

Innovative Approaches

for today's food analysis challenges

Agilent SPE Food Safety Applications Notebook
Volume 1



Our measure is your success.





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Reliable food safety testing begins with reliable SPE

Dear Valued Customer,

You are committed to producing foods and beverages of consistent quality and uncompromising safety. Your customers demand nothing less.

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From inspection and product development to quality assurance and packaging, Agilent instruments, systems, columns and supplies help your lab meet the toughest standards.

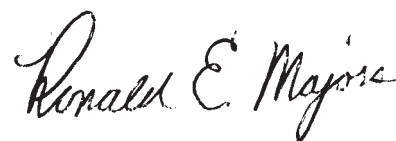
But that's only *part* of the story. Agilent also supports your analytical and business challenges with in-depth experience, broad knowledge, and creative people, plus our keen insight into industry trends and global regulations.

NEW Agilent SampliQ SPE products: your first step in food safety analysis

High-quality Agilent SampliQ SPE products help you confidently extract and concentrate samples from complex matrices, ensuring fast, accurate, and reproducible results from the very first step. Our family of products includes:

- **Agilent SampliQ QuEChERS kits** enable you to prepare food samples for multi-residue, multi-class pesticide analysis with just a few simple steps.
- **Agilent SampliQ polymers** allow the retention of target molecules over a wide pKa range. And unlike silica-based phases, SampliQ polymers yield the same exacting results if they inadvertently dry out during conditioning.

On the following pages, you'll discover leading-edge techniques and sample prep methods that can dramatically improve the reliability and throughput of your food safety analysis.



Ronald E. Majors, Ph. D., Senior Chemist





What is SPE?

Sample preparation is an essential part of successful chromatographic measurement, because it complements highly specific detectors and fast, high-resolution columns. However, if your sample contains compounds that are not of interest, the resulting interference can jeopardize your separation, detection, and quantification.

This problem can be remedied through Solid Phase Extraction (SPE), a fast, cost-effective technique for purifying extracts and ensuring accurate results.

Simply put, SPE reduces sample complexity. By harnessing the principles of HPLC, SPE selectively removes interferences and/or analytes from complex matrices such as foods, environmental samples, and biological specimens. SPE can also replace liquid-liquid extraction protocols, greatly reducing solvent consumption.

In short, SPE can make the difference between a definitive measurement and inaccurate, imprecise, irreproducible results.

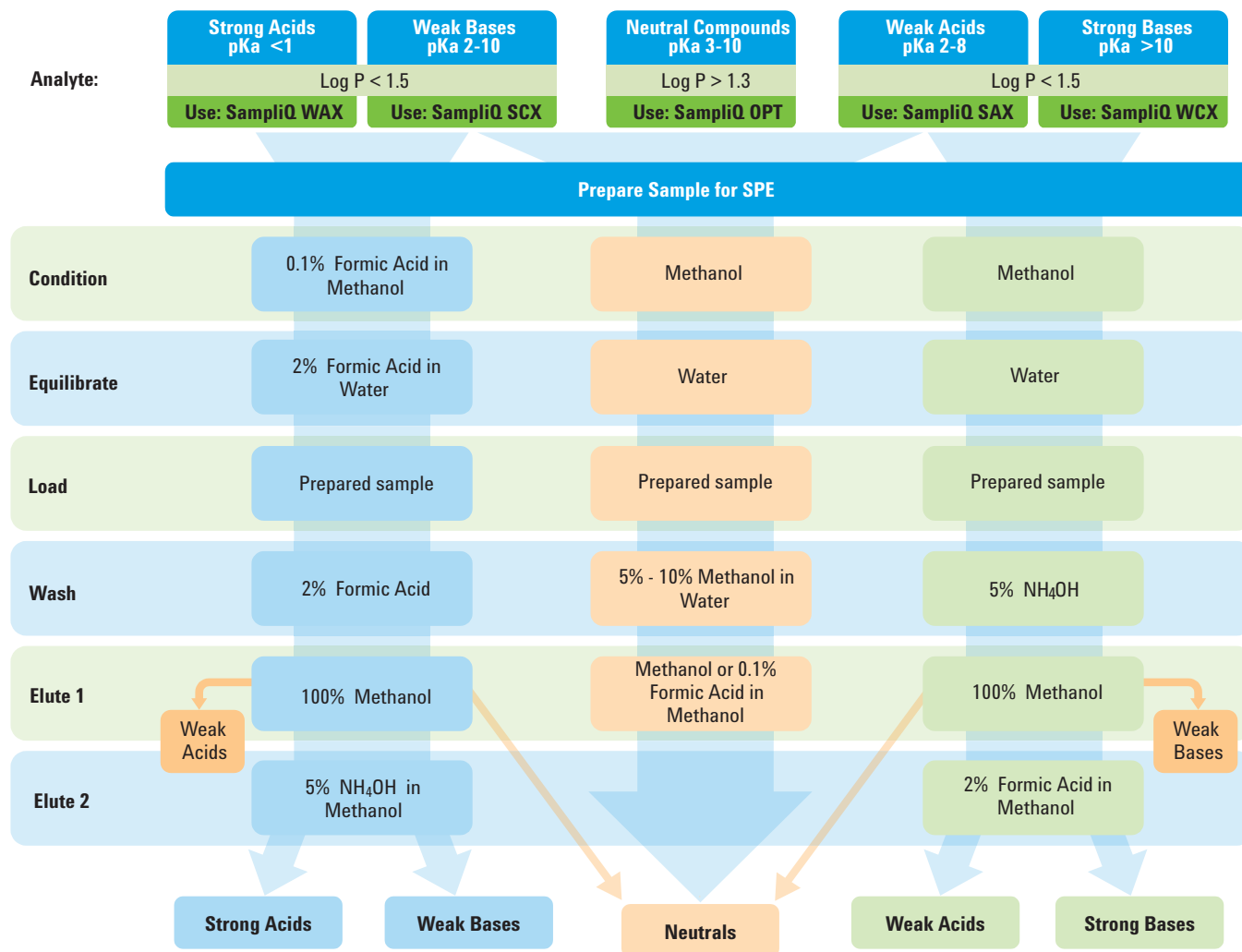
With the creation of our SampliQ line, Agilent has solidified our commitment to SPE as an integral part of your overall workflow. We are pleased to offer polymer, silica-based and other sorbents in a variety of configurations to address a wide array of extraction needs.





Method Development

Agilent SampliQ Polymeric A/N/B Method





Pesticides and Contaminants

Fast, confident pesticide detection and quantitation

The presence of certain pesticides and contaminants (such as glyphosate, benzimidazole fungicides, mycotoxins and melamine) in food can pose significant health risks. As a result, trace element analysis is quickly becoming a regulatory requirement for all food companies.

Agilent scientists work closely with major testing laboratories and regulatory agencies to develop strategies that can help you:

- Confidently monitor ultra-trace levels of target and non-target compounds
- Use multi-residue MS-based methods to achieve significantly lower LODs and LOQs for a wide range of food matrices
- Routinely screen for hundreds of compounds in a single analysis
- Significantly shorten your analysis time, boost your lab's productivity, and reduce your cost per sample
- Meet consumer and regulatory demands for origin and purity



Determination of Benzimidazole Fungicides in Apple Juice by SampliQ Polymer SCX Solid-Phase Extraction with High Performance Liquid Chromatography (Publication 5990-3235EN)

Introduction

Fungicides represent approximately 20-25% of all pesticides used for agricultural applications. As a class, Benzimidazole fungicides are used for pre- and post-harvest control of a wide range of pathogens. Two of the main compounds in the benzimidazole family are carbenazim and thiabendazole. SPE coupled with HPLC was optimized for the extraction and quantification of these fungicides in apple juice.

Sample Pretreatment

Weigh 10 g apple juice, dilute to 100 mL with water, and mix with a glass rod for 1 minute. Transfer the diluted sample to a 250 mL Erlenmeyer flask and adjust pH to 10 with 2 mM NaOH solution. Divide the sample between two or three 50 mL polypropylene centrifuge tubes and centrifuge for 10 minutes at 4,000 rpm. Recombine the supernatants into a glass beaker.

Instrument conditions

HPLC conditions

Column:	Agilent ZORBAX Eclipse Plus C18 LC Column, 100 mm x 2.1 mm, 3.5 μ m (Part No. 959793-902)
Flow rate:	1.0 mL/min
Injection volume:	20 μ L
Detection wavelength:	288 nm
Mobile phase:	Phosphate buffer-acetonitrile (73:27)

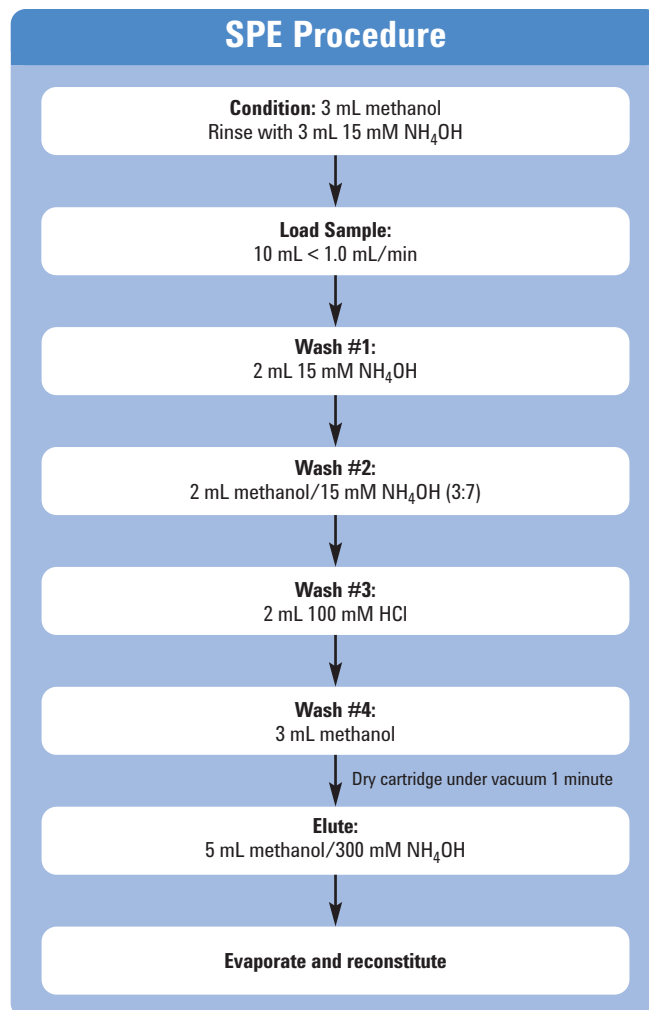


Figure 1. Fungicides in apple juice SPE procedure

Results

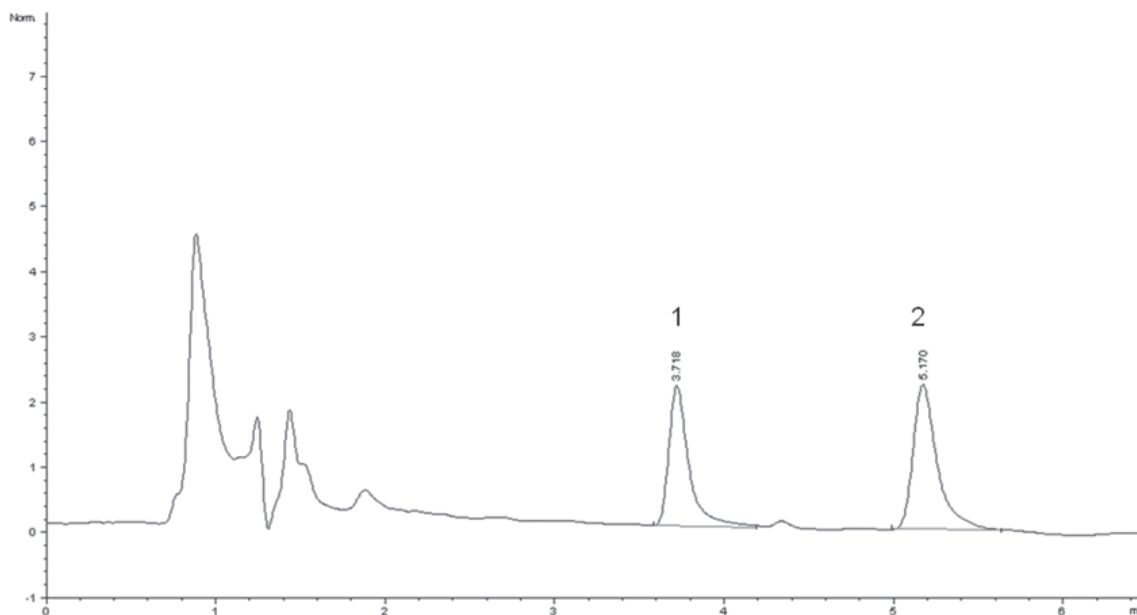


Figure 2. Chromatogram of apple juice sample spiked at 100 µg/kg (1 – Carbendazim, 2 – Thiabendazole)

Compound	Spiked level (µg/kg)	Recovery (%)	% RSD (n = 6)
Carbendazim	25	98.6	3.99
	50	99.4	3.24
	100	95.9	3.27
Thiabendazole	25	99.0	2.38
	50	92.1	4.90
	100	93.0	3.79

Table 1. Recoveries and RSDs of fungicides in apple juice by SPE

Ordering information

Agilent SampliQ SCX, 3 mL, 60 mg. Part No. 5982-3236.

Agilent ZORBAX Eclipse Plus C18 LC Column, 100 mm x 2.1 mm, 3.5 µm. Part No. 959793-902.

Agilent 0.45 µm Filter Membranes. Part No. 5185-5836.

Trace-level Analysis of Melamine in Milk Products on Agilent 7890A/5975 GC/MSD Using a New Agilent J&W DB-5ms Ultra Inert Column and SampliQ SCX Cartridges (Publication 5990-3282EN)

Introduction

A GC/MS method is presented for the quantitative determination and confirmation of melamine residues in milk products. The milk sample was cleaned up using Agilent's new SampliQ SCX SPE cartridges before derivatization. The derived extracts were analyzed by GC/MS with EI in synchronous SIM/scan mode on a new Agilent J&W DB-5ms Ultra Inert Column.

Instrument conditions

GC conditions

Instruments:	Agilent 7890A/5975C GC/MSD Agilent 7683 Automatic Liquid Sampler (ALS)
Column:	Agilent J&W DB-5ms Ultra Inert GC Column, 30 m x 0.25 mm, 0.25 µm (Part No. 122-5532UI)
Inlet temperature:	EPC, split/splitless at 250 °C
Injection volume:	1 µL, split 3:1
Carrier gas:	Helium, constant flow mode, 1.3 mL/min
Oven program:	75 °C (1 min); 30 °C/min to 300 °C (2 min)
Transfer line:	290 °C

MS conditions

MS:	EI, SIM/scan
Solvent delay:	4.2 min
MS temperature:	230 °C (source); 150 °C (quad)
Scan mode:	Mass range (40 to 450 amu)
SIM mode:	Ion (342, 327*, 171, 99)

*Quantitative ion

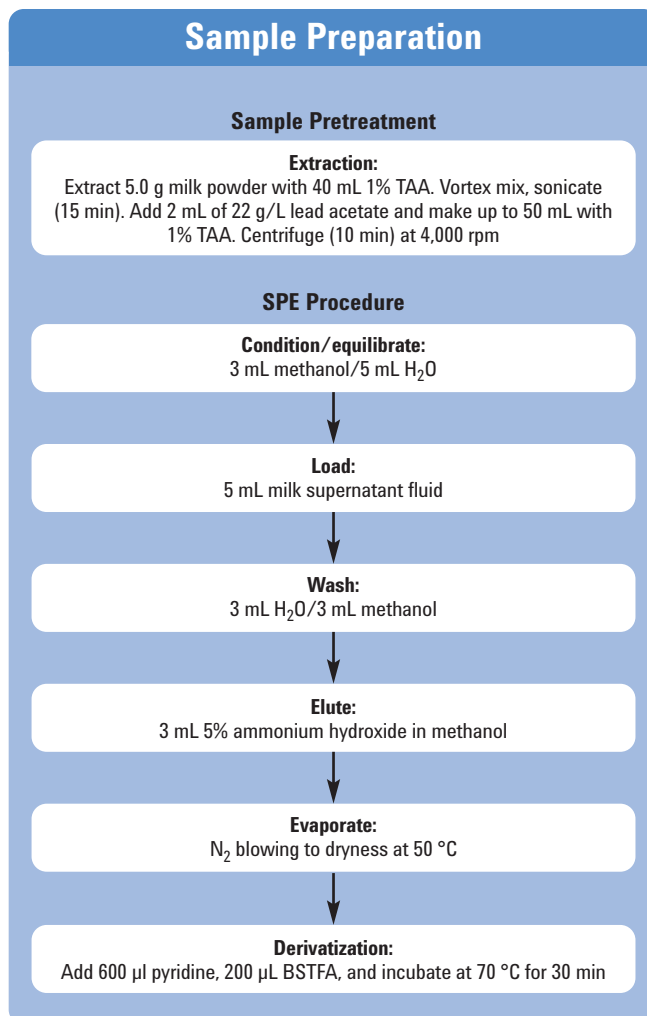


Figure 1. Scheme of sample preparation process

Results

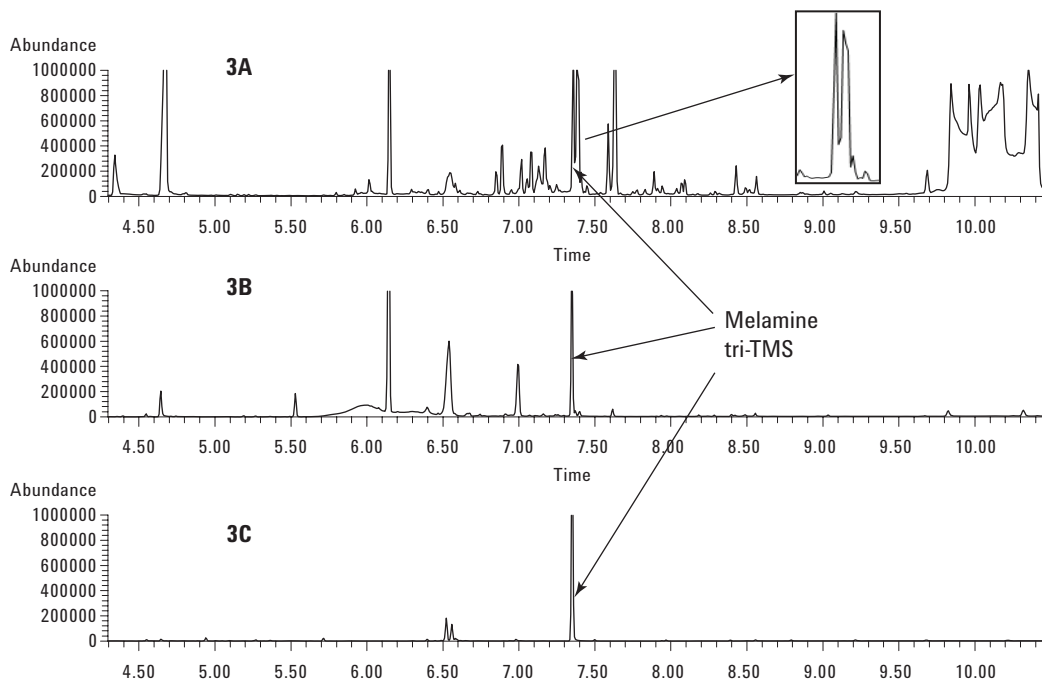


Figure 2. GC/MS SIM chromatogram of melamine tri-TMS. (3A: Sample without SPE cleanup; 3B: Sample with SPE cleanup; 3C: Standard)

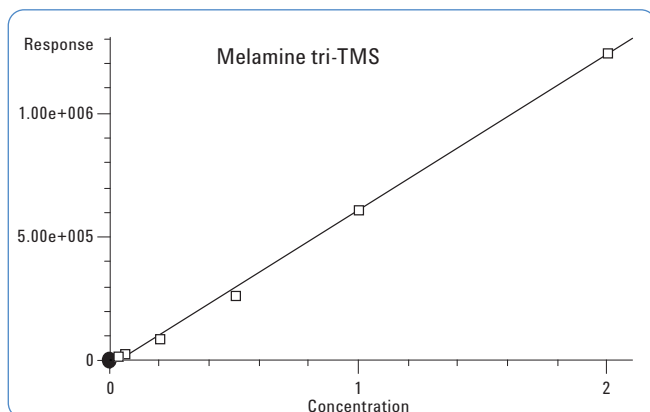


Figure 3. Calibration curve for melamine tri-TMS

Compound	Spiked level (mg/g)	Recovery (%)	RSD (%) (n = 6)
Melamine	0.080	82.1	2.04
tri-TMS	0.800	82.8	4.88
	1.600	80.8	3.58

Table 1. Recovery and repeatability of spiked samples

Ordering information

Agilent SampliQ SCX SPE Cartridge, 60 mg, 3 mL.
Part No. 5982-3236.

Agilent J&W DB-5ms Ultra Inert GC Column,
30 m x 0.25 mm, 0.25 μ m. Part No. 122-5532UI.

Rapid Screening and Confirmation of Melamine Residues in Milk and Its Products by Liquid Chromatography Tandem Mass Spectrometry (Publication 5989-9950EN)

Introduction

This rapid method uses the Agilent 6410 Triple Quadrupole (QQQ) with a cation ion exchange column for the liquid chromatography tandem mass spectrometry (LC/MS/MS) analysis of dairy products for melamine. Milk and milk products are prepared with a simple SPE cleanup method employing the new Agilent SampliQ SCX cartridge.

Instrument conditions

LC conditions

Column:	Agilent ZORBAX 300-SCX Column, 2.1 mm x 150 mm, 5 µm (Part No. 883700-704)
Injection volume:	10 µL
Flow rate:	0.2 mL/min
Temperature:	40 °C
Mobile phase:	A: 10 mM NH ₄ acetate/acetic acid pH adjusted to 3.0 B: ACN A:B = 20:80
Stop time:	10 min

MS conditions

Agilent 6410A LC/MS Triple Quadrupole	
Ion source:	Electrospray
Polarity:	Positive
Nebulizer gas:	Nitrogen
Ion spray voltage:	4,000 V
Dry gas temperature:	350 °C
Dry gas flow rate:	9 L/min
Nebulizer pressure:	40 psi
Resolution:	Q1 (unit) Q3 (unit)

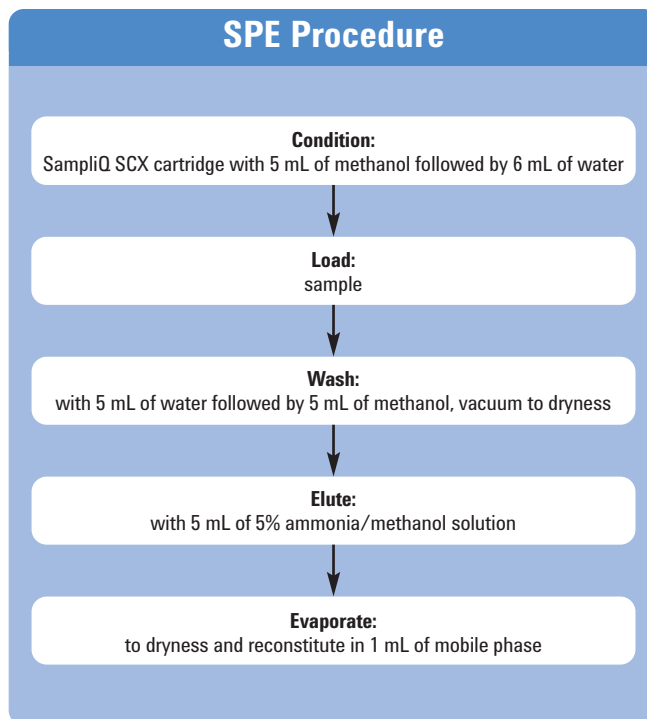


Figure 1. Scheme of sample preparation process

Results

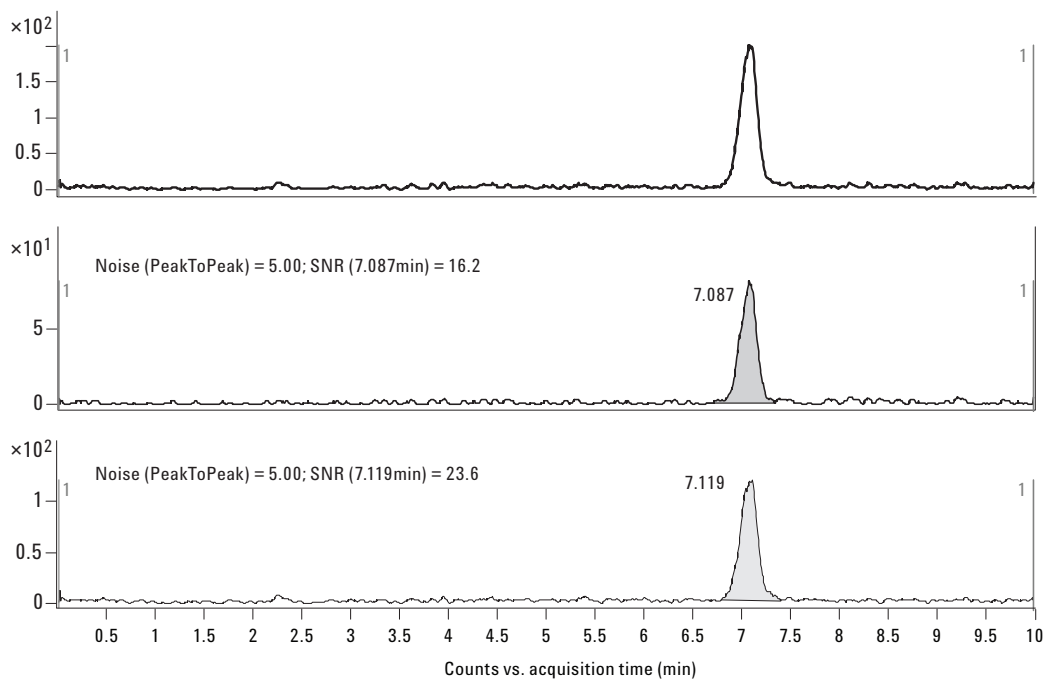


Figure 2. Response of melamine in a milk sample spiked at 1 ppb

	Conc. = 50 ppb (n = 3)	Conc. = 80 ppb (n = 3)
Recovery (%)	83.4	62.5
RSD (%)	2.78	1.02

Table 1. Recovery in milk powder – The recovery of 50 ppb and 80 ppb melamine spikes in milk powder using the external standard calculation is 83.4 and 62.5 percent, respectively, and the RSD is less than 3 percent

Ordering information

Agilent SampliQ SCX Polymeric SPE, 150 mg, 6 mL.
Part No. 5982-3267.

Agilent Regenerated Cellulose Membrane Filter, 0.2 μ m.
Part No. 5064-8222.

Agilent ZORBAX 300-SCX Column, 2.1 mm x 150 mm, 5 μ m.
Part No. 883700-704.

Determination of Melamine Residue in Milk Powder and Egg Using Agilent SampliQ Polymer SCX Solid Phase Extraction and the Agilent 1200 Series HPLC/UV (Publication 5990-3365EN)

Introduction

This method was developed for the determination of melamine in milk powder and egg. Solid phase extraction (SPE) and HPLC/UV are used consistent with the Chinese regulatory method. The sample preparation is performed using a polymeric mixed mode strong cation exchange resin. The separation and detection are performed by HPLC/UV.

Instrument conditions

HPLC conditions

Samples were analyzed on an Agilent 1200 Series HPLC with a diode array detector.

Column:	Agilent ZORBAX SB-C8 LC Column 250 mm x 4.6 mm, 5 µm (Part No. 880975-906)
Flow rate:	1.0 mL/min
Column temperature:	40 °C
Detector wavelength:	240 nm
Injection volume:	20 µL
Mobile phase:	acetonitrile-buffer (15:85)
Buffer:	10 mmol/L citric acid and 10 mmol/L sodium octanesulfonate solution with a pH 3.0
Chromatography:	Isocratic

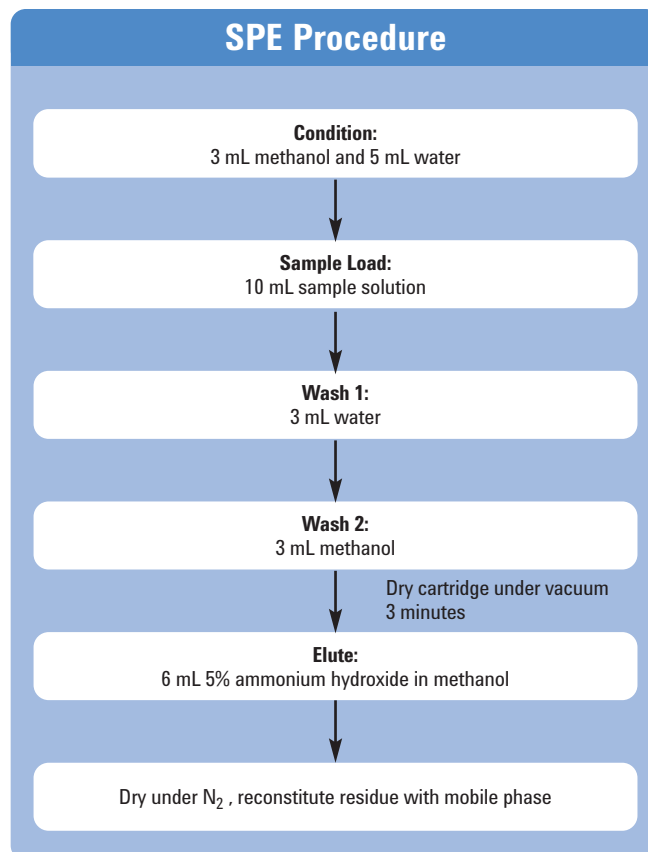


Figure 1: SPE schematic of melamine in milk and egg

Results

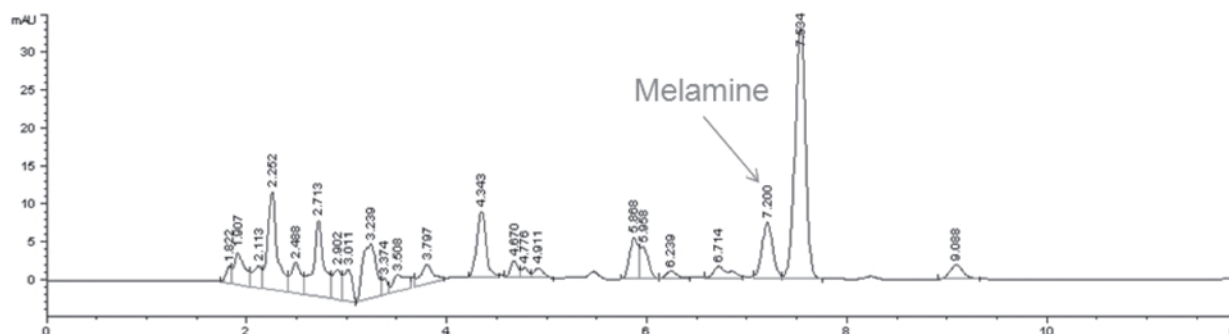


Figure 2. Chromatogram of a milk powder sample spiked at 2 mg/kg

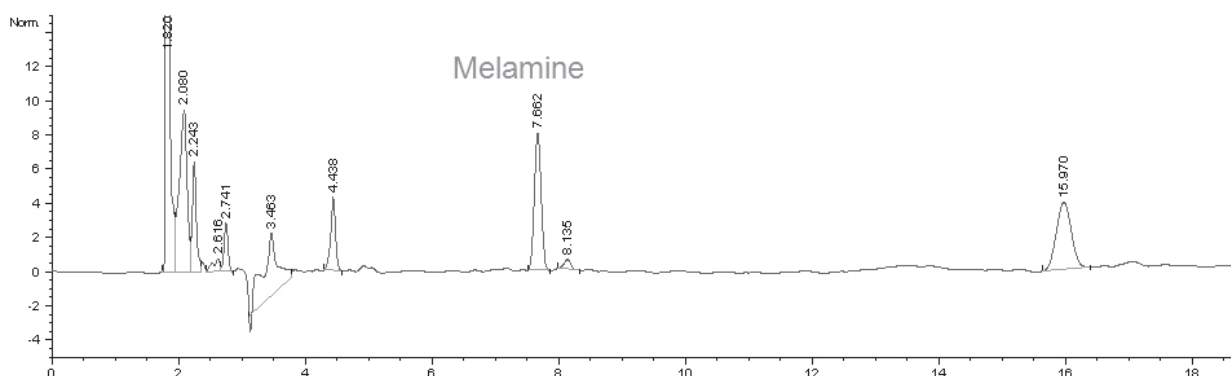


Figure 3. Chromatogram of an egg sample spiked at 2 mg/kg

Compound	Regression equation	Correlation coefficient	LOD (µg/kg)
Melamine	$Y = 77.4698x + 0.2117$	0.9999	10

Table 1. Linearity and LOD of melamine

Compound	Sample	Spiked level (mg/kg)	Recovery (%)	RSD (%)
Melamine	Milk powder	2	84.5	2.83
		5	85.3	2.56
		10	86.7	1.18
Melamine	Egg	2	95.2	3.00
		5	93.0	2.01
		10	95.7	2.89

Table 2. Recoveries and relative standard deviations of melamine in milk powder and egg using agilent SampliQ SCX SPE

Ordering information

Agilent SampliQ SCX Polymeric SPE Cartridges, 60 mg, 3 mL.
Part No. 5982-3236.

Agilent ZORBAX SB-C8 LC Column, 250 mm x 4.6 mm, 5 µm.
Part No. 880975-906.

Quantitative Liquid Chromatography Analysis of Melamine in Dairy Products Using Agilent's 1120 Compact LC and 1200 Rapid Resolution LC and SampliQ SCX SPE Cartridges (Publication 5989-9949EN)

Introduction

Melamine, originally an industrial use chemical, has found its way into the food chain as an illicit adulterant in milk and milk products. As global concern rises, widespread testing is proceeding. The following method illustrates successful removal of complex matrix interferences (protein, sugars and fats) for LC analysis of melamine in dairy products.

Instrument conditions

Conventional HPLC method using 1120 Compact LC or 1200 LC:

- Agilent 1120 Compact LC system with gradient pump (degasser inside), autosampler, Column compartment, and variable wavelength detector (VWD) or equivalent 1200 Series components
- EZChrom Elite Compact software or ChemStation software (Ver. B.04.01 or later)

Column:	Agilent ZORBAX SB-C8 LC Column (also known as Agilent ZORBAX Rx-C8), 4.6 mm x 250 mm, 5 µm (Part No. 880975-906)
Buffer:	10 mM citric acid, 10 mM sodium octane sulfonate, adjusted to pH 3.0
Mobile phase:	92:8 buffer: acetonitrile
Flow rate:	1.5 mL/min
Injection volume:	20 µL
Column temperature:	30 °C
Detection wavelength:	240 nm
Run time:	20 min

Sample Preparation

For liquid milk, milk powder, yogurt, ice cream, and creamy candy samples:

- Weigh 2 ± 0.01 g of sample and add to a 50 mL centrifuge tube, add 15 mL of 5% trichloroacetic acid in water and 5 mL of acetonitrile, then cap.
- Sonicate for 10 min and then place samples on vertical shaker for 10 min. Centrifuge for 10 min at 4,000 rpm.
- Wet filter paper with 5% trichloroacetic acid in water, then filter the supernatant into a 25.0 mL volumetric flask, and bring to volume with 5% trichloroacetic acid in water.
- Transfer a 5.0 mL aliquot of the extract into a glass tube, and then add 5.0 mL purified water. Vortex to mix thoroughly.

For cheese, cream, and chocolate samples:

- Weigh 2 ± 0.01 g of sample, grind with 8~12 g of sea sand in a mortar, and then transfer into a 50 mL centrifuge tube.
- Wash the used mortar with 5 mL of 5% trichloroacetic acid in water three times, transfer washings into a 50 mL centrifuge tube, and then add 5 mL of acetonitrile.
- Proceed with the sonication and other steps as described in the previous procedure.
- If the sample is very fatty, de-fat the extract using liquid-liquid extraction with hexane saturated with 5% trichloroacetic acid in water before cleanup by SPE.

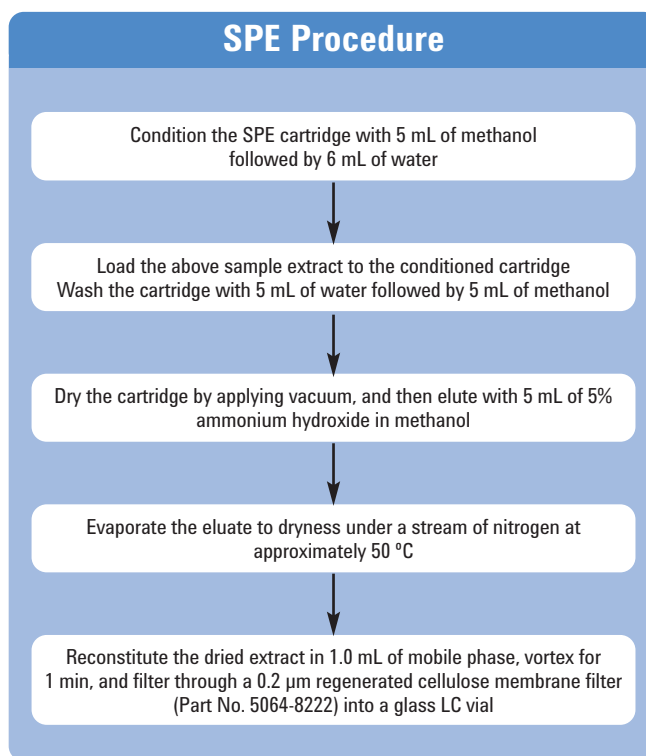


Figure 1 : SPE schematic of melamine in dairy products

Results

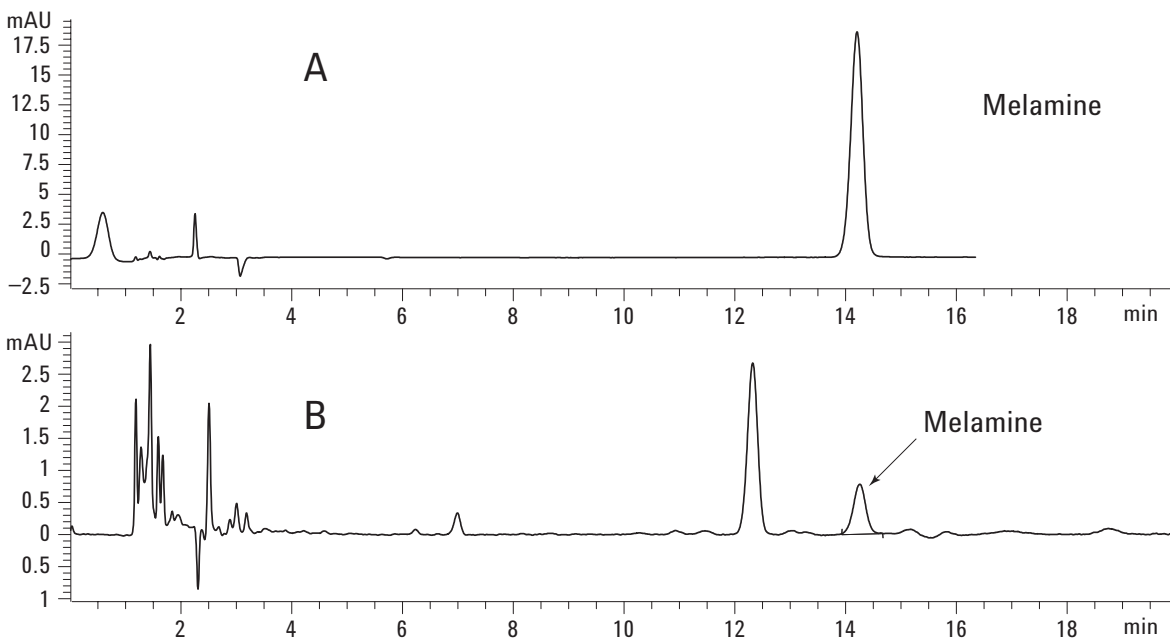


Figure 2. Separation of A: 20 µg/mL melamine standard, and B: positive yogurt sample after cleanup by SampliQ SCX SPE cartridge. Retention time of melamine is 14.2 minutes

Ordering information

Agilent SampliQ SCX SPE Cartridge,

3 mL, 60 mg. Part No. 5982-3236.

Agilent SampliQ SCX SPE Cartridge,

6 mL, 150 mg. Part No. 5982-3267.

Agilent ZORBAX SB-C8 LC Column (also known as Agilent ZORBAX Rx-C8), 4.6 mm x 250 mm, 5 µm. Part No. 880975-906.

To review this Application Note in its entirety, please search for 5989-9949EN at www.agilent.com/chem



Drugs/Antibiotics

Keeping antibiotics, hormones, and other chemicals out of the food supply

Animal diseases caused by viruses, bacteria, protozoa, or fungi can successfully be prevented and treated with antibiotics.

However, when trace amounts of antibiotics (or other drugs such as growth hormones) seep into milk, meat, eggs, fish, and honey, it can have serious long-term implications – including antibiotic-resistant strains of diseases once thought to be eradicated.



Determination of Hormones in Fish (*Carassius carassius*) by SampliQ-OPT Solid Phase Extraction with High Performance Liquid Chromatography (Publication 5990-3845EN)

Introduction

Hormones are a common food additive which, when consumed long-term, can possibly lead to human health concerns. Many countries' regulations clearly define residual limits for these compounds in food. Solid-phase extraction (SPE) coupled with high performance liquid chromatography (HPLC) was optimized for the extraction and determination of sixteen hormones in crucian carp meat.

Instrument conditions

HPLC conditions

Column:	Agilent ZORBAX Eclipse Plus C18 LC Column 250 mm x 4.6 mm, 5 µm, (Part No. 959990-902)		
Flow rate:	1.0 mL/min		
Injection volume:	5 µL		
Column temperature:	18 °C		
Detection wavelength:	230 nm		
Mobile phase:	water-acetonitrile gradient		
	Time (minutes)	% water	% acetonitrile
	0	70	30
	10	65	35
	23	50	50
	30	20	80

Sample Pretreatment

1. Weigh 200 grams of crucian meat, homogenize, and store in a clean, sealed container at -18 °C.
2. Place 1 g of homogeneous sample (accurate to 0.01 g) into a 10 mL polypropylene centrifuge tube with 5 mL of methanol.
3. Vortex for 1 minute.
4. Extract ultrasonically for 10 minutes in an ice bath.
5. Centrifuge the sample at a speed of 4,000 rpm for 5 minutes and remove the 3 mL of supernatant.
6. Save in a clean tube and evaporate with N₂ below 40 °C.
7. Reconstitute the residue in 5 mL of 5 % methanol in water.

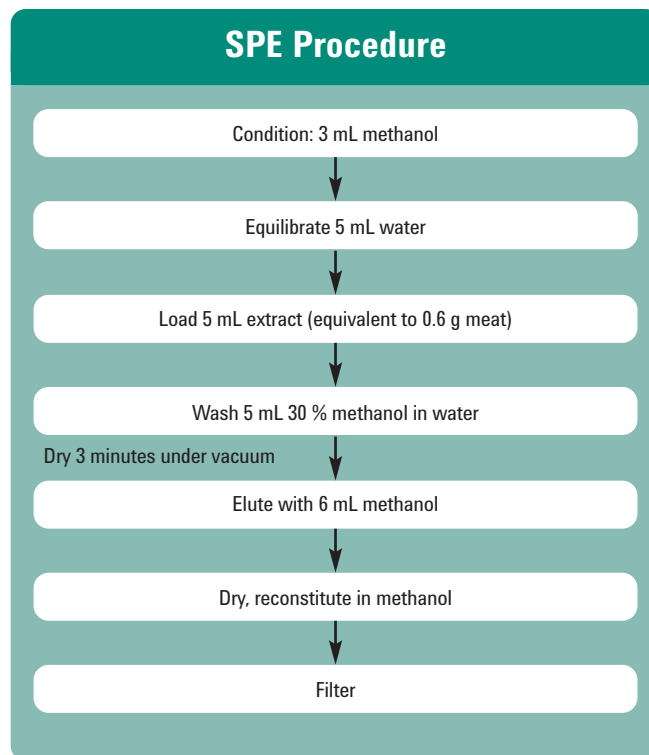


Figure 1. Hormones in crucian meat SPE procedure

Results

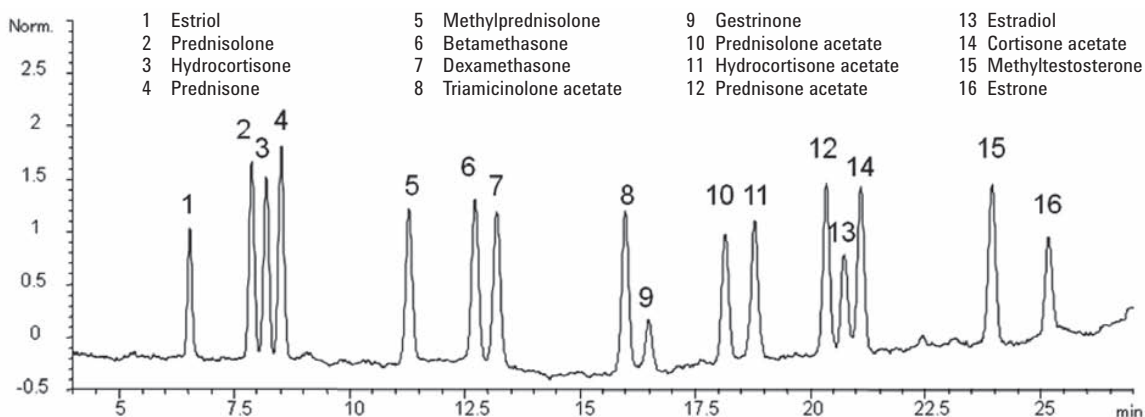


Figure 2. Chromatogram of crucian meat sample spiked hormone standards at 2 mg/kg

Compound	Spiked level (mg/kg)	Recovery (%)	RSD (n = 6, %)
Estriol	2	100.4	2.2
Prednisolone	2	89.4	3.8
Hydrocortisone	2	85.3	6.7
Prednisone	2	82.5	7.2
Methylprednisolone	2	83.2	8.3
Betamethasone	2	88.3	8.9
Dexamethasone	2	79.1	4.3
Triamcinolone acetate	2	86.7	8.4
Gestrinone	2	78.0	6.6
Prednisolone acetate	2	86.9	7.3
Hydrocortisone acetate	2	87.3	6.8
Prednisone acetate	2	76.7	7.7
Estradiol	2	78.7	4.2
Cortisone acetate	2	82.8	6.9
Methyltestosterone	2	82.9	3.4
Estrone	2	76.2	6.4

Table 1. Recoveries and RSDs of hormones in crucian meat by SPE

Ordering information

Agilent OPT Polymer Box, 30 x 6 mL tubes, 150 mg.
Part No. 5982-3067.

Agilent ZORBAX Eclipse Plus C18 LC Column, 250 mm x 4.6 mm,
5 µm. Part No. 95990-902.

Agilent PTFE 0.45 µm Premium Syringe Filter.
Part No. 5185-5836.

To review this Application Note in its entirety, please search for 5990-3845EN at www.agilent.com/chem

Determination of Tetracyclines in Chicken by Solid-Phase Extraction and High Performance Liquid Chromatography (Publication 5989-9735EN)

Introduction

A method for the simultaneous determination of the seven antibiotic residues of minocycline, oxytetracycline, tetracycline, demeclocycline, chlortetracycline, methacycline, and doxycycline in chicken has been developed. In this method, solid-phase extraction (SPE) and HPLC/UV are used consistent with Chinese regulatory methods.

Instrument conditions

HPLC conditions

Column: Agilent ZORBAX SB-C8 LC Column, 250 mm x 4.6 mm, 5 μ m, (Part No. 880975-906)
Flow rate: 1.5 mL/min
Column temperature: 30 $^{\circ}$ C
Injection volume: 100 μ L
Detector wavelength: 350 nm
Mobile phase: Methanol-acetonitrile-10 mM TFA solution, gradient elution

Time (minutes)	% methanol	% acetonitrile	% 10 mM TFA
0	1	4	95
7.5	6	24	70
13.5	7	28	65
15	1	4	95

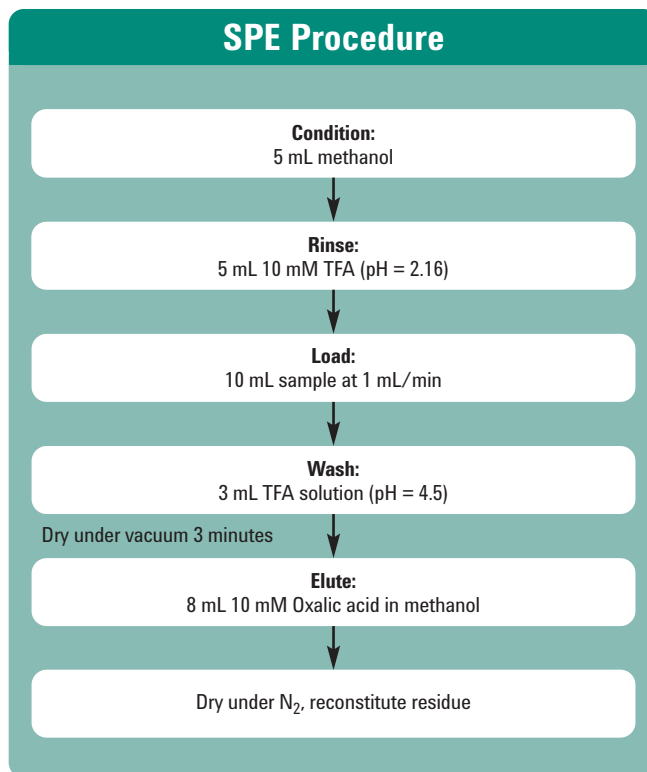


Figure 1. Tetracycline SPE procedure

Results

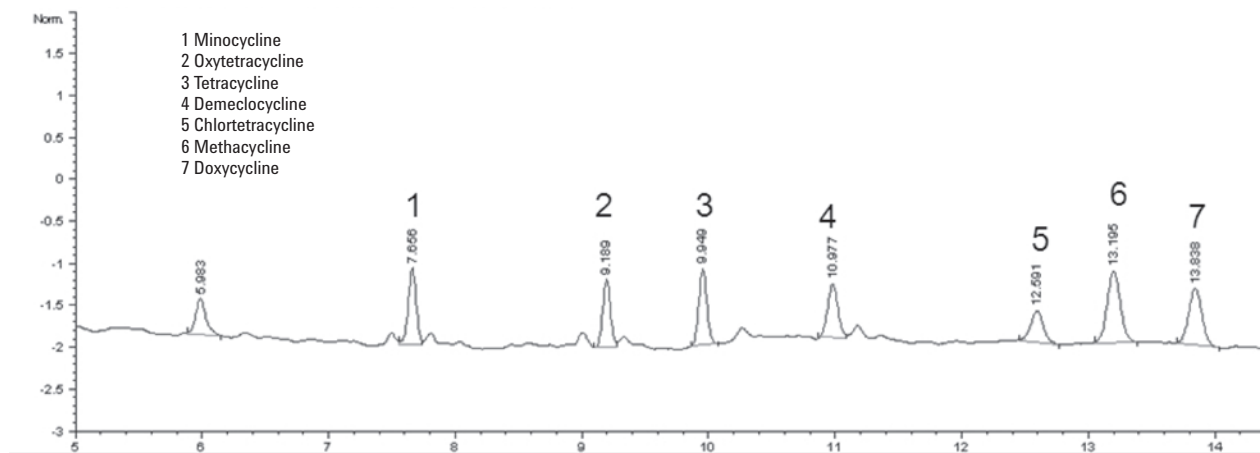


Figure 2. Chromatogram of a chicken sample spiked at 50 µg/kg

Compound	Spiked level (µg/kg)	Recovery (%)	RSD (%)
Minocycline	50	87.6	4.13
	100	80.8	5.68
	200	81.3	4.19
Oxytetracycline	50	68.8	6.49
	100	63.0	4.87
	200	59.4	4.35
Tetracycline	50	81.0	4.46
	100	70.0	3.47
	200	72.3	4.38
Demeclocycline	50	92.0	2.06
	100	94.8	3.78
	200	92.9	1.92
Chlortetracycline	50	93.3	3.16
	100	92.4	4.01
	200	87.7	2.54
Methacycline	50	93.3	2.89
	100	91.9	2.51
	200	86.6	3.39
Doxycycline	50	95.6	4.38
	100	96.4	1.00
	200	92.0	3.02

Table 1. Recoveries and RSDs of tetracyclines in chicken by SPE

Ordering information

Agilent SampliQ OPT SPE Cartridges, 60 mg 3 mL.
Part No. 5982-30360.

Agilent ZORBAX SB-C8 LC Column, 250 mm x 4.6 mm, 5 µm.
Part No. 880975-906.

To review this Application Note in its entirety, please search for 5989-9735EN at www.agilent.com/chem

Determination of Sulfonamides in Milk Using Solid Phase Extraction and Liquid Chromatography – Tandem Mass Spectrometry (Publication 5990-3713EN)

Introduction

The extraction of trace levels of nine nitrogen-containing sulfa drugs (sulfamethoxazole, sulfadiazine, sulfathiazole, sulfamerazine, sulfamethizole, sulfamethazine, sulfamethoxypridazine, sulfachloropyridazine, and sulfadimethoxine) in milk samples by solid-phase extraction was studied using Agilent SampliQ polymeric strong cation exchange (SCX) cartridges. An Agilent 6410 triple quadrupole LC/MS-MS System was used for the separation and determination of the sulfa drugs. For reversed-phase chromatography, an Agilent ZORBAX Eclipse Plus Column C18, (3.0 mm x 50 mm, 1.8 μm) with a 0.1% formic acid/acetonitrile gradient was used.

Sample Pretreatment

20 μL of a 45% solution of formic acid in water (prepared by mixing 10 mL of 90% formic acid with 10 mL of water) solution was added to each 1 mL of whole milk to precipitate proteins and lipids. The milk samples were then centrifuged at 8,000 rpm for 10 minutes. An aliquot of the supernatant (prepared whole milk extract) was removed and used to load onto SampliQ SCX cartridges.

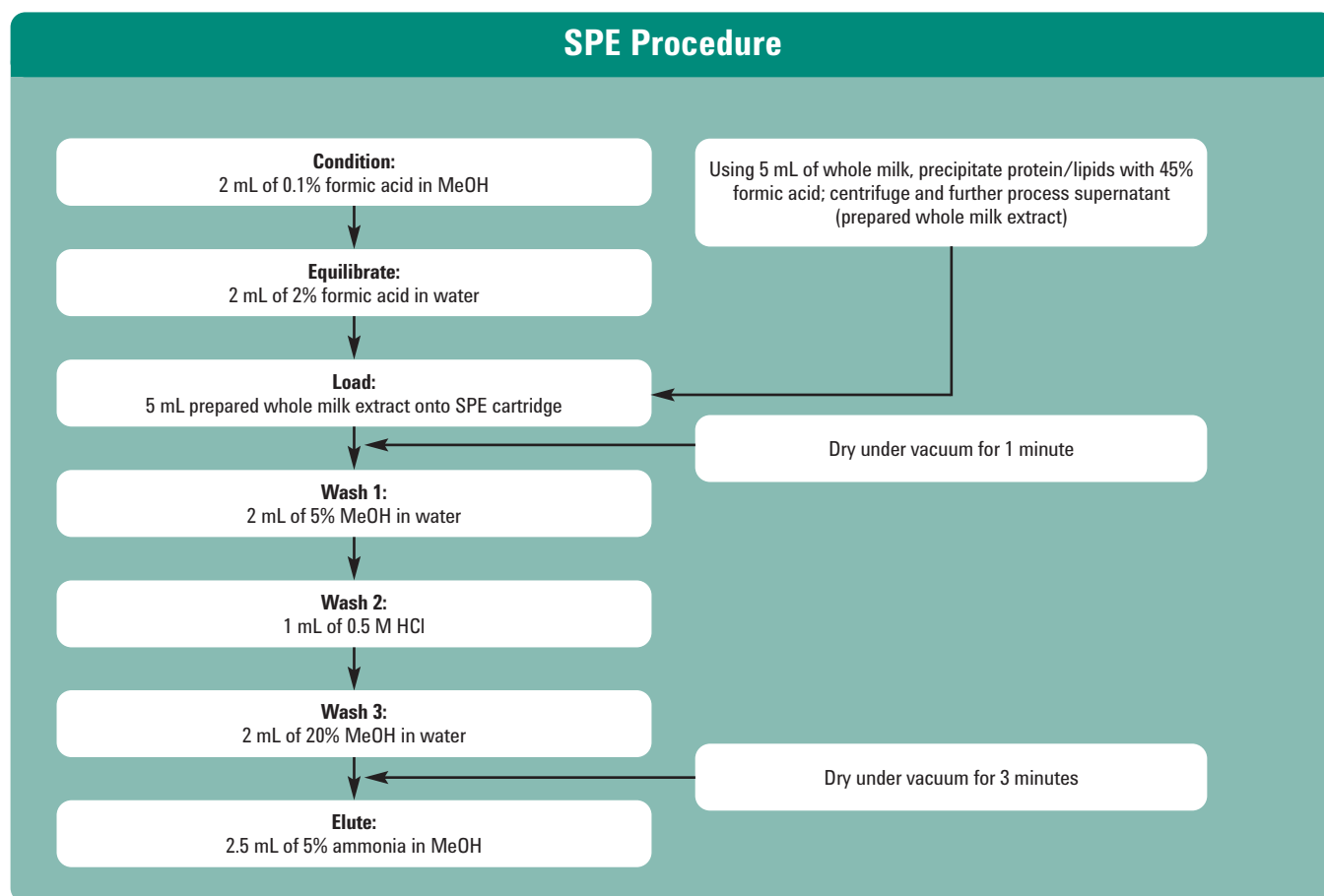


Figure 1. SPE procedure

Instrument conditions

HPLC Setup

Column:	Agilent ZORBAX Eclipse Plus C18 LC Column, 3.0 x 50 mm, 1.8 µm (Part No. 959941-302)		
Flow rate:	0.42 mL/min		
Column temperature:	35 °C		
Injection volume:	1.7 µL w/ needle wash; wash for 30 s in flush port with MeOH/H ₂ O (5:1)		
Mobile phase:	A: H ₂ O/acetonitrile (9:1) w/ 0.1% formic acid B: Acetonitrile w/ 0.1% formic acid		
Run time:	8 min		
Post time:	3 min		
Gradient:	Time	0	3.5
	%B	0	65

Conditions for Electrospray Ionization Source

Gas temperature:	350 °C
Gas flow:	12 L/min
Nebulizer:	40 psi
Capillary:	4,000 V

Results

Compound	Level spiked in milk (ng/mL)	Recovery	RSD (%)
Sulfadiazine	5	74.2	8.3
	10	99.7	5.7
Sulfathiazole	5	76.8	4.4
	10	83.2	4.7
Sulfamerazine	5	73.2	6.3
	10	84.8	0.6
Sulfamethazine	5	78.3	7.5
	10	89.0	3.1
Sulfamethizole	5	78.4	7.0
	10	94.5	5.3
Sulfamethoxypyridazine	5	76.3	6.2
	10	86.9	2.2
Sulfachloropyridazine	5	78.3	9.4
	10	84.3	6.0
Sulfamethoxazole	5	74.0	4.3
	10	87.7	6.4
Sulfadimethoxine	5	75.4	3.1
	10	82.5	5.4

Figure 1. Recovery and precision data for nine sulfa drugs used in this study

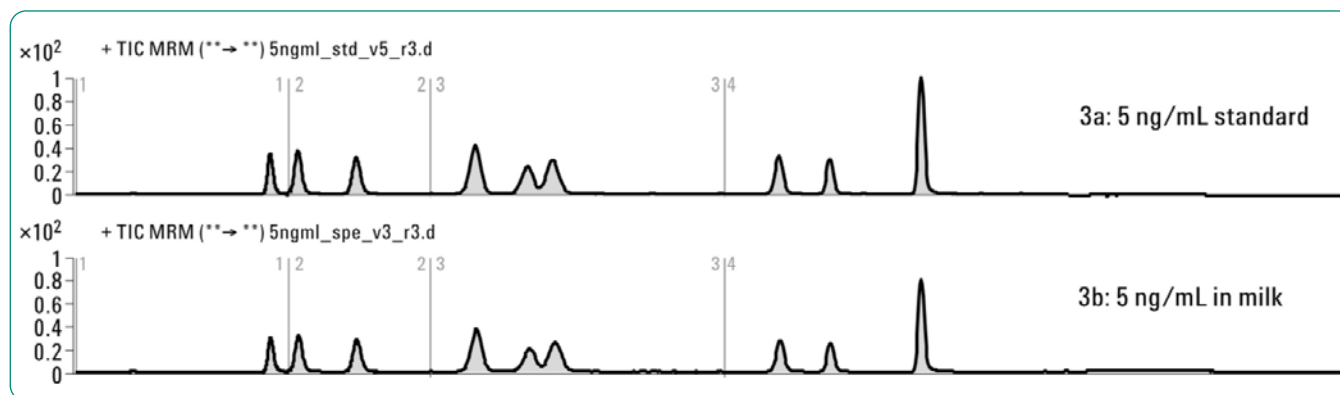


Figure 2. Total ion chromatograms of (3a) milk taken through extraction and cleanup, then spiked with sulfa drugs; (3b) milk spiked at 5 ng/mL, then taken through extraction and SPE cleanup

Ordering information

Agilent SampliQ SCX Polymeric SPE Cartridges, 60 mg, 3 mL.
Part No. 5982-3236.

Agilent ZORBAX Eclipse Plus C18 LC Column, 3.0 mm x 50 mm, 1.8 µm. Part No. 959941-302.

Determination of Chloramphenicol, Florfenicol and Thiamphenicol in Honey Using SampliQ OPT Solid-Phase Extraction Cartridges and Liquid Chromatography-Tandem Mass Spectrometry (Publication 5990-3615EN)

Introduction

A method for the simultaneous determination of three antibiotic residues of chloramphenicol (CAP), florfenicol (FF), and thiamphenicol (TAP) in honey has been developed and validated. The analytes are purified by liquid-liquid extraction and solid-phase extraction (SPE) and are quantified by liquid chromatography coupled to electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) operating in negative ion multiple reaction monitoring (MRM) mode. Chloramphenicol-D₅ is used as the internal standard. The method is validated by achieving reproducible, satisfactory, quantitative results.

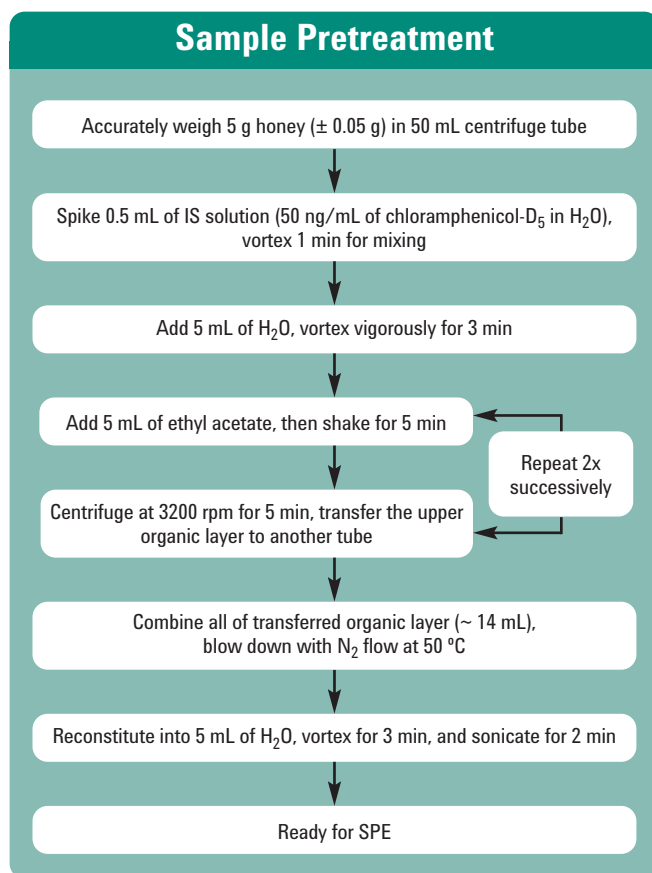


Figure 1. Sample preparation – liquid-liquid extraction of phenicols in honey

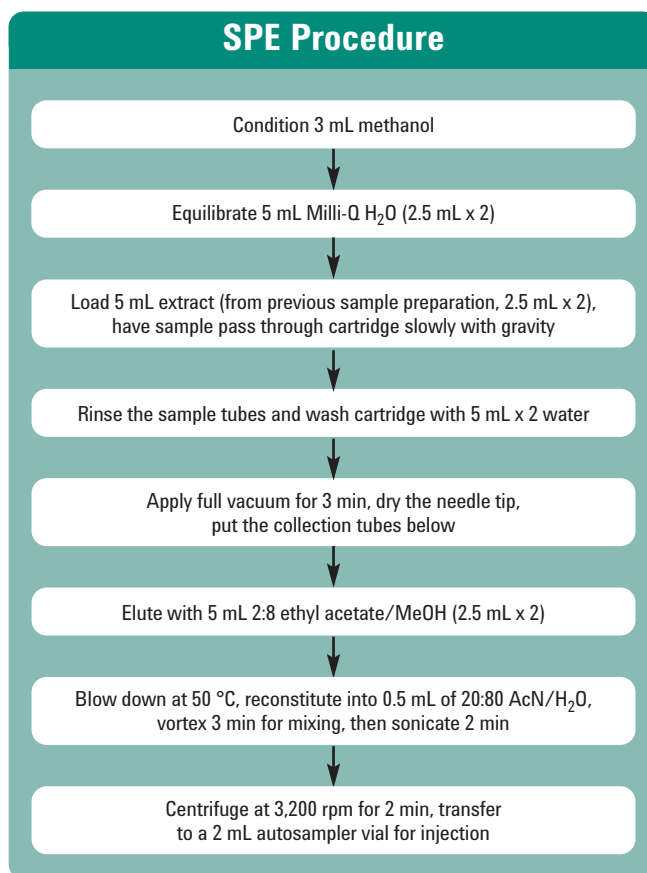


Figure 2. Sample clean-up – Agilent SampliQ solid-phase extraction

Instrument conditions

HPLC Conditions		Gradient:	Time	% acetonitrile	Flow rate (mL/min)
Column:	Agilent ZORBAX Eclipse Plus LC Column 150 mm x 2.1 mm, 5 μ m (Part No. 959701-906)		0	20	0.3
Flow rate:	0.3 mL/min		0.5	20	0.3
Column temperature:	30 °C		6.0	80	0.3
Injection volume:	20 μ L		6.01	100	0.5
Mobile phase:	pH 8.5 H ₂ O (A), acetonitrile (B)		6.50	100	0.5
			6.51	20	0.3
			7.00	STOP	

Results

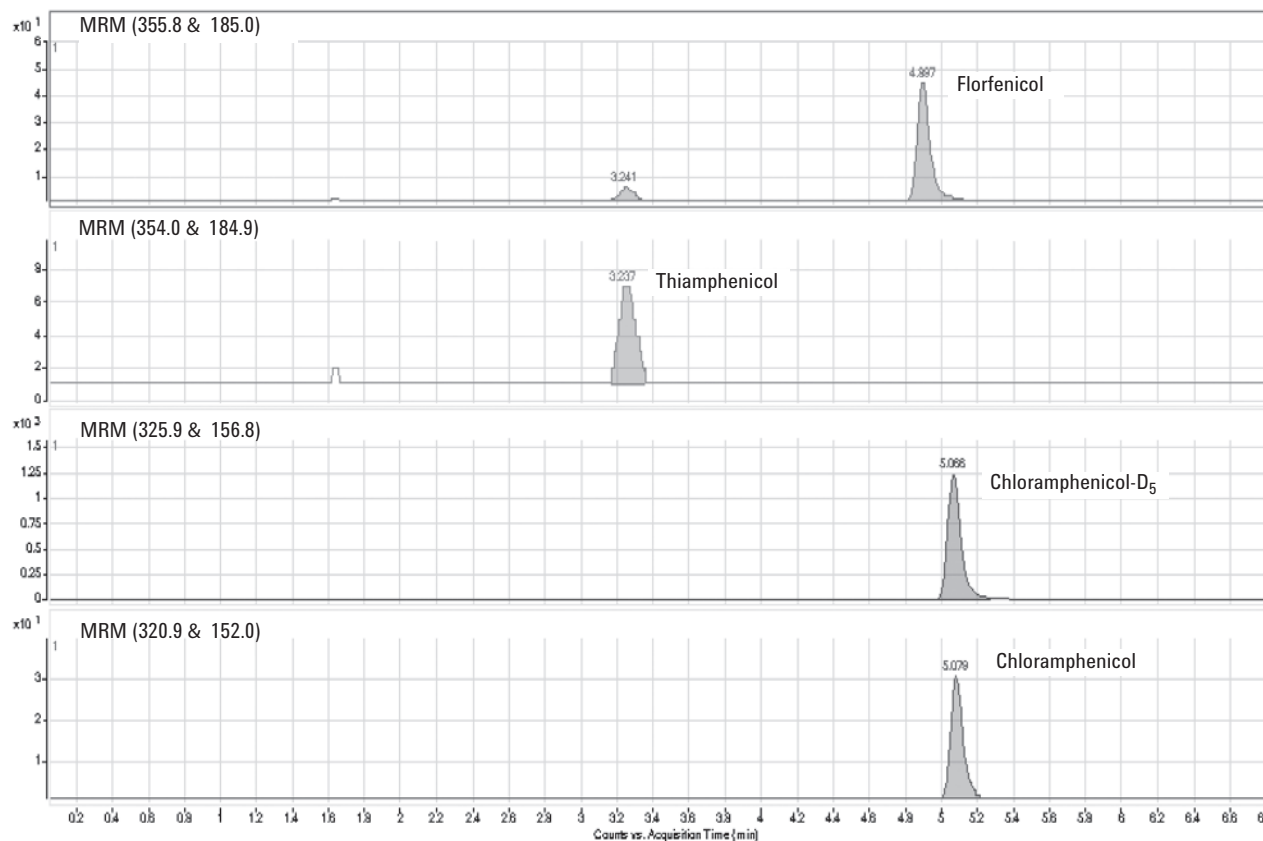


Figure 3. Chromatograms of 0.2 ng/g fortified honey extract

Analytes	Spiking Level (ng/g honey)	Recovery (%)	RSD (%) n = 6
Chloramphenicol	0.10	96.94	3.51
	5.00	98.88	0.87
	20.00	107.32	0.46
Florfenicol	0.10	100.67	9.77
	5.00	100.28	2.84
	20.00	107.49	2.55
Thiamphenicol	1.00	76.00	4.39*
	5.00	74.89	2.34
	20.00	89.81	3.83

* The experiment was done in replicates of four

Table 2. Recoveries and reproducibility of phenicols in fortified honey

Ordering information

Agilent SampliQ OPT Polymeric SPE Cartridges, 60 mg, 3 mL.
Part No. 5982-3036.

Agilent ZORBAX Eclipse Plus LC Column,
150 mm x 2.1 mm, 5 μ m. Part No. 959701-906.

Determination of Penicillins in Meat by High Performance Liquid Chromatography (HPLC/UV) and HPLC/MS/MS (Publication 5990-3364EN)

Introduction

Penicillins are antibiotics widely used to treat diseases in animals. In the method, the reversed phase column Agilent ZORBAX Eclipse Plus C18 (100 mm x 2.1 mm, 3.5 µm) and an Agilent mixed mode polymer solid phase extraction cartridge (Agilent SampliQ OPT) were combined to give a total solution to the analysis of residual penicillins. The performance of the solid phase extraction procedure on trace residues is quantitatively evaluated by HPLC/MS/MS.

Instrument conditions

HPLC conditions

Column	Agilent ZORBAX Eclipse Plus LC Column, 2.1 mm x 100 mm, 3.5 µm (Part No. 959793-902)	
Flow rate	0.6 mL/min	
Mobile phase	A: water/10 mM ammonium acetate B: acetonitrile	
Run time	12 minutes	
Post run	3 minutes	
Temperature	30 °C	
Injection	10 µL	
Gradient:	Time	% B
	0	2
	1.2	2
	2.0	10
	6.0	30
	8.0	40
	8.5	80
	11.9	80
	12.0	2

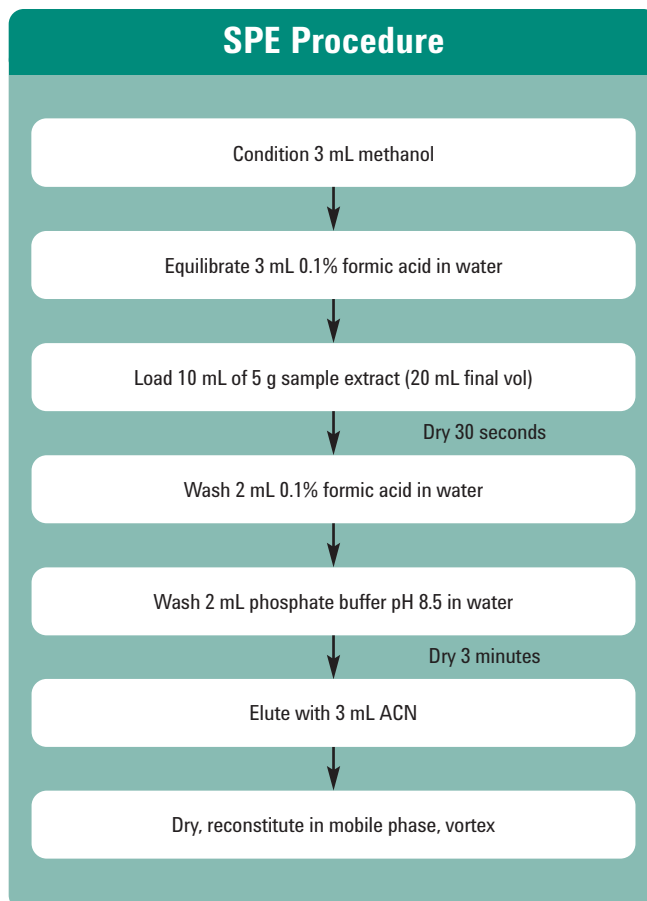


Figure 1. Agilent SampliQ OPT solid phase extraction of penicillins from pork

Results

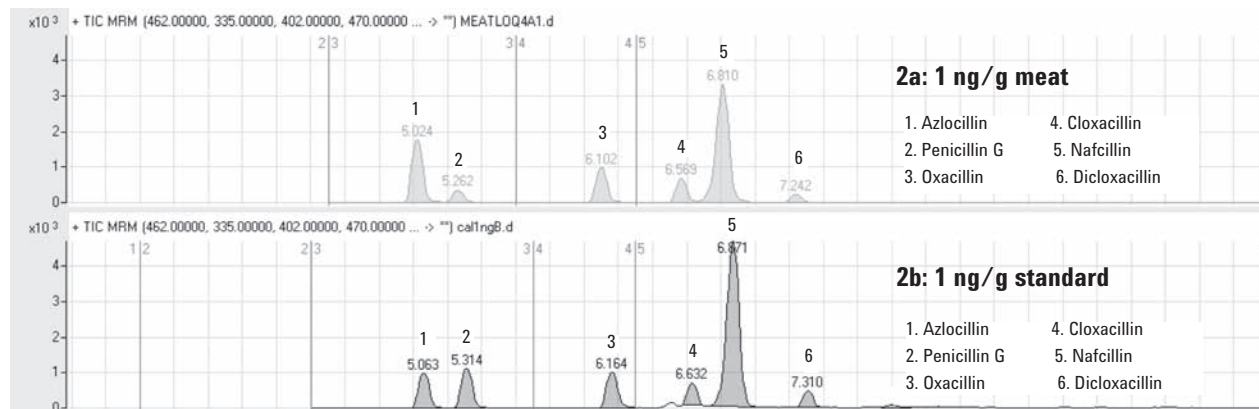


Figure 2. Meat spiked at 1 ng/g taken through extraction and SPE clean-up (2a), meat taken through extraction and clean-up then spiked at 1 ng/g (2b)

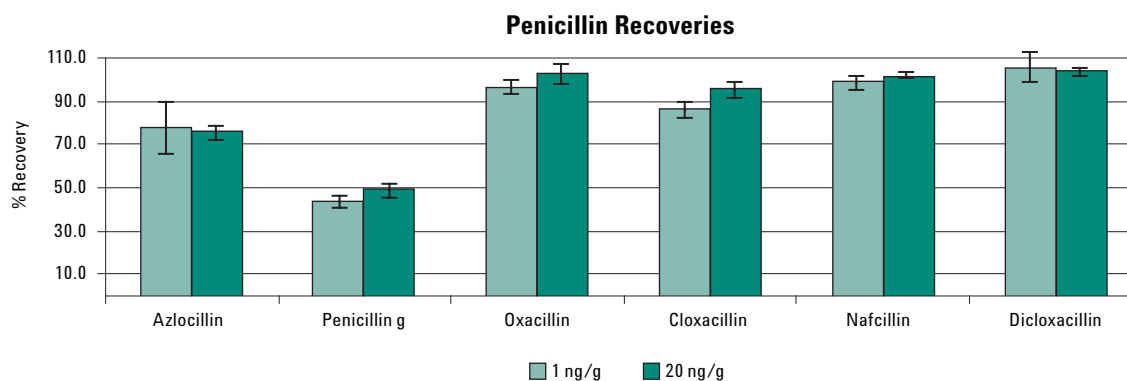


Figure 3. Recovery data for meat extracts at 1.0 and 20 ng/g

Ordering information

Agilent SampliQ OPT Polymeric SPE, 150 mg, 6 mL.
Part No. 5982-3067.

Agilent Syringe Filter, 13 mm, 45 μ m PTFE. Part No. 5185-5836.

Agilent ZORBAX Eclipse Plus LC Column, 2.1 mm x 100 mm,
3.5 μ m. Part No. 959793-902.

Determination of Multi Residue Tetracycline and their Metabolites in Milk by High Performance Liquid Chromatography – Tandem Mass Spectrometry (Publication 5990-3816EN)

Introduction

A high performance liquid chromatography – tandem mass spectrometric (HPLC /MS/MS) method is developed for the simultaneous determination of 10 antibiotic residues: Minocycline, 4-epioxytetracycline, 4-epitetracycline, Tetracycline, 4-epichlortetracycline, Demeclocycline, Chlortetracycline, Methacycline, Doxycycline, Oxytetracycline in milk and animal tissues. In the method, Agilent’s novel solid-phase extraction cartridge and a reversed phase Agilent ZORBAX RX-C8 Column (150 mm x 2.1 mm, 5 µm) are used for purification and separation. Overall recoveries are between 76.4% and 101% with a relative standard deviation (RSD, n = 6) less than 8.4%.

Instrument conditions

HPLC conditions

Column:	Agilent ZORBAX RX-C8 LC Column, 2.1 mm x 150 mm, 5 µm (Part No. 883700-906)
Flow rate:	0.3 mL/min
Mobile phase:	A: water/ 0.1 % formic acid, B: methanol
Gradient:	0-10 min, B from 5% to 30% 10-12 min, B from 30% to 40% 12.5-18 min, B 65% 18.5-25 min, B 95% 25.5 min, B 5.0%
Total run:	28 min
Post time:	5 min
Temp:	30 °C
Injection:	5 µL

MS Source settings

Source:	ESI
Ion polarity:	Positive
Drying Gas temp.:	350 °C
Drying gas flow rate:	10 L/min
Nebulizer:	45 psi
V _{cap} :	4,000V

Name	Frag.	Precursor ion	Product ion	CE	Rt. (min)
Minocycline	120	458	352	35	8.58
			441	20	
4-epitetracycline	120	445	410	20	8.60
			427	10	
4-epioxytetracycline	120	461	426	20	9.47
			444	15	
Tetracycline	120	445	410	20	9.90
			427	15	
Oxytetracycline	120	461	426	20	9.95
			443	10	
Demethylcyclocline	120	465	430	25	11.25
			448	15	
4-epichlortetracycline	120	479	444	22	11.59
			462	15	
Chlortetracycline	120	479	444	22	12.95
			462	15	
Methacycline	120	443	381	25	13.98
			426	15	
Doxycycline	120	445	154	30	14.08
			428	15	

SPE Procedure

Extraction:

Weigh 5 g milk sample (accurate to 0.01 g) into 50 mL colorimetric tube, dissolve with 0.1 mol/L Na₂EDTA-McIlvaine buffer solution. Bring volume to 50 mL

Vortex for 1 min and ultrasonicate in an ice water bath for 10 min

Transfer to 50 mL polypropylene centrifuge tube
Cool to 0 °C ~ 4 °C

Centrifuge at 5,000 rpm for 10 min (below 15 °C)

Filter with fast filter paper

Purification:

Draw 10 mL of the extract (equivalent to 1 g sample). Put it through the SampliQ OPT cartridge (Part No. 5982-3036) at a speed of 1 drop/s

After it elutes completely, clean the cartridge with 3 mL water adjusted to pH 4.5 with trifluoroacetic acid. Discard effluent

Under negative pressure below 2.0 kPa, drain cartridge for 5 min

Elute with 10 mL of 10 mmol oxalic acid in methanol

Collect the eluent and dry with nitrogen below 40 °C

Dissolve the residue with 1.0 mL of the initial mobile phase

Filter with 0.45 µm filter membrane and inject

Figure 1. Agilent SampliQ OPT solid phase extraction of penicillins from pork

Results

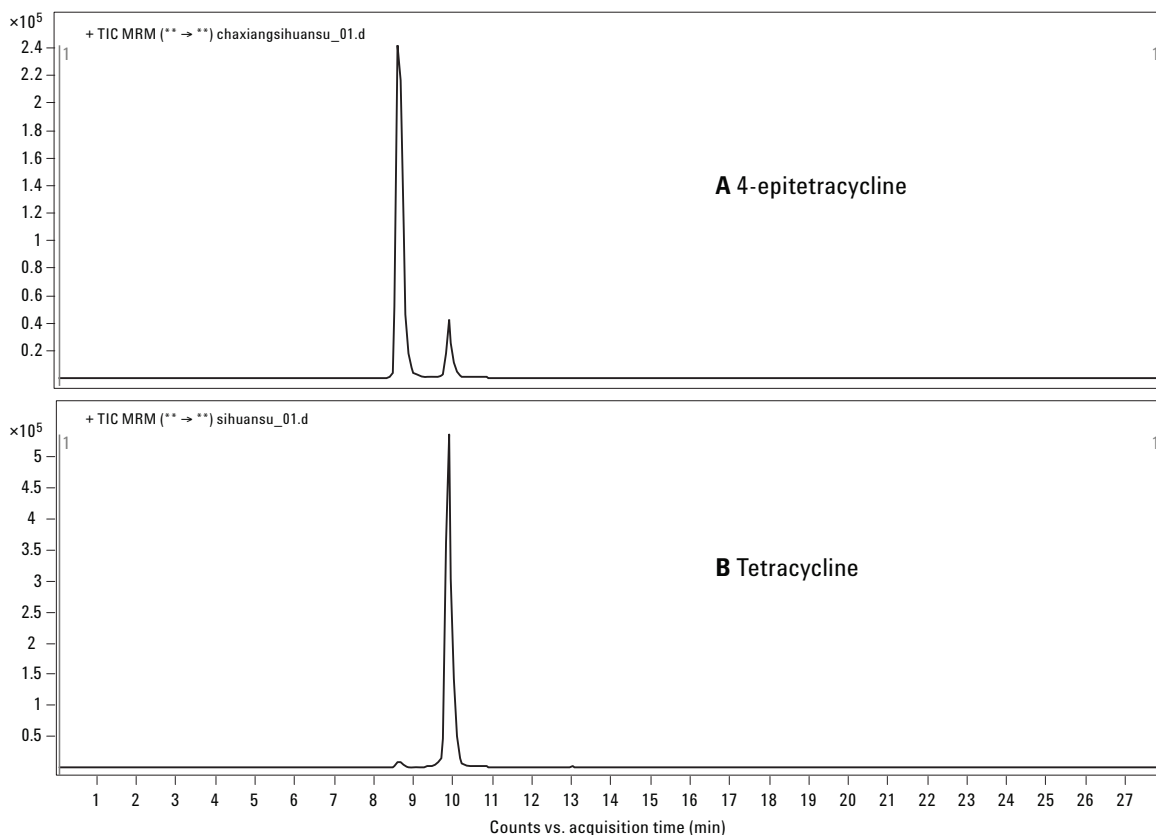


Figure 1. The separation of tetracycline and its degradation product 4-epitetracycline

Name	Recovery in milk (Conc. 50 ppb n=6)	RSD % (Signal response n=6)	RSD % (Ion ratio n=6)	Recovery in milk (Conc. 100 ppb n=6)	RSD % (Signal response n=6)	RSD % (Ion ratio n=6)
Minocycline	96.5	4.9	2.1	101.4	1.6	1.0
4-epitetracycline	89.2	3.8	1.5	96.3	1.6	0.9
4-epioxytetracycline	84.4	5.4	1.3	88.2	0.9	0.6
Tetracycline	86.1	2.5	1.2	90.7	1.1	1.2
Oxytetracycline	77.6	3.8	1.6	82.5	1.2	0.9
Demethylcyclocline	79.2	2.0	3.1	84.7	0.9	0.6
4-epichlortetracycline	76.4	5.5	5.4	84.3	1.1	0.5
Chlortetracycline	94.3	4.5	1.5	100.9	1.8	1.1
Methacycline	86.3	1.0	1.9	91.2	1.2	0.8
Doxycycline	78.7	3.6	6.7	82.4	1.0	0.8

Table 1. Recovery and repeatability in milk matrix

Ordering information

Agilent SampliQ OPT Polymeric SPE Cartridges, 60 mg, 3 mL. Part No. 5982-3036.

Agilent ZORBAX Rx-C8 LC Column, 2.1 mm x 150 mm, 5 µm. Part No. 883700-906.

To review this Application Note in its entirety, please search for 5990-3816EN at www.agilent.com/chem

Determination of β 2-Agonists in Pork Using Agilent SampliQ OPT Solid-Phase Extraction Cartridges and Liquid Chromatography-Tandem Mass Spectrometry (Publication 5990-4180EN)

Introduction

A method for simultaneous determination of four β 2-agonist residues of terbutaline, salbutamol, clenbuterol and formoterol in pork has been developed and validated. The analytes are purified by liquid-liquid extraction (LLE) and solid-phase extraction (SPE) and quantified by liquid chromatography coupled to electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) operating in positive ion multiple reaction monitoring (MRM) mode.

Instrument conditions

HPLC conditions

Column:	Agilent ZORBAX Eclipse Plus C18 LC Column, 50 mm x 2.1 mm 1.8 μ m (Part No. 959741-906)		
Flow rate:	0.4 mL/min		
Column temperature:	40 °C		
Injection volume:	5 μ L		
Mobile phase:	water (0.1% FA+2 mM NH ₄ Ac, A), acetonitrile (0.1% FA, B)		
Gradient:	Time (min)	%A	%B
	0	90	10
	0.5	90	10
	1.8	20	80
	2	90	10
	3.5	90	10

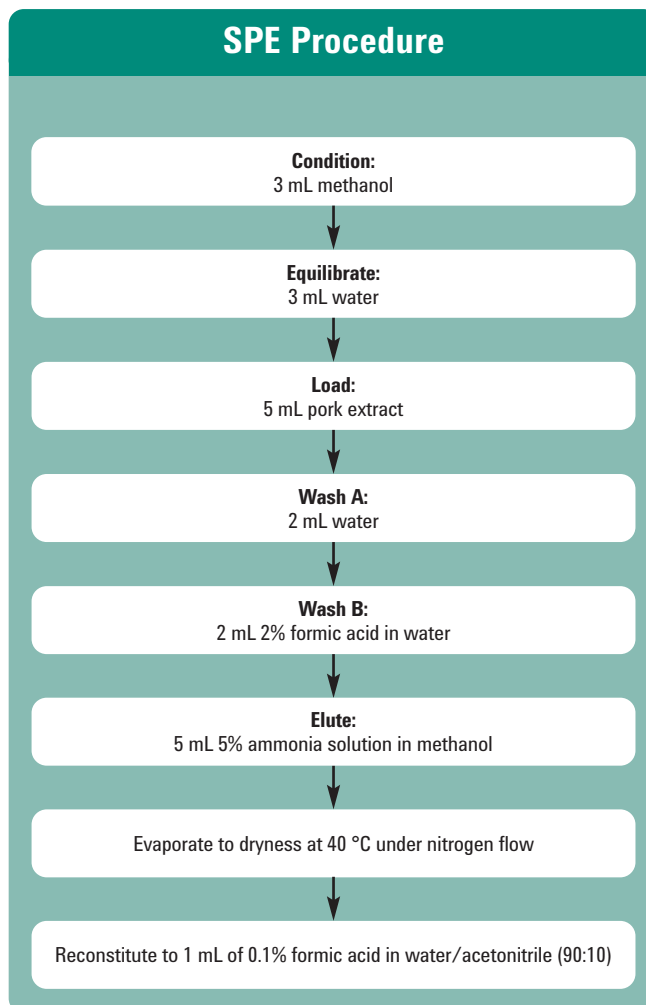


Figure 1. Pork clean up and enrichment – SPE procedure

Results

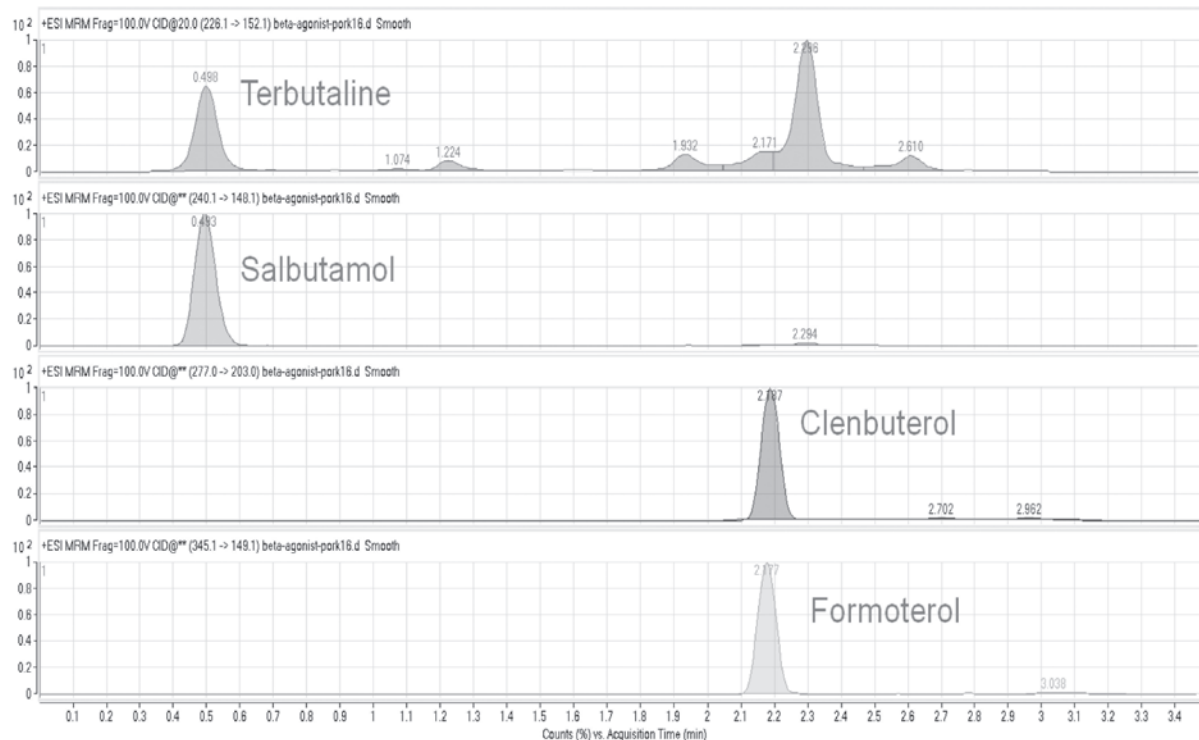


Figure 2. Chromatograms of 1.0 ng/g spiked pork sample extract

Compound	Spiked level (ng/g pork)	Recovery (%)	RSD (n=6)
Terbutaline	0.5	88.7	5.4
	1	98.0	7.2
	2	100.8	5.9
Salbutamol	0.5	100.6	1.8
	1	92.9	2.1
	2	97.4	3.9
Clenbuterol	0.5	82.3	5.0
	1	91.5	6.3
	2	90.6	4.3
Formoterol	0.5	85.1	1.9
	1	83.0	4.0
	2	77.9	2.5

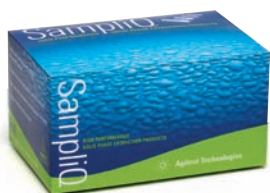
Table 1. Recoveries and reproducibility of β_2 -agonists in pork after SPE employing Agilent's SampliQ OPT; (Part No. 5982-3236), recovery 90% and RSD 4.4% on average

Ordering information

Agilent SampliQ OPT Polymer Cartridges, 50 x 3 mL tubes, 60 mg. Part No. 5982-3236.

Agilent ZORBAX Eclipse Plus C18 LC Column, 50 mm x 2.1 mm, 1.8 μ m. Part No. 959741-906.

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Agilent SampliQ QuEChERS Food Safety Applications Notebook

Volume 2



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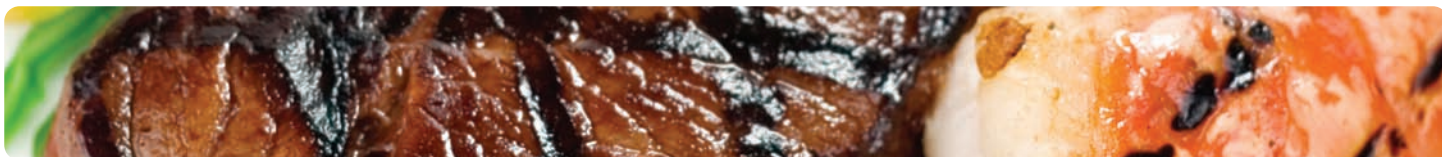
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On the following pages, you'll discover leading-edge techniques and sample prep methods that can dramatically improve the reliability and throughput of your food safety analysis.



Ronald E. Majors, Ph. D., Senior Chemist



What is QuEChERS?

Developed by United States Department of Agriculture in 2003, QuEChERS (pronounced “Catchers”) stands for **Q**uick **E**asy, **C**heap, **E**ffective, **R**ugged and **S**afe – the qualities that describe this sample preparation method for food substances. The technique is very simple, involves a minimum of steps, and is effective for the cleanup of complex samples.

QuEChERS is a technique that was developed for multi-class, multi-residue pesticides analysis in fruits and vegetables but more recently has expanded its scope to other trace contaminants in other non-vegetable foods such as meat and fish. Methods for hundreds of pesticides in a variety of fruits, vegetables, meat, and for dry materials such as beans and nuts have been published. “Official” methods are now available and a standardization of the technique on a worldwide basis is taking place. In the United States, the Association of Official Analytical Chemists (AOAC) has published its 2007.01 Method while the European equivalent, the EN 15662 2007, uses similar methodology.

The practice of QuEChERS involves two steps:

1. An extraction step that is based on partitioning via salting-out extraction involving an equilibrium between an aqueous and an organic layer, and
2. A dispersive solid-phase extraction (SPE) step that involves further cleanup using various combinations of salts and porous sorbents to remove interfering substances.

In the dispersive SPE step, the use of porous sorbents such as a primary-secondary amine (PSA), C18, and graphitized carbon black help to remove a variety of matrix compounds that are co-extracted in step 1. The most popular analytical methodology to measure extracted analytes is either LC/MS or GC/MS or their tandem equivalents.



Original QuEChERS Method



Analysis of Pesticide Residues in Apple by GC/MS using Agilent SampliQ QuEChERS Kits for Pre-injection Cleanup (Publication 5990-4468EN)

Introduction

This application note describes the use QuEChERS, a quick, easy, cheap, effective, rugged, and safe sample preparation approach to investigate the extraction of 15 multi-class pesticides in apples. The pesticides were chosen to represent typical types of volatile/semi-volatile pesticides that might be found in a typical fruit sample at levels normally encountered. The version of the QuEChERS non-buffered extraction method dates back to the original publication in 2003. For analysis, it uses GC/MS with selective ion monitoring (SIM) to measure pesticides down to the 10 ng/g levels.

Instrument conditions

GCMS conditions

Injection source	Manual
Inlet	Splitless
Column	Agilent J&W HP-5ms Ultra Inert GC Capillary Column, 30 m x 0.250 mm, 0.25 µm (Part No. 190915-433UI)
Carrier Gas	Helium in constant flow mode
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 (7 min)

Injection volume 1 µL

MS conditions

Tune File	Atune.u
Mode:	SIM
Source, Quad, Transfer line temperature	230 °C, 150 °C, 280 °C respectively
Solvent Delay	4.00 minutes
Multiplier Voltage	Autotune voltage

Ordering information

Agilent SampliQ QuEChERS Non-Buffered Extraction Kit. Part No. 5982-5550.

Agilent SampliQ QuEChERS Dispersive Kit for General Fruits and Vegetables, 2 mL. Part No. 5982-5022.

Agilent SampliQ QuEChERS AOAC Dispersive SPE Kit for General Fruits and Vegetables, 15 mL. Part No. 5982-5058.

Agilent J&W HP-5ms Ultra Inert GC Capillary Column, 30 m x 0.25 mm, 0.250 µm. Part No. 190915-433UI.

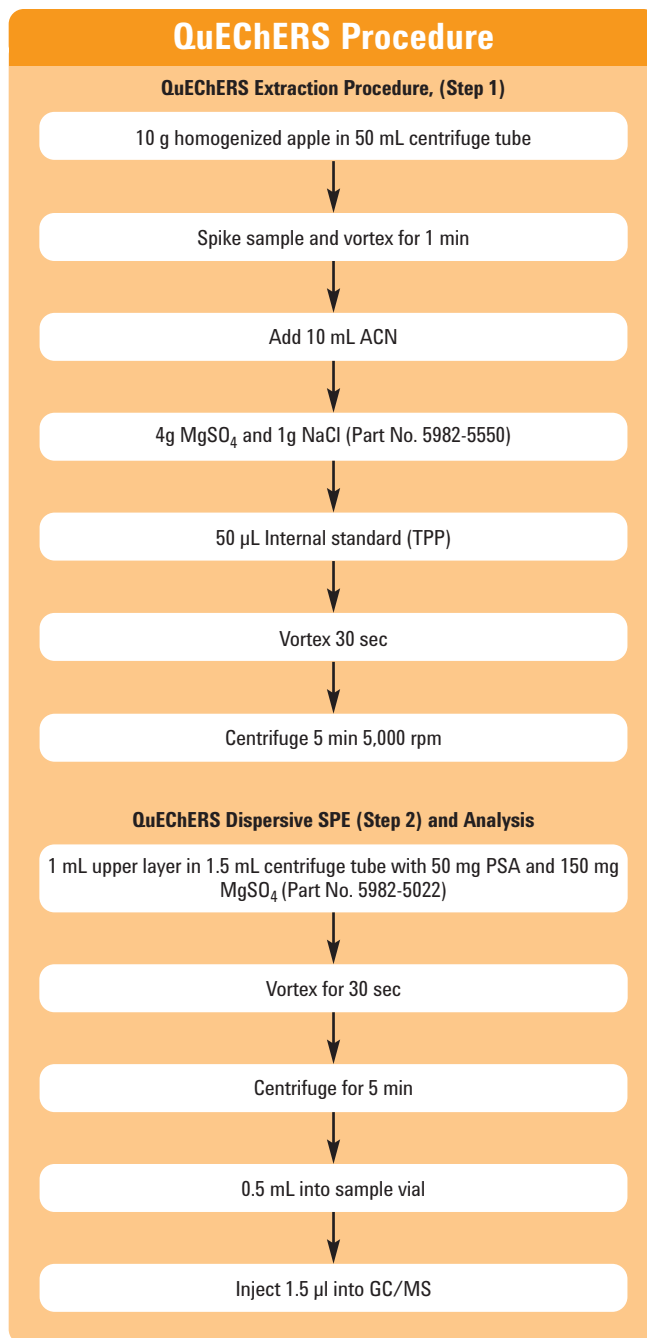


Figure 1: QuEChERS extraction procedure for general fruits and vegetables

Results

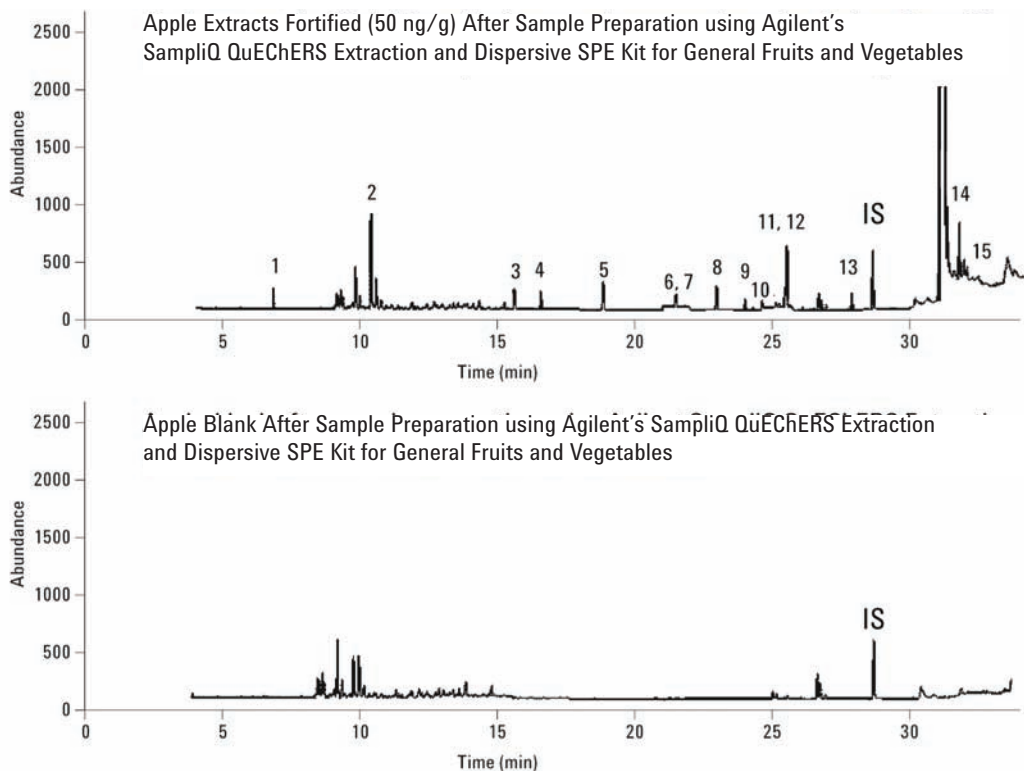


Figure 2: Comparison of blank apple extract to a fortified apple extract

Pesticide	Low-QC 10 ng/g		Mid-QC 50 ng/g		High-QC 200 ng/g	
	Recovery	RSD	Recovery	RSD	Recovery	RSD
Dichlorvos	102.8	5.0	96.7	10.8	99.4	2.8
<i>o</i> -phenylphenol	92.0	6.1	79.6	6.8	89.5	6.3
Lindane	97.9	2.0	88.5	9.7	92.6	4.2
Diazinone	90.5	9.1	98.8	5.5	102.1	4.4
Methyl-chlorpyrifos	88.7	7.1	90.0	4.3	98.5	3.1
Chlorpyrifos	93.5	6.5	95.6	4.0	100.2	1.2
Dichlorobenzophenone	90.3	5.0	89.1	6.4	99.4	0.6
Heptachlor-epoxide	87.0	3.2	85.6	5.4	95.4	3.9
γ -chlordane	92.3	3.5	90.0	6.8	95.9	2.0
α -chlordane	95.5	4.7	85.8	6.9	93.5	2.6
Dieldrin	99.4	4.2	93.6	5.3	99.9	1.8
DDE	94.5	4.2	87.1	5.7	92.7	1.9
Endosulfan Sulfate	97.8	2.3	90.8	2.8	99.5	2.3
Permethrin	100.7	4.8	93.0	3.4	97.6	2.1
Coumaphos	72.5	4.5	79.6	3.5	96.6	3.0

Table 1. Recovery and reproducibility of pesticides in apple using the original QuEChERS method (n=4)

To review this Application Note in its entirety, please search for 5990-4468EN at www.agilent.com/chem

EN Methods



Analysis of Pesticide Residues in Apple using Agilent SampliQ QuEChERS European Standard EN Kits by LC/MS/MS Detection (Publication 5990-3938EN)

Introduction

This application note describes the use of a quick, easy, cheap, effective, rugged, and safe (QuEChERS) sample preparation approach described in the European Committee Standard (EN) for extraction and cleanup of 16 multiple class pesticide residues of interest in apple. 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 (MRM) mode.

Instrument conditions

HPLC conditions

Column: Agilent ZORBAX Solvent Saver Plus Eclipse Plus Phenyl-Hexyl LC Column
Column 3.0 mm x 150 mm, 3.5 μ m
(Part No. 959963-312)

Flow rate: 0.3 mL/min

Column temperature: 30 °C

Injection volume: 10 μ L

Mobile phase: A: 5 mM ammonium acetate, pH 5.0 in 20:80 MeOH/H₂O

B: 5 mM ammonium acetate, pH 5.0 in ACN

Needle wash: 1:1:1 ACN/MeOH/IPA/H₂O (0.2% FA.)

Gradient:	Time	% B	Flow rate (mL/min)
	0	20	0.3
	0.5	20	0.3
	8.0	100	0.3
	10.0	100	0.3
	10.01	20	0.5
	12.0	100	0.5
	13.0	STOP	

Post run: 4 min

Total cycle time: 17 min

MS conditions

Positive mode

Gas temperature: 350 °C

Gas flow: 10 L/min

Nebulizer: 40 psi

Capillary: 4,000 V

QuEChERS Procedure

Weigh 10 g comminuted sample (\pm 0.05 g) in 50 mL centrifuge tube

Spike samples with 100 μ L of IS solution and vortex for 1 min

Add 10 mL of ACN, and shake 1 min

Add SampliQ EN extraction packet, and shake vigorously by hand for 1 min

Centrifuge at 4,000 rpm for 5 min

Transfer 1 mL of upper ACN layer to SampliQ EN dispersive SPE 2 mL tube, or 6 mL to SampliQ EN dispersive SPE 15 mL tube

Vortex 1 min, centrifuge at 13,000 rpm for 2 min for 2 mL tubes or at 4,000 rpm for 5 min for 15 mL tubes

Transfer 200 μ L extract to autosampler vial, add 10 μ L of 1% FA in ACN, and dilute with 800 μ L water

Samples are ready for LC/MS/MS analysis

Figure 1. QuEChERS EN sample preparation procedures flow chart

Results

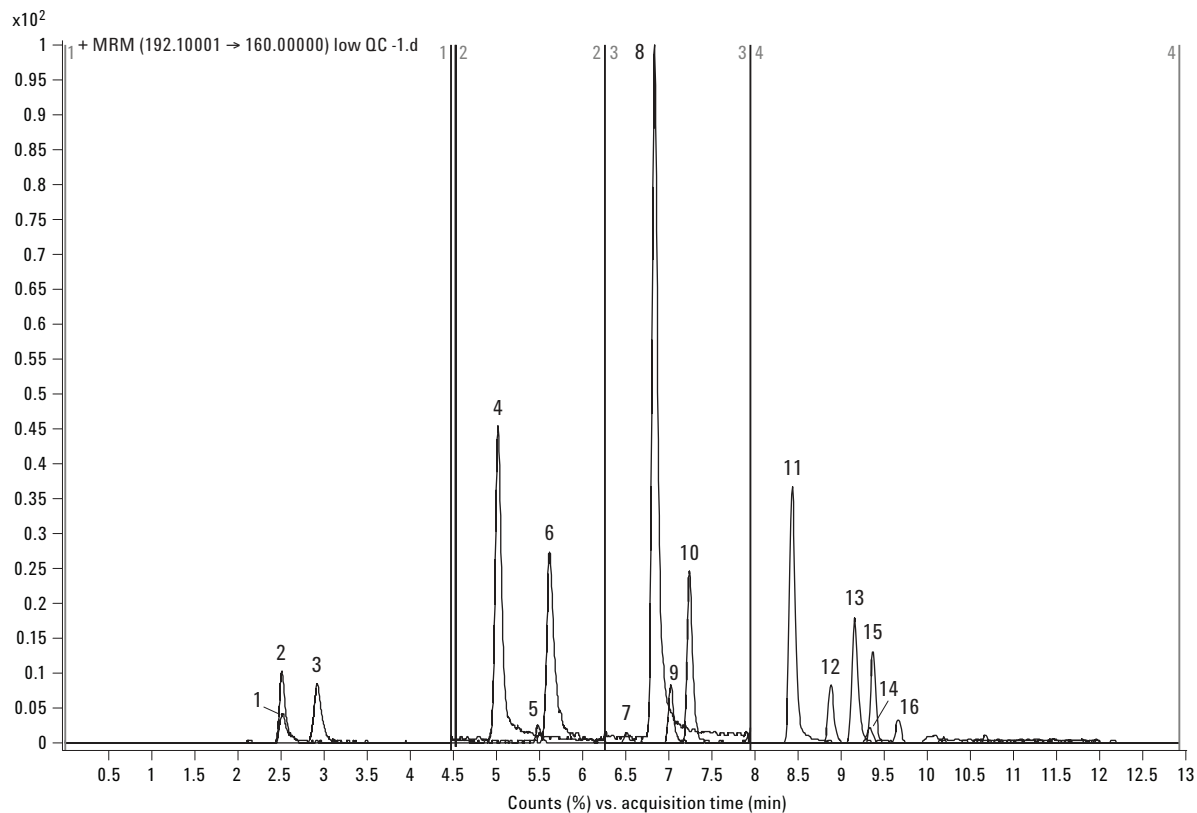


Figure 2. Chromatogram of 10 ng/g fortified apple extract. Peak identification: 1. Methamidophos, 2. Acephate, 3. Pymetrozine, 4. Carbendazim, 5. Imidacloprid, 6. Thiabendazole, 7. Dichlorvos, 8. Propoxur, 9. Thiophanate methyl, 10. Carbaryl, 11. Ethoprophos, 12. Penconazole, 13. Cyprodinil, 14. Dichlofluanid, 15. Kresoxim methyl, 16. Tolyfluanid

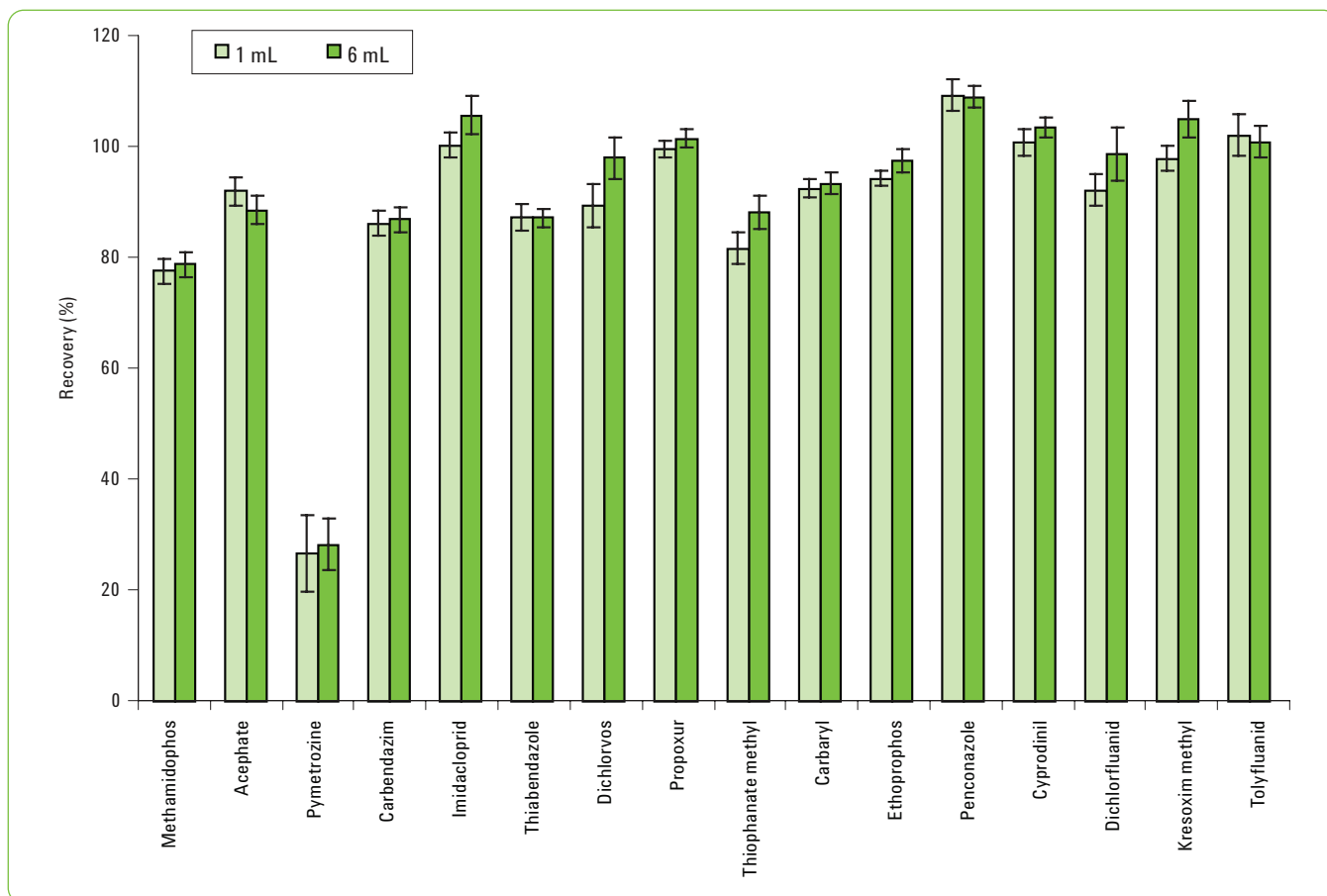


Figure 3. Results comparison of 1 mL dispersive SPE and 6 mL dispersive SPE

Ordering information

Agilent SampliQ QuEChERS EN Method Extraction Kit.
Part No. 5982-5755.

Agilent SampliQ QuEChERS EN Dispersive SPE Kit for General Fruits and Vegetables, 2 mL. Part No. 5982-5021.

Agilent SampliQ QuEChERS EN Dispersive SPE Kit for General Fruits and Vegetables, 15 mL. Part No. 5982-5056.

Agilent ZORBAX Solvent Saver Plus Eclipse Plus Phenyl-Hexyl LC Column, 3.0 mm x 150 mm, 3.5 μ m. Part No. 959963-312.

To review this Application Note in its entirety, please search for 5990-3938EN at www.agilent.com/chem

Analysis of Pesticide Residues in Apple Using Agilent SampliQ QuEChERS EN Kits by GC/MS (Publication 5990-4073EN)

Introduction

This application note describes the use of a quick, easy, cheap, effective, rugged, and safe (QuEChERS) sample preparation approach described in the European Committee (EN) for extraction and cleanup of 17 GC-amenable multiple pesticide class residues in apple. The method involves initial extraction in an aqueous/ acetonitrile system, an extraction/partitioning step after the addition of salt, and a cleanup step using dispersive solid phase extraction (dispersive SPE). The target pesticides in the apple extracts were then analyzed by gas chromatography/mass spectrometry (GC/MS) operating in selective ion monitoring (SIM) mode.

Instrument conditions

GC conditions

Inlet:	Splitless
Inlet liner:	Helix double taper, deactivated (Part No. 5188-5398)
Carrier gas:	Helium
Inlet pressure:	20.18 psi (constant pressure mode) during run 1.0 psi during backflush
Inlet temperature:	250 °C
Injection volume:	1.0 µL
Purge flow to split vent:	30 mL/min at 0.75 min
Oven temperature program:	70 °C (1 min), 50 °C/min to 150 °C (0 min), 6 °C /min to 200 °C (0 min), 16 °C/min to 280 °C (6 min)
Post run:	3 min
Capillary flow technology:	Purged Ultimate Union (Part No. G3186B) – used for backflushing the analytical column and inlet.
Aux EPC gas:	Helium plumbed to Purged Ultimate Union
Aux EPC pressure:	4.0 psi during run, 80.0 psi during backflush
Column:	Agilent J&W HP-5ms Ultra Inert GC Column, 15 m x 0.25 mm, 0.25 µm (Part No. 19091S-431UI)
Connections:	Between inlet and Purged Ultimate Union (Part No. G3186B)
Restrictor:	65 cm x 0.15 mm x 0.15 µm DB-5MS Ultra Inert.
Connections:	Between the Purged Ultimate Union and the MSD

MS conditions

Tune file:	Atune.u
Mode:	SIM (refer to Table 2 for settings in detail)
Source, quad, transfer line temperatures:	230 °C, 150 °C and 280 °C respectively
Solvent delay:	2.30 min
Multiplier voltage:	Autotune voltage

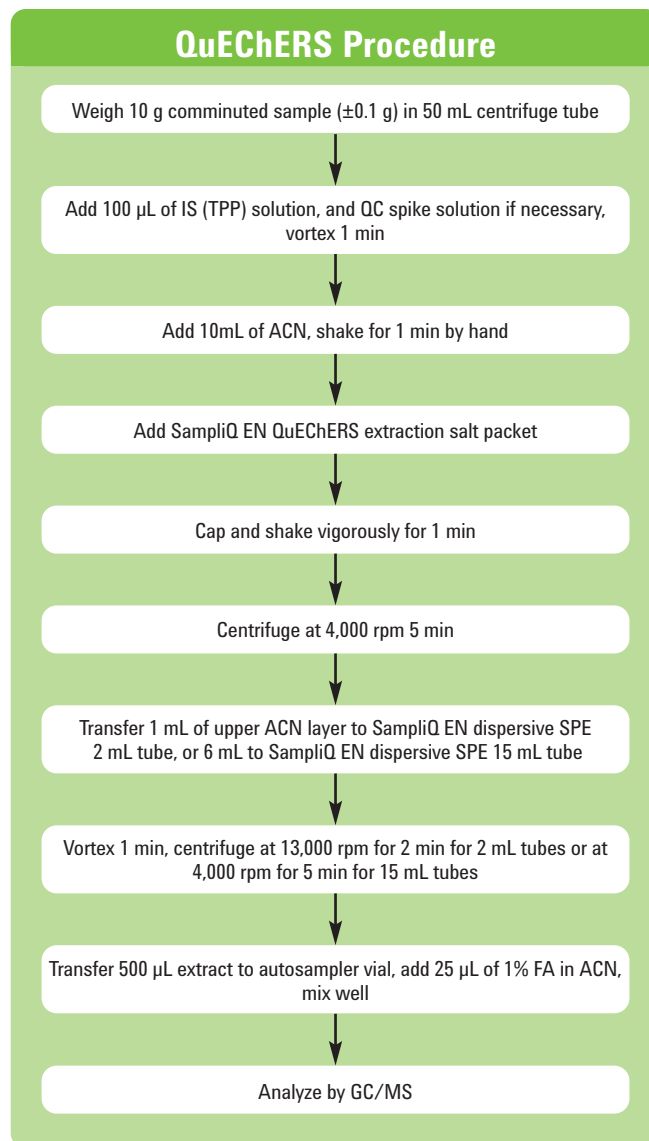


Figure 1. Flow chart of the Agilent SampliQ QuEChERS EN extraction procedure

Results

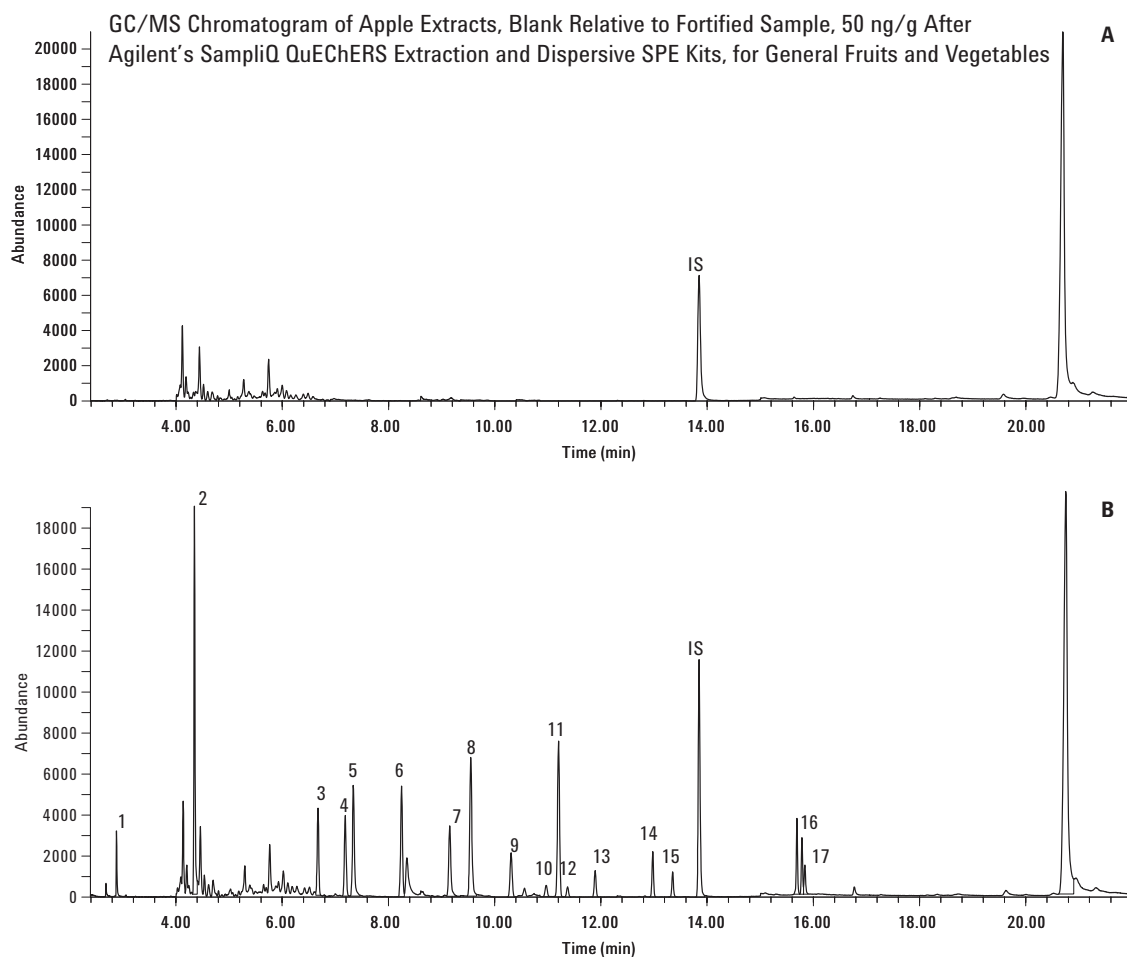


Figure 2. GC/MS chromatogram of apple extract. (A) apple extract blank; (B) 50 ng/g fortified apple extract. Peak Identification: 1. Dichlorvos, 2. *o*-Phenylphenol, 3. Lindane, 4. Diazinon, 5. Chlorothalonil, 6. Chlorpyrifos-methyl, 7. Dichlofluanid, 8. Dichlorobenzophenone, 9. Heptachlor epoxide, 10. γ -Chlordane, 11. DDE, 12. α -Chlordane, 13. Dieldrin, 14. Ethion, 15. Endosulfan sulfate, 16. Permethrin, 17. Coumaphos. IS: Triphenyl phosphate (TPP)

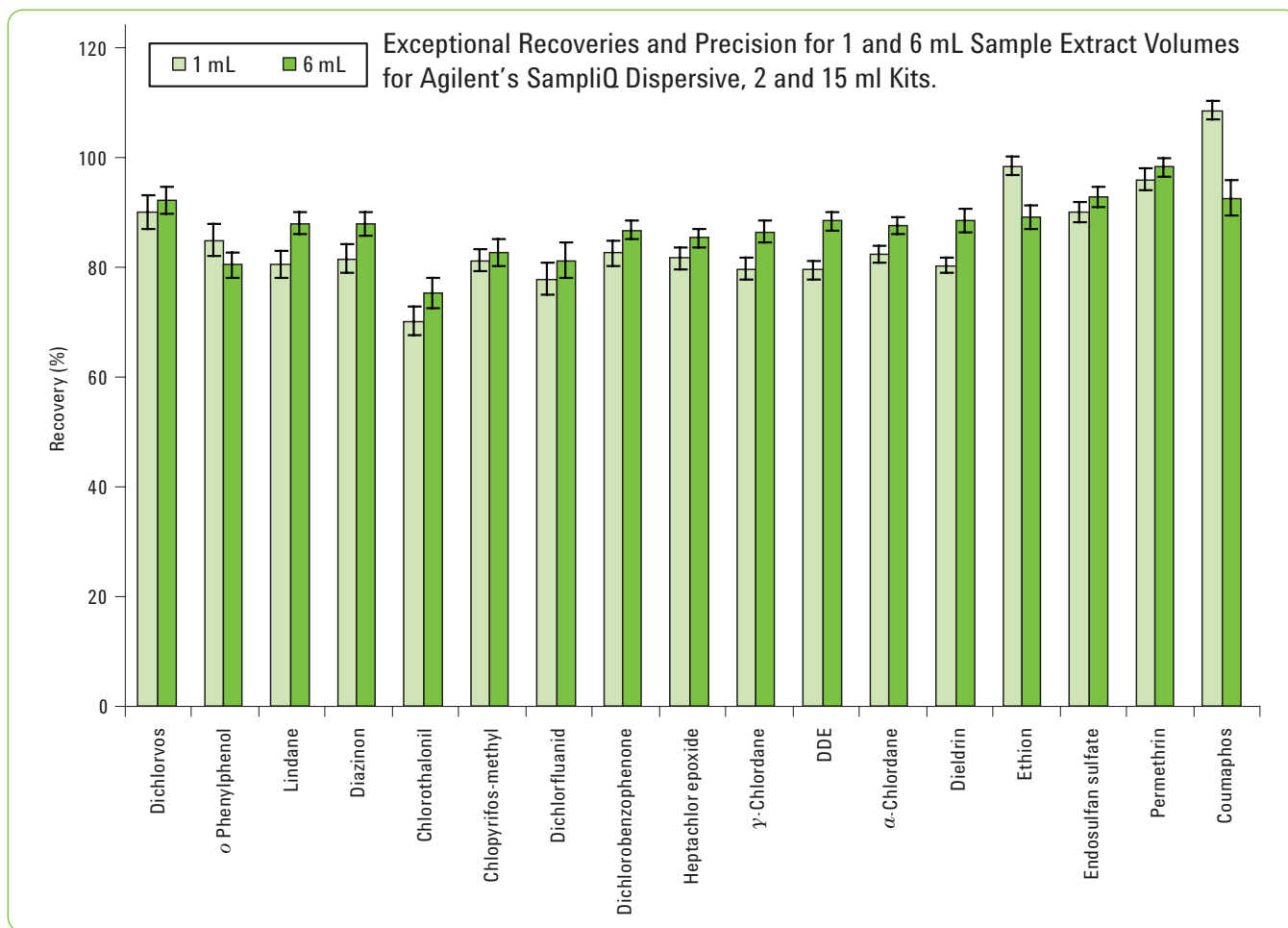


Figure 3. The recovery and precision results of 1 and 6 mL sample volumes employing Agilent's SampliQ Dispersive SPE, 2 and 15 mL Kits, respectively

Ordering information

Agilent SampliQ QuEChERS EN Method Extraction Kit.
Part No. 5982-5650.

Agilent SampliQ QuEChERS EN Method Dispersive SPE Kit for General Fruits and Vegetables, 2 mL. Part No. 5982-5021,
15 mL. Part No. 5982-5056.

Agilent J&W HP-5ms Ultra Inert GC Column,
15 m x 0.25 mm, 0.25 μm. Part No. 19091S-431UI.

To review this Application Note in its entirety, please search for 5990-4073EN at www.agilent.com/chem

Analysis of Pesticide Residues in Spinach Using Agilent SampliQ QuEChERS EN Kit by LC/MS/MS Detection (Publication 5990-4395EN)

Introduction

This application note describes the use of a quick, easy, cheap, effective, rugged, and safe (QuEChERS) EN sample preparation approach for extraction and cleanup of 13 pesticide residues representing various classes in spinach. Because spinach is considered a highly pigmented matrix, the EN dispersive SPE kit for highly pigmented fruits and vegetables is selected. Graphitized carbon black (GCB) in the amount of 7.5 mg/mL of ACN extract is added to the kit. The target pesticides in the spinach extracts are then determined by liquid chromatography coupled to an electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) operating in positive ion multiple reaction monitoring (MRM) mode.

Instrument conditions

HPLC conditions

Column:	Agilent ZORBAX Solvent Saver Plus Eclipse Plus Phenyl-Hexyl LC Column, 3.0 x 150 mm, 3.5 μ m (Part No. 959963-312)		
Flow rate:	0.3 mL/min		
Column Temperature:	30 °C		
Injection volume:	10 μ L		
Mobile Phase:	A, 5 mM ammonium acetate, pH 5.0 in 20:80 MeOH/H ₂ O B, 5 mM ammonium acetate, pH 5.0 in ACN		
Needle wash:	1:1:1 ACN/MeOH/IPA/H ₂ O w/0.2% FA.		
Gradient:	Time	% Acetonitrile	Flow rate (mL/min)
	0	20	0.3
	0.5	20	0.3
	8.0	100	0.3
	10.0	100	0.3
	13.0	STOP	
Post run:	4 min		
Total cycle time:	17 min		

MS conditions

Positive mode	
Gas temp.:	350 °C
Gas flow:	10 L/min
Nebulizer:	40 Psi
Capillary:	4,000 V

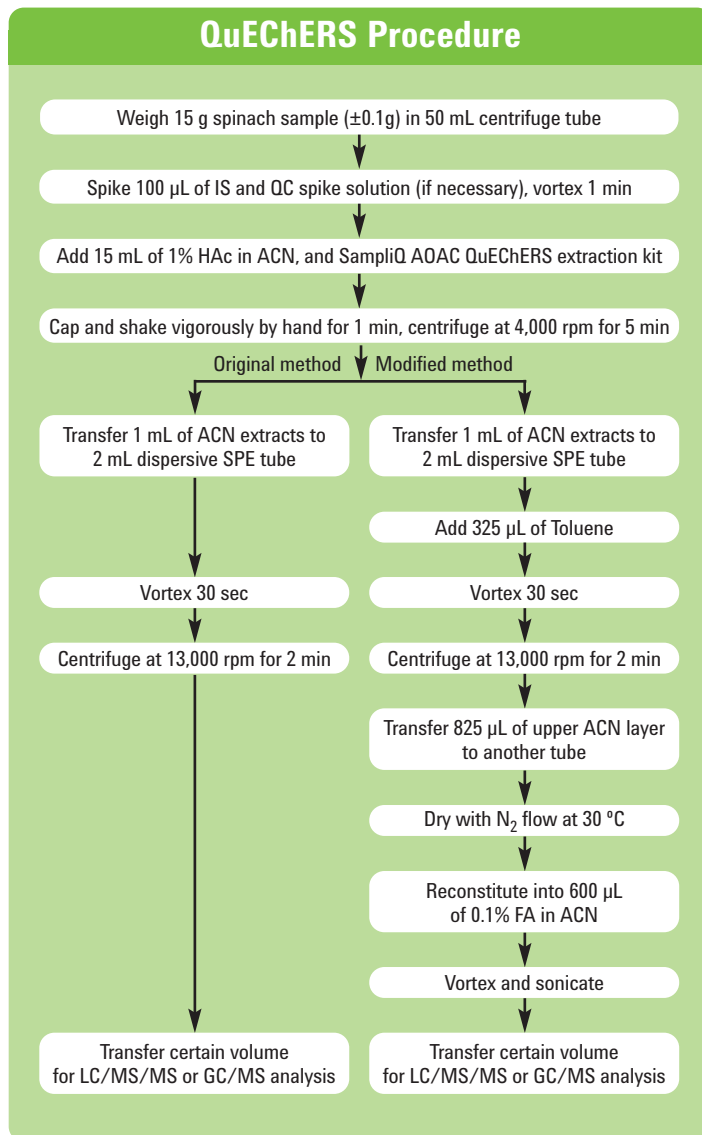


Figure 1. Flow chart of the QuEChERS AOAC extraction procedure (original and modified dispersive SPE, 2 mL size)

Results

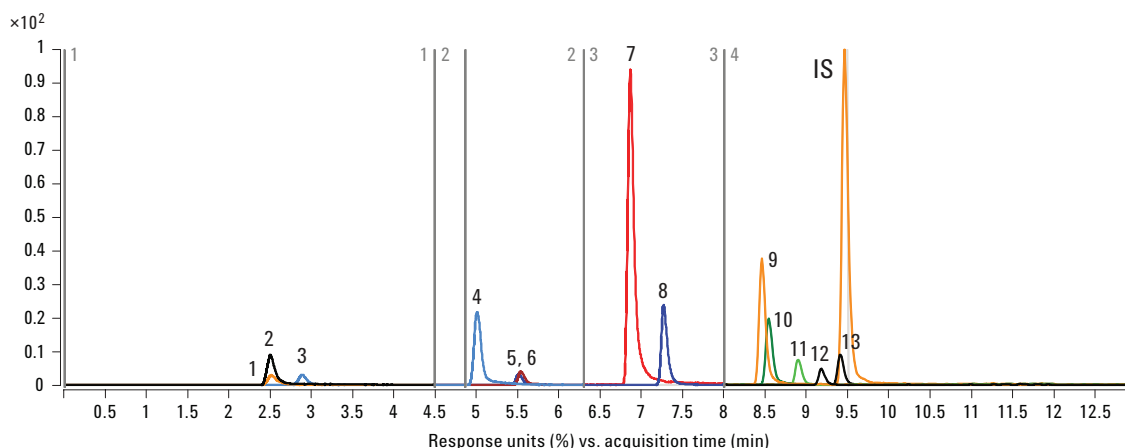


Figure 2. MRM chromatogram of 50 ng/g fortified sample processed by EN method. Peak identification: 1. Methamidophos, 2. Acephate, 3. Pymetrozine, 4. Carbendazim, 5. Imidacloprid, 6. Thiabendazole, 7. Propoxur, 8. Carbaryl, 9. Ethoprophos, 10. Imazalil, 11. Penconazole, 12. Cyprodinil, 13. Kresoxim methyl, IS: Internal Standard, TPP

Analytes	10 ng/g fortified QC		50 ng/g fortified QC		200 ng/g fortified QC	
	Recovery	RSD (n=6)	Recovery	RSD (n=6)	Recovery	RSD (n=6)
Methamidophos	85.0	8.3	87.7	2.7	95.0	9.4
Acephate	88.6	5.1	84.6	3.1	94.6	9.3
Pymetrozine*	68.7	3.7	65.7	1.5	71.9	10.8
Carbendazim*	94.0	5.4	91.4	2.7	53.5	9.3
Imidacloprid	102.0	8.9	85.4	6.1	100.1	7.7
Thiabendazole*	77.2	4.4	77.6	2.4	79.2	9.7
Propoxur	98.2	5.7	96.3	1.8	93.9	7.2
Carbaryl	98.5	3.6	94.0	1.7	97.4	7.2
Ethoprophos	102.3	6.0	95.3	1.7	91.0	6.8
Imazalil	88.8	6.4	86.8	2.8	93.5	7.7
Penconazole	104.5	2.5	96.4	2.0	84.6	5.5
Cyprodinil*	101.5	4.2	92.2	2.4	86.8	7.6
Kresoxim methyl	99.7	6.1	97.4	1.6	95.3	6.9

* Pesticides with planar structure.

Table 1. Recovery and reproducibility of pesticides in fortified spinach with 6 mL dispersive SPE tube (Part No. 5982-5356)

Ordering information

Agilent SampliQ QuEChERS AOAC Extraction Kits.

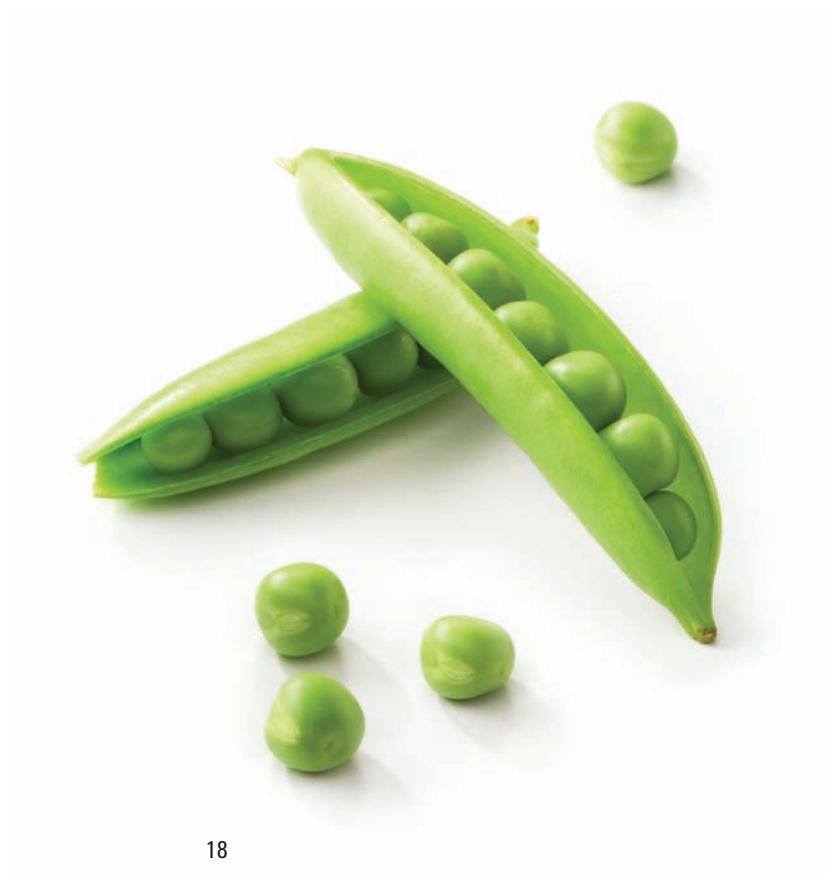
Part No. 5982-5755.

Agilent SampliQ QuEChERS AOAC Dispersive SPE Kits for Pigmented Fruits and Vegetables. Part Nos. 5982-5222 and 5982-5258.

Agilent ZORBAX Solvent Saver Plus Eclipse Plus Phenyl-Hexyl LC Column, 3.0 x 150 mm, 3.5 µm. Part No. 959963-312.

To review this Application Note in its entirety, please search for 5990-4395EN at www.agilent.com/chem

AOAC Methods



Analysis of Pesticide Residues in Apples using Agilent SampliQ QuEChERS AOAC Kit by LC/MS/MS Detection (Publication 5990-3937EN)

Introduction

This application note describes the use of a quick, easy, cheap, effective, rugged, and safe (QuEChERS), Association of Analytical Communities (AOAC) Official Method 2007.01; sample preparation approach for extraction and cleanup of 16 pesticide residues in apple.

The 5 ng/g limit of quantitation (LOQ) for pesticides in apple shown in this application was well below the maximum residue limits (MRLs). The spiking levels for the recovery experiments were 10, 50, and 200 ng/g. Mean recoveries ranged between 76 and 117% (95.4% on average), with RSD below 15% (4.3% on average).

Instrument conditions

HPLC conditions

Column: Agilent ZORBAX Solvent Saver Plus Eclipse Plus Phenyl-Hexyl LC Column
3.0 mm x 150 mm, 3.5 μ m (Part No. 959963-312)

Flow rate: 0.3 mL/min

Column Temperature: 30 °C

Injection volume: 10 μ L

Mobile Phase: A: 5mM NH₄OAc, pH 5.0 in 20:80 MeOH/H₂O

B: 5 mM NH₄OAc, pH 5.0 in ACN

Needle wash: 1:1:1 ACN/MeOH/IPA/H₂O (0.2% FA)

Gradient	Time	% B	Flow rate (mL/min)
	0	20	0.3
	0.5	20	0.3
	8.0	100	0.3
	10.0	100	0.3
	10.01	20	0.5
	12.0	100	0.5
	13.0	STOP	

Post run: 4 min

Total cycle time: 17 min

MS conditions

Positive mode

Gas Temperature: 350 °C

Gas Flow: 10 L/min

Nebulizer: 40 psi

Capillary: 4,000 V

QuEChERS Procedure

Accurately weigh 15 g homogenized sample (\pm 0.05 g) in 50 mL centrifuge tubes

Spike samples with 100 μ L of IS solution and vortex for 1 min

Add 15 mL of 1% acetic acid in ACN, shake vigorously for 1 min

Add SampliQ QuEChERS AOAC salt packet, cap tubes and shake vigorously for 1 min

Centrifuge at 4,000 rpm for 5 min

Transfer upper ACN layer to SampliQ QuEChERS dispersive-SPE tube, 1 mL/2 mL tube or 8 mL/15 mL tube

Vortex 1 min then centrifuge

Transfer 200 μ L extract to autosampler vial, dilute with 800 μ L appropriate solution if necessary

Samples are ready for LC/MS/MS analysis

Figure 1. QuEChERS AOAC sample preparation procedures flow chart

Ordering information

Agilent SampliQ QuEChERS Buffered AOAC Extraction Kit.
Part No. 5982-5755.

Agilent SampliQ QuEChERS AOAC Dispersive SPE Kit for General Fruits and Vegetables, 2 mL. Part No. 5982-5022.

Agilent SampliQ QuEChERS AOAC Dispersive SPE Kit for General Fruits and Vegetables, 15 mL. Part No. 5982-5058.

Agilent ZORBAX Solvent Saver Plus Eclipse Plus Phenyl-Hexyl LC Column, 3.0 mm x 150 mm, 3.5 μ m. Part No. 959963-312.

Results

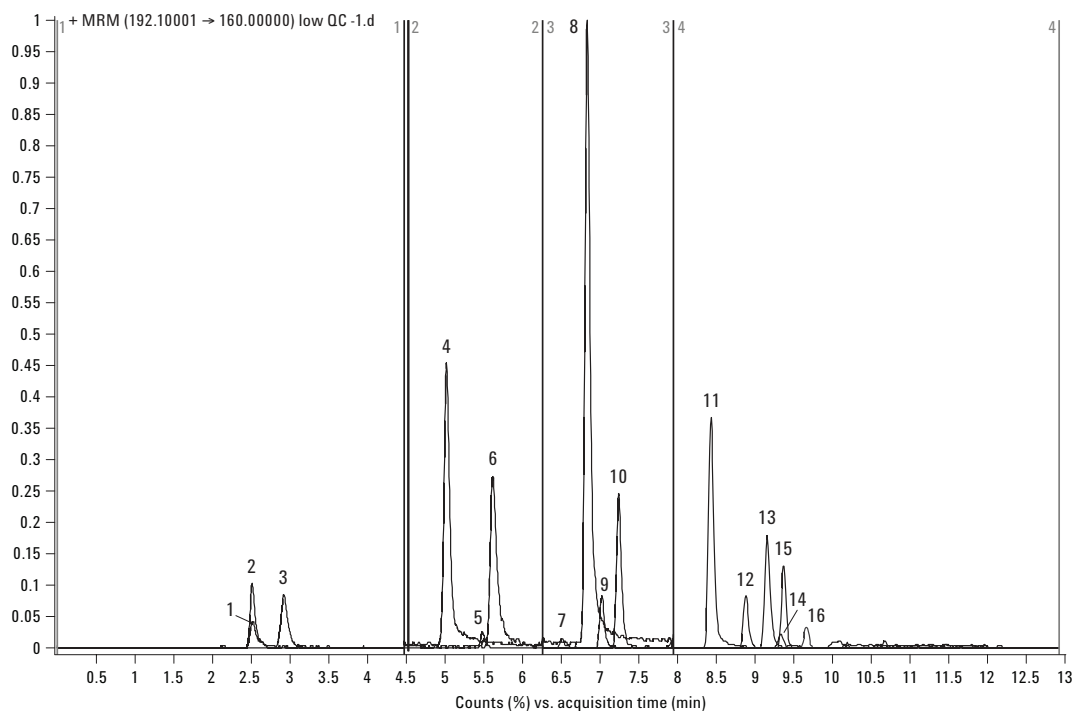


Figure 2. Chromatogram of 10 ng/g fortified apple extract. Peak identification: 1. Methamidophos, 2. Acephate, 3. Pymetrozine, 4. Carbendazim, 5. Imidacloprid, 6. Thiabendazole, 7. Dichlorvos, 8. Propoxur, 9. Thiophanate methyl, 10. Carbaryl, 11. Ethoprophos, 12. Penconazole, 13. Cyprodinil, 14. Dichlorfluandil, 15. Kresoxim methyl, 16. Tolyfluandil

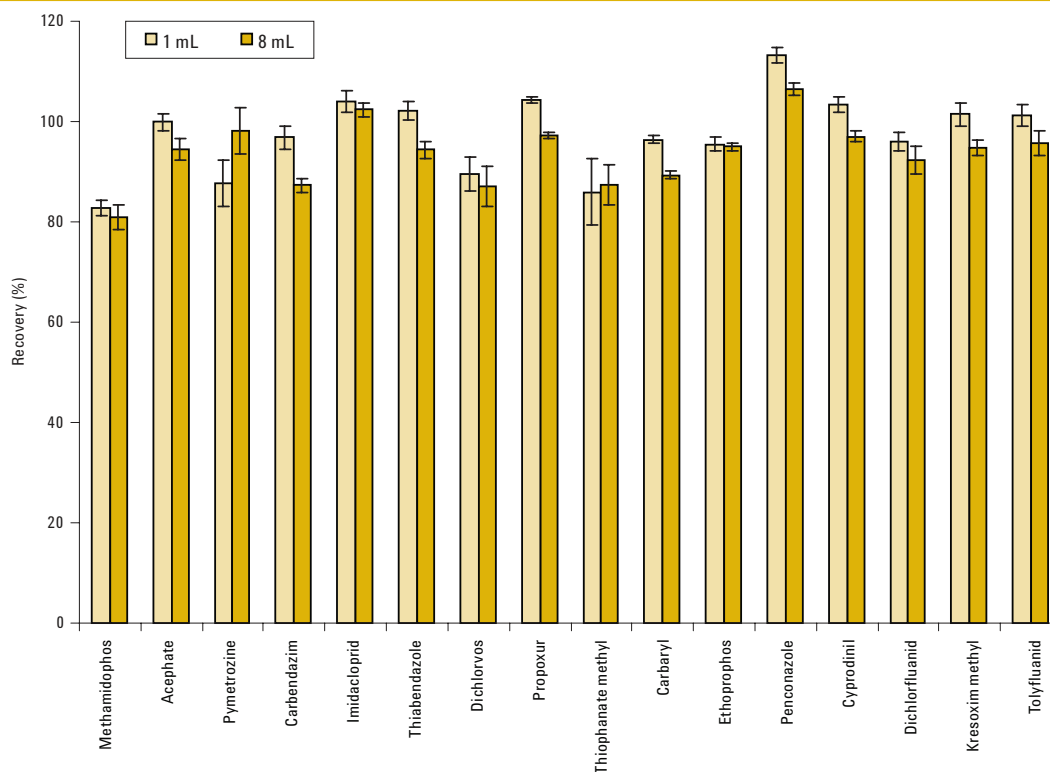


Table 1. Results comparison of 1 mL and 8 mL dispersive SPE sample volume

To review this Application Note in its entirety, please search for 5990-3937EN at www.agilent.com/chem

Analysis of Pesticide Residues in Apple Using Agilent SampliQ QuEChERS AOAC Kits by GC/MS (Publication 5990-4068EN)

Introduction

This application note describes the use of a quick, easy, cheap, effective, rugged, and safe (QuEChERS) sample preparation approach for extraction and cleanup of 17 GC-amenable pesticide residues from multiple classes, in apple. The target pesticides in the apple extracts were then analyzed by gas chromatography/mass spectrometry (GC/MS) operating in selective ion monitoring (SIM) mode.

Instrument conditions

GC conditions

Auto-sampler:	Agilent 7683 automatic liquid
Inlet:	Splitless
Column:	Agilent J&W HP-5ms Ultra Inert GC Column 30 m x 0.25 mm, 0.25 μ m (Part No. 19091S-433UI)
Carrier gas:	Helium in the constant pressure
Retention time locking:	Chlorpyrifos-methyl locked to 16.596 min (nominal Column head pressure=22.0 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 (11.5 min)
Injection volume:	1.0 μ L

MS conditions

Tune file:	Atune.u
Mode:	SIM (refer to Table 2 for settings in detail)
Source, quad, transfer line temperature:	230 °C, 150 °C and 280 °C respectively
Solvent delay:	3.00 min
Multiplier voltage:	Autotune voltage

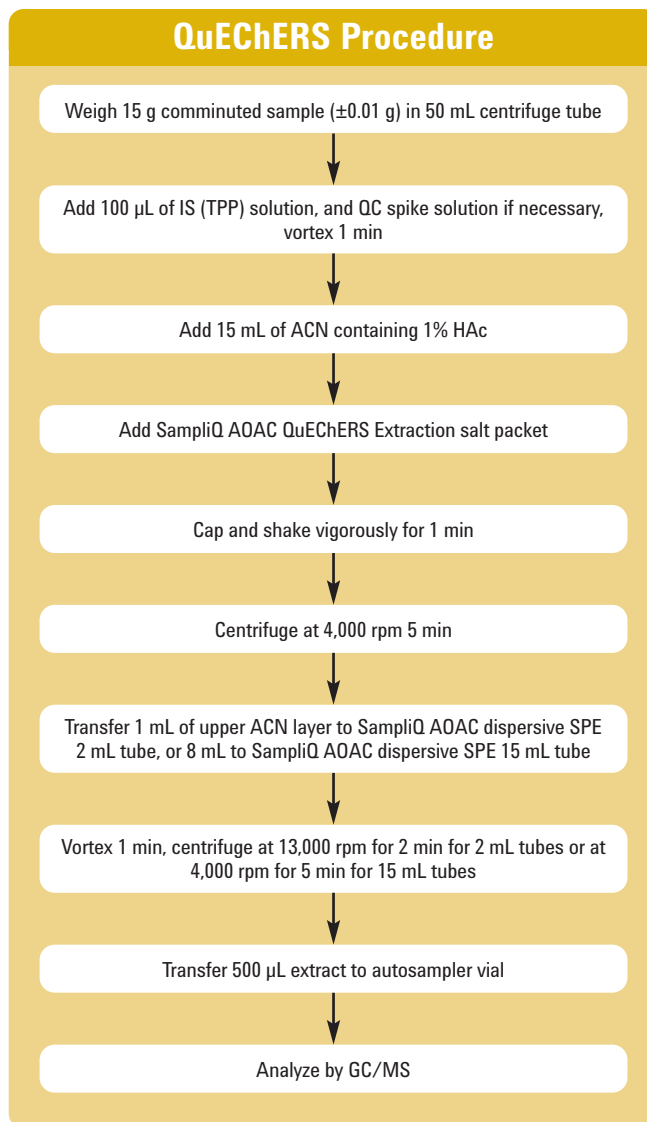


Figure 1. Flow chart of the Agilent SampliQ QuEChERS AOAC extraction procedure

Ordering information

Agilent SampliQ QuEChERS Buffered AOAC Extraction Kit.
Part No. 5982-5755.

Agilent SampliQ QuEChERS AOAC Dispersive SPE Kit for General Fruits and Vegetables, 2 mL. Part No. 5982-5022.

Agilent SampliQ QuEChERS AOAC Dispersive SPE Kit for General Fruits and Vegetables, 15 mL. Part No. 5982-5058.

Agilent J&W HP-5ms Ultra Inert GC Column, 30 m x 0.25 mm, 0.25 μ m. Part No. 19091S-433UI.

Results

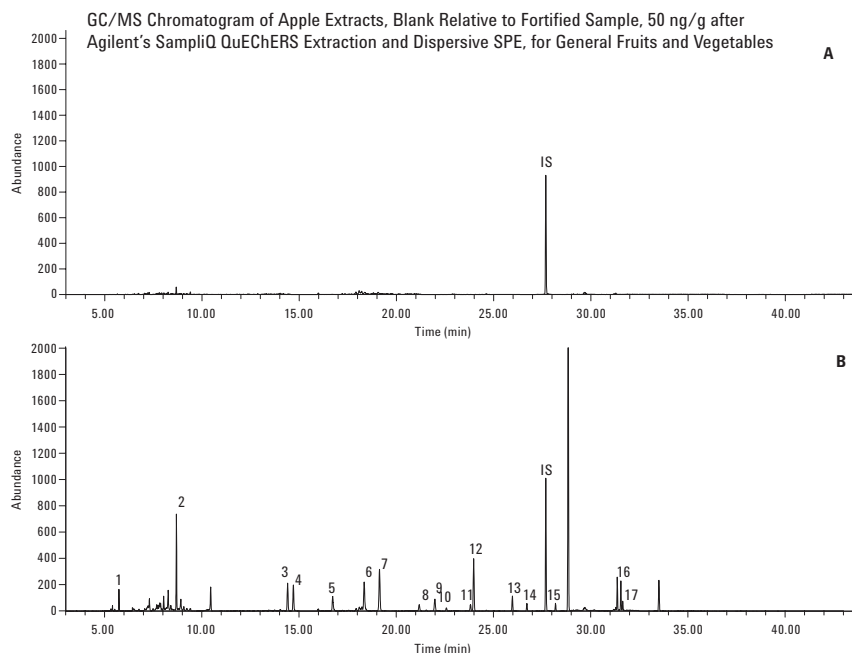


Figure 2. GC/MS chromatogram of apple extract. (A) apple extract blank; (B) 50 ng/g fortified apple extract. Peak Identification: 1. Dichlorvos, 2. σ -Phenylphenol, 3. Diazinon, 4. Chlorothalonil, 5. Carbaryl, 6. Dichlorfluaniid, 7. Dichlorobenzophenone, 8. Folpet, 9. α -Chlordane, 10. Endosulfan, 11. Dieldrin, 12. DDE, 13. Ethion, 14. Endosulfan sulfate, 15. Endrin ketone, 16. Permethrin, 17. Coumaphos. IS: Triphenyl phosphate (TPP)

Exceptional Recoveries and Precision for 1 and 8 mL Volumes for Agilent SampliQ Dispersive SPE, 2 and 15 mL Kits

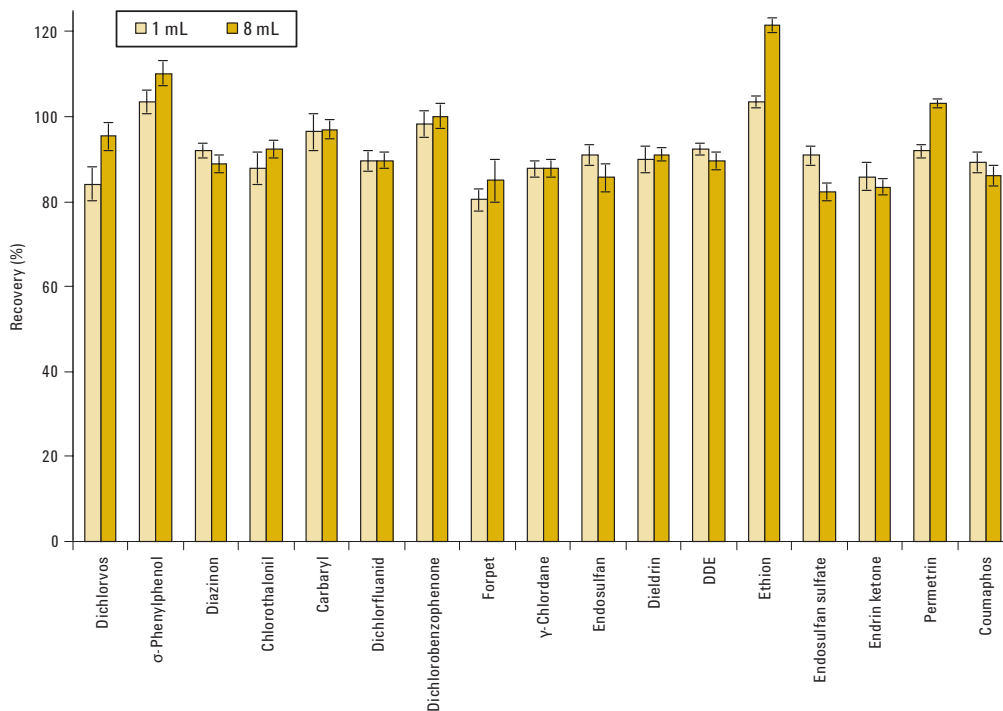


Figure 3. Recoveries and precision for 1 and 8 mL sample volumes employing Agilent SampliQ dispersive SPE, 2 and 15 mL kits, respectively

To review this Application Note in its entirety, please search for 5990-4068EN at www.agilent.com/chem

Analysis of Pesticide Residues in Spinach Using Agilent SampliQ QuEChERS AOAC Kits by GC/MS (Publication 5990-4305EN)

Introduction

This application note describes the use of a quick, easy, cheap, effective, rugged, and safe (QuEChERS) AOAC sample preparation approach for extraction and cleanup of 18 GC-amenable multiple pesticide class residues in spinach. In order to address the significant loss of planar pesticides caused by graphitized carbon black (GCB) in dispersive SPE, a modified method with addition of toluene was employed for the planar pesticides. The target pesticides in the spinach extracts were then analyzed by gas chromatography/mass spectrometry (GC/MS) operating in selective ion monitoring (SIM) mode.

Instrument conditions

GC conditions

Inlet:	Splitless
Inlet liner:	Helix double taper, deactivated (Part No. 5188-5398)
Carrier gas:	Helium
Inlet pressure:	19.6 psi (constant pressure mode) during run 1.0 psi during backflush
Inlet temperature:	250 °C
Injection volume:	1.0 µL
Purge flow to split vent:	30 mL/min at 0.75 min
Oven temperature program:	70 °C (1 min), 50 °C/min to 150 °C (0 min), 6 °C/min to 200 °C (0 min), 16 °C/min to 280 °C (6 min)
Post run:	3 min
Capillary flow technology:	Purged Ultimate Union (Part No. G3186B) - used for backflushing the analytical column and inlet.
Aux EPC gas:	Helium plumbed to Purged Ultimate Union
Aux EPC pressure:	4.0 psi during run, 80.0 psi during backflush
Column:	Agilent J&W HP-5ms Ultra Inert GC Column 15 m x 0.25 mm, 0.25 µm (Part No. 19091S-431UI)
Connections:	Between inlet and Purged Ultimate Union (Part No. G3186B)
Restrictor:	65 cm x 0.15 mm, 0.15 µm DB-5ms Ultra Inert
Connections:	Between the Purged Ultimate Union and the MSD

MS conditions

Tune file	Atune.u
Mode	SIM
Source, quad, transfer line temperature	230 °C, 150 °C and 280 °C respectively
Solvent delay	2.30 min
Multiplier voltage	Autotune voltage

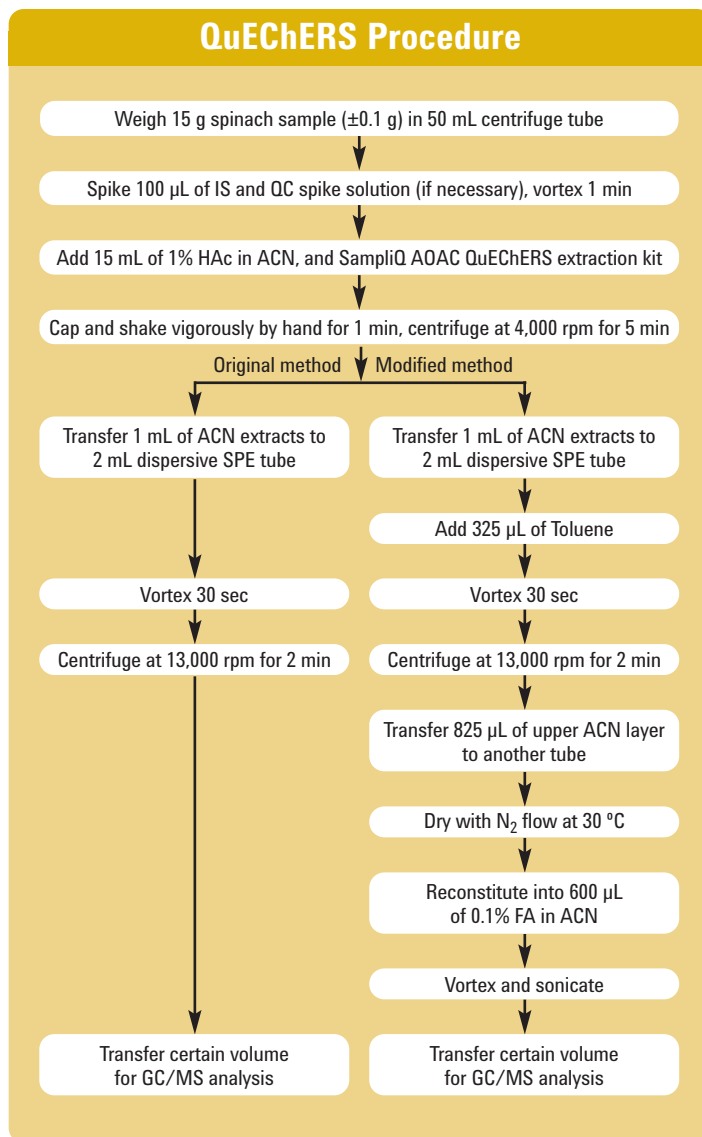


Figure 1. Flow chart of the QuEChERS AOAC extraction procedure (original and modified dispersive SPE, 2 mL size) for spinach sample

Results

GC/MS Chromatograms of 50 ng/g Fortified Spinach Samples Implementing the Original and Modified AOAC Dispersive Method

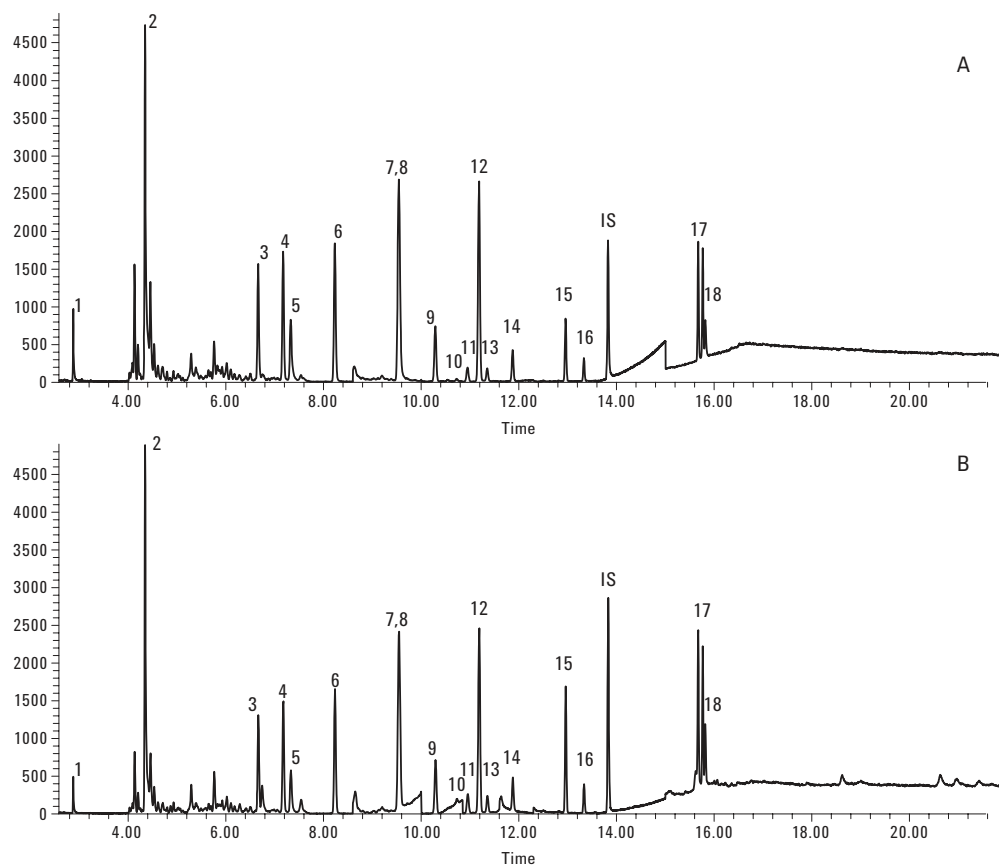


Figure 2. GC/MS chromatograms of 50 ng/g fortified spinach sample extracts processed by original dispersive SPE (A) and modified dispersive SPE (B). Peak identification: 1. Diachlorvos, 2. o-Phenylphenol, 3. Lindane, 4. Diazinon, 5. Chlorothalonil 6. Chlorpyrifos methyl 7. Dichlorobenzophenone, 8. Chlorpyrifos, 9. Heptachlor epoxide, 10. Folpet, 11. α -Chlordane, 12. DDE, 13. γ -Chlordane, 14. Dieldrin, 15. Ethion, 16. Endosulfan sulfate, 17. Permethrin, 18. Coumaphos. IS: Internal Standard, TPP

Pesticide	Low QC (10 ng/g)		Mid QC (50 ng/g)		High QC (200 ng/g)	
	Recovery	RSD	Recovery	RSD	Recovery	RSD
Dichlorvos	94.0	3.0	91.7	10.5	80.9	4.6
<i>o</i> -Phenylphenol	95.0	2.2	92.0	7.9	78.7	3.8
Lindane	83.7	3.1	93.9	12.2	91.8	3.3
Diazinon	97.3	4.3	95.6	9.9	91.8	3.3
Chlorothalonil*	47.5	6.8	44.9	6.6	49.4	4.3
Chlorpyrifos methyl	74.1	4.6	71.7	4.5	72.2	5.8
Dichlorobenzo Phenone*	97.5	7.6	66.8	3.9	68.8	6.8
Chlorpyrifos	88.3	3.0	79.6	3.5	77.0	3.5
Heptachlor epoxide	74.9	1.9	81.6	11.7	78.2	3.9
Folpet*	NA	NA	98.8	6.0	77.7	6.7
γ -Chlordane	106.0	4.9	112.2	3.3	93.6	5.3
DDE	80.3	2.2	86.8	9.6	75.4	3.5
α -Chlordane	107.6	4.2	108.4	3.5	91.6	3.7
Dieldrin	99.7	2.6	93.7	9.6	78.9	3.4
Ethion	91.4	3.4	100.0	5.0	107.4	7.6
Endosulfan sulfate	93.7	4.8	97.3	8.8	89.8	4.3
Permethrin	84.7	5.7	74.8	9.9	84.6	6.0
Coumaphos*	98.4	5.5	84.2	9.5	81.2	3.2

* Results from modified dispersive SPE method

Table 1. Spinach AOAC dispersive, 1 mL sample volume, 2 mL tube, LC/MS/MS results

Ordering information

Agilent SampliQ QuEChERS Buffered AOAC Extraction Kit.
Part No. 5982-5755.

Agilent SampliQ QuEChERS AOAC Dispersive SPE Kit for Pigmented Fruits and Vegetables, 2 mL. Part No. 5982-5222.

Agilent SampliQ QuEChERS AOAC Dispersive SPE Kit for Pigmented Fruits and Vegetables, 15 mL. Part No. 5982-5258.

Agilent J&W HP-5ms Ultra Inert GC Column, 15 m x 0.25 mm, 0.25 μ m. Part No. 19091S-431UI.

Agilent Ultimate Union. Part No. G3186B.

To review this Application Note in its entirety, please search for 5990-4305EN at www.agilent.com/chem

Analysis of Pesticide Residues in Spinach Using Agilent SampliQ QuEChERS AOAC Kit by LC/MS/MS Detection (Publication 5990-4248EN)

Introduction

This application note describes the use of a quick, easy, cheap, effective, rugged, and safe (QuEChERS) AOAC sample preparation approach for the extraction and cleanup of 13 pesticide residues representing various pesticide classes in spinach. In order to address the significant loss of planar pesticides caused by graphitized carbon black (GCB) in dispersive SPE, a modified method with the addition of toluene was employed. With the combination of original and modified dispersive SPE, the method was validated in terms of recovery and reproducibility for all of the analytes of interest.

Instrument conditions

HPLC conditions

Column: Agilent ZORBAX Solvent Saver Plus Eclipse Plus Phenyl-Hexyl LC Column 3.0 mm x 150 mm, 3.5 μ m (Part No. 959963-312)

Flow rate: 0.3 mL/min

Column Temperature: 30 °C

Injection volume: 10 μ L

Mobile Phase: A: 5 mM NH_4OAc , pH 5.0 in 20:80 MeOH/ H_2O
B: 5 mM NH_4OAc , pH 5.0 in ACN

Needle wash: 1:1:1:1 ACN/MeOH/isopropyl alcohol (IPA)/ H_2O w/0.2% FA.

Gradient:	Time	% B	Flow rate (mL/min)
	0	20	0.3
	0.5	20	0.3
	8.0	100	0.3
	10.0	100	0.3
	10.01	20	0.5
	13.0	STOP	

Post run: 4 min

Total cycle time: 17 min

MS conditions

Positive mode

Gas temperature: 350 °C

Gas flow: 10 L/min

Nebulizer: 40 psi

Capillary: 4,000 V

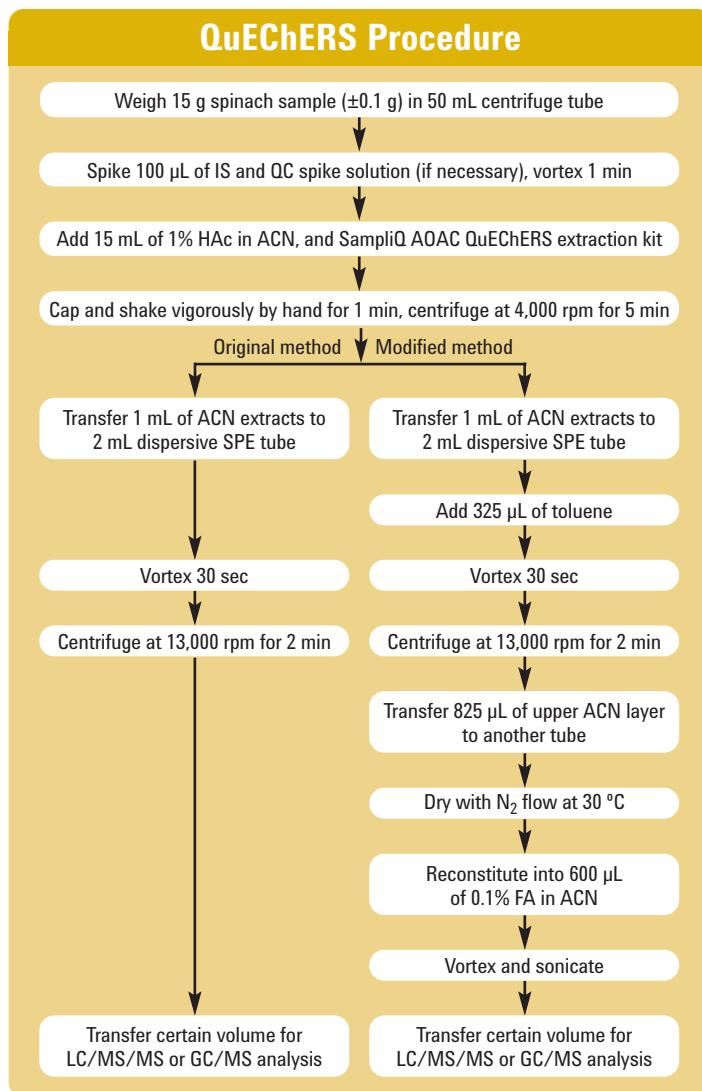


Figure 1. Flow chart of the QuEChERS AOAC extraction procedure (original and modified dispersive SPE, 2 mL size) for a spinach sample

Results

Comparison of LC/MS/MS Chromatograms Representing Improved Planar Pesticide Recovery with Toluene Addition

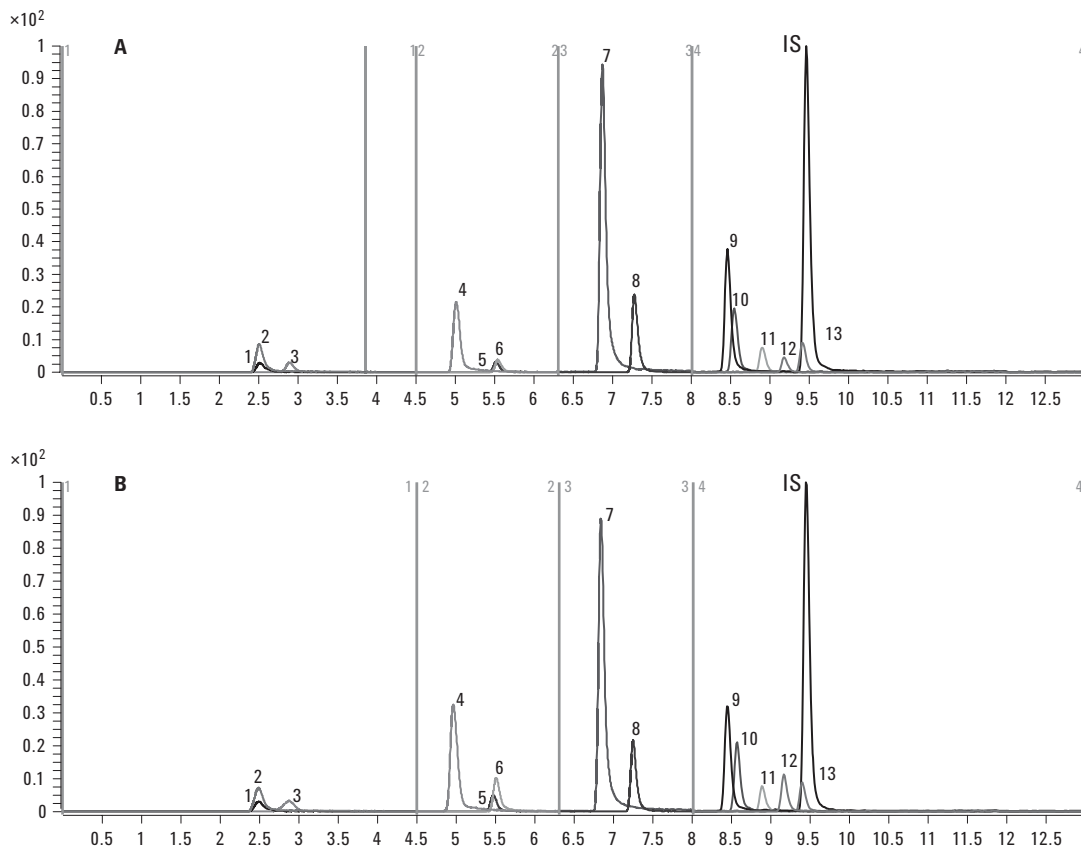


Figure 2. LC/MS/MS chromatograms of 50 ng/g fortified spinach sample extracts processed by original dispersive SPE (A) and modified dispersive SPE (B). Peak identification: 1. Methamidophos, 2. Acephate, 3. Pymetrozine, 4. Carbendazim, 5. Imidacloprid, 6. Thiabendazole, 7. Propoxur, 8. Carbaryl, 9. Ethoprophos, 10. Imazalil, 11. Penconazole, 12. Cyprodinil, 13. Kresoxim methyl IS: Internal Standard, TPP

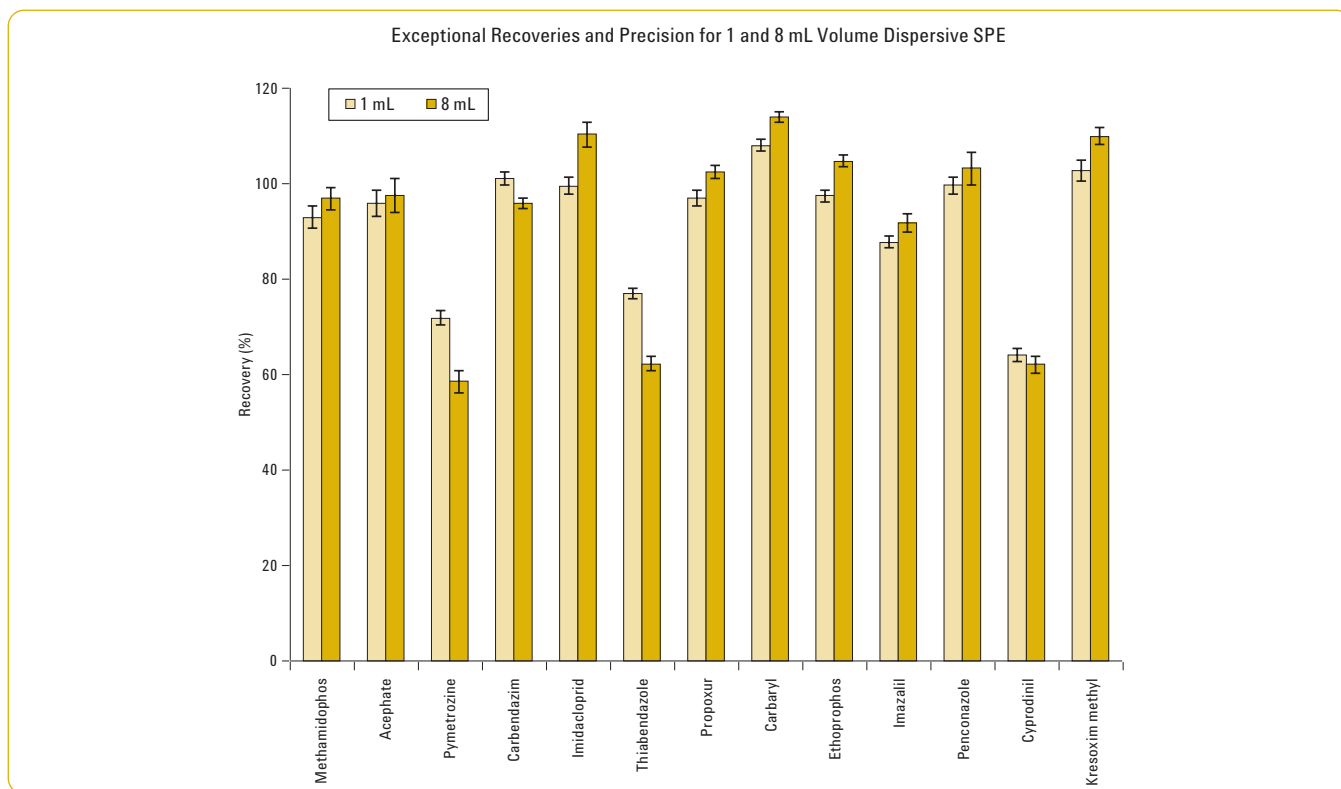


Figure 3. The recovery and precision results for 1 mL dispersive SPE and 8 mL dispersive SPE

Ordering information

Agilent SampliQ QuEChERS Buffered AOAC Extraction Kit.
Part No. 5982-5755.

Agilent SampliQ QuEChERS AOAC Dispersive SPE Kit for Pigmented Fruits and Vegetables, 2 mL. Part No. 5982-5222.

Agilent SampliQ QuEChERS AOAC Dispersive SPE Kit for Pigmented Fruits and Vegetables, 15 mL. Part No. 5982-5258.

Agilent ZORBAX Solvent Saver Plus Eclipse Plus Phenyl-Hexyl LC Column, 3.0 mm x 150 mm, 3.5 μ m.
Part No. 959963-312.

Optimizing Recoveries of Planar Pesticides in Spinach Using Toluene and Agilent SampliQ AOAC QuEChERS Kits with Graphitized Carbon (Publication 5990-4247EN)

Introduction

This application note describes the impact of toluene addition in the dispersive solid phase extraction (SPE) step on the analysis of pesticides in spinach using Agilent SampliQ QuEChERS AOAC kits for highly pigmented fruits and vegetables. With the modified AOAC method, the eight problematic pesticides generated substantially improved recoveries, 50% to 300%, and < 10% RSD.

Instrument conditions

HPLC conditions

Column:	Agilent ZORBAX Solvent Saver Plus Eclipse Plus Phenyl-Hexyl LC Column, 3.0 x 150 mm, 3.5 µm (Part No. 959963-312)		
Flow rate:	0.3 mL/min		
Column temperature:	30 °C		
Injection volume:	10 µL		
Mobile phase:	A: 5 mM ammonium acetate, pH 5.0 in 20:80 MeOH/H ₂ O; B: 5 mM ammonium acetate, pH 5.0 in ACN		
Needle wash:	1:1:1 ACN/MeOH/IPA/H ₂ O w/0.2% FA.		
Gradient:	Time	% B	Flow rate (mL/min)
	0	20	0.3
	0.5	20	0.3
	8.0	100	0.3
	10.0	100	0.3
	10.01	20	0.5
	13.0	STOP	
Post run:	4 min		
Total cycle time:	17 min.		

GC conditions

Inlet:	Splitless
Inlet liner:	Helix double taper, deactivated (Part No. 5188-5398)
Carrier gas:	Helium
Inlet pressure:	19.6 psi (constant pressure mode) during run 1.0 psi during back flush
Inlet temperature:	250 °C
Injection volume:	1.0 µL
Purge flow to split vent:	30 mL/min at 0.75 min
Oven temperature program:	70 °C (1 min), 50 °C/min to 150 °C (0 min), 6 °C/min to 200 °C (0 min), 16 °C/min to 280 °C (6 min)
Post run:	3 min
Capillary flow technology:	Purged Ultimate Union (Part No. G3186B) – used for backflushing the analytical column and inlet.
Aux EPC gas:	Helium plumbed to Purged Ultimate Union
Aux EPC pressure:	4.0 psi during run, 80.0 psi during backflush
Column:	Agilent J&W HP-5ms Ultra Inert GC Column 15 m x 0.25 mm, 0.25 µm (Part No. 19091S-431UI)
Connections:	Between inlet and Purged Ultimate Union (Part No. G3186B)
Restrictor:	65 cm x 0.15 mm, 0.15 µm DB-5 ms Ultra Inert.
Connections:	Between the Purged Ultimate Union and the MSD.

QuEChERS Procedure

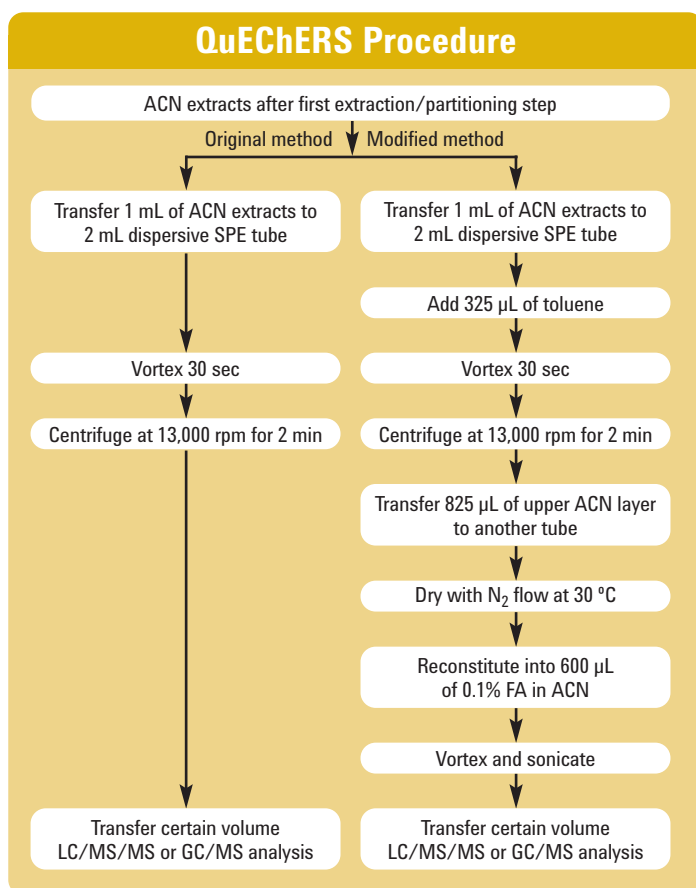


Figure 1. Dispersive SPE procedures of original method (w/o toluene) and modified method (w/toluene)

Ordering information

Agilent SampliQ QuEChERS Buffered AOAC Extraction Kit. Part No. 5982-5755.

Agilent SampliQ QuEChERS AOAC Dispersive SPE Kit for Pigmented Fruits and Vegetables, 2 mL. Part No. 5982-5222.

Agilent SampliQ QuEChERS AOAC Dispersive SPE Kit for Pigmented Fruits and Vegetables, 15 mL. Part No. 5982-5258.

Agilent J&W HP-5ms Ultra Inert GC Column, 15 m x 0.25 mm x 0.25 µm. Part No. 19091S-431UI.

Agilent ZORBAX Solvent Saver Plus Eclipse Plus Phenyl-Hexyl LC Column, 15 m x 0.25 mm, 0.25 µm. Part No. 959963-312.

Results

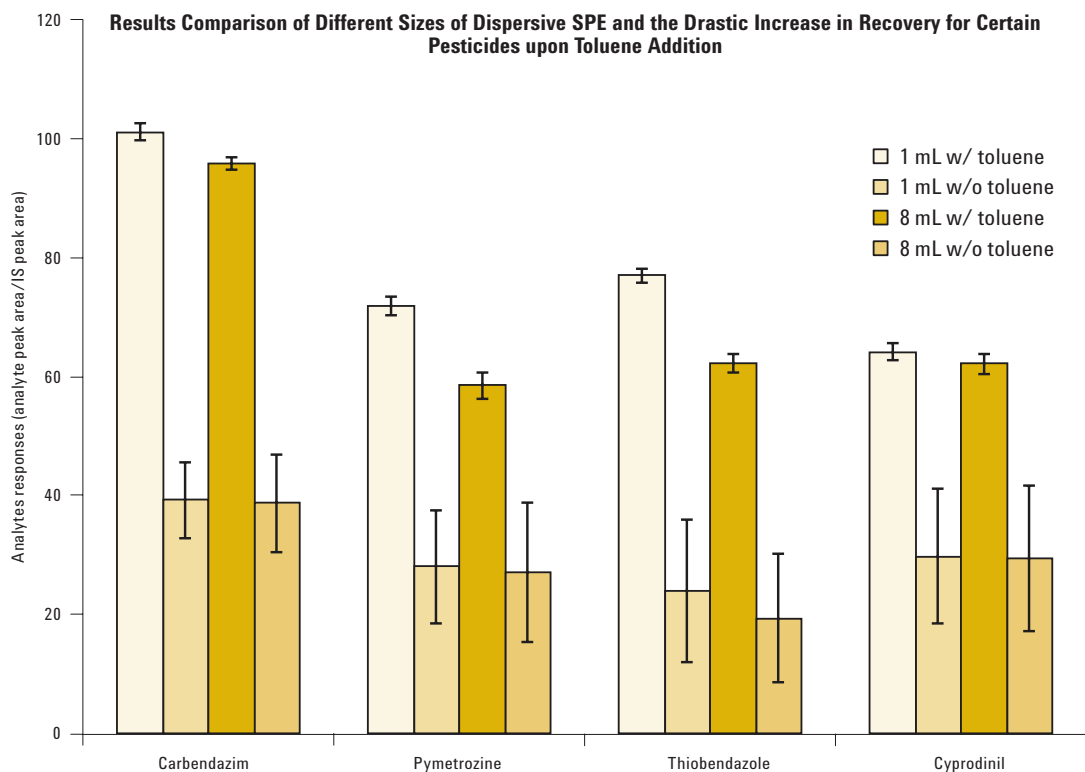


Figure 2. Results comparison of 1 mL and 8 mL dispersive SPE with the modified method (w/ toluene) and the original method (w/o toluene)

Analytes	Original method (w/o toluene)		Modified method (w/ toluene)		Impact with modified method	Detection method
	Recovery	RSD (n=6)	Recovery	RSD (n=6)		
Carbendazim	38.9	14.6	98.5	2.5	Positive	LC/MS/MS
Thiabendazole	21.8	19.7	69.7	2.7	Positive	LC/MS/MS
Pymetrozine	27.6	21.2	65.2	3.7	Positive	LC/MS/MS
Cyprodinil	29.6	23.4	63.1	3.2	Positive	LC/MS/MS
Chlorthalonil	21.1	16.4	47.3	5.9	Positive	GC/MS
Coumaphos	30.1	24.0	87.9	6.1	Positive	GC/MS
Dichlorobenzophenone	53.7	4.5	77.7	6.1	Positive	GC/MS
Folpet	62.0	14.6	88.2	6.3	Positive	GC/MS
Dichlorvos	88.8	6.0	20.4	89.8	Greatly negative	GC/MS
o-Phenylphenol	88.6	4.6	73.7	7.4	Slightly negative	GC/MS
Diazinon	94.9	5.9	81.3	4.0	Slightly negative	GC/MS
Chlordane	103.9	4.5	101.3	4.5	None	GC/MS
Permethrin	81.4	7.2	83.3	5.1	None	GC/MS
Acephate	95.5	5.6	99.8	4.7	None	LC/MS/MS
Carbaryl	108.0	2.5	109.1	1.9	None	LC/MS/MS
Propoxur	97.0	3.19	6.7	2.5	None	LC/MS/MS

Table 1. The impact on certain pesticides by the modified dispersive SPE with addition of toluene

To review this Application Note in its entirety, please search for 5990-4247EN at www.agilent.com/chem

Other Food Methods



Determination of Quinolone Residues in Bovine Liver Using Agilent SampliQ QuEChERS Kit by LC/MS/MS (Publication 5990-4974EN)

Introduction

A method for the determination of 11 Quinolone antibiotics in bovine liver has been established:

- Analytes were extracted and cleaned up from bovine liver with Agilent SampliQ QuEChERS kits
- Extraction was performed using SampliQ EN extraction kits and 5%FA in Acetonitrile
- Clean up was performed using SampliQ dispersive SPE kits Part no. 5982-4921 (25 mg C18 and 150 mg MgSO₄)
- Extracted samples were then analyzed by LC/MS/MS
- Limits of Quantitation (LOQ) were 5.0 ng/g
- Calibration curves were linear over the range of 5.0 to 400 ng/g
- The sample pre-fortified recoveries were between 62.0% and 113.1% with RSD (n=6) values between 2.2% and 13.4%

Instrument conditions

HPLC conditions

Column:	Agilent ZORBAX Solvent Saver Plus Eclipse Plus Phenyl-Hexyl LC Column, 150 mm x 3.0 mm, 3.5 μm (Part No. 959963-312)		
Flow rate:	0.3 ml/min		
Column Temperature:	30° C		
Injection volume:	10 μl		
Mobile phase:	A) 5 mM ammonium acetate in H ₂ O, pH3.0, B) 1:1 methanol/acetonitrile.		
Post Time:	4min		
Gradient:	time (minutes)	% B	Flow Rate (mL/min)
	0	15	0.3
	0.2	15	0.3
	8.0	75	0.3
	9.0	100	0.3
	11.5	Stop	

MS conditions

Polarity:	Positive
Gas Temperature:	325° C
Gas Flow:	8 L/min
Nebulizer:	50 psi
Capillary :	4,000 V

QuEChERS extraction procedure

Weigh 2 g homogenized liver sample (±0.05g) in 50 mL centrifuge tube

Spike 100 μL of IS spike solution, 50 μL of QC spike solution if necessary vortex 30 s

Add 8 mL of 30 mM KH₂PO₄, pH 7.0 buffer, vortex

Add 10 mL of 5% FA in ACN, and shake vigorously for 30 s

Add SampliQ EN QuEChERS extraction kit, and shake vigorously for 1 min

Centrifuge at 4,000 rpm for 5 min

Transfer 1 mL of upper ACN layer to SampliQ QuEChERS dispersive SPE 2 mL tube

Vortex 1 min, centrifuge at 13,000 rpm for 3 min with micro-centrifuge

Transfer 800 μL extract to another tube, blow down at 40° C with N₂

Reconstitute into 800 μL 1:9 MeOH/H₂O w/ 0.1% FA, vortex and sonicate

Filter samples w/ 0.22 μm cellulose acetate spin filter

Sample are ready for LC/MS/MS analysis

Figure 1: Agilent's QuEChERS flow chart procedure for antibiotics

Results

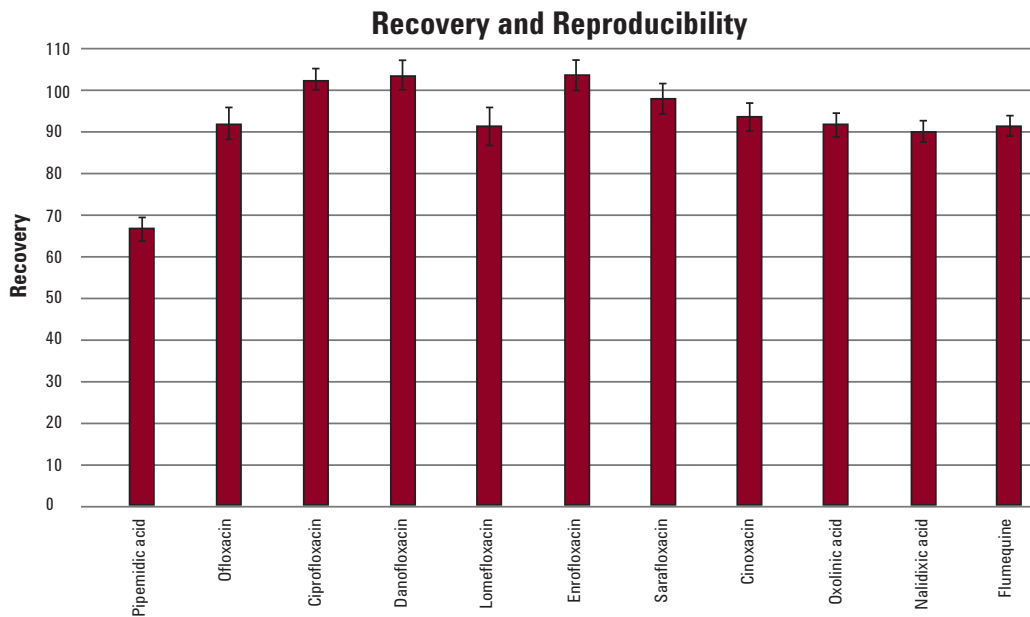


Figure 2: Recovery for 11 quinolone antibiotics in bovine liver

Ordering information

Agilent SampliQ QuEChERS EN Extraction Kit. Part No. 5982-5650.

Agilent SampliQ QuEChERS Dispersive SPE Kit. Part Nos. 5982-4921, 5982-4956.

Agilent ZORBAX Solvent Saver Plus Eclipse Plus Phenyl-Hexyl LC Column, 150 mm x 3.0 mm, 3.5 μ m. Part No. 959963-312.

Agilent Spin Filters, 0.22 μ m Cellulose Acetate. Part No. 5185-5990.

Determination of Sulfonamide Residues in Bovine Liver Using SampliQ QuEChERS EN Kit by LC/MS/MS (Publication 5990-4975EN)

Introduction

A method for the determination of 9 Sulfonamide antibiotics in bovine liver has been established:

- Analytes were extracted and cleaned up from bovine liver with Agilent SampliQ QuEChERS kits
- Extraction was performed using SampliQ EN extraction kits and 1% AA in Acetonitrile
- Clean up was performed using SampliQ EN fatty dispersive SPE kits, 6 mL (150 mg PSA, 150 mg C18 and 900 mg MgSO₄)
- Extracted samples were then analyzed by LC/MS/MS
- Limits of Quantitation (LOQ) were 2.0 ng/g
- Calibration curves were linear over the range of 2.0 to 400 ng/g
- The sample pre-fortified recoveries were between 53.0% and 92.8% with RSD (n=6) values between 2.1% and 16.8%

Instrument conditions

HPLC conditions

Column: Agilent ZORBAX Eclipse Rapid Resolution HT Plus C18 LC Column, 50 X 3.0 mm, 1.8 µm (Part No. 959941-302)

Flow rate: 0.3 ml/min

Column Temperature: 30° C

Injection volume: 10 µl

Mobile phase: A) 5 mM ammonium acetate in H₂O, pH 3.0,
B) 1:1 methanol/acetonitrile

Post Time: 3.5min

Gradient:	time (minutes)	% B	Flow Rate (mL/min)
	0	15	0.3
	0.2	15	0.3
	6.0	60	0.3
	6.01	100	0.3
	7.0	Stop	

MS conditions

Polarity: Positive

Gas Temperature: 325° C

Gas Flow: 8 L/min

Nebulizer: 50 psi

Capillary : 4,000 V

QuEChERS extraction procedure

Weigh 2 g homogenized liver sample (±0.05g) in 50 mL centrifuge tube

Spike 50 µL of IS spike solution, 50 µL of QC spike solution if necessary
vortex 30 s

Add 8 mL of water, vortex

Add 10 mL of 1% AA in ACN, and shake vigorously for 30 s

Add SampliQ EN QuEChERS extraction kit, and shake vigorously for 1 min

Centrifuge at 4,000 rpm for 5 min

Transfer 6 mL of upper ACN layer to SampliQ EN QuEChERS fatty dispersive SPE 2 mL tube

Vortex 2 min, centrifuge at 4,000 rpm for 5 min

Transfer 4 mL extract to another tube, blow down at 40° C with N₂

Reconstitute into 800 µL 1:9 MeOH/H₂O w/ 0.1% FA, vortex and sonicate

Filter samples w/ 0.22 µm cellulose acetate spin filter

Sample are ready for LC/MS/MS analysis

Figure 1: Flow chart for Agilent's QuEChERS procedure

Ordering information

Agilent SampliQ QuEChERS EN Extraction Kit. Part No. 5982-5650.

Agilent SampliQ QuEChERS EN Fatty Dispersive SPE Kit. Part No. 5982-5156.

Agilent ZORBAX Eclipse Rapid Resolution HT Plus C18 LC Column, 50 X 3.0 mm, 1.8 µm. Part No. 959941-302.

Agilent Spin Filters, 0.22 µm Cellulose Acetate. Part No. 5185-5990.

Results

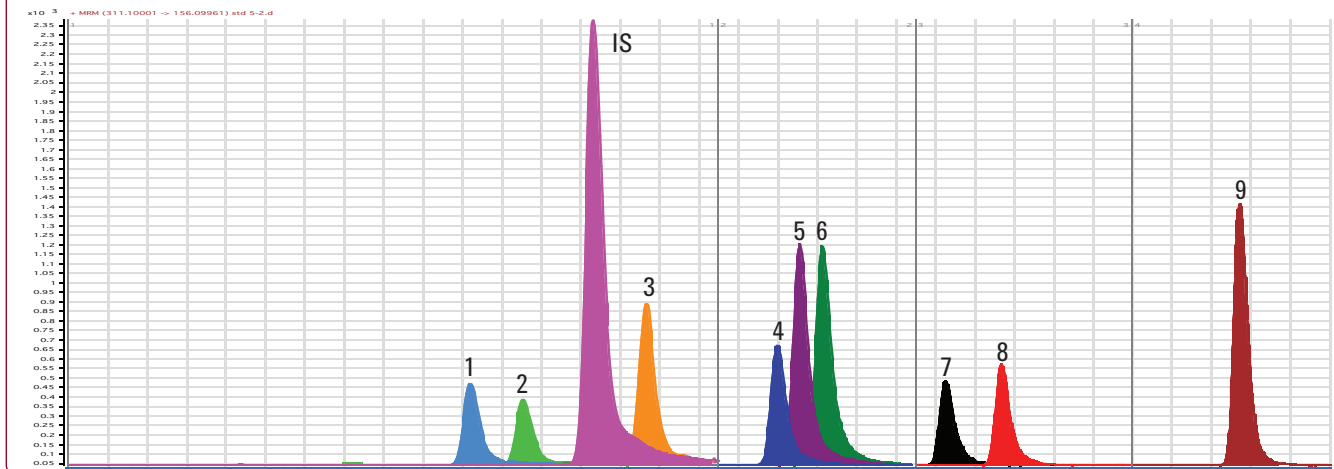


Figure 2. LC/MS/MS Chromatogram of 100 ng/g fortified liver extract. Peaks identification: 1. Sulfadizine, 2. Sulfathiazole, 3. Sulfamerazine, 4. Sulfamethizole, 5. Sulfamethazine, 6. Sulfamethoxyipyridazine, 7. Sulfachloropyridazine, 8. Sulfamethoxazole, 9. Sulfadimethoxin, IS (internal standard)

Compound	Low QC (5 ng/g)		Mid QC (100 ng/g)		High QC (400 ng/g)	
	Recovery	RSD	Recovery	RSD	Recovery	RSD
Sulfadizine	73.9	15.6	90.0	13.7	81.9	5.3
Sulfathiazole	62.9	16.8	75.3	8.4	67.9	5.8
Sulfamerazine	77.6	11.5	92.8	6.6	82.0	4.2
Sulfamethizole	62.8	4.7	60.7	6.5	53.0	2.1
Sulfamethazine	87.4	6.9	90.0	10.7	83.4	3.4
Sulfamethoxyipyridazine	81.8	9.4	84.8	8.1	76.4	2.9
Sulfachloropyridazine	84.2	10.0	78.6	6.3	73.8	3.6
Sulfamethoxazole	85.9	7.6	82.3	5.9	78.1	3.3
Sulfadimethoxin	77.8	8.4	80.9	4.9	75.6	3.3

Table 1. Quantitation results – recovery and reproducibility (n=6)

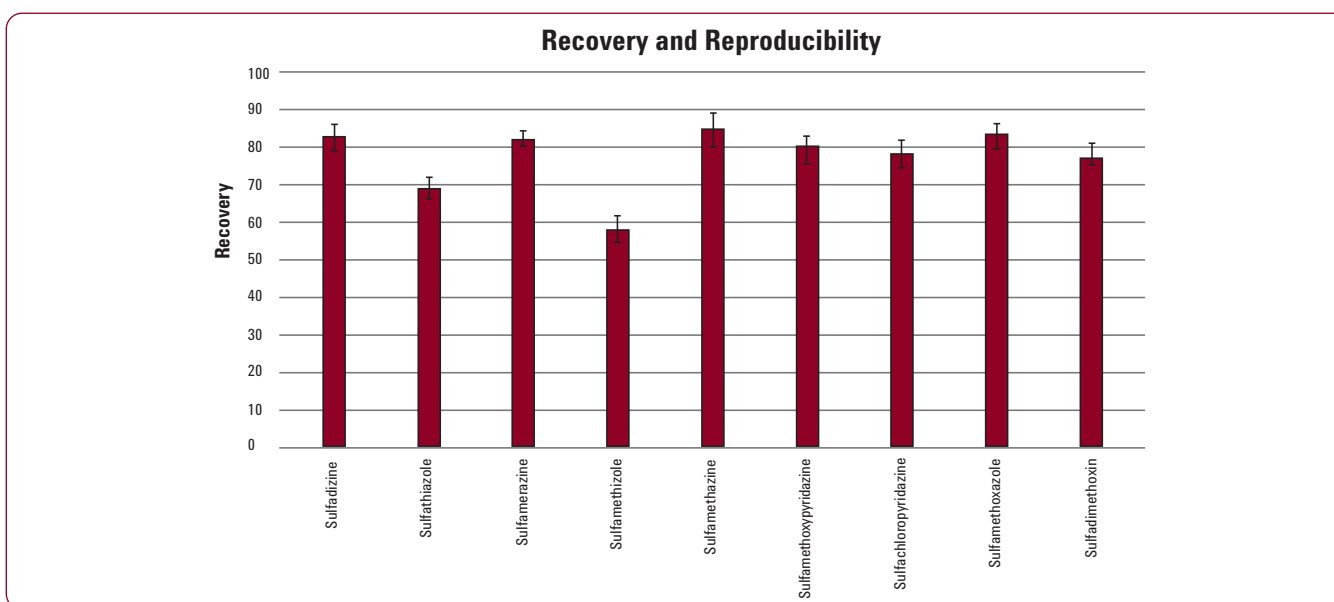


Figure 3. Recovery and reproducibility for 9 sulfonamides in bovine liver

To review this Application Note in its entirety, please search for 5990-4975EN at www.agilent.com/chem

Agilent SampliQ SPE – A full line of sample preparation products to support your lab



The QuEChERS kits featured in this brochure are part of the Agilent SampliQ family of SPE products. Manufactured in the US to strict ISO-9001 standards – the same process Agilent uses for its world-famous ZORBAX HPLC column packing material – Agilent SampliQ SPE products deliver the high quality and performance you expect from the industry's leading manufacturer of chromatography instruments, columns and supplies.

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