Separation of Pharmaceutical Excipients

Reduce interference and improve accurate mass measurement

Introduction

Identification and quantification of pharmaceutical analytes is often complicated by the presence of high concentrations of excipients such as lactose, starches, PEGs and many more. A suitable LC method may separate much of the background interference from the analyte of interest, but this is at the cost of additional run time and in difficult cases some interference with key peaks may still remain.

Field-asymmetric waveform ion mobility spectrometry (FAIMS) is a separation technique that is highly orthogonal to LC – in other words, it will often separate substances that LC does not. In addition, recent advances in FAIMS mean that separation can happen within the timescale of an LC peak; hence no additional time is required to achieve the improved separation. In fact, the addition of FAIMS separation may allow for a shorter LC gradient to be used.

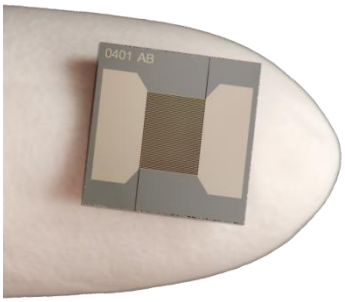

Figure 1: The miniature UltraFAIMS microchip (35µm gap design)

This application note describes the use of FAIMS to separate an example analyte (2-hydroxy-4-octyloxy benzophenone, HOBP) from a strong PEG background with no prior LC clean-up, enabling fast accurate mass measurement. In addition, the FISCID (FAIMS-in source CID) technique is applied to fragment the HOBP ion to provide additional confidence in identification without the need for tandem mass spectrometry.

UltraFAIMS background

UltraFAIMS is an evolution of standard FAIMS/DMS (differential mobility spectrometry, an alternative name for FAIMS) that uses a miniaturized microchip (Figure 1) as the separation device. The miniaturization allows ultra-high electric fields (up to 55 kV/cm) to be used for separation and this enables the separation to be performed in milliseconds[1]. Due to the extremely short ion transit time, transmission with and without dispersion fields applied is higher than in conventional planar DMS systems, typically well over 20% at the required dispersion field, for all but the most mobile ions[2]. UltraFAIMS is a quantitative technique that preserves the linearity of the analysis over several orders of magnitude[3,4].


Figure 2: UltraFAIMS device installed on Agilent 6230 TOF

Instruments and Methods

The Owlstone ultraFAIMS system was installed on an Agilent 6230 TOF MS with JetStream ESI source (Figure 2). The FAIMS microchip forms an array of parallel channels (35 µm x 300 µm) across which an asymmetric dispersion field was applied. Selected ions were transmitted through the chip by application of an appropriate compensation voltage (CV). FAIMS separation was carried out at a 48 kV/cm DF, over a CV range of -1 V to +4 V at 0.5 V/s CV sweep rate. For LC-FISCID-MS analysis, quasi-static scanning was performed to selectively allow analytes into the mass spectrometer using a 0.1 V window around the optimum CV for

transmission (optimum CVs are analyte specific and are indicated in the results and figures below). "Non-FAIMS" data was also collected for comparison purposes by setting the dispersion field to 0kV/cm and the CV to 0V. ESI was carried out in positive mode. Full MS conditions are shown in Table 1.

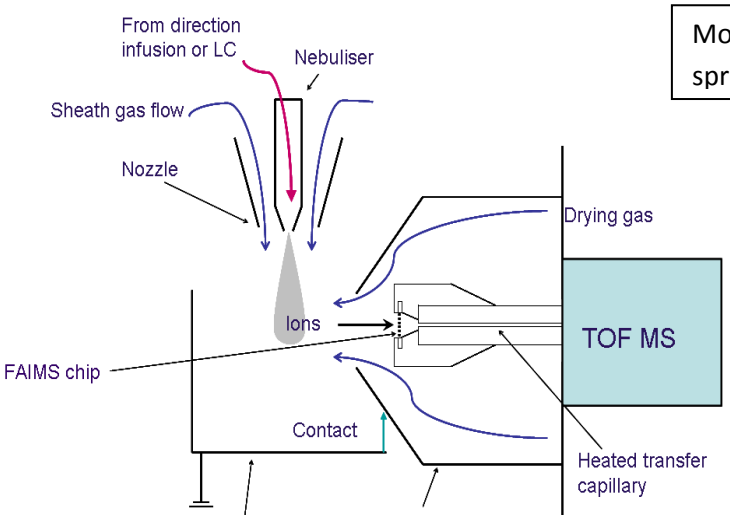

Figure 3: Schematic of Agilent JetStream ionization source with ultraFAIMS interface installed

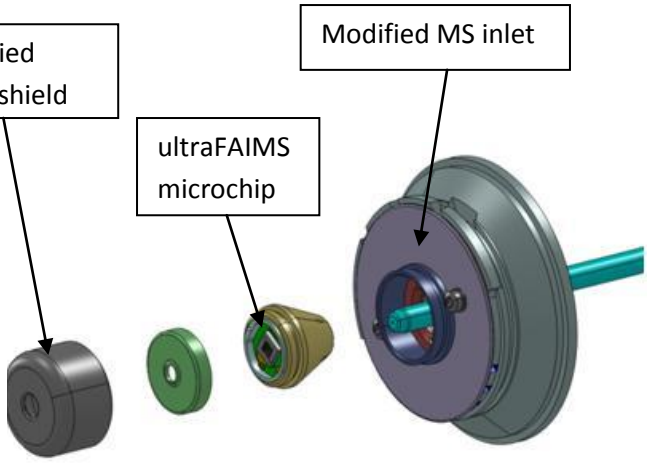

Figure 4: Exploded view of ultraFAIMS interface to Agilent 6230 TOF

Sample Preparation

HPLC grade methanol (MeOH) and formic acid (FA) were purchased from Fisher Scientific (Loughborough, UK). 2-hydroxy-4-octyloxy benzophenone and PEG 400, obtained from Sigma Aldrich (Gillingham, UK), were prepared in 50/50 (v/v) methanol/water with 0.1% FA at respective concentrations of 5.1 and 104 pmol/µl (1:20 molar ratio).

Table 1: MS and LC conditions

Parameter	Setting
TOF acquisition rate	10 scans/sec
Infusion rate	50µL/min
Nebulizer voltage	1.5kV
Spray shield voltage	400V
Skimmer voltage	65V
Drying gas temperature	150^{O}C
Sheath gas temperature	250^{O}C
Nebulizer pressure	25psig
Capillary voltage	-29V
Drying gas flow	5L/min
Sheath gas flow	7L/min
Fragmentor voltage	150V for intact ions; 400V for in-source CID

Results

Ions derived from HOBP and PEG 400 excipients were chosen as test analytes because the protonated HOBP ion (m/z 327.1955) and the PEG n=7 oligomer (m/z 327.2013) are sufficiently close in mass (17.7 ppm mass difference) that these ions could not be resolved by the reflectron TOF mass analyzer (required resolution ~130K). Robust accurate mass measurement of these ions is therefore not possible without separation prior to mass analysis.

The two components were analysed as a mixture containing a 20 fold molar excess of the PEG. The selected ion response for m/z 327.2 (Figure 5) shows that the protonated PEG n=7 and HOBP ions are resolved by FAIMS.

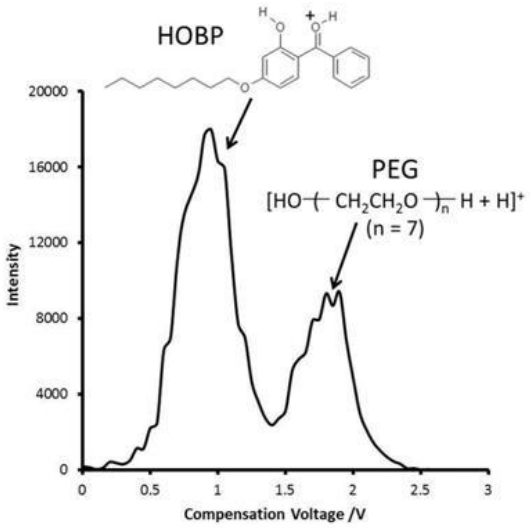

Figure 5: FAIMS-MS CV spectrum (m/z 327.2) of HOBP and PEG 400

The mass spectrum of the mixture without FAIMS separation (Figure 6a) shows the typical polymer distribution of PEG ions, with the HOPB unresolved from the protonated PEG (n=7) ion. The measured mass of the overlapping peaks is m/z 327.1994, a mass difference of 11.9 and -5.8ppm from HOBP and PEG respectively. In-source CID-MS of this mixture gives a complex product ion spectrum dominated by PEG fragment ions (Figure 6b). Quasi-static FAIMS filtering (CV range 0.6 – 0.7 V) was used to transmit the HOPB ion selectively, which appears as the base peak in the resulting mass spectrum (Figure 6c), with an observed mass of 327.1966 (3.3 ppm) The FISCID-MS product ion spectrum of the FAIMS-selected HOBP ion (Figure 6d) shows the HOBP fragment ions free of interference from PEG fragment ion peaks, reducing the complexity of the mass spectrum and allowing unambiguous identification of the HOBP fragments.

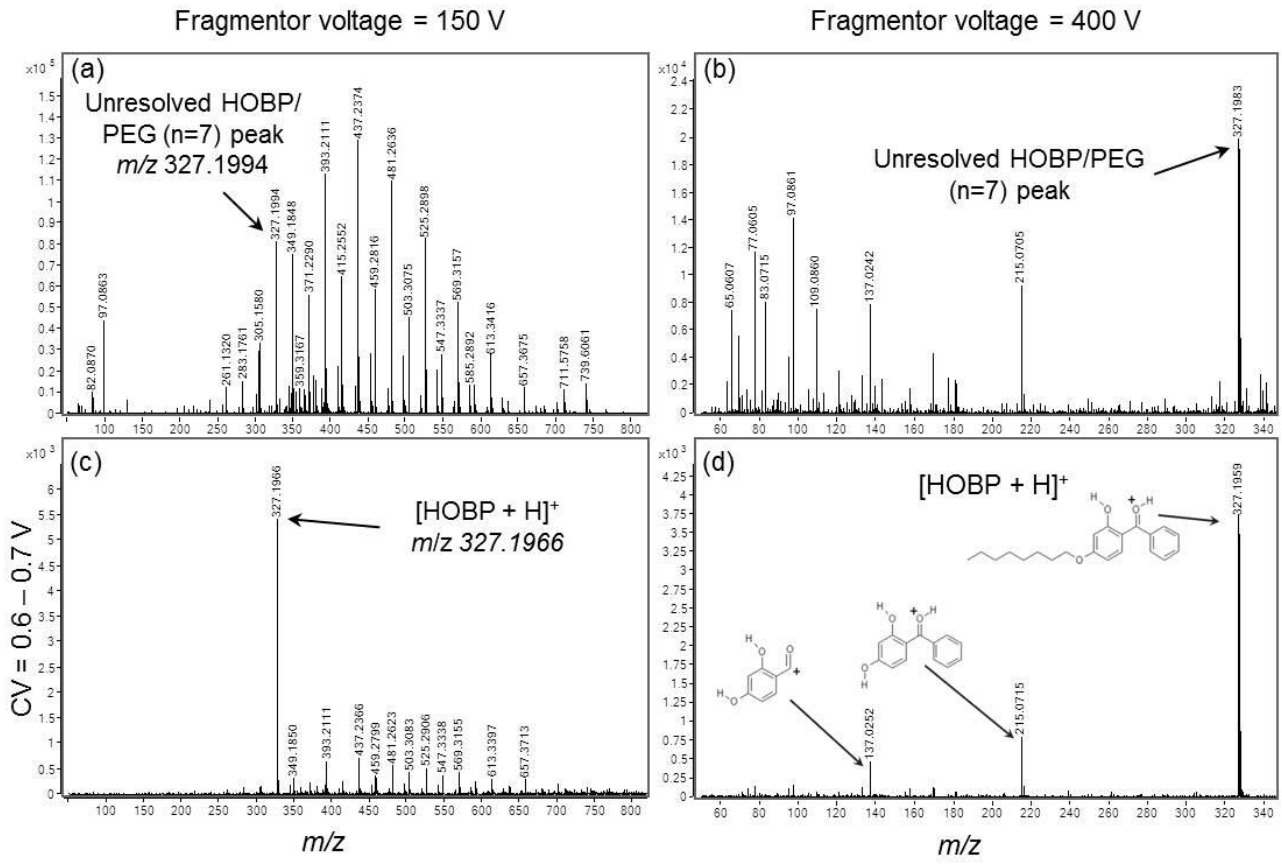

Figure 6: MS, FAIMS-MS and FISCID-MS mass spectra of a mixture of HOBP and PEG 400 (1:20 molar ratio): (a) without FAIMS separation or in-source CID, (b) In- source CID-MS without FAIMS separation, (c) FAIMS-selected HOBP ion (CV = 0.6–0.7 V) without in-source CID; (d) FISCID-MS product ion spectrum of the FAIMS-selected HOBP ion with in-source CID

Conclusions

The ultraFAIMS microchip has been used to separate an example pharmaceutical analyte from a 20x more concentrated PEG background (containing an unresolved isobaric ion) without the need for LC.

- The mass error of the analyte peak was reduced from 11.9 ppm to 3.3 ppm.
- With FAIMS selection prior to in-source CID (FISCID), the PEG fragment ions were eliminated from the mass spectrum, leaving a clean product ion spectrum.

These observations show the benefit that ultraFAIMS can bring to both qualitative and quantitative pharmaceutical analysis through the reduction or elimination of isobaric or near-isobaric background matrix ions from mass spectra.

References

1. *Shvartsburg A, Tang K, Smith RD, Holden M, Rush M, Thompson A and Toutoungi DE, Anal. Chem. 2009, 81, 8048–8053*
2. *Ugarov M, Dai Y, Bunting H, Fraser W, Optimization and characterization of a new ultra-fast FAIMS MS interface, oral presentation, AMS 2012*
3. *Brown LJ, Smith RW, Toutoungi DE, Reynolds JC, Bristow AW, Ray A, Sage A, Wilson ID, Weston DJ, Boyle B, Creaser CS. Anal Chem. 2012 May 1;84(9):4095-103*
4. *Smith R, Toutoungi DE, Reynolds JC, Bristow AWT, Ray A, Sage A, Wilson ID, Weston DJ, Boyle B, Creaser CS. Enhanced Performance in the Determination of Ibuprofen 1-β-O acyl glucuronide in Urine by Combining Ultra High Field Asymmetric Waveform Ion Mobility Spectrometry with Liquid Chromatography-Time-of-Flight Mass Spectrometry, Journal of Chromatography A, 2013, 1278:76–81*

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