## HPLC Application

### Vitamin D tablet assay (Up & Up) on a Kinetex 2.6 µm Biphenyl, 150 x 4.6

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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	Step No.	Time (min)	Pct A	Pct B	
Profile:	1	0	35	65	
Flow Rate:	1.75 mL/min				
Col. Temp.:	40 °C				
Detection:	UV-Vis AbsDiode 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 (KSO-8916) Spike in 20 $\mu$ L of triphenylene internal standard (1 mg/mL) and standard additions (if applicable) Add 800 $\mu$ L of water Sonicate until dissolved (approx. 5 min) Add 800 $\mu$ L of acetonitrile Shake for 10 min using mechanical shaker Centrifuge at 15000 rpm for 3 min Aspirate 100 $\mu$ L of supernatant and transfer to an autosampler			
	2.				
	3.				
	4.				
	5.				
	6.				
	7.				
	8.				
	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 $\mu$ L of triphenylene internal standard (1 mg/mL) and				
	3.	standard additions (if applicable) Add 2 mL of water			
	4.	Sonicate until dissolved (approx. 5 min) Add 400 mg of Quechers salt (AH0-9044) Add 2 mL of acetonitrile			
	5.				
	6.				
	7.	Shake for 10 min using mechanical shaker			
	8.	Centrifuge at 6000 rpm for 3 min Aspirate 100 $\mu\text{L}$ of supernatant and transfer to an autosampler vial for analysis			
	9.				

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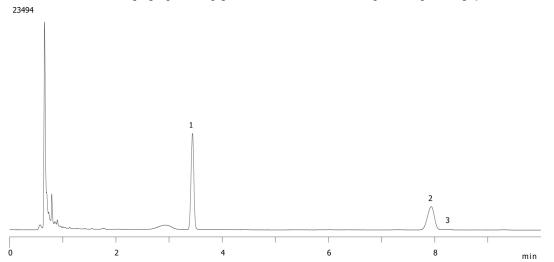
) KINETEX.

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# HPLC Application ID No.: 23494



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#### **ANALYTES:**

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

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