Application Note

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Quantitative Screening of Multiresidue Veterinary Drugs in Milk and Egg Using the Agilent 6495C 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 210 target residues in milk and egg matrices. The workflow specifies conditions for chromatographic separation, MS detection, and data processing, using a slightly modified sample preparation procedure. Workflow performance was assessed based on limit of detection (LOD), limit of quantitation (LOQ), calibration curve linearity, accuracy, precision, recovery, and repeatability. Over 93% of veterinary drugs showed LOD of ≤1 µg/kg in milk samples. Calibration curves for all targets ranged from the LOQ to 100 µg/kg with a coefficient of correlation (R^2) ≥0.99. Target peak area response (%RSD) was <15%, and retention time (RT) %RSD was <0.5%. Method accuracy values, based on matrix-matched calibration were within 87 to 117%. The average recovery of 95% of targets was within 60 to 120%, with repeatability %RSD of ≤15%. Both milk and egg matrices showed similar quantitative results. Injection-to-injection robustness results demonstrated excellent target peak area and RT reproducibility across 400 injections, confirming the workflow capability for routine multiresidue screening with large-scale sample sets.

Introduction

The Agilent Comprehensive Veterinary Drug dMRM Solution is an end-to-end workflow solution for targeted screening or quantitation of 210 veterinary drug residues in animal origin matrices, which accelerates and simplifies routine laboratory testing. The solution includes comprehensive sample preparation, chromatographic separations, optimized MS detection method conditions, data analysis methods, and reporting templates for 210 veterinary drugs in various food matrices. The Comprehensive Veterinary Drug dMRM Solution minimizes method development time and combines multiple food matrix analyses into one easy-to-follow protocol. Agilent MassHunter data acquisition software, together with the dMRM database offers easy customization of dMRM submethods based on preferred target list or regulation, as determined by the user. The solution is available and has been verified with two mass spectrometers (Agilent 6470 triple quadrupole LC/MS and the Agilent 6495C triple quadrupole LC/MS) to address diverse sensitivity demands based on the choice of sample matrix and specific regulations that vary globally.

The solution was originally developed for the quantitative screening of 210 multiclass veterinary drugs in chick, beef, and pork.[1] It was then demonstrated to be effective for seafood using salmon and shrimp as example matrices.[2] This study further demonstrates the applicability for milk and eggs using the 6495C triple quadrupole LC/MS. For the 210 target analytes screened in this study, 103 of them had maximum residue limits (MRL) established in milk regulated by the AOAC[3]—with an additional 16 targets regulated by US FDA-CFR[4], US FSIS[5], or EU[6] regulations/guidelines.

The MRL values are typically lower in milk compared to meat and seafood, thus requiring a higher MS detection sensitivity. Additionally, the high fat and protein content in milk demands effective sample preparation and a sensitive detector to monitor trace levels of drug residues. Compared to milk, the number of MRL-established targets for the egg matrix is fewer and the residue limits are more relaxed.

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, part number 5191-4496), methanol (MeOH, part number 5191-4497), and water (part number 5191-4498) 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, dimethyl sulfoxide (DMSO), 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

Milk and egg samples were purchased from a local grocery. For the analysis of milk, a 2.0 ±0.1 mL portion of milk was transferred in a 50 mL conical polypropylene tube. For the analysis of egg, a 2.0 ±0.1 g portion of the homogenized sample was weighed in a 50 mL conical polypropylene tube. If not analyzed immediately, the samples were stored at −20 °C.

Sample preparation was performed as per the procedure defined in the Comprehensive Veterinary Drug dMRM Solution (G5368AA) using solvent extraction followed by Agilent Captiva EMR—Lipid cleanup (part number 5190-1003), aided by the Agilent positive pressure manifold processor (PPM-48, part number 5191-4101).[7] The sample preparation procedure is summarized in Figure 1. The aqueous extraction step was modified to adjust the target dilution due to increased water content in milk and egg.

The following deviations from the protocol defined in the Comprehensive Veterinary Drug dMRM Solution are recommended for the aqueous extraction step:

- **Milk:** Concentration of EDTA solution: 1 M, volume added: 200 µL.
- **Egg:** Concentration of EDTA solution: 0.1 M (same as workflow guide), volume added: 1 mL

Matrix-spiked (pre-extraction) QC samples were prepared by spiking the appropriate veterinary standard solution into the milk and egg matrices at various levels: 1 µg/kg for low-range QC (LQC), 10 µg/kg for mid-range QC (MQC), and 25 µg/kg for high-range QC (HQC), respectively. An additional QC level lower than the LQC of 0.1 µg/kg (LLQC) was included in the milk analysis to verify the analytical characteristics of a few targets, and to meet the very low MRL requirement. After spiking standards, the samples were vortexed for 30 seconds, then equilibrated for 15 to 20 minutes to allow the spiked standards to infiltrate the sample matrix before sample extraction.

Matrix-matched calibration standards

Matrix-matched (postextraction) calibration standards were prepared as per the workflow protocol by spiking appropriate standards into the blank matrix extract.[7] The targeted concentrations of matrix-matched calibration levels were 0.1, 0.25, 0.5, 1.0, 2.5, 5.0, 10.0, 25.0, 50.0, and 100.0 µg/kg (10 levels). An additional matrix-matched calibration level of 0.05 µg/kg was added for milk analysis for similar consideration of few targets with very low MRL requirement. Considering the 10x dilution factor introduced during sample preparation, the actual spiking concentrations of postextraction calibration standards were 0.005, 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 the milk 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 postextraction-spiked calibration standards.

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, including Agilent 1290 Infinity II flexible pump (G7104C), Agilent 1290 Infinity II multisampler (G7167A), and 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 see the workflow guide included with the Agilent Comprehensive Veterinary Drug dMRM Solution (G5368AA) for additional details.[7]

The "6495 Veterinary Drug Comprehensive" method included in the Comprehensive Veterinary Drug dMRM Solution for the 6495C triple quadrupole LC/MS (G6495C) was used directly for acquisition. The 6495C LC/MS triple quadrupole with an Agilent Jet Stream (AJS) ion source was operated in dynamic MRM (dMRM) mode. Autotune was performed in unit resolution with report *m/z* below 100 mode enabled. MassHunter acquisition software version 10.0 was used for data acquisition, and MassHunter quantitative analysis software version 10.0 was used to process the data.

Results and discussion

Workflow performance in milk

Chromatographic separation using the Agilent InfinityLab Poroshell EC-C18 column resulted in good separation and RT distribution of 210 veterinary drugs within a 13-minute elution window. Target-specific MRM transitions included in the dynamic MRM method helped to meet regulatory requirements for compound identification and confirmation. The default dynamic MRM method utilized a cycle time of 750 ms, and dwell times for each dMRM transition ranged from 7 to 370 ms, offering more than 10 data points across any given peaks. Figure 2 shows a representative MRM chromatogram for all 210 veterinary drug targets, postextraction spiked at 1.0 µg/L in the milk blank matrix extract. Considering the dilution factor during sample preparation was 10x, this 1.0 µg/L postextraction spike was equivalent to a 10 µg/kg spike in milk. The symmetrically sharp peaks demonstrate the efficient chromatographic separation of targets within the elution window. Table 1 lists the name, chemical class, CAS number, and RT of all 210 targets covered in this work.

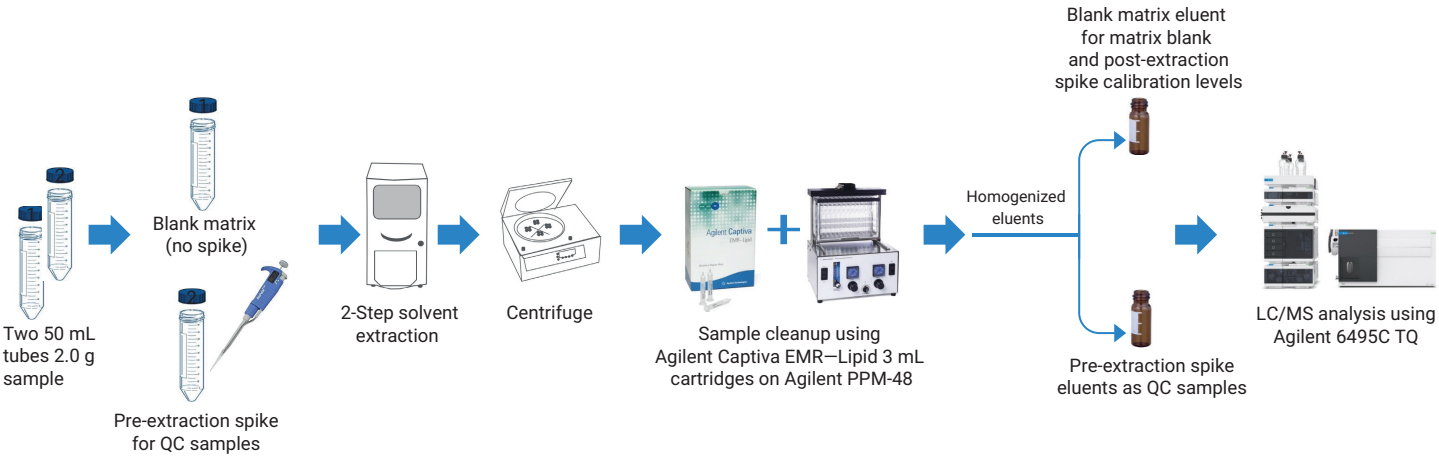


Figure 1. Sample extraction procedure using solvent extraction followed with Agilent Captiva EMR—Lipid cleanup.

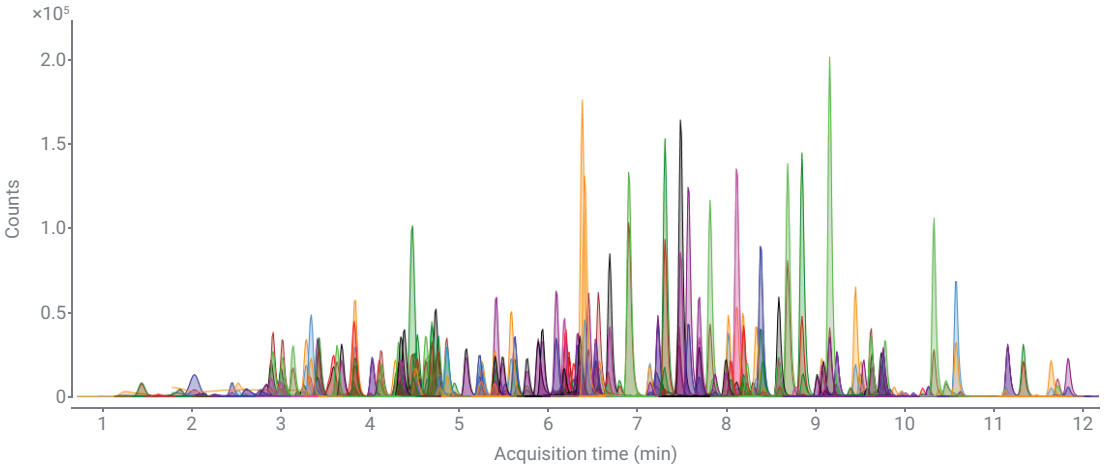


Figure 2. Representative MRM chromatogram of 210 veterinary drug targets postextraction spiked at 1.0 µg/L in the milk blank matrix extract using the Agilent 6495C triple quadrupole LC/MS).

From the AOAC MRL established list, the early eluted analytes, including amoxicillin, baquiloprim, deacetylcefapirin, diminazene, imidocarb, norgestomet, sulfaguanidine, and tilmicosin showed split peaks due to solvent effects. The "spectrum summation" integrator algorithm was used to reliably and automatically integrate these targets for consistent RT, and thus eliminated the need for manual reintegrations.[8] The peak shape for these targets can be improved by converting samples into a higher aqueous mixture prior to LC/TQ injection.

LOD, LOQ and calibration curve linearity

LOD and LOQ were established using various low level matrix-matched calibration standards.[1,2] The signal-to-noise ratio (S/N) was calculated using the peak height for signal and an auto-RMS algorithm for noise, included in the MassHunter quantitative analysis software. The method sensitivity using the 6495C LC/TQ system offered a LOD ≤1 µg/kg for over 93% of analytes tested in both milk and egg. The low detection limits achieved allowed the high sensitivity demand for screening trace level veterinary drug residues in milk. As an example, AOAC regulated MRL of 0.05 µg/kg for clenbuterol in milk. The 6495C TQ-based workflow provided a clean, symmetrical peak with S/N of 32 at the 0.05 µg/kg matrix-matched calibration level, thus enabling confident target identification and quantitation (Figure 3).

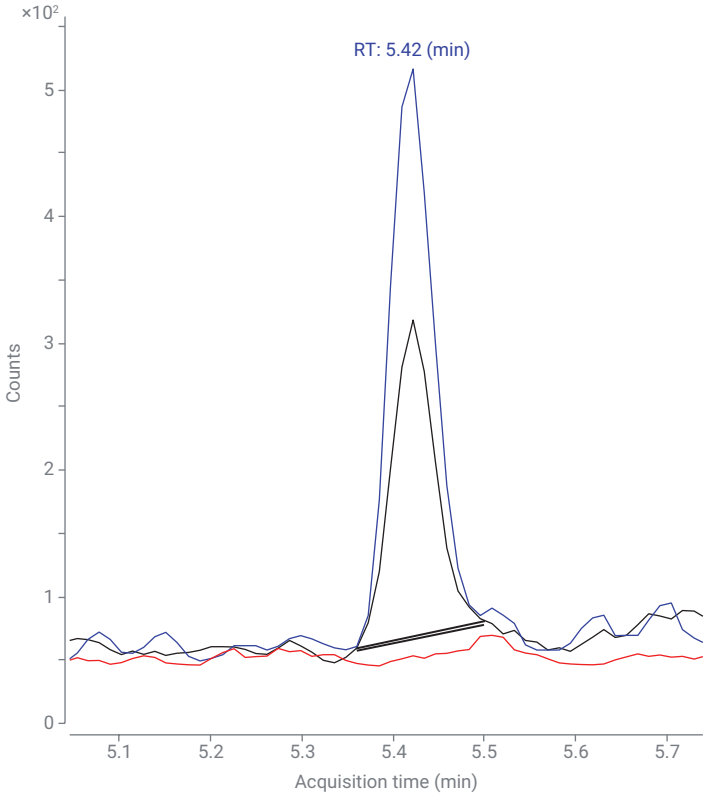


Figure 3. MRM chromatogram of clenbuterol (MRM 277.1 → 202.9) postextraction-spiked at 0.005 µg/L (black trace) and 0.01 µg/L (blue trace) in the milk matrix extract, overlaid with matrix blank (red trace). The defined LOD of clenbuterol is 0.05 µg/kg (S/N: 32) and LOQ is 0.1 µg/kg (S/N: 76).

A calibration curve for each target was generated using matrix-matched calibration standards at levels ranging from the defined LOQ to the highest-spiked level. The linear regression was used with ignored origin and $1/x$ or $1/x^2$ weight. All targets met the calibration curve linearity requirement of $R^2 \geq 0.99$. The LOD, LOQ, and calibration curve data of all targets in the milk are shown in Table1.

Instrument method precision and accuracy

Precision was determined by calculating the %RSD of the target response and RT using triplicate injections of the matrix-matched calibration levels. The average accuracy value for each matrix-matched calibration level was also calculated from the triplicate injections.

Good precision and accuracy values were obtained for all targets in milk. Target response %RSD for all targets in the milk matrix at 10 µg/kg was <15%, and the RT %RSD of all targets were within 0.5%.[9] The accuracy values of all targets at 10 µg/kg within a range of 87 to 117%. These results confirm the reproducibility of chromatographic separation and MS detection.

Target recovery and intrabatch repeatability

The impact of sample preparation on target recovery was assessed using matrix-spiked QC samples. Each QC level was prepared with four technical preparations and was injected for instrument analysis in duplicates. An appropriate level of matrix-spiked QC sample based on MRL was selected to evaluate target-specific recovery and repeatability. Recovery was calculated using target response in matrix-spiked QCs, and measured response using matrix-matched 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, calculated using four technical preparations of matrix-spiked QC samples.

The results showed that recoveries of about 93% of MRL-established targets reached the acceptable range of 60 to 120% with an excellent intrabatch RSD ≤20%.[9] Recoveries of the remaining seven targets, baquiloprim, chlortetracycline, deacetylcefapirin, diclofenac, imidocarb, oxytetracycline, and trichlorfon [DEP], were within a range of 30 to <60% or >120 to 124%. However, for these targets, the workflow still provided good recovery repeatability values within a %RSD of 9%, demonstrating consistent extraction behavior. These results confirmed the entire workflow reproducibility using Captiva EMR—Lipid sample extraction and cleanup protocol in the 6495-TQ-based instrument detection. The recovery and repeatability results of all 210 targets are included in Table 1 (see Appendix).

The workflow performance combined with the 6495C LC/TQ detection helped confident recovery and repeatability assessment at trace levels in milk. Figure 4 shows an example of workflow recovery and repeatability for clenbuterol at 0.1 µg/kg in milk. The average recovery of this target using the LLQC sample is 118% with good recovery repeatability of %RSD <5%.

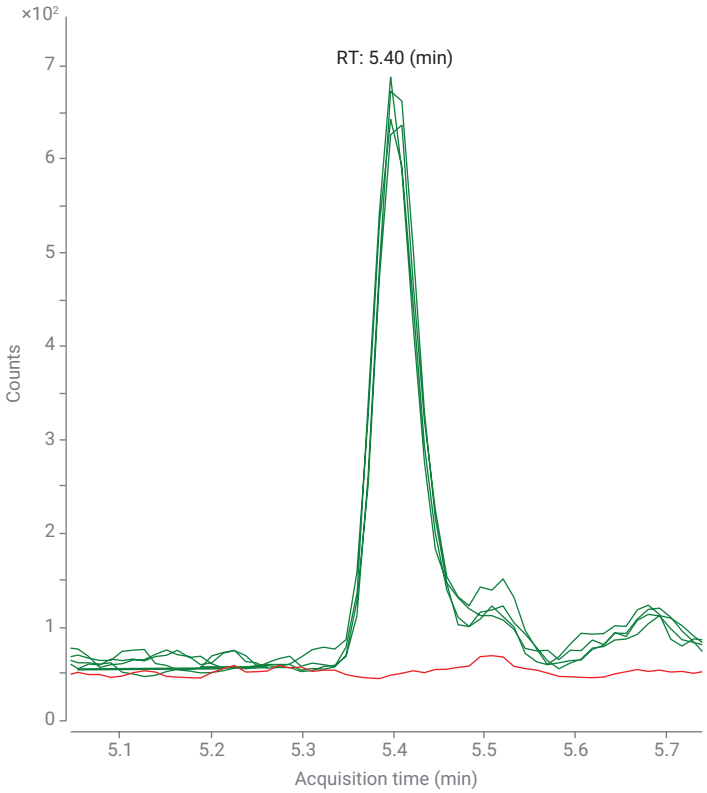


Figure 4. MRM chromatograms of clenbuterol (MRM 277.1 → 202.9) using four technical preparations of LLQC samples in milk (green traces) overlaid with matrix blank (red trace).

Matrix effect assessment

Matrix effect (ME) is an important parameter for method sensitivity and reliability assessments. ME is defined as the ratio of analyte area response (I) in matrix-matched samples with those in the corresponding neat standards (see Equation 1). The closer the ME value is to 100%, the less the matrix effect presents; results lower than 100% indicate matrix suppression, while results >100% indicate potential enhancement.

Equation 1.

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

In this study, ME was investigated using target response from postextraction-spiked calibration levels at 2.5 µg/L in blank matrix extract, compared with corresponding neat standards.

In the milk matrix, within a total of 103 MRL established analytes, >95% of targets showed an ME of >75%, indicating negligible matrix suppression. Four targets (amoxicillin, cefalonium, nafcillin, and sulfamethizole) resulted in an ME of 50 to 75%, indicating moderate ion suppression. Target deacetylcefapirin showed an ME of 48%, indicating significant ion suppression.

Method verification in egg matrix

The method sensitivity in the egg was similar to that of the milk matrix. Linear matrix-matched calibration curves ranging from LOQ to 100 µg/kg were demonstrated with R2 ≥ 0.99. Instrument method precision (%RSD) for target responses and RTs were <15% and <0.5%, respectively. Instrument method accuracy for the matrix-matched calibration level at 10.0 µg/kg were within 80 to 113% (n = 3). Recoveries of over 94% targets in the egg matrix were within 60 to 120% acceptance criteria, and recovery repeatability with %RSD values ≤15%. Targets amprolium, baquiloprim, chlortetracycline, deacetylcefapirin, doxycycline, erythromycin, oxytetracycline, tetracycline, and rafoxanide showed <60% recoveries, but recovery repeatability values were within an acceptable limit of <15%RSD. Severe ion suppression and poor recovery (<20%) was observed for the dipyrone hydrate-metabolite, however, no MRL is established for this veterinary drug in the egg.

AOAC MRL-based residue screening in milk and egg

The MRL values of 103 AOAC-listed targets range from 0.05 to 200 µg/kg in milk.[3] The method sensitivity in milk using the 6495C LC/TQ enabled confident screening of all targets, except for diclofenac and norgestomet. Table 1 summarizes the MRL requirement and observed results for all targets. Among the comprehensive target list, 22 have MRL established for egg under AOAC guidelines, and the values range from 0.7 to 4,000 µg/kg.[3] The method sensitivity easily met the screening requirement for all MRL-established targets in egg per the AOAC guidelines.

High blank contribution was observed for the analysis of chlorhexidine, clindamycin, progesterone, and gonadotropin in both milk and egg, indicating the potential positive incurrence in the used sample matrix. Trace residues of ethopabate, oxibendazole, piperonyl butoxide ammonia, and tripelennamine affected the LOQ determination in milk. Alternatively, the residues from imidocarb, oxyphenbutazone, piperonyl butoxide ammonia, and testosterone affected the LOQ determination in egg.

Method robustness

The method robustness was assessed by 400 continuous injections of Agilent Veterinary Drug System Suitability test mix (part number 5799-0015) postspiked in milk matrix. Peak responses and RT consistency were monitored for all 25 targets over time. The 25 veterinary drug targets are from 10 different chemical classes, with a broad range of molecular weight, eluted evenly across the elution window, and cover both positive and negative polarity ionization. The dMRM peak area %RSD and RT %RSD of all 25 targets were calculated from the 400 injections of 1.0 µg/L postspiked milk blank matrix extract. The data acquisition was continuous, and the instrument was operated without readjusting any tune parameters. The entire run lasted for >120 hours.

The elution profile using the InfinityLab Poroshell column was extremely consistent over 400 injections. A good response reproducibility with %RSD <4.0% and RT %RSD of <0.2% were observed for all 25 targets. The response reproducibility of all 25 standards over 400 injections is summarized in Figure 5, and an overlay of five total ion chromatograms (TIC) of Agilent Veterinary Drug System Suitability test mix MRM (spread across 400 injections) are shown in Figure 6. The innovative ion transfer optics design of Agilent triple quadrupole mass spectrometers minimizes the source contamination from the matrix, thus providing a robust analytical platform for the confident analysis of trace veterinary drug residues (Figure 7). The sample preparation procedure here provided efficient sample matrix cleanup, greatly reduced the matrix residue accumulation on the ion source interface, and provided extended column lifetime and detection consistency. The method robustness, calculated from a 5-day continuous data acquisition, confirmed the sustainable performance of the LC/TQ workflow for day-to-day operations.

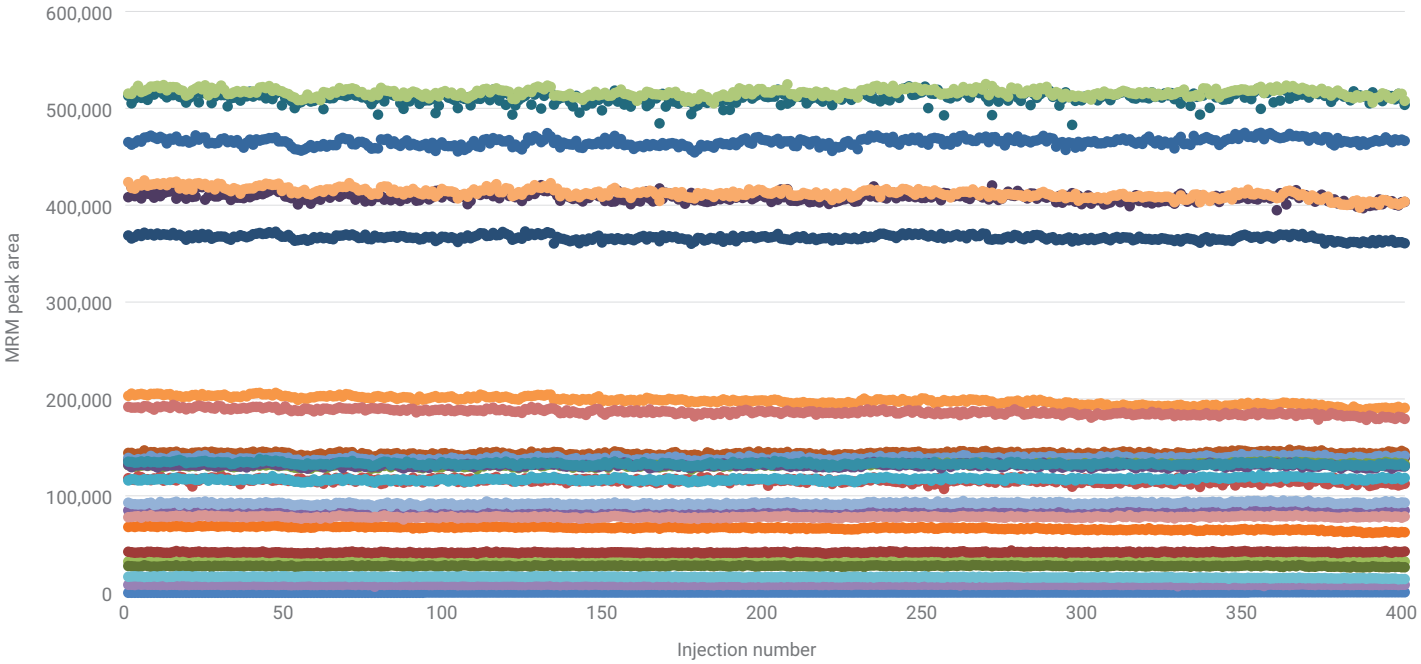


Figure 5. The response reproducibility of 25 targets included in the Agilent Veterinary Drug System Suitability test mix over 400 continuous injections. Concentration: postspiked at 1.0 µg/L in milk blank matrix extract (equivalent to a 10 µg/kg matrix spike in milk). Please refer to Table 1 for the list of 25 targets in the Veterinary Drug System Suitability test mix.

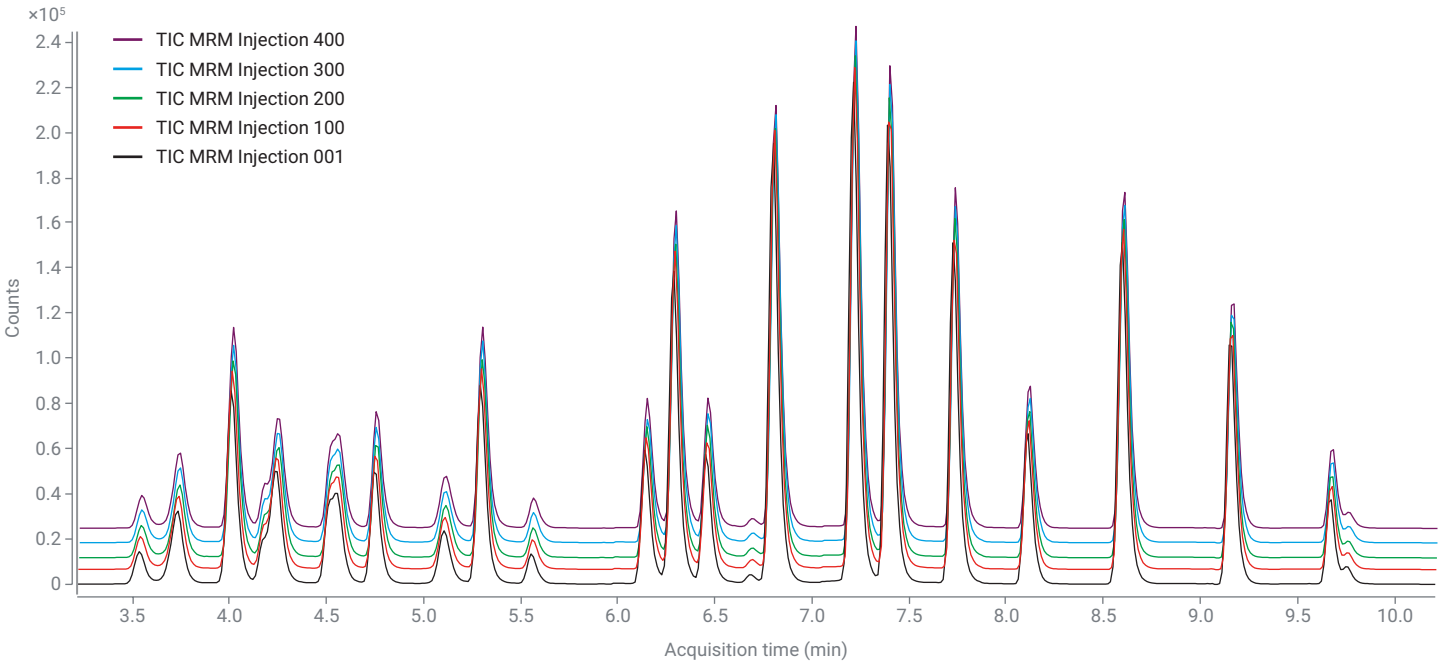
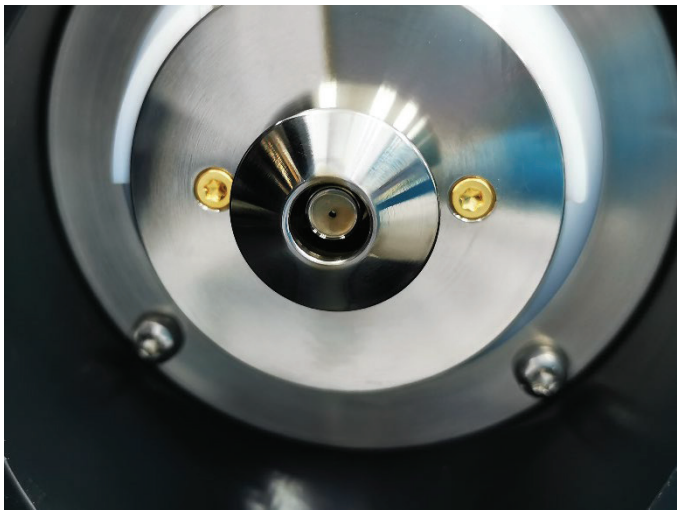


Figure 6. Overlay of five selected system suitability mix TIC MRM chromatograms, spread across 400 continuous replicate injections demonstrating the target elution consistency. Concentration: postextraction spiked at 1.0 µg/L in milk blank matrix extract, LC separation column: Agilent InfinityLab Poroshell 120 EC-C18 (part number 695575-302). (offset X, Y-axis values, injections: 1, 100, 200, 300, and 400).

A



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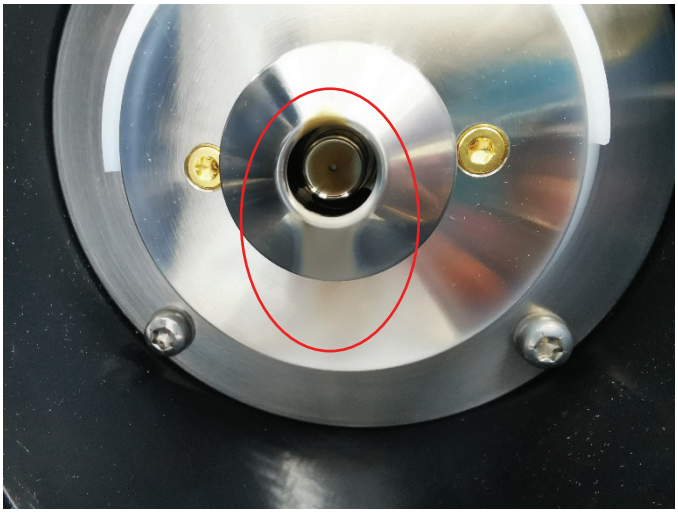

Figure 7. The Agilent Jet Stream technology ion source (AJS) of the Agilent 6495C triple quadrupole LC/MS before (A) and after (B) 400 continuous injections of milk matrix.

Conclusion

This study demonstrates the applicability of the Agilent Comprehensive Veterinary Drug dMRM Solution for the screening and quantitation of 210 multiclass veterinary drug residues in milk and egg matrices. The workflow-recommended sample preparation protocol, using solvent extraction followed by Agilent Captiva EMR—Lipid cleanup, was shown to be efficient for target extraction and matrix cleanup from milk and egg. The workflow performance was characterized by good results in terms of linearity, accuracy, recovery, and repeatability, allowing sensitive detection of multiclass veterinary drug residues. The Agilent 6495C triple quadrupole LC/MS-based workflow provided sub-1 µg/kg (ppb) LODs for most analytes, and exceeded the sensitivity requirements set by global regulatory agencies for screening trace veterinary residues in complex matrices like milk and eggs. The results demonstrated the method reliability for routine screening of over 98% of AOAC-listed veterinary drug targets from the milk matrix, and 100% of AOAC-listed targets from the egg matrix. The robustness of 400 continuous injections confirmed the method consistency and reliability, with minimized sample residue accumulation on the ion source interface.

References

1. Siji Joseph *et al.* An End-To-End Workflow for Quantitative Screening of Multiclass, Multiresidue Veterinary Drugs in Meat Using the Agilent 6470 Triple Quadrupole LC/MS, *Agilent Technologies application note*, publication number 5994-1932EN.
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8. Steven J. Lehotay, Utility of the Summation Chromatographic Peak Integration Function to Avoid Manual Reintegrations in the Analysis of Targeted Analytes, *LCGC North America* June **2017**, *35(6)*, 391.
9. Guidelines for Standard Method Performance Requirements, AOAC Official Methods of Analysis (**2016**) Appendix F.

Appendix

Table 1. Target screening results using milk matrix based on AOAC guidelines. The results were generated based on the Agilent 1290 Infinity II LC and Agilent 6495C triple quadrupole LC/MS systems. Note that these compounds may be obtained from Agilent, and those highlighted in bold are included in the Agilent Veterinary Drug System Suitability test mix (part number 5799-0015).

No.	Compound Name	RT (min)	Functional Use/ Chemical Class	CAS Number	AOAC[3] 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	1.62	Insecticide	465531-97-9	N/A	2.5	5 to 100	105	9
2	2,4-DMA [Amitraz Metabolite]	4.45	Insecticide	33089-74-6	10	0.5	1 to 100	108	3
3	2-Quinoxalinecarboxylic acid [QCA]	4.20	Quinoxalines	879-65-2	N/A	2.5	5 to 100	108	4
4	4-epi-oxytetracycline	4.36	Antibiotic/Tetracycline	14206-58-7	100	0.25	0.5 to 100	84	5
5	4-epi-tetracycline	4.28	Antibiotic/Tetracycline	79-85-6	100	0.25	0.5 to 100	82	4
6	5-Hydroxy thiabendazole	3.59	Anthelmintic/Benzimidazoles	948-71-0	50	0.1	0.25 to 100	110	2
7	5-Hydroxyflunixin	8.36	NSAIDs	75369-61-8	2	0.05	0.1 to 100	112*	10*
8	Acepromazine	7.44	Tranquilizer	61-00-7	N/A	0.05	0.1 to 100	100	2
9	Acetyl isovaleryl tylosin [Tylvalosin]	8.80	Antibiotic/Macrolides	63409-12-1	N/A	0.5	1 to 100	106	5
10	Albendazole	8.09	Anthelmintic/Benzimidazoles	54965-21-8	100	0.05	0.1 to 100	110	2
11	Albendazole sulfone	6.22	Anthelmintic/Benzimidazoles	75184-71-3	100	0.1	0.25 to 100	118	2
12	Albendazole sulfoxide	5.62	Anthelmintic/Benzimidazoles	54029-12-8	100	0.1	0.25 to 100	115	3
13	Albendazole-2-aminosulfone	3.81	Anthelmintic/Benzimidazoles	80983-34-2	100	0.25	0.5 to 100	105	2
14	Alpha Zearalanol	8.33	Hormones	26538-44-3	N/A	1	2.5 to 100	110	4
15	Altrenogest	9.05	Hormones	850-52-2	N/A	0.1	0.25 to 100	112	2
16	Aminoflubendazole	6.19	Anthelmintic/Benzimidazoles	82050-13-3	10	0.05	0.1 to 100	112	2
17	Amoxicillin	2.76	Antibiotic/Beta-lactam	26787-78-0	4	1	2.5 to 100	110*	7*
18	Ampicillin	4.00	Antibiotic/Beta-lactam	69-53-4	4	0.5	1 to 100	115*	7*
19	Amprolium	1.22	Antimicrobial	13082-85-4	N/A	1	2.5 to 100	9	4
20	Azaperone	5.87	Tranquilizer	1649-18-9	N/A	0.1	0.25 to 100	96	3

No.	Compound Name	RT (min)	Functional Use/ Chemical Class	CAS Number	AOAC[3] MRL (µg/kg)	LOD (µg/kg)	Linear Calibration Curve Range (µg/kg) with R^2 >0.99	MQC Recovery (%)	MQC RSD (%)
21	Azithromycin	6.27	Antibiotic/Macrolides	83905-01-5	N/A	0.1	0.25 to 100	76	3
22	Baquiloprim	2.74	Antimicrobial	102280-35-3	30	0.25	0.5 to 100	33	4
23	Betamethasone	7.83	Growth promoters/Corticosteroids	378-44-9	0.3	0.25	0.5 to 100	119*	3*
24	Cabergoline	4.72	Dopamine receptor	81409-90-7	0.1	0.05	0.1 to 100	111•	15•
25	Carazolol	6.16	Tranquilizer	57775-29-8	1	0.05	0.1 to 100	119*	5*
26	Carbadox	4.47	Antimicrobial	6804-07-5	N/A	0.25	0.5 to 100	113	3
27	Carprofen	9.08	NSAIDs	53716-49-7	N/A	1	2.5 to 100	106	3
28	Cefalexin	4.00	Antibiotic/Beta-lactam	15686-71-2	100	2.5	5 to 100	87	8
29	Cefalonium	3.98	Antibiotic/Beta-lactam	5575-21-3	20	1	2.5 to 100	73	8
30	Cefapirin	3.28	Antibiotic/Beta-lactam	21593-23-7	60	0.1	0.25 to 100	81	4
31	Cefazolin	4.39	Antibiotic/Beta-lactam	25953-19-9	50	1	2.5 to 100	112	2
32	Cefoperazone	5.21	Antibiotic/Beta-lactam	62893-19-0	50	1	2.5 to 100	114	8
33	Cefquinome	3.75	Antibiotic/Beta-lactam	84957-30-2	20	0.5	1 to 100	63	4
34	Ceftiofur	6.35	Antibiotic/Beta-lactam	80370-57-6	100	0.5	1 to 100	117	3
35	Cefuroxime	4.47	Antibiotic/Beta-lactam	55268-75-2	N/A	2.5	5 to 100	112	6
36	Chloramphenicol	6.34	Antibiotic/Amphenicols	56-75-7	N/A	0.5	1 to 100	113	5
37	Chlorhexidine	7.20	Antimicrobial	55-56-1	N/A	5	10 to 100	95	4
38	Chlormadinone	9.51	Hormones	1961-77-9	2.5	0.5	1 to 100	114*	6*
39	Chlorpromazine	8.16	Tranquilizer	50-53-3	N/A	0.05	0.1 to 100	97	2
40	Chlortetracycline	6.04	Antibiotic/Tetracycline	57-62-5	100	0.25	0.5 to 100	42	9
41	Ciprofloxacin	4.52	Antibiotic/Quinolones	85721-33-1	N/A	0.1	0.25 to 100	95	3
42	Clenbuterol	5.41	Growth promoters/Beta-agonists	37148-27-9	0.05	0.05	0.1 to 100	118•	5•
43	Clindamycin	6.55	Antibiotic/Macrolides	18323-44-9	N/A	10	25 to 100	106#	1#
44	Clopidol	3.61	Coccidiostats	2971-90-6	20	0.25	0.5 to 100	113	2
45	Closantel	10.60	Anthelmintic	57808-65-8	45	0.25	0.5 to 100	93	3
46	Colchicine	6.78	NSAIDs	64-86-8	N/A	0.1	0.25 to 100	105	4
47	Cotinine	2.23	Insecticide	486-56-6	N/A	0.1	0.25 to 100	90	3
48	Coumaphos	9.64	Anthelmintic	56-72-4	N/A	0.5	1 to 100	108	5
49	Cyromazine	2.52	Anthelmintic	66215-27-8	N/A	1	2.5 to 100	94	7
50	Danofloxacin	4.73	Antibiotic/Quinolones	112398-08-0	30	0.05	0.1 to 100	95	2
51	Dapson	4.76	Antibiotic/Sulfonamides	80-08-0	N/A	0.05	0.1 to 100	117	2
52	Dapson N-Acetyl	5.51	Antibiotic/Sulfonamides	565-20-8	N/A	0.25	0.5 to 100	115	3
53	Deacetylcefapirin	2.37	Antibiotic/Beta-lactam	104557-24-6	60	1	2.5 to 100	41	8
54	Diaveridine	3.83	Antimicrobial	5355-16-8	N/A	0.05	0.1 to 100	111	2
55	Diazinon	9.71	Insecticide	333-41-5	20	0.1	0.25 to 100	108	2
56	Diclofenac	9.21	NSAIDs	15307-86-5	0.1	0.25	0.5 to 100	123*	8*
57	Dicloxacillin	8.18	Antibiotic/Beta-lactam	3116-76-5	30	2.5	5 to 100	118	7
58	Dicyclanil	2.98	Insecticide	112636-83-6	N/A	0.25	0.5 to 100	108	1
59	Difloxacin	5.39	Antibiotic/Quinolones	98106-17-3	N/A	0.1	0.25 to 100	106	4
60	Diflubenzuron	9.18	Insecticide	35367-38-5	N/A	0.5	1 to 100	108	5
61	Dimetridazole	3.74	Coccidiostats	551-92-8	N/A	2.5	5 to 100	108	2
62	Diminazene	3.06	Coccidiostats	536-71-0	150	2.5	5 to 100	60	4
63	Dinitolmide [Zoalene]	5.66	Coccidiostats	148-01-6	N/A	0.5	1 to 100	115	3
64	Dipyrone hydrate- metabolite [4-Methylaminoantipyrine]	3.40	NSAIDs	519-98-2	N/A	0.25	0.5 to 100	54	7

No.	Compound Name	RT (min)	Functional Use/ Chemical Class	CAS Number	AOAC[3] MRL (µg/kg)	LOD (µg/kg)	Linear Calibration Curve Range (µg/kg) with R^2 >0.99	MQC Recovery (%)	MQC RSD (%)
65	Doxycycline	6.36	Antibiotic/Tetracycline	564-25-0	N/A	0.25	0.5 to 100	32	4
66	Emamectin B1a benzoate	10.18	Anthelmintic/Avermectins	121124-29-6	N/A	0.25	0.5 to 100	89	3
67	Emamectin B1b benzoate	9.99	Anthelmintic/Avermectins	121424-52-0	N/A	0.5	1 to 100	95	5
68	Enrofloxacin	4.85	Antibiotic/Quinolones	93106-60-6	100	0.05	0.1 to 100	102	2
69	Erythromycin	7.52	Antibiotic/Macrolides	114-07-8	N/A	0.5	1 to 100	97	13
70	Ethopabate	6.68	Coccidiostats	59-06-3	N/A	0.1	0.25 to 100	115	3
71	Famphur	8.25	Insecticide	52-85-7	N/A	0.25	0.5 to 100	116	7
72	Febantel	9.22	Anthelmintic/Benzimidazoles	58306-30-2	10	0.1	0.25 to 100	115	4
73	Fenbendazole	8.67	Anthelmintic/Benzimidazoles	43210-67-9	10	0.05	0.1 to 100	112	2
74	Fenbendazole Sulfoxide [Oxfendazole]	6.53	Anthelmintic/Benzimidazoles	53716-50-0	10	0.1	0.25 to 100	118	3
75	Firocoxib	8.04	NSAIDs	189954-96-9	N/A	2.5	5 to 100	111	14
76	Florfenicol	5.64	Antibiotic/Amphenicols	73231-34-2	N/A	0.25	0.5 to 100	115	4
77	Fluazuron	10.24	Insecticide	86811-58-7	N/A	0.25	0.5 to 100	108	6
78	Flubendazole	7.80	Anthelmintic/Benzimidazoles	31430-15-6	10	0.05	0.1 to 100	115	2
79	Flugestone acetate	8.42	Hormones	2529-45-5	1	0.5	1 to 100	120*	4*
80	Flumequine	7.47	Antibiotic/Quinolones	42835-25-6	50	0.05	0.1 to 100	119	2
81	Flunixin	8.83	NSAID's	38677-85-9	N/A	0.05	0.1 to 100	117	2
82	Fluralaner	9.95	Insecticide	864731-61-3	N/A	0.5	1 to 100	111	6
83	Furazolidone	4.77	Antimicrobial/Furans	67-45-8	N/A	1	2.5 to 100	118	5
84	Gamithromycin	6.56	Antibiotic/Aminoglycosides	145435-72-9	N/A	0.1	0.25 to 100	76	4
85	Gonadotropin	7.65	Hormones	33515-09-2	N/A	5	10 to 100	116	7
86	Halofuginone	6.55	Coccidiostats	55837-20-2	N/A	0.25	0.5 to 100	102	5
87	Haloperidol	7.21	Tranquilizer	52-86-8	N/A	0.05	0.1 to 100	116	2
88	Haloxon	8.65	Anthelmintic	321-55-1	N/A	2.5	5 to 100	112	12
89	Imidocarb	3.32	Coccidiostats	27885-92-3	50	1	2.5 to 100	55	5
90	Ipronidazole	6.13	Anthelmintic/Nitroimidazoles	14885-29-1	N/A	1	2.5 to 100	115	3
91	Ipronidazole-OH	4.93	Anthelmintic/Nitroimidazoles	35175-14-5	N/A	0.25	0.5 to 100	114	3
92	Isometamidium	6.09	Anthelmintic	20438-03-3	100	1	2.5 to 100	73	4
93	Josamycin	8.32	Antibiotic/Macrolides	16846-24-5	N/A	0.25	0.5 to 100	110	4
94	Ketamine	4.86	Anesthetic	6740-88-1	N/A	0.05	0.1 to 100	107	2
95	Ketoprofen	8.28	NSAIDs	22071-15-4	50	0.5	1 to 100	118	5
96	Kitasamycin A5 [Leucomycin A5]	7.79	Antibiotic/Aminoglycosides	18361-45-0	N/A	0.25	0.5 to 100	108	4
97	Lasalocid A	11.13	Coccidiostats	25999-31-9	N/A	0.05	0.1 to 100	106	2
98	Leuco Crystal violet	10.44	Fungicides and Dyes	603-48-5	N/A	0.25	0.5 to 100	79	3
99	Leucomalachite green	10.55	Fungicides and Dyes	129-73-7	N/A	0.05	0.1 to 100	96	2
100	Levamisole	3.67	Anthelmintic	14769-73-4	N/A	0.1	0.25 to 100	106	2
101	Lincomycin	3.81	Antibiotic/Aminoglycosides	154-21-2	150	0.05	0.1 to 100	79	3
102	Lufenuron	10.16	Insecticide	103055-07-8	N/A	1	2.5 to 100	113	5
103	Maduramicin Ammonium	11.69	Coccidiostats	79356-08-4	N/A	0.1	0.25 to 100	84	2
104	Malachite green	8.31	Fungicides and Dyes	10309-95-2	N/A	0.05	0.1 to 100	81	2
105	Malathion	9.00	Insecticide	121-75-5	N/A	0.1	0.25 to 100	117	3
106	Marbofloxacin	4.10	Antibiotic/Quinolones	115550-35-1	75	0.1	0.25 to 100	97	2
107	Mebendazole	7.55	Anthelmintic/Benzimidazoles	31431-39-7	N/A	0.05	0.1 to 100	114	2
108	Mefenamic acid	9.75	Anti-inflammatory	61-68-7	N/A	0.1	0.25 to 100	120	3

No.	Compound Name	RT (min)	Functional Use/ Chemical Class	CAS Number	AOAC[3] MRL (µg/kg)	LOD (µg/kg)	Linear Calibration Curve Range (µg/kg) with R^2 >0.99	MQC Recovery (%)	MQC RSD (%)
109	Megestrol acetate	9.49	Hormones	595-33-5	N/A	0.1	0.25 to 100	111	3
110	Melengestrol acetate	9.61	Hormones	2919-66-6	N/A	0.1	0.25 to 100	111	4
111	Meloxicam	8.17	NSAIDs	71125-38-7	15	0.05	0.1 to 100	120	2
112	Methylprednisolone	7.86	Growth promoters/Corticosteroids	83-43-2	2	0.25	0.5 to 100	117*	4*
113	Metoserpate	6.66	Tranquilizer	1178-28-5	N/A	0.1	0.25 to 100	113	3
114	Metronidazole	3.28	Anthelmintic/Nitroimidazoles	443-48-1	N/A	0.1	0.25 to 100	116	2
115	Metronidazole-OH	2.84	Anthelmintic/Nitroimidazoles	4812-40-2	N/A	0.25	0.5 to 100	118	2
116	Monensin	11.30	Coccidiostats	17090-79-8	2	0.1	0.25 to 100	98*	5*
117	Monepantel	9.52	Anthelmintic	851976-50-6	N/A	0.1	0.25 to 100	120	3
118	Morantel tartrate	5.39	Anthelmintic	20574-50-9	50	0.1	0.25 to 100	107	2
119	Moxidectin	11.09	Anthelmintic/Avermectins	113507-06-5	40	0.25	0.5 to 100	115	5
120	Nafcillin	8.10	Antibiotic/Beta-lactam	147-52-4	30	0.25	0.5 to 100	100	4
121	Nalidixic acid	7.29	Antibiotic	389-08-2	N/A	0.05	0.1 to 100	117	2
122	Narasin	11.80	Coccidiostats	55134-13-9	N/A	0.1	0.25 to 100	69	7
123	Neo-Spiramycin	5.75	Antibiotic/Macrolides	70253-62-2	200	0.25	0.5 to 100	70	2
124	Nequinate	9.43	Anthelmintic	13997-19-8	N/A	0.05	0.1 to 100	112	3
125	Netobimin	7.11	Anthelmintic	88255-01-0	100	1	2.5 to 100	108	7
126	Nicarbazine	8.84	Coccidiostats	587-90-6	N/A	0.1	0.25 to 100	116	3
127	Nicotine	1.54	Anti-herbivore	54-11-5	N/A	5	10 to 100	67	8
128	Niflumic Acid	9.14	Anti-inflammatory	4394-00-7	N/A	0.05	0.1 to 100	117	2
129	Nitroxynil	6.77	Anthelmintic	1689-89-0	N/A	0.5	1 to 100	114	5
130	Norfloxacin	4.38	Antibiotic/Quinolones	70458-96-7	N/A	0.1	0.25 to 100	95	2
131	Norgestomet	9.44	Hormones	472-54-8	0.12	1	2.5 to 100	117	3
132	Novobiocin	9.82	Antibiotic	303-81-1	50	0.25	0.5 to 100	120	4
133	Olaquindox	3.03	Growth promoters/Anabolic steroids	23696-28-8	N/A	0.25	0.5 to 100	104	2
134	Oleandomycin	7.13	Antibiotic/Aminoglycosides	3922-90-5	50	0.1	0.25 to 100	112	3
135	Orbifloxacin	5.07	Antibiotic/Quinolones	113617-63-3	20	0.1	0.25 to 100	115	3
136	Ormetoprim	4.49	Antibiotic	6981-18-6	N/A	0.1	0.25 to 100	112	2
137	Oxacillin	7.56	Antibiotic/Beta-lactam	66-79-5	30	1	2.5 to 100	114	9
138	Oxibendazole	6.89	Anthelmintic/Benzimidazoles	20559-55-1	50	0.1	0.25 to 100	116	2
139	Oxolinic acid	6.37	Antibiotic/Quinolones	14698-29-4	N/A	0.25	0.5 to 100	112	2
140	Oxyclozanide	9.56	Anthelmintic	2277-92-1	10	0.25	0.5 to 100	112	4
141	Oxyphenbutazone	8.16	NSAIDs	129-20-4	N/A	0.25	0.5 to 100	105	6
142	Oxytetracycline	4.54	Antibiotic/Tetracycline	79-57-2	100	0.25	0.5 to 100	46	9
143	Penicillin G	7.00	Antibiotic/Beta-lactam	61-33-6	N/A	0.5	1 to 100	90	7
144	Penicillin V [Phenoxymethylpenicillin]	7.41	Antibiotic/Beta-lactam	87-08-1	N/A	1	2.5 to 100	117	2
145	Phenylbutazone	9.09	NSAIDs	50-33-9	N/A	0.25	0.5 to 100	106	3
146	Phosalone	9.77	Insecticide	2310-17-0	N/A	0.5	1 to 100	113	9
147	Phoxim	9.70	Insecticide	14816-18-3	N/A	1	2.5 to 100	110	10
148	Piperonyl butoxide Ammonia	10.31	Insecticide	51-03-6	50	0.1	0.25 to 100	116	1
149	Pirlimycin	6.10	Antibiotic/Aminoglycosides	79548-73-5	100	1	2.5 to 100	96	4
150	Praziquantel	8.57	Anthelmintic	55268-74-1	N/A	0.25	0.5 to 100	116	2
151	Prednisolone	7.29	Growth promoters/Corticosteroids	50-24-8	6	0.1	0.25 to 100	120*	2*
152	Prednisone	7.13	Growth promoters/Corticosteroids	53-03-2	N/A	0.25	0.5 to 100	117	3

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153	Progesterone	9.60	Hormones	57-83-0	N/A	10	25 to 100	117#	4#
154	Propionylpromazin	8.00	Antiemetic	3568-24-9	N/A	0.05	0.1 to 100	98	2
155	Propyphenazone	7.68	NSAIDs	479-92-5	N/A	0.05	0.1 to 100	118	3
156	Pyrantel	4.29	Anthelmintic	15686-83-6	N/A	0.1	0.25 to 100	105	2
157	Pyrimethamine	6.31	Antimicrobial	58-14-0	N/A	0.05	0.1 to 100	112	2
158	Ractopamine	4.66	Growth promoters/Beta-agonists	97825-25-7	N/A	0.1	0.25 to 100	117	1
159	Rafoxanide	11.11	Anthelmintic	22662-39-1	10	0.25	0.5 to 100	70	4
160	Rifaximin	9.07	Antibiotic	80621-81-4	60	0.1	0.25 to 100	104	4
161	Robenidine	8.58	Coccidiostats	25875-51-8	N/A	0.25	0.5 to 100	101	4
162	Ronidazole	3.40	Anthelmintic/Nitroimidazoles	7681-76-7	N/A	0.1	0.25 to 100	119	2
163	Salbutamol [Albuterol]	3.03	Growth promoters/Beta-agonists	18559-94-9	N/A	0.05	0.1 to 100	105	2
164	Salinomycin	11.62	Coccidiostats	53003-10-4	N/A	0.1	0.25 to 100	77	6
165	Sarafloxacin	5.39	Antibiotic/Quinolones	98105-99-8	N/A	0.1	0.25 to 100	107	3
166	Spiramycin I	6.13	Antibiotic/Macrolides	24916-50-5	200	0.25	0.5 to 100	82	2
167	Sulfabenzamide	6.07	Antibiotic/Sulfonamides	127-71-9	100	0.05	0.1 to 100	120	2
168	Sulfacetamide	3.13	Antibiotic/Sulfonamides	144-80-9	100	0.1	0.25 to 100	115	2
169	Sulfachloropyridazine	5.25	Antibiotic/Sulfonamides	80-32-0	100	0.1	0.25 to 100	116	2
170	Sulfaclozine	6.30	Antibiotic/Sulfonamides	102-65-8	100	0.25	0.5 to 100	116	4
171	Sulfadiazine [Silvadene]	3.42	Antibiotic/Sulfonamides	68-35-9	100	0.1	0.25 to 100	120	2
172	Sulfadimethoxine	6.44	Antibiotic/Sulfonamides	122-11-2	100	0.05	0.1 to 100	119	2
173	Sulfadimidine [Sulfamethazine]	4.62	Antibiotic/Sulfonamides	57-68-1	100	0.1	0.25 to 100	114	2
174	Sulfadoxine	5.58	Antibiotic/Sulfonamides	2447-57-6	100	0.05	0.1 to 100	119	2
175	Sulfaethoxypyridazine	5.93	Antibiotic/Sulfonamides	963-14-4	100	0.05	0.1 to 100	117	1
176	Sulfaguanidine	1.72	Antibiotic/Sulfonamides	57-67-0	100	0.25	0.5 to 100	107	1
177	Sulfamerazine	4.02	Antibiotic/Sulfonamides	127-79-7	100	0.1	0.25 to 100	118	1
178	Sulfameter [sulfamethoxydiazine]	4.48	Antibiotic/Sulfonamides	651-06-9	100	0.1	0.25 to 100	113	2
179	Sulfamethizole	4.50	Antibiotic/Sulfonamides	144-82-1	100	0.1	0.25 to 100	115	3
180	Sulfamethoxazole	5.47	Antibiotic/Sulfonamides	723-46-6	100	0.1	0.25 to 100	116	2
181	Sulfamethoxypyridazine	4.68	Antibiotic/Sulfonamides	80-35-3	100	0.1	0.25 to 100	114	1
182	Sulfamonomethoxine	5.23	Antibiotic/Sulfonamides	1220-83-3	100	0.1	0.25 to 100	118	2
183	Sulfamoxole	4.31	Antibiotic/Sulfonamides	729-99-7	100	0.05	0.1 to 100	112	2
184	Sulfanitran	7.33	Antibiotic/Sulfonamides	122-16-7	100	1	2.5 to 100	115	7
185	Sulfaphenazole	6.34	Antibiotic/Sulfonamides	526-08-9	100	0.1	0.25 to 100	115	3
186	Sulfapyridine	3.83	Antibiotic/Sulfonamides	144-83-2	100	0.1	0.25 to 100	115	1
187	Sulfaquinoxaline	6.51	Antibiotic/Sulfonamides	59-40-5	100	0.1	0.25 to 100	119	3
188	Sulfathiazole	3.62	Antibiotic/Sulfonamides	72-14-0	100	0.1	0.25 to 100	115	2
189	Sulfisomidine	3.34	Antibiotic/Sulfonamides	515-64-0	100	0.05	0.1 to 100	111	1
190	Sulfisoxazole	5.76	Antibiotic/Sulfonamides	127-69-5	100	0.1	0.25 to 100	113	3
191	Sulindac	8.03	Antibiotic/Sulfonamides	38194-50-2	100	0.1	0.25 to 100	117	3
192	Teflubenzuron	10.08	Insecticide	83121-18-0	N/A	0.5	1 to 100	117	3
193	Testosterone	8.56	Growth promoters/Anabolic steroids	58-22-0	N/A	0.1	0.25 to 100	119	4
194	Tetracycline	4.78	Antibiotic/Tetracycline	60-54-8	100	0.25	0.5 to 100	64	4
195	Thiabendazole	4.34	Anthelmintic/Benzimidazoles	148-79-8	50	0.05	0.1 to 100	112	2
196	Thiamphenicol	4.33	Antibiotic/Amphenicols	15318-45-3	50	0.25	0.5 to 100	120	3

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197	Tiamulin	7.68	Antibiotic	55297-95-5	N/A	0.05	0.1 to 100	114	2
198	Tilmicosin	6.87	Antibiotic/Macrolides	108050-54-0	50	1	2.5 to 100	90	7
199	Tolfenamic acid	9.94	NSAIDs	13710-19-5	50	1	2.5 to 100	114	8
200	Trenbolone	7.98	Growth promoters/Anabolic steroids	10161-33-8	N/A	0.25	0.5 to 100	111	4
201	Trichlorfon [DEP]	5.29	Tranquilizer	52-68-6	50	0.5	1 to 100	124	3
202	Triclabendazole	9.74	Anthelmintic/Benzimidazoles	68786-66-3	10	0.1	0.25 to 100	113	2
203	Trimethoprim	4.12	Antibiotic	738-70-5	50	0.1	0.25 to 100	108	3
204	Tripelennamine	6.39	Anthelmintic	91-81-6	20	0.05	0.1 to 100	105	2
205	Tylosin	7.64	Antibiotic/Macrolides	1401-69-0	50	0.5	1 to 100	109	5
206	Valnemulin	8.39	Antibiotic	101312-92-9	N/A	0.1	0.25 to 100	113	3
207	Vedaprofen	9.14	NSAIDs	71109-09-6	N/A	0.1	0.25 to 100	114	2
208	Virginiamycin M1	8.21	Antibiotic/Macrolides	21411-53-0	N/A	0.1	0.25 to 100	114	5
209	Xylazine	5.24	Tranquilizer	7361-61-7	N/A	0.1	0.25 to 100	109	2
210	Zilpaterol	2.98	Growth promoters/Beta-agonists	119520-05-7	N/A	0.1	0.25 to 100	96	3

• Data using LLQC, * Data using LQC, # Data using HQC

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