IMPROVED EXTRACTION AND CLEANUP OF BOVINE TISSUE SAMPLES PRIOR TO MULTIRESIDUE VETERINARY DRUG LC-MS/MS ANALYSIS

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INTRODUCTION

Tissue samples, such as bovine liver, are typically extracted with an acetonitrile based solvent for LC-MS determination of veterinary drug residues. Among the most significant interfering co-extractable substances are fats and polar lipids, particularly phospholipids (lecithin). A gram of bovine liver typically contains about 45 mg of fat and about 25 mg of phospholipids. Fats can be removed from the acetonitrile based tissue extracts by liquid extraction with hexane or with SPE with octadecyl silica (C18). Although C18 is effective for removal of most non-polar lipids, it does not remove phospholipids. Excessive amounts of phospholipids can shorten LC column life and contribute to ion-suppression and contamination in the mass-spectrometer. In this study a novel reversed-phase sorbent, Oasis PRIME HLB, is used for effective removal of phospholipids and fats from bovine liver extracts prior to LC-MS analysis. With the new sorbent, greater than 95% of both fat and phospholipids were effectively removed from the tissue extracts after a simple pass-through SPE procedure.

SAMPLE PREPARATION

Initial Extraction

A 1 g sample of liver is transferred to a 15 mL centrifuge tube containing ceramic homogenizer balls (Precellys). 5 mL of extraction solvent is added (0.1 % formic acid in 80:20 acetonitrile/water). The sample is homogenized for 1.5 min (see at right). The tubes are then centrifuged for 5 minutes @ 4000 rpm.

Pass-Through Cleanup

Cartridge: Oasis PRIME HLB (6 cc, 200 mg, see below)
Pass 0.6 mL of extract through cartridge to waste
Pass 1.0 mL of extract through cartridge and collect
Take 200 mL of pass thru fraction, dilute with 600 mL of 10 mM of ammonium formate in water (pH 4.5)

UPLC-MS/MS ANALYSIS

UPLC Conditions

LC system: ACQUITY UPLC I-Class
Column: Cortecs UPLC T3, 1.6 µm, 100 mm x 2.1 mm ID
Mobile phase:
A: 0.1% formic in water
B: 0.1% formic acid in acetonitrile
Injection volume: 7 µL
Injection mode: partial loop injection
Column temperature 35 °C
Weak Needle Wash: 10/90 acetonitrile/water
Strong Needle Wash: 50:30:20 water:acetonitrile:IPA
Seal wash: 10/90 acetonitrile: water

Gradient:

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Table 1. Matrix matched calibration data, MRM transitions (primary transition first, instrument parameters, and observed retention times (RT) for this study (*Acetylation of quinoxalin is the target metabolite for enrofloxacin).