

Application

Note
21

Separation of Organic Acids in Beverages Using a SUPELCOGEL™ C-610H HPLC Column

SUPELCOGEL C-610H columns are crosslinked polystyrene resin HPLC columns prepared specifically for ion exchange analysis of organic acids. Sample preparation is minimal, and the chromatography can be performed using a simple, isocratic mobile phase. A table of retention times for commonly analyzed organic acids is provided to aid the analyst in method development. (ChromFax No.: 394021)

Key Words:

• organic acids • ion exchange chromatography • HPLC

Organic acids can easily be isolated, with a simple isocratic mobile phase and minimal sample preparation, using a SUPELCOGEL C-610H HPLC column. SUPELCOGEL C-610H columns are crosslinked polystyrene divinylbenzene resin HPLC columns prepared specifically for ion exchange analysis of organic acids. A SUPELCOGEL C-610H column is also ideal for separating sugars, alcohols, and other fermentation products, making it a versatile HPLC column for the food and beverage analyst.

Analyses are best performed at low pH (a 0.1% phosphoric acid mobile phase is commonly used). The same mobile phase conditions are suitable for a wide variety of samples. Organic acids were detected in a standards mix, in grape juice cocktail, and in apple extract, at 210nm UV (Figure A). Little sample preparation is required — all of the liquid samples shown here were simply filtered through a 0.45µm syringe-tip filter and injected directly onto the column.

Care should be taken in the selection of column temperature to assure the best overall separation of the components of interest. When we separated a standard mix containing oxalic, citric, tartaric, malic, succinic, formic, acetic, and fumaric acids at temperatures ranging from 30°C to 60°C, we found that the retention time for fumaric acid shifted drastically. At 60°C, fumaric acid coeluted with formic acid, while it coeluted with acetic acid at 40°C. A satisfactory separation was obtained at 30°C, with fumaric acid eluting at 20 minutes. Retention time shifts for the other components were negligible. Table 1 lists retention times obtained at 30°C for 20 commonly analyzed organic acids.

Figure A. Organic Acids

Column: SUPELCOGEL C-610H, 30cm x 7.8mm ID
Cat. No.: 59320-U
Mobile Phase: 0.1% H₃PO₄
Col. Temp.: 30°C
Flow Rate: 0.5mL/min
Det.: 210nm UV
Inj.: A1 — 10µL organic acid standards mix
A2 — 10µL filtered grape juice cocktail
A3 — 10µL of 20g apple extract in 40mL deionized water

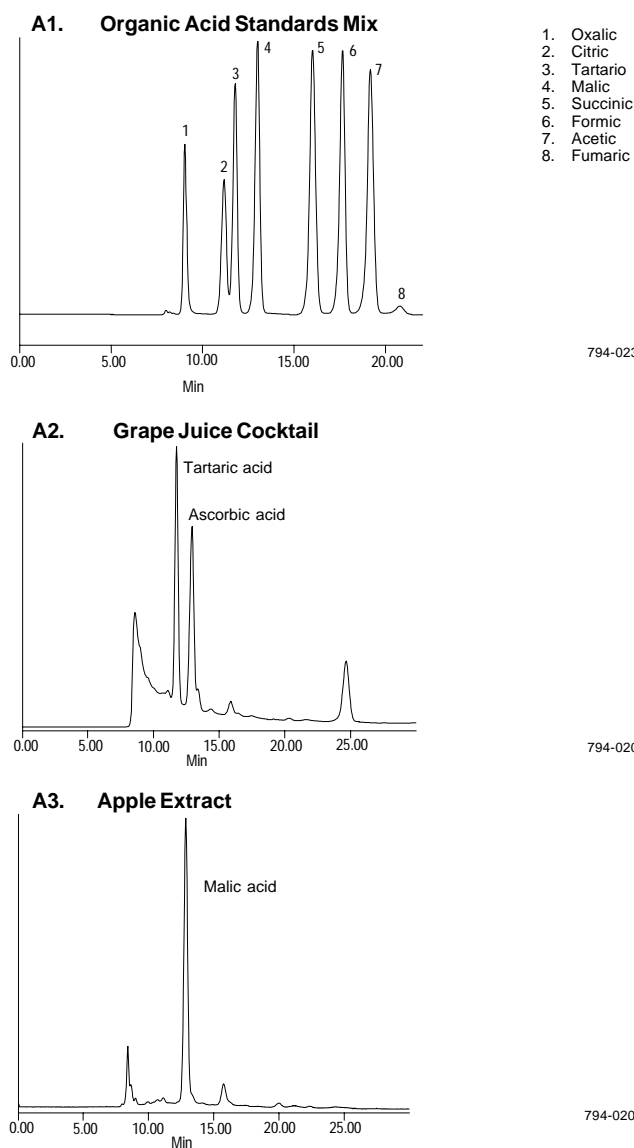


Table 1. Retention Times of Organic Acids on a SUPELCOGEL C610H Column

(See Figure A for conditions.)

Alphabetic Order		Elution Order	
Acid	Retention Time (min)	Acid	Retention Time (min)
Acetic	19.0	Phytic	8.3
Adipic	22.5	Oxalic	9.0
Ascorbic	13.1	Maleic	10.4
Benzoic	26.7	Citric	11.0
Citric	11.0	Isocitric	11.2
Formic	17.5	Tartaric	11.7
Fumaric	19.8	Malic	12.9
Isobutyric	25.6	Ascorbic	13.1
Isocitric	11.2	Quinic	13.3
Lactic	16.0	Malonic	13.4
Maleic	10.4	Shikimic	15.5
Malic	12.9	Succinic	15.7
Malonic	13.4	Lactic	16.0
Oxalic	9.0	Formic	17.5
Phytic	8.3	Acetic	19.0
Propionic	22.5	Fumaric	19.8
Quinic	13.3	Adipic	22.5
Shikimic	15.5	Propionic	22.5
Succinic	15.7	Isobutyric	25.6
Tartaric	11.7	Benzoic	26.7

Except for tartaric acid, the acids in the mix showed good efficiency at all column temperatures evaluated. Tartaric acid showed a decrease in number of theoretical plates as temperature increased, while theoretical plate values for the other acids increased with temperature. Likewise, all compounds exhibited good peak symmetry across the range of temperatures evaluated, except for tartaric acid, which exhibited slightly fronting peak shape at column temperatures of 40°C and 50°C.

The SUPELCOGEL C-610H column is a versatile column that can be used for a variety of food and beverage applications. Sample preparation is minimal, and the chromatography can be performed using simple isocratic mobile phase conditions.

Ordering Information:

SUPELCOGEL C610H HPLC column

30cm x 7.8mm ID **59320-U**

Iso-Disc N-254 Syringe-Tip Filters, pk. of 50

25mm diameter, nylon, 0.45µm pores **59230-U**

Contact our Technical Service Department (phone 800-359-3041 or 814-359-3041, FAX 814-359-5468) for expert answers to your questions.

Trademarks:

Iso-Disc and SUPELCOGEL are trademarks of Supelco, Inc.

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. • Selangor
 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. • 61-663 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.