

# Oasis PRiME HLB Cartridge for Effective Cleanup of Meat Extracts Prior to Multi-Residue Veterinary Drug UPLC-MS Analysis

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## GOAL

To demonstrate the effectiveness of the Oasis® PRiME HLB Cartridge for cleanup of meat extracts prior to UPLC®-MS analysis.

## BACKGROUND

Waters has developed an optimized sample preparation and analysis protocol for multi-class, multi-residue LC-MS/MS screening of veterinary drug residues in meat. 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-extraction, 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 meat extracts using cumbersome hexane defatting steps or by the use of reversed-phase sorbents such as C<sub>18</sub>-silica. Although these techniques may be effective for fat removal, neither of these procedures removes phospholipids.

Fats and phospholipids are significant potential instrument and column contaminants. Oasis PRiME HLB Cartridges provide a rapid cleanup to remove these substances from meat extracts prior to LC-MS analysis.

## THE SOLUTION

Pass-through cleanup with the Oasis PRiME HLB Cartridge. This procedure is highly effective for removal of both fat and phospholipid from meat extracts. Just as important, the recoveries of the veterinary drugs are not compromised with Oasis PRiME HLB Cartridge cleanup. The recoveries are similar to those obtained using hexane defatting or C<sub>18</sub>-silica cleanup but Oasis PRiME HLB Cartridge cleanup is more effective.

### Experimental

*Initial Extraction.* Typical pork samples (5 g, 15% fat) were fortified with representative compounds chosen from major classes of veterinary drugs. The homogenized meat samples were extracted with 10 mL of 80:20 acetonitrile/water with 0.2% formic acid. The samples were vortexed for 30 seconds, shaken for 30 minutes, and then centrifuged at 12000 rpm for 5 minutes.

*Oasis PRiME HLB Cartridge Cleanup.* An Oasis PRiME HLB Cartridge (3 cc, 60 mg) was mounted on a pre-cleaned vacuum manifold. No cartridge conditioning is required or was performed. A 0.5 mL aliquot of the supernatant was passed-through the Oasis PRiME HLB Cartridge and collected. The collected sample was diluted three-fold with aqueous 10 mM ammonium formate buffer (pH 4.5) prior to UPLC-MS/MS analysis.

## Results

Little or no recovery loss was observed in the pass-through cleanup step for any of the tested compounds. Absolute recoveries (measuring mostly the effectiveness of the initial liquid extraction) averaged over 80% for the tested compounds except for phenylbutazone (32%). These recoveries are consistent with C<sub>18</sub>-silica cleanup but no phospholipids are removed with C<sub>18</sub>-silica. UPLC-MS/MS conditions and chromatograms are presented in Figure 1. Figure 2 shows chromatograms that illustrate the effectiveness of the Oasis PRiME HLB Cartridge for phospholipid removal; greater than 90% more phospholipid is removed compared with C<sub>18</sub>-silica cleanup. Using gravimetric analysis it was also determined that the Oasis PRiME HLB Cartridge removed more than 90% of the co-extracted fat from the pork extract.

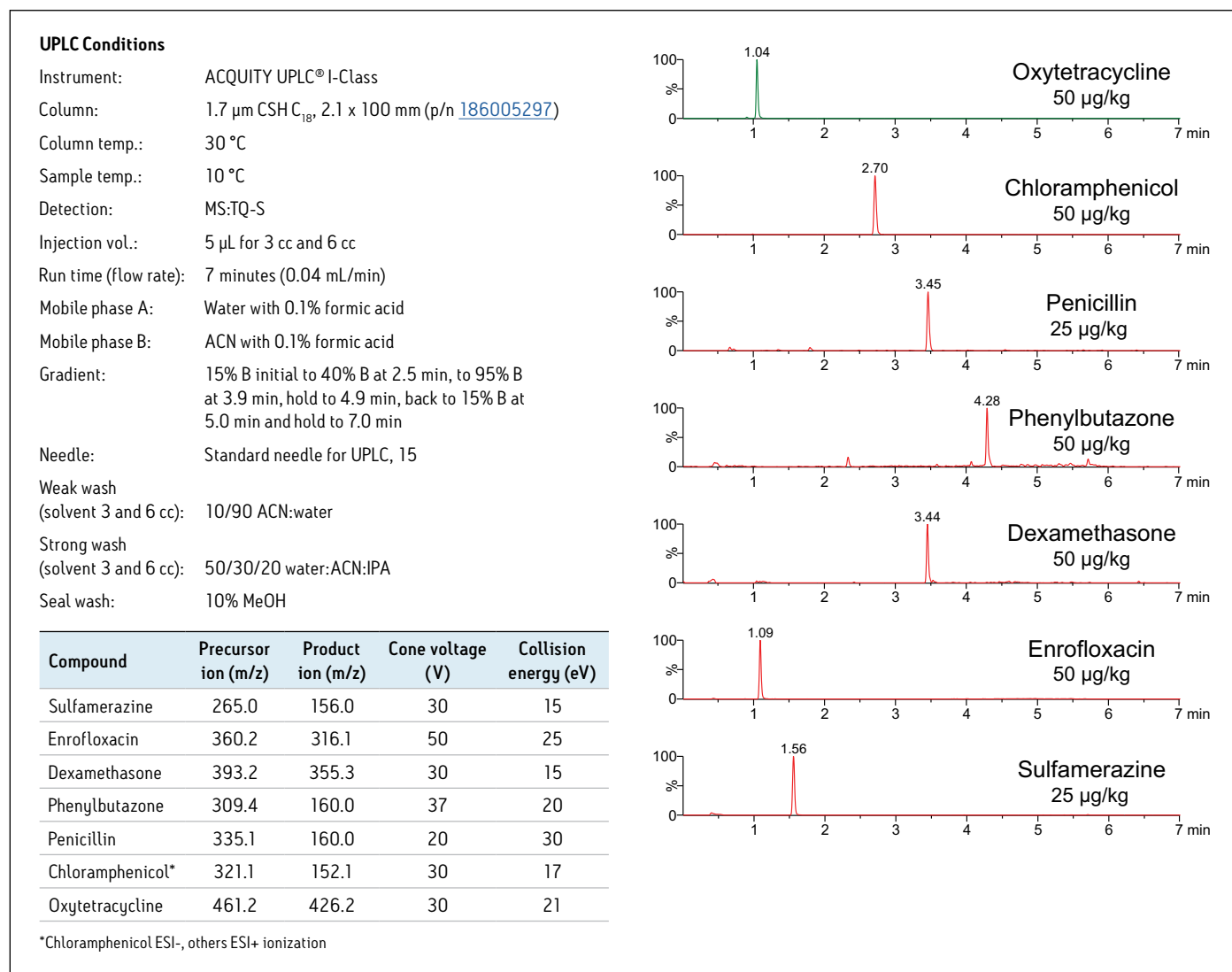


Figure 1. UPLC-MS/MS conditions and resulting chromatograms from a typical pork sample spiked at the levels indicated.

## CONCLUSIONS

- Oasis PRiME HLB Cartridges did not require conditioning or equilibration prior to use; a simple one-step SPE cleanup was effective
- Oasis PRiME HLB Cartridges removed greater than 90% of fats and greater than 90% of phospholipids from acetonitrile based extracts of pork
- When used in the pass-through mode, Oasis PRiME HLB Cartridges did not affect the recovery of the test compounds but gave significant removal of fats and phospholipids from the extract

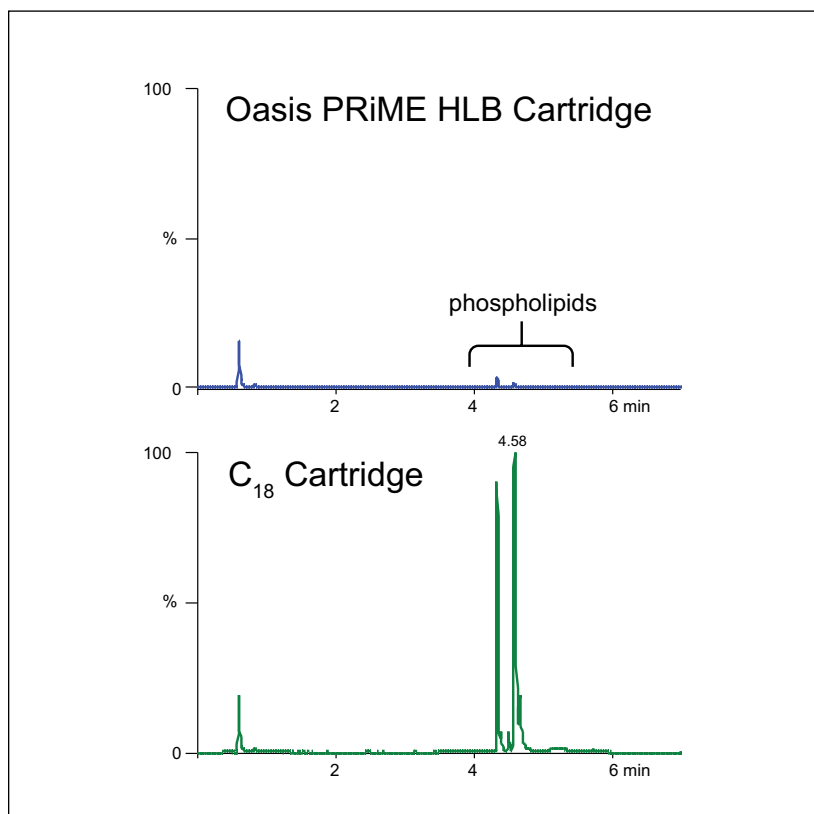


Figure 2. Removal of phospholipids from meat extracts comparing Oasis PRiME HLB cleanup (upper trace) with C<sub>18</sub>-silica cleanup (lower trace).

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