DETERMINATION OF THE RELATIVE CCS OF PESTICIDE PROTOMERS AND CONFORMERS USING LINEAR AND MULTI-PASS CYCLIC TWIM ION MOBILITY SPECTROMETRY

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OVERVIEW

- Linear TWIM and cIM cross platform correlation of pesticide protomer, sodimer and potassimer charge-site isomer $^{TW}CCSN_2$ values.
- Investigation into the utility and robustness of an in-house exploratory strategy to determine $^{TW}CCSN_2$ values and their relative differences from multi-pass cIM data.
- Characterisation of conformer formation and impact upon product ion ratios.
- Generation of pesticide conformer estimated $^{TW}CCSN_2$ values with increasing cyclic ion mobility resolution.

INTRODUCTION

Ion mobility-based measurement/separation of compounds is based on size, shape, charge differentiation as well as on its dipole moment in cases where polarizable buffer gas is used. It provides an added dimension of separation, producing increased peak capacity and $^{TW}CCSN_2$ as an additional analyte identification metric.

Linear travelling wave ion mobility (TWIM) mass spectrometry (MS) coupled with liquid chromatography (LC), has been used to screen food extracts for pesticide residues. The ability of TWIM-MS to distinguish protomeric charged isomer species (ions different only by their protonation site), and sodimers/potassimers (sodium and potassium charge bearing adducts) has been evaluated.[1] Additionally TWIM separation of the enantiomer charge-site isomers of indoxacarb and metaflumizone (*E)/(Z)*-isomers has been investigated.

Using linear TWIM broad arrival time distributions (ATDs) were observed for some analytes. Using a high-resolution cyclic ion mobility (cIM) device (see Figure 1), the multi-pass relative $^{TW}CCSN_2$ values of unresolved linear TWIM charged isomer/conformer species were characterised.[1] cIM provides a longer mobility separation path length and, consequently, higher ion mobility resolution than the standard linear TWIM cell. For the pesticides shown in Figure 2, charged isomer $^{TW}CCSN_2$ values have been compared cross-platform. Highly specific pesticide charge-site isomer and conformer $^{TW}CCSN_2$ physicochemical descriptor fingerprints have been characterised, as well as the impact upon product ion formation.

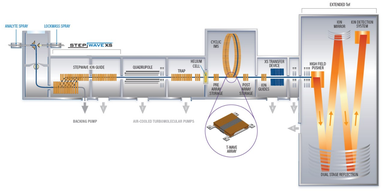

Figure 1. *Schematic of Cyclic IMS, with the cIM device embedded in a Q-cIM-ToF geometry.*

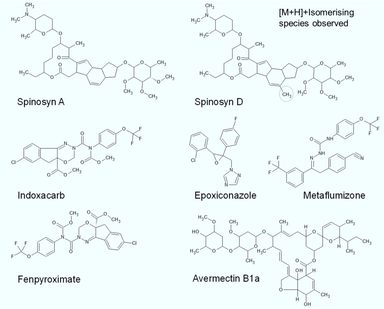

Figure 2. *Pesticides for which linear TWIM (standard resolution) and cIM (high-resolution) separation of charged isomers has been performed.*

METHODS

Positive electrospray ionisation (ESI) with linear TWIM LC-IM-MS data acquisition was performed using a quadrupole-IM-time-of-flight (ToF) mass spectrometer and a cyclic ion mobility device. Also, positive ESI direct infusion analysis with a cyclic ion mobility device. Fortified rice, green tea, black tea, orange, coriander and leek extracts were analysed using LC and reverse phase separation performed using a C_{18} (1.7 µm, 2.1x100 mm) analytical column. The chromatographic conditions were comprised of a 17-minute (0.1% *v/v* formic acid in H_2O) and (0.1% *v/v* formic acid in acetonitrile) gradient at 0.45 mL/min. Sample injection volumes of 5µL were used.

RESULTS AND DISCUSSION

Pesticide charged-site isomers (protomers, sodimers, and potassimers) have been characterised using linear TWIM. It was possible to separate the protomers, determine their respective collision cross section and individual protomer product ion spectra. Fortified extracts of rice, green tea, black tea, orange, coriander and leek were screened against the pesticide charged-site isomer reference data base to detect the spinosyn sodimer and potassimer species, where Δ $^{TW}CCSN_2$ <0.7% (RMS= 0.35 %) were obtained. In the case of metaflumizone (*E)-* and *(Z)*-isomers, two equivalent isomerising species, $^{TW}CCSN_2$ value pairs were determined and the enantiomers of indoxacarb were resolved using chiral LC coupled with IM, where two analogous $^{TW}CCSN_2$ value pairs were observed.[1]

The linear TWIM reference $^{TW}CCSN_2$ and cIM $^{TW}CCSN_2$ values determined for pesticides, forming charged-site isomers showed good correlation (Δ $^{TW}CCSN_2$ <1.69 % (RMS= 0.85 %), (R^2=0.99)), illustrating that charge-site isomers may provide an additional and robust identification descriptor to enhance analytical specificity (see Figure 3).

In the case of spinosyn A, spinosyn D, avermectin B1a and epoxiconazole, multiple ion mobility-separated conformer species were observed using cIM. CCS calibration was performed as described by Bush *et al.*[2] using a Major Mix IMS/ToF Calibration Kit (Waters Corp, Wilmslow UK). The exploratory multi-pass CCS calibration strategy has been described elsewhere.[1]

Our investigations were able to estimate the relative CCS differences between species separated in multi-pass experiments (2-5 passes, R ~92-145 Ω/ΔΩ).[1] The strategy has been shown to be robust, utilising previously characterised charged-site isomer species of the fluoroquinolone class of antibiotics determined using linear TWIM and cIM.[3]

CCS values obtained using cIM are compared to linear TWIM data. For ≥3 passes, Δ CCS for species one of avermectin B1a (<0.8%), spinosyn A (0.5%), spinosyn D (0.2%), and epoxiconazole (-1.9%) were obtained. Using cIM and multi-pass estimated CCS measurements, in the case of spinosyn A and D, the relative difference between the least and the most mobile species is ~17$Å^2$ (see Figure 4 and 5). For the pesticide spinosad (spinosyn A+D), a total of 16 $^{TW}CCSN_2$ values were determined of which eight protonated species have been characterised. For avermectin B1a the relative difference between the least and the most mobile species is ~11 $Å^2$ (see Figure 6 and 7) and for epoxiconazole ~8 $Å^2$. An example of the impact of conformer formation upon product ion ratios is shown for avermectin B1a and can be seen in Figure 8.

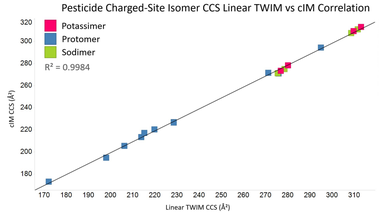

Figure 3. *Pesticide charge-site isomer reference $^{TW}CCSN_2$ values observed using Synapt G2-Si (linear TWIM separator) and Cyclic IMS instrument.*

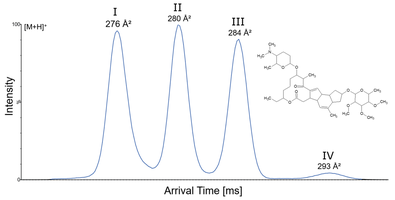

Figure 4. *[M+H]$^+$ spinosyn D species separation, (5 passes/ R ~145 Ω/ΔΩ) and estimated relative CSS.*

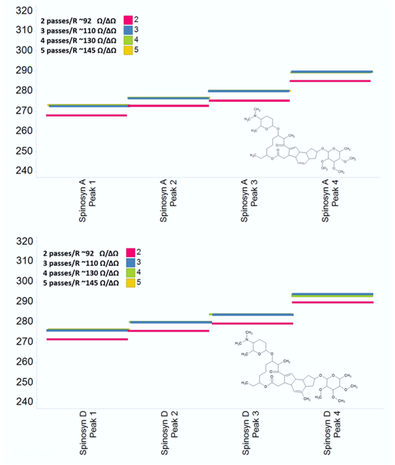

Figure 5. *Plot of estimated CCS values and relative differences for cIM resolved [M+H]$^+$ spinosyn A and spinosyn D conformers.*

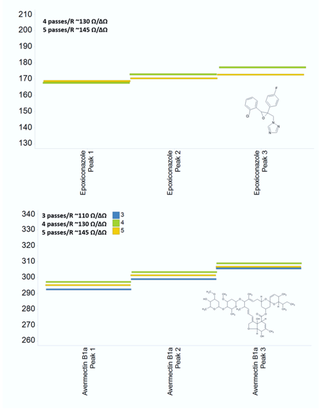

Figure 6. *Plot of estimated CCS values and relative differences for cIM resolved [M+H]$^+$ avermectin B1a and epoxiconazole conformers.*

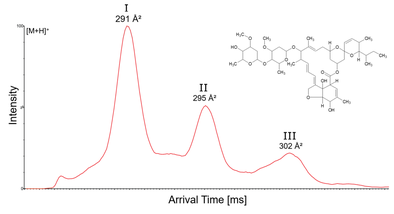

Figure 7. *[M+H]$^+$ avermectin B1a conformer species cIM separation (5 passes/R ~145 Ω/ΔΩ) and estimated relative $^{TW}CCSN_2$ values.*

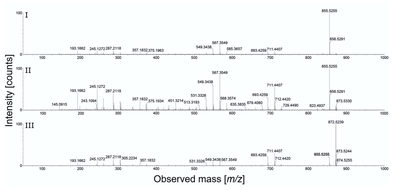

Figure 8. *Avermectin B1a cIM resolved conformer species product ion spectra.*

CONCLUSION

- Charged-site isomers CCS values provide an additional robust identification descriptor and have been integrated into a routine screening workflow to identify pesticide residues in complex matrices.
- Using an exploratory multi-pass calibration strategy, relative estimated CCS values have been determined for pesticide conformers and illustrate the potential to enhance analytical specificity using high resolution ion mobility conformational CCS fingerprints.
- Our investigations using high resolution cIM show that charged-site isomer formation and conformer formation can produce variation in product ion intensities.
- Initial investigations to determine high resolution cIM pesticide conformer $^{TW}CCSN_2$ values facilitated a robust evaluation of the in-house multi-pass calibration strategy utilised.

References

1. M McCullagh, S Goscinny, M Palmer, J Ujma. Investigations into pesticide charge site isomers using conventional IM and cIM systems. Talanta, 2021, 234, 122604.
2. M F Bush, IDG. Campuzano, CV Robinson. Ion mobility mass spectrometry of peptide ions: effects of drift gas and calibration strategies, Anal. Chem. 2012, 84, 7124–7130.
3. M McCullagh, J Fox, M Palmer, A Muck and D Higton. Further Investigations into Charged Isomer Species of the Fluoroquinolone Class of Antibiotics Using Linear TWIM and Cyclic Ion Mobility. ASMS Poster Number:FP 418. 69th ASMS Conference on Mass Spectrometry and Allied Topics. October 31 - November 4, 2021. Pennsylvania Convention Center, Philadelphia, PA.

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