

Screening of 25-OH Vitamin D2,D3 using Shimadzu LCMS-8040

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1. Introduction

Hypovitaminosis D is a relatively common problem traditionally manifesting in the elderly population and in people with severe liver or kidney disease. Inadequate levels of vitamin D can lead to increased risk of cancer, diabetes mellitus, chronic pain, and hypertension. Vitamin D2 (ergocalciferol) and D3 (cholecalciferol) may come from exogenous sources, but only vitamin D3 is produced in the skin from 7-dehydrocholesterol upon exposure to sunlight. Vitamin D2 and D3 are metabolized to 25-hydroxyvitamin D [25(OH)D2 and 25(OH)D3] in the liver and subsequently converted to the biologically active 1,25-dihydroxyvitamin

D form in the kidneys. Circulating levels of 25-hydroxyvitamin D have been demonstrated to be the most precise marker of a patient's vitamin D status. Various methodologies including HPLC-UV, HPLC-EC, RIA, automated chemiluminescence immunoassays have been described for the measurement of 25-OH-VitD but just LCMSMS offers a simple, linear and reproducible result. We evaluated Shimadzu Triple Quadrupole LCMS-8040 instrument using different commercial kit available for detection of 25-OH VitaminD and a home-brew method using a considerable number of biological sample.

2. Methods

We performed the analysis of 25-OH Vitamin D2 and D3 using PerkinElmer kit.

The analysis were performed using Shimadzu Triple Quadrupole LCMS-8040 instrument with APCI source in positive mode and following these conditions:

Flow : 0.5 mL/min
 Run : 6 min
 Injection Volume : 35 µL
 Mobile Phase A : Water
 Mobile Phase B : Methanol
 Gradient : 0 min B 50%
 3 min B 100%
 4 min B 100%
 4.1 min B 50%

DL 150 °C; Nebulizing Gas 2.5 L/min; Heat Block 250 °C; Drying Gas 5 L/min
 Column Polaris3 C18 50 mm x 2 mm 3 µm

Sample Preparation

The sample were prepared according to the guidelines of the kit used:

The transitions monitored, and calibration levels (noml/L) are reported in following table:

ID#	Name	Type	m/z	Ret. Time	ISTD Group	Group#	Conc.(1)	Conc.(2)	Conc.(3)	Conc.(4)	Conc.(5)	Conc.(6)	Event
1	D2 ISTD	ISTD	416.30>358.30	2.311	1	3	1	1	1	1	1	1	1:MRM(+)
2	D3 ISTD	ISTD	404.30>162.10	2.214	2	4	1	1	1	1	1	1	2:MRM(+)
3	CalD6 D2	Target	419.30>355.30	2.316	1	1	10.7	20	43.4	90.3	185	351	3:MRM(+)
4	CalD6 D3	Target	407.30>159.10	2.205	2	2	10.4	20.9	43.2	88.6	184	333	4:MRM(+)
5	VitD2	Target	413.20>355.50	2.310	1	1	10.7	20	43.4	90.3	185	351	5:MRM(+)
6	VitD3	Target	401.20>159.10	2.204	2	2	10.4	20.9	43.2	88.6	184	333	6:MRM(+)

Key feature of LCMS-8040 triple quadrupole mass spectrometer

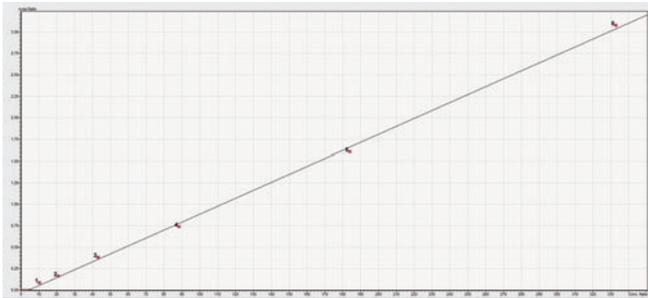
- * ultra fast polarity switching of 15 msec
- * Dwell time of 0.8 msec and Pause time of 1 msec
- * UFsweeperII™ technology dramatically minimizes cross talk
- * excellent linearity with wide dynamic range



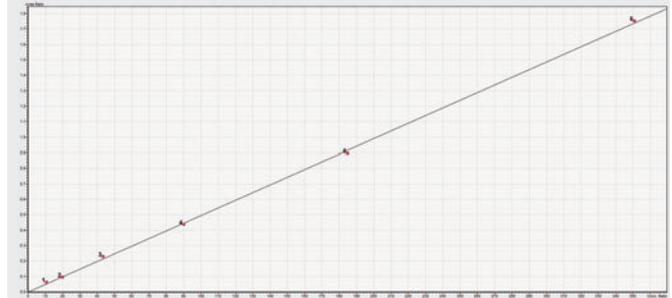
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3. Results

The data reported show the calibration curves developed for 25-OH-VitaminD2 and 25-OH-VitaminD3:

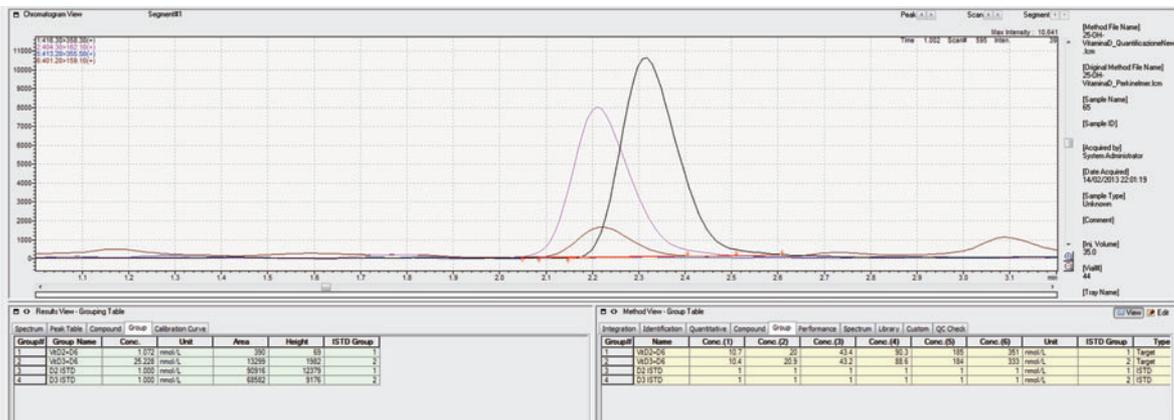
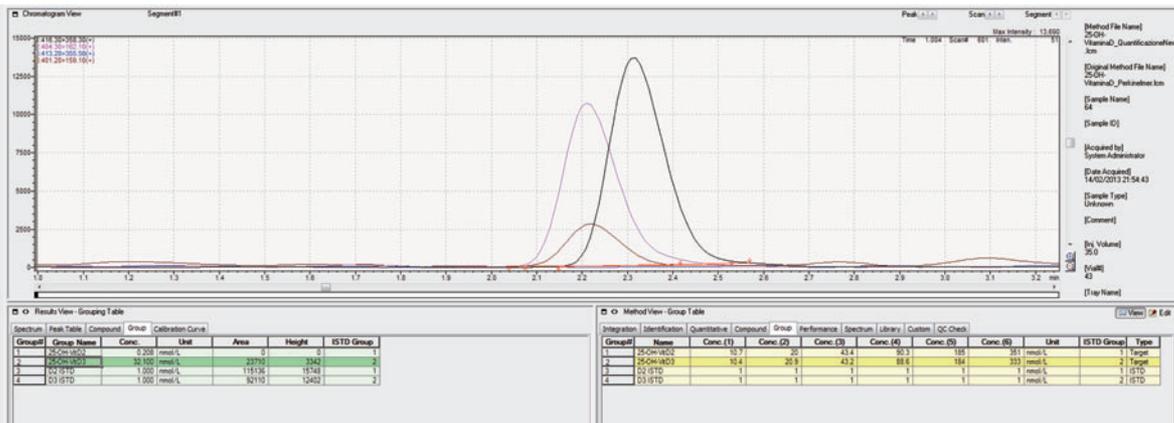


Calibration Curves of 25-OH-VitaminD2 $Y = (0.00923889)X + (-0.0391599)$
 $R^2 = 0.9982$



Calibration Curves of 25-OH-VitaminD3 $Y = (0.00495885)X + (-0.00102974)$
 $R^2 = 0.9995$

Example of real samples injected and quantify using Shimadzu LCMS-8040.



4. Conclusion

The data reported show that Shimadzu LCMS-8040 is suitable for identification and quantification of 25-OH-VitaminD2 & D3, without any derivatization.

LabSolution software allows the directly quantification of compound of interested (not marked) using a calibration curves developed on marked molecules.