OASIS®
SAMPLE EXTRACTION PRODUCTS

Oasis PRiME HLB Food Applications Notebook

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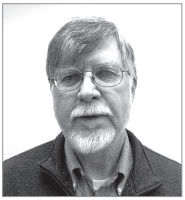

Michael S. Young, Ph.D.
Principal Applications Chemist
Waters Corporation

Dr. Young is a Principal Chemist and Applications Manager in the Scientific Operations division of Waters Corporation. He and his team of chemists develop sample preparation methodology and LC-MS and GC-MS analysis methodology for food safety and environmental applications. Among his recent applications are innovative methods for veterinary drugs, mycotoxins, pesticides and related contaminants in foodstuffs. Dr Young received his B.S. and Ph.D. in chemistry from the University of Massachusetts/Amherst. Prior to joining Waters in 1994, Dr. Young served as an GC-MS applications specialist, project manager, and laboratory manager at several private environmental testing and consulting firms. He is the author of numerous technical publications and a frequent speaker at technical meetings and symposia.

Dr. Jeremy Shia
Sr. Product Marketing Manager
Waters Corporation

Dr. Jeremy Shia is the Product Manager of Sep-Pak® and DisQuE™ sample preparation devices. He is also responsible for the Food & Environment market for the Consumables Group. Prior to this he was a Senior Applications Chemist at Waters responsible for development of analytical methods for determination of contaminants in food with focus on sample preparation. Dr. Shia had published several application notes in food analysis with subjects ranging from carbohydrate and vitamin analysis, to multi-residue analysis of veterinary drugs, pesticides, and mycotoxins in various food matrices.

Dr. Jeremy Shia has a Ph.D. degree in Chemistry from University of Massachusetts at Lowell. Before coming to Waters, he worked for at Millennium Pharmaceuticals as scientist in analytical development department. Prior to working in pharmaceutical industry, He served as chemist and organic laboratory manager in environmental laboratory.

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Dimple Shah
Senior Scientist
Waters Corporation

Dimple Shah has a M.S. degree in Organic Chemistry from St.Xavier's college, Gujarat University, India. She also has a M.S. degree in Chemistry from University of Massachusetts at Lowell. She is working at Waters Corporation, Milford as a Senior Scientist in Food and Environment market. She is responsible for development of analytical methods for determination of contaminants in food and water on LC and Mass spectrometers. Her areas of expertise are LC, tandem quadruple, high resolution mass spectrometry and various chromatographic and mass spectrometry softwares. She has published several application notes on pesticides, veterinary drugs, mycotoxins, sugars, sweeteners, vitamins, and dyes.

Kim Tran
Application Chemist II
Waters Corporation

Ms.Tran joined Waters Corporation in 1999 and is currently an Application Chemist II in the Scientific Operations division. She develops sample preparation methodology, LC-MS and GC-MS analysis methodology for food safety and environmental applications. Ms. Tran received her B.S in Biology from Chestnut Hill College. After, she worked in a laboratory at a biotechnology company for 10 years. She designed, executed, and interpreted experiments that contributed to the drug development of PGG-Glucan. She also developed Standard Operating Procedures and trained personnel in the Quality Control Department. She provided technical assistance in product development, evaluation characterization, transfer, validation and quality control testing of products.

She performed and validated bio-analytical assays to determine the concentration of the drugs and their metabolites in biological samples for clinical and pre-clinical studies by using LC-MS.

SCIENTIST BIOS

Defeng Huang
Senior Application Specialist
Waters Corporation AHQ team

Mr. Huang joined Waters in 2013,with a focus on food and environmental applications, spending the majority of his time on technical support and application development. As a graduate of Shanghai Ocean University, with a major in Food science and engineering, include theory and practice experience, Mr. Huang brings over 10 years of experience focusing on Food & Environment analysis, sample preparation, SPE knowledge and application, specifically on LC-MS/MS application. When he is not in the lab, Mr. Huang enjoys reading, swimming, as well as cheering on his favorite soccer team Manchester United.

INTRODUCTION

Oasis PRiME HLB Food Applications and Technical Briefs

When testing for different classes of contaminants, food safety and testing laboratories must develop sensitive and robust analytical methods for a variety of complex food matrices. Historically, scientists have attempted to reduce matrix effects by removing interferences such as lipids, phospholipids, and pigments from the extracts by using traditional solid phase extraction (SPE) sorbents such as silic-based C18. While traditional SPE products have been successful at removing fats, it becomes very challenging to remove multiple major interferences from a complex sample matrix.

Oasis PRiME HLB has recently been used to quickly and efficiently remove all major interferences including fats and phospholipids, as well as pigments from food matrices, using the simple and fast pass-through protocol. This enables Oasis PRiME HLB to be used as a powerful matrix removal tool for multi-residue analysis. Food contaminants, such as veterinary drugs, pesticides, mycotoxins, or other compounds pass through the sorbent without retention, while the Oasis PRiME HLB holds back interferences leading to matrix effects reduction. In this booklet, we have collected application notes to demonstrate how Oasis PRiME HLB cleans up challenging food samples, resulting in better accuracy, system uptime and method robustness.

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Oasis PRiME HLB Cartridge for Effective Cleanup of Meat Extracts Prior to Multi-Residue Veterinary Drug UPLC-MS Analysis

Michael S. Young and Kim Tran

Fats and phospholipids are significant potential instrument and column contaminants. Oasis PRiME HLB Cartridges provide a rapid cleanup to remove these substances from meat extracts prior to LC-MS analysis.

GOAL

To demonstrate the effectiveness of the Oasis® PRiME HLB Cartridge for cleanup of meat extracts prior to UPLC®-MS analysis.

BACKGROUND

Waters has developed an optimized sample preparation and analysis protocol for multi-class, multi-residue LC-MS/MS screening of veterinary drug residues in meat. The major constituents of a typical meat sample are water (up to 70%), protein (15–25%), fat (5–25%) and phospholipid (1–3%). During the sample pre-extraction, the protein is removed from the extract by precipitation and centrifugation. However, significant amounts of fat and phospholipid are co-extracted along with the target veterinary drugs. The presence of these co-extracted substances can lead to interference in the LC-MS analysis, contamination of the analytical column and other components of the UPLC System, and contamination of the mass spectrometer itself. Fats have traditionally been removed from meat extracts using cumbersome hexane defatting steps or by the use of reversed-phase sorbents such as C_{18}- silica. Although these techniques may be effective for fat removal, neither of these procedures removes phospholipids.

THE SOLUTION

Pass-through cleanup with the Oasis PRiME HLB Cartridge. This procedure is highly effective for removal of both fat and phospholipid from meat extracts. Just as important, the recoveries of the veterinary drugs are not compromised with Oasis PRiME HLB Cartridge cleanup. The recoveries are similar to those obtained using hexane defatting or C_{18}-silica cleanup but Oasis PRiME HLB Cartridge cleanup is more effective.

EXPERIMENTAL

Initial Extraction. Typical pork samples (5 g, 15% fat) were fortified with representative compounds chosen from major classes of veterinary drugs. The homogenized meat samples were extracted with 10 mL of 80:20 acetonitrile/water with 0.2% formic acid. The samples were vortexed for 30 seconds, shaken for 30 minutes, and then centrifuged at 12000 rpm for 5 minutes.

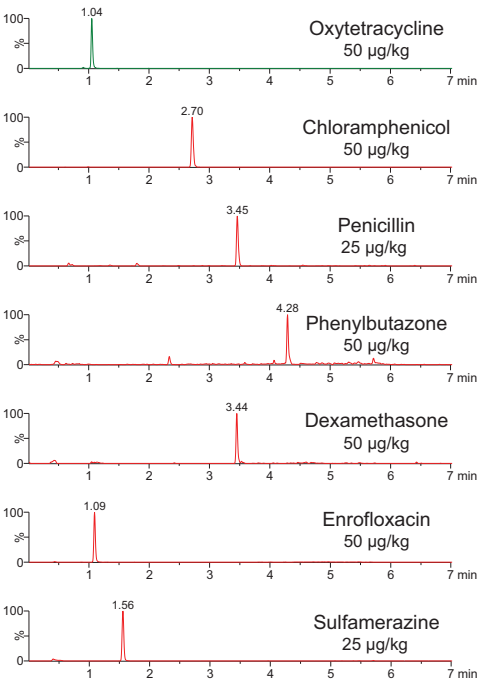
Oasis PRiME HLB Cartridge Cleanup. An Oasis PRiME HLB Cartridge (3 cc, 60 mg) was mounted on a pre-cleaned vacuum manifold. No cartridge conditioning is required or was performed. A 0.5 mL aliquot of the supernatant was passed-through the Oasis PRiME HLB Cartridge and collected. The collected sample was diluted three-fold with aqueous 10 mM ammonium formate buffer (pH 4.5) prior to UPLC-MS/MS analysis.

RESULTS

Little or no recovery loss was observed in the pass-through cleanup step for any of the tested compounds. Absolute recoveries (measuring mostly the effectiveness of the initial liquid extraction) averaged over 80% for the tested compounds except for phenylbutazone (32%). These recoveries are consistent with C_{18}-silica cleanup but no phospholipids are removed with C_{18}-silica. UPLC-MS/MS conditions and chromatograms are presented in Figure 1. Figure 2 shows chromatograms that illustrate the effectiveness of the Oasis PRiME HLB Cartridge for phospholipid removal; greater than 90% more phospholipid is removed compared with C_{18}-silica cleanup. Using gravimetric analysis it was also determined that the Oasis PRiME HLB Cartridge removed more than 90% of the co-extracted fat from the pork extract.

UPLC Conditions

Instrument:	ACQUITY UPLC® I-Class
Column:	1.7 µm CSH C_{18}, 2.1 x 100 mm (p/n 186005297)
Column temp.:	30 °C
Sample temp.:	10 °C
Detection:	MS:TQ-S
Injection vol.:	5 µL for 3 cc and 6 cc
Run time (flow rate):	7 minutes (0.04 mL/min)
Mobile phase A:	Water with 0.1% formic acid
Mobile phase B:	ACN with 0.1% formic acid
Gradient:	15% B initial to 40% B at 2.5 min, to 95% B at 3.9 min, hold to 4.9 min, back to 15% B at 5.0 min and hold to 7.0 min
Needle:	Standard needle for UPLC, 15
Weak wash (solvent 3 and 6 cc):	10/90 ACN:water
Strong wash (solvent 3 and 6 cc):	50/30/20 water:ACN:IPA
Seal wash:	10% MeOH



Compound	Precursor ion (*m/z*)	Product ion (*m/z*)	Cone voltage (V)	Collision energy (eV)
Sulfamerazine	265.0	156.0	30	15
Enrofloxacin	360.2	316.1	50	25
Dexamethasone	393.2	355.3	30	15
Phenylbutazone	309.4	160.0	37	20
Penicillin	335.1	160.0	20	30
Chloramphenicol*	321.1	152.1	30	17
Oxytetracycline	461.2	426.2	30	21

**Chloramphenicol ESI-, others ESI+ ionization*

Figure 1. UPLC-MS/MS conditions and resulting chromatograms from a typical pork sample spiked at the levels indicated.

CONCLUSIONS

- Oasis PRiME HLB Cartridges did not require conditioning or equilibration prior to use; a simple one-step SPE cleanup was effective.
- Oasis PRiME HLB Cartridges removed greater than 90% of fats and greater than 90% of phospholipids from acetonitrile based extracts of pork.
- When used in the pass-through mode, Oasis PRiME HLB Cartridges did not affect the recovery of the test compounds but gave significant removal of fats and phospholipids from the extract.

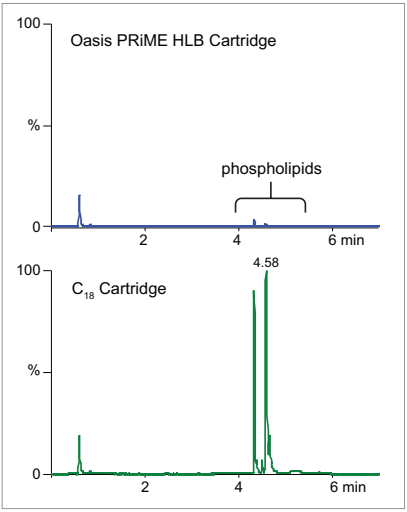


Figure 2. Removal of phospholipids from meat extracts comparing Oasis PRiME HLB cleanup (upper trace) with C_{18}-silica cleanup (lower trace).

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A Simple Cleanup Protocol Using a Novel SPE Device for UPLC-MS/MS Analysis of Multi-Residue Veterinary Drugs in Milk

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APPLICATION BENEFITS

- Enable simultaneously determination of multi-class of veterinary drugs using an innovative solid phase extraction device.
- Simple, fast, pass-through SPE cleanup prior to UPLC-MS/MS analysis.
- The matrix interference from fatty/non-polar materials and phospholipids are removed together in one straightforward SPE cleanup for longer column life and less maintenance of the mass spectrometer.

WATERS SOLUTIONS

ACQUITY UPLC® I-Class System

Xevo® TQ-S Mass Spectrometer

ACQUITY UPLC BEH C_{18} Column

Oasis® PRiME HLB 3 cc 60 mg cartridges

TruView™ LCMS Certified Vials

MassLynx® v4.1 data system with Quanpedia™ database

KEY WORDS

Oasis PRiME HLB, multi-residue, veterinary drug, SPE, milk, UPLC-MS/MS

SUMMARY

In this experiment a new solid phase extraction (SPE) device, the Oasis PRiME HLB Cartridge, was used in the sample preparation of milk samples as a cleanup method for multi-residue veterinary drug analysis. The initial extraction and protein precipitation was done by adding acidified acetonitrile. The extract was cleaned up by pass-thru SPE using the Oasis PRiME HLB Cartridge prior to UPLC-MS/MS analysis. Sample extraction, chromatographic and mass parameters were all optimized. As a result, within the ranges of 0.1 to 10.0 µg/mL spiking concentrations, 9 classes of 72 veterinary drugs including sulfonamides, fluoroquinolones, β-agonists, macrolides, glucocorticoids, amphenicols, β-lactams, cephalosporins, penicillin, and tetracyclines, the percent recoveries are all within 50% to 130%, and the RSD <20% (n=5). This method is simple, rapid, and accurate, suitable for multi-residue veterinary drug analysis of milk.

INTRODUCTION

Many veterinary drugs are used to treat animals grown for human consumption. The presence of excessive amounts of drug residues in animal products such as milk may represent a health hazard. Therefore, effective and reliable analytical methods are required to identify and quantify drug residues in animal products. Among the frequently used veterinary drugs in the animal farms are sulfonamides, fluoroquinolones, β-agonists, macrolides, glucocorticoids, amphenicols, β-lactams, cephalosporins, and tetracyclines. Drug residues in an animal's bloodstream can be introduced into the milk of lactating animals and eventually transferred to humans by consumption of the milk. Among the consequences are possible allergic reactions and the induced side effect of drug resistance. Therefore the monitoring of residual veterinary drugs of milk plays a significant role in the assurance of food safety of dairy products. Currently the published methods from official agencies and literatures are individual methods based on individual classes of compounds.

The goal of combining these individual methods into one multi-class method is difficult to accomplish due to the unavailability of a robust universal sample preparation procedure. Creating one single LC-MS/MS instrumental method for multiple classes of veterinary drugs poses certain degree of challenge as well.

EXPERIMENTAL

UPLC Conditions

System:	ACQUITY UPLC I-Class
Column:	ACQUITY UPLC BEH C_{18}, 1.7 µm, 2.1 x 100 mm
Injection volume:	5 µL
Temperature:	45 °C
Mobile phase A:	10 mM ammonium acetate in water (pH 5.0)
Mobile phase B:	10 mM ammonium acetate in methanol
Flow rate:	0.45 mL/min
Gradient:	2 %B initial and hold to 0.25 minutes, linear gradient to 99 %B at 12.25 minutes, hold to 13.0 minutes, back to 2 %B at 13.01 minutes, hold and re-equilibrate until 17 minutes

MS conditions for UPLC

Instrument:	Xevo TQ-S
Mode:	Electrospray (ES+ and ES-)
Capillary:	3.5 kV
Source temp.:	150 °C
Cone gas:	150 L/hr
Desolvation temp.:	600 °C
Desolvation gas:	1000 L/hr
Collision gas (Argon):	0.15 mL/min

UPLC-MS/MS cone and collision parameters, as well as MRM transitions used for this study are presented in Table 1.

This method utilizes Waters® new and novel Oasis PRiME HLB Solid Phase Extraction Device. This new SPE can retain the majority of phospholipids and fats in milk. By combining with protein precipitation technique, it can effectively remove most interference from the milk matrix.

Using the Xevo TQ-S System and including the veterinary drug analysis parameters in the Quanpedia Database establishes a highly efficient total solution for the multi-residue analysis of veterinary drugs in milk.

SAMPLE PREPARATION

Sample extraction:
In 1 mL of milk, add 4 mL of 0.2% formic acid (FA) in acetonitrile (ACN), mix well. Centrifuge for 5 min at 10,000 rpm. Aliquots of the supernatant are used for SPE cleanup.

Solid phase extraction (SPE) cleanup:
Prepare the 3 cc Oasis PRiME HLB Cartridge (p/n 186008056) by passing through 3 mL 0.2% FA in ACN. Note: this conditioning step is only required to facilitate subsequent gravity loading, it is not necessary if a sample is processed with minimal vacuum.

Pass the supernatant through the cartridge and collect. Evaporate to dryness under a gentle nitrogen stream. Reconstitute the solution in 1 mL 5% methanol in water. Filter the extract and transfer to a vial for UPLC-MS/MS analysis.

Name	Ion Mode	Precursor (*m/z*)	CV (V)	Product (*m/z*)	CE (V)	RT (min)
Cimaterol	ESI+	220.1	25	143.0	24	2.2
	ESI+	220.1	25	160.1	15	
Clenbuterol	ESI+	277.1	25	140.0	46	4.65
	ESI+	277.1	25	168.1	25	
Ractopamine	ESI+	302.2	25	164.1	15	4.21
	ESI+	302.2	25	284.2	12	
Salbutamol	ESI+	240.2	25	148.1	20	2.46
	ESI+	240.2	25	222.1	12	
Terbutaline	ESI+	226.1	25	107.0	26	2.33
	ESI+	226.1	25	125.0	26	
Tulobuterol	ESI+	228.2	30	118.0	25	5.11
	ESI+	228.2	30	154.1	15	
Zilpaterol	ESI+	262.2	25	185.1	22	2.41
	ESI+	262.2	25	202.1	18	
Clindamycin	ESI+	425.2	20	125.9	25	7.52
	ESI+	425.2	20	377.2	18	
Erythromycin	ESI+	734.5	30	158.1	30	7.74
	ESI+	734.5	30	576.5	20	
Kitasamycin	ESI+	786.4	20	108.9	35	
	ESI+	786.4	20	174.0	30	
Lincomycin	ESI+	407.4	40	126.2	25	3.86
	ESI+	407.4	40	359.4	20	
Spiramycin	ESI+	422.2	30	101.0	20	6.36
	ESI+	422.2	30	174.1	20	
Tilmicosin	ESI+	869.5	25	174.2	45	7.17
	ESI+	869.5	25	696.5	40	
Tylosin	ESI+	916.5	60	101.1	45	7.89
	ESI+	916.5	60	174.1	40	
Oxytetracycline	ESI+	460.7	34	426.2	18	4.28
	ESI+	460.7	34	444.2	18	
Tetracycline	ESI+	444.7	30	410.3	18	4.16
	ESI+	444.7	30	427.3	14	
Sulfabenzamide	ESI+	277.1	30	92.0	25	3.61
	ESI+	277.1	30	156.0	15	
Sulfachlorpyridazine	ESI+	285.0	20	92.0	28	3.77
	ESI+	285.0	20	155.9	15	
Sulfaclozine	ESI+	285.0	20	92.0	28	4.61
	ESI+	285.0	20	155.9	15	
Sulfadiazine	ESI+	251.0	30	92.0	27	2.34
	ESI+	251.0	30	156.0	15	
Sulfadimethoxine	ESI+	311.1	36	92.0	32	5.2
	ESI+	311.1	36	156.0	20	
Sulfaguanidine	ESI+	215.0	20	91.8	22	0.96
	ESI+	215.0	20	156.0	13	
Sulfamerazine	ESI+	265.1	35	92.0	25	2.99
	ESI+	265.1	35	156.0	15	

Table. 1 Analyte MS parameters and retention time.

Name	Ion Mode	Precursor (*m/z*)	CV (V)	Product (*m/z*)	CE (V)	RT (min)
Sulfameter	ESI+	281.0	20	91.8	27	3.34
	ESI+	281.0	20	155.9	15	
Sulfamethazine	ESI+	279.1	35	124.1	25	3.61
	ESI+	279.1	35	186.0	15	
Sulfamethizole	ESI+	271.1	30	92.0	25	3.31
	ESI+	271.1	30	156.0	15	
Sulfamethoxazole	ESI+	254.1	30	92.0	25	3.92
	ESI+	254.1	30	156.0	15	
Sulfamethoxypyridazine	ESI+	281.1	35	92.0	25	4.03
	ESI+	281.1	35	156.0	15	
Sulfamonomethoxine	ESI+	281.0	35	92.0	35	3.7
	ESI+	281.0	35	156.0	22	
Sulfamoxol	ESI+	268.0	20	91.8	26	3.47
	ESI+	268.0	20	155.9	15	
Sulfanilacetamide	ESI+	215.0	20	91.8	22	1.71
	ESI+	215.0	20	156.0	13	
Sulfaphenazole	ESI+	315.0	20	108.0	25	4.85
	ESI+	315.0	20	156.0	18	
Sulfapyridine	ESI+	250.0	33	108.0	25	2.87
	ESI+	250.0	33	156.0	16	
Sulfaquinoxaline	ESI+	301.1	32	92.2	30	5.38
	ESI+	301.1	32	156.1	16	
Sulfapyridine	ESI+	250.0	33	108.0	25	2.87
	ESI+	250.0	33	156.0	16	
Sulfaquinoxaline	ESI+	301.1	32	92.2	30	5.38
	ESI+	301.1	32	156.1	16	
Sulfathiazole	ESI+	256.0	31	92.0	25	2.68
	ESI+	256.0	31	156.0	15	
Sulfisomidine	ESI+	279.1	20	123.9	20	2.54
	ESI+	279.1	20	186.0	15	
Sulfisoxazole	ESI+	268.0	30	92.0	28	3.91
	ESI+	268.0	30	156.0	13	
Trimethoprim	ESI+	291.3	40	123.0	30	3.79
	ESI+	291.3	40	230.2	30	
Cinoxacin	ESI+	263.2	35	189.1	30	4.33
	ESI+	263.2	35	245.1	15	
Ciprofloxacin	ESI+	332.1	42	288.1	18	4.13
	ESI+	332.1	42	314.1	22	
Danofloxacin	ESI+	358.2	38	96.0	25	4.31
	ESI+	358.2	38	314.1	20	
Difloxacin	ESI+	400.3	30	356.2	20	5.39
	ESI+	400.3	30	382.2	20	
Enoxacin	ESI+	321.1	40	232.0	30	3.86
	ESI+	321.1	40	303.1	35	
Enrofloxacin	ESI+	360.3	25	316.3	20	4.85
	ESI+	360.3	25	342.3	20	

Table. 1 Analyte MS parameters and retention time.

Name	Ion Mode	Precursor (*m/z*)	CV (V)	Product (*m/z*)	CE (V)	RT (min)
Flumequine	ESI+	262.1	35	202.0	35	6.67
	ESI+	262.1	35	244.0	15	
Lomefloxacin	ESI+	352.1	39	265.1	22	4.32
	ESI+	352.1	39	308.1	16	
Marbofloxacin	ESI+	363.1	35	72.0	20	3.69
	ESI+	363.1	35	320.0	15	
Nalidixic acid	ESI+	233.1	30	187.0	25	6.33
	ESI+	233.1	30	215.0	15	
Norfloxacin	ESI+	320.1	40	233.0	25	4.00
	ESI+	320.1	40	276.1	20	
Ofloxacin	ESI+	362.3	25	261.3	30	3.99
	ESI+	362.3	25	318.3	20	
Orbifloxacin	ESI+	396.1	40	295.1	22	4.39
	ESI+					
Oxolinic acid	ESI+	262.0	32	216.0	30	5.44
	ESI+	262.0	32	244.0	19	
Pefloxacin	ESI+	334.1	42	290.1	19	4.45
	ESI+	334.1	42	316.1	19	
Sarafloxacin	ESI+	386.2	45	299.1	27	4.59
	ESI+	386.2	45	342.1	18	
Chloramphenicol	ESI-	321.2	25	152.2	15	4.94
	ESI-	321.2	25	257.2	10	
Florfenicol	ESI-	356.0	30	185.0	17	4.07
	ESI-	356.0	30	336.0	10	
Thiamphenicol	ESI-	354.1	20	184.9	20	3.20
	ESI-	354.1	20	290.0	12	
Amoxicillin	ESI+	366.2	27	114.0	20	1.37
	ESI+	366.2	27	349.1	8	
Penicillin V	ESI+	351.1	23	114.0	35	6.24
	ESI+	351.1	23	160.1	10	
Betamethasone	ESI-	361.2	40	307.2	18	7.78
	ESI-	361.2	40	325.2	20	
Cortisone	ESI+	361.3	40	163.1	25	6.93
	ESI+	361.3	40	342.2	20	
Dexamethasone	ESI+	393.3	20	355.2	10	7.83
	ESI+	393.3	20	373.2	10	
Hydrocortisone	ESI+	363.4	35	121.1	25	7.19
	ESI+	363.4	35	327.3	15	
Meprednisone	ESI+	373.2	20	355.1	11	7.78
	ESI+	373.2	20	357.1	12	
Methylprednisolone	ESI+	375.2	25	357.3	10	7.92
	ESI+					
Prednisolone	ESI+	361.2	25	147.0	20	7.21
	ESI+	361.2	25	343.2	10	
Triamcinolone	ESI+	435.4	25	397.3	15	5.3
	ESI+	435.4	25	415.3	5	

Table. 1 Analyte MS parameters and retention time.

Name	Ion Mode	Precursor (*m/z*)	CV (V)	Product (*m/z*)	CE (V)	RT (min)
Triamcinolone acetonide	ESI+	395.4	30	357.0	30	7.95
	ESI+	395.4	30	375.0	10	
Cefalexin	ESI+	348.2	40	139.9	35	3.36
	ESI+	348.2	40	158.0	20	
Cefotaxime	ESI+	456.1	30	167.0	20	3.43
	ESI+	456.1	30	396.2	10	
Ceftiofur	ESI+	524.2	35	241.1	16	5.25
	ESI+					
Cephapirin	ESI+	424.2	35	152.0	20	3.68
	ESI+	424.2	35	292.2	16	
Ceftiofur	ESI+	524.2	35	241.1	16	5.25
	ESI+					
Cephapirin	ESI+	424.2	35	152.0	20	3.68
	ESI+	424.2	35	292.2	16	

Table. 1 Analyte MS parameters and retention time.

RESULTS AND DISCUSSION

THE OPTIMIZATION OF SAMPLE PREPARATION

Milk matrix is complicated. It contains large amounts of proteins and phospholipids, which interferes with the detection of target analytes. It is essential to cleanup the complex matrix of milk and to release the analytes from the effect of matrix.

For the initial extraction and protein precipitation, 3:1 and 4:1 ratios of acetonitrile and milk were evaluated at both 0.2% and 1.0% formic acid concentrations. Results indicated that 1.0% formic acid in acetonitrile and milk at ratio of 4:1 gives the best effect of protein precipitation. However, 1% formic in acetonitrile has negative impact on the recoveries especially for the basic analytes like sulfonamides (see Figure 1). This could be because the more acidic condition gives rise to a higher degree of ionization of the sulfonamides with resulting solubility decrease in the high organic solvent. Therefore, 0.2% formic acid in acetonitrile mixed with milk at a 4:1 ratio was chosen for the final extraction and protein precipitation.

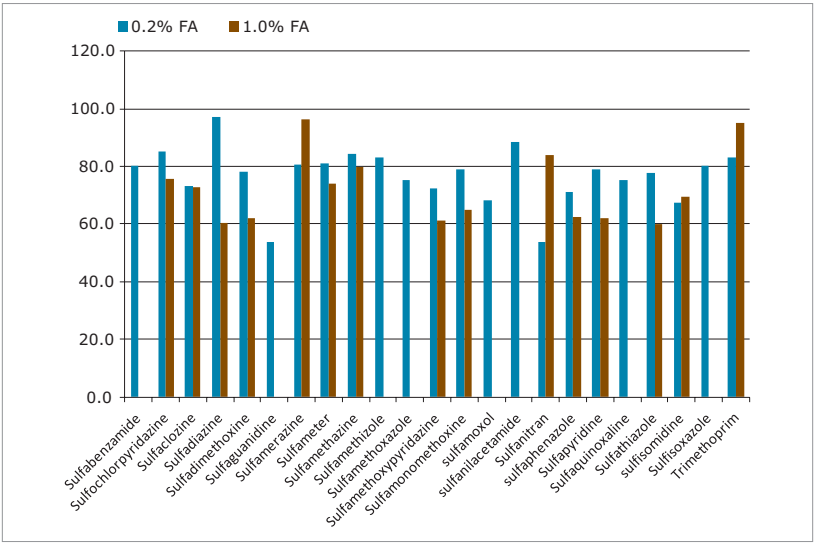

Figure 1. The comparison of recovery of sulfonamides for using 0.2% and 1.0% formic acid.

The large amount of phospholipids in milk not only becomes matrix interference for target analyte analysis, but also increases the cost and time of instrument maintenance. The use of Oasis PRiME HLB can remove the fat and phospholipids in sample matrix. As a result, the numbers of samples that could be analyzed are greatly increased before maintenance. In Figure 2, the effect of phospholipids removal by using SPE cleanup is compared to the milk sample only by protein precipitation. The sample after protein precipitation still has significant phospholipids present in the matrix that are mostly removed by the SPE cleanup.

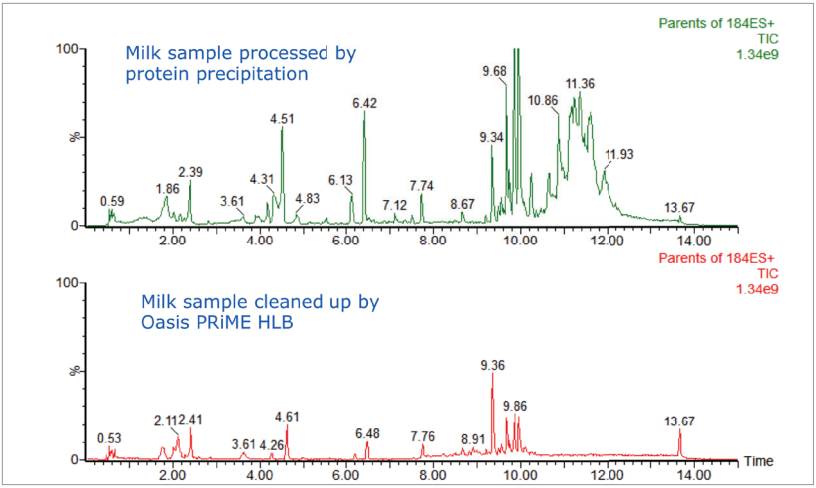

Figure 2. The chromatograms of phospholipids removal between milk samples processed by protein precipitation and cleanup by Oasis PRiME HLB.

Name	0.1 ug/L		0.5 ug/L		1.0 ug/L		10.0 ug/L		Matrix Effect at 10.0 ug/L
	Recovery (%)	RSD (%) n=5	Recovery (%)	RSD (%) n=5	Recovery (%)	RSD (%) n=5	Recovery (%)	RSD (%) n=5	
Cimaterol	95.0	2.5	94.0	8.5	77.2	18.0	98.1	3.2	0.17
Clenbuterol	81.0	15.6	84.4	3.5	92.6	5.3	113.0	5.8	0.11
Ractopamine	93.8	7.3	92.4	7.7	95.7	11.4	121.3	1.0	0.03
Salbutamol	93.8	5.5	93.2	6.9	90.0	3.3	111.3	2.6	0.11
Terbutaline	90.4	7.2	90.4	4.8	97.9	11.2	108.0	3.0	0.14
Tulobuterol	89.4	5.5	84.8	4.9	92.7	9.9	113.7	1.0	0.16
Zilpaterol	90.2	9.8	79.6	8.8	72.1	10.0	94.9	1.5	0.27
Clindamycin	–	–	111.2	12.6	73.0	18.0	86.5	5.4	0.10
Erythromycin	–	–	–	–	–	–	81.7	9.7	0.96
Kitasamycin	–	–	53.2	11.8	66.2	8.1	80.1	2.2	0.12
Lincomycin	85.6	9.0	71.2	7.1	70.8	3.2	70.8	3.2	0.25
Spiramycin	–	–	–	–	60.3	17.5	71.1	6.2	0.77
Tilmicosin	60.0	6.1	63.6	4.1	50.0	5.5	91.6	9.6	1.01
Tylosin	58.0	13.1	55.2	9.4	63.2	2.7	73.4	11.9	0.11
Oxytetracycline	–	–	75.2	17.1	72.0	8.9	69.0	3.1	0.08
Tetracycline	57.2	18.3	42.0	17.2	44.0	13.1	59.1	3.3	0.17
Sulfabenzamide	80.8	19.0	80.4	6.9	80.2	4.2	67.1	6.4	0.06
Sulfachlor-pyridazine	62.4	8.9	86.0	10.5	75.9	16.3	70.4	5.5	0.01
Sulfaclozine	61.6	30.6	76.4	22.9	72.9	6.4	71.1	13.0	0.04
Sulfadiazine	–	–	126.0	6.2	60.5	11.1	69.4	2.6	0.02
Sulfa-dimethoxine	96.8	6.3	78.8	8.5	62.2	9.5	80.0	6.7	0.00
Sulfaguanidine	90.4	10.2	69.6	13.4	53.8	13.0	70.9	11.5	0.46
Sulfamerazine	87.4	8.3	87.2	8.2	96.4	15.9	116.7	2.2	1.13
Sulfameter	–	–	80.4	8.7	74.1	1.7	75.5	5.4	0.51
Sulfamethazine	93.0	13.4	85.2	11.3	79.7	9.4	84.5	10.4	0.06
Sulfamethizole	82.4	36.9	74.8	7.0	83.2	11.5	72.8	5.6	0.04
Sulfa-methoxazole	74.8	12.4	77.2	9.1	75.2	2.9	63.6	5.4	0.05
Sulfamethoxy-pyridazine	73.4	11.1	79.2	17.4	61.4	17.4	73.4	8.7	0.04
Sulfamono-methoxine	93.4	10.7	80.8	2.8	64.8	9.0	69.9	5.6	0.05
Sulfamoxol	76.0	17.7	70.0	7.3	68.2	4.1	51.3	3.7	0.06
Sulfanil-acetamide	108.0	4.1	95.6	7.2	88.6	7.6	78.6	4.7	0.12
Sulfaphenazole	72.4	24.3	68.8	17.3	62.4	20.0	74.4	14.4	0.03
Sulfapyridine	87.4	17.0	78.0	1.8	62.1	1.4	70.3	3.5	0.10
Sulfathiazole	93.0	7.4	82.8	10.9	60.2	0.7	69.0	0.8	0.06
Sulfisomidine	87.2	3.8	80.8	10.3	69.5	4.2	79.6	5.4	0.13
Sulfisoxazole	71.0	20.3	92.8	9.2	80.2	7.8	66.5	7.0	0.15

Table. 2 The spike recoveries and precision (%RSD) of antibiotics in milk.

Name	0.1 ug/L		0.5 ug/L		1.0 ug/L		10.0 ug/L		Matrix Effect at 10.0 ug/L
	Recovery (%)	RSD (%) n=5	Recovery (%)	RSD (%) n=5	Recovery (%)	RSD (%) n=5	Recovery (%)	RSD (%) n=5	
Trimethoprim	77.6	25.2	82.4	6.0	95.0	11.5	113.7	1.8	0.23
Cinoxacin	89.8	30.0	94.8	13.6	75.3	7.7	113.7	5.9	0.12
Ciprofloxacin	–	–	90.4	31.3	87.1	17.3	86.3	13.9	0.53
Danofloxacin	73.2	16.1	64.4	8.9	12.9	62.4	104.1	14.0	0.31
Difloxacin	49.6	11.5	61.6	15.6	66.6	13.5	88.7	11.4	0.47
Enoxacin	–	–	–	–	78.9	15.1	91.1	12.7	0.45
Enrofloxacin	82.0	6.7	74.4	7.0	77.3	3.2	106.5	11.6	0.54
Flumequine	69.4	8.4	79.6	5.1	75.0	8.9	92.3	3.1	0.11
Lomefloxacin	66.0	16.8	66.0	6.4	67.8	5.3	105.6	10.0	0.16
Marbofloxacin	–	–	67.2	8.0	85.8	7.9	99.9	9.8	0.65
Nalidixic acid	75.6	9.1	82.8	2.2	86.1	8.4	106.5	6.4	0.27
Norfloxacin	68.8	14.6	70.4	16.4	62.6	4.6	92.9	18.1	0.24
Ofloxacin	92.0	9.1	91.6	8.7	70.4	17.0	86.3	13.9	0.45
Orbifloxacin	88.8	11.2	78.8	4.9	74.8	16.3	100.7	2.9	0.24
Oxolinic acid	79.4	11.5	79.6	6.5	97.3	7.2	118.7	4.0	0.08
Pefloxacin	65.8	14.9	70.0	6.1	75.4	10.2	87.4	7.3	0.69
Sarafloxacin	79.6	8.0	71.6	5.4	83.4	8.3	91.7	10.8	0.19
Chloramphenicol	85.4	16.8	97.2	12.4	80.2	15.0	113.7	2.7	0.03
Florfenicol	95.2	26.3	96.0	12.1	69.8	16.9	101.5	10.6	0.05
Thiamphenicol	40.0	18.3	68.0	39.6	63.2	4.7	123.3	3.4	0.18
Amoxicillin	–	–	–	–			54.1	3.2	0.15
Penicillin v	–	–	103.2	4.9	100.0	9.7	72.0	17.2	0.50
Betamethasone	–	–	–	–	58.0	15.7	84.3	1.9	0.02
Cortisone	118.4	13.2	92.8	7.6	71.5	16.2	86.1	2.8	0.12
Dexamethasone	–	–	–	–	72.8	13.3	82.6	6.4	0.18
Hydrocortisone	–	–	100.4	11.1	71.9	13.0	83.4	3.9	0.17
Meprednisone	–	–	78.0	10.9	79.6	7.6	83.0	3.0	0.01
Methyl–prednisolone	–	–	85.6	8.6	84.8	12.4	82.3	12.0	0.14
Prednisolone	–	–	74.4	12.7	84.8	12.4	84.8	5.3	0.08
Triamcinolone	–	–	–	–	–	–	84.7	13.4	0.55
Triamcinolone acetonide	70.2	7.5	79.6	10.3	61.7	18.7	101.2	7.6	0.38
Cefalexin	–	–	–	–	–	–	63.1	18.8	0.49
Cefotaxime	97.5	24.5	77.2	47.7	79.2	13.8	75.6	9.4	0.11
Ceftiofur	76.0	33.6	70.4	7.1	69.4	10.7	77.0	8.4	0.11
Cephapirin	–	–	46.8	21.3	71.5	15.4	91.5	13.2	0.09

CONCLUSIONS

- An analytical method was created for determination of multi-residue veterinary drugs in milk including 72 compounds in 9 drug classes.
- Reasonable recoveries were obtained in the range of 50% to 130% with precision (RSD) <20% (n=5) for all compounds.
- The Oasis PRiME HLB Cartridge was shown to effectively remove phospholipids and fats from milk. The sample preparation is simple, effective, and suitable for handling large numbers of samples in daily routine analysis.
- Waters Quanpedia Database contains all the liquid chromatographic methods, mass parameters, and quantitation method for veterinary drug analysis. It was very useful for developing this method.

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Rapid, Simple, and Effective Cleanup of Seafood Extracts Prior to UPLC-MS/MS Multiresidue Veterinary Drugs Analysis

Michael S Young and Kim Van Tran
Waters Corporation, Milford, MA, USA

APPLICATION BENEFITS

- Efficient, timesaving multiclass/multiresidue methodology.
- Simple, rapid and effective sample cleanup suitable for a diverse range of analytes.
- Fast, sensitive UPLC-MS/MS analysis.

WATERS SOLUTIONS

ACQUITY UPLC® I-Class System

Xevo® TQ-S Mass Spectrometer

Oasis® PRiME HLB Cartridge for SPE Cleanup

KEY WORDS

UPLC-MS/MS, Oasis PRiME HLB Cartridges, veterinary drugs, shrimp, salmon

OVERVIEW

In order to insure public health and safety, reliable analytical methods are necessary to determine veterinary drug residue levels in edible tissue samples such as fish and shellfish. The compounds of interest range from highly polar water-soluble compounds to very non-polar fat-soluble compounds. In order to maximize throughput and minimize costs it is desirable to determine the widest possible range of veterinary drug residues in tissue samples with a single analytical method. Seafood and meat tissue for human consumption typically contains up to 20% fat and up to 3% phospholipid.

INTRODUCTION

The major constituents of a typical meat sample are water (up to 70%), protein (15–25%), fat (5–25%) and phospholipid (1–3%). During the sample pre-treatment, the protein is removed from the extract by precipitation and centrifugation. However, significant amounts of fat and phospholipid are co-extracted along with the target veterinary drugs. The presence of these co-extracted substances can lead to interference in the LC-MS analysis, contamination of the analytical column and other components of the UPLC® System, and contamination of the mass spectrometer itself. Fats have traditionally been removed from tissue extracts using cumbersome hexane defatting steps or by the use of reversed-phase sorbents such as C_{18}-silica. Although these techniques may be effective for fat removal, neither of these procedures removes phospholipids. In this study, sample preparation, cleanup, and analysis protocols were developed for tandem LC-MS determination of a wide variety of veterinary drug residues in seafood tissue samples. This cleanup protocol was effective for removal of both fats and phospholipids. Two types of tissue samples, shrimp (prawn) and salmon, were chosen to demonstrate the suitability of the methodology. Samples were treated with an acidified acetonitrile/water solvent to precipitate proteins and to extract the veterinary drugs of interest. Then, a simple cleanup was performed using a novel SPE device, the Oasis PRiME HLB Cartridge. Representative compounds were chosen from major classes of veterinary drugs including tetracyclines, fluoroquinolones, sulfonamides, macrolides, beta-lactams, NSAIDS, steroids and beta-andrenergics. These compounds were spiked into the seafood samples prior to extraction and cleanup.

EXPERIMENTAL

UPLC conditions

LC system:	ACQUITY UPLC I-Class
Column:	ACQUITY UPLC CSH™ C_{18}, 1.7 µm, 100 mm x 2.1 mm ID
Mobile phase A:	0.1% formic in water
Mobile phase B:	0.1% formic acid in acetonitrile
Injection vol.:	5 µL
Injection mode:	Partial loop injection
Column temp.:	30 °C
Weak needle wash:	10:90 acetonitrile: water (600 µL)
Strong needle wash:	50:30:40 water: acetonitrile:IPA (200 µL)
Seal wash:	10:90 acetonitrile: water

Gradient:

Time (min)	Flow (mL/min)	%A	%B	Curve
Initial	0.4	85	15	Initial
2.5	0.4	60	40	6
3.9	0.4	5	95	6
4.9	0.4	5	95	6
5.0	0.4	85	15	6
7.0	0.4	85	15	6

MS conditions

Mass spectrometer:	Xevo TQ-S
Positive ion electrospray (negative ion for chloramphenicol)	
Source temp.:	150 °C
Desolvation temp.:	500 °C
Desolvation gas flow:	1000 L/Hr
Cone gas flow:	30 L/Hr
Collision gas flow:	0.15 mL/Min
Data management:	MassLynx® v4.1

Table 1 summarizes the MRM transitions and instrument parameters used for this study. Also presented in Table 1 are typical matrix matched calibration data for each compound (calculated using the primary transition in shrimp matrix; salmon data were similar) and retention times (RT).

SAMPLE PREPARATION

Initial Extraction/Precipitation

Place a 2.5 g sample of homogenized tissue into a 50 mL centrifuge tube. For standards or QC samples spike with appropriate amounts of desired analytes. Add 10 mL 0.2% formic acid in 80:20 acetonitrile/water. Vortex for 30 seconds and place on mechanical shaker for 30 minutes. Centrifuge at 12000 rpm for 5 minutes.

Note: The extraction/precipitation step gives good recovery of most compounds of interest but also extracts significant amounts of fat and phospholipid.

SPE Cleanup

An Oasis PRiME HLB Cartridge (3cc, 60mg) was mounted on a pre-cleaned vacuum manifold. Cartridge conditioning is NOT required, and was NOT performed. The vacuum was set to 1–2 psi. Approximately 0.5 mL of the supernatant was passed-through the Oasis PRiME Cartridge and collected. A 0.3 mL aliquot of the pass-thru cleanup sample was taken and diluted three-fold with aqueous 10 mM ammonium formate buffer (pH 4.5) prior to UPLC-MS/MS analysis.

Compounds	MRM	Cone (V)	Collision (eV)	Spike Level (low, high) µg/kg	Calibration Range µg/kg	Corr (R^2)	RT
Amoxicillin	366.2>349.1	30	8	12.5, 50	6.25–100	0.9978	0.70
	366.2>114.0	30	20				
Carbadox	263.0>231.0	25	15	25, 100	12.5–200	0.9978	1.43
	263.0>145.0	25	20				
Ceftiofur	524.3>241.1	30	16	250, 1000	125–2000	0.9975	2.84
	524.3>285.0	30	16				
Chloramphenicol	321.0>152.1	30	17	25, 100	12.5–200	0.9943	1.64
	321.0>257.1	30	15				
Chlortetracycline	479.3>444.2	30	21	25, 100	12.5–200	0.9955	0.97
	479.3>462.2	30	18				
Ciprofloxacin	332.1>288.1	30	18	25, 100	12.5–200	0.9918	2.99
	332.1>231.1	30	40				
Cortisol	363.2>121.0	42	52	50, 200	25–400	0.9989	3.45
	363.2>91.03	30	22				
Dexamethasone	393.2>373.2	30	10	25, 100	12.5–200	0.9980	1.09
	393.2>355.3	30	15				
Enrofloxacin	360.4>245.0	50	25	50, 200	25–400	0.9961	2.26
	360.4>316.1	50	25				
Erythromycin	734.4>158.1	30	32	2.5, 10	1.25–20	0.9982	0.61
	734.4>576.5	30	20				
Lincomycin	407.2>126.1	36	34	12.5, 50	6.25–100	0.9931	1.03
	407.2>359.3	36	20				
Lomefoxacin	352.1>265.1	31	22	50, 200	25–400	0.9960	3.79
	352.1>308.1	31	16				
Oxacillin	402.2>160.0	30	12	25, 100	12.5–200	0.9974	1.06
	402.2>243.1	30	15				
Oxytetracycline	461.2>426.2	30	21	25, 100	12.5–200	0.9952	1.06
	461.2>443.1	30					
Penicillin	335.2>160.1	20	30	12.5, 50	6.25–100	0.9903	3.46
	335.2>176.1	20	30				
Phenylbutazone	309.4>160.0	37	20	25, 100	12.5–200	0.9915	4.29
	309.4>103.9	37	20				
Ractopamine	302.2>164.1	30	15	75, 300	37.5–600	0.9915	1.03
	302.2>107.0	30	27				
Salbutamol	240.2>148.1	30	20	25, 100	12.5–200	0.9907	0.61
	240.2>222.1	30	12				
Sulfamerazine	265.0>92.0	30	28	25, 100	12.5–200	0.9918	0.91
	265.0>156.0	30	15				
Sulfamethazine	279.1>186.0	30	16	25, 100	12.5–200	0.9971	1.56
	279.1>92.0	30	28				
Sulfanilamide	156.0>92.0	30	15	25, 100	12.5–200	0.9977	1.73
	156.0>65.0	30	25				
Tetracycline	445.3>154.0	30	26	25, 100	12.5–200	0.9970	1.15
	445.3>410.2	30	21				
Tylosin	916.5>174.1	57	40	5, 20	2.5–40	0.9938	2.48
	916.5>101.1	57	45				

Table 1. Matrix matched calibration data, MRM transitions (primary transition first), instrument parameters, and retention times (RT) used for this study.

RESULTS

Table 2 shows the recovery data obtained from replicate analysis of spiked tissue samples. Matrix effects averaged about 40% for both shrimp and salmon. The chromatograms shown in Figure 1 show the effectiveness of the Oasis PRiME HLB Cartridge for removal of ≥95% of phopholipids from the shrimp extracts. The cartridge also removes more than 90% of hexane extractable fat.[1]

	Shrimp				Salmon			
	Low level		High Level		Low Level		High Level	
Compounds	**Recovery %**	**RSD(%) n=6**	**Recovery %**	**RSD(%) n=6**	**Recovery %**	**RSD(%) n=6**	**Recovery %**	**RSD(%) n=6**
Amoxicillin	BLOQ	–	67	18	BLOQ	–	59	17
Carbadox	113	9	75	10	85	5	84	7
Ceftiofur	111	7	84	6	64	4	67	4
Chloramphenicol	106	7	77	12	79	7	69	10
Chlortetracyclin	79	7	63	17	67	5	65	7
Ciprofloxacin	190	14	103	15	109	9	95	4
Cortisol	99	8	80	6	82	4	82	4
Dexamethasone	112	9	79	7	89	8	79	6
Enrofloxacin	90	12	71	12	86	4	84	8
Erythromycin	110	7	83	8	85	9	86	7
Lincomycin	104	6	99	6	90	4	92	3
Lomefloxacin	126	11	90	11	97	4	92	5
Oxacillin	115	5	86	2	71	2	74	5
Oxytetracyline	125	11	92	7	83	5	76	4
Penicillin	112	10	86	6	70	10	71	6
Phenylbultazone	78	10	51	8	51	7	51	3
Ractopamine	102	9	87	8	87	3	90	4
Salbutamol	115	7	89	4	92	12	93	3
Suflanilamide	BLOQ	–	82	17	BLOQ	–	95	12
Sulfamerazine	107	7	91	7	83	3	77	12
Sulfamethazine	102	8	85	9	82	3	78	8
Tetracyline	106	7	77	12	79	7	69	10
Tylosin	116	10	98	4	76	7	87	3

Table 2. Recovery data obtained from replicate analysis of spiked tissue samples (BLOQ – below quantitation limits).

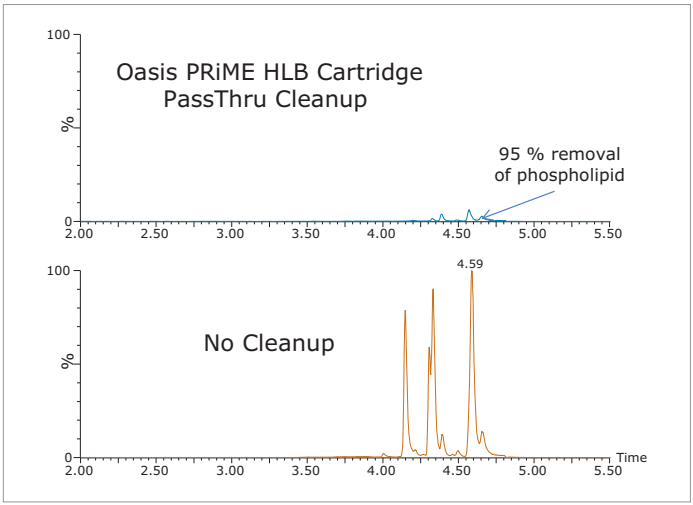

Figure 1. LC-MS/MS chromatograms showing effective removal of ≥95% of phosholipids from shrimp extract.

DISCUSSION

The procedure utilized in this study was developed from methods presented by Lehotay[2] and refined by Tran.[3] The overall method recoveries are generally above 70% but significantly lower recovery was observed for some of the more polar compound classes, such as tetracyclines. However, the Oasis PRiME HLB Cartridge cleanup contributes very little to any method recovery losses. As shown in Table 3, the measured recovery for the SPE cleanup step, specifically, is better than 80% in shrimp and better than 90% in salmon for all analytes except phenylbutazone.

Compounds	Shrimp %REC (%RSD) n=5	Salmon %REC (%RSD) n=5
Amoxicillin	81 (23)	97 (37)
Carbadox	102 (3)	99 (3)
Ceftiofur	102 (2)	99 (1)
Chloramphenicol	84 (17)	87 (5)
Chlortetracyclin	98 (3)	95 (1)
Ciprofloxacin	93 (4)	103 (5)
Cortisol	89 (2)	91 (2)
Dexamethasone	84 (3)	90 (4)
Enrofloxacin	94 (1)	97 (3)
Erythromycin	83 (11)	104 (4)
Lincomycin	101 (4)	103 (2)
Lomefloxacin	98 (2)	93 (4)
Oxacillin	100 (1)	95 (2)
Oxytetracyline	104 (4)	101 (4)
Penicillin	98 (3)	97 (3)
Phenylbultazone	55 (4)	60 (1)
Ractopamine	98 (1)	97 (2)
Salbutamol	107 (2)	99 (6)
Suflanilamide	109 (9)	95 (10)
Sulfamerazine	93 (2)	93 (2)
Sulfamethazine	93 (2)	93 (2)
Tetracyline	99 (3)	98 (5)
Tylosin	84 (5)	103 (5)

Table 3. SPE % recovery (percent recovered from spiked shrimp or salmon extracts after pass-through cleanup).

CONCLUSIONS

- A simple and effective extraction/protein precipitation procedure was applied to the analysis of shrimp and salmon tissue.
- A simple one-step pass-thru cleanup protocol using Oasis PRiME HLB Cartridges was employed to remove greater than 90% of fats and phospholipids from the initial extracts.
- The sample preparation methodology produced an extract that was free of particulates and required no subsequent filtration prior to LC-MS analysis.
- High and consistent recoveries were observed for a wide range of veterinary drugs using the simple one-step pass-thru cleanup protocol with Oasis PRiME HLB Cartridges.

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1. M. Young and K. Tran, *Oasis PRiME HLB Cartridge for Effective Cleanup of Meat Extracts Prior to Multi-Residue Veterinary Drug UPLC-MS Analysis*, Waters Application Brief, 2015.
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Oasis PRiME HLB Cartridge for Cleanup of Infant Formula Extracts Prior to UPLC-MS/MS Multiresidue Veterinary Drugs Analysis

Michael S. Young and Kim Van Tran
Waters Corporation, Milford, MA, USA

APPLICATION BENEFITS

- Efficient, timesaving multiclass/multiresidue methodology.
- Simple, rapid and effective sample cleanup suitable for a diverse range of analytes.
- Fast, sensitive UPLC-MS/MS analysis.

WATERS SOLUTIONS

ACQUITY UPLC® I-Class System

Xevo® TQ-S Mass Spectrometer

Oasis® PRiME HLB Cartridge for SPE Cleanup

KEY WORDS

UPLC-MS/MS, Oasis PRiME HLB Cartridges, veterinary drugs, infant formula

OVERVIEW

In order to insure public health and safety, reliable analytical methods are necessary to determine veterinary drug residue levels in foods. Of particular importance is such residue analysis in foods for infants. The compounds of interest range from highly polar water-soluble compounds to very non-polar fat-soluble compounds. In order to maximize throughput and minimize costs it is desirable to determine the widest possible range of veterinary drug residues in such samples with a single analytical method. Powdered infant formula typically contains significant amounts of proteins, fats, and lecithin (phospholipids). These components can be detrimental to good instrumental performance and should be reduced or eliminated prior to LC-MS analysis.

INTRODUCTION

Infant formula powder contains significant amounts of protein, about 20% fat and 1–3 % phospholipids. During the sample pre-treatment, the protein is removed from the extract by precipitation and centrifugation. However, significant amounts of fat and phospholipids are co-extracted along with the target veterinary drugs. The presence of these co-extracted substances can lead to interference in the LC-MS analysis, contamination of the analytical column and other components of the UPLC® System, and contamination of the mass spectrometer itself. Fats have traditionally been removed from tissue extracts using cumbersome hexane defatting steps or by the use of reversed-phase sorbents such as C_{18}-silica in pass-through or dispersive cleanup. Although these techniques may be effective for fat removal, neither of these procedures removes phosholipids. In a prior study, sample preparation, cleanup, and analysis protocols were developed for tandem LC-MS determination of a wide variety of veterinary drug residues in seafood tissue samples. This cleanup protocol was effective for removal of both fats and phospholipids. In this study similar extraction and cleanup protocols were applied to the analysis of infant formula powder. Representative compounds were chosen from major classes of veterinary drugs including tetracyclines, fluoroquinolones, sulfonamides, macrolides, beta-lactams, NSAIDS, steroids, and beta-andrenergics. These compounds were spiked into the infant formula samples prior to extraction and cleanup.

EXPERIMENTAL

UPLC conditions

LC system:	ACQUITY UPLC I-Class
Column:	ACQUITY UPLC CSH™ C_{18}, 1.7µm, 100 mm x 2.1 mm ID
Mobile phase A:	0.1% formic in water
Mobile phase B:	0.1% formic acid in acetonitrile
Injection volume:	5 µL
Injection mode:	partial loop injection
Column temp.:	30 °C
Weak needle wash:	10:90 acetonitrile:water (600 µL)
Strong needle wash:	50:30:40 water:acetonitrile: IPA (200 µL)
Seal wash:	10:90 acetonitrile: water

Gradient:

Time (min)	Flow (mL/min)	%A	%B	Curve
Initial	0.4	85	15	Initial
2.5	0.4	60	40	6
3.9	0.4	5	95	6
4.9	0.4	5	95	6
5.0	0.4	85	15	6
7.0	0.4	85	15	6

MS conditions

Mass spectrometer:	Xevo TQ-S
Positive ion electrospray source temp.:	150 °C
Desolvation temp.:	500 °C
Desolvation gas flow:	1000 L/Hr
Cone gas flow:	30 L/Hr
Collision gas flow:	0.15 mL/Min
Data management:	MassLynx® v4.1

SAMPLE PREPARATION

Initial Extraction/Precipitation

Place a 0.5 g sample of infant formula into a 50 mL centrifuge tube. For standards or QC samples spike with appropriate amounts of desired analytes. Add 3 mL extraction solvent (0.2% formic acid in 70:30 acetonitrile/water). Vortex for 30 seconds and place on mechanical shaker for 30 minutes. Centrifuge at 3220 rcf for 5 minutes.

Note: The extraction/precipitation step gives good recovery of most compounds of interest but also extracts significant amounts of fat and phospholipid.

SPE Cleanup:

Mount an Oasis PRiME HLB Cartridge (3 cc, 60 mg) on a pre-cleaned vacuum manifold. Cartridge conditioning is NOT required and is not performed. The vacuum is set to 1–2 psi. Approximately 0.5 mL of the supernatant is passed-through the Oasis PRiME Cartridge and collected. A 0.3 mL aliquot of the pass-thru cleanup sample is taken and diluted three-fold with aqueous 10 mM ammonium formate buffer (pH 4.5) prior to UPLC-MS/MS analysis.

Compounds	MRM	Cone (V)	Collision (eV)	Spike Level (low, high) µg/kg	Calibration Range µg/kg	Corr (R^2)	RT
Carbadox	263.0>231.0 263.0>145.0	25 25	15 20	25, 100	12.5–200	0.9990	1.37
Ceftiofur	524.3>241.1 524.3>285.0	30 30	16 16	250, 1000	125–2000	0.9980	2.79
Chlortetracycline	479.3>444.2 479.3>462.2	30 30	21 18	25, 100	12.5–200	0.9977	1.53
Ciprofloxacin	332.1>288.1 332.1>231.1	30 30	18 40	25, 100	12.5–200	0.9929	0.84
Cortisol	363.2>121.0 363.2>91.03	42 30	52 22	50, 200	25–400	0.9961	2.93
Dexamethasone	393.2>373.2 393.2>355.3	30 30	10 15	25, 100	12.5–200	0.9917	3.39
Enrofloxacin	360.4>245.0 360.4>316.1	50 50	25 25	50, 200	25–400	0.9991	0.98
Erythromycin	734.4>158.1 734.4>576.5	30 30	32 20	2.5, 10	1.25–20	0.9978	2.16
Lincomycin	407.2>126.1 407.2>359.3	36 36	34 20	12.5, 50	6.25–100	0.9962	0.57
Lomefoxacin	352.1>265.1 352.1>308.1	31 31	22 16	50, 200	25–400	0.9991	0.90
Oxacillin	402.2>160.0 402.2>243.1	30 30	12 15	25, 100	12.5–200	0.9990	3.75
Oxytetracyline	461.2>426.2 461.2>408.11	30 30	21 13	25, 100	12.5–200	0.9971	0.96
Pennicillin	335.16>160.1 335.15>176.1	20 20	30 30	12.5, 50	6.25–100	0.9956	3.41
Phenylbutazone	309.4>160.0 309.4>103.9	37 37	20 20	25, 100	12.5–200	0.9962	4.22
Ractopamine	302.2.164.1 302.2>107.0	30 30	15 27	75, 300	37.5–600	0.9990	0.90
Salbutamol	240.2>148.1 240.2>222.1	30 30	20 12	25, 100	12.5–200	0.9941	0.55
Sulfamerazine	265>92.0 265>156.0	30 30	28 15	25, 100	12.5–200	0.9998	1.50
Sulfamethazine	279.1>186.0 279.1>92.0	30 30	16 28	25, 100	12.5–200	0.9996	1.67
Sulfanilamide	156>92.0 156>65.0	30 30	15 25	25, 100	12.5–200	0.9964	0.86
Tetracycline	445.3>154.0 445.3.410.2	30 30	26 21	25, 100	12.5–200	0.9964	1.05
Tylosin	916.5>174.1 916.5>101.1	57 57	40 45	5, 20	2.5–40	0.9920	2.38

Table 1. Matrix matched calibration data, MRM transitions (primary transition first), instrument parameters, and retention times (RT) used for this study.

RESULTS

Table 2 shows the recovery data obtained from replicate analysis of spiked tissue samples. Matrix effects averaged about 40% for infant formula. The chromatograms shown in Figure 1 show the effectiveness of the Oasis PRiME HLB Cartridge for removal of ≥95% of phopholipids from the infant formula extracts.

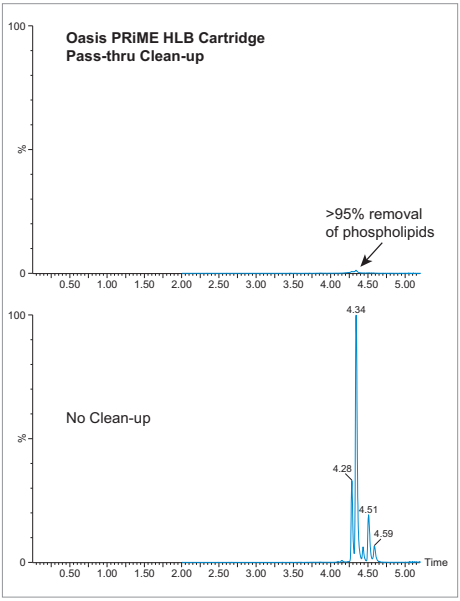

Figure 1. LC-MS/MS chromatograms showing effective removal of ≥95% of phospholipids from shrimp extract.

	Low Level		High Level	
Compounds	**Recovery %**	**RSD(%) n=6**	**Recovery %**	**RSD(%) n=6**
Carbadox	110	5	115	3
Ceftiofur	84	4	71	10
Chlortetracycline	47	9	46	9
Ciprofloxacin	107	8	102	6
Cortisol	111	8	117	5
Dexamethasone	113	14	121	5
Enrofloxacin	113	4	110	5
Erythromycin	126	6	125	6
Lincomycin	50	7	52	5
Lomefloxacin	120	2	111	2
Oxacillin	117	6	114	4
Oxytetracycline	29	13	27	13
Penicillin	120	10	116	7
Phenylbutazone	105	13	94	6
Ractopamine	115	2	117	5
Salbutamol	82	7	83	4
Sulfamerazine	130	4	126	4
Sulfamethazine	128	2	129	3
Sulfanilamide	105	12	120	5
Tetracyline	41	13	40	17
Tylosin	110	13	116	7
Carbadox	110	5	115	3
Ceftiofur	84	4	71	10

Table 2. Recovery data obtained from replicate analysis of spiked infant formula samples (n = 6).

DISCUSSION

The procedure utilized in this study was developed from methods presented previously.[1,2] The overall method recoveries are generally above 70% but lower recovery was observed for some of the more polar compound classes, such as tetracyclines. However, the Oasis PRiME HLB Cartridge cleanup contributes very little to any method recovery losses. As shown in Figure 2, the measured recovery for the SPE cleanup step is better than 80%.

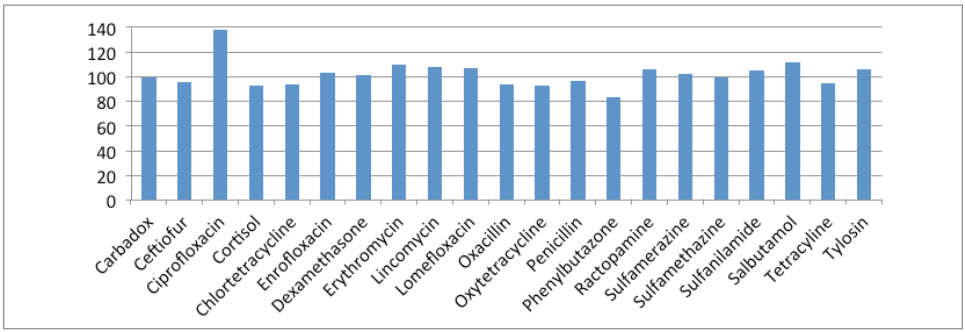

Figure 2. Recovery of veterinary compounds from prepared extracts subjected to Oasis PRiME HLB pass-through cleanup.

CONCLUSIONS

- A simple and effective extraction/protein precipitation procedure was applied to the analysis of infant formula.
- A simple one-step pass-thru cleanup protocol using Oasis PRiME HLB Cartridges was employed to remove greater than 90% of fats and phospholipids from the initial extracts.
- The sample preparation methodology produced an extract that was free of particulates and required no subsequent filtration prior to LC-MS analysis.
- High and consistent recoveries were observed for a wide range of veterinary drugs using the simple one-step pass-through cleanup protocol with Oasis PRiME HLB Cartridges.

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Simple and Effective Cleanup for UPLC-MS/MS Determination of Veterinary Drug Residues in Egg

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APPLICATION BENEFITS

- Efficient, timesaving multiclass/multiresidue methodology.
- Simple, rapid and effective sample cleanup suitable for a diverse range of analytes.
- Fast, sensitive UPLC-MS/MS analysis.

WATERS SOLUTIONS

ACQUITY UPLC® I-Class System

ACQUITY UPLC Columns

Xevo® TQ-S Mass Spectrometer

Oasis® PRiME HLB Cartridge for SPE Cleanup

KEY WORDS

UPLC-MS/MS, Oasis PRiME HLB Cartridges, veterinary drugs, eggs

OVERVIEW

In order to insure public health and safety, reliable analytical methods are necessary to determine veterinary drug residue levels in foods. The compounds of interest range from highly polar, water-soluble compounds to very non-polar, fat-soluble compounds. In order to maximize throughput and minimize costs it is desirable to determine the widest possible range of veterinary drug residues in food samples with a single analytical method. Eggs contains significant amounts of proteins, fats, and, lecithin (phospholipids). These components can be detrimental to good instrumental performance and should be reduced or eliminated prior to LC-MS analysis.

INTRODUCTION

Veterinary drugs are used in chicken farms to control diseases of laying hens. However, these compounds can be transferred to and accumulate in the eggs. The presence of veterinary drug residues in eggs is a potential health risk for the consumer because the residual drugs can provoke allergic reactions or induce pathogen resistance to antibiotics used in human medicine.[1] Sixteen representative veterinary drugs from twelve classes, most of which have MRLs established in US or China, were chosen for this study.[1,2] Figure 1 presents the structures of a subset of these veterinary drugs.

Sample preparation is a challenging task for the multi-residue determination of veterinary drugs in eggs. The analyst must recover a wide variety of drug classes with different physico-chemical properties. Some of the target compounds may bind to proteins or other matrix components. Eggs are among the highest food sources of lecithin (phospholipids) and also have significant amounts of fats; these co-extracted substances can lead to interference and ion suppression in the LC-MS analysis, contamination of the analytical column, and other components of the UPLC system, and contamination of the mass spectrometer itself.

In this work, sample extraction, cleanup, and analysis methods were developed for UPLC-MS/MS determination of a wide variety of veterinary drugs in eggs. Samples were treated with an acidified acetonitrile/water solvent to precipitate proteins, release bound residues, and to extract the veterinary drugs of interest. Then, to remove fats and phospholipids, a simple pass-through cleanup was performed using a novel SPE device, the Oasis PRiME HLB Cartridge.

STANDARD COMPOUNDS

Sixteen veterinary drugs from different classes were chosen for this study. Table 1 lists their formulas, MWs, and MRLs established in USA or China.

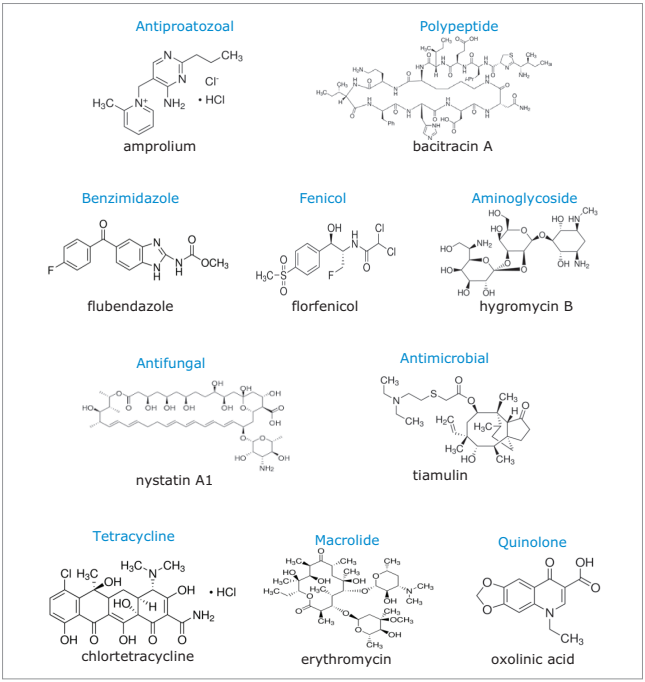

Figure 1. Structures of representative compounds from this study.

Compounds	Formula	Monoisotopic MW	Market	MRL (ng/g)
Amprolium	$C_{14}H_{19}ClN_4$	278.130	USA	4000
Bacitracin A	$C_{66}H_{103}N_{17}O_{16}S$	1421.749	USA/China	500
Hygromycin in B	$C_{20}H_{37}N_3O_{13}$	527.233	USA/China	No Residue Allowed
Nystatin A1	$C_{47}H_{75}NO_{17}$	925.503	USA	No Residue Allowed
Colistin B	$C_{52}H_{98}N_{16}O_{13}$	1154.750	China	300
Florfenicol	$C_{12}H_{14}Cl_2FNO_4S$	357.000	China	No Residue Allowed
Flubendazole	$C_{16}H_{12}FN_3O_3$	313.086	China	400
Oxolinic Acid	$C_{13}H_{11}NO_5$	261.063	China	50
Tiamulin	$C_{28}H_{47}NO_4S$	493.323	China	1000
Chlortetracycline	$C_{22}H_{23}ClN_2O_8$	478.114	USA/China	400/200
Erythromycin	$C_{37}H_{67}NO_{13}$	733.461	USA/China	25/150
Lincomycin	$C_{18}H_{34}N_2O_6S$	406.214	China	50
Oxytetracycline	$C_{22}H_{24}N_2O_9$	460.148	China	200
Penicillin G	$C_{16}H_{18}N_2O_4S$	334.099	USA	No Residue Allowed
Tetracycline	$C_{22}H_{24}N_2O_8$	444.153	China	200
Tylosin	$C_{46}H_{77}NO_{17}$	915.519	USA/China	200

Table 1. Veterinary drugs in this study (Bacitracin, Colistin, and Nystatin all contain a mixture of more than two components; one major component was chosen for analysis).

EXPERIMENTAL

UPLC conditions

System: ACQUITY UPLC I-Class

Column: ACQUITY UPLC BEH C_{18}, 2.1 x 100 mm, 1.7 µm (p/n 186003555)

Column temp.: 30 °C

Injection vol.: 10 µL

Flow rate: 0.4 mL/min

Mobile phase A: 0.1% Formic acid in water

Mobile phase B: 0.1% Formic acid in acetonitrile

Gradient: The initial composition was 85% A and 15% B. Phase B was increased linearly to 40% in the first 2.5 min, and then linear ramp to 95% B in 1.4 min, maintained for 2.3 min, then returned to the initial composition and equilibrated for 2 min.

MS conditions

System: Xevo TQ-S

Ionization mode: ES+ (ES-for Florfenicol)

Capillary voltage: 3.00 kV (2.50 kV for negative mode)

Source temp.: 150 °C

Desolvation temp.: 600 °C

Cone gas flow: 150 L/hr

Desolvation gas flow: 1000 L/hr

Collision gas flow: 0.15 mL/min

Nebulizer gas flow: 7.00 Bar

	MRM Transition 1				MRM Transition 2		
Compounds	Precursor Ion (m/z)	Product Ion (m/z)	Cone (V)	Collision (eV)	Product Ion (m/z)	Cone (V)	Collision (eV)
Amprolium	243.26	94.06	20	14	150.17	20	12
Bacitracin A	712.22	199.10	68	40	110.10	68	70
Hygromycin B	528.49	352.20	48	22	177.14	48	32
Nystatin A1	926.82	297.24	22	28	107.13	48	60
Colistin B	578.66	101.07	64	28	86.06	64	40
Florfenicol	356.03	335.96	52	10	184.94	52	22
Flubendazole	314.25	282.19	90	18	123.08	90	36
Oxolinic add	262.20	244.20	20	12	160.17	50	32
Tiamulin	494.45	119.10	40	42	192.17	40	20
Chlortetracycline	479.27	444.19	12	18	154.06	12	26
Erythromycin	734.72	158.08	48	26	576.52	48	18
Lincomycin	407.20	126.10	40	34	359.30	40	20
Oxytetracycline	461.36	426.22	20	18	201.07	64	36
Penicillin G	335.27	176.05	14	20	159.99	14	16
Tetracycline	445.30	410.20	40	21	154.00	40	26
Tylosin	916.88	174.13	80	36	101.10	45	45

Table 2. MRM transition parameters for 16 veterinary drugs.

Compounds	RT (min)	LOD (ng/g)	Linear range (ng/g)	R^2
2 Amprolium	0.61	0.5	80–40,000	0.998
9 Bacitracin A	2.52	1	10–5,000	0.992
1 Hygromycin B	0.48	4	4–1,000	0.990
15 Nystatin A1	3.30	10	40–1,000	0.992
4 Colistin B	1.73	30	90–600	0.990
10 Florfenicol	2.69	4	4–1,000	0.991
14 Flubendazole	3.29	0.5	8–240	0.993
11 Oxolinic acid	2.83	1	1–500	0.993
16 Tiamulin	3.88	0.5	20–1,000	0.990
8 Chlortetracycline	2.49	0.5	4–2,000	0.995
12 Erythromycin	2.95	0.5	0.5–250	0.995
3 Lincomycin	1.59	0.5	1–500	0.996
5 Oxytetracycline	1.89	0.5	4–2,000	0.995
6 Penicillin G	1.91	1	2–1,000	0.991
7 Tetracycline	2.04	0.5	4–2,000	0.994
13 Tylosin	3.06	0.5	20–800	0.991

Table 3. UPLC-MS retention times and calibration data.

SAMPLE PREPARATION

Extraction:
Two grams of homogenized whole chicken eggs were weighed into a 50 mL polypropylene centrifuge tube. Recovery samples were fortified with the appropriate amount of standards before 8 mL of 0.2% formic acid in 80:20 acetonitrile/water were added. The samples were vortexed for 30 s, placed on a mechanical shaker for 30 mins, and then centrifuged at 4500 rpm for 10 min. An aliquot of the supernatant was taken for the SPE cleanup.

Pass-through SPE cleanup:
An Oasis PRiME HLB 3 cc Vac Cartridge, 60 mg, (p/n 186008056), was mounted on a precleaned vacuum manifold. Cartridge conditioning is not required and was not performed. A 0.5 mL aliquot of the supernatant was passed through the cartridge and collected using 1~2 psi vacuum. 0.2 mL of the collected extract was taken and diluted to 0.6 mL with aqueous 10 mM ammonium formate buffer (pH 4.5) prior to UPLC-MS/MS analysis. Figure 2 shows a typical chromatographic separation obtained for a matrix-matched standard.

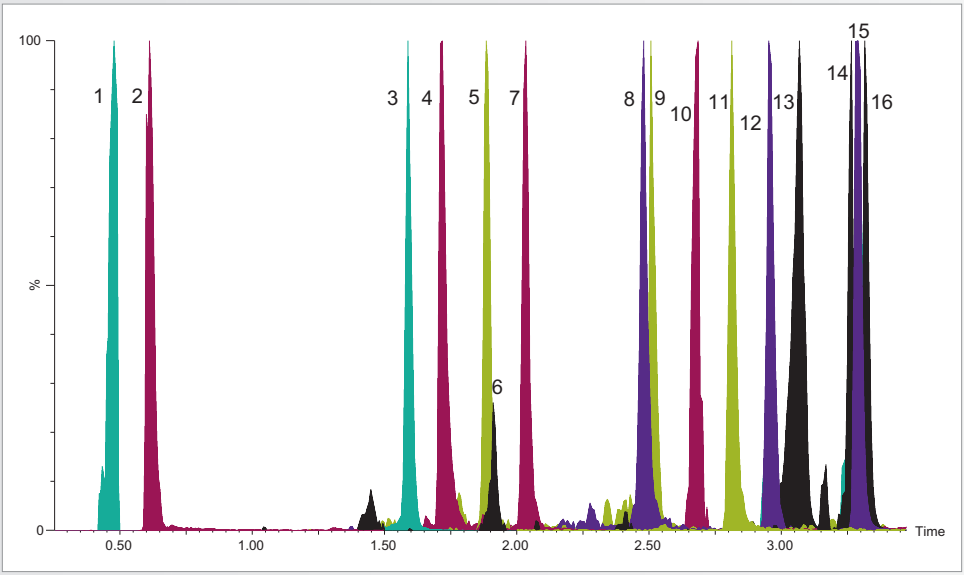

Figure 2. Overlay of MRM chromatograms of 16 veterinary drugs (matrix-matched standard at MRL level).

RESULTS AND DISCUSSION

OASIS PRIME HLB CARTRIDGE PASS-THROUGH CLEANUP

The Oasis PRiME HLB Cartridge was evaluated with respect to analyte recovery and phospholipids removal from egg matrix. The total method recoveries ranged from 50–97%. However, the Oasis PRiME HLB Cartridge cleanup contributes little to any method recovery losses. As shown in Figure 3, the measured recovery for the SPE cleanup step is better than 80% for all compounds, with recovery for most compounds greater than 90%.

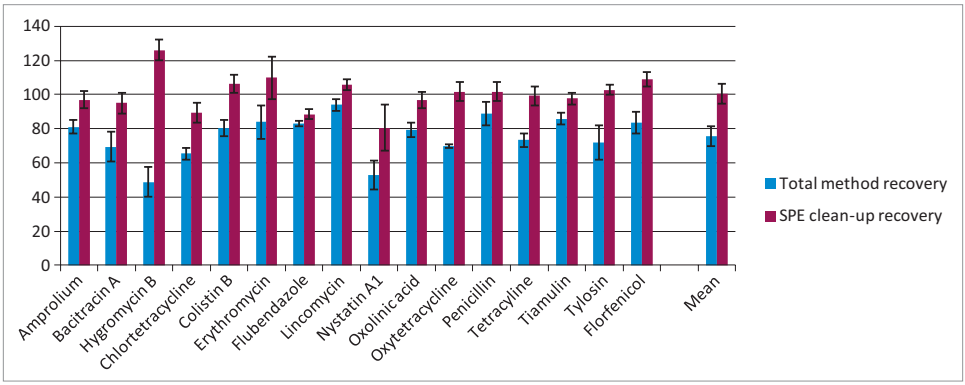

Figure 3. Recovery data for target veterinary drugs obtained using the Oasis PRiME HLB Cartridge cleanup procedure (at MRL level).

Whole eggs contain significant amounts of fat and are among the highest sources of dietary lecithin (phospholipids). The total lipid content of chicken egg is about 11% by weight (excluding the shell) and the phospholipids content is about 0.35%.[4] Significant amounts of these potential interfering substances are extracted along with the target drugs in the initial sample preparation extraction step. Greater than 84% of total lipids were removed from the egg extract after pass-through cleanup with the Oasis PRiME HLB Cartridge. The cleanup step was even more effective for removal of phospholipids. Figure 4 shows that the Oasis PRiME HLB Cartridge cleanup removed greater than 95% of phospholipids from the egg extract.

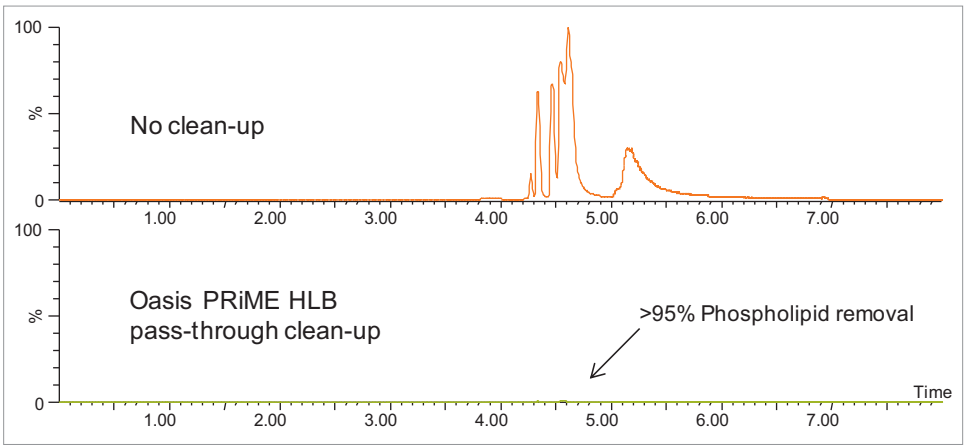

Figure 4. Effective removal of phospholipids from egg extracts with Oasis PRiME HLB cleanup.

METHOD RECOVERY AND PRECISION

Recovery studies were carried out at three concentration levels (0.4MRL, 1MRL, 2MRL), six replicates per level. Matrix-matched standard calibration curves were used. Figure 5 shows the results. Recovery was greater than 70% for most target compounds (>70%) except for Nystatin and Hygromycin. Reproducibility was acceptable (RSD<20%) for all compounds except for Hygromycin at 0.4 MRL (RSD=34%).

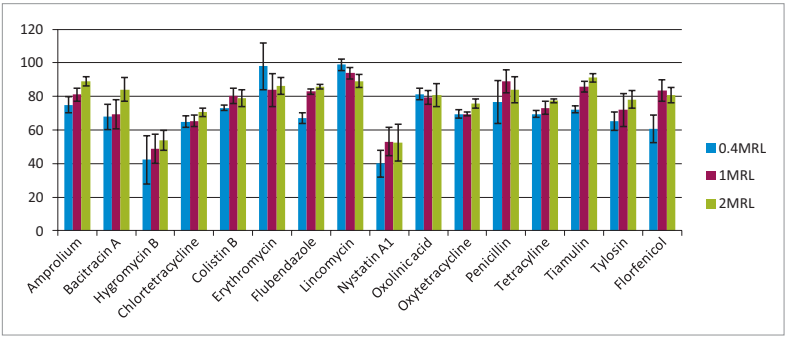

Figure 5. Summary of recovery data (blank eggs samples spiked at 0.4 MRL, 1MRL, 2MRL levels (Hygromycin B, Florfenicol, Penicillin G, and Nystatin A1 have no corresponding MRLs, so they were studied at 40, 100, 200 ppb levels).

CONCLUSIONS

- An analytical method has been developed for the simultaneous determination of several classes of veterinary drugs in eggs.
- A simple pass-through cleanup procedure using Oasis PRiME HLB Cartridge can remove more than 95% phospholipids from egg extracts.
- The Oasis PRiME HLB Cartridge cleanup procedure provided effective cleanup and good recoveries for the target veterinary drugs in egg.
- The ACQUITY UPLC I-Class System coupled with Xevo TQ-S MS offered good sensitivity for the veterinary drug residues in this study.

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Oasis PRiME HLB Cartridge for Rapid and Effective Cleanup of Avocado, a High Fat Matrix, Prior to APGC-MS/MS Analysis

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APPLICATION BENEFITS

- Efficient, time-saving multi-class/multi-residue methodology.
- Simple, rapid, and effective sample cleanup suitable for a diverse range of analytes in a fatty matrix.
- Fast, sensitive APGC-MS/MS analysis.

WATERS SOLUTIONS

Xevo® TQ-S Mass Spectrometer with APGC interface

Oasis® PRiME HLB Cartridge for SPE Cleanup

DisQuE™ pouch for AOAC QuEChERS

Qsert Vials for GC-MS

MassLynx® Software

KEY WORDS

APGC-MS/MS, Oasis PRiME HLB Cartridges, pesticides, avocado, QuEChERS

OVERVIEW

In order to ensure public health and safety, reliable analytical methods are necessary to determine pesticide residue levels in foods. Many of the compounds are well suited for gas chromatography (GC) and are often determined in fruits and vegetables using GC with mass spectrometry (GC-MS). This application note demonstrates an effective cleanup protocol for pesticide analysis in avocado, a highly fatty matrix. After a modified QuEChERS extraction, cleanup is performed using an Oasis PRiME HLB Cartridge. Analysis is performed using GC coupled with atmospheric pressure ionization mass-spectrometry (APGC-MS/MS).

INTRODUCTION

In recent years, food safety laboratories have adopted new and simplified sample preparation methods designed to reduce analysis time and related costs, as well as to increase throughput. For example, the QuEChERS methods for fruits and vegetables require only minutes for sample preparation and replace prior methods that took hours or days. In this study, this type of simplified sample preparation is applied to pesticide analysis in avocado, a fruit matrix of very high lipid content. A typical avocado contains 10–15% fat and about 1% total phospholipids. In the QuEChERS extraction, significant amounts of the fat and phospholipids are co-extracted along with the target pesticides. The presence of these co-extracted substances, particularly the phospholipids, can lead to chromatographic interference, contamination of the GC injector and column, and contamination of the mass spectrometer itself. To avoid these complications, a cleanup step is recommended prior to the instrumental analysis. This is typically performed using dispersive SPE with mixed sorbents, often with cumbersome multi-step centrifugation. In this study an Oasis PRiME HLB Cartridge was used for a simple pass-through cleanup to effectively remove fats and phospholipids. This method was applied to a number of pesticides registered for use on avocado in various world markets and suitable for GC-MS analysis. The APGC methodology was used for quantitative analysis in this study (APGC-MS/MS).

EXPERIMENTAL

GC conditions

GC system:	Agilent 7890
Column:	Restek Rxi-5 ms, 30 m x 0.25 mm x 0.25 µm
Flow rate:	1.0 mL/min Helium
Injection vol.:	1 µL (15:1 split)
Temperature program:	80 °C initial, hold for 0.5 min, 12 °C/min to 320 °C and hold for 8 min.

MS conditions

Mass spectrometer	Xevo TQ-S
Ion mode:	API+ (charge transfer mode)
Corona:	2.8 µA
Source temp.:	150 °C
Probe temp.:	450 °C
Cone gas:	170 L/Hr
Auxiliary gas:	250 L/Hr
Collision gas:	0.15 mL/min (Ar)
Nebulizer:	4.0 bar
Data management:	MassLynx v4.1

Other instrument parameters are presented in Table 1.

Compound	MRM	Cone (V)	Collision (eV)	RT (min)
Azoxystrobin	403.0>344.2 403.0>329.1	20 20	12 32	20.9
Carfentrazone-ethyl	410.9>312.2 410.9>340.2	20 20	20 10	16.1
Chlorothalonil	265.9>170.0 265.9>230.9	20 20	27 27	12.0
Cypermethrin	163.1>127.0 163.1>127.0	20 20	10 10	19.3*
µ-Cyhalothrin	449.0>181.2 449.0>197.3	20 20	20 14	17.8*
Cyprodinil	225.1>210.1 225.1>93.1	20 20	20 32	13.9
Dichlorvos	184.9>93.0 220.9>109.0	20 20	20 15	6.1
Fenpropathrin	349.1>265.2 349.1>210.2	20 20	10 20	17.1
Fludioxonil	248.0>127.1 248.0>182.1	20 20	25 20	14.9
Folpet	259.9>130.0 294.9>259.9	20 20	13 10	14.3
Malathion	173.1>127.1 173.1>99.0	20 20	6 10	13.2
Metalaxyl	206.1>132.1 206.1>162.1	20 20	20 8	12.8
Oxyfluorfen	361.0>300.1 361.0>252.2	20 20	10 30	15.1
Permethrin	183.1>153.0 183.1>168.0	20 20	15 15	18.6*
Pyriproxyfen	136.1>78.0 136.1>96.0	20 20	20 20	17.6
Simazine	201.1>173.1 201.1>186.1	20 20	10 8	11.2

*Table 1. MRM transitions (primary transition first), instrument parameters, and observed retention times (RT) for APGC-MS compounds (*signifies compounds with multiple isomers; the most abundant isomer was used for quantification).*

SAMPLE PREPARATION

AOAC QuEChERS Extraction:

Avocado is so high in fat, the AOAC QuEChERS method is modified to reduce the sample size from 15 g to 5 g. Weigh 5 g sample into a 50 mL centrifuge tube (for a spiked sample, add the required volume of spiking standard solution). Add 5 mL water and 15 mL 99:1 acetonitrile/acetic acid. Vortex for 30 seconds and shake well for 2 minutes. Add QuEChERS salts (contents of DisQuE pouch for AOAC, p/n 186006812). Shake the tube vigorously by hand for 1 minute and centrifuge at approximately 2500 rcf for 5 minutes. An aliquot of the supernatant extract (top layer) is taken for analysis.

Pass-through SPE Cleanup:

Install an Oasis PRiME HLB Cartridge (3 cc, 60 mg, p/n 186008056) on a vacuum manifold. Set to minimal vacuum (~2 in Hg). Pass 0.4 mL of the QuEChERS extract through the cartridge to waste. Install collection vessels. Pass 0.6 mL of the QuEChERS extract and collect. In this study for APGC-MS analysis 200 µL of the collected extract is transferred to a Qsert Vial and analyzed directly. Alternatively, a portion of the collected extract can be evaporated and reconstituted in toluene for splitless GC injection.

RESULTS AND DISCUSSION

AOAC QUECHERS EXTRACTION

It is important to distinguish any recovery losses resulting from the SPE cleanup from losses resulting from the initial QuEChERS extraction. Therefore, the modified QuEChERS procedure was evaluated for recovery of the target compounds prior to any SPE recovery experiments. All compounds (spiked at 40 ng/g) were recovered at greater than 80% with the exception of folpet (70%), fenpropathrin (65%), and pyriproxyfen (75%).

OASIS PRIME HLB CLEANUP

SPE cleanup recovery data (see Figure 1) were determined using blank avocado samples obtained using the modified QuEChERS protocol. Blank extracts were spiked at the 9 and 40 µg/kg (ppb) levels and were subjected to the pass-through SPE cleanup protocol. Response for each compound was compared with response obtained from identical blank sample extracts spiked after the SPE cleanup. Only folpet, a thermal and pH labile substance, showed recovery losses greater than 20% resulting from the cleanup protocol. Figure 2 shows that the Oasis PRiME HLB Cartridge cleanup removed greater than 95% of phospholipids from the avocado QuEChERS extract. Also, greater than 90% of chlorophyll and approximately 80% of fat was removed in the cleanup. Cleanup obtained in only seconds with this protocol was comparable to traditional dispersive SPE (dSPE) cleanups that often require multiple cumbersome centrifugation steps.

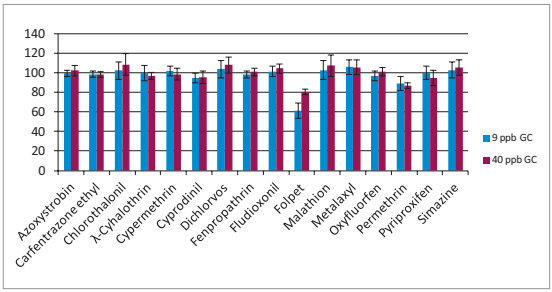

Figure 1. SPE cleanup recovery results.

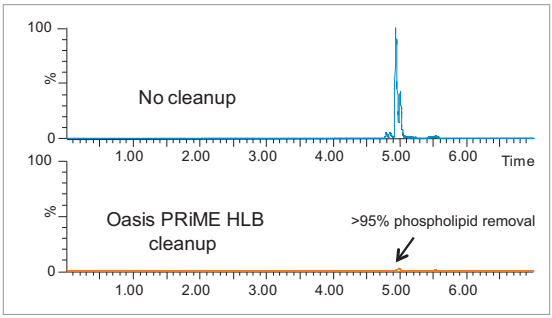

Figure 2. Effective removal of phospholipids from avocado QuEChERS extract with Oasis PRiME HLB cleanup.

APGC-MS/MS

Compared with traditional electron-impact mass spectrometry (EI-MS), the API ionization used for APGC is a softer form of ionization and is often a superior technique for tandem MS. The softer ionization results in less in-source fragmentation and a greater likelihood of obtaining a molecular ion for subsequent fragmentation in the collision cell. An example is shown in Figure 3, a comparison of the API+ and EI+ mass spectra obtained for λ-cyhalothrin. Note the high abundance of the molecular ion (*m/z* 449) in the API spectrum compared with the EI spectrum. In this study, two MRM transitions were monitored for determination of λ-cyhalothrin (see Table 1). Each of these transitions resulted from fragmentation of the *m/z* 449 molecular ion; these transitions would not be possible using EI-MS. Figure 4 shows the APGC-MS/MS determination of the isomers of λ-cyhalothrin spiked at 9 ng/g in avocado after QuEChERS extraction and Oasis PRiME HLB cleanup. Similar low ng/g (ppb) detection limits were observed for all the target compounds in this study.

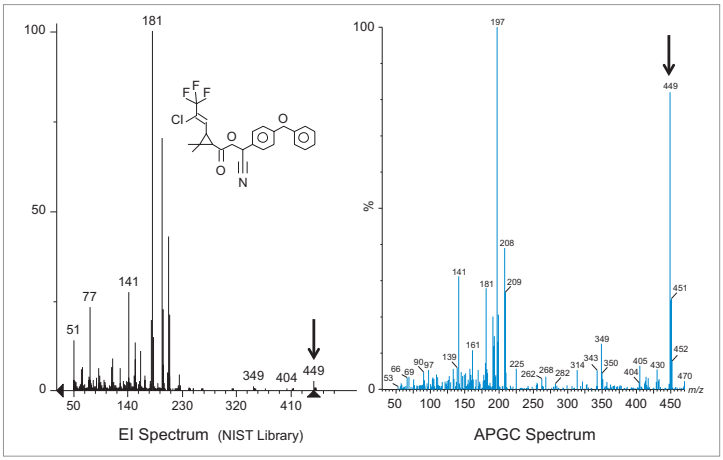

Figure 3. Comparison of mass-spectra for λ-cyhalothrin; EI+ (left) nearly devoid of any molecular ion but API+ (right) shows significant abundance of the molecular ion at m/z 449.

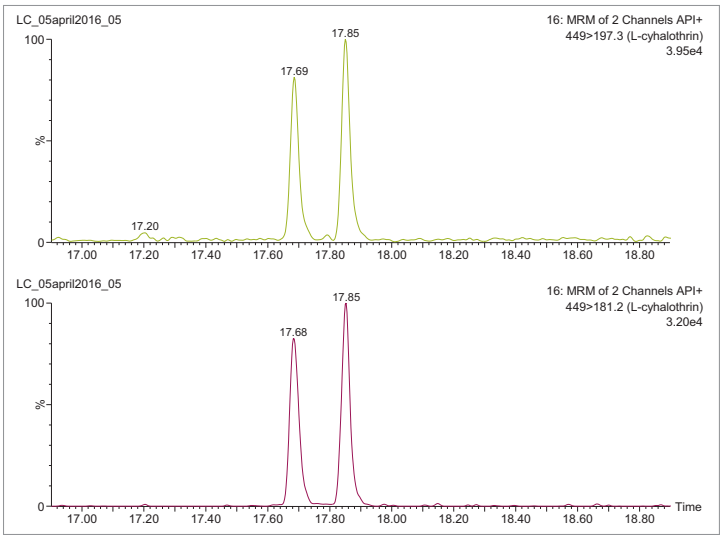

Figure 4. APGC-MS/MS chromatograms showing λ-cyhalothrin isomers from an avocado sample spiked at 9 ng/g (ppb); MRM transitions were obtained from fragmentation of the molecular ion at m/z 449.

CONCLUSIONS

- A modified QuEChERS procedure was effective for extraction of pesticides from avocado prior to APGC-MS/MS analysis.
- Oasis PRiME HLB pass-through cleanup was effective for removal of fats and phospholipids from the QuEChERS extracts.
- Cleanup and recovery obtained using the Oasis PRiME HLB Cartridge was comparable to that obtained from cumbersome and time-consuming multi-step dispersive SPE.
- The Xevo TQ-S Mass Spectrometer operated in APGC-MS/MS mode was effective for low ppb determination of pesticides in avocado.

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Oasis PRiME HLB Cartridges and DisQuE QuEChERS Products for UPLC-MS/MS Mycotoxin Analysis in Cereal Grains

Michael S. Young and Kim Tran
Waters Corporation, Milford, MA, USA

The Oasis PRiME HLB Cartridge and DisQuE Products for QuEChERS provide simple and effective extraction and cleanup of cereal grains prior to UPLC-MS/MS analysis.

GOAL

A simple modified QuEChERS extraction protocol and simple cleanup strategies suitable for multiresidue mycotoxins analysis by UPLC-MS/MS.

BACKGROUND

Mycotoxins are toxic compounds produced by molds or other fungi that can grow on foodstuffs intended for domestic animal or human consumption. Ingestion of food containing only parts-per-billion (µg/kg) concentration of some mycotoxins may cause severe illness. Therefore, sensitive and reliable analytical methods are required to determine mycotoxins in foods and feeds. Cereal grains, such as wheat, rice, and maize are important examples of these types of foods. Many of the natural constituents of these grains are potential interferences for LC-MS/MS analysis. Although proteins and starches are removed during the QuEChERS extraction by partition, precipitation, and centrifugation, significant amounts of fat and lecithins (phospholipids) are co-extracted along with the target mycotoxins.

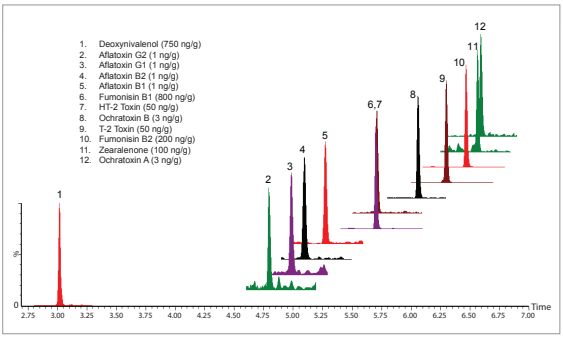

Figure 1. Ion chromatograms obtained from a wheat flour sample fortified with 12 mycotoxins at the indicated levels.

The presence of these co-extracted substances can lead to interference in the LC-MS analysis, contamination of the analytical column and other components of the UPLC system, and contamination of the mass-spectrometer itself. Fats have traditionally been removed from QuEChERS extracts using cumbersome hexane defatting steps or by the use of reversed-phase sorbents such as C_{18}– silica. Although these techniques may be effective for fat removal, neither of these procedures removes phosholipids.

THE SOLUTION

Pass-through cleanup with Oasis® PRiME HLB cartridge for fat and phospholipid removal and dSPE (dispersive SPE) for removal of residual sugars and other polars. The recoveries of the mycotoxins are not compromised using these cleanup protocols.

EXPERIMENTAL

QuEChERS Extraction. Cereal grain flours were purchased at a local grocery store. A 2 g sample was weighed into a 50 mL centrifuge tube. 10 mL water and 10 mL 10:90 formic acid/acetonitrile were added and the sample was placed on automated shaker for 1 hour. Then, QuEChERS salts (contents of DisQuE pouch for CEN, p/n 186006813) were added and the tube was shaken vigorously by hand for 1 minute. After centrifugation (3200 rcf for 5 minutes), a portion of the supernatant was taken for cleanup.

Cleanup. An Oasis PRiME HLB Cartridge (3 cc, 150 mg, p/n 186008717) was mounted on a pre-cleaned vacuum manifold set to minimal vacuum (approx 2 psi). **No cartridge conditioning is required or was performed.** A 0.4 mL aliquot of the supernatant was passed-through the Oasis PRiME Cartridge and discarded. Then a 1 mL portion of the supernatant was passed through the cartridge and collected. The collected extract was then transferred to a 2 mL dSPE tube (p/n 186008081) containing a mixture of sorbents. After centrifugation (1 minute at 13500 rcf), a 500 µL aliquot was taken, evaporated under a gentle nitrogen stream, and reconstituted in 250 µL of 15:85 acetonitrile/water.

INSTRUMENTAL CONDITIONS

UPLC-MS/MS conditions are presented below. Table 1 presents the target compound list, MRM transitions, and mass-spectral conditions used for this study. Six point matrix-matched calibration curves run bracketing the target levels showed good linearity (R2>0.99) for all compounds.

UPLC

LC system:	ACQUITY UPLC® I-Class (FTN)
Column:	CORTECS® UPLC T3, 1.6 µm, 100 x 2.1 mm
Mobile phase A:	0.5% formic acid, 5 mM ammonium formate in water
Mobile phase B:	0.5% formic acid, 5 mM ammonium formate in 50:50 methanol/acetonitrile

Gradient:

Time	%A	%B
Initial	95	5
1	95	5
6	30	70
6.5	5	95
7.5	1	99
9.7	1	99
10	95	5
11	95	5

Injection vol.:	10 µL
Column temp.:	30 °C
Needle wash and Sample manager purge:	1% formic acid, 10 mm citric acid in 1:1:1:1 water/methanol/isopropanol/acetonitrile
Seal wash:	10:90
methanol:	10:90 methanol:water

MS

Mass spectrometer:	Xevo® TQ-S micro
Mode:	Positive ion electrospray
Source temp.:	150 °C
Desolvation temp.:	500 °C
Desolvation gas flow:	1000 L/Hr
Cone gas flow:	30 L/Hr
Data management:	MassLynx® v4.1

RESULTS

Recoveries were measured for 12 mycotoxins at a low and high level. The high level was consistent with EU maximum permitted levels for aflatoxins, fumonisins, ochratoxin A, and zearalenone, and recommended levels for T2 and HT2 toxins (see Figure 1). The low level was 1/4 X the high level (0.25 ng/g for aflatoxins). In wheat flour, total method recoveries for both the low and high level spiked samples were better than 80% for all target compounds except for zearalenone (73%). Similar performance was observed for whole rice and maize flours.

Very little of any recovery loss for any of the toxins was caused by the pass-through or dSPE cleanup steps. Figure 1 shows ion chromatograms obtained from a sample spiked at 0.25 ng/g (ppb) in wheat flour. Figure 2 shows chromatograms that illustrate the effectiveness of the Oasis PRiME Cartridge for phospholipid removal; greater than 95% of phospholipids and greater than 80% of total fats were removed using the Oasis PRiME Cartridge.

Mycotoxin	Retention (min)	MRM transitions	Cone (V)	Collision (eV)
Deoxynivalenol	3.02	297.1 > 249.1 297.1 > 231.1	15 15	15 18
Aflatoxin G2	4.80	331.2 > 245.1 331.2 > 257.1	20 20	25 30
Aflatoxin G1	4.99	329.2 > 283.1 329.2 > 243.1	20 20	25 35
Aflatoxin B2	5.10	315.2 > 259.1 315.2 > 287.1	20 20	33 30
Aflatoxin B1	5.27	313.2 > 241.1 313.2 > 285.1	15 15	40 28
Fumonisin B1	5.70	722.4 > 334.2 722.4 > 352.2	30 30	40 35
HT-2 Toxin	5.72	442.2 > 263.1 442.2 > 215.2	20 20	15 15
Ochratoxin B	6.06	370.1 > 205.1 370.1 > 205.2	20 20	25 24
T-2 Toxin	6.30	484.2 > 305.1 484.2 > 245.1	25 25	9 9
Fumonisin B2	6.47	706.4 > 318.2 706.4 > 336.2	35 35	40 40
Zearalenone	6.57	319.2 > 187.1 319.2 > 283.1	15 15	15 13
Ochratoxin A	6.60	404.2 > 239.1 404.2 > 358.2	20 20	25 16

Table 1. Target compounds, MRM transitions, and mass spectral conditions used for this study (note: For HT-2 and T-2 toxins the ammonium adducts were used for MRMs; to enhance this response, ammonium formate was incorporated in the mobile phase).

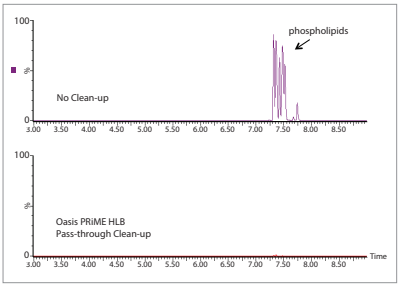

Figure 2. Pass-through cleanup using Oasis PRiME HLB; nearly complete removal of phospholipids from QuEChERS extract of wheat flour.

CONCLUSIONS

- An improved QuEChERS extraction procedure was shown to be effective for simultaneous extraction of 12 mycotoxins from wheat, rice, and maize flours.
- Pass-through cleanup with an Oasis PRiME HLB Cartridge effectively removed greater than 80% of fats and greater than 95% of phospholipids from the QuEChERS extract.
- Polar co-extractables were effectively removed from the QuEChERS extract using a mixed sorbent dSPE cleanup.
- LOQ acceptable for meeting EU regulations was achieved using the sample preparation protocol prior to LC-MS/MS analysis using Xevo TQS micro Mass Spectrometer.

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Rapid, Simple, and Effective Cleanup of Bovine Liver Samples Prior to UPLC-MS/MS Multiresidue Veterinary Drugs Analysis

Michael S. Young and Kim Van Tran
Waters Corporation, Milford, MA, USA

APPLICATION BENEFITS

- Efficient, timesaving multiclass/multiresidue methodology
- Simple, rapid, and effective sample cleanup suitable for a diverse range of analytes
- Fast, sensitive UPLC-MS/MS analysis.

WATERS SOLUTIONS

ACQUITY UPLC® I-Class System

Xevo® TQ-XS Mass Spectrometer

Oasis® PRiME HLB Cartridge for SPE Cleanup

KEY WORDS

UPLC-MS/MS, Oasis PRiME HLB Cartridges, Veterinary Drugs, Beef Liver

OVERVIEW

In order to ensure public health and safety, reliable analytical methods are necessary to determine veterinary drug residue levels in edible tissue samples such as beef liver. The compounds of interest range from highly polar water-soluble compounds to very non-polar fat-soluble compounds. In order to maximize throughput and minimize costs it is desirable to determine the widest possible range of veterinary drug residues in tissue samples with a single analytical method.

INTRODUCTION

Tissue samples, such as bovine muscle and liver, are typically extracted with an acetonitrile based solvent for LC-MS determination of veterinary drug residues. Among the most significant co-extracted substances are fats and polar lipids, particularly phospholipids (lecithin). A gram of bovine liver typically contains about 45 mg of fat, about half the amount usually present in muscle tissue, but still significant. Bovine liver is also a very good source of dietary lecithin (phospholipids); a gram of liver contains about 25 mg of phospholipids, about four times the amount typically found in muscle. Fats can be removed from the acetonitrile based tissue extracts by liquid extraction with hexane or with SPE with octadecyl silica (C_{18}). Although C_{18} is effective for removal of most non-polar lipids, it does not remove phospholipids. Excessive amounts of phospholipids can shorten LC column life, contribute to ion-suppression, and contaminate the mass spectrometer. In this study a novel reversed-phase sorbent, Oasis PRiME HLB, is used for highly effective removal of both phospholipids and fats from bovine liver extracts prior to LC-MS/MS analysis. With the new sorbent recoveries of veterinary drugs were similar to results obtained using C_{18} for cleanup. However, greater than 95% of phospholipids and greater than 85% of fats were effectively removed from the tissue extracts after the simple pass-through SPE procedure.

EXPERIMENTAL

UPLC conditions

LC system: ACQUITY UPLC I-Class with Fixed-Loop Sample Manager

Column: ACQUITY UPLC CSH™ C_{18}, 1.7 µm, 2.1 mm x 100 mm I.D.

Mobile phase: A: 0.1% formic in water

B: 0.1% formic acid in 50:50 acetonitrile/methanol

Injection vol.: 7 µL

Injection mode: Partial loop injection

Column temp.: 30 °C

Weak needle wash: 10:90 acetonitrile:water (600 µL)

Strong needle wash: 50:30:40 water:acetonitrile: IPA (200 µL)

Seal wash: 10:90 acetonitrile: water

Gradient:

Time	Flow (mL/min)	%A	%B
0.00	0.400	99.0	1.0
4.00	0.400	80.0	20.0
5.00	0.400	50.0	50.0
7.00	0.400	1.0	99.0
10.00	0.400	1.0	20.0
10.10	0.400	99.0	1.0
12.00	0.400	99.0	1.0

MS conditions

Mass spectrometer: Xevo TQ-XS

Mode: Positive Ion Electrospray

Source temp.: 150 °C

Desolvation temp.: 400 °C

Desolvation gas flow: 1000 L/Hr

Cone gas flow: 30 L/Hr

Collision gas flow: 0.15 mL/Min

Data management: MassLynx® v4.1

Compound	MRM	Con (V)	Collision (eV)	RT (min)
Amocixicillin	366.2>349.1 366.2>114.1	30 30	8 20	2.46
Ampicillin	350.2>106.1 350.2>160.1	30 30	18 12	4.14
Amprolium	243.3>150.2 243.3>94.1	20 20	12 14	0.54
Bacitracin A	712.2>110.1 712.2>191.1	68 68	70 40	5.72
Ceftiofur	524.3>241.1 524.3>285.0	30 30	16 16	5.98
Chlorotetracycline	479.3>444.2 479.3>462.2	15 15	22 18	5.28
Clopidol	192.1>100.9 192.1>128.0	40 40	26 24	4.10
Clorsulon	378>342.0 378>344.0	22 22	12 12	5.76
Cloxacillin	436.2 >160.0 36.2>277.1	27 27	15 15	6.67
Danofloxacin	358.2>314.1 358.2>96.0	38 38	20 25	4.65
Desethlylene Ciprofloxacin	305.9>268.1 305.9>288.1	32 32	25 18	3.90
Erythromycin	734.7>158.1 734.7>576.5	48 48	26 18	5.72
Eprinomectin	915.6>186.0 915.6154.0	30 30	35 20	7.78
Famphur	326.0>217.0 326.0>93.0	32 32	20 31	6.60
Fenbendazole	300.0>268.0 300.0>159.0	40 40	23 24	6.52
Flunixin	297.2>264.1 297.2>279.0	35 35	34 34	7.19
Ivermection	892.6>307.2 892.6>569.4	15 15	14 25	8.18
Levamisole	205.0>123.0 205.0>90.8	40 40	27 34	2.31
Melengestrol Acetate	397.4>337.3 397.4>279.0	10 10	15 15	7.30
Monesin	693.7>675.3 693.7>461.1	70 70	35 50	8.13
Morantel	221.2>186.1 221.2>108.0	20 20	20 25	5.44
Moxidectin	640.0>528.4 640.0>498.3	30 30	10 10	7.96
Noviobiocin	613.10>188.9 613.1>396.0	45 45	20 15	7.45
n-methyl-1 3-propanediamine	89.1>72.2 89.1>58.2	42 42	5 5	0.41
Oxfendazole	316.2>191.1 316.2>284.0	40 40	18 18	5.76
Oxteracyline	461.4>426.2 461.4>365.0	48 48	30 15	4.36
Penicillin G	335.2>289.1 335.2 >158.1	40 40	25 25	5.54
Progesterone	315.2>109.0 315.2>97.0	38 38	24 22	7.30
Ractopamine	302.2>164.1 302.2>284.2	35 35	15 12	4.30
Sulfachlorpyridazine	285.0>156.0 285.0>92.1	35 35	16 26	5.44
Sulfadimethoxine	311.1>156.0 311.1>92.0	36 36	32 32	5.89
Sulfamethazine	279.1>186.0 279.1>124.1	40 40	15 25	4.92
Sulfaquinoxaline	301.1>156.1 301.1>92.2	32 32	16 30	5.93
Tetracycline	445.1>154.0 445.1>410.1	40 40	26 22	4.43
Thiabendazole	202.0>175.0 202.0>131.0	15 15	25 30	3.46
Tilmicosin	869.5 >174.2 869.5>696.5	25 25	45 40	5.35
Tripelennamine	256.1>211.1 256.1>91.0	21 21	17 33	3.87
Tylosin	916.5>174.1 916.5>101.1	45 45	40 45	5.78
Zilpaterol	262.2>202.1 262.2>185.1	25 25	18 22	0.79

Table 1. MRM transitions (primary transition first) and instrument parameters used for this study; also listed are the observed retention times (RT) for the compounds.

SAMPLE PREPARATION

1. Initial Extraction/Precipitation:
A 2 g sample of tissue was placed into a 15 mL centrifuge tube containing ceramic homogenizer balls (a Bertin Technologies Precellys Evolution Homogenizer was used for this step). For standards or QC samples the samples were spiked with appropriate amounts of desired analytes. 10 mL 0.2% formic acid in 85:15 acetonitrile/water was added and the samples were homogenized/extracted for 1.5 minutes. The tubes were then centrifuged at 3200 rcf for 5 minutes.

Note: The extraction/precipitation step gives good recovery of most compounds of interest but also extracts significant amounts of fats and phospholipids.

2. Pass-through SPE cleanup:
An Oasis PRiME HLB Cartridge (6 cc, 200 mg) was mounted on a pre-cleaned vacuum manifold. Cartridge conditioning is NOT required, and was NOT performed. The vacuum was set to 2 psi. A 0.6 mL portion of the supernatant was passed-through the Oasis PRiME Cartridge and discarded. Collection tubes were then installed and a 1 mL portion of the supernatant was passed-through the Oasis PRiME Cartridge and collected. A 200 µL aliquot of the pass-through cleanup sample was taken and diluted with 400 µL of 10 mM ammonium formate buffer (pH 4.5) prior to UPLC-MS/MS analysis.

RESULTS

Figure 1 shows the recovery data obtained from replicate analysis of spiked tissue samples (n = 6). Matrix effects averaged about 40%. The chromatograms shown in Figure 2 show the effectiveness of the Oasis PRiME HLB Cartridge for removal of ≥95% of phospholipids from the shrimp extracts. The cartridge also removes more than 90% of hexane extractable fat.

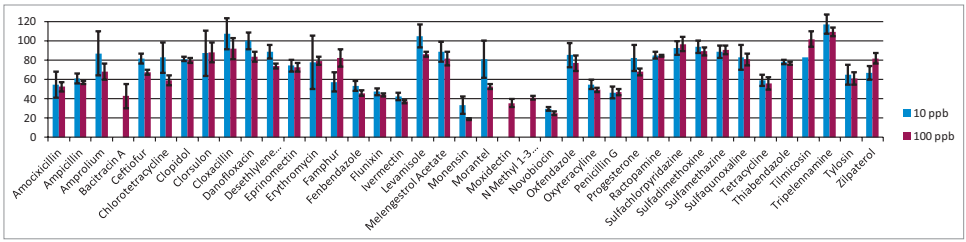

Figure 1. Recovery data from spiked beef liver sample for low level (10 ng/g in blue) and high level (100 ng/g) in red.

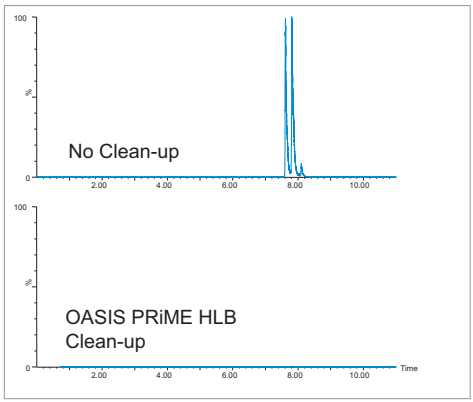

Figure 2. LC-MS/MS chromatograms showing effective removal of ≥95% of phospholipids from beef liver extract

DISCUSSION

The procedure utilized in this study was developed from methods presented previously.[1,2] Although the overall method recoveries averaged above 70 percent, lower recovery was observed for some of the more polar compound classes, such as tetracyclines. Unfortunately, no single solvent extraction step will be highly efficient for all target compounds. For most of the lower recovered compounds the signal response and reproducibility are acceptable for target screening analysis. It is important to understand the contribution of the sample cleanup to any observed recovery losses. The SPE recovery data shown in Figure 3 were obtained from beef liver samples spiked after solvent extraction and prior to SPE cleanup. These data indicate that, for most of the compounds, the Oasis PRiME HLB Cartridge cleanup contributes little to the observed recovery losses. However, for ivermectin, monensin, moxidectin, and novabiocin, the post extraction cleanup did introduce measurable recovery losses. More information on these analytes will be presented in future work.

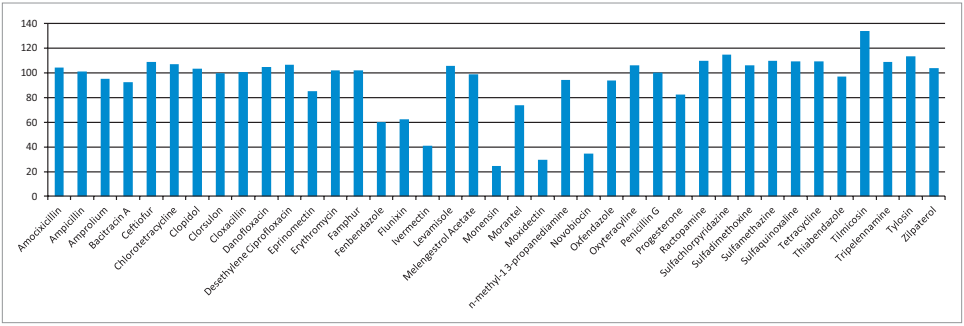

Figure 3. Recovery of veterinary compounds from blank beef liver extracts spiked after initial extraction and prior to Oasis PRiME HLB pass-through cleanup.

CONCLUSIONS

- A simple and effective extraction/protein precipitation procedure was developed for screening analysis of bovine liver tissue for a wide range of veterinary drugs
- A simple pass-through cleanup protocol using Oasis PRiME HLB Cartridges was employed to remove greater than 90% of fats and phospholipids from the initial extracts
- The sample preparation methodology produced an extract that was free of particulates and required no subsequent filtration prior to LC-MS analysis
- Consistent recoveries were observed for a wide range of veterinary drugs using the simple one-step pass-through cleanup protocol with Oasis PRiME HLB Cartridges

References

1. M. Young and K. Tran, "Oasis PRiME HLB Cartridge for Effective Cleanup of Meat Extracts Prior to Multi-Residue Veterinary Drug UPLC-MS Analysis", Waters Application Brief, 720005411EN, 2015.
2. S. Lehotay, "High-Throughput Screening Analysis by UHPLC-MS/MS of >60 Veterinary Drugs in Animal Tissues", 125th AOAC Annual Meeting, Presentation 2303, 21 September, 2011.

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Oasis PRiME HLB Cartridges for Rapid and Effective Removal of Chlorophyll from QuEChERS Spinach Extracts

Michael S. Young and Jeremy C. Shia
Waters Corporation, Milford, MA, USA

Oasis PRiME HLB Cartridges provide rapid removal of chlorophyll from spinach samples after QuEChERS extraction. This technology brief demonstrates Oasis PRiME HLB Sorbent in both Vac and Plus Formats.

GOAL

A simple, quick, and effective simple cleanup strategy to remove chlorophyll from QuEChERS extracts prior to GC-MS/MS analysis.

Demonstrating the flexible use of Oasis® PRiME HLB Plus Short and Plus Light Cartridge formats suitable for processing samples with or without the need for a vacuum or positive pressure manifold.

BACKGROUND

The QuEChERS method is highly effective for extraction of a wide range of pesticides from fruits and vegetables. After initial QuEChERS extraction, dispersive SPE (dSPE) is typically used for cleanup prior to chromatographic analysis. A combination of various sorbents is dispersed into an aliquot of the acetonitrile based extract for selective removal of potential interferences. Among the interfering substances found in many vegetables, chlorophyll is particularly bad for gas chromatography; only a few injections of a high chlorophyll extract can result in severe contamination of the injection port and column head.

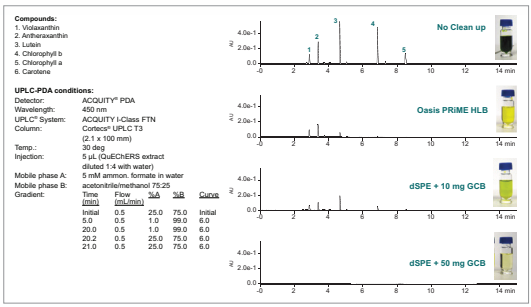

Figure 1. UPLC-PDA chromatograms showing removal of pigments from spinach extracts using the three cleanup protocols.

The most common sorbent used for removal of chlorophyll is graphitized carbon black (GCB). Although GCB is effective for removal of chlorophyll, recovery losses can occur for some pesticides, particularly those that have a planar geometry. Therefore, an alternative to GCB is desirable.

THE SOLUTION

Pass-through cleanup using Oasis PRiME HLB Cartridges is an effective alternative to dSPE with GCB with no loss of planar pesticides.

EXPERIMENTAL

Test Compounds. In a previous study,[1] good recoveries were shown for a wide variety of pesticides after cleanup using Oasis PRiME HLB Cartridges. Among those pesticides, there are three planar pesticides commonly used on high chlorophyll commodities, cyprodinil, chlorothalonil, and thiabendazone. These three compounds were chosen for this study and were spiked into the spinach sample at a concentration of 50 µg/kg (ppb).

QuEChERS Extraction. Raw spinach purchased at a local grocery store. A 15 g sample of homogenized sample was weighed into a 50 mL centrifuge tube and spiked with the test compounds. 15 mL 1:99 acetic acid/acetonitrile were added and the sample was manually shaken for 1 minute. Then, QuEChERS salts (contents of DisQuE™ pouch for AOAC QuEChERS, p/n 186006812) were added and the tube was shaken vigorously by hand for 1 minute. After centrifugation (3200 rcf for 5 minutes), portions of the supernatant were taken for cleanup using these two technique: by dSPE and by pass-through cleanup with Oasis PRiME HLB Cartridges.

Cleanup (dSPE). Into a 2 mL centrifuge tube was weighed 150 mg anhydrous sodium sulfate, 50 mg C_{18} silica, 50 mg PSA (primary/secondary amine silica) and 50 mg GCB. A second 2 mL tube was prepared with the same sorbents except with 10 mg GCB. A 1 mL portion of supernatant was transferred to each tube and the tubes were shaken by hand for 1 minute. After centrifugation (1 minute at 13500 rcf), a portion of sample was transferred to an auto-sampler vial for analysis by APGC-MS. Another portion of the sample (100 µL) was transferred to a separate vial and diluted with 400 µL water for UPLC-MS analysis.

Cleanup (Using Oasis PRiME HLB Vac Style Cartridges). An Oasis PRiME HLB Cartridge (3 cc, 150 mg) was mounted on a pre-cleaned vacuum manifold set to minimal vacuum (approximately 2 psi). **No cartridge conditioning is required or was performed**. A 0.8 mL aliquot of the supernatant was passed-through the Oasis PRiME HLB Cartridge and discarded. Then a 1.5 mL portion of the supernatant was passed through the cartridge and collected. Samples were then taken for APGS-MS and UPLC-MS analysis in the same manner as the dSPE samples.

Cleanup (Using Oasis PRiME HLB Plus Style Cartridges). No cartridge conditioning was performed. A 3 mL syringe was attached to the Oasis PRiME HLB Plus Light Cartridge used for cleanup. A 0.7 mL aliquot of the supernatant was passed through the Oasis PRiME HLB Cartridge and discarded. Then a 0.8 mL portion of the supernatant was passed through the cartridge and collected. A similar procedure was performed with the Plus Short format using a 6 mL syringe. A 2 mL aliquot of the supernatant was passed-through the Oasis PRiME HLB cartridge and discarded. Then a 3 mL portion of the supernatant was passed through the cartridge and collected. Samples were then prepared for APGS-MS and UPLC-MS analysis in the same manner as the dSPE samples.

APGC conditions:

GC system:	Agilent 7890
Column:	Restek Rxi-5 ms, 30 m x 0.25 mm x 0.25 µm
Flow rate:	1.0 mL/min Helium
Injection vol.:	1 µL (15:1 split)
Temp. program:	80 °C initial, hold for 0.5 min, 12 °C /min to 320 °C and hold for 8 min

MS conditions:

Mass spectrometer:	Xevo® TQ-S
Ion mode:	API+ (MRM mode)
Corona:	2.8 µA
Source temp.:	150 °C
Probe temp.:	450 °C
Cone gas:	170 L/Hr
Auxiliary gas:	170 L/Hr
Collision gas:	0.15 mL/min (Ar)
Nebulizer:	4.0 bar
Data Management:	MassLynx v4.1

MRM transitions:

Cyprodinil:	225.1>210.1 (20 V cone, 20 eV collision) 225.1>93.1 (20 V cone, 32 eV collision)
Chlorothalonil:	265.9>170.0 (20 V cone, 27 eV collision) 265.9>230.9 (20 V cone, 27 eV collision)

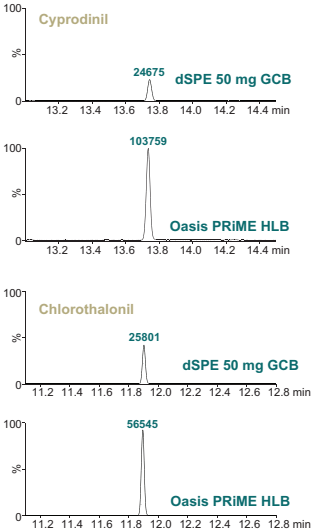

Figure 2. APGC-MS/MS ion chromatograms showing improved recovery for planar pesticides cyprodinil and chlorothalonil after cleanup with the Oasis PRiME HLB Cartridge compared with dSPE cleanup with graphitized carbon.

UPLC conditions:

UPLC system:	ACQUITY® UPLC® I-Class
Column:	ACQUITY UPLC BEH™ C_{18}, 1.7 µm, 100 x 2.1 mm
Mobile phase A:	10 mm ammonium acetate in water (pH 5.0)
Mobile phase B:	10 mm ammonium acetate in 99:1 methanol/water
Injection vol.:	5 µL
Column temp.:	45°C
Gradient:	2 % B initial, hold to 0.25 min, to 99 % B at 12.25 min, hold to 13.0 min, back to 2 % B at 13.01 min and hold to 17.0 min

MS conditions:

Mass spectrometer:	Xevo TQ-S micro
Ion mode:	ESI+ (MRM mode)
Source temp.:	150 °C
Desolvation temp.:	400 °C
Desolvation gas:	650 L/Hr (N_2)
Collision gas:	0.18 mL/min (Ar)
Data management:	MassLynx® v4.1

MRM transitions:

Thiabendazole:	202.0>175.0 (51 V cone, 25 eV collision) 202.0>131.0 (51 V cone, 30 eV collision)

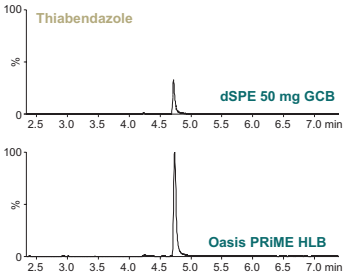

Figure 3. UPLC-MS/MS ion chromatograms showing improved recovery for planar pesticide thiabendazole after cleanup with the Oasis PRiME HLB Cartridge compared with dSPE cleanup with graphitized carbon.

INSTRUMENTAL ANALYSIS

Chlorophyll removal from the extracts was monitored using UPLC coupled to a photo-diode array detector (PDA). Pesticide concentrations were measured using APGC-MS/MS for cyprodinil and chlorothalonil, and using UPLC-MS/MS for thiabendazole.

RESULTS

All three cleanup methods were effective for removal of the majority of chlorophyll and carotenes from the QuEChERS extract of spinach. Pass-through cleanup with the Oasis PRiME HLB Cartridge was slightly better than dSPE with 10 mg GCB for removal of chlorophyll. dSPE with 50 mg GCB per mL extract was the only cleanup that effectively remove all pigments from the QuEChERS extract. However, significant losses of planar pesticides were observed using dSPE with 50 mg GCB. In contrast, little or no recovery losses were observed for the three planar pesticides with Oasis PRiME HLB cleanup or using dSPE cleanup with 10 mg GCB per mL extract. Figure 1 shows UPLC-PDA chromatograms illustrating removal of the pigments using the three cleanup protocols. Figure 2 shows APGC-MS/MS ion chromatograms illustrating the recovery losses for cyprodinil and chlorothalonil. Figure 3 shows a UPLC-MS/MS ion chromatogram illustrating recovery loss for thiabendazole.

The Oasis PRiME HLB Cartridge is available in various sizes and formats. The "vac" type cartridges are most convenient for use with vacuum/positive pressure manifold while the "plus" type cartridges are suitable for use with a syringe (similar to a syringe filter) or with a vacuum/positive pressure manifold. The choice of cartridge size is made based on the volume of extract required by the analyst. Figure 4 illustrates this cartridge choice; no difference was seen in total pigment removal or pesticide recovery among the three cartridge choices.

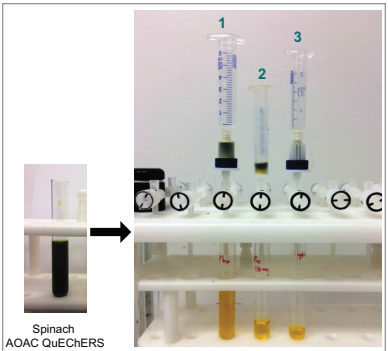

Figure 4. Oasis PRiME HLB in plus type cartridges provide identical cleanup compared with the traditional vac style cartridge.

CONCLUSIONS

- For the QuEChERS spinach extraction, significant amounts of chlorophyll and other pigments are co-extracted along with the target pesticides.
- Pass-through clean-up with an Oasis PRiME HLB Cartridge effectively removed greater than 99% of chlorophyll and greater than 95% of lutein from the QuEChERS extract.
- dSPE clean-up with 10 mg GCB (per mL extract) was less effective compared with the Oasis PRiME HLB Cartridge for removal of chlorophyll and lutein from the QuEChERS extract.
- dSPE cleanup with 50 mg GCB (per mL extract) removed all pigments from the QuEChERS extract, but significant loss of planar pesticides was observed.

References

1. Oasis PRiME HLB Cartridge for Rapid and Effective Cleanup of Avocado, A High Fat Matrix, Prior to APGC-MS/MS Analysis, Waters Application Note 720005816EN, 2016.

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Oasis PRiME HLB Cartridges Now Available in Syringe Compatible Plus Format

Michael S. Young and Jeremy C. Shia
Waters Corporation, Milford, MA, USA

Oasis PRiME HLB Cartridges provide rapid sample cleanup of many types of food matrices in food safety analysis. Now, Oasis PRiME HLB cartridges are available in "Plus" format with luer fittings. These versatile cartridge formats are compatible with standard syringes or can be fitted with appropriate reservoirs for use with vacuum or positive pressure manifolds.

GOAL

To demonstrate Oasis® PRiME HLB Cartridges in formats suitable for processing samples with or without the need for a vacuum/positive pressure manifold.

BACKGROUND

Oasis PRiME HLB Cartridges are effective for rapid pass-through cleanup of various food matrices infood safety analyses. These include pesticides in fruits and vegetables, as well as antibiotic residues in meats and fish. For these cleanups, the traditional "Vac" style cartridges are most conveniently used with vacuum manifolds. These cartridges are available in many sizes; the choice is made based on the volume of extract required by the analyst. In addition, the sample cleanup is performed without the cumbersome centrifugation steps required with dispersive cleanup procedures. However, there is also a need for cartridges that can be used for pass-through cleanup without a processing manifold.

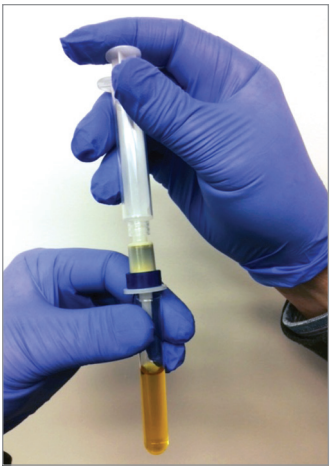


Figure 1. Oasis PRiME HLB Cartridge used in pass-through cleanup of a QuEChERS spinach extract.

THE SOLUTION

The Oasis PRiME HLB Sorbent is now available in "Plus" type cartridges. These cartridges are easily connected to a syringe (in a manner similar to a syringe filter). Alternatively, when fitted with an appropriate reservoir, they can be used with vacuum manifolds. Figure 1 shows an Oasis PRiME HLB Cartridge used for pass-through cleanup of QuEChERS spinach extract in the manual mode with a syringe.

EXPERIMENTAL

In previous studies, good recoveries were shown for a wide variety of pesticides in avocado[1] and in spinach[2] after cleanup using Oasis PRiME HLB Cartridges in 'Vac" formats. In this application brief, similar cleanups were performed using Oasis PRiME HLB Cartridges in the "Plus" formats.

QuEChERS Extraction. A 15 g homogenized sample was weighed into a 50 mL centrifuge tube. 15 mL 1:99 acetic acid/acetonitrile were added and the sample was manually shaken for 1 minute. Then, QuEChERS salts (contents of DisQuE Pouch for AOAC QuEChERS, p/n 186006812) were added and the tube was shaken vigorously by hand for 1 minute. After centrifugation (3200 rcf for 5 minutes), portions of the supernatant were taken for cleanup with Oasis PRiME HLB Cartridges.

Cleanup. **No cartridge conditioning was performed.** A 3 mL syringe was connected for cleanup using Oasis PRiME HLB in the Plus Light format and a 6 mL syringe was connected for cleanup using Oasis PRiME HLB in the Plus Short format. The extract was delivered by syringe in a manner to obtain a dropwise flow through the cartridge. A vacuum manifold was used for the "Vac" style cartridge formats. For all cartridge types, an initial portion of the QuEChERS extract (supernatant) was sent to waste after passing through the cartridge and a second portion was passed through the cartridge and collected. The volumes used for each type of cartridge are presented in Table 1.

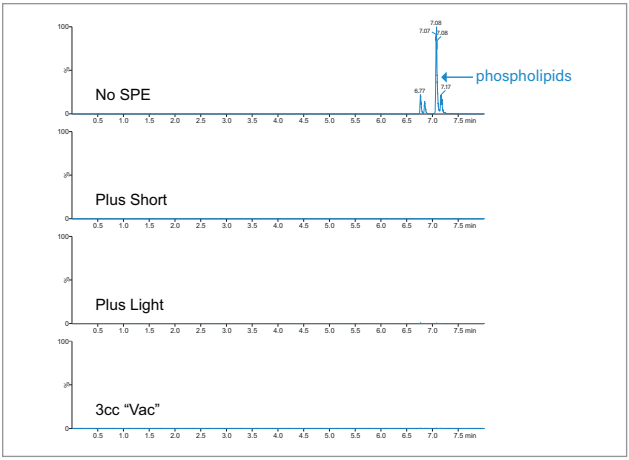


Figure 2. Equivalent removal of phospholipids from avocado QuEChERS extract using various Oasis PRiME HLB Cartridge formats (UPLC-MS/MS).

Compounds:
1. Violaxanthin
2. Antheraxanthin
3. Lutein
4. Chlorophyll b
5. Chlorophyll a
6. Carotene

UPLC-PDA Conditions:

Detector:	ACQUITY® PDA®
Monitored wav:	450 nm
UPLC system:	ACQUITY UPLC I-Class FTN
Column:	CORTECS® UPLC T3 (2.1 x 100 mm)
Temp.:	30 deg
Injection Vol.:	5 µL (QuEChERS extract diluted 1:4 with water)
Mobile phase A:	5 mM ammon. formate in water
Mobile phase B:	acetonitrile/methanol 75:25

Gradient:

Time (min)	Flow (mL/min)	%A	%B	Curve
Initial	0.5	25	75	Initial
5.0	0.5	1	99	6
20.0	0.5	1	99	6
20.2	0.5	25	75	6
21.0	0.5	25	75	6

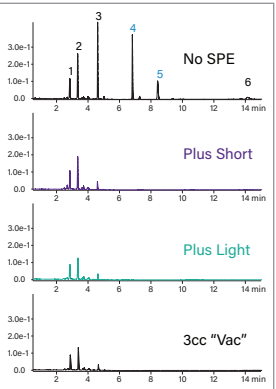
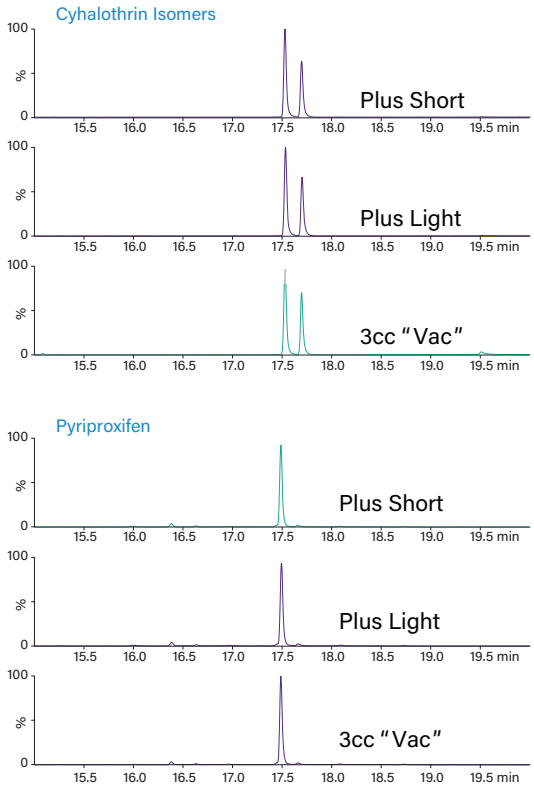


Figure 3. Effective and equivalent removal of chlorophyll from spinach QuEChERS extract using various Oasis PRiME HLB Cartridge formats.

Cartridge	Discard volume	Collect volume
3 cc 60 mg "Vac"	0.4 mL	0.6 mL
6 cc 150 mg "Vac"	0.8 mL	1.5 mL
Plus Light	0.6 mL	1 mL
Plus Short	2 mL	3 mL

Table 1. Volumes used for pass-through cleanup for each type of cartridge.



APGC conditions:

GC system:	Agilent 7890
Column:	Restek Rxi-5ms, 30 m x 0.25 mm x 0.25 µm
Flow rate:	1.0 mL/min Helium
Injection vol.:	1 µL (15:1 split)
Temperature program:	80 °C initial, hold for 0.5 min, 12 °C /min to 320 °C and hold for 8 min

MS conditions:

Mass spectrometer:	Xevo® TQ-S
Ion mode:	API+ (MRM mode)
Corona:	2.8 µA
Source temp.:	150 °C
Probe temp.:	450 °C
Cone gas:	170 L/Hr
Auxiliary gas:	170 L/Hr
Collision gas:	0.15 mL/min (Ar)
Nebulizer:	4.0 Bar
Data management:	MassLynx® v4.1

MRM transitions:

Cyhalothrin:	449.0>181.2 (20 V cone, 20 eV collision)
	449.0>197.3 (20 V cone, 14 eV collision)
Pyriproxifen:	136.1>78.0 (20 V cone, 20 eV collision)
	136.1>96.0 (20 V cone, 20 eV collision)

Figure 4. Equivalent recovery of pesticides from spinach QuEChERS extract using various Oasis PRiME HLB Cartridge formats.

INSTRUMENTAL ANALYSIS

Chlorophyll removal from spinach extracts was monitored using UPLC coupled to a photodiode array detector (PDA). Phospholipid removal from avocado extracts was monitored using UPLC-MS/MS. Pesticide analysis was accomplished using APGC-MS/MS. Details for these analyses are given in references 1 and 2.

RESULTS

No significant difference was seen among all cartridge types tested for any of the relevant cleanup or recovery parameters measured in this study. Phospholipid removal (see Figure 2), chlorophyll removal (see Figure 3), and pesticide recovery (see Figure 4) was virtually the same for "Vac" type cartridges processed using a vacuum manifold, or for "Plus" type cartridges processed by hand via syringe.

The choice of cartridge size is made based on the volume of extract required by the analyst. Figure 5 illustrates this cartridge choice.

CONCLUSIONS

- In addition to traditional "Vac" formats, Oasis PRiME HLB Cartridges are available in two "Plus" formats, Plus Light/100 mg and Plus Short/335 mg, suitable for manual syringe cleanup.
- No difference was seen for cleanups using either cartridge format.
- Pass-through cleanup with an Oasis PRiME HLB Cartridge effectively removes greater than 99% of chlorophyll and 95% of phospholipids from QuEChERS extracts.
- Pass-through cleanup with an Oasis PRiME HLB Cartridge is an effective alternative cleanup for QuEChERS and similar acetonitrile based extraction methods.

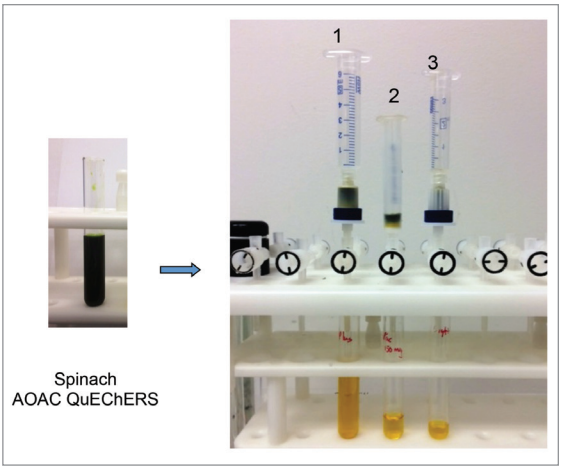


Figure 5. Oasis PRiME HLB in plus type cartridges provide identical cleanup compared with the traditional vac style cartridge.

References

1. Oasis PRiME HLB Cartridge for Rapid and Effective Cleanup of Avocado, A High Fat Matrix, Prior to APGC-MS/MS Analysis, Waters Application Note 720005816EN, 2016.
2. Oasis PRiME HLB Cartridges for Rapid and Effective Removal of Chlorophyll From QuEChERS Spinach Extracts, Waters Technology Brief 720005994EN, 2017.

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