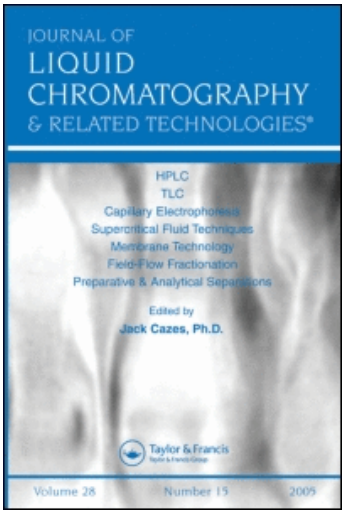

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Analysis of Mixtures of Glucose, Fructose and Mannose by HPLC

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ANALYSIS OF MIXTURES OF GLUCOSE, FRUCTOSE AND MANNOSE BY HPLC

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ABSTRACT

A reliable HPLC analysis of glucose-fructose-mannose mixtures, occurring in the commerical alkali catalysed production of High Fructose Syrup (HFS) from glucose is developed. As stationary phase a mechanically stable, cheap and easily available unmodified silica is used. The mobile phase is acetonitrile containing 0.1% w of water. The separation is complete within 25 minutes, at a pressure drop of 100 bar over the column.

INTRODUCTION

A commericial route for the production of High Fructose Syrup (HFS) from glucose is by alkali catalysed isomerization. The main monosaccharide by-product is mannose. To control the process it is important to have a reliable method of analysis of the glucose-fructose-mannose mixture. In the literature various systems for the analysis of mixtures of monosaccharides by liquid chromography are given (1-6), all having certain disadvantages. In some systems ion exchange resins are used as the stationary phase (1-3). This has the disadvantage that the allowable pressure drop is relatively small. Others apply modified silica (4,5). This is mechanically stable, but with the mobile phases indicated in the literature the separation of especially the mannose from glucose and fructose is poor

(own measurements). In one publication the use of unmodified silica is indicated (6). No information is given on the separation of mannose from glucose and fructose, however.

We have developed a reliable HPLC analysis of a glucose-fructose-mannose mixture, using cheap, easily available and mechanically stable unmodified silica as the stationary - and acetonitril containing a small amount of water as the mobile phase. A separation is complete within 25 minutes at a pressure drop of 100 bar over the column.

EXPERIMENTAL

A Du Pont 820 liquid chromatograph was used with a thermostated differential refractometer detector (Fresnel system). The stainless steel column was 250 mm long and had an I.D. of 2.1. mm. It was placed in the thermostat bath of the chromatograph.

The stationary phase was Lichrosorb SI 60 (5 µm particles, ex-Merck). The column had been packed by the "balanced slurry" method, using as slurry medium a mixture of tetrabromoethane, carbon tetrachloromethane and dioxane (2 : 1 : 1). Samples were injected by a Rheodyne injection valve, model 70-10, using a 20 µl loop.

The mobile phase was acetonitril, in which the water concentration was varied in the experiments (0.1-1.0% w). After a change in water content, due time was allowed for the stabilization of the column. The flow rate of the mobile phase was varied between 0.1 and 0.2 ml/min.

The samples were prepared by dissolving weighed amounts of monosaccharides in water. The chemicals were purchased from Merck or Baker.

RESULTS AND DISCUSSION

The capacity factor k' of each component was determined at various temperatures and water contents of the mobile phase. Here k' is defined as:

$$k'_i = \frac{t_{R,i} - t_0}{t_0}$$

where $t_{R,i}$ is the retention time of component i and t_0 the dead time of the column. Results are given in the Table. The values of k'_i were independent of the mobile phase flow rate.

From the data it is clear that the separation between the glucose and mannose peak is the main difficulty. The separation is enhanced by a low temperature and low water content. However, at water contents below 0.1% w and temperatures below 50°C the peaks begin to tail, which reduces the efficiency of the separation. Because of this we consider 0.1% w water the minimum allowable water content. The separation between glucose and fructose is favoured by a relatively high water content. At 0.1% w water, however, the separation is still sufficient. The optimal conditions for separation of the mixture of the three monosaccharides are therefore 50°C and a water content of the acetonitril of 0.1% w. Fig. 1 gives an example of the separation under these conditions. In our column the resolution factor Rs for fructose-mannose is 2.2, for fructose-glucose 1.3, and for glucose-mannose 1.0. Here Rs is defined as

TABLE

Capacity Factors for Glucose, Fructose and Mannose at various Temperatures and Water Contents in the Mobile Phase

% w water in acetonitril	k' fructose	k' glucose	k' mannose	temperature °C
1.00	4.4	5.5	5.8	40
0.75	4.8	6.0	6.3	40
0.50	4.75	5.8	6.3	40
0.25	5.1	6.1	6.9	40
0.10	5.2	6.1	7.0	40
1.00	3.9	4.9	5.0	50
0.75	4.1	5.2	5.4	50
0.50	3.9	4.8	5.2	50
0.25	4.2	5.0	5.5	50
0.10	4.3	5.1	5.7	50
0.50	3.45	4.2	4.4	60
0.25	3.7	4.4	4.8	60
0.10	3.6	4.4	4.7	60

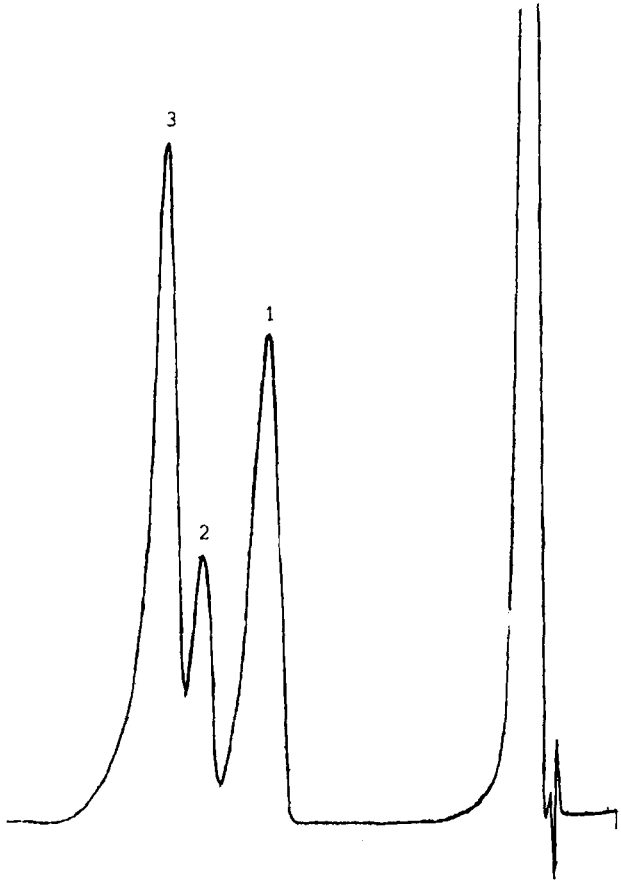

FIGURE 1.
Column: 250 x 2.1 Ø mm
Lichrosorb Si 60 5 µm
Column temperature: 50°C
Eluens: 0.1% w water in acetonitril
Eluens flow rate: 0.2 ml/min
Recorder sensitivity: 5 mV full scale
Sample: 20 µl, 6 gr/l of each monosaccharide
Detector: differential-refractometer.

Peak 1: fructose
Peak 2: glucose
Peak 3: mannose

$$Rs_{i,j} = \frac{\Delta t_{i,j}}{\bar{w}_{i,j}}$$

with $\Delta t_{i,j}$ the difference in the retention times of the components i and j and $\bar{w}_{i,j}$ the average value of the bandwidth of the two peaks.

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