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Evaluation of a multi-class, multi-residue liquid chromatography – tandem mass spectrometry method for analysis of 120 veterinary drugs in bovine kidney

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Traditionally, regulatory monitoring of veterinary drug residues in food animal tissues involves the use of several single-class methods to cover a wide analytical scope. Multi-class, multi-residue methods (MMMs) of analysis tend to provide greater overall laboratory efficiency than the use of multiple methods, and liquid chromatography–tandem mass spectrometry (LC-MS/MS) of targeted drug analytes usually provides exceptional performance even for complicated sample extracts. In this work, an LC-MS/MS method was optimized and validated in a test of 120 drug analytes from 11 different classes in bovine kidney. The method used 10 ml of 4/1 acetonitrile/water for extraction of 2 g samples and cleanup with hexane partitioning. Quantitative and qualitative performance was assessed for the analytes at fortification levels of 10, 50, 100, and 200 ng/g. With the method, 66 drugs gave 70–120% recovery with ≤20% RSD at all levels over the course of 3 days. At the 200 ng/g level, 89 drugs met these same standards. Limits of detection were ≤10 ng/g for 109 of the analytes in the kidney matrix in validation experiments. Qualitatively, MS/MS identification criteria were set that ion ratios occur within ±10% (absolute value) from those of the analyte reference standards. At the 10 ng/g level, 57% of the drugs met the identification criteria, which improved to 84% at the 200 ng/g level. The method serves as an efficient and useful additional option among the current monitoring methods available. Published 2012. This article is a U.S. Government work and is in the public domain of the USA.

Keywords: multi-class veterinary drug residues analysis; LC-MS/MS; MS identification; bovine kidney

Introduction

Administration of veterinary drugs in animals used for food can potentially lead to the presence of residues in the food products. In the USA, the Food and Drug Administration (FDA) Center for Veterinary Medicine (CVM) establishes tolerance levels for veterinary drug residues and the USDA Food Safety and Inspection Service (FSIS) is responsible for monitoring animal tissues in slaughter establishments to ensure that any such residues, if present, are at levels below the set tolerances. USDA-FSIS has the authority to condemn violative carcasses, and FDA-CVM has the authority to take enforcement actions at farm level. Current monitoring process and plans for improvement have been described elsewhere. [1]

Methods for residue monitoring have generally evolved over the years from single-analyte methods, to multi-analyte methods. In recent years, multi-class multi-residue methods (MMMs) have been introduced to further increase monitoring efficiency. Typically, such methods utilize liquid chromatography-tandem mass spectrometry (LC-MS/MS) due to the exceptional selectivity and sensitivity of the modern instrumentation. For example, LC-MS/MS has been demonstrated to perform well in the analysis of 62 drugs in bovine kidney, [1] 27 drug analytes from 5 classes in milk, [2] 41 drugs from 7 classes in eggs, [3] and 24–53 analytes from 4–8 classes in animal tissues. [4–7] LC-MS/MS is capable only of monitoring targeted analytes, and a few newer methods have been reported for even larger numbers of targeted analytes (≥100) from 9–12 classes in milk or animal tissues coupling LC to time of flight mass spectrometry (TOF-MS), or ultra high performance liquid chromatography (UHPLC) with high resolution MS, [8–10] with the added potential to find non-targeted chemical contaminants.

Recently, our laboratory used an LC-MS/MS method developed earlier for β-lactams [11] to screen bovine kidney juice and serum samples for 56 drugs from 6 classes. [12] Kidney is a commonly selected tissue in screening for veterinary drug residues, as evidenced by implementation of the Kidney Inhibition Swab (KIS™) test by FSIS. In this current work, we aimed to optimize our extraction protocol and quantitatively and qualitatively assess this approach in bovine kidney samples at fortification levels of 10, 50, 100, and 200 ng/g for 120 analytes from 11 veterinary drug families.

Materials and methods

Materials

Veterinary drug standards were obtained from sources described previously.[12] In addition, flumequine, spiramycin adipate,

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clindamycin hydrochloride, semicarbazide hydrochloride, AOZ, AMOZ, furaltadone, 6-propyl-2-thiouracil, 2-mercaptobenzamidazole, cefadroxil, cefalonium, cefoperazone sodium salt, flunixin-d_3, pyrantel citrate salt, thiabendazole, albendazole, oxfendazole, fenbendazole, flubendazole, levamisole, morantel citrate salt, oxibendazole, salbutamol, terbutaline hemisulfate, tulobuterol hydrochloride, clenbuterol, dipyrone, xylazine, acetopromazine maleate salt, haloperidol, promethazine hydrochloride, propionylpromazine hydrochloride, chlorpromazine hydrochloride, triflupromazine hydrochloride, ketoprofen, oxyphenylbutazone hydrate, diclofenac sodium, phenylbutazone, tolfenamic acid, triamcinolone, prednisolone, prednisone, methyl prednisolone, betamethasone, flumethasone, triamcinolone acetonide, virginiamycin, and novobiocin sodium were obtained from Sigma-Aldrich (Milwaukee, WI, USA), sulfaethoxypyridazine was from Cyanamid (Florham Park, NJ, USA), mebendazole was from Janssen Animal Health (Beerse, Belgium), gamithromycin was from the FSIS (St Louis, MO, USA) tulathromycin was from Pfizer (New York, NY, USA), nitrofurazone, nitrofurantoin, furazolidone, and azaperone were from USP (Rockville, MD, USA), 2-mercapto-1-methylimidazole and 6-methyl-2-thiouracil were from Lancaster synthesis (Windham, NH, USA), 6-phenyl-2-thiouracil was from VWR (Radnor, PA, USA), 5-hydroxythiabendazole was from the U.S. Environmental Protection Agency (Fort Meade, MD, USA), ractopamine hydrochloride was from Lilly (Indianapolis, IN, USA), zilpaterol hydrochloride was from Aventis Pharmaceuticals (Bridgewater, NJ, USA), cimaterol and fenoterol hydrobromide were from MP biomedicals (Solon, OH, USA), carazolol and mabuterol hydrochloride was from Dr Ehrenstorfer GmbH (Augsburg, Germany), melengestrol acetate was from Pharmacia and Upjohn (Bridgewater, NJ, USA), and triclabendazole was from Witega Laboratories Berlin-Aldershof GmbH (Berlin, Germany) Stock solutions of high concentration (~500–2000 μg/ml) were prepared for each analyte and stored at −20°C. Samples were dissolved in acetonitrile (MeCN) and/or methanol (MeOH) whenever possible, except for β-lactams (H_2O), a few quinolones (0.03 M NaOH), a few anthelminthics (addition of dimethyl formamide required), and novobiocin (ethanol). Two fortification mixture solutions of 15 β-lactams (50 μg/ml) and the 104 other veterinary drugs (5 μg/ml) were prepared by pipetting the required volumes of each stock solution and adding H_2O to the desired volume. These fortification mixtures were aliquoted into small vials and stored at −20°C. An internal standard (IS) stock solution (50 μg/ml) containing penicillin V, $^{13}C_6$-sulfamethazine, and flunixin-d_3 in water was prepared, aliquoted into microfuge tubes and stored at −20°C. In general, solutions prepared in organic solvents were stored in glass, while aqueous solutions were stored in plastic.

MeCN-saturated hexane was prepared by addition of MeCN to hexane and mixing until a second phase appeared; the upper hexane layer was decanted and stored for use in experiments. MeCN and MeOH were HPLC grade (Fisher, Fair Lawn, NJ, USA), and formic acid was MS grade (Fluka, St Louis, MO, USA). C-18 sorbent (40 μm) was obtained from JT Baker (Phillipsburg, NJ, USA) for use in experiments involving dispersive solid-phase extraction (d-SPE) clean-up. All aqueous solutions used deionized water prepared with a Barnstead E-pure system (Dubuque, IA, USA). Organic bovine kidneys used as controls were shipped frozen from Blackwing, Inc. (Antioch, IL, USA), homogenized, and then stored at −20°C.

Fortification and sample preparation of bovine kidney

Homogenized control (blank) bovine kidney tissue (2.0 g) was placed in a disposable 50-ml centrifuge tube. The IS mixture was added at a level of 100 ng/g, followed by the β-lactam and other veterinary drug fortification mixtures, at the desired level. None of these solutions were added to the blank kidney samples used for matrix-matched calibration. To each tube was added 10-mL of either 4/1 (*v/v*) MeCN/H_2O or 4/1 (*v/v*) MeCN/H_2O containing 1% formic acid. Tubes were vortex-mixed (30 s) and then centrifuged (3716 x g, 5 min). The supernatants (extracts) were then decanted into 50 mL polypropylene centrifuge tubes with either (a) addition of 10 mL MeCN-saturated hexane, (b) 0.5 g C-18 sorbent, or (c) both 0.5 g C-18 sorbent and addition of 10 ml MeCN-saturated hexane, and all tubes were vortexed (15 s) and centrifuged (3716 x g, 5 min). When present, the hexane layer was removed via aspiration. Extracts (5 ml) were pipetted into 15-ml graduated glass centrifuge tubes and evaporated to <0.7 ml at 40°C under N_2 flow (Zymark Turbovap LV, Hopkinton, MA, USA).

For matrix-matched calibration standards, the IS mixture, followed by the β-lactam and other veterinary drug fortification solutions, were added at the desired levels to the blank extracts. To all tubes were added 0.5% formic acid (100 μl) and water to yield a final volume of 1 ml. Samples were then transferred to 0.2 μm PVDF filter autosampler vials (Thomson Instrument Co., Oceanside, CA, USA) for LC-MS/MS analysis.

Instrumentation

LC-MS/MS was conducted with an Agilent 1100 HPLC system equipped with a degasser, binary pump, autosampler and column heater, coupled with an Applied Biosystems API 3000 triple quadrupole mass spectrometer. Chromatography involved a Prodigy ODS-3 (3.0 x 150 mm, 5 μ) column with an ODS (4.0 x 2.0 mm) Security Guard column (Phenomenex, Torrance, CA, USA). The column was maintained at 30°C, with a flow rate of 300 μl/min. The mobile phase consisted of (A) 0.1% aqueous formic acid and (B) 0.1% formic acid in MeCN, and the gradient programme was 2% B to start and ramped to 100% B until 15 min, where it was held for an additional 5 min. Sample injection volume was 20 μl. In order to reduce potential carryover, a solvent blank was injected after each sample, during which the column was re-equilibrated to 2% B over the course of 10 min. Thus samples could be injected every 30 min. A divert valve was used at the beginning and end of each chromatographic run to minimize contamination of the mass spectrometer, which was operated in positive ion electrospray mode. The spray shield was cleaned daily.

Data were collected using scheduled selected reaction monitoring (sSRM) software with a 40 s window centered on the expected retention time (t_R). Precursor and product ions from two transitions, along with retention times, are listed in Table 1, and a sample SRM from a fortified sample is shown in Figure 1. All other parameters, such as declustering potential, focusing potential, collision energy, and collision exit potential were optimized for each analyte using Analyst 1.5 optimization software.

Evaluation study

Matrix-matched calibration standards were prepared each day by spiking blank extracts over a range of 6 tissue equivalent concentrations. These concentrations consisted of 1, 5, 10, 20, 50, and 100 ng/g for kidney fortification levels of 10 and 50 ng/g, and 10, 50, 100, 150, 200, and 250 ng/g for fortification levels of 100 and 200 ng/g. Each range of calibration standards included four matrix-matched

Table 1. Drug analytes listed by class and their retention times, quantifier and qualifier ions, and the ranges of lowest calibrated level (LCL) for the evaluation experiments in the bovine kidney matrix. Qual. ions in bold text signify when the precursor ion was monitored at two different collision energies

CLASS/Drug	t_R (min)	Precursor Ion (*m/z*)	Quant. Ion (*m/z*)	Qual. Ion (*m/z*)	LCL (ng/g)
QUINOLONES					
Desethyleneciprofloxacin	8.0	305.9	288.1	268.0	5-10
Norfloxacin	8.1	320.0	302.1	276.2	10-50
Ofloxacin	8.1	362.1	318.1	261.0	1-1
Ciprofloxacin	8.1	332.0	288.2	314.0	5-10
Lomefloxacin	8.2	352.0	237.0	309.1	1-1
Danofloxacin	8.2	358.0	340.1	314.1	20-50
Enrofloxacin	8.3	360.0	316.2	245.1	1-1
Sarafloxacin	8.7	386.0	367.9	342.0	10-50
Difloxacin	8.7	400.0	356.0	299.1	1-1
Oxolinic acid	11.6	262.1	244.0	216.0	1-1
Flumequine	13.1	262.0	244.0	126.0	1-1
SULFONAMIDES					
Sulfaguanidine	6.5	215.0	156.0	108.0	10-50
Sulfanilamide	6.8	173.1	156.0	92.0	1-10
Sulfacetamide	8.9	215.0	155.9	108.1	5-50
Sulfadiazine	9.1	250.9	156.0	92.0	1-5
Sulfathiazole	9.1	256.0	156.0	108.1	5-5
Sulfapyridine	9.2	250.0	156.0	108.0	1-1
Sulfamerazine	9.6	265.0	156.0	108.1	1-1
Sulfamethizole	10.0	271.0	156.0	108.1	1-5
Sulfamethazine	10.2	279.0	186.0	124.0	1-5
^{13}C-Sulfamethazine (IS)	10.2	285.0	185.8	124.1	N/A
Sulfamethoxypyridazine	10.2	281.0	156.0	108.1	1-1
Sulfachloropyridazine	11.0	285.0	156.0	108.1	1-5
Sulfaethoxypyridazine	11.2	295.0	156.0	108.1	1-1
Sulfadoxine	11.2	310.9	156.0	92.0	1-1
Sulfamethoxazole	11.4	254.1	156.0	108.0	1-1
Sulfisoxazole	11.6	268.1	156.0	113.1	1-1
Sulfaquinoxaline	12.0	300.9	156.0	108.1	1-1
Sulfadimethoxine	12.1	311.0	156.0	108.1	1-1
Sulfaphenazole	12.2	315.0	160.0	159.0	1-1
Sulfabromomethazine	13.2	359.0	156.0	108.1	1-1
Sulfasalazine	15.1	399.1	381.0	119.0	1-5
TETRACYCLINES					
Oxytetracycline	8.2	460.9	426.0	443.1	1-1
Tetracycline	8.4	445.0	410.0	427.0	1-1
Chlortetracycline	9.3	479.0	462.0	444.0	1-5
Doxycycline	9.3	445.0	428.0	410.0	1-1
MACROLIDES					
Lincomycin	7.5	407.2	126.0	359.1	1-5
Tulathromycin	7.7	806.5	577.2	158.0	10-10
Spiramycin	8.3	422.2	174.1	101.0	5-5
Gamithromycin	8.6	777.5	83.0	158.2	5-10
Pirlimycin	8.7	411.1	112.0	363.0	1-1
Tilmicosin	9.0	869.7	174.1	696.5	5-10
Clindamycin	9.0	425.0	126.1	377.0	1-1
Erythromycin	9.7	734.6	158.0	576.4	1-1
Tylosin	10.0	916.7	173.9	772.5	1-5
NITROFURANS					
Semicarbazide	2.5	76.0	59.0	31.1	>250
AMOZ	2.5	202.1	129.0	126.0	10-100
AOZ	3.5	103.0	59.0	42.0	20-150
Furaltadone	7.7	325.0	281.0	252.0	1-5
Nitrofurazone	9.8	199.0	156.0	108.0	100-200
Nitrofurantoin	10.4	239.1	122.0	139.0	50- > 100

Table 1. *(Continued)*

CLASS/Drug	t_R (min)	Precursor Ion (*m/z*)	Quant. Ion (*m/z*)	Qual. Ion (*m/z*)	LCL (ng/g)
Furazolidone	11.0	226.0	121.9	139.0	20-50
THYREOSTATS					
2-Mercapto-1-methylimidazole	7.0	115.0	88.0	73.9	10-50
6-Methyl-2-thiouracil	7.1	143.0	84.0	125.9	100-100
6-Propyl-2-thiouracil	9.3	171.0	154.0	112.0	5-10
2-Mercaptobenzimidazole	9.9	151.0	93.0	118.0	5-5
6-Phenyl-2-thiouracil	10.5	204.9	188.0	146.1	100-100
β-LACTAMS					
Desacetyl cephapirin	6.6	382.0	151.9	225.9	1-10
Amoxicillin	6.9	366.0	349.0	114.0	1-50
Cefadroxil	7.2	364.0	113.9	207.8	5-50
Cephapirin	7.6	424.0	291.8	151.9	1-5
DCCD	7.7	549.1	182.9	240.8	10-50
Ampicillin	7.9	350.0	106.0	174.1	1-5
Cephalexin	8.0	348.0	157.8	173.9	1-5
Cefalonium	8.7	458.9	337.0	152.1	1-1
Cefazolin	10.1	455.0	322.9	155.9	1-1
Cefoperazone	11.0	646.1	530.1	143.2	1-1
Penicillin G	12.7	335.0	159.9	176.0	1-1
Penicillin V (IS)	13.2	351.0	159.9	114.0	N/A
Oxacillin	13.6	402.1	159.9	242.9	1-1
Cloxacillin	14.0	436.0	276.9	159.9	1-1
Nafcillin	14.2	415.1	198.9	170.9	1-1
Dicloxacillin	14.7	470.0	159.9	310.9	1-1
ANTHELMINTHICS					
5-Hydroxythiabendazole	7.5	217.9	191.1	147.0	1-10
Levamisole	7.7	205.0	178.2	91.0	1-5
Pyrantel	8.1	206.9	150.0	136.0	1-5
Thiabendazole	8.0	202.0	175.0	130.9	1-5
Morantel	8.7	221.0	123.0	164.0	1-1
Oxibendazole	9.6	250.0	218.2	176.1	1-1
Oxfendazole	10.4	346.0	159.1	283.9	1-1
Albendazole	11.4	266.0	234.0	191.0	1-5
Mebendazole	11.9	296.0	264.0	105.0	1-1
Flubendazole	12.4	314.0	282.0	123.1	1-1
Fenbendazole	13.0	299.9	267.9	159.1	1-50
Triclabendazole	16.5	360.9	274.0	171.1	20-100
β-AGONISTS					
Salbutamol	6.7	240.1	222.2	148.2	1-5
Terbutaline	6.7	226.0	152.0	170.1	1-5
Zilpaterol	6.7	262.0	185.2	202.1	1-5
Cimaterol	7.2	220.0	160.2	143.1	1-5
Fenoterol	7.5	304.0	135.1	286.1	1-10
Ractopamine	8.1	302.1	284.1	164.1	5-5
Tulobuterol	8.5	228.0	154.1	172.1	1-1
Clenbuterol	8.6	277.0	203.0	259.0	1-1
Mabuterol	9.2	311.0	237.0	293.0	1-1
TRANQUILIZERS/NSAIDS					
Dipyrone	7.1	218.1	187.0	125.1	5-50
Azaperone	8.1	328.1	165.0	122.9	1-1
Xylazine	8.6	221.0	90.0	77.2	1-5
Carazolol	9.2	299.1	116.1	**299.1**	1-1
Acetopromazine	10.0	327.1	253.9	222.0	10-50
Haloperidol	10.1	376.0	165.1	123.0	5-10
Promethazine	10.0	285.0	198.0	239.9	5-50
Propionylpromazine	10.5	341.1	86.1	267.9	10-50
Chlorpromazine	10.6	319.0	86.1	**319.0**	20-100

Table 1. *(Continued)*

CLASS/Drug	t_R (min)	Precursor Ion (*m/z*)	Quant. Ion (*m/z*)	Qual. Ion (*m/z*)	LCL (ng/g)
Triflupromazine	10.9	353.0	86.1	**353.0**	20-100
Ketoprofen	14.1	255.0	209.0	105.0	1-5
Oxyphenylbutazone	14.1	325.0	204.0	120.0	1-5
Flunixin	15.1	297.0	278.9	263.9	1-1
Flunixin-d_3 (IS)	15.1	300.1	282.1	264.0	N/A
Diclofenac sodium	15.7	295.9	249.9	214.0	5-5
Phenylbutazone	16.2	309.0	120.0	188.1	5-5
Tolfenamic acid	17.1	262.0	243.9	209.0	5-20
OTHER					
Florfenicol amine	5.2	248.0	230.0	130.0	1-50
Carbadox	9.1	263.0	231.0	130.0	1-5
Triamcinolone	10.5	395.1	375.0	357.0	1-1
Prednisolone	11.4	361.1	343.0	147.1	5-10
Prednisone	11.6	359.1	341.0	147.0	10-50
Methyl prednisolone	12.2	375.1	357.1	161.0	5-5
Betamethasone	12.4	393.1	373.1	355.2	1-1
Flumethasone	12.5	411.1	253.0	121.1	5-5
Triamcinolone acetonide	13.0	435.1	415.2	213.0	1-1
Virginiamycin	13.1	526.3	508.3	109.1	5-50
Novobiocin	16.0	613.2	189.3	217.9	1-1
Melengestrol acetate	17.0	397.1	337.1	279.0	50-100

N/A = not applicable

replicates at each studied fortification level. Recoveries were calculated based on comparison of the integrated peak areas for the spiked analytes with the average peak areas for the matrix-matched calibration samples at the given fortification level. The lowest calibrated level (LCL) was determined each day as the minimum concentration at which the signal height for matrix-matched calibration standards was at least twice that of peak-to-peak background noise (LCL equaled or exceeded the limit of quantification).

Results and discussion

Selection of drug analytes and LC-MS/MS method

The 120 analytes examined in this study were chosen as representative of 10 major veterinary drug classes, and also included a group of 12 assorted ‘other’ veterinary drugs. A generic mobile phase gradient was used, and analyte t_R values distributed well

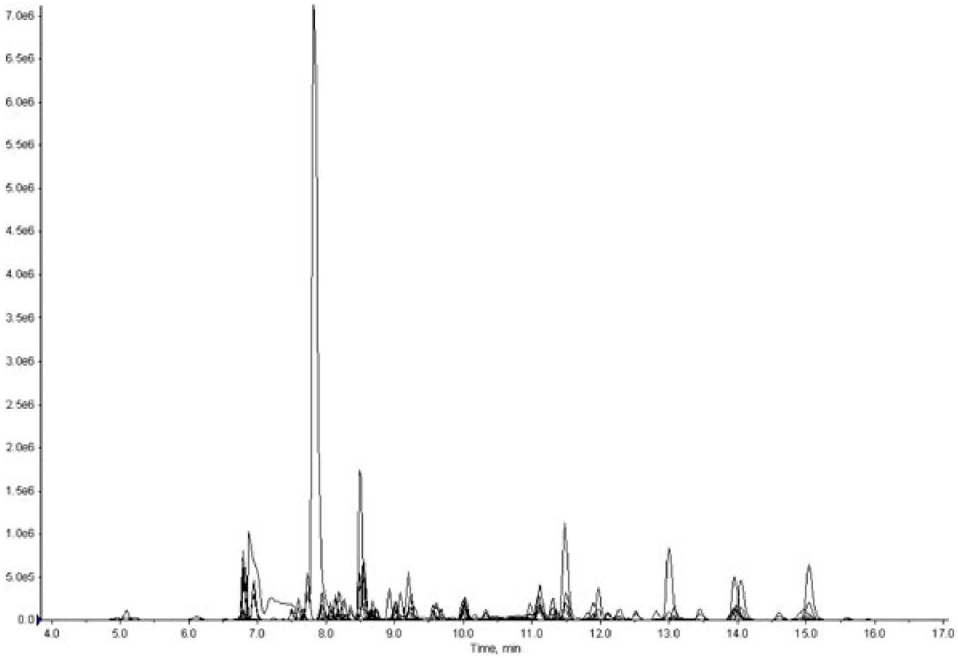

Figure 1. SRM from beef kidney fortified at 100 ng/g with 120 drug residues.

Table 2. Average recoveries (Rec.) and precisions for the drug analytes in spiked bovine kidney samples (n = 5 at each level each day for 3 days); IS = internal standard used (P = penicillin V, S = sulfamethazine-$^{13}C_6$; F = flunixin-d_3), rsd = relative standard deviation within day (repeatability), and RSD = reproducibility among days. Bold text highlights values outside of 70-120% recovery, rsd > 15%, and RSD >20%. Cut-off peaks and statistical outliers were not included

CLASS/Analyte		10 ng/g Level				50 ng/g Level				100 ng/g Level				200 ng/g Level			
	IS	n	Rec. (%)	rsd (%)	RSD (%)	n	Rec. (%)	rsd (%)	RSD (%)	n	Rec. (%)	rsd (%)	RSD (%)	n	Rec. (%)	rsd (%)	RSD (%)
QUINOLONES																	
Desethyleneciprofloxacin	P	12	85	9	**21**	12	80	**29**	**47**	14	99	9	**35**	10	72	2	4
Norfloxacin	P	15	90	11	11	15	100	6	7	15	86	5	8	15	79	7	8
Ofloxacin	P	14	97	9	9	13	99	4	4	15	101	5	12	15	91	8	10
Ciprofloxacin	P	14	87	13	13	15	91	7	7	15	86	9	11	15	81	6	8
Lomefloxacin	P	15	88	**17**	17	15	90	6	7	15	90	7	8	15	83	5	7
Danofloxacin	S	14	99	**37**	**34**	15	108	**18**	**25**	15	86	**16**	**21**	15	85	11	10
Enrofloxacin	P	15	98	8	10	15	103	3	4	15	94	4	5	15	91	5	6
Sarafloxacin	P	15	105	12	20	12	100	8	11	15	94	8	9	15	84	8	11
Difloxacin	P	15	102	5	8	15	105	7	6	15	95	7	6	15	98	4	5
Oxolinic acid	P	15	98	3	6	15	100	3	4	15	99	3	6	15	96	3	3
Flumequine	P	15	104	4	5	15	103	4	4	15	98	3	6	15	97	3	3
SULFONAMIDES																	
Sulfaguanidine	S	14	92	**24**	**24**	15	83	**36**	**33**	15	95	**37**	**34**	14	104	**35**	**50**
Sulfanilamide	P	15	109	**30**	**32**	13	120	**26**	**45**	15	105	**25**	**28**	14	**125**	**26**	**40**
Sulfacetamide	S	15	99	10	11	15	99	10	10	15	99	9	10	15	94	10	10
Sulfadiazine	S	15	99	4	6	15	99	6	7	15	102	5	5	15	97	5	6
Sulfathiazole	S	15	95	4	4	15	97	6	6	15	99	5	5	15	98	4	4
Sulfapyridine	S	15	100	4	4	15	98	6	6	15	101	5	6	15	98	3	3
Sulfamerazine	S	15	103	3	4	15	99	6	6	15	102	5	6	15	99	4	5
Sulfamethizole	S	15	94	2	5	15	97	5	5	15	95	3	5	15	94	3	6
Sulfamethazine	S	15	98	2	3	15	99	4	4	15	99	3	3	15	100	3	4
Sulfamethoxypyridazine	S	15	95	2	3	15	97	3	3	15	98	3	5	15	97	3	4
Sulfachloropyridazine	S	15	97	4	5	15	96	5	5	15	99	6	8	15	97	3	6
Sulfaethoxypyridazine	S	15	90	4	4	15	93	6	8	15	92	6	8	15	92	4	5
Sulfadoxine	S	15	98	5	5	15	99	6	6	15	98	5	7	15	101	3	4
Sulfamethoxazole	S	15	98	4	4	15	99	6	8	15	98	7	8	15	99	4	4
Sulfisoxazole	S	15	96	4	6	15	98	5	6	15	100	5	9	15	96	3	3
Sulfaquinoxaline	S	15	93	5	8	15	93	6	9	15	88	7	8	15	86	6	8
Sulfadimethoxine	S	15	96	4	6	15	96	5	5	15	97	6	8	15	96	3	7
Sulfaphenazole	S	15	95	5	6	15	97	6	7	15	94	6	7	15	93	5	6
Sulfabromomethazine	S	14	91	9	9	14	90	5	10	15	83	7	8	15	83	6	7
Sulfasalazine	S	15	98	5	6	15	100	7	7	15	91	7	9	15	93	4	6
TETRACYCLINES																	
Oxytetracycline	P	15	**62**	7	6	15	**66**	7	8	15	**66**	4	5	15	**64**	7	10
Tetracycline	P	15	**68**	6	6	15	71	5	8	15	71	4	4	15	70	5	6
Chlortetracycline	P	15	**60**	13	14	15	**56**	7	8	15	**53**	5	6	15	**52**	4	4
Doxycycline	P	15	71	5	6	15	76	5	6	15	72	6	9	15	70	4	4
MACROLIDES																	
Lincomycin	S	12	97	11	14	9	97	11	**26**	15	**122**	7	**31**	15	104	**33**	**35**
Tulathromycin	F	14	**53**	**21**	**24**	12	72	12	14	15	70	12	**21**	15	**67**	**16**	**31**
Spiramycin	F	14	96	11	11	13	104	9	11	15	94	14	19	13	102	8	10
Gamithromycin	S	15	97	12	14	12	106	13	18	15	92	11	**26**	15	98	13	12
Pirlimycin	P	15	84	9	10	15	87	6	9	15	89	10	13	15	87	8	11
Tilmicosin	S	14	93	14	15	12	113	10	13	15	88	12	16	14	99	10	15
Clindamycin	P	15	97	7	8	14	102	4	6	15	96	8	9	15	99	4	6
Erythromycin	S	15	101	10	12	14	102	9	8	15	97	10	10	15	99	7	7
Tylosin	S	15	114	9	**47**	14	108	**16**	20	15	83	11	13	14	86	16	20
NITROFURANS																	
Semicarbazide	**S**	0	--	--	--	0	--	--	--	5	**129**	6	**N/A**	5	113	12	**N/A**
AMOZ	S	15	114	**26**	**23**	15	93	15	**21**	15	93	**16**	**29**	14	89	16	19
AOZ	P	15	91	**86**	**100**	15	**61**	**48**	**90**	15	94	**24**	**33**	15	97	**33**	**31**
Furaltadone	S	12	106	7	**36**	10	83	14	**24**	15	111	3	**27**	15	86	**39**	**35**

Table 2. *(Continued)*

CLASS/Analyte	10 ng/g Level				50 ng/g Level				100 ng/g Level				200 ng/g Level				
	IS	n	Rec. (%)	rsd (%)	RSD (%)	n	Rec. (%)	rsd (%)	RSD (%)	n	Rec. (%)	rsd (%)	RSD (%)	n	Rec. (%)	rsd (%)	RSD (%)
Nitrofurazone	S	15	**140**	**81**	**137**	15	81	**37**	**44**	15	97	14	**25**	15	82	14	18
Nitrofurantoin	S	14	**129**	**17**	**21**	14	**129**	13	14	14	**122**	13	15	15	119	14	15
Furazolidone	P	14	80	**22**	**29**	15	93	14	17	15	96	8	8	15	97	7	17
THYREOSTATS																	
2-Mercapto-1-methylimidazole	S	15	118	**60**	**78**	12	78	**30**	**33**	14	87	**39**	**39**	15	94	**46**	**54**
6-Methyl-2-thiouracil	S	15	**192**	**48**	**45**	14	**175**	**69**	**55**	15	**123**	**44**	**42**	15	**144**	**40**	**49**
6-Propyl-2-thiouracil	S	15	93	14	14	15	102	11	13	15	98	11	12	15	97	5	9
2-Mercaptobenzamidazole	P	15	92	10	11	15	93	8	8	15	92	10	10	14	84	10	10
6-Phenyl-2-thiouracil	P	13	97	**44**	**71**	15	101	**30**	**27**	15	104	17	18	15	89	12	16
β-LACTAMS																	
Desacetyl cephapirin	S	14	**132**	**32**	**53**	11	**162**	8	**31**	15	115	19	18	15	115	**49**	**61**
Amoxicillin	P	15	**146**	**28**	**49**	13	99	9	18	15	90	11	15	15	98	15	**23**
Cefadroxil	P	14	74	**52**	**61**	15	**60**	**42**	**68**	15	84	**25**	**25**	14	93	**33**	**41**
Cephapirin	P	13	**64**	15	**25**	15	**55**	15	19	15	88	4	5	15	81	8	13
DCCD	P	14	84	**29**	**28**	15	90	11	10	15	87	6	9	15	83	6	9
Ampicillin	P	14	93	7	7	14	98	4	7	15	104	8	16	13	89	7	8
Cephalexin	P	14	95	7	8	15	91	5	6	15	89	8	9	15	80	9	14
Cefalonium	P	15	100	5	5	15	103	3	5	15	95	6	6	15	95	6	6
Cefazolin	P	15	106	6	6	15	107	4	6	15	102	4	4	15	98	3	3
Cefoperazone	P	15	106	5	5	15	110	3	3	15	103	3	4	15	101	3	3
Penicillin G	P	15	107	3	5	15	108	2	3	15	102	4	5	15	101	2	3
Oxacillin	P	15	102	3	5	15	103	3	4	15	100	2	2	15	101	2	3
Cloxacillin	P	15	101	4	6	15	104	3	3	15	98	3	3	15	99	2	2
Nafcillin	P	15	104	3	4	15	105	2	4	15	102	3	3	15	102	2	3
Dicloxacillin	P	15	101	4	6	15	99	3	4	15	97	3	3	15	96	4	4
ANTHELMINTHICS																	
5-Hydroxythiabendazole	S	10	101	12	**55**	11	**56**	10	**70**	14	108	14	**33**	13	87	**56**	**50**
Levamisole	P	14	93	**27**	**28**	14	98	7	7	15	104	5	7	15	101	6	6
Pyrantel	P	13	99	9	11	10	96	12	10	15	107	7	19	15	97	7	12
Thiabendazle	S	13	87	5	12	11	85	9	11	15	109	8	**29**	13	86	9	14
Morantel	P	15	86	11	11	15	83	9	9	15	82	7	7	15	83	8	7
Oxibendazole	F	15	**66**	11	12	14	**65**	6	7	15	70	**17**	**27**	15	**68**	6	13
Oxfendazole	P	15	98	8	12	15	96	6	9	15	91	6	6	15	84	5	6
Albendazole	F	15	**66**	14	13	15	**66**	13	14	15	**67**	**18**	**26**	15	70	6	14
Mebendazole	F	15	73	10	12	14	71	5	7	15	75	**16**	**26**	15	70	6	14
Flubendazole	F	15	72	10	11	14	70	4	6	15	72	**17**	**26**	15	**69**	5	15
Fenbendazole	F	15	86	**30**	**35**	15	82	**26**	**34**	14	89	**77**	**96**	15	76	11	13
Triclabendazole	S	15	110	**32**	**65**	15	119	**62**	**90**	13	**66**	**25**	**58**	15	101	**32**	**59**
β-AGONISTS																	
Salbutamol	S	15	95	10	11	12	100	8	10	15	95	9	15	14	110	8	**21**
Terbutaline	S	14	93	7	8	12	98	7	9	14	97	6	14	14	103	12	19
Zilpaterol	S	15	101	10	11	12	104	10	12	15	91	13	**21**	14	112	12	**31**
Cimaterol	S	11	76	**68**	**69**	14	110	**17**	**27**	14	106	6	10	14	102	8	10
Fenoterol	S	12	118	**104**	**100**	8	104	8	**22**	15	120	8	**39**	13	97	**47**	**43**
Ractopamine	P	15	100	7	7	15	106	6	5	15	96	5	6	15	100	5	7
Tulobuterol	S	15	96	4	9	15	98	6	7	15	98	6	8	15	99	6	7
Clenbuterol	P	15	102	5	6	15	102	6	5	15	96	5	6	15	97	5	6
Mabuterol	P	15	97	5	6	15	102	2	7	15	97	6	7	15	94	4	4
TRANQUILIZERS/NSAIDS																	
Dipyrone	P	15	**162**	**122**	**158**	13	71	**80**	**95**	12	**6**	**94**	**153**	14	**63**	**139**	**143**
Azaperone	P	14	90	11	12	14	89	8	13	15	74	12	15	15	85	10	14
Xylazine	P	14	88	7	10	15	86	7	8	15	90	8	9	14	86	5	8
Carazolol	P	15	88	8	11	14	88	4	8	15	78	7	8	15	77	3	6
Acetopromazine	F	15	92	**16**	18	15	78	9	9	14	72	**19**	**23**	15	76	8	14
Haloperidol	F	15	83	11	17	15	78	4	6	15	75	**17**	20	15	76	8	11

Table 2. *(Continued)*

CLASS/Analyte	**10 ng/g Level**				**50 ng/g Level**				**100 ng/g Level**				**200 ng/g Level**				
	IS	**n**	**Rec. (%)**	**rsd (%)**	**RSD (%)**	**n**	**Rec. (%)**	**rsd (%)**	**RSD (%)**	**n**	**Rec. (%)**	**rsd (%)**	**RSD (%)**	**n**	**Rec. (%)**	**rsd (%)**	**RSD (%)**
Promethazine	P	15	112	**18**	**37**	15	84	12	15	15	**59**	**16**	**27**	15	**52**	6	10
Propionylpromazine	F	15	85	**25**	**22**	15	78	10	**22**	15	73	**32**	**37**	15	74	7	9
Chlorpromazine	F	15	111	12	17	15	82	8	12	14	**67**	**20**	**38**	15	**67**	8	12
Triflupromazine	F	14	**63**	**36**	**40**	14	**63**	**19**	**32**	15	**66**	**91**	**106**	15	**64**	10	11
Ketoprofen	P	15	107	3	5	15	108	4	4	15	106	5	4	15	100	4	5
Oxyphenylbutazone	F	15	**47**	**25**	**26**	15	**47**	**18**	**42**	15	**47**	**28**	**30**	15	**63**	**29**	**37**
Flunixin	F	15	100	2	4	15	104	2	4	15	112	4	17	15	115	1	16
Diclofenac sodium	P	15	105	14	**22**	15	95	9	14	15	89	12	13	15	82	9	9
Phenylbutazone	F	15	**29**	**38**	**34**	15	**26**	**25**	**50**	13	**26**	**27**	**35**	15	**32**	**42**	**44**
Tolfenamic acid	S	15	104	**20**	19	15	93	**22**	**27**	13	**68**	14	19	15	74	12	14
OTHER																	
Florfenicol amine	S	15	**121**	**21**	**32**	14	**131**	**49**	**47**	13	**126**	**31**	**46**	11	**174**	14	**21**
Carbadox	P	15	89	10	9	15	88	6	7	15	85	7	9	15	83	10	10
Triamcinolone	S	15	100	6	7	15	99	6	8	15	102	5	5	15	99	4	6
Prednisolone	S	15	102	6	6	15	102	7	10	15	99	6	8	15	97	4	6
Prednisone	S	15	104	11	15	15	100	9	9	15	100	5	8	15	98	3	4
Methyl prednisolone	S	15	95	6	8	15	98	7	7	15	96	6	5	15	95	5	6
Betamethasone	S	15	97	4	7	15	101	8	7	15	99	5	5	15	98	4	5
Flumethasone	S	15	102	9	11	15	99	7	9	15	101	9	10	15	96	6	6
Triamcinolone acetonide	S	15	99	5	6	15	100	4	6	15	98	5	5	15	98	4	5
Virginiamycin	P	15	75	11	14	15	70	6	13	15	**68**	7	7	15	**67**	7	7
Novobiocin	F	14	**49**	**18**	19	14	**51**	15	16	15	**50**	**40**	**48**	15	**49**	**22**	**26**
Melengestrol acetate	F	15	107	**51**	**72**	14	91	10	12	15	103	**43**	**67**	15	81	12	20

throughout the chromatographic run. To optimize data collection, the method used sSRM, which automatically set MS/MS ion transition segments based on individual analyte retention times. In retrospect, the 40 s sSRM segments were set too narrow for some of the analytes because peaks sometimes fell at the edges of the time segments. Data were excluded when the full analyte peak could not be integrated in experiments and when, as a result, peak areas were identified as outliers using a *t*-test.[13] Early-eluting analytes were more likely to display problematic t_R shifts, but most analytes gave quite consistent t_R within analytical sequences from day to day.

Two transitions were monitored for each drug from the precursor ion to yield quantifier and qualifier product ions for each analyte as listed in Table 1. A few drugs (carazolol, chlorpromazine, and triflupromazine) did not yield adequate signals for a qualifier ion, and in these cases, the qualitative ion ratio for identification purposes was generated by monitoring the precursor ion exposed to reduced collision energy.

Extraction and clean-up optimization

First of all, the effect of 1% formic acid on the extraction of the drugs from kidney was examined. Clean-up of these samples was performed using d-SPE with C-18 sorbent. Recoveries indicated that formic acid worked well for some drug classes, and did not work as well for others. Specifically, quinolones, macrolides, and sedatives provided better recoveries when formic acid was present during extraction, while recoveries of β-lactams, β-agonists, and NSAIDS were generally better in the absence of formic acid. Sulfonamide and tetracycline recoveries were not significantly influenced by the presence or absence of formic acid. Given the importance of β-lactams in monitoring schemes, [1] the absence of formic acid

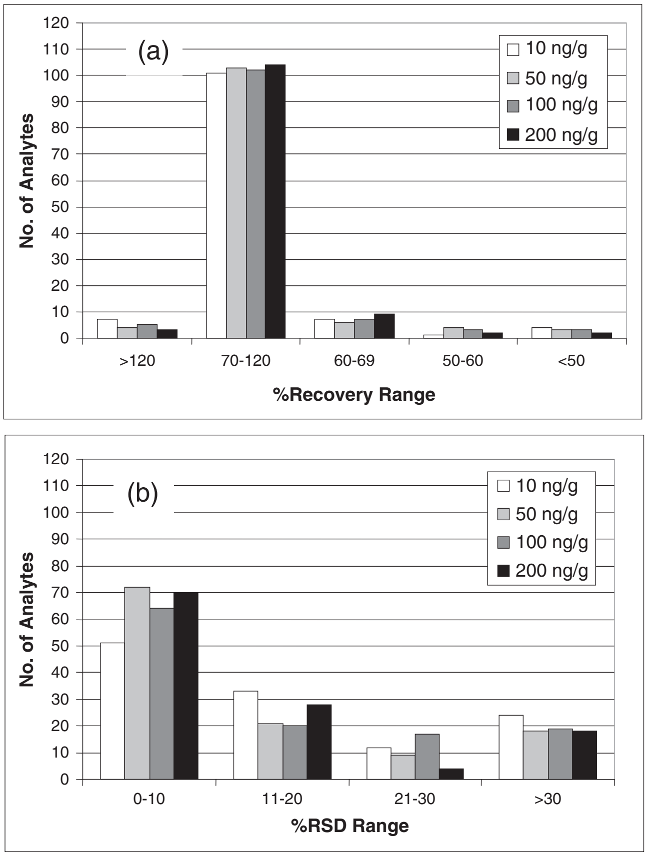

Figure 2. Compilation of recovery and reproducibility results for the 120 drugs tested in the method.

Table 3. Qualitative analysis of the drug analytes in bovine kidney: percentage of spiked samples (n = 15) at each spiking level that met identification criteria of RRT within ±4 standard deviations and ±10% absolute ion ratio difference of the averages for the reference standards. Values <93% are given in bold text

CLASS/ANALYTE	**10 ng/g**		**50 ng/g**		**100 ng/g**		**200 ng/g**	
	RRT	**ion ratio**	**RRT**	**ion ratio**	**RRT**	**Ion ratio**	**RRT**	**ion ratio**
QUINOLONES								
Desethyleneciprofloxacin	100	93	100	100	100	100	100	100
Norfloxacin	100	**53**	100	**80**	100	**80**	100	**87**
Ofloxacin	100	93	100	100	100	100	100	100
Ciprofloxacin	100	**53**	100	100	100	93	100	100
Lomefloxacin	100	100	100	100	100	100	100	100
Danofloxacin	100	**47**	100	73	100	**80**	100	100
Enrofloxacin	100	100	100	100	100	100	100	100
Sarafloxacin	100	**73**	100	**87**	100	100	100	100
Difloxacin	100	100	100	100	100	100	100	100
Oxolinic acid	100	100	100	100	100	100	100	100
Flumequine	100	100	100	100	100	100	100	100
SULFONAMIDES								
Sulfaguanidine	100	**40**	100	**27**	100	**60**	100	**60**
Sulfanilamide	100	100	100	100	100	100	100	100
Sulfacetamide	100	100	100	100	100	100	100	100
Sulfadiazine	100	100	100	100	100	100	100	100
Sulfathiazole	100	100	100	100	100	100	100	100
Sulfapyridine	100	100	100	100	100	100	100	100
Sulfamerazine	100	100	100	100	100	100	100	100
Sulfamethizole	100	100	100	100	100	100	100	100
Sulfamethazine	100	100	100	100	100	100	100	100
Sulfamethoxypyridazine	100	100	100	100	100	100	100	100
Sulfachloropyridazine	100	100	100	100	100	100	100	100
Sulfaethoxypyridazine	100	100	100	100	100	100	100	100
Sulfadoxine	100	100	100	100	100	100	100	100
Sulfamethoxazole	100	100	100	100	100	100	100	100
Sulfisoxazole	100	100	100	100	100	100	100	100
Sulfaquinoxaline	100	100	100	100	100	100	100	100
Sulfadimethoxine	100	100	100	100	100	100	100	100
Sulfaphenazole	100	100	100	100	100	100	100	100
Sulfabromomethazine	100	100	100	100	100	100	100	100
Sulfasalazine	100	100	100	100	100	100	100	100
TETRACYCLINES								
Oxytetracycline	100	**67**	100	100	100	100	100	100
Tetracycline	100	**87**	100	100	100	100	100	100
Chlortetracycline	100	**37**	93	**67**	100	100	100	100
Doxycycline	100	100	100	100	100	100	100	100
MACROLIDES								
Lincomycin	93	100	93	100	100	100	100	100
Tulathromycin	100	**67**	100	100	100	100	100	93
Spiramycin	100	**53**	100	**73**	100	100	100	100
Gamithromycin	100	**37**	100	**87**	100	100	100	100
Pirlimycin	100	100	100	100	100	100	100	100
Tilmicosin	100	**53**	100	**80**	100	93	93	**80**
Clindamycin	100	100	93	100	100	100	100	100
Erythromycin	100	100	100	100	100	100	100	100
Tylosin	100	100	100	100	100	100	100	100
NITROFURANS								
Semicarbazide	**67**	**47**	**67**	**40**	93	**73**	100	**67**
AMOZ	100	**47**	100	**87**	100	93	100	100
AOZ	100	**13**	100	**27**	100	**53**	100	**87**
Furaltadone	100	**80**	100	100	100	100	100	100
Nitrofurazone	100	**7**	100	**7**	100	**20**	100	**40**

Table 3. *(Continued)*

CLASS/ANALYTE	**10 ng/g**		**50 ng/g**		**100 ng/g**		**200 ng/g**	
	RRT	**ion ratio**	**RRT**	**ion ratio**	**RRT**	**Ion ratio**	**RRT**	**ion ratio**
Nitrofurantoin	93	**53**	100	**67**	100	**20**	100	**27**
Furazolidone	**87**	**0**	100	**60**	100	**60**	100	**67**
THYREOSTATS								
2-Mercapto-1-methylimidazole	100	100	100	100	100	100	100	100
6-methyl-2-thiouracil	93	**20**	100	**13**	100	**67**	100	**67**
6-propyl-2-thiouracil	100	**7**	100	**60**	100	**80**	100	**87**
2-mercaptobenzamidazole	100	**87**	100	100	100	100	100	100
6-phenyl-2-thiouracil	100	**20**	100	**7**	100	**13**	100	**20**
β-LACTAMS								
Desacetyl cephapirin	100	**47**	100	**47**	100	**80**	100	**80**
Amoxicillin	100	**27**	100	93	100	100	100	100
Cefadroxil	100	**53**	100	**40**	100	100	100	100
Cephapirin	100	100	93	100	100	100	100	100
DCCD	100	**27**	100	**40**	100	93	100	93
Ampicillin	100	100	100	100	100	100	100	100
Cephalexin	100	100	100	100	100	100	100	100
Cefalonium	100	100	100	100	100	100	100	100
Cefazolin	100	100	100	100	100	100	100	100
Cefoperazone	100	100	100	100	100	100	100	100
Penicillin G	100	100	100	100	100	100	100	100
Oxacillin	100	100	100	100	100	100	100	100
Cloxacillin	100	100	100	100	100	100	100	100
Nafcillin	100	100	100	100	100	100	100	100
Dicloxacillin	100	100	100	100	100	100	100	100
ANTHELMINTHICS								
5-Hydroxythiabendazole	100	**87**	100	**73**	100	100	100	100
Levamisole	100	100	93	100	100	100	100	100
Pyrantel	100	**87**	100	93	100	100	100	100
Thiabendazole	100	93	93	100	100	100	100	100
Morantel	100	100	100	100	100	100	100	100
Oxibendazole	100	93	100	100	100	100	100	100
Oxfendazole	100	100	100	100	100	100	100	100
Albendazole	100	**87**	100	100	100	100	100	100
Mebendazole	100	**53**	100	100	100	100	100	100
Flubendazole	100	**40**	100	100	100	100	100	100
Fenbendazole	100	**40**	100	100	100	100	100	100
Triclabendazole	100	**20**	100	**87**	100	**67**	100	**80**
β-AGONISTS								
Salbutamol	100	100	100	100	100	100	100	100
Terbutaline	100	100	100	100	100	100	100	100
Zilpaterol	100	**73**	100	**80**	100	100	100	100
Cimaterol	100	**40**	100	**47**	100	100	100	93
Fenoterol	100	**40**	100	**53**	100	93	100	**73**
Ractopamine	100	**80**	100	93	100	100	100	100
Tulobuterol	100	100	100	100	100	100	100	100
Clenbuterol	100	100	100	100	100	100	100	100
Mabuterol	100	100	93	100	100	100	100	100
TRANQUILIZERS/NSAIDS								
Dipyrone	100	**7**	100	**13**	100	**33**	100	**80**
Azaperone	100	**80**	100	100	100	100	100	100
Xylazine	100	100	100	100	100	100	100	100
Carazolol	100	**73**	100	100	100	100	100	100
Acetopromazine	100	**47**	100	**53**	100	**87**	100	100
Haloperidol	100	**80**	100	100	100	100	100	100
Promethazine	100	**40**	100	100	100	**67**	93	100

Table 3. *(Continued)*

CLASS/ANALYTE	**10 ng/g**		**50 ng/g**		**100 ng/g**		**200 ng/g**	
	RRT	**ion ratio**	**RRT**	**ion ratio**	**RRT**	**Ion ratio**	**RRT**	**ion ratio**
Propionylpromazine	100	100	100	100	100	100	100	100
Chlorpromazine	93	**13**	100	**67**	100	**67**	100	**87**
Triflupromazine	100	100	100	100	100	**20**	100	**17**
Ketoprofen	100	100	100	100	100	100	100	100
Oxyphenylbutazone	100	**67**	100	93	100	93	100	100
Flunixin	100	100	100	100	100	100	100	100
Diclofenac sodium	100	100	100	100	100	100	100	100
Phenylbutazone	100	**7**	100	**47**	100	**73**	100	**87**
Tolfenamic acid	100	**87**	100	100	100	100	100	100
OTHER								
Florfenicol amine	100	**7**	100	**13**	100	**47**	100	**33**
Carbadox	100	**13**	100	**67**	100	100	100	100
Triamcinolone	100	93	100	100	100	100	100	100
Prednisolone	100	100	100	100	100	100	100	100
Prednisone	100	**87**	100	100	100	100	100	100
Methyl prednisolone	100	100	100	100	100	100	100	100
Betamethasone	100	100	100	100	100	100	100	100
Flumethasone	100	93	100	100	100	100	100	100
Triamcinolone acetonide	100	100	100	100	100	100	100	100
Virginiamycin	100	100	100	100	100	100	100	100
Novobiocin	100	100	100	100	100	100	100	100
Melengestrol acetate	93	**20**	100	**47**	100	**67**	100	93

in the extraction solvent was deemed preferable for further experiments.

Next, the impact of cleanup on recoveries was studied. After extraction of analytes with 4/1 (v/v) MeCN/H_2O, three different clean-up protocols were examined: (1) use of C-18 in d-SPE alone; (2) use of hexane liquid-liquid partitioning alone; and (3) use of C-18 along with hexane partitioning. Results showed that analyte recoveries were not significantly affected by the three different clean-up protocols. Given the ease of use and lower expense of hexane partitioning, that cleanup method was chosen in the final method.

Quantification

Matrix-matched calibration was conducted on three days for each fortification level in the evaluation study, and the LCLs were assessed on each day. The range of LCLs obtained for each analyte is presented in Table 1. All but 11 of the drug analytes, 7 of which were thyreostats and nitrofurans or its markers, gave LCL ≤10 ng/g in at least one of the validation experiments. Sensitivity of the instrument varied somewhat depending on the analyte and condition of the instrument, but detection limits were not an issue for the majority of drugs, which was reflected in the recovery and precision results.

The optimized method was used to determine recoveries for 120 veterinary drugs in kidney tissue at levels of 10, 50, 100, and 200 ng/g. Five replicates for each level were fortified, extracted, and analyzed on each of three different days. As a practical matter, an analyst could perform two spiking levels per day. Recoveries were determined using each of the three added internal standards, and the most appropriate one for each analyte as labeled in Table 2 was empirically determined. Recoveries, %rsd (average within day repeatability) and overall %RSD (reproducibility over 3 days) are also listed in Table 2 for each fortification level.

In terms of MMMs for diverse veterinary drugs in complicated food matrices, this method was found to give very good overall results, and Figure 2 provides a graphical demonstration. At the lowest fortification level (10 ng/g), 101 (84%) of the 120 analytes provided recoveries between 70 and 120%, and 104 drugs (87%) were similarly recovered at the 200 ng/g level. This consistency indicated how the same drugs generally gave similar quality of results independent of concentration (Figure 2a). Figure 2b also shows how a similar number of analytes tended to yield similar degrees of precision vs. concentration, except somewhat higher variability for a small number of drugs at the 10 ng/g level.

The details can be deciphered from Table 2, but the general conclusion is that the method met typical quantitative criteria of 70–120% recoveries and ≤20% RSD for the majority of drugs. In particular, 76 of the drugs met these standards at 10 ng/g and 90 out of the 120 drugs met the criteria at the 200 ng/g level. Overall, 66 of the analytes met the given quantitative criteria at all spiking levels, and for levels ≥ 50 ng/g, this number rose to 72. Several drug classes gave exceptionally good results, particularly the quinolones, sulfonamides, β-agonists, most macrolides, and β-lactams. Even among the more problematic classes, there were well-performing individual analytes.

Among the different classes of drugs examined, some proved to be more difficult than others, such as the nitrofurans and thyreostats. Members of these classes frequently had higher LCLs, as shown in Table 1, and their worse detection sensitivities resulted in poor precision, especially at the lower fortification levels. In particular, 1-aminohydantoin did not give a satisfactory response and was not further pursued. Tetracyclines typically provided a lower recovery, which is expected due to the typical use of McIlvaine/EDTA buffer for extraction. [14] It is considered a benefit for screening and identification purposes that the tetracyclines be included and consistent recoveries and reproducibilities makes quantification possible depending on acceptability criteria. Similarly, selected anthelminthics, tranquilizers/NSAIDS, and 'other' vet drugs displayed lower recoveries, but often with higher variability, making quantification unreliable. They can still be included in the method for qualitative purposes.

Qualitative analysis

Qualitative identification of each analyte relied on relative retention time (RRT), vs. ^{13}C-sulfamethazine, and ion ratios (from integrated peak areas) between the quantifier and qualifier ions. An RRT was deemed acceptable if it was ±4 standard deviations from the average RRT of the matrix-matched calibration standards. According to FDA-CVM and FSIS criteria, [15] acceptable ion ratios were ±10% (absolute, not relative) from the average ion ratios determined from the matrix-matched calibration standards. Over three days, there were 15 analyses for each analyte at a given fortification level. For an analyte to meet our qualitative identification criteria, at least 14 of the 15 analyses ($\geq 93\%$) needed to meet the above criteria.

Identification results are summarized in Table 3. Nearly all analytes successfully met the t_R criteria at all fortification levels, with semicarbazide and furazolidone being the only exceptions. The former failed the RRT criterion 33% of the time at the 10 and 50 ng/g levels, and the latter failed 13% of the time at the lowest level, 10 ng/g In terms of MS/MS, 68 (57%) of the 120 analytes successfully met the ±10% ion ratio criterion at the 10 ng/g fortification level. This percentage increased to 74%, 82%, and 84% vs. increasing drug concentrations at the 50, 100, and 200 ng/g fortification levels, respectively. Again, nitrofurans and thyreostats gave the worst result due to their poor detection sensitivity, greater polarity, and smaller molecular weights. Remaining classes typically posed less difficulty, although even the normally non-problematic sulfonamides had one analyte, sulfaguanidine, which did not meet the ion ratio criterion even at the highest fortification level. Despite these difficult cases, it is clear that the majority of the analytes could be successfully identified the majority of the time in bovine kidney matrix using this method.

Conclusions

In this work, the use of extraction without 1% formic acid added was found to be preferable to the use of the acid in 4/1 (*v/v*) MeCN/H_2O for certain key analytes, such as β-lactams, from bovine kidney. Clean-up approaches using hexane partitioning, d-SPE using C-18, or a combination of both, did not significantly differ in their effect on recoveries. Acceptable quantitative and qualitative results were obtained for the vast majority of the 120 veterinary drugs tested from a variety of different analyte classes. LCLs were assessed to be 1–10 ng/g for most drug analytes. This MMM has been shown to be a useful tool for those involved in monitoring veterinary drug residues in animal tissues.

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Conflicts of interest

The authors have no conflicts of interest to declare.

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