APPLICATION NOTE

1599-9841

Author:
PerkinElmer, Inc.
Waltham, MA

Rapid Quantitative Analysis of Vitamin D Metabolism with Nanogram Detection (25-Hydroxy D_2 and 25-Hydroxy D_3 in Human Serum)

Introduction

The metabolites of vitamin D have a critical physiological function to maintain calcium and phosphate homeostasis. In order to determine levels vitamin D, both 25-Hydroxy Vitamin D2 and D3 are assessed.

A fast, sensitive LC-MS/MS method using the QSight® 220 mass spectrometer was developed. This method can obtain an LLOQ of <0.1 ng/mL for 25-Hydroxy Vitamin D_3 and <0.3 ng/mL for 25-Hydroxy Vitamin D_2 demonstrating the excellent quantitative capability and accuracy of PerkinElmer triple quadrupole mass spectrometers.

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2. Method

25-hydroxyvitamin D_2 and 25-hydroxyvitamin D_3 were purchased from Sigma-Aldrich (Milwaukee, WI). The stock solution, 1.0 mg/mL of 25-hydroxyvitamin D_2 and 25-hydroxyvitamin D_3 in methanol was prepared and stored at -15°C. Vitamin D free human serum was purchased from Golden Western Biologicals, Inc. and cleaned up using protein precipitation.

The mixture was vortexed for 1 min and then centrifuged for 30 min. The supernatant was transferred and used as a matrix for 25-hydroxyvitamin D_2 and 25-hydroxyvitamin D_3 quantitation. Vitamin D standards were then spiked into clean vitamin D free human serum to a concentration of 200 ng/mL. The solution was sequentially diluted by a factor 2 to 0.01 ng/mL. This solution was used to generate the calibration curves with three replicate injections for each concentration.

All of the solvents used in this application were HPLC grade.

2.1. Mass Spectrometry Conditions

Table 1: Conditions used on the QSight® 220 during the method.

ESI Voltage (Volts)	5000
HSID Temp (°C)	225
Nebulizer Gas Setting	200
Drying Gas Setting	120
Heating Gas Setting	250
Source Temp. (°C)	175
Dwell Time (ms)	100
Pause Time (ms)	5

The Q1 and Q2 mass filter were set to unit resolution. Table 2 provides the settings for each monitored MRM transition of vitamin D.

Quick Facts:

- Method for rapid quantitation of both 25-OH-Vitamin D_2 and D_3 using the QSight® 220 triple quadrupole mass spectrometer
- LLOQ of <0.1 ng/mL for 25-OH-Vitamin D_3
- LLOQ of <0.3 ng/mL for 25-OH-Vitamin D_2
- Linearity of R^2 >0.9999 for concentrations ranging from 0.4 ng/mL to 125 ng/mL

Table 2: Selected MRM Operating Conditions

Compound	MRM	CE	CCL 2	CCL 4
25-OH-D_3	401.3/257.2	-23	-55	-73
25-OH-D_2	413.2/355.2	-16	-51	-75

2.2 LC Conditions

The separation was performed using HPLC analysis. A 2 µL sample was loaded on a Fortis C18 column (50x2.1mm, 3µ) at 40 °C. The flow-rate was 400 µL/min with a total LC cycle time of 3 min. The composition of solvent B was 5% water in methanol with 0.1% formic acid and 5 mM ammonium acetate and the composition of solvent A is 5% methanol in water, 0.1% formic acid and 5 mM ammonium acetate.

3. Results

3.1 Extracted Ion Chromatograms (EICs)

Comparing the EIC chromatogram of MRM at 401.3/257.2 in serum blank with that in the serum spiked with 1 ng/mL of 25 hydroxyvitamin D3, there is no interference from the serum matrix.

Figure 1 (a): EIC of 401-257 (25-OH-Vitamin D_3) Serum Blank

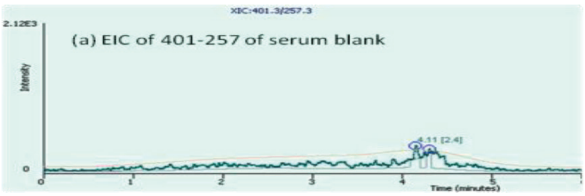


Figure 1 (b): EIC of 401-257 (25-OH-Vitamin D_3) Serum Blank

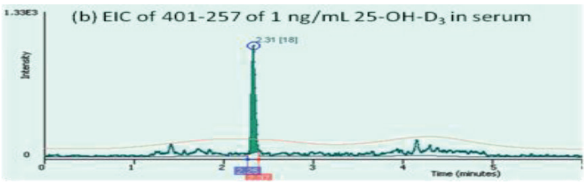


Figure 2 (a): EIC of 413-355 (25-OH-Vitamin D_2) Serum Blank

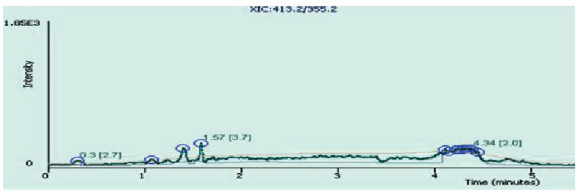
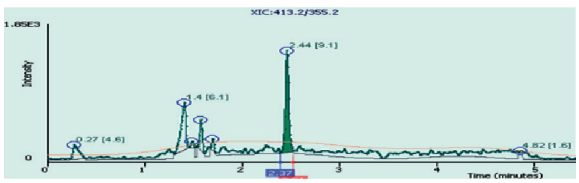


Figure 2(b): EIC of 413-355 (25-OH-Vtamin D_2) at 1ng/mL in Serum



3.2 Linearity

This method covers three orders of magnitude from 0.1 to 100 ng/mL, which includes the concentration at the LLOQ with a linearity of $R^2 > 0.9999$. The calibration curves for 25-hydroxyvitamin D_3 and 25-hydroxyvitamin D_2 are shown in the Figure 3a and 3b. The linear regression has a weighting factor, 1/x2. Figure 3a shows that 25-OH-D_2 in human serum has an R^2 value of 0.9999 from 0.4 ng/mL to 125 ng/mL.

Figure 3 (a): Calibration curve of 25-OH-D_2 in human serum from 0.4-125 ng/mL

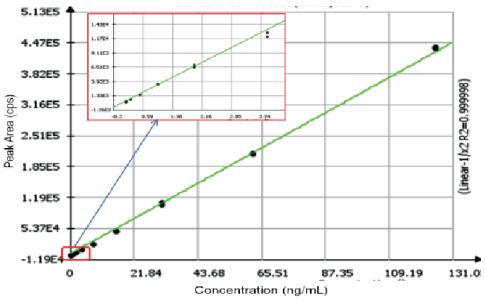
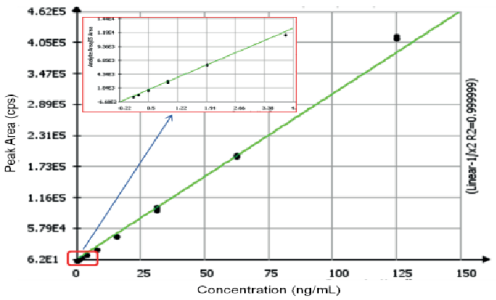


Figure 3 (b): Calibration curve of 25-OH-D_3 in human serum from 0.4-125 ng/mL

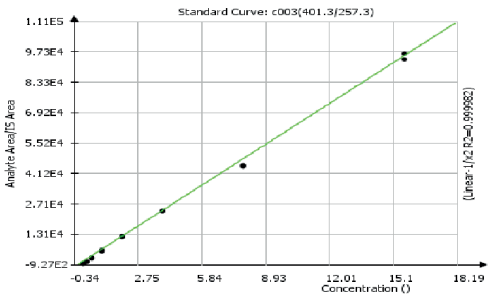


3.3 Quantitation Results

Between the concentrations of 0.4 to 125 ng/mL of 25-OH-D_2 and 25-OH-D_3 standard solution spiked in human serum, the LLOQ for the 25-OH-D2 in serum was 0.24 ng/mL at which accuracy was 94.5% and CV was 2.6%. The accuracy and CV values for the non-LLOQ range of concentrations vary from 91.1 to 98.1% and 0.5 to 5% respectively. Within the 0.4 to 125 ng/mL concentration range, the LLOQ for 25-OH-D_3 in serum was 0.12 ng/mL with an accuracy of 85.4% and CV at 19.9%, while other accuracies and CVs except at the LLOQ level range from 88.2 to 98.2% and 0.5 to 6.7% respectively.

Analyzing a low concentration range (from 0.05 to 16ng/mL), the LLOQ of the 25-OH-D_3 in human serum is reduced to 0.06ng/mL, approximately half of that found when monitoring the 0.4 to 125 ng/mL range. At the LLOQ level of 0.06 ng/mL there was an accuracy of 93.2% and a CV at 4.6%. The accuracies and CVs except those at the LLOQ change from 91.2 to 96.3% and 0.7 to 7.9% respectively.

Figure 4: Low concentration (0.05 to 15.6 ng/mL) calibration curve of 25-OH-D_3 in human serum.



4. Conclusion

This fast, sensitive LC-MS/MS method utilizing the QSight® 220 can achieve LLOQs of 0.06 ng/mL for 25-OH-D_3 and 0.24 ng/mL for 25-OH-D_2 in human serum while maintaining a linearity of R^2=0.9999 for both, illustrating the exceptional quantitative ability of the PerkinElmer QSight® 220 triple quadrupole mass spectrometer for 25-OH-Vitamin D measurements.

5. Contact Information

To learn more about PerkinElmer Mass Spectrometry, our products or services please visit our website or contact us directly.

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
Phone: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

PerkinElmer, Inc.
Wallac Oy
PO Box 10
20101 Turku, Finland
Phone: + 358 2 2678 111
Fax: + 358 2 2678 357

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