

Separating Fat-Soluble Vitamins by Reversed Phase HPLC, Using Discovery Columns

Food manufacturers routinely analyze vitamins in their products to determine quantitative levels and to monitor changes that have occurred in food processing. In our study, various mixtures of fat-soluble vitamins in A, D, and E groups were studied by reversed-phase HPLC using Discovery columns. These columns yielded excellent resolution and unique selectivity.

Key Words

- fat-soluble vitamins
- Discovery HPLC columns
- reversed phase HPLC

Vitamins are an extremely diverse range of organic compounds present in minute amounts in natural foodstuffs. They are vital in the enzyme reactions that are necessary for carbohydrate, fat, and protein metabolism. Vitamins are relatively unstable, affected by factors such as heat, light, air, other food components, and food processing conditions (1,2).

Because of their critical role in nutrition and their relative instability, qualitative and quantitative analyses are an important issue as well as a challenging task for food manufacturers. HPLC is preferred for vitamin separation because of its high selectivity (3). Recent studies show various applications in determining vitamins in different sample sources (4-6).

Fat-soluble vitamins are very hydrophobic and must be dissolved in organic solvents. In reversed phase HPLC, in order to elute the vitamins, a very high concentration of methanol or acetonitrile is needed.

We used 95% methanol to dissolve vitamin A acetate, three vitamin E isomers, and vitamin E acetate for separation on a Discovery® C18 column (Figure A), and 90% acetonitrile for analysis of a similar mixture on a Discovery C8 column (Figure B). The two analyses yielded almost identical results. We separated a mixture of these same vitamins and vitamin D using the Discovery C8 column (Figure C). All three chromatograms exhibited complete separation, excellent resolution, and good peak shape.

In our analysis of the fat-soluble vitamins in a sample of Centrum® multivitamin liquid, using a Discovery C8 column (Figure D), vitamin E acetate and vitamin A palmitate were clearly identified, while the vitamin D peak was barely obvious on the baseline. In a similar sample spiked with vitamin D (Figure E), the vitamin D was completely resolved.

We used a Discovery C18 column to separate the two main forms of vitamin D. Ergocalciferol (D₂) and cholecalciferol (D₃) were clearly separated using 100% acetonitrile (Figure F).

Discovery HPLC columns are excellent for separation of real samples of fat-soluble vitamins. They provide excellent resolution, peak shape, and reproducibility as well as unique selectivity.

Figure A. Vitamins A and E on a Discovery C18 Column

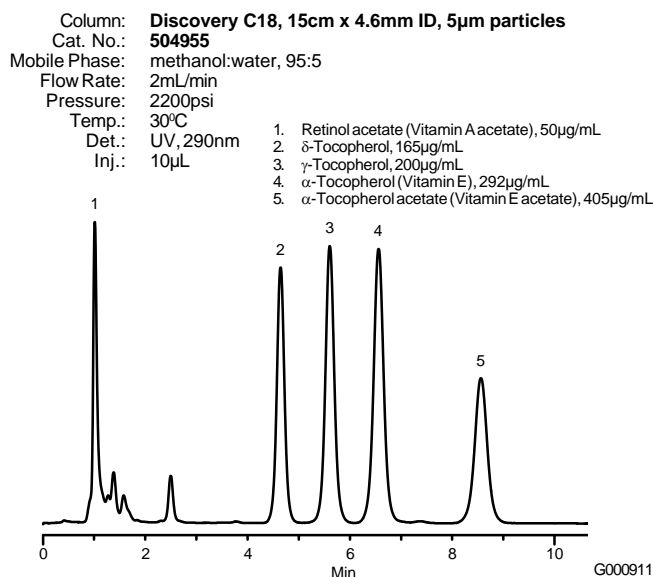


Figure B. Vitamins A and E on a Discovery C8 Column

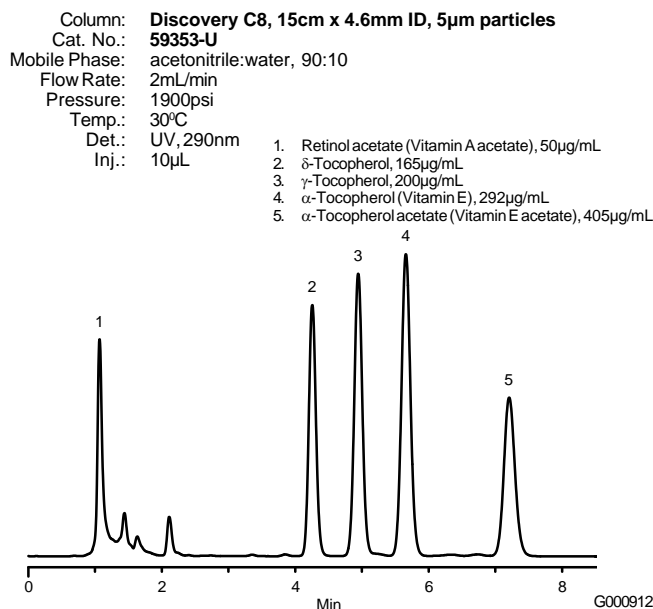


Figure C. Vitamins A, D, and E on a Discovery C8 Column

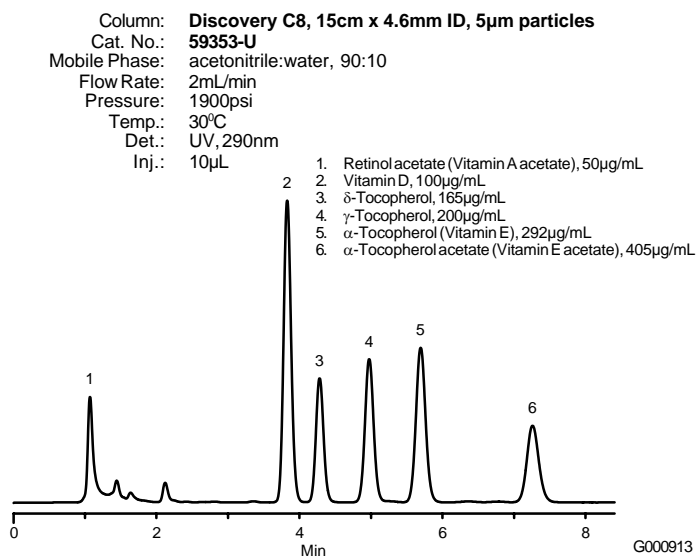


Figure E. Fat-Soluble Vitamins in Centrum Multivitamin Liquid, Spiked with Vitamin D

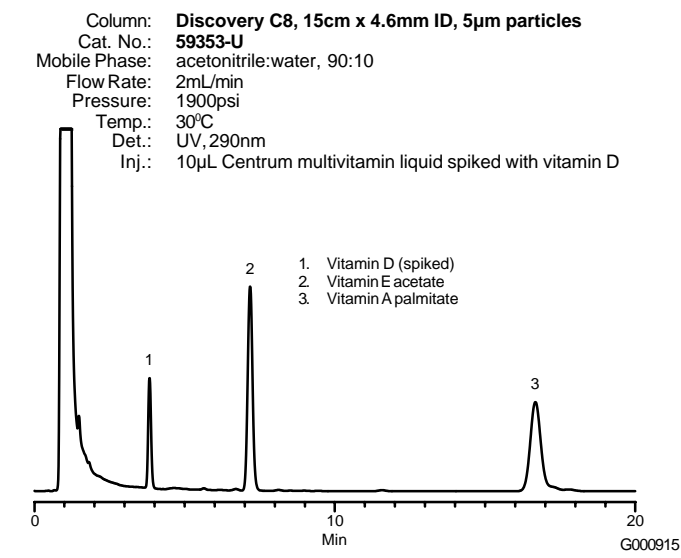


Figure D. Fat-Soluble Vitamins in Centrum Multivitamin Liquid

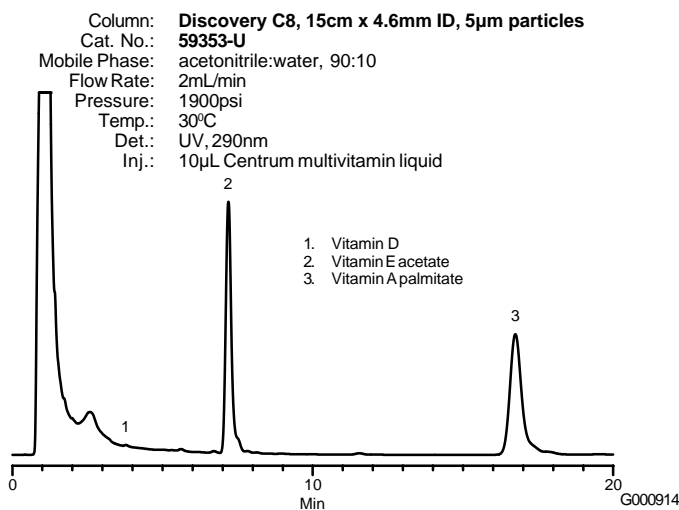
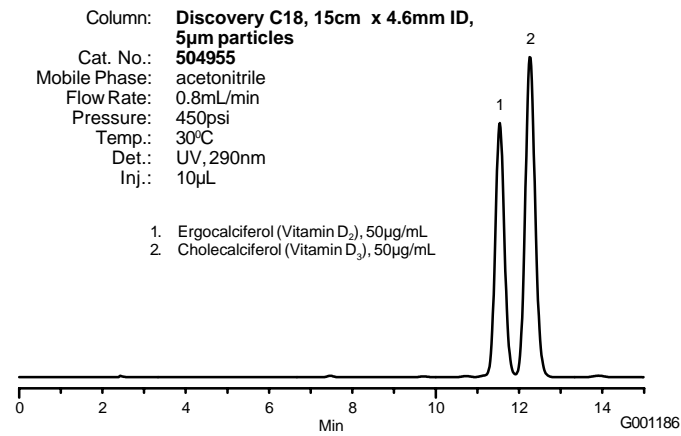


Figure F. Vitamins D₂ and D₃ on a Discovery C18 Column



Ordering Information:

Description	Cat. No.
Discovery HPLC Columns	
15cm x 4.6mm ID, 5µm particles	
Discovery C8	59353-U
Discovery C18	504955
Selectivity Pack	
Four columns, one of each Discovery phase (C8, C18, RP-AmideC16, Cyano), dimensions same as above	55722-U

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Trademarks

Centrum – American Home Products Corp.
 Discovery – Sigma-Aldrich Co.

Note 149

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