

Tips for Faster Sample Preparation

- Focusing on Multi-residue Analysis

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Agenda

- Sample Preparation for Residue analysis
 - Workflow
 - Compound/Class specific versus multi-residue
- Dealing with Sample Matrix in Foods
 - Cleanup of common matrix interference
- Modern Pass-through SPE cleanup
 - Simple pass-through protocol
 - Two-step pass-through protocol
- Summary

Sample Preparation for Residue Analysis

- Workflow

Sample Preparation

- Homogenization
- Liquid-liquid extraction
- Solvent extraction
- Protein precipitation
- Sonication
- pH adjustment
- QuEChERS
- Digestion
- Derivatization

Sample Pretreatment

Sample Cleanup

- Dilution
- Filtration
- GPC
- Supported liquid extraction
- SPME
- SPE

- LC-(MS/MS)
- GC-(MS/MS)
- SFC/UPC2
- IC
- AA/ICP
- UV/Vis

Analyte Determination

Methodology Comparison

Compound/Class Specific vs Multi-Residue/Multi-Class

	Compound/ Class Specific	Multi-Residue/ Multi-Class
Analytes	Specific for one compound or class of compounds	Generic to a diverse set of analytes
Sample Preparation	Multi-step	Simple (one or two steps)
Goal of Sample Cleanup	Maximizing Recovery Minimizing Matrix Interference	Speed Balancing Max. Recovery & Min. Interference
Level of Sample Cleanup	Maximum	Minimum/moderate
Detection Techniques	Non-MS (UV, FLD, ELS), GC, Single quad MS	Tandem MS, Time-of-Flight
SPE (if used)	Retained: analytes Pass-thru: interference	Retained: interference Pass-thru: analytes

Residue Analysis Trend: Moving toward Multi-Residue/Multi-Class Analysis

Major factors enabling the Multi-residue analysis

Analytes

Sample Preparation

Goal of Sample Cleanup

Level of Sample Cleanup

Detection Techniques

SPE (if used)

- Streamlined and simplified workflow combining sample pretreatment & sample cleanup
 - e.g. QuEChERS (Extraction + Liq-Liq Partitioning) + dSPE cleanup
- Development MS technology
 - High sensitivity
 - High resolution
 - Fast scan rate (narrow peaks in UHPLC/UPLC chromatogram)

Multi-Residue/ Multi-Class

Generic to a **diverse** set of analytes

Simple (one or two steps)

Speed
Balancing Max. Recovery & Min. Interference

Minimum/moderate

Tandem MS, Time-of-Flight

Retained: interference
Pass-thru: analytes

Most Successful Multi Pesticide Residues Analysis

- QuEChERS

- Acronym of **Quick, Easy, Cheap, Effective, Rugged, Safe**
- A sample preparation method of multi-residue pesticides analysis for fruits and vegetables

2003

412 ANASTASSIADES ET AL. JOURNAL OF AOAC INTERNATIONAL VOL. 86, NO. 2, 2003

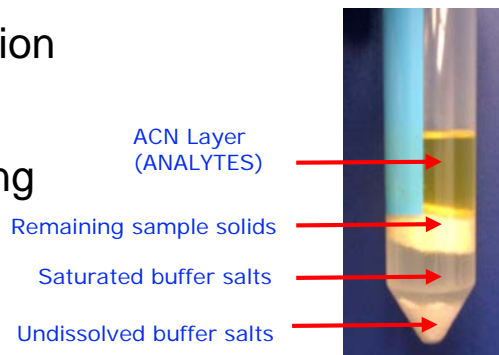
RESIDUES AND TRACE ELEMENTS

Fast and Easy Multiresidue Method Employing Acetonitrile Extraction/Partitioning and “Dispersive Solid-Phase Extraction” for the Determination of Pesticide Residues in Produce

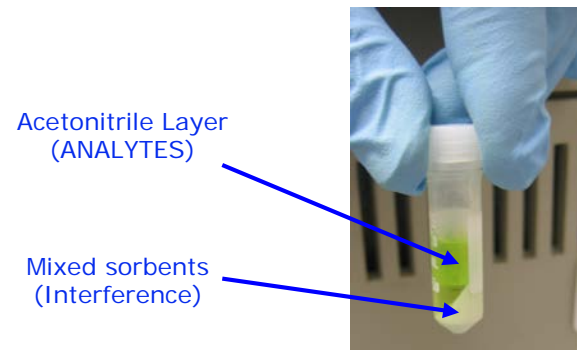
MICHELANGELO ANASTASSIADES¹ and STEVEN J. LEHOTAY²
U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, 600 E. Mermaid Ln, Wyndmoor, PA 19038
DARENKA ŠTAINBAHER
Public Health Institute, Environmental Protection Institute, Prvomajska 1, 2000 Maribor, Slovenia
FRANK J. SCHENCK
U.S. Food and Drug Administration, Office of Regulatory Affairs, Southeastern Regional Laboratory, 60 Eighth St. Atlanta, GA 30309

QuEChERS Extraction

- Acetonitrile extraction
- Salting out
- Liq – Liq Partitioning



dSPE Cleanup



What's in the Matrix? (How do I get rid of it?)

Food Samples



- Sugars
 - With QuEChERS most sugars partition into aqueous/salt phase



- Proteins
 - Protein precipitation
 - Acetonitrile extraction
 - Strong acid

Protein rich foods

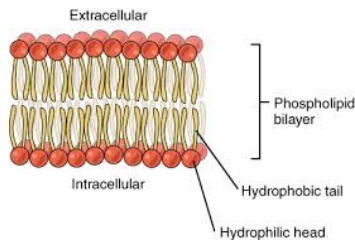
Most of them could be removed by liq-liq extraction or protein precipitation during sample pretreatment

What's in the Matrix? (How do I get rid of it?)

Food Samples



- **Fats**
 - Hexane liquid/liquid partition
 - SPE with suitable sorbent (C18)



- **Phospholipids (lecithins), free fatty acids**
 - SPE with PSA (cannot be used for acidic analytes)



- **Pigments**
 - SPE with GCB (graphitized carbon)

- Partially or little removed by extraction step
- All 3 interference can be efficiently removed **simultaneously** by Pass-thru SPE using PRiME HLB

Oasis PRiME HLB – What is it?

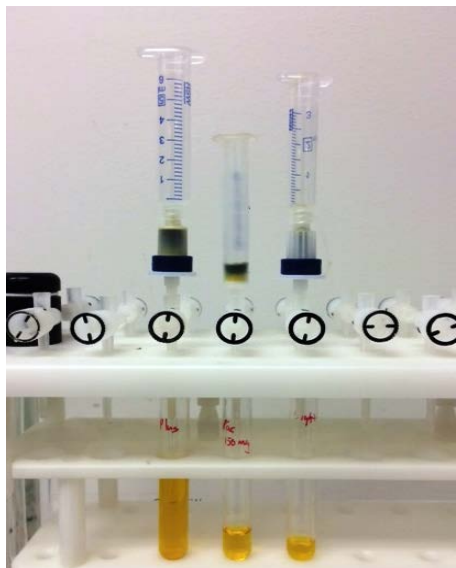
- A **reversed-phase** solid phase extraction device designed to eliminate phospholipids and other interference during MS analysis
- For multi-residue analysis, using pass-through mode to remove common interferences, **fats** and **phospholipids, pigments**

PROCESS ROBUSTNESS improvements
in... MATRIX EFFECTS EASE of USE

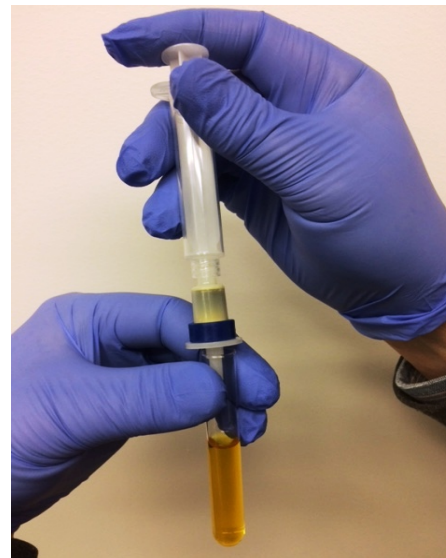


Flexibility in Usage

With vacuum manifold



Without vacuum manifold Manually operation using syringe



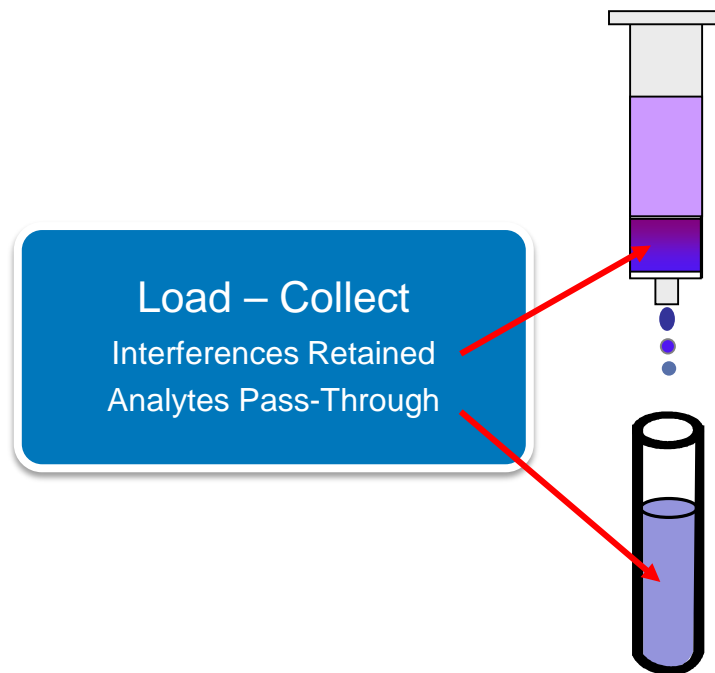
Please the demo at Waters booth

Simple Pass-Through Protocol

Simple Pass-Through Protocol

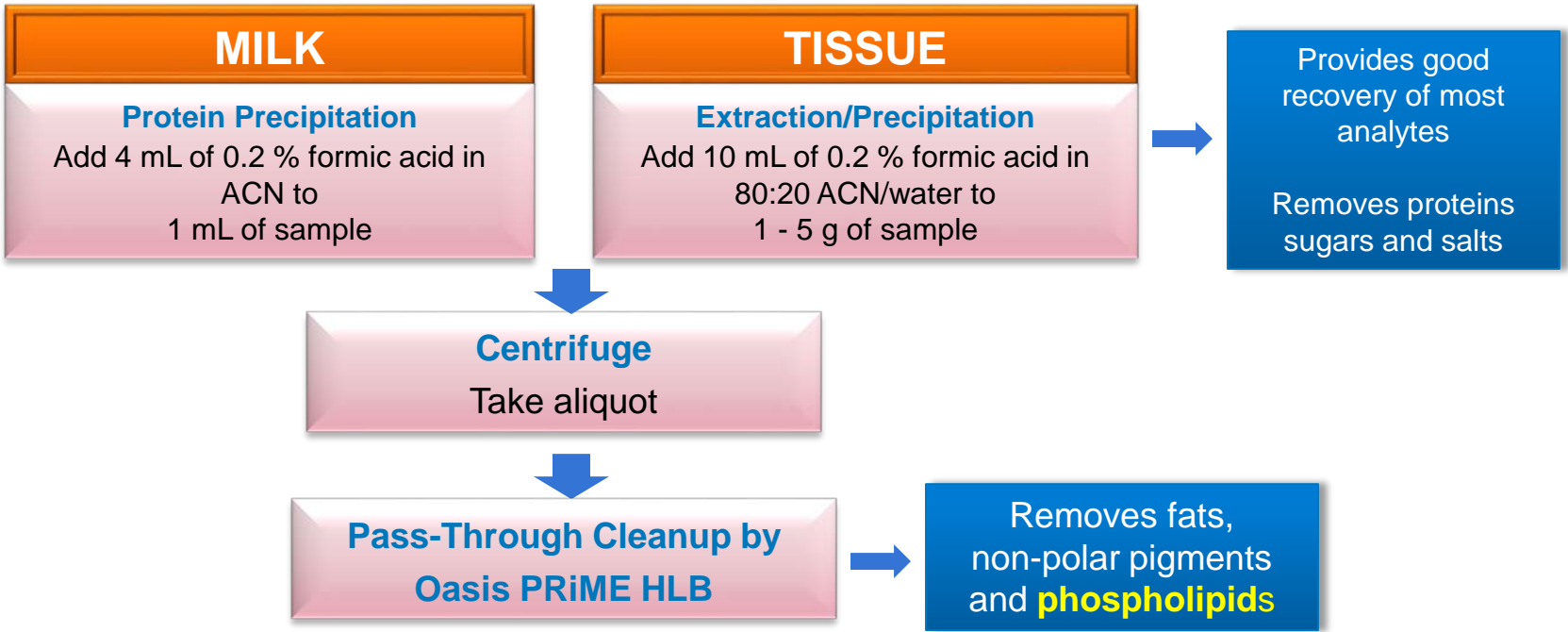
- Analytes must be in strong solvent
 - Preferred solvent is acetonitrile
 - For most veterinary drugs, > ~ 75%
 - For pesticides, > ~ 95% from QuEChERS extract
- Interference in weak (relative) solvent
 - Fat retained by reversed phase retention
 - Phospholipids retained by HILIC interaction

Pass-Through Protocol



Multi-Residues Veterinary Drug Analysis

- Sample Extraction & Pass-Through SPE Cleanup

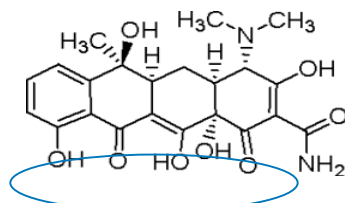


Veterinary Drug Classes

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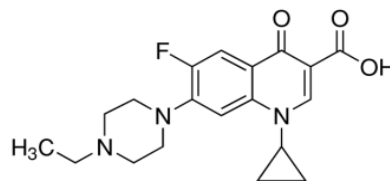
Tetracycline



tetracycline

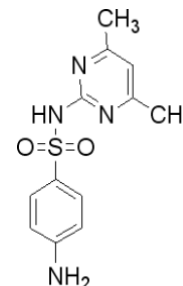
LogP -1.3, pKa 2.2

Fluoroquinolone



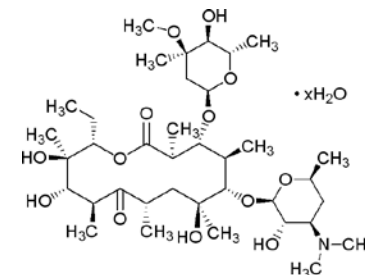
enrofloxacin

Sulfonamide



sulfamethazine

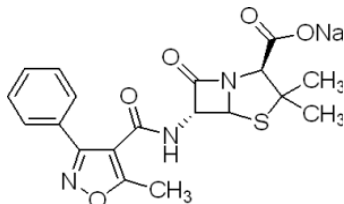
Macrolide



erythromycin

LogP 2.37 , pKa(basic)8.3

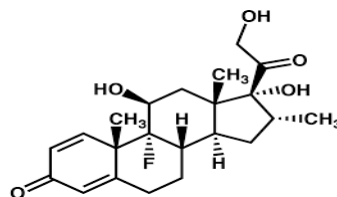
Beta-Lactam



oxacillin

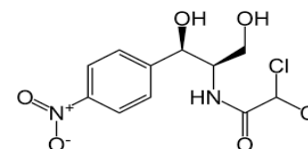
LogP 2.05, pKa 3.75

Steroid



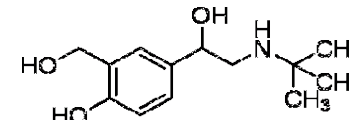
dexamethasone

Amphemicol



Chloramphenicol

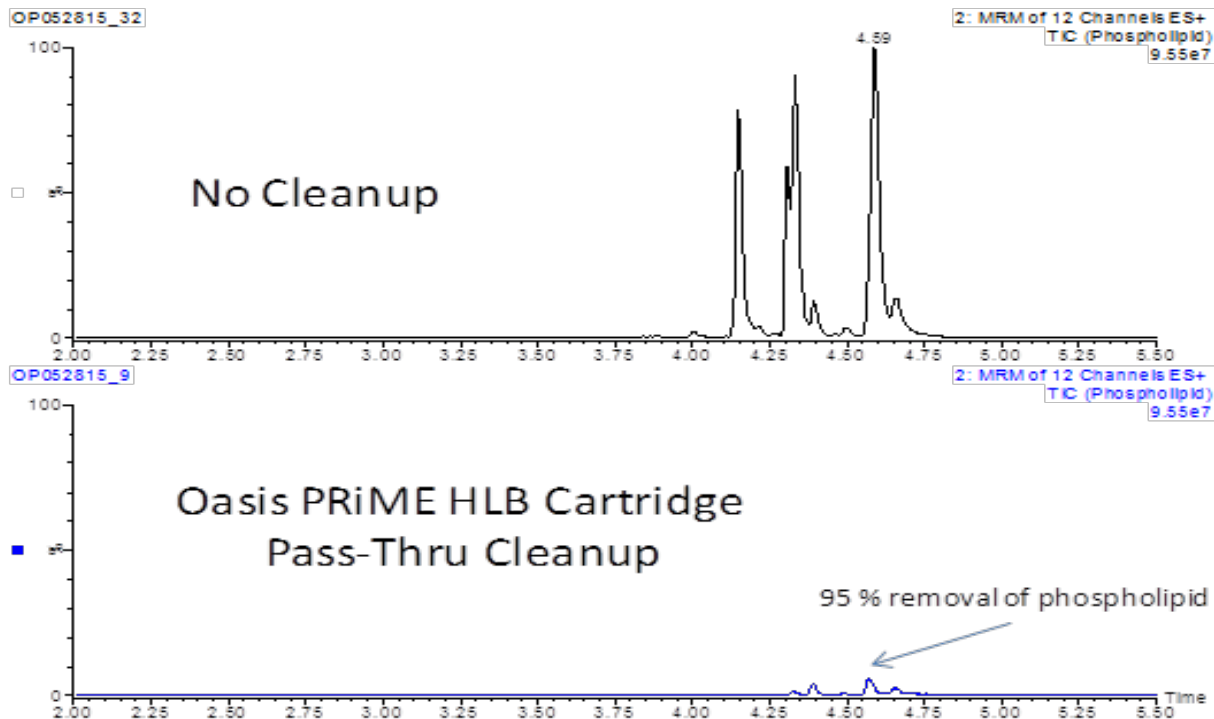
Beta-adrenergic



salbutamol

LogP 0.44, pKa (basic) 9.4

Phospholipid Removal from Shrimp Extract

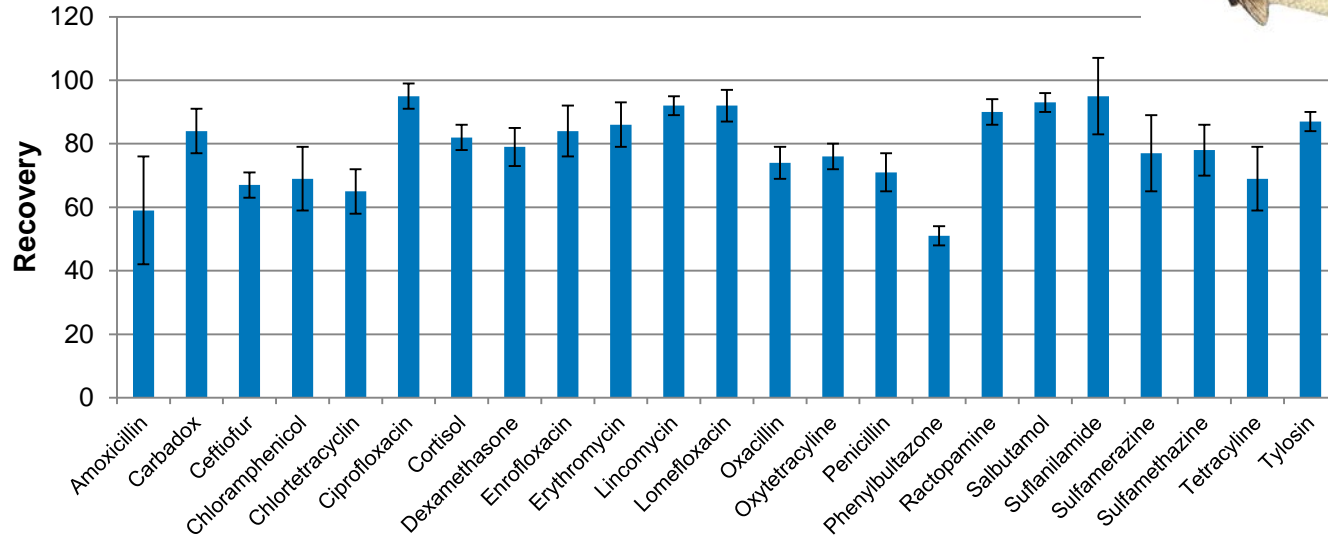


Recovery of Veterinary Drugs from Salmon Tissue (9 Classes of Drugs)

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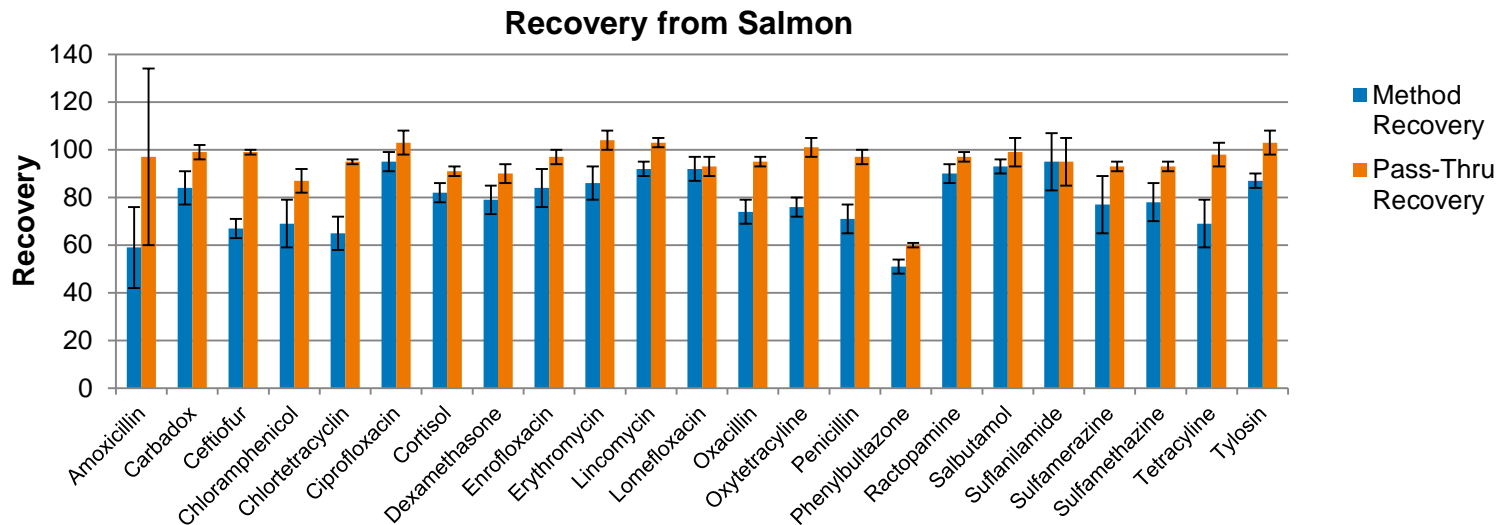
Total Method Recovery from Salmon



Waters Application Notes

Rapid, Simple and Effective Cleanup of Seafood Extracts Prior to UPLC-MS/MS Analysis of Multi-Residue Veterinary Drugs Method (720005488en)

Oasis PRiME HLB Pass-Thru Cleanup Recovery



- Salmon and Shrimp matrix blank extracts were spiked with the veterinary drugs and then cleaned up by passing through the Oasis PRiME HLB cartridge.
- This experiment eliminates the recovery loss contribution from the initial protein precipitation/extraction .
- This salmon data presented below shows the pass-thru cleanup efficiency versus the total method recovery. Shrimp data were similar.

Applications using Oasis PRiME HLB for Multi-Residue Analyses

- Residues: veterinary drugs, pesticides, mycotoxins
- Samples: milk, meat, eggs, grains, seafoods, infant formula, bovine liver, avocado, spinach
 - Application notebook 720005932en
- New applications could be found on Waters website

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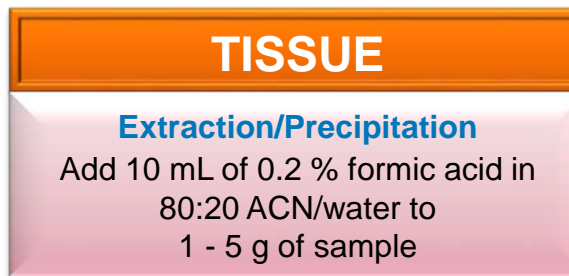
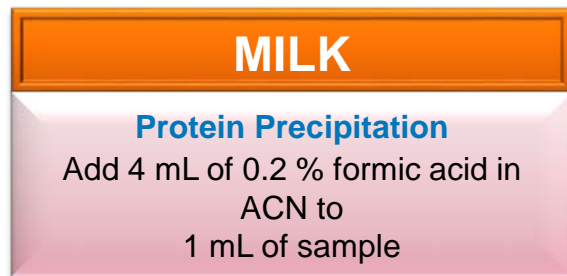


720005932en

Two-Step Pass-Through Protocol

Multi-Residues Veterinary Drug Analysis

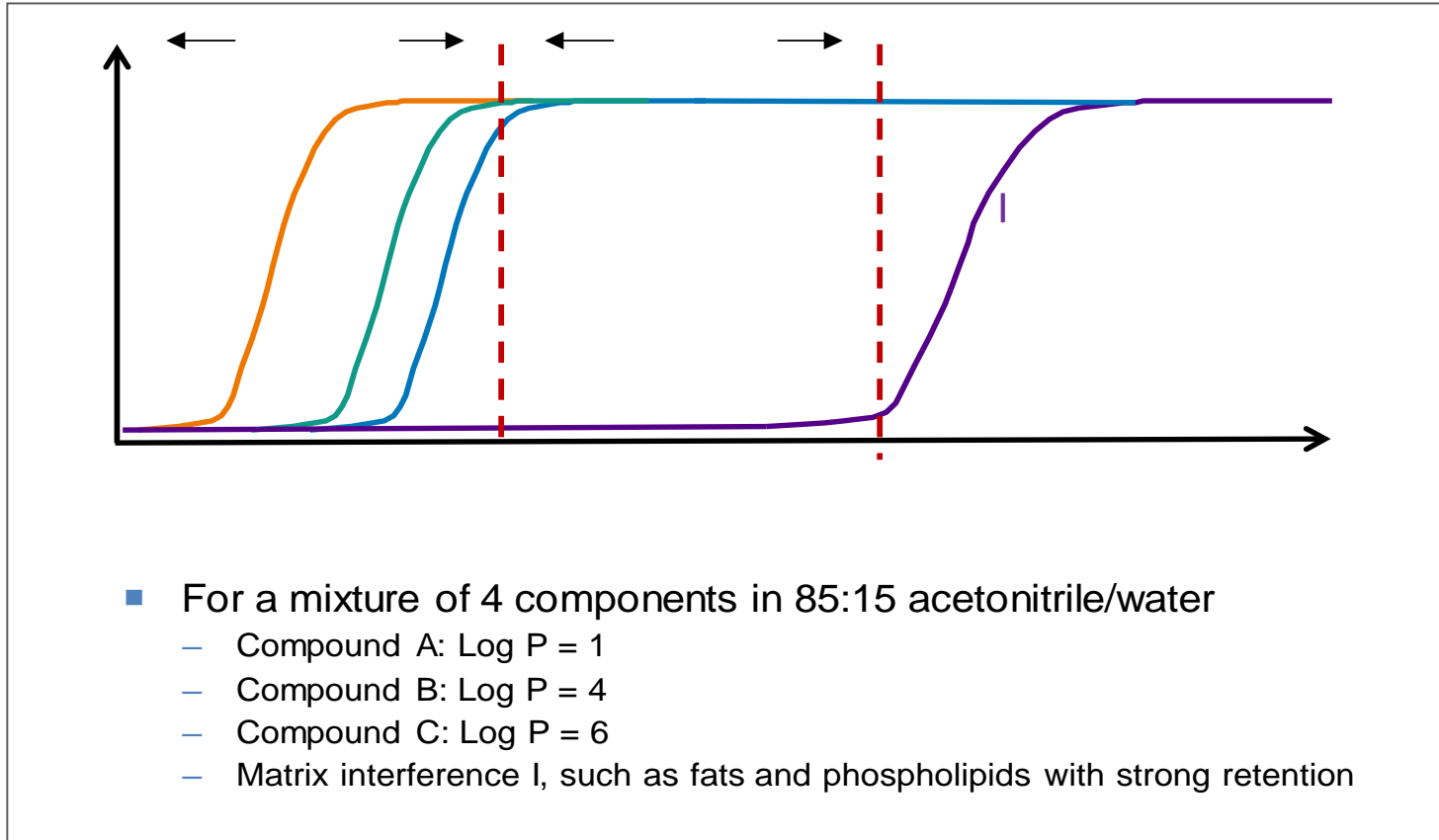
- Sample Extraction & Pass-Through SPE Cleanup



Overall acetonitrile is
between 70% to 80%

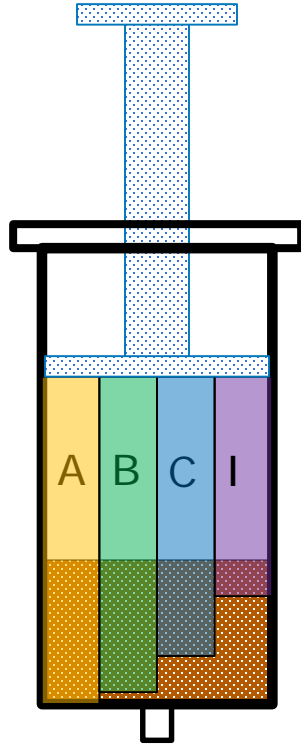
- Low recoveries for very high hydrophobic analytes (Log P >> 4)
- Need to increase the solvent strength to min. 85% acetonitrile
- Two-step pass-through protocol will be required

The Basis for Pass-Thru Protocol - Frontal Elution Chromatography



Two-Step Pass-thru Protocol

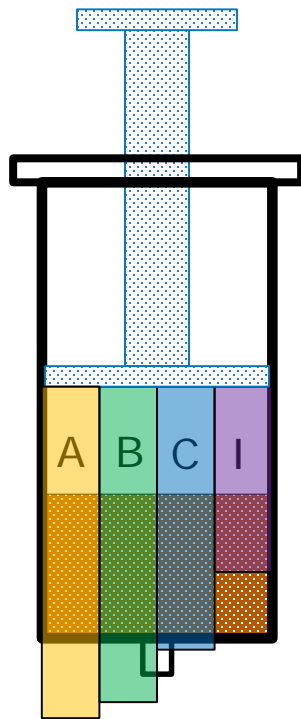
- At the Beginning stage



- As the band of sample continuously flow through sorbent, all analytes are making contact and interact with sorbent
 - Compound A moves along with solvent front (smallest k')
 - Compound B elutes closely in second
 - Compound C with large k' , elutes in third, but significant behind A & B
 - Interference I, retained by sorbent

Two-Step Pass-thru Protocol

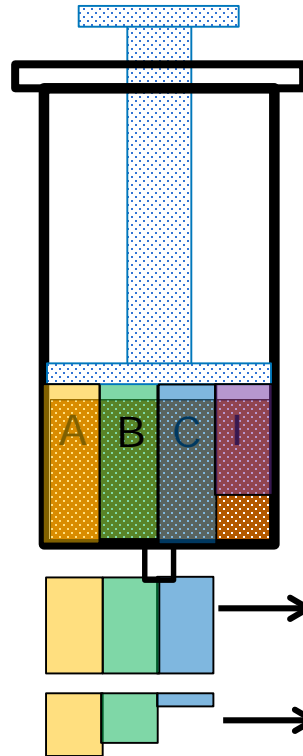
- “PRIME” the SPE device



- As plunger continues to push the band of sample continuously moving toward the tip. The SPE device now has been filled or “**PRIMED**” by the analytes
 - After compound A & B elutes for some time
 - Compound C, just starting to elute out of the tip, but significant behind A & B
 - Interference I, still retained by sorbent

Two-Step Pass-thru Protocol

- Collect the middle fraction



- As most sample pass-thru sorbent
 - Discard the beginning fraction which contains some B, but with little C
 - Collect the middle fraction which contain all three fractions at full strength.
 - Interference I should still be retained by sorbent

Collect the middle fraction

Discard the first fraction

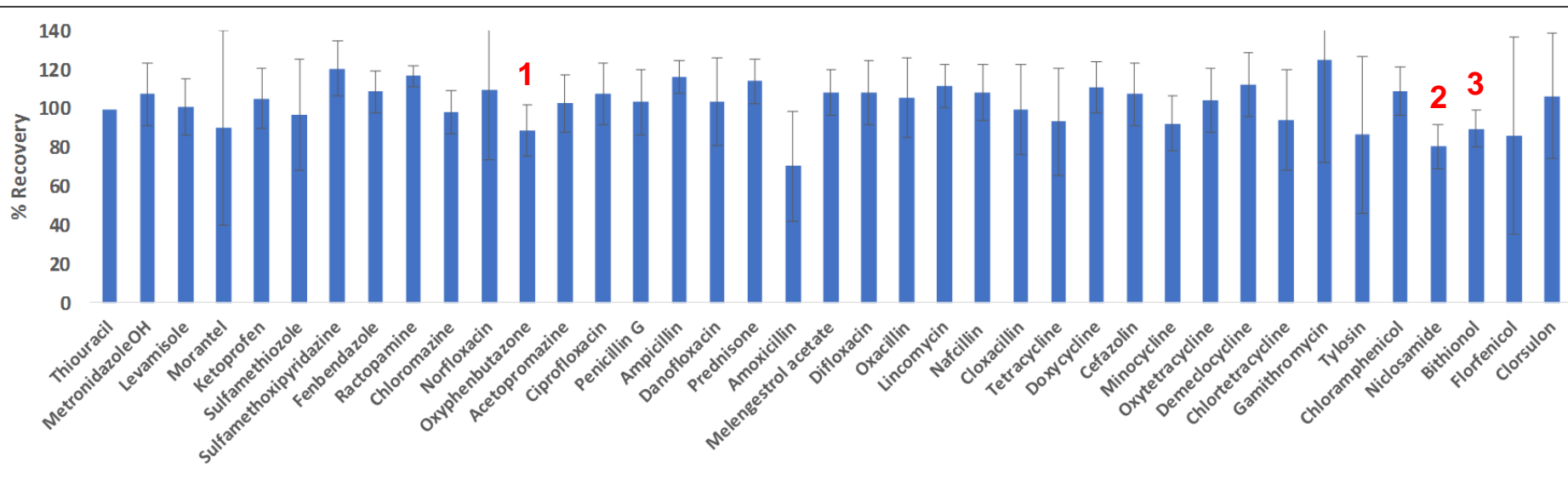
Extraction

- 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**

Recovery Results



Recovery of 39 drugs after optimized Oasis PRiME HLB pass-thru cleanup, $n = 18$, spike concentrations from 10 - 100 $\mu\text{g}/\text{kg}$

1. Oxyphenbutazone (Log P 3);

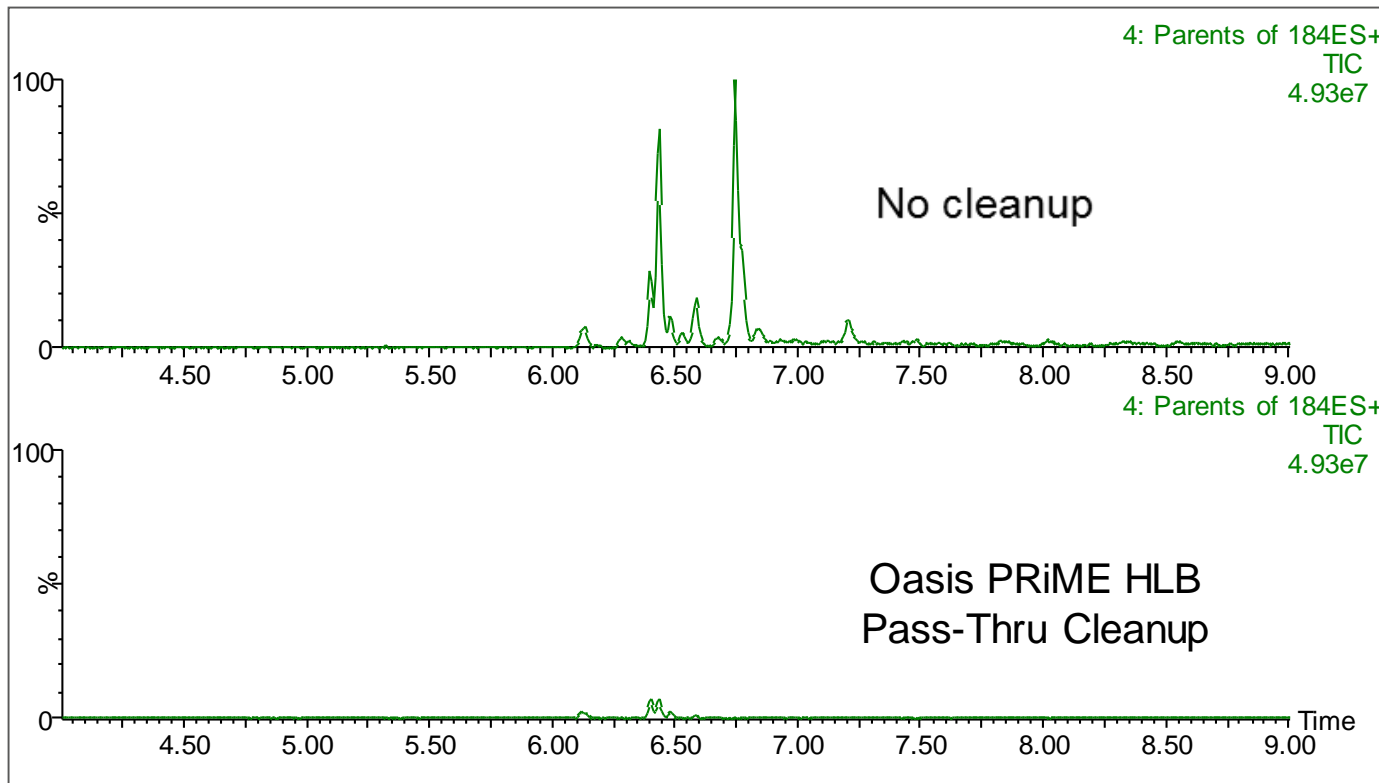
2. Niclosamide (Log P 4);

3. Bithionol (Log P 6)

Phospholipid Cleanup Result

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- Multi-residue/multi-class analysis is the main methodology for contaminant analysis
 - The level of cleanup is determined by the property of analytes, detection technique and their specificity and sensitivity
- Fast and optimized cleanup for pesticides can be achieved by using pass-through SPE cleanup
 - Dispersive SPE (dSPE) for pesticide analysis (QuEChERS)
 - Acidic pesticides by pass-through cleanup such as Oasis PRiME HLB
- Residual Veterinary drug analysis
 - Analyte list contains only low/moderate hydrophobic compounds ($\text{Log } P \leq 3$) uses simple pass-thru protocol
 - Analyte list contains very high hydrophobic compounds ($\text{Log } P > 4$) uses two-step pass-thru protocol with Min. 85% acetonitrile

Questions?

