

LC-MS analysis of vitamin D2/D3 in human plasma and serum

Application details

- Analysis and quantification of vitamin D2/D3 metabolites 25-hydroxyvitamin D2/D3 in two human patient plasma and serum samples via reversed phase LC-MS/MS.
- Samples were prepared via simple protein precipitation and two internal standards 2H3-25-OH vitamin D2/D3 were utilized.
- As complete elimination of endogenous levels of 25-OH Vitamin D2/D3 in human serum/plasma is impossible, deuterated 2H6-25-OH Vitamin D2/D3 were used.
- Standard curves were created from standard samples where ratio of deuterated 25-OH vitamin D2/D3 and two internal standards 2H3-25-OH vitamin D2/D3 were plotted against ratio of concentrations of the same.

Chromatographic Conditions

Column Purospher® STAR RP-18 endcapped (2µm) Hibar® HR 100-2.1

Injection volume 10 µL

Detection APCI-MS/MS, MRM transitions: *m/z* 419.3/355.1, 416.3/358.1 (vitamin D2) and *m/z* 407.3/159.0, 404.3/162.0 (vitamin D3)

Flow Rate 0.4 - 0.5 mL/min

Mobile Phase A: Water
B: Methanol

Gradient see table

Temperature 50 °C

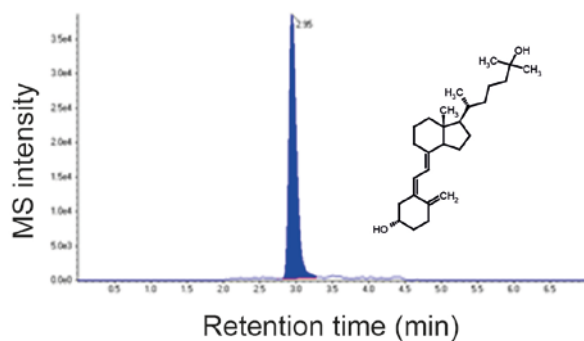
Sample diluent (v/v) Water

Sample/sample preparation Patient plasma and serum prepared with commercially available vitamin D kit.

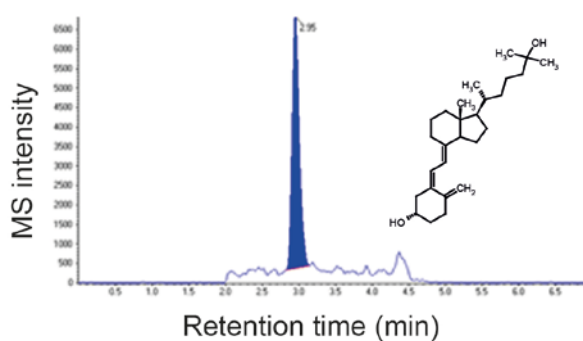
Ordering information

- Purospher® STAR RP-18 endcapped (2µm) Hibar® HR 100-2.1 (Cat. No. 150648)
- Methanol hypergrade for LC-MS LiChrosolv® (Cat. No. 106035)
- Water for chromatography LiChrosolv® (LC-MS) (Cat. No. 115333 or Milli-Q® water from water purification systems)

25-Hydroxyvitamin D3 content
55.7 nMol/L



25-Hydroxyvitamin D3 content
10.0 nMol/L



Chromatographic Data

No.	Compound	Retention time (min)
1	25-Hydroxyvitamin D3	2.95

Time (min)	Eluent A (%)	Eluent B (%)	Flow rate (mL/min)
0.00	15	85	0.40
4.50	15	85	0.40
4.51	0	100	0.50
5.53	0	100	0.50
5.55	15	85	0.40
6.50	15	85	0.40

