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Rapid, Simple, and Effective Clean-up of Bovine Liver Samples 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 clean-up 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 Clean-up

KEYWORDS

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 clean-up. 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	Cone (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 clean-up:

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 clean-up 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 beef liver extracts. The cartridge also removes more than 90% of hexane extractable fat.

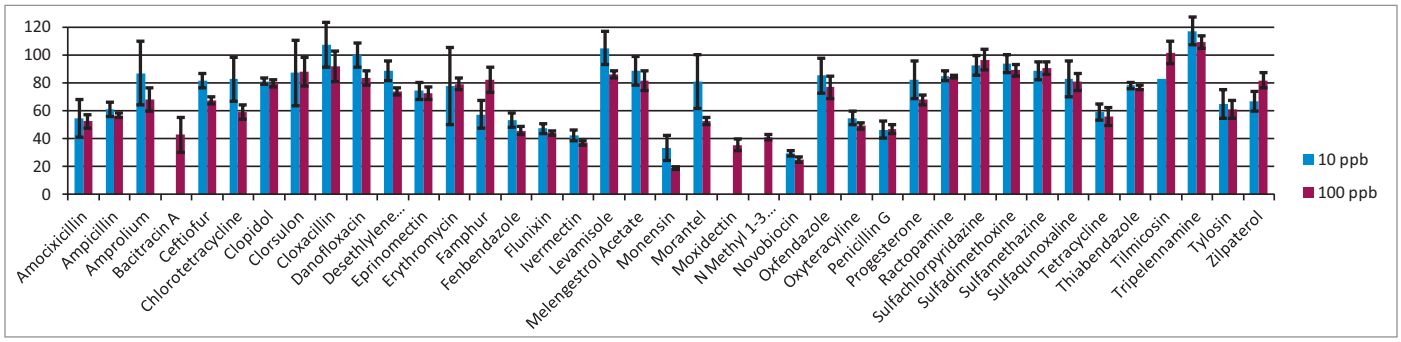

Figure 1. Recovery data from spiked beef liver sample for low level (10 ng/g in blue) and high level (100 ng/g) in red.

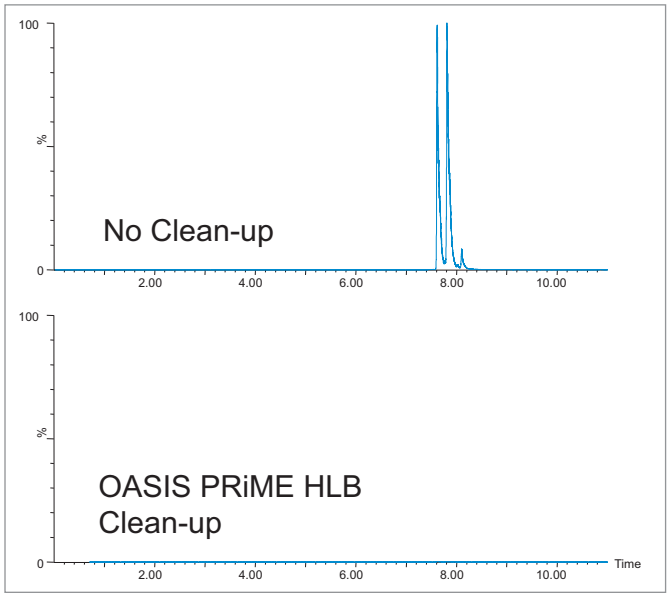

Figure 2. LC-MS/MS chromatograms showing effective removal of ≥95% of phospholipids from beef liver extract

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 clean-up. These data indicate that, for most of the compounds, the Oasis PRiME HLB Cartridge clean-up 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.

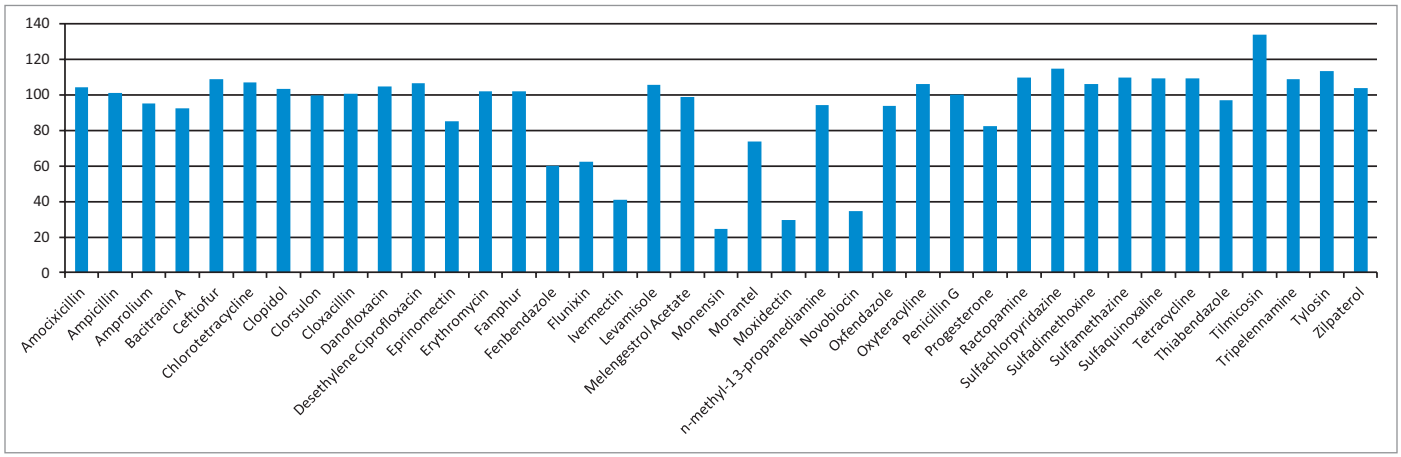

Figure 3. Recovery of veterinary compounds from blank beef liver extracts spiked after initial extraction and prior to Oasis PRiME HLB pass-through clean-up.

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 clean-up 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 clean-up protocol with Oasis PRiME HLB Cartridges

References

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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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