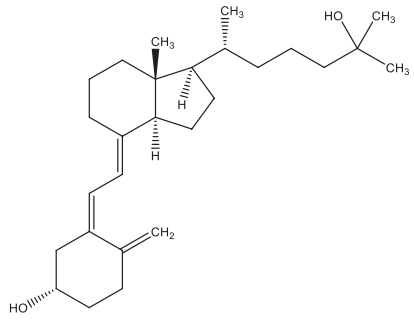
Supelco®
Analytical Products

Application Note

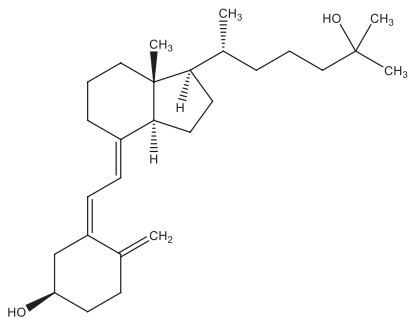
UHPLC Analysis of Vitamin D2 & D3 Metabolites and Epimers on Supel™ Carbon LC Column

Introduction

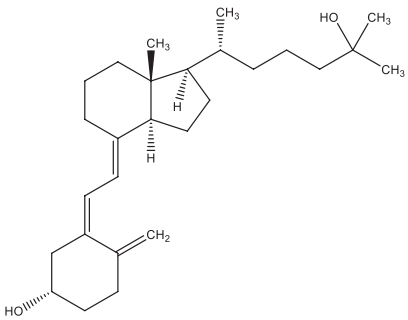
Analysis of vitamin D metabolites has continued to be a topic of interest in recent publications, primarily as biomarkers for possible disease states and vitamin deficiency. While vitamin D is present in two forms, vitamin D3 and vitamin D2, current ELISA methods demonstrate different cross-reactivities and cannot distinguish between D2 and D3 forms of the vitamin metabolites resulting in erroneous reporting of total 25-hydroxyvitamin D concentrations (**Figure 1**). Further, there is interest in an analytical means to differentiate the D2 and D3 forms from the D2 and D3 epimers because of their different degrees of bioactivity. This application demonstrates the use of the Supel™ Carbon LC UHPLC column, with its ability to resolve structural isomers, to baseline separate all four analytes.



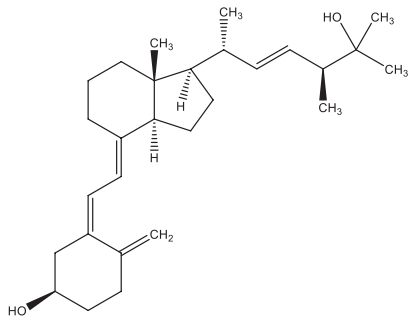
25-hydroxyvitamin D3



3-epi-25-hydroxyvitamin D3



25-hydroxyvitamin D2

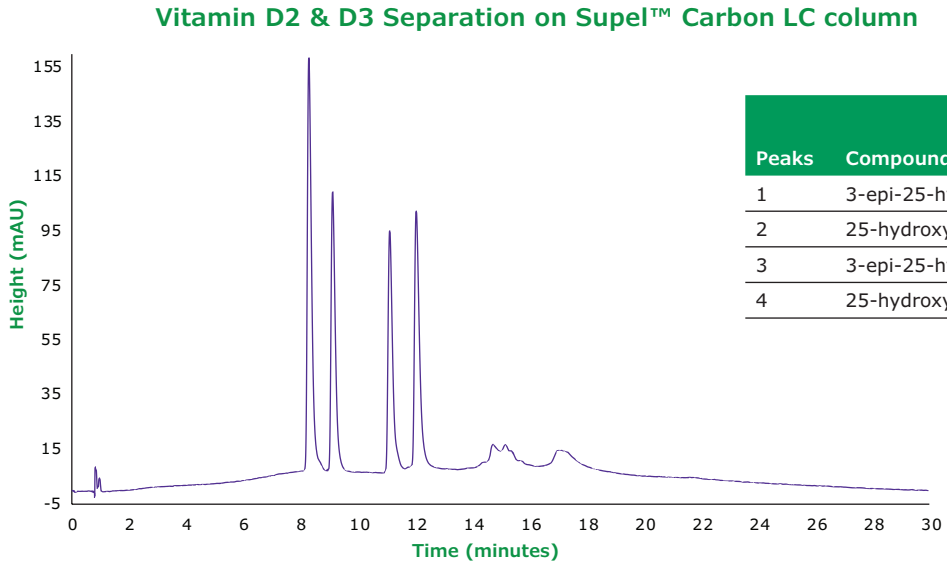


3-epi-25-hydroxyvitamin D2

Figure 1: Structures of Vitamin D2 and D3 metabolites.

Results/Conclusion

Figure 2 shows the results of the separation of the Vitamin D2 and D3 metabolites. Note the sharp peak shape and baseline resolution between all four analytes.



Peaks	Compound	Retention Time (min)
1	3-epi-25-hydroxyvitamin D3 (50 µg/mL)	8.294
2	25-hydroxyvitamin D3 (25 µg/mL)	9.125
3	3-epi-25-hydroxyvitamin D2 (50 µg/mL)	11.126
4	25-hydroxyvitamin D2 (100 µg/mL)	12.052

Figure 2: Chromatographic separation of Vitamin D2 and D3 metabolites on Supel™ Carbon LC. Conditions: Column: Supel™ Carbon LC, 10 cm x 2.1 mm I.D., 2.7 µm; Mobile Phase: [A] 2-Propanol; [B] Tetrahydrofuran; Gradient: 0% B to 70% B in 15 min; hold at 70% B for 5 min; Flow Rate: 0.3 mL/min; Column Temp.: 25 °C; Detector: UV, 275 nm; Injection: 2.0 µL; Sample: Vitamin D2 and D3 metabolites mix, varied concentration, ethanol

This application demonstrated the use of the Supel™ Carbon LC column to resolve vitamins D2 and D3 metabolites and their epimers. Baseline separation of all four analytes was achieved with excellent peak shape and sensitivity.

Product list	PN
Supel™ Carbon LC Column, 10 cm x 2.1mm I.D., 2.7 µm	**59986-U**
2-Propanol for HPLC, 99.5%	**439207**
Tetrahydrofuran for HPLC, > 99.9%, Inhibitor-free	**439215**
25-hydroxyvitamin D3 solution, 100 µg/mL in ethanol	**H-083**
25-hydroxyvitamin D2 solution, 50 µg/mL in ethanol	**H-073**
3-epi-25-hydroxyvitamin D3, 50 µg/mL in ethanol	**E-086**
3-epi-25-hydroxyvitamin D2, 98%	**753149**

Merck KGaA
Frankfurter Strasse 250
64293 Darmstadt, Germany

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