Analysis of Veterinary Drugs in Meat with the Agilent 6495 Triple Quadrupole LC/MS

Application Note

Food

Authors

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Abstract

A method using an Agilent 1290 Infinity II LC coupled to an Agilent 6495 Triple Quadrupole LC/MS for the rapid and sensitive analysis of 120 veterinary drugs in bovine meat has been developed. The analytical run time is 12 minutes, while limits of detection and quantification range between 0.1–2 ng/mL and 0.1–5 ng/mL, respectively. Three optimized MRM transitions were selected for all but three veterinary drugs, ensuring selectivity and robustness. Quantification of real samples was possible with most compounds having R^2 >0.99 when two sets of matrix-matched calibration curves were performed. The method is reproducible and repeatable as indicated by the results of intra- and interday variability tests that produce relative standard deviations of <15 % for more than 90 % of the compounds tested.

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Introduction

The monitoring of veterinary drugs in food is critical due to contamination and the possibility of increased antimicrobial resistance by pathogenic microorganisms [1]. Veterinary drug administration in animals is important to treat diseases and promote growth. However, improper dosing or illegal practices can lead to contamination in meat for human consumption. As a result, veterinary drugs in food are regulated in several regions including the US, Europe, China, Australia, and others [2-4].

Analysis of veterinary drugs is challenging due to their many classes with diverse structures and varying chemical properties. To meet the needs of analytical labs, rapid and efficient techniques using multiclass, multiresidue methods analyzing >100 veterinary drugs in a single run are required. Additional goals are detection limits of low µg/kg, with good reproducibility and high sample throughput. The use of ultrahigh performance liquid chromatography (UHPLC) coupled to tandem mass spectrometers (MS/MS) is the gold standard for this analysis. This technique offers the requisite analytical sensitivity and robustness while allowing for time and labor savings compared to other techniques for analysis of veterinary drugs.

This application note describes the development of a rapid UHPLC/MS/MS method with the Agilent 1290 Infinity II UHPLC and an Agilent 6495 Triple Quadrupole LC/MS for the analysis of 120 veterinary drugs in animal meat. The method used three transitions for each analyte (except three) satisfying US and EU specifications for identification. The sensitivity of the method was determined by calculating the limits of detection and quantification in kidney and liver. Other method validation protocols such as linearity, robustness, and reproducibility were also evaluated in this study.

Experimental

Standards and reagents

All native veterinary drug standards were bought from Sigma-Aldrich (St. Louis, MO), and prepared between 300 and 1,000 µg/mL in solvent (either acetonitrile, methanol, dimethyl sulfoxide, or water depending on solubility). The three internal standards used in this study (flunixin-d_3, nafcillin-d_5, and doxycycline-d_3) were acquired from Toronto Research Chemicals (Toronto, ON). LC/MS grade acetonitrile and water were procured from Burdick and Jackson (Muskegon, MI), while formic acid (>98 %, Suprapur) was obtained from EMD Millipore (Darmstadt, Germany).

Instrumentation

Separation of analytes for this method was performed using an Agilent 1290 Infinity II LC with a 20 µL injection loop and multiwash capability. An Agilent 6495 Triple Quadrupole LC/MS with the iFunnel and Jet Stream technology was used as the detector. Analysis was performed in simultaneous positive and negative electrospray ionization mode. All data acquisition and processing was performed using Agilent MassHunter software (Version 07.00). Tables 1 and 2 show the instrument conditions.

Table 1. Optimized LC Conditions

Parameter	Value	
Instrument	Agilent 1290 Infinity II with 20 µL flex loop and multiwash	
Column	Agilent ZORBAX C-18 Eclipse Plus 2.1 × 150 mm, 1.8 µm (p/n 959759-902)	
Guard column	Agilent ZORBAX C-18 Eclipse Plus 2.1 × 5 mm, 1.8 µm (p/n 821725-901)	
Column temperature	30 °C	
Injection volume	15 µL	
Mobile phase	A) Water + 0.1 % formic acid B) Acetonitrile	
Run time	12 minutes	
Equilibration time	2 minutes	
Flow rate	0.5 mL/min	
Gradient	Time (min)	A (%)
	0.0	98
	1.0	98
	1.5	85
	2.5	70
	6.0	55
	8.5	20
	10.0	0
	11.0	0
	11.2	98

Table 2. Optimized MS conditions

Parameter	Value	
Mass spectrometer	Agilent 6495 Triple Quadrupole LC/MS	
Gas temperature	150 °C	
Gas flow rate	18 L/min	
Sheath gas temperature	300 °C	
Sheath gas flow rate	11 L/min	
Nebulizer pressure	35 psi	
Capillary voltage	4,000 V (3,000 V)	
Nozzle voltage	500 V (1,500 V)	
Ion funnel HPRF	200 v (90 V)	
Ion funnel LPRF	100 V (60 V)	
Delta EMV	200 V	
Time segments	Time (min)	Flow
	0.0	Waste
	0.7	MS

Sample preparation

The Agilent Enhanced Matrix Removal—Lipid (EMR—L) product was used for sample extraction of veterinary drugs in this study. The EMR—L selectively removes lipids while not trapping contaminants of interest, and has been shown to be effective in extracting several classes of compounds including pesticides, toxins, and PAHs in food [5,6]. Details of the procedure followed for veterinary drug extraction using EMR—L, and product information can be found in previously published literature [7,8]. Briefly, 2 g samples of homogenized bovine kidney and liver were weighed and placed into 50-mL polypropylene tubes. A 10 mL solution of acetonitrile with 5 % formic acid was added to the sample and mixed with an orbital shaker for 5 minutes, followed by centrifugation at 4,000 rcf for 5 minutes. After this, 5 mL of the supernatant was added to the 1 g EMR—L tube, which had been activated previously with 5 mL of 5 mM ammonium acetate solution. The tube was then vortexed and centrifuged at 4,000 rcf for 5 minutes. The 5 mL of supernatant from this solution was transferred to a 15-mL centrifuge tube to which 2 g of $MgSO_4$ were added from the EMR—L pouch with vortexing and centrifugation, as before. Finally, a 100 µL extract was collected from the tube and diluted with 400 µL of ultrapure water in a 1-mL polypropylene vial, ready for LC/MS analysis.

Results and Discussion

Compound selection and optimization

The 120 veterinary drugs analyzed in this study were selected based on a monitoring list used by the United States Department of Agriculture's Agricultural Research Service (USDA-ARS) [9]. The compound-specific parameters including precursor ion, three most abundant unique product ions, and collision energy were determined by running each standard through the Agilent Optimizer software. Three specific transitions were selected for each compound (except thiouracil, metronidazole, and clindamycin) to satisfy both US and EU regulations for identification by mass spectrometry. Table 3 shows the optimized transitions, retention times, and other relevant parameters for each compound. The tolerance levels for each veterinary drug were obtained from the USDA-ARS, and were used to prepare calibration curves, and perform spike studies, described later. Care was taken to select transitions that did not have matrix interferences. Cimaterol had matrix interferants for the 220.1 → 202.1 and 220.1 → 160.1 transitions, therefore, extra transitions were obtained. The ion ratio intensities were helpful in identifying these issues (as opposed to reporting cimaterol as incurred). Figure 1 represents a chromatogram of cimaterol in standard and liver blank with the different MRM transitions that indicate the presence of two of the transitions in matrix but at different ion ratios than would be expected based on the standard.

Table 3. Optimized Compound Parameters and Tolerance Levels with Retention Times for 120 Veterinary Drugs

Compound	Class	Tolerance (ng/g)	Precursor ion	Product ion	Collision energy	RT (min)	Delta RT
Thiouracil	Thyreostat	400	129	90.1	8	0.95	1.0
			129	82.3	16		
Florfenicol amine	Phenicol	300	248.1	230.1	8	0.99	0.8
			248.1	130.1	28		
			248.1	91.1	50		
Florfenicol	Phenicol	300	358	241	16	1	0.6
			358	206	28		
			358	170	32		
Sulfanilamide	Sulfonamide	100	173	156	5	2	0.6
			173	92	25		
			173	76	5		
Methyl-thiouracil	Thyreostat	400	143	126	20	2.5	0.6
			143	86	20		
			143	84	20		
Amoxicillin	β-Lactam	10	367	349.1	4	2.56	0.6
			367	208	8		
			367	114	56		

Table 3. Optimized Compound Parameters and Tolerance Levels with Retention Times for 120 Veterinary Drugs (continued)

Compound	Class	Tolerance (ng/g)	Precursor ion	Product ion	Collision energy	RT (min)	Delta RT
Salbutamol	β-Agonist	10	240.2	222.2	4	2.6	0.6
			240.2	166.1	4		
			240.2	148.1	16		
Tildipirosin	Macrolide	100	734.5	561.5	36	2.65	0.6
			734.5	174	44		
			734.5	98.2	56		
Cimaterol	β-Agonist	10	220.1	202.1*	4	2.66	0.6
			220.1	160.1*	12		
			220.1	143.1	14		
			220.1	116.1	20		
Hydroxy- metronidazole	Coccidiostat	10	188.1	126.1	16	2.7	0.6
			188.1	123.1	8		
			188.1	68.0	22		
Lincomycin	Lincosamide	100	407.2	359.2	16	2.7	0.6
			407.2	126.1	24		
			407.2	42.2	68		
Hydroxy-dimetridazole	Coccidiostat	50	158.1	140	8	2.8	0.6
			158.1	55.2	20		
			158.1	42.2	40		
Metronidazole	Coccidiostat	10	172.1	128	12	2.83	0.6
			172.1	82.1	24		
Dipyrone metabolite	Anti- inflammatory	200	218.1	187.1	18	2.85	0.6
			218.1	125	16		
			218.1	97	14		
Levamisole	Anthelmintic	100	205.1	178.1	20	2.9	0.6
			205.1	123	32		
			205.1	91.1	44		
Albendazole-2-aminosulfone	Anthelmintic	50	240.1	198	20	2.97	0.6
			240.1	133.1	20		
			240.1	105	40		
Ampicillin	β-Lactam	10	350	160	4	3	0.6
			350	114	36		
			350	106	16		
Dimetridazole	Coccidiostat	10	142.1	96.1	16	3	0.6
			142.1	81.1	28		
			142.1	54.1	36		
Thiabendazole	Anthelmintic	100	202	175	24	3	0.6
			202	131	36		
			202	65	52		
Ronidazole	Coccidiostat	10	201.1	140.1	8	3.09	0.6
			201.1	55.2	20		
			201.1	154.9	8		
Desethylene Ciprofloxacin	Fluoroquinolone	100	306.1	288.2	20	3.1	0.6
			306.1	268.1	28		
			306.1	217	44		

* Potential matrix interferants in liver extract.

Table 3. Optimized Compound Parameters and Tolerance Levels with Retention Times for 120 Veterinary Drugs (continued)

Compound	Class	Tolerance (ng/g)	Precursor ion	Product ion	Collision energy	RT (min)	Delta RT
Norfloxacin	Fluoroquinolone	50	320.1	302.1	20	3.11	0.6
			320.1	282.1	40		
			320.1	231.1	40		
Ciprofloxacin	Fluoroquinolone	50	332.1	314.1	20	3.15	0.6
			332.1	288.2	20		
			332.1	231.1	40		
Sulfadiazine	Sulfonamide	100	251.1	108.1	20	3.16	0.6
			251.1	92.1	28		
			251.1	65.1	48		
Danofloxacin	Fluoroquinolone	200	358.2	340.1	20	3.19	0.6
			358.2	314.2	16		
			358.2	82.1	48		
Oxytetracycline	Tetracycline	1000	461.2	443.1	6	3.2	0.6
			461.2	426.1	14		
			461.2	201.1	48		
Ractopamine	β-Agonist	30	302.2	284.2	8	3.21	0.6
			302.2	164.1	12		
			302.2	107.1	24		
Orbifloxacin	Fluoroquinolone	50	396.2	352.1	20	3.22	0.6
			396.2	295	28		
			396.2	226	44		
Enrofloxacin	Fluoroquinolone	100	360.2	342.2	20	3.25	0.6
			360.2	316.2	16		
			360.2	245.1	32		
Carbadox	Miscellaneous	30	263.1	230.9	12	3.26	0.6
			263.1	129.1	32		
			263.1	102	50		
Azaperone	Tranquilizer	10	328.2	165.1	20	3.27	0.6
			328.2	123	40		
			328.2	121.1	20		
Sulfapyridine	Sulfonamide	100	250.1	156	20	3.28	0.6
			250.1	108	20		
			250.1	92	20		
Propylthiouracil	Thyreostat	50	171.1	154	20	3.3	0.6
			171.1	60	40		
			171.1	54	40		
Sulfathiazole	Sulfonamide	100	256	156	12	3.4	0.6
			256	92.1	28		
			256	65.1	52		
Sulfamerazine	Sulfonamide	100	265.1	156	12	3.41	0.6
			265.1	92.1	28		
			265.1	65.1	60		
Quinoxaline 2-carboxylic acid	Miscellaneous	30	175	131.2	16	3.43	0.6
			175	129.1	16		
			175	75.2	50		

Table 3. Optimized Compound Parameters and Tolerance Levels with Retention Times for 120 Veterinary Drugs (continued)

Compound	Class	Tolerance (ng/g)	Precursor ion	Product ion	Collision energy	RT (min)	Delta RT
Xylazine	Tranquilizer	10	221.1	105.1	40	3.43	0.6
			221.1	90	40		
			221.1	72	40		
Clenbuterol	β-Agonist	10	277.1	259.1	4	3.44	0.6
			277.1	203	12		
			277.1	132.1	32		
Chlortetracycline	Tetracycline	1000	479.1	462	12	3.45	0.65
			479.1	444	20		
			479.1	154.1	36		
Thiamphenicol	Phenicol	10	354	290	12	3.46	0.6
			354	227	18		
			354	184.9	34		
Cefapirin	β-Lactam	100	424.1	364	8	3.48	0.6
			424.1	124.1	48		
			424.1	112	24		
Mercaptobenzimi dazole	Thyreostat	25	151	118.1	28	3.47	0.6
			151	93	24		
			151	65.1	48		
Cefazolin	β-Lactam	100	455	323	4	3.49	0.6
			455	156	16		
			455	124	32		
Difloxacin	Fluoroquinolone	50	400.1	382.1	20	3.5	0.6
			400.1	356.2	16		
			400.1	299.1	32		
Gamithromycin	Macrolide	100	777.5	619.4	36	3.52	0.6
			777.5	158.1	54		
			777.5	116	54		
Sarafloxacin	Fluoroquinolone	50	386.1	368.1	20	3.44	0.6
			386.1	342.1	20		
			386.1	299.1	40		
Amino-mebendazole	Anthelmintic	10	238.1	133.1	44	3.54	0.6
			238.1	105.1	28		
			238.1	77.1	40		
Morantel	Anthelmintic	100	221.1	150	40	3.54	0.6
			221.1	123	40		
			221.1	111	40		
Bacitracin	Miscellaneous	500	711.9	669.3	20	3.55	0.6
			711.9	227.1	40		
			711.9	199.1	40		
Sulfamethazine	Sulfonamide	100	279.1	186.1	12	3.58	0.6
			279.1	124.1	24		
			279.1	92.1	32		
Clindamycin	Lincosamide	100	425.2	377.2	20	3.60	0.6
			425.2	126.1	20		
Sulfamethizole	Sulfonamide	100	271	156	10	3.62	0.6
			271	108	20		
			271	92	40		

Table 3. Optimized Compound Parameters and Tolerance Levels with Retention Times for 120 Veterinary Drugs (continued)

Compound	Class	Tolerance (ng/g)	Precursor ion	Product ion	Collision energy	RT (min)	Delta RT
Sulfamethoxypyr idazine	Sulfonamide	100	281.1	156	12	3.64	0.6
			281.1	92.1	32		
			281.1	65.1	60		
Aminoflubendazole	Anthelmintic	10	256.1	123	40	3.66	0.6
			256.1	95	40		
			256.1	75	40		
Hydroxy-ipronidazole	Coccidiostat	10	186.1	168.1	8	3.68	0.6
			186.1	122.1	20		
			186.1	106.1	44		
Tilmicosin	Macrolide	100	869.6	696.4	44	3.68	0.6
			869.6	174.1	48		
			869.6	88.1	70		
Cambendazole	Anthelmintic	10	303.1	261	16	3.73	0.6
			303.1	217.1	32		
			303.1	190	44		
Doxycycline	Tetracycline	100	445.2	428.1	16	3.78	0.6
			445.2	410	24		
			445.2	321.1	28		
Doxycycline-d_3	Internal Standard	–	448.1	431.2	16	3.78	0.8
			448.1	155.1	36		
Carazolol	Tranquilizer	10	299.2	222.1	20	3.81	0.6
			299.2	116.1	20		
			299.2	56	40		
Tetracycline	Tetracycline	1000	445.2	427.1	10	3.85	0.6
			445.2	410.1	20		
			445.2	154	40		
Phenyl-thiouracil	Thyreostat	400	205	188	20	3.86	0.6
			205	103	28		
			205	86.2	28		
Oxibendazole	Anthelmintic	10	250.1	218.1	16	3.87	0.6
			250.1	176.1	28		
			250.1	148	40		
Oxfendazole	Anthelmintic	800	316.1	284	16	3.97	1.0
			316.1	191.1	16		
			316.1	159	32		
Albendazole sulfone	Anthelmintic	50	298.1	266.1	20	4.1	0.6
			298.1	224	20		
			298.1	159	40		
Sulfadimethoxine	Sulfonamide	100	311.1	156	16	4.21	0.6
			311.1	92.1	36		
			311.1	65.1	60		
Sulfaethoxypyrid azine	Sulfonamide	100	298.1	158	16	4.25	0.6
			298.1	108.1	32		
			298.1	92.1	32		
Sulfachloropyrid azine	Sulfonamide	100	285	156	12	4.26	0.6
			285	92.1	24		
			285	65.1	60		

Table 3. Optimized Compound Parameters and Tolerance Levels with Retention Times for 120 Veterinary Drugs (continued)

Compound	Class	Tolerance (ng/g)	Precursor ion	Product ion	Collision energy	RT (min)	Delta RT
Sulfamethoxazole	Sulfonamide	100	254.1	156	12	4.35	0.6
			254.1	92.1	24		
			254.1	65.1	48		
Erythromycin	Lincosamide	100	734.5	576.3	16	4.38	0.6
			734.5	158.1	32		
			734.5	83.1	60		
Chloramphenicol	Phenicol	10	321.1	257	18	4.43	0.6
			321.1	151.9	26		
			321.1	80	50		
Ipronidazole	Coccidiostat	10	170.1	124	16	4.49	0.6
			170.1	109	24		
			170.1	81.1	36		
Tylosin	Macrolide	200	916.5	174.1	44	4.67	0.6
			916.5	101	56		
			916.5	83	60		
Acepromazine	Tranquilizer	10	327.2	222.1	40	4.73	0.6
			327.2	86.1	20		
			327.2	58.1	40		
Haloperidol	Tranquilizer	10	376.2	165.1	24	4.75	0.6
			376.2	123	50		
			376.2	95.1	50		
Promethazine	Tranquilizer	10	285.1	198	28	4.78	0.6
			285.1	86.2	20		
			285.1	71.3	48		
Prednisone	Anti- inflammatory	100	359.2	341.2	10	4.84	0.6
			359.2	237.1	20		
			359.2	147.1	40		
Clorsulon	Anthelmintic	100	377.9	341.9	0	4.91	0.8
			377.9	242	40		
			377.9	142	40		
Sulfadoxine	Sulfonamide	100	311.1	156	16	4.96	0.6
			311.1	108	28		
			311.1	92.1	32		
Sulfaquinoxaline	Sulfonamide	100	301.1	156	16	4.95	0.6
			301.1	108	28		
			301.1	92	32		
Albendazole	Anthelmintic	50	266.1	234.1	16	5.01	0.6
			266.1	191	32		
			266.1	159	44		
Mebendazole	Anthelmintic	10	296.1	264.1	20	5.16	0.8
			296.1	105	36		
			296.1	77	48		
Penicillin G	β-Lactam	10	335.0	114.0	35	5.29	0.6
			335.0	160.0	18		
			335.0	176.1	20		

Table 3. Optimized Compound Parameters and Tolerance Levels with Retention Times for 120 Veterinary Drugs (continued)

Compound	Class	Tolerance (ng/g)	Precursor ion	Product ion	Collision energy	RT (min)	Delta RT
Propionylpromaz ine	Tranquilizer	10	341.2	268.1	24	5.46	0.6
			341.2	86.2	20		
			341.2	58.2	52		
Flubendazole	Tranquilizer	10	314.1	282.1	20	5.58	0.6
			314.1	123	36		
			314.1	95.1	56		
Betamethasone	Anti-inflammatory	100	393.2	373.2	4	5.71	0.6
			393.2	237.2	12		
			393.2	147.1	32		
Chlorpromazine	Tranquilizer	10	319.1	246	28	5.77	0.6
			319.1	86.1	20		
			319.1	58.2	50		
Sulfanitran	Sulfonamide	100	334.1	137	40	6.18	0.6
			334.1	136	40		
			334.1	134.1	40		
Sulfabromomethazine	Sulfonamide	100	357	156	24	6.23	0.6
			357	108	36		
			357	92.1	36		
Zeranol	Miscellaneous	100	321.1	303.2	34	6.3	0.6
			321.1	277.2	34		
			321.1	259.1	36		
Oxacillin	β-Lactam	100	402	243	8	6.49	0.6
			402	160	8		
			402	114	40		
Triflupromazine	Tranquilizer	10	353.1	248.1	40	6.28	0.6
			353.1	86.1	20		
			353.1	58.1	40		
Fenbendazole	Anthelmintic	10	300.1	268.1	20	6.54	0.6
			300.1	159	36		
			300.1	131	56		
Virginiamycin M1	Miscellaneous	100	526.3	508.3	12	6.74	0.8
			526.3	355.2	16		
			526.3	109.1	32		
Nitroxynil	Anthelmintic	50	288.91	162	20	6.78	0.8
			288.91	127	28		
			288.91	89	44		
Cloxacillin	β-Lactam	10	436	358.2	0	7.15	0.6
			436	277	12		
			436	160	12		
Nafcillin-d_5	Internal Standard	–	420.1	204	16	7.41	0.8
			420.1	172	52		
Ketoprofen	Anti-inflammatory	10	255.1	209.1	8	7.4	0.8
			255.1	105.1	24		
			255.1	77.1	48		
Nafcillin	β-Lactam	10	415	199.1	8	7.41	0.6
			415	171	36		
			415	115	20		

Table 3. Optimized Compound Parameters and Tolerance Levels with Retention Times for 120 Veterinary Drugs (continued)

Compound	Class	Tolerance (ng/g)	Precursor ion	Product ion	Collision energy	RT (min)	Delta RT
Flunixin	Anti-inflammatory	25	297.1	279.1	24	7.46	0.8
			297.1	259.1	32		
			297.1	236	48		
Flunixin-d_3	Internal Standard	–	300.1	282.1	29	7.46	0.8
			300.1	264.1	45		
Oxyphenbutazone	Anti-inflammatory	100	325.2	204.1	20	7.51	0.8
			325.2	148	40		
			325.2	120	20		
Meloxicam	Anti-inflammatory	100	352	140.9	16	7.68	0.8
			352	115	16		
			352	73	44		
Emamectin B1a	Anthelmintic	10	886.5	158.1	40	8.07	0.8
			886.5	126.1	40		
			886.5	82.2	54		
Haloxon	Anthelmintic	100	415	352.9	24	8.24	0.8
			415	352.9	24		
			415	211	44		
Triclabendazole sulfoxide	Anthelmintic	50	375	356.9	20	8.25	0.8
			375	313	28		
			375	242	48		
Diclofenac	Anti- inflammatory	200	296	278	4	8.54	0.8
			296	250	8		
			296	215.1	16		
Phenylbutazone	Anti- inflammatory	100	309.2	160.2	20	8.89	0.8
			309.2	120	28		
			309.2	77.1	68		
Triclabendazole	Anthelmintic	50	359	343.9	24	9.01	1
			359	274	40		
			359	171	60		
Oxyclozanide	Anthelmintic	10	397.87	361.9	20	9.06	0.8
			397.87	201.9	20		
			397.87	175.8	28		
Melengestrol acetate	Miscellaneous	25	397.2	337.3	8	9.22	0.8
			397.2	279.2	20		
			397.2	236.2	28		
Niclosamide	Anthelmintic	10	324.99	289	16	9.24	0.8
			324.99	170.9	36		
			324.99	135.1	44		
Tolfenamic acid	Anti- inflammatory	200	262.1	244.1	12	9.27	0.8
			262.1	209.1	28		
			262.1	180.1	48		
Bithionol	Anthelmintic	10	355	193.7	32	9.74	0.8
			355	162.9	28		
			355	160.9	28		

Table 3. Optimized Compound Parameters and Tolerance Levels with Retention Times for 120 Veterinary Drugs (continued)

Compound	Class	Tolerance (ng/g)	Precursor ion	Product ion	Collision energy	RT (min)	Delta RT
Eprinomectin B1a	Anthelmintic	100	914.5	330.1	28	10.22	1.5
			914.5	186.1	28		
			914.5	112.1	60		
Abamectin	Anthelmintic	20	895.5	449.3	44	10.5	1.5
			895.5	751.5	48		
Closantel	Anthelmintic	50	660.9	344.9	44	10.88	1.2
			660.9	315	40		
			660.9	278.9	44		
Moxidectin	Anthelmintic	50	640.4	528.3	20	10.99	1.2
			640.4	498.2	18		
			640.4	496.2	20		
Doramectin	Anthelmintic	30	921.1	770.1	62	11.1	1.5
			921.1	449.2	66		
			921.1	353.1	66		
Selamectin	Anthelmintic	200	770.5	276	24	11.81	2.0
			770.5	203.2	28		
			770.5	113.2	40		
Rafoxanide	Anthelmintic	10	625.8	372.8	36	11.23	1.0
			625.8	252.9	28		
			625.8	127	36		
Ivermectin B1a	Anthelmintic	10	892.5	551.4	16	11.86	1.0
			892.5	307.3	24		
			892.5	145	36		

LC/MS Method optimization

The goal of this work was to achieve adequate separation of as many veterinary drugs as possible while having a rapid and robust method for analysis. Figure 2 shows the primary MRM transition for the 13 classes of veterinary drugs tested in this method using a 12-minute gradient with UHPLC in a kidney tissue at 50 ng/g. The most polar compounds such as thiouracil, florfenicol, and sulfanilamide elute early in the chromatogram with fairly good peak shapes. Several of the mectins however, such as abamectin, ivermectin, moxidectin, and selemectin eluted at the end of the run, with typical peak widths of 9–12 seconds. A dynamic MRM method with a cycle time of 550 ms was used with a minimum dwell time of 3.2 ms and a maximum dwell time of 274 ms.

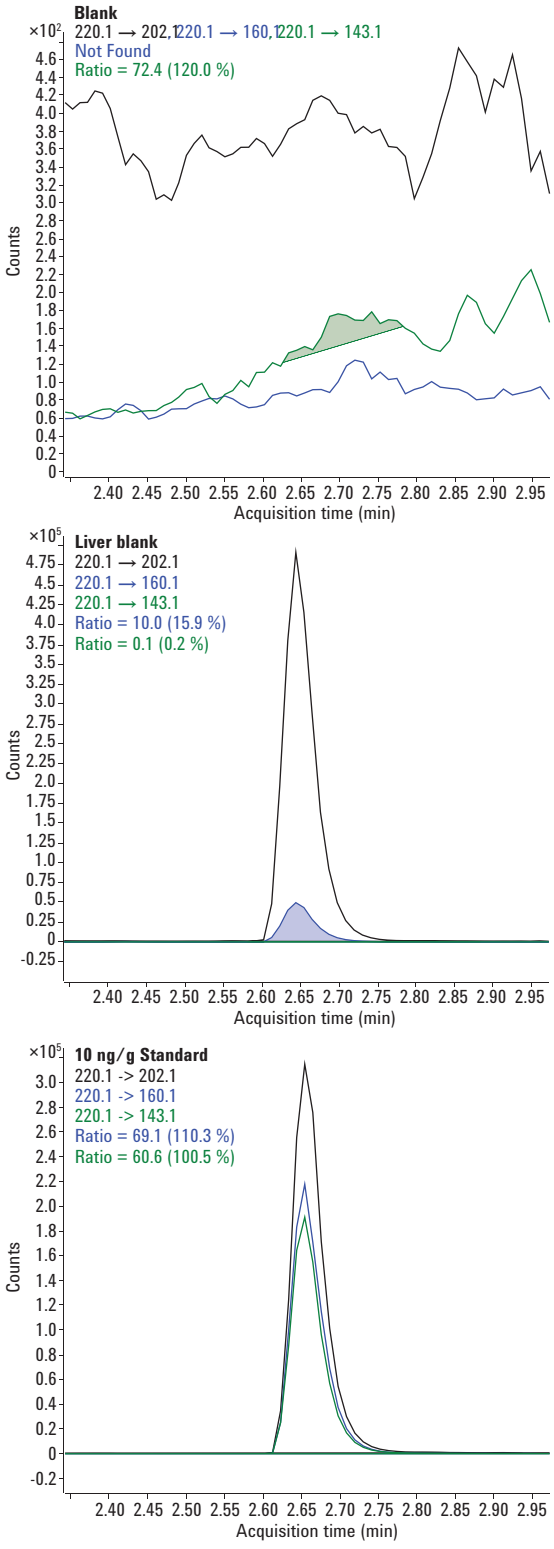

Figure 1. Potential matrix interferences for two cimaterol transitions (220.1 → 202.1; 220.1 → 160.1).

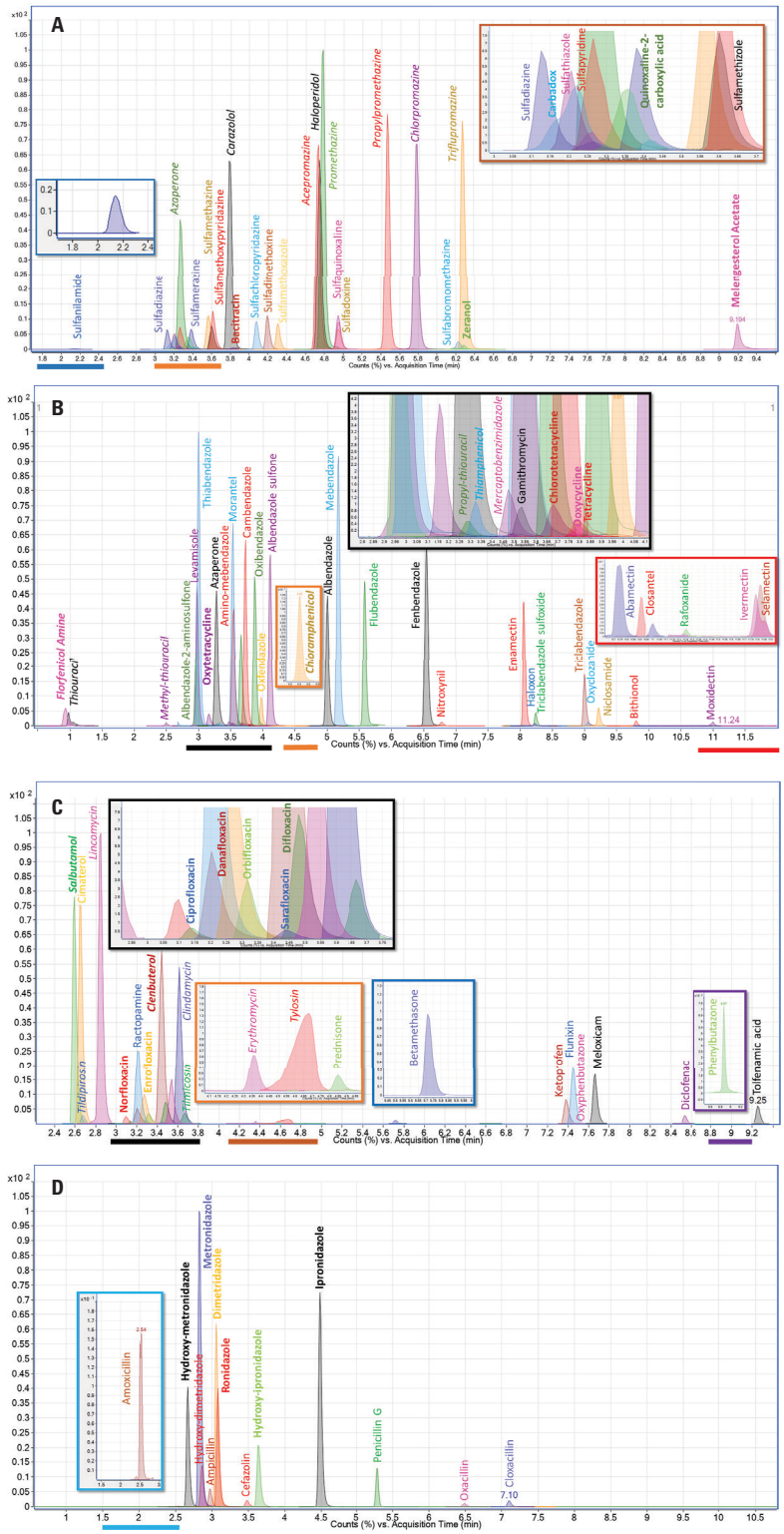

Figure 2. Representative chromatogram of veterinary drug classes at 50 ng/g in Kidney tissue. A) Sulfonamides, tranquilizers, miscellaneous; B) anthelmintics, thyreostats, tetracyclines, phenicols; C) anti-inflammatories, macrolides/lincosamides, fluoroquinolones; β-agonists D) β-lactams, coccidiostats.

Limits of detection and quantification

The limit of detection (LOD) was defined as the lowest concentration at which the signal-to-noise ratio (S/N) was greater than 3. Meanwhile, the limit of quantification (LOQ) was the lowest concentration at which the S/N was greater than 10 for a compound. Blank kidney and liver tissue samples were extracted through the EMR—L procedure. The resulting extract was spiked with different concentrations of veterinary drugs to determine the LOD and LOQ, thus accounting for matrix effects encountered in the instrument. The corresponding results showed no difference between the liver and kidney, and are detailed in Table 4. Several compounds have LODs (and LOQs) lower than the smallest spike concentration of 0.1 ng/mL. The LODs for the analytes tested varied from 0.1–2 ng/mL, while the LOQs ranged between 0.1 and 5 ng/mL. Most of the compound classes (sulfonamides, fluoroquinolones, tranquilizers, and so forth) had LOQs in the sub 1 ng/mL region, while the β-lactams ranged between 1 and 5 ng/mL. Figure 3 illustrates that 89 compounds had LODs of 0.1 ng/mL (and many would be lower) while 61 compounds had LOQs at 0.1 ng/mL. All compounds had LODs and LOQs at or lower than 5 ng/mL. Most importantly, all 120 veterinary drugs had LOQs lower than the tolerance levels presented in Table 3.

Table 4. LOD, LOQ, Inter- and Intraday Variability

Compounds	LOD (ng/mL)	LOQ (ng/mL)	Intraday variability RSD (%)	Interday variability RSD (%)
Abamectin	1	2	9.5	9.9
Acepromazine	0.1	0.1	3.6	1.9
Albendazole sulfone	0.1	0.5	2.4	2.6
Albendazole	0.1	0.1	2.7	1.3
Albendazole-2-aminosulfone	0.1	0.5	8.9	2.6
Aminoflubendazol	0.1	0.1	13.6	**22.0**
Amino-Mebendazole	0.1	0.1	9.7	13.7
Amoxicillin	2	5	4.2	8.8
Ampicillin	0.5	1	7.6	*16.1*
Azaperone	0.1	0.1	5.1	8.0
Bacitracin	2	5	6.3	9.2
Betamethasone	0.1	0.5	3.6	4.5
Bithionol	0.1	0.1	1.9	9.8
Cambendazole	0.1	0.1	2.5	7.2
Carazolol	0.1	0.1	6.6	9.4
Carbadox	0.5	1	5.6	9.6
Cefapirin	1	2	**20.3**	*17.1*
Cefazolin	1	2	9.8	13.5
Chloramphenicol	0.5	1	6.7	8.5
Chlorpromazine	0.1	0.1	2.3	3.3
Chlortetracycline	1	2	10.7	11.1
Cimaterol	0.1	0.1	7.5	10.5
Ciprofloxacin	0.5	1	2.3	*15.6*
Clenbuterol	0.1	0.1	5.3	5.2
Clindamycin	0.1	0.1	1.1	2.9
Clorsulon	0.5	1	3.3	3.3
Closantel	0.1	0.1	3.8	4.1
Cloxacillin	2	5	6.2	7.8
Danofloxacin	0.5	1	1.5	6.4
Desethylene ciprofloxacin	1	2	5.7	12.3
Diclofenac	0.5	1	1.1	3.4
Difloxacin	0.1	0.1	8.2	8.1
Dimetridazole	0.1	0.1	4.2	8.8
Dipyrone metabolite	NA	NA	5.4	*16.2*
Doramectin	1	2	6.5	9.3
Doxycycline	0.1	0.5	10.6	6.1
Emamectin	0.1	0.1	3.1	6.3
Enrofloxacin	0.1	0.5	10.0	6.2
Eprinomectin B1a	0.5	1	5.3	7.7
Erythromycin	0.1	0.5	3.2	13.8
Fenbendazole	0.1	0.1	3.2	2.3
Florfenicol Amine	0.5	0.5	1.6	6.2
Florfenicol	1	2	4.2	*15.1*
Flubendazole	0.1	0.1	1.8	3.8
Flunixin	0.1	0.1	1.5	7.3
Gamithromycin	0.1	0.5	8.0	*15.9*
Haloperidol	0.1	0.1	2.4	1.6
Haloxon	0.5	0.5	9.1	5.9
Hydroxydimetridazole	0.5	1	4.9	6.8
Hydroxy-Ipronidazole	0.1	0.5	8.3	5.2
Hydroxy-metronidazole	0.1	0.1	8.3	12.1
Ipronidazole	0.1	0.1	1.8	6.3
Ivermectin B1a	0.5	1	4.2	9.4
Ketoprofen	0.1	0.5	5.1	3.8
Levamisole	0.1	0.1	1.6	7.2
Lincomycin	0.1	0.1	5.5	4.2
Mebendazole	0.1	0.1	1.9	2.8
Melengestrol acetate	0.1	0.1	3.5	3.4
Meloxicam	0.1	0.1	1.0	3.8
Mercaptobenzimidazole	0.1	0.5	10.8	11.0

RSDs >15 % in *italic*, RSDs >20 % in **bold**

Table 4. LOD, LOQ, Inter- and Intraday Variability (continued)

Compounds	LOD (ng/mL)	LOQ (ng/mL)	Intraday variability RSD (%)	Interday variability RSD (%)
Methylthiouracil	0.5	1	5.1	*16.9*
Metronidazole	0.1	0.5	6.1	3.2
Morantel	0.1	0.1	9.5	3.1
Moxidectin	1	2	14.4	*16.2*
Nafcillin	2	5	5.6	9.0
Niclosamide	0.1	0.1	2.1	7.8
Nitroxynil	0.1	0.1	2.1	3.8
Norfloxacin	0.1	0.1	2.8	5.6
Orbifloxacin	0.5	0.5	9.7	8.3
Oxacillin	0.5	1	8.5	11.3
Oxfendazole	0.1	0.1	1.6	2.4
Oxibendazole	0.1	0.1	2.7	6.2
Oxyclozanide	0.1	0.1	3.0	6.7
Oxyphenbutazone	0.1	0.5	3.9	6.7
Oxytetracycline	0.1	1	6.5	4.2
Penicillin G	NA	NA	NA	NA
Phenyl Thioracil	0.5	1	5.9	5.9
Phenylbutazone	0.1	0.5	0.5	6.0
Prednisone	0.5	0.5	4.3	10.1
Promethazine	0.1	0.1	1.8	1.4
Propionylpromazine	0.1	0.1	1.7	6.3
Propylthiouracil	0.1	0.5	7.5	8.1
Quinoxaline 2- carboxylic acid	0.5	1	6.8	11.0
Ractopamine	0.1	0.1	2.3	11.4
Rafoxanide	0.5	2	3.3	6.0
Ronidazole	0.1	0.5	1.9	6.0
Salbutamol (Albuterol)	0.1	0.5	2.1	6.5
Sarafloxacin	0.5	0.5	8.7	12.9
Selamectin	0.5	1	5.8	7.8
Sulfabromomethazine	0.1	0.1	3.0	4.0
Sulfachloropyridazine	0.1	0.1	3.8	4.1
Sulfadiazine	0.1	0.1	3.4	10.2
Sulfadimethoxine	0.1	0.1	3.9	2.7
Sulfamethazine	0.1	0.1	4.6	7.2
Sulfadoxine	0.1	0.1	2.6	3.3
Sulfaethoxypyridazine	0.1	0.1	3.9	7.1
Sulfamerazine	0.1	0.1	9.7	7.6
Sulfamethizole	0.1	0.1	4.2	5.6
Sulfamethoxazole	0.1	0.1	2.5	4.4
Sulfamethoxypyridazine	0.1	0.1	7.0	9.1
Sulfanilamide	0.1	0.1	11.6	9.2
Sulfanitran	0.1	0.1	2.0	2.5
Sulfapyridine	0.1	0.1	3.5	13.6
Sulfaquinoxaline	0.1	0.1	4.0	5.3
Sulfathiazole	0.1	0.1	8.6	7.8
Tetracycline	0.5	1	6.3	6.2
Thiabendazole	0.1	0.1	2.2	9.7
Thiamphenicol	0.1	0.5	6.5	12.7
Thiouracil	1	2	10.3	10.9
Tildipirosin	0.1	0.1	2.5	9.3
Tilmicosin	0.5	0.5	7.8	8.0
Tolfenamic acid	0.1	0.1	1.1	4.7
Triclabendazole sulfoxide	0.1	0.1	2.5	9.4
Triclabendazole	0.1	0.1	2.2	9.0
Triflupromazine	0.1	0.1	2.6	9.7
Tylosin	0.5	1	3.0	6.4
Virginiamycin M1	1	2	4.1	2.3
Xylazine	0.1	0.1	12.2	14.7
Zeranol	0.1	0.5	1.7	4.5

RSDs >15 % in *italic*, RSDs >20 % in **bold**

Linearity

The linearity of the methods was determined by creating two matrix-matched calibration curves each in kidney and liver. The first calibration curve was prepared to examine the ability to quantify across the range of tolerance levels that would be of interest to regulatory and monitoring agencies. This entailed creating a four-point calibration curve in liver and kidney at 0.5x, 1.0x, 1.5x, and 2.0x of the tolerance levels listed in Table 3. The second calibration curve was prepared at the low end to test the linearity for sensitive measurements, with a range of 1 to 100 ng/g in kidney and liver tissue (for compounds with LOQs >1 ng/mL, the point above the LOQ was selected as the first calibration level). Table 5 shows the linearity of all veterinary drugs for both types of calibration curves in kidney and liver.

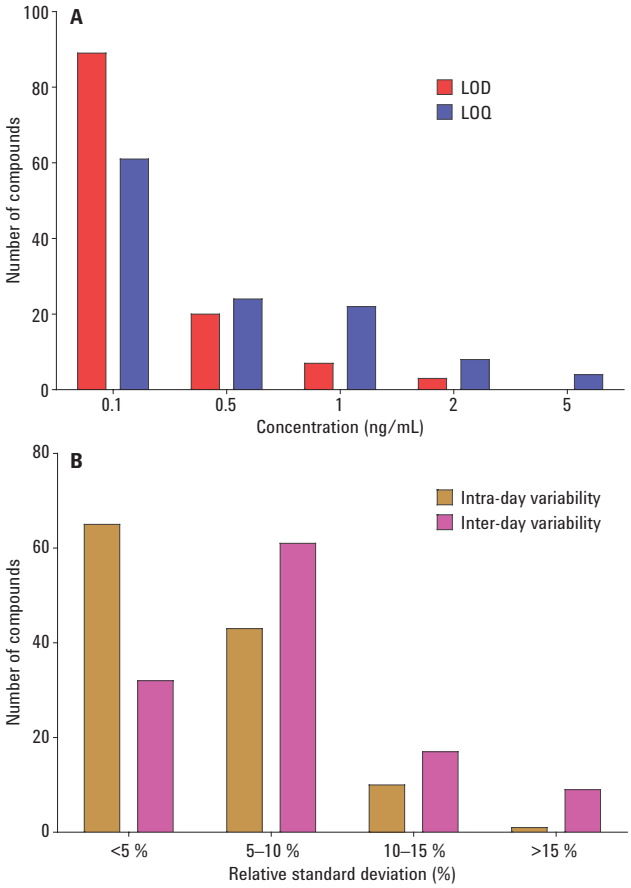

Figure 3. Distribution of (A) LODs and LOQs; (B) intraday and interday variability for the veterinary drugs tested in kidney.

Table 5. Linearity for Two Sets of Calibration Curves Tested

	0.5X, 1.0X, 1.5X, 2.0X				1.0, 2.0, 5.0, 10, 25, 50, 100 ng/g			
	Kidney		Liver		Kidney		Liver	
Compound	**R^2**	**Fit**	**R^2**	**Fit**	**R^2**	**Fit**	**R^2**	**Fit**
Abamectin	0.9977	Linear	**0.9788**	Linear	**0.9782**	Quadratic	0.999	Quadratic
Acepromazine	0.9997	Linear	0.9982	Linear	0.9928	Linear	0.9983	Linear
Albendazole sulfone	0.9974	Linear	0.9986	Linear	0.9982	Linear	0.9971	Linear
Albendazole	0.9966	Linear	0.9935	Linear	0.9981	Linear	0.9998	Linear
Albendazole-2-aminosulfone	**0.9841**	Linear	0.9988	Linear	0.997	Linear	0.9975	Linear
Aminoflubendazol	**0.9882**	Linear	0.9999	Linear	0.9987	Linear	**0.9861**	Linear
Amino-Mebendazole	0.9926	Linear	**0.984**	Linear	0.9959	Linear	0.9911	Linear
Amoxicillin	**0.899**	Linear	0.9965	Linear	**0.9665**	Linear	0.9997	Linear
Ampicillin	0.9957	Linear	0.995	Linear	0.9983	Linear	0.9948	Linear
Azaperone	0.9981	Linear	**0.921**	Linear	0.9928	Quadratic	**0.9876**	Quadratic
Bacitracin	0.9913	Linear	**0.9825**	Linear	0.9941	Linear	0.9913	Linear
Betamethasone	0.9998	Linear	0.9984	Linear	0.9995	Linear	0.9995	Linear
Bithionol	0.9938	Linear	0.996	Linear	**0.9894**	Quadratic	0.994	Quadratic
Cambendazole	0.9938	Linear	**0.9692**	Linear	0.9977	Linear	0.9995	Linear
Carazolol	0.9925	Linear	0.9994	Linear	0.9996	Linear	**0.9898**	Linear
Carbadox	0.9973	Linear	0.9993	Linear	0.994	Linear	**0.987**	Linear
Cefapirin	0.9908	Linear	0.9962	Linear	0.9956	Linear	0.9987	Linear
Cefazolin	0.9919	Linear	0.9999	Linear	0.9966	Linear	0.9986	Linear
Chloramphenicol	0.9945	Linear	0.9981	Linear	0.9972	Linear	0.9943	Linear
Chlorpromazine	**0.9879**	Linear	0.9977	Linear	0.9807	Linear	0.9891	Linear
Chlortetracycline	0.9972	Linear	0.9957	Linear	0.9924	Linear	0.9915	Linear
Cimaterol	0.9986	Linear	0.9986	Linear	**0.9894**	Linear	0.9965	Linear
Ciprofloxacin	0.9967	Linear	0.9918	Linear	**0.9349**	Linear	**0.9771**	Linear
Clenbuterol	0.9999	Quadratic	0.9909	Linear	0.9906	Linear	0.9985	Linear
Clindamycin	0.9969	Linear	0.9908	Linear	0.9973	Linear	0.9993	Linear
Clorsulon	0.9965	Linear	0.9982	Linear	0.9933	Linear	0.9984	Linear
Closantel	**0.9554**	Linear	**0.9895**	Linear	**0.9004**	Linear	0.995	Linear
Cloxacillin	0.9998	Linear	0.9958	Linear	0.996	Linear	0.992	Linear
Danofloxacin	0.9945	Linear	0.9933	Linear	0.9987	Linear	0.9997	Linear
Desethylene ciprofloxacin	0.9959	Linear	0.9998	Linear	**0.9815**	Linear	**0.9486**	Linear
Diclofenac	0.9987	Linear	0.9967	Linear	0.9996	Linear	0.9995	Linear
Difloxacin	0.9905	Linear	0.9919	Linear	0.9955	Linear	0.9957	Linear
Dimetridazole	0.9938	Linear	0.996	Linear	0.9958	Linear	0.9965	Linear
Dipyrone metabolite	0.998	Linear	0.9912	Linear	0.9916	Linear	0.9981	Linear
Doramectin	NA		NA		NA		NA	
Doxycycline	**0.9899**	Linear	0.9999	Linear	0.9999	Linear	0.9989	Linear
Emamectin	**0.9549**	Linear	0.9901	Linear	0.9951	Quadratic	0.9972	Quadratic
Enrofloxacin	0.9993	Quadratic	0.9963	Linear	0.9938	Linear	0.9913	Linear
Eprinomectin B1a	0.9913	Linear	0.9936	Linear	0.9932	Linear	0.9911	Linear
Erythromycin	0.9999	Linear	0.9978	Linear	0.9995	Linear	0.9982	Linear
Fenbendazole	0.9989	Linear	0.9962	Quadrati c	0.9998	Linear	0.9933	Linear
Florfenicol Amine	**0.9535**	Linear	**0.9555**	Linear	**0.8648**	Linear	**0.9614**	Linear
Florfenicol	**0.9842**	Linear	**0.984**	Linear	**0.9818**	Linear	**0.9191**	Linear

R^2 <0.99 in bold

Table 5. Linearity for Two Sets of Calibration Curves Tested (continued)

	0.5X, 1.0X, 1.5X, 2.0X				1.0, 2.0, 5.0, 10, 25, 50, 100 ng/g			
	Kidney		Liver		Kidney		Liver	
Compound	**R^2**	**Fit**	**R^2**	**Fit**	**R^2**	**Fit**	**R^2**	**Fit**
Flubendazole	0.999	Linear	0.9914	Linear	0.9924	Linear	0.9975	Linear
Flunixin	0.9922	Linear	0.9996	Linear	0.9935	Linear	0.9968	Linear
Gamithromycin	0.9925	Linear	0.9902	Linear	0.9998	Linear	**0.987**	Linear
Haloperidol	0.9931	Linear	0.9995	Linear	0.9921	Quadratic	0.9967	Quadratic
Haloxon	0.9942	Linear	0.9934	Linear	0.999	Linear	0.9997	Linear
Hydroxydimetridazole (Dimetridazol-OH)	**0.9846**	Linear	0.9998	Linear	0.9996	Linear	0.9934	Linear
Hydroxy-Ipronidazole	0.995	Linear	1	Quadratic	0.9969	Linear	0.9987	Linear
Hydroxy-metronidazole	0.9979	Linear	0.9937	Linear	0.9962	Linear	0.9998	Linear
Ipronidazole	0.9952	Linear	0.9922	Linear	0.9919	Linear	0.9975	Linear
Ivermectin B1a	**0.9357**	Linear	0.9914	Linear	0.9964	Linear	0.9986	Linear
Ketoprofen	0.9956	Linear	0.998	Linear	0.9953	Linear	0.9997	Linear
Levamisole	0.991	Linear	0.9949	Linear	0.9984	Linear	0.9941	Linear
Lincomycin	0.9916	Linear	**0.9891**	Linear	**0.9816**	Linear	0.9936	Linear
Mebendazole	0.9972	Linear	0.9985	Linear	0.9981	Linear	0.9941	Linear
Melengestrol acetate	0.9968	Linear	0.9931	Linear	**0.9747**	Linear	0.9918	Linear
Meloxicam	0.9998	Linear	0.9996	Linear	0.9961	Linear	0.9998	Linear
Mercaptobenzimidazole	0.9982	Linear	0.9921	Linear	**0.9794**	Linear	0.9937	Linear
Methylthiouracil	0.9967	Linear	0.9959	Linear	0.9927	Linear	0.9997	Linear
Metronidazole	0.9982	Linear	0.9941	Linear	0.9995	Linear	0.9994	Linear
Morantel	0.9962	Linear	0.9936	Linear	0.9903	Linear	0.9979	Linear
Moxidectin	0.9904	Linear	0.9903	Linear	NA		NA	
Nafcillin	0.9917	Linear	0.9901	Linear	0.9982	Linear	0.9971	Linear
Niclosamide	0.9999	Linear	0.9917	Linear	0.9911	Quadratic	0.998	Quadratic
Nitroxynil	0.9988	Linear	**0.9898**	Linear	0.9967	Linear	0.9992	Linear
Norfloxacin	0.9906	Linear	0.9946	Linear	**0.924**	Linear	**0.9827**	Linear
Orbifloxacin	**0.9699**	Linear	0.9992	Linear	**0.987**	Linear	0.9994	Linear
Oxacillin	0.9995	Linear	0.9924	Linear	0.9935	Linear	0.9938	Linear
Oxfendazole	0.9998	Linear	0.9999	Quadratic	0.9997	Linear	0.993	Linear
Oxibendazole	0.9978	Linear	0.9985	Linear	0.9965	Quadratic	0.9975	Quadratic
Oxyclozanide	0.9959	Linear	0.9992	Linear	0.9964	Linear	0.998	Linear
Oxyphenbutazone	0.9958	Linear	0.9986	Linear	0.9971	Linear	0.9941	Linear
Oxytetracycline	0.9987	Linear	**0.9885**	Linear	0.9969	Linear	0.9976	Linear
Penicillin G	NA		NA		NA		NA	
Phenyl Thioracil	0.9988	Linear	0.9987	Linear	0.9977	Linear	0.9983	Linear
Phenylbutazone	0.999	Linear	0.9969	Linear	0.997	Linear	0.9922	Linear
Prednisone	0.9958	Linear	0.9902	Linear	0.9973	Linear	0.9986	Linear
Promethazine	0.9994	Linear	0.9901	Linear	**0.9574**	Linear	0.9966	Linear
Propionylpromazine	0.9971	Linear	0.9927	Linear	0.9981	Linear	0.996	Linear
Propylthiouracil	0.9922	Linear	0.9982	Linear	0.9969	Linear	0.9986	Linear
Quinoxaline 2-carboxylic acid	0.9994	Linear	0.9904	Linear	0.9974	Linear	0.9959	Linear
Ractopamine	0.9984	Linear	0.998	Linear	**0.9886**	Linear	0.992	Linear
Rafoxanide	0.9993	Linear	0.9974	Linear	0.9911	Linear	0.9937	Linear
Ronidazole	0.9983	Linear	**0.9894**	Linear	0.9993	Linear	0.9988	Linear

R^2 <0.99 in bold

Table 5. Linearity for Two Sets of Calibration Curves Tested (continued)

	0.5X, 1.0X, 1.5X, 2.0X				1.0, 2.0, 5.0, 10, 25, 50, 100 ng/g			
	Kidney		Liver		Kidney		Liver	
Compound	**R^2**	**Fit**	**R^2**	**Fit**	**R^2**	**Fit**	**R^2**	**Fit**
Salbutamol (Albuterol)	**0.9865**	Linear	1	Linear	0.9955	Linear	0.9968	Linear
Sarafloxacin	0.9989	Linear	0.9917	Linear	0.9901	Linear	**0.989**	Linear
Selamectin	0.9989	Linear	0.9999	Quadratic	0.9944	Linear	0.998	Linear
Sulfabromomethazine	0.9900	Linear	0.9998	Linear	0.9988	Linear	0.9995	Linear
Sulfachloropyridazine	0.9945	Linear	0.9957	Linear	0.9995	Linear	0.9999	Linear
Sulfadiazine	0.9931	Linear	0.9992	Linear	0.9969	Linear	0.9945	Linear
Sulfadimethoxine	0.9959	Linear	0.9946	Linear	0.9994	Linear	0.9999	Linear
Sulfamethazine	0.9991	Linear	0.9975	Linear	0.9900	Linear	0.9964	Linear
Sulfadoxine	0.9979	Linear	0.9936	Linear	0.9997	Linear	0.9998	Linear
Sulfaethoxypyridazine	0.9957	Linear	0.9938	Linear	0.9989	Linear	0.9935	Linear
Sulfamerazine	0.9984	Linear	0.9949	Linear	0.9979	Linear	0.9971	Linear
Sulfamethizole	0.9986	Linear	0.9999	Linear	0.9937	Linear	0.9962	Linear
Sulfamethoxazole	0.9995	Linear	0.9924	Linear	0.9997	Linear	0.998	Linear
Sulfamethoxypyridazine	0.9948	Linear	0.9983	Linear	0.9955	Linear	0.9963	Linear
Sulfanilamide	0.9945	Linear	0.9945	Linear	0.9937	Linear	0.9983	Linear
Sulfanitran	0.9967	Linear	0.9987	Linear	**0.969**	Linear	0.9935	Linear
Sulfapyridine	0.9962	Linear	0.9941	Linear	0.9942	Linear	0.9957	Linear
Sulfaquinoxaline	0.9964	Linear	0.9983	Linear	0.9965	Linear	0.9998	Linear
Sulfathiazole	0.9998	Linear	0.9921	Linear	0.9903	Linear	0.9962	Linear
Tetracycline	0.993	Linear	0.9992	Linear	0.9989	Linear	0.9977	Linear
Thiabendazole	0.9998	Linear	0.9918	Linear	0.9971	Linear	0.9991	Linear
Thiamphenicol	0.9914	Linear	0.9992	Linear	**0.9783**	Linear	**0.985**	Linear
Thiouracil	0.9941	Linear	**0.957**	Linear	0.9948	Linear	0.9991	Linear
Tildipirosin	0.9979	Linear	0.9984	Linear	0.9974	Linear	**0.9843**	Linear
Tilmicosin	0.9903	Linear	0.9994	Linear	0.9993	Linear	0.9926	Linear
Tolfenamic acid	0.9944	Linear	0.9977	Linear	0.9918	Linear	0.9974	Linear
Triclabendazole sulfoxide	0.9992	Linear	0.9994	Linear	0.9985	Linear	0.9989	Linear
Triclabendazole	**0.9807**	Linear	0.9983	Linear	0.9997	Linear	0.9999	Linear
Triflupromazine	0.9994	Linear	0.9979	Linear	0.9978	Linear	**0.9884**	Linear
Tylosin	0.9986	Linear	0.9995	Linear	0.9908	Linear	0.9901	Linear
Virginiamycin M1	0.9984	Linear	0.999	Linear	0.9913	Linear	0.9954	Linear
Xylazine	0.9942	Linear	0.9929	Linear	0.9964	Linear	0.9985	Linear
Zeranol	0.9965	Linear	0.9923	Linear	0.9931	Linear	0.9952	Linear

R^2 <0.99 in bold

For the calibration curve based on the tolerance levels, more than 89 % of the compounds had R^2 >0.99. In fact, only azaperone had R^2 < 0.95 in liver, while only ivermectin and amoxicillin had R^2 < 0.95 in kidney tissue. This was despite the fact that only three internal standards were used to correct the data (doxycycline-d_3 to correct for tetracyclines, nadcillin-d_5 for β-lactams, and flunixin-d_3 for the remaining veterinary drugs). When looking at the low-end calibration curve, more than 85 % of the compounds still had R^2 >0.99 in both the liver and kidney tissue, and azaperone, ivermectin, and amoxicillin looked much better. In this case, it was norfloxacin and florfenicol amine that had R^2 < 0.95 in the kidney, while florfenicol was the only compound in the liver. The behavior of florfenicol and florfenicol amine could be because they eluted extremely early, which may have caused matrix effects that could not be accounted for by the flunixin-d_3. Nonetheless, this method had excellent linearity for most of the veterinary drugs tested while using a limited set of internal standards. This further illustrates the benefits of using matrix-matched calibrations for this analysis. Figure 4 illustrates typical calibration curves in kidney for the two types of calibrations performed.

Reproducibility and repeatability

The repeatability of the method was estimated by calculating the intraday variability based on relative standard deviation (%RSD) of five replicate injections of kidney tissue spiked at 1.0x tolerance level of each veterinary drug injected throughout a 24-hour period. Similarly, the reproducibility was determined as the %RSD of a sample injected on four consecutive days. Table 4 shows the %RSDs for all veterinary drugs tested in this method. Only one compound (cefapirin) had an RSD greater than 15 % for the intraday variability. Nine compounds (amino-flubendazole, ampicillin, cefapirin, ciprofloxacin, dipyrone metabolite, florfenicol, gamithromycin, methyl-thiouracil, and moxidectin) had RSDs greater than 15 % (less than 23 %) during the interday RSD tests. The interday variabilities were understandably a little higher than the corresponding intraday variability, probably due to standard preparation and potential compound degradation across the four-day period. Figure 3 shows that most compounds had both inter- and intraday RSDs of less than 10 %, proving that the method is robust and reproducible.

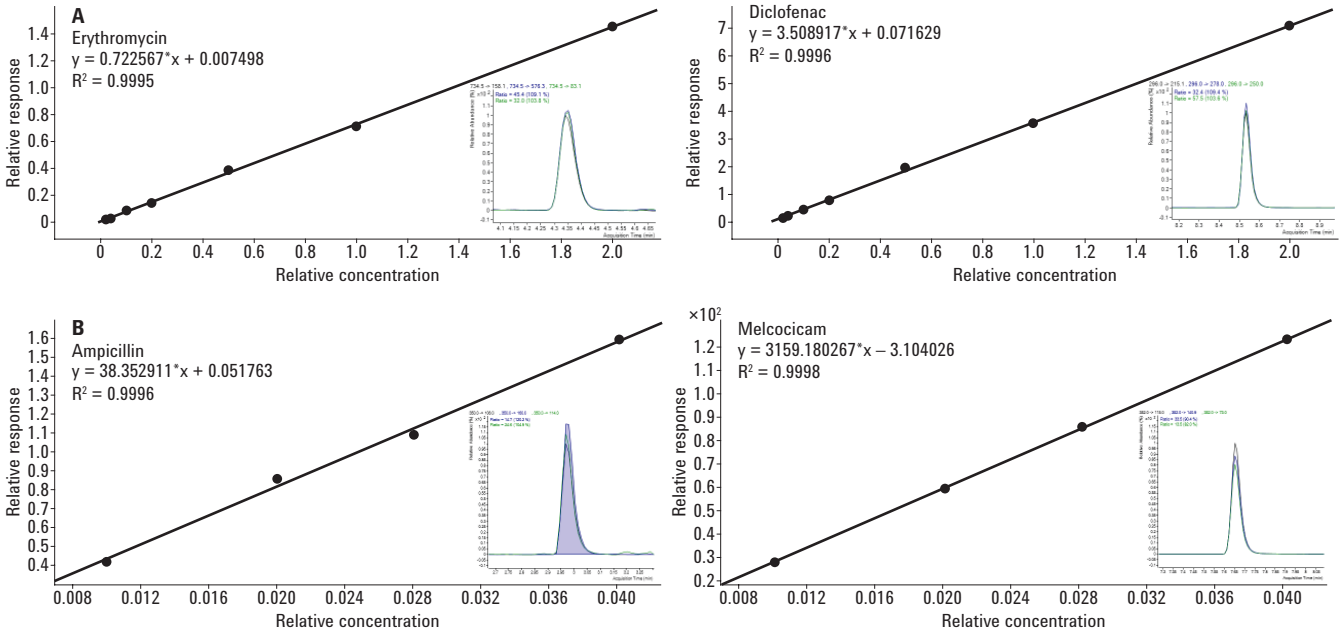

Figure 4. Typical calibration curves for veterinary drugs in two ranges: A) 1–100 ng/mL; B) 0.5–2.0x TLs in liver.

Conclusions

This method shows the analysis of 120 veterinary drugs in meat using the Agilent 1290 Infinity II UHPLC coupled to an Agilent 6495 Triple Quadrupole LC/MS in 12 minutes. It is common practice within analytical surveillance laboratories to be able to validate an analytical method down to half a compound's maximum tolerance level. For all analytes in this method, both LODs and LOQs were in line with this requirement when compared to tolerance levels for liver and kidney in the USA. In fact, this method is sensitive enough to achieve sub-1 ng/mL LODs and LOQs for most analytes. The method is robust and selective with the use of three transitions for almost all veterinary drugs tested, while being reproducible and repeatable. Quantitative performance was excellent with good linearity for most compounds by using matrix-matched calibration curves. The method was also cost-effective since there was limited use of expensive internal standards.

References

1. J. L. Martinez. "Environmental pollution by antibiotics and by antibiotic resistance determinants" *Environmental Pollution* **157(11)**, 2893-2902 (2009).
2. EuropeanCommision Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results (**2002**).
3. CFR Title 21 - Food And Drugs, Part 556 Tolerances for residues of new animal drugs in food (http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?cfrpart=556) (2015) United States Food and Drug Administration. Accessed Apr 1, 2016.
4. Health Canada Administrative Maximum Residue Limits (AMRLs) and Maximum Residue Limits (MRLs) set by Canada (**2012**).
5. L. Han, *et al.* "Evaluation of a recent product to remove lipids and other matrix co-extractives in the analysis of pesticide residues and environmental contaminants in foods" *J. Chromatog. A* **1449**, 17-29 (2016).
6. D. Lucas, L. Zhao, PAH Analysis in Salmon with Enhanced Matrix Removal, *Agilent Technologies Application Note*, publication number 5991-6088EN (**2015**).
7. L. Zhao, D. Lucas, Multiresidue analysis of veterinary drugs in bovine liver by LC/MS/MS, *Agilent Technologies Application Note*, publication number 5991-6096 (**2015**).
8. T. Anumol, *et al.*, Analysis of 122 Veterinary Drugs in Meat Using All Ions MS/MS with an Agilent 1290/6545 UHPLC-Q-TOF System, *Agilent Technologies Application Note*, publication number 5991-6651EN (**2016**).
9. M. J. Schneider, S. J. Lehotay, A. R. Lightfield. "Evaluation of a multi-class, multi-residue liquid chromatography – tandem mass spectrometry method for analysis of 120 veterinary drugs in bovine kidney" *Drug Testing and Analysis* **4**, 91-102 (2012).

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