

A QUALITY ASSURANCE PROGRAMME FOR THE WEEKLY ANALYSIS OF SUGARS IN CANE FINAL MOLASSES BY GAS CHROMATOGRAPHY

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Abstract

A quality control programme for the GC analysis of sugars in cane molasses is described. Multi-level standard calibration reduces systematic bias. Repeatability and target values for check molasses samples are used as the control parameters to evaluate day to day precision and accuracy.

Introduction

The 1982–83 season saw the official implementation of GC-based sugar data for chemical control of S.A. raw sugar factories. The SMRI undertook the analysis of weekly molasses samples. This necessitated a quality assurance scheme for monitoring GC measurement errors so that these could be maintained within reliable limits. Results thus have a high probability of complying with acceptable quality standards.

A quality control programme incorporating multi-level calibration, with the use of pure sugar solutions (of known concentration) and molasses check samples to evaluate the precision and accuracy of the procedure has been introduced. The precision of the method is tested using the repeatability concept and the accuracy with the aid of target values for the check molasses samples.

Calibration

Details of the experimental procedures for determining fructose (F), glucose (G) and sucrose (S) in cane molasses have been published.^{1,2} The internal standard technique is used to calibrate the GC and to measure sugar levels in molasses. Many chemists and most commercial integrators assume a linear response from the GC. Although this assumption is very often valid it should be verified since various factors can produce a deviation from linearity. Typical examples are silica build-up in the detector, septum leaks, carrier gas pressure drops, vent line blockages, incorrect integrator settings and low or very high sugar levels in the samples. Shatkey has investigated systematic errors in quantitative GC analysis using the internal standard technique.³ Schäffler and Morel du Boil have also pointed out several limitations of this technique.⁴ To reduce linearity errors, it is necessary to incorporate two analytical concepts. Firstly three calibration standards are always prepared with each batch of molasses samples. These standards bracket the expected concentration range for each of the three sugars. Secondly bias or drift with time can be monitored by chromatographing the standards in duplicate both *before and after* the molasses samples. The usefulness of multi-level calibration is illustrated diagrammatically in Fig. 1.

The six calibration standards are coded one through to six. One and four, two and five, and three and six are replicates. These standards contain different amounts of F, G and S bracketing the ranges of these sugars in molasses. The sucrose response factor is plotted for each standard in Fig. 1A. No significant drift or systematic bias can be observed. In Fig. 1B however it is obvious that the response factors *after* all the molasses samples have been run are lower than the original factors. (On this particular occasion the drift was due to incomplete silylation as a repeat run produced the results in Fig.

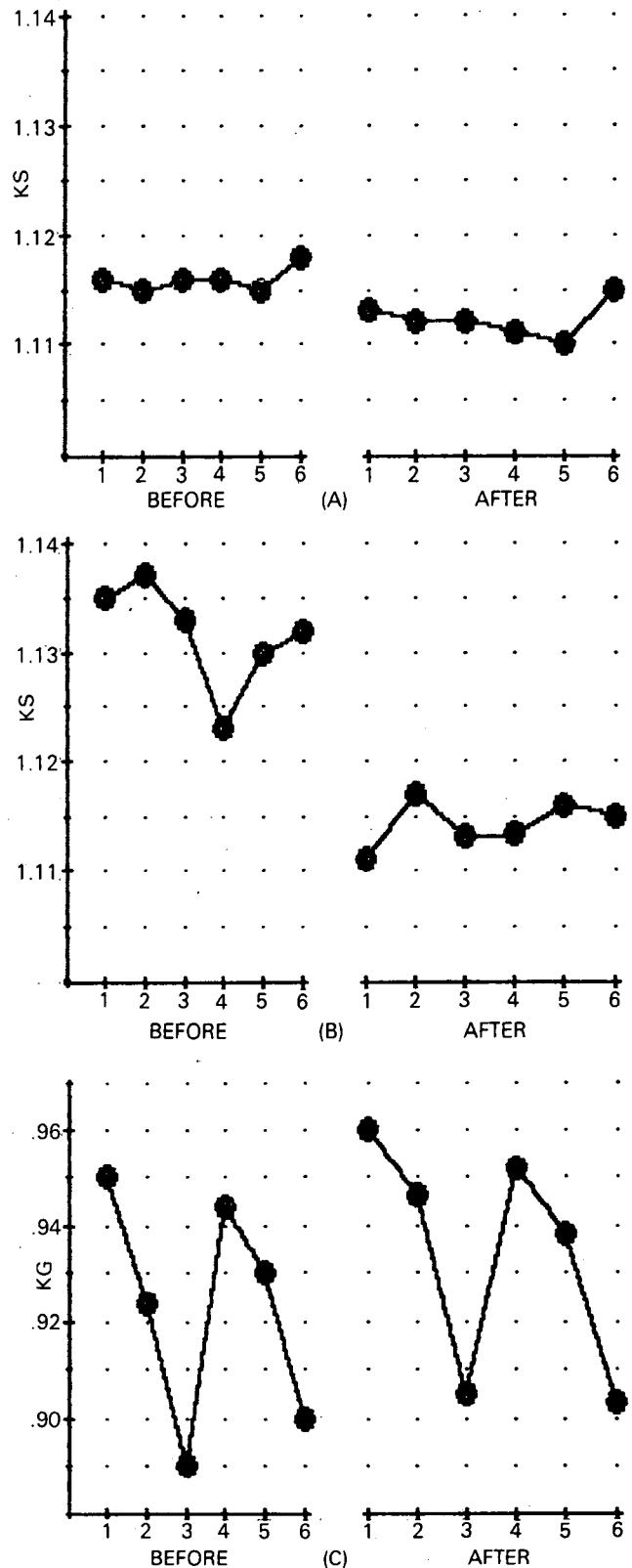


FIGURE 1 Diagrammatic illustration of the use of multi-level GC calibration of sugars in molasses. (See text for details).

1A). The glucose response factor in Fig. 1C does not show any drift with time. However as the glucose concentration drops from standards 1 to 3 so does the response factor. (This was attributed to a small leak at the column inlet.)

Control Samples

Control samples are prepared by weighing known amounts of F, G and S. The concentrations of these sugars are similar to those found in molasses. These synthetic molasses solutions are used to verify the response factors. The incorporation of these controls will highlight faulty calibration and the appropriate correction step(s) can be taken. These solutions are freshly prepared with each run.²

Precision

Repeatability

A lack of precision will impair a result as surely as a systematic error, therefore a statistical measure of the variability of the GC method was required. We decided to standardise on the repeatability concept recently introduced to the S.A. sugar industry by Lionnet.⁴ Repeatability is also used for statistical comparison by the Association of Official Analytical Chemists,⁵ although the definition and formula of this organisation are different from those used in this paper. Repeatability (r) of an analytical method can be defined as that value below which the *absolute* difference between replicate analytical results obtained from the same method on identical test material under the same conditions may be expected to lie within a 95% probability.

Calculation of repeatability

The estimation of a target value for r was determined before the start of the milling season. This was done by analysing a large number of samples, to increase the number of degrees of freedom. Briefly three molasses samples were each subsampled seven times. Each subsample was analysed in triplicate on two separate occasions. The calculated repeatabilities are listed in Table 1. (See appendix for calculation of r)

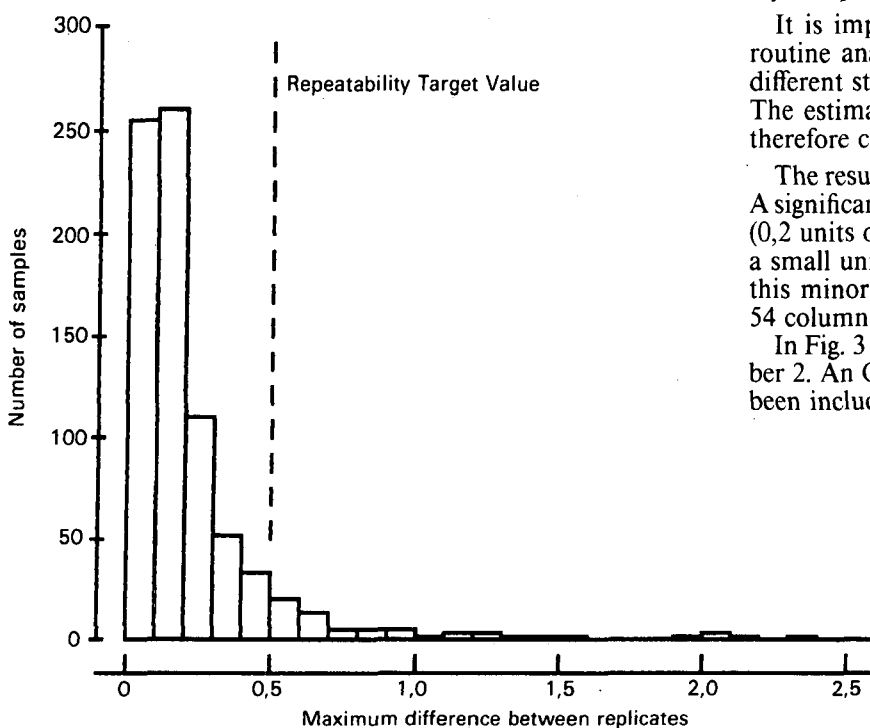


FIGURE 2 Frequency distribution of the maximum difference between replicates molasses samples for sucrose during the 1982/83 season.

TABLE 1
Repeatability target values for sugars in molasses by GC (n = 126)

Sugar	r (in g/100g)
Fructose	0,3
Glucose	0,4
Sucrose	0,5

Use of repeatability target values to reject outliers

It is extremely difficult to judge whether or not 1 of 3 replicates is an outlier, however for practical reasons this is sometimes necessary. Our approach has been to determine the maximum differences between triplicates for a sample and if this difference is greater than r for a particular sugar, the suspect value is rejected. It must be remembered that r has been derived statistically and is not simply an experimental value.

Verification of the repeatability target values

Maximum differences are plotted routinely for monitoring day-to-day precision. A frequency distribution has been prepared from the data obtained throughout the season. This is illustrated in Fig. 2. The maximum difference between triplicates for 92% of the samples lay within the target repeatability value (0,5). This is very close to the theoretical value (95%) indicating that r as determined prior to mill startup is a realistic yardstick for monitoring precision and rejecting outliers. Similar findings were obtained for F and G. It is important to note that for the calculation of r suspect data must *not* be eliminated.

Accuracy

This is a difficult term to define; for many chemists however it is a measure of the degree of bias or how close the results are to the actual value of a particular component.

Estimating target values for F, G and S in check samples

Estimates of the true concentrations of all three sugars for 3 cane molasses check samples were obtained prior to mill startup. All seven subsamples were subjected to the complete analytical procedure on two separate occasions.

It is important to standardise experimental conditions for routine analysis. In previous years capillary columns with 2 different stationary phases were used for F, G and S analysis. The estimation of target values for these check samples was therefore carried out on both these columns.

The results were subjected to a two-way analysis of variance. A significant difference was found for fructose. The higher result (0,2 units on average) on the OV-17 column was attributed to a small unidentified co-eluting impurity. It would appear that this minor component is separated from fructose on the SE-54 column.

In Fig. 3 this component is tentatively depicted as peak number 2. An OV-17 separation of the monosaccharide region has been included for comparative purposes (Fig. 4).

For this reason we standardised on SE-54 and only used quantitative data from this column to determine target values for the 3 molasses samples. These are presented in Table 2.

TABLE 2

Target values for F, G & S concentrations in the three check molasses samples

Sample	X	Y	Z
F	6,68	6,92	6,54
G	5,23	5,57	4,55
S	29,10	28,80	35,65

Monitoring the check samples routinely

The 3 check samples were chromatographed with each weekly batch of molasses samples. Control charts offer an easy way for keeping a running check on possible bias. As the data ac-

cumulates the amount of scatter and any persistent displacement of the results from the target values becomes plainly visible. The upper and lower confidence limits for the difference between target values for each sugar and the weekly results were obtained from the repeatability data in Table 1. Comparisons between target values and on-line results have been summarised in histogram form (Fig. 5).

Fructose

A total of 112 check samples was analysed throughout the season, the differences for fructose between the target value for each check sample and these 112 samples are shown in Fig. 5A. It is obvious that the differences are normally distributed around zero with only about 5% of the results outside the confidence limits.

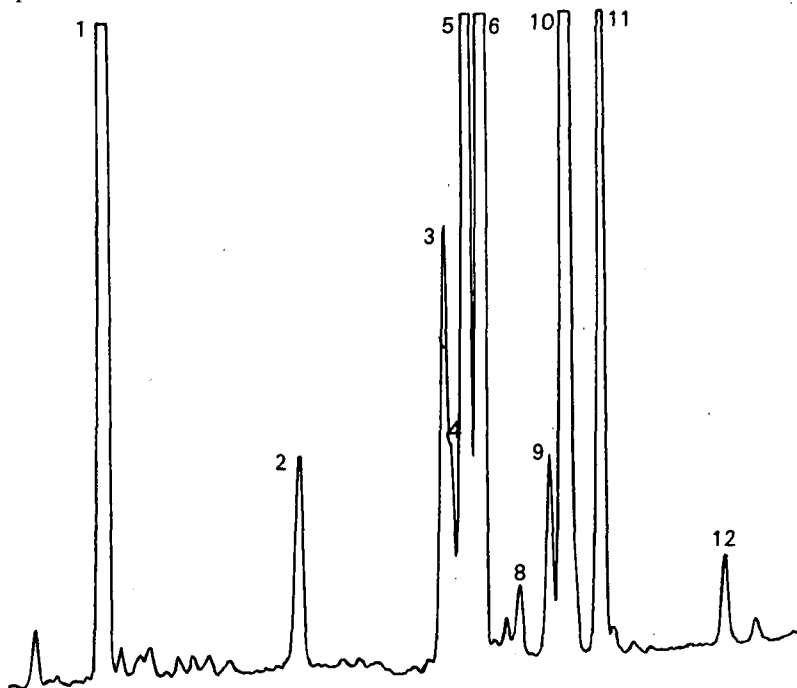


FIGURE 3 Separation of oxime-trimethylsilyl derivatives of monosaccharides in molasses on a fused silica capillary column coated with SE-54. (Xylose = 1, unknown = 2, mannitol = 3, psicose = 4, fructose = 5 and 6, unknown = 8, mannose = 9, glucose = 10 and 11, inositol = 12).

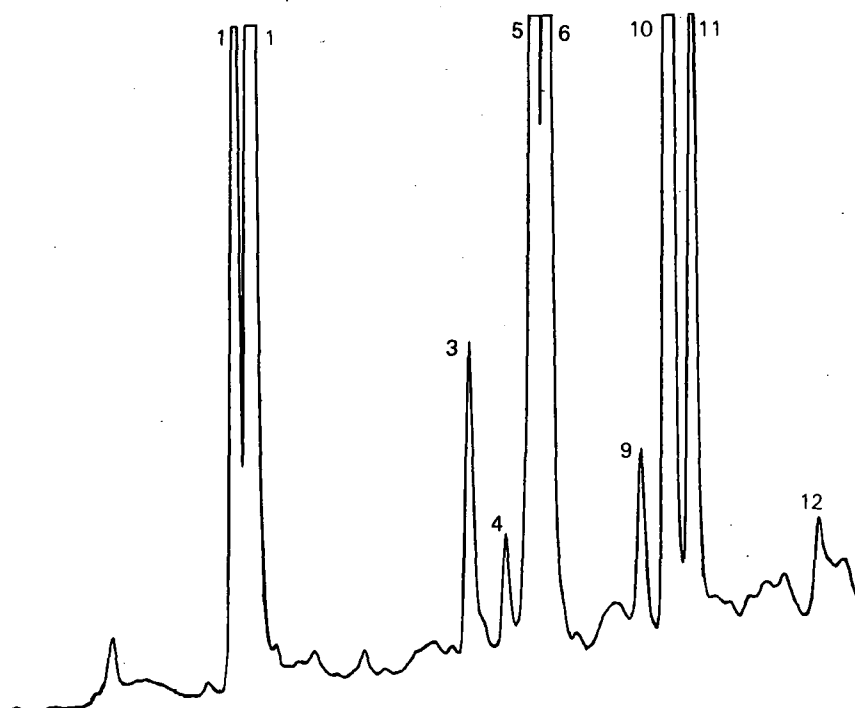


FIGURE 4 Separation of oxime-trimethylsilyl derivatives of monosaccharides in molasses on a glass capillary column coated with OV-17. (See legend of Figure 3 for further details).

Glucose

Again a normal distribution is obtained however the target value was consistently about 0,07 units higher than the routine results. We could find no reason for this bias. It is important to note that 97% of the differences lay within the confidence limits.

Sucrose

Once again the differences are distributed around zero. The distribution does tail to the right of zero with only 81% of the results within the limits. This slight positive bias was due to difficulties experienced with one of the check samples. Anomalies concerning this sample will be discussed later.

Interlaboratory Comparison

In-house quality control programmes are essential to monitor the validity of routine analyses. Any systematic bias produced in this laboratory is best detected by asking outside laboratories to collaborate in a comparative ring test. This was done by sending two independent laboratories ten molasses samples. The SMRI results were obtained by using the check molasses data determined immediately prior to dispatching the samples to the other laboratories. Excellent results were obtained for both fructose and glucose, the means from the three laboratories were extremely close ($\pm 0,03$ units).⁵ There were significant differences between the laboratories for the sucrose analyses. This disparity was traced to one of the check samples (Table 3).

TABLE 3

Interlab comparison for sucrose, check sample Z.	
Laboratories	Sucrose
Target Value	35,61
SMRI	35,31
Lab 1	35,80
Lab 2	35,44

The target value for this sample sat midway between the actual results obtained by the 3 laboratories, estimates ranging from 35,3 to 35,8. As agreement for the other 2 samples (with lower sucrose levels) was excellent it is apparent that deviation from linearity at higher levels was occurring. The GC at the Institute was leak tested and a small crack in the injector/carrier gas weldment was found and repaired. A control chart in Fig. 6 illustrates that correction of this fault during week 24 ensured that check values for sample Z were within specifications for the rest of the season.

Effect of linearity bias for sucrose on the weekly molasses results.

Monthly minimum, mean and maximum sucrose levels for S.A. mills for the period May to September are shown in Table 4.

TABLE 4

Sucrose % Final Molasses for S.A. Sugar Mills, 1982 Season			
Month	Minimum	Mean	Maximum
May	27,0	29,5	33,7
June	27,6	29,6	32,2
July	26,4	29,6	32,2
Aug	26,1	29,4	31,7
Sept	26,8	29,6	32,0

With the exception of Entumeni (highest at 33,7 during May) all mills produced a sucrose level below 32%. It is therefore unlikely that any significant errors occurred this season as a result of the small deviation from linearity at high sucrose concentrations earlier in the season.

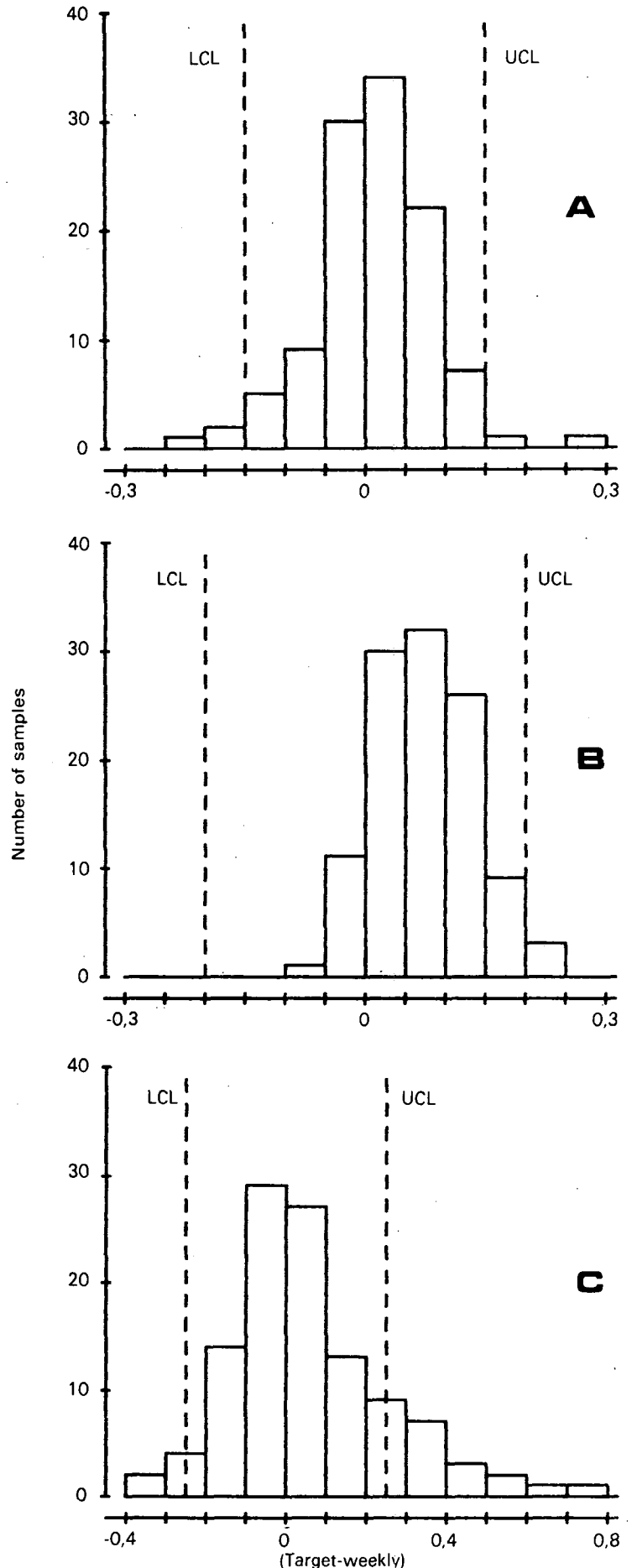


FIGURE 5 Comparison between target values and routine GC data for F, G and S for 3 molasses check samples for 1982/83 season. (A = Fructose, B = Glucose, C = Sucrose, UCL and LCL = upper and lower confidence limits respectively).

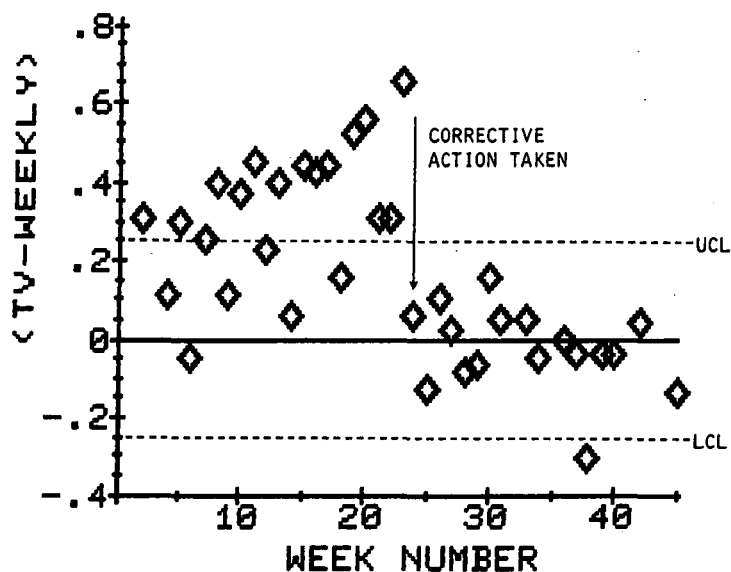


FIGURE 6 Control chart of the differences between the target value and routine GC data for sucrose for sample Z. (Vertical line indicates where corrective action was taken to repair GC. TV = Target Value).

Conclusions

Multi-level calibration provides both a statistically reliable response factor for sugars and is extremely useful in locating drift or systematic bias. The introduction of a repeatability yardstick has given us the opportunity of continuously monitoring the precision of routine GC data and confidently rejecting replicate outliers. The use of target values for molasses check samples has proved useful in monitoring accuracy and correcting for systematic bias before this offset becomes both significant and embarrassing. The quality assurance scheme described in this paper is obviously a prototype and will be improved upon in future milling seasons.

Acknowledgements

The co-operation of the staff of the GC laboratories of the Sugar Industry Central Board and Hulett R&D in the inter-laboratory comparison is gratefully acknowledged. The authors also wish to express their thanks to R. Lionnet for his assistance with the compilation of Anova computer programs.

APPENDIX I

Calculation of Repeatability

Repeatability of the molasses replicates was calculated by a one-way analysis of variance procedure. This technique results in the following breakdown of variances.

Source of Variations	Degrees of Freedom	Sum of Squares	Mean Squares	F-Value
Between Samples	n-1	SS _B	MS _B	
Between Replicates	N-n	SS _R	MS _R	
Total	N-1			

where N = total number of observations
n = number of molasses samples

The within replicate variance = MS_R and the repeatability variance = 2 × MS_R.
Repeatability = $t \times \sqrt{2 \times MS_R}$,
where $t = t_{N-n; \alpha/2}$ and t is approximated by 2.

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