SCREENING VETERINARY DRUGS IN PRODUCTS OF ANIMAL ORIGIN

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INTRODUCTION

Veterinary drugs are widely used to treat or prevent disease in animals which can result in trace levels of drug residues in products of animal origin such as meat, fish, milk, eggs or honey. The presence of drug residues in the food chain is of concern due to their potential detrimental effect on human health. To protect consumer health and to ensure the high quality of animal products, maximum residue limits (MRLs) to set allowed maximum levels for drugs residues in animal products have been established worldwide[1-3].

As regulations became more stringent with respect to MRLs, the need to develop qualitative methods as well as confirmation and identification techniques becomes important in order to minimize false positives.

Time of flight mass spectrometry (ToF MS) screening has gained popularity due to benefits such as historical data interrogation, simplified instrumental method set-up and reduced compromise in method performance when increasing the scope of the method.

Processing and reviewing TOF screening data is often a complex workflow where positive peaks are first identified then quantified to assess the risk posed to the consumer. Frequently the transfer from qualitative to quantitative processes is performed manually, which places a significant drain on data review resource and introduces a high probability for errors.

METHODS

Sample preparation

The sample preparation method for milk has been reported previously[4]. For liver, blood, fish, and meat samples, a 5 g sample was mixed with 20 mL acetonitrile and 5 g anhydrous Na_2SO_4. After centrifugation, 0.4 mL DMSO was added to 4 mL supernatant. Acetonitrile was then evaporated and samples were reconstituted to 0.8 g with water and ultracentrifuged before injection.

UPLC conditions

LC System:	ACQUITY UPLC
Column:	ACQUITY UPLC BEH C_{18} 1.7 µm, 2.1 x 100 mm
Column temp:	40˚C
Mobile phase A:	0.1% formic acid (aqueous)
Mobile phase B:	Acetonitrile + 0.1% formic acid
Gradient:	0.00 min 95% A
	0.25 min 95% A
	6.00 min 5% A
	7.00 min 5% A
	7.20 min 95% A
	9.00 min 95% A
Flow rate:	0.40 mL/min
Injection volume:	20 µL full loop

MS conditions

MS system:	XEVO QTof MS
Acquisition mode:	MS^E
Ionization mode:	ESI positive
Capillary voltage:	2.4 kV
Cone voltage:	30 V
MS collision energy:	6 V
MS^E energy ramp:	25 to 35 V
Source temp:	120 °C
Desolvation temp:	400 °C
Desolvation gas flow:	800 L/hr
Cone gas flow:	20 L/hr
Acquisition range:	*m/z* 50 to 1000 for 0.1 s

Xevo QTof MS setup (mass calibration using sodium formate and lock mass checks) was automated using IntelliStart™ Software. The data were acquired using Waters MassLynx™ Software, v.4.1 and processed using POSI±IVE Software. In MS^E acquisition mode, data were collected in two channels all of the time; low collision energy (CE) for molecular ion information; and high CE for product ions. The TOF screening workflow is illustrated in Figure 1.

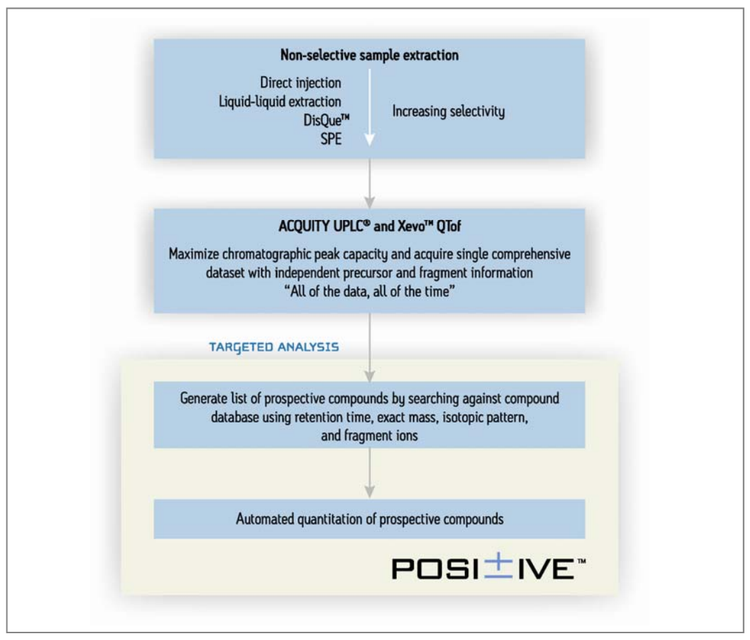

Figure 1. Targeted TOF screening workflow.

RESULTS AND DISCUSSION

The selectivity in complex matrices comes from the high chromatographic resolution of ACQUITY UPLC and the high mass spectral resolution of Xevo QTof MS.

High resolution is very useful for achieving selectivity and enhancing confidence in results. However, in some cases, extra information such as product ions would be of significant benefit to unambiguously identify compounds of interest. The added confidence is illustrated in Figure 2 for xylazine and morantel, which share the same exact mass and elemental composition and have similar retention times. They share one product ion but can have their identities assigned with unique product ions. Very high mass spectral resolution would be unable to separate these compounds so structural information must be used to unambiguously assign an identity to the residue of interest.

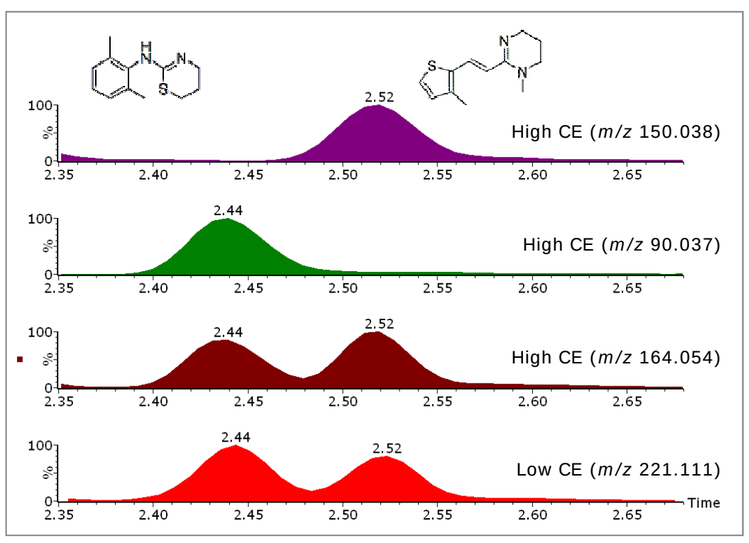

Figure 2. Assigning an unambiguous identity to residues that share the same exact mass, xylazine and morantel with MS^E.

Product ion information can be obtained by performing MS^E:

MS^E is a patented data-independent acquisition technique that provides a simple, unbiased, parallel route to delivering exact mass molecular and product ions from every component, without the need for multiple injections.

Figure 3 shows the low CE and high CE mass chromatograms from MS^E mode for sulfadoxine in a cow blood extract showing that product ions can be detected at relevant levels in matrix.

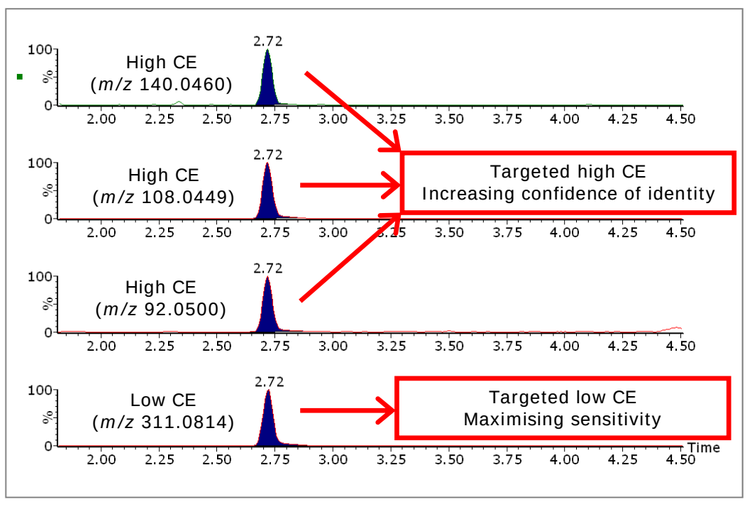

Figure 3. Mass chromatograms of sulfadoxine in low CE and high CE from MS^E mode in cow blood.

The samples were analyzed so that a total cycle time of 9 min (including equilibration time) was achieved. This would allow a realistic sample throughput of more than 100 samples per day. However, this sample throughput, together with the 150 residues per sample traditionally yields significant problems in data handling and processing.

POSI±IVE™ Software has been developed to specifically reduce the data processing time for review of TOF MS screening data, using mass accuracy, retention time and MS^E product ions, by ensuring that only positive or tentative detections are quantified automatically. A target compound list containing the compound name, formula, and retention time is all that is needed. The list can be of unlimited length.

All positive and tentative detections are then automatically quantified and listed within a TargetLynx browser report with a measure of their isotope fit (iFit™). Figure 4 shows the POSI±IVE™ Xevo QTof MS screening results for a cow blood extract where more than 150 veterinary drugs have been qualitatively and quantitatively reduced to just three: hydrocortisone, sulfadoxine, and trimethoprim. The displayed sample shows the positive identification and quantification of trimethoprim in a blood extract.

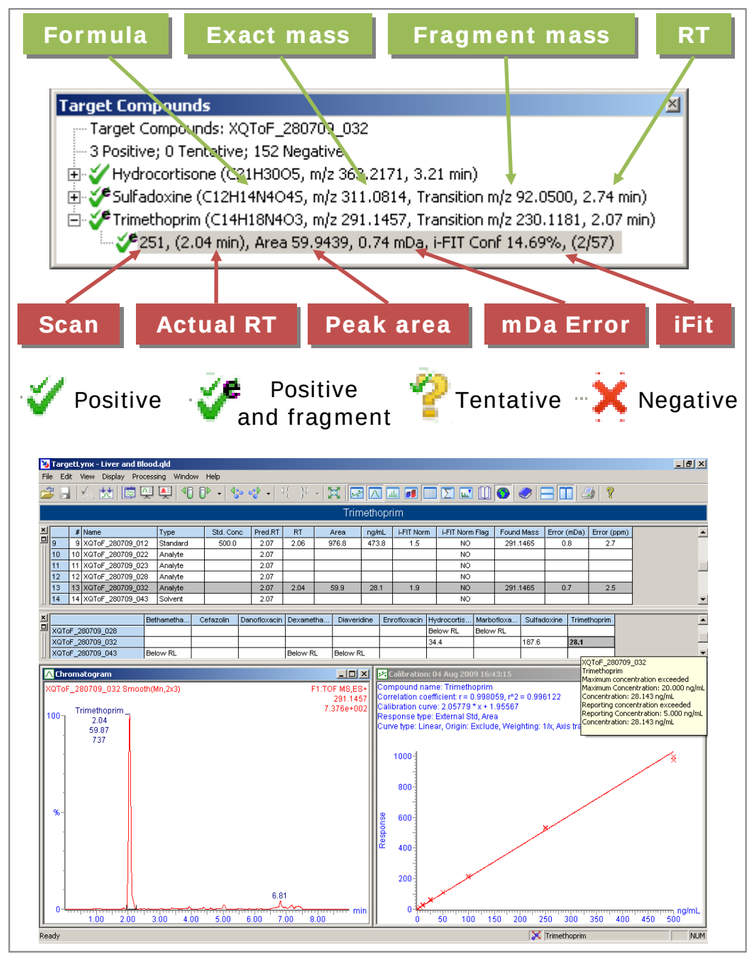

Figure 4. POSI±IVE™ Xevo QTof MS screening results for trimethoprim in cow blood. .

CONCLUSION

- **The ACQUITY UPLC and Xevo QTof MS solution facilitated the screening of more than 150 veterinary drug residues at the appropriate MRLs in products of animal origin.**
- **MS^E acquisition adds extra confidence when assigning identity to the residue of interest and overcomes the limitations of conventional data-dependent approaches.**
- **The information-rich nature of TOF MS data increases demands upon data processing software, therefore reducing manual processing and automating repetitive tasks are necessary for improving the quality of results and accessibility to TOF MS.**
- **POSI±IVE™ significantly reduces the bottleneck of data processing for reviewing TOF MS screening data, by ensuring that only the positive and tentative detections are quantified automatically. The automated nature of processing also reduces the possibility of errors by removing manual transcription steps from the workflow.**

References

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2. http://www.foodsafety.gov/%7Efsg/animal.html
3. http://www.mhlw.go.jp/english/topics/foodsafety/positivelist/index.html
4. D Ortelli, E Cognard, P Jan, and P Edder. J Chrom B. 877 (2009) 2363-2374.

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