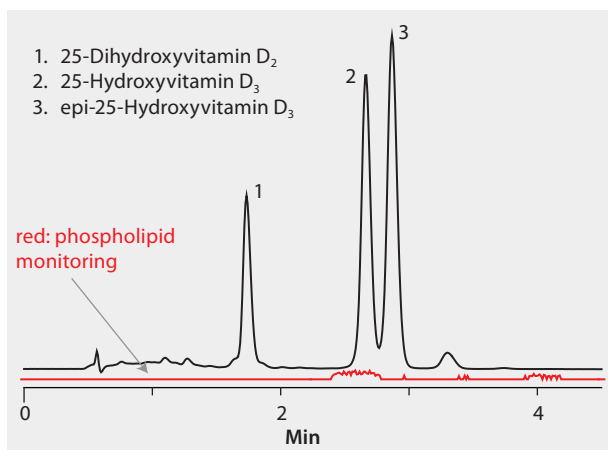


LC/MS/MS of 25-Dihydroxyvitamin D₂, 25-Hydroxyvitamin D₃, and 3-epi-25-Hydroxyvitamin D₃ in Serum or Plasma on Ascentis® Express F5 after SPE with HybridSPE®-Phospholipid, Minimization of Matrix Effects

Chromatographic resolution of the various homologs of vitamin D₂ 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 D₃ and the 3-epi-25-hydroxyvitamin D₃ 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.	G1006521



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 D₃ ([Aldrich 705993](#))

25-Hydroxyvitamin D₂ solution ([Cerilliant H-073](#))

25-Hydroxyvitamin D₃ solution ([Cerilliant H-083](#))