# A COMPARISON OF THE ESTIMATION OF SUCROSE IN SUGAR CANE MIXED JUICE BY POLARIMETRIC AND GAS LIQUID CHROMATOGRAPHIC METHODS

By M. A. BROKENSHA and R. H. NIEMEYER
*Sugar Industry Central Board*
and K. SCHÄFFLER
*Huletts Research and Development*

**Abstract**

Parallel analysis of mixed juice samples for pol and sucrose content (the latter analysis by gas liquid chromatography) has been conducted at two factories for most of the 1977/78 milling season. Results from the one factory show the season to-date pol/sucrose ratio (expressed as a percentage) to be 99,7 per cent with a standard deviation of the weekly average ratios of ± 0,64 per cent. At the second factory pol underestimated sucrose by a wider margin, the pol/sucrose ratio being 98,4 per cent with a standard deviation of the weekly average ratios of ± 0,47 per cent. The relatively small variation in pol/sucrose ratio within any one week permitted a considerable reduction in the frequency of determination without serious loss of precision. At a 20 per cent analytical frequency the standard error of the weekly measure of the pol/sucrose ratio was ± 0,2 per cent.

The gas liquid chromatography (gc) method for the determination of sucrose in mixed juice has proved to be a practical procedure applicable to routine sugar laboratories.

## Introduction

In the South African sugar industry the determination of the total sucrose input to the factory for both cane payment and control purposes is obtained by the analysis of mixed juice and final bagasse using the single polarisation measurement. Pol is a hypothetical material which approximates sucrose but significant variations in the difference between pol and sucrose will have implications for factory control and cane payment.

Schäffler[1] has reported on a rapid and accurate method for the analysis of sucrose using a gas liquid chromatographic procedure, thus opening the way for routine sucrose analyses. Accordingly, at the request of the South African Sugar Technologists' Associations' Factory Control Advisory Committee, the Sugar Industry Central Board has for the past season been involved at two factories in the determination of sucrose in mixed juice by gas chromatography and the comparison of these results with the Horne's dry lead method of single polarisation analysis. Besides the comparison of pol with sucrose, the practicality of the gc method as applied under routine laboratory conditions has also been assessed.

## Procedure

1. General.

A Hewlett Packard 5721A gas chromatograph and ancillary equipment was purchased and installed in the Central Board laboratory at the Huletts Empangeni mill.

A parallel project, conducted with the co-operation of Huletts Research and Development, was initiated at the Huletts Mount Edgecombe mill. In this case the Central Board carried out all the operations up to and including the preparation of the samples for injection into the gas chromatograph; thereafter the prepared samples were sent to Huletts Research and Development for gc analysis.

(Refer Table I under analytical for gas chromatographic conditions.)

2. Staff Training.

The Central Board laboratory staff comprises semi skilled personnel on the bench working in three shifts around the clock, with one chemist in attendance during the day. The analytical procedures necessary for gc call for a delicacy of handling not required of the staff in the course of their normal routine but they readily adapted themselves to the new techniques. It was clear after the first week that practice was all that was necessary for the attainment of an acceptable standard of proficiency. Steady progress was made during the first month of the season at each mill and at the end of this period the results could be accepted with confidence.

3. Analytical.

Frequency of analysis – Mixed juice was sampled continuously and analysed hourly by single polarisation using the Horne's dry lead method whilst analysis for sucrose by gc was performed routinely but at a reduced frequency.

The frequency of gc analysis was limited by the additional work load which the laboratory staff could accommodate over and above their normal routine. Target was two mixed juice samples analysed per 8 hour shift i.e. 1 in 4 but this was not attained and the average turned out to be approximately 1 in 5. At Empangeni mill there is the further factor in that the mixed juice is routinely sampled and analysed as two separate streams viz., primary juice and secondary (diffuser) juice. Consideration was given to performing the sucrose analysis on a combined primary and secondary juice sample but it was considered preferable that in a fundamental investigation, the two streams be analysed separately.

Preparation of sample for silylation – For the gc analysis 50 $cm^3$ of the mixed juice was added to a 250 $cm^3$ beaker containing 1 g filter aid (Johns-Manville Celite 577). After thorough agitation the mixture was filtered through a Schleicher and Schull No. 3000 fluted filter paper into a 150 mm x 14 mm test tube, the funnel being capped to prevent evaporation. After discarding the initial runnings, duplicate 2 $cm^3$ aliquots were pipetted and massed into 6 $cm^3$ hypovials containing trehalose (140 - 400 mg). The quantity of trehalose used was dependent on the sucrose concentration prevailing at the time in order that the chromatogram peak heights for sucrose and trehalose would be approximately the same. After massing of the juice sample the hypovial was sealed with parafilm and mechanically shaken for 10 minutes.

Silylation – An 8 µl aliquot was silylated in a 2 $cm^3$ Hewlett Packard sampling vial by adding pyridine (0,50 $cm^3$) hexamethyldisilylazane (HMDS)(0,45 $cm^3$) and trifluoroacetic acid (T F A) (0,05 $cm^3$) and heating the capped vial in an ultrasonic water bath for 10 minutes at 80°C.

Calibration Standards – Sucrose (120 - 360 mg), (Aristar sucrose dried in vacuo under $P_2O_5$) and trehalose (140 - 400 mg) were dissolved in 1,8 $cm^3$ of water and silylated as above – such standards being prepared once per shift and analysed in duplicate.

However, for convenience, the procedure was modified in that a stock solution preserved with sodium benzoate (0,25%;pH adjusted to 6,9 ±0,1) was prepared at the beginning of each week and aliquots then withdrawn for silylation as required. This procedure was followed at Empangeni for the whole season without adverse effect whilst at Mount Edgecombe it was

abandoned during the latter half of the season, individual standards were prepared once a shift due to occasional T F A contamination and subsequent sucrose hydrolysis.

Gas Chromatography – At Empangeni laboratory the samples were injected manually in triplicate. Calibration standards were injected after each sample.

The gas chromatographic conditions at Empangeni are given below in Table 1.

**Table 1**

**Gas Chromatographic conditions for the anlysis of sucrose in mixed juice.**

**Empangeni laboratory**

| | |
|---|---|
| Gas chromatograph | H P 5721 A |
| Injector temperature | 245°C |
| Oven temperature | 245°C |
| Detector temperature | 245°C |
| Carrier ($N_2$) flow rate | 23 cm³/min |
| Hydrogen flow rate | 30,5 cm³/min |
| Air flow rate | 260 cm³/min |
| Column | 20" x 1/8" O.D. stainless steel, packed with U.C.W. – 982 (10%) on Chromosorb W (H P), 80 - 100 mesh. |
| Detection | flame ionization detection |
| Amount injected | 1 µl |
| Electrometer range | 10 |
| Integration | HP 3380 S |
| Slope sensitivity | 0,3 mV/min |
| Attenuation | X8 |

At Mount Edgecombe conditions differed in certain respects viz., a Hewlett Packard 7671 automatic liquid sampler injected the samples automatically into a Hewlett Packard 5711 gas chromatograph and the integrator used was the Autolab System I. For the rest, conditions were basically similar to those at Empangeni.

Results and discussion.

1. Pol/Sucrose Ratios.

Results of the comparison between pol and sucrose per cent mixed juice at the two factories are given in Table 2. In addition, the breakdown into primary and secondary juice as analysed at Empangeni is also given.

Weekly pol/sucrose ratios for mixed juice are shown in Figure 1 and for primary and secondary juices in Figure 2.

It is seen that the to-date pol/sucrose ratio for mixed juice at Empangeni is 99,7 whilst that for Mount Edgecombe is significantly lower at 98,4; these averages being the means of the individual weekly ratios and not of the individual analyses. As regards the pol/sucrose ratios for primary and secondary juice at Empangeni it is seen that the to-date figures are virtually on a par, this trend having been evident for the whole period reported.

Individual week standard error of pol minus sucrose differences adveraged ±0,02, showing that reliance can be placed on the pol/sucrose ratio for a particular week despite the reduced frequency of analysis (approximately 1 in 5).

2. Accuracy of analysis.

Recovery tests – Results reported by Schäffler[1] indicate that the analysis is accurate.

Slope sensitivity – Tests[2] reported by Schäffler show that for the 20" packed column (as used in these experiments) a slope sensitivity equal to or less than 0,5 mV /min gave acceptable accuracy. Analysis of pure sucrose solutions ranging in concentration from 5 Bx to 20 Bx (n = 14) for pol and sucrose showed a mean pol/sucrose ratio of 100,1 (standard deviation ± 0,2) at a slope sensitivity of 0,3 mV/min.

Purity of chromatogram –

(i) mixed juice with no trehalose was silylated and

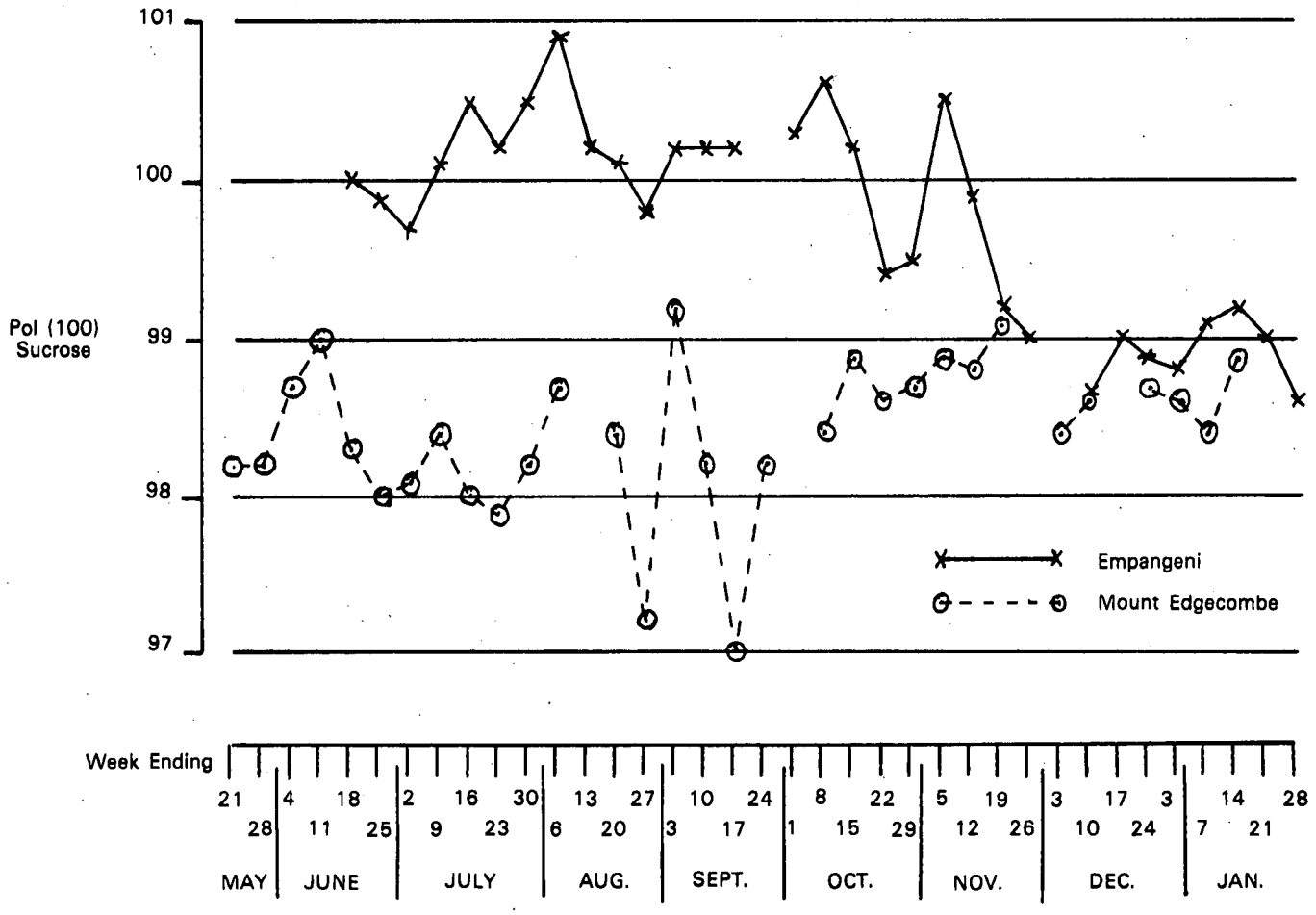


**Figure 1: Pol/sucrose ratios for Empangeni and Mount Edgecombe mixed juice samples for 1977/78 season.**

chromatographed. No peak was detected at the trehalose retention time position, even at an attenuation four times less than normal;

(ii) a trehalose solution was silylated and chromatographed and no sucrose peak detected;

(iii) water alone was silylated and chromatographed and no sucrose or trehalose peaks detected;

(iv) mixed juice samples chromatographed on an open tabular column with a column efficiency of 40 000 plates (nearly sixty times that of the 20" column) showed no new peaks compared with the shorter packed column. Results of nine mixed juice samples chromatographed on both types of column showed a mean difference of 0,01 units of sucrose per cent, this difference having no statistical significance.

Chromatograms with the open tubular and packed columns are shown in figures 3 and 4 respectively.

Linearity – Checks on the variation in response factor (K) for different ratios of sucrose to trehalose were carried out. The response factor

$$K = \frac{Mt}{Ms} \times \frac{Cs}{Ct}$$

where Mt and Ms are the masses of trehalose and sucrose respectively and Cs and Ct the counts of sucrose and trehalose reported by the integrater. Results recorded at Empangeni for the sucrose concentration ranges encountered in primary and secondary juices are shown in Table 3.

Results at Mount Edgecombe are shown in Table 4.

In all cases it is seen that linearity is excellent. Further evidence of satisfactory linearity is that the to-date K factors determined for primary and secondary juices (at an average of 16 and 8 per cent sucrose respectively) show a difference of less than 0,1 per cent relative.

Checks using standard solutions – Accuracy checks were conducted by comparing the gc sucrose analysis of a refined sugar solution with the polarisation of that solution as found on

**Table 2**

**Comparison of pol with sucrose**

| Mill | $n_1$ | $n_2$ | °Bx | Pol°S | Suc% | Diff Pol-Suc | S.E. Pol-Suc | Pol (100) / Sucrose |
|---|---|---|---|---|---|---|---|---|
| EM (m.j.) ..... | 647 | 31 | 13,59 | 11,61 | 11,64 | - 0,03 | ± 0,00 | 99,7 |
| ME (m.j.) ..... | 785 | 31 | 13,88 | 11,62 | 11,81 | - 0,19 | ± 0,00 | 98,4 |
| EM (p.j.) ..... | 660 | 31 | 18,56 | 16,30 | 16,33 | - 0,03 | ± 0,00 | 99,8 |
| EM (s.j.) ..... | 651 | 31 | 10,09 | 8,29 | 8,32 | - 0,03 | ± 0,00 | 99,6 |

**m.j. = mixed juice; p.j. = primary juice; s.j. = secondary juice**

**$n_1$ = number of individual analyses; $n_2$ = number of weeks**

**S.E. = standard error**

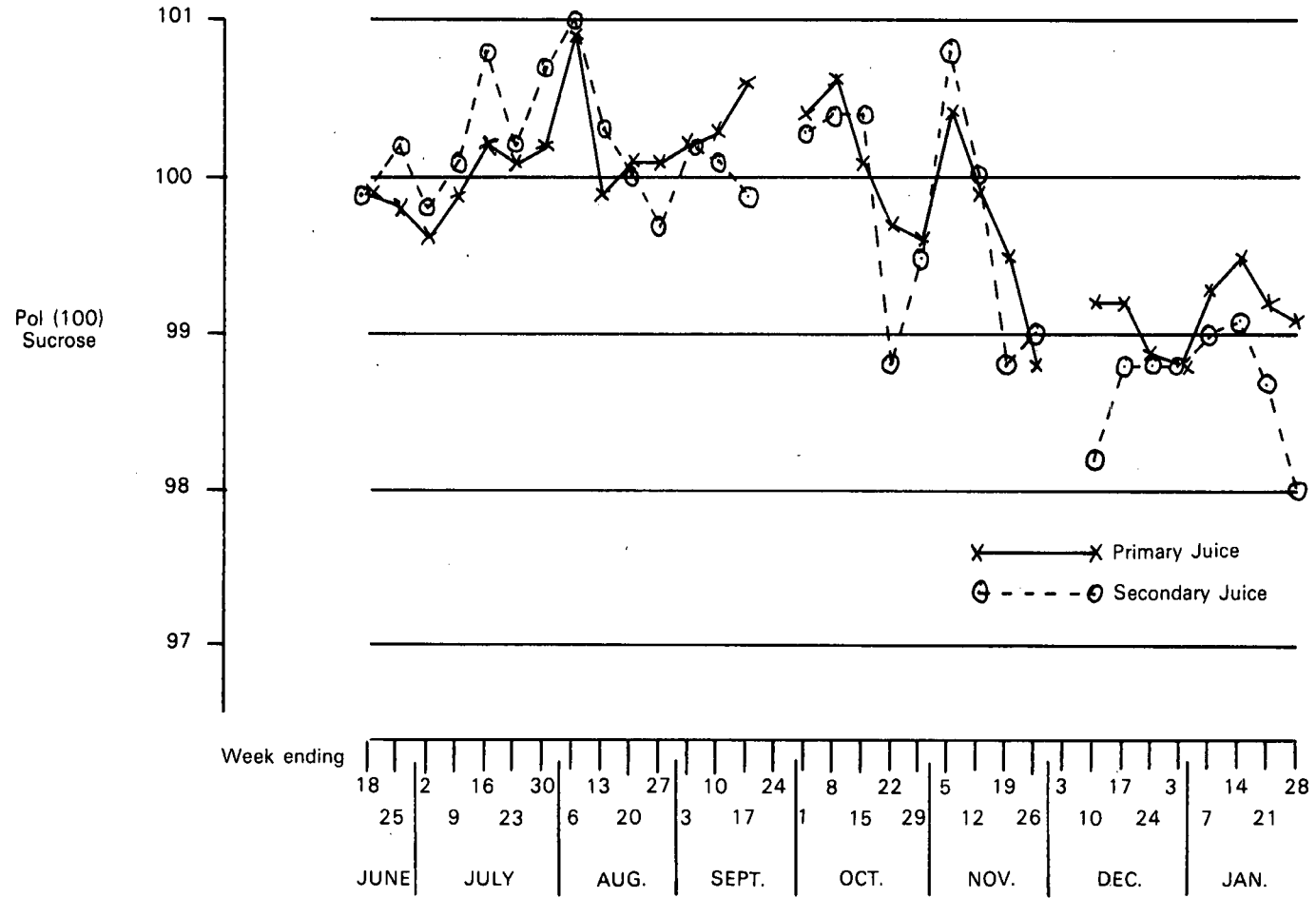


**Figure 2: Pol/sucrose ratios for Empangeni primary and secondary juice samples for 1977/78 season.**

a Schmidt and Haensch Saccharomat III calibrated with quartz plates. Results of these tests are shown in Table 5.

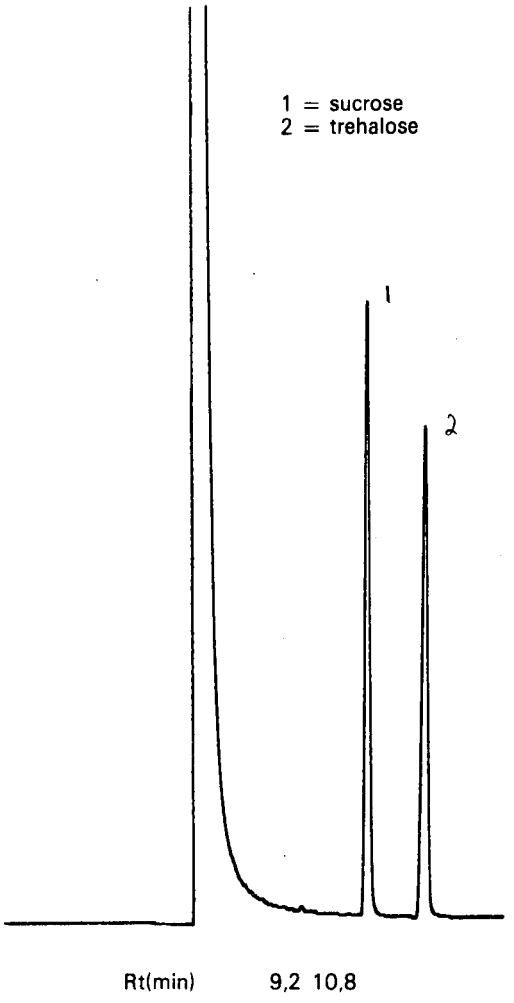


**Figure 3: TMS derivatives of sucrose and trehalose in a mixed juice sample separated at 240°C on a 25 m long by 0,5 mm internal diameter glass capillary (Scot-Silanox) coated with OV-101.**

**Table 3**

**Linearity checks – Empangeni**

| Trehalose / sucrose | Approx sucrose % | Response factor |
|---|---|---|
| **Primary Juice** | | |
| 0,91 | 20,2 | 1,1036 |
| 1,00 | 18,6 | 1,1008 |
| 1,08 | 17,3 | 1,1053 |
| 1,34 | 14,2 | 1,1029 |
| **Secondary Juice** | | |
| 0,80 | 11,6 | 1,1062 |
| 1,04 | 9,1 | 1,1049 |
| 1,38 | 6,8 | 1,1076 |

**Table 4**

**Linearity checks – Mt. Edgecombe**

| Trehalose / sucrose | Approx. sucrose % | Response factor |
|---|---|---|
| 0,37 | 17 | 1,106 |
| 2,66 | 6 | 1,108 |

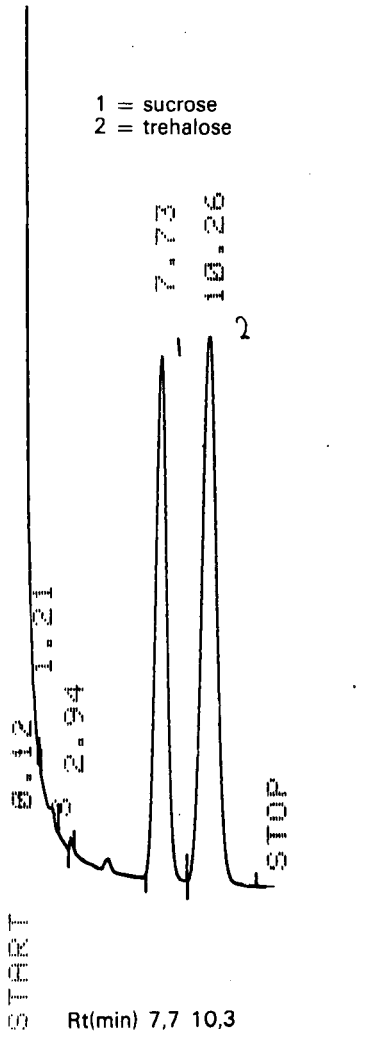


**Figure 4: TMS derivatives of sucrose and trehalose in a mixed juice sample separated at 240°C on a 20" x 1/8" stainless steel column packed with UCW - 982 (10%) on Chromosorb W (HP).**

**Table 5**

**Accuracy checks**

| | (Pol (100) / Sucrose | Standard Deviation | Standard Error | n |
|---|---|---|---|---|
| Empangeni | 99,96 | ± 0,29 | ± 0,04 | 66 |
| Mt. Edgecombe | 99,85 | ± 0,51 | ± 0,05 | 112 |

Initially these checks were conducted intermittently but later in the season were introduced as part of the analyst's routine.

To investigate any gc instrumental bias at either laboratory a set of pure sucrose standards was analysed on both instruments. The procedure was to prepare sucrose solutions ranging in concentration from 5 brix to 19 brix (n = 14) and silate them in duplicate. One set was analysed on the Empangeni instrument and the other on the Mount Edgecombe instrument. Results of the comparison are given in Table 6.

**Table 6**

**Comparative analyses between Empangeni and Mt. Edgecombe**

| | EM | ME | Diff | S.D. of diff | n |
|---|---|---|---|---|---|
| Sucrose % | 11,64 | 11,64 | 0,00 | ± 0,04 | 14 |

Detector response – As discussed by Schäffler[1], the problem of rapid coating of the flame ionisation detector with silicon dioxide reduces sensitivity and a loss of linearity is observed. The use of a gas chromatograph with rapid purging and combustion has overcome the problem. As a routine the detector was inspected daily and cleaned whenever silicon dioxide build-up was detected. A check was run by deliberately allowing the detector to build-up with silica and then to analyse a sucrose solution before and after cleaning. Results shown in Table 7 show no change in analysis before and after cleaning.

**Table 7**

**Flame ionisation detector response**

| | Before cleaning | After cleaning |
|---|---|---|
| Sucrose % | 19,46 | 19,45 |
| K factor | 1,1051 | 1,1051 |
| C.V. of K factor | ± 0,27 | ± 0,19 |

C.V. = coefficient of variation

3. Precision of analysis

At Empangeni injections were performed manually and results showed an injection precision of well under 0,5 coefficient of variation which is most satisfactory. Samples at both factories were analysed in duplicate and the precision of duplicate analyses is given in Table 8. The percentage of analyses rejected (R) on account of the coefficient of variation for duplicate analyses being greater than 1 per cent is also shown.

**Table 8**

**Precision of sample analysis and rejection level (R)**

| Mill | C.V. | R |
|---|---|---|
| EM (PJ) | ± 0,31 | 2% |
| EM (SJ) | ± 0,38 | 4% |
| ME | ± 0,41 | 7% |

PJ = primary juice; SJ = secondary juice

CV = coefficient of variation;
R = percentage of reject analyses

Here again the precision of analysis is well within 0,5 coefficient of variation which can be considered very satisfactory.

The to-date averages for the response factors with intra and inter-week variation data are shown in Table 9.

**Table 9**

**Response factor (K) data**

| Mill | To date K factor | To date CV | |
|---|---|---|---|
| | | Intra-week | Inter-week |
| EM (PJ) | 1,1039 | ± 0,3% | ± 0,3% |
| EM (SJ) | 1,1047 | ± 0,4% | ± 0,3% |
| ME | 1,1091 | ± 0,8% | ± 0,9% |

It is seen that the variation at Mount Edgecombe is more than twice that encountered at Empangeni.

4. Equipment Performance

(i) Empangeni

In all, just over three weeks down time was experienced on account of problems with the gas chromatograph or integrator; twice because of a faulty electrometer cable, three times because of partial blocking of the injection column liner when a portion of the septum became detached and fell into the liner, twice on account of the integrater paper drive motor/gears giving trouble and once on account of cause unknown which corrected itself before it could be traced. Down time in some cases would have been shorter but for lack of experience in trouble shooting.

Ancillary equipment was virtually trouble free save for the rather high consumption of syringes, particularly that used for the trifluoracetic acid which seized on numerous occasions and had to be replaced close to a dozen times.

(ii) Mount Edgecombe

At Mount Edgecombe only the sample preparation equipment was in the routine laboratory; the gas chromatograph being owned and operated by Huletts Research and Development on their premises.

Trouble was experienced at one stage with the pan arrester rod in the analytical balance sticking and giving rise to mass determination errors. This necessitated rejection of a few days' results.

5. Practicality of gas liquid chromatography analysis in a routine laboratory.

This investigation has shown that the gc procedure can be applied in a routine laboratory. However, it is a relatively time consuming and laborious procedure compared with pol determination and cannot replace the latter on a one for one basis at the high frequencies encountered in a cane testing laboratory without considerable additional expenditure on equipment and manpower. Its role in the cane testing laboratory is seen rather as it was applied in this investigation viz., the provision of sucrose analyses at a reduced frequency compared with pol and the calculation of the weekly average pol minus sucrose difference applied to all the individual pol analyses for the week.

Experience at Empangeni has also showed that if glc became standard routine, greater automation by way of automatic dispensers, an automatic injector and a more sophisticated data computation system is required in order to relieve manpower requirements – these additional features all being readily available.

## Conclusion

(i) The results show that at Empangeni the to-date difference between mixed juice pol and sucrose is minimal (0,3 per cent relative) whereas at Mount Edgecombe pol persistently under-estimates sucrose with the to-date difference being 1,6 per cent relative.

(ii) The variation in the difference between pol and sucrose during a week is small, thus permitting a considerably reduced frequency of analysis (20 per cent) without incurring significant loss of precision in the measure of the difference between pol and sucrose for the week; the average weekly standard error of pol minus sucrose difference being ± 0,02 units per cent.

(iii) At Empangeni the to-date pol/sucrose ratios for primary and secondary juice are virtually the same; the to-date difference being 0,2 per cent relative.

(iv) Experience has shown that the gas liquid chromatographic method of sucrose determination can be applied in a routine laboratory.

## Acknowledgements

The authors wish to thank the Sugar Industry Central Board and Huletts Sugar Limited for permission to publish the paper.

## REFERENCES

1. Schäffler, K. J. (1976). Preliminary comparison of polarimetric and gas chromatographic methods for the estimation of sucrose in sugar cane mixed juice and molasses – SASTA Proc 50 : 220-223.
2. Schäffler, K. J. (1978). Unpublished data.