

HPLC Application

ID No.: 21868

Extraction of Mono Hydroxy Vitamin D2 and D3 from Phree on Kinetex C18 2.6u 30x3 mm

Column: Kinetex® 2.6 µm C18 100 Å, LC Column 30 x 3 mm, Ea

Dimensions: 30 x 3 mm ID

Order No: 00A-4462-Y0

Elution Type: Gradient

Eluent A: 0.1% formic acid/water

Eluent B: 0.1% Formic acid/methanol

Gradient Profile:	Step No.	Time (min)	Pct A	Pct B
	1	0	40	60
	2	0.5	5	95
	3	2	5	95
	4	2.01	40	60
	5	3.5	40	60

Flow Rate: 0.6 mL/min

Col. Temp.: ambient

Detection: Electrospray Mass Spec (ESMS) @ 0.0000000000 (ambient)

Analyst Note: Instrument: API 5000 (AB SCIEX)

Ionization Source: APCI, Positive Polarity

Ionization Source Parameters

Curtain Gas: 25.0 psi

Gas 1 (Neb Gas): 40.00 psi

ihe: ON

CAD (Collision Gas): 0.0

NC: 5.00 µA

TEM: 360 °C

Note: Gas 2 is not available in APCI source

MRM Transitions and Mass Dependent Parameters:

Compound	Q1, Da	Q3, Da	Dwell, msec	DP, V	EP, V	CE, V	CXP, V
Vit D2 (1)	395.3	209.3	45	69	10	25	10
Vit D2 (2)	395.3	269.2	45	69	10	25	10
IS (D3-3H2)	386.2	257.2	45	69	10	25	10
Vit D3 (1)	383.2	257.2	45	69	10	23	10
Vit D3 (2)	383.2	211.1	45	69	10	34	10

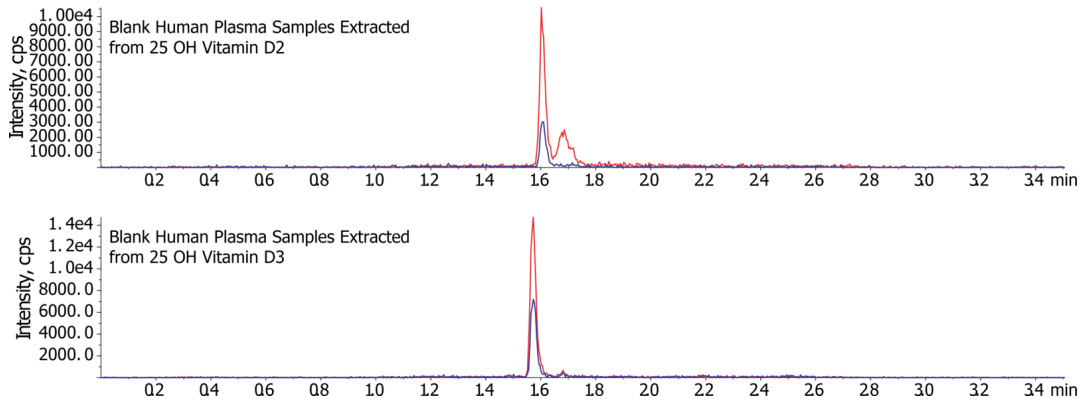


Products used in this application:



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ANALYTES:

- 1 25 Hydroxy vitamin D3-2H3

Retention Time: 1.59 min

- 2 25 Hydroxy vitamin D3

Retention Time: 1.59 min

- 3 25 Hydroxy vitamin D2

Retention Time: 1.62 min



Sample Preparation Details

for HPLC Application ID No.: 21868

Extraction of Mono Hydroxy Vitamin D2 and D3 from Phree on Kinetex C18 2.6u 30x3 mm

PRODUCT DESCRIPTION:

Phree™ Phospholipid Removal, 30 mg / well, 96-Well Plates , 2/Pk

Order No.: 8E-S133-TGB

SOLID PHASE EXTRACTION (SPE) PROCEDURE:

Note: The solvent volumes shown below are for a 30 mg bed mass.

The solvent volumes will need to be adjusted for a smaller or larger bed mass.

Condition:

Load:

Dispense 300 uL of 85%ACN/15% MeOH in the Phree plate.

Add 100uL of Analyte & IS spiked (Vitamin D2 & D3 mix, to yield conc. = 75ng/mL) Human Plasma sample in each well with manual aspiration 2-3x (to ensure complete PPT).

Wait for 30 secs. Apply vacuum for 1-2 mins at high vacuum (15" Hg) and collect filtrate.

Dispense 200 uL more of 85%ACN/15% MeOH. Wait for 30 secs followed by vacuum application for 1-2 mins at 15" Hg and collect filtrate.

Inject the extract directly on to the column (no need to dry down the sample).

Wash:

Dry:

Elute:

Final Prep and Analysis:

Inject: 50 µL on HPLC Electrospray Mass Spec (ESMS) @ 0.000000000 (ambient)

ANALYTES:	Spiked Conc. (ng/mL)	Log P	pKa	% Rec	%RSC (n=0)
1 25 Hydroxy vitamin D3-2H3	200				
2 25 Hydroxy vitamin D3	75			98	
3 25 Hydroxy vitamin D2	75			90	

Note: This method is designed as a convenient starting point for further investigation and can be tailored to meet your extraction goals.
Call your local Phenomenex Representative for assistance in method development and optimization techniques.

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For more information contact your Phenomenex Representative at support@phenomenex.com



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support@phenomenex.com