Determination of N-methylpyrrolidine in cefepime hydrochloride using field asymmetric waveform ion mobility spectrometry combined with mass spectrometry

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Introduction and Background

- Cefepime is a cephalosporin antibiotic with low toxicity and broad range activity.
- N-methyl pyrrolidine (NMP),[1,2] may be present as an impurity in Cefepime and must not exceed 0.3 %, because of its toxicity.[3]
- The determination of NMP in Cefepime presents a challenge because, (i) Cefepime rapidly degrades to form NMP in aqueous solution during the analysis, particularly outside the pH range 4-6, and above 4 ˚C, and (ii) the protonated Cefepime generated by ESI readily fragments in the mass spectrometer interface to form protonated NMP by 'in-source' collision-induced dissociation (in-source CID).
- The hyphenation of high-field asymmetric waveform ion mobility spectrometry with mass spectrometry (FAIMS-MS) allows ions to be separated rapidly on the basis of differential ion mobility and *m/z*.[4-6]
- The determination of NMP in Cefepime using FAIMS-MS by the direct infusion of aqueous samples into the ESI source is demonstrated and the quantitative performance evaluated.

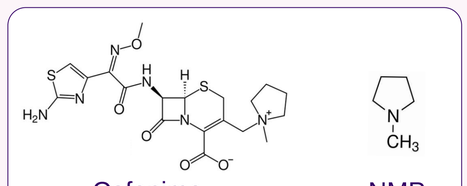

Figure 1. Structures of cefepime and impurity NMP

Instrumentation and Experimental

Instrumentation:

- A chip-based prototype FAIMS device (Owlstone Ltd.) was inserted in front of the capillary inlet of an orthogonal acceleration TOF MS (Agilent Technologies 6230 TOF) fitted with a Jet Stream ESI source (Figure 2).
- The FAIMS device has parallel electrode pairs with a 100 μm electrode gap and 700 μm path length. Devices with trench lengths of 78.1 mm and 97.0 mm were; the latter for the quantitative studies.

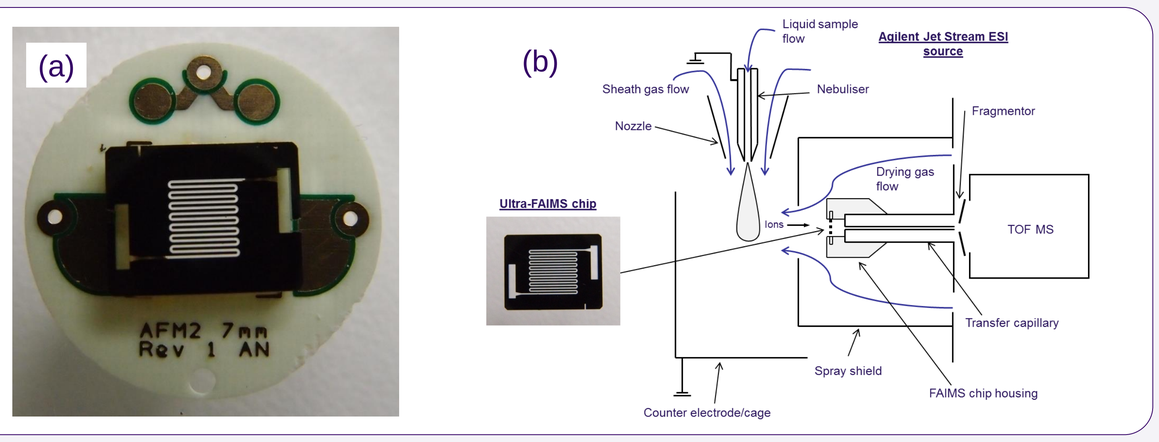

Figure 2. (a) Photograph of the miniaturised chip-based FAIMS device and (b) Schematic diagram of FAIMS interfaced with the TOF-MS.

Experimental:

- Solutions of NMP and Cefepime prepared in methanol/water (50:50) + 0.1% formic acid were directly infused (10 μL/min) into the ESI source, which was operated in positive ion mode
- ESI-FAIMS-MS conditions: sheath and drying gas flows, 9 and 7 L/min; sheath and drying gas temperatures, 250°C and 150°C; nebuliser pressure, 35 psig; spray shield, 2500 V; transfer capillary voltage and nozzle voltage, 3000 V and 2000 V respectively
- Dispersion fields (DF) of 180 - 300 Td were used for optimising separation and DF 180 Td used for quantitative studies.
- The 'fragmentor' voltage applied in the TOF inlet was varied between 75 and 375 V (25 V steps), but set to 200 V for quantification measurements. The MS acquisition scan rate was 10 scans/s for FAIMS scanning and 1 scan/s for quantification with static FAIMS

Results: In-source CID Fragmentation Studies

- The effect of fragmentor voltage on the intensities of the NMP (*m/z* 86.07) and cefepime (*m/z* 481.13) ions was investigated, to determine the extent of cefepime fragmentation by in-source CID.
- NMP and cefepime were infused separately and the fragmentor voltage varied in the range 75-375 V (Figure 3).
- The maximum response for transmission of the NMP ion was at a fragmentor voltage of 200 V (Figure 3a). However, infusion of a Cefeime standard also resulted in a response for NMP as a result of in-source CID at all fragmentor voltages in the range 75-375 V.
- The NMP response in the Cefepime sample directly infused into the ESI source arises from a combination of NMP in the Cefepime sample and in-source CID, preventing the direct determination of NMP.

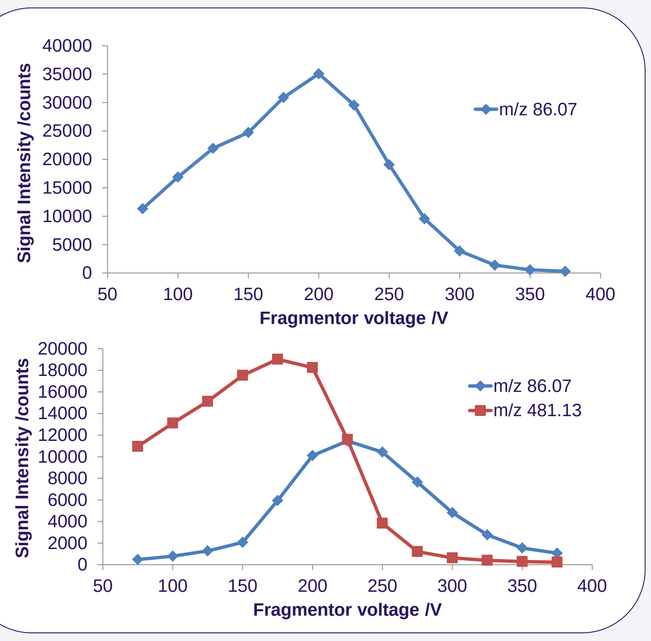

Figure 3. a plot of intensity of *m/z* 86.07 (blue) and 481.13 (red) in (a) NMP standard; and (b) Cefepime sample without NMP added

Results: ESI-FAIMS-MS of NMP and Cefepime

- Figure 4 shows the mass-selected ion responses for Cefepime (*m/z* 481) and NMP (*m/z* 86) in the FAIMS compensation field (CF) spectrum of a solution of Cefepime containing 0.1 % (w/w) NMP.
- The NMP selected ion response (blue trace) shows two peaks, at CF ~ 0 Td, arising from NMP in the Cefepime sample, and at CF 0.7 Td, arising from the transmission of intact Cefepime, followed by in-source CID to form NMP.
- Cefepime and NMP were resolved by FAIMS at a DF of 180 Td (Figure 4, insert).
- Singly and doubly protonated cefepime are the main peaks observed in the mass spectrum of a Cefipime solution without FAIMS separation, (Figure 5a)
- The NMP ion is the base peak in the mass spectrum obtained with FAIMS selection (DF 180 Td, CF 0 Td) (Figure 5b).

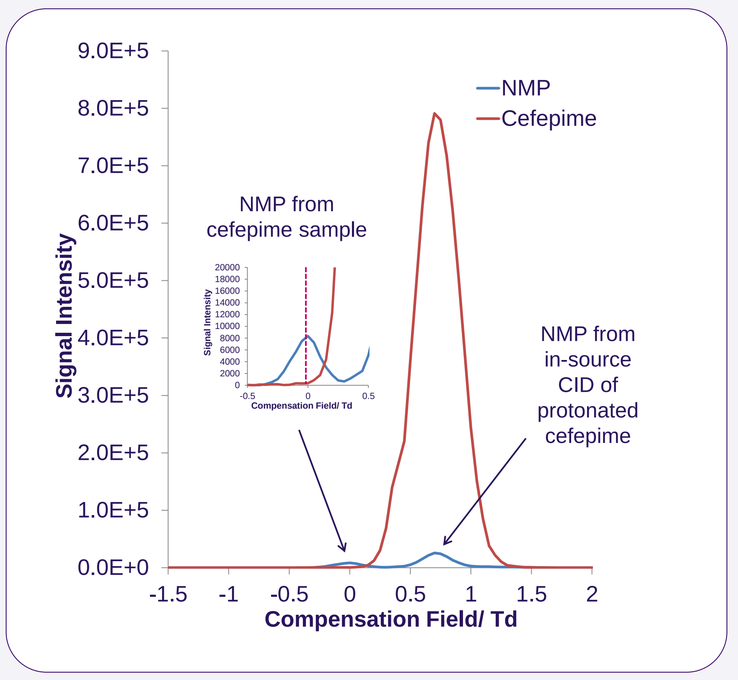

Figure 4. FAIMS CF spectrum of NMP (*m/z* 86.07, blue trace) and cefepime (*m/z* 481.13, red trace) for a Cefepime sample spiked with NMP (0.1% w/w) at a DF of 180 Td. Insert, the spectrum in the CF range -0.5 to +0.5 Td.

- FAIMS selection allows NMP in Cefipime to be separated from NMP formed by in-source CID.

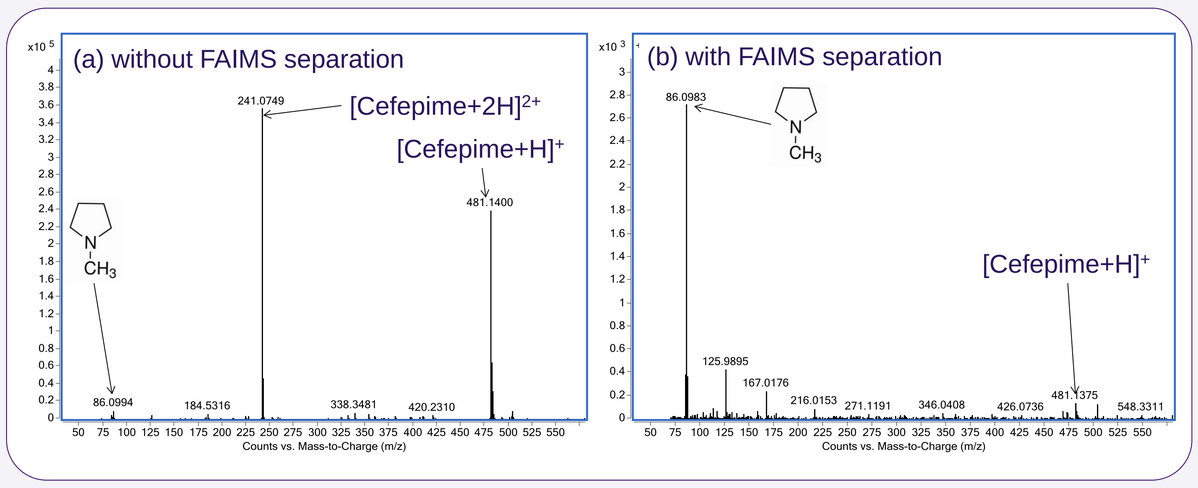

Figure 5. Mass spectra of NMP (0.05 µg/ml) in cefepime (50 µg/ml): (a) without FAIMS separation and (b) with the FAIMS set to CF 0 Td, DF 180 Td to transmit NMP.

Results: Determination of NMP in Cefepime by ESI-FAIMS-MS

- The FAIMS was operated in 'static' mode (CF 0 Td, DF 180 Td), so that NMP was selectively transmitted through the device for the quantification of NMP in Cefepime. The quantitative performance of direct ESI-FAIMS-MS is summarised in Table 1.
- The LOQ was 0.013 µg/ml, equivalent to 0.027% (w/w) in the Cefepime sample, which is well below the 0.3% threshold
- Good linearity and precision were observed for NMP making direct infusion ESI-FAIMS-MS suitable for the determination of NMP in Cefepime

Table 1. Quantitative performance for the ESI-FAIMS-MS determination of NMP (0.1% w/w) in cefepime (n = 6)

LOD /µg.ml^{-1}	LOQ /µg.ml^{-1}	LDR /µg.ml^{-1}	R^2	%RSD
0.004	0.013	0.005-0.5	0.9979	3.85

Conclusions

- Solution degradation and in-source CID of Cefepime to NMP presents an analytical challenge for the determination of NMP in Cefepime.
- The FAIMS separation of NMP and Cefepime allows NMP in Cefepime to be distinguished from NMP ions generated by in-source CID.
- Direct infusion of a cefepime solution using ESI-FAIMS-MS is a rapid, direct ambient ionization method for quantifying NMP in Cefepime.
- Quantitative determination by ESI-FAIMS-MS was found to be sensitive and precise, with an LOQ well below the required 0.3 % threshold.

References

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