

Multiclass Veterinary Drugs Analysis in Animal Muscle and Eggs Using Ultivo Triple Quadrupole LC/MS System

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Introduction

Veterinary drugs, consisting mainly of antimicrobials, have been widely used to treat or prevent diseases and enhance growth and production. There have been world-wide concerns that veterinary drug residues in the products of animal origin at the time of consumption can cause adverse effects to humans. In order to protect public health, governmental agencies have set limits for allowable veterinary drugs.

Due to the complexity of the matrices and characteristics of different classes of veterinary drugs, such as hydrophobicity, stability and pH sensitivity, it is very challenging to detect multiple-class analytes in one run. A typical standard method covers one class or a small cohort of similar classes of veterinary drugs. In order to effectively monitor veterinary drugs with short turn around time, the detection of multiple-class analytes in one run is highly desired.

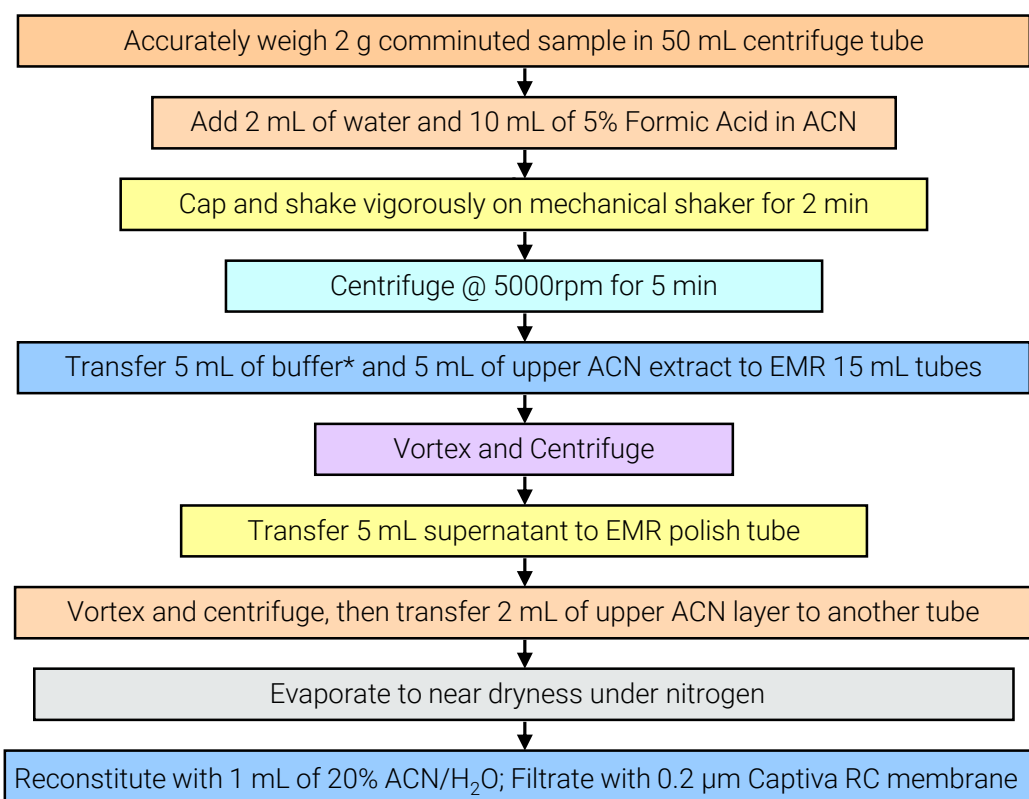
In this study, we demonstrated a screening method that could analyze 151 veterinary drugs across 27 classes in pork muscle and hen eggs using Agilent Ultivo Triple Quadrupole LC/MS (LC/TQ) in combination with 1290 infinity II UHPLC. We also demonstrated the wide applicability of Agilent EMR-Lipid sample preparation kit. EMR-Lipid kit could efficiently remove high lipid contents from matrices of animal origin, and provided fair recovery of the veterinary drugs.



Figure 1. Ultivo Triple Quad LC/MS

Experimental

Sample Preparation



LC and Mass Spectrometer parameters

Table 1. LC Conditions

Column	Eclipse Plus C18 3.0 x 150 mm, 1.8µm (p/n: 959759-302)			
Column temp	40°C			
Injection volume	15 µL			
Mobile phase	A: Water, 0.2% Formic Acid B: ACN, 0.2% Formic Acid			
Flow rate	0.5 mL/min			
Gradient	Time (min.)	B%	Time (min.)	B%
	0	2	7	30
	0.5	2	11	35
	1.8	15	16	100
	3.5	20	26	100
	6	25		
Stop Time	26min, Post Time 4min			

Table 2. Source and Mass Spec Parameters

Ionization mode	Positive/Negative
Scan type	Dynamic MRM
Dry gas temp. and Flow	200 °C; 11 L/min
Sheath gas temp. and Flow	275 °C; 11 L/min
Capillary voltage	3000 V(+); 3500 V(-)
Nozzle voltage	200 V(+); 1500 V(-)

Results and Discussion

Analytes Separation and Responses

Very good chromatographic separation was achieved with the analysis method. Figure 2 shows the chromatogram of 2 ng/g analytes spiked in hen egg.

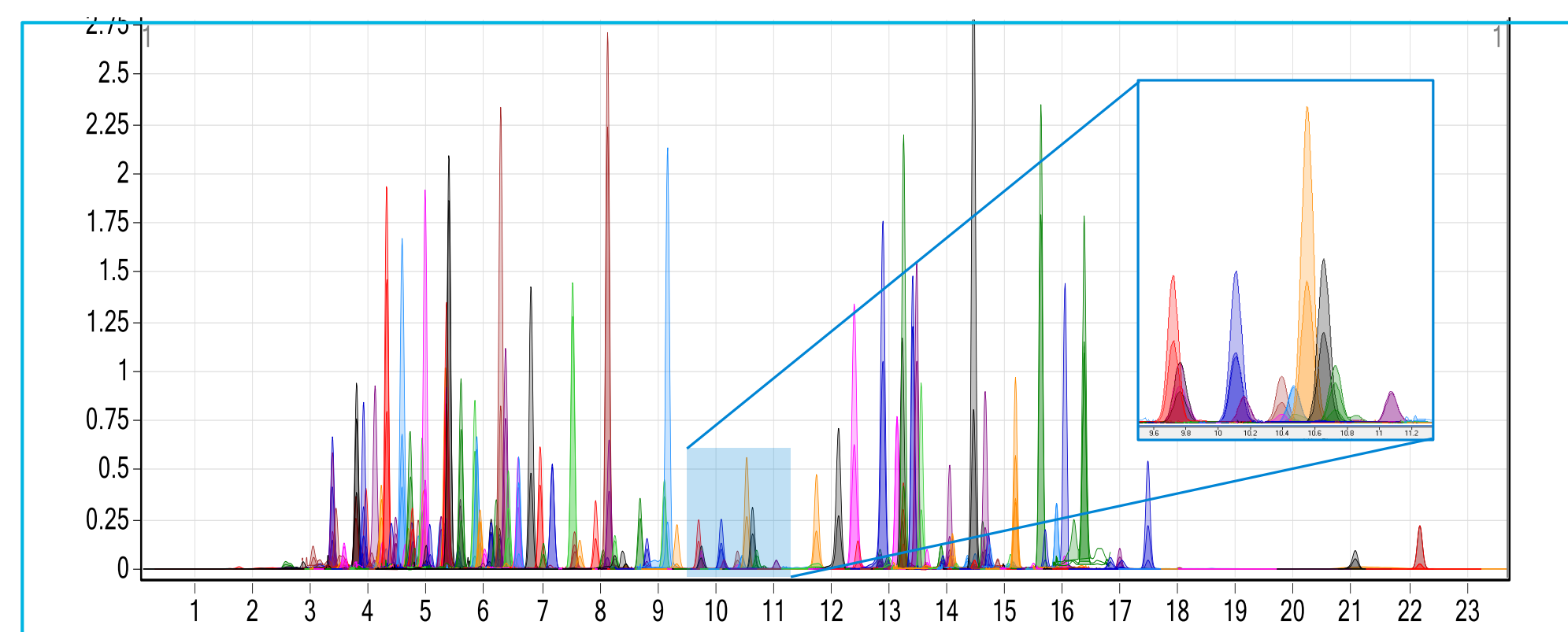


Figure 2. Chromatogram of an egg sample fortified with 151 veterinary drugs at 2 ng/g

EMR-Lipid

Agilent Bond Elut Enhanced Matrix Removal-Lipid (EMR-Lipid) is a unique sorbent that selectively removes major lipid classes from the sample without undesired analyte loss. The mechanisms of removing lipids are the combinations of size exclusion and hydrophobic interaction.

Figure 3 shows the precursor ion scan of *m/z* 184, a representative of phospholipid. It clearly indicates the efficient lipid removal of EMR-Lipid kit for pork matrix.

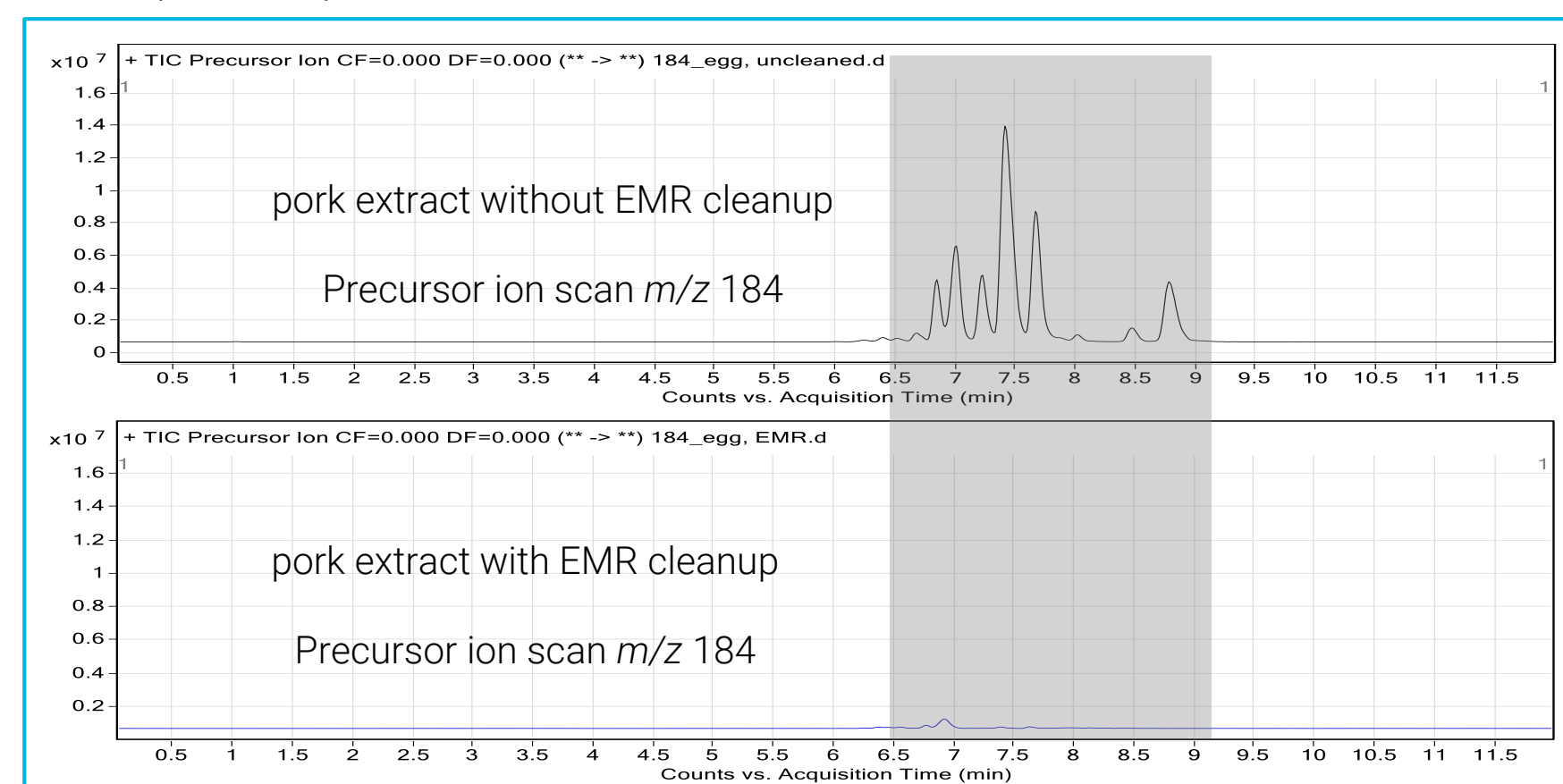


Figure 3. Precursor ion scan of *m/z* 184 for pork extract with and without EMR-Lipid cleanup

Results and Discussion

Detection Sensitivity

Ultivo showed excellent sensitivity. 130 out of 151 analytes could be detected at 1 ng/g in egg with signal-to-noise ratios (S/N) greater than 10 for both qualifier and quantifier transitions. Figure 4 illustrates the numbers of compounds that have S/N >10 for both qualifier and quantifier transitions at different concentrations in pork and egg.

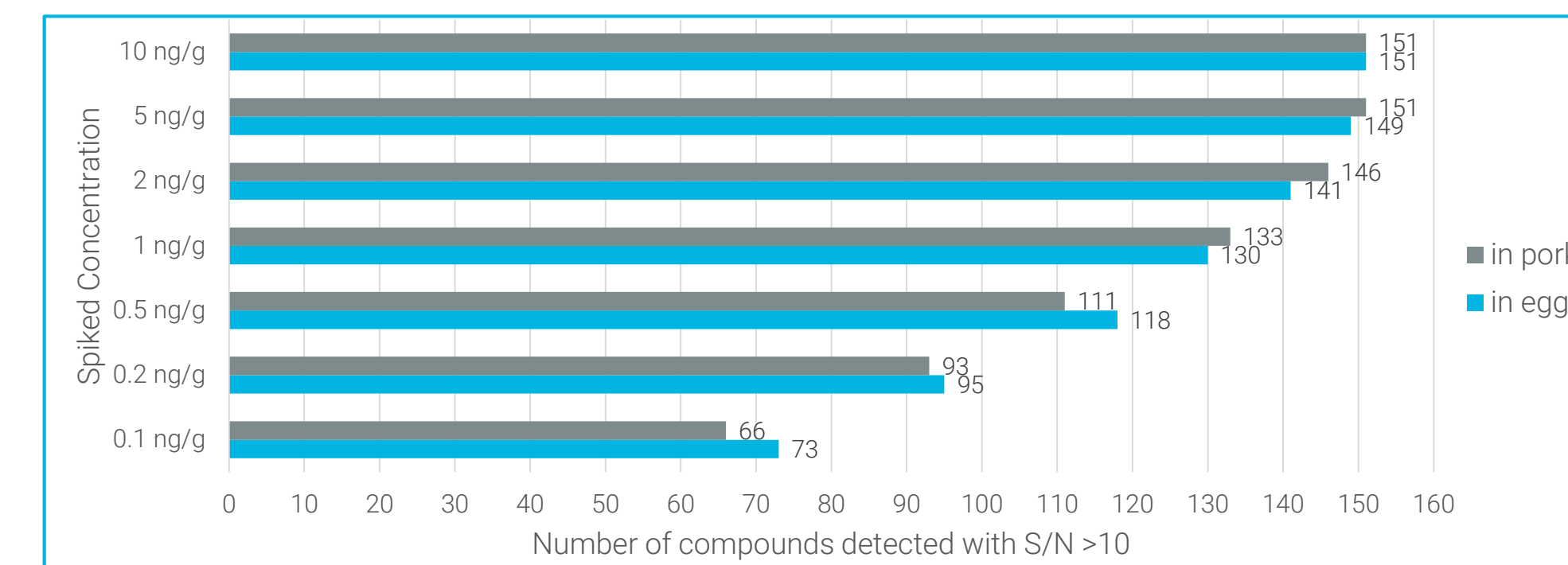


Figure 4. Number of compounds detected (S/N >10) at different spiked concentrations in matrices.

Precision

Exceptional precision was achieved for majority of compounds. Figure 5 shows the distribution of %RSD at 5 ng/g in pork and hen egg with n=7.

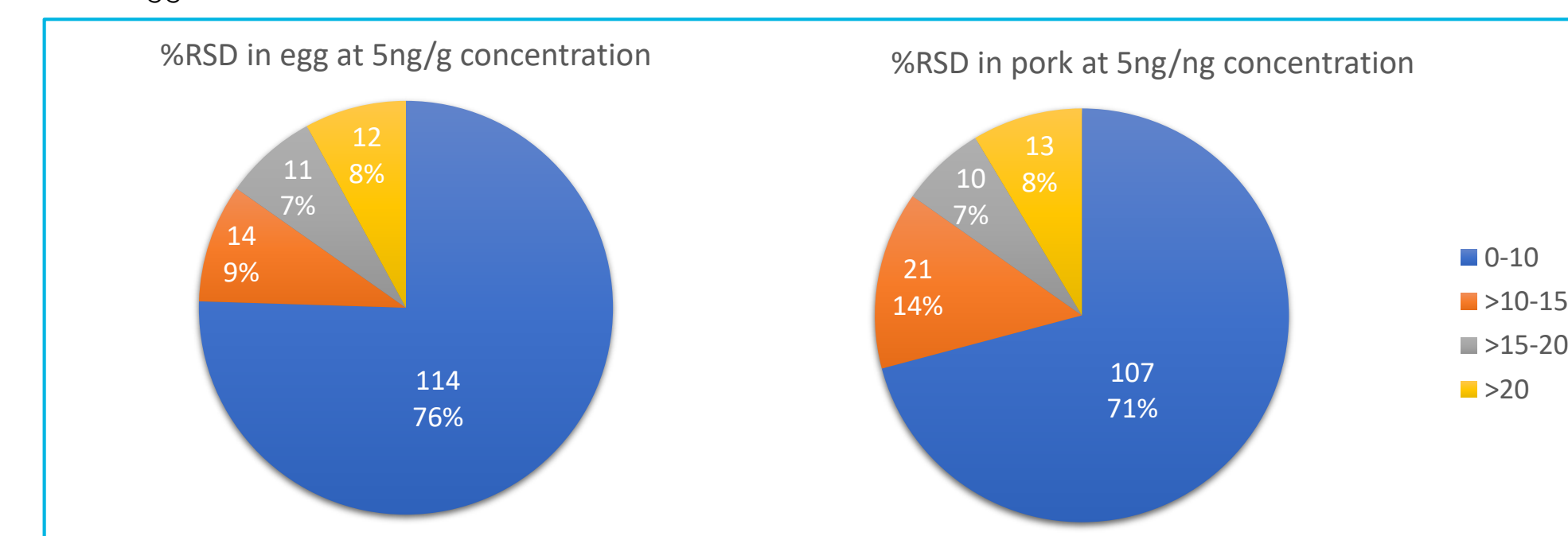


Figure 5. Distribution of %RSD in pork and egg at 5ng/g spiking level (n = 7).

Conclusions

- A quick and easy solution was developed to analyze 151 veterinary drugs across 27 classes in pork and eggs. Ultivo triple quadrupole LC/MS provided excellent sensitivity, accuracy and precision.
- Ultivo Triple Quad LC/MS delivers the ultimate performance of an analytical instrument with a minimized footprint and provides significant advantages in routine testing laboratories with enhanced features for non-expert LC/MS users.

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