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SEPARATION AND DETERMINATION OF SOME ORGANIC ACIDS AND THEIR SODIUM SALTS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

A liquid chromatographic method for the determination of gluconic acid is presented. The method has been used for the determination of gluconic acid and sodium gluconate as well as other acids formed in biochemical or catalytic oxidations of glucose. The acids are separated on a column of cation-exchange resin and eluted with dilute sulphuric acid. The effluent is monitored by an ultraviolet detector at 210 nm. The only pretreatment necessary is filtration of the samples through a membrane in order to prolong column life. Other column materials such as amino and hexyl phases have also been tested. The amino column is suitable for the determination of gluconates. The properties of the hexyl phase column make it especially useful for the separation of citric acid cycle acids.

INTRODUCTION

Gluconic acid as well as its salts and lactones are mild non-corrosive, non-toxic organic compounds. They have important industrial uses primarily due to their ability to form chelates with metal ions in caustic solutions. They are physiologically compatible and can therefore be used in foodstuffs without risk.

The oxidation of carbohydrates has been widely studied, but quantitative and even qualitative analytical data are often lacking[1–3]. However, some analytical methods for the separation of organic acids have been reported. Many of these are related to the determination of the acids of the citric cycle. Samuelson and co-workers[4–8] have reported the analysis of aldonic acids by chromatography on a strong basic anion-exchange resin. Recently, other workers[9–12] have used a high-performance liquid chromatographic (HPLC) procedure which is based on the principle of ion exclusion and partition. Gas chromatography has also been used for the determination of organic acids. However, since most organic acids are not sufficiently volatile, the preparation of suitable volatile derivatives is required. The most frequently used derivatives involve the conversion of carboxyl groups into methyl esters or trimethylsilyl esters and of hydroxyl groups into trimethylsilyl ethers[12–15].

In this paper is described a liquid chromatographic procedure which separates gluconic acid from the other acids which are formed in the biochemical conversion of glucose into gluconic acid. This method has also been used for the determination of

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arabinonic and formic acids which are formed in the chemical oxidation of glucose in alkaline media.

The method is based on ion exclusion and partition chromatography with UV monitoring of the column effluent at 210 nm. The acids are separated on a column packed with a strong cation-exchange resin (sulphonated polystyrene–divinylbenzene copolymer, H^+) using dilute sulphuric acid as eluent. Some alternative column materials for organic acid analysis are also presented.

EXPERIMENTAL

Apparatus

A Varian 5000 Series liquid chromatograph (Varian Aerograph, Walnut Greek, CA, U.S.A.) was used together with a Perkin-Elmer LC 75 variable-wave-

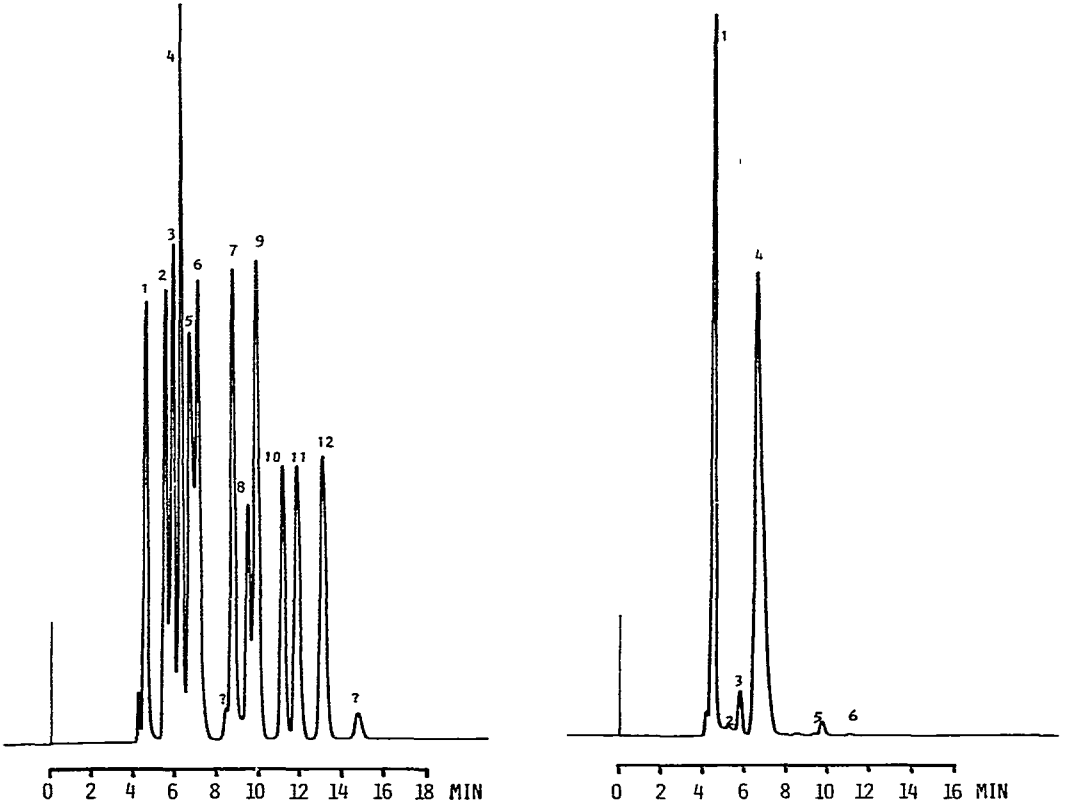


Fig. 1. Separation of standard acid mixture. Chromatographic conditions: column, Aminex HPX-87, 9 μm, 300 × 7.8 mm I.D., temperature 65°C; UV monitoring at 210 nm, 0.16 absorbance units full scale; eluent, 0.006 *N* H_2SO_4 at flow-rate 0.8 ml/min. Peaks: 1 = oxalic; 2 = maleic; 3 = citric; 4 = tartaric; 5 = gluconic; 6 = malic; 7 = succinic; 8 = lactic; 9 = glutaric; 10 = acetic; 11 = levulinic; 12 = propionic acid.

Fig. 2. Chromatogram of the products formed in biochemical oxidation of D-glucose. Conditions as in Fig. 1. 1 = oxalic; 2 = maleic; 3 = citric; 4 = gluconic; 5 = unknown; 6 = acetic acid.

length spectrophotometric detector (Perkin-Elmer, Norwalk, CT, U.S.A.) and a Goerz Servogor 321 recorder (Goerz Electro, Vienna, Austria). The columns were as follows: self-packed Hamilton HC-X8.00 strong cation-exchange resin column, H^+ (particle size 10–15 μm, 500 × 9 mm I.D.); Aminex HPX-87 strong cation-exchange resin column, H^+ (particle size 9 μm, 300 × 7.8 mm I.D.) (Bio-Rad Labs, Richmond, CA, U.S.A.); self-packed Nucleosil 10 NH_2, particle size 10 μm (250 × 4.6 mm I.D.) and self-packed Spherisorb hexyl (5 μm, 170 × 6.2 mm I.D.). The Rheodyne 7125 injector was provided with 20-μl loop (Rheodyne, Cotati, CA, U.S.A.).

Reagents

Dilute sulphuric acid solutions were prepared by making appropriate dilutions of reagent grade sulphuric acid (Lääketehdas Orion, Espoo, Finland) in ultra pure water. Acetonitrile (Art. 30) was obtained from E. Merck (Darmstadt, G.F.R.) and the analytical grade acids (minimum purity 99 %) were obtained from Fluka (Buchs, Switzerland), BDH (Poole, Great Britain) and E. Merck.

Liquid chromatographic separation and quantitation

Standards of the acids were prepared individually in ultra pure water and chromatographed separately in order to determine the retention time for each acid. The acids were then chromatographed as a mixture (Fig. 1).

The samples were diluted to about 1 g/100 ml and filtered through a 0.22-μm membrane. This solution was injected into the chromatographic system. Three different columns were used for the analysis of oxidation products of D-glucose. Two of

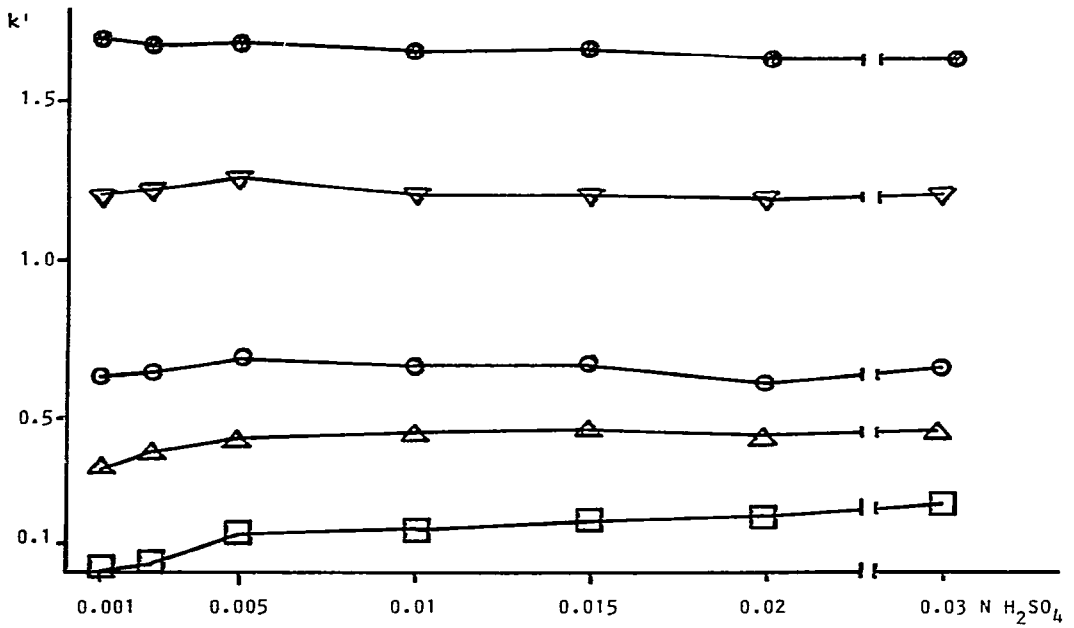


Fig. 3. Effect of sulphuric acid concentration on retention of some organic acids. Column: Aminex HPX-87. Flow-rate: 1.0 ml/min. Temperature: 75°C. Acids: □, oxalic; △, citric; ○, gluconic; ▽, glycolic; ●, acetic.

these were packed with strong cation-exchange resins (Hamilton HC-X8.00 and Aminex HPX-87, both H^+) and operated at elevated temperatures (338–353°K) using dilute sulphuric acid as eluent at flow-rates of 0.8–1.5 ml/min (see Fig. 2). The amino column (Nucleosil 10 NH_2), with phosphate buffer–acetonitrile (75:25) as eluent at a flow-rate of 2 ml/min, was operated at room temperature.

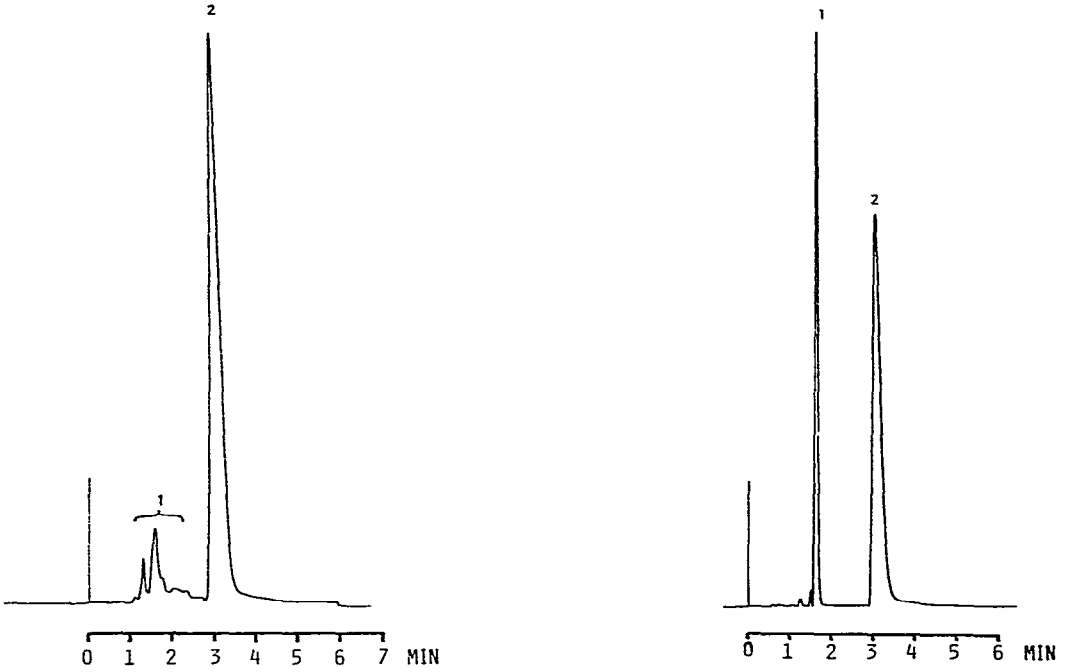


Fig. 4. Determination of sodium gluconate formed in biochemical oxidation of D-glucose. Chromatographic conditions: column, Nucleosil 10 NH_2, 10 μm, 250 × 4.6 mm I.D., ambient temperature; eluent, 0.01 *M* KH_2PO_4 (pH 2.5)–acetonitrile (75:25), flow-rate 2 ml/min; UV detection at 210 nm, 0.16 a.u.f.s. Peaks: 1 = unknowns; 2 = sodium gluconate.

Fig. 5. Separation of glucono delta lactone (1) and gluconic acid (2). Chromatographic conditions as in Fig. 4.

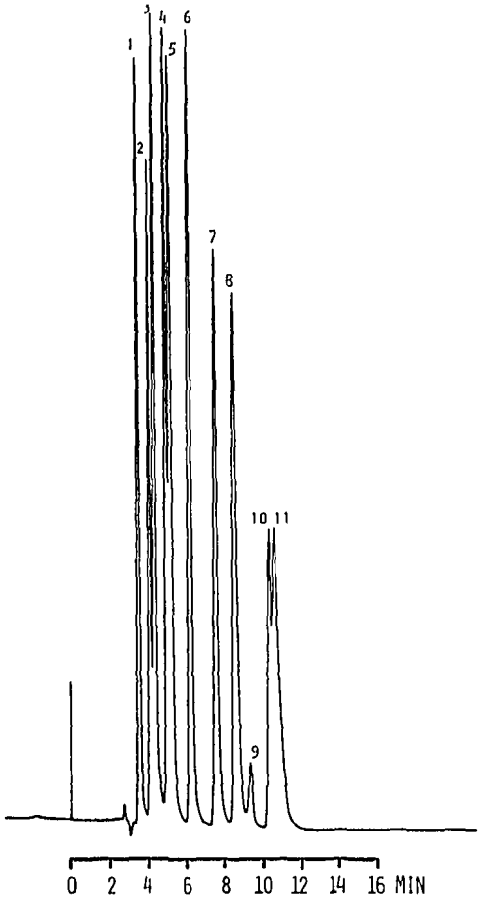
For the separation of organic acids, a hexyl phase column (Spherisorb hexyl) was also tested. The eluent was dilute phosphoric acid at a flow-rate of 1 ml/min.

In all cases the column effluents were monitored by an UV spectrophotometric detector at 210 nm. Peak height measurements with an external standard were used for quantitation.

RESULTS AND DISCUSSION

Effect of eluent concentration on capacity factors of some acids

The concentration of the eluent (sulphuric acid) seems to have only a slight



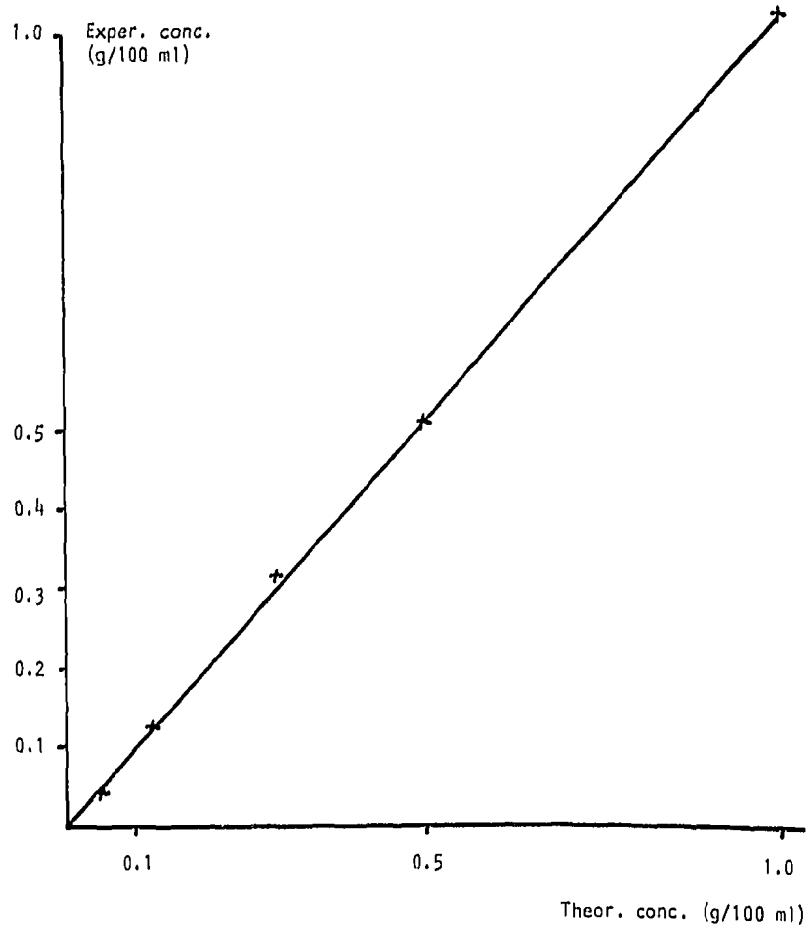


Fig. 6. Separation of standard acid mixture. Chromatographic conditions: column, 5 μm Spherisorb hexyl, 170 × 6.2 mm I.D., ambient temperature; eluent, 0.01 *N* H_3PO_4, flow-rate 1.0 ml/min; UV detection at 210 nm, 0.16 a.u.f.s. Peaks: 1 = tartaric; 2 = malic; 3 = maleic; 4 = acetic; 5 = citric; 6 = succinic; 7 = unknown; 8 = propionic; 9 = unknown; 10 = glutaric; 11 = levullinic acid.

Fig. 7. Linearity curve for sodium gluconate. Column: Aminex HPX-87, temperature 65°C. Eluent: 0.015 *N* H_2SO_4, flow-rate 1.0 ml/min. Detection: UV at 210 nm.

TABLE I

DETERMINATION OF SODIUM GLUCONATE IN FERMENTATION SAMPLES USING THREE DIFFERENT COLUMNS

Sample	*Sodium gluconate content (%) on dry substance*		
	Aminex HPX-87	*Hamilton HC-X8.00*	*Nucleosil 10 NH_2*
1	94.4	94.0	94.5
2	91.9	91.2	91.5
3	87.2	87.0	86.8
4	92.0	91.8	91.3
5	88.6	88.3	88.3

effect on the retention time of gluconic acid sodium salt (see Fig. 3) on a column of strong cation-exchange resin. The effect of eluent composition on the resolution and retention times of the acids on NH_2 or hexyl columns has not yet been fully investigated (see Figs. 4–5). However, these silica-based materials offer different selectivities from that of styrene-based strong cation-exchange resins.

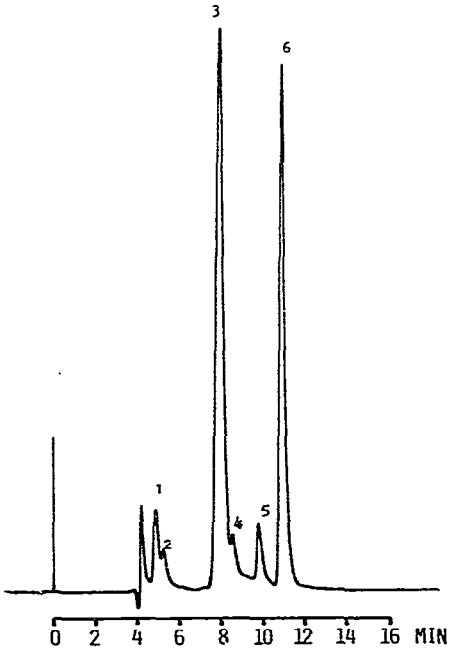


Fig. 8. Separation of oxygen-oxidation products of D-glucose (alkaline solution). Conditions: column, Hamilton HC-X8.00, 10–15 μm, 500 × 9 mm I.D., temperature 80°C; eluent, 0.003 *N* H_2SO_4, flow-rate 1.5 ml/min; UV detection at 210 nm. Peaks: 1 = oxalic; 2 = unknown; 3 = arabinonic; 4 = ribonic; 5 = glycolic; 6 = formic acid.

Detection limits and linearity

The detectable amount of gluconic acid sodium salt is 0.3–0.5 μg at 210 nm, but 0.1–0.2 μg at 210 nm.

The quantitation and linearity of the method were tested by means of a series of standard sodium gluconate solutions. Glycolic acid was used as internal standard (Fig. 7). The results for three different columns are shown in Table I.

Column life

The Hamilton HC-X8.00 column is very durable. It has been used for routine analysis of sodium gluconate, gluconic acid and arabinonic acid samples for 14 months without any noticeable deterioration of the column performance (see Fig. 8).

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