

## 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:** Mass Spectrometer (MS) @ amu (ambient)**Detector Info:** <a target="\_blank"**Analyst Note:** href="https://sciex.com/products/mass-spectrometers?utm\_campaign=2019%20application%20search&utm\_source=phenomenex&utm\_medium=referral">SCIEX<

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     |

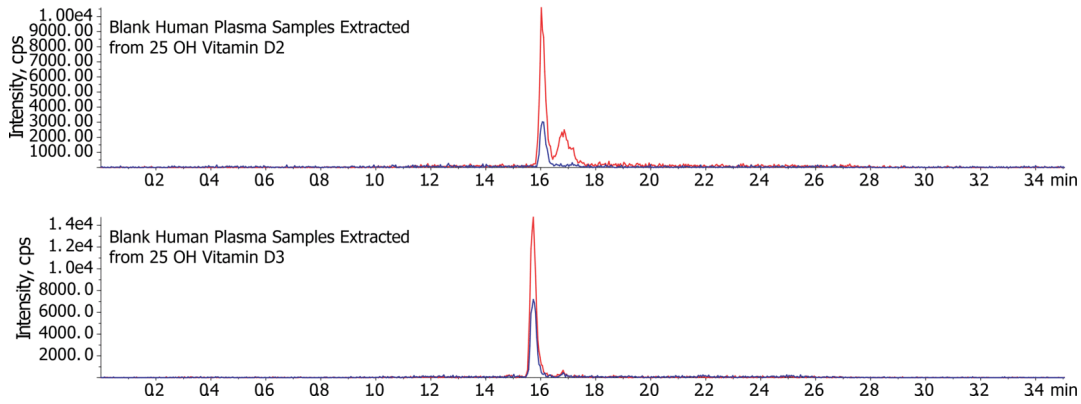


Products used in this application:



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

21868



### 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 Mass Spectrometer (MS) @ amu (ambient)

| ANALYTES:                   | Spiked Conc.<br>(ng/mL) | Log P | pKa | % Rec | %RSC<br>(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.

©2025 Phenomenex Inc. All rights reserved.

For more information contact your Phenomenex Representative at [info@phenomenex.com](mailto:info@phenomenex.com)



Phenomenex products are available worldwide.

[www.phenomenex.com.cn](http://www.phenomenex.com.cn)

[info@phenomenex.com](mailto:info@phenomenex.com)