

APPLICATION

A Fast Approach of a Supported Liquid Extraction (SLE) Method to Determine 25-OH Vitamin D₂/D₃ in Human Serum Using LC-MS/MS

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Introduction

In this technical note, we proposed a simple and fast extraction method using Strata[®] DE supported liquid extraction (SLE), 400 μ L 96-well plate coupled with LC-MS/MS analysis for the characterization of 25-OH Vitamin D₂/D₃ in human serum. The chemical structures of 25-OH Vitamin D₂ and D₃ are similar, so the chromatographic separations of these molecules is one of the challenges of the assay. We use a shorter analytical column of Kinetex[®] 2.6 μ m C18, 30 x 3.0 mm, which maintains the separation and reduces the run time to 2 minutes. The method improves the high-throughput capabilities and reduces the cost in the lab significantly by reducing solvent loss and unreliable results. The assay evaluation test shows the accuracy and precision across three level QCs (n=6) from 98.7 - 110 % with CV % from 3.30 - 7.62 %, respectively. The linear dynamic range of the assay is 2-100 ng/mL.

Materials

Standards were purchased from Cerilliant[®] (Round Rock, TX). Double charcoal stripped human serum was purchased from BioreclamationIVT[®] (Westbury, NY). All other reagents and chemicals were obtained from Sigma-Aldrich[®].

Experimental Conditions

Sample Pre-treatment

Dilute 200 μ L of human serum* with 100 μ L of 5 % Ammonium hydroxide (w/v), add 25 μ L of 25-OH Vitamin-D₃-²H₆ (1 μ g/mL) and mix.

* Double Charcoal-stripped human serum was used to prepare all standards and QCs

SLE Protocol

96-Well Plate: Strata DE 400 μ L

Part No.: 8E-S325-5GB

Load: Pre-treated sample and wait for 5 minutes

Elute: Sample with 600 μ L MTBE by gravity, wait for 5 minutes

Repeat: Elution step twice by gravity, and after the final elution, apply 5-10 Hg vacuum to finish elution

Dry: 40 °C under N₂

Reconstitute: 200 μ L 0.1 % Formic acid in Water/0.1 % Formic acid in Methanol (30:70)

LC-MS/MS Conditions

Column: Kinetex 2.6 μ m C18

Dimensions: 30 x 3.0 mm

Part No.: 00A-4462-Y0

Mobile Phase: 0.1 % Formic acid in Water /
0.1 % Formic acid in Methanol (15:85)

Flow Rate: 0.75 mL/min

Injection Volume: 30 μ L

Detection: MS/MS (SCIEX 4000 QTRAP[®]), APCI +

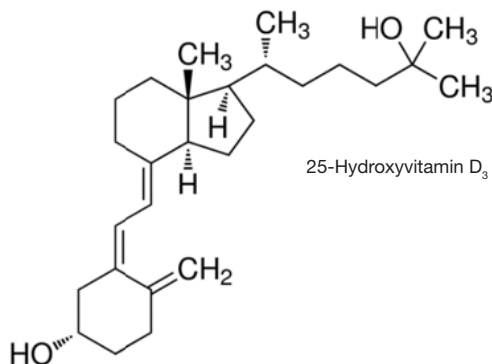
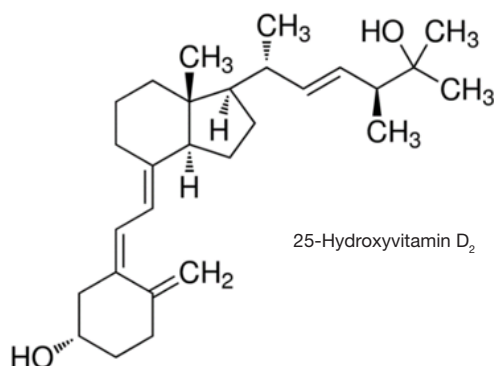


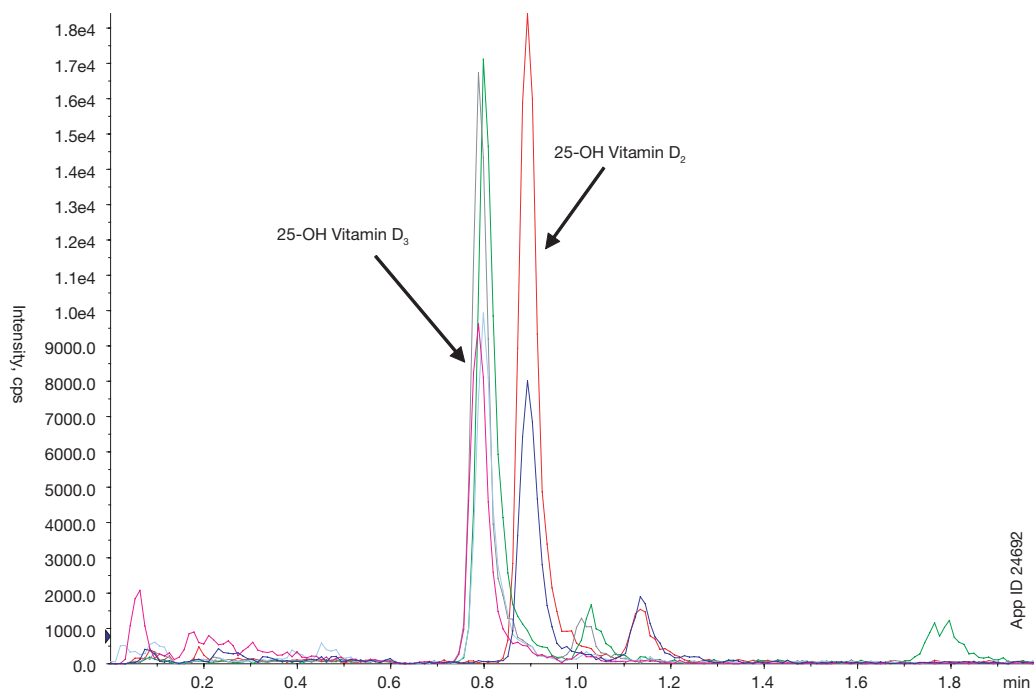
Table 1.
MRM Transitions

ID	Q1 Mass (DA)	Q3 Mass (DA)	Dwell (msec)	CE
25-OH D ₂ 1	395.4	209	100	36
25-OH D ₂ 2	395.4	269.1	100	28
25-OH D ₃ 1	383.6	257.2	100	23
25-OH D ₃ 2	383.6	229.4	100	28
D6-25-OH-D ₃ 1	389.5	263.3	100	23
D6-25-OH-D ₃ 2	389.5	229.4	100	28

Table 2.
Accuracy and precision

	QCL	QCM	QCH
Target Conc. (ng/mL)	6	50	80
	25-OH-D₂		
Mean Conc. Found	5.92	53.0	80.8
STDV	4.09	2.21	5.55
CV%	6.90	4.18	6.86
Accuracy (%)	98.7	3	101
n	6	6	6
	25-OH-D₃		
Mean Conc. Found (ng/mL)	6.59	52.7	87.2
STDV	0.50	1.74	5.50
CV%	7.62	3.30	6.31
Accuracy (%)	110	105	109
n	6	6	6

Figure 1.
Representative of chromatogram of human serum at 100 ng/mL



Results and Discussion

Table 1 presents the mass transitions with APCI, positive mode on mass spectrometry. The molecular weight of 25-OH vitamin D₂ is 412.65 Da and 25-OH vitamin D₃ is 400.64 Da. In **Table 1**, the Q1 mass that we selected are representative of water loss plus one proton in positive mode, which is -17 Da of analyte mass. Those transitions provide cleaner background and better sensitivity of target compounds. **Table 2** shows the mini assay evaluation run results to demonstrate the accuracy and precision of the assay, the three QC levels (QCL, QCM and QCH) were used in the run, accuracy and precision across all QCs are from 98.7 – 110 % with CV % from 3.30 -7.62 %, respectively.

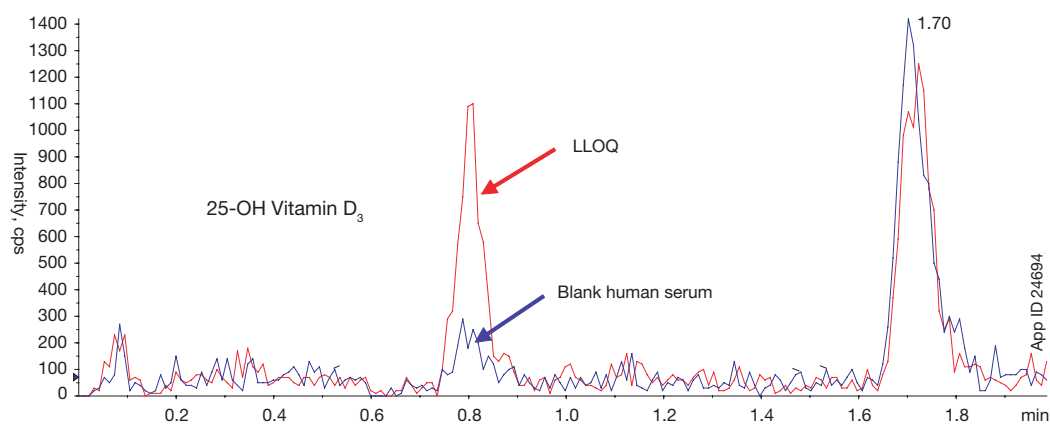
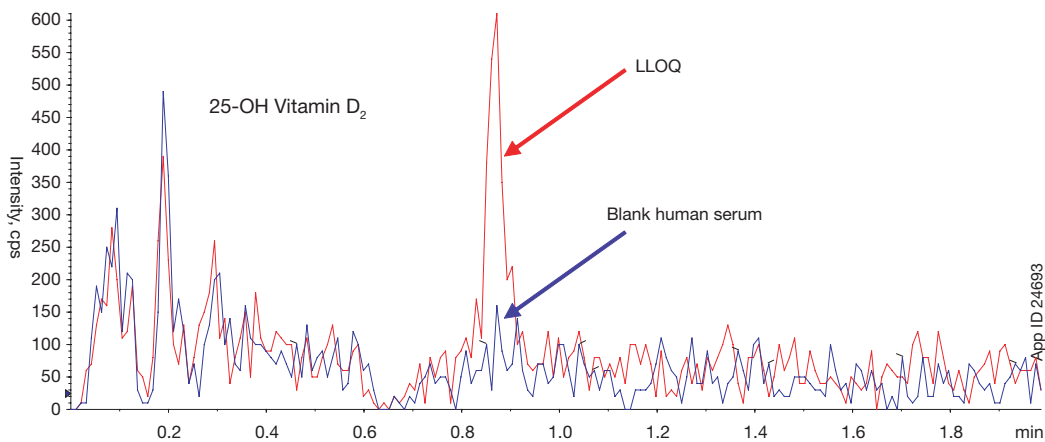
The linear dynamic range of this method was tested with seven calibrators (n=2) from 2-100 ng/mL and the linearity of the curve of 25-OH vitamin D₂ is shown in **Figure 3** which shows r=0.9985. The chromatogram of ULOQ at concentration of 100 ng/mL is shown in **Figure 1**. The chromatogram for the blank matrix, double charcoal-stripped serum, and LLOQ at 2 ng/mL in matrix, were overlaid as shown in **Figure 2**, indicating that there is no affect endogenous level background of 25-OH Vitamin D₂/D₃, respectively.

Conclusion

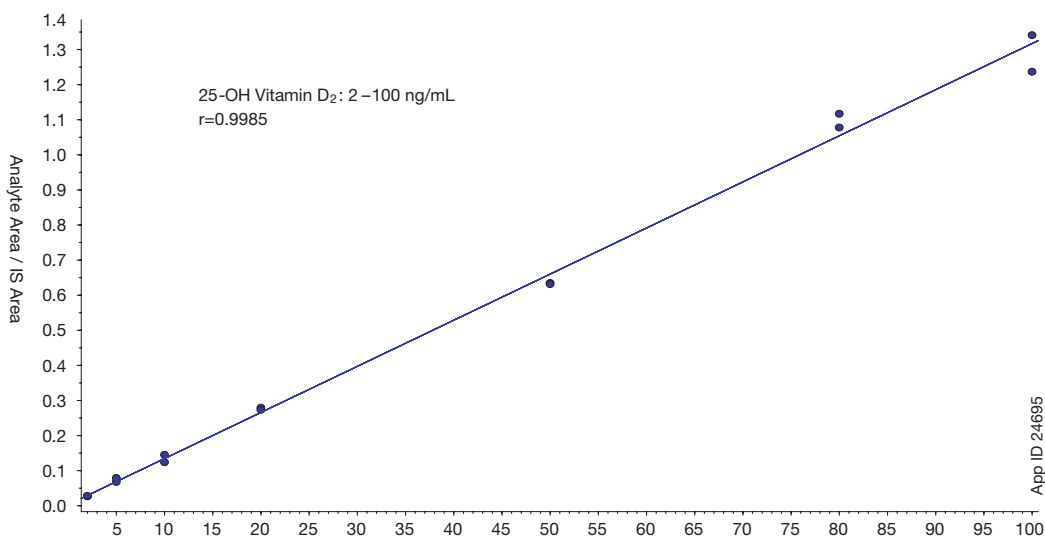
The assay is associated with a faster and more simple SLE 96-well format extraction method, the total separation time on LC-MS/MS system with a shorter analytical column takes only 2 minutes, which is ideal for cost savings and high-throughput analysis in research and production environment.

Figure 2.

Representative of chromatograms of blank human serum vs human serum at 2 ng/mL (LLOQ)

**Figure 3.**

Representative of Assay Dynamic Range



APPLICATION

Ordering Information

Strata® DE Supported Liquid Extraction

Part No.	Description	Unit
8E-S325-FGB	Strata DE SLE 200 µL 96-Well Plate	2/pk
8E-S325-5GB	Strata DE SLE 400 µL 96-Well Plate	2/pk
8B-S325-KDG	Strata DE SLE 12 cc Tube	20/pk
8B-S325-VFF	Strata DE SLE 60 cc Tube	16/pk

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Kinetex® Core-Shell HPLC/UHPLC Columns

2.6 µm Minibore Columns (mm)

Phases	30 x 2.1	50 x 2.1	75 x 2.1	100 x 2.1	150 x 2.1	SecurityGuard TM ULTRA Cartridges [†]
C18	00A-4462-AN	00B-4462-AN	00C-4462-AN	00D-4462-AN	00F-4462-AN	3/pk AJ0-8782 for 2.1 mm ID

2.6 µm MidBoreTM Columns (mm)

Phases	30 x 3.0	50 x 3.0	75 x 3.0	100 x 3.0	150 x 3.0	SecurityGuard TM ULTRA Cartridges [†]
C18	00A-4462-YO	00B-4462-YO	00C-4462-YO	00D-4462-YO	00F-4462-YO	3/pk AJ0-8775 for 3.0 mm ID

2.6 µm Analytical Columns (mm)

Phases	30 x 4.6	50 x 4.6	75 x 4.6	100 x 4.6	150 x 4.6	SecurityGuard TM ULTRA Cartridges [†]
C18	00A-4462-E0	00B-4462-E0	00C-4462-E0	00D-4462-E0	00F-4462-E0	3/pk AJ0-8768 for 4.6 mm ID

[†] SecurityGuard ULTRA Cartridges require holder, Part No.: AJ0-9000

PresstonTM 100 Positive Pressure Manifold

Part No.	Description
AH0-9334	Presston 100 Positive Pressure Manifold, 96-Well Plate
AH0-9342	Presston 100 Positive Pressure Manifold, 1 mL Tube Complete Assembly
AH0-9347	Presston 100 Positive Pressure Manifold, 3 mL Tube Complete Assembly
AH0-9343	Presston 100 Positive Pressure Manifold, 6 mL Tube Complete Assembly

Presston 100 Tube Adapter Kits (for AH0-9334)

Part No.	Description
AH0-9344	1 mL Tube Adapter Kit
AH0-9345	3 mL Tube Adapter Kit
AH0-9346	6 mL Tube Adapter Kit



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