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# Oasis PRiME HLB Cartridge for Cleanup of Infant Formula 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

## WATERS SOLUTIONS

ACQUITY UPLC® I-Class

Xevo® TQ-S Mass Spectrometer

Oasis® PRiME HLB Cartridge for SPE Clean-up

## KEYWORDS

UPLC-MS/MS, Oasis PRiME HLB Cartridges, veterinary drugs, infant formula

## OVERVIEW

In order to insure public health and safety, reliable analytical methods are necessary to determine veterinary drug residue levels in foods. Of particular importance is such residue analysis in foods for infants. 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 such samples with a single analytical method. Powdered infant formula typically contains significant amounts of proteins, fats, and lecithin (phospholipids). These components can be detrimental to good instrumental performance and should be reduced or eliminated prior to LC-MS analysis.

## INTRODUCTION

Infant formula powder contains significant amounts of protein, about 20% fat and 1–3 % phospholipids. During the sample pre-treatment, the protein is removed from the extract 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 reversed-phase sorbents such as $C_{18}$-silica in pass-through or dispersive cleanup. Although these techniques may be effective for fat removal, neither of these procedures removes phosholipids. In a prior 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. In this study similar extraction and cleanup protocols were applied to the analysis of infant formula powder. 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 infant formula samples prior to extraction and cleanup.

## EXPERIMENTAL

### UPLC conditions

| | |
|---|---|
| LC system: | ACQUITY UPLC I-Class |
| Column: | ACQUITY UPLC CSH™ $C_{18}$, 1.7µm, 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 volume: | 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 |

| 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 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 |

### Sample preparation

1. Initial Extraction/Precipitation:

Place a 0.5 g sample of infant formula into a 50 mL centrifuge tube. For standards or QC samples spike with appropriate amounts of desired analytes. Add 3 mL extraction solvent (0.2% formic acid in 70:30 acetonitrile/water). Vortex for 30 seconds and place on mechanical shaker for 30 minutes. Centrifuge at 3220 rcf 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:

Mount an Oasis PRiME HLB Cartridge (3 cc, 60 mg) on a pre-cleaned vacuum manifold. Cartridge conditioning is NOT required and is not performed. The vacuum is set to 1–2 psi. Approximately 0.5 mL of the supernatant is passed-through the Oasis PRiME Cartridge and collected. A 0.3 mL aliquot of the pass-thru cleanup sample is 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 |
|---|---|---|---|---|---|---|---|
| Carbadox | 263.0>231.0<br>263.0>145.0 | 25<br>25 | 15<br>20 | 25, 100 | 12.5–200 | 0.9990 | 1.37 |
| Ceftiofur | 524.3>241.1<br>524.3>285.0 | 30<br>30 | 16<br>16 | 250, 1000 | 125–2000 | 0.9980 | 2.79 |
| Chlortetracycline | 479.3>444.2<br>479.3>462.2 | 30<br>30 | 21<br>18 | 25, 100 | 12.5–200 | 0.9977 | 1.53 |
| Ciprofloxacin | 332.1>288.1<br>332.1>231.1 | 30<br>30 | 18<br>40 | 25, 100 | 12.5–200 | 0.9929 | 0.84 |
| Cortisol | 363.2>121.0<br>363.2>91.03 | 42<br>30 | 52<br>22 | 50, 200 | 25–400 | 0.9961 | 2.93 |
| Dexamethasone | 393.2>373.2<br>393.2>355.3 | 30<br>30 | 10<br>15 | 25, 100 | 12.5–200 | 0.9917 | 3.39 |
| Enrofloxacin | 360.4>245.0<br>360.4>316.1 | 50<br>50 | 25<br>25 | 50, 200 | 25–400 | 0.9991 | 0.98 |
| Erythromycin | 734.4>158.1<br>734.4>576.5 | 30<br>30 | 32<br>20 | 2.5, 10 | 1.25–20 | 0.9978 | 2.16 |
| Lincomycin | 407.2>126.1<br>407.2>359.3 | 36<br>36 | 34<br>20 | 12.5, 50 | 6.25–100 | 0.9962 | 0.57 |
| Lomefoxacin | 352.1>265.1<br>352.1>308.1 | 31<br>31 | 22<br>16 | 50, 200 | 25–400 | 0.9991 | 0.90 |
| Oxacillin | 402.2>160.0<br>402.2>243.1 | 30<br>30 | 12<br>15 | 25, 100 | 12.5–200 | 0.9990 | 3.75 |
| Oxytetracyline | 461.2>426.2<br>461.2>408.11 | 30<br>30 | 21<br>13 | 25, 100 | 12.5–200 | 0.9971 | 0.96 |
| Pennicillin | 335.16>160.1<br>335.15>176.1 | 20<br>20 | 30<br>30 | 12.5, 50 | 6.25–100 | 0.9956 | 3.41 |
| Phenylbutazone | 309.4>160.0<br>309.4>103.9 | 37<br>37 | 20<br>20 | 25, 100 | 12.5–200 | 0.9962 | 4.22 |
| Ractopamine | 302.2.164.1<br>302.2>107.0 | 30<br>30 | 15<br>27 | 75, 300 | 37.5–600 | 0.9990 | 0.90 |
| Salbutamol | 240.2>148.1<br>240.2>222.1 | 30<br>30 | 20<br>12 | 25, 100 | 12.5–200 | 0.9941 | 0.55 |
| Sulfamerazine | 265>92.0<br>265>156.0 | 30<br>30 | 28<br>15 | 25, 100 | 12.5–200 | 0.9998 | 1.50 |
| Sulfamethazine | 279.1>186.0<br>279.1>92.0 | 30<br>30 | 16<br>28 | 25, 100 | 12.5–200 | 0.9996 | 1.67 |
| Sulfanilamide | 156>92.0<br>156>65.0 | 30<br>30 | 15<br>25 | 25, 100 | 12.5–200 | 0.9964 | 0.86 |
| Tetracycline | 445.3>154.0<br>445.3.410.2 | 30<br>30 | 26<br>21 | 25, 100 | 12.5–200 | 0.9964 | 1.05 |
| Tylosin | 916.5>174.1<br>916.5>101.1 | 57<br>57 | 40<br>45 | 5, 20 | 2.5–40 | 0.9920 | 2.38 |

*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 infant formula. The chromatograms shown in Figure 1 show the effectiveness of the Oasis PRiME HLB Cartridge for removal of ≥95% of phopholipids from the infant formula extracts.

| Compounds | Low level | | High Level | |
|---|---|---|---|---|
| | Recovery (%) | RSD (%) | Recovery (%) | RSD (%) |
| | | n=6 | | n=6 |
| Carbadox | 110 | 5 | 115 | 3 |
| Ceftiofur | 84 | 4 | 71 | 10 |
| Chlortetracycline | 47 | 9 | 46 | 9 |
| Ciprofloxacin | 107 | 8 | 102 | 6 |
| Cortisol | 111 | 8 | 117 | 5 |
| Dexamethasone | 113 | 14 | 121 | 5 |
| Enrofloxacin | 113 | 4 | 110 | 5 |
| Erythromycin | 126 | 6 | 125 | 6 |
| Lincomycin | 50 | 7 | 52 | 5 |
| Lomefloxacin | 120 | 2 | 111 | 2 |
| Oxacillin | 117 | 6 | 114 | 4 |
| Oxytetracycline | 29 | 13 | 27 | 13 |
| Penicillin | 120 | 10 | 116 | 7 |
| Phenylbutazone | 105 | 13 | 94 | 6 |
| Ractopamine | 115 | 2 | 117 | 5 |
| Salbutamol | 82 | 7 | 83 | 4 |
| Sulfamerazine | 130 | 4 | 126 | 4 |
| Sulfamethazine | 128 | 2 | 129 | 3 |
| Sulfanilamide | 105 | 12 | 120 | 5 |
| Tetracyline | 41 | 13 | 40 | 17 |
| Tylosin | 110 | 13 | 116 | 7 |
| Carbadox | 110 | 5 | 115 | 3 |
| Ceftiofur | 84 | 4 | 71 | 10 |

*Table 2. Recovery data obtained from replicate analysis of spiked infant formula samples (n = 6).*

## DISCUSSION

The procedure utilized in this study was developed from methods presented previously.[1,2] The overall method recoveries are generally above 70% but 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 Figure 2, the measured recovery for the SPE cleanup step is better than 80%.

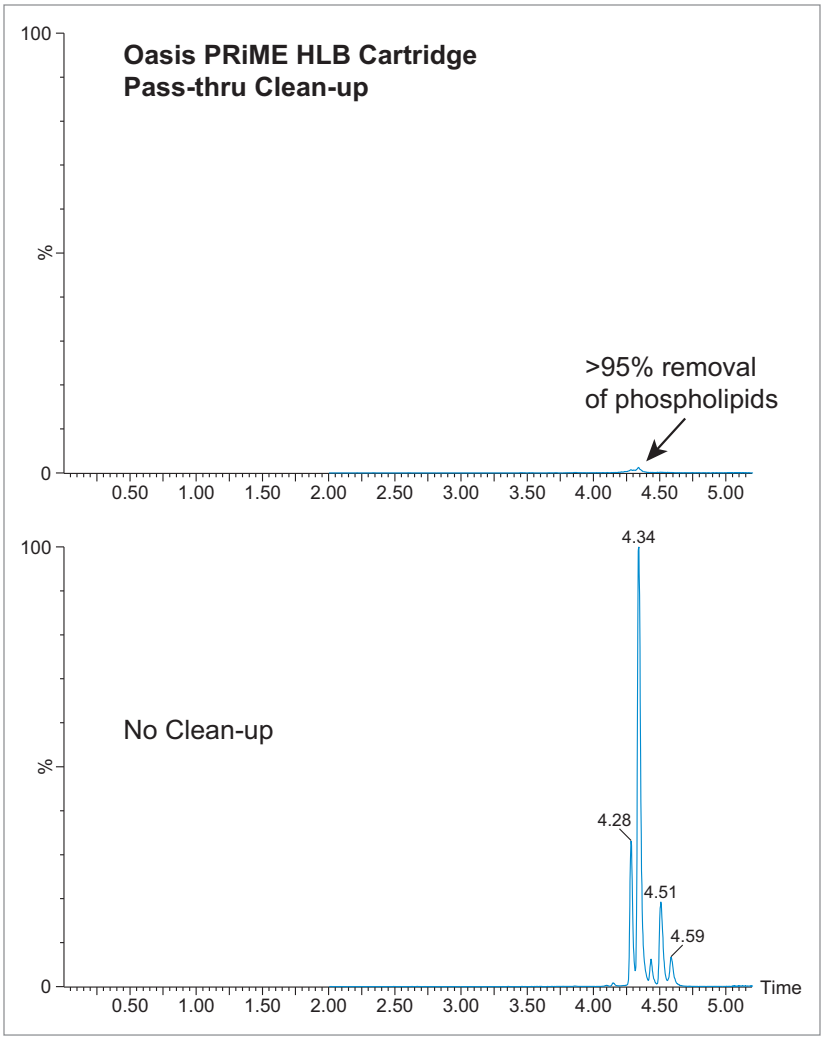


*Figure 1. LC-MS/MS chromatograms showing effective removal of ≥95% of phospholipids from shrimp extract.*

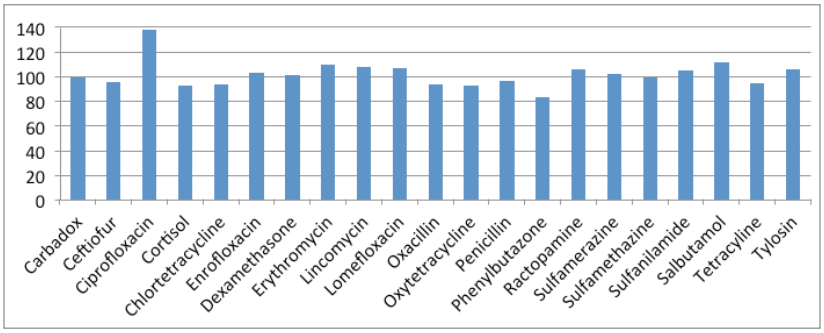


*Figure 2. Recovery of veterinary compounds from prepared extracts subjected to Oasis PRiME HLB pass-through clean-up.*

## CONCLUSIONS

- A simple and effective extraction/protein precipitation procedure was applied to the analysis of infant formula
- 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-through clean-up 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 Technology Brief 720005411EN, 2015.
2. M. Young and K. Tran, Rapid, Simple, and Effective Cleanup of Seafood Extracts Prior to UPLC-MS/MS Multiresidue Veterinary Drugs Analysis, Waters Application Note 720005488EN, 2015.

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