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Note

Separation and quantitative determination of fructose as the O-methyloxime by gas–liquid chromatography using glass capillary columns

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The main difficulty in the gas chromatographic separation of different mono- and oligosaccharides is the formation of multiple peaks due to tautomeric forms of reducing sugars. The possibility of reducing the number of tautomers by converting the sugars into oximes before forming the trimethylsilyl (TMS) ethers has been investigated by Sweeley and co-workes[1–3]. Mason and Slover[4] made it possible to separate in one run arabinose, ribose, sucrose, lactose and raffinose, which gave well resolved single peaks on a column packed with 1% SE-30 on Gas-Chrom Q. Under the same conditions, fructose gave only partially separated peaks. Moreover, unseparated peaks of isomers were observed for a sample of glucose and galactose.

The problem of the separation of different isomers can be solved by using high-efficiency capillary columns[5,6]. Wall-coated glass capillary columns of length 50–120 m have been used successfully for the separation of a complex mixture of carbohydrate radiolysis products[7–11].

The results of earlier investigations[4,12] on the chromatography of carbohydrates in the form of their TMS ethers showed the suitability of these derivatives for analytical applications. In this work, fructose and other monosaccharides were converted into O-methyloximes before trimethylsilylation. Good separations of fructose, galactose and glucose have been achieved using glass capillary columns.

EXPERIMENTAL

Materials

The silylating reagents, namely chlorotrimethylsilane (TMCS), hexamethyldisilazane (HMDS), N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and pyridine and methoxylammonium chloride, were purchased from Pierce (Rockford, Ill., U.S.A.).

The monosaccharides were purchased from Merck (Darmstadt, G.F.R.). Research-grade cyclohexane, benzene and octane were obtained from POCH (Gliwice, Poland).

Apparatus

Gas–liquid chromatographic (GLC) analyses were performed on a Perkin-

Elmer F-11 gas chromatograph equipped with a flame-ionization detector (FID) and a split restrictor needle assembly for venting the carrier gas flow. Two different glass capillary columns were tested, 50 m × 0.3 mm I.D. coated with OV-101 and 35 m × 0.3 mm I.D. coated with SE-30 containing Silanox 101. The glass capillary columns were prepared as described by Szafranek *et al.*[13].

Preparation of O-methyloximes and trimethylsilylation

A modified method described by Mason and Slover[4] for the preparation of sugar oximes was used. To the sample containing 0.5–3 mg of fructose or other monosaccharide was added 1 mg of aqueous glucitol solution as an internal standard. The sample was evaporated to dryness at 40°, then 1 ml of a 25 mg/ml solution of methoxylammonium chloride in pyridine was added and the mixture was heated at 70–80° for 1 h. Then 2–3 drops of benzene were injected and the solvent was removed by evaporation at 60°. The mixture of sugar O-methyloximes was dissolved in 0.5 ml of dry pyridine and silylated by adding 0.5 ml of pyridine–HMDS–TMCS (1:0.9:0.6). The solution was allowed to stand for 1 h, then centrifuged. To 0.3 ml of the clear supernatant was added 0.3 ml of cyclohexane in order to promote the silylation of carbohydrates[14]. Finally, 0.1 ml of BSTFA was injected to protect O-methyloxime trimethylsilyl (MO-TMS) derivatives from air moisture. Samples prepared in this way can be kept for 3–4 weeks.

Calculations

The peak areas were measured by triangulation. The relative response factor or relative sensitivity of the MO-TMS derivative of fructose with respect to TMS-glucitol was determined experimentally.

RESULTS AND DISCUSSION

Quantitative determination of fructose

For the quantitative determination of fructose by GLC as the MO-TMS derivative, a good separation of the *syn* and *anti* forms is required. The separation of these two forms achieved on the glass capillary column coated with OV-101 is shown in Fig. 1. Glucitol was used as an internal standard.

For the FID (*i.e.*, a mass-sensitive detector) the amount of fructose in the sample was calculated as follows:

$$W_F = \frac{W_G \cdot A_{F/G}}{S_{F/G}}$$

where W_F is the weight of fructose, W_G is the weight of the internal standard (glucitol), $A_{F/G}$ is the area of both peaks of fructose relative to the peak area of glucitol and $S_{F/G}$ is the relative response factor.

The relative response factor, $S_{F/G}$, was measured for mixtures containing various amounts of fructose and a fixed amount of glucitol, which were derivatized and analysed under identical conditions. Good linearity of the detector response was found for weight ratios of fructose to glucitol from 0.4 to 3.5 (Fig. 2). The slope of the graph was calculated by the method of least squares. The value of $S_{F/G} = 0.80$ was

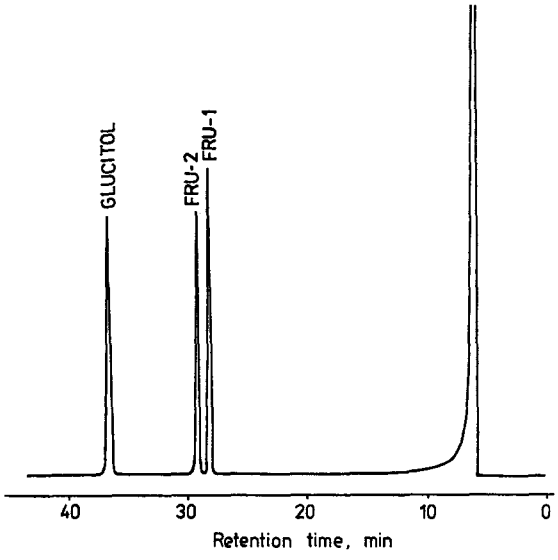

Fig. 1. Chromatogram of TMS ethers of O-methyloximes of fructose and glucitol. Column, 50 m, coated with OV-101; temperature, 175° (isothermal); carrier gas, helium at a flow-rate of 0.85 ml/min. FRU = fructose.

nearly identical with the correction factor found for TMS ethers of fructose oxime with respect to TMS-inositol[6]. The recovery of added amounts of fructose evaluated for several samples ranged from 94% to 107%.

Separation of fructose in mixtures with other monosaccharides

Fig. 3 shows a gas chromatogram of a mixture of fructose and some other monosaccharides. Three of the sugars (2-deoxyribose, arabinose and 2-deoxyglucose) gave single peaks under the GLC conditions used. Rhamnose, galactose, glucose and fructose each gave well separated double peaks that were easily distinguishable. Table I

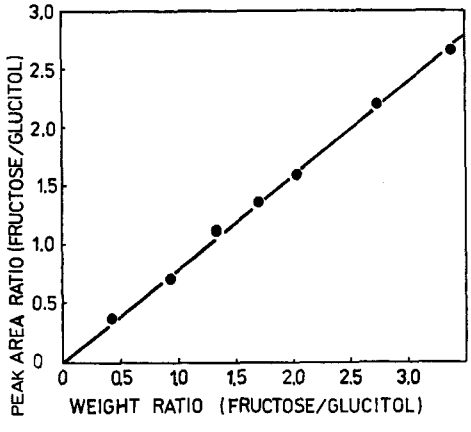

Fig. 2. GLC response plots for fructose in the form of MO-TMS ethers with respect to TMS-glucitol used as an internal standard.

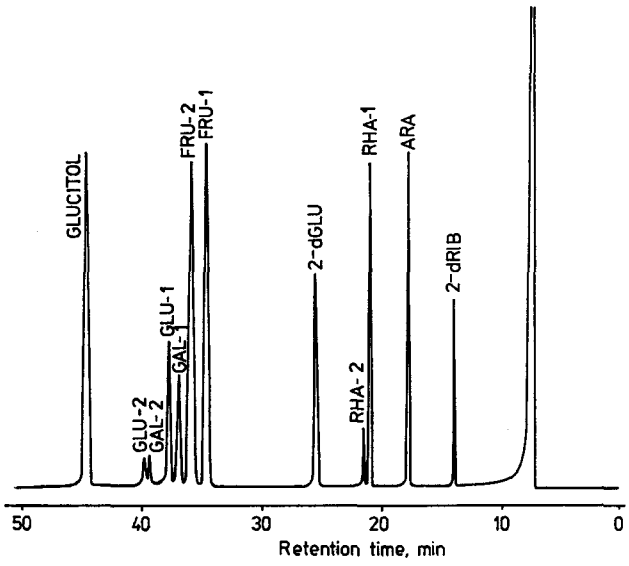

Fig. 3. Chromatogram of TMS ethers of O-methyloximes of a mixture of monosaccharides. Column as in Fig. 1; temperature, 175°; carrier gas, argon at a flow-rate of 0.7 ml/min. 2-dRIB = 2-deoxyribose; ARA = arabinose; RHA = rhamnose; GAL = galactose; GLU = glucose.

summarizes the retention data for different monosaccharides tested on OV-101 and SE-30 columns. The retention data are presented either as Kováts retention indices, *I*, or as relative retention times, *t*, with respect to glucitol. For the determination of retention indices, a solution of *n*-alkanes ($C_{15}H_{32}$ to $C_{20}H_{42}$) was prepared in *n*-octane and co-injected with the sample.

To illustrate the applicability of the capillary columns to the separation of

TABLE I

RETENTION DATA FOR TMS DERIVATIVES OF MONOSACCHARIDES ON OV-101 AND SE-30 COLUMNS

Derivative	*OV-101, 175°*		*SE-30, 165°*	
	*I**	*t***	*I**	*t***
2-Deoxyribose O-methyloxime	1570	0.31	1570	0.28
Arabinose O-methyloxime	1680	0.39	1690	0.36
Rhamnose O-methyloxime-1	1750	0.46	1755	0.43
Rhamnose O-methyloxime-2	1760	0.48	1760	0.44
2-Deoxyglucose O-methyloxime	1825	0.57	1830	0.54
Fructose O-methyloxime-1	1925	0.77	1935	0.76
Fructose O-methyloxime-2	1935	0.80	1950	0.79
Galactose O-methyloxime-1	1945	0.82	1955	0.81
Galactose O-methyloxime-2	1965	0.88	1975	0.87
Glucose O-methyloxime-1	1950	0.84	1960	0.83
Glucose O-methyloxime-2	1970	0.89	1980	0.88
Glucitol	2005	1.00	2020	1.00

* Kováts retention index.

** Retention time relative to glucitol.

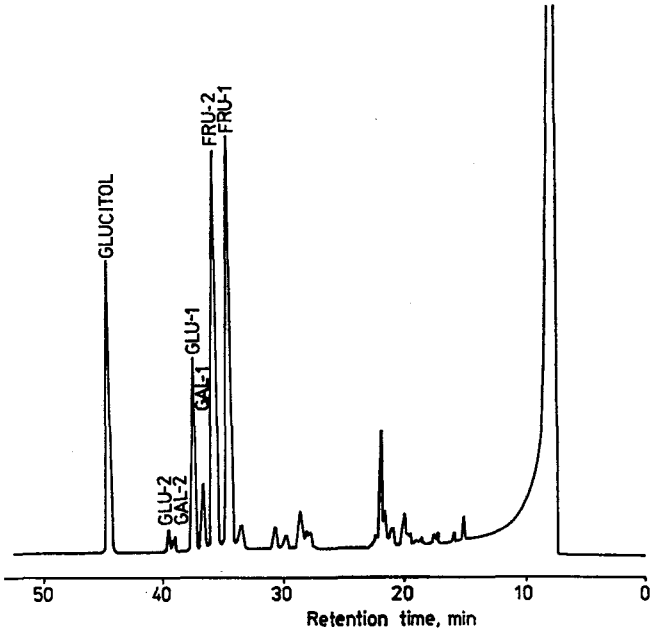

Fig. 4. Chromatogram of TMS ethers of the products of raffinose radiolysis analysed as O-methyloximes. GLC conditions and abbreviations as in Fig. 3.

MO-TMS derivatives of sugars, the monosaccharide fraction of the products of raffinose radiolysis was separated, and the results are presented in Fig. 4.

Raffinose was irradiated with a dose 0.6 Mrad in deaerated aqueous solution (10^{-2} *M*). To 20 ml of the irradiated solution 1 ml of aqueous glucitol solution (1 mg/ml) was added. The MO-TMS derivatives were prepared as described under Experimental. Fructose is the major product of raffinose radiolysis, in addition to glucose, galactose and 6-deoxyhexose, which could be identified from their retention times.

It is evident from the results that the proposed method permits the separation and quantitative determination of complex mixtures of monosaccharides.

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REFERENCES

1 C. C. Sweeley, R. Bentley, M. Makita and W. W. Wells, *J. Amer. Chem. Soc.*, 85 (1963) 2497.
2 R. A. Laine and C. C. Sweeley, *Carbohydr. Res.*, 27 (1973) 199.
3 R. A. Laine and C. C. Sweeley, *Anal. Biochem.*, 43 (1971) 533.
4 S. Mason and H. T. Slover, *J. Agr. Food Chem.*, 19 (1971) 551.
5 G. Eklund, B. Josefsson and C. Roos, *J. Chromatogr.*, 142 (1977) 575.

6 G. Gerwig, J. P. Camerling and J. F. G. Vliegenthart, *Carbohydr. Res.*, 62 (1978) 349.
7 M. Dizdaroglu and C. von Sonntag, *Z. Naturforsch. B.*, 28 (1973) 635.
8 M. Dizdaroglu, D. Henneberg, G. Schomburg and C. von Sonntag, *Z. Naturforsch. B.*, 30 (1975) 416.
9 M. Dizdaroglu, J. Leitich and C. von Sonntag, *Carbohydr. Res.*, 47 (1976) 15.
10 H. Żegota and C. von Sonntag, *Z. Naturforsch. B.*, 32 (1977) 1060.
11 S. Adam, *Int. J. Radiat. Isotopes*, 28 (1977) 903.
12 G. Peterson, *Carbohydr. Res.*, 33 (1974) 47.
13 J. Szafranek, A. Wisniewski, J. Gajdus and J. Kusmierz, *J. Chromatogr.*, 168 (1979) 445.
14 D. J. Leblanc and A. J. S. Ball, *Anal. Biochem.*, 84 (1978) 574.