Application Note

Food Testing

Agilent

Trusted Answers

Veterinary Drug Detection in Pork and Milk

Using an Ultivo LC/TQ with a standard ESI ion source

Figure 1. Agilent Ultivo LC/TQ with ESI source.

Author

Theresa Sosienski
Agilent Technologies, Inc.

Abstract

This Application Note highlights a 10-minute analytical method for the precise quantification of 12 regulated veterinary drug compounds in pork and milk. The method uses an Agilent 1260 Infinity II Prime LC and an Agilent Ultivo triple quadrupole LC/MS with an ESI source. The 12 veterinary drug compounds selected for evaluation in this method have maximum residue limits (MRLs) up to 1,000 µg/kg as defined by global regulations, and need to be analyzed at levels up to 5× the MRL. The intuitive design and easy maintenance of Ultivo make the system well suited for high-throughput detection of these veterinary drugs. Ground pork and milk were chosen to represent broad matrices with high fat and water content. This method exceeds the sensitivity requirements defined by global regulations, with high precision (RSD% <14 %) for all veterinary drug compounds included in this method.

Introduction

Veterinary drugs are mainly used in livestock to prevent diseases and parasites, and promote growth. Improper use of veterinary drugs in livestock operations can result in the accumulation of these drugs in animal tissues and other animal-derived foods such as milk or eggs. Because of global concern for the presence of veterinary drugs in livestock products for human consumption, an AOAC working group recently proposed Standard Method Performance Requirements (SMPRs) for an extensive list of veterinary drug compounds. These included detection limits based on US[1], Codex[2], China[3], and Canada[4] regulations for veterinary drug residues in meat and milk. Detection limit requirements were proposed at half the MRL with the lowest MRL between the regulatory agencies always being chosen as the default.

The 12 veterinary drugs included in this method represent a group of veterinary drugs that have relatively high MRLs in milk and meat. The veterinary drug compounds each have target testing levels, defined as 1/2 the MRL of the compound. The target testing levels of the 12 studied compounds range from 22.5–100 µg/kg in milk and from 50–500 µg/kg in meat (Table 1). The Ultivo triple quadrupole LC/MS with a standard ESI source can be the perfect system for such measurements.

This Application Note demonstrates the precise quantification of 12 regulated veterinary drug compounds in pork and milk using a 1260 Infinity II Prime LC and an Ultivo LC/TQ equipped with an ESI source. The Ultivo-ESI inherited the outstanding performance of Ultivo.

Table 1. Target testing levels of the 12 veterinary drugs in milk and meat.

Compound	Milk target testing level (µg/kg)	Meat target testing level (µg/kg)
Ceftiofur	50	500
Chlortetracycline	50	50
Closantel	22.5	500
Dihydrostreptomycin	62.5	250
Diminazene	75	250
Fenbendazole	50	50
Lincomycin	75	50
Novobiocin	25	500
Oxytetracycline	50	50
Spiramycin	100	100
Streptomycin	62.5	250
Tetracycline	50	50

Experimental

Reagents and chemicals

All reagents used were HPLC or LC/MS grade. Acetonitrile and methanol were purchased from Honeywell (Morristown, NJ, USA), and ultrapure water was sourced from a Milli-Q Integral System with a LC-Pak Polisher and a 0.22-µm point-of-use membrane filter cartridge (EMD Millipore, Billerica, MA, USA). Formic acid was purchased from Fisher Scientific (Fair Lawn, NJ, USA), and ammonium fluoride (solid powder) was purchased from Aldrich (Sigma-Aldrich Corp., St. Louis, MO, USA), a 5 M stock solution was made. Veterinary drug standards were also purchased from Sigma-Aldrich.

Sample preparation

Fresh 2 % pasteurized organic milk and antibiotic-free ground pork (80 % lean, 20 % fat) were obtained from local grocery stores. Samples of 2 g of pork or milk were weighed in 50-mL polypropylene tubes, and frozen until analysis. The sample preparation procedure for pork was sourced from a method previously evaluated by Zhao; *et al*.[5]. The method was further modified for the milk extraction, and is summarized in Figure 2. A Heidolph Hei-MIX Multi Reax system was used to vortex the samples. Agilent Captiva EMR–Lipid cartridges, 6 mL, 600 mg (p/n 5190-1004) were used in the final cleanup of the pork extraction. Captiva EMR—Lipid cartridges provide efficient and selective lipid removal with exceptional recovery of hydrophobic analytes.

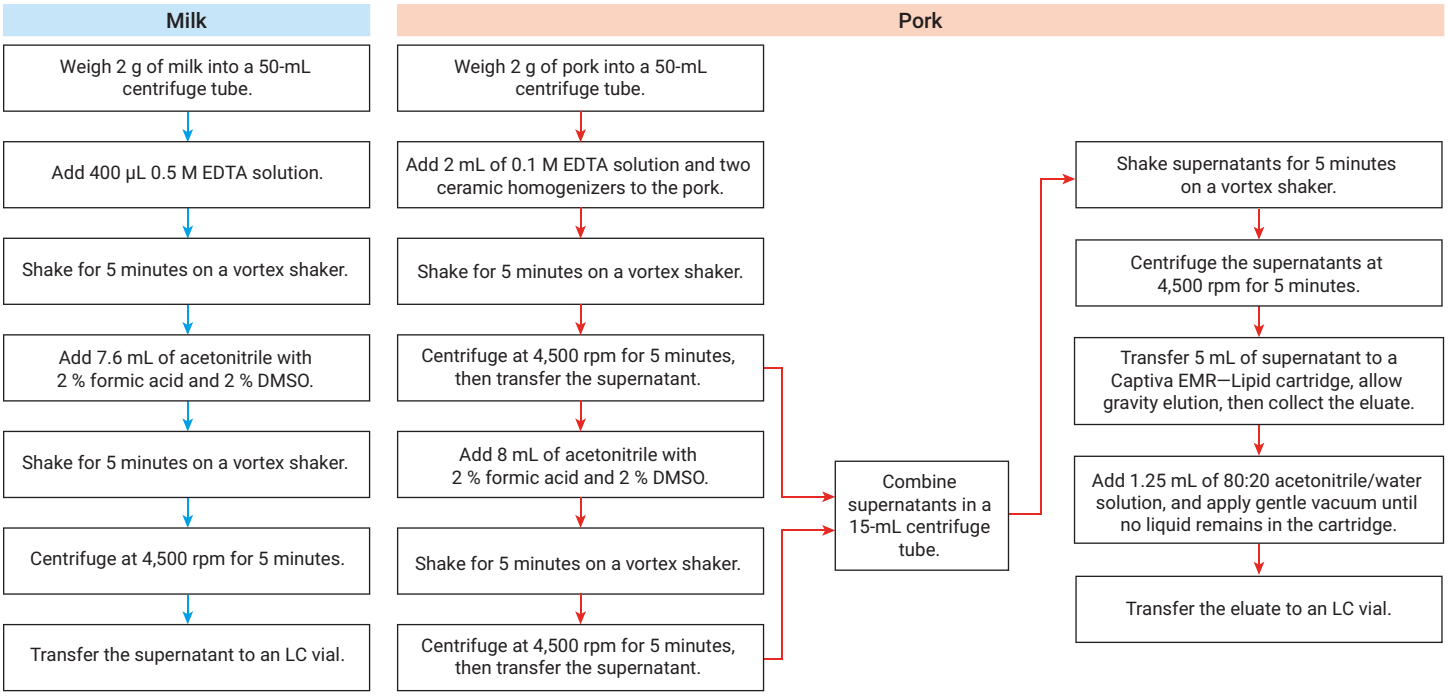

Figure 2. Sample preparation procedure for veterinary drugs in pork and milk.

Instrumentation

Agilent 1260 Infinity II Prime LC

- 1260 Infinity II Prime flexible pump (G7104C)
- 1260 Infinity II multisampler with cooler (G7167A)
- 1260 Infinity II multicolumn thermostat (G7116A)

Agilent Ultivo triple quadrupole LC/MS system

- Electrospray ionization source (G1948B)

Method

Table 2 summarizes the 1260 Infinity II Prime LC conditions, and Table 3 summarizes the Ultivo ion source and instrument parameters. Table 4 shows the optimized MS parameters for the compounds of interest. Dynamic multiple reaction monitoring (dMRM) was used for data collection. MassHunter Quantitative Analysis software B.09 with the Quant-My-Way feature was used to accelerate and streamline the data analysis and review process.

Table 2. Agilent 1260 Infinity II Prime LC parameters.

Column	Agilent InfinityLab Poroshell 120 EC-C8, 2.1 × 100 mm, 2.7 µm (p/n 695775-906)
Column temperature	40 °C
Observed column backpressure range	170–370 bar
Injection volume	4 µL
Mobile phase	A) 0.2 % Formic acid in water B) 0.5 mM Ammonium fluoride in methanol
Flow rate	0.350 mL/min
Gradient	Time (min) %B 0 2 1.5 2 2.5 70 5.0 100 7.0 100 7.1 2 9.0 2
Stop time	9.0 minutes
Post time	1.0 minutes

Table 3. Ultivo ion source and mass analyzer parameters.

Gas temperature	325 °C
Gas flow	8 L/min
Nebulizer pressure	40 psi
Capillary voltage	2,000 V (+)
Cycle time	500 ms

Experimental design

Pork and milk spiked after the extraction procedure (post spiked) were used for the sensitivity, precision, and linearity studies. For recovery evaluation, pork and milk were spiked with a stock solution of veterinary drugs before extraction (prespiked), and compared to post spiked pork and milk extracts for recovery (%) calculations after analysis.

Results and discussion

Method sensitivity and precision

All veterinary drugs could accurately be quantified at 1/2 MRL, while most could be quantified at 1/10 MRL, the lowest level tested in this study. Figure 3 shows the excellent signal response for all analytes at the target testing level in pork extract. The veterinary drugs also showed excellent precision at the lowest testing level, with RSD% below 14 % for all compounds tested, along with each compound's lowest testing level, as shown in Table 5. Accurate quantification at the lowest testing level was defined as having four out of six replicate injections with accuracy of 80–120 %, and a signal-to-noise ratio (S/N) greater than 10 for both quantifiers and qualifiers. Several veterinary drug compounds had a very strong signal response at 1/10 MRL, indicating that the quantitation limit is considerably lower than 1/10 MRL (a few examples are included in Figure 4).

Table 4. Optimized transitions for veterinary drug detection in dynamic MRM mode.

Compound	Precursor (*m/z*)	Product (*m/z*)	RT (min)	RT Window (min)	Fragmentor (V)	CE (V)	Polarity
Dihydrostreptomycin	584.3	204	0.67	0.89	100	44	Positive
Dihydrostreptomycin	584.3	200	0.67	0.89	100	32	Positive
Streptomycin	599.3	582.3	0.67	0.69	160	12	Positive
Streptomycin	599.3	263	0.67	0.69	160	32	Positive
Diminazine	282.2	254.1	4.5	0.77	90	0	Positive
Diminazine	282.2	118.9	4.5	0.77	90	12	Positive
Lincomycin	407.2	126	4.78	1.1	150	28	Positive
Lincomycin	407.2	82.2	4.78	1.1	150	80	Positive
Tetracycline	445.2	427.1	4.86	0.81	130	4	Positive
Tetracycline	445.2	410.1	4.86	0.81	130	8	Positive
Oxytetracycline	461.2	443.1	4.88	1.05	130	0	Positive
Oxytetracycline	461.2	426	4.88	1.05	130	12	Positive
Spiramycin	422.2	100.9	5	0.93	70	20	Positive
Spiramycin	422.2	83	5	0.93	70	20	Positive
Chlortetracycline	479.1	444.1	5.09	1.1	140	12	Positive
Chlortetracycline	479.1	260.1	5.09	1.1	140	60	Positive
Ceftiofur	524	241	5.21	0.97	140	8	Positive
Ceftiofur	524	124.9	5.21	0.97	140	68	Positive
Fenbendazole	300.1	268.1	5.98	1.05	140	16	Positive
Fenbendazole	300.1	159	5.98	1.05	140	36	Positive
Novobiocin	613.2	133.1	6.6	0.85	120	68	Positive
Novobiocin	613.2	132.5	6.6	0.85	120	72	Positive
Closantel	662.9	264	7.29	0.8	180	28	Positive
Closantel	662.9	194.1	7.29	0.8	180	80	Positive

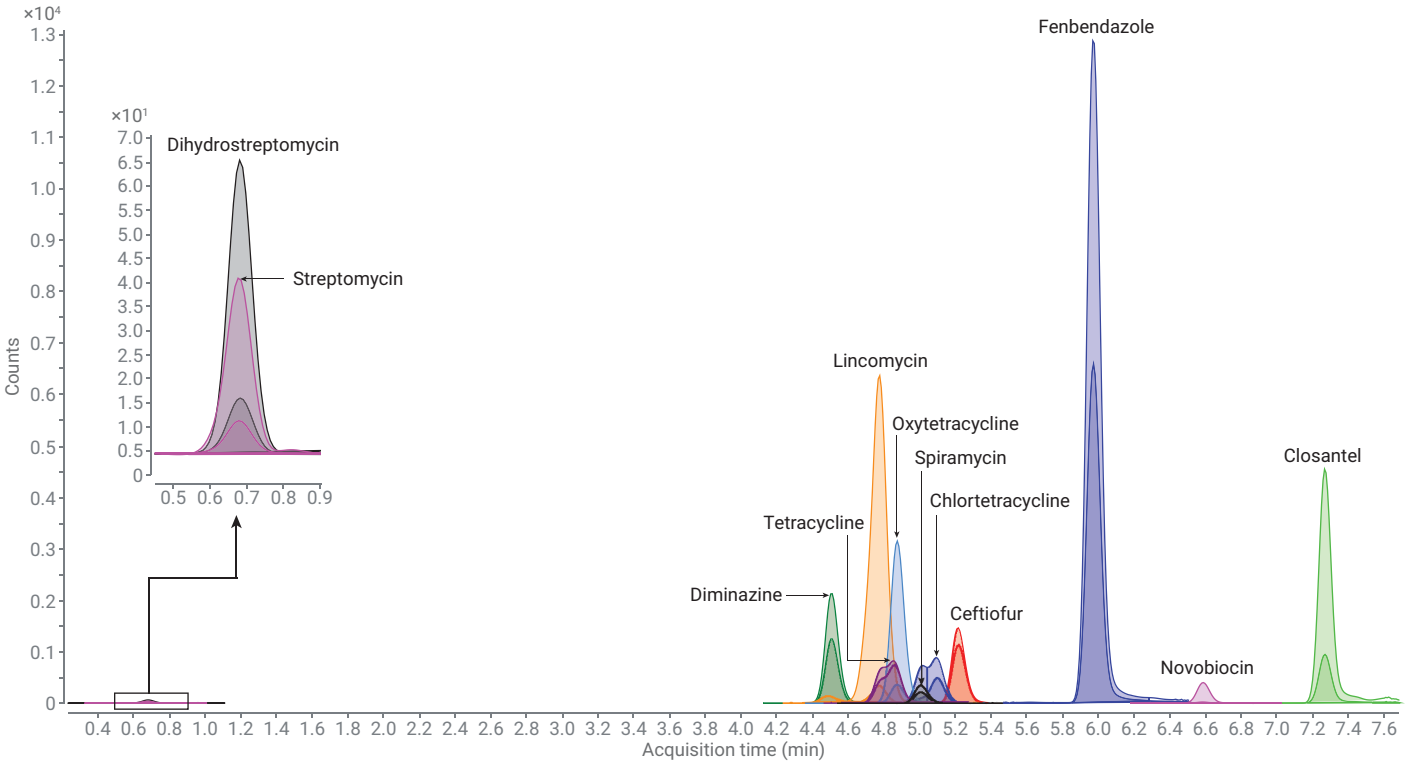

Figure 3. Chromatogram of veterinary drug analytes spiked into pork extract at the target testing level (1/2 MRL).

Table 5. Lowest testing level and precision for all veterinary drugs studied in pork and milk extract. All could accurately be quantified at or below the target testing level (1/2 MRL).

Compound	Milk			Pork		
	Lowest testing level (µg/kg)		RSD% (n = 6)	Lowest testing level (µg/kg)		RSD% (n = 6)
Streptomycin	1/2 MRL	62.5	13.74	1/5 MRL	100	6.92
Dihydrostreptomycin	1/2 MRL	62.5	7.76	1/5 MRL	100	6.52
Diminazine	1/10 MRL	15	5.79	1/10 MRL	50	3.74
Lincomycin	1/10 MRL	15	2.02	1/10 MRL	10	0.83
Tetracycline	1/10 MRL	10	3.26	1/10 MRL	10	3.48
Oxytetracycline	1/10 MRL	10	4.16	1/10 MRL	10	3.60
Spiramycin	1/10 MRL	20	4.34	1/10 MRL	20	5.39
Chlortetracycline	1/10 MRL	10	3.38	1/10 MRL	10	3.46
Ceftiofur	1/10 MRL	10	11.56	1/10 MRL	100	2.22
Fenbendazole	1/10 MRL	10	1.10	1/10 MRL	10	1.26
Novobiocin	1/5 MRL	10	10.93	1/10 MRL	100	4.13
Closantel	1/10 MRL	4.5	4.75	1/10 MRL	100	3.60

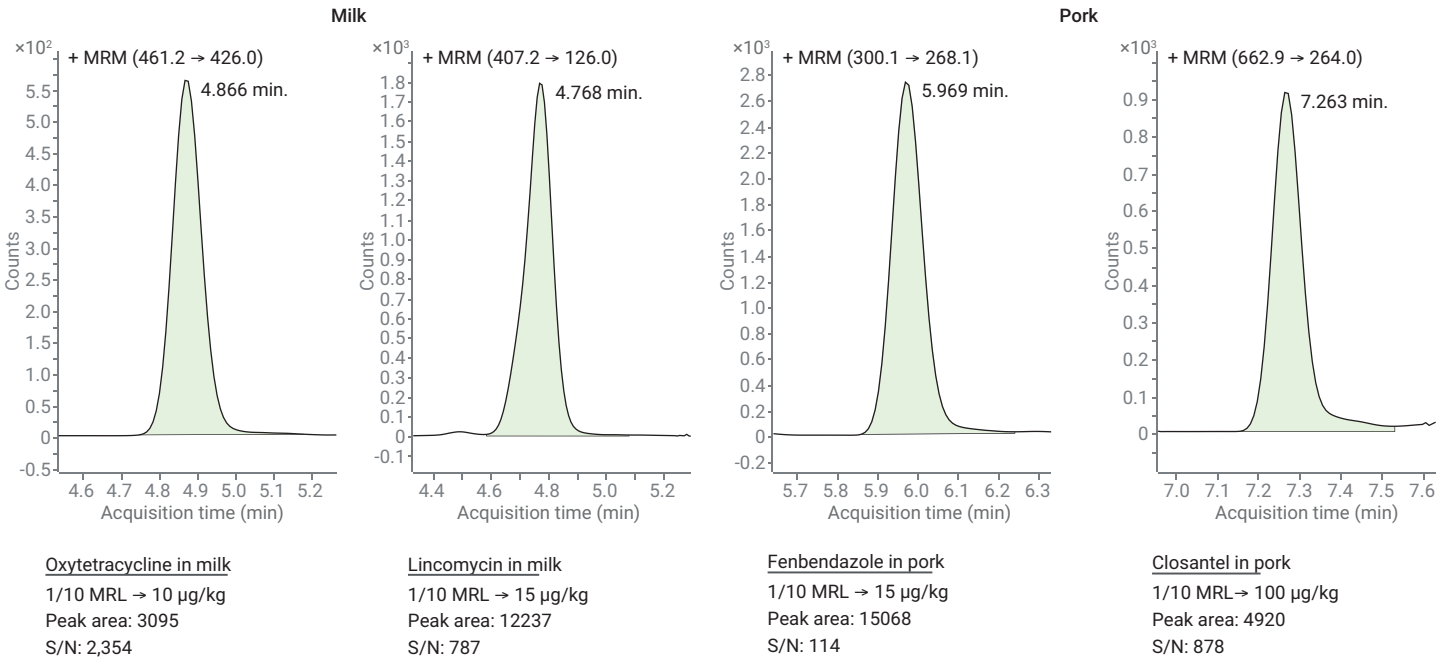

Figure 4. Select veterinary drug compounds with strong signal response at 1/10 MRL, indicating that the quantitation limit is much lower than 1/10 MRL.

Method linearity

All veterinary drugs showed good linearity with 1/x weighting, and all calibration curves have R^2 values greater than 0.98. Calibration levels ranged from 1/10 MRL to 5× MRL for all analytes. Figure 5 shows examples of some of the calibration curves.

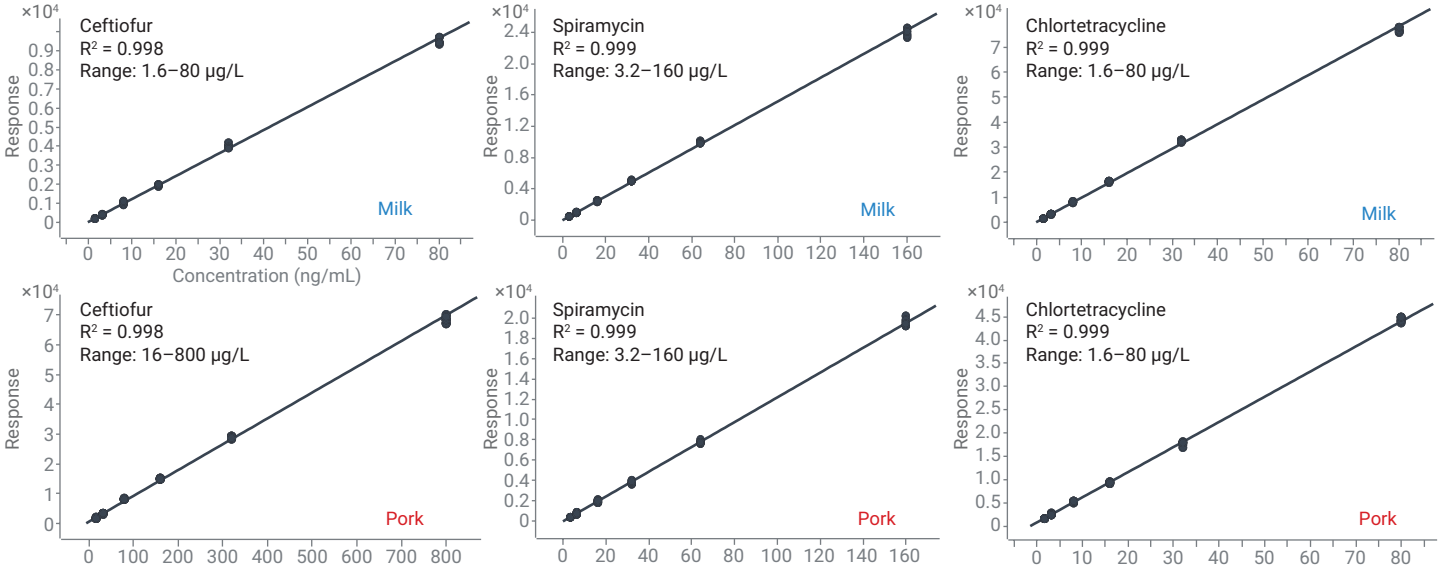

Figure 5. Select calibration curves of veterinary drugs spiked into pork and milk matrices at concentrations ranging from 1/10 MRL to 5 × MRL.

Method recovery

The recovery of all veterinary drugs was evaluated in both milk and pork at three levels: 1/2 MRL, MRL, and 2× MRL. Six replicates of each spiking level were evaluated in the recovery study. For 10 of the compounds, the recovery was between 60 and 120 % at all levels in both matrices (Figure 6). Dihydrostreptomycin and streptomycin had poor recovery with this extraction method, but could be detected at or below the target testing level (1/2 MRL) in post spiked matrix. For these two very hydrophilic compounds, a different extraction method may be used, but this analysis method is suitable for screening. For accurate quantitation, internal standards should be used to correct the loss of these two compounds during extraction.

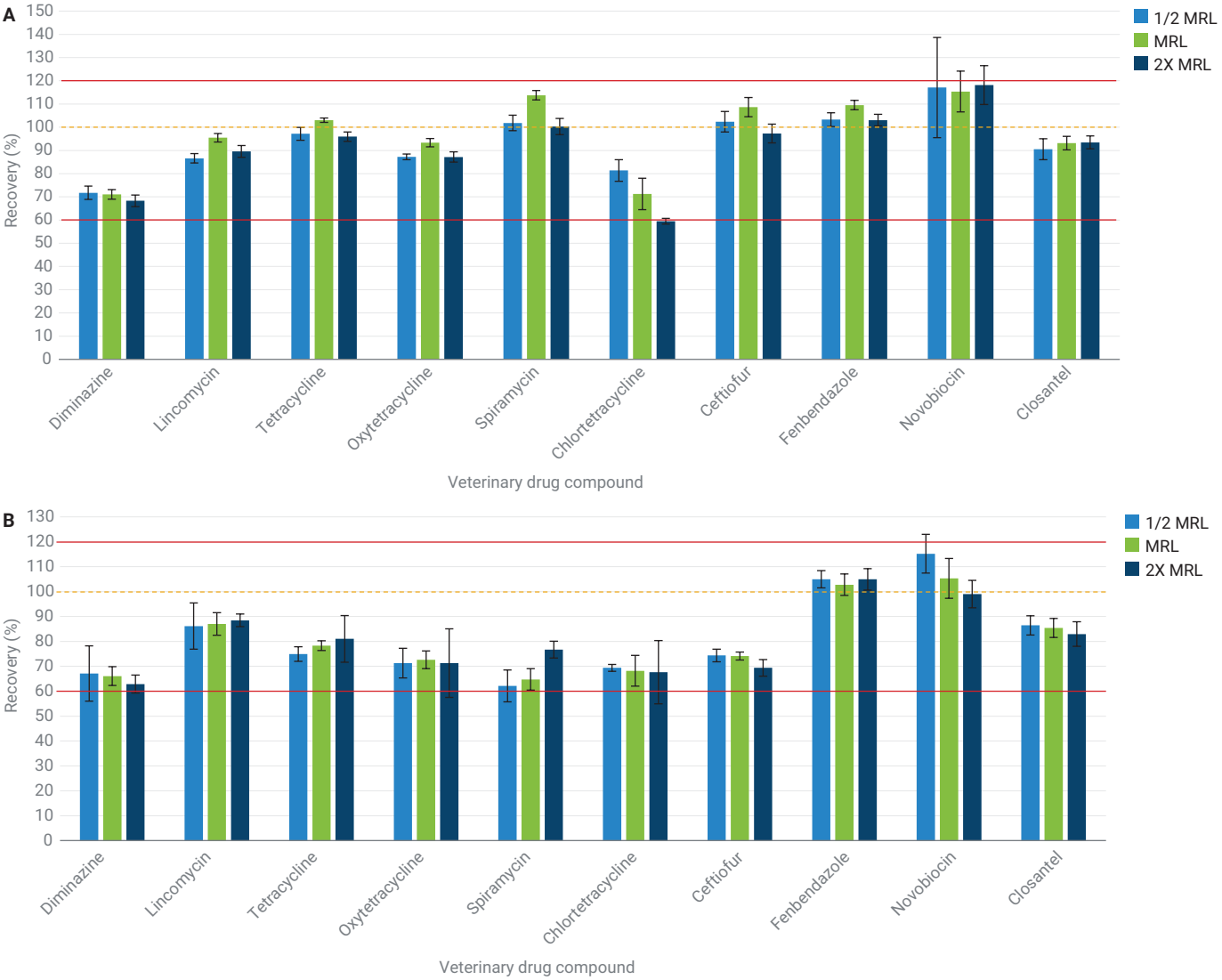

Figure 6. Recovery of veterinary drugs in milk (A) and pork (B) at 1/2 MRL, MRL, and 2× MRL spiking levels. Error bars denote the standard deviation of six replicates. Dihydrostreptomycin and streptomycin are not included.

Conclusions

The use of an Ultivo triple quadrupole LC/MS equipped with an ESI ion source exceeded the MRL requirements set by global regulatory agencies for veterinary drugs in meat and milk, with excellent precision. Captiva EMR–Lipid cartridges provided adequate extra cleanup of the fat-laden pork matrix, assisting the method sensitivity. The 1260 Infinity II Prime LC was a perfect separation tool for the low backpressure observed with this method. In applications where the sensitivity requirements can be relaxed, this configuration of Ultivo with an ESI source is an excellent fit-for-purpose choice.

References

1. Department of Health and Human Services, Food and Drug Administration, 21 CFR Parts 514 and 558, FDA-2010-N-0155.
2. Proposal for a regulation of the European Parliament and of the council on veterinary medicinal products, European Commission, **2014**/0257 (COD).
3. Maximum Residue Limits in animal derived foods, Announcement No. 235, Ministry of Agriculture, China, **2002**.
4. List of Maximum Residue Limits (MRLs) for Veterinary Drugs in Foods, Health Canada, Government of Canada. August 2, **2017**. https://www.canada.ca/content/dam/hc-sc/migration/hc-sc/dhp-mps/alt_formats/pdf/vet/mrl-lmr/mrl-lmr_versus_new-nouveau-20170802-eng.pdf
5. Zhao, L.; Lucas, D. Multiclass Multiresidue Veterinary Drug Analysis in Beef Using Agilent Captiva EMR–Lipid Cartridge Cleanup and LC/MS/MS. *Agilent Technologies Application Note*, publication number 5991-8598EN, **2017**.

www.agilent.com/chem

Printed in the USA, December 13, 2018
5994-0480EN

Agilent
Trusted Answers