected sample with mobile phase when the binary pump flow is split between the injector and the 2-mixer system and (B) post-mixing when the binary pump gradient starts. Components: A–G the same as in Figure 2; H: 6-port, 2-position valve (1,200 bar).

Final LC/MS/MS method optimization

The final method optimization involved mainly selection of the method MRMs for each analyte and optimization of the tMRM conditions, LC gradient (analyte separation), MS source conditions, and injection volume.

The initial MRM optimization usually provided 10 MRMs per analytes. These MRMs were then ranked based on their sensitivity and also selectivity, which was evaluated in multiple challenging matrices. Two (in some cases three) top-ranked MRMs were included in the tMRM draft method as the primary MRMs (that is, to be collected throughout the entire analyte acquisition window). The draft method was then supplemented with triggered MRMs to create a total of four MRMs per compound in the majority of cases. The distribution of MRMs throughout the run was then evaluated using histograms. This approach permitted optimization of the final mobile phase gradient to spread the analytes and MRMs evenly throughout the run. Special attention was paid to the separation of critical pairs to make sure that those compounds could be resolved using MS/MS and/or chromatography. Using the optimized separation conditions, further triggered MRMs were added to some compounds for the final method. In some cases this meant as many as 6–7 total MRMs per analyte (see Table 1). Only two MRMs were used for internal standards. The total MRMs for individual analytes depended on several factors, including the actual number of viable transitions or amenability to the GC/MS/MS analysis (more MRMs were added for compounds amenable only to LC/MS/MS). Figure 6 shows the chromatographic separation and also histograms (obtained in the MassHunter acquisition software) illustrating the distribution of 968 primary MRMs and 2,070 total MRMs.

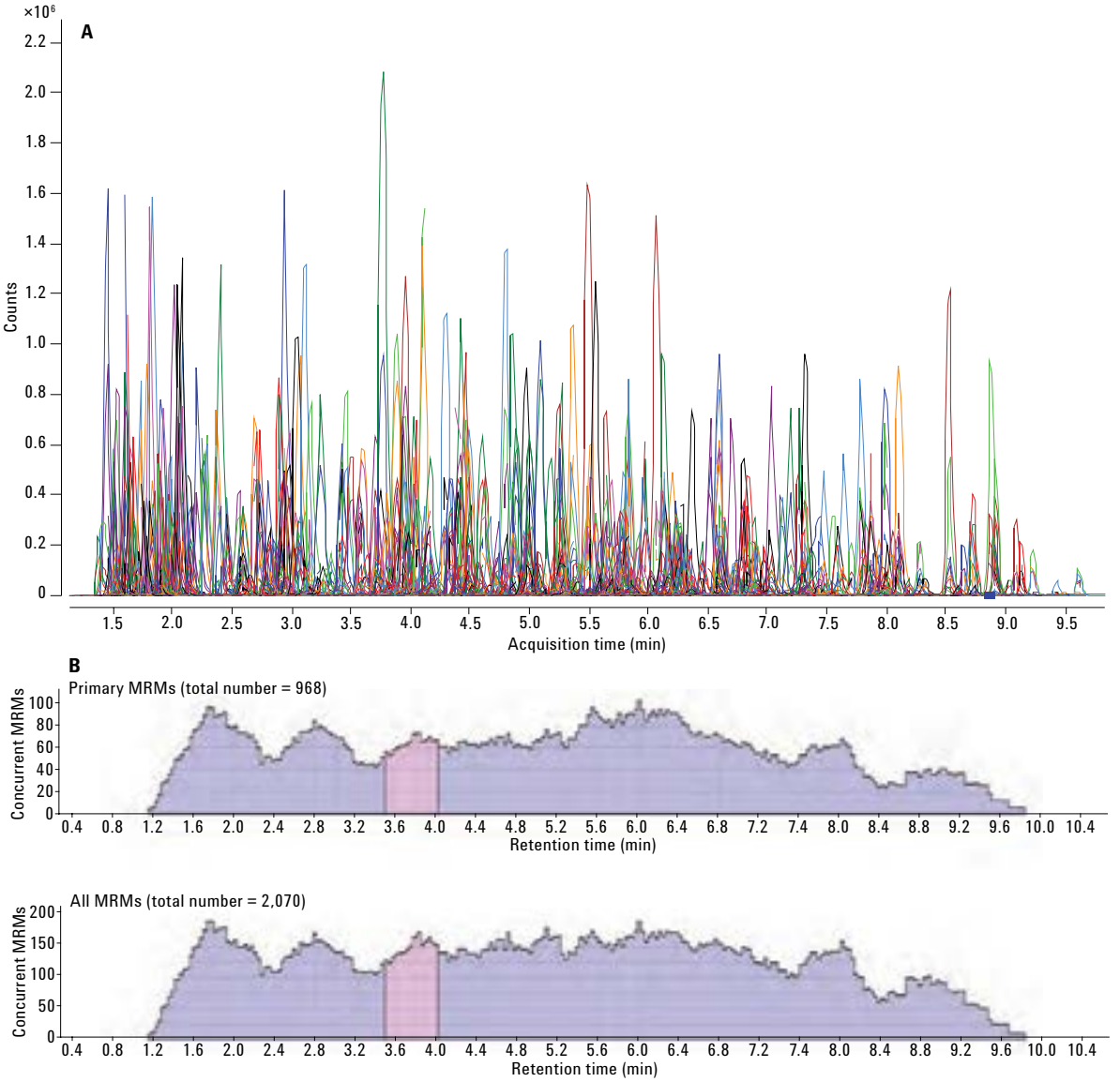

Figure 6. Extracted ion chromatograms of the analytes included in the method, and MRM histograms showing distribution of the analytes including their primary MRMs and all MRMs.

Using the final method MRMs, different cycle times were tested to evaluate repeatability of quantification (peak areas) at different settings. The cycle time parameter affects the number of data points across a peak and dictates minimum dwell time for the MRM acquisition. A cycle time of 650 ms was selected for the final method, providing a minimum primary MRM dwell time of 5.22 ms, and at least seven to eight data points above baseline for good analyte quantitation. This translated to good repeatability obtained in the method validation throughout the chromatographic run, as demonstrated in Figure 7.

Source conditions and injection volume were fine-tuned using the final method LC gradient and MRM program. We initially targeted 3 µL as the injection volume for undiluted QuEChERS extracts to replace a previously used 10 µL injection of three-fold diluted extracts analyzed on a different LC/MS/MS system. However, the sensitivity of the 6490 triple quadrupole LC/MS allowed us to use a lower injection volume of 2 µL, which has the benefit of a reduced matrix introduction into the system.

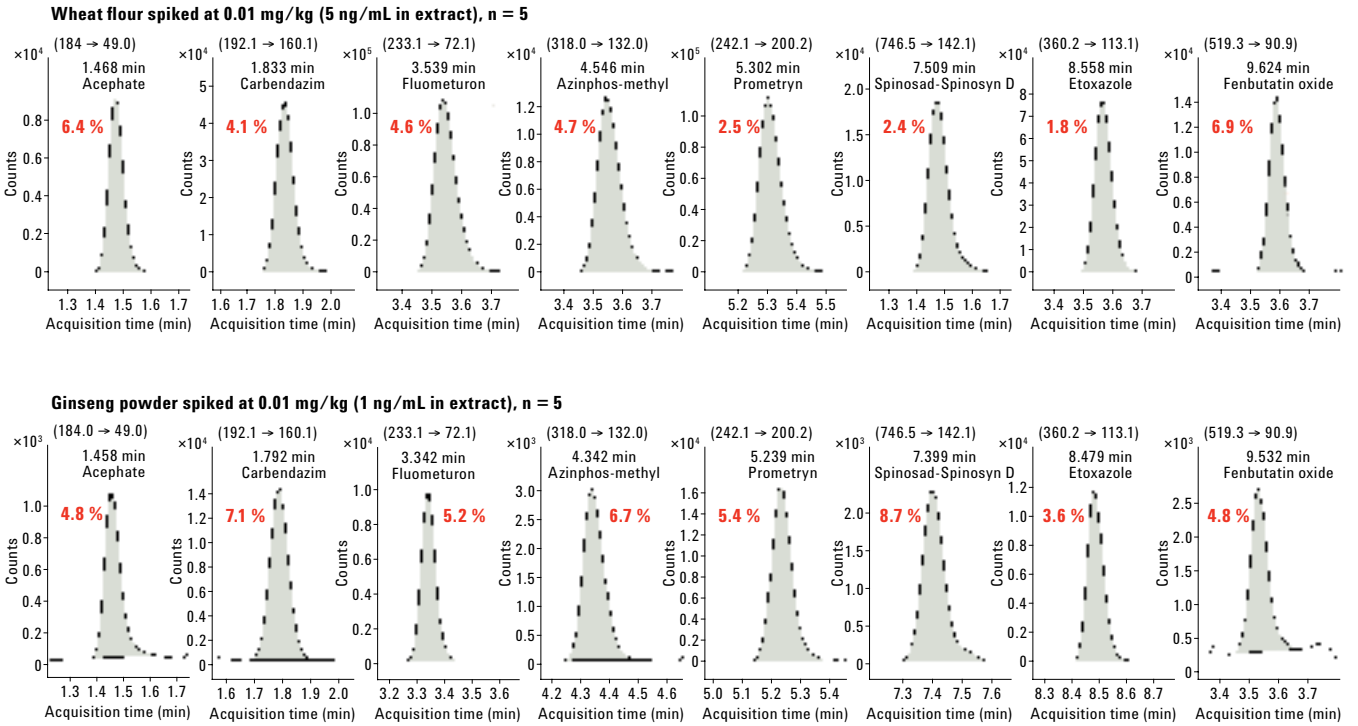

Figure 7. Chromatograms of quantitation MRMs and repeatability results (% RSD, five replicates) obtained during the method validation in wheat flour and ginseng powder for selected analytes from different pesticide classes eluting throughout the chromatographic run.

Interlaboratory method transfer and validation

The method development, especially the MRM optimization, was done in four different laboratories. The final method was assembled at one site, then transferred onto multiple identically configured 1290 Infinity II LC systems with 6490 triple quadrupole LC/MS instruments in three different pesticide routine testing laboratories in the US, Europe, and Asia, which then conducted the method validation. The method transfer mainly involved verification or an update of the analyte retention times using MassHunter software.

The method was validated in multiple commodity types/matrices using the SANTE method validation guidelines and criteria [1]. A method-validated limit of quantitation (LOQ) of 0.01 mg/kg was achieved for the majority analyte-matrix combinations. The evaluated matrices included representative matrices from the following SANTE commodity groups:

- High water content
- High water content, high acid content
- High sugar content, low water content
- High oil content, low water content
- High starch/protein, low water/fat
- Difficult/unique commodities
- Milk and milk products.

Examples of the validation results are provided in the supplemental information to this application note [7], which shows recoveries and RSDs of pesticides included in Agilent LC/MS mixes one to eight (p/n 5190-0551), obtained during the method validation in tomato, orange juice, spinach, and wheat flour.

Analyte identification using triggered MRM

The tMRM function provides additional information beyond the minimum identification criteria, providing increased identification confidence. It also serves as a useful tool for eliminating false positive results and effectively dealing with suspect results. Figure 8 gives an example of a suspect result for a pesticide, fenhexamid, in a highly complex botanical extract sample. The retention time in the sample matches the retention time of the analyte in the reference standard analyzed in the same sample batch. The qualifier transition *m/z* 302.1 → 97.1 is present in the sample at the same retention time, but the ion ratio is not within the tolerance.

However, this is a highly complex sample where potential matrix interferences could affect the ion ratio. Therefore, having the additional MRM information is helpful for fast decision making and dismissing this as suspect because the additional tMRMs do not match, with one of them (*m/z* 302.1 → 143.1) actually missing.

The same botanical extract sample contained an herbicide, imazathepyr. Figure 9 shows that, in this case, there were five well matching MRMs, providing confidence in the identification of imazathepyr in this complex sample.

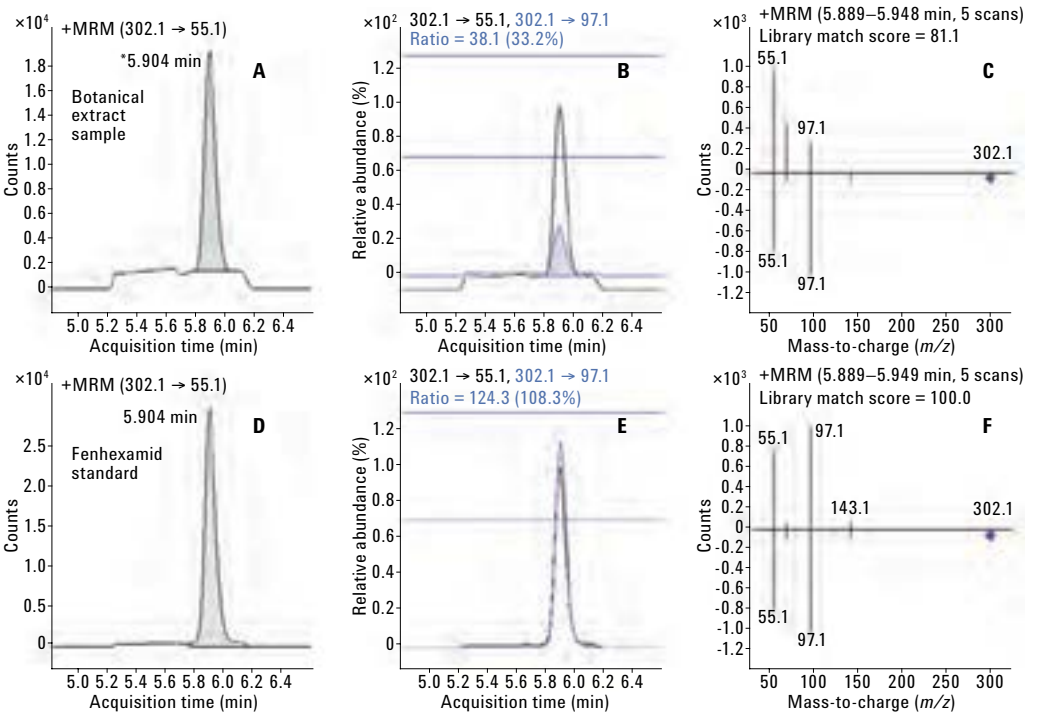

Figure 8. Example of a suspect result for fenhexamid in a botanical extract sample, comparing its quantitation MRM (A), overlay of quantitation and qualification MRMs (B), and tMRM library match (C) in the sample with those obtained for a fenhexamid reference standard analyzed in the same sample batch (D, E, and F, respectively). The suspect result was dismissed due to the missing m/z 302.1 → 143.1 transition, and two additional MRMs with an incorrect ion ratio in the sample.

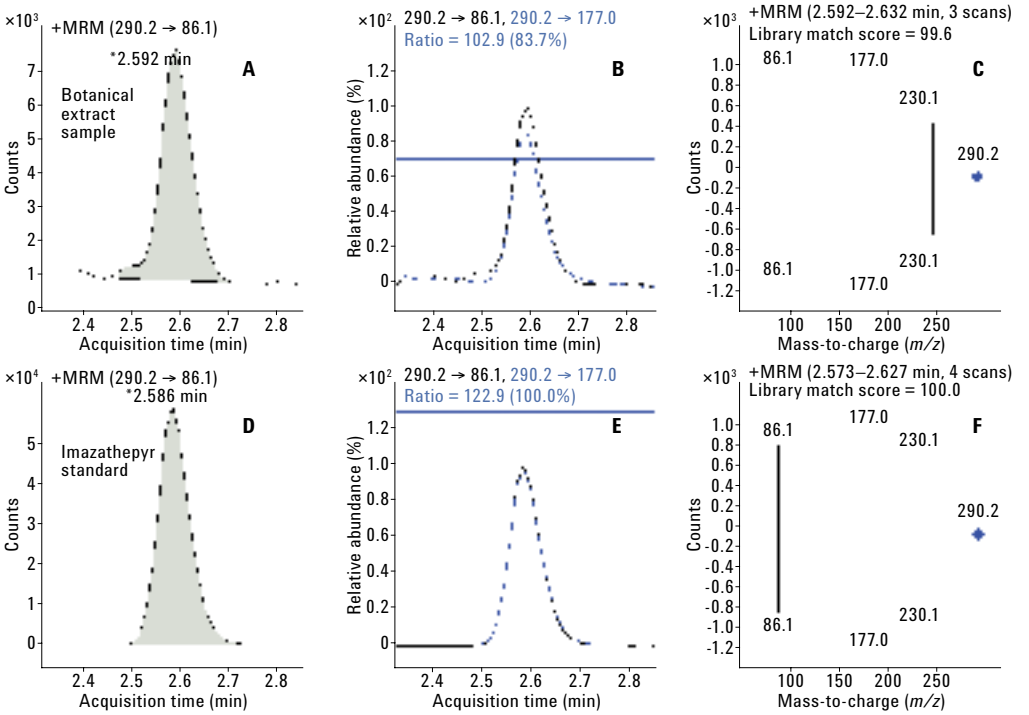

Figure 9. Example of positive identification of imazathepyr in a botanical extract sample, comparing its quantification MRM (A), overlay of quantitation and qualification MRMs (B), and tMRM library match (C) in the sample with those obtained for a imazathepyr reference standard analyzed in the same sample batch (D, E, and F, respectively). A high identification confidence was achieved due to five matching MRMs (two primary and three triggered).

Conclusions

This LC/MS/MS method provides fast and reliable analysis of about 450 globally important pesticides in various food commodities. It uses the tMRM function for increased identification confidence and effective dealing with suspect results. Robust online dilution setup provides excellent peak shapes and retention of early eluting (more polar) analytes, which are notorious troublemakers in other multiresidue pesticide LC/MS/MS methods. The method was successfully transferred and validated in three different laboratories using Agilent 1290 Infinity II LC systems coupled to Agilent 6490 triple quadrupole LC/MS instruments.

View the full validated results:

Validation Results for LC/MS/MS Pesticide Multiresidue Analysis using Triggered MRM and Online Dilution

Acknowledgements

The authors wish to acknowledge Laura Harrison, Camilla Midtlien, and Max Chang from Covance Laboratories for their contribution to the method development, and thanks to Agilent Technologies, specifically John Lee, Steve Royce, and Andre Santos, for their support of this collaborative project between Covance Food Solutions and Agilent Technologies.

Table 1. List of Compounds Included in the Method Together with Their Chemical Abstract Service (CAS) Numbers, Retention Times (RT), Primary Transitions, Collision Energies (CE), and Total Number of Transitions in the tMRM Program. For Compounds Provided in Agilent LC/MS mixes 1–8 (p/n 5190-0551), the Respective Mix Number is Aso Listed

Compound	CAS	Agilent Mix No.	RT (min)	Primary transition (CE)	No. of transitions
Abamectin - Avermectin B1a	71751-41-2	7	9.09	890.5 → 305.1 (28); 890.5 → 307.1 (16); 890.5 → 567.4 (12)	5
Acephate	30560-19-1	1	1.50	184.0 → 143.0 (4); 184.0 → 49.0 (20)	4
Acetamiprid	135410-20-7	5	1.96	223.1 → 125.9 (16); 223.1 → 89.9 (36)	4
Acetochlor	34256-82-1		5.89	270.1 → 59.2 (28); 270.1 → 148.2 (20); 270.1 → 224.2 (12)	4
Acibenzolar-S-methyl	135158-54-2		4.80	211.0 → 135.9 (32); 211.0 → 139.9 (24)	5
Aclonifen	74070-46-5		6.26	265.0 → 182.2 (28); 265.0 → 218.1 (24)	4
Acrinathrin	101007-06-1		8.91	559.2 → 208.0 (16); 559.2 → 181.0 (32); 559.2 → 82.8 (14)	4
Alachlor	15972-60-8		5.89	270.1 → 45.0 (32); 270.1 → 238.0 (12); 270.1 → 162.3 (20)	4
Alanycarb	83130-01-2	5	6.52	400.1 → 238.1 (8); 400.1 → 91.1 (56)	4
Aldicarb	116-06-3	5	2.42	208.1 → 116.0 (8); 208.1 → 89.0 (12)	5
Aldicarb sulfone (Aldoxycarb)	1646-88-4		1.57	240.1 → 76.2 (12); 240.1 → 148.2 (16)	4
Aldicarb sulfoxide	1646-87-3		1.54	207.1 → 89.0 (12); 207.1 → 65.0 (20)	4
Allethrin	584-79-2		8.04	303.2 → 135.0 (8); 303.2 → 107.0 (20)	4
Ametryn	834-12-8		4.46	228.1 → 186.2 (16); 228.1 → 68.0 (52)	5
Amidosulfuron	120923-37-7	4	3.24	370.1 → 218.1 (24); 370.1 → 69.1 (56)	5
Aminocarb	2032-59-9	4	1.60	209.1 → 137.0 (24); 209.1 → 122.0 (48)	4
Amitraz	33089-61-1		8.85	294.2 → 163.0 (12); 294.2 → 122.0 (32)	6
Amitraz metabolite DMF	60397-77-5		2.66	150.1 → 107.1 (20); 150.1 → 106.1 (40)	5
Amitraz metabolite DMPF	33089-74-6		1.72	163.1 → 122.1 (16); 163.1 → 106.1 (48)	4
Anilofos	64249-01-0		6.57	368.0 → 198.9 (12); 368.0 → 124.9 (44)	4
Atrazine	1912-24-9		3.91	216.1 → 174.0 (16); 216.1 → 68.1 (36)	5
Azaconazole	60207-31-0	1	4.21	300.0 → 159.1 (44); 300.0 → 231.1 (20)	5
Azamethiphos	35575-96-3	2	2.75	325.0 → 112.1 (40); 325.0 → 76.1 (60)	4
Azinphos-ethyl	2642-71-9	1	5.81	346.1 → 132.0 (16); 346.1 → 160.1 (4); 346.1 → 289.1 (4)	4
Azinphos-methyl	86-50-0	1	4.51	318.0 → 125.0 (24); 318.0 → 260.9 (4); 318.0 → 132.0 (16)	5
Azoxystrobin	131860-33-8	5	4.94	404.1 → 372.0 (12); 404.1 → 329.1 (36)	5
Beflubutamid	113614-08-7	8	6.41	356.1 → 91.1 (40); 356.1 → 65.1 (68)	4
Benalaxyl	71626-11-4	2	6.63	326.2 → 148.2 (24); 326.2 → 91.2 (52)	4
Bendiocarb	22781-23-3		2.90	224.1 → 109.0 (12); 224.1 → 167.0 (4)	4
Benfuracarb	82560-54-1	4	7.72	411.2 → 195.1 (36); 411.2 → 252.1 (12)	5
Benoxacor	98730-04-2		4.55	260.0 → 149.0 (16); 260.0 → 134.0 (36)	5
Bensulide	741-58-2		6.30	398.1 → 77.0 (60); 398.1 → 157.8 (24)	4
Bentazone	25057-89-0		2.42	241.1 → 198.9 (8); 241.1 → 80.0 (56)	4
Benzoximate	29104-30-1	7	6.95	364.1 → 199.0 (8); 364.1 → 105.1 (36)	5
Bifenazate	149877-41-8	8	5.63	301.2 → 198.1 (4); 301.2 → 170.1 (24)	4
Bifenthrin	82657-04-3	2	9.27	440.2 → 181.2 (20); 440.2 → 166.2 (52)	4
Bispyribac	125401-92-5	7	5.17	431.1 → 275.1 (12); 431.1 → 118.9 (48)	5
Bitertanol	55179-31-2	3	6.91	338.2 → 70.3 (4); 338.2 → 269.3 (4)	5
Bixafen	581809-46-3		6.31	414.0 → 265.9 (28); 414.0 → 394.1 (16)	4
Boscalid	188425-85-6	4	5.20	343.0 → 307.0 (20); 343.0 → 140.0 (20)	5
Bromacil	314-40-9		2.97	261.0 → 204.9 (12); 261.0 → 187.8 (32)	4
Bromuconazole I	116255-48-2	2	5.56	378.0 → 159.1 (44); 378.0 → 70.1 (20)	2
Bromuconazole II	116255-48-2	2	6.25	378.0 → 159.1 (40); 378.0 → 70.1 (24)	2
Bupirimate	41483-43-6	2	5.99	317.2 → 166.1 (18); 317.2 → 108.1 (28)	4
Buprofezin	69327-76-0	1	7.87	306.2 → 201.1 (12); 306.2 → 116.0 (12)	5

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Compound	CAS	Agilent Mix No.	RT (min)	Primary transition (CE)	No. of transitions
Butachlor	23184-66-9		8.04	312.2 → 57.0 (24); 312.2 → 238.0 (8)	5
Butafenacil	134605-64-4		5.83	492.1 → 179.9 (52); 492.1 → 330.9 (24)	4
Butocarboxim	34681-10-2	4	2.37	213.1 → 75.1 (12); 213.1 → 47.1 (48)	3
Butocarboxim sulfoxide	34681-24-8		1.51	207.1 → 74.9 (12); 207.1 → 87.9 (8)	4
Butoxycarboxim (butocarboxim sulfone)	34681-23-7		1.57	240.1 → 106.0 (4); 240.1 → 44.1 (24)	4
Butylate	2008-41-5		7.56	218.2 → 57.1 (12); 218.2 → 156.1 (8); 218.2 → 100.1 (12)	4
Cadusafos	95465-99-9		7.14	271.1 → 97.0 (40); 271.1 → 158.9 (12)	5
Carbaryl	63-25-2	6	3.24	202.1 → 145.1 (4); 202.1 → 127.0 (28)	5
Carbendazim	10605-21-7	5	1.87	192.1 → 160.1 (16); 192.1 → 132.1 (36)	4
Carbetamide	16118-49-3		2.63	237.1 → 118.0 (12); 237.1 → 72.0 (36)	5
Carbofuran	1563-66-2	8	2.96	222.1 → 123.0 (20); 222.1 → 165.1 (8)	4
Carbofuran-3-hydroxy-	16655-82-6		1.94	238.1 → 163.2 (12); 238.1 → 181.0 (4)	4
Carbosulfan	55285-14-8	6	9.14	381.2 → 118.0 (20); 381.2 → 160.1 (12)	6
Carboxin	5234-68-4	5	3.23	236.1 → 143.1 (12); 236.1 → 87.1 (28)	5
Carfentrazone-ethyl	128639-02-1	4	6.39	412.1 → 346.1 (24); 412.1 → 366.1 (16)	4
Chlorantraniliprole	500008-45-7	8	4.59	484 → 452.9 (20); 484 → 286.1 (20)	5
Chlorbromuron	13360-45-7		5.03	293.0 → 181.9 (12); 293.0 → 203.8 (16)	5
Chlordimeform	6164-98-3		1.78	197.1 → 46.2 (20); 197.1 → 116.9 (28)	4
Chlorfenvinphos I	470-90-6	2	6.67	359.0 → 155.1 (12); 359.0 → 99.1 (36)	4
Chlorfenvinphos II	470-90-6	2	6.92	359.0 → 155.1 (12); 359.0 → 99.1 (36)	4
Chlorfluazuron	71422-67-8		8.76	540.0 → 382.9 (28); 540.0 → 158.0 (24)	6
Chloridazon (Pyrazon)	1698-60-8	4	2.05	222.0 → 104.1 (28); 222.0 → 92.1 (36)	4
Chlorimuron-ethyl (Classic)	90982-32-4		5.28	415.1 → 185.9 (24); 415.1 → 184.9 (28)	5
Chlorotoluron (Chlortoluron)	15545-48-9	7	3.69	213.1 → 72.0 (20); 213.1 → 46.1 (16)	5
Chloroxuron	1982-47-4	7	5.59	291.1 → 72.0 (20); 291.1 → 46.1 (20)	4
Chlorpyrifos	2921-88-2	2	8.21	349.9 → 97.0 (40); 349.9 → 197.8 (28)	5
Chlorpyrifos-methyl	5598-13-0	2	7.07	321.9 → 47.1 (48); 321.9 → 125.0 (20)	2
Chlorsulfuron	64902-72-3	4	3.16	358.0 → 141.1 (24); 358.0 → 167.1 (20)	4
Clethodim I	99129-21-2	3	5.48	360.1 → 164.1 (16); 360.1 → 240.1 (12)	4
Clethodim II	99129-21-2	3	7.51	360.1 → 164.1 (16); 360.1 → 166.1 (32)	5
Clodinafop-propargyl	105512-06-9		6.35	350.1 → 265.9 (12); 350.1 → 90.9 (36)	4
Clofentezine	74115-24-5	4	6.82	303.0 → 138.1 (12); 303.0 → 102.1 (44)	5
Clomazone	81777-89-1	8	4.59	240.1 → 125.1 (32); 240.1 → 89.1 (68)	5
Cloquintocet-mexyl	99607-70-2		8.01	336.1 → 237.9 (16); 336.1 → 178.9 (36)	5
Clothianidin	210880-92-5		1.86	250.0 → 132.1 (16); 250.0 → 169.2 (12)	4
Coumaphos	56-72-4	2	6.64	363.0 → 227.1 (28); 363.0 → 307.1 (16)	4
Cyanazine	21725-46-2		2.64	241.1 → 214.2 (16); 241.1 → 103.9 (28)	5
Cyanofenphos	13067-93-1		6.48	304.1 → 156.9 (20); 304.1 → 119.9 (20); 304.1 → 62.9 (44)	3
Cyazofamid	120116-88-3	4	5.96	325.1 → 108.1 (20); 325.1 → 44.1 (36)	4
Cycloate	1134-23-2	1	7.13	216.1 → 83.1 (12); 216.1 → 55.1 (36)	5
Cycloxydim	101205-02-1		7.46	326.2 → 180.0 (24); 326.2 → 100.9 (24)	5
Cycluron	2163-69-1	7	4.24	199.2 → 89.1 (12); 199.2 → 69.1 (20)	5
Cyflufenamid	180409-60-3		6.92	413.1 → 222.9 (20); 413.1 → 202.9 (40)	5
Cyhexatin	91465-08-6		9.02	365.0 → 201.0 (16); 365.0 → 81.2 (28)	4

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Compound	CAS	Agilent Mix No.	RT (min)	Primary transition (CE)	No. of transitions
Cymiazole	61676-87-7	1	1.99	219.1 → 171.2 (28); 219.1 → 100.0 (30)	4
Cymoxanil	57966-95-7	4	2.14	199.1 → 128.1 (8); 199.1 → 111.1 (16)	4
Cyproconazole I	94361-06-5	1	5.65	292.1 → 70.1 (48); 292.1 → 125.1 (40)	3
Cyproconazole II	94361-06-5	1	6.07	292.1 → 70.1 (48); 292.1 → 125.1 (40)	3
Cyprodinil	121552-61-2	8	6.17	226.1 → 93.0 (36); 226.1 → 77.1 (56)	4
Cyromazine	66215-27-8		1.41	167.1 → 85.0 (16); 167.1 → 68.0 (40)	4
DEET (Diethyltoluamide)	134-62-3	4	4.03	192.1 → 119.1 (20); 192.1 → 91.1 (40)	5
Demeton-S (disulfoton oxon)	126-75-0		4.67	259.1 → 89.0 (4); 259.1 → 61.0 (44)	2
Demeton-S-methyl	919-86-8		3.05	231.0 → 89.0 (8); 231.0 → 61.0 (40)	2
Demeton-S-methyl sulfone	17040-19-6		1.66	263.0 → 109.1 (32); 263.0 → 169.0 (12)	4
Desmedipham	13684-56-5	8	4.42	318.1 → 182.1 (16); 318.1 → 136.1 (36)	5
Dialifos (Dialifor)	10311-84-9		7.06	394.0 → 207.9 (20); 394.0 → 186.9 (8)	5
Diazinon	333-41-5	2	6.65	305.1 → 97.1 (36); 305.1 → 169.1 (20)	4
Diazinon oxon	962-58-3		4.56	289.1 → 135.1 (28); 289.1 → 233.0 (20)	5
Dichlofluanid	1085-98-9		5.73	333.0 → 123.0 (32); 333.0 → 224.0 (16); 350.0 → 123.0 (32)	5
Dichlorvos	62-73-7	2	2.85	221.0 → 109.1 (16); 221.0 → 79.1 (28)	4
Diclobutrazol	75736-33-3		6.36	328.1 → 69.9 (36); 328.1 → 158.9 (40)	4
Diclocymet I	139920-32-4		5.92	313.1 → 172.9 (16); 313.1 → 101.9 (52)	4
Diclocymet II	139920-32-4		6.15	313.1 → 172.9 (16); 313.1 → 101.9 (52)	4
Dicrotophos	141-66-2		1.75	238.1 → 112.2 (12); 238.1 → 72.0 (32)	4
Diethofencarb	87130-20-9	6	4.89	268.2 → 124.0 (32); 268.2 → 226.1 (4)	5
Difenoconazole	119446-68-3	3	7.13	406.1 → 251.1 (24); 406.1 → 188.1 (48)	5
Diflubenzuron	35367-38-5	4	6.08	311.0 → 158.1 (24); 311.0 → 141.1 (56)	4
Diflufenican	83164-33-4	1	7.31	395.1 → 266.0 (28); 395.1 → 246.0 (40)	5
Dimethachlor	50563-36-5	1	4.38	256.1 → 224.1 (24); 256.1 → 148.1 (36)	5
Dimethametryn	22936-75-0		6.09	256.2 → 95.9 (28); 256.2 → 90.9 (32)	4
Dimethenamid	87674-68-8		5.03	276.1 → 244.0 (16); 276.1 → 168.1 (28)	5
Dimethoate	60-51-5	8	2.00	230.0 → 125.0 (24); 230.0 → 47.0 (56)	4
Dimethomorph I	110488-70-5	5	5.06	388.1 → 301.1 (24); 388.1 → 165.1 (36)	5
Dimethomorph II	110488-70-5	5	5.37	388.1 → 301.1 (24); 388.1 → 165.1 (36)	5
Dimetilan	644-64-4		2.03	241.1 → 71.9 (24); 241.1 → 196.0 (8)	3
Dimoxystrobin	149961-52-4	1	6.32	327.2 → 205.1 (12); 327.2 → 116.1 (40)	4
Diniconazole	83657-24-3	2	7.01	326.1 → 70.0 (36); 328.1 → 70.1 (36)	4
Dinitramine	29091-05-2		6.78	323.1 → 289.3 (16); 323.1 → 194.9 (44)	4
Dinotefuran	165252-70-0	7	1.55	203.1 → 129.1 (12); 203.1 → 114.0 (12)	4
Dioxacarb	6988-21-2	7	1.99	224.1 → 123.1 (20); 224.1 → 167.1 (12)	4
Diphenamid	957-51-7		4.38	240.1 → 134.0 (28); 240.1 → 91.0 (48)	5
Dipropetryn	4147-51-7		6.12	256.2 → 213.9 (20); 256.2 → 101.9 (48)	4
Disulfoton	298-04-4	1	7.00	275.1 → 89.0 (8); 275.1 → 61.0 (40)	2
Disulfoton sulfone	2497-06-5		3.81	307.0 → 96.9 (36); 307.0 → 125.1 (20)	5
Disulfoton sulfoxide	2497-07-6		3.67	291.0 → 185.0 (12); 291.0 → 96.8 (44)	5
Diuron	330-54-1	5	4.11	233.0 → 72.0 (20); 233.0 → 46.0 (16); 235.0 → 72.1 (20)	4
DMSA (Dimethylphenylsulfamide)	4710-17-2		2.41	201.1 → 92.0 (16); 201.1 → 65.0 (36)	4
DMST (Dimethylaminosulfotoluidide)	66840-71-9		3.08	215.1 → 77.0 (52); 215.1 → 51.1 (60)	3

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Compound	CAS	Agilent Mix No.	RT (min)	Primary transition (CE)	No. of transitions
Dodemorph I	1593-77-7		4.40	282.3 → 116.0 (16); 282.3 → 98.0 (24)	5
Dodemorph II	1593-77-7		4.60	282.3 → 116.0 (16); 282.3 → 98.0 (24)	5
Dodine	2439-10-3		6.77	228.3 → 59.9 (24); 228.3 → 56.9 (24)	5
Doramectin	117704-25-3		9.27	916.5 → 331.2 (24); 916.5 → 113.1 (52)	4
Emamectin B1a benzoate	117704-25-3		8.20	886.5 → 157.9 (44); 886.5 → 81.9 (72)	5
Emamectin B1b benzoate	117704-25-3		7.87	872.5 → 157.9 (40); 872.5 → 81.9 (56)	4
Epoxiconazole	133855-98-8	2	5.96	330.1 → 121.1 (24); 330.1 → 101.1 (48)	4
Eprinomectin B1a	123997-26-2		9.00	914.5 → 186.1 (24); 914.5 → 153.9 (44)	5
Ethaboxam	162650-77-3		3.71	321 → 183.1 (24); 321 → 200.1 (28)	5
Ethidimuron (Sulfadiazole)	30043-49-3	8	1.85	265.1 → 208.0 (12); 265.1 → 113.9 (20)	4
Ethiofencarb	29973-13-5		3.48	226.1 → 107.2 (12); 226.1 → 77.1 (56)	6
Ethiofencarb sulfone	53380-23-7		1.80	275.1 → 106.9 (20); 275.1 → 200.9 (8)	4
Ethiofencarb sulfoxide	53380-22-6		1.82	242.1 → 106.9 (16); 242.1 → 163.9 (8)	4
Ethion	563-12-2	2	8.10	385.0 → 199.1 (12); 385.0 → 97.1 (52)	5
Ethiprole	181587-01-9		5.08	397.0 → 351.1 (20); 397.0 → 255.1 (44)	5
Ethirimol	23947-60-6	4	2.80	210.2 → 98.1 (32); 210.2 → 140.1 (28)	4
Ethofumesate	26225-79-6	4	4.88	287.1 → 121.1 (16); 287.1 → 161.1 (20)	5
Ethoprophos (Ethoprop)	13194-48-4	2	5.88	243.1 → 97.1 (32); 243.1 → 131.1 (20)	4
Ethoxyquin	91-53-2	8	4.96	218.2 → 160.2 (36); 218.2 → 174.0 (32)	5
Etofenprox	80844-07-1	3	9.23	394.2 → 177.1 (16); 394.2 → 107.0 (44)	6
Etoxazole	153233-91-1		8.54	360.2 → 141.0 (32); 360.2 → 113.1 (60)	6
Famoxadone	131807-57-3	4	6.70	392.1 → 331.0 (8); 392.1 → 93.0 (44)	5
Fenamidone	161326-34-7	5	5.06	312.1 → 92.1 (24); 312.1 → 236.2 (8)	5
Fenamiphos	22224-92-6	1	6.18	304.1 → 217.0 (24); 304.1 → 202.0 (40)	4
Fenamiphos sulfone	31972-44-8		3.13	336.1 → 308.1 (12); 336.1 → 265.8 (12)	4
Fenamiphos sulfoxide	31972-43-7		2.97	320.1 → 108.0 (48); 320.1 → 171.0 (24)	4
Fenarimol	60168-88-9	2	5.79	331.0 → 81.1 (28); 331.0 → 139.1 (36)	4
Fenazaquin	120928-09-8	5	8.90	307.2 → 57.1 (28); 307.2 → 161.1 (14)	6
Fenbuconazole	114369-43-6	2	6.08	337.1 → 125.1 (40); 337.1 → 70.1 (28)	4
Fenbutatin oxide	13356-08-6		9.62	519.3 → 90.9 (76); 519.3 → 196.9 (56); 517.3 → 90.9 (72); 517.3 → 194.9 (56)	6
Fenchlorphos oxon	3983-45-7		5.75	304.9 → 109.1 (20); 306.9 → 109.0 (20)	4
Fenhexamid	126833-17-8	3	5.79	302.1 → 97.1 (28); 302.1 → 55.1 (48)	4
Fenobucarb	3766-81-2	5	4.77	208.1 → 95.1 (12); 208.1 → 77.1 (48)	5
Fenoxanil I	115852-48-7		6.22	329.1 → 86.0 (24); 329.1 → 189.0 (24)	4
Fenoxanil II	115852-48-7		6.22	329.1 → 86.0 (24); 329.1 → 189.0 (24)	4
Fenoxycarb	72490-01-8	6	6.21	302.1 → 88.0 (20); 302.1 → 116.2 (8)	4
Fenpropathrin	39515-41-8		8.65	350.2 → 125.1 (12); 350.2 → 55.1 (44)	2
Fenpropidin	67306-00-7	5	4.49	274.3 → 147.1 (28); 274.3 → 86.1 (28)	5
Fenpropimorph	67564-91-4		4.99	304.3 → 147.2 (32); 304.3 → 117.1 (64)	5
Fenpyroximate	111812-58-9	5	8.72	422.2 → 366.1 (16); 422.2 → 135.1 (36)	6
Fensulfothion	115-90-2		4.07	309.0 → 157.0 (28); 309.0 → 173.0 (24)	5
Fensulfothion oxon	6552-21-2		2.23	293.1 → 94.0 (52); 293.1 → 140.0 (44)	5
Fensulfothion oxon sulfone	6132-17-8		2.32	309.1 → 253.0 (16); 309.1 → 175.0 (28)	5
Fensulfothion sulfone	14255-72-2		4.27	325.0 → 268.9 (12); 325.0 → 191.0 (24)	5

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Compound	CAS	Agilent Mix No.	RT (min)	Primary transition (CE)	No. of transitions
Fenthion	55-38-9		6.47	279.0 → 169.0 (16); 279.0 → 105.2 (24)	4
Fenthion oxon	6552-12-1		4.52	263.1 → 231.2 (16); 263.1 → 216.0 (24)	5
Fenthion oxon sulfone	14086-35-2		1.97	295.0 → 217.1 (16); 295.0 → 104.2 (24)	4
Fenthion oxon sulfoxide	6552-13-2		1.91	279.1 → 104.1 (32); 279.1 → 264.2 (16)	4
Fenthion sulfone	3761-42-0		3.34	311.0 → 125.0 (24); 311.0 → 109.1 (32)	5
Fenthion sulfoxide	3761-41-9		3.10	295.0 → 109.1 (36); 295.0 → 280.1 (16)	4
Fentin	76-87-9		4.31	351.0 → 197.0 (32); 351.0 → 120.0 (60); 349.0 → 195.0 (32)	4
Fentrazamide	158237-07-1		6.58	350.1 → 83.0 (24); 350.1 → 154.1 (12)	4
Fenuron	101-42-8	7	2.01	165.1 → 72.0 (24); 165.1 → 46.1 (8)	4
Fipronil	120068-37-3	4	6.20	454.0 → 368.1 (26); 454.0 → 255.0 (44)	4
Flazasulfuron	104040-78-0	4	4.50	408.1 → 182.1 (24); 408.1 → 83.1 (60)	5
Flonicamid	158062-67-0	6	1.69	230.1 → 203.1 (16); 230.1 → 174.1 (16)	4
Fluazifop-butyl	69806-50-4		7.80	384.1 → 282.2 (20); 384.1 → 328.2 (16)	5
Flubendiamide	272451-65-7	7	6.47	683.0 → 408.0 (8); 683.0 → 273.9 (40)	3
Flucarbazone-sodium	181274-17-9		2.04	414.0 → 129.9 (24); 414.0 → 114.9 (56)	4
Fludioxonil	131341-86-1	2	5.06	266.1 → 229.1 (8); 266.1 → 158.1 (36)	5
Flufenacet	142459-58-3	1	5.85	364.1 → 152.0 (20); 364.1 → 194.1 (8)	4
Flufenoxuron	101463-69-8	4	8.53	489.1 → 158.1 (16); 489.1 → 141.1 (56)	4
Flumethrin	69770-45-2		9.15	527.1 → 267.0 (12); 527.1 → 238.9 (24); 527.1 → 202.9 (36)	5
Flumetsulam	98967-40-9	8	1.84	326.1 → 129.1 (36); 326.1 → 109.1 (68)	4
Flumioxazin	103361-09-7	6	4.61	355.1 → 299.1 (32); 355.1 → 107.0 (36)	5
Fluometuron	2164-17-2	8	3.55	233.1 → 72.1 (24); 233.1 → 46.1 (16)	4
Fluopicolide	239110-15-7	1	5.34	383.0 → 173.1 (36); 383.0 → 109.1 (68)	5
Fluopyram	658066-35-4		5.70	397.1 → 207.9 (24); 397.1 → 144.9 (60)	4
Fluoxastrobin	361377-29-9	8	5.82	459.1 → 427.1 (16); 459.1 → 188.1 (44)	4
Fluquinconazole	136426-54-5	2	5.71	376.0 → 108.1 (52); 376.0 → 307.1 (28)	4
Fluridone	59756-60-4		4.69	330.1 → 309.0 (40); 330.1 → 310.0 (32)	5
Flusilazole	85509-19-9	2	6.18	316.1 → 165.1 (28); 316.1 → 247.1 (16)	4
Flutolanil	66332-96-5		5.31	324.1 → 242.0 (28); 324.1 → 262.0 (20)	5
Flutriafol	76674-21-0	8	3.90	302.1 → 70.1 (12); 302.1 → 123.1 (32)	5
Foramsulfuron	173159-57-4	3	3.34	453.1 → 182.2 (28); 453.1 → 83.1 (68)	4
Forchlorfenuron	68157-60-8	7	4.16	248.1 → 129.0 (16); 248.1 → 93.1 (40)	5
Formetanate hydrochloride	23422-53-9		1.47	222.1 → 165.1 (12); 222.1 → 46.2 (28)	4
Formothion	2540-82-1		2.52	258.0 → 124.9 (24); 258.0 → 198.9 (8)	6
Fosthiazate	98886-44-3	1	3.58	284.1 → 104.1 (24); 284.1 → 228.1 (8)	5
Fuberidazole	3878-19-1	4	2.15	185.1 → 156.0 (28); 185.1 → 65.0 (48)	5
Furalaxyl	57646-30-7	7	4.88	302.1 → 95.1 (40); 302.1 → 242.1 (12)	4
Furathiocarb	65907-30-4	6	7.82	383.2 → 195.0 (24); 383.2 → 252.0 (8)	5
Griseofulvin	126-07-8		3.89	353.1 → 164.9 (16); 353.1 → 214.9 (24)	5
Halofenozide	112226-61-6	8	5.04	331.1 → 104.9 (16); 331.1 → 138.9 (20)	5
Halosulfuron-methyl	100784-20-1	8	5.45	435.1 → 182.1 (24); 435.1 → 83.1 (56)	4
Haloxyfop	69806-34-4		6.16	362.0 → 287.9 (32); 362.0 → 90.9 (28)	4
Haloxyfop-methyl	69806-40-2		7.22	376.1 → 316.0 (16); 376.1 → 90.9 (40)	5
Hexaconazole	79983-71-4	2	6.73	314.1 → 70.1 (24); 314.1 → 159.1 (40)	5
Hexaflumuron	86479-06-3	7	7.40	461.0 → 158.1 (20); 461.0 → 141.1 (48)	4

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Compound	CAS	Agilent Mix No.	RT (min)	Primary transition (CE)	No. of transitions
Hexazinone	51235-04-2		3.00	253.2 → 171.1 (12); 253.2 → 71.0 (36)	4
Hexythiazox	78587-05-0	4	8.29	353.1 → 228.0 (16); 353.1 → 168.0 (28)	6
Hydramethylnon	67485-29-4	7	7.33	495.2 → 323.2 (32); 495.2 → 151.1 (56)	5
Hydroprene-S-	65733-18-8		9.58	267.2 → 94.9 (24); 267.2 → 55.1 (40)	4
Imazalil	35554-44-0	2	3.78	297.1 → 159.1 (24); 297.1 → 201.1 (16)	5
Imazamethabenz-methyl	81405-85-8		3.02	289.2 → 143.9 (40); 289.2 → 85.9 (24)	4
Imazethapyr	81335-77-5		2.55	290.2 → 177.0 (28); 290.2 → 86.1 (28)	5
Imidacloprid	138261-41-3	5	1.82	256.1 → 209.0 (16); 256.1 → 175.0 (28)	4
Indoxacarb	144171-61-9	3	7.33	528.1 → 203.1 (36); 528.1 → 150.1 (32)	5
Ipconazole	125225-28-7	2	7.41	334.2 → 70.1 (24); 334.2 → 125.1 (60)	4
Iprodione	36734-19-7		6.10	330.0 → 244.9 (16); 332.0 → 246.9 (16)	2
Iprovalicarb	140923-17-7	5	5.73	321.2 → 119.0 (20); 321.2 → 91.0 (60)	4
Isocarbamid	30979-48-7		2.20	186.1 → 86.9 (16); 186.1 → 129.9 (12)	4
Isofenphos	25311-71-1		6.97	346.1 → 216.9 (24); 346.1 → 245.0 (8)	5
Isofenphos-methyl	99675-03-3	1	6.43	332.1 → 231.1 (16); 332.1 → 120.9 (44)	4
Isoprocarb	2631-40-5		3.87	194.1 → 95.1 (12); 194.1 → 137.0 (4)	5
Isoprothiolane	50512-35-1	1	5.33	291.1 → 85.1 (60); 291.1 → 231.1 (12); 291.1 → 145.1 (40)	5
Isoproturon	34123-59-6		4.05	207.1 → 72.1 (24); 207.1 → 46.1 (24)	5
Isoxaben	82558-50-7	4	5.33	333.2 → 165.0 (20); 333.2 → 107.1 (60)	5
Isoxadifen-ethyl	163520-33-0		6.32	296.1 → 203.9 (24); 296.1 → 231.9 (12)	4
Isoxaflutole	141112-29-0	3	4.11	360.1 → 250.9 (12); 360.1 → 220.1 (44)	5
Isoxathion	18854-01-8		6.82	314.1 → 104.9 (36); 314.1 → 96.9 (56)	5
ISTD/QC - Atrazine-d5	163165-75-1		3.87	221.1 → 101.1 (20); 221.1 → 179.0 (20)	2
ISTD/QC - BDMC	672-99-1		5.18	258.0 → 122.1 (28); 258.0 → 201.0 (8)	2
ISTD/QC - Chlorpyrifos-d10	285138-81-0		8.15	360.0 → 99.1 (32); 360.0 → 199.1 (16)	2
ISTD/QC - Fentin-d15	358731-94-9		4.18	366.0 → 202.0 (32); 366.0 → 119.9 (60)	2
ISTD/QC - Imidacloprid-d4	1015855-75-0		1.82	260.1 → 213.0 (16); 260.1 → 179.1 (24)	2
ISTD/QC - Simazine-d10	220621-39-6		2.90	212.1 → 105.0 (32); 212.1 → 76.2 (28)	2
ISTD/QC - Triphenyl phosphate (TPP)	115-86-6		6.73	327.1 → 215.0 (44); 327.1 → 152.1 (44)	2
Ivermectin B1a	70288-86-7	7	9.46	892.5 → 307.2 (24); 892.5 → 569.3 (12)	4
Kresoxim-methyl	143390-89-0	4	6.38	314.1 → 223.0 (15); 314.1 → 116.0 (32)	4
Lactofen	77501-63-4		7.92	479.1 → 343.9 (28); 479.1 → 222.9 (52)	5
Lenacil	2164-08-1	1	4.03	235.1 → 153.1 (20); 235.1 → 136.1 (36)	5
Linuron	330-55-2	4	4.81	249.0 → 160.0 (20); 249.0 → 182.1 (12)	5
Lufenuron	103055-07-8	4	8.15	511.0 → 158.1 (20); 511.0 → 141.1 (44)	4
Malaoxon	1634-78-2	3	2.99	315.1 → 99.1 (36); 315.1 → 127.2 (12)	4
Malathion	121-75-5	3	5.33	331.1 → 127.1 (8); 331.1 → 125.1 (36)	5
Mandipropamid	374726-62-2	4	5.28	412.1 → 328.1 (16); 412.1 → 125.1 (44)	5
Mecarbam	2595-54-2	3	5.84	330.1 → 97.0 (40); 330.1 → 227.0 (8); 330.1 → 199.0 (12)	4
Mepanipyrim	110235-47-7	3	5.65	224.1 → 106.0 (24); 224.1 → 104.1 (32)	4
Mepanipyrim-2-hydroxypropyl	204571-52-8		4.03	244.2 → 200.1 (16); 244.2 → 226.0 (20)	5
Mephosfolan	950-10-7		2.79	270.0 → 139.9 (24); 270.0 → 195.9 (12)	5
Mesosulfuron-methyl	208465-21-8	6	3.87	504.1 → 182.1 (28); 504.1 → 83.1 (68)	5
Metaflumizone	139968-49-3	4	7.99	507.1 → 178.0 (36); 507.1 → 116.0 (52)	5

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Compound	CAS	Agilent Mix No.	RT (min)	Primary transition (CE)	No. of transitions
Metalaxyl	139968-49-3	3	4.12	280.2 → 220.1 (12); 280.2 → 160.2 (24)	5
Metamitron	41394-05-2	4	2.03	203.1 → 104.1 (24); 203.1 → 77.1 (24)	4
Metazachlor	67129-08-2	3	3.99	278.1 → 134.1 (28); 278.1 → 210.1 (8)	5
Metconazole	125116-23-6	2	6.81	320.1 → 70.1 (36); 320.1 → 125.1 (48)	4
Methabenzthiazuron	18691-97-9	5	3.86	222.1 → 165 (16); 222.1 → 150 (36)	5
Methamidophos	10265-92-6	1	1.45	142.0 → 47.1 (28); 142.0 → 94.0 (12); 142.0 → 125.1 (10)	4
Methidathion	950-37-8	3	4.34	303.0 → 85.1 (24); 303.0 → 145.1 (8)	3
Methiocarb	2032-65-7	7	4.97	226.1 → 121.0 (16); 226.1 → 169.2 (6)	5
Methiocarb sulfone	2179-25-1		1.98	275.1 → 122.2 (28); 275.1 → 201.2 (12)	4
Methiocarb sulfoxide	2635-10-1		1.85	242.1 → 122.2 (32); 242.1 → 170.1 (24)	4
Methomyl	16752-77-5	5	1.68	163.1 → 88.0 (8); 163.1 → 58.2 (20)	4
Methoprotryne	841-06-5	7	4.56	272.2 → 198.0 (24); 272.2 → 170.0 (28)	5
Methoxyfenozide	161050-58-4	5	5.47	369.2 → 149.1 (16); 369.2 → 91.1 (60)	4
Metobromuron	3060-89-7	8	3.75	259.0 → 169.9 (20); 259.0 → 91.1 (56)	5
Metolachlor	51218-45-2	3	6.01	284.1 → 176.2 (24); 284.1 → 134.1 (32)	4
Metolcarb	1129-41-5		2.62	166.1 → 109.1 (8); 166.1 → 94.0 (36); 166.1 → 91.1 (24)	5
Metosulam	139528-85-1		3.07	418.0 → 174.9 (24); 418.0 → 139.9 (60)	4
Metoxuron	19937-59-8		2.42	229.1 → 71.9 (20); 229.1 → 46.2 (20)	4
Metrafenone	220899-03-6	4	7.01	409.1 → 209.1 (28); 409.1 → 227.0 (24)	5
Metribuzin	21087-64-9	4	2.99	215.1 → 49.1 (28); 215.1 → 84.1 (20)	4
Metsulfuron-methyl	74223-64-6	4	2.84	382.1 → 167.0 (16); 382.1 → 56.0 (48)	4
Mevinphos I	7786-34-7	3	1.94	225.0 → 127.1 (20); 225.0 → 193.1 (8)	4
Mevinphos II	7786-34-7	3	2.18	225.0 → 127.1 (16); 225.0 → 193.1 (8)	4
Mexacarbate	315-18-4	7	3.20	223.1 → 136.1 (44); 223.1 → 151.1 (28)	5
MGK 264 I	113-48-4		7.17	276.2 → 210.0 (12); 276.2 → 97.9 (24)	3
MGK 264 II	113-48-4		7.61	276.2 → 210.0 (12); 276.2 → 97.9 (24)	3
Molinate	2212-67-1	3	5.39	188.1 → 126.1 (8); 188.1 → 55.1 (28)	2
Monocrotophos	6923-22-4	4	1.70	224.1 → 127.1 (16); 224.1 → 98.1 (12)	4
Monolinuron	1746-81-2		3.42	215.1 → 125.9 (16); 215.1 → 98.9 (44)	6
Moxidectin	113507-06-5	7	9.27	640.4 → 528.0 (4); 640.4 → 199.1 (28); 640.4 → 81.1 (60); 640.4 → 98.1 (60); 640.4 → 478.1 (8)	5
Myclobutanil	88671-89-0	1	5.48	289.1 → 70.2 (24); 289.1 → 125.1 (40)	4
Naled (Dibrom)	300-76-5		4.28	380.8 → 126.9 (12); 382.8 → 127.0 (12); 380.8 → 108.9 (44)	5
Napropamide	15299-99-7		5.89	272.2 → 129.0 (16); 272.2 → 171.1 (20)	4
Naptalam	132-66-1		2.93	292.1 → 148.9 (40); 292.1 → 255.9 (40); 292.1 → 64.9 (60)	4
Neburon	555-37-3		6.28	275.1 → 88.0 (12); 275.1 → 57.1 (20)	4
Nicosulfuron	111991-09-4	4	2.79	411.1 → 182.1 (28); 411.1 → 106.1 (44)	5
Nitenpyram	150824-47-8	7	1.61	271.1 → 225.1 (8); 271.1 → 126.1 (36)	4
Norflurazon	27314-13-2		4.27	304.1 → 284.0 (24); 304.1 → 88.1 (52)	5
Norflurazon-desmethyl	23576-24-1		3.67	290.0 → 269.9 (28); 290.0 → 145.0 (48)	5
Novaluron	116714-46-6	4	7.55	493.0 → 158.0 (20); 493.0 → 141.0 (52)	4
Nuarimol	63284-71-9		4.93	315.1 → 251.9 (20); 315.1 → 138.9 (44)	5
Ofurace	58810-48-3		2.97	282.1 → 160.0 (36); 282.1 → 148.0 (40)	4
Omethoate	1113-02-6	6	1.52	214.0 → 125.0 (24); 214.0 → 183.0 (8)	4
Oxadiazon	19666-30-9	3	8.01	345.1 → 220.1 (20); 345.1 → 185.1 (28)	5

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Compound	CAS	Agilent Mix No.	RT (min)	Primary transition (CE)	No. of transitions
Oxadixyl	77732-09-3	3	2.51	279.1 → 219.1 (8); 279.1 → 132.1 (32)	6
Oxamyl	23135-22-0	5	1.59	237.1 → 72.1 (12); 237.1 → 90.1 (12)	4
Oxamyl oxime	30558-43-1		1.54	163.1 → 72.1 (16); 163.1 → 47.1 (28)	4
Oxasulfuron	144651-06-9	4	2.66	407.1 → 150.1 (20); 407.1 → 107.0 (56)	4
Oxycarboxin	5259-88-1		2.13	268.1 → 174.9 (24); 268.1 → 146.9 (32)	4
Oxydemeton-methyl	301-12-2		1.63	247.0 → 169.0 (12); 247.0 → 125.0 (22)	4
Paclobutrazol	76738-62-0	3	5.27	294.1 → 70.1 (16); 294.1 → 125.1 (48)	4
Paraoxon	311-45-5		3.80	276.1 → 94.0 (40); 276.1 → 220.0 (16)	5
Paraoxon-methyl	950-35-6		2.47	248.0 → 202.1 (16); 248.0 → 90.1 (28)	5
Penconazole	66246-88-6	3	6.40	284.1 → 70.1 (24); 284.1 → 123.1 (52)	4
Pencycuron	66063-05-6	6	7.05	329.1 → 125.2 (24); 329.1 → 89.1 (64)	5
Pendimethalin	40487-42-1	3	8.27	282.1 → 212.0 (8); 282.1 → 194.0 (16)	3
Penoxsulam	219714-96-2		3.38	484.1 → 194.9 (36); 484.1 → 164.0 (36)	6
Phenmedipham	13684-63-4	4	4.57	318.1 → 93.0 (56); 318.1 → 136.0 (24); 318.1 → 168.0 (12)	5
Phenthoate	2597-03-7	3	6.32	321.0 → 247.1 (8); 321.0 → 163.1 (12)	4
Phorate sulfone	2588-04-7		3.82	293.0 → 171.0 (8); 293.0 → 96.8 (44)	5
Phorate sulfoxide	2588-03-6		3.66	277.0 → 96.9 (44); 277.0 → 143.0 (20)	5
Phosalone	2310-17-0	3	6.89	368.0 → 182.1 (12); 368.0 → 110.9 (56)	5
Phosmet	732-11-6	6	4.60	318.0 → 159.9 (16); 318.0 → 133.0 (40)	5
Phosmet oxon	3735-33-9		2.44	302.0 → 160.0 (24); 302.0 → 77.0 (56); 302.0 → 133.1 (40)	5
Phosphamidon I	13171-21-6	3	2.49	300.1 → 127.0 (16); 300.1 → 174.0 (12)	5
Phosphamidon II	13171-21-6	3	2.60	300.1 → 127.0 (16); 300.1 → 174.0 (12)	2
Phoxim	14816-18-3	4	6.77	299.1 → 77.1 (32); 299.1 → 129.1 (16)	5
Picolinafen	137641-05-5	3	7.90	377.1 → 238.1 (28); 377.1 → 145.1 (68)	5
Picoxystrobin	117428-22-5	5	6.24	368.1 → 145.1 (24); 368.1 → 205.1 (12)	4
Piperonyl butoxide	51-03-6		8.05	356.2 → 119.1 (44); 356.2 → 177.1 (32)	5
Piperophos	24151-93-7		7.29	354.1 → 170.9 (28); 354.1 → 212.9 (16)	5
Pirimicarb	23103-98-2	3	3.12	239.1 → 72.1 (20); 239.1 → 182.3 (16)	4
Pirimicarb-desmethyl	30614-22-3		2.05	225.1 → 72.0 (24); 225.1 → 168.1 (12)	4
Pirimiphos-methyl	29232-93-7	3	6.85	306.1 → 108.0 (36); 306.1 → 164.1 (28)	5
Prallethrin	23031-36-9		7.26	301.2 → 133.0 (12); 301.2 → 105.0 (20)	5
Pretilachlor	51218-49-6		7.45	312.2 → 176.0 (32); 312.2 → 251.9 (16)	5
Primisulfuron-methyl	86209-51-0		5.39	469.1 → 253.9 (20); 469.1 → 198.9 (24)	5
Prochloraz	67747-09-5	1	6.76	376.0 → 307.9 (12); 376.0 → 70.1 (20)	5
Prodiamine	29091-21-2		7.95	351.1 → 249.9 (32); 351.1 → 266.9 (20)	5
Profenofos	41198-08-7	3	7.60	375.0 → 304.8 (20); 373.0 → 302.8 (20); 373.0 → 96.9 (44)	7
Promecarb	2631-37-0	7	5.16	208.1 → 109.0 (12); 208.1 → 151.0 (4)	5
Prometon	1610-18-0	4	3.93	226.2 → 184.1 (16); 226.2 → 142.1 (20)	5
Prometryn	7287-19-6		5.39	242.1 → 158.1 (24); 242.1 → 200.2 (16)	5
Propamocarb	24579-73-5	5	1.54	189.2 → 102.0 (16); 189.2 → 74.0 (24)	4
Propanil	709-98-8		4.98	218.0 → 127.2 (24); 218.0 → 162.0 (14); 218.0 → 57.1 (20)	5
Propaquizafop	111479-05-1	4	7.92	444.1 → 100.1 (24); 444.1 → 56.1 (36)	5
Propargite	2312-35-8	4	8.55	368.1 → 175.1 (16); 368.1 → 57.1 (24)	5
Propetamphos (Safrotin)	31218-83-4	3	5.52	282.1 → 138.1 (20); 282.1 → 156.1 (8)	4
Propham	122-42-9	3	3.79	180.1 → 120.0 (16); 180.1 → 92.1 (28)	2

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Compound	CAS	Agilent Mix No.	RT (min)	Primary transition (CE)	No. of transitions
Propiconazole	60207-90-1	2	6.64	342.1 → 69.1 (20); 342.1 → 159.1 (44)	4
Propoxur	114-26-1	6	2.91	210.1 → 111.1 (12); 210.1 → 65.1 (40)	4
Propyzamide (Pronamide)	23950-58-5	3	5.31	256.0 → 173.1 (28); 256.0 → 190.1 (16)	5
Proquinazid	189278-12-4	1	8.75	373.0 → 330.9 (12); 373.0 → 288.9 (24)	6
Prosulfocarb	52888-80-9	4	7.49	252.1 → 91.1 (24); 252.1 → 65.1 (64)	5
Prothioconazole-desthio	120983-64-4		5.98	312.1 → 70.1 (28); 312.1 → 125.1 (32)	4
Pymetrozine	123312-89-0	7	1.55	218.1 → 105.0 (24); 218.1 → 78.0 (48)	4
Pyracarbolid	24691-76-7	7	3.11	218.1 → 125.1 (20); 218.1 → 97.1 (28)	4
Pyraclostrobin	175013-18-0	5	6.82	388.1 → 194.2 (8); 388.1 → 162.9 (20)	5
Pyraflufen-ethyl	129630-19-9		6.62	413.0 → 338.9 (20); 413.0 → 252.9 (32)	4
Pyrazophos	13457-18-6		6.95	374.1 → 222.1 (24); 374.1 → 194.1 (32)	5
Pyrethrum - Cinerin I	13457-18-6		8.62	317.2 → 107.0 (20); 317.2 → 149.1 (8)	6
Pyrethrum - Cinerin II	13457-18-6		7.25	361.2 → 107.1 (24); 361.2 → 149.1 (8)	5
Pyrethrum - Jasmolin I	13457-18-6		8.92	331.2 → 163.2 (8); 331.2 → 107.0 (24)	6
Pyrethrum - Jasmolin II	13457-18-6		7.86	375.2 → 163.1 (8); 375.2 → 107.1 (36); 375.2 → 77.1 (60)	5
Pyrethrum - Pyrethrin I	13457-18-6		8.66	329.2 → 161.1 (8); 329.2 → 133.1 (16)	6
Pyrethrum - Pyrethrin II	13457-18-6		7.38	373.2 → 161.1 (8); 373.2 → 133.1 (24)	5
Pyridaben	96489-71-3	5	8.93	365.2 → 147.1 (28); 365.2 → 309.1 (12)	6
Pyridalyl	179101-81-6		9.44	490.0 → 108.9 (40); 490.0 → 182.9 (20)	6
Pyridaphenthion	119-12-0		5.56	341.1 → 188.9 (28); 341.1 → 91.9 (48)	4
Pyridate	55512-33-9	5	9.10	379.1 → 207.0 (12); 379.1 → 351.1 (8)	6
Pyrifenox I	88283-41-4		5.40	295.0 → 92.9 (28); 295.0 → 91.9 (76)	4
Pyrifenox II	88283-41-4		5.70	295.0 → 92.9 (28); 295.0 → 91.9 (76)	4
Pyrimethanil	53112-28-0	6	4.68	200.1 → 107.2 (24); 200.1 → 82.0 (28)	5
Pyriproxyfen	95737-68-1	5	8.10	322.2 → 96.1 (20); 322.2 → 78.1 (56)	5
Pyroquilon	57369-32-1		2.81	174.1 → 117.0 (36); 174.1 → 132.0 (28)	5
Pyroxsulam	422556-08-9		2.85	435.1 → 194.9 (28); 435.1 → 193.9 (36)	4
Quinalphos	95737-68-2	3	6.29	299.1 → 97.1 (40); 299.1 → 163.1 (28)	4
Quinmerac	90717-03-6	7	2.03	222.0 → 203.9 (20); 222.0 → 141.0 (44)	4
Quinoclamine	2797-51-5	4	2.71	208.0 → 105.1 (28); 208.0 → 77.1 (40)	5
Quinoxyfen	124495-18-7	3	8.14	308.0 → 197.1 (40); 308.0 → 162.1 (56)	5
Quizalofop	82-68-8		5.93	345.1 → 298.9 (16); 345.1 → 272.9 (24)	4
Quizalofop-ethyl	76578-14-8		7.66	373.1 → 299.0 (20); 373.1 → 90.9 (36)	5
Resmethrin	10453-86-8		9.04	339.2 → 171.1 (12); 339.2 → 128.1 (52)	6
Rimsulfuron	122931-48-0	4	3.31	432.1 → 182.1 (32); 432.1 → 325.1 (16)	5
Rotenone	83-79-4	7	6.20	395.2 → 213.0 (24); 395.2 → 192.0 (20)	4
Schradan (Octamethylpyrophosphoramide)	152-16-9		2.10	287.1 → 135.0 (32); 287.1 → 241.9 (16)	4
Secbumeton	26259-45-0	7	4.11	226.2 → 170.1 (16); 226.2 → 113.9 (24)	5
Sethoxydim I	74051-80-2		5.27	328.2 → 178.0 (20); 328.2 → 282.3 (8)	5
Sethoxydim II	74051-80-2		7.84	328.2 → 178.0 (20); 328.2 → 282.3 (8)	5
Siduron	1982-49-6		4.97	233.2 → 94.0 (40); 233.2 → 55.1 (48)	5
Silthiofam	175217-20-6	4	6.28	268.1 → 139.1 (20); 268.1 → 73.1 (36)	4
Simazine	122-34-9		2.98	202.1 → 96.1 (24); 202.1 → 104.1 (30)	4
Simeconazole	149508-90-7		5.84	294.2 → 70.0 (24); 294.2 → 134.9 (28)	4

Table 1. List of Compounds Included in the Method Together with Their Chemical Abstract Service (CAS) Numbers, Retention Times (RT), Primary Transitions, Collision Energies (CE), and Total Number of Transitions in the tMRM Program (continued). For Compounds Provided in Agilent LC/MS mixes 1–8 (p/n 5190-0551), the Respective Mix Number is Aso Listed

Compound	CAS	Agilent Mix No.	RT (min)	Primary transition (CE)	No. of transitions
Simetryn	1014-70-6		3.47	214.1 → 68.1 (40); 214.1 → 124.1 (20)	6
Spinetoram - Spinosyn J	187166-40-1		7.56	748.5 → 142.1 (36); 748.5 → 98.1 (72)	5
Spinetoram - Spinosyn L	187166-40-1		7.99	760.5 → 142.1 (36); 760.5 → 98.1 (72)	5
Spinosad - Spinosyn A	168316-95-8	7	7.03	732.5 → 142.0 (32); 732.5 → 98.3 (52)	5
Spinosad - Spinosyn D	168316-95-8	7	7.47	746.5 → 142.1 (32); 746.5 → 98.4 (60)	5
Spirodiclofen	148477-71-8	1	8.76	411.1 → 313.1 (8); 411.1 → 71.1 (32)	6
Spiromesifen	283594-90-1	6	8.52	388.2 → 273.0 (8); 388.2 → 255.0 (28)	6
Spiromesifen enol	148476-30-6		4.77	273.2 → 186.9 (16); 273.2 → 67.0 (40)	5
Spirotetramat	203313-25-1	6	5.85	374.2 → 216.1 (40); 374.2 → 302.2 (20)	4
Spiroxamine I	118134-30-8	1	4.97	298.3 → 144.1 (20); 298.3 → 100.1 (36)	5
Spiroxamine II	118134-30-8	1	5.07	298.3 → 144.1 (20); 298.3 → 100.1 (36)	5
Sulfentrazone	122836-35-5	6	3.17	404.0 → 306.9 (28); 404.0 → 273.1 (40)	4
Sulprofos	35400-43-2		8.33	323.0 → 218.8 (12); 323.0 → 139.2 (32)	6
Tebuconazole	107534-96-3	2	6.49	308.1 → 70.0 (20); 308.1 → 124.9 (52)	4
Tebufenozide	112410-23-8	5	6.25	353.2 → 133.1 (20); 353.2 → 105.1 (52)	4
Tebufenpyrad	119168-77-3	3	7.86	334.1 → 145.0 (28); 334.1 → 117.0 (40)	5
Tebupirimfos	96182-53-5		7.95	319.1 → 153.1 (32); 319.1 → 276.9 (12)	5
Tebuthiuron	34014-18-1	7	3.15	229.1 → 172.1 (12); 229.1 → 116.0 (24)	4
Teflubenzuron	83121-18-0	4	7.89	381.0 → 141.0 (48); 381.0 → 158.0 (16)	5
Temephos	3383-96-8	7	8.02	467.0 → 125.0 (44); 467.0 → 418.9 (20)	5
Tepraloxydim I	149979-41-9	3	3.34	342.2 → 250.0 (8); 342.2 → 166.0 (16)	5
Tepraloxydim II	149979-41-9	3	5.70	342.2 → 250.0 (8); 342.2 → 166.0 (16)	4
Terbufos	13071-79-9	3	7.82	289.1 → 103.1 (4); 289.1 → 57.0 (24); 289.1 → 232.9 (0)	4
Terbufos sulfone	56070-16-7		4.80	338.1 → 171.0 (12); 338.1 → 97.1 (60)	5
Terbufos sulfoxide	10548-10-4		4.83	305.1 → 187.1 (6); 305.1 → 96.8 (52)	5
Terbumeton	33693-04-8		4.15	226.2 → 170.1 (20); 226.2 → 114.2 (26)	5
Terbuthylazine	5915-41-3		5.07	230.1 → 174.1 (16); 230.1 → 96.1 (32)	5
Terbutryn	886-50-0		5.68	242.1 → 186.2 (20); 242.1 → 71.1 (36)	4
Tetrachlorvinphos	961-11-5		6.27	366.9 → 127.0 (16); 364.9 → 127.0 (16)	2
Tetraconazole	112281-77-3	2	5.85	372.0 → 70.1 (24); 372.0 → 159.1 (44)	4
Thiabendazole	148-79-8	5	2.07	202.0 → 131.0 (40); 202.0 → 175.0 (28)	4
Thiabendazole-5-hydroxy-	948-71-0		1.68	218.0 → 190.9 (28); 218.0 → 147.0 (36)	4
Thiacloprid	111988-49-9	5	2.12	253.0 → 126.1 (24); 253.0 → 90.1 (48)	4
Thiamethoxam	153719-23-4	5	1.68	292.0 → 211.0 (20); 292.0 → 131.9 (44)	5
Thiazopyr	117718-60-2		6.49	397.1 → 377.0 (24); 397.1 → 335.0 (36)	4
Thidiazuron	51707-55-2	7	2.93	221.0 → 101.9 (12); 221.0 → 93.9 (8)	4
Thifensulfuron-methyl	79277-27-3	4	2.71	388.0 → 166.9 (16); 388.0 → 56.0 (44); 388.0 → 140.9 (20)	4
Thiobencarb (Benthiocarb)	28249-77-6		6.93	258.1 → 124.9 (16); 258.1 → 88.9 (64)	5
Thiodicarb	59669-26-0	5	3.50	355.0 → 88.1 (24); 355.0 → 108.1 (12)	6
Thiofanox	39196-18-4	5	3.62	241.0 → 184.0 (8); 241.0 → 57.0 (20); 241.0 → 98.0 (8)	4
Thiofanox sulfone	39184-59-3		1.89	268.1 → 57.1 (12); 268.1 → 75.9 (8)	4
Thiofanox sulfoxide	39184-27-5		1.84	235.1 → 103.9 (12); 235.1 → 56.9 (12)	4
Thionazin (Zinophos)	297-97-2		3.99	249.0 → 97.0 (24); 249.0 → 192.9 (12)	5
Thiophanate-methyl	23564-05-8		2.86	343.1 → 151.1 (16); 343.1 → 93.1 (56)	4
Tolfenpyrad	129558-76-5		7.99	384.2 → 197.0 (28); 384.2 → 116.9 (40)	5

Table 1. List of Compounds Included in the Method Together with Their Chemical Abstract Service (CAS) Numbers, Retention Times (RT), Primary Transitions, Collision Energies (CE), and Total Number of Transitions in the tMRM Program (continued). For Compounds Provided in Agilent LC/MS mixes 1–8 (p/n 5190-0551), the Respective Mix Number is Aso Listed

Compound	CAS	Agilent Mix No.	RT (min)	Primary transition (CE)	No. of transitions
Tolylfluanid	731-27-1	3	6.44	347.0 → 137.0 (36); 347.0 → 237.9 (8)	4
Topramezone	210631-68-8		1.68	364.1 → 334.0 (12); 364.1 → 235.9 (24)	4
Tralkoxydim	87820-88-0	1	8.30	330.2 → 284.2 (12); 330.2 → 96.1 (32)	6
Triadimefon	43121-43-3	3	5.43	294.1 → 197.0 (12); 294.1 → 69.0 (20)	4
Triadimenol	55219-65-3	6	5.61	296.1 → 70.0 (12); 298.1 → 70.0 (12); 296.1 → 99.2 (12)	4
Triasulfuron	82097-50-5	4	2.73	402.1 → 141.1 (36); 402.1 → 167.0 (36)	5
Triazophos	24017-47-8	3	5.62	314.1 → 162.1 (20); 314.1 → 97.1 (36)	4
Tribenuron-methyl	101200-48-0	4	3.93	396.1 → 155.1 (28); 396.1 → 181.1 (28)	5
Tribufos (DEF)	78-48-8		8.93	315.1 → 57.1 (32); 315.1 → 169.0 (14)	6
Trichlorfon (Metrifonate)	52-68-6	6	2.01	256.9 → 109.0 (12); 256.9 → 220.9 (8)	4
Tricyclazole	41814-78-2	2	2.31	190.0 → 136.1 (28); 190.0 → 163.1 (20)	5
Trietazine	1912-26-1	6	5.72	230.1 → 99.0 (24); 230.1 → 132.0 (20)	4
Trifloxystrobin	141517-21-7	5	7.33	409.1 → 186.1 (20); 409.1 → 145.1 (56)	5
Trifloxysulfuron (sodium)	199119-58-9		3.91	438.07 → 182.1 (24); 438.07 → 139.1 (56)	5
Triflumizole	68694-11-1	3	7.34	346.1 → 73.0 (16); 346.1 → 55.0 (12)	5
Triflumuron	64628-44-0	4	6.86	359.0 → 156.1 (16); 359.0 → 139.0 (40)	5
Triforine	26644-46-2		4.54	434.9 → 389.9 (12); 434.9 → 97.9 (44)	5
Trimethacarb	2655-15-4	6	4.10	194.1 → 137.1 (12); 194.1 → 122.1 (32)	5
Triticonazole	131983-72-7	2	5.87	318.1 → 70.1 (16); 320.1 → 70.1 (16); 318.1 → 125.1 (40)	4
Uniconazole	83657-17-4	2	6.07	292.1 → 70.1 (20); 294.1 → 70.0 (20); 292.1 → 125.1 (28)	4
Vamidothion	2275-23-2	2	1.92	288.1 → 146.1 (8); 288.1 → 118.1 (28)	4
Zoxamide	156052-68-5	6	6.63	336.0 → 186.9 (28); 336.0 → 158.9 (52)	4

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Agilent Technologies

Routine Multiresidue Pesticide Analysis using the Agilent 6470 Triple Quadrupole Mass Spectrometer

Application Note

Food safety

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Abstract

This application note describes a UHPLC/MS/MS-based method for the screening and quantification of more than 250 pesticides and pesticide metabolites in food samples. The method leverages the:

- Increased chromatographic resolution of the Agilent 1290 Infinity UHPLC System
- Versatile ionization capabilities of the Agilent Jet Stream ionization source
- Innate sensitivity of the Agilent 6470 Triple Quadrupole LC/MS System.

The method was applied to the analysis of pesticide residues in complex food matrices. Sample dilution prior to injection was used as a means of maximizing method robustness and minimizing matrix effects.

Our results demonstrate that the increased sensitivity of the 6470 Triple Quadrupole LC/MS System enables the accurate and precise quantification of targeted pesticides below the maximum residue limits (MRLs) specified by the European Commission, even in 1:10 and up to 1:20 dilutions of black tea extracts.

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Introduction

An important application in food safety is the screening and quantitation of hundreds of pesticides from a wide variety of food commodities using multiresidue methods. Major challenges in achieving accurate and precise quantitation include diversity of compound classes, matrix effects, low concentrations and poor ionization efficiencies of some pesticides.

This application note describes the development of an UHPLC/MS/MS method for the screening and quantitation of hundreds of pesticides in complex food matrices using the Agilent Pesticide tMRM LC/MS Application Kit (p/n G1733BA). Transitions for all compounds in the comprehensive pesticide standard mixture (p/n 5190-0551) and a few additional pesticides of interest were included in the method. An Agilent 1290 Infinity UHPLC system was coupled to an Agilent 6470 Triple Quadruple LC/MS system operated in dynamic MRM (DMRM) and fast polarity switching mode. Several hardware modifications to previous designs resulted in increased quantitative performance. Improvements were achieved by optimized mass filter one (MS1) ion optics, an improved curved and tapered collision cell, a detector operating at dynode accelerating voltages of up to ±20 kV, and a new autotune optimized for speed and sensitivity. In addition, the use of a curved collision cell resulted in a smaller physical footprint of the instrument.

Enhanced sensitivity achieved by the design translates into enhanced peak area response and improved area precision, leading ultimately to lower detection limits compared to previous designs. We achieved rugged and high performance quantitation at low levels in tomato, orange, and black tea. Moreover, we evaluated the use of dilution as a means of minimizing matrix effects, and demonstrated that the increased sensitivity achieved by this design allowed a high degree of sample dilution while still allowing to achieve the maximum residue level (MRL) stipulated by the European Union.

Experimental

Reagents and chemicals

The Agilent comprehensive pesticide mixture (p/n 5190-0551) was used, and several additional pesticides were purchased from Fluka (Sigma-Aldrich Corp., St. Louis, MO, USA). The eight submixes of the comprehensive pesticide mixture and the additional pesticides were combined and further diluted with acetonitrile to a final pesticide working solution containing more than 250 pesticides at a concentration of 10 µg/mL (10 ppm). This solution was used for spiking the QuEChERS extracts and for the preparation of the calibration samples. For instrument detection limit (IDL) and low limit of quantitation (LLOQ) determination in solvent, 13 calibration levels with concentrations ranging from 1 ppt to 100 ppb were prepared in pure acetonitrile. For recovery calculations in matrices, a solvent calibration set ranging from 10 ppt to 100 ppb was prepared in acetonitrile.

All reagents and solvents were HPLC or LC/MS grade. Acetonitrile and methanol were purchased from Honeywell (Morristown, NJ, USA). Ultrapure water was produced with a Milli-Q Integral system equipped with a LC-Pak Polisher and a 0.22-µm point-of-use membrane filter cartridge (EMD Millipore, Billerica, MA, USA). Formic acid and ammonium formate were from Fluka (Sigma-Aldrich Corp., St. Louis, MO, USA).

Sample preparation

Organic tomato, orange, and black tea samples were obtained from a local grocery store. Two grams of tea samples were wetted with 8 mL of water and incubated for 2 hours at room temperature. Well-blended homogenized fruit samples were prepared with a ceramic homogenizer (p/n 5982-9312), and 10 g were weighed. The fruit and tea samples were extracted with 10 mL acetonitrile for 1 minute with vigorous shaking. One pouch of Agilent EN extraction salts (p/n 5982-6650) was added to each mixture, and shaken for 1 minute followed by centrifugation at 3,000xg for 5 minutes. Six mL of tea supernatant were added into Agilent QuEChERS Dispersive SPE for high pigment EN (p/n 5982-5356), 6 mL of tomato supernatant were added into Agilent QuEChERS Dispersive SPE for general fruits and vegetables EN (p/n 5982-5056), and 6 mL of orange supernatant were added into Agilent QuEChERS Dispersive SPE for fruits and vegetables with fats and waxes EN (p/n 5982-5156), followed by shaking for 1 minute and centrifuged at 3,000xg for 5 minutes. Supernatant was

collected and passed through a 0.45 µm syringe filter. Final extracts were spiked at 10 ng/g in relation to starting matrix quantity with the comprehensive pesticide working solution and diluted 1:2, 1:5, 1:10, and 1:20 with acetonitrile. Matrix matched standards and dilutions were prepared immediately before injection and measured with five technical replicates.

Equipment

Separation was carried out using an Agilent 1290 Infinity UHPLC system consisting of:

- Agilent 1290 Infinity Binary Pump (G4220A)
- Agilent 1290 Infinity High Performance Autosampler (G4226A)
- Agilent sample cooler (G1330B)
- Agilent 1290 Infinity Thermostatted Column compartment (G1316C)

The UHPLC was coupled to an Agilent 6470 Triple Quadrupole Mass Spectrometer equipped with an Agilent Jet Stream electrospray ionization source. Agilent MassHunter Acquisition (ver. B.08.00) and Agilent MassHunter Quantitative Analysis (Ver B.07.00) software was used for data acquisition and analysis.

Methods

The LC/MS conditions and parameters are provided below. MRM parameters, such as polarity, precursor, and product ions as well as optimal collision energies were imported from the Agilent Pesticide tMRM LC/MS Application Kit, and source conditions were optimized for selected poor-responding analytes using Agilent Source Optimizer Software. Data acquisition was carried out in fast polarity switching DMRM mode. A 2-µL amount of the final extract was injected into the LC/MS system.

Data was evaluated using the Agilent MassHunter Quantitative Analysis Software. Calibration was done using neat standard solutions. For calibration curves, linear fit with weight = $1/x$ or $1/x^2$ was used.

Chromatography

Agilent 1290 Infinity UHPLC System

Column	Agilent EclipsePlus C18, RRHD, 2.1 × 150 mm, 1.8 µm (p/n 959759-902)
Column temperature	40 °C
Injection volume	2 µL
Autosampler temperature	4 °C
Needle wash	8 seconds in wash port (75:25 methanol/H_2O)
Mobile phase	A) 5 mM ammonium formate + 0.1% formic acid B) 5 mM ammonium formate + 0.1% formic acid in methanol
Flow rate	0.400 mL/min
Gradient program	Time B (%) 0.00 5 0.50 5 3.50 40 17.00 98 20.00 98 20.10 5
Post time	3 minutes

Mass spectrometry

Agilent 6470 Triple Quadrupole Mass Spectrometer

Ion source	Agilent Jet Stream
Polarity	Positive and negative switching
Gas temperature	140 °C
Drying gas (nitrogen)	5 L/min
Nebulizer gas	30 psi
Sheath gas	375 °C
Sheath gas flow	12 L/min
Capillary voltage	4,000 V/–3,000 V
Nozzle voltage	0 V
Scan type	Dynamic MRM (DMRM)
Q1/Q2 Resolution	Unit (0.7 amu)
Delta EMV	200 V
Cell acceleration voltage	3–7 V
Cycle time	500 ms
Total number of MRMs	525 (positive: 505, negative: 20)
Min/max dwell time	2.3/246.5 ms

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Results and Discussion

Development and performance of the UHPLC/MS/MS method

The pesticide screening method developed for the Agilent Pesticide tMRM LC/MS Application Kit was transferred to the 6470 Triple Quadrupole LC/MS system. The method was extended to include several relevant acidic herbicides. DMRM and fast polarity switching mode were employed. Sheath gas temperature, drying gas temperature, capillary voltage, and nozzle voltage were optimized using the MassHunter Source Optimizer Software to produce the highest abundance for a selected subset of labile and poor-responding analytes. Figure 1 shows the overlapped MRM chromatograms of a black tea extract spiked with more than 250 pesticides to a concentration of 10 µg/kg, and diluted 1:10 with acetonitrile prior to injection.

The improved ion optics and detector allowed the quantitation of the majority of pesticides at an LLOQ of 10% of their MRL. The precision and accuracy of measurements were evaluated at 10 standard concentrations ranging from the LLOQ as low as 10 ppt to the upper limit of quantitation (ULOQ) of 100 ppb, and were calculated from five replicate injections at each level. Excellent assay precision (RSD (%) <20% at LLOQ and <15% at the rest of the levels) as well as average accuracy (80–125% at LLOQ and 85–115% at the rest of the levels) were obtained. Correlation coefficients (R^2) for calibration curves were higher than 0.99 over up to four orders of linear dynamic range. These results are well within the criteria set by bioanalytical method validation guidelines.

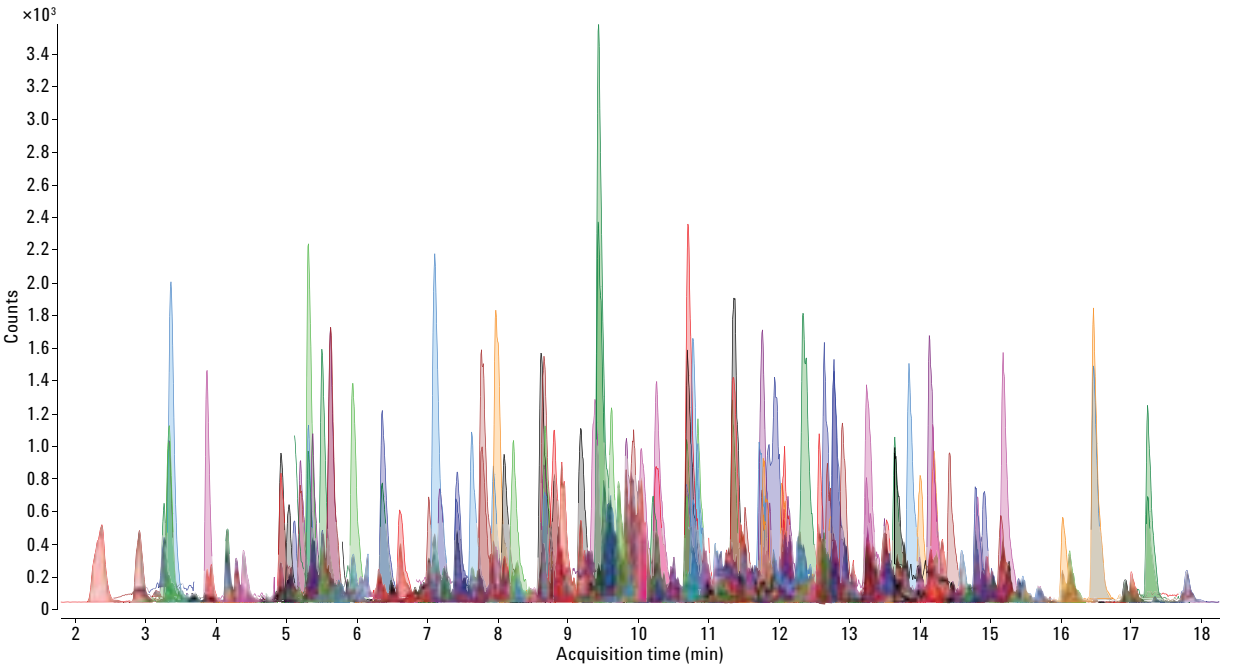

Figure 1. Overlapped MRM chromatograms of more than 250 pesticides spiked into black tea at the MRL (10 µg/kg) and diluted 1:10 with acetonitrile (corresponding to a concentration of 0.2 ng/mL).

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Evaluation of increased instrument performance

The improved hardware design of the 6470 Triple Quadrupole LC/MS system showed sensitivity gains of up to factor 4 compared to the previous 6460 Triple Quadrupole model. The observed sensitivity gain was compound-dependent.

Enhanced ion transmission not only resulted in increased area reponse but also in improved area precision. Area relative standard deviation (RSD) can be used as an indirect measure of the relative number of ions under a chromatographic peak and therefore can be used to estimate the instrument detection limit (IDL) [1]. Performance based on signal response precision gives a much clearer indication of sensitivity compared to signal to noise ratios in quantitative applications. Table 1 shows the fold-improvement in IDL for individual relevant pesticides comparing the 6470 to the previous instrument design. A median fold-improvement of 3.6x was observed across all >250 pesticides.

The increased sensitivity of the 6470 LC/MS system enabled the quantitation of most targeted pesticides in tomato, orange, and black tea extracts below the default MRL of 10 µg/kg specified by the European Commission. Figure 2 shows the histogram of LLOQs in the solvent standard and food extracts. LLOQs in the black tea extract were found to be higher due to the 5-fold lower sample amount and the complex matrix. Even in this challenging matrix, the majority of pesticides achieved an LLOQ of ≤10% of the default MRL.

Table 1. IDL for 50 Relevant Pesticides in Black Tea Based on a Dilution Series Prepared in Acetonitrile

	IDL (ppt)				IDL (ppt)		
Pesticide	Agilent 6470	Agilent 6460	Fold improvement	Pesticide	Agilent 6470	Agilent 6460	Fold improvement
Acephate	7.1	10.4	1.5x	Fenarimol	264.5	4023.8	15.2x
Acetamiprid	1.8	32.9	18.0x	Fipronil	74.5	339.7	4.6x
Aldicarb	645.0	43.3	0.1x	Flufenoxuron	58.0	521.6	9.0x
Azinphos-methyl	165.1	278.9	1.7x	Flusilazole	56.1	204.5	3.6x
Bifenthrin	267.5	67.8	0.3x	Hexaflumuron	1003.8	20771	20.7x
Bosclid	220.2	665.8	3.0x	Imazalil	30.1	203.2	6.7x
Buprofezin	6.2	36.0	5.8x	Imidacloprid	57.6	191.1	3.3x
Butocarboxim	645.0	107.3	0.2x	Isocarbophos	14.6	129.5	8.9x
Carbendazim	3.7	13.5	3.6x	Metamitron	172.6	558.4	3.2x
Chloroxuron	7.4	98.4	13.3x	Methamidophos	1.7	43.1	25.9x
Chlorpyriphos	68.9	401.1	5.8x	Methidathion	71.9	340.1	4.7x
Cycluron	8.4	71.1	8.4x	Methomyl	4.1	21.5	5.2x
Cyprodinil	182.1	61.9	0.3x	Monocrotophos	10.8	52.3	4.8x
Desmedipham	11.2	32.4	2.9x	Myclobutanil	10.3	356.2	34.5x
Diazinon	32.1	31.4	1.0x	Omethoat	2.1	6.1	2.9x
Diethofencarb	11.1	59.0	5.3x	Oxamyl	1.0	5.8	5.7x
Difenoconazole	315.1	620.7	2.0x	Phosalone	36.9	2044.3	55.3x
Dimethoate	2.8	32.8	11.8x	Pirimicarb	1.3	2.8	2.2x
Dimethomorph	59.0	310.7	5.3x	Pyridaben	0.8	0.6	0.8x
Dimoxystrobin	6.3	23.6	3.8x	Tebuconazole	45.8	41.9	0.9x
Diniconazole	30.9	237.4	7.7x	Tebufenozid	175.4	52.3	0.3x
Dioxacarb	103.7	139.1	1.3x	Teflubenzuron	291.6	2012.1	6.9x
Diuron	22.6	37.4	1.7x	Thiacloprid	1.5	6.0	4.1x
Epoxyconazol	21.4	144.8	6.8x	Thiamethoxam	8.7	38.8	4.5x
Ethion	11.0	166.3	15.2x	Triazophos	10.1	65.9	6.6x

Five injection replicates were used for calculation.

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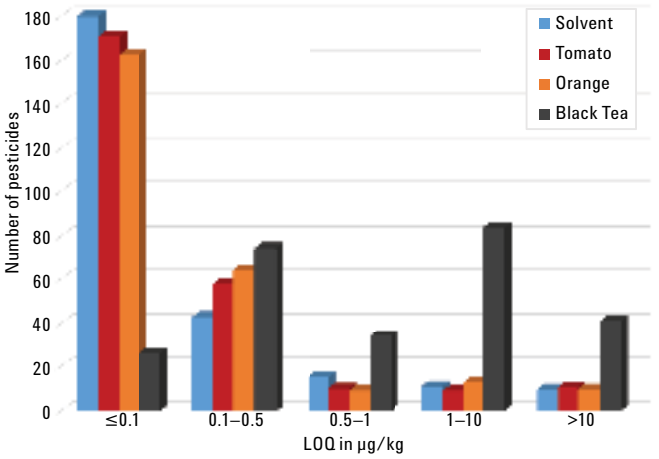

Figure 2. LLOQs for the >250 pesticides in solvent and spiked tomato, orange, and black tea extracts. Results were classified in five relevant concentration ranges and are shown as a histogram.

Minimizing matrix effects by dilution of extracts

Evaluation of matrix effects (suppression and enhancement) was done by comparing the response of the targeted compound in a solvent calibration series against the spiked black tea sample extract. Primarily, signal suppression was observed, with a significant number (n = 104) of target compounds exhibiting a suppressed signal, where the analyte response in undiluted black tea was less than 70% of the analyte response in neat solvent.

The ability to dilute complex sample extracts to minimize matrix effects enables more accurate quantification against a solvent calibration, and is an attractive capability to many routine testing labs. A possible cause for matrix effects in electrospray ionization is the limited number of excess charges, and the limited space on the surface of the charged droplet. The dilution of the matrix frees up space at the surface, resulting in more efficient ionization of the target compounds. In addition, the amount of matrix injected to the LC/MS system is limited, which results in minimization of instrument contamination, translating into increased instrument uptime and robustness of the analytical method. Table 2 shows the beneficial effect of dilution, where pesticide recoveries in black tea improve as the sample is further diluted. For compounds such as chloroxuron and myclobutanil, only weak matrix effects were observed, whereas compounds such as aldicarb and methomyl experienced significant signal suppression, and required a dilution of 1:20 to achieve acceptable recoveries. There were a few of compounds, such as monocrotophos, that would require a higher degree of sample dilution to achieve acceptable recovery, previously shown to be achieved with the Agilent 6495 Triple Quadrupole LC/MS System [2].

Table 2. Recoveries for Selected Relevant Pesticides in Black Tea, Calculated for Different Dilution Ratios Based on a Solvent Calibration

Analyte	No Dilution	Dilution 1:2	Dilution 1:5	Dilution 1:10	Dilution 1:20
Acephate	57 ± 1.6	68.9 ± 1.9	78.5 ± 3.9	83.6 ± 3.4	90 ± 1.9
Aldicarb	24.1 ± 5.8	37.5 ± 4.6	58.3 ± 8.9	68.5 ± 4	84 ± 6.1
Carbofuran	45.3 ± 0.5	60.6 ± 2.9	74.5 ± 4	81 ± 1.6	96.9 ± 7.2
Chloroxuron	75.4 ± 2.3	78.5 ± 9.5	81.8 ± 5.8	83.9 ± 9.4	97.8 ± 7
Dimethoate	25.1 ± 2.2	37.9 ± 2.7	57.8 ± 3.8	70.4 ± 6.5	84.9 ± 6.4
Epoxyconazol	66.1 ± 3.6	75.5 ± 6.4	73.4 ± 12.6	84.6 ± 11	89.3 ± 11.9
Ethion	54 ± 3.1	73.3 ± 4.3	81 ± 5.8	83 ± 5.3	88.2 ± 9
Methamidophos	42.6 ± 0.8	54.9 ± 1	67.9 ± 1	76.7 ± 1	88.2 ± 1.2
Methidathion	67.3 ± 4.1	79.3 ± 7.8	83.3 ± 9.5	88.8 ± 5	108.6 ± 3.3
Methomyl	10.4 ± 3.2	20.9 ± 2.7	42.1 ± 1.2	60.7 ± 2.6	76.4 ± 11.2
Monocrotophos	5.5 ± 5.6	9.9 ± 9.4	18.7 ± 6.9	31.4 ± 14.5	48.4 ± 8.7
Myclobutanil	84.6 ± 4.8	84.6 ± 3.8	86.8 ± 9	90.3 ± 13.4	99.7 ± 9.7
Oxamyl	14.1 ± 1.6	23.4 ± 2.2	44.1 ± 1.8	60.2 ± 1.7	76.8 ± 4
Pirimicarb	50.7 ± 1.2	62.9 ± 2	75.2 ± 1.7	81.9 ± 1.5	88.9 ± 2.4
Pyridaben	50.8 ± 1	60.4 ± 1.7	71.3 ± 1	79.4 ± 2	89.8 ± 2.9
Thiacloprid	24.1 ± 0.5	37.3 ± 0.7	56.8 ± 1.9	69.3 ± 2.7	82.4 ± 1.2

Cells shaded in green comply with requirements of SANCO/12571/2013.

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Figure 3 demonstrates that most pesticides spiked in black tea achieve acceptable recoveries at the 1:20 dilution. In the 1:20 dilution, 93% of the detectable pesticides showed acceptable recoveries, basically showing minimal signal suppression.

The enhanced sensitivity of the 6470 LC/MS system enabled analysis of the desired black tea dilution level while still maintaining the ability to detect the majority of pesticides. Figure 4 shows the detection rates of pesticides for different dilution levels in the black tea matrix. Under the applied experimental conditions, approximately 67% of the spiked pesticides were easily detected in the 1:20 dilution with RSD values below 20%, corresponding to a concentration of 0.1 ng/mL. In addition approximately 7% were detected with acceptable precision in the 1:10 dilution, and another ~9% in the 1:5 dilution.

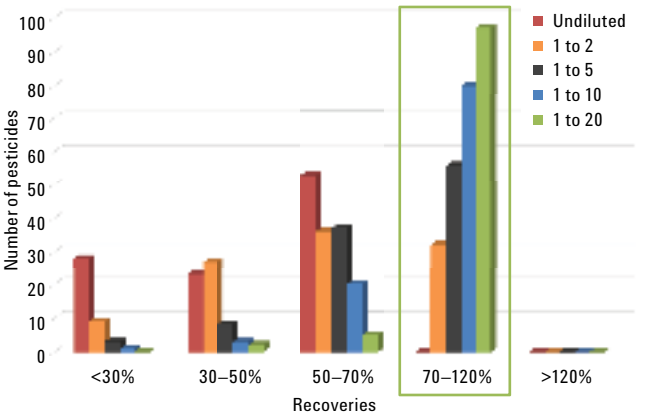

Figure 3. Histogram of recoveries for pesticides spiked into black tea at the MRL of 10 µg/kg and diluted with acetonitrile. 104 pesticides showed strong ion suppression and significantly better recoveries were observed after dilution. The green box denotes acceptable recoveries according to SANCO specifications.

Conclusion

An UHPLC/MS/MS based multiresidue method for the determination of more than 250 pesticides and pesticide metabolites has been developed. The obtained results demonstrate the increased chromatographic resolution of the Agilent 1290 Infinity LC System, the high sensitivity of the Agilent 6470 Triple Quadrupole LC/MS System, and the proven ionization enhancement capabilities of the Agilent Jet Stream Ionization Source.

Acquisition in DMRM, and fast polarity switching mode allowed maximized dwell times for each compound. Source parameters were optimized to improve detection of poor-responding analytes.

The method was applied to the analysis of pesticides in complex matrices including black tea, and the enhanced sensitivity enabled the appropriate dilution of the sample, which is required to minimize ionization suppression effects. With any dilution, a lower matrix amount is introduced into the LC/MS system leading not only to fewer matrix effects, but also improved method robustness, increased instrument uptime, and lab productivity. Dilution of sample extracts was applied to minimize matrix effects, while still allowing quantification of the majority of pesticides with acceptable recovery ranges of 70 to 120% based on a solvent calibration. The increased sensitivity of the 6470 Triple Quadrupole LC/MS System allowed the quantification of the majority of all targeted pesticides below the maximum residue limits specified by the European Commission, even in 1:20 diluted extracts with improved precision and method robustness.

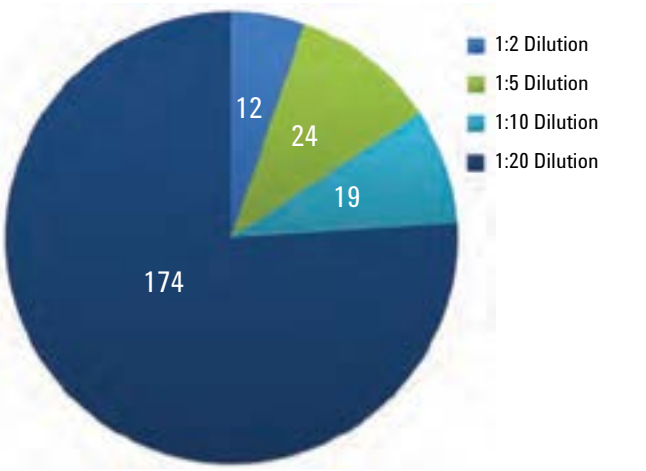

Figure 4. Detection rates of pesticides spiked into black tea extracts at the MRL of 10 µg/kg and diluted with acetonitrile. 174 pesticides were detected at the 1:20 dilution level with an area RSD <20%. Additional compounds are detected at lower dilution levels (higher concentrations).

References

1. N. P. Parra, L. Taylor, Why Instrument Detection Limit (IDL) is a Better Metric for Determining the Sensitivity of Triple Quadrupole LC/MS Systems. *Agilent Technologies Technical Overview*, publication number 5991-4089EN (**2014**).
2. D. D. Yang, *et al*. Multi-Residue Pesticide Screening and Quantitation in Difficult Food Matrixes Using the Agilent 6495 Triple Quadrupole Mass Spectrometer. *Agilent Technologies Application Note*, publication number 5991-4687EN (**2014**).

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Multi-Residue Pesticide Screening and Quantitation in Difficult Food Matrixes Using the Agilent 6495 Triple Quadrupole Mass Spectrometer

Application Note

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Abstract

This Application Note describes a UHPLC/MS/MS-based multi-residue method for the determination of more than 250 pesticides and pesticide metabolites in food samples. The method benefits from the increased chromatographic resolution of the Agilent 1290 Infinity UHPLC System, the versatile ionization capabilities of the Agilent Jet Stream ionization source, and the innate sensitivity of the Agilent 6495 Triple Quadrupole LC/MS System. The method has been applied to the analysis of pesticide residues in complex matrixes such as black tea. Matrix effects in the ionization were controlled by extensive dilution of the sample extracts prior to injection.

Our results demonstrate that the increased sensitivity of the 6495 Triple Quadrupole LC/MS System enables the accurate quantitation of targeted pesticides below the maximum residue limits (MRLs) specified by the European Commission, most of them even in the 1:100 diluted extracts, with improved precision and excellent robustness.

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Introduction

The screening and quantitation of pesticide residues in food products is one of the most important and demanding applications in food safety. There are more than 1,000 pesticides and pesticide metabolites that can be present in food and, thus, are regulated and controlled. The European Commission regulation (EC) 396/2005 and its annexes set maximum residue limits (MRLs) for more than 170,000 matrix-pesticide combinations for food[1]. Similar regulations are in place in other regions. Most pesticides are analyzed with multi-residue methods covering hundreds of compounds, which are applied to various food commodities for both screening and quantitation. Therefore, fast and reliable analytical methods are required to allow identification and quantitation of hundreds of pesticides at low concentrations in a broad range of food matrixes with confidence. Criteria for the identification of pesticide residues and requirements for method validation and quality control procedures for quantitation are specified in guidance documents such as SANCO/12571/2013[2].

Matrix effects in electrospray ionization, which change considerably between different food samples, present a significant challenge to the accurate quantitation of pesticides. There are different strategies to compensate for matrix effects such as matrix matched calibrations, standard addition, or the use of internal standards. However, matrix matched calibrations do not fully compensate for variations in matrix effects within a commodity or a commodity group. Standard addition requires multiple injections for each sample, which reduces productivity. The use of isotopically labelled internal standards is probably the most attractive approach, however, it is not applicable for all target compounds in a multi-residue pesticide method. Sample dilution is another approach to minimize matrix effects[3] but requires the use of highly sensitive analytical instruments due to the need to detect contaminants below the MRLs stipulated by the EC. Furthermore, the extensive dilution of sample extracts requires very high precision, as even small deviations result in considerable inaccuracies when multiplied with high dilution factors.

This Application Note shows the development of an UHPLC/MS/MS method for the screening and quantitation of hundreds of pesticides in food samples. The method was developed using the Pesticide tMRM LC/MS Application Kit (p/n G1733BA). Transitions for all compounds in the comprehensive pesticide standard mix (p/n 5190-0551) and a few additional pesticides of interest were included in the method. An Agilent 1290 Infinity UHPLC System was coupled to the highly sensitive Agilent 6495 Triple Quadrupole LC/MS System operated with dynamic MRM mode with fast polarity switching. Several modifications to the previous high-end triple quadrupole mass spectrometer design resulted in higher analytical performance.

- New mass filter one (MS1) ion optics for increased precursor ion transmission
- An improved curved and tapered collision cell providing enhanced MS/MS spectral fidelity
- A new ion detector operating at dynode accelerating voltages of up to 20 kV
- A new autotune optimized for speed and sensitivity

In addition, the 6495 Triple Quadrupole LC/MS System uses the proven Agilent Jet Stream Ionization source and the dual stage ion funnel. Enhanced sensitivity gives enhanced peak area response and improved peak area precision, which ultimately leads to lower detection limits compared to previous designs. The enhanced sensitivity was used for the extensive dilution of complex food sample extracts to minimize matrix effects in the electrospray ionization. The improved precision of the analytical method is demonstrated for diluted black tea samples.

Experimental

Reagents and chemicals

All reagents and solvents were HPLC or LC/MS grade. Acetonitrile and methanol were purchased from Honeywell (Morristown, NJ, USA). Ultrapure water was produced using a Milli-Q Integral system equipped with a LC-Pak Polisher and a 0.22-µm point-of-use membrane filter cartridge (EMD Millipore, Billerica, MA, USA). Formic acid was from Fluka (Sigma-Aldrich Corp., St. Louis, MO, USA) and ammonium formate solution (5 M) was from Agilent (p/n G1946-85021). Pesticides were included in the Agilent comprehensive pesticide mixture (p/n 5190-0551). A limited number of additional pesticides were purchased from Fluka (Sigma-Aldrich Corp., St. Louis, MO, USA). Immediately before use, the eight submixes of the comprehensive pesticide mixture and the mixed stock solution of the additional pesticides were combined and further diluted with acetonitrile to a final pesticide working solution containing more than 250 pesticides at a concentration of 10 µg/mL. This solution was used for spiking the QuEChERS extracts and for the preparation of the calibration samples. Eight calibration samples with concentrations ranging from 0.02 to 100 ng/mL were prepared in pure acetonitrile.

Sample preparation

Tea, orange, and tomato samples were obtained from a local grocery store. Samples were extracted according to the citrate buffered QuEChERS protocol using Agilent BondElut QuEChERS kits (p/n 5982-5650). Ten grams of homogenized fruit and vegetable or 2 g of tea were weighed into 50-mL polypropylene tubes and extracted with 10 mL acetonitrile for 1 minute while shaking vigorously by hand. The tea samples were wetted with 8 mL ultrapure water for 2 hours prior to extraction. Raw extracts were cleaned up by dispersive SPE using primary

secondary amine (PSA, p/n 5982-5256). In black tea samples, graphitized carbon black (GCB) contained in the Agilent BondElut QuEChERS EN dispersive SPE tubes (p/n 5982-5356H) was also used for cleanup. Final extracts of blank samples were spiked in five relevant concentrations with the comprehensive pesticide working solution and then diluted 1:5, 1:10, 1:20, 1:50, and 1:100 with acetonitrile. Matrix matched standards and dilutions were prepared immediately before injection, and were measured with five technical replicates.

Equipment

Separation was carried out using an Agilent 1290 Infinity UHPLC system consisting of an Agilent 1290 Infinity Binary Pump (G4220A), an Agilent 1290 Infinity High Performance Autosampler (G4226A), a sample cooler (G1330B), and an Agilent 1290 Infinity Thermostatted Column compartment (G1316C). The UHPLC system was coupled to an Agilent G6495 Triple Quadrupole LC/MS System equipped with an Agilent Jet Stream electrospray ionization source. Agilent MassHunter Workstation Software was used for data acquisition and analysis (v. B.07.00).

Method

The 1290 Infinity UHPLC System conditions are summarized in Table 1, and a summary of the 6495 Triple Quadrupole parameters are shown in Table 2. Identification of polarity, precursor and product ions, as well as optimization of collision energies, was taken from the Agilent Pesticide tMRM LC/MS Application Kit, and was further optimized using Agilent MassHunter Optimizer Software. Analysis was carried out with positive and negative electrospray ionization in dynamic multiple reaction monitoring (dMRM) in a single analytical run. A 2-µL amount of the final extract was injected into the UHPLC/MS/MS.

Data were evaluated using the Agilent MassHunter Quantitative Analysis Software. Calibration was done using neat standard solutions and linear, 1/x weighted calibration curves. The lower limits of quantitation (LLOQs) correlate with the instrument detection limits (IDLs) in black tea matrix. IDL is calculated based on the relative standard deviation of a series of replicates of a low level sample that is not higher in concentration as 2 to 5 times the detection limit. The IDL is defined as the minimum amount of analyte required to produce a signal that is statistically distinguishable from background noise with a confidence level of 99 %. This approach has much more relevance for routine operation as it avoids ambiguity related to the variation in the chemical noise and subjectivity in the way signal-to-noise (S/N) is determined[4]. In addition, it is directly correlated to the precision of the analytical method, which is important when doing an extensive dilution of sample extracts.

Table 1. Instrument parameters.

Agilent 1290 Infinity UHPLC System	
Column	Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 150 mm, 1.8 µm (p/n 959759-902)
Column temperature	40 °C
Injection volume	2 µL
Speed	Draw 100 µL/min, Eject 200 µL/min
Autosampler temp	6 °C
Needle wash	10 s with acetonitrile/water (50/50; v/v)
Mobile phase	A) 5 mM ammonium formate + 0.1 % formic acid B) 5 mM ammonium formate + 0.1 % formic acid in methanol
Flow rate	0.4 mL/min
Gradient program	Time B % 0 5 0.5 5 3.5 50 17.0 100 20.0 100 20.1 5
Stop time	20.1 minutes
Post time	3 minutes
Agilent 6495 Triple Quadrupole LC/MS System	
Ion mode	Positive and negative ESI with Agilent Jet Stream
Scan type	Dynamic MRM
Drying gas temperature	120 °C
Drying gas flow	17 L/min
Sheath gas temperature	300 °C
Sheath gas flow	12 L/min
Nebulizer pressure	30 psi
Capillary voltage	3,500 (pos/neg)
Nozzle voltage	300 V (pos); 500 V (neg)
Cycle time	500 msec
Total number of MRMs	532 (positive: 509/negative: 23)
Maximum number of concurrent MRMs	68
Minimum dwell time	5.10 ms
Maximum dwell time	249.09 ms
MS1 and MS2 resolution	Unit

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Results and Discussion

Development of the UHPLC/MS/MS method

The pesticide screening method developed for the Agilent Pesticide tMRM LC/MS Application Kit was transferred to the 6495 Triple Quadrupole LC/MS System. The method was extended to include several relevant acidic herbicides, and fast polarity switching was employed. Compound-dependent parameters such as collision energy and cell acceleration voltage were fine optimized but only minor deviation from optimized values for previous models was observed. The prefilter and the detector were adjusted according to mass during the instrument's autotune. Sheath gas temperature was optimized using the MassHunter Source Optimizer Software to produce the highest abundance for the majority of target compounds, and to not compromise labile and ammonium adduct-forming compounds.

Figure 1 shows the chromatogram of a tea extract spiked with more than 250 pesticides at a concentration of 10 µg/kg, and diluted 1:20 with acetonitrile prior to injection.

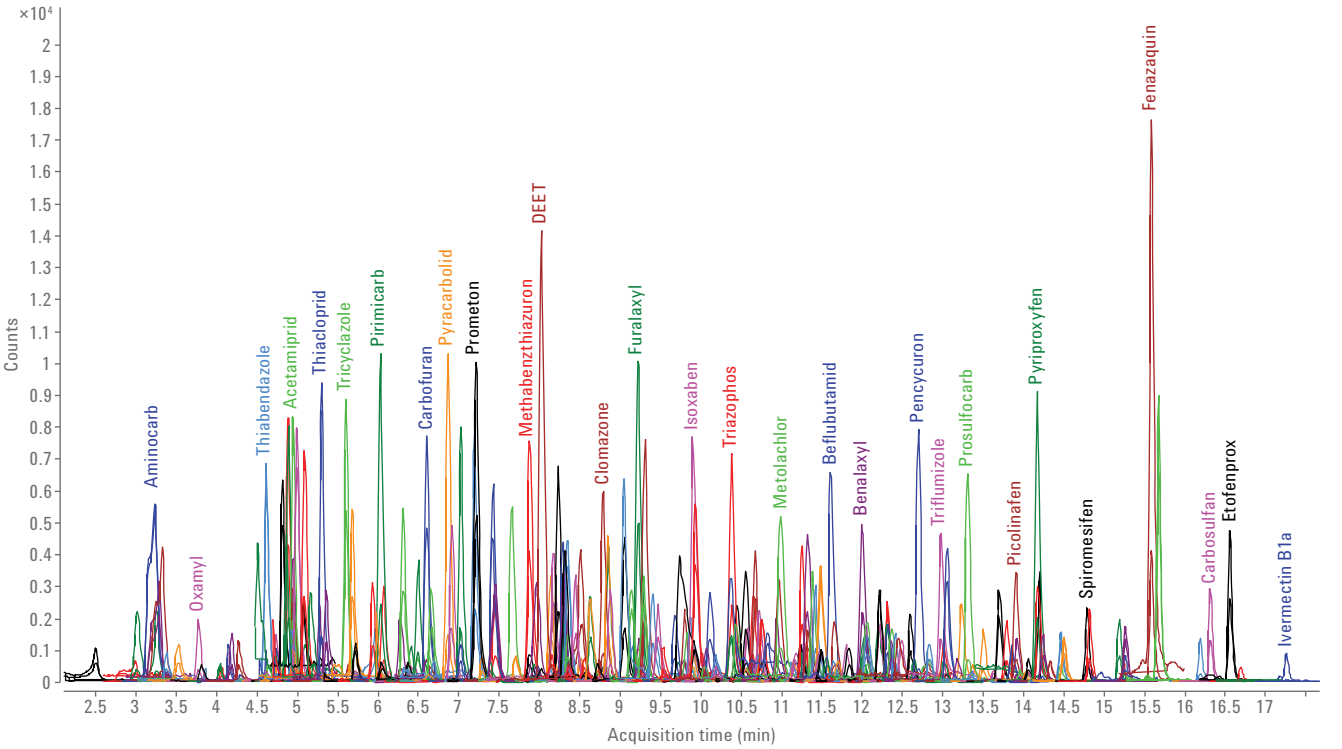

Figure 1. Chromatograms of more than 250 pesticides spiked into black tea at the MRL and diluted 1:20 with acetonitrile (corresponding to a concentration of 0.1 ng/mL). For the sake of clarity, only part of the chromatographic peaks are labelled.

Evaluation of increased instrument performance

The MS1 ion optics has demonstrated a noticeable increase in the precursor ion transmission, and an increase up to a factor of 3 has been observed, depending on the ion mass. In addition, the detector design results in signal gains especially for large fragment ions and negative ions across a broad mass range. When comparing the area response of pesticides acquired with the 6495 Triple Quadrupole System to results from the earlier model, a compound dependent area gain of up to a factor of 5 was observed.

Enhanced ion transmission not only resulted in increased peak areas but also in improved peak area precision.

These enhancements ultimately lowered detection limits compared to previous designs. The empirical observation that supports this hypothesis is shown in Figure 2, which compares the obtained area RSDs on a 6495 Triple Quadrupole System versus a 6490 Triple Quadrupole for 50 pesticides spiked into black tea at the MRL and diluted in different ratios with acetonitrile. The selection of these 50 pesticides was based on relevance. Several of those compounds were found in official control samples above the MRL and thus, the import of tea into the European Union was blocked. The area of the blue polygon is considerably smaller than the area of the red polygon, which indicates that the improved ion statistics of the 6495 Triple Quadrupole System instrument translates into considerably lower RSD values for most pesticides at the same dilution levels. The relative standard deviation (RSD) of a series of replicates at a low concentration level is a universal measure of the ion efficiency, and can be used for the estimation of the quantitation limits. A low RSD value has much more relevance than S/N measurements, which can change based on the selected noise region and the software algorithm used for calculation. A particular RSD can be defined as the minimum amount that can be reliably detected, as long as the noise level does not significantly contribute to RSD values. For pesticide residues, a maximum RSD of 20 % has been specified as the minimum performance requirement in SANCO/12571/2013.

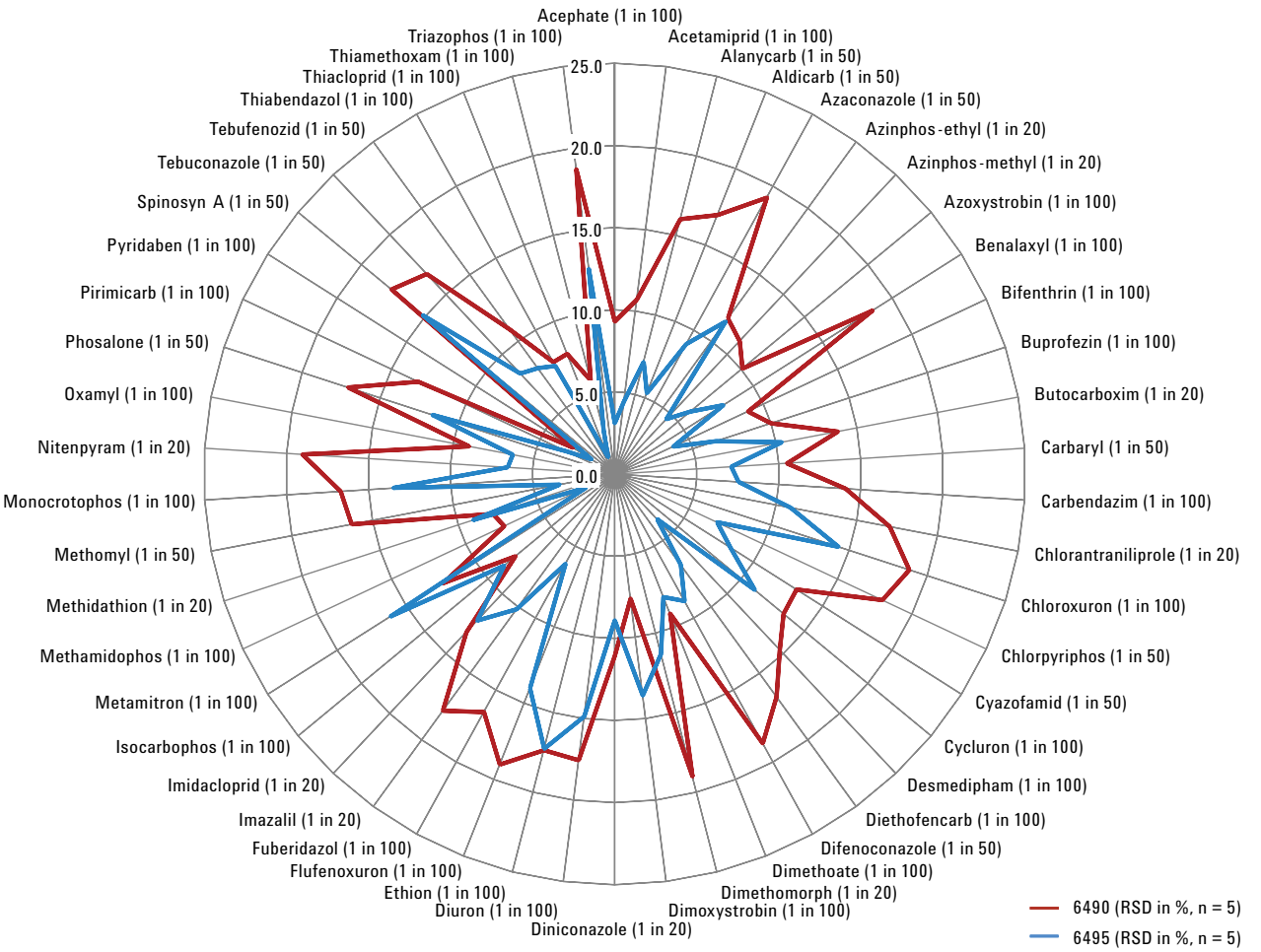

Figure 2. Comparison of area RSDs for pesticides spiked into black tea at the MRL and diluted in different ratios with acetonitrile for the Agilent 6495 LC/MS (blue) and the Agilent 6490 Triple Quadrupole (red).

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With the updated design of the 6495 Triple Quadrupole LC/MS System, more pesticides can be detected at low concentrations in QuEChERS extracts of different food commodities according to the quality criteria specified in the SANCO guidelines. In tomato and orange extracts, all pesticides were easily detected at the lowest spiked concentrations of 1 ng/g. The tea matrix at this concentration has a slightly smaller detection rate due to the 5-fold lower sample amount and the more complex matrix. Figure 3 shows the detection rate of the pesticides for different dilution levels in the black tea matrix.

Under the applied experimental conditions, approximately 67 % of all the spiked pesticides were easily detected with an RSD below 20 % in the 1:100 dilution, corresponding to a concentration of 0.02 ng/mL. In addition, approximately 20 % were detected with acceptable precision in the 1:50 dilution, and another ~10 % in the 1:20 dilution. Excellent precision was observed for replicate injections of these samples within a 72-hour worklist.

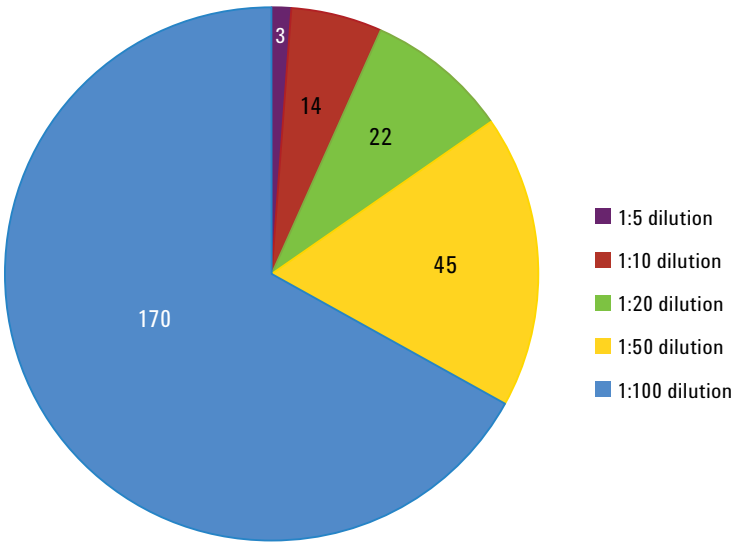

Figure 3. Pesticides spiked in black tea extract to 2 ng/mL and diluted at different levels. 170 pesticides can be detected at the 1:100 dilution level with an area RSD < 20 %. Additional compounds are detected at higher concentrations, that is, at lower dilution levels.

Minimizing matrix effects by dilution of sample extracts

The ability to extensively dilute sample extracts to remove matrix effects is an attractive capability to many routine testing labs. It enables quantitation of complex samples against a solvent calibration. A possible cause for matrix effects in electrospray ionization is the limited number of excess charges, and the limited space on the surface of the charged droplet. The dilution of the matrix frees up space at the surface, resulting in more efficient ionization of the target compounds. In addition, the amount of matrix injected to the LC/MS system is limited, which results in increased robustness of the analytical method, minimization of instrument contamination, and increased instrument uptime.

Figure 4 shows the chromatograms of alanycarb and oxamyl spiked in black tea extract corresponding to 10 µg/kg and diluted with acetonitrile prior to injection in different dilution ratios.

Upon 1:5 dilution, the signal for alanycarb increased. For oxamyl and the further dilution levels of alanycarb, the peak areas decreased less than the extent to which the target compounds were diluted. Dilution typically causes the final concentrations of the pesticides to increase until the point at which complete recovery is achieved. Table 3 shows the recoveries in black tea extract for 10 pesticides and different dilution ratios.

While a weak matrix effect was observed for diuron and flufenoxuron, the signal suppression for monocrotophos and alanycarb in the nondiluted tea was substantial. However, when diluting the final extract 1:10, more than half of the compounds showed adequate recoveries of over 70 %. Very few compounds required a larger dilution of 1:50, or even 1:100 to minimize the matrix effects to achieve acceptable recoveries based on a solvent calibration. In the 1:100 dilution, all detectable pesticides showed full recovery and basically no signal suppression. This is in agreement with published results, which showed that the Agilent Jet Stream Ionization required less dilution to eliminate matrix effects compared to equivalent techniques[3].

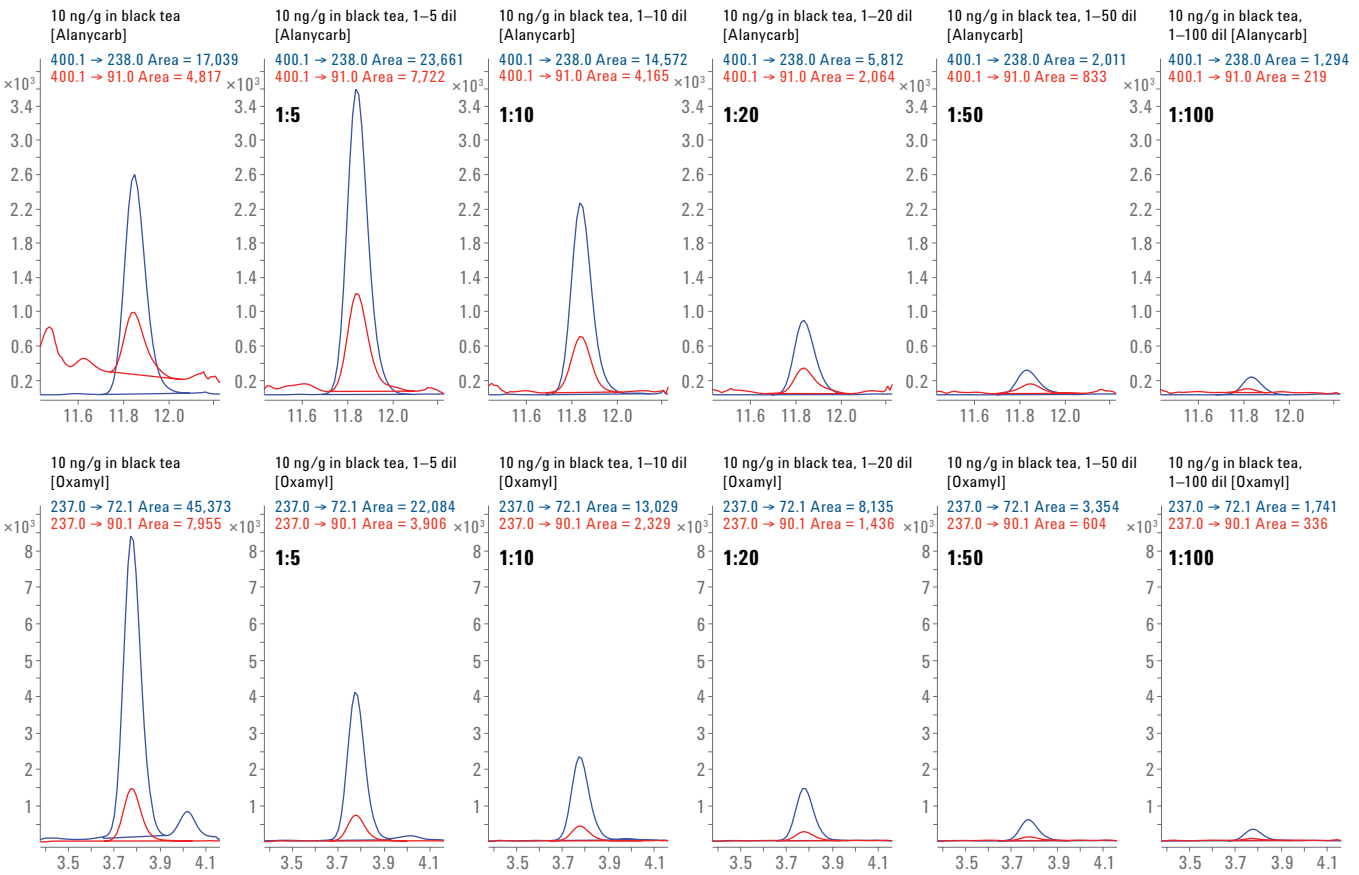

Figure 4. Comparison of peak areas for alanycarb and oxamyl spiked in black tea and diluted with acetonitrile 1:5, 1:10, 1:20, 1:50, and 1:100 prior to injection.

Table 3. Recoveries for selected pesticides calculated for different dilution ratios. Cells shaded in green comply with requirements of SANCO/12571/2013.

Analytes	No dilution (n = 5)	Dilution 1:5 (n = 5)	Dilution 1:10 (n = 5)	Dilution 1:20 (n = 5)	Dilution 1:50 (n = 5)	Dilution 1:100 (n = 5)
Acetamiprid	29.4 ± 0.8	57.3 ± 1.4	67.5 ± 3.7	79.9 ± 2.9	91.8 ± 5.2	109.5 ± 3.4
Alanycarb	10.4 ± 1.3	73.9 ± 2.2	81.5 ± 14.3	85.7 ± 11.1	87.6 ± 4.7	121.7 ± 10.8
Aldicarb	36.9 ± 1.0	69.9 ± 1.4	78.0 ± 3.5	91.0 ± 4.2	95.2 ± 8.8	104.9 ± 14.1
Carbaryl	56.9 ± 1.8	80.1 ± 3.8	80.8 ± 4.1	96.1 ± 7.2	102.6 ± 6.6	116.4 ± 9.6
Dimethoate	33.9 ± 1.7	68.6 ± 2.4	84.1 ± 5.4	89.0 ± 7.9	88.2 ± 8.8	84.7 ± 7.5
Diuron	79.7 ± 4.0	90.4 ± 7.0	91.7 ± 4.9	94.9 ± 7.2	89.2 ± 7.3	100.9 ± 13.5
Flufenoxuron	95.4 ± 1.1	88.8 ± 1.6	89.4 ± 3.8	93.3 ± 5.8	100.0 ± 6.1	119.2 ± 13.9
Monocrotophos	4.6 ± 0.3	13.9 ± 0.3	21.8 ± 0.8	33.8 ± 1.1	58.5 ± 2.0	95.1 ± 5.7
Oxamyl	20.8 ± 0.7	52.6 ± 1.9	65.0 ± 2.0	79.7 ± 3.0	91.2 ± 4.6	110.6 ± 5.2
Thiamethoxam	40.0 ± 1.4	45.9 ± 0.9	46.6 ± 3.8	52.2 ± 1.7	70.9 ± 2.9	97.3 ± 2.0

Conclusions

An UHPLC/MS/MS based multi-residue method for the determination of more than 250 pesticides and pesticide metabolites has been developed. It takes full advantage of the low delay volumes of the Agilent 1290 Infinity LC System and its ability to handle high back pressures in UHPLC separations for increased chromatographic resolution. The method benefits from the highly sensitive Agilent 6495 Triple Quadrupole LC/MS System and from the versatile ionization capabilities of the Agilent Jet Stream ionization source. Dynamic MRM acquisition and fast polarity switching were used to maximize dwell times for each individual compound. Source parameters were optimized to achieve good sensitivity across the suite of target compounds.

The method was applied to the analysis of pesticides in complex matrixes such as black tea. Enhanced sensitivity allowed for more flexibility in the degree of sample dilution. With any dilution, a lower matrix amount is introduced into the LC/MS system leading not only to fewer matrix effects, but also to improved method robustness and increased instrument uptime. Extensive dilution of sample extracts was applied to minimize matrix effects, and to allow quantitation of all pesticides within the acceptable recovery range of 70 to 120 % based on a solvent calibration. The increased sensitivity of the 6495 Triple Quadrupole LC/MS System allowed the quantitation of the majority of all targeted pesticides below the maximum residue limits specified by the European Commission, even in the 1:100 diluted extracts with improved precision and excellent robustness.

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1. Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin (including amendments as of 18 March 2008) and complying with regulation (EC) 1107/2009.

2. Guidance document on analytical quality control and validation procedures for pesticide residues analysis in food and feed. Document N° SANCO/12571/2013. Implemented by 01/01/2014. http://www.eurl-pesticides.eu/library/docs/allcrl/AqcGuidance_Sanco_2013_12571.pdf

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Application of a Triggered MRM Database and Library for the Quantitation and Identification of Pesticides in Food Extracts

Application Note

Food

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Abstract

This application note describes the development of a triggered MRM database and library for more than 300 pesticides. It illustrates its use to analyze a range of food commodities with an LC/MS method developed for a specific suite of 120 pesticide residues. An Agilent 1290 Infinity LC System was coupled to an Agilent 6460 Triple Quadrupole LC/MS System and operated in positive electrospray using Agilent Jet Stream Technology. The triggered MRM acquisition mode was used for quantitation and verification and to eliminate potential false detects. A short, in-house, validation done for three commodity groups with five representative matrices showed that the developed triggered MRM method was appropriate for the analysis of pesticides in food extracts with regards to the required limits of quantitation (LOQs), linearity, and reproducibility. Several examples are shown where a high risk of an interfering matrix peak being incorrectly assigned as a pesticide, was mitigated through triggered MRM. Automatic reference library matching, displayed alongside quantitation results allows data to be reviewed efficiently and for suspect cases to be flagged automatically.

Introduction

Food commodities, designated for human consumption need to be analyzed for pesticide residues. Commission regulation (EC) 396/2005 and its annexes which were implemented in September 2008 set maximum residue limits (MRLs) for more than 170,000 matrix-pesticide combinations for food and feed products produced in, or imported into the European Union [1]. Criteria for method validation and quality control procedures for pesticide residue analysis in food and feed are set in the SANCO/12495/2011 guideline [2].

Multiresidue methods based on LC/MS are used to monitor and quantify an increasing number of pesticides. It is generally accepted that, due to their selectivity and sensitivity, triple-quadrupole instruments are the best instruments for the quantitation of hundreds of pesticide residues in a wide variety of food matrices at very low levels. However, in complex matrices, several cases have been reported where matrix constituents were interfering on the MRM traces of pesticides with similar or identical retention times, eventually resulting in false positives [3]. In some examples, the identification criteria laid down in SANCO/12495/2011 requiring two MRM transitions with a constant ion ratio, and the retention time have not been selective enough for an unambiguous identification. Consequently, it is desirable to acquire additional transitions or a full compound spectrum. Typically, this does compromise the number of compounds which can be included in the method.

Triggered MRM enables both, fast cycle times allowing for methods with hundreds of compounds and full spectrum acquisition. Based on the response of one or more primary transitions, the acquisition of up to nine additional transitions can be triggered resulting in a full compound spectrum which can be compared against a spectral library. Therefore, confirmation of an analyte is not only based on the area ratio of the two primary MRM transitions but also on a reference library match score. Since each fragment is acquired with the optimized collision energy, and since dwell times for a fragment in triggered MRM are considerably longer than in a full scan cycle, spectral quality of the triggered MRM spectra is significantly better than data dependent product ion scans [4]. Furthermore, triggered MRM is managed by the Agilent dynamic MRM algorithm ensuring constant cycle times for the primary (quantitative) transitions. Therefore, the collection of triggered MRM spectra does not affect the data collection rate and area of the quantitative chromatographic peak.

This application note shows the development of a triggered MRM database and library for more than 300 of the most important pesticides amenable to LC/MS according to the "Check-your-Scope" list of the European reference laboratory (EURL) for pesticide residues. The triggered MRM acquisition mode was applied to the analysis of 120 pesticide residues in different food commodities (lemon, tomato, green tea, chamomile, and ginger). QuEChERS extracts of the selected matrices were spiked in several levels ranging from 1 µg/kg to 100 µg/kg. An in-house validation according to SANCO/12495/2011 was done for three commodity groups using tomato, lemon, and green tea as representative commodities. Method performance was characterized for the triggered MRM method and a corresponding dynamic MRM method including the evaluation of matrix effects due to signal suppression or enhancement (SSE), the determination of linearity, limits of quantitation (LOQs) based on the signal-to-noise ratio, and repeatability derived from five replicates on different spiking levels. Several examples of complex matrices are shown which show spectral interferences in either one or both primary MRM traces and for which triggered MRM adds valuable information to avoid false positives.

Experimental

Reagents and Chemicals

All reagents and solvents were HPLC or LCMS grade. Acetonitrile and methanol were purchased from Baker (Mallinckrodt Baker, Deventer, Netherlands). Ultrapure water was produced using a Milli-Q Integral system equipped with a 0.22 µm point-of-use membrane filter cartridge (EMD Millipore, Billerica, MA, USA). Formic acid was from Fluka (Fluka AG, Buchs, Switzerland) and ammonium formate solution (5 M) was from Agilent (p/n G1946-85021). The majority of pesticide analytical standards were purchased from Dr. Ehrenstorfer (Augsburg, Germany).

Solutions and standards

The individual pesticide standard solutions were combined to eight mixtures containing 30 to 40 compounds of similar physicochemical properties at a concentration of 10 µg/mL in acetonitrile and were stored at –20 °C. Immediately before use, the eight sub-mixes were combined to a final pesticide mixture containing more than 300 pesticides at a concentration of 1 µg/mL in acetonitrile. This solution was used for spiking the QuEChERS extracts and for the preparation of the calibration samples. Eight calibration samples in a concentration range from 0.1 to 100 ng/mL were prepared in pure acetonitrile.

Sample preparation

Fruit and vegetables, dried chamomile flowers, and green tea were obtained from a local greengrocery. Samples were prepared according to the official citrate buffered QuEChERS method [5] using an Agilent BondElut QuEChERS kit (p/n 5982-5650). Ten grams of homogenized fruit and vegetable samples or 2 g of chamomile flowers or green tea were weighed in 50-mL plastic tubes. The chamomile flowers and the green tea were wetted with 10 mL ultrapure water. All samples were extracted with 10 mL acetonitrile for 1 minute while shaking vigorously by hand. Only the lemon homogenate was neutralized afterwards by adding 600 µL of a 5 M sodium hydroxide solution. An extraction salt packet containing 4 g anhydrous $MgSO_4$, 1 g NaCl, and 1.5 g buffering citrate salts was added to each tube for partitioning. The tube was again shaken for 1 minute by hand. Sample tubes were centrifuged at 3,000 rpm for 5 minutes.

A 6-mL aliquot of the upper acetonitrile layer was transferred into an Agilent BondElut QuEChERS EN dispersive SPE tube (p/n 5982-5256) containing 150 mg primary secondary amine (PSA) and 15 mg graphitized carbon black (GCB) for sample cleanup, and 900 mg $MgSO_4$ for water removal. The tubes were closed and vortexed for 1 minute. Afterwards, the tubes were centrifuged at 3,000 rpm for 5 minutes. The clear extracts were transferred in glass vials and 10 µL of 5% formic acid in acetonitrile were added to each mL extract to improve the stability of the target pesticides.

For the evaluation of repeatability and matrix effects, blank tomato, ginger, lemon, green tea, and chamomile samples were extracted and matrix-matched standards were prepared in three concentration levels by adding the required amount of the final pesticide mixture to an aliquot of the final QuEChERS extract. The matrix matched standards were prepared immediately before injection and were measured with five technical replicates.

Equipment

Separation was carried out using an Agilent 1290 Infinity UHPLC system consisting of an Agilent 1290 Infinity Binary Pump (G4220A), an Agilent 1290 Infinity High Performance Autosampler (G4226A), and an Agilent 1290 Infinity Thermostatted Column compartment (G1316C). The UHPLC system was coupled to an Agilent G6460A Triple Quadrupole LC/MS System equipped with an Agilent Jet Stream electrospray ionization source. MassHunter workstation software was used for data acquisition and analysis.

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Method

The 1290 Infinity UHPLC conditions are summarized in Table 1 and the G6460A Triple Quadrupole parameters are summarized in Table 2 and Table 3. Analysis was carried out in positive electrospray ionization in dynamic MRM mode using two major transitions per compound and in triggered MRM mode using two primary transitions and up to eight confirmatory ions. The confirmatory ions were measured over five acquisition cycles once the primary transition set as the trigger (typically the quantifier trace) was over a given threshold. The thresholds were compound specific and were set to 50% of the lowest calibration standard. No trigger entrance delay or trigger delay were set and the trigger window was set to the full width of the MRM window to allow for the multiple triggering in case of matrix interferences. The Pesticide MRM data base kit (p/n G1733AA) was used to populate the method with two major (primary) transitions and conditions [6]. Further transitions used as confirmatory ions in the triggered MRM method were optimized for each individual pesticide using the Mass Hunter Optimizer software. A Dynamic MRM method was then automatically produced from the primary transitions and was run both with and without triggered MRM. Table 5 summarizes the primary transitions and the number of additional confirmatory ions for all investigated pesticides.

Table 1. UHPLC Parameters

UHPLC column	Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 150 mm, 1.8 µm (p/n 959758-902) at 30 °C	
Mobile phase	A: 0.1% formic acid + 5 mM ammonium formate in water	
	B: 0.1% formic acid + 5 mM ammonium formate in methanol	
Gradient program	Min	%B
	0	5
	0.5	5
	3	40
	17	100
	19	100
	19.1	5
	Post time 2 minutes	
Flow rate	0.4 mL/min	
Injection volume	3 µL	

Table 2. Agilent G6460A Triple Quadrupole Parameters Operated in Dynamic MRM Mode

Ionization mode	Positive ESI with Agilent Jet Stream
Scan type	Dynamic MRM
Gas temperature	300 °C
Gas flow	9 L/min
Nebulizer pressure	35 psi
Sheath gas temperature	350 °C
Sheath gas flow	12 L/min
Capillary voltage	+4,000 V
Nozzle voltage	0 V
Cycle time	800 ms
Total number of MRMs	240
Maximum number of concurrent MRMs	36
Minimum dwell time	18.72 ms
Maximum dwell time	396.5 ms

Table 3. Agilent G6460A Triple Quadrupole Parameters Operated in Triggered MRM Mode

Ionization mode	Positive ESI with Agilent Jet Stream
Scan type	Triggered MRM with five repeats
Gas temperature	300 °C
Gas flow	9 L/min
Nebulizer pressure	35 psi
Sheath gas temperature	350 °C
Sheath gas flow	12 L/min
Capillary voltage	+4,000 V
Nozzle voltage	0 V
Cycle time	800 ms
Total number of MRMs	818
Maximum number of concurrent MRMs	117
Minimum dwell time	3.34 ms
Maximum dwell time	196.5 ms

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Results and Discussion

Development of a triggered MRM database and library

Most commercially available MRM databases as well as public domain collections of MRM transitions for pesticides typically contain only two major transitions. The value of triggered MRM arises from the availability of several MRM transitions per compound and a spectral library including those transitions acquired under optimized conditions. A major part of this work was the development of a triggered MRM database and library containing more than 300 pesticides. For each compound, all MRM transitions which showed a reasonable response were optimized using the Mass Hunter Optimizer software. The precursor and product ions as well as the fragmentor voltages and collision energies were optimized by flow injection of single analyte solutions into the UHPLC-MS/MS system. In the default configuration, Optimizer automatically optimizes the four most abundant fragments per compound. If further fragments were observed in the product ion spectra which showed an intensity of more than 5% relative to the most abundant fragment, the additional fragments were optimized in a second Optimizer experiment. Figure 1 shows the sum of the spectra of napropamide resulting from collisionally induced dissociation (CID) and acquired at collision energies of 0, 15, 30, 45, and 60 eV. The fragments marked with a circle were automatically picked by the Optimizer algorithm; the fragments marked with a triangle were optimized in a second Optimizer experiment.

The optimized transitions were stored in an Optimizer database which finally contained more than 2,000 transitions and conditions for more than 300 pesticides. Depending on the fragmentation behavior of the individual compounds the triggered MRM database contained transitions and conditions of 2 to 10 fragments per pesticide.

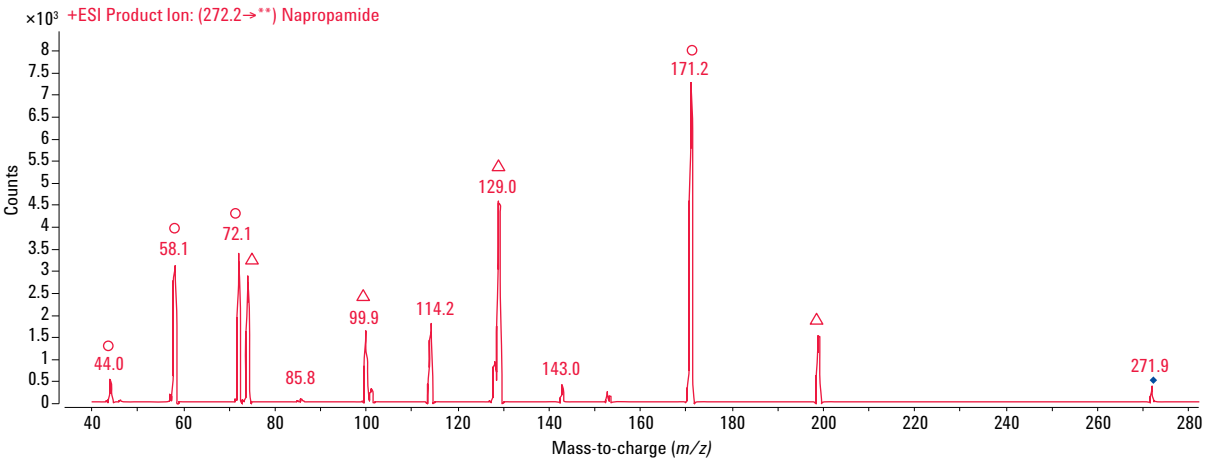

Figure 1. Sum of CID spectra of napropamide acquired at collision energies of 0, 15, 30, 45, and 60 eV. Fragments marked with a circle were optimized during the first optimization experiment, fragments marked with a triangle were optimized in a second experiment.

These optimized conditions were then used for the creation of a triggered MRM spectral library. Spectra were acquired under the UHPLC and Agilent Jet Stream conditions specified in Tables 1 and 3 for the eight pesticide sub-mixes diluted to a concentration of 100 ng/mL. Using Mass Hunter Quantitative Analysis software, the spectra could then be easily populated into a triggered MRM library. The library can be browsed with the Library Editor which shows the spectra along with the name, the CAS number, the molecular formula, weight, and the structure (an example is shown in Figure 2).

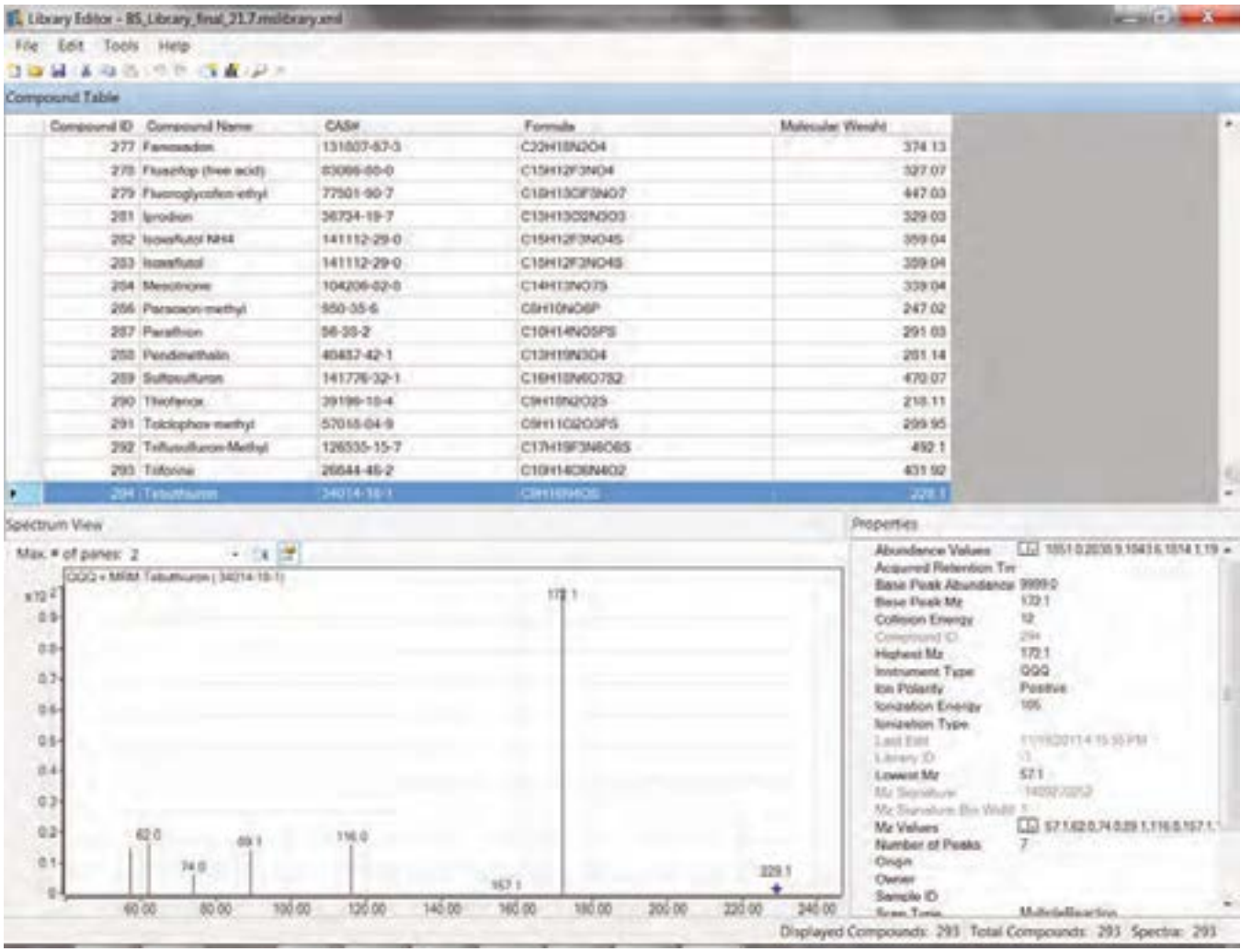

Figure 2. Mass Hunter Quantitative Analysis Library Editor showing a section of the triggered MRM library and the triggered MRM spectrum of tebuthiuron acquired in positive ESI.

Setup and optimization of the triggered MRM acquisition method

An analytical method using UHPLC and triggered MRM acquisition was set up for 120 relevant pesticides covering the full polarity range and the most important compound classes. The chromatographic method was optimized to fully resolve pesticides sharing isobaric transitions and the Agilent Jet Stream parameters were optimized to produce the highest abundance for the target compounds. Depending on the compounds, the $[M+H]^+$ or $[M^+NH_4]^+$ species were used as the precursor ion. The two most abundant fragments were defined as primary transitions which were acquired over the full retention time window and were used as the quantifier and qualifier ion. The quantifier transition was typically used as the triggering transition and the threshold for the data dependent triggering of the additional four to eight fragment ions was set on a compound by compound basis between 100 and 5,000 counts corresponding to 50% of the response of the lowest calibration sample. This approach allowed the acquisition of product ion spectra for most compounds in all matrices and even at the lowest spiking levels.

Figure 3 shows the chromatogram of a lemon extract spiked with more than 120 pesticides at a concentration of 10 µg/kg measured with triggered MRM with two primary transitions and up to eight additional confirmatory transitions (not shown).

While the primary transitions were measured during the whole observation window and were used for quantitation, the additional transitions were measured only for a certain number of repeats once the intensity of the triggering transition exceeded the given threshold. Triggered MRM is managed as part of the dynamic MRM algorithm so constant cycle times for the primary transitions are maintained throughout. Therefore, the acquisition of additional transitions does not compromise the peak shape of the quantifier or qualifier trace, and does not affect the signal intensities.

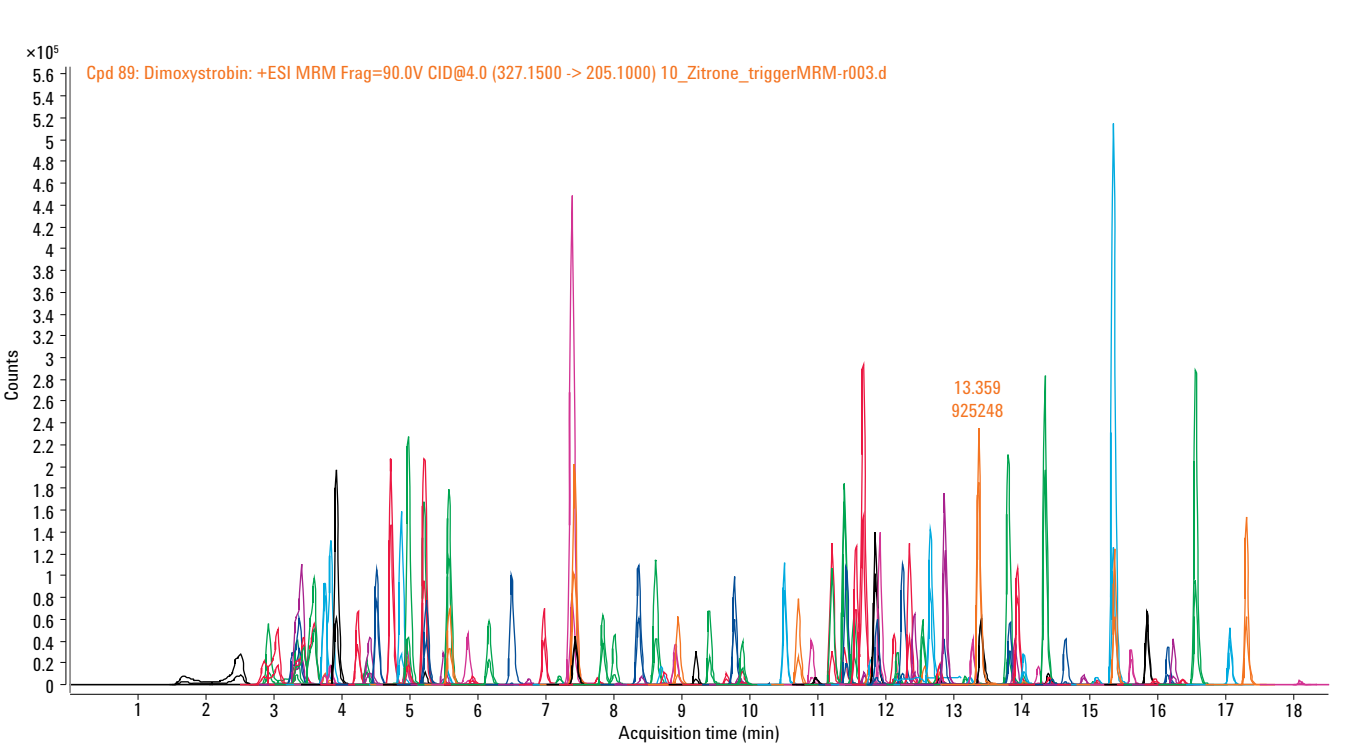

Figure 3. Chromatogram of the primary transitions of 120 pesticides spiked into lemon extract at a concentration corresponding to 10 µg/kg and acquired with triggered MRM.

Figure 4A shows the non-smoothed MRM chromatograms of napropamide spiked into lemon extract to a concentration of 1 µg/kg acquired with the triggered MRM method. Although napropamide elutes in the most crowded region of the chromatogram with 34 primary transitions and additionally 86 confirmatory ions acquired at the tip of the peak, the peak shape of the quantifier and first qualifier is not impinged. Even at a concentration 50 times below the maximum residue limit (MRL) for lemons, the observed area ratio of the two primary transitions was in good agreement with the expected ratio. The triggered MRM spectra of napropamide in lemon extract acquired for spiking concentrations of 1, 10, and 100 µg/kg are shown in Figure 4B. Across the different concentration levels the in spectrum ratio of the fragments were extremely reproducible with RSDs well below 5% for five replicate injections. Consequently, Reference Library Match Scores above 90 were observed even for the lowest spiking levels. This was verified for several other pesticides within the test suite. The high quality spectra acquired with triggered MRM even at very low concentrations are a result of an improved ion statistics due to the use of optimized collision energies for each transition and reasonably long dwell times.

For standard dynamic MRM, the average dwell time of a transition is constant for different samples. When data dependent triggering is added to a method this will result in lower dwell times for the primary transitions when the confirmatory ions are triggered. This might be different for various samples or calibration standards. It is essential that these differences in the average dwell times are not reflected in the peak areas and do not have negative effects on the quantitation and the reproducibility.

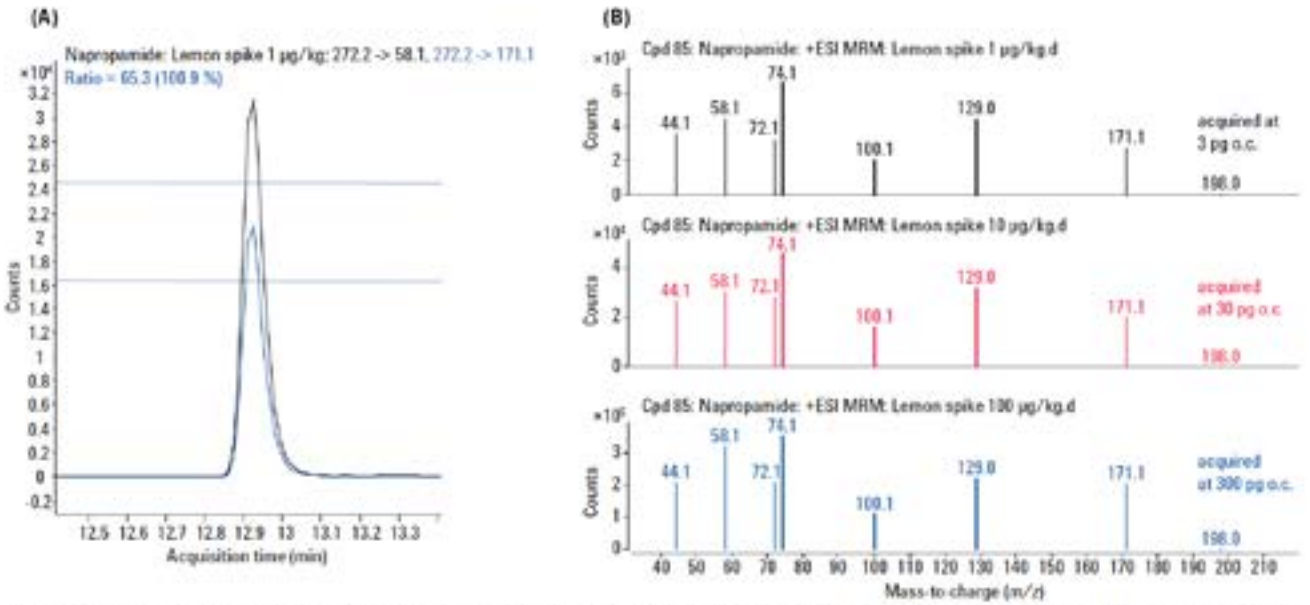

Figure 4. MRM chromatograms for the primary transitions for napropamide spiked into lemon extract at a concentration corresponding to 1 µg/kg (A) and triggered MRM spectra of napropamide spiked into lemon extract (B) at concentrations of 1 (black), 10 (red), and 100 µg/kg (blue).

To demonstrate this, a dynamic MRM method was compared to a triggered MRM method for the analysis of a complex standard (representing a worst case scenario). Figure 5 shows the calibration curves for oxamyl, a pesticide rated high in the Check-your-scope ranking of the EURL for pesticides acquired with dynamic MRM (A) and triggered MRM (B). The average dwell times for the transitions of oxamyl in the dynamic MRM method were 44 ms. In comparison, the dwell times for the primary transitions of oxamyl in the triggered MRM method was only 12 ms since product ion spectra for all co-eluting target pesticides were triggered during this peak. Nevertheless, the calibration functions as well as the correlation coefficients were very similar.

Figure 6 compares the slopes of all targeted pesticides acquired with dynamic MRM and triggered MRM. For both acquisition modes, the calibration slopes are closely correlated with a slope of 0.9987 and a correlation coefficient of $R^2 = 0.9975$. This shows that peak areas for both acquisition modes were comparable at all levels and for differently

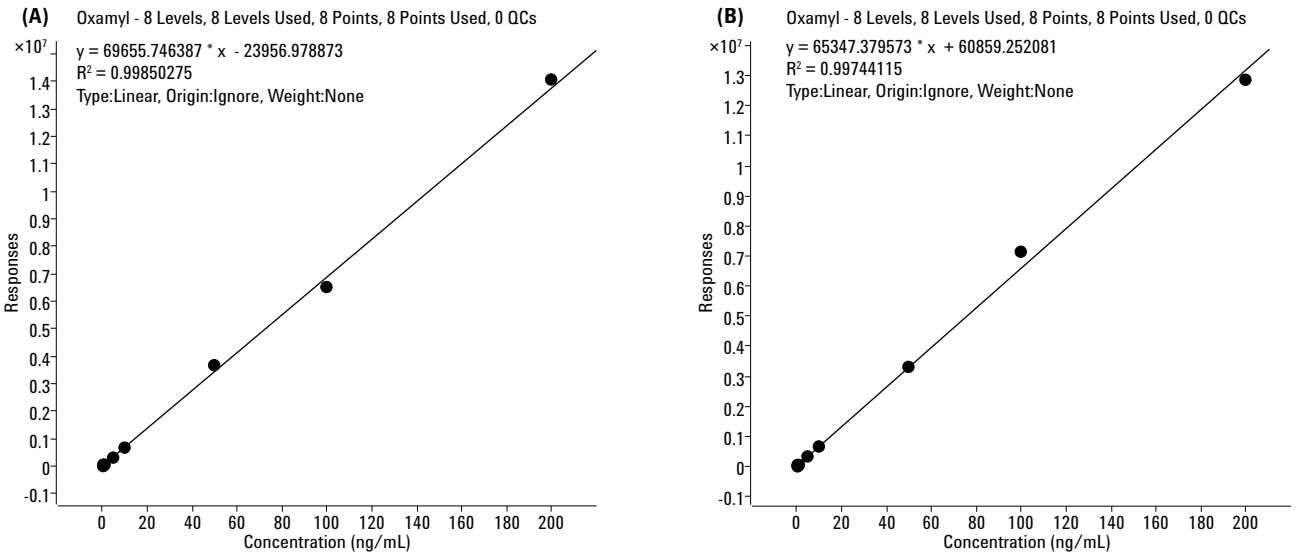

Figure 5. Calibration curves for the pesticide oxamyl acquired with dynamic MRM (A) and triggered MRM (B).

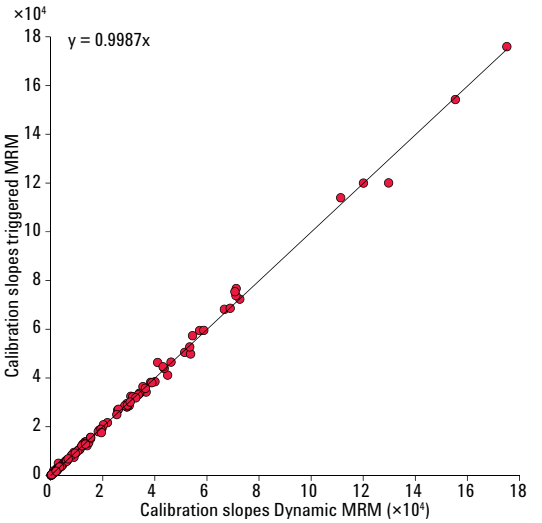

Figure 6. Comparison of the calibration curve slopes of triggered MRM versus dynamic MRM for all pesticides included in the methods. The linear range of the 7-point calibration curves were selected on a compound by compound basis and the equal calibration points were compared for both acquisition modes.

responding compounds. For 97% of all target compounds, the slope deviation was less than 20% which allows the quantitation of samples acquired with triggered MRM based on a calibration acquired with dynamic MRM.

In-house validation of triggered MRM for the quantitation of pesticides in different matrices

Method performance was characterized by the linear working range, limits of quantitation, and the repeatability of the method. Matrix effects were evaluated for spiked QuEChERS extracts of tomato, ginger, chamomile, green tea, and lemon. Validation experiments were done and evaluated based on the guideline SANCO/12495/2011.

LOQs of the triggered MRM method were derived from a signal-to-noise ratio of 10:1 (peak-to-peak noise algorithm; based on signal height) of the quantifier transition and were below 5 µg/kg for all target compounds. More than 100 compounds could be quantified in all tested matrices well below 1 µg/kg. Figure 7 shows the histogram of the LOQs in the solvent standard and in the lemon extract. As expected, LOQs in the lemon matrix were slightly higher due to matrix effects. A similar distribution was observed for the ginger, green tea, and chamomile matrix.

The repeatability was determined for all matrices at three different concentration levels (n = 5) and was below 5% for more than 80% of all compounds at a spiking level corresponding to 1 µg/kg independent of the matrix. At this concentration even in the lemon matrix, 95% of all compounds showed RSDs below 20% and could be successfully validated according to the SANCO guidelines.

Evaluation of matrix suppression and enhancement was done by comparing the response of the target compound in a solvent standard against a spiked sample extract. Depending on the commodity, up to 90% of the target compounds were affected by matrix effects. Primarily, signal suppression was observed, but for the ginger matrix, more than 10% of the target compounds showed an enhanced signal of more than 120% of the solvent response. When using matrix matched calibrations, accurate quantitation of the target compounds in each matrix could be achieved.

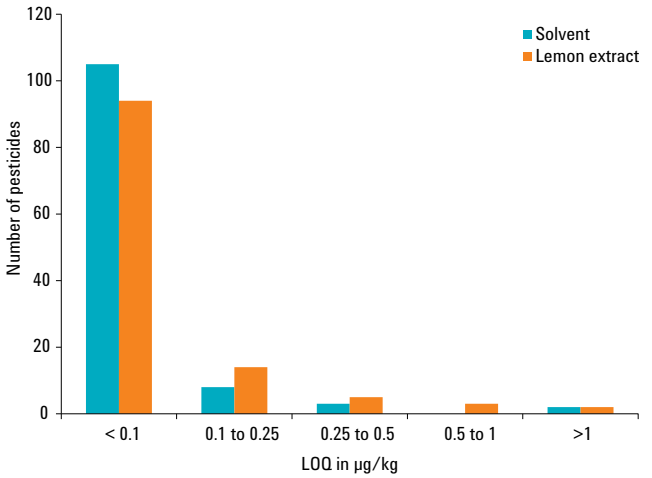

Figure 7. LOQ for the 120 evaluated pesticides in solvent and in the spiked lemon extracts. Results were classified in five relevant concentration ranges and are shown as histogram.

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Analysis of real samples

During the validation runs several matrix-pesticide combinations were observed for which natural compounds showed high analogies to the targeted pesticides, as for example, identical precursors and fragments and similar retention times. By using a single qualifier/quantifier ratio, this could result in false detects, especially in a high-throughput environment. The key advantage of using triggered MRM is the acquisition of additional information allowing for the unequivocal verification of compounds by the comparison of a compound spectrum with spectra saved in a reference library.

Figure 8 shows the chromatograms and triggered MRM spectra of a natural compound in a QuEChERS extract of chamomile flowers (A) which has a similar retention time and qualifier/quantifier ratio as the herbicide tebuthiuron (B) in a solvent standard (10 ng/mL). The triggered MRM spectra are shown in comparison to the reference library spectrum. While the spectrum of the calibration sample (B) shows a perfect match and consequently results in a match score of 100.0, the fragment spectrum of the chamomile constituent (A) shows low abundances for the low mass fragments 57.1, 62.0, 74.0, and 89.1 (red arrows), the fragments 116.0 and 157.1 show high abundances (green arrows) compared to the quantifier transition.

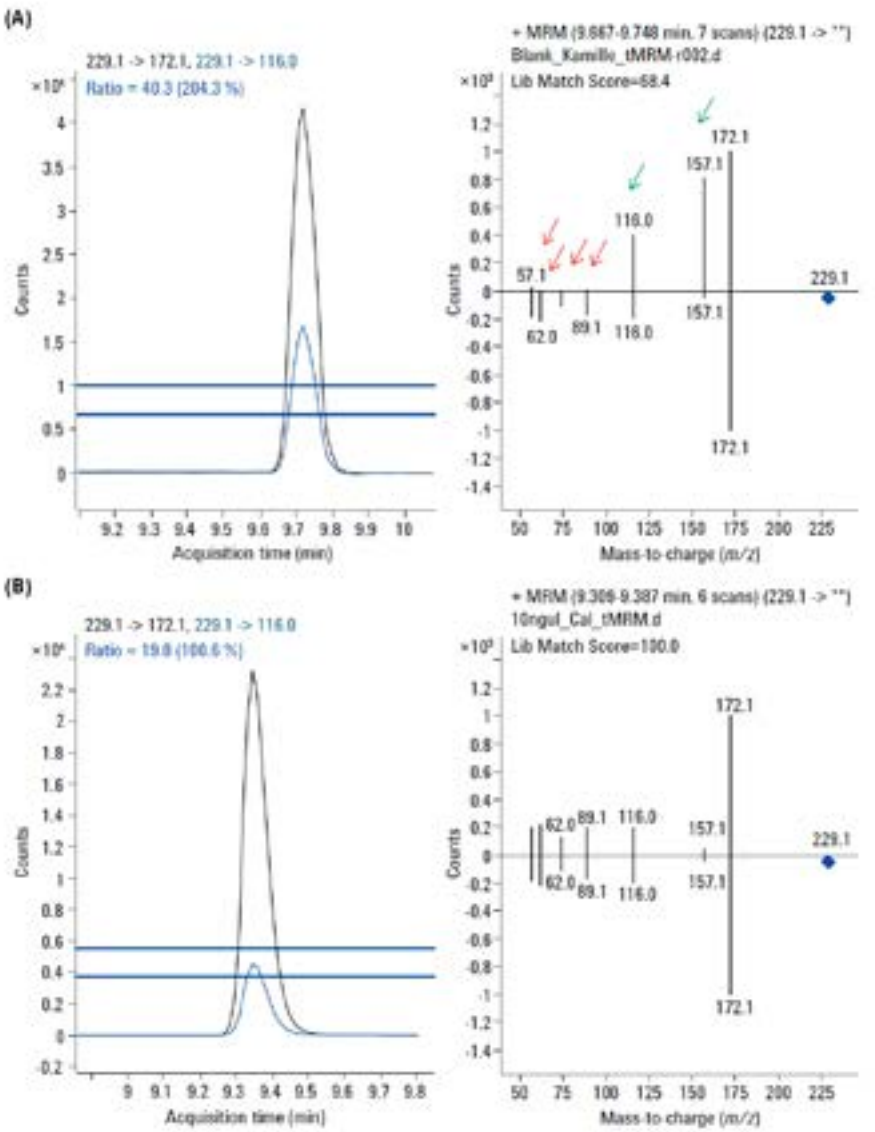

Figure 8. Chromatograms and triggered MRM spectra of a natural chamomile constituent (A) and the herbicide tebuthiuron (B). Spectra are shown in comparison to the reference library spectrum of tebuthiuron.

The resulting match score was only 68.4. From the validation results, it was shown that positive identification required a match score above 75.0 out of 100.0.

Without this additional qualitative filter there is a risk that, sooner or later, such a peak from a chamomile extract might be assigned as tebuthiuron. In this example, that would have produced a result of 0.67 mg/kg which would have been well over the default MRL of 0.01 mg/kg.

Within the tested matrix-pesticide combinations the example of tebuthiuron in chamomile extract was only one out of several where matrix interferences may appear as pesticides. Table 4 summarizes the commodities together with the suspected target analytes and the observed library match scores. In a high throughput environment, these interferences might result in false positives. Additional information such as product ion spectra and minimum required reference library match scores can help prevent reporting false detects.

Table 4. Reference Library Match Scores for Matrix Compounds Showing High Analogies to Targeted Pesticides

		Reference Library Match Score	
Pesticide	**Matrix**	**Target compound**	**Matrix interference**
Dichlorovos	Lemon	94.5%	78.1%
Thifensulfuron-methyl	Green tea	96.6%	71.5%
Tebufenpyrad	Ginger	99.8%	55.9%
Tebuthiuron	Chamomile	97.8%	58.0%
Imazalil	Chamomile	99.8%	58.1%
Terbutylazin	Chamomile	99.6%	82.1%

Table 5. Primary Transitions and Number of Additional Confirmatory Ions Included in the Triggered MRM Method

Compound name	CAS number	Precursor species	Primary transitions	Additional confirmatory ions
Acephate	30560-19-1	$[M+H]^+$	184.0 → 143.0; 184.0 → 49.1	6
Acetamiprid	135410-20-7	$[M+H]^+$	223.0 → 126.0; 223.0 → 90.1	4
Aclonifen	74070-46-5	$[M+H]^+$	265.0 → 182.1; 265.0 → 218.0	5
Aldicarb	116-06-3	$[M+NH_4]^+$	208.1 → 116.2; 208.1 → 89.1	7
Aldicarb sulfone	1646-88-4	$[M+H]^+$	223.0 → 86.1; 223.0 → 76.1	7
Aldicarb sulfoxide	1646-87-3	$[M+H]^+$	207.1 → 131.9; 207.1 → 89.1	7
Alloxydim	55634-91-8	$[M+H]^+$	324.2 → 178.1; 324.2 → 234.1	6
Amidosulfuron	120923-37-7	$[M+H]^+$	370.0 → 261.1; 370.0 → 218.1	4
Amitraz	33089-61-1	$[M+H]^+$	294.2 → 163.1; 294.2 → 122.1	5
Azinphos-ethyl	2642-71-9	$[M+H]^+$	346.0 → 77.0; 346.0 → 132.2	6
Bifenazate	149877-41-8	$[M+H]^+$	301.1 → 198.2; 301.1 → 170.1	6
Bispyribac	125401-75-4	$[M+H]^+$	431.1 → 275.1; 431.1 → 413.1	6
Bitertanol	55179-31-2	$[M+H]^+$	338.2 → 99.1; 338.2 → 269.1	5
Bromacil	314-40-9	$[M+H]^+$	261.0 → 205.0; 261.0 → 187.9	4
Butocarboxim	34681-10-2	$[M+NH_4]^+$	208.1 → 116.1; 208.1 → 75.0	7
Butocarboxim sulfoxide	34681-24-8	$[M+H]^+$	207.1 → 132.0; 207.1 → 75.0	4
Butoxycarboxim	34681-23-7	$[M+H]^+$	223.0 → 106.1; 223.0 → 166.1	6
Buturon	3766-60-7	$[M+H]^+$	237.1 → 84.1; 237.1 → 53.1	6
Cadusafos	95465-99-9	$[M+H]^+$	271.1 → 159.0; 271.1 → 97.0	5
Carbaryl	63-25-2	$[M+H]^+$	202.1 → 145.1; 202.1 → 127.1	6
Carbendazim	10605-21-7	$[M+H]^+$	192.1 → 160.1; 192.1 → 105.0	5
Carbosulfan	55285-14-8	$[M+H]^+$	381.2 → 118.1; 381.2 → 76.0	5
Chlorflurazurone	71422-67-8	$[M+H]^+$	539.9 → 158.0; 539.9 → 383.0	4
Chloridazone	1698-60-8	$[M+H]^+$	222.0 → 77.0; 222.0 → 87.9	6
Chlorsulfuron	64902-72-3	$[M+H]^+$	358.0 → 141.1; 358.0 → 167.0	5
Clomazone	81777-89-1	$[M+H]^+$	240.1 → 223.1; 240.1 → 44.1	6
Cyhexatin	13121-70-5	$[M+H-H_2O]^+$	369.2 → 205.0; 369.2 → 287.0	2
Cymoxanil	57966-95-7	$[M+H]^+$	199.1 → 128.0; 199.1 → 110.9	2
DEET	134-62-3	$[M+H]^+$	192.1 → 91.1; 192.1 → 119.0	4
Desmedipham	13684-56-5	$[M+NH_4]^+$	318.1 → 182.1; 318.1 → 108.0	8
Dichlorvos	62-73-7	$[M+H]^+$	221.0 → 109.0; 221.0 → 127.0	3
Diclofop-methyl	51338-27-3	$[M+NH_4]^+$	358.1 → 281.0; 358.1 → 120.0	6
Dicrotophos	3735-78-3	$[M+H]^+$	238.1 → 72.1; 238.1 → 112.1	6
Diflubenzuron	35367-38-5	$[M+H]^+$	311.0 → 158.0; 311.0 → 141.0	2
Dimethoate	60-51-5	$[M+H]^+$	230.0 → 125.0; 230.0 → 198.8	4
Dimoxystrobin	149961-52-4	$[M+H]^+$	327.2 → 205.1; 327.2 → 116.0	4
Diniconazole	83657-24-3	$[M+H]^+$	326.1 → 70.0; 326.1 → 159.0	7
N,N-Dimethyl-N'-phenylsulfamide (DMSA)	4710-17-2	$[M+H]^+$	201.0 → 92.1; 201.0 → 65.1	5
O-ethyl O-(4-nitrophenyl) P-phenylphosphonothioate (EPN)	2104-64-5	$[M+H]^+$	324.0 → 156.9; 324.0 → 296.1	5
Ethiofencarb	29973-13-5	$[M+H]^+$	226.1 → 107.0; 226.1 → 77.0	5
Ethiofencarb sulfone	53380-23-7	$[M+H]^+$	258.0 → 201.0; 258.0 → 106.9	6
Ethiofencarb sulfoxide	53380-22-6	$[M+H]^+$	242.1 → 185.0; 242.1 → 107.0	6

Table 5. Primary Transitions and Number of Additional Confirmatory Ions Included in the Triggered MRM Method (Continued)

Compound name	CAS number	Precursor species	Primary transitions	Additional confirmatory ions
Ethion	563-12-2	$[M+H]^+$	385.0 → 199.1; 385.0 → 142.8	6
Ethirimol	23947-60-6	$[M+H]^+$	210.2 → 140.1; 210.2 → 43.1	6
Ethofumesate	26225-79-6	$[M+NH_4]^+$	304.1 → 121.1; 304.1 → 161.2	5
Etofenprox	80844-07-1	$[M+NH_4]^+$	394.2 → 177.3; 394.2 → 107.1	2
Fenazaquin	120928-09-8	$[M+H]^+$	307.2 → 57.1; 307.2 → 161.1	5
Fenbutatin oxide	13356-08-6	$[M+H\text{-}C_{30}H_{40}SnO]^+$	519.2 → 91.1; 519.2 → 196.9	6
Fenhexamid	126833-17-8	$[M+H]^+$	302.1 → 97.1; 302.1 → 55.1	4
Fenobucarb	3766-81-2	$[M+H]^+$	208.1 → 95.0; 208.1 → 77.1	3
Fenpyroximate	111812-58-9	$[M+H]^+$	422.2 → 366.2; 422.2 → 107.0	6
Fluopicolid	239110-15-7	$[M+H]^+$	382.9 → 172.9; 382.9 → 144.9	6
Fluroxypyr	69377-81-7	$[M+H]^+$	255.0 → 209.1; 255.0 → 181.1	7
Flurtamone	96525-23-4	$[M+H]^+$	334.1 → 178.1; 334.1 → 247.1	6
Formothion	2540-82-1	$[M+H]^+$	258.0 → 199.0; 258.0 → 125.0	5
Fuberidazole	3878-19-1	$[M+H]^+$	185.1 → 157.1; 185.1 → 156.0	6
Hexaconazole	79983-71-4	$[M+H]^+$	314.1 → 70.1; 314.1 → 159.0	7
Hexythiazox	78587-05-0	$[M+H]^+$	353.1 → 168.1; 353.1 → 227.9	4
Imazalil	35554-44-0	$[M+H]^+$	297.1 → 159.0; 297.1 → 201.0	6
Indoxacarb	144171-61-9	$[M+H]^+$	528.1 → 150.0; 528.1 → 203.0	6
Ipconazole	125225-28-7	$[M+H]^+$	334.1 → 70.0; 334.1 → 125.0	4
Iprodione	36734-19-7	$[M+H]^+$	330.0 → 245.0; 330.0 → 56.1	4
Mepanipyrim	110235-47-7	$[M+H]^+$	224.1 → 77.0; 224.1 → 42.1	5
Mesotrione	104206-82-8	$[M+H]^+$	340.0 → 228.0; 340.0 → 104.0	3
Metamitron	41394-05-2	$[M+H]^+$	203.1 → 77.0; 203.1 → 175.1	4
Methamidophos	10265-92-6	$[M+H]^+$	142.0 → 94.0; 142.0 → 125.0	5
Methiocarb	2032-65-7	$[M+H]^+$	226.1 → 121.1; 226.1 → 169.0	6
Methiocarb sulfone	2179-25-1	$[M+H]^+$	258.0 → 122.0; 258.0 → 201.1	7
Methiocarb sulfoxide	2635-10-1	$[M+H]^+$	242.1 → 185.1; 242.1 → 122.1	7
Methomyl	16752-77-5	$[M+H]^+$	163.1 → 88.0; 163.1 → 106.0	3
Methoxyfenozide	161050-58-4	$[M+H]^+$	369.2 → 149.0; 369.2 → 313.1	6
Metoxuron	19937-59-8	$[M+H]^+$	229.0 → 72.1; 229.0 → 46.1	5
Monocrotophos	6923-22-4	$[M+H]^+$	224.1 → 127.0; 224.1 → 193.0	6
Monuron	150-68-5	$[M+H]^+$	199.1 → 72.0; 199.1 → 46.1	2
Myclobutanil	88671-89-0	$[M+H]^+$	289.1 → 70.1; 289.1 → 125.1	2
Napropamide	15299-99-7	$[M+H]^+$	272.2 → 58.1; 272.2 → 171.1	5
Neburon	555-37-3	$[M+H]^+$	275.1 → 88.1; 275.1 → 57.1	5
Ofurace	58810-48-3	$[M+H]^+$	282.0 → 160.1; 282.0 → 148.1	6
Omethoate	1113-02-6	$[M+H]^+$	214.0 → 125.0; 214.0 → 109.0	5
Oxamyl	23135-22-0	$[M+NH_4]^+$	237.1 → 72.0; 237.1 → 90.0	4
Phenmedipham	13684-63-4	$[M+NH_4]^+$	318.1 → 136.0; 318.1 → 168.0	8
Phorate	298-02-2	$[M+H]^+$	261.0 → 75.1; 261.0 → 199.0	3
Phosalone	2310-17-0	$[M+H]^+$	368.0 → 182.0; 368.0 → 110.9	3
Phosmet	732-11-6	$[M+H]^+$	318.0 → 160.0; 318.0 → 133.0	8
Phosphamidon	13171-21-6	$[M+H]^+$	300.0 → 127.1; 300.0 → 174.1	6
Piperonyl butoxide	51-03-6	$[M+NH_4]^+$	356.2 → 177.1; 356.2 → 119.1	2

Table 5. Primary Transitions and Number of Additional Confirmatory Ions Included in the Triggered MRM Method (Continued)

Compound name	CAS number	Precursor species	Primary transitions	Additional confirmatory ions
Pirimicarb	23103-98-2	$[M+H]^+$	239.2 → 72.1; 239.2 → 182.1	4
Pirimicarb, Desmethyl-	30614-22-3	$[M+H]^+$	225.1 → 72.1; 225.1 → 168.1	5
Pirimiphos-methyl	29232-93-7	$[M+H]^+$	306.2 → 108.1; 306.2 → 164.1	4
Propamocarb	24579-73-5	$[M+H]^+$	189.2 → 102.0; 189.2 → 144.0	2
Propargite	2312-35-8	$[M+NH_4]^+$	368.1 → 231.2; 368.1 → 175.2	3
Propoxur	114-26-1	$[M+H]^+$	210.1 → 168.1; 210.1 → 153.1	5
Proquinazid	189278-12-4	$[M+H]^+$	373.0 → 331.0; 373.0 → 289.0	6
Pymetrozine	123312-89-0	$[M+H]^+$	218.1 → 105.0; 218.1 → 51.0	2
Pyrifenox	88283-41-4	$[M+H]^+$	295.0 → 93.0; 295.0 → 66.1	4
Pyrimethanil	53112-28-0	$[M+H]^+$	200.1 → 82.0; 200.1 → 106.9	8
Pyroxsulam	422556-08-9	$[M+H]^+$	435.1 → 195.1; 435.1 → 124.1	4
Quizalofop-ethyl	76578-14-8	$[M+H]^+$	373.1 → 271.2; 373.1 → 255.1	6
Rimsulfuron	122931-48-0	$[M+H]^+$	432.1 → 182.0; 432.1 → 324.9	6
Rotenone	83-79-4	$[M+H]^+$	395.0 → 213.1; 395.0 → 192.1	6
Spinosad (Spinosyn A)	131929-60-7	$[M+H]^+$	732.5 → 142.1; 732.5 → 98.1	4
Spirotetramat	203313-25-1	$[M+H]^+$	374.2 → 216.1; 374.2 → 302.2	6
Spiroxamine	118134-30-8	$[M+H]^+$	298.3 → 144.1; 298.3 → 100.1	3
Sulfosulfuron	141776-32-1	$[M+H]^+$	471.0 → 211.0; 471.0 → 261.0	5
Tebufenpyrad	119168-77-3	$[M+H]^+$	334.2 → 117.0; 334.2 → 145.0	7
Tebuthiuron	34014-18-1	$[M+H]^+$	229.1 → 172.1; 229.1 → 116.0	5
Terbutylazine	5915-41-3	$[M+H]^+$	230.1 → 174.1; 230.1 → 104.0	4
Tetraconazole	112281-77-3	$[M+H]^+$	372.0 → 70.0; 372.0 → 159.0	5
Thiabendazole	148-79-8	$[M+H]^+$	202.0 → 175.0; 202.0 → 131.0	6
Thiacloprid	111988-49-9	$[M+H]^+$	253.0 → 126.0; 253.0 → 186.0	3
Thiamethoxam	153719-23-4	$[M+H]^+$	292.0 → 211.1; 292.0 → 181.1	4
Thifensulfuron-methyl	79277-27-3	$[M+H]^+$	388.0 → 167.0; 388.0 → 205.0	5
Thiofanox sulfone	39184-59-3	$[M+H]^+$	251.1 → 57.0; 251.1 → 75.9	5
Thiofanox sulfoxide	39184-27-5	$[M+NH_4]^+$	252.1 → 104.0; 252.1 → 57.2	6
Topramezone	210631-68-8	$[M+H]^+$	364.1 → 334.1; 364.1 → 125.1	4
Tralkoxydim	87820-88-0	$[M+H]^+$	330.2 → 216.1; 330.2 → 244.1	7
Trichlorfon	52-68-6	$[M+H]^+$	256.9 → 109.0; 256.9 → 221.0	2
Trinexapac-ethyl	95266-40-3	$[M+H]^+$	253.1 → 69.1; 253.1 → 207.1	3
Triticonazole	131983-72-7	$[M+H]^+$	318.1 → 70.2; 318.1 → 125.2	2
Zoxamide	156052-68-5	$[M+H]^+$	336.0 → 187.0; 336.0 → 159.0	4

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Conclusion

False positive identifications of pesticides in food products are a major concern for official control labs. Several pesticide-matrix combinations exist for which false positive identifications could occur when only two MRM transitions are acquired. In this application note, we showed that triggered MRM in combination with library searching against a reference spectra library reliably eliminated potential false positives. Due to the use of optimized collision energies for each MRM and due to reasonably long dwell times per transition, triggered MRM produced authentic compound spectra even at very low concentrations and in complex matrices. Linear calibration curves and excellent precision data for replicate injections showed that quantitation was not compromised when triggering additional transitions for confirmation. Triggered MRM allowed the accurate quantification and confirmation of a large number of pesticides in a single analytical run. Using only one primary transition triggered MRM potentially extends the scope of multiresidue methods to up to twice as many compounds as currently possible when using identification criteria based on the concept of a quantifier and a qualifier transition.

The developed data base and library is available from Agilent as part of the Triggered MRM library and database. It contains transitions, conditions and spectra for more than 600 pesticides and is available as Agilent product p/n G1733CA or p/n G1733BA which also contains a column, a comprehensive pesticide standard and application support.

References

1. Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin (including amendments as of 18 March 2008) and complying with regulation (EC) 1107/2009.

2. European Guideline SANCO/12495/2011: Method validation and quality control procedures for pesticides residue analysis in food and feed.

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4. Triggered MRM: Simultaneous Quantification and Confirmation Using Agilent Triple Quadrupole LC/MS Systems, Agilent Technical Overview 2011, Publication number 5990-8461EN.

5. European Committee for Standardization, EN 15662:2008.

6. Pesticide Dynamic MRM Compound Database for Screening and Identification Using the Agilent Triple Quadrupole LC/MS Systems, Technical Note, Publication number 5990-4255EN.

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Agilent Technologies

Pesticide Dynamic MRM Compound Database for Screening and Identification Using the Agilent Triple Quadrupole LC/MS Systems

Technical Note

Introduction

Over the last century more than 1000 pesticides have been in common use for crop protection. Beyond approved and recommended usage, there always exists the possibility that any of these chemicals can be found in the environment and make their way into the food supply. To protect both the environment and human health, detection and identification in survey type monitoring is very important. Liquid chromatography/tandem mass spectrometry with a triple quadrupole LC/MS meets this need by providing the most sensitive and highly selective detection in complex samples. The system must be run in multiple reaction monitoring (MRM) mode, in order to obtain the maximum sensitivity and selectivity from this technology.

Although the triple quadrupole LC/MS is the most sensitive method for multiresidue analysis, the technique can only detect the pesticides that have been included in the methodology. Each pesticide must contain its predetermined precursor ion and an indicative product ion. This single precursor/product ion pair or transition is required for screening in the MRM mode. For confirmation of a compound at least two transitions must be included in the method so that their presence and correct ratio in a sample can be determined along with the correct chromatographic retention time. Because every compound is different there are specific instrument conditions that will provide an appropriate response for each transition. On the Agilent systems this includes both fragmentor voltage, optimizing transmission of the precursor ion into the mass spectrometer, and the collision energy, optimizing the maximum intensity for a specific product ion. Excellent results are obtained using all other mass spectrometer settings provided by the system's Autotune program.

A powerful tool called MassHunter Optimizer has been added to the Agilent 6400 Series triple quadrupole LC/MS systems. This software allows automated optimization of compound specific parameters, and it is within this tool that the Pesticide Database operates. Any compound that the user optimizes can be saved to a Project or to a Database. Agilent has created a pesticide database containing the operating parameters for over 700 pesticides. The Pesticide Database is read-only and can be saved to any name for customization by re-optimization of compounds in

the database or addition/deletion of those present. Presently, modifications can be done only in MassHunter Optimizer or by saving method conditions in MassHunter Acquisition, not in the database browser. The power of the database is due to the fact that the conditions included will provide good results without the user needing to optimize each and every compound. In addition, methods are included that now contain retention times for Dynamic MRM [1,2] acquisition. For analysts needing customized methods with hundreds of pesticides per method, the database will allow fast startup and provide good results without the need to optimize the compounds in the database. This does not negate the need to run standards and validate results with good QC/QA procedures. Compounds that the user needs to analyze but are not in the database will need to be optimized. This is readily facilitated by MassHunter Optimizer, and the compounds can then be added to the user's customized database.

Description

The Pesticide Dynamic MRM Database requires MassHunter Acquisition and MassHunter Optimizer 3.01 or later. The link to the database is from the MassHunter Acquisition software or MassHunter Optimizer. It is here that the user can import selected compounds to rapidly develop a customized method, which meets the analytical needs of a specific analysis. This can include compounds in the supplied Pesticide MRM Database and those added by the user, importing to a custom designed chromatographic method for a specific matrix, or a host of other needs for customization. Figure 1 is a screen capture of the MS QQQ Acquisition setup tab for controlling any of the Agilent 6400 Series triple quadrupole LC/MS systems. Right-clicking on the white or grey area of the "Scan Segments" section of the screen displays the pull-down menu where "Import from optimizer" is accessed. When this is selected, the database browser is opened with the default database. The Agilent pesticide database or a customized version of it can be made the default (please note again that the supplied database is read-only). The user can then select the compounds and product ions to import into the acquisition method of his or her choice. The user can also save the compounds in the method to the database with the retention times used in Dynamic MRM.

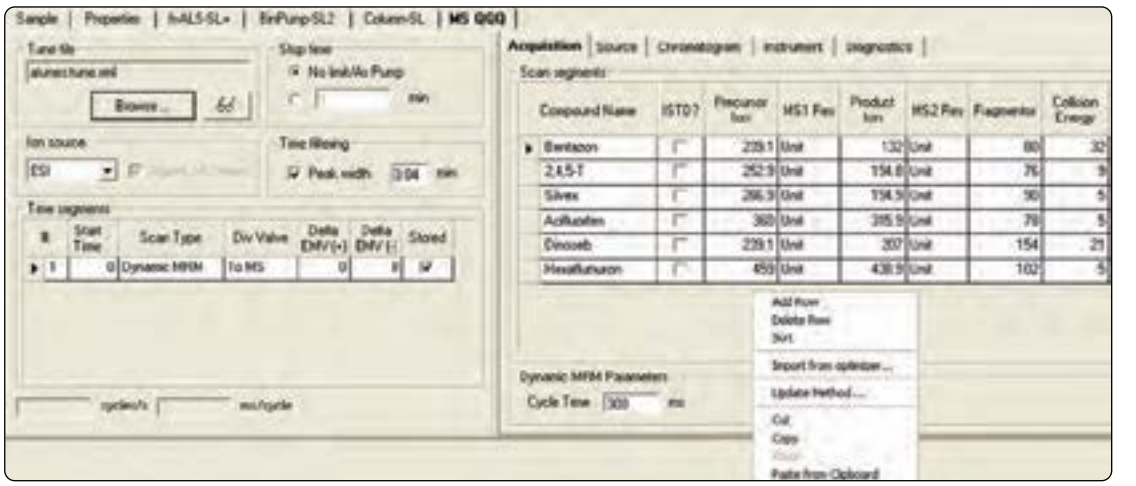

Figure 1. MS QQQ Acquisition tab of MassHunter showing the "Import from optimizer" function.

Once in the Database Browser the Pesticide Database can be opened. Figure 2 shows the Database Browser with the Pesticide Database loaded. The pull-down menu at the top left of the screen in Figure 2 or the Database Save icon allows the user to save the database to a new name. This is a necessary step for changes to be made because the Pesticide Dynamic MRM Database is read-only. Compounds can be deleted from the database in the Database Browser, but they cannot be added. That must be done from MassHunter Optimizer (see below). The copied database can now be customized and set to the default. The database contains the compound name, its formula, the nominal monoisotopic mass (nominal mass plus one decimal place where the second is not significant) of the compound, and the method(s) that were used for analyses. The parameters for analysis include the precursor that gave the optimal signal and its associated fragmentor voltage, at least two product ions (if the compound did produce two significant product ions), and the optimized collision energy for each product ion. In addition, the abundance and response factor of each ion is shown so that the user can distinguish between the quantitation ion and the qualifier by their response. In addition, acquisition methods and retention times with retention time windows are given for Dynamic MRM. The user may select a method and import compounds and their associated retention times with that method. The LC portion of this method must be used or the retention times become invalid. If not all the compounds desired are found in a specific method, the user must find the retention times for those compounds using the desired method and then import the other compounds from the database with the same method. The method must also use the same LC configuration used in the database method. Therefore, if a user has a 1200 SL pump, a method using the Agilent Infinity 1290 LC should not be used without expecting to modify the retention times. If only some of the compounds in a method or compounds from various methods are desired, they can be added to the "Import List" as shown in Figure 3. This shopping cart of compounds allows mixing sources of the compounds found by the various search filters provided (Figure 2).

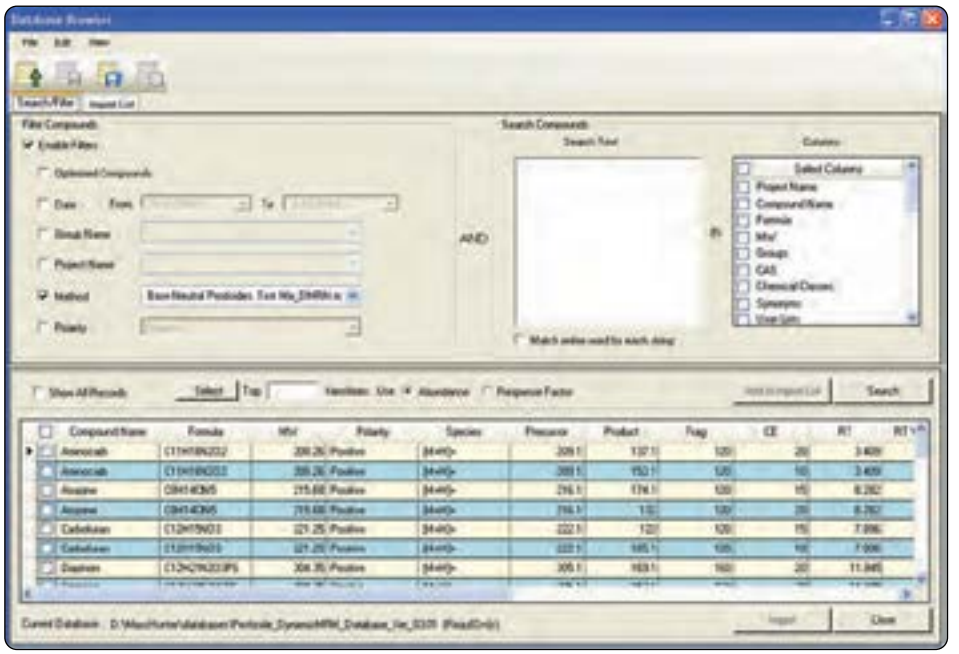

Figure 2. Database Browser view of Pesticide Database.

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A good way to search for compounds is to make a list in Excel and copy that list to the search text window (Figure 3). However, the search filter desired MUST be checked. For example, if the user has a list of compound names, then the compound name box must be checked. Likewise if the list is CAS numbers then the "CAS" box must be checked. Selected compounds can be added to the Import list and removed from that list if necessary. However the database itself cannot be edited from the database browser, even in a user-created database.

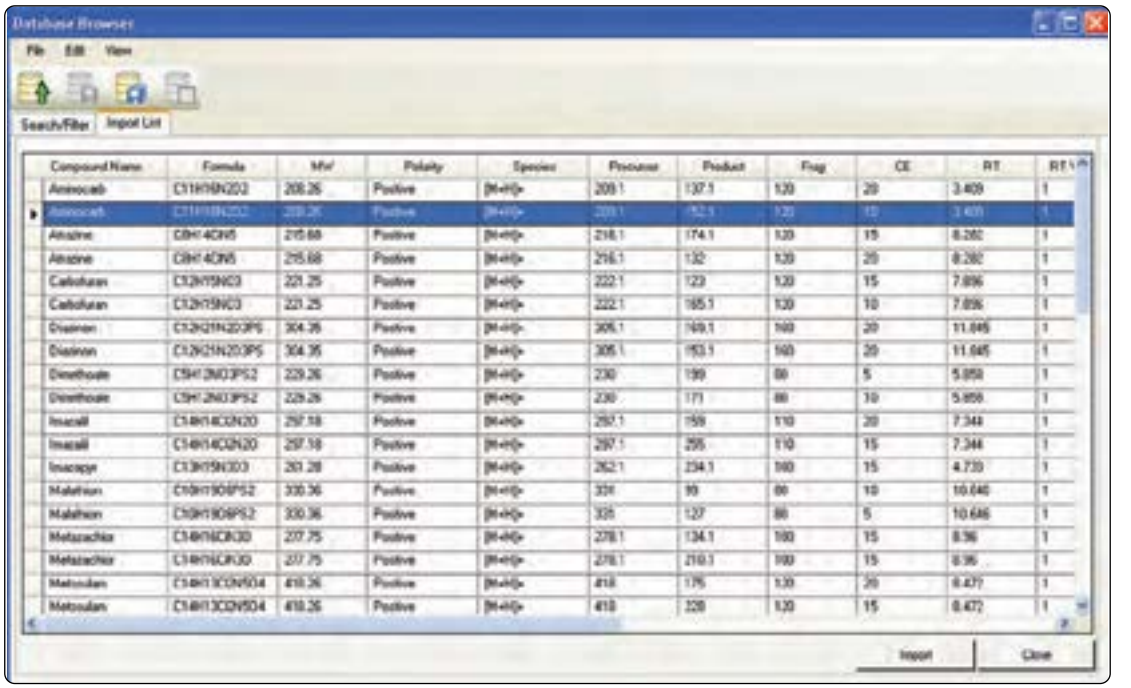

Figure 3. Import list where compounds selected from different search filters can be added to a Dynamic MRM method.

Finally, the other access point for the database is from MassHunter Optimizer program. It is from this access that the user can add compounds to their customized database. Figure 4 shows the initial screen for the MassHunter Optimizer. The icons across the top allow import and export to and from Excel, naming and saving projects and compounds, starting an optimization or breakdown profile, and access to the databases. The circled icon in Figure 4 allows the import from an acquisition method. Figure 5 with "show results" from an import demonstrates that not only the operating parameters, but the retention times and the retention time windows are imported from the method. By selecting the icon circled in Figure 5 the user can then export this to the default database (their custom database). Again, the Pesticide Dynamic MRM database is read-only but once accessed it can be saved as a customized database and then set as the default. As the default, compounds and projects can be saved from MassHunter Optimizer to the database. Compounds can be deleted directly from the Database Browser but can only be added or changed using MassHunter Optimizer. It will be useful for the user to save compounds not in the provided database and at times to re-optimize certain compounds for specific user conditions.

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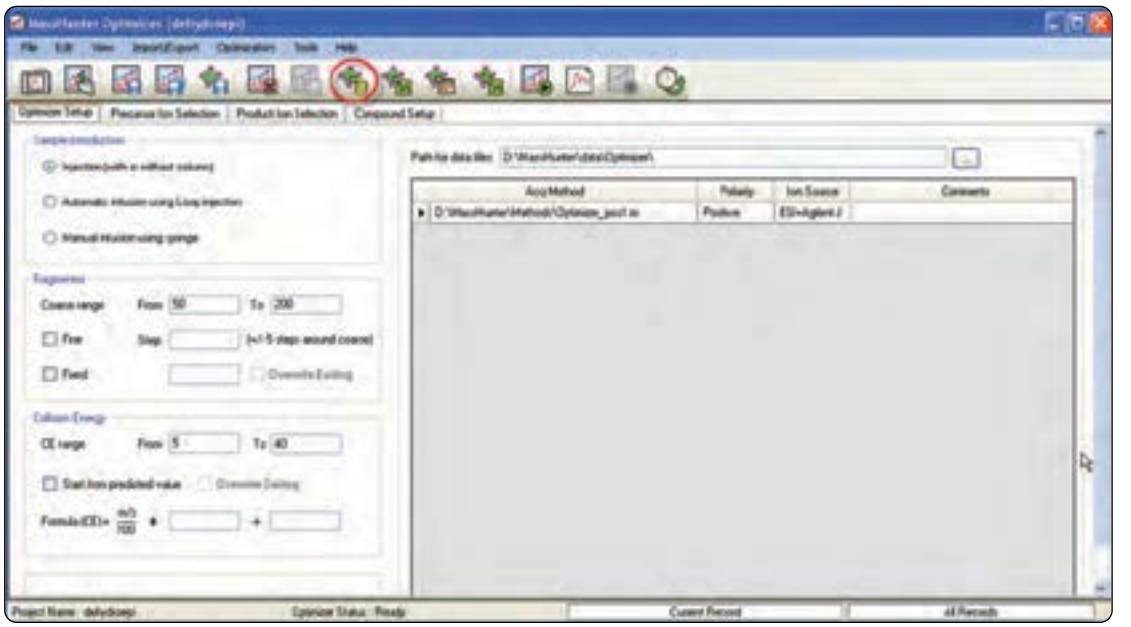

Figure 4. First tab of MassHunter Optimizer where parameter range and method settings are made. Note icon "Import from Database" provides access to Database Browser.

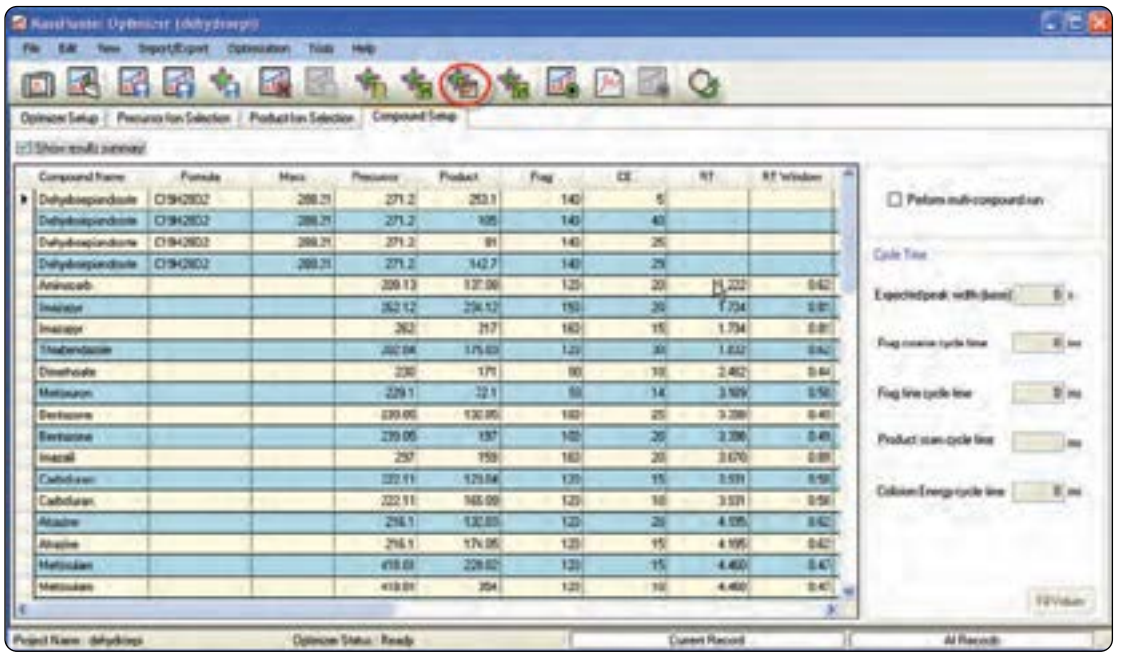

Figure 5. Import from an Acquisition method with "Show Results" displayed. The circled icon allows export of all compound information to the default database.

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Summary

The Dynamic MRM Pesticide Database provides over 700 compounds with specific parameters for all Agilent 6400 Series triple quadrupole LC/MS systems. It is designed to meet the needs of laboratories analyzing hundreds of pesticides in one analysis. It allows re-optimization of compounds through the MassHunter Optimizer program and incorporation of the compounds into data acquisition methods for multi-residue analysis where Dynamic MRM is most useful. Its benefits to the analyst are:

- Fast method development with compound-specific parameters for hundreds of compounds
- Storage and retrieval of compounds added to the supplied database
- Customization to meet specific needs of laboratories and their analyses

The database and its functionality will continue to evolve to provide greater search and retrieval capabilities and faster development for Dynamic MRM methods.

For More Information

The Dynamic MRM Pesticide Database is included in only the Pesticide Application kit (Agilent part number, G1733AA). Details of how to use the database are given in the Quick Start Guide that is included in the application kit.

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Multi-Residue Pesticide Analysis with Dynamic Multiple Reaction Monitoring and Triple Quadrupole LC/MS/MS

Fast and Effective Method Development Using an Application Kit and a Pesticides Compound Parameter Database

Application Note

Food Safety and Environmental

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Abstract

The analysis of pesticide residues in food and environmental samples is challenging due to the low concentrations and large number of analytes that need to be monitored and quantified. In addition, method development for Liquid Chromatography/Mass Spectrometry/Mass Spectrometry (LC/MS/MS) with a triple quadrupole instrument is laborious and time consuming because of the compound dependent parameters that need to be optimized. This application note describes how pesticide residue LC/MS/MS methods can be set up quickly and efficiently using the Agilent Pesticides Application Kit. This Application Kit contains a pesticide test mix, a 600-compound pesticide MRM database, a quick start guide and several dynamic Multiple Reaction Monitoring (MRM) methods, which can easily be incorporated into a specific method for pesticide residue analysis. The Pesticides Dynamic MRM database contains compounds commonly monitored around the world and provides fast, customized method development of the analysts' list of pesticides. Results from a 100 and 300-compound mixture are demonstrated with an Agilent 1200 SL Series Rapid Resolution LC and the Agilent 6460 Series Triple Quadrupole LC/MS System with Agilent Jet Stream Technology. The 300-compound mixture was also analyzed using an Agilent 1290 Infinity Ultra High Pressure Liquid Chromatograph (UHPLC) and a 6460 LC/MS. With the higher pressure capabilities of the Agilent 1290 Infinity UHPLC, rapid separations with higher peak capacity and less peak overlap than the Agilent 1200 Series RRLC were produced. Using a spinach matrix spiked with 16 pesticides, the performance of a complete method with the SampliQ extraction and dispersive SPE kits and the Agilent LC/MS/MS triple quadrupole on a typical food matrix was

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Introduction

The analysis of target pesticide residues has traditionally been performed using Gas Chromatography/Mass Spectrometry (GC/MS) or Liquid Chromatography/Mass Spectrometry (LC/MS) methods. Because of the number of pesticides used and the sensitivity needed for monitoring hundreds of pesticides in a single analysis, both techniques are a requirement. GC/MS is needed for the less polar, more volatile pesticides and LC/MS for pesticides that are more polar or thermally labile and there is much overlap between them. However, many of the pesticides developed over the last 20 years are most amenable to LC/MS. The method of choice for trace analysis in complex matrices uses a triple quadrupole (QQQ) mass spectrometer incorporating multiple reaction monitoring (MRM). During an MRM analysis the QQQ monitors the product ions produced by collisions of precursor ions in the central quadrupole (the collision cell) of the mass spectrometer, as seen in Figure 1. An MRM analysis can generate a very sensitive and specific analysis of target

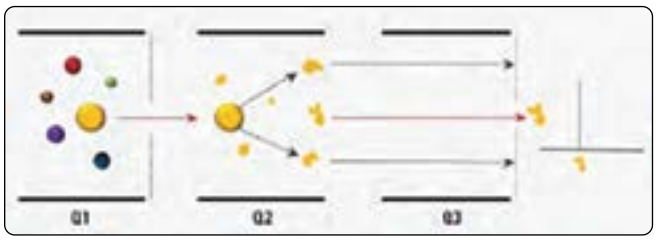

Figure 1. A schematic diagram of MRM mode on a triple quadrupole instrument. The precursor ion is selected in Q1, fragmentation occurs in Q2, and the product is selected by Q3. Since two stages of mass selectivity are used, there is very little interference from background matrix resulting in excellent sensitivity.

compounds.

Over time regulating agencies have continually increased the number of pesticides and residues that must be monitored. It is now common that hundreds of residues need to be analyzed in a single LC/MS analysis. To address this challenge the MRM transitions that need to be monitored are switched using programmed time segments. This is called time segmented MRM. It is accomplished by programming the QQQ to monitor specific product ions in time segments during the LC/MS analysis. However, the method requires well defined elution time boundaries and must avoid time segment switches when compounds elute from the LC. If a time segmented MRM analysis is generated for a sample that contains hundreds of residues, the time segmented MRM analysis becomes subject to cycle and dwell time limitations that affect the sensitivity and specificity of the analysis. A new technique, Dynamic Multiple Reaction Monitoring (MRM) alleviates these limitations and also allows easier method development and future modifications of the method, such as the addition of new pesticides to be analyzed. Using Dynamic MRM, analyte ions are only monitored while they are eluting from the LC. This significantly improves the MS duty cycle time for very complex samples when compared with the time segment method and improves the sensitivity and specificity of an analysis.[1]

One of the challenges in developing an MRM method, whether it is a time segment or Dynamic MRM, is creating the time sequence of MS/MS events and mass spectrometer conditions necessary to maximize sensitivity and specificity. It is essential to generate a list of two or more MRM transitions and compound specific parameters, fragmentor voltage and collision energy for each compound being analyzed. The availability of a database containing over 600 pesticides with the MS/MS instrumental information that can be used with all Agilent triple quadrupoles eliminates the need to create this information via tedious manual procedures. The database allows easy import of selected compounds into the user's analytical method. A portion of this database is shown in Figure 2. In addition to creating custom methods, the read-only database allows the user to copy their customized database to meet his or her specific needs. A technical note describes this database in detail. [2] The Agilent Pesticides Application Kit also includes a pesticide test mixture that is used to demonstrate the performance of the system and pretested methods, allowing faster method development. Neither the kit nor the test mixture diminishes the need for each laboratory to define suitable QC/QA procedures and perform validation. Each laboratory must have QC tests fit-for-purpose and run analytical standards to validate analytical results.

This application note will demonstrate the use of the Agilent Pesticide Application Kit with a 600-compound parameter database and Dynamic MRM for the analysis of complex pesticide mixtures. The liquid chromatographic separations are performed using an Agilent 1200 SL Series RRLC or an Agilent 1290 Infinity UHPLC with an Agilent 6460 QQQ incorporating Jet Stream technology.[3] The methods described in the note are straightforward to generate using the Agilent MassHunter data analysis software and the Pesticide Dynamic MRM Database. Some limits of detection (LOD) of 100 fg or less were achieved using these methods with the Agilent 6460 Series QQQ LC/MS system. These methods are also compatible with all Agilent 6400 series LC/MS systems.

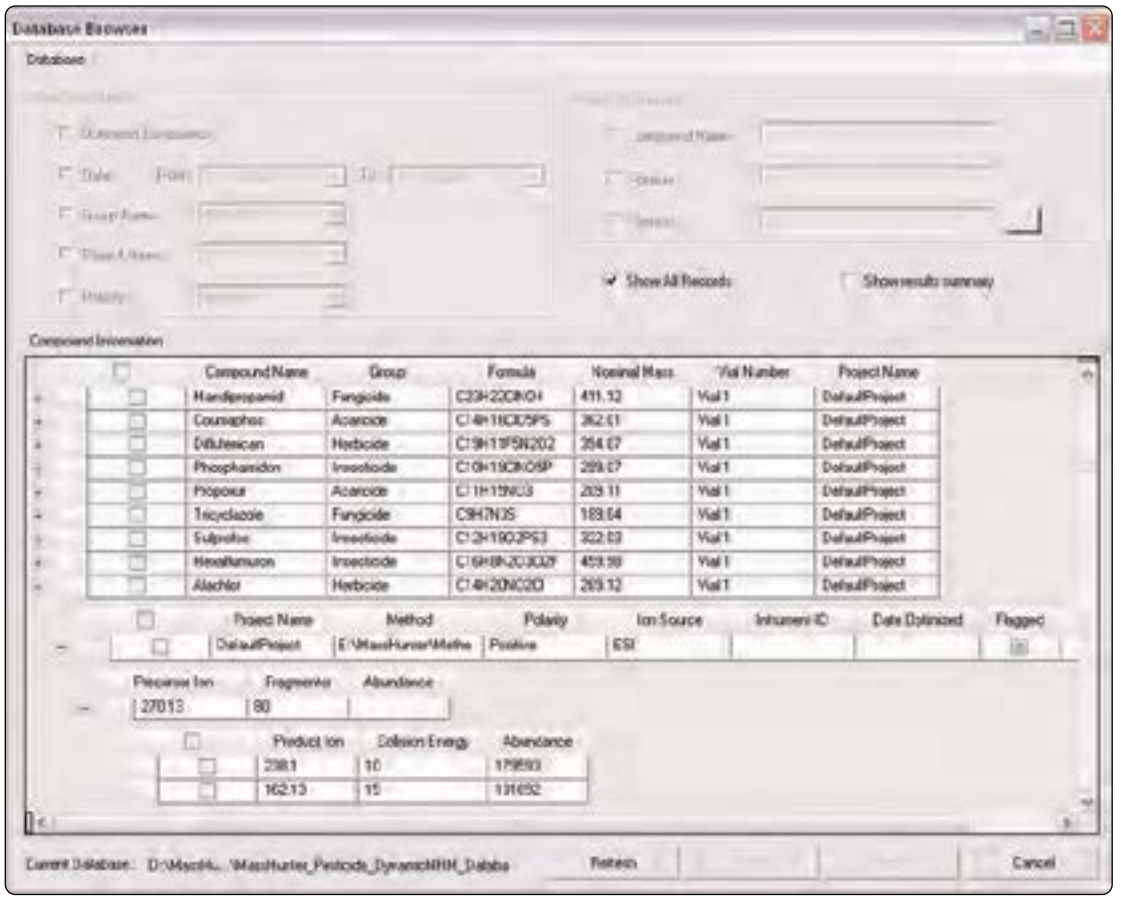

Figure 2. Compound Parameter Database with over 600 pesticides entries.

Experimental

Reagents and Chemicals

- Agilent Pesticide Test Mix, p/n 5190-0469 acid and base diluted separately as instructed to 10 ppb in 10% acetonitrile/90% water
- An Agilent SampliQ QuEChERS AOAC Extraction kit, p/n 5982-5755. Agilent SampliQ QuEChERS AOAC Dispersive SPE kits for Highly Pigmented Fruits and Vegetables, p/n 5982-5321 (2 mL) and p/n 5982-5356 (15 mL)
- Multiple pesticide standards were obtained from Sigma, Chemservice, and Dr. Erhenstofer

Instrument Settings

- *Appendix I: LC/MS/MS Conditions for Test mix Positive and Negative Ion Samples
- Appendix II: LC/MS/MS Conditions for a 100 Pesticide Methods
- *Appendix III: LC/MS/MS Conditions for 300-Pesticide Methods using the Agilent 1200 Series SL
- Appendix IV: LC/MS/MS Conditions for the 300-Pesticide Methods using the Agilent 1290 Infinity LC
- Appendix V: LC/MS/MS Conditions for Pesticides in Spinach using QuEChERS Extraction.
- *Appendix VI: LC/MS/MS Conditions for the 165-Pesticide Methods using the Agilent 1200 Series SL
- *Appendix VII: LC/MS/MS Conditions for the 224-Pesticide Methods using the Agilent 1200 Series SL
- Appendix VIII: LC/MS/MS Conditions for the 224-Pesticide Methods using Agilent 1290 Infinity LC

*Each of these methods are included with the Application Kit

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Spinach Sample Preparation

- Weigh 15 g (±0.1 g) of homogenized spinach sample.
- Spike standards or IS solution if necessary.
- Vortex 30 s.
- Add 15 mL of 1% acetic acid in acetonitrile.
- Add 1 bag of extraction kit (p/n 5982-5982-5755) buffered QuEChERS extraction tubes, AOAC Method 2007.01 to 6 g $MgSO_4$ and 1.5 g NaAc.
- Cap and hand-shake vigorously for 1 min.
- Centrifuge at 4000 rpm for 5 min.
- Transfer 1 mL or 8 mL upper layer to the dispersive SPE kit (p/n 5982-5321 or p/n 5982-5356) for highly pigmented fruits and vegetables.
- Vortex 1 min.
- Centrifuge 2-mL tubes at 13000 rpm for 2 min, or 15 mL tubes at 4000 rpm for 5 min.
- Transfer 200 µL of the upper layer to the autosampler vial.
- Add 800 µL of water or appropriate standard spiking solution.
- Vortex 1 min, to prepare for LC/MS/MS analysis.

Results and Discussion

Positive and Negative Ion Test Mix

In addition to the 600-compound database, the Agilent Application Kit for pesticide residue analysis also includes a positive and negative ion test mix, with their analysis methods shown in Appendix I. The methods contain compound names, MRM transitions, fragmentor voltages, collision energies, and retention times for the Dynamic MRM. The test mix and the supplied method allow the analyst to demonstrate that the system is operating properly for pesticide analysis immediately after installation. The LC/MS/MS extracted ion chromatograms (EIC) from the test mix analyzed in the positive and negative ion mode using Dynamic MRM is shown in Figures 3 and 4.

The Application Kit Quick Start Guide [4] shows the analyst how to run the test mixes and create a Dynamic MRM method. To create new methods, standards are analyzed at higher concentrations with a one segment MRM method. The data is processed using the Agilent MassHunter Quantitative Data Analysis software to generate a custom report that now includes analyte retention times. A Dynamic MRM method is generated by importing the results from the custom report and specifying a delta retention time window. This process will be automated in the near future. Table 1 shows a partial listing of

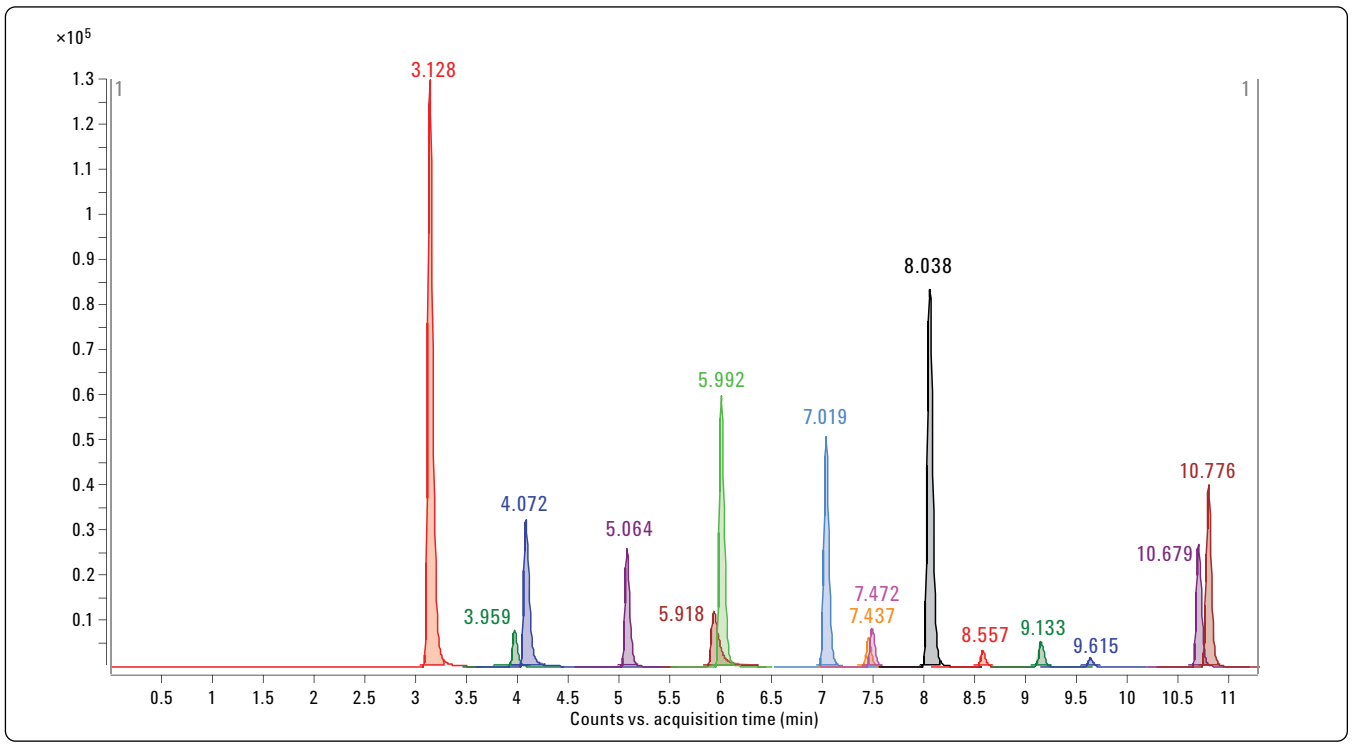

Figure 3. Positive ion test mix extracted ion chromatogram (see Appendix 1 for list of compounds matching retention times given in chromatogram).

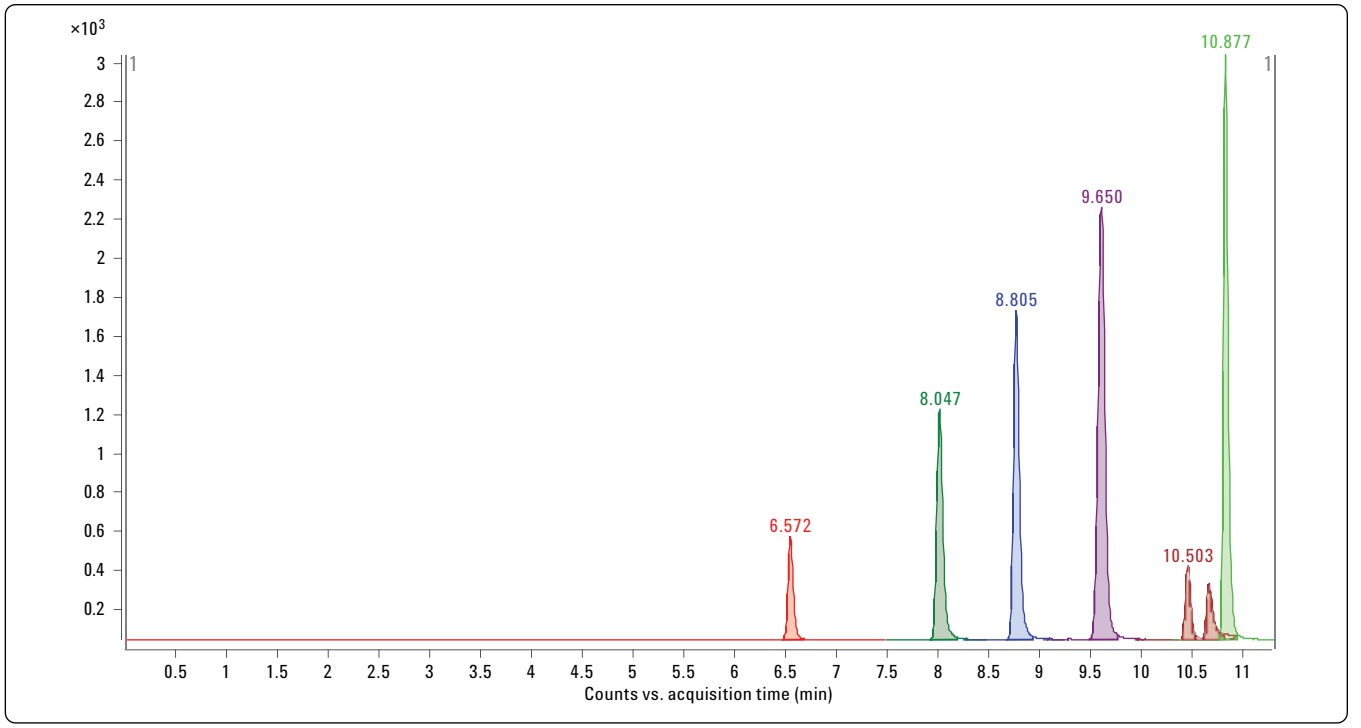

Figure 4. Negative Ion Test Mix extracted ion chromatogram (see Appendix 1 for list of compounds matching retention times given in chromatogram).

the acquisition parameters from a Dynamic MRM method. Note in this example the retention time window (Delta RT) is 2 min which is large for narrow peaks. A window this wide can be used to run standards where retention times have shifted and need to be updated in the users' customized method.

Table 1. Dynamic MRM Screen Capture of Acquisition Parameters

Acquisition | Source | Chromatogram | Instrument | Diagnostics

Scan segments

Compound Name	ISTD?	Precursor Ion	MS1 Res	Product Ion	MS2 Res	Fragmentor	Collision Energy	Ret Time (min)	Delta Ret Time
Acephate	☐	184	Unit	125	Unit	80	10	1.212	2
Aminocarb	☐	209	Unit	137	Unit	120	20	1.251	2
Atrazine	☐	216	Unit	132	Unit	120	20	7.602	2
Azinphos-methyl	☐	318	Unit	132	Unit	80	10	9.346	2
Carbofuran	☐	222	Unit	123	Unit	120	15	7.13	2
Chlorpyrifos methyl	☐	322	Unit	125	Unit	80	15	12.168	2
Diazinon	☐	305	Unit	153	Unit	160	20	11.822	2
Dimethoate	☐	230	Unit	171	Unit	80	10	4.645	2
Imazalil	☐	297	Unit	159	Unit	160	20	6.498	2

Dynamic MRM Parameters

Cycle Time 500 ms

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Fast and effective screening of a 100-compound pesticide mix using Dynamic MRM

A 100-compound mix of pesticides was used to demonstrate the effectiveness of the Dynamic MRM. Appendix II contains the LC/MS/MS conditions and a partial listing of the Dynamic MRM method used to analyze a 100-pesticide mixture at the 100 pg/compound level. Note that the column used was 50 mm in length so faster analysis and less efficiency is obtained. The LC/MS/MS extracted ion chromatogram shown in Figure 5 illustrates the performance of the system. The complete LC analysis took less than 15 minutes. Figure 6 shows a 1-min time window where 11 compounds (22 MRM's) are eluting. Figure 7 shows the 1-min delta retention time window for each Dynamic MRM transition. Note the many peak overlaps in the chromatograms. This necessitates the use of dynamic transitions instead of time segmented transitions in order to achieve the needed cycle time so that each peak can have enough data points to adequately describe the peak for quantitation. Furthermore time segmented MRM has an inherent "dead time" data loss when monitoring analyte peaks eluting near or between time segment boundaries. Time segmented MRM methods may require duplicate monitoring of specific analytes which elute over adjoining time segments. In addition, Dynamic MRM maximizes the dwell times for overlapping peaks enhancing the signal-to-noise while maintaining constant cycle time. Note that the cycle time selected should ideally provide about 20 data points across a peak with a minimum of 64 data points in the retention time window (Delta Ret Window).

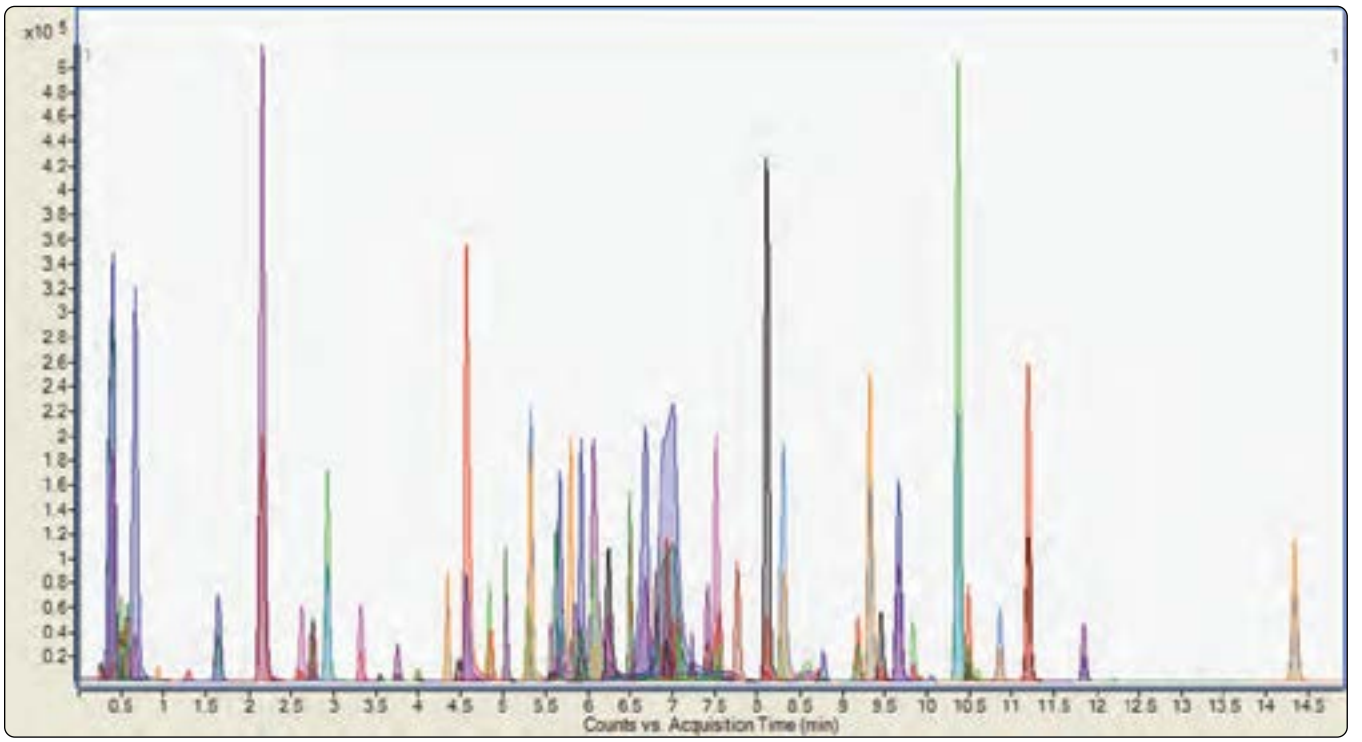

Figure 5. Extracted Ion Chromatograms of 100 compound pesticide mixture (100 pg level).

Compound name	Precursor ion	Product ion	Retention time
Cinosulfuron	414.1	183	5.579
Cinosulfuron (Q)	414.1	157	5.579
Chlorotoluron	213.1	72	5.642
Chlorotoluron (Q)	213.1	140	5.642
Atrazine	216.1	174	5.682
Atrazine (Q)	216.1	132	5.682
Carbaryl	202.1	145	5.736
Carbaryl (Q)	202.1	117	5.734
Carboxin	236.1	143	5.836
Carboxin (Q)	236.1	87	5.836
Chlorsulfuron	358.0	167	5.896
Chlorsulfuron (Q)	358.0	141	5.896
Ethiofencarb	226.1	107	5.937
Ethiofencarb (Q)	226.1	164	5.936
Dodemorph	283.3	116	6.073
Dodemorph (Q)	282.3	98	6.074
Diuron (Q)	233.0	160	6.101
Cyprodinil	226.1	108	6.245
Cyprodinil (Q)	226.1	93	6.246
Difenoxurone	287.1	123	6.509
Difenoxurone (Q)	287.1	72	6.509

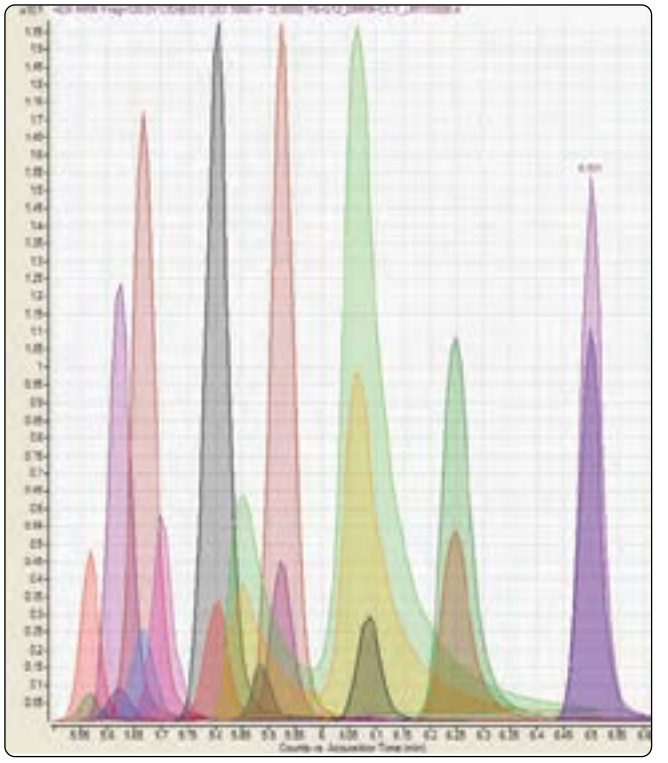

Figure 6. Left: Table of 11 compounds monitored during a 1 minute time window. Right: Dynamic MRM of compounds being monitored.

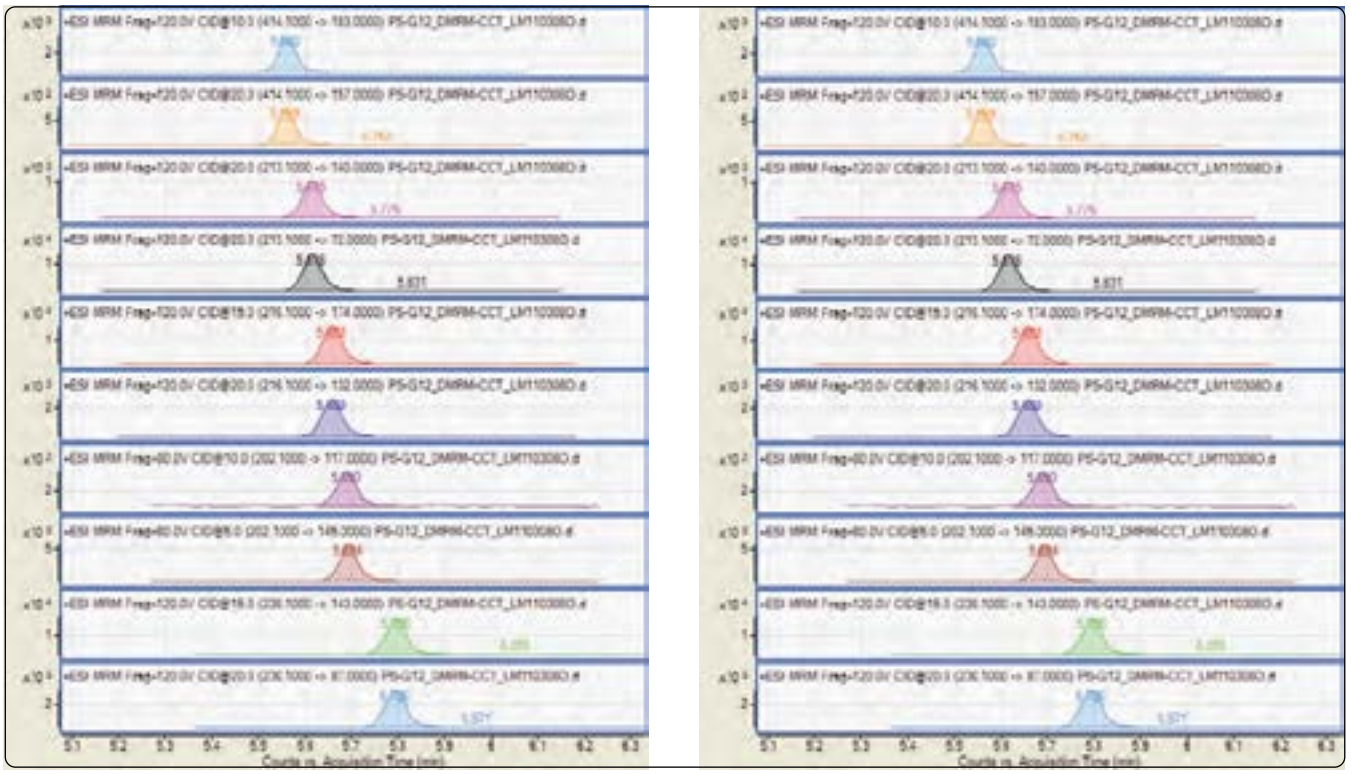

Figure 7. Dynamic MRM windows for each MRM transition.

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Typical results achieved with the method are shown in Figure 8. It illustrates the results from one of the compounds, atrazine, in the 100-compound mixture. Note the 20 data points that were collected during the elution of atrazine. This provides a sufficient number of data points to assure quantitative accuracy and shows the effectiveness of Dynamic MRM.

Average signal height:	15,650
Average signal area:	50,966
RSD:	3.2%
Estimated LOQ:	100 fg or less

6–7 data points above FWHM

3 sec FWHM, 6 sec at 10% valley

20 data points baseline-to-baseline

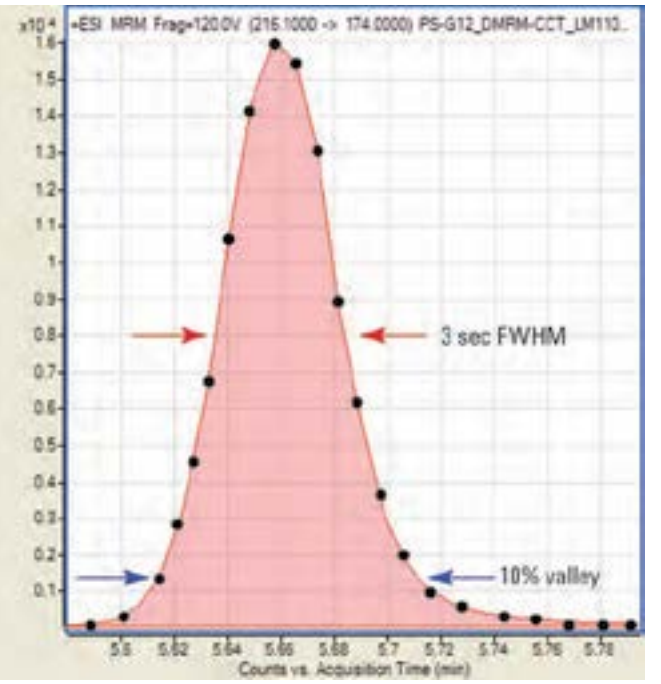

Figure 8. Typical analytical results shown with 10 pg of atrazine visualizing the effectiveness of Dynamic MRM.

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The calibration data from four compounds in the mixture are illustrated in Figure 9. $R^{2'}$s = 0.0998 are achieved for each pesticide. With constant cycle time maintained, the quantitative results with Dynamic MRM are excellent.

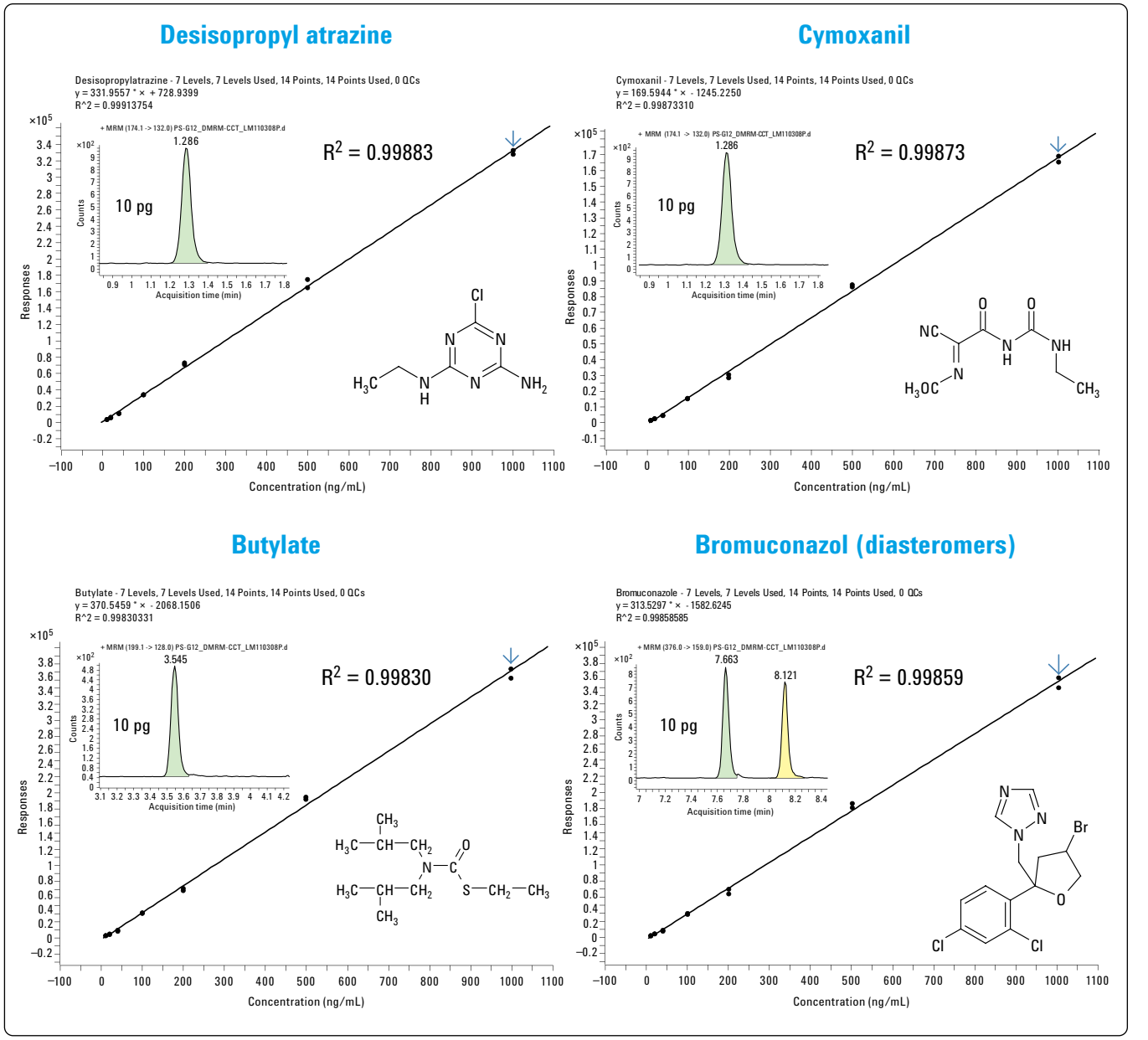

Figure 9. Dynamic MRM Calibration Plots, 10 pg–1 ng (7 levels).

Sharper peaks are produced with a 300-pesticide mix using the new Agilent 1290 Infinity LC

Appendix III contains a partial listing of the Dynamic MRM method used to analyze a 300-pesticide mixture at the 100 pg/compound level. The LC/MS/MS extracted ion chromatogram is shown in Figure 10. The analysis took less than 20 minutes using the Agilent 1200 Series SL RRLC and an Eclipse Plus C18 2.1 mm × 100 mm, 1.8 µm column at a flow rate of 0.5 mL/min. The same mixture was separated using an Agilent 1290 Infinity UHPLC with an Eclipse-Plus C18, 2.1 mm × 150 mm, 1.8 µm column. Figure 11, an extracted ion chromatogram and Figure 12, an expanded portion of the chromatogram, demonstrate that this complex mixture has been analyzed in about 15 minutes which is approximately 25% faster than with the Agilent 1200 Series SL RRLC. The Agilent 1290 Infinity UHPLC also produced a separation with higher peak capacity and less peak overlap than the Agilent 1200 Series SL RRLC. Typical peak ½ heights using atrazine as an example with the Agilent 1290 Infinity UHPLC are 1.8 s. This is because the longer column provides higher efficiency and the Agilent 1290 Infinity LC can operate at the pressure these conditions incurred (~900 bar).

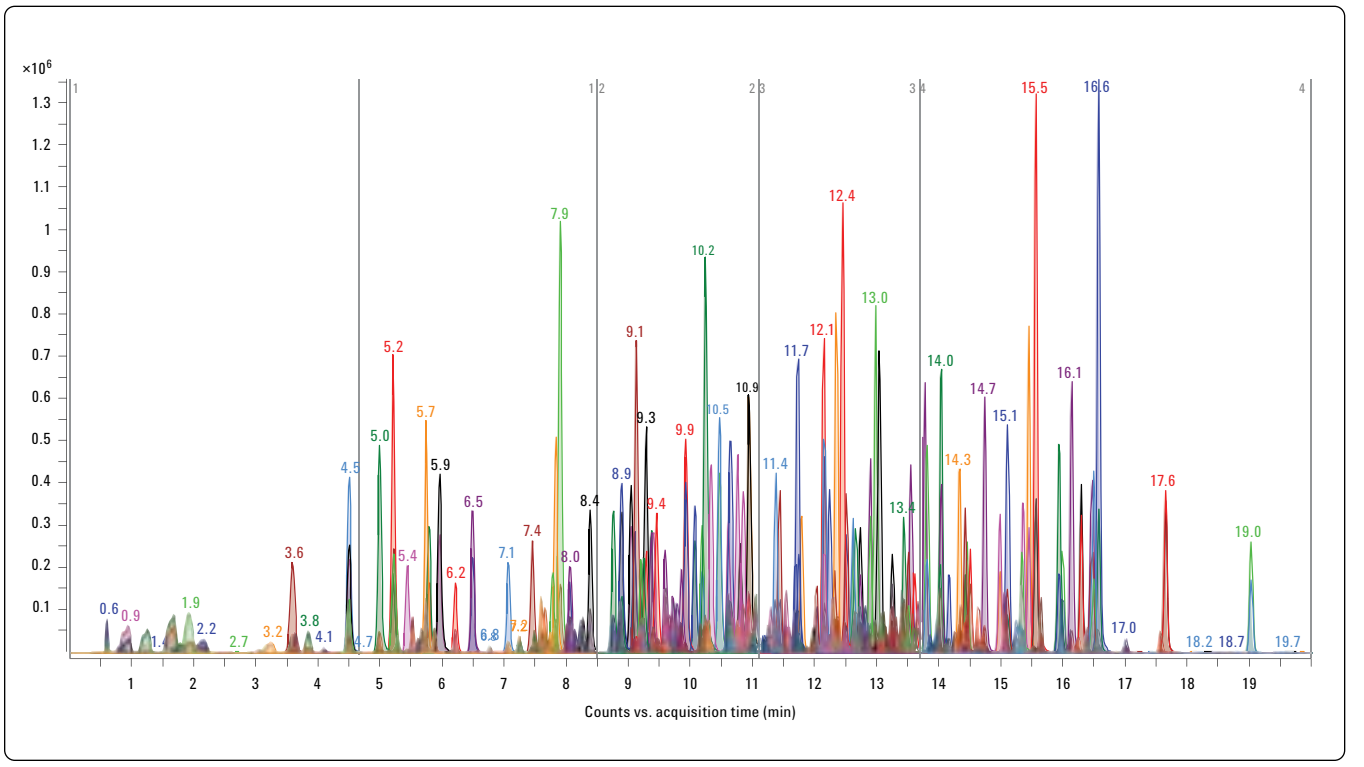

Figure 10. EIC of 300 compound pesticide mixture using an Agilent 1200 Series SL RRLC.

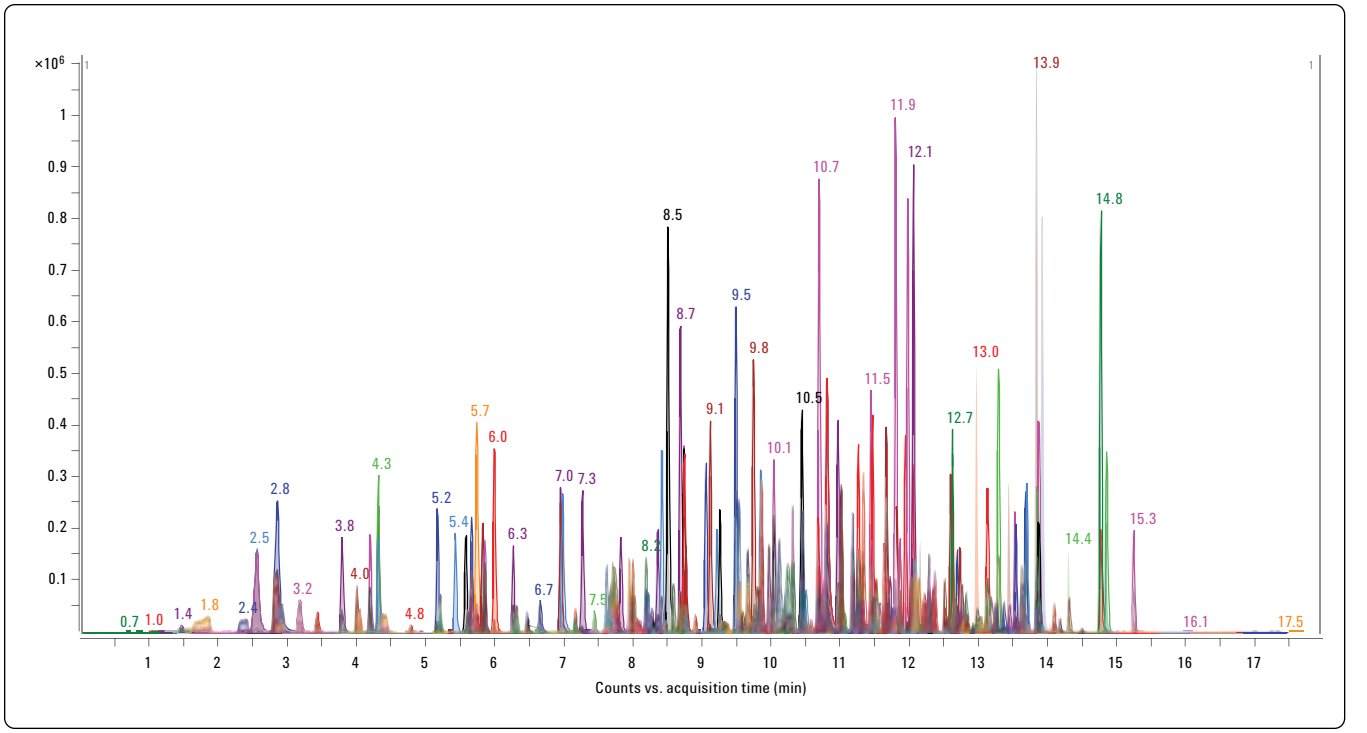

Figure 11. EIC of 300-compound pesticide mixture using the Agilent 1290 Infinity UHPLC.

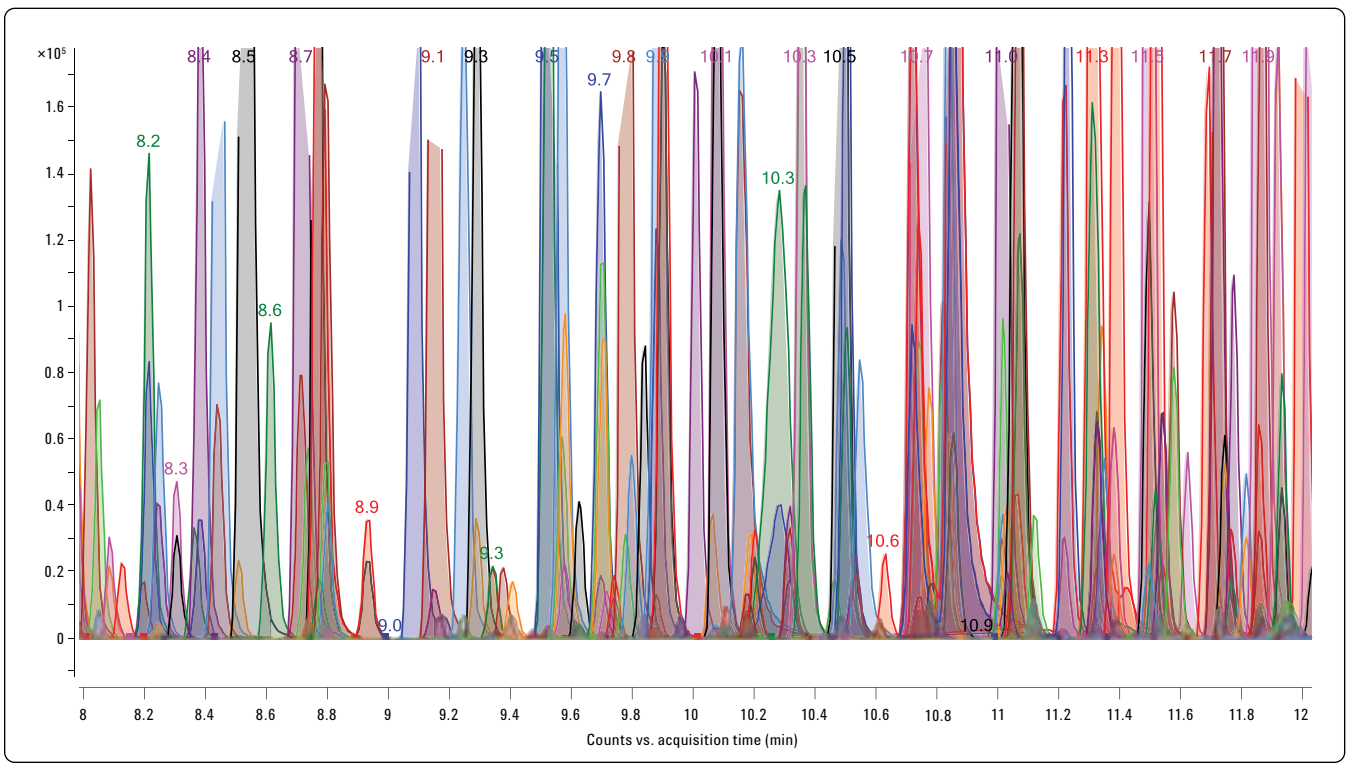

Figure 12. Expanded EIC of 300-compound pesticide mixture using an Agilent 1290 Infinity UHPLC illustrating the high peak capacity of the Agilent 1290 Infinity.

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Faster analysis with a 224-pesticide mix using the new Agilent 1290 Infinity LC

Another advantage of the Agilent 1290 Infinity LC with the Agilent 6460 Series QQQ LC/MS is the ability to increase flow and decrease analysis time. Using the 1200 Series SL the analysis of 225 pesticides is performed in 15 min and shown in Figure 13. The method for this analysis is given in Appendix IV. With the Agilent 1290 Infinity LC the flow can be doubled and the gradient completed in half the time. This provides the same separation in less than 7 min as shown in Figure 14. The method for this analysis is given in Appendix V. Analyzing hundreds of pesticides in one run, it is best to obtain the highest peak capacity as shown in the 300-pesticide example. However, if speed of analysis is absolutely necessary, it is shown that the higher pressure capability of the Agilent 1290 Infinity LC and the higher pressure capability of the HD columns provide the performance needed.

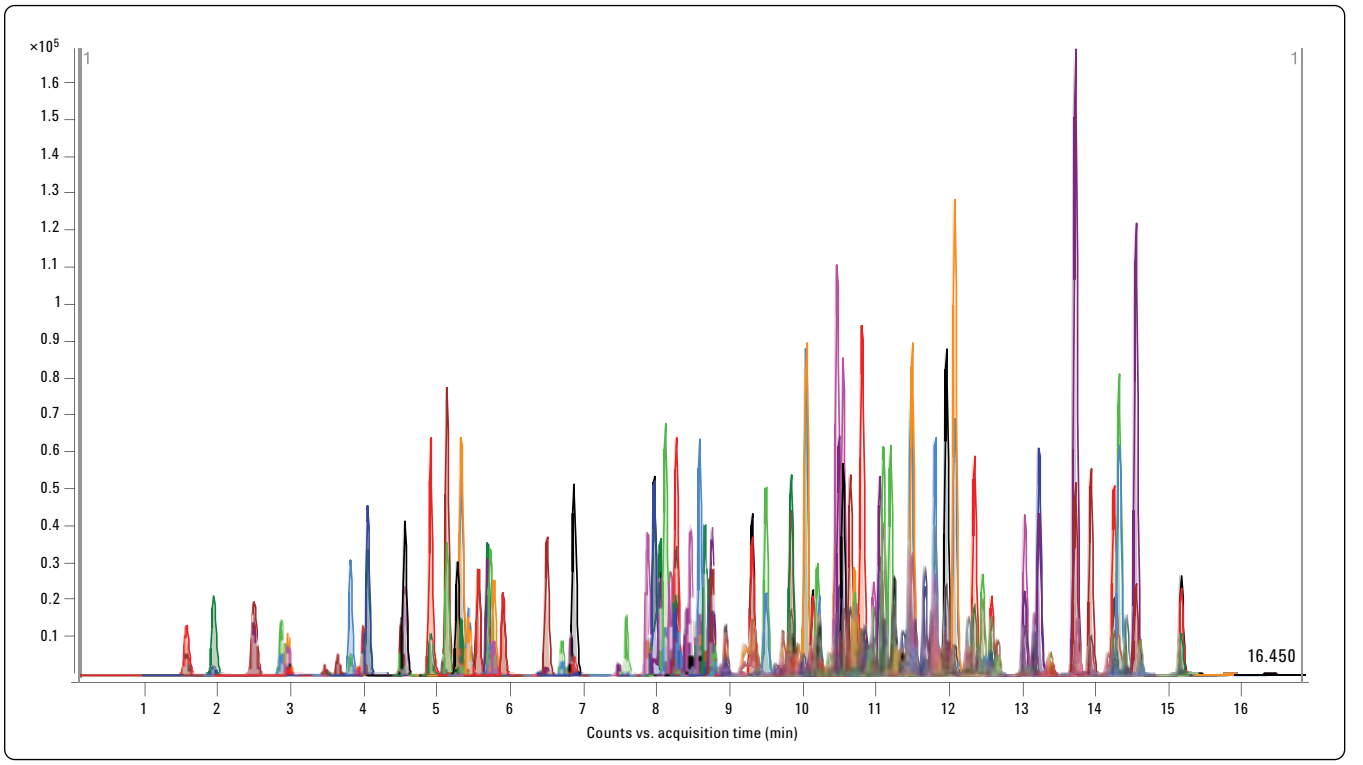

Figure 13. EIC of 224 pesticides using the Agilent 1200 Series SL LC and the Agilent 6460 QQQ LC/MS.

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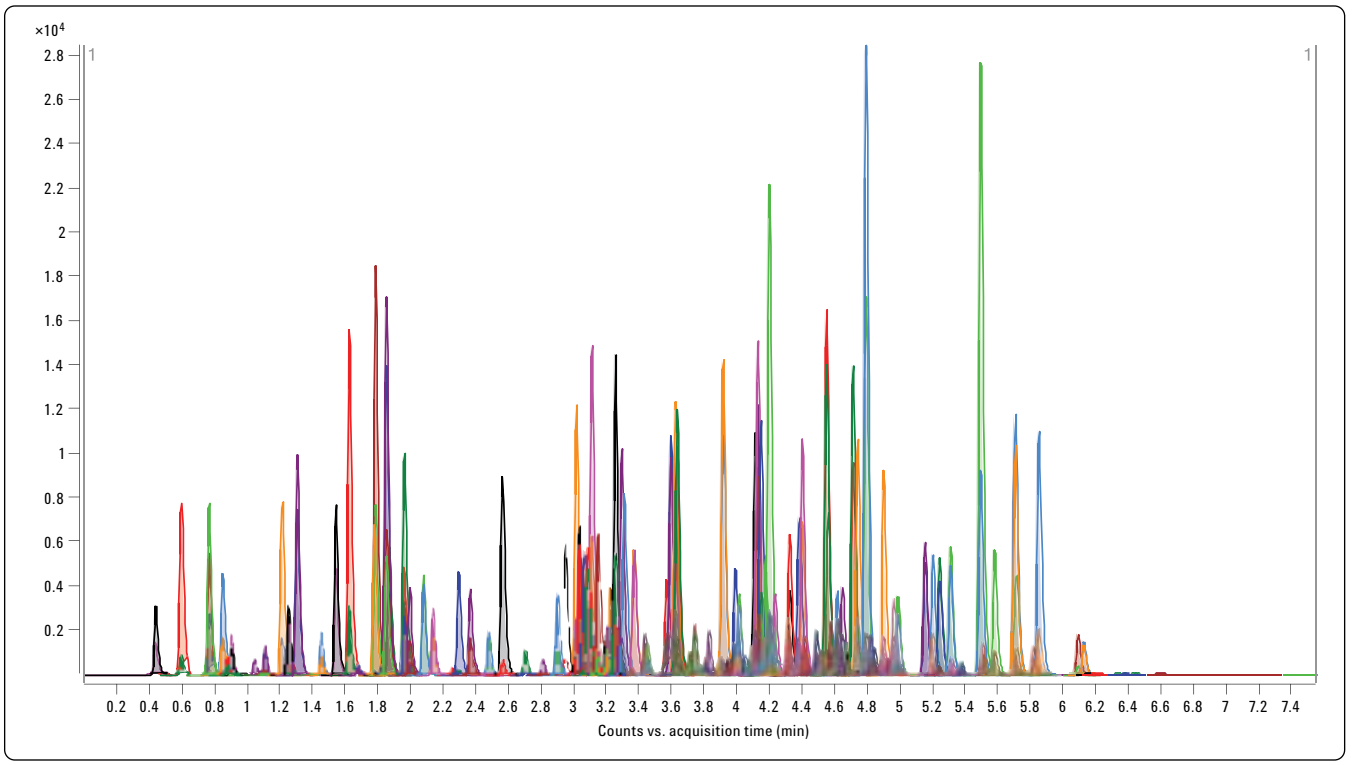


Figure 14. EIC of 224-pesticide mix analyzed with Agilent 1290 Infinity LC and the Agilent 6460 QQQ LC/MS.

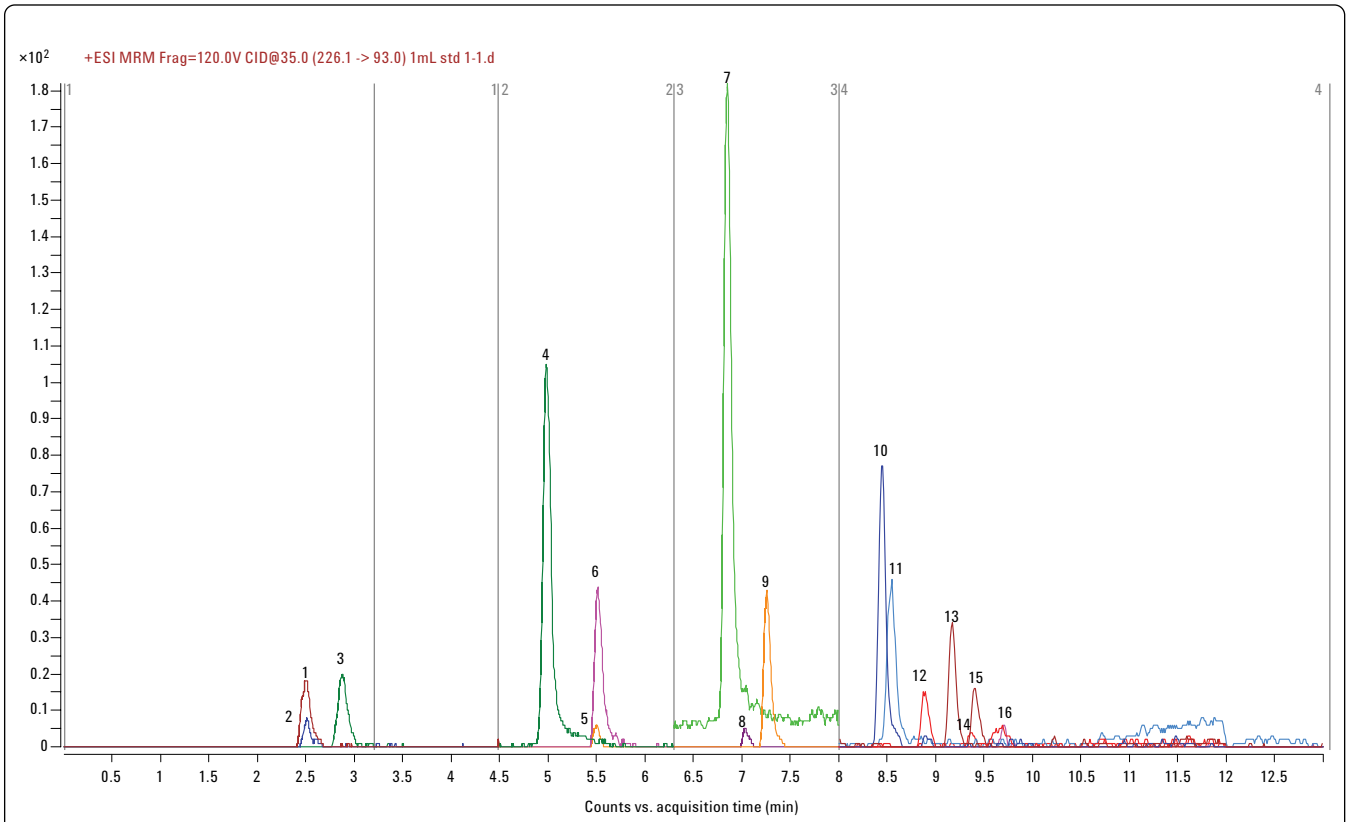
Pesticides Application Kit in a food matrix-Spinach using SampliQ Extraction and Dispersive SPE Kits

To demonstrate the use of the Agilent application kit for the analysis of a typical food product with Agilent's easy to use SampliQ extraction and dispersive SPE kits, a spinach matrix was spiked with 10 ppb of the 16 pesticides listed in Table 2. Triphenylphosphate (TPP) is the internal standard.

Table 2. List of 16 Pesticides and Instrument Parameters Spiked into Spinach Matrix at 10 ppb

Analyte	MRM channel (*m/z*) Quantifier	Qualifier	Fragmentor (V)	Collision energy (V) Quantifier	Qualifier	Retention Time (min)
Acephate	184.0 > 94.9	184.0 > 110.0	60	3	15	2.55
Methamidophos	142.0 > 94.0	142.0 > 124.9	60	8	8	2.54
Pymetrozine	218.1 > 105.0	218.1 > 78.0	115	20	50	2.97
Carbendazim	192.1 > 160.0	192.1 > 105.0	95	18	40	5.07
Imidacloprid	256.1 > 209.1	256.1 > 175.0	60	12	18	5.53
Thiabendazole	202.1 > 175.0	202.1 > 131.0	110	27	38	5.65
Propoxur	210.1 > 111.0	210.1 > 92.9	50	12	15	6.89
Thiophanate methyl	343.1 > 151.0	343.1 > 117.9	105	17	65	7.08
Carbaryl	202.0 > 145.0	202.0 > 115.0	50	3	40	7.30
Ethoprophos	243.1 > 130.9	243.1 > 172.9	80	15	15	8.50
Imazalil	297.1 > 158.9	297.1 > 200.9	80	22	15	8.52
Penconazole	284.1 > 158.9	284.1 > 172.9	80	32	32	8.95
Cyprodinil	226.1 > 93.0	226.1 > 108.0	120	35	35	9.23
Dichlorfluanid	333.0 > 123.0	333.0 > 223.9	85	28	5	9.40
Kresoxim methyl	314.0 > 222.1	314.0 > 235.0	70	10	10	9.44
Tolyfluanid	347.0 > 136.9	347.0 > 238.0	60	25	3	9.73
TPP (IS)	327.1 > 77.0	327.1 > 151.9	70	45	45	9.49

Figure 15 shows the EIC of the spinach sample spiked at the 10-ppb pesticide level. All the pesticides are easily detected at this level with a total analysis time less than ten minutes.



10 ppb of pesticides spiked into the spinach matrix.

Peak identification:

1. Acephate
2. Methamidophos
3. Pymetrozine
4. Carbendazim
5. Imidacloprid
6. Thiabendazole
7. Propoxur
8. Thiophanate methyl
9. Carbaryl
10. Ethoprophos
11. Imazalil
12. Penconazole
13. Cyprodinil
14. Dichlorfluanid
15. Kresoxim methyl,
16. Tolyfluanid

Internal standard peak (TPP) is not shown to get clear peak profile of other small peaks.

Figure 15. EIC of 10 ppb pesticides into spinach matrix..

An example of the linearity achieved for the spiked spinach matrix is shown in Figure 16. The calibration range was 5 – 250 ng/g and seven levels were used to generate the curve, 5, 10, 25, 100, and 250 ng/g. The curve was generated by plotting the ratio of the analyte peak area, carbaryl, to the internal standard (IS) peak area with the ratio of the analytes concentration to IS concentration. The R^2 = 0.998.

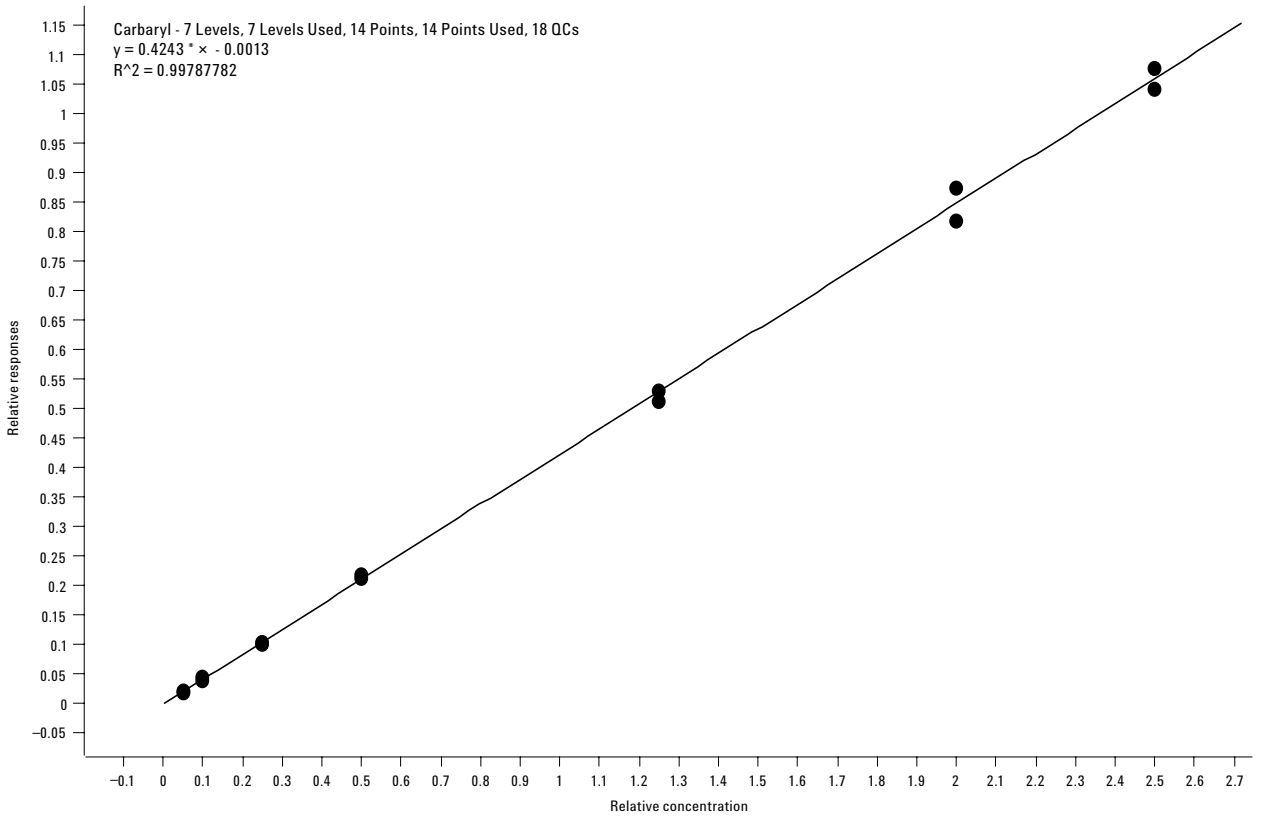


Figure 16. Carbaryl calibration curve.

Conclusions

The **Agilent Pesticide Application Kit** for LC/QQQ provides the user with fast method development for hundreds of pesticides with multiple transitions and the ability to develop those methods customized to his or her specific analytical needs.

This application note demonstrates the use of the Agilent Application Kit for Pesticides using several Agilent technologies for screening large numbers of compounds. The following technologies are used:

- ***600 compound pesticide MRM database*** and the **Agilent MassHunter Data Acquisition and Analysis software.** The combination gives users the ability to generate acquisition and analysis methods quickly. The methods can be easily customized and rapidly modified to meet the needs of future analyses.
- **Dynamic MRM** which maximizes the detection capability of the QQQ when hundreds of residues are being analyzed.
- **Agilent 1200 Series SL RRLC** interfaced to the **Agilent 6400 series triple quadrupoles** for fast and high resolution LC/MS/MS analysis. Use of the Agilent 6460 QQQ with Agilent's Jet Stream Electrospray Ion Source ensures lowest levels of detection of the pesticides. However, any of the Agilent 6400 series LC/QQQ will provide excellent results.
- Easy to use **SampliQ QuEChERS sample preparation kits** included in the Application Kit provide a fast and reproducible method to extract pesticide residues from complex food matrixes in a few simple steps.
- **Ready to use methods** with retention times for Dynamic MRM using the Agilent 1200 Series SL LC system. See all * Appendix methods.[4]

Use of these technologies allows methods to be quickly developed and enables screening of complex matrices containing hundreds of potential residues at femtomole concentrations.

This kit is compatible with all Agilent 1200 Series LC and 6400 series QQQ MS systems and will enable the user to quickly get started running multi-residue pesticides. For the most demanding analyses, the Agilent 1290 Infinity LC with the 6460 QQQ should be considered. Additional methods for this system should be available in the near future.

References

1. Application Note 5990-3595EN, New Dynamic MRM Mode Improves Data Quality and Triple Quad Quantification in Complex Samples.
2. Technical Note 5990-4255EN Pesticide Dynamic Multireaction monitoring Database.
3. Technical Note 5990-3494EN Agilent Jet Stream Thermal GradienFocusing Technology.
4. Agilent Publication 5990-4262EN Pesticide analysis with DRMRM database quick start guide.

For More Information

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Appendix I

LC/MS/MS Conditions for Test mix Positive and Negative Ion Samples

Agilent 1200 Series SL LC Parameters

Column:	Agilent ZORBAX Eclipse Plus C18, 2.1 mm × 100 mm 1.8 µm Agilent p/n 959764-902
Column temperature:	35
Injection volume:	5
Autosampler temperature:	Ambient
Needle wash:	5 s with methanol
Mobile phase:	A = 5 mM acetic acid in water B = 100% acetonitrile
Flow Rate:	0.3 mL/min
Gradient:	5% B at t = 0 to 95% B at t = 12 min
Stop Time:	12 min
Post:	Time 3 min

Jet Stream Conditions

Gas temperature:	250 °C
Gas flow:	7 L/min
Nebulizer:	40 psi
Sheath gas temperature:	325 °C
Sheath gas flow:	1 L/min
Capillary + ion:	3500 V
Nozzle voltage:	0 V
Capillary – ion:	2500 V
Nozzle voltage:	1500 V

MS/MS Scans for positive ions

Compound Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Frag (V)	CE (V)	Ret Time	Ret Window	Polarity
Aminocarb	❑	209	Unit	137	Unit	120	20	3.128	1	Positive
Imazapyr	❑	262	Unit	217	Unit	160	15	3.959	1	Positive
Thiabendazole	❑	202	Unit	131	Unit	120	30	4.072	1	Positive
Dimethoate	❑	230	Unit	171	Unit	80	10	5.064	1	Positive
Imazalil	❑	297	Unit	159	Unit	160	20	5.918	1	Positive
Metoxuron	❑	229.1	Unit	72.1	Unit	93	14	5.992	1	Positive
Carbofuran	❑	222	Unit	123	Unit	120	15	7.019	1	Positive
Atrazine	❑	216	Unit	132	Unit	120	20	7.437	1	Positive
Metosulam	❑	418	Unit	175	Unit	144	26	7.472	1	Positive
Metazachlor	❑	278.1	Unit	134.1	Unit	75	18	8.038	1	Positive
Molinate	❑	188.1	Unit	55.1	Unit	78	22	9.113	1	Positive
Malathion	❑	331	Unit	99	Unit	80	10	9.615	1	Positive
Pyraclostrobin	❑	388	Unit	163	Unit	120	20	10.679	1	Positive
Diazinon	❑	305	Unit	153	Unit	160	20	10.776	1	Positive

MS/MS Scans for negative ions

Compound Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Frag (V)	CE (V)	Ret Time	Ret Window	Polarity
Bentazon	❑	239.1	Unit	132	Unit	80	32	6.572	1	Negative
2,4,5-T	❑	252.9	Unit	194.8	Unit	76	9	8.047	1	Negative
Silvex	❑	266.9	Unit	194.9	Unit	90	5	8.805	1	Negative
Acifluorfen	❑	360	Unit	315.9	Unit	78	5	9.650	1	Negative
Dinoseb	❑	239.1	Unit	207	Unit	154	21	10.503	1	Negative
Hexaflumuron	❑	459	Unit	438.9	Unit	102	5	10.877	1	Negative

Appendix II

LC/MS/MS Conditions for 100-Pesticide Methods

Agilent 1200 Series LC Parameters

Column:		Agilent ZORBAX Eclipse Plus-C18, 2.1 mm × 50 mm, 1.8 µm Agilent p/n 959741-902
Column temperature:		35 °C
Injection volume:		1.0 µL
Autosampler temperature:		6 °C
Needle wash:		Flushport (MeOH:H_2O 75:25), 5 s
Mobile phase:		A = 0.1% formic acid in water B = 0.1% formic acid in 95:5 acetonitrile:water
Flow rate:		0.6 mL/min
Gradient	Time	%B
	0	10
	10	70B
	15	90B
Stop time	20	10B
Post time	5	

Note that example transitions, fragmentor voltages, and collision energies for this method are shown in Figure 7.

Jet Stream Conditions

Drying gas temperature:	325 °C
Drying gas flow (nitrogen):	6 L/min
Nebulizer gas pressure (nitrogen):	35 psig
Capillary voltage:	4000 V
Sheath gas temperature:	400 °C
Sheath gas flow:	12 L/min
Nozzle voltage:	Off

Agilent 6460A QQQ settings

MS1 and MS2 resolution:	Unit
Time Filtering:	Peak width = 0.03 min
Dynamic MRM transitions:	200
Constant cycle time:	373 ms
Delta EMV:	400 V

Appendix III

LC/MS/MS Conditions for 300-Pesticide Methods using the Agilent 1200 Series SL

Agilent 1200 Series LC Parameters

Column:	Agilent ZORBAX Eclipse Plus-C18, 2.1 mm × 100 mm, 1.8 µm Agilent p/n 959764-902
Column temperature:	35 °C
Injection volume:	1.0 µL
Autosampler temperature:	6 °C
Needle wash:	Flushport (MeOH:H_2O 75:25), 5 s
Mobile phase:	A = H_2O w/5 mM ammonium formate + 0.01% formic acid B = 5 mM ammonium formate + 0.01% formic acid in 95:5 acetonitrile:water
Flow rate:	0.5 mL/min

Gradient pump time table

Time	Flow	Pressure	Solv ratio B
0.5	No change	600	6
18	No change	600	95
20	No change	600	95
20.01	No change	600	6
Stop time	20 min	10%B	
Post time	5 min		

Jet Stream Conditions

Drying gas temperature:	325 °C
Drying gas flow (nitrogen):	6 L/min
Nebulizer gas pressure (nitrogen):	35 psig
Capillary voltage:	4000 V
Sheath gas temperature:	400 °C
Sheath gas flow:	12 L/min
Nozzle voltage:	Off

Ten representative MS/MS Transitions from 300-Compound Methods

Compound Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Frag (V)	CE (V)	Ret Time
Promecarb	❑	208.1	Unit	151	Unit	80	5	11.635
Promecarb	❑	208.1	Unit	109	Unit	80	10	11.635
Flurtamone	❑	334.1	Unit	303	Unit	120	20	11.644
Flurtamone	❑	334.1	Unit	247	Unit	120	30	11.644
Isoxaflutole	❑	377.1	Unit	360.1	Unit	100	5	11.669
Isoxaflutole	❑	360.1	Unit	251	Unit	120	10	11.669
Dimethenamide	❑	276.1	Unit	244	Unit	120	10	11.683
Dimethenamide	❑	276.1	Unit	168	Unit	120	15	11.683
Diethofencarb	❑	268.2	Unit	226	Unit	80	5	11.706
Diethofencarb	❑	268.2	Unit	152	Unit	80	20	11.706

Appendix IV

LC/MS/MS Conditions for 300-Pesticide Methods using the Agilent 1290 Infinity LC

Agilent 1290 LC Parameters

Column:	Agilent ZORBAX Eclipse Plus-C18, 2.1 mm × 150 mm, 1.8 µm RRHD 1200 Series bar columns Agilent p/n 959759-902
Column temperature:	60 °C
Injection volume:	35 µL (stacked injection, 5 µL sample + 30 µL H_2O
Autosampler temperature:	6 °C
Needle wash:	Flushport (MeOH:H_2O 75:25 + 0.01% formic acid), 10 s
Mobile phase:	A = H_2O w/5 mM ammonium formate + 0.01% formic acid B = MeOH w/5 mM ammonium formate + 0.01% formic acid
LC flow rate:	0.5 mL/min
LC gradient:	6% B (T = 0) to 98% B (T = 15 min), hold 3 min

MS Parameters

Sheath gas flow:	11 L/min
Sheath gas heater:	375 °C
Charging Electrode:	300 V (pos ion mode)
Capillary voltage:	–4 kV (pos ion mode)
Nebulizer pressure:	35 psig
Drying gas temperature:	325 °C
Drying gas flow:	8 L/min

Ten representative MS/MS Scan Segments from 300-Compound Methods

Compound Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Frag (V)	CE (V)	Ret Time
Chloridazon	❑	222	Unit	104	Unit	120	25	5.841
Chloridazon	❑	222	Unit	92	Unit	120	30	5.841
Aminocarb	❑	209.1	Unit	152.1	Unit	120	10	5.841
Aminocarb	❑	209.1	Unit	137	Unit	120	20	5.841
Fluroxypyr	❑	255	Unit	209	Unit	80	10	5.845
Fluroxypyr	❑	255	Unit	181	Unit	80	15	5.845
Acetamiprid	❑	223.1	Unit	126	Unit	80	15	5.858
Acetamiprid	❑	223.1	Unit	56	Unit	80	15	5.858
Vamidothion	❑	288	Unit	146	Unit	80	10	5.996
Vamidothion	❑	288	Unit	118	Unit	80	20	5.996

Appendix V

LC/MS/MS Conditions for Pesticides in Spinach using QuECHERS Extraction

Agilent 1200 Series HPLC conditions

Column:	Agilent ZORBAX Eclipse Plus Phenyl-hexyl, 150 mm × 3 mm, 3.5 µm Agilent p/n 959963-312		
Column temperature:	30 °C		
Injection volume:	10 µL		
Mobile phase:	A = 5 mM ammonium acetate, pH 5.0 in 20:80 MeOH/H_2O B = 5 mM ammonium acetate, pH 5.0 in ACN		
Needle wash:	1:1:1:1 ACN/MeOH/IPA/H_2O w/0.2% FA		
Gradient:	Time (min)		Flow rate
	(min)	% B	(mL/min)
	0	20	0.3
	0.5	20	0.3
	8.0	100	0.3
	10.0	100	0.3
	10.1	20	0.5
	12.0	100	0.5
Stop time:	13.0 min		
Post run:	4 min		
Total cycle time:	17 min		

Agilent 6410 MS conditions

Positive mode	
Gas temperature:	350 °C
Gas flow:	10 L/min
Nebulizer:	40 psi
Capillary:	4000 V

Appendix VI

LC/MS/MS Conditions for 165-Pesticide Methods using the Agilent 1200 Series Infinity SL

Agilent 1200 Series Infinity SL LC Parameters

Column:	Agilent ZORBAX Eclipse Plus C18, 2.1 mm × 100 mm 1.8 µm Agilent p/n 959764-902
Column temperature:	35 °C
Injection volume:	5.0 µL
Autosampler temperature:	6 °C
Needle wash:	Flushport (MeOH:H2O 75:25) 5 s
Mobile phase:	A = H2O w/5mM ammonium formate + 0.01% formic acid B = 5 mM ammonium formate + 0.01% formic acid in methanol

Gradient — Pump Time Table

Time (min)	Solv ratio B (%)
0.00	10
1.00	10
18.00	100
20.00	100
20.10	10
25.00	10

Jet Stream Conditions

Spray Chamber Conditions

Gas temperature:	200 °C
Dry gas :	6 L/min
Nebulizer:	35 psi
Sheath gas temperature:	250 °C
Sheath gas flow:	12 L/min
Positive cap voltage:	4000 V
Nozzle voltage:	300 V

Ten Representative MS/MS Transitions from 167-Compound Methods

Compound Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Frag (V)	CE (V)	Ret Time	Ret Window
Ethiofencarb-sulfon	❑	275	Unit	201	Unit	80	0	6.89	1
Ethiofencarb-sulfon	❑	275	Unit	107	Unit	80	10	6.89	1
Clothianidin	❑	250	Unit	169	Unit	90	5	7.064	1
Clothianidin	❑	250	Unit	132	Unit	90	15	7.064	1
Imidacloprid	❑	256.1	Unit	209	Unit	80	15	7.071	1
Imidacloprid	❑	256.1	Unit	175.1	Unit	80	20	7.071	1
Ethiofencarb-sulfoxid	❑	242	Unit	185	Unit	80	15	7.153	1
Ethiofencarb-sulfoxid	❑	242	Unit	107	Unit	80	5	7.153	1
Monalide	❑	257.1	Unit	200.1	Unit	105	4	7.165	1
Monalide	❑	257.1	Unit	137.1	Unit	105	8	7.165	1

Appendix VII

LC/MS/MS Conditions for 224-Pesticide Methods using the Agilent 1200 Series SL

Agilent 1200 Series LC Parameters

Column:	Agilent ZORBAX Eclipse Plus-C18, 2.1 mm × 100 mm, 1.8 µm Agilent p/n 959764-902
Column temperature:	55 °C
Injection volume:	5.0 µL
Autosampler temperature:	6 °C
Needle wash:	Flushport (MeOH:H_2O 75:25), 5 s
Mobile phase:	A = H_2O w/5 mM ammonium formate + 0.01% formic acid B = 5 mM ammonium formate + 0.01% formic acid in 95:5 acetonitrile:water
Flow rate:	0.3 mL/min

Gradient pump time table

Time	Flow	Pressure	Solv ratio B
0.5	No change	600	6
14	No change	600	95
17	No change	600	95

Stop time 17 min
Post time 3 min

Jet Stream Conditions

Drying gas temperature:	225 °C
Drying gas flow (nitrogen):	10 L/min
Nebulizer gas pressure (nitrogen):	25 psig
Capillary voltage:	4500 V
Sheath gas temperature:	350 °C
Sheath gas flow:	11 L/min
Nozzle voltage:	500 V

Ten representative MS/MS Transitions from 224-Compound Methods

Compound Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Frag (V)	CE (V)	Ret Time	Ret Window
Buprofezin	❑	306.2	Unit	201.1	Unit	115	4	14.321	1
Buprofezin	❑	306.2	Unit	57.2	Unit	115	16	14.321	1
Sulprofos	❑	323	Unit	247.1	Unit	130	5	14.327	1
Sulprofos	❑	323	Unit	219	Unit	130	12	14.327	1
Eprinomectin B1a	❑	914.6	Unit	468.3	Unit	150	5	14.372	1
Eprinomectin B1a	❑	914.6	Unit	330.3	Unit	150	10	14.372	1
Chlorfluazuron	❑	540	Unit	383	Unit	115	16	14.402	1
Chlorfluazuron	❑	540	Unit	158	Unit	115	16	14.402	1
Fenpyroximat	❑	422.2	Unit	366.2	Unit	130	15	14.428	1
Fenpyroximat	❑	422.2	Unit	135	Unit	130	40	14.428	1

Appendix VIII

LC/MS/MS Conditions for 224-Pesticide Methods using the Agilent 1290 Infinity LC

Agilent 1200 Series LC Parameters

Column:	Agilent ZORBAX Eclipse Plus-C18, 2.1 mm × 100 mm, 1.8 µm Agilent p/n 959764-902
Column temperature:	55 °C
Injection volume:	5.0 µL
Autosampler temperature:	6 °C
Needle wash:	Flushport (MeOH:H_2O 75:25), 5 s
Mobile phase:	A = H_2O w/5mM ammonium formate + 0.01% formic acid B = 5 mM ammonium formate + 0.01% formic acid in 95:5 acetonitrile:water
Flow rate:	0.6 mL/min

Gradient pump time table

Time	Flow	Pressure	Solv ratio B
0.5	No change	600	6
7	No change	600	95
10	No change	600	95

Stop time 10 min
Post time 3 min

Jet Stream Conditions

Drying gas temperature:	225 °C
Drying gas flow (nitrogen):	10 L/min
Nebulizer gas pressure (nitrogen):	25 psig
Capillary voltage:	4500 V
Sheath gas temperature:	350 °C
Sheath gas flow:	11 L/min
Nozzle voltage:	500 V

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- Increase the number of targets screened
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- Reduce the analysis time required per sample
- Perform a complete screening and quantitation in 2-3 minutes
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- Retention Time Locked application-specific column, ensuring reliable database matching
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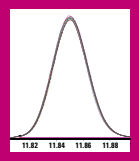
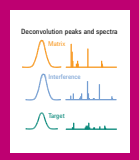
Multimode Inlet (MMI) with large-volume injection enhances trace-level detection and adds flexibility by including standard split/splitless capabilities.



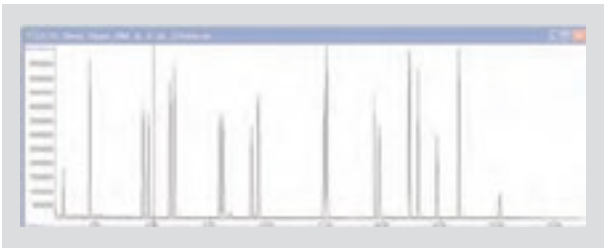
Retention Time Locking (RTL) for consistent retention times after column maintenance and easy matching with the 927-compound Pesticides and Endocrine Disruptors database.



Deconvolution Reporting Software (DRS) for fast data review in 2-3 minutes per sample, with screening and quantitation in one run.

Capillary Flow Technology (CFT) and backflush shorten run times, reduce chemical background, extend column life, and reduce frequency of source cleaning.

The Pesticide Screener will get your lab on the *fast track* to better broad-range screening



Running the pesticides checkout sample: A quick-start guide included with Agilent's GC/MS DRS Pesticide Screener shows you how to load the pesticide method, relock the method to get consistent retention times, and run the checkout sample.

A DRS Checkout Report: You can analyze your results quickly using Deconvolution Reporting Software (DRS) and the RTL Pesticides database.

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Identifying Pesticides with Full Scan, SIM, µECD, and FPD from a Single Injection

Application

Food Safety, Environmental

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Abstract

In this application note, a gas chromatography/mass spectrometry (GC/MS) system capable of providing up to four signals from a single injection is described. When a three-way micro-fluidic splitter is added to the end of the column, two additional signals from GC detectors can be acquired together with the MS data from a single injection. This multi-signal configuration provides: full-scan data for library searching, selective ion monitoring (SIM) data for trace analysis, micro-electron capture detector and flame photometric detector data for excellent selectivity and sensitivity from complex matrices. A combination of element selective detectors, SIM/Scan, and deconvolution reporting software makes a very powerful pesticide analysis system. Examples for trace-level compound quantitation/confirmation or for screening are discussed.

Introduction

Many laboratories in the world are analyzing pesticide residue levels in both foods and the environment to protect human health. The process usually involves homogenizing the sample, extracting the pesticides, and analyzing the target compounds with a Gas Chromatograph (GC) or a Liquid Chromatograph (LC) depending on the nature of the compounds. For GC amenable compounds, the traditional detectors are NPD (Nitrogen Phosphorus Detector), µECD (micro-Electron Capture Detector), and FPD (Flame Photometric Detector) for their excellent sensitivity and selectivity. However, even with dual-column confirmation analysis, these GC detectors cannot be used to verify the identity of the compounds with high confidence.

Full scan mass spectral data and library searching are typically used for final compound verification. However, full-scan analysis has a worse (higher) detection limit (DL) compared to selective detectors on a GC. To improve the DL, the technique selective ion monitoring (SIM) is often used. With SIM, the MS monitors only a few characteristic ions for each target compound within the retention time (RT) range that the target elutes from the column. By monitoring only a few specific ions, the signal-to-noise ratio (S/N) improves significantly. The ions monitored are time programmed in groups corresponding to the RTs of the targets. SIM analyses with closely eluting targets require precise alignment of chromatographic RTs with the time programming of SIM groups. The Retention Time Locking (RTL) technique can be applied to eliminate the need to adjust SIM group time-windows after column maintenance or replacement.

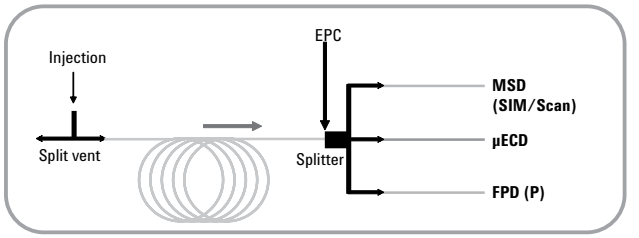
In this application note, a GC/MS system capable of providing up to four signals from a single injection is described. The benefits of the multi-signal detection include:

- Confirmatory information – Full-scan data for library search capability

- Maximum sensitivity – SIM data enables trace analysis
- Excellent selectivity – µECD and FPD detect trace-level hetero-compounds from complex matrices

Experimental

A recent technical note describes “Synchronous SIM/Scan”, which takes advantages of the Performance Electronics in the 5975 inert MSD to get both SIM and full-scan signals in a single run without sacrificing performance [1]. The SIM method can be easily developed automatically using the ChemStation’s AutoSIM tool [2]. By simply selecting a checkbox in the method, the SIM and full-scan data can be acquired together. The trade-off is giving up some cycles per second but gaining an additional signal (full-scan data or SIM data) for the whole analysis. With properly chosen acquisition parameters, for example, increasing the scan speed, the decrease of cycles per second is usually not significant and does not affect peak quantitation or the quality of results (for example, S/N).



At the end of the column, effluent flow is split three ways according to the length and diameter of the capillary tubing (restrictor) used.

Figure 1. A schematic of the multi-signal configuration. Note: the EPC flow adds to the column flow into the splitter.

Besides the SIM/Scan data, the ChemStation software can simultaneously acquire up to two additional GC detector signals, for example, FPD (in phosphorus- or sulfur- mode) and NPD (nitrogen-phosphorus detector) signals or both P- and S- signals from a dual-wavelength FPD (DFPD). See Figure 1.

Figure 1 is a schematic for multi-signal detection. At the end of the column, a three-way micro-fluidic splitter was used to split the column effluent to different detectors [3]. For this study, an FPD and a µECD were installed. Notice on the figure that an Auxiliary Electronic Pneumatics Control (Aux EPC) gas channel was connected to the splitter to maintain the pressure at the end of the column so that the split ratios/flows are kept constant throughout a run. Figure 2 shows a close-up view of the micro-fluidic splitter installed in the GC oven.

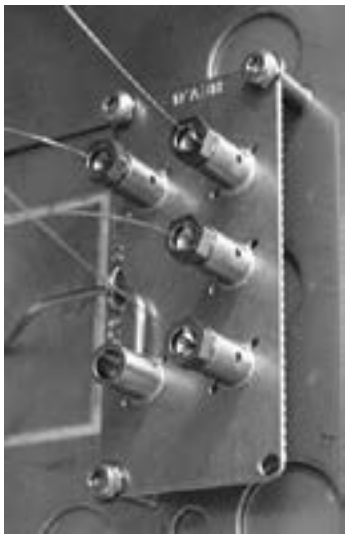


Figure 2. A close-up view of the micro-fluidic three-way splitter in the 6890 GC oven.

The size of the micro-fluidic plate is 1.25 inches (3.2 cm) wide and 2.5 inches (6.4 cm) tall. The device was designed to eliminate the common problems of large thermal mass, excess dead volume, and leaky connection due to oven temperature cycling etc. The splitter's flow paths and connection points are laid out and etched onto a thin, stainless steel plate using photolithography and chem-milling technologies. The plate is diffusion bonded, mounted with column connectors, and surface deactivated, resulting in an integrated and compact micro-fluidic splitter. Metal ferrules are used at the connectors that are leak-free after temperature cycling and will not absorb solvents or sample matrix, improving sensitivity for trace analysis applications.

Deactivated capillary tubing between the splitter and each detector was used as a flow restrictor. Aux EPC pressure and the restrictor dimensions were determined using a spreadsheet-like calculator program to achieve the proper split ratio among all detectors. The three-way splitter can easily turn into a two-way splitter when a connector is capped.

Other advantages of a splitter include back-flushing [3] and quick-swapping. The Aux EPC flow can be run-time programmed to a higher pressure, while at the same time the inlet pressure is lowered to near ambient. This causes the column flow to reverse direction, back-flushing the less volatile materials out of the split vent of the inlet. The Aux EPC on the splitter also allows column changing and inlet maintenance without cooling and venting the MSD. The splitter’s flow paths and connection points were designed in such a way

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that when the column fitting is removed, the helium gas from the Aux EPC purges the fitting, preventing air from entering the splitter/MSD. See Table 1 for hardware details and settings.

Table 1. Gas Chromatograph, Mass Spectrometer, and Three-Way Splitter Operating Parameters

GC	Agilent Technologies 6890		
Inlet	EPC Split/Splitless		
Mode	Splitless, 1.0 µL injected (7683 ALS)		
Inlet temp	280 °C		
Pressure	~27 psi (chlorpyrifos methyl RT locked to 16.596 min)		
Purge flow	50.0 mL/min		
Purge time	0.75 min		
Total flow	55.3 mL/min		
Gas saver	Off		
Gas type	Helium		
Inlet liner	Siltek Cyclosplitter, 4-mm id, Restek p/n 20706-214.1		
Oven			
Oven ramp	°C/min	Final (°C)	Hold (min)
Initial		70	2.00
Ramp 1	25	150	0.00
Ramp 2	3	200	0.00
Ramp 3	8	280	15
Total run time		46.87 min (last standard elutes around 35 min)	
Equilibration time		0.5 min	
Oven max temp		325 °C	
Column		Agilent Technologies HP 5-ms, p/n 19091S-433	
Length		30.0 m	
Diameter		0.25 mm	
Film thickness		0.25 µm	
Mode		Constant pressure	
Nominal initial flow		2.5 mL/min	
Outlet		Unspecified	
Outlet pressure		3.8 psi (Aux EPC pressure to splitter)	
Front detector (FPD)			
Phosphorus mode		**Sulfur mode**	
Hydrogen flow:	75.0 mL/min	Hydrogen flow:	50.0 mL/min
Oxidizer flow:	100.0 mL/min	Oxidizer flow:	60.0 mL/min
Temperature:	250 °C		
Oxidizer gas type:	Air		
Mode:	Constant makeup flow		
Makeup flow:	60.0 mL/min		
Makeup gas type:	Nitrogen		
Lit offset:	2.00		
Data rate:	5 Hz		

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Table 1. Gas Chromatograph, Mass Spectrometer, and Three-Way Splitter Operating Parameters (Continued)

Back detector (µECD)	
Temperature:	300 °C
Mode:	Constant makeup flow
Makeup flow:	60.0 mL/min
Makeup gas type:	Nitrogen
Date rate:	5 Hz
Thermal AUX 2	
Use:	MSD Transfer line heater
Initial temp:	280 °C
Pressure AUX 5	
Gas type:	Helium
Initial pressure:	3.80 psi
Initial time:	0.00 min (this value will follow oven ramp)
MSD	Agilent Technologies 5975 inert MSD
Tune file	Atune.U
Mode	Scan
Solvent delay	3.00 min
EM voltage	Atune voltage
Low mass	45 amu
High mass	555 amu
Threshold	100
Sampling	2
A/D Samples	4
Scans/s	2.89
Quad temp	150 °C
Source temp	230 °C
Three-way splitter	Agilent 6890N Option 890, when installed on the GC during factory assembly
Split ratio	10:10:1 MSD:FPD:µECD
MSD restrictor	1.444 m × 0.18-mm id Deactivated fused silica tubing
FPD restrictor	0.532 m × 0.18-mm id Deactivated fused silica tubing
µECD restrictor	0.507 m × 0.10-mm id Deactivated fused silica tubing
Flow to MSD (at 280 °C)	1.53 mL/min
Flow to FPD (at 280 °C)	1.53 mL/min
Flow to µECD (at 280 °C)	0.153 mL/min
Makeup flow (at 280 °C)	1.38 mL/min

Software Used in this Application Note	
GC/MSD ChemStation	G1701DA
Deconvolution Reporting Software (DRS)	G1716AA
NIST Library	G1033A
AMDIS (included for free with the NIST library CD)	

Results and Discussion

Figure 3 shows four signals that were simultaneously acquired from a single injection of a pesticide mixture. Due to the high sensitivity of the µECD, the split ratios for the three detectors was set to MSD:FPD:µECD = 10:10:1. This split ratio distributes the sample of a 1-µL splitless injection of a 1-ppm (1000 pg/µL) sample to the different detectors as labeled in Figure 3.

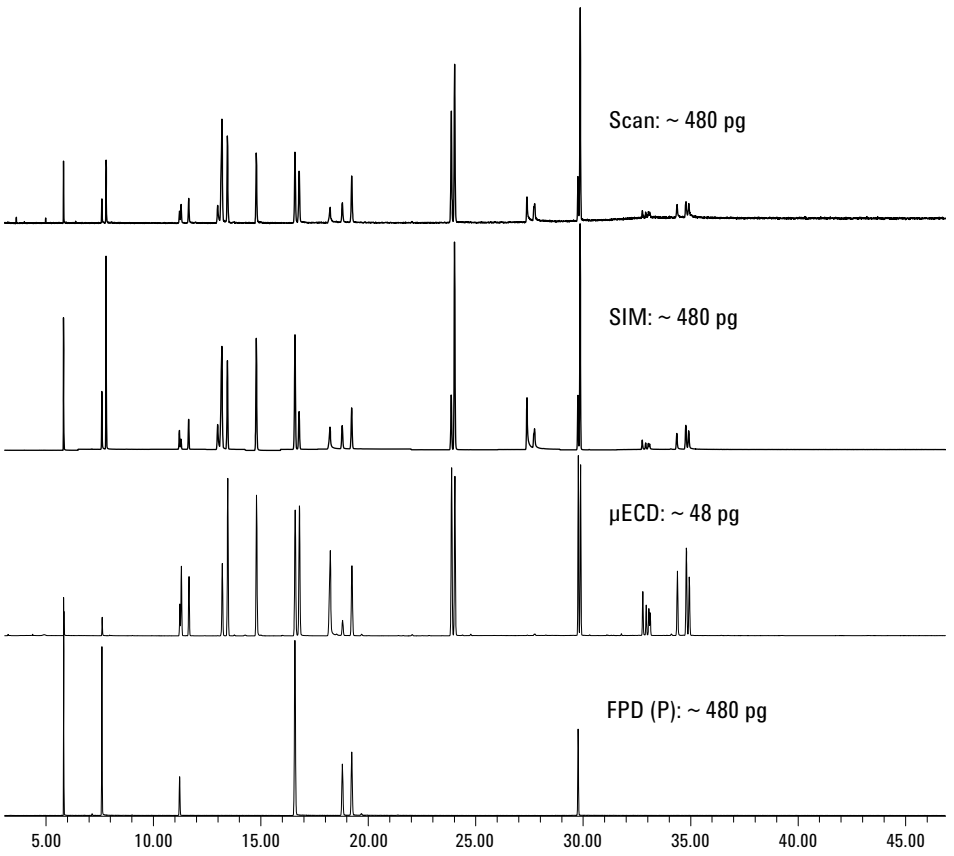

Figure 3. Signals acquired simultaneously from a 1-µL splitless injection of 1-ppm standard. The split ratios were MSD:FPD:µECD = 10:10:1.

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Figure 4 shows the signals when the pesticide standard was diluted 100-fold in a produce matrix. The total ion chromatogram (TIC) from full scan was not shown due to the lack of sensitivity. The FPD(P) and µECD were able to detect all the pesticides spiked in this extract. For trace-level target compound analysis, the SIM signal can be used for quantitation and the GC signals used for further confirmation.

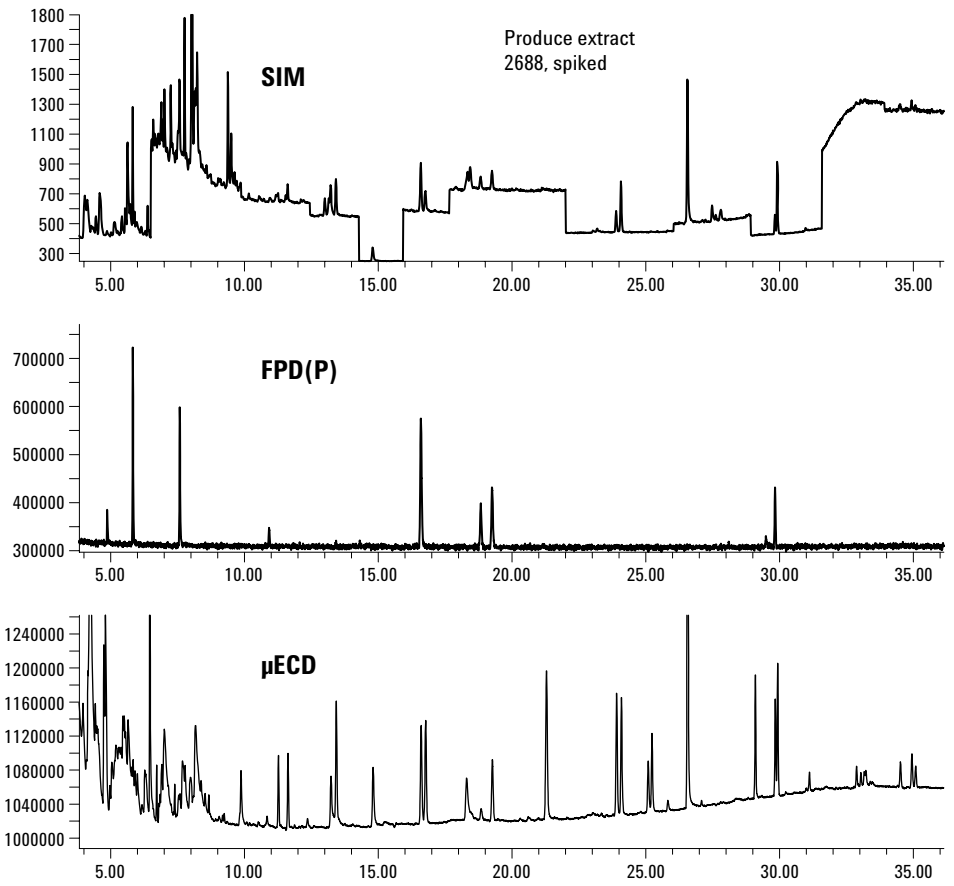

Figure 4. Data of a produce extract spiked at 10 ppb. FPD and µECD were able to detect the respective standards spiked into the extract.

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Another application for this multi-signal system is for screening. In screening, no target list is available for the analysis; therefore, SIM acquisition or MS/MS is not possible. Figure 5 shows three signals (no SIM) from a produce extract.

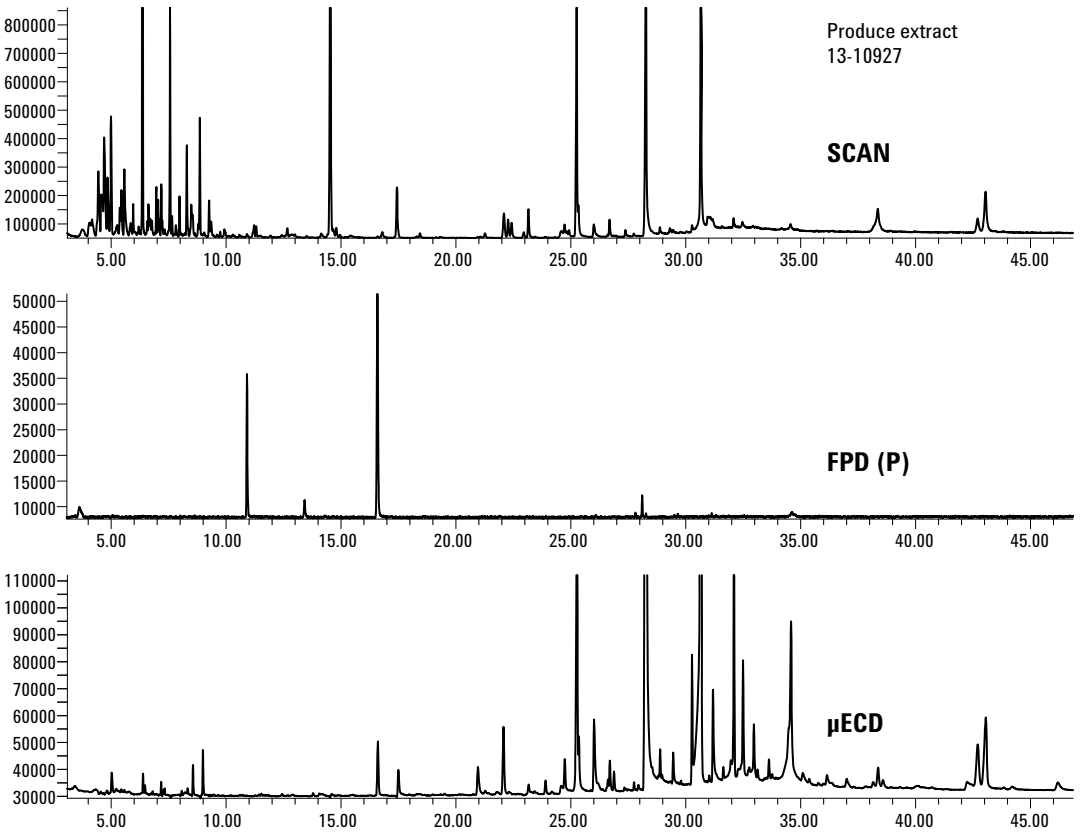
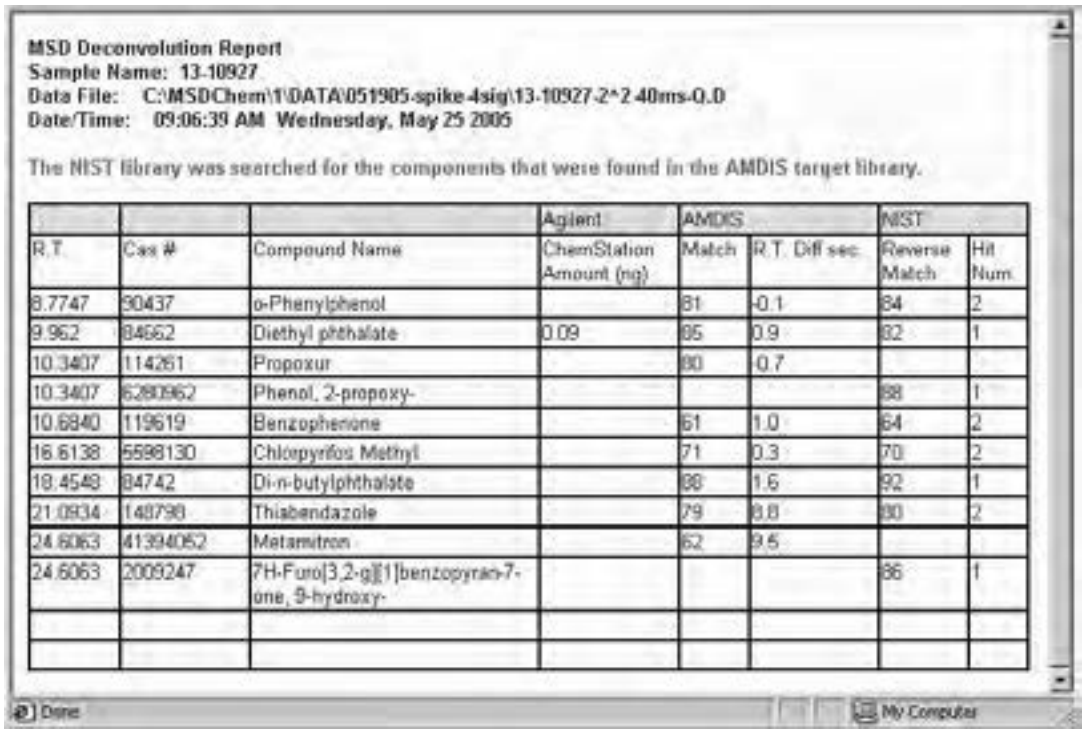


Figure 5. Full-scan, FPD(P), and µECD data for extract 13-10927.

The Deconvolution Reporting Software (DRS) [3, 4] found several pesticides in the TIC as shown in Figure 6.



MSD Deconvolution Report
Sample Name: 13-10927
Data File: C:\MSDChem\1\DATA\051905-spike-4sig\13-10927-2^2.40ms-Q.D
Date/Time: 09:06:39 AM Wednesday, May 25 2005

The NIST library was searched for the components that were found in the AMDIS target library.

			Agilent	AMDIS		NIST	
R.T.	Cas #	Compound Name	ChemStation Amount (ng)	Match	R.T. Diff sec	Reverse Match	Hit Num
8.7747	90437	o-Phenylphenol		81	-0.1	84	2
9.962	84662	Diethyl phthalate	0.09	85	0.9	82	1
10.3407	114261	Propoxur		80	-0.7		
10.3407	6280962	Phenol, 2-propoxy-				88	1
10.6840	119619	Benzophenone		61	1.0	64	2
16.6138	5598130	Chlorpyrifos Methyl		71	0.3	70	2
18.4548	84742	Di-n-butylphthalate		88	1.6	92	1
21.0934	148798	Thiabendazole		79	8.8	80	2
24.6063	41394052	Metamitron		62	9.5		
24.6063	2009247	7H-Furo[3,2-g][1]benzopyran-7-one, 9-hydroxy-				86	1

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Figure 6. Report for extract 13-10927 generated from DRS.

The possible pesticides in the sample were benzophenone, chlorpyrifos methyl, and thiabendazole. Propoxur and metamitron were not confirmed by both AMDIS and NIST; therefore, they were most likely false positives.

Due to the complexity of the sample matrix and other interferences, it is sometimes difficult to get a high library match factor from peaks in the TIC, even after background subtraction. Therefore, element selective detectors would be very useful in providing the supporting information for compound confirmation. The multi-signal system was retention time locked, therefore, from the RT and the aligned peaks from the FPD(P) and the µECD responses, chlorpyrifos methyl ($C_7H_7Cl_3NO_3PS$) was confirmed.

It usually takes less than 3 minutes to turn off the FPD photomultiplier, swap the P-filter with the S-filter, and turn the photomultiplier back on. After the swap, adjust the detector gas flows to optimize the response in either P- or S- mode. A new injection of the same extract was made in FPD(S) mode. The FPD(S) result is shown with previously acquired signals in Figure 7. Two major peaks were seen on the FPD(S) chromatogram. From the peak RTs, they supported the presence of chlorpyrifos methyl and thiabendazole ($C_{10}H_7N_3S$) respectively. Note that the full-scan TIC barely showed a peak for either compound, which made it impossible for traditional data analysis to identify both compounds. The FPD(S) mode is very selective, but it is not as sensitive as the FPD(P) mode. Although the µECD is very sensitive, it is not as selective as the FPD. A combination of GC detectors, SIM/Scan, and DRS makes a very powerful pesticide analysis system.

Conclusion

The Synchronous SIM/Scan provides users with library searchable full-scan spectra as well as trace level SIM data in a single analysis. When a three-way micro-fluidic splitter is added to the end of the column, two additional signals from element selective detectors can be acquired together with the MS data from a single injection. This configuration makes it very attractive for the analysis of trace-level pesticide residues in foods or environmental samples.

This multi-signal configuration provides: full-scan data for library searching, SIM data for trace analysis, µECD and FPD data for excellent selectivity and sensitivity from complex matrices. In this application note, examples of µECD signal and FPD signal (P- or S- mode) were acquired together with the SIM/Scan data from a single injection for trace-level compound quantitation/confirmation, or for screening.

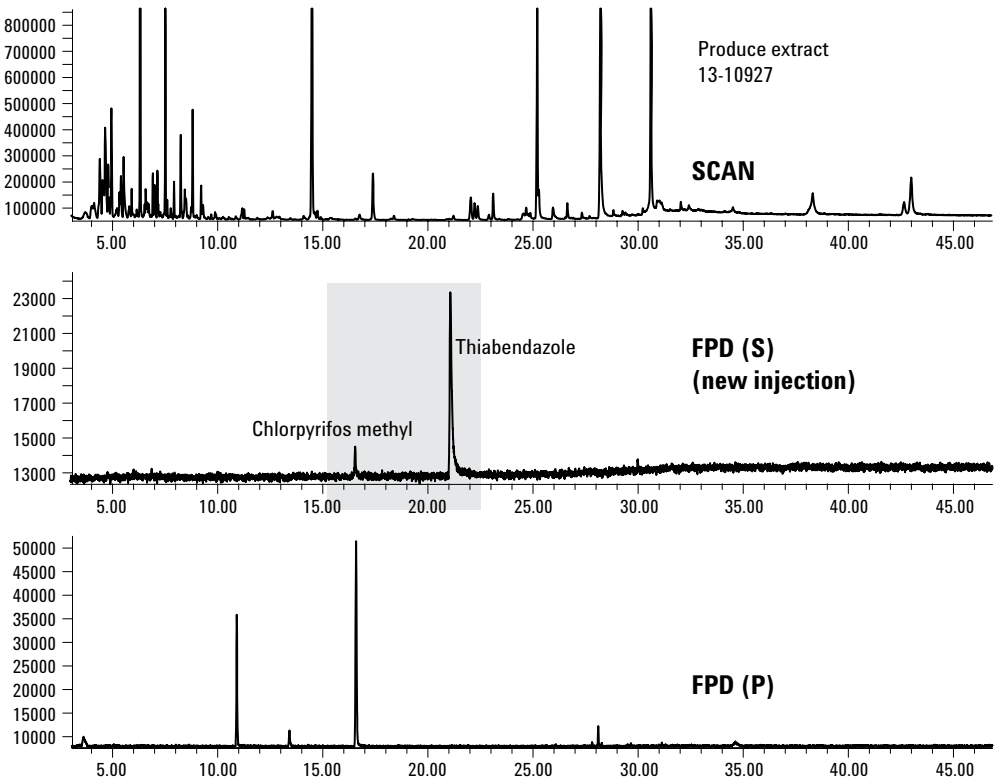

Figure 7. Full-scan, FPD(S), and FPD(P) data for extract 13-10927.

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Screening for 926 Pesticides and Endocrine Disruptors by GC/MS with Deconvolution Reporting Software and a New Pesticide Library

Application Note

Food and Environmental

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Abstract

An updated and greatly expanded collection of mass spectral libraries has been introduced, replacing Agilent's RTL Pesticide Library and DRS pesticide solution. The new library contains 926 pesticides, endocrine disruptors, and related compounds – 359 more than the original library. Included are all compounds specified for GC/MS analysis in the new Japanese "Positive List" regulations. All compounds have locked retention times that can be accurately reproduced using an Agilent GC/MS system with the ChemStation's Retention Time Locking software. The new Database can be used as a standard GC/MS library for compound identification or with Agilent's Screener software for identifications based upon retention time and mass spectral matching. The greatest benefit accrues when these libraries are used with Agilent's new version of Deconvolution Reporting Software (part number G1716AA version A.03.00). This solution allows one to screen GC/MS files for all 926 pesticides and endocrine disrupters in about two minutes per sample. Deconvolution helps identify pesticides that are buried in the chromatogram by co-extracted materials. The new database was compared to the smaller one for the DRS analysis of 17 surface water samples. With the new database, DRS found 99 pesticides, metabolites, fire retardants, and related contaminants that were not contained in the original RTL Pesticide and Endocrine Disruptor Library.

Introduction

Several years ago Agilent Technologies introduced Retention Time Locking (RTL) for gas chromatography (GC) and GC with mass spectral detection (GC/MS). RTL software makes it possible to reproduce retention times from run-to-run on any Agilent GC or GC/MS, in any laboratory in the world, so long as the same nominal method and GC column are used (1). Since any laboratory can reproduce retention times generated in another, it is possible to create mass spectral libraries that contain locked retention times. By locking their method to the published database, users can screen GC/MS files for all of the library's compounds. "Hits" are required to have the correct retention time as well as the correct spectrum, which eliminates many false positives and gives more confidence in compound identifications (2).

More recently, Agilent introduced Deconvolution Reporting Software (DRS) that incorporates mass spectral deconvolution with conventional library searching and quantification. DRS results from a marriage of three different GC/MS software packages:

1) The Agilent GC/MS ChemStation,
2) The National Institute of Standards and Technology (NIST) Mass Spectral Search Program with the NIST '05 MS Library, and
3) The Automated Mass Spectral Deconvolution and Identification System (AMDIS) software, also from NIST.

The original DRS software was intended to be a comprehensive solution for pesticide analysis and, therefore, included the mass spectra (in AMDIS format) and locked retention times for 567 pesticides and suspected endocrine disrupters (3).

Recently, Agilent introduced an updated and greatly expanded Pesticide and Endocrine Disruptor Database (part number G1672AA) that now contains 926 entries. This represents the addition of 359 new compounds to the original library. At the same time, Agilent introduced a new version of the DRS software (part number G1716AA version A.03.00) that can be used with any Agilent-provided or user-developed DRS library.

Pesticide and Endocrine Disruptor Database Contents

The G1672AA Pesticide and Endocrine Disruptor Database contains virtually all GC-able pesticides, including those introduced very recently. In addition, the database includes numerous metabolites, more endocrine disruptors, important PCBs and PAHs, certain dyes (for example, Sudan Red), synthetic musk compounds, and several organophosphorus fire retardants.

This new database includes:

- A conventional mass spectral library for use with Agilent GC/MS ChemStations
- A screener database for use with Agilent's powerful screener software that is integrated into the GC/MS ChemStation
- Locked Retention Times for all 926 compounds that any Agilent 5975 or 5973 GC/MS user can reproduce in their laboratory
- Files for use with Agilent's G1716AA (A.03.00) Deconvolution Reporting Software
- An e-method that can be loaded into Agilent's G1701DA (version D.02.00 SP1 or higher) with instrument parameters for acquiring GC/MS files and analyzing the data with DRS. These parameters are listed in Table 1.
- Example files
- Application notes

On November 29, 2005, the Japanese Government published a "Positive List" system for the regulation of pesticides, feed additives, and veterinary drugs. Maximum Residue Limits (MRL) have been set for 758 chemicals while 65 others have been exempted from regulation. Fifteen substances must have no detectable residues. Other agricultural chemicals not mentioned have a uniform MRL of 0.01 ppm (4). This new regulation is scheduled to take effect on May 29, 2006.

Of the pesticides in the Japanese Positive List, 265 are to be analyzed by GC/MS. The new G1672AA Pesticide library contains mass spectra and locked retention times for all of these compounds. Thus, a laboratory could screen for all 265 "positive list" compounds and several hundred more pesticides in just 1–3 minutes after the GC/MS run.

Experimental

Table 1 lists the instrumentation, software, and analytical parameters used by Agilent for pesticide analysis. Depending upon the desired injection volume, a PTV inlet or split/splitless inlet can be used.

Table 1. Instrumentation and Conditions of Analysis

Gas Chromatograph	**Agilent 6890N**
Automatic Sampler	Agilent 7683 Injector and AutoSampler
Inlet	Agilent PTV operated in the solvent vent mode or Split/Splitless
Column	Agilent 30 m × 0.25 mm × 0.25 µm HP-5MSi (part number 19091S-433i)
Carrier gas	Helium in the constant pressure mode
Retention time locking	Chlorpyrifos-methyl locked to 16.596 min (nominal column head pressure = 17.1 psi)
Oven temperature program	70 °C (2 min), 25 °C/min to 150 °C (0 min), 3 °C /min to 200 °C (0 min), 8 °C /min to 280 °C (10–15 min)
PTV inlet parameters	Temp program: 40 °C (0.25 min), 1600 °C/min to 250 °C (2 min); Vent time: 0.2 min; Vent flow: 200 mL/min; Vent pressure: 0.0 psi; Purge flow: 60.0 mL/min; Purge time: 2.00 min
Injection volume	15 µL (using a 50-µL syringe)
Mass Selective Detector	**Agilent 5975 inert**
Tune file	Atune.u
Mode	Scan (or SIM with SIM DRS library)
Scan range	50–550 u
Source, quad, transfer line temperatures	230, 150, and 280 °C, respectively
Solvent delay	4.00 min
Multiplier voltage	Autotune voltage
Software	
GC/MSD ChemStation	Agilent part number G1701DA (version D02.00 sp1 or higher)
Deconvolution Reporting Software	Agilent part number G1716AA (version A.03.00) Deconvolution Reporting Software
Library searching software	NIST MS Search (version 2.0d or greater) (comes with NIST '05 mass spectral library – Agilent part number G1033A)
Deconvolution software	Automated Mass Spectral Deconvolution and Identification Software (AMDIS_32 version 2.62 or greater; comes with NIST '05 mass spectral library – Agilent part number G1033A)
MS Libraries	NIST '05 mass spectral library (Agilent part number G1033A) Agilent RTL Pesticide and Endocrine Disruptor Libraries in Agilent and NIST formats (part number G1672AA)

Results and Discussion

DRS, which has been described in preceding papers (3,5,6), can be summarized as follows:

Three separate, but complimentary, data analysis steps are combined into the DRS. First, the GC/MS ChemStation software performs a normal quantitative analysis for target pesticides using a target ion and up to three qualifiers. An amount is reported for all calibrated compounds that are detected. For other compounds in the database, an estimate of their concentration can be reported based upon an average pesticide response factor that is supplied with the DRS software. The DRS then sends the data file to AMDIS, which deconvolutes the spectra and searches the Agilent RTL Pesticide Library using the deconvoluted full spectra. A filter can be set in AMDIS, which requires the analyte's retention time to fall within a user-specified time window. Because RTL is used to reproduce the RTL database retention times with high precision, this window can be quite small – typically 10–20 seconds. Finally, the deconvoluted spectra for all of the targets found by AMDIS are searched against the 147,000-compound NIST mass spectral library for confirmation; for this step, there is no retention time requirement.

This approach was rapidly adopted by many laboratories because of its ability to identify pesticides in complex chromatograms containing high levels of co-extracted interferences. Indeed, the solution proved to be so useful that users began to create their own DRS libraries (7). Therefore, the DRS was unbundled from the pesticide database so that it could be used with any agilent-provided or user-created database.

The original 567-compound RTL Pesticide Library (G1049A) included pesticides, a few metabolites, and most of the GC-amenable endocrine disruptors that were known at the time. The new version of the library includes many more pesticides, endocrine disruptors, and metabolites. This update also contains important compounds from other classes of contaminants that have been found in food and water supplies. Included are eighteen polychlorinated biphenyls (PCBs), four polybrominated biphenyls (PBBs), several polynuclear aromatic hydrocarbons (PAHs), several organophosphorus fire retardants, three important toxaphene congeners, and three Sudan dyes.

Advantages of Deconvolution

Figure 1 shows a screen from AMDIS that illustrates the power of this deconvolution software. The white trace in Figure 1A is the total ion chromatogram while the other three are extracted ions of a deconvoluted peak (a “component” in AMDIS terminology). Note that the TIC and extracted ions are not scaled to each other and this component is actually obscured by co-eluting compounds. Figure 1B juxtaposes the deconvoluted component spectrum (white) with the complete “undeconvoluted” spectrum (black). Clearly, this component is buried under co-eluting peaks that would ordinarily obscure the analyte. Figure 1C shows that the deconvoluted peak (white spectrum) is a good library match for norflurazon (black spectrum). The locked retention time for norflurazon in the RTL Pesticide Database is 26.933 min, which is just 2.3 seconds away from its observed RT in this chromatogram. Confidence in peak identifications is greatly enhanced by the combination of spectral deconvolution and locked retention time filtering.

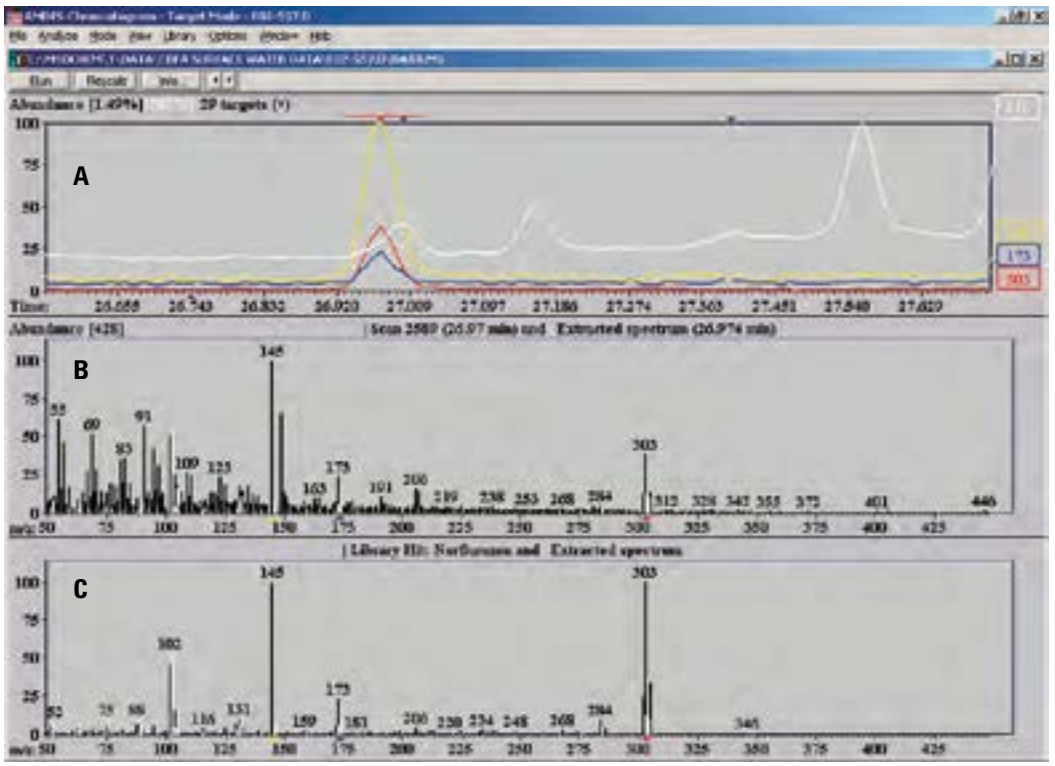

Figure 1. AMDIS screen showing the identification of norflurazon.
A) The total ion and extracted ion chromatograms where norflurazon elutes.
B) The deconvoluted component spectrum (white) juxtaposed with the spectrum at 26.972 min (black).
C) The deconvoluted component matched to the library spectrum of norflurazon.

Surface Water Analysis - Revisiting an Earlier Study

In an earlier study, a comparison was made between Agilent's DRS and conventional pesticide analysis (3). The California Department of Food and Agriculture (CDFA) provided data files for 17 surface water extracts that had been analyzed in their laboratory. Since the GC/MS chromatograms were locked to the Agilent pesticide method, it was possible to analyze these data files using DRS without having to re-run the samples. The original DRS analysis was made using the 567-compound RTL Pesticide Database. For comparison, these same data files were re-analyzed using the new 926-compound RTL Pesticide Database. The chromatogram (Figure 2) and the DRS report (Figure 3) from one of these samples are shown below.

Excluding phthalates, seven new compounds (shown with bold type in Figure 3) were identified using the 926-compound database: 4-chlorophenyl isocyanate (a phenylurea herbicide metabolite); 3,4-dichlorophenyl isocyanate (diuron metabolite); tris(2-chloroethyl) phosphate (a fire retardant); caffeine (a stimulant); Cyprodinil (a fungicide); desmethyl-norflurazon (a metabolite of norflurazon, an herbicide); and tris(2-butoxyethyl) phosphate (a fire retardant). Although caffeine is not generally considered to be dangerous, it is included in the database because it has been found frequently in sewage effluent and in numerous waterways together with a various pharmaceuticals and pesticides (8).

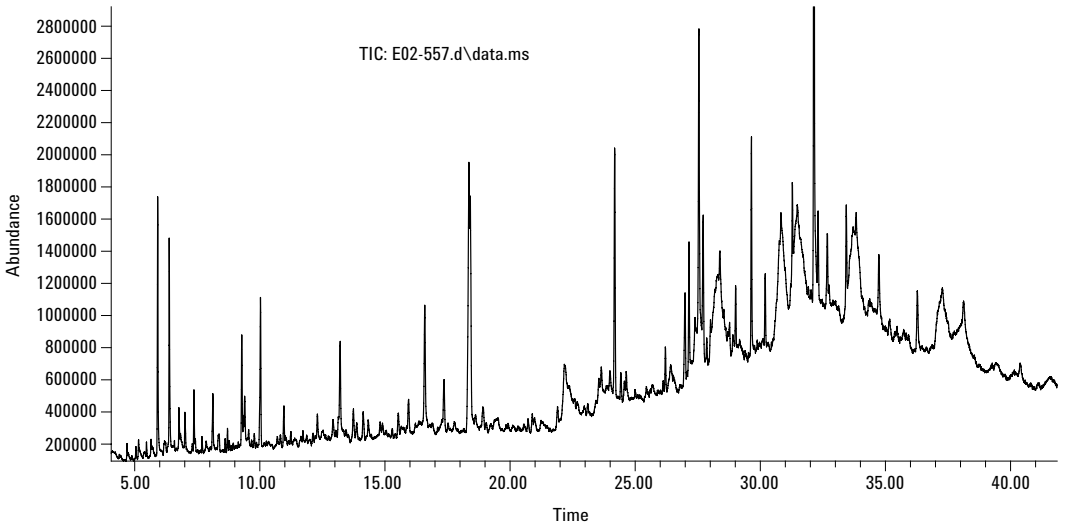

Figure 2. Chromatogram of a surface water extract that was analyzed by DRS using the new RTL Pesticide and Endocrine Disrupter Database. The results of this analysis are shown in Figure 3.

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MSD Deconvolution Report
Sample Name: E02-557
Data File: C:\MSDChem\1\DATA\CDFA surface water data\E02-557.d
Date/Time: 11:24 AM Tuesday, Apr 4 2006

The NIST library was searched for the components that were found in the AMDIS target library.

			Agilent			NIST	
RT	Cas #	Compound name	ChemStation amount (ng)	AMDIS match	RT Diff (sec.)	reverse match	Hit number
4.4689	106445	4-Methylphenol		62	3.2		
4.4689	0000	3-Carbobenzyloxy-4-ketoproline				48	1
4.8840	104121	**4-Chlorophenyl isocyanate**		84	–1.8	86	2
6.3879	102363	**Diuron Metabolite [3,4-Dichlorophenyl isocyanate]**		99	3.1	95	1
6.8357	759944	EPTC		84	2.0	85	1
7.6988	95761	3,4-Dichloroaniline		93	2.1	89	2
7.9342	131113	Dimethylphthalate		67	1.7	84	2
8.1112	25013165	Butylated hydroxyanisole		63	–7.7		
8.1112	0000	7-Methoxy-2,2,4,8-tetramethyltricyclo [5.3.1.0(4,11)]undecane				62	1
8.941	29878317	Tolyltriazole [1H-Benzotriazole, 4-meth-]	1.29				
9.7903	134623	N,N-Diethyl-m-toluamide		85	2.2	84	2
10.0019	84662	Diethyl phthalate		98	2.6	92	1
10.7109	119619	Benzophenone		86	2.6	88	2
10.9684	126738	Tributyl phosphate		96	3.0	90	1
11.6491	1582098	Trifluralin		83	0.7	74	1
12.9326	122349	Simazine		88	1.4	86	2
13.4309	115968	**Tris(2-chloroethyl) phosphate**		79	1.0	78	1
13.7478	1517222	Phenanthrene-d10		95	1.3	83	1
15.4048	58082	**Caffeine**		80	1.6	74	1
15.9474	84695	Diisobutyl phthalate		90	3.2	88	4
16.5988	5598130	Chlorpyrifos Methyl		97	0.4	90	1
17.3653	7287196	Prometryn		90	1.5	84	1
18.4213	84742	Di-n-butylphthalate		99	0.4	94	1
18.9214	51218452	Metolachlor		90	0.7	87	1
20.5633	121552612	**Cyprodinil**		69	–0.1		
20.5633	76470252	9,9-Dimethoxy-9-sila-9, 10-dihydroanthracene				70	1
26.4247	23576241	**Norflurazon, Desmethyl-**		87	–4.5	69	2
26.9700	27314132	Norflurazon		87	1.5	79	1
26.9992	85687	Butyl benzyl phthalate		94	–0.5	94	1
27.3984	51235042	Hexazinone		89	0.8	83	1
28.0127	78513	**Tris(2-butoxyethyl) phosphate**		75	3.3	83	1
29.6537	117817	Bis(2-ethylhexyl)phthalate		98	0.3	90	3
33.9298	84764	Di-n-nonyl phthalate		65	–1.9		
33.9298	0000	Phthalic acid, 3,4-dichlorophenyl propyl ester				71	1
13.739		Phenanthrene-d10	10				

Figure 3. DRS report from the analysis of a surface water sample. The compounds shown in bold type were found by the new RTL Pesticide Database but not the original one because these compounds were not included.

For this sample, the ChemStation identified only tolyltriazole at 8.941 min, but AMDIS did not confirm this assignment, nor could it be confirmed manually. Butylated hydroxyanisole was tentatively identified by AMDIS with a low match value, but the retention time is off by –7.7 seconds which is considerably more than most other hits. This compound is not in the NIST library so it could not be confirmed. The ChemStation method used for this analysis required that all three qualifier ions fall within ±20% (relative) which is a rigorous requirement for such a complex sample. This explains why so few compounds were found by the ChemStation.

Cyprodinil (20.563 min) was identified by AMDIS but the NIST library search failed to confirm its presence. The next line shows that the best NIST library match is an anthracene derivative that is nothing like cyprodinil. This result was obtained when AMDIS was configured to “use uncertain peaks” as shown in Figure 4. When this feature is turned off in DRS Compound Identification Configuration, the best NIST library hit for this spectrum is, indeed, cyprodinil. When a compound's identity is ambiguous, as with cyprodinil, it may be useful to perform the DRS search both ways and compare the results.

In the comparison described earlier (3), DRS was able to identify all 37 pesticides found by the CDFA chemist. However, DRS completed the task for all 17 samples in about 20 minutes compared to ~8 hours for the manual procedure (Table 2). Moreover, DRS identified one false positive in the CDFA report and found 34 additional pesticides and related compounds.

Using the new 926-compound Database, it took 32 minutes to analyze all of the samples and DRS was able to find an additional 99 pesticides, metabolites, fire retardants, and related compounds (Table 2).

Figure 4. DRS configuration screen for the method called Tri_Pest. When the box labeled “Use Uncertain Peaks” is checked, AMDIS will use uncertain peaks for library searches. When unchecked, AMDIS ignores uncertain mass spectral peaks. Sometimes, this can affect the quality of a library match.

Table 2. Comparison of the Results Obtained by Screening 17 Surface Water Extracts Using Traditional Methods (CDFA) and Using DRS With Two Different Databases – the G1049A With 567 Compounds and the G1672AA With 926 Entries

	CDFA	Agilent DRS (Original G1049A database)	Agilent DRS (G1672 AA database)
Targets found (not counting ISTD)	37	Same 37 +34 more	Same 37 +99 more
False positives	1	0	0
Processing time	~8 hrs (ChemStation only)	20 minutes	32 min

Handling Stereoisomers

Many pesticides have multiple stereoisomers with virtually identical mass spectra. For example, cyfluthrin has four diastereomers arising from its three chiral centers. It is very difficult and sometimes impossible to determine the elution order of these isomers and most analysts report them as a sum of the isomer amounts. Agilent's G1049A RTL Pesticide database arbitrarily assigned each isomer a Roman numeral with I for the earliest eluting isomer, II for the next, and so on. The same Chemical Abstracts Service number (CAS #) was assigned to all of the isomers. Generally, it was a CAS # for the compound with "unstated stereochemistry." This caused some incompatibility with AMDIS as explained below.

AMDIS software differentiates among compounds using a "chemical identification number." The easiest and most consistent approach is to use each compound's CAS #. The default setting for AMDIS is to allow each CAS # to be used only once when analyzing a GC/MS data file. While this seems logical, it requires that each database entry have a different CAS #. It is possible to allow multiple hits per compound by checking the box in AMDIS found in the drop down menu under Analyze/Settings/Identif. However, this allows multiple peaks to be assigned the same compound name.

In the new RTL Pesticide Database (G1672AA), the Roman numeral designations remain and the first isomer in the series is given its genuine CAS #. Subsequent isomers in the series are given unique, but fictitious "CAS #s" generated by Agilent. The compound's real CAS # appears in braces after the compound name. For example, the cyfluthrin isomers are entered into the database as shown in Table 3.

Table 3. Method for Listing Compounds with Multiple Stereoisomers in the New G1672AA RTL Pesticide Database

RT	Compound name*		CAS #**
32.218	Cyfluthrin I		68359-37-5
32.359	Cyfluthrin II	{CAS # 68359-37-5}	999028-03-4
32.477	Cyfluthrin III	{CAS # 68359-37-5}	999029-03-7
32.536	Cyfluthrin IV	{CAS # 68359-37-5}	999030-03-4

* In a series, the earliest eluting isomer is identified with "I" and is assigned its legitimate CAS #. Subsequent isomers are assigned unique, but fictitious CAS #s (see footnote **). Their actual CAS # is put in braces behind the compound name.

** Cyfluthrin I has been given it's genuine CAS #. Cyfluthrin II-IV have been given unique numbers that can be distinguished from actual CAS numbers because they all have six digits before the first hyphen (9 total) and all begin with the series 999.

Figure 5 shows how permethrin was identified in a spinach sample using both databases with AMDIS configured to allow one hit per compound. Using the older 567-compound database (G1049A) only one permethrin isomer was identified because its CAS # could be used only once. With the new format used in the 926-compound RTL Pesticide Database (G1672AA), both isomers of permethrin were identified. Not surprisingly, the NIST library search found no hits with the same fictitious CAS # assigned to permethrin II. So, the software printed the best match on the following line. This compound, a cyclopropanecarboxylic acid derivative, is a permethrin isomer.

So long as the NIST library search is turned on in DRS, it will always print another line after reporting a compound with a fictitious CAS #. Note that these fictitious CAS #s always contain 9 digits and begin with 999.

A)

			Agilent			NIST	
RT	**Cas #**	**Compound name**	**ChemStation amount (ng)**	**AMDIS match**	**RT Diff (sec.)**	**reverse match**	**Hit number**
31.6158	52645531	Permethrin II		88	3.9	91	3

B)

			Agilent			NIST	
RT	**Cas #**	**Compound name**	**ChemStation amount (ng)**	**AMDIS match**	**RT Diff (sec.)**	**reverse match**	**Hit number**
31.4127	52645531	Permethrin I		78	2.6	81	3
31.6088	999046036	Permethrin II {CAS # 52645-53-1}		65	3.5		
31.6088	51877748	Cyclopropanecarboxylic acid, 3-(2,2-dichlorovinyl)-2,2-dimethyl-, (3-phenoxyphenyl)methyl ester, (1R-trans)-				95	1

Figure 5. A) A single isomer of permethrin was identified by DRS using the G1049A 567-compound database when AMDIS was not allowed to use multiple hits per compound.
B) Two permethrin isomers are identified by DRS with the G1672AA 926-compound database under the same circumstances.

Conclusions

The new G1672AA RTL Pesticide and Endocrine Disruptor library contains substantially more target analytes than its predecessor. With the addition of 359 new compounds, it is the most comprehensive library of its type available today. Many new pesticides, metabolites, and endocrine disruptors were added along with important PCBs, PBBs, PAHs, synthetic musk compounds, Sudan dyes, and organophosphorus fire retardants. The database contains all of the analytes specified for GC/MS analysis in the new Japanese "Positive List" regulations.

When combined with the complete DRS solution, one can screen GC/MS data files for all 926 compounds in about two minutes per sample. This is the fastest, most comprehensive, most accurate, and least tedious method for screening food and environmental samples for these compounds.

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Lists of Compounds in Databases

1,2,4-Trichlorobenzene
1,2-Dibromo-3-chloropropane
1,3,5-Tribromobenzene
1,3-Dichlorbenzene
17a-Ethynylestradiol
1-naphthalenol
2-(1-naphthyl)acetamide
2-(2-Butoxyethoxy)ethyl thiocyanate
2-(Octylthio)ethanol
2,3,4,5-Tertrachloronitrobenzene
2,3,4,5-Tetrachlorophenol
2,3,4,6-Tetrachlorophenol
2,3,5,6-Tetrachlorophenol
2,3,5,6-Tetrachloro-p-terphenyl
2,3,5-Trichlorophenol
2,3,5-Trimethacarb
2,3,6-Trichloroanisole
2,3,7,8-Tetrachlorodibenzofuran
2,3,7,8-Tetrachlorodibenzo-p-dioxin
2,4,5,6-Tetrachloro-m-xylene
2,4,5-T methyl ester
2,4,5-Trichloroaniline
2,4,5-Trichlorophenol
2,4,5-Trichloro-p-terphenyl
2,4,5-Trimethylaniline
2,4,6-Tribromoanisole
2,4,6-Tribromophenol
2,4,6-Trichloroanisole
2,4,6-Trichlorophenol
2,4-D methyl ester
2,4-D sec-butyl ester
2,4-DB methyl ester
2,4'-Dichlorobenzophenone (2,4'-Dicofol decomposition product)
2,4-Dichlorophenol
2,4-Dichlorophenyl benzenesulfonate
2,4-Dimethylaniline
2,4-Dimethylphenol
2,6-Dichlorobenzamide
2,6-Dichlorobenzonitrile
2,6-Dimethylaniline
2-[3-Chlorophenoxy]propionamide
2-Chlorophenol
2-Ethyl-1,3-hexanediol
2-ethyl-6-methylaniline
2-Hydroxyestradiol
2-Methyl-4,6-dinitrophenol
2-Methylphenol
2-Nitrophenol
2-Phenoxypropionic acid
3,4,5-Trimethacarb
3,4-Dichloroaniline
3,5-Dichloroaniline
3-Aminophenol
3-Chloro-4-fluoroaniline
3-Chloro-4-methoxyaniline
3-Chloroaniline
3-Hydroxycarbofuran
3-Indolylacetonitrile
3-Trifluormethylaniline
4,4'-Dichlorobenzophenone
4,4'-Oxydianiline
4,6-Dinitro-o-cresol (DNOC)
4-Aminodiphenyl
4-Bromoaniline
4-Chloro-2-methylaniline
4-Chloro-3-methylphenol
4-Chloroaniline
4-Chlorophenyl isocyanate
4-Isopropylaniline
4-Methylphenol
4-Nitrophenol
4-Nonylphenol
5,7-Dihydroxy-4'-methoxyisoflavone
9,10-Anthraquinone
Acenaphthene
Acenaphthylene
Acephate
Acequinocyl
acetamiprid
Acetochlor
Acifluorfen methyl ester
Aclonifen
Acrinathrin
Alachlor
Aldrin
Allidochlor
Ametryn
Amidithion
Aminocarb
Amitraz
Amitraz metabolite [Methanimidamide, N-(2,4-dimethylphenyl)-N'-methyl-]
Ancymidol
Anilazine
Aniline
Anilofos
Anthracene
Aramite I
Aramite II {CAS # 140-57-8}
Atraton
Atrazine
Atrazine-desethyl
Azaconazole
Azamethiphos
Azibenzolar-S-methyl
Azinphos-ethyl
Azinphos-methyl
Aziprotryn metabolite [2-Amino-4-isopropylamino-6-methylthio-1,3,5-triazine]
Aziprotryne
Azobenzene
Azoxybenzene
Azoxystrobin
Barban
Beflubutamid
Benalaxyl
Benazolin-ethyl
Bendiocarb
Benfluralin

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Benfuresate
Benodanil
Benoxacor
Bentazone
Bentazone methyl derivative
Benthiocarb
Benzene, 1,3-*bis*(bromomethyl)-
Benzenesulfonamide
Benzidine
Benzo(a)anthracene
Benzo(a)pyrene
Benzo[b]fluoranthene
Benzo[g,h,i]perylene
Benzo[k]fluoranthene
Benzophenone
Benzoximate metabolite
Benzoylprop ethyl
Benzyl benzoate
b-Estradiol
BHC alpha isomer
BHC beta isomer
BHC delta isomer
BHC epsilon isomer
Bifenazate metabolite (5-Phenyl-o-anisidine)
Bifenox
Bifenthrin
Binapacryl
Bioallethrin
Bioallethrin S-cyclopentenyl isomer
Bioresmethrin
Biphenyl
Bis(2,3,3,3-tetrachloropropyl) ether
Bis(2-butoxyethyl) phthalate
Bis(2-ethylhexyl)phthalate
Bisphenol A
Bitertanol I
Bitertanol II {CAS # 55179-31-2}
Boscalid (Nicobifen)
Bromacil
Bromfenvinphos-(E)
Bromfenvinphos-(Z)
Bromobutide
Bromocyclen
Bromophos
Bromophos-ethyl
Bromopropylate
Bromoxynil
Bromoxynil octanoic acid ester
Bromuconazole I
Bromuconazole II {CAS # 116255-48-2}
Bufencarb
Bupirimate
Buprofezin
Butachlor
Butafenacil
Butamifos
Butoxycarboxim
Butralin
Butyl benzyl phthalate
Butylate
Butylated hydroxyanisole
Cadusafos
Cafenstrole
Caffeine
Captafol
Captan
Carbaryl
Carbetamide
Carbofuran
Carbofuran-3-keto
Carbofuran-7-phenol
Carbophenothion
Carbosulfan
Carboxin
Carfentrazone-ethyl
Carpropamid
Carvone
Cashmeran
Cekafix
Celestolide
Chinomethionat
Chloramben methyl ester
Chloranocryl
Chlorbenside
Chlorbenside sulfone
Chlorbicyclen
Chlorbromuron
Chlorbufam
Chlordecone
Chlordene, *trans*-
Chlordimeform
Chlorethoxyfos
Chlorfenapyr
Chlorfenethol
Chlorfenprop-methyl
Chlorfenson
Chlorfenvinphos
Chlorfenvinphos, *cis*-
Chlorfenvinphos, *trans*-
Chlorflurecol-methyl ester
Chlormefos
Chlornitrofen
Chlorobenzilate
Chloroneb
Chloropropylate
Chlorothalonil
Chlorotoluron
Chlorpropham
Chlorpyrifos
Chlorpyrifos Methyl
Chlorthal-dimethyl
Chlorthiamid
Chlorthion
Chlorthiophos
Chlorthiophos sulfone
Chlorthiophos sulfoxide
Chlozolinate
Chrysene
Cinerin I
Cinerin II
Cinidon-ethyl
cis-Chlordane
Clodinafop-propargyl
Clomazone
Cloquintocet-mexyl
Coumaphos
Crimidine
Crotoxyphos
Crufomate
Cyanazine
Cyanofenphos
Cyanophos
Cyclafuramid
Cycloate
Cyclopentadecanone
Cycluron

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Cyflufenamid
Cyfluthrin I
Cyfluthrin II {CAS # 68359-37-5}
Cyfluthrin III {CAS # 68359-37-5}
Cyfluthrin IV {CAS # 68359-37-5}
Cyhalofop-butyl
Cyhalothrin I (lambda)
Cyhalothrin (Gamma)
Cymiazole
Cymoxanil
Cypermethrin I
Cypermethrin II {CAS # 52315-07-8}
Cypermethrin III {CAS # 52315-07-8}
Cypermethrin IV {CAS # 52315-07-8}
Cyphenothrin *cis*-
Cyphenothrin *trans*- {CAS # 39515-40-7}
Cyprazine
Cyproconazole
Cyprodinil
Cyprofuram
Cyromazine
d-(*cis-trans*)-Phenothrin-I
d-(*cis-trans*)-Phenothrin-II {CAS # 260002-80-2}
Dazomet
DDMU [1-Chloro-2,2-*bis*(4'-chlorophenyl)]
Decachlorobiphenyl
Deltamethrin
Demephion
Demeton-S
Demeton-S-methylsulfon
Desbromo-bromobutide
Desmedipham
Desmetryn
Dialifos
Di-allate I
Di-allate II {CAS # 2303-16-4}
Diamyl phthalate
Diazinon
Diazinon-oxon
Dibenz[a,h]anthracene
Dicamba
Dicamba methyl ester
Dicapthon
Dichlofenthion
Dichlofluanid
Dichlofluanid metabolite (DMSA)
Dichlone
Dichlormid
Dichlorophen
Dichlorprop
Dichlorprop methyl ester
Dichlorvos
Diclobutrazol
Diclocymet I
Diclocymet II {CAS # 139920-32-4}
Diclofop methyl
Dicloran
Dicrotophos
Dicyclohexyl phthalate
Dicyclopentadiene
Dieldrin
Diethatyl ethyl
Diethofencarb
Diethyl dithiobis(thionoformate) (EXD)
Diethyl phthalate
Diethylene glycol
Diethylstilbestrol
Difenoconazol I
Difenoconazol II {CAS # 119446-68-3}
Difenoxuron
Diflufenican
Diisobutyl phthalate
Dimefox
Dimepiperate
Dimethachlor
Dimethametryn
Dimethenamid
Dimethipin
Dimethoate
Dimethomorph-(E)
Dimethomorph-(Z) {CAS # 110488-70-5}
Dimethylphthalate
Dimethylvinphos(z)
Dimetilan
Dimoxystrobin
Di-n-butylphthalate
Di-n-hexyl phthalate
Diniconazole
Dinitramine
Di-n-nonyl phthalate
Dinobuton
Dinocap I
Dinocap II {CAS # 39300-45-3}
Dinocap III {CAS # 39300-45-3}
Dinocap IV {CAS # 39300-45-3}
Di-n-octyl phthalate
Dinoseb
Dinoseb acetate
Dinoseb methyl ether
Dinoterb
Dinoterb acetate
Di-n-propyl phthalate
Diofenolan I
Diofenolan II {CAS # 63837-33-2}
Dioxabenzofos
Dioxacarb
Dioxathion
Diphacinone
Diphenamid
Diphenyl phthalate
Diphenylamine
Dipropetryn
Dipropyl isocinchomeronate
Disulfoton
Disulfoton sulfone
Ditalimfos
Dithiopyr
Diuron
Diuron Metabolite [3,4-Dichlorophenyl isocyanate]
Dodemorph I
Dodemorph II {CAS # 1593-77-7}
Drazoxolon
Edifenphos
Empenthrin I
Empenthrin II {CAS # 54406-48-3}
Empenthrin III {CAS # 54406-48-3}
Empenthrin IV {CAS # 54406-48-3}
Empenthrin V {CAS # 54406-48-3}
Endosulfan (alpha isomer)
Endosulfan (beta isomer)
Endosulfan ether
Endosulfan lactone
Endosulfan sulfate
Endrin
Endrin aldehyde
Endrin ketone

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EPN
Epoxiconazole
EPTC
Erbon
Esfenvalerate
Esprocarb
Etaconazole
Ethalfluralin
Ethidimuron
Ethiofencarb
Ethiolate
Ethion
Ethofenprox
Ethofumesate
Ethofumesate, 2-Keto
Ethoprophos
Ethoxyfen-ethyl
Ethoxyquin
Ethylenethiourea
Etoxazole
Etridiazole
Etridiazole, deschloro- (5-ethoxy-3-dichloromethyl-1,2,4-thiadiazole)
Etrimfos
Eugenol
Exaltolide [15-Pentadecanolide]
Famoxadon
Famphur
Fenamidone
Fenamiphos sulfoxide
Fenamiphos-sulfone
Fenarimol
Fenazaflor
Fenazaflor metabolite
Fenazaquin
Fenbuconazole
Fenchlorazole-ethyl
Fenchlorphos
Fenchlorphos-oxon
Fenclorim
Fenfuram
Fenhexamid
Fenitrothion
Fenitrothion-oxon
Fenobucarb
Fenoprop
Fenoprop methyl ester
Fenothiocarb
Fenoxanil
Fenoxaprop-ethyl
Fenoxycarb
Fenpiclonil
Fenpropathrin
Fenpropidin
Fenson
Fensulfothion
Fensulfothion-oxon
Fensulfothion-oxon -sulfone
fensulfothion-sulfone
Fenthion
Fenthion sulfoxide
Fenthion-sulfone
Fenuron
Fenvalerate I
Fenvalerate II {CAS # 51630-58-1}
Fepropimorph
Fipronil
Fipronil, desulfinyl-
Fipronil-sulfide
Fipronil-sulfone
Flamprop-isopropyl
Flamprop-methyl
Fluacrypyrim
Fluazifop-p-butyl
Fluazinam
Fluazolate
Flubenzimine
Fluchloralin
Flucythrinate I
Flucythrinate II {CAS # 70124-77-5}
Fludioxonil
Flufenacet
Flumetralin
Flumiclorac-pentyl
Flumioxazin
Fluometuron
Fluoranthene
Fluorene
Fluorodifen
Fluoroglycofen-ethyl
Fluoroimide
Fluotrimazole
Fluoxastrobin *cis*-
Fluquinconazole
Flurenol-butyl ester
Flurenol-methylester
Fluridone
Flurochloridone I
Flurochloridone II {CAS # 61213-25-0}
Flurochloridone, deschloro-
Fluroxypyr-1-methylheptyl ester
Flurprimidol
Flurtamone
Flusilazole
Fluthiacet-methyl
Flutolanil
Flutriafol
Fluvalinate-tau-I
Fluvalinate-tau-II {CAS # 102851-06-9}
Folpet
Fonofos
Formothion
Fosthiazate I
Fosthiazate II {CAS # 98886-44-3}
Fuberidazole
Furalaxyl
Furathiocarb
Furilazole
Furmecyclox
Halfenprox
Haloxyfop-methyl
Heptachlor
Heptachlor epoxide isomer A
Heptachlor exo-epoxide isomer B
Heptenophos
Hexabromobenzene
Hexachlorobenzene
Hexachlorophene
Hexaconazole
Hexazinone
Hexestrol
Hydroprene
Imazalil
Imazamethabenz-methyl I
Imazamethabenz-methyl II {CAS # 81405-85-8}
Imibenconazole
Imibenconazole-desbenzyl

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Indeno[1,2,3-cd]pyrene
Indoxacarb and Dioxacarb decomposition product [Phenol, 2-(1,3-dioxolan-2-yl)-]
Ioxynil
Ioxynil octanoate
Ipconazole
Iprobenfos
Iprodione
Iprovalicarb I
Iprovalicarb II {CAS # 140923-25-7}
Irgarol
Isazophos
Isobenzan
Isobornyl thiocyanoacetate
Isocarbamide
Isocarbophos
Isodrin
Isofenphos
Isofenphos-oxon
Isomethiozin
Isoprocarb
Isopropalin
Isoprothiolane
Isoproturon
Isoxaben
Isoxadifen-ethyl
Isoxaflutole
Isoxathion
Jasmolin I
Jasmolin II
Jodfenphos
Kinoprene
Kresoxim-methyl
Lactofen
Lenacil
Leptophos
Leptophos oxon
Lindane
Linuron
Malathion
Malathion-o-analog
MCPA methyl ester
MCPA-butoxyethyl ester
MCPB methyl ester
m-Cresol
Mecarbam
Mecoprop methyl ester
Mefenacet
Mefenpyr-diethyl
Mefluidide
Menazon
Mepanipyrim
Mephosfolan
Mepronil
Metalaxyl
Metamitron
Metasystox thiol
Metazachlor
Metconazole I
Metconazole II {CAS # 125116-23-6}
Methabenzthiazuron [decomposition product]
Methacrifos
Methamidophos
Methfuroxam
Methidathion
Methiocarb
Methiocarb sulfone
Methiocarb sulfoxide
Methomyl
Methoprene I
Methoprene II {CAS # 40596-69-8}
Methoprotryne
Methoxychlor
Methoxychlor olefin
Methyl (2-naphthoxy)acetate
Methyl paraoxon
Methyl parathion
Methyl-1-naphthalene acetate
Methyldymron
Metobromuron
Metolachlor
Metolcarb
Metominostrobin (E)
Metominostrobin (Z) {CAS # 133408-50-1}
Metrafenone
Metribuzin
Mevinphos
Mirex
Molinate
Monalide
Monocrotophos
Monolinuron
Musk amberette
Musk Ketone
Musk Moskene
Musk Tibetene (Moschustibeten)
Musk xylene
Myclobutanil
N,N-Diethyl-m-toluamide
N-1-Naphthylacetamide
Naled
Naphthalene
Naphthalic anhydride
Naproanilide
Napropamide
Nicotine
Nitralin
Nitrapyrin
Nitrofen
Nitrothal-isopropyl
N-Methyl-N-1-naphthyl acetamide
Nonachlor, *cis*-
Nonachlor, *trans*-
Norflurazon
Norflurazon, desmethyl-
Nuarimol
o,p'-DDD
o,p'-DDE
o,p'-DDT
Octachlorostyrene
o-Dianisidine
o-Dichlorobenzene
Ofurace
Omethoate
o-Phenylphenol
Orbencarb
ortho-Aminoazotoluene
Oryzalin
Oxabetrinil
Oxadiazon
Oxadixyl
Oxamyl
Oxycarboxin
Oxychlordane
Oxydemeton-methyl
Oxyfluorfen

p,p'-DDD
p,p'-DDE
p,p'-DDM [*bis*(4-chlorophenyl)methane]
p,p'-DDT
p,p'-Dibromobenzophenone
p,p'-Dicofol
Paclobutrazol
Paraoxon
Parathion
PBB 52 Tetrabrombiphenyl
PBB 101
PBB 15
PBB 169 Hexabrombiphenyl
PCB 101
PCB 105
PCB 110
PCB 118
PCB 126
PCB 127
PCB 131
PCB 136
PCB 138
PCB 153
PCB 169
PCB 170
PCB 180
PCB 30
PCB 31
PCB 49
PCB 77
PCB 81
p-Dichlorobenzene
Pebulate
Penconazole
Pendimethalin
Pentachloroaniline
Pentachloroanisole
Pentachlorobenzene
Pentachloronitrobenzene
Pentachlorophenol
Pentanochlor
Permethrin I
Permethrin II {CAS # 52645-53-1}
Perthane
Phantolide
Phenamiphos
Phenanthrene
Phenanthrene-d10
Phenkapton
Phenol
Phenothiazine
Phenothrin I
Phenothrin II
Phenoxyacetic acid
Phenthoate
Phorate
Phorate sulfone
Phorate sulfoxide
Phorate-oxon
Phosalone
Phosfolan
Phosmet
Phosphamidon I
Phosphamidon II {CAS # 13171-21-6}
Phthalide
Phthalimide
Picloram methyl ester
Picolinafen
Picoxystrobin
Pindone
Piperalin
Piperonyl butoxide
Piperophos
Pirimicarb
Pirimiphos-ethyl
Pirimiphos-methyl
Plifenat
p-Nitrotoluene
Potasan
Prallethrin, *cis*-
Prallethrin, *trans*- {CAS # 23031-36-9}
Pretilachlor
Probenazole
Prochloraz
Procymidone
Prodiamine
Profenofos
Profenofos metabolite (4-Bromo-2-chlorophenol)
Profluralin
Prohydrojasmon I
Prohydrojasmon II {CAS # 158474-72-7}
Promecarb
Promecarb artifact [5-isopropyl-3-methylphenol]
Prometon
Prometryn
Propachlor
Propamocarb
Propanil
Propaphos
Propargite
Propargite metabolite [Cyclohexanol, 2-(4-tert-butylphenoxy)]
Propazine
Propetamphos
Propham
Propiconazole-I
Propiconazole-II {CAS # 60207-90-1}
Propisochlor
Propoxur
Propyzamide
Prosulfocarb
Prothioconazole-desthio
Prothiofos
Prothoate
Pyracarbolid
Pyraclofos
Pyraflufen-ethyl
Pyrazon
Pyrazophos
Pyrazoxyfen
Pyrene
Pyrethrin I
Pyrethrin II
Pyributicarb
Pyridaben
Pyridaphenthion
Pyridate
Pyridinitril
Pyrifenox I
Pyrifenox II {CAS # 88283-41-4}
Pyriftalid
Pyrimethanil
Pyrimidifen
Pyriminobac-methyl (E)
Pyriminobac-methyl (Z) {CAS # 136191-64-5}

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Pyriproxyfen
Pyroquilon
Quinalphos
Quinoclamine
Quinoxyfen
Quintozene metabolite (pentachlorophenyl methyl sulfide)
Quizalofop-ethyl
Rabenzazole
Resmethrin
Resmethrine I
Resmethrine II {CAS # 10453-86-8}
Rotenone
S,S,S-Tributylphosphorotrithioate
Schradan
Sebuthylazine
Sebuthylazine-desethyl
Secbumeton
Silafluofen
Silthiopham
Simazine
Simeconazole
Simetryn
Spirodiclofen
Spiromesifen
Spiroxamine I
Spiroxamine II {CAS # 118134-30-8}
Spiroxamine metabolite (4-tert-butylcyclo-hexanone)
Sudan I
Sudan II
Sudan Red
Sulfallate
Sulfanilamide
Sulfentrazone
Sulfotep
Sulfur (S8)
Sulprofos
Swep
Tamoxifen
TCMTB
Tebuconazole
Tebufenpyrad
Tebupirimifos
Tebutam
Tebuthiuron
Tecnazene
Tefluthrin, *cis*-
Temephos
Terbacil
Terbucarb
Terbufos
Terbufos-oxon-sulfone
Terbufos-sulfone
Terbumeton
Terbuthylazine
Terbuthylazine-desethyl
Terbutryne
Tetrachlorvinphos
Tetraconazole
Tetradifon
Tetraethylpyrophosphate (TEPP)
Tetrahydrophthalimide, *cis*-1,2,3,6-
Tetramethrin I
Tetramethrin II {CAS # 7696-12-0}
Tetrapropyl thiodiphosphate
Tetrasul
Thenylchlor
Theobromine
Thiabendazole
Thiazopyr
Thifluzamide
Thiofanox
Thiometon
Thionazin
Thymol
Tiocarbazil I
Tiocarbazil II {CAS # 36756-79-3}
Tolclofos-methyl
Tolfenpyrad
Tolylfluanid
Tolylfluanid metabolite (DMST)
Tolyltriazole [1H-Benzotriazole, 4-methyl-]
Tolyltriazole [1H-Benzotriazole, 5-methyl-]
Tonalide
Toxaphene Parlar 26
Toxaphene Parlar 50
Toxaphene Parlar 62
trans-Chlordane
Transfluthrin
Traseolide
Triadimefon
Triadimenol
Tri-allate
Triamiphos
Triapenthenol
Triazamate
Triazophos
Tributyl phosphate
Tributyl phosphorotrithioite
Trichlamide
Trichlorfon
Trichloronate
Triclopyr methyl ester
Triclosan
Triclosan-methyl
Tricresylphosphate, *meta*-
Tricresylphosphate, *ortho*-
Tricresylphosphate, *para*
Tricyclazole
Tridemorph, 4-tridecyl-
Tridiphane
Trietazine
Triethylphosphate
Trifenmorph
Trifloxystrobin
Triflumizole
Trifluralin
Triphenyl phosphate
Tris(2-butoxyethyl) phosphate
Tris(2-chloroethyl) phosphate
Tris(2-ethylhexyl) posphate
Triticonazole
Tryclopyrbutoxyethyl
Tycor (SMY 1500)
Uniconizole-P
Vamidothion
Vernolate
Vinclozolin
XMC (3,4-Dimethylphenyl N-methylcarbama
XMC (3,5-Dimethylphenyl N-methylcarbama
Zoxamide
Zoxamide decomposition product

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COMPREHENSIVE PESTICIDE SURVEILLANCE USING A SINGLE SYSTEM

MassHunter Pesticides PCDL and Workflow for GC/Q-TOF

Monitoring pesticide residues is crucial to ensuring a safe food supply. There are currently over 1000 registered pesticides; the latest Agilent GC/TQ and LC/TQ instruments can each now deliver analysis of hundreds of these compounds per run. However, comprehensive calibration of hundreds of pesticides can be time consuming and cost prohibitive, especially considering it is often necessary to create different calibrations for different matrices or sample preparation procedures.

There is therefore a strong demand for the capability to qualitatively screen for a broad array of pesticides and quickly determine whether identified pesticides are in compliance with the regulated maximum residue limits (MRLs).

Confidently use accurate mass to perform target and suspect screening

The Agilent MassHunter Pesticides Personal Compound Database and Library (PCDL) and Workflow for GC/Q-TOF provide a comprehensive pesticide surveillance solution for both quantitative and qualitative screening. This solution combines a comprehensive PCDL and detailed qualitative and quantitative screening workflows for use with the Agilent 7200 Series High Resolution Accurate Mass GC/Q-TOF to ensure laboratories meet expanding demand and productivity goals.

The Agilent MassHunter Pesticides PCDL and Workflow for GC/Q-TOF includes:

- Easy adoption and start-up of dual qualitative and quantitative workflow using the included comprehensive workflow guide, PCDL, and methods
- Expertly curated accurate mass PCDL for GC/Q-TOF with more than 850 pesticides and environmental contaminants
- Customize the PCDL exactly for your analysis—easily add or update compounds, spectra, and retention times (RTs) through the latest PCDL Manager Software
- Optimized data collection using two sets of industry standard acquisition GC methods
- Confident Retention Time Locking (RTL)-based compound identification for use with the accompanying acquisition methods
- Keep your experiments current with 3-years of free PCDL upgrades

Flexibility to perform quantification only when needed

Combining the Agilent MassHunter Pesticides PCDL and Workflow for GC/Q-TOF with the accurate mass capabilities of the 7200 Series GC/Q-TOF enables:

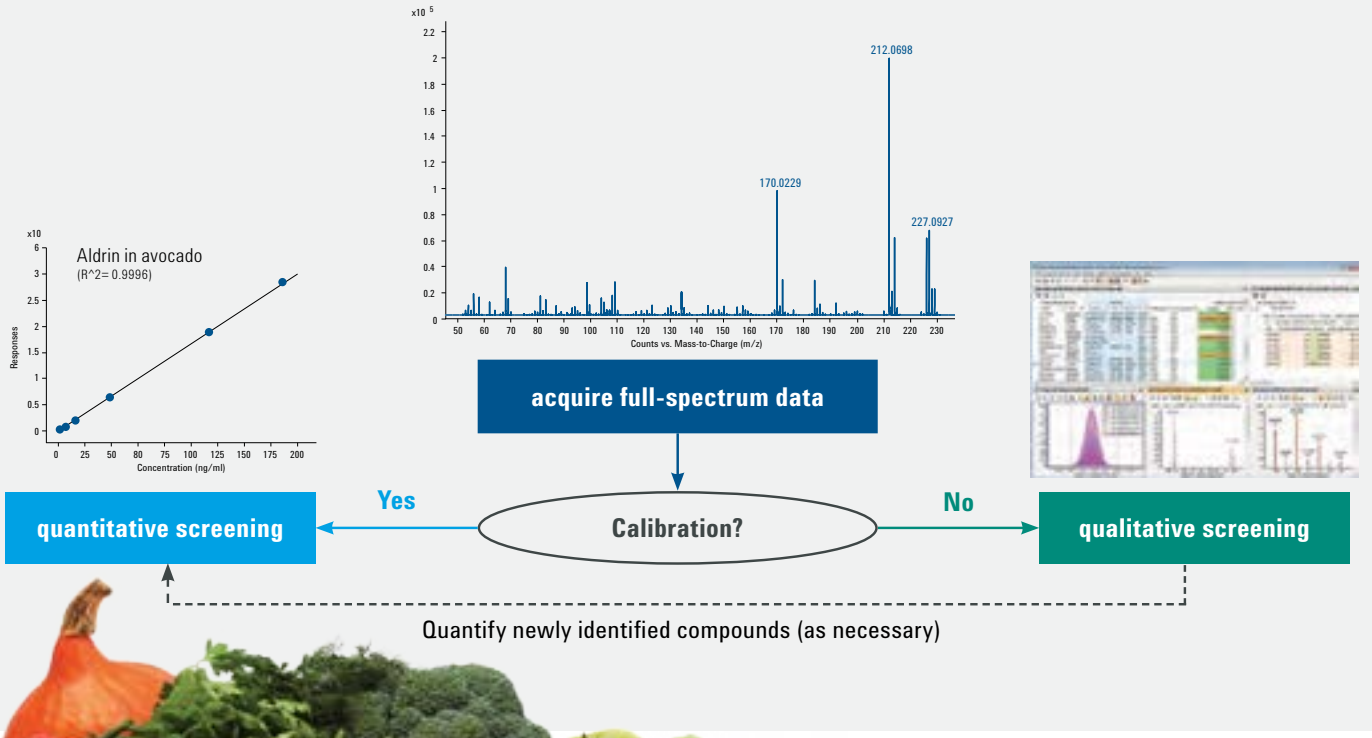
- Quantitative screening for pesticides employing either comprehensive multi-level or fast quantitation
- Qualitative (suspect) screening against the PCDL

Unrivaled confidence in identification

Find by Fragments—a MassHunter Qualitative Analysis All Ions data processing workflow—provides an automated process for identifying compounds against PCDL input. MassHunter Data Analysis software also offers a comprehensive review of screening results, assisted by delta RT, EIC alignment, fragment ratio score, and mass accuracy, to verify the compound's identification with even greater confidence.

When target compounds are available, a comprehensive multiple-level calibration curve can be generated with increased linear range, thanks to the SureMass feature in MassHunter Quantitative Analysis software. Fast quantitative screening, using a one- or two-level calibration, can be employed for rapid estimation of whether a broad range of pesticides are in compliance with certain MRLs.

Workflow for quantitative and qualitative screening



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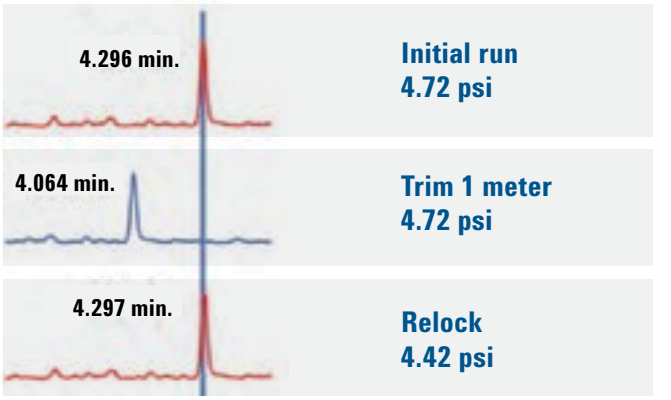
Developed in collaboration with leading pesticides laboratories

Transform your results from acceptable to exceptional using expert-developed acquisition methods and the MassHunter Pesticides PCDL and Workflow for GC/Q-TOF:

- Identify compounds through accurate mass, retention time, isotope pattern, and fragment confirmation
- Readjustment of RT in the library is never required—RTL ensures that the method always delivers the same RT regardless of column age, maintenance, or replacement
- 20-minute acquisition method with shorter cycle times for higher-throughput requirements
- 40-minute acquisition method when enhanced chromatographic separation is required
- Unique capillary flow technology enables backflushing, which extends column life and reduces run time through removal of late-eluting matrix components
- Add your own compounds and library spectra using the MassHunter Qualitative Data Acquisition auto-curate workflow to create PCDLs specific to your analyses
- Perform retrospective data analysis using newly added PCDL compounds—without the need to re-run samples



Auto RTL tuning following inlet or column maintenance enables your laboratory to start analyzing samples faster.

MassHunter Quantitative Analysis result and data review

Ordering information

The MassHunter Pesticides PCDL and Workflow for GC/Q-TOF can be purchased with a new instrument order or as an add-on to an existing 7200 Series GC/Q-TOF.

Description	Part Number
MassHunter Pesticides PCDL and Workflow for GC/Q-TOF	G3892AA

A full portfolio of customized products, services, and support

Sample preparation for the most challenging food matrices
Matrix effects are of increasing concern, especially given the number of pesticides being monitored and their low detection limits. The innovative Bond Elut Enhanced Matrix Removal-Lipid, a new addition to Agilent's comprehensive QuEChERS products, delivers the most complete matrix removal and analyte recovery of any sample prep product.

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5991-7837EN

Agilent Technologies

GC/Q-TOF MS Surveillance of Pesticides in Food

A Combined Workflow for Quantitative and Qualitative Screening of Pesticides using the Agilent MassHunter GC/Q-TOF Pesticide Personal Compound Database and Library

Application Note

Food Safety

Authors

Kai Chen, Sofia Nieto, and Joan Stevens

Agilent Technologies, Inc.

Abstract

High resolution accurate mass GC/Q-TOF mass spectrometry has become an increasingly promising technique to routinely perform both quantitative and qualitative screening for a wide range of pesticide residues in food samples with a single injection. The Agilent 7200 Series high-resolution accurate mass GC/Q-TOF, together with Agilent MassHunter Software tools, and an updated Agilent MassHunter GC/Q-TOF Pesticides Personal Compound Database and Library (PCDL) offers pesticide surveillance laboratories a combined workflow to achieve:

- Quantitative screening for pesticides whose standards will be used for calibration of response when running the analysis to perform comprehensive multilevel calibration or fast quantitation.
- Qualitative (suspect) screening against the PCDL for those pesticides whose standards will not be used when running the analysis for reasons of availability, cost, or likelihood of occurrence.

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Six different organic food extracts were prepared using the QuEChERS methods, and spiked with a mixture of 120 pesticides at multiple concentration levels (ng/mL). A midcolumn backflushing GC configuration provided excellent stability and precision of results. Six levels of matrix-matched calibration was demonstrated, with the majority of pesticides yielding a linear calibration curve fitting coefficient (R^2) of ≥ 0.99 from 5 to 200 ng/mL. Fast quantitative screening of 10 ng/mL spiking levels permitted quantification of more than 117 pesticides within a variation range of ±20 % in all food extracts. The same pesticide mixture was also used to evaluate a qualitative screening approach in which over 116 pesticides at spiking level of 10 ng/mL were identified in all studied food matrices. The intention was to show that laboratories using GC/Q-TOF for pesticide surveillance in food can flexibly choose which pesticides to quantify, and which pesticides can be screened qualitatively with a view to subsequent precise quantitation based on need.

Introduction

Monitoring pesticide residues is crucial to ensure a safe food supply. More than 1,000 pesticides are in use today, and the number continues to increase. Thus, there is a strong demand to screen a broad scope of pesticides, and determine whether residual levels of those pesticides are in compliance with the regulated maximum residue limits (MRLs). There is also increasing global emphasis on reliable validation of methods for pesticide screening as reflected by the guideline advised in the European Union (EU) through SANTE/11945/2015 [1].

For pesticides amenable to gas chromatography, triple quadrupole mass spec detection has been shown to be an effective way to perform precise quantitative screening with a wide scope of up to 400 pesticides. However, with increasing demands for a broader scope, some laboratories are questioning whether precise quantitation is required for rarely occurring pesticides. Calibration of GC/MS methods with wide scope can be time-consuming and expensive, and it is often necessary to create different calibrations for different matrices or sample prep procedures. Qualitative screening without extensive in-batch calibration is an attractive way to increase scope without increasing time and cost. If this strategy is implemented with untargeted full-spectrum detection, it can allow laboratories to look for things they previously might not have considered, or to add further compounds to the targets without extensive additional method development.

For pesticides detected in this manner, a subsequent precise quantitation will be required, and in some cases additional confirmation of identity. Either way, it makes sense to use technology that can perform a simple screen with inherently high confidence in identifications. This way, only reliable results move forward for extra work, and laboratory efficiency is kept high.

Gas chromatography coupled to high-resolution accurate mass quadrupole time-of-flight (GC/Q-TOF) MS serves as a fit-for-purpose solution to address these challenges. Benefitting from the full scan accurate mass spectra acquired for all GC-amenable pesticides, GC/Q-TOF in electron ionization (EI) mode can screen pesticides with very high identification confidence. Furthermore, high resolution data enable the use of a narrow mass window to be extracted if the accurate masses of characteristic ions from target pesticides are known. The resulting extracted ion chromatograms (EICs) from high resolution data suffer significantly less from interference by complex food matrices, and lower screening detection limits can be achieved. Therefore, a library containing accurate mass spectra of target pesticides is also essential to streamline analysis of high resolution mass spectrometry data when it comes to qualitative screening workflows.

For those compounds that a lab might still wish to quantitate on first injection, it is extremely useful to have verification of results from full scan accurate mass spectra, particularly since (unlike with triple quadrupoles) quantitation with a Q-TOF is typically performed in MS domain for the selectivity reasons explained above.

The qualitative screening of pesticides in various foodstuffs by GC/Q-TOF MS has been studied previously [2,3]. Compound identification results can be reviewed comprehensively through enhanced software compound verification features [4]. This application note looks at performance (compound by compound) for both quantitative and qualitative pesticide screening, using the Agilent 7200 Series GC/Q-TOF system, and an updated Agilent MassHunter GC/Q-TOF pesticide PCDL.

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Experimental

Reagents and standards

All pesticide standards were obtained as multiple mix stock solutions (100 mg/L of each pesticide in acetonitrile) from ULTRA Scientific (North Kingstown, RI, USA). The mixture of 120 pesticide standards contains diversified pesticide categories including carbamates, organochlorines, organophosphorus, triazoles, pyrethroids, and so forth. The standard mix solution was further diluted to appropriate concentrations in acetonitrile before being spiked into food extracts. Acetonitrile was obtained from Honeywell (Muskegon, MI, USA). Ultrapure water was produced using a Milli-Q Integral system equipped with an LC-Pak Polisher and a 0.22 µm point-of-use membrane filter cartridge (EMD Milllipore, Billerica, MA, USA).

Sample preparation

Organic apple, avocado, cucumber, peach, tomato, and salmon were obtained from a local grocery store. Ten grams of homogenized food samples (except peach) were extracted based on the buffered EN 15662 method using an Agilent QuEChERS Extraction Kit (p/n 5982-5650CH). The extraction of peach sample (15 g) followed the buffered AOAC 2007.1 method using an Agilent QuEChERS Extraction Kit (p/n 5982-5755CH). The fruit and vegetable samples were cleaned up with a dedicated Agilent Bond Elut QuEChERS Dispersive Kit (p/n 5982-5058 for AOAC method, p/n 5982-5056 for EN method). To remove the high-lipid content in avocado and salmon, the extracts were cleaned up with Agilent Bond Elut EMR—Liquid tubes (p/n 5982-1010) and Polish Pouch (p/n 5982-0102) with dry steps. The final extracts of food matrices were spiked with the mix of standards (120 pesticides) at various concentrations in a range of 5–200 ng/mL. Sample solutions spiked with pesticides were subsequently analyzed by GC/Q-TOF.

Instrumental analysis

All samples were analyzed in EI full-spectrum acquisition mode using an Agilent 7890B GC system coupled to an Agilent 7200B high resolution accurate mass Q-TOF system. The instrument was configured with a midcolumn backflush setup (Figure 1). The constant flow acquisition method was retention time locked (RTL) with chlorpyrifos-methyl to 9.143 minutes. Table 1 lists the conditions and parameters of GC/Q-TOF operation.

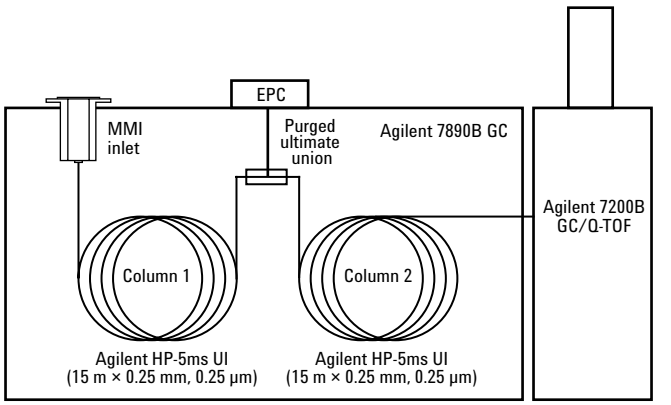

Figure 1. Agilent 7200 Series GC/Q-TOF System configuration depicting midcolumn backflush. The Agilent 7890B GC was coupled to an Agilent 7200B Q-TOF Mass Spectrometer.

Table 1. Agilent 7890B GC and Agilent 7200B GC/Q-TOF MS Conditions

GC	
Columns	Agilent HP-5ms UI, 15 m × 0.25 mm, 0.25 µm film (two each)
Carrier gas	Helium
Column 1 flow	1.0 mL/min
Column 2 flow	1.2 mL/min
Injection volume	2 µL cold splitless
Inlet liner	4 mm id Agilent Ultra Inert Liner Single Taper w wool (p/n 5190-2293)
MMI temperature program	60 °C for 0.2 minutes 600 °C/min to 300 °C, hold 330 °C, post run
Oven temperature program	60 °C for 1 minute 40 °C/min to 170 °C, 0 minutes 10 °C/min to 310 °C, 3 minutes
Run time	20.75 minutes
Backflush conditions	5 minutes (post run) 310 °C (oven temperature) 50 psi (aux EPC pressure), 2 psi (inlet pressure)
Retention time locking	Chlorpyrifos-methyl locked to 9.143 minutes
Transfer line temperature	280 °C
Q-TOF MS	
Ionization mode	EI
Source temperature	300 °C
Quadrupole temperature	180 °C
Mass range	45 to 550 *m/z*
Spectral acquisition rate	5 Hz, collecting both in centroid and profile modes
Acquisition mode	4 GHz high resolution

Data analysis

Data analysis relies on Agilent MassHunter software, Qualitative Analysis B.08 and Quantitative Analysis B.08. Agilent MassHunter GC/Q-TOF pesticide PCDL (p/n G3892AA) contains RTs, and full accurate mass EI spectra of 850+ compounds were used as input to set up data analysis. MassHunter offers an integrated workflow for pesticide screening from method development to routine implementation (Figure 2).

Results and Discussion

Quantitative screening

Evaluation of controlled sample data (for example, validation samples) helps to create quantitation methods with lowest interference. This is a necessary evaluation when developing a method to look at new food types, or when adding a new compound to a quant method, because it is difficult to predict appropriate quantifier and qualifier ions for all compounds of interest with no preknowledge of matrix background ion interferences [5]. In this study, food sample data (with pesticides spiked at 20 ng/mL) were used for this evaluation.

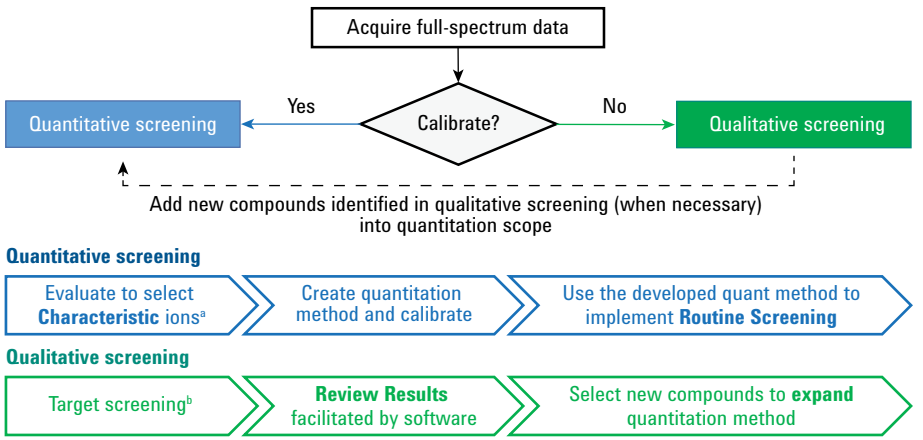

Figure 2. Workflow for quantitative and qualitative screening. [a]Evaluate is only applied to the method development stage with curated accurate mass spectra from the PCDL as an input for ion selection (a subset of compounds with standards for calibration). [b]Suspect screening against a PCDL subset including compounds without authentic standards.

Quantitative screening methods developed in this manner can be used with comprehensive multiple-level calibration, or where desired with a one or two-level calibration if only a rapid estimation on whether a broad range of pesticides is in compliance with certain MRLs. Figure 3 shows matrix-matched calibration curves of three example pesticides in peach and avocado. The matrix-matched calibrations of peach and avocado samples with pesticides spiked at 5–200 ng/mL (triplicates) yielded excellent linearity ($R^2 \geq 0.99$) for over 105 pesticides in these two complex matrices.
To evaluate the accuracy of a two-level fast quantitative screening approach, we used sample data (triplicates) with each pesticide spiked at 5 and 20 ng/mL to set up calibration and quantitate pesticides at 10 ng/mL in food extracts. Figure 4 shows the accuracy of fast qualitative screening analyses. The number of pesticides quantified at 10 ng/mL within a deviation of ±20 % exceeds 117 in all matrices, with detailed results shown in Table 2.

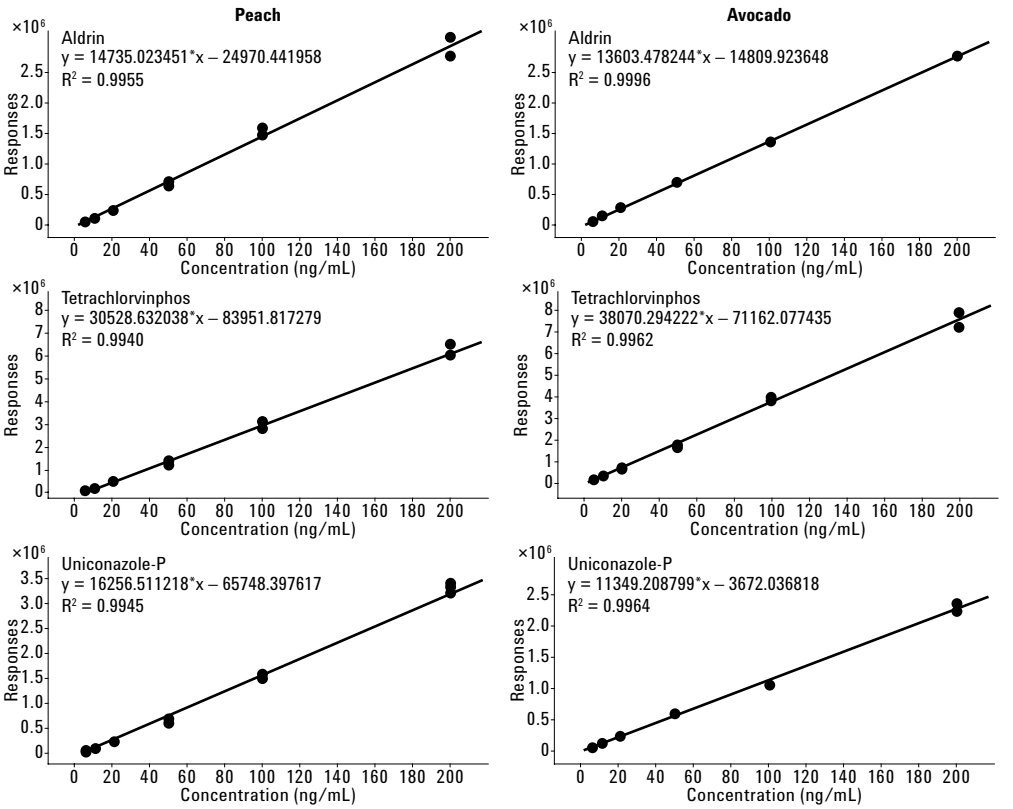

Figure 3. Matrix-matched calibration with concentrations of 5–200 ng/mL in peach and avocado.

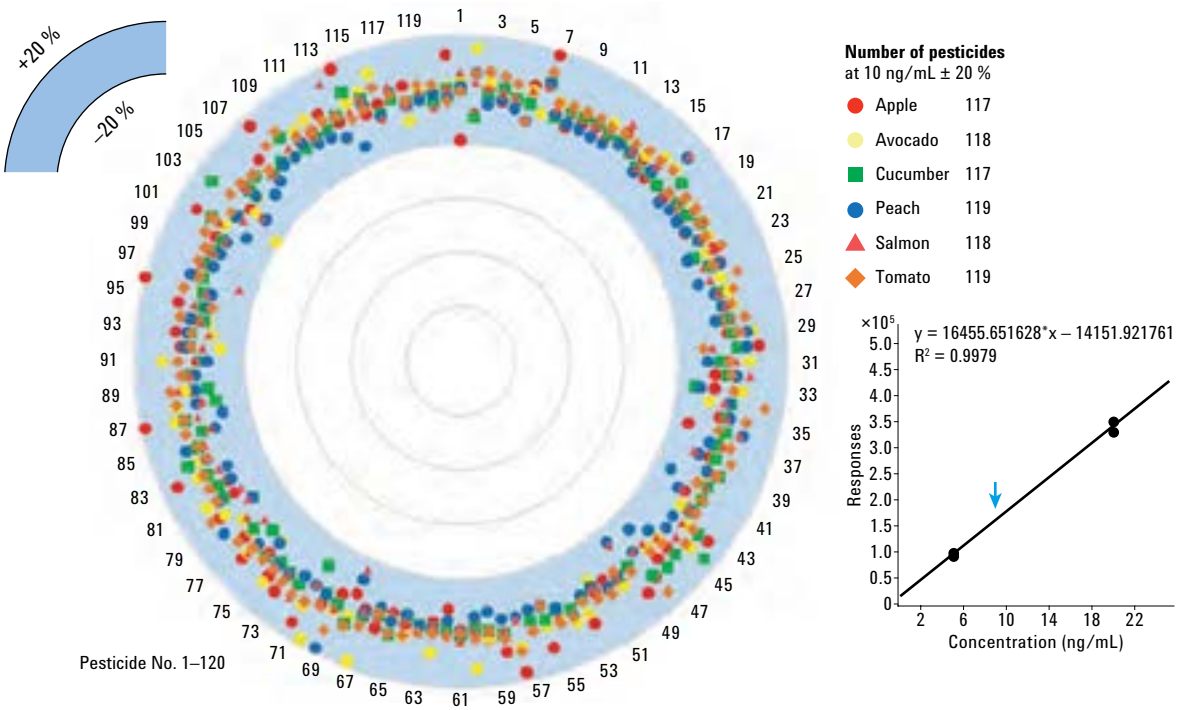

Figure 4. Fast quantitation of 10 ng/mL pesticides spiked in all food matrices. The inserted example plot shows quantitation result of cis-Permethrin in salmon based on a 2-level calibration.

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Table 2. Results of Fast Quantitative Screening and Detectability of Qualitative Screening in Food Matrices

		Apple		Avocado		Cucumber		Peach		Salmon		Tomato	
No.	Name	Quant	Qual	Quant	Qual	Quant	Qual	Quant	Qual	Quant	Qual	Quant	Qual
1	1,2-Dibromo-3-chloropropane	8.1	○ ●	10.4	○ ●	10.0	○ ●	10.1	○ ●	10.6	○ ●	9.9	○ ●
2	Acephate	9.1	○ ●	11.5	○ ●	9.0	○ ●	10.6	○ ●	10.3	○ ●	10.6	○ ●
3	Acibenzolar-S-methyl (BTH)	10.1	○ ●	10.3	○ ●	9.9	○ ●	9.5	○ ●	10.1	○ ●	10.7	○ ●
4	Alachlor	9.7	○ ●	9.8	○ ●	10.2	○ ●	9.7	○ ●	10.0	○ ●	10.6	○ ●
5	Aldrin	9.7	○ ●	9.9	○ ●	9.9	○ ●	9.5	○ ●	9.9	○ ●	10.4	○ ●
6	Azoxystrobin	10.5	○ ●	10.3	○ ●	9.8	○ ●	9.2	○ ●	10.3	○ ●	9.1	●
7	Benalaxyl	11.8	○ ●	9.6	○ ●	10.8	○ ●	10.8	○ ●	10.6		11.2	○ ●
8	Benfluralin	9.6	○ ●	10.2	○ ●	9.6	○ ●	9.7	○ ●	10.0	○ ●	10.2	○ ●
9	BHC-*alpha*	9.4	○ ●	10.1	○ ●	10.0	○ ●	9.6	○ ●	10.1	○ ●	10.3	○ ●
10	BHC-*beta*	9.8	○ ●	10.2	○ ●	10.1	○ ●	9.6	○ ●	10.2	○ ●	10.3	○ ●
11	BHC-*delta*	10.0	○ ●	10.1	○ ●	10.1	○ ●	9.6	○ ●	10.0	○ ●	10.3	○ ●
12	Lindane	9.7	○ ●	10.4	○ ●	10.0	○ ●	9.5	○ ●	10.1	○ ●	10.2	○ ●
13	Bromacil	10.4	○ ●	10.1	○ ●	10.0	○ ●	9.9	○ ●	10.8	○ ●	10.3	○ ●
14	Bromophos	9.8	○ ●	10.2	○ ●	9.9	○ ●	10.0	○ ●	10.0	○ ●	10.3	○ ●
15	Butralin	10.0	○ ●	10.2	○ ●	9.6	○ ●	9.4	○ ●	9.7	○ ●	10.4	○ ●
16	Cadusafos	9.6	○ ●	10.6	○ ●	9.8	○ ●	9.9	○ ●	10.2	○ ●	10.4	○ ●
17	Carbofuran	9.7	○ ●	10.6	○	9.7	○ ●	11.2	○ ●	11.3	○ ●	10.5	○ ●
18	Chlorantraniliprole	9.5	○ ●	9.3	○ ●	10.4	○ ●	9.3	○ ●	10.0	●	9.8	○ ●
19	Chlordane-*cis*	9.7	○ ●	10.2	○ ●	9.9	○ ●	9.5	○ ●	10.0	○ ●	10.3	○ ●
20	Chlordane-*trans*	9.6	○ ●	10.2	○ ●	9.9	○ ●	9.4	○ ●	10.2	○ ●	10.3	○ ●
21	Chlordimeform	9.5	○ ●	10.3	○ ●	10.2	○ ●	9.6	○ ●	10.4	○ ●	10.1	○ ●
22	Chlorfenvinphos	9.9	○ ●	10.2	○ ●	9.8	○ ●	9.8	○ ●	9.9	○ ●	10.1	○ ●
23	Chlornitofen	10.2	○ ●	9.9	○ ●	9.4	○ ●	9.1	○ ●	10.1	○ ●	10.5	○ ●
24	Chlorobenzilate	10.2	○ ●	9.8	○ ●	9.8	○ ●	10.2	○ ●	9.6	○ ●	10.0	○ ●
25	Chlorothalonil	>12.0	○ ●	10.0	●	<8.0	○ ●	9.2	○ ●	9.7	●	10.7	○ ●
26	Chlorpyrifos	10.0	○ ●	10.2	○ ●	9.7	○ ●	9.7	○ ●	9.8	○ ●	10.4	○ ●
27	Chlorpyrifos-methyl	9.9	○ ●	10.2	○ ●	9.8	○ ●	9.8	○ ●	10.0	○ ●	10.2	○ ●
28	DCPA	9.8	○ ●	9.9	○ ●	10.0	○ ●	9.5	○ ●	10.1	○ ●	10.6	○ ●
29	Clomazone	9.8	○ ●	10.8	○ ●	10.0	○ ●	10.6	○ ●	10.5	○ ●	10.3	○ ●
30	Deltamethrin	11.0	○ ●	10.3	○ ●	10.2	●	10.5	○ ●	9.3	○ ●	9.0	●
31	Demeton-O	9.7	○ ●	10.6	○ ●	9.6	○ ●	9.1	○ ●	10.2	○ ●	9.9	○ ●
32	Demeton-S	9.4	○ ●	9.9	○ ●	9.8	○ ●	10.2	○ ●	10.7	○ ●	10.0	○ ●
33	Demeton-S-methyl	9.4	○ ●	10.4	○ ●	8.7	○ ●	8.9	○ ●	10.1	○ ●	10.1	○ ●

Quant – fast quantitation result of each pesticide at 10 (ng/mL). Average of triplicate injections is presented.

Qual – detectability by automated compound identification in qualitative screening.

○ = pesticide identified at 5 (ng/mL) spiking level

● = pesticide identified at 10 (ng/mL) spiking level

Blank cell = not detected

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Table 2. Results of Fast Quantitative Screening and Detectability of Qualitative Screening in Food Matrices (Continued)

		Apple		Avocado		Cucumber		Peach		Salmon		Tomato	
No.	**Name**	**Quant**	**Qual**	**Quant**	**Qual**	**Quant**	**Qual**	**Quant**	**Qual**	**Quant**	**Qual**	**Quant**	**Qual**
34	Demeton-S-methylsulfone	>12.0	○ ●	9.3	●	10.1	○	10.0	○ ●	9.8	○ ●	11.3	○ ●
35	Diazinon	9.7	○ ●	10.1	○ ●	10.0	○ ●	10.1	○ ●	10.0	○ ●	10.8	○ ●
36	Dichlorvos	8.7	○ ●	10.1	○ ●	10.1	○ ●	10.7	○ ●	10.6	○ ●	9.8	○ ●
37	Dicloran (Dichloran)	9.9	○ ●	10.1	○ ●	9.9	○ ●	9.6	○ ●	10.2	○ ●	10.5	○ ●
38	Dieldrin	9.8	○ ●	10.0	○ ●	10.0	○ ●	9.4	○ ●	9.2	○ ●	10.6	○ ●
39	Dimethoate	9.9	○ ●	10.1	○ ●	10.4	○ ●	9.9	○ ●	10.2	○ ●	10.4	○ ●
40	Dimethomorph (E)	10.1	○ ●	9.9	○ ●	10.2	○ ●	8.7	○ ●	10.1	○ ●	9.8	○ ●
41	Diphenamid	9.5	○ ●	10.1	○ ●	10.2	○ ●	9.2	○ ●	9.4	○ ●	10.3	○ ●
42	Disulfoton	9.8	○ ●	9.7	○ ●	10.1	○ ●	10.2	○ ●	10.0	○ ●	10.3	○ ●
43	Disulfoton-sulfone	11.2	○ ●	10.3	○ ●	10.8	○ ●	9.6	○ ●	10.1	○ ●	10.6	○ ●
44	Endosulfan (*alpha* isomer)	10.8	○ ●	10.8	○ ●	11.5	○ ●	9.7	○ ●	10.2	○ ●	10.6	○ ●
45	Endosulfan (*beta* isomer)	10.2	○ ●	9.7	○ ●	10.6	○ ●	9.3	○ ●	9.7	○ ●	10.0	○ ●
46	Endosulfan sulfate	10.2	○ ●	9.7	○ ●	10.7	○ ●	8.7	○ ●	9.4	○ ●	9.9	○ ●
47	Endrin	10.7	○ ●	9.4	○ ●	10.6	○ ●	9.4	○ ●	9.5	○ ●	11.4	○ ●
48	EPN (Tsumaphos)	11.0	○ ●	9.7	○ ●	9.7	○ ●	8.5	○ ●	8.7	○ ●	10.0	○ ●
49	Ethion	10.0	○ ●	10.2	○ ●	9.7	○ ●	9.7	○ ●	9.7	○ ●	9.5	○ ●
50	Ethoprophos (Ethoprop)	9.6	○ ●	10.1	○ ●	9.9	○ ●	9.9	○ ●	10.0	○ ●	10.2	○ ●
51	Fenamiphos	9.8	○ ●	9.3	○ ●	9.5	○ ●	9.6	○ ●	9.7	○ ●	10.1	○ ●
52	Fenamiphos-sulfone	10.8	○ ●	10.4	○ ●	9.8	○ ●	9.6	○ ●			9.9	○ ●
53	Fenchlorphos (Ronnel)	10.0	○ ●	10.5	○ ●	9.9	○ ●	10.2	○ ●	10.1	○ ●	10.3	○ ●
54	Fenitrothion	10.1	○ ●	10.1	○ ●	9.7	○ ●	9.7	○ ●	10.1	○ ●	10.3	○ ●
55	Fenvalerate	11.1	●	10.2	○ ●	10.0	○ ●	9.4	●	9.3	●	9.5	○ ●
56	Fonofos	9.7	○ ●	9.6	○ ●	9.8	○ ●	9.4	○ ●	10.2	○ ●	10.3	○ ●
57	Formothion	11.7	○ ●	10.7	○ ●	<8.0	○ ●	9.6	○ ●	9.7	○ ●	10.9	○ ●
58	Heptachlor	10.8	○ ●	10.1	○ ●	10.1	○ ●	9.6	○ ●	10.0	○ ●	10.4	○ ●
59	heptachlor endo-epoxide isomer A	10.2	○ ●	10.2	○ ●	9.9	○ ●	9.2	○ ●	10.0	○ ●	10.0	○ ●
60	Heptachlor exo-epoxide isomer B	9.6	○ ●	11.3	○ ●	9.8	○ ●	9.4	○ ●	9.9	○ ●	10.2	○ ●
61	Heptenophos	9.9	○ ●	10.2	○ ●	10.1	○ ●	10.0	○ ●	10.3	○ ●	10.0	○ ●
62	HCB	9.1	○ ●	9.8	○ ●	9.8	○ ●	9.5	○ ●	10.0	○ ●	10.1	○ ●
63	Iprobenfos	9.6	○ ●	10.8	○ ●	9.9	○ ●	9.9	○ ●	9.7	○ ●	10.1	○ ●
64	Isazofos (Miral)	10.0	○ ●	10.0	○ ●	9.8	○ ●	9.8	○ ●	10.1	○ ●	10.1	○ ●
65	Isopropalin	9.9	○ ●	10.3	○ ●	9.6	○ ●	9.5	○ ●	9.8	○ ●	10.3	○ ●

Quant – fast quantitation result of each pesticide at 10 (ng/mL). Average of triplicate injections is presented.

Qual – detectability by automated compound identification in qualitative screening.

○ = pesticide identified at 5 (ng/mL) spiking level

● = pesticide identified at 10 (ng/mL) spiking level

Blank cell = not detected

Table 2. Results of Fast Quantitative Screening and Detectability of Qualitative Screening in Food Matrices (Continued)

		Apple		Avocado		Cucumber		Peach		Salmon		Tomato	
No.	**Name**	**Quant**	**Qual**	**Quant**	**Qual**	**Quant**	**Qual**	**Quant**	**Qual**	**Quant**	**Qual**	**Quant**	**Qual**
66	Isoprothiolane	9.6	○ ●	9.8	○ ●	10.4	○ ●	9.9	○ ●	9.5	○ ●	10.4	○ ●
67	Leptophos	10.3	○ ●	9.5	○ ●	9.8	○ ●	9.5	○ ●	10.0	○ ●	9.7	○ ●
68	Malathion	9.7	○ ●	11.8	○ ●	10.0	○ ●	9.9	○ ●	9.9	○ ●	10.4	○ ●
69	Metalaxyl	9.4	○ ●	10.2	○ ●	10.6	○ ●	8.8	○ ●	8.4	○ ●	10.6	○ ●
70	Methamidophos	9.6	○ ●	10.2	○ ●	10.0	○ ●	11.8	○ ●	10.2	○ ●	11.1	○ ●
71	Methidathion	10.2	○ ●	11.8	○ ●	9.9	○ ●	9.9	○ ●	10.2	○ ●	10.4	○ ●
72	Methiocarb	11.4	○ ●	10.7	○ ●	8.9	○ ●	9.8	○ ●	9.7	○ ●	10.5	○ ●
73	Metolachlor	10.1	○ ●	10.1	○ ●	10.1	○ ●	9.7	○ ●	10.7	○ ●	10.6	○ ●
74	Mevinphos	10.9	○ ●	10.2	○ ●	10.0	○ ●	10.1	○ ●	10.1	○ ●	10.5	○ ●
75	Mexacarbate	10.6	○ ●	10.9	○ ●	9.9	○ ●	10.1	○ ●	10.3	○ ●	10.3	○ ●
76	Mirex	9.9	○ ●	9.3	○ ●	10.2	○ ●	9.4	○ ●	10.0	○ ●	10.3	○ ●
77	Monocrotophos	10.8	○ ●	10.6	○ ●	9.3	○ ●	10.0	○ ●	10.5	○ ●	10.2	○ ●
78	Myclobutanil	10.2	○ ●	9.9	○ ●	9.8	○ ●	>12.0	○ ●	9.2	○ ●	10.5	○ ●
79	Naled	>12.0	○ ●	9.9	○ ●			10.8		9.7	●		
80	Nitrofen	10.6	○ ●	10.3	○ ●	9.1	○ ●	9.2	○ ●	9.3	○ ●	10.9	○ ●
81	*o,p′*-DDD	9.6	○ ●	10.8	○ ●	10.1	○ ●	10.3	○ ●	9.3	○ ●	10.3	○ ●
82	*o,p′*-DDE	9.6	○ ●	10.2	○ ●	10.0	○ ●	9.5	○ ●	10.3	○ ●	10.3	○ ●
83	*o,p′*-DDT	11.4	○ ●	10.6	○ ●	10.2	○ ●	9.4	○ ●	9.7	○ ●	10.3	○ ●
84	Omethoate	10.8	○ ●	10.3	○ ●	10.8	○ ●	10.0	○ ●	10.0	○ ●	9.9	○ ●
85	*p,p′*-DDD	10.3	○ ●	10.6	○ ●	10.2	○ ●	10.6	○ ●	9.9	○ ●	10.1	○ ●
86	*p,p′*-DDE	9.8	○ ●	9.8	○ ●	9.9	○ ●	9.4	○ ●	9.5	○ ●	10.5	○ ●
87	*p,p′*-DDT	11.9	○ ●	10.7	○ ●	10.4	○ ●	9.0	○ ●	9.9	○ ●	10.4	○ ●
88	Parathion	9.8	○ ●	10.6	○ ●	9.1	○ ●	9.9	○ ●	9.6	○ ●	10.6	○ ●
89	Parathion-methyl	10.0	○ ●	10.2	○ ●	9.3	○ ●	9.8	○ ●	10.4	○ ●	10.7	○ ●
90	Penconazole	9.8	○ ●	10.0	○ ●	9.9	○ ●	9.8	○ ●	10.0	○ ●	10.3	○ ●
91	Pendimethalin	9.7	○ ●	11.0		9.6	○ ●	9.3	○ ●	9.7	○ ●	10.4	○ ●
92	Permethrin, *cis*-	10.3	○ ●	8.9	●	10.0	○ ●	10.1	○ ●	9.5	●	9.9	○ ●
93	Permethrin, *trans*-	10.5	○ ●	9.2	●	10.0	○ ●	10.1	○ ●	9.3	○ ●	9.9	○ ●
94	Phorate	10.0	○ ●	9.4	○ ●	9.6	○ ●	10.1	○ ●	9.6	○ ●	10.4	○ ●
95	Phosalone	10.6	○ ●	9.4	○ ●	9.7	○ ●	9.5	○ ●	10.0	○ ●	9.3	○ ●
96	Phosphamidon	12.0	○ ●	9.9	●	9.8	○ ●	9.5	○ ●	9.7	○ ●	10.5	○ ●
97	Piperonyl butoxide	10.3	○ ●	10.0	○ ●	10.0	○ ●	10.5	○ ●	8.6		10.1	○ ●
98	Pirimicarb	9.9	○ ●	9.8	○ ●	10.1	○ ●	9.7	○ ●	10.2	○ ●	10.3	○ ●

Quant – fast quantitation result of each pesticide at 10 (ng/mL). Average of triplicate injections is presented.

Qual – detectability by automated compound identification in qualitative screening.

○ = pesticide identified at 5 (ng/mL) spiking level

● = pesticide identified at 10 (ng/mL) spiking level

Blank cell = not detected

Table 2. Results of Fast Quantitative Screening and Detectability of Qualitative Screening in Food Matrices (Continued)

		Apple		Avocado		Cucumber		Peach		Salmon		Tomato	
No.	Name	Quant	Qual	Quant	Qual	Quant	Qual	Quant	Qual	Quant	Qual	Quant	Qual
99	Pirimiphos-methyl	10.0	○ ●	9.7	○ ●	9.9	○ ●	9.7	○ ●	9.9	○ ●	10.3	○ ●
100	Profenofos	10.1	○ ●	9.7	○ ●	9.8	○ ●	9.6	○ ●	9.7	○ ●	10.5	○ ●
101	Propoxur	11.2	○ ●	10.1	●	10.6	○ ●	9.1		10.2	○ ●	10.5	○ ●
102	Prothiofos	9.8	○ ●	8.1	○ ●	9.8	○ ●	9.8	○ ●	9.5	○ ●	10.1	○ ●
103	Pyrazophos	9.6	○ ●	9.3	○ ●	11.3	○ ●	8.9	○ ●	9.0	●	10.5	○ ●
104	Quinalphos	10.2	○ ●	9.6	○ ●	10.0	○ ●	9.7	○ ●	10.1	○ ●	10.4	○ ●
105	Quinomethionate	10.1	○ ●	>12	○ ●	10.2	○ ●	9.2	○ ●	10.4	○ ●	10.3	○ ●
106	Quizalofop-ethyl	10.5	○ ●	9.8	○ ●	9.7	○ ●	9.4	○ ●	9.7	○ ●	10.0	○ ●
107	Schradan (OMPA)	11.6	○ ●			9.9	○ ●	9.8	○ ●			10.6	○ ●
108	Tefluthrin	9.8	○ ●	9.8	○ ●	10.6	○ ●	9.7	○ ●	10.1	○ ●	10.9	○ ●
109	Terbufos	9.8	○ ●	10.5	○ ●	9.7	○ ●	9.9	○ ●	10.2	○ ●	10.2	○ ●
110	Terbufos sulfone	10.1	○ ●	10.6	○ ●	9.8	○ ●	9.5	○ ●	9.9	○ ●	10.1	○ ●
111	Tetrachlorvinphos	10.5	○ ●	10.2	○ ●	9.8	○ ●	9.5	○ ●	10.1	○ ●	10.2	○ ●
112	Tetradifon	9.9	○ ●	9.2	○ ●	10.2	○ ●	9.3	○ ●	11.5	○ ●	10.1	○ ●
113	Thiamethoxam	11.8	○ ●	10.4	○ ●	10.8	○ ●	8.7	○ ●	10.0	○ ●	9.7	○ ●
114	Thionazine	9.7	○ ●	10.6	○ ●	10.3	○ ●	10.1	○ ●	10.4	○ ●	9.9	○ ●
115	Triadimefon	10.0	○ ●	11.1	○ ●	9.6	○ ●	9.5	○ ●	9.4	○ ●	10.6	○ ●
116	Triadimenol	10.0	○ ●	10.2	○ ●	10.0	○ ●	10.2	○ ●	10.5	○ ●	10.1	○ ●
117	Triazophos	10.4	○ ●	9.1	○ ●	9.8	○ ●	9.7	○ ●	9.6	○ ●	9.8	○ ●
118	Trifluralin	9.7	○ ●	10.1	○ ●	9.8	○ ●	9.8	○ ●	10.1	○ ●	10.3	○ ●
119	Uniconazole-P	10.0	○ ●	10.1	○ ●	9.6	○ ●	9.9	○ ●	9.4	○ ●	10.2	○ ●
120	Vamidothion	11.3	●	10.2	○ ●	10.0		9.7	○ ●	10.0	○ ●	9.6	

Quant – fast quantitation result of each pesticide at 10 (ng/mL). Average of triplicate injections is presented.

Qual – detectability by automated compound identification in qualitative screening.

○ = pesticide identified at 5 (ng/mL) spiking level

● = pesticide identified at 10 (ng/mL) spiking level

Blank cell = not detected

Qualitative screening

Qualitative screening was set up to automatically extract up to six ions per pesticide from the PCDL, and to require at least two of these to produce EICs with a coelution score ≥70 and an S/N ≥3. If a compound passing these requirements had an RT within ±0.15 minutes, it was considered identified. The same mixture of 120 pesticides used in the quantitative assessment was used to evaluate the effectiveness of this approach.

Over 110 spiked pesticides at 5 ng/mL, and 116 in 10 ng/mL were identified in all investigated food matrices. Table 2 lists the detailed results for each pesticide. The latest Qualitative Analysis (Workflows) offers a comprehensive review of qualitative screening results, assisted by delta RT, EIC coelution, fragment ratio score, and mass accuracy to verify the compound identification with enhanced confidence. The methodology using software to review and verify the automated identification results on target analytes and unexpected compounds has been discussed elsewhere [4].

Retention time and response repeatability

The RTL backflushing capability ensured the retention time and response repeatability of the method. Six replicate injections of peach, avocado, and salmon samples spiked with 5 and 10 ng/mL pesticides were used to evaluate RT and response repeatability. The standard deviation (SD) of RT was less than 0.01 minutes for every identified pesticide. The response repeatability was demonstrated by the percentage relative standard deviation (%RSD) of identified pesticides at these low spike levels, as shown in Figure 5. Most of the pesticides yielded single digit %RSD. EICs are shown for two example compounds (Figure 5).

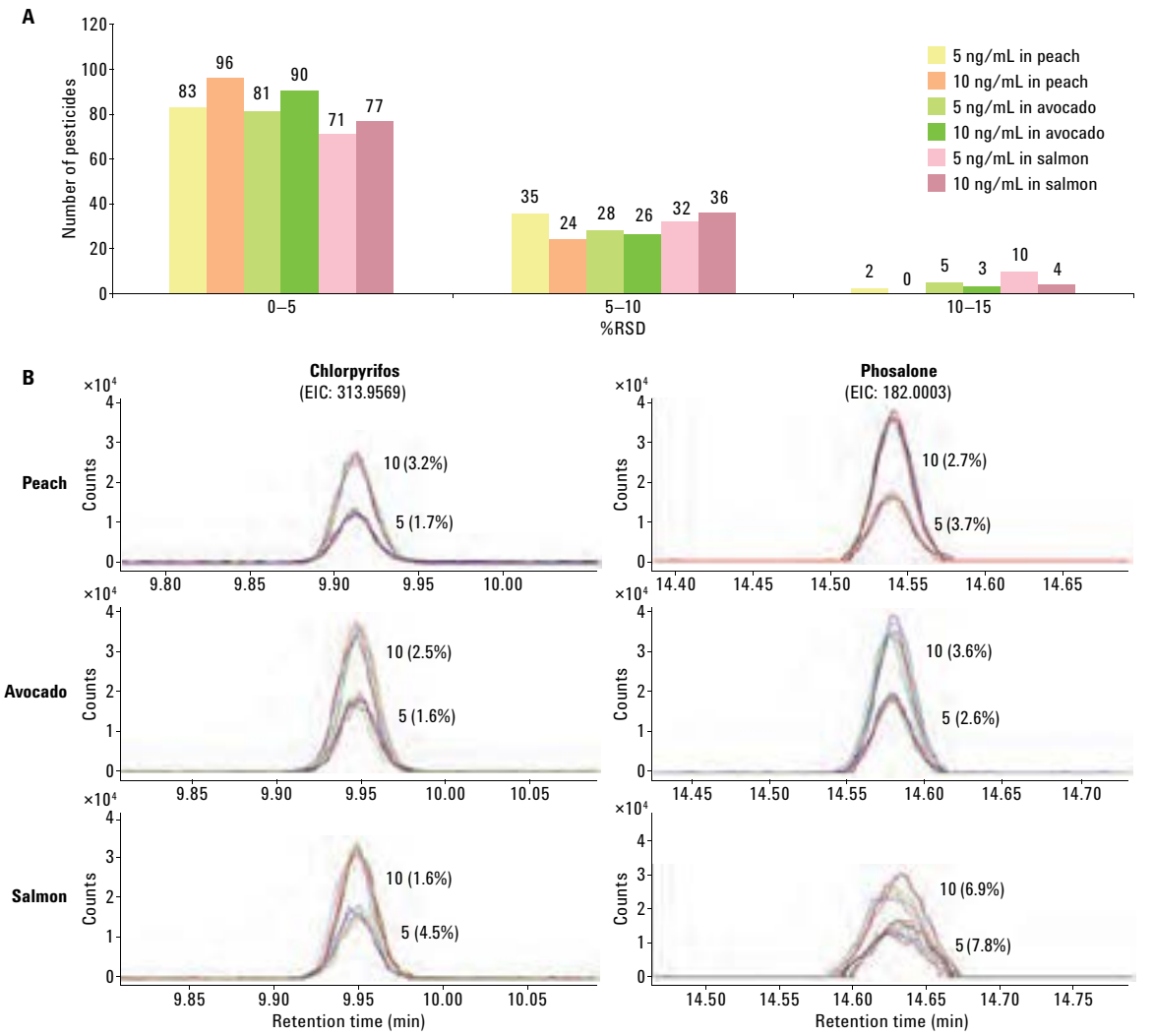

Figure 5. Response RSD% of pesticides in food matrices (A) and EICs of example compounds (B) from six replicate injections. EICs were extracted with a window of ±25 ppm. Numbers inserted in example EICs follow the format: concentration with the unit of ng/mL and (%RSD).

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Backflushing also ensures long term system stability, and this was evaluated by a sequence of alternate injections of 5 and 10 ng/mL pesticides spiked in avocado, with 36 injections. Figure 6 shows the long term response stability of five example pesticides of various categories. These compounds also span a wide RT range, from mevinphos, which eluted at 5.6 minutes to deltamethrin at 18.12 minutes.

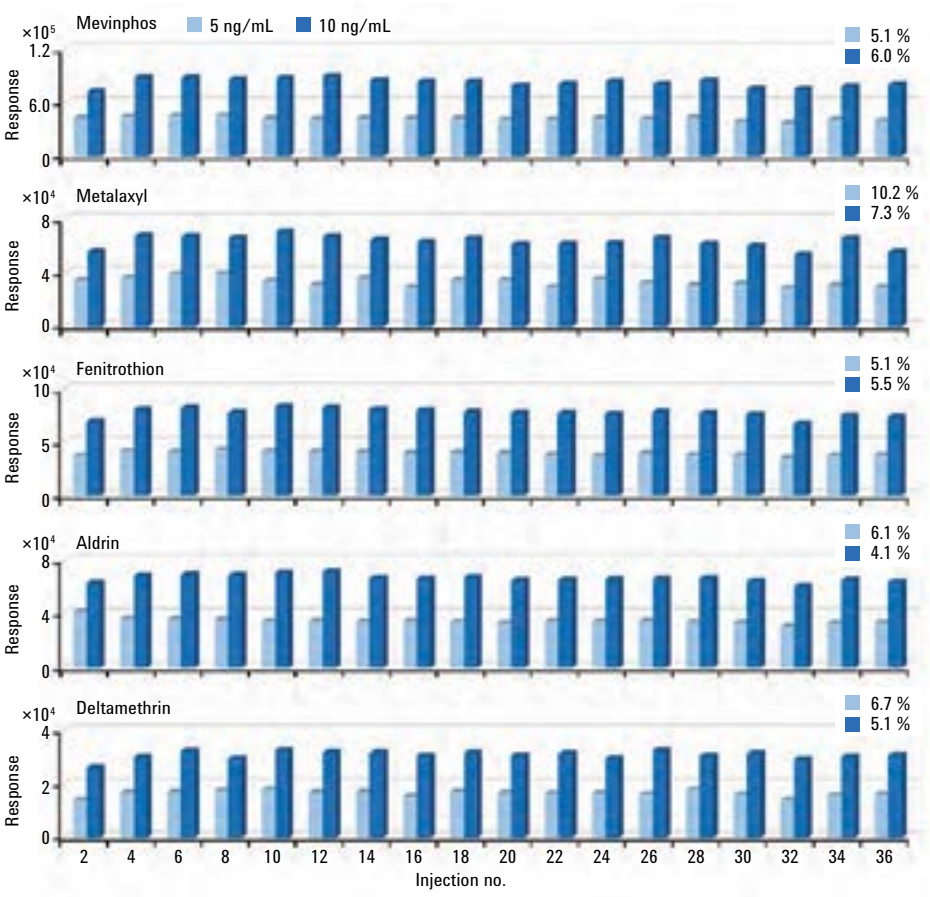

Figure 6. Long term response stability in avocado, with %RSD indicated in each plot.

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Ion ratio

The relative intensity or ratio of selective ions is an important aspect for compound identification. The EI accurate mass GC/Q-TOF spectrum of each pesticide in the PCDL offers relative abundances of ion peaks to serve as an initial reference value of ion ratio. Over 90 % of identified pesticides possessed at least one pair of identified ions with a relative ion ratio within 30 % variance to that in the corresponding library spectrum. The relative ion ratio of almost all identified pesticides deviates <30 % when it is compared to the measured spectrum using matrix-matched calibration solutions. Figure 7 illustrates the stability of ion ratio by examples from different pesticide categories.

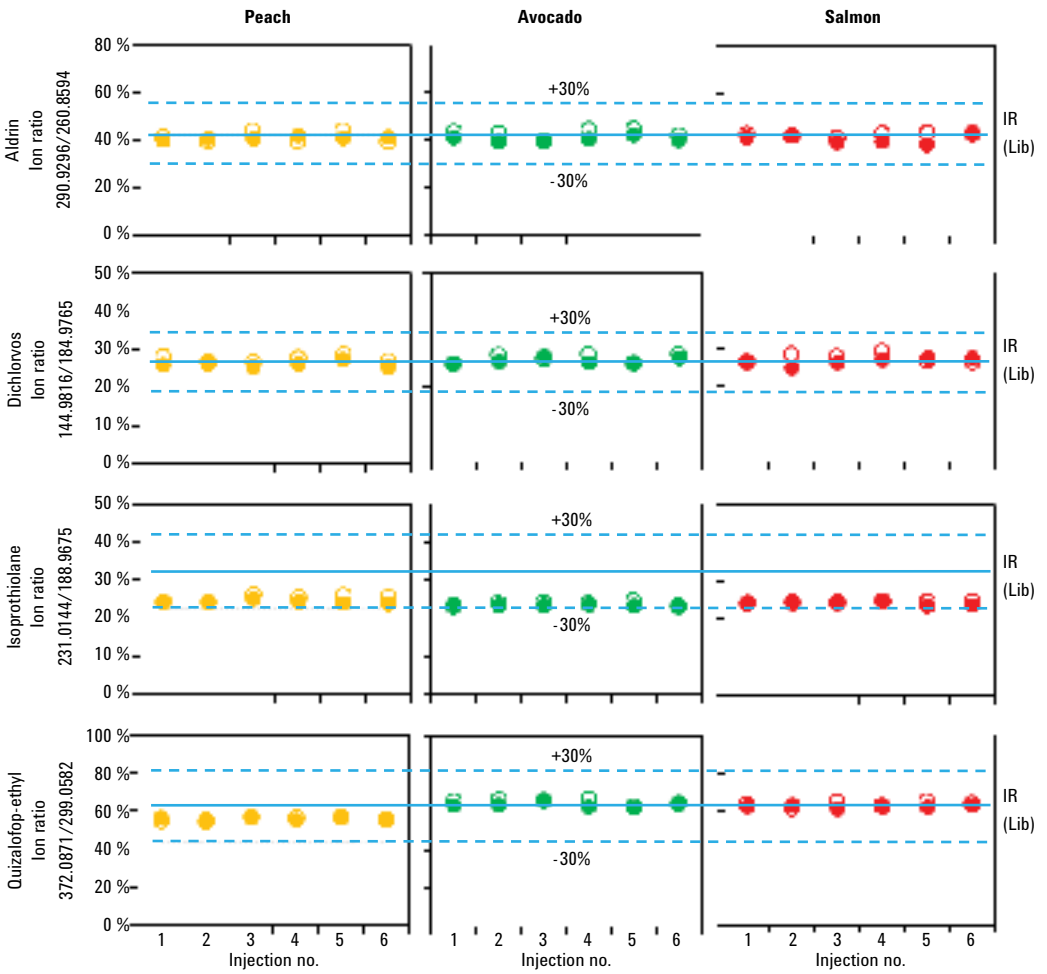

Figure 7. Ion ratio (IR) stability in food matrices.

Mass accuracy

The analysis of these pesticides by GC/Q-TOF provided excellent mass accuracy for all the investigated matrices (Table 3). The mass accuracy of each pesticide was calculated using the average spectrum extracted over its entire chromatographic peak. For those pesticides with mass accuracy >5 ppm, the majority had at least three ions identified with an S/N ≥3 for the corresponding EICs, and had relative ion ratio variance <30 % compared to their reference spectra, thus meeting identification criteria in major guidelines.

Table 3. Summary of Mass Accuracy at 10 ng/mL in Food Matrices

Matrix	Number of pesticides (mass accuracy <5 ppm)
Apple	120
Avocado	108
Peach	117
Salmon	107
Tomato	118

Conclusion

Workflows for both quantitative and qualitative screening by high resolution accurate mass GC/Q-TOF has successfully been applied to screen pesticides in diverse food matrices. This illustrates that laboratories can use flexible strategies when performing wide scope screening, mixing both quantitative and qualitative approaches depending on need.

The confidence in identification of pesticides is enhanced by stable RT, repeatable response, and excellent mass accuracy as a result of using an RTL backflush method and high resolution accurate mass measurement. An increased calibration linearity range was also achieved with a new data processing algorithm in the latest Agilent MassHunter Quantitative software (with the SureMass feature). The GC/Q-TOF system and workflow together serve as a promising fit-for-purpose solution to routinely screen for a wide scope of pesticide residues in food samples.

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GC/Q-TOF Screening of Pesticides in Food

Searching and Verification using Agilent MassHunter GC/Q-TOF Pesticide Personal Compound Database and Library

Application Note

Food Safety

Author

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Agilent Technologies, Inc.

Abstract

The use of high-resolution accurate mass GC/Q-TOF mass spectrometry is of growing interest for the development of multiresidue pesticide screening methods. The electron ionization (EI) full spectrum acquisition mode of the Agilent 7200 Series high-resolution accurate mass GC/Q-TOF system provides rich accurate mass information on fragment ions of analytes. Used with Agilent MassHunter Software tools and the updated Agilent MassHunter GC/Q-TOF Pesticides Personal Compound Database and Library (PCDL), it enables suspect screening for pesticides in complex food matrices. Coelution of extracted ions, relative abundance ratio of fragment ions, and mass error of each qualified ion are used to verify compound identifications with highest confidence. Accurate mass spectra of new pesticides of interest can also be added into a customized PCDL directly from the Agilent MassHunter Qualitative Software, allowing continuous expansion of the surveillance scheme.

Introduction

The screening of a broad scope of pesticides in food commodities is one of the most demanding applications in modern food safety laboratories. High-resolution quadrupole time of flight (Q-TOF) instruments enable the measurement of accurate mass trace contaminants for low screening detection limits and improved spectral confirmation. Using the Agilent 7200 series GC/Q-TOF system, spectra can be collected in full acquisition mode. The benefit of this untargeted approach is that it captures spectral information of all GC amenable components for more comprehensive data analysis, particularly in cases when unexpected or new contaminants emerge. Thus, the use of high-resolution GC/Q-TOF instrumentation is of growing interest for the development of multiresidue pesticide screening methods with high identification confidence [1].

The analytical method and workflow using the 7200 Series GC/Q-TOF system to screen pesticides in various foodstuffs has been previously discussed [2,3]. In that work, it was shown that a PCDL can be implemented to perform suspect screening for a large scope of pesticides using Agilent MassHunter Software. The Agilent workflow chooses the most specific ions from each compound with MS spectra in the PCDL, and extracts them from the total ion chromatogram. The qualification of hits is based on a coelution score, which determines the covariance of extracted ions in the reference retention time (RT) window. These studies demonstrated that more than 95% of a wide range of spiked pesticides at various concentrations can be identified in various food matrices.

This application note focuses on the review and verification of screening results of pesticides in food matrices using accurate mass spectral information. The enhanced Target/Suspect Screening workflow and the updated Agilent MassHunter GC/Q-TOF Pesticide Personal Compound Database and Library (PCDL) (p/n G3892AA), were used to perform suspect screening of a list of representative pesticides in a blend of food matrices. The RT difference, relative abundance ratio of characteristic fragment ions generated by electron ionization (EI), and the mass error of these ions were used to increase identification confidence. The GC/Q-TOF pesticide PCDL can be customized. This customization allows users to add new compounds into the PCDL, then add their own curated accurate mass spectra directly from Agilent MassHunter Qualitative Software.

Experimental

Instruments

This study was performed using an Agilent 7890B GC system coupled to an Agilent 7200B Q-TOF system. Figure 1 shows the instrument configuration, and Table 1 lists the instrument conditions. The GC operation enabled retention time locking (RTL) with a constant flow midcolumn backflushing full screening method.

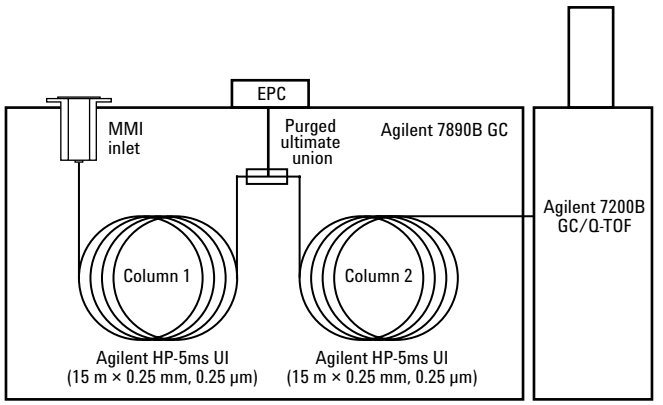

Figure 1. Agilent 7200 GC/Q-TOF System configuration depicting midcolumn backflush. The Agilent 7890B GC was coupled to an Agilent 7200B Q-TOF Mass Spectrometer.

Table 1. Agilent 7890B GC and Agilent 7200B GC/Q-TOF Mass Spectrometer Conditions

GC	
Columns	Agilent HP-5ms UI, 15 m × 0.25 mm, 0.25 µm (two each)
Carrier gas	Helium
Column 1 flow	1.0 mL/min
Column 2 flow	1.2 mL/min
Injection volume	2 µL cold splitless
Inlet liner	2 mm id Ultra Inert Dimpled (p/n 5190-2296)
MMI temperature program	60 °C for 0.2 minutes
	600 °C/min to 300 °C, hold
	330 °C, post run
Oven temperature program	60 °C for 1 minute
	40 °C/min to 170 °C, 0 minutes
	10 °C/min to 310 °C, 3 minutes
Run time	20.75 minutes
Backflush	5 minutes (post run)
Retention time locking	Chlorpyrifos-methyl locked to 9.143 minutes
Transfer line temperature	280 °C
Q-TOF MS	
Ionization mode	EI
Source temperature	300 °C
Quadrupole temperature	180 °C
Mass range	45 to 550 *m/z*
Spectral acquisition rate	5 Hz, collecting both in centroid and profile modes
Acquisition mode	4 GHz high resolution

Sample preparation

An extract of blended food matrices was used for the evaluation in this study. The food samples were obtained from local markets. Extract preparation was based on the European Standard (EN) version of the Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) method using Agilent extraction salts and disperse kits, with extraction and cleanup steps as previously outlined [2]. The extract was spiked with the Agilent Pesticide Checkout Standard Mix (p/n 5190-0494) at a concentration of 10 ng/mL, then analyzed using the GC/Q-TOF system.

Results and Discussion

Compound identification using the Find by Fragments tool

The suspect screening was performed with the latest Agilent MassHunter GC/Q-TOF Pesticide PCDL (p/n G3892AA). The PCDL contains more than 850 compounds, each with high-resolution accurate mass spectra and reference RT for the RT locked chromatographic method used in this study. Data analysis for suspect screening relies on the Find by Fragments algorithm in the MassHunter Qualitative Analysis Software (B.08.00). The software picks accurate mass ions of each compound from the PCDL EI MS spectra to extract ion chromatograms around each analyte's reference RT. One ion is chosen as the reference ion. The software then evaluates the covariance of each fragment ion by calculating a Coelution Score (value 0–100) based on the intensity ratio between the fragment ion and the reference ion across the elution time range of the reference ion. Any RT shift difference in peak width or peak symmetry (fronting, tailing) will negatively impact the coelution score. To identify each compound (indicated in software as Qualified) requires a user-defined number of other fragment ions to meet coelution criteria to the reference ion (for example, coelution score ≥70). The user can also set the maximum allowed RT deviation (for example, ≤0.1 minutes) on both sides of the reference RT in the PCDL. Table 2 shows that all 17 pesticides in the pesticide checkout standard mix could be successfully identified in the blend of food matrices, at 10 ng/mL, in all six replicate injections. The RTs of these pesticides obtained by the RTL acquisition method match the reference values well within a window of ±0.03 minutes. Thus demonstrating that, when an RTL method is used for acquisition, the reference RTs in the PCDL serve as valuable analyte identifiers, in particular for isobaric compounds that can be chromatographically separated. Each of the 17 pesticides has at least two qualified ions with mass error no greater than 5 ppm, as indicated in Table 2.

*Table 2. Summary of Compound Identification in the Blend of Food Extracts**

					Qual ion (I)		Qual ion (II)	
Name	**Formula**	**Ref RT (min)**	**ΔRT (min)**	**Qual ions**	***m/z***	**Mass diff**	***m/z***	**Mass diff**
Dichlorvos	$C_4H_7Cl_2O_4P$	4.679	0.013	6	219.9464	3.6	184.9744	2.2
Mevinphos	$C_7H_{13}O_6P$	5.610	0.005	4	192.0198	2.8	164.0233	2.8
Ethalfluralin	$C_{13}H_{14}F_3N_3O_4$	7.139	0.005	5	316.0911	1.8	292.0548	2.7
Trifluralin	$C_{13}H_{16}F_3N_3O_4$	7.247	0.003	6	306.0709	2.3	290.0755	3.3
Atrazine	$C_8H_{14}ClN_5$	7.887	0.000	6	215.0932	3.7	202.068	3.2
Chlorpyrifos-methyl	$C_7H_7Cl_3NO_3P_S$	9.143	0.002	6	287.9236	3.8	285.9267	2.3
Heptachlor	$C_{10}H_5Cl_7$	9.339	0.000	6	336.8496	2.8	271.8106	2.9
Malathion	$C_{10}H_{19}O_6PS_2$	9.729	0.003	5	124.9824	3.0	99.0077	3.6
DDE, *p,p'*-	$C_{14}H_8Cl_4$	11.612	0.006	6	317.9349	3.9	315.9375	1.9
Dieldrin	$C_{12}H_8Cl_6O$	11.717	0.005	6	276.8722	1.3	260.8595	1.1
Hexazinone	$C_{12}H_{20}N_4O_2$	13.195	0.012	5	172.0896	5.0	171.0877	1.7
Propargite	$C_{19}H_{26}O_4S$	13.318	–0.001	4	173.0955	3.9	136.0835	2.9
Mirex	$C_{10}Cl_{12}$	14.874	0.005	6	269.8127	2.1	236.8409	2.1
Fenarimol	$C_{17}H_{12}Cl_2N_2O$	15.084	0.009	5	219.0317	2.9	138.9941	2.6
Coumaphos	$C_{14}H_{16}ClO_5PS$	15.853	0.012	5	362.0139	2.6	333.9822	2.4
Etofenprox	$C_{25}H_{28}O_3$	16.777	0.019	3	164.1155	3.0	163.1124	2.0
Deltamethrin	$C_{22}H_{19}Br_2NO_3$	18.117	0.023	3	252.9053	3.8	171.9882	4.2

* ΔRT and mass error reflect average values of replicated injections (n = 6)

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Compound verification provides added confidence

An EI full spectrum acquisition high-resolution accurate mass measurement provides rich fragment ion information for each analyte. To take full advantage of this rich information for pesticide identification, the Agilent MassHunter Find by Fragments algorithm can generate a fragment ratio score based on matching the relative abundance ratios of qualified ions for each identified analyte with the reference spectra in the library. The identification confidence of each compound can be further increased by verifying the fragment ratio score, mass error of qualified ions, and the RT difference to the expected RT in the PCDL. Figure 2 shows an example where the identification of the pesticide atrazine has been verified.

The measured RT of this pesticide was 7.887 minutes, identical to the reference RT in the PCDL. The EIC plots of the fragment ions show good coelution (score 98.64), and the mass errors of all qualified ions (including both molecular and fragment ions) were <5 ppm. The characteristic ions of atrazine can clearly be distinguished in the raw spectrum, even in the presence of a complex background originating from the blend of food matrices. The fragment ratio score of over 99.62 (out of 100) indicates that the abundance ratios of all six identified ions are similar to those in the MS spectral library. The high score can be confirmed by a comparison between the reconstructed ion plot shown as part of the screening results for the food sample and the library spectrum of atrazine.

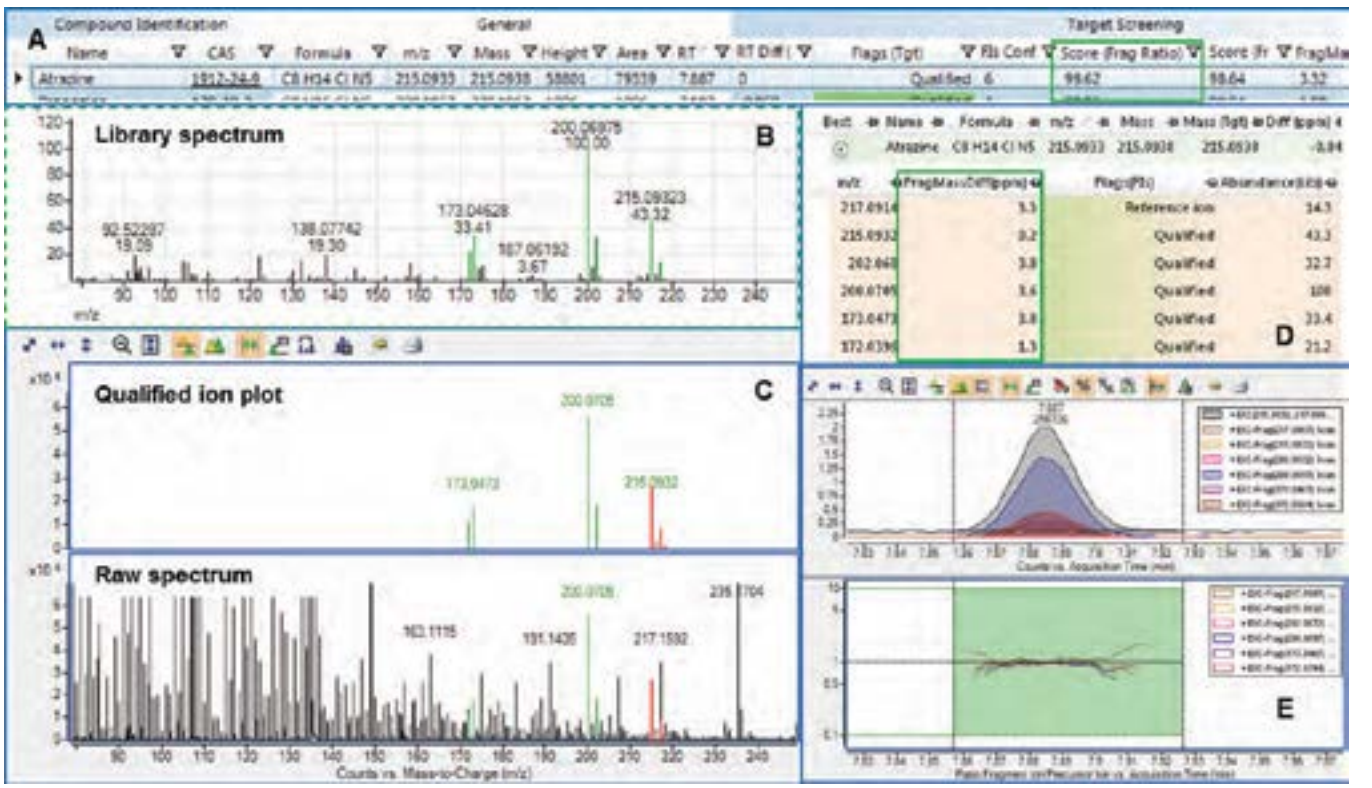

Figure 2. Identification results of atrazine were verified for increased identification confidence. The fragment ratio score of over 99 (A) reflects a similar relative abundance ratio of qualified ions between the library spectrum (B) and measured spectrum (C). The mass error of all qualified ions is <5 ppm (D), and EICs of these ions are well aligned (E).*

** Library spectra can be viewed in Agilent MassHunter PCDL Manager Software.*

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Detection of the unexpected

Another advantage of the full spectrum acquisition mode of GC/Q-TOF is that the untargeted acquisition enables the detection of unexpected compounds. The identification of unexpected compounds can be comprehensively evaluated through fragment ratio score, mass error, and RT difference for verification purposes. Figure 3 shows that the pesticide *o,p′*-DDE was identified in the food sample with a trace signal, however, it was not present in the Pesticide Checkout Standard Mix compound list. The fragment ratio score of >80, together with good EIC alignment and small mass errors of each qualified ion (< 5 ppm), confirm the presence of the pesticide *o,p′*-DDE. To understand the origin of this compound, comparison of EIC results (*m/z* ion 245.9998) from the blank matrix with samples spiked with two different concentrations of the Pesticide Checkout Standard Mix was performed. The ion at *m/z* 245.9998, when combined with the reference RT for each isomer, is a unique identifier for the presence of both *o,p′*-DDE and *p,p′*-DDE. The absence of a peak in the EIC for this characteristic ion at a RT of 11.1 and 11.6 minutes shows that both compounds, *o,p′*-DDE and *p,p′*-DDE, are not present in the blank matrix. The EIC signal at a RT of 11.6 minutes confirms that *p,p′*-DDE is, as expected, present in the pesticide standard mix. It can also be seen that the isomer *o,p′*-DDE is also present (RT of 11.1 minutes) at a much lower concentration. Furthermore, a similar response ratio (approximate 1.5%) of *o,p′*-DDE to *p,p′*-DDE was observed at both concentration levels of the Pesticide Checkout Standard Mix. This confirmed that *o,p′*-DDE is a trace impurity in the standard.

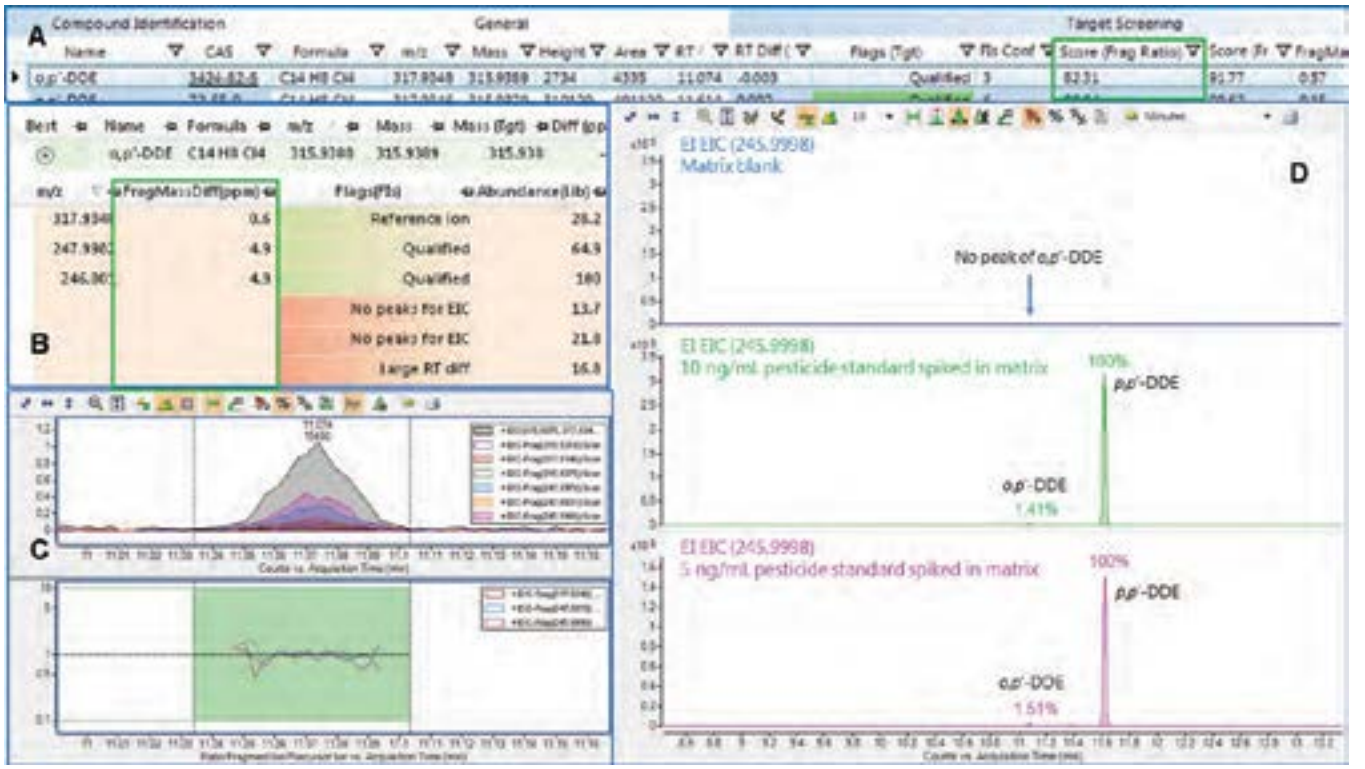

Figure 3. The unexpected peak of o,p′-DDE can be verified by fragment ratio score (A), mass error of qualified fragment ions (B) and alignment of EICs (C). No signal of o,p′-DDE was detected in a matrix blank (D top). Samples with the Pesticide Checkout Standard Mix spiked at two concentrations (D middle and bottom panels) show similar response ratios of o,p′-DDE compared to reference peak of p,p′-DDE, which confirms that the unexpected peak is a trace impurity of spiking standard.

The scalable PCDL

In full spectrum acquisition mode, the GC/Q-TOF system records the mass spectra of all GC amenable compounds present in each sample. The resulting data can be archived, then reanalyzed at a future date for any new analytes of interest. Large numbers of highly diverse pesticides are applied globally each year. Therefore, some pesticides may not be included in the current surveillance scheme or commercially available libraries. The MassHunter software provides a workflow that allows users to add curated EI GC/Q-TOF accurate mass spectra of new compounds into a custom PCDL. These new PCDL entries can also be used for retrospective analysis of historic data. During the curation process, the software determines formulas of fragment masses, and verifies if they are valid subformulas of the neutral formula of any given compound. The software then corrects the measured accurate mass values to the corresponding theoretical values. Ions for which no valid subformula can be determined will be considered as not originating from the compound, and eliminated. The curated spectra can then be sent to the designated PCDL using the Send Spectra to PCDL function as new spectral entries for the identified compound. Figure 4 illustrates the curation process of a pesticide EI GC/Q-TOF spectrum using an acquired accurate mass spectrum of cyprazine ($C_9H_{14}ClN_5$). The fragment ion mass peaks in the measured mass spectrum were automatically annotated by the software.

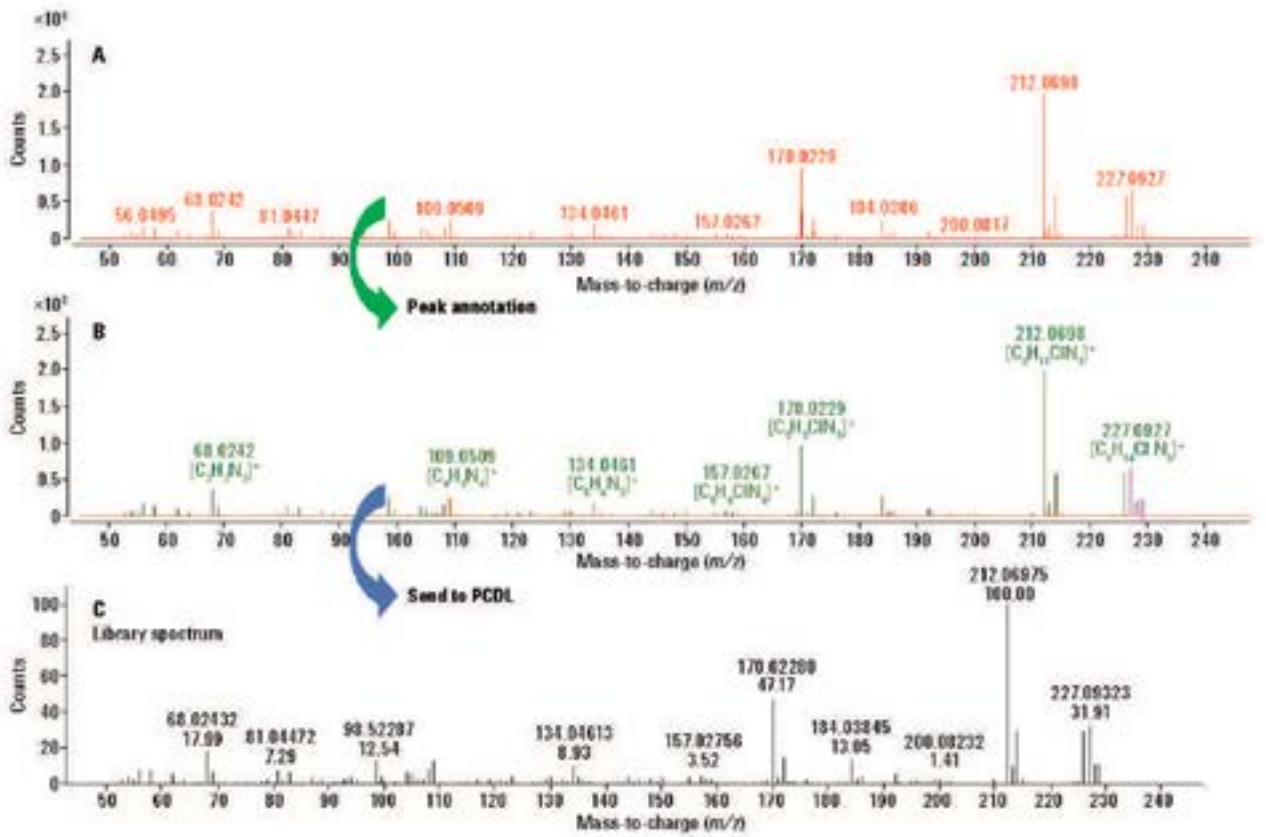

Figure 4. The MS spectrum for the pesticide cyprazine was curated and added to the PCDL using the Send Spectra to PCDL function in Agilent MassHunter Qualitative Analysis. Measured spectrum with background subtracted (A), fragment peak annotation based on its formula $C_9H_{14}ClN_5$ (B), and curated spectrum in PCDL (C) are depicted.

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Conclusion

Pesticide residues at concentrations typically specified by regulatory agencies down to 10 ng/mL equivalent in food matrix extracts can be screened using the Agilent 7200 Series GC/Q-TOF system. This shows that this is a fit-for-purpose screening solution with the EI full spectrum acquisition accurate mass Agilent MassHunter GC/Q-TOF Pesticide PCDL and Agilent MassHunter Qualitative Analysis Software. The confidence in identification of pesticides can be greatly enhanced by reviewing the fragment ratio score, mass error, and RT difference. These are available in the compound results of the Find by Fragments algorithm. The PCDL can be customized, and spectra for new compounds of interest can be curated and added to the PCDL directly from the MassHunter Qualitative Analysis Software. These new PCDL entries can be used for the expansion of the surveillance scheme. While MassHunter Qualitative Analysis can be used to build the method and conduct a qualitative screening, the compound information can also easily be transferred to Quantitative Analysis for combined calibrated quantitation and confirmation.

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1. N. Belmonte-Valles, *et al.* "Analysis of pesticide residues in fruits and vegetables using gas chromatography-high resolution time-of-flight mass spectrometry" *Anal. Methods* **7**, 2162–2171 (2015).
2. P. L. Wylie, J. Stevens, S. Nieto, Screening for More Than 740 Pesticide Residues in Food Using an Agilent GC/Q-TOF and an Exact Mass Pesticide Library". *Agilent Technologies Application Note*, publication number 5991-5633EN (**2015**).
3. A. R. Fernández-Alba; S. Uclés, J. Riener, Screening for Hundreds of Pesticide Residues Using a GC/Q-TOF with an Exact Mass Pesticide Database in Food, *Agilent Technologies Application Note*, publication number 5991-5894EN (**2015**).

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DETECT AND REPORT
TRACE-LEVEL DIOXINS AND DIOXIN-LIKE PCBs

Agilent GC/MS/MS Dioxins in Feed and Food Analyzer

Dioxin and dioxin-like PCBs are environmental pollutants and persistent organic pollutants (POPs) that originate as by-products of industrial processes such as paper bleaching, pesticide manufacturing, and waste incineration. These compounds accumulate in the food chain, mainly in the fatty tissue of animals. Humans can ingest these highly toxic compounds from eating meat, dairy, fish, and other animal products.

Regulatory agencies, particularly the European Union (EU) Commission, have imposed strict limits on dioxin levels in feed and food. Gas chromatography/tandem mass spectrometry (GC/MS/MS) has become an accepted confirmatory method for EU regulations 589/2014 and 709/2014.

Reliably detect and confirm low levels of dioxins and furans – from *day one*

The NEW Agilent GC/MS/MS Dioxins in Feed and Food Analyzer technology is based on the Agilent 7010C GC/MS/MS system. With up to *ten times* more sensitivity than competitive tandem quad analyzers, it allows you to observe ***below*** EU regulated levels of dioxins for ultimate confidence in your results.

In addition, the Analyzer software streamlines reporting by combining the results of two sample fractions: dioxins/furans and PCBs. It also automatically performs complex calculations, including individual and summed concentrations, and consolidates the data into a single report that conforms to EU regulations. This report organizes the compounds into four groups: dioxins, furans, dioxin-like PCBs, and non-dioxin-like PCBs.

The Agilent Dioxins in Feed and Food Analyzer leverages the latest GC and MS/MS innovations and reflects our focus on quality and performance and includes:

Factory
- System setup and leak testing
- Instrument checkout
- Installation of validated DB-5ms UI GC column
- Factory-run checkout method using a custom Dioxin Analyzer checkout mix

Delivery
- User's manual for running the method
- Supplemental software to automate reporting
- CD-ROM with method parameters and checkout data files for easy out-of-the-box operation
- Application-related consumables included—no separate ordering required
- Easy consumables re-ordering information

Installation
- Duplicate factory checkout with checkout sample—onsite by factory-trained support engineer
- Optional application consulting available

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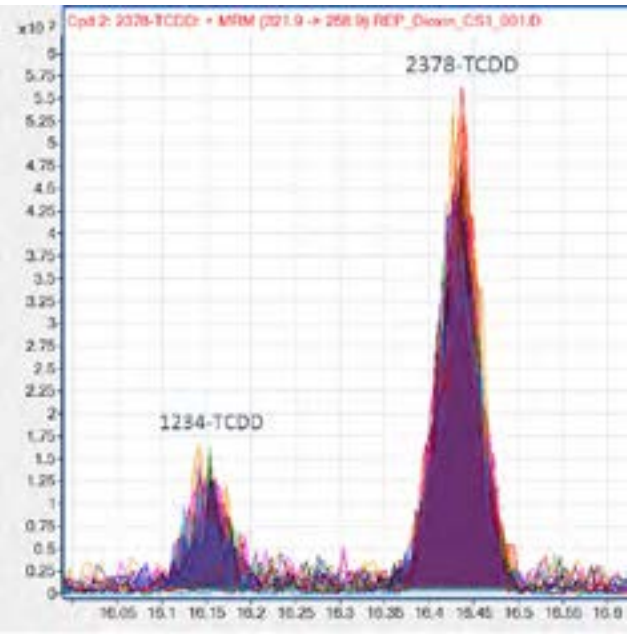
Follow strict maximum-level dioxin regulations

Built around the Agilent 7010C Triple Quadropole GC/MS, the Dioxins in Feed and Food Analyzer is configured and chemically tested for this application in the Agilent factory. This ensures that your team can reduce start-up time and deliver results quickly after delivery.

Advanced features include:

- Configured with the Multi Mode Inlet (MMI)
- The *same* GC parameters for dioxin and dioxin-like PCB fractions for easy operation and higher productivity
- Retention time locking to PCB 105
- Heated quadrupoles for improved sensitivity
- Automatic performance of complex calculations required by EU regulations
- Leading-edge reporting that combines results from dioxin/furan and PCB fractions

Excellent repeatability and femtogram-level sensitivity



Ten replicate injections of 0.05 ppb TCDD in nonane

Inlet Flexibility with The Multimode Inlet (MMI)

The MMI allows for flexibility with injection techniques and volumes. The injection modes that the MMI offers includes: hot and cold split and splitless, pulsed split and splitless, solvent vent, and direct inject mode.

In dioxin analysis, some users might be accustomed to using larger injection volumes due to its recognized benefit of signal-to-noise improvement and lower system detection limits. One significant benefit of this dioxin analyzer system, and the 7010C MS/MS, is the ability to inject and introduce a small sample volume onto the column (1 µL) and maintain the ability to quantitate these trace compounds at low concentrations. Using the Solvent Vent Mode, the user has the option of larger injection volumes.

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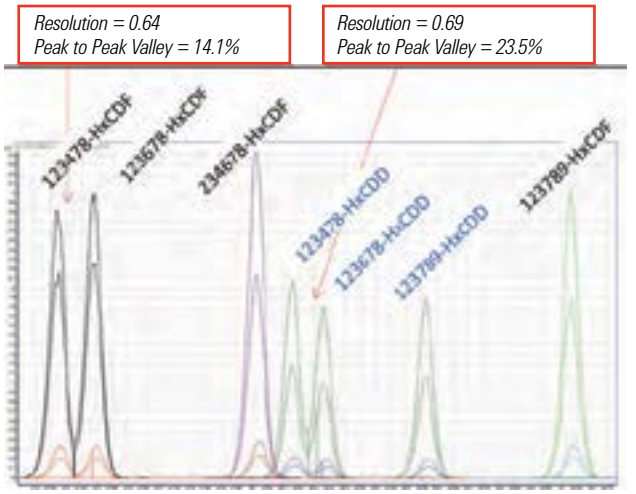
Reliable separation of hexa-PCDD/F isomers per EU regulations

The separation of PCDD/PCDFs from interfering substances and coelutions is a monitored criteria in the EU Regulations. Each congener can be confirmed and individually quantified by GC/MS/MS.

Agilent offers a factory checkout of two customized standard mixes that display the analytes found in each sample fraction (Dioxin/Furan/DL-PCB and DL/NDL-PCB) and their separations.



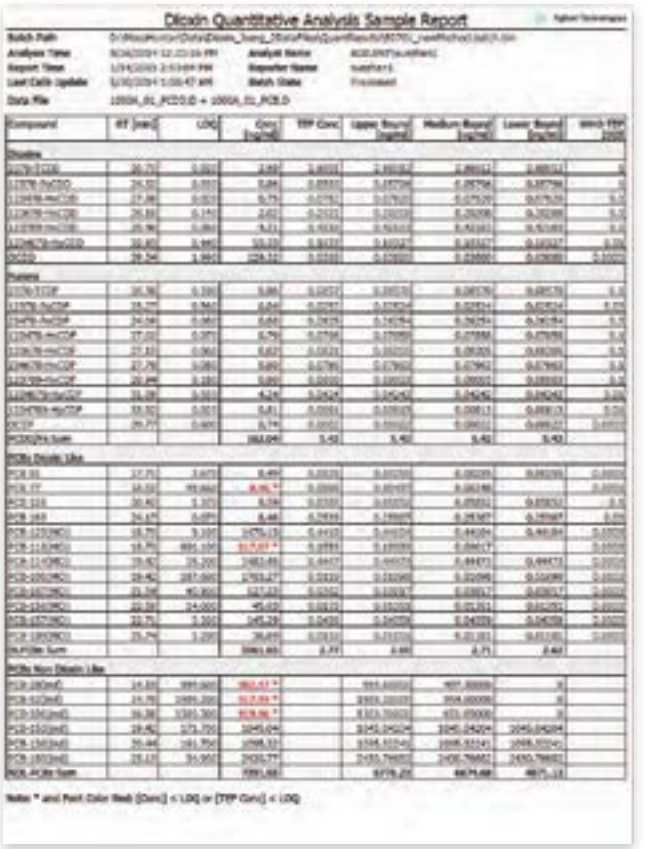
Chromatogram of the hexa-PCDD/F isomers (2 transitions for both native and C13 labeled compounds; at a mid-point calibration level) and their separations, compliant with EU regulations.

Simplify customized reporting

Data review and mandatory reporting requirements are time consuming and complicated.

The EU regulations state that the reporting of the results shall include maximum information, thereby enabling their interpretation. This includes factoring in blank subtraction, determining the limit of quantitation (LOQ), and calculating the lower/upper/medium-bound limits of the individual PCDD/PCDF and dioxin-like PCB congeners.

Agilent has developed specific software scripts and a customized report for the analysis of dioxin and dioxin-like PCBs in feed and food, in compliance with EU regulations.



The reporting software combines the measurements of dioxins, furans, and PCBs (both dioxin-like and non-dioxin-like) into one report in compliance with EU regulations.

To find out more about this analyzer, visit **www.agilent.com/chem/dioxins_analyzer**

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Greater sensitivity... not just lower noise

MS sensitivity depends on the number of ions measured. The 7010C Triple Quadrupole GC/MS system's ultra-efficient EI source maximizes the number of ions that are created and transferred out of the source body and into the quadrupole analyzer—giving you the advantages of:

- Increased response and better precision at all levels
- Lower detection limits
- More precise ion ratios and better qualitative information

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Validation of a Confirmatory GC/MS/MS Method for Dioxins and Dioxin-like PCBS to Meet the Requirements of EU Regulation 709/2014

Application Note

Food

Author

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Agilent Technologies GmbH
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Germany

Abstract

Using the Agilent 7890B GC and the Agilent 7000C Series Triple Quadrupole GC/MS System, a GC/MS/MS method has been developed and fully validated to meet the requirements of EU Regulation 709/2014 for the monitoring of polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs) in animal feedstuffs. It provides similar performance to GC/HRMS, the analytical platform required by previous EU regulations, in spite of the difference in mass analyzer technologies.

Introduction

Polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs) are highly toxic Persistent Organic Pollutants (POPs). As such, they were regulated after the Stockholm convention for POPs in 2001 to safeguard the environment and human health [1]. Many of these compounds have been linked to cancer, endocrine disruption, and reproductive disorders. They are created as byproducts of industrial processes, pesticide manufacturing, combustion processes, and other sources.

These toxic compounds are very stable in the environment, and their lipophilic nature allows them to accumulate in the fat tissues of animals. Therefore, the European Commission requires that any food or animal feedstuffs released on the market must be monitored to not exceed assigned maximum levels (MLs) for these pollutants. European regulations also require enforcement of continuous food and feed monitoring of these compounds. These regulations enable efficient reduction in human exposure over time, and decreased daily human intake of these toxic compounds [1].

Historically, high resolution mass spectrometry (HRMS) was needed to confirm and quantify trace levels of dioxins. However, as of June 2014, the European Union (EU) has instituted regulation (709/2014) governing the levels of PCDDs, PCDFs, dioxin-like PCBs, and non-dioxin-like (NDL) PCBs in food and feed that enables the use of gas chromatography/tandem mass spectrometry (GC/MS/MS) systems in confirmatory testing for compliance with EU MLs. This change was due to the realization that triple quadrupole mass spectrometers could provide performance similar to that seen with HRMS systems [2].

This application note describes a published study that validated the use of GC/triple quadrupole MS for the confirmatory analysis of dioxins and dioxin-like PCBs in vegetable oil [1]. Using the Agilent 7890GC and Agilent 7000 Triple Quadrupole GC/MS System, a method was validated that met the strict requirements for analytical criteria (for example, selectivity, accuracy, and reproducibility) set by the EU regulation. Results were similar to those that can be attained with GC/HRMS, thus providing a viable and economical alternative to the GC/HRMS approach.

Experimental

Reagents and standards

Solvents and reagents were obtained as described [1]. Quantitation of all congeners of PCDD/Fs (2,3,7,8-substituted) and non-ortho (NO-)PCBs (PCBs 81, 77, 126, and 169) was carried out using the corresponding ^{13}C-labeled internal standards (EDF-4144, Cambridge Isotope Laboratories (CIL)). Recovery standards (EDF-4145, syringe standard, CIL) were used for determination of recoveries. Calibration curve standards were also purchased from CIL for PCDD/Fs and NO-PCBs (EDF-4143). Internal standard spiking solution (MBP-MKX) of ^{13}C-labeled mono-ortho (MO-)PCBs (including PCBs 105, 114, 118, 123, 156, 157, 167, and 189) was obtained from Wellington Laboratories. The EC-4987, EC-5179, EC-4058 (CIL), and MBP-MKX standards were used to construct the calibration curve for MO-PCBs and NDL-PCBs (PCBs 28, 52, 101, 138, 153, and 180).

Instruments

This study was performed using an Agilent 7890B GC system coupled to an Agilent 7000C Series Triple Quadrupole GC/MS System. The instrument conditions are listed in Table 1. Every 10 days, calibration and tune of the instrument were repeated using the EI high sensitivity autotune mode, and instrument performance was verified.

Table 1. GC/MS Run Conditions

GC Conditions	
Column	Agilent DB-5 MS UI 60 m × 250 µm × 0.25 µm (p/n 122-5562UI) inlet PTV, outlet vacuum
Injection volume	PCDD/Fs and NO-PCBs: 5 µL MO and NDL-PCBs: 2 µL
Injection port	PTV cooled with liquid CO2
Injection port liner	Multibaffle, deactivated, PTV liner (p/n 5183-2037)
Injection mode	Solvent vent 45 °C (3 minutes), ramp at 720 °C/min to 320 °C Vent flow 100 mL/min pressure of 10 psi for 2.8 minutes Purge flow was set to 1200 mL/min after 5 minutes.
Carrier gas	Helium
Carrier gas mode	Constant flow
Column flow	0.96 mL/min
Retention time locking	PCB-105 locked to 19.66 minutes
Oven program	120 °C (5 minutes) 25 °C/min to 250 °C (5 minutes) 3 °C/min to 285 °C (15 minutes) The same program was used for the MO-PCB fraction, with the exception of 0 minutes hold at 285 °C
Total run time	41.6 minutes
MS Conditions	
Operation mode	Electron ionization (EI), Multiple Reaction Monitoring (MRM)
Transfer line temperature	280 °C
Source temperature	280 °C
Quadrupole temperature	150 °C

Multiple Reaction Monitoring (MRM) mode was used for data acquisition, with acquisition windows and dwell times adjusted to optimize acquisition frequency to obtain 10 data points for each peak. For each target, two MRM transitions were used, one for quantitation and one for qualification. The two transitions used two different and specific precursor ions (usually 2 Da offset) and two distinct product ions. Table 2 gives a full list of the analyte retention times and MRM transitions.

Quantitation was performed with the quantitative transition only, while the qualitative transition was used to verify the ion ratio between the two transitions. This procedure limited the risk of integrating wrong peaks or interferences. If the ratio did not fall between acceptable limits for Regulation 709/2014 (±15%), the chromatogram was inspected to ensure that the appropriate peaks were being integrated. This approach minimized the risk of integrating interferences or the wrong peaks. Retention time locking was employed, using PCB-105.

Sample preparation

Preparation of vegetable oil samples was performed as described [1].

Data acquisition and analysis

The data were acquired with Agilent MassHunter Acquisition Software (B.07.02). Data analysis was performed with Agilent MassHunter Quantitative Analysis Software (B.07.01).

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Table 2. Acquisition Parameters for Native PCDD, PCDF Mono-Ortho, Non-ortho and NDL-PCB Congeners, and ^{13}C-Internal Standards

			Quantifier			Qualifier		
Name	**Type**	**RT***	**Precursor ion**	**Product ion**	**CE†(V)**	**Precursor ion**	**Product ion**	**CE†(V)**
^{13}C-PCB 28	ndl-PCB	14.19	268.0	198.0	26	270.0	200.0	26
PCB 28	ndl-PCB	14.19	256.0	186.0	26	258.0	188.0	26
^{13}C-PCB 52	ndl-PCB	14.79	301.9	231.9	28	303.9	233.9	28
PCB 52	ndl-PCB	14.79	289.9	219.9	28	291.9	221.9	28
^{13}C-PCB 101	ndl-PCB	16.81	337.9	267.9	28	339.9	269.9	28
PCB 101	ndl-PCB	16.81	325.9	255.9	28	327.9	257.9	28
^{13}C-PCB 123	MO-PCB	18.62	337.9	267.9	28	339.9	269.9	28
PCB 123	MO-PCB	18.62	325.9	255.9	28	327.9	257.9	28
^{13}C-PCB 118	MO-PCB	18.74	337.9	267.9	28	339.9	269.9	28
PCB 118	MO-PCB	18.74	325.9	255.9	28	327.9	257.9	28
^{13}C-PCB 114	MO-PCB	19.12	337.9	267.9	28	339.9	269.9	28
PCB 114	MO-PCB	19.12	325.9	255.9	28	327.9	257.9	28
^{13}C-PCB 153	ndl-PCB	19.43	371.9	301.9	28	373.9	303.8	28
PCB 153	ndl-PCB	19.43	359.9	289.9	28	361.9	291.8	28
^{13}C-PCB 105	MO-PCB	19.66	337.9	267.9	28	339.9	269.9	28
PCB 105	MO-PCB	19.66	325.9	255.9	28	327.9	257.9	28
^{13}C-PCB 138	ndl-PCB	20.46	371.9	301.9	28	373.9	303.8	28
PCB 138	ndl-PCB	20.46	359.9	289.9	28	361.9	291.8	28
^{13}C-PCB 167	MO-PCB	21.56	371.9	301.9	28	373.9	303.8	28
PCB 167	MO-PCB	21.56	359.9	289.9	28	361.9	291.8	28
^{13}C-PCB 156	MO-PCB	22.51	371.9	301.9	28	373.9	303.8	28
PCB 156	MO-PCB	22.51	359.9	289.9	28	361.9	291.8	28
^{13}C-PCB 157	MO-PCB	22.71	371.9	301.9	28	373.9	303.8	28
PCB 157	MO-PCB	22.71	359.9	289.9	28	361.9	291.8	28
^{13}C-PCB 180	ndl-PCB	23.14	405.8	335.8	30	407.8	337.8	30
PCB 180	ndl-PCB	23.14	393.8	323.8	30	395.8	325.8	30
^{13}C-PCB 189	MO-PCB	25.76	405.8	335.8	30	407.8	337.8	30
PCB 189	MO-PCB	25.76	393.8	323.8	30	395.8	325.8	30
^{13}C-PCB 80	non-Ortho PCB	16.23	301.9	231.9	28	303.9	233.9	28
^{13}C-PCB 81	non-Ortho PCB	17.72	301.9	231.9	28	303.9	233.9	28
PCB 81	non-Ortho PCB	17.73	289.9	219.9	28	291.9	221.9	28
^{13}C-PCB 77	non-Ortho PCB	18.04	301.9	231.9	28	303.9	233.9	28
PCB 77	non-Ortho PCB	18.05	289.9	219.9	28	291.9	221.9	28
^{13}C-2378-TCDF	PCDF	20.32	315.9	251.9	33	317.9	253.9	33
2378-TCDF	PCDF	20.34	303.9	240.9	33	305.9	242.9	33
^{13}C6-1234-TCDD	PCDD	20.44	325.9	262.9	28	327.9	264.9	28
^{13}C-2378-TCDD	PCDD	20.74	331.9	267.9	24	333.9	269.9	24
2378-TCDD	PCDD	20.75	319.9	256.9	24	321.9	258.9	24
^{13}C-PCB 126	non-Ortho PCB	20.93	335.9	265.9	28	337.9	267.9	28
PCB 126	non-Ortho PCB	20.95	323.9	253.9	28	325.9	255.9	28
^{13}C-12378-PeCDF	PCDF	23.29	351.9	287.9	35	349.9	285.9	35
12378-PeCDF	PCDF	23.29	339.9	276.9	35	337.9	274.9	35

			Quantifier			Qualifier		
Name	Type	RT*	Precursor ion	Product ion	CE†(V)	Precursor ion	Product ion	CE†(V)
^{13}C-23478-PeCDF	PCDF	24.08	351.9	287.9	35	349.9	285.9	35
23478-PeCDF	PCDF	24.10	339.9	276.9	35	337.9	274.9	35
^{13}C-PCB 169	non-Ortho PCB	24.19	371.9	301.9	28	369.9	299.9	28
PCB 169	non-Ortho PCB	24.20	359.9	289.9	28	357.8	287.9	28
^{13}C-12378-PeCDD	PCDD	24.34	365.9	301.9	25	367.9	303.9	25
12378-PeCDD	PCDD	24.36	355.9	292.9	25	353.9	290.9	25
^{13}C-123478-HxCDF	PCDF	27.04	385.8	321.9	35	387.8	323.9	35
123478-HxCDF	PCDF	27.05	373.8	310.9	35	375.8	312.9	35
^{13}C-123678-HxCDF	PCDF	27.18	385.8	321.9	35	387.8	323.9	35
123678-HxCDF	PCDF	27.19	373.8	310.9	35	375.8	312.9	35
^{13}C-234678-HxCDF	PCDF	27.83	385.8	321.9	35	387.8	323.9	35
234678-HxCDF	PCDF	27.85	373.8	310.9	35	375.8	312.9	35
^{13}C-123478-HxCDD	PCDD	28.00	403.8	339.8	25	401.8	337.9	25
123478-HxCDD	PCDD	28.02	389.8	326.9	25	391.8	328.8	25
^{13}C-123678-HxCDD	PCDD	28.12	403.8	339.8	25	401.8	337.9	25
123678-HxCDD	PCDD	28.14	389.8	326.9	25	391.8	328.8	25
^{13}C-123789-HxCDD	PCDD	28.49	403.8	339.8	25	401.8	337.9	25
123789-HxCDD	PCDD	28.50	389.8	326.9	25	391.8	328.8	25
^{13}C-123789-HxCDF	PCDF	28.98	385.8	321.9	35	387.8	323.9	35
123789-HxCDF	PCDF	29.00	373.8	310.9	35	375.8	312.9	35
^{13}C-1234678-HpCDF	PCDF	31.13	419.8	355.8	36	421.8	357.8	36
1234678-HpCDF	PCDF	31.14	407.8	344.8	36	409.8	346.8	36
^{13}C-1234678-HpCDD	PCDD	32.97	437.8	373.8	25	435.8	371.8	25
1234678-HpCDD	PCDD	33.01	423.8	360.8	25	425.8	362.8	25
^{13}C-1234789-HpCDF	PCDF	33.97	419.8	355.8	36	421.8	357.8	36
1234789-HpCDF	PCDF	34.00	407.8	344.8	36	409.8	346.8	36
^{13}C-OCDD	PCDD	39.38	469.7	405.8	26	471.7	407.8	26
OCDD	PCDD	39.41	457.7	394.8	26	459.7	396.8	26
^{13}C-OCDF	PCDF	39.83	453.7	389.8	35	455.7	391.8	35
OCDF	PCDF	39.84	441.7	378.8	35	443.7	380.8	35

*RT=Retention time
†CE=Collision energy

Results and Discussion

Validation criteria

EU Regulation 709/2014 lists specific compliance requirements for GC/MS confirmatory methods for PCDDs, PCDFs, dioxin-like PCBs, and non-dioxin-like (NDL) PCBs [1]. Some of these criteria are dependent on the type of MS analyzer. For example, GC/triple quadrupole MS performs in tandem (MS/MS) mode, while GC/HRMS performs in selected ion monitoring (SIM) mode. Other requirements such as selectivity, upper- bound, and lower-bound differences are the same for both instrumental methodologies.

Each of these criteria was considered in this study, and a full method validation was performed for official control of dioxins in feed in accordance with the regulation, systematically investigating all parameters and performances on this instrumentation [1]. This validation could easily be transposed to other feed and foodstuffs, because it was performed using vegetable oil, which has maximum limits (MLs) that are amongst the lowest for these compounds (1.5 picograms WHO2005-PCDD/F-PCB-TEQ/g (parts per trillion)) [3], and the analytical criteria are the same. The World Health Organization 2005 toxic equivalent quantity per gram (WHO2005-TEQ) ML is the sum of the concentration of each individual congener corrected by a toxic equivalency factor (TEF) established by WHO in 2005. The measurement criteria for NDL-PCBs in food and feed are generally less stringent than those for PCDD/Fs and DL-PCBs since the MLs are in the ng/g (ppb) range and usually easier to attain [4]. However, for this validation study, the criteria for PCDD/Fs and DL-PCBs measurement were also applied to NDL-PCBs. For example, EU Regulation 709/2014 [5] stipulates only one precursor ion for quantitative and qualitative MRM transitions of NDL-PCBs, but two distinct precursor ions are required for PCDD/F and DL-PCB measurements. In this study, two specific precursor ions were also used for NDL-PCBs.

Instrumental limit of quantitation (iLOQ)

One of the major differences between the GC/triple quadrupole MS/MS and GC/HRMS methods is the proper establishment of the limit of quantitation (LOQ). Efficient ion filtering and the consequent significant reduction of noise are key advantages of GC/triple quadrupole MS/MS. A noise-free signal and flat baseline are characteristic of this instrumental approach. As a result, any signal-to-noise (S/N) calculation determined using such a baseline would produce S/N values that are unrealistic.

Therefore, a distinction was made for this validation study between a method limit of quantitation (mLOQ), which is the real-LOQ that takes possible matrix effects and blank levels into account, and the instrumental limit of quantitation (iLOQ), which is a performance- LOQ. This study used a statistical approach to assess the iLOQ of the GC/Triple Quadrupole MS/MS method, based on a report from an EU core working group composed of members from expert laboratories and EU national reference laboratories (NRLs) [6].

Eight replicate injections of the lowest acceptable calibration point were used to calculate the iLOQs, defined as 10 times the standard deviation (SD) associated with these replicates. To qualify as the lowest acceptable calibration point, the calculated relative standard deviations (RSDs) of the lowest level for all congeners had to be ≤15%. Although this 15% criterion was not included in Regulation 709/2014, it was chosen as a typical value for acceptable RSDs at such low analyte levels.

In addition, the regulation stipulates that the acceptable deviation to the relative response factor, which is the difference between the average response factors (RFs) obtained for the lowest calibration point versus the average RFs obtained for all points, is required to be ≤30%. The linearity of calibration was acceptable only when these two criteria were met. The iLOQ could then be determined as explained in the previous paragraph, using the resulting lowest calibration level.

Some exceptions were made in the calculation of the iLOQs shown in Table 3, when most criteria were acceptable. For example, 1,2,3,6,7,8-HxCDF had an RSD of 17.9% for triplicates of the lowest calibration point, which should have excluded it from calculation of iLOQ. However, the calibration coefficient (R^2) was very good (0.9990), and the difference between the average RF of the lowest point and the average RF of all points was only –1.21%, so a decision was made to use this lowest calibration point for the iLOQ calculation.

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Table 3. iLOQs, Calibration Curve Data, and mLOQs

	iLOQ (pg/pL)	R^2	Lowest calibration point (pg/pL)	RSD of the lowest calibration point (%)	RF Difference (%)	Average blank level (ng/kg)	mLOQ (ng WHO2005 TEQ/kg)
PCDFs							
2,3,7,8-TCDF	0.010	0.9919	0.016	5.5	-11.82	33	0.010
1,2,3,7,8-PeCDF	0.022	0.9969	0.016	13.7	-9.38	92	0.017
2,3,4,7,8-PeCDF	0.021	0.9922	0.016	7.7	-3.25	8	0.025
1,2,3,4,7,8-HxCDF	0.016	0.9990	0.016	3.8	8.99	17	0.007
1,2,3,6,7,8-HxCDF	0.009	0.9990	0.016	17.9	-1.21	25	0.006
2,3,4,6,7,8-HxCDF	0.007	0.9993	0.016	9.3	7.17	33	0.008
1,2,3,7,8,9-HxCDF	0.020	0.9993	0.016	14.2	-7.19	50	0.018
1,2,3,4,6,7,8-HpCDF	0.053	0.9946	0.080	9.8	-8.94	92	0.005
1,2,3,4,7,8,9-HpCDF	0.020	0.9990	0.016	14.9	-2.23	25	0.000
OCDF	0.027	0.9933	0.016	12.4	18.92	83	0.000
						Sum mLOQ	0.096
PCDDs							
2,3,7,8-TCDD	0.018	0.9960	0.016	2.5	-1.94	0	0.005
1,2,3,7,8-PeCDD	0.029	0.9949	0.016	12.8	-3.72	0	0.007
1,2,3,4,7,8-HxCDD	0.022	0.9949	0.016	8.9	0.11	8	0.001
1,2,3,6,7,8-HxCDD	0.032	0.9996	0.040	4.6	8.23	25	0.014
1,2,3,7,8,9-HxCDD	0.062	0.9962	0.080	4.0	-6.60	17	0.004
1,2,3,4,6,7,8-HpCDD	0.053	0.9990	0.400	3.4	2.32	100	0.004
OCDD	0.465	0.9900	4.000	2.3	-11.83	100	0.001
						Sum mLOQ	0.036
						Sum PCDD/F mLOQ	0.132.
NO-PCBs							
PCB 81	0.030	0.9933	0.320	1.7	-10.65	75	0.001
PCB 77	0.037	0.9931	0.320	1.4	-10.31	100	0.005
PCB 126	0.077	0.9905	0.320	1.7	-9.96	92	0.137
PCB 169	0.071	0.9935	0.320	2.1	-7.19	0	0.001
						Sum mLOQ	0.144
MO-PCBs							
PCB-105	2.109	0.9958	1.000	9.8	-0.38	100	0.006
PCB-114	1.504	0.9938	1.000	8.8	-4.89	100	0.000
PCB-118	1.930	0.9994	1.000	8.7	15.32	100	0.018
PCB-123	1.537	0.9945	1.000	11.8	-4.86	100	0.000
PCB-156	1.897	0.9892	1.000	5.6	-5.38	100	0.000
PCB-157	1.287	0.9882	1.000	4.2	-7.09	100	0.000
PCB-167	2.067	0.9925	1.000	10.0	-6.32	100	0.001
PCB-189	1.626	0.9893	1.000	2.5	-7.48	100	0.000
						Sum mLOQ	0.025
						Sum PCDD/F-PCB mLOQ	0.300
NDL-PCBs							
PCB-28	3.928	0.9944	4.000	2.0	8.39	100	994.601*
PCB-52	6.530	0.9983	4.000	1.5	16.98	100	1909.234
PCB-101	2.733	0.9964	4.000	2.4	11.11	100	1303.266
PCB-138	1.587	0.9817	4.000	4.3	-5.59	100	161.674
PCB-153	1.469	0.9780	4.000	2.0	-6.03	100	171.672
PCB-180	0.904	0.9723	4.000	0.4	-1.64	100	34.941
						Sum mLOQ	4575.388

*Reported as ng/kg.

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The iLOQs for the NDL-PCBs were much higher than those for the PCDDs, PCDFs, and non-ortho (NO-)PCBs, and some of them were higher than the iLOQs for the mono-ortho (MO-)PCBs as well (Table 3). This was due to the fact that the 1 pg/µL lowest calibration point for congeners was excluded from the iLOQ calculation for NDL-PCBs due to RF differences >30% and an RSD >15%. The 4 pg/µL calibration point was then used for all NDL-PCB iLOQ calculations, since all of its RSD values were far below 15%. Although the R^2 values were slightly below 0.9900 for three of the six NDL-PCBs (PCB-138,PCB-153, and PCB-180), the differences between RFs were very low, and the 4 pg/µL calibration point was used to calculate the iLOQs for these three NDL-PCBs as well. The calculated iLOQ values for all of the analytes were similar to those attained for GC/HRMS (data not shown).

Method limit of quantitation (mLOQ)

The mLOQ is a measure of the real analytical sensitivity of the method in a real environment. It is determined for the congeners by analyzing blank replicates, in this case 12 procedural blanks for each congener, from which an average value and an SD were calculated. The mLOQs are defined such that levels higher than the mLOQs are statistically proven to be due to the presence of congener in the sample, and not due to background noise. In this case, the mLOQs are defined as the average value of the blank plus six times the standard deviation.

Table 3 shows the average blank levels found in 12 individual procedure blanks for each of the 35 congeners, as well as mLOQs (ng WHO2005TEQ/kg). Most of the 35 congeners analyzed in our study, based on a 4-g sample size, gave measurable blank levels which were used to calculate the mLOQs (Table 3). For those congeners not present in blanks (2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, and PCB-169), the mLOQS were determined by adjusting the iLOQs to the sample amount.

The EU Regulation MLs are 0.75 ng/kg for the sum of the PCDDs and PCDFs (WHO2005-PCDD/F-TEQ/kg), 1.50 ng/kg for the sum of the PCDDs, PCDFs, NO-PCBS, and MO-PCBs (WHO2005-PCDD/F-PCB-TEQ/kg), and10 µg/kg for the sum of the 6 NDL-PCBs. The regulation requires that mLOQs must be <20% of MLs. Table 3 shows the sums of the mLOQs for each congener group for direct comparison with the MLs. The sum of 0.132 ng WHO2005-PCDD/F-TEQ/kg, is 18% of the ML, and the sum of 0.30 ng WHO2005-PCDD/F-PCB-TEQ/kg is 20% of the ML. This method is compliant with the regulation for these two congener groups. For the NDL-PCBs, the sum was 4.6 µg/kg, which was above 20% of the ML. This was due to a high level of contamination in the laboratory, as was also seen with the GC/HRMS method.

Selectivity and quantitative/qualitative transitions

The EU regulation does not call for specific criteria for the selectivity of GC/triple quadrupole MS/MS methods. However, triple quadrupole ion filtering produces a flat baseline, making these chromatograms look very different from those generated using GC/HRMS. To avoid any artificial enhancement of signals and keep as close as possible to raw data, unsmoothed chromatograms were used in this study. Baseline separation was observed for the 1,2,3,4,7,8- and 1,2,3,6,7,8-hexachlorinated furans congeners (HxCDF), which are the most difficult to separate. No improvement in results was observed using smoothed chromatogram correction in terms of either accuracy or precision (RSD).

The intensity ratio of quantitation to qualification ions (Quant/Qual) was used to ensure absence of interference and correct peak integration. The lower response for an analyte was observed for the qualification transition (quantitation transition with a +2 Da offset), while the quantitation MRM transition gave the higher response. Using the same MS parameters such as collision energy, Quant/Qual ratios were established experimentally from the calibration curve (Table 4). The allowed tolerance was ±15% for PCDD/Fs and DL-PCBs, and more for NDL-PCBs [1]. To guarantee an accurate result, a closer look at raw data is required whenever a congener Quant/Qual ratio is out of range.

Table 4. Measured Average Quantitative/Qualitative Ion Ratio

	Mean	RSD%	Tolerance (%)
PCDFs			
2,3,7,8-TCDF	94.0	14	15
1,2,3,7,8-PeCDF	81.7	14	15
2,3,4,7,8-PeCDF	88.0	26	15
1,2,3,4,7,8-HxCDF	62.3	19	15
1,2,3,6,7,8-HxCDF	60.8	10	15
2,3,4,6,7,8-HxCDF	62.7	10	15
1,2,3,7,8,9-HxCDF	62.6	16	15
1,2,3,4,6,7,8-HpCDF	76.1	11	15
1,2,3,4,7,8,9-HpCDF	82.3	29	15
OCDF	93.0	20	15
PCDDs			
2,3,7,8-TCDD	96.4	10	15
1,2,3,7,8-PeCDD	81.6	21	15
1,2,3,4,7,8-HxCDD	64.7	15	15
1,2,3,6,7,8-HxCDD	64.4	17	15
1,2,3,7,8,9-HxCDD	73.3	19	15
1,2,3,4,6,7,8-HpCDD	79.7	18	15
OCDD	94.3	12	15
NO-PCBs			
PCB 81	64.3	14	15
PCB 77	62.4	1	15
PCB 126	95.1	9	15
PCB 169	73.3	8	15
MO-PCBs			
PCB-105	30.5	4	15
PCB-114	30.0	3	15
PCB-118	30.3	2	15
PCB-123	29.9	3	15
PCB-156	46.8	2	15
PCB-157	47.6	3	15
PCB-167	47.0	2	15
PCB-189	62.7	2	15
NDL-PCBs			
PCB-28	31.8	2	25
PCB-52	63.2	1	20
PCB-101	30.5	3	25
PCB-138	47.3	2	25
PCB-153	47.4	2	25
PCB-180	62.9	2	20

Background subtraction

Measured concentrations of an analyte can be corrected by subtracting an individual blank of the same kind of sample prepared in the same way. Such a blank is used for each series of samples (for example, one blank per 10 samples). Alternatively, an average blank value from a series of controlled blanks measured over time can be used for the correction. Two advantages are provided by the latter approach, which was used in this study. A chart of control levels kept over time enables detection of trends, can highlight contamination problems, and provides a proactive approach to contamination control. Secondly, this approach reduces the effect of a statistical outlier blank in a single sample series. Averaging blank levels that are controlled proactively within a confidence interval enables the rejection of such an outlier, and instead includes the statistical variation of the blank in the measurement uncertainty, which is monitored in the chart of control levels. This is a key point for determination of mLOQs.

Accuracy

The bias of the method for PCDD/Fs, NO-PCBs, and MO-PCBs was assessed using fortified samples in sunflower oil. No congener was found in the unfortified vegetable oil matrix blank. Six series of samples spiked at twice ML (2ML), ML, and half ML (ML/2) were injected over three days (two series per day), and all were within acceptable reproducibility limits (Table 5). The results were well within the requirements of the EU Regulation, which are method bias <20% and random error (%RSD) <15% [1].

Table 5. Bias of the Method for DL-PCBs and PCDD/Fs Using Six Series of Samples Spiked at Three Levels Around the ML

	Target*	Average*	SD	RSD%	Bias%
DL-PCBs					
2ML	1.3	1.26	0.02	1.6	–3.42
ML	0.65	0.59	0.02	3.4	–8.53
ML/2	0.33	0.31	0.03	9	–7.00
PCDD/Fs					
2ML	1.58	1.6	0.03	2.2	1.3
ML	0.79	0.78	0.04	5.7	–1.54
ML/2	0.4	0.41	0.03	7.1	2.36

* ng WHO2005TEQ/kg

Quality control and robustness

Two blank and two QC samples (pork fat) prepared in the lab were injected twice each week during September–October 2013, and again in April and May of 2014 (~6 months later). All of the QC sample values fell within the 95% confidence interval (Figure 1). That included QC samples that were run after the system was used for other purposes, including system venting and several column changes, over a 6-month period.

Conclusions

A GC/triple quadrupole MS/MS method has been developed and fully validated in accordance with criteria in EU Regulation 709/2014 that allows the use of GC/triple quadrupole MS/MS as a confirmatory method for official control of PCDDs, PCDFs, and DL-PCBs in animal feedstuffs. This method, developed using the Agilent 7890B GC system coupled to an Agilent 7000C Series Triple Quadrupole GC/MS system, meets the requirements of the regulation, and can achieve similar performance to GC/HRMS. Realistic measurement uncertainty in the typical range of the HRMS method was achieved, along with very similar analytical parameters, despite the difference in mass analyzer technology. The most stringent criteria were followed to demonstrate that this method provides accurate, consistent, and reliable results in the context of maximum level (ML) measurements.

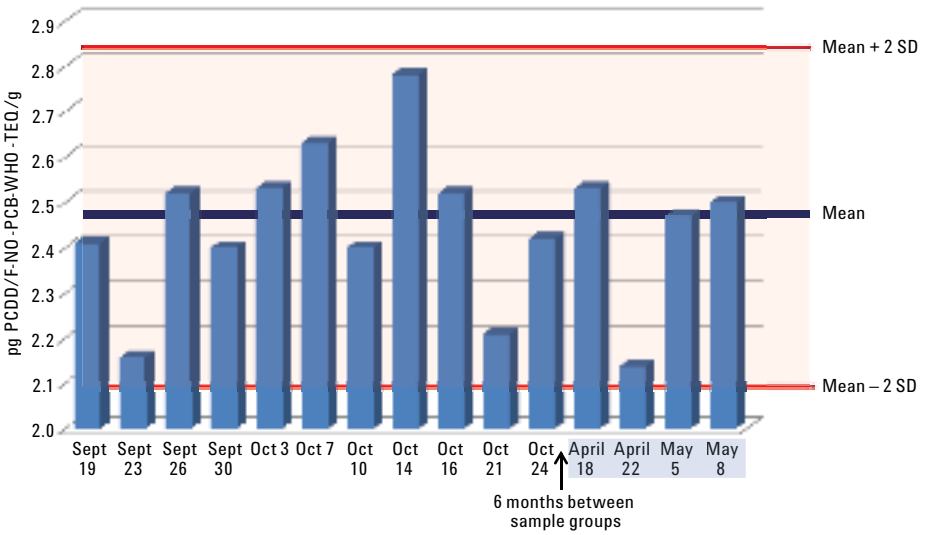

Figure 1. QC chart over September–October and April–May validation periods. All values fell within the 95% confidence interval (± 2SD.)

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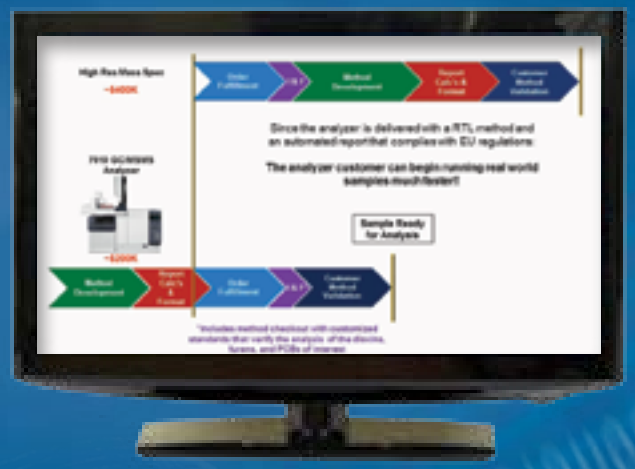

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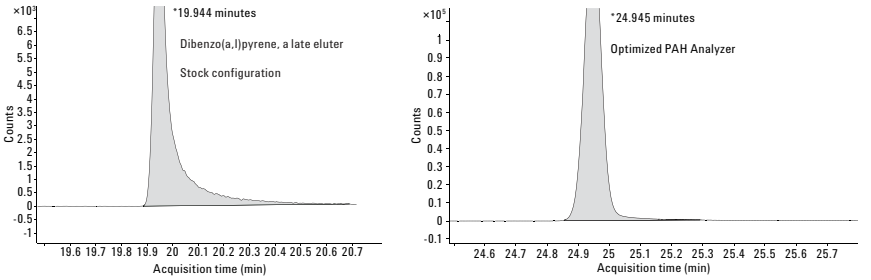

PAH analysis performed on a stock configuration vs. an Enhanced PAH Analyzer with JetClean Self-Cleaning Ion Source. Note the improvement in peak shape for Dibenzo (a,l) pyrene – a late-eluting PAH.

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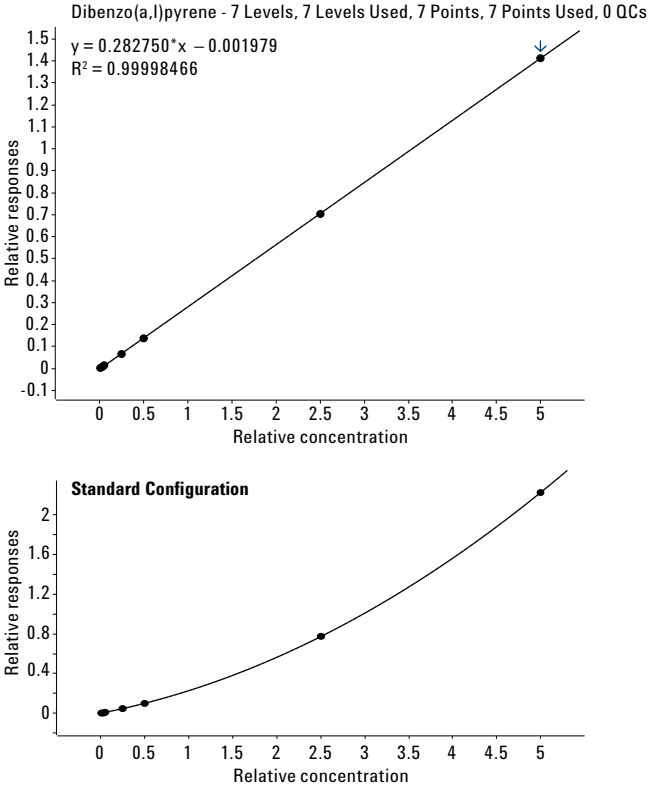
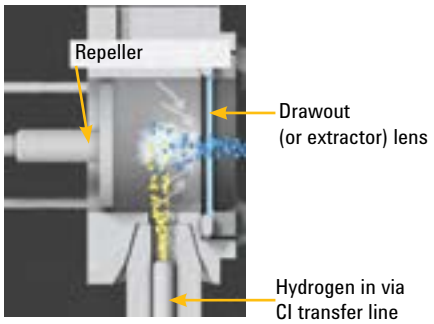


	Initial data R^2	CCM R^2
Naphthlene	0.9982	0.9999
1-Methyl naphthalene	0.9981	1.0000
2-Methyl naphthalene	0.9977	1.0000
1,2-Dimethyl naphthalene	0.9974	1.0000
1,6-Dimethyl naphthalene	0.9976	1.0000
Acenaphthylene	0.9975	0.9999
Acenaphthene	0.9983	1.0000
Fluorene	0.9976	1.0000
Phenanthrene	0.9972	0.9999
Anthracene	0.9959	0.9999
2-Methyl phenanthrene	0.9846	0.9999
2-Methyl anthracene	0.9846	0.9999
1-Methyl phenanthrene	0.9969	1.0000
3,6-Dimethyl phenanthrene	0.9851	1.0000
2,3-Dimethyl anthracene	0.9648	0.9999
Fluoranthene	0.9978	0.9999
9,10 Dimethyl anthracene	0.9726	1.0000
Pyrene	0.9846	1.0000
1-Methyl pyrene	0.9927	0.9997
Benz (a) anthracene	0.9976	0.9998
Chrysene	0.9976	0.9999
6-Methyl chrysene	0.9690	0.9998
Benzo (k) fluoranthene	0.9954	1.0000
Benzo (a) pyrene	0.9576	1.0000
Dibenz (a,h) anthracene	0.9581	0.9999
Indeno (1,2,3-c,d) pyrene	0.9642	0.9999
Benzo (g,h,i) perylene	0.9965	1.0000
Dibenzo (a,l) pyrene	0.9788	1.0000

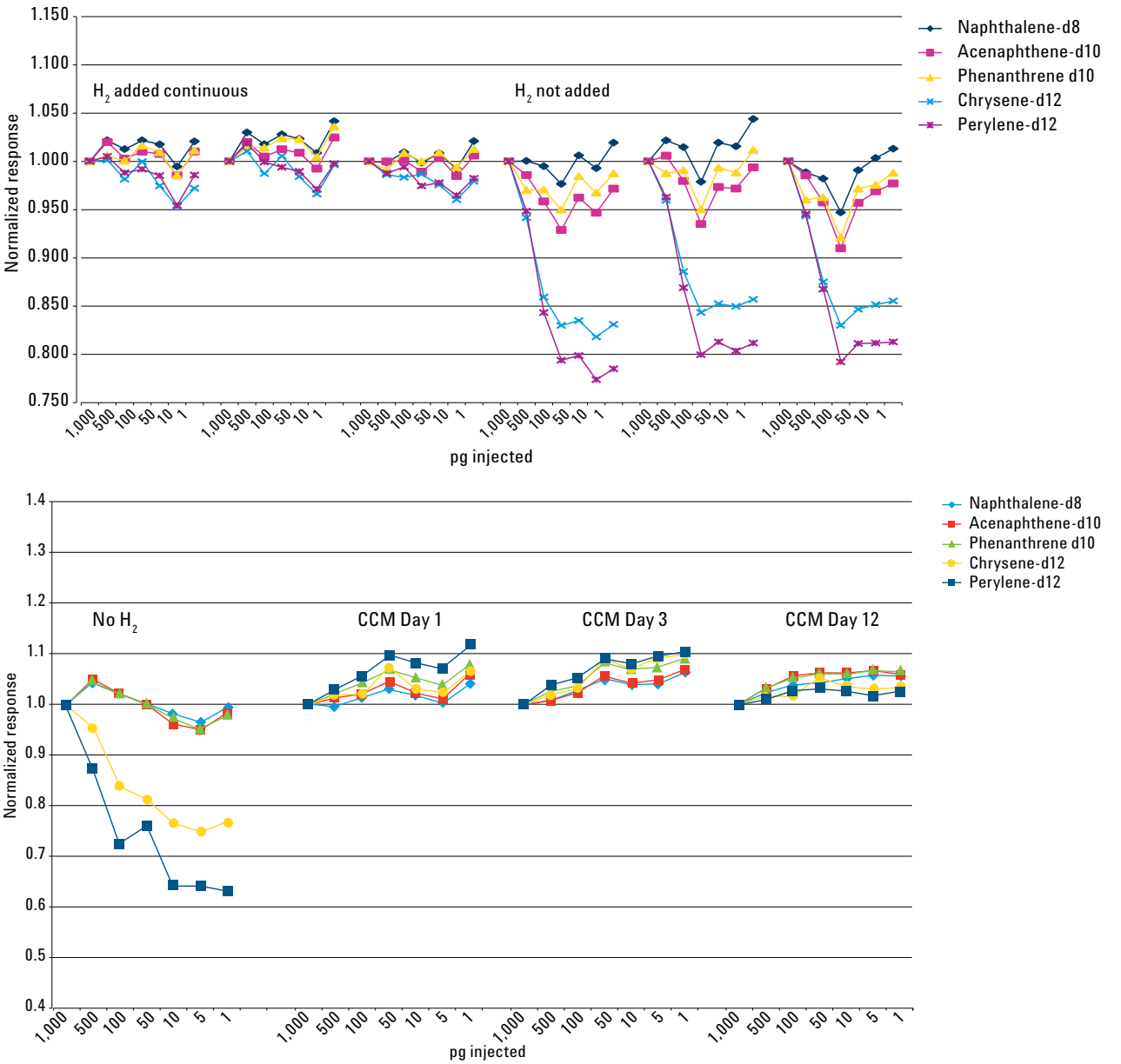
7-level calibration curve comparison for the latest-eluting PAH analyzed by GC/MS/MS. The JetClean Self-Cleaning Ion Source improved linearity for the calibration curve of Dibenzo (a,l) pyrene to R^2=0.99998. Correlation coefficients ranged from R^2=0.9997-1.0000 for all PAHs analyzed by the Enhanced PAH GC/MS/MS Analyzer.

Stability of internal standard response factors

Inconsistent ISTD response across the calibration range makes accurate quantitation both difficult and dependent on the amount of other analytes in the sample. It can also lead to linearity that falls short of performance criteria for regulatory methods. GC/MS and GC/MS/MS systems optimized for PAH analysis—and equipped with the JetClean Self-Cleaning Ion Source—demonstrate substantial improvement in the consistency of ISTD response.



Normalized plot (highest to lowest) of ISTD response across a seven-level calibration of a 30 PAH mix with five ISTDs



With hydrogen addition to the source, ISTD responses for the GC/MS analysis improved from ±25% to within 5%. For GC/MS/MS analysis of PAHs, the addition of hydrogen improved ISTD RF from ±35% to within 8% – well within method reporting requirements.

Ordering information:

- M7431AA: Enhanced PAH Analyzer with Const. Flow; Post Col. BF Method by GC/MS/MS
- M7466AA Enhanced PAH Analyzer by GC/MS

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Leverage these technological advances and start performing sensitive PAH analysis immediately after installation

Pre-configured analytical method, retention time locked with Capillary Flow Technology (CFT) backflush. CFT backflush promotes shorter analysis times, lower chemical background, longer column life, and less frequent source cleaning to improve uptime.

JetClean Self-Cleaning Ion Source introduces a low hydrogen flow continuously during data acquisition. This keeps the source clean from run to run, improving analytical results with:

- Greater dynamic range: on-column sensitivity ensures reproducible and linear calibration from 1-1000 pg
- Excellent calibration linearity with r^2 values of 0.999 – 1.0000 (compound specific)

Multimode inlet (MMI) lets you choose from several injection options, such as large-volume injection or cold splitless injection (for thermally labile compounds).

Productivity tools help you make the most of every analysis: Agilent Autotune, Batch-at-a-Glance data review, and parameter-less integrator streamline your data review and processing.

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VETERINARY DRUGS

Detect and quantify veterinary drugs in foods of animal origin

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Agilent Veterinary Drug LC/MS Personal Compound Database and Library

Triple quadrupole LC/MS methods are considered the "gold standard" for multiclass veterinary drug analysis of meat or other foods. These methods are ideal for a pre-defined set of target compounds.

Accurate mass high resolution time-of-flight (TOF) mass spectrometers enable you to analyze *both* targets and unknowns. Their full-spectrum data acquisition ensures that signals from *all* ionizing compounds in your sample will be captured.

Confidently use accurate mass to perform target and suspect screening

You can screen for more than 2,100 veterinary drugs when you combine TOF or Q-TOF LC/MS with the Agilent Veterinary Drug Personal Compound Database and Library (PCDL).

This comprehensive PCDL includes accurate mass MS/MS spectra for more than 1,500 compounds, and can expand to accommodate new compounds of emerging concern. In addition, surveillance using LC/Q-TOF allows you to re-analyze or mine your data at any time—without reruns—to investigate samples further.

The following components are included in the Agilent Veterinary Drug PCDL—saving you time and maximizing performance

- Curated accurate-mass database with more than 2,100 compounds
- Accurate-mass MS/MS spectra for more than 1,500 compounds—over 5,200 spectra total
- Searchable user notes containing compound class and regulation tags
- Retention time information added to more than 120 compounds
- Quick-start guide with data examples and familiarization exercises
- Method Setup Guide that shows you how to create acquisition methods
- Application note with detailed LC/MS method information
- Latest version of PCDL Manager Software
- Free database upgrades for 3 years

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Combine the Agilent Veterinary Drug PCDL with the accurate mass capabilities of LC/TOF and Q-TOF instruments.

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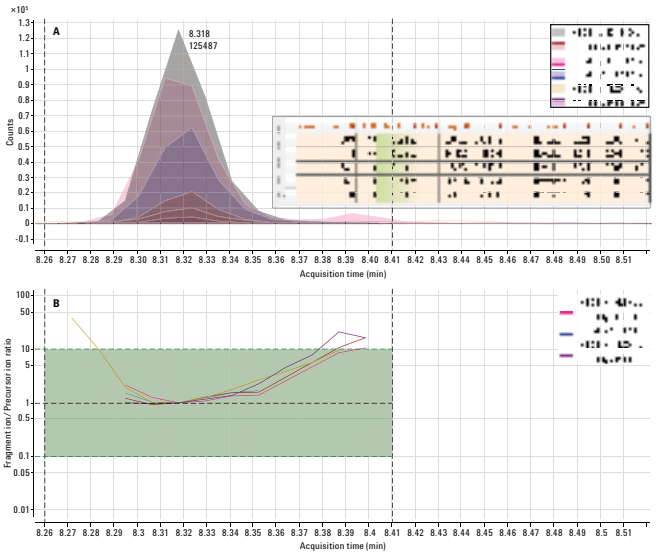
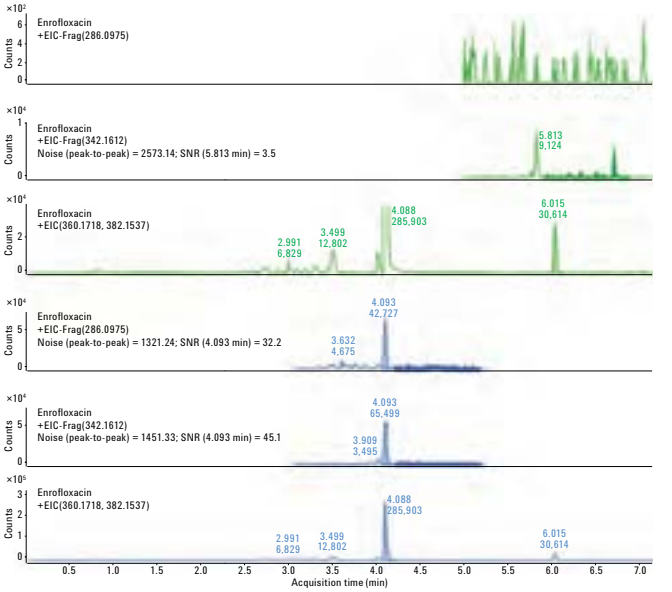
- **Perform presumptive matching** of acquired and library spectra—without the need to source standards
- **Create a custom PCDL** for more focused screening
- **Propose a suspect list** based on MS data and the "Find by Formula" algorithm
- **Confirm contaminants and eliminate false positives** with targeted MS/MS and library search
- **Mine data from Auto MS/MS experiments** using "Molecular Feature Extraction," and search for proposed compounds against the PCDL
- **Add your own compounds and library spectra** to create PCDLs specific to your needs
- **Perform retrospective data analysis** using newly added PCDL compounds—without the need to re-run samples

Simply put, the Veterinary Drug PCDL makes compound confirmation and data mining easier, even for high-throughput labs.

PCDL Manager Software makes it easy to control and edit the database and library.



Straightforward data mining and unambiguous identification using All Ions Software.



Potential false positive of enrofloxacin identified through fragment ion confirmation using the Agilent All Ions workflow.

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The Agilent Veterinary Drug PCDL can help you meet the strict requirements established by global food regulatory agencies, including the following:

EU
Council Directive 96/23/EC

U.S.
CFR Title 21 Part 556,
USDA Anal Bioanal Chem 2014

China/Japan
MOA Notice 235

Available class tags
Insecticides, beta-agonists, antibiotics, anti-inflammatories, anti-psychotics, tetracyclines, dyes, anti-parasitics, pesticides, sedatives, herbicides, fungicides, equine drugs

Maximize your data quality with database and library curation

- Compound common name and IUPAC name
- Accurate mass of neutral molecule
- Molecular formula and structure
- Ion type (anion, cation, or neutral)
- CAS number/PubChem link (if existing)
- ChemSpider ID and hyperlink (if existing)
- Precursor and product ion peaks corrected to theoretical accurate mass
- Spectra acquired at 10, 20, and 40 V collision energy
- Spectra measured in positive and/or negative ion mode, where applicable
- Spectra filtered for signal intensity and curated for spectrum noise, chemical impurities, and incorrectly set instrument parameters
- Includes adduct & loss spectra

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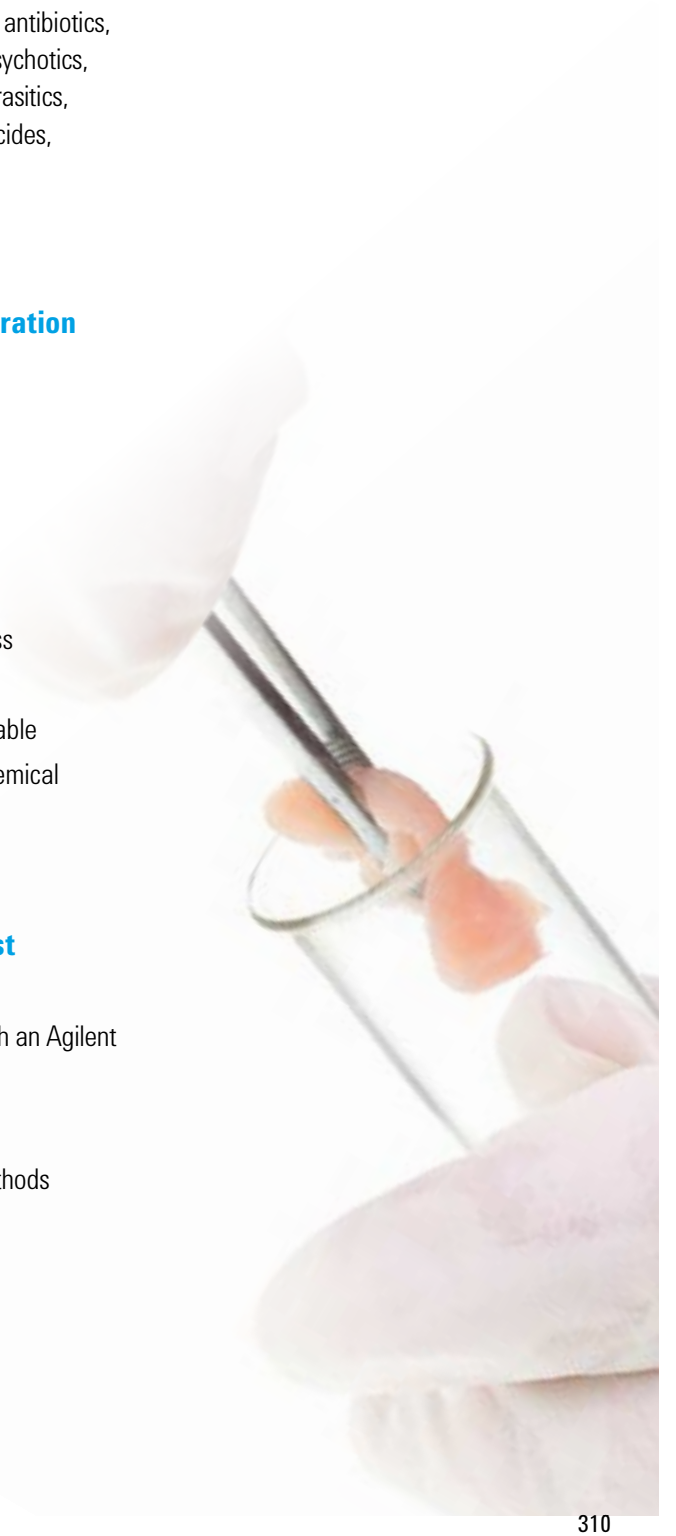
Advanced application consulting (optional):

Let us help you get the most out of your PCDL by setting up screening methods for your samples of interest.



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Agilent 1290 Infinity II LC system

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The full-scan capability and mass accuracy of All Ions MS/MS lets you access all the data, all the time, so you can screen for large numbers of suspect and unknown veterinary drugs. What's more, the Agilent Jet Stream electrospray ion source dramatically lowers your detection limits.

Agilent LC columns, supplies, and sample prep products

Increase your uptime and achieve the best scientific outcomes.

Ordering Information:

Veterinary Drug Personal Compound Database and Library (G3879CA)
Required but not included with the Veterinary Drug PCDL:
Agilent 1260 or 1290 Infinity II LC
Agilent 6200 Series TOF or 6500 Series Q-TOF LC/MS
Agilent MassHunter Acquisition Software (B.05 or higher) and Windows 7 (64-Bit)
Agilent MassHunter Qualitative Analysis Software (B.07 SP1 or higher)
Agilent MassHunter Quantitative Analysis Software (B.07 or higher)
OPTIONAL: G3878CA #001 Installation and Familiarization Service
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Analysis of 122 Veterinary Drugs in Meat Using All Ions MS/MS with an Agilent 1290/6545 UHPLC-Q-TOF System

Application Note

Authors

Tarun Anumol, Joan Stevens, Dan-Hui Dorothy Yang, and Jerry Zweigenbaum
Agilent Technologies Inc., Wilmington, DE

Abstract

The presence of veterinary drugs (VDs) in meat may pose a health risk to humans during consumption; therefore, monitoring of VD residues for regulatory enforcement and risk assessment is commonly conducted. Several hundred VDs are available spanning a number of classes with very different chemical characteristics. This typically requires sophisticated analytical methods and instruments, generally based on LC-MS/MS, sophisticated workflows, and often tedious data processing. In this study, a 12-minute analytical method was developed for 122 priority VDs in meat. The method uses Agilent All Ions MS/MS on an Agilent Q-TOF LC/MS instrument along with the Agilent Veterinary Drugs personal compound database and library (PCDL) to test the method. All 122 VDs were spiked into bovine liver, kidney, and muscle tissue at levels of 0.5, 1, and 2 times the maximum tolerance levels for each drug. These spikes were then analyzed using All Ions data acquisition mode in an Agilent 6545 Q-TOF LC/MS. The Agilent MassHunter Find by Formula software was used to detect and verify the presence of these compounds. The PCDL provides MS/MS spectral and retention time information about each compound enabling a data review process to quickly and reliably filter out false positives. At all three spike levels, >92% of VDs were detected in every matrix. To demonstrate the ability of this system to deliver quantitative results, calibration curves were generated for ground beef and liver starting at low ng/g levels. With >85% VDs having an $R^2 > 0.99$ without correction using any internal standard, this method can be used to do screening and quantification of VDs in animal matrices in one analytical run.

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Introduction

The breeding of livestock for food requires the controlled use of veterinary drugs (VDs) as a means to prevent diseases or promote rapid growth. However, poor management strategies and improper administration of these drugs to livestock can lead to drug residues being present in the animal meat and other organs, which can pose a human health risk on consumption. Antimicrobial resistance is another concern that arises from the use of antibiotics in agriculture. Therefore, the levels of VDs in meat and other foods are regulated with maximum residue levels (MRLs) or tolerance levels that vary significantly from one drug to another [1-3]. There are several hundred VDs known to be used in livestock, varying vastly in class, chemical structure, and polarity, making them difficult to analyze in the same method. Furthermore, the MRLs are often low, and must be achieved in complex matrices, requiring sensitive and robust analytical equipment [4,5].

LC/MS technologies have been shown to offer sensitivity and selectivity, along with time, labor, and cost savings, through multiclass multiresidue methods [2,6]. However, the use of accurate mass high resolution time-of-flight (TOF) mass spectrometers can give the user some extra capabilities. Full spectrum data acquisition ensures that signals from all ionizing compounds in the sample are captured. Therefore, it is possible for a surveillance scheme using this technology to grow to accommodate new compounds of emerging concern without the need for any method development. Moreover, it is possible to perform retrospective data mining for new analytes without rerunning samples. In addition, TOF spectra permit the detection and elucidation of new VDs and metabolites for which analytical standards may be unavailable.

This study sought to develop a rapid screening method for >120 commonly monitored VDs across multiple classes. The list of VDs analyzed was based on previous work performed by the US Department of Agriculture's Agricultural Research Service (USDA-ARS) and Food Safety and Inspection Service (USDA-FSIS) [2,7]. The VDs were analyzed in bovine muscle, kidney, and liver using an Agilent Q-TOF LC/MS operating with All Ions MS/MS acquisition. This mode of data collection provides high resolution accurate mass spectra of both molecular ions (low energy channels) and fragment ions (high energy channels).

MS/MS spectra in the Agilent PCDL were then used to verify if the molecular ions and corresponding fragment ions match those in the sample. This study also included the use of commercialized Agilent QuEChERS Enhanced Matrix Removal (EMR—Lipid) material for cleanup of meat extracts. This has previously been shown to effectively and selectively remove lipids from high fat food commodities.

The quantitative capability in this overall method was evaluated by generating matrix matched calibration curves in ground beef and liver at ng/g levels.

Experimental

Standards and Reagents

A significant number of veterinary drug standards were provided by the USDA-ARS Eastern Regional Research Center (Wyndmoor, PA) as solutions in acetonitrile (MeCN), methanol, water, or a combination thereof between 214 and 1,200 mg/L. Abamectin, ivermectin, thiouracil, and the β-lactams (amoxicillin, ampicillin, cefazolin, desacetyl cephapirin, cloxacillin, nafcillin, oxacillin, and penicillin) were purchased from Sigma-Aldrich (St. Louis, MO). Ultrapure water was obtained from a Millipore (Billerica, MA) system and was >18.2 MΩ-cm. MeCN (LC-MS grade) was purchased from VWR International (Radnor, PA), and formic acid (88% double distilled) was purchased from GFS Chemicals (Powell, OH).

Sample extraction

Prehomogenized samples (2 g each) of bovine muscle, liver, and kidney were spiked with 122 VDs and two internal standards (flunixin-d_3 and sulfamethazine-$^{13}C_6$) as selected previously [2]. The spiking concentrations were 0.5, 1, and 2 times the USDA tolerance levels (x, shown in Table 1), in all three matrices. Sample preparation was conducted using the Agilent Bond Elut EMR—Lipid procedure as described in a previous application note (5991-6096EN) [8]. The final extracts were diluted to 80/20 water/MeCN ratio, and stored in 2-mL polypropylene autosampler vials before being injected into the LC/MS. Spiking solution was added to solvent and blank matrix extracts at 0.5x, 1x, and 2x levels (post-extraction) to evaluate instrument and analytical method performance, as well as matrix effects. All extracts were stored in a –10 °C freezer, and analyzed two weeks after sample preparation.

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Table 1. Veterinary Drugs Monitored in PCDL Along with Tolerance Levels

Veterinary drug	Class	1x Tolerance level (ng/g)
2-Amino Flubendazole	Anthelmintic	10
2-Amino Mebendazole	Anthelmintic	10
2-Hydroxy Dimetridazole	Coccidiostat	50
2-Mercaptobenzimidazole	Thyreostat	25
2-Thiouracil	Thyreostat	400
5-hydroxy thiabendazole	Anthelmintic	100
6-Methylthiouracil	Thyreostat	400
6-Phenylthiouracil	Thyreostat	400
6-Propyl-2-thiouracil	Thyreostat	50
Abamectin	Anthelmintic	20
Acetopromazine	Tranquilizer	10
Albendazole	Anthelmintic	50
Albendazole sulfoxide	Anthelmintic	50
Albendazole sulphone	Anthelmintic	50
Albendazole-2-aminosulphone	Anthelmintic	50
Amoxicillin	β-Lactam	10
Ampicillin	β-Lactam	10
Azaperone	Tranquilizer	10
Bacitracin	Miscellaneous	500
Betamethasone	Anti-inflammatory	100
Cambendazole	Anthelmintic	10
Carazolol	Tranquilizer	10
Carbadox	Miscellaneous	30
Cefazolin	β-Lactam	100
Cephapirin	β-Lactam	100
Chloramphenicol	Phenicol	10
Chlorpromazine (thorazine)	Tranquilizer	10
Chlortetracycline	Tetracycline	1,000
Cimaterol	β-Agonist	10
Ciprofloxacin	Fluoroquinolone	50
Clenbuterol	β-Agonist	10
Clindamycin	Macrolide/Lincosamide	100
Cloxacillin	β-Lactam	10
Danofloxacin	Fluoroquinolone	200
DCCD (marker for ceftiofur)	β-Lactam	400
Desacetyl cephapirin	β-Lactam	100
Desethylene ciprofloxacin	Fluoroquinolone	100
Diclofenac	Anti-inflammatory	200
Dicloxacillin	β-Lactam	100
Difloxacin	Fluoroquinolone	50
Dimetridazole	Coccidiostat	10
Dipyrone (metabolite)	Anti-inflammatory	200
Doramectin	Anthelmintic	30
Doxycycline	Tetracycline	100
Emamectin	Anthelmintic	10
Enrofloxacin	Fluoroquinolone	100
Eprinomectin B1a	Anthelmintic	100
Erythromycin A	Macrolide/Lincosamide	100
Fenbendazole	Anthelmintic	400
Fenbendazole sulphone	Anthelmintic	400
Florfenicol	Phenicol	300
Florfenicol amine	Phenicol	300
Flubendazole	Anthelmintic	10
Flunixin	Anti-inflammatory	25
Flunixin-d3	Internal Standard	250
Gamithromycin	Macrolide/Lincosamide	100
Haloperidol	Tranquilizer	10
Haloxon	Anthelmintic	100
Hydroxy-Ipronidazole	Coccidiostat	10
Ipronidazole	Coccidiostat	10
Ivermectin B1a	Anthelmintic	10
Ketoprofen	Anti-inflammatory	10
Levamisole	Anthelmintic	100
Lincomycin	Macrolide/Lincosamide	100
Mebendazole	Anthelmintic	10
Melengesterol acetate	Miscellaneous	25
Meloxicam	Anti-inflammatory	100
Metronidazole	Coccidiostat	10
Morantel	Anthelmintic	100
Moxidectin	Anthelmintic	50
Nafcillin	β-Lactam	100
Norfloxacin	Fluoroquinolone	50
Novobiocin	Miscellaneous	1,000
Orbifloxacin	Fluoroquinolone	50
Oxacillin	β-Lactam	100
Oxfendazole	Anthelmintic	800
Oxibendazole	Anthelmintic	10
Oxyphenylbutazone	Anti-inflammatory	100
Oxytetracycline	Tetracycline	1000
Penicillin G	β-Lactam	50
Phenylbutazone	Anti-inflammatory	100
Pirlimycin	Macrolide/Lincosamide	300
Prednisone	Anti-inflammatory	100
Promethazine	Tranquilizer	10
Propionylpromazine	Tranquilizer	10
Quinoxaline-2-carboxylic acid	Miscellaneous	30

Veterinary drug	Class	1x Tolerance level (ng/g)
Ractopamine	B-Agonist	30
Ronidazole	Coccidiostat	10
Salbutamol	B-Agonist	10
Sarafloxacin	Fluoroquinolone	50
Selamectin	Anthelmintic	200
Sulfabromomethazine	Sulfonamide	100
Sulfachloropyridazine	Sulfonamide	100
Sulfadiazine	Sulfonamide	100
Sulfadimethoxine	Sulfonamide	100
Sulfadoxine	Sulfonamide	100
Sulfaethoxypyridazine	Sulfonamide	100
Sulfamerazine	Sulfonamide	100
Sulfamethazine	Sulfonamide	100
Sulfamethazine-$^{13}C_6$	Internal Standard	250
Sulfamethizole	Sulfonamide	100
Sulfamethoxazole	Sulfonamide	100
Sulfamethoxypyridazine	Sulfonamide	100
Sulfanilamide	Sulfonamide	100
Sulfanitran	Sulfonamide	100
Sulfapyridine	Sulfonamide	100
Sulfaquinoxaline	Sulfonamide	100
Sulfathiazole	Sulfonamide	100
Tetracycline	Tetracycline	1,000
Thiabendazole	Anthelmintic	100
Thiamphenicol	Phenicol	10
Tildipirosin	Macrolide/Lincosamide	100
Tilmicosin	Macrolide/Lincosamide	100
Tolfenamic acid	Anti-inflammatory	200
Triclabendazole	Anthelmintic	50
Triclabendazole sulfoxide	Anthelmintic	50
Triflupromazine	Tranquilizer	10
Troleandomycin	Macrolide/Lincosamide	1,000
Tulathromycin A	Macrolide/Lincosamide	5,500
Tylosin	Macrolide/Lincosamide	200
Virginiamycin	Miscellaneous	100
Xylazine	Tranquilizer	10
Zeranol (β-Zearalanol)	Miscellaneous	100
Zilpaterol	B-Agonist	12

Instrumental analysis

An Agilent 1290 Infinity ultrahigh-performance liquid chromatograph (UHPLC) with a 40 µL loop HiPALS autosampler was used for this method. Separation was performed with an Agilent ZORBAX Eclipse Plus C-18 (2.1 × 150 mm, 1.8 µm) column using a gradient of water + 0.1% formic acid (A) and MeCN + 0.1% formic acid (B). An Agilent inline filter (p/n 5067-4638) was installed after the autosampler and an Agilent ZORBAX Eclipse plus guard column (p/n 959757-902) was used before the analytical column to protect and enhance column lifetime. Table 2 lists the LC conditions used for this analysis. Figure 1 illustrates a sample chromatogram of a VD standard at 50 ng/mL in 80/20 water/MeCN.

Table 2. LC Conditions

Parameter	Value	
Instrument	Agilent 1290 Infinity LC	
Column	Agilent ZORBAX Eclipse Plus C-18, 2.1 × 150 mm, 1.8 µm (p/n 959759-902)	
Mobile phase	A) Water + 0.1% formic acid B) Acetonitrile + 0.1% formic acid	
Gradient	Time (min)	B (%)
	0.0	2
	1.0	2
	10	100
	11	100
	11.1	2
Flow rate	0.5 mL/min	
Post time	3.0 minutes	
Column temperature	30 °C	
Injection volume	15 µL	

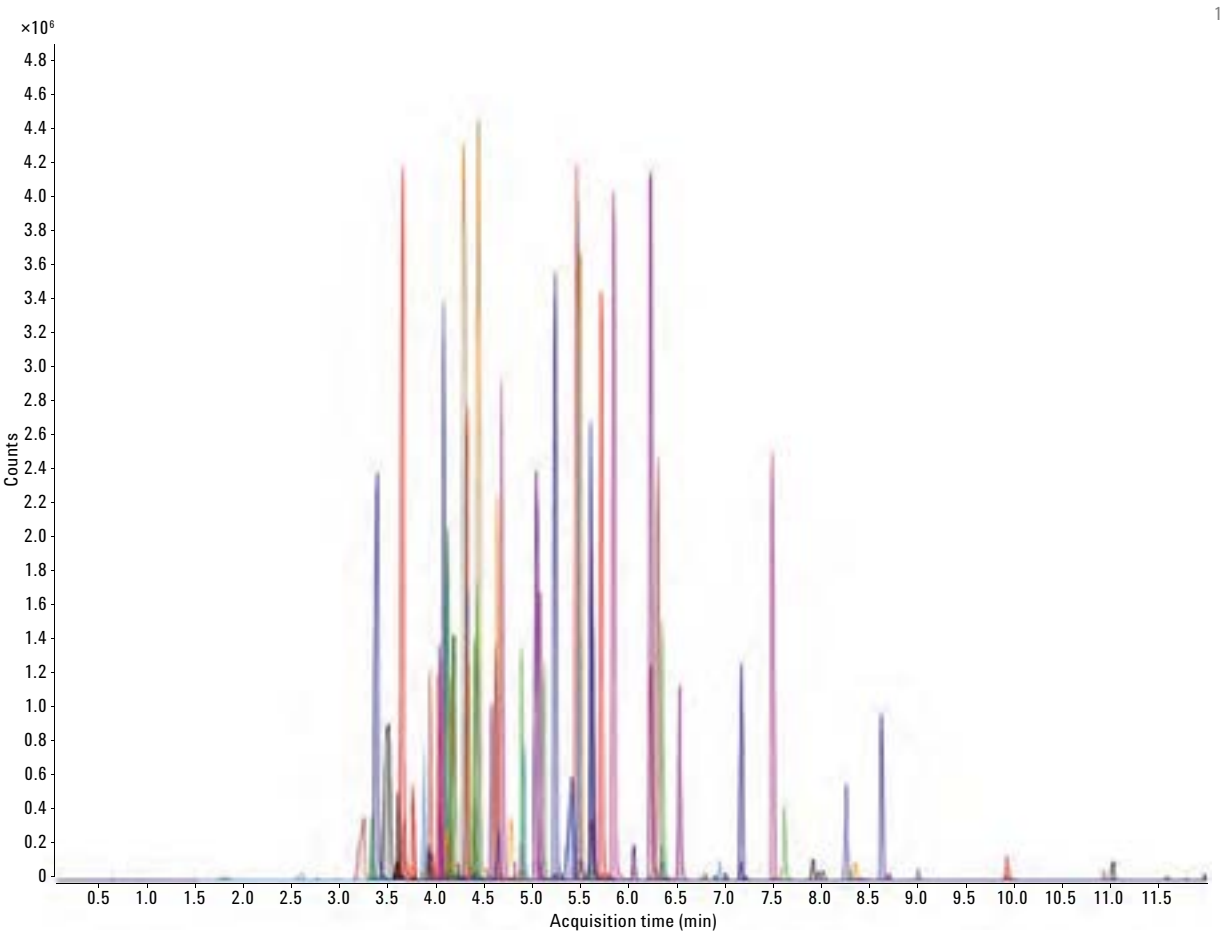

Figure 1. Sample chromatogram at 50 ng/g for 122 veterinary drugs.

An Agilent 6545 Accurate-Mass Quadrupole Time-of-Flight LC/MS system with Agilent Jet Stream dual electrospray source operating in positive mode was used for this analysis. The mass spectrometer operation conditions are detailed in Table 3. The 6545 Q-TOF LC/MS was tuned using the Agilent tune solution (p/n G1969-85000) over the entire mass range. Using the SWARM tune capability in the 6545 Q-TOF LC/MS, the instrument was tuned with the fragile ion tune for a mass range of *m/z* 50–750 in the 2 GHz extended dynamic range. During analysis, the reference ions consisting of purine (*m/z* 122.0509) and HP-921 (*m/z* 922.0098) were delivered to the mass spectrometer from reference bottle A on the mass spectrometer.

Table 3. Mass Spectrometer Conditions

Parameter	Value
Instrument	Agilent 6545 Accurate-Mass Q-TOF LC/MS
Ionization mode	Positive electrospray ionization with jet stream
Instrument mode	2 GHz extended dynamic range
Instrument tune range	SWARM tune with fragile ion (*m/z* 50–750)
Mass range	*m/z* 50–1,000
Drying gas temperature	200 °C
Drying gas flow	11 L/min
Sheath gas temperature	375 °C
Sheath gas flow	11 L/min
Nebulizer gas	35 psi
Fragmentor	135 V
Capillary	3,500
Nozzle voltage	300 V
Skimmer	45
Collision energy	0, 10, 40 V

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All Ions MS/MS workflow and data analysis

The All Ions MS/MS workflow is designed to acquire high resolution MS data simultaneously in low and high collision energy channels by collecting: (A) pseudo-molecular ion or precursor ion data from the low collision energy (CE) channel, and (B) fragment ion information from the high collision energy channel. For this experiment, the instrument was set at CEs of 0 V, 10 V, and 40 V. The 0 V setting was used to acquire precursor information, while 10 V was sufficient to get good fragment ion information for the majority of VDs. Some larger VDs, such as mectins, require higher CEs to fragment the precursor; therefore, a 40 V channel was also used to collect a third channel of data.

Data acquisition was performed using Agilent MassHunter software (Ver. B.06.01), while Agilent MassHunter Qualitative software (Ver B.07.00) was used for data analysis. The Find by Formula feature in MassHuter Qualitative Analysis was used with the database search function to take advantage of the PCDL information. The PCDL consisted of all the VDs necessary for this study with information on molecular formulas, exact monoisotopic mass, CAS number, MS/MS spectra collected at 0, 10, 20, and 40 V for the $[M+H]^+$ ion and retention times obtained from running standards against the developed LC method. This allowed significant increase in specificity of identification. Figure 2 illustrates the All Ions MS/MS workflow including data processing filters used for analysis of VDs in this study.

Results and Discussion

Identification of VDs in meat

The All Ions MS/MS workflow identifies precursor masses and uses the spectral data available in the Agilent PCDL to look for coeluting fragments in the high collision energy channel. Before showing how such data can be reviewed in a high throughout scenario, Figure 3 illustrates the component pieces of data that are used to automatically verify the results in MassHunter Qualitative with the All Ions MS/MS approach. The inset 3A indicates the mass spectrum for novobiocin identified in bovine muscle at the 1x tolerance level with the expected isotope abundances and spacing in red boxes matched up against actual data (vertical red sticks). The mass accuracy, isotope spacing, isotope abundance, and retention time (RT) matching with the PCDL accounted for a total score of 98.41. The inset in Figure 3B is the MS/MS spectrum for novobiocin at CE of 10 V, available in the Agilent PCDL, with the $[M+H]^+$ precursor (I) and the three most abundant fragment ions (II, III, and IV) denoted. Figure 3C shows the actual chromatographic peaks seen in the muscle sample at the correct RT for the four ions. Fragment II (*m/z* 189.0910) and III (*m/z* 218.1023) were qualified, but IV (*m/z* 396.1442) had an S/N of <9.0 and was not included for further qualification (based on the S/N threshold set in the data analysis method: Figure 2). Data for the fragments were assessed in MassHunter Qualitative through the coelution score and plot.

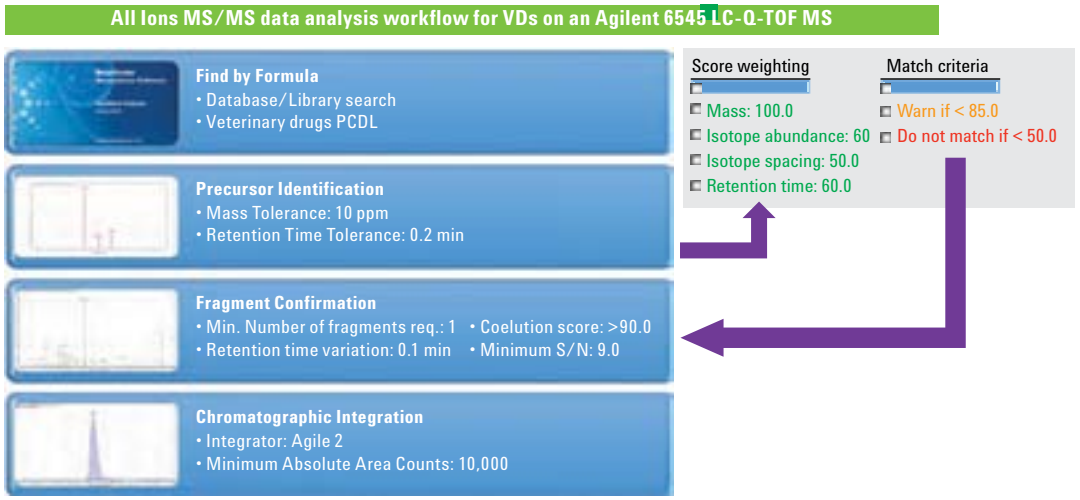

Figure 2. Agilent All Ions MS/MS data analysis workflow.

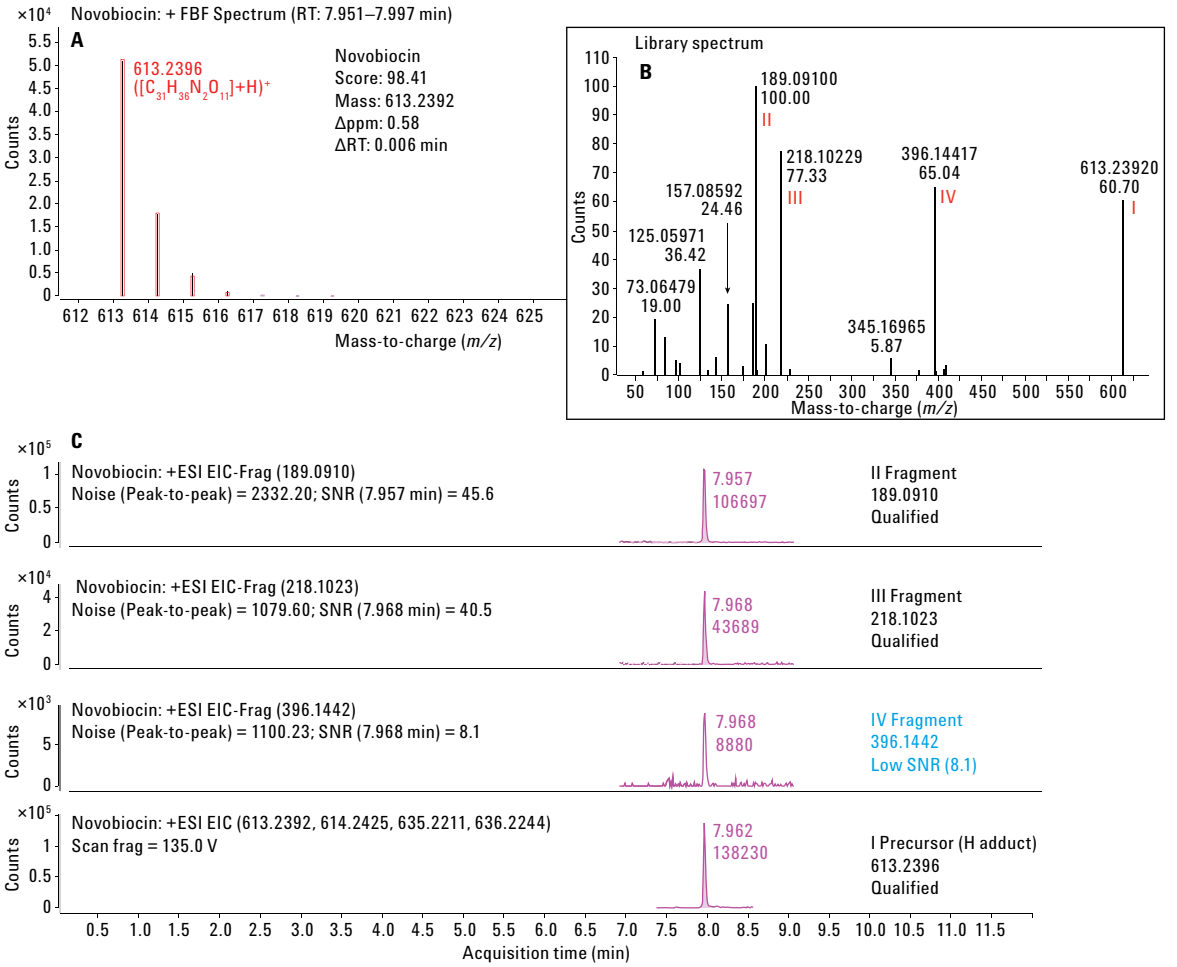

Figure 3. *A) Mass spectra and isotope spacing of Novobiocin in muscle. B) Library spectra in Agilent PCDL at CE: 10 eV for Novobiocin with precursor (I) and the three most abundant fragments (II, III, and IV), compared to (C) actual sample data. Compound positively identified.*

In Figure 4A, the plot overlays the extracted ion chromatogram of the molecular ion peak over the fragment ion peaks for novobiocin in the bovine muscle. The coelution score for each fragment ion (value 0–100, where 100 is the highest possible score) was calculated based on its intensity ratio to the reference ion across the elution time range after normalization, and applying a weighting to de-emphasize the contribution at the beginning and end of the reference ion peak. An RT shift, different peak widths, or different peak symmetry (fronting, tailing) will all negatively impact the coelution score. Figure 4B shows the ion coelution plot of novobiocin in the muscle sample. The coelution plot was prepared by overlaying the chromatogram of each fragment ion with the reference ion (precursor ion in LC/MS) after normalizing to the maximum intensity of both within the elution time range of the reference ion, and plotting the intensity ratios within that time range. Ratios of 1 or close to 1 across the center of the reference ion peak indicate that a fragment ion exhibits strong coelution. The plot provides a powerful visual assessment of the validity of a fragment signal.

The related ions coelution score is also a productive route to verifying the reliability of a hit occurring with the software. In addition, a threshold can be set to help filter out potential false positives. In this method, the data analysis required that that at least one fragment provided a coelution score of over 90.0. Consequently, novobiocin in Figure 4 was positively identified in this sample.

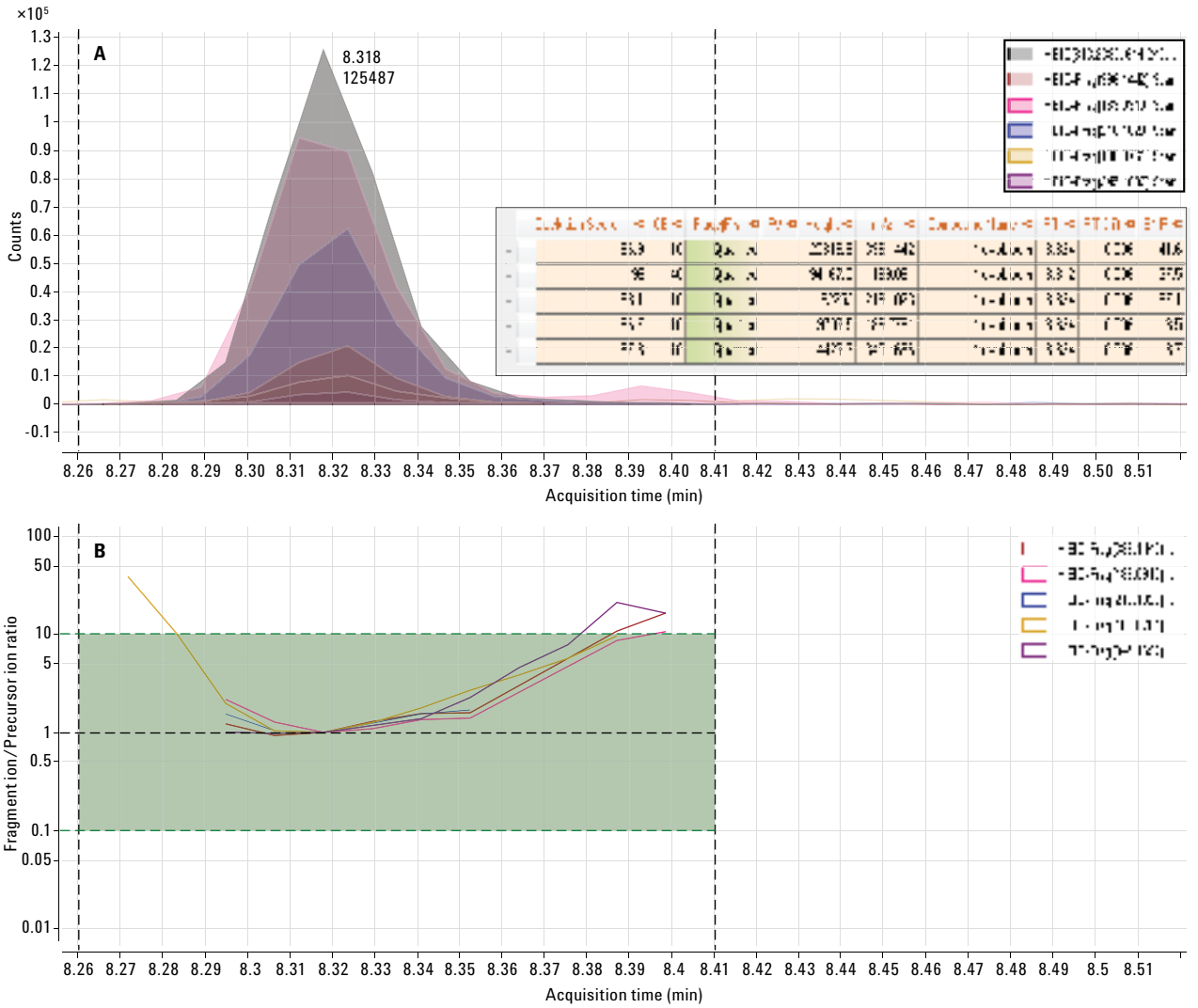

Figure 4. A) EICs of precursor and fragment ions for novobiocin in bovine muscle. B) Normalized fragment to precursor ion ratio plotted against retention time.

Reduction in false positives

The presence of isomers, isobaric compounds, interferences, and coeluting matrix elements in real samples can often mean that the measurement of accurate mass for a precursor ion alone is not a definitive compound identification. The All Ions MS/MS workflow described above is a powerful tool to achieve this, but it is most powerful when combined with a RT requirement. For this reason, we used the Veterinary Drugs AMRT PCDL, which includes the RTs specific to the LC method described in this work. The availability of high resolution fragment ion spectra that include retention times in the Agilent PCDL dramatically reduces the detection of false positives without the need to continually inject analytical standards.

Figure 5 shows an example where the use of RT matching and fragment ion verification available through the Agilent VD PCDL prevented a false positive. Enrofloxacin, a fluoroquinolone, was detected in a spiked sample of kidney extract at 4.088 and 6.015 minutes. Both species had a mass error of <2.0 ppm compared to the $[M+H]^+$ ion for enrofloxacin (360.1718), and would both have been characterized as a detect for the compound even with a tight mass tolerance window of 5.0 ppm. However, using the **verify with fragment ions** option in MassHunter Qualitative software, none of the four most abundant fragments of enrofloxacin were present in the 6.015 minutes peak, while all ion fragments were detected in the 4.088 minutes peak.

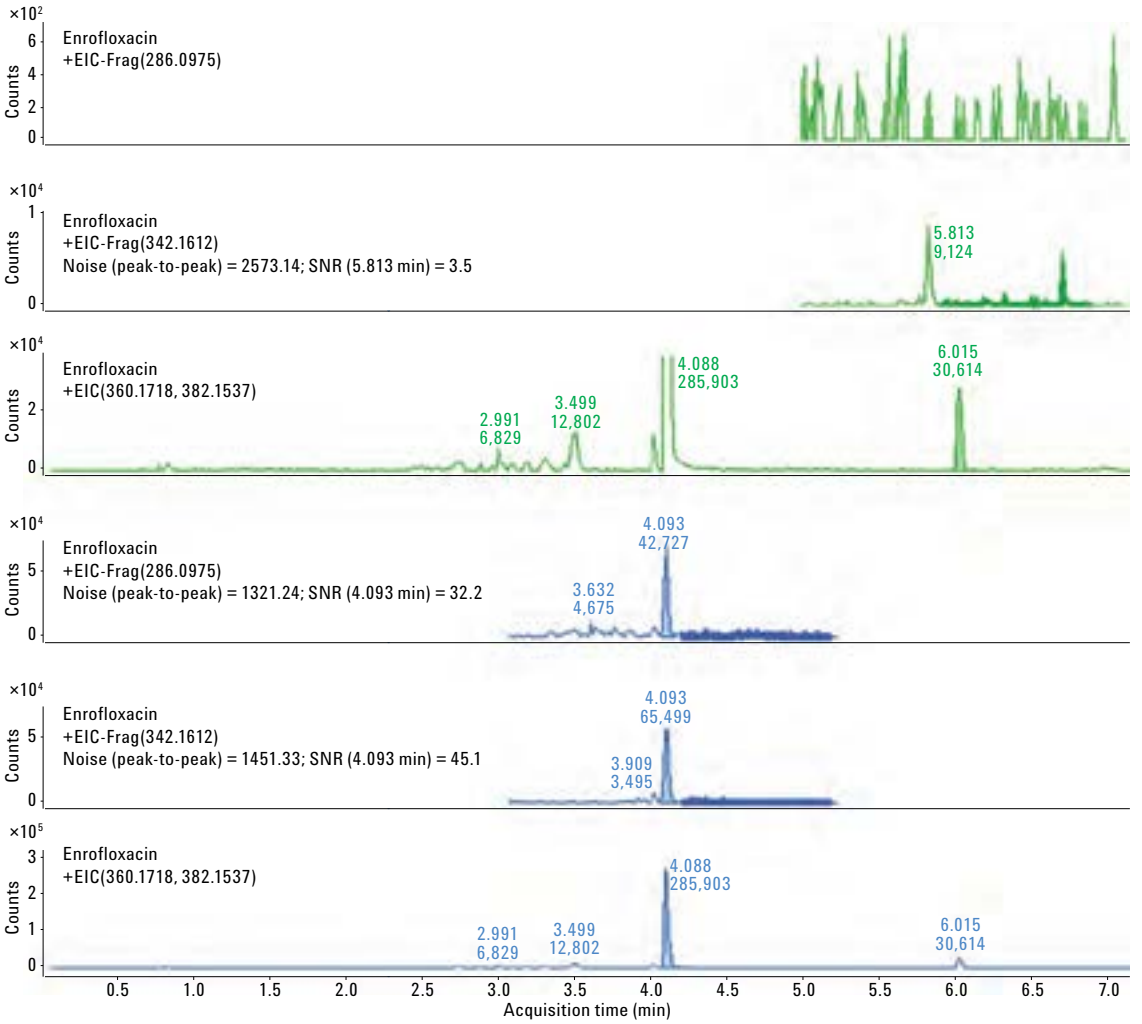

Figure 5. Potential false positive of enrofloxacin dentified through fragment ion confirmation using the Agilent All Ions workflow.

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It was stated earlier that retention time was also seen as a powerful qualifier and was also used to verify results through inclusion in the PCDL. The Agilent Veterinary Drugs PCDL with the RT information for these compounds included was used to verify that the enrofloxacin peak is expected at 4.23 minutes in this method. This was done by selecting the quality ions with the **Mass and Retention time** feature in the MassHunter Qualitative software with a ±0.2 minute RT setting. It was therefore unnecessary to inject all analytical standards with each run, which is a welcome benefit when over 100 compounds are involved. This also means that this method and it's associated PCDL with retention times can be implemented very easily in other labs and is very cost-effective and straightforward to run.

Sensitive detection of VDs in bovine kidney, liver, and muscle extracts

The VDs were classified as being detected if there was a match within the tolerance limits for the molecular mass (<10.0 ppm), the presence of at least one coeluting fragment ion at an S/N ratio >9.0, and a RT within 0.2 minutes. Compounds were characterized as being tentatively identified if the precursor ion was within the 10.0 ppm tolerance, but either the RT was off by >0.2 minutes, or fragment ions were not found or had an ions coelution score of <90.0. Figure 6 presents the results for all three matrices and reagent blanks at the three spiking levels. At least 92% of VDs were either positively or tentatively identified in all samples. In the standards, 98% (2x), 96% (1x), and 94% (0.5x) of VDs were detected. When looking at the matrix spiked samples, the detects ranged from 94–96% in the liver, 94–97% in the muscle, and 93–97% in the kidney. The VDs positively detected (with fragment ion and RT agreement) in the standards were between 88% and 90% across the three spike levels. Similarly, 81–88% (liver), 82–88% (muscle), and 79–86% (kidney) of the VDs were positively identified in the matrix samples.

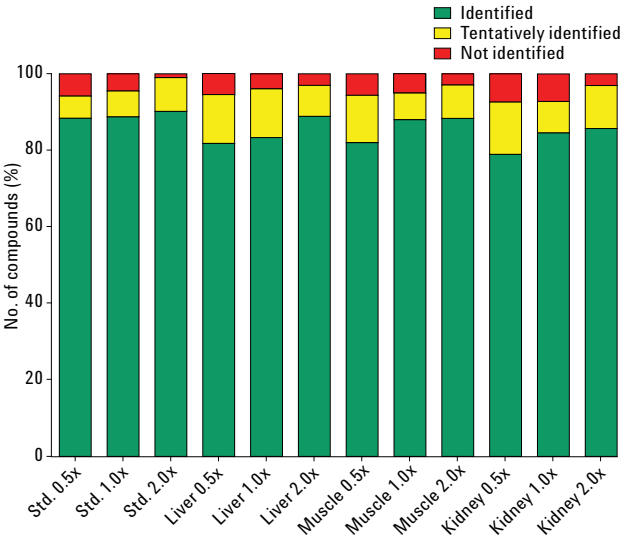

Figure 6. Percentage of veterinary drugs identified in spiked samples of reagent blank and matrix at three different levels (x: tolerance levels).

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Only two compounds (cephapirin and cimaterol) were not detected in any sample or the standards at all spike levels. Table 4 lists a few compounds that were not identified in some of the samples. These nine compounds require some extra study to determine their feasibility of analysis with the current method. Most, if not all, of these cases are probably the result of degradation in the spiked samples and in the standards themselves, some of which were only available as mixtures in solution. In both cases, there was an unavoidable 2-week delay between preparing the spiked extracts and standards to the time of injection on the instrument. In a separate project carried out on another Agilent Q-TOF instrument, all six β-lactams in question were reliably detectable at half the spiking level for the matrices described using the same method. In this case, much fresher samples and standards were used. Further work is planned to determine if all these nine compounds can join the others as being detectable and identifiable at their half tolerance level, using the above screening method.

Approximately 10% of compounds were tentatively identified in each of the three matrices. We plan to revisit some of these compounds to investigate whether the analysis of fresher spikes and standards can result in not only the detection of precursor ions, but also in the diagnostic fragments required for full verification according to the previously described requirements set by this method.

Overall, the results in the three matrices were similar, and detection rates were high for all in a single run of less than 15 minutes.

Table 4. List of VDs Not Identified in the Samples

Class	Std. 0.5x	Std. 1.0x	Std. 2.0x	Liver 0.5x	Liver 1.0x	Liver 2.0x
β-Agonist	Cimaterol	Cimaterol	Cimaterol	Cimaterol	Cimaterol	Cimaterol
β-Lactam	Cephapirin	Cephapirin	Cephapirin	Cephapirin	Cephapirin	Cephapirin
β-Lactam	Cloxacillin	Cloxacillin	✓	Cloxacillin	Cloxacillin	Cloxacillin
β-Lactam	Amoxicillin	Amoxicillin	✓	Amoxicillin	Amoxicillin	Amoxicillin
β-Lactam	Ampicillin	Ampicillin	✓	Ampicillin	Ampicillin	✓
β-Lactam	Nafcillin	✓	✓	Nafcillin	✓	✓
β-Lactam	Oxacillin	✓	✓	Oxacillin	✓	✓
All other compounds	✓	✓	✓	✓	✓	✓

Class	Kindney 0.5x	Kindney 1.0x	Kindney 2.0x	Muscle 0.5x	Muscle 1.0x	Muscle 2.0x
β-Agonist	Cimaterol	Cimaterol	Cimaterol	Cimaterol	Cimaterol	Cimaterol
β-Lactam	Cephapirin	Cephapirin	Cephapirin	Cephapirin	Cephapirin	Cephapirin
β-Lactam	Cloxacillin	Cloxacillin	Cloxacillin	Cloxacillin	Cloxacillin	Cloxacillin
β-Lactam	Amoxicillin	Amoxicillin	Amoxicillin	Amoxicillin	Amoxicillin	Amoxicillin
β-Lactam	Ampicillin	Ampicillin	✓	Ampicillin	Ampicillin	✓
β-Lactam	Nafcillin	Nafcillin	✓	Nafcillin	✓	✓
β-Lactam	Oxacillin	Oxacillin	✓	Oxacillin	✓	✓
Misc.	Zeranol	Zeranol	✓	✓	Zeranol	✓
Thyreostat	Propyl-thiouracil	Propyl-thiouracil	✓	✓	✓	✓
All other compounds	✓	✓	✓	✓	✓	✓

Quantification of VDs using Q-TOF LC/MS

To determine the linearity of the LC Q-TOF in the All Ions MS/MS method developed for the VDs, a four or five point matrix-matched calibration curve of 113 VDs were prepared in ground beef and liver samples extracted with the EMR—Lipid procedure. Levels were between 2 (or 10) and 100 ng/g.

Quantification of a large number of disparate analytes in complex matrices is difficult, and almost always requires the use of several surrogates and internal standards to correct for variable ion suppression effects through the extraction and analytical run. Selection of good internal standards depends on various factors including analytics and economics. To avoid bias, raw data without correction with an internal standard have been provided in this section. To determine the linearity for quantification, the coefficient of determination (R^2) was calculated for each analyte in ground beef and liver matrices. Over 95% and 93% of target analytes had $R^2 > 0.90$ (85% and 86% $R^2 > 0.99$) in ground beef and liver, respectively. Only 5% and 7% of the VDs analyzed had $R^2 < 0.90$ in beef and liver. Figure 7 depicts the calibration curves for ipronidazole and enrofloxacin in ground beef and liver between 2 ng/g and 100 ng/g. All calibration curves were linearly fitted with no weighting.

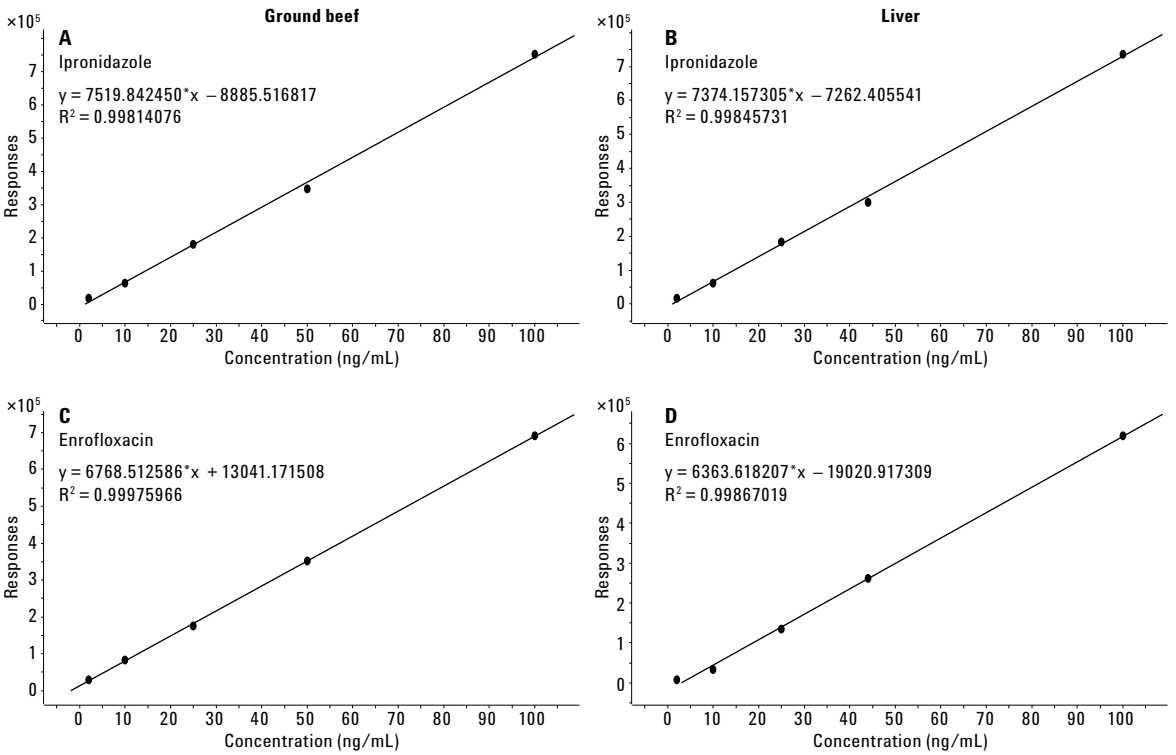

Figure 7. Calibration curves for Ipronidazole (A nd B) and Enrofloxacin (C and D) in ground beef and liver; (2–100 ng/g).

Conclusions

This application note demonstrates the ability of the Agilent 6545 Q-TOF LC/MS, with its high resolution and sensitivity, to analyze over 120 VDs in relevant matrices including bovine muscle, liver, and kidney at ng/g levels. The use of a simple and efficient Agilent All Ions MS/MS workflow allows for analyte detection and identification using fragment ions in the same analytical run. This dramatically reduces potential false positives. The availability of accurate mass, MS/MS spectra, and updated retention times under specific LC conditions in the Agilent Veterinary Drugs PCDL further improves compound identification and robustness in complex matrices. The ability to perform quantification was demonstrated with calibration curve generation. As a result, sensitive qualitative and quantitative information for VDs in meat can be performed using the 6545 Q-TOF LC/MS instrument in a single analytical run.

Acknowledgment

We thank Steven Lehotay of the USDA-ARS Eastern Regional Research Center for providing veterinary drug solutions and feedback in this study and application note.

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- Method Setup Guide that shows you how to create MRM, dMRM, and tMRM methods
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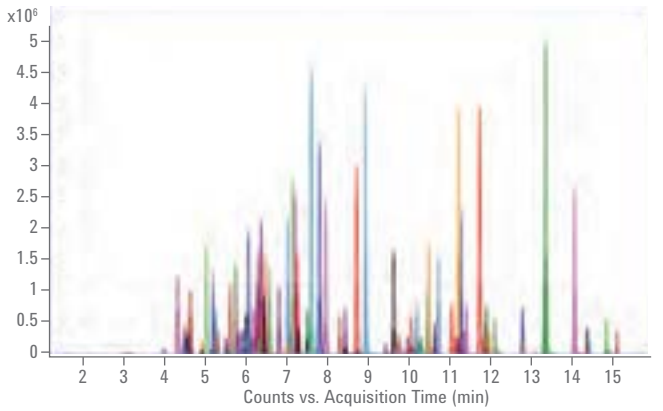

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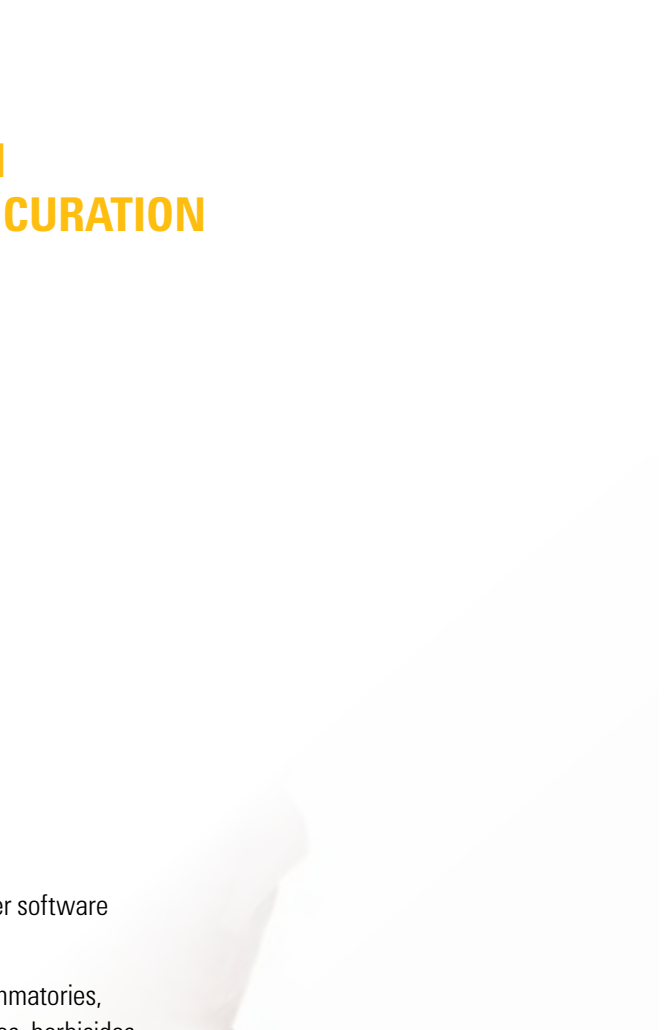

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Analysis of Veterinary Drugs in Meat with the Agilent 6495 Triple Quadrupole LC/MS

Application Note

Food

Authors

Tarun Anumol, Joan M. Stevens, and Jerry Zweigenbaum
Agilent Technologies Inc.

Abstract

A method using an Agilent 1290 Infinity II LC coupled to an Agilent 6495 Triple Quadrupole LC/MS for the rapid and sensitive analysis of 120 veterinary drugs in bovine meat has been developed. The analytical run time is 12 minutes, while limits of detection and quantification range between 0.1–2 ng/mL and 0.1–5 ng/mL, respectively. Three optimized MRM transitions were selected for all but three veterinary drugs, ensuring selectivity and robustness. Quantification of real samples was possible with most compounds having R^2 >0.99 when two sets of matrix-matched calibration curves were performed. The method is reproducible and repeatable as indicated by the results of intra- and interday variability tests that produce relative standard deviations of <15 % for more than 90 % of the compounds tested.

Introduction

The monitoring of veterinary drugs in food is critical due to contamination and the possibility of increased antimicrobial resistance by pathogenic microorganisms [1]. Veterinary drug administration in animals is important to treat diseases and promote growth. However, improper dosing or illegal practices can lead to contamination in meat for human consumption. As a result, veterinary drugs in food are regulated in several regions including the US, Europe, China, Australia, and others [2-4].

Analysis of veterinary drugs is challenging due to their many classes with diverse structures and varying chemical properties. To meet the needs of analytical labs, rapid and efficient techniques using multiclass, multiresidue methods analyzing >100 veterinary drugs in a single run are required. Additional goals are detection limits of low µg/kg, with good reproducibility and high sample throughput. The use of ultrahigh performance liquid chromatography (UHPLC) coupled to tandem mass spectrometers (MS/MS) is the gold standard for this analysis. This technique offers the requisite analytical sensitivity and robustness while allowing for time and labor savings compared to other techniques for analysis of veterinary drugs.

This application note describes the development of a rapid UHPLC/MS/MS method with the Agilent 1290 Infinity II UHPLC and an Agilent 6495 Triple Quadrupole LC/MS for the analysis of 120 veterinary drugs in animal meat. The method used three transitions for each analyte (except three) satisfying US and EU specifications for identification. The sensitivity of the method was determined by calculating the limits of detection and quantification in kidney and liver. Other method validation protocols such as linearity, robustness, and reproducibility were also evaluated in this study.

Experimental

Standards and reagents

All native veterinary drug standards were bought from Sigma-Aldrich (St. Louis, MO), and prepared between 300 and 1,000 µg/mL in solvent (either acetonitrile, methanol, dimethyl sulfoxide, or water depending on solubility). The three internal standards used in this study (flunixin-d_3, nafcillin-d_5, and doxycycline-d_3) were acquired from Toronto Research Chemicals (Toronto, ON). LC/MS grade acetonitrile and water were procured from Burdick and Jackson (Muskegon, MI), while formic acid (>98 %, Suprapur) was obtained from EMD Millipore (Darmstadt, Germany).

Instrumentation

Separation of analytes for this method was performed using an Agilent 1290 Infinity II LC with a 20 µL injection loop and multiwash capability. An Agilent 6495 Triple Quadrupole LC/MS with the iFunnel and Jet Stream technology was used as the detector. Analysis was performed in simultaneous positive and negative electrospray ionization mode. All data acquisition and processing was performed using Agilent MassHunter software (Version 07.00). Tables 1 and 2 show the instrument conditions.

Table 1. Optimized LC Conditions

Parameter	Value
Instrument	Agilent 1290 Infinity II with 20 µL flex loop and multiwash
Column	Agilent ZORBAX C-18 Eclipse Plus 2.1 × 150 mm, 1.8 µm (p/n 959759-902)
Guard column	Agilent ZORBAX C-18 Eclipse Plus 2.1 × 5 mm, 1.8 µm (p/n 821725-901)
Column temperature	30 °C
Injection volume	15 µL
Mobile phase	A) Water + 0.1 % formic acid B) Acetonitrile
Run time	12 minutes
Equilibration time	2 minutes
Flow rate	0.5 mL/min
Gradient	Time (min) A (%) 0.0 98 1.0 98 1.5 85 2.5 70 6.0 55 8.5 20 10.0 0 11.0 0 11.2 98

Table 2. Optimized MS conditions

Parameter	Value
Mass spectrometer	Agilent 6495 Triple Quadrupole LC/MS
Gas temperature	150 °C
Gas flow rate	18 L/min
Sheath gas temperature	300 °C
Sheath gas flow rate	11 L/min
Nebulizer pressure	35 psi
Capillary voltage	4,000 V (3,000 V)
Nozzle voltage	500 V (1,500 V)
Ion funnel HPRF	200 v (90 V)
Ion funnel LPRF	100 V (60 V)
Delta EMV	200 V
Time segments	Time (min) Flow 0.0 Waste 0.7 MS

Sample preparation

The Agilent Enhanced Matrix Removal—Lipid (EMR—L) product was used for sample extraction of veterinary drugs in this study. The EMR—L selectively removes lipids while not trapping contaminants of interest, and has been shown to be effective in extracting several classes of compounds including pesticides, toxins, and PAHs in food [5,6]. Details of the procedure followed for veterinary drug extraction using EMR—L, and product information can be found in previously published literature [7,8]. Briefly, 2 g samples of homogenized bovine kidney and liver were weighed and placed into 50-mL polypropylene tubes. A 10 mL solution of acetonitrile with 5 % formic acid was added to the sample and mixed with an orbital shaker for 5 minutes, followed by centrifugation at 4,000 rcf for 5 minutes. After this, 5 mL of the supernatant was added to the 1 g EMR—L tube, which had been activated previously with 5 mL of 5 mM ammonium acetate solution. The tube was then vortexed and centrifuged at 4,000 rcf for 5 minutes. The 5 mL of supernatant from this solution was transferred to a 15-mL centrifuge tube to which 2 g of $MgSO_4$ were added from the EMR—L pouch with vortexing and centrifugation, as before. Finally, a 100 µL extract was collected from the tube and diluted with 400 µL of ultrapure water in a 1-mL polypropylene vial, ready for LC/MS analysis.

Results and Discussion

Compound selection and optimization

The 120 veterinary drugs analyzed in this study were selected based on a monitoring list used by the United States Department of Agriculture's Agricultural Research Service (USDA-ARS) [9]. The compound-specific parameters including precursor ion, three most abundant unique product ions, and collision energy were determined by running each standard through the Agilent Optimizer software. Three specific transitions were selected for each compound (except thiouracil, metronidazole, and clindamycin) to satisfy both US and EU regulations for identification by mass spectrometry. Table 3 shows the optimized transitions, retention times, and other relevant parameters for each compound. The tolerance levels for each veterinary drug were obtained from the USDA-ARS, and were used to prepare calibration curves, and perform spike studies, described later. Care was taken to select transitions that did not have matrix interferences. Cimaterol had matrix interferants for the 220.1 → 202.1 and 220.1 → 160.1 transitions, therefore, extra transitions were obtained. The ion ratio intensities were helpful in identifying these issues (as opposed to reporting cimaterol as incurred). Figure 1 represents a chromatogram of cimaterol in standard and liver blank with the different MRM transitions that indicate the presence of two of the transitions in matrix but at different ion ratios than would be expected based on the standard.

Table 3. Optimized Compound Parameters and Tolerance Levels with Retention Times for 120 Veterinary Drugs

Compound	Class	Tolerance (ng/g)	Precursor ion	Product ion	Collision energy	RT (min)	Delta RT
Thiouracil	Thyreostat	400	129	90.1	8	0.95	1.0
			129	82.3	16		
Florfenicol amine	Phenicol	300	248.1	230.1	8	0.99	0.8
			248.1	130.1	28		
			248.1	91.1	50		
Florfenicol	Phenicol	300	358	241	16	1	0.6
			358	206	28		
			358	170	32		
Sulfanilamide	Sulfonamide	100	173	156	5	2	0.6
			173	92	25		
			173	76	5		
Methyl-thiouracil	Thyreostat	400	143	126	20	2.5	0.6
			143	86	20		
			143	84	20		
Amoxicillin	β-Lactam	10	367	349.1	4	2.56	0.6
			367	208	8		
			367	114	56		

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Table 3. Optimized Compound Parameters and Tolerance Levels with Retention Times for 120 Veterinary Drugs (continued)

Compound	Class	Tolerance (ng/g)	Precursor ion	Product ion	Collision energy	RT (min)	Delta RT
Salbutamol	β-Agonist	10	240.2	222.2	4	2.6	0.6
			240.2	166.1	4		
			240.2	148.1	16		
Tildipirosin	Macrolide	100	734.5	561.5	36	2.65	0.6
			734.5	174	44		
			734.5	98.2	56		
Cimaterol	β-Agonist	10	220.1	202.1*	4	2.66	0.6
			220.1	160.1*	12		
			220.1	143.1	14		
			220.1	116.1	20		
Hydroxy- metronidazole	Coccidiostat	10	188.1	126.1	16	2.7	0.6
			188.1	123.1	8		
			188.1	68.0	22		
Lincomycin	Lincosamide	100	407.2	359.2	16	2.7	0.6
			407.2	126.1	24		
			407.2	42.2	68		
Hydroxy-dimetridazole	Coccidiostat	50	158.1	140	8	2.8	0.6
			158.1	55.2	20		
			158.1	42.2	40		
Metronidazole	Coccidiostat	10	172.1	128	12	2.83	0.6
			172.1	82.1	24		
Dipyrone metabolite	Anti- inflammatory	200	218.1	187.1	18	2.85	0.6
			218.1	125	16		
			218.1	97	14		
Levamisole	Anthelmintic	100	205.1	178.1	20	2.9	0.6
			205.1	123	32		
			205.1	91.1	44		
Albendazole-2-aminosulfone	Anthelmintic	50	240.1	198	20	2.97	0.6
			240.1	133.1	20		
			240.1	105	40		
Ampicillin	β-Lactam	10	350	160	4	3	0.6
			350	114	36		
			350	106	16		
Dimetridazole	Coccidiostat	10	142.1	96.1	16	3	0.6
			142.1	81.1	28		
			142.1	54.1	36		
Thiabendazole	Anthelmintic	100	202	175	24	3	0.6
			202	131	36		
			202	65	52		
Ronidazole	Coccidiostat	10	201.1	140.1	8	3.09	0.6
			201.1	55.2	20		
			201.1	154.9	8		
Desethylene Ciprofloxacin	Fluoroquinolone	100	306.1	288.2	20	3.1	0.6
			306.1	268.1	28		
			306.1	217	44		

* Potential matrix interferants in liver extract.

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Table 3. Optimized Compound Parameters and Tolerance Levels with Retention Times for 120 Veterinary Drugs (continued)

Compound	Class	Tolerance (ng/g)	Precursor ion	Product ion	Collision energy	RT (min)	Delta RT
Norfloxacin	Fluoroquinolone	50	320.1	302.1	20	3.11	0.6
			320.1	282.1	40		
			320.1	231.1	40		
Ciprofloxacin	Fluoroquinolone	50	332.1	314.1	20	3.15	0.6
			332.1	288.2	20		
			332.1	231.1	40		
Sulfadiazine	Sulfonamide	100	251.1	108.1	20	3.16	0.6
			251.1	92.1	28		
			251.1	65.1	48		
Danofloxacin	Fluoroquinolone	200	358.2	340.1	20	3.19	0.6
			358.2	314.2	16		
			358.2	82.1	48		
Oxytetracycline	Tetracycline	1000	461.2	443.1	6	3.2	0.6
			461.2	426.1	14		
			461.2	201.1	48		
Ractopamine	β-Agonist	30	302.2	284.2	8	3.21	0.6
			302.2	164.1	12		
			302.2	107.1	24		
Orbifloxacin	Fluoroquinolone	50	396.2	352.1	20	3.22	0.6
			396.2	295	28		
			396.2	226	44		
Enrofloxacin	Fluoroquinolone	100	360.2	342.2	20	3.25	0.6
			360.2	316.2	16		
			360.2	245.1	32		
Carbadox	Miscellaneous	30	263.1	230.9	12	3.26	0.6
			263.1	129.1	32		
			263.1	102	50		
Azaperone	Tranquilizer	10	328.2	165.1	20	3.27	0.6
			328.2	123	40		
			328.2	121.1	20		
Sulfapyridine	Sulfonamide	100	250.1	156	20	3.28	0.6
			250.1	108	20		
			250.1	92	20		
Propylthiouracil	Thyreostat	50	171.1	154	20	3.3	0.6
			171.1	60	40		
			171.1	54	40		
Sulfathiazole	Sulfonamide	100	256	156	12	3.4	0.6
			256	92.1	28		
			256	65.1	52		
Sulfamerazine	Sulfonamide	100	265.1	156	12	3.41	0.6
			265.1	92.1	28		
			265.1	65.1	60		
Quinoxaline 2-carboxylic acid	Miscellaneous	30	175	131.2	16	3.43	0.6
			175	129.1	16		
			175	75.2	50		

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Table 3. Optimized Compound Parameters and Tolerance Levels with Retention Times for 120 Veterinary Drugs (continued)

Compound	Class	Tolerance (ng/g)	Precursor ion	Product ion	Collision energy	RT (min)	Delta RT
Xylazine	Tranquilizer	10	221.1	105.1	40	3.43	0.6
			221.1	90	40		
			221.1	72	40		
Clenbuterol	β-Agonist	10	277.1	259.1	4	3.44	0.6
			277.1	203	12		
			277.1	132.1	32		
Chlortetracycline	Tetracycline	1000	479.1	462	12	3.45	0.65
			479.1	444	20		
			479.1	154.1	36		
Thiamphenicol	Phenicol	10	354	290	12	3.46	0.6
			354	227	18		
			354	184.9	34		
Cefapirin	β-Lactam	100	424.1	364	8	3.48	0.6
			424.1	124.1	48		
			424.1	112	24		
Mercaptobenzimi dazole	Thyreostat	25	151	118.1	28	3.47	0.6
			151	93	24		
			151	65.1	48		
Cefazolin	β-Lactam	100	455	323	4	3.49	0.6
			455	156	16		
			455	124	32		
Difloxacin	Fluoroquinolone	50	400.1	382.1	20	3.5	0.6
			400.1	356.2	16		
			400.1	299.1	32		
Gamithromycin	Macrolide	100	777.5	619.4	36	3.52	0.6
			777.5	158.1	54		
			777.5	116	54		
Sarafloxacin	Fluoroquinolone	50	386.1	368.1	20	3.44	0.6
			386.1	342.1	20		
			386.1	299.1	40		
Amino-mebendazole	Anthelmintic	10	238.1	133.1	44	3.54	0.6
			238.1	105.1	28		
			238.1	77.1	40		
Morantel	Anthelmintic	100	221.1	150	40	3.54	0.6
			221.1	123	40		
			221.1	111	40		
Bacitracin	Miscellaneous	500	711.9	669.3	20	3.55	0.6
			711.9	227.1	40		
			711.9	199.1	40		
Sulfamethazine	Sulfonamide	100	279.1	186.1	12	3.58	0.6
			279.1	124.1	24		
			279.1	92.1	32		
Clindamycin	Lincosamide	100	425.2	377.2	20	3.60	0.6
			425.2	126.1	20		
Sulfamethizole	Sulfonamide	100	271	156	10	3.62	0.6
			271	108	20		
			271	92	40		

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Table 3. Optimized Compound Parameters and Tolerance Levels with Retention Times for 120 Veterinary Drugs (continued)

Compound	Class	Tolerance (ng/g)	Precursor ion	Product ion	Collision energy	RT (min)	Delta RT
Sulfamethoxypyr idazine	Sulfonamide	100	281.1	156	12	3.64	0.6
			281.1	92.1	32		
			281.1	65.1	60		
Aminoflubendazole	Anthelmintic	10	256.1	123	40	3.66	0.6
			256.1	95	40		
			256.1	75	40		
Hydroxy-ipronidazole	Coccidiostat	10	186.1	168.1	8	3.68	0.6
			186.1	122.1	20		
			186.1	106.1	44		
Tilmicosin	Macrolide	100	869.6	696.4	44	3.68	0.6
			869.6	174.1	48		
			869.6	88.1	70		
Cambendazole	Anthelmintic	10	303.1	261	16	3.73	0.6
			303.1	217.1	32		
			303.1	190	44		
Doxycycline	Tetracycline	100	445.2	428.1	16	3.78	0.6
			445.2	410	24		
			445.2	321.1	28		
Doxycycline-d_3	Internal Standard	–	448.1	431.2	16	3.78	0.8
			448.1	155.1	36		
Carazolol	Tranquilizer	10	299.2	222.1	20	3.81	0.6
			299.2	116.1	20		
			299.2	56	40		
Tetracycline	Tetracycline	1000	445.2	427.1	10	3.85	0.6
			445.2	410.1	20		
			445.2	154	40		
Phenyl-thiouracil	Thyreostat	400	205	188	20	3.86	0.6
			205	103	28		
			205	86.2	28		
Oxibendazole	Anthelmintic	10	250.1	218.1	16	3.87	0.6
			250.1	176.1	28		
			250.1	148	40		
Oxfendazole	Anthelmintic	800	316.1	284	16	3.97	1.0
			316.1	191.1	16		
			316.1	159	32		
Albendazole sulfone	Anthelmintic	50	298.1	266.1	20	4.1	0.6
			298.1	224	20		
			298.1	159	40		
Sulfadimethoxine	Sulfonamide	100	311.1	156	16	4.21	0.6
			311.1	92.1	36		
			311.1	65.1	60		
Sulfaethoxypyrid azine	Sulfonamide	100	298.1	158	16	4.25	0.6
			298.1	108.1	32		
			298.1	92.1	32		
Sulfachloropyrid azine	Sulfonamide	100	285	156	12	4.26	0.6
			285	92.1	24		
			285	65.1	60		

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Table 3. Optimized Compound Parameters and Tolerance Levels with Retention Times for 120 Veterinary Drugs (continued)

Compound	Class	Tolerance (ng/g)	Precursor ion	Product ion	Collision energy	RT (min)	Delta RT
Sulfamethoxazole	Sulfonamide	100	254.1	156	12	4.35	0.6
			254.1	92.1	24		
			254.1	65.1	48		
Erythromycin	Lincosamide	100	734.5	576.3	16	4.38	0.6
			734.5	158.1	32		
			734.5	83.1	60		
Chloramphenicol	Phenicol	10	321.1	257	18	4.43	0.6
			321.1	151.9	26		
			321.1	80	50		
Ipronidazole	Coccidiostat	10	170.1	124	16	4.49	0.6
			170.1	109	24		
			170.1	81.1	36		
Tylosin	Macrolide	200	916.5	174.1	44	4.67	0.6
			916.5	101	56		
			916.5	83	60		
Acepromazine	Tranquilizer	10	327.2	222.1	40	4.73	0.6
			327.2	86.1	20		
			327.2	58.1	40		
Haloperidol	Tranquilizer	10	376.2	165.1	24	4.75	0.6
			376.2	123	50		
			376.2	95.1	50		
Promethazine	Tranquilizer	10	285.1	198	28	4.78	0.6
			285.1	86.2	20		
			285.1	71.3	48		
Prednisone	Anti- inflammatory	100	359.2	341.2	10	4.84	0.6
			359.2	237.1	20		
			359.2	147.1	40		
Clorsulon	Anthelmintic	100	377.9	341.9	0	4.91	0.8
			377.9	242	40		
			377.9	142	40		
Sulfadoxine	Sulfonamide	100	311.1	156	16	4.96	0.6
			311.1	108	28		
			311.1	92.1	32		
Sulfaquinoxaline	Sulfonamide	100	301.1	156	16	4.95	0.6
			301.1	108	28		
			301.1	92	32		
Albendazole	Anthelmintic	50	266.1	234.1	16	5.01	0.6
			266.1	191	32		
			266.1	159	44		
Mebendazole	Anthelmintic	10	296.1	264.1	20	5.16	0.8
			296.1	105	36		
			296.1	77	48		
Penicillin G	β-Lactam	10	335.0	114.0	35	5.29	0.6
			335.0	160.0	18		
			335.0	176.1	20		

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Table 3. Optimized Compound Parameters and Tolerance Levels with Retention Times for 120 Veterinary Drugs (continued)

Compound	Class	Tolerance (ng/g)	Precursor ion	Product ion	Collision energy	RT (min)	Delta RT
Propionylpromaz ine	Tranquilizer	10	341.2	268.1	24	5.46	0.6
			341.2	86.2	20		
			341.2	58.2	52		
Flubendazole	Tranquilizer	10	314.1	282.1	20	5.58	0.6
			314.1	123	36		
			314.1	95.1	56		
Betamethasone	Anti-inflammatory	100	393.2	373.2	4	5.71	0.6
			393.2	237.2	12		
			393.2	147.1	32		
Chlorpromazine	Tranquilizer	10	319.1	246	28	5.77	0.6
			319.1	86.1	20		
			319.1	58.2	50		
Sulfanitran	Sulfonamide	100	334.1	137	40	6.18	0.6
			334.1	136	40		
			334.1	134.1	40		
Sulfabromomethazine	Sulfonamide	100	357	156	24	6.23	0.6
			357	108	36		
			357	92.1	36		
Zeranol	Miscellaneous	100	321.1	303.2	34	6.3	0.6
			321.1	277.2	34		
			321.1	259.1	36		
Oxacillin	β-Lactam	100	402	243	8	6.49	0.6
			402	160	8		
			402	114	40		
Triflupromazine	Tranquilizer	10	353.1	248.1	40	6.28	0.6
			353.1	86.1	20		
			353.1	58.1	40		
Fenbendazole	Anthelmintic	10	300.1	268.1	20	6.54	0.6
			300.1	159	36		
			300.1	131	56		
Virginiamycin M1	Miscellaneous	100	526.3	508.3	12	6.74	0.8
			526.3	355.2	16		
			526.3	109.1	32		
Nitroxynil	Anthelmintic	50	288.91	162	20	6.78	0.8
			288.91	127	28		
			288.91	89	44		
Cloxacillin	β-Lactam	10	436	358.2	0	7.15	0.6
			436	277	12		
			436	160	12		
Nafcillin-d_5	Internal Standard	–	420.1	204	16	7.41	0.8
			420.1	172	52		
Ketoprofen	Anti-inflammatory	10	255.1	209.1	8	7.4	0.8
			255.1	105.1	24		
			255.1	77.1	48		
Nafcillin	β-Lactam	10	415	199.1	8	7.41	0.6
			415	171	36		
			415	115	20		

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Table 3. Optimized Compound Parameters and Tolerance Levels with Retention Times for 120 Veterinary Drugs (continued)

Compound	Class	Tolerance (ng/g)	Precursor ion	Product ion	Collision energy	RT (min)	Delta RT
Flunixin	Anti-inflammatory	25	297.1	279.1	24	7.46	0.8
			297.1	259.1	32		
			297.1	236	48		
Flunixin-d_3	Internal Standard	–	300.1	282.1	29	7.46	0.8
			300.1	264.1	45		
Oxyphenbutazone	Anti-inflammatory	100	325.2	204.1	20	7.51	0.8
			325.2	148	40		
			325.2	120	20		
Meloxicam	Anti-inflammatory	100	352	140.9	16	7.68	0.8
			352	115	16		
			352	73	44		
Emamectin B1a	Anthelmintic	10	886.5	158.1	40	8.07	0.8
			886.5	126.1	40		
			886.5	82.2	54		
Haloxon	Anthelmintic	100	415	352.9	24	8.24	0.8
			415	352.9	24		
			415	211	44		
Triclabendazole sulfoxide	Anthelmintic	50	375	356.9	20	8.25	0.8
			375	313	28		
			375	242	48		
Diclofenac	Anti- inflammatory	200	296	278	4	8.54	0.8
			296	250	8		
			296	215.1	16		
Phenylbutazone	Anti- inflammatory	100	309.2	160.2	20	8.89	0.8
			309.2	120	28		
			309.2	77.1	68		
Triclabendazole	Anthelmintic	50	359	343.9	24	9.01	1
			359	274	40		
			359	171	60		
Oxyclozanide	Anthelmintic	10	397.87	361.9	20	9.06	0.8
			397.87	201.9	20		
			397.87	175.8	28		
Melengestrol acetate	Miscellaneous	25	397.2	337.3	8	9.22	0.8
			397.2	279.2	20		
			397.2	236.2	28		
Niclosamide	Anthelmintic	10	324.99	289	16	9.24	0.8
			324.99	170.9	36		
			324.99	135.1	44		
Tolfenamic acid	Anti- inflammatory	200	262.1	244.1	12	9.27	0.8
			262.1	209.1	28		
			262.1	180.1	48		
Bithionol	Anthelmintic	10	355	193.7	32	9.74	0.8
			355	162.9	28		
			355	160.9	28		

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Table 3. Optimized Compound Parameters and Tolerance Levels with Retention Times for 120 Veterinary Drugs (continued)

Compound	Class	Tolerance (ng/g)	Precursor ion	Product ion	Collision energy	RT (min)	Delta RT
Eprinomectin B1a	Anthelmintic	100	914.5	330.1	28	10.22	1.5
			914.5	186.1	28		
			914.5	112.1	60		
Abamectin	Anthelmintic	20	895.5	449.3	44	10.5	1.5
			895.5	751.5	48		
Closantel	Anthelmintic	50	660.9	344.9	44	10.88	1.2
			660.9	315	40		
			660.9	278.9	44		
Moxidectin	Anthelmintic	50	640.4	528.3	20	10.99	1.2
			640.4	498.2	18		
			640.4	496.2	20		
Doramectin	Anthelmintic	30	921.1	770.1	62	11.1	1.5
			921.1	449.2	66		
			921.1	353.1	66		
Selamectin	Anthelmintic	200	770.5	276	24	11.81	2.0
			770.5	203.2	28		
			770.5	113.2	40		
Rafoxanide	Anthelmintic	10	625.8	372.8	36	11.23	1.0
			625.8	252.9	28		
			625.8	127	36		
Ivermectin B1a	Anthelmintic	10	892.5	551.4	16	11.86	1.0
			892.5	307.3	24		
			892.5	145	36		

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LC/MS Method optimization

The goal of this work was to achieve adequate separation of as many veterinary drugs as possible while having a rapid and robust method for analysis. Figure 2 shows the primary MRM transition for the 13 classes of veterinary drugs tested in this method using a 12-minute gradient with UHPLC in a kidney tissue at 50 ng/g. The most polar compounds such as thiouracil, florfenicol, and sulfanilamide elute early in the chromatogram with fairly good peak shapes. Several of the mectins however, such as abamectin, ivermectin, moxidectin, and selemectin eluted at the end of the run, with typical peak widths of 9–12 seconds. A dynamic MRM method with a cycle time of 550 ms was used with a minimum dwell time of 3.2 ms and a maximum dwell time of 274 ms.

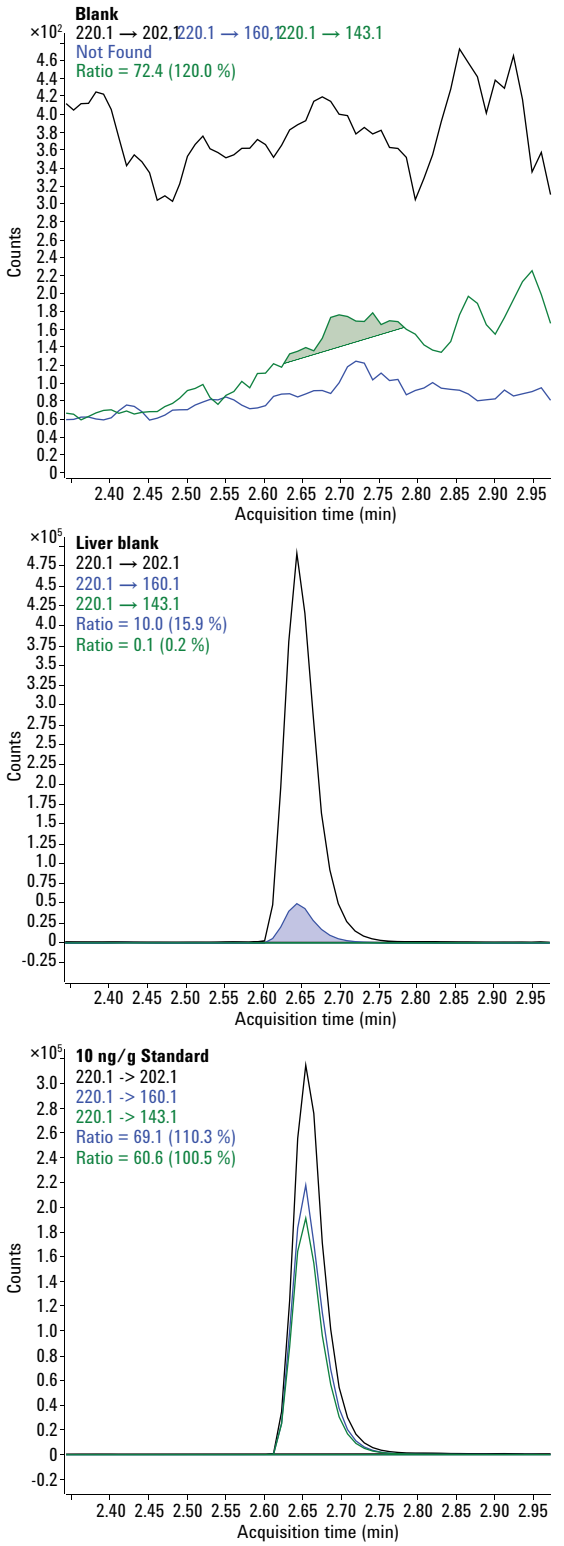

Figure 1. Potential matrix interferences for two cimaterol transitions (220.1 → 202.1; 220.1 → 160.1).

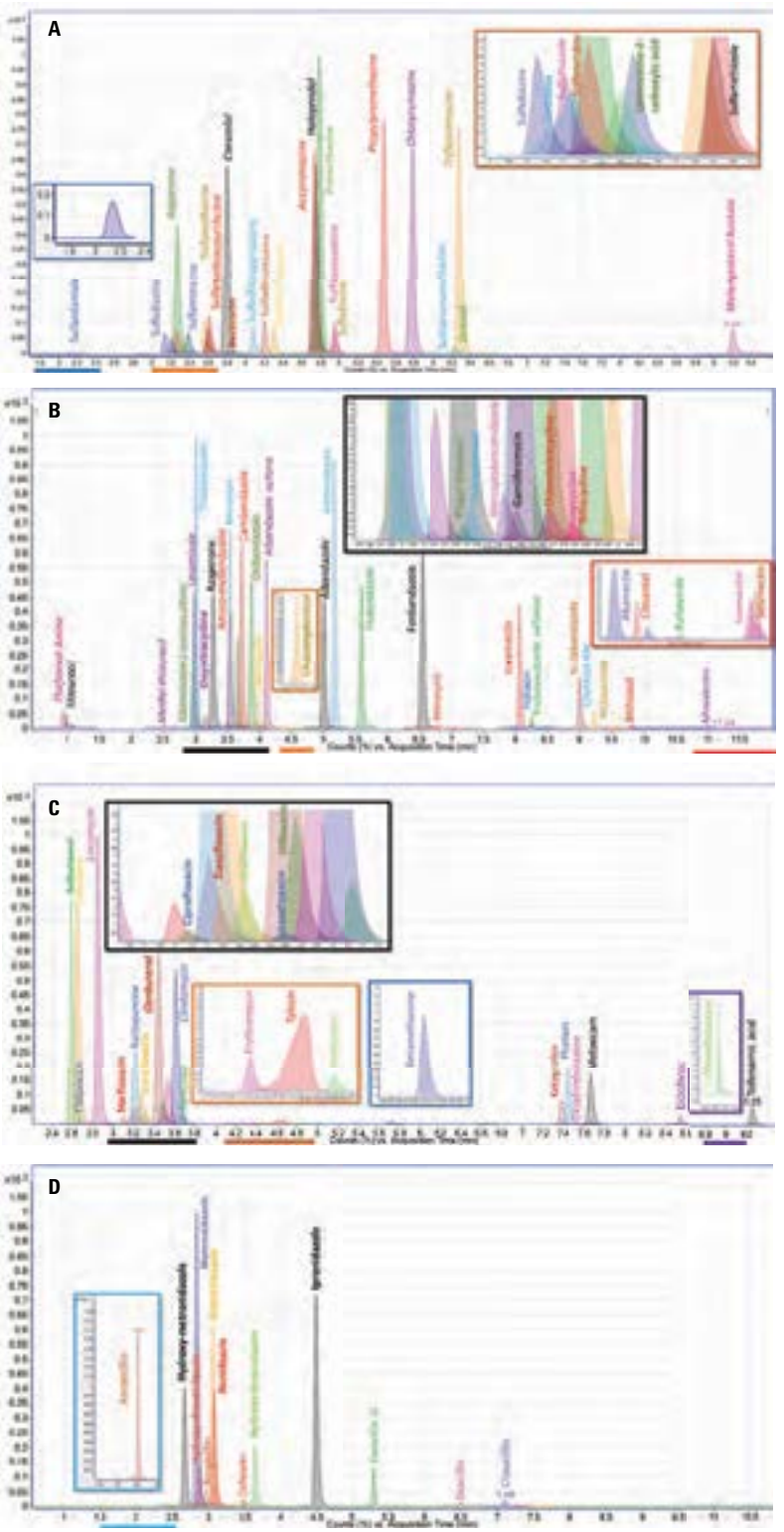

Figure 2. *Representative chromatogram of veterinary drug classes at 50 ng/g in Kidney tissue. A) Sulfonamides, tranquilizers, miscellaneous; B) anthelmintics, thyreostats, tetracyclines, phenicols; C) anti-inflammatories, macrolides/lincosamides, fluoroquinolones; β-agonists D) β-lactams, coccidiostats.*

Limits of detection and quantification

The limit of detection (LOD) was defined as the lowest concentration at which the signal-to-noise ratio (S/N) was greater than 3. Meanwhile, the limit of quantification (LOQ) was the lowest concentration at which the S/N was greater than 10 for a compound. Blank kidney and liver tissue samples were extracted through the EMR—L procedure. The resulting extract was spiked with different concentrations of veterinary drugs to determine the LOD and LOQ, thus accounting for matrix effects encountered in the instrument. The corresponding results showed no difference between the liver and kidney, and are detailed in Table 4. Several compounds have LODs (and LOQs) lower than the smallest spike concentration of 0.1 ng/mL. The LODs for the analytes tested varied from 0.1–2 ng/mL, while the LOQs ranged between 0.1 and 5 ng/mL. Most of the compound classes (sulfonamides, fluoroquinolones, tranquilizers, and so forth) had LOQs in the sub 1 ng/mL region, while the β-lactams ranged between 1 and 5 ng/mL. Figure 3 illustrates that 89 compounds had LODs of 0.1 ng/mL (and many would be lower) while 61 compounds had LOQs at 0.1 ng/mL. All compounds had LODs and LOQs at or lower than 5 ng/mL. Most importantly, all 120 veterinary drugs had LOQs lower than the tolerance levels presented in Table 3.

Table 4. LOD, LOQ, Inter- and Intraday Variability

Compounds	LOD (ng/mL)	LOQ (ng/mL)	Intraday variability RSD (%)	Interday variability RSD (%)
Abamectin	1	2	9.5	9.9
Acepromazine	0.1	0.1	3.6	1.9
Albendazole sulfone	0.1	0.5	2.4	2.6
Albendazole	0.1	0.1	2.7	1.3
Albendazole-2-aminosulfone	0.1	0.5	8.9	2.6
Aminoflubendazol	0.1	0.1	13.6	**22.0**
Amino-Mebendazole	0.1	0.1	9.7	13.7
Amoxicillin	2	5	4.2	8.8
Ampicillin	0.5	1	7.6	*16.1*
Azaperone	0.1	0.1	5.1	8.0
Bacitracin	2	5	6.3	9.2
Betamethasone	0.1	0.5	3.6	4.5
Bithionol	0.1	0.1	1.9	9.8
Cambendazole	0.1	0.1	2.5	7.2
Carazolol	0.1	0.1	6.6	9.4
Carbadox	0.5	1	5.6	9.6
Cefapirin	1	2	**20.3**	*17.1*
Cefazolin	1	2	9.8	13.5
Chloramphenicol	0.5	1	6.7	8.5
Chlorpromazine	0.1	0.1	2.3	3.3
Chlortetracycline	1	2	10.7	11.1
Cimaterol	0.1	0.1	7.5	10.5
Ciprofloxacin	0.5	1	2.3	*15.6*
Clenbuterol	0.1	0.1	5.3	5.2
Clindamycin	0.1	0.1	1.1	2.9
Clorsulon	0.5	1	3.3	3.3
Closantel	0.1	0.1	3.8	4.1
Cloxacillin	2	5	6.2	7.8
Danofloxacin	0.5	1	1.5	6.4
Desethylene ciprofloxacin	1	2	5.7	12.3
Diclofenac	0.5	1	1.1	3.4
Difloxacin	0.1	0.1	8.2	8.1
Dimetridazole	0.1	0.1	4.2	8.8
Dipyrone metabolite	NA	NA	5.4	*16.2*
Doramectin	1	2	6.5	9.3
Doxycycline	0.1	0.5	10.6	6.1
Emamectin	0.1	0.1	3.1	6.3
Enrofloxacin	0.1	0.5	10.0	6.2
Eprinomectin B1a	0.5	1	5.3	7.7
Erythromycin	0.1	0.5	3.2	13.8
Fenbendazole	0.1	0.1	3.2	2.3
Florfenicol Amine	0.5	0.5	1.6	6.2
Florfenicol	1	2	4.2	*15.1*
Flubendazole	0.1	0.1	1.8	3.8
Flunixin	0.1	0.1	1.5	7.3
Gamithromycin	0.1	0.5	8.0	*15.9*
Haloperidol	0.1	0.1	2.4	1.6
Haloxon	0.5	0.5	9.1	5.9
Hydroxydimetridazole	0.5	1	4.9	6.8
Hydroxy-Ipronidazole	0.1	0.5	8.3	5.2
Hydroxy-metronidazole	0.1	0.1	8.3	12.1
Ipronidazole	0.1	0.1	1.8	6.3
Ivermectin B1a	0.5	1	4.2	9.4
Ketoprofen	0.1	0.5	5.1	3.8
Levamisole	0.1	0.1	1.6	7.2
Lincomycin	0.1	0.1	5.5	4.2
Mebendazole	0.1	0.1	1.9	2.8
Melengestrol acetate	0.1	0.1	3.5	3.4
Meloxicam	0.1	0.1	1.0	3.8
Mercaptobenzimidazole	0.1	0.5	10.8	11.0

RSDs >15 % in *italic*, RSDs >20 % in **bold**

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Table 4. LOD, LOQ, Inter- and Intraday Variability (continued)

Compounds	LOD (ng/mL)	LOQ (ng/mL)	Intraday variability RSD (%)	Interday variability RSD (%)
Methylthiouracil	0.5	1	5.1	*16.9*
Metronidazole	0.1	0.5	6.1	3.2
Morantel	0.1	0.1	9.5	3.1
Moxidectin	1	2	14.4	*16.2*
Nafcillin	2	5	5.6	9.0
Niclosamide	0.1	0.1	2.1	7.8
Nitroxynil	0.1	0.1	2.1	3.8
Norfloxacin	0.1	0.1	2.8	5.6
Orbifloxacin	0.5	0.5	9.7	8.3
Oxacillin	0.5	1	8.5	11.3
Oxfendazole	0.1	0.1	1.6	2.4
Oxibendazole	0.1	0.1	2.7	6.2
Oxyclozanide	0.1	0.1	3.0	6.7
Oxyphenbutazone	0.1	0.5	3.9	6.7
Oxytetracycline	0.1	1	6.5	4.2
Penicillin G	NA	NA	NA	NA
Phenyl Thioracil	0.5	1	5.9	5.9
Phenylbutazone	0.1	0.5	0.5	6.0
Prednisone	0.5	0.5	4.3	10.1
Promethazine	0.1	0.1	1.8	1.4
Propionylpromazine	0.1	0.1	1.7	6.3
Propylthiouracil	0.1	0.5	7.5	8.1
Quinoxaline 2- carboxylic acid	0.5	1	6.8	11.0
Ractopamine	0.1	0.1	2.3	11.4
Rafoxanide	0.5	2	3.3	6.0
Ronidazole	0.1	0.5	1.9	6.0
Salbutamol (Albuterol)	0.1	0.5	2.1	6.5
Sarafloxacin	0.5	0.5	8.7	12.9
Selamectin	0.5	1	5.8	7.8
Sulfabromomethazine	0.1	0.1	3.0	4.0
Sulfachloropyridazine	0.1	0.1	3.8	4.1
Sulfadiazine	0.1	0.1	3.4	10.2
Sulfadimethoxine	0.1	0.1	3.9	2.7
Sulfamethazine	0.1	0.1	4.6	7.2
Sulfadoxine	0.1	0.1	2.6	3.3
Sulfaethoxypyridazine	0.1	0.1	3.9	7.1
Sulfamerazine	0.1	0.1	9.7	7.6
Sulfamethizole	0.1	0.1	4.2	5.6
Sulfamethoxazole	0.1	0.1	2.5	4.4
Sulfamethoxypyridazine	0.1	0.1	7.0	9.1
Sulfanilamide	0.1	0.1	11.6	9.2
Sulfanitran	0.1	0.1	2.0	2.5
Sulfapyridine	0.1	0.1	3.5	13.6
Sulfaquinoxaline	0.1	0.1	4.0	5.3
Sulfathiazole	0.1	0.1	8.6	7.8
Tetracycline	0.5	1	6.3	6.2
Thiabendazole	0.1	0.1	2.2	9.7
Thiamphenicol	0.1	0.5	6.5	12.7
Thiouracil	1	2	10.3	10.9
Tildipirosin	0.1	0.1	2.5	9.3
Tilmicosin	0.5	0.5	7.8	8.0
Tolfenamic acid	0.1	0.1	1.1	4.7
Triclabendazole sulfoxide	0.1	0.1	2.5	9.4
Triclabendazole	0.1	0.1	2.2	9.0
Triflupromazine	0.1	0.1	2.6	9.7
Tylosin	0.5	1	3.0	6.4
Virginiamycin M1	1	2	4.1	2.3
Xylazine	0.1	0.1	12.2	14.7
Zeranol	0.1	0.5	1.7	4.5

RSDs >15 % in *italic*, RSDs >20 % in **bold**

Linearity

The linearity of the methods was determined by creating two matrix-matched calibration curves each in kidney and liver. The first calibration curve was prepared to examine the ability to quantify across the range of tolerance levels that would be of interest to regulatory and monitoring agencies. This entailed creating a four-point calibration curve in liver and kidney at 0.5x, 1.0x, 1.5x, and 2.0x of the tolerance levels listed in Table 3. The second calibration curve was prepared at the low end to test the linearity for sensitive measurements, with a range of 1 to 100 ng/g in kidney and liver tissue (for compounds with LOQs >1 ng/mL, the point above the LOQ was selected as the first calibration level). Table 5 shows the linearity of all veterinary drugs for both types of calibration curves in kidney and liver.

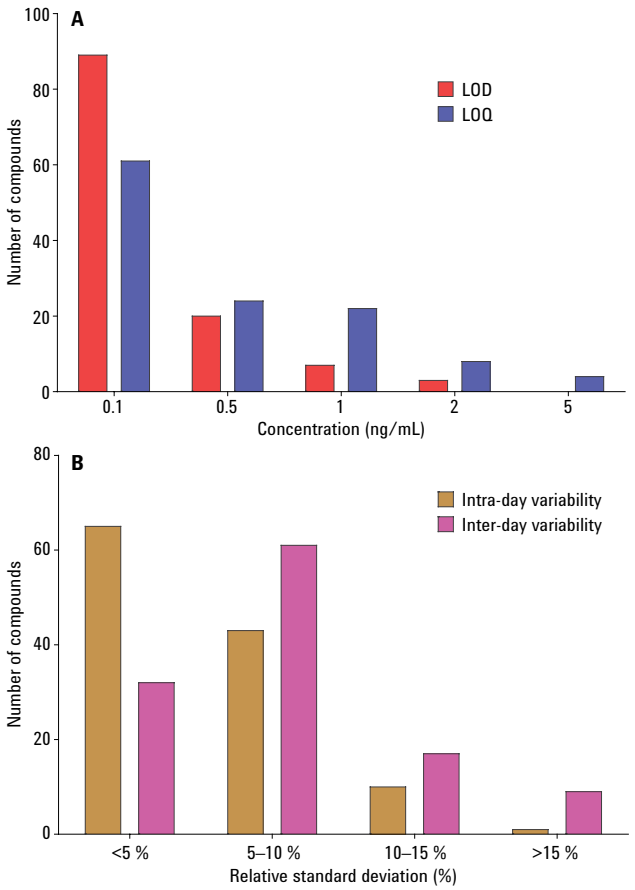

Figure 3. Distribution of (A) LODs and LOQs; (B) intraday and interday variability for the veterinary drugs tested in kidney.

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Table 5. Linearity for Two Sets of Calibration Curves Tested

	0.5X, 1.0X, 1.5X, 2.0X				1.0, 2.0, 5.0, 10, 25, 50, 100 ng/g			
	Kidney		Liver		Kidney		Liver	
Compound	R^2	Fit	R^2	Fit	R^2	Fit	R^2	Fit
Abamectin	0.9977	Linear	**0.9788**	Linear	**0.9782**	Quadratic	0.999	Quadratic
Acepromazine	0.9997	Linear	0.9982	Linear	0.9928	Linear	0.9983	Linear
Albendazole sulfone	0.9974	Linear	0.9986	Linear	0.9982	Linear	0.9971	Linear
Albendazole	0.9966	Linear	0.9935	Linear	0.9981	Linear	0.9998	Linear
Albendazole-2-aminosulfone	**0.9841**	Linear	0.9988	Linear	0.997	Linear	0.9975	Linear
Aminoflubendazol	**0.9882**	Linear	0.9999	Linear	0.9987	Linear	**0.9861**	Linear
Amino-Mebendazole	0.9926	Linear	**0.984**	Linear	0.9959	Linear	0.9911	Linear
Amoxicillin	**0.899**	Linear	0.9965	Linear	**0.9665**	Linear	0.9997	Linear
Ampicillin	0.9957	Linear	0.995	Linear	0.9983	Linear	0.9948	Linear
Azaperone	0.9981	Linear	**0.921**	Linear	0.9928	Quadratic	**0.9876**	Quadratic
Bacitracin	0.9913	Linear	**0.9825**	Linear	0.9941	Linear	0.9913	Linear
Betamethasone	0.9998	Linear	0.9984	Linear	0.9995	Linear	0.9995	Linear
Bithionol	0.9938	Linear	0.996	Linear	**0.9894**	Quadratic	0.994	Quadratic
Cambendazole	0.9938	Linear	**0.9692**	Linear	0.9977	Linear	0.9995	Linear
Carazolol	0.9925	Linear	0.9994	Linear	0.9996	Linear	**0.9898**	Linear
Carbadox	0.9973	Linear	0.9993	Linear	0.994	Linear	**0.987**	Linear
Cefapirin	0.9908	Linear	0.9962	Linear	0.9956	Linear	0.9987	Linear
Cefazolin	0.9919	Linear	0.9999	Linear	0.9966	Linear	0.9986	Linear
Chloramphenicol	0.9945	Linear	0.9981	Linear	0.9972	Linear	0.9943	Linear
Chlorpromazine	**0.9879**	Linear	0.9977	Linear	0.9807	Linear	0.9891	Linear
Chlortetracycline	0.9972	Linear	0.9957	Linear	0.9924	Linear	0.9915	Linear
Cimaterol	0.9986	Linear	0.9986	Linear	**0.9894**	Linear	0.9965	Linear
Ciprofloxacin	0.9967	Linear	0.9918	Linear	**0.9349**	Linear	**0.9771**	Linear
Clenbuterol	0.9999	Quadratic	0.9909	Linear	0.9906	Linear	0.9985	Linear
Clindamycin	0.9969	Linear	0.9908	Linear	0.9973	Linear	0.9993	Linear
Clorsulon	0.9965	Linear	0.9982	Linear	0.9933	Linear	0.9984	Linear
Closantel	**0.9554**	Linear	**0.9895**	Linear	**0.9004**	Linear	0.995	Linear
Cloxacillin	0.9998	Linear	0.9958	Linear	0.996	Linear	0.992	Linear
Danofloxacin	0.9945	Linear	0.9933	Linear	0.9987	Linear	0.9997	Linear
Desethylene ciprofloxacin	0.9959	Linear	0.9998	Linear	**0.9815**	Linear	**0.9486**	Linear
Diclofenac	0.9987	Linear	0.9967	Linear	0.9996	Linear	0.9995	Linear
Difloxacin	0.9905	Linear	0.9919	Linear	0.9955	Linear	0.9957	Linear
Dimetridazole	0.9938	Linear	0.996	Linear	0.9958	Linear	0.9965	Linear
Dipyrone metabolite	0.998	Linear	0.9912	Linear	0.9916	Linear	0.9981	Linear
Doramectin	NA		NA		NA		NA	
Doxycycline	**0.9899**	Linear	0.9999	Linear	0.9999	Linear	0.9989	Linear
Emamectin	**0.9549**	Linear	0.9901	Linear	0.9951	Quadratic	0.9972	Quadratic
Enrofloxacin	0.9993	Quadratic	0.9963	Linear	0.9938	Linear	0.9913	Linear
Eprinomectin B1a	0.9913	Linear	0.9936	Linear	0.9932	Linear	0.9911	Linear
Erythromycin	0.9999	Linear	0.9978	Linear	0.9995	Linear	0.9982	Linear
Fenbendazole	0.9989	Linear	0.9962	Quadrati c	0.9998	Linear	0.9933	Linear
Florfenicol Amine	**0.9535**	Linear	**0.9555**	Linear	**0.8648**	Linear	**0.9614**	Linear
Florfenicol	**0.9842**	Linear	**0.984**	Linear	**0.9818**	Linear	**0.9191**	Linear

R^2 <0.99 in bold

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Table 5. Linearity for Two Sets of Calibration Curves Tested (continued)

	0.5X, 1.0X, 1.5X, 2.0X				1.0, 2.0, 5.0, 10, 25, 50, 100 ng/g			
	Kidney		Liver		Kidney		Liver	
Compound	**R^2**	**Fit**	**R^2**	**Fit**	**R^2**	**Fit**	**R^2**	**Fit**
Flubendazole	0.999	Linear	0.9914	Linear	0.9924	Linear	0.9975	Linear
Flunixin	0.9922	Linear	0.9996	Linear	0.9935	Linear	0.9968	Linear
Gamithromycin	0.9925	Linear	0.9902	Linear	0.9998	Linear	**0.987**	Linear
Haloperidol	0.9931	Linear	0.9995	Linear	0.9921	Quadratic	0.9967	Quadratic
Haloxon	0.9942	Linear	0.9934	Linear	0.999	Linear	0.9997	Linear
Hydroxydimetridazole (Dimetridazol-OH)	**0.9846**	Linear	0.9998	Linear	0.9996	Linear	0.9934	Linear
Hydroxy-Ipronidazole	0.995	Linear	1	Quadratic	0.9969	Linear	0.9987	Linear
Hydroxy-metronidazole	0.9979	Linear	0.9937	Linear	0.9962	Linear	0.9998	Linear
Ipronidazole	0.9952	Linear	0.9922	Linear	0.9919	Linear	0.9975	Linear
Ivermectin B1a	**0.9357**	Linear	0.9914	Linear	0.9964	Linear	0.9986	Linear
Ketoprofen	0.9956	Linear	0.998	Linear	0.9953	Linear	0.9997	Linear
Levamisole	0.991	Linear	0.9949	Linear	0.9984	Linear	0.9941	Linear
Lincomycin	0.9916	Linear	**0.9891**	Linear	**0.9816**	Linear	0.9936	Linear
Mebendazole	0.9972	Linear	0.9985	Linear	0.9981	Linear	0.9941	Linear
Melengestrol acetate	0.9968	Linear	0.9931	Linear	**0.9747**	Linear	0.9918	Linear
Meloxicam	0.9998	Linear	0.9996	Linear	0.9961	Linear	0.9998	Linear
Mercaptobenzimidazole	0.9982	Linear	0.9921	Linear	**0.9794**	Linear	0.9937	Linear
Methylthiouracil	0.9967	Linear	0.9959	Linear	0.9927	Linear	0.9997	Linear
Metronidazole	0.9982	Linear	0.9941	Linear	0.9995	Linear	0.9994	Linear
Morantel	0.9962	Linear	0.9936	Linear	0.9903	Linear	0.9979	Linear
Moxidectin	0.9904	Linear	0.9903	Linear	NA		NA	
Nafcillin	0.9917	Linear	0.9901	Linear	0.9982	Linear	0.9971	Linear
Niclosamide	0.9999	Linear	0.9917	Linear	0.9911	Quadratic	0.998	Quadratic
Nitroxynil	0.9988	Linear	**0.9898**	Linear	0.9967	Linear	0.9992	Linear
Norfloxacin	0.9906	Linear	0.9946	Linear	**0.924**	Linear	**0.9827**	Linear
Orbifloxacin	**0.9699**	Linear	0.9992	Linear	**0.987**	Linear	0.9994	Linear
Oxacillin	0.9995	Linear	0.9924	Linear	0.9935	Linear	0.9938	Linear
Oxfendazole	0.9998	Linear	0.9999	Quadratic	0.9997	Linear	0.993	Linear
Oxibendazole	0.9978	Linear	0.9985	Linear	0.9965	Quadratic	0.9975	Quadratic
Oxyclozanide	0.9959	Linear	0.9992	Linear	0.9964	Linear	0.998	Linear
Oxyphenbutazone	0.9958	Linear	0.9986	Linear	0.9971	Linear	0.9941	Linear
Oxytetracycline	0.9987	Linear	**0.9885**	Linear	0.9969	Linear	0.9976	Linear
Penicillin G	NA		NA		NA		NA	
Phenyl Thioracil	0.9988	Linear	0.9987	Linear	0.9977	Linear	0.9983	Linear
Phenylbutazone	0.999	Linear	0.9969	Linear	0.997	Linear	0.9922	Linear
Prednisone	0.9958	Linear	0.9902	Linear	0.9973	Linear	0.9986	Linear
Promethazine	0.9994	Linear	0.9901	Linear	**0.9574**	Linear	0.9966	Linear
Propionylpromazine	0.9971	Linear	0.9927	Linear	0.9981	Linear	0.996	Linear
Propylthiouracil	0.9922	Linear	0.9982	Linear	0.9969	Linear	0.9986	Linear
Quinoxaline 2-carboxylic acid	0.9994	Linear	0.9904	Linear	0.9974	Linear	0.9959	Linear
Ractopamine	0.9984	Linear	0.998	Linear	**0.9886**	Linear	0.992	Linear
Rafoxanide	0.9993	Linear	0.9974	Linear	0.9911	Linear	0.9937	Linear
Ronidazole	0.9983	Linear	**0.9894**	Linear	0.9993	Linear	0.9988	Linear

R^2 <0.99 in bold

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Table 5. Linearity for Two Sets of Calibration Curves Tested (continued)

	0.5X, 1.0X, 1.5X, 2.0X				1.0, 2.0, 5.0, 10, 25, 50, 100 ng/g			
	Kidney		Liver		Kidney		Liver	
Compound	**R^2**	**Fit**	**R^2**	**Fit**	**R^2**	**Fit**	**R^2**	**Fit**
Salbutamol (Albuterol)	**0.9865**	Linear	1	Linear	0.9955	Linear	0.9968	Linear
Sarafloxacin	0.9989	Linear	0.9917	Linear	0.9901	Linear	**0.989**	Linear
Selamectin	0.9989	Linear	0.9999	Quadratic	0.9944	Linear	0.998	Linear
Sulfabromomethazine	0.9900	Linear	0.9998	Linear	0.9988	Linear	0.9995	Linear
Sulfachloropyridazine	0.9945	Linear	0.9957	Linear	0.9995	Linear	0.9999	Linear
Sulfadiazine	0.9931	Linear	0.9992	Linear	0.9969	Linear	0.9945	Linear
Sulfadimethoxine	0.9959	Linear	0.9946	Linear	0.9994	Linear	0.9999	Linear
Sulfamethazine	0.9991	Linear	0.9975	Linear	0.9900	Linear	0.9964	Linear
Sulfadoxine	0.9979	Linear	0.9936	Linear	0.9997	Linear	0.9998	Linear
Sulfaethoxypyridazine	0.9957	Linear	0.9938	Linear	0.9989	Linear	0.9935	Linear
Sulfamerazine	0.9984	Linear	0.9949	Linear	0.9979	Linear	0.9971	Linear
Sulfamethizole	0.9986	Linear	0.9999	Linear	0.9937	Linear	0.9962	Linear
Sulfamethoxazole	0.9995	Linear	0.9924	Linear	0.9997	Linear	0.998	Linear
Sulfamethoxypyridazine	0.9948	Linear	0.9983	Linear	0.9955	Linear	0.9963	Linear
Sulfanilamide	0.9945	Linear	0.9945	Linear	0.9937	Linear	0.9983	Linear
Sulfanitran	0.9967	Linear	0.9987	Linear	**0.969**	Linear	0.9935	Linear
Sulfapyridine	0.9962	Linear	0.9941	Linear	0.9942	Linear	0.9957	Linear
Sulfaquinoxaline	0.9964	Linear	0.9983	Linear	0.9965	Linear	0.9998	Linear
Sulfathiazole	0.9998	Linear	0.9921	Linear	0.9903	Linear	0.9962	Linear
Tetracycline	0.993	Linear	0.9992	Linear	0.9989	Linear	0.9977	Linear
Thiabendazole	0.9998	Linear	0.9918	Linear	0.9971	Linear	0.9991	Linear
Thiamphenicol	0.9914	Linear	0.9992	Linear	**0.9783**	Linear	**0.985**	Linear
Thiouracil	0.9941	Linear	**0.957**	Linear	0.9948	Linear	0.9991	Linear
Tildipirosin	0.9979	Linear	0.9984	Linear	0.9974	Linear	**0.9843**	Linear
Tilmicosin	0.9903	Linear	0.9994	Linear	0.9993	Linear	0.9926	Linear
Tolfenamic acid	0.9944	Linear	0.9977	Linear	0.9918	Linear	0.9974	Linear
Triclabendazole sulfoxide	0.9992	Linear	0.9994	Linear	0.9985	Linear	0.9989	Linear
Triclabendazole	**0.9807**	Linear	0.9983	Linear	0.9997	Linear	0.9999	Linear
Triflupromazine	0.9994	Linear	0.9979	Linear	0.9978	Linear	**0.9884**	Linear
Tylosin	0.9986	Linear	0.9995	Linear	0.9908	Linear	0.9901	Linear
Virginiamycin M1	0.9984	Linear	0.999	Linear	0.9913	Linear	0.9954	Linear
Xylazine	0.9942	Linear	0.9929	Linear	0.9964	Linear	0.9985	Linear
Zeranol	0.9965	Linear	0.9923	Linear	0.9931	Linear	0.9952	Linear

R^2 <0.99 in bold

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For the calibration curve based on the tolerance levels, more than 89 % of the compounds had R^2 >0.99. In fact, only azaperone had R^2 < 0.95 in liver, while only ivermectin and amoxicillin had R^2 < 0.95 in kidney tissue. This was despite the fact that only three internal standards were used to correct the data (doxycycline-d_3 to correct for tetracyclines, nadcillin-d_5 for β-lactams, and flunixin-d_3 for the remaining veterinary drugs). When looking at the low-end calibration curve, more than 85 % of the compounds still had R^2 >0.99 in both the liver and kidney tissue, and azaperone, ivermectin, and amoxicillin looked much better. In this case, it was norfloxacin and florfenicol amine that had R^2 < 0.95 in the kidney, while florfenicol was the only compound in the liver. The behavior of florfenicol and florfenicol amine could be because they eluted extremely early, which may have caused matrix effects that could not be accounted for by the flunixin-d_3. Nonetheless, this method had excellent linearity for most of the veterinary drugs tested while using a limited set of internal standards. This further illustrates the benefits of using matrix-matched calibrations for this analysis. Figure 4 illustrates typical calibration curves in kidney for the two types of calibrations performed.

Reproducibility and repeatability

The repeatability of the method was estimated by calculating the intraday variability based on relative standard deviation (%RSD) of five replicate injections of kidney tissue spiked at 1.0x tolerance level of each veterinary drug injected throughout a 24-hour period. Similarly, the reproducibility was determined as the %RSD of a sample injected on four consecutive days. Table 4 shows the %RSDs for all veterinary drugs tested in this method. Only one compound (cefapirin) had an RSD greater than 15 % for the intraday variability. Nine compounds (amino-flubendazole, ampicillin, cefapirin, ciprofloxacin, dipyrone metabolite, florfenicol, gamithromycin, methyl-thiouracil, and moxidectin) had RSDs greater than 15 % (less than 23 %) during the interday RSD tests. The interday variabilities were understandably a little higher than the corresponding intraday variability, probably due to standard preparation and potential compound degradation across the four-day period. Figure 3 shows that most compounds had both inter- and intraday RSDs of less than 10 %, proving that the method is robust and reproducible.

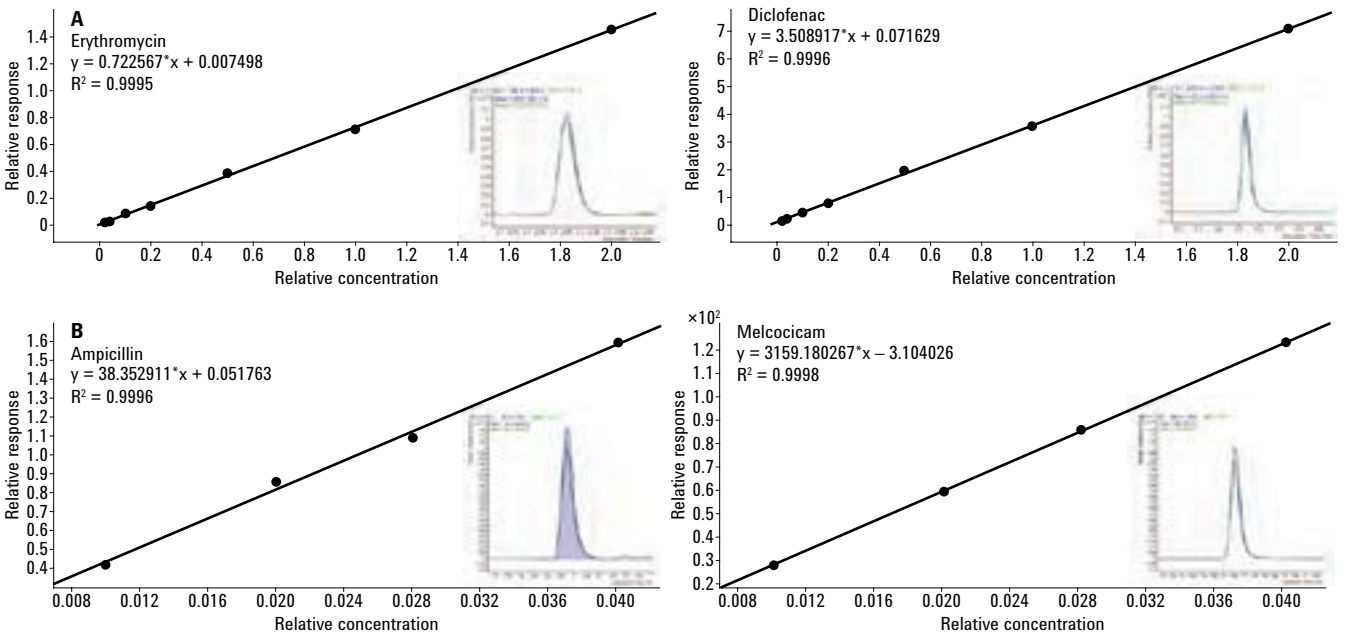

Figure 4. Typical calibration curves for veterinary drugs in two ranges: A) 1–100 ng/mL; B) 0.5–2.0x TLs in liver.

Conclusions

This method shows the analysis of 120 veterinary drugs in meat using the Agilent 1290 Infinity II UHPLC coupled to an Agilent 6495 Triple Quadrupole LC/MS in 12 minutes. It is common practice within analytical surveillance laboratories to be able to validate an analytical method down to half a compound's maximum tolerance level. For all analytes in this method, both LODs and LOQs were in line with this requirement when compared to tolerance levels for liver and kidney in the USA. In fact, this method is sensitive enough to achieve sub-1 ng/mL LODs and LOQs for most analytes. The method is robust and selective with the use of three transitions for almost all veterinary drugs tested, while being reproducible and repeatable. Quantitative performance was excellent with good linearity for most compounds by using matrix-matched calibration curves. The method was also cost-effective since there was limited use of expensive internal standards.

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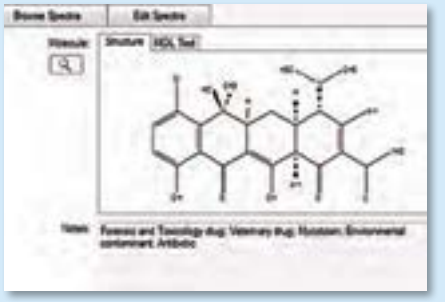

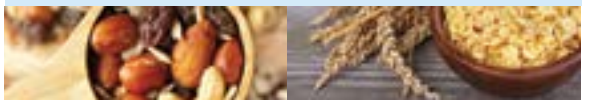

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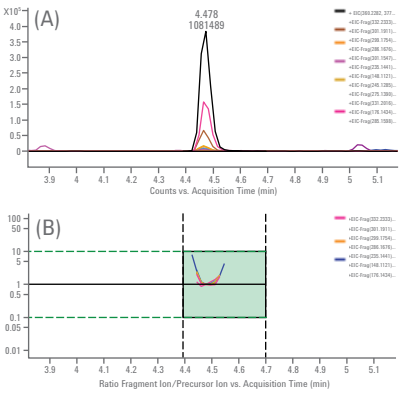

Overlay of precursor and fragment ion traces for aspergillimide in a spiked maize sample (A), coelution plot (B) and compound identification results including the co-elution score.

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Screening and Verifying Mycotoxins in Food with Q-TOF LC/MS and an Accurate Mass Library

Application Note

Food and Agriculture

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Abstract

This application note describes the creation of an accurate mass library for mycotoxins and related metabolites and its application for the screening of mycotoxins in food. An Agilent 1290 Infinity LC coupled to an Agilent 6550 iFunnel Q-TOF LC/MS was operated in positive and negative electrospray with dual-spray Agilent Jet Stream Technology. Accurate mass spectra were acquired for a large collection of mycotoxins and related metabolites in one or both ionization modes and for all relevant ion species.

Three different matrices were extracted and spiked with 44 indicator compounds. Samples were analyzed using target MS/MS and All Ions MS/MS acquisition. This work demonstrates the value of both acquisition modes, combined with an efficient data analysis workflow and the Mycotoxins and Related Metabolites Personal Compound Database and Library (PCDL), for the screening and verification of mycotoxins in complex matrices.

Introduction

Mycotoxins are toxic secondary metabolites of fungi, which can occur in various food and feed products including cereals, nuts, fruits, spices, wine, and coffee [1]. The several hundreds of mycotoxins and secondary fungal metabolites belong to different chemical classes with very different physicochemical properties. Currently, only about a dozen compounds are regulated in food and feed. In Europe, Commission Regulation (EC) 1881/2006 and its amendments specify maximum levels for aflatoxins, deoxynivalenol, fumonisins, ochratoxin A, patulin, and zearalenone in food [2]. In addition, there are indicative levels for T-2 and HT-2 toxin specified in Commission Recommendation 2013/165/EU [3].

Comprehensive data on the occurrence of mycotoxins apart from the regulated compounds is limited, especially in food matrices other than raw cereals [4]. This is one reason why, in recent years, single mycotoxin methods have been increasingly replaced by LC/MS-based multitarget methods [4,5]. The harmonization of methods for different commodities, the identification of mycotoxins in unlikely matrices, and the increase in knowledge of emerging mycotoxins from *Aspergillus*, *Penicillium*, *Fusarium*, or *Alternaria* species are just a few reasons for this trend. These developments were aided by the increase in performance of modern LC/MS instruments over the last few years, and the development of software tools that enhance productivity. Modern high-resolution, accurate-mass LC/Q-TOF instruments can analyze a virtually unlimited number of contaminants [6]. They also allow retrospective data analysis to find contaminants that were not considered at the time of the measurement [7].

While most multitarget methods were developed for screening contaminants, they also allow acquisition of quantitative information for regulated mycotoxins. The challenges are the efficient extraction of analytes with largely different physicochemical properties from many food products, and the huge differences in naturally occurring toxin concentrations.

This application note describes the creation and use of an accurate mass LC/MS/MS database and library. The library contains spectra for more than 300 LC/MS-amenable mycotoxins and fungal or bacterial metabolites, to screen and identify these compounds in food samples. The sample preparation method comprises a single extraction with an acidified acetonitrile-water mixture. Two different screening strategies are used. In the conventional approach, the Q-TOF LC/MS system is first operated in TOF mode and a database search is conducted. Using a second injection, a targeted MS/MS method of the list of suspects was used and the obtained spectra were compared to the MS/MS library. In a second approach, the Q-TOF operates in the All Ions MS/MS mode with two collision energies. The All Ions technique features easy setup of the acquisition method and verification of the mycotoxins using the MS/MS spectral library. This method produces chromatographic coelution of the precursor and product ions. We show method performance parameters for 44 representative indicator compounds in maize, hazelnuts, and wine.

Experimental

Reagents and standards

All reagents and solvents were HPLC or LC/MS grade. Acetonitrile, methanol, and formic acid were purchased from VWR International (Vienna, Austria). Ammonium formate solution (p/n G1946-85021) came from Agilent. Ultrapure water was produced using a Purelab Ultra system (ELGA LabWater, Celie, Germany). Analytical standards of the fungal and bacterial metabolites were purchased from Enzo Life Sciences (Lausen, Switzerland), Bioviotica Naturstoffe GmbH (Dransfeld, Germany), Bioaustralis (distributed by Tebu-Bio, Germany), Iris Biotech GmbH (Marktredwitz, Germany), Romer Labs (Tulln, Austria), or Sigma-Aldrich Corp. (Vienna, Austria). Other standards were provided as isolates from research groups around the world.

Stock standard solutions were prepared by dissolving the reference compounds in acetonitrile, methanol, water, or mixtures of these, depending on the physicochemical properties of the substance. The individual standard solutions were combined to a multi-analyte working solution that was used for calibration and for spiking blank samples. Stock standard solutions and the multi-analyte working solution were stored until use at –20 °C. Calibration samples were prepared by diluting the working solution with a mixture of acetonitrile:water:formic acid (79:20.9:0.1, v:v:v). The extraction solvent had the same composition.

Sample preparation

Blank maize and hazelnut samples for spiking experiments were purchased and were checked for the absence of any compounds of interest with an LC/MS/MS method. The samples were ground and homogenized using an electric blender. A 5 g (± 0.01 g) portion of the samples was weighed in a 50 mL polypropylene tube and 20 mL extraction solvent was added. The samples were extracted at room temperature for 90 minutes on a rotary shaker. After settling of the solid residue, an aliquot of the extract was transferred to an HPLC vial. The raw extracts were spiked with the multianalyte working solution at three different concentration levels.

LC/MS/MS analyses

Separation was carried out using an Agilent 1290 Infinity UHPLC, consisting of:

- Agilent 1290 Infinity Binary Pump (G4220A),
- Agilent 1290 Infinity High Performance Autosampler (G4226A), and
- Agilent1290 Infinity Thermostatted Column Compartment (G1316C)

The UHPLC system was coupled to an Agilent 6550 iFunnel Quadrupole Time-of-Flight LC/MS equipped with a dual-spray Agilent Jet Stream electrospray ionization source. Reference mass ions were delivered using an Agilent 1260 Infinity Isocratic Pump (G1310B) operated at 1.0 mL/min and using a 1 in 100 flow splitter (p/n G1607-60000). The final flow rate to the reference sprayer was 10 µL/min. The Q-TOF LC/MS instrument was operated with Agilent MassHunter Data Acquisition Software, rev. B.05.01, in 2 GHz extended dynamic range mode with positive or negative ionization with two different methods. In target MS/MS acquisition, a data rate of three scans/s in MS and MS/MS mode was used. All Ions MS/MS acquisition used three scans/s with two discrete collision energies. The use of two collision energies resulted in alternating spectra with a low-energy channel containing the precursor ion and two high-energy channels containing the precursor and product ions.

Chromatographic conditions

Conditions for chromatography

Column:	Agilent Poroshell 120 EC-C18, 2.1 × 100 mm, 2.7 µm (p/n 695775-902)
Mobile phase:	A) 5 mM NH_4 formate + 0.1% formic acid B) 5 mM NH_4 formate + 0.1% formic acid in methanol
Gradient:	Time (min) % B 0.0 10 0.5 10 10.0 98 15.0 98 15.1 10 17.0 10
Stop time:	17.0 min
Temperature:	30 °C
Flow rate:	0.40 mL/min
Injection volume:	2 µL

Conditions for Dual AJS

Gas temperature:	130 °C
Gas flow:	16 L/min
Nebulizer:	30 psig
Sheath gas temperature:	300 °C
Sheath gas flow:	11 L/min
Capillary voltage:	+ve 4,000 V; –ve 4,000 V
Nozzle voltage:	+ve 500 V; –ve 500 V
Reference mass correction:	+ve 121.05087 and 922.00980; –ve 112.98559 and 966.00073

All-Ions MS/MS

Mass range:	40 to 1,300 amu
Scan rate:	3 spectra/s
Collision energies:	0 – 10 – 40 V

Target MS/MS

MS mass range:	80 to 1,300 amu
MS/MS mass range:	40 to 1,300 amu
Scan rate:	3 spectra/s for MS and MS/MS
Collision energy:	20 V
Target masses:	45 (positive and negative), Delta RT 0.5 min

Data were evaluated using MassHunter Qualitative Analysis Software B.07.00. Positive identifications of mycotoxins were reported if the compound was detected by the Find by Formula data-mining algorithm with a mass error below 5 ppm and with a sufficient score (including isotope abundance and isotope spacing). A retention time window of ± 1 minute was specified for peak detection to compensate for retention time shifts due to system-to-system variability.

Creation of the mycotoxins and related metabolites PCDL

Accurate mass spectra were acquired with flow injection or through a short column of single-analyte solutions in target MS/MS mode with collision energies of 10, 20, and 40 eV. All relevant compound species including $[M+H]^+$, $[M-H]^-$, $[M+NH_4]^+$, and $[M+HCOO]^-$ were used as target masses. If precursor ion stability required higher collision energies, extra spectra were acquired in a second run. In either positive or negative ionization mode, meaningful MS/MS spectra were acquired for more than 300 mycotoxins and other fungal or bacterial metabolites. For several compounds, MS/MS library spectra were captured in both ionization modes and for more than one precursor ion species. Solid standards or stock solutions were collected over more than a decade. Most compounds were purchased from different suppliers, the others were either isolated at IFA-Tulln, BOKU or were provided by other research groups. To eliminate mass assignment errors, fragment masses in the acquired spectra were compared to the theoretical fragment formulas and corrected to their theoretical masses. All MS/MS spectra were curated for spectral noise. A minimum base peak threshold was applied to ensure good ion statistics for all fragment ions. The corrected spectra were included in the Agilent Personal Compound Database and Library for Mycotoxins and Related Metabolites (p/n G5883CA), which was used for the screening and verification of mycotoxins in food samples. For the 44 indicator compounds, retention time information was added to the library by analyzing a comprehensive mycotoxin standard with the given UHPLC method.

Figure 1 shows a screen capture of the MassHunter PCDL Manager Software, along with the accurate mass spectrum of the $[M+NH_4]^+$ species of T-2 toxin acquired with a collision energy of 10 eV.

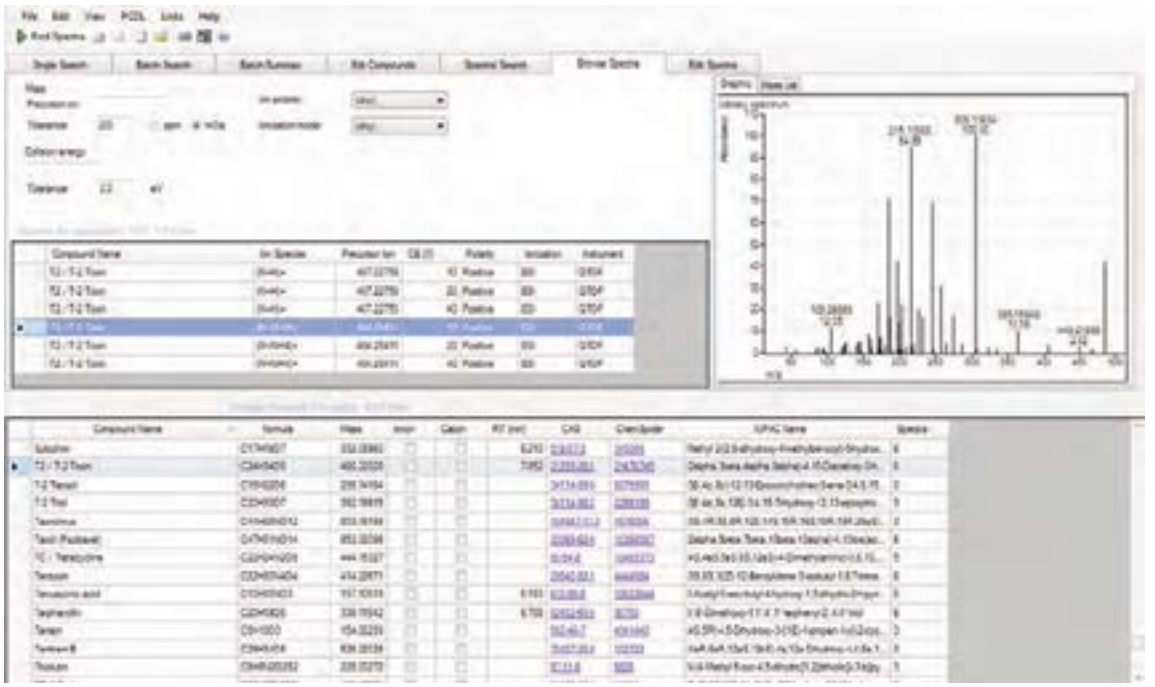

Figure 1. Agilent MassHunter PCDL Manager Software showing the Mycotoxins and Related Metabolites PCDL and the accurate mass spectrum for the $[M+NH_4]^+$ species of T-2 toxin at a collision energy of 10 eV.

Results and Discussion

All Ions MS/MS acquisition for simultaneous screening and verification

Maize and hazelnut extracts, and red wine samples, were spiked with 44 indicator compounds from the group of mycotoxins and fungal metabolites. All regulated compounds, some polar and nonpolar analytes, and poor ionizing compounds were selected. In the All Ions MS/MS workflow, accurate mass data were collected without fragmentation in a low-energy channel. At the same time, compounds were fragmented with two different collision energies without precursor selection. Accurate mass fragment data were recorded in two high-energy channels. When the data are analyzed using the Find by Formula algorithm, precursor information is derived from the Mycotoxins and Related Metabolites PCDL and compound chromatograms are extracted for all specified ion species. For putative identifications, the spectra stored in the PCDL are used, and for a specified number of the most abundant fragments chromatograms are automatically extracted from the high-energy channels. For example, Figure 2A shows the accurate mass library spectrum of aspergillimide from the Mycotoxins and Related Metabolites PCDL compared to the cleaned high-energy spectrum from a spiked maize sample (Figure 2B). The red triangles indicate the fragment ions automatically selected from the library spectrum for evaluation. While the library spectrum is based on a collision energy of 40 eV, the cleaned high-energy spectrum combines information from both high-energy channels acquired with 10 and 40 eV.

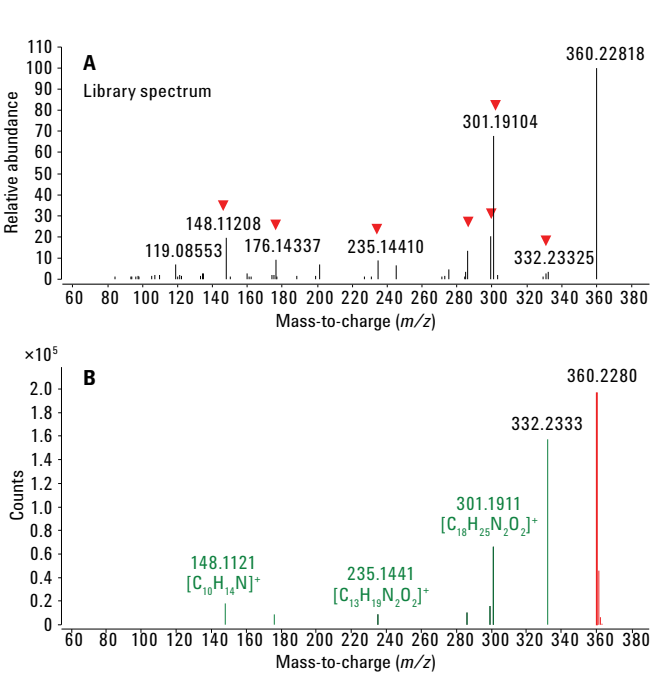

Figure 2. Accurate-mass library spectrum for aspergillimide at a collision energy of 40 eV (A) compared to the acquired high-energy spectrum (B) from a spiked maize sample (cleaned spectrum). Red triangles in the library spectrum indicate ions automatically selected for the All Ions MS/MS evaluation.

By overlaying chromatograms for both precursor and fragment ions and the calculation of a coelution score, the identity of the fungal metabolite aspergillimide was confirmed. The coelution score accounts for factors such as abundance, peak shape (symmetry), peak width, and retention time. The scores were plotted and made available for inspection in a coelution plot. Figure 3A shows the overlay of the precursor chromatogram with the fragment chromatograms from the high-energy channels. From the automatically extracted fragments, six fragment chromatograms showed coelution with the precursor ion, as revealed by the coelution plot in Figure 3B. Figure 3C shows the detailed identification results in the compound table.

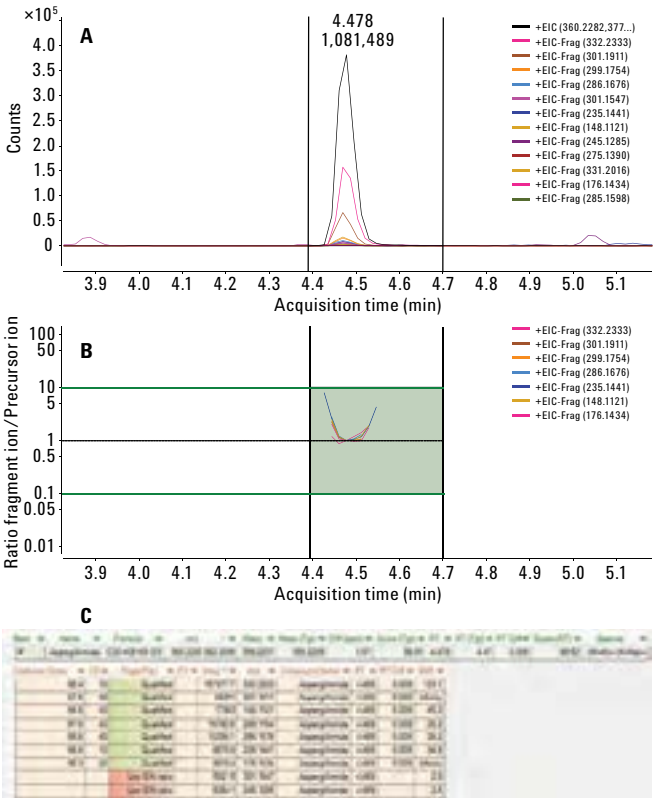

Figure 3. Overlay of precursor and fragment ion traces for aspergillimide in a spiked maize sample (A), coelution plot (B), and compound identification results including the coelution score (C).

Table 1 lists all 44 mycotoxins and fungal metabolites spiked into a maize extract at 30 ng/mL. Compounds were measured with positive or negative ionization and the predominant species for each analyte are given. Several compounds were detected and qualified in both polarities. In these cases, results for the more sensitive ionization mode are shown. At this concentration, most compounds were found by automatically searching using the Find by Formula algorithm. The mass deviations of the measured masses compared to the theoretical masses were generally below 1 ppm. We observed a mass deviation between 2 and 5 ppm for only 11 compounds. Therefore, target scores, including retention time, mass accuracy, isotope abundance, and isotope spacing, were typically above 90 (out of 100). Most of the compounds were verified with at least one extra fragment ion in either positive or negative ion mode. A minimum coelution score of 80 (out of 100) has been specified as the criterion for compound verification.

Screening and verification of mycotoxins in food by target MS/MS acquisition

The same samples were also analyzed using the same chromatographic method, but with target MS/MS acquisition. The Mycotoxins and Related Metabolites PCDL was used with the Find by Formula data-mining algorithm to find the compounds. Chromatograms for the expected ion species, MS and MS/MS spectra, were extracted automatically for the identified compounds. The results were scored based on the agreement of the accurate monoisotopic mass, the isotope ratio, the isotope spacing, and the retention time.

Figure 4 shows the compound chromatogram and peak spectrum of T-2 toxin spiked into a maize extract. The predominant ion species for T-2 toxin were $[M+NH_4]^+$ and $[M+Na]^+$ ions. The measured *m/z* signals for these species (blue) were in good agreement with the expected isotope ratio (red boxes). In total, the software assigned 10 ions to the $[M+H]^+$, $[M+NH_4]^+$, and $[M+Na]^+$ species of T-2 toxin, including their isotope signals. The good mass accuracy and isotope pattern matching was reflected in a good target score of 98.5 (out of 100). Target scores for the other compounds (not shown) were comparable to the values in Table 1 for the All Ions MS/MS workflow.

The red diamond indicates that an MS/MS spectrum was acquired for that *m/z*. MS/MS spectra were extracted automatically over the peak range and matched against the library spectra contained in the PCDL.

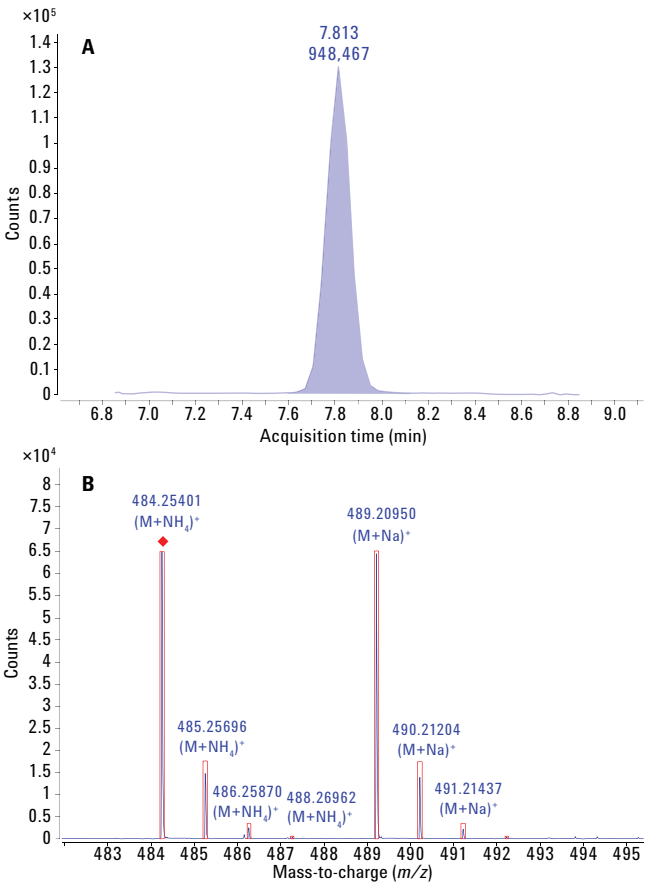

Figure 4. Compound chromatogram (A) and peak spectrum (B) obtained by the Find by Formula algorithm for T-2 toxin spiked in a maize sample at 30 ng/mL.

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Table 1. Analysis of 44 mycotoxins and fungal metabolites spiked into maize extract at 30 ng/mL, and measured with positive or negative All Ions MS/MS acquisition.

Compound	Retention time (min)	Formula	Ion species	Mass	Mass deviation (ppm)	Target score	Coelution score
15-Monoacetoxyscirpenol	5.61	$C_{17}H_{24}O_6$	$[M+NH_4]^+$	324.1573	-2.52	96.8	96.3
16-Keto-Aspergillimide	5.85	$C_{20}H_{27}N_3O_4$	$[M+H]^+$	373.2002	-1.61	94.9	94.9
3-Acetyldeoxynivalenol	4.88	$C_{17}H_{22}O_7$	$[M+HCOO]^-$	338.1366	-2.73	98.0	
Aflatoxin B_1	6.36	$C_{17}H_{12}O_6$	$[M+H]^+$	312.0634	-0.59	98.2	90.2
Aflatoxin B_2	6.12	$C_{17}H_{12}O_6$	$[M+H]^+$	314.0790	0.86	98.5	96.9
Aflatoxin G_1	5.83	$C_{17}H_{12}O_7$	$[M+H]^+$	328.0583	-0.65	99.6	97.0
Aflatoxin G_2	5.56	$C_{17}H_{14}O_7$	$[M+H]^+$	330.0740	1.06	98.3	96.5
Aflatoxin M_1	5.62	$C_{17}H_{12}O_7$	$[M+H]^+$	328.0583	-0.14	97.7	95.3
Agroclavine	4.77	$C_{16}H_{18}N_2$	$[M+H]^+$	238.1470	-0.41	99.2	96.5
Alternariol	7.48	$C_{14}H_{10}O_5$	$[M-H]^-$	258.0528	0.27	97.5	96.6
Alternariolmethylether	8.72	$C_{15}H_{12}O_5$	$[M-H]^-$	272.0685	0.10	98.6	97.0
Aspergillimide	4.48	$C_{20}H_{29}N_3O_3$	$[M+H]^+$	359.2209	-0.57	99.1	97.2
Beauvericin	10.09	$C_{45}H_{57}N_3O_9$	$[M+NH_4]^+$	783.4095	0.24	99.2	95.5
Brevianamid F	4.99	$C_{16}H_{17}N_3O_2$	$[M+H]^+$	283.1321	-0.63	96.8	98.7
Curvularin	7.10	$C_{16}H_{20}O_5$	[M+H]+	292.1311	-0.86	99.1	97.8
Cyclopiazonic acid	8.86	$C_{20}H_{20}N_2O_3$	$[M+H]^+$	336.1474	-3.01	97.3	96.7
Cyclosporin A	10.45	$C_{62}H_{111}N_{11}O_{12}$	$[M+HCOO]^-$	1201.8414	-4.30	85.5	96.4
Diacetoxyscirpenol	6.34	$C_{19}H_{26}O_7$	$[M+NH_4]^+$	366.1679	-1.48	96.9	92.3
Deoxynivalenol	2.99	$C_{15}H_{20}O_6$	$[M+Na]^+$	296.1260	-1.80	97.6	
Emodin	7.39	$C_{15}H_{10}O_5$	$[M-H]^-$	270.0528	-2.46	91.1	
Enniatin B	9.90	$C_{33}H_{57}N_3O_9$	$[M+NH_4]^+$	639.4095	-2.96	95.2	92.5
Ergosine	6.29	$C_{30}H_{37}N_5O_5$	$[M+H]^+$	547.2795	-3.30	94.2	82.2
Ergosinine	6.19	$C_{30}H_{37}N_5O_5$	$[M+H]^+$	547.2795	-1.18	95.9	93.5
Ergotaminine/ergotamine	6.46	$C_{33}H_{35}N_5O_5$	$[M+H]^+$	581.2638	-1.29	97.5	86.3
Fumonisin B_1	7.78	$C_{34}H_{59}NO_{15}$	$[M+H]^+$	721.3885	-3.12	87.2	
Fumonisin B_2	8.69	$C_{34}H_{59}NO_{14}$	$[M+H]^+$	705.3936	-2.42	97.3	96.6
Fusarenon-X	3.91	$C_{17}H_{22}O_8$	$[M+Na]^+$	354.1315	-4.80	89.0	
HT-2 toxin	7.30	$C_{22}H_{32}O_8$	$[M+NH_4]^+$	424.2097	-2.05	97.8	97.8
Macrosporin	9.36	$C_{16}H_{12}O_5$	$[M-H]^-$	284.0685	0.33	97.6	96.6
Moniliformin	0.72	$C_4H_2O_3$	$[M-H]^-$	98.0004	-3.01	91.2	
Mycophenolic acid	7.75	$C_{17}H_{20}O_6$	$[M+H]^+$	320.1260	-0.09	97.7	97.3
Nivalenol	2.15	$C_{15}H_{20}O_7$	$[M+HCOO]^-$	312.1209	-3.22	87.4	
Ochratoxin A	8.38	$C_{20}H_{18}ClNO_6$	$[M+H]^+$	403.0823	-1.81	91.7	97.7
Paraherquamide A	6.26	$C_{28}H_{35}N_3O_5$	$[M+H]^+$	493.2577	-0.85	96.7	96.8
Patulin	2.38	$C_7H_6O_4$	$[M-H]^-$	154.0266	-1.07	99.7	92.0
Roquefortine C	7.35	$C_{22}H_{23}N_5O_2$	$[M+H]^+$	389.1852	-0.36	98.9	97.3
Skyrin	10.23	$C_{30}H_{18}O_{10}$	$[M-H]^-$	538.0900	-1.19	98.0	99.1
Stachybotrylactam	9.22	$C_{23}H_{31}NO_4$	$[M+H]^+$	385.2253	-1.10	99.5	97.0
Sulochrin	6.21	$C_{17}H_{16}O_7$	$[M-H]^-$	332.0896	-0.71	98.1	97.8
T-2 toxin	7.83	$C_{24}H_{34}O_9$	$[M+NH_4]^+$	466.2203	-0.18	97.4	97.7
Tenuazonic acid	6.19	$C_{10}H_{15}NO_3$	$[M+H]^+$	197.1052	0.96	99.1	
Terphenyllin	6.75	$C_{20}H_{18}O_5$	$[M+H]^+$	338.1154	-1.79	98.3	95.4
Viridicatin	7.97	$C_{15}H_{11}NO_2$	$[M+H]^+$	237.0790	1.26	99.0	96.2
Zearalenone	8.36	$C_{18}H_{22}O_5$	$[M-H]^-$	318.1467	0.10	99.3	96.0

Figure 5 shows the MS/MS spectrum for T-2 toxin acquired in the maize extract spiked at 30 ng/mL (top panel) in comparison to the library spectrum from the PCDL (bottom panel). The middle panel is a mirror representation of the difference spectrum. All major fragment ions in the library spectrum of T-2 toxin were found in the measured spectrum within a narrow mass extraction window and in a similar ratio as in the reference spectrum, for a collision energy of 20 eV. Thus, the reverse search against the accurate mass library resulted in a score of 91.7 out of 100 and verified the presence of T-2 toxin in the sample. The detailed compound identification results are shown in the compound table in Figure 6. MS/MS scores for all individual compounds are not shown, but had to be above 60 to achieve verification (see Figure 7).

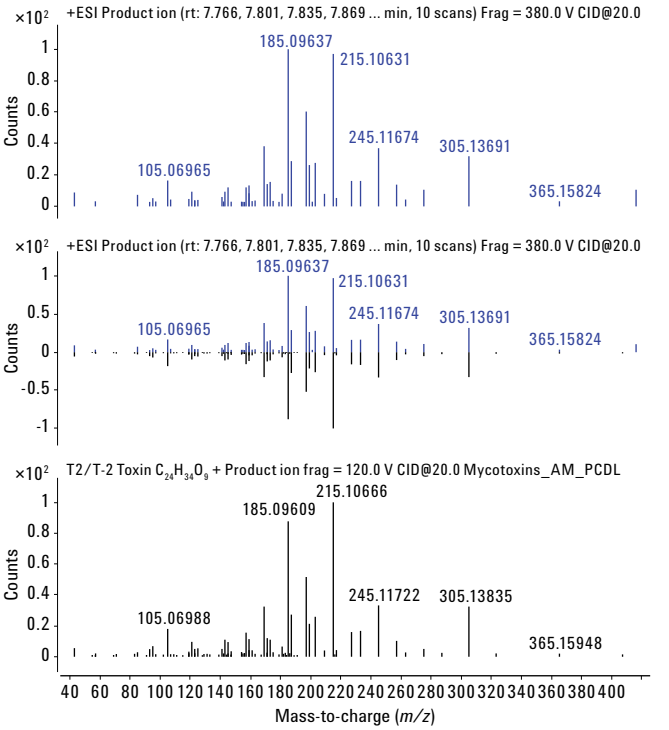

Figure 5. Comparison of the measured spectrum of T-2 toxin in the spiked maize extract with the reference spectrum from the Agilent Mycotoxins and Related Metabolites PCDL.

Comparison of the two workflows

Accurate-mass screening for mycotoxins and fungal metabolites, combined with verification of the identified contaminants by MS/MS library searching or All Ions MS/MS acquisition, was applied to spiked food extracts. Figure 7 shows the comparison of both acquisition modes for the three different matrices at 30 ng/mL. In general, red wine showed strong suppression effects, resulting in lower detection and verification rates compared to the other matrices. Target MS/MS acquisition with a single collision energy combined with library matching resulted in similar verification rates as the All Ions MS/MS acquisition with fragment coelution. In particular, the precursor isolation improved identification compared to the All Ions MS/MS fragmentation in the heavier matrix and for low mass contaminants. Conversely, All Ions MS/MS acquisition was very fast and allowed the differentiation of closely eluting isomers such as ergosine and ergosinine.

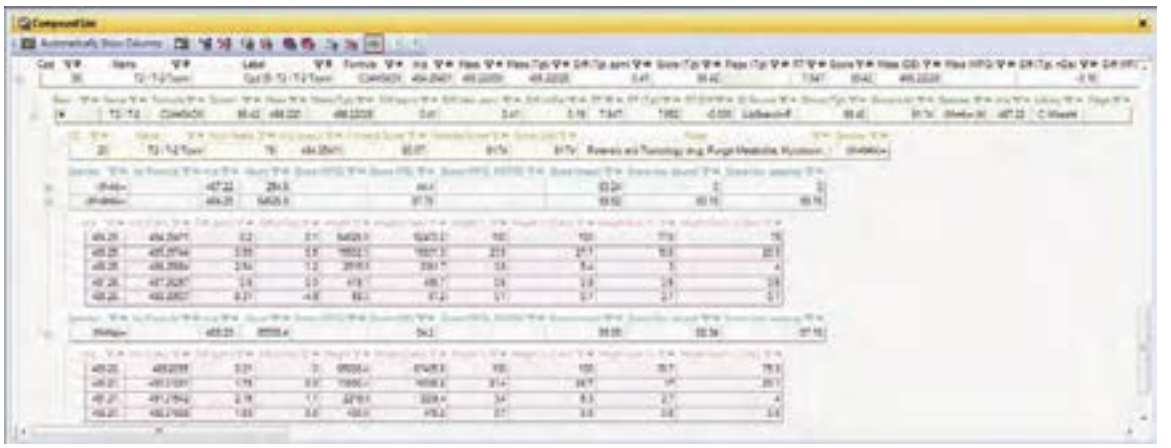

Figure 6. Compound identification results for T-2 toxin in a spiked maize sample including mass accuracy and isotope information from MS spectra, and MS/MS spectrum comparison.

Target MS/MS				All Ions MS/MS		
Red wine	Maize	Hazelnut	Compound name	Red wine	Maize	Hazelnut
			15-Monoacetoxyscirpenol			
			16-Keto-Aspergillimide			
			3-Acetyldeoxynivalenol			
			Aflatoxin B_1			
			Aflatoxin B_2			
			Aflatoxin G_1			
			Aflatoxin G_2			
			Aflatoxin M_1			
			Agroclavine			
			Alternariol			
			Alternariolmethylether			
			Aspergillimide			
			Beauvericin			
			Brevianamid F			
			Curvularin			
			Cyclopiazonic acid			
			Cyclosporin A			
			Diacetoxyscirpenol			
			Deoxynivalenol			
			Emodin			
			Enniatin B			
			Ergosine			
			Ergosinine			
			Ergotaminine/Ergotamine			
			Fumonisin B_1			
			Fumonisin B_2			
			Fusarenon-X			
			HT-2 toxin			
			Macrosporin			
			Moniliformin			
			Mycophenolic acid			
			Nivalenol			
			Ochratoxin A			
			Paraherquamide A			
			Patulin			
			Roquefortine C			
			Skyrin			
			Stachybotrylactam			
			Sulochrin			
			T-2 toxin			
			Tenuazonic acid			
			Terphenyllin			
			Viridicatin			
			Zearalenone			

Compound found by FBF and verified by library matching or coelution of fragments

Compound found by FBF, not verified

Compound not found by FBF

Figure 7. Results of screening and verification of mycotoxins and fungal metabolites in three different matrices at 30 ng/mL, using target MS/MS acquisition and library searching or All Ions MS/MS acquisition. Green: compound automatically found and presence verified by MS/MS library matching or fragment coelution; yellow: compound automatically found but no qualified MS/MS spectrum acquired.

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Analysis of a real sample

In addition to the spiked matrices, a contaminated hazelnut sample was extracted according to the method and was injected into the Q-TOF LC/MS system using All Ions MS/MS in positive and negative modes. The precursor and fragment information from the All Ions MS/MS evaluation of a calibration sample was exported to MassHunter Quantitative Analysis for the rapid creation of a quantitative data processing method. After data processing, results were reviewed by sample and compound in the Compounds-at-a Glance module. Figure 8 shows the chromatograms of six fungal metabolites found as contaminants in the hazelnut sample. Alternariol, alternariolmethylether, zearalenone, and macrosporin were analyzed in negative mode. Brevianamid F and mycophenolic acid were analyzed in positive mode. For all compounds, the mass deviation of the precursor *m/z* and at least one fragment ion was below 5 ppm, which is required for the identification of the compound.

Conclusions

The method presented here comprises fast, easy, and cheap solvent extraction and the subsequent injection of the diluted raw extract into the UHPLC/Q-TOF/MS system. It takes full advantage of the low delay volumes of the Agilent 1290 Infinity LC and its ability to handle high backpressures in UHPLC separations to increase chromatographic resolution. The method benefits from the sensitivity of the Agilent 6550 iFunnel Q-TOF, and from the versatile ionization capabilities of the Agilent Jet Stream ionization source.

An accurate mass PCDL for Mycotoxins and Related Metabolites was created and applied for screening and verification of mycotoxins in food samples. Target MS/MS and All Ions MS/MS acquisition were evaluated by analyzing food samples containing 44 fungal metabolites. Both acquisition modes, with Agilent MassHunter Software and the unique built-in identification criteria, effectively verified the presence of mycotoxins in the sample. While the target MS/MS information gave more confidence for low molecular weight compounds, the All Ions MS/MS data can be reinterrogated later for compounds that were not in the scope of the analysis during initial measurement. For efficient data review, the quantitative analysis software was used. This allowed visualization of quantifier and qualifier ions, including quality criteria such as mass accuracy, qualifier ratios, library match scores, and retention time matching.

The method is an appropriate supplement to single analyte or analyte-group detection methods, to increase knowledge of the occurrence of mycotoxins in various food commodities.

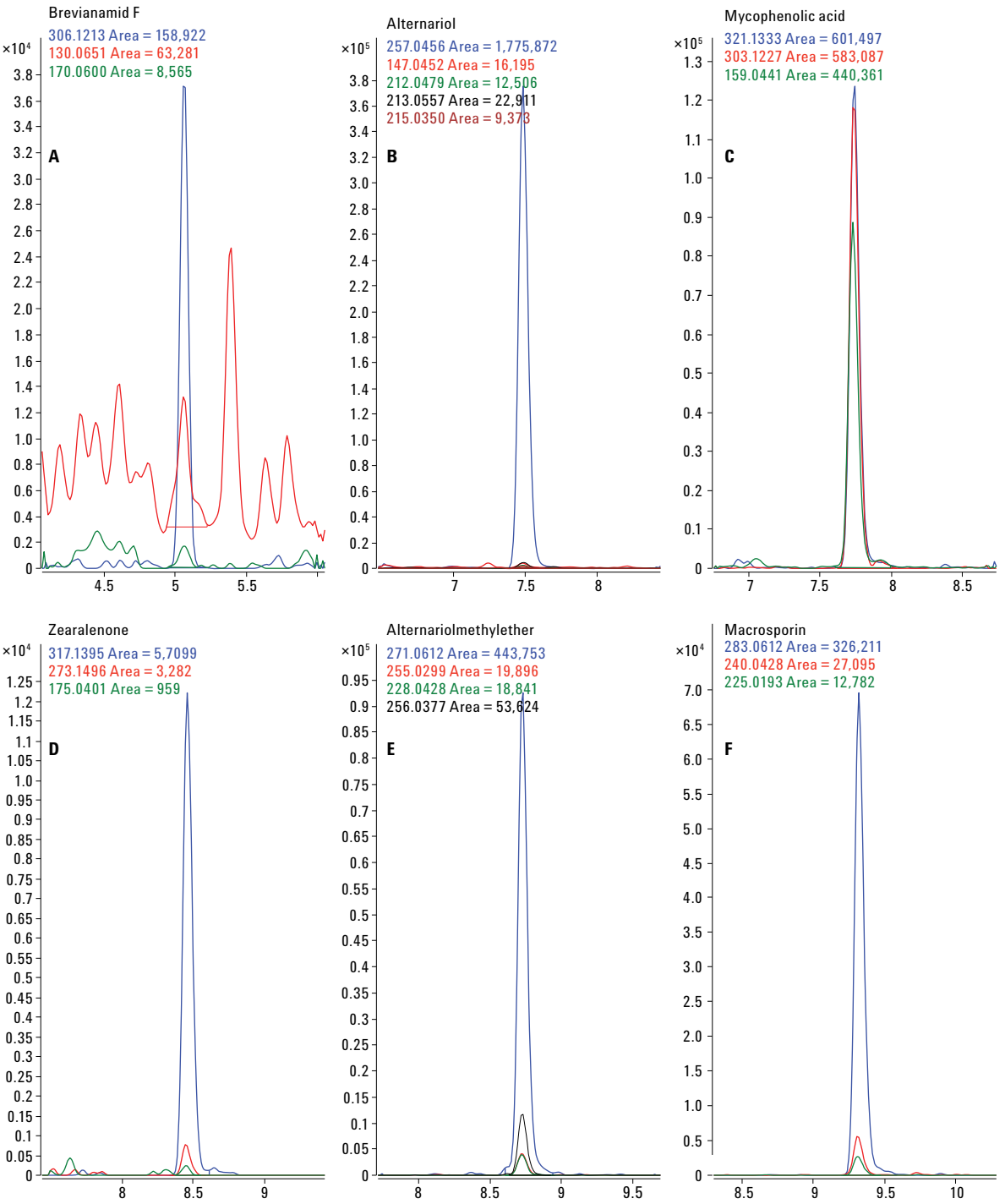

Figure 8. Chromatograms of mycotoxins detected in a naturally contaminated hazelnut sample. (A) brevianamid F (<LLOQ), (B) alternariol (310 µg/kg), (C) mycophenolic acid (6,100 µg/kg), (D) zearalenone (21 µg/kg), (E) alternariolmethylether (220 µg/kg), (F) macrosporin (520 µg/kg).

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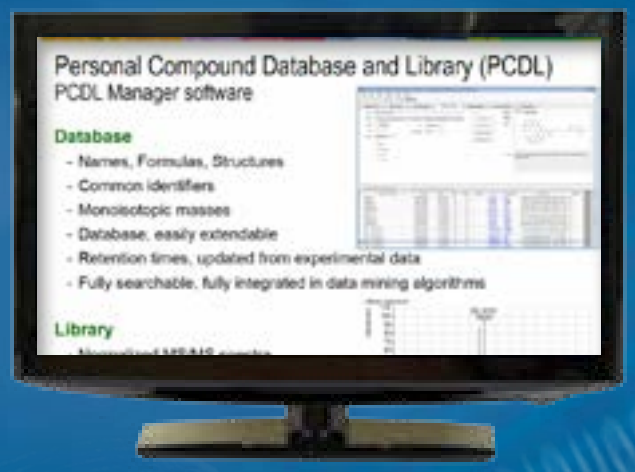

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