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Analysis of sugars by supercritical fluid chromatography using polar packed columns and light-scattering detection

B. HERBRETEAU, M. LAFOSSE, L. MORIN-ALLORY and M. DREUX*
Laboratoire de Chimie Bioorganique et Analytique (LCBA), UFR Sciences, Université d'Orléans, B.P. 6759, F-45067 Orléans Cedex 2 (France)

SUMMARY

The application of supercritical fluid chromatography (SFC) with polar packed columns and light-scattering detection for the analysis of sugars is reported. Cyano-, diol- and nitro-bonded silicas were used with carbon dioxide–methanol mobile phases and a comparison of sugar retention was carried out. These SFC systems showed different selectivities from that found in high-performance liquid chromatography. The association of a constant flow-rate of carbon dioxide and a variable flow-rate of methanol affords the elution of mono-, di- and trisaccharides in the same analysis without baseline drift.

INTRODUCTION

Many papers have been published on the analysis of sugars by high-performance liquid chromatography (HPLC)[1–4]. This interest is due to the difficulties of these analyses, resulting from the instability and relatively short life of the systems[5] and the need for a simple and sensitive detection method[6].

Amino-bonded silicas are often used, but a loss of sugars is observed with reducing sugars that form Schiff bases[7], necessitating accurate calibration for quantitative analysis. In contrast, diol-bonded silicas do not show this problem and afford the advantage of a very low hydrolysis rate with aqueous eluents[5]; consequently their use in gradient elution with light-scattering detection (LSD) is to be preferred. On the other hand, diol-bonded silicas offer a lower selectivity for sugars than do amino-bonded silicas. Moreover, peak broadening occurs with these aqueous eluents due to the anomeric forms of reducing sugars at the time of mutarotation[2,4].

To avoid these difficulties and to obtain better selectivity, we used certain polar bonded silicas in combination with supercritical fluid chromatography (SFC). Sugar analysis was previously carried out by SFC with capillary columns and carbon dioxide after derivatization in order to render the solutes less polar[8,9].

With packed columns, the addition of a polar modifier to the eluent increases the solubility of polar solutes, which is necessary for an acceptable elution time. However, the use of flame ionization detection, often used with capillary columns and carbon

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dioxide, becomes impossible with packed columns and a polar modifier. For non-UV-absorbing compounds we achieved the first coupling of SFC on packed columns using carbon dioxide with LSD[10,11]. This constitutes a universal detection method using SFC with packed columns and polar modifiers. Recently we have described an SFC method with apolar packed columns for the determination of sugars in tobacco[12].

In this paper we show how columns packed with polar bonded silicas can be used to analyse sugars by SFC with greater selectivity than HPLC.

EXPERIMENTAL

Apparatus

Carbon dioxide, kept in a cylinder with an eductor tube connected to a Waters Assoc. (Milford, MA, U.S.A.) Model M 45 pump, was passed through an ethanol cooling bath. The pump head was cooled to improve efficiency. The flow-rates and the weight percentages of carbon dioxide and methanol given in tables and figures have been corrected to take into account the pumping yield and the pump head temperature. The polar modifier (methanol) was added using a Jasco (Tokyo, Japan) Model 2510 pump. The two solvents were mixed in a Knauer (Berlin, F.R.G.) mixer. The column temperature (40°C) was controlled by a water-bath. The loop of a Rheodyne (Cotati, CA, U.S.A.) Model 7125 valve was immersed in the same water-bath.

A Model Sedex 45 evaporative light-scattering detector (Sedere, Vitry sur Seine, France) was used. A fused-silica capillary tube (160 × 0.075 mm I.D.) was chosen as a restrictor instead of the conventional nebulizer for HPLC[10,11]. Consequently, with such a restrictor, variations in flow-rate provide a pressure gradient. The polarity of the mobile phase increases with increase in the flow-rate of methanol. Some experiments with large amounts of methanol in the mobile phase were performed under sub-critical conditions.

Columns

The following columns were used: 10-μm Lichrospher Diol (250 × 4.6 mm I.D.), 5-μm LiChrosorb CN (150 × 4.6 mm I.D.), both from Merck (Darmstadt, F.R.G.), 7-μm Zorbax CN (150 × 4.6 mm I.D.) from DuPont (Wilmington, DE, U.S.A.), 5-μm RSil NO_2 (250 × 4.6 mm I.D.) from RSL (Eke, Belgium) and 10-μm μBondapak CN (150 × 3.9 mm I.D.) from Waters Assoc.

Chemicals and reagents

Carbon dioxide (Air Liquide, Paris, France) was of B 50 grade and was flushed through molecular sieves before the pump. Pestipur-grade methanol was purchased from SDS (Vitry, France). Only a few grades of methanol are suitable as polar modifiers. With others a high baseline noise is observed; HPLC- and spectroscopic-grade methanol must be tested before use. The noise may be partly caused by the amount of dry residue, but this is not the only cause. Studies on the quality of solvents for HPLC and SFC suitable for this detector are currently in progress.

The solutes (analytical-reagent grade) were dissolved in methanol–water, chloroform–methanol or pure methanol. Chloroform–methanol was preferred in order to avoid problems such as band broadening and peak splitting resulting from injection of solvents of high elution strength.

RESULTS AND DISCUSSION

In order to compare the capabilities of the HPLC and SFC separation systems for the analysis of sugars and polyols on polar stationary phases, it was necessary to explore a large number of solvent mixtures as mobile phases.

In this SFC study, we only investigated carbon dioxide–methanol mixtures in order to determine the influence of the methanol concentration on the retention of carbohydrates and corresponding polyols on some polar bonded silicas. The results should be compared with those obtained using organic HPLC mobile phases (*e.g.*, chloroform–methanol) having a polarity similar to that of carbon dioxide–methanol mixtures in SFC. Such a study is currently in progress; so far only the acetonitrile–water mobile phase has been investigated and consequently useful comparisons cannot be given in this paper.

With the carbon dioxide–methanol systems, peak broadening occurs only for certain sugars and as all solutes were injected separately we do not show the chromatograms of mixtures but report for discussion the retention times in two tables.

For the different stationary phases the mobile phase compositions and the pressures were chosen in order to maintain a similar retention for all the compounds.

TABLE I

RETENTION TIMES (min) OF MONOSACCHARIDES AND POLYOLS ON POLAR BONDED SILICAS IN SFC AT 40°C AND WITH CO_2–METHANOL MIXTURES

Compound	*Column No.*[a]				
	1	*2*	*3*	*4*	*5*
2-Deoxy-D-ribose	2.4	2.1	1.1	3.9	3.3
L-Rhamnose	3.6	3.2	4.5	5.8	4.1
D-Ribose	3.8	3.4	4.8	5.6	4.7
meso-Erythritol	3.9	3.5	3.6	5.0	4.6
L-Arabinose	4.4	3.7	3.5	6.2	5.0
D-Xylose	4.5	4.0	4.5	6.4	4.6
D-Fructose	6.0	5.2	3.7	7.6	6.5
L-Sorbose	6.2	5.2	3.7	9.0	6.2
Xylitol	7.0	5.1	8.7	7.3	7.6
D-Galactose	8.0	7.5	7.0	–	8.0
D-Mannose	8.0	4.5	6.7	9.4	7.0
D-Glucose	8.5	7.8	6.5	10.6	8.0
meso-Inositol	13.0	12.0	9.8	19.0	–
D-Mannitol	14.0	8.0	15.0	10.8	10.2
D-Sorbitol	–	–	–	11.0	11.7

[a] 1 = Zorbax CN (150 × 4.6 mm I.D.), CO_2–methanol (93.5:6.5, w/w), 4.35 ml min^{-1}, 3700 p.s.i.;
2 = μBondapak CN (150 × 3.9 mm I.D.), CO_2–methanol (95.9:4.1, w/w), 3.37 ml min^{-1}, 3900 p.s.i.;
3 = LiChrosorb CN (150 × 4.6 mm I.D.), CO_2–methanol (96.4:3.6, w/w), 3.35 ml min^{-1}, 3900 p.s.i.;
4 = Lichrospher Diol (250 × 4.6 mm I.D.), CO_2–methanol (83.7:16.3, w/w), 1.79 ml min^{-1}, 3900 p.s.i.;
5 = RSil NO_2 (250 × 4.6 mm I.D.), CO_2–methanol (87.0:13.0, w/w), 3.8 ml min^{-1}, 3500 p.s.i.

Influence of methanol content

As seen in Table I, the mobile phase for Zorbax CN (column 1) requires a higher methanol content than that for μBondapak (column 2) and LiChrosorb CN (column 3) to elute sugars with similar retentions. Using Lichrospher Diol (column 4) and RSil NO_2 (column 5), the methanol content is much higher, indicating strong interactions of sugars with these packings.

The data in Table I may be compared with those in Table II. The retention times decrease with increase in the amount of methanol in the mobile phase and consequently the disaccharides are more easily eluted (sucrose, trehalose, lactose and maltose; see Table II). As shown in Fig. 1, by increasing the flow-rate of methanol while maintaining a constant flow-rate of carbon dioxide, a composition and flow-rate gradient can be realized, allowing the elution of mono-, di- and trisaccharides in the same analysis. This promising technique, which is easily compatible with LSD, will subsequently be applied to other samples.

Comparison of selectivities on cyano-bonded silicas

Sugars are not retained in HPLC on cyano-bonded silicas using an acetonitrile–water eluent. As seen in Table I, the elution sequence in SFC follows the order of molecular size (*i.e.*, the number of carbon atoms) on Zorbax and μBondapak CN. This sequence is changed on LiChrosorb CN: fructose and sorbose show lower retentions than molecules having fewer carbon atoms (xylose, ribose).

Different selectivities of the three cyano-bonded silicas are noted. Galactose and mannose are not separated on Zorbax and LiChrosorb CN, but are separated on μBondapak CN. In contrast, galactose and mannitol have similar retention times on μBondapak CN, whereas Zorbax and LiChrosorb CN provide a good selectivity. Only LiChrosorb CN easily separates fructose and xylitol; poor results were obtained using

TABLE II

RETENTION TIMES (min) OF MONO- AND DISACCHARIDES ON POLAR BONDED SILICAS IN SFC AT 40°C AND WITH CO_2–METHANOL MIXTURES

Compound	*Column No.*[a]		
	1	*2*	*3*
2-Deoxyribose	1.5	–	–
L-Rhamnose	1.9	–	–
D-Ribose	–	–	3.8
D-Fructose	2.7	2.5	4.0
D-Glucose	3.3	3.2	–
meso-Inositol	6.0	4.9	9.1
D-Sorbitol	–	–	5.9
Sucrose	8.3	7.6	9.7
Trehalose	14.1	15.1	–
Lactose	16.6	17.2	–
Maltose	–	14.1	13.3

[a] 1 = Zorbax CN (150 × 4.6 mm I.D.), CO_2–methanol (90.2:9.8, w/w), 4.09 ml min^{-1};
2 = μBondapak CN (150 × 3.9 mm I.D.), CO_2–methanol (94.7:5.3, w/w), 3.85 ml min^{-1};
3 = Lichrospher Diol (250 × 4.6 mm I.D.), CO_2–methanol (78.3:21.7, w/w), 1.94 ml min^{-1}.

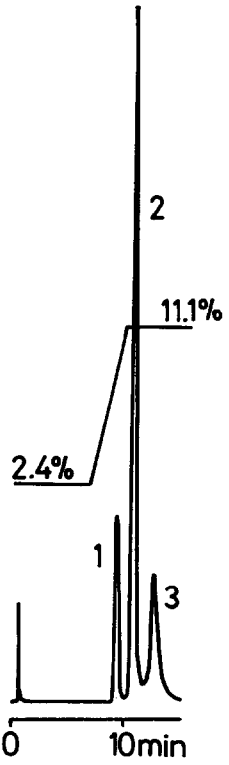

Fig. 1. Gradient elution chromatogram of sugars. Column, μBondapak CN. Mobile phase, CO_2–methanol. Gradient conditions: t = 0 min, 97.6:2.4 (w/w), 3.3 ml min^{-1}; t = 7 min, 97.6:2.4 (w/w), 3.3 ml min^{-1}; t = 10 min, 88.9:11.1 (w/w), 3.7 ml min^{-1}; flow-rate of CO_2 constant at 3.2 ml min^{-1}. Solutes: 1 = fructose; 2 = sucrose; 3 = raffinose.

Zorbax CN for the separation of *meso*-inositol and mannitol whereas μBondapak CN and LiChrosorb CN lead to a good selectivity but to reverse elution orders.

Different selectivity of polar bonded silicas

Table I illustrates the different selectivities of polar bonded silicas in SFC and the larger retention of polyols with regard to the corresponding sugars.

Mannose and glucose are poorly resolved in HPLC on aminopropylsilica. In SFC with a similar selectivity a better resolution is obtained owing to a good efficiency on Lichrospher Diol (Fig. 2) and on RSil NO_2, whereas μBondapak CN provides a large selectivity (Table I). Glucose, mannitol and sorbitol are not separated on aminopropylsilica in HPLC and on Lichrospher Diol in SFC. Although it is not useful for the HPLC of sugars, as it gives very short retention times, RSil NO_2 permits a good separation of these three compounds in SFC (Fig. 2).

The change in the elution sequence of sorbose and xylitol on Lichrospher Diol, RSil NO_2 and LiChrosorb CN columns may be noted from Table I. *meso*-Erythritol, xylose and rhamnose are not separated in HPLC on a Lichrospher Diol column. In

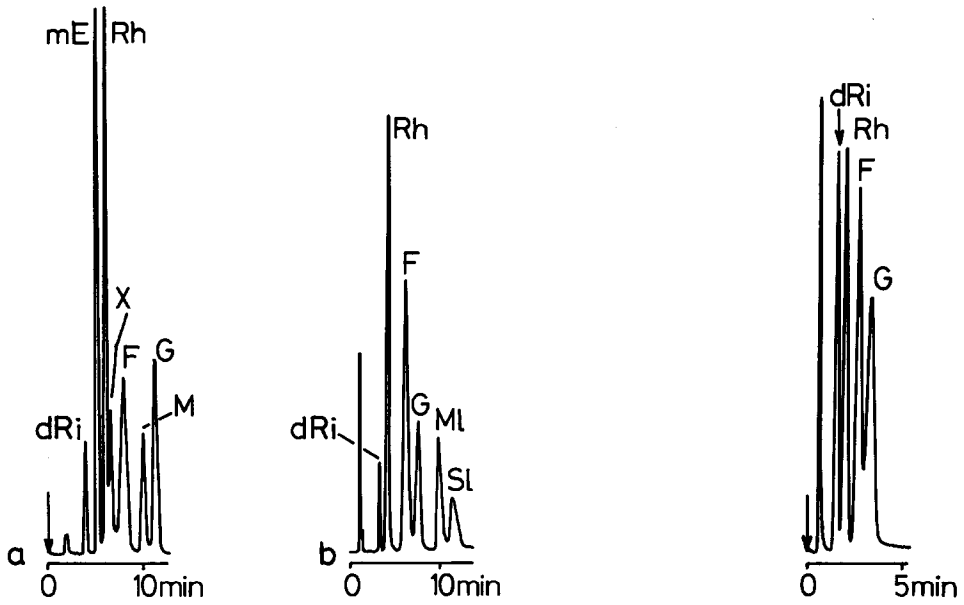

Fig. 2. Separation of sugars in SFC packed columns. (a) Lichrospher Diol column. Eluent: CO_2–methanol (84.5:15.5, w/w), 1.77 ml min^{-1}, 3900 p.s.i. (b) RSil NO_2 column. Eluent: CO_2–methanol (87.0:13.0, w/w), 3.8 ml min^{-1}, 3500 p.s.i. dRi = 2-Deoxy-D-ribose; mE = *meso*-erythritol; Rh = rhamnose; X = xylose; F = fructose; M = mannose; G = glucose; Ml = mannitol; Sl = sorbitol.

Fig. 3. Separation of sugars in SFC on Zorbax CN column. Eluent: CO_2–methanol (91.1:8.9, w/w), 4.49 ml min^{-1}, 3500 p.s.i.

contrast, this column is the only one that permits the separation of these solutes in SFC (Table I and Fig. 2).

Figs. 2 and 3 illustrate good separations of sugars with polar bonded silicas in SFC. In HPLC polyols and corresponding sugars generally show similar retentions on aminopropylsilica[1]. In SFC, and more especially on RSil NO_2, polyols show greater retention than do sugars (*e.g.*, mannitol and mannose, sorbitol and glucose, xylitol and xylose). Finally, *meso*-inositol, a cyclic polyol, yields a high retention as in HPLC.

CONCLUSION

The analysis of polar compunds such as sugars can easily be carried out by SFC using polar bonded phases and with carbon dioxide–methanol as the mobile phase. Light-scattering detection is presented as a universal method using SFC with packed columns and polar modifiers. This system provides a greater range of selectivity than in HPLC.

The possibility of changing the modifier flow-rate permits the elution of mono-, di- and trisaccharides in the same analysis without baseline drift. The chromatographic behaviour of sugars will be investigated in SFC with other apolar and polar bonded silicas. A comparison of selectivities will be presented later.

Research is in progress on other cyano-bonded silicas. Factor analysis of the chromatographic results[13] will emphasize the factors that affect SFC selectivity.

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