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

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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

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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

1. 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.

2. 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).

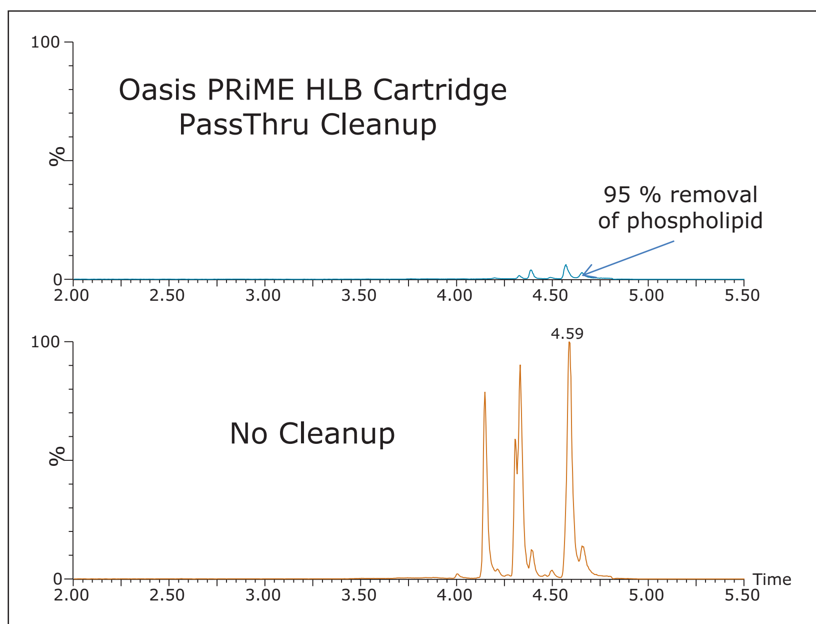

Figure 1. LC-MS/MS chromatograms showing effective removal of ≥95% of phosholipids from shrimp extract.

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

References

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