Vitamin Analysis with Vydac Reversed-Phase Columns

Water-Soluble Vitamins

Vydac SP reversed-phase columns are recommended for the analysis of water-soluble vitamins. They are based on a 90Å pore-size spheroidal silica with a high surface area. Two reversed-phase chemistries are available: 201SP, a C18 phase, and 208SP, a C8. Both are monomeric-bonded and exhaustively end-capped.

SP columns have inherently high performance that does not depend on deactivation or solvent modifiers. They are very stable, with long lifetimes in normal use. Quality tests include packed-column efficiency and selectivity for water-soluble vitamins.

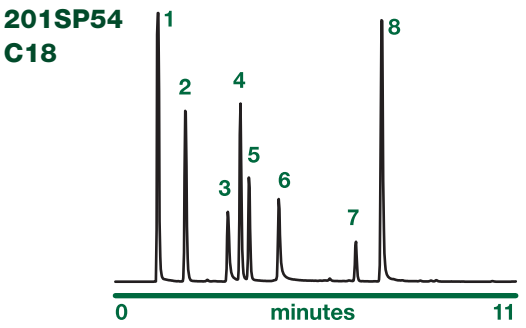


Figure 1. Vitamin selectivity test for 201SP. Column: Vydac 201SP54 (C18, 5µm, 90Å, 4.6mmID x 250mmL). Mobile phase: A = 0.1M KOAc adjusted to pH 4.9 to 5.2 with formic acid. B = 50:50 ACN:water. Gradient: 5% to 60% B over 15 minutes. Flow rate: 1.5 mL/min. Detection: 254 nm. Peaks: (1) ascorbic acid (C), (2) nicotinic acid (Niacin), (3) pyridoxine (B6), (4) thiamine (B1), (5) nicotinamide (B3), (6) folic acid (M), (7) cyanocobalamin (B12), (8) riboflavin (B2). All peaks must be baseline resolved.

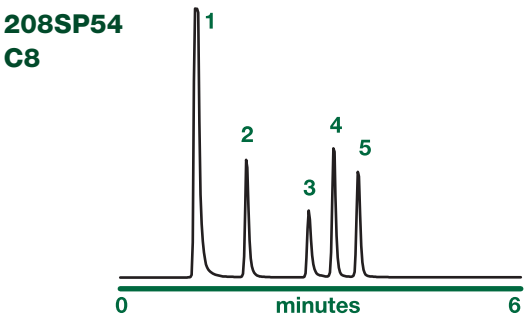
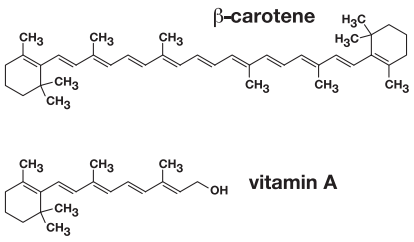


Figure 2. Vitamin selectivity test for 208SP. Column: Vydac 208SP54 (C8, 5µm, 90Å, 4.6mmID x 250mmL). Mobile phase: A = 0.1M KOAc adjusted to pH 4.9 to 5.2 with formic acid. B = 50:50 ACN:water. Gradient: 5% to 60% B over 15 minutes. Flow rate: 1.5 mL/min. Detection: 254 nm. Peaks: (1) ascorbic acid (C), (2) nicotinic acid (Niacin), (3) pyridoxine (B6), (4) thiamine (B1), (5) nicotinamide (B3). All peaks must be baseline resolved.

Fat-Soluble Vitamins

Vitamins A, E, and β-carotene have been separated from interfering non-active geometric isomers and quantified by reversed-phase chromatography on Vydac 201TP54 columns



(Refs. 2 and 3). Vydac 201TP is a wide-pore (300 Å) specialty C18 reversed-phase adsorbent originally developed for optimal separation of polyaromatic hydrocarbons in environmental analysis. As noted in reference 2, the large pores (300Å) of the TP silica are thought to contribute to selectivity in separating the long, rigid molecules of vitamins A, E, and β-carotene by assuring they are not excluded from active adsorbent surfaces.

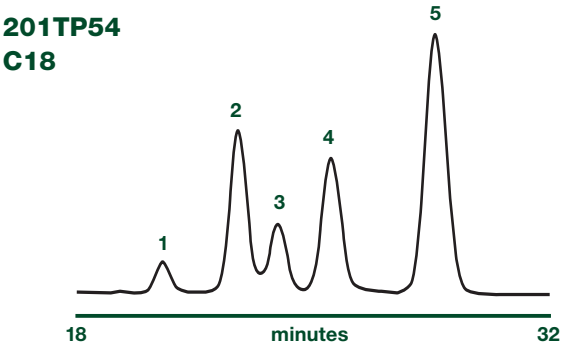


Figure 3. Separation of retinol isomers. Geometric isomers were easily resolved on Vydacs 201TP 300Å C18 reversed-phase material. Column: Vydac 201TP54 (C18, 5µm, 300Å, 4.6mmID x 250mmL). Flow: 1.0 mL/min. Mobile phase = 65:10:25 methanol:n-butanol:water containing 10mM ammonium acetate, pH 3.2. Isocratic. Peaks: 1. di-cis-retinol; 2. 11-cis-retinol; 3. 9-cis-retinol; 4. 13-cis-retinol; 5. all-trans-retinol (vitamin A).
Chromatogram reproduced with author's permission from Ref. 3.

Figures 4 and 5 illustrate the use of Vydac201TP columns for determination of vitamins A, E, and β-carotene in serum and analysis of vitamins D.

VYDAC • 17434 Mojave Street • Hesperia, CA 92345 • USA • Web: http://www.vydac.com
Toll-Free: 800-247-0924 • Tel: 760-244-6107 • Fax: 760-244-1984 • EMail: experts@vydac.com

201TP54
C18

Peaks:
1. all-*trans*-retinol (vitamin A)
2. tocol
3. γ-tocopherol
4. α-tocopherol (vitamin E)
5. lutein
6. zeaxanthin
7. cryptoxanthin
8. α-carotene
9. all-*trans*-β-carotene
10. *cis*-β-carotene

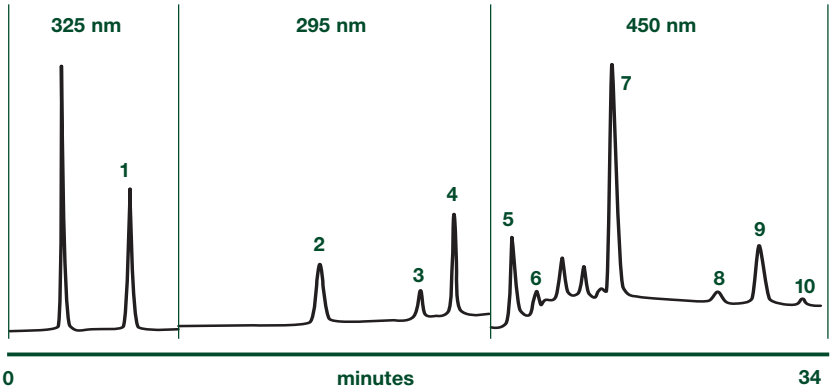
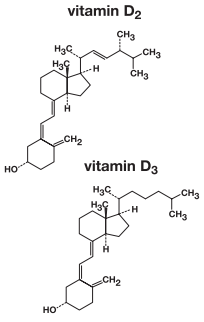


Figure 4. Determination of vitamin A, vitamin E, and β-carotene in serum. Reliable analysis depends on chromatographic separation to distinguish these molecules from isomers and interfering matrix constituents. The analytes can be quantified by electrochemical detection or UV absorbance. The UV absorbance trace with programmed wavelengths is shown here. Column: Vydac 201TP54 (C18, 5µm, 300Å, 4.6mmID x 250mmL). Flow: 1.5 mL/min. Detection: programmed wavelength absorbance. Mobile phase: A = 15:75:10 water:methanol:n-butanol. B = 2:88:10 water:methanol:n-butanol. Both mobile phase mixtures included ammonium acetate buffer, pH 3.5, at a final concentration of 0.02M. Gradient: Hold 100% A for three minutes after injection. Then linear to 100% B over 15 minutes and maintain 100% B for 17 minutes.

Chromatogram reproduced with author's permission from Ref. 2.

Vitamins D2 and D3

The highly water-insoluble vitamins D2 (calciferol) and D3 (cholecalciferol) have been analyzed by HPLC using a Vydac 201TP54 specialty reversed-phase column with a totally nonaqueous mobile phase (Ref. 8). This method superceded the use of normal-phase HPLC, for which reproducible retention was more difficult to achieve.



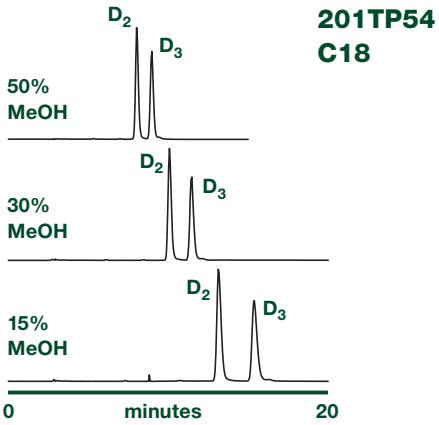


Figure 5. Separations of vitamins D2 and D3. Elution times can be adjusted while maintaining resolution by varying methanol concentration in the mobile phase. This can be useful for assuring separation from interfering peaks, depending on the matrix being analyzed. Column: Vydac 201TP54 (C18, 5µm, 300Å, 4.6mmID x 250mmL). Flow: 0.7 mL/min. Detection: 265 nm. Temperature: 27°C. Isocratic mobile phase: Methanol:acetonitrile totalling 100% with methanol concentration as indicated. Column is washed between runs with 100% ethyl acetate at 2.5 mL/min for 3.5 minutes, then reequilibrated with methanol:acetonitrile mixture before sample injection.

Ordering Information

Cat.No.	Description
201SP54	Column, Reversed-Phase, C18, 90Å, 5µm, 4.6mm ID x 250mm L
201SP5415	Column, Reversed-Phase, C18, 90Å, 5µm, 4.6mm ID x 150mm L
208SP54	Column, Reversed-Phase, C8, 90Å, 5µm, 4.6mm ID x 250mm L
208SP5415	Column, Reversed-Phase. C8, 90Å, 5µm, 4.6mm ID x 150mm L
201TP54	Column, Reversed-Phase, C18, 300Å, 5µm, 4.6mmID x 250mmL

Other column sizes are available for analytical and preparative applications.

References

General:

1. Review: Chromatographic Determination of Vitamins in Foods, A. Rizzolo and S. Polesello, J. Chrom., 624, 103-152 (1992)

Vitamin A and Vitamin E:

2. Determination of Retinol, α-Tocopherol, and β-Carotene in Serum by Liquid Chromatography with Absorbance and Electrochemical Detection, W.A. MacCrehan and E. Schonberger, Clin. Chem., 33(9), 1585-1592 (1987)
3. Determination of Retinol, α-Tocopherol, and β-Carotene in Serum by Liquid Chromatography, W.A. MacCrehan, Methods in Enzymology, VIII, 189, 172 (Academic Press, 1990)
4. Chromatographic and Electrophoretic Analysis of Biomedically Important Retinoids, R. Wyss, J. Chrom. B, 671, 381-425 (1995)
5. Improved Reversed-Phase Liquid Chromatographic Determination of Vitamins A and D in Fortified Milk, A.F. Wickroski and A.L. McLean, JAOAC, 67(1), 62-65 (1984)
6. Changes of Carotenoids, Color, and Vitamin A Contents during Processing of Carrot Juice, B.H. Chen, H.Y. Peng, and H.E. Chen, J. Agric. and Food Chem., 43, 1912-1918 (1995)
7. Simultaneous Profiling and Identification of Carotenoids, Retinols, and Tocopherols by High Performance Liquid Chromatography Equipped with Three-Dimensional Photodiode Array Detection, A. Ben-Amotz, J. Liquid Chromatog., 18(14), 2813-2825 (1995)

Vitamin D:

8. An Improved Method for Routine Determination of Vitamin D and its Hydroxylated Metabolites in Serum from Childern and Adults, M.T. Parviainen, K.E. Savolainen, P.H. Korhonen, E.M. Alhava, and J.K. Visakorpi, Clin. Chim. Acta, 114, 233-247 (1981)
9. Reversed-Phase Liquid Chromatographic Determination of Vitamin D in Infant Formulas and Enteral Nutritionals, M.G. Sliva, A.E. Green, J.K. Sanders, J.R. Euber, and J.R. Saucerman, JAOAC Intl., 75(3), 566-571 (1992)