



**VITAMIN D AND METABOLITES CONFIRMATIONS USING: 200 mg  
CLEAN SCREEN<sup>®</sup> VITAMIN D EXTRACTION COLUMN**

Part #: CEC30126  
LC-UV (PDA), LC-MS/MS

**1. PREPARE SAMPLE:**

To 0.2 to 0.5 mL of sample add an equal volume of deproteinating agent<sup>+</sup>  
(containing internal standard)\*\*

Vortex mix and centrifuge as appropriate.

Transfer organic phase to clean, dry glass tube.

Evaporate to approximately 100  $\mu$ L at  $< 40$  °C.

Dilute with 3 mL of DI H<sub>2</sub>O.

Vortex mix.

**2. CONDITION COLUMN:**

1 x 3 mL CH<sub>3</sub>OH

1 x 3 mL D.I. H<sub>2</sub>O

**Note:** aspirate at  $< 3$  inches Hg to prevent sorbent drying out

**3. APPLY SAMPLE:**

Load sample at 1-2 mL / minute.

**4. WASH COLUMN:**

1x 3 mL DI H<sub>2</sub>O

Dry column (5 minutes at  $> 10$  inches Hg).

**5. ELUTE VITAMIN D AND METABOLITES:**

1 x3 mL CH<sub>3</sub>OH

**6. EVAPORATION:**

Evaporate eluates to dryness at  $< 40$  °C under a gentle stream of nitrogen

## 7. LC-MS/MS

Reconstitute sample in 50  $\mu$ L of CH<sub>3</sub>OH.  
Inject 10  $\mu$ L.

LC-UV (includes PDA)

Reconstitute sample in 100  $\mu$ L of DI H<sub>2</sub>O  
Inject 50  $\mu$ L

### INSTRUMENTAL CONDITIONS:

**Column:** 150 x 2.1 mm (3 $\mu$ m) SB-aq (Agilent Technologies)

**Mobile phase:** 90: 10 CH<sub>3</sub>OH (containing 0.1% Formic acid): 0.1% Formic acid aqueous

**Flowrate:** 0.2 mL/ minute

**UV (PDA):** 260 nm

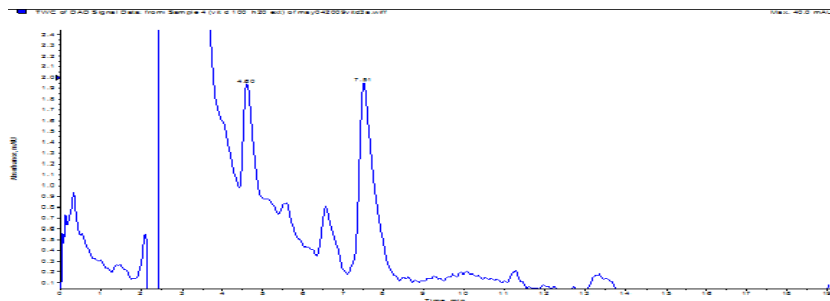
### MS/MS

Vitamin D3  
25Hydroxy Vitamin D3  
25Hydroxy Vitamin D2

### MRM Transition (M-H<sub>2</sub>O)

386.5/256.7  
383.1/211.1  
395.4/209.1

### PDA Chromatogram of Vitamin D ( R<sub>t</sub>=7.3 min)



<sup>†</sup>: Deproteinating agents include acetonitrile, acetone, or methanol.

<sup>\*\*</sup>: Internal Standard: 25 Hydroxy Vitamin D3-D6: MRM Transition: 389.1/211.1

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