Agilent Food Safety Applications Notebook:
Volume 1 – SPE, SPME, SLE, and Captiva Filtration

PROVEN APPROACHES FOR TODAY'S FOOD ANALYSIS CHALLENGES

The Measure of Confidence




Agilent Technologies

Table of Contents

Introduction

Dietary Supplements

Food and Flavors

Food Testing and Agriculture

Other

Personal Care Products

Tips and Tricks

Approaches to sample preparation of food are widely varied, and Agilent offers a range of options that may be suitable for each application. In many cases, more than one sample preparation tool may be available for the particular commodity or analyte type. Tips for selecting the right method from available options are available in this guide. Achieving the right level of cleanup for your lab's needs requires striking the perfect balance between your sample prep investment, required method ruggedness, and any mandated selectivity and sensitivity.

Looking for QuEChERS applications?

Our Agilent QuEChERS application notebook offers a comprehensive reference of QuEChERS approaches to sample preparation for food analysis.

Please view Publication #5990-4977EN

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 comprehensive analytical solutions deliver on that promise.

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

Agilent Sample Preparation products: Your first step in food safety analysis.

Agilent sample preparation products help you confidently extract and concentrate samples from complex matrices, to deliver fast, accurate, and reproducible results.

Our products support your food sample preparation needs with a broad set of formats and chemistries manufactured to strict quality standards.

- **Agilent Bond Elut solid phase extraction (SPE)**, comprising over 40 different polymeric and silica-based functionalities, available in a variety of cartridge and plate formats.
- **Agilent Chem Elut solid supported liquid extraction (SLE)**, for easy and reproducible cleanup using the same principles as liquid-liquid extractions, without the complications.
- **Agilent Captiva filtration**, for mechanical and chemical filtration in a variety of formats to simplify sample preparation methods and deliver the best sample hygiene.

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

Trisa Robarge
Sample Preparation Product Manager

Interference Guide:

Select your sample preparation technique based on the type(s) of interferences you need to remove.

	More Selective	*Instrument Separation and Detection Selectivity*					Less Selective
	Less Selective	*Sample Preparation Selectivity*					More Selective
Interference Removed / Sample Prep Technique	Dilute & Shoot	Filtration	Supported Liquid Extraction (SLE)	Precipitation	QuEChERS	Lipid Removal 'Hybrid' Filtration	Solid Phase Extraction
Lipids	No	No	No	No	Yes	Yes	Yes
Oligomeric Surfactants	No	No	No	No	No	Yes	Yes
Particulates	No	Yes	Some	Yes	Yes	Yes	Yes
Pigments	No	No	Some	No	Yes	No	Yes
Polar Organic Acids	No	No	Yes	No	Yes	No	Yes
Proteins	No	No	Yes	Yes	Yes	Yes	Yes
Salts	No	No	Yes	No	Yes	No	Yes
Suggested Agilent Products	**Agilent Autosampler Vials**	**Captiva Filtration**	**Chem Elut Hydromatrix**	**Captiva Non-Drip (ND)**	**Bond Elut QuEChERS**	**Captiva ND Lipids**	**Bond Elut Silica and Polymeric SPE**

Application Guide:

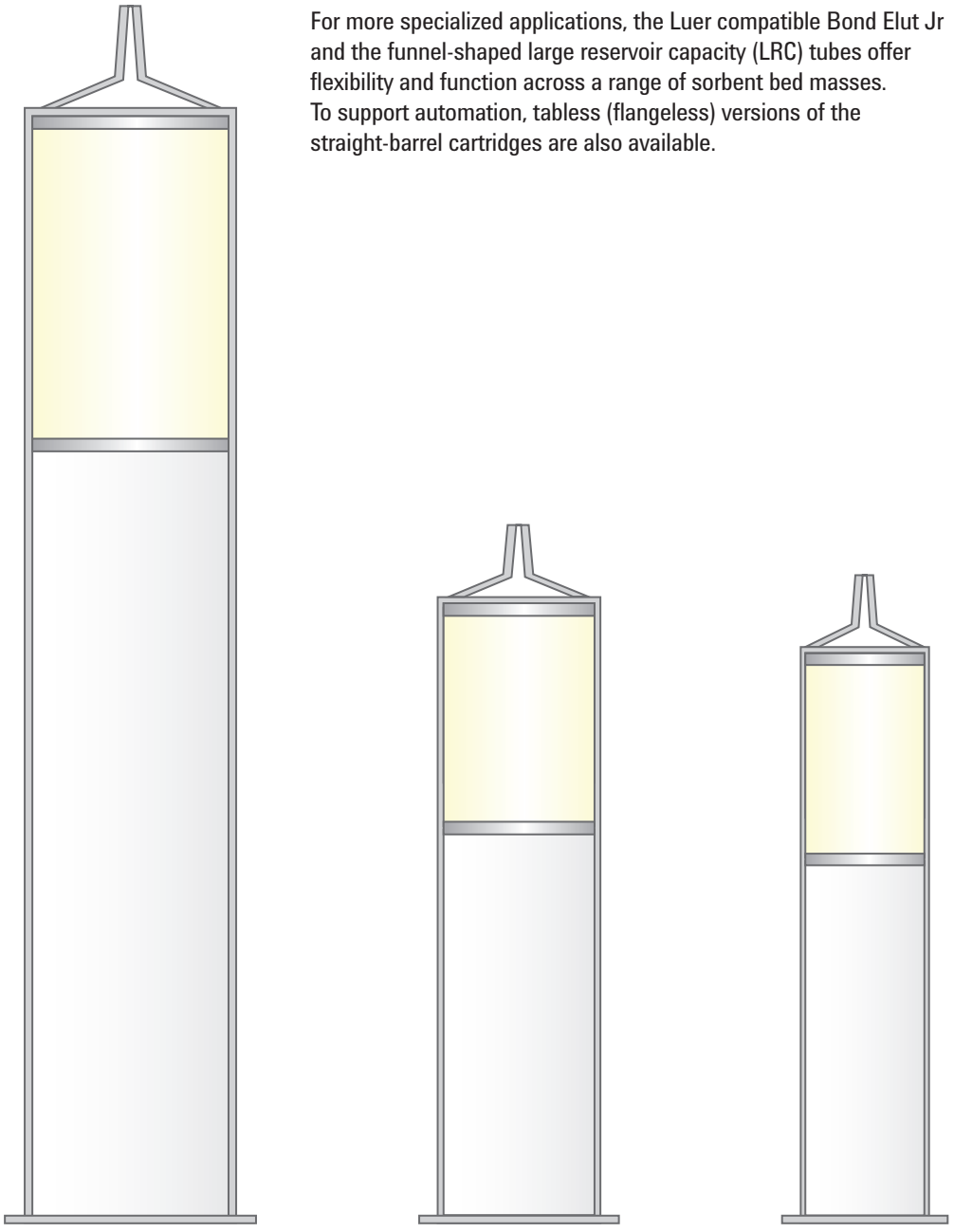
Select the sample preparation product best suited for your analysis needs

Industry	Application	Technique	Product
Biotechnology	Protein/Peptide Purification	Lysate Filtration	Captiva
		Micro-volume SPE	OMIX
Clinical Research and Forensics	Bioanalysis	Solid Phase Extraction	Bond Elut
			Bond Elut Plexa
			Bond Elut Plexa PCX
		Micro-volume SPE	OMIX
		Supported Liquid Extraction (SLE)	Chem Elut
		Protein Precipitation Filtration	Captiva ND
			Captiva ND Lipids
			Captiva
Environmental Monitoring	Semi-volatiles	Solid Phase Extraction	Bond Elut
			SPEC
	Oils and Grease	Solid Phase Extraction	Bond Elut
			SPEC
		Water Removal	Bond Elut
			Na_2SO_4
	Emerging Contaminants	Solid Phase Extraction	Bond Elut
		Supported Liquid Extraction (SLE)	Chem Elut
	Textile Analysis	Supported Liquid Extraction (SLE)	Chem Elut
Food and Beverage	Pesticides and Herbicides	Filtration	Captiva ND
			Captiva ND Lipids
			Captiva
		Solid Phase Extraction	Bond Elut
			Bondesil
			QuEChERS
		Supported Liquid Extraction (SLE)	Chem Elut
Pharmaceutical	Bioanalysis	Solid Phase Extraction	Bond Elut Plexa
			Bond Elut Plexa PCX
			Bond Elut Plexa PAX
			Bond Elut
			SPEC
		Micro-volume SPE	OMIX
		Protein Precipitation Filtration	Captiva ND
			Captiva ND Lipids
			Captiva
		Supported Liquid Extraction (SLE)	Chem Elut
	Veterinary Drugs	Solid Phase Extraction	QuEChERS

Agilent Offers a Broad Range of Tube Formats and 96-well Plate Designs

We have a full set of straight barrel tubes ranging from 1-150 mL in a wide range of bonded silica and polymeric chemistries, sorbent particle sizes and bed masses.

For more specialized applications, the Luer compatible Bond Elut Jr and the funnel-shaped large reservoir capacity (LRC) tubes offer flexibility and function across a range of sorbent bed masses. To support automation, tabless (flangeless) versions of the straight-barrel cartridges are also available.



Diagrams are to scale

60 mL

20 mL

12 mL

Bond Elut 96-well Plates

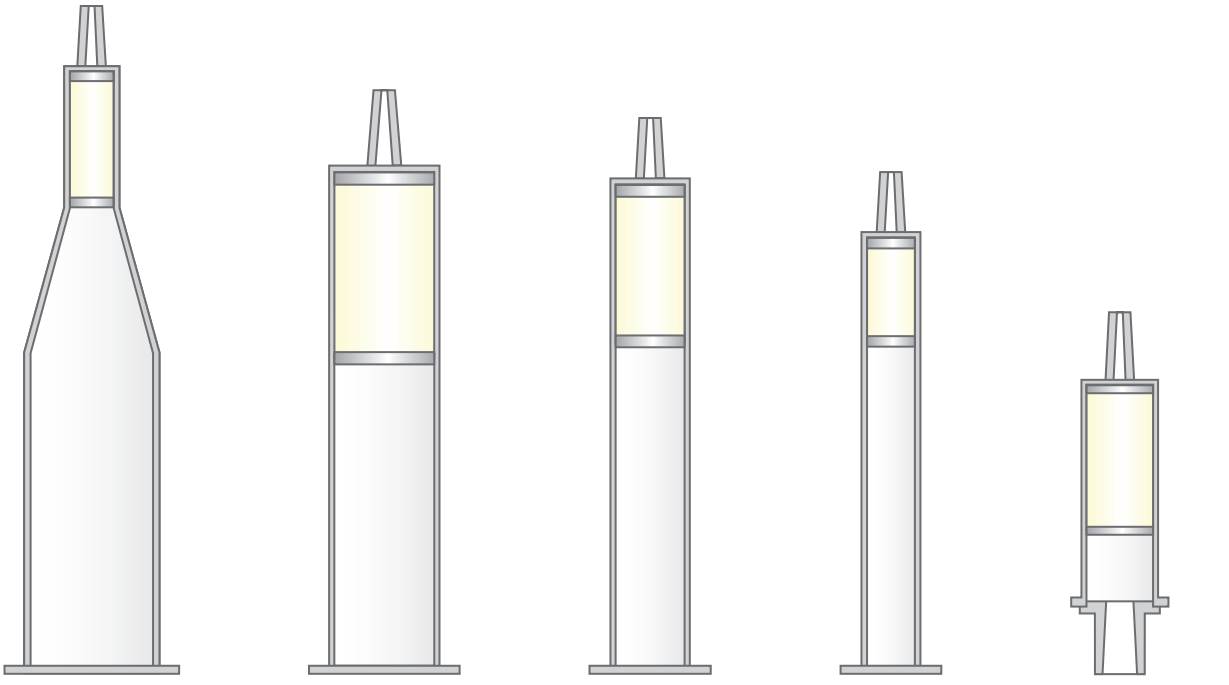
Bond Elut 96-well plate formats are best in class for flow performance and well-to-well reproducibility. These specially designed plates are available with well volumes of 1 mL and 2 mL and in a large range of different sorbent chemistries.

VersaPlate

VersaPlate is an innovative, versatile design that lets you customize plates. Insert tubes packed with different phases for sorbent screening, or insert only enough tubes to match the number of samples to be extracted for minimal waste. Luer tip of Versaplate tubes can also fit VacElut 12, VacElut 20, and VacElut SPS 24 vacuum manifolds. VersaPlate can be purchased in a pre-packed 96 position format or as loose tubes.

Packed Formats for Automation

Bond Elut sorbents are also available in packed bed formats for automation platforms, such as the Spark Holland Symbiosis, Gilson ASPEC and Gerstel MPS systems. Agilent's unique OMIX pipette format is also used with a wide range of liquid handling devices, ranging from hand-held pipettors to high-throughput automated systems.



Cross Reference of Comparable Phases by Manufacturer

Different chemistries and manufacturing processes create sorbents that exhibit differences in selectivity, so there is no universal equalivent for every application. However, the performance of products can be similar in many applications. This table provides suggestions for using Agilent Bond Elut products in comparison to products from other manufacturers.

Polymers

If you are using...				Try this...
Phenomenex Strata	**Waters Oasis**	**Supelco Supelclean/Discovery**	**UCT**	**Agilent Bond Elut**
Strata-X	HLB			Plexa
SDB-L		ENVI-ChromP	Styre Screen	ENV or LMS
Strata-X-C	MCX			Plexa PCX
	MAX			Plexa PAX

Silica-Based and Other Sorbents

If you are using...				Try this...
Phenomenex Strata	**Waters Sep-Pak**	**Supelco Supelclean/Discovery**	**UCT**	**Agilent Bond Elut**
C18-E	tC18	ENVI-18, DSC-C18, LC-18	C18-E	C18
C18-U	C18		C18-U	C18 OH
C8	C8	DSC-8, Envi-8, LC-8	C8	C8
	tC2			C2
Phenyl (PH)		DSC-Ph, LC-Ph	Phenyl	PH
Screen-C			Clean Screen	Certify
Si-1	Silica	DSC-Si, LC-SI	Silica	SI
FL-PR	Florisil	LC and ENVI Florisil	Florisil PR	FL
NH2	Amino Propyl	DSC-NH2, LC-NH	Amino Propyl	NH2
		DSC-Diol, LC-Diol	Diol	2OH
CN	Cyano Propyl	DSC-CN, LC-CN	Cyano Propyl	CN-E
	Alumina A, B, N	LC-Alumina A, B, N	Alumina A, B, N	Alumina A, B, N
SAX	AccellPlus QMA	DSC-SAX, LC-SAX, Quat amine with Cl	Quat amine with Cl	SAX
SCX	AccellPlus CM	DSC-SCX, LC-SCX	Benzenesulfonic acid	SCX
		ENVI-Carb	Carbon	Carbon
		ENVICarb-II/NH2		Carbon/NH2
		ENVICarb-II/PSA		Carbon/PSA

General Protocol for Trouble-Free SPE Applications with Bond Elut Plexa Polymeric SPE

Regardless of your application or sample type, you will appreciate the difference the Bond Elut Plexa range makes. Plexa delivers simple methods and superior flow characteristics that effectively eliminate common matrix background that can cause interference and ion suppression, resulting in improved analytical sensitivity and data quality.

	Acids	Neutrals		Bases
Analyte	**Log P > 1.0 pKa < 5**	**Log P > 1.5 pKa 3-6**	**pKa 6-10**	**Log P > 0.8 pKa 6-10**
	Plexa PAX	**Plexa Acid Load Method**	**Plexa Base Load Method**	**Plexa PCX**
Sample Treatment	2% NH_4OH	1% HCO_2H	2% NH_4OH	2% H_3PO_4
Sorbent Condition	100% MeOH	100% MeOH		100% MeOH
Equilibrate	100% H_2O	100% H_2O		100% H_2O
Load	Apply pre-treated sample			
Wash	100% H_2O	5% MeOH in H_2O		2% HCO_2H in H_2O
Elution 1	100% MeOH **Neutrals**	100% MeOH **Neutrals**		1:1 MeOH/ACN **Acids, Neutrals**
Elution 2	5% HCO_2H in MeOH **Acids**			5% NH_3 in 1:1 MeOH/ACN **Bases**
Analysis	Prepare extracts for instrumental analysis			

Determination of Flavonoids in Ginkgo Biloba Using Bond Elut Plexa Solid Phase Extraction Sorbent for Cleanup and HPLC-DAD Analysis

(Publication 5990-9547EN)

Introduction

Characterization of the active flavonoids in Gingko biloba leaves and supplements is an important means of controlling for consistency in the final product as well as for understanding active constituents for research. This application compares a simple sample preparation process to one in which solid phase extraction (SPE) using Agilent Bond Elut Plexa polymeric SPE is used to perform an additional cleanup. The extracts following Plexa cleanup were free from interferences, provided excellent linearity and detection limits, and were compatible with HPLC and diode array detection (DAD). Recoveries ranged from 73-88% for isorhamnetin to 103-109% for kaempferol, with precision of less than 5% RSD for all analytes.

HPLC/MS conditions

Column:	ZORBAX Eclipse Plus C18 959933-902 4.6 mm x 75 mm, 3.5 µm
Mobile phase:	A: 0.5% phosphoric acid B: methanol
Flow rate:	1 mL/min
Volume:	5 µL
Temperature:	35 °C
Detector:	UV, 370 nm
Isocratic:	40% A:60% B
Run time:	4 min

LOD and LOQ for Quercetin, Kaempferol, and Isorhamnetin

	LOD (µg/mL)	LOQ (µg/mL)
Quercetin	1.47	4.67
Kaempferol	0.80	2.65
Isorhamnetin	3.25	10.8

SPE Procedure

Homogenize Ginkgo biloba capsules or loose leaves.

Reflux 2 g homogenized capsule or 1 g loose leaves with 40 mL methanol and 40 mL 5.5% HCl (V:V) while stirring continuously for 1 hour. Cool to room temperature.

Filter sample with filter paper and dilute 1:3 with 2% ammonia solution. Adjust sample pH to 7 with 1M KOH.

Condition Plexa SPE tube with 500 µL methanol followed by 500 µL H_2O.

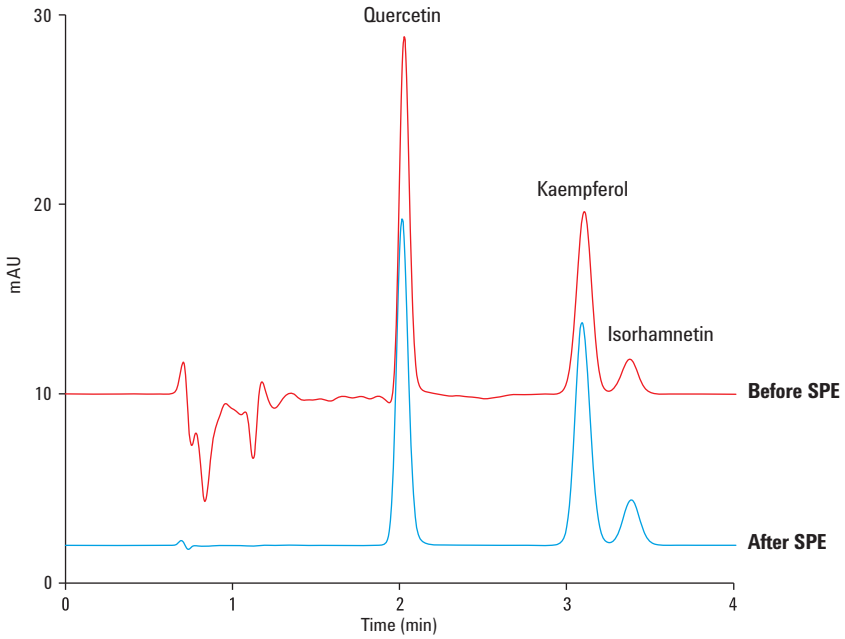
Apply treated sample to SPE tube and extract under low to no vacuum.

Wash the SPE cartridge with 500 µL 45% methanol. Repeat with a second 500 µL 45% methanol wash.

Elute flavonoids with 1 mL methanol.

Filter the extract and transfer to an autosampler vial for analysis.

Results



Recovery and Reproducibility Data of Quercetin, Kaempferol, and Isorhamnetin from Spiked Samples

Analyte	Spiking level (µg/mL) n=6	% Recovery	% RSD
Quercetin	10	107	4.35
	20	106	3.35
Kaempferol	10	109	2.53
	20	103	1.14
	40	108	4.39
Isorhamnetin	10	88	4.11
	20	73	4.5
	40	79	1.73

Products used in the above application

Agilent Bond Elut Plexa Cartridge, 30 mg, 1 mL, 100/pk, Part No. 12109301

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

Recommended Filter:
Agilent Captiva PES Premium Syringe Filter, 25 mm, 0.45 µm, Part No. 5190-5099

To review this Application Note in its entirety, please view **5990-9547EN**

Determination of Alkaloids in Goldenseal Using Agilent Bond Elut Plexa Solid Phase Extraction Sorbent for Cleanup and HPLC-DAD Analysis

(Publication 5990-9563EN)

Introduction

Two alkaloids believed to play a central role as active constituents in herbal remedies made from goldenseal (Hydrastis canadensis), berberine and hydrastine, can be extracted from goldenseal supplements for identification and quantification. Controlling for the levels of these alkaloids can provide product consistency. A simple sample extraction technique was compared to sample extraction followed by cleanup with Agilent Bond Elut Plexa solid phase extraction, with analysis by HPLC and diode array detection (DAD). The combination of steps resulted in a sample with reduced matrix background. Recoveries for hydrastine were between 76-102%, and recoveries for berberine ranged from 99-104%, with precision of less than 5% RSD, reflecting a simple and rugged method.

HPLC Conditions

Column:	ZORBAX Eclipse Plus C18 959933-902 4.6 mm x 75 mm, 3.5 µm
Mobile phase:	A: 0.5% phosphoric acid B: methanol
Flow rate:	1.00 mL/min
Volume:	5 µL
Temperature:	35 °C
Run time:	4 min

Gradient:

Time	0	0.5	3
% B	25	25	50

SPE Procedure

Homogenize 200 mg goldenseal root.

Reflux homogenized sample with 200 mL dionized water while stirring continuously for 1 hour. Cool to room temperature.

Filter sample with filter paper and dilute 1:3 with 2% ammonia solution. Adjust sample pH to 7 with 0.01M HCl.

Condition Plexa SPE tube with 500 µL methanol followed by 500 µL H_2O.

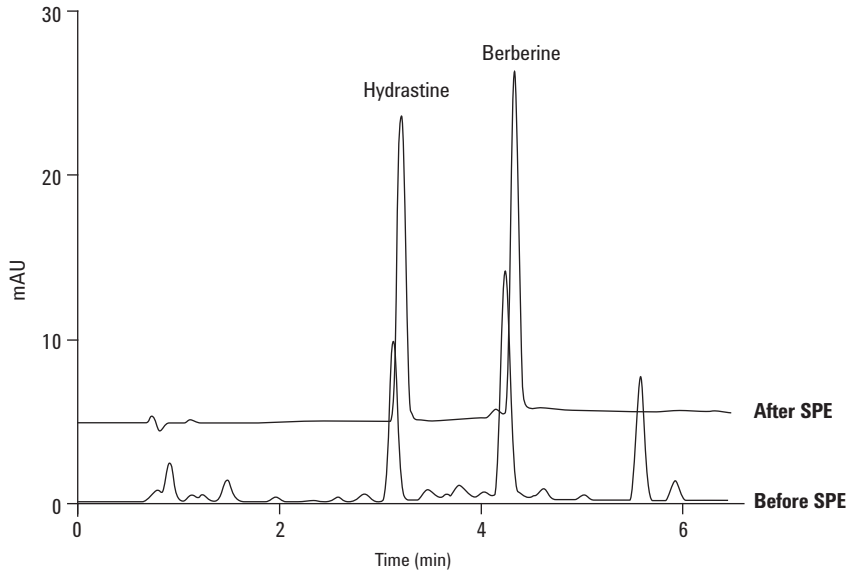
Apply treated sample to SPE tube and extract under low to no vacuum.

Wash the SPE cartridge with 500 µL 45% methanol.

Elute alkaloids with 1 mL methanol.

Transfer sample to an autosampler vial for analysis.

Results



Recovery and Reproducibility Data for Hydrastine and Berberine (n = 6)

Alkaloid	Spiking level (µg/mL)	% Recovery	%R.S.D.
Hydrastine	10	76	3.94
	50	83	4.98
	100	102	2.40
Berberine	5	99	4.68
	75	104	3.21

HPLC-DAD chromatograms of hydrastine and berberine from goldenseal roots extract before and after SPE.

Products used in the above application

Agilent Bond Elut Plexa Cartridge, 30 mg, 1 mL, 100/pk, Part No. 12109301

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

Recommended Filter:
Agilent Captiva PES Premium Syringe Filter, 25 mm, 0.45 µm, Part No. 5190-5099

To review this Application Note in its entirety, please view **5990-9563EN**

Sensitive Detection of Trichloroanisole (TCA) in Wine Using Triple Quadrupole GC/MS

(Publication 5990-4968EN)

Introduction

This application describes a method for detecting and quantifying 2,4,6-trichloroanisole (TCA) in wine using the Agilent 7000 Series Triple Quadrupole GC/MS and headspace solid phase microextraction (HS-SPME). TCA in wine is also known as "cork taint" that causes an off-flavor, even at very low concentrations. Using HS-SPME for sample preparation, coupled with tandem GC/MS and backflushing, yielded detection limits as low as 1 ppt, comparable to high resolution MS methods and compatible with the olfactory threshold for this compound.

Instrument Conditions

GC Run Conditions

Column:	HP-5ms Ultra Inert 19091S-431UI 15 m x 0.25 mm, 0.25 µm
Instrument:	• Agilent 7890A Gas Chromatograph equipped with a split/splitless inlet • Agilent 7000 Series Triple Quadrupole GC-MS/MS
Injection:	SPME; 2 min; 250 °C; 50 mL/min purge at 2 min
Carrier:	Helium, constant flow, 3 mL/min
Oven:	40 °C (2 min hold), 25 °C/min to 215 °C
Transfer line temperature:	280 °C

GC Post-Run Conditions

Backflush device:	Purged Ultimate Union (P/N G3186-60580) controlled by a Pressure Control Module (P/N G3476-60501)
Backflush conditions:	-5 mL/min at 250 °C for 2 min

MS Conditions

Tune:	Autotune
Delta EMV:	20
Acquisition parameters:	EI; selected reaction monitoring
Collision gas flows:	Nitrogen at 1.5 mL/min, helium at 2.35 mL/min
Solvent delay:	6.5 minutes
MS temperatures:	Source 300 °C; quadrupoles 150 °C

HS-SPME Procedure

To a 20 mL clean headspace vial, add 2 mL of wine sample.

Add 80 ng/L isotopically labeled MIBP internal standard to each vial.

Add 2 g NaCl to each vial, mix, and cap.

At room temperature, insert SPME fiber into headspace vial and perform HS-SPME extraction for 30 minutes.

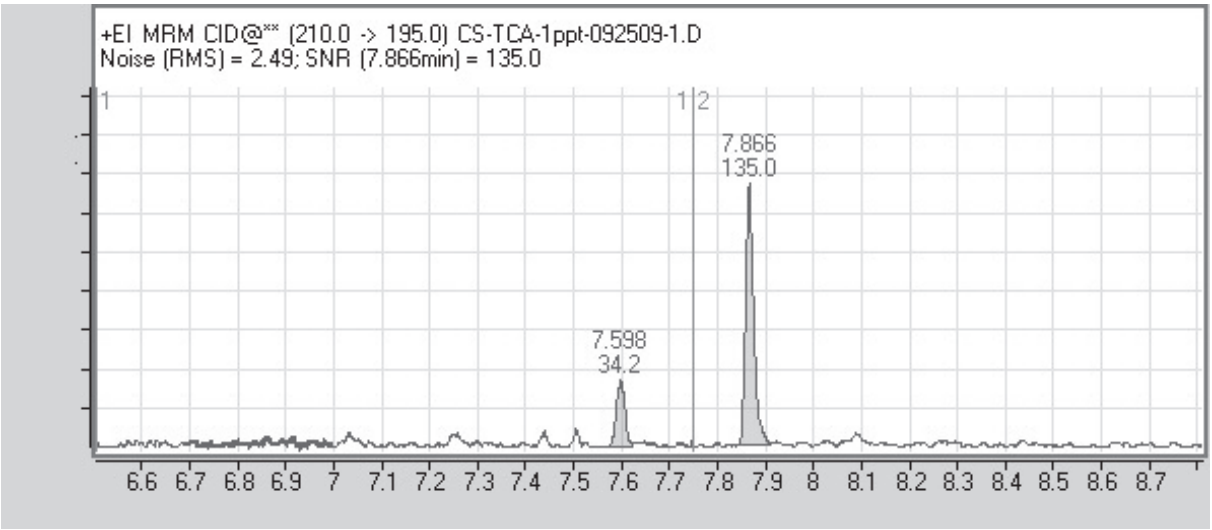
Remove fiber and desorb in GC inlet at 250 °C.

Triple Quadrupole GC/MS Analysis Parameters

The parameters used in the analysis of 2,3,6- and 2,4,6-TCA

Compound	RT (min)	SRM	Dwell Time (ms)	Collision Energy (EV)
2,4,6-Trichloroanisole	7.594	210→195	25	15
		167→83	25	20
2,3,6-Trichloroanisole	7.867	210→195	25	10
		210→167	25	20
		167→83	25	20

Results



Reconstructed Total Ion Current Chromatogram (RTICC) resulting from SRM analysis, showing the separation of 2,3,6- and 2,4,6-TCA in a sample of Cabernet Sauvignon wine spiked with 10 ng/L 2,3,6-TCA and 1 ng/L 2,4,6-TCA. The 2,4,6-TCA spiked sample elutes at 7.598 minutes, and the 2,3,6-TCA internal standard elutes at 7.866 minutes.

Products used in the above application

Agilent SPME Fiber Carboxen/DVB/PDMS 80U Cartridge, 1 cm, Part No. SU57329U

Agilent SPME Fiber Holder for Manual Sampling, Part No. 391896401

Agilent J&W HP-5ms Ultra Inert GC Column, 15 m x 0.25 mm, 0.25 µm, 7 inch cage, Part No. 19091S-431UI

Agilent Non-Stick Long-Life Septa, 11 mm, Part No. 5183-4761

Agilent Liner, Splitless, Single Taper, Deactivated, 4 mm id, Part No. 5181-3316

To review this Application Note in its entirety, please view **5990-4977EN**

Sensitive Detection of 2-Methoxy-3-Isobutylpyrazine (MIBP or IBMP) in Wine Using Triple Quadrupole GC/MS in PCI Mode

(Publication 5990-4935EN)

Introduction

This application describes a method for detecting and quantifying 2-methoxy-3-isobutylpyrazine (MIBP) in wine using the Agilent 7000 Series Triple Quadrupole GC/MS and headspace solid phase microextraction (HS-SPME). Utilization of positive chemical ionization and backflushing in combination with SPME yielded detection of MIBP down to 2 ng/L (2 ppt). HS-SPME was performed at room temperature and was easily optimized for extraction efficiency.

GC-MS/MS Run Conditions

Column:	HP-5ms Ultra Inert 19091S-431UI 15 m x 0.25 mm, 0.25 µm
Instrument:	• Agilent 7890A Gas Chromatograph equipped with a split/splitless inlet • Agilent 7000 Series Triple Quadrupole GC-MS/MS
Inlet temperature:	250 °C
Inlet pressure:	9.5 psi
Carrier:	Helium, constant flow mode, 1.2 mL/min
Splitless:	Purge 50 mL/min @ 2 min
Oven:	45 °C (2.25 min hold), 8 °C/min to 130 °C
Column velocity:	39.8 cm/s
Injection:	SPME; 2 min; 250 °C
Transfer line temperature	250 °C
GC Post-Run Conditions	
Backflush device:	Purged Ultimate Union (P/N G3186-60580) controlled by a Pressure Control Module (P/N G3476-60501)
Backflush conditions:	-1.2 mL/min @ 200 °C for 2 min
MS Conditions	
Tune:	PCI autotune
Delta EMV:	800 V
Acquisition parameters:	PCI; selected reaction monitoring
Reagent gas flow:	20% methane
Solvent delay:	3.75 minutes
MS temperatures:	Source 300 °C; quadrupoles 150 °C

HS-SPME Procedure

To a 20 mL clean headspace vial, add 10 mL of wine sample.

Add 2,3,6-TCA internal standard to each vial for a concentration of 10 ng/L.

Add 2 g NaCl to each vial, mix, and cap.

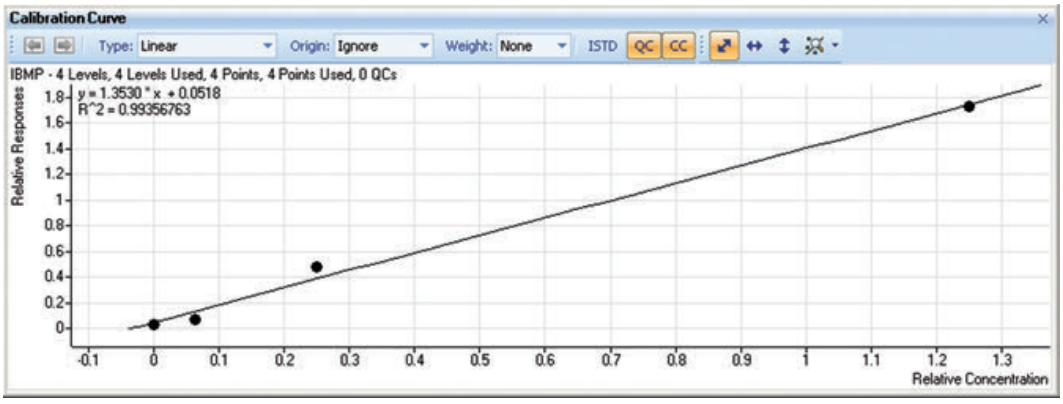
At room temperature, insert SPME fiber into headspace vial and perform HS-SPME extraction for 30 minutes.

Remove fiber and desorb in GC inlet at 250 °C.

MRM Analysis Parameters

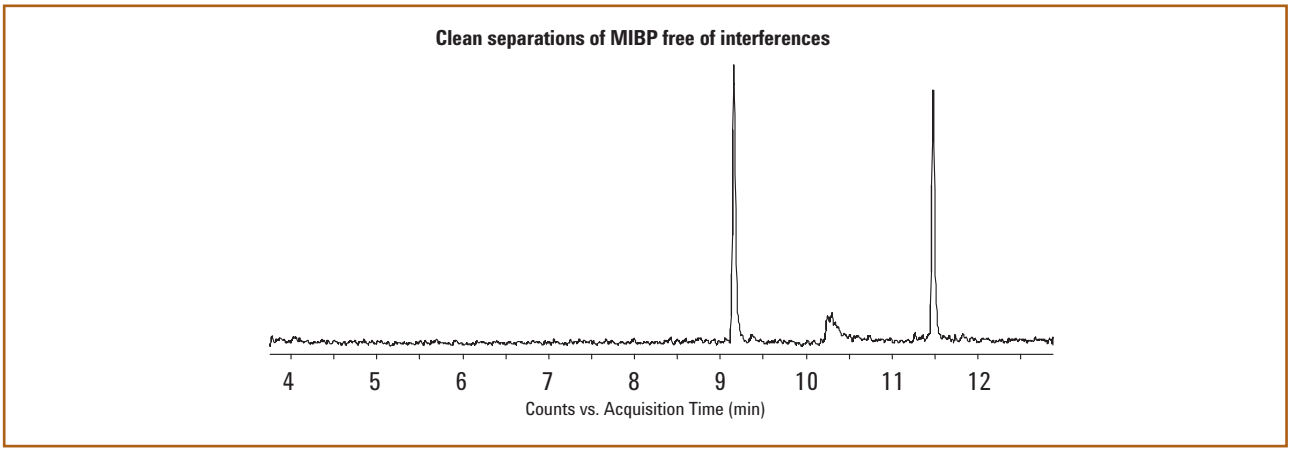
Triple Quadrupole GC/MS				
Compound	**RT (min)**	**Transition**	**Dwell Time (ms)**	**Collision Energy (EV)**
MIBP	11.5	167→94	60	35
		195→124	60	30
		195→106	60	35
Isotopically Labeled-MIBP (Internal Standard)	11.5	170→127	20	30
		170→128	20	30
		170→100	20	30

Quick and easy calibration



Calibration curve for quantification of MIBP. Samples containing 0, 5, 20 and 100 ng/L of MIBP in model wine were used to construct the curve.

Results



Reconstructed Total Ion Current Chromatogram (RTICC) resulting from SRM analysis, showing the separation of MIBP in a sample of Cabernet Sauvignon wine spiked with 5 ng/L MIBP. Both the isotopically labeled internal standard and MIBP standard elute at 11.5 minutes, and both are well resolved from the interference peaks at 9.2 and 10.4 minutes.

Products used in the above application

Agilent SPME Fiber Carboxen/DVB/PDMS 80U Cartridge, 1 cm, Part No. SU57329U

Agilent SPME Fiber Holder for Manual Sampling, Part No. 391896401

Agilent J&W HP-5ms Ultra Inert GC Column, 15 m x 0.25 mm, 0.25 µm, 7 inch cage, Part No. 19091S-431UI

Agilent Non-Stick Long-Life Septa, 11 mm, Part No. 5183-4761

Agilent Liner, Splitless, Single Taper, Deactivated, 4 mm id, without Glass Wool, Part No. 5181-3316

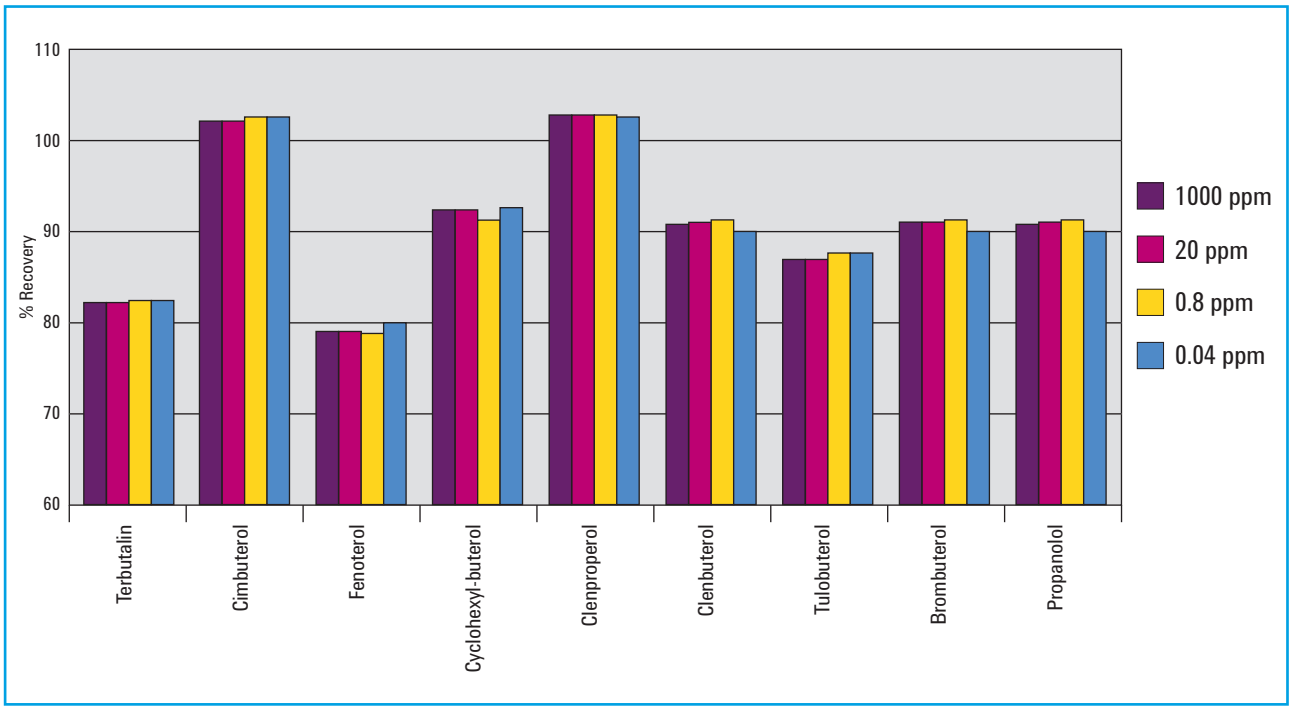
To review this Application Note in its entirety, please view **5990-4935EN**

Improved SPE for the Analysis of Beta-Agonist Residues from Animal Tissue

(Publication 5990-7687EN)

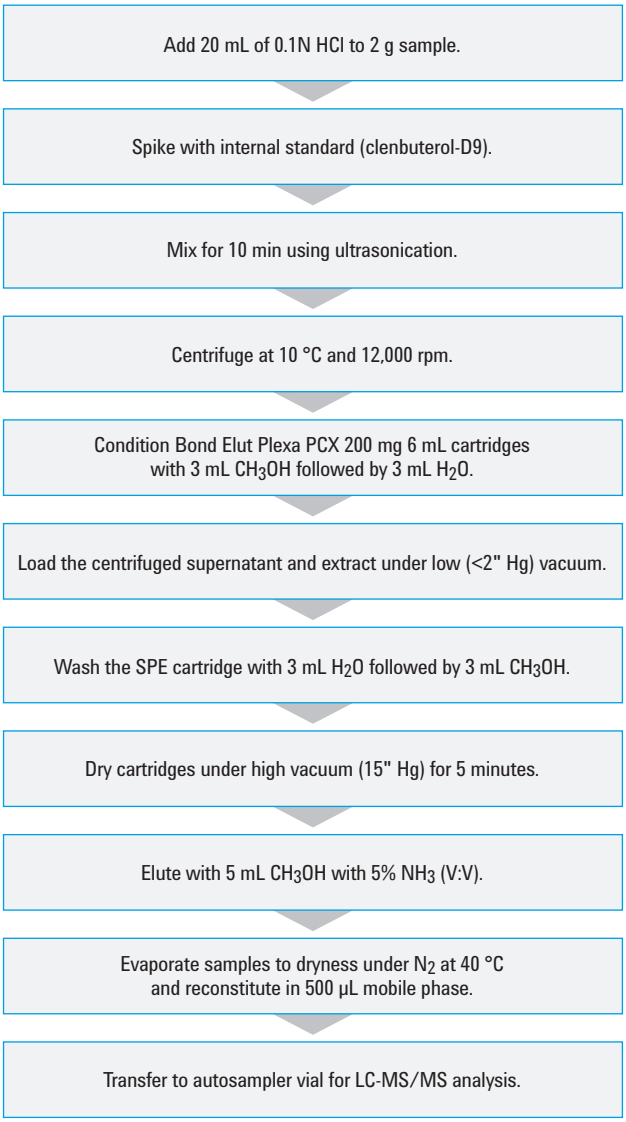
Introduction

Veterinary drug residues in animal tissues are challenging but important to verify suitability of products for consumption. This application illustrates the use of Bond Elut Plexa PCX cation exchange SPE for the sample cleanup and extraction of selected beta-agonist residues using LC-MS/MS for analysis. Good recoveries were achieved with a simple, time-saving method.



Recoveries of spiked beta-agonists from vitreous humor and retina extracts of pigs. SPE cleanup with Bond Elut Plexa PCX. Samples are analyzed with LC-MS/MS.

SPE Procedure



Products used in the above application

Agilent Bond Elut Plexa PCX Cartridge, 200 mg, 6 mL, 30/pk, Part No. 12108206

To review this Application Note in its entirety, please view **5990-7687EN**

Multiresidue Screening of Veterinary Drugs (I) and (II) in Meat According to the Japan Positive List Using Cartridge-based SPE and LC-MS/MS

(Publication 5990-8986EN)

Introduction

Extraction and analysis of veterinary drugs from animal tissue remains challenging due to the variability of the sample matrix plus the range of chemical properties reflected in the drugs themselves. This application describes the use of a multi-step approach to the analysis of veterinary drugs in meat according to the Japan Positive List (JPL). Extraction includes liquid-liquid extraction with Agilent Chem Elut Hydromatrix sorbent-based filtration followed by cleanup with Agilent Bond Elut Plexa SPE. The resulting extracts were analyzed using LC-MS/MS, utilizing two analytical methods depending on the analyte properties, and the methods delivered extraction capabilities and necessary precision to meet a 10 ppb detection limit and MRLs as required. The LC column provided unique separation of three pairs of isomers, while the SPE method was reproducible and removed turbidity.

LC Protocol for Method (I)

Mobile Phase:: A: CH_3CN + 0.1% formic acid
B: H_2O + 0.1% formic acid

Column temperature: 40 °C

Gradient:

Time (min)	%A	%B	Flow Rate (µL/min)
0	5	95	200
2	5	95	200
30	80	20	200
34	80	20	200
35	5	95	200
40	5	95	200

LC Protocol for Method (II)

Mobile Phase: A: CH_3CN + 0.1% formic acid
B: H_2O + 0.1% formic acid

Column temperature: 40 °C

Gradient:

Time (min)	%A	%B	Flow Rate (µL/min)
0	5	95	200
28	99	1	200
33	99	1	200
34	5	95	200
40	5	95	200

Note: *Detailed MRM transitions are available in the complete application note.*

SPE Procedure

Weigh 5 g of meat sample. Add 100 mL acetonitrile/methanol/ 0.2% metaphosphoric acid (1:1:3 V:V:V) and homogenize.

Filter under vacuum with filter paper coated with 2-3 mm of Hydromatrix diatomaceous earth. Collect filtrate.

Rinse filter with 20 mL ACN/Methanol/0.2% metaphosphoric acid (1:1:3 V:V:V) and collect filtrate.

Pool filtrates and concentrate through evaporation to 20 mL.

Condition Bond Elut Plexa SPE cartridges with 5 mL methanol followed by 5 mL 2% ammonium hydroxide, using low vacuum.

Load sample under low vacuum.

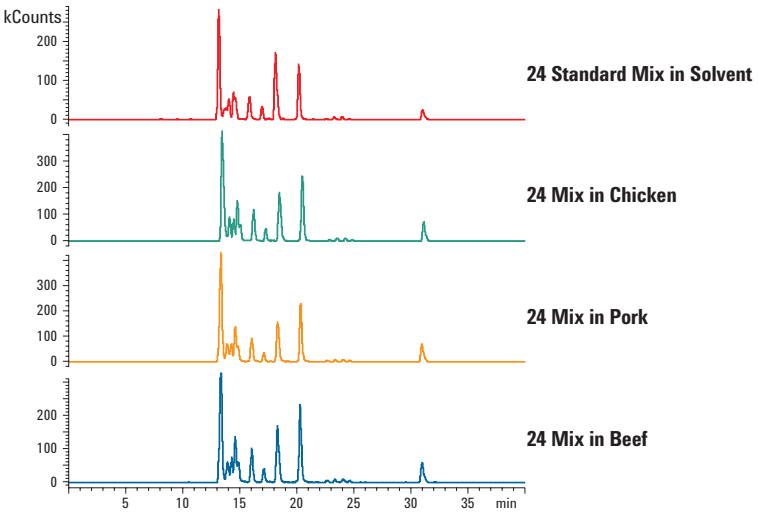
Wash SPE cartridges with 5 mL 2% ammonium hydroxide.

Elute samples with 5 mL methanol and collect in clean tubes. Evaporate the extracts to dryness at 40 °C under nitrogen.

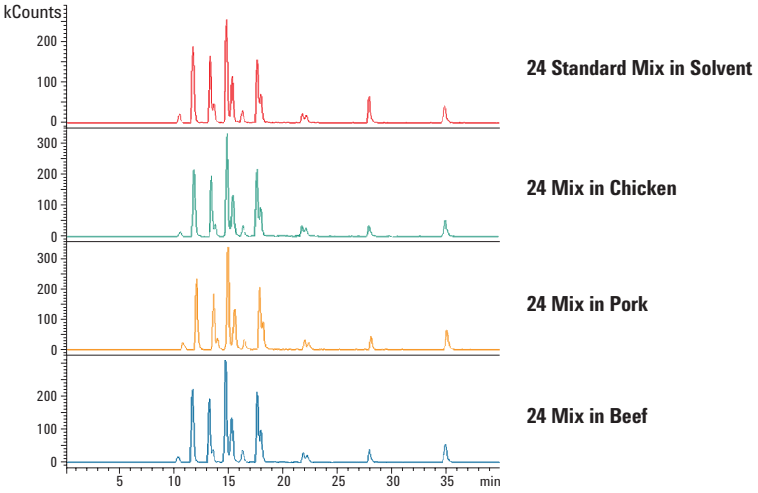
Reconstitute samples with 1 mL acetonitrile:water (1:9 V:V).

Analyze using LC-MS/MS and the appropriate method for the drug classes.

Results



Total ion chromatograms of Method (I) compounds in standard and spiked matrices (chicken, pork, and beef) at 10 ppb for quantitation.



Total ion chromatograms of Method (II) compounds in standard and spiked matrices (chicken, pork, and beef) at 10 ppb for quantitation.

Products used in the above application

Agilent Chem Elut Hydromatrix Bulk Sorbent, 1 kg, Part No. 198003

Agilent Bond Elut Plexa Cartridge, 3 mL, 60 mg, 50/pk, Part No. 12109603

Agilent Pursuit C18 Column, 3.0 mm x 150 mm, 3 µm, Part No. A3001150X030

To review this Application Note in its entirety, please view **5990-8986EN**

LC-MS/MS of Trichothecenes and Zearalenone in Wheat Using Different Sample Prep Methods

(Publication 5990-9107EN)

Introduction

Mycotoxins in food products, when consumed, can be toxic even at very low concentrations. Analytical methods for mycotoxins must deliver the required sensitivity to detect at these low levels, as well as provide the capability to extract and quantify a wide range of potential mycotoxins. This application compares two approaches to the extraction of mycotoxins from wheat, using Agilent QuEChERS extraction and dispersive SPE (dSPE) cleanup and Agilent Bond Elut Mycotoxin SPE cartridges. Samples prepared using both methods were used to identify nine trichothecenes and zearalenone in wheat by LC-MS/MS, with good recoveries and detection limits. Bond Elut Mycotoxin SPE delivers cleaner extracts and lower detection and quantification limits, while QuEChERS offers shorter processing times while using less solvent and smaller sample sizes. Both approaches are viable options for mycotoxin analysis.

HPLC/MS Conditions

Column:	Agilent ZORBAX Rapid Resolution HT Eclipse Plus C18 959764-902 2.1 mm × 100 mm, 1.8 µm
Instrument:	Agilent 6460 Triple Quadrupole LC/MS, Agilent 1290 Infinity LC System
Mobile phase:	A: Water + 0.2% acetic acid, 5 mM ammonium acetate B: Methanol + 0.2% acetic acid, 5 mM ammonium acetate
Flow rate:	0.25 mL/min
Temperature:	30 °C
Volume:	10 µL

Agilent Jet Stream Parameters

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

For MRM details, including precursor and product ions, fragmentor voltages, collision energies, and polarities, please reference the complete Application Note #5990-9107EN.

QuEChERS Procedure

Weigh 5 g of milled wheat sample. Add 10 mL methanol:acetonitrile (85:15 V:V) and one packet Original QuEChERS extraction salts (P/N 5982-5550). Shake and centrifuge.

Transfer 2 mL aliquot to dispersive SPE tube (P/N 5982-5022). Shake, then centrifuge.

Remove supernatant and evaporate under N_2.

Reconstitute in 1 mL H_2O:ACN (80:20) and transfer to autosampler vial for analysis by LC-MS/MS.

SPE Procedure

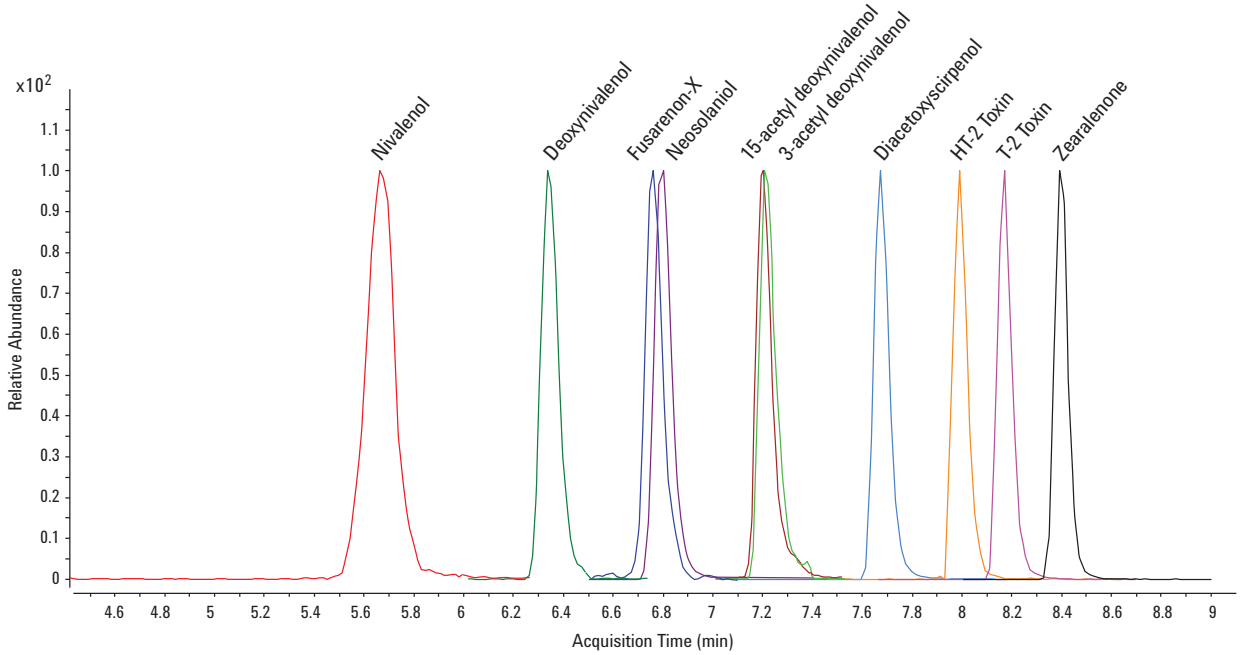
Weigh 25 g of milled wheat sample.
Add 100 mL H_2O:acetonitrile (20:80 V:V) and shake for 1 hour. Centrifuge.

Apply 8 mL aliquot of supernatant to Bond Elut Mycotoxin SPE cartridge. Collect eluent.

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

Filter through 0.02 µm membrane and collect in autosampler vial for analysis by LC-MS/MS.

Results

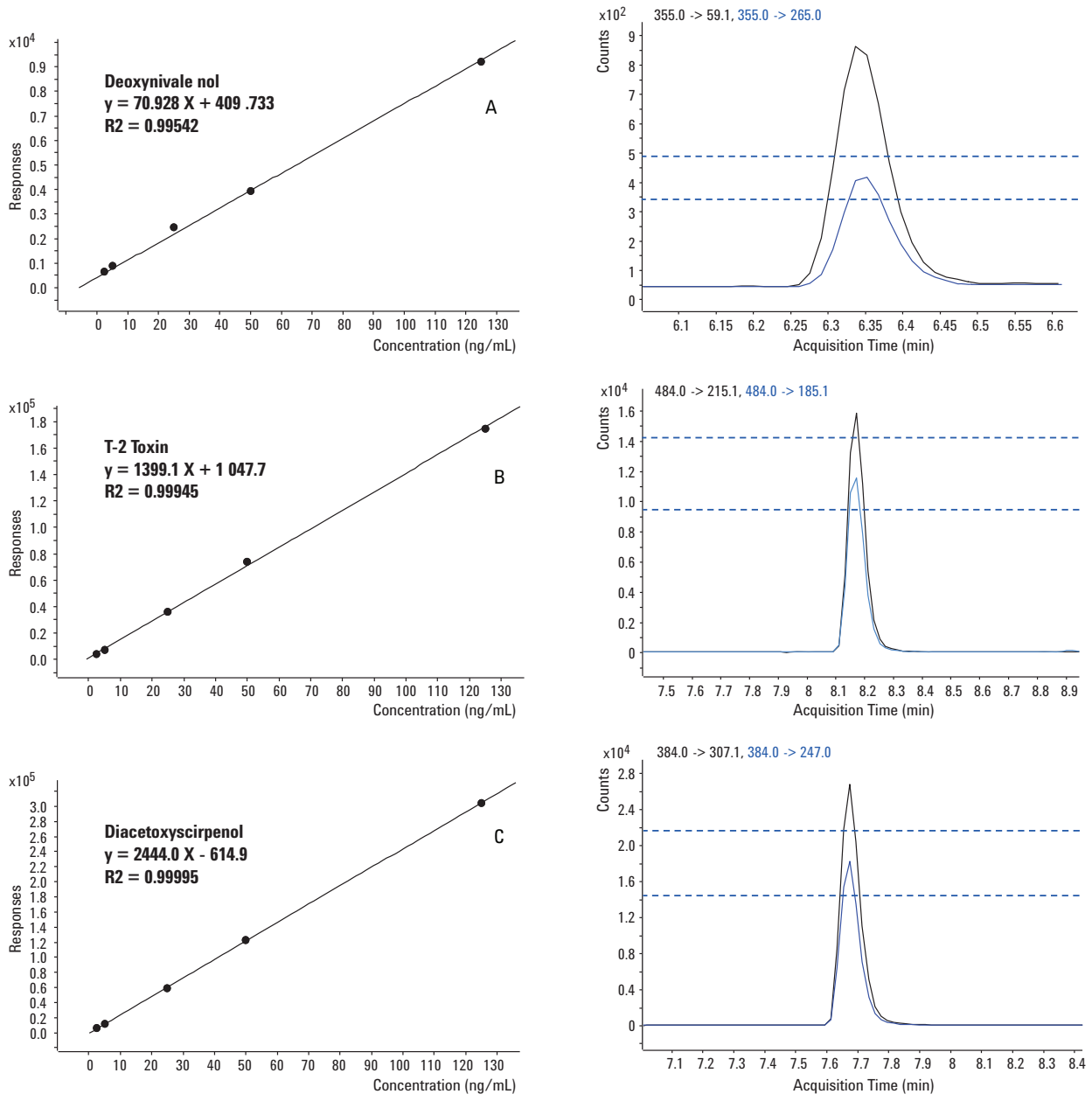


50 ppb Wheat Matrix Standard: normalized chromatogram for primary transitions. Please note that this method does not require chromatographic resolution between the isomers 15-acetyl DON and 3-acetyl DON because they are distinguished through measurement under different mass spec polarities.

Trichothecene and Zearalenone Limits of Detection and Quantification in Wheat Samples

Analyte	Modified QuEChERS LOD (µg/kg)	Modified QuEChERS LOQ (µg/kg)	BE Mycotoxin SPE LOD (µg/kg)	BE Mycotoxin SPE LOQ (µg/kg)
Nivalenol	0.31	1.04	0.07	0.24
Deoxynivalenol	0.04	0.12	0.4	0.12
Fusarenon-X	0.09	0.3	0.08	0.26
Neosolaniol	0.13	0.4	0.03	0.1
15-acetyl deoxynivalenol	0.2	0.66	0.02	0.66
3-acetyl deoxynivalenol	0.1	0.34	0.1	0.33
Diacetoxyscirpenol	0.001	0.003	0.0006	0.002
HT-2	0.05	0.17	0.03	0.1
T-2 toxin	0.01	0.04	0.006	0.02
Zearalenone	0.02	0.06	0.02	0.06

LOD = limit of detection (S/N >3), LOQ = limit of quantification (S/N >10)



Calibration curves, (A) Deoxynivalenol, (B) T-2 toxin, (C) Diacetoxyscirpenol, with corresponding chromatograms.

This figure shows the calibration curves for three selected mycotoxins acquired for matrix matched calibration standards with corresponding chromatograms.

Products used in the above application

Agilent Bond Elut Mycotoxin Cartridge, 500 mg, 3 mL, 50/pk, Part No. 12102167

Agilent QuEChERS Original Extraction Salts with Tubes, 50/pk, Part No. 5982-5550

Agilent QuEChERS AOAC 2007.01 Dispersive Kits for Fruits and Vegetables, 2 mL, 50/pk, Part No. 5982-5022

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

To review this Application Note in its entirety, please view **5990-9107EN**

Rapid, Sensitive, and Robust Detection of Phthalates in Food Using GC/MS or LC/MS

(Publication 5990-9510EN)

Introduction

Phthalate contamination of food, whether intentionally or through contact with packaging materials, is a concern because of the potential health hazards of these contaminants. Due to the ubiquity of phthalates from plasticizers, analytical methods must provide clean extracts that do not contribute phthalates, and the instrumentation must be contaminant-free as well. This application describes the use of Agilent Chem Elut diatomaceous earth for solid-supported liquid extraction (SLE) of phthalates from beverages including drinking water, sports drinks, and orange juice. A simple dilution extraction method for analysis of solids by LC/MS and LC-MS/MS is also described. Beverage sample analysis was performed using gas chromatography paired with single quadrupole or tandem quadrupole mass spectrometers. Limits of detection from 50-100 ppb were achieved using SLE and GC/MS, and sample extracts were free from contamination to ensure optimal sensitivity.

GC/MS and GC-MS/MS Conditions

Column:	DB-5ms Ultra Inert 122-5532UI 30 m x 0.25 mm, 0.25 µm
Volume:	1 µL
Inlet temperature:	Isothermal at 290 °C
Injection mode:	Splitless
Carrier:	Helium at 1.2 mL/min
Oven:	120 °C for one minute 120 °C to 300 °C at 20 °C/min Hold at 300 °C for 5 min
Post run:	300 °C for 5 min
Transfer line temperature:	300 °C

Agilent 5975C Series GC/MSD (Single Quadrupole) Conditions

Acquisition parameters:	EI, SIM/Scan
Scan mode:	50-500 amu mass range

Agilent 7000B Triple Quadrupole GC/MS Conditions

Mode:	EI, MRM
Source temperature:	230 °C
Quadrupole temperature:	Q1 and Q2 = 150 °C
Tune file:	atunes.eiextune.xml
Collision gas flows:	Nitrogen at 1.5 mL/min, Helium at 2.25 mL/min
Detector gain:	15

Solid Supported Lliquid Extraction (SLE) Procedure

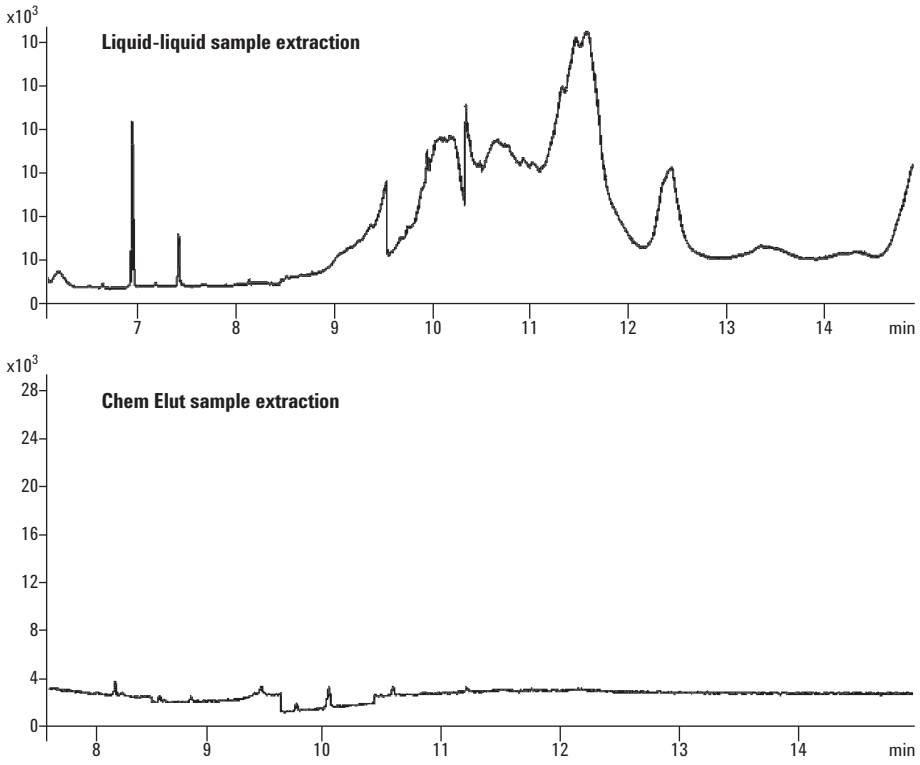
Prepare glass cartridges by loading with 5 g Chem Elut Hydromatrix bulk sorbent.

Apply 5 mL beverage sample to the cartridge, allowing sample to adsorb into the sorbent under gravity.

Apply 5 mL dichloromethane ($MeCl_2$) and collect the extract. Repeat for a total of 3 elutions.

Concentrate the extract under N_2 to 2.5 mL final volume and transfer to autosampler vial for analysis.

Results



Comparison of injections of a blank after a GC/MS sample run, using either the liquid-liquid sample extraction (top), or the Chem Elut sample preparation procedure (bottom). Notice the heavy carryover in the liquid extracted blank, even in the second blank injection after sample. Contrast this with the virtual absence of background in the blank prepared with Chem Elut, only one injection after a sample prepared the same way.

Products used in the above application

Agilent Chem Elut Hydromatrix Bulk Sorbent, 1 kg, Part No. 198003

Agilent J&W DB-5MS Ultra Inert Capillary GC Column, 30 mm x 0.25 mm, 0.25 µm, Part No. 122-5532UI

Agilent ZORBAX Eclipse Plus Column, 2.1 mm x 50 mm, 1.8 µm, Part No. 959741-912

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

To review this Application Note in its entirety, please view **5990-9510EN**

Simple and Quick Detection of Melamine and Its Analogues from Powdered Infant Milk

(Publication 5990-9591EN)

Introduction

This application describes a straightforward and rapid method for preparing powdered infant milk samples for analysis using HLPC and tandem mass spectrometry (LC-MS/MS). Melamine and its analogues were extracted from powdered milk using Agilent Captiva ND filtration. Captiva ND offers a unique non-drip (ND) membrane that removes matrix and therefore reduces ion suppression. Sample preparation using the non-drip membrane for filtration plus LC-MS/MS analysis resulted in a method that delivers sufficient extraction recoveries for melamine and analogs ammeline, cyanuric acid, and ammelide, with a short analysis time. 1 µg/g levels of detection were easily achieved for melamine, and the processing time is dramatically decreased relative to other methods.

HPLC/MS Conditions

Column:	Pursuit XRs Ultra 2.8 Diphenyl A7521100X020 2.0 mm x 100 mm, 2.8 µm
Instrument:	• Agilent 1290 Infinity LC system • Agilent 6460 Triple Quadrupole LC/MS system
Mobile phase:	A: 0.1% Formic Acid in H_2O B: MeOH
Flow rate:	0.4 mL/min
Volume:	5 µL
Temperature:	Ambient
Run time:	3 min
EMV:	± 300
Dwell:	300
Voltage:	7

Gradient:

Time	0.00	0.50	2.00	3.00	3.01
% B	2	5	5	80	2

MRM Transitions

Compound	Precursor Ion	Product Ion	Fragment	Collision Energy	Polarity
Melamine	127	85.1	100	18	+
Cyanuric acid	128	42.1	60	14	-
Ammeline	128	69.1	140	34	+
Ammelide	127	84	100	6	-

Agilent 6460 Triple Quadrupole MS Source Conditions

Source	
Gas temperature	300 °C
Gas flow	5 mL/min
Nebulizer	20 psi
Sheath gas temperature	275 °C
Sheath gas flow	7 mL/min
Capillary	+3500 -2000
Nozzle	+0 -500

Sample Preparation Procedure

Weigh out 2.0 ± 0.01 g powdered milk sample.

Spike the milk powder with analytes to 1.0 µg/g or 2.5 µg/g as applicable for calibration and controls.

Add 20 mL H_2O (10 mL per g of powder).

Vortex or shake the sample, ensuring that there is no unreconstituted powder.

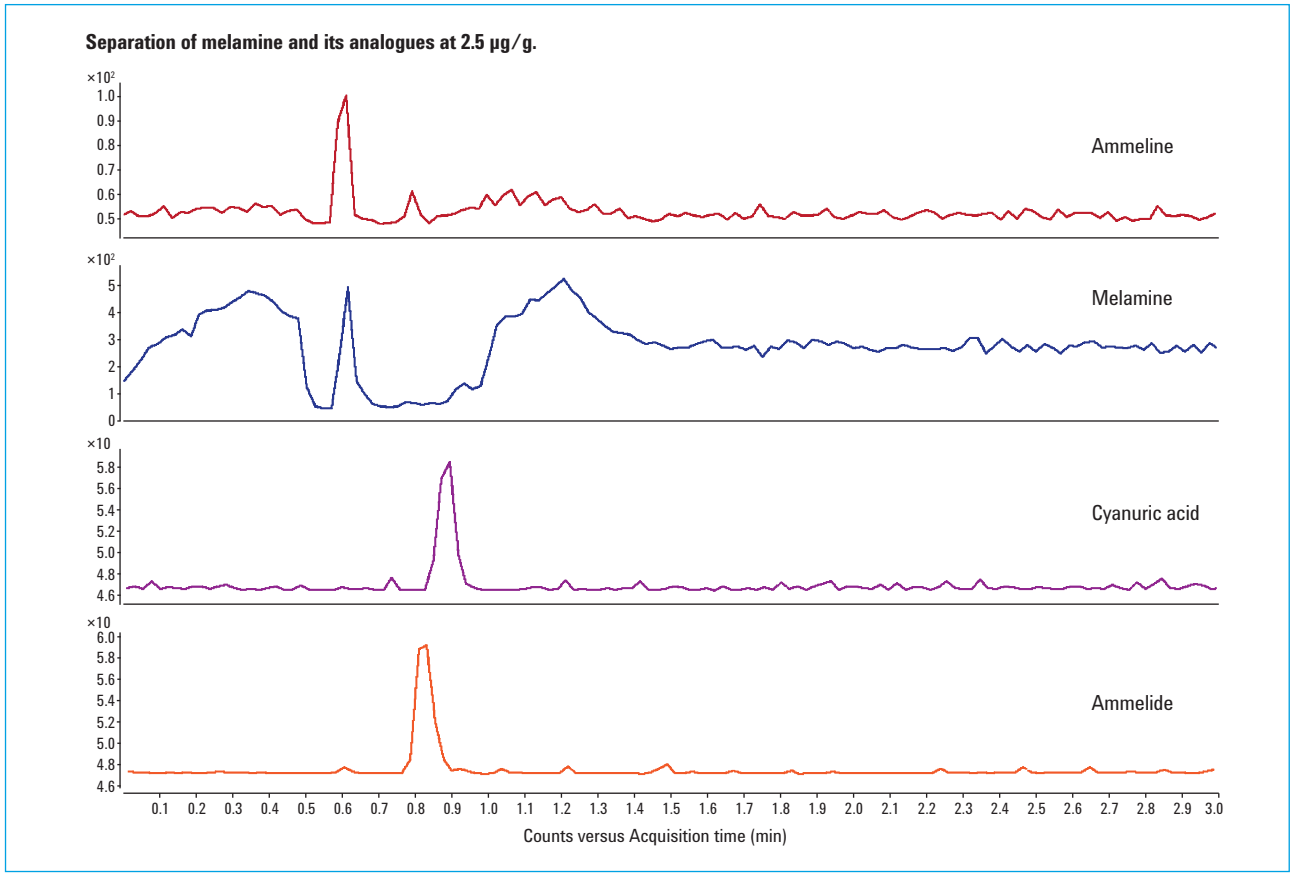
Add 400 µL of acetonitrile to the corresponding wells of the Captiva ND filtration plate. (Acetonitrile will not drip).

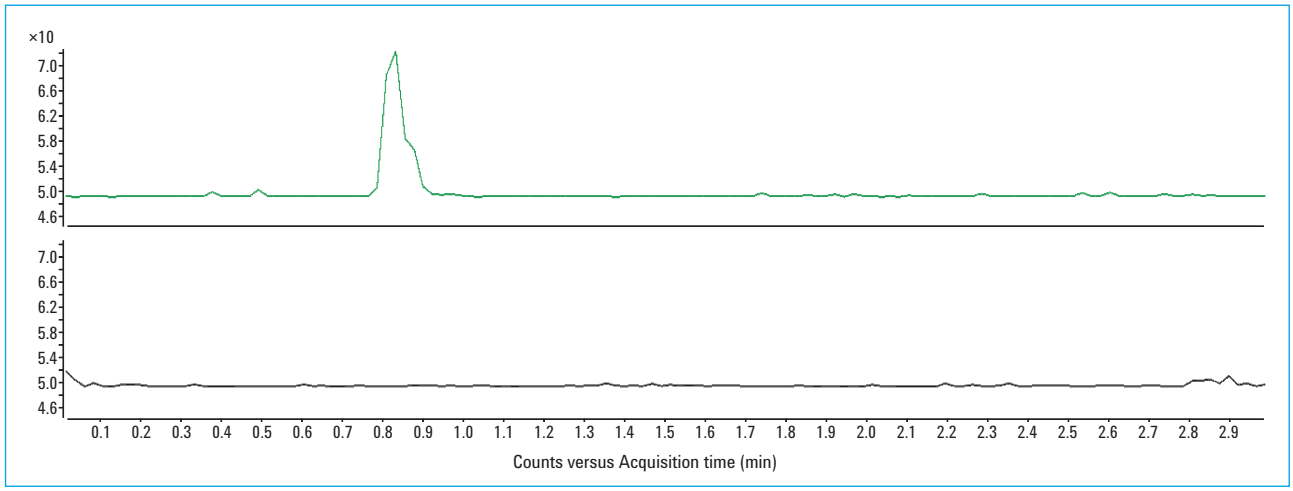
Add 100 µL of the prepared milk sample to the appropriate well. Use a pipettor to perform 5 cycles of in-well mixing, with the pipettor set to 300 µL.

Ensure that a Captiva collection plate is in position under the Captiva ND filtration plate, and apply vacuum to filter the sample and collect the extracts.

Transfer filtrate to an autosampler vial or analyze directly from the collection plate.

Results





Top: Captiva ND processed spiked sample, Bottom: 1:5 diluted spiked sample. Ammelide peak is completely suppressed by matrix interferences.

Recoveries of Melamine and its Analogues from Fortified Powdered Infant Formula (n = 6)

Average % Recovery ± RSD			
Compound	**1.0 µg/g**	**10 µg/g**	**25 µg/g**
Melamine	94 ± 12.4	89.5 ± 9.1	107 ± 9.9
Cyanuric acid	n/a	105 ± 8.3	102 ± 7.7
Ammeline	n/a	90.1 ± 5.6	110 ± 9.4
Ammelide	n/a	108 ± 9.4	92.4 ± 6.3

Products used in the above application

Agilent Captiva ND Plate, 0.2 µm, Polypropylene, 5/pk, Part No. A5969002

Agilent Captiva 96-Deep Well Collection Plate, 1 mL, 10/pk, Part No. A696001000

Agilent Pursuit XRs Ultra 2.8 Diphenyl Column, 2.0 mm x 100 mm, 2.8 µm, Part No. A7521100X020

To review this Application Note in its entirety, please view **5990-9591EN**

Multiresidue Screening of Agricultural Chemicals (I) and (II) in Food According to the Japan Positive List Using Agilent Cartridge-Based SPE and LC-MS/MS

(Publication 5990-9895EN)

Introduction

This application details a complete approach to the extraction and analysis of chemical residues in food using solid phase extraction and liquid chromatography coupled with tandem mass spectrometry. Agilent Bond Elut Silica SPE and Bond Elut Carbon/Amino (NH2) dual-phase SPE were used to develop two separate cleanup approaches, which were coupled to the appropriate HPLC separation method. Detection using multiple reaction monitoring (MRM) creates two complete approaches for sensitive confirmation of residues in food.

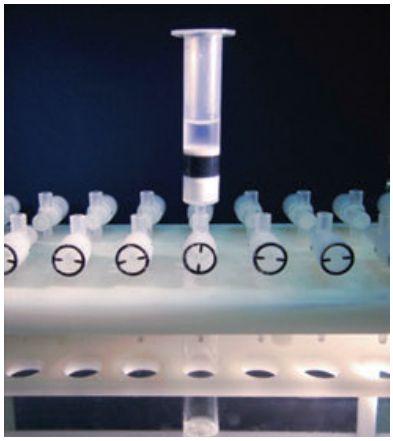
HPLC/MS Conditions

Instrument:	Agilent Triple Quadrupole LC-MS/MS
Column:	Method I: Agilent Pursuit XRs C18 A6001150X020 2.0 mm x 150 mm, 3 µm Method II: Agilent Pursuit C18 A3001150X020 2.0 mm x 150 mm, 3 µm
Mobile phase:	A: H_2O + 3 mM ammonium acetate B: CH_3OH + 3 mM ammonium acetate
Flow rate:	0.2 mL/min
Temperature:	Ambient (Method I), 40 °C (Method II)
Source:	ESI
Ionization mode:	Positive/Negative
Collision gas:	Argon

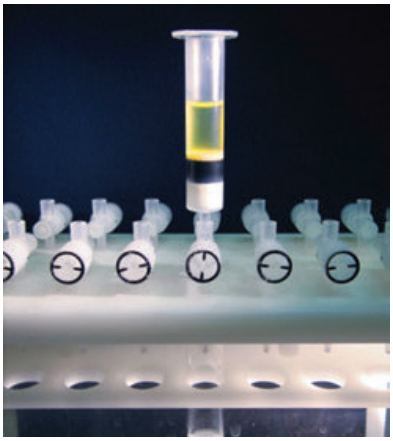
Gradient:

Time	0:00	1:00	3.5	6:00	8:00	17:50	30:00	30:06	40:00
% A	85	60	60	50	45	5	5	85	85
% B	15	40	40	50	55	95	95	15	15

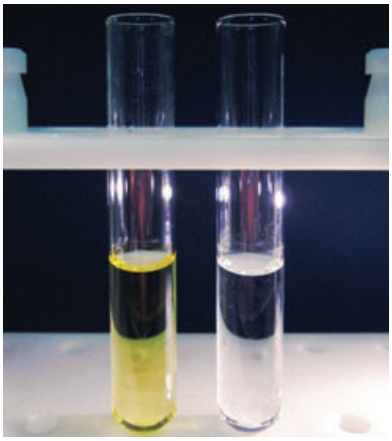
Note: *Detailed MRM transitions are available in the complete application note.*



Conditioning



Cleanup



Before and after cleanup

Pigment removal and cleanup offered by Agilent Bond Elut Carbon/NH2 dual phase SPE cartridge.

SPE Procedure

For each of the two methods, there is a 2-step protocol to process the samples before analysis: step 1 involves a liquid-liquid extraction with acetonitrile, followed by an SPE cleanup in step 2. The cleanup sorbents involved for both methods were, however, different. Bond Elut Carbon/NH2 and Bond Elut Silica cartridges were used in Methods I and II respectively.

Step 1: Liquid-liquid extraction for Methods I and II

For fruits and vegetables, weigh out 20.0 g of the sample.

Add 50 mL of acetonitrile, and homogenize the sample.
Filter by suction. Add 20 mL of acetonitrile to the residue on the filter paper, mix, and filter. Mix and vortex both filtrates.
Add acetonitrile to the filtrate to make a 100 mL solution.

Method I

Take 20 mL of the extracted solution. Add 10 g of sodium chloride and 20 mL of 0.5 mol/L phosphate buffer (pH 7.0) and vigorously shake. Once the solution has separated into 2 layers, transfer the acetonitrile (top layer), dry over sodium sulfate (anhydrous), and filter.

Concentrate the filtrate to dryness at 40 °C.
Dissolve the residue in 2 mL of acetonitrile/toluene (3:1).

Method II

Take 20 mL of the extracted solution. Add 10 g of sodium chloride and 20 mL of 0.01 mol/L hydrogen chloride and vigorously shake. Once the solution has separated into 2 layers, transfer the acetonitrile (top layer), dry over sodium sulfate (anhydrous), and filter.

Concentrate the filtrate to dryness at 40 °C.
Dissolve the residue in 2 mL of acetone/triethylamine/n-hexane (20:0.5:80).

Step 2: SPE clean-up with Agilent Bond Elut Carbon/NH2 (Method I) and Agilent Bond Elut Silica (Method II)

Method I

Condition an Agilent Bond Elut dual phase SPE cartridge containing graphite carbon black/aminopropyl (500 mg/500 mg) with 10 mL of acetonitrile/toluene (3:1). Load the solution obtained from the extraction step (method I) to the column, and allow the solution to pass through the column (do not collect). Elute the sample from the column with 20 mL of acetonitrile/toluene (3:1).

After collecting the effluent, concentrate the effluent to about 1 mL at 40 °C. Add 10 mL of acetone and concentrate to about 1 mL at 40 °C. Add 5 mL of acetone to the concentrated solution and concentrate to dryness.

Dissolve the residue in methanol to make a 4 mL solution, and analyze by LC/MS.

Method II

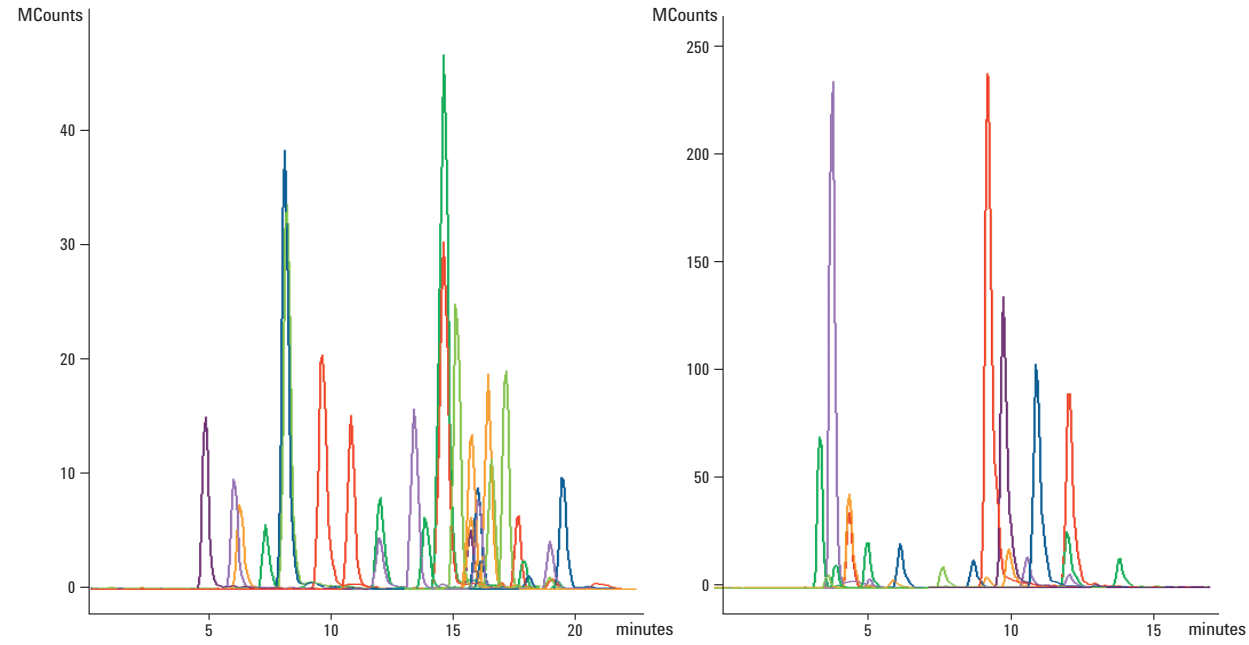
Condition an Agilent Bond Elut SPE silica cartridge (500 mg) with 5 mL of methanol, 5 mL of acetone, and then 10 mL of n-hexane. Load the solution obtained from the extraction step (method II), and allow the solution to pass through the column (do not collect).

Wash the column with 10 mL of acetone/triethylamine/n-hexane (20:0.5:80), and discard the effluent.

Elute the sample from the column with 20 mL of acetone/methanol (1:1), and collect.

Concentrate the effluent to dryness at 40 °C. Dissolve the residue in methanol to make a 4 mL solution, and analyze by LC/MS.

Results



Analysis of multiresidue pesticide standards by Japanese Positive List Method I (A, 42 compounds) and Method II (B, 25 compounds).

Products used in the above application

Agilent Bond Elut Carbon/NH2 Cartridge, 500 mg, 6 mL, 30/pk, Part No. 12252202

Agilent Bond Elut High-Flow Si LRC Cartridge, 120 µm, 500 mg, 50/pk, Part No. 14113036

Agilent Pursuit XRs C18 Column, 2.0 mm x 150 mm, 3 µm, Part No. A6001150X020

Agilent Pursuit C18 Column, 2.0 mm x 150 mm, 3 µm, Part No. A3001150X020

To review this Application Note in its entirety, please view **5990-9895EN**

LC-MS/MS of Fungicides and Metabolites in Apple Juice with Agilent Bond Elut Plexa and Poroshell 120

(Publication 5991-0050EN)

Introduction

This application offers a complete method for the extraction, identification, and quantification of four fungicides that may be found in apple juice. Using Agilent Bond Elut Plexa solid phase extraction (SPE), fungicides from two classes – benzimidazoles and imidazoles – were selectively removed from apple juice and analyzed using liquid chromatography and tandem mass spectrometry (LC-MS/MS). Minimal sample pretreatment was required, and relative extraction recoveries expressed as accuracy ranged from 93.6 to 107.3%, with good linearity and sensitivity. The combination of sample prep, HPLC method and column selection, and selective detection using multiple reaction monitoring (MRM) provides a complete solution for fungicide analysis.

HPLC/MS Conditions

Instrument:	• Agilent 1200 Infinity Series HPLC • Agilent 6460 Triple Quadrupole LC-MS/MS with Electrospray Ionization with Agilent JetStream Source
Mobile phase:	A: 0.1% formic acid in water B: 0.1% formic acid in methanol
Flow rate:	0.5 mL/min
Temperature:	5 µL
Stop time:	7.2 min
Post time:	2.5 min
Max pump pressure:	400 bar
Needle wash:	Flush port 75 methanol:25 water for 10 s Disable overlapped injection No automatic delay volume reduction

Gradient:

Time	0.0	0.5	2.0	3.0	75	7.1
% B	10	10	50	95	95	10

MS Conditions

ESI Source Parameters:

Ionization mode:	Positive
Capillary voltage:	2,800 V
Dyring gas flow:	12/min
Drying gas temperature:	350 °C
Nebulizer gas:	40 psi
Sheath gas flow:	12/min
Sheath gas temperature:	300 °C
Nozzle voltage:	0 V

MS Parameters	
Scan Type	Dynamic MRM
Pre-Run Script	SCP_MSDiverterValveToWaste(){MH_Acq_ Scripts.exe}
Time Segment #1	1.5 min – diverter valve to MS
Delta EMV	(+) 400 V

Note: *Detailed MRM transitions are available in the complete application note.*

SPE Procedure

Measure 0.5 mL of juice and add internal standard (triphenyl phosphine - TPP) for a final concentration of 50 ppb of TPP.

Dilute sample 1:3 (V:V) with HPLC-grade deionized water, vortex mix, and centrifuge if cloudy.

Condition Bond Elut Plexa SPE cartridges with 0.5 mL methanol. Allow methanol to soak into sorbent, then let drip.

Load the pre-treated sample, and allow to extract under gravity.

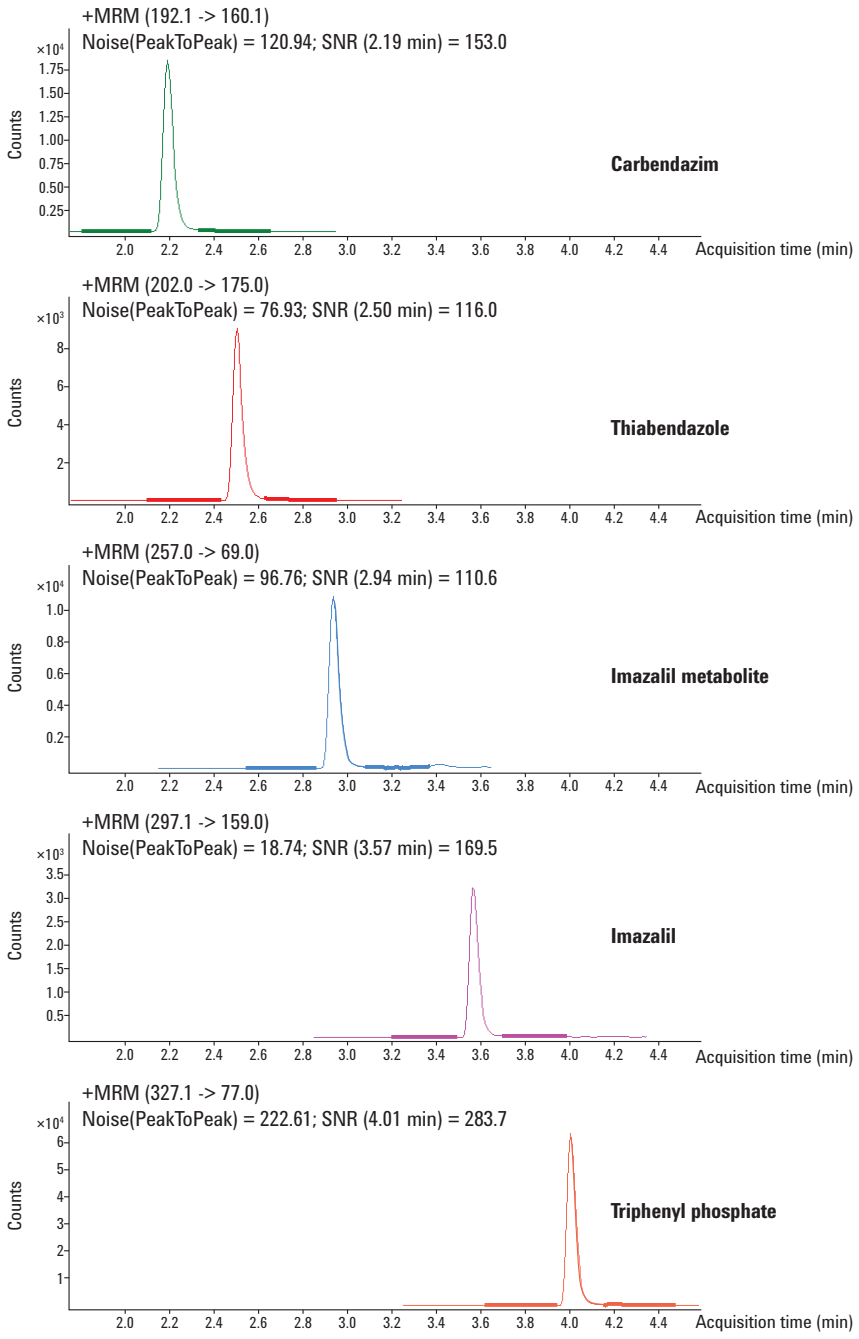
Wash the SPE cartridges with 1 mL water, followed by 1 mL of 30% methanol (30:70 V:V methanol with water).

Dry SPE cartridges for 5-10 minutes under high vacuum (10-15" Hg).

Ensure that collection vials or tubes are in place, and add 1 mL 80:20 ethyl acetate:isopropyl alcohol (V:V). Collect under gravity. Apply low vacuum after elution to remove remaining elution solvent.

Evaporate to dryness under N_2 at 55 °C and reconsititute in 0.5 mL mobile phase (10% methanol, 90% water, 0.1% formic acid).

Results



MRM extracted ion chromatograms for four fungicides (2 ppb) and TPP (50 ppb) in apple juice extract. Agilent Poroshell 120 EC-C18, 2.1 × 50 mm, 2.7 µm column. Noise regions are shown in bold.

Compound Name	R^2	10 ppb Accuracy (%)	CV (%)	50 ppb Accuracy (%)	CV (%)	250 ppb Accuracy (5)	CV (%)
Carbendazim	0.996	95.7	1.1	94.1	0.4	107.3	1.2
Thiabendazole	0.995	94.7	1.7	93.6	1	104.8	0.9
Imazalil	0.994	95.7	0.4	98.5	1.3	108	0.9
Imazalil degradate	0.995	94.1	2.1	100.2	0.8	106.7	1.4

Products used in the above application

Agilent Bond Elut Plexa Cartridge, 30 mg, 3 mL, Part No. 12109303

Agilent Vac Elut 20 Vacuum Manifold, 2.1 mm x 50 mm, 2.7 µm, Part No. 12234100

Agilent Silanized Autosampler Vials, 2 mL, 100/pk, Part No. 5183-2072

Agilent Screw Caps for Autosampler Vials, Blue, PTFE/Red Silicone Septa, 100/pk, Part No. 5182-0717

Agilent Poroshell 120 EC-C18 Column, 2.1 mm x 50 mm, 2.7 µm, Part No. 699775-902

To review this Application Note in its entirety, please view **5991-0050EN**

LC-MS/MS of Fungicides and Metabolites in Orange Juice with Agilent Bond Elut Plexa and Poroshell 120

(Publication 5991-0051EN)

Introduction

This application offers a complete method for the extraction, identification, and quantification of four fungicides that may be found in orange juice. Using Agilent Bond Elut Plexa solid phase extraction (SPE), fungicides from two classes – benzimidazoles and imidazoles – were selectively removed from orange juice and analyzed using liquid chromatography and tandem mass spectrometry (LC-MS/MS). Minimal sample pretreatment was required, and relative extraction recoveries expressed as accuracy ranged from 96.3 to 105.3%, with good linearity and sensitivity. The combination of Plexa SPE sample cleanup, HPLC method and column selection, and selective detection using multiple reaction monitoring (MRM) provides a complete solution for fungicide analysis.

HPLC/MS Conditions

Instrument:	• Agilent 1200 Infinity Series HPLC • Agilent 6460 Triple Quadrupole LC-MS/MS with Electrospray Ionization and Agilent JetStream Source
Mobile phase:	A: 0.5% phosphoric acid B: methanol
Flow rate:	1 mL/min
Volume:	5 µL
Stop time:	7.2 min
Post time:	2.5 min
Max pump pressure:	400 bar
Needle wash:	Flush port 75 methanol:25 water for 10 s Disable overlapped injection No automatic delay volume reduction

Gradient:

Time	0.0	0.5	2.0	3.0	7.0	7.1
% B	10	10	50	95	95	10

MS Conditions

ESI Source Parameters

Ionization mode:	Positive
Capillary voltage:	2,800 V
Dyring gas flow:	12/min
Drying gas temperature:	350 °C
Nebulizer gas:	40 psi
Sheath gas flow:	12/min
Sheath gas temperature:	300 °C
Nozzle voltage:	0 V

MS Parameters	
Scan type	Dynamic MRM
Pre-run script	SCP_MSDiverterValveToWaste(){MH_Acq_Scripts.exe}
Time segment #1	1.5 min – diverter valve to MS
Delta EMV	(+) 400 V

Note: Detailed MRM transitions are available in the complete application note.

Sample Prep Procedure

Measure 0.5 mL of juice and add internal standard (triphenyl phosphine - TPP) for a final concentration of 50 ppb of TPP.

Dilute sample 1:3 (V:V) with HPLC-grade deionized water, vortex mix, and centrifuge for 15-20 min at 6000 rpm. No pH adjustment is necessary.

Condition Bond Elut Plexa SPE cartridges with 0.5 mL methanol. Allow methanol to soak into sorbent, then let drip.

Load the pre-treated sample, and allow to extract under gravity.

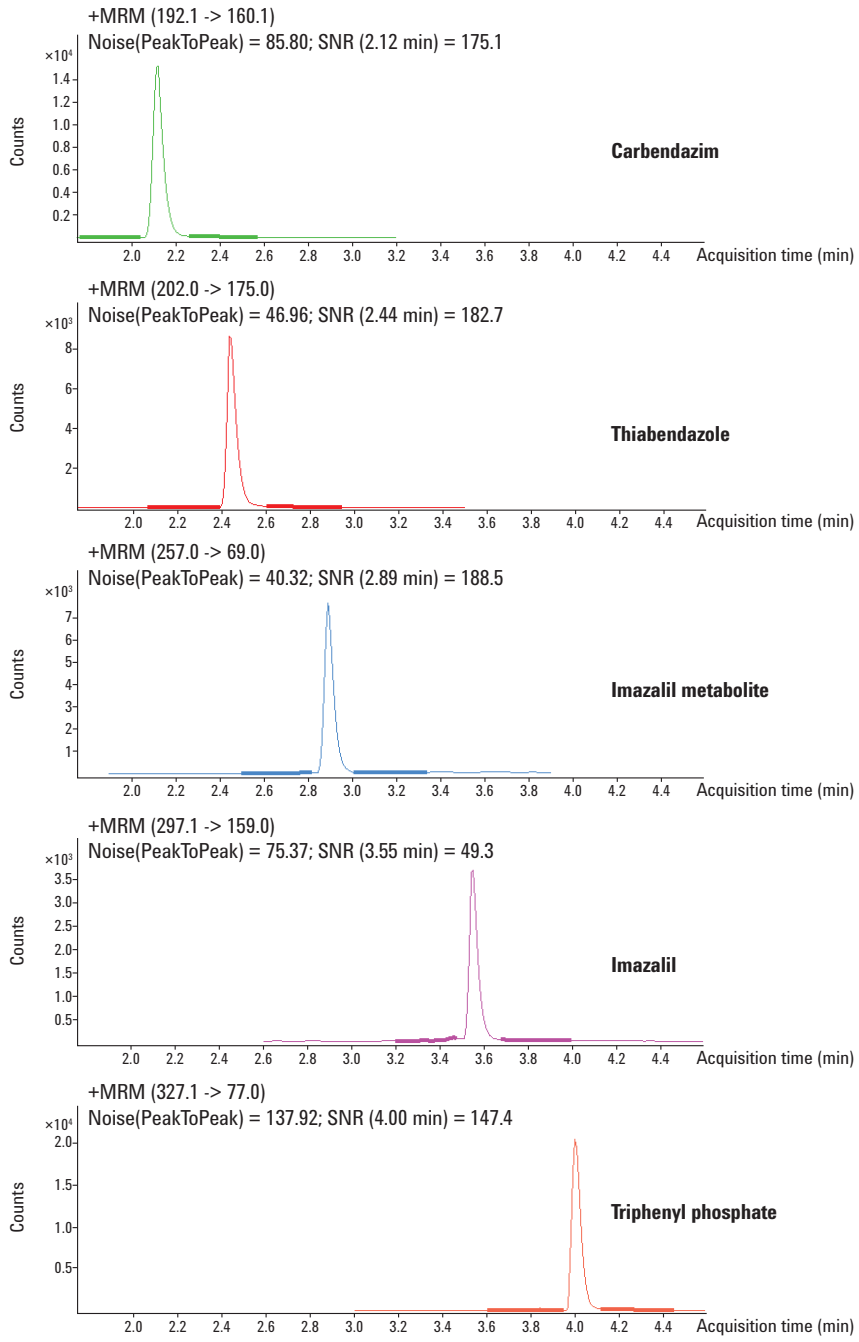
Wash the SPE cartridges with 1 mL water, followed by 1 mL of 30% methanol (30:70 V:V methanol with water).

Dry SPE cartridges for 5-10 minutes under high vacuum (10-15 inches Hg).

Ensure that collection vials or tubes are in place, and add 1 mL 80:20 ethyl acetate:isopropyl alcohol (V:V). Collect under gravity. Apply low vacuum after elution to remove remaining elution solvent.

Evaporate to dryness under N2 at 55 °C and reconsititute in 0.5 mL mobile phase (10% methanol, 90% water, 0.1% formic acid).

Results



MRM extracted ion chromatograms for four fungicides (2 ppb) and TPP (50 ppb) in orange juice extract. Agilent Poroshell 120 EC-C18, 2.1 x 50 mm, 2.7 µm column. Noise regions are shown in bold.

Compound Name	R^2	10 ppb Accuracy (%)	CV (%)	10 ppb Accuracy (%)	CV (%)	10 ppb Accuracy (%)	CV (%)
Carbendazim	0.999	103.5	1.7	100.1	0.7	99	2.5
Thiabendazole	0.998	101.2	1.4	96.3	3.2	99.9	2.9
Imazalil	0.999	105.3	1.3	100.2	0.9	101.2	1.8
Imazalil degradate	0.998	101.7	1.3	103.3	2.8	101.6	3.1

Products used in the above application

Agilent Bond Elut Plexa Cartridge, 30 mg, 3 mL, Part No. 12109303

Agilent Vac Elut 20 Vacuum Manifold, 2.1 mm x 50 mm, 2.7 µm, Part No. 12234100

Agilent Silanized Autosampler Vials, 2 mL, 100/pk, Part No. 5183-2072

Agilent Screw Caps for Autosampler Vials, Blue, PTFE/Red Silicone Septa, 100/pk, Part No. 5182-0717

Agilent Poroshell 120 EC-C18 Column, 2.1 mm x 50 mm, 2.7 µm, Part No. 699775-902

To review this Application Note in its entirety, please view **5991-0051EN**

LC-MS/MS of Malachite Green and Crystal Violet in Fish with Agilent Bond Elut PCX and Poroshell 120

(Publication 5991-0091EN)

Introduction

Extraction of malachite green, crystal violet, and their primary metabolites in fish demonstrates the effectiveness of Agilent Bond Elut Plexa PCX solid phase extraction for use in sample cleanup of marine products prior to LC-MS/MS analysis. Plexa PCX effectively removed the complex matrix components found in fish tissue, while delivering consistent and high recoveries of these antibacterial dyes. A 1 g sample size was sufficient to achieve limits of quantitation of 0.5 ng/g. Using a mixed-mode cation exchange SPE method resulted in final extracts that had minimal matrix interference, and the combination of sample cleanup, HPLC method, and tandem MS detection of the dyes offers a rugged and reliable solution for this application.

HPLC/MS Conditions

Column:	Poroshell 120 EC-C18 699775-902 2.1 mm x 50 mm, 2.7 µm
Instrument:	• Agilent 1200 Infinity Series HPLC • Agilent 6460 Triple Quadrupole LC-MS/MS with Electrospray Ionization and Agilent JetStream Source
Mobile phase:	A: Water (5 mM NH_4Ac):acetonitrile B: 0.1% FA
Flow rate:	0.4 mL/min
Volume:	5 µL
Temperature:	Ambient

Gradient:

Time	0	5	6	6.5	7
% B	30	80	80	30	30

MS Source Parameters

Gas temperature:	300 °C
Gas flow:	5 L/min
Nebulizer gas:	45 psi
Sheath gas flow:	11 L/min
Sheath gas temperature:	400 °C
Nozzle voltage:	Positive: 0 V, Negative: 0 V
Capillary voltage:	Positive: 3500 V, Negative: 2500 V

Analyte	MRM channels (m/z)	Fragmentor (V)	CE (V)
Malachite green	1) 329.3>313.3	175	38
	2) 329.3>208.3		38
Crystal violet	1) 372.3>356.2	175	42
	2) 372.3>251.1		36
Leuco-malachite green	1) 331.3>316.2	175	26
	2) 331.3>238.2		16
Leuco-crystal violet	1) 374.3>358.3	175	30
	2) 374.3>238.2		26
MG-d5	334.3>318.3	175	38
LMG-d6	337.3>240.2	175	30

SPE Procedure

Homogenize small pieces of fish meat, and weigh 1.0 g of homogenized sample into a centrifuge tube. Add 50 µL of internal standard solution and 10 mL of McIlvaine's buffer:acetonitrile (1:1 V:V). Vortex mix 1 min, then centrifuge 5 min at 4,500 rpm.

Transfer supernatant to clean tube.
Add 5 mL of McIlvaine's buffer:acetonitrile 1:1 V:V to the pellet, vortex mix for 1 min, then centrifuge for 5 min at 4,500 rpm.
Remove the supernatant and combine it with the first extraction.

Condition the Bond Elut Plexa PCX SPE tubes with 2 mL methanol followed by 2 mL of 2% formic acid in water.

Load the collected supernatants and extract under low to no vacuum.

Wash the SPE cartridges with 2 mL 2% formic acid in water, followed by 2 mL methanol.

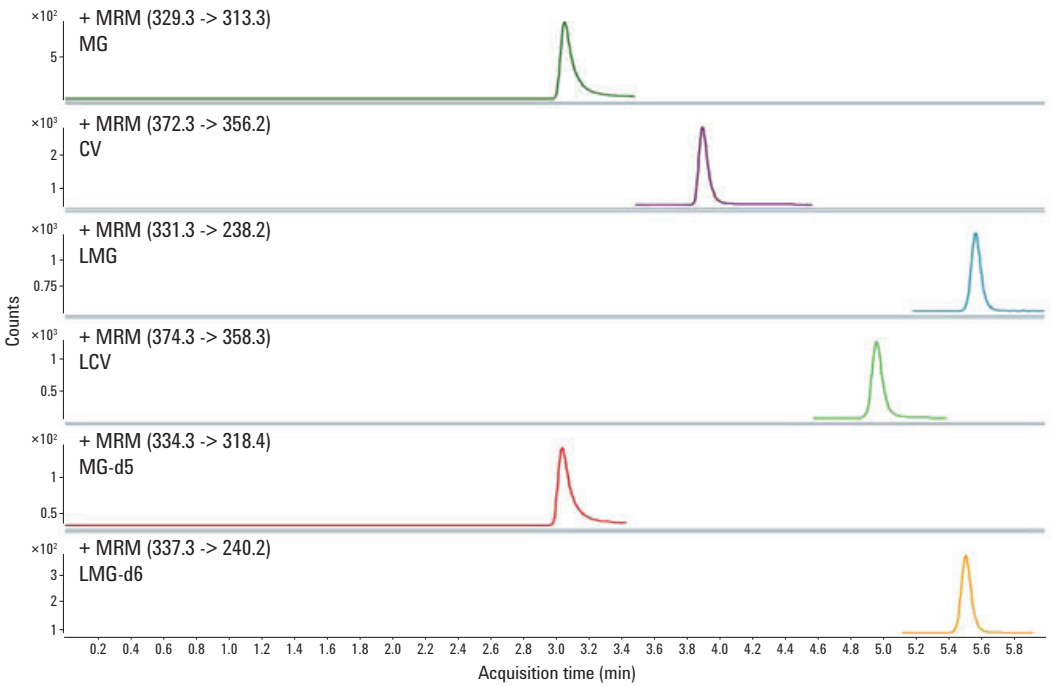
Dry SPE cartridges for 5 minutes under high vacuum (10-15" Hg).

Wash the SPE cartridges with 2 mL hexane.

Ensure that collection tubes are in place, and apply 4 mL elution buffer (see Tips).

Bring eluate to 5 mL volume with water.
Mix and transfer 2 mL to autosampler vial for analysis.

Results



Recovery and precision results for three spiking levels

Compound	Spiked Level (ng/g)	Recovery (%)	RSD (n=6)
Malachite green	1	102.1	3.5
	10	102.8	1.8
	50	99.2	1.9
Crystal violet	1	96.9	2.1
	10	102.8	2.6
	50	97.4	1.7
Leuco-malachite green	1	103.5	2.6
	10	108.9	3.4
	50	96.7	3.7
Leuco-crystal violet	1	99.4	3.8
	10	106.1	3.8
	50	102.7	4.5

Chromatograms of 10 ng/g spiked sample extracts of antibacterial agents in fish on an Agilent Poroshell 120 EC-C18 column.

Products used in the above application

Agilent Bond Elut Plexa PCX Cartridge, 60 mg, 3 mL, Part No. 12108603

Agilent Vac Elut 20 Manifold, Part No. 12234101

Agilent Poroshell 120 EC-C18 Column, 2.1 mm x 50 mm, 2.7 µm, Part No. 699775-902

To review this Application Note in its entirety, please view **5991-0091EN**

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

(Publication 5991-0868EN)

Introduction

Efficient extraction of a wide range of pesticides is demonstrated with the use of Agilent Chem Elut solid-supported liquid-liquid extraction (SLE) followed by LC-MS/MS analysis. Chem Elut SLE is straightforward, and unlike traditional liquid-liquid extraction, Chem Elut SLE can be automated. Filtration of the final sample prior to LC-MS/MS analysis using Agilent Captiva PTFE syringe filters ensures removal of particulates that may form upon reconstitution of the sample. The sample preparation method and use of LC-MS/MS results in a workflow that can be applied to a broad multiresidue approach for confirming pesticides in apples.

HPLC/MS Conditions

Instrument:	• Agilent 1200 Infinity Series HPLC • Agilent 6460 Triple Quadrupole LC-MS/MS with Electrospray Ionization
Column:	Poroshell 120 SB-C18 685775-902 2.1 mm x 50 mm, 2.7 µm
Sample prep:	Chem Elut cartridges, unbuffered, 5.0 mL 12198006
Eluent:	A: 0.1% FA in water B: 0.1% FA in ACN
Flow rate:	0.4 mL/min
Volume:	5 µL
Temperature:	30 °C
Post run:	2 minutes
Total cycle time:	12 minutes

Gradient:

Time	0	1	4	8	9	9.2	10
% B	5	5	50	90	90	5	5

MS Source Parameters

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

Note: *Detailed MRM transitions are available in the complete application note.*

SLE Procedure

Measure 10 g (± 0.1g) of homogenized apple sample into a 50 mL centrifuge tube and add 1 mL water to bring to 10 mL total volume.

Add 100 µL internal standard solution to yield a 10 ng/g final concentration of internal standard. Cap tubes and vortex mix for 1 minute.

Add 20 mL methanol (MeOH) to each tube. Homogenize 2 minutes using a high-speed blender, then centrifuge 5 minutes at 4,000 rpm.

Add 2.5 mL NaCl solution (20% w:w in H_2O) to a 10 mL volumetric flask. Add supernatant from centrifuged sample to bring the total volume to 10 mL and mix well.

Apply 5 mL of the sample solution to a 5 mL Chem Elut cartridge, and allow the sample to slowly pass through the sorbent layer, eluting to waste.

Apply 15 mL dichloromethane to the Chem Elut cartridge, and collect the eluate in a 50 mL round-bottom flask. Repeat elution with 15 mL dichloromethane for a total of 30 mL of eluate.

Reduce the eluate nearly to dryness using a rotary evaporator. Under a gentle stream of nitrogen, evaporate the remaining solvent.

Add 0.5 mL MeOH to the flask, then swirl in an ultrasonic bath to dissolve any residue.

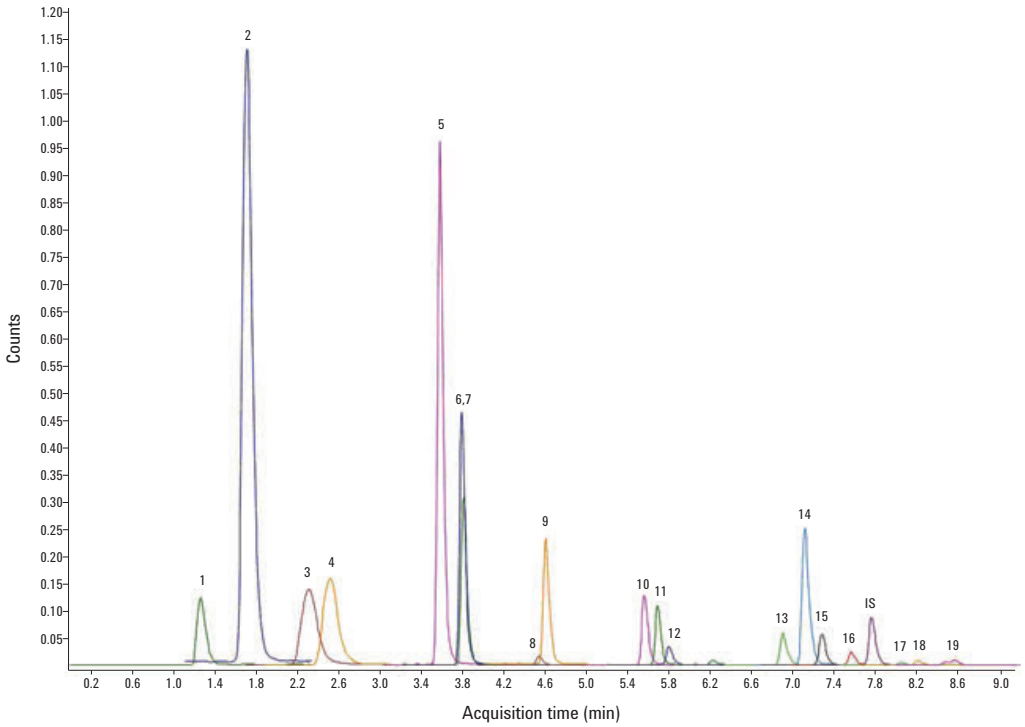
Add 0.5 mL water and vortex-mix for 1 minute.

Measure 10 g (± 0.1g) of homogenized apple sample into a 50 mL centrifuge tube and add 1 mL water to bring to 10 mL total volume.

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

No.	Analytes	Recovery (%)	RSD (%) (n=6)	Recovery (%)	RSD (%) (n=6)
1	Methamidophos	75.3	6.2	71.8	5.3
2	Acephate	93.9	3.7	86.3	4
3	Pymetrozine	103.8	3.3	107.8	3.8
4	Omethoate	95.9	8.1	94.8	7.5
5	Carbendazim	96.1	2.5	91.2	1.9
6	Monocrotophos	98.2	6.4	73.7	8.1
7	Thiabendazole	94.5	6.9	89.6	5.4
8	Imidacloprid	93.6	1.7	102	2.7
9	Dimethoate	89.4	8.6	83.2	6.6
10	Propoxur	62.4	7.3	69.3	9.3
11	Imazalil	88.8	9.2	83.6	9.6
12	Carbaryl	92.7	2.7	109.3	2.9
13	Ethoprophos	29.2	11	28.6	12.7
14	Cyprodinil	95.6	1.8	91.1	4.4
15	Penconazole	119	10.5	119.2	9.6
16	Kresoxim-methyl	102.2	0.8	110.9	2.1
17	Phosalone	106.4	5.4	116	4.2
18	Profenofos	78.3	2.6	89.7	4.2
19	Tetramethrin	78.5	3.8	84.8	8

Results



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

Products used in the above application

Agilent Chem Elut Cartridges, Unbuffered, 5.0 mL, 100/pk, Part No. 12198006

Agilent Captiva PTFE Premium Syringe Filters, 25 mm x 0.45 µm, 1,000/pk, Part No. 5190-5087

Agilent Poroshell 120 SB-C18 Column, 2.1 mm x 50 mm, 2.7 µm, Part No. 685775-902

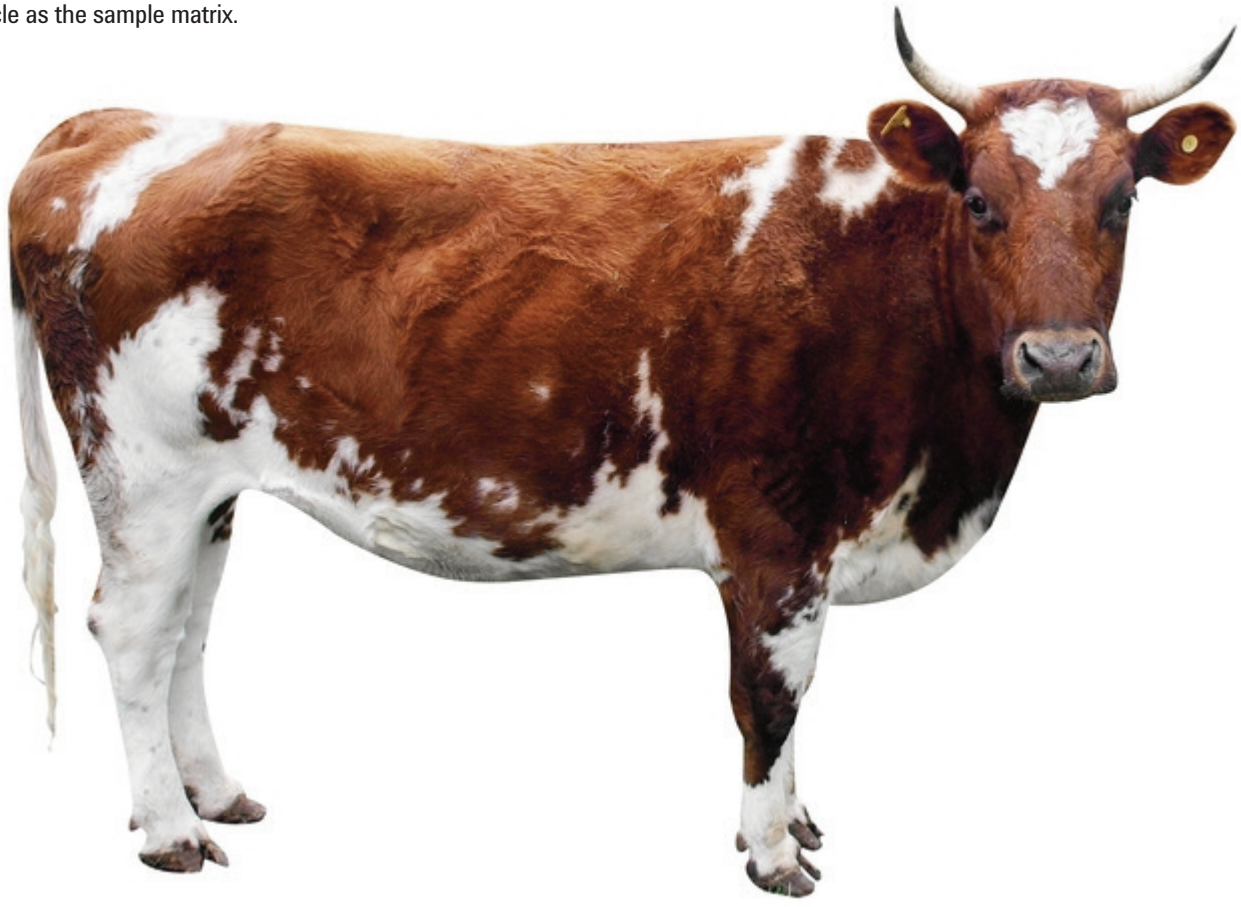
To review this Application Note in its entirety, please view **5991-0868EN**

Aminoglycosides in Bovine Muscle Using Agilent Bond Elut Plexa SPE, an Agilent Poroshell 120 Column, and LC/Tandem MS

(Publication 5991-1321EN)

Introduction

Aminoglycosides are a broad-spectrum class of antibiotics commonly administered to farm animals to prevent and control disease. This application demonstrates a method for effective extraction of a range of AG target compounds from beef using Agilent Bond Elut Plexa solid phase extraction, Agilent Captiva filtration, and analysis using liquid chromatography and tandem mass spectrometry (LC-MS/MS). The resulting method provides high recoveries with good precision and low detection limits, demonstrating the effectiveness of this approach using bovine (beef) muscle as the sample matrix.



HPLC/MS Conditions

Column:	Poroshell 120 SB-C18 685775-902 2.1 mm x 50 mm, 2.7 µm
Sample prep:	Bond Elut Plexa, 500 mg, 6 mL 12259506
Mobile phase:	A: water:acetonitrile (950:50, 20 mmol/L HFBA), B: acetonitrile:water (800:200, 20 mmol/L HFBA)
Mobile phase:	0.3 mL/min
Volume:	20 µL
Temperature:	Ambient
Instrument:	Agilent 1200 Infinity Series LC System Agilent 6460 Triple Quadrupole LC-MS/MS System
Manifold:	Agilent Vac Elut 20 Manifold

Gradient:

Time	0	3	9.5	9.55	10
A%	85	85	25	85	85
% B	15	15	75	15	15

MS Source Parameters

Gas temperature:	350 °C
Gas flow:	5 L/min
Nozzle voltage:	Positive, 0 V
Nebulizer gas:	45 psi
Capillary voltage:	3,500 V

Mass monitored by multiple-reaction monitoring

Compound	Precursor ion	Product ion	Fragmentor (V)	Collision energy (V)
Spectinomycin	351.2	333.2	170	15
		207.1	170	18
Hygromycin B	528.3	177.1	170	25
		352	170	20
Streptomycin	582.4	263.2	180	30
		245.8	180	35
Dihydrostreptomycin	584.4	263.3	180	30
		246.2	180	40
Amikacin	586.4	163.1	170	30
		425.2	170	15
Kanamycin	485.3	163.1	150	20
		324.2	150	10
Apramycin	540.3	217.1	140	25
		378.2	140	12
Tobramycin	468.3	163.2	125	20
		324.2	125	8
Gentamicin	478.3	322.3	125	8
		157.2	125	15
Neomycin	615.3	161.1	175	30
		293.1	175	20

SPE Procedure

Measure 5 g homogenized bovine muscle into a 50 mL polypropylene centrifuge tube, and add 10 mL of 5% trichloroacetic acid (TCA).

Homogenize thoroughly for 1 minute. Centrifuge 5 minutes at 4,000 rpm, and transfer supernatant to a separate 50 mL centrifuge tube.

Repeat extraction by adding 10 mL of 5% TCA solution, homogenize thoroughly for 1 minute, and centrifuge 5 minutes at 4,000 rpm.

Combine supernatants into a single tube, and add 5 mL of 0.2 M heptafluorobutyric acid (HFBA) to the extract. Vortex-mix for 1 minute, and then centrifuge for 5 minutes at 4,000 rpm.

Adjust sample pH to 4.0 ± 0.5 using 5% ammonia in water. Add water to bring total volume to 30 mL. Vortex-mix for 1 minute.

Prepare Bond Elut Plexa SPE tubes for extraction by placing them on a vacuum manifold prepared for waste removal.

Condition the SPE cartridges with 3 mL ACN, followed by 3 mL water, then 5 mL 0.2 M HFBA.

Load 6 mL of pre-treated sample solution and extract slowly, at 1-2 mL/min.

Wash the SPE cartridges with 5 mL water, then dry the SPE cartridges for 5 minutes at high vacuum (15" Hg).

Prepare the vacuum manifold for collection, ensuring that clean tubes are in place.

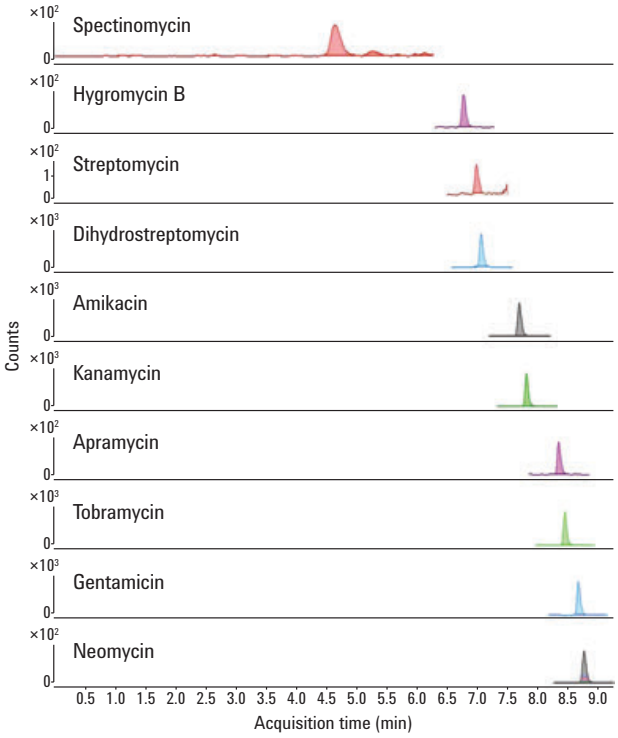
Apply 3 mL of elution solvent (ACN:0.2M HFBA 8:2 V:V).

Evaporate to dryness under nitrogen at 40 °C, and reconstitute with 1 mL of 0.02M HFBA.

Vortex-mix and use an ultra-sonic bath to thoroughly combine the sample. Filter through a 0.2 µm filter, and collect sample in an autosampler vial. Transfer to LC-MS/MS system for analysis.

Compound	Spiked Level (ng/g)	Recovery (%)	RSD (n=6, %)
Streptomycin	20	87.7	2.1
	100	79.7	2.4
	500	91.2	3.2
Hydromycin B	20	75.9	3.9
	100	82.1	3.4
	500	85.6	4
Streptomycin	20	71.5	11.2
	100	80	9.4
	500	74.5	8
Dihydrostreptomycin	20	89.1	4.5
	100	91.2	2.3
	500	93.3	3.6
Amikacin	20	85.9	1.8
	100	90.1	2.4
	500	96.5	3.8
Kanamycin	20	86.7	1.4
	100	90	2.2
	500	97.6	2.8
Apramycin	20	84.6	4.9
	100	87.6	3.1
	500	95.4	5.4
Tobramycin	20	89.3	4.4
	100	88.1	3.4
	500	97.7	5.8
Gentamicin	20	82.4	3.5
	100	81.2	4.6
	500	95.8	6.8
Neomycin	20	72.1	2.6
	100	82.8	5.6
	500	90.3	5.5

Results



Chromatograms of 20 ng/g spiked bovine muscle sample extract.

Products used in the above application

Agilent Bond Elut Plexa Cartridge, 500 mg, 6 mL, 30/pk, Part No. 12259506

Agilent Captiva Premium Syringe Filters, PES, 25 mm, 0.2 µm, LC/MS certified, Part No. 5190-5098

Agilent Poroshell 120 SB-C18 Column, 2.1 mm x 50 mm, 2.7 µm, Part No. 685775-902

To review this Application Note in its entirety, please view **5991-1321EN**

Aminoglycosides in Milk Using Agilent Bond Elut Plexa SPE, an Agilent Poroshell 120 Column, and LC/Tandem MS

(Publication 5991-1758EN)

Introduction

Aminoglycosides are a broad-spectrum class of antibiotics commonly administered to farm animals to prevent and control disease. This application demonstrates a method for effective extraction of a range of AG target compounds from bovine milk using Agilent Bond Elut solid phase extraction, Agilent Captiva filtration, and analysis using liquid chromatography and tandem mass spectrometry (LC-MS/MS). The resulting method offers high recoveries with good precision and low detection limits, demonstrating the effectiveness of this approach using bovine milk as the sample matrix.

HPLC/MS Conditions

Column:	Poroshell 120 SB-C18 685775-902 2.1 mm x 50 mm, 2.7 µm
Sample prep:	Bond Elut Plexa, 500 mg, 6 mL 12259506
Mobile phase:	A: water:acetonitrile (950:50, 20 mmol/L HFBA), B: acetonitrile:water (800:200, 20 mmol/L HFBA)
Flow rate:	0.3 mL/min
Volume:	20 µL
Temperature:	Ambient
Instrument:	Agilent 1200 Infinity Series LC System Agilent 6460 Triple Quadrupole LC-MS/MS System
Manifold:	Agilent Vac Elut 20 Manifold

Gradient:

Time	0	3	9.5	9.55	10
A%	85	85	25	85	85
% B	15	15	75	15	15

MS Source Parameters

Gas temperature:	350 °C
Gas flow:	5 L/min
Sheath gas temperature:	400 °C
Sheath gas flow:	11 L/min
Nozzle voltage:	Positive, 0 V
Nebulizer gas:	45 psi
Capillary voltage:	3,500 V

Compound	Precursor ion	Product ion	Fragmentor (V)	Collision energy (V)
Streptomycin	351.2	333.2	170	15
		207.1	170	18
Hydromycin B	528.3	177.1	170	25
		352	170	20
Streptomycin	582.4	263.2	180	30
		245.8	180	35
Dihydrostreptomycin	584.4	263.3	180	30
		246.2	180	40
Amikacin	586.4	163.1	170	30
		425.2	170	15
Kanamycin	485.3	163.1	150	20
		324.2	150	10
Apramycin	540.3	217.1	140	25
		378.2	140	12
Tobramycin	468.3	163.2	125	20
		324.2	125	8
Gentamicin	478.3	322.3	125	8
		157.2	125	15
Neomycin	615.3	161.1	175	30
		293.1	175	20

SPE Procedure

Measure 5 g homogenized bovine milk into a 50 mL polypropylene centrifuge tube, and add 10 mL of extraction solution (5% trichloroacetic acid, 0.6 mM Na_2EDTA, and 15 mM KH_2PO_4).

Shake thoroughly for 5 minutes. Centrifuge 5 minutes at 4,000 rpm, and transfer supernatant to a separate 50 mL centrifuge tube

To the pellet, add 10 mL of extraction solution, shake thoroughly for 5 minutes, and centrifuge 5 minutes at 4,000 rpm.

Combine supernatants into a single tube, and add 5 mL of 0.2 M heptafluorobutyric acid (HFBA) to the extract. Vortex-mix for 1 minute, and then centrifuge for 5 minutes at 4,000 rpm.

Adjust sample pH to 4.0 ± 0.5 using 5M NaOH. Vortex-mix for 1 minute.

Prepare Bond Elut Plexa SPE tubes for extraction by placing them on a vacuum manifold prepared for waste removal.

Condition the SPE cartridges with 3 mL ACN, followed by 3 mL water, then 5 mL 0.02 M HFBA.

Load pre-treated sample solution and extract slowly, at 1-2 mL/min.

Wash the SPE cartridges with 5 mL water, then dry the SPE cartridges for 5 minutes at high vacuum (15" Hg).

Prepare the vacuum manifold for collection, ensuring that clean collection tubes are in place

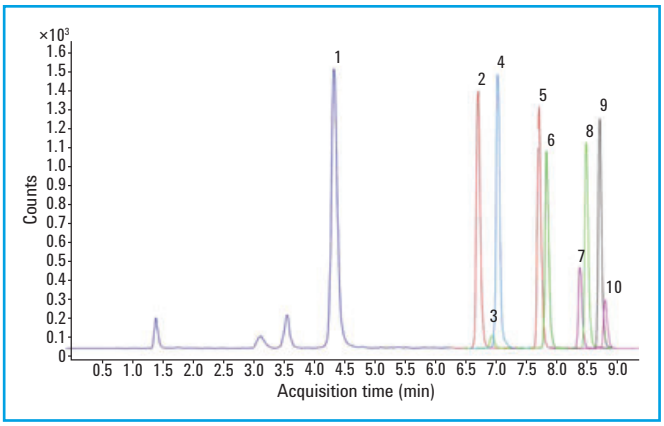
Apply 3 mL of elution solvent (ACN:0.2M HFBA 8:2 v:v).

Evaporate to dryness under nitrogen at 40 °C, and reconstitute with 1 mL of 0.02M HFBA.

Vortex-mix and use an ultrasonic bath to thoroughly combine the sample. Filter through a 0.2 µm filter, and collect sample in an autosampler vial. Transfer to LC-MS/MS system for analysis.

Compound	Spiked Level (ng/g)	Recovery (%)	RSD (n=6, %)
Streptomycin	0.01	78.7	3.8
	0.02	82.5	5.6
	.1	87.3	4.1
Hydromycin B	0.01	73.1	8.7
	0.02	69.7	6.3
	.1	77.3	5.9
Streptomycin	0.01	78.1	7.7
	0.02	66.5	10.1
	.1	71.8	7.1
Dihydrostreptomycin	0.01	84.2	2.1
	0.02	88.2	3.1
	.1	91.5	5.4
Amikacin	0.01	102.3	2.4
	0.02	97.2	2.7
	.1	99.4	3.6
Kanamycin	0.01	98.7	4.5
	0.02	92.1	3.9
	.1	93.6	6.8
Apramycin	0.01	97.1	4.8
	0.02	101.9	6.6
	.1	89.6	7.1
Tobramycin	0.01	92.5	2.9
	0.02	98.5	4.9
	.1	94.8	1.7
Gentamicin	0.01	107.3	3.9
	0.02	101.4	3.1
	.1	105.8	4.5
Neomycin	0.01	88.2	6.7
	0.02	97.4	7.2
	.1	87.6	5.4

Results



Chromatogram of 0.02 mg/kg spiked milk sample extract. 1. spectinomycin, 2. hygromycin B, 3. streptomycin, 4. dihydrostreptomycin, 5. amikacin, 6. kanamycin, 7. apramycin, 8. tobramycin, 9. gentamicin, and 10. neomycin.

Products used in the above application

Agilent Bond Elut Plexa Cartridge, 500 mg, 6 mL, 30/pk, Part No. 12259506

Agilent Captiva Premium Syringe Filters, PES, 25 mm, 0.2 µm, LC/MS Certified, Part No. 5190-5098

Agilent Poroshell 120 SB-C18 Column, 2.1 mm x 50 mm, 2.7 µm, Part No. 685775-902

To review this Application Note in its entirety, please view **5991-1758EN**

Pesticides Analysis Using the Agilent 5977A Series GC/MSD
(Publication 5991-2212EN)

Introduction

Gas chromatography combined with mass spectrometry (GC-MSD) for pesticide analysis provides a sensitive and comprehensive means of testing for pesticide residues in food. This application features the Agilent 5977A Series GC-MSD with Ultra Inert flow path components and the Agilent 7696A Sample Prep WorkBench for automated calibration curve preparation. Agilent Bond Elut Carbon/Amino (NH2) dual-phase SPE was used to effectively clean up apple extracts for use in matrix-matched calibration. The method delivers excellent precision and low detection limits for 42 commonly tested pesticide residues.

GC/MS Conditions

Instrument:	• Agilent 7890B Gas Chromatograph equipped with a split/splitless inlet • Agilent 5977A GC/MSD
Column:	HP-5ms Ultra Inert 19091S-433UI 30 m x 0.25 mm, 0.25 µm
Volume	1 µL
Inlet temperature:	280 °C
Injection mode:	Splitless
Carrier:	Helium at 1.2 mL/min
Oven:	120 °C for one minute 120 °C to 300 °C at 20 °C/min Hold at 300 °C for 5 min
Liner:	Ultra Inert single taper splitless liner with glass wool, 5190-2293
Plated Seal Kit:	Ultra Inert gold seal and washer, 5190-6144
Transfer line temperature:	280 °C
GC/MS Conditions	
Acquisition parameters:	SIM, EI
Gain Factor:	5.00
Source temperature:	250 °C
Quadrupole temperature:	150 °C
Tune file:	Etune.u
Detector gain:	5
TID:	On

SPE Procedure

Weigh 20 g of apple sample and homogenize thoroughly.

Add 40 mL acetonitrile and vortex-mix for 1 minute.
Add 5 g NaCl, and vortex-mix for 1 minute.

Centrifuge sample for 5 minutes at 4,200 rpm.

Remove 20 mL of supernatant and concentrate to approximately 1 mL.

Apply the concentrated sample to the Bond Elut Carbon/NH2 cartridge, and collect the eluate in a clean collection tube.

Reproducibility RSDs and Calculated MDLs for a 50 ppb Standards Mix Sample in Solvent*

Compound	RSD (%)	MDL (ppb)	Target Compound	RSD (%)	MDL (ppb)
Dichlorvos	5	6.4	Fipronil	6	8.3
Phorate	5	6.3	Procymidone	4	4.7
BHC-alpha	3	4.3	Profenofos	10	12.0
Dimethoate	7	8.3	DDE-p,p'	3	4.5
BHC-beta	3	3.6	DDD-p,p'	5	6.7
BHC-gamma	2	3.0	DDT-o,p'	3	3.6
Pentachloronitrobenzene	3	3.8	Triazophos	8	9.9
Pyrimethanil	4	5.0	DDT-p,p'	3	4.0
Diazinon	4	4.7	Iprodione	10	12.2
BHC-delta	3	3.5	Phosmet	8	9.7
Chlorothalonil	3	4.1	Bifenthrin	6	8.2
Vinclozolin	3	4.2	Fenpropathrin	6	8.6
Parathion-methyl	5	5.9	Phosalone	7	9.1
Fenitrothion	5	6.6	Cyhalothrin	8	10.6
Malathion	5	6.2	Permethrin	8	9.6
Fenthion	5	6.6	Cyfluthrin	9	11.3
Chlorpyrifos	4	5.7	Cypermethrin	9	11.0
Parathion	5	5.6	Flucythrinate	11	13.1
Triadimefon	4	5.5	Fenvalerate	9	12.0
Isocarbophos	8	9.1	Fluvalinate-tau	11	14.3
Isofenphos-methyl	5	6.7	Deltamethrin	9	11.2

*Eight consecutive injections were used to calculate the RSDs.

Products used in the above application

Agilent Bond Elut Carbon/NH2 Cartridge, 500 mg, 6 mL, 30/pk, Part No. 12252202

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

Agilent Ultra Inert Single Taper Splitless Liner with Glass Wool, Part No. 5190-2293

To review the Application Note in its entirety, please view **5991-2212EN**

Determination of Sulfonamide Residues in Milk with Agilent Captiva ND Lipids Filtration and LC-MS/MS

(Publication 5991-2230EN)

Introduction

Sulfonamide antibiotics are commonly administered to cattle for disease control or prevention. Because these antibiotic residues can transfer to bovine milk and enter into the human food supply, methods to monitor for these residues are needed. Agilent Captiva ND Lipids can be used to successfully and simply prepare milk in advance of LC-MS/MS analysis for three sulfonamide residues. The Captiva ND Lipids in a cartridge format accommodates larger sample volumes for additional flexibility when preparing samples. The non-drip (ND) membrane supports simple precipitation in the cartridge, because the sample does not elute until vacuum is applied. The resulting extract is clear, with many of the matrix components removed. Low detection limits and good recoveries demonstrate that Captiva ND Lipids is a useful tool for this application.

HPLC/MS Conditions

Instrument:	• Agilent 1200 Infinity Series HPLC • Agilent 6460 Triple Quadrupole LC/MS with Agilent JetStream ESI source
Column:	Poroshell 120 SB-C18 685775-902 2.1 mm x 50 mm, 2.7 µm
Eluent:	A: 0.1% formic acid in water B: 0.1% formic acid in acetonitrile
Flow rate:	0.5 mL/min
Volume:	10 µL
Post run:	2.5
Stop time:	6.2
Max pump pressure:	400 bar
Needle wash:	Flush port with 75:25 acetonitrile:water for 10 s
Overlapped injections:	Disabled
Automatic delay volume reduction:	No

Gradient:

Time	0.0	0.5	1.0	1.5	2.5	6.0	6.1
% B	5	5	40	60	95	95	10

ES Source Parameters

Gas temperature:	350 °C
Gas flow:	7 L/min
Sheath gas flow:	9 L/min
Sheath gas temperature:	350 °C
Nebulizer gas:	40 psi
Nozzle voltage:	10
Capillary voltage:	3,600 V

MS Source Parameters

Scan type:	MRM
Delta EMV:	(+) 300 V

SPE Procedure

Prepare the Captiva ND Lipids cartridges for extraction by loading them on the Vac Elut vacuum manifold. Ensure that the manifold is set up with collection tubes or vials in place.

Add 1.3 mL acetonitrile to each Captiva ND Lipids cartridge. Add 50 µL of 0.5 µg/mL working internal standard solution to each cartridge.

Load 0.25 mL spiked whole milk to the cartridge.

Mix the contents of the cartridge using a 1 mL pipette, performing 5 aspiration/dispensing cycles. Dispose of the pipette tip and replace with a clean tip for each cartridge.

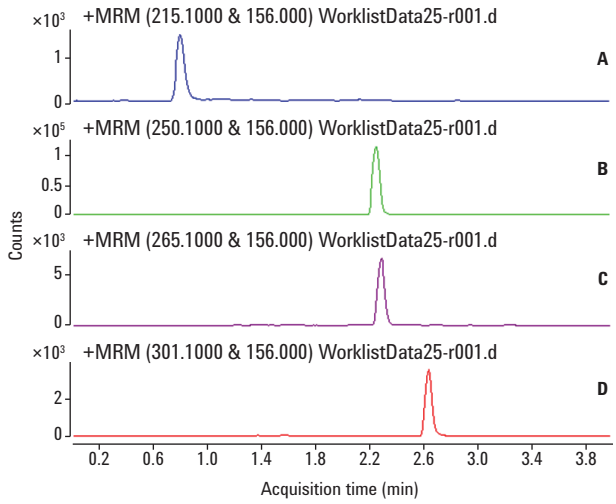
Apply high vacuum (15" Hg) and collect eluate in clean collection tubes or autosampler vials

Evaporate the extracts under nitrogen to dryness at 40 °C.

Reconstitute with 0.125 mL (125 µL) of mobile phase (5% ACN:95% H_2O V:V), mix, and transfer to autosampler vial for LC-MS/MS analysis as applicable.

Compound	ISTD?	Precursor ion	MS1 res	Product ion	MS2 res	Fragmentor (V)	Collision energy (V)	Polarity
Sulfaquinoxaline	No	301.1	Unit	156	Wide	110	15	Positive
Sulfaquinoxaline	No	301.1	Unit	108	Wide	110	28	Positive
Sulfamerazine	No	265.1	Unit	156	Wide	105	15	Positive
Sulfamerazine	No	265.1	Unit	108	Wide	105	28	Positive
Sulfaguanidine	No	215.1	Unit	156	Wide	85	12	Positive
Sulfaguanidine	No	215.1	Unit	108	Wide	85	24	Positive
Sulfapyridine	Yes	250.1	Unit	156	Wide	105	14	Positive
Sulfapyridine	Yes	250.1	Unit	108	Wide	105	27	Positive

Results



MRM extracted ion chromatograms of sulfa drugs in milk extract; A, 10 ng/mL sulfaguanidine; B, 100 ng/mL sulfapyridine (ISTD); C, 10 ng/mL sulfamerazine; D, 10 ng/mL sulfaquinoxaline.

Method performance for sulfonamide drug residues in milk, n = 5

		10 ng/mL		50 ng/mL		200 ng/mL	
Compound	**R^2**	**Accuracy (%)**	**RSD (%)**	**Accuracy (%)**	**RSD (%)**	**Accuracy (%)**	**RSD (%)**
Sulfaguanidine	0.999	96	6.9	94.8	4	104.1	5
Sulfamerazine	0.998	98.7	3.8	107.3	3.8	98.6	5
Sulfaquinoxaline	0.997	105.2	3.1	91.3	9.4	94.4	14.5

Products used in the above application

Agilent Captiva ND Lipids Filter Cartridges, 3 mL, 100/pk, Part No. A5300635

Agilent Vac Elut 20 Vacuum Manifold, 2.1 mm x 50 mm, 2.7 µm, Part No. 12234100

Agilent Poroshell 120 SB-C18 Column, 2.1 mm x 50 mm, 2.7 µm, Part No. 685775-902

To review the Application Note in its eniirety, please view **5991-2230EN**

PAHs in Chocolate and Peanuts with Agilent J&W Select PAH and Longer GC Columns

(Publication 5991-2299EN)

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are environmental contaminants, but they may also be introduced into foods through the processing steps themselves. Because dietary exposure to PAHs is a concern, a method for analyzing 25 of the 15+1 European Food Safety Authority targets and nine targeted by the US Food and Drug Administration using gas chromatography and mass spectrometry (GC-MSD) was developed. Chocolate and roasted peanut samples were prepared using Agilent Bond Elut SI SPE, and a PAH-specific GC column, Agilent J&W Select PAH 15 m GC column, provided chromatographic separation of even the more challenging PAHs. The resulting method can be used to evaluate challenging matrices for PAH contamination at low detection limits.

HPLC/MS Conditions

Instrument:	• Agilent 7890A Gas Chromatograph • Agilent 5975C Series GC/MSD
Column:	Select PAH CP7461 15 m x 0.15 mm, 0.10 µm
Inlet temperature:	300 °C
Injection mode:	Splitless
Carrier:	MSD UHP Helium, FID Hydrogen, both at 1.2 mL/min constant flow
Oven:	70 °C (hold 0.4 min) to 180 °C at 70 °C/min, then to 230 °C at 7 °C/min (hold 7 min), then to 280 °C at 50 °C/min (hold 7 min), then to 350 °C at 30 °C/min (hold 24 min)
Detector:	FID at 350 °C
Sampler:	Agilent 7693A Automatic Liquid Sampler 1 µL injection volume
MSD transfer aux temperature:	350 °C

GC/MS Conditions

Solvent delay:	1.4 min
MS temperature:	300 °C (source), 150 °C (quad)
SIM mode:	

Group	Start Time	Ions	Dwell
1	1.40	128, 152, 153, 165	50
2	4.70	178	200
3	7.40	202, 216	100
4	12.50	226, 228, 242	50
5	18.30	252	200
6	23.00	276, 278	100
7	27.00	302	200

SPE Procedure

Slurry 1 g chocolate in 10 mL methanol (MeOH) and grind to fine solids using a PTFE-coated steel spatula.

Allow the cocoa solids to settle by sitting for 1 hour. Remove the methanol and evaporate to dryness under N_2.

Wash the solids in 2 mL MeOH.

Resuspend the oily residue in 10 mL deionized water.

Extract the oily residue/deionized water mixture with 5 mL n-pentane, remove the pentane layer to a separate tube, and repeat the extraction with another 5 mL n-pentane. Combine the two 5 mL portions and dry under N_2.

Dissolve the residue in 2 mL n-pentane and mix well.

Prepare the Bond Elut SI SPE cartridges for extraction by loading onto a vacuum manifold. Ensure that the manifold is set up to divert to waste.

Condition with 5 mL MeOH, followed by 5 mL THF. Apply 5 mL n-pentane.

Apply the 2 mL pentane sample extract and extract under low vacuum or gravity.

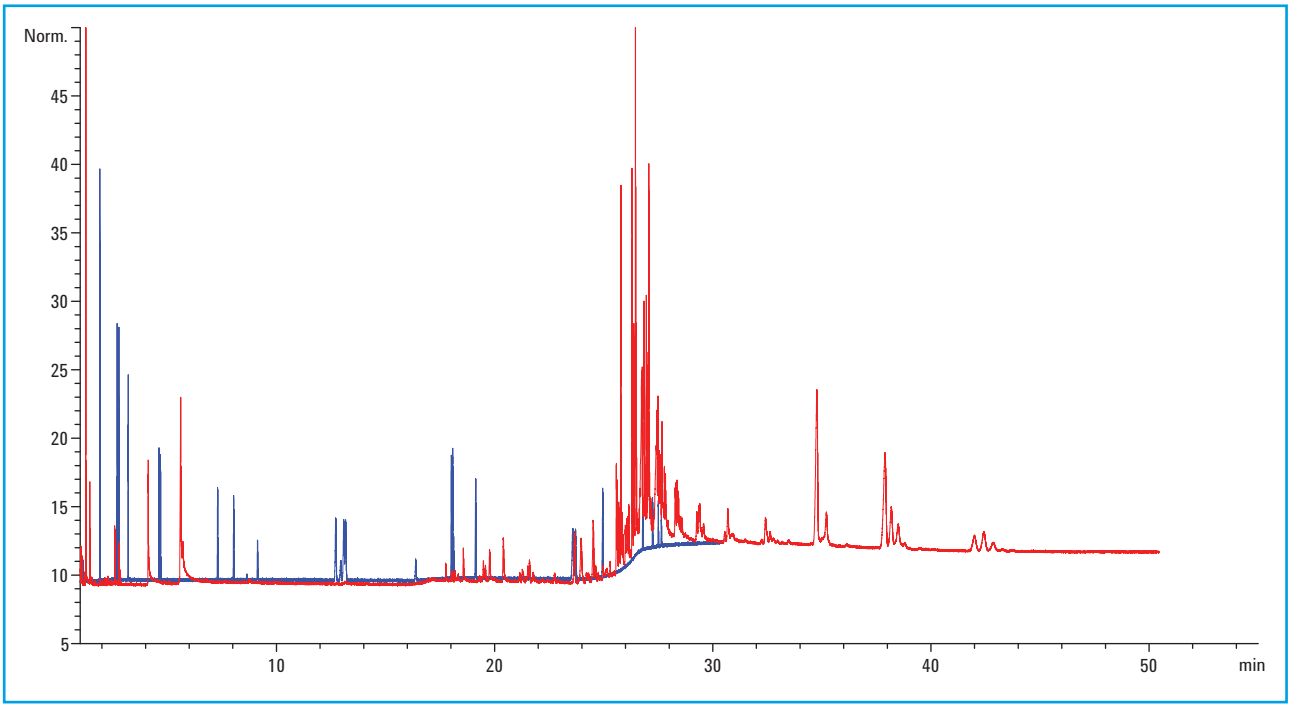
Wash the cartridges with 3 mL n-pentane, diverted to waste.

Prepare the manifold to collect samples.

Elute with 5 mL of 10% MeOH:90% THF (uninhibited) and collect the eluate. Repeat with an additional 5 mL application of 10% MeOH:90 % THF.

Evaporate the extracts to near dryness and reconstitute to a final volume of 1 mL with MeOH. Mix well and transfer to an autosampler vial for analysis.

Results



Overlay of milk chocolate extract (red trace) matrix with PAH standards (blue trace) showing interferences and need for bakeout at end of run.

Products used in the above application

Agilent Bond Elut SI Cartridges, 1 g, 6 mL, 30/pk, Part No. 12256008

Agilent J&W Select PAH Column, 15 m x 15 mm, 0.10 µm, Part No. CP7461

Agilent Ultra Inert Liner with Wool, Part No. 5190-2295

To review the Application Note in its entirety, please view **5991-2299EN**

GC/MS of Native Patulin in Apple Juice and Cider
(Publication 5991-2799EN)

Introduction

Patulin is a mycotoxin produced by several types of molds, including those that can be found on rotting apples. Patulin levels in apple products such as cider, juice, or applesauce, is monitored and regulated in several countries. This application describes a straightforward cleanup of apple juice and cider using Agilent Bond Elut LMS polymeric SPE, followed by analysis with gas chromatography and mass spectrometry (GC-MSD). Agilent Ultra Inert flow path components, the use of a selective GC column, and the Bond Elut LMS cleanup provide a method that separates the patulin from 5-hydroxymethylfurfural, a common by-product of overheated sugars that is also monitored by this method. Good precision and sensitivity were achieved, with recoveries greater than 90%.

GC/MS Conditions

Column:	DB-35ms Ultra Inert 122-3832UI 15 m x 0.15 mm, 0.10 µm
Inlet temperature:	300 °C
Injection mode:	Cold-splitless, 67 °C (hold 0.1 min), then to 160 °C at 720 °C/min, split vent on at 1 min (30 mL/min), gas saver on at 3 min (20 mL/min)
Carrier:	MSD helium, 1 mL/min constant flow
Oven:	50 °C (hold 5 min), then to 300 °C at 40 °C/min (hold 8.75 min)
GC:	Agilent 7890A GC
Sampler:	Agilent 7693 Automatic Liquid Sampler, 1 µL volume injection, 5190-6144
MSD transfer aux temperature:	300 °C
GC/MS Conditions	
MS:	Agilent 5975C Series MSD with inert EI 350 source, tandem axis detector
Solvent delay:	6 min
SIM mode:	Mass 55.00, 97.00, 110.00, 126.00 dwell 100 ms for each

SPE Procedure

Measure 10 g (10 mL) of juice or cider into a clean tube.

Add internal standard and spike as necessary for QC samples.

Prepare the Bond Elut LMS SPE cartridges by placing them on a vacuum extraction manifold.
Ensure that the manifold is set up to divert to waste.

Condition the cartridges by applying 4 mL methanol (MeOH), followed by 4 mL water.

Load the sample to the SPE cartridge, and allow the sample to extract under gravity.
(Note: Slight vacuum may be required for cider, depending on the level of suspended solids.)

Wash with 8 mL 1% sodium bicarbonate under gravity.

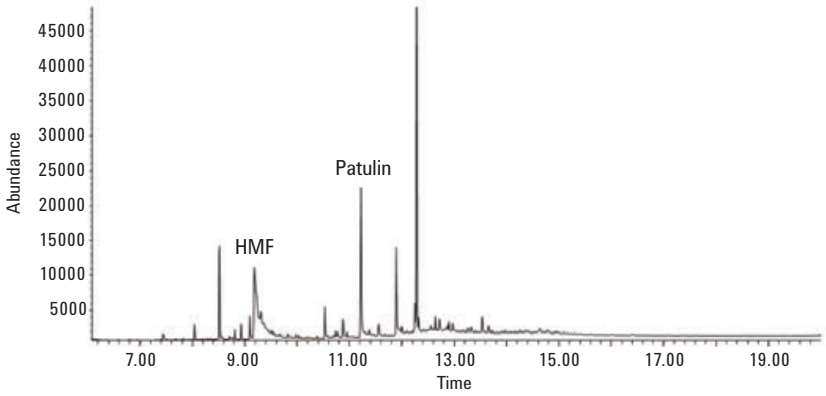
Wash with 8 mL 1% acetic acid under gravity.

Insert clean collection tubes and ensure that the vacuum manifold is set to collect samples.

Add 8 mL MeOH elution solvent and collect the eluate.

Dry the eluate under N_2 until approximately 4 mL remains.

Results



Scan mode of a 10 ng/g mix of HMF and patulin with acceptable peak shape and satisfactory resolution from the matrix components.

Products used in the above application

Agilent Bond Elut LMS SPE Cartridges, 1 g, 6 mL, 30/pk, Part No. 12255022

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

Agilent Ultra Inert Splitless Single Taper Liner, Part No. 5190-3162

To review the Application Note in its entirety, please view **5991-2799EN**

Multiresidue Confirmation of Pesticides in Honey Using Solid Supported Liquid Extraction

(Publication SI-01002)

Introduction

A simple method to test honey for pesticides and pesticide metabolites using liquid chromatography and tandem mass spectrometry (LC-MS/MS) was developed. This method uses Agilent Chem Elut solid supported liquid-liquid extraction (SLE) products for extraction and concentration of pesticide residues from honey. The SLE method was compared to a liquid-liquid method, and the Chem Elut SLE approach delivered higher recoveries for the range of pesticides and metabolites studied. Certain target compounds that were not found using LLE were recovered at high levels using Chem Elut SLE. Chem Elut SLE alleviated some of the issues encountered with the LLE approach, providing a rugged method for this analysis.

HPLC/MS Conditions

The method is based on HPLC coupled to mass spectrometry (MS) operating in tandem mode (MS/MS) according to EU advice 2002/657/EC [2].

Column:	Agilent Polaris C18-A A2001150X020 2.0 mm x 150 mm, 3 µm
Mobile phase:	A: Water + 0.1% acetic acid B: Acetonitrile + 0.1% acetic acid
Flow rate:	0.4 mL/min
Temperature:	40 °C
Linear gradient conditions:	Hold 10% B for 1 min, to 80% B in 14 min, to 100% B in 2 min, hold 100% B for 2 min

Before loading sample

Sample loaded

Extracting solvent added

Solid supported extraction on Agilent Chem Elut cartridges.

SLE Procedure

Measure 1 g honey. Spike with internal standard and surrogate standard as appropriate.

Mix the spiked honey with 1.25 mL water and 2.5 mL acetone.

Add 1.25 mL NaCl solution (20 g NaCl in 100 mL water). Mix the sample.

Set up the Chem Elut cartridges on a vacuum extraction manifold, ensuring that the manifold is set to divert to waste.

Apply the prepared samples to the Chem Elut cartridges, and allow the samples to flow through under gravity. Allow 15 minutes for complete extraction and adsorption to occur.

Insert collection tubes and ensure that the manifold is set to collect sample extracts.

Apply 10 mL ethyl acetate to the Chem Elut SLE cartridges and collect the eluate. Repeat with an additional 10 mL ethyl acetate for a total of 20 mL eluate.

Evaporate the sample extracts under N_2 at 30 °C.

Reconstitute the samples with 200 µL acetonitrile:water (10:90 v:v), and mix well.

Transfer samples to autosampler vials for LC-MS/MS analysis.

Results

Pesticide (ng/mL):

Am 0.4, At 0.4, Ca 0.4, Ch 20.0, De 2.0, Dm 2.0, Fi 10.0, Im 2.0, Is 2.0, Li 2.0, Mh 10.0, MhS 20.0, Mo 2.0, Pi 0.4, Ri 0.4, Si 2.0, TOH 1.0

Recovery comparison of pesticides between solid supported liquid-liquid extraction (SLE) on Agilent Chem Elut and classical liquid-liquid extraction (LLE).

Products used in the above application

Agilent Chem Elut Cartridges, Unbuffered, 5.0 mL, 100/pk, Part No. 12198006

Agilent Polaris C18-A Column, 2.0 mm x 150 mm, 3 µm, Part No. A2001150X020

To review the Application Note in its entirety, please view **SI-01002**

Analysis of Pesticide Residues in Spinach Using Bond Elut Carbon, Carbon/PSA, and Carbon/NH2 SPE Cartridges

(Publication 5990-6943EN)

Introduction

This technical overview describes the use of Agilent Bond Elut solid phase extraction (SPE) products using Bond Elut Carbon and dual-phase SPE cartridges for the extraction of pesticide residues from spinach. SPE enables a rapid cleanup of spinach with good recoveries and day-to-day reproducibility across the cartridges. Select Bond Elut Carbon SPE to remove chlorophyll and other pigments. Bond Elut NH2 removes fatty acids as well as pigments and sugars and is amenable to separating structural isomers. Bond Elut PSA also removes fatty acids and pigments, but it is more suitable for applications where polar compounds retain too strongly on NH2. Overall, the methods for each SPE cartridge are straightforward and support multiresidue pesticide analyses.

HPLC Conditions

Column:	Eclipse Plus C18 959963-902 4.6 mm x 150 mm, 3.5 µm	
Mobile phase:	A: H_2O 0.1% formic acid B: ACN 0.1% formic acid	
Detector:	DAD 254 nm	
Gradient:	Time (min)	B (%)
	0.0	15
	0.1	15
	5	21
	18	30
	30	67
	30.1	15

Target Analyte Information

Compounds	Log P	Type	pKa
Caffeine	-0.13	CNS stimulant	14
Tebuthiuron	1.79	herbicide	0.9
Sulfadimethoxine	1.48	sulfa drug	6.1
Bromacil	2.1	pesticide	9.1
Prednisone	1.57	steroid	n/a*
Warfarin	3.42	anticoagulant	4.9

*Not available

SPE Procedure

Spike 10 g homogenized spinach with 6-component mixture.

Extract with 20 mL acetonitrile (ACN).

Centrifuge and decant 10 mL of supernatant.
Concentrate under N_2 to 1 mL.

Condition SPE cartridge with 5 mL acetonitrile:toluene (3:1 V:V).

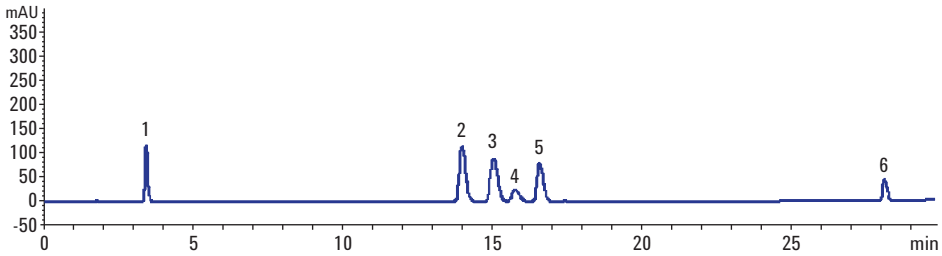
Apply the sample and extract under low to no vacuum.

Elute sample with 20 mL ACN:toluene (3:1 V:V).

Evaporate just to dryness and reconstitute with 1 mL mobile phase
(85:15 H_2O with 0.1% Formic acid:ACN with 0.1% Formic Acid).

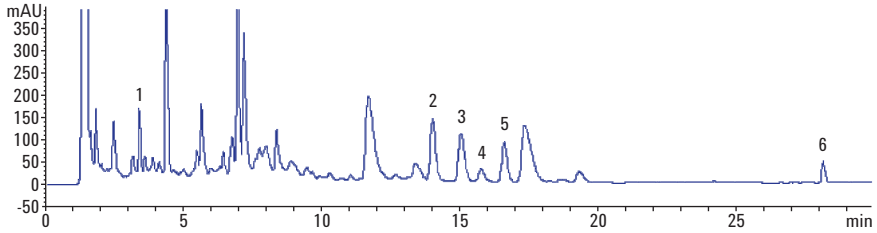
Analyze by HPLC with DAD detection.

Results

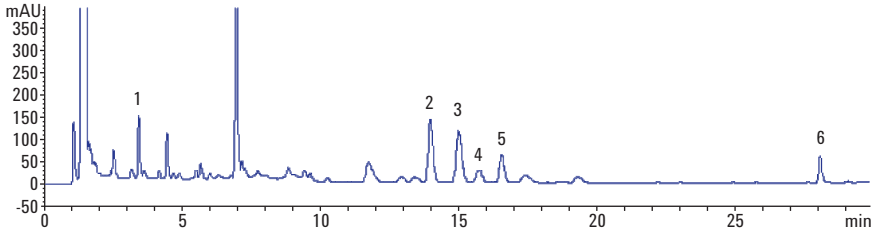


HPLC UV-Vis chromatogram of 10 ppm neat solution extract. Peak identification: 1. Caffeine, 2. Tebuthiuron, 3. Sulfadimethoxine, 4. Bromacil, 5. Prednisone, 6. Warfarin.

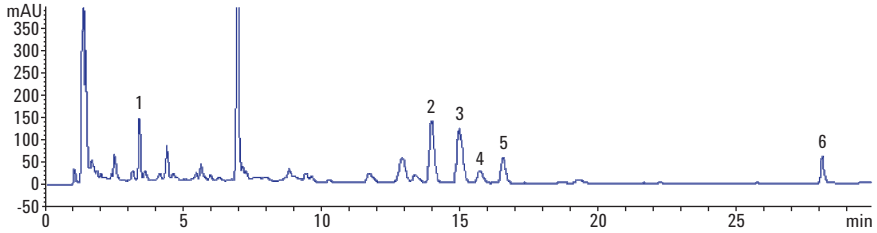
Relative comparison of matrix absorption at 254 nm.



HPLC UV-Vis chromatogram of spinach sample extracts processed by Bond Elut Carbon 500 mg/6mL (P/N 12252201).



HPLC UV-Vis chromatogram of spinach sample extracts processed by Bond Elut Carbon/PSA 500, 500 mg/6 mL (P/N 12102042C500).



HPLC UV-Vis chromatogram of spinach sample extracts processed by Bond Elut Carbon/NH_2 500, 500 mg/6 mL (P/N 12252202).

Products used in the above application

Agilent Bond Elut Carbon Cartridge, 500 mg, 6 mL, 30/pk, Part No. 12252201

Agilent Bond Elut Carbon/NH2 Cartridge, 500 mg, 6 mL, 30/pk, Part No. 12252202

Agilent Bond Elut Carbon/PSA Cartridge, 500 mg, 6 mL, 30/pk, Part No. 12102042C500

Agilent ZORBAX Rapid Resolution Eclipse Plus C18 Column, 4.6 mm x 150 mm, 3.5 µm, Part No. 959963-902

To review this Application Note in its entirety, please view **5990-6943EN**

Analysis of Carcinogenic Tobacco-specific Nitrosamines in Mainstream Cigarette Smoke Using an Agilent J&W DB-35ms Ultra Inert GC Column

(Publication 5990-8894EN)

Introduction

A complete method incorporating Agilent Bond Elut SPE sample preparation, Agilent Ultra Inert GC column, and the Agilent 7000 Series Triple Quadrupole GC/MS was developed to identify and quantify four tobacco-specific nitrosamines (TSNAs) in cigarettes. The resulting method delivered low detection limits, good linearity, and consistent recovery of the target TSNAs. An Agilent DB-35ms Ultra Inert GC column provided baseline separation of the TSNAs, with excellent peak shapes, while the Agilent Bond Elut Alumina B column supported a simple sample cleanup method. GC-tandem MS offered the required sensitivity compared to the traditional method for this analysis. This sample preparation technique and analysis method could be applied to analyzing the nitrosamines in other matrices.

Instrumentation and analytical conditions for the Agilent 7000A Triple Quadrupole GC/MS

Column:	DB-35ms Ultra Inert 122-3832UI 30 m x 0.25 mm, 0.25 µm
GC	Agilent 7890A Series
Autosampler:	Agilent 7683A Injector and sample tray
Inlet mode:	Pulsed splitless
Carrier:	Helium
Column flow:	1.2 mL/min constant flow
Inlet temperature:	250 °C
Injection:	1 µL
Oven:	50 °C (1 min), 30 °C/min to 170 °C, 5 °C/min to 250 °C, 30 °C/min to 300 °C (5 min)
Triple Quadrupole Mass Spectrometer:	Agilent 7000A Series
Mode:	Electron impact
Transfer line temperature:	250 °C
Solvent delay:	10 min
Source temperature:	280 °C
Quadrupole temperature:	Q1 and Q2 = 150 °C
MRM Mode Conditions	
Resolution:	Wide
Collision gas flows:	Nitrogen at 1.0 mL/min, helium at 2.25 mL/min
Detector gain:	15

SPE Procedure

Smoke particulate matter from 20 cigarettes was collected onto CFPs according to ISO 4387:2000.

Transfer CFP to 250 mL Erlenmeyer flask.

Add 20 mL of methylene chloride solution containing 20 ng/mL of D4-NNK internal standard.

Shake flask horizontally for 40 minutes.

Condition the Bond Elut Alumina B SPE cartridge with 3 mL methylene chloride.

Apply 3 mL of the CFP extract spiked with internal standard to the conditioned SPE tube.

Wash the SPE tube with 2 mL methylene chloride.

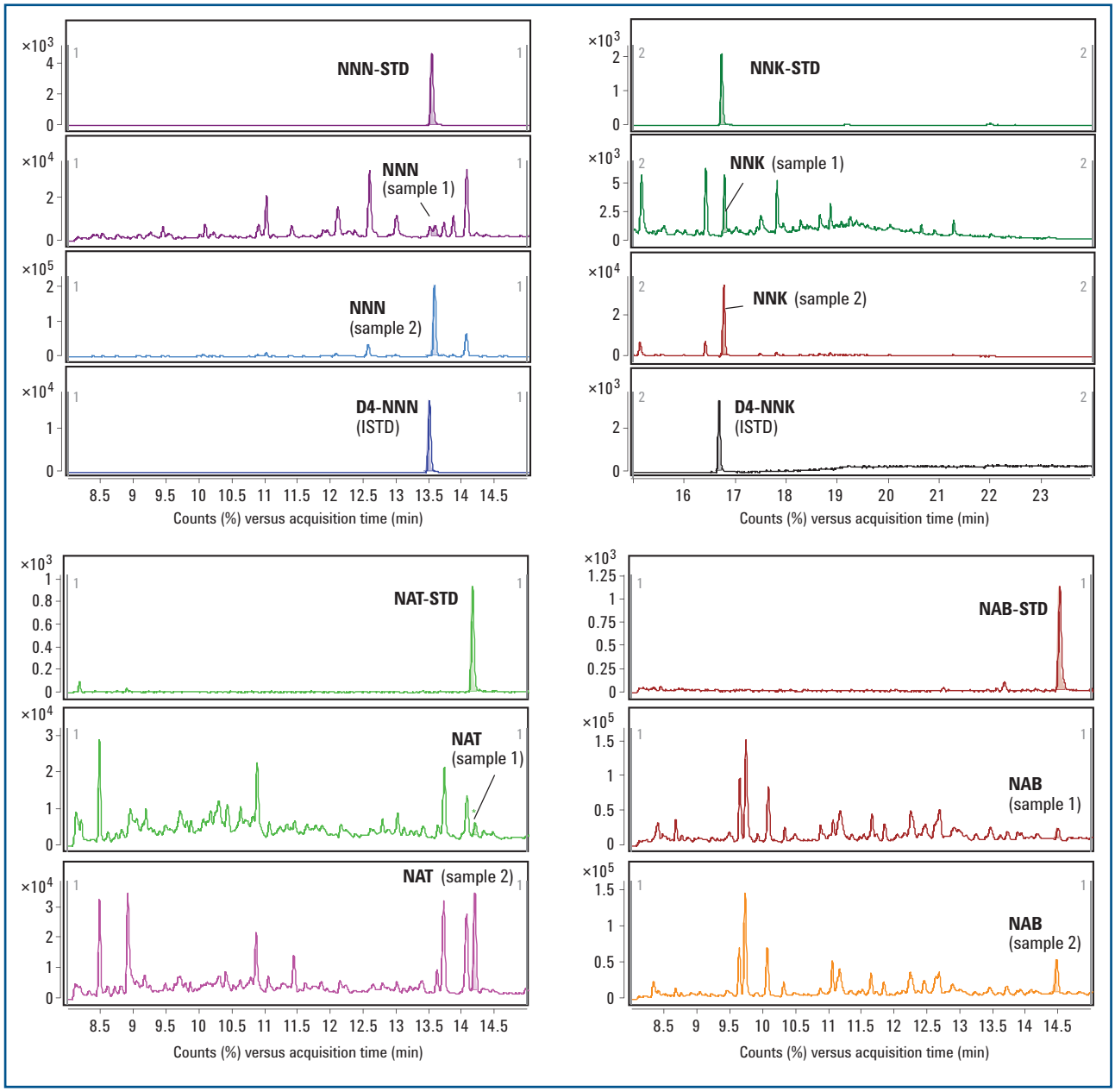
Extract with 3 mL of 8% methanol/92% methylene chloride (V:V).

Collect eluent and analyze by GC-MS/MS.

MRM Parameters

	Quantitation Transition			Confirmation Transition		
Compounds	Precursor Ion (m/z)	Product Ion (m/z)	CE (eV)	Precursor Ion (m/z)	Product Ion (m/z)	CE (eV)
NNN	177	147	5	105	104	10
D4-NNN	181	151	5	109	108	10
NAT	159	157	10	159	130	25
NAB	161	133	15	161	106	25
D4-NNK	181	150	5	181	122	15
NNK	177	146	5	177	118	15

Results



MRM chromatograms of TSNA standard solution and the real samples using an Agilent 7000A Triple Quadrupole GC/MS system and an Agilent J&W DB-35ms Ultra Inert 0.25 mm x 30 m, 0.25 µm column.

Products used in the above application

Agilent Bond Elut Alumina B Cartridge, 500 mg, 3 mL, 50/pk, Part No. 12102048

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

Agilent Liner, Splitless, Single Taper, Deactivated, 11 mm id, without Glass Wool, Part No. 5183-4761

Agilent Liner, Splitless, Single Taper, Deactivated, 4 mm id, without Glass Wool, Part No. 5181-3316

To review this Application Note in its entirety, please view **5990-8894EN**

Analyzing Synthetic Sweeteners in Waste Water with Robust Sample Preparation

(Publication 5990-8248EN)

Introduction

Solid phase extraction using Agilent Bond Elut Plexa SPE cartridges was employed for extraction and pre-concentration of artificial sweeteners from surface water, with high recoveries and good precision. This method may be extended to drinking water and similar matrices. The analysis was performed using LC-MS/MS, and looked at the artificial sweeteners acesulfame, cyclamate, saccharin, and sucralose.

HPLC/MS Conditions

Column:	Eclipse XDB-C18 927975-902 4.6 mm x 50 mm, 1.8 µm	
Mobile phase:	A: Water, 2 mM ammonium carbonate B: Methanol, 2 mM ammonium carbonate	
Flow rate:	0.6 mL/min	
Gradient:	Time (min)	B (%)
	0.0	2
	7.0	75
	9.0	75
	9.1	2
	15.0	2

MRM Transistions

Sweetener	RT (min)	Precursor Ion (m/z)	Product Ion (m/z)
Acesulfame	2.21	162	82
	2.21	162	78
Cyclamate	3.49	178.2	80
	3.49	178.2	81
Saccharin	2.96	182	42
	2.96	182	106
Sucralose	5.37	395.2	35
	5.37	397	37

SPE Procedure

Acidify 100 mL water samples using sulfuric acid to pH of 2.

Condition Bond Elut Plexa 200 mg 6 mL cartridges with 3 mL CH_3OH followed by 3 mL acidified HPLC water (sulfuric acid, pH2) at rate of 5 mL/min.

Load the acidified water sample at a rate of 5 mL/min.

Dry cartridges under high vacuum (15" Hg) for 5 minutes.

Elute with 5 mL CH_3OH under low vacuum (<2" Hg) at a rate of 2 mL/min.

Evaporate samples to near dryness under N_2 at 40 °C and reconstitute in 1 mL acetonitrile:water (5:95 V:V).

Transfer to autosampler vial for LC-MS/MS analysis and inject 20 µL.

Results

Percent recovery and RSD values of sweeteners in water using LC-MS/MS determination after SPE with Agilent Bond Elut Plexa. Spiked concentration of wweeteners was 1 ppb.

Recovery and RSD (%)

Injection volume	Acesulfame	Cyclamate	Saccharin	Sucralose
20 µL	86	74	91	86
RSD 20 µL	7	5	2	15
2 µL	92	77	92	nd*
RSD 2 µL	7	5	2	-

*No data

Products used in the above application

Agilent Bond Elut Plexa 200 mg 6 mL, 30/pk, Part No. 12109206

Agilent ZORBAX Eclipse XDB-C18 Column, 4.6 mm x 50 mm, 1.8 µm, Part No. 927975-902

To review this Application Note in its entirety, please view **5990-8248EN**

Analysis of Phthalates in Body Wash Using Solid-Supported Liquid-Liquid Extraction

(Publication 5991-2734EN)

Introduction

A comparison of liquid-liquid extraction (LLE) and solid-supported liquid-liquid-extraction (SLE) using Agilent Chem Elut SLE products was performed to assess the presence of phthalates in infant body wash and shampoo products. Exposure to phthalates through food and personal care products is a concern, and this method enabled the fast, sensitive identification and detection of phthalate residues. Chem Elut SLE delivered cleaner extracts with better performance at low concentrations than a standard LLE method, and provided a reliable means of extracting phthalates from infant shampoo and body wash.

HPLC-DAD Conditions

Column:	Eclipse Plus C18 959993-902 4.6 x 150 mm, 5 µm			
Instrument:	Agilent 1200 Infinity Series with a binary pump, autosampler, inline degasser, and an 80 Hz Diode Array Detector			
Eluent:	A: 90% water:10% acetonitrile B: acetonitrile			
Flow rate:	2.00 mL/min			
Volume:	1.7 µL			
Response time:	0.02 s			
Detection:	230 nm			
Gradient:	Time	0.00	3.00	5.00
	% B	50	65	70

SLE Procedure

Measure 1.00 ± 0.05 g of sample into a clean, phthalate-free tube, such as glass. Add internal standard solution.

Add 2.5 mL acetone and 1.25 mL NaCl solution. Vortex-mix for 30 seconds.

Prepare Chem Elut cartridges by loading them onto a vacuum extraction manifold. Ensure the manifold is set up to divert to waste.

Apply the prepared samples and allow the samples to pass through the sorbent layer. This will take approximately 15 minutes.

Set up the manifold to collect samples, and ensure that clean, phthalate-free tubes are in place for sample collection.

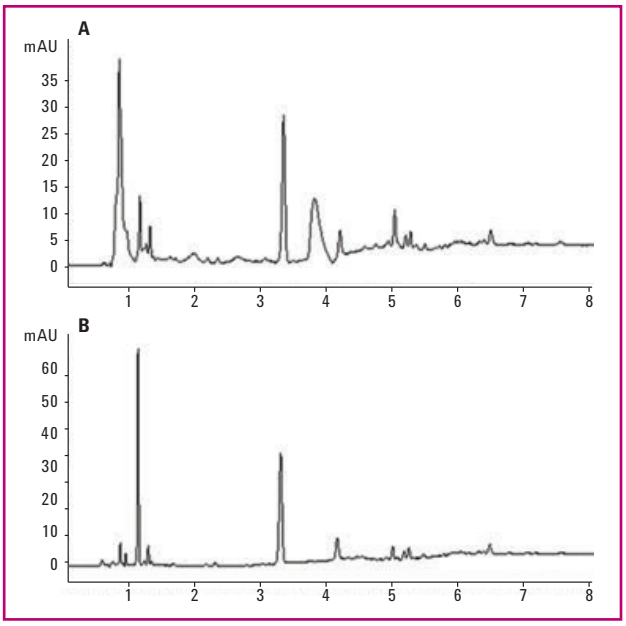
Apply 10 mL ethyl acetate to the Chem Elut tubes and collect the eluate.

Apply an addtional 10 mL ethyl acetate to the tubes and collect the eluate.

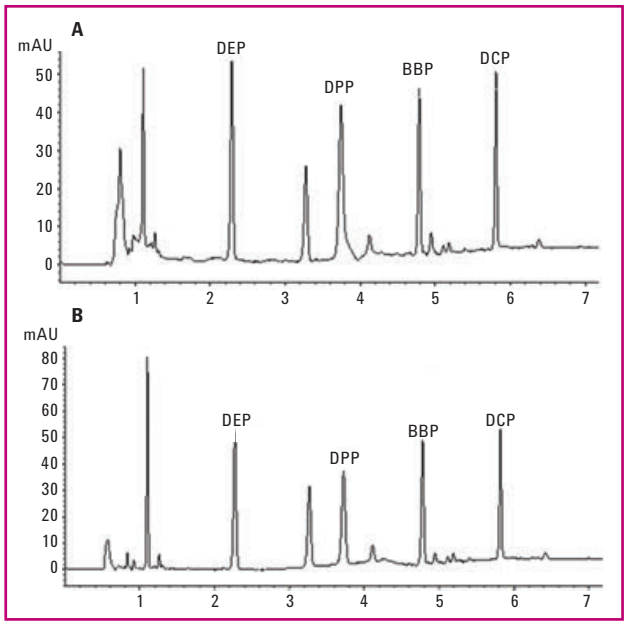
Dry the extracts under N_2.

Reconstitute the samples with 500 µL MeOH, vortex-mix, and transfer to certified clean autosampler vials for analysis on the HPLC system.

Results



Chromatograms of infant shampoo/body wash (not spiked) after A) LLE and B) SLE.



Chromatograms of extract from infant shampoo/body wash spiked with phthalates after A) LLE and B) SLE.

Products used in the above application

Agilent Chem Elut Cartridges, Unbuffered, 5.0 mL, 100/pk, Part No. 12198006

Agilent ZORBAX Eclipse Plus C18 Column, 4.6 mm x 150 mm, 5 µm, Part No. 959993-902

To review the Application Note in its entirety, please view **5991-2734EN**

Determination of Parabens in Body Wash Using Solid-Supported Liquid-Liquid Extraction

(Publication 5991-2735EN)

Introduction

A comparison of liquid-liquid extraction (LLE) and solid-supported liquid-liquid-extraction (SLE) using Agilent Chem Elut SLE products was performed to assess the presence of parabens in body wash and shampoo. Exposure to parabens in personal care products is a concern, and this method enabled the fast, sensitive identification and detection of parabens. Chem Elut SLE delivered cleaner extracts with better performance at low concentrations than a standard LLE method, and provided a reliable means of extracting parabens from infant body wash and shampoo.

HPLC-DAD Conditions

Column:	Eclipse Plus C18 959993-902 4.6 x 150 mm, 5 µm
Instrument:	Agilent 1200 Infinity Series with a binary pump, autosampler, inline degasser, and an 80 Hz Diode Array Detector
Sample prep:	Chem Elut cartridges, unbuffered, 5.0 mL 12198006
Eluent:	A: 90% water:10% acetonitrile B: acetonitrile
Flow rate:	2.00 mL/min
Volume:	1.7 µL
Response time:	0.02 s
Detection:	230 nm

Gradient:

Time	0.00	4.00	5.00
% B	30	65	70

SLE Procedure

Measure 1.00 ± 0.05 g of sample into a clean, phthalate-free tube, such as glass. Add internal standard solution.

Add 2.5 mL acetone and 1.25 mL NaCl solution. Vortex-mix for 30 seconds.

Prepare Chem Elut cartridges by loading them on to a vacuum extraction manifold. Ensure the manifold is set up to divert to waste.

Apply the prepared samples and allow the samples to pass through the sorbent layer. This will take approximately 15 minutes.

Set up the manifold to collect samples, and ensure that clean, phthalate-free tubes are in place for sample collection.

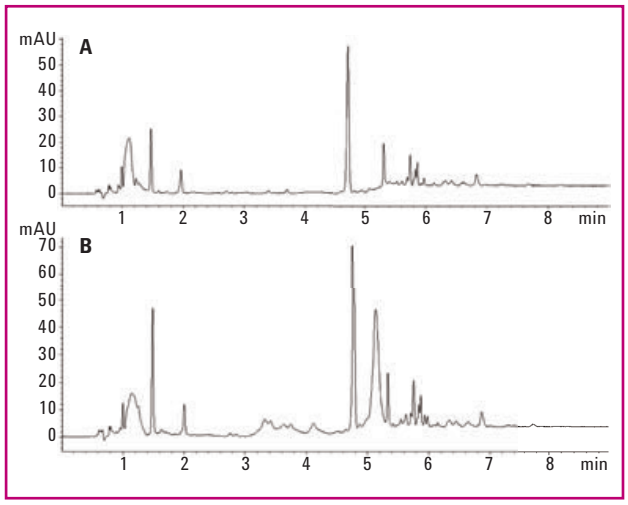
Apply 10 mL ethyl acetate to the Chem Elut tubes and collect the eluate.

Apply an addtional 10 mL ethyl acetate to the tubes and collect the eluate.

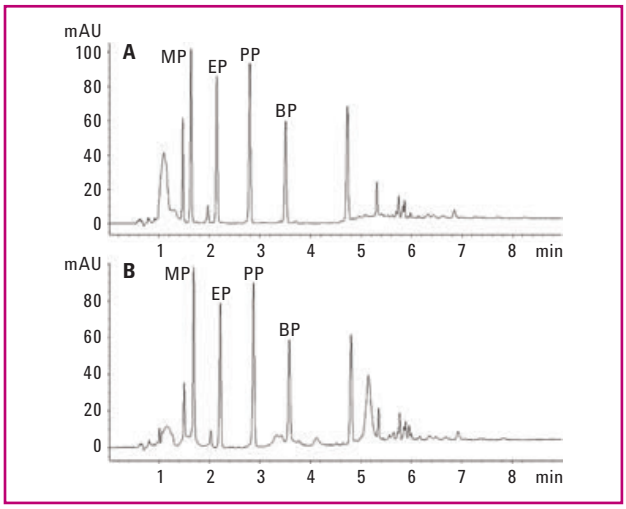
Dry the extracts under N_2.

Reconstitute the samples with 500 µL MeOH, vortex-mix, and transfer to certified clean autosampler vials for analysis on the HPLC system.

Results



Chromatograms of infant shampoo/body wash (not spiked) after A) SLE and B) LLE.



Chromatograms of spiked shampoo/body wash after A) SLE and B) LLE.

Calculated percent recoveries for the extraction of four phthalates from infant shampoo/body wash using SLE and LLE

	% Recovery (LLE)				% Recovery (SLE)			
	Spiked at 20 µg/mL		Spiked at 175 µg/mL		Spiked at 20 µg/mL		Spiked at 175 µg/mL	
	avg	std dev	avg	std dev	avg	std dev	avg	std dev
Methyl paraben	94.32	17.82	79.15	2.53	96.87	2.33	100.29	1.33
Ethyl paraben	83.14	7.63	81.80	2.95	87.78	3.68	101.04	0.78
Propyl paraben	81.95	6.16	83.93	3.08	82.53	3.94	99.87	1.42
Butyl paraben	97.36	26.54	82.94	4.86	84.26	3.79	99.41	1.21

Products used in the above application

Agilent Chem Elut Cartridges, Unbuffered, 5.0 mL, 100/pk, Part No. 12198006

Agilent ZORBAX Eclipse Plus C18 Column, 4.6 mm x 150 mm, 5 µm, Part No. 959993-902

To review the Application Note in its entirety, please view **5991-2735EN**

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Asia Pacific:
adinquiry_aplsca@agilent.com

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