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Short Communication

High-performance liquid chromatographic determination of sugars in an infusion and soft drinks using a silica-based 3-morpholinopropyl-bonded stationary phase

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ABSTRACT

A recently developed column packing material, 3-morpholinopropylsilyl-modified silica gel (MPS), was successfully applied to the separation of many kinds of sugars (rhamnose, xylose, arabitol, glucose, mannose, sucrose, maltose, melibiose, raffinose and maltotriose) and the determination of glucose or/and sucrose in an infusion, drinks for sports and soft drinks, with elution with acetonitrile–10 m*M* imidazole–borate buffer (pH 6.5) (90:10, v/v) by refractive index detection. The MPS column was found to be chemically stable under the elution conditions used and during continuous operation for over 500 h.

INTRODUCTION

In clinical medicine, routine biochemical analyses for sugars by high-performance liquid chromatography (HPLC) for the diagnosis of various diseases is widely employed, and the development of more rapid methods for the HPLC determination of sugars is desirable. Silica modified with chemically bonded primary amino groups, *e.g.* 3-aminopropylsilyl silica gel (APS), is widely used in the HPLC analyses of sugar mixtures [1]. However, APS columns have problems of frequently observed changes in their properties [2–7]. Recently, a study on the chromatography stability of silica-based aminopropyl-bonded stationary phases was reported and the causes of changes were investigated chromatographically [8].

In a trial of modifications of APS, a newly developed silica-based 3-morpholinopropyl-bonded column packing (MPS) was shown to be an excellent separation agent in analyses of water-soluble substances (*e.g.*, water-soluble vitamins in health beverages [9], the components of eye lotions [10] and 5′-ribonucleotidic components of seasonings [11]).

This paper describes a HPLC method using MPS that permitted the simultaneous analysis of a mixture of ten mono- and oligosaccharides [rhamnose (Rha), xylose (Xyl), arabitol (Ara), glucose (Glu), mannose (Man), sucrose (Suc), maltose (Mal), melibiose (Mel), raffinose (Raf) and maltotriose (Mat)]. The method was also applied to the determination of Glu or/and Suc in an infusion and soft drinks. This results with this method correlated well with those given by Somogyi's [12] and Bertrand's [13]

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reference titration methods. We also examined the durability of the MPS column.

EXPERIMENTAL

Materials

Silica gel for HPLC, Daiso gel SP 120, 200, 300 or 1200 Å (particle size 5 μm), was supplied by Daiso (Osaka, Japan). (3-Morpholinopropyl)trimethoxysilane was purchased from Shin-Etsu Chemical Industry (Japan).

Preparations of MPS

MPS was prepared as described previously [9]. Silica gel (10.0 g) was suspended in toluene (300 ml) and the mixture was refluxed for 24 h to remove water. (3-Morpholinopropyl)trimethoxysilane (12 g) was added to the reaction vessel and the mixture was refluxed for 24 h. After treatment with toluene, acetone and diethyl ether successively, it was then dried under reduced pressure at 100°C for 4 h. Elemental analysis data for MPS and the grafted value (G) [9] estimated from the result of nitrogen determination were as follows: found, C 7.89, H 15.4, N 1.20%; G = 0.86 mmol g^{-1}.

Reagents

Water was deionized and distilled. Acetonitrile was of HPLC grade from Wako (Osaka, Japan). All other reagents (Wako) were of analytical-reagent grade and were not further purified.

Instrumentation and chromatographic conditions

The liquid chromatograph consisted of a Tosoh CCPD pump with a Rheodyne Model 7125 injector with a 50-μl loop, a Tosoh RI 8010 refractive index detector, a Shimadzu R-111M recorder and a stainless-steel column (150 mm × 6.0 mm I.D.) of MPS (5 μm), packed by the slurry packing technique [glycerol–methanol, 4:6 (v/v)] [9] or a Daiso APS (5 μm) column (150 mm × 6.0 mm I.D.). The eluent for the separation of sugars was 10 mM imidazole–borate buffer (pH 6.5)–acetonitrile (10:90, v/v). The flow-rate was set at 1.0 ml/min. All separations were carried out at 30°C.

RESULTS AND DISCUSSION

The effects of the pore diameter of MPS (particle size 5 μm) (120, 200, 300 and 1200 Å) were examined for the separation of equal volume mixtures of Glu, Mal, Xyl and lactose (Lac) (each present as a 10^{-3} M solution). As shown in Fig. 1, the column packed with the particles of 120 Å gave the most

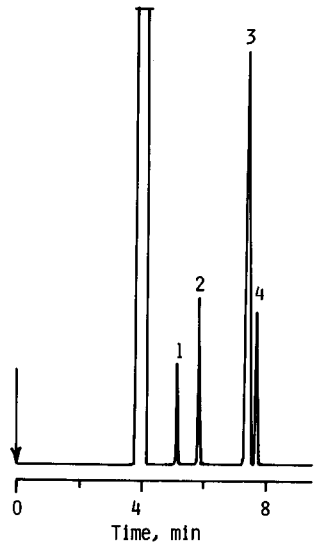

Fig. 1. Chromatogram of monosaccharides obtained by MPS. Peaks: 1 = Xyl; 2 = Glu; 3 = Mal; 4 = Lac. Mobile phase, acetonitrile–water (80:20, v/v); injection volume, 50 μl.

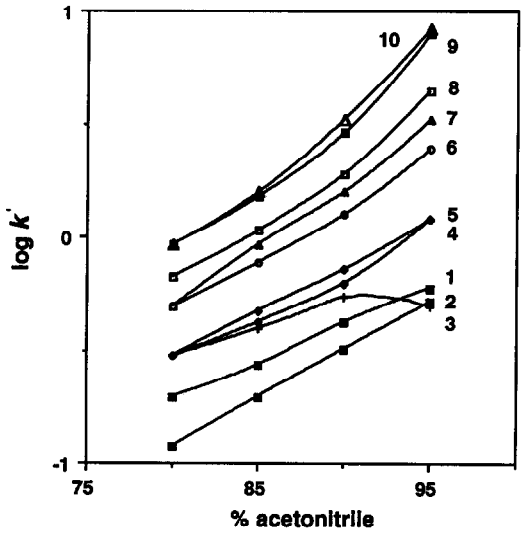

Fig. 2. Effect of acetonitrile concentration in the mobile phase on the separation of saccharides by MPS. 1 = Rha; 2 = Xyl; 3 = Ara; 4 = Glu; 5 = Man; 6 = Suc; 7 = Mal; 8 = Mel; 9 = Raf; 10 = Mat. For HPLC conditions, see Experimental.

efficient separation on elution with phosphate buffer (0.07 *M*, pH 5.5–7.5)–acetonitrile (20:80, v/v).

The effects of acetonitrile concentration in the mobile phase (acetonitrile–10 m*M* imidazole–borate buffer) on the separation of ten saccharides (Glu, Xyl, Rha, Mal, Suc, Mel, Mat, Raf, Ara and Man) are shown in Fig. 2. An increase in acetonitrile concentration led to an increase in k', except for Ara. The best separation was obtained at 90% (v/v). A partition mechanism is probably involved in this separation. Fig. 3 shows a chromatogram obtained under the above conditions.

With a fixed acetonitrile concentration (90%, v/v), the effects of the pH of imidazole–borate buffer were examined for the separation of the same saccharides. As illustrated in Fig. 4, a pH of 6.5 was optimum in the separation of these sugars.

Generally, it seems that APS columns often cause a decrease in efficiency and a shortening of the column lifetime. For the examination of the stability of our MPS columns, the changes in the retention times of each of five kinds of standard sugars were recorded over 500 h with continuous pumping of acetonitrile–10 m*M* imidazole–borate buffer (pH 6.5) (90:10, v/v). Fig. 5 shows that each retention time was almost unchanged, except for Mat in the first stage. A chromatogram produced subsequent to 500 h of continuous elution proved to be adequate for practical use.

As a practical application, determinations of sugars in a commercially available infusion, drinks for sports and soft drinks were successfully carried out. In the infusion, a resolved peak assigned to Glu was observed and the calibration graph was linear over the range 62.5–500 nmol (r = 0.999). The deter-

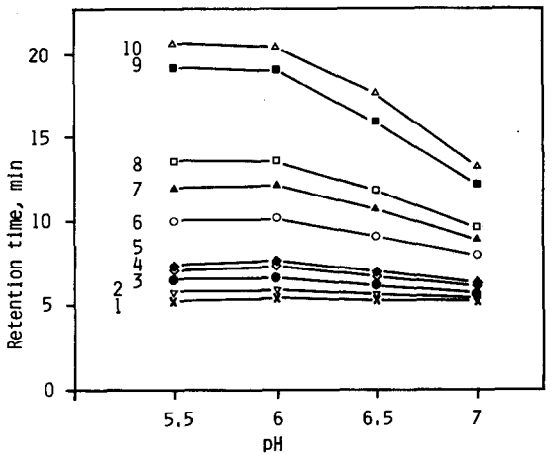

Fig. 4. Effect of pH on separation of saccharides by MPS. The order of retention times and the HPLC conditions are as in Figs. 2 and 3.

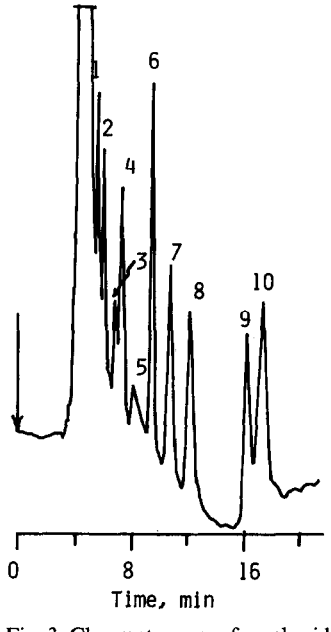

Fig. 3. Chromatogram of saccharides obtained by MPS. Peaks: 1 = Rha; 2 = Xyl; 3 = Ara; 4 = Glu; 5 = Man; 6 = Suc; 7 = Mal; 8 = Mel; 9 = Raf; 10 = Mat. Injection volume, 50 μl.

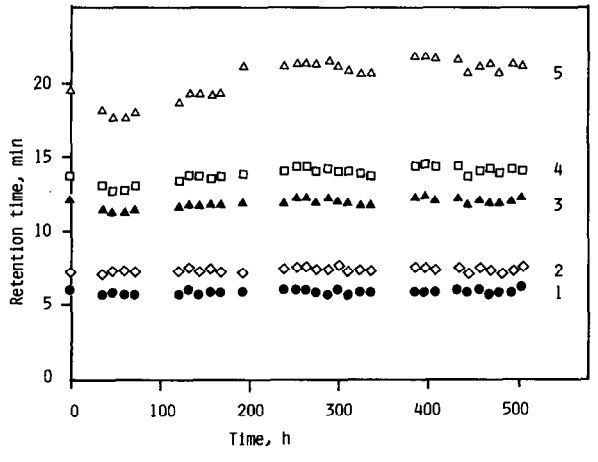

Fig. 5. Durability of MPS. 1 = Xyl; 2 = Glu; 3 = Mal; 4 = Mel; 5 = Mat. See Fig. 3 for conditions.

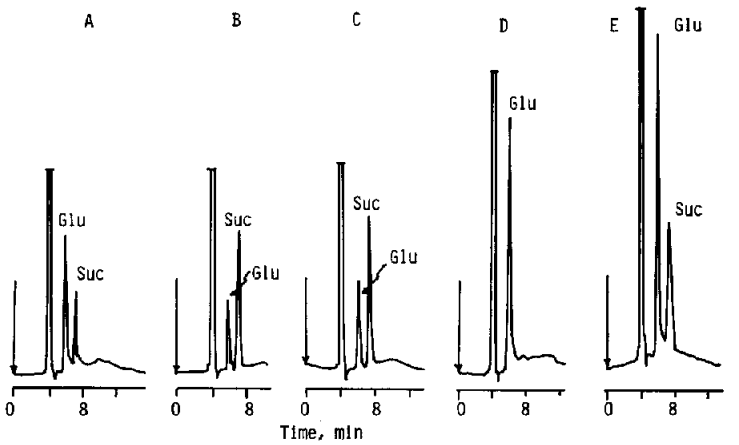

Fig. 6. Chromatograms of saccharides in commercially available soft drinks. The samples (A–E) were diluted 20-fold with eluent. A 50-μl aliquot of each diluted samples was injected on to the HPLC column. See Fig. 3 for conditions.

mined value (1.34 *M*) calculated from the calibration graph agreed well with the indicated value (1.40 *M*) and the reproducibility (R.S.D. = 1.5%, n = 5) was satisfactory.

The values determined by the standard addition method and recoveries of sugars for two commercially available drinks for sports were as follows: Company a, Glu 1.1 g per 100 ml (107.4%) and Suc 4.1 g per 100 ml (78.5%); and Company b, Glu 2.6 g per 100 ml (128.5%).

TABLE I

DETERMINATION OF SACCHARIDES IN SOFT DRINKS

All the drinks are packed in cans. Results are means of triplicate measurements.

Method	Concentration (g per 100 ml)				
	A[a]	B[b]	C[c]	D[d]	E[e]
HPLC	3.0	7.8	4.2	9.9	2.4
Somogyi[f]	4.6	9.3	8.1	10.6	3.4
Bertrand[g]	3.2	8.2	5.9	9.3	3.8

[a] Soda pop (carbonated drink).
[b] Carbonated orange juice.
[c] Orange juice.
[d] Healthy drink containing some vitamins.
[e] Tea.
[f] Ref. 12.
[g] Ref. 13.

Chromatograms of saccharides in commercially available soft drinks (A–E) are shown in Fig. 6. The calibration graphs for Suc and Glu were prepared under the HPLC conditions and then used for the determination. Because no indicated values of sugar (s) were given for any of the soft drinks analysed, the values determined by this HPLC method were correlated with both Somogyi's [12] and Bertrand's [13] methods, applied frequently for the determination of reducing sugars. Linear regressions are as follows: $y = 1.40 + 0.98x$ ($r = 0.987$) (g per 100 ml) (Somogyi's *vs.* HPLC method); $y = 1.36 + 0.83x$ ($r = 0.980$) (Bertrand's *vs.* HPLC method); and $y = 0.28 + 0.83x$ ($r = 0.976$) (Bertrand's *vs.* Somogyi's method). The contents of saccharides determined by three methods are given in Table I. From these results, the HPLC method proposed is considered to be reliable, simple and rapid for the determination of the sugars.

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