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Application News

No.C107

Liquid Chromatography Mass Spectrometry

Quantitative Analysis of Vitamin D Metabolite Using Triple Quadrupole LC/MS/MS

Vitamin D is a fat-soluble vitamin that functions as a hormone in the body to regulate calcium metabolism. In addition, there is a report that vitamin D has a number of other physiological roles, including maintaining muscle strength, modulating immune function, and regulating blood pressure. Moreover, vitamin D functions to help regulate cellular differentiation, the biological process by which cells become specialized for a specific function.

Due to these physiological functions, vitamin D has been implicated in protection against muscle pain and weakness, certain autoimmune diseases, hypertension, and even some forms of cancer.
This sheet describes analysis of 25 hydroxy vitamin D_2 and D_3 in human serum using LC/MS/MS with ISOLUTE® SLE+.

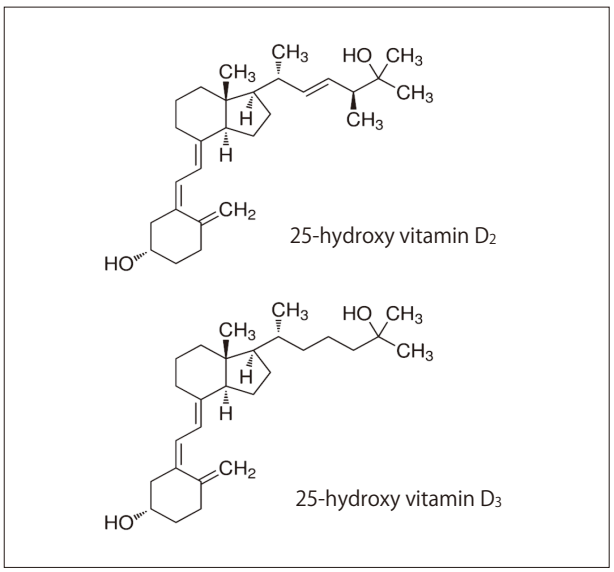

Fig. 1 Structure of Vitamin D Metabolites

[Sample Preparation Procedure]

1. Sample Pre-treatment:
   To a mixture of 150 µL water and 150 µL isopropanol with ISTD add 100 µL of sample and mix for 10 seconds.
2. Sample Load:
   Load pre-treated sample (400 µL) to plate followed by a pulse of positive pressure (2-3 seconds) to initiate flow and leave to flow under gravity for five minutes.
3. Analyte Elution:
   Elute with heptane (700 µL × 2) directly in to a deep well collection plate. Leave each aliquot to flow under gravity for 5 minutes then apply positive pressure to completely remove the final volume.
4. Post extraction:
   Transfer extraction solvent to glass vials and evaporate to dryness at 40 °C.
   Reconstitute in 100 µL of solution containing: 33 % of mobile phase A and 67 % of mobile phase B v/v. Vortex the aliquot, centrifuge and transfer supernatant to new deep well plate.

Table 1 Analytical Contditions

[LC] NexeraX2 System	
Column	: YMC Triart C18 (50 mm L. × 2 mm I.D., 1.9 µm)
Column Temp.	: 40 ˚C
Mobile Phase A	: Water : Methanol : Formic Acid = 50 : 50 : 0.025
Mobile Phase B	: Methanol
Time Program	: 67 %B (0 - 1 min) - 67 %B - 95 %B (1 - 3 min) - 95 %B (3 - 4 min) - 67 %B (4 - 6 min)
Flowrate	: 0.5 mL/min
Injection Volume	: 10 µL
[MS] LCMS-8050	
Ionization	: ESI Positive
DL Temp.	: 150 ˚C
Block Heater Temp.	: 400 ˚C
Interface Temp.	: 180 ˚C
Nebulizing Gas Flow	: 3 L/min
Drying Gas Flow	: 10 L/min
Heating Gas Flow	: 10 L/min

A seven point calibration curve was generated. The calibrant levels for the 25-hydroxy vitamin D_2/D_3 were from 2.4 to 150 ng/mL. Typical calibration curves for analytes post extraction from stripped serum are shown in Fig. 2. The calibration curves that were generated had linear regression values of r^2 >0.998 for each curve.

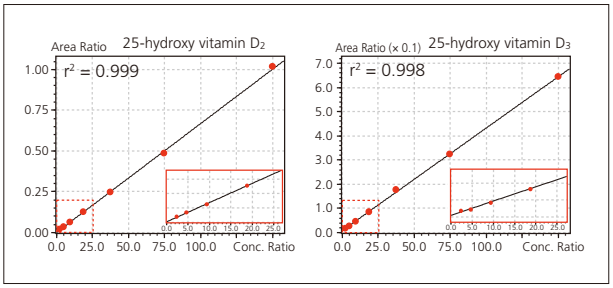

Fig. 2 Typical Calibration Curves for 25-OH Vitamin D_2/D_3

Table 2 Comparison of ISOLUTE® SLE+ Treatment with Protein Precipitaion

25-hydroxy vitamin D_2 — ISOLUTE® SLE+ treatment

Level	STD		Protein precipitation		Pre- spiked		Post- spiked		Matrix	Recovery
(ng/mL)	area	%RSD	area	%RSD	area	%RSD	Area	%RSD	Effect	
2.4	27,168	5.49	1,619	24.34	4,846	9.29	9,612	3.16	35 %	50 %
4.7	52,991	2.32	2,585	17.35	9,837	7.77	10,786	4.09	20 %	91 %
9.4	100,374	3.52	6,997	4.68	24,595	4.85	24,699	3.45	25 %	100 %
18.8	198,154	0.59	12,484	4.05	45,006	9.11	50,536	1.06	26 %	89 %
37.5	368,606	1.91	28,593	8.64	89,217	2.47	107,515	2.40	29 %	83 %
75	831,880	2.15	65,483	2.12	168,910	4.74	209,480	2.52	25 %	81 %
150	1,694,370	1.47	127,188	2.50	338,857	7.88	441,717	4.31	26 %	77 %
								Average	27 %	82 %

25-hydroxy vitamin D_3 — ISOLUTE® SLE+ treatment

Level	STD		Protein precipitation		Pre- spiked		Post- spiked		Matrix	Recovery
(ng/mL)	area	%RSD	area	%RSD	area	%RSD	area	%RSD	Effect	
2.4	29,924	6.26	8,398	2.63	17,122	3.80	28,245	1.33	94 %	61 %
4.7	53,757	2.43	7,263	9.05	25,982	1.31	45,086	3.44	84 %	58 %
9.4	111,778	5.32	15,255	3.53	76,775	0.48	85,577	1.10	77 %	90 %
18.8	217,082	3.13	15,189	7.29	159,245	1.52	172,609	1.35	80 %	92 %
37.5	409,324	2.02	31,658	6.81	340,455	1.23	349,433	1.64	85 %	97 %
75	930,622	2.53	66,674	7.15	619,011	0.15	679,384	3.06	73 %	91 %
150	1,906,127	2.31	114,775	5.84	1,197,905	0.85	1,356,054	0.50	71 %	88 %
								Average	81 %	82 %

Recovery of analytes was determined by compare pre-spiked with post-spiked. The recoveries for analytes ranged above 80 % with RSDs <10 % (N=3).
Fig. 3 shows MRM chromatograms of 25-hydroxy vitamin D_2/D_3 (each 4.7 ng/mL) treated by protein precipitation (A and B) or ISOLUTE® SLE+ (C and D). It is clear that ISOLUTE® SLE+ treatment provide a significantly more efficient extract than protein precipitation, leading to more reliable quantitation due to minimization of matrix effects.

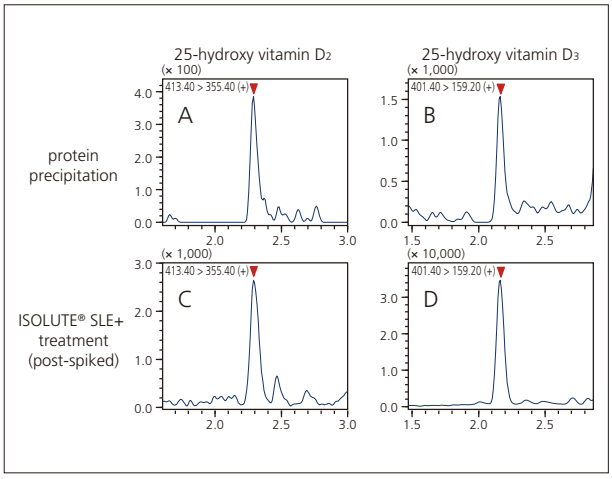

Fig. 3 MRM Chromatograms of 25-OH Vitamin D_2/D_3 (Each 4.7 ng/mL) Comparing ISOLUTE® SLE+ Treatment with Protein Precipitation

[Acknowledgement]
We appreciate cooperation from Biotage Japan, Ltd..

First Edition: May. 2015

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