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Agilent Bond Elut QuEChERS Food Safety
Applications Notebook: Volume 2

PROVEN APPROACHES FOR TODAY'S FOOD ANALYSIS CHALLENGES

The Measure of Confidence




Agilent Technologies

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Food Safety Overview

Reliable food safety testing begins with reliable sample preparation

Dear Valued Customer,

Today's consumers demand foods and beverages that are safe, high-quality, and nutritious. Your food laboratory's work lays the foundation for meeting that demand.

Agilent's comprehensive analytical solutions deliver on that promise

From inspection and product development to quality assurance and packaging, Agilent instruments, systems, columns, and supplies help your lab meet the toughest standards. In-depth experience, broad knowledge, and creative people, along with our insight into industry trends and global regulations, address your challenges.

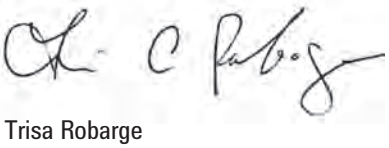
Agilent Bond Elut QuEChERS and SPE Sample Preparation products: your first step in food safety analysis

Agilent Bond Elut Sample Preparation products help you confidently extract and concentrate samples from complex matrixes, to deliver fast, accurate, and reproducible results.

The Bond Elut family of products supports your food sample preparation needs with a broad set of formats and products manufactured to strict quality standards:

- **Bond Elut QuEChERS Pre-Packed Extraction and Dispersive SPE Kits** enable preparation of food samples for multi-residue, multi-class organic contaminant analysis in just a few simple steps, with consistent quality and high performance built into the kits.
- **The Bond Elut SPE product range**, comprising over 40 different polymeric and silica-based functionalities, comes in a variety of cartridge and plate formats.
- **Bulk sorbents** support method development and lab-level customization of QuEChERS sample prep approaches.

Our team of scientists continues to support your food testing needs, so be sure to check our QuEChERS page for new applications and product developments at **agilent.com/chem/QuEChERS**. Accuracy starts here.



Trisa Robarge
Sample Preparation Product Manager

What is QuEChERS?

Developed by United States Department of Agriculture in 2003, QuEChERS (pronounced "catchers") stands for **Qu**ick, **E**asy, **Ch**eap, **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 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.

Tips and Tricks

To support the wide range of QuEChERS product options, we offer a number of applications featuring the QuEChERS approach. This guide includes many of these applications. You'll find the applications grouped according to the standard method associated with the application, such as AOAC, EN, or the original method, along with a section for other approaches. A handy index also lets you search this guide based on matrix type and analyte class.

QuEChERS Extraction Kits

Step 1: Extraction

Choose the extraction salt packet based on your method of analysis, AOAC or EN. The buffered extraction salts are amenable for more labile pesticides. Adding solvent and then salts to a homogenized fruit or vegetable sample (10 g or 15 g) enables you to extract the pesticides of interest into the organic layer. Agilent pre-packages its QuEChERS salts and buffers in anhydrous packages. This allows you to add them after adding your solvent to the sample, as specified in QuEChERS methodologies.

In the table below, the "CH" products contain the appropriately-sized ceramic homogenizers for those particular kits. For more information on ceramic homogenizers see page 13.

QuEChERS extraction kits are available with or without 50 mL centrifuge tubes and caps and includes $MgSO_4$, NcCl, or other salts for buffering; pre-weighed in anhydrous packets.

Ceramic homogenizers cut extraction time by up to 70%. They are available with QuEChERS kits or separately.

QuEChERS Extraction Kits

Method	Buffered	Contents	Ceramic Homogenizers	with 50 mL Tubes 50/pk	Packets Only 50/pk	Packets Only 200/pk
AOAC 2007.01	Yes	6 g $MgSO_4$; 1.5 g NaAcetate	Yes	5982-5755CH		
			No	5982-5755	5982-6755	5982-7755
Original (10 g samples)	No	4 g $MgSO_4$; 1 g NaCl	Yes	5982-5550CH		
			No	5982-5550	5982-6550	5982-7550
Original (15 g samples)	No	6 g $MgSO_4$; 1.5 g NaCl	Yes	5982-5555CH		
			No	5982-5555	5982-6555	5982-7555
EN 15662	Yes	4 g $MgSO_4$; 1 g NaCl; 1 g NaCitrate; 0.5 g disodium citrate sesquihydrate	Yes	5982-5650CH		
			No	5982-5650	5982-6650	5982-7650
Acrylamides*	No	4 g $MgSO_4$; 0.5 g NaCl	No	5982-5850		
Veterinary Drugs	No	4g $Na2SO_4$, 1 g NaCl	No	5982-0032		

** Procedure from K. Mastovska and S. J. Lehotay, J. Agric. Food Chem. 2006, 54, 7001-7008.*

QuEChERS Dispersive Kits

Step 2: Dispersive SPE Cleanup

Select the Dispersive SPE kit suited to the type of food being analyzed and the method you are following. In this step, an aliquot of the sample extract from Step One is added to a 2 mL or 15 mL centrifuge tube containing a small amount of SPE sorbent and $MgSO_4$. The sorbent will pull out interfering matrix materials from the sample, while the $MgSO_4$ helps remove excess water and improve analyte partitioning. Select kits are now available with ceramic homogenizers (two per tube). Their part numbers are designated by a CH.

QuEChERS Dispersive Kits, Fruits and Vegetables

			AOAC 2007.01 Method	European Method EN 15662
Kit	**Size**	**Unit**	**Kit Contents Part No.**	**Kit Contents Part No.**
General fruits and vegetables: Removes polar organic acids, some sugars and lipids	2 mL	100/pk	50 mg PSA 150 mg $MgSO_4$ 5982-5022 5982-5022CH	25 mg PSA 150 mg $MgSO_4$ 5982-5021 5982-5021CH
	15 mL	50/pk	400 mg PSA 1200 mg $MgSO_4$ 5982-5058 5982-5058CH	150 mg PSA 900 mg $MgSO_4$ 5982-5056 5982-5056CH
Fruits and vegetables with fats and waxes: Removes polar organic acids, some sugars, more lipids and sterols	2 mL	100/pk	50 mg PSA 50 mg C18EC 150 mg $MgSO_4$ 5982-5122 5982-5122CH	25 mg PSA 25 mg C18EC 150 mg $MgSO_4$ 5982-5121 5982-5121CH
	15 mL	50/pk	400 mg PSA 400 mg C18EC 1200 mg $MgSO_4$ 5982-5158 5982-5158CH	150 mg PSA 150 mg C18EC 900 mg $MgSO_4$ 5982-5156 5982-5156CH

Part numbers ending in CH indicate tubes containing ceramic homogenizers.

(Continued)

QuEChERS Dispersive Kits, Fruits and Vegetables

Kit	Size	Unit	AOAC 2007.01 Method Kit Contents Part No.	European Method EN 15662 Kit Contents Part No.
Pigmented fruits and vegetables: Removes polar organic acids, some sugars and lipids, and carotenoids and chlorophyll; not for use with planar pesticides	2 mL	100/pk	50 mg PSA 50 mg GCB 150 mg $MgSO_4$ 5982-5222 5982-5222CH	25 mg PSA 2.5 mg GCB 150 mg $MgSO_4$ 5982-5221 5982-5221CH
	15 mL	50/pk	400 mg PSA 400 mg GCB 1200 mg $MgSO_4$ 5982-5258 5982-5258CH	150 mg PSA 15 mg GCB 885 mg $MgSO_4$ 5982-5256 5982-5256CH
Highly pigmented fruits and vegetables: Removes polar organic acids, some sugars and lipids, plus high levels of carotenoids and chlorophyll; not for use with planar pesticides	2 mL	100/pk		25 mg PSA 7.5 mg GCB 150 mg $MgSO_4$ 5982-5321 5982-5321CH
	15 mL	50/pk		150 mg PSA 45 mg GCB 855 mg $MgSO_4$ 5982-5356 5982-5356CH
Fruits and vegetables with pigments and fats: Removes polar organic acids, some sugars and lipids, plus carotenoids and chlorophyll; not for use with planar pesticides	2 mL	100/pk	50 mg PSA 50 mg GCB 150 mg $MgSO_4$ 50 mg C18EC 5982-5421 5982-5421CH	
	15 mL	50/pk	400 mg PSA 400 mg GCB 1200 mg $MgSO_4$ 400 mg C18EC 5982-5456 5982-5456CH	

Part numbers ending in CH indicate tubes containing ceramic homogenizers.

QuEChERS Dispersive Kits: Other Food Methods

Kit	Size	Unit	AOAC 007.01 Method Kit Contents Part No.	European Method EN 15662 Kit Contents Part No.
Other Food Methods: Removes biological matrix interferences, including hydrophobic substances (fats, lipids) and proteins	2 mL	100/pk	25 mg C18 150 mg $MgSO_4$ 5982-4921 5982-4921CH	
	15 mL	50/pk	150 mg C18 900 mg $MgSO_4$ 5982-4956 5982-4956CH	
All Food Types: Removes all matrix interfering materials including polar organic acids, lipids, sugars, proteins, carotenoids and chlorophyll	2 mL	100/pk	50 mg PSA 50 mg C18 7.5 mg GCB 150 mg $MgSO_4$ 5982-0028 5982-0028CH	
	15 mL	50/pk	400 mg PSA 400 mg C18 45 mg GCB 1200 $MgSO_4$ 5982-0029 5982-0029CH	
Animal Origin Food: Removes matrix interferences such as polar organic salts, sugars, lipids and proteins	15 mL	50/pk	50 mg PSA 150 mg C18EC 900 mg Na_2SO_4 5982-4950	

Part numbers ending in CH indicate tubes containing ceramic homogenizers.

Suggested Bond Elut QuEChERS Dispersive Kit by Food Type and Method

Group commodity	Commodity	General fruits and vegetables; EN or AOAC	Fruits and vegetables with fats and waxes; EN or AOAC	Pigmented fruits and vegetables; EN or AOAC	Highly pigmented fruits and vegetables; EN	Fruits and vegetables with pigment and fats; AOAC only
Use with		Lightly colored samples	Samples containing > 1% fat/lipids	Colored samples (chlorophyll, carotinoids), no planar pesticides	Highly colored samples (chlorophyll, carotinoids), no planar pesticides	Colored samples that also contain fats or waxes
Fruits						
 Citrus fruits	citrus juices					
	grapefruit					
	lemon/lime					
	orange					
	orange peel					
	nectarine					
	tangerine					
 Pome fruits	apple					
	apple, dried					
	apple juice					
	apple sauce					
	pear					
	quince					
 Stone fruits	apricot					
	apricot, dried					
	apricot nectar					
	cherry					
	mirabelle					
	nectarine					
	peach					
	peach, dried					
	plum					
	plum, dried					
 Soft and small fruits	blackberry					
	blueberry					
	cranberry					
	currant					
	elderberry					
	gooseberry, red					
	grapes, green					
	grapes, red					
	raisin					
	raspberry					
	strawberry					
 Other fruits	avocado					
	banana					
	fig, dried					
	kiwi					
	mango					
	melon					
	olives					
	papaya					
	pineapple					

(Continued)

Suggested Bond Elut QuEChERS Dispersive Kit by Food Type and Method

Group commodity	Commodity	General fruits and vegetables; EN or AOAC	Fruits and vegetables with fats and waxes; EN or AOAC	Pigmented fruits and vegetables; EN or AOAC	Highly pigmented fruits and vegetables; EN	Fruits and vegetables with pigment and fats; AOAC only
Use with		Lightly colored samples	Samples containing > 1% fat/lipids	Colored samples (chlorophyll, carotinoids), no planar pesticides	Highly colored samples (chlorophyll, carotinoids), no planar pesticides	Colored samples that also contain fats or waxes
Vegetables						
 Root and tuber vegetables	beets					
	black salsify					
	carrot					
	celeriac					
	horseradish					
	parsley root					
	potato					
	radish					
 Leek plants	chive					
	garlic					
	leek					
	onion					
	scallion					
	shallot					
 Fruiting vegetables	aubergine/eggplant					
	cucumber					
	pepper, sweet green					
	pepper, sweet red					
	pumpkin					
	tomato					
	zucchini (courgette)					
 Broccoli	broccoli					
	brussel sprouts					
	cabbage, chinese					
	cabbage, red					
	cabbage, savoy					
	cabbage, white					
	cauliflower					
	kale					
	kohlrabi					
 Leafy vegetables and herbs	arugula, rucola					
	basil					
	cilantro					
	cress					
	endive					
	lamb's lettuce					
	lettuce varieties					
	parsley					
	spinach					
 Stem vegetables	artichokes					
	asparagus					
	celery					
	leek					
	rhubarb					
 Legumes	beans, lentils, peas, (dried)					
	beans, lentils, peas, (fresh)					

(Continued)

Suggested Bond Elut QuEChERS Dispersive Kit by Food Type and Method

Group commodity	Commodity	General fruits and vegetables; EN or AOAC	Fruits and vegetables with fats and waxes; EN or AOAC	Pigmented fruits and vegetables; EN or AOAC	Highly pigmented fruits and vegetables; EN	Fruits and vegetables with pigment and fats; AOAC only
Use with		Lightly colored samples	Samples containing > 1% fat/lipids	Colored samples (chlorophyll, carotinoids), no planar pesticides	Highly colored samples (chlorophyll, carotinoids), no planar pesticides	Colored samples that also contain fats or waxes
Animal-Sourced Foods						
 Meats	beef, chicken, pork, veal					
	kidney, liver					
 Seafood	bivalve, shellfish					
	finfish					
 Dairy	dairy					
Other Foods						
 Cereals	corn, rice, wheat					
	flour, grain, etc.					
 Tea/coffee	coffee beans					
	tea leaves					
 Dried spices	curry, peppers					
	leek plants					
	peppercorn seeds					
 Oils	canola, olive					
	citrus					
 Baby food	baby food					
Other						
 Agricultural products	cocoa solids					
	cotton, hemp					
	tobacco					
 Soil	soil					
 Whole blood	whole blood					

QuEChERS Ceramic Homogenizers

Ceramic homogenizers increase your overall lab productivity and give you greater confidence in your results. Make analyte extraction easier with these benefits:

- Cut the required extraction time from 60 seconds to as little as 20 seconds – a time savings of 70% per sample
- Maintain high, reproducible extractions in one-third of the time
- Minimize variation between technicians
- Break up salt agglomerates and maintain a consistent grinding of homogenized material

The same ceramic homogenizers available in our QuEChERS kits are also available for bulk purchase, providing excellent grinding capabilities of your samples.

QuEChERS ceramic homogenizers

Description	Unit	Part No.
Ceramic homogenizer for 50 mL tubes	100/pk	5982-9313
Ceramic homogenizer for 15 mL tubes	100/pk	5982-9312
Ceramic homogenizer for 2 mL tubes	200/pk	5982-9311

Standards for QuEChERS Products

In addition to our industry-leading QuEChERS kits, Agilent makes your analysis easier by providing standards for the most commonly used regulatory methods, including AOAC and EN.

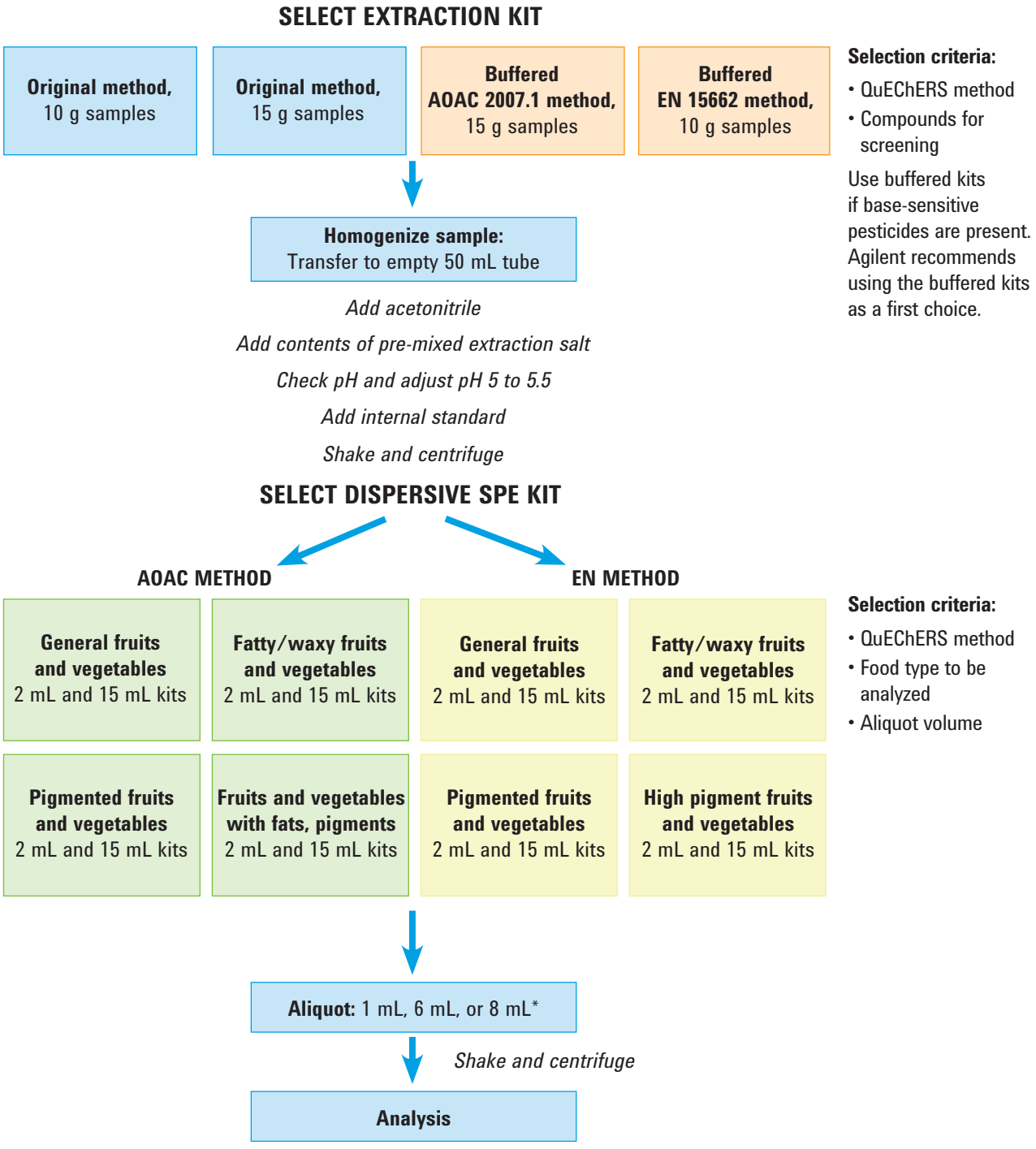
- Save time and avoid inconvenience of making standards
- Available for GC and LC instruments
- Ready to use for QuEChERS extractions – no dilutions required

QuEChERS Standards

Description	Concentration	Kit Contents	Part No.
HPLC and GC Internal Standard, AOAC Method	1,000 µg/mL	Parathion-d10 (diethyl-d10), alpha-BHC-d6 (alpha-HCH-d6)	5190-0502
QC Solution, AOAC Method	500 µg/mL	Triphenyl phosphate	5190-0503
HPLC Internal Standard, EN Method	100 µg/mL	Tris (1,3-dichloroisopropyl) phosphate, nicarbazin	5190-0500
GC Internal Standard, EN Method	5,000 µg/mL	(2,2'5,5'-tetrachlorobiphenyl), triphenylmethane, tris (1,3-dichloroisopropyl) phosphate	5190-0501
QC Surrogate for GC Standard, EN Method	500 µg/mL 1,000 µg/mL	(2,2',3,4,4',5'-hexachlorobiphenyl) Anthracene-d10	5190-0499

Agilent Bond Elut Recommended Standard Operating Procedure for QuEChERS

Here are the basic steps to complete the QuEChERS method on any fruit or vegetable sample. In just a few easy steps, you'll prepare your sample for multi-class, multi-residue pesticide analysis.



*Aliquot size is specified by the method, and kits are created for these specific amounts. For pesticides with acidic groups (phenoxyalcanoic acids), analyze directly by LC/MS/MS at this point (skip the dispersive SPE stage). These acidic groups interact with the PSA that is part of the dispersive SPE step.

Original QuEChERS Method

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

Introduction

This application describes the use of QuEChERS, an effective 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

GC/MS conditions

Column:	Agilent J&W HP-5ms Ultra Inert, 30 m x 0.25 mm, 0.25 µm (Part No. 190915-433UI)
Injection source:	Manual
Inlet:	Splitless
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 min
Multiplier voltage:	Autotune voltage

QuEChERS Procedure

QuEChERS Extraction Procedure (Step 1)

10 g homogenized apple in 50 mL centrifuge tube.

Spike sample and vortex for 1 min.

Add 10 mL ACN.

4g $MgSO_4$ and 1g NaCl (Part No. 5982-5550).

50 µL internal standard (TPP).

Vortex for 30 sec.

Centrifuge 5 min 5,000 rpm.

QuEChERS Dispersive SPE (Step 2) and Analysis

1 mL upper layer in 1.5 mL centrifuge tube with 50 mg PSA and 150 mg $MgSO_4$ (Part No. 5982-5022).

Vortex for 30 sec.

Centrifuge for 5 min.

0.5 mL into sample vial.

Inject 1.5 µL into GC/MS.

Figure 1. QuEChERS extraction procedure for general fruits and vegetables.

Products used in the above application

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

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

Agilent Bond Elut 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, 0.25 µm. Part No. 190915-433UI.

Results

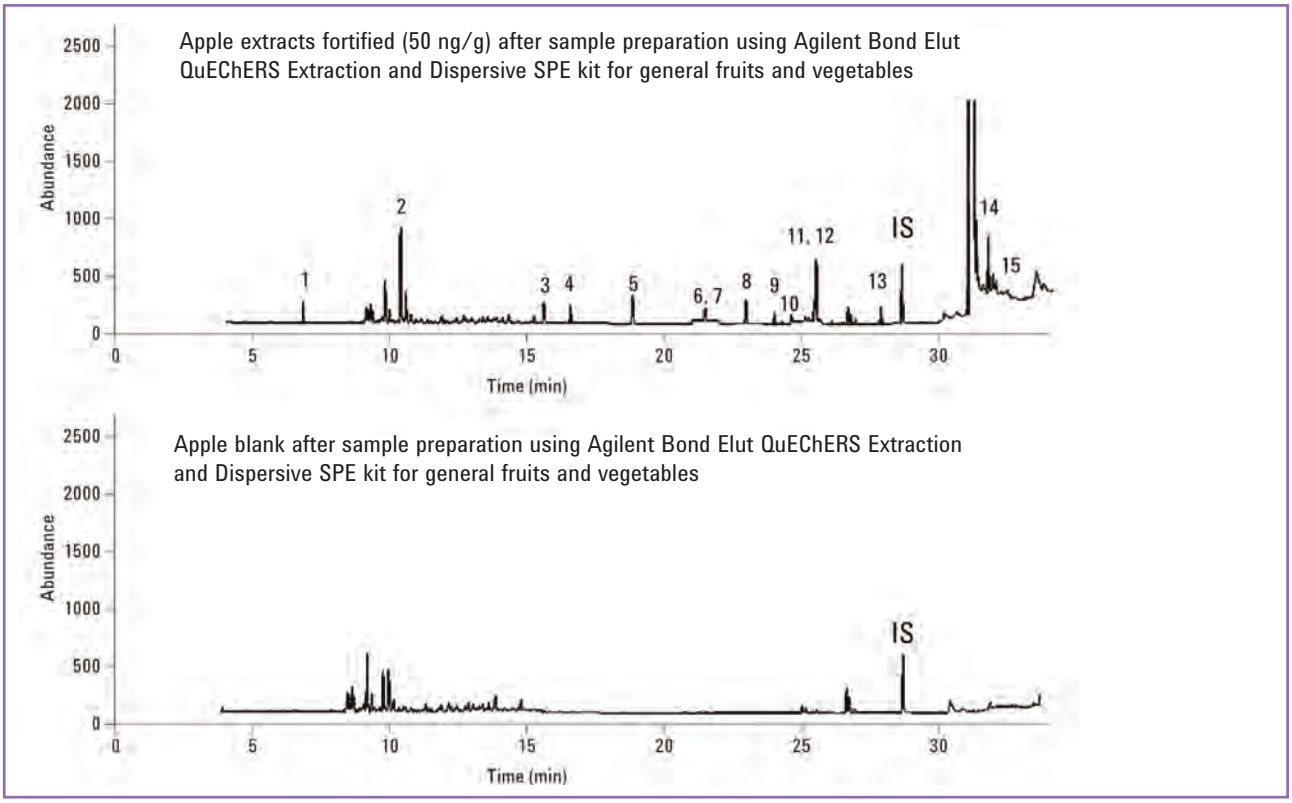


Figure 2. Comparison of blank apple extract to a fortified apple extract.

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

	Low-QC 10 ng/g		Mid-QC 50 ng/g		High-QC 200 ng/g	
Pesticide	Recovery	RSD	Recovery	RSD	Recovery	RSD
Dichlorvos	102.8	5.0	96.7	10.8	99.4	2.8
o-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

EN Methods

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

Introduction

This application describes the use of QuEChERS, an effective 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, 3.0 x 150 mm, 3.5 µm (Part No. 959963-312)
Flow rate:	0.3 or 0.5 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_2O B: 5 mM Ammonium acetate, pH 5.0 in ACN
Needle wash:	1:1:1:1 ACN:MeOH:IPA:H_2O (0.2% FA.)

Gradient:

Time (min)	% 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

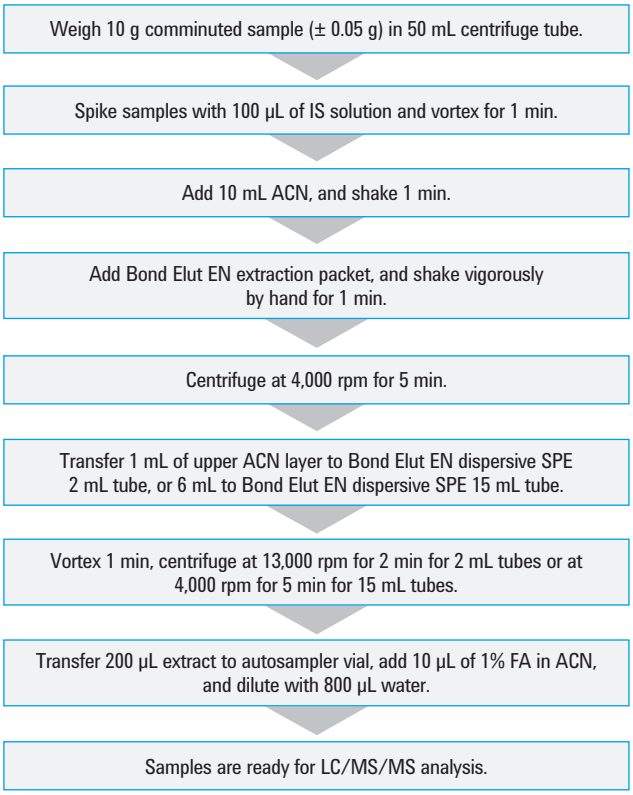


Figure 1. QuEChERS EN sample preparation procedure for pesticides in apple.

Products used in the above application

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

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

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

Results

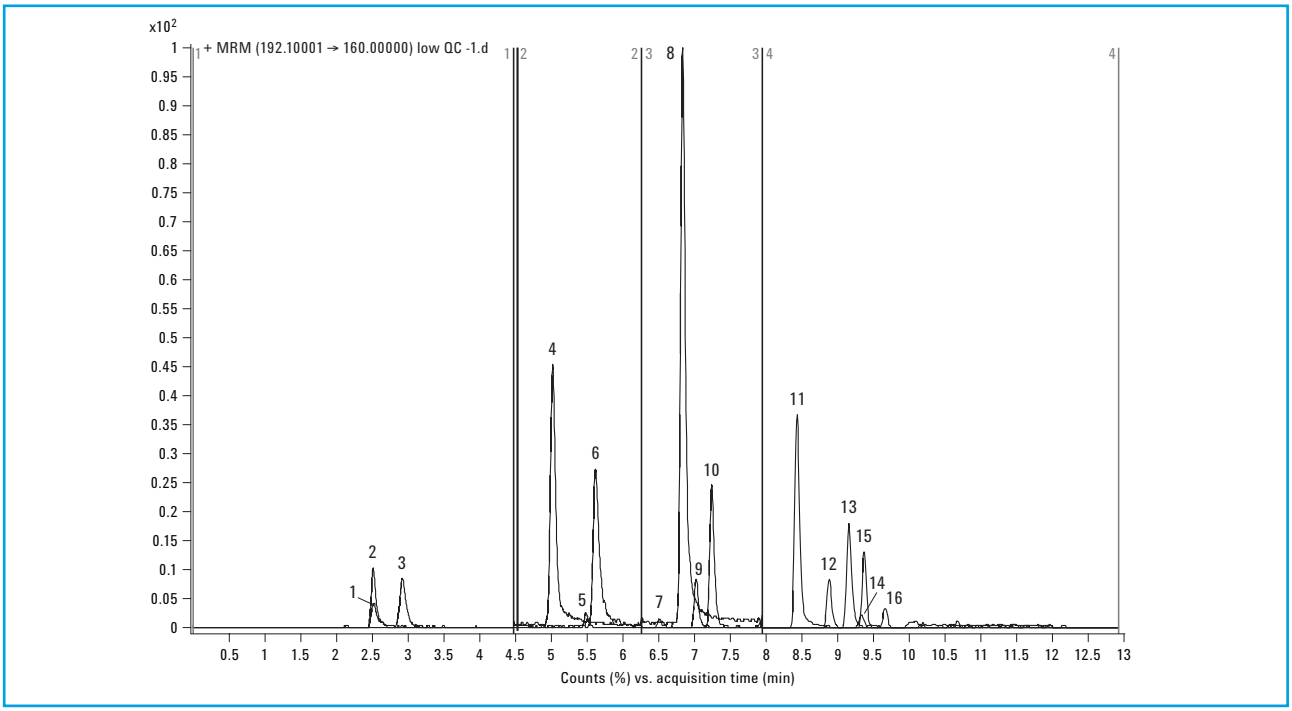


Figure 2. Chromatogram of 10 ng/g fortified apple extract. Peak identification: 1. Methamidophos, 2. Acephate, 3. Pymetrozine, 4. Carbendazim, 5. Imidacloprid, 6. Thiabendazole, 7. Dichlorvos, 8. Propoxur, 9. Thiophanate methyl, 10. Carbaryl, 11. Ethoprophos, 12. Penconazole, 13. Cyprodinil, 14. Dichlofluanid,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

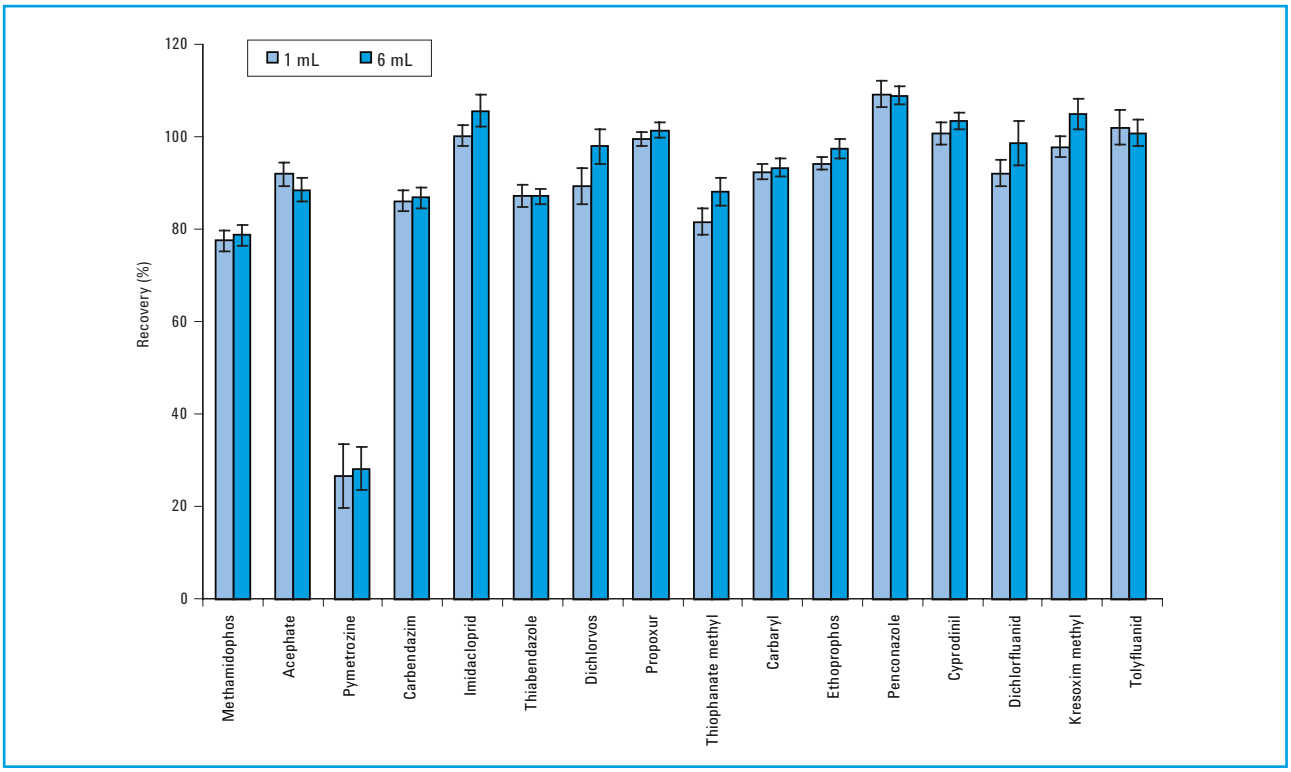


Figure 3. Results comparison of 1 mL dispersive SPE and 6 mL dispersive SPE.

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

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

Introduction

This application describes the use of QuEChERS, an effective 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

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

QuEChERS Procedure

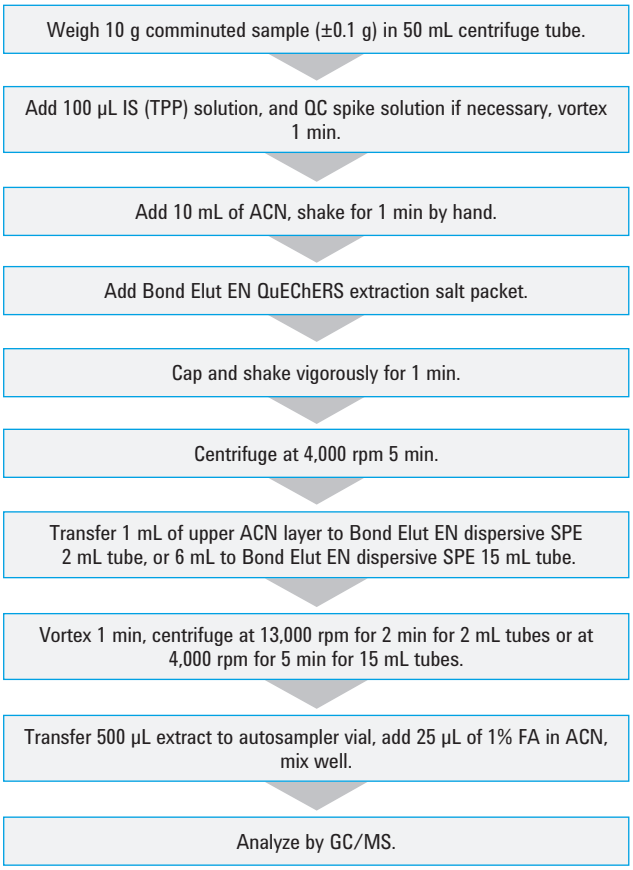


Figure 1. Agilent Bond Elut QuEChERS EN extraction procedure for pesticides in apple.

Results

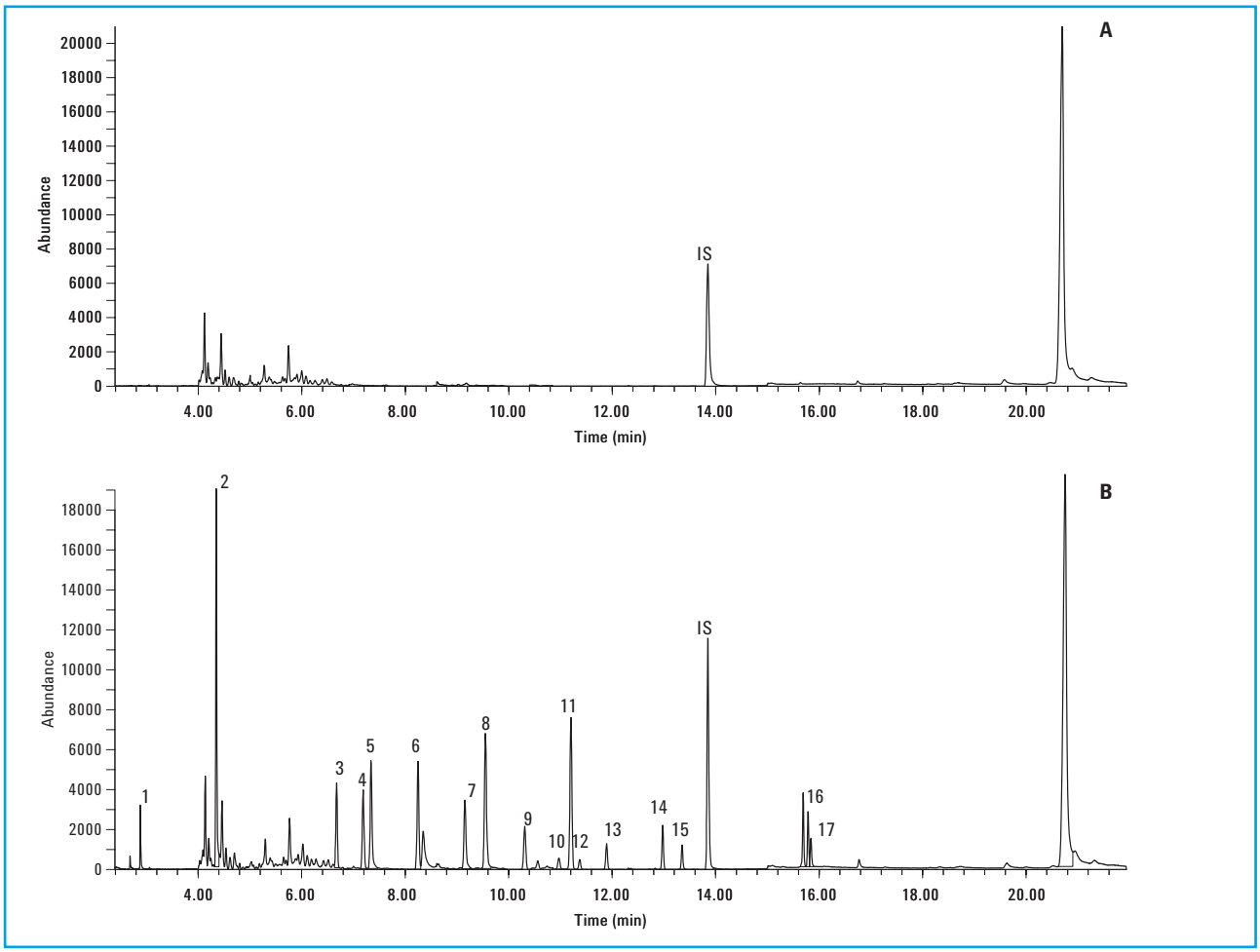


Figure 2. GC/MS chromatogram of apple extract. (A) apple extract blank; (B) 50 ng/g fortified apple extract. Peak Identification: 1. Dichlorvos, 2. o-Phenylphenol, 3. Lindane, 4. Diazinon, 5. Chlorothalonil, 6. Chlorpyrifos-methyl, 7. Dichlofluanid, 8. Dichlorobenzophenone, 9. Heptachlor epoxide, 10.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

Products used in the above application

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

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

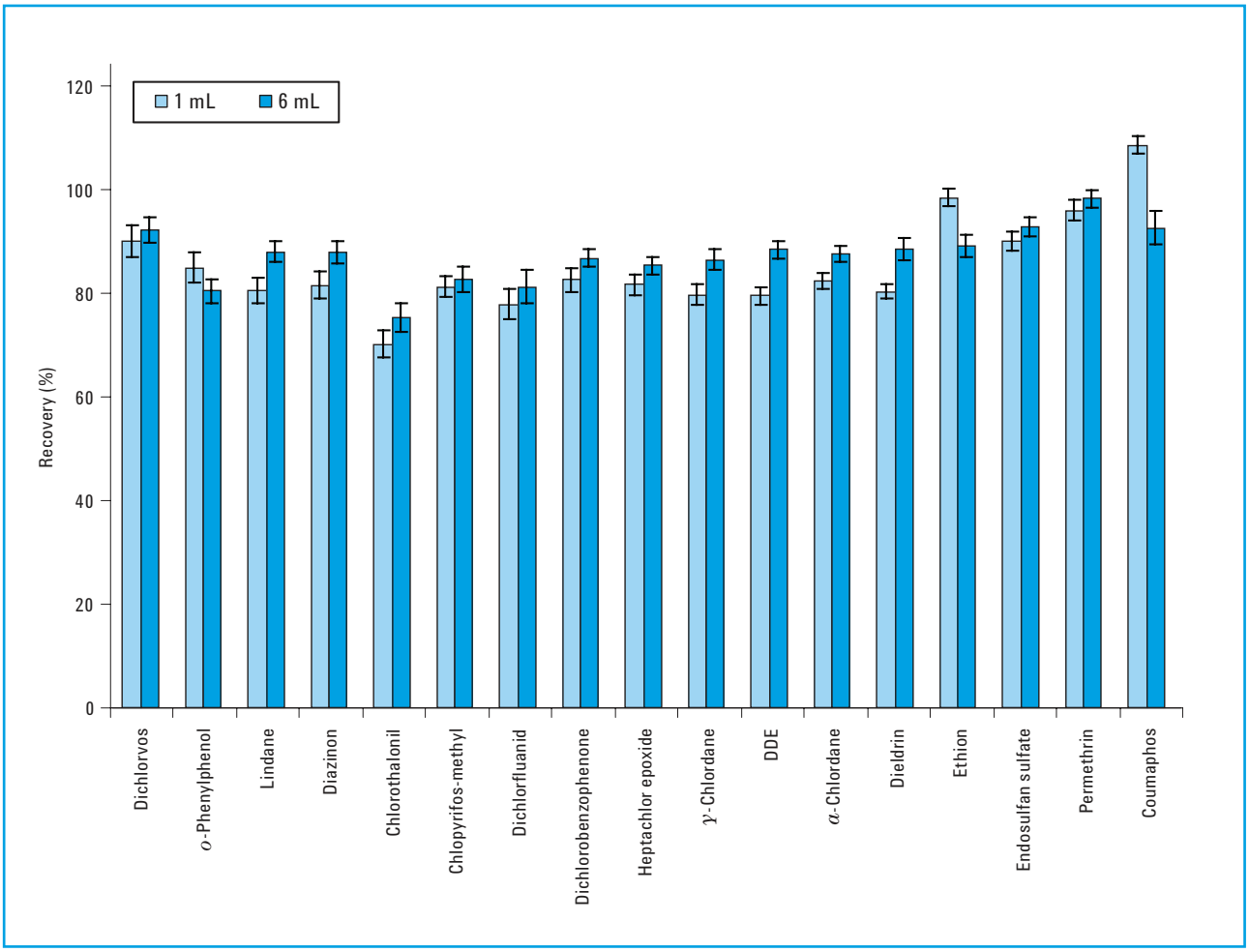


Figure 3. Recovery and precision results of 1 and 6 mL sample volumes employing Agilent Bond Elut Dispersive SPE, 2 and 15 mL kits, respectively.

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

Introduction

This application describes the use of the 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 pectrometry (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, 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_2O B: 5 mM Ammonium acetate, pH 5.0 in ACN
Needle wash:	1:1:1:1 ACN:MeOH:IPA:H_2O w/0.2% FA

Gradient:

Time (min)	% 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 temperature:	350 °C
Gas flow:	10 L/min
Nebulizer:	40 psi
Capillary:	4,000 V

QuEChERS Procedure

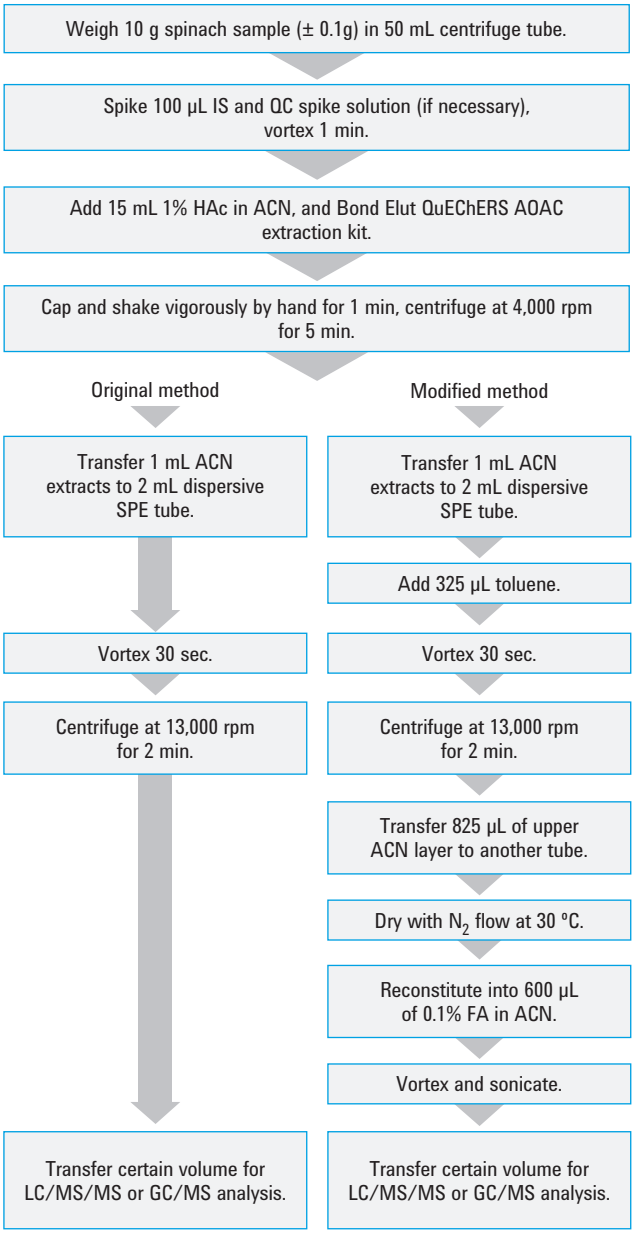


Figure 1. QuEChERS EN procedure (original and modified dispersive SPE, 2 mL size) for pesticide residues in spinach.

Results

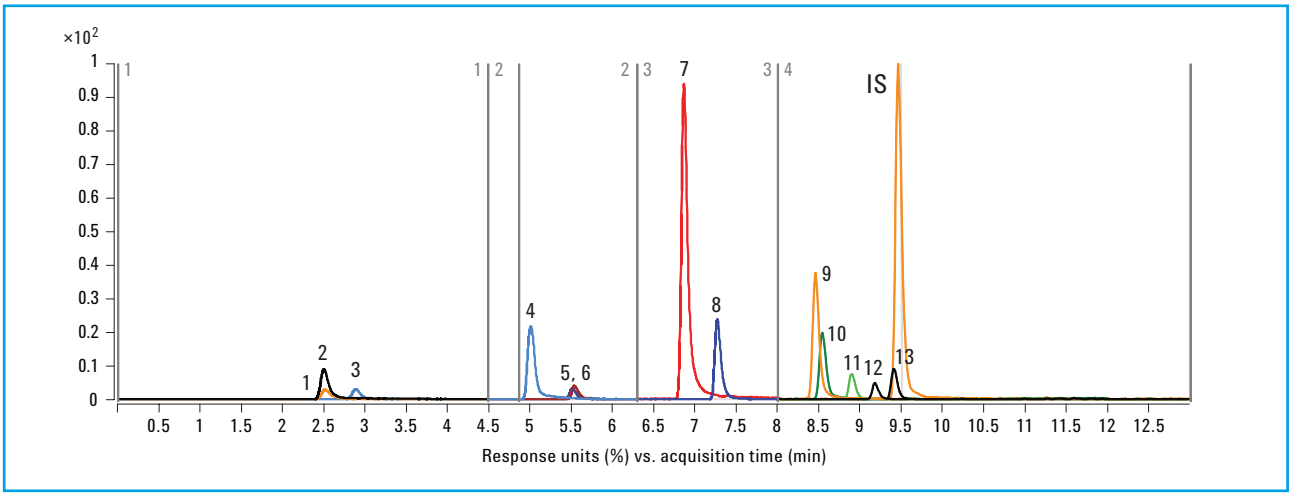


Figure 2. MRM chromatogram of 50 ng/g fortified sample of spinach processed by EN method. Peak identification: 1. Methamidophos, 2. Acephate, 3. Pymetrozine, 4. Carbendazim, 5. Imidacloprid 6. Thiabendazole, 7. Propoxur, 8. Carbaryl, 9. Ethoprophos, 10. Imazalil, 11. Penconazole, 12. Cyprodinil, 13.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

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

	10 ng/g fortified QC		50 ng/g fortified QC		200 ng/g fortified QC	
Analytes	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.

Products used in the above application

Agilent Bond Elut QuEChERS EN Extraction Kits. Part No. 5982-5650.

Agilent Bond Elut QuEChERS AOAC Dispersive SPE Kits for Pigmented Fruits and Vegetables. Part Nos. 5982-5321 and 5982-5356.

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 **agilent.com/chem**

Determination of Acrylamide in Cooking Oil by Agilent Bond Elut QuEChERS Acrylamide Kit and HPLC-DAD (Publication 5990-5988EN)

Introduction

This application describes the use of the QuEChERS sample preparation approach for the analysis of acrylamide in cooking oil, employing methacrylamide as the internal standard. Acrylamide occurs naturally as a byproduct of the cooking process and in heat-treated foods. The acrylamide recoveries ranged from 84 to 93.8%.

The analysis was performed on an Agilent 1200 Infinity Series equipped with a binary pump and a diode array detector (DAD) set at 210 nm.

Instrument conditions

HPLC conditions

Column:	Agilent ZORBAX HILIC Plus, 4.6 × 50 mm, 3.5 µm
Flow rate:	0.2 mL/min
Column temperature:	30 °C
Injection volume:	5 µL
Mobile phase:	Isocratic elution: A: 3% 5 mM Acetic acid B: 97% Acetonitrile
Run time:	10 min
Post time:	3 min
Detection:	DAD at 210 nm

QuEChERS Procedure

Weigh 1 g oil sample into a 50 mL centrifuge tube.

Spike sample with 1,000 µL 20 ng/mL IS, 1,000 µg/mL spiking solution. Shake vigorously 1 min.

Add 5 mL n-hexane. Shake vigorously 1 min.

Add 9 mL water and 10 mL ACN. Shake vigorously 1 min.

Add Bond Elut QuEChERS salt packet for acrylamide extraction. Shake 1 min, centrifuge at 4,000 rpm 5 min.

Discard the upper hexane layer.

Transfer 6 mL aliquot of CH_3CN layer to Bond Elut QuEChERS dispersive SPE 15 mL tube (containing PSA, C18EC and $MgSO_4$). Shake 1 min, centrifuge at 4,000 rpm 5 min.

Transfer 1,000 µL into autosampler vial.

Samples are ready for HPLC-DAD analysis.

Figure 1. QuEChERS sample preparation procedure for acrylamide in cooking oil.

Results

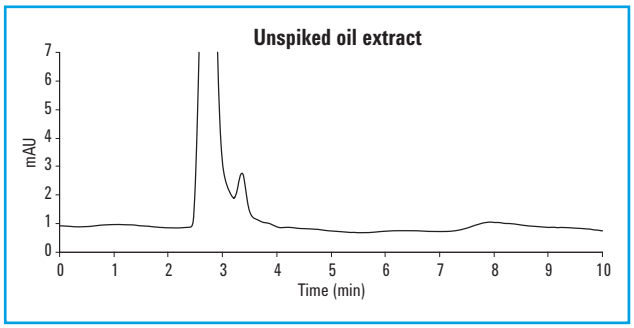


Figure 2. Chromatogram of the blank oil extract.

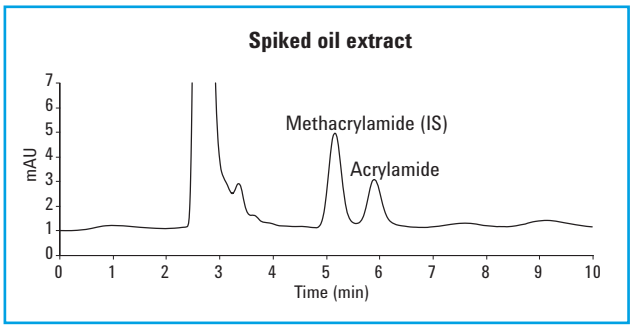


Figure 3. Chromatogram of the spiked oil extract.

Table 1. Recoveries and RSDs for the acrylamide in oil sample (n=6)

Concentration (ng/mL)	%Recovery (n=6)	%RSD (n=6)
500	84.0	3.2
1,000	93.8	2.2
2,000	92.2	1.5

Products used in the above application

Agilent Bond Elut QuEChERS Extraction Kit for Acrylamides. Part No. 5982-5850.

Agilent Bond Elut QuEChERS EN Dispersive SPE Kit. Part No. 5982-5156.

Agilent ZORBAX HILIC Plus Column, 4.6 × 50 mm, 3.5 µm. Part No. 959943-901.

To review this Application Note in its entirety, please search for 5990-5988EN at **agilent.com/chem**

A Blind Study of Pesticides in Fruiting Vegetables by Agilent Bond Elut QuEChERS Extraction Kits and Agilent 5975T LTM GC/MSD (Publication 5990-6323EN)

Introduction

This application describes the use of the QuEChERS EN sample preparation approach for the identification of target pesticides in fruiting vegetables. Cherry tomato and cucumber were used as pesticide matrixes. The analysis was performed on an Agilent 5975T LTM GC/MSD system equipped with an Agilent 7693 Automatic Liquid Sampler. RTL software is a feature of Agilent MSD ChemStation.

Instrument conditions

Instrumentation

Column:	Agilent J&W HP-5ms LTM, 30 m × 0.25 mm, 0.25 µm
Guard column:	1 m column with same phase as analytical column, connected to the injector
GCMS system:	5975T LTM
Inlet:	Split/splitless
Autosampler:	Agilent 7693

Experimental conditions

Inlet temperature:	280 °C
Injection volume:	1 µL
Injection mode:	Splitless
Carrier gas:	Helium
Head pressure:	26.878 psi, constant pressure mode
Method:	RT locked to chlorpyrifos methyl at 16.593 min
LTM oven temperature:	70 °C (2 min), 25 °C/min, 150 °C (0 min), 3 °C/min, 200 °C (0 min), 8 °C/min, 280 °C (10 min)
Transfer line temperature:	270 °C
MSD interface:	270 °C
Ion source:	230 °C
Quad temperature:	150 °C
Ionization mode:	EI
Scan mode:	Full scan, 50–550 u
EMV mode :	Gain factor
Gain factor :	5.00
Resulting EM voltage:	1129 V
Solvent delay:	3 min

QuEChERS Procedure

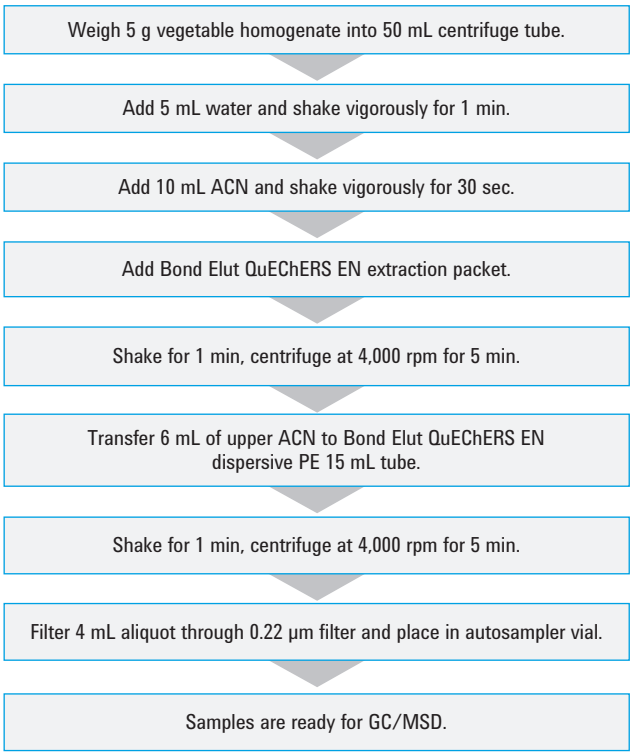


Figure 1. QuEChERS EN procedure for pesticides in fruiting vegetables.

Results

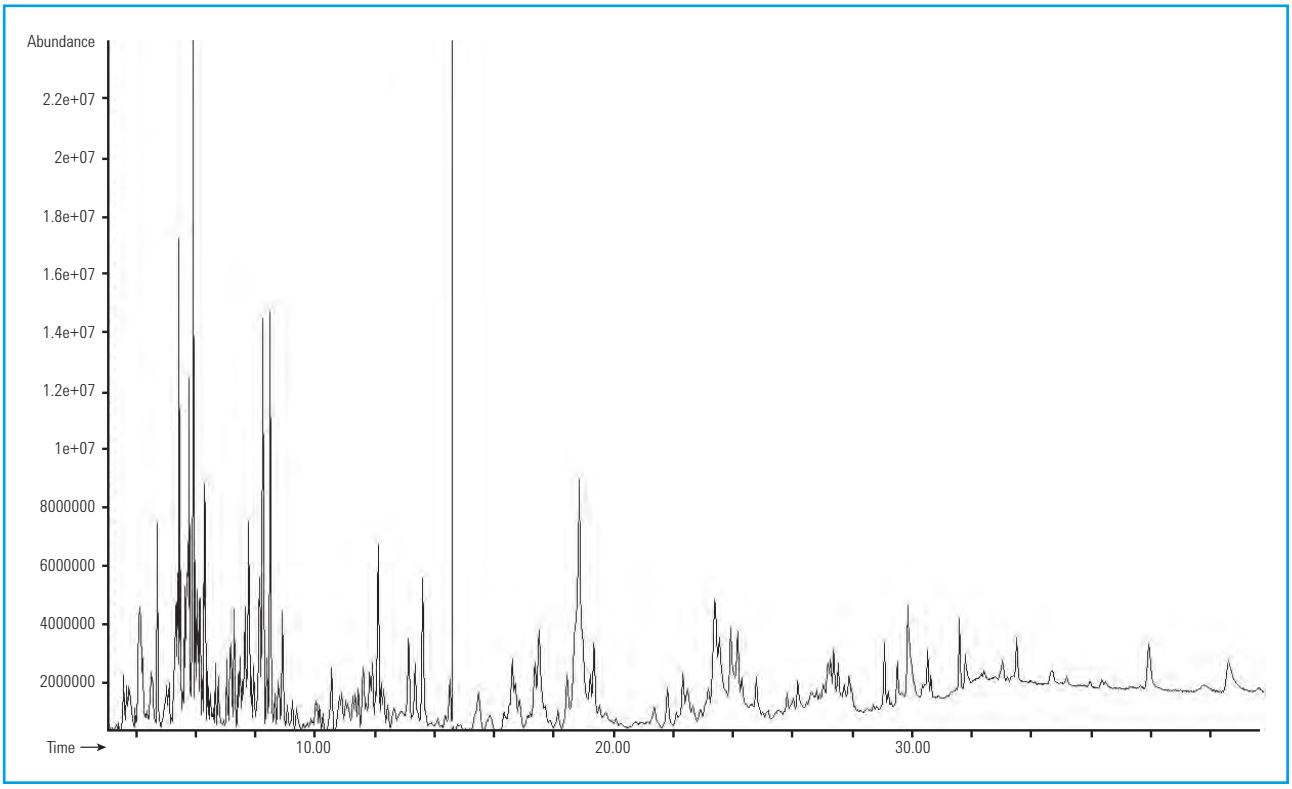


Figure 2. MA 0.2 to 1.0 µg/g amount of pesticide spiked in cherry tomato. All of the targets could be found by DRS software within 2 min.

Products used in the above application

Agilent Bond Elut QuEChERS EN Extraction Kits. Part No. 5982-5650.

Agilent Bond Elut QuEChERS EN Dispersive SPE Kit. Part No. 5982-5156.

Agilent J&W HP-5ms LTM Column, 30 m x 0.25 mm, 0.25 µm. Part No. 29091S-433LTM.

To review this Application Note in its entirety, please search for 5990-6323EN at **agilent.com/chem**

Analysis of Pesticide Residues in Green Tea Using Agilent Bond Elut QuEChERS EN Kit by LC/MS/MS Detection (Publication 5990-6400EN)

Introduction

This application describes the use of the QuEChERS EN sample preparation approach for the extraction and cleanup of 12 pesticide residues representing various pesticide classes in green tea. Green tea is considered to be a highly pigmented sample since it contains high levels of chlorophyll. The presence of target pesticides was determined by liquid chromatography with an Agilent 1200 Infinity Series coupled to an Agilent 6410 Triple Quadrupole MS system with electrospray ionization operating in MRM mode. Mean recoveries ranged between 87% and 108% (average of 93.5%).

Instrument conditions

HPLC conditions

Column:	Agilent Poroshell 120 EC-C18, 2.1 × 100 mm, 2.7 µm, (Part No. 695775-902)
Flow rate:	0.4 mL/min
Column temperature:	30 °C
Injection volume:	10 µL
Mobile phase:	A: 5 mM FA in water B: 5 mM FA in ACN
Needle wash:	1:1:1:1 ACN:MeOH:IPA:H_2O w/0.2% FA

Gradient:

Time (min)	% Acetonitrile
0	5
1	5
3	50
7	90
8	90
8.2	5
9	5

Post run:	2 min
Total cycle time:	11 min

MS conditions
Positive mode

Gas temperature:	350 °C
Gas flow:	10 L/min
Nebulizer:	40 psi
Capillary:	3,500 V

QuEChERS Procedure

Weigh 2 g homogenized sample (+/- 0.1 g) in a 50 mL centrifuge tube.

Add 100 µL IS solution (10 µg/mL of TPP) to samples to yield a 50 ng/g concentration.

Cap and vortex for 1 min.

Add 8 mL water to each tube; cap and vortex for 1 min.

Add two ceramic homogenizers and 10 mL ACN to each tube.

Cap and shake for 1 min.

Add contents of Bond Elut EN extraction salt packet.

Shake vigorously for 20 seconds and centrifuge at 4,000 rpm for 5 min.

Transfer a 6 mL aliquot of upper ACN layer to Bond Elut EN dispersive SPE 15 mL tube.

Cap and vortex for 1 min; centrifuge at 4,000 rpm for 5 min.

Transfer 1 mL extract into a 10 mL tube and dry under nitrogen below 40 °C.

Dissolve residue to a constant volume of 1 mL using ACN/water (1/9).

Filter residue through a 0.45 µm membrane.

Analyze by LC/MS/MS.

Figure 1. QuEChERS EN procedure for pesticides in green tea.

Results

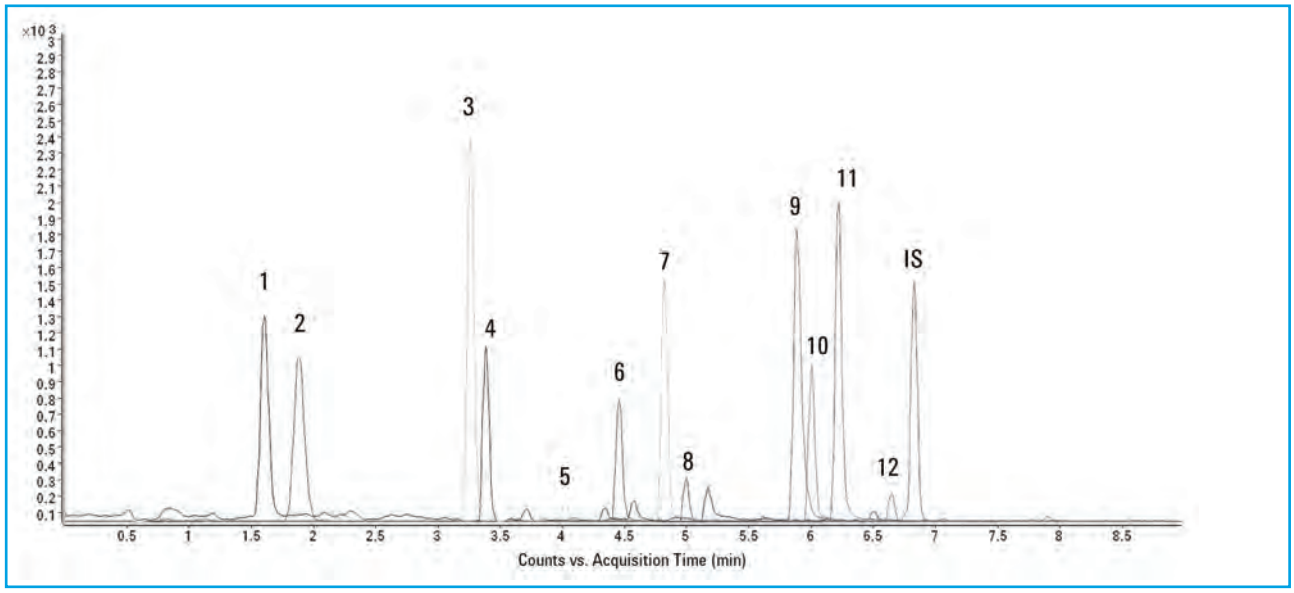


Figure 2. MRM chromatograms of 50 ng/g fortified sample processed by EN method. Peak identification: 1. Acephate, 2. Pymetrozine, 3. Carbendazim, 4. Thiabendazole, 5. Imidacloprid, 6. Imazalil, 7. Propoxur, 8. Carbaryl, 9. Cyprodinil, 10. Ethoprophos, 11. Penconazole, 12. Kresoxim-methyl, IS: TPP.

Table 1. Recovery and reproducibility of pesticides in fortified green tea with Agilent Bond Elut QuEChERS

	10 ng/g fortified QC		50 ng/g fortified QC		200 ng/g fortified QC	
Analytes	Recovery	RSD (n=6)	Recovery	RSD (n=6)	Recovery	RSD (n=6)
Acephate	80.5%	5.4%	91.7%	2.9%	88.9%	8.2%
Pymetrozine	43.1%	3.0%	42.2%	3.4%	43.4%	9.8%
Carbendazim	114.6%	11.6%	97.6%	2.0%	105.0%	6.2%
Thiabendazole	98.1%	6.9%	90.4%	2.4%	81.7%	5.8%
Imidacloprid	104.3%	11.7%	108.6%	2.5%	93.9%	7.9%
Imazalil	97.5%	4.4%	87.8%	5.6%	92.4%	4.6%
Propoxur	98.1%	2.4%	110.2%	1.7%	107.8%	3.9%
Carbaryl	89.7%	11.4%	104.9%	3.3%	108.1%	5.2%
Cyprodinil	84.9%	2.1%	92.5%	3.7%	93.9%	5.5%
Ethoprophos	103.4%	3.1%	111.2%	3.2%	104.9%	5.7%
Penconazole	108.7%	2.9%	94.3%	4.5%	89.8%	3.3%
Kresoxim-methyl	105.7%	12.4%	96.4%	2.5%	99.2%	5.5%

Products used in the above application

Agilent Bond Elut QuEChERS EN Extraction Kits. Part No. 5982-5650.

Agilent Bond Elut QuEChERS AOAC Dispersive SPE Kits for Pigmented Fruits and Vegetables. Part No. 5982-5356.

Agilent Poroshell 120 EC-C18 Column, 2.1 x 100 mm, 2.7 µm, Part No. 695775-902.

To review this Application Note in its entirety, please search for 5990-6400EN at **agilent.com/chem**

Determination of Hormones in Shrimp by Agilent 1290 Infinity LC with Agilent Poroshell 120 LC Column and Agilent Bond Elut QuEChERS for Sample Preparation (Publication 5990-6589EN)

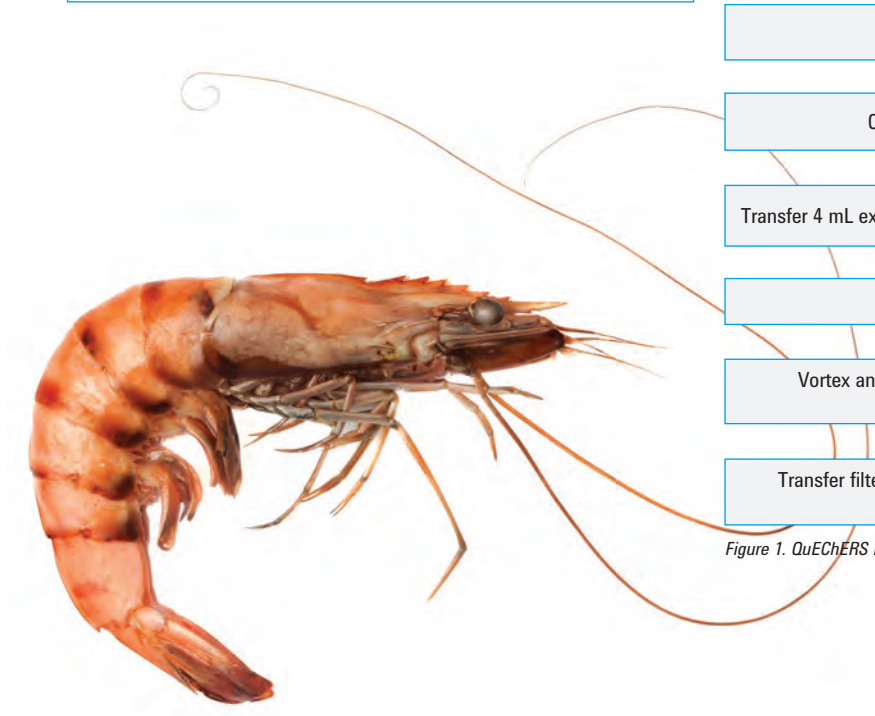
Introduction

This application describes the use of the QuEChERS EN sample preparation approach for the extraction of 13 hormones in shrimp. A method for the determination of hormones in shrimp was developed using an Agilent 1290 Infinity LC with an Agilent Poroshell 120 EC-C18 column. Method recoveries ranged from 91.6 to 107.2%.

Instrument conditions

HPLC conditions

Column:	Agilent Poroshell 120 EC-C18, 3.0 x 100 mm, 2.7 µm (Part No. 695975-302)		
Instrument:	Agilent 1290 Infinity LC with DAD detector		
Flow rate:	0.8 mL/min		
Column temperature:	30 °C		
Injection volume:	10 µL		
Detection wavelength:	230 nm		
Mobile phase:	Water-acetonitrile gradient		
Gradient:	Time (min)	% Water	% Acetonitrile
	0	80	20
	6	50	50
	8	10	90



QuEChERS Procedure

Weigh 5 g (+/- 0.05 g) homogenized sample in 50 mL centrifuge tube.

Centrifuge 30 seconds to move the sample to the bottom of tube, then add appropriate QC spiking solution.

Cap and vortex sample for 30 seconds and add 5 mL water.

Cap and vortex for 10 seconds and add 10 mL ACN to each tube.

Cap and shake for 30 sec.

Add Bond Elut EN extraction packet.

Cap and shake vigorously for 1 min.

Centrifuge at 4,000 rpm for 5 min at 4 ˚C.

Transfer 6 mL aliquot of upper ACN layer to Bond Elut dispersive SPE 15 mL tube.

Cap and vortex for 1 min.

Centrifuge at 13,000 rpm for 3 min at 4 ˚C.

Transfer 4 mL extract into another tube and dry by nitrogen flow at 35 ˚C.

Reconstitute into 2 mL of 1:4 ACN/H_2O.

Vortex and sonicate for 10 min, then filter through a 0.2 µm cellulose acetate spin filter.

Transfer filtered sample into an autosampler vial; cap and vortex for HPLC analysis.

Figure 1. QuEChERS EN procedure for hormones in shrimp.

Results

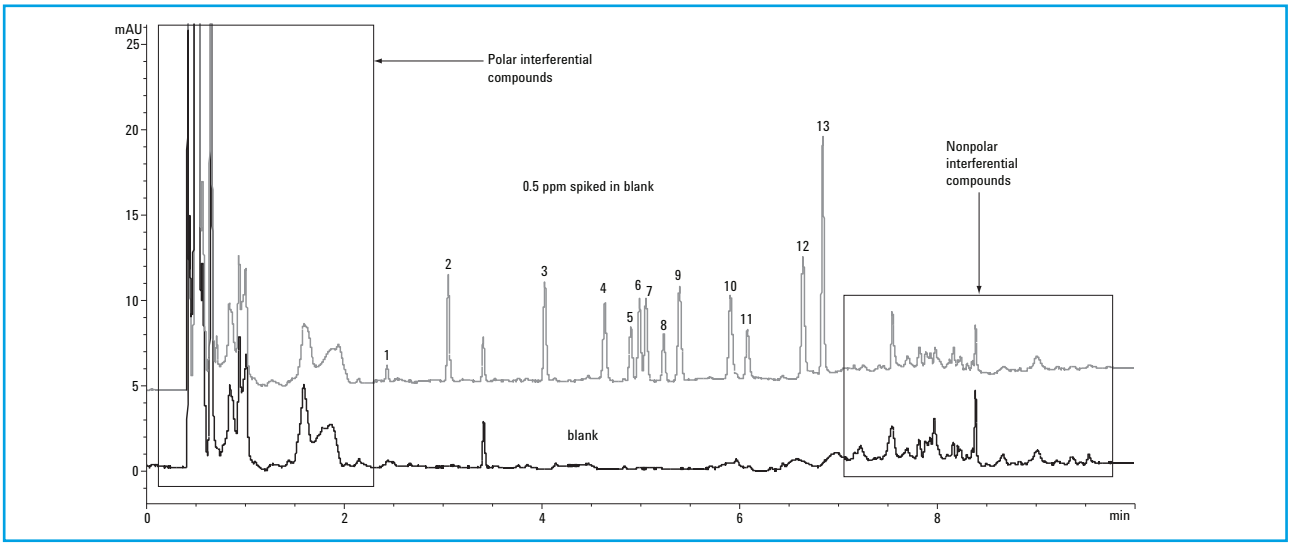


Figure 2. Chromatograms of blank sample and 0.5 ppm spiked in blank sample.

Table 1. Recovery and reproducibility for the QuEChERS method developed for hormone determination in shrimp

Analytes	Concentration	Recovery (%)	% RSD (n=6)
Estriol	0.5	107.2	3.5
	10	98.2	0.98
Prednisone	0.5	97.6	2.3
	10	101.7	0.58
Dexamethasone	0.5	101.8	0.96
	10	96.1	1.2
Boldenone	0.5	98.9	1.5
	10	96.2	1.8
Hydrocortisone	0.5	103.5	1.5
	10	92.3	0.23
Fludrocortisone acetate	0.5	104.3	1.9
	10	91.8	0.17
Metandienone	0.5	100.0	1.4
	10	95.6	0.25
Estradiol	0.5	99.4	1.3
	10	97.8	0.54
Testosterone	0.5	98.0	0.85
	10	98.2	0.15
Methyltestosterone	0.5	97.1	0.99
	10	92.1	0.63
Estrone	0.5	103.4	1.2
	10	92.5	0.68
Diethylstilbestrol	0.5	100.9	1.9
	10	97.3	0.79
Hexestrol	0.5	98.5	1.6
	10	91.6	0.81

Products used in the above application

Agilent Bond Elut QuEChERS EN Extraction Kits. Part No. 5982-5650.

Agilent Bond Elut QuEChERS AOAC Dispersive SPE Kits for Drug Residues in Meat, 15 mL. Part No. 5982-4956.

Agilent Poroshell 120 EC-C18 Column, 3.0 x 100 mm, 2.7 µm. Part No. 695975-302.

To review this Application Note in its entirety, please search for 5990-6589EN at **agilent.com/chem**

Analysis of Pesticide Residues in Rice with Bond Elut QuEChERS Extraction Kits and Agilent J&W HP-5ms Ultra Inert GC Column (Publication 5990-8108EN)

Introduction

This application describes the use of the QuEChERS EN sample preparation approach for the extraction and cleanup of 57 GC-amenable multiple pesticide class residues in rice. Agilent developed GC and GC/MS RTL databases (Part No. G1672AA) that include 962 pesticides, metabolites, and suspected endocrine disrupters. The experiments were performed on an Agilent 7890 GC equipped with 5975C inert MSD, and Agilent 7683 Automatic Liquid Sampler (ALS). Separation of the compounds was achieved on an Agilent J&W HP-5ms Ultra Inert GC column. Most of the recoveries ranged between 80 and 110%.

Instrument conditions

GC conditions

Column:	Agilent J&W HP-5ms Ultra Inert, 30 m × 0.25 mm, 0.25 µm (Part No. 19091S-433UI)
Inlet temperature:	250 °C
Carrier gas:	Helium, constant pressure mode
Retention time locking:	Chropyrifos-methyl locked to 16.596 min
Injection mode:	Splitless, purge flow 50 mL/min at 0.75 min
Injection volume:	1 µL
Oven:	70 °C (2 min), 25 °C/min to 150 °C (0 min), 3 °C/min to 200 °C, 8 °C/min to 280 °C (10 min), postrun: 320 °C (5 min)

MS conditions

Solvent delay:	4 min
MS temp:	230 °C (source); 150 °C (quad)
Transfer line:	280 °C
MS libraries:	Agilent RTL Pesticide Library (G1672AA) and NIST08 Mass Spec Library
MS:	EI, SIM/Scan
Scan mode:	Mass range (50-550 amu)

QuEChERS Procedure

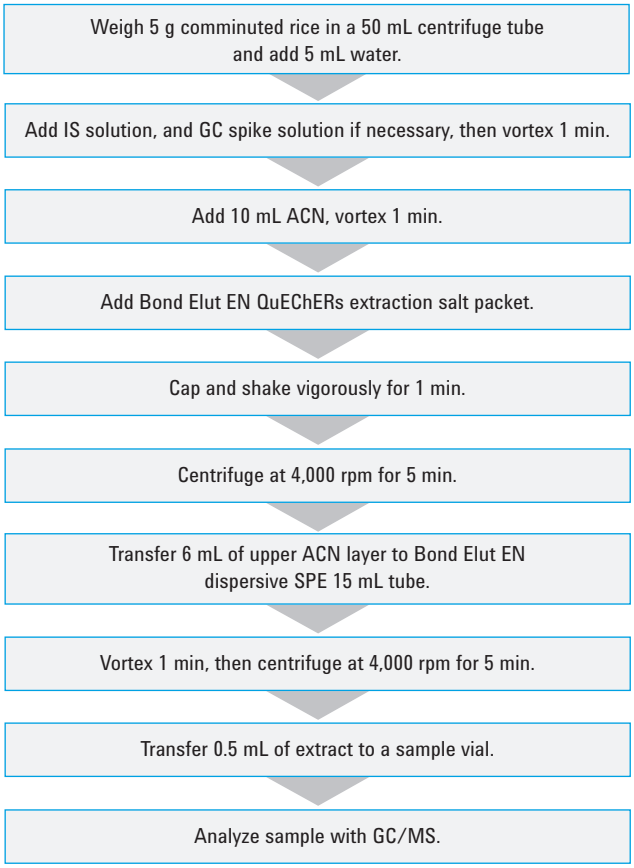
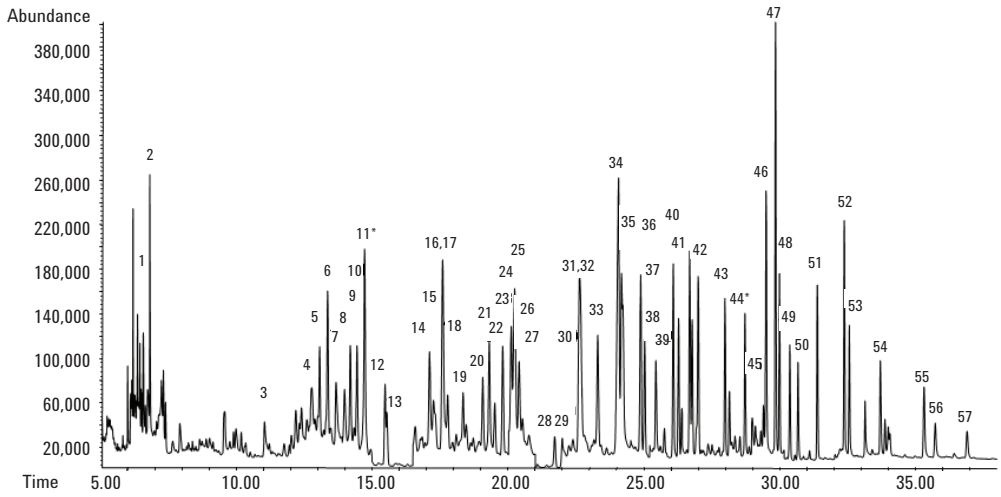


Figure 1. Agilent Bond Elut QuEChERS EN extraction procedure for pesticides in rice.

Results



1 Methamidophos	13 BHC delta isomer	25 Chlorpyrifos	37 p,p′-DDE	49 Tetradifon
2 Dichlorvos	14 Propanil	26 Parathion	38 Myclobutanil	50 Phosalone
3 Omethoate	15 Methyl parathion	27 Triadimefon	39 Endrin	51 Lambda-cyhalothrin
4 Monocrotophos	16 Chlorpyrifos methyl	28 Heptachlor exo-epoxide	40 p,p′-DDD	52 Permethrin I
5 BHC alpha isomer	17 Vinclozolin	29 Pendimethalin	41 o,p′-DDT	53 Permethrin II
6 Hexachlorobenzene	18 Heptachlor	30 Isofenphos	42 Ethion	54 Cypermethrin I
7 Dimethoate	19 Metalaxyl	31 Quinalphos	43 p,p′-DDT	55 Fenvalerate
8 BHC beta isomer	20 Fenitrothion	32 Triadimenol	44 Triphenyl phosphate*	56 Cypermethrin II
9 Lindane	21 Pirimiphos-methyl	33 Methidathion	45 Iprodione	57 Deltamethrin
10 Pentachloronitrobenzene	22 Aldrin	34 Butachlor	46 Phosmet	
11 Phenanthrene-d10*	23 Malathion	35 Dieldrin	47 Bifenthrin	* Internal standard
12 Diazinon	24 Fenthion	36 Isoprothiolane	48 Fenpropathrin	

Figure 2. Total ion chromatogram (TIC) of pesticides in rice at 200 ng/mL.

Products used in the above application

Agilent Bond Elut QuEChERS EN Extraction Kits. Part No. 5982-5650.

Agilent Bond Elut QuEChERS AOAC Dispersive SPE Kits. Part No. 5982-5156.

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

To review this Application Note in its entirety, please search for 5990-8108EN at **agilent.com/chem**

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

Introduction

This application describes the use of 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, 3.0 x 150 mm, 3.5 µm (Part No. 959963-312)
Flow rate:	0.3 or 0.5 mL/min
Column temperature:	30 °C
Injection volume:	10 µL
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:IPA:H_2O (0.2% FA)

Gradient:

Time (min)	% 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

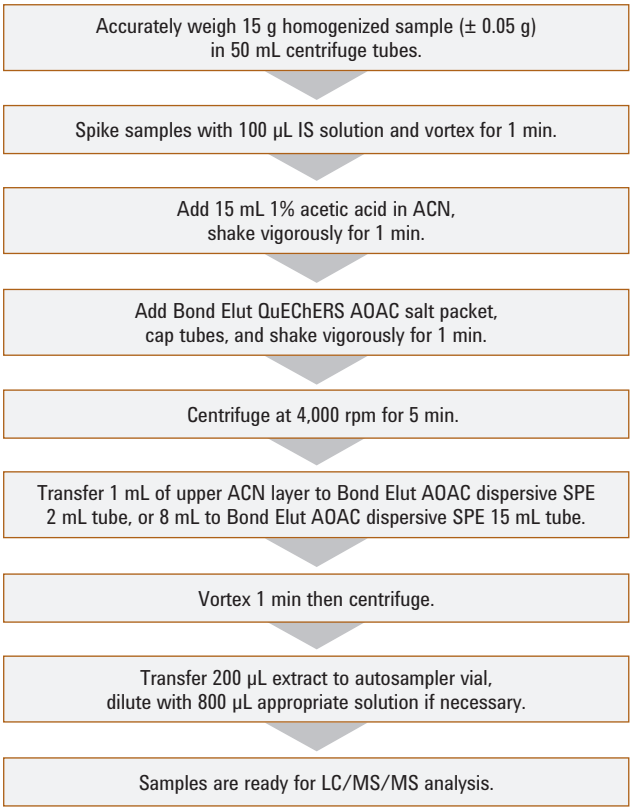


Figure 1. QuEChERS AOAC sample preparation procedures for pestcides in apple.

Products used in the above application

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

Agilent Bond Elut QuEChERS AOAC Dispersive SPE Kit for General Fruits and Vegetables, 2 mL. Part No. 5982-5022 or 15 mL. Part No. 5982-5058.

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

Results

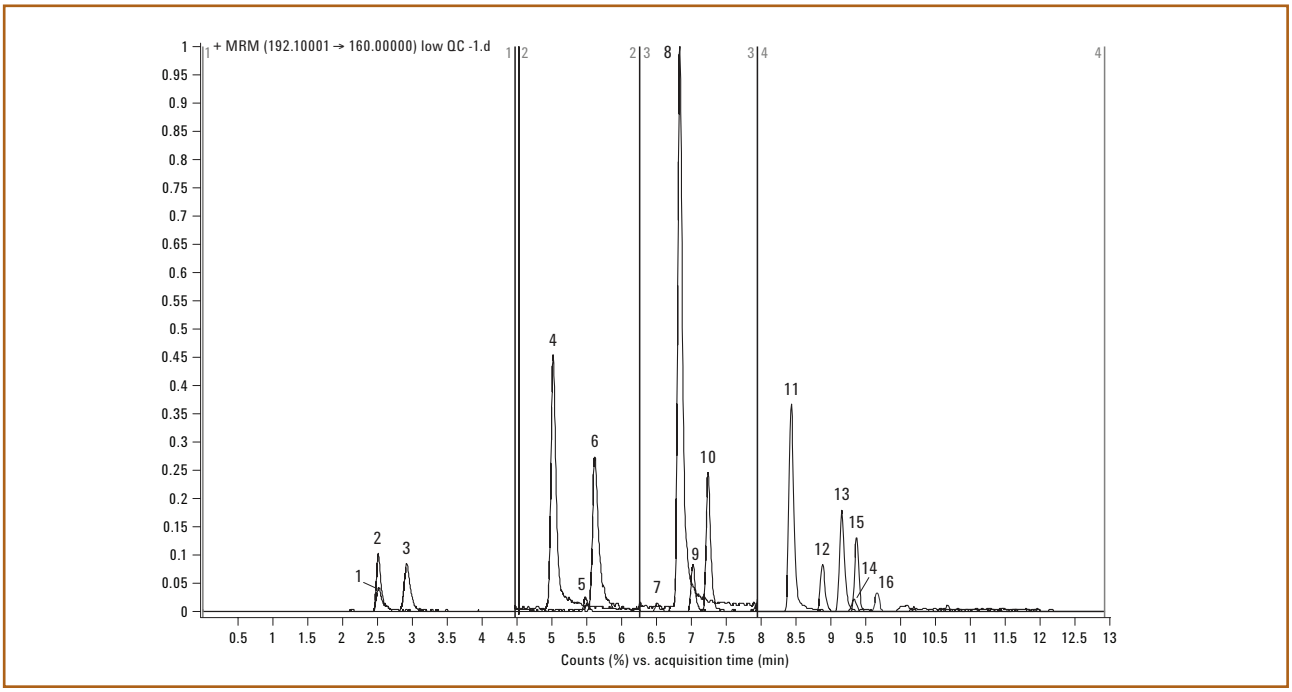


Figure 2. Chromatogram of 10 ng/g fortified apple extract. Peak identification: 1. Methamidophos, 2. Acephate, 3. Pymetrozine, 4. Carbendazim, 5. Imidacloprid, 6. Thiabendazole, 7. Dichlorvos, 8. Propoxur, 9. Thiophanate methyl, 10. Carbaryl, 11. Ethoprophos, 12. Penconazole, 13. Cyprodinil, 14. Dichlofluanid,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

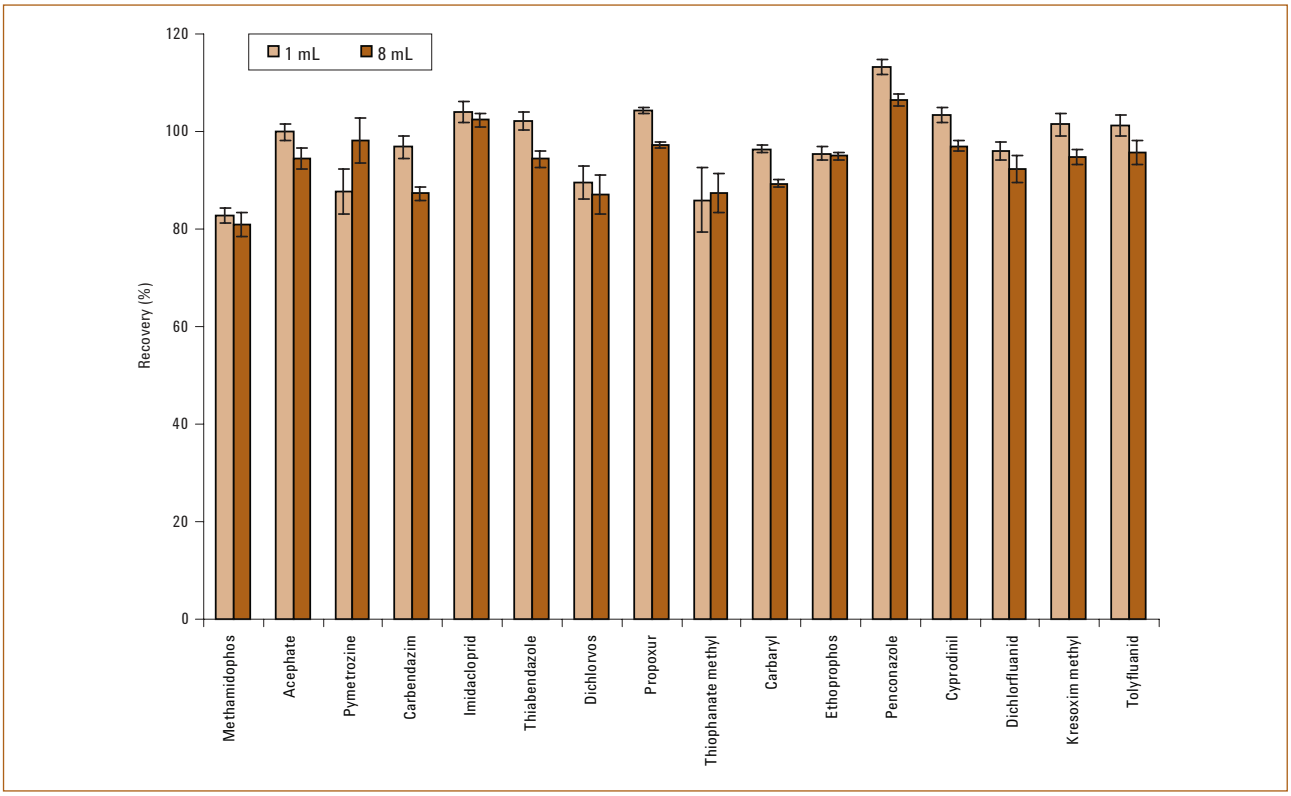


Figure 3. Results comparison of 1 mL and 8 mL dispersive SPE sample volume.

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

Introduction

This application describes the use of the 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

Column:	Agilent J&W HP-5ms Ultra Inert, 30 m x 0.25 mm, 0.25 µm (Part No. 19091S-433UI)
Autosampler:	Agilent 7683 Automatic Liquid Sampler
Inlet:	Splitless
Carrier gas:	Helium in 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 µL

MS conditions

Tune file:	Atune.u
Mode:	SIM (refer to Table 2 of App Note for settings in detail)
Source, quad, transfer line temperature:	230 °C, 150 °C, and 280 °C, respectively
Solvent delay:	3 min
Multiplier voltage:	Autotune voltage

QuEChERS Procedure

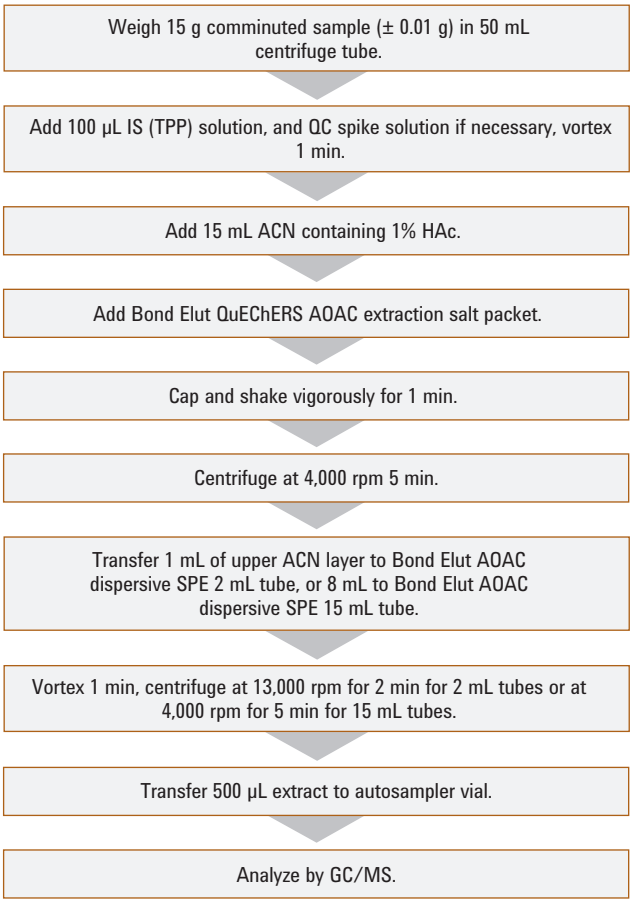


Figure 1. Agilent Bond Elut QuEChERS AOAC extraction procedure for pesticide residues in apple.

Products used in the above application

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

Agilent Bond Elut QuEChERS AOAC Dispersive SPE Kit for General Fruits and Vegetables, 2 mL. Part No. 5982-5022 or 15 mL. Part No. 5982-5058.

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

Results

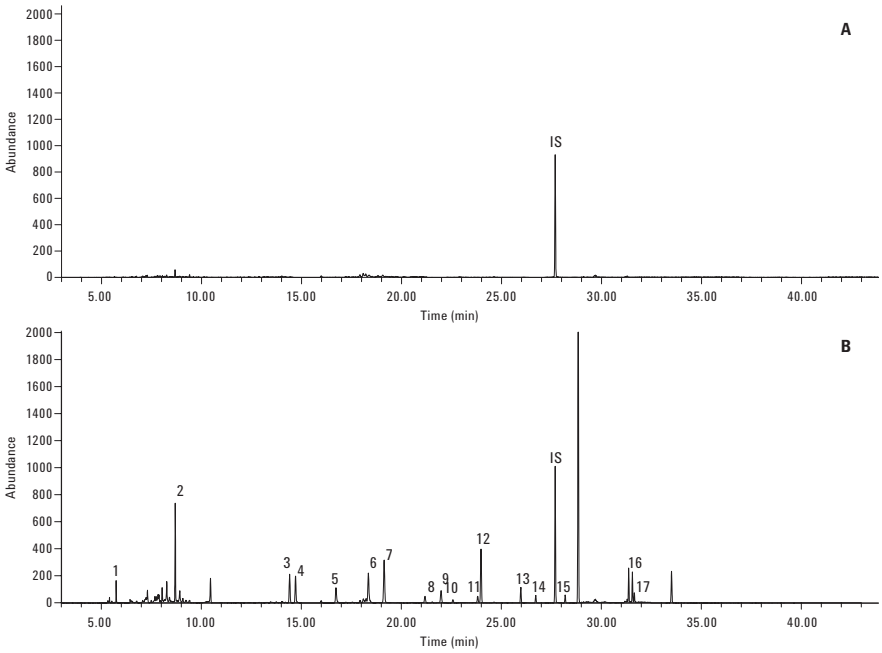


Figure 2. GC/MS chromatogram of apple extract. (A) apple extract blank; (B) 50 ng/g fortified apple extract. Peak Identification: 1. Dichlorvos, 2. o-Phenylphenol, 3. Diazinon, 4. Chlorothalonil, 5. Carbaryl, 6. Dichlofluanid, 7. Dichlorobenzophenone, 8. Folpet, 9. α-Chlordane, 10. Endosulfan, 11. Dieldrin,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

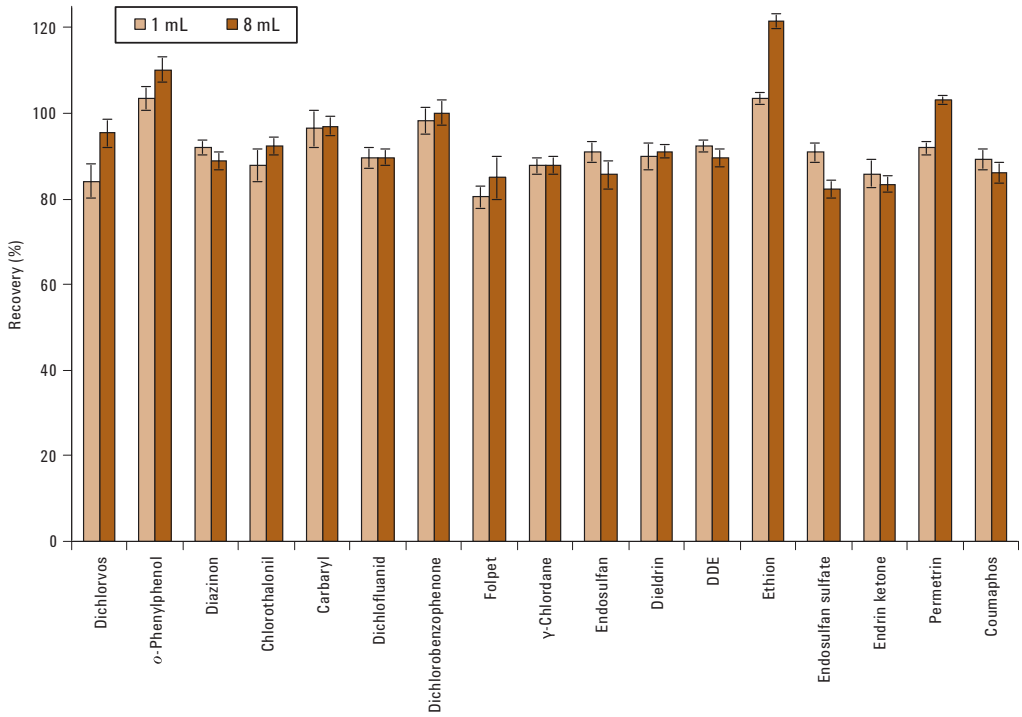


Figure 3. Exceptional recoveries and precision for 1 and 8 mL sample volumes employing Agilent Bond Elut dispersive SPE, 2 and 15 mL kits, respectively.

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

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

Introduction

This application describes the use of the QuEChERS AOAC sample preparation approach for extraction and cleanup of 18 GC-amenable multiple pesticide class residues in spinach. 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

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

QuEChERS Procedure

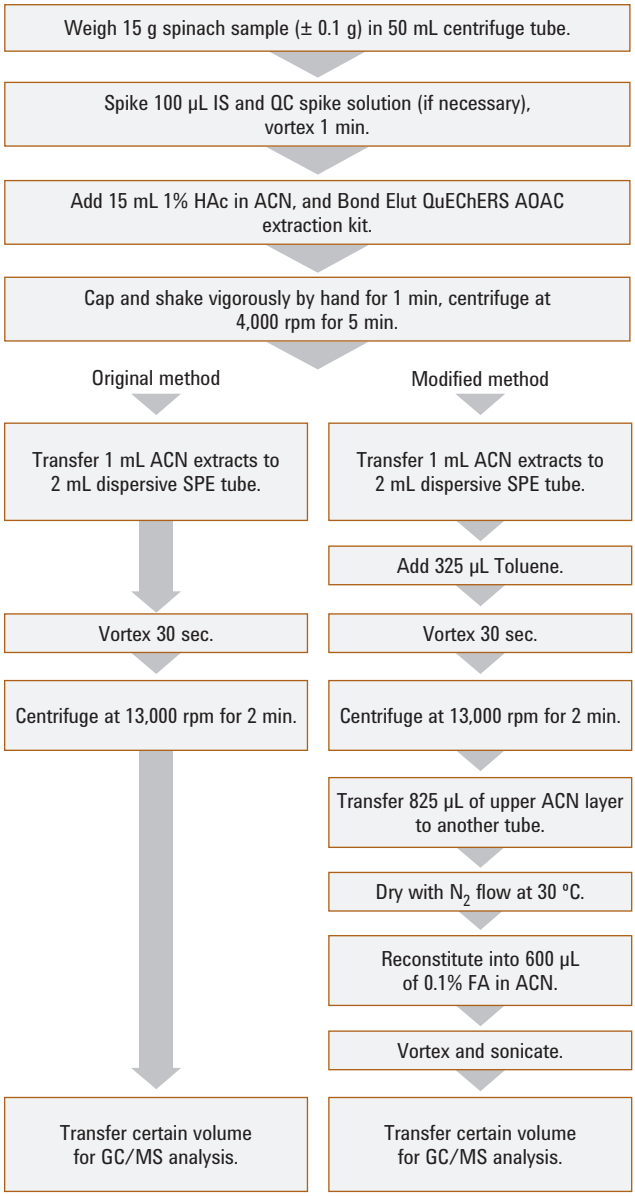


Figure 1. QuEChERS AOAC extraction procedure (original and modified dispersive SPE, 2 mL size) for spinach sample.

Results

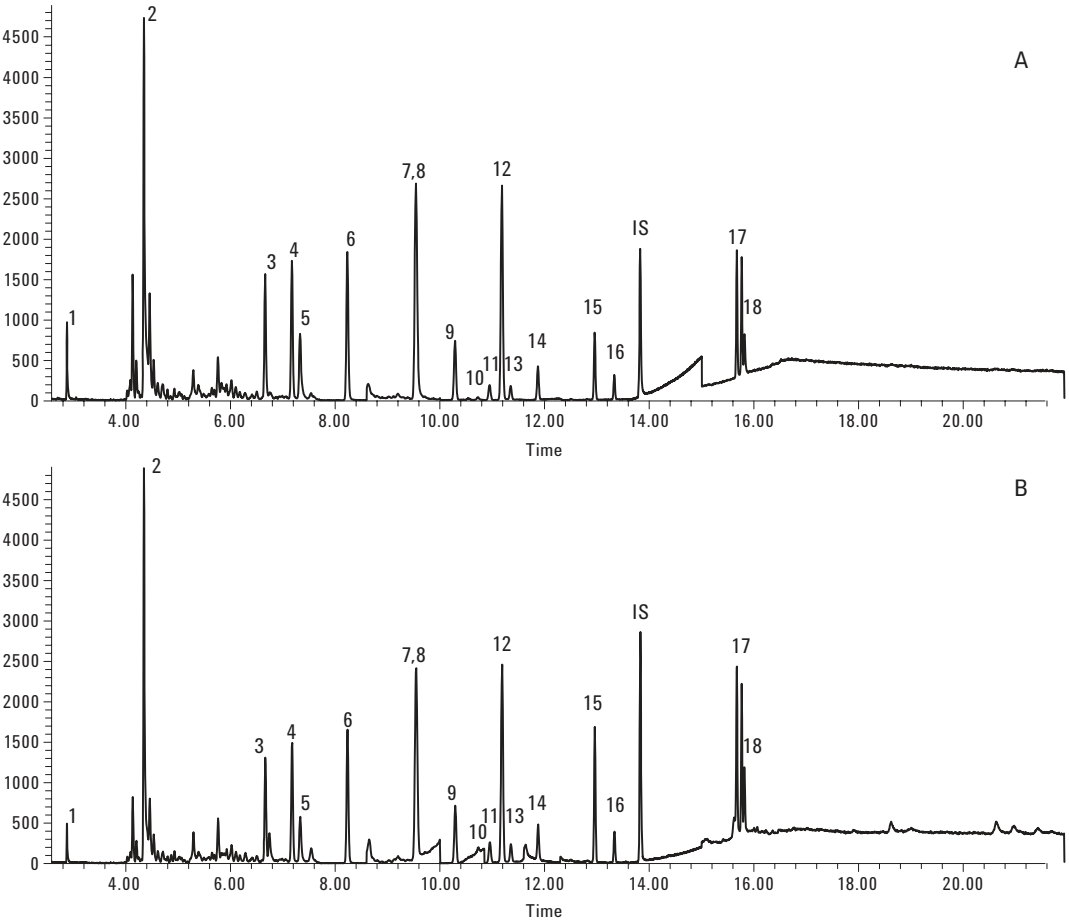


Figure 2. GC/MS chromatograms of 50 ng/g fortified spinach sample extracts processed by original dispersive SPE (A) and modified dispersive SPE (B). Peak identification: 1. Diachlorvos, 2. o-Phenylphenol, 3. Lindane, 4. Diazinon, 5. Chlorothalonil 6. Chloropyrifos methyl 7. Dichlorobenzophenone, 8. Chlorpyrifos, 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.

Products used in the above application

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

Agilent Bond Elut QuEChERS AOAC Dispersive SPE Kit for Pigmented Fruits and Vegetables, 2 mL. Part No. 5982-5222 or 15 mL. Part No. 5982-5258.

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

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

	Low QC (10 ng/g)		Mid QC (50 ng/g)		High QC (200 ng/g)	
Pesticide	Recovery	RSD	Recovery	RSD	Recovery	RSD
Dichlorvos	94.0	3.0	91.7	10.5	80.9	4.6
o-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.

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

Introduction

This application describes the use of the QuEChERS AOAC sample preparation approach for the extraction and cleanup of 13 pesticide residues representing various pesticide classes in spinach. 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 Eclipse Plus Phenyl-Hexyl, 3.0 x 150 mm, 3.5 µm (Part No. 959963-312)
Flow rate:	0.3 or 0.5 mL/min
Column temperature:	30 °C
Injection volume:	10 µL
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 (min)	% 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

QuEChERS Procedure

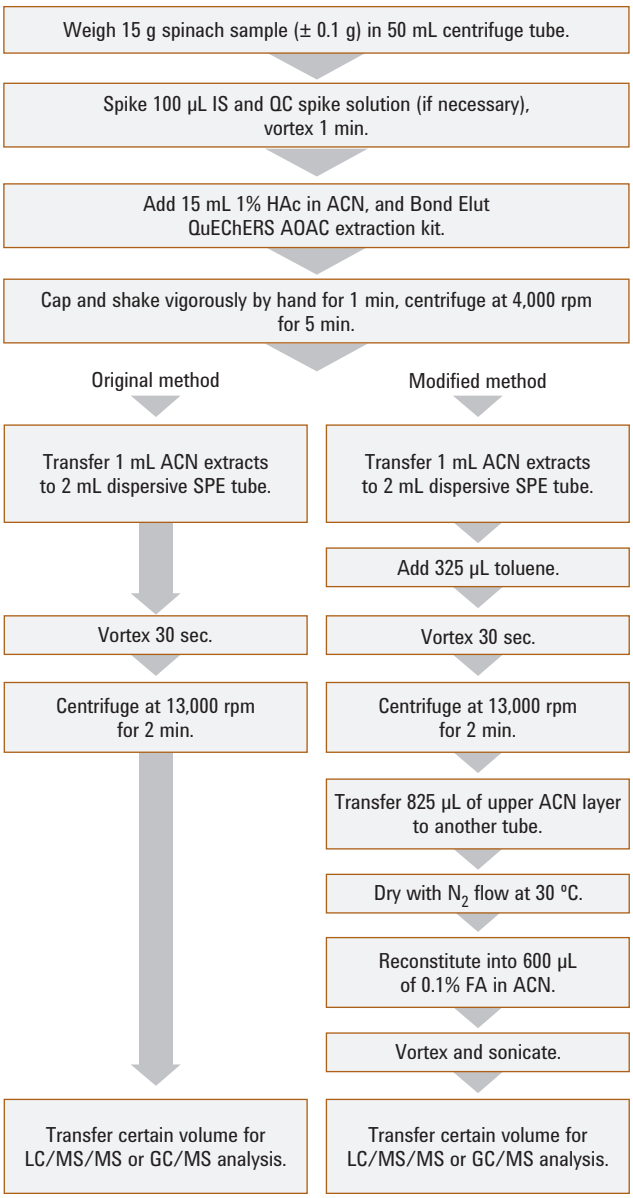


Figure 1. QuEChERS AOAC extraction procedure (original and modified dispersive SPE, 2 mL size) for a spinach sample.

Results

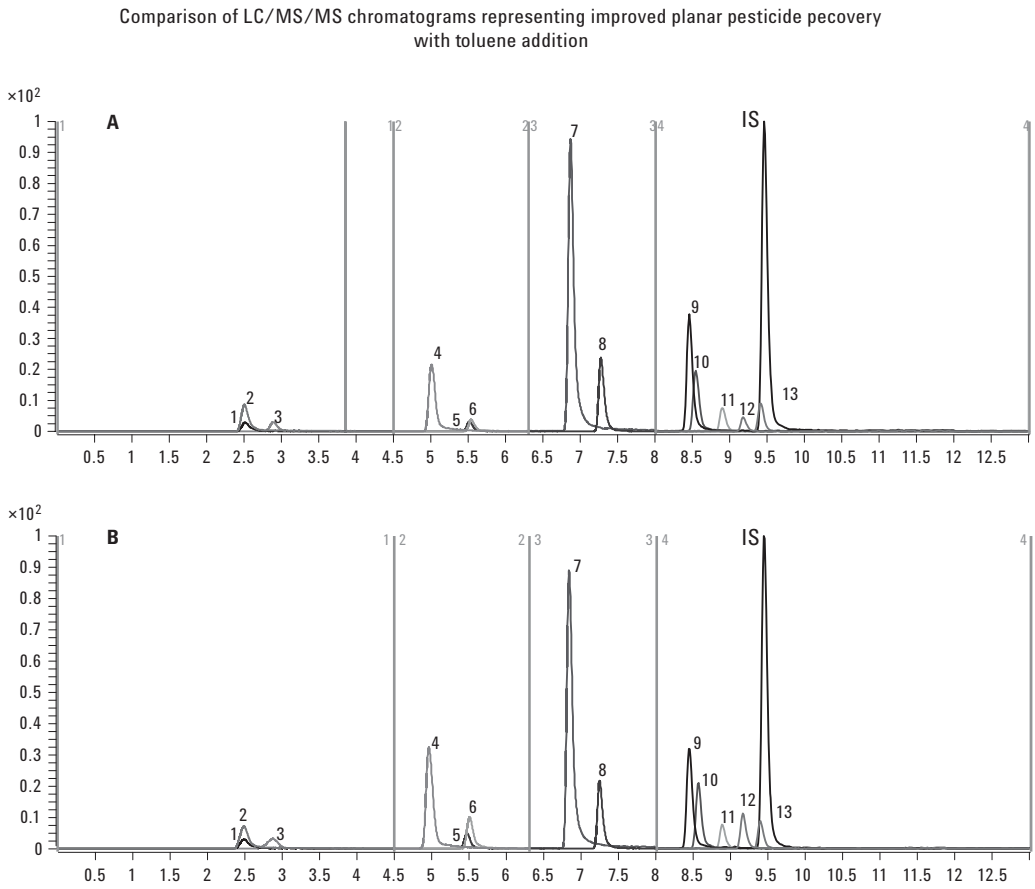


Figure 2. LC/MS/MS chromatograms of 50 ng/g fortified spinach sample extracts processed by original dispersive SPE (A) and modified dispersive SPE (B). Peak identification: 1. Methamidophos, 2. Acephate, 3. Pymetrozine, 4. Carbendazim, 5. Imidacloprid 6. Thiabendazole, 7. Propoxur, 8. Carbaryl, 9. Ethoprophos,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

Products used in the above application

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

Agilent Bond Elut QuEChERS AOAC Dispersive SPE Kit for Pigmented Fruits and Vegetables, 2 mL. Part No. 5982-5222 or 15 mL. Part No. 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.

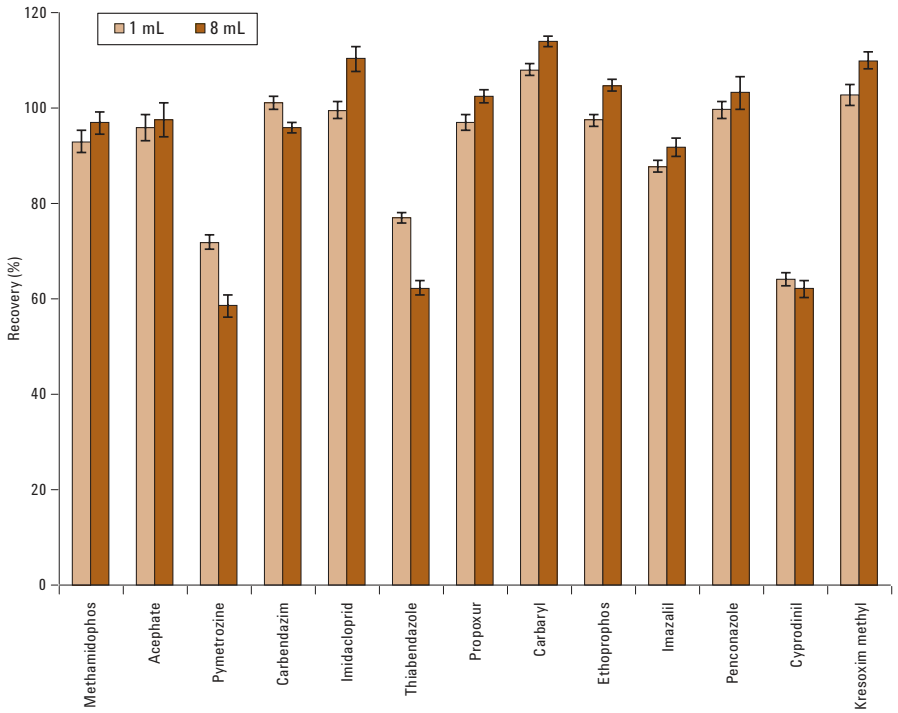


Figure 3. Recovery and precision results for 1 mL dispersive SPE and 8 mL dispersive SPE.

To review this Application Note in its entirety, please search for 5990-4248EN at **agilent.com/chem**

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

Introduction

This application describes the impact of toluene addition in the dispersive solid phase extraction (SPE) step on the analysis of pesticides in spinach using Agilent Bond Elut 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. Using GC/MS and LC/MS/MS, the method provides an alternative extraction process for challenging pesticides in highly pigmented foods.

QuEChERS Procedure

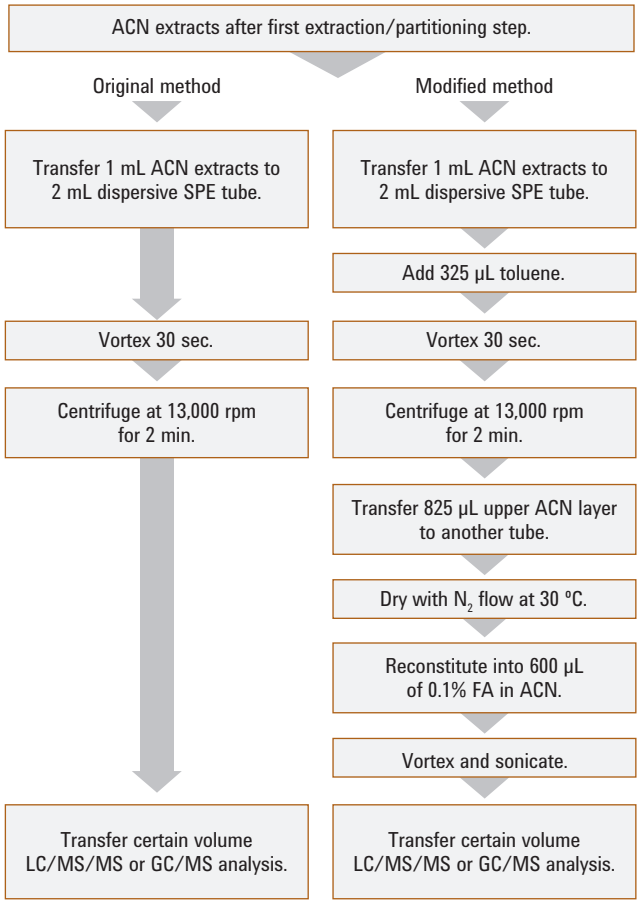


Figure 1. Dispersive SPE procedures of original method (without toluene) and modified method (with toluene).

Instrument conditions

HPLC conditions

Column:	Agilent ZORBAX Solvent Saver Plus Eclipse Plus Phenyl-Hexyl, 3.0 x 150 mm, 3.5 µm (Part No. 959963-312)
Flow rate:	0.3 or 0.5 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_2O B: 5 mM ammonium acetate, pH 5.0 in ACN
Needle wash:	1:1:1:1 ACN/MeOH/IPA/H_2O w/0.2% FA

Gradient:

Time (min)	% 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

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

Results

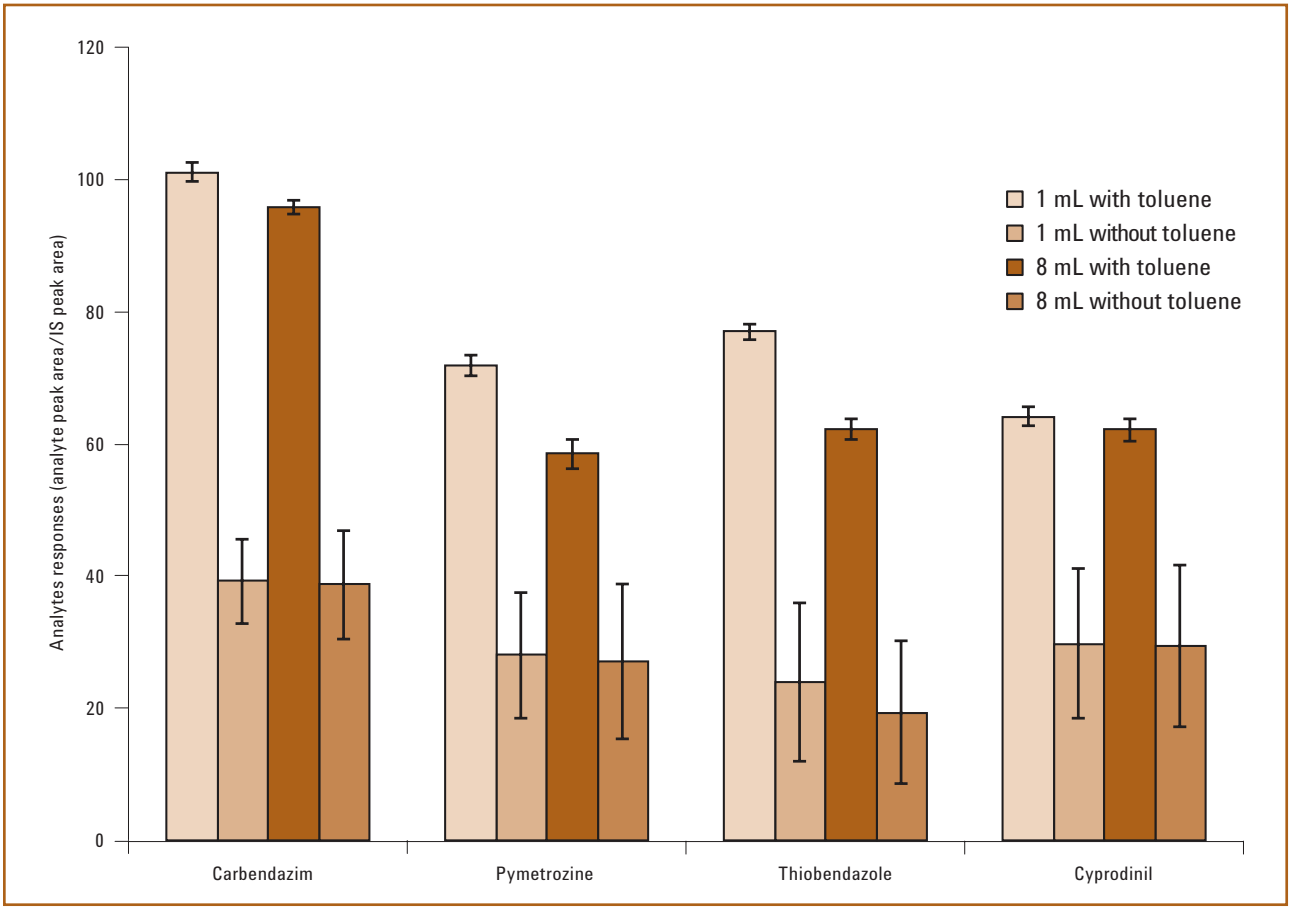


Figure 2. Results comparison of 1 mL and 8 mL dispersive SPE with the modified method (with toluene) and the original method (without toluene).

Products used in the above application

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

Agilent Bond Elut QuEChERS AOAC Dispersive SPE Kit for Pigmented Fruits and Vegetables, 2 mL. Part No. 5982-5222 or 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, 3.0 x 150 mm, 3.5 µm. Part No. 959963-312.

Table 1. Effects on certain pesticides using a modified dispersive SPE cleanup with addition of toluene

Analytes	Original method (without toluene) Recovery	RSD (n=6)	Modified method (with toluene) Recovery	RSD (n=6)	Impact with modified method	Detection method
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

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

Determination of Pesticides in Baby Food by UHPLC/MS/MS using the Agilent 1290 Infinity LC and the Agilent 6460 Triple Quadrupole LC/MS (Publication 5990-5028EN)

Introduction

This application describes the use of the QuEChERS sample preparation approach for extraction and cleanup of 40 pesticides in baby food at levels below the maximum residue level (MRL) (10 µg/kg fruit or vegetable) specified in EC Regulation 396/2005. The qualitative and quantitative analysis of pesticides at trace levels in baby food matrixes using UHPLC and triple quadrupole MS was demonstrated.

The method and extraction performance were evaluated in terms of repeatability, linearity and sensitivity. Moreover the influence of the additional dispersive SPE cleanup was investigated. Detection limits were between 500 ng/kg and 10 ng/kg (ppt), which is much lower than the MRL of 10 µg/kg (ppb) imposed by the European Union.

Instrument conditions

Method parameters

Column:	Agilent ZORBAX Eclipse Plus RRHD C18, 2.1 x 150 mm, 1.8 µm
Mobile phase:	A: 0.05% (w/v) Ammonium formate + 0.01% (v/v) Formic acid in water B: Methanol
Flow rate:	0.5 mL/min
Gradient:	Time (min) % B 0 to 5 10 to 65 5 to 6.5 65 to 95 6.5 to 8.5 95 8.5 to 10 10
Temperature:	45 °C
Injection:	2 µL, with needle wash (flushport, 5 s, water/methanol 1/1)
Detection:	MS/MS
Ionization:	Electrospray, positive ionization

Jet Stream parameters

Drying gas temperature:	250 °C
Drying gas flow:	10 L/min
Nebulizer pressure:	30 psig
Sheath gas temperature:	340 °C
Sheath gas flow:	11 L/min
Capillary voltage:	4500 V
Nozzle voltage:	500 V

Acquisition

Dynamic MRM:	See Table 1 of app note
Delta EMV:	50
Cycle time:	200 ms

QuEChERS Procedure

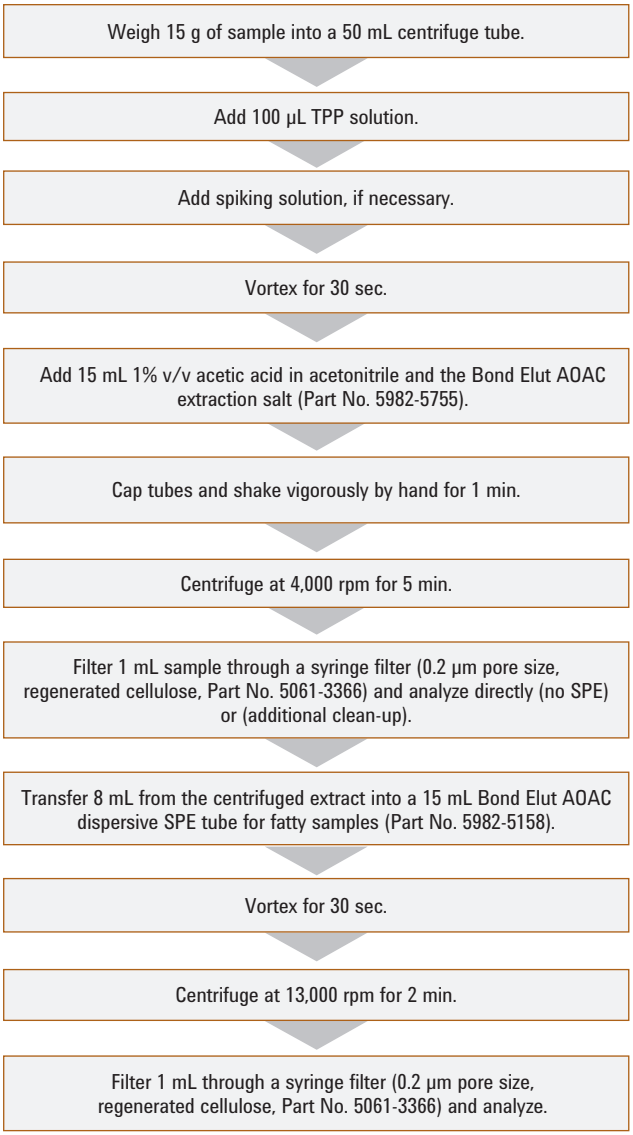


Figure 1. Agilent Bond Elut QuEChERS AOAC extraction procedure for pesticides in baby food.

Results

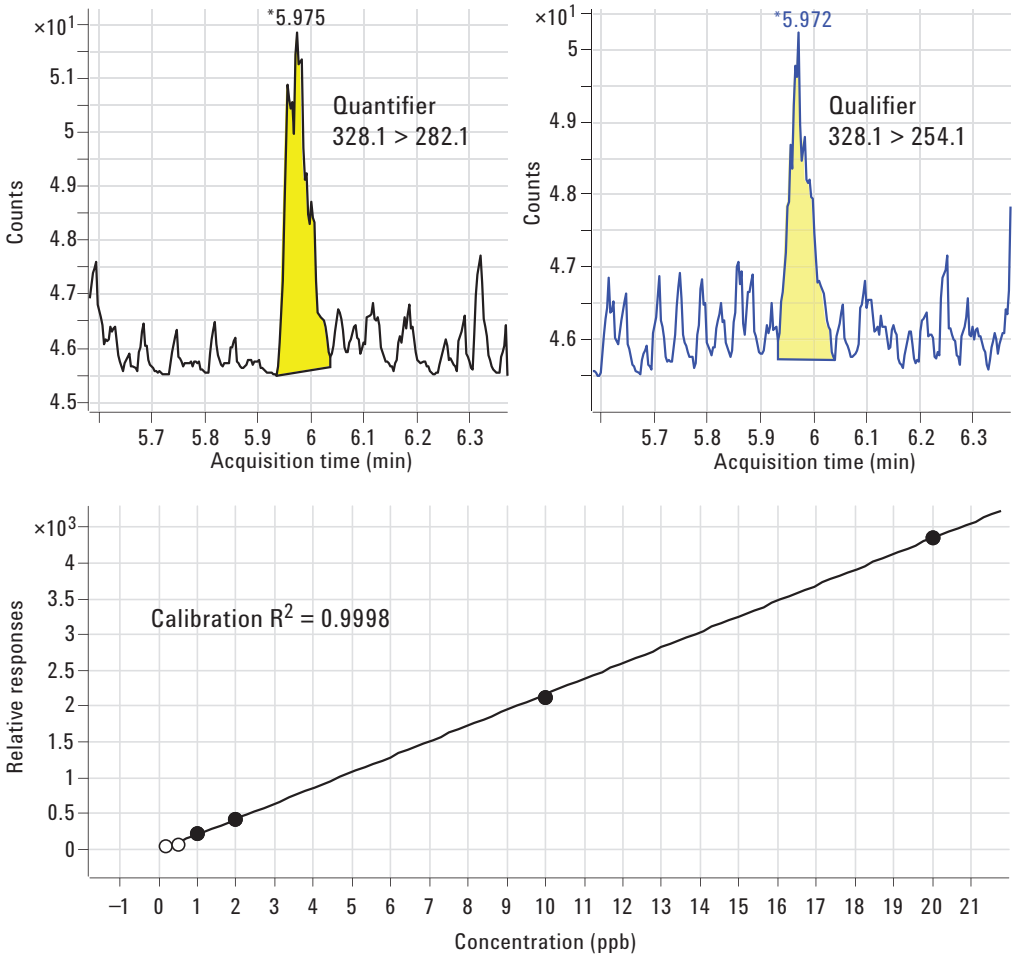


Figure 2. Ion traces for two transitions at the LOD (0.5 ppb standard solution) and calibration curve for fluazifop in baby food.

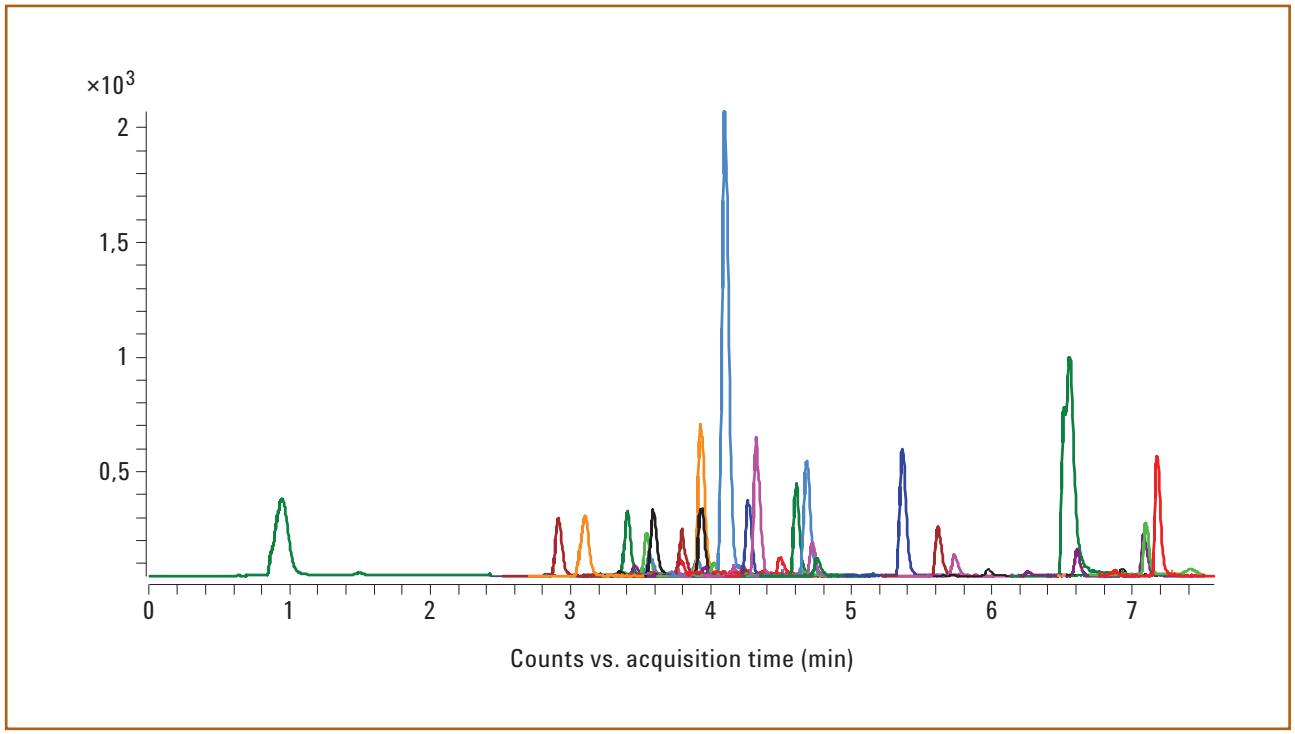


Figure 3. MRM of an extract of baby food sample 2 spiked with 1 ppb (only quantifier transitions are shown). No dispersive SPE performed on the sample. The transition for the internal standard is not shown.

Products used in the above application

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

Agilent Bond Elut AOAC Dispersive SPE Tube for Fatty Samples. Part No. 5982-5158.

Agilent ZORBAX Eclipse Plus RRHD C18 Column, 2.1 x 150 mm, 1.8 µm. Part No. 959759-902

To review this Application Note in its entirety, please search for 5990-5028EN at **agilent.com/chem**

Determination of Sulfonamide Residues in Chicken Muscle by Agilent Bond Elut QuEChERS AOAC Kit and HPLC-FLD (Publication 5990-5395EN)

Introduction

This application describes the use of the QuEChERS sample preparation approach for the determination of sulfonamide drugs in chicken muscle with HPLC-FLD after a pre-column derivatization with fluorescamine. The recoveries ranged from 76.8% to 95.2% and an HPLC-Fluorescence detection (FLD) method was developed and validated for the determination of nine sulfonamides.

The analysis was performed on an Agilent 1200 Infinity Series equipped with a binary pump and a fluorescence detector (FLD) set at λ_{ex} = 405 nm and λ_{em} = 495 nm. Separation of the compounds was achieved on an Agilent ZORBAX Eclipse Plus C18 column. The data was processed by HPLC 2D ChemStation Software.

Instrument conditions

HPLC conditions

Column:	Agilent ZORBAX Eclipse Plus C18, 4.6 × 75 mm, 3.5 µm
Flow rate:	1 mL/min
Column temperature:	25 °C
Injection volume:	5 µL
Mobile phase:	A: 0.05 M Sodium acetate pH 4.5 B: MeOH
Gradient:	Time (min) % B 0 35 35 41 50 55
Detection:	Ex = 405 nm Em = 495 nm

QuEChERS Procedure

Weigh 2 g homogenized chicken muscle into a 50 mL centrifuge tube.

Spike samples with 1,000 µL 20 µg/mL IS, 1,000 µL of 10 µg/mL spiking solution. Shake vigorously 1 min.

Add 8 mL water. Shake vigorously 30 sec.

Add 10 mL 1% HOAc in ACN. Shake vigorously 1 min.

Add Bond Elut QuEChERS AOAC salt packet. Shake 1 min, centrifuge at 4,000 rpm 5 min.

Transfer 6 mL aliquot to Bond Elut QuEChERS dispersive SPE 15 mL tube. Shake 1 min, centrifuge at 4,000 rpm 5 min.

Transfer 4 mL extract to a tube; blow down at 35 °C with N_2.

Reconstitute 200 µL into 600 µL 0.05M NaOAc pH 3.5; add 200 µL 0.02% fluorescamine. Shake 1 min, incubate 60 min.

Samples are ready for HPLC-FLD analysis.

Figure 1. QuEChERS AOAC sample preparation procedure for sulfonamides in chicken.

Results

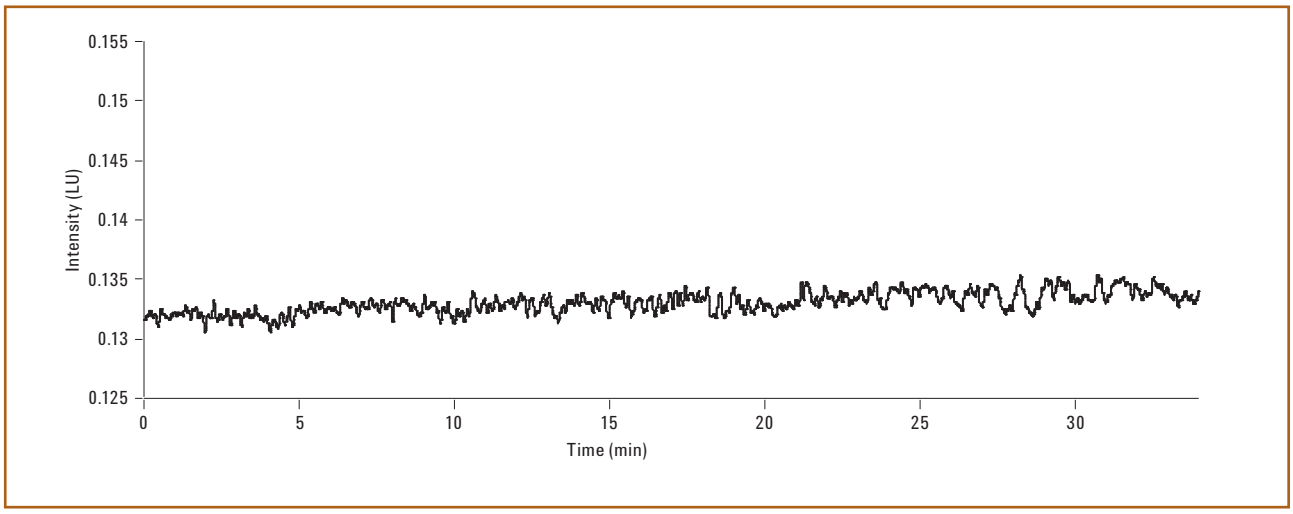


Figure 2. Chromatogram of the blank chicken muscle extract.

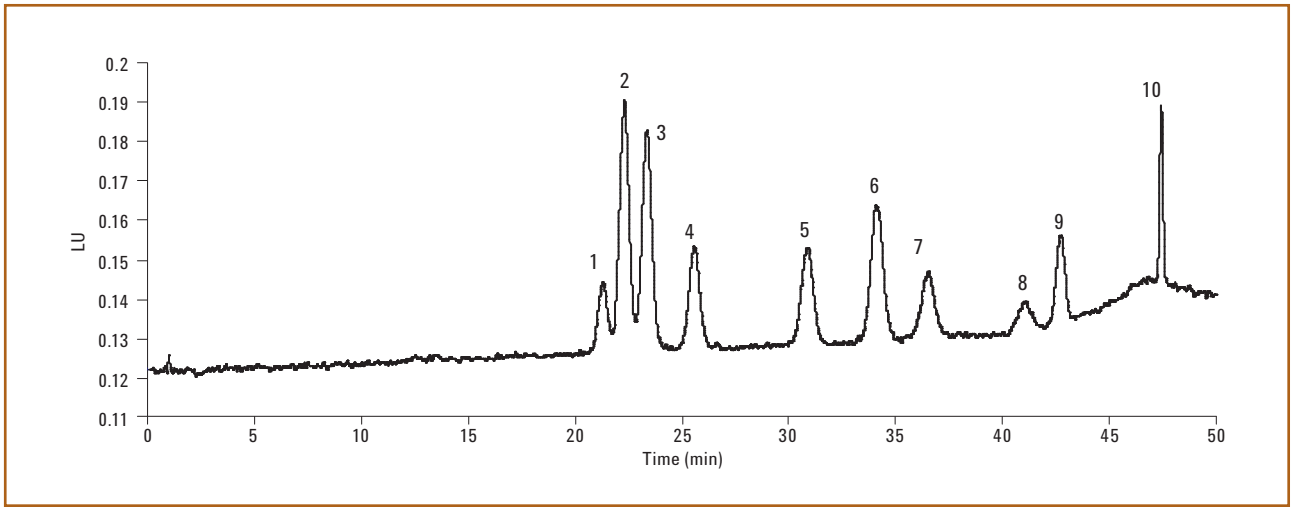


Figure 3. Chromatogram of the spiked chicken muscle extract at 50 ng/g level: 1. Sulfadiazine ; 2. Sulfathiazole; 3. Sulfapyridine (IS); 4. Sulfamerazine; 5. Sulfamethazine; 6. Sulfamethizole; 7. Sulfamethoxypyridazine; 8. Sulfachloropyridazine; 9. Sulfamethoxazole; 10. Sulfadimethoxine.

Products used in the above application

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

Agilent Bond Elut QuEChERS AOAC Dispersive SPE Kit. Part No. 5982-5158.

Agilent ZORBAX Eclipse Plus C18 Column, 4.6 x 75 mm, 3.5 µm. Part No. 959933-902.

To review this Application Note in its entirety, please search for 5990-5395EN at **agilent.com/chem**

Analysis of Polycyclic Aromatic Hydrocarbons in Fish with Agilent Bond Elut QuEChERS AOAC Kit and HPLC-FLD (Publication 5990-5441EN)

Introduction

This application describes the use of the QuEChERS AOAC sample preparation approach for the determination of sixteen polycyclic aromatic hydrocarbons (PAHs) in fish fillets. The analyte recoveries ranged from 83.4% to 101% with relative standard deviations ranging from 0.6 to 1.9% at three different fortification levels.

The analysis was performed on an Agilent 1200 Infinity Series equipped with a binary pump and a fluorescence detector (FLD) and data was processed by HPLC 2D ChemStation software. High recoveries with excellent precision were attained. Therefore, this method is suitable for quality control testing for PAHs in real samples.

Instrument conditions

HPLC conditions

Column:	Agilent ZORBAX Eclipse PAH C18, 4.6 × 50 mm, 1.8 µm
Flow rate:	0.8 mL/min
Column temperature:	18 °C
Injection volume:	5 µL
Mobile phase:	A: Deionized H_2O B: CH_3CN
Gradient:	Time (min) % B 0 60 1.5 60 7 90 13 100
Detection:	UV at 230 nm (Acy) and varying fluorescence excitation (Ex) and emission (Em) wavelengths

Wavelengths:

Time (min)	Ex/Em wavelengths (nm)	PAH detected
0 - 5 (dark blue)	260/352	Nap, Ace, Flu, Phe, Chr
0 - 14 (red)	260/420	Ant, Pyr, BeP, DahA, BghiP
0 - 14 (light blue)	260/460	Fln, 1,2-BaA,BeA, BkF, InP

QuEChERS Procedure

Weigh 5 g homogenized fish sample into a 50 mL centrifuge tube.

Spike samples with 2,000 µL spiking solution. Shake vigorously 1 min.

Add 8 mL ACN. Shake vigorously 1 min.

Add 10 mL 1% HOAc in ACN. Shake vigorously 1 min.

Add Bond Elut QuEChERS AOAC salt packet. Shake 1 min, centrifuge at 4,000 rpm 5 min.

Transfer 6 mL aliquot to Bond Elut QuEChERS dispersive SPE 15 mL tube. Shake 1 min, centrifuge at 4,000 rpm 5 min.

Filter through a 0.45 µm PVDF syringe filter.

Transfer 1 mL extract to an autosampler vial.

Samples are ready for HPLC-FLD analysis.

Figure 1. QuEChERS AOAC sample preparation procedure for PAHs in fish.

Results

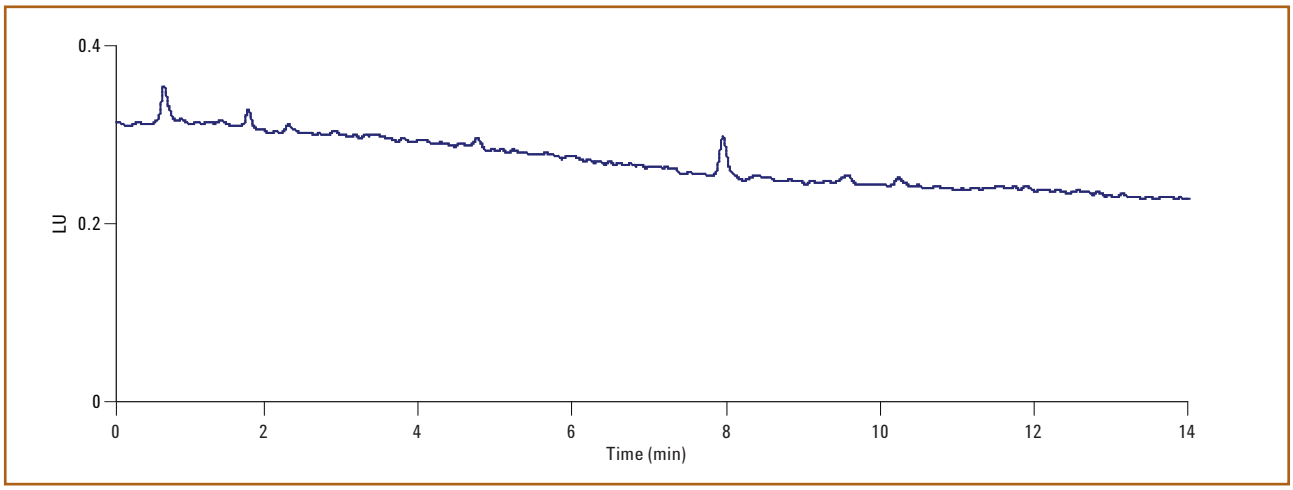


Figure 2. Chromatogram of the blank fish extract. Chromatographic conditions are shown in Table 1 of the app note. The baseline chromatogram used the following excitation/emission wavelengths: 260-nm/352-nm. The other excitation/emission conditions showed no other interferences.

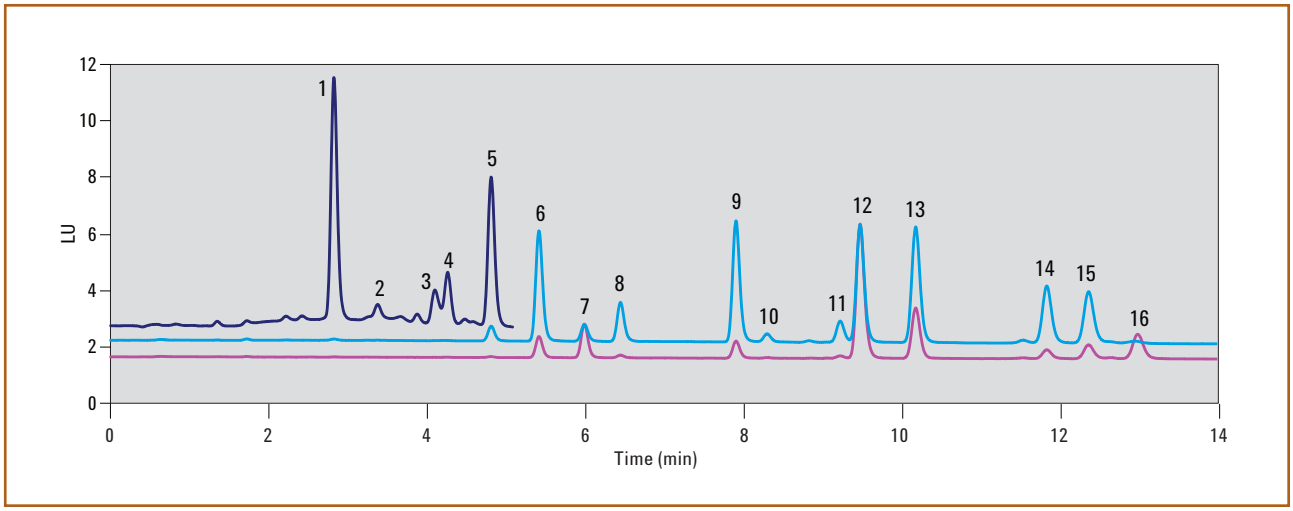


Figure 3. Overlay HPLC – FLD chromatograms of the spiked fish sample containing: 1. Nap; 2. Acy; 3. Ace; 4. Flu; 5. Phe; 6. Ant; 7. Fln; 8. Pyr; 9. BaA; 10. Chr; 11. BeP; 12. BeA; 13. BkF; 14. DahA; 15. BghiP; 16. InP. The spiking level for this sample was level 1 (see Table 3 of the app note). The blue portion of the chromatogram used the following excitation/emission wavelengths: 260-nm/352-nm; the red portion: 260-nm/420-nm; the light blue portion: 260-nm/440-nm. For acenaphthylene, UV detection at 230-nm was used.

Products used in the above application

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

Agilent Bond Elut AOAC Fatty Dispersive SPE Kit, 15 mL. Part No. 5982-5158.

Agilent ZORBAX Eclipse PAH C18 Column, 4.6 × 50 mm, 1.8 µm. Part No. 959941-918.

To review this Application Note in its entirety, please search for 5990-5441EN at **agilent.com/chem**

Analysis of Acrylamide in Fried Potatoes using Agilent Bond Elut QuEChERS and LC/MS/MS (Publication 5990-5940EN)

Introduction

This application describes the use of a QuEChERS Acrylamide extraction step combined with an AOAC dSPE cleanup sample preparation approach for the analysis of acrylamide in fried potatoes, using LC/MS/MS for detection and $^{13}C_3$-acrylamide as the internal standard. The acrylamide recoveries ranged from 97% to 116%. An Agilent 1200 Infinity Series equipped with an Agilent 6460 Triple Quadrupole LC/MS was used to perform the analysis.

A simple and fast method based on Agilent Bond Elut QuEChERS for acrylamide extraction and cleanup with LC/MS/MS analysis was developed with high extraction yields and excellent precision.

Instrument conditions

LC/MS/MS conditions

Column:	Reversed C18, 2.1 × 150 mm, 3 µm
Column temperature:	30 °C
Isocratic mode (%B):	2.5% Methanol/97.5% of 0.1% Formic acid
Flow rate:	0.2 mL/min
Injection volume:	10 µL
Run time:	7 min
Post run time:	3 min
Mass spectrometer:	Positive electrospray ionization mode with jet stream technology
Capillary voltage:	4,000
Nozzle voltage:	500 V
Sheath gas temperature:	325 °C at 5 L/min
Drying gas temperature:	350 °C at 11 L/min

QuEChERS Procedure

Weigh 1 g fried potatoes in a 50 mL centrifuge tube.

Spike sample with 500 µL 1,000 ng/mL $^{13}C_3$-acrylamide.

Add 5 mL hexane, vortex.

Add 10 mL water, 10 mL ACN, Bond Elut QuEChERS Acrylamide salt packet. Shake vigorously for 1 min.

Centrifuge for 5 min at 5,000 rpm.

Discard the hexane layer.

Transfer 1 mL upper ACN layer to 2 mL microcentrifuge vial packed with 50 mg PSA and 150 mg $MgSO_4$.

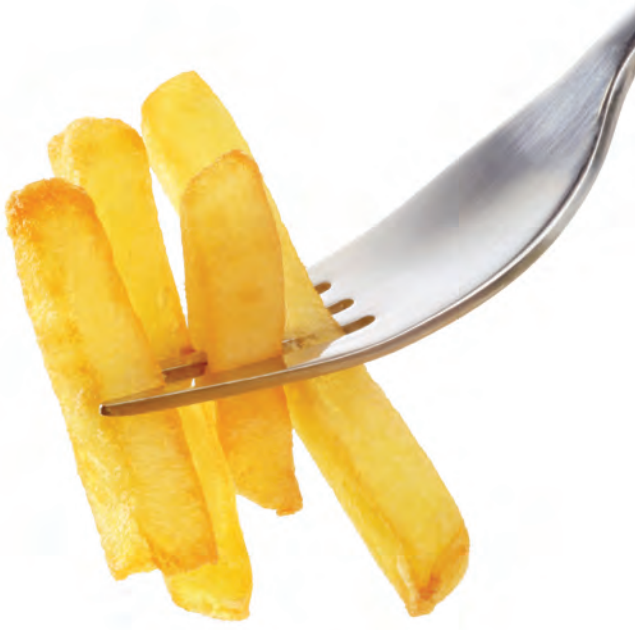
Vortex for 30 sec.

Centrifuge for 1 min at 5,000 rpm.

Transfer 500 µL extract to an autosampler vial.

Analyze by LC/MS/MS.

Figure 1. QuEChERS sample preparation procedure for acrylamides in fried potatoes.



Results

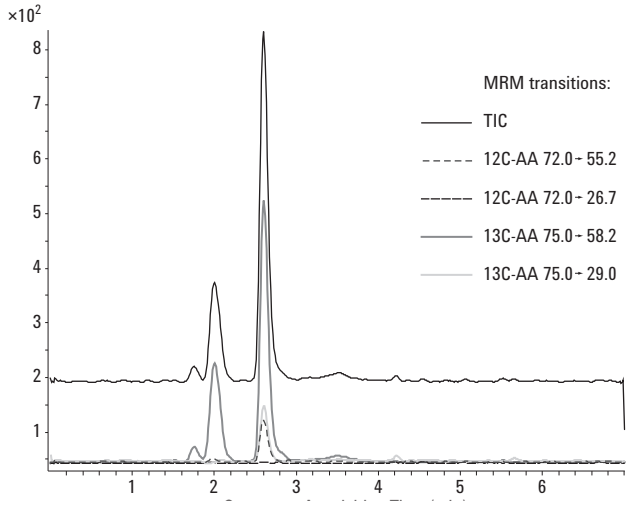


Figure 2. Chromatogram of the 10 ng/mL acrylamide standard and 500 ng/m: internal standard $^{13}C_3$-acrylamide.

Table 1. Recoveries and RSDs for acrylamide in spiked fried potato samples and 1:1 water:acetonitrile (n=3)

Matrix	Concentration of acrylamide spike (ng/mL)	%Recovery (n=3)	%RSD (n=3)
1:1 Water:ACN	50	116.6	4.07
1:1 Water:ACN	100	114.06	4.85
Fried potatoes	100	97.14 (after blank correction)	5.04
Fried potatoes	200	97.50 (after blank correction)	2.55

Products used in the above application

Agilent Bond Elut QuEChERS Extraction Kit for Acrylamides. Part No. 5982-5850.

Agilent Bond Elut AOAC Dispersive SPE Kit, 2 mL. Part No. 5982-5022.

To review this Application Note in its entirety, please search for 5990-5940EN at **agilent.com/chem**

GC/μECD Analysis and Confirmation of PCBs in Fish Tissue with Agilent J&W DB-35ms and DB-XLB GC Columns (Publication 5990-6236EN)

Introduction

This application describes the use of the QuEChERS sample preparation approach for the extraction and cleanup of 19 PCB (polychlorinated biphenyl) congeners in fish tissue. Spiked recoveries ranged between 72 and 116%. An Agilent 7890A GC system equipped with dual μECD detection and dual capillary GC column was used, enabling simultaneous identification and confirmation from a single injection. The GC was also fitted with an unpurged two-way splitter capillary flow technology (CFT) device.

QuEChERS Procedure

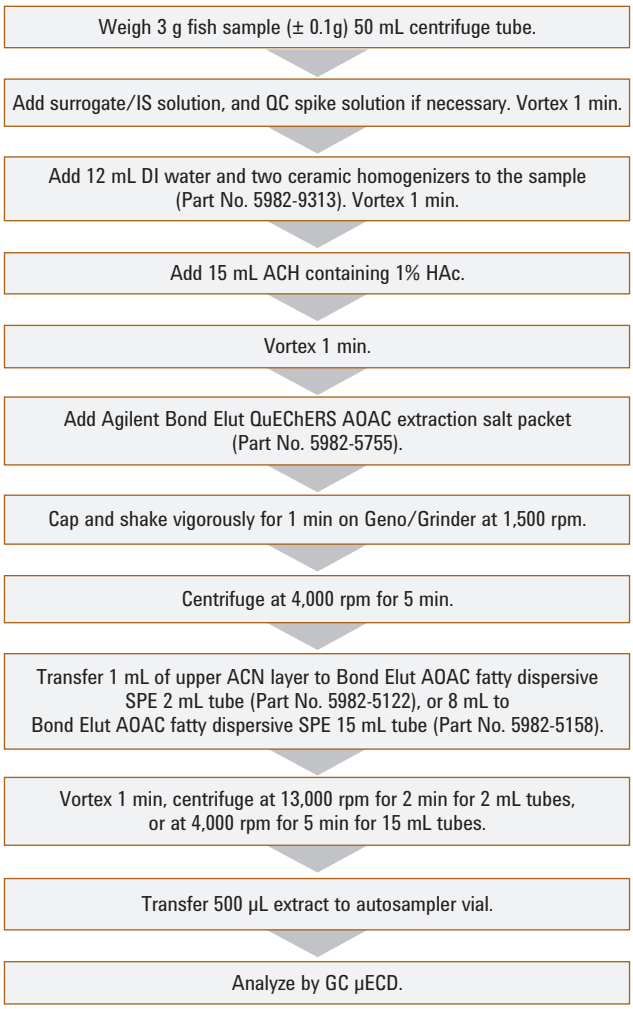


Figure 1. Agilent Bond Elut QuEChERS modified AOAC extraction procedure for fish.

Instrument conditions

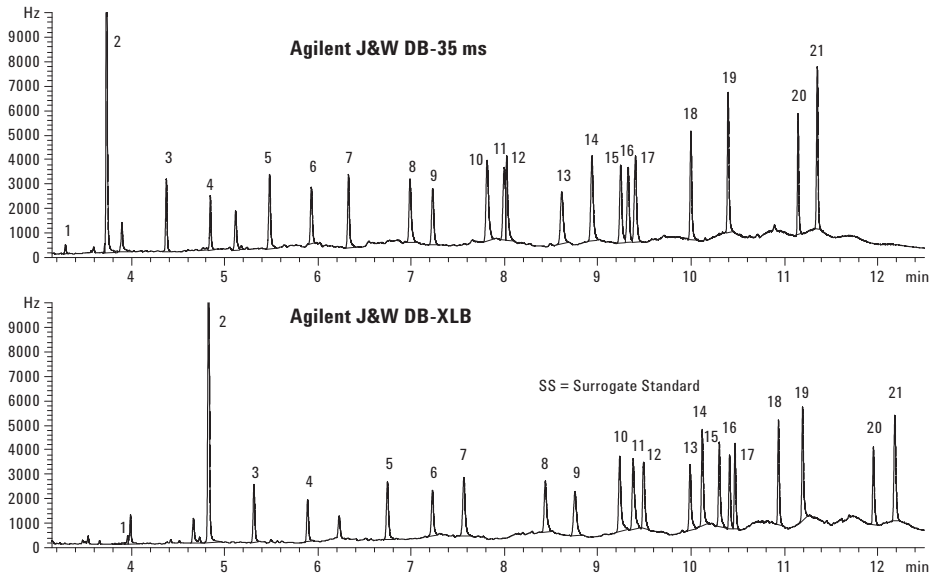
HPLC conditions

Column 1:	Agilent J&W DB-35 ms, 30 m × 0.25 mm, 0.25 μm (Part No. 122-3832)
Column 2:	Agilent J&W DB-XLB, 30 m × 0.25 mm, 0.50 μm (Part No. 122-1236)
GC:	Agilent 7890A equipped with dual μECD detection
Sampler:	Agilent 7873B 5.0 μL syringe (Part No. 5181-1273)
CFT device:	2-way unpurged splitter capillary flow technology (Part No. G3181B)
Carrier:	Hydrogen 85 cm/s, constant flow 3.5 mL/min
Injection:	1.0 μL splitless; 250 °C, Purge flow 50 mL/min at 0.3 min, Gas saver 50 mL/min at 2 min
Oven:	110 °C (0.1 min), 25 °C/min to 200 °C (0.5 min), 10 °C/min to 240 °C (0.5 min), 30 °C/min to 325 °C (1.5 min)
Injection:	1 μL, 250 °C splitless, purge 50 mL/min at 0.3 min, gas saver 50 mL/min on at 2 min
Dual μECD:	350 °C, N_2 makeup; constant column + makeup = 30 mL/min

Flow path supplies

Vials:	Amber screw top glass vials (Part No. 5183-2072)
Vial caps:	Blue screw caps (Part No. 5182-0717)
Vial inserts:	100 μL glass/polymer feet (Part No. 5181-8872)
Syringe:	5 μL (Part No. 5181-1273)
Septum:	Advanced Green (Part No. 5183-4759)
Inlet seal:	Gold plated inlet seal (Part No. 5188-5367)
Inlet liners:	Deactivated dual-taper direct connect (Part No. G1544-80700)
Ferrules:	0.4 mm id short; 85/15 Vespel/graphite (Part No. 5181-3323)
CFT fittings:	Internal nut (Part No. G2855-20530)
CFT ferrules:	SilTite ferrules, 0.25 mm id (Part No. 5188-5361)
20x Magnifier:	20x Magnifier loop (Part No. 430-1020)

Results



1. IUPAC 1
2. Trichloro-m-xylene (SS)
3. IUPAC 5
4. IUPAC 18
5. IUPAC 31
6. IUPAC 52
7. IUPAC 44
8. IUPAC 66
9. IUPAC 101
10. IUPAC 87
11. IUPAC 110
12. IUPAC 151
13. IUPAC 153
14. IUPAC 141
15. IUPAC 138
16. IUPAC 187
17. IUPAC 183
18. IUPAC 180
19. IUPAC 170
20. IUPAC 206
21. Decachlorobiphenyl (SS)

Figure 2. GC/μECD chromatogram of the 50 ng/mL fortified fish extract analyzed on Agilent J&W DB-35ms and DB-XLB GC columns. Chromatographic conditions are listed in Table 1 of app note.

Products used in the above application

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

Agilent Bond Elut QuEChERS AOAC Fatty Sample Dispersive SPE,
2 mL Tube, Part No. 5982-5122 or 15 mL Tube. Part No. 5982-5158.

Agilent J&W DB-35ms Column, 30 m x 0.25 mm, 0.25 µm. Part No. 122-3832.

Agilent J&W DB-XLB Column, 30 m x 0.25 mm, 0.50 µm. Part No. 122-1236.

To review this Application Note in its entirety, please search for 5990-6236EN at **agilent.com/chem**

Analysis of Pesticide Residues in Lettuce and Apple Samples using Agilent Bond Elut QuEChERS AOAC Extraction Kit with Universal Dispersive SPE (Publication 5990-6558EN)

Introduction

This application describes the use of the AOAC buffered extraction method followed by the use of the universal dispersive SPE method for preparing lettuce and apple samples for residue analysis by GC/MS. Twenty-six pesticides of different classes were studied. The analysis was performed using an Agilent 7890 GC System with an Agilent 5975C Series GC/MSD using selective ion monitoring (SIM) mode. All compounds were free of interferences and gave excellent linearity.

Instrument conditions

GC conditions

Column:	Agilent J&W HP-5ms Ultra Inert, 30 m x 0.25 mm, 0.25 µm (Part No. 190915-433UI)
Injection source:	Agilent 7683 Automatic Liquid Sampler with 100 sample tray
Inlet:	Splitless
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 min
Multiplier voltage:	Autotune voltage

QuEChERS Procedure

Weigh 15 g sample into 50 mL centrifuge tube.

Spike sample with IS and spiking solution. Vortex for 1 min.

Add 15 mL ACN (1% acetic acid). Shake vigorously for 30 sec.

Add salt packet for AOAC buffered extraction.
Shake for 1 min, centrifuge at 3,000 rcf for 2 min.

Transfer 1 mL aliquot ACN layer to universal dispersive SPE tube (containing $MgSO_4$, PSA, C18 and GCB).
Vortex for 30 sec, centrifuge for 2 min.

Transfer 0.5 mL to autosampler vial.

Figure 1. QuEChERS sample preparation procedure for pesticides in lettuce and apple.

Results

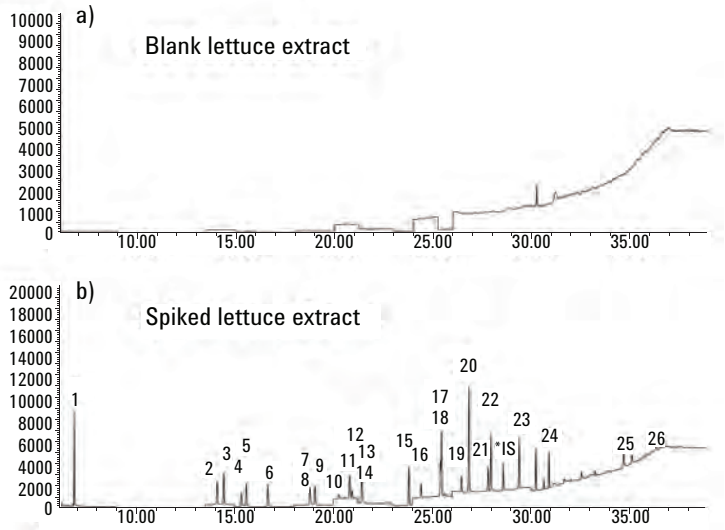


Figure 2. GC/MS of lettuce extracts. (a) blank lettuce extract and (b) spiked lettuce extract after QuEChERS sample preparation.

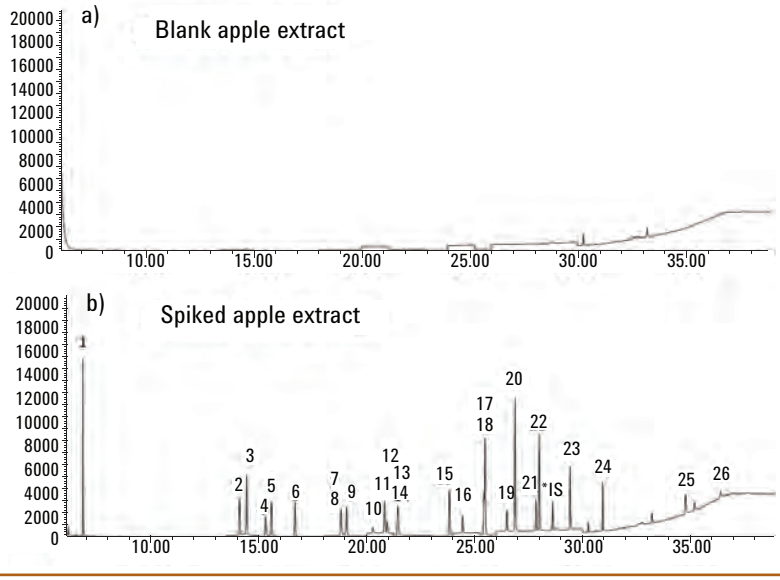


Figure 3. GC/MS of apple extracts (a) blank apple extract and (b) spiked apple extract after QuEChERS sample preparation.

Products used in the above application

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

Agilent Bond Elut QuEChERS AOAC Dispersive Kit for All Food Types. Part No. 5982-0029.

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

To review this Application Note in its entirety, please search for 5990-6558EN at **agilent.com/chem**

Organophosphorus Pesticides in Apple Matrix by GC/MS/FPD using an Agilent J&W DB-35ms Ultra Inert GC Column (Publication 5990-7165EN)

Introduction

This application details a quick and effective analytical method for the determination of low ppm and trace level organophosphorus (OP) pesticides residues in apple extract. Using an ultra-inert mid-polarity GC column, an inert flow path with backflushing, and QuEChERS sample cleanup, good results were achieved, even for the more polar OP pesticides. An Agilent 7890 GC with an Agilent 5975C MSD equipped with a flame photometric detector and Agilent 7683B Automatic Liquid Sampler were used for this series of experiments.

QuEChERS Procedure

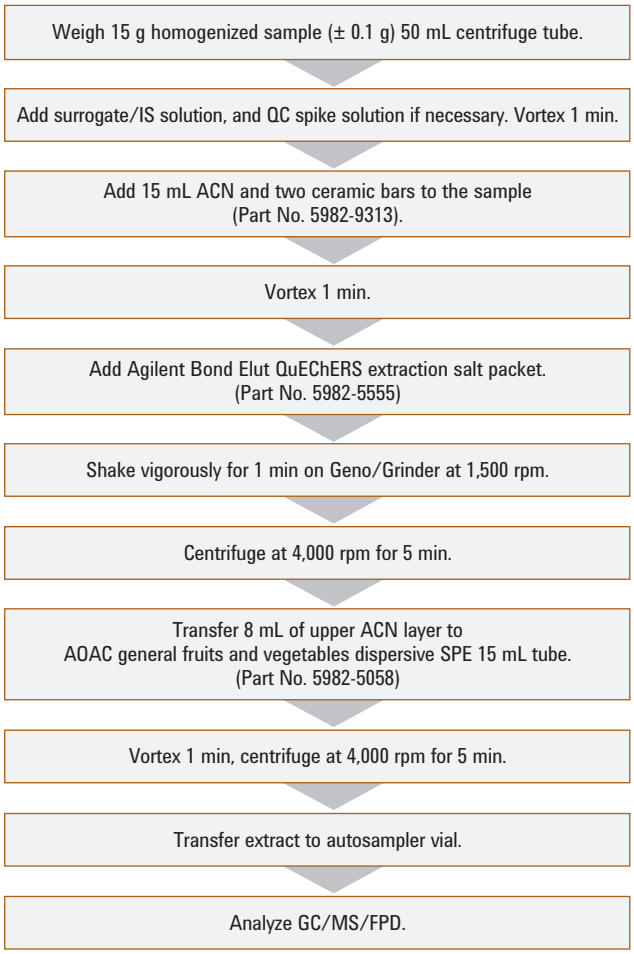


Figure 1. Agilent QuEChERS extraction procedure for apple samples.

Instrument conditions

GC conditions

Column:	Agilent J&W DB-35ms Ultra Inert, 20 m × 0.18 mm, 0.18 µm (Part No.121-3822UI)
GC/MSD:	Agilent 7890 GC/Agilent 5975C Series GC/MSD
Sampler:	Agilent 7683B Automatic Liquid Sampler, 5.0 µL syringe (Part No. 5181-1273)
CFT device:	Purged 2-way splitter (Part No. G3180B) Split ratio 3:1 MSD:FPD
MSD restrictor:	1.2 m × 0.15 mm id deactivated fused silica tubing
FPD restrictor:	1.4 m × 0.15 mm id deactivated fused silica tubing
PCM 1:	3.8 psi constant pressure
Inlet:	1 µL splitless; 250 °C, purge flow 60 mL/min at 0.25 min, gas saver on at 2 min 20 mL/min
Carrier:	Helium, constant pressure 43.5 psi at 95 °C
Oven:	95 °C (1.3 min), 15 °C/min to 125 °C, 5 °C/min to 165 °C, 2.5 °C/min to 195 °C, 20 °C/min to 280 °C (3.75 min)
Postrun backflush:	5 min at 280 °C, PCM 1 pressure 70 psi during backflush, 2 psi inlet pressure during backflush
MSD:	310 °C transfer line, 310 °C source, 150 °C quad

Flow path supplies

Vials:	Amber crimp top glass vials (Part No. 5183-4496)
Vial caps:	Crimp caps (Part No. 5181-1210)
Vial inserts:	250 µL glass/polymer feet (Part No. 5181-8872)
Syringe:	5 µL (Part No. 5181-1273)
Septum:	Advanced Green (Part No. 5183-4759)
Inlet liner:	Deactivated dual-taper helix liner (Part No. 5188-5398)
Ferrules:	0.4 mm id short; 85/15 Vespel/graphite (Part No. 5181-3323)
PCT fittings:	Internal nut (Part No. G2855-20530)
PCT ferrules:	SilTite ferrules, 0.25 mm id (Part No. 5188-5361)
20x Magnifier:	20x Magnifier loop (Part No. 430-1020)

Results

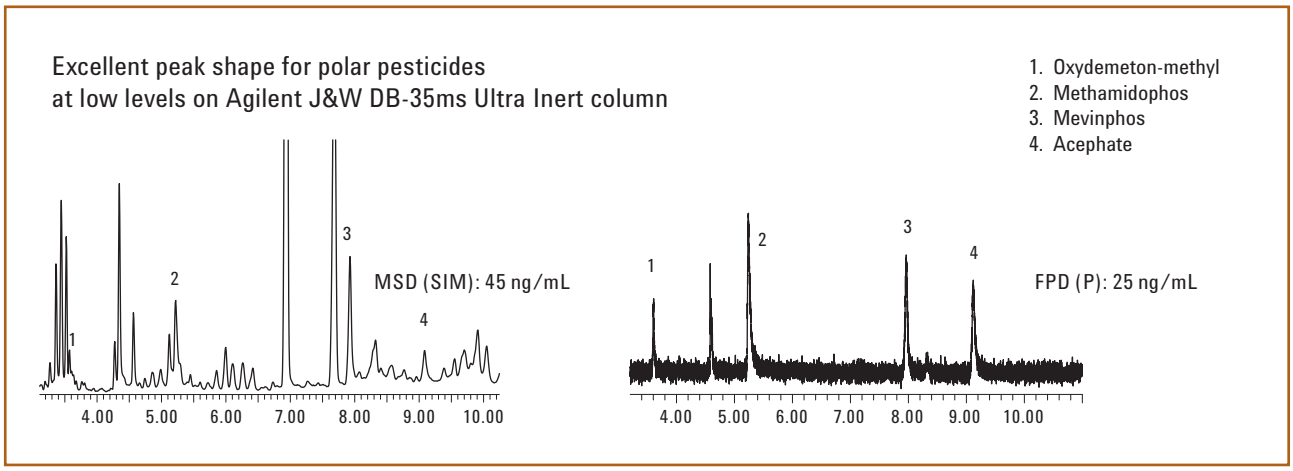


Figure 2. Enlarged section of GC/MS/SIM and FPD chromatograms of the more problematic polar pesticides analyzed on an Agilent J&W DB-35ms Ultra Inert capillary column. Chromatographic conditions are listed in Table 1 of the app note. The effluent split ratio was MSD:FPD = 3:1.

Products used in the above application

Agilent Bond Elut QuEChERS Extraction Salt Packet. Part No. 5982-5555.

Agilent Bond Elut QuEChERS General Fruits and Vegetables Dispersive SPE, 15 mL Tube. Part No. 5982-5058.

Agilent J&W DB-35ms Ultra Inert Column, 20 m x 0.18 mm, 0.18 µm. Part No. 121-3822UI.

To review this Application Note in its entirety, please search for 5990-7165EN at **agilent.com/chem**

Analysis of Pesticide Residues in Rice using Agilent Bond Elut QuEChERS AOAC Kit by LC/MS/MS Detection (Publication 5990-8034EN)

Introduction

This application describes the use of the QuEChERS AOAC sample preparation approach for the extraction and cleanup of 12 pesticide residues representing various pesticides classes in rice. The analysis was performed on an Agilent 1200 Infinity Series and an Agilent 6460 Triple Quadrupole LC/MS with electrospray ionization. The mean recoveries ranged between 76% and 108% (average of 97.8%).

Instrument conditions

HPLC conditions

Column:	Agilent Poroshell 120 EC-C18, 2.1 × 100 mm, 2.7 µm (Part No. 695775-902)
Flow rate:	0.4 mL/min
Column temperature:	30 °C
Injection volume:	5 µL
Mobile phase:	A: 0.1% FA in water
	B: 0.1% FA in ACN

Gradient:

Time (min)	%B
0	5
1	5
3	50
7	90
8	90
8.2	5
9	5

Post run:	2 min
Total cycle time:	11 min

MS conditions
Positive mode

Gas temperature:	350 °C
Gas flow:	10 L/min
Nebulizer:	40 psi
Capillary:	3,500 V

QuEChERS Procedure

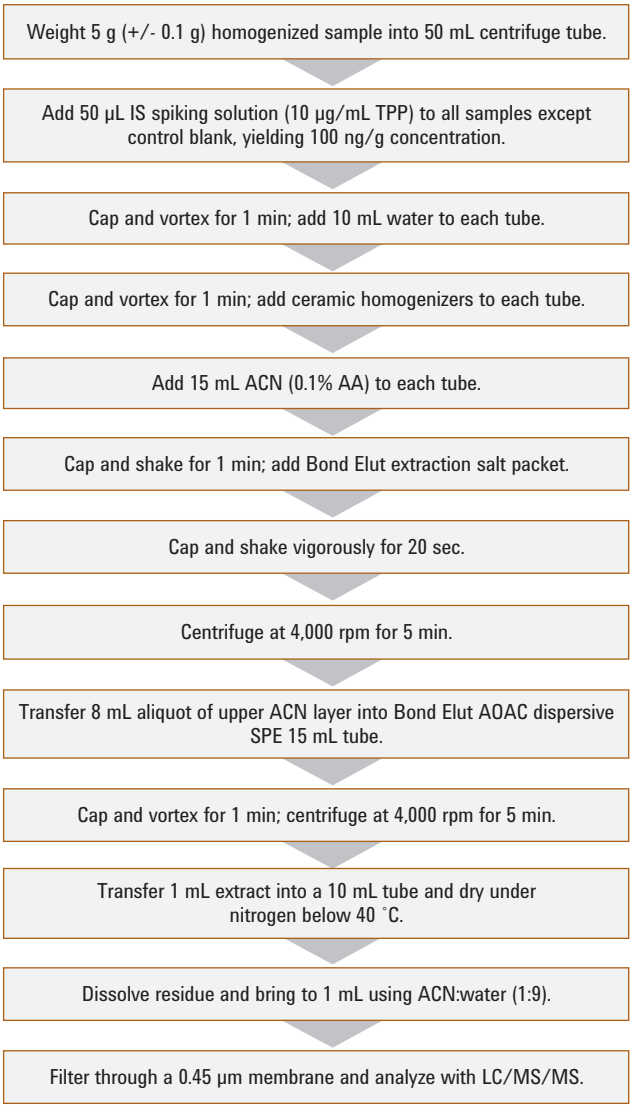


Figure 1. Agilent QuEChERS extraction procedure for rice samples.

Results

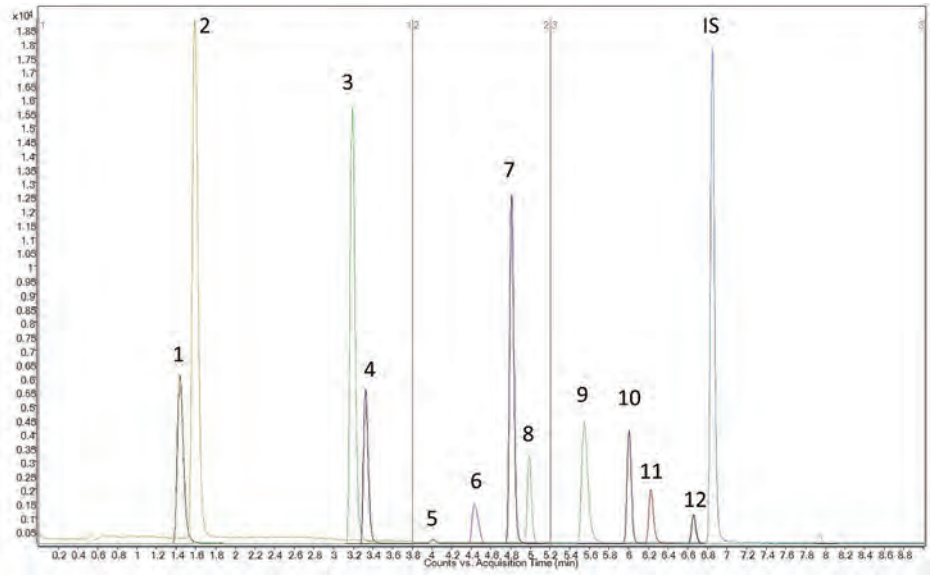


Figure 2. MRM chromatograms of 10 ng/g fortified sample processed by AOAC method. Peak identification: 1. Pymetrozine, 2. Acephate, 3. Carbendazim, 4. Thiabendazole, 5. Imidacloprid, 6. Imazalil, 7. Propoxur, 8. Carbaryl, 9. Cyprodinil, 10. Ethoprophos, 11. Penconazole, 12. Kresoxim-methyl, IS: TPP.

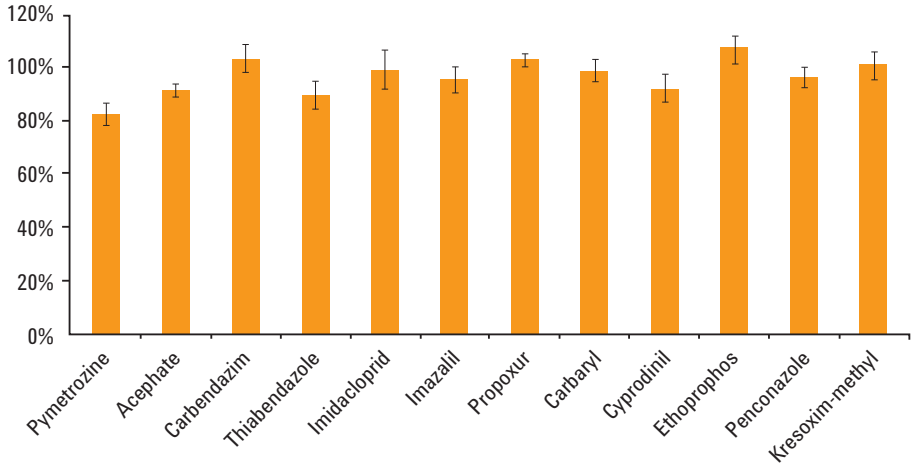


Figure 3. The recovery and precision results of 12 pesticides in rice.

Products used in the above application

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

Agilent Bond Elut QuEChERS AOAC Dispersive SPE Kits for Fruits and Vegetables with Fats and Waxes. Part No. 5982-5158.

Agilent ceramic homogenizers, 50 mL tubes. Part No. 5982-9313.

Agilent Poroshell 120 EC-C18 Column, 2.1 × 100 mm, 2.7 µm. Part No. 695775-902.

To review this Application Note in its entirety, please search for 5990-8034EN at **agilent.com/chem**

Quantitative and Repeatability Analysis of Trace Level Pesticides in Produce and Grains by GC/MS/MS (Publication 5990-9317EN)

Introduction

This application describes the use of the QuEChERS sample preparation approach for the extraction of 33 representative pesticides in six different types of produce and grains, including pear, orange, strawberry, flour, pepper, and spinach. This analysis was performed on the Agilent 7890 GC and the Agilent 7000 Triple Quadrupole GC/MS/MS Analyzer, which has a Pesticides and Environmental Pollutants MRM database.

QuEChERS Procedure

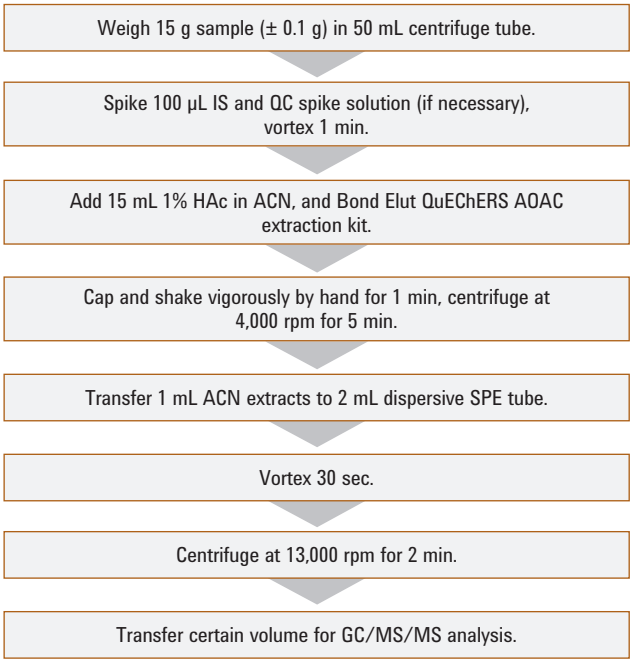


Figure 1. QuEChERS AOAC extraction procedure for produce and grains.

Instrument conditions

GC conditions

Analytical column:	Agilent J&W HP-5ms Ultra Inert, 15 m x 0.25 mm, 0.25 µm (Part No. 19091-431UI)
Column connections:	Between inlet and Purged Ultimate Union (Part No. G3182-61580)
GC:	Agilent 7890 Series GC
Autosampler:	Agilent 7693 Autosampler and sample tray 5 µL syringe (Part No. 5181-5246), 1 µL injection volume Postinj solvent A (acetone) washes: 3 Postinj solvent B (acetonitrile) washes: 3 Sample pumps: 3
Carrier gas:	Helium, constant pressure
Inlet:	Multimode Inlet (MMI)
Inlet temperature:	280 °C
Injection mode:	Pulsed splitless mode
Injection pulse pressure:	36 psi until 1 min
Purge flow to split vent:	50 mL/min at 1 min
Inlet pressure:	18.35 psi (RT locked) during run, and 1.0 psi during backflush
RT locking:	Chlorpyrifos methyl at 8.298 min
Oven profile:	100 °C for 2 min, to 150 °C at 50 °C/min, to 200 °C at 6 °C/min, to 280 °C at 16 °C/min and hold for 6 min
Post run:	2 min at 280 °C
Capillary flow technology:	Purged Ultimate Union (Part No. G3182-61580) – used for backflushing the analytical column and inlet. Aux EPC gas: helium plumbed to Purged Ultimate Union
Bleed line:	0.0625 in od × 0.010 in id × 100 cm, 316 SS tubing, on top of the oven
Aux pressure:	4 psi during run, 75 psi during backflushing
Restrictor:	Inert fused silica tubing, 0.65 m × 0.15 mm (Part No. 160-7625-5)
Restrictor connections:	Between Purged Ultimate Union and the MS
MS:	Agilent 7000 Triple Quadrupole GC/MS
Mode:	MRM
Database:	Agilent Pesticides and Environmental Pollutants database (Part No. G9250AA)
Transfer line temperature:	280 °C
Source temperature:	300 °C
Quad temperature:	Q1 and Q2 = 150 °C
Solvent delay:	2.3 min
Collision gas flows:	Helium quench gas at 2.35 mL/min, N_2 collision gas at 1.5 mL/min
MS resolution:	MS1 and MS2 = 1.2 amu (Low resolution or Wide setting)

Results

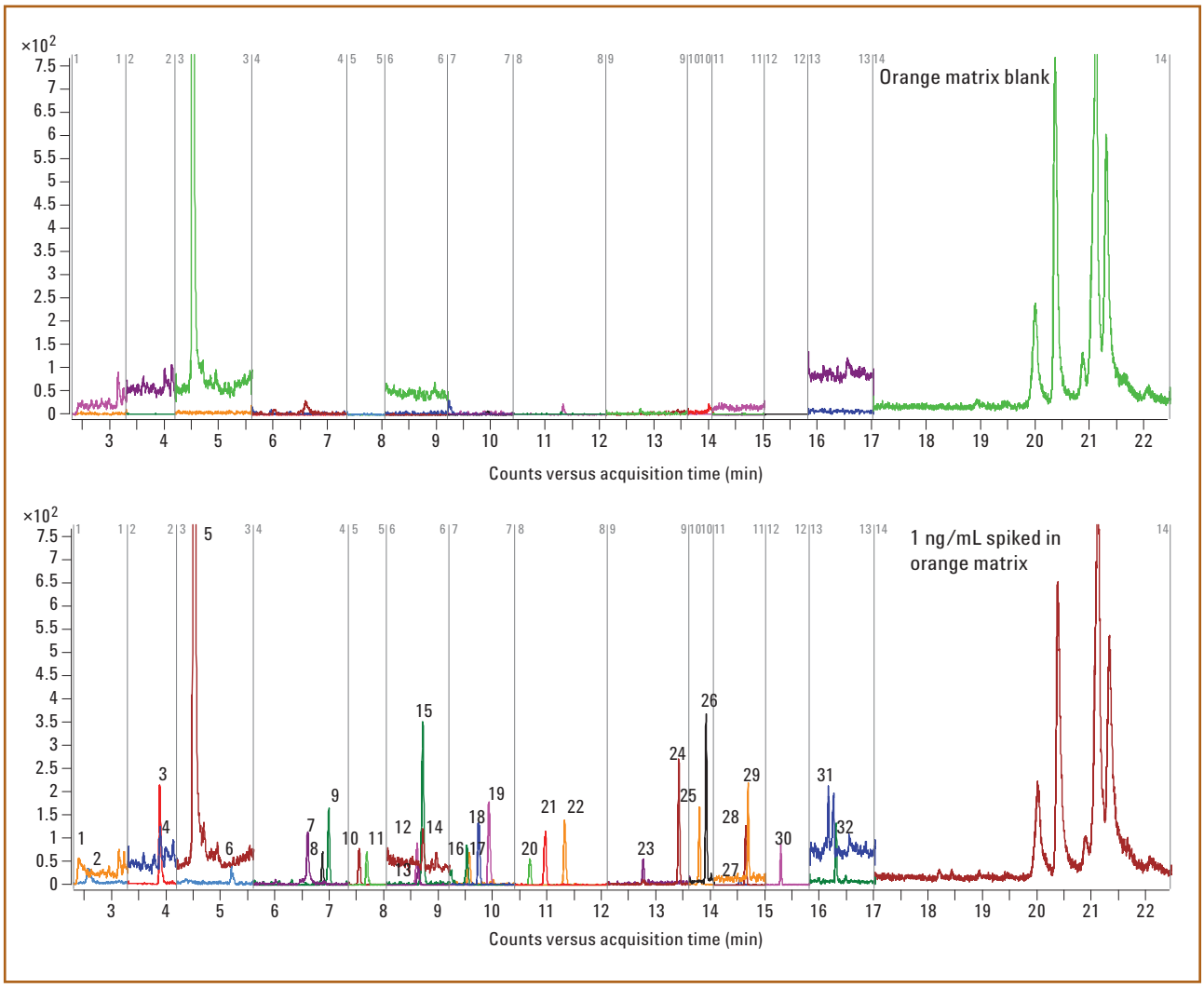


Figure 2. GC/QQQ MRM chromatograms for orange matrix blank and orange matrix spiked with 1 ng/mL pesticides. Refer to Table 3 in the app note for peak identification. Deltamethrin (33) was not identified at 1 ng/mL in orange matrix due to low responses.

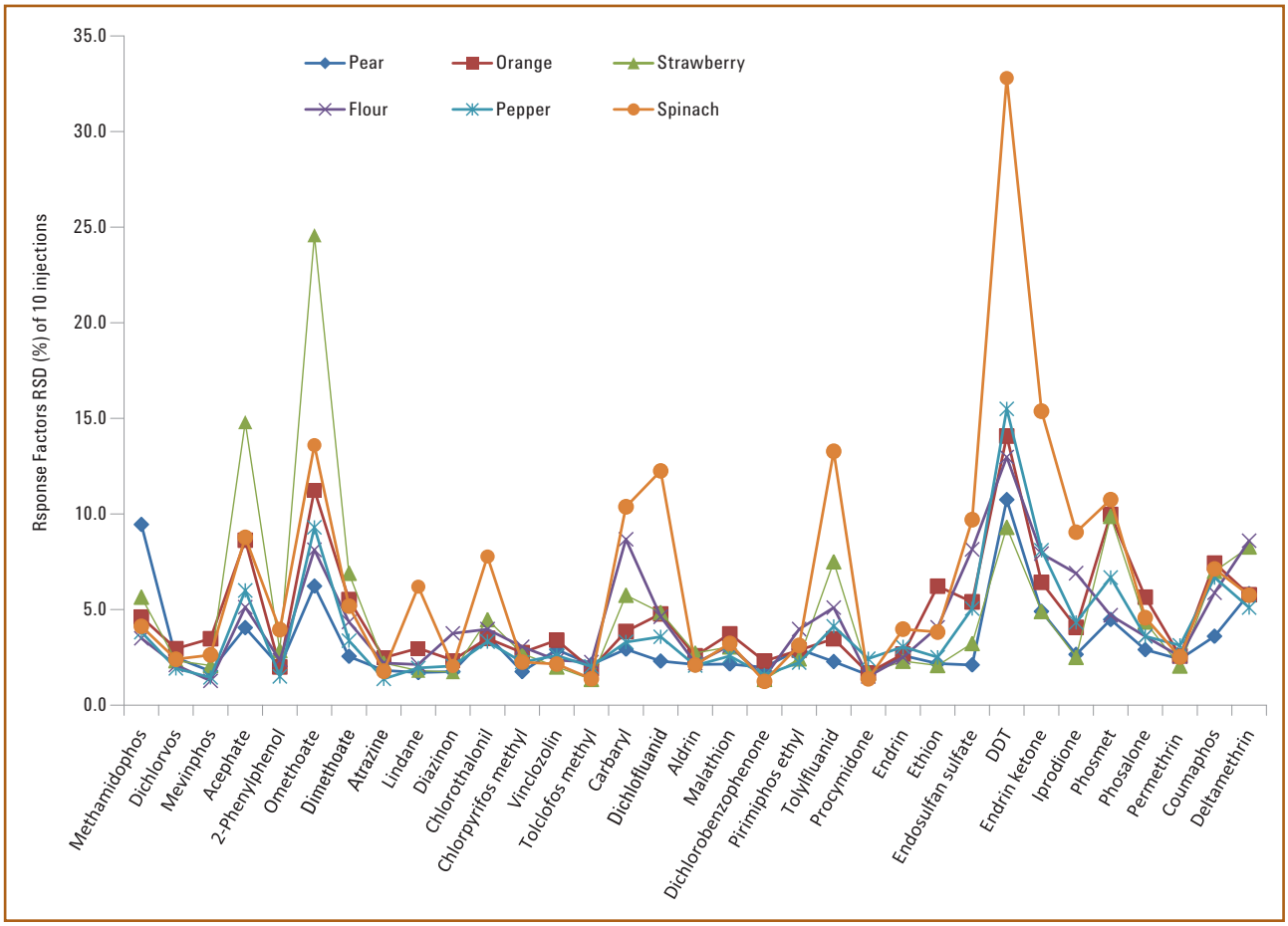


Figure 3. Repeatability (% RSD in response factors) of 10 injections in different matrixes.

Products used in the above application

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

Agilent Bond Elut QuEChERS General Dispersive SPE Kit. Part No. 5982-5022.

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

Agilent Pesticides and Environmental Pollutants MRM Database. Part No. G9250AA.

To review this Application Note in its entirety, please search for 5990-9317EN at **agilent.com/chem**

Comprehensive Pesticide Analysis in Juice using a Combination of GC/MS and LC/MS Methods (Publication 5990-9924EN)

Introduction

This application describes the use of the QuEChERS sample preparation approach for the effective use of three mass spectral techniques – GC/MS, GC/MS/MS, and LC/MS/MS – for the comprehensive analysis of 39 pesticide residues in vegetable juice. The GC/MS experiments were performed on an Agilent 7890A Series GC coupled to an Agilent 5975C Series GC/MS inert XL MSD with triple axis detector and operated in electron ionization (EI) mode. The GC/MS/MS experiments were performed on an Agilent 7890A Series GC coupled to an Agilent 7000B Triple Quadrupole GC/MS operated in EI mode. The LC/MS/MS experiments were performed on an Agilent 1200 Infinity Series coupled to an Agilent 6460 Series Triple Quadrupole LC/MS System with Jet Stream technology. This comprehensive approach covers a wide range of pesticide classes for confident screening and confirmation of 39 pesticides in vegetable juice.

Note: The procedure below represents a general approach to the extraction of beverages using QuEChERS extraction and dSPE cleanup steps.

QuEChERS Procedure

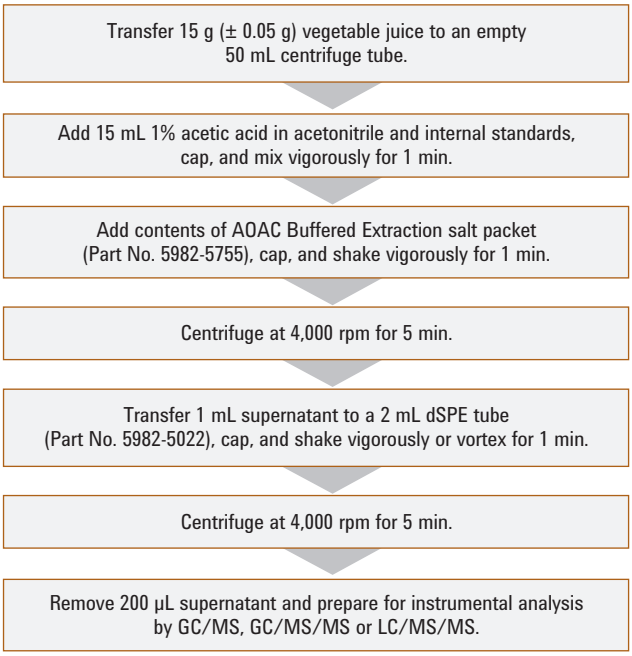


Figure 1. The Agilent QuEChERS extraction procedure. Note: This represents a general approach to the extraction of beverages using QuEChERS extraction and dispersive SPE clean-up steps.

GC/MS conditions

GC conditions

Columns:	Agilent J&W HP-5ms Ultra Inert, 15 m × 0.25 mm, 0.25 µm (Part No. 19091S-431UI)
Injection volume:	1 µL
Injection mode:	Cold splitless using a Multimode Inlet
Inlet temperature program:	60 °C (0.35 min hold); 900 °C/min to 280 °C (15 min hold); 900 °C/min at 300 °C.
Oven program:	**Scan mode** 70 °C for 1 min 70 °C to 150 °C at 50 °C/min 150 °C to 200 °C at 6 °C/min 200 °C to 280 °C at 16 °C/min, 5 min hold 4 min added on to the run at 290 °C for column backflush **SIM mode** 60 °C for 1.5 min 60 °C to 150 °C at 50 °C/min 150 °C to 240 °C at 8 °C/min 240 °C to 280 °C at 50 °C/min, 2.5 min hold 280 °C to 290 °C at 100 °C/min, 2.05 min hold 4 min added on to the run at 290 °C for column backflush
Flow rate for SIM:	1 mL/min constant flow mode
Initial flow rate for scan:	2.7 mL/min (nominal, constant pressure mode)
Retention time locking:	Chlorpyrifos-methyl locked to 8.298 min for scan runs
Restrictor:	0.7 m x 0.15 mm deactivated, (Part No. 160-7625-5)
Transfer line temperature:	280 °C
Backflush configuration:	The analytical column was connected between the Multimode Inlet and a Purged Ultimate Union. The 0.7 m restrictor was connected between the Purged Ultimate Union and the MSD. Pressure at the Purged Ultimate Union was set to 4 psig using an auxiliary EPC module.

MSD conditions

Scan mode:	Scan and SIM run separately
Mode:	Electron ionization (EI)
Source temperature:	300 °C
Quadrupole temperature:	200 °C

GC/MS/MS conditions

GC conditions

Columns:	Two Agilent J&W HP-5ms Ultra Inert, 15 m × 0.25 mm, 0.25 µm (Part No. 19091S-431UI) columns joined by a Purged Ultimate Union
Injection volume:	1 µL
Injection mode:	Cold splitless using a Multimode Inlet
Inlet temperature program:	60 °C (0.35 min hold); 600 °C/min to 270 °C
Oven program:	60 °C for 1 min 60 °C to 170 °C at 40 °C/min 170 °C to 310 °C at 10 °C/min, 1.25 min hold
Flow rate:	1.224 mL/min (constant flow)
Solvent delay:	2.3 min
Flow mode:	Constant flow, chlorpyrifos methyl retention time locked to 9.143 min
Transfer line temperature:	300 °C
Run time:	19 min
Backflush configuration:	A Purged Ultimate Union (PUU) was connected between the two 15 m analytical columns. Column 1 was backflushed for 4 min at the end of the run with the GC oven at 310 °C, the inlet pressure at 1 psi, and the pressure at the PUU held at 60 psi.

Triple Quadrupole MS conditions

Mode:	Electron ionization (EI), MRM
Source temperature:	300 °C
Quadrupole temperatures:	Both at 180 °C

LC/MS/MS conditions

LC conditions

Column:	Agilent ZORBAX Eclipse Plus C18, 2.1 × 100 mm, 1.8 µm (Part No. 959758-902)
Column temperature:	40 °C
Injection volume:	5 µL
Mobile phase:	A: 0.1% Formic acid in H_2O B: 0.1% Formic acid in acetonitrile
Run time:	15 min
Flow rate:	0.3 mL/min
Gradient:	Initial 5% B; 10 min gradient to 95% B, then step to 100% B for 5 min

Triple Quadrupole MS conditions

Mode:	ESI, positive, MRM
Sheath gas:	350 °C, 11 L/min
Drying gas flow:	11 L/min
Nebulizer pressure:	40 psi
Capillary voltage:	4,000 V
Nozzle voltage:	1,000 V

Results

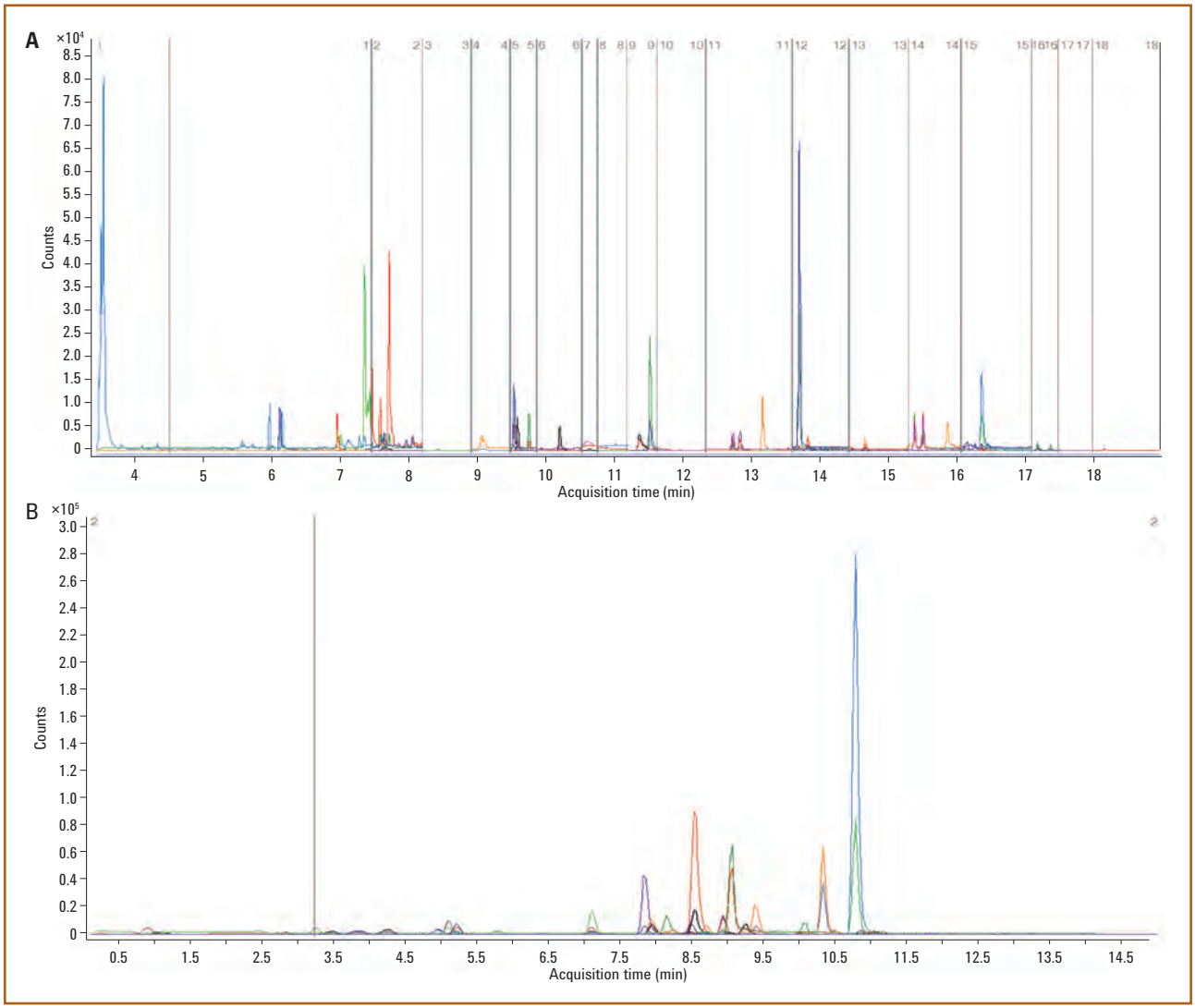


Figure 2. The MRM transitions (in overlay) of the pesticides spiked at 10 ppb (ng/mL) and detected in the vegetable juice blend matrix with GC/MS/MS (Figure 2A) and LC/MS/MS (Figure 2B).

Products used in the above application

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

Agilent Bond Elut QuEChERS AOAC Dispersive SPE Kit for Fruits and Vegetables. Part No. 5982-5022.

Agilent ZORBAX Eclipse Plus C18 Column, 2.1 x 100 mm, 1.8 µm. Part No. 959758-902.

Agilent J&W HP-5ms Ultra Inert 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 5990-9924EN at **agilent.com/chem**

Determination of Quinolone Antibiotics in Bovine Liver using Agilent Bond Elut QuEChERS Kits by LC/MS/MS (Publication 5990-5085EN)

Introduction

A method for the determination of 11 quinolone antibiotics in bovine liver was established:

- Analytes were extracted and cleaned up from bovine liver with Agilent Bond Elut QuEChERS kits
- Extraction was performed using Bond Elut EN extraction kits and 5% formic acid (FA) in acetonitrile
- Cleanup was performed using Bond Elut dispersive SPE kits Part No. 5982-4921 (25 mg C18 and 150 mg $MgSO_4$)
- 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, 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 in H_2O, pH 3.0 B: 1:1 Methanol:acetonitrile
Post time:	4 min

Gradient:

Time (min)	% 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 Procedure

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

Spike 100 µL IS spike solution, 50 µL QC spike solution.
If necessary vortex 30 sec.

Add 8 mL 30 mM KH_2PO_4, pH 7.0 buffer, vortex.

Add 10 mL 5% FA in ACN, and shake vigorously for 30 sec.

Add Bond Elut 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 Bond Elut QuEChERS dispersive SPE 2 mL tube.

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

Transfer 800 mL extract to another tube, blow down at 40 °C with N_2.

Reconstitute into 800 µL 1:9 MeOH:H_2O with 0.1% FA, vortex and sonicate.

Filter samples with 0.22 µm cellulose acetate spin filter.
(Part No. 5185-5990)

Sample are ready for LC/MS/MS analysis.

Figure 1: Agilent QuEChERS flow chart procedure for antibiotics.

Results

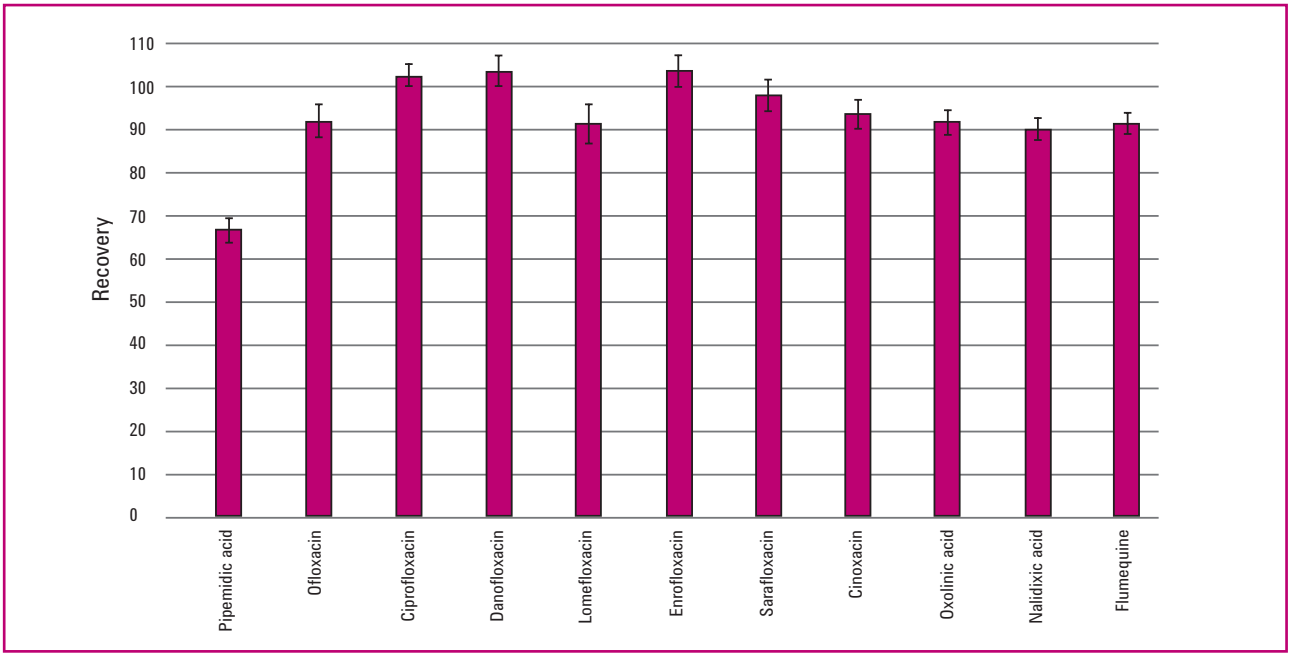


Figure 2: Recovery and reproducibility of eleven quinolone antibiotics from bovine liver.

Products used in the above application

Agilent Bond Elut QuEChERS EN Extraction Kit. Part No. 5982-5650.

Agilent Bond Elut QuEChERS Dispersive SPE Kit, 2 mL. Part No. 5982-4921 or 15 mL. Part No. 5982-4956

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 5990-5085EN at **agilent.com/chem**

Determination of Sulfonamide Antibiotics in Bovine Liver using Bond Elut QuEChERS EN Kits by LC/MS/MS (Publication 5990-5086EN)

Introduction

A method for the determination of nine sulfonamide antibiotics in bovine liver was established:

- Analytes were extracted and cleaned up from bovine liver with Agilent Bond Elut QuEChERS kits
- Extraction was performed using Bond Elut EN extraction kits and 1% acetic acid (AA) in acetonitrile
- Cleanup was performed using Bond Elut EN fatty dispersive SPE kits, 6 mL (150 mg PSA, 150 mg C18 and 900 mg $MgSO_4$)
- 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, 3.0 x 50 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 H_2O, pH 3.0 B: 1:1 Methanol:acetonitrile	
Post time:	3.5 min	
Gradient:	Time (min)	% B
	0	15
	0.2	15
	6.0	60
	6.01	100
	7.0	STOP

MS conditions

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

QuEChERS Procedure

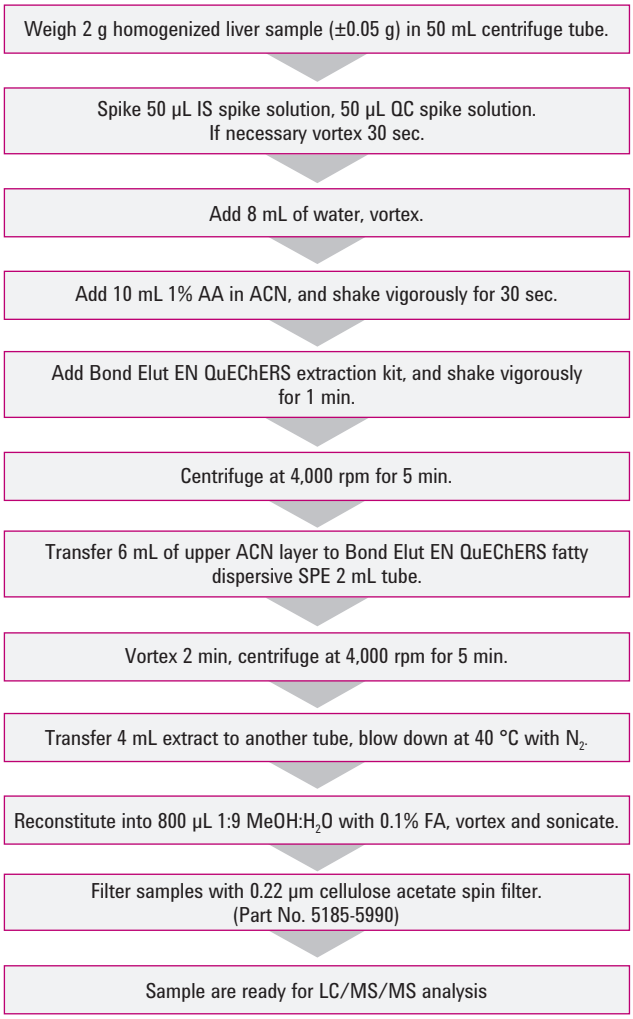


Figure 1. Agilent QuEChERS procedure for sulfonamide antibiotics in bovine liver.

Products used in the above application

Agilent Bond Elut QuEChERS EN Extraction Kit.
Part No. 5982-5650.

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

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

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

Results

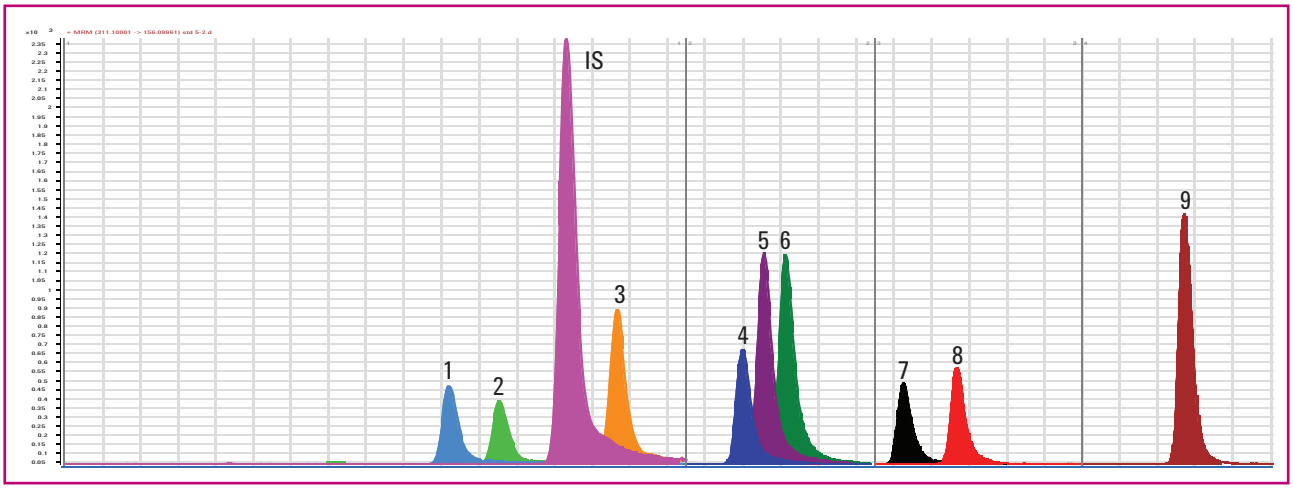


Figure 2. LC/MS/MS chromatogram of 100 ng/g fortified liver extract. Peaks identification: 1. Sulfadizine, 2. Sulfathiazole, 3. Sulfamerazine, 4. Sulfamethizole, 5. Sulfamethazine, 6. Sulfamethoxypyridazine, 7. Sulfachloropyridazine, 8. Sulfamethoxazole, 9. Sulfadimethoxin, IS (internal standard)

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

	Low QC (5 ng/g)		Mid QC (100 ng/g)		High QC (400 ng/g)	
Compound	**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
Sulfamethoxypyridazine	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

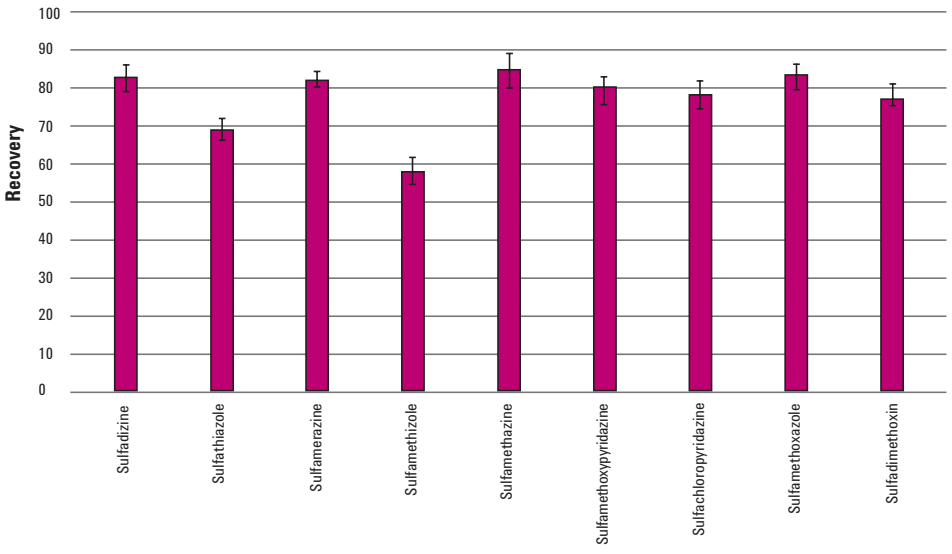


Figure 3. Recovery and reproduciblity of nine sulfonamides in bovine liver.

To review this Application Note in its entirety, please search for 5990-5086EN at **agilent.com/chem**

GC-µECD Analysis and Confirmation of Contract Laboratory Protocol Pesticides in Olive Oil (Publication 5990-5553EN)

Introduction

This application describes the use of the QuEChERS sample preparation approach to analyze an olive oil sample from a local grocery store for 20 contract laboratory protocol (CLP) pesticides. A dual µECD and dual capillary GC column approach accomplished simultaneous primary and confirmatory analysis where endosulfan sulfate and endosulfan 1 were present in the oil sample. This note successfully shows a robust, inexpensive analytical method to monitor CLP pesticides in olive oil, to address food safety concerns. The method demonstrates the feasibility of using a dual column µECD approach for routine olive oil screening as an alternative to GC/MS.

QuEChERS Procedure

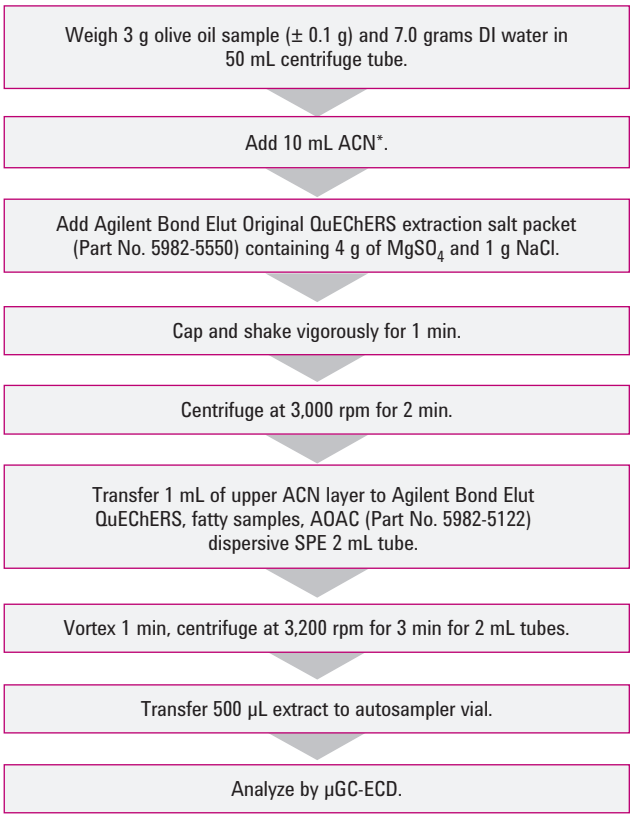


Figure 1. Agilent QuEChERS procedure for pesticides in olive oil.

**Spike sample addition.*

Instrument conditions

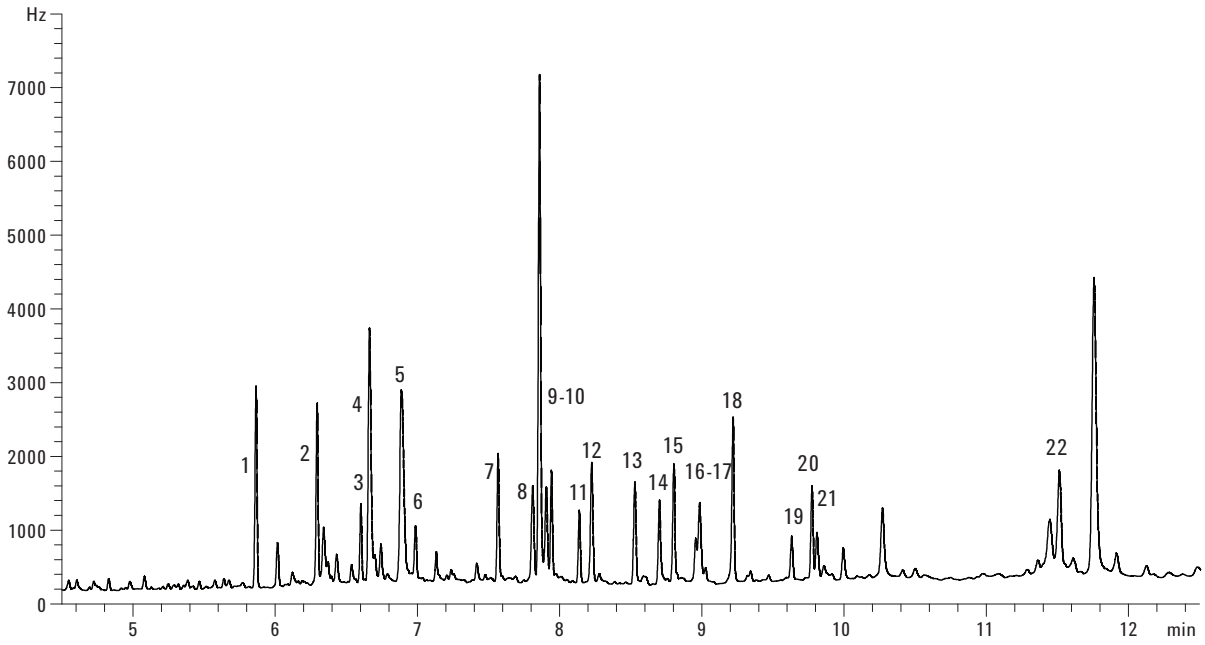
GC conditions

Column 1:	Agilent J&W DB-35 ms 30 m × 0.25 mm, 0.25 µm (Part No. 122-3832)
Column 2:	Agilent J&W DB-XLB 30 m × 0.25 mm, 0.50 µm (Part No. 122-1236)
GC/dual µECD:	7890A equipped with dual µECD detection and a 7873B auto sampler
CFT device:	2-way unpurged splitter capillary flow technology (Part No. G3181B)
Carrier gas:	Hydrogen 56 cm/sec
Oven:	110 °C (1.4 min), 21 °C/min to 285 °C (1 min), 30 °C/min to 300 °C (2 min)
Injection:	1 µL, 250 °C splitless, purge 50 mL/min at 0.3 min, gas saver 50 mL/min on at 2 min
Dual µ-ECD:	350 °C, N_2 makeup; constant column + makeup = 30 mL/min

Flow path supplies

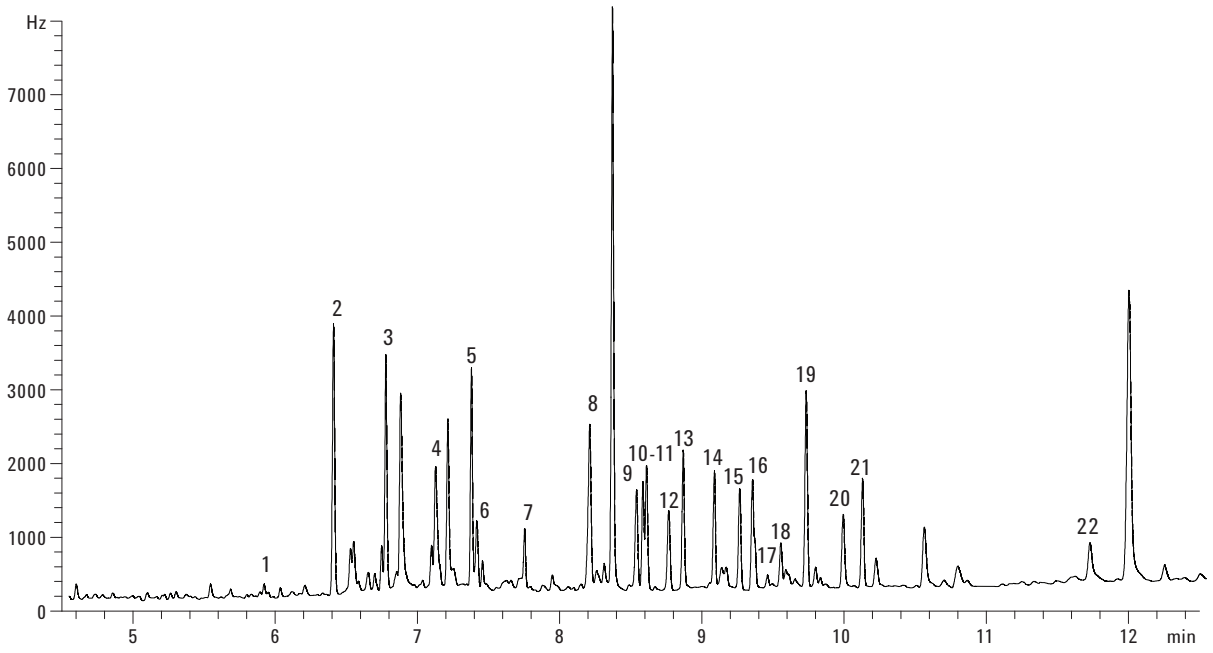
Vials:	Amber screw top glass vials (Part No. 5183-2072)
Vial caps:	Screw caps (Part No. 5182-0723)
Vial inserts:	100 µL glass/polymer feet (Part No. 5181-8872)
Syringe:	5 µL (Part No. 5183-4729)
Septum:	Advanced Green (Part No. 5183-4759)
Inlet seal:	Gold-plated inlet seal (Part No. 5188-5367)
Inlet liners:	Dual-taper direct connect liner (Part No. G1544-80700)
Ferrules:	0.4 mm id short, 85/15 Vespel/graphite (Part No. 5181-3323)
CFT fittings:	Internal nut (Part No. G2855-20530)
CFT ferrules:	SilTite ferrules, 0.25 mm id (Part No. 5188-5361)
20x Magnifier:	20x Magnifier loop (Part No. 430-1020)

Results



1. Tetrachloro-m-xylene (surrogate standard)
2. α-BHC
3. g-BHC
4. b-BHC
5. Heptachlor
6. d-BHC
7. Aldrin
8. Heptachlor epoxide
9. g-Chlordane
10. α-Chlordane
11. Endosulfan I
12. Dieldrin
13. p,p-DDE
14. Endrin
15. p,p-DDD
16. Endosulfan II
17. p,p-DDT
18. Endrin aldehyde
19. Endosulfan sulfate
20. Methoxychlor
21. Endrin ketone
22. Decachlorobiphenyl (surrogate standard)

Figure 2. GC-μECD chromatogram of olive oil sample spiked with 80 ng/mL of CLP pesticides and 20 ng/mL surrogates standard.



1. Tetrachloro-m-xylene (surrogate standard)
2. α-BHC
3. g-BHC
4. b-BHC
5. Heptachlor
6. d-BHC
7. Aldrin
8. Heptachlor epoxide
9. g-Chlordane
10. α-Chlordane
11. Endosulfan I
12. p,p-DDE
13. Dieldrin
14. Endrin
15. p,p-DDD
16. Endosulfan II
17. Endrin aldehyde
18. p,p-DDT
19. Endosulfan sulfate
20. Methoxychlor
21. Endrin ketone
22. Decachlorobiphenyl (surrogate standard)

Figure 3. GC-µECD chromatogram of olive oil sample spiked with 80 ng/mL of CLP pesticides and 20 ng/mL surrogates standard.

Products used in the above application

Agilent Bond Elut Original QuEChERS Extraction Salt Packet. Part No. 5982-5550.

Agilent Bond Elut QuEChERS, Fatty Samples, AOAC Dispersive SPE, 2 mL Tube. Part No. 5982-5122.

Agilent J&W DB-35ms Column, 30 m x 0.25 mm, 0.25 µm. Part No. 122-3832.

Agilent J&W DB-XLB Column, 30 m x 0.25 mm, 0.50 µm. Part No. 122-1236.

To review this Application Note in its entirety, please search for 5990-5553EN at **agilent.com/chem**

Determination of Pesticides in Lemon Oil via Modified Agilent Bond Elut QuEChERS Method (Publication 5990-6432EN)

Introduction

This application describes a simple and rapid extraction procedure for pesticide residues in lemon essential oils using Agilent Bond Elut QuEChERS extraction and modified dispersive SPE coupled with Agilent gas chromatography and Agilent 5975C Series GC/MSD. Pesticide extraction in essential oil is considered difficult due to the complicated oily matrix. The approach presented here uses multiple dispersive SPE steps along with hexane in the initial extraction step, which results in cleaner extracts amenable with GC/MS analysis using selected ion monitoring (SIM) in electron ionization mode.

Instrument conditions

GC conditions

Column:	Agilent J&W HP-5ms Ultra Inert, 15 m × 0.25 mm, 0.25 µm
Inlet:	Splitless
Inlet liner:	Helix double-taper, deactivated (Part No. 5188-5398)
Carrier gas:	Helium
Inlet pressure:	19.6 psi (constant pressure mode)
Inlet temperature:	250 °C
Injection volume:	1.0 µL
Purge flow to split vent:	30 mL/min at 0.75 min
Over 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)

MS conditions

Tune file:	Atune.u
Mode:	SIM (refer to Table 3 in app note for settings in detail)
Source, quad, transfer:	230 °C, 150 °C, and 280 °C, respectively line temperature
Solvent delay:	2.30 min
Multiplier voltage:	Autotune voltage

QuEChERS Procedure

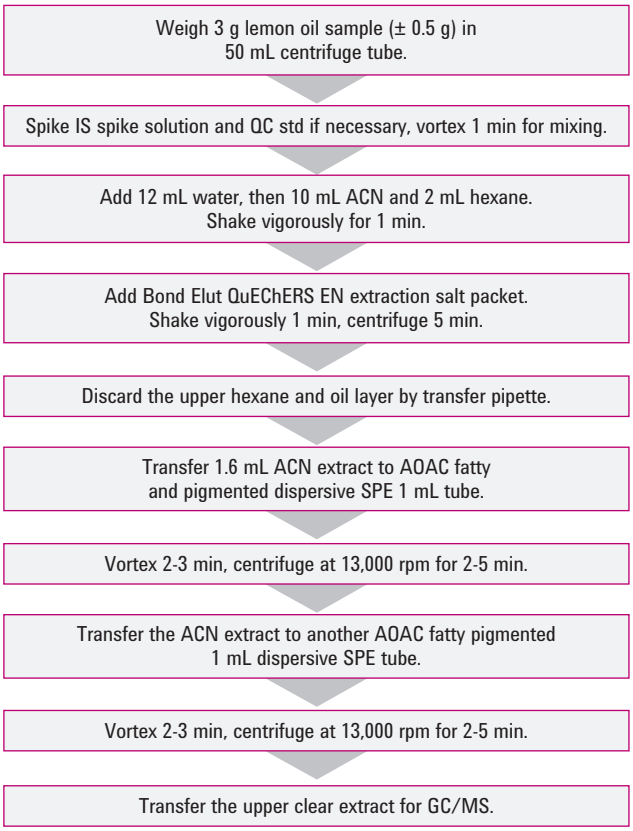


Figure 1. Agilent modified QuEChERS EN extraction procedure for pesticides in lemon oil.

Results

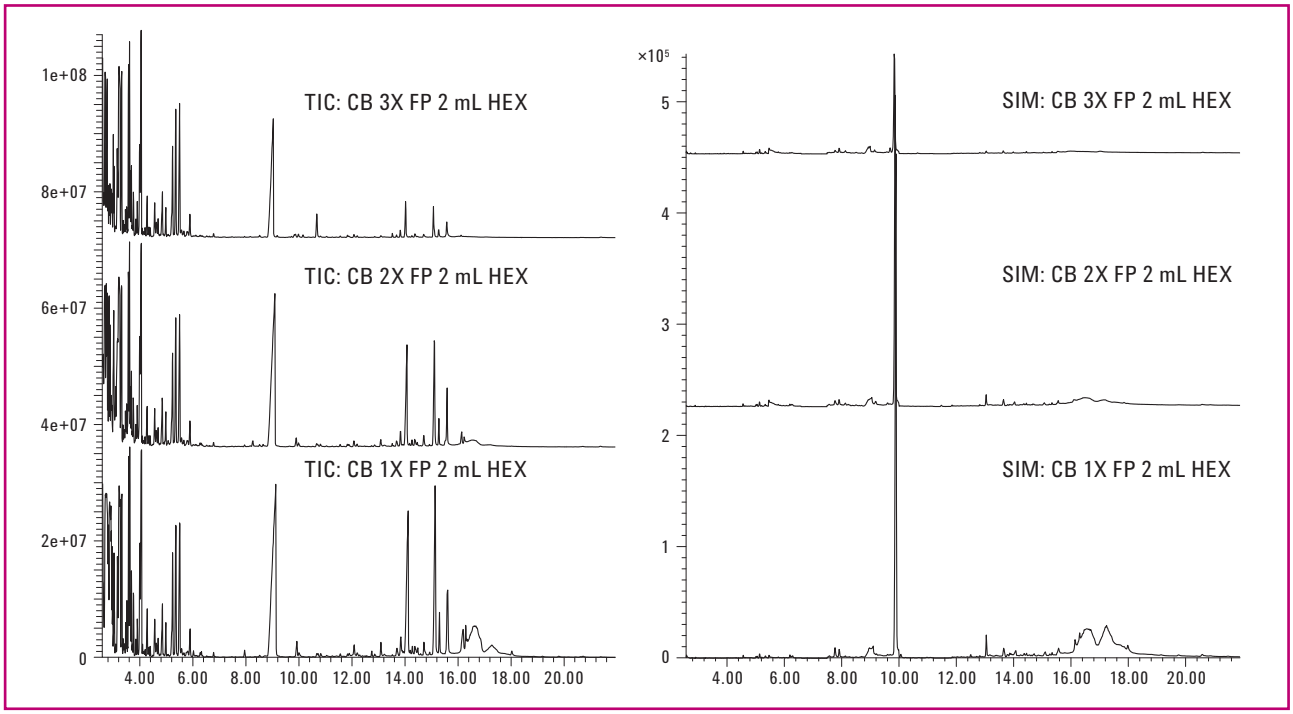
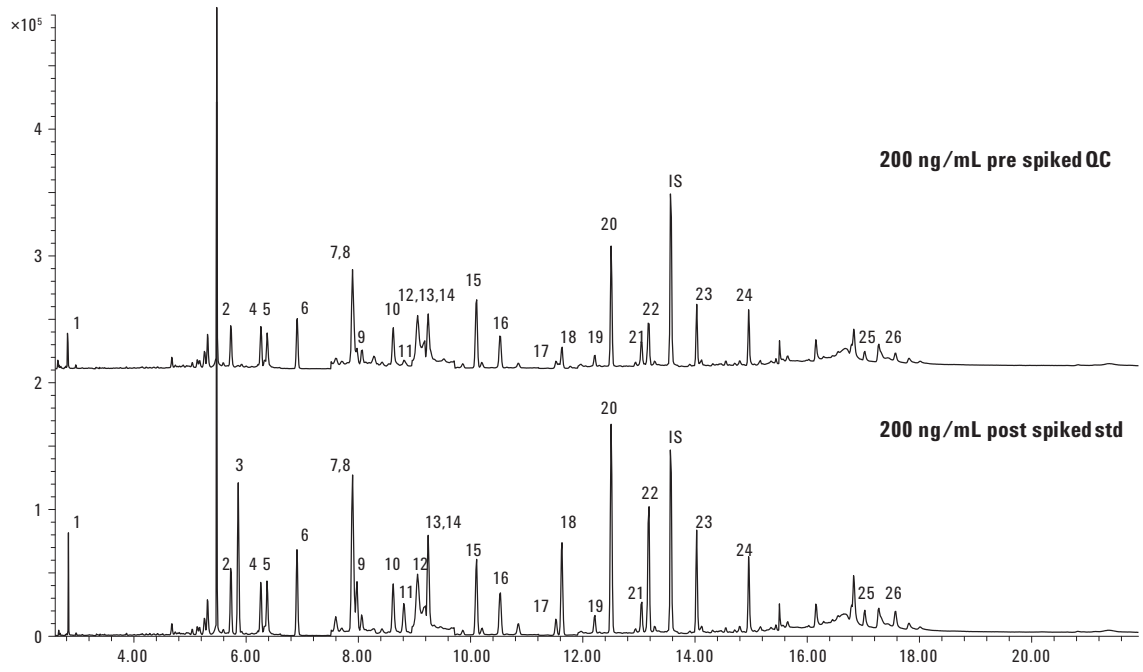


Figure 2. Optimized extraction from the method development experiments involving the addition of hexane and multiple dispersive SPE steps. CB (control blank), FP (AOAC fatty pigmented dispersive SPE), HEX (hexane).



1. Dichlorvos
2. α-BHC
3. Hexachlorobenzene
4. β-BHC
5. γ-HCH
6. Disulfoton
7. Chlorpyrifos methyl
8. Parathion methyl
9. Heptachlor
10. Fenitrothion
11. Aldrin
12. Malathion
13. Chlorpyrifos
14. Parathion
15. Procymidone
16. Endosulfan I
17. Dieldrin
18. 4,4′-DDE
19. Endosulfan II
20. 4,4′-DDD
21. Endosulfan sulfate
22. 4,4′-DDT
23. Bromopropylate
24. λ-Cyhalothrin
25. Fenvalerate
26. Deltmethrin

Figure 3. GC/MS chromatograms of 200 ng/mL pre and postmatrix fortified samples. This shows the chromatograms for a prespiked and postspiked lemon oil extract. Twenty-five of the twenty-six pesticides gave repeatable results with RSD < 5% on average.

Products used in the above application

Agilent Bond Elut QuEChERS Buffered EN Extraction Kit. Part No. 5982-5650.

Agilent Bond Elut QuEChERS AOAC Dispersive SPE Kit for Fatty and Pigmented Produce. Part No. 5982-5421.

Agilent J&W HP-5MS Ultra Inert 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 5990-6432EN at **agilent.com/chem**

Polycyclic Aromatic Hydrocarbon (PAH) Analysis in Fish by GC/MS using Agilent Bond Elut QuEChERS dSPE Sample Preparation and a High Efficiency DB-5ms Ultra Inert GC Column (Publication 5990-6668EN)

Introduction

This application describes the use of the QuEChERS dispersive solid phase extraction (dSPE) sample preparation approach for the determination of low and trace level polycyclic aromatic hydrocarbons (PAHs) in fish. The analysis was performed on an Agilent 7890 GC and Agilent 5975B GC/MS system equipped with a Multimode Inlet (MMI) and Agilent 7693 Automatic Liquid Sampler. The GC was also fitted with a pressure controlled tee (PCT) post-column for automated backflush. Recoveries ranged from 80% to 139%.

QuEChERS Procedure

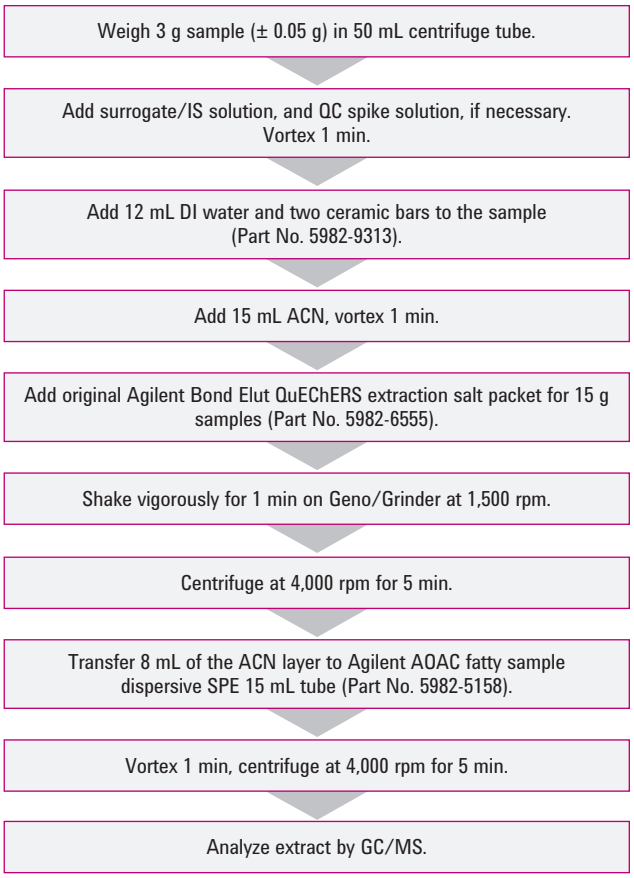


Figure 1. Agilent Bond Elut QuEChERS modified extraction procedure for fish samples.

Instrument conditions

GC conditions

Column:	Agilent J&W DB-5ms UI 20 m × 0.18 mm, 0.18 µm (Part No. 122-5522UI)
GC/MSD:	Agilent 7890 GC/Agilent 5975B GC/MS System
Sampler:	Agilent 7693 Automatic Liquid Sampler, 5.0 µL syringe (Part No. 5181-1273)
PCT device:	Purged Ultimate Union (Part No. G3186-60580)
Carrier:	Helium, constant flow 1.7 mL/min
Restrictor:	0.7 m × 0.15 mm id deactivated silica tubing
PCM 1:	3.8 psi constant pressure
MMI:	0.5 µL splitless; 320 °C, purge flow 50 mL/min at 0.8 min; Gas saver 30 mL/min at 2 min
Oven:	50 °C (0.4 min), 25 °C/min to 195 °C (1.5 min), 8 °C/min to 265 °C, 20 °C/min to 315 °C (1.25 min)
Postrun backflush:	7 min at 315 °C, backflush pressure 70 psi, 2 psi inlet pressure during backflush
MSD:	340 °C transfer line, 340 °C source, 150 °C quad

Flow path supplies

Vials:	Amber screw top glass vials (Part No. 5183-2072)
Vial caps:	Blue screw caps (Part No. 5182-0717)
Vial inserts:	100 µL glass/polymer feet (Part No. 5181-8872)
Syringe:	5 µL (Part No. 5181-1273)
Septum:	Advanced Green (Part No. 5183-4759)
Inlet liners:	Deactivated dual-taper helix liner (Part No. G5188-5398)
Ferrules:	0.4 mm id short, 85/15 Vespel/graphite (Part No. 5181-3323)
PCT fittings:	Internal nut (Part No. G2855-20530)
PCT ferrules:	SilTite ferrules, 0.25 mm id (Part No. 5188-5361)
20x Magnifier:	20x Magnifier loop (Part No. 430-1020)

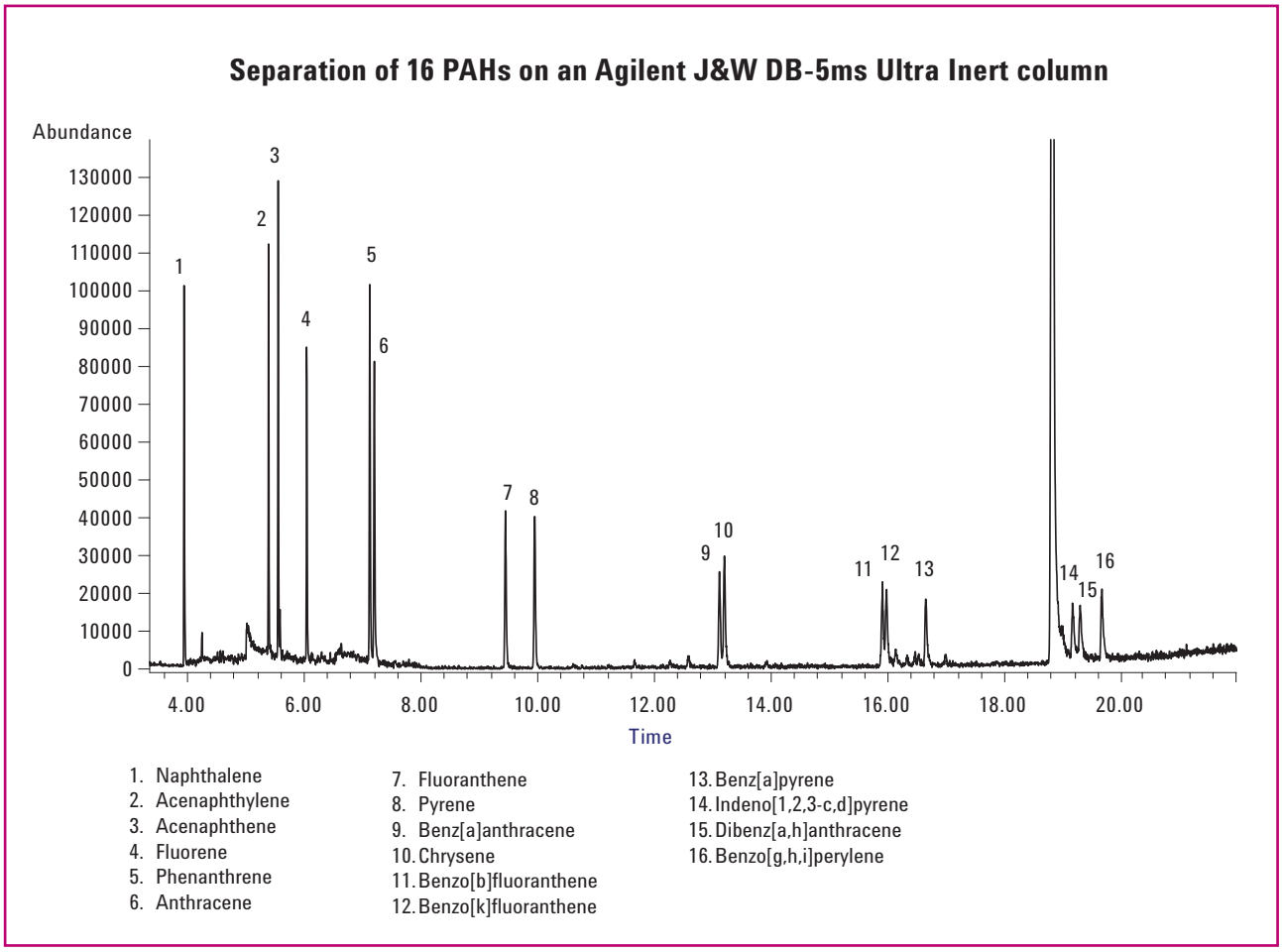
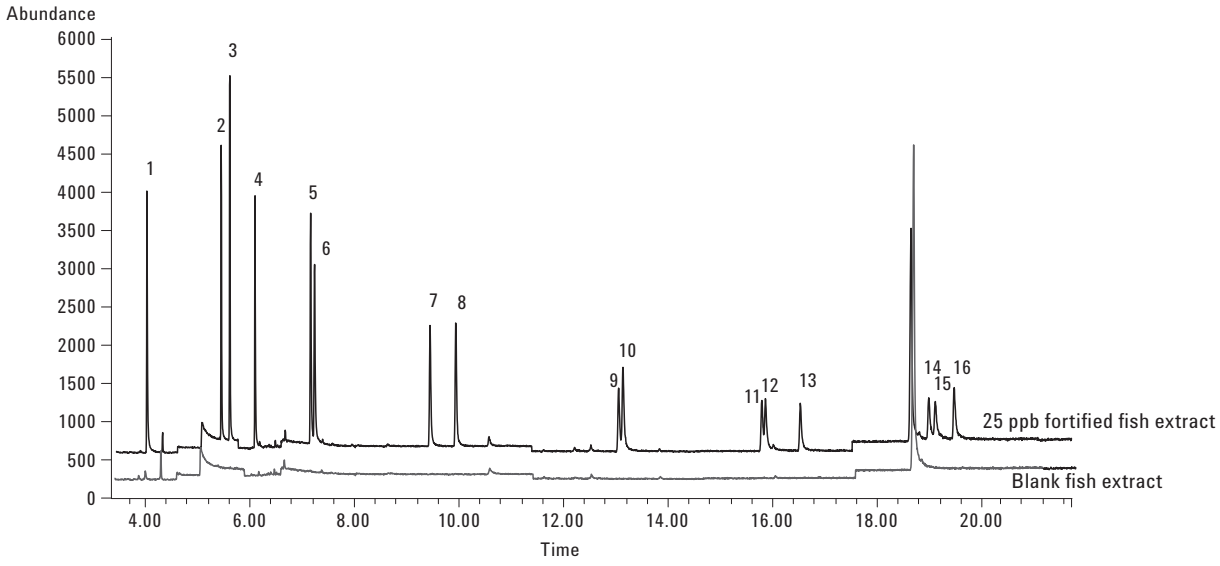


Figure 2. GC/MS chromatogram of the 500 ng/mL PAH standard prepared in sample matrix analyzed on an Agilent J&W DB-5ms Ultra Inert GC capillary column (Part No. 122-5522UI). Chromatographic conditions are listed in Table 1 of the app note.



1. Naphthalene
2. Acenaphthylene
3. Acenaphthene
4. Fluorene
5. Phenanthrene
6. Anthracene
7. Fluoranthene
8. Pyrene
9. Benz[a]anthracene
10. Chrysene
11. Benzo[b]fluoranthene
12. Benzo[k]fluoranthene
13. Benz[a]pyrene
14. Indeno[1,2,3-c,d]pyrene
15. Dibenz[a,h]anthracene
16. Benzo[g,h,i]perylene

Figure 3. GC/MS SIM chromatogram of the fish extract blank and the 25 ng/mL spiked fish extract analyzed on Agilent J&W DB-5ms Ultra Inert GC capillary column. Chromatographic conditions are listed in Table 1 of the app note.

Products used in the above application

Agilent Bond Elut QuEChERS Extraction Salt Packet. Part No. 5982-6555.

Agilent Bond Elut QuEChERS Fatty Sample Dispersive SPE, 15 mL Tubes. Part No. 5982-5158.

Agilent Bond Elut QuEChERS Ceramic Homogenizers, 50 mL Tubes. Part No. 5982-9313.

Agilent J&W DB-5ms Ultra Inert Column, 20 m × 0.18 mm, 0.18 µm. Part No. 122-5522UI.

To review this Application Note in its entirety, please search for 5990-6668EN at **agilent.com/chem**

Analysis of Pesticides in Food by GC/MS/MS using the Ultra Inert Liners with Wool (Publication 5990-7706EN)

Introduction

This application describes the use of the QuEChERS sample preparation approach for analyzing multi-residue pesticides. Five kinds of fruits and vegetables were selected: white flowers, banana, strawberry, pear, and lettuce. A representative group of 33 difficult pesticides were selected to evaluate the Agilent Ultra Inert liner. The pesticides standard was spiked in fruit and vegetables matrix blank samples extracted by QuEChERS AOAC method. All testing was done on an Agilent 7890A GC equipped with a 7693B Autosampler and 7000 Series Triple Quadrupole MSD system. The extracted samples were used to demonstrate the performance advantages of Agilent Ultra Inert liners with wool.

QuEChERS Procedure

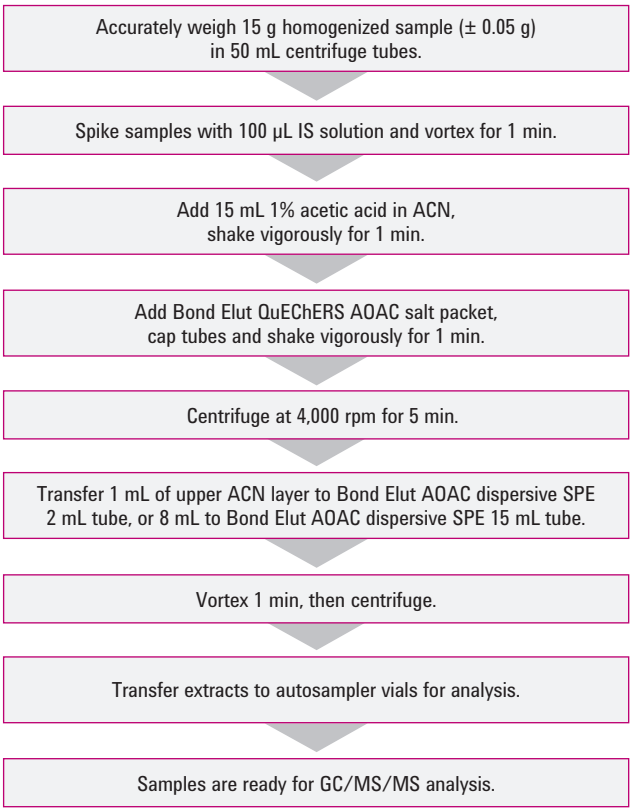


Figure 1. QuEChERS AOAC sample preparation procedures for pesticides in food.

Instrument conditions

GC conditions

Analytical column:	Agilent J&W HP-5ms Ultra Inert, 15 m × 0.25 mm, 0.25 µm (Part No. 19091-431UI)
GC:	Agilent 7890A Series
Autosampler:	Agilent 7693 Autosampler and sample tray, 5 µL syringe (Part No. 5181-5246), 1 µL injection volume. Postinj solvent A (acetone) washes: 3 Sample pumps: 3 Postinj solvent B (acetonitrile) washes: 3
Carrier gas:	Helium, constant pressure
Inlet:	MMI inlet at pulsed splitless mode: 280 °C,
Injection pulse pressure:	36 psi until 1 min
Purge flow to split vent:	50 mL/min at 1 min
Inlet pressure:	18.35 psi (RT locked) during run, and 1.0 psi during back flushing
RT locking:	Chlorpyrifos methyl at 8.298 min
Oven profile:	100 °C for 2 min, then to 150 °C at 50 °C/min, to 200 °C at 6 °C/min, to 280 °C at 16 °C/min and hold for 6 min (for sample run); 100 °C for 1 min, then to 280 °C at 100 °C/min and hold for 5.2 min (for matrix blank run)
Post run:	2 min at 280 °C
Capillary flow technology:	Purged Ultimate Union (Part No. G3182-61580) – used for backflushing the analytical column and inlet
Aux EPC gas:	Helium plumbed to Purge Ultimate Union
Bleed line:	0.0625 in od × 0.010 in id × 100 cm, 316 SS tubing, on top of the oven
Aux pressure:	4 psi during run, 75 psi during backflushing
Connections:	Between inlet and Purged Ultimate Union (Part No. G3182-61580)
Restrictor:	Inert fused silica tubing, 0.65 m × 0.15 mm (Part No. 160-7625-5)
Connections:	Between Purged Ultimate Union and the MSD
MSD:	Agilent 7000 Triple Quadrupole Inert with performance electronics
Vacuum pump:	Performance turbo
Mode:	MRM
Tune file:	Atune.u
Transfer line temperature:	280 °C
Source temperature:	300 °C
Quad temperature:	Q1 and Q2, 150 °C
Solvent delay:	2.3 min
Collision gas flows:	He quench gas at 2.35 mL/min, N_2 collision gas at 1.5 mL/min
MS resolution:	MS1 and MS2, 1.2 u

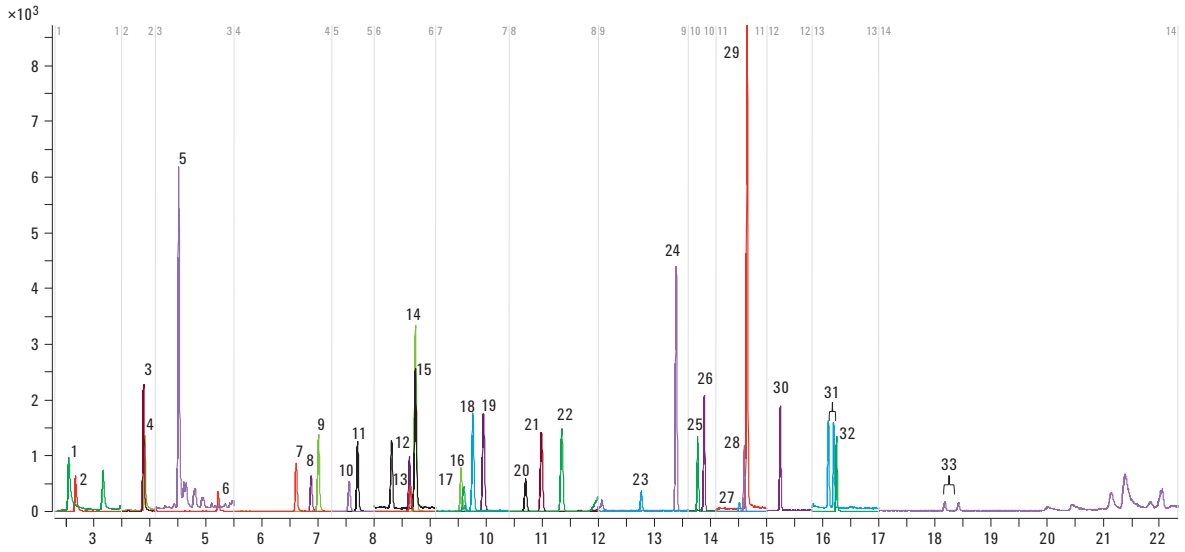


Figure 2. GC/MS/MS chromatogram (MRM) for 10 ppb spiked QuEChERS sample using Ultra Inert liner with wool.

Products used in the above application

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

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

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

To review this Application Note in its entirety, please search for 5990-7706EN at **agilent.com/chem**

Determination of Chemical Contaminants in Marine Shellfish using the Agilent 7000 Triple Quadrupole GC/MS System (Publication 5990-7714EN)

Introduction

This application describes the use of the QuEChERS sample preparation approach for the determination of selected organochlorine pesticides, polyaromatic hydrocarbons, and polychlorinated biphenyl congeners in marine mussel tissue. The analysis was performed on an Agilent 7890 GC configured with a carbon-dioxide-cooled Multimode Inlet (MMI) with an Agilent 7693A Automatic Liquid Sampler and an Agilent 7000 Triple Quadrupole GC/MS system. In this application, a QuEChERS extraction is coupled with solvent and tube-based SPE clean-up steps to result in final extracts that are suitable for use with GC/QQQ confirmation of the target analytes.

Instrument conditions

GC conditions

Column (1):	Agilent J&W DB-5ms Ultra Inert, 15 m × 0.25 mm id, 0.25 µm (Part No. 122-5512UI)
Column (2):	Agilent J&W DB-5ms Ultra Inert, 0.65 m × 0.15 mm id, 0.15 µm (cut from Part No. 165-6626)
Injection mode (1):	1 µL cold pulsed splitless using CO_2 cooled Multimode Inlet (MMI) and a 10 µL syringe
Inlet temperature program:	50 °C (0.05 min), 600 °C/min to 325 °C
Inlet pressure pulse:	13.0 psig for 0.75 min
Purge flow to split vent:	50 mL/min at 1.0 min
Injection port liner:	2 mm id, multi-baffled (Part No. 5190-2296)
Injection mode (2):	10 µL solvent vent using CO_2-cooled Multimode Inlet (MMI) and a 25 µL syringe
Inlet temperature program:	40 °C (0.31 min), 600 °C/min to 325 °C
Inlet vent pressure:	5.0 psig
Inlet vent flow:	100 mL/min
Inlet vent time:	0.31 min
Outlet pressure:	0 psig
Injection speed:	100 µL/min
Purge flow to split vent:	50 mL/min at 1.0 min
Injection port liner:	2 mm id, multi-baffled (Part No. 5190-2296)
Carrier gas:	Helium, constant flow 1.2 mL/min
Oven temperature program:	50 °C (1 min), 20-200 °C/min (0 min), 10 °C/min, 300 °C (1.5 min)
RTL compound:	PCB 118, locked at 12.370 min
Pressure controlled tee:	G3186B, operated at 2.0 psig constant pressure
Back flush conditions:	Inlet pressure

MC conditions

MS transfer line temp:	325 °C
MS source:	300 °C
MS quad 1, 2 temp:	150 °C , 150 °C
Collision cell gases:	Nitrogen 1.5 mL/min, helium 2.25 mL/min
MS1/MS2 resolution:	Wide/wide
MRM settings:	See Table 3 in the app note
Electron energy:	-70 eV
Ionization mode:	Electron impact (EI)
EI Autotune:	Gain normalized
Gain factor:	5

QuEChERS Procedure

Weigh 2 g homogenized mussel tissue into a QuEChERS extraction tube.

Add labelled ISTDs (prepared in acetone).

Vortex mix sample for 30 sec. Add 13 mL of DI water and two ceramic homogenizers.

Vortex mix sample for 1 min Add 15 mL of extraction solvent (1% acetic acid in acetonitrile). Vortex mix for 1 min.

Add the QuEChERS AOAC salt mix (5982-5755). Shake the tube by hand for 1 min Vortex mix for 1 min.

Centrifuge the tube for 5 min at 3,900 rpm, then cool the tube in a freezer at -20 °C for 30 min.

Transfer the acetonitrile layer to a clean and dry centrifuge tube containing 1 g of anhydrous sodium sulphate.

Shake by hand for 1 min, then place the tube in a freezer at -20 °C overnight.

Pipette 10 mL clear extract into a clean turbovap tube and evaporate to 0.5 mL.

Add 20 mL dichlorometheane (DCM). Evaporate to 0.5 mL, then add 10 mL DCM. Evaporate to 0.5 mL.

Add 10 mL hexane and evaporate to 0.5 mL.

Activate silica at 180 °C overnight. Add 1 g to an empty 15 mL SPE tube.

Condition 1 g of silica in an SPE tube with 10 mL DCM and 20 mL hexane.

Apply the concentrated extract to the silica SPE tube; elute with 13 mL 40:60 DCM:hexane.

Evaporate the extract to 0.5 mL. Repeat steps 13 and 14.

Evaporate the extract to 0.5 mL and trasfer to a 2 mL autosampler vial.

Figure 1. QuEChERS sample extraction and clean-up procedure for contaminants in shellfish.

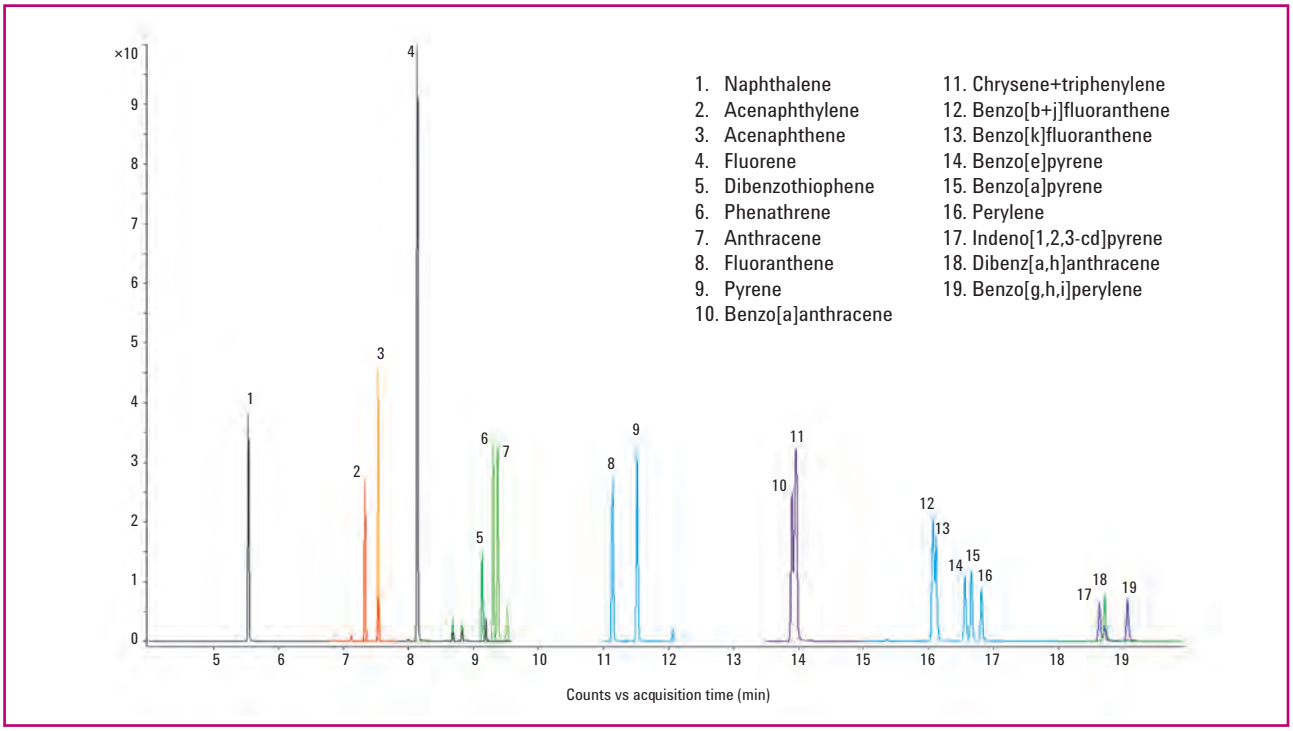


Figure 2. TIC MRM chromatogram for PAH analytes.

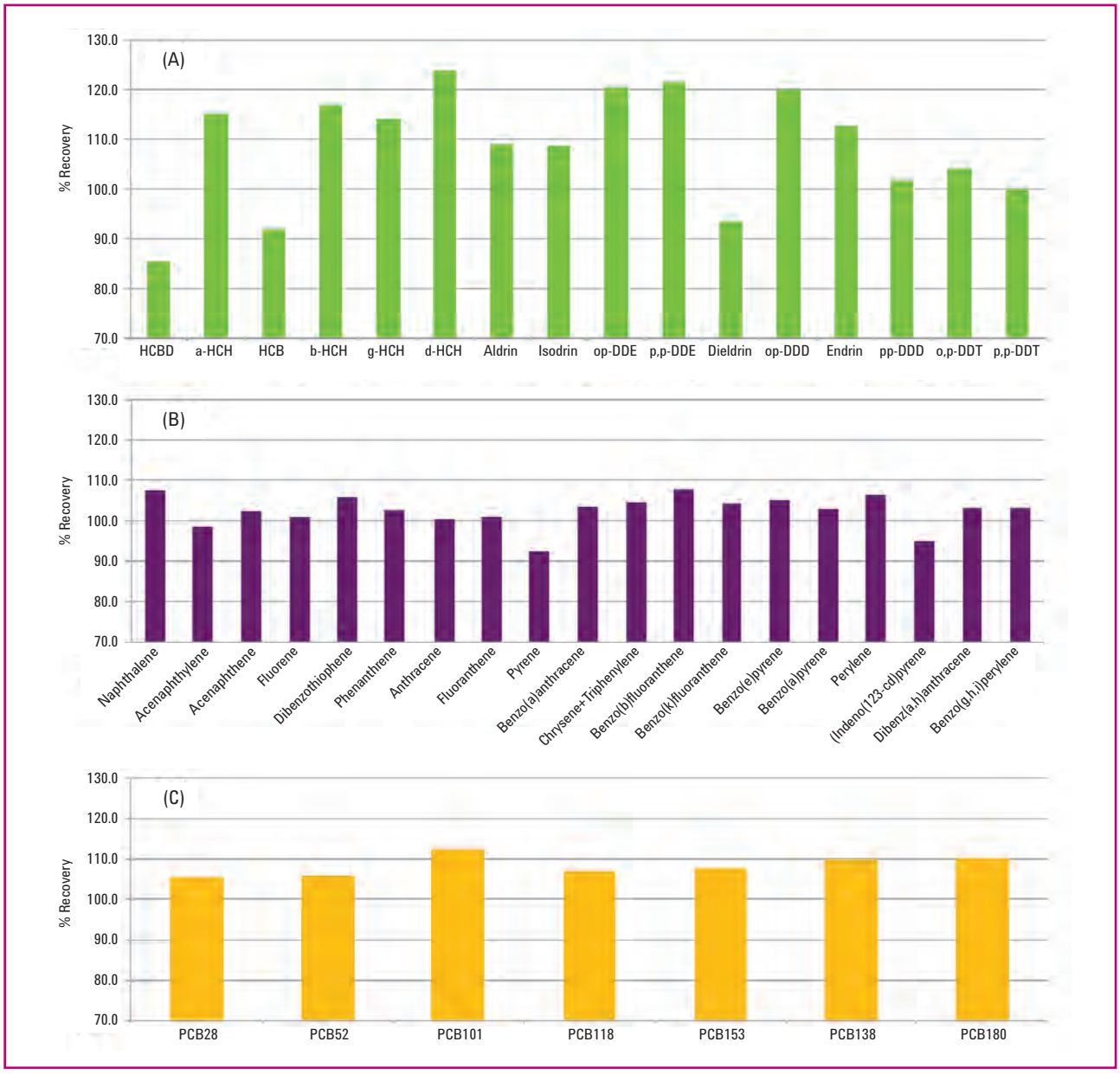


Figure 3. Graphical representation of analyte percent recovery values for (a) OCPs, (b) PAHs and (c) PCB congeners in spiked mussel tissue.

Products used in the above application

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

Agilent J&W DB-5ms Ultra Inert Column, 15 m x 0.25 mm, 0.25 µm. Part No. 122-5512UI.

Agilent J&W DB-5ms Ultra Inert Column, 0.65 m x 0.15 mm, 0.15 µm. Cut from Part No. 165-6626.

To review this Application Note in its entirety, please search for 5990-7714EN at **agilent.com/chem**

LC/MS/MS of Trichothecenes and Zearalenone in Wheat using Different Sample Prep Methods (Publication 5990-9107EN)

Introduction

This application describes a modified QuEChERS method and Agilent Bond Elut Mycotoxin SPE for sample preparation and then subsequent detection of trichothecenes and zearalenone by LC/MS/MS. An Agilent 6460 Triple Quadrupole LC/MS with MassHunter Optimizer software was used for this analysis. Recoveries were within 72 to 105% relative to immuno-affinity extractions. Both the QuEChERS approach and the traditional SPE cleanup using the Mycotoxin columns provided good recoveries. Samples prepared using SPE cleanup were cleaner and resulted in lower detection limits, while those prepared using the QuEChERS approach were processed more quickly.

Instrument conditions

HPLC conditions

Columns:	Agilent ZORBAX Rapid Resolution HT Eclipse Plus C18, 2.1 × 100 mm, 1.8 µm (Part No. 959764-902)
Instrument:	Agilent 6460 Triple Quadrupole LC/MS, Agilent 1290 Infinity LC
Flow rate:	0.25 mL/min
Column temperature:	30 °C
Injection volume:	10 µL
Mobile phase:	A: Water + 0.2% acetic acid, 5 mM ammonium acetate B: Methanol + 0.2% acetic acid, 5 mM ammonium acetate

MS/MS conditions

ESI with Agilent Jet Stream parameters, pos/neg fast polarity switching.

Drying gas temperature:	200 °C
Drying gas flow:	8 L/min
Nebulizer pressure:	45 psi
Sheath gas temperature:	400 °C
Sheath gas flow:	12 L/min
Capillary voltage:	± 3,000 V
Nozzle voltage:	± 500 V
Delta EMV:	500 V
Resolution:	Unit, unit

QuEChERS Procedure

Mill 5 g of grain product and add to 50 mL empty centrifuge tube.

Add 10 mL MeOH:acetonitrile (85:15 v:v), cap, and mix.

Add Bond Elut non-buffered extraction packet (Part No. 5982-5550), cap, shake, and centrifuge.

Transfer 1 mL of upper layer to 2 mL dispersive SPE tube (Part No. 5982-5022).

Cap, vortex mix, and centrifuge.

Filter through 0.2 µm membrane.

Evaporate under N_2 and reconstitute in 1 mL H_2O:ACN (80:20 v:v).

Figure 1. QuEChERS AOAC sample preparation procedures for grains.

Bond Elut Mycotoxin Method

Mill 25 g of grain product

Add 100 mL MeOH:acetonitrile (85:15 v:v), cap, and shake for one hour.

Apply 8 mL aliquot to Bond Elut Mycotoxin tube (Part No. 12102167) and collect filtrate.

Evaporate under N_2 and reconstitute in 1 mL H_2O:ACN (80:20 v:v).

Filter through 0.2 µm membrane.

Figure 2. Bond Elut Mycotoxin SPE sample preparation method for grain mycotoxins.

Table 1. MS conditions for the analysis of trichothecenes and zearalenone

Mycotoxin	Precursor ion	Product ion	Fragmentor	Collision energy	Polarity
15-acetyl Deoxynivalenol	356	321	95	5	Positive
15-acetyl Deoxynivalenol	356	137	95	8	Positive
15-acetyl Deoxynivalenol	339	137	105	12	Positive
3-acetyl Deoxynivalenol	397	337	95	4	Negative
3-acetyl Deoxynivalenol	397	59	95	20	Negative
Diacetoxyscirpenol (DAS)	384	307	105	4	Positive
Diacetoxyscirpenol (DAS)	384	247	105	6	Positive
Deoxynivalenol (DON)	355	265	95	4	Negative
Deoxynivalenol (DON)	355	59	95	20	Negative
Fusarenone-X	413	263	95	8	Negative
Fusarenone-X	413	59	95	28	Negative
HT-2 toxin	442	263	105	4	Positive
HT-2 toxin	442	215	105	4	Positive
Neosolaniol	400	215	95	16	Positive
Neosolaniol	400	185	95	16	Positive
Nivalenol	371	311	108	4	Negative
Nivalenol	371	281	108	8	Negative
Nivalenol	371	59	108	24	Negative
T-2 toxin	484	215	120	16	Positive
T-2 toxin	484	185	120	14	Positive
Zearalenone	317	175	190	16	Negative
Zearalenone	317	131	190	24	Negative
Zearalanone	319	275	185	16	Negative
Zearalanone	319	205	185	16	Negative

Table 2. Comparison of modified QuEChERS and cartridge SPE recovery data spiked in wheat samples at 50 µg/kg (n=9)

Analyte	Analyte retention time (min)	Modified QuEChERS recovery (%)	Modified QuEChERS RSD (%)	BE Mycotoxin SPE recovery (%)	BE Mycotoxin SPE RSD (%)
Nivalenol	5.6	73	7	93	11
Deoxynivalenol	6.4	85	8	84	11
Fusarenon-X	6.8	81	9	89	9
Neosolaniol	6.8	94	9	77	9
15-acetyl Deoxynivalenol	7.2	88	9	72	10
3-acetyl Deoxynivalenol	7.2	100	9	92	11
Diacetoxyscirpenol	7.7	105	2	104	3
HT-2	8.0	83	8	99	4
T-2 toxin	8.2	83	8	100	4
Zearalenone	8.4	87	8	79	9

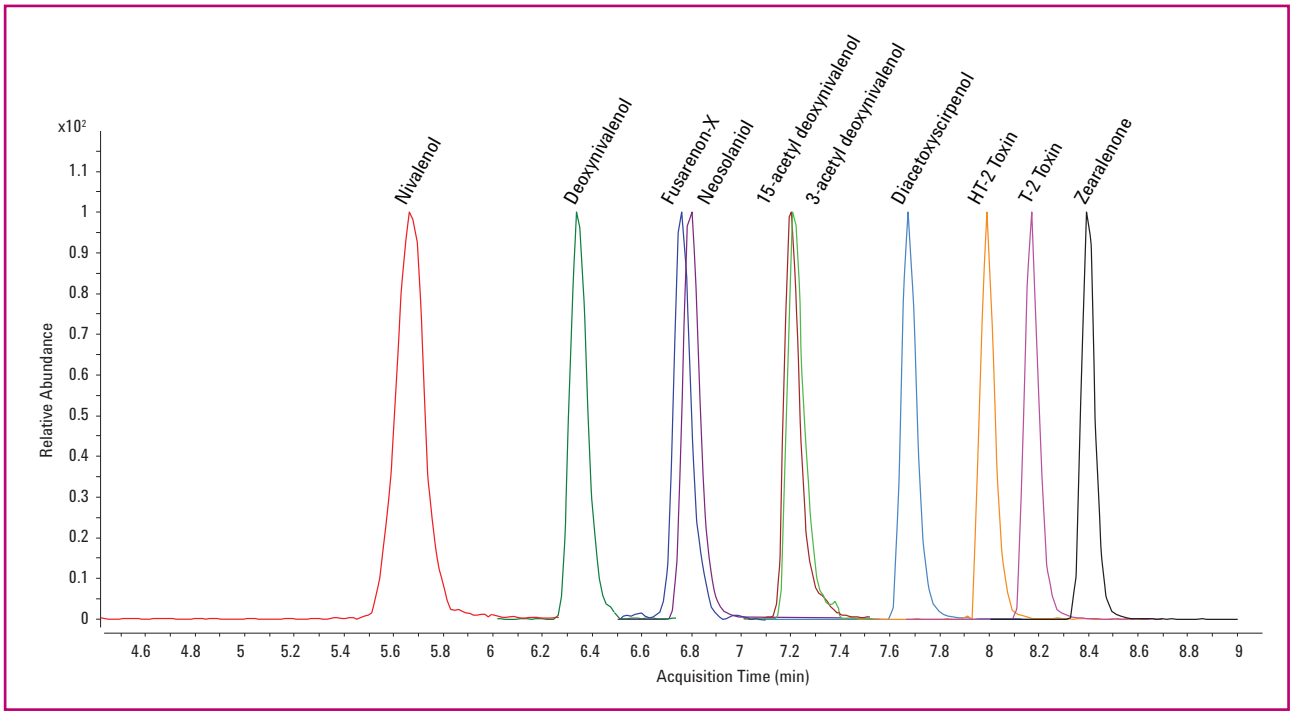


Figure 3. 50 ppb wheat matrix standard: normalized chromatogram for primary transitions. Please note that this method does not require chromatographic resolution between the isomers 15-acetyl DON and 3-acetyl DON because they are distinguished through measurement under different mass spec polarities.

Products used in the above application

Agilent Bond Elut QuEChERS Nonbuffered Extraction Packet. Part No. 5982-5550.

Agilent Bond Elut QuEChERS Dispersive SPE Kit. Part No. 5982-5022.

Agilent Bond Elut Mycotoxin SPE Cartridge, 500 mg, 3 mL. Part No. 12102167.

Agilent ZORBAX Rapid Resolution HT Eclipse Plus C18 Column, 2.1 x 100 mm, 1.8 µm. Part No. 959764-902.

To review this Application Note in its entirety, please search for 5990-9107EN at **agilent.com/chem**

Determination of Multi-Pesticide Residues in Dried Tea Samples using an Optimized Extraction/Cleanup Regime and the Agilent 7000 Series Triple Quadrupole GC/MS System (Publication 5990-9865EN)

Introduction

This application describes the use of an optimized QuEChERS sample preparation approach to effectively extract pesticides from tea, while at the same time minimizing the extraction of caffeine and other co-extractives, which can cause degenerative effects on chromatographic peak shape, analyte retention time shifts and loss of sensitivity. The tea extracts were analyzed by GC/MS/MS using MRM mode on an Agilent 7890 GC with an Agilent 7000B Triple Quadrupole GC/MS system. This novel approach uses a QuEChERS extraction followed by clean-up using liquid extraction for consistent results.

QuEChERS Procedure

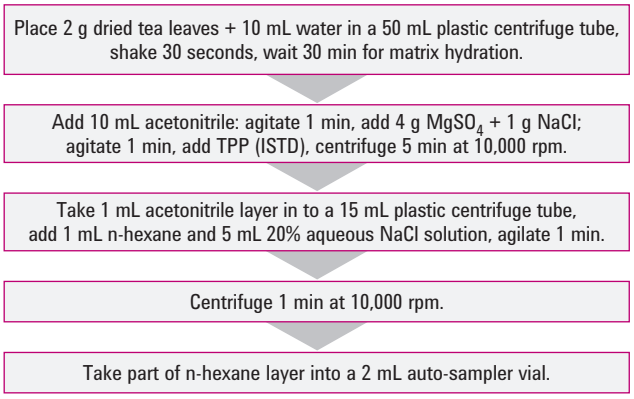


Figure 1. QuEChERS sample extraction and clean-up regime for tea.

Instrument conditions

GC conditions

Column (1):	Agilent J&W HP-5ms Ultra Inert, 15 m × 0.25 mm, 0.25 µm (Part No. 19091S-431UI)
Column (2):	Agilent J&W DB-5ms Ultra Inert, 0.50 m × 0.15 mm, 0.15 µm (cut from Part No. 165-6626)
Capillary flow device:	Pressure controlled tee (PCT) with pneumatics control module (PCM)
Auto-sampler:	Agilent 7693A Automatic Liquid Sampler
Injection:	2 µL cold splitless using CO_2 -cooled Multimode Inlet (MMI)
Splitless period:	1 min
Injection:	Port liner 2 mm id dimpled deactivated liner (Part No. 5190-2296)
Inlet temperature program:	50 °C (0.1 min), 600 °C/min to 300 °C
Purge flow to split vent:	50 mL/min at 1.0 min
RTL compound:	Trifluralin, locked to 6.219 min
Carrier gas:	Helium
Inlet pressure:	17.460 psig constant pressure mode (during run)
PCM pressure:	2.0 psig constant pressure mode (during run)
Oven program:	50 °C (1.0 min), 50 °C/min to 150 °C, 6 °C/min to 200 °C, 16 °C/min to 280 °C (4.07 min)
Post-run time:	2.0 min
Post-run temperature:	280 °C
Post-run pressures:	Inlet 1.0 psig, PCM 60.0 psig
MS transfer line temperature:	280 °C

MS conditions

Ionization mode:	Electron ionization
Electron energy:	-70 eV
Tune:	EI autotune
EM gain:	10
MS1 resolution:	1.2 amu full width at half maximum
MS2 resolution:	1.2 amu full width at half maximum
Transitions:	See reference [2] on app note
Collision energies:	See reference [2] on app note
Dwell times:	2-28 ms depending on the number of transitions per time window to achieve 5 cycles/sec
Collision cell gas flows:	Nitrogen at 1.5 mL/min, helium at 2.25 mL/min
MS temperature zones:	Ion source 280 °C, Q1 150 °C, Q2 150 °C

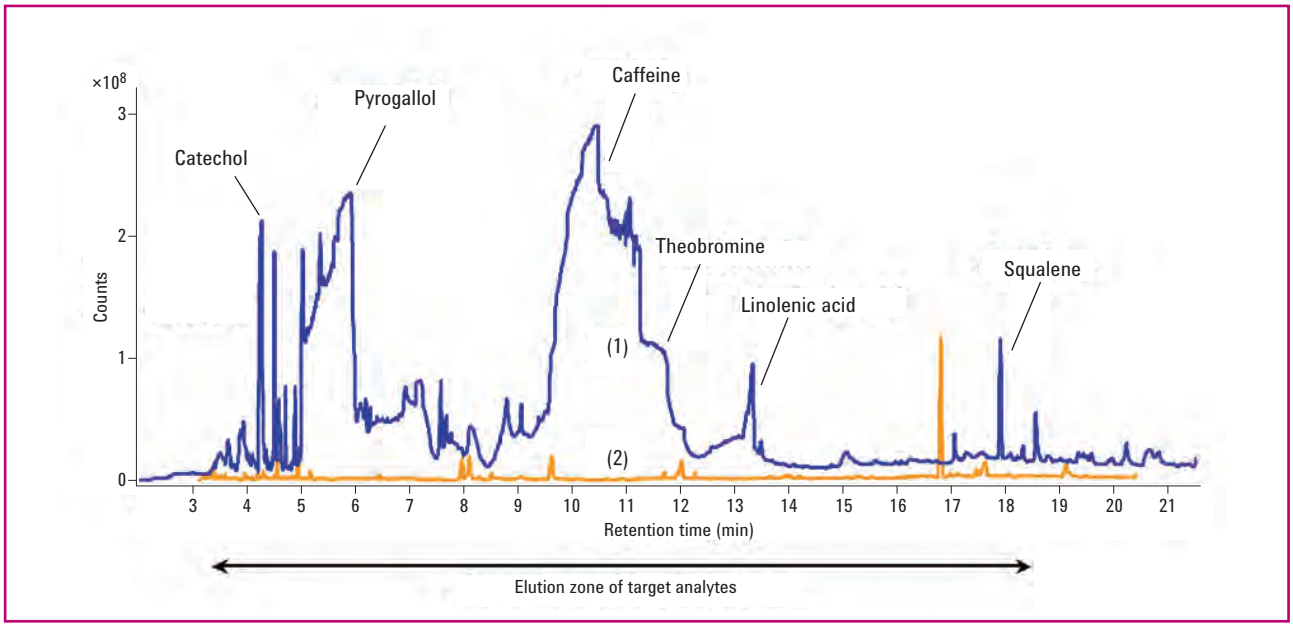


Figure 2. Full scan MS chromatograms showing very high abundance of caffeine and other co-extractives remaining in the acetonitrile extract after the QuEChERS extraction (1 blue trace) compared to their significant reduction when subsequent liquid–liquid extraction is employed (2 yellow trace).

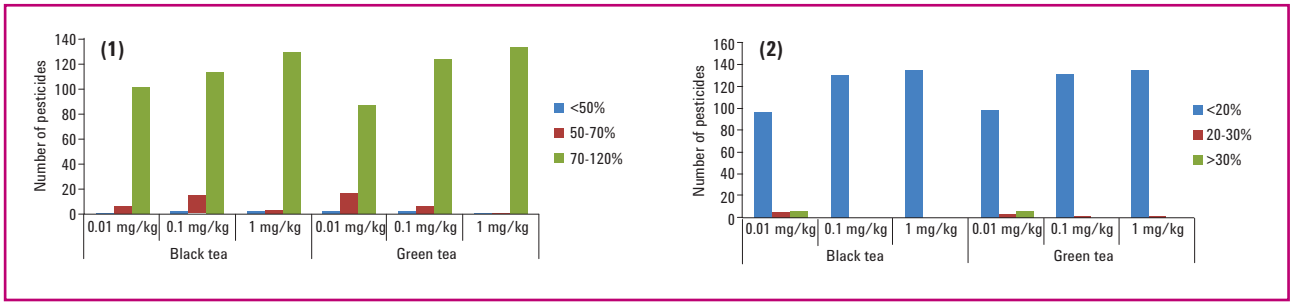


Figure 3. Distribution of (1) overall recoveries and (2) RSDs for the pesticide residues in green and black tea at spiking levels of 0.01, 0.1 and 1 mg/kg.

Products used in the above application

Agilent Bond Elut QuEChERs Nonbuffered Extraction Packet. Part No. 5982-5550.

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

Agilent J&W DB-5ms Ultra Inert Column, 0.50 m x 0.15 mm, 0.15 µm. Cut from Part No. 165-6626.

To review this Application Note in its entirety, please search for 5990-9865EN at **agilent.com/chem**

Screening 36 Veterinary Drugs in Animal Origin Food by LC/MS/MS Combined with Modified QuEChERS Method (Publication 5991-0013EN)

Introduction

This application introduces a modified QuEChERS method that screens food for four classes of veterinary drugs: sulfanilamides, macrocyclic lactones, quinolones, and clopidols. Satisfactory recoveries were achieved by this method for all four classes of veterinary drugs. Instrumental analysis was performed using the Agilent 1260 Infinity LC with Diode Array Detector and the Agilent 6460 Triple Quadrupole LC/MS with the Agilent Jet Stream technology electrospray ionization source. In addition to the meat matrix, the method was successfully used on egg, milk, and honey samples.

QuEChERS Procedure

Transfer 2 g (+/- 0.05 g) of sample, homogenized if necessary, to an empty 50 mL centrifuge tube.

Add 4 mL H_2O, cap and vortex for 1 min.

Add 10 mL 1% acetic acid in ACN, cap, and vortex for 1 min.

Add contents of QuEChERS extraction salt packet (Part No. 5982-0032).

Cap and shake vigorously for 1 min Centrifuge 5 min at 5,000 rpm in a temperature-controlled centrifuge set to 4 °C. Let stand for 30 min.

Transfer a 6 mL aliquot of the upper layer to a 15 mL dispersive SPE tube (Part No. 5982-4950), cap, and vortex for 1 min.

Centrifuge at 5,000 rpm for 5 min.

Remove 4 mL of the upper layer and transfer to a clean tube.

Dry under N_2 at 40 °C.

Reconstitute in 1 mL of 2:8 ACN:H_2O and then centrifuge at 10,000 rpm for 10 min.

Transfer the upper layer to an autosampler vial.

Figure 1. QuEChERS AOAC sample preparation procedures for veterinary drugs in foods of animal origin.

Instrument conditions

HPLC conditions

Column:	Agilent ZORBAX Solvent Saver HD Eclipse Plus C18, 3.0 × 100 mm, 1.8 µm
Flow rate:	0.5 mL/min
Column temperature:	30 °C
Injection volume:	5 µL
Mobile phase:	A: H_2O 0.1% formic acid B: ACN

Gradient:

Time (min)	%A	%B
0.0	90	10
0.5	90	10
1.0	80	20
4.0	75	25
8.0	40	60
9.0	5	95
12.0	5	95
12.1	90	10
15.0	90	10

MS conditions

Polarity:	Positive
Gas temperature:	300 °C
Gas flow:	7 L/min
Nebulizer:	50 psi
Capillary:	3,000 V
Sheath gas temperature:	350 °C
Sheath gas flow:	10 L/min
Scan mode:	DMRM

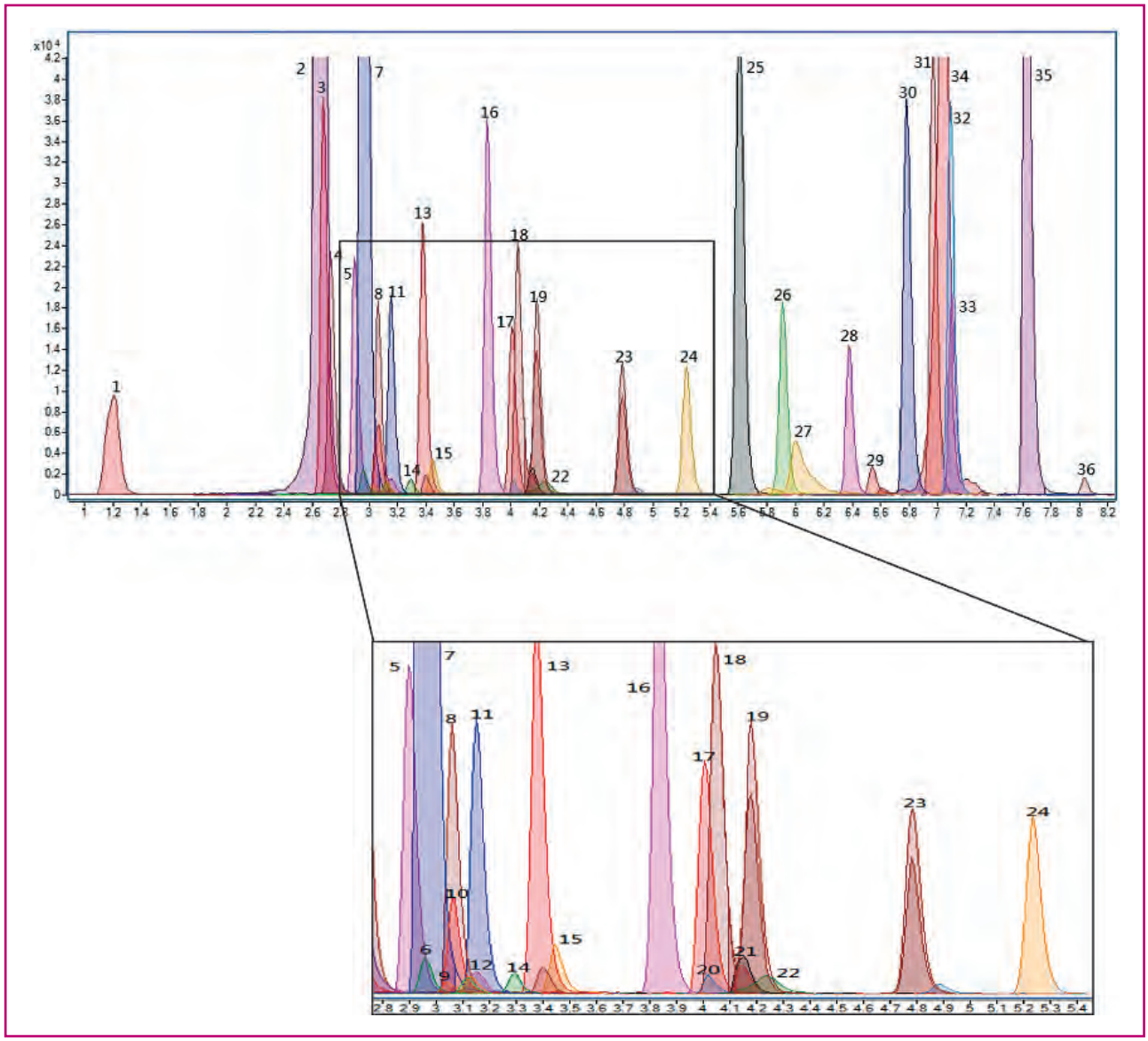


Figure 2. MRM extracted chromatogram for veterinary drugs.

Table 1. Results of optimization of the extraction step. An extraction salt mixture including Na_2SO_4 was selected based on better recoveries

Method	1	2	3	4
Extract salt	$MgSO_4$+NaCl	Na_2SO_4+NaCl	Na_2SO_4+NaCl	Na_2SO_4+NaCl
Dispersive-SPE mix	C18EC+$MgSO_4$	C18EC+Na_2SO_4	C18EC+Na_2SO_4	C18EC+Na_2SO_4
Extract solvent	1% formic acid acetonitrile	1% formic acid acetonitrile	1% formic acid acetonitrile	1% formic acid acetonitrile
Water	8 mL	8 mL	4 mL	4 mL
Average recovery of macrocyclic lactones	22.95%	43.66%	45.12%	70.46%
Average recovery of sulfanilamides	10.86%	25.96%	33.25%	54.35%
Average recovery of quinolones	86.79%	47.69%	62.69%	64.44%
Average recovery of clopidols	55.12%	38.02%	49.89%	65.37%
Zearalenone	8.4	87	8	79

Table 2. Results of dispersive SPE parameter optimization. A dSPE mixture including PSA was selected for greater versatility.

Method	1	2	3	4	5
Extract salt	Na_2SO_4+NaCl	Na_2SO_4+NaCl	Na_2SO_4+NaCl	Na_2SO_4+NaCl	Na_2SO_4+NaCl
Dispersive-SPE mix	50 mg PSA+ 150 mg C18EC+ 900 mg Na_2SO_4	100 mg PSA+ 150 mg C18EC+ 900 mg Na_2SO_4	50 mg SAX+ 150 mg C18EC+ 900 mg Na_2SO_4	100 mg NH2+ 150 mg C18EC+ 900 mg Na_2SO_4	300 mg C18EC+ 900 mg Na_2SO_4
Extract solvent	1% acetic acid	1% acetic acid	1% acetic acid	1% acetic acid	1% acetic acid
	acetonitrile	acetonitrile	acetonitrile	acetonitrile	acetonitrile
Water	4 mL	4 mL	4 mL	4 mL	4 mL
Average recovery of macrocyclic lactone	54.57%	42.23%	59.87%	33.70%	66.10%
Average recovery of sulfanilamide	64.37 %	63.27 %	77.35%	51.71%	71.80%
Average recovery of quinolone	73.88%	88.34 %	76.82%	97.03%	84.66%
Average recovery of clopidol	85.12%	100.11%	71.57%	70.27%	91.17%

Products used in the above application

Agilent Bond Elut Extraction Tubes. Part No. 5982-0032.

Agilent Bond Elut QuEChERS Dispersive SPE Kits for Drug Residues. Part No. 5982-4950.

Agilent ZORBAX Solvent Saver HD Eclipse Plus C18 Column, 3.0 x 100 mm, 1.8 µm. Part No. 959757-302.

To review this Application Note in its entirety, please search for 5990-0013EN at **agilent.com/chem**

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