MULTI-RESIDUE SCREENING APPROACH FOR THE DETECTION OF VETERINARY DRUGS IN ANIMAL TISSUES

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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 cleanup steps.

This poster highlights work to investigate the advantages of using a simple dSPE protocol or a pass-through SPE approach in combination with a highly sensitive LC-MS/MS system.

METHODS

Extraction

A generic extraction protocol was used to extract out bovine tissue samples. The protocol followed is listed below:

1. Weigh 5 g (± 10%) of sample into 50 mL plastic falcon tube.
↓
2. Add 15 mL of 1% oxalic acid in acetonitrile.
↓
3. Homogenise or shake the sample as appropriate.
↓
4. Add 5 (± 0.2) g sodium sulfate (Na_2SO_4) and shake for 5 minutes.
↓
5. Centrifuge (4,500 rpm, 10 min, 5 °C)

Cleanup options

After samples had been extracted the extracts were then split and taken through either cleanup option A using OASIS PRiME HLB cartridge, option B dSPE using a C18 product with the remaining extract used as the reference extract to compare the results against.

Cleanup A: dSPE using Bondapak HC18 HA 37-55µm 125A

6. Transfer 7.5 mL of supernatant to clean 50 mL polypropylene tube containing 0.5 g of C18 dSPE material:
↓
7. Shake for 15 minutes.
↓
8. Centrifuge (4,500 rpm, 5 min, 5 °C)
↓
9. Transfer 500 µL extract to an HPLC vial and 500µL of MeOH.
↓
10. Determination by LC-MS/MS

Cleanup B: OASIS PRiME HLB Plus Short Cartridge

6. Transfer 5mL of supernatant to 6mL syringe and pass the first 2mL through the PRiME device and collect the next 3mL for analysis
↓
7. Transfer 500 µL extract to an HPLC vial and 500µL of MeOH
↓
8.Determination by LC-MS/MS

Instrumentation

UPLC Conditions

LC System:	ACQUITY HPLC I-Class Plus (FL SM)
Column:	ACQUITY UPLC T3 (2.1 x 100mm)
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:

Time (min)	Flowrate (mL/min)	%A	%B	Curve
Initial	0.4	100	0	-
0.2	0.4	100	0	6
9.4	0.4	1	99	6
12.0	0.4	1	99	1
12.1	0.4	100	0	6
15.0	0.4	100	0	6

MS Conditions

MS System:	Waters Xevo TQ-XS
Ionisation:	Electrospray
Acquisition:	MRM with polarity switching
MS Parameters:	Optimised transitions and instrument parameters available on request

RESULTS

Initial experiments focused on response comparisons between standards prepared from crude extracts from bovine muscle tissue and standards prepared using extracts that had been through either cleanup A or B. A sample of the results are presented in Figure 1.

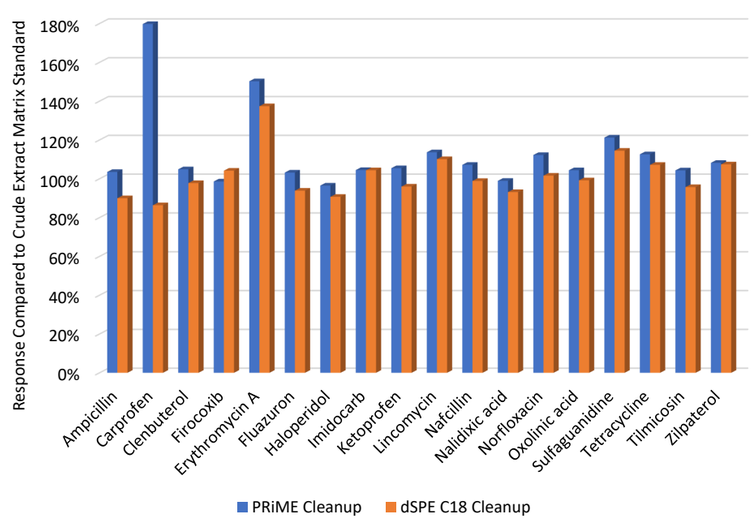

Figure 1. Response comparison of PRiME vs dSPE cleanup techniques compared to crude extract

Spiked extracts were then used to assess the effectiveness of each cleanup technique for over 150 veterinary drugs at three concentration levels (0.1, 1.0 and 10 µg/kg, n=7) when compared to matrix blanks (n=7). A sample of these results are presented in Figures 2,3 and 4.

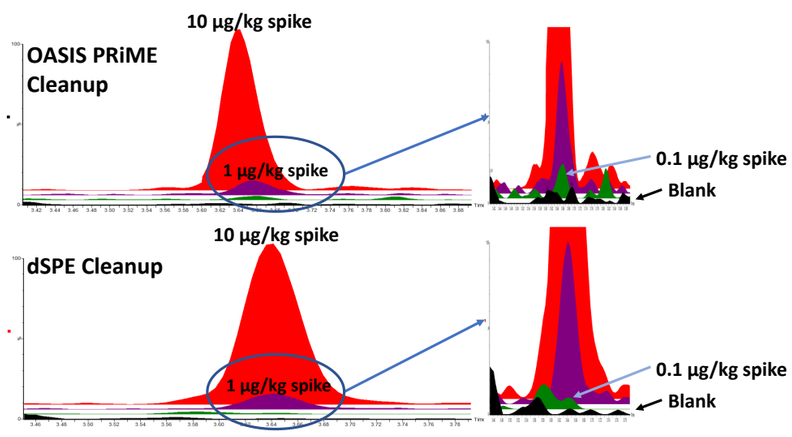

Figure 2. Lincomycin spiked bovine muscle extracts after both cleanup options normalised to the 10 ug/kg spike level

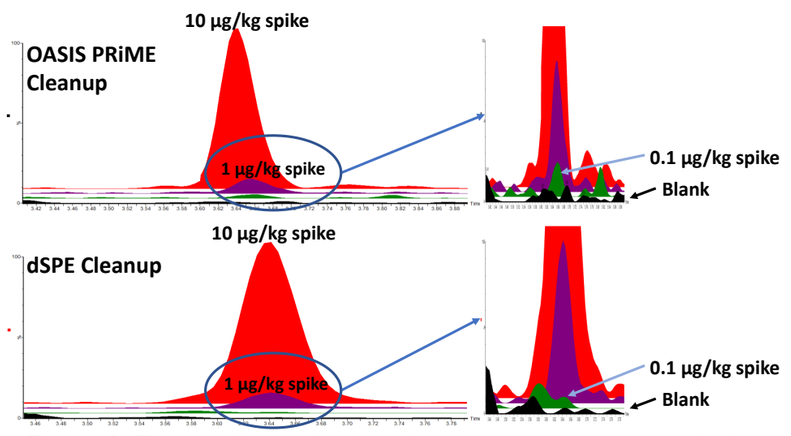

Figure 3. Tetracycline spiked bovine muscle extracts after both cleanup options normalised to the 10 ug/kg spike level

Figure 4. Lincomycin in bovine muscle LOD calculations

DISCUSSION

The key requirement for a screening method is its ability to reliably detect the analyte in question at the chosen Screening Target Concentration (STC) and to avoid false-compliant results. The STC is the concentration at which a screening test categorises the sample as potentially non-compliant and triggers a confirmatory test. For authorised analytes, the STC should preferably be set at one half of the MRL wherever possible but for prohibited and unauthorised analytes, the STC must be at or less than the Reference Point for Action (RPA). However, the further the STC is below the regulatory limit, the lower the probability of obtaining a false compliant result in samples containing the drug at the regulatory limit so evaluation of different concentrations is important. When using methods based on LC-MS/MS, sensitivity and selectivity are key so ion suppression and losses during cleanup need to be minimised and/or impact mitigated.

A comparison of the responses of the different spikes was conducted to highlight any significant differences between crude bovine muscle tissue extract and extracts after HLB PRiME and dSPE cleanup. A selection of results is presented in Figure 1. Most of the 150 veterinary drugs in the method showed that, for bovine muscle tissue, there was no significant difference in the response of the analyte after either type of cleanup when compared to the crude extract. This assessment includes the impact of any matrix effects and losses during cleanup. Figure 2 and 3 show the response for two of the compounds, in blank muscle tissue and the same spiked at 3 concentrations, using the different cleanup options. As bovine muscle represents one of the less challenging matrices, these experiments will be repeated using other sample types including bovine liver and fatty fish tissue.

Validation of screening methods requires identification of a Cut-Off factor at, or above which the sample is categorised as 'screen positive' and liable to physicochemical confirmation. The Cut-off factor is the response (or concentration) from a screening test which indicates that a sample contains an analyte at or above the STC. The Cut-off factor may be established through analysis of matrix blank samples and replicates of those same samples spiked at different concentrations to investigate which STC is valid for each compound. This initial work was restricted to 7 replicates but for full validation, when STC is half the regulatory/action limit or lower, at least 20 replicates would be required.

Threshold value T:

$T = B + 1.64 \times SDb$

B the mean response and "SDb" the standard deviation of the blank samples.

Cut-off factor Fm:

$Fm = M - 1.64 \times SD$

M the mean response and "SD" the standard deviation of the spiked samples.

Figure 4 shows the Cut-off factor (Fm) and Threshold value (T) plotted for lincomycin. For validation, one needs to identify the number of spiked samples at each concentration with results below the Cut-off factor. If more than 5% are below the Cut-off factor, the STC (0.1, 1 or 10 µg/kg) chosen for the spiking study is too low as this STC will not give a response above the cut off level and therefore be judged 'screen positive'. The data in Figure 4 shows that for lincomycin, when using either cleanup options, there is no clear distinction between the Fm and T value at the spike level 0.1 ug/kg, but validation is successful at 1 µg/kg. This shows that for lincomycin, a STC of 1 µg/kg is valid for analysis of bovine muscle.

CONCLUSION

- The initial investigation using bovine muscle tissue indicates that there is no significant difference in method performance for screening between the two cleanup techniques studied but this may change with assessment of more challenging matrices
- The high sensitivity of the XEVO TQ-XS allows for a low injection volume of 1 µL to be used, which minimises the amount of co-extractives introduced into the system
- The method in development, with the choice of two complimentary cleanup options, has demonstrated sufficient performance to allow screening to take place at concentrations lower than the current MRLs for the veterinary drugs in this study
- Further work is required to assess the performance of this method for the determination of a large number of veterinary drug compounds in different tissue matrices such as shrimp/fish, chicken and bovine liver

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