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Note

Multi-step time program for the rapid gas–liquid chromatography of carbohydrates

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Following previously used methods[1,2], the gas–liquid chromatography (GLC) of carbohydrates ranging from C_4 compounds to tetrasaccharides resulted in retention times far longer than 1 h. A reduction in retention time can be achieved by (i) a more rapid temperature program, (ii) high initial temperatures, (iii) higher final temperatures and (iv) the use of more volatile derivatives, but none of these possibilities alone can be applied without causing difficulties. High final temperatures tend to exaggerate column bleeding, higher heating rates can result in a poor resolution of components and high initial temperatures and the use of more volatile derivatives[3,4] give inadequate separations of early emerging substances such as pentoses and tetroses from solvent peaks.

By using a reasonably high initial temperature, a thermally stable stationary phase, not too volatile derivatives and a multi-step time program, excellent resolution of numerous components and short analysis times were achieved.

EXPERIMENTAL

Materials and equipment

N,O-Bis(trimethylsilyl)acetamide (BSA) was obtained from Pierce (Rockford, Ill., U.S.A.). Pyridine was of silylation grade and carbohydrates were obtained from various commercial sources. Phenyl β-D-glucopyranoside (Fluka, Buchs, Switzerland) was used as an internal standard.

Analysis was carried out on a Hewlett-Packard 5835-A terminal-operated gas chromatograph with flame-ionization detectors using nitrogen as the carrier gas (flow-rate 20 ml/min) on 6 ft. × 2.0 mm I.D. glass columns equipped for on-column injection (all-glass system). The stationary phase was 3% Dexsil 300 GC (Applied Science Labs., State College, Pa., U.S.A.) on Chromosorb W AW DMCS (80–100 mesh). The injector and detector temperatures were 275° and 345°, respectively. The initial temperature was 160°. The temperature program was 10°/min for 8 min, 30°/min for 3.66 min, isothermal at 350° for 11.34 min. The run time was 23 min. The injection frequency (run time plus cooling time) was 32.5 min.

Reactions were carried out in 0.2-ml vials (made from ordinary glass-tubing) fitting 1-ml glass flasks (Hewlett-Packard, 62311-S29) and capped with PTFE–rubber

laminated seals (Hewlett-Packard, 5080-8713/1540-0132). Samples of 5 μl were injected using a Hewlett-Packard 7671-A Automatic Sampler (Hamilton 701 syringe).

Derivatization procedure

Approximately 0.5–1.0 mg of sugar mixture in a 200-μl vial was dissolved in 70 μl of pyridine containing 0.21 mg of phenyl β,D-glucopyranoside as internal standard. After 5 min, 130 μl of BSA were added. The vial was capped immediately and kept at room temperature for 30 min. Reactions were complete by that time and the mixture was stable for at least 9 h at room temperature. A 5-μl volume of the reaction product was taken for analysis.

RESULTS

BSA, which is normally used as a silylating agent for organic acids, was introduced into sugar analysis[5] in order to simplify the procedures involved as the GLC separation of both organic acids and carbohydrates is carried out simultaneously on separate instruments in our laboratory. As BSA derivatives of sugars are only moderately volatile, Dexsil, a thermally stable stationary phase which can be heated to 350° without severe column bleeding, was used in detection of tetrasaccharides.

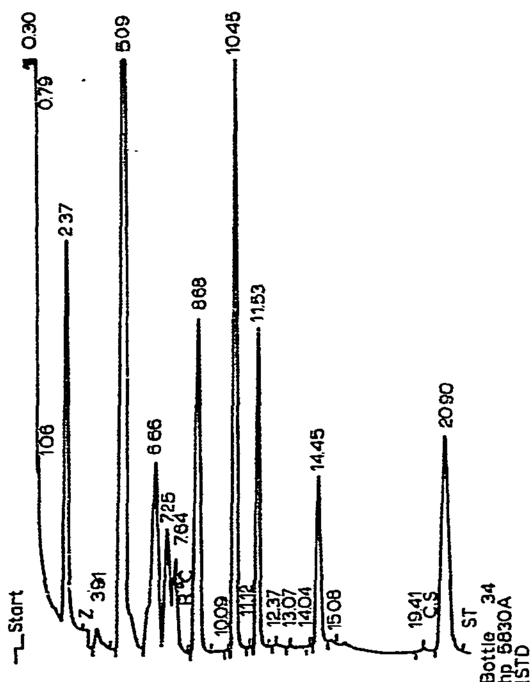

Fig. 1. GLC separation of carbohydrates. Initial temperature, 160°; heating rate, 10°/min for 8 min, 30°/min for 3.66 min; final temperature, 350° (held for 11.34 min); carrier gas (nitrogen) flow-rate, 20 ml/min. Column: 6 ft. × 2.0 mm I.D. all-glass system; 3% Dexsil on Chromosorb W AW DMCS (80–100 mesh). Retention times (min) of peaks: 2.37, erythritol; 5.09, ribose; 6.66, fructose; 7.25, α-glucose; 7.64, β-glucose; 8.68, inositol; 10.45, phenyl β-D-glucopyranoside; 11.53, sucrose; 14.45, raffinose; 20.90, stachyose.

Using our former GLC conditions (initial temperature 130°, heating rate 10°/min, final temperature 350°), the retention times obtained were still too long. Therefore, the initial temperature was raised to 160° and the heating rate was changed to 30°/min. This reduced the retention times of tetrasaccharides to less than 20 min, but had an adverse effect on early emerging components, as could be foreseen. As there was evidence that with a one-phase temperature program satisfactory separation of C_4 and C_5 compounds on the one hand and shorter retention times for tetrasaccharides on the other could not be obtained, a multi-phase time program was adopted.

Starting at 160° with a heating rate of 10°/min minimized the influence of solvent peak tailing on erythritol (Fig. 1). Such a good resolution of a C_4 compound was due to the lower volatility of the BSA derivatives compared with that of the solvent. The low heating rate also improved the separation of all C_6 compounds. After 8 min, elution of all slowly emerging compounds were speeded up by adopting a heating rate of 30°/min. Finally, an isothermal phase at 350° yielded retention times for stachyose close to 21 min without damaging the column. The flat base-line should be noted. After 23 min the heating was shut off and the column oven cooled to the initial temperature. After thermal equilibration, a new run was started automatically. The total run time (heating period, cooling and equilibration) was 32.5 min, allowing 15 runs to be carried out during the time period when the derivatives remained stable (9 h). More than 600 samples have been analysed with the method described without any signs of ageing of the column. Relative retention times of a number of compounds are given in Table I and absolute retention times are shown in Fig. 1.

TABLE I

RELATIVE RETENTION TIMES OF CARBOHYDRATES

RRT = mean relative retention time; n = number of observations; S.D. = standard deviation.

Compound	*RRT*	*n*	*S.D.*
Erythritol	0.228	7	0.0008
α-Xylose	0.450	1	—
β-Xylose	0.510	1	—
Ribose	0.488	7	0.0008
α-Arabinose	0.460	1	—
β-Arabinose	0.530	1	—
Fructose	0.638	12	0.0031
α-Glucose	0.694	12	0.0027
β-Glucose	0.730	12	0.0028
Sedoheptulose	0.710	1	—
Inositol	0.831	12	0.0018
Phenyl β-D-glucopyranoside (internal standard)	1.000	12	0.0131
Sucrose	1.103	12	0.0007
Arbutine	1.131	1	—
Trehalose	1.152	1	—
Raffinose	1.383	12	0.0010
Stachyose	2.000	7	0.0029

As for the wide molecular range the method is well suited for application on complex carbohydrate mixtures like plant extracts and other biological fluids.

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