

# The VetDrugs Explorer Collection: screening and quantitation of multi-class veterinary drug residues in animal matrices with a comprehensive workflow solution

## Authors

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## Keywords

VetDrugs Explorer Collection, Veterinary Medicines, Triple Quadrupole Mass Spectrometry, [TSQ Altis MS](#), [TraceFinder Software](#)

## Goal

To present a LC-MS/MS workflow solution for multi-class analysis of veterinary drug residues in animal matrices that is robust, rapid, easy to use, while having the sensitivity, accuracy, and precision that is required in order to meet regulatory guidelines around the world.

## Introduction

Veterinary drugs are broadly defined as chemicals that are used to protect animals from contracting disease, promote growth, and in some cases provide aesthetic qualities in food production. The inappropriate use of veterinary drugs can have adverse effects on animals, the environment, and human health. Antimicrobial resistance, or the ability of certain microorganisms (bacteria or viruses) to eliminate or reduce the effectiveness of a drug, can be promoted in the environment by overuse of some of these medicines.

As a result, the determination and efficient analysis of veterinary drugs is an important part of routine food quality control. The European Union (EU) and other countries have developed specific regulations to address these growing concerns. Decision 2002/657/EC (and amendments) specifies method validation guidelines, while maximum residue limit (MRL) definitions and limits are specified in EU Commission Regulation 37/2010 and EU Council Regulation 2377/90 (as amended), respectively. The requirements of low limits of quantification in diverse matrices, along with a wide variety of chemical

classes and properties of veterinary drugs pose significant analytical challenges. Consequently, several analytical methodologies have emerged which are typically limited in scope to specific chemical classes, are labor intensive, and require extensive sample preparation and clean-up.

This study presents a multi-residue, multi-class analytical workflow solution enabled by the Thermo Scientific™ VetDrugs Explorer Collection. It encompasses everything that is required from sample preparation to final reporting for over 160 veterinary drugs using liquid chromatography-triple stage mass spectrometry (LC-MS/MS) and a rapid sample preparation procedure based on modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) extraction. The method is sensitive, and is able to identify, confirm, and quantify the veterinary drugs below their required EU maximum residue limits (MRLs). The VetDrugs Explorer Collection includes the QuEChERS sample preparation supplies, a quality control (QC) check sample, the vetdrugs analytical standard solutions, the Thermo Scientific™ Vanquish™ Flex Binary UHPLC system, Thermo Scientific™ Accucore™ VDX LC column, a Thermo Scientific™ TSQ Altis™ triple quadrupole mass spectrometer, Thermo Scientific™ TraceFinder™ software, and complete compound data base with acquisition and processing methods.

## Experimental

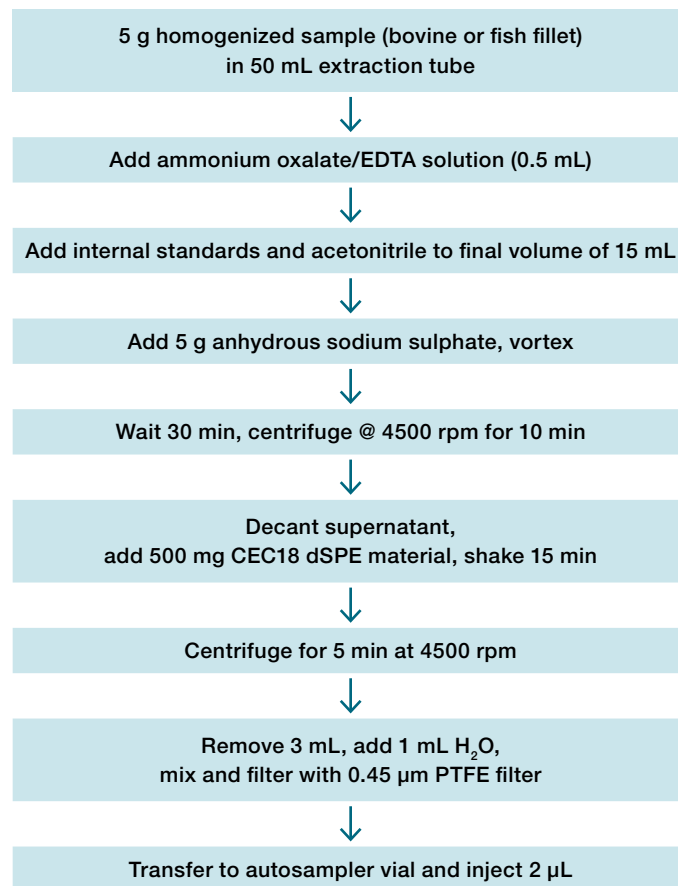
The VetDrugs Explorer Collection workflow is designed to address the essential elements of a complex multi-class veterinary drug residue analysis that can be applied to a variety of matrices. Bovine muscle, salmon (fillet), and milk (dairy) were processed and analyzed to test the core methodology—from sample preparation to analysis by LC-MS/MS.

### Sample preparation reagents and supplies

- Buffer: 0.2 M ammonium oxalate monohydrate/  
0.1 M disodium EDTA dihydrate
- 5 g Sodium Sulfate, slim pouch—50 pk  
(P/N 60105-368-SP)
- 500 mg CEC18, Slim Line Pouch—50 pk  
(P/N 60105-367-SP)
- Falcon tubes (50 mL)—50 pk (P/N 60106-425)
- 0.45 µm PTFE filters, 17 mm—100 pk (P/N F2513-3)
- 10 mL luer-lock syringe—100 pk (P/N S7515-10)

### Sample preparation procedure

Sample preparation involves a modified QuEChERS preparation protocol that was optimized to be easy for laboratories to implement and also reduce matrix co-extractives, resulting in enhanced sensitivity in electrospray ionization LC-MS/MS. The basic elements of the preparation procedure are described in Figure 1.



**Figure 1: Extraction procedure for bovine muscle and salmon (fillet).** Further details for milk are included in the VetDrugs Explorer Collection.

### Spiking solution mixtures for Matrix Extracted Spikes (MES)

When developing spiking solutions for a multi-class veterinary method, several considerations need to be addressed. These include the stability of the spiking mixtures (stock solutions) and the final concentration of the target analytes required as they relate to the regulatory MRLs in given matrices. Laboratories will often adopt a specific SOP to address these issues and periodically check the stability of the stock mixtures. The VetDrugs Explorer Collection includes suggested spiking mixtures for the three matrices to help laboratories perform this task. In addition, the veterinary drugs standards in this application are included in the kit.

## LC-MS/MS analysis

The assays in this study were carried out using a Vanquish Flex Binary UHPLC system and a TSQ Altis MS. TraceFinder software was used for instrument control, analysis, data review, and reporting. The LC conditions and gradient are shown in Tables 1 and 2, respectively. The gradient was optimized for analysis time and to ensure chromatographic separation of the compounds. Note that all LC and MS of methods and conditions for acquisition and processing are included in the VetDrugs Explorer Collection.

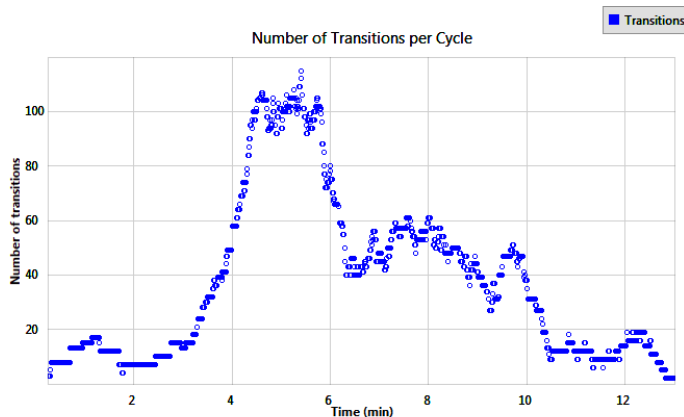
**Table 1. LC column and mobile phase conditions.**

Injection Volume	2 $\mu$ L
Column Temperature	40 $^{\circ}$ C
Analytical Column	Accucore VDX, 100 $\times$ 2.1 mm $\times$ 2.6 $\mu$ m
Run Time	17 minutes
Tray Temperature	15 $^{\circ}$ C
Mobile Phases	A) Water with 0.05% Formic Acid B) 50% Acetonitrile 50% Methanol 5% Water with 0.05% Formic Acid

**Table 2. LC pump gradient.**

Time (minutes)	Flow rate (mL/min)	%B
0.0	0.30	2
2.0	0.30	2
3.0	0.30	20
11.0	0.30	100
13.0	0.40	100
14.4	0.40	100
14.5	0.35	2
16.0	0.30	2
17.0	0.30	2

The TSQ Altis MS was operated in timed-SRM mode to ensure enough data points were collected for proper quantitation of each analyte. The conditions for each SRM transition (RF lens, collision energy, precursor and product ions) were automatically optimized using a new Compound Optimization Tool within the instrument control software. The total number of individual SRM transitions used in the method was 546, and they are easily visualized in terms of number of transitions per cycle versus time across the chromatographic run, as shown in Figure 2.



**Figure 2: A total of 546 SRMs in the method are easily visualized in the instrument software tune page**

The TSQ Altis MS was operated in positive/negative switching mode. The ion source for the analysis was Heated Electrospray (H-ESI). The mass spectrometer's atmospheric pressure ionization (API) settings are shown in Table 3. For fast method implementation, details of the complete method including the LC and SRM settings are included in the VetDrugs Explorer Collection.

**Table 3. Mass spectrometer API settings.**

Negative Voltage	2500 V
Positive Voltage	3500 V
Sheath Gas	50 Arb Units
Auxiliary Gas	13 Arb Units
Sweep Gas	1 Arb Unit
Ion Transfer Tube Temperature	310 $^{\circ}$ C
Vaporizer Temperature	350 $^{\circ}$ C

## Quality Control Check Standard

Before analyzing samples with the full VetDrugs Explorer Collection LC-MS/MS analytical method, users can analyze a QC Check Standard sample to ensure that the system is ready for analysis. The QC Check Standard is included in the kit and is packaged in a box containing four, 1-mL flame-sealed ampoules.

The QC Check Standard, which contains twenty compounds with multiple classes of veterinary drugs, challenges the analytical system for sensitivity, inertness, and retention time (RT) stability. Full instructions for dilution of the standard, LC and MS conditions, as well as criteria for passing the QC Check, are provided in the VetDrugs Explorer Collection.

## Results and discussion

### Scope

The VetDrugs Explorer Collection method applies to the following veterinary drug compound classes: Cefalosporins, macrolides, penicillins, quinolones, sulfonamides, tetracyclines, anthelmintics, nitroimidazoles, non-steroidal anti-inflammatory drugs (NSAIDs), sedatives, avermectins and coccidiostats, dyes (applied to fish), and steroids (milk) (Figure 3).

## Method performance

To evaluate the method performance, a series of multi-component spiking solutions were developed. Over 160 veterinary drugs were added directly to the homogenized matrix during QuEChERS sample preparation to create matrix extracted spikes (MES) at concentration levels that reference a screening target concentration, or STC. The STC relates back to the relevant MRL for each compound in a given matrix. For method development, the STC level was chosen to be  $\frac{1}{3}$  to  $\frac{1}{4}$  of the concentration of the EU-based MRL. In order to observe the quantitative performance of the method, a series of MES were prepared and used to construct calibration curves at 0.0 (MES blank), 0.2, 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0. Eight replicates at each STC spike level were injected, along with the MES blank (injected three times). Additionally, a “post spike” sample was prepared in the blank matrix extract. The target analytes were added after a blank matrix was extracted. It was spiked at the 3× STC level. Spike recovery was then estimated by the ratio of the peak areas observed in the 3× STC MES and post spike sample, using the following formula: % Recovery = Peak area 3× STC MES/Peak area 3× STC Post Spike × 100.

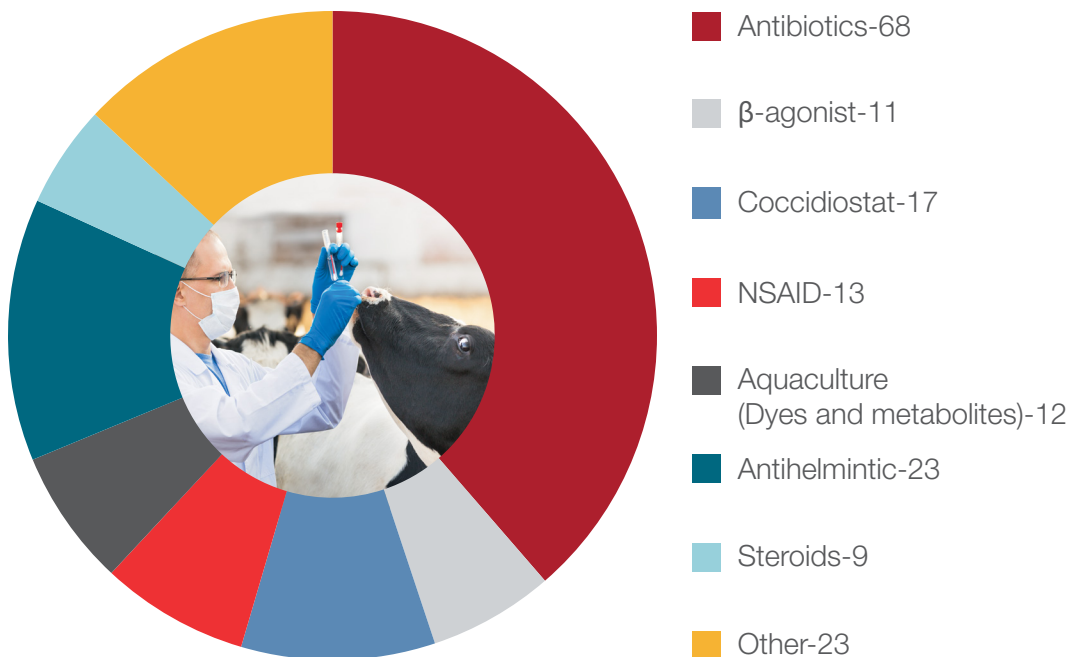


Figure 3: Compound classes with number of analytes evaluated during the development of the VetDrugs Explorer Collection.

To calculate a method detection limit (MDL), the results of the 8 replicate injections at each level were evaluated. The criteria used to determine the STC level at which the MDL was calculated was based upon the lowest level where the %RSD was less than or equal to 15%. Once that level had been determined, the MDL was calculated by using the formula  $MDL = S * 2.998$ , where S equals the standard deviation of calculated concentration of the 8 replicate injections and 2.998 is from the Student's *t*-test value for eight degrees of freedom. The MDLs calculated for each compound, the recoveries, and the retention times for all three matrices are listed in Table 4 in the appendix.

An extracted total ion chromatogram showing all the compounds in a salmon MES at 1x STC is shown in Figure 4. Figure 5 is an example of a chromatogram showing the quantitation ion for the dye Ethyl Violet in salmon matrix at 1x STC as well as confirmation ion chromatograms, and its calibration line. The calibration range the curve represents in this case is from 0.2 ng/g to 5 ng/g (STC = 1.0 ng/g for Ethyl Violet) and demonstrates confidence in the results well below the minimum required performance limit (MRPL). A similar set of data with other veterinary drugs is shown for bovine muscle and for a steroid hormone in milk (Figures 6 and 7).

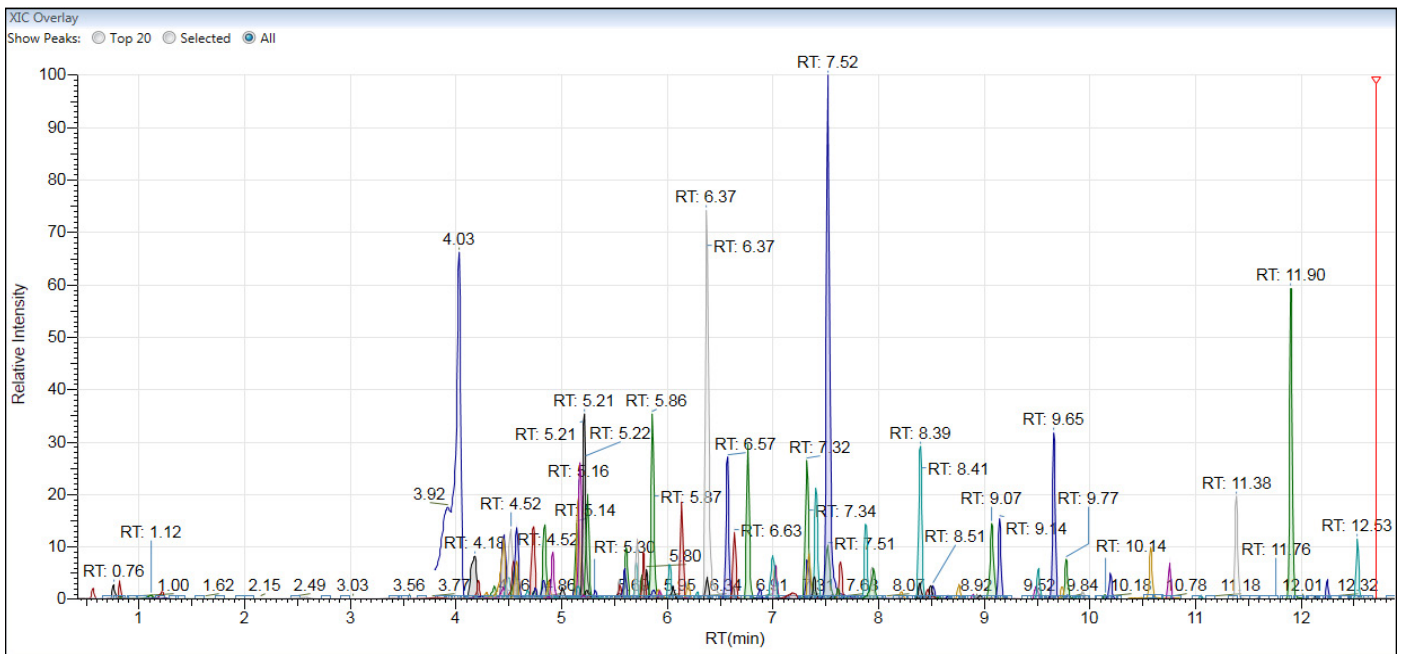


Figure 4: Total extracted ion chromatogram of salmon extract at 1x STC.

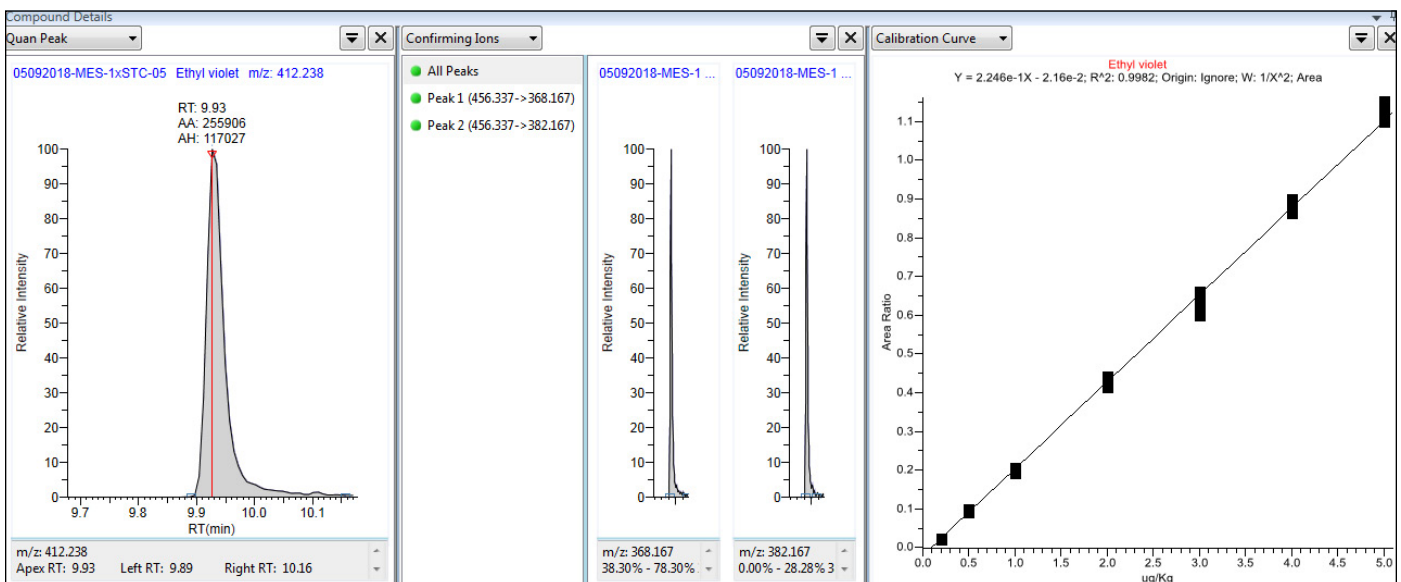


Figure 5: Ethyl Violet in salmon extract at 1x STC, with a calibration curve from 0.2–5 ng/g.

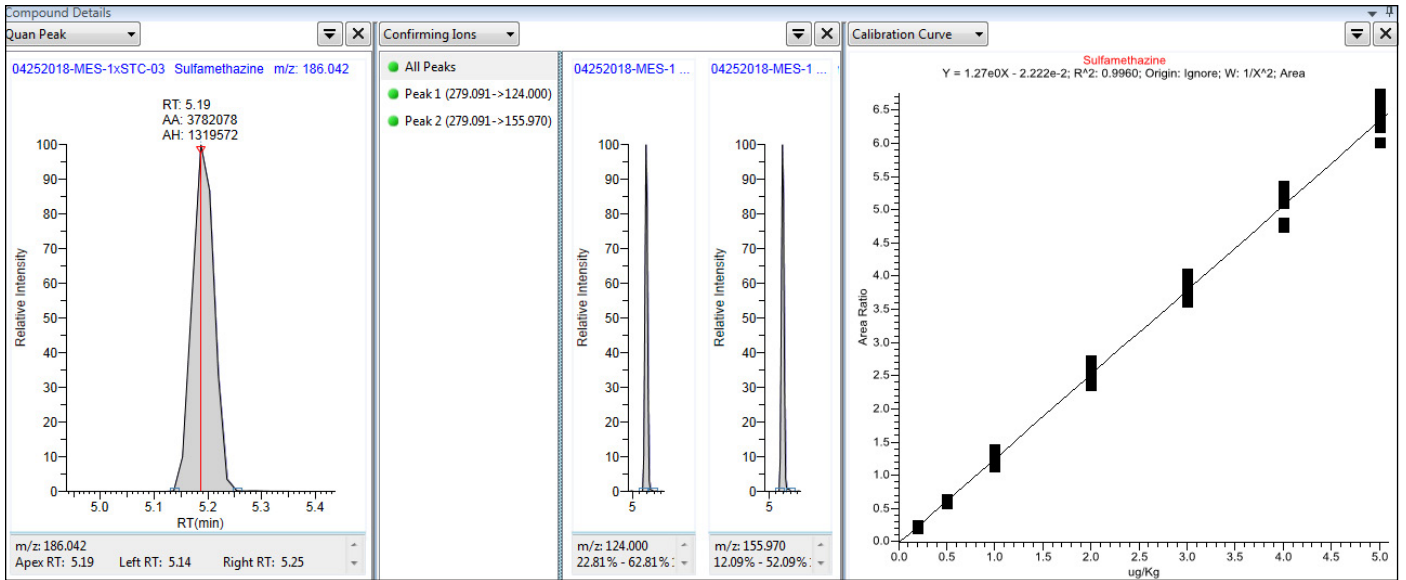


Figure 6: Sulfamethazine in bovine extract at 1x STC, with a calibration curve from 10–250 ng/g.

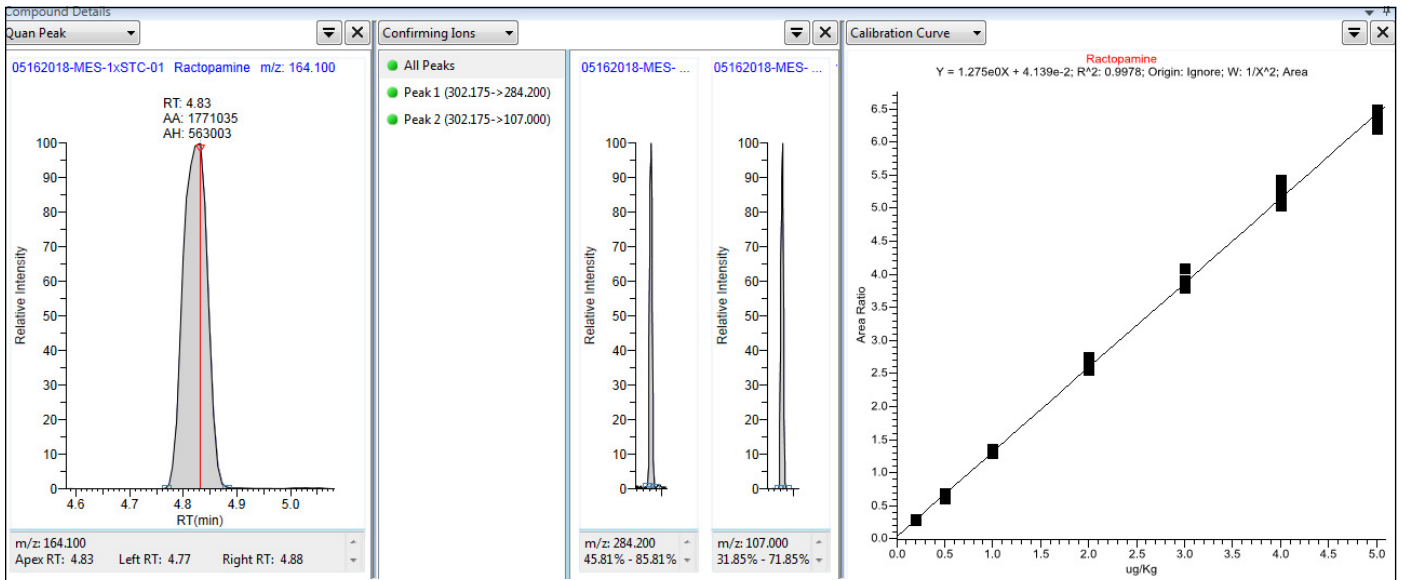
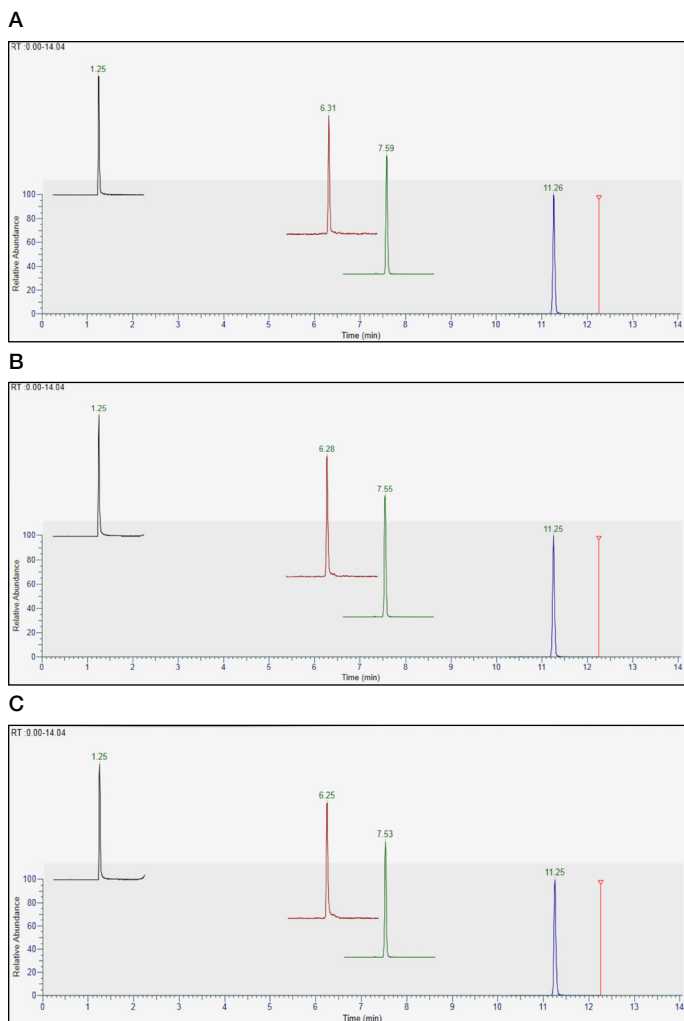


Figure 7:  $\beta$ -agonist Ractopamine in milk extract at 1x STC, with a calibration curve from 2–50 ng/g.



**Figure 8A–C: Comparison of injections of MES of bovine muscle extract at 3× STC over 500 injections. Peaks from left to right: cyromazine, ciprofloxacin, sulfamethoxazole, and flunixin.** A) Injection #20. B) Injection #260. C) Injection #500. Peak shape and RT stability are maintained, indicating robust method operation for routine testing.

A large number of injections of a bovine extract at 3× STC over a period of 1 week were performed to demonstrate the robustness of the analytical column in terms of maintaining peak shape, response, and retention time stability. A comparison of chromatograms is shown in Figure 8.

## Conclusion

Regulations of the European Union and the United States pose significant challenges to the analytical laboratories invested in methods quantifying veterinary drugs in complex matrices. This application brief described a robust, sensitive, and reliable multi-residue LC-MS/MS method workflow solution that uses the TSQ Altis triple quadrupole mass spectrometer for analysis and quantitation of more than 160 veterinary drugs with the VetDrugs Explorer Collection solution in salmon (fillet) bovine muscle, and milk. For convenience and fast method implementation, the complete instrument and data processing method including SRM settings is included with the VetDrugs Explorer Collection start-to-finish workflow solution. In 17 minutes, the target veterinary drugs were detected and quantified in food matrices. Quantitation of the veterinary drugs was evaluated by matrix extracted spikes between 0.2 and 5 × STC. Method detection limits were established, along with estimated extraction recovery based upon post-extraction spikes. The extraction process and high sensitivity of the LC-MS allowed for the injection of only 2 µL of sample, with increased robustness and throughput.

## Appendix

**Table 4: Calculated percent recoveries and method detection limits (MDLs) for select compounds in the multi-class VetDrugs Explorer Collection method.**

Retention Time	Compound	Class	Salmon Fillet MDL (ng/g)	Salmon Fillet Recovery	Bovine Muscle MDL (ng/g)	Bovine Muscle Recovery	Milk MDL (ng/g)	Milk Recovery
9.13	1,3-bis(4-nitrophenyl) urea	Cocciostat	0.23	86.6	0.25	84.5	0.04	87.1
0.75	2,4,6-Triamino-pyrimidine-5-carbonitrile	Dicyclanil Marker Residue	2.71	81.7	3.67	68.0	0.70	82.3
8.65	5-Hydroxyflunixin	Flunixin metabolite	0.07	79.6	0.08	52.1	NA	NA
4.21	5-hydroxythiabendazole	Thiabendazole metabolite	0.73	102.5	0.23	79.4	0.22	92.5
11.76	Abamectin B1a	Anthelmintic	1.29	79.1	2.01	85.8	0.45	82.6
6.91	Acepromazine	Sedative and antiemetic	0.20	69.0	0.46	69.9	NA	NA
7.41	Albendazole	Anti parasitic worm	0.21	86.7	0.46	86.0	0.17	90.8
4.36	Albendazole 2-aminosulfone	Albendazole metab	0.32	86.9	0.65	74.4	0.21	87.9
6.2	Albendazole sulfone	Albendazole metab	0.75	81.9	0.72	85.5	0.30	82.7
5.55	Albendazole sulfoxide	Albendazole metab	0.27	77.2	0.82	82.6	0.44	83.3
9.52	Altrenogest	Steroid	0.26	78.4	0.42	92.5	0.02	88.7
5.25	Amoxicillin	Antibiotic (b-lactam)	0.56	51.4	1.19	50.7	0.13	50.2
5.25	Ampicillin	Antibiotic (b-lactam)	0.36	52.4	0.39	52.4	0.07	51.4
4.84	Azaperol	Azaperone metabolite	0.22	78.8	0.24	70.4	NA	NA
5.17	Azaperone	Sedative and antiemetic	0.07	70.4	0.20	72.3	NA	NA
5.71	Azure B	Aquaculture (Dye)	0.33	67.3	0.66	67.3	NA	NA
7.95	Betamethazone	Steroid	0.29	104.2	1.29	96.1	0.06	95.9
9.01	Brilliant Green	Fungicide	0.05	57.0	0.05	59.6	NA	NA
5.8	Carazolol	Beta blocker	0.03	65.5	0.11	82.6	NA	NA
9.51	Carprofen	NSAID	3.25	76.7	5.10	78.8	NA	NA
4.25	Cefapirin	Antibiotic (b-lactam)	1.70	91.8	1.06	67.5	0.42	94.9
5.29	Cefazolin	Antibiotic (b-lactam)	7.53	88.4	8.19	74.9	1.70	92.8
5.79	Cefoperazone	Antibiotic (b-lactam)	2.26	82.4	8.61	82.7	2.10	88.9
4.52	Cefquinome	Antibiotic (b-lactam)	7.87	96.6	8.55	83.7	5.40	99.9
6.56	Ceftiofur	Antibiotic (b-lactam)	0.54	69.6	0.76	71.2	0.34	87.3
4.71	Cephalonium	Antibiotic (b-lactam)	14.33	90.6	69.90	88.6	2.40	91.0
10.04	Chlormadinone acetate	Progestin	**	**	**	**	0.14	86.8
5.73	Chlorpromazine	Sedative and antiemetic	0.06	55.7	0.14	59.2	NA	NA
7.6	Chlortetracycline	Antibiotic (tetracycline)	1.51	53.5	1.75	55.2	1.20	67.1
4.17	Cimbuterol	$\beta$ -agonist	0.06	102.2	0.07	76.8	0.08	82.0
4.75	Ciprofloxacin	Antibiotic	0.35	51.2	0.95	50.9	0.42	65.3
5.22	Clenbuterol	$\beta$ -agonist	**	**	**	**	0.16	81.3
5.71	Clenpenterol	$\beta$ -agonist	0.06	74.3	0.23	85.9	0.13	80.1
4.8	Clenproperol	$\beta$ -agonist	0.27	67.7	0.15	70.2	0.11	75.9
4.34	Clopidol	Cocciostat (antiprotozoal)	0.59	86.1	0.77	81.9	0.13	85.3
6.28	Clorsulon	Anthelmintic	1.83	86.1	0.78	83.8	0.47	81.6
11.89	Closantel	Anthelmintic	10.78	90.0	9.15	83.6	0.31	37.7
8.21	Cloxacillin	Antibiotic (b-lactam)	4.87	81.1	20.05	83.1	2.90	90.0
8.54	Crystal violet	Aquaculture (Dye)	0.10	61.1	0.24	69.7	NA	NA
0.82	Cyromazine	Ectoparasiticide	3.78	67.6	2.29	62.1	0.14	78.4
4.84	Danofloxacin	Antibiotic	0.66	61.1	0.76	59.2	0.45	68.2
5.3	Dapsone	Antibiotic	0.61	84.7	0.46	83.5	0.33	89.3
11.38	Decoquinatate	Cocciostat (antiprotozoal)	0.08	72.6	0.15	76.9	0.10	62.8
6.53	Derquantel	Anthelmintic	0.10	72.4	0.05	81.6	0.02	81.2



Table 4. Continued

Retention Time	Compound	Class	Salmon Fillet MDL (ng/g)	Salmon Fillet Recovery	Bovine Muscle MDL (ng/g)	Bovine Muscle Recovery	Milk MDL (ng/g)	Milk Recovery
1.84	Desacetylcefapirin	Metabolite	2.59	66.2	6.42	38.7	0.81	82.0
7.95	Dexamethasone	Steroid anti-inflammatory	0.23	89.3	0.37	100.9	0.10	89.3
9.78	Diclazuril	Coccidiostat (antiprotozoal)	0.23	81.4	0.26	82.3	0.43	85.5
9.72	Diclofenac	NSAID	0.28	70.4	0.55	71.1	NA	NA
8.46	Dicloxacillin	Antibiotic (b-lactam)	1.25	82.6	1.18	81.4	**	**
2.58	Dicyclanil	Insecticide/larvacide	4.80	81.9	3.31	82.5	0.41	89.4
5.22	Difloxacin	Antibiotic (quinolone)	0.74	75.0	5.81	76.5	0.85	74.1
9.63	Diflubenzuron	Insecticide	1.73	95.3	1.92	84.7	0.22	84.7
4.07	Dimetridazole	Coccidiostat (antiprotozoal)	0.14	93.4	0.12	84.6	NA	NA
12	Doramectin	Anthelmintic	3.83	81.0	8.65	87.8	1.30	83.4
6.07	Doxycycline	Antibiotic (tetracycline)	0.85	54.4	2.18	52.5	1.19	68.7
9.79	Emamectin B1a	Anthelmintic	0.21	82.2	0.12	80.7	0.06	79.6
4.92	Enrofloxacin	Antibiotic (quinolone)	0.30	71.1	0.33	65.4	0.50	68.9
5.33	Epichlortetracycline	Antibiotic (tetracycline)	1.10	49.9	14.22	47.6	0.92	71.2
4.68	Epioxytetracycline	Antibiotic (tetracycline)	17.77	53.0	18.87	30.4	1.95	65.4
4.62	Epitetracycline	Antibiotic (tetracycline)	4.43	62.1	7.33	58.6	0.82	69.0
11.59	Eprinomectin B1a	Anthelmintic	0.30	81.7	0.70	92.3	0.13	81.0
7.04	Erythromycin	Antibiotic (macrolide)	1.62	87.5	1.17	84.7	0.28	95.4
9.98	Ethyl violet	Aquaculture (Dye)	0.03	45.5	0.07	61.1	NA	NA
9.65	Febantel	Anthelmintic	0.25	89.6	0.39	95.9	NA	NA
8.4	Fenbendazole	Anthelmintic	0.22	83.7	0.21	82.9	0.08	86.7
7.02	Fenbendazole Sulfone	Fenbendazole metabolite	0.15	85.4	0.41	85.1	0.06	88.4
8.34	Firocoxib	NSAID	28.84	78.2	9.82	92.5	3.87	85.4
11.05	Fluazuron	Insecticide	1.32	72.9	3.14	113.0	0.21	86.7
7.64	Flubendazole	Anthelmintic	0.24	88.3	0.28	83.1	0.16	89.8
5.78	Flubendazole amine	Flubendazole metabolite	0.14	63.5	0.56	69.1	0.17	80.7
7.52	Flumequine	Antibiotic (quinolone)	1.47	80.7	2.48	84.1	0.56	62.6
9.07	Flunixin	NSAID	0.07	58.2	0.21	83.4	NA	NA
3.9	Furaltadone	Antibiotic (nitrofurantoin)	0.15	86.7	0.07	78.6	NA	NA
5.94	Gamithromycin	Antibiotic (macrolide)	0.75	61.8	3.11	79.3	0.33	76.2
6.07	Halofuginone	Coccidiostat (antiprotozoal)	0.07	49.0	0.34	55.6	0.34	70.2
6.8	Haloperidol	Sedative and antiemetic	0.26	81.4	0.41	82.9	NA	NA
5.36	Hydroxyipronidazole	Ipronidazole metabolite	0.56	82.6	0.24	81.7	NA	NA
2.3	Hydroxymetronidazole	Metronidazole metabolite	0.12	91.9	0.15	77.1	NA	NA
6.17	Ipronidazole	Coccidiostat (antiprotozoal)	0.09	71.9	0.10	82.8	NA	NA
5.66	Isoxsuprine	$\beta$ -agonist	0.06	75.3	0.09	62.7	0.11	80.9
12.34	Ivermectin B1a	Anthelmintic	0.55	69.5	2.27	76.2	**	**
7.99	Josamycin	Antibiotic (macrolide)	1.33	80.1	3.26	81.7	0.24	86.0
8.56	Ketoprofen	NSAID	0.14	80.2	0.16	81.0	NA	NA
12.12	Lasalocid	Coccidiostat (antiprotozoal)	0.12	59.7	0.23	61.1	0.45	57.9
7.06	Leucocrystal violet	Aquaculture (Dye)	0.29	75.0	0.14	64.5	NA	NA
8.93	Leucomalachite green	Aquaculture (Dye)	0.05	71.8	0.05	74.6	NA	NA
4.45	Levamisole	Anti parasitic worm	0.23	84.1	0.06	103.9	0.33	81.8
4.45	Lincomycin	Antibiotic (macrolide)	0.69	78.1	0.68	53.1	0.74	70.9

Table 4. Continued

Retention Time	Compound	Class	Salmon Fillet MDL (ng/g)	Salmon Fillet Recovery	Bovine Muscle MDL (ng/g)	Bovine Muscle Recovery	Milk MDL (ng/g)	Milk Recovery
4.88	Lomefloxacin	Antibiotic (quinolone)	0.84	61.0	1.33	58.7	NA	NA
5.74	Mabuterol	$\beta$ -agonist	0.04	54.1	0.05	99.8	0.08	83.0
12.53	Maduramicin	Coccidiostat (antiprotozoal)	0.01	67.8	0.03	72.0	0.06	69.8
7.79	Malachite green	Aquaculture (Dye)	0.06	37.0	0.06	47.3	NA	NA
6.19	Mapenterol	$\beta$ -agonist	0.04	70.5	0.04	75.3	0.07	81.7
4.54	Marbofloxacin	Antibiotic (quinolone)	1.14	66.5	1.49	68.0	1.60	75.6
7.34	Mebendazole	Anthelmintic	0.26	84.7	0.49	82.5	0.16	89.7
5.57	Mebendazole amine	Anthelmintic	0.30	70.3	0.63	65.3	0.27	80.9
5.72	Mebendazol-hydroxy	Mebendazole metabolite	0.27	80.5	0.77	82.0	0.43	84.7
10.12	Medroxyprogesterone acetate	$\beta$ -agonist	0.31	69.2	0.22	86.7	0.14	88.1
10.02	Megestrol acetate	Steroidal progestin	0.15	79.9	0.03	83.7	0.09	86.8
8.51	Meloxicam	NSAID	0.33	110.4	0.29	92.9	NA	NA
5.89	Methylene Blue	Aquaculture (Dye)	0.31	77.4	0.43	77.4	NA	NA
3.74	Metronidazole	Antibiotic (nitroimidazole)	0.10	99.2	0.13	80.2	NA	NA
12.22	Monensin	Antibiotic	0.03	72.2	0.13	78.5	0.08	72.7
12.13	Moxidectin	Anthelmintic	0.36	82.9	0.43	83.8	0.36	73.7
7.32	Nalidixic acid	Antibiotic (quinolone)	0.28	74.1	0.63	79.7	0.27	44.7
12.71	Narasin	Coccidiostat (antiprotozoal)	0.03	60.3	0.15	65.1	0.02	73.6
5.24	Neospiramycin I	Major metabolite of Spiramycin I	4.23	47.0	7.52	47.0	3.90	47.4
7.08	New methylene blue	Aquaculture (Dye)	0.12	70.9	0.13	73.3	NA	NA
9.77	Niflumic acid	NSAID	0.06	77.5	0.20	80.5	NA	NA
7.66	Nile blue A	Aquaculture (Dye)	0.08	63.6	0.10	68.6	NA	NA
7.49	Nitroxynil	Anthelmintic/antiparasitic	0.12	85.9	0.11	82.5	0.13	89.6
4.67	Norfloxacin	Antibiotic (quinolone)	0.48	50.9	0.76	45.4	1.04	65.5
8.21	Oxacillin	Anthelmintic	1.90	81.9	2.18	82.4	0.49	90.6
6.37	Oxfendazole	Anthelmintic	0.11	72.3	0.42	84.6	0.12	83.0
6.15	Oxibendazole	Anthelmintic	0.15	72.3	0.32	84.6	0.29	83.1
6.37	Oxolinic acid	Antibiotic (quinolone)	0.25	69.7	0.23	84.3	0.55	68.9
10.14	Oxyclozanide	Anthelmintic	0.07	85.0	0.08	82.0	0.04	53.6
4.83	Oxytetracycline	Antibiotic (tetracycline)	2.07	50.7	2.92	35.9	2.10	66.6
5.72	Pararosaniline base	Aquaculture (Dye)	0.23	68.6	0.32	74.7	NA	NA
7.43	Penicillin G	Antibiotic b-lactam	0.23	76.4	1.09	85.6	0.17	85.9
7.78	Penicillin V	Antibiotic b-lactam	4.45	85.7	3.66	78.3	0.57	95.7
9.64	Phenylbutazone	NSAID	7.63	80.6	2.11	88.0	NA	NA
5.96	Pirlimycin	Antibiotic (lincosamide)	0.98	39.3	2.17	39.3	0.36	64.6
7.43	Propionylpromazine	Sedative and antiemetic	0.16	65.0	0.14	69.3	NA	NA
4.84	Ractopamine	$\beta$ -agonist	0.06	77.8	0.03	74.5	0.19	78.6
12.24	Rafoxanide	Anthelmintic	0.07	78.9	0.08	66.3	0.11	16.0
8.44	Rhodamine-6g	Aquaculture	0.02	73.0	0.05	79.4	NA	NA
8.79	Rifampicin	Antibiotic	1.06	65.3	2.02	76.6	0.25	69.9
9.49	Rifaximin	Antibiotic	0.54	79.7	1.24	80.7	0.65	85.9
4.02	Ronidazole	Coccidiostat	0.14	95.7	0.20	83.7	NA	NA
3.79	Salbutamol	$\beta$ -agonist	0.51	62.7	2.79	55.8	0.17	67.5
12.45	Salinomycin	Coccidiostat (ionophore)	0.05	68.5	0.04	68.0	0.06	67.1

Table 4. Continued

Retention Time	Compound	Class	Salmon Fillet MDL (ng/g)	Salmon Fillet Recovery	Bovine Muscle MDL (ng/g)	Bovine Muscle Recovery	Milk MDL (ng/g)	Milk Recovery
5.22	Salmeterol	β-agonist	0.47	80.5	0.29	80.1	0.13	86.3
5.22	Sarafloxacin	Antibiotic (quinolone)	0.66	62.8	1.23	71.5	0.85	77.0
5.57	Spiramycin I	Antibiotic (macrolide)	0.89	67.1	3.54	64.7	0.32	68.4
5.6	Sulfachlorpyridazine	Antibiotic (sulfonamide)	0.80	80.4	2.12	85.6	0.26	82.6
4.17	Sulfadiazine	Antibiotic (sulfonamide)	0.72	99.9	0.44	87.4	0.17	91.6
6.56	Sulfadimethoxine	Antibiotic (sulfonamide)	0.91	74.7	0.86	88.5	0.11	86.6
5.85	Sulfadoxine	Antibiotic (sulfonamide)	0.76	73.5	0.96	89.6	0.66	84.4
1.11	Sulfaguanadine	Antibiotic (sulfonamide)	1.27	90.1	1.25	75.3	0.23	87.3
4.72	Sulfamerazine	Antibiotic (sulfonamide)	0.63	85.3	0.69	79.5	0.31	85.9
5.17	Sulfamethazine	Antibiotic (sulfonamide)	0.60	86.0	2.25	88.2	0.10	92.6
5.13	Sulfamethizole	Antibiotic (sulfonamide)	1.01	84.0	2.69	81.4	0.16	86.5
5.8	Sulfamethoxazole	Antibiotic (sulfonamide)	0.60	75.1	1.07	90.4	0.24	83.5
5.23	Sulfamethoxypyridazine	Antibiotic (sulfonamide)	1.24	80.4	2.98	89.7	0.30	84.9
5.62	Sulfamonomethoxine	Antibiotic (sulfonamide)	1.00	83.5	1.61	85.5	0.32	86.5
1.12	Sulfanilamide	Antibiotic (sulfonamide)	1.76	96.4	3.08	80.0	0.24	93.3
4.57	Sulfapyridine	Antibiotic (sulfonamide)	0.90	89.3	0.86	82.0	0.24	86.5
6.63	Sulfaquinoxaline	Antibiotic (sulfonamide)	0.82	77.1	0.52	83.3	0.36	86.9
4.44	Sulfathiozole	Antibiotic (sulfonamide)	0.87	100.3	0.81	90.2	0.31	94.8
6.01	Sulfisoxazole	Antibiotic (sulfonamide)	0.68	77.0	1.01	88.7	0.28	91.9
10.75	Teflubenzuron	Aquaculture	0.39	83.2	0.56	89.9	0.06	82.1
3.42	Terbutaline	β-agonist	0.23	61.8	0.25	46.4	0.06	71.2
8.91	Testosterone	Steroid hormones	0.63	92.3	0.31	66.9	0.10	81.4
4.94	Tetracycline	Antibiotic (tetracycline)	2.94	63.8	2.76	56.0	2.40	70.1
4.52	Thiabendazole	Antiparasitic; antiroundworm	0.13	84.1	0.16	84.9	0.25	88.5
5.02	Thiamphenicol	Antibiotic	3.24	77.1	2.25	78.8	NA	NA
4.23	Tildipirosin	Antibiotic (macrolide)	7.20	40.1	10.34	26.7	1.20	24.2
6.2	Tilmicosin	Antibiotic (macrolide)	0.86	63.6	2.47	80.9	1.01	75.6
10.58	Tolfenamic acid	NSAID	0.41	76.2	0.20	90.1	NA	NA
9.88	Triclabendazole sulfone	Triclabendazole metabolite	0.10	74.8	0.50	86.4	0.11	87.7
9.73	Triclabendazole sulfoxide	Triclabendazole metabolite	0.60	86.3	0.55	82.5	0.21	97.2
10.19	Triclabendazole	Anthelmintic	0.15	83.0	0.24	80.9	0.08	86.2
4.58	Trimethoprim	Antibiotic	0.43	79.3	0.24	80.9	0.50	75.1
7.24	Tylosin A	Antibiotic	2.24	81.4	0.38	76.2	0.38	82.2
7.46	Tylosin-3-acetate	Metabolite	3.34	81.1	1.37	82.9	0.09	86.4
8.56	Tylvalosin	Antibiotic (macrolide)	0.78	73.1	0.12	82.3	0.11	85.0
7.92	Valnemulin	Antibiotic (pleuromutilin)	1.19	79.9	0.79	79.7	0.13	86.4
9.21	Victoria blue b	Aquaculture (Dye)	0.65	59.0	0.42	76.8	NA	NA
9.79	Victoria blue bo	Aquaculture (Dye)	1.47	52.1	1.57	68.3	NA	NA
8.41	Virginiamycin_M1	Antibiotic (streptogramin)	1.46	100.2	1.33	97.4	0.23	90.7
5.14	Xylazine	Sedative and anesthetic	0.20	60.9	0.58	68.6	NA	NA
3.67	Zilpaterol	β-agonist	0.98	51.5	0.92	42.6	0.39	64.1
	<b>Average</b>		<b>1.34</b>	<b>75.20</b>	<b>2.05</b>	<b>75.6</b>	<b>0.52</b>	<b>79.2</b>

NA = Not spiked into sample

\*\* = Not calculated

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