Multiclass Multi-Residue 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

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

Homogenized Sample Preparation

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.

Fig 1. Minimizes 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

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.

Validation

- 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

Fig 2. 1X Spiking Levels for the 133 Analytes

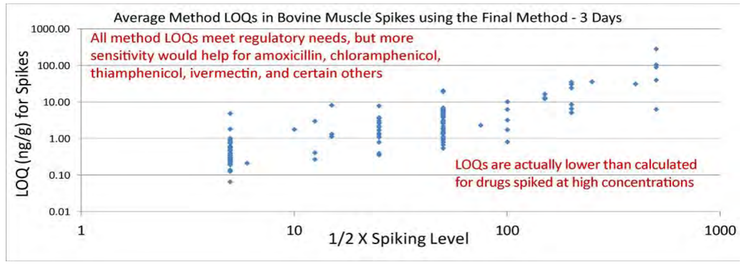
Conc.(ng/g)	Veterinary Drug Analytes	No.
10 -12	amoxicillin, ampicillin, cloxacillin, zilpaterol, salbutamol, cimaterol, clenbuterol, chloramphenicol, thiamphenicol, ivermectin,emamectin, bithionol, azaperone, xylazine, carazolol,haloperidol, ketoprofen, (aceto)(chlor)(triflu)(propionyl)promazine, promethazine, 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

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

Fig. 3 Limits of Quantification (LOQs) for the Final Method



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Conclusion

- 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 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

Comparison of updated and new methods for Veterinary Drugs

FSIS Method Logistics (UPLC-TQD)

- 1 chemist was able to process 60 pre-homogenized samples in an 8-hr day
- Waste = 10 mL hexane, 5mL CAN, two 50 mL tubes, one 15 mL PP tubes, and one autosampler vial/cap

New Method Logistics

- 1 chemist was able to process 60 pre-homogenized samples in 3 hours
- Waste = 10mL ACN, one 50mL tube and one Thomson eXtractor|3D Filter Vial™