Comprehensive vitamin D analysis: LC-MS/MS profiling of eight vitamin D metabolites for enhanced biochemical evaluation of vitamin D (patho)physiology in the clinical laboratory

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BACKGROUND

Vitamin D is crucial in calcium and phosphate balance, bone metabolism, and various physiological processes. It exists in two forms: vitamin D3 (cholecalciferol) and vitamin D2 (ergocalciferol). Both undergo hydroxylation, resulting in 25-hydroxyvitamin D (25(OH)D) and the active 1α.25-dihydroxyvitamin D (1α.25$(OH)_2$D). Alongside this main activation pathway, around 50 different vitamin D metabolites (VDM) exist. In endocrine disorders like obesity and hyperparathyroidism, **precise biochemical evaluation of vitamin D status is vital**. Immunoassays are commonly used for total 25(OH)D concentrations, but interference and cross-reactivity, e.g. 3-epi-25(OH)D, can disrupt quantification. Moreover, for certain VDM, e.g. 24.25$(OH)_2$D, no immunoassays are available. In addition, in chronic kidney disease (CKD), 25(OH)D alone is not an adequate reflection of vitamin D status. In CKD, measurement of 1α.25$(OH)_2$D and 24.25$(OH)_2$D is recommended. Therefore, accurate assessment, especially of less abundant VDM, requires **liquid chromatography tandem mass spectrometry** (LC-MS/MS).

VITAMIN D PANEL

Development of a novel LC-MS/MS method for use in a **clinical setting** for comprehensive assessment of vitamin D status. A single run **maps vitamin D metabolism** by chromatographic separation and quantification of

- 25(OH)D2
- 25(OH)D3
- 3-epi-25(OH)D3
- 1.25$(OH)_2$D2
- 1α.25$(OH)_2$D3
- 1β.25$(OH)_2$D3
- 24.25$(OH)_2$D3
- 3-epi-1.25$(OH)_2$D3

METHODOLOGY

Sample preparation is automated on the **Andrew+** (Waters, USA). Proteins are precipitated with ZnSO4 and methanol and samples are further purified by solid-phase extraction followed by **derivatization** with 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD). Subsequently, 25µL of sample is injected in the Acquity UPLC I-Class equipped with a CORTECS Phenyl column (1.6 µm, 2.1x150 mm) coupled to a Xevo TQ-XS IVD (Waters, USA). LC runs gradient elution with mobile phase A, ultra-pure water (MilliQ), and mobile phase B, acetonitrile, both supplemented with 0.1mM NH4F, for a total run time of 15.5 minutes. MS runs positive mode with a type of atmospheric pressure ionization (**UniSpray**, Waters, USA).

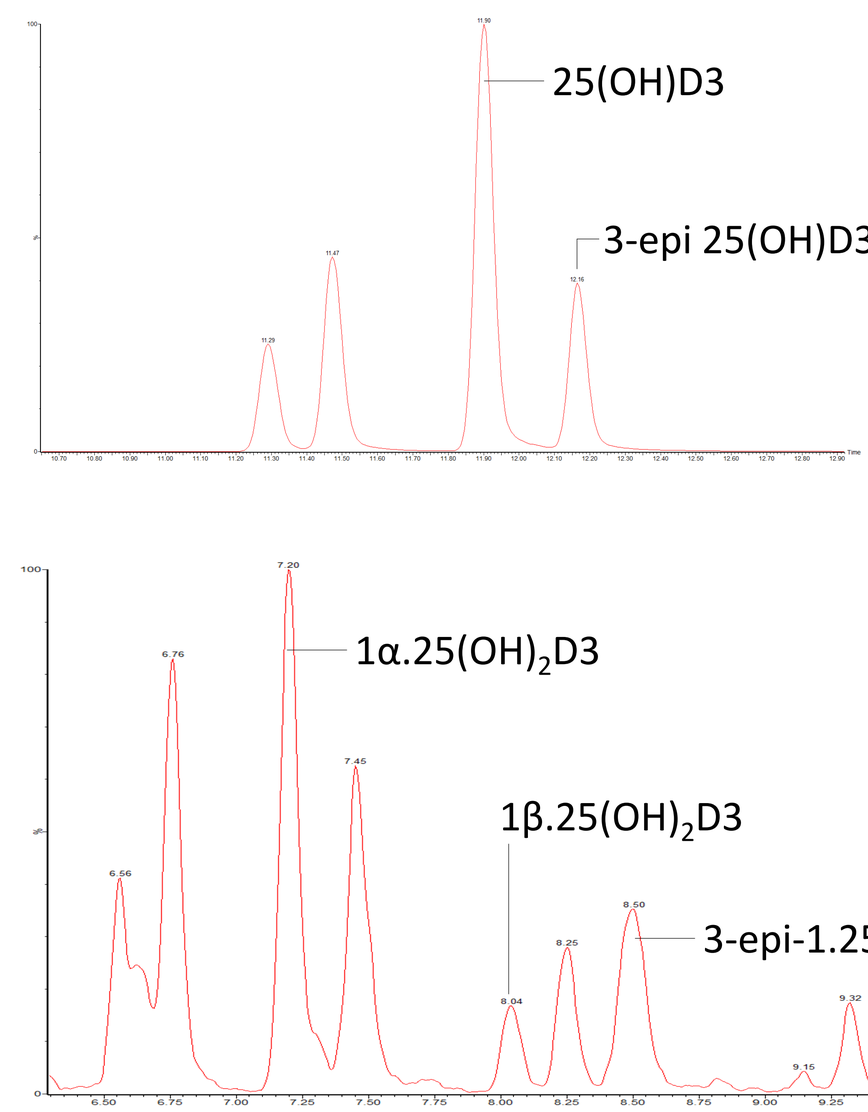

Figure – Serum sample from a healthy volunteer, spiked with stock VDM. Apart from the 8 quantifiable VDM, and 3-epi-24.25$(OH)_2$D3, following VDM(-related) products showed no interference for the quantification of the 8 quantifiable VDM in the LC-MS/MS method:

- 5,6trans$(OH)_2$D3
- 4α,25$(OH)_2$D3
- 4β,25$(OH)_2$D3
- 1,20$(OH)_2$D3
- 20S,24R$(OH)_2$D3
- 20,22$(OH)_2$D3
- 20S,25$(OH)_2$D3
- 20,23$(OH)_2$D3
- 25,26$(OH)_2$D3
- Paracalcitol

CONCLUSION

This LC-MS/MS method is the first **clinical application** to separate known VDM interferences for a **robust diagnostic insight into vitamin D (patho)physiology**, including quantification of eight VDM. This novel LC-MS/MS method is expected to be launched in clinical routine at UZ Leuven, Belgium, after clinical validation, in Q2 2024.

CONTACT INFORMATION

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