Vitamin Analysis:
201TP™ Reversed-Phase Columns

Vitamins A, E, and β-Carotene

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 (see Refs. 2-3 on page 59). As noted in reference 2, the large pores (300 Å) of the TP silica are thought to contribute to selectivity in separating these long, rigid molecules by assuring they are not excluded from active adsorbent surfaces.

Retinol isomers
Geometric isomers were easily resolved on Vydac’s 201TP 300 Å C18 reversed-phase material.

Conditions
Column: Vydac 201TP54 (C18, 5 µm, 300 Å, 4.6 mm i.d. x 250 mm).
1.0 mL/min. Mobile phase = 65:10:25 methanol:n-butanol:water containing 10 mM ammonium acetate, pH 3.2. Isocratic.
Peaks: 1. di-cis-retinol; 2. 11-cis-retinol; 3. 9-cis-retinol; 4. 13-cis-retinol; and 5. all-trans-retinol (vitamin A).
Chromatogram reproduced with author’s permission (see Ref. 3 on page 59).

Determination of retinol, α-tocopherol, and β-carotene in serum
Reliable determination of vitamin A, vitamin E, and β-carotene in serum 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.

Conditions
Column: Vydac 201TP54 (C18, 5 µm, 300 Å, 4.6 mm i.d. x 250 mm).
1.5 mL/min. Programmed wavelength.
Both mobile phase mixtures included ammonium acetate buffer, pH 3.5, at a final concentration of 0.02 M. Hold 100% A for three minutes after injection, then linear gradient to 100% B over 15 minutes and maintain 100% B for 17 minutes.
Chromatogram reproduced with author’s permission (see Ref. 2 on page 59).
**Vitamin A separations run in acetic acid and ammonium acetate, pH 4.7, with two different solvent systems**

The separation of vitamin A from all-trans-retinoic acid on a large-pore (300 Å) polymerically-bonded C₁₈ column (Vydac 201TP54) was investigated in two different solvent systems as a function of pH. Retinoic acid, more polar than vitamin A, elutes earlier. Higher pH improves resolution by ionizing the acid and accentuating the polarity difference.

**Vitamin E**

Resolution of vitamin E (α-tocopherol) from γ-tocopherol and vitamin E acetate was investigated in mixtures of methanol and n-propanol. The n-propanol was used in this work instead of n-butanol used in some references because it is less toxic, less viscous, and provides an alternative system.

Inclusion of 15 mM ammonium acetate, pH 4.7, instead of 25 mM acetic acid in the mobile phases had no discernable effect on these separations, as would be expected from the absence of ionizable groups in the structures of the analytes. Only the separations done with acetic acid are shown. Mobile phases with acetic acid are easier to make and would thus normally be preferred.

**Separation of vitamin E, γ-tocopherol, and vitamin E acetate.**

**Conditions**

Column: Vydac 201TP54 (C₁₈, 5μ, 300 Å, 4.6 mm i.d. x 250 mm). Isocratic. Flow: 1.5 mL/min. Mobile phase: 79:15:6 methanol:n-propanol:water with 25 mM acetic acid.
**Water-Insoluble Vitamins: D₂ and D₃**

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

![Vitamin D2 and D3](image)

**Ordering Information**

<table>
<thead>
<tr>
<th>Cat.No.</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>201TP54</td>
<td>Specialty Reversed-Phase Column, C₁₈, 5 µm, 300 Å, 4.6 mm i.d. x 250 mm</td>
</tr>
<tr>
<td>201SP54</td>
<td>Specialty Reversed-Phase Column, C₁₈, 5 µm, 90 Å, 4.6 mm i.d. x 250 mm</td>
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<td>(See page 32 for other 201SP column sizes.)</td>
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**Water-soluble vitamins**

Water-soluble vitamins can be separated on a Vydac 201SP column.

**Conditions**

Vydac 201SP54 (C₁₈, 5 µm, 4.6 mm i.d. x 250 mm). Flow: 1.5 mL/min. Detection: 254 nm. Mobile phase: Gradient from 2.5 to 50% ACN with 0.1 M KOAc, pH 5.4, over 15 min. Peaks: 1. vitamin C; 2. niacin; 3. pyridoxine (B6); 4. thiamine (B1); 5. nicotinamide (B3); 6. folic acid (M); 7. cyanocobalamin (B12); and 8. riboflavin (B2).

**Vitamin D₂ and D₃**

Elution times of vitamins D₂ and D₃ can be adjusted while maintaining resolution by varying the methanol concentration in the mobile phase. This can be useful for providing separation from interfering peaks, depending on the matrix being analyzed.

![Vitamin D2 and D3](image)

**Conditions**

Column: Vydac 201TP54 (C₁₈, 5 µm, 300 Å, 4.6 mm i.d. x 250 mm). Flow: 0.7 mL/min. Detection: 265 nm, 27°C. Mobile phase: Isocratic. Methanol:acetonitrile totalling 100% with methanol concentration as indicated on chromatogram. 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.

**Vitamin Analysis References**

General:

Vitamin A and Vitamin E: