A NEW SORBENT FOR CLEANUP OF SEAFOOD, INFANT FORMULA, AND OTHER FATTY MATRIX EXTRACTS

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INTRODUCTION

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 and infant formula powder. The compounds of interest range from polar water-soluble compounds to 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.

The major constituents of a typical meat or seafood sample are water (up to 70%), protein (15-25%), fat (5-25%) and phospholipids (lecithin, 1-3%). Infant formula powder contains protein (16%), fat (12-16%) and lecithin (phospholipids) 0.3 %.

The protein is removed in an initial solvent extraction step 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 C18-silica. Although these techniques may be effective for fat removal, neither of these procedures removes phosholipids. Recently sample preparation, cleanup, and analysis protocols were developed for tandem LC/MS determination of a wide variety of veterinary drug residues in pork[1] and milk[2]. This cleanup protocol was effective for removal of both fats and phospholipids. In this study the methodology was applied to the analysis of salmon and shrimp (prawn) tissue samples and also to infant formula powder. 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 (see Figures 1 and 3, right). Representative compounds were chosen from major classes of veterinary drugs including tetracyclines, fluoroquinolones, sulfonamides, macrolides, beta-lactams, NSAIDS, steroids and beta-andrenergics (see Figure 2, below). These compounds were spiked into the seafood samples prior to extraction and cleanup

Oasis PRiME HLB is a patent-pending reversed-phase SPE sorbent
- Ideal for samples that contain proteins, fats, and phospholipids (lecithins)

SIMPLER: Streamlined protocols, **no conditioning and equilibration steps required!**

FASTER: Faster and more even flows through devices with less plugging and faster overall processing

CLEANER: Reduced matrix effects, phospholipid *and* fat removal, less column and/or instrument contamination

1-Step Pass-Thru
Pass-Thru and Collect
Matrix Interferences Retained
for acetonitrile based tissue extracts
(like this study)

3-Step Catch and Release
Load
Analytes Retained
Wash
Interferences Removed
Elute
for aqueous samples
(like plasma)

Figure 1. Oasis PRiME HLB cartridge/plate protocols

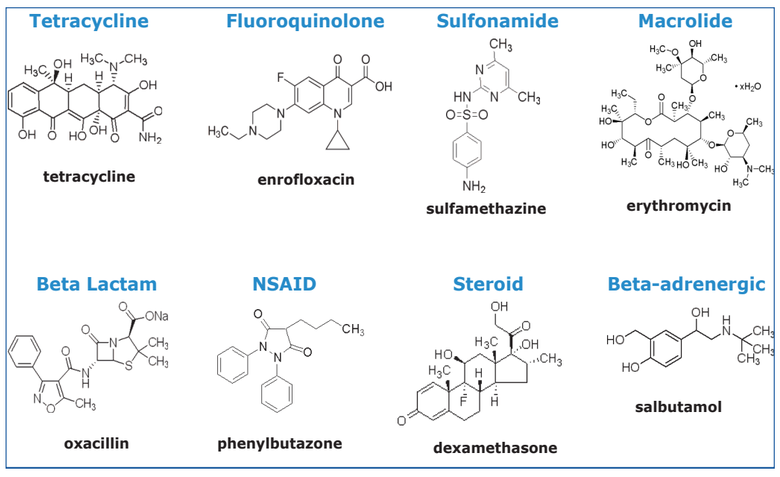

Figure 2. Examples of various classes of veterinary drugs included in this study

METHODS

SAMPLE PREPARATION

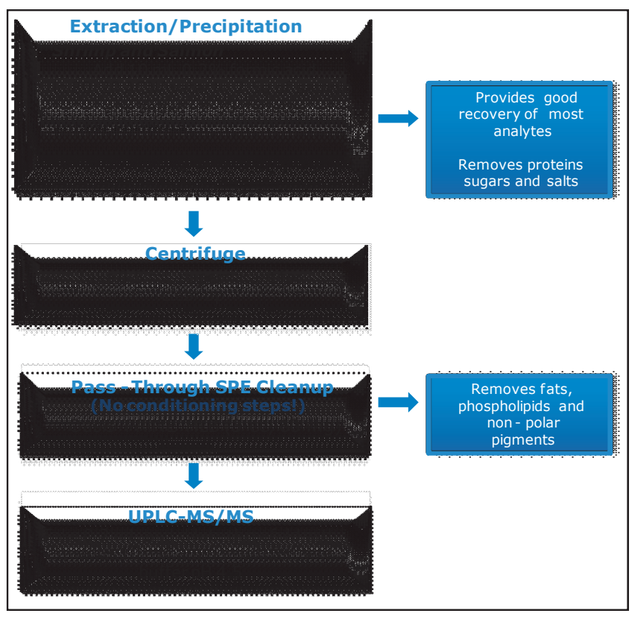

Figure 3. Sample preparation and cleanup for tissue and infant formula samples

UPLC-MS/MS ANALYSIS

UPLC Conditions

LC system: ACQUITY UPLC I-Class
Column: ACQUITY UPLC CSH™ C18, 1.7µm, 100 mm x 2.1 mm ID
Mobile phase:
A: 0.1% formic in water
B: 0.1% formic acid in acetonitrile
Injection volume: 5 µL
Injection mode: partial loop injection
Column temperature 30 °C
Weak Needle Wash: 10:90 acetonitrile:water
Strong Needle Wash: 50:30:20 water:acetonitrile:IPA
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: Waters Xevo TQ-S
Positive Ion Electrospray (negative ion for chloramphenicol)
Source Temperature: 150°C
Desolvation Temperature: 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

Other instrument and calibration pararameters are presented in Table 1 (below).

Compounds	MRM	Cone (V)	Collision (eV)	Spike Level (low,high) (µg/kg)	Calibration Range (µg/kg)	Corr R^2	RT (min)
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	26	25, 100	12.5-200	0.9952	1.06
	461.2>443.1	30	21				
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 observed retention times (RT) for this study

RESULTS

Figure 4 shows the overall method recovery data obtained from replicate analysis of spiked salmon samples (shrimp data were similar). Matrix effects averaged about 40% for both shrimp and salmon. Figure 5 shows the overall method recovery data obtained from replicate analysis of spiked of infant formula powder. Matrix effects averaged about 30% for infant formula powder.

The chromatograms shown in Figure 6 show the effectiveness of the Oasis PRiME HLB cartridge for removal of ≥95% of phopholipids from the salmon extract. Based on gravimetric measurements, the cartridge also removes more than 90% of hexane extractable fat from the extract. Similar performance was observed for shrimp and infant formula.

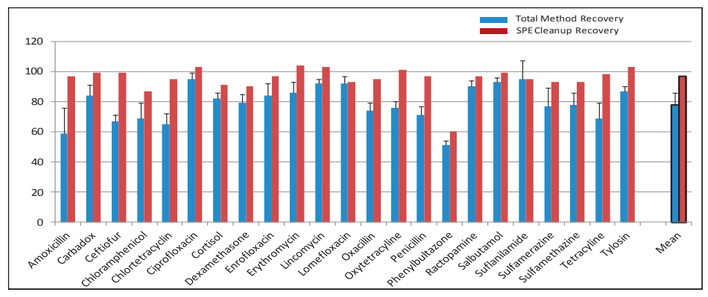

Figure 4. Recovery of veterinary drugs from salmon tissue (% RSD indicated with error bars)

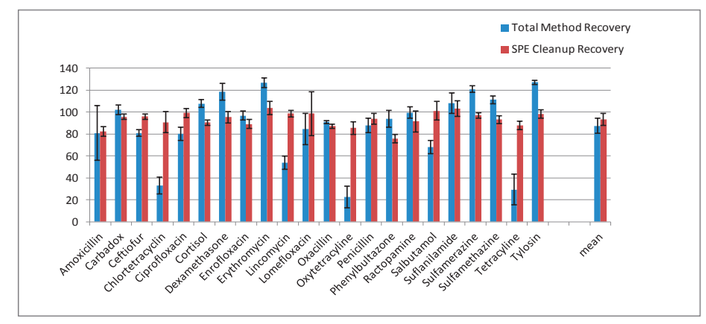

Figure 5. Recovery of veterinary drugs from infant formula powder (% RSD indicated with error bars)

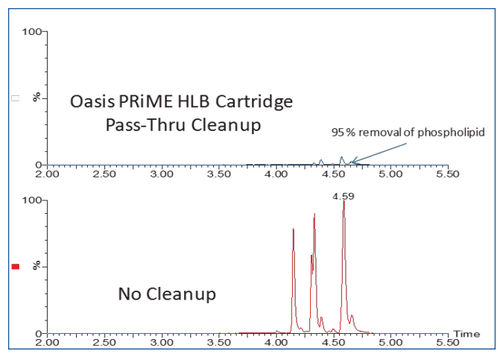

Figure 6. LC-MS/MS chromatograms showing effective removal of ≥95% of phospholipids from shrimp extract

DISCUSSION

Although the tissue extraction protocol used in this study is generally effective, this is a multi-class, multi-residue method and overall method recoveries for some compounds are below 70% (see blue bars on Figures 4 and 5). However, the Oasis PRiME HLB cartridge cleanup contributes very little to the overall method recovery losses (see red bars on Figure 3 and 4). The recovery for the SPE cleanup step is better than 80% in shrimp and better than 90% in salmon for all analytes except phenylbutazone. Unlike seafood tissue (approx 70 % water) there is little water in infant formula powder; therefore, more water was used in the extraction solution for this matrix. For optimal cleanup, the extract applied to the cleanup cartridge should be close to 70 % acetonitrile for all matrices.

CONCLUSIONS

- **A simple and effective extraction and protein precipitation procedure was applied to the analysis of shrimp, salmon tissue and 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**
- **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**

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, 2015 (720005411en)

2. D. Huang , K. Tran, and M. Young, A simple Cleanup Protocol Using a Novel SPE Device for UPLC-MS/MS Analysis of Multi-Residue Veterinary Drugs in Milk, Waters Application Note, 2015 (720005414en)

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