FURTHER INVESTIGATIONS INTO CHARGED ISOMER SPECIES OF THE FLUOROQUINOLONE CLASS OF ANTIBIOTICS USING LINEAR TWIM AND CYCLIC ION MOBILITY

Michael McCullagh, Jonathan Fox, Martin Palmer, Alexander Muck and David Higton.
Waters Corporation, Stamford Avenue, Altrincham Road, Wilmslow, SK9 4AX. UK.

OVERVIEW

- Linear TWIM and cIM cross platform correlation of fluoroquinolone protomer $^{TW}CCSN_2$ values.
- Characterisation of protomer formation for eighteen fluoroquinolones.
- Investigation into the utility and robustness of an in-house exploratory strategy to determine estimated $^{TW}CCSN_2$ values and their relative differences for multi-pass cIM data.
- Generation of fluoroquinolone protomer estimated $^{TW}CCSN_2$ values with increasing cyclic ion mobility resolution.

INTRODUCTION

Fluoroquinolones (FLQs) are a class of antimicrobial agents which have been administered to livestock for different purposes, (a) prevention and control of infections and (b) growth promotion. Concerns regarding the spread of resistant microorganisms in the human population, resulted in a USA FDA ban on enrofloxacin/ciprofloxacin use in livestock production in 2005. Since 2006, use of antibiotic growth promoting agents in animal husbandry has been forbidden in the EU. The FLQs are a chemically diverse zwitterionic species possessing a basic 4-quinolone ring structure; functional group modifications around the quinolone ring (benzopyridone nucleus) improve the antimicrobial potency. Kaufmann *et al.*[1] reported protomers (charge-site isomers) separation of FLQs for the first time using ion mobility (IM).

Ion mobility spectrometry (IMS) is a fast, gas phase separation technique which can resolve charge-site isomers based on differences in their collisional cross sections (CCSs). We previously used the IM device embedded in a LC -IM-MS geometry to generate a FLQ charge-site isomer CCS database, which has subsequently been expanded. Investigations into the linear travelling wave ion mobility (TWIM) protomer CCS values provided a highly specific physicochemical descriptor fingerprint to identify fluoroquinolone residues.[2]

The use of a cyclic ion mobility (cIM) device (see Figure 1), which affords a longer mobility separation path length, provides increased resolution to fully resolve the FLQ charge-site isomer species. Using high resolution cIM we determined the CCS values of multi-pass resolved isomerising species at increasing IM resolution for fluoroquinolones which had previously been characterised using linear TWIM.[2]

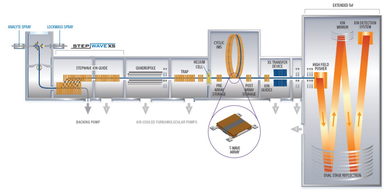

Figure 1. *Schematic of the cIM device embedded in a Q-cIM-ToF geometry.*

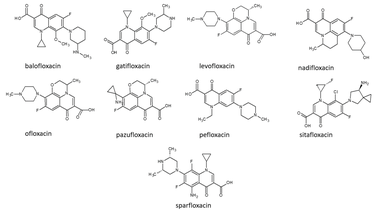

Figure 2. *FLQs investigated to characterise charged isomer formation.*

METHODS

FLQ standards were analysed using liquid chromatography (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 (0.1% *v/v* formic acid in H_2O) and (0.1% v/v formic acid in acetonitrile) gradient. Sample injection volumes of 5µL were used. Positive ion electrospray (ESI) with precursor/product ion data acquisition was performed using a quadrupole-IM-time-of-flight (ToF) mass spectrometer (Resolution (R)~40 Ω/ΔΩ) and a cyclic ion mobility device (R~65 to ~145 Ω/ΔΩ). Multi-pass ESI direct infusion analysis was also performed with cIM.

RESULTS AND DISCUSSION

LC linear TWIM data was acquired for a mixture of nine fluoroquinolone solvent standards (ciprofloxacin, enrofloxacin, danofloxacin, lomefloxacin, sarafloxacin, marbofloxacin, norfloxacin, enoxacin and difloxacin).[2] Ion mobility separated, charged-site isomer precursor/product ion spectra were generated. Additionally for the single component $[M+H]^+$ species, $^{TW}CCSN_2$ values were determined. Each charged-site isomer species produced characteristic product ion spectra.

The presence of the multiple sites of protonation were observed and 18 $^{TW}CCSN_2$ values obtained. For a second set of FLQS (see Figure 2), 7/9 FLQS formed charge-site isomer species and $^{TW}CCSN_2$ values determined (see Figure 3). In the case of nadifloxacin and sitafloxacin a single $[M+H]^+$ species was observed. For nadifloxacin and sitafloxacin, a neutral loss of *m/z* 18 (H_2O) was observed in the respective product ion spectra (typically characteristic of FLQ charged-site isomer 1 (more mobile species)), however a neutral loss of *m/z* 44 (typically characteristic of FLQ charged-site isomer 2 (lesser mobile species)) was not observed and would suggest that the observed ATDs are not comprised of two unresolved charge-site isomers.

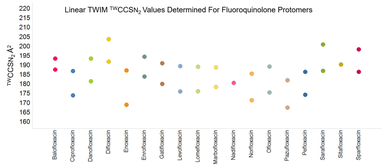

Figure 3. Linear TWIM $^{TW}CCSN_2$ values determined for fluoroquinolone protomers (R ~40 Ω/ΔΩ).

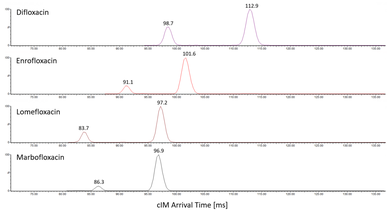

Figure 4. cIM separation of fluoroquinolone protomers pairs (5 passes/R ~145 Ω/ΔΩ)).

Using Linear TWIM (R~40 Ω/ΔΩ), not all the FLQ protomer pairs were fully resolved. However, baseline resolution was obtained using cIM (R~65 Ω/ΔΩ) and the $^{TW}CCSN_2$ values determined. FLQ charged isomer resolution at R~145 Ω/ΔΩ, is illustrated in Figure 4. Comparing the linear TWIM reference $^{TW}CCSN_2$ values and cIM (R~65 Ω/ΔΩ) $^{TW}CCSN_2$ values, Δ CCS <1.1 % (RMS= 0.54 %) have been obtained.

Further investigations were performed into the utility and robustness of an in-house exploratory strategy to determine estimated $^{TW}CCSN_2$ values and the relative differences of multi-pass cIM separated FLQ species. CCS calibration was performed as described by Bush *et al.*[3] using a Major Mix IMS/ToF Calibration Kit (Waters Corp, Wilmslow UK). The exploratory multi-pass CCS calibration strategy has been described elsewhere.[4]

Positive ESI direct infusion analysis with cIM of individual FLQ standards was performed for 1 to 5 passes (R~65 to ~145) of the cyclic IMS device. Compared to the linear TWIM reference $^{TW}CCSN_2$ values, using a linear calibration fit, for 85 $^{TW}CCSN_2$ estimates, Δ CCS <2.1 % (RMS 0.97%) have been obtained. The correlation and reproducibility of linear TWIM and cIM CCS values determined at 1-5 passes (R ~ 65-145 Ω/ΔΩ) around the cIM device are presented in Figure 5 and 6.

During our investigations of FLQs using cIM, for danofloxacin the observed arrival time distribution (ATD) comprised three ion mobility separated species. The in-house exploratory strategy,[3] has been applied to determine estimated CCS values and relative differences of the multi-pass cIM separated species. For the first time the relative $^{TW}CCSN_2$ values have been determined for all three charged isomer species of danofloxacin (see Figure 7).

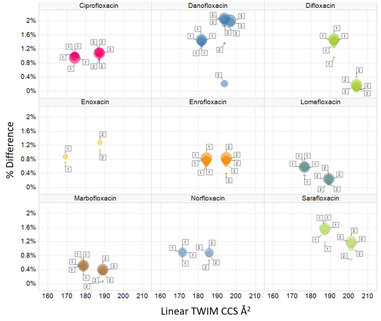

Figure 5. Comparison of linear TWIM measured CCS and estimated multi-pass cIM CCS for pairs of fluoroquinolone protomers using increasing cIM resolution.

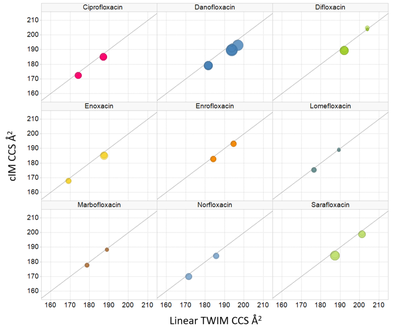

Figure 6. Plot of linear TWIM fluoroquinolone protomers pairs CCS (R ~40 Ω/ΔΩ) versus cIM estimated CCS observed with increasing IM resolution (R ~65 to ~145 Ω/ΔΩ).

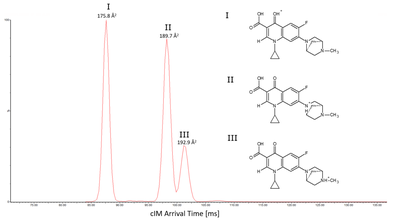

Figure 7. Separation of three charge-site isomers of danofloxacin (5 passes/R ~145 Ω/ΔΩ)) and relative estimated multi-pass CCS values.

CONCLUSION

- Ion mobility mass spectrometry provides additional peak capacity and new insights into analyte molecular characteristics.
- Detection and elucidation of multiple sites of protonation within a single compound have been characterised.
- The FLQ charged isomer $^{TW}CCSN_2$ library has been extended to eighteen characterised analytes.
- For a FLQ charged isomer cross platform comparison Δ CCS RMS= 0.54 % was obtained and using the exploratory multi-pass strategy Δ CCS RMS 0.97% determined.
- Previously characterised FLQ protomer pair $^{TW}CCSN_2$ reference values facilitated a robust evaluation of an in-house strategy to generate cIM multi-pass relative $^{TW}CCSN_2$ values.
- Three charged isomer CCS values have been determined for danofloxacin.

Acknowledgements

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