SHIMADZU

MP002

Determination of vitamin A, 25-hydroxyvitamin D2/D3 and vitamin E in human serum by UPLC-MS/MS

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1. Overview

Vitamin A, 25-hydroxyvitamin D2/D3 and vitamin E are all fat-soluble vitamins. Monitoring the content of fat-soluble vitamins in human serum is very important for reflecting human health and diagnosing related diseases. But it is very difficult to determine that four fat-soluble vitamins in serum at one method because of the instability in light and heat, the large amount of interfering substances in serum and low concentration in vivo of 25-hydroxyvitamin D2/D3.

2. Introduction

We developed and validated a rapid and precise analytical method based on liquid-liquid extraction and high performance liquid chromatography separation coupled to tandem mass spectrometry for measuring vitamin A, 25-hydroxyvitamin D2/D3 and vitamin E in human serum.

3. Methods and Materials

Following liquid-liquid extraction purification of vitamin A, 25-hydroxyvitamin D2/D3 and vitamin E from human serum, chromatographic separation and quantitative detection were performed using HPLC-MS/MS. The analyses were conducted on a shimadzu LCMS-8050CL operated in electrospray positive mode. The chromatographic separation was achieved with a Shim-pack GIST C18（2.1 mm I.D. × 50 mm L., 2 μm）column. The mobile phases consisted of 0.1% formic acid in water and 0.1% formic acid in methanol. The linearity of four compounds was evaluated by using five analyte standard mixture solutions prepared in blank matrix. The ratios of the signal intensity of each analyte to its corresponding ISTD were plotted against the known concentrations of the standard mixtures to build the calibrations curves.

Analysis conditions :

LC condition:

Column：Shim-pack GIST C18（2.1 mm I.D. × 50 mm L., 2 μm）;

Mobile phase: A-water (contain 0.1% formaic acid and 1 mmol/L ammonium acetate)；B-methanol (contain 0.1% formaic acid and 1 mmol/L ammonium acetate);

Binary gradient: 75%B (0 min)-98%B (3.5 min)-98%B (5.5 min)-75%B (5.51 min- Stop (7 min);

Flow rate: 0.4 mL/min;

Column temperature: 40 ℃;

Injection volume: 10 μL.

Analysis conditions :

MS condition:

Ion type : ESI; Scan mode :MRM;

Interface temp.: 350℃ DL temp.: 150℃

Heating block temp.: 500℃ Nebulizing gas :3 L/min

Drying gas :15 L/min Heating gas :5 L/min

Detector voltage: Tuning result

Figure 1. Shimadzu LCMS-8050 CL

4. Result

Table 1. Parameters for mass spectrometry

名称	Precursor ion	Product ions	Q1 Pre Bias (V)	CE (V)	Q3 Pre Bias (V)
VA	269.25	93.20	-18	-21	-10
d6-VA	275.30	96.20	-16	-11	-34
25OHD2	413.30	337.30	-14	-11	-23
d6-25OHD2	419.35	355.45	-24	-11	-18
25OHD3	401.40	365.35	-24	-11	-26
d6-25OHD3	407.35	371.45	-24	-12	-18
VE	431.40	165.10	-14	-35	-28
d6-VE	437.40	171.20	-14	-21	-28

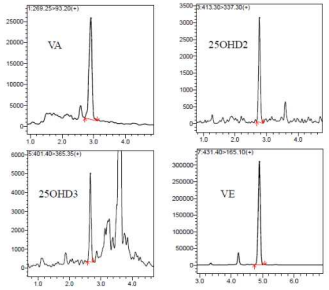

Figure 2. MRM chromatogram of lowest standard solution

A method for simultaneous determination of vitamins A, 25-hydroxyvitamin D2/D3 and vitamin E in human serum was established by using shimadzu clinical triple quadrupole mass spectrometer LCMS-8050CL. The experiment was repeated three times in three consecutive days. The linear correlation of VA was good in the range of 80-1349 ng/mL, 25-hydroxyvitamin D2 in the range of 2.6-39.9 ng/mL, 25-hydroxyvitamin D3 in the range of 2.9-51.1 ng/mL and VE in the range of 678-11253 ng/mL, and the correlation coefficients were above 0.998.

Table 2. Calibration curve parameters

Target compound	linear equation	correlation coefficient	Accuracy (%)
VA	Y = (0.121185)X + (-1.61691)	0.9995	97.0~103.8
25OHD2	Y = (0.0368731)X + (-0.0158060)	0.9989	94.9~104.3
25OHD3	Y = (0.0551478)X + (-0.0407025)	0.9989	94.6~104.0
VE	Y = (0.00792136)X + (-1.12147)	0.9987	93.2~103.7

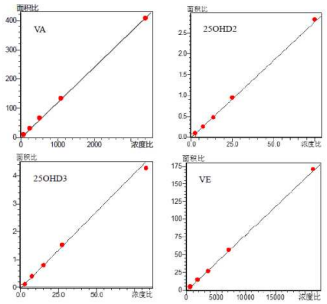

Figure 2. Calibration curve

The human serum samples were analyzed for 5 times, and the precision of the four substances is 2.6%, 4.0%, 2.9% and 3.5% respectively, which meets the needs of routine detection. The results of the quality control products were close to the theoretical values and fully met the requirements of 85%~115% accuracy of the quality control products. The accuracy of three-day determination of low concentration quality control products ranged from 88.8% to 109.7%, and that of high concentration quality control products ranged from 87.2% to 111.9%.

Table 3. Precision data （ng/mL）

NO.	VA	25OHD2	25OHD3	VE
1	610.4	13.3	15.3	4537.0
2	601.5	14.3	15.3	4807.6
3	623.2	13.7	14.5	4708.8
4	628.4	14.5	15.7	4788.7
5	642.9	14.7	15.3	5003.0
Average	621.3	14.1	15.2	4769.0
CV%	2.6	4.0	2.9	3.5

Table 4. Accuracy of controlled samples （ng/mL）

NO.	DAY1		DAY2		DAY3	
	Measured value	Accuracy	Measured value	Accuracy	Measured value	Accuracy
C1-VA	410.6	90.6	451.2	99.6	402.5	88.8
C1-25-OHD2	9.33	89.7	10.6	101.8	10.1	96.8
C1-25-OHD3	11.96	109.7	10.8	99.2	10.7	97.7
C1-VE	3561.9	95.4	3674.4	98.4	4077.2	109.2
C2-VA	1226.8	90.9	1297.4	96.2	1266.6	93.9
C2-25-OHD2	43.3	108.5	44.6	111.9	42.0	105.4
C2-25-OHD3	51.4	100.6	52.4	102.6	53.6	105.0
C2-VE	9812.0	87.2	10246.0	91.1	11000.1	97.8

Table 5. Detection results of human serum clinical samples

NO.	VA(ng/mL)	25OHD2(ng/mL)	25OHD3(ng/mL)	VE(ng/mL)
Sample-1	610.4	13.3	15.3	4537.0
Sample-2	1851.3	1.3	25.8	14709.0
Sample-3	1216.9	0.9	27.2	9823.5

5. Conclusions

A method for simultaneous determination of vitamins A, 25-hydroxyvitamin D2, 25-hydroxyvitamin D3 and vitamin E in human serum was established by using Shimadzu clinical triple quadrupole mass spectrometer LCMS-8050CL and Guangzhou Darui brand fat-soluble vitamin detection kit (tandem mass spectrometry). The linearity, accuracy, precision and stability of the method were investigated using the internal standard, standard and quality control products of the kit. The results showed that the method had good specificity, the correlation coefficients of standard curves were greater than 0.994, and the accuracy and precision met the requirements of the kit. This method is helpful for clinical judgement, treatment management and physiological evaluation of vitamin A/D/E deficiency.