# Evaluation of the performance of microscale FAIMS for the enhancement of the quantitative analysis of metabolites and peptides

Robert W. Smith[1], Lauren J. Brown[1], Danielle E. Toutoungi[2], James C. Reynolds[1], Ashley Sage[3], Anthony W.T. Bristow[4], Andrew Ray[4], Daniel J. Weston[5], Ian Wilson[5], Billy Boyle[2], Colin S. Creaser[1]

[1]Centre for Analytical Science, Loughborough University, Loughborough, U.K.; [2]Owlstone Ltd, Cambridge, U.K.; [3]Agilent Technologies, Stockport, U.K.; [4]AstraZeneca, Pharmaceutical Development, Macclesfield, U.K.; [5]AstraZeneca, DMPK Innovative Medicines, Alderley Park, U.K.

## Introduction

- High-field asymmetric waveform ion mobility spectrometry (FAIMS) is an atmospheric pressure technique that separates ions by their differential mobility under high and low electric fields. In microscale FAIMS, the ions travel through channels with sub-millimetre dimensions, which enables substantially higher electric fields and faster separation than macroscale differential mobility devices. Separation on sub-second timescales becomes possible, allowing the device to be used during real-time LC-MS analysis.[1,2] The extra separation dimension can be used to improve signal-to-noise in real-time quantitative analysis.
- This study evaluated the quantitative performance of LC-microscale FAIMS-TOFMS systems for the determination of the metabolite (R/S)-ibuprofen 1-β-O acyl glucuronide (IAG) in urine and gramicidin S in a human plasma tryptic digest.[3] A tandem FAIMS-in source CID-MS approach, termed FISCID-MS, allows the acquisition of fragment ion data from, FAIMS-selected intact electrospray (ESI) generated ions to enhance selectivity

## Methods

- Microscale FAIMS devices (Owlstone Ltd, Cambridge, UK) were interfaced to an Agilent 6230 series TOF MS with a Jet Stream ESI source and an Agilent 1200 series LC (Figure 1). The ion channels in these devices consist of an array of parallel electrodes with either 35 *µ*m or 100 *µ*m gaps, to which an asymmetric dispersion field (DF) is applied. Ions with a selected differential ion mobility are transmitted through the device by application of a compensation field (CF). At this scale, DFs of up to 300Td can be applied, and with a typical TOF MS capillary inlet gas flow rate of 1.7 L/min, with ion residence times of 50-250 *µ*s.

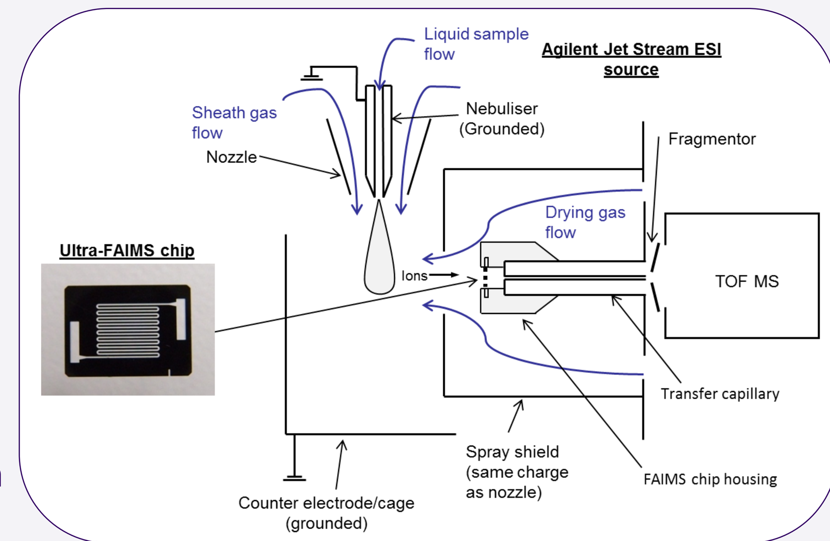


**Figure 1.** The miniaturised chip-based FAIMS interfaced with the TOF-MS.

Determination of IAG in urine:

- IAG was spiked into filtered (0.45 µm), diluted (2x) urine (200 µL). LC separation was carried out on C18 column (Agilent Zorbax Rapid Resolution HT, 2.1 x 50 mm, 1.8 µm) using an isocratic 0.2 mL/min flow of 50:50 ACN:aqueous ammonium acetate (10mM) at pH 3 to achieve rapid elution of the metabolite (< 2 min).
- The FAIMS device was set at a fixed DF and CF (CF= 2.1-2.2 Td, DF=260 Td) that had been previously determined to give good transmission of IAG. Negative ESI conditions were used with fragmentor voltage at -150 V for transmission of the $[M-H]^-$ ion and -250 V for in-source CID experiments

Determination of gramicidin S in plasma:

- ACN (400 *µ*L) was added to human plasma (200 *µ*L) to precipitate proteins prior to digestion with trypsin (Promega, UK) in 100 mM $NH_4HCO_3$ at 37 °C and cleaned-up using a C18 SPE cartridge. Aliquots of the plasma digest were spiked with the exogenous peptide gramicidin S. LC separation was carried out on a Poroshell 300SB-C18 column with a flow rate of 0.4 ml/min using a gradient elution: linear increase from 95:5 water/ACN (v/v, 0.1% FA) to 60:40 water/ACN (v/v) in 10 min, then to 10:90 water/ACN in 2 min and returned to initial conditions (total run time 15 min)
- A DF of 275 Td and CF of 3.0 Td was applied to pre-select the gramicidin $[M+2H]^{2+}$ precursor ion (*m/z* 571). The LC-FISCID-MS analysis of the four FAIMS-selected in-source CID product ions (*m/z* 311, 424, 685 and 798) was carried out with a fragmentor voltage of 340 V.

## Results: LC-FAIMS-MS of IAG Metabolite in Urine

- The quantitative performance of the prototype LC-FAIMS-MS system was evaluated by the analysis of IAG metabolite spiked into urine
- Direct infusion of an IAG standard was used to optimise FAIMS transmission of IAG (Figure 2)
- The incorporation of a FAIMS separation in the LC-MS analysis significantly reduced chemical interference from urine (Figures 3.a and b)
- The absolute intensity of the $[IAG-H]^-$ peak is reduced ~50% because of lower FAIMS transmission, but is compensated by an improvement in signal to noise ratio, which reduces the LOQ from 0.018 to 0.010 *µ*g/ml (Table 1)
- Improved intra-day reproducibility was observed with FAIMS pre-selection of the $[IAG-H]^-$ ion
- Higher LDR observed with FAIMS separation, over 3 orders of magnitude (Table 1)

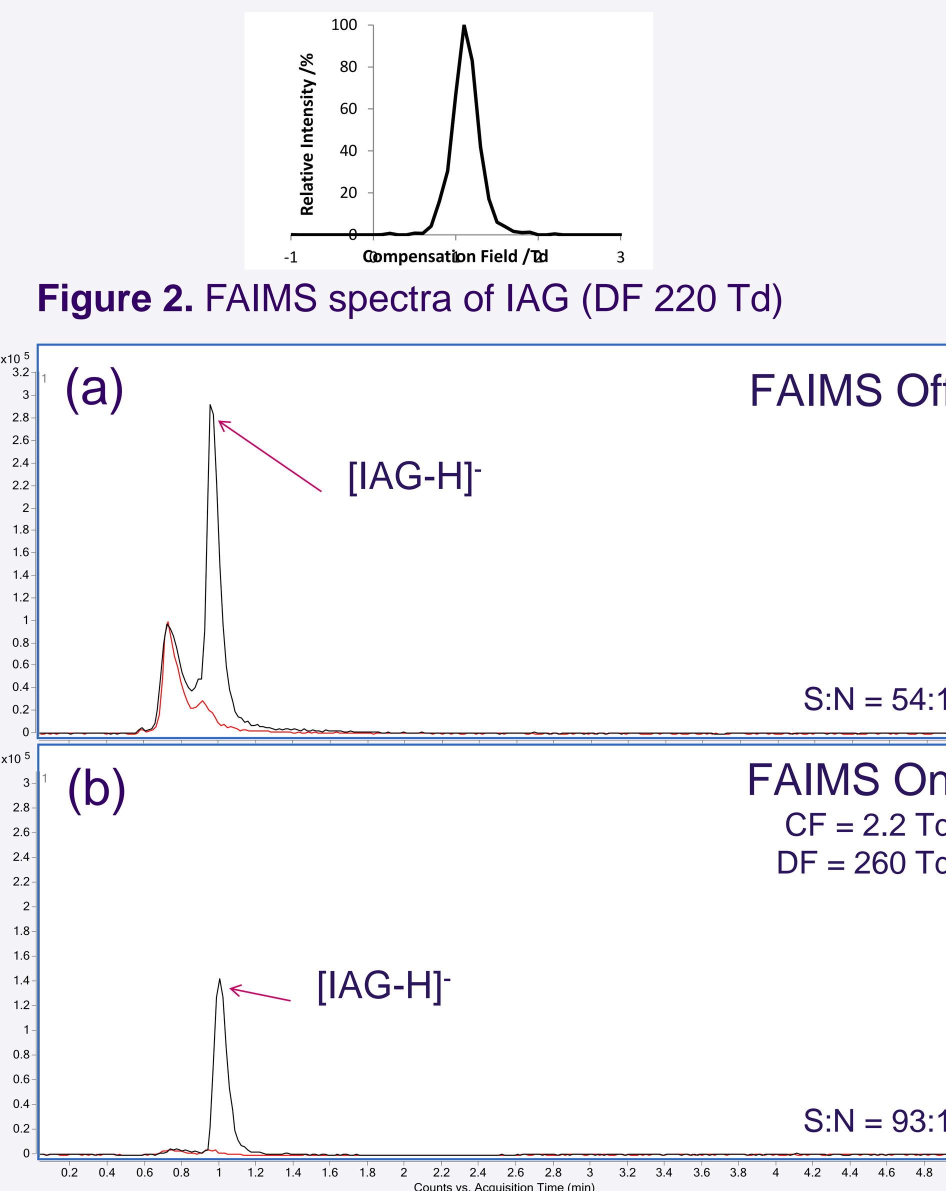


**Figure 2.** FAIMS spectra of IAG (DF 220 Td)

**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

**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 (*µ*g/ml) | 0.018 | 0.010 |
| LDR (*µ*g/ml) | 0.018-11 | 0.010-11 |
| $R^2$ | 0.9991 | 0.9987 |
| Intra-day (% RSD) | 5.0 | 2.7 |

## LC-FISCID-MS analysis

The LC-FISCID-MS method is shown in Figure 4. In this technique, the FAIMS device is used to pre-select ions of a selected differential mobility, which are then fragmented by setting the TOF fragmentor voltage to induce in-source CID. The LC-MS spectrum of target analyte ions Figure 4a) is simplified by LC-FISCID-MS with FAIMS pre-selection (Figure 4b).

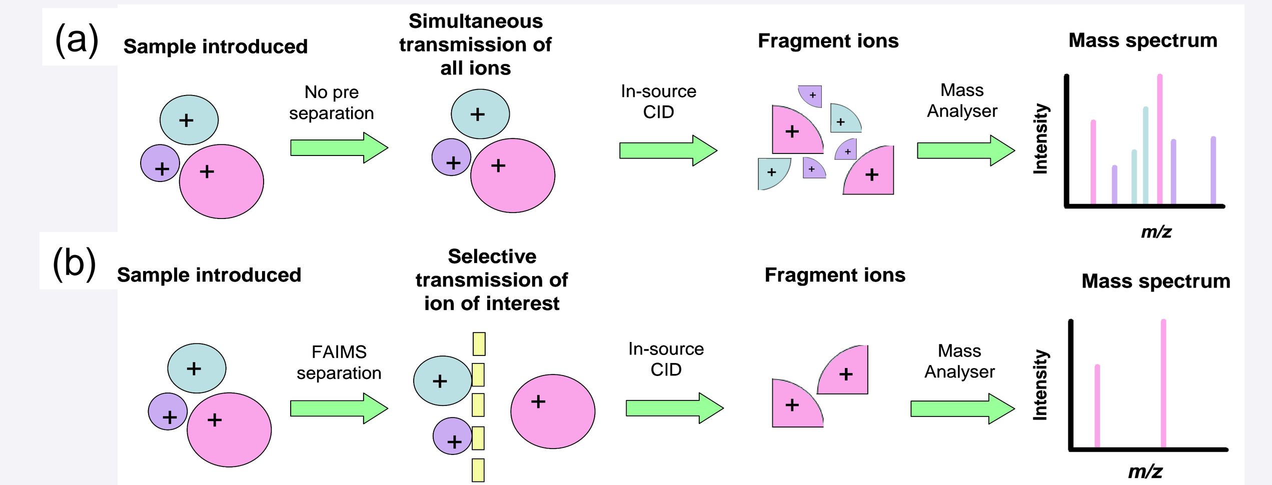


**Figure 4.** LC-MS and LC-FISCID-MS analysis of target analyte ions, (a) in-source CID-MS without FAIMS separation, (b) In-source CID-MS with FAIMS separation.

## Results: LC-FISCID-MS of a Peptide in Plasma Digest

- The quantitative potential of LC-FISCID-MS was evaluated for the determination of an exogenous peptide, gramicidin S, spiked into human plasma. Calibration graphs for the LC-FAIMS-MS analysis of the gramicidin $[M+H]^+$ ion (*m/z* 571) and the LC-FISCID-MS analysis of the four FAIMS-selected in-source CID product ions *m/z* 311, 424, 685 and 798) show a linear response ($R^2$ > 0.99) in the range 0.45-9.0 *µ*g/mL (Figure 5). The selected ion response for the *m/z* 311 fragment of the $[M+2H]^{2+}$ ion of gramicidin S obtained from the LC-FISCID-MS analysis of the plasma is shown in Figure 5b.
- The %RSD for the peak area of the FAIMS-selected [M+2H]2+ precursor ion (m/z 571) without in-source fragmentation was 5.1% (n=6; 0.45 µg/mL). LC-FISCID-MS precision for the fragment ions at m/z 311, m/z 424, m/z 685 and m/z 798, was 13.3 %, 14.6 %, 13.1 % and 8.2 % respectively

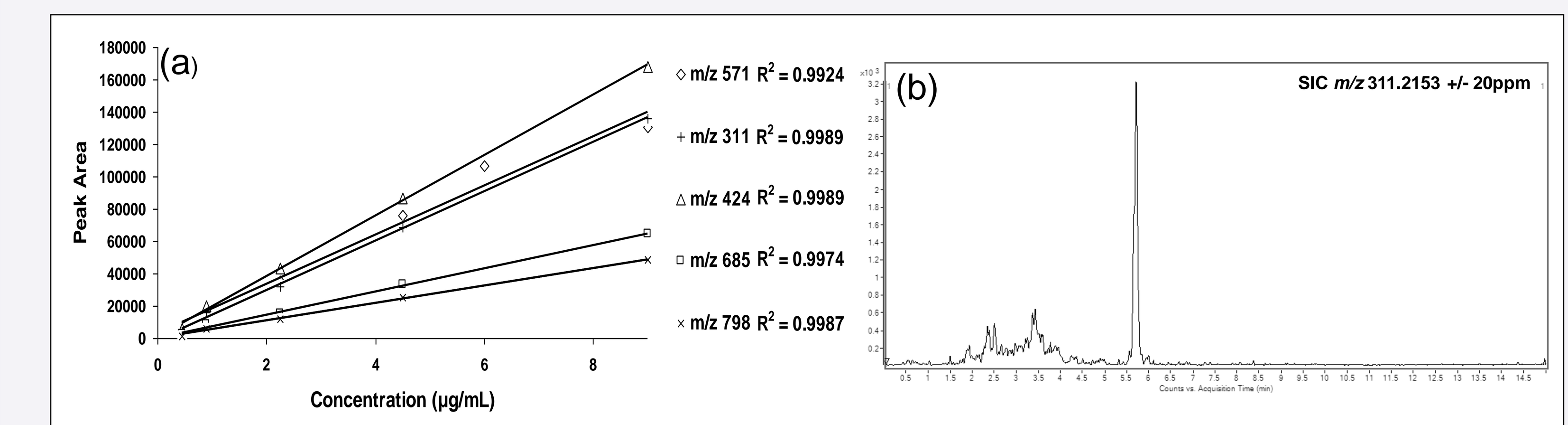


**Figure 5.** (a) Calibration graphs for the LC-FISCID-MS analysis of the FAIMS-selected (CV 1.75-1.85 V) $[M+2H]^{2+}$ precursor ion (*m/z* 571) and four product ions (*m/z* 311, 424, 685 and 798) of gramicidin S spiked in human plasma tryptic digest and (b) LC-FISCID-MS chromatogram for *m/z* 311 fragment ion.

## Conclusions

- LC-FAIMS-MS and LC-FISCID-MS using microscale FAIMS has been demonstrated to be a quantitative technique applicable to metabolite and peptide determination in complex matrices
- A significant reduction in chemical noise was observed for the IAG metabolite in urine with FAIMS pre-selection, giving a linear response over three orders of magnitude ($R^2$ >0.99), an improvement in LOQ of a factor of 2 and improved precision.
- The selective FAIMS transmission of a peptide precursor ion prior to in-source CID-MS (LC-FISCID-MS) was used for the determination of gramicidin S in a plasma tryptic digest with good linearity ($R^2$ >0.99) and precision.

## References

**1.** Brown, L.J.; Toutoungi, D.E.; Devenport, N.A.; Reynolds, J.C.; Kaur-Atwal, G.; Boyl, P.; Creaser, C.S.; Anal. Chem., **2010**, 82, 9827. **2.** Shvartsburg, A.A.; Smith, R.D.; Wilks, A.; Koehl, A.; Ruiz-Alonso, D.; Boyle, B.; Anal. Chem., 2009, 81, 6289. **3.** Brown, L.J.; Smith, R.W.; Toutoungi, D.E.; Reynolds, J.C.; Bristow, A.W.T.; Ray, A.; Sage, A; Wilson, I.D; Weston, D.J.; Boyle, B.; Creaser, C.S.; Anal. Chem., **2012**, 84, 4095.

## Acknowledgements

The authors thank Owlstone Ltd. and Loughborough University for financial support. We thank Owlstone Ltd. and Agilent Technologies for the provision of instrumentation and technical support and AstraZeneca for the provision of chemicals used in the study.

Agilent Technologies
OWLSTONE
AstraZeneca
Loughborough University