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# DETERMINATION OF SUGARS (AND BETAINE) IN MOLASSES BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

## COMPARISON OF THE RESULTS WITH THOSE OBTAINED BY THE CLASSICAL LANE–EYNON METHOD

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SUMMARY

The adaptability of four different high-performance liquid chromatographic (HPLC) columns (ion exchangers in the sodium and calcium forms, ODS and $NH_2$) for the analysis of sugars in molasses was studied. Some sample pre-treatment procedures were also examined. A strong cation-exchange column in the sodium form was the most suitable for the determination of both sugars in cane molasses and sugars and betaine in beet molasses. The only necessary pre-treatment was filtration of the diluted molasses sample through a 0.22-$\mu$m membrane before injection.

HPLC gave lower sugar contents than the classical Lane–Eynon method. The lower values are more reliable, because the HPLC method eliminates interferents from the sugars.

## INTRODUCTION

Molasses is a by-product of either manufacture or refining of raw cane sugar. It is a dark, heavy, viscous liquid from which no further sugar can be crystallized by normal methods. The sugar content of molasses is the reason for its extensive use in the fermentation industry, in animal feeds and recently also in liquid sugar production[1]. The sugars in molasses that have commercial importance are sucrose, glucose and fructose, the last two often being referred to as reducing sugars and usually reported as such in conventional analyses. The remainder of molasses consists of organic non-sugar matter, inorganic constituents and water.

Molasses is an agricultural product and its composition varies with the variety and maturity of the cane and with climatic and soil conditions. In addition, processing conditions in sugars factories may also bring about changes in the composition of molasses. Therefore, it is not possible to establish a typical analysis of molasses, but a broad range of composition may be given. The sucrose content may be from 25 to 40% or slightly more, reducing sugars from 12 to 35% and the total sugar content, both sucrose and reducing sugars, is usually about 50% or even more.

Beet molasses may be defined in a similar way to cane molasses, but as the

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cultivation of the sugar beet is different from that of cane, there are differences in composition with regard to both the sugar and the non-sugar contents.

The classical, official Lane–Eynon method for the determination of the total sugar content in molasses is based on a copper reduction method before and after inversion[2]. The volumetric Lane–Eynon method is well known in the sugar industry. It is simple and does not require expensive instrumentation, but it is time consuming. It is based on emprically derived constants and excess acid or heat may cause errors in the inversion procedure.

Although many methods for the high-performance liquid chromatographic (HPLC) determinations of carbohydrates in natural or other products have been published, few have related to sugars in molasses[3,4]. This study was undertaken in order to compare the results obtained by our HPLC method (sodium-form ion-exchange column) with those obtained by other LC methods[3,4] or by the Lane–Eynon method. Different kinds of sample pre-treatment procedures were also examined. A quantitative HPLC method for the determination of betaine in beet molasses is described.

## EXPERIMENTAL

### *Apparatus*

A Varian 5000 liquid chromatograph (Varian Aerograph, Walnut Creek, CA, U.S.A.) was used, equipped with a Rheodyne 7125 injector, 20-$\mu$l loop, a refractive index detector (Knauer RI, type 98.00, Dr. Herbert Knauer Wissenschaftliche Geräte KG, Bad Homburg, F.R.G.), a variable-wavelength UV detector (LC 75; Perkin-Elmer, Norwalk, CT, U.S.A.) and Goerz Servogor 321 recorder (Goerz Electro, Vienna, Austria). The following columns were used: a Resolution Carbohydrate $Na^+$, 150 × 6.4 mm I.D., packed with Toyo Soda strong cation-exchange resin, 6–11 $\mu$m; a Resolution Carbohydrate $Na^+$, 300 × 7.8 mm I.D., packed with Interaction strong cation-exchange resin, 6% cross-linked, 7–11 $\mu$m; a Resolution Carbohydrate $Ca^{2+}$, 250 × 6.4 mm I.D., packed with Interaction strong-cation exchange resin, 8% cross-linked, 7–11 $\mu$m; a Resolution ODS 5, 150 × 4.6 mm I.D.; and a Resolution $NH_2$ 5, 250 × 4.6 mm I.D.

### *Eluents and reagents*

HPLC-grade water and acetonitrile were used. Sugar standards were purchased from E. Merck (Darmstadt, G.F.R.) and from Fluka (Buchs, Switzerland).

Reagents for total sugar measurements were prepared according to the Lane–Eynon volumetric method[2].

Sep-Pak $C_{18}$ cartridges (Waters Assoc., Milford, MA, U.S.A.), Bond-Elut ion-exchange (mixed) extraction columns (Analytichem International, Harbor City, CA, U.S.A.) and Zerolit 225 and Zerolit FF ion exchangers (Zerolit, U.K.) were used for sample pre-treatment procedures.

### *Sample preparation procedures*

The molasses samples were mixed in a homogeniser. The samples were accurately weighed (0.5–2 g) into 100-ml volumetric flasks and diluted to the mark with deionized water. The following procedures were used: (1) the samples were filtered

through 0.22-$\mu$m membranes; (2) the samples were passed through Sep-Pak $C_{18}$ cartridges; (3) the samples were passed through Bond-Elut ion-exchange (mixed) extraction columns; and (4) the samples were passed through mixed ion-exchange resin cartridges.

*Chromatographic conditions and quantitation*

After the sample clean-up procedures, the samples were analysed on four different HPLC columns, the column effluents being monitored with a refractive index detector. Quantitation was effected by an external standard method. The chromatographic conditions were as follows: Resolution Carbohydrate $Na^+$, eluent water, flow-rate 0.4 ml//min, temperature 85°C; Resolution Carbohydrate $Ca^{2+}$, eluent water, flow-rate 0.8 ml/min, temperature 85°C; Resolution ODS 5, eluent water, flow-rate 0.5 ml/min, room temperature; Resolution $NH_2$ 5, eluent acetonitrile–0.01 *M* $KH_2PO_4$, pH 7 (74:26), flow-rate 2.0 ml/min, room temperature.

## RESULTS AND DISCUSSION

*Effect of sample pre-treatment on chromatography*

A very effective way of purifying molasses samples has been published[3,4]. In addition, we have also used Sep-Pak $C_{18}$ cartridges, Bond-Elut ion-exchange extraction columns and membrane filtration for sample pre-treatment. Fig. 1 shows that only a good ion-exchange procedure removes all the interferents. However, there is also the danger of inverting sucrose to glucose and fructose by this procedure if ion exchange is not performed under controlled conditions (time and temperature).

Table I shows how the sample pre-treatments affect the results obtained on different columns. It can be seen that proper clean-up procedures are necessary for all except the sodium-form ion-exchange column. However, we also recommend the use of Sep-Pak $C_{18}$ cartridges in order to protect the column. Fig. 2 shows typical chromatogram obtained on different columns.

*Comparison of the total sugar content in molasses determined by the HPLC and Lane–Eynon methods*

Nine molasses samples were analysed for fructose, glucose (= invert sugar) and sucrose by the HPLC methods described after sample preparation procedure 1 for the sodium-form ion-exchange column and sample preparation method 4 for the other columns. These methods are compared in Table II.

The results show that the differences between the HPLC methods are not significant, but the results obtained by the Lane–Eynon method differ from the HPLC results by 2–4% on average. These results are similar those published earlier[3,5].

*Reproducibility of the HPLC method*

As mentioned earlier, the differences between our sodium-form ion-exchange column and the other HPLC methods are minimal. The reproducibility of our HPLC method was tested by analysing one came molasses sample ten times. Membrane filtration was used as the sample pre-treatment method. The results are given in Table III.

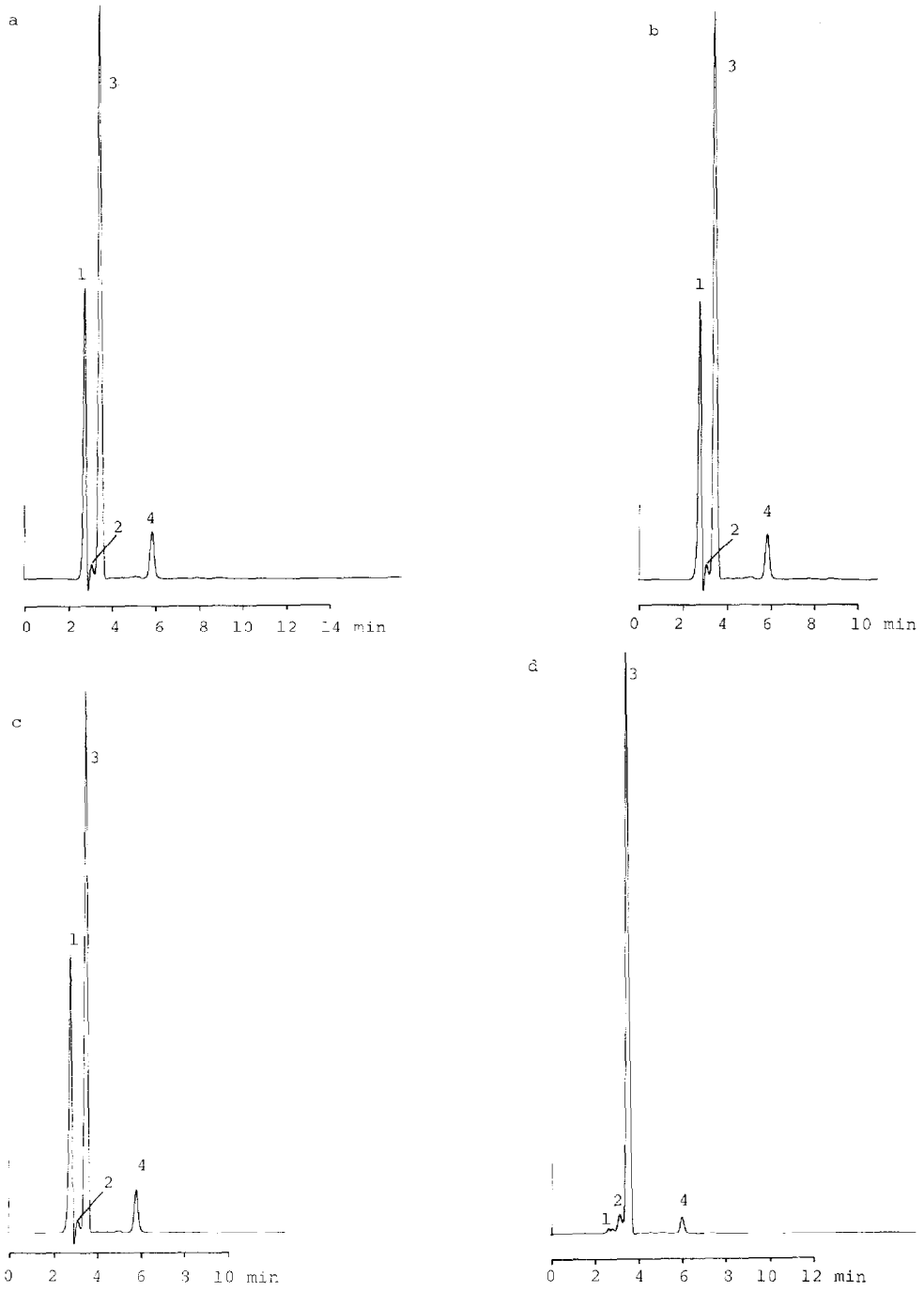


Fig. 1. Effect of sample pre-treatment on the chromatogram. Chromatographic conditions: column, 150 × 6.4 mm I.D., Resolution Carbohydrate $Na^+$; column temperature, 85°C; eluent, water; flow-rate, 0.4 ml/min; detector, refractive index × 32. (a) Membrane filtration; (b) Sep-Pak $C_{18}$; (c) Bond Elut; (d) ion exchange. Peaks: 1 = "non-sugars"; 2 = trisaccharides (raffinose); 3 = sucrose; 4 = betaine.

TABLE I

EFFECT OF SAMPLE PRE-TREATMENT PROCEDURES ON THE CHROMATOGRAPHIC DATA OBTAINED WITH DIFFERENT COLUMNS

| *Sample clean-up procedure* | *Sugars analysed (%)* | *Column* | | | |
|---|---|---|---|---|---|
| | | *$Na^+$* | *$Ca^{2+}$* | *ODS* | *$NH_2$* |
| Filtration through 0.22-μm membrane | Sucrose | 37.1 | 38.2 | 36.9 | 36.9 |
| | Glucose | 11.2 | 11.2 | 24.6 | 11.4 |
| | Fructose | 9.8 | 9.2 | | 10.2 |
| Sep-Pak $C_{18}$ | Sucrose | 37.2 | 38.2 | 36.9 | 37.0 |
| | Glucose | 11.1 | 11.0 | 25.4 | 11.4 |
| | Fructose | 9.8 | 9.0 | | 10.3 |
| Bond-Elut | Sucrose | 37.2 | 37.7 | 36.8 | 37.0 |
| | Glucose | 11.3 | 11.2 | 24.7 | 11.6 |
| | Fructose | 10.0 | 9.4 | | 10.3 |
| Ion exchange | Sucrose | 37.1 | 36.8 | 36.8 | 37.2 |
| | Glucose | 11.2 | 11.0 | 22.0 | 11.1 |
| | Fructose | 10.0 | 9.5 | | 10.5 |

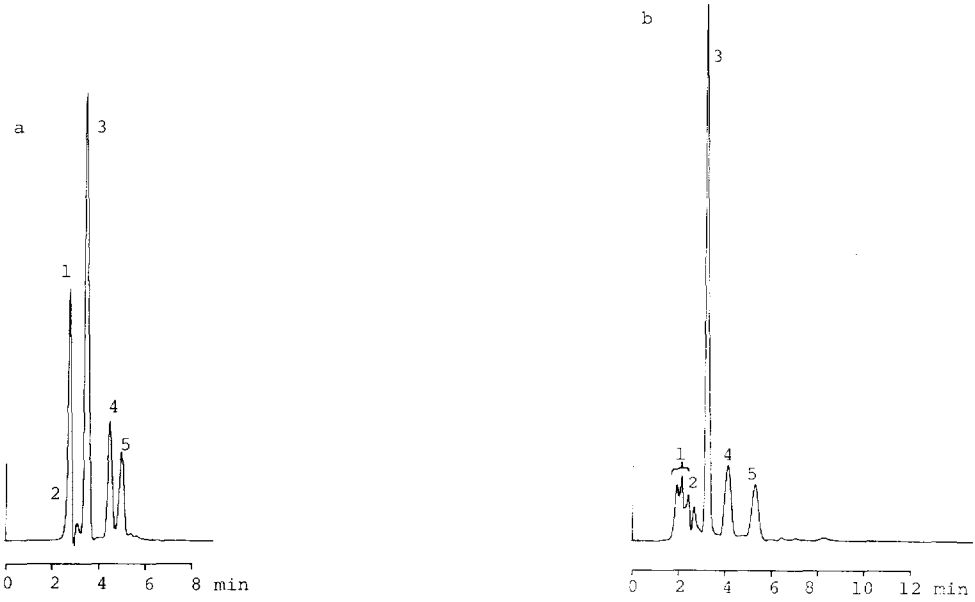


Fig. 2.

(*Continued on p. 600*)

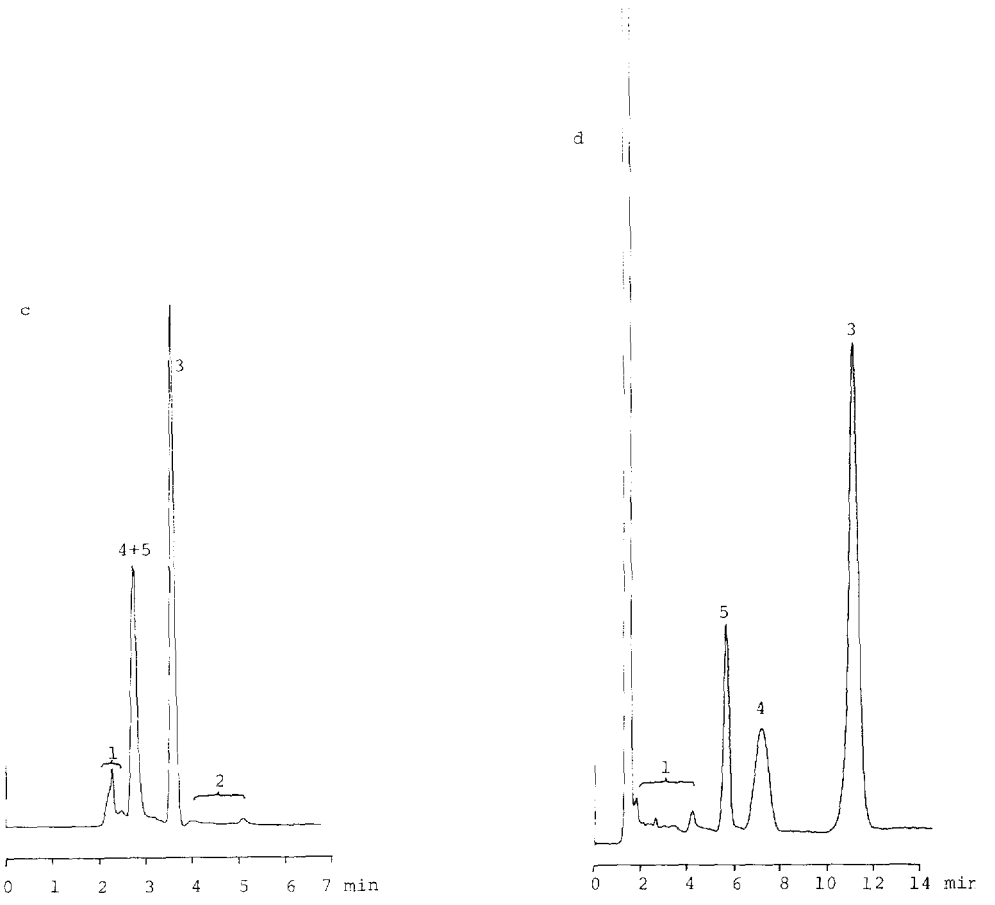


Fig. 2. Determination of sugars in cane molasses on four different columns. For conditions, see text. (a) $Na^+$; (b) $Ca^{2+}$; (c) ODS; (d) $NH_2$. Peaks: 1 = "non-sugars"; 2 = trisaccharides; 3 = sucrose; 4 = glucose; 5 = fructose.

TABLE II

COMPARISON OF TOTAL SUGAR CONTENT IN MOLASSES BY THE HPLC AND OFFICIAL LANE–EYNON METHODS

| *Sample* | *Total sugar content (%)* | | | | |
|---|---|---|---|---|---|
| | *Lane–Eynon method* | *HPLC method* | | | |
| | | *$Na^+$* | *$Ca^{2+}$* | *ODS* | *$NH_2$* |
| Beet | 55.4 | 51.9 | 51.1 | – | – |
| Beet | 55.5 | 52.3 | 51.8 | – | – |
| Beet | 52.9 | 51.6 | 50.9 | – | – |
| Beet | 50.7 | 48.8 | 47.1 | 47.9 | 48.4 |
| Cane | 63.9 | 60.9 | 61.6 | – | – |
| Cane | 59.6 | 58.1 | 57.3 | 58.8 | 58.8 |
| Cane | 56.6 | 52.7 | 52.0 | – | – |
| Cane | 52.4 | 52.9 | 52.3 | – | – |
| Cane | 53.5 | 52.7 | 51.4 | – | – |

*Determination of betaine in beet molasses*

Betaine has been analysed by using an amino-bonded phase as the column material[6,7]. We improved these methods by using as the eluent: acetonitrile–0.01 *M* $KH_2PO_4$, pH 7 (74:26). The alternative (possibly a better one) is to use sodium-form cation-exchange resins as the column material and water as the eluent. Either refractive index or variable-wavelength (at 190 nm) detectors can be used. Figs. 3 and 4 show typical chromatograms for an analysis of betaine in beet molasses by the methods described.

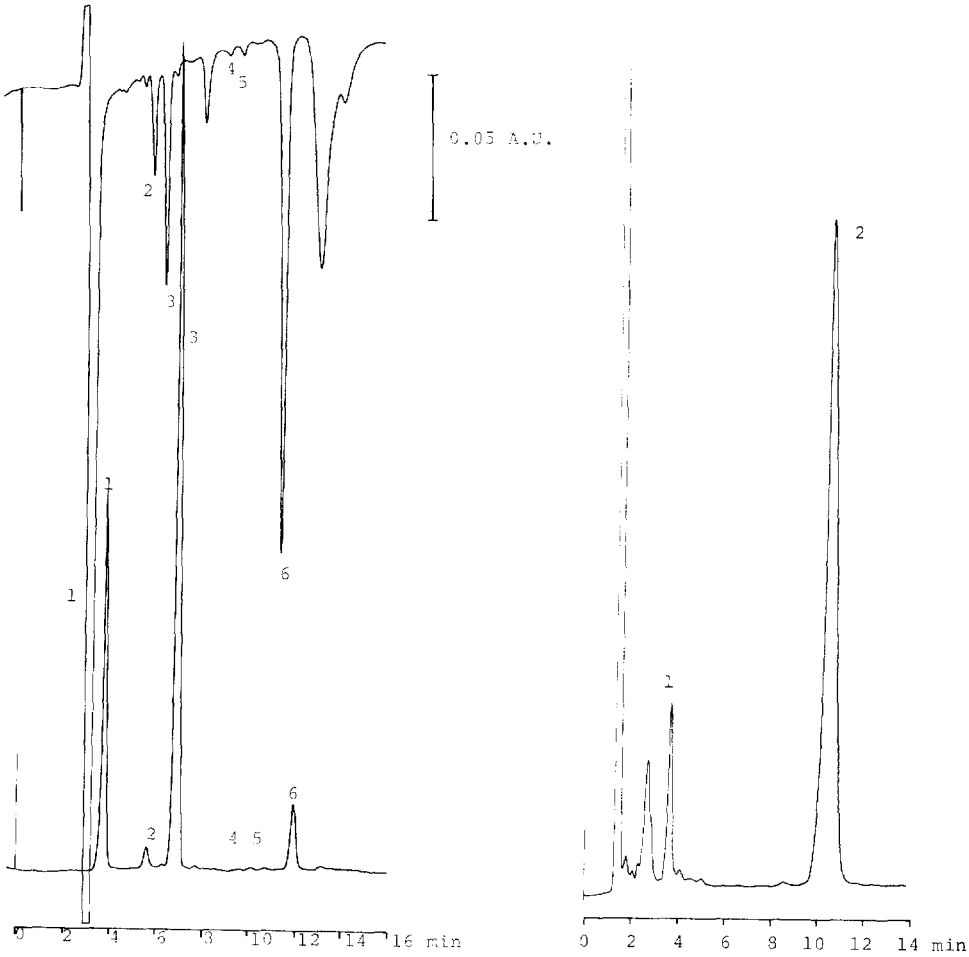


Fig. 3. Determination of betaine in beet molasses. Column, 300 × 7.8 mm I.D., Resolution Carbohydrate $Na^+$; temperature, 85°C; eluent, water; flow-rate, 0.7 ml/min; detectors, UV at 190 nm (above) and refractive index, ×8 (below). Peaks: 1 = "non-sugars"; 2 = trisaccharides; 3 = sucrose; 4 = glucose; 5 = fructose; 6 = betaine.

Fig. 4. Determination of betaine in beet molasses on a Resolution $NH_2$ column. Eluent, acetonitrile 0.01 *M* $KH_2PO_4$, pH 7 (74:26); flow-rate, 2 ml/min; column temperature, ambient; detector, refractive index, ×8. Peaks: 1 = betaine; 2 = sucrose.

TABLE III

REPRODUCIBILITY OF THE DETERMINATION OF SUGARS IN MOLASSES BY HPLC WITH A SODIUM-FORM ION-EXCHANGE COLUMN AND WATER AS THE ELUENT

| *Sample No.* | *Trisaccharides (%)* | *Sucrose (%)* | *Glucose (%)* | *Fructose (%)* |
|---|---|---|---|---|
| 1 | 1.1 | 37.7 | 11.3 | 10.0 |
| 2 | 1.1 | 37.7 | 11.4 | 9.9 |
| 3 | 1.1 | 37.8 | 11.5 | 10.1 |
| 4 | 1.1 | 37.3 | 11.3 | 9.9 |
| 5 | 1.0 | 37.0 | 11.2 | 9.8 |
| 6 | 1.1 | 37.3 | 11.4 | 10.2 |
| 7 | 1.0 | 38.0 | 11.6 | 10.4 |
| 8 | 1.1 | 37.2 | 11.6 | 10.5 |
| 9 | 1.1 | 37.4 | 12.0 | 10.3 |
| 10 | 1.0 | 36.9 | 10.9 | 9.9 |
| $\bar{x}$ | 1.07 | 37.43 | 11.36 | 10.10 |
| Relative S.D. (%) | 4.5 | 1.0 | 2.7 | 2.4 |

## CONCLUSIONS

The chromatographic methods presented are rapid, reproducible and give similar results. Although the results differ from those obtained by the Lane–Eynon method, the HPLC technique gives more specific and reliable information about the sugar composition in molasses, and we recommend its adoption as an official method for determing sugars in molasses along with the Lane–Eynon method. Comparison of the different HPLC methods shows that the simplest is the use of a strong cation-exchange resin in the sodium form as the column material and water as the eluent. In addition, this column is durable, cheaper than other commercial ion-exchange columns and very simple to regenerate. The only disadvantage is that on elevated column temperature must be used.

We have successfully used this HPLC method for routine process control in the sugar refinery process, in the fructose–glucose process and in other liquid sugar processes for several years.

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