THE DIRECT DETERMINATION OF LACTIC ACID IN CANE MOLASSES BY GAS-LIQUID CHROMATOGRAPHY

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Abstract

A method for the direct determination of lactic acid in cane molasses by gas-liquid chromatography is described. Data has been presented to show that the method is both precise and accurate. The method overcomes the lengthy ion-exchange separation of lactic acid, currently in use.

Introduction

The quantification of lactic acid in molasses is important as a means of determining sugar losses during the manufacturing process. Lactic acid can be formed during processing by the chemical breakdown of sucrose, glucose and fructose[1, 2, 3] and also by microbiological attack by bacteria on sugars.[4, 5, 6]. Therefore a simple rapid and accurate technique for determining lactic acid would be valuable for the optimisation of sucrose yields.

There have been numerous papers devoted to the determination of lactic acid.[7, 8, 9, 10]. At present, at Huletts Research and Development, lactic acid is determined by a lengthy ion-exchange separation followed by a colorimetric determination. The accuracy of the ion-exchange method is questionable and the concentration of lactic acid is reported with an eighty percent confidence limit.

The purpose of this investigation was to determine lactic acid in molasses, (after derivatisation of the lactic acid present), directly by gas-liquid chromatography using glass capillary columns. Various procedures have been published concerning gas-liquid chromatography to determine lactic acid in various process streams,[11, 12] and these differ from the proposed method in terms of the sample preparation procedure.

Initial research into a direct gas chromatographic method for lactic acid was carried out by workers from Braunschweig,[13] where they first mention the use of bis (trimethylsilyl) trifluoroacetamide as a derivatising agent. Work done at Huletts Research and Development in 1976[14] showed that the introduction of trifluoroacetic acid improved the derivatisation step. The author developed the following techniques which vastly improve the precision of this method for lactic acid :—

- performing the analysis by the internal standard method.
- introduction of acetonitrile as the solvent for the derivatisation step which ensures rapid quantitative derivatisation and also improves integration of the peaks since acetonitrile shows no serious tailing, and
- performing the analysis on a capillary column which has a greater ability to separate components as compared to a packed column.

Experimental

1. All reagents used were of analytical grade.
   (a) Bis (trimethylsilyl) trifluoroacetamide, (BSTFA); Ohio Valley Speciality Chemical Inc.
   (b) Acetonitrile; BDH Chemicals Ltd.
   (c) Trifluoroacetic acid; BDH Chemicals Ltd.
   (d) Lithium lactate; BDH Chemicals Ltd., and
   (e) Heptanoic acid; Sigma Chemical Company.

2. *Apparatus*

   Gas-liquid chromatography was performed on a fused silica, wall coated open tubular column, 0,31 mm x 15 m coated with SE-54 as stationary phase using a Hewlett Packard 5840 gas chromatograph with flame ionisation detection. Sample injection was in the split mode.

3. *Initial Recovery Trial*

   In order to evaluate whether quantification was possible by gas-liquid chromatography, a number of synthetic samples were analysed using the internal standard heptanoic acid. Prior to injection the sample required derivatisation of the carboxyl and hydroxyl groups. The general derivatisation procedure is outlined in the Appendix.

4. *Results*

   Two synthetic samples were analysed in duplicate using four calibration standards. Table 1 lists the amount of lactic acid added to the samples and the calculated amount of lactic acid recovered.

TABLE 1
Analysis of two synthetic lactic acid samples

Sample	Lactic acid added (%)	Lactic acid calculated (%)
1A	0,24	0,24 } 0,24
1B	0,24	0,24 }
2A	0,25	0,25 } 0,25
2B	0,25	0,25 }

   Samples 1A, 1B and 2A, 2B refer to duplicate determinations of the same sample. As the agreement between the expected and calculated percentage of lactic acid is excellent, the quantification of lactic acid in molasses appeared feasible provided that no interferences occurred.

5. *Analysis of final molasses samples taken from the Darnall, Felixton and Mount Edgecombe Mills*

   The general sample preparation procedure adopted for these molasses samples is outlined in the Appendix. All the molasses samples were analysed in duplicate using six calibration standards. Also, a synthetic sample was prepared to provide an external check on the recovery of the method. The results are listed in Table 2, which shows excellent agreement on duplicate samples and also excellent recoveries on the synthetic sample.

   These results show the method to be precise. To show that the method is also accurate we decided to spike a molasses sample with varying amounts of lactic acid which would then give an internal check on the accuracy of the method. This contrasts with the synthetic

sample in Table 2 which is an external check on the accuracy of the method.

TABLE 2
Analysis of final molasses samples

Sample	Lactic acid added (%)	Lactic acid calculated (%)	
DL A	—	0,12	0,12
DL B	—	0,12	
FX A	—	0,22	0,23
FX B	—	0,23	
ME A	—	0,23	0,23
ME B	—	0,22	
Synthetic 1A	0,18	0,18	0,18
Synthetic 1B	0,18	0,18	

The results are listed in Table 3. Samples FX1A and FX1B refer to the original molasses sample, whereas samples FX2A and FX2B and FX3A and FX3B refer to the original molasses sample to which was added 0,03 and 0,06 percent by mass of lactic acid respectively.

TABLE 3
Internal accuracy check results

Sample	Lactic acid added (%)	Lactic acid calculated (%)	
FX 1A	—	0,23	0,23
FX 1B	—	0,23	
FX 2A	0,03	0,25	0,26
FX 2B	0,03	0,27	
FX 3A	0,06	0,31	0,30
FX 3B	0,06	0,29	

From Table 3 it can be concluded that the method is precise and accurate since the calculated percentages agree with the theoretical percentages.

6. *Further Quantitative Data*

As the method had produced reliable data, it could now be used on a routine basis. Lactic acid in molasses was determined for all five Huletts mills and these results compared with the present ion-exchange colorimetric method in use. The samples used were final molasses monthly composite samples.

The results for December 1981 are listed in Table 4. Samples A and B refer to duplicate determinations for each mill's sample.

For gas-liquid chromatography each sample was determined in duplicate whereas they were determined once by the ion-exchange method. The synthetic samples were only used for the analysis by gas-liquid chromatography.

TABLE 4
Analysis of final molasses composites by gas-liquid chromatography (g l c) and ion-exchange procedures

Sample	Lactic acid (%)		
	By g l c		By ion-exchange
ME A	0,30	0,30	0,36
ME B	0,29		
EM A	0,22	0,22	0,26
EM B	0,22		
AK A	0,17	0,17	0,22
AK B	0,17		
FX A	0,25	0,26	0,32
FX B	0,26		
DL A	0,26	0,26	0,30
DL B	0,26		
			Lactic acid added (%)
Synthetic 1A	0,25	0,25	0,25
Synthetic 1B	0,25		0,25
Synthetic 2A	0,26	0,26	0,26
Synthetic 2B	0,26		0,26

Table 4 highlights two points :

(1) The ion-exchange method consistently overestimates lactic acid in molasses, probably because this method is not specific to lactic acid as is an analysis by gas-liquid chromatography, and

(2) the excellent agreement between duplicate samples analysed by gas-liquid chromatography.

Further quantitative data is presented in the Appendix, in Tables 5, 6, 7, and 8.

Discussion

Clearly the method developed for the quantification of lactic acid is precise and accurate. Sample preparation time has been kept to a minimum thus reducing analyst time. If the analysis is performed on an automatic gas chromatograph with data reduction facilities, then the total analyst time required for a batch of samples (5 here) is approximately three hours. This is a significant improvement on the ion-exchange procedure currently in use in this laboratory.

Figure 1 shows the chromatogram of a standard prepared as described. As BSTFA will derivatise all compounds with

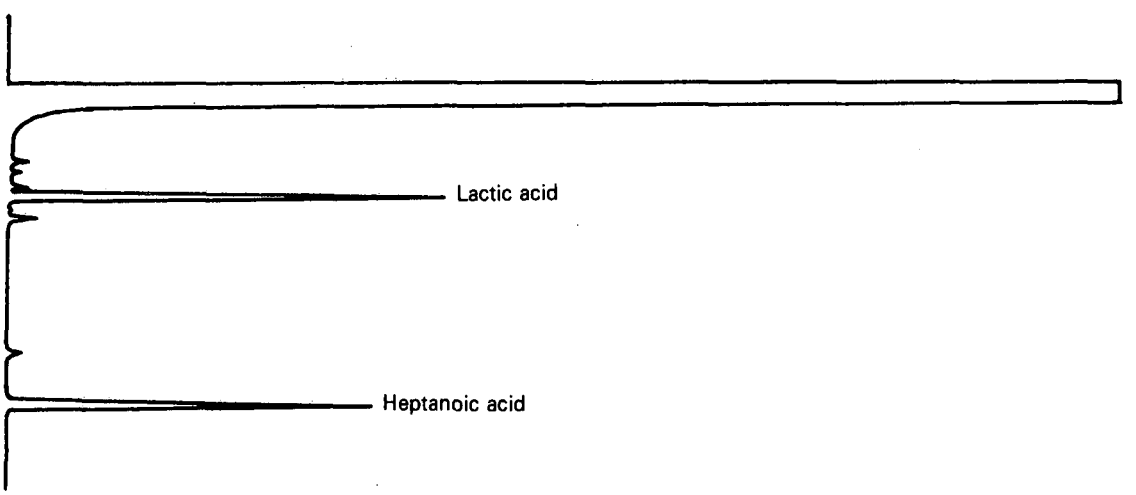

FIGURE 1 Chromatogram of lactic acid and heptanoic acid.

active hydrogen, all the sugars present will also become derivatised and thus volatile. Therefore, when analysing samples it is necessary to allow sufficient time for the sugars to elute before analysing the next sample.

It was shown that the response to varying concentrations of lactic acid was linear by a plot of

$$\frac{\text{area lactic acid}}{\text{area heptanoic acid}} \text{ versus } \frac{\text{mass lactic acid}}{\text{mass heptanoic acid}}$$

Conclusions

Accurate quantitative results are possible for samples containing 0,1% lactic acid. This lower limit can be decreased by derivatising larger aliquots of sample.

The use of packed columns is not possible for this analysis because the analysis required high resolution. For this reason capillary columns giving high resolution were used.

The choice of solvent was found to be important. Pyridine showed excessive tailing and thus acetronitrile was used.

The analysis of mixed juice is, in my opinion, not feasible by this method, because of the much lower concentrations of lactic acid present. A number of modifications are envisaged for this analysis and this will be the subject of a further report.

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Appendix

1. Chromatographic conditions

The following program was used for the the samples. Initial temperature was 95°C for eight minutes, thereafter programmed at 30°C per minute to 270°C with an analysis cycle of 30 minutes. The injection port temperature was 250°C and that of the flame ionisation detector was 270°C.

2. General derivatisation procedure

All samples were derivatised in the following manner. Each sample (5 $\mu\ell$) was dissolved in acetonitrile (0,1 ml) and to this solution was added BSTFA (0,5 ml) and trifluoroacetic acid (50 $\mu\ell$). The resulting mixture was heated at 80°C for ten minutes, cooled and chromatographed.

3. Sample preparation procedure

Molasses samples were prepared as outlined below. To the molasses sample (10 g) was added heptanoic acid (0,045 g) and water (5 g). Owing to the insolubility of heptanoic acid, a small quantity of concentrated sodium hydroxide solution (ca. five drops) was added and the resulting solution mixed. Five $\mu\ell$ was withdrawn in duplicate and derivatised as in (2) above. Three calibration standards plus two synthetic samples were also prepared with concentrations bracketing the molasses samples.

TABLE 5
Lactic acid in molasses — September, 1981

Sample	Lactic acid (%)		
	By g l c		By ion-exchange
ME A	0,22	0,22	0,29
ME B	0,21		
EM A	0,18	0,18	0,25
EM B	0,18		
AK A	0,41	0,41	0,45
AK B	0,40		
FX A	0,22	0,22	0,30
FX B	0,21		
DL A	0,11	0,11	0,28
DL B	0,11		

TABLE 6
Lactic acid in molasses - October, 1981

Sample	Lactic acid (%)		
	By g l c		By ion-exchange
ME A	0,29	0,29	0,29
ME B	0,29		
EM A	0,15	0,15	0,19
EM B	0,15		
AK A	0,23	0,23	0,26
AK B	0,23		
FX A	0,25	0,25	0,27
FX B	0,24		
DL A	0,27	0,27	0,31
DL B	0,26		

TABLE 7
Lactic acid in molasses - November, 1981

Sample	Lactic acid (%)		
	By g l c		By ion-exchange
ME A	0,23	0,23	0,31
ME B	0,23		
EM A	0,13	0,13	0,21
EM B	0,13		
AK A	0,15	0,15	0,22
AK B	0,15		
FX A	0,23	0,23	0,29
FX B	0,23		
DL A	0,23	0,23	0,29
DL B	0,22		

TABLE 8
Lactic acid in molasses - January, 1982

Sample	Lactic acid (%)		
	By g l c		By ion-exchange
ME A	0,30	0,30	0,35
ME B	0,29		
EM A	0,21	0,21	0,27
EM B	0,20		
AK A	0,17	0,17	0,25
AK B	0,17		
FX A	0,23	0,23	0,30
FX B	0,23		
DL A	0,21	0,21	0,28
DL B	0,21		

4. Further quantitative data

Data for all five Huletts Mills for the months September, 1981, October, 1981, November, 1981 and January, 1982 are listed in tables 5, 6, 7 and 8. Comparisons are made with the ion-exchange method.

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