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.

Agilent Technologies

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)

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).

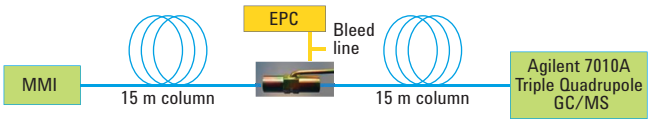


Figure 1. Column configuration for an optimal MRM application.

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

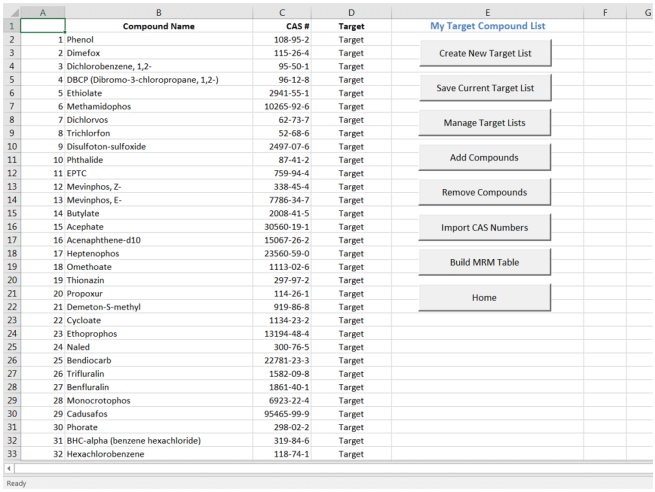
* All dwells in each TS were given the same value (no value under 10 was set) to attain a scan rate of ~5 scans/sec for the TS.

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

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.

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.



	Compound Name	CAS #	Target
1	Phenol	108-95-2	Target
2	Dimefox	115-26-4	Target
3	Dichlorobenzene, 1,2-	95-50-1	Target
4	DBCP (Dibromo-3-chloropropane, 1,2-)	96-12-8	Target
5	Ethiolate	2941-55-1	Target
6	Methamidophos	10265-92-6	Target
7	Dichlorvos	62-73-7	Target
8	Trichlorfon	52-68-6	Target
9	Disulfoton-sulfoxide	2497-07-6	Target
10	Phthalide	87-41-2	Target
11	EPTC	759-94-4	Target
12	Mevinphos, Z-	338-45-4	Target
13	Mevinphos, E-	7786-34-7	Target
14	Butylate	2008-41-5	Target
15	Acephate	30560-19-1	Target
16	Acenaphthene-d10	15067-26-2	Target
17	Heptenophos	23560-59-0	Target
18	Omethoate	1113-02-6	Target
19	Thionazin	297-97-2	Target
20	Propoxur	114-26-1	Target
21	Demeton-S-methyl	919-86-8	Target
22	Cycloate	1134-23-2	Target
23	Ethoprophos	13194-48-4	Target
24	Naled	300-76-5	Target
25	Bendiocarb	22781-23-3	Target
26	Trifluralin	1582-09-8	Target
27	Benfluralin	1861-40-1	Target
28	Monocrotophos	6923-22-4	Target
29	Caduasfos	95465-99-9	Target
30	Phorate	298-02-2	Target
31	BHC-alpha (benzene hexachloride)	319-84-6	Target
32	Hexachlorobenzene	118-74-1	Target

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).

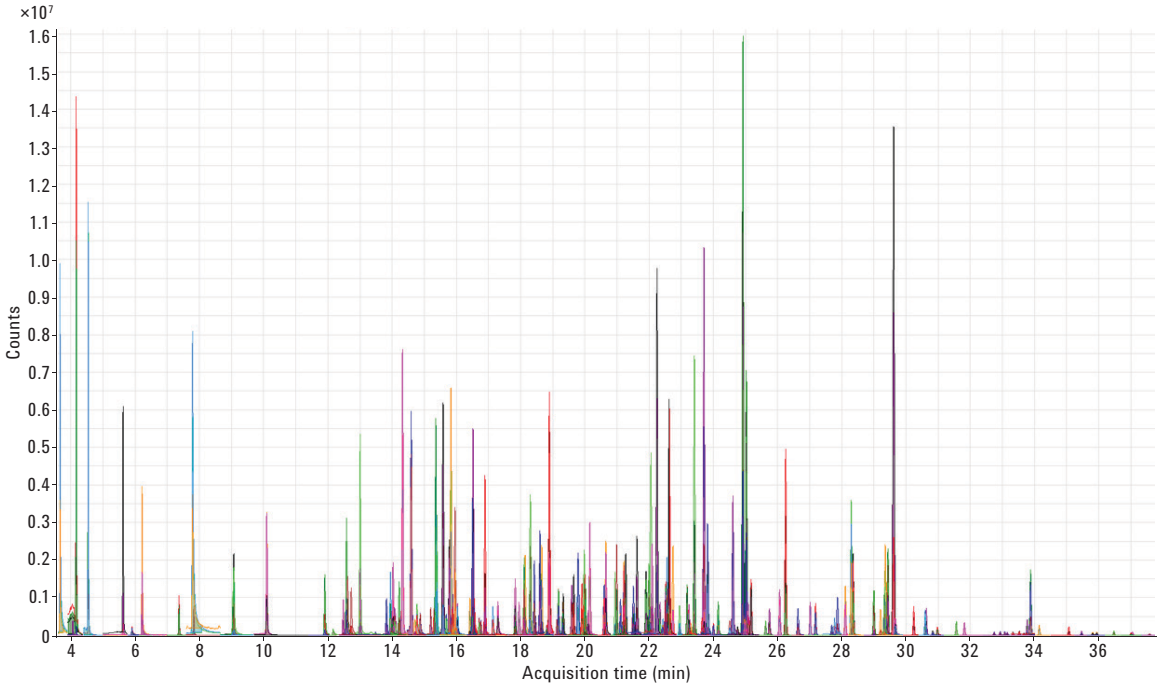


Figure 3. Chromatogram of all target compounds in acetonitrile (~200-400 ppb; compound dependent).

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.

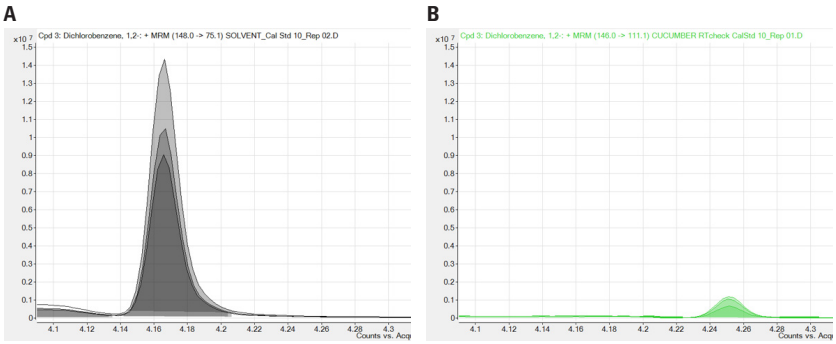


Figure 4. Chromatogram of MRM transitions of 1,2-dichlorobenzene in ACN (A) and cucumber (B).

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

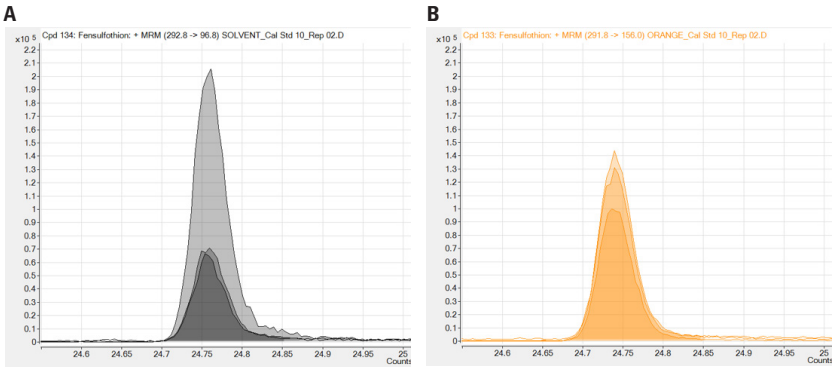


Figure 5. Chromatogram of MRM transitions of fensulfothion in ACN (A) and navel orange (B).

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

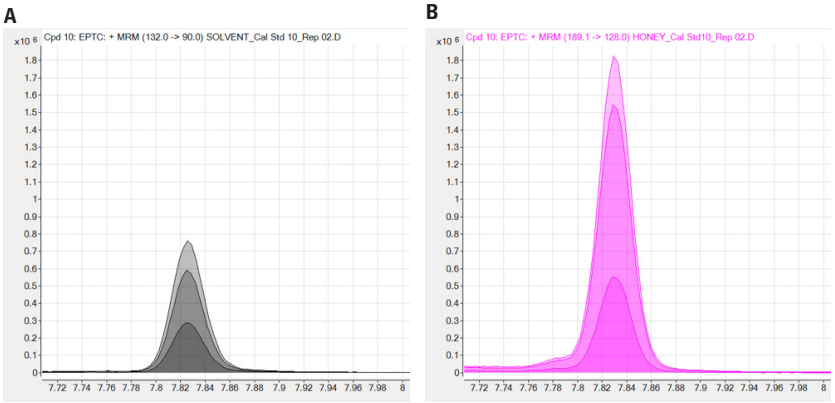


Figure 6. Chromatogram of MRM transitions of EPTC in ACN (A) and organic honey (B).

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

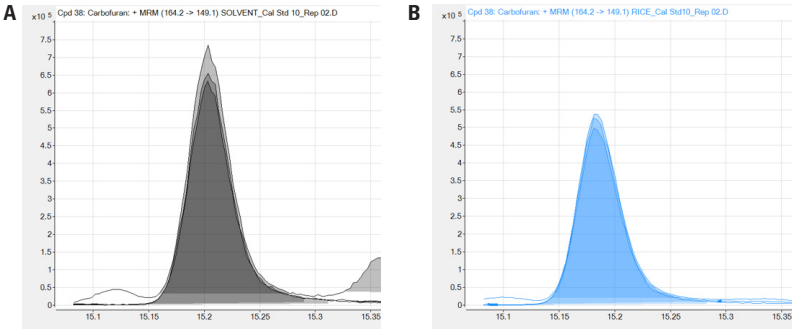


Figure 7. Chromatogram of MRM transitions of carbofuran (A) in ACN and jasmine rice (B).

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

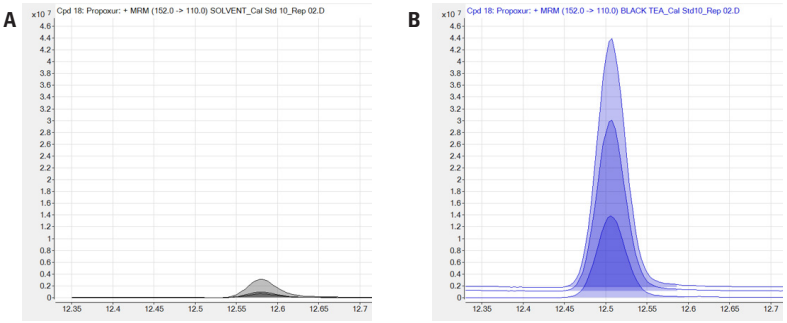


Figure 8. Chromatogram of MRM transitions of propoxur in ACN (A) and black tea (B).

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

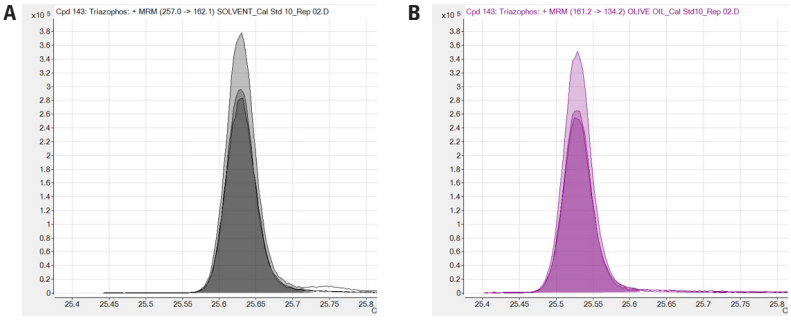


Figure 9. Chromatogram of MRM transitions of aldrin in ACN (A) and yellow onion (B).

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

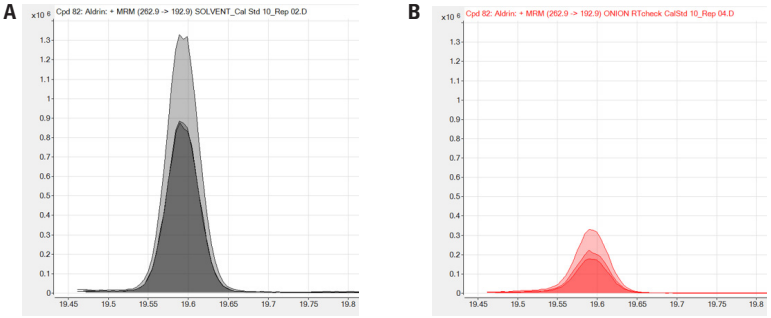


Figure 10. Chromatogram of MRM transitions of triazophos in ACN (A) and extra virgin olive oil (B).

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

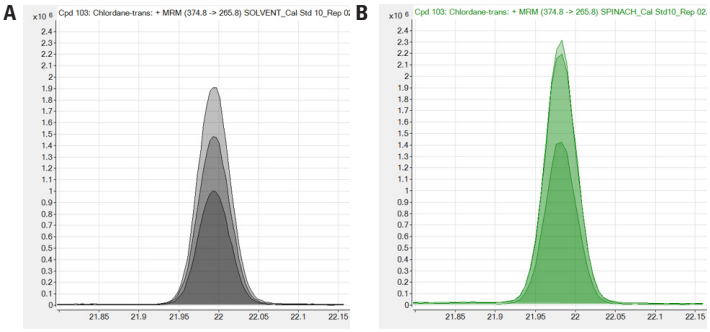


Figure 11. Chromatogram of MRM transitions of *trans*-chlordane in ACN (A) and spinach (B).

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

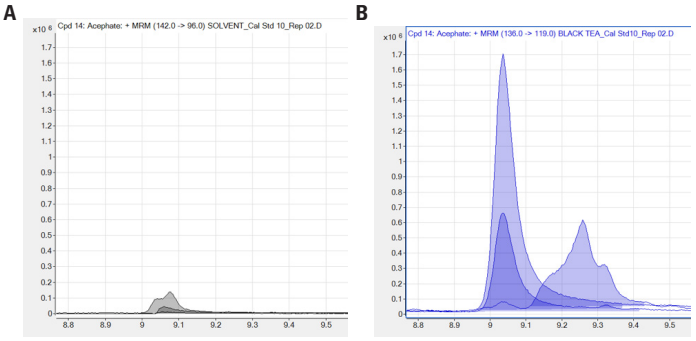


Figure 12. Chromatogram of MRM transitions of acephate in ACN (A) and in black tea (B).

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

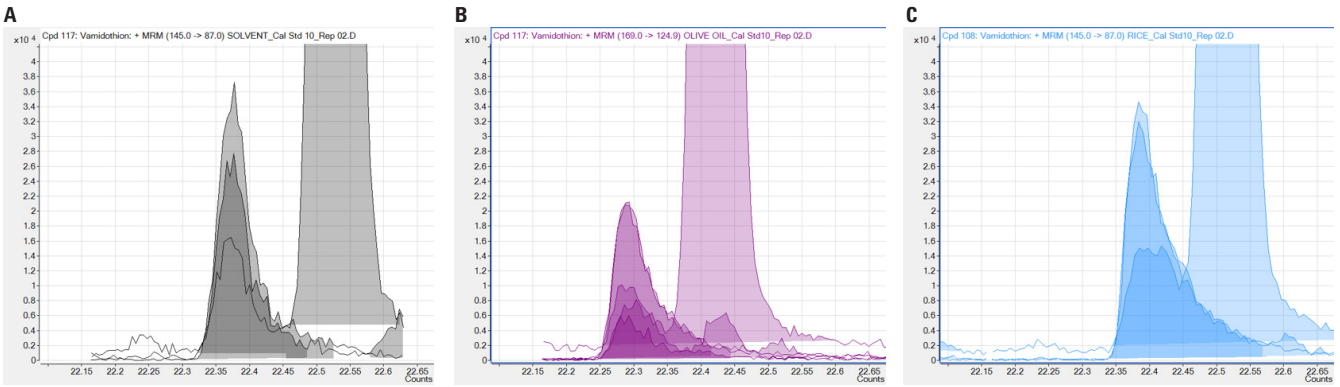


Figure 13. Chromatograms of MRM transitions of vamidothion in ACN (A), extra virgin olive oil (B), and jasmine rice (C).

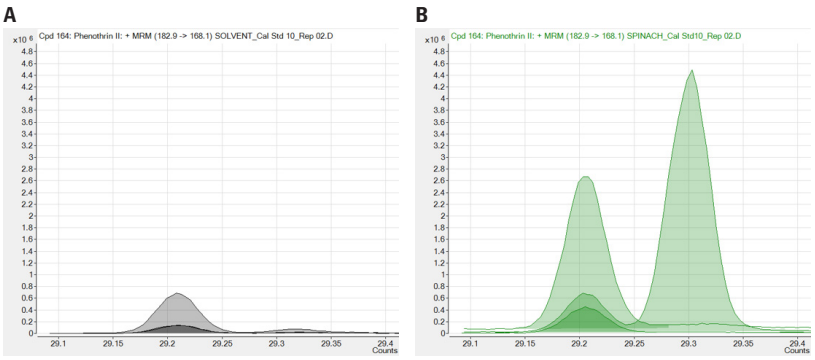


Figure 14. Chromatogram of MRM transitions of phenothrin II in ACN (A) and spinach (B).

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

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.

For More Information

These data represent typical results. For more information on our products and services, visit our Web site at www.agilent.com/chem.

www.agilent.com/chem

Printed in the USA
May 11, 2017
5991-7303EN

Agilent Technologies