Analysis of 122 Veterinary Drugs in Meat Using All Ions MS/MS with an Agilent 1290/6545 UHPLC-Q-TOF System

Application Note

Authors

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Abstract

The presence of veterinary drugs (VDs) in meat may pose a health risk to humans during consumption; therefore, monitoring of VD residues for regulatory enforcement and risk assessment is commonly conducted. Several hundred VDs are available spanning a number of classes with very different chemical characteristics. This typically requires sophisticated analytical methods and instruments, generally based on LC-MS/MS, sophisticated workflows, and often tedious data processing. In this study, a 12-minute analytical method was developed for 122 priority VDs in meat. The method uses Agilent All Ions MS/MS on an Agilent Q-TOF LC/MS instrument along with the Agilent Veterinary Drugs personal compound database and library (PCDL) to test the method. All 122 VDs were spiked into bovine liver, kidney, and muscle tissue at levels of 0.5, 1, and 2 times the maximum tolerance levels for each drug. These spikes were then analyzed using All Ions data acquisition mode in an Agilent 6545 Q-TOF LC/MS. The Agilent MassHunter Find by Formula software was used to detect and verify the presence of these compounds. The PCDL provides MS/MS spectral and retention time information about each compound enabling a data review process to quickly and reliably filter out false positives. At all three spike levels, >92% of VDs were detected in every matrix. To demonstrate the ability of this system to deliver quantitative results, calibration curves were generated for ground beef and liver starting at low ng/g levels. With >85% VDs having an $R^2 > 0.99$ without correction using any internal standard, this method can be used to do screening and quantification of VDs in animal matrices in one analytical run.

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Introduction

The breeding of livestock for food requires the controlled use of veterinary drugs (VDs) as a means to prevent diseases or promote rapid growth. However, poor management strategies and improper administration of these drugs to livestock can lead to drug residues being present in the animal meat and other organs, which can pose a human health risk on consumption. Antimicrobial resistance is another concern that arises from the use of antibiotics in agriculture. Therefore, the levels of VDs in meat and other foods are regulated with maximum residue levels (MRLs) or tolerance levels that vary significantly from one drug to another [1-3]. There are several hundred VDs known to be used in livestock, varying vastly in class, chemical structure, and polarity, making them difficult to analyze in the same method. Furthermore, the MRLs are often low, and must be achieved in complex matrices, requiring sensitive and robust analytical equipment [4,5].

LC/MS technologies have been shown to offer sensitivity and selectivity, along with time, labor, and cost savings, through multiclass multiresidue methods [2,6]. However, the use of accurate mass high resolution time-of-flight (TOF) mass spectrometers can give the user some extra capabilities. Full spectrum data acquisition ensures that signals from all ionizing compounds in the sample are captured. Therefore, it is possible for a surveillance scheme using this technology to grow to accommodate new compounds of emerging concern without the need for any method development. Moreover, it is possible to perform retrospective data mining for new analytes without rerunning samples. In addition, TOF spectra permit the detection and elucidation of new VDs and metabolites for which analytical standards may be unavailable.

This study sought to develop a rapid screening method for >120 commonly monitored VDs across multiple classes. The list of VDs analyzed was based on previous work performed by the US Department of Agriculture's Agricultural Research Service (USDA-ARS) and Food Safety and Inspection Service (USDA-FSIS) [2,7]. The VDs were analyzed in bovine muscle, kidney, and liver using an Agilent Q-TOF LC/MS operating with All Ions MS/MS acquisition. This mode of data collection provides high resolution accurate mass spectra of both molecular ions (low energy channels) and fragment ions (high energy channels).

MS/MS spectra in the Agilent PCDL were then used to verify if the molecular ions and corresponding fragment ions match those in the sample. This study also included the use of commercialized Agilent QuEChERS Enhanced Matrix Removal (EMR—Lipid) material for cleanup of meat extracts. This has previously been shown to effectively and selectively remove lipids from high fat food commodities.

The quantitative capability in this overall method was evaluated by generating matrix matched calibration curves in ground beef and liver at ng/g levels.

Experimental

Standards and Reagents

A significant number of veterinary drug standards were provided by the USDA-ARS Eastern Regional Research Center (Wyndmoor, PA) as solutions in acetonitrile (MeCN), methanol, water, or a combination thereof between 214 and 1,200 mg/L. Abamectin, ivermectin, thiouracil, and the β-lactams (amoxicillin, ampicillin, cefazolin, desacetyl cephapirin, cloxacillin, nafcillin, oxacillin, and penicillin) were purchased from Sigma-Aldrich (St. Louis, MO). Ultrapure water was obtained from a Millipore (Billerica, MA) system and was >18.2 MΩ-cm. MeCN (LC-MS grade) was purchased from VWR International (Radnor, PA), and formic acid (88% double distilled) was purchased from GFS Chemicals (Powell, OH).

Sample extraction

Prehomogenized samples (2 g each) of bovine muscle, liver, and kidney were spiked with 122 VDs and two internal standards (flunixin-d_3 and sulfamethazine-$^{13}C_6$) as selected previously [2]. The spiking concentrations were 0.5, 1, and 2 times the USDA tolerance levels (x, shown in Table 1), in all three matrices. Sample preparation was conducted using the Agilent Bond Elut EMR—Lipid procedure as described in a previous application note (5991-6096EN) [8]. The final extracts were diluted to 80/20 water/MeCN ratio, and stored in 2-mL polypropylene autosampler vials before being injected into the LC/MS. Spiking solution was added to solvent and blank matrix extracts at 0.5x, 1x, and 2x levels (post-extraction) to evaluate instrument and analytical method performance, as well as matrix effects. All extracts were stored in a –10 °C freezer, and analyzed two weeks after sample preparation.

Table 1. Veterinary Drugs Monitored in PCDL Along with Tolerance Levels

Veterinary drug	Class	1x Tolerance level (ng/g)
2-Amino Flubendazole	Anthelmintic	10
2-Amino Mebendazole	Anthelmintic	10
2-Hydroxy Dimetridazole	Coccidiostat	50
2-Mercaptobenzimidazole	Thyreostat	25
2-Thiouracil	Thyreostat	400
5-hydroxy thiabendazole	Anthelmintic	100
6-Methylthiouracil	Thyreostat	400
6-Phenylthiouracil	Thyreostat	400
6-Propyl-2-thiouracil	Thyreostat	50
Abamectin	Anthelmintic	20
Acetopromazine	Tranquilizer	10
Albendazole	Anthelmintic	50
Albendazole sulfoxide	Anthelmintic	50
Albendazole sulphone	Anthelmintic	50
Albendazole-2-aminosulphone	Anthelmintic	50
Amoxicillin	β-Lactam	10
Ampicillin	β-Lactam	10
Azaperone	Tranquilizer	10
Bacitracin	Miscellaneous	500
Betamethasone	Anti-inflammatory	100
Cambendazole	Anthelmintic	10
Carazolol	Tranquilizer	10
Carbadox	Miscellaneous	30
Cefazolin	β-Lactam	100
Cephapirin	β-Lactam	100
Chloramphenicol	Phenicol	10
Chlorpromazine (thorazine)	Tranquilizer	10
Chlortetracycline	Tetracycline	1,000
Cimaterol	β-Agonist	10
Ciprofloxacin	Fluoroquinolone	50
Clenbuterol	β-Agonist	10
Clindamycin	Macrolide/Lincosamide	100
Cloxacillin	β-Lactam	10
Danofloxacin	Fluoroquinolone	200
DCCD (marker for ceftiofur)	β-Lactam	400
Desacetyl cephapirin	β-Lactam	100
Desethylene ciprofloxacin	Fluoroquinolone	100
Diclofenac	Anti-inflammatory	200
Dicloxacillin	β-Lactam	100
Difloxacin	Fluoroquinolone	50
Dimetridazole	Coccidiostat	10
Dipyrone (metabolite)	Anti-inflammatory	200
Doramectin	Anthelmintic	30
Doxycycline	Tetracycline	100
Emamectin	Anthelmintic	10
Enrofloxacin	Fluoroquinolone	100
Eprinomectin B1a	Anthelmintic	100
Erythromycin A	Macrolide/Lincosamide	100
Fenbendazole	Anthelmintic	400
Fenbendazole sulphone	Anthelmintic	400
Florfenicol	Phenicol	300
Florfenicol amine	Phenicol	300
Flubendazole	Anthelmintic	10
Flunixin	Anti-inflammatory	25
Flunixin-d3	Internal Standard	250
Gamithromycin	Macrolide/Lincosamide	100
Haloperidol	Tranquilizer	10
Haloxon	Anthelmintic	100
Hydroxy-Ipronidazole	Coccidiostat	10
Ipronidazole	Coccidiostat	10
Ivermectin B1a	Anthelmintic	10
Ketoprofen	Anti-inflammatory	10
Levamisole	Anthelmintic	100
Lincomycin	Macrolide/Lincosamide	100
Mebendazole	Anthelmintic	10
Melengesterol acetate	Miscellaneous	25
Meloxicam	Anti-inflammatory	100
Metronidazole	Coccidiostat	10
Morantel	Anthelmintic	100
Moxidectin	Anthelmintic	50
Nafcillin	β-Lactam	100
Norfloxacin	Fluoroquinolone	50
Novobiocin	Miscellaneous	1,000
Orbifloxacin	Fluoroquinolone	50
Oxacillin	β-Lactam	100
Oxfendazole	Anthelmintic	800
Oxibendazole	Anthelmintic	10
Oxyphenylbutazone	Anti-inflammatory	100
Oxytetracycline	Tetracycline	1000
Penicillin G	β-Lactam	50
Phenylbutazone	Anti-inflammatory	100
Pirlimycin	Macrolide/Lincosamide	300
Prednisone	Anti-inflammatory	100
Promethazine	Tranquilizer	10
Propionylpromazine	Tranquilizer	10
Quinoxaline-2-carboxylic acid	Miscellaneous	30

Veterinary drug	Class	1x Tolerance level (ng/g)
Ractopamine	B-Agonist	30
Ronidazole	Coccidiostat	10
Salbutamol	B-Agonist	10
Sarafloxacin	Fluoroquinolone	50
Selamectin	Anthelmintic	200
Sulfabromomethazine	Sulfonamide	100
Sulfachloropyridazine	Sulfonamide	100
Sulfadiazine	Sulfonamide	100
Sulfadimethoxine	Sulfonamide	100
Sulfadoxine	Sulfonamide	100
Sulfaethoxypyridazine	Sulfonamide	100
Sulfamerazine	Sulfonamide	100
Sulfamethazine	Sulfonamide	100
Sulfamethazine-$^{13}C_6$	Internal Standard	250
Sulfamethizole	Sulfonamide	100
Sulfamethoxazole	Sulfonamide	100
Sulfamethoxypyridazine	Sulfonamide	100
Sulfanilamide	Sulfonamide	100
Sulfanitran	Sulfonamide	100
Sulfapyridine	Sulfonamide	100
Sulfaquinoxaline	Sulfonamide	100
Sulfathiazole	Sulfonamide	100
Tetracycline	Tetracycline	1,000
Thiabendazole	Anthelmintic	100
Thiamphenicol	Phenicol	10
Tildipirosin	Macrolide/Lincosamide	100
Tilmicosin	Macrolide/Lincosamide	100
Tolfenamic acid	Anti-inflammatory	200
Triclabendazole	Anthelmintic	50
Triclabendazole sulfoxide	Anthelmintic	50
Triflupromazine	Tranquilizer	10
Troleandomycin	Macrolide/Lincosamide	1,000
Tulathromycin A	Macrolide/Lincosamide	5,500
Tylosin	Macrolide/Lincosamide	200
Virginiamycin	Miscellaneous	100
Xylazine	Tranquilizer	10
Zeranol (β-Zearalanol)	Miscellaneous	100
Zilpaterol	B-Agonist	12

Instrumental analysis

An Agilent 1290 Infinity ultrahigh-performance liquid chromatograph (UHPLC) with a 40 µL loop HiPALS autosampler was used for this method. Separation was performed with an Agilent ZORBAX Eclipse Plus C-18 (2.1 × 150 mm, 1.8 µm) column using a gradient of water + 0.1% formic acid (A) and MeCN + 0.1% formic acid (B). An Agilent inline filter (p/n 5067-4638) was installed after the autosampler and an Agilent ZORBAX Eclipse plus guard column (p/n 959757-902) was used before the analytical column to protect and enhance column lifetime. Table 2 lists the LC conditions used for this analysis. Figure 1 illustrates a sample chromatogram of a VD standard at 50 ng/mL in 80/20 water/MeCN.

Table 2. LC Conditions

Parameter	Value	
Instrument	Agilent 1290 Infinity LC	
Column	Agilent ZORBAX Eclipse Plus C-18, 2.1 × 150 mm, 1.8 µm (p/n 959759-902)	
Mobile phase	A) Water + 0.1% formic acid B) Acetonitrile + 0.1% formic acid	
Gradient	Time (min)	B (%)
	0.0	2
	1.0	2
	10	100
	11	100
	11.1	2
Flow rate	0.5 mL/min	
Post time	3.0 minutes	
Column temperature	30 °C	
Injection volume	15 µL	

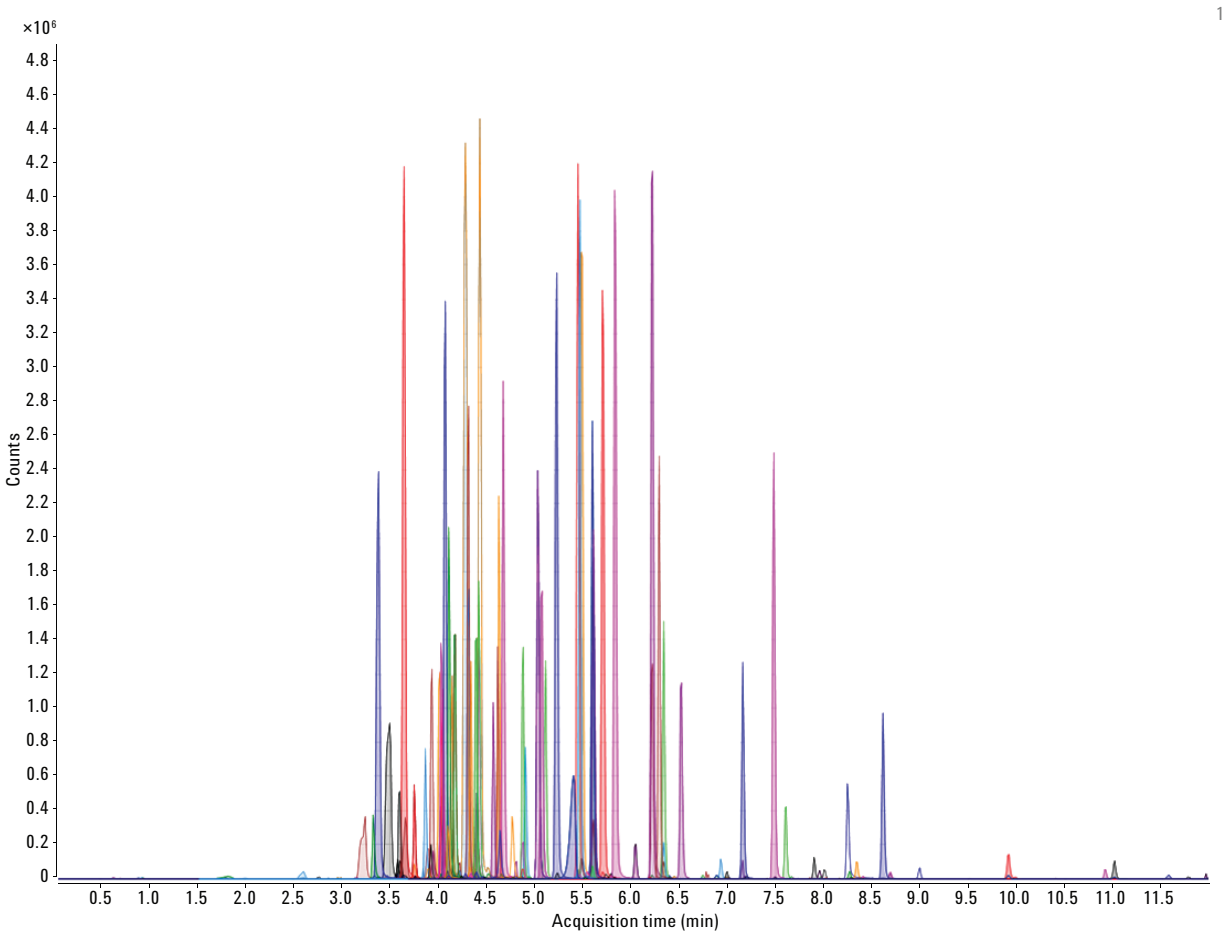

Figure 1. Sample chromatogram at 50 ng/g for 122 veterinary drugs.

An Agilent 6545 Accurate-Mass Quadrupole Time-of-Flight LC/MS system with Agilent Jet Stream dual electrospray source operating in positive mode was used for this analysis. The mass spectrometer operation conditions are detailed in Table 3. The 6545 Q-TOF LC/MS was tuned using the Agilent tune solution (p/n G1969-85000) over the entire mass range. Using the SWARM tune capability in the 6545 Q-TOF LC/MS, the instrument was tuned with the fragile ion tune for a mass range of *m/z* 50–750 in the 2 GHz extended dynamic range. During analysis, the reference ions consisting of purine (*m/z* 122.0509) and HP-921 (*m/z* 922.0098) were delivered to the mass spectrometer from reference bottle A on the mass spectrometer.

Table 3. Mass Spectrometer Conditions

Parameter	Value
Instrument	Agilent 6545 Accurate-Mass Q-TOF LC/MS
Ionization mode	Positive electrospray ionization with jet stream
Instrument mode	2 GHz extended dynamic range
Instrument tune range	SWARM tune with fragile ion (*m/z* 50–750)
Mass range	*m/z* 50–1,000
Drying gas temperature	200 °C
Drying gas flow	11 L/min
Sheath gas temperature	375 °C
Sheath gas flow	11 L/min
Nebulizer gas	35 psi
Fragmentor	135 V
Capillary	3,500
Nozzle voltage	300 V
Skimmer	45
Collision energy	0, 10, 40 V

All Ions MS/MS workflow and data analysis

The All Ions MS/MS workflow is designed to acquire high resolution MS data simultaneously in low and high collision energy channels by collecting: (A) pseudo-molecular ion or precursor ion data from the low collision energy (CE) channel, and (B) fragment ion information from the high collision energy channel. For this experiment, the instrument was set at CEs of 0 V, 10 V, and 40 V. The 0 V setting was used to acquire precursor information, while 10 V was sufficient to get good fragment ion information for the majority of VDs. Some larger VDs, such as mectins, require higher CEs to fragment the precursor; therefore, a 40 V channel was also used to collect a third channel of data.

Data acquisition was performed using Agilent MassHunter software (Ver. B.06.01), while Agilent MassHunter Qualitative software (Ver B.07.00) was used for data analysis. The Find by Formula feature in MassHuter Qualitative Analysis was used with the database search function to take advantage of the PCDL information. The PCDL consisted of all the VDs necessary for this study with information on molecular formulas, exact monoisotopic mass, CAS number, MS/MS spectra collected at 0, 10, 20, and 40 V for the $[M+H]^+$ ion and retention times obtained from running standards against the developed LC method. This allowed significant increase in specificity of identification. Figure 2 illustrates the All Ions MS/MS workflow including data processing filters used for analysis of VDs in this study.

Results and Discussion

Identification of VDs in meat

The All Ions MS/MS workflow identifies precursor masses and uses the spectral data available in the Agilent PCDL to look for coeluting fragments in the high collision energy channel. Before showing how such data can be reviewed in a high throughout scenario, Figure 3 illustrates the component pieces of data that are used to automatically verify the results in MassHunter Qualitative with the All Ions MS/MS approach. The inset 3A indicates the mass spectrum for novobiocin identified in bovine muscle at the 1x tolerance level with the expected isotope abundances and spacing in red boxes matched up against actual data (vertical red sticks). The mass accuracy, isotope spacing, isotope abundance, and retention time (RT) matching with the PCDL accounted for a total score of 98.41. The inset in Figure 3B is the MS/MS spectrum for novobiocin at CE of 10 V, available in the Agilent PCDL, with the $[M+H]^+$ precursor (I) and the three most abundant fragment ions (II, III, and IV) denoted. Figure 3C shows the actual chromatographic peaks seen in the muscle sample at the correct RT for the four ions. Fragment II (*m/z* 189.0910) and III (*m/z* 218.1023) were qualified, but IV (*m/z* 396.1442) had an S/N of <9.0 and was not included for further qualification (based on the S/N threshold set in the data analysis method: Figure 2). Data for the fragments were assessed in MassHunter Qualitative through the coelution score and plot.

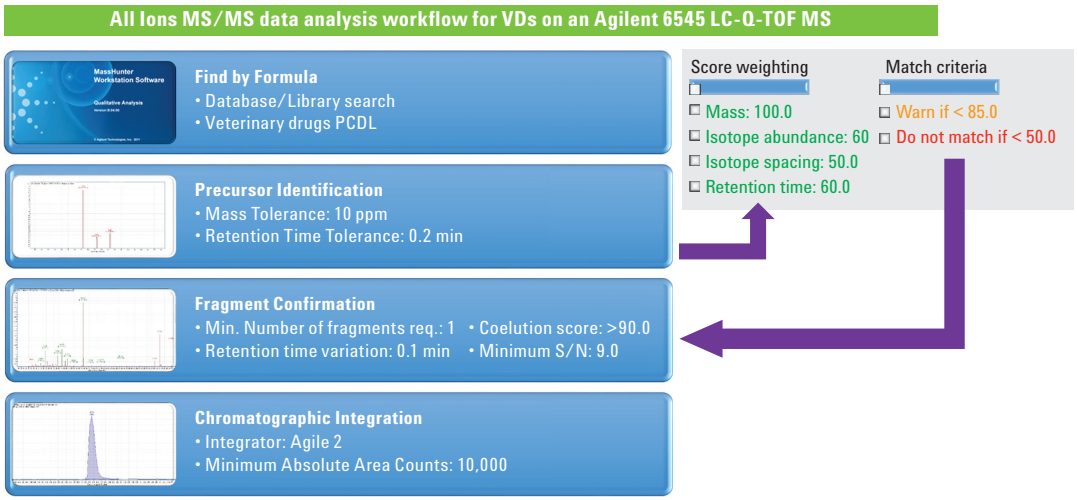

Figure 2. Agilent All Ions MS/MS data analysis workflow.

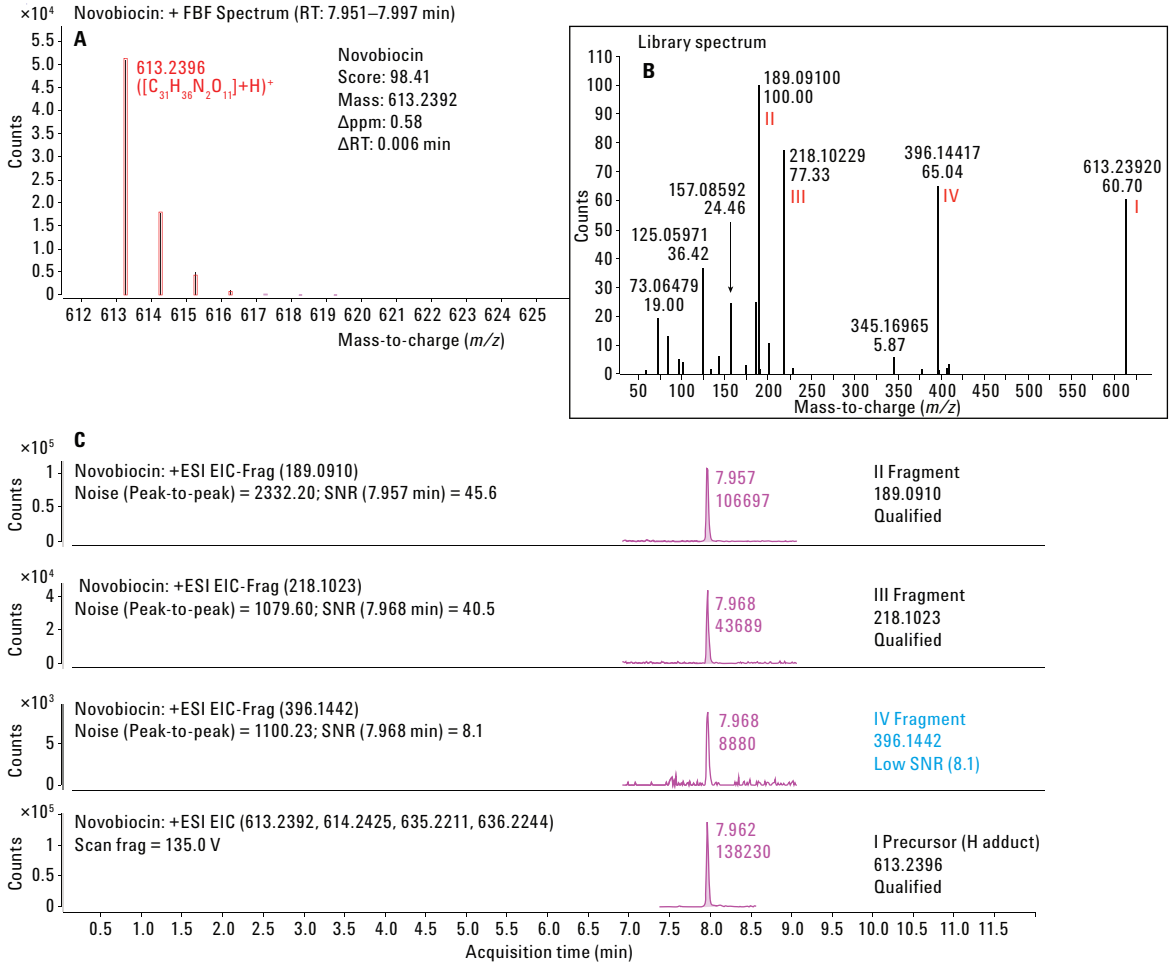

Figure 3. A) Mass spectra and isotope spacing of Novobiocin in muscle. B) Library spectra in Agilent PCDL at CE: 10 eV for Novobiocin with precursor (I) and the three most abundant fragments (II, III, and IV), compared to (C) actual sample data. Compound positively identified.

In Figure 4A, the plot overlays the extracted ion chromatogram of the molecular ion peak over the fragment ion peaks for novobiocin in the bovine muscle. The coelution score for each fragment ion (value 0–100, where 100 is the highest possible score) was calculated based on its intensity ratio to the reference ion across the elution time range after normalization, and applying a weighting to de-emphasize the contribution at the beginning and end of the reference ion peak. An RT shift, different peak widths, or different peak symmetry (fronting, tailing) will all negatively impact the coelution score. Figure 4B shows the ion coelution plot of novobiocin in the muscle sample. The coelution plot was prepared by overlaying the chromatogram of each fragment ion with the reference ion (precursor ion in LC/MS) after normalizing to the maximum intensity of both within the elution time range of the reference ion, and plotting the intensity ratios within that time range. Ratios of 1 or close to 1 across the center of the reference ion peak indicate that a fragment ion exhibits strong coelution. The plot provides a powerful visual assessment of the validity of a fragment signal.

The related ions coelution score is also a productive route to verifying the reliability of a hit occurring with the software. In addition, a threshold can be set to help filter out potential false positives. In this method, the data analysis required that that at least one fragment provided a coelution score of over 90.0. Consequently, novobiocin in Figure 4 was positively identified in this sample.

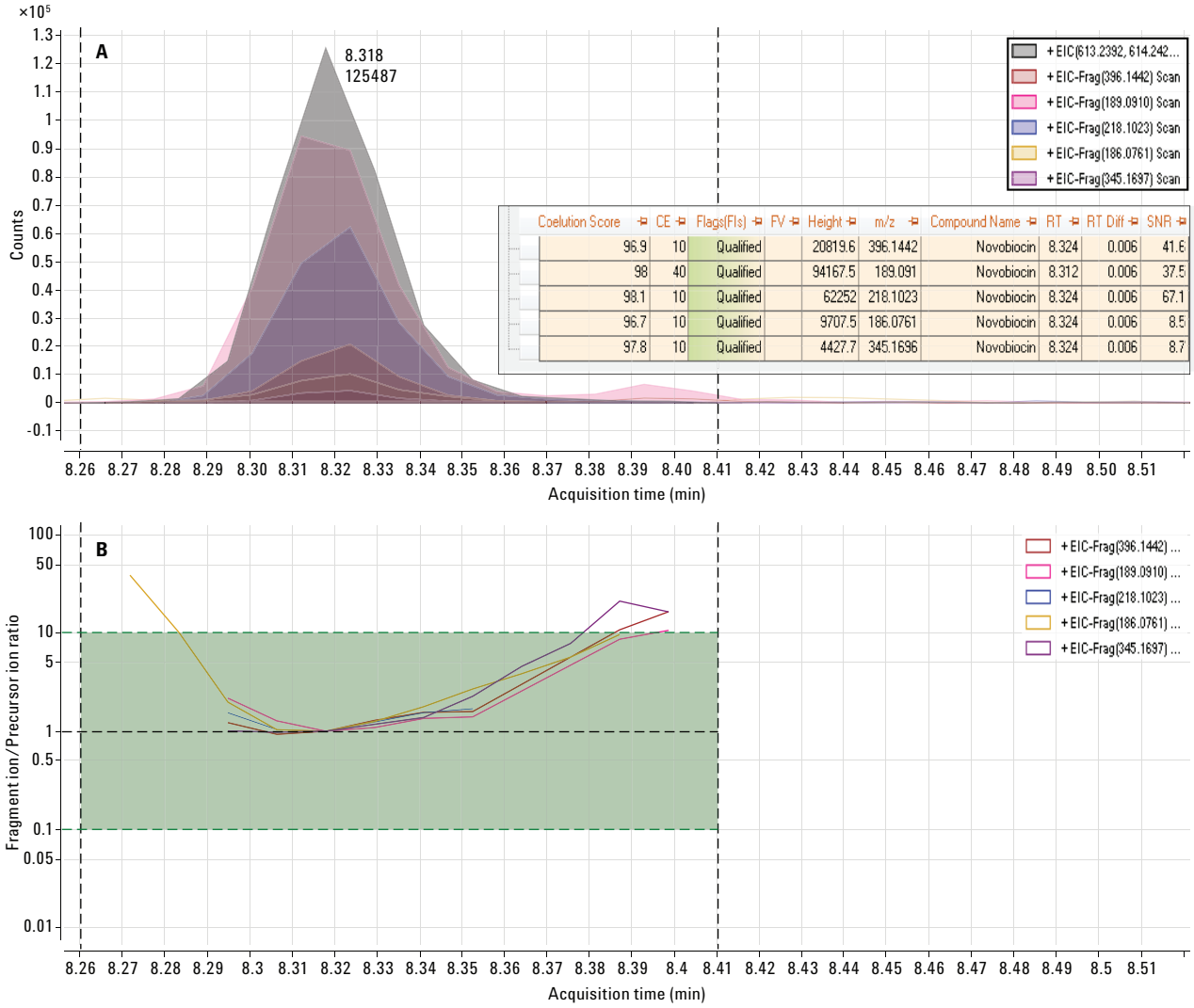

Figure 4. A) EICs of precursor and fragment ions for novobiocin in bovine muscle. B) Normalized fragment to precursor ion ratio plotted against retention time.

Reduction in false positives

The presence of isomers, isobaric compounds, interferences, and coeluting matrix elements in real samples can often mean that the measurement of accurate mass for a precursor ion alone is not a definitive compound identification. The All Ions MS/MS workflow described above is a powerful tool to achieve this, but it is most powerful when combined with a RT requirement. For this reason, we used the Veterinary Drugs AMRT PCDL, which includes the RTs specific to the LC method described in this work. The availability of high resolution fragment ion spectra that include retention times in the Agilent PCDL dramatically reduces the detection of false positives without the need to continually inject analytical standards.

Figure 5 shows an example where the use of RT matching and fragment ion verification available through the Agilent VD PCDL prevented a false positive. Enrofloxacin, a fluoroquinolone, was detected in a spiked sample of kidney extract at 4.088 and 6.015 minutes. Both species had a mass error of <2.0 ppm compared to the $[M+H]^+$ ion for enrofloxacin (360.1718), and would both have been characterized as a detect for the compound even with a tight mass tolerance window of 5.0 ppm. However, using the **verify with fragment ions** option in MassHunter Qualitative software, none of the four most abundant fragments of enrofloxacin were present in the 6.015 minutes peak, while all ion fragments were detected in the 4.088 minutes peak.

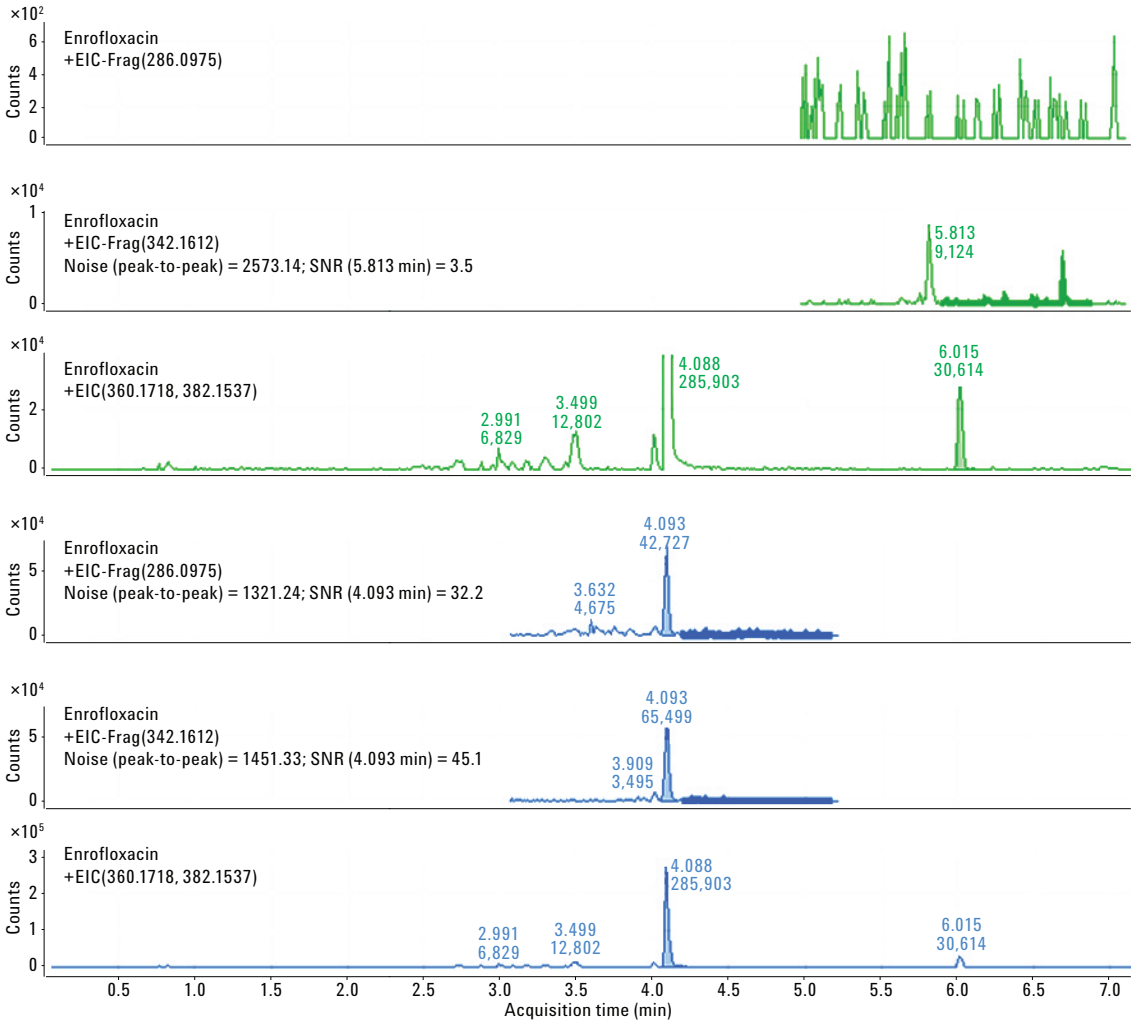

Figure 5. Potential false positive of enrofloxacin dentified through fragment ion confirmation using the Agilent All Ions workflow.

It was stated earlier that retention time was also seen as a powerful qualifier and was also used to verify results through inclusion in the PCDL. The Agilent Veterinary Drugs PCDL with the RT information for these compounds included was used to verify that the enrofloxacin peak is expected at 4.23 minutes in this method. This was done by selecting the quality ions with the **Mass and Retention time** feature in the MassHunter Qualitative software with a ± 0.2 minute RT setting. It was therefore unnecessary to inject all analytical standards with each run, which is a welcome benefit when over 100 compounds are involved. This also means that this method and it's associated PCDL with retention times can be implemented very easily in other labs and is very cost-effective and straightforward to run.

Sensitive detection of VDs in bovine kidney, liver, and muscle extracts

The VDs were classified as being detected if there was a match within the tolerance limits for the molecular mass (<10.0 ppm), the presence of at least one coeluting fragment ion at an S/N ratio >9.0, and a RT within 0.2 minutes. Compounds were characterized as being tentatively identified if the precursor ion was within the 10.0 ppm tolerance, but either the RT was off by >0.2 minutes, or fragment ions were not found or had an ions coelution score of <90.0. Figure 6 presents the results for all three matrices and reagent blanks at the three spiking levels. At least 92% of VDs were either positively or tentatively identified in all samples. In the standards, 98% (2x), 96% (1x), and 94% (0.5x) of VDs were detected. When looking at the matrix spiked samples, the detects ranged from 94–96% in the liver, 94–97% in the muscle, and 93–97% in the kidney. The VDs positively detected (with fragment ion and RT agreement) in the standards were between 88% and 90% across the three spike levels. Similarly, 81–88% (liver), 82–88% (muscle), and 79–86% (kidney) of the VDs were positively identified in the matrix samples.

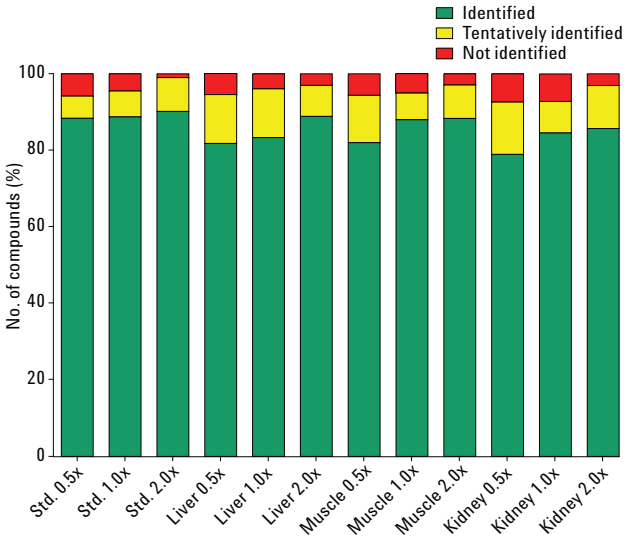

Figure 6. Percentage of veterinary drugs identified in spiked samples of reagent blank and matrix at three different levels (x: tolerance levels).

Only two compounds (cephapirin and cimaterol) were not detected in any sample or the standards at all spike levels. Table 4 lists a few compounds that were not identified in some of the samples. These nine compounds require some extra study to determine their feasibility of analysis with the current method. Most, if not all, of these cases are probably the result of degradation in the spiked samples and in the standards themselves, some of which were only available as mixtures in solution. In both cases, there was an unavoidable 2-week delay between preparing the spiked extracts and standards to the time of injection on the instrument. In a separate project carried out on another Agilent Q-TOF instrument, all six β-lactams in question were reliably detectable at half the spiking level for the matrices described using the same method. In this case, much fresher samples and standards were used. Further work is planned to determine if all these nine compounds can join the others as being detectable and identifiable at their half tolerance level, using the above screening method.

Approximately 10% of compounds were tentatively identified in each of the three matrices. We plan to revisit some of these compounds to investigate whether the analysis of fresher spikes and standards can result in not only the detection of precursor ions, but also in the diagnostic fragments required for full verification according to the previously described requirements set by this method.

Overall, the results in the three matrices were similar, and detection rates were high for all in a single run of less than 15 minutes.

Table 4. List of VDs Not Identified in the Samples

Class	Std. 0.5x	Std. 1.0x	Std. 2.0x	Liver 0.5x	Liver 1.0x	Liver 2.0x
β-Agonist	Cimaterol	Cimaterol	Cimaterol	Cimaterol	Cimaterol	Cimaterol
β-Lactam	Cephapirin	Cephapirin	Cephapirin	Cephapirin	Cephapirin	Cephapirin
β-Lactam	Cloxacillin	Cloxacillin	✓	Cloxacillin	Cloxacillin	Cloxacillin
β-Lactam	Amoxicillin	Amoxicillin	✓	Amoxicillin	Amoxicillin	Amoxicillin
β-Lactam	Ampicillin	Ampicillin	✓	Ampicillin	Ampicillin	✓
β-Lactam	Nafcillin	✓	✓	Nafcillin	✓	✓
β-Lactam	Oxacillin	✓	✓	Oxacillin	✓	✓
All other compounds	✓	✓	✓	✓	✓	✓

Class	Kindney 0.5x	Kindney 1.0x	Kindney 2.0x	Muscle 0.5x	Muscle 1.0x	Muscle 2.0x
β-Agonist	Cimaterol	Cimaterol	Cimaterol	Cimaterol	Cimaterol	Cimaterol
β-Lactam	Cephapirin	Cephapirin	Cephapirin	Cephapirin	Cephapirin	Cephapirin
β-Lactam	Cloxacillin	Cloxacillin	Cloxacillin	Cloxacillin	Cloxacillin	Cloxacillin
β-Lactam	Amoxicillin	Amoxicillin	Amoxicillin	Amoxicillin	Amoxicillin	Amoxicillin
β-Lactam	Ampicillin	Ampicillin	✓	Ampicillin	Ampicillin	✓
β-Lactam	Nafcillin	Nafcillin	✓	Nafcillin	✓	✓
β-Lactam	Oxacillin	Oxacillin	✓	Oxacillin	✓	✓
Misc.	Zeranol	Zeranol	✓	✓	Zeranol	✓
Thyreostat	Propyl-thiouracil	Propyl-thiouracil	✓	✓	✓	✓
All other compounds	✓	✓	✓	✓	✓	✓

Quantification of VDs using Q-TOF LC/MS

To determine the linearity of the LC Q-TOF in the All Ions MS/MS method developed for the VDs, a four or five point matrix-matched calibration curve of 113 VDs were prepared in ground beef and liver samples extracted with the EMR—Lipid procedure. Levels were between 2 (or 10) and 100 ng/g.

Quantification of a large number of disparate analytes in complex matrices is difficult, and almost always requires the use of several surrogates and internal standards to correct for variable ion suppression effects through the extraction and analytical run. Selection of good internal standards depends on various factors including analytics and economics. To avoid bias, raw data without correction with an internal standard have been provided in this section. To determine the linearity for quantification, the coefficient of determination (R^2) was calculated for each analyte in ground beef and liver matrices. Over 95% and 93% of target analytes had $R^2 > 0.90$ (85% and 86% $R^2 > 0.99$) in ground beef and liver, respectively. Only 5% and 7% of the VDs analyzed had $R^2 < 0.90$ in beef and liver. Figure 7 depicts the calibration curves for ipronidazole and enrofloxacin in ground beef and liver between 2 ng/g and 100 ng/g. All calibration curves were linearly fitted with no weighting.

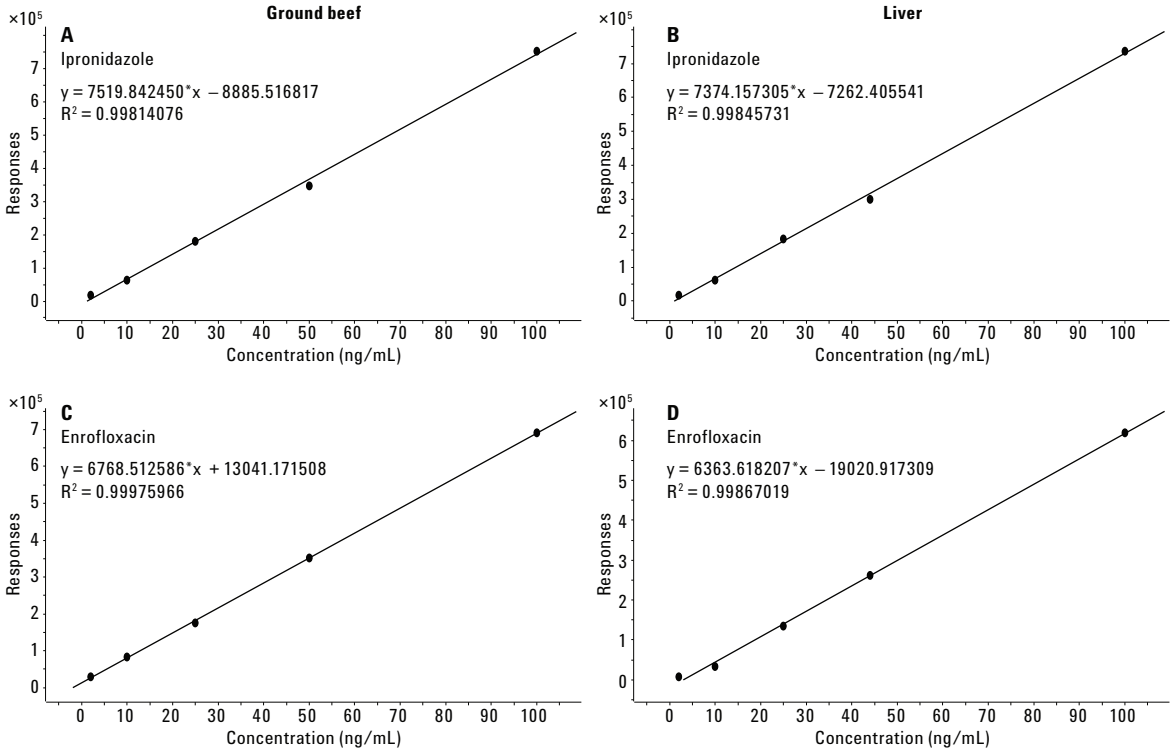

Figure 7. Calibration curves for Ipronidazole (A nd B) and Enrofloxacin (C and D) in ground beef and liver; (2–100 ng/g).

Conclusions

This application note demonstrates the ability of the Agilent 6545 Q-TOF LC/MS, with its high resolution and sensitivity, to analyze over 120 VDs in relevant matrices including bovine muscle, liver, and kidney at ng/g levels. The use of a simple and efficient Agilent All Ions MS/MS workflow allows for analyte detection and identification using fragment ions in the same analytical run. This dramatically reduces potential false positives. The availability of accurate mass, MS/MS spectra, and updated retention times under specific LC conditions in the Agilent Veterinary Drugs PCDL further improves compound identification and robustness in complex matrices. The ability to perform quantification was demonstrated with calibration curve generation. As a result, sensitive qualitative and quantitative information for VDs in meat can be performed using the 6545 Q-TOF LC/MS instrument in a single analytical run.

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References

1. EuropeanCommision, Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results, O.J.E.C. L221, Editor. (2002).
2. M. J. Schneider, S. J. Lehotay, A. R. Lightfield. "Validation of a streamlined multiclass, multiresidue method for determination of veterinary drug residues in bovine muscle by liquid chromatography–tandem mass spectrometry" *Analytical and Bioanalytical Chemistry* (2014).
3. Health Canada. Administrative Maximum Residue Limits (AMRLs) and Maximum Residue Limits (MRLs) set by Canada. (2012).
4. J. A. Park, *et al*. "Single-step multiresidue determination of ten multiclass veterinary drugs in pork, milk, and eggs using liquid chromatography with tandem mass spectrometry" *Journal of Separation Science* **38(16)**, 2772-2780 (2015).
5. H. Wei, *et al*. "Development and validation of a multiresidue screening method for veterinary drugs, their metabolites and pesticides in meat using liquid chromatography-tandem mass spectrometry" *Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment* **32(5)**, 686-701 (2015).
6. R. Yamada, *et al*. "Simultaneous determination of residual veterinary drugs in bovine, porcine, and chicken muscle using liquid chromatography coupled with electrospray ionization tandem mass spectrometry" *Bioscience, Biotechnology and Biochemistry* **70(1)**, 54-65 (2006).
7. L. Geis-Asteggiante, *et al*. "Structural characterization of product ions by electrospray ionization and quadrupole time-of-flight mass spectrometry to support regulatory analysis of veterinary drug residues in foods" *Rapid Communications in Mass Spectrometry* **28(10)**, 1061-1081 (2014).
8. L. Zhao, D. Lucas, Multiresidue analysis of veterinary drugs in bovine liver by LC/MS/MS, *Agilent Technologies Application Note*, publication number 5991-6096 (2015).

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