Determination of a Urinary Drug Metabolite using Liquid Chromatography Combined with FAIMS-MS and FAIMS-In Source CID-MS

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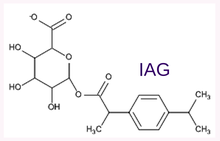
Introduction and Overview

- Field Asymmetric Waveform Ion Mobility Spectrometry (FAIMS) separation is orthogonal to liquid chromatography (LC) and mass spectrometry (MS), making the interfacing of these techniques a powerful combination for the rapid, selective and sensitive analysis of drug metabolites in biological matrices.
- The quantitative determination of (R/S) Ibuprofen 1-β-O acyl glucuronide (IAG) in urine by LC-FAIMS-MS is reported using a miniaturised FAIMS device
- The use of FAIMS reduces matrix chemical noise and improves quantitation limits for IAG
- FAIMS pre-selection of the $[IAG\text{-}H]^-$ ion, based on differential mobility, followed by in-source CID (FISCID-MS) [2,3] is shown to enhance selectivity for IAG when selected fragment ions are monitored using a single mass analyser



Methods

A prototype miniaturised chip-based FAIMS device (Owlstone Ltd.) has been interfaced to an Agilent 6230 series TOF MS with a Jet Stream ESI source and an Agilent 1200 series LC (Figure 1b), allowing FAIMS separation of gas phase ions, based on differences in ion mobility under alternating high and low electric fields.

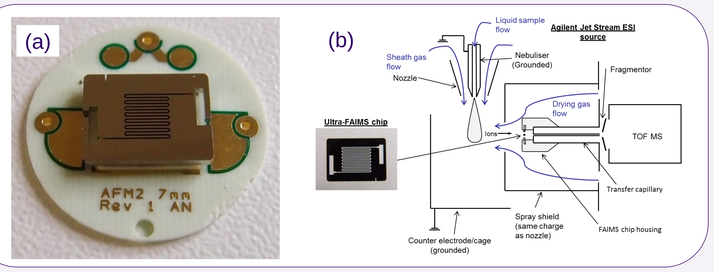


Figure 1. (a) The miniaturised chip-based FAIMS and **(b)** interfaced with the TOF-MS.

- The miniaturised FAIMS chip consists of multiple planar electrode channels with a 100 µm gap and 700 µm depth (Figure 1a). Higher dispersion fields (< 300 Td) with short residence times (50-250 µs) enable fast scan rates compatible with LC.
- The serpentine length of the channel in the FAIMS chip (trench length) can be varied in the FAIMS device, which modifies resolution vs. transmission characteristics.
- Dispersion fields (DF) of 220 - 260 Td were used in all experiments (RT 0.5 – 2.5 min). LC-FAIMS-MS and LC-FISCID-MS studies used a static compensation field (CF) of 2.2 Td and 1.7 Td, respectively.
- Urine samples (200 µl) were filtered (0.45 µm), diluted (2x) in water and spiked with IAG metabolite.
- LC separation: Zorbax C18 column (2.1 x 50 mm, 1.8 µm) with an isocratic 0.2 ml/min flow of 50:50 acetonitrile:aqueous ammonium acetate (10 mM) at pH 3.
- Negative ESI-FAIMS-MS conditions: sheath and drying gas flows, 11 and 10 L/min; sheath and drying gas temperatures, 150°C and 350°C; nebuliser pressure, 25 psig; MS scans rate, 1 scan/s; transfer capillary voltage and nozzle voltage, 4000 V and 2000 V respectively; fragmentor voltage, -150 V.
- ESI-FISCID-MS settings: sheath gas flow and temperature, 12 L/min and 250°C; capillary voltage, 3500 V; MS scan rate 10 scan/s; fragmentor voltage, -250 V.

Results: FAIMS-MS of metabolite and parent drug

- The compensation field (CF) spectra of ibuprofen and IAG at DF 220 Td using FAIMS devices with different trench lengths are shown in Figure 2
- The resolution vs. sensitivity relationship can be exploited by changing the trench length

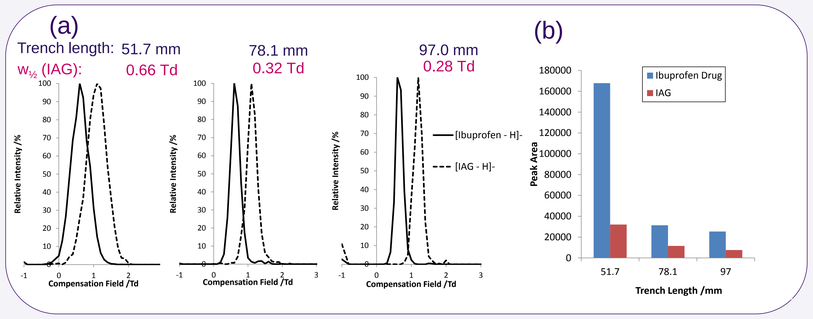


Figure 2. (a) FAIMS-MS CF scans of ibuprofen and the IAG metabolite with different trench lengths and **(b)** a plot of CF peak area against trench lengths (n = 6)

Results: LC-FAIMS-MS of metabolite in urine

- The incorporation of a FAIMS separation in the LC-MS analysis significantly reduced chemical interference from urine (Figures 3a and b)
- The absolute intensity of the $[IAG\text{-}H]^-$ peak is reduced to ~50% because of the lower FAIMS transmission, but this is compensated by an improvement in signal to noise ratio (Table 1)
- FAIMS-selective transmission of the IAG makes the metabolite peak (*m/z* 381) the base peak in mass spectrum (Figure 3d)

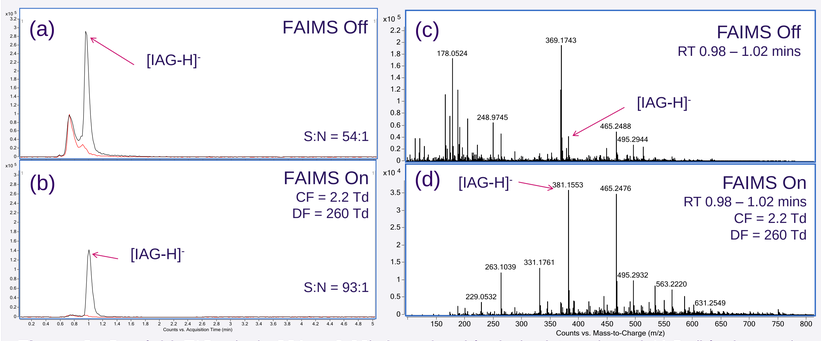


Figure 3. Overlaid EICs (*m/z* 381 ± 0.02) for urine blank (red trace) and IAG (black trace) spiked into urine (0.55 µg/ml) with **(a)** FAIMS off, and **(b)** FAIMS on. Mass spectra of $[IAG - H]^-$ LC peak with **(c)** FAIMS off, and **(d)** FAIMS pre-selection of $[IAG - H]^-$

Table 1. Quantitative LC-MS and LC-FAIMS-MS determination of IAG in urine (15.5 µg/ml, n = 5)

	FAIMS off	FAIMS on
LOQ (µg/ml)	0.018	0.010
LDR (µg/ml)	0.018-11	0.010-11
R^2	0.9991	0.9987
Intra-day (% RSD)	5.0	2.7

- Lower LOQ with optimised FAIMS transmission of IAG metabolite
- Similar LDR observed with, and without FAIMS separation
- Good intra-day reproducibility

Results: LC-FISCID-MS of metabolite in urine

- LC-FISCID-MS analysis of IAG in urine, monitoring the *m/z* 205 fragment of the FAIMS-selected $[IAG\text{-}H]^-$ ion, shows enhanced selectivity compared to FAIMS off LC-CID-MS (Figure 4a and b).
- The ability to select the metabolite based on differential mobility results in the enhancement of IAG derived fragment ion peaks in mass spectrum compared with LC-CID-MS without FAIMS (Figures 4c and d)

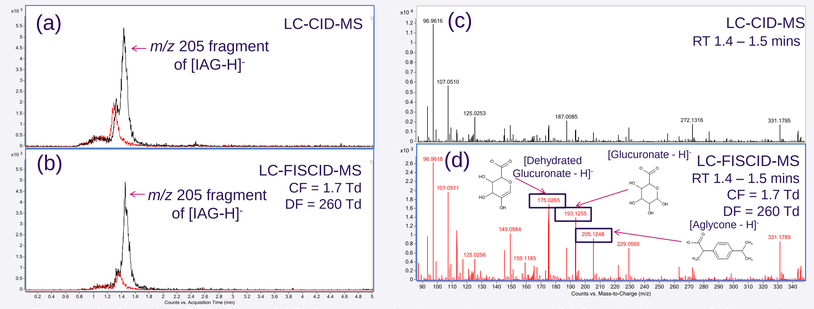
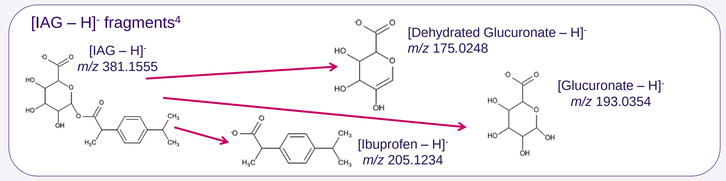


Figure 4. Overlaid EICs (*m/z* 205) for urine blank (red trace) and IAG spiked urine (3.9 µg/ml) (black trace) using **(a)** LC-CID-MS and **(b)** LC-FISCID-MS (FAIMS-selected $[IAG\text{-}H]^-$ ion). Mass spectra of $[IAG\text{-}H]^-$ LC peak with **(c)** FAIMS off, and **(d)** FAIMS selecting $[IAG\text{-}H]^-$



Conclusions

- The miniaturised FAIMS separation of Ibuprofen and IAG has been achieved in negative ion mode and the influence of trench length on resolution and transmission has been demonstrated.
- A significant reduction in chemical noise for IAG in urine was observed under optimum LC-FAIMS-MS conditions.
- Lower LOQ, good linearity and precision were observed with the use of LC-FAIMS-MS
- Enhanced fragmentation data is produced using a single mass analyser by FAIMS pre-selection of the IAG followed by in-source CID-MS (FISCID-MS)
- The potential to improve quantitative determination of a metabolite in biological samples has been demonstrated

References

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