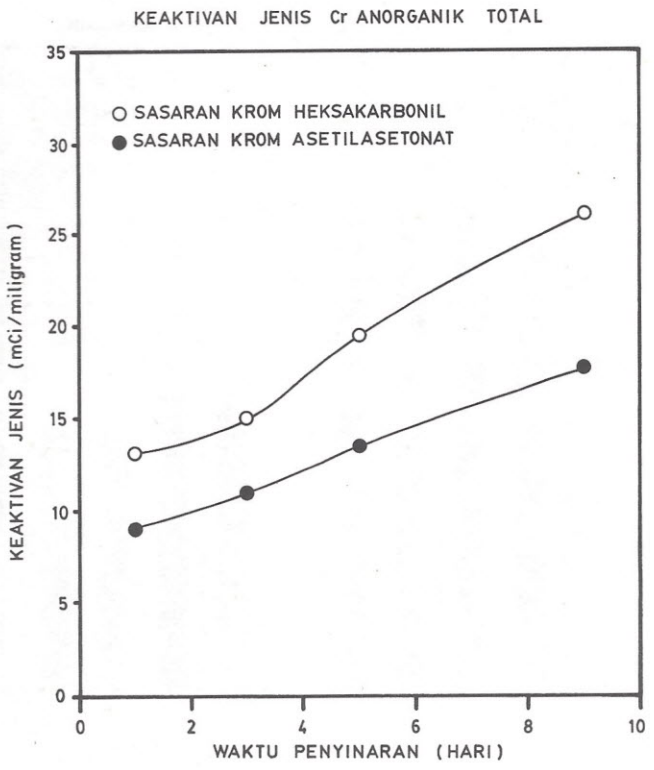

Gambar 4. Keaktivan jenis Cr-5 anorganik total.

memberikan gambaran bahwa pertukaran isotopik antara Cr*(VI) anorganik dengan $Cr(CO)_6$ berlangsung lebih mudah dibandingkan dengan pertukaran isotopik antara Cr*(III) anorganik dengan $Cr(C_5H_7O_2)_3$. Perbedaan kemudahan pertukaran isotopik tersebut dimungkinkan oleh adanya perbedaan efek ruang dan perbedaan efek elektron ikatan pada struktur molekul sasaran dimana pada $Cr(CO)_6$ atom Cr berikatan dengan atom C sedang pada $Cr(C_5H_7O_2)_3$ atom Cr berikatan dengan atom O.

Gambar 4 menunjukkan bahwa sasaran $Cr(CO)_6$ menghasilkan keaktivan jenis radiokrom anorganik secara keseluruhan lebih tinggi dari pada sasaran $Cr(C_5H_7O_2)_3$. Hal ini mudah dipahami mengingat radiokrom anorganik dari penyinaran $Cr(CO)_6$ mengandung spesi trivalen yang tinggi dengan keaktivan jenis yang tinggi pula seperti terlihat pada Gambar 2 dan Gambar 3. Di sisi lain terlihat kecenderungan semakin tingginya keaktivan jenis radiokrom anorganik yang dihasilkan dengan semakin lamanya waktu penyinaran (Gambar 3 dan Gambar 4). Namun pada pelaksanaannya ternyata penyinaran yang lebih lama akan menimbulkan pengarangan bahan sasaran. Hal ini akan mempersulit proses pemisahan karena produk pengarangan tidak larut dalam kloroform maupun air.

KESIMPULAN

Penyinaran/radiasi sasaran $Cr(CO)_6$ dan $Cr(C_5H_7O_2)_3$ dengan neutron termal menghasilkan radiokrom (Cr-51) anorganik dengan tingkat oksidasi +3 (trivalen) dan +6 (heksavalen). Pada penyinaran $Cr(CO)_6$ keradioaktivan total spesi radiokrom trivalen lebih tinggi dari spesi heksavalennya, tetapi pada penyinaran $Cr(C_5H_7O_2)_3$ spesi radiokrom heksavalen mempunyai keradioaktivan total yang lebih tinggi. Hal ini menunjukkan bahwa retensi keradioaktivan radiokrom pada penyinaran kedua bahan sasaran adalah relatif kecil.

Kedua bahan sasaran menghasilkan keaktivan jenis untuk spesi trivalen lebih besar dari pada spesi heksavalennya. Hal ini memberikan gambaran bahwa pertukaran isotopik radiokrom heksavalen anorganik dengan $Cr(CO)_6$ berlangsung lebih mudah dari pada pertukaran isotopik radiokrom trivalen anorganik dengan $Cr(C_5H_7O_2)_3$.

Secara keseluruhan keaktivan jenis Cr anorganik yang berasal dari sasaran $Cr(CO)_6$ adalah lebih tinggi dibandingkan dengan yang berasal dari sasaran $Cr(C_5H_7O_2)_3$.

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THE DETERMINATION OF SUGARS BY CHROMATOGRAPHIC METHOD*)

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ABSTRACT

Experiments have been carried out to analyse sugars using TLC and HPLC methods, In the TLC method, separation of sugars was performed on silica plates impregnated with monosodium phosphate and using mixture of ethylacetate/ pyridine/water as an eluent. Whilst in the HPLC method, the use of three column types i.e. diol, RP-18 and modified silica column were tested. The results showed that TLC method was able to measure three sugars i.e. sucrose, glucose and fructose with standard deviations of 11.6%, 7,6% and 1,9%, respectively. On the other hand, the HPLC method with silica column modified by polyamine and compressed with WATERS RCM-100, showed the best results, in which mixtures of nine sugars were well separated and measured quantitatively with good precision.

INTISARI

Telah dilakukan percobaan untuk menganalisa gula menggunakan metoda KLT (kromatografi lapisan tipis) dan KCKT (kromatografi cairan kinerja tinggi). Pada KLT, pemisahan gula dilakukan pada pelat silika yang telah diimpregnasi dengan natrium fosfat, menggunakan campuran pelarut etilasetat/piridin/air sebagai eluen. Sedangkan pada metoda KCKT, telah dicobakan 3 macam kolom yaitu kolom diol, RP-18 dan kolom silika yang sudah dimodifikasi. Hasil percobaan menunjukkan bahwa metoda KLT dapat menentukan kandungan tiga macam gula, yaitu sukrosa, glukosa dan fruktosa dengan simpangan baku berturut-turut 11,6%,7,6% dan 1,9%. Sedangkan pada metoda KCKT hasil yang terbaik ditunjukkan oleh kolom silika yang dimodifikasi dengan poliamina dan ditekan dengan RCM-100 WATERS. Campuran dari sembilan macam gula dapat dipisahkan dan dapat diukur secara kuantitatif dengan presisi cukup baik.

INTRODUCTION

Determination of sugars by chromatograpic techniques, particularly by Thin Layer Chromatography (TLC) and High Performance Liquid Chromatography (HPLC) methods, have been studied extensively to find rapid methods with good accuracy and precision. Gas Liquid Chromatography (GLC) is rarely used since this technique need prior derivatization reaction.

TLC separation is usually carried out on an impregnated silica plates and eluted by aqueous eluent systems. Kwan et. al (1) determined sugars simultaneously by HPTLC (High Performance Thin Layer Chromatography) using eluent system of ethylacetate-pyridine-water on silica gel plate impregnated with mono basic potassium phosphate. The relative standard deviations obtained by this method are 1.1% for sucrose, 2.2% for fructose and 4.3% for glucose. Doner et al (2) separated sugars using silica gel G HPTLC plates impregnated with monosodiium phosphate and 3-aminopropyl-triethoxysilan (3-APTS) as stationary phase and acetonitrile-water as an eluent.

Amino bonded phase silica columns such as Lichrosorb-NH_2, μ-Bondapak Carbohydrate and Partisil PAC have been widely employed while using acetonitrile-water as mobile phase (3,4,5,6). The method gate an effective resolution, but doubtfull quantitative results. Brons & Olieman (3) reporter that the formation of Schiff bases between amine groups of the stationary phase and carbonyl groups in the sugar, causes the loss of reducing sugars in the range of 0–100%, depending on type, age and temperature of the column. The formation of Schiff bases could be overcome by using a non reactive amine column, such as Nucleosil [$N(CH_3)_2$]. However, some of the sugars could not be separated by a mixture of acetonitrile/water (90:10). Eluents having a higher acetonitrile to water ratio should not be used due to very low solubility of sugars. Similar investigations were also carried out by WATERS group to improve the column life and performance by modification of eluent using some amines (7).

In the present work, a study was carried out to find a suitable column for sugars separation by HPLC. The results were then compared to those of TLC method in term of precision and resolution. In the TLC method used, an impregnated silica plate was used in combination with the eluent similar that used by Kwan et al (10). Whilst in the HPLC method, three kinds of column i.e. diol, RP-18 and silica compressed column were tested as stationary phases.

EXPERIMENTAL

All sugar standards and chemicals were analytical grade and purchased from E. Merck.

1. TLC

The HPTLC plates from E Merck were cut into 10 x 10 cm and impregnated by eluting three times with 0.2 M aqueous solution of monobasic sodium phosphate and then dried at 85 °C for 45 minutes. The developing solvent system was ethylacetate/ pyridine/water (8:2:1).

A solution containing 4 gram diphenylamine, 4 ml aniline, 30 ml of 85% H_3PO_4 in 200 ml acetone was made for visualization. Sugar standard solutions containing 0.8 – 8 gram fructose, glucose and sucrose in 1 L of 20% ethanol were prepared. 1 – 2 μl solution were taken for spotting on the TLC plate.

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The plates were eluted three times and then after spraying with the visualization reagent, the plates were allowed in the laboratory atmosphere for 15 minutes for initial drying and then placed in the oven at 110 °C for 20 minutes. The plates were scanned with Camag TLC-Scanner with the following parameters:

- wavelength : 395 nm
- slit length : 4 mm
- slit width : 0.6 mm
- chart speed : 40 mm/min
- scanning speed : 2 mm/sec.

2. HPLC

A liquid chromatograph (Waters Associates) consists of a solvent delivery system (Model 6000 A), universal injector (Model U6K) and differential refractometer detector (Model R-401). The column used was Radial-Pak Silica Cartridge (10 cm x 8 mm 1.D) which was compressed using Waters RCM-100 (Radial Compression Module), Lichrosorb DIOL E. Merck and Lichrosorb RP-18, from E. Merck. A Spectra Physics Integrator (SP 4920) was applied to measure the peak areas.

Water used for the mobile phases or for the preparation of mobile phases was purified using a Millipore (Bedford, M.A., USA) Milli Q Water purification system. Mobile phases and sugar solutions were filtered through a 0.45 μm membrane filter and the mobile phases were degassed in an ultrasonic bath before use. Sugar solutions were prepared with concentrations of 8 mgr/ml, 16 mgr/ml, 24 mgr/ml, 29 mgr/ml, 32 mgr/ml and 40 mgr/ml in 20% alcohol and 20 μl were used for each sample injection.

RESULT AND DISCUSSION

1. Chromatographic Characteristics of Sugars on TLC plates.

Separation of sucrose, glucose and fructose was carried out using conditions described above and Figure 1 shows the chromatogram of sucrose, glucose and fructose separated in an overnight saturation chamber. In sugar concentrations a range of 4–8 mg/ml, the relative standard deviations were found to be 11.6%, 7.6% and 1.9% for sucrose, glucose and fructose, respectively (n=5).

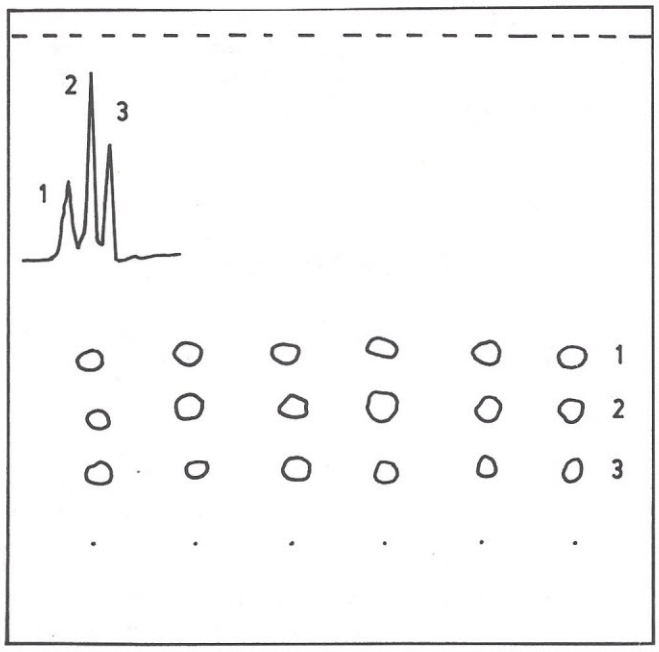

Figure 1. Chromatogram of fructose, glucose and sucrose in saturation chamber of TLC. Spots: (1) fructose, (2) glucose and (3) sucrose.

2. Chromatographic Characteristics of Sugars in HPLC Column.

Separation of galactose, sucrose and lactose on diol column were been reported by Brons & Olieman (3), using acetonitrile/water (85:15) and 0,1% tertiary amine diisopropylethyl amine (DIPEA) as an eluent system. From our experiments using a similar solvent (acetonitrile/water 80:20), it was found that diol column tended to swelling if water was used as a part of the eluent system. This phenomenon was indicated by pressure fluctuation occured during analysis. Such phenomenon was not reported by Brons and Olieman. Decreasing water content in the mobile phase was impossible, since it will produce peak broadening i.e. an ineffective resolution and column blocking due to precipitation of the sugars. The capacity factor of sugars examined, were 0.55; 0.72; 0.93; 1.17; 1.24; 1.88 for rhamnose, xylose, fructose, glucose, galactose and sucrose, respectively.

The capacity factor obtained on C_{18} column for sucrose, raffinose and invert sugar were similar to Palla's results (8). However, each monosaccharide can not be separated effectively.

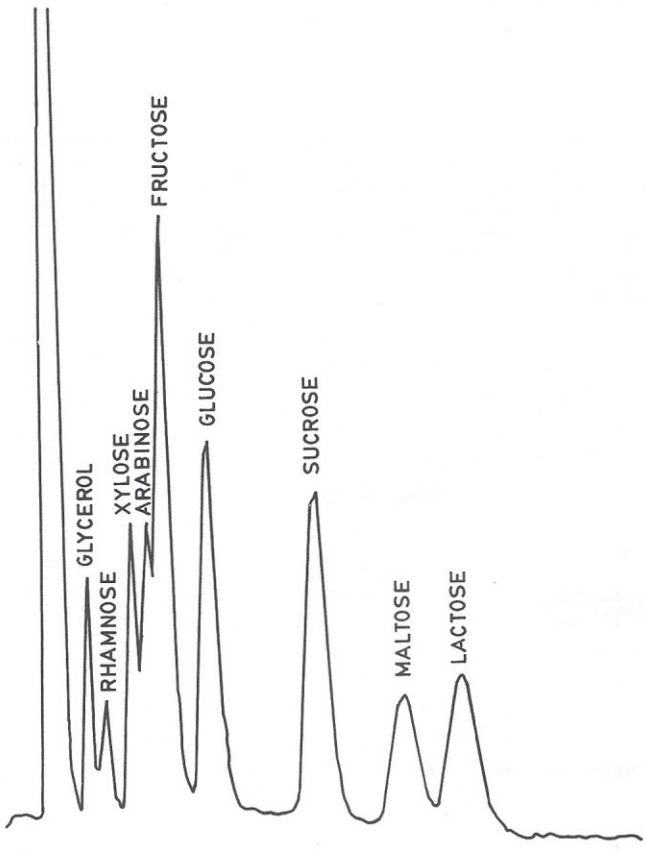

Figure 2. Separation of sugars on Radial Pak Silica Cartridge in RCM-100. Column conditioning: 500 ml acetonitril-water (385:15) and 5 vials of WATERS SAM reagent-1. Eluent: acetonitril-water (770:210) and 1 vial SAM reagent-1. Flow rate: 3 ml/min and differential refractometer was used as detection system.

They give similar capacity factors (0.31 – 0.34) near to t_o (retention time of mobile phase or other unretained molecules). $NaNO_3$ solution was used for determining t_o. From these experiment it can be derived that C_{18} column could not be used for separating sugars with the same atom C numbers. The other capacity factors of sugars examined were found 0.87, 0.23, 0.39, 0.48, 0.74, 0,35, 0.42, 0.48, 0.58, 0.74 and 1.38 for D-glyseraldehyde, xylose, ribose, rhamnose, digitose, lactose, melibiose, trehalose, cellibiose, saccarose, and raffinose, respectively. For further investigation, reverse phase column chromatography may be applied using specific ion pairing reagent in order to retain sugars longer in the column. It is expected that the sugars separation will be more effective.

A Radial-Pak Silica Cartridge column with an eluent containing polyamine reagent, which was introduced by WATERS was tried. The polyamine reagent in the eluent act as a modifier which impregnates the silica column in situ. This technique was termed as a Dynamic Exchange Separation or Silica Amine Modification (SAM). The capability of the method to separate mono– and disaccharides is demonstrated in Figure 2. All peaks are shown to be well separated. Identification of the sugars based on retention times, and qualitative results are given in the figure. The condition could also be used for arabinose, galactose, mannit and sorbose separation (Figure 3). However, if all sugars were mixed together and analyzed, galactose and glucose were not separated from each other. Mannit and glucose showed similar results.

Often only a few dilute solutions of sugars are available for quantitative analysis, so the linear correlation between peak areas and concentration needs a investigation. In this experiment the linear correlation between peak areas and concentrations were indicated by the correlation coefficients of 0.9608, 0.9970, 0.9988, 0.9750, 0.9992, 0.9992, 0.9986, 0.9933, 0.9937 for glycerol, rhamnose, xylose, arabinose, fructose, glucose, sucrose, maltose and lactose, respectively (Figure 4). The relative standard deviations were found to be 1.7%, 2.5% and 0.8% for fructose, glucose and sucrose, respectively.

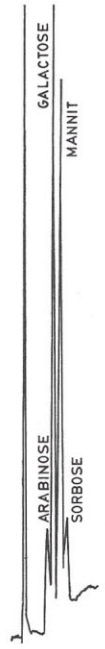

Figure 3. Separation of arabinose, galactose, mannit and sorbose in Radial Pak Silica Cartridge in RCM-100. Column conditioning: 500 ml acetonitril-water (385:15) and 5 vials of WATERS SAM reagent-1. Eluent: acetonitril-water (770:210) and 1 vial SAM reagent 1. Flow rate: 3 ml/min and detection system was differential refractometer.

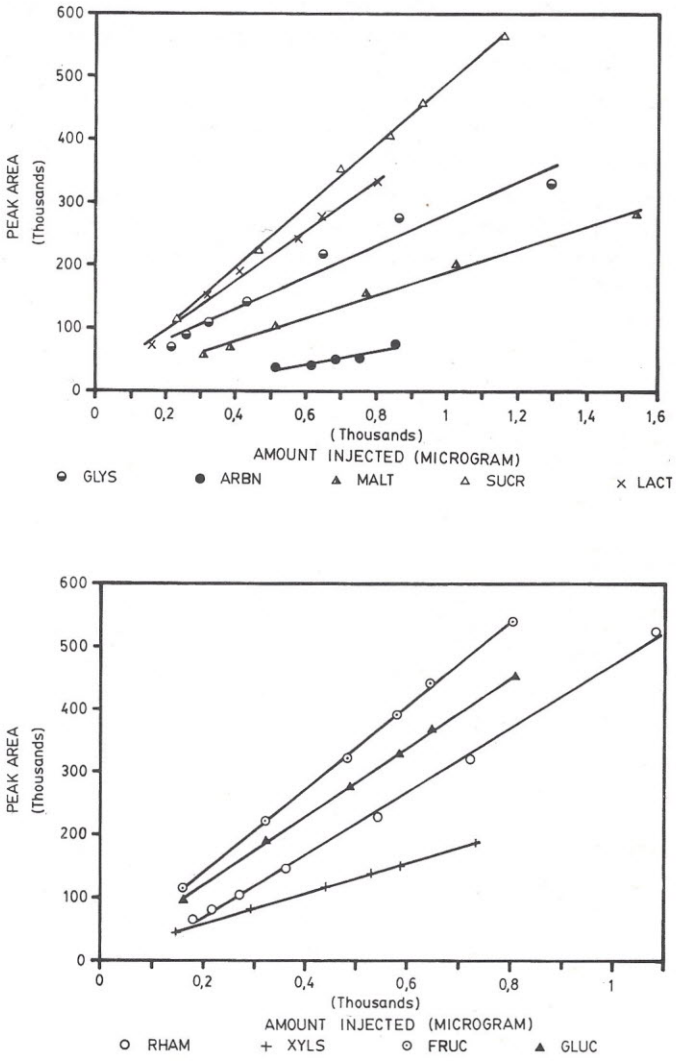

Figure 4. Calibration curves of sugars which is separated by Radial-Pak Column.

CONCLUSION

By comparing the results obtained from the examination of the three columns used, it was found that the Radial-Pak Silica Cartridge column compressed with RCM-100 shows the best result in terms of resolution and precision. Monosaccharides such as rhamnose, xylose, arabinose, fructose, glucose, and dissacharides such as sucrose, maltose and lactose may be separated from each other except for galactose and glucose separation. Mannit and glucose showed similar results as galactose and glucose. The results were much better than the those from TLC method, which have relative standard deviations of 11.6%, 7.65% and 1.9% for sucrose, glucose and fructose respectively.

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