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If you have questions about applying methodology described in this article to a current application, please contact our technical service chemists.

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Fat Soluble Vitamin Analyses by HPLC

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Supelco, Bellefonte, PA USA

Analyses of fat soluble vitamins are performed to mon-itor changes which may occur during food processing, to provide data for food composition studies, and to check the effects of geographic, environmental, and climatic conditions on vitamins. The SUPELCOSIL LC-NH_2 column, used under normal phase HPLC conditions, and the SUPELCOSIL LC-18 reversed phase column offer unique separation capabilities.

Vitamins are necessary in small amounts in the human diet. These nutrients function in enzyme systems which facilitate the metabolism of proteins, carbohydrates, and fats. Vitamins are classified into two major groups: fat soluble and water soluble. Fat soluble vitamins include vitamins A, D, E, and K.

Food products such as milk, margarine, breakfast cereals, and dietetic foods commonly are fortified with vitamins A, D, and E. (Vitamin K currently is added only to infant formula.) Fortification is necessary to ensure that vitamins are present in sufficient amounts, as they tend to be destroyed by light, air, and food processing conditions. Fortification must be conducted with a stable form of the vitamin. The addition of vitamins also satisfies a portion of the recommended daily allowance (RDA).

Food manufacturers routinely perform analyses of fat soluble vitamins in their products to determine quantitative levels, to monitor changes that may have occurred during food processing, to provide data for food composition studies, and to check the effects of geographic, environmental, and climatic changes. Fat soluble vitamins can be analyzed by HPLC using either a SUPELCOSIL™ LC-NH_2 column, under normal phase conditions, or a reversed phase SUPELCOSIL LC-18 column.

Retinol palmitate, a stabilized form of vitamin A, is rapidly analyzed using normal phase HPLC (Figure A). Retinol acetate, which is more water soluble, is best analyzed by reversed phase HPLC.

The two main forms of vitamin D — ergocalciferol (D2) and cholecalciferol (D3) — can be separated using non-aqueous reversed phase HPLC (Figure B). Separation of four vitamin E alcohols, the tocopherol isomers, can be performed using normal phase HPLC (Figure C). A reversed phase separation of these compounds would yield a coelution of the β- and γ-tocopherols. Figure D shows a reversed phase analysis of various forms of vitamin E.

By using a wavelength switching technique to accommodate the differing absorption maxima of these compounds, an analyst can detect retinol and retinol acetate, tocopherol isomers, and tocopherol acetate in a single separation, with excellent resolution and peak shape (Figure E).

Figure A. Retinol Palmitate by Normal Phase HPLC

Column: **SUPELCOSIL LC-NH_2, 25cm x 4.6mm ID, 5µm particles**
Cat. No.: **58338**
Mobile Phase: hexane:ethyl acetate (99:1)
Flow Rate: 1.5mL/min
Temp.: 30°C
Det.: UV, 325nm
Inj.: 10µL, 1.0mg/mL in hexane

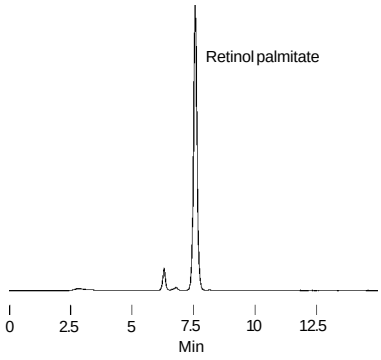

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Figure B. Ergocalciferol (D2) and Cholecalciferol (D3) by Reversed Phase HPLC

Column: **SUPELCOSIL LC-18, 25cm x 4.6mm ID, 5µm particles**
Cat. No.: **58298**
Mobile Phase: acetonitrile:methanol (95:5)
Flow Rate: 1.5mL/min
Temp.: 30°C
Det.: UV, 265nm
Inj.: 10µL, 1.0mg/mL in methanol

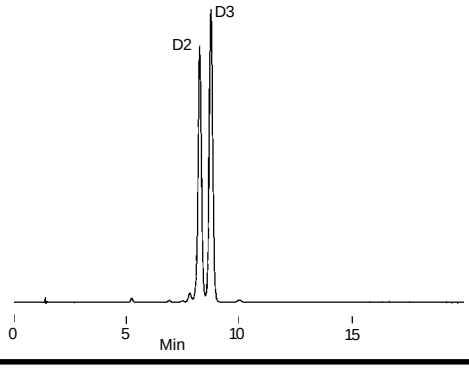

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Figure C. Tocopherol Isomers by Normal Phase HPLC

Column: **SUPELCOSIL LC-NH$_2$, 25cm x 4.6mm ID, 5µm particles**
Cat. No.: **58338**
Mobile Phase: hexane:ethyl acetate (70:30)
Flow Rate: 1.0mL/min
Det.: UV, 295nm
Inj.: 5µL hexane (0.75mg/mL each analyte)

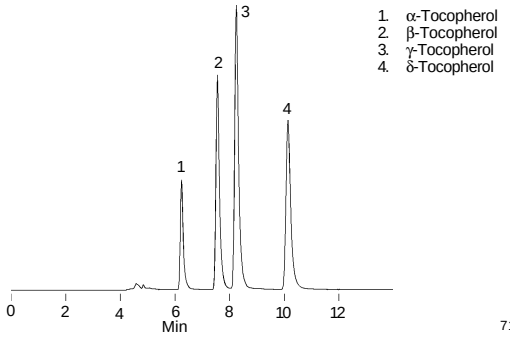


Figure D. Tocopherols by Reversed Phase HPLC

Column: **SUPELCOSIL LC-18, 25cm x 4.6mm ID, 5µm particles**
Cat. No.: **58298**
Mobile Phase: acetonitrile:methanol (75:25)
Flow Rate: 1.5mL/min
Temp.: 30°C
Det.: UV, 280nm
Inj.: 10µL

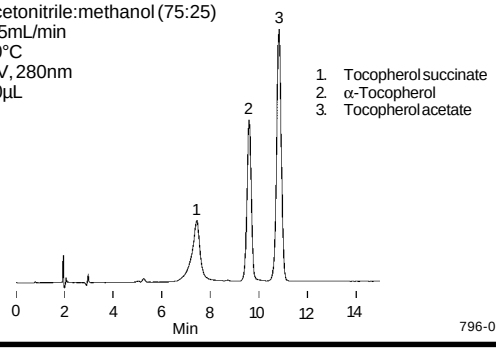


Figure E. Vitamins A and E by Reversed Phase HPLC

Column: **SUPELCOSIL LC-18, 25cm x 4.6mm ID, 5µm particles**
Cat. No.: **58298**
Mobile Phase: methanol:deionized water (98:2)
Flow Rate: 2mL/min
Det.: UV, 325nm (retinol, retinol acetate) or 290nm (tocopherols, tocopherol acetate)
Inj.: 20µL methanol (0.2-1mg/mL each analyte)

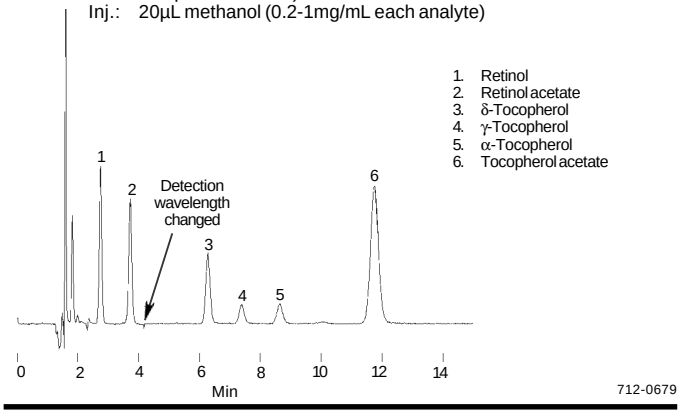
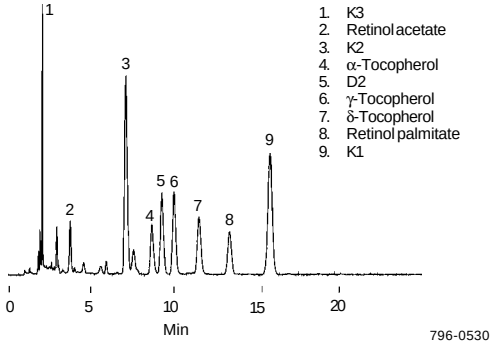


Figure F. Fat Soluble Vitamins by Reversed Phase HPLC

Column: **SUPELCOSIL LC-18, 25cm x 4.6mm ID, 5µm particles**
Cat. No.: **58298**
Mobile Phase: acetonitrile:methanol (75:25)
Flow Rate: 1.5mL/min
Temp.: 30°C
Det.: UV, 280nm
Inj.: 10µL



Analyses of fat soluble multivitamins can be performed using reversed phase HPLC. The analysis of vitamins A, D, E, and K in Figure F was completed in slightly more than 15 minutes.

Supelco now offers vitamin standards and analytical columns for fat soluble vitamin analyses. Our standards are tested for purity and packaged neat to ensure compound integrity. For more information, contact our Technical Service chemists.

Ordering Information:

Fat Soluble Vitamin Standards (Neat)

Compound	Qty.	Cat. No.
Retinol acetate	100mg	**46958**
Retinol palmitate	100mg	**46959-U**
D-α-Tocopherol succinate	100mg	**47782**
DL-α-Tocopherol	100mg	**47783**
δ-Tocopherol	100mg	**47784**
γ-Tocopherol	10mg	**47785**
DL-α-Tocopherol acetate	100mg	**47786**
Cholecalciferol (D3)	100mg	**47763**
Ergocalciferol (D2)	100mg	**47768**
Phylloquinone (K1)	100mg	**47773**
Menaquinone (K2)	100mg	**47774**
Menadione (K3)	1000mg	**47775**

Description	Cat. No.
HPLC Columns	
SUPELCOSIL LC-NH$_2$ 25cm x 4.6mm ID, 5µm particles	**58338**
SUPELCOSIL LC-18 25cm x 4.6mm ID, 5µm particles	**58298**

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