Quantitative Analysis of Vitamin D Metabolites in Plasma Using the TSQ Quantiva Triple-Stage Quadrupole MS

Claudio De Nardi, Thermo Fisher Scientific, Dreieich, Germany
Katarina Hartmann, Barbara Hoyer, Immundiagnostik, Bensheim, Germany

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Key Words

Vitamin D, liquid chromatography, tandem mass spectrometry, TSQ Quantiva, 25-hydroxy vitamin D_2, 25-hydroxy vitamin D_3, 1,25-dihydroxy vitamin D_2, 1,25-dihydroxy vitamin D_3, 25-D_2, 25-D_3, 1,25-D_2, 1,25-D_3

Goal

To evaluate the KM1000 kit from Immundiagnostik AG for the quantification of vitamin D metabolites in human plasma on a Thermo Scientific™ TSQ Quantiva™ triple quadrupole mass spectrometer for research purposes. The analyte panel includes 25-hydroxy vitamin D_2 (25-D_2) and D_3 (25-D_3) and 1,25-dihydroxy vitamin D_2 (1,25-D_2) and D_3 (1,25-D_3).

Introduction

In the last decade, liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) has become increasingly popular in the analysis of vitamin D metabolites in plasma. A sensitive and robust mass spectrometer is a valuable tool for clinical researchers involved in vitamin D assessment.

Methods

Sample Preparation

Vitamin D was stripped from a large pool of plasma using a proprietary approach from Immunodiagnostik AG. Six calibrators for 25-D_2 and 25-D_3 and eight calibrators for 1,25-D_2 and 1,25-D_3 were prepared in replicates of six by spiking the stripped human plasma to the final nominal concentrations reported in Table 1.

Hexadeuterated 25-hydroxy vitamin D_3 was used as an internal standard for 25-D_2 and 25-D_3. Hexadeuterated 1,25-dihydroxy vitamin D_3 was used as an internal standard for 1,25-D_2 and 1,25-D_3. Calibrators were extracted using Immundiagnostik's approach described in their LC-MS/MS kit KM1000. Then, 50 µL were injected onto an LC-MS/MS system.

Liquid Chromatography

The LC conditions were as follows:

LC system	Thermo Scientific™ UltiMate™ 3000 Rapid Separation LC (RSLC)
LC column	Provided with the kit
Mobile phase A	0.1% formic acid in water
Mobile phase B	0.1% formic acid in methanol
Injection volume	50 µL
LC gradient	See Table 2

Table 1. Nominal concentration of calibrators.

Analyte	CAL 1	CAL 2	CAL 3	CAL 4	CAL 5	CAL 6	CAL 7	CAL 8
25-D_2 (nmol/L)	1.90	3.80	7.60	15.2	30.3	60.5	N/A	N/A
25-D_3 (nmol/L)	2.00	3.90	7.80	15.6	31.3	62.5	N/A	N/A
1,25-D_2 (pg/mL)	2.50	5.00	10.0	20.0	40.0	80.0	160	320
1,25-D_3 (pg/mL)	2.50	5.00	10.0	20.0	40.0	80.0	160	320

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Table 2. LC gradient.

Time (min)	Flow Rate (mL/min)	A (%)	B (%)	Curve
0.0	0.500	95	5	5
7.0	0.500	20	80	3
7.1	0.500	0	100	5
9.0	0.500	0	100	5
9.1	0.500	95	5	5
10.0	0.500	95	5	5

Mass Spectrometry

The LC system was connected to a TSQ Quantiva triple quadrupole mass spectrometer. The following MS parameters were used:

Source type	Heated electrospray ionization (HESI)
Ionization mode	Positive
RF lens	100 V
Vaporizer temp	300 °C
Capillary temp	350 °C
Spray voltage	4000 V
Sheath gas	50 AU
Sweep gas	2 AU
Auxiliary gas	20 AU
Data acquisition mode	Selected-reaction monitoring (SRM)
Chrom filter peak width	3.0 s
Collision gas pressure	1.5 mTorr
Cycle time	0.35 s
Q1 (FWMH)	0.7 u
Q3 (FWMH)	0.7 u

Data Processing

Data were quantitated using a linear regression, and 1/x weighting was used to build the calibration curves. A maximum percentage bias between nominal and calculated concentration of 15% was set as acceptance criterion for all the calibrators. The percentage RSD for each analyte at each level was also examined.

Results

Linear calibration curves were obtained for all the analytes in the concentration ranges specified in the kit. A summary of calibration range, intercept, slope, and correlation factor (R^2) for each analyte is reported in Table 3.

The calibration curves for each analyte are reported from Figure 1 to Figure 4. The percentage bias between nominal and calculated concentration for the calibrators was well within the ±15% acceptance range. The maximum percentage RSD on the six replicates at each level was 9.2%. Representative chromatograms at the limit of quantitation (LOQ) for each analyte, including the internal standards, are reported in Figure 5.

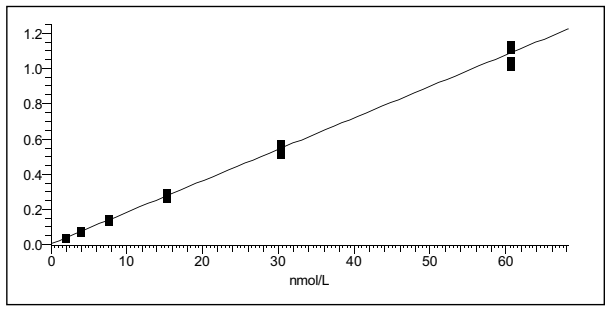

Figure 1. Calibration curve for 25-hydroxy vitamin D_2.

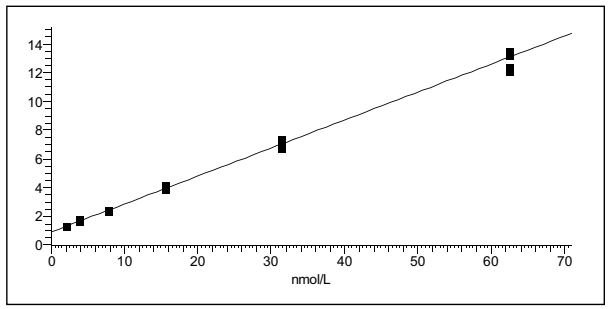

Figure 2. Calibration curve for 25-hydroxy vitamin D_3.

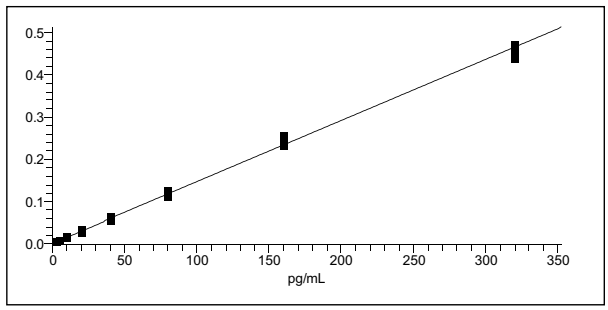

Figure 3. Calibration curve for 1,25-dihydroxy vitamin D_2.

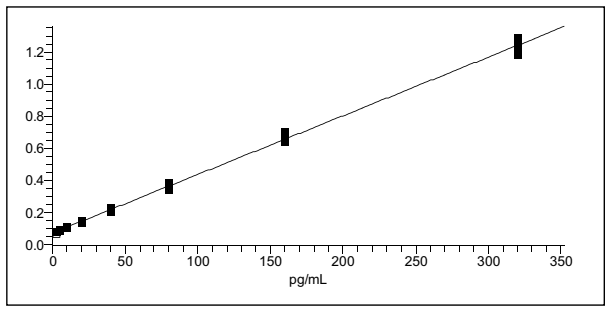

Figure 4. Calibration curve for 1,25-dihydroxy vitamin D_3.

Table 3. Calibration range, intercept, slope, and correlation factor (R^2).

Analyte	Calibration range	Intercept	Slope	R^2
25-hydroxy vitamin D_2	1.9 – 60.5 (nmol/L)	0.0054	0.0179	0.997
25-hydroxy vitamin D_3	2.0 – 62.5 (nmol/L)	0.9209	0.1949	0.997
1,25-dihydroxy vitamin D_2	2.5 – 320 (pg/mL)	0.0018	0.0015	0.998
1,25-dihydroxy vitamin D_3	2.5 – 320 (pg/mL)	0.0732	0.0037	0.998

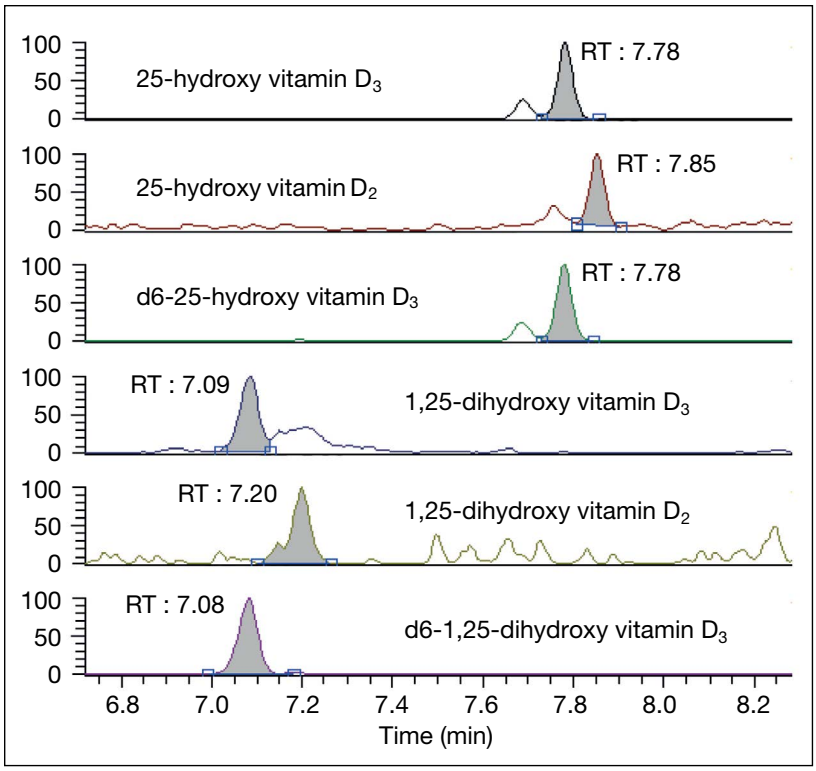

Figure 5. Representative chromatograms for each analyte at the LOQ; internal standards are also included.

Conclusion

The TSQ Quantiva triple quadrupole mass spectrometer was used to evaluate sensitivity, linearity, and reproducibility of an analytical method for the research analysis of vitamin D_2 and D_3 metabolites (namely 25-hydroxy vitamin D_2 and D_3 and 1,25-dihydroxy vitamin D_2 and D_3) in human plasma. The obtained LOQs are below most of the reported LOQs for the same analytes using different methods. The TSQ Quantiva mass spectrometer represents a valuable and sensitive analytical tool to support research.

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