

# ultraFAIMS-MS for High Throughput Separation and Quantification of Vitamin D Metabolite Stereoisomers

Rapid ultraFAIMS separation of stereoisomers can replace slow LC steps

Combining Ultra Field Asymmetric Ion Mobility Spectrometry (ultraFAIMS) with mass spectrometry (MS) and ionic modifiers enables quantification of Vitamin D metabolite 25-hydroxy  $D_3$  by removing isomeric interference from epimer 3-epi-25-hydroxy  $D_3$ , avoiding a lengthy liquid chromatography separation step.

The miniaturised ultraFAIMS system provides separation at timescales suitable for rapid sample introduction enabling high-throughput Vitamin D analysis in clinical settings.

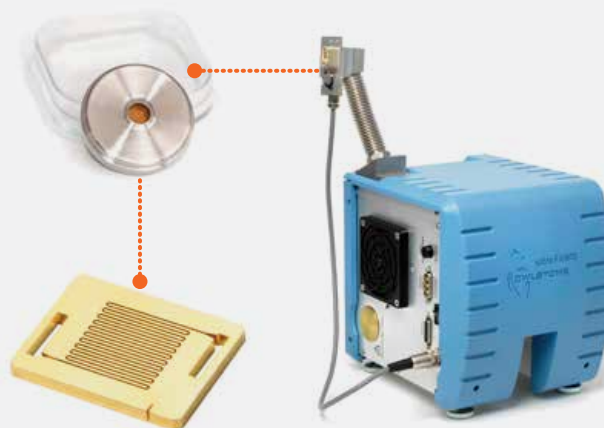


Figure 1: ultraFAIMS A1 device. FAIMS filter system integrates easily into commercial MS systems.

## Introduction

Vitamin D, along with calcium, promotes bone growth in children and aids in the prevention of osteoporosis in older adults. The major source of vitamin D in humans is the photoconversion (from exposure of skin to sunlight) of 7-dihydrocholesterol to pre-vitamin  $D_3$ , which then isomerizes to vitamin  $D_3$ . Vitamin  $D_3$  undergoes hydroxylation by enzymes in the liver to produce 25-hydroxy vitamin  $D_3$  (25-OH vitamin  $D_3$ ) which is generally believed to be the most abundant form circulating in the body.

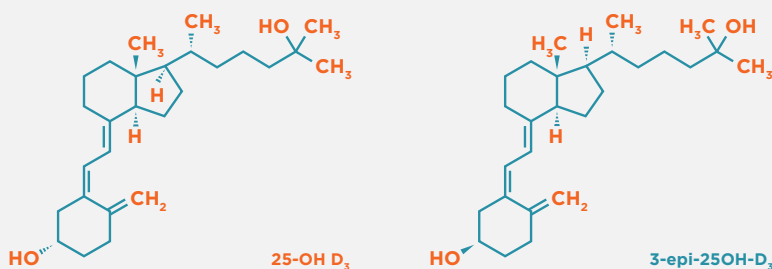


Figure 2: Structures of Vitamin D metabolites 25-OH  $D_3$  and 3-epi-25OH- $D_3$

Simultaneous and accurate measurements of circulating vitamin D metabolites are critical to studies of the metabolic regulation of vitamin D and its impact on health and disease. Accurate quantification is also vital for routine diagnostic assessment of vitamin D related diseases.

However, occurrence of the biologically inactive 3-epi analog of 25-OH  $D_3$  (3-epi-25-OH  $D_3$ ) has been reported; interference from this inactive 3-epi stereoisomer during measurement of vitamin D may lead to inaccurate information used for treatment and prevention of hypovitaminosis D.

At present, separation and quantification of 25-OH  $D_3$  and its epimer is achieved using High Performance Liquid Chromatography (HPLC)-MS/MS<sup>1</sup>. The liquid chromatography step in this process is slow, preventing high-throughput analysis of samples, making it unsuitable for a clinical setting. In this application note, we demonstrate that by using ultraFAIMS-MS combined with an ionic modifier, Vitamin D metabolites 25-OH  $D_3$  and 3-epi-25OH- $D_3$  can be separated and quantified at timescales that allow rapid sample introduction and therefore high-throughput analysis of clinical samples.

## Methods

### Instrumentation

Measurements were performed on an Agilent 6230 Time-of-flight (TOF) MS combined with an Owlstone UltraFAIMS A1 device (Figure 3)<sup>2-4</sup>. The ultraFAIMS microchip, which acts as a filter for specific ions, is composed of an array of parallel channels across which an asymmetric dispersion field (DF) is applied. Selected ions are transmitted through the chip by application of an appropriate compensation field (CF). Each epimer was analysed individually over a CF range of -1 to +5 V at 240 to 290 Townsend (Td) DF (1Td =  $10^{-17}$  V.cm<sup>2</sup>). The time taken to produce a scan of each epimer was 72 seconds (720 data points recorded).

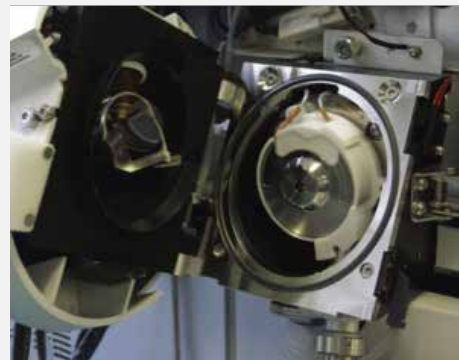


Figure 3: ultraFAIMS A1 device installed on Agilent 6230 ToF-MS

### Choice of ionic modifier

A range of ionic modifying group I element salts were studied to assess their ability to cause separation of the ultraFAIMS signal. Example CF spectra are shown in Figure 4, below.

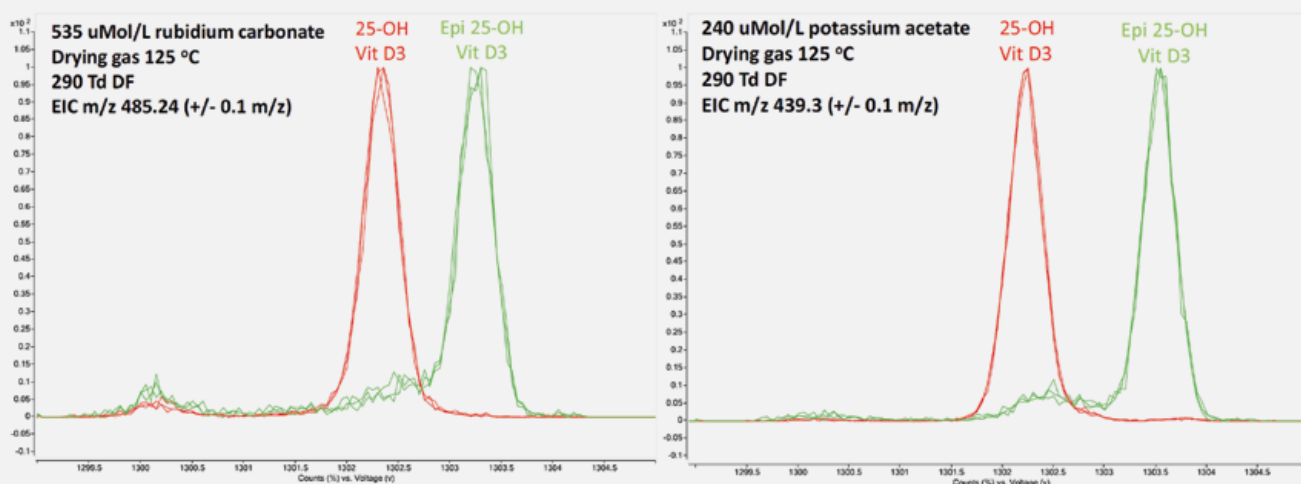


Figure 4: Extracted ion chromatograms for 25-OH D<sub>3</sub> and 3-epi-25-OH D<sub>3</sub> adducts, left - rubidium, right - potassium ionic modifier

Further details of all successful adduct-based separations of the epimers are detailed in Table 1. One interesting observation was that separation of epimers appeared to correlate with ionic radii e.g., potassium>sodium>lithium.

Table 1: Separation of epimers observed using ionic modifiers with UltraFAIMS and another commercial FAIMS-MS platform

Anion	ultraFAIMS					Other commercial FAIMS-MS system				
	25-OH D <sub>3</sub> CF position (Td)	3-epi-25-OH D <sub>3</sub> CF position (Td)	Peak position difference (Td)	Peak width (Td)	Resolution	25-OH D <sub>3</sub> CF position (Td)	3-epi-25-OH D <sub>3</sub> CF position (Td)	Peak position difference (Td)	Peak width (Td)	Resolution
Li <sup>+</sup>	2.3	3	0.7	0.5	1.4	5	10	5	6	0.8
Na <sup>+</sup>	2.2	3.5	1.3	0.5	2.6	5	15	10	8	1.3
K <sup>+</sup>	2.2	3.6	1.4	0.5	2.8	6	17	11	7	1.6
Rb <sup>+</sup>	2.2	3.3	1.1	0.5	2.2	5	13	8	6	1.3
Cs <sup>+</sup>	1.8	2.4	0.6	0.5	1.2	6	12	6	8	0.8

The experiments were repeated on another commercial FAIMS-MS system with the ultraFAIMS-MS system found to provide greater resolution between 25-OH D<sub>3</sub> & 3-epi-25-OH D<sub>3</sub> epimers (resolution defined as peak position difference / peak width) measurement).

### Separation optimisation

Rubidium acetate was chosen for the remainder of the study due to its ability to yield a relatively large separation, and the apparent reduced interference between 25-OH D<sub>3</sub> & 3-epi-25-OH D<sub>3</sub> epimers (Figure 4). The interference was assessed in more detail, comparing the ion counts from the tailing of each peak in relation to the ion counts from the apex of the FAIMS peak from each epimer; 2.05 Td CF for 25-OH D<sub>3</sub> and 2.95 Td CF for 3-epi 25-OH D<sub>3</sub> (Figure 5). Interference from peak tailing was ~2% based on elevated baseline for both 25-OH D<sub>3</sub> and 3-epi 25-OH D<sub>3</sub> from equimolar solutions.

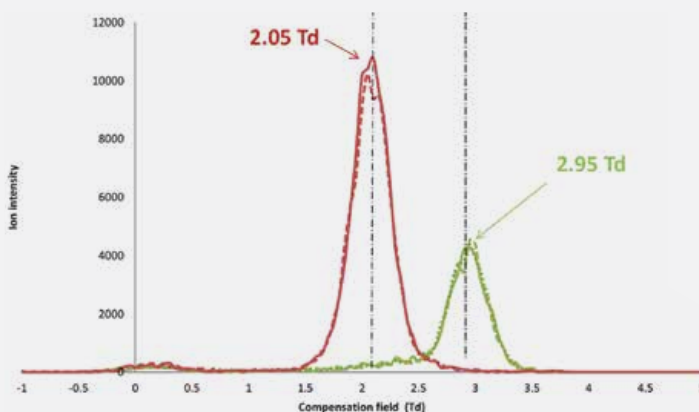


Figure 5: Optimised separation of 25-OH D<sub>3</sub> (red) and 3-epi-25-OH D<sub>3</sub> (green) Rb<sup>+</sup> adducts

### Calibration

A 535 μMol/L rubidium acetate salt solution was prepared in methanol/water (80:20). This was then used to prepare a stock solution of 5 μMol/L 25-OH D<sub>3</sub> and 500 nMol/L 3-epi-25-OH D<sub>3</sub>. The stock solution was then diluted further in rubidium salt solution to prepare a range of 10:1 calibration standards over a 25-OH D<sub>3</sub> concentration range of 50 nMol/L to 1μMol/L. The 10:1 ratio was selected as it was within the biologically relevant ratio quoted in the literature<sup>1</sup>. Data was collected at fixed FAIMS conditions of CF +2.05 Td (25-OH D<sub>3</sub>) and +2.95 Td (3-epi 25-OH D<sub>3</sub>), 30s for each epimer. Ion intensities were measured by averaging across each 30s time period and the data plotted as a calibration curve (Figure 6).

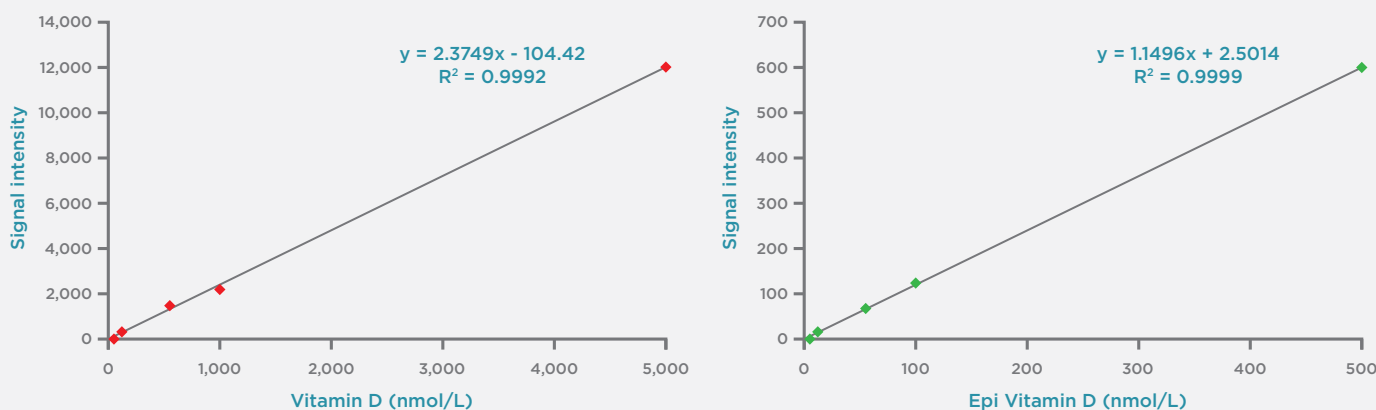


Figure 6: Calibration curves of 25-OH D<sub>3</sub> (red) and 3-epi-25-OH D<sub>3</sub> (green) Rb<sup>+</sup> adducts.

### Results

A 50 nMol/L 25-OH D<sub>3</sub> sample was analysed to test the calibration. Experimental results gave a calculated concentration of 53 nmol/L, +6% of actual concentration (50 nMol/L).

### Conclusions

- The ultraFAIMS device is highly-compatible with, and easily integrates into, standard commercial MS systems.
- Combining ultraFAIMS-MS with use of ionic modifiers enables accurate quantification of 25-OH D<sub>3</sub> by removing isomeric interference from naturally-occurring epimer 3-epi 25-OH D<sub>3</sub>.

- This method avoids lengthy LC separation steps making it suitable for rapid sample introduction techniques and high throughput clinical analyses.
- Combining ultraFAIMS-MS with rapid, online sample preparation techniques is an exciting prospect for high-throughput vitamin D analysis in the clinical environment.

### Keywords

*Stereoisomer separation, Vitamin D, epimer, metabolite, liquid chromatography, mass spectrometry, FAIMS, ultraFAIMS, ion mobility, differential mobility, DMS, IMS-MS, fast, rapid*

### References

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