Identification of Amino-Sulfonamide Isomers using Chip-Based FAIMS-MS Approaches

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Overview: Chip-based FAIMS with modifier vapours is used to distinguish between isobaric pairs of isomers, with downstream in-source CID used to provide additional confirmation of identity

1. Introduction

Isomeric substitutions of active pharmaceutical ingredients (APIs) are commonly found in counterfeit drugs. Accurate mass cannot provide unique identification, since the elemental composition may match several isomeric compounds. Whilst chromatographic separation or tandem mass spectrometry (MS/MS) can aid identification, isomeric compounds are likely to exhibit similar fragmentation patterns and in some cases, similar chromatographic behaviour. In this study, we explore the use of chip-based FAIMS [1] methods to distinguish sulfonamide isomers, a frequently encountered substitute API in counterfeit medicines.

2. Methods

Analytes were infused into an Agilent 6230 TOFMS equipped with chip-based FAIMS system (Owlstone Ltd) (Figure 1).

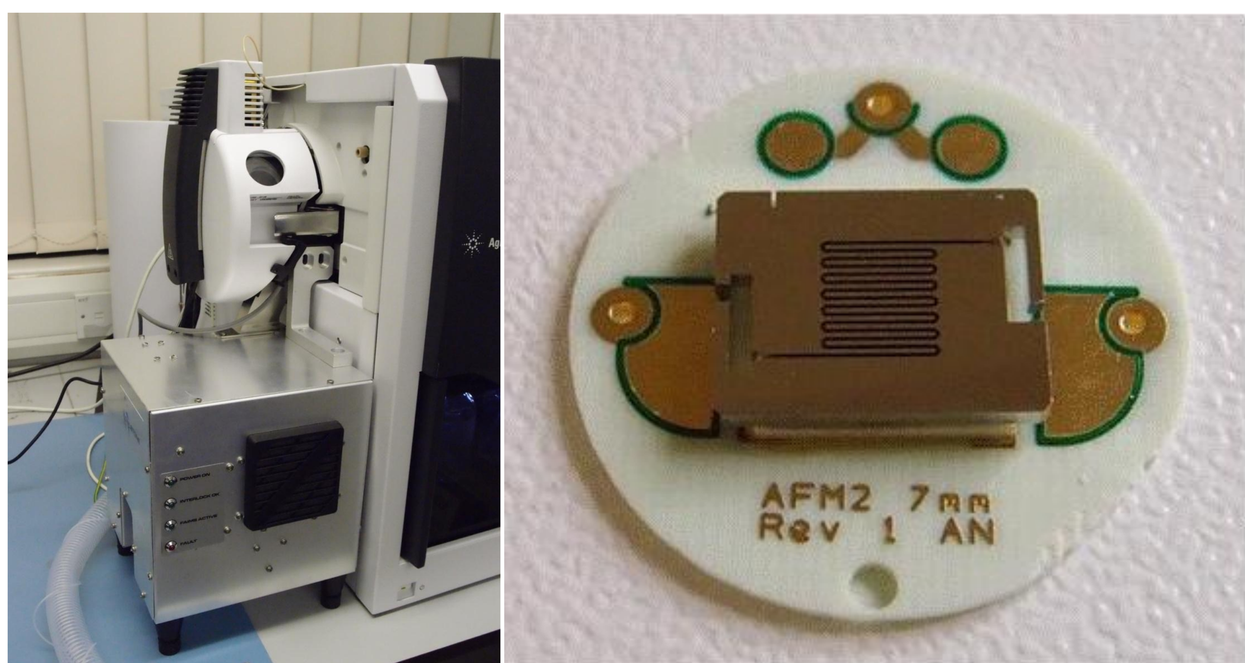


Figure 1 Chip-based FAIMS device set up on Agilent 6230 TOFMS (left) and close up of 100µm gap chip (right)

Isobaric amino-sulfonamides (Table 1) were prepared at 0.1mg/ml in 85:15 H_2O:MeOH with 0.1% formic acid. Each analyte was then subsequently diluted to give similar peak heights. Samples were directly infused and ionised by electrospray ionisation (ESI).

Test analyte	Formula	m/z	Structure
sulfadimethoxine	$C_{12}H_{14}N_4O_4S$	311.07	
sulfadoxine	$C_{12}H_{14}N_4O_4S$	311.07	
sulfisomidine	$C_{12}H_{14}N_4O_2S$	279.33	
sulfamethazine	$C_{12}H_{14}N_4O_2S$	279.33	

Table 1: Analyte details

Experiments were carried out with a chip with an ion residence time of ~235µs. Initial experiments used no added solvent modifier, then the effect of acetone and methanol vapour added to the drying gas as a modifier at different concentration levels was explored.

FAIMS sweeps were performed at dispersion fields (DFs) of 200 to 300Td in 10Td steps with compensation field (CF) swept from -5Td to +5Td.

3. Results

3.1. FAIMS separation

Sulfadimethoxine was analysed with varying % concentrations of acetone modifier. A shift in peak position from positive CF to negative CF values was observed (indicating a change from Type C to Type A ion behaviour). Concentrations above 1.5% did not seem to lead to further CF shift.

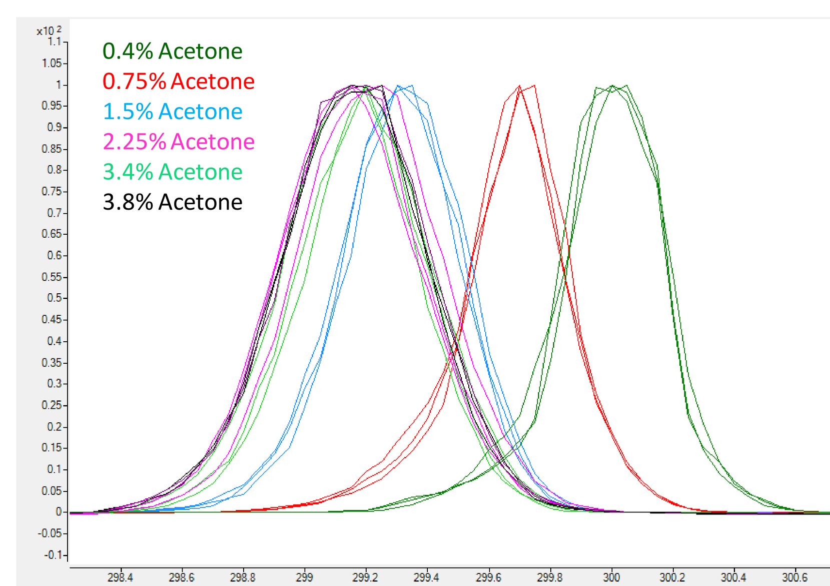


Figure 2 EIC for sulfadimethoxine (*m/z* 311.08 +/-0.1mu) with 0.4% to 3.8% acetone modifier (3 repeats for each concentration) at 210Td

Optimal conditions for separation of sulfadimethoxine and sulfadoxine were then investigated. With no modifier, the maximum separation was around 0.1Td at 210Td. At DFs >210Td, both analytes continue to shift CF positions, but the amount of separation did not increase further. With the presence of 0.75% acetone in the drying gas, separation was increased to 0.6Td.(Figure 3).

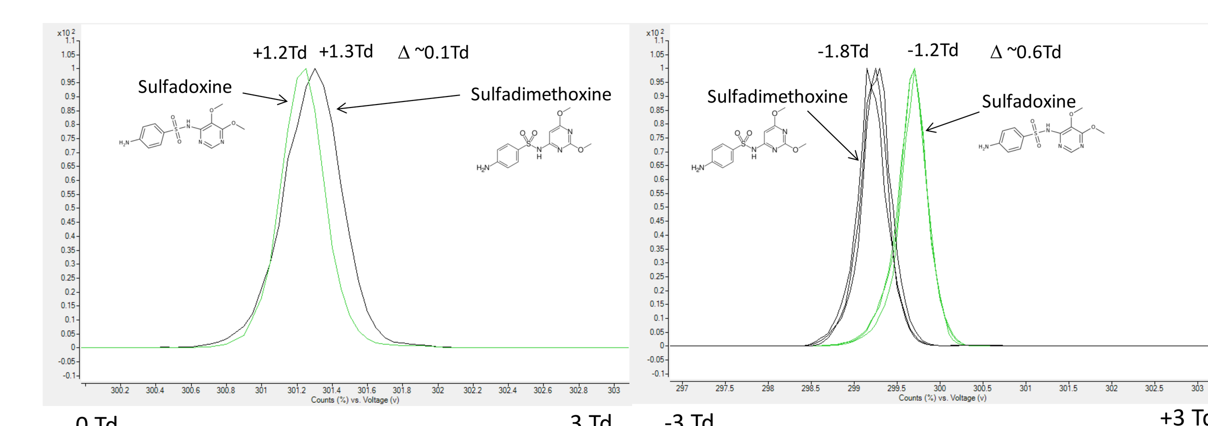


Figure 3 EIC for *m/z* 311.08 +/-0.1mu at 210Td, with no modifier (left) and at 210Td, with acetone modifier at ~0.75% (right)

Analysis was repeated for sulfasomidine and sulfamethazine. With no modifier, maximum separation was 0.3Td at 240Td DF. At DFs higher than 240Td, as with the previous isomer pair, the amount of separation did not increase further. With 2% acetone, separation was increased to 0.6Td (Figure 4). This allowed all the studied sulfonomide isomers to be identified based on CF position.

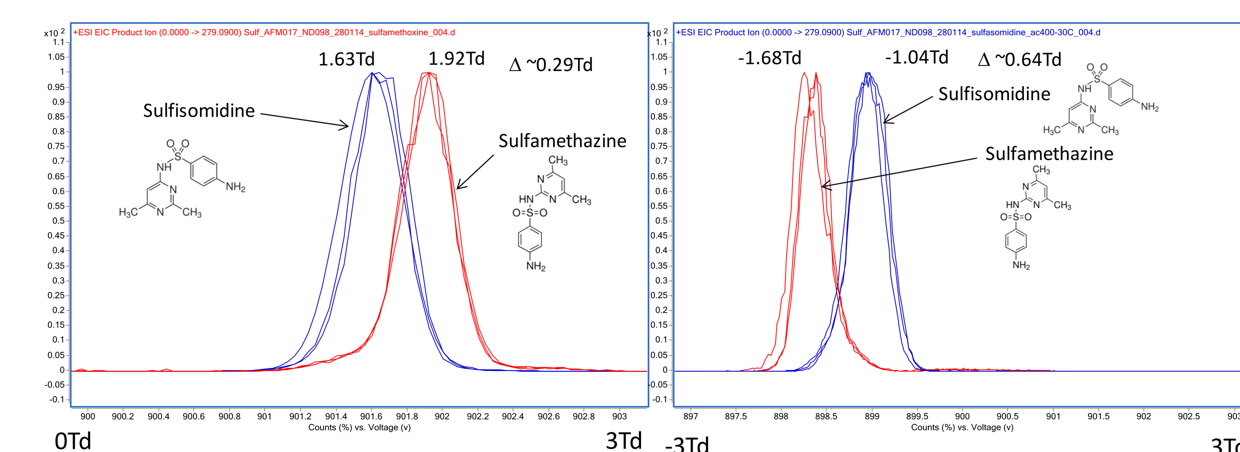
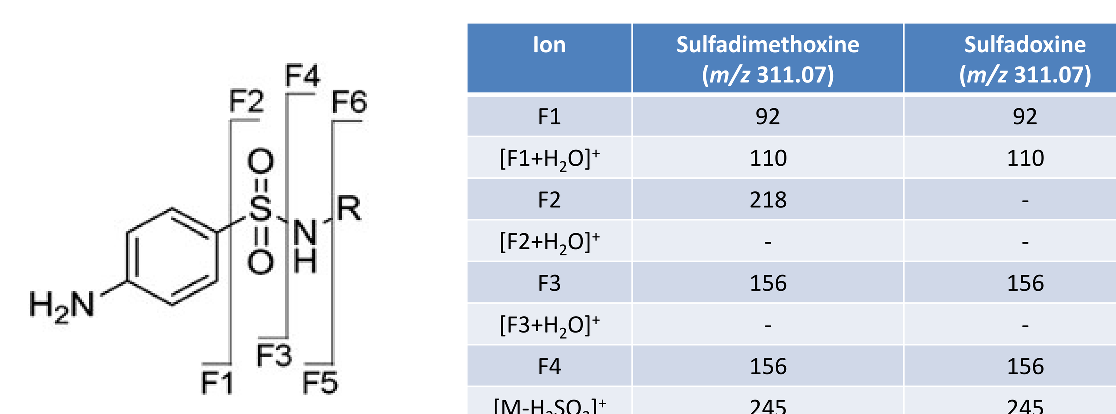


Figure 4 EIC for *m/z* 279.09 +/-0.5mu at 240Td, with no modifier (left) and at 240Td, with acetone modifier at ~2% (right)

3.2. In-source CID data

In-source CID allows the acquisition of fragment data from intact ESI-generated ions, but in the absence of precursor ion selection, complex mixtures yield overlapping product ion spectra. Previously, in-source CID combined with FAIMS pre-selection (FISCID) has been applied to the analysis of peptides [2] and metabolites [3].

Identification of amino-sulfonomide isomers has previously been carried out using MS^n [4]. Reported product ions formed by CID are shown in Figure 5, with further details in Table 2.



Ion	Sulfadimethoxine (*m/z* 311.07)	Sulfadoxine (*m/z* 311.07)
F1	92	92
$[F1+H_2O]^+$	110	110
F2	218	-
$[F2+H_2O]^+$	-	-
F3	156	156
$[F3+H_2O]^+$	-	-
F4	156	156
$[M-H_2SO_2]^+$	245	245

Figure 5 and Table 2 Reported fragmentation of amino-sulfonomide isomers

With acetone as a modifier, the resulting product ion spectra were not as expected. Predicted fragments based on previously reported data were not observed. Instead, the spectra was dominated by what appeared to be protonated and sodiated clusters of acetone (Figure 6).

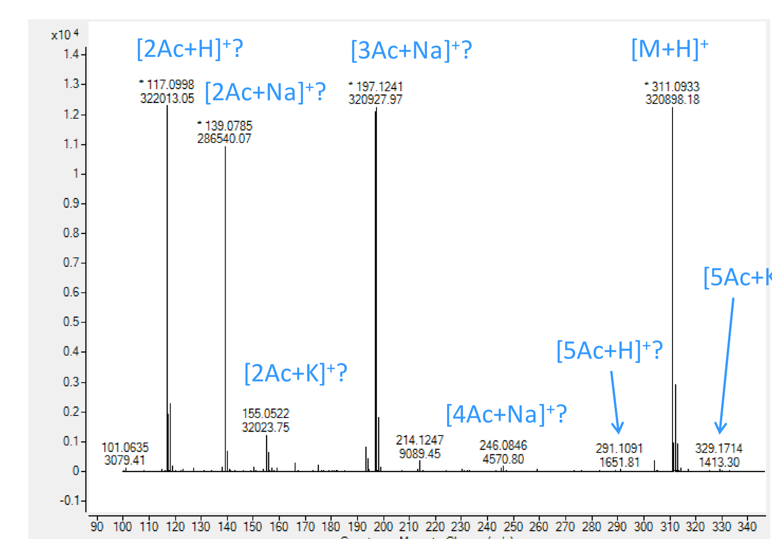


Figure 6 FISCID spectra of sulfadimethoxine using a 0.75% acetone and a 350V fragmentor voltage

The use of methanol as a modifier was therefore investigated. With the presence of ~0.25% methanol in the drying gas, separation was increased to 0.4Td, compared to 0.1Td without modifier (Figure 7). Whilst this separation was not as great as with acetone, it was sufficient to allow the selective transmission of one isomer prior to in-source CID to confirm isomer identity.

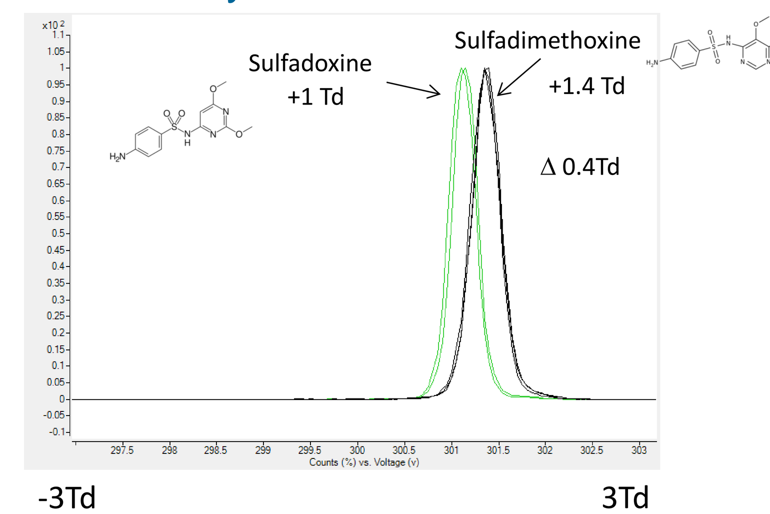


Figure 7 Separation of sulfadimethoxine and sulfadoxine at 210Td in the presence of ~0.4% methanol

The FISCID mass spectrum underneath each peak could be extracted (Figure 8). Differences in intensity of F3 and F4 product ions (*m/z* 156) were observed (Figure 8). In addition, the F2 product ion (*m/z* 218) previously reported to be the only unique identifier for sulfadimethoxine, was also observed only for that isomer and was not present in the analysis of sulfadoxine (Figure 8).These differences in fragmentation could be used to confirm the identity of the isomer.

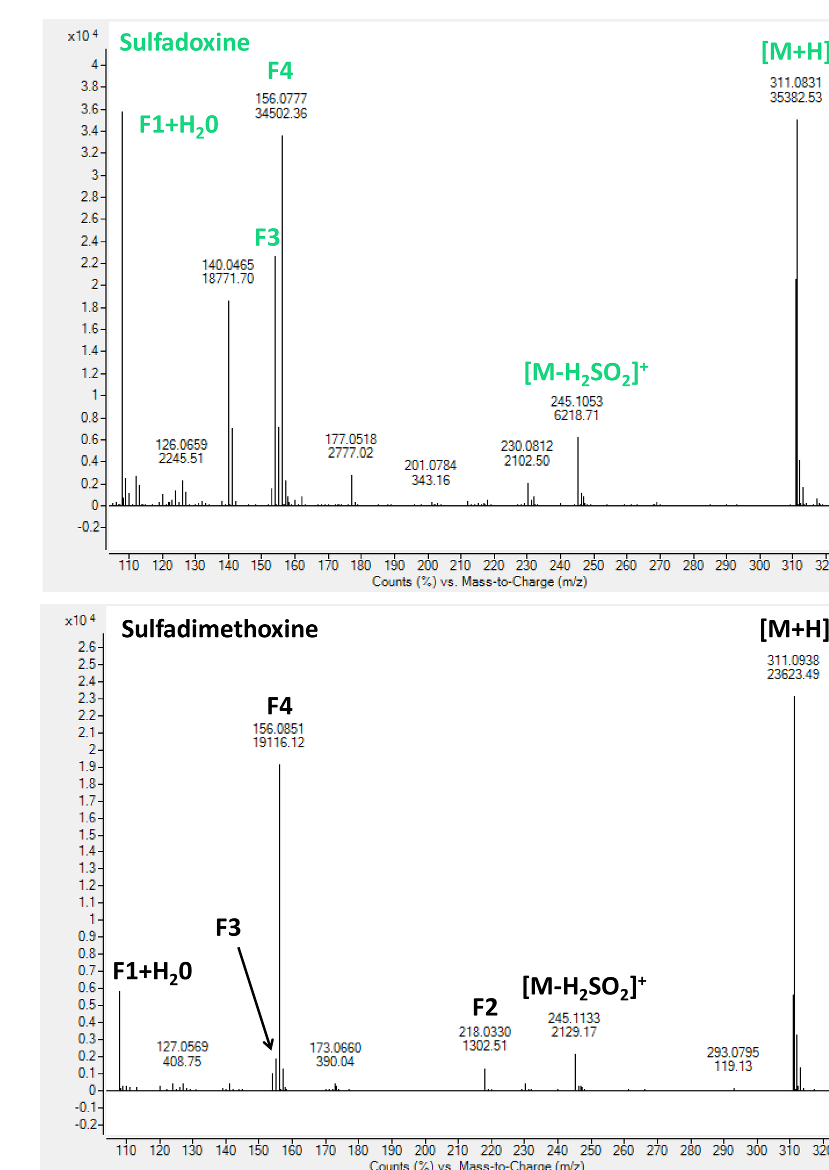


Figure 8 FISCID spectra of sullfadoxine (top) and sulfadimethoxine (bottom) using a 0.4% methanol and a 275V fragmentor voltage

4. Conclusions

- Chip-based FAIMS-MS can be used to distinguish isomeric substitute APIs based on their optimal CF position
  - Some separation is achieved with nitrogen carrier gas alone, and this separation can be enhanced by the addition of acetone or methanol vapours at low percentage level concentrations
- In-source CID can be applied after separation to confirm identification
  - Product ion spectra comparable to MS/MS were observed with nitrogen alone and with the use of methanol modifier
  - Unusual product ion spectra were seen with the acetone modifier, potentially indicative of differences in clustering behaviour compared to methanol
- The FISCID-MS approach offers an alternative to LC/MS/MS for counterfeit drug analysis when direct analysis methods are needed or when increased throughput is required

References

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