Journal of Chromatography, 322 (1985) 220–222
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 17,464

Note

High-performance liquid chromatographic separation of glucose-1-phosphate, fructose, sucrose and inorganic orthophosphate

R. M. STIKKELMAN*, T. T. TJIOE, J. P. VAN DER WIEL and F. VAN RANTWIJK
Laboratory of Organic Chemistry, Delft University of Technology, P.O. Box 5045, 2600 GA Delft (The Netherlands)
(Received December 6th, 1984)

The phosphorylysis of sucrose (I) by means of sucrose phosphorylase[1,2] (E.C. 2.4.1.7) obtained from *Leuconostoc mesenteroides* (ATCC 12291) results in the formation of equimolar amounts of glucose-1-phosphate (α-D-glucopyranosylphosphate, II) and fructose (III). In the course of our investigation[3] of this reaction we needed a simple method for the quantitative analysis of I–III and inorganic phosphate (IV).

Ion-exchange chromatography of II using an anionic resin and aqueous potassium acetate as the mobile phase[4,5] does not allow the analysis of I and III. Analytical scale chromatographic separations of phosphate esters, with anion-exchange resins or gels, *e.g.*, in enzyme assays, are well known[6–9], but these methods generally employ complex salt and pH gradients which require post-column colorimetric analysis.

Ion-moderated partitioning (IMP) chromatography[10] is increasingly being used for the analysis of organic acids, carbohydrates and alditols. For organic acids, a cation-exchange resin in the protonated form is used. The ionization of the solutes is suppressed by utilizing an acidic mobile phase (pH < pK_a − 1). In the present note we describe the application of IMP chromatography for the analysis of I–IV.

MATERIALS

Chemicals

Glucose-1-phosphate (dipotassium salt) was obtained from Janssen Chimica (Beerse, Belgium); glucose and fructose were supplied by Merck (Darmstadt, F.R.G.). Aminex A-7 HPLC resin was obtained from Bio-Rad (Richmond, CA, U.S.A.).

Chromatography

Chromatography was carried out using a Waters Assoc. M 6000A pump, a Waters Assoc. R401 refractive index detector and a Rheodyne 7125 injection valve. The stationary phase (Aminex A-7 resin, 8% divinylbenzene cross-linked sulphonated polystyrene, particle size 9 ± 2 μm) was slurry-packed[11] into a 200 × 9 mm I.D. column. The mobile phase was filtered and degassed by sonication under low pressure before use.

0021-9673/85/$03.30

TABLE I

RETENTION TIMES (min) ON AMINEX A-7 OF GLUCOSE-1-PHOSPHATE, INORGANIC PHOSPHATE, SUCROSE, FRUCTOSE, AND GLUCOSE IN SEVERAL MOBILE PHASES

Chromatographic conditions: 21°C; flow-rate 0.3 ml/min.

Component	*Mobile phase*			
	0.06 M TFA	*0.04 M TFA*	*0.01 M TFA*	*0.03 M H_2SO_4*
Solvent front	12.2	12.2	12.2	11.8
Glucose-1-phosphate	13.7	13.4	13.0	12.7
Sucrose	16.0	15.9	–	15.4
Inorganic phosphate	19.1	19.0	–	18.0
Glucose	19.2	18.9	–	18.4
Fructose	21.2	21.2	–	20.5

RESULTS AND DISCUSSION

The retention times for I–IV, and for glucose with 0.03 *M* aqueous sulphuric acid and 0.01, 0.04 and 0.06 *M* aqueous trifluoroacetic acid (TFA) as the mobile phase are compiled in Table I. Aqueous sulphuric acid gives a satisfactory separation. However, corrosion of the steel parts of the detector cell occurs upon prolonged use of this mobile phase. Aqueous TFA, which is increasingly being used in reversed-phase ion-pair chromatography[12,13], performed equally satisfactory, as shown in Fig. 1 and Table I. The aqueous TFA solutions used do not corrode 316 stainless steel.

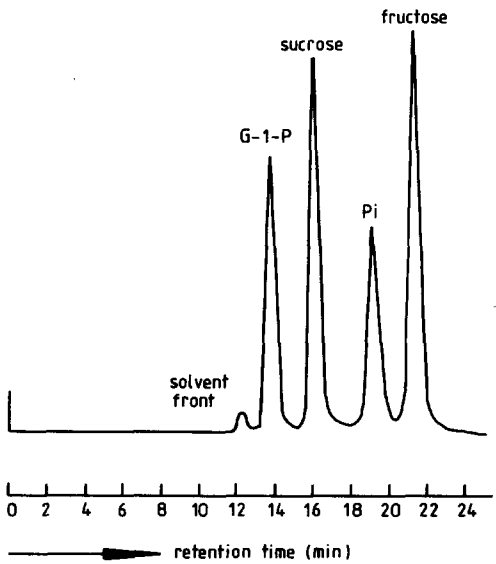

Fig. 1. The chromatographic separation of glucose-1-phosphate (G-1-P), inorganic phosphate (Pi), sucrose and fructose on Aminex A-7. Chromatographic conditions: 21°C; flow-rate 0.3 ml/min; mobile phase 0.06 *M* TFA.

In IMP chromatography, neutral polar compounds are retarded by sorption and negative ions are excluded. The retention times of conjugated bases will increase with decreasing pH of the mobile phase. IMP chromatography of II constitutes a problem because of the low pK_a value (1.10) of II. This necessitates a strong acidic mobile phase in order to prevent Donnan-type exclusion. On the other hand, the pH value of the mobile phase should not be too low, because of the hydrolysis of II into glucose and IV[14]. Treatment of II with 0.06 *M* aqueous TFA for 20 min at 21°C resulted in no detectable formation of glucose. Therefore analyses could be carried out at room temperature without hydrolysis of II.

As shown in Table I, the retention time of II increases with increasing concentration of TFA. An 0.06 *M* aqueous TFA solution gives a good separation between the peak of II and the solvent front. The sequence of the peaks is not affected by changing the concentration of TFA.

CONCLUSION

A simple quantitative analysis of mixtures of glucose-1-phosphate, inorganic phosphate, fructose and sucrose can be performed by HPLC on an Aminex A-7 column with an 0.06 *M* aqueous TFA solution as the mobile phase, without detectable hydrolysis of glucose-1-phosphate.

REFERENCES

1 B. M. Chassy and M. I. Krichewsky, *Anal. Biochem.*, 49 (1972) 232.
2 A. De Laporte, L. de Valk and E. Vandamme, *Antonie van Leeuwenhoek*, 48 (1982) 516.
3 R. M. Stikkelman, A. J. J. Straathof, T. T. Tjioe and J. P. van der Wiel, *Graduate Research Project*, Internal Report.
4 H. Hokse, *Stärke*, 35 (1983) 101.
5 K. Brunt and H. Hokse, *J. Chromatogr.*, 268 (1983) 131.
6 H. J. Duncan, *J. Chromatogr.*, 62 (1971) 391.
7 P. J. Geiger, S. Ahn and S. P. Bessman, *Methods Carbohyd. Chem.*, 8 (1980) 21.
8 S. Tabata, S. Hizukuri and K. Nagata, *Carbohyd. Res.*, 67 (1978) 189.
9 E. Li, I. Jabas and S. Kornfeld, *J. Biol. Chem.*, 253 (1978) 7762.
10 R. Pecina, G. Bonn, E. Burtscher and O. Bobleter, *J. Chromatogr.*, 287 (1984) 245.
11 L. A. Th. Verhaar and B. F. M. Kuster, *J. Chromatogr.*, 210 (1981) 279.
12 H. P. J. Bennett, A. M. Hudson, C. McMartin and G. E. Purdon, *Biochem. J.*, 168 (1977) 9.
13 D. Voskamp, C. Olieman and H. C. Beyerman, *Rec. Trav. Chim. Pays-Bas*, 99 (1980) 105.
14 C. A. Bunton, D. R. Llewellyn, K. G. Oldham and C. A. Vernon, *J. Chem. Soc., London*, (1958) 3588.