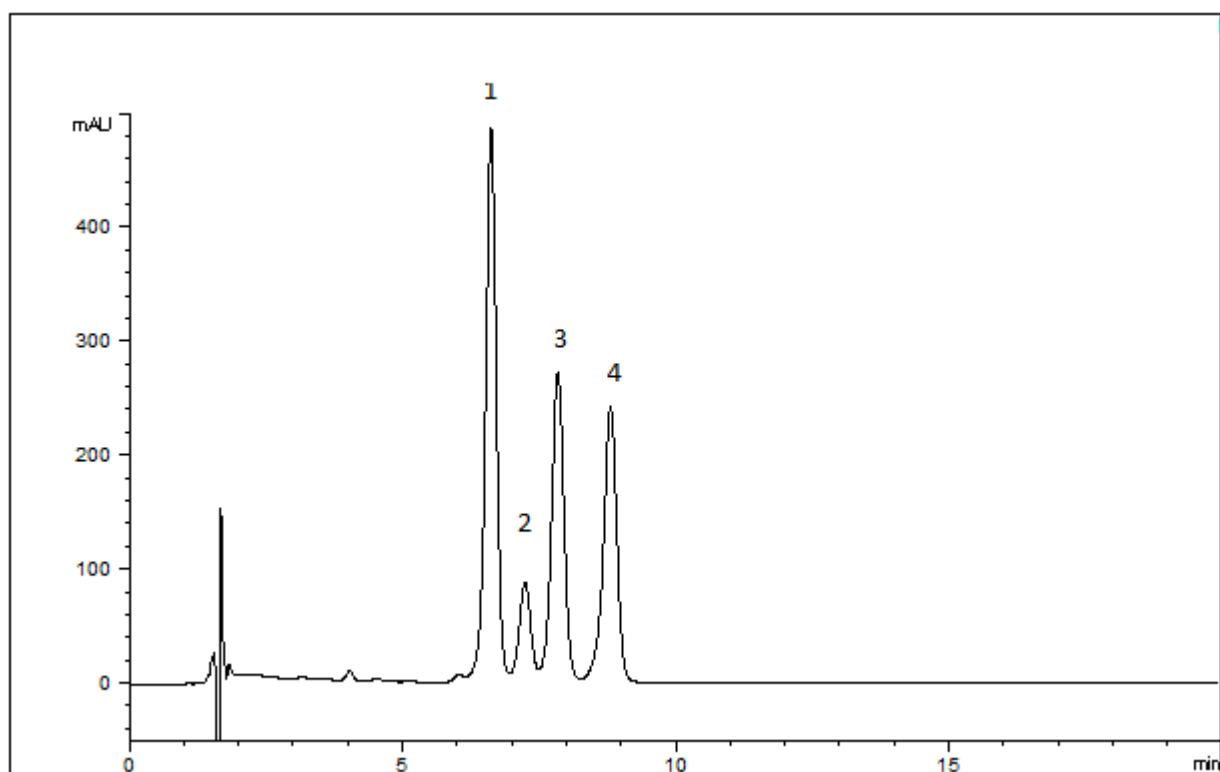


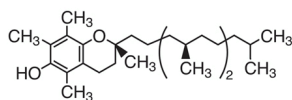
Vitamins D2 D3 E and K1 Analyzed by HPLC - AppNote

Date: 8-OCTOBER-2026 Last Updated: 13-OCTOBER-2026

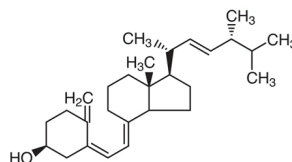
Introduction

Fat-soluble vitamins D2 (Ergocalciferol), D3 (Cholecalciferol), E (α -Tocopherol), and K1 (Phylloquinone) are commonly found in dietary supplements and fortified products. Due to their hydrophobic nature and similar properties, their analysis can be challenging using conventional C18 columns. This application note demonstrates the simultaneous HPLC analysis of these vitamins using a C30 column, which provides enhanced selectivity and resolution.

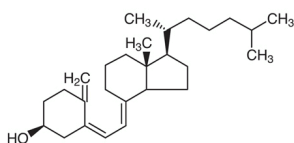




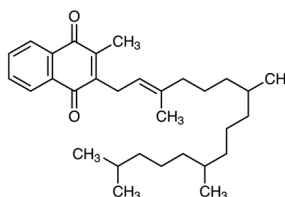
1



2



3



4

Peaks: 1. α -Tocopherol (Vitamin E), CAS 59-02-9
 2. Ergocalciferol (Vitamin D₂), CAS 50-14-6
 3. Cholecalciferol (Vitamin D₃), CAS 67-97-0
 4. Phylloquinone (Vitamin K₁), CAS 84-80-0

Method Conditions

Column: Cogent C30™, 5 μ m, 200 Å

Catalog No. : [72530-25P](#)

Dimensions : 4.6 x 250 mm

Mobile Phase : 80:20 Acetonitrile / Methanol (80:20) containing 0.1% formic acid

Injection vol. : 4 μ L

Flow rate : 1.0 mL/minute

Detection : UV @ 210 nm

Sample Preparation : Phylloquinone (Vitamin K₁), CAS 84-80-0, 1.7 mg/mL, α -Tocopherol, CAS 59-02-9, 2.0 mg/mL, Ergocalciferol (Vitamin D₂), CAS 50-14-6, 2.0 mg/mL, Cholecalciferol (Vitamin D₃), CAS 67-97-0, 2.15 mg/mL- all prepared in ethanol

Mixed Standard: 100 μ L of each stock solution.

Notes: Vitamins D₂ and D₃ are structurally similar secosteroids, while Vitamin E (α -tocopherol) and Vitamin K₁ (phylloquinone) are fat-soluble vitamins with distinct chromophores. Due to their hydrophobic nature, these compounds are well-suited for separation using C30 stationary phases.