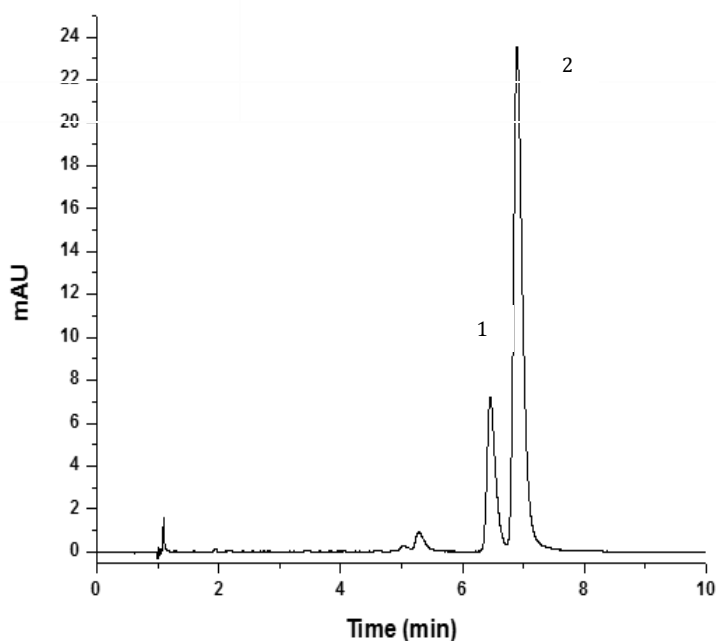


## FLARE C18 MM Column: Vitamin D<sub>2</sub> and Vitamin D<sub>3</sub>

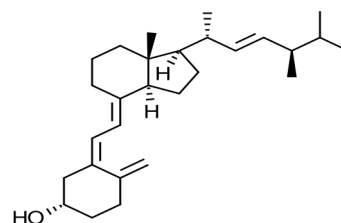
### HPLC Conditions

|                           |                               |
|---------------------------|-------------------------------|
| <b>Column Name:</b>       | FLARE C18 MM                  |
| <b>Column Dimensions:</b> | 150 x 4.6 mm (SN: 15698.35-1) |
| <b>HPLC System:</b>       | Agilent 1290                  |
| <b>Injection Volume:</b>  | 0.5 µl in ACN                 |
| <b>Detection:</b>         | UV at 254 nm                  |
| <b>Flow Rate:</b>         | 1.0 ml/min                    |
| <b>Solvents:</b>          | A: H <sub>2</sub> O<br>B: ACN |
| <b>Elution Method:</b>    | Isocratic 20% A/ 80% B        |
| <b>Temperature:</b>       | 35 °C                         |

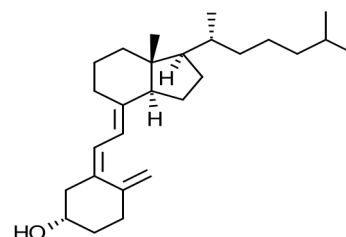
|                        | Rt (mins) | Rs   | $\alpha$ | N     |
|------------------------|-----------|------|----------|-------|
| Vit D <sub>2</sub> (1) | 6.453     |      |          | 10830 |
| Vit D <sub>3</sub> (2) | 6.891     | 1.73 | 1.09     | 11417 |



1. Vitamin D<sub>2</sub> (ergocalciferol)



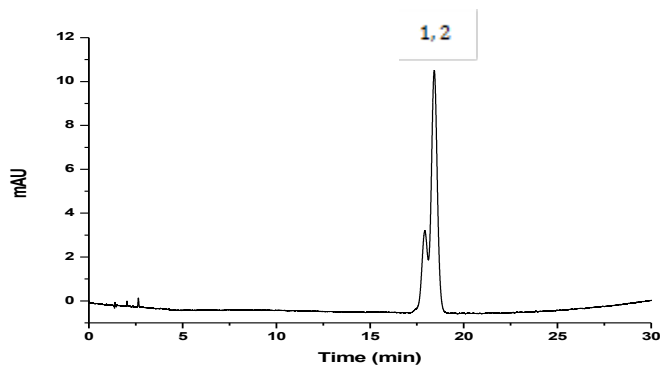
2. Vitamin D<sub>3</sub> (cholecalciferol)



### Separation on leading Silica C18 Column

Diamond Analytics, 1260 S. 1600 W., Orem, Utah  
 P: (801) 235-9001, F: (801) 235-9141  
 info@diamond-analytics.com  
 www.diamond-analytics.com





*On silica column, separation is long (about 20mins) and resolution is 0.83 using a 150 x 4.6mm column containing 3.6 um fully porous silica particles.*

### Notes

Vitamin D is essential for adsorption of minerals in the intestines, for bone and muscular health as well as the prevention of chronic conditions such as autoimmune disorders, diabetes and heart disease. This essential vitamin is obtained from supplements and food such as meat and eggs and from exposure to sunlight. Chromatographic analysis of vit D<sub>2</sub> and vit D<sub>3</sub> is difficult on a silica C18 column.. The method presented here using the FLARE diamond core-shell column is MS-compatible and can be reproduced on any traditional LC system.

### References

1. Hymoller, Lone; Jensen, Soren Krogh. Vitamin D analysis in plasma by high performance liquid chromatography (HPLC) with C30 reversed phase column and UV detection – Easy and acetonitrile-free. J. Chrom A, 2011, 1218(14), 1835-1841
2. Lensmeyer, Gary L; Wiebe, Donald A.,; Binkley, Neil; Drezner, Marc K. HPLC Method for 25-Hydroxyvitamin D Measurement: Comparison with Contemporary Assays. Clinical Chemistry, 2006, 52(6), 1120-1126

