

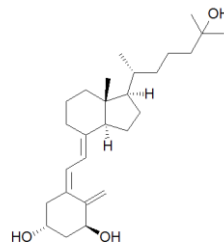
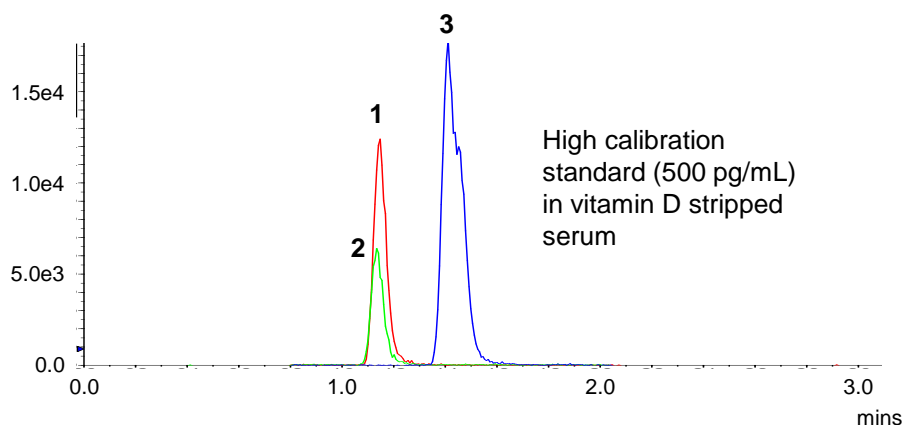
1,25-Dihydroxyvitamins D2 and D3 in Serum by LC-MS/MS

Application #AN4070

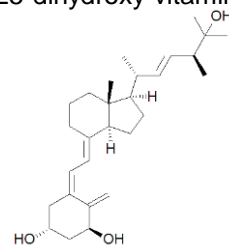
Conditions

Column: ACE UltraCore 2.5 SuperC18
Dimensions: 50 x 2.1 mm
Part Number: CORE-25A-0502U
Mobile Phase: MeCN/H₂O (50:50 v/v) containing 30 µL methylamine per 500 mL
Flow Rate: 0.5 mL/min
Injection: 20 µL
Temperature: 40 °C
Detection: AB Sciex 5500 triple quad MS
ESI in positive ion mode
IonSpray Voltage: 5500 V
Temperature: 550 °C

Sample: 1,25 diOH vitamin D2 and 1,25 diOH vitamin D3 metabolites are extracted from serum using supported liquid extraction. Sensitivity of LC-MS/MS analysis is maximised through use of PTAD (9-phenyl-1,2,4-triazole-3,5-dione) derivatisation and complexation with methylamine.

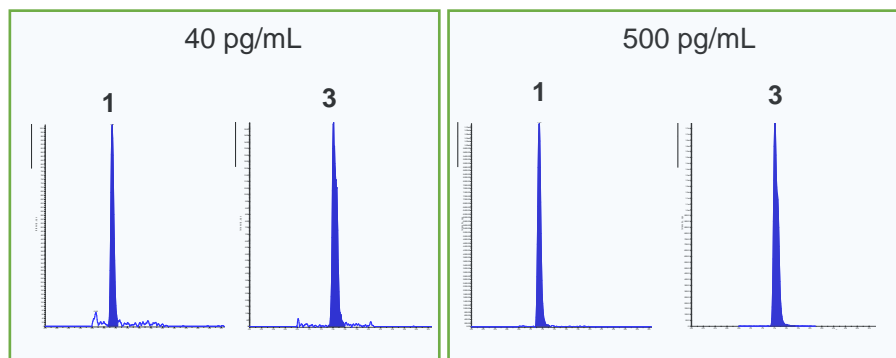


1,25-dihydroxy vitamin D3



1,25-dihydroxy vitamin D2

Low and high calibration standards in charcoal stripped (vitamin D free) serum, analysed as PTAD-methylamine complexes



- 1,25-diOH vitamin D3-PTAD-methylamine complex (m/z 623.4 \rightarrow 314.1)
- d3-1,25-diOH vitamin D3-PTAD-methylamine complex (I.S.) (m/z 626.4 \rightarrow 317.1)
- 1,25-diOH vitamin D2-PTAD-methylamine complex (m/z 635.4 \rightarrow 314.1)

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