The VetDrugs Explorer Collection: A comprehensive multi-class veterinary drug analytical workflow for a variety of animal matrices

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ABSTRACT

Purpose: To present a LC/MS/MS workflow solution for multi-class analysis of veterinary drug residues in animal matrices that is robust, rapid, easy to use, and has the sensitivity, accuracy, and precision that is required in order to meet regulatory guidelines.

Methods: The Thermo Scientific™ VetDrugs Explorer Collection is a solution designed to address the essential elements of a complex multi-class veterinary drug residue analysis that can be applied to a variety of matrices. Bovine muscle, salmon (fillet), and milk (dairy) were processed and analyzed to test the core methodology- from sample preparation using a modified QuEChERS protocol (Quick, Easy, Cheap, Effect, Rugged, and Safe) to analysis, data processing, and reporting with LC/MS/MS and comprehensive data handling software.

Results: Results demonstrate that the method is fit-for-purpose as a comprehensive semi-quantitative screening workflow that can be easily implemented in a residue testing laboratory. Screening target concentrations (STCs) established for each matrix meet regulatory requirements based on MRL definitions and limits specified in EU Commission Regulation 37/2010 and EU Council Regulation 2377/90, as well as USDA/FDA guidelines.

INTRODUCTION

Veterinary drugs are broadly defined as chemicals that are used to protect animals from contracting disease, promote growth, and in some cases provide aesthetic qualities in food production. The inappropriate use of veterinary drugs can have adverse effects on animals, the environment, and human health. Antimicrobial resistance, or the ability of certain microorganisms (bacteria or viruses) to eliminate or reduce the effectiveness of a drug, can be promoted in the environment by overuse of some of these medicines.

As a result, the determination and efficient analysis of veterinary drugs is an important part of routine food quality control. The European Union (EU) and others globally have developed specific regulations to address these growing concerns. The requirements of low limits of quantification in diverse matrices, along with a wide variety of chemical classes and properties of veterinary drugs pose significant analytical challenges. Consequently, several analytical methodologies have emerged which are typically limited in scope to specific chemical classes, are labor intensive, and require extensive sample preparation and clean-up.

A multi-class analytical workflow will streamline this screening process in the lab. We present a solution that encompasses everything that is required from sample preparation to final reporting for over 160 veterinary drugs using liquid chromatography-triple stage mass spectrometry (LC-MS/MS) and a rapid sample preparation procedure based on QuEChERS. The solution is sensitive and robust, and is able to detect, confirm, and quantify the veterinary drugs below their required EU and/or US FDA/USDA maximum residue limits (MRLs).

MATERIALS AND METHODS

Sample Preparation

Sample preparation involves a protocol that was optimized to be easy for laboratories to implement and also reduce matrix co-extractives, resulting in enhanced sensitivity in electrospray ionization LC-MS/MS. The basic elements of the preparation procedure are described in Figure 1, and are used for preparation of bovine muscle, salmon fillet, and liquid milk samples.

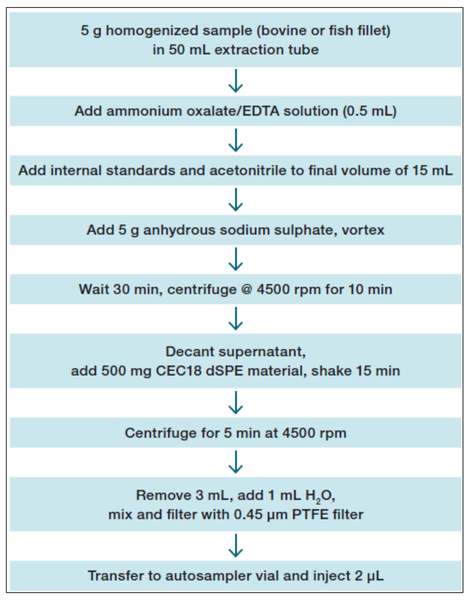


Figure 1: Extraction procedure for bovine muscle and salmon (fillet). For liquid milk extraction, 4g sample was used and extracted with 10mL of acetonitrile, with supernatant after centrifugation cleaned up with 500 mg CEC18 dSPE, evaporated to near dryness. 1 mL acetonitrile was added, centrifuged, and filtered before analysis.

When developing spiking solutions for a multi-class veterinary method, several considerations need to be addressed. These include the stability of the spiking mixtures (stock solutions) and the final concentration of the target analytes required as they relate to the regulatory MRLs in given matrices. Laboratories will often adopt a specific SOP to address these issues and periodically check the stability of the stock mixtures.

Test Method(s)

The veterinary drug compound classes and number of compounds tested for this method are shown in Figure 2. Over 160 veterinary drugs were added directly to the homogenized matrix during QueChERS sample preparation to create matrix extracted spikes (MES) at concentration levels that reference a screening target concentration, or STC. The STC relates back to the relevant maximum residue limit (MRL) for each compound in a given matrix. For method development, the STC level (i.e. STC level 1) was chosen to be 1/3 to 1/4 of the concentration of the EU-based MRL. In order to observe the quantitative performance of the method, a series of MES were prepared and used to construct calibration curves at 0.0 (MES blank), 0.2, 0.5, 1, 3, and 5× STC.

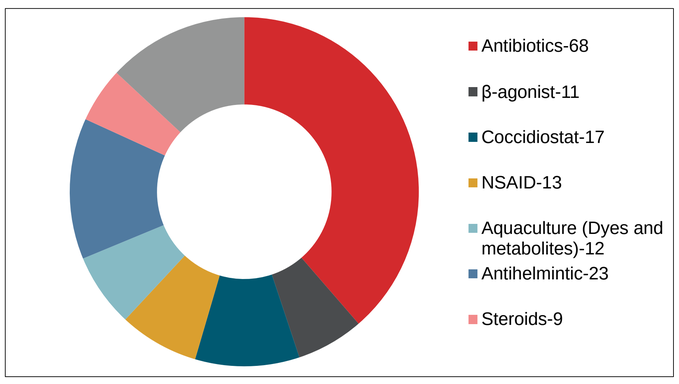
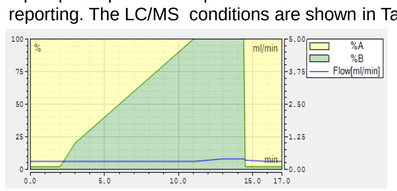


Figure 2: Compound classes studied for applicability to the multi-class method. Spiking mix 'cocktails' were used to prepare MES based upon the regulatory MRL of a given compound and matrix combination.

Data Analysis

The assays in this study were carried out using a Thermo Scientific™ Vanquish™ Flex Binary UHPLC system and a Thermo Scientific™ TSQ Altis™ triple quadrupole mass spectrometer. Thermo Scientific™ TraceFinder™ software was used for instrument control, analysis, data review, and reporting. The LC/MS conditions are shown in Tables 1 and 2.



No	Time	Flow [ml/min]	%B	Curve
1	0.000	Equilibration		
2	0.000	0.300	2.0	5
3	0.000	0.300	2.0	5
4	*New Row*			
5	0.000	Run		
6	0.000	0.300	2.0	5
7	0.000	0.300	2.0	5
8	2.000	0.300	2.0	5
9	3.000	0.300	20.0	5
10	11.000	0.300	100.0	5
11	13.000	0.400	100.0	5
12	14.400	0.400	100.0	5
13	14.500	0.350	2.0	5
14	16.000	0.300	2.0	5
15	17.000	0.300	2.0	5
16	*New Row*			
17	17.000	Stop Run		

Negative Voltage	2500 V
Positive Voltage	3500 V
Sheath Gas	50 units
Auxiliary Gas	13 units
Sweep Gas	1 unit
Ion Transfer Tube Temperature	310 C
Vaporizer Temperature	350 C

Table 1. Mass Spectrometer API source conditions

Injection Volume	2 µL
Column Temperature	40 C
Analytical Column	Thermo Scientific™ Accucore™ VDX, 100 x 2.1 mm, 2.6 µm
Run Time	17 min
Mobile Phase A	Water with 0.05% Formic Acid
Mobile Phase B	50% Acetonitrile 50% Methanol 5% Water with 0.05% Formic Acid

Table 2. LC Gradient (left) and column with mobile phase conditions (right)

RESULTS

The steps for evaluating the method are described in Figure 3. Eight replicates at each screening target concentration (STC) spike level were injected, along with the matrix extracted spikes (MES) blank (injected three times). Additionally, a "post spike" sample was prepared in the blank matrix extract, i.e., the target analytes were added after a blank matrix was extracted. It was spiked at the 3× STC level. Spike recovery was then estimated by the ratio of the peak areas observed in the 3× STC MES and post spike sample, using the following formula: % Recovery = Peak area 3× STC MES/Peak area 3× STC Post Spike × 100.

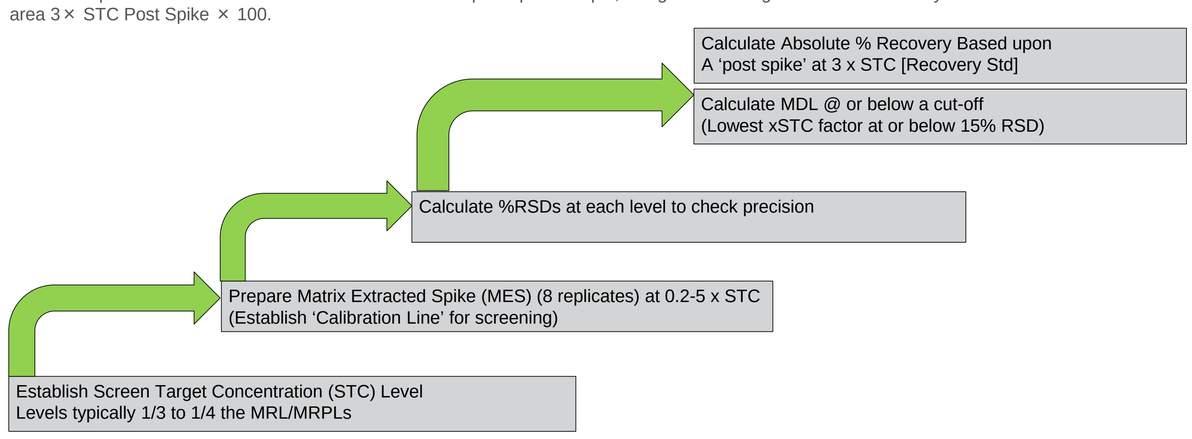
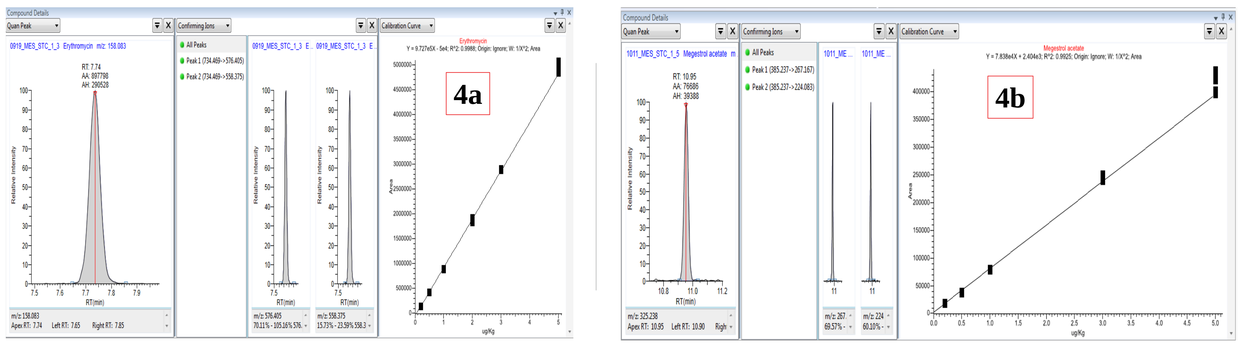


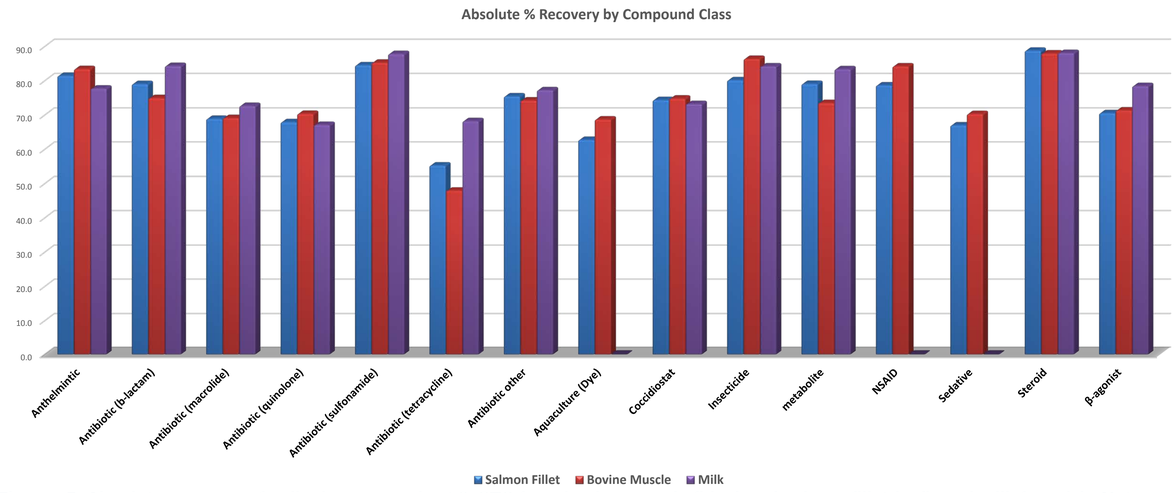
Figure 3: Steps for initial evaluation of method performance of the multi-class method in milk, bovine muscle, and salmon fillet samples

Screening levels of 0.2. 0.5, 1, 2, 3, 4, and 5 X STC were established with two examples shown in the following figure 4a and 4b .



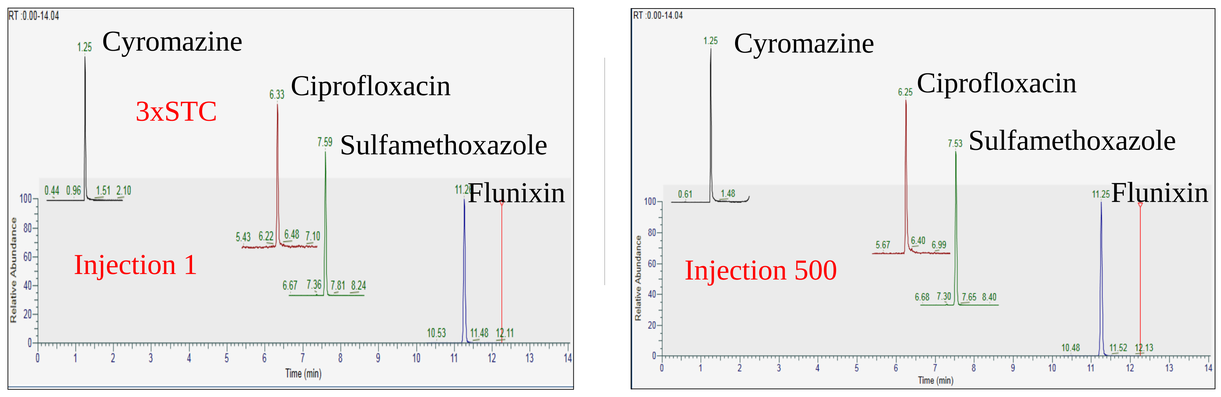
Figures 4a and 4b: Quantitation ions, confirming ions, and STC range shown in TraceFinder software for Erythromycin in cattle muscle (left) and Megestrol acetate in milk (right). The technique allows for confident screening with confirmation surrounding the MRL concentration for each analyte/matrix combination.

Absolute recovery of the compounds was measured in the three matrices. Overall averages for salmon fillet, bovine muscle and milk were 75, 76, and 79 % recovery respectively. Figure 5 summarizes recoveries observed across the different chemical classes studied. The method detection limit was calculated for each analyte, and they ranged from an average value of 0.5 to 2 ng/g across all the matrices.



Figures 5: Absolute recovery of spiked compounds at 3xSTC from bovine muscle (blue) and salmon fillet (red), and milk (purple). Note: The Dyes, NSAIDs, and Sedatives were not spiked in the milk samples .

Column and API source robustness were demonstrated by injecting a bovine muscle spike sample 500 x over a period of 1 week, showing good peak shapes and intensities.



Figures 6: LC/MS/MS system test components spiked in bovine muscle extract demonstrate good robustness of the analytical system and API source. Left: Injection # 1; Right: Injection # 500

CONCLUSIONS

- A multi-class, semi-quantitative method for veterinary drugs has been presented, using screening target concentrations (STCs) in matrix extracted spikes, based upon the MRLs established for analyte/matrix combinations in beef muscle, milk, and salmon fillet.
- The workflow solution is useful for labs that wish to consolidate multiple single-class methods, and it provides excellent sensitivity using the TSQ Altis triple quadrupole mass spectrometer.
- The modified QuEChERS extraction demonstrated good recovery and precision, with robust performance below the MRLs/MRPLs listed in the EU and US regulations.
- More work is required to evaluate the performance of the method with incurred residues

REFERENCES

1. European Medicine Agency Commission Regulation (EU) No 37/2010
2. Code of Federal Regulations (CFR) –Title 21 Part 556
3. Collection of Maximum Residue Limits for Veterinary Drug of Major Countries and Regions (China)

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