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High-Throughput Analysis of Drugs in Biological Matrices with Enhanced Selectivity for Quantitation Using FAIMS SPE/MS/MS

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Introduction

High selectivity is essential for reliable quantitation of analytes of interest in biological samples. This is especially true in cases of in-source dissociation in which parent or metabolite drug levels may be overestimated due to the conversion of one compound to the other. The conversion of a metabolite into the parent drug, for example, can interfere with the assay if chromatographic separation is limited between the metabolite and its parent molecule.

High-Field Asymmetric Waveform Ion Mobility Spectrometry (FAIMS) can effectively separate ions based on variation of ion drift velocity in atmospheric pressure environments at very high electric fields. Therefore FAIMS is an attractive candidate for enhancing the separation provided by liquid chromatography or introducing separation to high-throughput technologies like SPE/MS/MS for quantitation of drugs in biological matrices. In this work, we demonstrate the ability to separate and measure two drug pairs using the combined technologies of fast-scan chip FAIMS and the Agilent RapidFire High-Throughput Mass Spectrometry System, an ultrafast SPE/MS/MS.

Experimental

Hardware

The Owlstone (Cambridge, UK) microscale chip-FAIMS device was coupled to a RapidFire/MS/MS system which consisted of the following modules: Agilent RapidFire 360, Agilent 6460 Triple Quadrupole Mass Spectrometer, and Mass Hunter Qualitative Analysis (B.05.00). Samples were analyzed at a rate of 12 seconds or less per sample using conditions shown in Table 1 below.

Chemicals and reagents

Acetaminophen, phenacetin, morphine, morphine-d3, morphine-6-β-D-glucuronide, and morphine-6-β-D-glucuronide-d3 were purchased from Cerilliant, Round Rock, TX. All other LC/MS grade solvents and reagents were purchased from Sigma-Aldrich St. Louis, MO.

Sample preparation

Acetaminophen and phenacetin were spiked into a solution of 50% acetonitrile and 50% water at 500 ng/mL. Morphine and morphine-6-β-D-glucuronide were spiked into drug-free human urine at 5000 ng/mL. A set of calibration standards was created using serial dilutions to achieve a concentration range of 500-5000 ng/mL. Urine samples were diluted 1/50 using 10mM ammonium formate, pH 9 containing morphine-d3 and morphine-6-β-D-glucuronide-d3 as internal standards.

Table 1. RF/MS/MS Conditions

RapidFire Conditions	Acetaminophen-Phenacetin			Morphine-Glucuronide		
Buffer A	Water with 0.09% formic acid, 0.01% trifluoroacetic acid			10 mM ammonium acetate		
Buffer B	Acetonitrile with 0.09% formic acid, 0.01% trifluoroacetic acid			50% methanol, 50% isopropanol, 0.09% formic acid, 0.01% trifluoroacetic acid		
Injection volume	10 µL			10 µL		
SPE cartridge	Agilent RapidFire cartridge C (reversed-phase C18 chemistry, G9205A)					
Triple Quadrupole Conditions						
Gas temperature	150 °C			150 °C		
Gas flow	10 L/min			8 L/min		
Nebulizer	45 psi			35 psi		
Sheath gas temperature	400 °C			350 °C		
Sheath gas flow	11 L/min			11 L/min		
Analyte	**Q1**	**Q3**	**Dwell**	**Fragmentor**	**CE**	**CAV**
Acetaminophen	152.1	110	50	125	17	6
Phenacetin	180.1	110.1	50	140	21	6
Morphine	286.1	201	30	155	25	7
Morphine-d3	289.1	201	30	155	25	7
Morphine-6-β-D-glucuronide	462.1	286.1	30	175	33	5
Morphine-6-β-D-glucuronide-d3	465.4	289.2	30	140	33	5

Results and Discussion

Two parent and metabolite drug pairs were investigated for enhanced selectivity by separation using FAIMS. For this purpose compensation field (CF) scans of chip based FAIMS were performed in under 100 ms with switching between CF settings in less than 2 ms. This speed is enabled by using high values of dispersion field (DF) inside miniature channels and allows real-time optimization while running a series of RapidFire injections. Several CF ramps at varying DF settings were applied while injecting analyte standards by RapidFire to determine optimum FAIMS method parameters for separation.

The best separation of acetaminophen and phenacetin was achieved when using DF of 170 Td (Figure 1). This separation allowed for enhanced selectivity of acetaminophen by suppressing its parent drug phenacetin's signal. Figure 2 shows RapidFire MS/MS injection series for two chip FAIMS settings – ON and OFF. While the signal levels are comparable when FAIMS is not active, the phenacetin signal is drastically reduced when the FAIMS device parameters are parked at optimum values for acetaminophen ion transmission. While there is a certain loss to acetaminophen signal, phenacetin is suppressed 37^{x} more so this is a compromise that one would be willing to take given a very significant improvement in specificity.

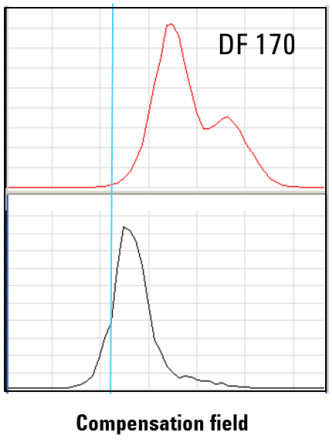

Figure 1. Optimized Separation of Acetaminophen (bottom) from Phenacetin (top)

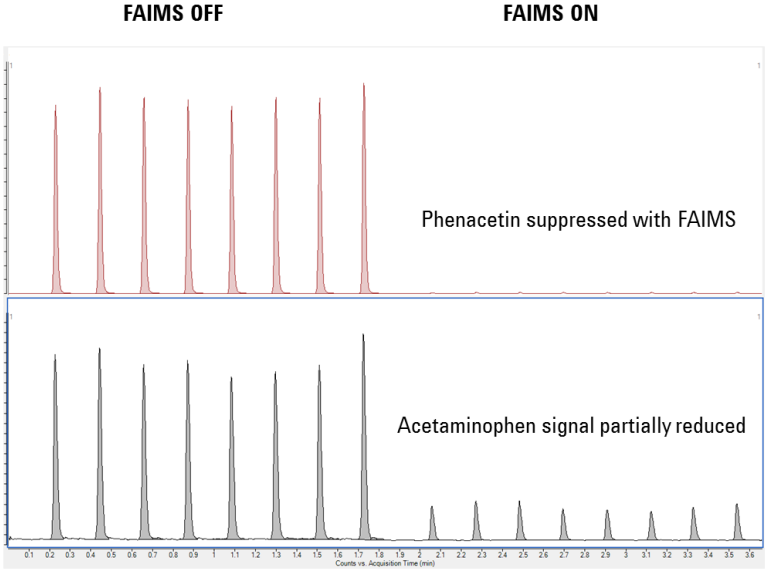

Figure 2. Suppression of Phenacetin Using FAIMS and RapidFire/MS/MS

The best separation between morphine and one of its metabolites morphine-6-β-D-glucuronide in urine was completed by the use of methanol (about 0.4% by volume) as a vapor modifier for FAIMS. Figure 3 demonstrates the optimization plot for CF at DF level of 230 Td. Addition of methanol drastically improves both separation and transmission efficiency of the FAIMS device. As a result, an enhancement of selectivity (about 25^{x}) for morphine was demonstrated (Figure 4) with relatively small reduction of the target analyte absolute signal intensity.

The same FAIMS parameters were applied during injections of a calibration curve of morphine and its metabolite in urine. In the absence of interference from morphine-6-β-D-glucuronide, morphine had excellent linearity in concentration range measured (500-5000 ng/ml) with an R2 of 0.999 (Figure 5).

Results and Discussion

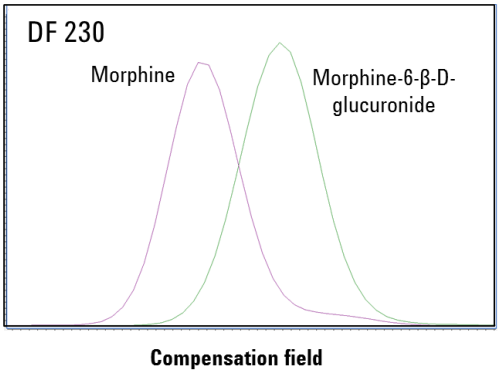

Figure 3. Optimized Separation of Morphine From Morphine-6-β-D-glucuronide

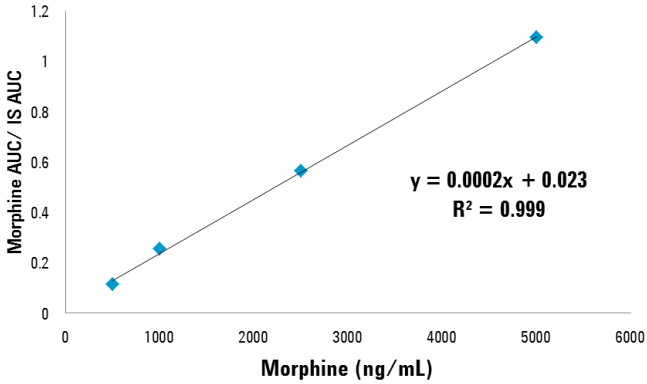

Figure 5. Standard Curve of Morphine in Urine

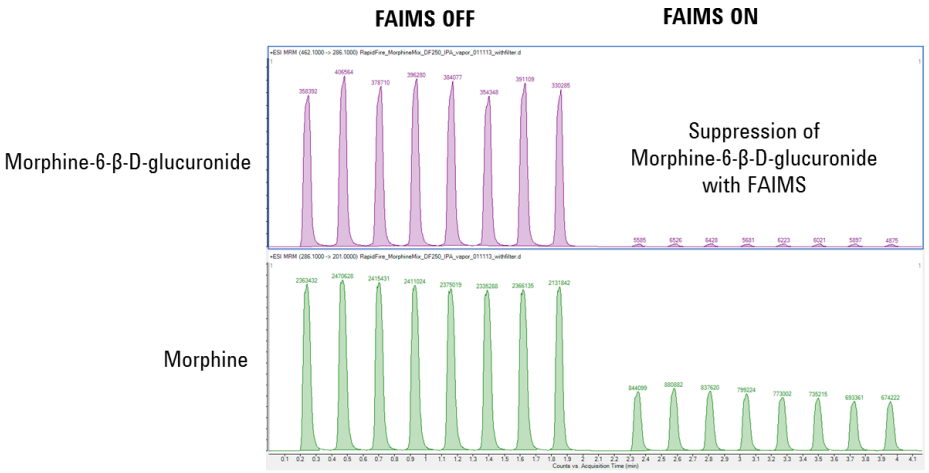

Figure 4. Enhanced Selectivity of Morphine Using FAIMS and RapidFire/MS/MS

Conclusions

Two parent drug and metabolite pairs of compounds were successfully separated using FAIMS and RapidFire/MS/MS. The ultrafast speed of CF scanning by FAIMS and seconds per sample speed of RapidFire enabled real-time method development for selectivity of analytes using FAIMS. The separation introduced by FAIMS enabled enhanced selectivity of acetaminophen from its parent drug phenacetin and morphine from its glucuronide metabolite without significantly compromising signal intensity. All samples were analyzed at a rate of 12 seconds or less, allowing throughputs of greater than 300 samples per hour. This methodology may be useful for enhanced selectivity and quantitation of other analytes that are susceptible to interference from in-source fragmentation.