Robust and Verified LC-MS/MS Workflow for Screening of Multi-class Veterinary Drug Residues from Various Food Matrices

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Introduction

Veterinary drugs are commonly used to treat diseases and improve the growth and health outcomes of farm animals. Improper use of veterinary drugs in animal farming can result in accumulation of these drugs in animal-derived foods, causing adverse effects to consumers. Global regulations define limits for vet-drugs in food of animal origin to protect public health. Triple quadrupole LC/MS (LC/TQ) is a widely accepted modern technique for this analysis, however laboratories traditionally run individual analyses based on compound class. This can be inefficient and result in high operating costs. In this poster we describe a comprehensive screening and quantitative analytical workflow (Figure 1) for highly sensitive, and reproducible analysis of >200 multi-class veterinary drugs in various animal origin food matrices using LC-MS/MS.
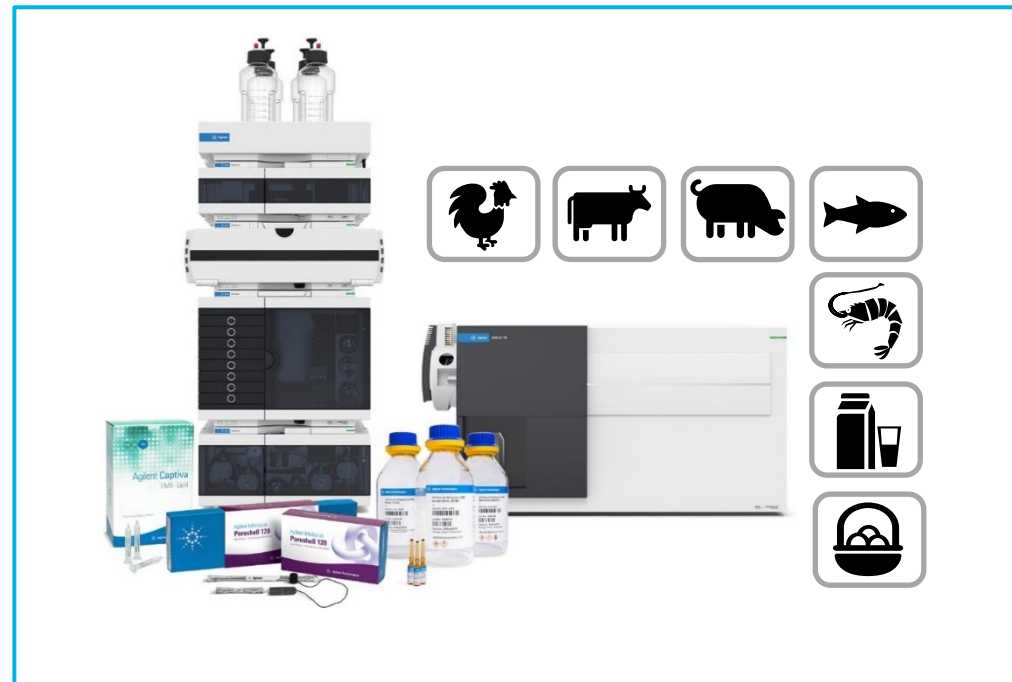
Experimental

Figure 1: Agilent Comprehensive Veterinary Drug dMRM Solution

Experimental

Target Selection and classification:

The 210 targeted veterinary drugs included in the workflow solution were selected based on combinatory study of recommendations by AOAC[1], US FDA-CFR[2], US FSIS[3], and EU[4]. These targets are from >28 different chemical classes (Figure 2).

Workflow Solution Protocol:

A simple sample preparation protocol based on solvent extraction and Captiva EMR-Lipid cleanup helped to extract wide range of veterinary drugs (Figure 3). Based on AOAC recommendations, the analytical performance was assessed using 9 different matrices (chicken, beef, pork salmon, shrimp, milk, egg, kidney, and liver) using two different triple quadrupole LC/MS models (6470 LC/TQ and 6495 LC/TQ)[5, 6].
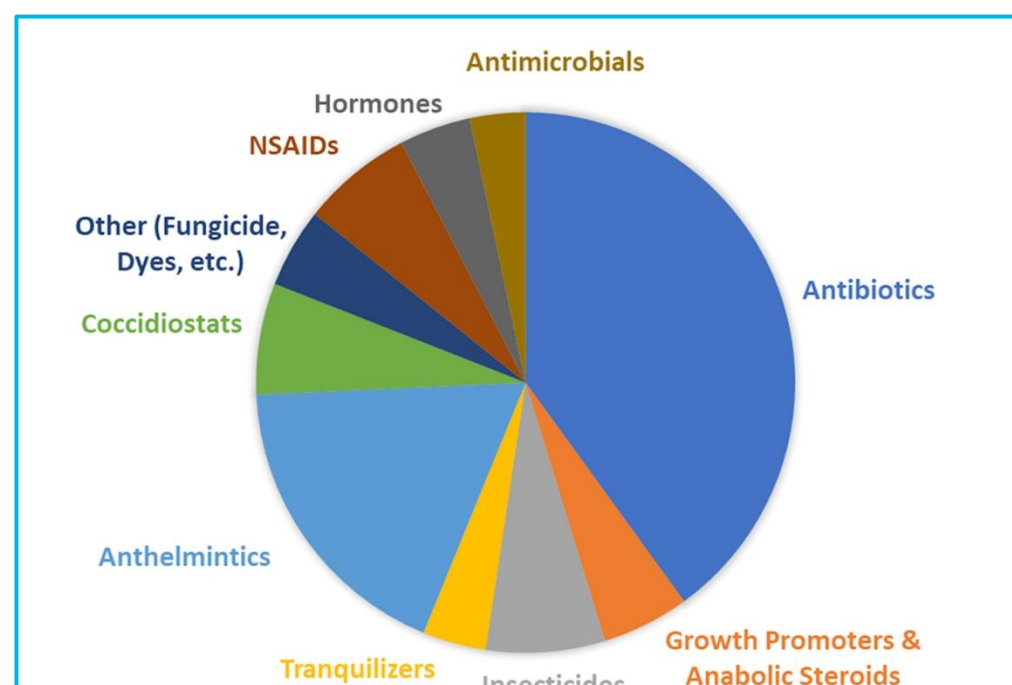

Figure 2: Chart describing chemical classification of 210 targets

2g Sample (Spike, Blank) → 2 x liquid extraction (EDTA + Acidified Acetonitrile) → Centrifuge → Clean up: Agilent Captiva EMR-Lipid cartridge (p/n 5190-1003) and Agilent PPM-48 (p/n 5191-4101) → Analysis using Agilent MassHunter and 6470 LC/TQ or 6495C LC/TQ
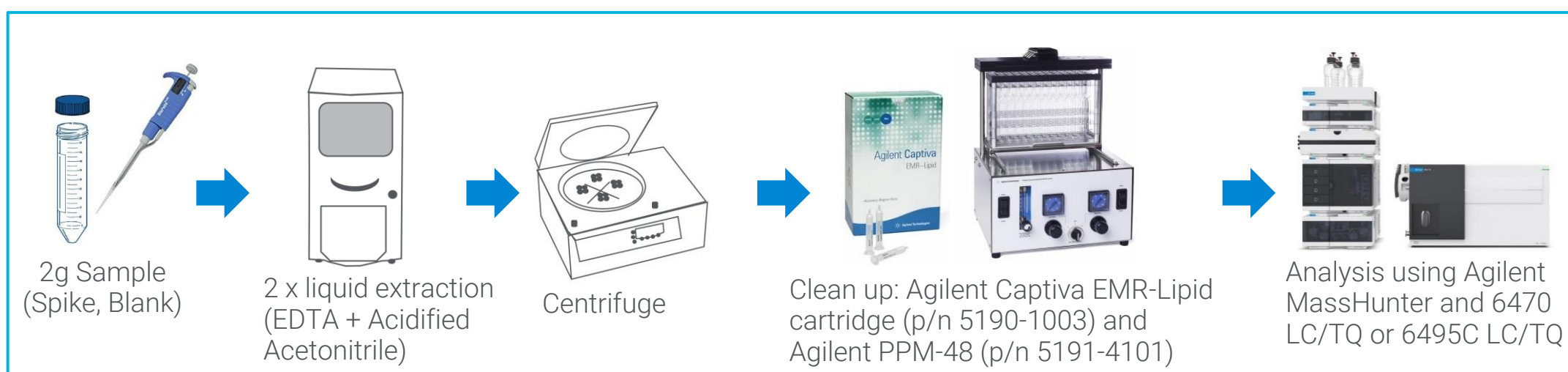
Figure 3: Analytical flow-chart

Method sensitivity, linearity, accuracy, and precision using matrix-matched samples

A 13-minute chromatographic method using Poroshell 120 superficially porous columns resulted acceptable analyte separation for reliable MS/MS quantitation (Figure 4). Over 90% of targets showed LOD of ≤ 5 µg/kg in all matrices (Figure 5). The calibration curves with linearity R^2 ≥0.99 were established from LOQ to 100 µg/kg. Accuracy results were well within the range of 70–120%. Target analyte response and retention time precision were evaluated at 10ppb concentration and observed %RSD values were <20% and <1.0% respectively.

Recovery using matrix-spiked QCs

Applicability for routine veterinary drug screening is verified by performing recovery analysis using matrix-spiked QC samples.

QC levels: LLQC: 0.1 µg/kg, LQC: 1µg/kg, MQC: 10 µg/kg, and HQC: 25 µg/kg. LLQC only for milk matrix to meet very low MRL requirements.

Based on the MRL value, one of the QC levels was chosen to demonstrate target recovery. The average recovery of 92% targets was within 60-120% (Figure 6).
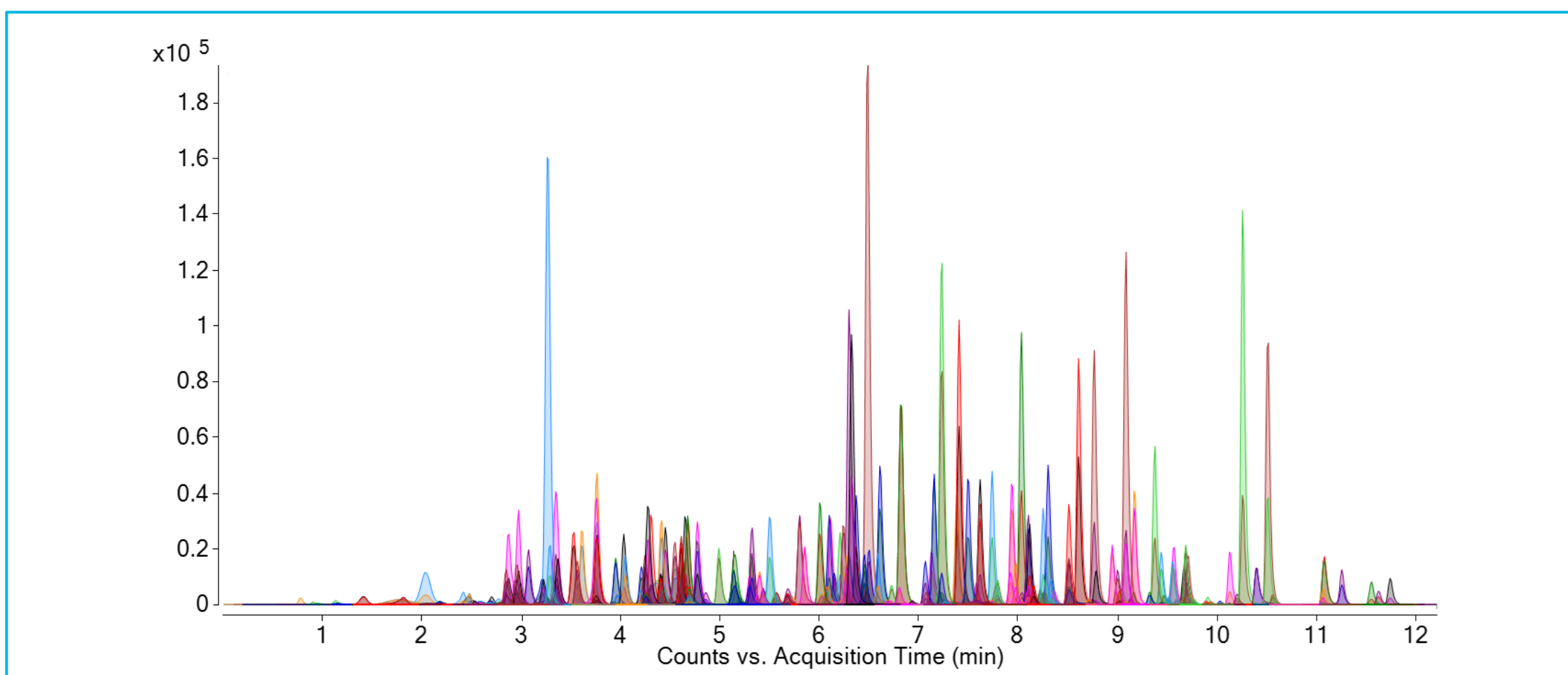

Figure 4: Representative dynamic MRM chromatogram of 210 veterinary drug targets postextraction spiked at 2.5 µg/L in chicken blank matrix
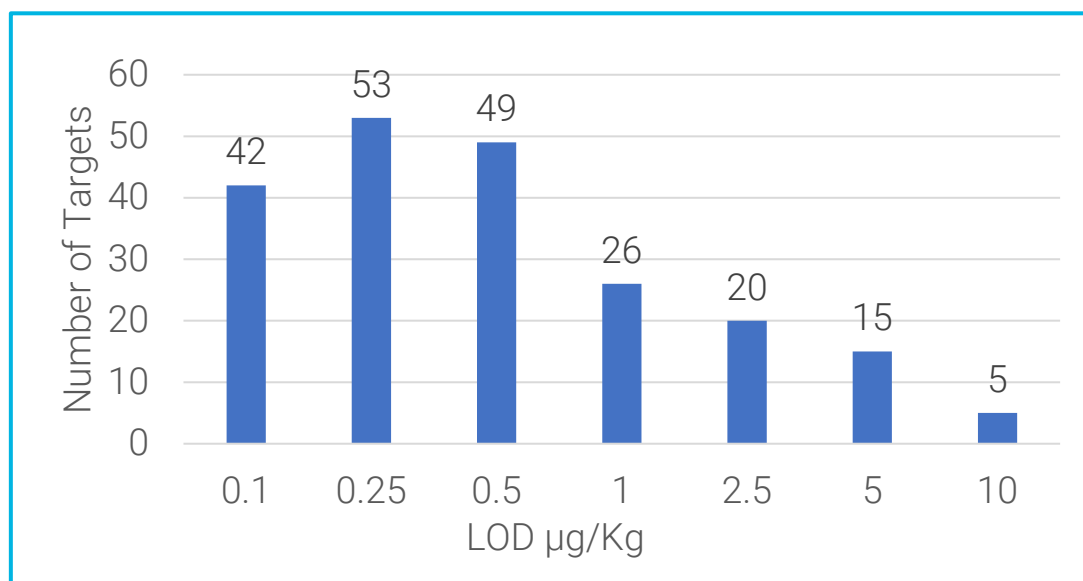

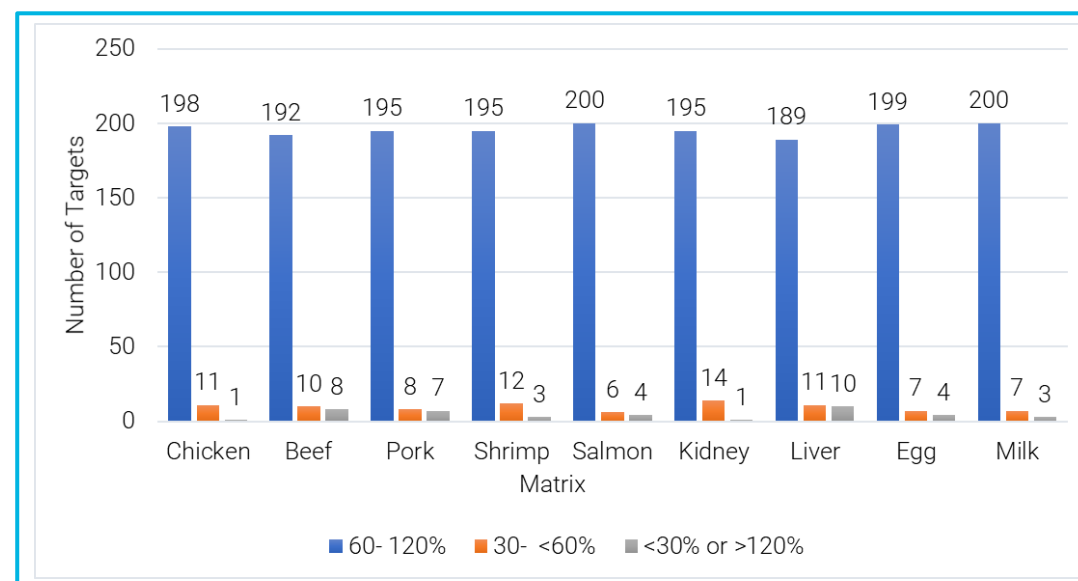Figure 5: LOD distribution of 210 targets in chicken matrix

Figure 6: Recovery results summary from 9 different matrices

Intra-batch repeatability, Inter-batch reproducibility using matrix-spiked QCs

The intra-batch recovery repeatability of all targets were evaluated using technical replicates of matrix-spiked QC samples. Recovery reproducibility was evaluated from multiple batches run in different days.

The repeatability %RSD values of majority of targets were within the acceptable limits[6]. All 210 targets met the recovery reproducibility limit of <32% RSD (Figure 7). Intra- and inter-batch results confirmed the workflow solution applicability for regulatory-based routine analysis of veterinary drug residues in all listed matrices[6].
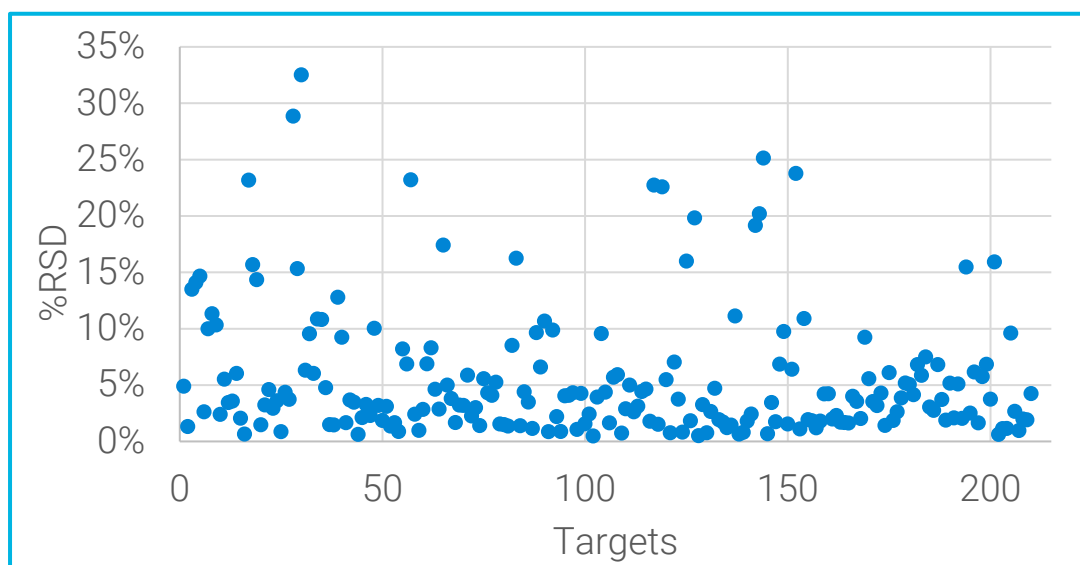

Figure 7: Inter-batch reproducibility of 210 targets using matrix-spiked QCs. All 210 targets met the limit of <32% RSD. Reproducibility of 194 targets were <15%.

Method robustness for confident routine operation

The method robustness results using 400 continuous injections of Agilent Veterinary Drug System Suitability test mix (part number 5799-0015) confirmed the method consistency and reliability for day-to-day operations, with minimal sample residue accumulation on the ion source interface (Figure 8).
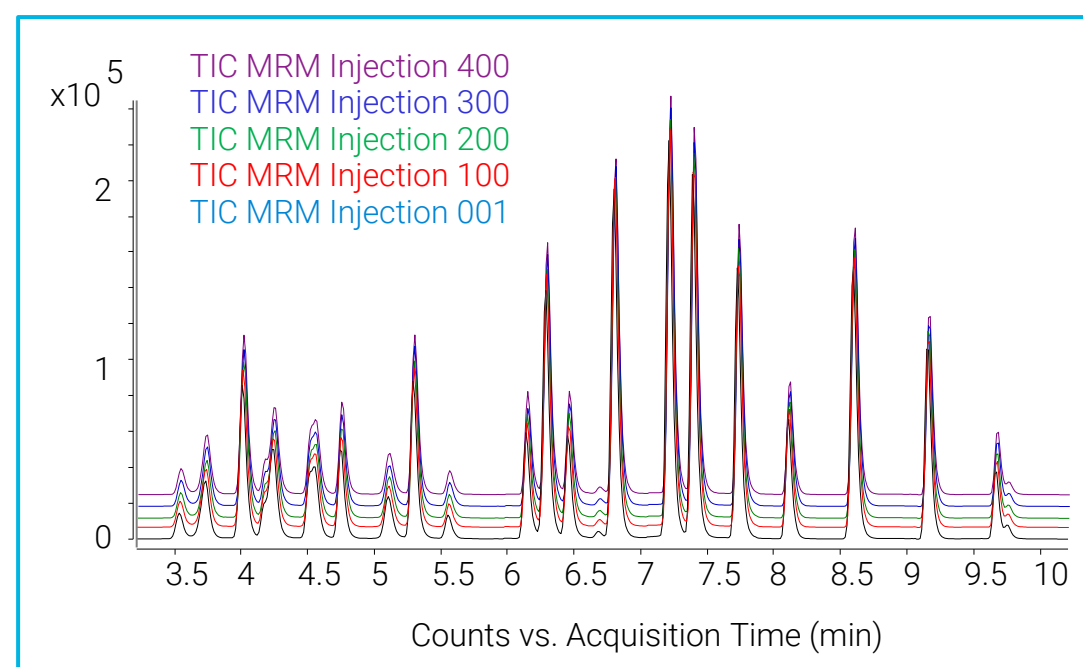

Figure 8: Overlay of five selected system suitability mix TIC MRM chromatograms, spread across 400 continuous replicate injections. Concentration: postextraction spiked at 1.0 µg/L in milk blank matrix extract. (offset X, Y-axis values)

Conclusions

- Demonstrates a robust LC/MS-MS workflow solution for routine analysis of veterinary drug residues which overcomes challenges of many single-class methods and extraction procedures.

- High throughput Sample cleanup (48 samples in 30 min) & fast analysis (18 minute Inj-to-Inj)

- The workflow analytical performance is verified in 9 different matrices by following AOAC guidelines including sensitivity, linearity, precision, accuracy, recovery, Intra- & Inter-lab recovery analysis.

- The workflow sensitivity is exceeding the requirements set by global regulatory agencies for screening trace veterinary residues in complex matrices.

- Offers flexibility to customize the solution to meet local laboratory/ regulatory requirements.

References

1. Screening and identification method for regulated veterinary drug residues in food, AOAC guidelines, Version 7, June 20, 2018

2. The United States, Code of Federal Regulations (CFR) - Title 21, Tolerance of Residues in New Animal Drugs in Food, Part 556, volume 6, April 1, 2019.

3. The United States, Chemical contaminants of public health concern used by the Food Safety and Inspection Service (FSIS), 2017.

4. Official Journal of the European Union, Pharmacologically active substances and their classification regarding maximum residue limits (MRL), Commission Regulation (EU) No 37/2010.

5. Guidelines for Standard Method Performance Requirements, AOAC Official Methods of Analysis 2016, Appendix F.

6. Agilent App Notes 5994-1932EN, 5994-2832EN, 5994-3124EN, and 5994-3680EN.

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