Poster Reprint

ASMS 2020
WP 171

An End-to-End Workflow Solution For Quick and Easy Quantitative Analysis of Multiclass Veterinary Drug Residues in Meat Using LC-MS/MS

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Introduction

Veterinary drugs are commonly used to improve the growth and health outcomes of farm animals. Improper use of vet-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 ensure Food Safety.

LC-MS/MS is a widely accepted 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 veterinary drug dMRM workflow solution (Figure 1) for highly sensitive, reproducible screening and /or quantitative analysis of >200 multi-class veterinary drugs in various animal origin food matrices using LC-MS/MS.

Figure 1:
Agilent Comprehensive Veterinary Drug dMRM Solution

Experimental

Target Selection

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

A Venn diagram of 210 target distribution across various organizations is given in Figure 2. Out of 210 targets, 168 of them have Maximum Residue Limits (MRL) established in three muscle matrices defined by the AOAC, EU, or US regulation/guidelines.

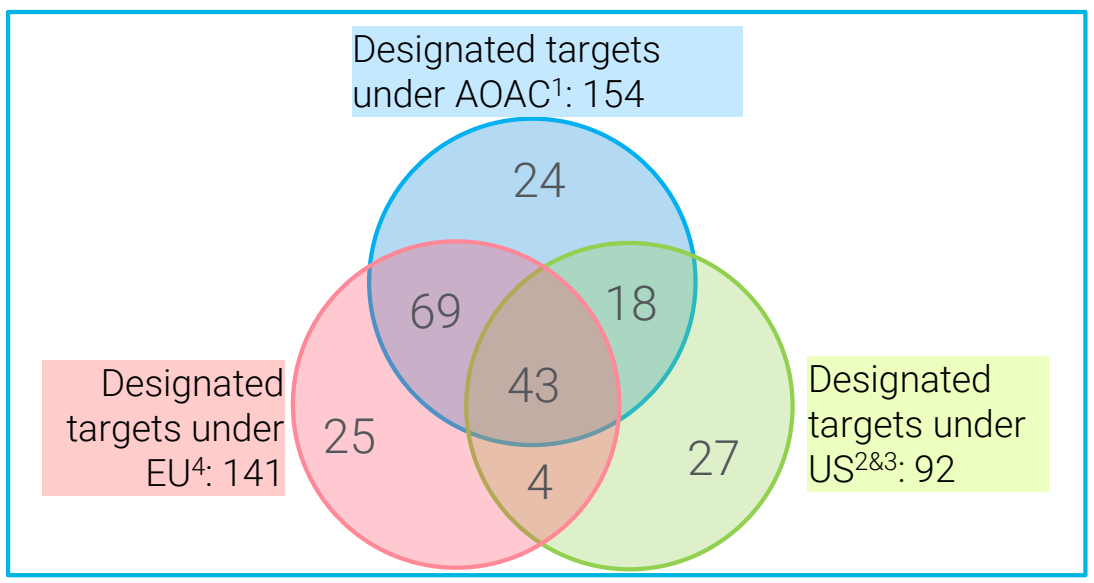

Figure 2: Venn Diagram of 210 targets distribution across various regulations.

Workflow Solution Protocol

The analytical workflow utilized for this work is summarized in Figure 3.

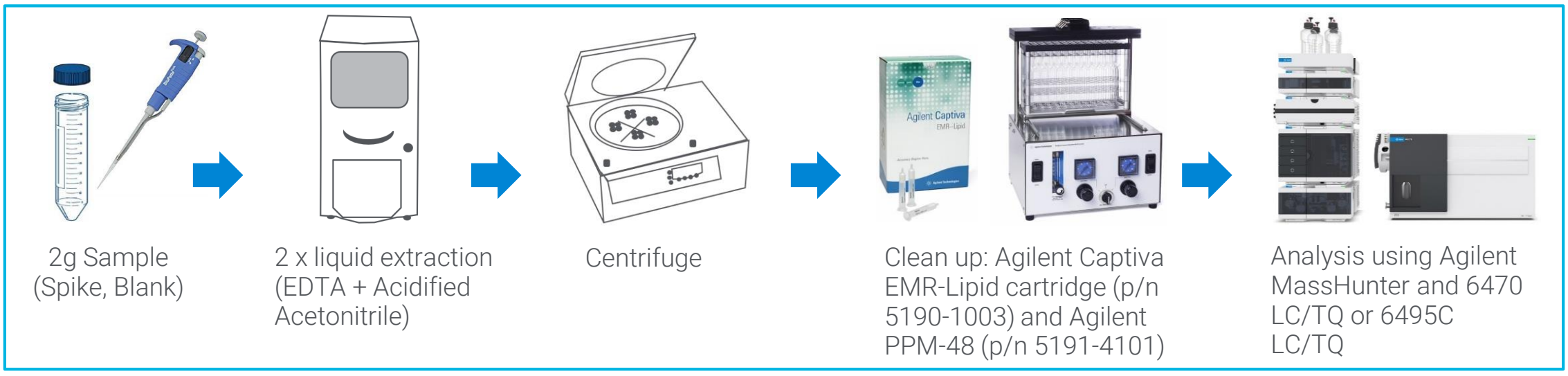

Figure 3: Analytical flow-chart

LC-MS/MS MRM Overlay

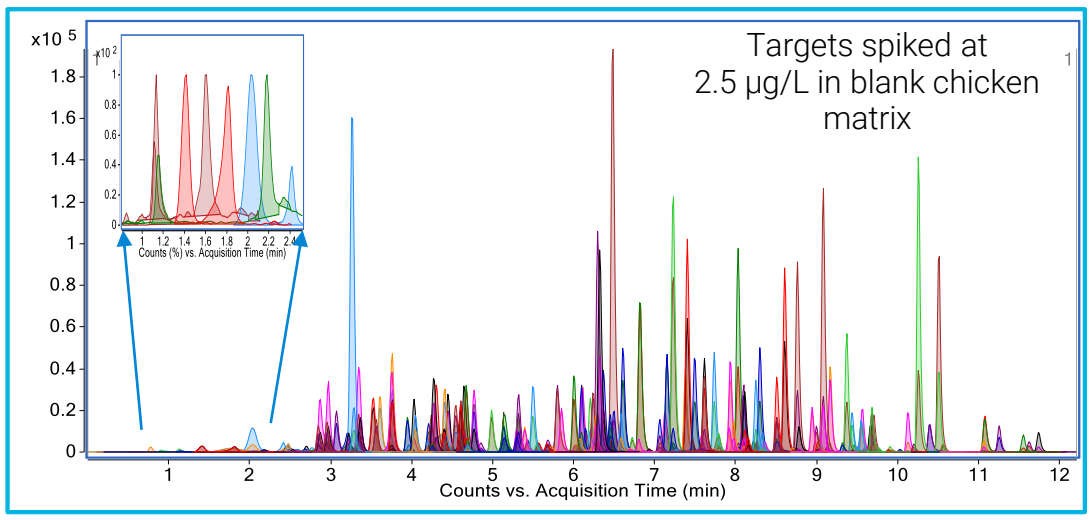

Figure 4: MRM chromatogram of 210 veterinary drug targets with zoom view of six early-eluting targets.. Column: Agilent InfinityLab Poroshell 120 EC-C18 column (p/n: 695575-302).

LC-MS/MS Method Performance Evaluation

Method sensitivity, linearity, accuracy, and precision data were measured using matrix-matched spike samples from 0.1 to 100 µg/kg. Method recovery analysis was performed using matrix-spiked samples at 1 (Low QC), 10 (Mid QC), and 25 (High QC) µg/kg concentrations.

The limit of detection (LOD) of all targets ranged between 0.1 -10 µg/kg. Calibration curves for all targets were plotted from limit of quantitation (LOQ) to 100 µg/Kg. The sensitivity and linearity results are summarized in the below table.

# of Targets	LOD (µg/kg)	Linear calibration curve Range with R2> 0.99 (µg/kg)
42	0.1	0.25 - 100
53	0.25	0.5 - 100
49	0.5	1.0 - 100
26	1	2.5 - 100
20	2.5	5.0 - 100
15	5	10.0 -100
5	10	25.0 - 100

Instrument Method Accuracy and Precision

The average accuracy was calculated from triplicate injections and observed results were well within the range of 70–120%.[5]

Precision was determined as %RSD of target response and retention time (RT) using triplicate injections of matrix sample. Response %RSD for all targets in the chicken matrix was <20% and RT %RSD of all targets was within 0.5%.

Applicability For Routine Screening

Applicability for routine veterinary drug screening is verified by performing recovery analysis using QC samples. The target recoveries from chicken muscle matrices are shown in Figure 5. The successful application of this workflow solution to screen all MRL established targets in chicken matrix as per AOAC guidelines is demonstrated in Agilent publication 5994-1932EN.[6]

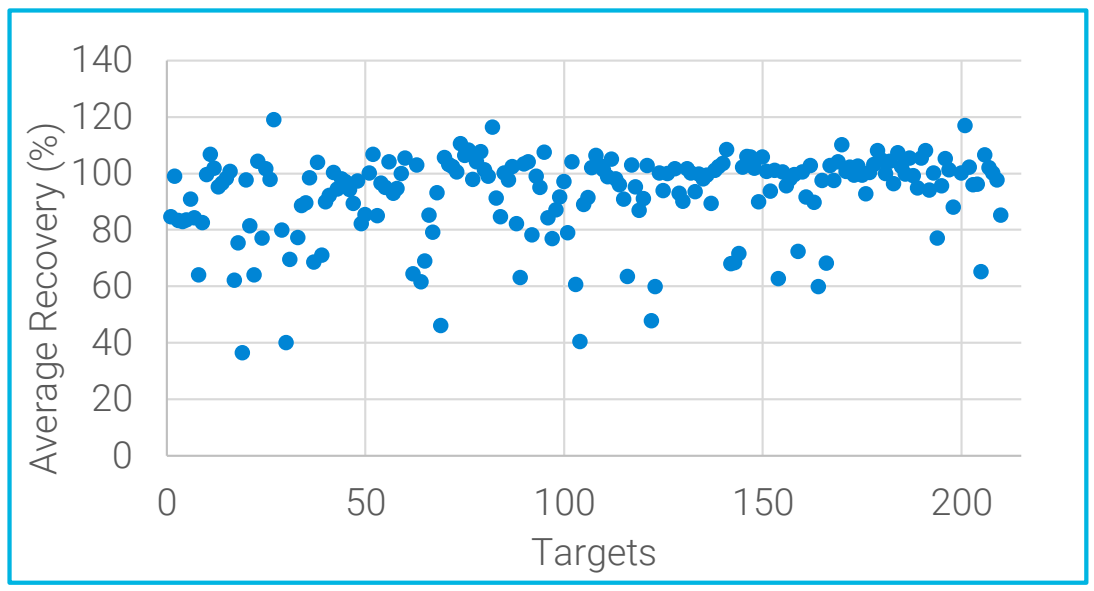

Figure 5: Target recoveries from chicken muscle spiked with 10 µg/kg standard

Intrabatch Recovery Repeatability

The intrabatch recovery repeatability of all targets were evaluated by running n=3 replicates of spiked chicken samples within a batch (Figure 6). The recovery value of a few targets was less than 60%; however, the recovery reproducibility for these targets was within 10% RSD.

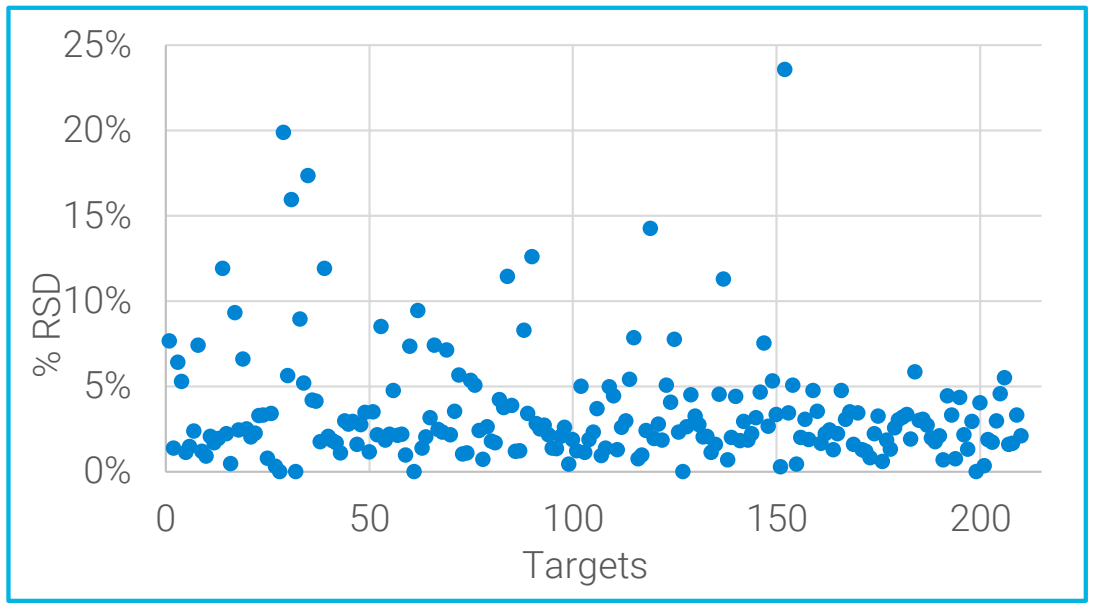

Figure 6: Intrabatch recovery repeatability of all targets using chicken matrix. Recovery repeatability of >98% targets were within 15% RSD.

Results and Discussion

Interbatch Recovery Reproducibility

Interbatch recovery reproducibility was evaluated by running n=3 replicates of spiked chicken samples prepared in different days and run in different batches (Figure 7).

Consistent and reproducible results on intrabatch repeatability and interbatch reproducibility confirmed the workflow solution applicability for confident day-to-day screening analysis.

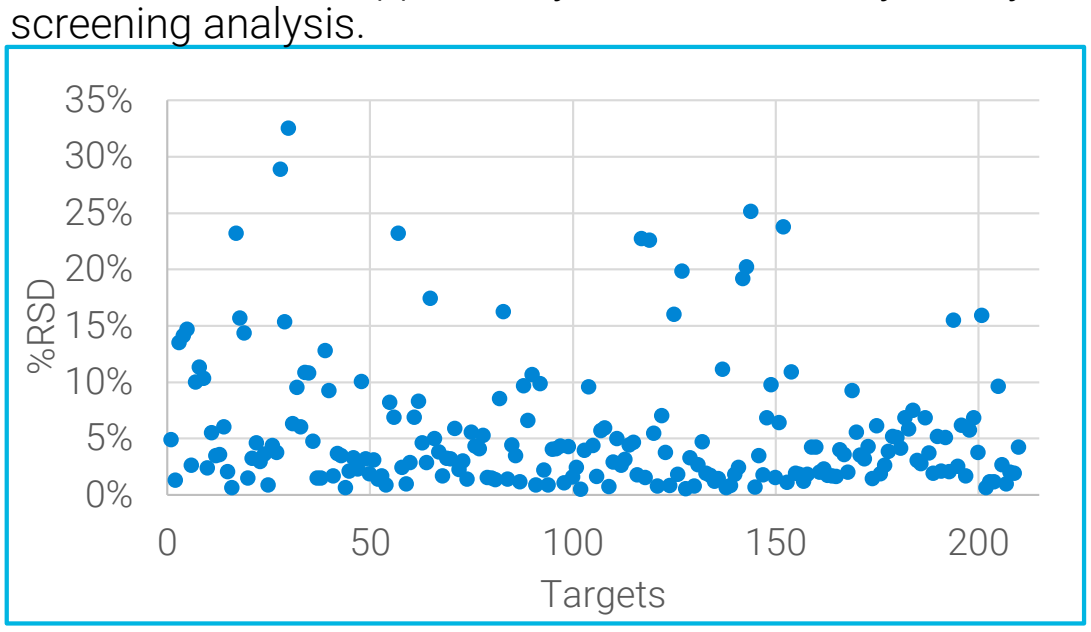

Figure 7: Interbatch reproducibility of 210 targets using prespiked QCs. All 210 targets met the limit of <32% RSD. Reproducibility of 194 targets were <15%.

Workflow Performance in Other Matrices

The workflow solution applicability in beef and pork muscles were also evaluated and results were in good agreement with that of chicken matrix. [6]

VetDrug dMRM Database for Easy Sub-methods

A dMRM database was created that includes all the settings for acquisition of 210 targets. The database helps to easily customize dMRM sub-methods based on target list of interest or regulation in a region (Figure 8).

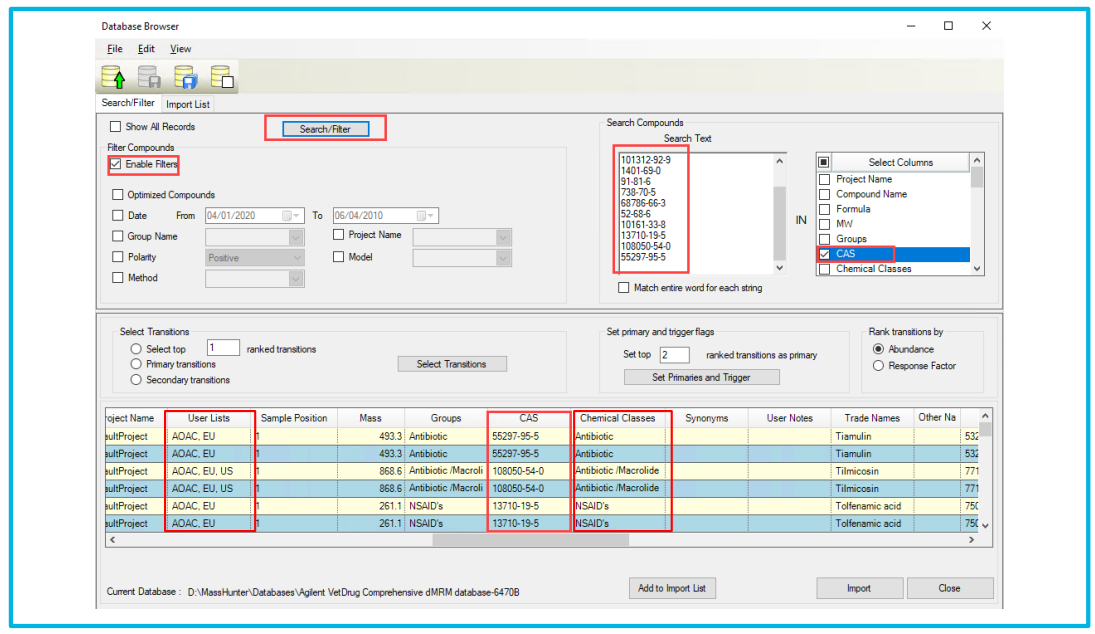

Figure 8: VetDrug dMRM database in Data Browser .

System Suitability STD Mix for Confident Performance

Vet drug System Suitability Test-Mix (Agilent p/n: 5799-0015) is available to support the workflow solution. The 25 targets are from 10 different chemical classes, with broad range of molecular weight, eluted evenly across the elution time, and covers both positive and negative polarity ionization. This standard simplifies installation verification and, when used as a regular QC sample, ensures confident day-to-day operation of workflow solution.

Conclusions

- Demonstrates a rapid, sensitive, and robust end-to-end LC/MS-MS workflow solution to analyze >200 multi-class veterinary drug residues in meat using Agilent LC/TQ.
- The applicability of the workflow solution for routine veterinary drug screening is demonstrated by performing screening of AOAC-listed targets in chicken matrix.
- The performance of Agilent Comprehensive Veterinary Drug dMRM Solution (G5368A) is verified using two different triple quadrupole models (6470 LC/TQ and 6495C LC/TQ).
- Workflow applicability verified for beef and pork muscle, and will extend to seafood, milk products, etc. in future.

References

1. AOAC guidelines on “Screening and identification method for regulated veterinary drug residues in food”, Version 7; June, 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 Note, “An End-To-End Workflow for Quantitative Screening of Multiclass, Multiresidue Veterinary Drugs in Meat Using the Agilent 6470 Triple Quadrupole LC/MS”, 5994-1932EN

Published in USA, June 1, 2020

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