

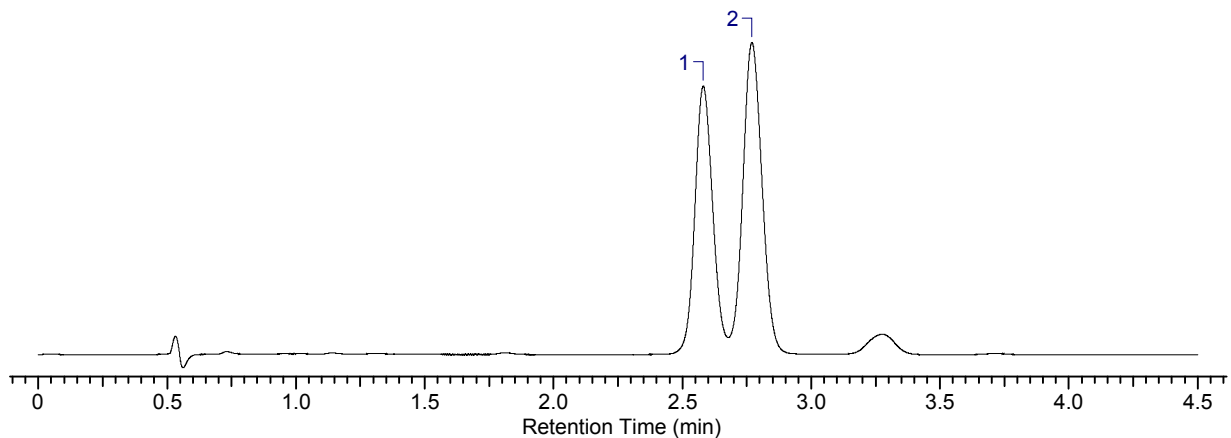
## Application Report

### Analysis of 25-hydroxyvitamin D3 and 3-epi-25-hydroxyvitamin D3 on Ascentis Express F5

Bonded Phase pentafluorophenylpropyl  
Column Name Ascentis Express F5  
Instrument Name Agilent 1200SL

#### Key Words

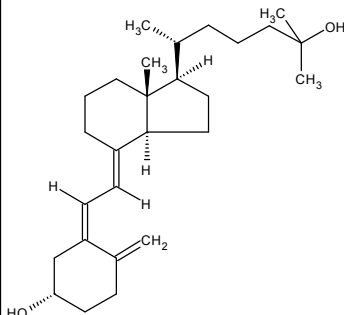
25 hydroxyvitamin D3, 3-epi-25-hydroxyvitamin D3, Ascentis Express F5, 63283-36-3, 73809-05-9, 53569-U



#### Conditions

column: Ascentis Express F5, 10 cm x 2.1 mm I.D.  
2.7  $\mu\text{m}$  particles (53569-U)  
mobile phase A: 5 mM ammonium formate (75:25 methanol:water)  
flow rate: 0.4 mL/min.  
initial pressure: 300 bar (2900 psi)  
temp.: 40  $^{\circ}\text{C}$   
det.: UV 265 nm  
MS det.: ESI(+), m/z 100-1000  
injection: 1  $\mu\text{L}$   
sample: 300  $\mu\text{g}$  /mL in 25:75, water:methanol

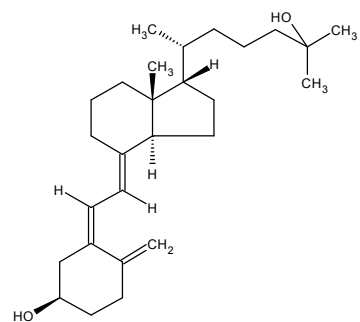
#### Structures



25-Hydroxyvitamin D3

Monoisotopic Mass = 400.334131 Da

Molecular Formula =  $\text{C}_{27}\text{H}_{44}\text{O}_2$



3-epi-25-Hydroxyvitamin D3

Monoisotopic Mass = 400.334131 Da

Molecular Formula =  $\text{C}_{27}\text{H}_{44}\text{O}_2$

#### Peak IDs

No.	Name	tR
1	25-hydroxyvitamin D3	2.6
2	epi 25-hydroxyvitamin D3	2.8