MULTI-RESIDUE SCREENING APPROACH FOR THE DETECTION OF VETERINARY DRUGS IN ANIMAL TISSUES USING LC-MS/MS

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INTRODUCTION

Veterinary drugs are widely used at therapeutic levels for livestock breeding for treating different diseases but are also misused to promote animal growth. The presence of residues in tissues and products of animal origin is of concern, so analytical methods for their determination in animal tissues and associated foodstuffs are needed to check regulatory compliance and ensure consumer safety. An effective approach to this challenge is to use a generic method, which can monitor many compounds, belonging to different drug classes, with a wide range of chemical properties. The use of sensitive and selective LC-MS/MS instrumentation can avoid the need for lengthy and costly clean up steps.

This poster demonstrates how a multi-residue method utilizing a generic extraction and clean-up method coupled with a LC-MSMS method can achieve and in almost all cases surpass required method limited of detection for over 150 veterinary drugs. Animal tissue was used to demonstration method performance.

SAMPLE PREPARATION

Sample Preparation and Extraction

Samples of minced muscle tissue from various animal species were purchased from local supermarkets. Muscle tissues were extracted using a generic liquid extraction using oxalic acid in acetonitrile followed by a dispersive solid-phase extraction (dSPE) clean-up. Figure 1 shows the extraction method workflow. Extracts were stored at -20°C and were analyzed by LC-MS/MS within 2 days of extraction due to concerns over stability of some of the analytes.

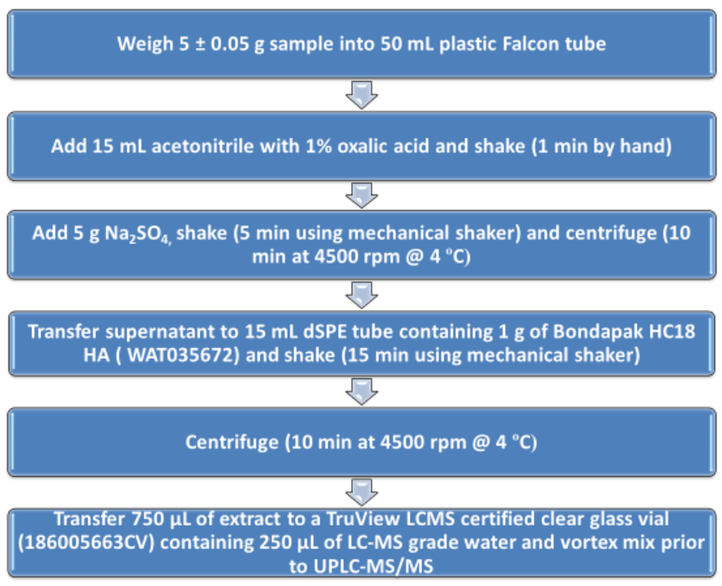

Figure 1. Generic extraction and clean-up workflow

MS CONDITIONS

MS System	Waters Xevo TQ-XS
Ionization	Electrospray
Acquisition	MRM with polarity switching
MS Parameters	Optimized transitions available at https://marketplace.waters.com

CHROMATOGRAPHY

LC System:	ACQUITY UPLC I-Class Plus (FL SM)
Column:	ACQUITY UPLC T3 (2.1 x 100mm), (186003539) with ACQUITY
Column	In-line Filter (205000343)
Mobile Phase:	A: 0.1% Formic acid + 0.1 mM Ammonium formate (aq)
	B: 0.1% Formic acid in 50/50 (v/v) methanol/acetonitrile
Injection volume:	1 µL
Column temp:	40°C
LC Separation Method:	details are presented in our app note (see QR code below)

During the initial method development several columns were trialed using solvent and then matrix standards. The HSS T3 column selected provides excellent retention and peak shape for all the analytes and separation of isobaric compounds (see Figure 2). All peaks in the method eluted between 1.65 and 11 minutes with a total run time of 15 minutes.

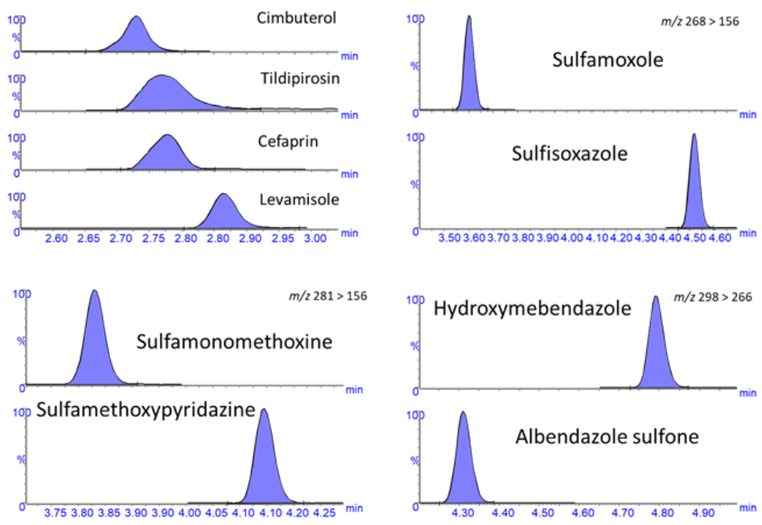

Figure 2. Examples of chromatographic performance of HSS T3 column for veterinary drug analysis

METHOD VALIDATION STUDY

The protocol from the guideline document that supplements Commission Decision 2002/657/EC regarding the validation of screening methods to assess the performance of the method was used to evaluate method performance. The principle of this validation was to evaluate the range of analytical responses in un-spiked versus spiked samples and to set a cut off level ensuring that the lowest response for the spiked samples does not overlap with the highest response for the un-spiked samples. The cut off level is the response from a screening test which indicates that a sample contains an analyte at or above the STC. Screening methods do not have to fulfil requirements of Commission Decision 2002/657/EC with respect to repeatability, reproducibility or trueness. The validation study used is outlined in Figure 4 that was used to establish the method STC. In the case of drugs with complex MRL definitions, the parent and/or metabolites have been sought as marker residues, but no attempt was made to include any chemical conversion step to a single marker residue (such as hydrolysis or oxidation) as this would adversely affect performance for other compounds. Calculation details are presented in our application note.

	Blank	0.1 µg/kg	1.0 µg/kg	10 µg/kg
Batch #1	4 bovine muscle	4 bovine muscle	4 bovine muscle	4 bovine muscle
	2 turkey muscle	2 turkey muscle	2 turkey muscle	2 turkey muscle
	1 porcine muscle	1 porcine muscle	1 porcine muscle	1 porcine muscle
Batch #2	4 bovine muscle	4 bovine muscle	4 bovine muscle	4 bovine muscle
	2 turkey muscle	2 turkey muscle	2 turkey muscle	2 turkey muscle
	1 porcine muscle	1 porcine muscle	1 porcine muscle	1 porcine muscle
Batch #3	4 bovine muscle	4 bovine muscle	4 bovine muscle	4 bovine muscle
	2 turkey muscle	2 turkey muscle	2 turkey muscle	2 turkey muscle
	1 porcine muscle	1 porcine muscle	1 porcine muscle	1 porcine muscle
	n=21	n=21	n=21	n=21

Table 1. Method Validation Study Protocol

RESULTS – DATA INTERPRETATION

Results from all 3 validation batches were assessed as a whole with response values assessed as measured concentration from a matrix matched calibration standard curve. This data assessment was used to calculate detection capability (CCß) of the method. This is done by comparing the Threshold value (T), which is the minimum analytical response above which the sample can be considered positive and the Cut-off factor (Fm) from which the sample can be considered negative. This is explained in more detail in our application note (link). Figure 3 shows what data set for ciprofloxin below the CCß (0.1 µg/kg) and Figure 4 above the CCß (1.0 µg/kg) of the method. These calculations were performed for over 150 veterinary drugs. A summary of the overall method performance is displayed in Figure 5.

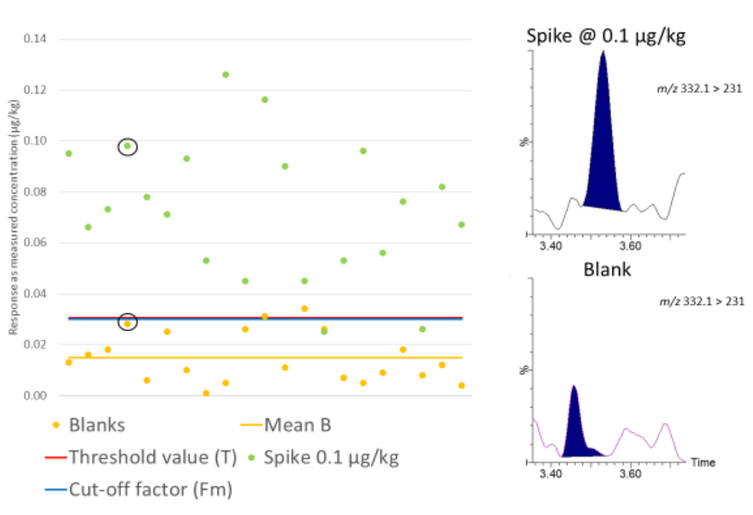

Figure 3. Ciprofloxacin, CCß assessment at 0.1 µg/kg level

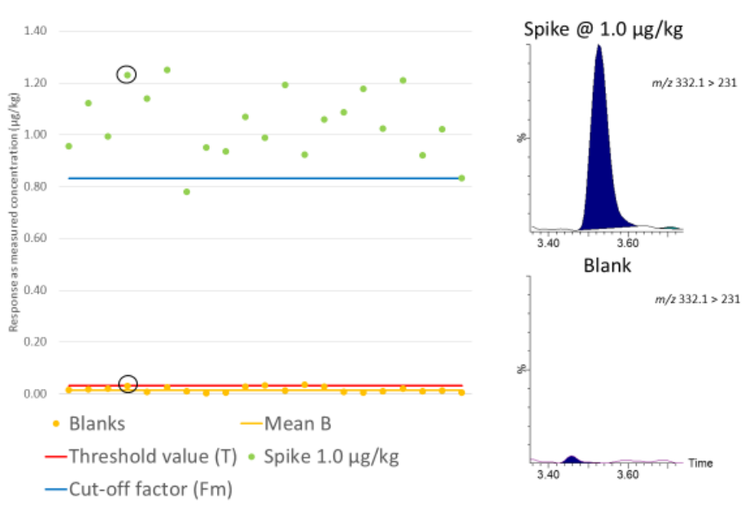

Figure 4. Ciprofloxacin, CCß assessment at 1.0 µg/kg level

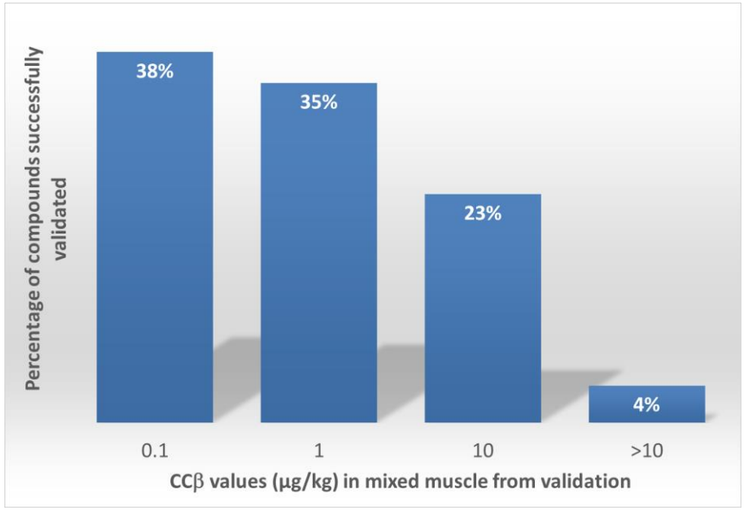

Figure 5. Overall method performance for veterinary drugs studied

CONCLUSIONS

- Single extraction and simple clean-up method with combination with a low injection volume LC-MS/MS method suitable for the determination of various classes of veterinary drugs in animal tissue to facilitate screening of samples for MRL
- Offers method performance in line with method validation criteria as set out in Commission Decision 2002/657/EC and supporting document
- The high sensitivity of the XEVO TQ-XS allows for a low injection volume of 1 µL to be used, which minimizes the amount of co-extractives introduced into the system
- Provides sufficient sensitivity to detect veterinary drugs at concentrations as low as 1 µg/kg in over 70% of the veterinary drugs studied and, in almost all cases, exceeds required MRLs

SCAN ME

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