

Application

Note
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Separation of Carbohydrates and Fermentation Products in Beverages, Using HPLC

A SUPELCOGEL C-610H ion exchange HPLC column, used with refractive index detection, provides a versatile means of detecting and quantifying a wide range of carbohydrates, including alcohols, sugars, and fermentation products, in foods and beverages. Sample preparation is minimal, and the chromatography can be performed using a simple, isocratic mobile phase. Tables of retention times for commonly analyzed alcohols, sugars, and sugar alcohols are provided to aid the analyst in method development. (ChromFax No.: 394020)

Key Words:

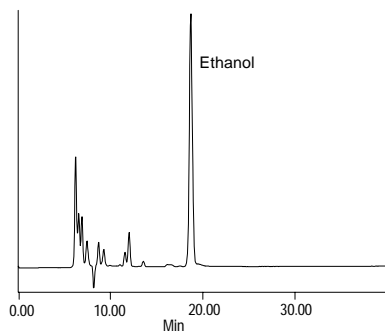
alcohols • fermentation products • HPLC • sugars

SUPELCOGEL™ C-610H columns are crosslinked polystyrene divinylbenzene resin HPLC columns prepared specifically for ion exchange analysis of organic acids. When used with refractive index detection (RI), 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. Little sample preparation is required — the liquid samples analyzed in this study were simply filtered through a 0.45µm syringe filter and injected directly onto the column.

Figure A. Beer

Column: SUPELCOGEL C-610H, 30cm x 7.8mm ID
Cat. No.: 59320-U
Mobile Phase: 0.1% H₃PO₄
Col. Temp.: 50°C
Flow Rate: 0.7mL/min
Det.: RI
Inj.: 10µL of beer



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The SUPELCOGEL C-610H column provides excellent resolution of alcohols and sugar alcohols commonly found in fermentation products. An analysis of beer shows a large, symmetrical ethanol peak (Figure A). Table 1 lists retention times obtained for alcohols analyzed under the conditions used in Figure A.

Table 1. Retention Times of Fermentation Products

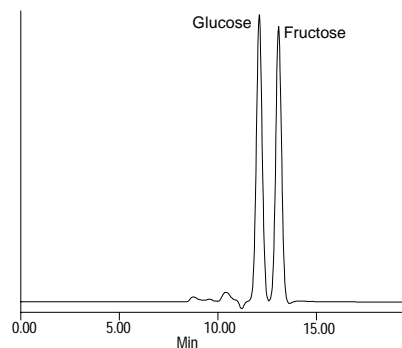
(See Figure A for conditions.)

Alphabetic Order		Elution Order	
Compound	Retention Time (min)	Compound	Retention Time (min)
1-Butanol	32.0	Glycerol	12.1
2-Butanol	26.8	Methanol	16.5
3-Butanol	21.8	Ethanol	18.5
Ethanol	18.5	2-Propanol	20.4
Glycerol	12.1	3-Butanol	21.8
Isobutanol	28.4	1-Propanol	23.2
Methanol	16.5	2-Butanol	26.8
1-Propanol	23.2	Isobutanol	28.4
2-Propanol	20.4	1-Butanol	32.0

Sugars can also be detected using this method, as shown in the separation of glucose and fructose in grape juice cocktail (Figure B). Retention times for a wide range of sugars and sugar alcohols are listed in Table 2. A flow rate of 0.7mL/min and a column temperature of 50°C were used to shorten the analysis time for the alcohol separations. However, care should be taken when analyzing sugars, since inversion of sucrose to glucose and fructose can occur.

Figure B. Grape Juice Cocktail

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.: RI
Inj.: 10µL of grape juice cocktail



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Table 2. Retention Times of Carbohydrates and Sugar Alcohols

(See Figure B for conditions.)

Alphabetic Order		Elution Order	
Compound	Retention Time (min)	Compound	Retention Time (min)
Arabinose	13.9	Maltoheptaose	8.8
Arabitol	14.1	Maltohexaose	8.9
Dulcitol	13.4	Maltopentaose	9.1
Erythritol	15.0	Maltotetraose	9.3
Ethanol	25.6	Stachyose	9.3
Fructose	13.1	Isomaltotriose	9.5
Galactose	12.9	Maltotriose	9.7
Glucose	12.1	Melzitose	9.7
Glycerol	16.8	Raffinose	9.7
Inositol	12.6	Isomaltose	10.3
Isomaltose	10.3	Maltose	10.5
Isomaltotriose	9.5	Sucrose	10.6
Lactose	10.8	Lactose	10.8
Maltitol	11.0	Maltitol	11.0
Maltoheptaose	8.8	Glucose	12.1
Maltohexaose	8.9	Inositol	12.6
Maltopentaose	9.1	Mannose	12.8
Maltose	10.5	Xylose	12.8
Maltotetraose	9.3	Galactose	12.9
Maltotriose	9.7	Fructose	13.1
Mannitol	13.2	Mannitol	13.2
Mannose	12.8	Dulcitol	13.4
Melzitose	9.7	Psicose	13.4
Psicose	13.4	Sorbitol	13.4
Raffinose	9.7	Ribitol	13.7
Ribitol	13.7	Arabinose	13.9
Ribose	14.2	Arabitol	14.1
Sorbitol	13.4	Ribose	14.2
Stachyose	9.3	Xylitol	14.4
Sucrose	10.6	Erythritol	15.0
Xylitol	14.4	Glycerol	16.8
Xylose	12.8	Ethanol	25.6

The SUPELCOGEL C-610H column provides versatility for a wide range of food and beverage applications. Combined with the proper mode of detection, this column can be used to analyze organic acids, sugars, sugar alcohols, and fermentation products. Sample preparation is minimal, and the chromatography can be performed using simple isocratic mobile phase conditions.

Ordering Information:

SUPELCOGEL C-610H 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**

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