Improved Sample Prep of Meat & Fish for Contaminate Analysis by LC/MS using Thomson eXtractor3D|FV™

Multiclass multiresidue analysis of >100 veterinary drug residues in bovine tissues by filter-vial dispersive-SPE & LC-MS/MS

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Abstract

High-throughput analysis is needed to meet demand for monitoring of veterinary drug residues in food animal tissues. The current veterinary drug residue monitoring method used by the USDA Food Safety and Inspection Service (FSIS) uses a combination of hexane-partitioning, dispersive-SPE, and solvent evaporation to achieve adequate cleanup for 20 mg equivalent sample injections to meet regulatory detection limit needs. This extra effort adds to the time and cost of the method and limits sample throughput. An improved method is developed and validated to streamline the sample preparation and LC-MS/MS method for identification and quantification of >120 veterinary drugs in bovine animal tissues for use in high throughput monitoring in the FSIS National Residue Program.

Experimental

Sample Preparation and Analysis

1. Weigh 2g homogenized tissue sample into 50mL tube. Add internal standard and spike solutions as needed.
2. Dispense 10 mL 4/1 (v/v) ACN/water and shake 5min on a platform shaker, then centrifuge 5min at 3700rcf.
3. Transfer 0.4 mL extracts to filter-vial shell containing 25 mg C18, insert plunger halfway and shake 30s, then fully depress plunger to filter final extract into autosampler vial.
4. Inject 1µL in LC-MS/MS.

Equipment

LC: Agilent 1100
MS/MS: AB Sciex 6500 Q-Trap, Electrospray ionization (positive/negative switching)
Column: Phenomenex Kinetex C18 (50 x 3.0mm, 2.6µm)
Mobile phase: (A) water; (B) ACN both with 0.1% HCO2H
Gradient: 2% A 100% B over 8.0min, hold for 2.7min
Flow rate: 0.3 mL/min
Column temperature: 40˚C

Sample Preparation

Minimize sample Size Minimizes Effort & Expense

Freeze Sample (-20˚C) add dry ice, homogenize to produce a flow able powder (-25˚ to -30˚C)

Improved homogeneity

Comparison of updated and new methods for Veterinary Drugs

	QuEChERS LC/MS Method Anastassiades et al. 2007	New Method for Veterinary Drugs
Homogenization	10-15 g tissue in a 50 mL tube add IS mix	2 g tissue in a 50 mL tube add IS mix (SMZ-IS; flunixin-d3)
Extraction	add 10-15 mL of 4/1 (v/v) Acetonitrile/water, shake	add 10 mL of 4/1 (v/v) Acetonitrile/water vortex briefly, shake for 5 min centrifuge for 5 min >3500 rcf
Clean-up	supernatant + 500 mg C18 + 10 mL hexane sat'd w/Acetonitrile; mix for 30 s, centrifuge for 5 min > 3500 rcf; aspirate hexane to waste evaporate 5 mL extract and dilute with water to a final volume of 1 mL filter extract with the PVDF filter vial	0.6 mL supernatant + 30 mg C18 in filter-vial d-SPE; vibrate AS tray for 30 s and filter through 0.2 µm PVDF by pressing plungers to seal the vials
Injection	Inject 1 µL in LC-MS/MS	Inject 1 µL in LC-MS/MS

Filter Vial Dispersive SPE using Thomson eXtractor|3D®

1. Weigh sorbents into bottom half of device, add 0.5 mL extract.
2. Shake then compress filter plunger into sample chamber.
3. Place the vial into an autosampler tray.

Results

Compounds were analyzed to determine the optimum extraction, sample size, and solvent ratio.

- Spikes made of 18 different tissue blank samples at 0X, 0.5X, 1X, and 2X levels (n=10 each) repeated over 3 days by 3 chemists
- Matrix-matched and reagent-only calibration standards prepared at equivalent tissue levels of 0X, 0.25X, 0.5X, 1X, 2X, and 3X
- Internal standards were added
- Method LOQs determined for spiked samples in matrix

1X Spiking Levels for the 133 Analytes

Conc.(ng/g)	Veterinary Drug Analytes	No.
10 -12	amoxicillin, ampicillin, cloxacillin, zilpaterol, salbutamol, cimaterol, clenbuterol, chloramphenicol, thiamphenicol, ivermectin,emamectin, bithionol, azaperone, xylazine, carazolol,haloperidol, ketoprofen, (aceto)(chlor)(triflu)(propionyl)promazine, promethazine, flubendazole+amino, mebendazole+amino, cambendazole, oxibendazole, ronidazole, metronidazole+hydroxy, ipronidazole+hydroxy, dimetridazole, niclosamide, oxyclosanide, rafoxanide	38
20-50	ciprofloxacin, difloxacin, norfloxacin, orbifloxacin,sarafloxacin, flunixin, melengestrolacetate, 2-mercaptobenzimidazole, 6-propyl-2-thitheacil, ractopamine, penicillin, carbadox+metabolite, abamectin, doramectin, albendazole+(amino)sulfone+sulfoxide,2-hydroxydimetridazole, triclabendazole+sulfoxide,closantel, moxidectin, nitroxynil	25
100	sulfonamides (16), desethyleneciprofloxacin, enrofloxacin, doxycyline, clindamycin, erythromicin, gamithromycin, lincomycin, tildipirosin, tilmicosin, cefazolin, cephapirin& metabolite, dicloxacillin, oxacillin, nafcillin, levamisole, (5-hydroxy)thiabendazole, morantel,clorsulon, haloxon,(oxy)phenbutazone, eprinomectin, meloxicam,betamethasone, prednisone, lasalosid, virginiamycin, zeranol	46
150-300	danofloxacin, tylosin, troleandomycin, methimazole, dipyronemetabolite, selamectin, diclofenac, tolfenamicacid, florfenicol+amine, pirlimycin	11
400-1000	(6-methyl)(6-phenyl)(2-)thitheacil, bacitracin, fenbendazole+sulfone, (oxy)(chlor)tetracycline, oxfendazole, novobiocin, tulathromycin, DCCD	13
250 (fixed)	$^{13}C_6$-sulfamethazine, flunixin-d_3, DCCD-d_3	3

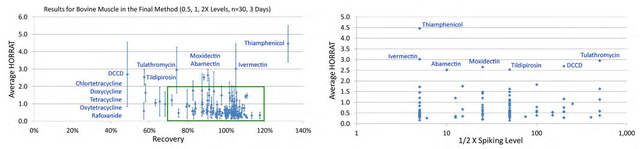
Validation and Results

- Spikes made of 18 different tissue blank samples at 0X, 0.5X, 1X, and 2X levels (n=10 each) repeated 3 days by 3 chemists
- Matrix-matched and reagent-only calibration stds prepared at equiv. tissue levels of 0X, 0.25X, 0.5X, 1X, 2X, and 3X
- Internal standards were added, but not needed nor used
- Method LOQs determined for spiked samples in matrix

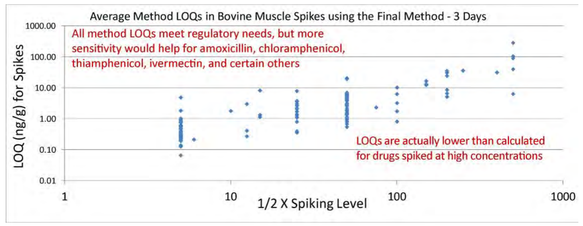
Among-Day Reproducibility of Recoveries

HorwitzRatio = HORRAT = $RSD_R/(2C^{-0.1505})$

in which RSD_R is reproducibility and C is concentration (g/g)



Limits of Quantification (LOQs) for the Final Method



Conclusion

Updated method logistics compared to previous

FSIS Method Logistics (UPLC-TQD)

- 1 chemist was able to process 60 pre-homogenized samples in an 8-hr day for an overnight sequence
- Longest step was 1 hr to evaporate Acetonitrile
- Cost of materials = $3/sample (using bulk C18)
- Waste = 10 mL hexane and 5 mL ACN & two 50 mL, one 15 mL PP tubes, and AS vial

New Method Logistics

- 1 chemist was able to process 60 pre-homogenized samples in 3 hthes
- (longest steps involved labeling tubes/vials, weighing, and preparing calibration standards)
- No glassware to be cleaned afterwards
- Waste = 10 mL ACN and one 50 mL tube and an autosampler vial
- Review of results for 135 drugs x 3 transitions x 67 injections (>27,000 data points) took 8 hthes

- The previous MRM for vet drug residues was streamlined and improved by using modern LC-MS/MS and eliminating hexane partitioning cleanup and solvent evaporation steps, and using filter-vial d-SPE for cleanup
- This method is more quantitative than the previous method
- LOQ <10 ng/g for nearly all drug analytes tested
- Sample throughput was 60 samples per day per 2 chemists for both sample prep and analysis/review of results
- Qualitative screening and identification results still need to be evaluated for method implementation by USDA-FSIS

Comparison of different methods of extraction for incurred contaminants in fish using the eXtractor3D|FV® & analyzed by LPGC-MS/MS

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Introduction

The goal of this study was to investigate variables impacting extraction yields of incurred pesticides and environmental contaminants: polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and flame retardants (FRs) in fish samples of white croaker and salmon. We sought to compare extraction efficiencies of different shakers and extraction devices including the determination of the optimum shaking/extraction time, sample size, and sample-to-solvent ratio. In Filter-Vial Dispersive Solid-Phase Extraction with MgSO4, Z-Sep, C18, and primary secondary amine sorbents were used for sample clean-up in the Thomson eXtractor3D|FV®. Samples were analyzed by Low Pressure Vacuum Outlet Gas Chromatography coupled to a Triple Quadrupole Tandem Mass Spectrometry (LPGC-MS/MS).

Equipment

- Agilent LPGC-MS/MS System with Multi-Mode Inlet (MMI)
- Carrier Gas: He+ @ 2mL/min constant flow rate
- MMI @ 80ºC
- Injection Volume: 5µL
- Thomson eXtractor3D, 0.2um PVDF (p/n 95531)
- Vortex
- Centrifuge

Sample Preparation:

Approximate Fish Composition

Sample	%H2O	%Lipid	%Protein
Croaker	78	3-4	18
Salmon	68-75	5-10	20-22
SRM 1947	73	10	17

Homogenization

Fish Type	Homogenizer Type	Size (g)	Acetonitrile(mL)	Time (min)	n
Croaker / Salmon	Vortex & Vibrate	10	10	1,10,30,60	4
SRM 1947	Vortex & Vibrate	5	5	1,10,30,60	1
Croaker / Salmon	Blender	10	10	1	4
SRM 1947	Blender	5	5	1	1
Croaker / Salmon	Vortex & Vibrate	2,4,4	2,4,8	10	4
SRM 1947	Vortex & Vibrate	2,4,4	2,4,8	10	1

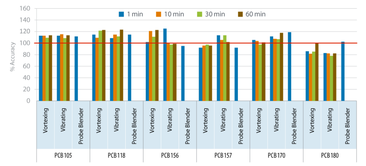
Sample Extraction and Clean Up:

1. Add 10g of homogenized fish and Internal Standard to a conical tube.
2. Add 10mL of Acetonitrile to the homogenate mix
3. Vortex for 10 minutes at 80% with max pulsing
4. Add 5g HCO_2NH_4 to the conical tube and shake for 1 minute
5. Centrifuge for 2 minutes at 3700RCF
6. Add 75mg of each: $MgSO_4$, 1:1:1 PSA:C18:Z-Sep to the outer shell of the Thomson eXtractor3D
7. Add 0.5mL of the fish extract to the sorbents in the outer shell of the Thomson eXtractor3D
8. Partially depress the eXtractor3D plunger into the outer shell
9. Shake for 30's
10. Fully depress the plunger.

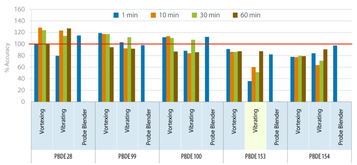
Result

Compounds were analyzed to determine the optimum extraction, sample size, and solvent ratio. Please see the following tables for the compounds that were analyzed: the pesticides analyzed can be found in Table 1; Table 2 shows the PCB Congeners; Table 3 shows the PBDE Congeners; Table 4 shows the PAH's and Table 5 shows the isotopically-labeled internal standards.

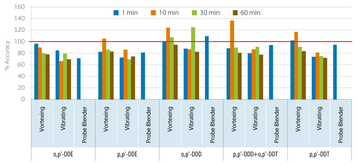
SMR 1947 results for PCBs



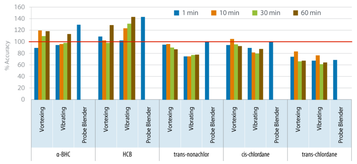
SMR 1947 results for PBDEs



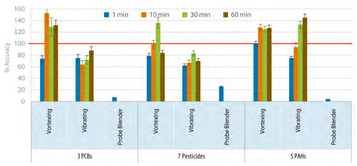
SMR 1947 results for pesticides (part 1)



SMR 1947 results for pesticides (part 2)



Salmon results



Conclusion

- A 1 minute extraction with the pulsed vortex shaker is sufficient for extraction of many but not all of the incurred contaminants in homogenized fish tissues. A 10 min extraction time was better and worked well for all the incurred contaminants in homogenized fish tissues.
  - Extraction with the prototype vibration shaker often took 60 minutes to achieve 100% extraction efficiency
  - Extraction with a probe blender was rapid and complete, but it limited sample throughput and was inconvenient
- The 1:1 Sample:Acetonitrile (g:mL) ratio was sufficient to achieve full extraction.
  - 2g of homogenized sample gave equivalent results as 4g and 10g samples.

New and Improved Final Sample Prep Method

1. Add 10g of homogenized fish and Internal Standard to a conical tube.
2. Add 10mL of Acetonitrile to the homogenate mix
3. Vortex for 10 minutes at 80% with max pulsing
4. Add 5g HCO_2NH_4 to the conical tube and shake for 1 minute
5. Centrifuge for 2 minutes at 3700 RCF
6. Add 75mg of each: $MgSO_4$, 1:1:1 PSA:C18:Z-Sep to the outer shell of the Thomson eXtractor3D
7. Add 0.5mL of the fish extract to the sorbents in the outer shell of the Thomson eXtractor3D
8. Partially depress the eXtractor3D plunger into the outer shell
9. Shake for 30's
10. Fully depress the plunger