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Veterinary Drug Detection in Pork and Milk Using a Small, Innovative Triple Quad with an ESI Source

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Introduction

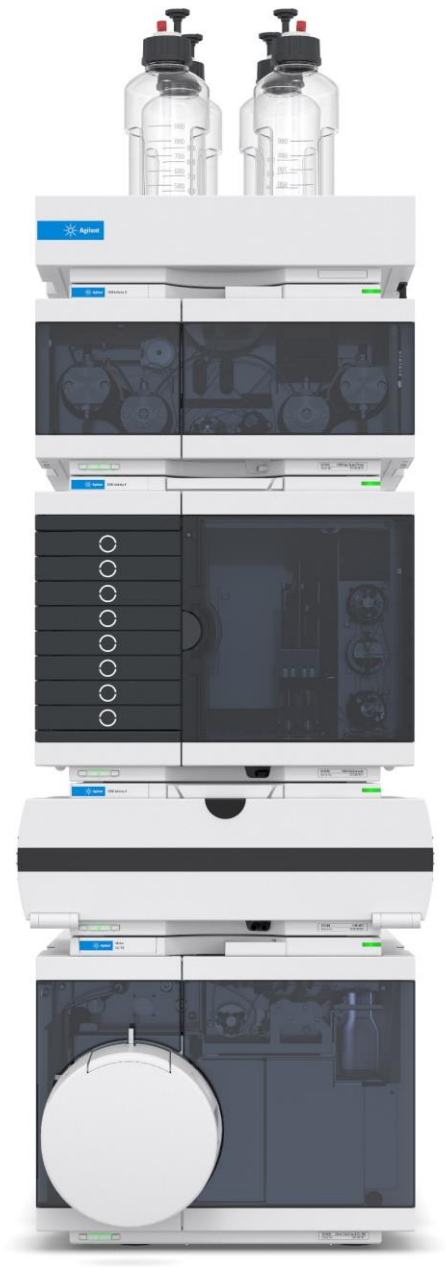


Figure 1: Ultivo LC/TQ

Veterinary drugs are used in livestock operations to prevent diseases and parasites, and to promote growth. Improper use of veterinary drugs can result in their accumulation in animal tissues and other animal-derived foods. 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 Maximum Residue Limit (MRL) with lowest MRL between the regulatory agencies chosen as the default.

The following method includes 12 veterinary drugs which have relatively high MRLs in milk and meat. 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).

Here we demonstrate the precise quantitation of 12 regulated veterinary drug compounds in pork and milk using an Agilent 1260 Infinity II Prime LC and the Ultivo LC/TQ equipped with an ESI source. The Ultivo-ESI inherited the outstanding performance of the Ultivo LC/TQ.

Compound	Milk (µg/kg)	Meat (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

Table 1: Target testing levels of the 12 vet drugs in milk and meat

Experimental

Sample Preparation

Fresh 2% organic milk and ground pork were obtained from local grocery stores. The sample preparation procedure for pork was sourced from Zhao et al. [5] and modified for the milk extraction (Figure 2). Agilent Captiva EMR–Lipid cartridges, 6 mL, 600 mg were used in the final clean-up of the pork extraction.

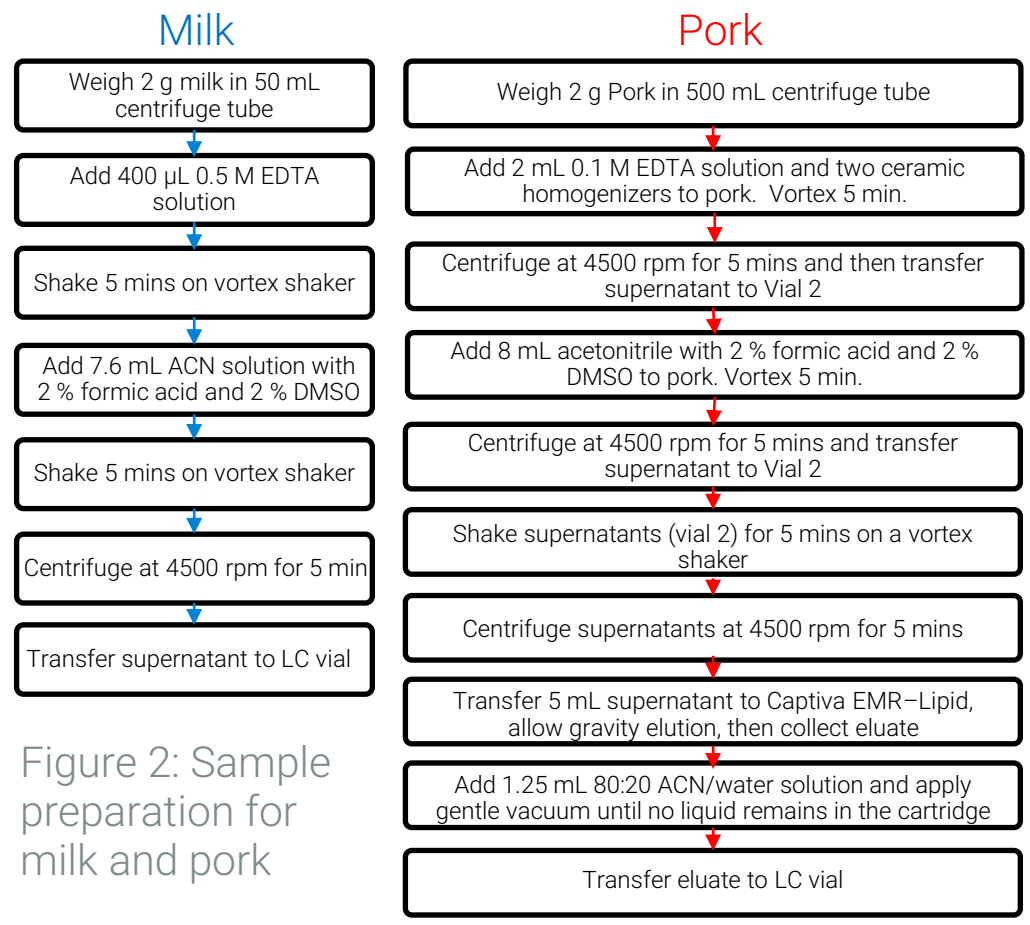


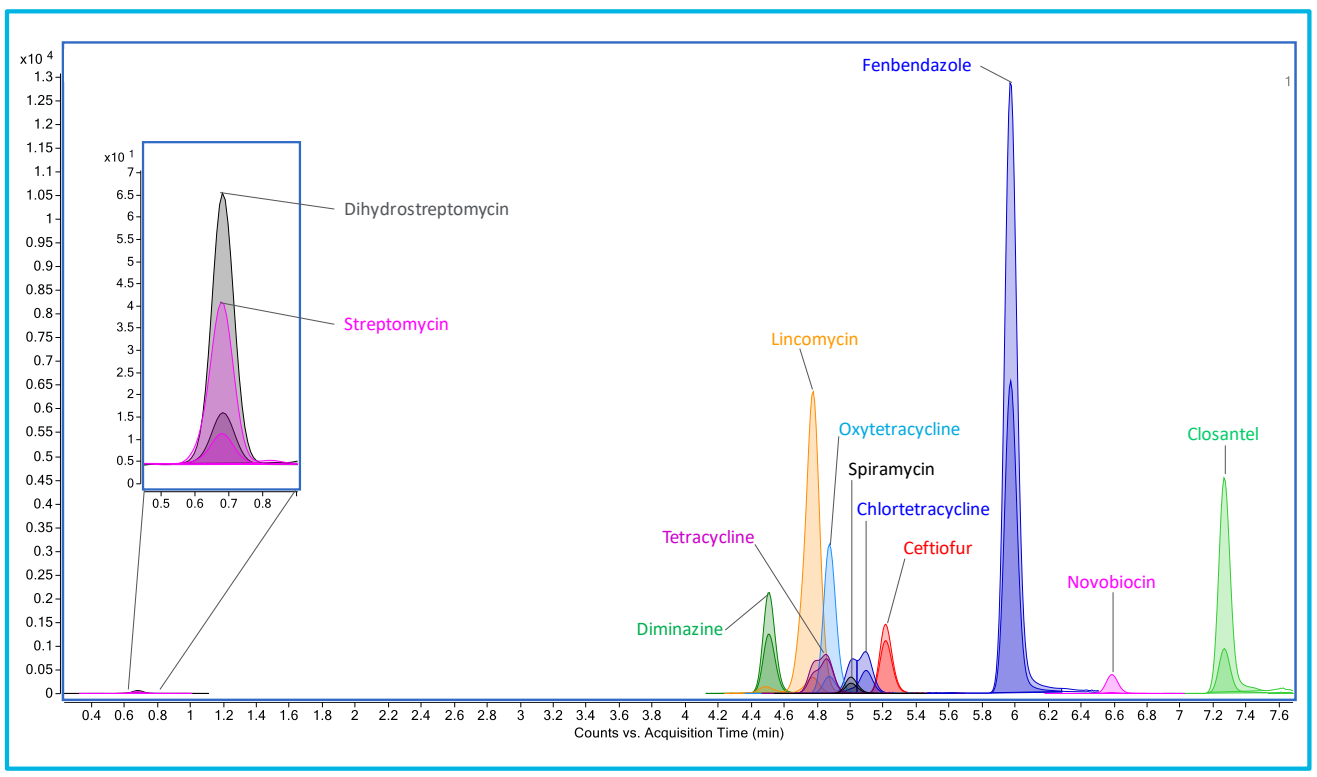
Figure 2: Sample preparation for milk and pork

Instrument Parameters

Agilent 1260 Infinity II Prime UHPLC Parameters		
Column	Poroshell 120 EC-C8 2.1 x 100 mm, 2.7µm	
Column temp	40°C	
Injection volume	4 µL	
Mobile phase	A: 0.2% formic acid in water B: 0.5mM ammonium fluoride in methanol	
Flow rate	0.350 mL/min	
Gradient	Time (min) 0 1.5 2.5 5.0 7.0 7.1 9.0	B% 2 2 70 100 100 2 2
Stop-time, Post-time	9.0 min, 1.0 min	

Agilent Ultivo Triple Quad MS and ESI Source Parameters	
Gas temp	325 °C
Gas flow	8 L/min
Nebulizer pressure	40 psi
Capillary voltage	2000V(+)
Cycle Time	500 ms

Table 2: LC and MS Parameters



Chromatography and Method Recovery

Figure 3 shows the excellent signal response for all analytes at the target testing level in pork extract. The recovery of all veterinary drugs was evaluated in both milk and pork at three levels: ½ MRL, MRL, and 2 × MRL. Six replicates of each spiking level were evaluated. Method recovery was 60 - 120 % at all levels in both matrices (Figure 4). Dihydrostreptomycin and streptomycin have poor recovery with this extraction method; for these two very hydrophilic compounds, a different extraction method may be employed, but this analysis method is suitable for screening, as the compounds can be detected at ½ MRL in post-spiked matrix.

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

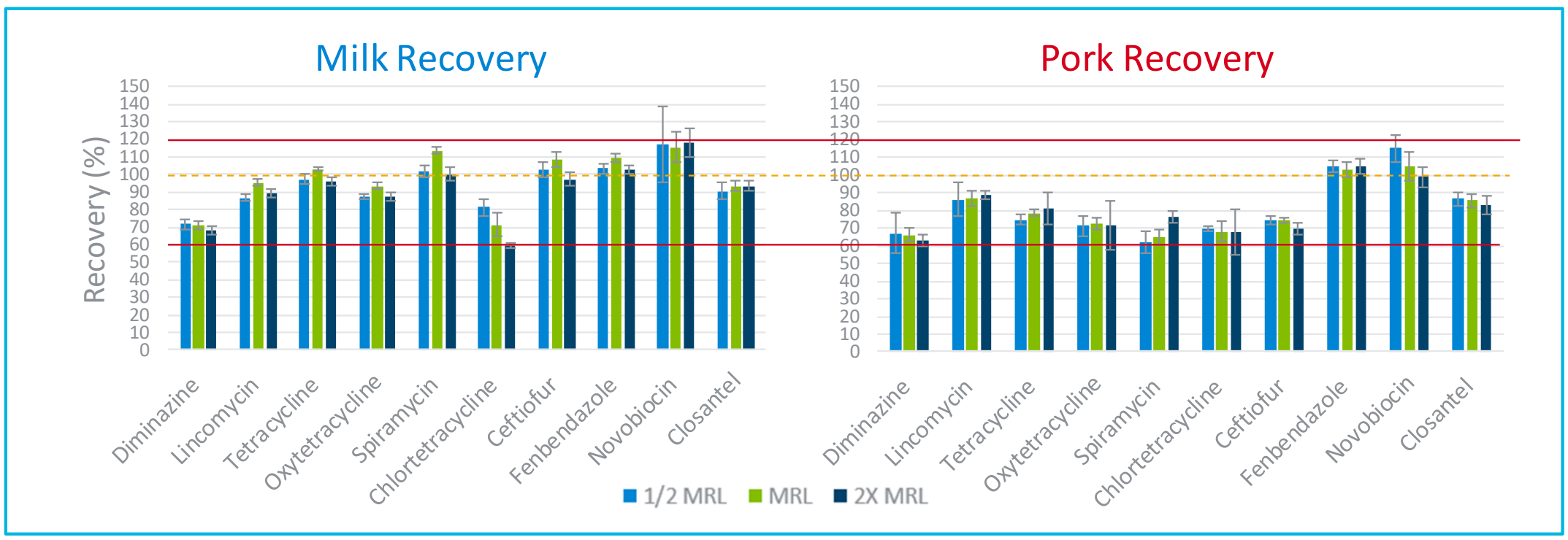


Figure 4: Recovery of veterinary drugs in pork and milk at ½ MRL, MRL, and 2 × MRL spiking levels. Error bars denote the standard deviation of six replicates. Does not include dihydrostreptomycin and streptomycin.

Method Precision at the Lowest Testing Level

All veterinary drugs could be accurately quantified at ½ MRL, while most could be quantified at 1/10 MRL, the lowest level tested in this study. The veterinary drugs also showed excellent precision at the lowest testing level, with RSD% below 14 % for all compounds tested, as demonstrated along with each compound's lowest testing level in Table 3.

Table 3: Lowest testing level and precision for all veterinary drugs studied in pork and milk extract

	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

Results and Discussion

Excellent Sensitivity and Linearity

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 (Figure 5). 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 x MRL (Figure 6).

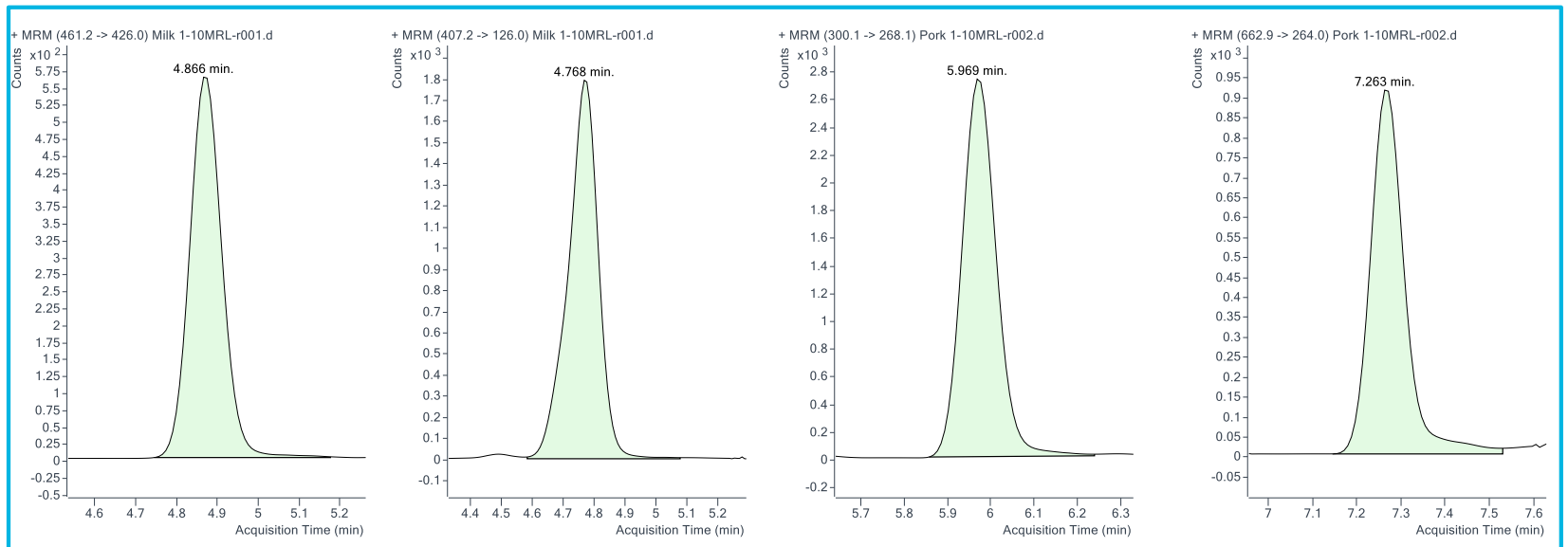


Figure 5: Select veterinary drug compounds with strong signal response at 1/10 MRL

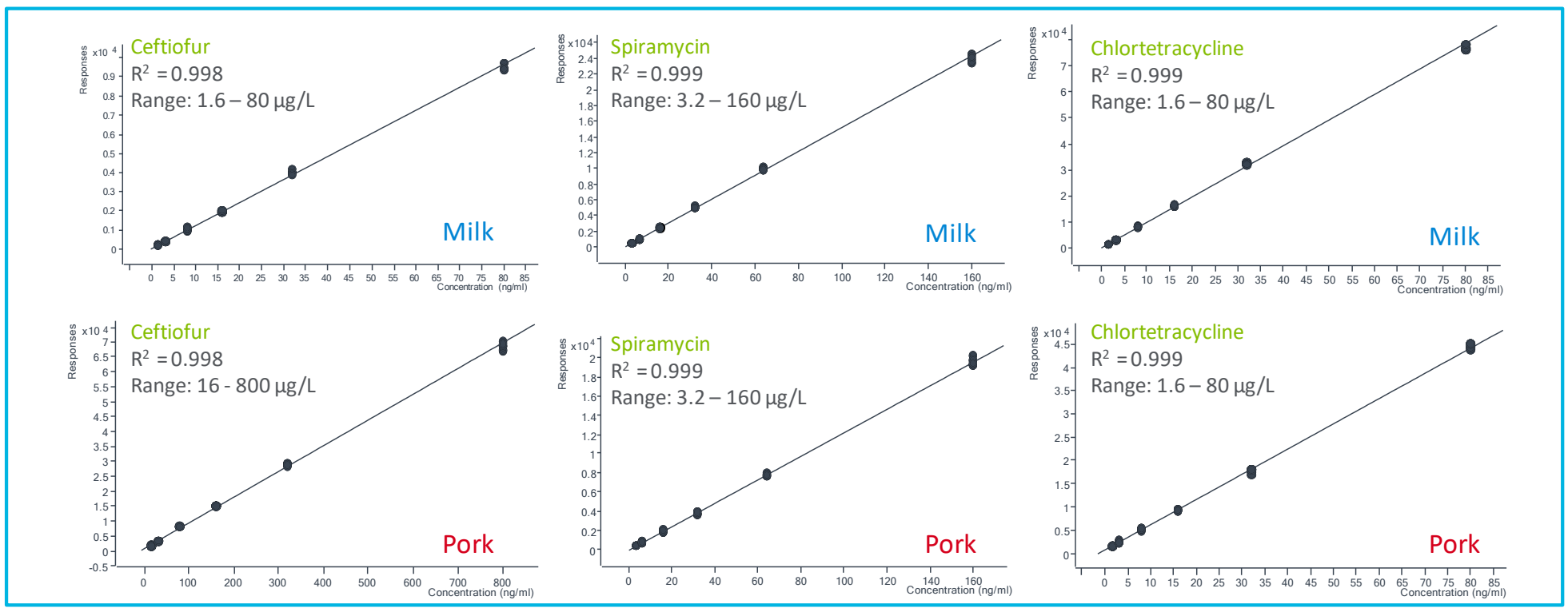


Figure 6: Select calibration curves of veterinary drugs spiked into pork and milk matrices from 1/10 MRL to 5 × MRL.

Conclusions

Ultivo with an ESI source is an excellent fit-for-purpose choice when sensitivity requirements are relaxed

- Ultivo 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 cleanup of the fatty pork matrix, assisting the method sensitivity.

References

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