Featured Application: *LC Analysis of Vitamin D Epimers on Raptor FluoroPhenyl Columns*

Fast, Accurate LC-MS/MS Analysis of the C3 Epimer of 25-Hydroxyvitamin D on Raptor FluoroPhenyl Columns

- 5-minute analysis time allows more samples per day.
- Complete separation of vitamin D epimers ensures accurate results.
- Long-lasting, rugged Raptor FluoroPhenyl LC columns reduce cost per sample.

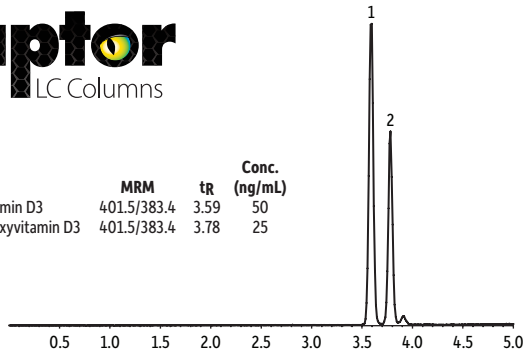
Vitamin D analysis has increased dramatically in clinical practice due to its association with multiple human diseases including cancer, rheumatoid arthritis, and diabetes. It also has an important role in calcium and phosphorous absorption in relation to bone strength, so there is growing concern about the prevalence of vitamin D deficiency worldwide. Accurate LC analysis of vitamin D epimers is critical for quantitative determination of the bioactive forms of vitamin D.

Vitamin D exists in two forms, vitamin D2 and vitamin D3, which undergo metabolism to form 25-hydroxyvitamins D2 and D3, respectively. These metabolites are used as biomarkers to assess vitamin D status; however, both have epimeric forms (3-epi-25-hydroxyvitamins D2 and D3) that contribute significantly to the total 25-hydroxyvitamin D2 and D3 concentrations. Studies have shown that these vitamin D epimers have much lower bioactivity than the primary metabolites; therefore, they must be separated in order to prevent overestimating vitamin D levels.

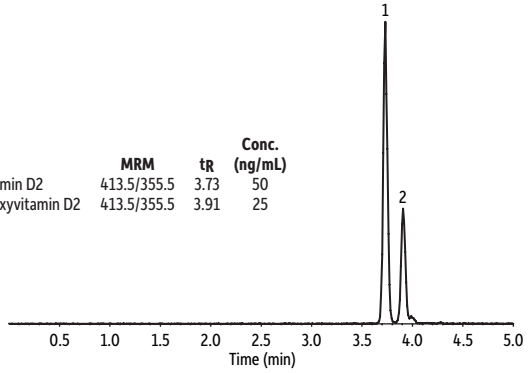
LC-MS/MS is a common technique for vitamin D analysis; however, because the biomarkers and their epimers are isobaric, chromatographic separation is essential for accurate quantitation. Many published methods employ a C18 column, but its utility is limited by incomplete resolution of the biomarkers and their epimers as well as by analysis times of 10 minutes or more. The Raptor FluoroPhenyl column is a better choice as it provides alternate selectivity to a C18 column and completely separates the isobaric epimers and biomarkers in just 5 minutes. As shown below, Raptor FluoroPhenyl columns improve LC analysis of vitamin D epimers, reliably providing accurate measurement of vitamin D content for disease and deficiency investigation and monitoring.

Raptor LC Columns

Peaks	MRM	t_R	Conc. (ng/mL)
1. 25-Hydroxyvitamin D3	401.5/383.4	3.59	50
2. 3-Epi-25-hydroxyvitamin D3	401.5/383.4	3.78	25



Peaks	MRM	t_R	Conc. (ng/mL)
1. 25-Hydroxyvitamin D2	413.5/355.5	3.73	50
2. 3-Epi-25-hydroxyvitamin D2	413.5/355.5	3.91	25



LC_CF0644

Column	Raptor FluoroPhenyl (cat.# 9319A1E)
Dimensions:	100 mm x 3 mm ID
Particle Size:	2.7 µm
Temp.:	30 °C
Sample	
Diluent:	Water:methanol (50:50)
Conc.:	25-50 ng/mL
Inj. Vol.:	5 µL
Mobile Phase	
A:	0.1% Formic acid in water
B:	Methanol

Time (min)	Flow (mL/min)	%A	%B
0.00	0.6	25	75
4.00	0.6	15	85
4.10	0.6	0	100
5.00	0.6	0	100
5.01	0.6	25	75
7.00	0.6	25	75

Detector	MS/MS
Ion Mode:	ESI+
Mode:	MRM
Instrument	HPLC

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