LC/MS/MS Analysis of Pesticide Residues in Apples Using Agilent Chem Elut Cartridges

Application Note

Food Testing & Agriculture

Author

Andy Zhai
Agilent Technologies
Shanghai Co. Ltd.

Abstract

This application note describes a method for the analysis of pesticide residues in fruit, such as apples. The sample is extracted with a methanol water mixture. After elution with dichloromethane from Agilent Chem Elut cartridges, the organic phase is evaporated, and the residue is reconstituted with methanol. The presence of the target pesticides in the apple extracts are then determined by liquid chromatography coupled to an electrospray ionization tandem mass spectrometer (LC/ESI/MS/MS) operating in positive ion multiple reaction monitoring mode. The method is validated in terms of recovery and reproducibility for all of the analytes of interest. The 5 ng/g limit of quantitation (LOQ) for pesticides in apples is well below maximum residue limits (MRLs). The spiking levels for the recovery experiments are 5 and 10 ng/g. Most of the mean recoveries range between 70 and 120% (average of 89.2%), with RSD below 15% (average of 5.4%).

Introduction

Liquid-liquid extraction (LLE) is the most popular technique for sample preparation for pesticide residues in food matrixes. However, LLE requires large amounts of solvent, is time consuming, is laborious, and is not well suited for automation. As an alternative, solid-supported LLE was developed to provide a simple, robust, and rapid method for sample preparation using an Agilent Poroshell 120 LC column and Agilent Chem Elut cartridges. These cartridges contain a high-purity diatomaceous earth with a high capacity for aqueous adsorption. This is an exceptional medium for solid-supported liquid/liquid extraction (SLE), which is suggested as a standard technique in EN methods for pesticide residues in foods [1].

In this study, 19 pesticides were used for evaluating the performance of Agilent Chem Elut cartridges. The method was validated in terms of recovery and reproducibility. Table 1 shows the chemical and regulatory information for these pesticides in apples.

Table 1. Chemical and regulatory information of pesticides [2-4].

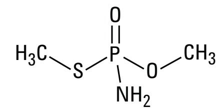
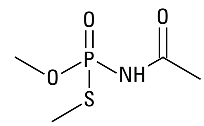
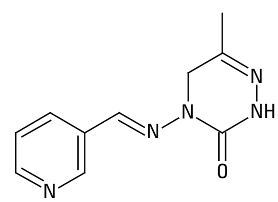
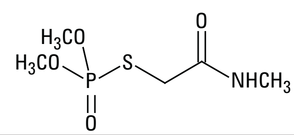
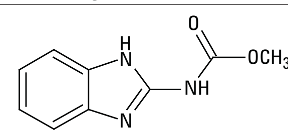
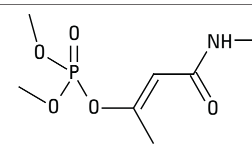
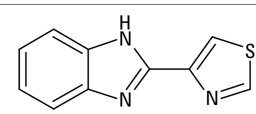
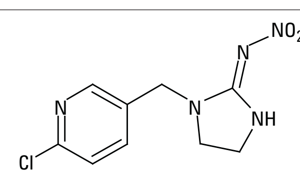
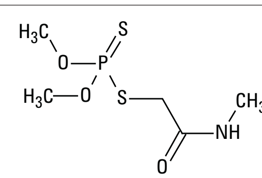
No.	Name	Class	Log P	pKa	Structure	MRLs (ng/g)
1	Methamidophos	Organophosphate	-0.79	NA		10
2	Acephate	Organophosphate	-0.89	8.35		20
3	Pymetrozine	Pyridine	-0.19	4.06		600
4	Omethoate	Organophosphate	-0.74	NA		50
5	Carbendazim	Benzimidazole	1.48	4.2		100
6	Monocrotophos	Organophosphate	-0.22	NA		50
7	Thiabendazole	Benzimidazole	2.39	4.73 12.00		50
8	Imidacloprid	Neonicotinoid	0.57	NA		1000
9	Dimethoate	Organophosphate	0.704	NA		50

Table 1. Chemical and regulatory information of pesticides [2-4] (continued).

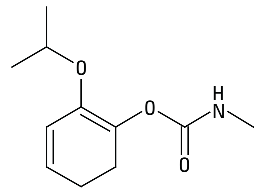
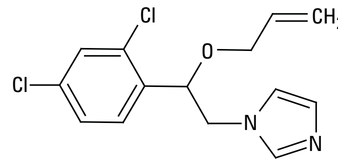
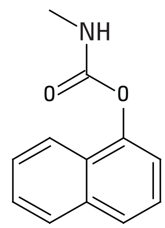
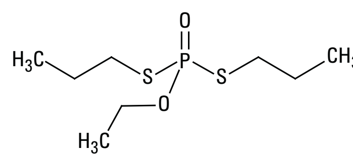
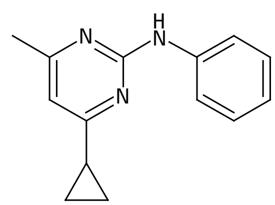
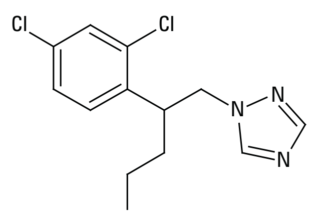
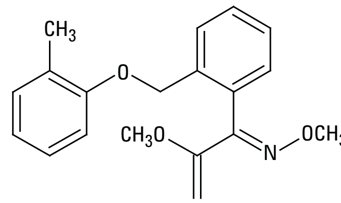
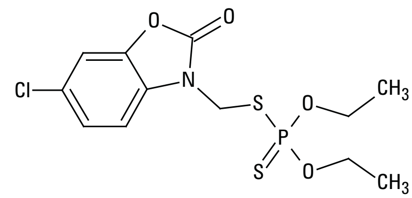
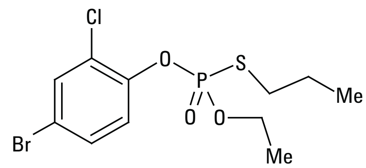
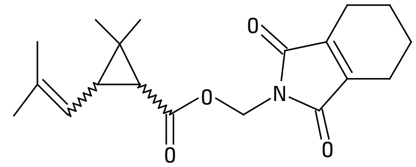
No.	Name	Class	Log P	pKa	Structure	MRLs (ng/g)
10	Propoxur	Carbamate	0.14	NA		2000
11	Imazalil	Imidazole	3.82	6.53		20
12	Carbaryl	Carbamate	2.36	10.4		50
13	Ethoprophos	Organophosphate	2.99	NA		5
14	Cyprodinil	Anilinopyrimidine	4	4.44		500
15	Penconazole	Triazole	3.72	1.51		50
16	Kresoxim-methyl	Strobilurin	3.4	NA		50

Table 1. Chemical and regulatory information of pesticides [2-4] (continued).

No.	Name	Class	Log P	pKa	Structure	MRLs (ng/g)
17	Phosalone	Organophosphate	4.01	NA		50
18	Profenofos	Organophosphate	1.7	NA		10
19	Tetramethrin	Pyrethoid	4.6	NA		10

Materials and Methods

All reagents and solvents were HPLC or analytical grade. Water, methanol (MeOH), and acetonitrile (ACN) were from Honeywell (Muskegon, MI, USA). Formic acid (FA) was from Fluka (Sleinheim, Germany). The pesticide standards were purchased from Sigma-Aldrich (St Louis, MO, USA). The internal standard (triphenyl phosphate, TPP) was from Agilent Technologies, Inc. (Wilmington, DE, USA).

Standard and internal standard (IS) stock solutions (0.5 mg/mL for all) were made in MeOH, 0.1% FA in ACN, or DMSO, and stored at -20 °C. QC spiking solutions were made fresh daily in 1:1 MeOH:water with 0.1% FA.

Equipment

Agilent 1200 Infinity Series

Agilent 6460 Triple Quadrupole LC/MS with Electrospray Ionization

Flying Pigeon Centrifuge (Anting Science Instrument, Shanghai, P.R. China)

Conditions

Column:	Agilent Poroshell 120 SB-C18, 2.1 × 100 mm, 2.7 µm (p/n 685775-902)	
Sample prep:	Agilent Chem Elut Cartridges, 5 mL (p/n 12198006)	
Eluent:	A, 0.1% FA in water B, 0.1% FA in ACN	
Injection volume:	5 µL	
Flow rate:	0.4 mL/min	
Gradient:	Time (min)	%B
	0	5
	1	5
	4	50
	8	90
	9	90
	9.2	5
	10	5
Temperature:	30 °C	
Post run:	2 minutes	
Total cycle time:	12 minutes	

MS conditions

Positive mode

Gas temperature:	300 °C
Gas flow:	10 L/min
Nebulizer:	40 psi
Capillary:	3,500 V

Other conditions relating to the analytes are listed in Table 2.

Table 2. Instrument acquisition data used for the analysis of 19 pesticides by LC/MS/MS.

No.	Analyte	MRM channels (*m/z*)	Fragmentor (V)	CE (V)	RT (min)
1	Methamidophos	142.0>94.0 142.0>125.0	80	15 10	1.30
2	Acephate	184.0>143.0 184.0>95.1	65	3 20	1.73
3	Pymetrozine	218.1>105.1 218.1>78.1	130	20 50	2.31
4	Omethoate	214.0>124.9 214.0>182.9	92	17 5	2.50
5	Carbendazim	192.1>160.1 192.1>132.1	110	15 30	3.62
6	Monocrotophos	224.1>127.0 224.1>193.0	100	20 5	3.82
7	Thiabendazole	202.0>175.1 202.0>131.1	160	25 35	3.84
8	Imidacloprid	256.1>209.1 256.1>175.1	140	10 15	4.55
9	Dimethoate	230.0>125.0 230.0>199.0	92	17 5	4.62
10	Propoxur	210.2>111.1 210.2>93.1	70	10 25	5.57
11	Imazalil	297.1>159.0 297.1>69.1	150	20 15	5.79
12	Carbaryl	202.0>145.0 202.0>127.1	70	15 40	5.82
13	Ethoprophos	243.0>96.9 243.0>130.9	115	35 15	6.92
14	Cyprodinil	226.1>93.1 226.1>108.1	150	37 52	7.23
15	Penconazole	284.0>70.1 284.0>159.0	125	10 30	7.28
16	Kresoxim-methyl	314.1>222.1 314.1>235.1	70	3 5	7.57
17	Phosalone	368.0>182.0 368.0>322.0	80	10 5	8.06
18	Profenofos	373.0>303.0 373.0>345.0	80	15 1	8.22
19	Tetramethrin	332.2>164.1 332.2>135.2	87	20 20	8.57
IS	TPP	327.1>77.1 327.1>152.1	170	45 40	7.77

Sample preparation

Organically grown, pesticide-free apples were purchased from a local supermarket. The apples were cut coarsely and placed into a clean plastic bag and frozen at -20 °C overnight. The following day, the required amount of frozen apple was removed and blended. Samples were comminuted thoroughly to homogenize the sample. No pieces of apple were visible in the final sample.

A 10 g (±0.1g) amount of homogenized apple was placed into a 50 mL centrifuge tube. One mL of water was added, so that a total volume of 10 mL water was obtained; apples contain approximately 10% water. QC samples were fortified with 100 µL of the appropriate QC spiking solution. IS spiking solution (100 µL) was added to all the samples, except the control blank, to yield a 10 ng/g concentration. Tubes were capped and vortexed for 1 minute. MeOH (20 mL) was added to each tube using the dispenser and homogenized for 2 minutes using a high-speed blender. Sample tubes were centrifuged at 4,000 rpm for 5 minutes. NaCl solution (2.5 mL, 20% w/w) was pipetted into a 10 mL measuring flask, which was filled up to the mark with the supernatant, and mixed well. This was the sample solution.

Chem Elut cleanup

Sample solution (5 mL) was added to a 5 mL Chem Elut cartridge. After 5 minutes, the cartridge was eluted with 15 mL of dichloromethane, and the eluate was collected in a 50 mL round-bottom flask. Elution was repeated with another 15 mL of dichloromethane, and the eluate was added to the same flask. The combined eluates were reduced almost to dryness using a rotary evaporator. The remaining dichloromethane was removed with a stream of nitrogen. MeOH (0.5 mL) was added to the flask, and the residue was carefully dissolved by swirling the flask in an ultrasonic bath. Water (0.5 mL) was then added and vortexed for 1 minute. Finally, the solution was filtered through a PTFE filter into a sample vial for LC/MS/MS analysis.

Results and Discussion

Figures 1, 2, and 3 show the LC/MS/MS chromatograms of matrix blank, 5 ng/g fortified apple extract processed by Chem Elut, and standard solution with matrix blank.

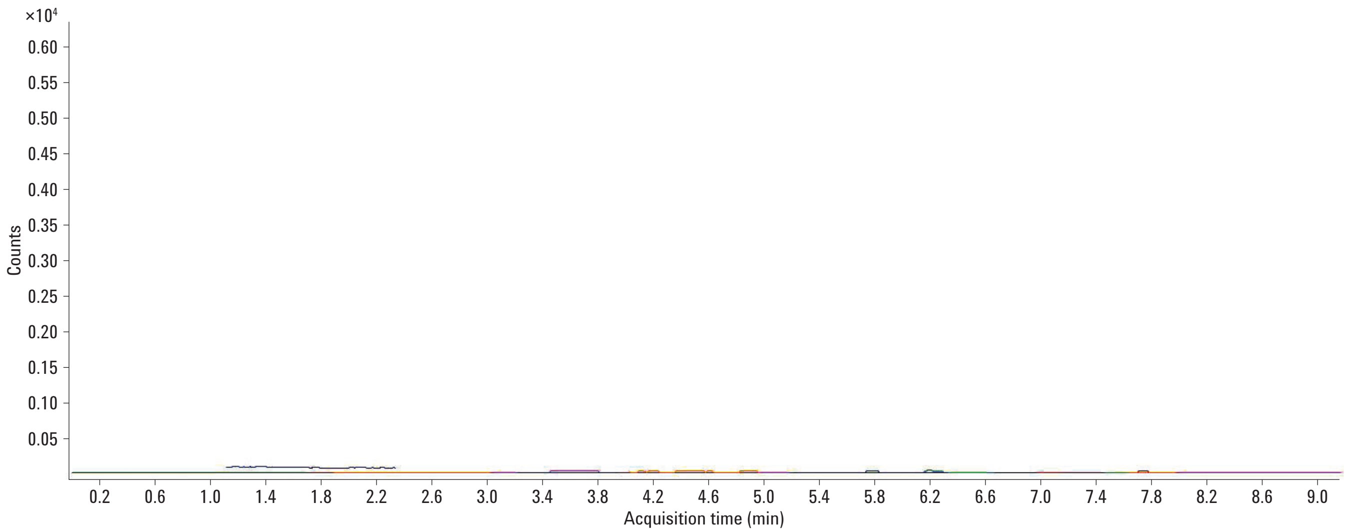


Figure 1. Multiple reaction monitoring chromatogram of apple matrix blank.

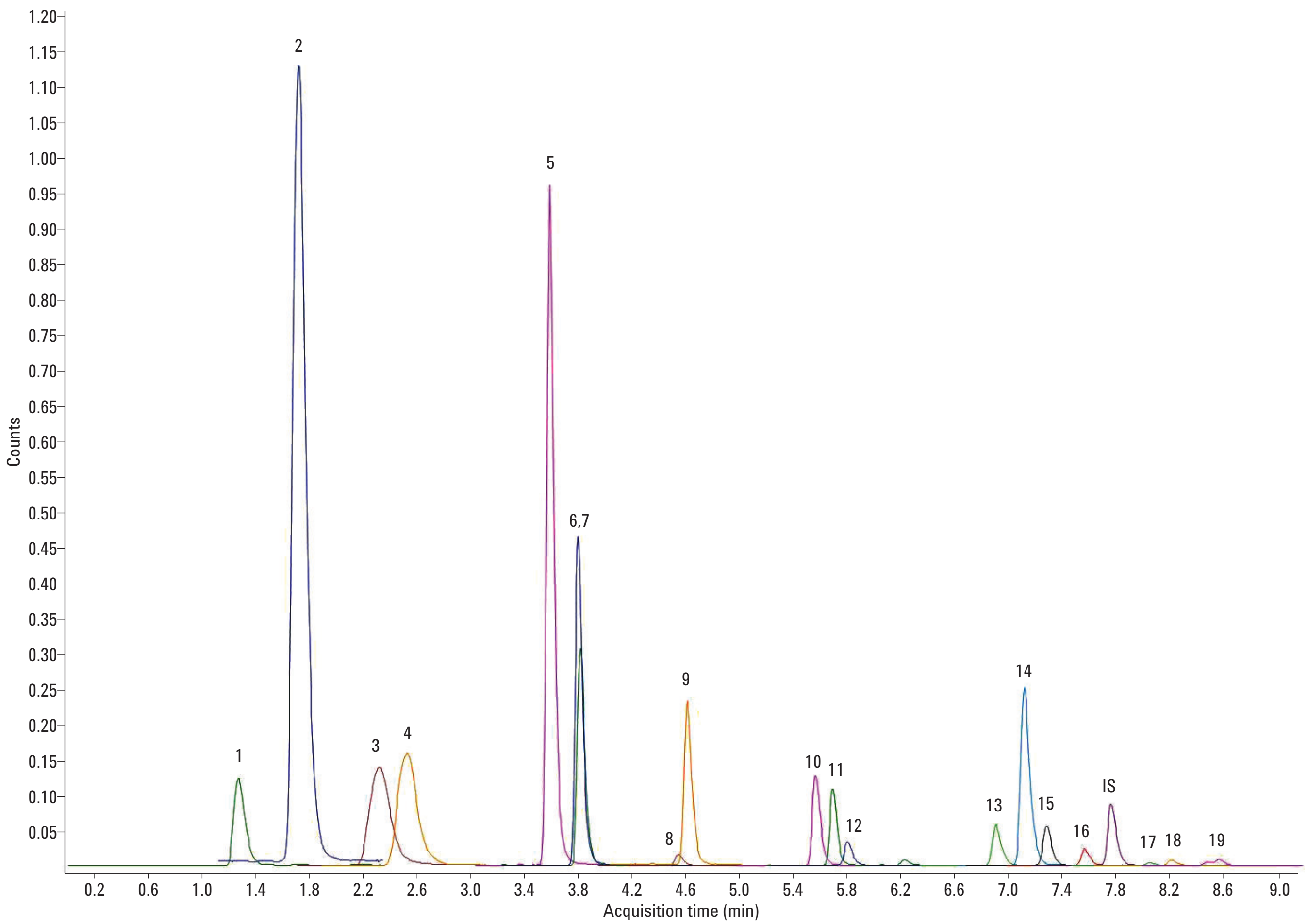


Figure 2. Multiple reaction monitoring chromatograms of 5 ng/g fortified apple sample processed using an Agilent Chem Elut cartridge. Peaks identified in Table 1.

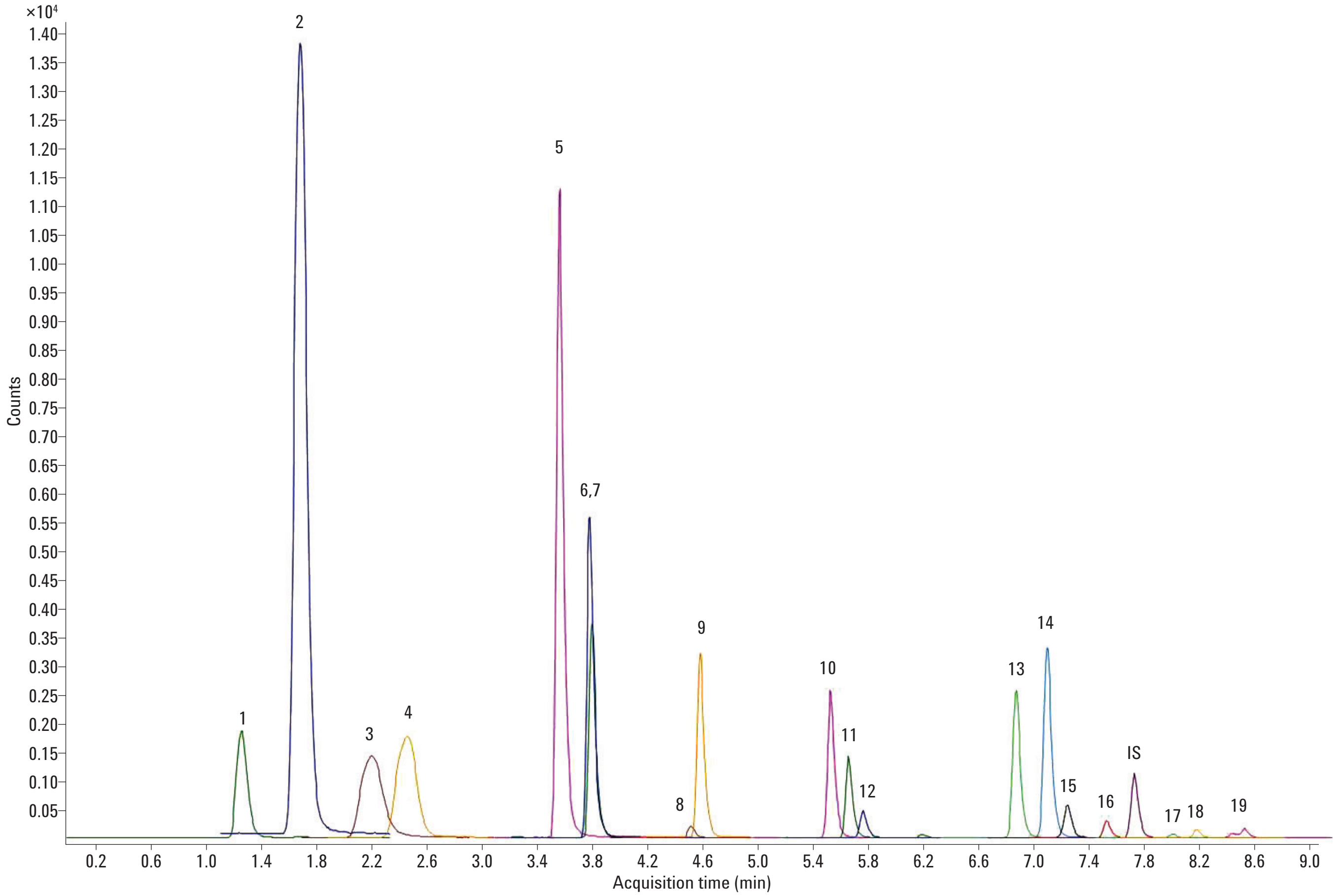


Figure 3. Multiple reaction monitoring chromatograms of standards solution with matrix blank. Peaks identified in Table 1.

Linearity and limit of quantification

The linearity calibration range for all of the pesticides tested was 5 to 100 ng/g. Calibration curves, spiked in matrix blanks, were made at 5, 10, 20, 50, and 100 ng/g. TPP was used as an internal standard at 10 ng/g. The calibration curves were generated by plotting the relative responses of analytes (peak area of analyte/peak area of IS) to the relative concentration of analytes (concentration of analyte/concentration of IS). The 5 ng/g quantification limit established for all pesticides was at or lower than the MRLs of these pesticides in fruits and vegetables. Table 3 shows the linear regression equations and correlation coefficients.

Table 3. Linearity of pesticides in apples extracted with Agilent Chem Elut.

No.	Name	Regression equation	R^2
1	Methamidophos	Y = 1.0236x + 0.0347	0.999
2	Acephate	Y = 2.3861x – 0.1255	0.999
3	Pymetrozine	Y = 1.9630x + 0.1412	0.999
4	Omethoate	Y = 1.4163x – 0.0127	0.998
5	Carbendazim	Y = 0.0724x – 0.0097	0.998
6	Monocrotophos	Y = 0.8779x + 0.0213	0.997
7	Thiabendazole	Y = 1.5729x + 0.0445	0.998
8	Imidacloprid	Y = 0.4215x – 0.0035	0.999
9	Dimethoate	Y = 2.3146x + 0.0711	0.999
10	Propoxur	Y = 1.2570x + 0.0025	0.998
11	Imazalil	Y = 3.0021x + 0.0772	0.999
12	Carbaryl	Y = 0.1475x + 0.0068	0.996
13	Ethoprophos	Y = 1.8301x + 0.0362	0.999
14	Cyprodinil	Y = 0.3677x – 0.0014	0.999
15	Penconazole	Y = 3.2473x + 0.2007	0.999
16	Kresoxim-methyl	Y = 1.2881x + 0.0425	0.999
17	Phosalone	Y = 2.8005x + 0.0259	0.998
18	Profenofos	Y = 0.1732x + 0.0103	0.999
19	Tetramethrin	Y = 0.1524x + 0.0201	0.996

Recovery and reproducibility

The recovery and reproducibility were evaluated by spiking pesticide standards in comminuted samples at 5 and 10 ng/g. These QC samples were quantitated against the matrix spike calibration curve. The analysis was performed in replicates of six at each level. The recovery and reproducibility (shown as RSD) data are shown in Table 4. It can be seen from the results that most of the pesticides give excellent recoveries and precision.

Conclusions

All of the pesticides were separated and well detected with the Agilent Poroshell 120 column and Agilent 6460 Triple Quadrupole LC/MS. After cleanup with Agilent Chem Elut cartridges, there were no interferences in the chromatograms. Chem Elut cartridges provided a simple and effective method for the extraction of representative pesticides in apples. The recovery and reproducibility, based on matrix-spiked standards, were acceptable for multiclass, multiresidue pesticide determination in apples.

Table 4. Recovery and reproducibility of pesticides in fortified apple with Agilent Chem Elut.

		5 ng/g fortified QC		10 ng/g fortified QC	
No.	**Analytes**	**Recovery (%)**	**RSD (%) (n=6)**	**Recovery (%)**	**RSD (%) (n=6)**
1	Methamidophos	75.3	6.2	71.8	5.3
2	Acephate	93.9	3.7	86.3	4.0
3	Pymetrozine	103.8	3.3	107.8	3.8
4	Omethoate	95.9	8.1	94.8	7.5
5	Carbendazim	96.1	2.5	91.2	1.9
6	Monocrotophos	98.2	6.4	73.7	8.1
7	Thiabendazole	94.5	6.9	89.6	5.4
8	Imidacloprid	93.6	1.7	102.0	2.7
9	Dimethoate	89.4	8.6	83.2	6.6
10	Propoxur	62.4	7.3	69.3	9.3
11	Imazalil	88.8	9.2	83.6	9.6
12	Carbaryl	92.7	2.7	109.3	2.9
13	Ethoprophos	29.2	11.0	28.6	12.7
14	Cyprodinil	95.6	1.8	91.1	4.4
15	Penconazole	119.0	10.5	119.2	9.6
16	Kresoxim-methyl	102.2	0.8	110.9	2.1
17	Phosalone	106.4	5.4	116.0	4.2
18	Profenofos	78.3	2.6	89.7	4.2
19	Tetramethrin	78.5	3.8	84.8	8.0

References

1. DIN EN 15637:2009-02. “Foods of plant origin - determination of pesticide residues using LC-MS/MS following methanol extraction and clean-up using diatomaceous earth.” Deutsches Institut für Normung, Berlin.
2. http://sitem.herts.ac.uk/aeru/footprint/en/index.htm
3. http://www.m5.ws001.squarestart.ne.jp/foundation/search.html
4. http://www.mrldatabase.com/?selectvetdrug=0

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
July 23, 2012
5991-0868EN

Agilent Technologies