Multiclass multiresidue analysis of >100 veterinary drug residues in bovine tissues by filter-vial dispersive-SPE and 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. In this study, a new LC-MS/MS Q-Trap instrument was used which met the same detection limit needs with merely 0.17 mg equivalent sample injection. This obviated the hexane-partitioning and solvent evaporation steps, and filter-vial dispersive-SPE was used to eliminate a centrifugation step. In this way, sample turnaround time was 20 min for an individual sample, and sample throughput was doubled for a batch of 60 samples. More than 100 of the 130 tested drug analytes achieved 70-120% recoveries and <20% RSD for 90 replicate injections at 3 spiking levels over the course of 3 days in each of bovine kidney and muscle sample types. This method outperforms the current FSIS method and may be used in the future to provide quantitative as well as qualitative screening results in routine monitoring programs.

INTRODUCTION

The USDA FSIS is responsible for conducting a monitoring and surveillance program of animal tissues in processing plants to ensure that veterinary drug residues are not present at levels above the US tolerances. Multiclass, multiresidue methods are increasingly used to provide more efficient monitoring, akin to pesticide residue monitoring of foods. However, veterinary drug residues are typically more diverse and animal-derived foods are more complicated than in pesticide applications, which makes their multiclass multiresidue analysis more challenging.

Goal

Develop and validate a highly streamlined 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 2 g homogenized tissue sample into 50 mL tube. Add internal standard and spike solutions as needed.
2. Dispense 10 mL 4/1 (v/v) MeCN/water and shake 5 min on platform shaker, then centrifuge 5 min at 3700 rcf.
3. Transfer 0.4 mL extracts to filter-vial shell containing 25 mg C_{18}, insert plunger halfway and shake 30 s, then fully depress plunger to filter final extract into autosampler vial.
4. Inject 1 µL in LC-MS/MS.

Filter-vial dispersive-SPE

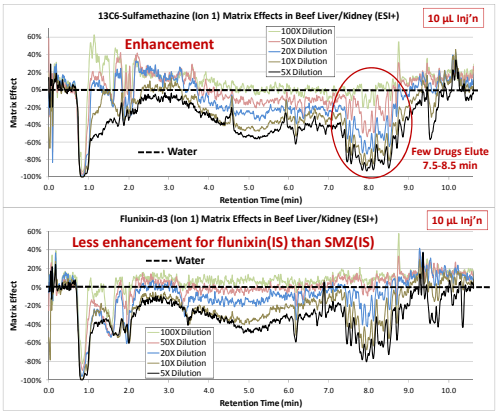
LC: Agilent 1100
MS/MS: AB Sciex 6500 Q-Trap
Column: Phenomenex Kinetex C18 (50 x 3.0 mm, 2.6 µm)
Mobile phase: (A) water; (B) MeCN both with 0.1% HCO_2H
Gradient: 2% → 100% B over 8.0 min, hold for 2.7 min
Flow rate: 0.3 mL/min Column temperature: 40°C
Electrospray ionization (positive/negative switching)
Scheduled MRM acquisition – 3 ions/drug w/60 s t_R window

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, melengestrol acetate, 2-mercaptobenzimidazole, 6-propyl-2-thiouracil, ractopamine, penicillin, carbadox+metabolite, abamectin, doramectin, albendazole+(amino)sulfone+sulfoxide, 2-hydroxydimetridazole, triclabendazole+sulfoxide, closantel, moxidectin, nitroxynil	25
100	sulfonamides (16), desethylene ciprofloxacin, enrofloxacin, doxycycline, 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, dipyrone metabolite, selamectin, diclofenac, tolfenamic acid, florfenicol+amine, pirlimycin	11
400 – 1,000	(6-methyl)(6-phenyl)(2-)thiouracil, bacitracin, fenbendazole+sulfone, (oxy)(chlor)tetracycline, oxfendazole, novobiocin, tulathromycin, DCCD	13
250 (fixed)	$^{13}C_6$-sulfamethazine, flunixin-d_3, DCCD-d_3	3

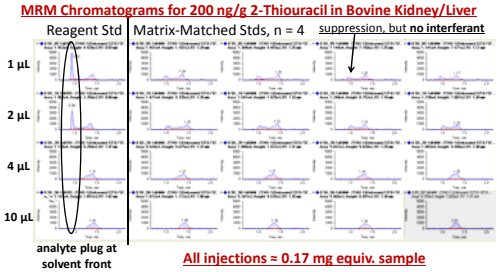
RESULTS and DISCUSSION

How much to dilute extracts?



Effect of Injection Volume on Chromatography

MRM Chromatograms for 200 ng/g 2-Thiouracil in Bovine Kidney/Liver

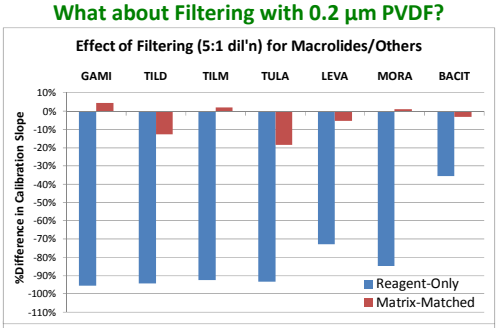


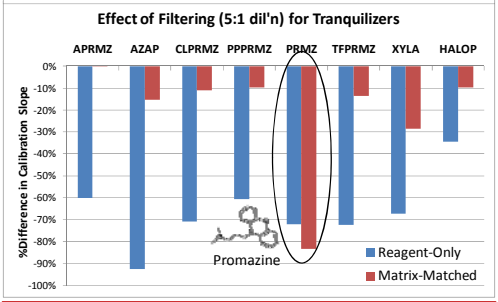
All injections = 0.17 mg equiv. sample

Inj. Vol (µL)	Dilution	%MeCN in Extracts	Avg Peak Area (RSD)	Matrix Effect
1	5X	≈70	13,000 (7%)	-41%
2	10X	≈35	14,000 (11%)	-36%
4	20X	≈17	10,000 (6%)	-10%
10	50X	≈8	9,800 (6%)	-14%

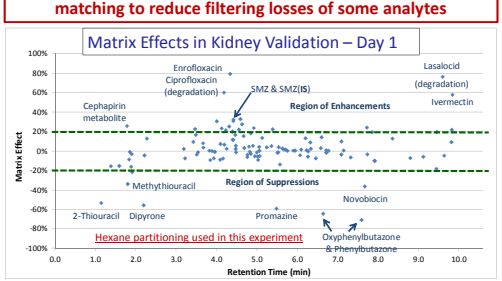
Final Conditions:
1 µL inj'n of 5X diluted extracts (≈70% MeCN into 2% MeCN mobile phase)

What about Filtering with 0.2 µm PVDF?



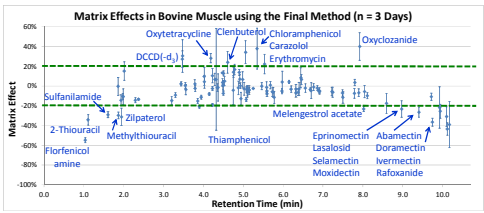


Can avoid matrix effects by dilution, but still needed matrix-matching to reduce filtering losses of some analytes

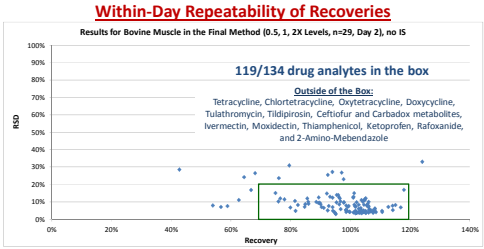


Validation and Results

- 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

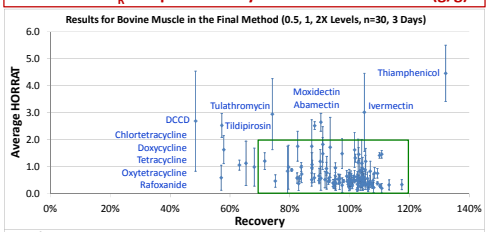


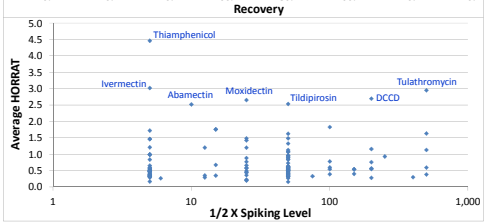
Within-Day Repeatability of Recoveries



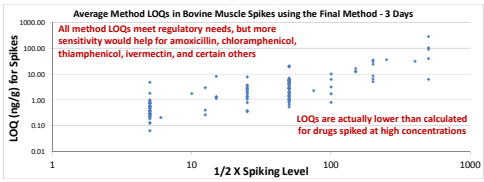
Among-Day Reproducibility of Recoveries

Horwitz Ratio = 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



CONCLUSIONS

- Our 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 our 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

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