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Title: Screening and Confirmation of Animal Drug Residues by UHPLC-HRMS-MS		
Revision: Original	Replaces: NA	Effective: 08/21/2017

Contents

A. INTRODUCTION

1. Summary of Procedure

   Animal drug residues are extracted from tissue using dispersive SPE for both extraction and sample clean up. The extracted residues are examined by UHPLC-HRMS using a quadrupole Orbitrap mass spectrometer under electrospray ionization (ESI) conditions.

2. Applicability

   This method is suitable for the screening and confirmation of animal drug residues in bovine and porcine kidney and muscle tissue at the levels listed in Tables 18A, 18B, 19A and 19B in Appendix J.3.

   Note: Refer to 21CFR for tolerance values set by FDA and 40CFR for tolerance values set by EPA.

B. EQUIPMENT

Note: Equivalent equipment may be substituted.

1. Apparatus
   a. Platform shaker - Cat. No. 6010, Eberbach
   b. Centrifuge - Thermo IEC, Sorvall RC-6 capable of 3720 rcf
   c. Balance - Mettler Top Loading Model PB300 Balance capable of weighing 2 ± 0.01 g
   d. Balance Analytical - Mettler Model X-205 Dualrange
   e. Turbovap LV Concentration Workstation - Biotage Corp
   f. C18 BakerBond Octadecyl (C18) 40 µm Prep LC Packing
   g. Centrifuge tubes - Polypropylene (PP), 50 mL, Falcon Part number 352070
   h. Centrifuge tubes - Polypropylene (PP), 15 mL, Falcon Part Number 352096
   i. Whatman Mini-UniPrep Syringless filter vials - VWR 0.2 micron, PVDF, Cat. No. 12000-524.

      Note: Avoid glass if the Mini-UniPrep filter vials are substituted with syringe filters and autosampler vials, and substitutes must be checked for possible retention of analytes.

   j. Magnetic stirrer and stirbars, freezer, volumetric flasks, graduated cylinders, Pasteur pipettes, repeating pipettes and tips, beakers, bottles, weigh boats, spatulas, funnels, and bottle top volumetric dispensors.

k. LC vials with screw cap lids - Amber glass, 4 mL, Cat. No. 1232 P 49, Thomas Scientific

l. Plastic screw cap vials - Polypropylene, 4 mL, Cat. No. 1708 H 01, Thomas Scientific

2. Instrumentation

a. UHPLC column - Waters UHPLC HSS T3, 2.1 x 100 mm, 1.8 µm column with VanGuard Precolumn UHPLC HSS T3 2.1 x 5.0 mm, 1.8 µm.

b. Thermo Scientific Q Exactive Focus/Ultimate 3000 UHPLC system with Xcalibur operating software and TraceFinder processing software.

C. REAGENTS AND SOLUTIONS

Note: Equivalent reagents / solutions may be substituted. The stability time frame of the solution is dependant on the expiration date of the components used or the listed expiration date, whichever is soonest. The maximum length of time that a working reagent shall be used is 1 year unless the laboratory has produced extension data.

1. Reagents

a. Hexane - HPLC Grade, Fisher Optima Cat. No. H303-4

b. Acetonitrile (ACN) - HPLC Grade, Spectrum Chemical Co. Cat. No. HP412

c. Formic acid - Sigma Chemical Co., Cat. No. F0507-500ML

d. Water LC-MS Grade, House deionized water passed through an ELGA Pure Lab Ultra Filtration System.

e. Sodium hydroxide (NaOH) - pellets, Fisher, Item No. S318-3.

f. Methanol – Fisher Scientific – Cat. No. A456-4

g. Dimethyl Sulfoxide (DMSO) - Spectrum Chemical – Cat. No. HP412

h. Acetonitrile (ACN) – LCMS Grade, Honeywell Burdick & Jackson - Product Number LC015

i. Formic Acid – LCMS Grade, Thermo Scientific - Product Number 85178 or 28905

2. Solutions

a. 80:20 Acetonitrile/Water:

Measure 800 mL of acetonitrile using a graduated cylinder and transfer to a 1 L volumetric flask. Measure 200 mL of deionized water using a graduated cylinder and add to the volumetric flask containing the acetonitrile. Mix this solution and transfer to a dispenser bottle.

b. 50:50 Acetonitrile/Methanol:

Add equal volumes of acetonitrile and methanol and mix.

c. Hexane (saturated with acetonitrile):

Add 40 mL of acetonitrile to approximately 1 L of hexane in a separatory funnel. Mix this solution vigorously. Allow the layers to separate and discard the acetonitrile layer. Transfer the saturated hexane to a dispenser bottle for storage. Mix this solution prior to use each day.

d. 0.1% Formic Acid in water:

Add 1.0 mL of LCMS grade formic acid to a 1 L volumetric flask. Dilute to volume with deionized water.

e. 0.03 M Sodium Hydroxide:

Add 0.12 g of NaOH to a 100 ml volumetric flask containing 80 mL of deionized water. Mix and allow solution to cool. Adjust to final volume using deionized water. Store in a plastic container.

f. UHPLC Aqueous Mobile Phase (5% ACN, 95% Water, 0.1% Formic Acid):

Measure 50 mL of LCMS grade acetonitrile and add to a 1 L volumetric flask. Add 1.0 mL of LCMS grade formic acid to the flask using a glass Class A Pasteur pipette or a premeasured 1 mL ampule of this acid may be used. Bring to volume with deionized water. Mix and transfer to the aqueous reservoir of the LC.

g. UHPLC Organic Mobile Phase (Acetonitrile, 0.1% Formic Acid):

Add 1.0 mL of LCMS grade formic acid using a glass Class A Pasteur pipette (a premeasured 1 mL ampule of this acid may be used instead) into a 1 L volumetric flask. Bring to volume using LCMS grade acetonitrile. Mix and transfer to the organic reservoir of the LC.

D. STANDARD(S)

Note: Equivalent standards / solutions may be substituted. Purity and counterions are to be taken into account when calculating standard concentrations. The stability time frame of the solution is dependant on the expiration date of the components used or the listed expiration date, whichever ends sooner. *The maximum length of time that an in-house prepared standard shall be used is 1 year unless the laboratory has produced extension data.*

1. Standard Information

Table 1 – Standard information

Standard Analyte	Manufacturer	Catalog Number	Standard Analyte	Manufacturer	Catalog Number
2-amino-Flubendazole	Sigma Aldrich	32841-10MG	Nafcillin	US Pharmacopeia	1450007
2-Aminosulfone Albendazole	Toronto Research Co.	A580950	Norfloxacin	Sigma Aldrich	N9890
Acepromazine	Sigma Aldrich	A7111-50MG	Orbifloxacin	Sigma Aldrich	34041-100MG-R
Albendazole	Sigma Aldrich	A4673-10G	Oxacillin	US Pharmacopeia	1481000
Amoxicillin	US Pharmacopeia	1031503	Oxyphenylbutazone	Toronto Research	876950
Ampicillin	Sigma Aldrich	A1593	Oxytetracycline	Sigma Aldrich	O5875
Azaperone	Sigma Aldrich	34223-100MG	Penicillin G	US Pharmacopeia	1502508
Butorphanol	Sigma Aldrich	B9156-100MG	Phenylbutazone	MP Biochemicals	153567
Carazolol	Sigma Aldrich	53787-10MG	Pirlimycin	Pfizer1	Not applicable
Cefazolin	Sigma Aldrich	C5020	Prednisone	Sigma Aldrich	P6254
Chlortetracycline	US Pharmacopeia	1129007	Promethazine	Sigma Aldrich	P4651-25G
Cimaterol	Tocris Bioscience	435	Propionylpromazine	Sigma Aldrich	P7780-500MG
Ciprofloxacin	US Pharmacopeia	1134313	Ractopamine	Sigma Aldrich	34198
Clenbuterol	Sigma Aldrich	C5423	Salbutamol	Sigma Aldrich	S8260
Clindamycin	Sigma Aldrich	C5269	Sarafloxacin	Abbott Labs5	Not applicable
Cloxacillin	US Pharmacopeia	1142005	Sulfachloropyridazine	Sigma Aldrich	46778
Danofloxacin	Pfizer1	Not applicable	Sulfadiazine	Sigma Aldrich	S8626
DCCD	Pfizer1	Not applicable	Sulfadimethoxine	Sigma Aldrich	46794
Desacetyl Cephapirin	Acs Dorfar SpA	Not applicable	Sulfadoxine	US Pharmacopeia	1626500
Desethylene Ciprofloxacin	Bayer Healthcare2	Not applicable	Sulfaethoxypyridazine	Fluka	2743
Diclofenac	Sigma Aldrich	D6899-10G	Sulfamerazine	Sigma Aldrich	S8876
Dicloxacillin	US Pharmacopeia	1189009	Sulfamethazine	Sigma Aldrich	S6256
Difloxacin	Abbott Labs5	Not applicable	Sulfamethizole	Sigma Aldrich	S5632
Dipyrone	Sigma Aldrich	46232-250MG	Sulfamethoxazole	Sigma Aldrich	S7507
Doxycycline	Sigma Aldrich	D9891-1G	Sulfamethoxypyridazine	Sigma Aldrich	S7257
Emamectin Benzoate	Sigma Aldrich	31733-250 MG	Sulfanitran	Sigma Aldrich	46882
Enrofloxacin	Bayer Healthcare2	Not applicable	Sulfapyridine	Sigma Aldrich	S6252
Eprinomectin	Sigma Aldrich	32526-100MG	Sulfaquinoxaline	Sigma Aldrich	45662
Erythromycin A	Sigma Aldrich	E0774	Sulfathiazole	Sigma Aldrich	S9876
Fenbendazole	Sigma Aldrich	F5396-5G	Tetracycline	US Pharmacopeia	1651009
Fenbendazole sulphone	Sigma Aldrich	32544-10MG	Thiabendazole	Sigma Aldrich	T8904-100G
Florfenicol	Sigma Aldrich	F1427	Tildipirosin	Lonza	RS-STD0512
Florfenicol Amine	Sigma Aldrich	32492	Tilmicosin	Lilly4	Not applicable

Standard Analyte	Manufacturer	Catalog Number	Standard Analyte	Manufacturer	Catalog Number
Flubendazole	Sigma Aldrich	34091-100MG	Tolfenamic Acid	Sigma Aldrich	T0535-5G
Flunixin	US Pharmacopeia	1274607	Tulathromycin A	Pfizer1	CP-472,295
Gamithromycin	Hovione Farma3	Not applicable	Tylosin	Sigma Aldrich	T6134
Haloperidol	Sigma Aldrich	H1512-5G	Tylvalosin	ECO Animal Health	N/A
Ketamine	Sigma Aldrich	K2753-1G	Virginiamycin	Sigma Aldrich	V2753-10MG
Ketoprofen	Sigma Aldrich	K1751-1G	Xylazine	Sigma Aldrich	X1126-5G
Levamisole	Sigma Aldrich	31742-250MG	Zeranol (B-Zearalanol)	Sigma Aldrich	Z0417
Lincomycin	Sigma Aldrich	L6004	Zilpaterol	Sigma Aldrich	32379
Melengestrol Acetate	MP Biochemicals	158952	13C6 Sulfamethazine Phenyl	Sigma Aldrich6	32519 (Vetranal)
Meloxicam	Sigma Aldrich	M3935-100MG	Flunixin d3	Sigma Aldrich6	34083 (Vetranal)
Morantel tartrate	Sigma Aldrich	M5529-25G	d7 Penicillin G	Toronto Research6	B288600

[1] - Pfizer, Groton, CT

[2] - Bayer Healthcare, AG Business Group Pharma, PH-GDD-PT, Clinical Supplies Ops, Wuppertal, Germany.

[3] - Hovione FarmaCiencia SA, Sete Casa, Loures, Portugal

[4] - Lilly Corporate Center, Indianapolis, Indiana

[5] - Abbott Labs, Chicago, IL

[6]- Internal Standard (IS) – optional

[7]- Lonza, Schwabenheim, Germany

[8]- ECO Animal Health, London, England

2. Preparation of Standard Solution(s)

   a. Animal Drug Stock Solutions and internal standard stock solutions

      Prepare animal drug stock solutions and internal standard stock solutions at approximately 1.0 mg/mL when adequate material is available. Other concentrations are used based on two criteria:

      i. Solubility of the drug in the solvent

      ii. Cost and availability of the drug

      For each stock solution, calculate the amount of base material needed (ex. accounting for purity and/or water and salt content) to prepare at the concentration listed below using the appropriate solvent listed.

Note: When DMSO is referenced in solvent used, dissolve weighed standard with appropriate volume of DMSO and dilute to volume with appropriate solvent.

Table 2 – Stock standard concentrations

Standard Analyte	Category	Solvent used	Stock Standard Concentration (ng/µL)
2-amino-Flubendazole	Acetonitrile Mix	12.5% DMSO in Acetonitrile	1000
2-Aminosulfone Albendazole	Acetonitrile Mix	12.5% DMSO in Methanol	1000
Acepromazine	Acetonitrile Mix	Acetonitrile	1000
Albendazole	Acetonitrile Mix	12.5% DMSO in Acetonitrile	1000
Amoxicillin	Beta Lactam Mix	Water	350
Ampicillin	Beta Lactam Mix	Water	250
Azaperone	Acetonitrile Mix	Methanol	1000
Butorphanol	Acetonitrile Mix	Methanol	1000
Carazolol	Acetonitrile Mix	Acetonitrile	1000
Cefazolin	Beta Lactam Mix	Water	400
Chlortetracycline	Acetonitrile Mix	Methanol	500
Cimaterol	Acetonitrile Mix	Acetonitrile	1000
Ciprofloxacin	Acetonitrile Mix	0.03 M NaOH	1000
Clenbuterol	Acetonitrile Mix	Acetonitrile	1000
Clindamycin	Acetonitrile Mix	Acetonitrile	1000
Cloxacillin	Beta Lactam Mix	Water	200
Danofloxacin	Acetonitrile Mix	0.03 M NaOH	1000
DCCD	Beta Lactam Mix	Water	300
Desacetyl Cephapirin	Acetonitrile Mix	Water	250
Desethylene Ciprofloxacin	Acetonitrile Mix	0.03 M NaOH	300
Diclofenac	Acetonitrile Mix	6% DMSO in Acetonitrile	1000
Dicloxacillin	Beta Lactam Mix	Water	200
Difloxacin	Acetonitrile Mix	50%ACN/MeOH	500
Dipyrone	Acetonitrile Mix	Methanol	1000
Doxycycline	Acetonitrile Mix	Methanol	1000
Emamectin Benzoate	Acetonitrile Mix	Acetonitrile	1000
Enrofloxacin	Acetonitrile Mix	Acetonitrile	500
Eprinomectin	Acetonitrile Mix	Acetonitrile	1000
Erythromycin A	Acetonitrile Mix	Acetonitrile	1000
Fenbendazole	Acetonitrile Mix	12.5% DMSO in Methanol	1000
Fenbendazole sulphone	Acetonitrile Mix	12.5% DMSO in Acetonitrile	1000
Florfenicol	Acetonitrile Mix	Acetonitrile	1000
Florfenicol Amine	Acetonitrile Mix	Acetonitrile	1000
Flubendazole	Acetonitrile Mix	12.5% DMSO in Acetonitrile	1000
Flunixin	Acetonitrile Mix	Methanol	1000
Gamithromycin	Acetonitrile Mix	Acetonitrile	500
Haloperidol	Acetonitrile Mix	Methanol	1000
Ketamine	Acetonitrile Mix	Purchased	1000
Ketoprofen	Acetonitrile Mix	Acetonitrile	1000
Levamisole	Acetonitrile Mix	12.5% DMSO in	1000

Standard Analyte	Category	Solvent used	Stock Standard Concentration (ng/µL)
		Methanol	
Lincomycin	Acetonitrile Mix	50%ACN/MeOH	500
Melengestrol Acetate	Acetonitrile Mix	Acetonitrile	1000
Meloxicam	Acetonitrile Mix	Acetonitrile	1000
Morantel tartrate	Acetonitrile Mix	Milli-Q Water	1000
Nafcillin	Beta Lactam Mix	Water	300
Norfloxacin	Acetonitrile Mix	Acetonitrile	1000
Orbifloxacin	Acetonitrile Mix	Methanol	500
Oxacillin	Beta Lactam Mix	Water	200
Oxyphenylbutazone	Acetonitrile Mix	Acetonitrile	1000
Oxytetracycline	Acetonitrile Mix	Methanol	1000
Penicillin G	Beta Lactam Mix	Water	250
Phenylbutazone	Acetonitrile Mix	Acetonitrile	1000
Pirlimycin	Acetonitrile Mix	50%ACN/MeOH	1000
Prednisone	Acetonitrile Mix	Methanol	1000
Promethazine	Acetonitrile Mix	Acetonitrile	1000
Propionylpromazine	Acetonitrile Mix	Acetonitrile	1000
Ractopamine	Acetonitrile Mix	Water	1000
Salbutamol	Acetonitrile Mix	Acetonitrile	1000
Sarafloxacin	Acetonitrile Mix	Methanol	1000
Sulfachloropyridazine	Acetonitrile Mix	Acetonitrile	1000
Sulfadiazine	Acetonitrile Mix	Acetonitrile	1000
Sulfadimethoxine	Acetonitrile Mix	Acetonitrile	1000
Sulfadoxine	Acetonitrile Mix	Acetonitrile	1000
Sulfaethoxypyridazine	Acetonitrile Mix	Acetonitrile	1000
Sulfamerazine	Acetonitrile Mix	Acetonitrile	1000
Sulfamethazine	Acetonitrile Mix	Acetonitrile	1000
Sulfamethizole	Acetonitrile Mix	Acetonitrile	1000
Sulfamethoxazole	Acetonitrile Mix	Acetonitrile	1000
Sulfamethoxypyridazine	Acetonitrile Mix	Acetonitrile	1000
Sulfanitran	Acetonitrile Mix	Acetonitrile	500
Sulfapyridine	Acetonitrile Mix	Acetonitrile	1000
Sulfaquinoxaline	Acetonitrile Mix	Acetonitrile	500
Sulfathiazole	Acetonitrile Mix	Acetonitrile	1000
Tetracycline	Acetonitrile Mix	Methanol	500
Thiabendazole	Acetonitrile Mix	Methanol	1000
Tildipirosin	Acetonitrile Mix	Methanol	1000
Tilmicosin	Acetonitrile Mix	Acetonitrile	1000
Tolfenamic Acid	Acetonitrile Mix	Acetonitrile	1000
Tulathromycin A	Acetonitrile Mix	Acetonitrile	1000
Tylosin	Acetonitrile Mix	Acetonitrile	1000
Tylvalosin	Acetonitrile Mix	Methanol	1000
Virginiamycin	Acetonitrile Mix	Methanol	250
Xylazine	Acetonitrile Mix	Acetonitrile	1000
Zeranol (B-Zearalanol)	Acetonitrile Mix	Methanol	1000
Zilpaterol	Acetonitrile Mix	Water	1000
13C6 Sulfamethazine Phenyl	Internal Standard Mix	Acetonitrile	1000
Flunixin d3	Internal Standard Mix	Methanol	1000
Benzyl Penicillin d7	Internal Standard Mix	Water	1000

"Acetonitrile Mix" stock standards will expire in 6 months when stored at ≤ -10 ºC or at the time of the earliest expiring component. Stock standards containing Oxyphenylbutazone and Phenylbutazone will expire in two months. The Tilmicosin standard will be stable for three months when stored at < -10 ºC. "Beta Lactam Mix" stock standards will expire in 2 months when stored at ≤ -10 ºC or at the time of the earliest expiring component. "Internal Standard Mix" stock standards will expire by 6 months for Flunixin-d3,13C6 Sulfamethazine Phenyl, and Benzyl Penicillin-d7 when stored at ≤ -10 ºC.

Note: Internal standards are optional in this method and can be used to monitor injection sequence performance within a set. For issues observed, such as inconsistent internal standard area counts, samples may be reinjected or reanalyzed as needed. If internal standards are not used, appropriate volumes and chemicals must be adjusted as outlined in appropriate method steps below.

b. Intermediate standard solutions

Prepare individual intermediate standard solutions as described for the analytes below and dilute to a volume of 4 mL. Intermediate stock standards will expire in 6 months when stored at ≤ -10 ºC or at the time of the earliest expiring component. All standards will use acetonitrile as the diluent.

Table 3 – Intermediate standard solutions

Standard Analyte	Category	Solvent used	Intermediate Standard Concentration (ng/µL)
Azaperone	Acetonitrile Mix	Methanol	100
Butorphanol	Acetonitrile Mix	Methanol	100
Carazolol	Acetonitrile Mix	Acetonitrile	100
Cimaterol	Acetonitrile Mix	Acetonitrile	50
Flunixin	Acetonitrile Mix	Methanol	100
Haloperidol	Acetonitrile Mix	Methanol	100
Melengestrol Acetate	Acetonitrile Mix	Acetonitrile	50
Promethazine	Acetonitrile Mix	Acetonitrile	100
Propionylpromazine	Acetonitrile Mix	Acetonitrile	100
Ractopamine	Acetonitrile Mix	Water	50
Salbutamol	Acetonitrile Mix	Acetonitrile	50
Xylazine	Acetonitrile Mix	Acetonitrile	100
Zeranol (B-Zearalanol)	Acetonitrile Mix	Methanol	50
Zilpaterol	Acetonitrile Mix	Water	100

c. Antibiotic drug composite working (spiking) and internal standard (spiking) solutions

i. Prepare the composite “Acetonitrile Mix” working solution(s) for the veterinary drugs contained in the acetonitrile spiking solutions using the stock and intermediate standard solutions above and the volumes listed in the tables below.

(a) Calculate or use the volume of stock or intermediate stock solution required to give the concentration listed for each standard type in the table below.

(b) Pipet the calculated volume of stock into a 10 mL volumetric flask.

(c) Dilute to 10 mL volume with acetonitrile.

(d) Cap flask and mix.

(e) Transfer solution into amber glass LC vials with screw cap lids.

(f) Composite working solutions will expire based on the earliest expiration date of an individual component.

Table 4A – “Acetonitrile Mix” kidney working solution preparation, for screening

Standard Analyte	Stock or Intermediate Standard Concentration (ng/µL)	Bovine Kidney Screening Volume (µL)	Porcine Kidney Screening Volume (µL)
2-amino-Flubendazole	1000	5	5
2-Aminosulfone Albendazole	1000	12.5	12.5
Acepromazine	1000	2	2
Albendazole	1000	12.5	12.5
Azaperone	100		5
Butorphanol	100	5	5
Carazolol	100	5	5
Chlortetracycline	500	1000	1000
Cimaterol	50	60	120
Ciprofloxacin	1000	12.5	12.5
Clenbuterol	1000	3	1.5
Clindamycin	1000	25	25
Danofloxacin	1000	50.0	12.5
Desacetyl Cephapirin	250	100	100
Desethylene Ciprofloxacin	300		
Diclofenac	1000	2.5	2.5
Difloxacin	500	50	100
Dipyrone	1000		12.5
Doxycycline	1000	12.5	12.5
Emamectin Benzoate	1000	3.75	3.75
Enrofloxacin	500	25	25
Eprinomectin	1000	15	15
Erythromycin A	1000	25	25
Fenbendazole	1000	100	100
Fenbendazole sulphone	1000	100	100
Florfenicol	1000	50	50
Florfenicol Amine	1000	75	75
Flubendazole	1000	5	5
Flunixin	100	62.5	62.5

Standard Analyte	Stock or Intermediate Standard Concentration (ng/µL)	Bovine Kidney Screening Volume (µL)	Porcine Kidney Screening Volume (µL)
Gamithromycin	500	50	50
Haloperidol	100	5	5
Ketamine	1000	20	10
Ketoprofen	1000	2.5	2.5
Levamisole	1000	25	25
Lincomycin	500	50	50
Melengestrol Acetate	50	200	200
Meloxicam	1000	5	5
Morantel tartrate	1000	175.0	175.0
Norfloxacin	1000	12.5	12.5
Orbifloxacin	500	50	25
Oxyphenylbutazone	1000	25	25
Oxytetracycline	1000	250	250
Phenylbutazone	1000	25	25
Pirlimycin	1000	125	125
Prednisone	1000	25	25
Promethazine	100		20
Propionylpromazine	100		5
Ractopamine	50		
Salbutamol	50	30	30
Sarafloxacin	1000		12.5
Sulfachloropyridazine	1000	25	25
Sulfadiazine	1000	25	25
Sulfadimethoxine	1000	25	25
Sulfadoxine	1000	25	25
Sulfaethoxypyridazine	1000	25	25
Sulfamerazine	1000	25	25
Sulfamethazine	1000	25	25
Sulfamethizole	1000	25	25
Sulfamethoxazole	1000	25	25
Sulfamethoxypyridazine	1000	50	25
Sulfanitran	500	50	50
Sulfapyridine	1000	25	25
Sulfaquinoxaline	500	50	50
Sulfathiazole	1000	25	25
Tetracycline	500	1000	1000
Thiabendazole	1000	50	25
Tildipirosin	1000	500	250
Tilmicosin	1000	30	30
Tolfenamic Acid	1000	12.5	12.5
Tulathromycin A	1000	2000	500
Tylosin	1000	50	
Tylvalosin	1000	12.5	12.5
Virginiamycin	250	100	100
Xylazine	100	5	10
Zeranol (B-Zearalanol)	50		
Zilpaterol	100	15	15

Table 4B – "Acetonitrile Mix" kidney working solution preparation, for confirmation

Standard Analyte	Stock or Intermediate Standard Concentration (ng/µL)	Bovine Kidney Confirmation Volume (µL)	Porcine Kidney Confirmation Level (µL)
2-amino-Flubendazole	1000	5	5
2-Aminosulfone Albendazole	1000	12.5	12.5
Acepromazine	1000	2	2
Albendazole	1000	12.5	12.5
Azaperone	100		5
Butorphanol	100	5	5
Carazolol	100	5	20
Chlortetracycline	500	1000	1000
Cimaterol	50		
Ciprofloxacin	1000	12.5	12.5
Clenbuterol	1000	3	1.5
Clindamycin	1000	25	25
Danofloxacin	1000	50.0	12.5
Desacetyl Cephapirin	250	100	100
Desethylene Ciprofloxacin	300		
Diclofenac	1000	10	
Difloxacin	500	50	100
Dipyrone	1000		
Doxycycline	1000	12.5	12.5
Emamectin Benzoate	1000	3.75	3.75
Enrofloxacin	500	25	25
Eprinomectin	1000		
Erythromycin A	1000	25	25
Fenbendazole	1000	100	100
Fenbendazole sulphone	1000	100	100
Florfenicol	1000	50	50
Florfenicol Amine	1000	75	75
Flubendazole	1000	5	5
Flunixin	100	62.5	62.5
Gamithromycin	500	50	50
Haloperidol	100	5	5
Ketamine	1000	20	10
Ketoprofen	1000	2.5	5
Levamisole	1000	25	25
Lincomycin	500	50	50
Melengestrol Acetate	50	200	400
Meloxicam	1000	5	5
Morantel tartrate	1000	175	175
Norfloxacin	1000	12.5	12.5
Orbifloxacin	500	50	25
Oxyphenylbutazone	1000	25	25
Oxytetracycline	1000	250	250
Phenylbutazone	1000	25	25
Pirlimycin	1000	125	125
Prednisone	1000	25	25
Promethazine	100		20
Propionylpromazine	100		5
Ractopamine	50		

Standard Analyte	Stock or Intermediate Standard Concentration (ng/µL)	Bovine Kidney Confirmation Volume (µL)	Porcine Kidney Confirmation Level (µL)
Salbutamol	50	30	30
Sarafloxacin	1000		12.5
Sulfachloropyridazine	1000	25	25
Sulfadiazine	1000	25	25
Sulfadimethoxine	1000	25	25
Sulfadoxine	1000	25	25
Sulfaethoxypyridazine	1000	25	25
Sulfamerazine	1000	25	25
Sulfamethazine	1000	25	25
Sulfamethizole	1000	25	25
Sulfamethoxazole	1000	25	25
Sulfamethoxypyridazine	1000	50	25
Sulfanitran	500	50	50
Sulfapyridine	1000	25	25
Sulfaquinoxaline	500	50	50
Sulfathiazole	1000	25	25
Tetracycline	500	1000	1000
Thiabendazole	1000		
Tildipirosin	1000	500	250
Tilmicosin	1000	30	30
Tolfenamic Acid	1000	25	
Tulathromycin A	1000	2000	500
Tylosin	1000	50	
Tylvalosin	1000	12.5	12.5
Virginiamycin	250	100	100
Xylazine	100		
Zeranol (B-Zearalanol)	50		
Zilpaterol	100	15	15

Table 5A – “Acetonitrile Mix” muscle working solution preparation, for screening

Standard Analyte	Stock or Intermediate Standard Concentration (ng/µL)	Bovine Muscle Screening Volume (µL)	Porcine Muscle Screening Volume (µL)
2-amino-Flubendazole	1000	5	5
2-Aminosulfone Albendazole	1000	12.5	12.5
Acepromazine	1000	2	
Albendazole	1000	12.5	12.5
Azaperone	100	5	5
Butorphanol	100	5	5
Carazolol	100	5	5
Chlortetracycline	500	1000	1000
Cimaterol	50	60	30
Ciprofloxacin	1000	12.5	12.5
Clenbuterol	1000	1.5	1.5
Clindamycin	1000	25	25
Danofloxacin	1000	12.5	12.5
Desacetyl Cephapirin	250	100	100
Desethylene Ciprofloxacin	300	41.7	41.7
Diclofenac	1000	2.5	2.5

Standard Analyte	Stock or Intermediate Standard Concentration (ng/µL)	Bovine Muscle Screening Volume (µL)	Porcine Muscle Screening Volume (µL)
Difloxacin	500	25	25
Dipyrone	1000	50	50
Doxycycline	1000	12.5	12.5
Emamectin Benzoate	1000		
Enrofloxacin	500	25	25
Eprinomectin	1000		7.5
Erythromycin A	1000	25	25
Fenbendazole	1000	100	100
Fenbendazole sulphone	1000	100	100
Florfenicol	1000	50	50
Florfenicol Amine	1000	75	75
Flubendazole	1000	5	5
Flunixin	100	62.5	62.5
Gamithromycin	500	50	50
Haloperidol	100	5	5
Ketamine	1000	10	10
Ketoprofen	1000	2.5	2.5
Levamisole	1000	25	25
Lincomycin	500	50	50
Melengestrol Acetate	50		200
Meloxicam	1000	5	5
Morantel tartrate	1000	175.0	175.0
Norfloxacin	1000	12.5	12.5
Orbifloxacin	500	25	25
Oxyphenylbutazone	1000		100
Oxytetracycline	1000	250	250
Phenylbutazone	1000		
Pirlimycin	1000	125	125
Prednisone	1000	25	25
Promethazine	100		
Propionylpromazine	100		
Ractopamine	50		60
Salbutamol	50	30	30
Sarafloxacin	1000	12.5	12.5
Sulfachloropyridazine	1000	25	25
Sulfadiazine	1000	25	25
Sulfadimethoxine	1000	25	25
Sulfadoxine	1000	25	25
Sulfaethoxypyridazine	1000	25	25
Sulfamerazine	1000	25	25
Sulfamethazine	1000	25	25
Sulfamethizole	1000	25	25
Sulfamethoxazole	1000	25	25
Sulfamethoxypyridazine	1000	25	25
Sulfanitran	500	50	50
Sulfapyridine	1000	25	25
Sulfaquinoxaline	500	50	50
Sulfathiazole	1000	25	25
Tetracycline	500	500	500

Standard Analyte	Stock or Intermediate Standard Concentration (ng/µL)	Bovine Muscle Screening Volume (µL)	Porcine Muscle Screening Volume (µL)
Thiabendazole	1000	25	50
Tildipirosin	1000	250	250
Tilmicosin	1000	30	30
Tolfenamic Acid	1000		12.5
Tulathromycin A	1000	500	500
Tylosin	1000	50	50
Tylvalosin	1000		12.5
Virginiamycin	250	100	100
Xylazine	100	5	5
Zeranol (B-Zearalanol)	50	240	
Zilpaterol	100	15	15

Table 5B – “Acetonitrile Mix” muscle working solution preparation, for confirmation

Standard Analyte	Stock or Intermediate Standard Concentration (ng/µL)	Bovine Muscle Confirmation Volume (µL)	Porcine Muscle Confirmation Level (µL)
2-amino-Flubendazole	1000	5	5
2-Aminosulfone Albendazole	1000	12.5	12.5
Acepromazine	1000	2	
Albendazole	1000	12.5	12.5
Azaperone	100	5	5
Butorphanol	100	5	5
Carazolol	100	5	10
Chlortetracycline	500	1000	1000
Cimaterol	50	60	
Ciprofloxacin	1000	12.5	12.5
Clenbuterol	1000	1.5	1.5
Clindamycin	1000	25	25
Danofloxacin	1000	12.5	12.5
Desacetyl Cephapirin	250	100	100
Desethylene Ciprofloxacin	300	83.3	166.7
Diclofenac	1000	5	
Difloxacin	500	25	25
Dipyrone	1000		
Doxycycline	1000	12.5	12.5
Emamectin Benzoate	1000		
Enrofloxacin	500	25	25
Eprinomectin	1000		
Erythromycin A	1000	25	25
Fenbendazole	1000	100	100
Fenbendazole sulphone	1000	100	100
Florfenicol	1000	50	50
Florfenicol Amine	1000	75	75
Flubendazole	1000	5	5
Flunixin	100	62.5	62.5
Gamithromycin	500	50	50
Haloperidol	100	5	5
Ketamine	1000	10	10
Ketoprofen	1000	2.5	2.5

Standard Analyte	Stock or Intermediate Standard Concentration (ng/µL)	Bovine Muscle Confirmation Volume (µL)	Porcine Muscle Confirmation Level (µL)
Levamisole	1000	50	100
Lincomycin	500	50	50
Melengestrol Acetate	50		400
Meloxicam	1000	5	5
Morantel tartrate	1000	175.0	175.0
Norfloxacin	1000	12.5	12.5
Orbifloxacin	500	25	25
Oxyphenylbutazone	1000		100
Oxytetracycline	1000	250	250
Phenylbutazone	1000		
Pirlimycin	1000	125	125
Prednisone	1000	25	25
Promethazine	100		
Propionylpromazine	100		
Ractopamine	50		60
Salbutamol	50	30	30
Sarafloxacin	1000	12.5	12.5
Sulfachloropyridazine	1000	25	25
Sulfadiazine	1000	25	25
Sulfadimethoxine	1000	25	25
Sulfadoxine	1000	25	25
Sulfaethoxypyridazine	1000	25	25
Sulfamerazine	1000	25	25
Sulfamethazine	1000	25	25
Sulfamethizole	1000	25	25
Sulfamethoxazole	1000	25	25
Sulfamethoxypyridazine	1000	25	25
Sulfanitran	500	50	50
Sulfapyridine	1000	25	25
Sulfaquinoxaline	500	50	50
Sulfathiazole	1000	25	25
Tetracycline	500	500	500
Thiabendazole	1000		
Tildipirosin	1000	250	250
Tilmicosin	1000	30	30
Tolfenamic Acid	1000		50
Tulathromycin A	1000	500	500
Tylosin	1000	50	50
Tylvalosin	1000		12.5
Virginiamycin	250	100	100
Xylazine	100		
Zeranol (B-Zearalanol)	50	240	
Zilpaterol	100	15	15

Using these volumes yield the following concentrations as found in the tables below:

Table 6A – "Acetonitrile Mix" kidney working standard concentrations, for screening

Standard Analyte	Bovine Kidney Screening Level (ng/µL)	Porcine Kidney Screening Level (ng/µL)
2-amino-Flubendazole	0.5	0.5
2-Aminosulfone Albendazole	1.25	1.25
Acepromazine	0.2	0.2
Albendazole	1.25	1.25
Azaperone		0.05
Butorphanol	0.05	0.05
Carazolol	0.05	0.05
Chlortetracycline	50	50
Cimaterol	0.3	0.6
Ciprofloxacin	1.25	1.25
Clenbuterol	0.3	0.15
Clindamycin	2.5	2.5
Danofloxacin	5	1.25
Desacetyl Cephapirin	2.5	2.5
Desethylene Ciprofloxacin		
Diclofenac	0.25	0.25
Difloxacin	2.5	5
Dipyrone		1.25
Doxycycline	1.25	1.25
Emamectin Benzoate	0.375	0.375
Enrofloxacin	1.25	1.25
Eprinomectin	1.5	1.5
Erythromycin A	2.5	2.5
Fenbendazole	10	10
Fenbendazole sulphone	10	10
Florfenicol	5	5
Florfenicol Amine	7.5	7.5
Flubendazole	0.5	0.5
Flunixin	0.625	0.625
Gamithromycin	2.5	2.5
Haloperidol	0.05	0.05
Ketamine	2	1
Ketoprofen	0.25	0.25
Levamisole	2.5	2.5
Lincomycin	2.5	2.5
Melengestrol Acetate	1	1
Meloxicam	0.5	0.5
Morantel tartrate	17.5	17.5
Norfloxacin	1.25	1.25
Orbifloxacin	2.5	1.25
Oxyphenylbutazone	2.5	2.5
Oxytetracycline	25	25
Phenylbutazone	2.5	2.5
Pirlimycin	12.5	12.5
Prednisone	2.5	2.5
Promethazine		0.2

Standard Analyte	Bovine Kidney Screening Level (ng/µL)	Porcine Kidney Screening Level (ng/µL)
Propionylpromazine		0.05
Ractopamine		
Salbutamol	0.15	0.15
Sarafloxacin		1.25
Sulfachloropyridazine	2.5	2.5
Sulfadiazine	2.5	2.5
Sulfadimethoxine	2.5	2.5
Sulfadoxine	2.5	2.5
Sulfaethoxypyridazine	2.5	2.5
Sulfamerazine	2.5	2.5
Sulfamethazine	2.5	2.5
Sulfamethizole	2.5	2.5
Sulfamethoxazole	2.5	2.5
Sulfamethoxypyridazine	5	2.5
Sulfanitran	2.5	2.5
Sulfapyridine	2.5	2.5
Sulfaquinoxaline	2.5	2.5
Sulfathiazole	2.5	2.5
Tetracycline	50	50
Thiabendazole	5	2.5
Tildipirosin	50	25
Tilmicosin	3	3
Tolfenamic Acid	1.25	1.25
Tulathromycin A	200	50
Tylosin	5	
Tylvalosin	1.25	1.25
Virginiamycin	2.5	2.5
Xylazine	0.05	0.1
Zeranol (B-Zearalanol)		
Zilpaterol	0.15	0.15

Table 6B – “Acetonitrile Mix” kidney working standard concentrations, for confirmation

Standard Analyte	Bovine Kidney Confirmation Level (ng/µL)	Porcine Kidney Confirmation Level (ng/µL)
2-amino-Flubendazole	0.5	0.5
2-Aminosulfone Albendazole	1.25	1.25
Acepromazine	0.2	0.2
Albendazole	1.25	1.25
Azaperone		0.05
Butorphanol	0.05	0.05
Carazolol	0.05	0.2
Chlortetracycline	50	50
Cimaterol		
Ciprofloxacin	1.25	1.25
Clenbuterol	0.3	0.15
Clindamycin	2.5	2.5
Danofloxacin	5	1.25
Desacetyl Cephapirin	2.5	2.5

Standard Analyte	Bovine Kidney Confirmation Level (ng/µL)	Porcine Kidney Confirmation Level (ng/µL)
Desethylene Ciprofloxacin		
Diclofenac	1	
Difloxacin	2.5	5
Dipyrone		
Doxycycline	1.25	1.25
Emamectin Benzoate	0.375	0.375
Enrofloxacin	1.25	1.25
Eprinomectin		
Erythromycin A	2.5	2.5
Fenbendazole	10	10
Fenbendazole sulphone	10	10
Florfenicol	5	5
Florfenicol Amine	7.5	7.5
Flubendazole	0.5	0.5
Flunixin	0.625	0.625
Gamithromycin	2.5	2.5
Haloperidol	0.05	0.05
Ketamine	2	1
Ketoprofen	0.25	0.5
Levamisole	2.5	2.5
Lincomycin	2.5	2.5
Melengestrol Acetate	1	2
Meloxicam	0.5	0.5
Morantel tartrate	17.5	17.5
Norfloxacin	1.25	1.25
Orbifloxacin	2.5	1.25
Oxyphenylbutazone	2.5	2.5
Oxytetracycline	25	25
Phenylbutazone	2.5	2.5
Pirlimycin	12.5	12.5
Prednisone	2.5	2.5
Promethazine		0.2
Propionylpromazine		0.05
Ractopamine		
Salbutamol	0.15	0.15
Sarafloxacin		1.25
Sulfachloropyridazine	2.5	2.5
Sulfadiazine	2.5	2.5
Sulfadimethoxine	2.5	2.5
Sulfadoxine	2.5	2.5
Sulfaethoxypyridazine	2.5	2.5
Sulfamerazine	2.5	2.5
Sulfamethazine	2.5	2.5
Sulfamethizole	2.5	2.5
Sulfamethoxazole	2.5	2.5
Sulfamethoxypyridazine	5	2.5
Sulfanitran	2.5	2.5
Sulfapyridine	2.5	2.5
Sulfaquinoxaline	2.5	2.5

Standard Analyte	Bovine Kidney Confirmation Level (ng/µL)	Porcine Kidney Confirmation Level (ng/µL)
Sulfathiazole	2.5	2.5
Tetracycline	50	50
Thiabendazole		
Tildipirosin	50	25
Tilmicosin	3	3
Tolfenamic Acid	2.5	
Tulathromycin A	200	50
Tylosin	5	
Tylvalosin	1.25	1.25
Virginiamycin	2.5	2.5
Xylazine		
Zeranol (B-Zearalanol)		
Zilpaterol	0.15	0.15

Table 7A – “Acetonitrile Mix” muscle working standard concentrations, for screening

Standard Analyte	Bovine Muscle Screening Level (ng/µL)	Porcine Muscle Screening Level (ng/µL)
2-amino-Flubendazole	0.5	0.5
2-Aminosulfone Albendazole	1.25	1.25
Acepromazine	0.2	
Albendazole	1.25	1.25
Azaperone	0.05	0.05
Butorphanol	0.05	0.05
Carazolol	0.05	0.05
Chlortetracycline	50	50
Cimaterol	0.3	0.15
Ciprofloxacin	1.25	1.25
Clenbuterol	0.15	0.15
Clindamycin	2.5	2.5
Danofloxacin	1.25	1.25
Desacetyl Cephapirin	2.5	2.5
Desethylene Ciprofloxacin	1.25	1.25
Diclofenac	0.25	0.25
Difloxacin	1.25	1.25
Dipyrone	5	5
Doxycycline	1.25	1.25
Emamectin Benzoate		
Enrofloxacin	1.25	1.25
Eprinomectin		0.75
Erythromycin A	2.5	2.5
Fenbendazole	10	10
Fenbendazole sulphone	10	10
Florfenicol	5	5
Florfenicol Amine	7.5	7.5
Flubendazole	0.5	0.5
Flunixin	0.625	0.625
Gamithromycin	2.5	2.5
Haloperidol	0.05	0.05

Standard Analyte	Bovine Muscle Screening Level (ng/µL)	Porcine Muscle Screening Level (ng/µL)
Ketamine	1	1
Ketoprofen	0.25	0.25
Levamisole	2.5	2.5
Lincomycin	2.5	2.5
Melengestrol Acetate		1
Meloxicam	0.5	0.5
Morantel tartrate	17.5	17.5
Norfloxacin	1.25	1.25
Orbifloxacin	1.25	1.25
Oxyphenylbutazone		10
Oxytetracycline	25	25
Phenylbutazone		
Pirlimycin	12.5	12.5
Prednisone	2.5	2.5
Promethazine		
Propionylpromazine		
Ractopamine		0.3
Salbutamol	0.15	0.15
Sarafloxacin	1.25	1.25
Sulfachloropyridazine	2.5	2.5
Sulfadiazine	2.5	2.5
Sulfadimethoxine	2.5	2.5
Sulfadoxine	2.5	2.5
Sulfaethoxypyridazine	2.5	2.5
Sulfamerazine	2.5	2.5
Sulfamethazine	2.5	2.5
Sulfamethizole	2.5	2.5
Sulfamethoxazole	2.5	2.5
Sulfamethoxypyridazine	2.5	2.5
Sulfanitran	2.5	2.5
Sulfapyridine	2.5	2.5
Sulfaquinoxaline	2.5	2.5
Sulfathiazole	2.5	2.5
Tetracycline	25	25
Thiabendazole	2.5	5
Tildipirosin	25	25
Tilmicosin	3	3
Tolfenamic Acid		1.25
Tulathromycin A	50	50
Tylosin	5	5
Tylvalosin		1.25
Virginiamycin	2.5	2.5
Xylazine	0.05	0.05
Zeranol (B-Zearalanol)	1.2	
Zilpaterol	0.15	0.15

Table 7B – "Acetonitrile Mix" muscle working standard concentrations, for confirmation

Standard Analyte	Bovine Muscle Confirmation Level (ng/µL)	Porcine Muscle Confirmation Level (ng/µL)
2-amino-Flubendazole	0.5	0.5
2-Aminosulfone Albendazole	1.25	1.25
Acepromazine	0.2	
Albendazole	1.25	1.25
Azaperone	0.05	0.05
Butorphanol	0.05	0.05
Carazolol	0.05	0.1
Chlortetracycline	50	50
Cimaterol	0.3	
Ciprofloxacin	1.25	1.25
Clenbuterol	0.15	0.15
Clindamycin	2.5	2.5
Danofloxacin	1.25	1.25
Desacetyl Cephapirin	2.5	2.5
Desethylene Ciprofloxacin	2.5	5
Diclofenac	0.5	
Difloxacin	1.25	1.25
Dipyrone		
Doxycycline	1.25	1.25
Emamectin Benzoate		
Enrofloxacin	1.25	1.25
Eprinomectin		
Erythromycin A	2.5	2.5
Fenbendazole	10	10
Fenbendazole sulphone	10	10
Florfenicol	5	5
Florfenicol Amine	7.5	7.5
Flubendazole	0.5	0.5
Flunixin	0.625	0.625
Gamithromycin	2.5	2.5
Haloperidol	0.05	0.05
Ketamine	1	1
Ketoprofen	0.25	0.25
Levamisole	5	10
Lincomycin	2.5	2.5
Melengestrol Acetate		2
Meloxicam	0.5	0.5
Morantel tartrate	17.5	17.5
Norfloxacin	1.25	1.25
Orbifloxacin	1.25	1.25
Oxyphenylbutazone		10
Oxytetracycline	25	25
Phenylbutazone		
Pirlimycin	12.5	12.5
Prednisone	2.5	2.5
Promethazine		
Propionylpromazine		
Ractopamine		0.3

Standard Analyte	Bovine Muscle Confirmation Level (ng/µL)	Porcine Muscle Confirmation Level (ng/µL)
Salbutamol	0.15	0.15
Sarafloxacin	1.25	1.25
Sulfachloropyridazine	2.5	2.5
Sulfadiazine	2.5	2.5
Sulfadimethoxine	2.5	2.5
Sulfadoxine	2.5	2.5
Sulfaethoxypyridazine	2.5	2.5
Sulfamerazine	2.5	2.5
Sulfamethazine	2.5	2.5
Sulfamethizole	2.5	2.5
Sulfamethoxazole	2.5	2.5
Sulfamethoxypyridazine	2.5	2.5
Sulfanitran	2.5	2.5
Sulfapyridine	2.5	2.5
Sulfaquinoxaline	2.5	2.5
Sulfathiazole	2.5	2.5
Tetracycline	25	25
Thiabendazole		
Tildipirosin	25	25
Tilmicosin	3	3
Tolfenamic Acid		5
Tulathromycin A	50	50
Tylosin	5	5
Tylvalosin		1.25
Virginiamycin	2.5	2.5
Xylazine		
Zeranol (B-Zearalanol)	1.2	
Zilpaterol	0.15	0.15

ii. Prepare the composite working solution(s) for the veterinary drugs contained in the “Beta Lactam mix” spiking solutions using the stock standard solutions above and the volumes listed in the table below.

(a) Calculate or use the volume of stock solution required to give the concentrations listed for each standard type in the table below.

(b) Pipet the calculated volume of stock into a 10 mL volumeteric flask.

(c) Dilute to 10 mL volume with water.

(d) Cap flask and mix.

(e) Transfer solution into plastic screw cap vials.

(f) Composite working solutions will expire in 2 months when stored at $\leq$ -10 ºC or at the time of the earliest expiring component.

Table 8A – Beta-lactam kidney working solutions preparation volumes, for screening

Standard Analyte	Stock or Intermediate Standard Concentration (ng/µL)	Bovine Kidney Screening Volume (µL)	Porcine Kidney Screening Volume (µL)
Amoxicillin	350	28.6	28.6
Ampicillin	250	10	10
Cefazolin	400	62.5	62.5
Cloxacillin	200	12.5	12.5
DCCD	300	83.3	83.3
Dicloxacillin	200	125	125
Nafcillin	300	83.3	83.3
Oxacillin	200	125	125
Penicillin G	250	50	50

Table 8B – Beta-lactam kidney working solutions preparation volumes, for confirmation

Standard Analyte	Stock or Intermediate Standard Concentration (ng/µL)	Bovine Kidney Confirmation Volume (µL)	Porcine Kidney Confirmation Level (µL)
Amoxicillin	350	28.6	28.6
Ampicillin	250	10	10
Cefazolin	400	62.5	62.5
Cloxacillin	200	12.5	12.5
DCCD	300	83.3	83.3
Dicloxacillin	200	125	125
Nafcillin	300	83.3	83.3
Oxacillin	200	125	125
Penicillin G	250	50	50

Table 9A – Beta-lactam muscle working solutions preparation volumes, for screening

Standard Analyte	Stock or Intermediate Standard Concentration (ng/µL)	Bovine Muscle Screening Volume (µL)	Porcine Muscle Screening Volume (µL)
Amoxicillin	350	28.6	28.6
Ampicillin	250	10	10
Cefazolin	400	62.5	62.5
Cloxacillin	200	12.5	12.5
DCCD	300	83.3	83.3
Dicloxacillin	200	125	125
Nafcillin	300	83.3	83.3
Oxacillin	200	125	125
Penicillin G	250	100	50

Table 9B – Beta-lactam muscle working solutions preparation volumes, for confirmation

Standard Analyte	Stock or Intermediate Standard Concentration (ng/µL)	Bovine Muscle Confirmation Volume (µL)	Porcine Muscle Confirmation Level (µL)
Amoxicillin	350	28.6	28.6
Ampicillin	250	10	10
Cefazolin	400	62.5	62.5
Cloxacillin	200	12.5	12.5
DCCD	300	83.3	83.3
Dicloxacillin	200	125	125
Nafcillin	300	83.3	83.3
Oxacillin	200	125	125
Penicillin G	250	100	50

Using these volumes yield the following concentrations as found in the tables below:

Table 10A – Beta-lactam kidney working solution levels for screening

Standard Analyte	Bovine Kidney Screening Level (ng/µL)	Porcine Kidney Screening Level (ng/µL)
Amoxicillin	1	1
Ampicillin	0.25	0.25
Cefazolin	2.5	2.5
Cloxacillin	0.25	0.25
DCCD	2.5	2.5
Dicloxacillin	2.5	2.5
Nafcillin	2.5	2.5
Oxacillin	2.5	2.5
Penicillin G	1.25	1.25

Table 10B – Beta-lactam kidney working solution levels, for confirmation

Standard Analyte	Bovine Kidney Confirmation Level (ng/µL)	Porcine Kidney Confirmation Level (ng/µL)
Amoxicillin	1	1
Ampicillin	0.25	0.25
Cefazolin	2.5	2.5
Cloxacillin	0.25	0.25
DCCD	2.5	2.5
Dicloxacillin	2.5	2.5
Nafcillin	2.5	2.5
Oxacillin	2.5	2.5
Penicillin G	1.25	1.25

Table 11A – Beta-lactam muscle working solution levels for screening

Standard Analyte	Bovine Muscle Screening Level (ng/µL)	Porcine Muscle Screening Level (ng/µL)
Amoxicillin	1	1
Ampicillin	0.25	0.25
Cefazolin	2.5	2.5
Cloxacillin	0.25	0.25
DCCD	2.5	2.5
Dicloxacillin	2.5	2.5
Nafcillin	2.5	2.5
Oxacillin	2.5	2.5
Penicillin G	2.5	1.25

Table 11B – Beta-lactam muscle working solution levels, for confirmation

Standard Analyte	Bovine Muscle Confirmation Level (ng/µL)	Porcine Muscle Confirmation Level (ng/µL)
Amoxicillin	1	1
Ampicillin	0.25	0.25
Cefazolin	2.5	2.5
Cloxacillin	0.25	0.25
DCCD	2.5	2.5
Dicloxacillin	2.5	2.5
Nafcillin	2.5	2.5
Oxacillin	2.5	2.5
Penicillin G	2.5	1.25

iii. Prepare the composite (spiking) solution for the isotopically-labeled veterinary drugs used for internal standards at 20 ng/uL if internal standards will be used. This solution can contain any number of the internal standards listed in the method.

(a) Calculate the volume of stock solution required to give the 20 ng/uL level (see the following table).

(b) Pipet the calculated volume of stock into a 5 mL volumeteric flask.

(c) Dilute to 5 mL with acetonitrile.

(d) Cap flask and mix.

(e) Transfer 1.25 mL of solution into 4 amber glass LC vials.

(f) Store at < -10 ºC. The stability of the solution is dependent on which internal standards are present. If the solution contains D7 Penicillin G, then the solution is stable for 2 months. Otherwise, the solution is stable for 6 months.

Table 12 – IS spiking solution

Standard Analyte	Stock or Intermediate Standard Concentration (ng/µL)	Volume (µL)	Final Volume (mL)	Working Standard Concentration (ng/µL)
13C6 Sulfamethazine Phenyl	1000	100	5	20
Benzyl Penicillin-d7	1000	100	5	20
Flunixin-d3	1000	100	5	20

3. Preparation of External Calibration Curve (Optional)

Use the following table to prepare external standards.

Table 13 – Preparation of external standards

Target Conc.	Volume Acetonitrile Standard Mix (µL)	Volume Beta Lactam Standard Mix (µL)	Volume Internal Standard Mix (µL)	Volume Acetonitrile (µL)	Volume of 0.1% Formic Acid in water
0 X	0	0	20	120	860
1/2 X	20	20	20	100	840
1 X	40	40	20	80	820
2 X	80	80	20	40	780

Note: The volume of acetonitrile will be increased by 20 µL to maintain a total volume of 1 mL if no internal standard solution is added.

E. SAMPLE PREPARATION

Samples collected fresh must be kept cold before and during shipping to the laboratory. Once received at the laboratory, samples must be frozen (< -10 °C) prior to grinding if they cannot be prepared on the day of receipt. Once frozen, the sample should be allowed to thaw, while keeping it as cold as possible. Dissect away fat and connective tissue. Grind tissue in blender or vertical cutter-mixer until homogeneous. Store samples frozen (< -10 °C) prior to analysis.

Sample preparation may also be done by dry ice grinding as follows:

a. Chop 0.5 - 1 lb of muscle tissue into small pieces and homogenize with an equal amount of dry ice in a large food processor. The resulting sample homogenate will be a frozen powder.

b. Transfer a portion of the homogenized sample into a loosely capped sample cup until the dry ice has sublimed. Excess sample from step E.a. may be discarded.

c. For any retained sample, tighten the caps and store in a freezer at -10 ºC.

F. ANALYTICAL PROCEDURE

1. Preparation of Controls and Samples

a. Weigh 2 ± 0.1 g of homogenized samples into labeled 50 mL polypropylene centrifuge tubes.

Note: Use corresponding blank tissue for controls for each specific species and tissue sample being analyzed.

i. Screening - Weigh one portion each for a blank (negative control), a 1/2 X recovery (positive control), a matrix matched standard, and a check sample, if necessary.

ii. Confirmation - Weigh six 2 g portions of blank tissue into 50 ml polypropylene centrifuge tubes. One for 1/2 X recovery (positive control), one for the blank (negative control) and four for the matrix matched standards (1/2X, 1X, 2X). Weigh one additional portion for a check sample, if necessary.

iii. Prepare recoveries, check samples, blank, matrix matched standard(s), and samples using the solutions and volumes in the table below:

Table 14 – Preparation of controls and samples

Sample Type	Acetonitrile Standard Mix (µL)	Beta Lactam Standard Mix (µL)	Internal Standard Mix (µL)	Acetonitrile (µL)	Water (µL)
Samples and Negative Controls			40	160	160
Matrix Matched Standards				200	160
½ X Recovery	40	40	40	120	120
1X Recovery	80	80	40	80	80
2X Recovery	160	160	40		

Note: If no internal standard mix is used, the volume of acetonitrile added to each tube must be increased by 40 µL.

Note: The “1/2 X Recovery” sample type represents the minimum level of applicability (refer to Section J.3).

2. Extraction Procedure

   a. Vortex all uncapped tubes 10 seconds each to mix chemicals with matrix and allow to stand 5 minutes.

      Note: Press down to start swirling action slowly and then increase speed to mix without splashing.

   b. Add 9.7 mL of 4/1 (v/v) acetonitrile/water to all tubes using a calibrated solvent dispenser. Cap tubes well.

   c. Place the racks of tubes in platform shaker on high for 5 minutes.

   d. Centrifuge the tubes at >3000 rcf for 5 minutes.

   e. Decant each extract into a pre-labeled 50 mL polypropylene centrifuge tubes containing 0.50 g of C18.

   f. Add 10 mL of hexane (saturated with acetonitrile) using a calibrated dispenser to all tubes. Cap all tubes well.

   g. Shake all tubes in the platform shaker on high for 1 minute.

   h. Centrifuge all tubes at approximately 3000 rcf for 5 minutes.

   i. Aspirate hexane to waste using a Pasteur pipette on a hose connected to a side-arm Erlenmeyer flask.

   j. Pipet a 5 mL aliquot of the extract into a pre-labeled 15 mL polypropylene centrifuge tubes.

   k. Evaporate extract to < 0.5 mL in TurboVap at 45 ± 2°C at 15 psi setting, changing to 20 psi after 10 minutes. Total time to evaporate to < 0.5 mL is 45 to 60 minutes.

   l. Per the table below, add the volumes of the standard mixes and solutions to the matrix matched standards, add 140 µL LCMS grade acetonitrile to all other tubes, and dilute to 1.0 mL with 0.1% formic acid in water. Vortex all tubes for 5 seconds.

      Note: After dilution to 1.0 mL with 0.1% formic acid in water, the extracts will contain ≈14% acetonitrile by volume.

Table 15 – Preparation of matrix matched standards

Sample Type	Acetonitrile Standard Mix (µL)	Beta Lactam Standard Mix (µL)	Internal Standard Mix (µL)	Acetonitrile (µL)
Samples, Recoveries, and Negative Controls				140
1/2X Matrix Matched Standard	20	20	20	100
1X Matrix Matched Standard	40	40	20	80
2X Matrix Matched Standard	80	80	20	40

Note: If no internal standard mix is used, the volume of acetonitrile added to each tube must be increased by 20 µL.

m. Pipet 500 µL of all final extracts and calibration standards into bottom portions of pre-labeled Whatman Mini UniPrep Syringless Filter Vials, PVDF, 0.2 micron.

n. Pressed filter/caps onto the vials and placed in autosampler tray for UHPLC-MS/MS analysis.

3. Instrumental Settings

a. Instrument Operating Parameters - UHPLC system

Note: The instrument parameters may be optimized to ensure system suitability.

i. Mobile phase for Residue analysis:

Mobile Phase A - 95% water / 5% ACN / 0.1% Formic Acid
Mobile Phase B - 100% ACN / 0.1% Formic Acid

Mobile phases must be prepared with LCMS grade chemicals only. Allow column to equilibrate with 100% A until the pressure reading is stable.

ii. UHPLC gradient program:

Flow rate: 0.5 mL/min
Pressure Limits: 200 psi minimum; 15,000 psi maximum
Run time: 12.9 minutes

Table 16 – UHPLC gradient program

Time (min)	*% Mobile Phase A*	*% Mobile Phase B*	*Gradient*
0.00	100	0	none
0.10	100	0	linear
8.00	0	100	linear
9.50	0	100	linear
9.60	100	0	linear
12.90	100	0	linear

iii. Injection volume:15 µL

iv. Autosampler temperature: 10 °C

v. Column oven temperature: 40 °C

vi. Draw speed: 5 µL/s

vii. Dispense speed: 20 µL/s

viii. Wash volume: 100 µL

ix. Wash speed: 20 µL/s

x. Needle flush: 50:50 Acetonitrile/Methanol

b. Instrument Operating Parameters - Mass Spectrometer

Note: The positive mass calibration must be performed a minimum of once per week when samples are being analyzed, according to the manufacturer's specification using the manufacturer's calibration solution.

i. Electrospray Source Parameters:

The following parameters should be optimized for ESI spray stability on each mass spectrometer used for this analysis.

(a) Capillary temperature: 375 °C

(b) Spray voltage: 3.5 kV

(c) Sheath gas: 53

(d) Auxilliary gas: 14

(e) S-lens: 65

(f) HESI2 Probe position: Centered, 1B

ii. MS Method Parameters:

(a) Chromatographic peak width: 5 seconds

(b) Method duration: 12.9 minutes

(c) Polarity: Positive

(d) Use divert valve to divert eluant to waste a minimum of 0.25 minutes prior to first peak and 0.25 minutes after last analyte peak.

iii. Full MS Settings

(a) Full MS resolution: 70,000

(b) Scan range: 130-1050 m/z

(c) AGC target: 3e6

(d) Max IT: Auto

(e) Microscans: 1

(f) Spectrum data type: Profile

iv. MS2 Settings

(a) MS2 Resolution: 17,500

(b) Isolation range 1: 130-232 m/z

(c) Isolation range 2: 230-332 m/z

(d) Isolation range 3: 330-432 m/z

(e) Isolation range 4: 430-532 m/z

(f) Isolation range 5: 530-1050 m/z

(g) Stepped CE: 10, 30, 60

(h) AGC target: 1e6

(i) Max IT: Auto

(j) Microscans: 1

(k) Spectrum data type: Profile

Table 17 – Analyte List

Analyte	CAS	Formula	Parent Exact Mass	Adduct	RT	Fragments
Florfenicol Amine	76639-93-5	C10H14FNO3S	248.07512	M+H	0.8	130.06526 131.07268 151.07861 230.06435
Desacetyl Cephapirin	38115-21-8	C15H15N3O5S2	382.05259	M+H	1.24	111.01402 112.02196 124.02162 152.01635 226.02239 292.05692
Amoxicillin	61336-70-7	C16H19N3O5S	366.11182	M+H	2.05	70.06619 114.00153 160.04330 208.04333 211.07201 321.09048 349.08551
Salbutamol	18559-94-9	C13H21NO3	240.15942	M+H	2.13	57.07081 148.07553 166.08621 222.14992
Zilpaterol	119520-05-7	C14H19N3O2	262.15500	M+H	2.13	60.08163 185.07051 202.09696 244.14373
Cimaterol	54239-37-1	C12H17N3O	220.14444	M+H	2.16	89.03936 116.04991 143.06053 145.07588 160.08698 202.13398

Analyte	CAS	Formula	Parent Exact Mass	Adduct	RT	Fragments
Dipyrone	5907-38-0	C12H15N3O	218.12879	M+H	2.25	97.07681 125.07181 187.08701
DCCD	158039-15-7	C17H20N6O7S4	549.03491	M+H	2.34	126.01223 183.03351 241.03865
Tildipirosin	328898-40-4	C41H71N3O8	734.53139	M+H	2.42	116.07062 132.10210 174.11234
Lincomycin	859-18-7	C18H34N2O6S	407.22100	M+H	2.48	126.12799 359.21722 389.20999
Levamisole	16595-80-5	C11H12N2S	205.07940	M+H	2.51	91.05486 123.02652 178.06837
2-Aminosulfone Albendazole	80983-34-2	C10H13N3O2S	240.08012	M+H	2.52	106.05311 133.06345 198.03304
Sulfadiazine	68-35-9	C10H10N4O2S	251.05972	M+H	2.55	96.05621 108.04487 156.01132
Thiabendazole	148-79-8	C10H7N3S	202.04334	M+H	2.61	65.03957 131.06062 175.03227
Ampicillin-1	69-52-3	C16H19N3O4S	350.11690	M+H	2.65	106.06554 114.00109 160.04243 174.05472 192.04729
Sulfathiazole	72-14-0	C9H9N3O2S2	256.02089	M+H	2.67	101.01727 108.04478 156.01118

Analyte	CAS	Formula	Parent Exact Mass	Adduct	RT	Fragments
Desethylene Ciprofloxacin	103222-12-4	C15H16FN3O3	306.12485	M+H	2.69	245.10828 288.11530 289.09802
Sulfapyridine	144-83-2	C11H11N3O2S	250.06447	M+H	2.76	92.05013 95.06104 108.04490 156.01141 184.08691
Norfloxacin	70458-96-7	C16H18FN3O3	320.14050	M+H	2.8	219.09258 233.10818 256.14401 276.15030
Tulathromycin A	217500-96-4	C41H79N3O12	806.57365	M+H	2.8	72.08162 116.10732 577.40521
Oxytetracycline	2058-46-0	C22H24N2O9	461.15546	M+H	2.84	154.04976 201.05443 226.07100 337.06979 365.06546 381.05975 426.11780
Ciprofloxacin	85721-33-1	C17H18FN3O3	332.14050	M+H	2.86	203.06110 245.10812 288.15027 314.12952
Sulfamerazine	127-79-7	C11H12N4O2S	265.07537	M+H	2.89	92.05006 108.04478 110.07164 156.01132 190.02785

Analyte	CAS	Formula	Parent Exact Mass	Adduct	RT	Fragments
Danofloxacin	112398-08-0	C19H20FN3O3	358.15615	M+H	2.9	96.08147 255.05603 283.12360 314.16613 340.14532
Ractopamine	90274-24-1	C18H23NO3	302.17507	M+H	2.92	91.05486 107.04960 121.06503 136.07570 164.10693 284.16403
Ketamine	1867-66-9	C13H16ClNO	238.09932	M+H	2.95	125.01568 179.06233 207.05731 220.08881
Tetracycline	64-75-5	C22H24N2O8	445.16054	M+H	3.01	154.04976 410.12250 427.15002 428.13376
Azaperone	1649-18-9	C19H22FN3O	328.18200	M+H	3.03	121.07632 123.02433 147.09160 149.10747 165.07101
Enrofloxacin	93106-60-6	C19H22FN3O3	360.17180	M+H	3.03	72.08162 203.06120 245.10817 316.18158
Orbifloxacin	113617-63-3	C19H20F3N3O3	396.15300	M+H	3.1	58.06614 267.03949 295.10516 352.16275

Analyte	CAS	Formula	Parent Exact Mass	Adduct	RT	Fragments
Xylazine	7361-61-7	C12H16N2S	221.11070	M+H	3.11	90.03771 105.07016 147.09137
13C6 Sulfamethazine Phenyl	1196157-77-3	C6[13C]6H14N4O2S	285.11115	M+H	3.12	98.07003 124.08701 186.03229
Sulfamethazine	57-68-1	C12H14N4O2S	279.09100	M+H	3.13	108.04467 124.08695 156.01106 204.04341 213.11313
Sulfamethizole	144-82-1	C9H10N4O2S2	271.03179	M+H	3.13	68.05027 92.05001 108.04473 156.01112
Cefazolin	27164-46-1	C14H14N8O4S3	455.03729	M+H	3.16	100.05093 112.02179 132.98882 153.04793 156.01111
Sulfamethoxypyridazine	80-35-3	C11H12N4O3S	281.07029	M+H	3.16	92.04995 108.04466 126.06615 156.01102
Clenbuterol	21898-19-1	C12H18Cl2N2O	277.08690	M+H	3.23	57.07083 132.06824 168.04472 203.01349
Sarafloxacin	91296-87-6	C20H17F2N3O3	386.13107	M+H	3.25	285.08298 299.09845 342.14078

Analyte	CAS	Formula	Parent Exact Mass	Adduct	RT	Fragments
Difloxacin	91296-86-5	C21H19F2N3O3	400.14672	M+H	3.28	58.06605 285.08237 299.09833 356.15604
Morantel	26155-31-7	C12H16N2S	221.11070	M+H	3.32	111.02652 123.0264 150.03705
Pirlimycin	78822-40-9	C17H31ClN2O5S	411.17150	M+H	3.4	110.06018 112.11233 363.16708
2-amino-Flubendazole	82050-13-3	C14H10FN3O	256.08807	M+H	3.45	95.02956 123.02417 133.06339
Chlortetracycline	64-72-2	C22H23ClN2O8	479.12157	M+H	3.5	98.06049 154.04965 444.08344 462.09384
Sulfachloropyridazine	80-32-0	C10H9ClN4O2S	285.02075	M+H	3.54	68.05025 92.04996 108.04467 156.01111
Clindamycin	21462-39-5	C18H33ClN2O5S	425.18715	M+H	3.56	126.12782 335.17261 377.18271 389.20804
Gamithromycin	145435-72-9	C40H76N2O12	777.54710	M+H	3.59	83.04978 116.10719 158.11734
Doxycycline	24390-14-5	C22H24N2O8	445.16054	M+H	3.64	98.06054 154.05021 321.07581 428.13411

Analyte	CAS	Formula	Parent Exact Mass	Adduct	RT	Fragments
Carazolol	57775-29-8	C18H22N2O2	299.17540	M+H	3.65	98.09705 116.10746 222.09137
Butorphanol	58786-99-5	C21H29NO2	328.22711	M+H	3.67	310.21637 157.06483 124.11242
Sulfadoxine	2447-57-6	C12H14N4O4S	311.08085	M+H	3.72	92.04996 108.04465 140.04524 156.01106
Tilmicosin	108050-54-0	C46H80N2O13	869.57332	M+H	3.75	88.07628 116.07083 132.10187 174.11218 696.46570
Sulfamethoxazole	723-46-6	C10H11N3O3S	254.05939	M+H	3.78	93.05789 99.05566 108.04469 147.07909 156.01106
Florfenicol	73231-34-2	C12H14Cl2FNO4S	358.00774	M+H	3.79	132.05687 206.03659 210.05816 241.00452 319.99065 339.99690
Sulfaethoxypyridazine	963-14-4	C12H14N4O3S	295.08594	M+H	3.8	108.04464 140.08154 156.01112
Erythromycin A	59319-72-1	C37H67NO13	734.46852	M+H	4.17	83.04994 116.07102 127.07558 158.11768 576.37354

Analyte	CAS	Formula	Parent Exact Mass	Adduct	RT	Fragments
Sulfadimethoxine	122-11-2	C12H14N4O4S	311.08085	M+H	4.2	92.05000 108.04476 156.07666 245.10283
Sulfaquinoxaline	59-40-5	C14H12N4O2S	301.07537	M+H	4.26	92.05000 108.04475 146.07117 156.01114
Haloperidol	52-86-8	C21H23ClFNO2	376.14741	M+H	4.29	95.02959 123.02421 165.07083 194.07280 358.13605
Prednisone	53-03-2	C21H26O5	359.18530	M+H	4.29	147.08032 237.12598 295.16754 341.17377
Acepromazine	3598-37-6	C19H22N2OS	327.15256	M+H	4.31	58.06606 86.09707 239.07690 254.06335
Promethazine	58-33-3	C17H20N2S	285.14200	M+H	4.33	71.07377 84.08143 86.09708 198.03690 240.08345
Tylosin	1405-54-5	C46H77NO17	916.52643	M+H	4.34	83.04981 101.06014 132.10187 145.08578 174.11234

Analyte	CAS	Formula	Parent Exact Mass	Adduct	RT	Fragments
Albendazole	54965-21-8	C12H15N3O2S	266.09577	M+H	4.38	159.04254 192.02222 234.06920
Fenbendazole sulphone	54029-20-8	C15H13N3O4S	332.07000	M+H	4.39	131.04794 159.04236 300.04315
PenG D7	352323-25-2	C16H11D7N2O4S	342.14994	M+H	4.49	98.09878 160.04263 183.11453
Penicillin G	113-98-4	C16H18N2O4S	335.10600	M+H	4.49	91.05487 114.03761 160.04254 176.07037
Flubendazole	31430-15-6	C16H12FN3O3	314.09355	M+H	4.63	95.02977 123.02438 282.06708
Propionylpromazine	7681-67-6	C20H24N2OS	341.16821	M+H	4.65	86.09716 236.10670 268.07877
Sulfanitran	122-16-7	C14H13N3O5S	336.06487	M+H	4.84	134.05992 156.01105
B-Zearalanol	42422-68-4	C18H26O5	323.18530	M+H	5	149.05971 189.09064 305.17477
Fenbendazole	43210-67-9	C15H13N3O2S	300.08012	M+H	5.06	109.01085 131.04764 159.04231 190.00638 268.05298

Analyte	CAS	Formula	Parent Exact Mass	Adduct	RT	Fragments
Oxacillin	7240-38-2	C19H19N3O5S	402.11182	M+H	5.06	114.03734 144.04410 160.04231 172.07524 243.07568
Virginiamycin M1	21411-53-0	C28H35N3O7	526.25478	M+H	5.2	109.10160 355.12808 508.24390
Cloxacillin	7081-44-9	C19H18ClN3O5S	436.07285	M+H	5.32	114.03743 160.04245 178.00520 206.03661 277.03683
Tyvalson	63409-12-1	C53H87NO19	1042.59451	M+H	5.37	83.04988 109.06527 174.11253
Ketoprofen	22071-15-4	C16H14O3	255.10157	M+H	5.4	105.03394 177.05429 194.07260 209.09587
Nafcillin	7177-50-6	C21H22N2O5S	415.13222	M+H	5.45	115.05415 171.04370 181.06438 199.07500 256.09561
Oxyphenylbutazone	129-20-4	C19H20N2O3	325.15467	M+H	5.45	120.04449 148.03900 160.11218 204.10153

Analyte	CAS	Formula	Parent Exact Mass	Adduct	RT	Fragments
Flunixin	42461-84-7	C14H11F3N2O2	297.08454	M+H	5.5	109.04523 259.06802 264.05014 277.07843 279.07376
Flunixin-d3	1015856-60-6	C14H8D3F3N2O2	300.10337	M+H	5.5	112.06416 264.05060 282.09268
Meloxicam	71125-39-8	C14H13N3O4S2	352.04200	M+H	5.63	115.03273 141.01158 184.05391 194.02629
Dicloxacillin	13412-64-1	C19H17Cl2N3O5S	470.03387	M+H	5.66	114.03751 160.04254 211.96626 239.99748 310.99786
Emamectin Benzoate	155569-91-8	C49H75NO13	886.53112	M+H	6.2	82.06572 126.09131 158.11732 302.19556
Diclofenac	15307-79-6	C14H11Cl2NO2	296.02400	M+H	6.24	215.04840 250.01833 278.01311
Phenylbutazone	50-33-9	C19H20N2O2	309.15975	M+H	6.52	92.04993 120.04441 160.11162 188.10651 190.12224 211.08609
Tolfenamic Acid	13710-19-5	C14H12ClNO2	262.06293	M+H	6.91	180.08041 229.02809 244.05173

Analyte	CAS	Formula	Parent Exact Mass	Adduct	RT	Fragments
Melengesterol Acetate	2919-66-6	C25H32O4	397.23734	M+H	6.93	221.13147 279.17285 337.21457
Eprinomectin	123997-26-2	C50H75NO14	914.52600	M+H	8.07	112.07594 144.10178 154.08611 186.11223

4. Sample Set
   a. Screening Set
      i. External Standard(s)
      ii. Blank (negative control)
      iii. Matrix matched standard
      iv. Recovery(ies) (positive controls)
      v. Check sample (if necessary)
      vi. Up to 27 Samples
      vii. External standard, matrix matched standard, or recovery
   b. Confirmation Set
      i. External Standard(s) (optional)
      ii. Blank (negative control)
      iii. Matrix matched standards
      iv. Recovery(ies) (positive control)
      v. Check sample (if necessary)
      vi. Up to 24 Samples
      vii. External standard, matrix matched standard, or recovery

Note: Placing solvent blanks in the sample injection sequence is prudent in case a high finding leads to carry-over. Additionally, one may want to include an additional external standard, matrix matched standard, or recovery within the sample injection sequence to verify retention time and instrument response stability.

G. CALCULATIONS / IDENTIFICATION

1. Screening

   a. The full scan precursor ion (screening ion) for a given analyte must be present in the extracted ion chromatograph (EIC) using a mass extraction window (MEW) of 5 ppm. The precursor exact mass is listed in Table 17.

   b. The retention time for the screening ions in the fortified recoveries must be within ± 0.1 minutes of the retention time of the screening ion in the matrix-matched standard. Retention time for the screening ions in the samples must match the retention time of the screening ions in a fortified recovery or the matrix matched standard within ± 0.1 minutes.

   c. The screening ion must have a signal-to-noise ratio ≥ 3. This may be verified by visual inspection. Visual inspection for detection may also include assessment of peak shape or drift in relation to standard peaks or evaluating the presence or absence of monitored fragment ions.

   d. A sample is screened positive for an analyte if the following criteria are met:

      i. The fortified recovery of the analyte must exceed 10% of the 1/2 X matrix matched standard level.

      ii. The sample response equals or exceeds the 1/2 X (or level of interest) fortified recovery level.

      iii. The level of the screening ion in the blank (negative control) must be less than 10% of the 1/2 X level for the matrix-matched standard

      Note: If a sample shows a positive response for a compound which did not meet screening criteria in the associated QC samples, then further testing of that sample is warranted.

2. Confirmation

   a. The full scan precursor ion (screening ion) for a given analyte must be present in the extracted ion chromatograph (EIC) using a mass extraction window (MEW) of 5 ppm. The precursor exact mass is listed in Table 17.

   b. The retention time for the screening ions in the fortified recoveries must match the retention time of the screening ions in the matrix-matched standard within ± 0.1 minutes. Retention time for the screening ions in the samples must match the retention time of the screening ions in a fortified recovery or the matrix matched standard within ± 0.1 minutes.

   c. The screening ion must have a signal-to-noise ratio ≥ 3. This may be verified by visual inspection. Visual inspection for detection may also include assessment of peak shape or drift in relation to standard peaks or evaluating the presence or absence of monitored fragment ions.

d. A minimum of one fragment ion must be present at the corresponding precursor retention time and isolation range with a mass accuracy of 10 ppm or less. Fragment ions for each analyte are specified in Table 17.

e. A sample is confirmed positive for an analyte if the following criteria are met:

i. The fortified recovery of the analyte must exceed 10% of the 1/2 X matrix matched standard level.

ii. The sample response equals or exceeds the 1/2 X (or level of interest) fortified recovery level.

iii. The blank (negative control) must be less than 10% of the 1/2 X level for the matrix matched standard.

Note: Additionally an analyst recovery curve (0X, 1/2X, 1X, and 2X) may be analyzed and used for assessement or identification purposes in addition to the matrix matched standards.

H. SAFETY INFORMATION AND PRECAUTIONS

1. Required Protective Equipment -Safety eyewear, protective gloves, and lab coat.

2. Hazards

Procedure Step	*Hazard*	*Recommended Safe Procedures*
Antibiotic standards	Some individuals may have allergic reactions to certain β-lactams, sulfa, or other drugs.	Wear appropriate personal protective equipment to avoid dermal contact.
Acetonitrile, Methanol	Flammable	Keep in well-closed containers away from ignition sources. Avoid contact or prolonged exposure to vapors. Work in fume hood. Keep away from flame or heat.
Formic acid	Corrosive, Caustic	Wear personal protective equipment, avoid skin contact.

3. Disposal Procedures

Follow local, state and federal guidelines for disposal.

I. QUALITY ASSURANCE PLAN

1. Performance Standard

   a. Screening Criteria

      i. For set acceptance, 90% of the monitored analytes in the fortified recovery (positive control) must meet screening criteria. For sample reporting purposes, the analytes of interest in the fortified recovery (positive control) must meet screening criteria.

      ii. For set acceptance, 95% of the monitored analytes in the blank (negative control) must not meet screening criteria. The blank (negative control) must be negative using the criteria in Section G for samples containing corresponding presumptive positive analytes.

   b. Confirmation Criteria

      i. For set acceptance, nine of the following ten analytes must meet confirmation criteria (Oxytetracycline, Enrofloxacin, Florfenicol, Sulfadimethoxine, Penicillin G, Erythromycin A, Dicloxacillin, Flunixin, Meloxicam, and Fenbendazole Sulphone). For sample reporting purposes, the analytes of interest in the fortified recovery (positive control) must meet confirmation criteria.

      ii. The blank (negative control) must be negative using the criteria in Section G for the analytes of interest.

2. Critical Control Points and Specifications

Record	*Acceptable Control*
none known at this time	

3. Intralaboratory Check Samples

   a. System, minimum contents.

      i. Frequency: One per week per analyst when samples analyzed.

      ii. Records are to be maintained.

   b. Acceptability criteria.

      Refer to I. 1.

      If unacceptable values are obtained, then:

      i. Investigate following established procedures.

      ii. Take correction or corrective action as warranted.

4. Sample Condition upon Receipt

   Cool or frozen

J. APPENDIX

1. References

   [Reserved]

2. Chromatograms/spectra

   [Reserved]

3. Minimum Level of Applicability

Table 18A - Minimum Level of Applicability for kidney screening

Standard Analyte	Bovine Kidney Screening Level (ng/g)	Porcine Kidney Screening Level (ng/g)
2-amino-Flubendazole	10	10
2-Aminosulfone Albendazole	25	25
Acepromazine	4	4
Albendazole	25	25
Amoxicillin	20	20
Ampicillin	5	5
Azaperone		1
Butorphanol	1	1
Carazolol	1	1
Cefazolin	50	50
Chlortetracycline	1000	1000
Cimaterol	6	12
Ciprofloxacin	25	25
Clenbuterol	6	3
Clindamycin	50	50
Cloxacillin	5	5
Danofloxacin	100	25
DCCD	50	50
Desacetyl Cephapirin	50	50
Diclofenac	5	5
Dicloxacillin	50	50
Difloxacin	50	100
Dipyrone		25
Doxycycline	25	25
Emamectin Benzoate	7.5	7.5
Enrofloxacin	25	25
Eprinomectin	30	30
Erythromycin A	50	50
Fenbendazole	200	200
Fenbendazole sulphone	200	200
Florfenicol	100	100

Standard Analyte	Bovine Kidney Screening Level (ng/g)	Porcine Kidney Screening Level (ng/g)
Florfenicol Amine	150	150
Flubendazole	10	10
Flunixin	12.5	12.5
Gamithromycin	50	50
Haloperidol	1	1
Ketamine	40	20
Ketoprofen	5	5
Levamisole	50	50
Lincomycin	50	50
Melengestrol Acetate	20	20
Meloxicam	10	10
Morantel tartrate	350	350
Nafcillin	50	50
Norfloxacin	25	25
Orbifloxacin	50	25
Oxacillin	50	50
Oxyphenylbutazone	50	50
Oxytetracycline	500	500
Penicillin G	25	25
Phenylbutazone	50	50
Pirlimycin	250	250
Prednisone	50	50
Promethazine		4
Propionylpromazine		1
Salbutamol	3	3
Sarafloxacin		25
Sulfachloropyridazine	50	50
Sulfadiazine	50	50
Sulfadimethoxine	50	50
Sulfadoxine	50	50
Sulfaethoxypyridazine	50	50
Sulfamerazine	50	50
Sulfamethazine	50	50
Sulfamethizole	50	50
Sulfamethoxazole	50	50
Sulfamethoxypyridazine	100	50
Sulfanitran	50	50
Sulfapyridine	50	50
Sulfaquinoxaline	50	50
Sulfathiazole	50	50
Tetracycline	1000	1000
Thiabendazole	100	50
Tildipirosin	1000	500
Tilmicosin	60	60
Tolfenamic Acid	25	25
Tulathromycin A	4000	1000
Tylosin	100	
Tylvalosin	25	25
Virginiamycin	50	50

Standard Analyte	Bovine Kidney Screening Level (ng/g)	Porcine Kidney Screening Level (ng/g)
Xylazine	1	2
Zilpaterol	3	3

Table 18B - Minimum Level of Applicability for kidney confirmation

Standard Analyte	Bovine Kidney Confirmation Level (ng/g)	Porcine Kidney Confirmation Level (ng/g)
2-amino-Flubendazole	10	10
2-Aminosulfone Albendazole	25	25
Acepromazine	4	4
Albendazole	25	25
Amoxicillin	20	20
Ampicillin	5	5
Azaperone		1
Butorphanol	1	1
Carazolol	1	4
Cefazolin	50	50
Chlortetracycline	1000	1000
Ciprofloxacin	25	25
Clenbuterol	6	3
Clindamycin	50	50
Cloxacillin	5	5
Danofloxacin	100	25
DCCD	50	50
Desacetyl Cephapirin	50	50
Diclofenac	20	
Dicloxacillin	50	50
Difloxacin	50	100
Doxycycline	25	25
Emamectin Benzoate	7.5	7.5
Enrofloxacin	25	25
Erythromycin A	50	50
Fenbendazole	200	200
Fenbendazole sulphone	200	200
Florfenicol	100	100
Florfenicol Amine	150	150
Flubendazole	10	10
Flunixin	12.5	12.5
Gamithromycin	50	50
Haloperidol	1	1
Ketamine	40	20
Ketoprofen	5	10
Levamisole	50	50
Lincomycin	50	50
Melengestrol Acetate	20	40
Meloxicam	10	10
Morantel tartrate	350	350
Nafcillin	50	50

Standard Analyte	Bovine Kidney Confirmation Level (ng/g)	Porcine Kidney Confirmation Level (ng/g)
Norfloxacin	25	25
Orbifloxacin	50	25
Oxacillin	50	50
Oxyphenylbutazone	50	50
Oxytetracycline	500	500
Penicillin G	25	25
Phenylbutazone	50	50
Pirlimycin	250	250
Prednisone	50	50
Promethazine		4
Propionylpromazine		1
Salbutamol	3	3
Sarafloxacin		25
Sulfachloropyridazine	50	50
Sulfadiazine	50	50
Sulfadimethoxine	50	50
Sulfadoxine	50	50
Sulfaethoxypyridazine	50	50
Sulfamerazine	50	50
Sulfamethazine	50	50
Sulfamethizole	50	50
Sulfamethoxazole	50	50
Sulfamethoxypyridazine	100	50
Sulfanitran	50	50
Sulfapyridine	50	50
Sulfaquinoxaline	50	50
Sulfathiazole	50	50
Tetracycline	1000	1000
Tildipirosin	1000	500
Tilmicosin	60	60
Tolfenamic Acid	50	
Tulathromycin A	4000	1000
Tylosin	100	
Tylvalosin	25	25
Virginiamycin	50	50
Zilpaterol	3	3

Table 19A - Minimum Level of Applicability for muscle screening

Standard Analyte	Bovine Muscle Screening Level (ng/g)	Porcine Muscle Screening Level (ng/g)
2-amino-Flubendazole	10	10
2-Aminosulfone Albendazole	25	25
Acepromazine	4	
Albendazole	25	25
Amoxicillin	20	20
Ampicillin	5	5
Azaperone	1	1

Standard Analyte	Bovine Muscle Screening Level (ng/g)	Porcine Muscle Screening Level (ng/g)
Butorphanol	1	1
Carazolol	1	1
Cefazolin	50	50
Chlortetracycline	1000	1000
Cimaterol	6	3
Ciprofloxacin	25	25
Clenbuterol	3	3
Clindamycin	50	50
Cloxacillin	5	5
Danofloxacin	25	25
DCCD	50	50
Desacetyl Cephapirin	50	50
Desethylene Ciprofloxacin	25	25
Diclofenac	5	5
Dicloxacillin	50	50
Difloxacin	25	25
Dipyrone	100	100
Doxycycline	25	25
Enrofloxacin	25	25
Eprinomectin		15
Erythromycin A	50	50
Fenbendazole	200	200
Fenbendazole sulphone	200	200
Florfenicol	100	100
Florfenicol Amine	150	150
Flubendazole	10	10
Flunixin	12.5	12.5
Gamithromycin	50	50
Haloperidol	1	1
Ketamine	20	20
Ketoprofen	5	5
Levamisole	50	50
Lincomycin	50	50
Melengestrol Acetate		20
Meloxicam	10	10
Morantel tartrate	350	350
Nafcillin	50	50
Norfloxacin	25	25
Orbifloxacin	25	25
Oxacillin	50	50
Oxyphenylbutazone		200
Oxytetracycline	500	500
Penicillin G	50	25
Pirlimycin	250	250
Prednisone	50	50
Ractopamine		6
Salbutamol	3	3
Sarafloxacin	25	25
Sulfachloropyridazine	50	50

Standard Analyte	Bovine Muscle Screening Level (ng/g)	Porcine Muscle Screening Level (ng/g)
Sulfadiazine	50	50
Sulfadimethoxine	50	50
Sulfadoxine	50	50
Sulfaethoxypyridazine	50	50
Sulfamerazine	50	50
Sulfamethazine	50	50
Sulfamethizole	50	50
Sulfamethoxazole	50	50
Sulfamethoxypyridazine	50	50
Sulfanitran	50	50
Sulfapyridine	50	50
Sulfaquinoxaline	50	50
Sulfathiazole	50	50
Tetracycline	500	500
Thiabendazole	50	100
Tildipirosin	500	500
Tilmicosin	60	60
Tolfenamic Acid		25
Tulathromycin A	1000	1000
Tylosin	100	100
Tylvalosin		25
Virginiamycin	50	50
Xylazine	1	1
Zeranol (B-Zearalanol)	24	
Zilpaterol	3	3

Table 19B - Minimum Level of Applicability for muscle confirmation

Standard Analyte	Bovine Muscle Confirmation Level (ng/g)	Porcine Muscle Confirmation Level (ng/g)
2-amino-Flubendazole	10	10
2-Aminosulfone Albendazole	25	25
Acepromazine	4	
Albendazole	25	25
Amoxicillin	20	20
Ampicillin	5	5
Azaperone	1	1
Butorphanol	1	1
Carazolol	1	2
Cefazolin	50	50
Chlortetracycline	1000	1000
Cimaterol	6	
Ciprofloxacin	25	25
Clenbuterol	3	3
Clindamycin	50	50
Cloxacillin	5	5
Danofloxacin	25	25
DCCD	50	50

Standard Analyte	Bovine Muscle Confirmation Level (ng/g)	Porcine Muscle Confirmation Level (ng/g)
Desacetyl Cephapirin	50	50
Desethylene Ciprofloxacin	50	100
Diclofenac	10	
Dicloxacillin	50	50
Difloxacin	25	25
Doxycycline	25	25
Enrofloxacin	25	25
Erythromycin A	50	50
Fenbendazole	200	200
Fenbendazole sulphone	200	200
Florfenicol	100	100
Florfenicol Amine	150	150
Flubendazole	10	10
Flunixin	12.5	12.5
Gamithromycin	50	50
Haloperidol	1	1
Ketamine	20	20
Ketoprofen	5	5
Levamisole	100	200
Lincomycin	50	50
Melengestrol Acetate		40
Meloxicam	10	10
Morantel tartrate	350	350
Nafcillin	50	50
Norfloxacin	25	25
Orbifloxacin	25	25
Oxacillin	50	50
Oxyphenylbutazone		200
Oxytetracycline	500	500
Penicillin G	50	25
Pirlimycin	250	250
Prednisone	50	50
Ractopamine		6
Salbutamol	3	3
Sarafloxacin	25	25
Sulfachloropyridazine	50	50
Sulfadiazine	50	50
Sulfadimethoxine	50	50
Sulfadoxine	50	50
Sulfaethoxypyridazine	50	50
Sulfamerazine	50	50
Sulfamethazine	50	50
Sulfamethizole	50	50
Sulfamethoxazole	50	50
Sulfamethoxypyridazine	50	50
Sulfanitran	50	50
Sulfapyridine	50	50
Sulfaquinoxaline	50	50
Sulfathiazole	50	50

Standard Analyte	Bovine Muscle Confirmation Level (ng/g)	Porcine Muscle Confirmation Level (ng/g)
Tetracycline	500	500
Tildipirosin	500	500
Tilmicosin	60	60
Tolfenamic Acid		100
Tulathromycin A	1000	1000
Tylosin	100	100
Tylvalosin		25
Virginiamycin	50	50
Zeranol (B-Zearalanol)	24	
Zilpaterol	3	3

K. APPROVALS AND AUTHORITIES

1. Approvals on file.
   a. Issuing Authority: Director, Laboratory Quality Assurance Staff.