Quantitative Analysis of 1,25-Dihydroxyvitamin D_2 and D_3 by LC-MS/MS Utilizing Ion Funnel Technology

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Introduction

A highly sensitive, and selective LC-MS/MS method for determination of 1,25-dihydroxyvitamin D_2 and D_3 is a powerful tool for clinical researchers. While 25 hydroxy vitamin D is found in the ng/ml concentration range, 1,25-dihydroxyvitamin D_2 and D_3 are typically found in the low pg/ml range, making quantitative analysis challenging except when employing highly sensitive analytical techniques. Additionally, extraction is a critical step for this analysis as removal of interfering analytes is required to quantify at the low pg/ml concentration range. Where previously published work (Casetta et al., 2010) demonstrates good sensitivity, such approaches require a complex 2D LC set-up, with post column infusion. The work presented in this poster illustrates the quantitative analysis of 1,25-dihydroxyvitamin D_2 and D_3 using the Agilent 1260 UHPLC & 6490 QQQ with Ion Funnel technology coupled with ImmunoTube LC-MS/MS Kit (an extraction kit from ImmunoDiagnostik) for the extraction of 1,25-dihydroxyvitamin D_2 and D_3 from plasma samples.

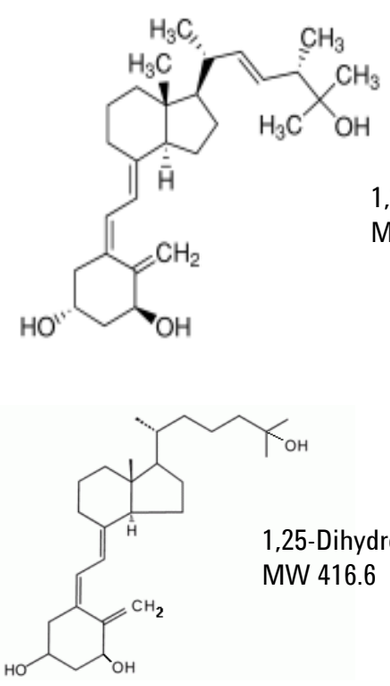

Figure 1. Structures and molecular weights of 1,25-dihydroxyvitamin D_2 and D_3

Sample Preparation

ImmunoTubes were spun down to ensure all the suspension was forced to the bottom of the tube. 500µl of calibrator / sample / control was added followed by 10µL of IS and mixed gently. ImmunoTubes were then mixed in a spiral rotator for 1hr at RT. The closed ImmunoTubes were then placed in a micro tube and centrifuged for 1min at 550 x g. Subsequently, the cover and the outlet of the ImmunoTubes were removed. The Immunotubes were then placed back into the micro tubes for centrifugation a further 2min at 550 x g. The waste collected in the micro tubes was discarded.

Casetta A, Jans I, Billen J, Vanderschueren, Bouillon R. (2010) Development of a method for the quantification of 1α,25$(OH)_2$-vitamin D3 in serum by liquid chromatography tandem mass spectrometry without derivatization *Eur. J Mass Spectrom 16(1):81-9.*

Sample Preparation

Continued...
500uL of WASHSOL was added to the ImmunoTubes and centrifuged for 2 min at 550 x g. This step was repeated twice. Each micro tube was replaced by a glass vial and 250µL of ELUREAG were added to each ImmunoTube which was centrifuged for 2min at 550 x g. The recovered eluent was evaporated under N2 at 37°C. Samples were reconstituted with 165µL of activated Solution A prior to analysis.

WASHSOL and ELUREA are solutions from the ImmunoDiagnostic extraction kit.

LC Method
An Agilent 1260 HPLC series binary pump with 56 vial sample tray, sampler with thermostat, temperature-controlled column compartment, 2 position/6 ports switching valve, was used.

Column : Zorbax Eclipse Plus 2.1x100 mm 1.8µm
Column temperature: 50 °C
Injection volume: 100 µL
Autosampler temperature: 4 °C
Needle wash: 3:1 MeOH:H2O, 10 seconds

Mobile Phase
A: ImmunoDiagnostik Mobile Phase A
B: ImmunoDiagnostik Mobile Phase B

Gradient	Flow	% Solvent B
0.00	0.3	0
6.00	0.3	100
6.50	0.3	100
6.51	0.3	0
8.00	0.3	0

Table 1. LC conditions

Compound	Prec Ion	Prod Ion	CE (V)
1,25$(OH)_2$ Vitamin D_2	411.1	150.7	20
1,25$(OH)_2$ Vitamin D_2	411.1	132.9	18
1,25$(OH)_2$ Vitamin D_3	399.1	150.9	12
1,25$(OH)_2$ Vitamin D_3	399.1	134.9	12
1,25$(OH)_2$ Vitamin $D_{3\text{ - d6}}$	405.1	150.6	12
1,25$(OH)_2$ Vitamin $D_{3\text{ - d6}}$	405.1	134.6	12

Table 2. MRM Parameters

Results and Discussion

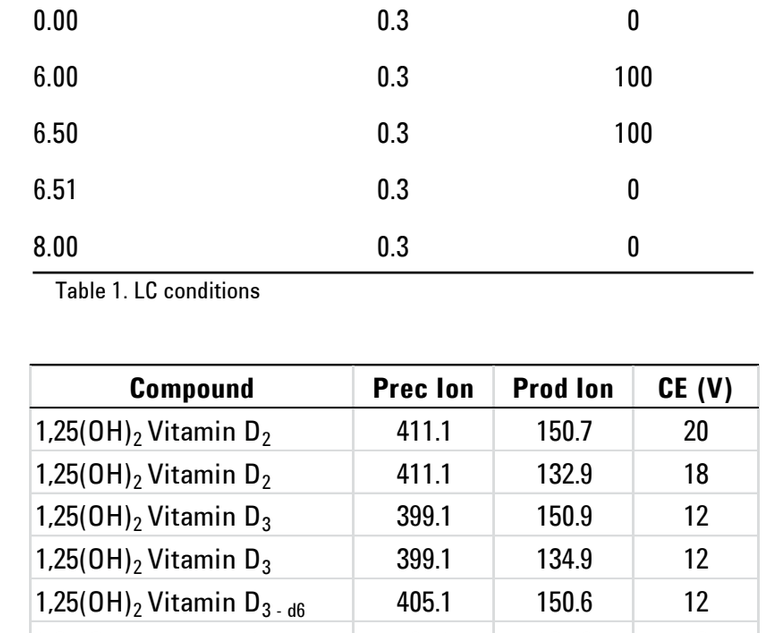

Figure 2. Lower limits of quantification (LLOQ) for neat, unextracted 1,25-dihydroxyvitamin D_2 and D_3.

Results and Discussion

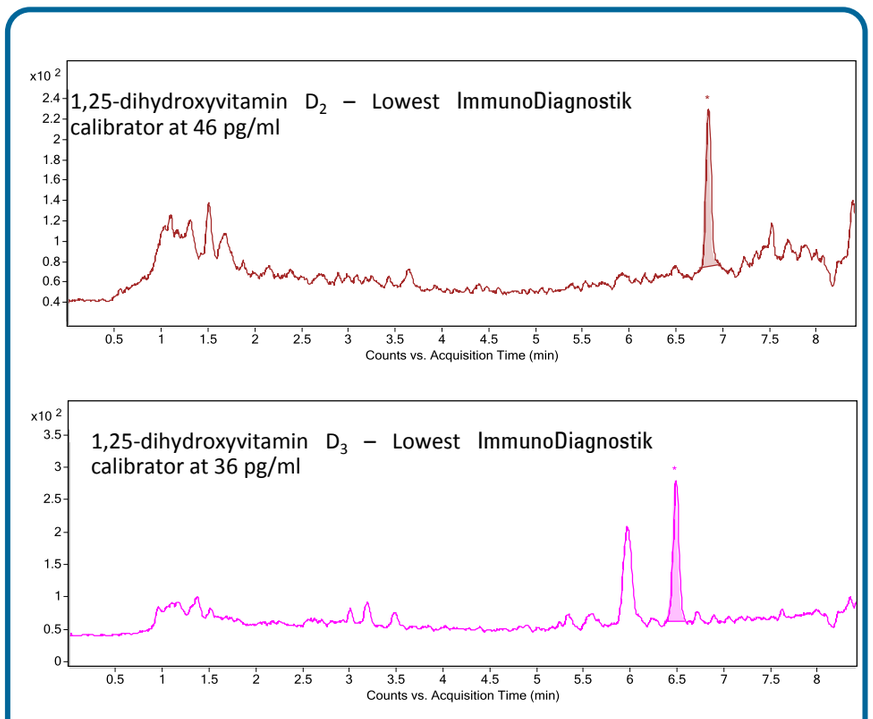

Figure 3. Chromatograms of extracted calibrators from ImmunoDiagnostik

Figure 4. Calibration curve for 1,25-dihydroxyvitamin D_2

Figure 5. Calibration curve for 1,25-dihydroxyvitamin D_3

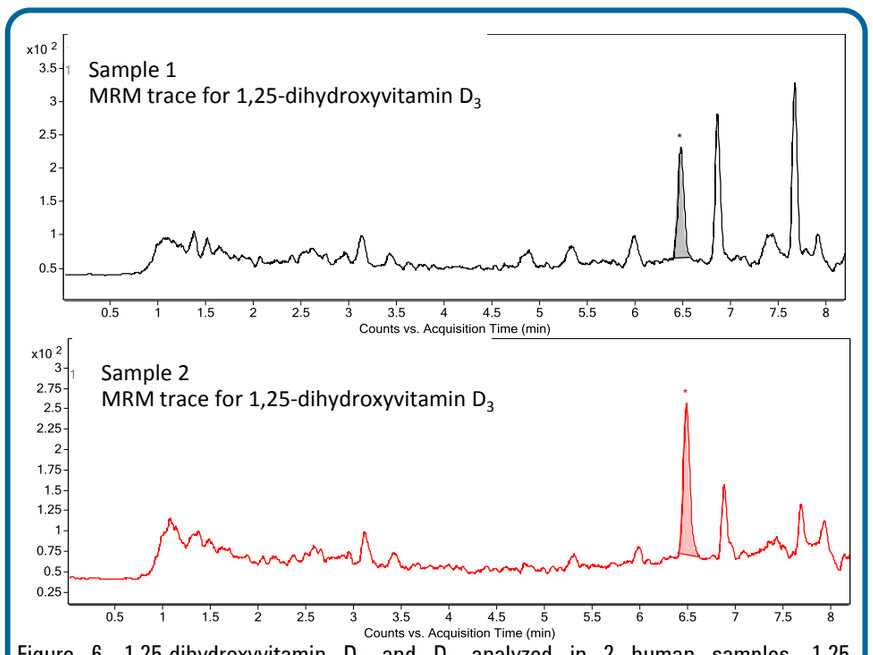

Figure 6. 1,25-dihydroxyvitamin D_2 and D_3 analyzed in 2 human samples. 1,25-dihydroxyvitamin D_2 was not detected in either sample but 1,25-dihydroxyvitamin D_3 was measured at 22 and 36 pg/ml in sample 1 and 2 respectively. Quantification of sample 1 required extrapolation of the calibration curve

Conclusion

A highly sensitive and selective method for quantifying 1,25-dihydroxyvitamin D_2 and D_3 from human plasma has been optimized. By combining the sensitivity of the 6490 QQQ with Ifunnel Technology and the ImmunoDiagnostik extraction method, quantification at low pg/ml levels has been achieved.