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. 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%). 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 precipitation and centrifugation. However, significant amounts of fat and phospholipids (lecithin, 1-3%) are removed in an initial solvent extraction step by Oasis PRiME HLB (3 cc, 60 mg) cartridges (see Figure 1). Representative compounds were chosen from major classes of drugs of interest. Then, a simple cleanup was performed using a novel SPE device, the Oasis PRiME HLB (see Figure 2). These compounds were spiked into the fluoroquinolones, sulfonamides, macrolides, beta-lactams, NSAIDS, steroids and beta-andrennergics (see Figure 2). These compounds were spiked into the seafood samples prior to extraction and cleanup.

METHODS

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 acid 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: 50:30:40 water/acetonitrile/IPA
Seal wash: 10:90 acetonitrile: water

Gradient:

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>A (%)</th>
<th>B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-1.0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>1.0-1.6</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>1.6-4.6</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>4.6-10.6</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

Other instrument and calibration parameters are presented in Table 1 below.

UPLC-MS Conditions

Column temperature 30 °C
Desolvation Temperature: 300°C
Desolvation Flow: 1000 L/Hr
Cone Gas Flow: 30 L/Hr
Collision Gas Flow: 0.15 mL/Min
Desolvation Temperature: 500°C
Source Temperature: 150°C
Positive Ion Electrospray (negative ion for chloramphenicol)

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

<table>
<thead>
<tr>
<th>Compound</th>
<th>MRM</th>
<th>Calibration (µg/mL)</th>
<th>Corr</th>
<th>RT (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-Step Pass-Thru Cleanup</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS Conditions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

RESULTS

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 Figure 3). However, the Oasis PRiME HLB cartridge cleanup contributes very little to the overall method recovery losses (see red bars on Figure 3). The recovery for the SPE cleanup step is better than 80% in shrimp and better than 90% in salmon for all analytes except phenylbutazone.

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 (72005411en)
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 (72008944en)

CONCLUSIONS

- A simple and effective extraction and 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

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 (72005411en)
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 (72008944en)