

# HPLC Application

ID No.: 23497

## Vitamin D tablet assay (Naturemade 2000 IU + 100 µg std add) on a Kinetex 2.6 µm Biphenyl, 150 x 4.6

**Column:** Kinetex® 2.6 µm Biphenyl 100 Å, LC Column 150 x 4.6 mm, Ea

**Dimensions:** 150 x 4.6 mm ID

**Order No:** 00F-4622-E0

**Elution Type:** Isocratic

**Eluent A:** 0.1% TFA

**Eluent B:** Acetonitrile

Gradient Profile:	Step No.	Time (min)	Pct A	Pct B
	1	0	35	65

**Flow Rate:** 1.75 mL/min

**Col. Temp.:** 40 °C

**Detection:** UV-Vis Abs.-Diode Array (PDA) @ 280 nm (ambient)

**Analyst Note:** Sample preparation: small tablets (<400 mg)

1. Grind tablet into powder and transfer to a 2 mL dSPE tube (KS0-8916)
2. Spike in 20 µL of triphenylene internal standard (1 mg/mL) and standard additions (if applicable)
3. Add 800 µL of water

4. Sonicate until dissolved (approx. 5 min)
5. Add 800 µL of acetonitrile
6. Shake for 10 min using mechanical shaker
7. Centrifuge at 15000 rpm for 3 min

8. Aspirate 100 µL of supernatant and transfer to an autosampler vial for analysis

Sample preparation: large tablets (>400 mg)

1. Grind tablet into powder and transfer to a 5 mL centrifuge tube
2. Spike in 20 µL of triphenylene internal standard (1 mg/mL) and standard additions (if applicable)
3. Add 2 mL of water

4. Sonicate until dissolved (approx. 5 min)
5. Add 400 mg of Quechers salt (AH0-9044)
6. Add 2 mL of acetonitrile
7. Shake for 10 min using mechanical shaker

8. Centrifuge at 6000 rpm for 3 min
9. Aspirate 100 µL of supernatant and transfer to an autosampler vial for analysis

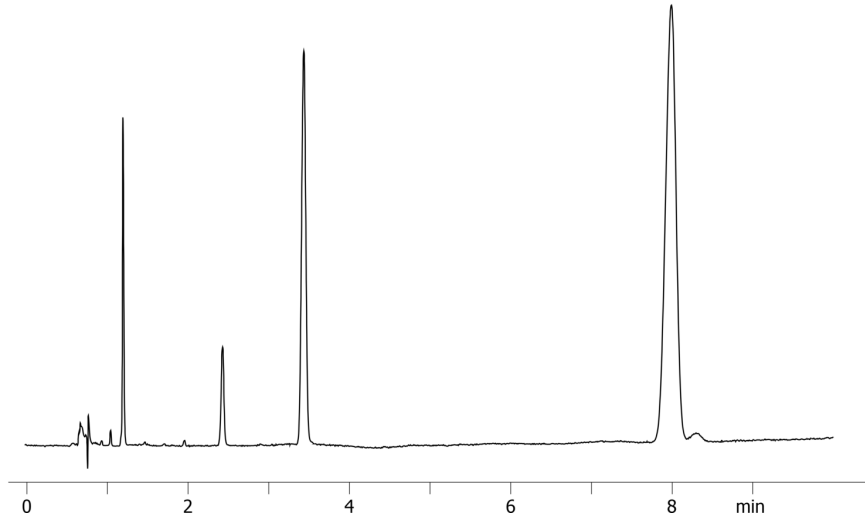


Products used in this application:



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### ANALYTES:

- 1 Triphenylene (I.S.)
- 2 Vitamin D3

