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Enhanced performance in the determination of ibuprofen 1-β-O-acyl glucuronide in urine by combining high field asymmetric waveform ion mobility spectrometry with liquid chromatography-time-of-flight mass spectrometry

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ABSTRACT

The incorporation of a chip-based high field asymmetric waveform ion mobility spectrometry (FAIMS) separation in the ultra (high)-performance liquid chromatography–high resolution mass spectrometry (UHPLC–HRMS) determination of the (R/S) ibuprofen 1-β-O-acyl glucuronide metabolite in urine is reported. UHPLC–FAIMS–HRMS reduced matrix chemical noise, improved the limit of quantitation approximately two-fold and increased the linear dynamic range compared to the determination of the metabolite without FAIMS separation. A quantitative evaluation of the prototype UHPLC–FAIMS–HRMS system showed better reproducibility for the drug metabolite (%RSD 2.7%) at biologically relevant concentrations in urine. In-source collision induced dissociation of the FAIMS-selected deprotonated metabolite was used to fragment the ion prior to mass analysis, enhancing selectivity by removing co-eluting species and aiding the qualitative identification of the metabolite by increasing the signal-to-noise ratio of the fragment ions.

1. Introduction

The performance of a liquid chromatographic separation is determined both by the on-column separation and by the selectivity of the detector. UHPLC has significantly reduced chromatographic run times, but the use of selective detection, such as mass spectrometry, is required if the highest throughput is to be achieved. Tandem mass spectrometry using a triple quadrupole mass spectrometer capable of mass-selecting precursor ions for selected reaction monitoring is widely used in the quantitative chromatographic 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 coupling UHPLC with HRMS is that HRMS provides both robust quantification and qualitative analysis using a single mass spectrometer platform. However, HRMS may lack the selectivity and sensitivity of selected reaction monitoring.

One approach to enhancing the selectivity of LC–MS analyses is the incorporation of a rapid gas-phase separation by ion mobility (IM) spectrometry between the LC and the mass spectrometer. Two ion mobility approaches are currently utilised: drift tube-ion mobility spectrometry, which separates ions based on time taken to traverse a drift tube [3], and field asymmetric waveform ion mobility spectrometry (FAIMS), also known as differential mobility spectrometry [4]. Drift-tube IM has been interfaced with ultra (high)-performance liquid chromatography-mass spectrometry (UHPLC–IM–MS) to enhance the quantitative determination of drugs and metabolites in urine by removing co-eluting interferences [5,6]. However, incorporation of a drift-tube ion mobility separation significantly reduces the linear dynamic range (LDR) compared to LC–MS alone [7–10].

FAIMS separation is orthogonal to MS and acts as an on-line filter for ions entering the MS. Ions are pre-selected by FAIMS based upon their differential ion mobility – the difference in mobility under low and high electric fields. In FAIMS, a dispersion field (DF) with an asymmetric waveform alternating between low and high fields is applied across the gap between two parallel electrodes, causing ions to oscillate between the electrodes. If high and low field mobilities are different, ions drift towards one of the electrodes as

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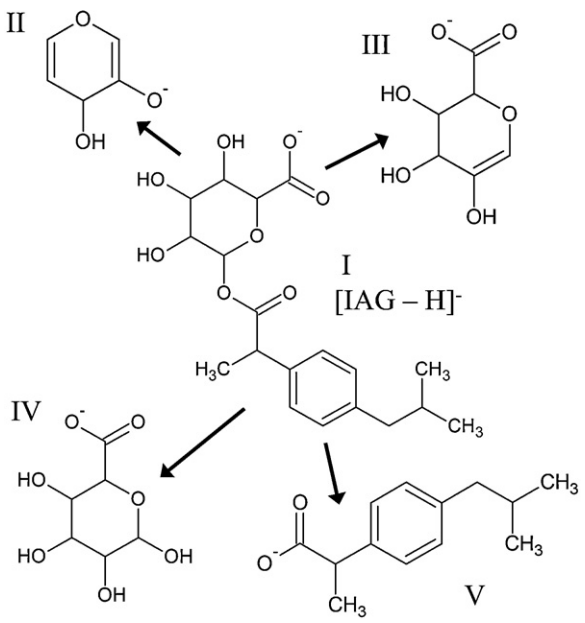

Fig. 1. Fragments produced from $[IAG-H]^-$ (I); decarboxylated di-dehydrated glucuronate (II); dehydrated glucuronate (III); glucuronate (IV); and aglycone (V).

they are carried through the electrode gap by a flow of nitrogen buffer gas at atmospheric pressure. The drift will result in a neutralising collision with one of the electrodes unless compensated for by a compensation field (CF). The CF for transmission of an ion is a characteristic of ion structure and is scanned at a fixed DF to determine the optimum CF at which the ion is preferentially transmitted, whilst other interfering ions are neutralised and filtered out [4,11]. FAIMS devices have been interfaced with MS to improve analytical selectivity by providing a differential mobility separation prior to MS detection of small molecules, metabolites and peptides [12–14].

Fragmentation of ions using a single mass analyser can be achieved by increasing voltages in the interface region between the ESI source and the vacuum of the mass spectrometer, called in-source CID, which will fragment all co-eluting ions generated in the ESI source [15]. The production of fragments from multiple precursors, without ion pre-selection, complicates fragmentation spectra making identification difficult. The effect of in-source CID on the analysis of glucuronide metabolites in biological matrices has been explored [16]. A comparison between in-source CID and collision cell CID using a triple quadrupole mass spectrometer for characterising microcystins in water samples showed that even though in-source CID proved to be more sensitive, CID in a collision cell was found to be more selective for identification. Fragmentation patterns were found to be similar for the two techniques [17]. Combining FAIMS with in-source CID, referred to FISCID–MS, has been used for the identification of an active pharmaceutical ingredient in a common pharmaceutical excipient and the qualitative and quantitative analysis of peptides in plasma by LC–FISCID–MS [12].

In this paper, we report enhanced chromatographic performance in the UHPLC–MS analysis of the drug metabolite (R/S) ibuprofen 1-β-O-acyl glucuronide, IAG (Fig. 1, I), in urine, without sample clean-up, by the incorporation of a FAIMS separation using a prototype chip-based FAIMS device. FAIMS pre-selection of the metabolite is shown to improve the limit of quantification, linear dynamic range and reproducibility, and allow a shorter chromatographic run time compared to UHPLC–MS by reducing chemical/matrix interference. The FAIMS device was also used to pre-select IAG for UHPLC–FISCID–MS, enhancing the qualitative identification of the metabolite.

2. Experimental

2.1. Chemicals

HPLC grade methanol (MeOH), acetonitrile (ACN), water, formic acid (FA) and ammonium acetate were purchased from Fisher Scientific (Loughborough, UK). (R/S) Ibuprofen 1-β-O-acyl glucuronide was supplied by AstraZeneca (Alderley Park, UK). Ibuprofen was extracted into methanol from an ibuprofen tablet (200 mg).

2.2. Sample preparation

Aliquots of urine (5 ml) from healthy adult males (2×) and females (2×) (AstraZeneca, UK) was pooled (20 ml) and filtered (0.45 μm), diluted (2×) with a solution of the IAG metabolite (0.055–44 μg/ml in 50:50 acetonitrile:aqueous ammonium acetate (10 mM) at pH 3), corresponding to urine concentrations in the range 0.028–22 μg/ml.

2.3. Instrumentation

UHPLC–FAIMS–MS and UHPLC–FISCID–MS analyses of IAG were carried out using an Agilent 1200 series HPLC interfaced with an Agilent 6230 time-of-flight mass spectrometer fitted with a JetStream ESI source operated in negative ion mode (Agilent Technologies, Santa Clara, CA, USA). The prototype chip-based FAIMS device (Owlstone, Cambridge, UK) has been previously described elsewhere [12] and was located in front of the transfer inlet capillary, behind a modified spray shield within a Jet Stream ESI source. The dispersion field (DF) feeder supplied asymmetric waveforms to the FAIMS device through a hole in the desolvation assembly. The FAIMS device has a 100 μm electrode gap and a depth of 700 μm with the FAIMS electrodes arranged as multiple parallel channels, linked by a serpentine channel – trench length (50–100 mm). Dispersion fields in the range 200–300 Td were applied at a 27 MHz frequency with an approximate low to high field ratio of 2:1. Nitrogen (99.5% purity) was used as the carrier gas for the FAIMS system and for the ESI source and mass spectrometer interface.

Samples were introduced into the ESI source either by direct infusion or from the liquid chromatograph. UHPLC separation was carried out, with a 5 μl sample injection volume on a Zorbax C18 column (2.1 mm × 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. The scan rate of the TOF MS was 10 scans/s for scanning FAIMS–MS experiments and 1 scan/s for UHPLC–FAIMS–MS and UHPLC–FISCID–MS experiments. Data were acquired with the instrument mode set to extended dynamic range (2 GHz) at a resolution of 5700. Source conditions for LC experiments were nozzle voltage, 2000 V; sheath gas temperature and flow, 350 °C and 11 L/min; drying gas temperature and flow, 150 °C and 10 L/min; nebuliser pressure, 25 psig; transfer capillary, 4000 V; skimmer voltage, 65 V; fragmentor voltage, −150 V and −250 V for transmission and in-source CID respectively. Data were processed using Mass Hunter Qualitative Software B.04.00 (Agilent Technologies, Santa Clara, CA, USA) and Microsoft Excel 2010 (Microsoft, Seattle, USA).

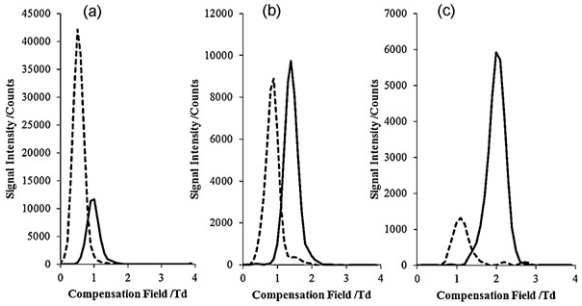

Fig. 2. FAIMS CF spectra of ibuprofen, m/z 205, (dashed line) and IAG, m/z 381 (solid line) at DF: (a) 200 Td, (b) 230 Td and (c) 260 Td.

3. Results and discussion

3.1. Direct infusion FAIMS analysis of IAG and ibuprofen

Direct infusion of ibuprofen and the IAG metabolite (0.65 μg/ml) was used to optimise the microscale FAIMS conditions for separation and sensitivity. FAIMS compensation field (CF) spectra of the $[M-H]^-$ ions of the analytes obtained at a dispersion field (DF) of 200, 230 and 260 Td are shown in Fig. 2. 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. A decrease in the absolute intensity of IAG was observed when the DF was increased from 200 to 260 Td, but with a significant increase in the response relative to ibuprofen, which falls sharply with increasing DF. The optimum CF for transmission of IAG was at 2.2 Td with a DF of 260 Td, based on the best separation without significant loss of signal intensity for IAG, and these conditions were used in the high resolution UHPLC–FAIMS–MS analyses.

3.2. Determination of IAG by UHPLC–FAIMS–TOF-MS

The UHPLC–MS (FAIMS off) and UHPLC–FAIMS–MS (FAIMS on) selected ion chromatograms ($[M-H]^-$; m/z 381) obtained for the analysis of IAG spiked into urine with a high resolution mass window of m/z 381 ± 0.02 (±50 ppm) is shown in Fig. 3a. 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 co-elution 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 (±20 ppm) was therefore investigated to

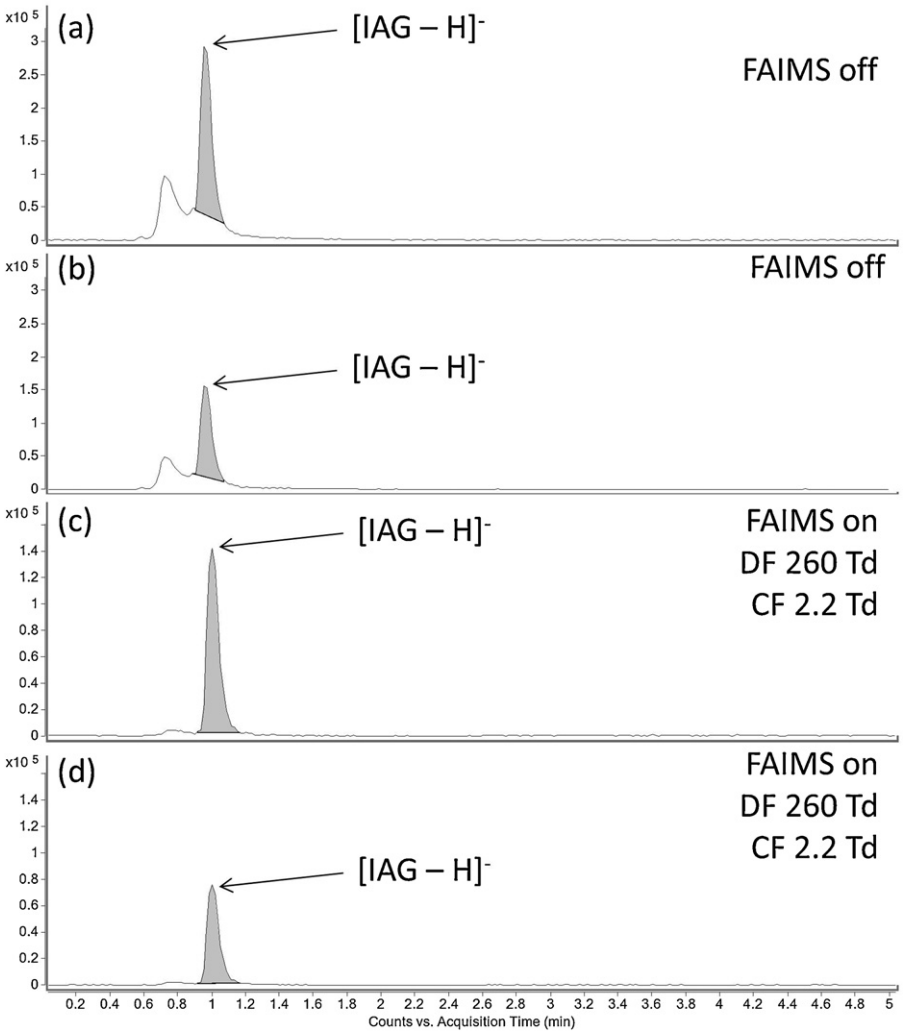

Fig. 3. Selected ion chromatograms (m/z 381) for IAG (highlighted) spiked into urine (0.55 μg/ml) analysed by UHPLC–MS (FAIMS off) using a mass window of (a) m/z 381 ± 0.02 and (b) m/z 381 ± 0.008; and by UHPLC–FAIMS–MS (FAIMS on) with selective transmission of IAG (DF 260 Td, CF 2.2 Td) using a mass window of (c) m/z 381 ± 0.02 and (d) m/z 381 ± 0.008.

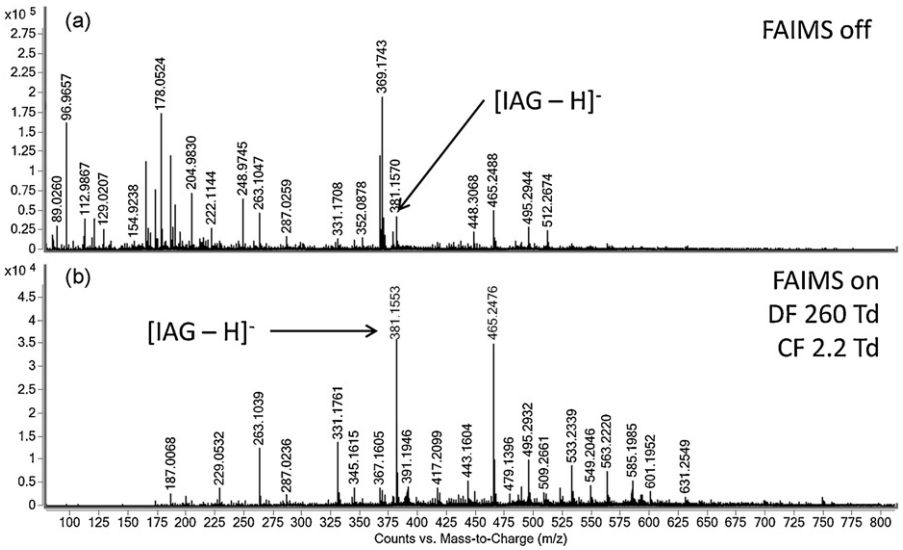

Fig. 4. Mass spectra at retention time (RT) 0.98–1.02 min with (a) FAIMS off, and (b) FAIMS on.

determine whether this would improve selectivity without extending the chromatographic run time. The absolute intensity of the IAG peak, with the FAIMS switched off, is reduced by a factor of two if the mass window is narrowed from ±50 ppm to ±20 ppm (Fig. 3b), but there 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 the incorporation of the FAIMS separation is demonstrated by a significant reduction in the co-eluting chemical noise from the urine matrix (Fig. 3c and d), whilst maintaining a rapid elution time. Ionisation suppression in the ESI source was not determined, but was the same in both FAIMS on and FAIMS off modes, allowing an evaluation of the improvement in the chromatographic performance offered by FAIMS. The removal of chemical noise resulted in an improvement in signal-to-noise ratio and better peak integration with FAIMS on, even though the absolute intensity of the IAG peak was reduced because of ion losses in the device. FAIMS 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. The ±0.02 mass window was therefore used in subsequent studies.

The mass spectrum taken from the IAG UHPLC peak with FAIMS off (Fig. 4a) showed IAG (m/z 381.1570; −3.9 ppm) to be a minor peak in a complex mass spectrum. The mass spectrum for IAG with FAIMS on (Fig. 4b) was simplified, with IAG as the base peak in the mass spectrum (m/z 381.1553; 0.52 ppm), as a result of discrimination against interferences from urine matrix.

Table 1 compares the quantitative performance of the UHPLC–MS and UHPLC–FAIMS–MS methods for the determination of IAG. The limit of quantitation (LOQ; signal-to-noise 10:1) for IAG was reduced from 18 ng/ml (FAIMS off) to 10 ng/ml (FAIMS on), based on the selected ion peak areas of IAG (m/z 381 ± 0.02) using UHPLC–FAIMS–MS. The upper limit of the linear dynamic range (LDR) was the same in both FAIMS off and on modes, giving an increased LDR of >3 orders of magnitude for the miniaturised FAIMS–MS, in contrast to ∼2 orders of magnitude with cylindrical FAIMS–MS [18,19] and <2.5 orders of magnitude for drift tube IM–MS [5,6]. The intra-day reproducibility of the prototype UHPLC–FAIMS–MS system was compared with UHPLC–MS by analysing IAG spiked into urine (15.5 μg/ml) and running FAIMS in on and off modes respectively (Table 1). %RSDs, sufficient for good quantification, were obtained for both UHPLC–MS (5.0%) and UHPLC–FAIMS–MS (2.7%), with the UHPLC–FAIMS–MS showing better reproducibility. These data demonstrate that the FAIMS device enhances quantitative performance compared to high resolution UHPLC and MS analysis.

Table 1
A comparison of LOQ; LDR (R^2) and intra-day reproducibility for the determination of IAG spiked into urine (15.5 μg/ml, $n = 5$).

	UHPLC–MS	UHPLC–FAMS–MS
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

3.3. Qualitative identification of IAG by UHPLC–FISCID–MS

The in-source CID fragmentation of IAG ion was used to assess the improvement offered by the UHPLC–FISCID–MS technique for the qualitative identification of IAG. The FAIMS-selected $[M-H]^-$ ion of IAG was subjected to in-source CID to produce the characteristic fragments of the ion (Fig. 1). A comparison of the selected ion chromatograms of the aglycone fragment (m/z 205.1234) for a urine sample spiked with IAG (3.9 μg/ml), with and without FAIMS pre-selection prior to in-source CID, was used to define the level of isobaric chemical interference present in the sample spiked with IAG (Fig. 5a and b). Peak integration of the UHPLC–FISCID–MS data was more reliable than UHPLC–MS data because chemical interference was reduced almost to baseline.

Mass spectra extracted from the aglycone peaks (Fig. 6) shows the effect of applying the FAIMS separation to the UHPLC–CID–MS analysis of IAG. Four diagnostic fragment ions for IAG are observed in the production mass spectrum (Fig. 1) [20]: decarboxylated dehydrated glucuronate (II, m/z 113); dehydrated glucuronate (III, m/z 175); glucuronate (IV, m/z 193); and the aglycone (V, m/z 205). In Fig. 6a, these fragments are difficult to locate due to the complexity of the mass spectrum and poor signal to noise as a result of other ions in the matrix. Pre-selecting IAG using FAIMS before in-source CID reduced the intensity of interfering ions, increasing the relative intensity of the IAG (Fig. 6b). The signal-to-noise ratios for the fragment ions increased by approximately 2-fold with FAIMS on (Table 2), enhancing the response of the IAG fragments relative to other interfering peaks to aid identification.

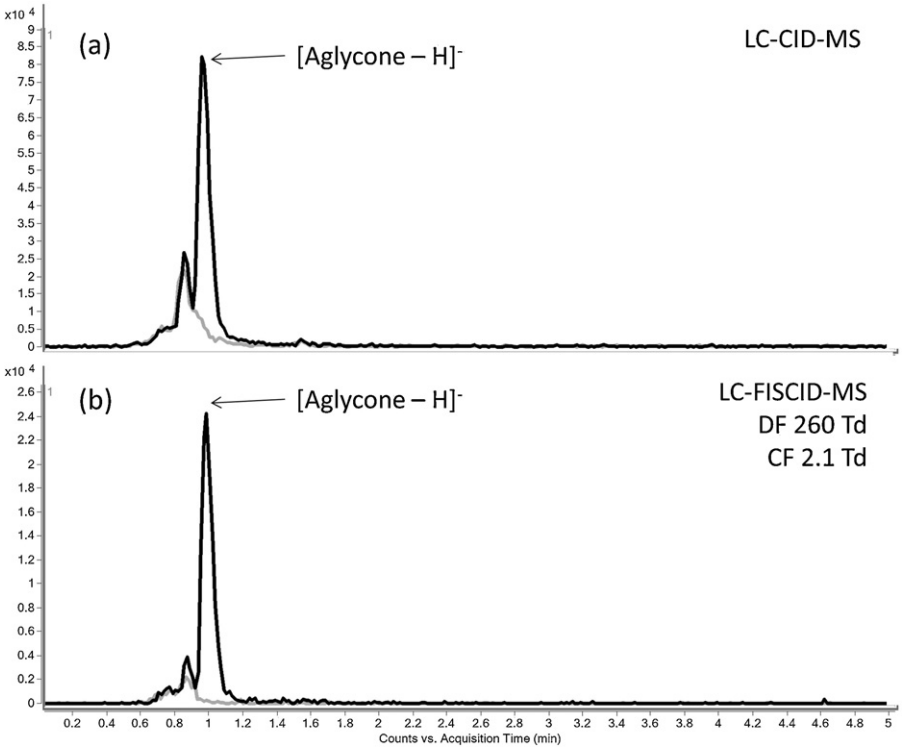

Fig. 5. Selected ion chromatograms (m/z 205 ± 0.02) of the [Aglycone−H]$^-$ for the urine blank (grey) and IAG (black) spiked into urine (3.9 μg/ml) by (a) UHPLC–CID–MS and (b) UHPLC–FISCID–MS.

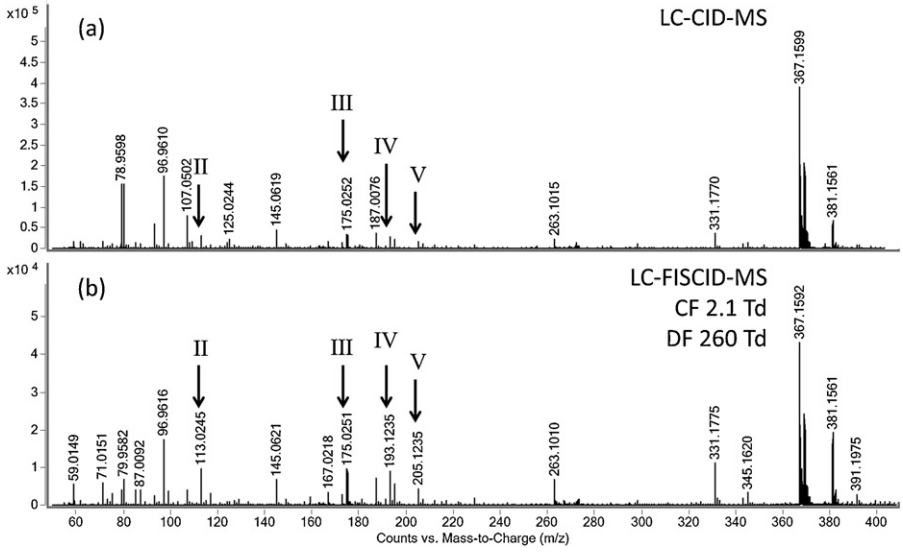

Fig. 6. Mass spectra at RT 0.97–1.02 min with (a) UHPLC–CID–MS, and (b) UHPLC–FISCID–MS.

Table 2
A comparison of signal-to-noise ratios for in-source CID generated IAG fragment ions from the analysis of a spiked urine sample with FAIMS off and FAIMS on.

Fragments	S:N ratio(FAIMS off)	S:N ratio(FAIMS on)
II	14.9	33.0
III	17.5	34.0
IV	13.9	31.6
V	8.6	16.4

4. Conclusions

The incorporation of the chip-based FAIMS in the ESI source of the UHPLC-TOF spectrometer is a novel approach for improving the qualitative and quantitative performance of the chromatographic analysis of IAG by reducing co-eluting chemical interference from the urine matrix. UHPLC–FAIMS–HRMS showed a lower LOQ, increased LDR and better reproducibility, compared to UHPLC–HRMS without a FAIMS separation. The FAIMS device was able to distinguish IAG from ibuprofen, preventing interference from the drug when observing the aglycone fragment. The UHPLC–FISCID–MS technique removed interference from urine in

the chromatogram for IAG and enhanced the qualitative identification of fragments of IAG by reducing the relative response of interfering peaks from the urine.

Acknowledgements

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