

Separation and Analysis of Some Sugars by Using Thin Layer Chromatography

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SUMMARY

Different sugars such as glucose, fructose, sucrose, raffinose, and others have been separated on silica gel pre-coated plates. The plates were doubly developed in one direction with chloroform, acetic system consisting of diphenylamine, aniline, and orthophosphoric acid in acetone. The technique was used to analyze different sugars in beet and juice samples.

INTRODUCTION

Great attention has been given recently to some relatively rapid techniques of analysis. Thin layer chromatography (T.L.C.) is already accepted as a laboratory tool for routine work. Its low cost, ease, and rapidity along with its capacity for separating and identifying small quantities of compound mixtures make the technique a prime tool for research as well. The objective of this investigation was to adapt a method for separation, identification, and approximation of different sugars in beet processing liquors, thick juice from storage, and beet storage samples.

REAGENTS AND MATERIALS

1. Glass plates precoated with 0.25 mm dry silica gel, EM reagents, EM Laboratories, Inc., 500 Exec. Blvd., Elmsford, NY 10523.

2. Solvent system which consists of a mixture of chloroform, acetic acid, and water (3:3.5:0.5) by volume, respectively.

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3. Spraying agent made from 1 gram diphenylamine and 1 ml of aniline in 100 ml acetone. This mixture is further mixed with 85% orthophosphoric acid prior to use (10:1 v/v, respectively).

PROCEDURE

1. ul is applied.
2. Dry the plate in air for approximately 30 minutes.
3. Irrigate with the solvent system in the ascending direction in a tight container.
4. Allow the solvent to move upward about 12.5 cm. This usually requires 90 minutes.
5. Remove the plate from the tank. Leave to dry in air for about 30 minutes.
6. Place the plate back in the same developing solvent and let the solvent move in the same direction to the same distance of 12.5 cm. This usually takes 45 minutes. The plates should then be dried in air for approximately 30 minutes.

RESULTS AND DISCUSSION

Figure 1 is a thin layer chromatogram of some standard sugar solutions which involve glucose, fructose, sucrose, raffinose, and a standard invert solution (0.5% each of glucose and fructose).

Figure 2 includes some standard sugar solutions (glucose, fructose, sucrose, and raffinose). It also includes a diluted thick juice sample (26 g in 100 ml) and a diluted diffusion juice sample (1:1 by volume).

Figure 3 is a thin layer chromatogram of standard sugar solution (0.5% of glucose, fructose, and sucrose); and

also some beet storage samples (diluted 1:1 by volume).

It should be pointed out that the conventional single development technique when using cellulose precoated plates resulted in sucrose tailing which interfered with the determination of other sugars. On the other hand, the combination of double development and silica gel plates gives separations with minimum or no sucrose tailing and with improved resolution of other sugars.

Direct visual comparison with known standards provided reliable semi-quantitative information. If greater accuracy is required on quantitative analyses, the intensity of the spots may be measured by transmission densitometry.

It should be pointed out that this method is most suitable for the detection of many carbohydrates present in beets and in factory juices. Because of the simplicity of the method and the low cost of the equipment used, it is recommended for use in support of beet storage studies, thick juice storage analysis, and screening of agriculture research samples.

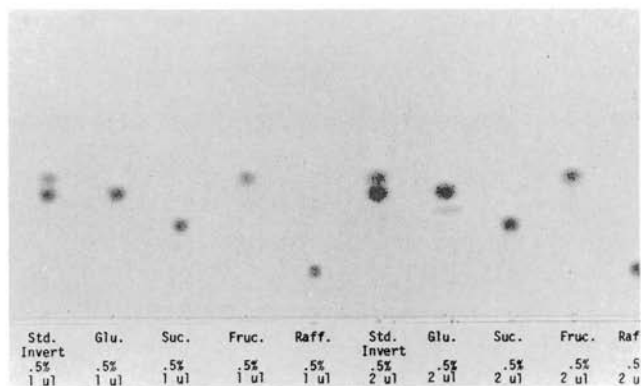


Figure 1. Chromatogram of standard sugar solutions.

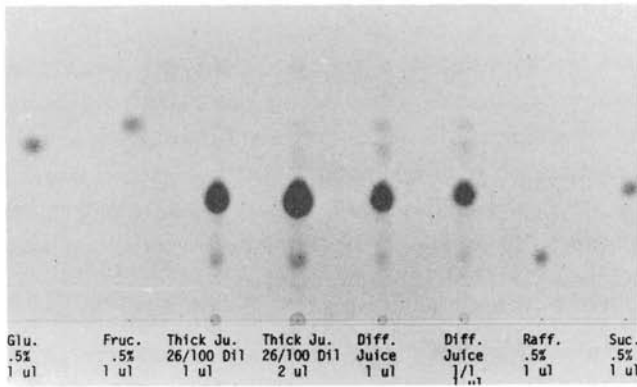


Figure 2. Chromatogram of some process juices.

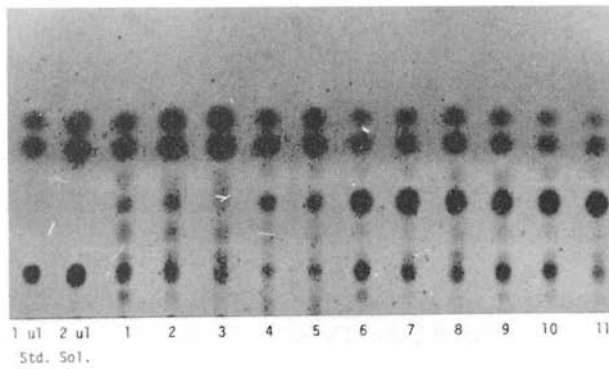


Figure 3. Chromatogram of some beet storage samples.