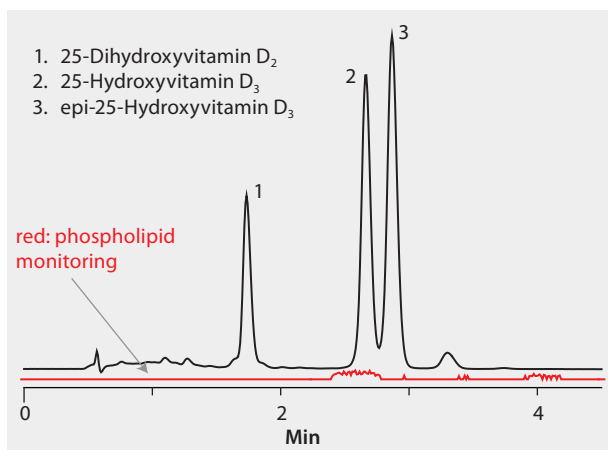


## LC/MS/MS of 25-Dihydroxyvitamin D<sub>2</sub>, 25-Hydroxyvitamin D<sub>3</sub>, and 3-epi-25-Hydroxyvitamin D<sub>3</sub> in Serum or Plasma on Ascentis® Express F5 after SPE with HybridSPE®-Phospholipid, Minimization of Matrix Effects

Chromatographic resolution of the various homologs of vitamin D<sub>2</sub> is necessary for accurate quantification, especially considering several key metabolites are isobaric and not distinguishable by MS alone. A pentafluorophenyl HPLC phase (Ascentis Express F5) was chosen because of its ability to rapidly resolve the vitamin D homologs tested, especially the 25-hydroxyvitamin D<sub>3</sub> and the 3-epi-25-hydroxyvitamin D<sub>3</sub> that coelute on C18 stationary phases. HybridSPE-Phospholipid selectively depleted the phospholipid matrix and precipitated proteins, providing no interference from the serum matrix. Cerilliant CRMs provided reliable quantification.

market focus	.....	Clinical
sample preparation	.....	SPE (Solid Phase Extraction)
sample/matrix	.....	plasma or serum spiked at 25 ng/mL with each vitamin D metabolite
SPE well plate	.....	575656-U (HybridSPE-Phospholipid 96-well plate, 50 mg/2 mL)
sample addition	.....	100 µL spiked rat plasma followed by 300 µL 1% formic acid in acetonitrile
elution	.....	apply vacuum at 10 in Hg for 4 min
eluate post-treatment	.....	collect filtrate and analyze directly
column	.....	Ascentis Express F5, 10 cm x 2.1 mm I.D., 2.7 µm particles (53569-U)
mobile phase	.....	(A) 5 mM ammonium formate in 75:25 (v/v) methanol:water
flow rate	.....	0.4 mL/min
column temp.	.....	40 °C
detector	.....	MS, ESI(+), m/z 100-1000; phospholipids monitored at 496.3, 524.3, 758.5, 786.5, 806.5, and 810.5 m/z
injection	.....	1 µL
Application No.	.....	<b>G1006521</b>



**Related Products**

- analytical column**  
Ascentis® Express F5, 2.7 Micron HPLC Column ([Supelco 53569-U](#))
- SPE tube or plate**  
HybridSPE®-Phospholipid ([Supelco 575656-U](#))
- standard**  
3-epi-25-Hydroxyvitamin D<sub>3</sub> ([Aldrich 705993](#))  
25-Hydroxyvitamin D<sub>2</sub> solution ([Cerilliant H-073](#))  
25-Hydroxyvitamin D<sub>3</sub> solution ([Cerilliant H-083](#))