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Investigation of Vitamin D Metabolites using Different Ion Mobility-Mass Spectrometry (IM-MS) Methods

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Introduction

Vitamin D is a secosteroid that regulates the absorption of calcium and phosphate in the body. It also plays roles in regulating the immune, endocrine, cardiovascular, and nervous systems. A deficiency in Vitamin D can cause brittle bones or muscle weakness, which can lead to health complications such as rickets, increased bone fractures, or osteoporosis.[1] In the body, Vitamin D is metabolized to 25-hydroxyvitamin D, and then to 24,25- or 1,25-dihydroxyvitamin D. C3 epimers of these metabolites have been shown to contribute less to calcium homeostasis (and have been implicated in certain disease states), but are not tested for clinically and thus interfere with measurement of accurate Vitamin D status in the body.[2] Currently, the active form of 25OHD (including its epimer) is used for clinical and laboratory testing of total Vitamin D levels. The most common methods used for analysis are immunoassays or chromatographic/mass spectrometric methods.[3] While fast and inexpensive, the main disadvantage of immunoassays is their lack of selectivity. However, with chromatographic/mass spectrometric methods such as LC-MS, issues arise with time consuming chromatographic separations (especially for those seeking to separate the epimers). Therefore, there is a need for more rapid analytical methods to quickly and accurately identify different Vitamin D metabolites in complex biosamples.

Ion mobility-mass spectrometry (IM-MS) rapidly (ms timescale) separates gas-phase ions based on differences in their size, shape, and charge. Since the shape of an ion can affect its mobility, this technique can be used to separate isomers or isotopologues.[4] Preliminary work from our group has shown promise for IM-MS in Vitamin D analysis.[5] In this project, I investigated the separation of various Vitamin D isomers and isotopologues using different IM-MS instruments including a low-resolution drift tube and a high-resolution Structures for Lossless Ion Manipulations (SLIM)-based traveling wave instrument. TWIMS was done in a short path and long path to increase resolution of the measurements.

Experimental Methods

Preparation of Vitamin D Metabolites

Solutions were prepared by combining Vitamin D metabolites (10 µg/mL), individually or as mixtures in 50% (v/v) aqueous methanol. The molecules used consisted of:

- 25 Hydroxyvitamin D2 and ($^{13}C_5$) isotopologue
- 25 Hydroxyvitamin D3 and ($^{13}C_5$) isotopologue
- 3-epi-25 Hydroxyvitamin D2 and ($^{13}C_5$) isotopologue
- 3-epi-25 Hydroxyvitamin D3 and ($^{13}C_5$) isotopologue
- 1,25 Dihydroxyvitamin D3 and ($^{13}C_5$) isotopologue
- 24R, 25 Dihydroxyvitamin D3 with d6 and ($^{13}C_5$) isotopologues

Data Processing

All data was visualized using Agilent MassHunter IM-MS Browser 10.0. Data was smoothed and drift spectra were converted to CCS values with the PNNL PreProcessor. SLIM HRIM data was manually fitted to gaussian peaks.

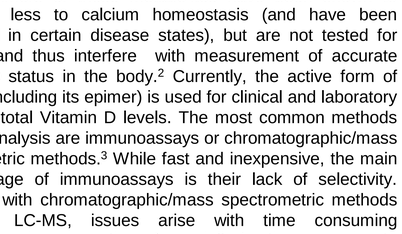

Figure 1. Schematic of the Agilent 6560 IM-QTOF instrument used.[6] The instrument contains a 78 cm drift tube maintained at 4 Torr nitrogen at 25 °C.

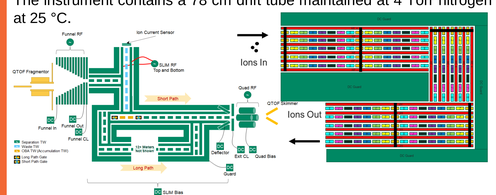

Figure 2. Schematic of the MOBILion TW-SLIM system.[7] This instrument features a 13 m SLIM design maintained at 2.5 Torr nitrogen and 25 °C.

Results & Discussion

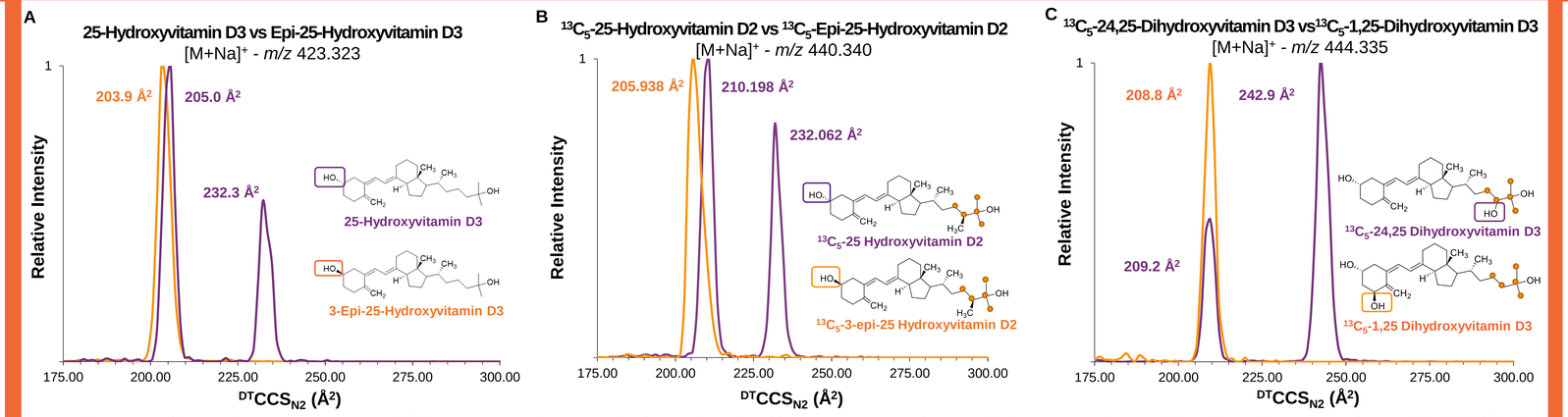

Figure 3. Ion mobility overlay comparison and CCS measurement of several Vitamin D metabolite isomer pairs including: (A) 25-hydroxyvitamin D3 vs. 3-epi-25-hydroxyvitamin D3; (B) 13C5-25-Hydroxyvitamin D2 vs 13C5-Epi-25-Hydroxyvitamin D2; and (C) 13C5-24,25-Dihydroxyvitamin D3 vs13C5-1,25-Dihydroxyvitamin D3. The results for 25OHD3 and epi25OHD3 show good agreement with previous measurements of the same compounds and demonstrate the ability to differentiate isomers.

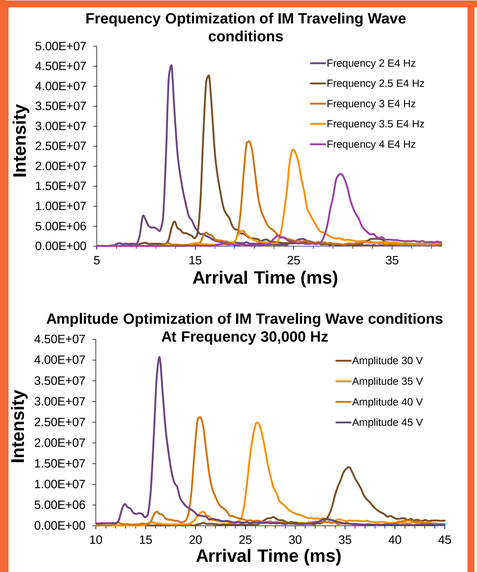

Figure 4. Optimization of traveling wave conditions in the MOBILion TW Slim system. Frequency and amplitude were adjusted to determine the best conditions for high signal intensity and good separation

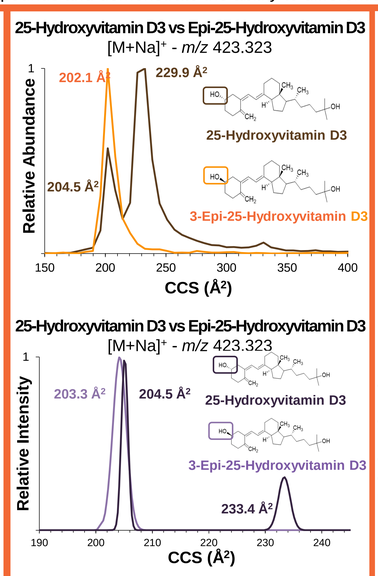

Figure 5. Overlay comparison and CCS measurement of 25-hydroxyvitamin D3 vs. 3-epi-25-hydroxyvitamin D3 under IM and HRIM conditions

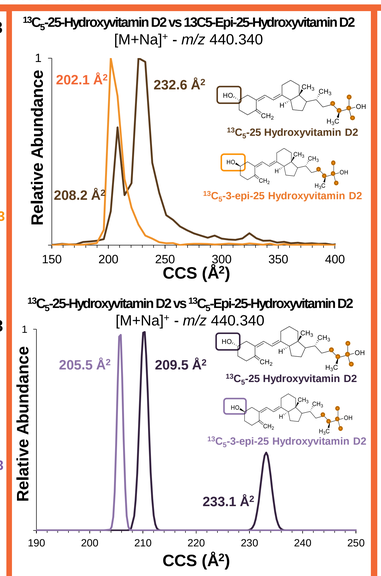

Figure 6. Overlay comparison and CCS measurement of $^{13}C_5$-25-Hydroxyvitamin D2 vs $^{13}C_5$-Epi-25-Hydroxyvitamin D2 under IM and HRIM conditions

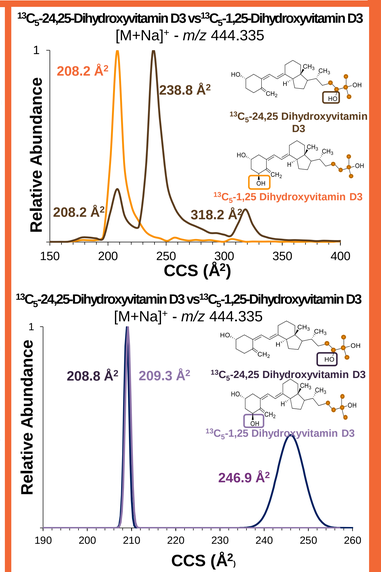

Figure 7. Overlay comparison and CCS measurement of $^{13}C_5$-24,25-Dihydroxyvitamin D3 vs$^{13}C_5$-1,25-Dihydroxyvitamin D3 under IM and HRIM conditions

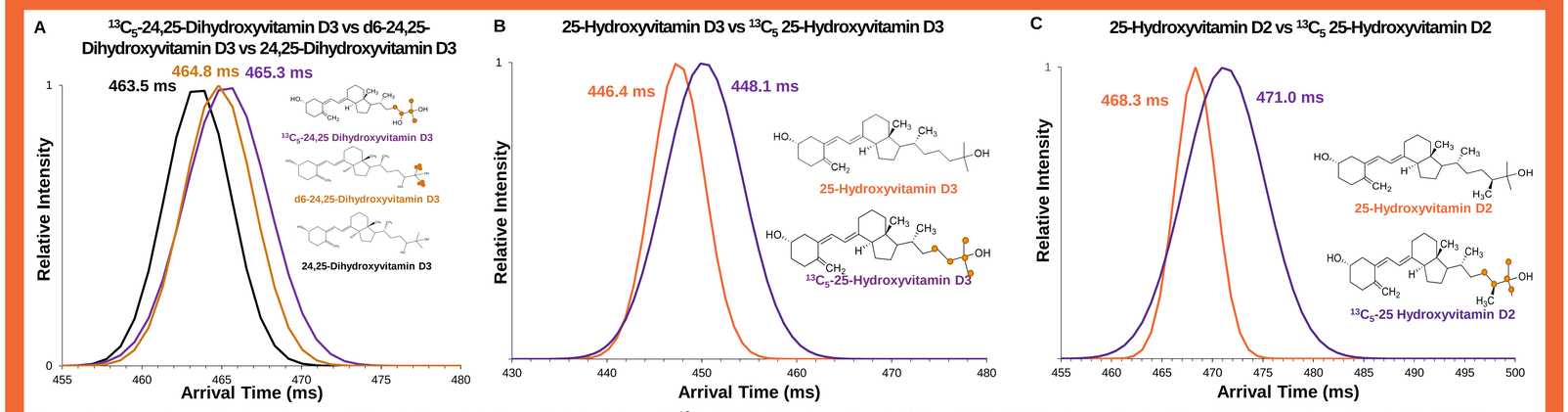

Figure 8. Comparison of isotopologues of Vitamin D metabolites which includes: (A)$^{13}C_5$-24,25 Dihydroxyvitamin D3 vs d6-24,25-Dihydroxyvitamin D3 vs 24,25-Dihydroxyvitamin D3; (B) 25-Hydroxyvitamin D3 vs $^{13}C_5$-25-Hydroxyvitamin D3; (C) 25-Hydroxyvitamin D2 vs $^{13}C_5$-25 Hydroxyvitamin D2

Summary & Conclusions

In this research, we investigated several IM-MS strategies for separation of Vitamin D metabolite isomers and isotopologues. First, drift tube IM measurements were made using an Agilent 6560 IM-QTOF, which demonstrated good separation of conformational differences between the isomers.

Next, we used SLIM IM, which required optimization of traveling wave (TW) conditions (amplitude and frequency) for sensitivity and selectivity. We observed that the best overall conditions involved setting the SLIM conditions to 30,000 Hz and 40 V. We used these parameters for the remainder of the measurements.

The current SLIM instrument can operate in low-resolution (0.4 m pathlength) and high-resolution (13 m pathlength) modes. We compared these two and observed that only very subtle separations between same isotope confirmers were observed in low-resolution mode. However, high-resolution mode resulted in much better separations amongst the isomer pairs and their conformers.

Finally, isotopic mobility shifts were investigated amongst different isotopologues in high-resolution mode. As expected, minor shifts in mobility were indeed observed between these isotopologues which were $^{13}C_5$- or d_6-labeled.

Overall, these different IM-MS strategies show promise for separation of clinically relevant Vitamin D metabolites.

References

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Acknowledgements

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