Innovative Approaches

for today's food analysis challenges

Agilent SPE Food Safety Applications Notebook

Volume 1

Our measure is your success.

products | **applications** | software | services

Agilent Technologies

Table of Contents

Reliable food safety testing begins with reliable SPE

Dear Valued Customer,

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

And now, Agilent can help you deliver on that promise

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

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

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

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

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

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

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

What is SPE?

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

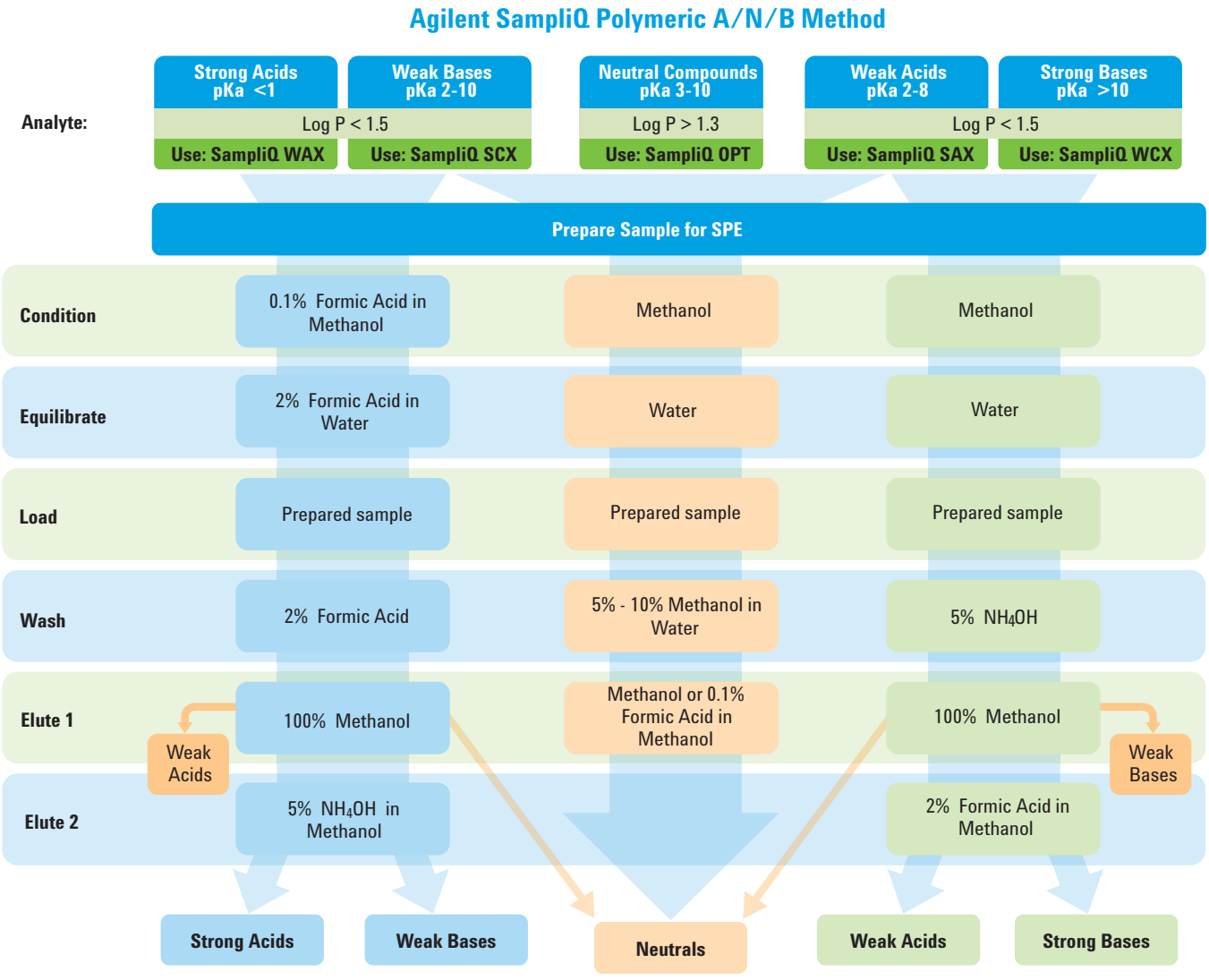
This problem can be remedied through Solid Phase Extraction (SPE), a fast, cost-effective technique for purifying extracts and ensuring accurate results.

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

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

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

Method Development



Pesticides and Contaminants

Fast, confident pesticide detection and quantitation

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

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

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

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

Introduction

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

Sample Pretreatment

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

Instrument conditions

HPLC conditions

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

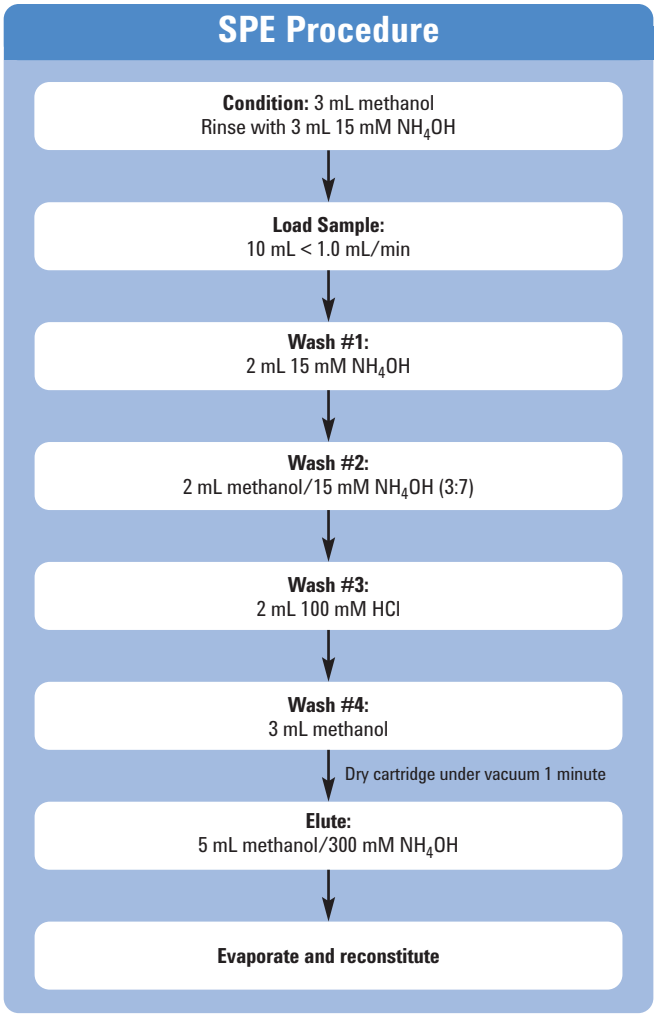


Figure 1. Fungicides in apple juice SPE procedure

Results

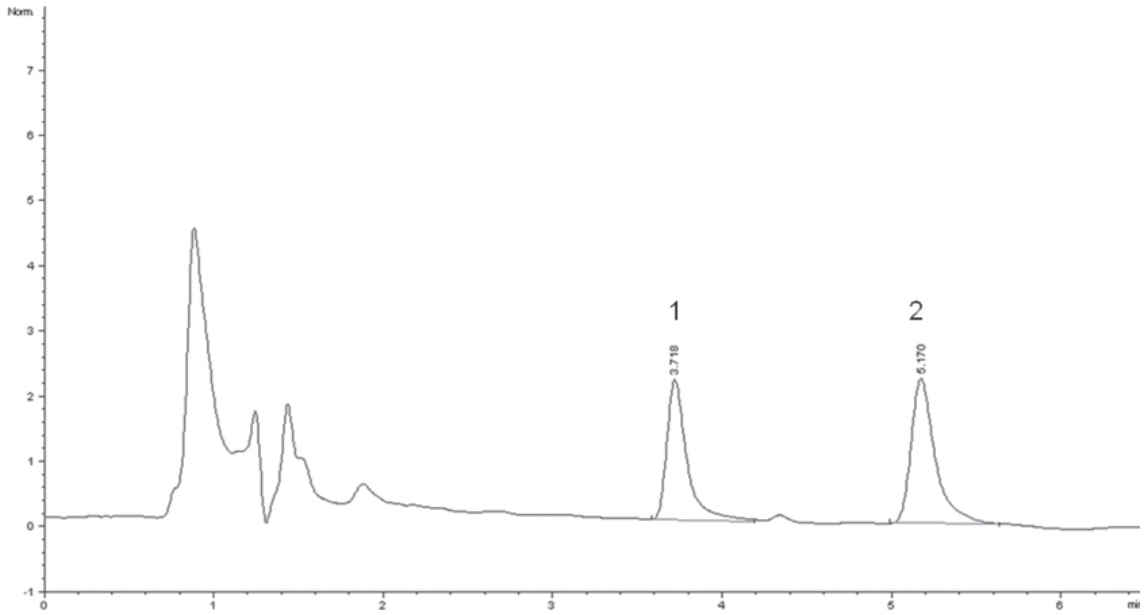


Figure 2. Chromatogram of apple juice sample spiked at 100 µg/kg (1 – Carbendazim, 2 – Thiabenzole)

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

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

Ordering information

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

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

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

To review this Application Note in its entirety, please search for 5990-3235EN at www.agilent.com/chem

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

Introduction

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

Instrument conditions

GC conditions

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

MS conditions

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

*Quantitative ion

Sample Preparation

Sample Pretreatment

Extraction:
Extract 5.0 g milk powder with 40 mL 1% TAA. Vortex mix, sonicate (15 min). Add 2 mL of 22 g/L lead acetate and make up to 50 mL with 1% TAA. Centrifuge (10 min) at 4,000 rpm

SPE Procedure

Condition/equilibrate:
3 mL methanol/5 mL H_2O

↓

Load:
5 mL milk supernatant fluid

↓

Wash:
3 mL H_2O/3 mL methanol

↓

Elute:
3 mL 5% ammonium hydroxide in methanol

↓

Evaporate:
N_2 blowing to dryness at 50 °C

↓

Derivatization:
Add 600 µl pyridine, 200 µL BSTFA, and incubate at 70 °C for 30 min

Figure 1. Scheme of sample preparation process

Results

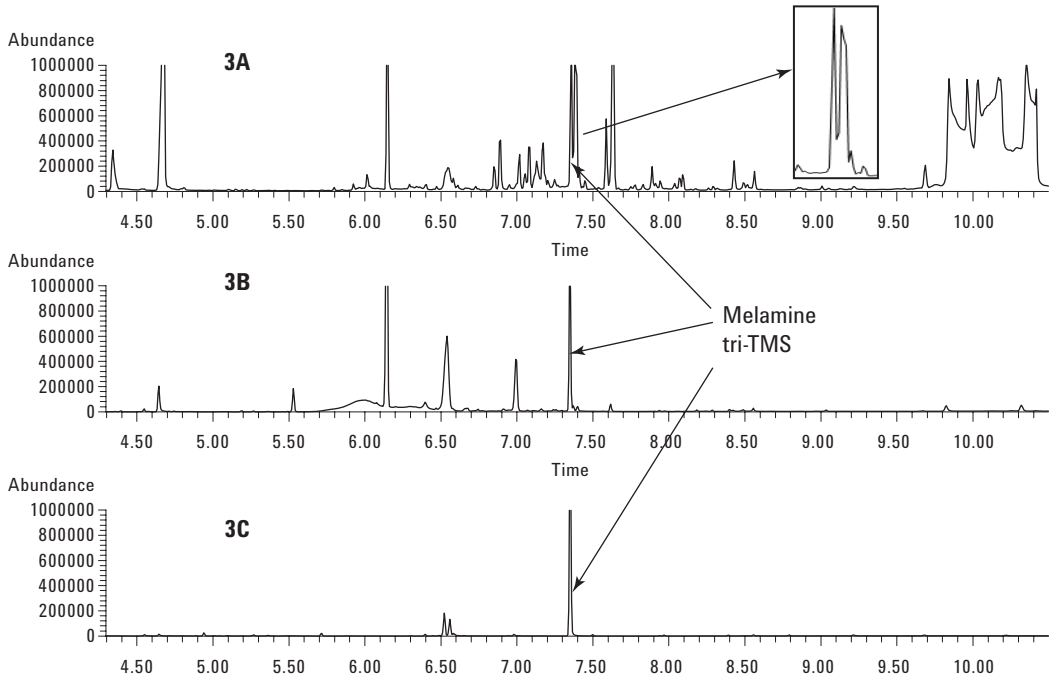


Figure 2. GC/MS SIM chromatogram of melamine tri-TMS. (3A: Sample without SPE cleanup; 3B: Sample with SPE cleanup; 3C: Standard)

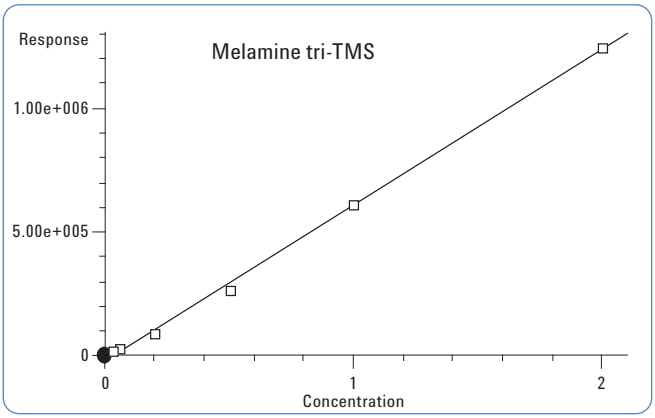


Figure 3. Calibration curve for melamine tri-TMS

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

Table 1. Recovery and repeatability of spiked samples

Ordering information

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

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

To review this Application Note in its entirety, please search for 5990-3282EN at www.agilent.com/chem

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

Introduction

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

Instrument conditions

LC conditions

Column:	Agilent ZORBAX 300-SCX Column, 2.1 mm x 150 mm, 5 µm (Part No. 883700-704)
Injection volume:	10 µL
Flow rate:	0.2 mL/min
Temperature:	40 °C
Mobile phase:	A: 10 mM NH_4 acetate/acetic acid pH adjusted to 3.0 B: ACN A:B = 20:80
Stop time:	10 min

MS conditions

Agilent 6410A LC/MS Triple Quadrupole

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

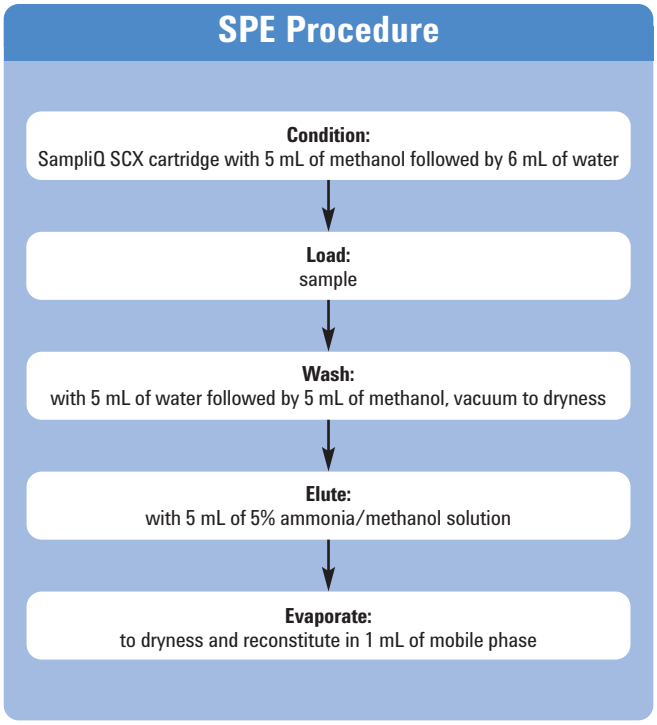


Figure 1. Scheme of sample preparation process

Results

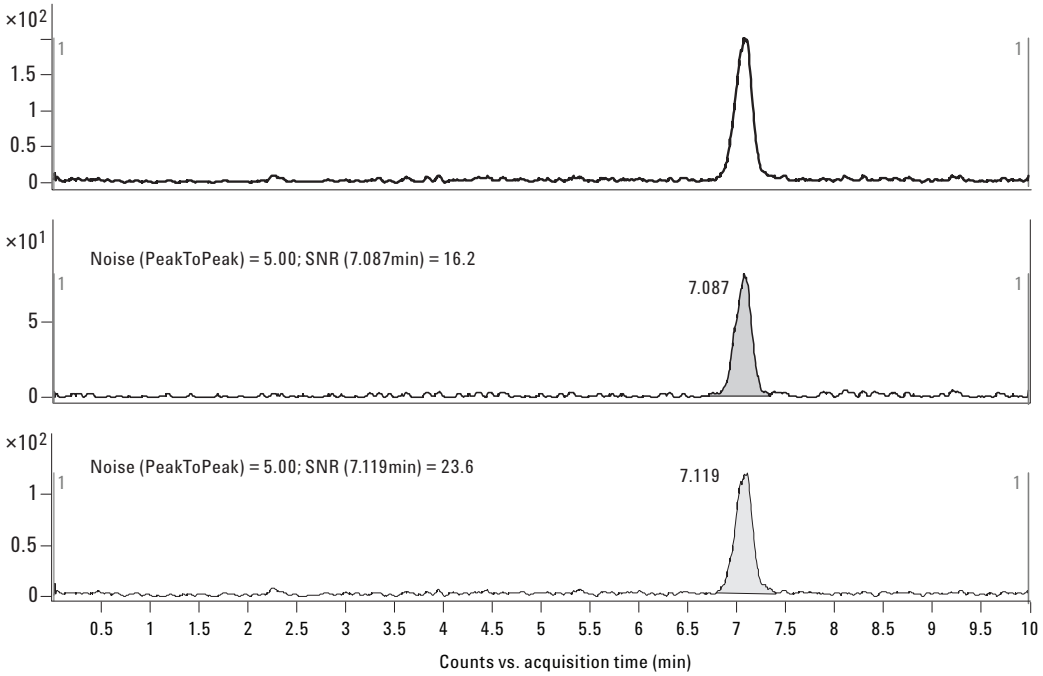


Figure 2. Response of melamine in a milk sample spiked at 1 ppb

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

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

Ordering information

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

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

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

To review this Application Note in its entirety, please search for 5990-3282EN at www.agilent.com/chem

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

Introduction

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

Instrument conditions

HPLC conditions

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

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

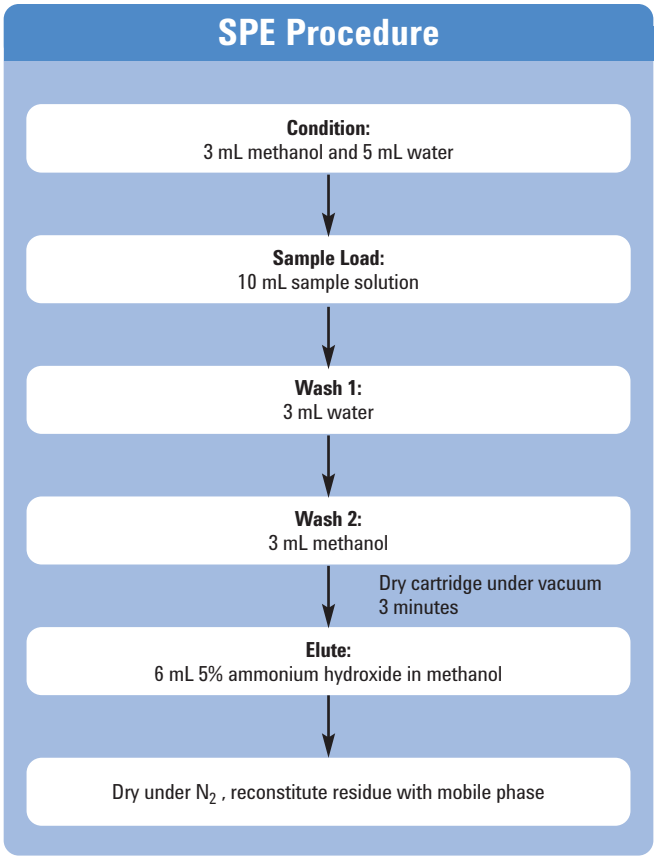


Figure 1: SPE schematic of melamine in milk and egg

Results

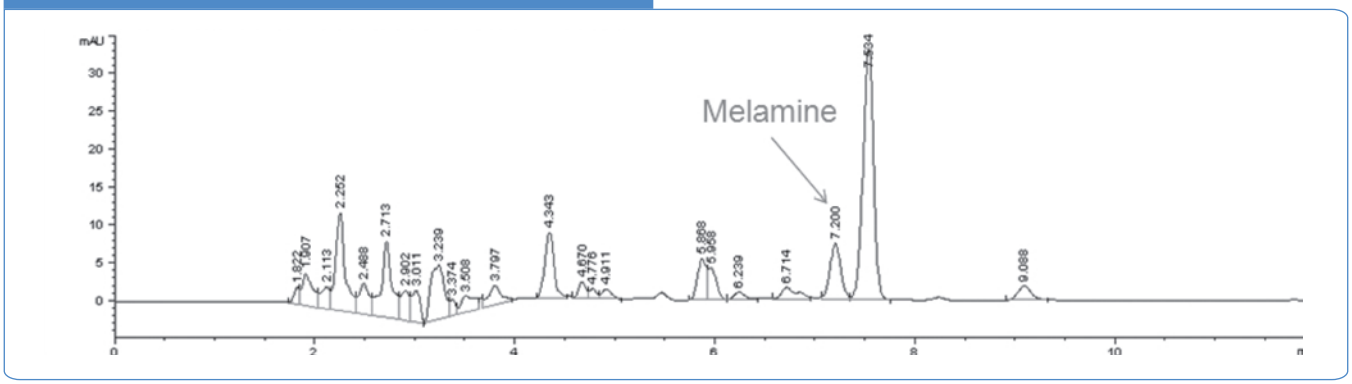


Figure 2. Chromatogram of a milk powder sample spiked at 2 mg/kg

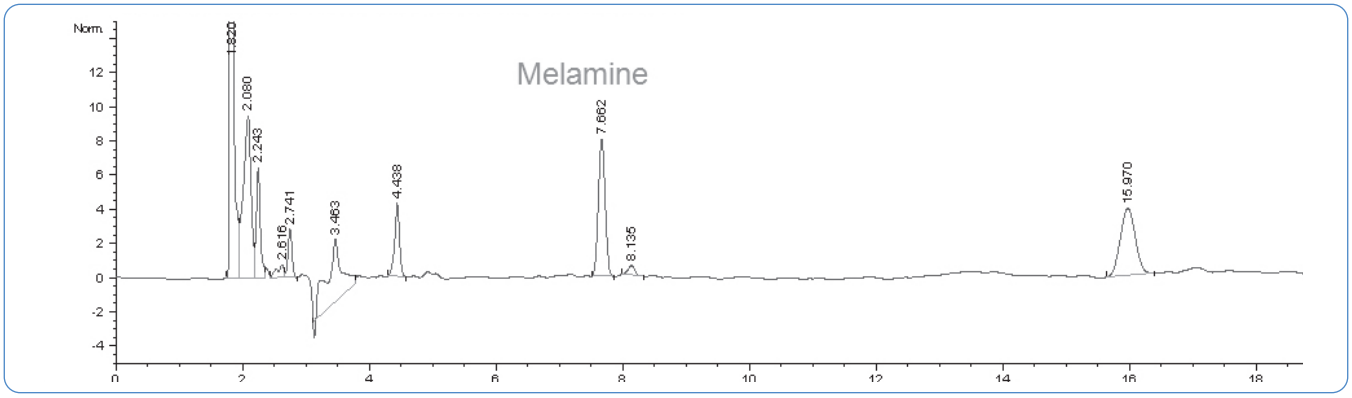


Figure 3. Chromatogram of an egg sample spiked at 2 mg/kg

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

Table 1. Linearity and LOD of melamine

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

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

Ordering information

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

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

To review this Application Note in its entirety, please search for 5990-3365EN at www.agilent.com/chem

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

Introduction

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

Instrument conditions

Conventional HPLC method using 1120 Compact LC or 1200 LC:

- Agilent 1120 Compact LC system with gradient pump (degasser inside), autosampler, Column compartment,and variable wavelength detector (VWD) or equivalent1200 Series components
- EZChrom Elite Compact software or ChemStation software (Ver. B.04.01 or later)

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

Sample Preparation

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

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

For cheese, cream, and chocolate samples:

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

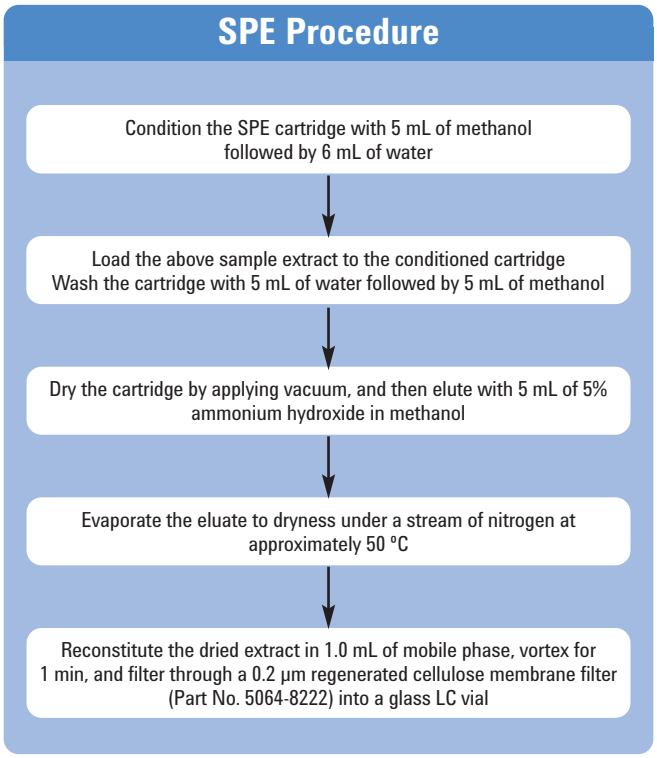


Figure 1 : SPE schematic of melamine in dairy products

Results

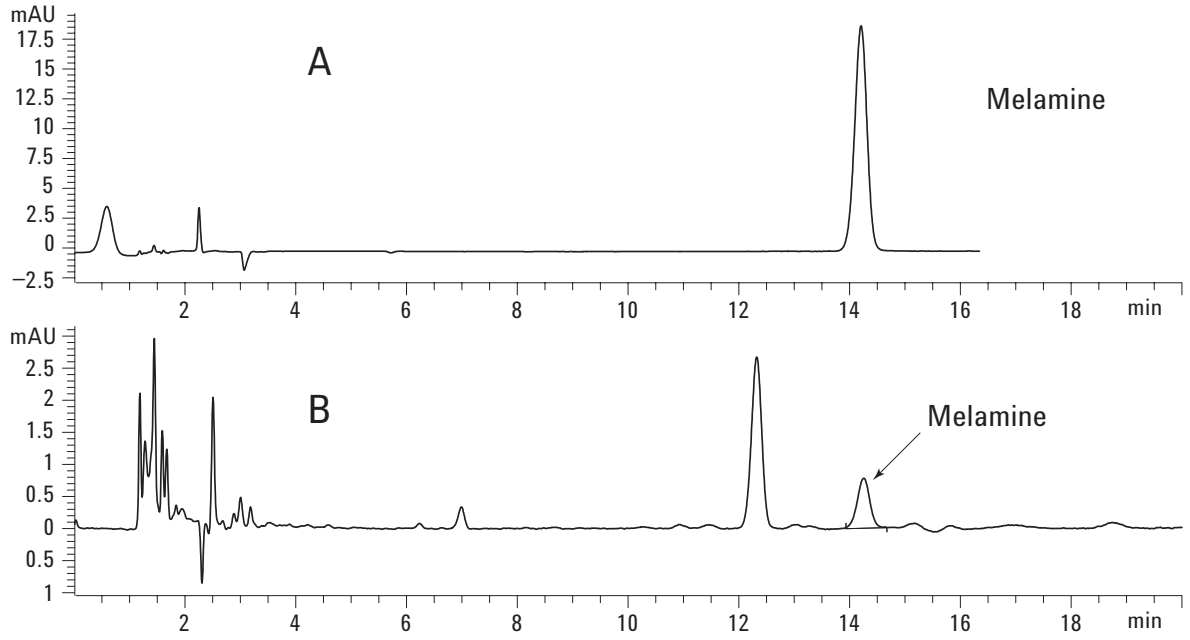


Figure 2. Separation of A: 20 µg/mL melamine standard, and B: positive yogurt sample after cleanup by SampliQ SCX SPE cartridge. Retention time of melamine is 14.2 minutes

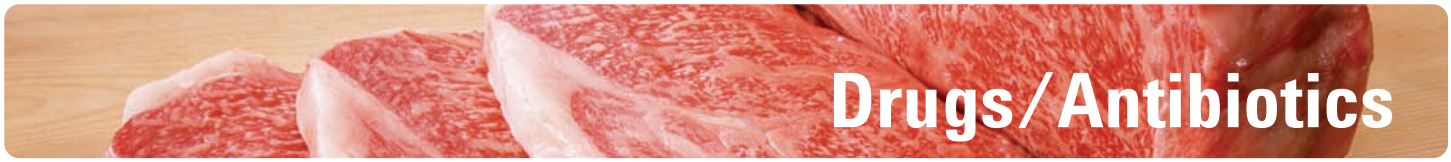
Ordering information

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

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

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

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



Drugs/Antibiotics

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

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

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

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

Introduction

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

Instrument conditions

HPLC conditions

Column:	Agilent ZORBAX Eclipse Plus C18 LC Column 250 mm x 4.6 mm, 5 µm, (Part No. 959990-902)
Flow rate:	1.0 mL/min
Injection volume:	5 µL
Column temperature:	18 °C
Detection wavelength:	230 nm
Mobile phase:	water-acetonitrile gradient

Time (minutes)	% water	% acetonitrile
0	70	30
10	65	35
23	50	50
30	20	80

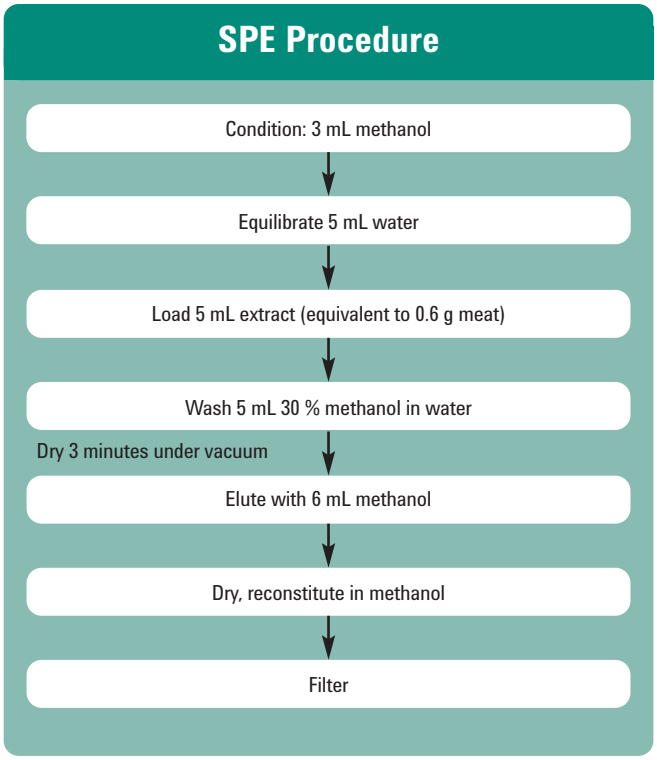


Figure 1. Hormones in crucian meat SPE procedure

Sample Pretreatment

1. Weigh 200 grams of crucian meat, homogenize, and store in a clean, sealed container at -18 °C.
2. Place 1 g of homogeneous sample (accurate to 0.01 g) into a 10 mL polypropylene centrifuge tube with 5 mL of methanol.
3. Vortex for 1 minute.
4. Extract ultrasonically for 10 minutes in an ice bath.
5. Centrifuge the sample at a speed of 4,000 rpm for 5 minutes and remove the 3 mL of supernatant.
6. Save in a clean tube and evaporate with N_2 below 40 °C.
7. Reconstitute the residue in 5 mL of 5 % methanol in water.

Results

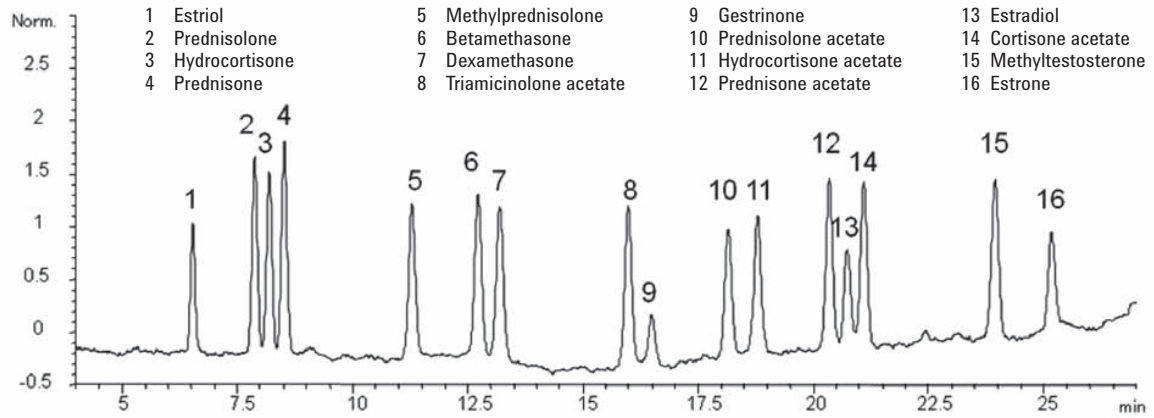


Figure 2. Chromatogram of crucian meat sample spiked hormone standards at 2 mg/kg

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

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

Ordering information

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

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

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

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

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

Introduction

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

Instrument conditions

HPLC conditions

Column:	Agilent ZORBAX SB-C8 LC Column, 250 mm x 4.6 mm, 5 µm, (Part No. 880975-906)
Flow rate:	1.5 mL/min
Column temperature:	30 °C
Injection volume:	100 µL
Detector wavelength:	350 nm
Mobile phase:	Methanol-acetonitrile-10 mM TFA solution, gradient elution

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

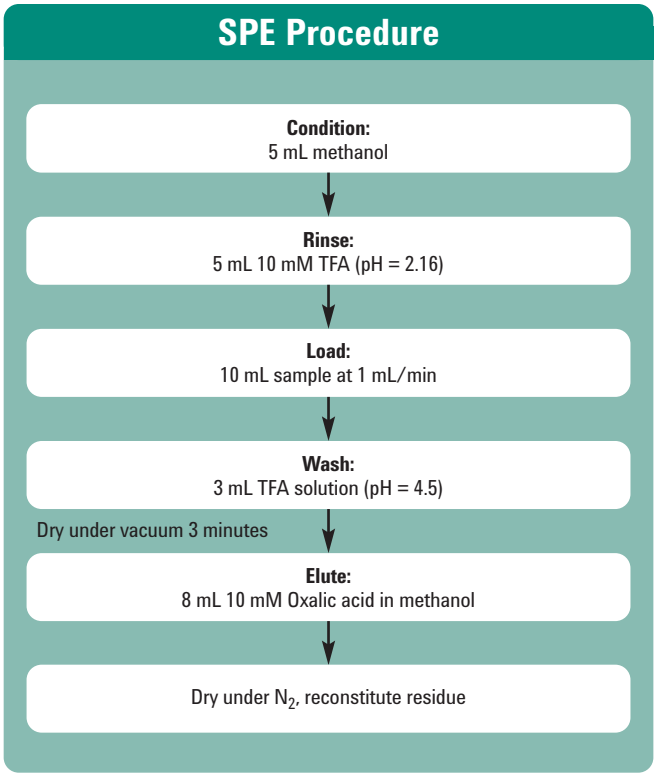


Figure 1. Tetracycline SPE procedure

Results

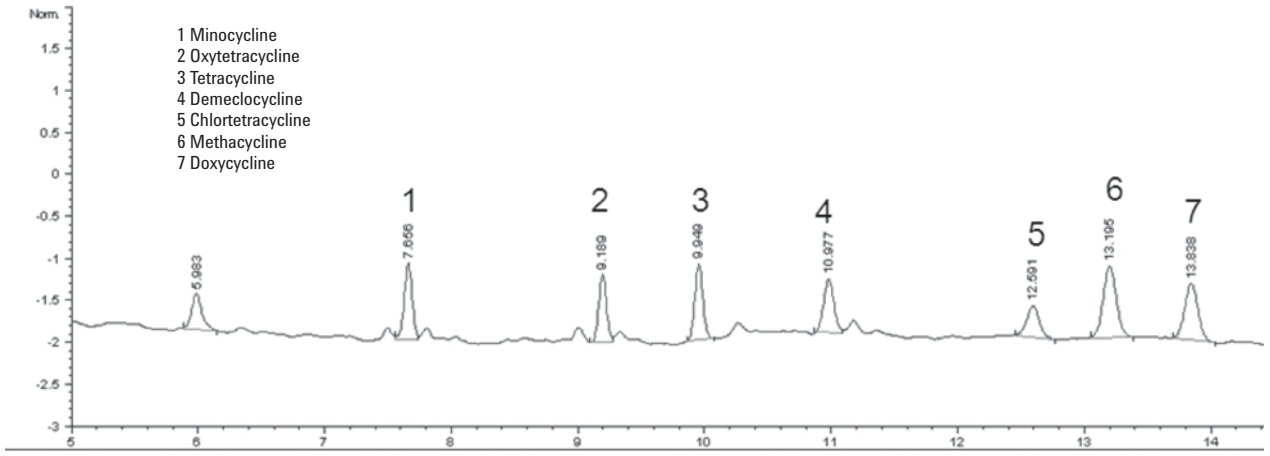


Figure 2. Chromatogram of a chicken sample spiked at 50 µg/kg

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

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

Ordering information

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

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

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

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

Introduction

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

Sample Pretreatment

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

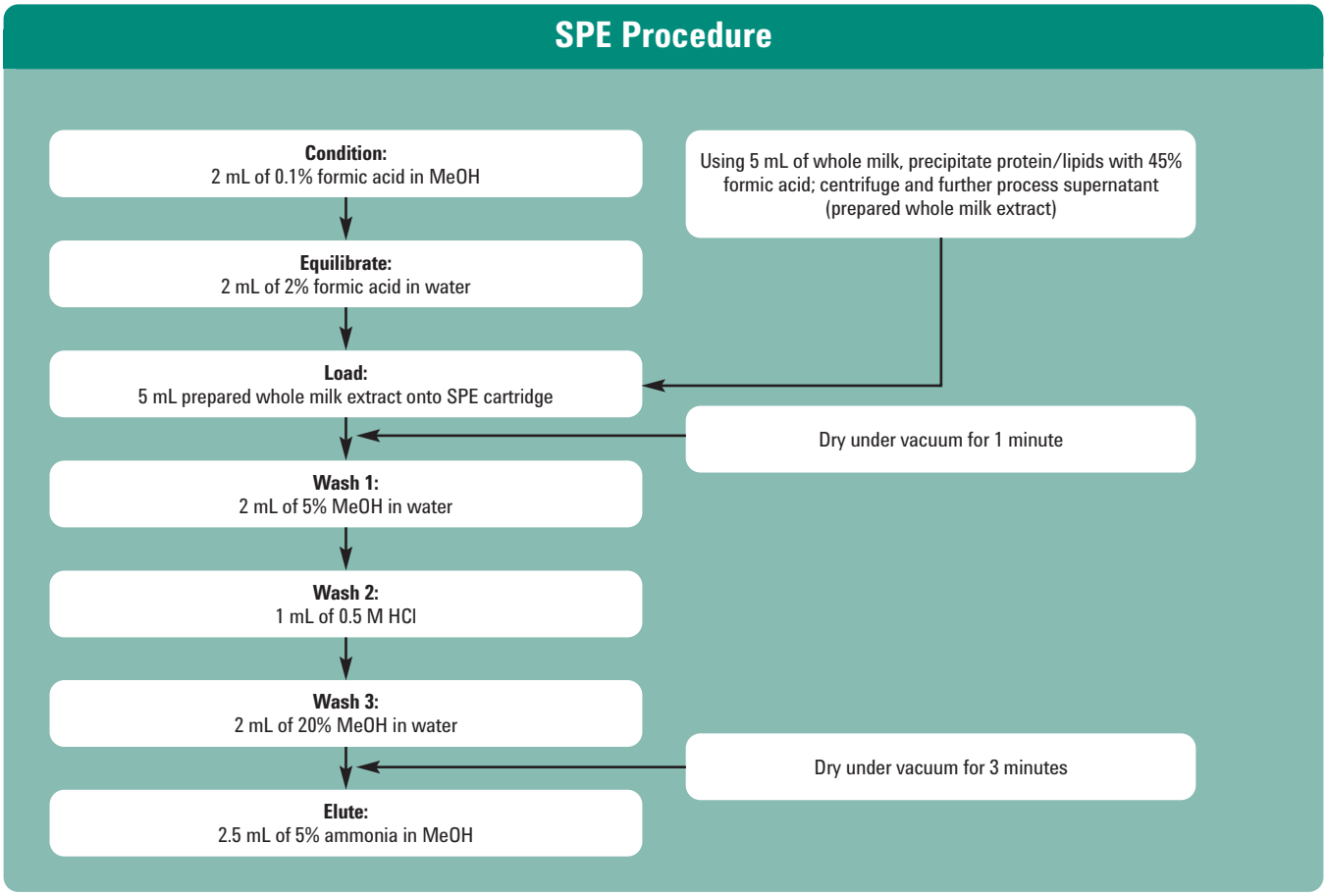


Figure 1. SPE procedure

Instrument conditions

HPLC Setup

Column:	Agilent ZORBAX Eclipse Plus C18 LC Column, 3.0 x 50 mm,1.8 µm (Part No. 959941-302)
Flow rate:	0.42 mL/min
Column temperature:	35 °C
Injection volume:	1.7 µL w/ needle wash; wash for 30 s in flush port with MeOH/H_2O (5:1)
Mobile phase:	A: H_2O/acetonitrile (9:1) w/ 0.1% formic acid B: Acetonitrile w/ 0.1% formic acid
Run time:	8 min
Post time:	3 min

Gradient:

Time	0	3.5	8
%B	0	0	65

Conditions for Electrospray Ionization Source

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

Results

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

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

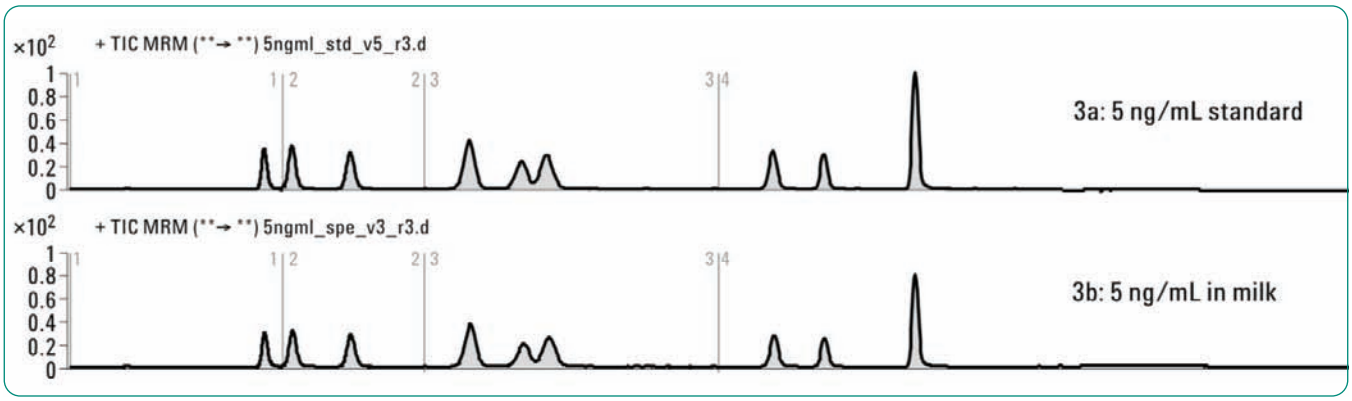


Figure 2. Total ion chromatograms of (3a) milk taken through extraction and cleanup, then spiked with sulfa drugs; (3b) milk spiked at 5 ng/mL, then taken through extraction and SPE cleanup

Ordering information

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

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

To review this Application Note in its entirety, please search for 5990-3713EN at www.agilent.com/chem

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

Introduction

A method for the simultaneous determination of three antibiotic residues of chloramphenicol (CAP), florfenicol (FF), and thiamphenicol (TAP) in honey has been developed and validated. The analytes are purified by liquid-liquid extraction and solid-phase extraction (SPE) and are quantified by liquid chromatography coupled to electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) operating in negative ion multiple reaction monitoring (MRM) mode. Chloramphenicol-D_5 is used as the internal standard. The method is validated by achieving reproducible, satisfactory, quantitative results.

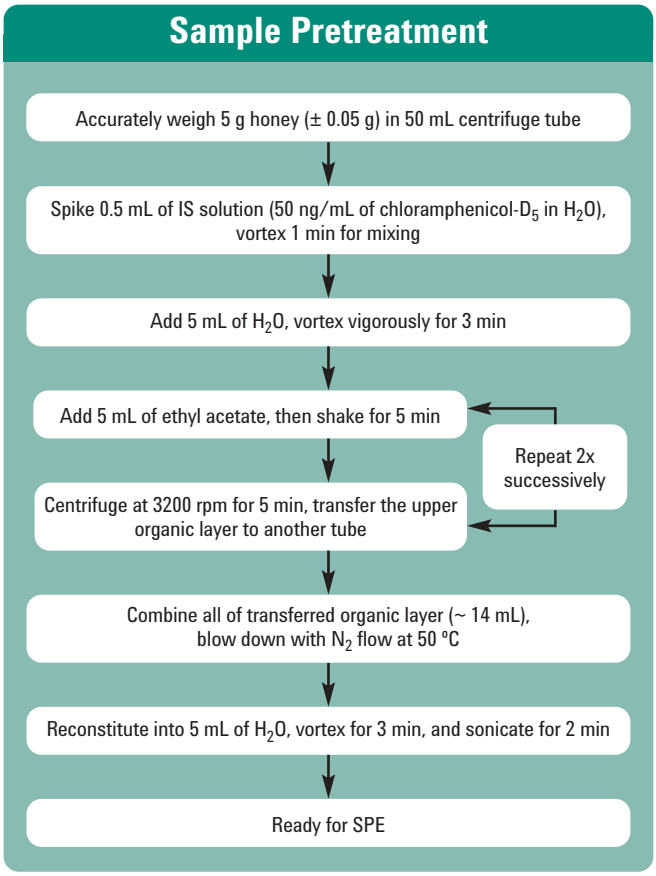


Figure 1. Sample preparation – liquid-liquid extraction of phenicols in honey

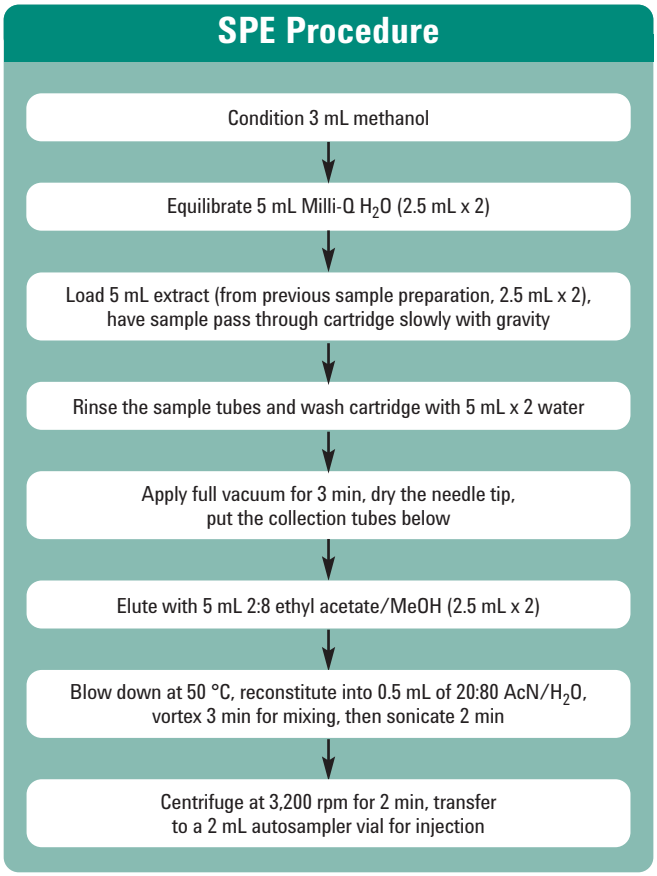


Figure 2. Sample clean-up – Agilent SampliQ solid-phase extraction

Instrument conditions

HPLC Conditions

Column:	Agilent ZORBAX Eclipse Plus LC Column 150 mm x 2.1 mm, 5 µm (Part No. 959701-906)
Flow rate:	0.3 mL/min
Column temperature:	30 °C
Injection volume:	20 µL
Mobile phase:	pH 8.5 H_2O (A), acetonitrile (B)

Gradient:

Time	% acetonitrile	Flow rate (mL/min)
0	20	0.3
0.5	20	0.3
6.0	80	0.3
6.01	100	0.5
6.50	100	0.5
6.51	20	0.3
7.00	STOP	

Results

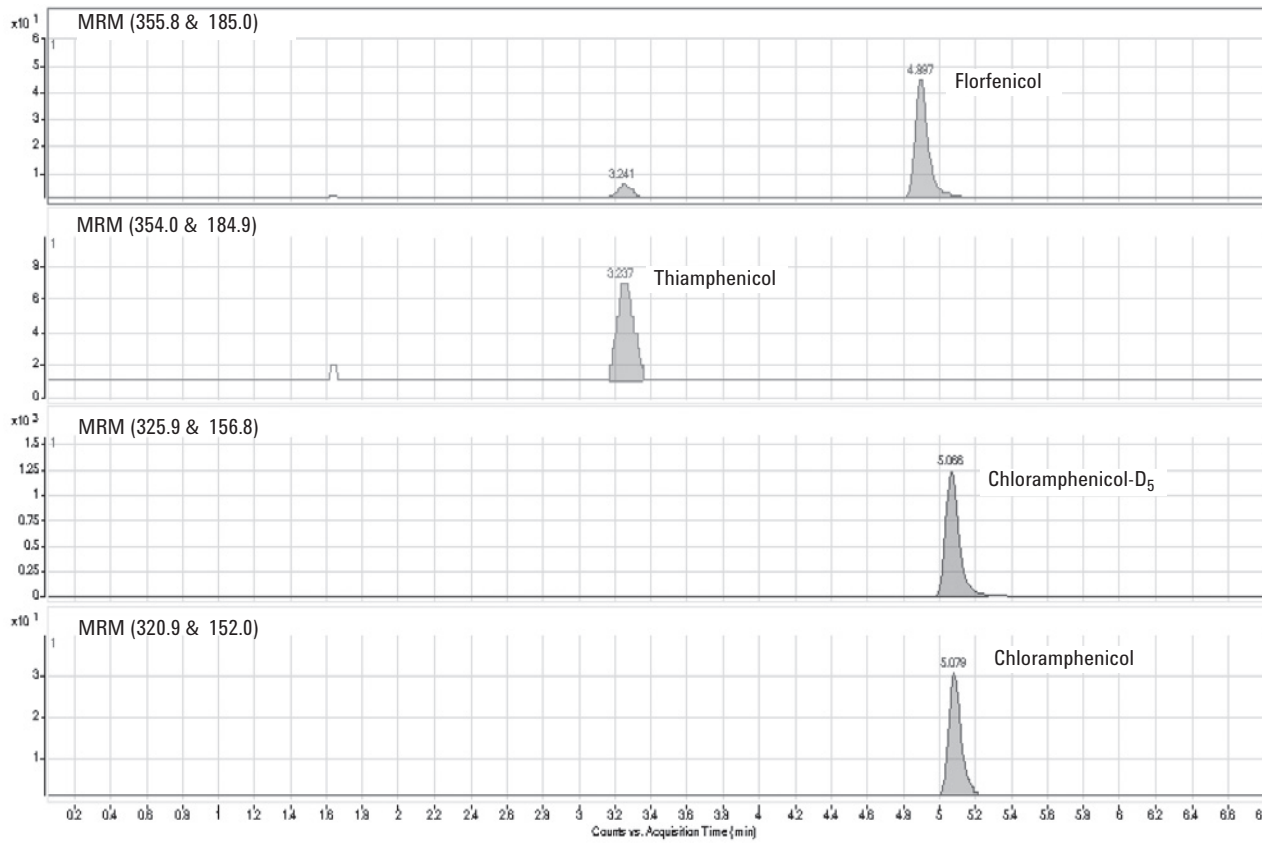


Figure 3. Chromatograms of 0.2 ng/g fortified honey extract

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

** The experiment was done in replicates of four*

Table 2. Recoveries and reproducibility of phenicols in fortified honey

Ordering information

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

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

To review this Application Note in its entirety, please search for 5990-3615EN at www.agilent.com/chem

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

Introduction

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

Instrument conditions

HPLC conditions

Column	Agilent ZORBAX Eclipse Plus LC Column, 2.1 mm x 100 mm, 3.5 µm (Part No. 959793-902)
Flow rate	0.6 mL/min
Mobile phase	A: water/10 mM ammonium acetate B: acetonitrile
Run time	12 minutes
Post run	3 minutes
Temperature	30 °C
Injection	10 µL

Gradient:

Time	% B
0	2
1.2	2
2.0	10
6.0	30
8.0	40
8.5	80
11.9	80
12.0	2

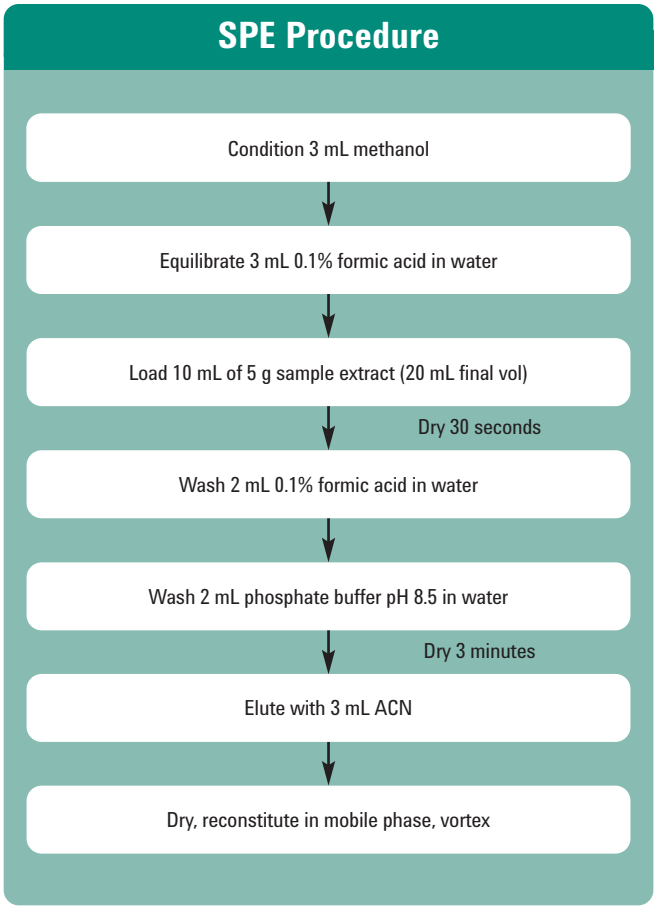


Figure 1. Agilent SampliQ OPT solid phase extraction of penicillins from pork

Results

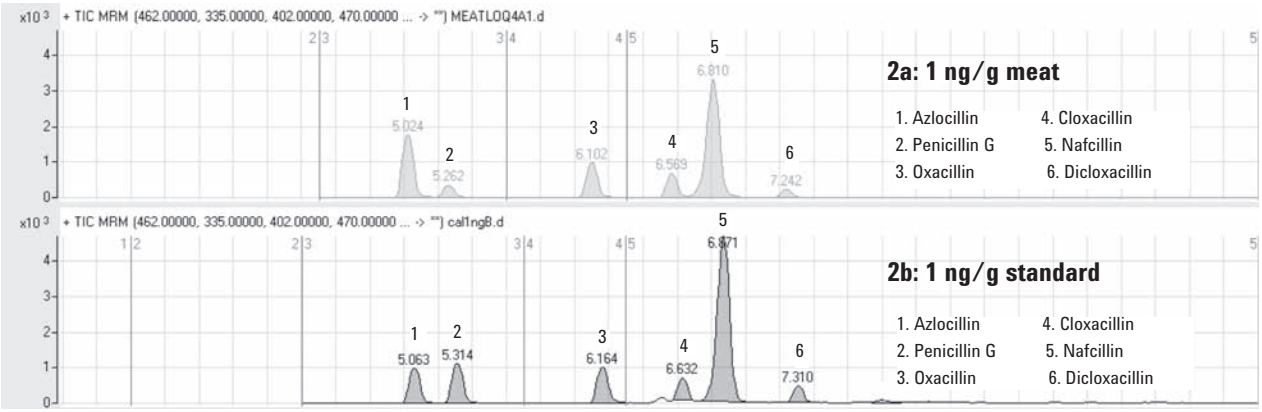


Figure 2. Meat spiked at 1 ng/g taken through extraction and SPE clean-up (2a), meat taken through extraction and clean-up then spiked at 1 ng/g (2b)

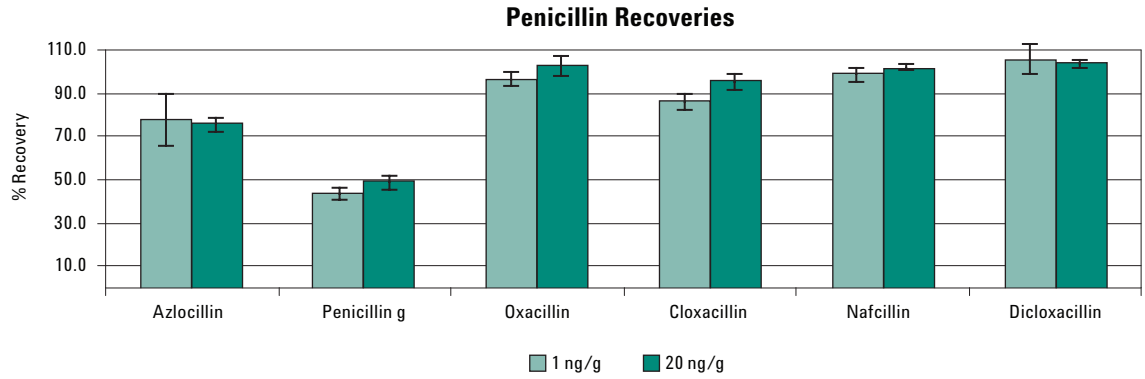


Figure 3. Recovery data for meat extracts at 1.0 and 20 ng/g

Ordering information

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

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

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

To review this Application Note in its entirety, please search for 5990-3364EN at www.agilent.com/chem

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

Introduction

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

Instrument conditions

HPLC conditions

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

MS Source settings

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

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

SPE Procedure

Extraction:

Weigh 5 g milk sample (accurate to 0.01 g) into 50 mL colorimetric tube, dissolve with 0.1 mol/L Na_2EDTA-McIlvaine buffer solution Bring volume to 50 mL

↓

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

↓

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

↓

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

↓

Filter with fast filter paper

Purification:

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

↓

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

↓

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

↓

Elute with 10 mL of 10 mmol oxalic acid in methanol

↓

Collect the eluent and dry with nitrogen below 40 °C

↓

Dissolve the residue with 1.0 mL of the initial mobile phase

↓

Filter with 0.45 µm filter membrane and inject

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

Results

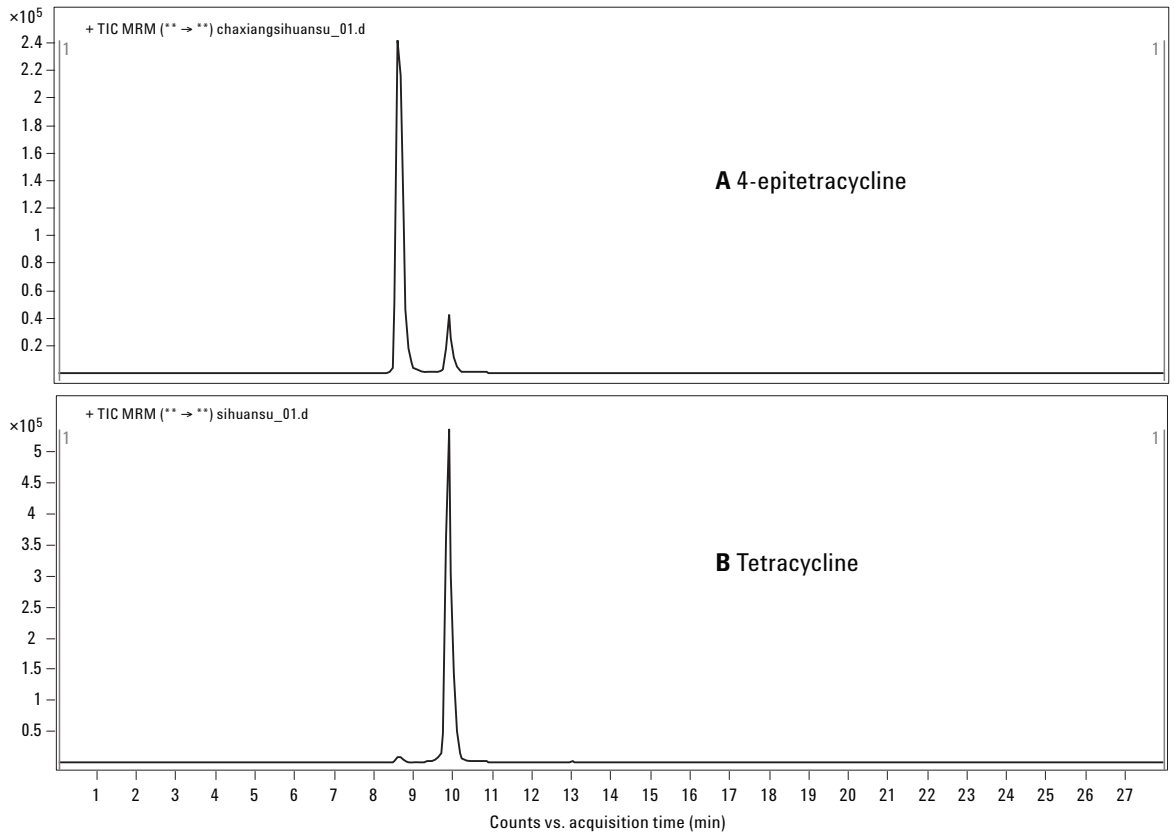


Figure 1. The separation of tetracycline and its degradation product 4-epitetracycline

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

Table 1. Recovery and repeatability in milk matrix

Ordering information

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

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

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

Determination of β2-Agonists in Pork Using Agilent SampliQ OPT Solid-Phase Extraction Cartridges and Liquid Chromatography-Tandem Mass Spectrometry (Publication 5990-4180EN)

Introduction

A method for simultaneous determination of four β2-agonist residues of terbutaline, salbutamol, clenbuterol and formoterol in pork has been developed and validated. The analytes are purified by liquid-liquid extraction (LLE) and solid-phase extraction (SPE) and quantified by liquid chromatography coupled to electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) operating in positive ion multiple reaction monitoring (MRM) mode.

Instrument conditions

HPLC conditions

Column:	Agilent ZORBAX Eclipse Plus C18 LC Column, 50 mm x 2.1 mm 1.8 µm (Part No. 959741-906)
Flow rate:	0.4 mL/min
Column temperature:	40 °C
Injection volume:	5 µL
Mobile phase:	water (0.1% FA+2 mM NH_4Ac, A), acetonitrile (0.1% FA, B)

Gradient:

Time (min)	%A	%B
0	90	10
0.5	90	10
1.8	20	80
2	90	10
3.5	90	10

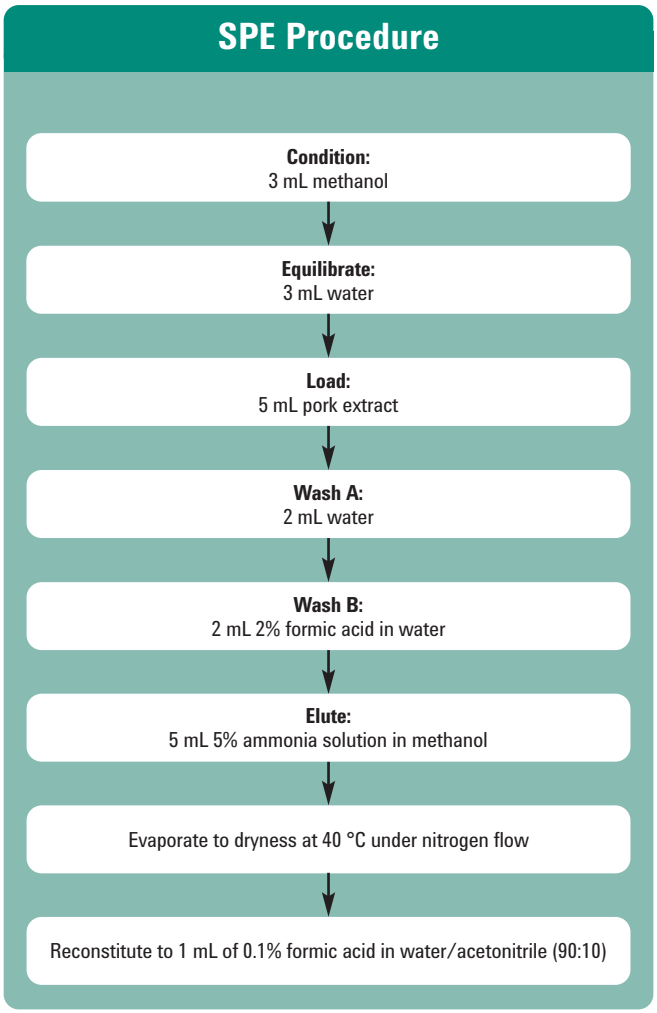


Figure 1. Pork clean up and enrichment – SPE procedure

Results

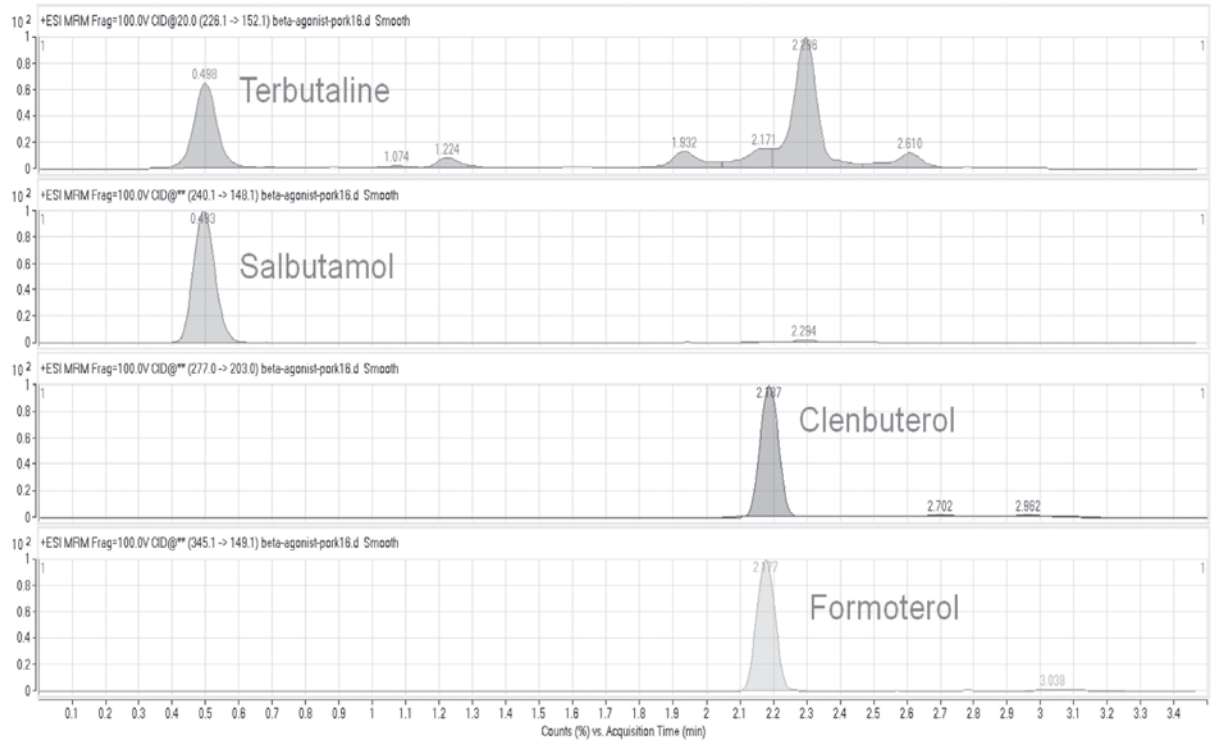


Figure 2. Chromatograms of 1.0 ng/g spiked pork sample extract

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

Table 1. Recoveries and reproducibility of β2-agonists in pork after SPE employing Agilent's SampliQ OPT; (Part No. 5982-3236), recovery 90% and RSD 4.4% on average

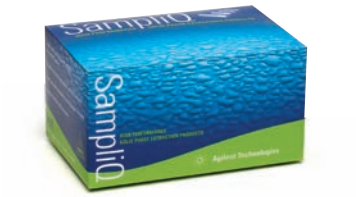
Ordering information

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

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

To review this Application Note in its entirety, please search for 5990-4180EN at www.agilent.com/chem

Simplify your sample prep and ensure quality results right from the start with Agilent SampliQ QuEChERS Kits



Agilent SampliQ QuEChERS Kits make it easier to save time and improve efficiency using QuEChERS methodologies. Unlike some QuEChERS extraction kits, SampliQ salts and buffers are pre-filled into anhydrous packages, so you can add them to your sample after the solvent, as specified by standard QuEChERS procedures.

In addition, all QuEChERS kits are part of Agilent's SampliQ family of SPE products. Manufactured in the US to strict ISO-9001 standards, SampliQ SPE products deliver the quality and performance you expect from the industry's leading manufacturer of chromatography instruments, columns, and supplies. To learn more, visit **www.agilent.com/chem/SampliQ**

To view a live demo of QuEChERS Standard Operating Procedures, visit **www.agilent.com/chem/quechersdemo**

Find out how to take your food safety analysis to the next level

Agilent SampliQ SPE Products:
www.agilent.com/chem/SampliQ

Agilent Solutions for Food Safety Testing:
www.agilent.com/chem/foodsafety

Buy Online:
www.agilent.com/chem/store

Find an Agilent center in your country:
www.agilent.com/chem/contactus

U.S. and Canada:
1-800-227-9970
agilent_inquiries@agilent.com

Europe:
info_agilent@agilent.com

Asia Pacific:
adinquiry_aplsca@agilent.com

Printed in USA November 20, 2009
5990-4917EN

Agilent Technologies