Journal of Chromatography, 261 (1983) 320–323
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 15,713

Note

Gas–liquid chromatographic determination of hydroxy carboxylic acids on a fused-silica capillary column

TAPANI HYPPÄNEN*, EERO SJÖSTRÖM and TAPANI VUORINEN
Laboratory of Wood Chemistry, Helsinki University of Technology, SF-02150 Espoo 15 (Finland)
(Received December 24th, 1982)

In conjunction with our studies on the oxidative alkaline degradation of carbohydrates, a reasonably simple method was needed for analysis of the reaction products (hydroxy carboxylic acids) in the presence of unreacted sugars. The method suggested by Petersson[1] was potentially attractive because in this the acids are converted into their trimethylsilyl derivatives as sodium salts and lactone peaks can thus be eliminated from the gas chromatogram. However, the solubility of sodium salts in the silylation reagent is low, occasionally causing difficulties in the determination. We now report a modification of this method in which the acids are converted into the more soluble ammonium salts by use of weakly acidic cation-exchange resin before silylation. The method has been applied to mixtures obtained after oxidation of glucose with oxygen. Capillary gas–liquid chromatography (GLC) was used for separation.

EXPERIMENTAL

Materials

D-Erythrono-1,4-lactone was obtained from D-arabinose[2] and recrystallized from acetic acid. Sodium D-arabinonate was prepared from D-glucose by oxygen oxidation in alkaline methanol–water solution[3] and recrystallized from 80% methanol. All the other chemicals were commercial products of analytical grade or the highest available purity. The weakly acidic cation-exchange resin was Amberlite IRC-50 (35–60 mesh, standard grade).

Ion exchange

Known amounts of alkaline reaction solution containing 1–100 mg of sugars and hydroxy carboxylic acids and a solution of the internal standard (D-xylitol) were mixed. The mixture was eluted through a column (10 mm I.D.) of weakly acidic cation-exchange resin (H^+; upper layer, 2 ml) and the same resin (NH_4^+; lower layer, 2 ml). The column was washed with 20 ml water.

In the determination of the response factors for the pure compounds, the solutions were made alkaline with 0.1 *M* sodium hydroxide 2 min before ion exchange to hydrolyze the lactones and esters.

0021-9673/83/$03.00

Preparation of trimethylsilyl derivatives

An aliquot of the effluent containing 1–5 mg of sugars and ammonium salts of hydroxy carboxylic acids was evaporated to dryness at reduced pressure at 35°C. A few glass balls, 0.5 ml pyridine and 0.25 ml of trifluorobis(trimethylsilyl)acetamide (BSTFA) containing 5% of chlorotrimethylsilane (TMCS) were added and the mixture was shaken for at least 30 min at room temperature.

Gas–liquid chromatography

The per(trimethylsilylated) compounds were analyzed with a Hewlett-Packard 5880 A gas chromatograph equipped with a flame ionization detector and an OV-101 (or OV-1701) fused-silica capillary column (25 m × 0.32 mm I.D.). The oven temperature was held for 2 min at 100°C, raised at 20°C/min to 200°C and held for 5 min at 200°C. The injection port and manifold were kept at 260°C. The injection volume was 0.1–1 μl and the splitting ratio was 20:1. The flow-rate of hydrogen carrier gas was 2.5 ml/min.

RESULTS AND DISCUSSION

When the sample solution was passed through the first resin bed layer (hydrogen form) only a part of the sodium salts was converted into free acids (effluent pH 4.0–4.5) and no significant lactonization took place. After passage through the second resin bed layer (ammonium form) the free acids and sodium salts were completely converted into ammonium salts (effluent pH 8.5–9.0). During evaporation, the pH of the solution decreased slightly below 7 but negligible lactonization was observed.

In separate experiments it was shown that the presence of TMCS in the silylation reagent was necessary because otherwise partial lactonization of acids and con-

TABLE I

MOLAR RESPONSE FACTORS OF HYDROXY CARBOXYLIC ACIDS AND GLUCOSE RELATIVE TO XYLITOL ON AN OV-101 COLUMN

The respective standard deviations have been calculated from at least four replicate determinations at different substrate concentrations.

Compounds	*Response factor*	
	Calc.[4,5]	*Found ± S.D.*
Lactic acid	0.42	0.43 ± 0.01
Glycolic acid	0.37	0.38 ± 0.01
Oxalic acid	0.32	0.21 ± 0.01
Glyceric acid	0.56	0.56 ± 0.02
Tartronic acid	0.52	0.49 ± 0.01
Erythronic acid	0.76	0.76 ± 0.02
Ribonic acid	0.96	0.92 ± 0.02
Arabinonic acid	0.96	0.93 ± 0.02
Mannonic acid	1.15	1.05 ± 0.03
Gluconic acid	1.15	1.05 ± 0.03
Glucose	0.99	0.89 ± 0.02

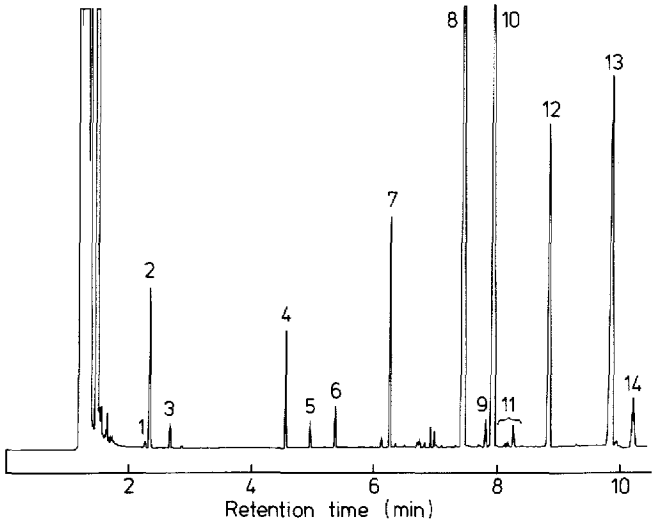

Fig. 1. Separation of per(trimethylsilylated) oxidation products of D-glucose on an OV-101 column. Peaks: 1 = lactic acid; 2 = glycolic acid; 3 = oxalic acid; 4 = glyceric acid; 5 = tartronic acid; 6 = 2-deoxytetronic acid; 7 = erythronic acid; 8 = xylitol (internal standard); 9 = ribonic acid; 10 = arabinonic acid; 11 = fructose; 12 = glucose; 13 = glucose and mannonic acid; 14 = gluconic acid.

version of neutral sugars occurred. Addition of 5% TMCS completely eliminated these undesirable reactions.

The trimethylsilylation was completed in less than 30 min. The relative molar responses determined for pure compounds on an OV-101 column differed somewhat (normally less than 10%) from the calculated[4,5] (Table I). In contrast, when an OV-1701 column was used the response factors were markedly lower. It is obvious that the trimethylsilyl derivatives are partially hydrolyzed on OV-1701, and therefore

TABLE II

COMPOSITION (mol% OF STARTING MATERIAL) OF AN OXYGEN OXIDATION PRODUCT MIXTURE OF D-GLUCOSE

The mean values and standard deviations have been calculated from four replicate determinations.

Compound	*Mean ± S.D.*
Glycolic acid	6.59 ± 0.09
Oxalic acid	2.05 ± 0.33
Glyceric acid	3.24 ± 0.03
Tartronic acid	1.02 ± 0.04
2-Deoxytetronic acid*	1.06 ± 0.01
Erythronic acid	4.64 ± 0.06
Ribonic acid	0.62 ± 0.01
Arabinonic acid	57.29 ± 0.51
Gluconic acid	1.57 ± 0.01
Unreacted glucose	25.81 ± 0.72

* The calculated[4,5] response factor has been used.

its use is not recommended, although better separation would occasionally be obtained.

The standard deviations of the response factors (at least four determinations at different concentrations) were usually 2–3% (*cf.*, Table I). In replicate (four) determinations of an oxidation product mixture of glucose (Fig. 1) the reproducibility of the method was often even better (Table II). For dicarboxylic acids, however, considerably higher standard deviations were recorded.

ACKNOWLEDGEMENTS

Financial support from the Academy of Finland is gratefully acknowledged.

REFERENCES

1 G. Petersson, *Carbohyd. Res.*, 33 (1974) 47–61.
2 W. B. Gleason and R. Barker, *Can. J. Chem.*, 49 (1971) 1425–1432.
3 T. Hyppänen, E. Sjöström and T. Vuorinen, in preparation.
4 R. G. Ackman, *J. Gas Chromatogr.*, 2 (1964) 173–179.
5 L. A. Th. Verhaar and H. G. J. de Wilt, *J. Chromatogr.*, 41 (1969) 168–179.