THE UTILITY OF ION MOBILITY IN AN ACCURATE MASS SCREENING WORKFLOW FOR THE DETECTION OF VETERINARY DRUG RESIDUES IN COMPLEX MATRICES

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INTRODUCTION

Screening is intended to offer a qualitative assessment of the presence of a large number of compounds in many samples; to provide a rapid, cost-effective analysis that aims to generate no false negative results with a manageable frequency of false positives. Although the benefits of full spectral MS acquisition and the specificity of high mass resolution with accurate mass measurements for non-targeted screening of residues are well characterised, fail-safe detection of trace concentrations of residues in complex samples in a non-targeted manner, often remains a challenge. Addition of ion mobility (IM) to the mass spectrometric (MS) parameters has been used for pesticide residue screening, reducing false detection rate, providing spectral cleanup, avoiding reliance on LC retention times for identification and improving confidence in identification by using Collision Cross Section (CCS) values **[1]** CCS is an important, robust distinguishing characteristic of an ion in the gas phase, being related to its chemical structure and three-dimensional conformation which gives information about the ionic shape of a molecule in the gas phase.
In this poster the evaluation of the performance of UPLC coupled to IM-MS for the comprehensive screening veterinary drug residues is presented. Matrix matched standards and extracts of bovine liver, urine and porcine muscle, some containing residues of undisclosed veterinary drug residues, generated for an inter-laboratory validation study, [2] were analysed.

EXPERIMENTAL

Sample Analysis
- Screening method: Banned and permitted substances spiked from a list of 100 analytes; spiking levels ranged from 0.5 to 150 $\mu g.kg^{-1}$
- Test samples:
  - Reference standard mix (containing 100 compounds);
  - 3 matrices of porcine muscle, bovine liver and bovine urine;
  - 3 samples provided per matrix

LC separation [2]
- Column: ACQUITY HSS T3 C_{18} 2.1 mm x 100 mm, 1.8 μm
- Gradient: 21 minute consisting of 0.016 % formic acid and 2 mM ammonium formate in A: Water and B: Methanol

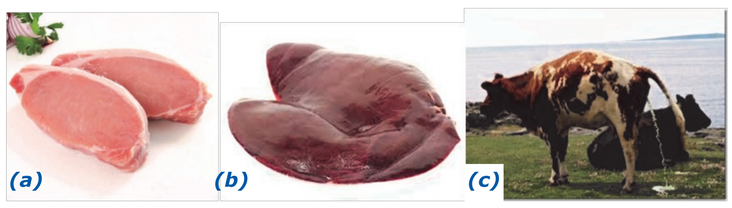

***Figure 1** Three matrices screened for 100 veterinary residue drug compounds (a) Porcine muscle; (b) Bovine liver; (c) Bovine urine by ACQUITY UPLC coupled with Ion Mobility enabled Time of Flight Mass Spectrometers.*

Instrument Parameters
- MS: Synapt G2 S, G2 *Si* and Vion IMS QToF
- Acquisition mode: HDMS^E ESI+ in Resolution mode
- Mass range: 50 to 1200 Da
- Scan speed: 10 spectra.sec^{-1}

UPLC- HDMS^E data was acquired for solvent standard mixtures and was used to generate mobility separated MS^E spectra for $[M+H]^+$ species.
The precursor ion, two fragment ions, mobility drift time and collision cross section (CCS) area were determined for the 100 compounds and entered into the UNIFI scientific library.

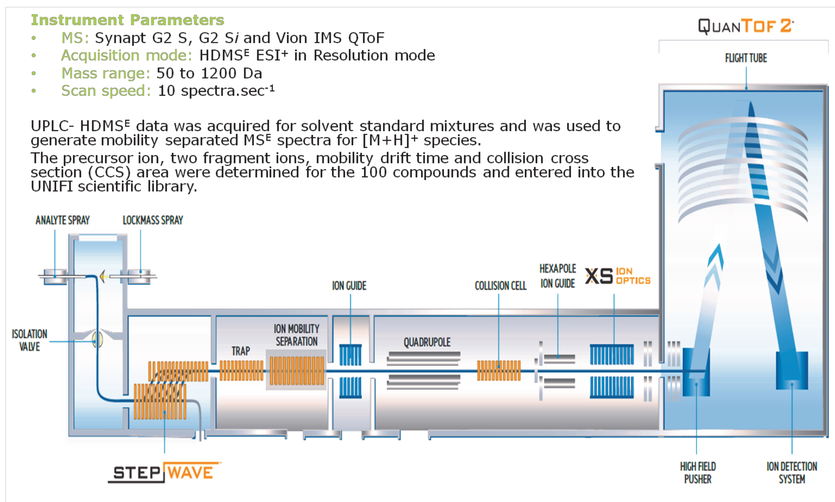

***Figure 2** Configuration of Vion IMS Q-ToF with summary of instrument parameters utilised in the screening method.*

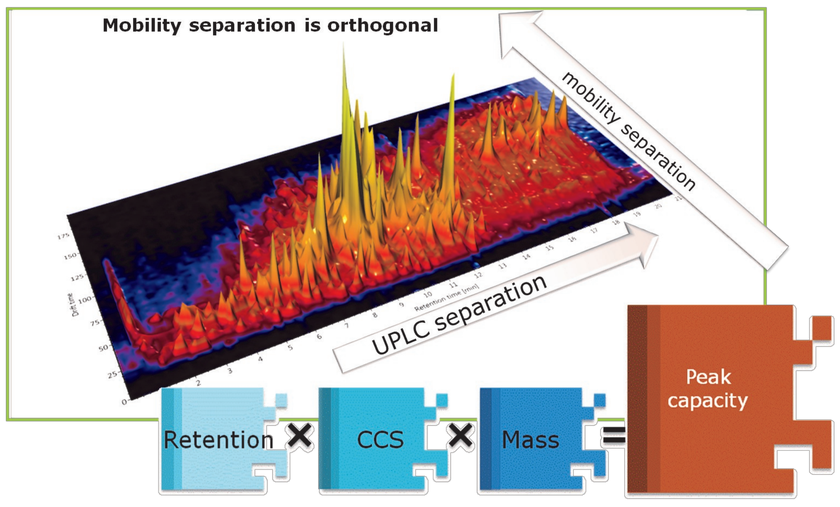

***Figure 3** Increased peak capacity afforded by IMS enabled Q-ToF technology; standardised retention time (LC), accurate mass (ToF), drift time and collision cross section (IMS)*

RESULTS

1. SCREENING METHOD

***Table 1** Summary of results achieved from UPLC- Synapt G2 S screening of 3 matrices (3 samples per matrix). A false positive rate of 3 % equates to one compound, which is in agreement with ≤ 5 % false positive rate typically permitted for fit for purpose screening methods.*

Sample extract	Number of compounds included	False positive rate (%)
Liver A	43	0
Liver B	44	0
Liver C	44	0
Muscle A	48	3
Muscle B	48	0
Muscle C	48	3
Urine A	42	0
Urine B	41	3
Urine C	42	0
Overall False Positive total		**1 %**

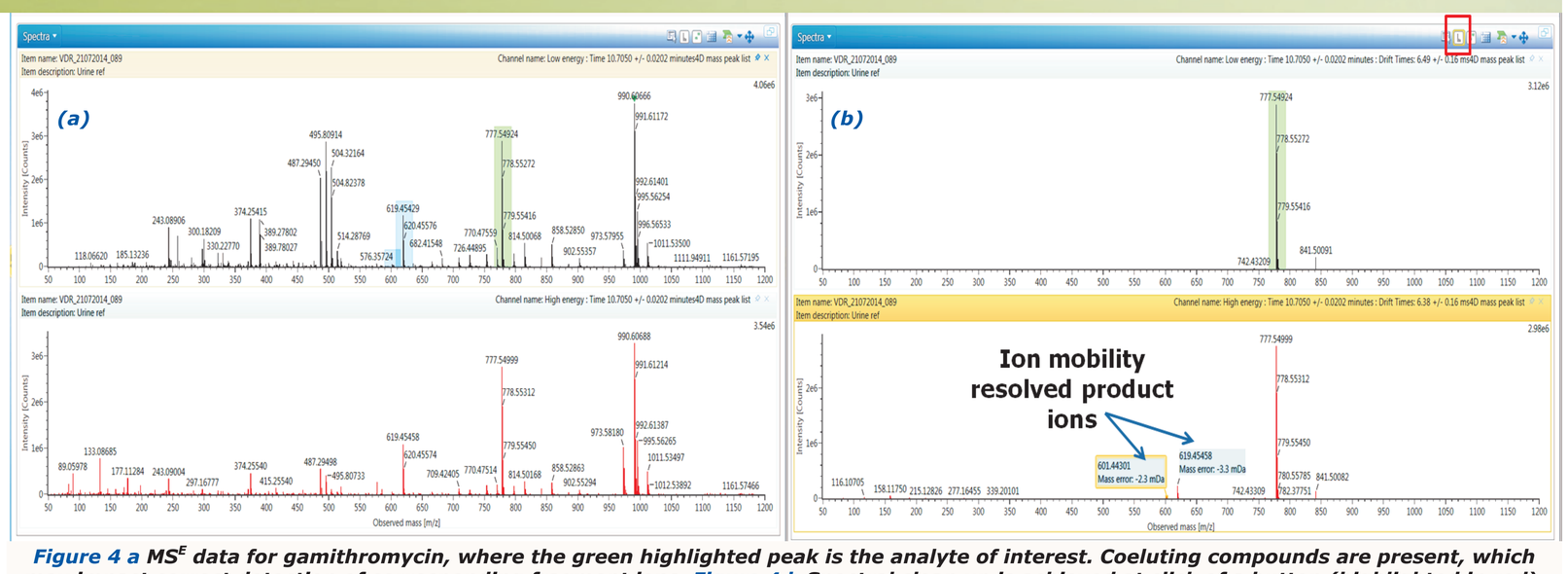

***Figure 4 a** MS^E data for gamithromycin, where the green highlighted peak is the analyte of interest. Coeluting compounds are present, which may impact correct detection of corresponding fragment ions. **Figure 4 b** Spectral clean up is achieved at click of a button (highlighted in red). By aligning ions on retention and drift times, increased specificity is achieved for gamithromycin precursor and fragment ions.*

2. COLLISION CROSS SECTION

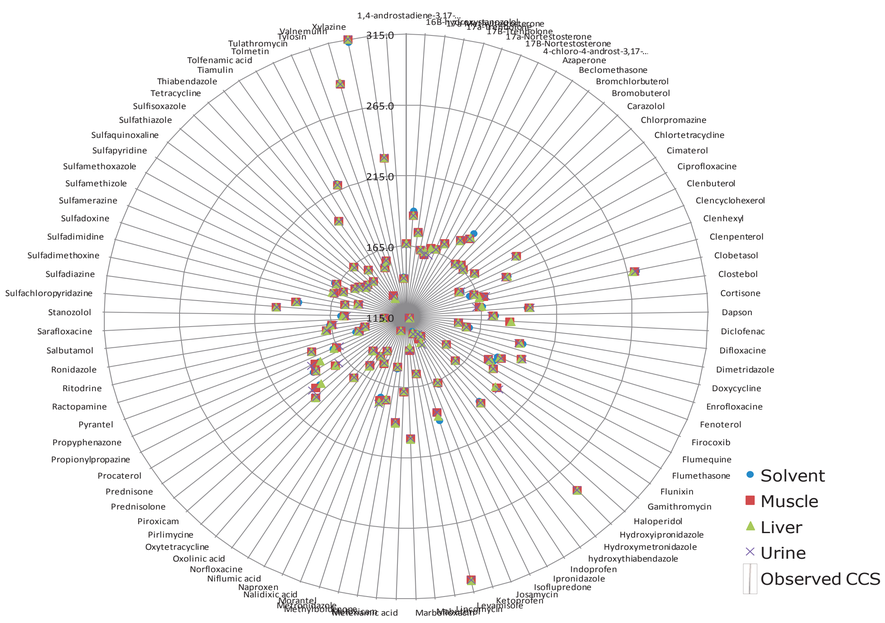

***Figure 5** Overlay of CCS achieved for vet drug compounds in solvent and the 3 matrices, where all CCS were within 2 % deviation, irrespective of matrix.*

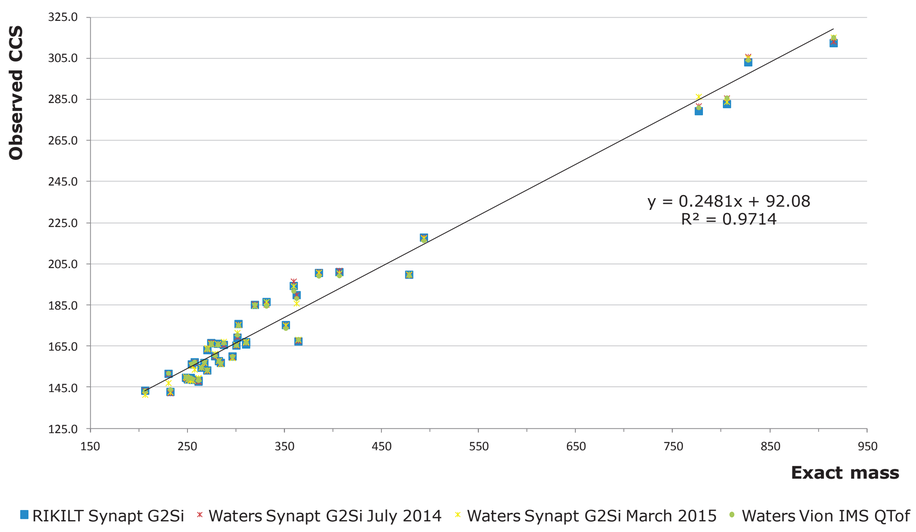

***Figure 6** Overall precision of CCS for a selection of vet drug compounds across 2 sites and 3 instruments including 2 different IMS platforms*

***Table 2** CCS values obtained using the travelling wave ion mobility cell (Synapt G2 S) and linear drift tube showed excellent agreement. Ion mobility mass spectrometry experiments demonstrate increased separation in more polarisable gases in the order CO_2>N_2>He [3]*

Compound	Observed CCS (Å²)		% Difference
	TWIM	*Linear drift tube*	
Ciprofloxacin			
Protomer N20	187.53	188.80	0.7
Protomer O16	173.98	174.98	0.6
Norfloxacin			
Protomer N4	185.96	187.36	0.8
Protomer O18	171.36	172.25	0.5
Enrofloxacin			
Protomer N1	195.06	198.27	1.6
Protomer O20	183.47	186.58	1.6

3. DECTECTION OF PROTOMERS

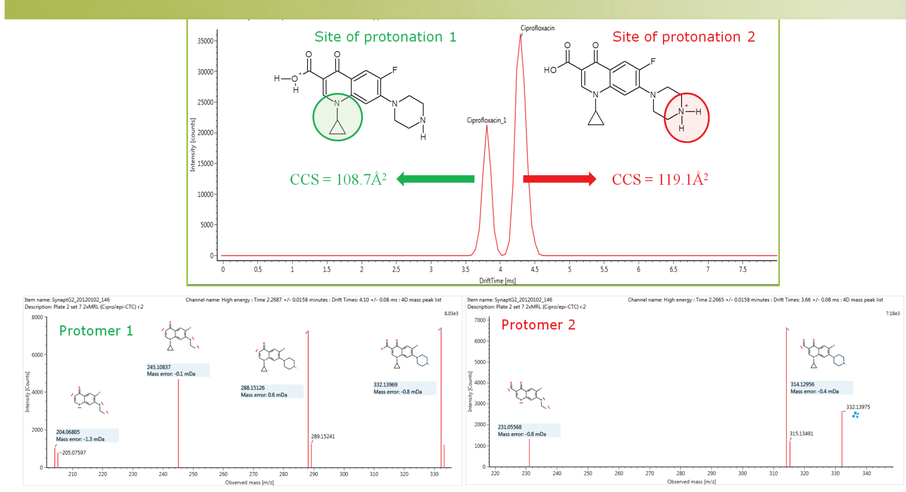

***Figure 7** Ion mobility trace for ciprofloxacin showing two mobility separated peaks by > 10 Å². The location of the charge is seen to impact upon the molecular conformation, drift time and fragmentation. Using the fragmentation of the mobility separated species the possible sites of intra-molecular protonation for the 2 predominant species have been determined.*

CONCLUSIONS

- **A fit for purpose screening method was developed on ACQUITY UPLC IClass coupled with Synapt G2 S, where an overall false positive detection rate of 1 % was achieved (n=9).**
- **The measured CCS values for target compounds present in solvent standards, matrix matched standards and validation samples were shown to agree (≤2% error).**
- **Reproducibility of CCS values was demonstrated across 2 laboratory sites (RIKILT and Waters) using 3 instruments and 2 IMS enabled platforms (Synapt and Vion)**
- **Ion mobility drift times are independent of matrix and can be utilised as an additional parameter to reduce false assignments and increase the confidence in non-targeted screening.**
- **Improve analytical selectivity of this otherwise non-selective acquisition mode (MS^E) using ion mobility clean-up prior to fragmentation thus monitor "drift time resolved product ions" originating from specific precursor ions**
- **The application of ion mobility to reduce the false assignment rate, reduce spectral complexity and overcome matrix related retention time shifts in multi-residue screening is demonstrated as routine.**

REFERENCES

1. Goscinny, *et al.*, 2015, J Chrom A, DOI: 10.1016/j.chroma.2015.05.057
2. Performance criteria for multi-veterinary drug-residue analytical methods; Joint DEFRA VMD and industry sponsored project (project number DEFRA R3; RIKILT 1237312301)
3. Lapthorn et al The importance of protomers in quantitation; ion mobility mass spectrometry of fluoroquinolone antibiotics, 2015

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