

Application Note 148

Separating Water-Soluble Vitamins by Reversed Phase HPLC Using a Discovery C18 Column

Manufacturers routinely analyze vitamins in their products to determine quantitative levels and to monitor changes that have occurred in processing. In our study, we separated water-soluble vitamins by reversed-phase HPLC on a Discovery C18 column. This column yielded excellent resolution.

Key Words

- water-soluble vitamins • Discovery C18 column
- 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 classified into two groups: water-soluble and fat-soluble. Vitamins are relatively unstable, affected by factors such as heat, light, air, other food components, and food processing conditions (1,2).

Because of the critical role of vitamins 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 the preferred technique for vitamin separation because of its high selectivity (3). Recent studies show various applications in determining vitamins in different sample sources (4-6).

We analyzed a standard mixture of water-soluble vitamins using a Discovery® C18 reversed-phase HPLC column, 15cm x 4.6mm ID, 5µm particles. Because each vitamin has its own maximum absorbance at a different wavelength, we used 220nm as a compromise to detect all vitamins in the sample. In the mobile phase, we attempted to use simple buffers without adding ion-pairing agents or competing amine modifiers.

Water-soluble vitamins are very hydrophilic. They can be eluted from reversed-phase columns with low concentrations of aqueous methanol or acetonitrile. A mixture of ten water-soluble vitamin standards, including ascorbic acid, niacin, pantothenic acid, pyridoxine, niacinamide, thiamine, folic acid, biotin, cyanocobalamin, and riboflavin, was analyzed by reversed-phase HPLC on a Discovery C18 column. Figure A shows the chromatogram of gradient elution of the ten vitamins at neutral pH. Excellent resolution and peak shape were achieved. Under the same condition, a multivitamin supplement – Centrum Multivitamin liquid - was separated and the peaks were identified (Figure B) by comparing to Figure A.

From the above example, it can be seen that these ten water-soluble vitamins can be well resolved within 25 minutes in one single run by an appropriate gradient elution. However, sometimes isocratic elution may be the preferred method, especially in

QA/QC laboratories. Efforts have been made to develop a universal isocratic elution method to separate these vitamins on Discovery columns. However, cyanocobalamin and riboflavin are relatively hydrophobic, while the other eight water-soluble vitamins are extremely hydrophilic. A mobile phase condition that can elute cyanocobalamin and riboflavin will cause other vitamins to co-elute. Therefore, a set of two mobile phase conditions with different organic compositions would be a practical solution. Figure C shows the chromatogram of eight well-resolved water-soluble vitamins on Discovery C18 column using 1% methanol in 50 mM K₂HPO₄ buffer (pH 7). The eight vitamins are ascorbic acid, niacin, pantothenic acid, pyridoxine, niacinamide, thiamine, biotin, and folic acid, respectively. Under this condition, cyanocobalamin and riboflavin are strongly retained on the column. Further study has confirmed that the presence of cyanocobalamin and riboflavin has no effect on elution of the other eight vitamins even with multiple injections. Cyanocobalamin and riboflavin can be eluted from a Discovery C18 column using a mobile phase containing 20 % methanol in 50 mM K₂HPO₄ buffer (pH 7). The chromatogram is illustrated in Figure D. These two conditions combined can be a valuable method in separation of all water-soluble vitamins for those who prefer an isocratic elution method.

Figure A. Gradient Elution of Ten Water-Soluble Vitamins

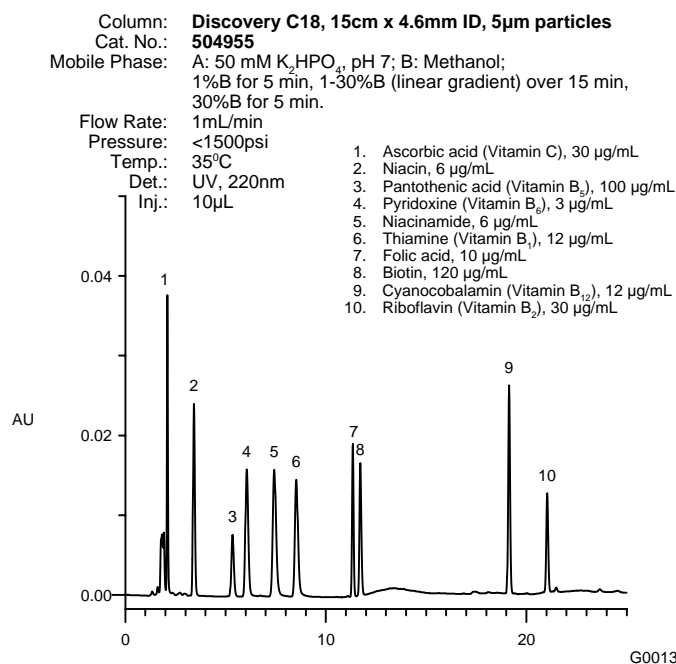


Figure B. Water-Soluble Vitamins in Centrum Multivitamin Liquid

Column: **Discovery C18, 15cm x 4.6mm ID, 5µm particles**
 Cat. No.: **504955**
 Mobile Phase: A: 50mM K₂HPO₄, pH 7; methanol; 1%B for 5 min, 1-30%B (linear gradient) over 15 min, 30%B for 5 min.
 Flow Rate: 1mL/min
 Pressure: <1500psi
 Temp.: 35°C
 Det.: UV, 220nm
 Inj.: 10µL

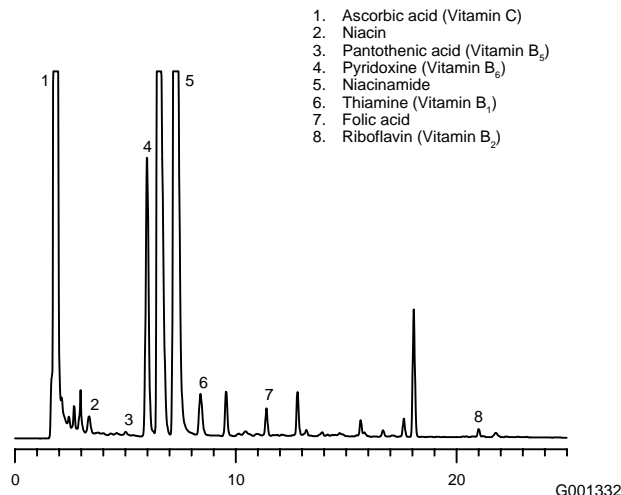


Figure C. Isocratic Elution of Water-Soluble Vitamins (I)

Column: **Discovery C18, 15cm x 4.6mm ID, 5µm particles**
 Cat. No.: **504955**
 Mobile Phase: A = 50mM K₂HPO₄, pH 7; B: Methanol; 99%A/1%B isocratically
 Flow Rate: 1mL/min
 Pressure: 1300psi
 Temp.: 35°C
 Det.: UV, 220nm
 Inj.: 10µL

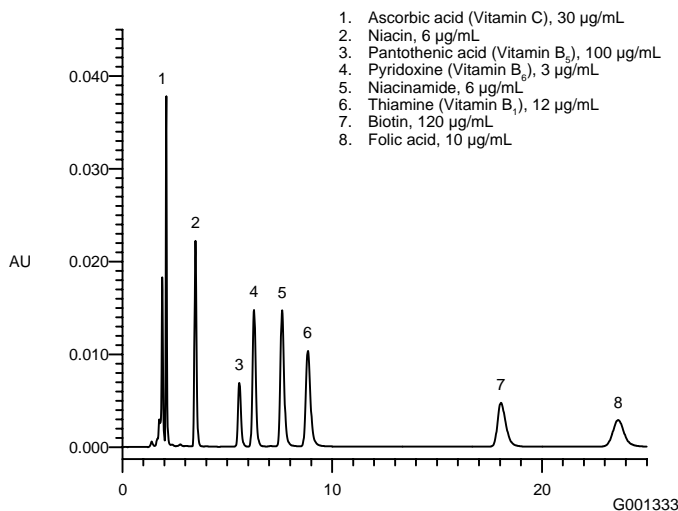
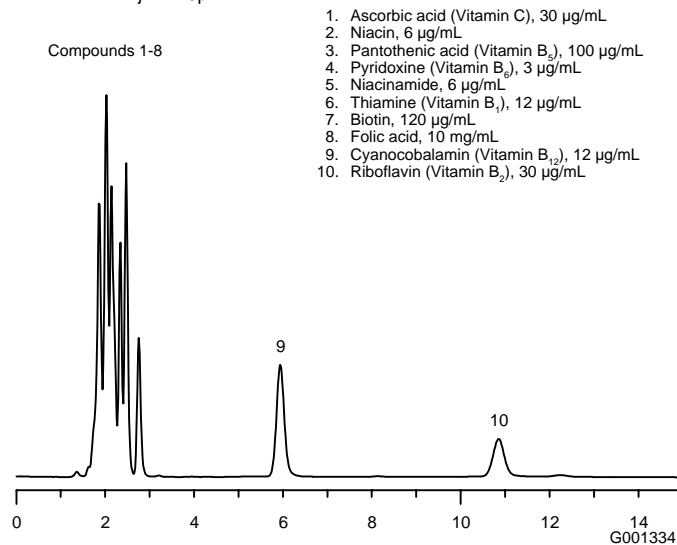


Figure D. Isocratic Elution of Water-Soluble Vitamins (II)

Column: **Discovery C18, 15cm x 4.6mm ID, 5µm particles**
 Cat. No.: **504955**
 Mobile Phase: A = 50mM K₂HPO₄, pH 7 B: Methanol; 80%A/20%B isocratically
 Flow Rate: 1mL/min
 Pressure: 1400psi
 Temp.: 35°C
 Det.: UV, 220nm
 Inj.: 10µL



Ordering Information:

Description	Cat. No.
Discovery C18 Column 15cm x 4.6mm ID, 5µm particles	504955

References

- Machlin, L.J. ed. *Handbook of Vitamins*, Marcel Dekker, Inc., New York (1991).
 - Ottaway, P.B. ed. *The Technology of Vitamins in Food*, Chapman & Hall, Inc., New York (1993).
 - De Leenheer, A.P., W.E. Lambert, M.G. De Ruyter, M Ed. *Modern Chromatographic Analysis of the Vitamins, Chromatographic Science Series, Vol. 30*, Marcel Dekker, Inc., New York (1985).
 - Burri, B.J., T.R. Neidlinger, A.O. Lo, C. Kwan, M.R. Wong, *J. Chromatogr. A* **762**: 201 (1997).
 - Qian, H., M. Sheng, *J. Chromatogr. A* **825**: 127 (1998).
 - Gratzfeld-Huesgen, A., R. Schuster, W. Haecker, *Tec. Lab* **14**: 277 (1992).
- References not available from Supelco.

Trademarks

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

For additional information about this application, contact our Applications Laboratory at aplab@sial.com

For more information, or current prices, contact your nearest Supelco subsidiary listed below. To obtain further contact information, visit our website (www.sigma-aldrich.com), see the Supelco catalog, or contact Supelco, Bellefonte, PA 16823-0048 USA.

ARGENTINA • Sigma-Aldrich de Argentina, S.A. • Buenos Aires 1119 AUSTRALIA • Sigma-Aldrich Pty. Ltd. • Castle Hill NSW 2154 AUSTRIA • Sigma-Aldrich Handels GmbH • A-1110 Wien
 BELGIUM • Sigma-Aldrich N.V./S.A. • B-2880 Bornem BRAZIL • Sigma-Aldrich Quimica Brasil Ltda. • 01239-010 São Paulo, SP CANADA • Sigma-Aldrich Canada, Ltd. • 2149 Winston Park Dr., Oakville, ON L6H 6J8
 CZECH REPUBLIC • Sigma-Aldrich s.r.o. • 186 00 Praha 8 DENMARK • Sigma-Aldrich Denmark A/S • DK-2665 Vallensbaek Strand FINLAND • Sigma-Aldrich Finland/YA-Kemia Oy • FIN-00700 Helsinki
 FRANCE • Sigma-Aldrich Chimie • 38297 Saint-Quentin-Fallavier Cedex GERMANY • Sigma-Aldrich Chemie GmbH • D-82041 Deisenhofen GREECE • Sigma-Aldrich (o.m.) Ltd. • Ilioupoli 16346, Athens
 HUNGARY • Sigma-Aldrich Kft. • H-1067 Budapest INDIA • Sigma-Aldrich Co. • Bangalore 560 048 IRELAND • Sigma-Aldrich Ireland Ltd. • Dublin 24 ISRAEL • Sigma Israel Chemicals Ltd. • Rehovot 76100
 ITALY • Sigma-Aldrich s.r.l. • 20151 Milano JAPAN • Sigma-Aldrich Japan K.K. • Chuo-ku, Tokyo 103 KOREA • Sigma-Aldrich Korea • Seoul MALAYSIA • Sigma-Aldrich (M) Sdn. Bhd. • 58200 Kuala Lumpur
 MEXICO • Sigma-Aldrich Quimica S.A. de C.V. • 50200 Toluca NETHERLANDS • Sigma-Aldrich Chemie BV • 3330 AA Zwijndrecht NORWAY • Sigma-Aldrich Norway • Torshov • N-0401 Oslo
 POLAND • Sigma-Aldrich Sp. z o.o. • 61-863 Poznań PORTUGAL • Sigma-Aldrich Quimica, S.A. • Sintra 2710 RUSSIA • Sigma-Aldrich Russia • Moscow 103062 SINGAPORE • Sigma-Aldrich Pte. Ltd.
 SOUTH AFRICA • Sigma-Aldrich (pty) Ltd. • Jet Park 1459 SPAIN • Sigma-Aldrich Quimica, S.A. • 28100 Alcobendas, Madrid SWEDEN • Sigma-Aldrich Sweden AB • 135 70 Stockholm
 SWITZERLAND • Supelco • CH-9471 Buchs UNITED KINGDOM • Sigma-Aldrich Company Ltd. • Poole, Dorset BH12 4QH
 UNITED STATES • Supelco • Supelco Park • Bellefonte, PA 16823-0048 • Phone 800-247-6628 or 814-359-3441 • Fax 800-447-3044 or 814-359-3044 • email: supelco@sial.com

Supelco is a member of the Sigma-Aldrich family. Supelco products are sold through Sigma-Aldrich, Inc. Sigma-Aldrich warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product for a particular use. Additional terms and conditions may apply. Please see the reverse side of the invoice or packing slip. CIK