### **UltraFAIMS Application Note**

**Quantitative Analysis** 

# Quantitative determination of an ibuprofen metabolite in urine

Improve LOQ and dynamic range using LC-ultraFAIMS-MS

Adding an ultraFAIMS separation stage to UHPLC-HRMS analysis can enhance the quantitation of target analytes in biological matrices. This application note describes the methodology, using the (R/S) ibuprofen 1-beta-O-acyl glucuronide metabolite in urine as an example. In this case, a 2x improvement in level of quantitation and dynamic range was achieved (see Table 1).

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#### Introduction



Figure 1: The miniature UltraFAIMS microchip (100µm gap design)

Tandem mass spectrometry using a triple quadrupole mass <sup>(100µm gap design)</sup> spectrometer capable of mass-selecting precursor ions for selected reaction monitoring is widely used in the quantitative determination of drugs and metabolites. However, recent years have seen the emergence of high resolution mass spectrometry (HRMS), using TOF or Orbitrap mass analysers, as an alternative to triple quadrupole instruments [1, 2]. The advantage of HRMS is that it provides both robust quantification and qualitative analysis using a single mass spectrometer platform. On the other hand, HRMS may lack the selectivity and sensitivity of selected reaction monitoring.

One approach to enhancing the selectivity of LC–MS analyses is to incorporate an ion mobility-based rapid gas-phase separation stage between the LC and the mass spectrometer. UltraFAIMS is a mobility-based pre-separator that uses high-field asymmetric waveform mobility separation (FAIMS) to add an extra dimension of selectivity. This application note describes how this technique has been used in conjunction with UHPLC-HRMS to increase level of quantitation (LOQ) and dynamic range by a factor of ~2 in the determination of the (R/S) ibuprofen 1-beta-O-acyl glucuronide (IAG) metabolite in urine.

#### **Experiments**

The experiments were carried out using an Agilent 1200 series HPLC interfaced with an Agilent 6230 time-

Table 1: Summary of performance	improvements using ultraFAIMS
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Parameter	UHPLC-MS	UHPLC-FAIMS-MS
LOQ (µg/ml)	0.018	0.010
LDR (µg/ml)	0.018-11	0.010-11
R <sup>2</sup>	0.9991	0.0087
Intra-day (%RSD)	5.0	2.7

of-flight mass spectrometer fitted with an Agilent JetStream ESI source (AJS) operated in negative ion mode. The ultraFAIMS device was located in front of the transfer inlet capillary, behind a modified spray shield within the AJS source (see Figure 2).

Aliquots of urine (5ml) from healthy adult males (2x) and females (2x) was

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pooled (20 ml) and filtered (0.45  $\mu$ m), diluted (2x) with a solution of the IAG metabolite (0.055–44 $\mu$ g/ml in 50:50 acetonitrile:aqueous ammonium acetate (10mM) at pH3), corresponding to urine concentrations in the range 0.028–22 $\mu$ g/ml.

Samples were introduced into the ESI source either by direct infusion or from the liquid chromatograph. LC and MS settings used are reported in Table 2.

## Step 1. Selection of optimum ultraFAIMS settings

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A direct infusion of ibuprofen and the IAG metabolite  $(0.65\mu g/ml)$  was used to optimise the ultraFAIMS conditions for separation and sensitivity. UltraFAIMS was operated in scanning mode, varying both compensation field (CF) and dispersion field (DF) in turn. CF spectra of the  $[M-H]^-$  ions of the analytes obtained at a DFs of 200, 230 and 260 Td are shown in Figure 3. The IAG response is separated from that of the parent drug ibuprofen as a result of structural differences between the two ions.

The separation of IAG and the parent drug improves with increased DF. As expected, the absolute



Figure 2: UltraFAIMS interface installed on Agilent 6230TOF

intensity of both IAG and ibuprofen decreases when the DF is increased from 200 to 260 Td, but the response of IAG relative to ibuprofen is enhanced, since the ibuprofen intensity falls more sharply with increasing DF. The optimum CF for transmission of IAG was at 2.2 Td with a

Table 2: MS and LC conditions

DF of 260 Td, based on the best separation without significant loss of signal

Setting
10 scans/sec for scanning FAIMS experiments; 1 scans/sec for UHPLC-FAIMS-MS
4kV
2kV
65V
-150V
150 <sup>0</sup> C
350 <sup>0</sup> C
25psig
10L/min
11L/min
Zorbax C18 column, 2.1x50mm, 1.8µm
0.2mL/min
Isocratic 50:50 ACN:acqueous ammonium acetate (10mM) at pH 3

intensity for IAG, and these conditions were used in the high resolution UHPLC–ultraFAIMS–MS analyses.

## Step 2. Determination of IAG by UHPLC-ultraFAIMS-TOF-MS

UHPLC-MS analysis of the samples of IAG spiked into urine was then carried out, first with no ultraFAIMS separation and then using the ultraFAIMS settings determined above.

The UHPLC–MS (ultraFAIMS off) and UHPLC–ultraFAIMS–MS (ultraFAIMS on) selected ion chromatograms ( $[M-H]^-$ ; m/z 381) obtained with a high resolution mass window of m/z 381 ± 0.02 (±50ppm) are shown in Fig. 4a. The UHPLC gradient was adjusted to minimise the run time for the IAG, but this resulted in a significant overlap between the IAG peak and urine matrix components. The observed coelution of IAG with matrix components could be reduced by changing the UHPLC



conditions to separate the metabolite from the urine matrix, but at the cost of a significantly increased chromatographic run time. A narrower mass window, m/z 381 ±0.008 (±20ppm) was therefore investigated to determine whether this would improve selectivity without extending the chromatographic run time. The absolute intensity of the IAG peak, with the ultraFAIMS switched off, is reduced by a factor of two if the mass window is narrowed from ±50ppm to ±20ppm (Fig. 4b), but there

Figure 3: FAIMS CF spectra of ibuprofen, m/z 205, (dashed line) and IAG, m/z 381 (solid line) at DF: (a) 200 Td, (b) 230 Td and (c) 260 Td.

is no additional discrimination against the chemical noise from the urine matrix at the mass resolution used in the analysis (5700 FWHM).

The advantage of incorporating the ultraFAIMS separation is demonstrated by a significant reduction in the co-eluting chemical noise from the urine matrix (Fig. 4c and d), whilst maintaining a rapid elution time.

The removal of chemical noise with ultraFAIMS on resulted in an improvement in signal-to-noise ratio and



better peak integration, even though the absolute intensity of the IAG peak was reduced because of ion losses in the device. UltraFAIMS pre-selection of IAG removes matrix ions to baseline for both the m/z 381 ±0.02 and ±0.008 mass windows, but at a cost of lower sensitivity and signal-to-noise ratio for the narrower window.

With ultraFAIMS off, the mass spectrum taken from the IAG UHPLC peak (Fig. 5a) showed IAG (m/z 381.1570; -3.9ppm) to be a minor peak in a complex mass spectrum. With ultraFAIMS on, the mass spectrum for IAG was simplified (Fig. 5b), with IAG as the base peak in the mass spectrum (*m*/*z* 381.1553; 0.52ppm), as a result of discrimination against interferences from urine matrix.

Figure 4: Selected ion chromatograms (m/z 381) for IAG (highlighted) spiked into urine (0.55  $\mu$ g/ml) analysed by UHPLC–MS (ultraFAIMS off) using a mass window of (a) m/z 381  $\pm$ 0.02 and (b) m/z 381  $\pm$ 0.008; and by UHPLC– FAIMS-MS (ultraFAIMS on) with selective transmission of IAG (DF 260 Td, CF 2.2 Td) using a mass window of (c) m/z 381  $\pm$ 0.02 and (d) m/z 381  $\pm$ 0.008.

Owlstone Limited, 127 Cambridge Science Park, Milton Road, Cambridge CB4 0GD, UK www.ultrafaims.com



#### **Quantitative Performance**

As summarized earlier in Table 1:

- The limit of quantitation (LOQ; signal-to-noise 10:1) for IAG was reduced from 18ng/ml (ultraFAIMS off) to 10ng/ml (ultraFAIMS on), based on the selected ion peak areas of IAG (m/z 381 ± 0.02).
- The upper limit of the linear dynamic range (LDR) was the same in both ultraFAIMS off and on modes, giving an increased LDR of >3 orders of magnitude for ultraFAIMS–MS, compared with 2 orders of magnitude with cylindrical FAIMS–MS [3, 4] and <2.5 orders of magnitude for drift tube IM–MS [5, 6].
- Intra-day reproducibility was also compared. %RSDs of 5.0% and 2.7%, sufficient for good quantification, were obtained for both UHPLC–MS and UHPLC–ultraFAIMS–MS, with the UHPLC–FAIMS–MS showing the better reproducibility.

These data demonstrate that the ultraFAIMS device enhances quantitative performance compared to high resolution UHPLC and MS analysis alone.

#### References

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This work was first published as Enhanced performance in the determination of ibuprofen 1-beta-O-acyl glucuronide in urine by combining high field asymmetric waveform ion mobility spectrometry with liquid chromatography-time-of-flight mass spectrometry, Smith RW, Toutoungi DE, Reynolds JC, Bristow AW, Ray A, Sage A, Wilson ID, Weston DJ, Boyle B, Creaser CS. Journal of Chromatography A, 1278 (2013) 76–81.

The (R/S) ibuprofen 1-beta-O-acyl glucuronide was supplied by Astra Zeneca UK.