OPTIMIZED PASS-THROUGH SPE CLEANUP FOR LC-MS/MS MULTI-RESIDUE VETERINARY DRUG ANALYSIS

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INTRODUCTION

Before the introduction of the highly sensitive and selective LC-MS instrumentation commonly used for today's multi-residue methods, compound or class specific analytical methods were required to meet regulatory requirements. These methods often involved cumbersome and time-consuming multi-step analyte isolation and enrichment followed by cumbersome and time-consuming multi-step cleanup. Today's residue methods require much less analytical rigor. A simple extraction procedure followed by a simple pass-through cleanup step may be suitable for accurate determination of hundreds of analytes from complex matrices. Although simple, such extraction and cleanup methods are not fool-proof; variables in the extracts, such as water content, protein content, pigment content, and lipid content, need to be considered to maximize cleanup and avoid recovery losses of individual compounds or compound classes. In this study, pass-through cleanup for multi-residue veterinary drug analysis was optimized for analysis of beef muscle.

EXTRACTION AND PASS-THROUGH CLEANUP FOR MULTI-RESIDUE ANALYSIS

Extraction

The tissue sample (2 g beef muscle in this study) is extracted with 15 mL of 85:15 acetonitrile/water (0.2 % formic acid) using an appropriate homogenizer. After centrifugation, a portion of the extract is diluted 1:1 with acetonitrile (taking account of the original water content of the beef, this adjusts the extract to approx. 85 % acetonitrile).

Cleanup (OASIS PRiME HLB Cartridge: 3 cc, 150 mg)

- 2 mL of extracted sample is applied to SPE Cartridge and allowed to elute dropwise **to waste**
- 3 mL of extracted sample is then applied to SPE Cartridge and allowed to elute dropwise **and is collected**

WHY DO WE DISCARD THE FIRST FRACTION?

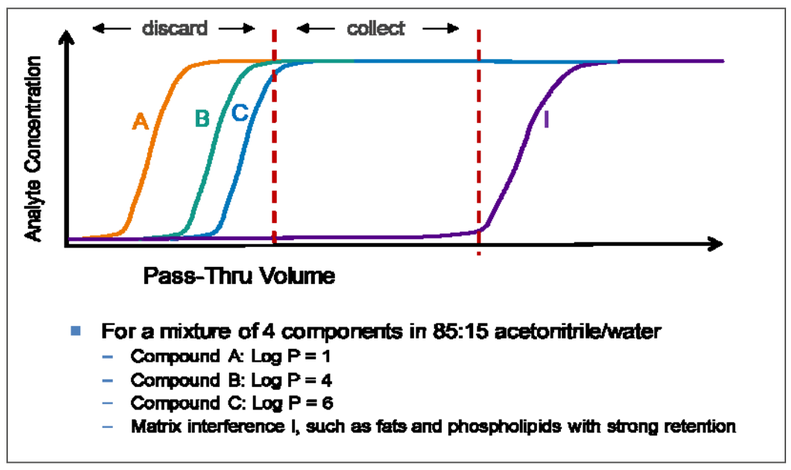

Figure 1. Graphical representation of pass-thru cleanup

Consider Figure 1 above:

- A pass-thru cleanup is performed on an Oasis PRiME HLB SPE cartridge that develops 10 or more theoretical plates. Because the cartridge behaves as a short chromatography column, pass-thru SPE is a rudimentary form of ***Frontal Elution Chromatography.***
- Polar analyte A has no retention and elutes in the first drops of sample extract eluting from the cartridge.
- Moderately non polar analyte B has some weak retention and elutes from the cartridge after about 1 mL is passed.
- Highly non-polar analyte C demonstrated stronger retention and elutes from the cartridge after about 2 mL are passed.
- Fats and phospholipids (I) are strongly retained and do not elute until after 5 mL are passed.

⇒ ***The first 2 mL are discarded because not all analytes have reached their maximum elution concentrations until this fraction has passed.***

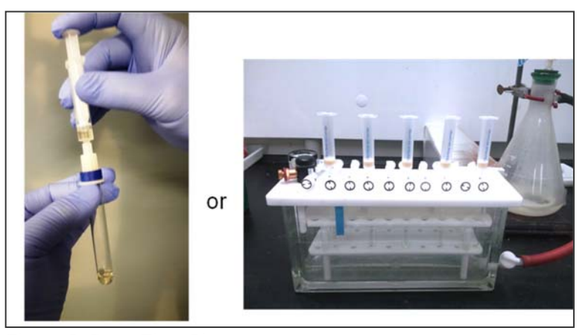

Figure 2. Pass-Thru Cleanup

Figure 2 at left illustrates the Pass-Thru cleanup procedure.

Cleanup can be accomplished by hand using a syringe and a Plus type cartridge (left photo).

Cleanup can be accomplished using a vacuum manifold (right photo).

EXPERIMENTAL

Instrumentation

UPLC Conditions

LC system:	ACQUITY UPLC H-Class
Column:	ACQUITY UPLC ® BEH (2.1x 100 mm)
Mobile phase:	A: 0.1% Formic acid in water B: 0.1% Formic acid in methanol
Injection volume:	7 µL
Column temperature:	40°C

Time	Flow (mL/min)	%A	%B
0.00	0.300	95.0	5.0
3.50	0.300	70	30
6.0	0.300	2	98
7.00	0.300	95.0	5.0
9.00	0.300	95.0	5.0

Gradient

MS Conditions

Mass Spectrometer:	Waters Xevo TQ-S micro
Mode:	MRM, ESI- for bithionol, niclosamide, chloramphenicol and florfenicol ESI+ for all others
MS Parameters:	Optimized transitions and instrument parameters available on request

Optimizing the Pass-Thru Cleanup

We considered a list of 39 analytes of varying polarity. We chose three non-polar analytes to optimize the pass-thru cleanup.

- Test compounds: bithionol (Log P 6), niclosamide (Log P 4) and oxyphenbutazone (Log P 3)
- Parameters tested
  - – Recovery of test compounds
  - – Phospholipid removal
  - – Fat removal
- Experiment design
  - – 3 mL of spiked meat extract was diluted to 6 mL with pure acetonitrile (to adjust to ~ 85% ACN)
  - – The sample was passed thru the cartridge in 1 mL increments (fractions 1-6)
  - – Each increment was analyzed for the test compounds and residual lipids
- Results
  - – oxyphenbutazone concentration reached maximum after fraction 2
  - – bithionol and niclosamide concentration reached maximum in fraction 4
  - – Significant phospholipid and fat breakthrough did not occur until after fraction 6

Optimized Pass-Thru Cleanup

- – Pass 2 mL of diluted extract through cartridge to waste
  - – Then pass 3 mL of diluted extract through cartridge and collect

RESULTS

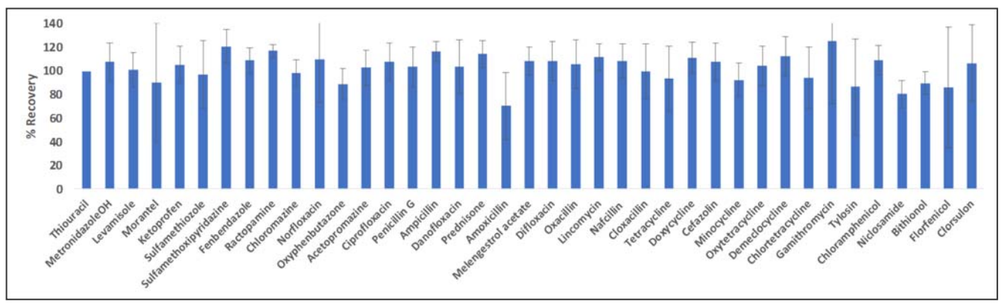

Figure 3. Recovery of 39 drugs after optimized Oasis PRiME HLB pass-thru cleanup, n = 18, spike concentrations from 10 - 100 µg/kg (error bars indicate standard deviation)

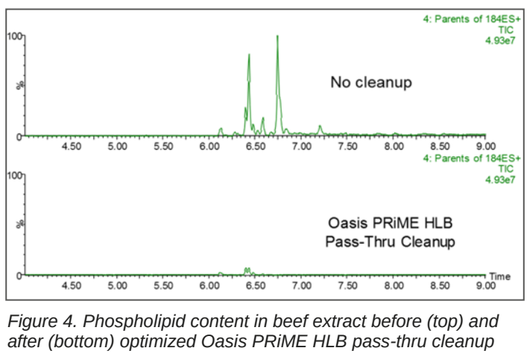

Figure 4. Phospholipid content in beef extract before (top) and after (bottom) optimized Oasis PRiME HLB pass-thru cleanup

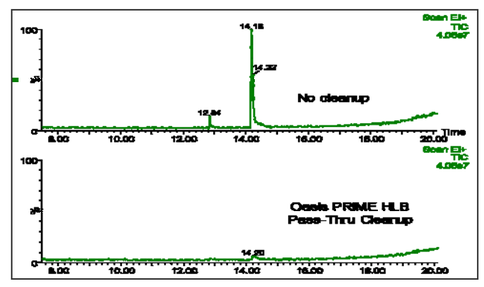

Figure 5. To investigate fat removal, 0.5 gm of phospholipid-free soybean oil was extracted with 85:15 acetonitrile/water; the extract was cleaned up with the optimized Oasis PRiME HLB protocol and then subjected to methanolysis/methylation prior to GC-MS analysis

CONCLUSIONS

- **Pass-Thru cleanup with the Oasis PRiME HLB cartridge is an effective procedure for removal of fat and phospholipid from beef muscle extract**
- **Good recovery is obtained across a wide range of compound polarity**
- **Optimized cleanup is obtained from acetonitrile based extracts containing ~ 85 % water,**
  - **- For 3 cc 150 mg cartridge pass 2 mL to waste, collect 3 mL**
  - **- For Plus Light cartridge pass 1.3 ml to waste and collect 2 mL**

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