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THE DETERMINATION OF SUCROSE IN MOLASSES BY HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY

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SUMMARY

A method is described for the determination of sucrose in molasses by high-performance thin-layer chromatography. A baseline separation of sucrose is achieved in about 40 min using acetone–water (90:10) as developing solvent. The relative standard deviation of determinations by this method is about 2.2%. Results agree with those obtained by isotope dilution analysis of the same molasses samples.

INTRODUCTION

The determination of sucrose in molasses is an important assay in the sugar industry. The conventional approach is to determine sucrose directly by polarimetry or indirectly by a chemical assay of reducing sugars before and after the hydrolysis of sucrose. Both of these methods are inaccurate due to the presence of other sugars that interfere with the determination. Other methods that have been proposed include isotope dilution analysis[1], which in principle should be a very accurate method of sucrose determination and gas chromatography which can yield precise results[2] that are usually of a lower value than those found by isotope dilution.

Thin-layer chromatography (TLC) has been used to analyze mixtures of sugars in which sucrose is a component[3–6] and has been used to determine sucrose in beet molasses[7]. The precision of saccharide determination by TLC–densitometry is moderate with a relative standard deviation of about 5–10%[8]. This value is inadequate for the needs of the sugar industry. The recent introduction of high-performance TLC (HPTLC) enables the determination to be performed with a high degree of precision. We report here on our results for the determination of sucrose by HPTLC in cane molasses.

EXPERIMENTAL

Chromatography

Chromatography was performed on 10 × 10 cm HPTLC plates (E. Merck, Darmstadt, G.F.R.) which were pre-washed by continuous development for 12 h using acetone–water (90:10) as solvent. The plates were dried in a vacuum oven for 1 h at 80° before use.

Two developing chambers were used: a 24 × 23 × 8 cm closed chamber and a chamber for continuous development made from a glass crystallizing dish 75 mm deep and 150 mm in diameter, fitted with an aluminum cover with a slit 2 × 11 cm.

Samples were applied to the plate 7 mm apart, using a 200 nl Pt/Ir capillary (Antech, Bad Dürkheim, G.F.R.) attached to an EVA-Chrom Applicator (W + W Electronic Co., Basle, Switzerland). The two outermost samples were applied 1 cm from the edge of the plate. All standards and molasses solutions were prepared immediately before analysis using ethanol–water (4:1) as solvent.

After chromatography the plate was dried in a stream of warm air, allowed to cool to room temperature, rapidly dipped (about 2 sec) into the visualizing reagent described by Hansen[9] and then heated at 120° for 30 min.

The plate was allowed to cool to room temperature and the spots scanned with the KM-3 Spectrophotometer (Carl Zeiss, New York, N.Y., U.S.A.) in the reflectance mode for visible absorption. The spectrophotometer parameters were: λ, 625 nm; slit width, 0.1 mm, slit length, 6 mm; scanning speed, 50 mm/min. Integration of spot areas was performed using a Spectra-Physics Minigrator (Spectra-Physics, Santa Clara, Calif., U.S.A.).

Isotope dilution analysis

The method has been described elsewhere[1].

RESULTS AND DISCUSSION

The separation of glucose and sucrose has been reported by Hezel[10] using dichloroethane–acetic acid–methanol–water (50:25:15:10) as developing solvent. Two 20-min developments are required. We have found that the following two solvent systems yield baseline separations between reducing sugars (glucose and fructose appearing as a single peak), sucrose and the higher saccharides. Ethyl acetate–acetone–water (13:75:12) requires three 18-min developments in the closed chromatography tank for a total development time of about 54 min. A faster separation could be achieved in the unsaturated chamber using the solvent system acetone–water (90:10). The same solvent system has been previously reported[3] for the separation of mono- and disaccharides on silica gel plates impregnated with monobasic phosphate and boric acid. Two 18-min developments are required during which the solvent front travels 6 cm. The separation is shown in Fig. 1. The width of the slit in the cover of the developing tank is important as it influences the degree of solvent saturation in the atmosphere of the developing tank. This in turn influences the separation. No attempt was made to optimize the width as the separation achieved was considered satisfactory. However, it should be noted that runs performed on a $2\frac{1}{2}$ × 10 cm plate using the same solvent system in a small jar with a slit 2 mm wide achieved adequate separation in two 11-min developments. This acetone–water solvent system could not be used in the closed chamber as it yielded poor separation due to spot elongation.

The plates were dried after development and the spots visualized by dipping in the reagent described by Hansen and then heated in an oven at 120° for 30 min. No attempt was made to optimize the heating period. Both the intensity of the spots and of the background increases with the time that the plate is left in the oven.

The spots were scanned by the spectrophotometer in the reflectance mode.

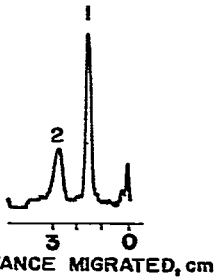

Fig. 1. Separation of sucrose (1) and reducing sugars (2) using acetone–water (90:10) as developing solvent.

Scanning the back of the plate yielded spots that were about three times more intense than when the front of the plate was scanned. The detection limit is below 5 ng. The plate may be scanned in a direction parallel to or perpendicular to the direction of solvent flow. The exact alignment of the spot with the scanning slit is a critical factor in obtaining satisfactory quantitative results. The plate need be aligned only once in the perpendicular mode whereas with parallel scanning each spot must be individually aligned. The respective times for the combination of alignment and scanning are 2½ and 10 min.

The spots are elliptical in shape with the length along the major axis (which is perpendicular to the direction of solvent flow) being about 3.5 mm visually. The slit length was set at 6 mm in order to cover the entire spot which is longer than 3.5 mm due to the high sensitivity of the spectrophotometer as compared to the eye. Samples were spotted at 7-mm intervals to eliminate the possibility of neighboring spot interference.

The plot of spectrophotometer response *vs.* weight sucrose spotted over the range 0–100 ng is shown in Fig. 2. The excellent agreement between parallel and perpendicular scanning is illustrated in the same figure. The relative standard deviation for the value of sucrose obtained from the same sample of molasses spotted 12 times on the same plate is about 1.5% with both parallel and perpendicular scanning Use of the data pair technique[11] on the same results reduced the value of relative standard deviation to 0.8%.

The good precision discussed above applied to comparing results on one plate only. The same sample applied to different plates yields different spectrophotometer readings even when all plates are subjected to an apparently identical experimental procedure. Possible causes of the problem are that the dipping time in the visualizing reagent could vary by about 10–20% and that the oven temperature fluctuated over a range of about 10°. Whatever the cause of this problem it may be overcome by including a sucrose calibration curve on each plate. When this precaution was taken, the same sample applied four times on each of four plates yielded results (data pair method) with a relative standard deviation of 2.2% when the plates were scanned in the parallel direction. Scanning in the perpendicular direction yielded results with a relative standard deviation of 3.7%. No attempt was made to investigate the causes for these differences in relative standard deviation. An uneven solvent front could effect the precision of perpendicular scanning.

The reproducibility between plates would most probably be improved by in-

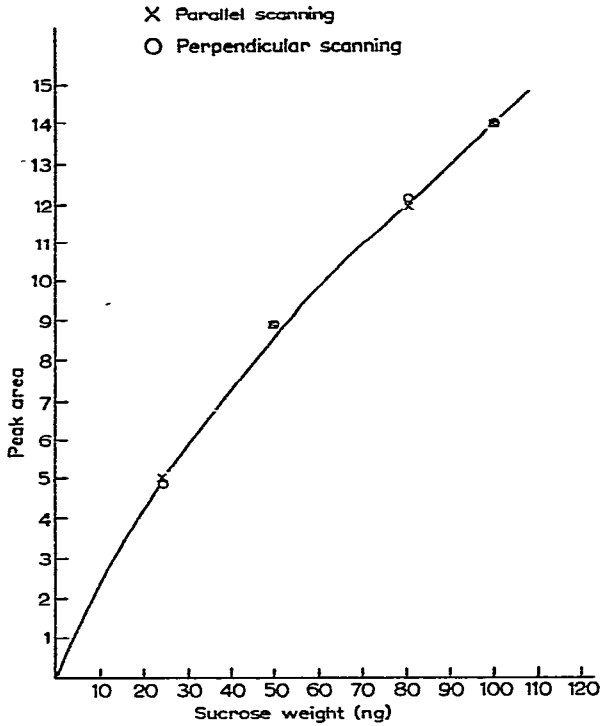

Fig. 2. Plot of spectrophotometer response *vs.* weight sucrose spotted.

cluding an internal standard in the molasses solution. Peak area ratios would be compared rather than absolute areas. However the inclusion of an internal standard causes additional separation problems.

The effect of spot aging after removal of the plate from the oven was tested in the following way. A sucrose spot in a molasses sample was scanned 4 min after removal of a plate from the oven and was then scanned repetitively over a period of 2 h for a total of 40 scans. The relative standard deviation for all 40 measurements of sucrose was 1.3%. A comparable value, 1.1%, was obtained for the same sample scanned 20 times over a period of 4 min. Thus there appears to be no effect of spot aging over a period of 2 h.

The accuracy of the method was investigated by comparing the results of a sucrose assay in molasses with those obtained by isotope dilution, a method that is considered to be of very high accuracy. The results listed in Table I show that there is good agreement between the isotope dilution and HPTLC determination.

Only 12 samples may be spotted 7 mm apart on a 10 × 10 cm plate. For the best results the data pair technique should be utilized which reduces the number of samples spotted to six samples per plate. The necessity of including a sucrose calibration curve on each plate reduces the number of unknown samples to 2 or 3 per plate depending on the number of points used for the calibration curve. More samples could be applied if the final spots were smaller. This could be achieved by using a different solvent system. It may possibly be achieved by reducing the initial spot size by heating the plate during spotting and at the same time blowing a stream of warm air

TABLE I

COMPARISON OF THE DETERMINATION OF SUCROSE IN MOLASSES BY ISOTOPE DILUTION AND HPTLC

ID = isotope dilution analysis.

Sample number	*Percent sucrose*		
	HPTLC	*ID*	*ID − HPTLC*
1	29.0	30.3	1.3
2	30.4	30.9	0.5
3	29.0	29.4	0.4
4	30.6	31.7	1.1
5	29.5	29.1	−0.4

across the surface of the plate to accelerate solvent evaporation. A 10 × 20 cm plate would allow an additional 6 samples to be applied without changing the technique.

Preliminary studies using the U-chamber indicate that it may be possible to achieve the separation of sucrose in molasses in about 6 min.

CONCLUSION

The determination of sucrose in molasses by HPTLC is a rapid and precise procedure. The accuracy of the method as determined by comparison with the isotope dilution technique is good.

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REFERENCES

1 J. Bruijn and R. A. Carreyett, *Proc. S.A. Sugar Tech. Ass., Durban*, 47 (1973) 44.
2 M. Kort, M. Matic, P. Mellet and D. Nurok, *Proc. S.A. Sugar Tech. Ass., Durban*, 49 (1975) 99.
3 M. Ghebregzabher, S. Rufini, B. Monaldi and M. Lato, *J. Chromatogr.*, 127 (1976) 133.
4 C. W. Raadsveld and H. Klomp, *J. Chromatogr.*, 57 (1971) 99.
5 A. Damonte, A. Lombard, M. L. Tourn and M. C. Cassone, *J. Chromatogr.*, 60 (1971) 203.
6 B. L. Welch and N. E. Martin, *J. Chromatogr.*, 72 (1972) 359.
7 S. Petrov, J. Gyura and D. Ignjacev, *Ind. Secera*, 28, Suppl. 3–4 (1974) 21; *C.A.*, 82 (1975) 126869b.
8 C. T. Mansfield, in J. C. Touchstone (Editor), *Quantitative Thin Layer Chromatography*, Wiley, New York, 1973, p. 79.
9 S. A. Hansen, *J. Chromatogr.*, 105 (1975) 388.
10 U. B. Hezel, in Z. Zlatkis and R. E. Kaiser (Editors), *HPTLC —High Performance Thin-Layer Chromatography*, Elsevier, Amsterdam and Institute for Chromatography, Bad Dürkheim, 1977, p. 147.
11 H. Beshke, W. Santi and R. W. Frei, *J. Chromatogr. Sci.*, 12 (1974) 392.