

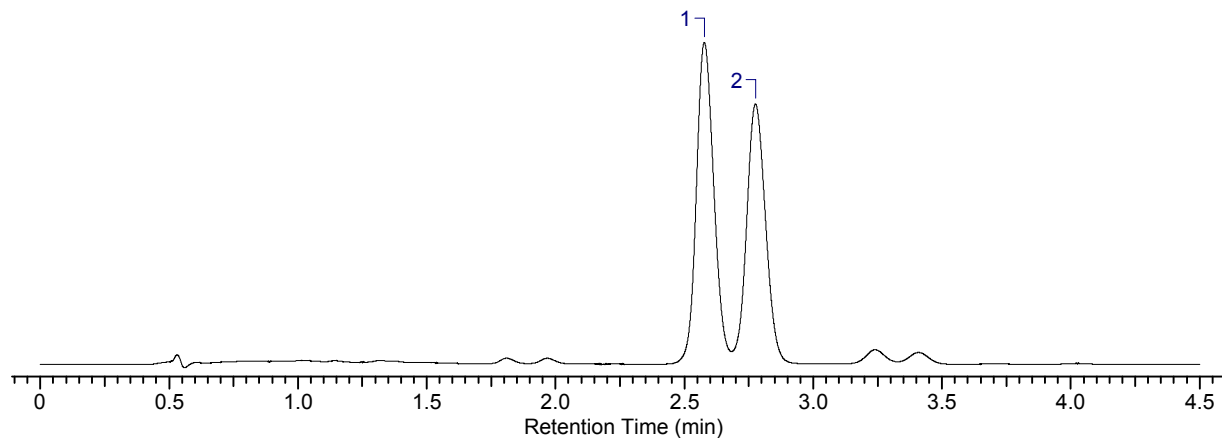
## Application Report

### Analysis of 25-hydroxyvitamin D3 and 25-hydroxyvitamin D2 on Ascentis Express F5

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

#### Key Words

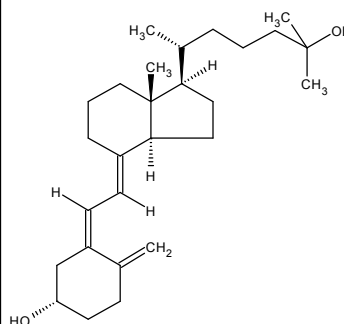
25 hydroxyvitamin D3, 25-hydroxyvitamin D2, Ascentis Express F5, 63283-36-3, 21343-40-8, 53569-U



#### Conditions

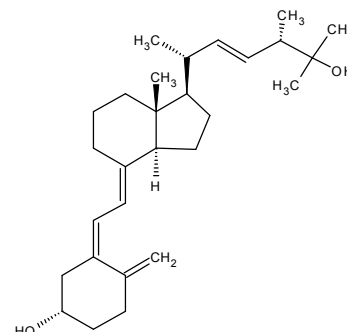
column: Ascentis Express F5, 10 cm x 2.1 mm I.D.  
2.7  $\mu$ m particles (53569-U)  
mobile phase A: 5mM ammonium formate (75:25 methanol:water)  
flow rate: 0.4 mL/min.  
initial pressure: 300 bar  
temp.: 40 °C  
det.: UV 265 nm  
MS det. ESI(+), m/z 100-1000  
injection: 1  $\mu$ L  
sample: 300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

#### Structures



25-Hydroxyvitamin D3

Monoisotopic Mass = 400.334131 Da  
Molecular Formula = C<sub>27</sub>H<sub>44</sub>O<sub>2</sub>



25-Hydroxyvitamin D2

Monoisotopic Mass = 412.334131 Da  
Molecular Formula = C<sub>28</sub>H<sub>44</sub>O<sub>2</sub>

#### Peak IDs

No.	Name	tR
1	25-hydroxyvitamin D3	2.6
2	25-hydroxyvitamin D2	2.8