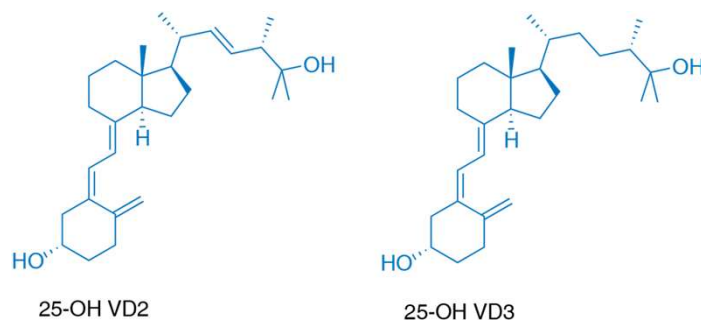


# Application Note: Vitamin D2/D3



25-Hydroxyvitamin D (abbreviated as 25-OH VD), including 25-hydroxyvitamin D2 (25-OH VD2) and 25-hydroxyvitamin D3 (25-OH VD3), as one of the main metabolic forms of vitamin D in the body, is considered a vitamin D nutrient level marker due to its long half-life and stable form of existence. Liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) is considered the "gold standard" assay for evaluating vitamin D nutritional status due to its strong specificity and high accuracy.

Serum sample preparation using SPE-HLB 96-well plates, UPLC-MS/MS for sample determination, and Horizon C18/PFP 1.6 $\mu$ m 100 $\times$ 2.1mm provided a robust and reliable solution for this project.



**Column: Horizon C18/PFP 1.6 $\mu$  100 $\times$ 2.1mm**

Mobile phase: A: 2 mM ammonium acetate-0.1% formic acid aqueous solution, B: 2 mM ammonium acetate-0.1% formate methanol solution

Time	A%	B%
0	32	68
0.80	32	68
2.80	28	72
3.50	2	98
4.80	32	68

Flow rate: 0.5mL/min  
Column temperature: 50°C  
Injection volume: 10 $\mu$ L  
Ionization mode: ESI  
Capillary voltage: 1.0 KV  
Solvent degassing temperature: 60°C  
Desolvation airflow: 1200L/h  
Source temperature: 350°C

