A Modified Orbitrap Exploris MS used for Multi-class Veterinary Drug Screening and Quantitation by High Resolution Mass Spectrometry (HRMS)

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ABSTRACT

Purpose: Demonstrate a HRMS method for screening and quantitation of a multi-class veterinary drug panel in bovine muscle matrix based upon AOAC Standard Method Performance Requirements (SMPR 2018.010)[1].

Methods: A QuEChERS sample preparation procedure was used to extract 5g of homogenized sample. 5 µL was injected into a UHPLC coupled to a modified Thermo Scientific™ Orbitrap Exploris™ mass spectrometer with data independent acquisition (DIA) and data dependent (DDA) workflows for screening and quantitation.

Results: Both DIA and DDA acquisition workflows showed a high degree of confidence for effective screening and confirmation in bovine muscle for target analytes based on probability of detection (POD) at ½ and 1 x the lowest global maximum residue limits (MRLs) as cited in the SMPR.

INTRODUCTION

There is considerable interest in maximizing the amount of information obtained from animal product analyses for the residues of veterinary medicines. One strategy to improve efficiency is to maximize the number of analytes that may be accurately screened and quantitated using a limited number of analytical methods. Commodities could then be checked to ensure compliance with regulatory maximum residue limits (MRLs), and ideally, the method(s) would be capable of simultaneously screening for other potential residues.

MATERIALS AND METHODS

Sample Preparation

1. Weigh 5 g of ground bovine muscle (50 mL Teflon tube)
2. Add 15 mL ACN + 0.5 mL NH4 Oxalate/EDTA solution
3. Shake 10 min; add NaSO4, mix, let stand 30 minutes
4. Centrifuge 10 min @ 3700 rpm

5. Decant supernatant into new tubes and add CEC18 reagent; shake on multi-tube vortexer for 15 minutes
6. Centrifuge 10 min @ 3700 ppm; Collect 3 mL
7. Evaporate 1 mL to near dryness at 30 C; reconstitute to 0.5 mL 75:25 mobile phase A:B; transfer to A/S vial

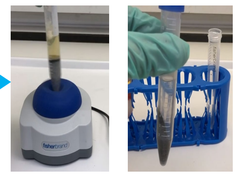


Table 1. UHPLC Conditions

Time	Flow (ml/min)	% B
0.0	0.300	2.0
0.0	0.300	2.0
2.0	0.300	2.0
3.0	0.300	20.0
11.0	0.300	100
13.0	0.400	100
14.4	0.400	100
14.5	0.35	2.0
16.0	0.300	2.0
17.0	0.300	2.0

Thermo Scientific™ Vanquish™ Flex UHPLC System

Column: Thermo Scientific™ Accucore™ VDX 100 x 2.1 x 2.6µm
Mobile Phase A: 0.05 % Formic Acid in water
Mobile Phase B: 0.05% Formic Acid in 1:1
Acetonitrile : Methanol + 5% Water
Run Time: 17 minutes
Injection Volume: 5 µL

HRMS Acquisition Workflows

Data Independent Acquisition (DIA)
No target list
Precursor isolation windows w/ stepped NCE
MS2 triggered across entire peak

Data Dependent Acquisition (DDA)
Target inclusion list with retention time
Specific precursor isolation and NCE
MS2 trigger on single apex scan

HRMS Parameters/Software

Full Scan Resolution: 60,000 FWHM
MS2 Resolution: 15,000 FWHM
Full Scan Range: 140-1100 da
NCE (DDA): Opt. per cmpd.
NCE (DIA): Stepped 30,80V

Spectral database | TraceFinder™ | mzVault™

RESULTS

Compounds, Chemical Classes, and POD Experiment

Instrument detection limits (IDLs) were first established with spikes directly into a bovine muscle extract for 170 compounds. Calibration ranged from 0.7-350 ppb. IDLs ranged from 0.02 to 8 ppb with r2 values >0.995 for most analytes. Good peak shapes as shown if Figure 1 were obtained under the method conditions.

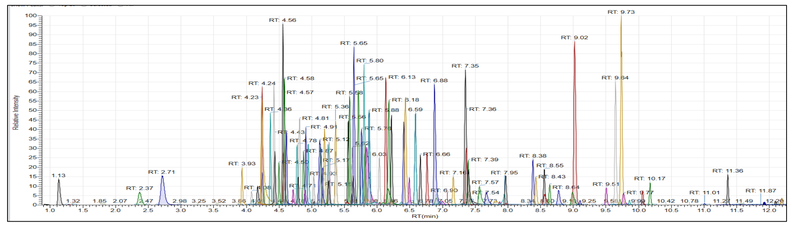


Figure 1. Extracted HRMS chromatogram of 170 VetDrugs in bovine muscle extract show excellent peak shapes for compounds in the multi-class VetDrugs method. Compound classes included benzimidazoles, macrolides, tetracyclines, avermectins, ß-lactams, non-steroidal anti-inflammatories, quinolones, coccidiostats and sulfonamides.

For the AOAC method, a mini-POD experiment was developed for 109 target compounds listed in the SMPR for bovine muscle. Matrix Extracted Spikes (MES) of 10 blank bovine samples, along with 10 samples spiked at ½ x MRL and 10 samples spiked at 1 x the MRL (from lowest global MRLs listed in the SMPR) were analyze by UHPLC-HRMS with both the DIA and DIA acquisition workflows.

POD is calculated as the ratio of the number positives (x) to total number tested (N): **POD = x/N.** A **detected and confirmed** result must have POD value of >/= 0.80, with both precursor ion in full scan and at least 1 fragment ion, both with mass accuracy </= 5 ppm with signal-to-noise >/= 3. (SANTE/12682/2019)[2]

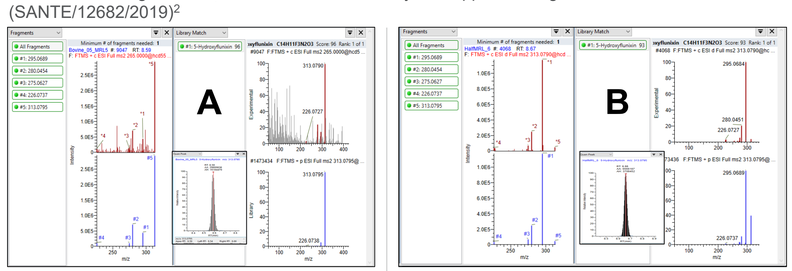


Figure 2. 5-Hydroxy-Flunixin detected and confirmed at ½ MRL (10 ng/g) in bovine muscle MES. A-Left (DIA workflow) and B-Right (DDA workflow). Full scan precursor and all MS2 fragments detected at <5 ppm mass accuracy with excellent library match scores, and POD =1.0.

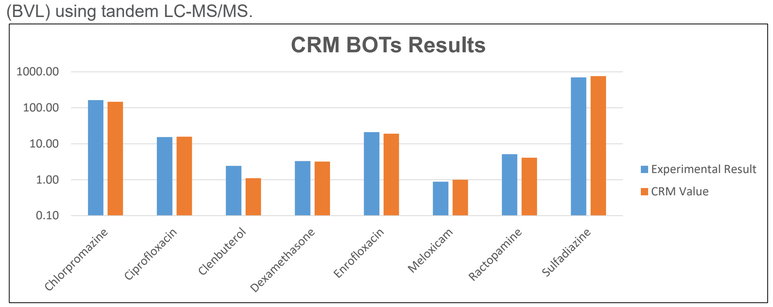
Compound	Class	1/2 MRL (ng/g)	DIA POD Score	Library Match Score (%)	# Fragments Match	ddMS2 POD Score	Library Match Score (%)	# Fragments Match
Albendazole	Benzimidazoles	50	1	99	5/5	1	93	5/5
Cefapirin	Cephalosporin Antibiotics	25	1	88	4/5	1	86	5/5
Ciprofloxacin	Floxacins	50	1	100	3/5	1	100	3/5
Imidocarb	Coccidiostats	150	1	95	2/5	1	97	4/5
Flunixin	NSAIDS	10	1	98	5/5	1	98	5/5
Levamisole	Anthelmintic	5	1	100	4/5	1	100	5/5
Lincomycin	Antibiotics	50	1	97	5/5	1	97	5/5
Oxytetracycline	Tetracyclines	100	1	96	5/5	1	90	5/5
Penicillin V	Penicillin Antibiotics	25	1	96	5/5	1	94	5/5
Ractopamine*	Beta-Agonists	5	1	89	2/2	1	no score*	1/2
Sulfamethazine	Sulfanilamides	3	1	100	5/5	1	100	5/5

Table 2. Probability of Detection (POD) for a selection of compounds representing several drug classes at 1/2x the MRL. The POD from two different acquisition methods (DIA, and DDA) is shown with the library match score and fragments. * Ractopamine not currently in library.

RESULTS

Certified Reference Material (CRM)

Quantitation with the HRMS DIA method was evaluated using a certified reference material known as BOTS-1, containing incurred veterinary drug residues in bovine muscle. The certified values are based on results from the National Research Council Canada (NRC), the Canadian Food Inspection Agency (CFIA), the USDA, and the German Federal Office of Consumer Protection and Food Safety (BVL) using tandem LC-MS/MS.



Compound	Experimental (ng/g)	True Value (ng/g)
Chlorpromazine	164.0	147
Ciprofloxacin	15.4	15.7
Clenbuterol	2.4	1.1
Dexamethasone	3.3	3.2
Enrofloxacin	21.0	19
Meloxicam	0.9	1
Ractopamine	5.1	4.1
Sulfadiazine	699.0	763

Figure 3 and Table 3. Average experimental results for 3 biological replicates vs. true value of incurred residues in BOTS-1. Results were confirmed with MS2 fragment matches and precursor ions with mass accuracies less than 5 ppm.

CONCLUSIONS

- The modified Oribtrap Exploris mass spectrometer provided high quality data in both DIA and ddMS2 scan operation modes for both screening and quantitation assays applied to veterinary drugs.
- This preliminary method development using the AOAC SMPR and POD guidelines clearly demonstrates the ability to confidently screen samples over a very wide range of MRLs.
- Quantitation accuracy and reproducibility were shown to be excellent from the analysis of incurred residues in the BOTs CRM sample.

REFERENCES

1. AOAC SMPR 2018.010 https://www.aoac.org/wp-content/uploads/2019/09/SMPR2018_010.pdf
2. https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_mrl_guidelines_wrkdoc_2019-12682.pdf

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TRADEMARKS/LICENSING

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