Differentiation of Vitamin D Epimers by Application of Trapped Ion Mobility Spectrometry (TIMS)

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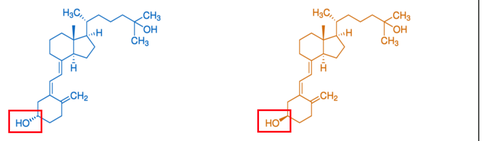
Introduction

Vitamin D deficiency is common in children and adults. It is associated with growth retardation, skeletal deformation, and osteomalacia and is presumed to play a role in chronic illnesses, such as common cancer, autoimmune diseases, and cardiovascular diseases. Hence, the interest in the analysis of its metabolites has seen a remarkable increase. However, difficulties arise from interfering epimers.

Vitamin D3 is photosynthesized in animals and enzymatically converted to 25-hydroxyvitamin D3 - one of the most abundant vitamin D metabolites. However, the common clinical target can erroneously be confused with its C3-epimer 3-epi-25-hydroxyvitamin D3 (see Fig. 1). Methods that fail to differentiate the two epimers lead to positively biased results. Therefore, clinical testing of vitamin D3 requires a technique with sufficient separation capabilities to resolve small molecule isomers with minor structural differences,.

In this study, the separation power of the Trapped Ion Mobility Spectrometry (TIMS) is explored for the vitamin D epimers 25-hydroxyvitamin D3 and 3-epi-25-hydroxyvitamin D3.



25-hydroxyvitamin D3
(25-OH Vit.D3)
Molar mass: 400.64 g/mol
$[M+Na]^+$: *m/z* 423.3234

3-epi-25-hydroxyvitamin D3
(3-epi-25-OH Vit.D3)
Molar mass: 400.64 g/mol
$[M+Na]^+$: *m/z* 423.3234

Fig. 1 Structures of 25-hydroxyvitamin D3 (left) and 3-epi-25-hydroxyvitamin D3 (right), indicating the major difference between the to epimers. The epimers differ in the chirality of the C3 hydroxyl group (red).

Methods

- Sample: 25-hydroxyvitamin D3, 3-epi-25-hydroxyvitamin D3
- Set up: Direct infusion experiment
- MS instrument: timsTOF Pro 2 (Bruker)
- Processing: DataAnalysis 5.3 software (Bruker).
- Data acquisition:
  - ESI-source operated in positive ionization mode
  - TIMS mode

Results

Two stable conformations (Table A & Figure 2)

- First, the TIMS data was collected for standard solutions of 25-OH Vit.D3 and 3-epi-25-OH Vit.D3.
- Both full mass spectra revealed the sodiated monomer $[M+Na]^+$ as major ion identified.
- The separation power of the TIMS device revealed distinctive mobility peaks for the sodiated monomer of 25-OH Vit.D3 and 3-epi-25-OH Vit.D3 which only differ in their C3 hydroxyl group.
- For 25-OH Vit.D3 a second mobility peak can be utilized for epimer identification: According to literature the sodiated monomer can result in two stable conformations [1].
- Depending on the coordination of the sodium a closed conformation or a more linear open conformation is generated for 25-OH Vit.D3. Thus, two CCS values can be measured – at 206 Å² and 232 Å², respectively.
- The experimentally measured CCS values and resolution in case of the single standards and the mixture of the sample are listed in the result table A.

Result Table A

	CCS [Å²]	Resolution $1/K_0$
25-OH Vit.D3 (Peak 1)	206.6	150.5
25-OH Vit.D3 (Peak 2)	233.0	146.2
3-epi-25-OH Vit.D3	204.7	99.2
Vit.D3 Mix (Peak 1)	204.8	122.6
Vit.D3 Mix (Peak 2)	206.9	138.3
Vit.D3 Mix (Peak 3)	232.7	134.2

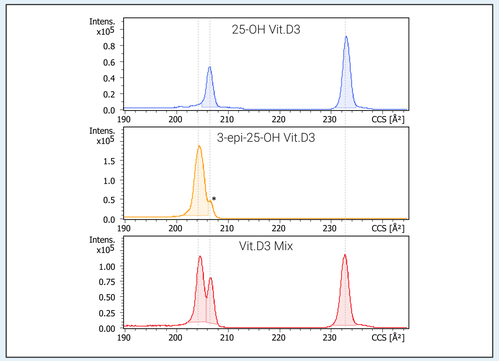


Fig. 2 Extracted ion mobilograms of the sodium adducts (*m/z* 423.3234): 25-OH Vit.D3 (blue), 3-epi-25-Vit.D3 (yellow) and the Vit.D3 Mix (red). The sodiated monomer of 25-OH Vit.D3 (blue) can result in two stable conformations. (The peak shoulder (*) can be allocated to impurity.)

Epimer separation (Table B & Figure 3)

- The second experiment was focused exclusively on the coeluting CCS peaks of 25-OH Vit.D3 and 3-epi-25-OH Vit.D3, which can be attributed to their according stable closed conformations.
- For the measurements in the focused mode, the scan range was adjusted according to the eluting profile of the compounds
- The extracted ion mobilograms depict the ability of an unambiguous differentiation of the similar eluting profiles in ion mobility.
- The CCS of vitamin D epimers measured agreed well with published values [1].

Result Table B

	CCS [Å²]	Resolution $1/K_0$
25-OH Vit.D3	206.2	159.6
3-epi-25-OH Vit.D3	204.1	137.0
Vit.D3 Mix (Peak 1)	204.1	152.5
Vit.D3 Mix (Peak 2)	206.3	163.4

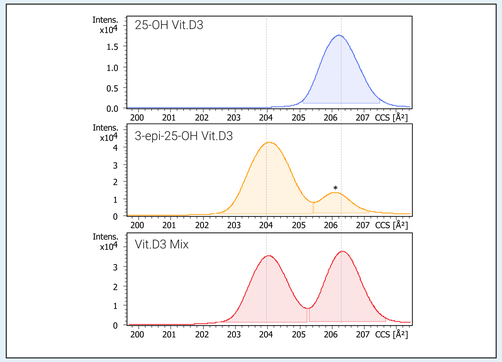


Fig. 3 Extracted ion mobilograms of the sodium adducts (*m/z* 423.3234): 25-OH Vit.D3 (blue), 3-epi-25-Vit.D3 (yellow) and the Vit.D3 Mix (red). Measurements were focused on the "closed confirmations". (The peak shoulder (*) can be allocated to impurity.)

References

[1] Chouinard, C.D., Cruzeiro, V.W.D., Beekman, C.R. et al.. J. Am. Soc. Mass Spectrom. 28, 1497–1505 (2017).

Conclusion

Trapped Ion Mobility Spectrometry presents a high resolving power, capable to **resolve epimers**:

- The separation power of the TIMS device revealed **distinctive mobility peaks** for the sodiated monomer of 25-hydroxyvitamin D3 and 3-epi-25-hydroxyvitamin D3.
- Their structures **only differ in their C3 hydroxyl group**.
- The investigation of the mixture the mobility dimension enabled a separation of both epimers by means of the **high resolving power**.
- The CCS of vitamin D epimers measured agreed well with published values.

Ion mobility for epimer separation