

APPLICATION

Extraction of 25-OH-Vitamin D2 and D3 from Serum by Novum™ Simplified Liquid Extraction (SLE) and LC/MS/MS Analysis using a Kinetex® 2.6µm F5 HPLC/UHPLC Column

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Overview

The aim of this application note is to introduce a fast and effective sample preparation technique using Novum Simplified Liquid Extraction (SLE) products that overcomes the challenges observed in a traditional liquid-liquid extraction (LLE). This rapid cleanup is then paired with an LC/MS/MS method using a Kinetex 2.6µm F5 core-shell HPLC column, resulting in >80% recoveries.

Sample Pre-treatment Step

To 200µL human serum spiked with 30µL 25-OH-Vitamin D3-d3 (1µg/mL in water) add 190µL of 2% Ammonium hydroxide in water and vortex for 30 seconds.

Extraction Procedure

Sample Loading

- Load the sample from pre-treatment step onto the Novum SLE 3 cc tube (Part No. 8B-S138-5BJ) and apply a short and gentle pulse of vacuum (~5-10 sec at 5" or less of Hg) until the sample has completely entered the media.
NOTE: Avoid prolonged or excessive vacuum. The sample must not leave the Novum bed.
- Wait for 5-6 minutes.
NOTE: Inadequate or excessive wait period will lead to variable recoveries and poor precision.

Elution

- Dispense 3x 600µL of ethyl acetate onto the Novum SLE sorbent and collect the solvent under force of gravity.
- Apply vacuum at 5" of Hg (or lower) for 20-30 secs to complete the extraction.

Dry Down

- Evaporate extracted samples to complete dryness under slow stream of N₂ and room temperature.
- Reconstitute the dry residue in 200µL of 0.1% Formic acid in Water/0.1% Formic acid in Methanol (30:70).

HPLC Conditions

Column: Kinetex 2.6µm F5
Dimensions: 100 x 4.6mm
Part No.: 00D-4723-E0
Mobile Phase: A: 0.1% Formic acid in Water
B: 0.1% Formic acid in Methanol
Gradient:

Time (min)	% B
0.00	85
5.00	85
5.01	100
6.50	100
6.51	85
8.00	85

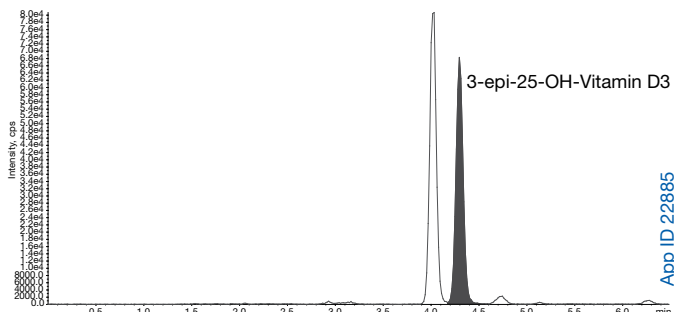
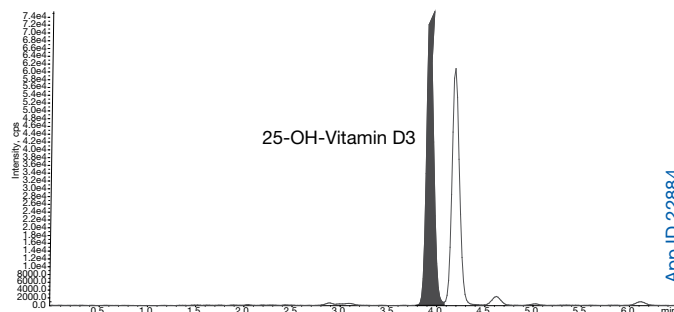
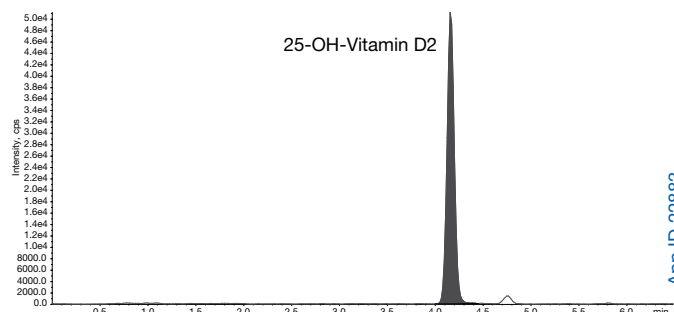
Flow Rate: 0.75 mL/min
Temperature: Ambient
Detection: MS/MS, API 5000™ (AB SCIEX), APCI+
Injection: 10µL

Analyte	RT, min	Q1, Da	Q3, Da	CE
25-OH-Vitamin D2	4.60	395.3	209.3	30
		395.3	269.1	22
25-OH-Vitamin D3	3.93	383.6	257.2	25
		383.6	159.1	30
3-epi-25-OH-Vitamin D3	4.30	383.6	257.2	25
		383.6	159.1	30
25-OH-Vitamin D3-d3 (IS)	4.00	386.6	257.2	23
		386.6	368.4	20

Analyte Recovery

Analyte	% Absolute recovery (100 ng/mL)	%CV (N=6)
25-OH-Vitamin D2	80.4	5.03
25-OH-Vitamin D3	92.6	6.03
3-epi-25-OH-Vitamin D3	87.2	5.22

Representative Chromatograms



APPLICATION

Ordering Information

Novum™ Simplified Liquid Extraction (SLE)

96-Well Plates

Part No.	Description	Unit/Box
8E-S138-FGA	Novum SLE MINI 96-Well Plate	1/Box
8E-S138-5GA	Novum SLE MAX 96-Well Plate	1/Box

Tubes

Part No.	Description	Unit
8B-S138-FAK	Novum SLE 1 cc tubes	100/box
8B-S138-5BJ	Novum SLE 3 cc tubes	50/box
8B-S138-JCH	Novum SLE 6 cc tubes	30/box
8B-S138-KDG	Novum SLE 12 cc tubes	20/box

Kinetex® Core-Shell HPLC/UHPLC Columns

2.6 µm Minibore Columns (mm)

Phases	50 x 2.1	100 x 2.1	150 x 2.1	SecurityGuard™ ULTRA Cartridges [†]
F5	00B-4723-AN	00D-4723-AN	00F-4723-AN	3/pk AJ0-9322 for 2.1 mm ID

2.6 µm MidBore™ Columns (mm)

Phases	50 x 3.0	100 x 3.0	150 x 3.0	SecurityGuard™ ULTRA Cartridges [†]
F5	00B-4723-Y0	00D-4723-Y0	00F-4723-Y0	3/pk AJ0-9321 for 3.0 mm ID

2.6 µm Analytical Columns (mm)

Phases	50 x 4.6	100 x 4.6	150 x 4.6	SecurityGuard™ ULTRA Cartridges [†]
F5	00B-4723-E0	00D-4723-E0	00F-4723-E0	3/pk AJ0-9320 for 4.6 mm ID

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

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