



Streamlined Method for the Determination of More Than 100 Veterinary Drugs in Animal Tissue Using Dispersive-SPE Clean-up and LC-MS/MS Detection

UCT Part Number:

- **ECC1850CT** (500 mg endcapped C18)

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More than 100 veterinary drugs can be extracted and analyzed using this fast and easy multi-class, multi-residue method.

Procedure:

1) Extraction

- a) Weigh 2 g of homogenized tissue sample into a 50 mL centrifuge tube
- b) Add 10 mL of acetonitrile/water (4/1, v/v)
- c) Shake or vortex for 5 min
- d) Centrifuge for 5 min at >3700 rcf

2) dSPE Clean-up

- a) Transfer the supernatant into product **ECC1850CT**
- b) Add 10 mL hexane that has been pre-saturated with acetonitrile
- c) Shake or vortex for 30 sec
- d) Centrifuge for 5 min at >3700 rcf
- e) Aspirate hexane to waste
- f) Evaporate 5 mL of the extract under nitrogen at 45°C to <0.7 mL
- g) Add water with 0.1% formic acid to reach a final volume of 1 mL (1 g/mL sample equivalent)
- h) Transfer sample to a HPLC vial (filter with 0.2µm PVDF membrane if desired)
- i) Sample is ready for LC-MS/MS analysis

3) LC-MS/MS analysis

MS: Waters TQD

HPLC: Waters Acquity UHPLC system

LC Parameters

LC column	Waters Acquity HSS T3 (C18), 1.8 μm , 2.1 x 100 mm
Guard column	Agilent Eclipse Plus C18, 5 μm , 4.6 x 12.5 mm
Flow rate	0.5 ml/min
Injection volume	20 μl
Oven temperature	40 $^{\circ}\text{C}$
Autosampler temperature	4 $^{\circ}\text{C}$
Equilibration time	3.3 min

Mobile Phase

A: 0.1 % formic acid in water/MeCN (95/5, v/v)

B: 0.1 % formic in MeCN

Time	%B
0.0	0.2
0.1	0.2
8.0	99.8
9.5	99.8
9.6	0.2
13.0	0.2

For analysis of late eluting compounds, 50 $\mu\text{L}/\text{min}$ of 27 mM ammonium formate in MeOH:MeCN (75:25) is infused from 5.05 to 9.45 min using the instrument's infusion syringe to enhance the signal of the late-eluting anthelmintics ($[\text{M}+\text{NH}_4]^+$ ion formation).

MS Instrument Settings

Capillary voltage	3000 V
Extractor voltage	3 V
Desolvation temperature	450 $^{\circ}\text{C}$
Source temperature	150 $^{\circ}\text{C}$
Dwell time	5 msec

Analyte	Drug class	RT (min)	Precursor ion	Cone V	Product 1	Collision energy (V)	Product 2	Collision energy (V)
Desacetyl Cephapirin	β -Lactam	0.69	382.1	32	152	28	124.2	48
Florfenicol Amine	Phenicol	0.68	248.1	25	230.2	10	130.1	35
Sulfanilamide	Sulfonamide	1.19	173	40	92.9	20	75.9	36
Amoxicillin	β -Lactam	1.47	366.1	20	114	22	349.3	10
Salbutamol	β -Agonist	1.46	240.2	20	148.2	20	222.3	10
Zilpaterol	β -Agonist	1.46	262.3	27	244.3	12	185.2	30
Cimaterol	β -Agonist	1.51	220	16	143	24	115.9	34
DCCD	β -Lactam	1.72	549.1	40	183	30	241.1	20
Lincomycin	Lincosamide	1.87	407.3	20	126.1	30	359.2	20
Sulfadiazine	Sulfonamide	2.00	251.1	30	156.1	15	108	20
Ampicillin	β -Lactam	2.01	350.1	26	106.1	24	114	30
Desethyle Ciprofloxacin	Fluoroquinolone	2.06	306.2	35	288.2	20	245.2	20
Sulfathiazole	Sulfonamide	2.10	256.1	25	156.1	15	108	25
Sulfapyridine	Sulfonamide	2.18	250.1	32	156.1	18	108.1	28
Norfloxacin	Fluoroquinolone	2.16	320.2	36	276.2	18	233.1	26
Tulathromycin	Macrolide	2.17	806.8	38	72	56	577.5	24
Oxytetracycline	Tetracycline	2.21	461.2	25	426.4	20	443.4	15
Ciprofloxacin	Fluoroquinolone	2.22	332.2	35	245.2	25	288.4	20
Ractopamine	β -Agonist	2.27	302.2	26	164	16	107	32
Sulfamerazine	Sulfonamide	2.30	265.1	28	91.9	28	155.9	16
Danofloxacin	Fluoroquinolone	2.31	358.1	28	96	26	314.2	18
Tetracycline	Tetracycline	2.35	445.2	30	154.1	30	410.2	20
Enrofloxacin	Fluoroquinolone	2.38	360.2	35	316.4	20	245.3	25
2-Quinoxalinecarboxylic Acid	Other	2.43	175	22	129	16	131	16
Sulfamethizole	Sulfonamide	2.55	271.1	28	156.1	16	92	30
Sulfamethazine	Sulfonamide	2.54	279.1	35	186.1	20	156.1	20
Sulfamethazine-13C6 (IS)	-	2.54	285.2	32	186.1	18	124.1	26
Cefazolin	Cephalosporin	2.56	455.1	20	156	16	323.2	12
Sulfamethoxy pyridazine	Sulfonamide	2.58	281.1	30	156.1	20	126.2	20
Difloxacin	β -Lactam	2.62	400.3	35	356.4	20	299.2	30
Sarafloxacin	Fluoroquinolone	2.58	386.1	20	342.2	20	299.2	30
Clenbuterol	β -Agonist	2.56	277.2	25	259.2	10	132.1	30
Pirlimycin	Lincosamide	2.74	411.3	30	112.2	40	363.3	20
Chlortetracycline	Tetracycline	2.84	479.2	30	154.1	30	444.3	20
Clindamycin	Lincosamide	2.89	425.3	45	126.2	40	377.4	20
Gamithromycin	Macrolide	2.91	777.8	62	83	54	116	50
Sulfachloropyridazine	Sulfonamide	2.95	285	28	156.1	16	108	26
Tilmicosin	Macrolide	3.06	869.8	45	174.2	35	696.6	35
Sulfadoxine	Sulfonamide	3.10	311.2	35	156.1	20	108.1	30
Sulfamethoxazole	Sulfonamide	3.11	254	26	92.1	30	156	18
Sulfaethoxy pyridazine	Sulfonamide	3.14	295.1	30	156.1	20	140.2	20
Florfenicol	Phenicol	3.15	358.1	24	241	18	206	28
Chloramphenicol	Phenicol	3.36	323.1	16	275	16	165	26
Erythromycin	Macrolide	3.49	734.8	30	158.2	36	115.9	54
Sulfadimethoxine	Sulfonamide	3.57	311.1	35	156.1	20	108	30
Sulfaquinoxaline	Sulfonamide	3.59	301.1	34	156.1	18	108	28
Prednisone	Corticosteroid	3.67	359.2	22	341.1	10	146.9	26
Tylosin	Macrolide	3.66	916.8	45	174.2	35	101.1	35
Penicillin G-d7 (IS)	-	3.86	342.1	46	183.1	26	160.1	24
Penicillin G	β -Lactam	3.86	335.1	18	176	16	160.1	18
Beta/Dexa-methasone	Corticosteroid	4.11	393.2	20	373.2	10	147.1	28
Sulfantran	Sulfonamide	4.16	336.2	26	156	12	134.1	28
Sulfabromomethazine	Sulfonamide	4.21	357.1	35	92	30	156.1	25
Zeranol ($\hat{\pm}$ -Zearalanol)	Other	4.37	323.2	16	305.2	10	189.1	24

Oxacillin	β -Lactam	4.39	402.1	22	160	20	243.1	18
Atrazine (QC)	-	4.49	216.1	34	174	18	103.9	30
Nafcillin	β -Lactam	4.79	415.2	20	199.1	14	171.1	38
Oxyphenylbutazone	NSAID	4.83	325.2	26	120.1	24	148.2	30
Flunixin	NSAID	4.86	297.1	42	279.1	22	109	50
Flunixin-d3 (IS)		4.82	300.1	40	282.1	24	112	54
Dicloxacillin	β -Lactam	5.03	470.2	22	160.1	14	311.1	16
Phenylbutazone	NSAID	5.93	309.1	28	120	20	91.8	30
Melengesterol Acetate	Other	6.30	397.4	30	279.3	20	337.5	15
2-thiouracil	Thyreostat	0.85	128.9	32	111.9	12	69.9	18
2-mercapto-1-methylimidazole	Thyreostat	1.14	114.9	40	87.9	16	73.9	16
6-methyl-2-thiouracil	Thyreostat	1.22	142.9	32	83.9	18	125.9	14
Metronidazole-OH	Nitroimidazole	1.42	188	22	123	14	126	18
Dipyrone	Tranquilizer	1.60	218.1	24	96.9	12	187	10
Dimetridazole-OH	Nitroimidazole	1.63	158	22	140	12	93.9	22
Metronidazole	Nitroimidazole	1.63	172	26	127.9	14	81.9	24
5-hydroxythiabendazole	Anthelmintic	1.70	218	50	190.9	26	147	32
Albendazole 2-amino-sulfone	Anthelmintic	1.85	240	36	133	28	198	20
Ronidazole	Nitroimidazole	1.85	201	18	139.9	10	54.8	20
Levamisole	Anthelmintic	1.86	205	40	178	22	90.9	34
Dimetridazole	Nitroimidazole	1.86	142	32	95.9	16	80.9	24
Thiabendazole	Anthelmintic	1.94	202	44	174.9	26	130.9	32
6-propyl-2-thiouracil	Thyreostat	2.15	171	38	154	18	112	20
2-mercaptobenzimidazole	Thyreostat	2.30	150.9	42	92.8	20	118	22
Azaperone	Tranquilizer	2.34	328.3	34	165	20	122.9	36
Orbifloxacin	Fluoroquinolone	2.39	396.2	36	352.2	18	295.1	24
Albendazole sulfoxide	Anthelmintic	2.44	282.1	28	240	14	207.3	24
Xylazine	Tranquilizer	2.48	221.1	42	164	26	147	24
Ipronidazole-OH	Nitroimidazole	2.54	186.1	22	168	14	121.8	20
Morantel	Anthelmintic	2.60	221.1	50	122.9	36	163.9	28
2-amino-Mebendazole	Anthelmintic	2.63	238.1	50	104.9	26	132.9	36
6-phenyl-2-thiouracil	Thyreostat	2.73	205	38	103	26	187.9	18
2-amino-Flubendazole	Anthelmintic	2.77	256	50	122.9	28	94.9	38
Cambendazole	Anthelmintic	2.83	303.1	34	261.1	18	217	28
Bacitracin	Other	2.87	475.3	26	85.9	24	199.1	30
Carazolol	Tranquilizer	2.90	299.3	34	116	20	97.9	22
Doxycycline	Tetracycline	2.91	445.3	28	428.2	20	97.9	46
Oxibendazole	Anthelmintic	2.95	250.1	34	218.1	18	175.9	28
Oxfendazole	Anthelmintic	3.01	316.1	40	158.9	32	191	22
Albendazole sulfone	Anthelmintic	3.02	298.1	38	266	20	159	36
Ipronidazole	Nitroimidazole	3.20	170.1	34	124	18	109	24
Clorsulon	Flukicide	3.39	377.7	24	341.8	12	241.9	20
Haloperidol	Tranquilizer	3.53	376.2	40	165	24	122.9	42
Acetopromazine	Tranquilizer	3.55	327.2	32	86	20	254	22
Promethazine	Tranquilizer	3.58	285.2	24	85.9	16	198	20
Fenbendazole sulfone	Anthelmintic	3.65	332.1	40	300	22	158.9	38
Albendazole	Anthelmintic	3.65	266.1	34	234	20	191.1	32
Mebendazole	Anthelmintic	3.70	296.1	36	264.1	20	104.9	36
Flubendazole	Anthelmintic	3.90	314.1	38	282	22	94.9	50
Propionylpromazine	Tranquilizer	3.91	341.2	32	85.9	22	268.1	24
Chlorpromazine	Tranquilizer	4.04	319.2	32	86	20	246	22
Triflupromazine	Tranquilizer	4.26	353.2	34	85.9	22	280	28
Fenbendazole	Anthelmintic	4.33	300.1	38	268	20	158.9	36
Oleandomycin triacetate	Macrolid	4.37	814.7	38	200.1	30	98	48
Nitroxynil	Flukicide	4.41	288.8	40	126.8	20	115.9	34
Virginiamycin M1	Other	4.49	526.4	26	508.3	12	108.9	44

Ketoprofen	Tranquilizer	4.71	255.1	28	104.9	24	209	14
Haloxon	Anthelmintic	5.28	415.1	44	272.9	34	210.9	36
Triclabendazole sulfoxide	Flukicide	5.37	372.8	36	357.8	18	212.9	30
Emamectin benzoate	Anthelmintic	5.49	886.8	52	158	40	126	46
Diclofenac	Tranquilizer	5.55	296	20	214.9	20	250	12
Triclabendazole	Flukicide	5.99	359	52	343.9	26	274	38
Novobiocin	Other	6.05	613.5	30	189	28	132.9	64
Oxyclozanide	Flukicide	6.08	399.6	38	363.8	14	175.9	24
Niclosamide	Flukicide	6.20	325	36	170.9	30	289	16
Tolfenamic acid	Tranquilizer	6.23	262.1	22	244	14	180	40
Bithionol	Flukicide	6.76	352.9	36	160.8	24	191.2	28
Eprinomectin	Anthelmintic	7.44	914.8	18	186.1	20	154	40
Abamectin	Anthelmintic	7.94	890.8	16	305.3	28	145	42
Closantel	Flukicide	8.07	660.9	70	126.8	54	344.8	32
Doramectin	Anthelmintic	8.30	916.9	22	331.3	26	113	56
Moxidectin	Anthelmintic	8.32	640.5	16	528.3	8	498.2	10
Rafoxanide	Flukicide	8.50	623.9	62	126.1	48	344.8	30
Selamectin	Anthelmintic	8.62	770.7	36	145	30	112.9	40
Ivermectin [M+Na] ⁺	Anthelmintic	8.77	897.8	82	183	58	329.2	56

Accuracy and Precision

A multi-day, multi-analyst validation demonstrated that the final method is suitable for screening of 113 analytes, identifying 98 and quantifying 87 out of the 127 tested drugs at or below US regulatory tolerance levels in bovine muscle. Overall, the method demonstrated reasonably good quantitative performance with recoveries ranging between 70–120% for 87 out of 127 analytes, and recovery of < 50% for only 20 analytes. 85 analytes gave RSDs ≤ 20% and 100 analytes gave RSDs ≤ 25%.

Adapted from: L. Geis-Asteggiante, S.J. Lehotay, A.R. Lightfield, T. Dutko, C. Ng, L. Bluhm, Ruggedness testing and validation of a practical analytical method for >100 veterinary drug residues in bovine muscle by ultrahigh performance liquid chromatography tandem mass spectrometry, *Journal of Chromatography A* 1258 (2012) 43-54.