

THE APPLICATION OF HIGH PRESSURE LIQUID CHROMATOGRAPHY FOR PROCESS CONTROL IN A SUGAR FACTORY AND ETHANOL PLANT

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Abstract

During the 1983 season, high pressure liquid chromatography (HPLC) has been used with great effect by Triangle Limited in their ethanol plant and sugar factory.

The advantages and disadvantages of the technique, compared with gas liquid chromatography (GLC) and classical methods of analysis and process control are discussed, and examples of actual and potential value to the industry are given.

It is concluded that whilst there are areas in which other techniques have advantages, HPLC will become an increasingly valuable and widely used analytical and process control tool.

Introduction

The application of chromatographic methods to chemical analysis has had an enormous and profound effect on the science of analytical chemistry. Coupled with other technological advances such as micro-processor control and data processing systems the capabilities of modern chromatographic instrumentation exceed by far those imagined possible ten or even five years ago.

The essential feature of chromatographic methods is that the component of interest is separated from other substances in the analytical sample before quantification. This separation from possible interference, contrasts sharply with almost all classical methods, in which precipitation, titration, distillation, optical rotation measurement, or any other procedure is carried out on a mixture of substances.

Gas liquid chromatography (GLC) has been applied with considerable success to the analysis of sugars in factory products.^{1, 2, 3} In GLC, volatile substances in a stream of inert gas are separated by passing them over a stationary liquid phase

of low volatility. As sugars are not volatile, they must first be converted into volatile derivatives, and several elegant and well documented procedures have been developed to achieve this.^{4, 5}

In high pressure liquid chromatography (HPLC) the moving phase is a liquid, and the stationary phase is a solid, and apart from the purely practical differences arising from the need to achieve and control the flow of a gas in the one case, and a liquid in the other, there are no fundamental differences between the two. An obvious practical advantage of HPLC in the analysis of sugars, however, is their ready solubility in water which can be used as the moving phase. Thus the need to prepare derivatives before HPLC, does not exist, and apart from filtration, no sample preparation is necessary.

Until the Triangle Limited ethanol plant was commissioned in July 1980, the analytical workload in its mill laboratory was no different to that of any other sugar factory. The need for a more accurate analysis for fermentable sugars in feedstocks for ethanol production soon became obvious, however, as did the need for more reliable methods of trace sugar analysis in stillage, and for ethanol in fermented beer and stillage. Classical methods for the analysis of sucrose, glucose and fructose in molasses are tedious and technically demanding. In addition, glucose and fructose cannot be distinguished from each other, or from other reducing substances either in the molasses, or in the even more complex mixture obtained during and after fermentation.

Chromatographic methods offer the only practical solution to an analytical problem of this nature and the decision was taken to purchase a high pressure liquid chromatograph. The principal reasons for the preference of HPLC over GLC were the simplicity of sample preparation and operation, and its relative cheapness both in capital and running costs. Added to

TABLE 1
Reproducibility of Sucrose, Glucose, Fructose and Ethanol by HPLC

Date	Time	Run no.	SUCROSE			GLUCOSE			FRUCTOSE			ETHANOL			
			Area	Mean area	% S.D.	Area	Mean area	% S.D.	Area	Mean area	% S.D.	Area	Mean area	% S.D.	
12.5.83	11:11 13:54	152 161	48 940 48 537	48 739	0,58	56 070 55 021	55 546	1,34	56 048 54 541	55 295	1,93	92 058 91 985	92 022	0,06	
27.5.83	09:55 11:57	250 257	78 916 78 927	78 922	0,01	92 215 91 460	91 838	0,58	87 315 87 447	87 381	0,11	76 423 78 740	77 582	2,11	
6.6.83	10:33 15:05	412 429	105 030 108 690	106 860	2,42	94 094 93 715	93 905	0,29	100 820 100 670	100 745	0,11	98 298 98 493	98 396	0,14	
21.6.83	09:35 13:41	597 613	42 817 42 655	42 736	0,27	57 211 57 727	57 469	0,63	61 177 62 222	61 700	1,20	53 732 52 772	53 252	1,27	
5.7.83	09:02 16:18	721 740	61 309 61 810	61 560	0,58	62 040 60 322	61 181	1,99	65 480 64 387	64 934	1,19	55 378 54 686	55 032	0,89	
15.7.83	11:58 15:21	28 45	82 074 82 286	82 180	0,18	90 764 93 197	91 981	1,87	86 889 87-188	87 039	0,24	57 150 56 054	56 602	1,37	
		Means													
						0,67				1,12				0,80	0,97

this was that the results would be available within minutes of samples being taken; that all components of interest (sucrose, glucose, fructose, ethanol and glycerol) would be quantified in one injection and that unskilled laboratory staff would be able to carry out HPLC analyses satisfactorily.

The HPLC system installed in the Triangle mill laboratory has exceeded all expectations and has been used to great advantage in the ethanol plant and sugar factory.

Accuracy and Precision

The reproducibility obtained using HPLC for the analysis of sugars is exceptionally good. Many studies have been reported^{6, 7, 8} and replication has been highly satisfactory in the mill laboratory. To illustrate this peak areas of sucrose, glucose, fructose and ethanol of standard solutions which were analysed at different times and on different days during routine operation of the instrument, are shown in Table 1.

The accuracy of HPLC results is more difficult to assess as no definitive method other than GLC exists. Recovery experiments were carried out by adding three different levels of sucrose, glucose, fructose and ethanol to final molasses. The recoveries obtained, which are shown in Table 2, are excellent and are in good agreement with similar published data.^{6, 8}

The possibility of interference by other sugars or non-sugars was investigated by analysing factory products (mixed juice, syrup, B-molasses and C-molasses) before and after the addition of invertase, and after fermentation with bakers yeast. No detectable peaks were found to be present under the sucrose peak which was removed completely by the invertase, or under the glucose and fructose peaks after fermentation. The production of small amounts of trehalose during fermentation is of importance however, as this sugar would be only partially resolved from sucrose if both were present in the same mixture.

Interference by inorganic species (K^+ , Mg^{++} , Ca^{++} , Na^+ , Cl^- , SO_4^{--} , PO_4^{--} , and NO_3^-) is not likely, and a mixed bed ion exchange resin guard column can be installed in the system just before the Sugarpak column. The possibility that some interference might occur was tested by the injection of sugar and ethanol standards made up with water containing those ions, and no evidence of interference was obtained.

Experimental Procedure

The equipment installed at Triangle and which was used for the work described in this paper was the Waters Sugaranalyser I Liquid Chromatograph with the R401 refractive index detector coupled to a Hewlett-Packard HP3390 integrator.

The Waters Sugarpak column was used at 90°C, with a solvent flow of 0,5 ml min⁻¹ and operated at a pressure of 5,17 MPa.

The solvent used was de-ionised distilled water filtered through a 0,45 μ membrane filter and containing 20 mg l⁻¹ of mono-calcium EDTA or calcium acetate.

Sample preparation consisted of dilution to suitable concentration and filtration through a 0,45 μ membrane filter. Injection of 20 μl samples were carried out using a rheodyne F010 manual sample injection valve. Calibration by external standardisation.

Integration parameters:

Attenuation 2⁷
 Threshold 5
 Area reject 5 000
 Peak width 0,16
 Retention time window 5(%)
 Variation 0,5(%)

Detector sensitivity was 8X and response was linear for all concentrations encountered (0,6 — 0,006 percent sugar in solution as injected).

After the injection of some 800 samples, *in-situ* regeneration was necessary to restore resolution, but the use of re-chargeable guard columns greatly reduces the need for such regeneration.

Applications

A comparison of the results obtained from analysis of various factory products by HPLC and by standard chemical methods is shown in Table 3.

These results show that for high purity products, classical methods are in good agreement with HPLC, but that this is not the case when products of low purity are analysed. This is true also of analysis by GLC^{7,9} and as mill laboratories are well equipped to carry out large numbers of routine pol and reducing sugar determinations, there is little advantage to be gained in

TABLE 2
 Recoveries of Sucrose, Glucose, Fructose and Ethanol added to final Molasses

	Sucrose g/100 ml			Glucose g/100 ml			Fructose g/100 ml			Ethanol g/100 ml		
	added	recovered	% recovered	added	recovered	% recovered	added	recovered	% recovered	added	recovered	% recovered
Initial	—	0.3549	—	—	0,0232	—	—	0,0572	—	—	—	—
Level 1	0.0219	0.0217	99,08	0,0189	0,0191	101,05	0,203	0,2010	99,01	0,0511	0,0508	99,41
Level 2	0.0438	0.0437	99,77	0,0378	0,0372	98,41	0,406	0,0411	101,23	0,1022	0,1025	100,29
Level 3	0.0657	0.0654	99,54	0,0567	0,0579	102,12	0,609	0,0607	99,67	0,1533	0,1527	99,61
	Mean % recovery		99,46			100,52			99,97			99,84

TABLE 3
 Comparison of HPLC and chemically determined sugars in factory products

Product	Number of analyses	HPLC sucrose %	Pol %	HPLC glucose %	HPLC fructose %	HPLC glucose + fructose	Reducing sugars	Purity
Mixed Juice	10	11.55	11,52	0,25	0,26	0,51	0,52	85,48
Syrup	4	53,16	53,43	1,29	1,29	2,58	2,73	84,81
B-Molasses	4	45,81	46,01	5,14	6,63	11,77	13,78	52,83
C-Molasses	8	32,50	29,72	2,51	5,50	8,01	13,12	37,23

considering chromatographic methods for routine analysis of products such as juices, unless information on glucose and fructose, rather than total reducing sugars, is required.

HPLC has been used for the analysis of mixed juice, clarified juice, filtrate and samples from evaporator vessels to obtain information on sucrose losses by microbiological and chemical pathways. The ease and rapidity of analysis has enabled useful information to be obtained.

After brief investigation¹⁰ it was established that HPLC could be used to monitor sugar levels in condensate, as levels as low as 5 ppm are detectable using the refractive index detector as a flow through cell. The possibility of dextran analysis in juices,

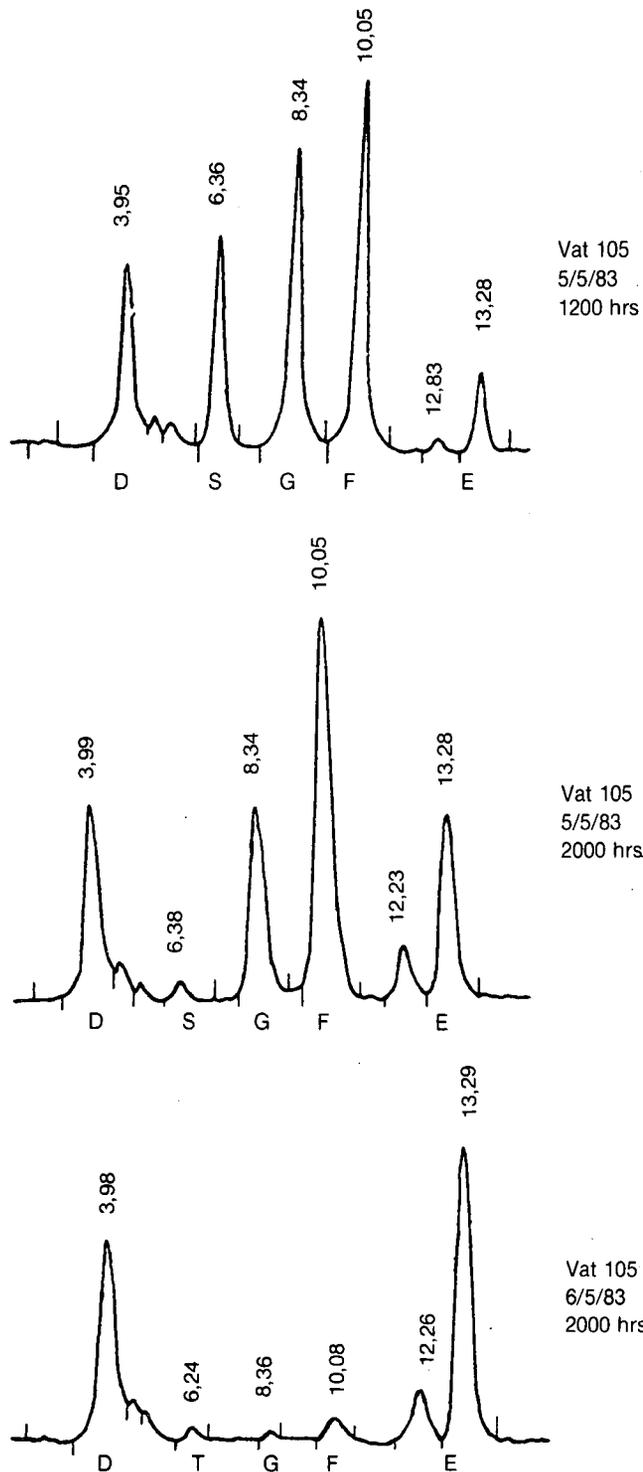


FIGURE 1 HPLC traces of fermentation 4, 12 and 20 hours after initiation.
(D = dextrans, polysaccharides and ionics, T = trehalose, G = glucose, F = fructose, E = ethanol)

pan floor products and sugars, is another area which has only cursorily been examined, but which would apparently be equally straightforward. Because dextrans cannot be rendered volatile by derivatisation, and pose very great problems to purely chemical methods, HPLC appears to be the most likely method by which this analytical challenge will be solved.

Most investigations into sugar factory problems using HPLC at Triangle have been curtailed by the more pressing need for accurate analysis of ethanol plant feedstock and wastes, and the majority of instrument time has been devoted to these problems.

In Figures 1 and 2, the great value of chromatographic analysis in fermentation plant operation is illustrated. HPLC traces obtained from samples taken 4, 12 and 20 hours after initiation of fermentation are shown in Figure 1 and the sucrose, glucose and fructose consumption and the ethanol production curves obtained by analysis of all four-hourly samples from the same vat are shown in Figure 2. The production of detailed information such as this has provided management with an excellent basis on which to plan and carry out changes in pre-fermentation (yeast growth), fermentation with stillage recycle and fermentation with yeast recycle.

One of the immediate benefits of such analysis is that information on completeness of fermentation is obtained within 15 minutes of sampling and this means that the decision to delay distillation on a vat can be made quickly, and with certainty.

The routine measurement of losses is illustrated in Figure 3 in which typical HPLC traces of stillage samples containing unfermented fructose and residual ethanol is shown. These chromatograms also illustrate the sensitivity of the technique as the fructose concentration is 0,05% and the ethanol content is 0,08%.

The benefits which have accrued to us from the use of HPLC in the ethanol plant are considerable, and loss controls as illustrated above have saved many thousands of dollars. The intangible value of rapid and reliable data acquisition with the benefits of increased confidence in process control decisions and alterations to operating procedures cannot be so easily calculated and is arguably far greater.

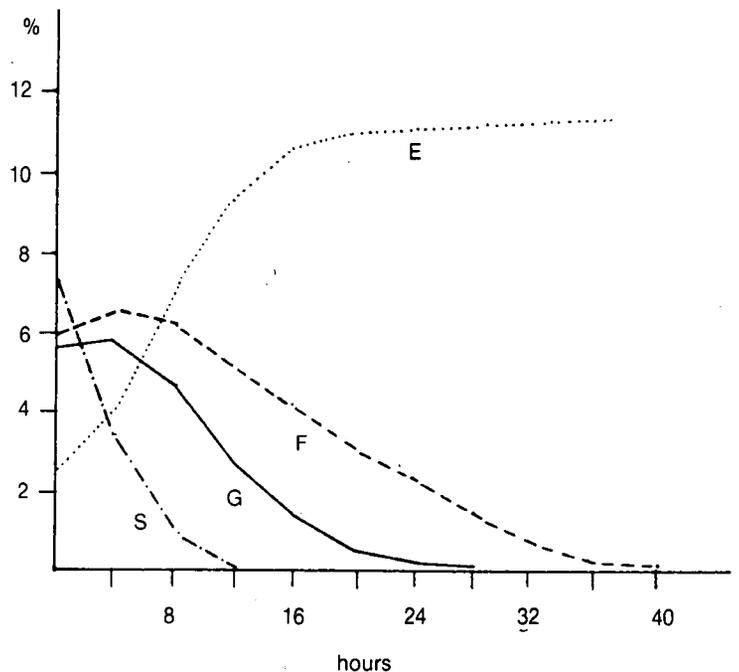


FIGURE 2 Sucrose (S), glucose (G) and fructose (F) consumption and ethanol (E) production during fermentation.

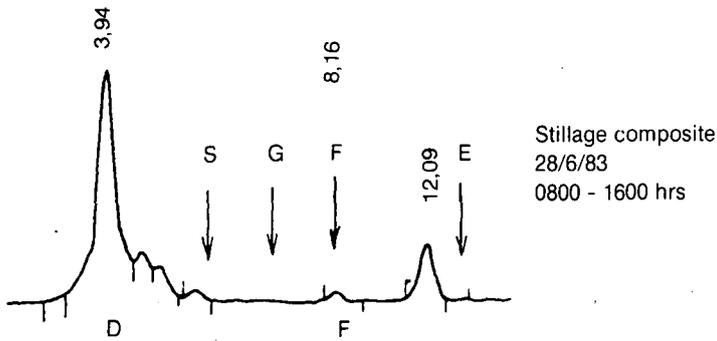
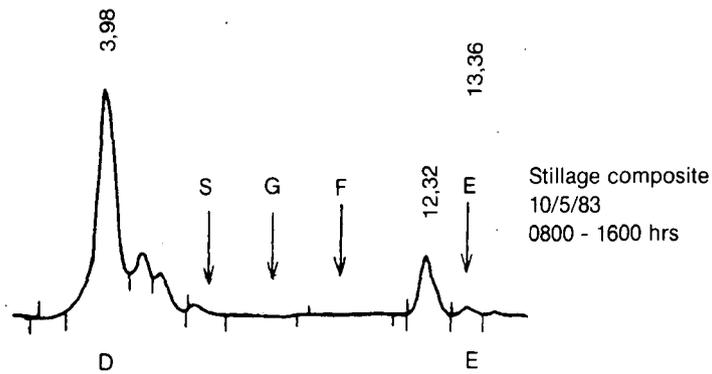


FIGURE 3 HPLC traces of stillage composite samples. (D = dextrans, polysaccharides and ionics, S = sucrose, G = glucose, F = fructose, E = ethanol)

Conclusions

The advantages of chromatographic methods for the analysis of sugars and ethanol in factory and fermentation products and distillation of plant waste are well known and widely accepted as being superior to classical chemical methods. Although there

is no great need to replace methods such as pol and reducing sugar determinations in high purity juices and factory products, chromatographic methods offer rapid and reliable analysis of low purity and fermentation products. The trend towards measurement of true sucrose rather than pol and of glucose and fructose rather than reducing sugars for factory and process control, will be accelerated by the advent of HPLC, which offers a more rapid and less expensive route than GLC. The simplicity of sample preparation and instrument operation in HPLC enables its use by unskilled laboratory staff and in the mill.

Many valuable applications of HPLC methods such as trace sugar and dextran analysis require further investigation, and the great and increasing interest in the application of the technique in the international sugar industry augurs well for its future.

The greater resolving power of capillary column GLC will ensure its continued use for research purposes, particularly when detailed information on complex sugar mixtures is required.

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