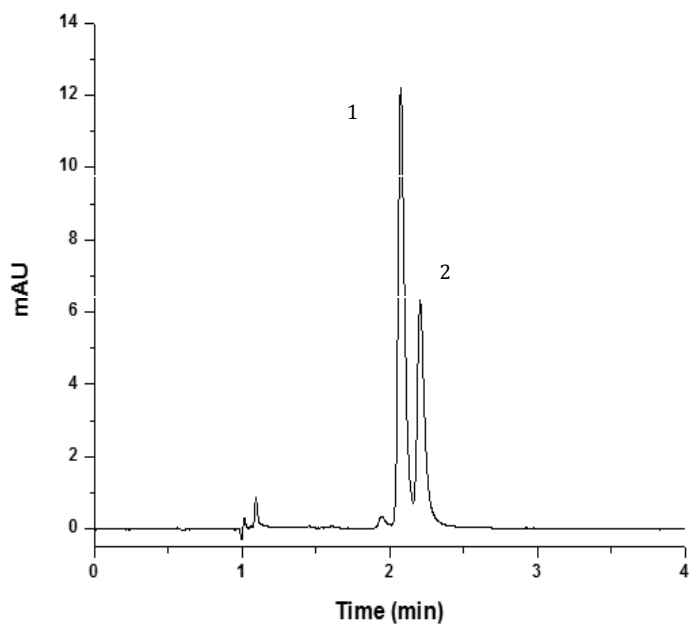


## FLARE C18 MM Column: Vitamin D<sub>2</sub> and Vitamin D<sub>3</sub>

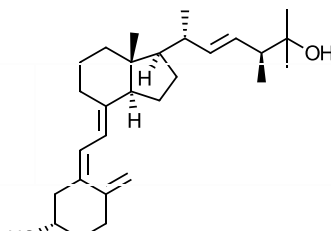
### HPLC Conditions

<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 B: ACN
<b>Elution Method:</b>	Isocratic 20% A/ 80% B
<b>Temperature:</b>	35 °C

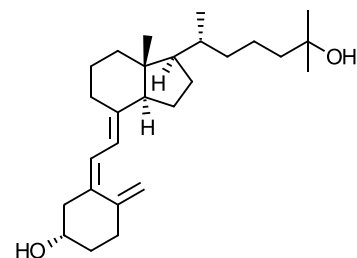
	Rt (mins)	Rs	$\alpha$	N
25 (OH) vit D <sub>2</sub> (1)	2.075			11773
25 (OH) vit D <sub>3</sub> (2)	2.205	1.6	1.23	10307



1. 25 (OH) Vitamin D<sub>2</sub> (ercalcidiol)



2. 25 (OH) Vitamin D<sub>3</sub> (calcifediol)



### Notes

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



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. In the human body, vitamins D<sub>2</sub> and D<sub>3</sub> are converted by the liver into various compounds which are more abundant and can be monitored to determine vitamin D levels. The amount of these compounds in human serum also indicates how well the body is metabolizing the vitamin. There are various metabolic products on the market, but among the most important are 25 (OH) Vitamin D<sub>2</sub> (ercalcidiol) and 25 (OH) vitamin D<sub>3</sub> (calcifediol). In this experiment, a baseline separation of these two metabolites is accomplished in less than 6 minutes on the FLARE C18 MM column. This method 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

