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Quantitative Screening of Multiresidue Veterinary Drugs in Seafood Using the Agilent 6470 Triple Quadrupole LC/MS

Authors

Siji Joseph, Aimei Zou,
Limian Zhao, Patrick Batoon,
and Chee Sian Gan
Agilent Technologies, Inc.

Abstract

This application note demonstrates the use of the Agilent Comprehensive Veterinary Drug dMRM Solution for the screening of target residues in seafood matrices. The quantitation performance for 210 veterinary drugs was evaluated in shrimp and salmon matrices. The workflow experimental conditions included sample preparation, chromatographic separation, MS detection, and data processing, in accordance with the Comprehensive Veterinary Drug dMRM Solution kit.

Method performance was assessed based on limit of detection (LOD), limit of quantitation (LOQ), calibration curve linearity, accuracy, precision, and recovery analysis. Method sensitivity was established using matrix-matched samples within the range of 0.1 to 100 µg/kg. All target analytes demonstrated a linearity of R^2 >0.99 with calibration curves plotted from the LOQ to 100 µg/kg. Target area response % RSD was <14%, and retention time % RSD was <0.4%. Analyte recovery and repeatability were assessed using two levels of fortified sample matrix quality control (QC) samples at 10 and 25 µg/kg. Over 93 percent of targets fell within 60 to 120% recovery, with recovery repeatability (calculated using intrabatch technical replicates) of % RSD ≤20%. Both shrimp and salmon matrices showed similar recovery and precision, demonstrating the applicability and capability of the Agilent Comprehensive Veterinary Drug dMRM Solution for routine multiresidue screening in seafood matrices.

Introduction

The Agilent Comprehensive Veterinary Drug dMRM Solution (G5368AA) is a complete end-to-end workflow solution that includes sample extraction and matrix cleanup, chromatographic separation and MS detection method conditions, and data analysis and quantitation methodology. The workflow was originally developed for the accurate and reliable analysis of more than 200 multiclass veterinary drugs in chicken, beef, and pork muscle.[1] This study aims to demonstrate that this solution can be applied to seafood matrices such as shrimp and salmon as well.

Of the total 210 target analytes in this study, 60 of them had maximum residue limits (MRL) established in seafood regulated by the AOAC[2]—with an additional 11 targets regulated by US FDA-CFR,[3] US FSIS,[4] or EU[5] regulations/guidelines.

Experimental

Standards and reagents

Veterinary drug standards were purchased from Sigma-Aldrich (St. Louis, MO, USA), Toronto Research Chemicals (Ontario, Canada), and Alta Scientific (Tianjin, China). Agilent LC/MS-grade acetonitrile (ACN), methanol (MeOH), and water were used for the study. All other solvents used were HPLC-grade from Sigma-Aldrich. LC/MS additives for mobile phases were also purchased from Sigma-Aldrich. Individual stock solutions of veterinary drugs were prepared from powdered or liquid veterinary drug standards at 1,000 or 2,000 µg/mL using an appropriate solvent (MeOH, or dimethyl sulfoxide (DMSO), or ACN, or water or solvent mixture). A few stock standard solutions (100 µg/mL) were obtained from the suppliers listed above.

A comprehensive standard mix (1 µg/mL of each target analyte in 50/50 ACN/water) was prepared from individual stock solutions and used for this experiment.

Sample preparation

Fresh samples of shrimp and salmon were purchased from a local grocery. Samples were homogenized using a domestic blender. A 2.0 ±0.1 g portion of blended meat was weighed in a 50 mL conical polypropylene tube. If not analyzed immediately, the homogenized meat samples were stored at −20 °C.

Sample preparation was performed in accordance with the procedure defined in the Agilent Comprehensive Veterinary Drug dMRM Solution (G5368AA) workflow guide shown in Figure 1.[6] Sample preparation was based on using solvent extraction followed by cleanup using Agilent Captiva EMR—Lipid cartridges (part number 5190-1003), aided by the Agilent positive pressure manifold (PPM-48, part number 5191-4101) processor.

Pre-extraction (matrix-spiked) QC samples were fortified by spiking the appropriate veterinary standard solution into the homogenized seafood matrices at two levels in each matrix, 10 µg/kg for mid-range QC (MQC), and 25 µg/kg for high-range QC (HQC), respectively. After spiking standards into the matrix, samples were vortexed for 30 seconds, then equilibrated for 15 to 20 minutes. Equilibration allowed the spiked standards to infiltrate the sample matrix before sample extraction.
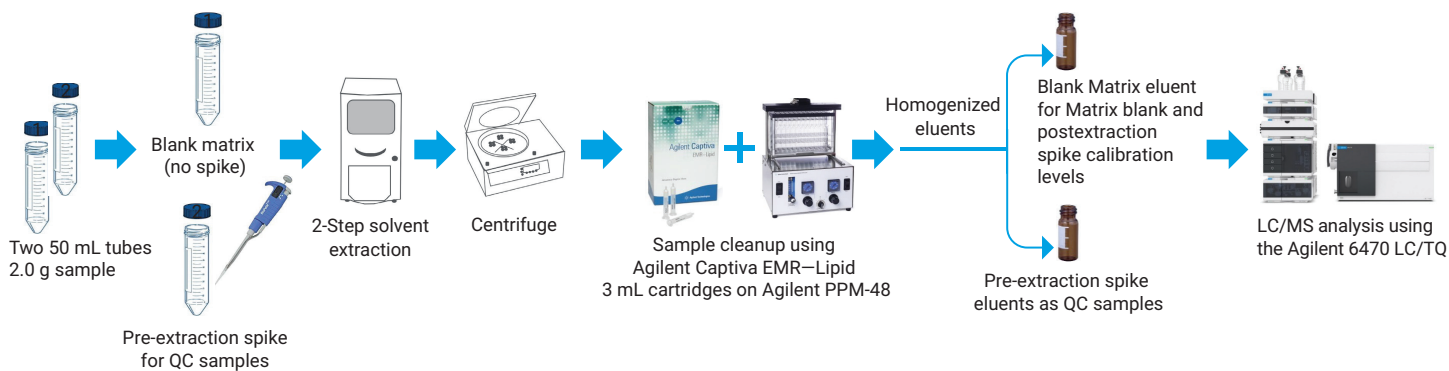
Figure 1. Sample extraction procedure using solvent extraction followed with Agilent Captiva EMR-Lipid cleanup. (The size of the images is not to scale).

Postextraction calibration standards

Blank matrix extract was prepared using unfortified matrix samples. Matrix-matched calibration standards were prepared by spiking appropriate standards into the blank matrix extract. The targeted concentrations of matrix-matched calibration levels in seafood matrix were 0.1, 0.25, 0.5, 1.0, 2.5, 5.0, 10.0, 25.0, 50.0, and 100.0 µg/kg. Considering the 10x dilution factor introduced during sample preparation, the actual postextraction spiked calibration standard levels were 0.01, 0.025, 0.05, 0.10, 0.25, 0.5, 1.0, 2.5, 5.0, and 10.0 µg/L (ppb) in blank matrix extract.

Neat standards at 2.5 µg/L in a 50/50 ratio of ACN/water was used to evaluate matrix effects by comparing the responses in the corresponding matrix-matched calibration standards.

Instrumentation

Chromatographic separation was performed using an Agilent InfinityLab Poroshell 120 EC-C18 column (part number 695575-302) installed on an Agilent 1290 Infinity II LC. The individual modules of the 1290 Infinity II LC were:

– Agilent 1290 Infinity II Flexible pump (G7104C)

– Agilent 1290 Infinity II Multisampler (G7167A)

– Agilent 1290 Infinity II Multicolumn Thermostat (G7116A)

Mobile phase A was water with 4.5 mM ammonium formate, 0.5 mM ammonium fluoride, and 0.1% formic acid; mobile phase B was 50/50 ACN/MeOH with 4.5 mM ammonium formate, 0.5 mM ammonium fluoride, and 0.1% formic acid. The LC system was equipped with a 20 µL injection loop and multiwash capability. Please refer to the workflow guide included with the Agilent Comprehensive Veterinary Drug dMRM Solution for additional details.[6]

The "6470_VetDrug_Comprehensive.m" method included in the Agilent Comprehensive Veterinary Drug dMRM Solution for the Agilent 6470 LC/TQ (G6470AA) was used directly for the acquisition. The LC/TQ with an Agilent Jet Stream (AJS) ion source was operated in dynamic MRM (dMRM) mode. The LC/TQ autotune was performed in unit resolution with report m/z below 100 mode enabled. Agilent MassHunter Acquisition software (version 10.0) was used for data acquisition and Agilent MassHunter Quantitative analysis software (version 10.0) was used to process the data.

Results and discussion

Simple workflow method for screening of multiclass veterinary drugs

Of the total 210 target analytes, 60 have maximum residue limits (MRLs) established for seafood under AOAC guidelines, and the results are summarized in Table 1. Method sensitivity was established using postextraction spiked calibration levels, and recovery analysis was demonstrated using pre-extraction spiked QC levels.

The MRL of all targets listed in the AOAC guidelines for seafood are greater than or equal to 10 µg/kg, and recovery analysis using MQC (10 µg/kg) was appropriate to screen all these targets. For lufenuron (MRL at 1,350 µg/kg), the established LOD using a matrix-matched calibration level was 10 µg/kg, and HQC (at 25 µg/kg) was used to assess the target performance. In summary, MRL requirements for all 60 targets in shrimp can be achieved per AOAC guidelines.

LC/TQ method

The LC/TQ method included in the Comprehensive Veterinary Drug dMRM Solution was used for performance evaluation. Chromatographic separation using the InfinityLab Poroshell EC-C18 column resulted in good separation and retention time distribution of 210 veterinary drugs with a 13-minute gradient. Two or three target-specific MRM transitions were included in the method for each compound to meet the regulatory requirements for identification and confirmation by the LC/TQ method. The default dMRM method used a cycle time of 750 ms, and dwell times for each dMRM transition ranged between 7 to 370 ms.

LOD, LOQ, and calibration curve linearity

LOD and LOQ were established using various lower levels of postextraction spiked (matrix-matched) calibration levels. For each compound, the signal-to-noise ratio (S/N) defined for LOD was S/N >3, and S/N >10 for LOQ, using the peak height for signal and an auto-RMS algorithm for noise, included in Agilent's MassHunter Quantitative Analysis software. For determining LOQ, an additional assessment like target selectivity from the sample matrix and analyte response reproducibility were also considered. The LOD and LOQ calculation based on only S/N may be impacted if there is matrix contribution due to the endogenous presence of targets in the matrix or matrix interferences. When there was a contribution from the matrix to the target analyte, LOD was defined as the three-fold peak area of matrix contribution, and LOQ was defined as the five-fold area of matrix contribution. Analyte response reproducibility calculated from three replicate injections was another important consideration for LOQ, and % RSD was less than the typical acceptance criteria of 20%. The lowest matrix-matched level corresponded to 0.1 µg/kg, which is considerably lower than the regulatory MRLs requirement (≥10 µg/kg) for most vet drugs in seafood. Additionally, the signal intensity of many target MRM signals showed the potential to reach lower LODs and LOQs.

A calibration curve for each target was generated using matrix-matched calibration levels from the defined LOQ to the highest spiked level. For example, for a target with LOD at 0.1 µg/kg, the calibration curve was constructed from 0.25 to 100 µg/kg; for a target with LOD at 1 µg/kg, the calibration curve range was 2.5 to 100 µg/kg; and for a target with LOD at 10 µg/kg, the calibration curve range was 25 to 100 µg/kg. The regression model used in this study was linear with ignored origin and $1/x$ weighting. All targets met the calibration curve linearity requirement of $R^2 > 0.99$. Table 1 shows the LOD, LOQ, and calibration curve data of all targets in the shrimp.

Instrument method accuracy and precision

The average accuracy value for each postextraction (matrix-matched) spiked calibration level was calculated from triplicate injections. The average accuracy values for all targets were well within the range of 70 to 120%.

Precision was determined by calculating the % RSD of the target response and retention time (RT) using triplicate injections for the postextraction spiked calibration levels. Good RTs and response precision values for all targets in both matrices were obtained. As an example, response % RSD for all targets in the shrimp at 25 µg/kg was <14%, and RT % RSD of all targets were within 0.3%. The precision results confirm the excellent reproducibility of chromatographic separation and MS detection.

Target recovery and intrabatch repeatability

In this experiment, the impact of sample preparation on target recovery was assessed using two levels of pre-extraction spiked QC samples (MQC and HQC). Each QC level was prepared with four technical preparations. Each technical preparation was injected for instrument analysis in duplicates. Recovery was calculated using target response in pre-extraction spiked QCs and measured response using postextraction spiked calibration curve equations. The average recovery was calculated from duplicate injections of four technical preparations. The intrabatch recovery repeatability was measured as % RSD of recovery values calculated using intraday technical preparations of pre-extraction spiked QC samples. An appropriate level of pre-extraction spiked QC sample was used to evaluate method recovery and repeatability.

Recoveries for over 93% of the targets met the acceptable range of 60 to 120% with intrabatch repeatability RSD ≤20%. For targets baquiloprim, chlortetracycline, deacetylcefapirin, doxycycline, erythromycin, haloxon, imidocarb, maduramicin, malachite green, monensin, narasin, nicotine, and salinomycin, recoveries were within a range of 30 to 60%; however, the recovery repeatability for these targets was within 20% RSD, demonstrating consistent behavior with each technical preparation. These results confirmed the repeatability of analyte recovery using Captiva EMR— Lipid sample cleanup. Poor recovery was observed for amprolium and dipyrone hydrate-metabolite (<30%), however, no MRL is established for these veterinary drug targets in seafood. The recovery repeatability % RSD values for these targets were within 23%.

Figure 2 summarizes the recovery repeatability of 25 representative targets from each technical preparation and injection replicates of MQC sample. These 25 standards correspond to Agilent's vet drug system suitability mix (part number 5799-0015), highlighted in Table 1. The consistent recovery values for all targets across four technical preparations of QC samples confirm the robustness of workflow. The average recovery and repeatability results for all targets are included in Table 1.

Matrix effect assessment

Matrix effect (ME) is defined as the ratio of analyte response (I) in matrix-matched samples with those in the corresponding neat standards (Equation 1). The closer the ME value to 100%, the less matrix effect presents.

Equation 1.

$$ME = \frac{I_{matrix}}{I_{solvent}} \times 100$$

Typically, there is no strict requirement on acceptable ME criteria, because the matrix effect can be corrected by the matrix-matched calibration curve. However, the matrix effect is an important parameter for method sensitivity and reliability assessments. In this study, ME was investigated using the matrix-matched levels at 2.5 µg/L in comparison to the corresponding neat standards.
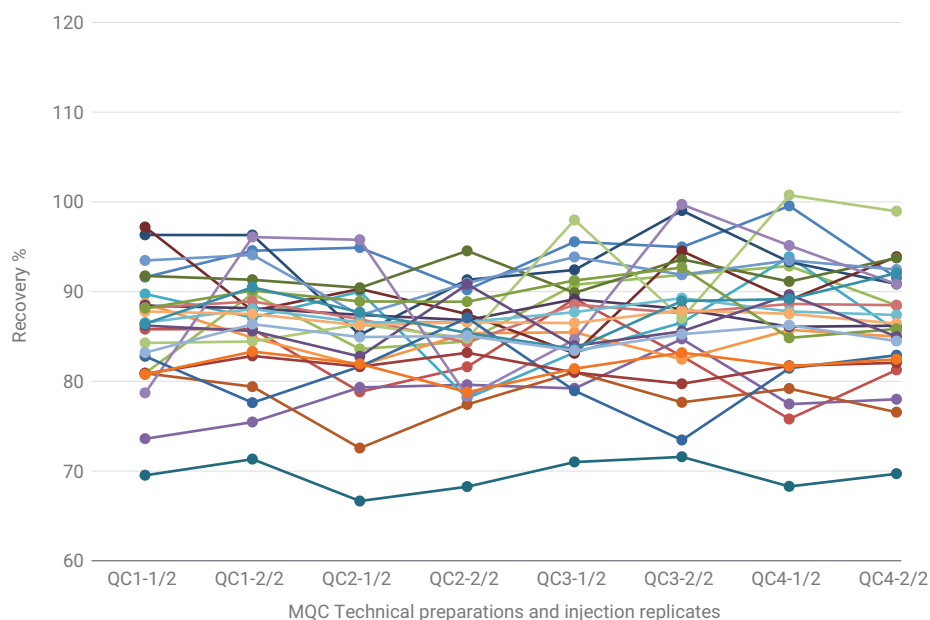
Figure 2. Recovery of 25 vet drug targets included in the Agilent vet drug system suitability mix using four technical preparations and duplicate injections. Please refer to Table 1 for the list of 25 targets in the Agilent vet drug system suitability mix.

In the shrimp matrix, within a total of 210 analytes, >96% of targets showed ME of over 75%, indicating minor matrix suppression. Five targets, cyromazine, dicloxacillin, dipyrone hydrate metabolite, narasin, and salinomycin, resulted in a ME of 50 to 75%, indicating moderate ion suppression. Two targets, 2,4,6-tri amino-pyrimidine-5-carbonitrile and metronidazole-OH, showed ME within 25 to 50%, indicating significant ion suppression; and one target (erythromycin) exhibited severe ion suppression with ME <25%.

Method verification in salmon matrix

The method sensitivity in the salmon matrix was similar to that in the shrimp matrix. Calibration curves were plotted from LOQ to 100 µg/kg, and all targets demonstrated linearity R^2 >0.99.

Instrument method precision results for target response and retention time precision % RSD values were <13% and <0.5% respectively. Instrument method accuracy values at 2.5 µg/L postextraction spiked calibration level was within 80 to 110%, with % RSD ≤13% (n = 3). The recoveries of over 94% of targets in salmon meet the 60 to 120% acceptance criteria, and recovery repeatability RSD for all targets was ≤20%. The method performance results using salmon matrix confirmed the workflow applicability for routine vet drug screening in other seafood matrices. Two targets, amprolium and rafoxanide, exhibited poor recovery (<30%) in this matrix. The matrix suppression for a few targets from the salmon matrix was slightly higher when compared to the shrimp matrix.

Figure 3 shows an MRM chromatogram overlay of two targets, trimethoprim, and febantel, for pre-extraction spiked QC samples at a concentration of 10 µg/kg in salmon. The average recoveries of these targets were 82% and 87%, respectively. The consistent response counts from a total of eight MRM traces; four technical preparations injected in duplicates indicate the good recovery repeatability (% RSD <5%) of these targets.
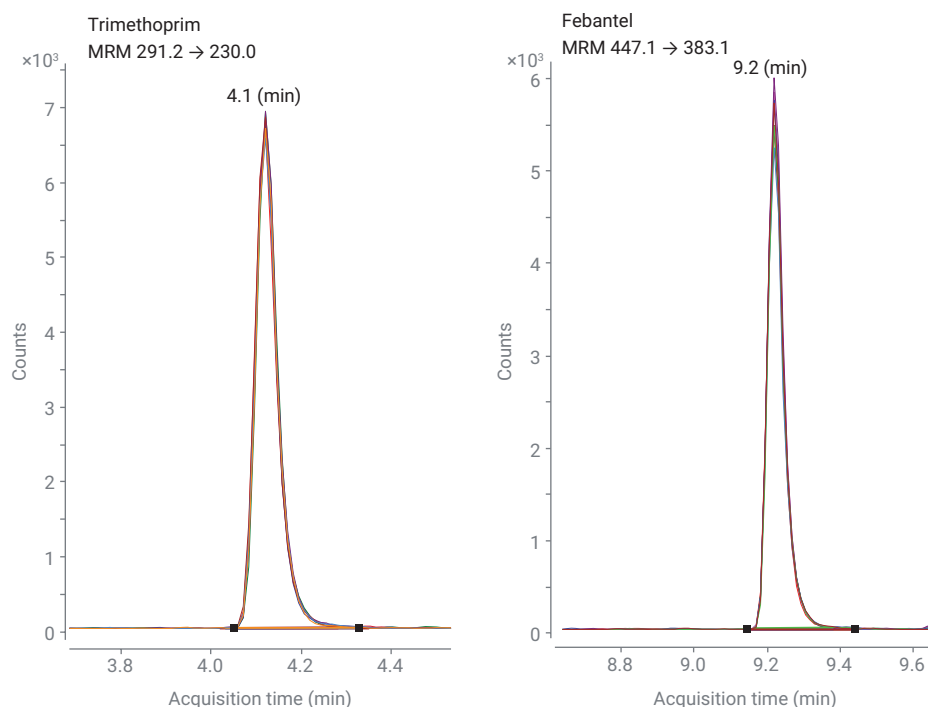
Interference was observed for the analysis of chlorhexidine, clindamycin, and gonadotropin in both shrimp and salmon matrices. Trace residues of carbadox, doxycycline, enrofloxacin, mebendazole, nalidixic acid, nicotine, and oxytetracycline affected the LOQ determinations in shrimp. Alternatively, the residues from dimetridazole, emamectin B1a benzoate, piperonyl butoxide ammonia, and sulfapyridine affected the LOQ determinations in salmon.

Figure 3. MRM chromatograms overlay (n = 8) of two selected veterinary drug targets corresponding to 10 µg/kg in salmon across the method retention window. The consistent MRM response promises the recovery repeatability of these targets.

Method transferability to the Agilent 1260 Prime LC method

The method was transferred from G5368AA based on the Agilent 1290 Infinity II LC to the Agilent 1260 Infinity II Prime LC configuration. The objective was to assess the method robustness and scalability across different front-end LC configurations. The individual modules of the 1260 Infinity II Prime LC System were:

– Agilent 1260 Infinity II Flexible pump (G7104C)

– Agilent 1260 Infinity II Multisampler (G7167A)

– Agilent 1260 Infinity II Multicolumn Thermostat (G7116A)

A 20 µL injection loop was used and 0.12 mm id tubings were included in the flow path from the autosampler to the detector. The 1260 Infinity II Prime LC gradient was appropriately modified to include the variations due to the pump mixing pattern and system volume changes. Typical MRM chromatograms for all 210 veterinary drug targets postextraction spiked at 2.5 µg/L concentration in a shrimp blank matrix extract (equivalent to a matrix-spiked concentration of 25 µg/kg) using the 1290 Infinity II LC System and 1260 Infinity II Prime LC System are illustrated in Figures 4 and 5, respectively. The elution profile from a 1260 Infinity II Prime LC is comparable to that from a 1290 Infinity II LC system with proper adjustments.

Targets such as 2,4,6-tri amino-pyrimidine-5-carbonitrile, amoxicillin, baquiloprim, cefapirin, cotinine, deacetylcefapirin, dicloxacillin, dicyclanil, diminazene, ractopamine, salbutamol (albuterol), sulfaguanidine, tilmicosin, and zilpaterol showed split peaks due to solvent effect. The spectrum summation integrator algorithm was used to integrate these targets. The peak shape for these targets can be improved by converting samples in a higher aqueous mixture for LC/TQ injection.

Workflow performance verification using Agilent 6495C LC/TQ

The workflow performance was also verified for Agilent 6495C LC/TQ (G6495CA) using the MS detection method included in the Agilent Comprehensive Veterinary Drug dMRM Solution (Results not shown). The method performance results from Agilent 6470 LC/TQ and 6495C LC/TQ models were in good alignment with additional benefits of improved sensitivity when using the latter.
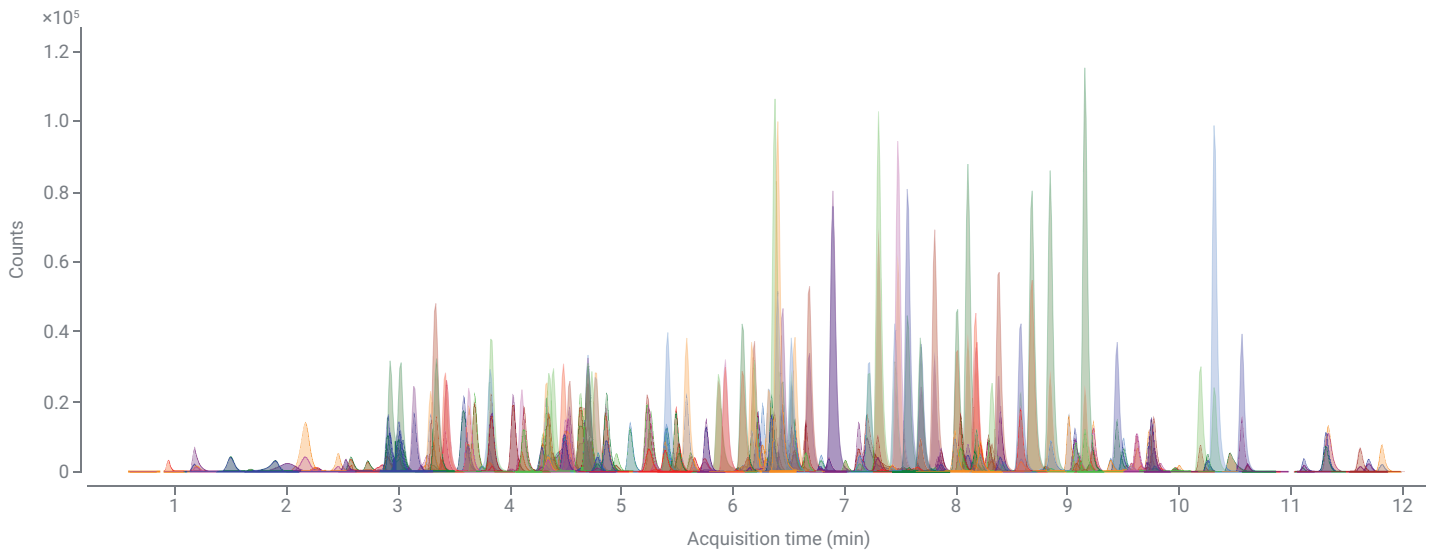
Figure 4. Representative MRM chromatogram of 210 veterinary drug targets postextraction spiked at 2.5 μg/L in the shrimp blank matrix extract.
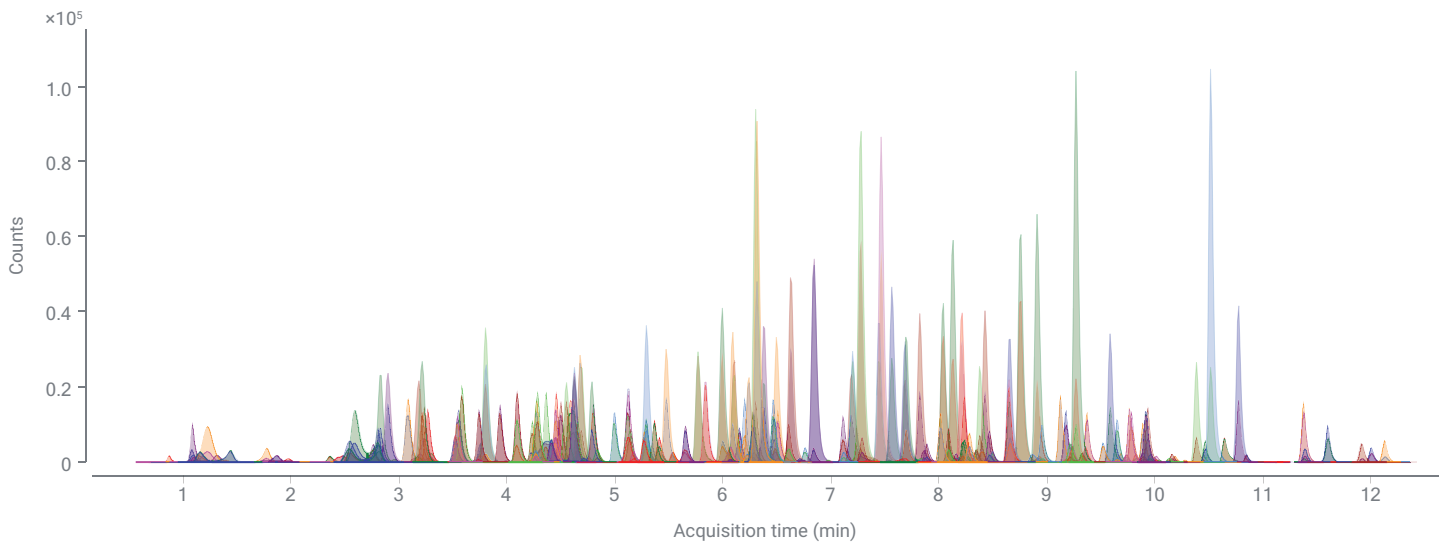
Figure 5. Representative MRM overlay of 210 veterinary drug targets postextraction spiked at 2.5 μg/L in shrimp blank matrix extract, separated using an Agilent InfinityLab Poroshell EC-C18 column installed on an Agilent 1260 Infinity II Prime LC System.

Conclusion

This study summarizes the efficient utilization of the Agilent Comprehensive Veterinary Drug dMRM Solution for the screening and quantitation of 210 multiclass veterinary drugs in shrimp and salmon matrices. The results from the shrimp matrix proved the reliability of the workflow solution for routine screening analysis of 60 AOAC-listed veterinary drug targets.

The workflow used a simple sample preparation protocol based on solvent extraction followed by Agilent Captiva EMR—Lipid cleanup. This provided highly efficient, selective, and reproducible matrix/lipid removal without impacting the target analyte recoveries. The 13-minute LC method using an Agilent InfinityLab Poroshell EC-C18 column offered good chromatographic separation and even RT distribution of all targets. LC/TQ data acquisition was in dMRM mode with fast polarity switching for the most efficient use of instrument cycle time. The method's sensitivity helped to achieve sub-5 µg/L (ppb) LODs for most analytes.

References

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2. AOAC guidelines on Screening and identification method for regulated veterinary drug residues in food, Version 7; June 20, **2018**.

3. The United States, Code of Federal Regulations (CFR) - Title 21, Tolerance of Residues in New Animal Drugs in Food, *Part 556, volume 6*, April 1, **2019**.

4. The United States, Chemical contaminants of public health concern used by the Food Safety and Inspection Service (FSIS), **2017**.

5. Official Journal of the European Union, Pharmacologically active substances and their classification regarding maximum residue limits (MRL), Commission Regulation (EU) No 37/2010.

6. G5368AA Comprehensive Veterinary Drug dMRM Solution, *Agilent Technologies workflow guide*, D0002979.

Table 1. Target screening results using shrimp matrix based on AOAC guidelines. The results were generated based on Agilent 1290 Infinity II LC and 6470 LC/TQ systems. Note that those highlighted in light gray are included in the vet drug test mix (part number 5799-0015).

No.	Compound Name	RT (min)	Functional Use/ Chemical Class	CAS Number	AOAC[2] MRL (µg/kg)	LOD (µg/kg)	Linear Calibration Curve Range (µg/kg) with R^2 >0.99	MQC Recovery (%)	MQC RSD (%)
1	2, 4, 6-triamino-pyrimidine-5-carbonitrile	1.58	Insecticide	465531-97-9	N/A	5	10 to 100	98	7
2	2,4-DMA [Amitraz Metabolite]	4.34	Insecticide	33089-74-6	N/A	0.5	1 to 100	85	2
3	2-Quinoxalinecarboxylic acid [QCA]	4.13	Quinoxalines	879-65-2	N/A	5	10 to 100	81	20
4	4-epi-oxytetracycline	4.26	Antibiotic/Tetracycline	14206-58-7	200	0.5	1 to 100	75	3
5	4-epi-tetracycline	4.17	Antibiotic/Tetracycline	79-85-6	200	0.25	0.5 to 100	79	5
6	5-Hydroxy thiabendazole	3.52	Anthelmintic/Benzimidazoles	948-71-0	N/A	0.25	0.5 to 100	83	3
7	5-Hydroxyflunixin	8.29	NSAIDs	75369-61-8	N/A	0.1	0.25 to 100	89	2
8	Acepromazine	7.34	Tranquilizer	61-00-7	N/A	0.1	0.25 to 100	77	4
9	Acetyl isovaleryl tylosin [Tylvalosin]	8.71	Antibiotic/Macrolides	63409-12-1	N/A	1	2.5 to 100	72	7
10	Albendazole	8.01	Anthelmintic/Benzimidazoles	54965-21-8	N/A	0.1	0.25 to 100	87	2
11	Albendazole sulfone	6.14	Anthelmintic/Benzimidazoles	75184-71-3	N/A	0.25	0.5 to 100	94	3
12	Albendazole sulfoxide	5.54	Anthelmintic/Benzimidazoles	54029-12-8	N/A	0.5	1 to 100	90	5
13	Albendazole-2-aminosulfone	3.71	Anthelmintic/Benzimidazoles	80983-34-2	N/A	0.5	1 to 100	82	5
14	Alpha Zearalanol	8.25	Hormones	26538-44-3	N/A	2.5	5 to 100	87	11
15	Altrenogest	8.96	Hormones	850-52-2	N/A	0.25	0.5 to 100	89	4
16	Aminoflubendazole	6.08	Anthelmintic/Benzimidazoles	82050-13-3	N/A	0.1	0.25 to 100	86	3
17	Amoxicillin	2.78	Antibiotic/Beta-lactam	26787-78-0	50	2.5	5 to 100	67	10
18	Ampicillin	3.94	Antibiotic/Beta-lactam	69-53-4	50	2.5	5 to 100	77	11
19	Amprolium	1.19	Antimicrobial	13082-85-4	N/A	1	2.5 to 100	13	11
20	Azaperone	5.76	Tranquilizer	1649-18-9	N/A	0.25	0.5 to 100	76	3
21	Azithromycin	6.16	Antibiotic/Macrolides	83905-01-5	N/A	0.25	0.5 to 100	68	3
22	Baquiloprim	2.63	Antimicrobial	102280-35-3	N/A	0.5	1 to 100	44	5
23	Betamethasone	7.77	Growth promoters/Corticosteroids	378-44-9	N/A	1	2.5 to 100	90	3
24	Cabergoline	4.58	Dopamine receptor	81409-90-7	N/A	0.5	1 to 100	64	4
25	Carazolol	6.06	Tranquilizer	57775-29-8	N/A	0.1	0.25 to 100	85	3
26	Carbadox	4.41	Antimicrobial	6804-07-5	N/A	0.5	1 to 100	91	3
27	Carprofen	9.00	NSAIDs	53716-49-7	12	5	10 to 100	103	16
28	Cefalexin	3.91	Antibiotic/Beta-lactam	15686-71-2	N/A	10	25 to 100	90 #	12 #
29	Cefalonium	3.91	Antibiotic/Beta-lactam	5575-21-3	N/A	5	10 to 100	91	19
30	Cefapirin	3.19	Antibiotic/Beta-lactam	21593-23-7	N/A	0.5	1 to 100	68	5
31	Cefazolin	4.31	Antibiotic/Beta-lactam	25953-19-9	N/A	5	10 to 100	93	15
32	Cefoperazone	5.14	Antibiotic/Beta-lactam	62893-19-0	N/A	5	10 to 100	92	12
33	Cefquinome	3.69	Antibiotic/Beta-lactam	84957-30-2	N/A	2.5	5 to 100	78	9
34	Ceftiofur	6.27	Antibiotic/Beta-lactam	80370-57-6	N/A	1	2.5 to 100	84	7
35	Cefuroxime	4.40	Antibiotic/Beta-lactam	55268-75-2	N/A	5	10 to 100	109	8
36	Chloramphenicol	6.24	Antibiotic/Amphenicols	56-75-7	N/A	5	10 to 100	84	13
37	Chlorhexidine	7.08	Antimicrobial	55-56-1	N/A	10	25 to 100	71 #	9 #
38	Chlormadinone	9.45	Hormones	1961-77-9	N/A	2.5	5 to 100	90	11
39	Chlorpromazine	8.06	Tranquilizer	50-53-3	N/A	0.1	0.25 to 100	73	2
40	Chlortetracycline	5.94	Antibiotic/Tetracycline	57-62-5	200	2.5	5 to 100	57	16
41	Ciprofloxacin	4.43	Antibiotic/Quinolones	85721-33-1	N/A	0.25	0.5 to 100	80	3
42	Clenbuterol	5.28	Growth promoters/ Beta-agonists	37148-27-9	N/A	0.1	0.25 to 100	86	2
43	Clindamycin	6.45	Antibiotic/Macrolides	18323-44-9	N/A	10	25 to 100	88 #	4 #

No.	Compound Name	RT (min)	Functional Use/ Chemical Class	CAS Number	AOAC[2] MRL (µg/kg)	LOD (µg/kg)	Linear Calibration Curve Range (µg/kg) with R[2] >0.99	MQC Recovery (%)	MQC RSD (%)
44	Clopidol	3.56	Coccidiostats	2971-90-6	N/A	0.5	1 to 100	83	5
45	Closantel	10.54	Anthelmintic	57808-65-8	N/A	1	2.5 to 100	86	4
46	Colchicine	6.72	NSAIDs	64-86-8	N/A	1	2.5 to 100	85	4
47	Cotinine	2.35	Insecticide	486-56-6	N/A	0.25	0.5 to 100	75	2
48	Coumaphos	9.58	Anthelmintic	56-72-4	N/A	1	2.5 to 100	86	10
49	Cyromazine	2.47	Anthelmintic	66215-27-8	N/A	2.5	5 to 100	73	3
50	Danofloxacin	4.63	Antibiotic/Quinolones	112398-08-0	100	0.25	0.5 to 100	73	3
51	Dapson	4.67	Antibiotic/Sulfonamides	80-08-0	N/A	0.1	0.25 to 100	86	5
52	Dapson N-Acetyl	5.40	Antibiotic/Sulfonamides	565-20-8	N/A	0.5	1 to 100	88	4
53	Deacetylcefapirin	2.30	Antibiotic/Beta-lactam	104557-24-6	N/A	2.5	5 to 100	52	5
54	Diaveridine	3.73	Antimicrobial	5355-16-8	N/A	0.1	0.25 to 100	83	2
55	Diazinon	9.64	Insecticide	333-41-5	N/A	0.25	0.5 to 100	79	3
56	Diclofenac	9.14	NSAIDs	15307-86-5	N/A	0.5	1 to 100	87	6
57	Dicloxacillin	8.11	Antibiotic/Beta-lactam	3116-76-5	300	5	10 to 100	102	10
58	Dicyclanil	2.93	Insecticide	112636-83-6	N/A	0.5	1 to 100	83	3
59	Difloxacin	5.29	Antibiotic/Quinolones	98106-17-3	300	0.25	0.5 to 100	88	5
60	Diflubenzuron	9.11	Insecticide	35367-38-5	N/A	2.5	5 to 100	85	16
61	Dimetridazole	3.66	Coccidiostats	551-92-8	N/A	10	25 to 100	85 #	13 #
62	Diminazene	2.96	Coccidiostats	536-71-0	500	5	10 to 100	72	10
63	Dinitolmide [Zoalene]	5.56	Coccidiostats	148-01-6	N/A	5	10 to 100	84	12
64	Dipyrone hydrate- metabolite [4-Methylaminoantipyrine]	3.34	NSAIDs	519-98-2	N/A	1	2.5 to 100	27	23
65	Doxycycline	6.26	Antibiotic/Tetracycline	564-25-0	10	2.5	5 to 100	49	14
66	Emamectin B1a benzoate	10.09	Anthelmintic/Avermectins	121124-29-6	100	0.1	0.25 to 100	68	1
67	Emamectin B1b benzoate	9.90	Anthelmintic/Avermectins	121424-52-0	100	2.5	5 to 100	72	9
68	Enrofloxacin	4.74	Antibiotic/Quinolones	93106-60-6	100	0.5	1 to 100	78	4
69	Erythromycin	7.40	Antibiotic/Macrolides	114-07-8	200	1	2.5 to 100	34	15
70	Ethopabate	6.60	Coccidiostats	59-06-3	N/A	0.1	0.25 to 100	91	2
71	Famphur	8.18	Insecticide	52-85-7	N/A	1	2.5 to 100	89	8
72	Febantel	9.15	Anthelmintic/Benzimidazoles	58306-30-2	N/A	0.25	0.5 to 100	87	5
73	Fenbendazole	8.59	Anthelmintic/Benzimidazoles	43210-67-9	N/A	0.1	0.25 to 100	85	2
74	Fenbendazole Sulfoxide [Oxfendazole]	6.44	Anthelmintic/Benzimidazoles	53716-50-0	N/A	0.25	0.5 to 100	93	5
75	Firocoxib	7.96	NSAIDs	189954-96-9	N/A	10	25 to 100	97 #	20 #
76	Florfenicol	5.55	Antibiotic/Amphenicols	73231-34-2	1000	1	2.5 to 100	90	5
77	Fluazuron	10.17	Insecticide	86811-58-7	N/A	0.5	1 to 100	89	3
78	Flubendazole	7.72	Anthelmintic/Benzimidazoles	31430-15-6	N/A	0.1	0.25 to 100	92	2
79	Flugestone acetate	8.35	Hormones	2529-45-5	N/A	1	2.5 to 100	88	5
80	Flumequine	7.39	Antibiotic/Quinolones	42835-25-6	500	0.1	0.25 to 100	88	1
81	Flunixin	8.75	NSAIDs	38677-85-9	N/A	0.1	0.25 to 100	88	2
82	Fluralaner	9.89	Insecticide	864731-61-3	N/A	2.5	5 to 100	87	4
83	Furazolidone	4.68	Antimicrobial/Furans	67-45-8	N/A	2.5	5 to 100	96	6
84	Gamithromycin	6.44	Antibiotic/Aminoglycosides	145435-72-9	N/A	0.25	0.5 to 100	66	8
85	Gonadotropin	7.57	Hormones	33515-09-2	N/A	10	25 to 100	86 #	16 #
86	Halofuginone	6.44	Coccidiostats	55837-20-2	N/A	0.5	1 to 100	80	6
87	Haloperidol	7.11	Tranquilizer	52-86-8	N/A	0.1	0.25 to 100	87	2
88	Haloxon	8.58	Anthelmintic	321-55-1	N/A	5	10 to 100	51	20
89	Imidocarb	3.20	Coccidiostats	27885-92-3	N/A	1	2.5 to 100	48	6

No.	Compound Name	RT (min)	Functional Use/ Chemical Class	CAS Number	AOAC[2] MRL (µg/kg)	LOD (µg/kg)	Linear Calibration Curve Range (µg/kg) with R[2] >0.99	MQC Recovery (%)	MQC RSD (%)
90	Ipronidazole	6.04	Anthelmintic/Nitroimidazoles	14885-29-1	N/A	5	10 to 100	113	12
91	Ipronidazole-OH	4.85	Anthelmintic/Nitroimidazoles	35175-14-5	N/A	1	2.5 to 100	88	5
92	Isometamidium	5.98	Anthelmintic	20438-03-3	N/A	2.5	5 to 100	71	7
93	Josamycin	8.22	Antibiotic/Macrolides	16846-24-5	N/A	1	2.5 to 100	85	5
94	Ketamine	4.74	Anesthetic	6740-88-1	N/A	0.5	1 to 100	81	7
95	Ketoprofen	8.20	NSAIDs	22071-15-4	N/A	0.5	1 to 100	91	3
96	Kitasamycin A5 [Leucomycin A5]	7.70	Antibiotic/Aminoglycosides	18361-45-0	50	1	2.5 to 100	75	11
97	Lasalocid A	10.99	Coccidiostats	25999-31-9	N/A	0.25	0.5 to 100	73	2
98	Leuco Crystal violet	10.36	Fungicides and Dyes	603-48-5	N/A	0.5	1 to 100	70	5
99	Leucomalachite green	10.48	Fungicides and Dyes	129-73-7	N/A	0.1	0.25 to 100	75	2
100	Levamisole	3.58	Anthelmintic	14769-73-4	N/A	0.25	0.5 to 100	81	2
101	Lincomycin	3.74	Antibiotic/Aminoglycosides	154-21-2	100	0.1	0.25 to 100	70	2
102	Lufenuron	10.11	Insecticide	103055-07-8	1350	10	25 to 100	99 #	10 #
103	Maduramicin Ammonium	11.59	Coccidiostats	79356-08-4	N/A	1	2.5 to 100	36	6
104	Malachite green	8.21	Fungicides and Dyes	10309-95-2	N/A	0.1	0.25 to 100	30	7
105	Malathion	8.92	Insecticide	121-75-5	N/A	0.25	0.5 to 100	83	2
106	Marbofloxacin	4.00	Antibiotic/Quinolones	115550-35-1	N/A	0.25	0.5 to 100	78	4
107	Mebendazole	7.49	Anthelmintic/Benzimidazoles	31431-39-7	N/A	0.5	1 to 100	91	1
108	Mefenamic acid	9.68	Anti-inflammatory	61-68-7	N/A	0.5	1 to 100	94	5
109	Megestrol acetate	9.43	Hormones	595-33-5	N/A	0.5	1 to 100	86	3
110	Melengestrol acetate	9.55	Hormones	2919-66-6	N/A	0.25	0.5 to 100	85	3
111	Meloxicam	8.10	NSAIDs	71125-38-7	N/A	0.25	0.5 to 100	92	2
112	Methylprednisolone	7.78	Growth promoters/Corticosteroids	83-43-2	N/A	1	2.5 to 100	91	5
113	Metoserpate	6.55	Tranquilizer	1178-28-5	N/A	0.5	1 to 100	86	3
114	Metronidazole	3.22	Anthelmintic/Nitroimidazoles	443-48-1	N/A	0.25	0.5 to 100	89	3
115	Metronidazole-OH	2.77	Anthelmintic/Nitroimidazoles	4812-40-2	N/A	1	2.5 to 100	90	4
116	Monensin	11.22	Coccidiostats	17090-79-8	N/A	0.25	0.5 to 100	41	5
117	Monepantel	9.45	Anthelmintic	851976-50-6	N/A	2.5	5 to 100	78	20
118	Morantel tartrate	5.27	Anthelmintic	20574-50-9	N/A	0.5	1 to 100	79	3
119	Moxidectin	11.04	Anthelmintic/Avermectins	113507-06-5	N/A	5	10 to 100	87	9
120	Nafcillin	8.02	Antibiotic/Beta-lactam	147-52-4	N/A	0.5	1 to 100	83	3
121	Nalidixic acid	7.21	Antibiotic	389-08-2	N/A	1	2.5 to 100	88	2
122	Narasin	11.71	Coccidiostats	55134-13-9	N/A	0.25	0.5 to 100	33	8
123	Neo-Spiramycin	5.71	Antibiotic/Macrolides	70253-62-2	200	1	2.5 to 100	62	5
124	Nequinate	9.35	Anthelmintic	13997-19-8	N/A	0.1	0.25 to 100	82	3
125	Netobimin	7.06	Anthelmintic	88255-01-0	N/A	5	10 to 100	106	11
126	Nicarbazine	8.76	Coccidiostats	587-90-6	N/A	0.5	1 to 100	82	3
127	Nicotine	1.44	Anti-herbivore	54-11-5	N/A	5	10 to 100	55	7
128	Niflumic Acid	9.07	Anti-inflammatory	4394-00-7	N/A	0.1	0.25 to 100	87	2
129	Nitroxynil	6.67	Anthelmintic	1689-89-0	N/A	2.5	5 to 100	91	8
130	Norfloxacin	4.28	Antibiotic/Quinolones	70458-96-7	N/A	0.25	0.5 to 100	78	2
131	Norgestomet	9.31	Hormones	472-54-8	N/A	1	2.5 to 100	89	7
132	Novobiocin	9.75	Antibiotic	303-81-1	N/A	1	2.5 to 100	90	9
133	Olaquindox	3.00	Growth promoters/Anabolic steroids	23696-28-8	N/A	0.5	1 to 100	88	2
134	Oleandomycin	7.03	Antibiotic/Aminoglycosides	3922-90-5	N/A	0.25	0.5 to 100	84	4
135	Orbifloxacin	4.97	Antibiotic/Quinolones	113617-63-3	N/A	0.25	0.5 to 100	85	3

No.	Compound Name	RT (min)	Functional Use/ Chemical Class	CAS Number	AOAC[2] MRL (µg/kg)	LOD (µg/kg)	Linear Calibration Curve Range (µg/kg) with R² >0.99	MQC Recovery (%)	MQC RSD (%)
136	Ormetoprim	4.39	Antibiotic	6981-18-6	100	0.25	0.5 to 100	84	2
137	Oxacillin	7.51	Antibiotic/Beta-lactam	66-79-5	300	2.5	5 to 100	92	18
138	Oxibendazole	6.79	Anthelmintic/Benzimidazoles	20559-55-1	N/A	0.1	0.25 to 100	87	1
139	Oxolinic acid	6.29	Antibiotic/Quinolones	14698-29-4	50	0.5	1 to 100	87	1
140	Oxyclozanide	9.49	Anthelmintic	2277-92-1	N/A	1	2.5 to 100	91	7
141	Oxyphenbutazone	8.09	NSAIDs	129-20-4	N/A	2.5	5 to 100	87	4
142	Oxytetracycline	4.46	Antibiotic/Tetracycline	79-57-2	200	1	2.5 to 100	68	5
143	Penicillin G	6.92	Antibiotic/Beta-lactam	61-33-6	50	1	2.5 to 100	77	6
144	Penicillin V [Phenoxymethylpenicillin]	7.33	Antibiotic/Beta-lactam	87-08-1	N/A	1	2.5 to 100	87	9
145	Phenylbutazone	9.01	NSAIDs	50-33-9	N/A	1	2.5 to 100	85	8
146	Phosalone	9.69	Insecticide	2310-17-0	N/A	1	2.5 to 100	95	7
147	Phoxim	9.63	Insecticide	14816-18-3	N/A	2.5	5 to 100	81	8
148	Piperonyl butoxide Ammonia	10.24	Insecticide	51-03-6	N/A	0.1	0.25 to 100	80	1
149	Pirlimycin	6.05	Antibiotic/Aminoglycosides	79548-73-5	N/A	2.5	5 to 100	76	12
150	Praziquantel	8.49	Anthelmintic	55268-74-1	N/A	0.25	0.5 to 100	91	2
151	Prednisolone	7.22	Growth promoters/Corticosteroids	50-24-8	N/A	0.5	1 to 100	88	5
152	Prednisone	7.06	Growth promoters/Corticosteroids	53-03-2	N/A	1	2.5 to 100	90	7
153	Progesterone	9.53	Hormones	57-83-0	N/A	0.5	1 to 100	87	4
154	Propionylpromazin	7.90	Antiemetic	3568-24-9	N/A	0.1	0.25 to 100	74	5
155	Propyphenazone	7.61	NSAIDs	479-92-5	N/A	0.1	0.25 to 100	88	2
156	Pyrantel	4.15	Anthelmintic	15686-83-6	N/A	0.5	1 to 100	81	5
157	Pyrimethamine	6.20	Antimicrobial	58-14-0	N/A	0.25	0.5 to 100	82	4
158	Ractopamine	4.55	Growth promoters/Beta-agonists	97825-25-7	N/A	0.25	0.5 to 100	82	1
159	Rafoxanide	11.03	Anthelmintic	22662-39-1	N/A	1	2.5 to 100	66	5
160	Rifaximin	9.00	Antibiotic	80621-81-4	N/A	0.5	1 to 100	91	4
161	Robenidine	8.48	Coccidiostats	25875-51-8	N/A	1	2.5 to 100	76	9
162	Ronidazole	3.34	Anthelmintic/Nitroimidazoles	7681-76-7	N/A	0.25	0.5 to 100	95	3
163	Salbutamol [Albuterol]	2.93	Growth promoters/Beta-agonists	18559-94-9	N/A	0.1	0.25 to 100	78	2
164	Salinomycin	11.52	Coccidiostats	53003-10-4	N/A	0.5	1 to 100	51	7
165	Sarafloxacin	5.29	Antibiotic/Quinolones	98105-99-8	10	0.25	0.5 to 100	89	3
166	Spiramycin I	6.03	Antibiotic/Macrolides	24916-50-5	200	0.5	1 to 100	69	6
167	Sulfabenzamide	5.99	Antibiotic/Sulfonamides	127-71-9	100	0.1	0.25 to 100	89	3
168	Sulfacetamide	3.06	Antibiotic/Sulfonamides	144-80-9	100	0.25	0.5 to 100	87	4
169	Sulfachloropyridazine	5.16	Antibiotic/Sulfonamides	80-32-0	100	0.25	0.5 to 100	96	2
170	Sulfaclozine	6.21	Antibiotic/Sulfonamides	102-65-8	100	1	2.5 to 100	92	5
171	Sulfadiazine [Silvadene]	3.36	Antibiotic/Sulfonamides	68-35-9	100	0.25	0.5 to 100	90	4
172	Sulfadimethoxine	6.39	Antibiotic/Sulfonamides	122-11-2	100	0.1	0.25 to 100	88	3
173	Sulfadimidine [Sulfamethazine]	4.54	Antibiotic/Sulfonamides	57-68-1	100	0.25	0.5 to 100	86	3
174	Sulfadoxine	5.49	Antibiotic/Sulfonamides	2447-57-6	100	0.1	0.25 to 100	87	3
175	Sulfaethoxypyridazine	5.84	Antibiotic/Sulfonamides	963-14-4	100	0.25	0.5 to 100	87	3
176	Sulfaguanidine	1.82	Antibiotic/Sulfonamides	57-67-0	100	0.25	0.5 to 100	85	3
177	Sulfamerazine	3.94	Antibiotic/Sulfonamides	127-79-7	100	0.25	0.5 to 100	86	5
178	Sulfameter [sulfamethoxydiazine]	4.40	Antibiotic/Sulfonamides	651-06-9	100	0.25	0.5 to 100	92	8
179	Sulfamethizole	4.43	Antibiotic/Sulfonamides	144-82-1	100	0.25	0.5 to 100	97	4
180	Sulfamethoxazole	5.39	Antibiotic/Sulfonamides	723-46-6	100	0.25	0.5 to 100	89	4
181	Sulfamethoxypyridazine	4.60	Antibiotic/Sulfonamides	80-35-3	100	0.25	0.5 to 100	88	4

No.	Compound Name	RT (min)	Functional Use/ Chemical Class	CAS Number	AOAC[2] MRL (µg/kg)	LOD (µg/kg)	Linear Calibration Curve Range (µg/kg) with R^2 >0.99	MQC Recovery (%)	MQC RSD (%)
182	Sulfamonomethoxine	5.14	Antibiotic/Sulfonamides	1220-83-3	100	0.25	0.5 to 100	88	3
183	Sulfamoxole	4.24	Antibiotic/Sulfonamides	729-99-7	100	0.25	0.5 to 100	89	4
184	Sulfanitran	7.25	Antibiotic/Sulfonamides	122-16-7	100	5	10 to 100	84	13
185	Sulfaphenazole	6.26	Antibiotic/Sulfonamides	526-08-9	100	0.25	0.5 to 100	87	2
186	Sulfapyridine	3.75	Antibiotic/Sulfonamides	144-83-2	100	0.25	0.5 to 100	85	4
187	Sulfaquinoxaline	6.44	Antibiotic/Sulfonamides	59-40-5	100	0.1	0.25 to 100	95	4
188	Sulfathiazole	3.55	Antibiotic/Sulfonamides	72-14-0	100	0.25	0.5 to 100	91	5
189	Sulfisomidine	3.27	Antibiotic/Sulfonamides	515-64-0	100	0.25	0.5 to 100	83	3
190	Sulfisoxazole	5.67	Antibiotic/Sulfonamides	127-69-5	100	0.25	0.5 to 100	89	2
191	Sulindac	7.97	Antibiotic/Sulfonamides	38194-50-2	100	0.25	0.5 to 100	89	5
192	Teflubenzuron	10.01	Insecticide	83121-18-0	300	5	10 to 100	100	10
193	Testosterone	8.49	Growth promoters/ Anabolic steroids	58-22-0	N/A	0.25	0.5 to 100	90	3
194	Tetracycline	4.67	Antibiotic/Tetracycline	60-54-8	200	0.5	1 to 100	61	7
195	Thiabendazole	4.26	Anthelmintic/Benzimidazoles	148-79-8	N/A	0.1	0.25 to 100	86	3
196	Thiamphenicol	4.25	Antibiotic/Amphenicols	15318-45-3	50	0.5	1 to 100	91	9
197	Tiamulin	7.56	Antibiotic	55297-95-5	N/A	0.1	0.25 to 100	85	3
198	Tilmicosin	6.76	Antibiotic/Macrolides	108050-54-0	50	1	2.5 to 100	79	5
199	Tolfenamic acid	9.86	NSAIDs	13710-19-5	N/A	5	10 to 100	83	17
200	Trenbolone	7.91	Growth promoters/Anabolic steroids	10161-33-8	N/A	0.5	1 to 100	88	3
201	Trichlorfon [DEP]	5.20	Tranquilizer	52-68-6	10	1	2.5 to 100	90	7
202	Triclabendazole	9.67	Anthelmintic/Benzimidazoles	68786-66-3	N/A	0.25	0.5 to 100	88	3
203	Trimethoprim	4.02	Antibiotic	738-70-5	50	0.25	0.5 to 100	82	2
204	Tripelennamine	6.28	Anthelmintic	91-81-6	N/A	0.1	0.25 to 100	77	2
205	Tylosin	7.56	Antibiotic/Macrolides	1401-69-0	100	1	2.5 to 100	71	9
206	Valnemulin	8.30	Antibiotic	101312-92-9	N/A	0.25	0.5 to 100	92	3
207	Vedaprofen	9.00	NSAIDs	71109-09-6	N/A	0.5	1 to 100	83	4
208	Virginiamycin M1	8.15	Antibiotic/Macrolides	21411-53-0	N/A	0.5	1 to 100	80	7
209	Xylazine	5.11	Tranquilizer	7361-61-7	N/A	0.25	0.5 to 100	85	1
210	Zilpaterol	2.93	Growth promoters/ Beta-Agonists	119520-05-7	N/A	0.25	0.5 to 100	81	2

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