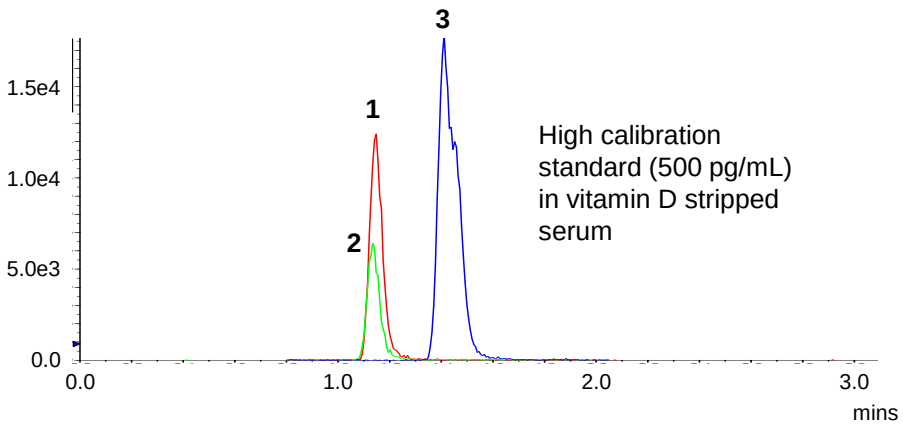
1,25-Dihydroxyvitamins D2 and D3 in Serum by LC-MS/MS

ACE®
Ultra-inert
UHPLC & HPLC Columns

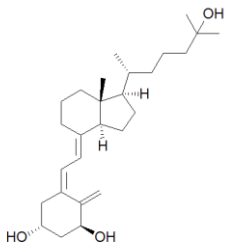
Application #**AN4070**

Conditions

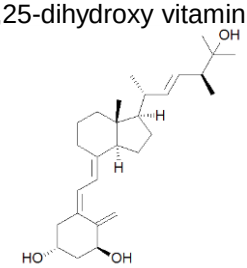
Column:	ACE UltraCore 2.5 SuperC18
Dimensions:	50 x 2.1 mm
Part Number:	CORE-25A-0502U
Mobile Phase:	MeCN/H_2O (50:50 v/v) containing 30 µL methylamine per 500 mL
Flow Rate:	0.5 mL/min
Injection:	20 µL
Temperature:	40 °C
Detection:	AB Sciex 5500 triple quad MS ESI in positive ion mode IonSpray Voltage: 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.



High calibration standard (500 pg/mL) in vitamin D stripped serum

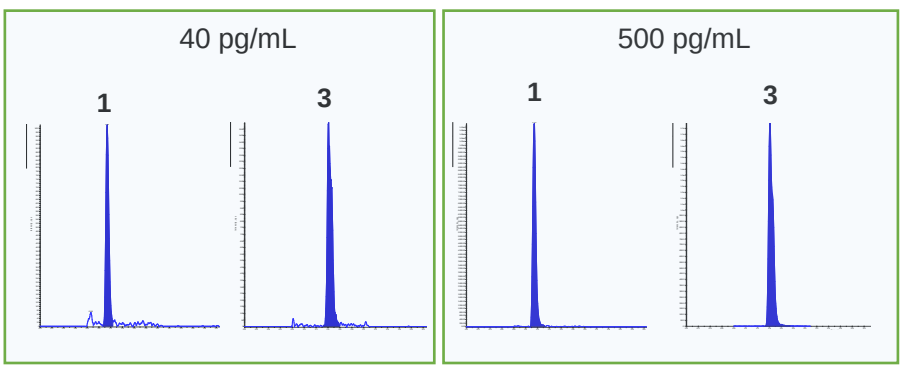


1,25-dihydroxy vitamin D3



1,25-dihydroxy vitamin D2

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



1. 1,25-diOH vitamin D3-PTAD-methylamine complex (*m/z* 623.4 → 314.1)
2. d3-1,25-diOH vitamin D3-PTAD-methylamine complex (I.S.) (*m/z* 626.4 → 317.1)
3. 1,25-diOH vitamin D2-PTAD-methylamine complex (*m/z* 635.4 → 314.1)

Reproduced with permission of Biotage GB Ltd, UK. For further details of sample preparation see Biotage App Note AN857

© 2015 ACT Ltd. All rights reserved. All products are available worldwide.