APPLICATION NOTE



Vitamin D Level Determination in Serum

Vitamin D_3 (cholecalciferol) is essential for the human body. Low levels of vitamin D_3 increase the risk of illness from a range of disorders. Babies, young children, pregnant mothers and old people are at a higher risk. Traditionally, vitamin D deficiency has been associated with rickets, a disease in which the bone tissue does not properly form, leading to soft bones and skeletal deformities. However, research is revealing the importance of vitamin D in protecting against a host of other health problems.



What to Detect in Serum

The serum level of vitamin D is monitored to exclude deficiency symptoms. As vitamin D products and nutritional supplements often contain vitamin D_3 and/or vitamin D_2 (ergocalciferol), usually the concentration of 25-hydroxy vitamin D_3 (25-OH-D₃, calcidiol) is determined. 25-OH-vitamin D₃ is the liver-generated storage form of vitamin D and provides a differentiated and meaningful impression of a patient's vitamin D level.

Challenging Separation

As vitamin D_2 , D_3 and 25-OH-vitamin D_3 are **structurally similar substances**, the (U)HPLC separation is therefore **very challenging**. Often, **special columns** in combination with **complex eluent mixtures** have to be used.

Using YMC-Triart C18 ExRS makes the separation much easier!

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Baseline Separation in less than 6 min

The use of an UHPLC YMC-Triart C18 ExRS column under isocratic conditions allows a baseline separation of these three substances in less than 6 min. THF / acetonitrile (10/90) was used as mobile phase with a flow rate of 0.65 mL/min.



	25-OH vitamin D ₃	Vitamin D ₂	Vitamin D ₃
	(calcidiol)	(ergocalciferol)	(cholecalciferol)
Retention Time	1.244 min	4.893 min	5.296 min

Column: YMC-Triart C18 ExRS 1.9 μm, 8 nm (100 x 2.0 mm ID)

Part No.: TAR08SP9-1002PT

Eluent: THF / acetonitrile (10/90)

Flow rate: 0.65 ml/min

Temperature: 30 °C

Detection: UV at 265 nm

Injection: 1 µL (0.2 mg/mL calcidiol

0.5 mg/mL ergocalciferol 0.5 mg/mL cholecalciferol)