ANALYTICAL CHROMATOGRAPHIC SOLUTIONS FOR SUGAR PROCESSING

S N WALFORD, K J SCHÄFFLER and P G MOREL DU BOIL

Sugar Milling Research Institute, University of KwaZulu-Natal, Durban, 4041, South Africa
E-mail: swalford@smri.org, kschaffler@smri.org and pduboil@smri.org

Abstract

An overview is given of some of the varied chromatographic techniques which have been used in the South African sugar industry over the past 30 years. These include accurate, precise analytical procedures used for cane payment and factory control. Chromatographic procedures for estimating cane delays, troubleshooting for bacterial infection, factory corrosion and measuring inversion in evaporators are reported. Other applications include the determination of polysaccharides, anions and cations in factory products such as juices, molasses and VHP and white sugars.

Keywords: chromatography, GC, HPLC, HPIC

Introduction

The South African sugar industry has successfully used analytical chromatographic separation techniques for cane payment, factory control and troubleshooting for more than 20 years. The use of these techniques arose from the desire of sugar technologists to determine, directly, the concentration of sucrose in process streams instead of by inference using indirect methods. Analytical chromatography has matured and few developments applicable to the sugar industry are likely to take place in the foreseeable future. This review thus serves to record most of the chromatographic applications in the South African industry over the past 30 years. The review will consider the routine analytical application of chromatography, as well as methods developed for troubleshooting in the factories and for use in research projects.

Discussion

Chromatography as an analytical tool

Chromatography is essentially a method for separating components present in a mixture. It can be used both to identify and determine the concentration of a constituent in the mixture. All chromatographic methods separate compounds based on their distribution between two phases, one of which is stationary (stationary phase or chromatographic bed), while the other (mobile phase) moves in a definite direction. Compounds in the mixture partition between these two phases at different rates, thus separating from each other. A variety of different chromatographic techniques has been used during this period.

These include:

- Thin-layer chromatography (TLC). This is a planar chromatographic technique. The stationary phase is present as a flat surface (the chromatographic bed can be paper or a thin layer of stationary phase bound to a glass or aluminium sheet). The mobile phase moves across the bed by capillary action. Detection of the separated compounds is

achieved by drying and spraying the plate with a reagent that reacts with the compounds of interest to form coloured spots. This method is useful for identification, isolation of compounds and, with the use of modern multi-wavelength densitometers, can be used for quantitative analysis.

- Gas chromatography (GC). Volatile substances are separated in a stream of inert gas (mobile phase) flowing over a film of non-volatile material (either coated packing in a glass or stainless steel column, or bonded onto the inside of a silica or glass column). Detection of the separated compounds is achieved using a detector placed at the exit of the column. The most common is the flame ionisation detector (FID).
- Liquid chromatography (LC). Soluble substances are separated in a flowing liquid stream (mobile phase or eluent) over a chemically bonded stationary phase. LC may be classified according to the mechanism of separation leading to many different acronyms, e.g. IC (ion chromatography), HPAEC (high performance anion exchange chromatography), IEC (ion exclusion chromatography), etc. Common detectors used in LC of sugar streams include refractive index (RI), ultraviolet (UV), conductivity and the pulsed amperometric detector (PAD).

It is not the purpose of this review article to describe these techniques. The reader is directed to standard chromatography textbooks for this information (Snyder and Kirkland, 1979; McNair and Miller, 1998).

Routine analytical chromatography

Cane payment and factory control

Research into the possible use of GC in South Africa for the accurate measurement of sucrose in sugar processing streams began in 1972. One of the drawbacks of GC is the requirement that the compounds to be analysed must be volatile. This requires derivatisation of the sugars to produce a GC compatible, volatile form. Silylation using a trimethylsilylation reagent is probably the most widely used derivatisation procedure. This procedure required anhydrous conditions, so samples were freeze-dried to remove water before derivatisation. Early work had shown that GC could be a specific and reproducible procedure for the determination of fructose, glucose and sucrose in molasses (Schäffler and Loker, 1974). The lengthy sample preparation precluded its use as a routine analysis method. Nurok and Reardon (1975) showed that the superior resolution on open tubular columns compared with that on packed columns allowed for greater confidence in the analysis. This resulted in subsequent sucrose and pol data for final molasses being published (Kort *et al.*, 1975).

Silylation in the presence of water was the breakthrough required for GC to become a routine method of analysis for sugars. This eliminated the need for drying of the samples (Schäffler and Morel du Boil, 1984). A comparison of the polarimetric and GC methods for sucrose estimation in mixed juice (MJ) and molasses showed the feasibility of calculating the pol/sucrose ratio and for its use in factory performance calculations (Schäffler, 1976). This led the South African Sugarcane Technologists' Association's Factory Advisory Control Committee to request the Sugar Industry Central Board (SICB) to determine sucrose routinely in MJ at two mills, and compare the results with the standard pol method (Brokensha *et al.*, 1978). The results showed that the GC method could be used routinely to determine the sucrose content of MJ, and to apply this to the individual pol analyses for each week. Freeze preservation of MJ samples allowed the compositing of hourly samples to produce weekly composite samples for the determination of sucrose by GC (Brokensha, 1979). Subsequently, the 1982-83 season saw the official implementation of GC based sugar data for chemical control in South African raw sugar factories. The Cane Testing Service

undertook analysis of weekly composite MJ samples at a central location, while the Sugar Milling Research Institute (SMRI) undertook analysis of weekly molasses samples. Brix and pol determinations on the weekly composite samples provided an accurate check on the juice preservation procedure and the accuracy of each individual cane testing laboratory.

To ensure the integrity and quality of the GC data, a quality assurance program was instituted for both MJ and molasses analyses (Brokensha *et al.*, 1978; Schäffler and Day-Lewis, 1983). This consisted of including both check samples and control samples, which were routinely analysed with the weekly samples, allowing the operators to monitor both precision and accuracy of the methods.

The original routine GC methods used by SICB were developed to analyse sucrose only in MJ. This gave a reasonably rapid analysis – approximately 10 minutes per analysis (Figure 1). An example of the use of GC instead of pol is shown in Table 1 (Schäffler and Smith, 1978).

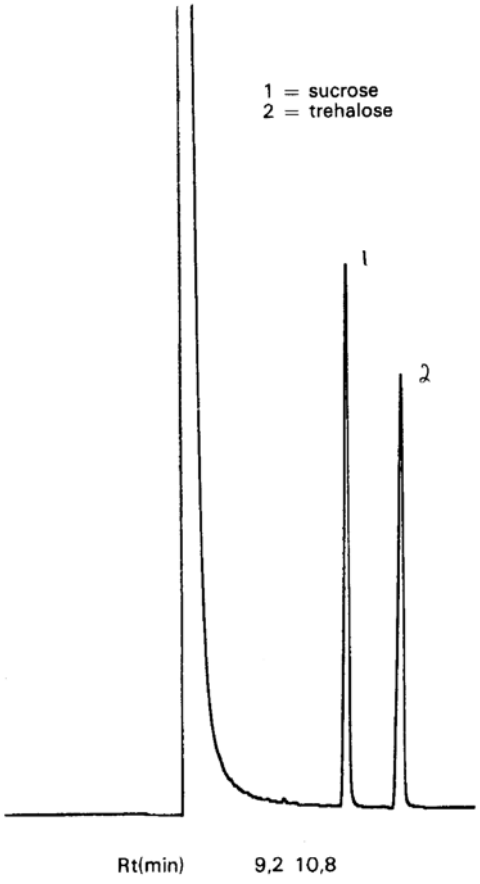

Figure 1. Separation of TMS derivatives of sucrose and trehalose (internal standard) in a mixed juice sample (from Brokensha *et al.*, 1978).

Due to the fact that pol/sucrose ratios in MJ are almost always less than 1.0, and because more glucose is destroyed than fructose in the backend (see later), pol underestimates sucrose losses during processing. Pol-based factories are therefore unaware of the significant losses occurring during sugar manufacture.

Table 1. Comparison of cane quality and factory loss data based on pol and sucrose.

	Basis	
	Pol	Sucrose
Mixed Juice (%)	11.7	11.9
Molasses (%)	25.5	28.2
Cane quality:		
Pol, sucrose % cane	13.0	13.2
Non-pol, non-sucrose % cane	2.8	2.6
Purity of mixed juice	83.6	85.0
Losses % in cane in:		
Bagasse	2.9	2.9
Filter cake	0.6	0.6
Molasses	7.7	8.4
Undetermined	1.6	2.4

Analysis of fructose and glucose in sugar streams is of interest for the following reasons:

- The Lane and Eynon titrimetric method can overestimate glucose and fructose in molasses samples (Schäffler and Loker, 1974).
- The fructose/glucose ratio could give indications of factory processing problems.
- The reducing sugar/ash ratio can be used in Target Purity Difference (TPD) calculations (Rein and Smith, 1981).

Direct silylation of fructose and glucose gives rise to multiple peaks due to the aldo- or keto-reducing group existing in solution in two tautomeric forms resulting in the α- and β-anomers when silylated. Schäffler and Morel du Boil (1981) described the use of a buffered oximation reagent to produce the oxime-TMS derivatives whilst avoiding sucrose hydrolysis. This procedure reduced the number of multiple peaks to two for each monosaccharide, making quantitation easier. The use of modern megabore capillary columns reduces the resolution between the two peaks for each monosaccharide, resulting in a single peak for each sugar (Figure 2). Xylose and trehalose are added to the sample as internal standards for quantitation purposes.

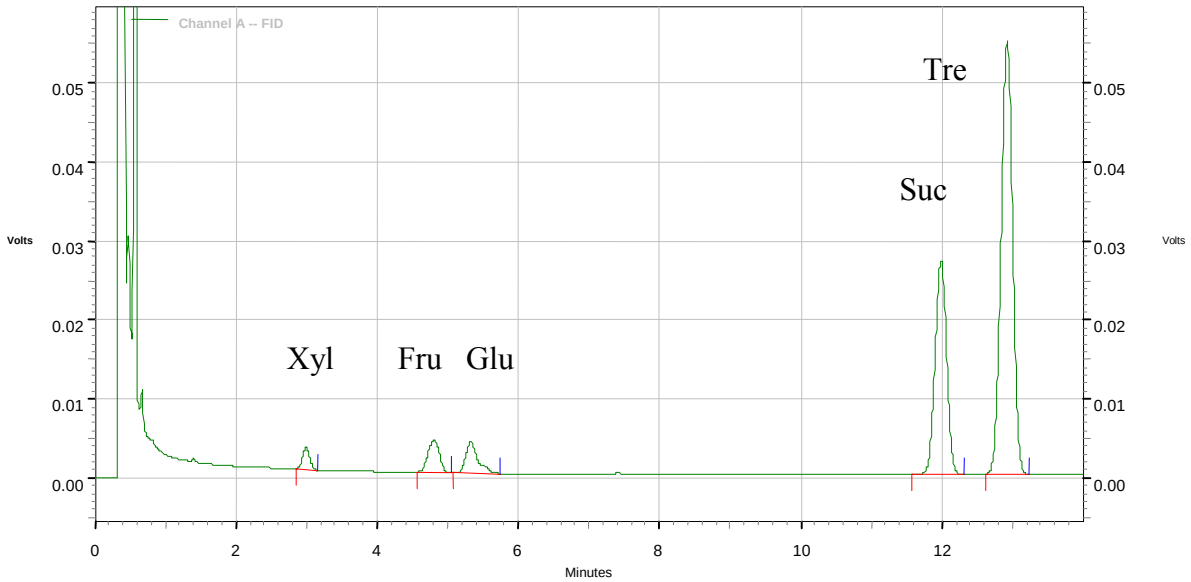

Figure 2. Separation of a standard mixture on a megabore column (Xyl = Xylose, Fru = Fructose, Glu = Glucose, Suc = Sucrose, Tre = Trehalose).

The use of GC procedures do have a number of advantages over the HPLC techniques described in the next section.

These include:
- The derivatisation procedure ensures that the chromatogram is relatively free from interfering impurities.
- Derivatisation results in sample stability prior to sample injection.
- GC maintenance is generally easier and less costly than HPLC.

Disadvantages of the GC method include:
- Sample preparation is tedious.
- Derivatisation chemicals are toxic.
- Sample derivatisation could lead to errors.

HPLC

HPLC does not suffer from the derivatisation problems associated with GC and would thus seem to be a logical successor to these methods. Initial success with GC was due to the high resolution achieved in the 1970s. In comparison, HPLC suffered from low resolution and insensitive detection methods. However, research into HPLC methods for the routine analysis of sugars in factory streams continued. Initial methods used reverse phase and amino-bonded silica columns for separation of the sugars, followed by RI detection. A major drawback with these columns is the necessity to use expensive or toxic solvents. Also, reducing sugars react with primary and secondary amino groups in the packing causing low glucose recoveries (Schäffler, 1981).

The introduction of cation exchange columns in the hydrogen, calcium or sodium form overcame some of these problems. Numerous studies showed that there was little difference in results between HPLC using these columns and GC across a range of samples including MJ, clear juice (CJ), syrup and molasses (Day-Lewis and Schäffler, 1990; Chorn and Hugo, 1984). This method allowed for the rapid determination of total ash, total oligosaccharides, sucrose, glucose, fructose and ethanol in a single run (Day-Lewis, 1994) and was used to monitor five factory streams at two mills throughout a season (Day-Lewis, 1995).

Cation columns nevertheless have a number of drawbacks, including:
- Limited separation power due to the size exclusion/ligand exchange separation mechanism.
- Non-specificity of the RI detector leading to possible over-estimation of carbohydrate concentrations (Anon, 1990).

Anion exchange chromatography using PAD to monitor the separation of the carbohydrates overcomes these problems. The PAD is more specific for sugars as the oxidation potential of the detector can be optimised for detection of the carbohydrates. Varying the alkalinity and/or ionic strength of the mobile phase can alter the separation power of the column. Initial results showed poor agreement between GC and HPAEC for sucrose (Morel du Boil and Schäffler, 1990). However, adding lactose as an internal standard and using a method of bracketing each sample with standards to correct for the PAD drift, improved the precision of the HPAEC method (Thompson, 1990). Subsequent comparison between GC and HPAEC for both MJ and molasses showed good repeatabilities and precision for the HPEAC method. This resulted in the South African industry replacing GC with HPAEC for mill weekly composite molasses samples from the 1992/93 season (Schäffler and Day-Lewis, 1992) (Figure 3). As with most analytical methods, attention to detail and the use of control and check samples ensured precise and reproducible results (Schäffler *et al*, 1996). The method

was considered as a possible alternative to analyse weekly MJ samples when using sodium azide as a microbial inhibitor. Comparison with the official GC method showed that agreement for the three sugars was good, but that precision for the GC sucrose was significantly better (Walford, 1996a). Some problems were encountered with inversion of the MJ samples during the long run times of the automated analysis. This was not a problem with the derivatised GC samples due to the use of toxic pyridine as a solvent.

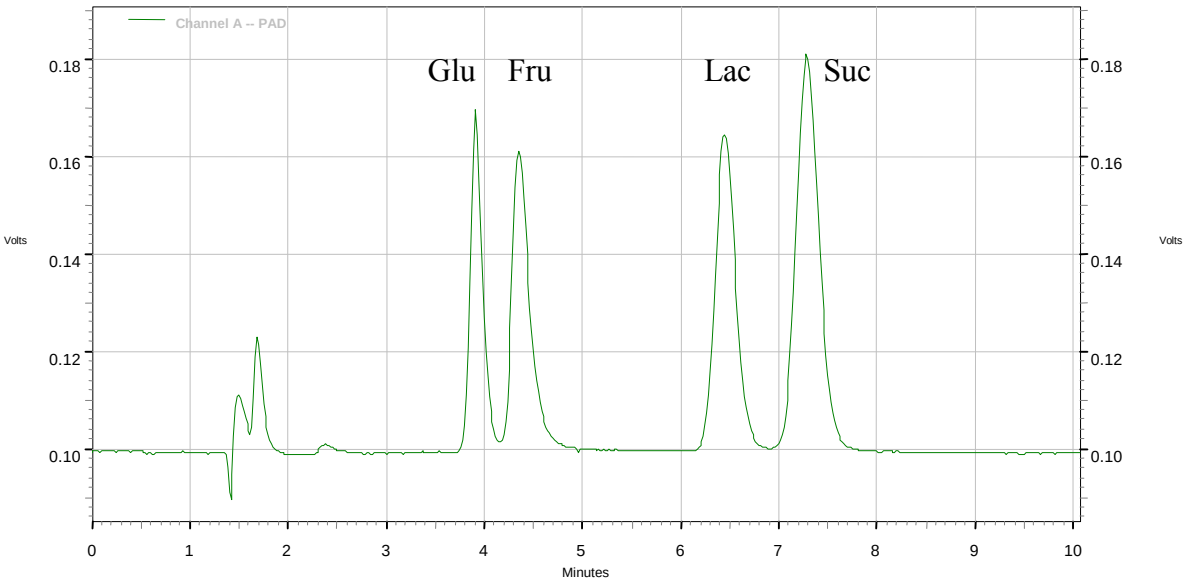

Figure 3. HPAEC chromatogram of molasses
(Glu = Glucose, Fru = Fructose, Lac = Lactose, Suc = Sucrose).

Good exhaustion of final molasses is necessary to maximise sucrose recovery, especially when impurities are high. The type of impurity present is known to affect the purity of molasses. A target purity is calculated based on the ratio between reducing sugars and ash for each mill's molasses samples. The difference between this target purity and actual purity represents the level of exhaustion performance. In South Africa the reducing sugars are measured by HPIC, and used to determine the target purity:

$$\text{Target purity} = 43.1 - 17.5(1 - e^{-0.74\,(\text{Fru} + \text{Glu}) / \text{Ash}})$$

This formula, using fructose and glucose measured by HPIC, does not show seasonal trends as do those based on reducing sugars determined by the Lane and Eynon method.

ICUMSA/AOAC

The use of collaborative studies under the auspices of the International Commission for the Uniform Methods of Sugar Analysis (ICUMSA) has been a feature throughout the development work and acceptance of these methods. The adoption of the various methods is shown in Table 2. The HPAEC method for analysis of sucrose, glucose and fructose in beet and cane molasses was given first action by AOAC International (Schäffler *et al*, 1997). A sub-section study (Schäffler, 2002) for determining glucose and fructose in raw sugar by HPAEC was also officially accepted by AOAC International.

Table 2. Chromatographic methods adopted by ICUMSA.

Subjects	Current ICUMSA method	Analysis method	Adoption
GS1/2/3-4	The determination of glucose and fructose in raw and white sugars using high performance anion-exchange chromatography (HPAEC)	HPAEC Ion exchange	1998
GS4/7/8/5-2	The determination of sucrose by gas chromatography (GC) in molasses and factory products	GC	1986
GS4-22	The determination of sucrose and betaine by HPLC in beet molasses	HPLC Cation exchange	2002
GS7/4/3-22	The determination of fructose, glucose and sucrose by gas chromatography (GC) in cane juices, syrups and molasses	GC	1998
GS7/4/8-23	The determination of sucrose, glucose and fructose by HPLC	HPLC Cation exchange	2002
GS7/8/4-24	The determination of glucose, fructose and sucrose in cane juices, syrups and molasses and sucrose in beet molasses by high performance ion chromatography	HPAEC Ion exchange	1998

Factory troubleshooting

Lactic acid

The quantifying of lactic acid in process streams is a means of determining possible sucrose losses during the milling process. Lactic acid can be formed by the chemical breakdown of sucrose, glucose and fructose and/or by thermophilic bacteria. It has been estimated that for every part of lactic acid produced by bacteria, approximately two parts of sugar have been lost in the mill (McMaster and Ravnö, 1977). Furthermore, these authors showed that the lactic acid produced ended up in the final molasses, resulting in further exhaustion losses. Initially, a lengthy ion-exchange extraction procedure to isolate the acid from the process streams was followed by a colorimetric analysis to determine the lactic acid. A direct derivatisation of molasses was used as the basis of a GC method (Chorn, 1982). A simpler, headspace procedure (Shore *et al.*, 1982) was investigated and adopted at the SMRI (Day-Lewis, 1983). In this method lactic acid is oxidised to acetaldehyde, which is measured directly on a packed GC column. It was found to be linear (0 to 200 ppm) and precise, even in the presence of large quantities of sugar.

The method has successfully been used to monitor losses around filter stations (Sahadeo, 1995). A method for the simultaneous headspace analysis of ethanol and lactic acid was also reported (Anon, 2000). Huletts refinery experienced high ash gains in the 1987/88 season which was found to be caused by the formation of calcium lactate (Cox *et al,* 1990). GC was used to measure the lactic acid, which was produced by chemical destruction of reducing sugars under alkaline conditions during liming.

Acetic acid

Acidic corrosion of V2 vapour lines and mild steel diffusers led to an investigation into the origin of acetic acid in condensates which required a routine, reliable acetic acid analyses method (Purchase *et al.,* 1987; Schäffler, 1988; Cox *et al.,* 1993). It was found that excessive liming in diffusers hydrolysed the acetyl groups present in bagasse. The GC method used a 50% formic acid trap to saturate the nitrogen carrier gas and required injection of formic acid solutions between samples to overcome acetic acid absorption and peak ghosting. The method was linear and reproducible for determining volatile organic acids (acetic, propionic,

butyric and valeric acids). Condensate samples could be analysed directly by GC, whereas sugar-containing samples required a vacuum micro-distillation technique prior to GC to isolate the acids from the sugars (Schäffler and Morel du Boil, 1984) (Figure 4). Other research samples analysed by this method included bagasse hydrolysates and monitoring the performance of anaerobic ponds for the treatment of factory effluent.

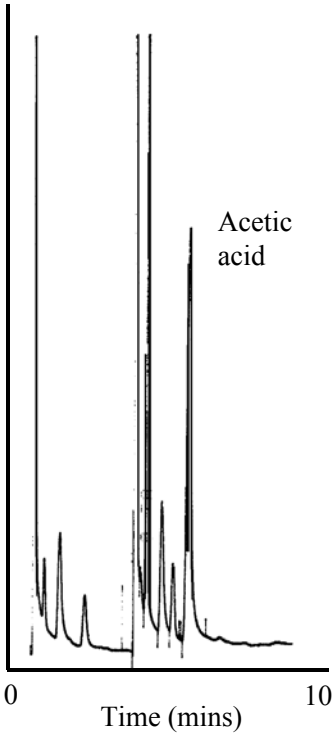

Figure 4. Chromatogram of acetic acid from a molasses sample.

Inversion and monosaccharide losses

Traditional reducing sugar methods cannot provide accurate measurements of the individual reducing sugars. The use of the GC gave the sugar technologist accurate measurements of sucrose, glucose and fructose in processing streams. This resulted in a greater understanding of the fate of these sugars during sugar mill processing. One of the first applications was to follow the changes in these sugars from the evaporator syrup stage through to final molasses (Morel du Boil and Schäffler, 1978).

In this and subsequent work (Schäffler and Morel du Boil, 1984) it was shown that:

- The F/G ratio increased from syrup to molasses.
- Glucose, more than fructose, was being destroyed.
- Approximately 20% of the reducing substances were neither fructose nor glucose.
- If fructose destruction took place, this occurred predominantly in the front end, whereas almost all the glucose loss occurred in the back end of the mill.

Glucose/brix or glucose/chloride ratios were found to be sensitive indicators of inversion (Schäffler *et al.,* 1985). The method required a sampling technique that was labour intensive and necessitated the analysis of a large number of samples to produce meaningful results. Further studies in this area confirmed that inversion correlates with individual evaporator retention times and heating surfaces (Purchase *et al.,* 1987). From this work and other research into the effect of high temperature on pH in sugar juices, a theoretical model of inversion was developed (Schäffler, 1987) which has been used extensively in the industry to estimate sucrose losses through the factory (Table 3).

Table 3. Example of the use of glucose determination to estimate inversion losses in an evaporator train.

INPUT DATA

	CJ	First	Second	Third	Fourth	Fifth
Brix or Solids (%)	12.5	20.5	33.1	43.1	48.8	68.1
Temperature (°C)		115	110	101	88	58
pH @ 25°C	7.0	6.8	6.6	6.4	6.3	6.2
Residence Time (min)		4	6	10	15	20

CALCULATED

	CJ	First	Second	Third	Fourth	Fifth
Mean Bx		16.5	26.8	38.1	46.0	58.5
density (gm/mL)		0.99	1.04	1.11	1.17	1.26
Water content (gm/mL)		0.83	0.76	0.69	0.63	0.53
Log (water)		-0.083	-0.117	-0.161	-0.199	-0.279
Mean pH		6.9	6.7	6.5	6.4	6.3
dpH/dT		-0.011	-0.010	-0.008	-0.007	-0.007
pH @ operating temperature		5.9	5.9	5.9	5.9	6.0
K		0.0002	0.0001	5E-05	1E-05	3E-07
Sucrose inverted (%)		0.09	0.08	0.05	0.02	0.00
					TOTAL LOSS	**0.24**

Ethanol

Ethanol as an indictor of burn-to-crush delay was first reported by Lionnet (1986) and Lionnet and Pillay (1987). A direct injection GC method was used to measure the ethanol content of the DAC samples. The method was found to be simple, reliable and quick. By 1988, GCs were being installed at some mills to measure ethanol in DAC samples to monitor the cut-to-crush delay. A typical set of results is shown in Figure 5 (Lionnet, 1995).

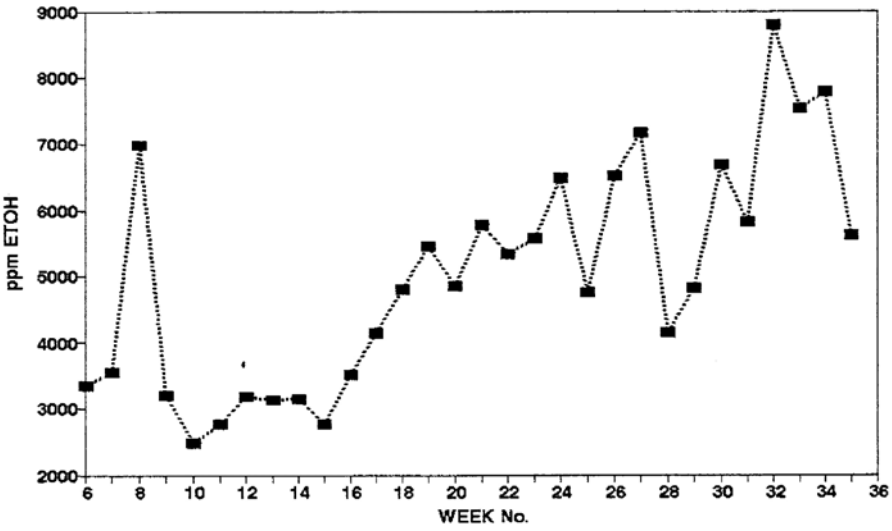

Figure 5. Average results of weekly DAC ethanol analysis for NB (from Lionnet, 1995).

Caramel – imidazole
When ammonia is used to produce caramel colours, trace quantities of 4-methyl imidazole can be formed. The Foodstuffs Act regulates the maximum amount of this toxic substance allowed in the caramel. A chloroform extraction method followed by gas chromatography has been used which is repeatable and linear over the range of 50 to 500 ppm (Day-Lewis, 1986).

Research applications

Soluble ash
Soluble inorganic and organic material present in a process stream is known as soluble ash. Factory process control is largely dependent on the measurement of the inorganics in factory streams. This can be either total ash or specific ions such as calcium. The use of techniques such as IC, in the cane sugar industry, has been limited even though it has the advantage of allowing the simultaneous separation of the cations, and both inorganic and organic anions present in a process stream. This advantage has been used in the study of clarification, tracer studies and refinery problems (Walford, 2000b).

An IC separation followed by suppressed detection has been used to measure acetic acid in process streams. The advantage over the micro-distillation/GC method was that it only required sample dilution and filtration and is specific for the acid (Figure 6). A simple IC method (dilute and inject) has also been used to measure dissolved silica present in process samples (Walford, 2001).

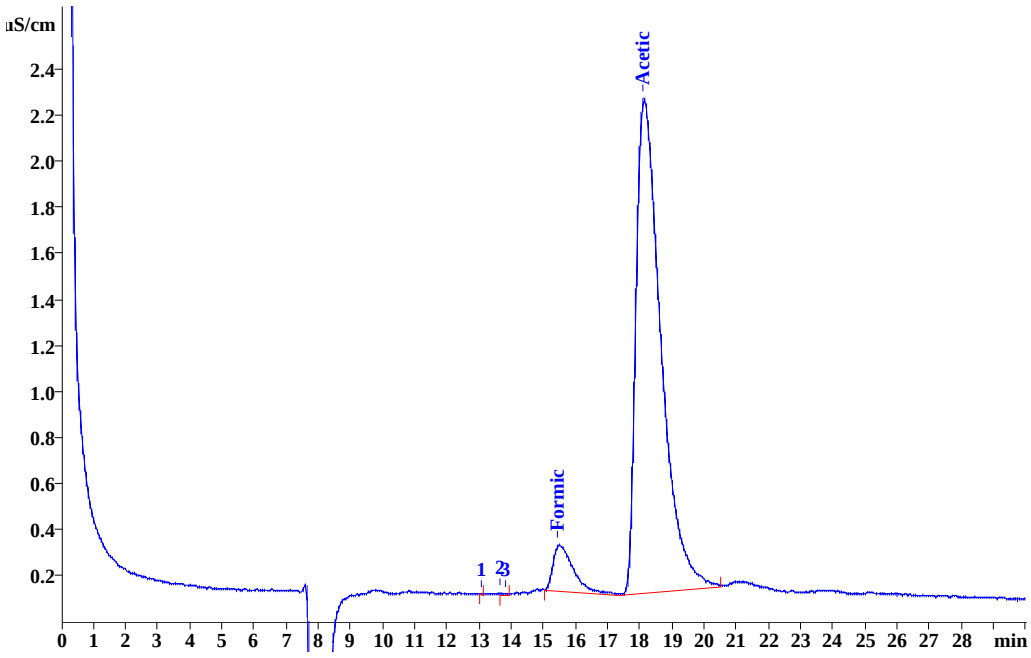

Figure 6. Chromatogram of a factory condensate sample using suppressed ion exchange (35 ppm acetic acid).

Aconitic acid
Aconitic acid is the major non-nitrogeneous acid found in cane juice and factory process streams. It exists in two geometrical forms, the *trans*- and *cis*-isomers. The former is the predominate form found in MJ. This acid gives the MJ its natural pH of approximately 5, the juice's natural buffering capacity, and accounts for approximately two-thirds of the quantity of lime added to neutralise MJ to $pH_{25°C}$ 7. This isomeric form changes during the factory processes and has been implicated in scale formation and gassing reactions (Walford, 1998a,

1998b, 2000a). Analysis was performed using a solid phase extraction (SPE) ion exchange isolation method followed by ion-exclusion HPLC analyses, using dual detection (UV and RI) so as to monitor the non-UV absorbing phosphate in addition to the organic acids. These included *cis*- and *trans*-aconitic acids, oxalic, tartaric, malic, succinic, glycolic, acetic and lactic acids (Figure 7) (Walford, 1995, 1996b).

The advantages of the IC method (mentioned in the previous section) were found to be useful in the study of molasses gassing. Molasses and C-massecuites are known to gas and foam. Although Newell (1979) showed that both high temperature and high total solids at low purity promoted gas evolution, the conditions under which this occur have not yet been well defined. The larger volumes occupied by this material can decrease factory throughput. In a laboratory study, molasses samples stored at elevated temperatures showed good correlation between the measured volume of CO_2 produced and the amount of aconitic acid decarboxylated to itaconic and other acids as measured by IC (Walford, 2002).

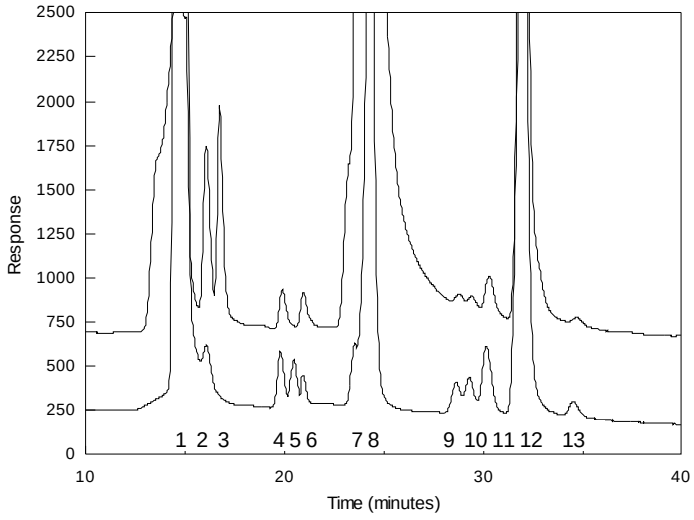

Figure 7. Chromatogram of a standard acid mixture showing UV (top trace) and RI (lowertrace) response. Peaks: 1 solvent; 2 oxalic; 3 cis-aconitic; 4 citric; 5 phosphoric; 6 tartaric; 7 malic; 8 trans-aconitic; 9 succinic; 10 glycollic; 11 lactic; 12 formic; 13 acetic.

Oligosaccharides – kestoses and theanderose

The first studies of oligosaccharide occurrence in South African sugar factories were reported by Morel du Boil *et al.* (1970). Raffinose and fructosylsucroses were found in cane refinery molasses. A quantitative TLC method showed that the kestoses constituted the major portion of the oligosaccharide content in final molasses (Schäffler and Juckes, 1971). Oligosaccharides derived from sucrose by substitution on the primary hydroxyl group attached to C_6 of glucose have been found to be inhibitors of sucrose crystal growth and result in crystal elongation (Morel du Boil, 1991). Silica gel TLC was used for isolation of the oligosaccharides and GC, HPLC and HPAEC were used as confirmatory techniques to establish their identity. The exceptional selectivity of HPAEC for oligosaccharides is illustrated in Figure 8 (Morel du Boil, 1998a). The five major oligosaccharides were 1-kestose, neo-kestose, theanderose, erlose and raffinose. The influence of raffinose on b-axis elongation is well documented, and it was demonstrated that theanderose (a glucosyl sucrose) promotes c-axis elongation. After analysing several beet sugars from various countries and a multitude of cane sugars by HPAEC (Figure 9), it has been proposed that the presence of theanderose is a better indicator for distinguishing cane from beet white sugars than is the

absence of raffinose (Morel du Boil, 1996).

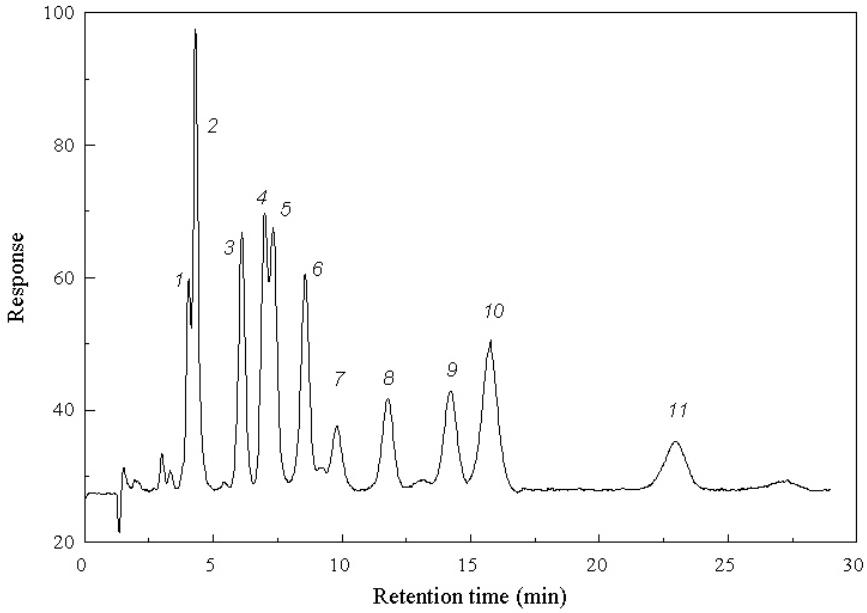

Figure 8. HPAEC of a mixture of di- and tri-oligosaccharides showing the exceptional selectivity of the anion exchange system (1=isomaltose; 2=sucrose; 3=raffinose +isomaltotriose; 4=1-kestose; 5=theanderose; 6=maltose; 7=isomaltotetraose; 8=6-kestose; 9=neo-kestose; 10=isomaltopentaose+panose; 11=matotriose).

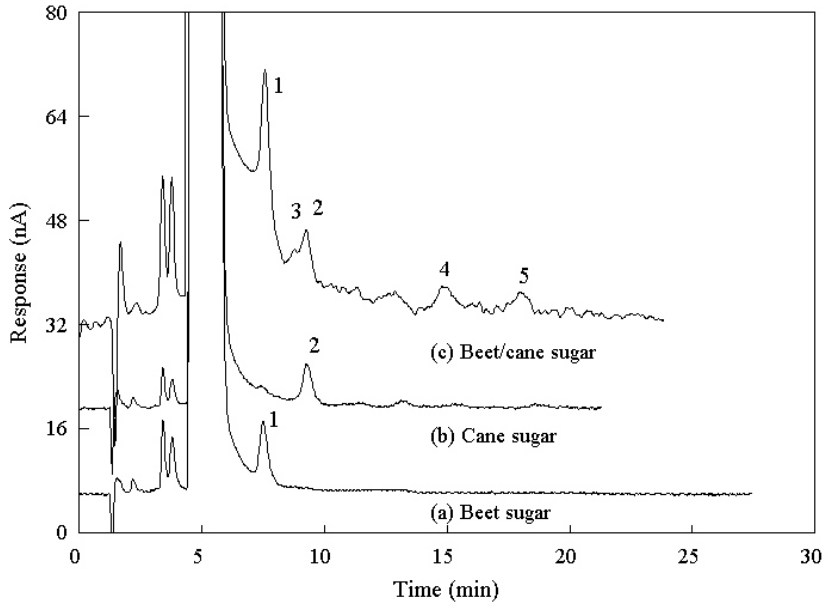

Figure 9. Chromatograms obtained for (a) beet sugar with raffinose, (b) cane sugar with theanderose, (c) white sugar from a refinery processing both cane and beet sugar. 1=raffinose, 2=theanderose, 3=1-kestose, 4=6-kestose, 5=neo-kestose (from Morel du Boil, 1996).

Preparative chromatography

Ion exclusion chromatography is a process for separating ionic from non-ionic solutes using ion exchange resin. On a preparative scale it is used extensively in the beet industry for desugarisation of molasses and for the isolation of fructose in the high fructose corn syrup

industry. Peacock (1995) described the recovery of sucrose from refinery jet 4 using a pilot scale preparative ion exclusion column. The process showed large reductions in colour, ash, monosaccharide and oligosaccharide concentrations and estimated that 78% of the jet 4 sucrose could be recovered as saleable material. A method of high test molasses production from final molasses was reported in 1997 by Davis *et al*. The process inverted sucrose and separated the resultant monosaccharides from the soluble ash present in the molasses using a pilot scale preparative ion exclusion column.

Polysaccharides

Polysaccharides are composed of long chain polymers of monosaccharides linked to each other in a variety of different forms. Dextran is produced in cane products as the direct result of cane deterioration. Its presence has an adverse effect on cane processing due to viscosity increases, and also indicates that sucrose has been destroyed. Sarkaran is a polysaccharide reported in both stale (as opposed to deteriorated) and standover cane. Typical problems reported with processing standover cane include very high syrup viscosities and poor crystallisation rates. Both of these polysaccharides have been analysed in sugarcane products ranging from MJ to molasses and VHP and refined sugars. A combination of enzymic hydrolysis followed by HPAEC separation and detection was used to determine the polysaccharides and study the seasonal and geographic differences in these polysaccharides (Figure 10) (Morel du Boil, 2000). Whilst the HPAEC dextran results were comparable to the Haze method, the former was not designed as a routine analytical procedure but as a research tool.

Other chromatographic analyses

On occasions, the SMRI has been contracted to determine carbohydrates in a variety of matrices. These have included analysing the inulin content (a fructose polysaccharide) of chicory. Inulin is prebiotic and a source of dietary fibre with a range of fructose repeating units with a Degree of Polymerisation (DP) of between DP 10 and DP 60. Processing conditions can lead to partial hydrolysis of the inulin, resulting in oligofructoses with lower molecular weight. These can be analysed and resolved by gradient HPAEC (changing the composition of the mobile phase as the peaks are eluting from the column) (Figure 11).

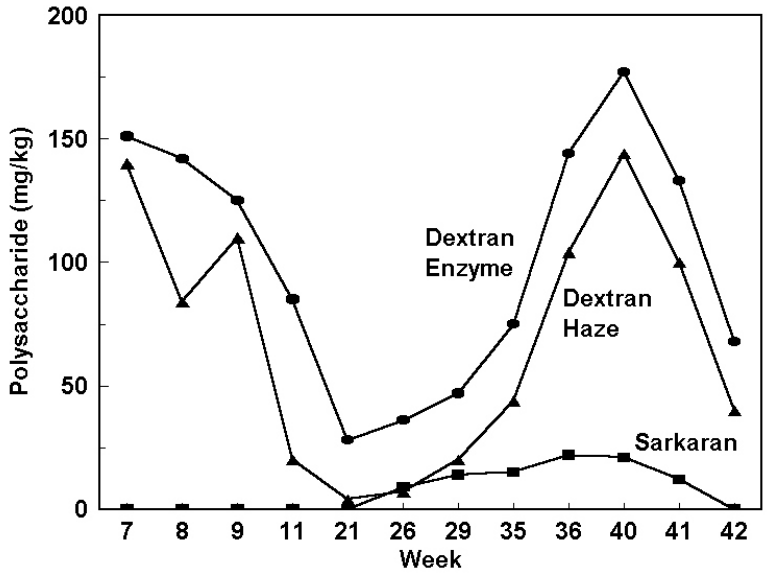

Figure 10. Seasonal trend in polysaccharides for NB refined sugar (from Morel du Boil, 2000).

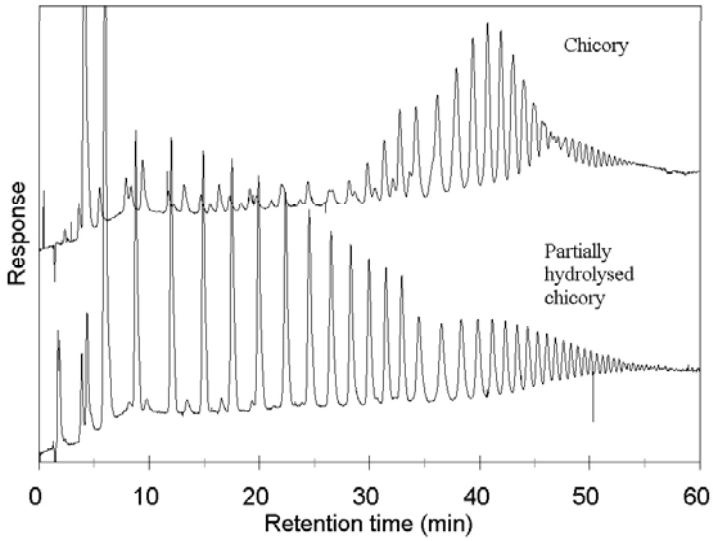

Figure 11. Gradient elution of chicory standard (upper) and partially hydrolysed chicory (lower).

Tequila is produced from the Agave plant. Alcohol yields are dependent on the hydrolysis or saccharification of the natural polysaccharides, which are present in the heart of the plant, to glucose and fructose for fermentation. In Mexico, the Agave plant produces inulin, which upon hydrolysis yields mainly fructose. A local producer approached the SMRI to help determine why yields were lower than expected. HPAEC indicated that the local Agave variety yielded a polysaccharide material that was not the same as inulin and did not give the expected profile after saccharification (Figure 12).

Trehalose is a disaccharide that is used as an internal standard in the determination of sucrose in MJ and is prepared from Bakers Yeast (*Sacchararomyces cerevisea*). During a routine analysis, it was found that the sucrose response factor was very poor. This was traced to a new batch of trehalose that was found to be contaminated with invertase – an enzyme that inverts or hydrolyses sucrose to glucose and fructose (Morel du Boil, 1998b). HPAEC was used to follow the hydrolysis of the sucrose. Approximately 11% of the sucrose to be determined was lost due to the enzyme. Inactivation of the enzyme activity was accomplished by autoclaving and sterilising the trehalose solution.

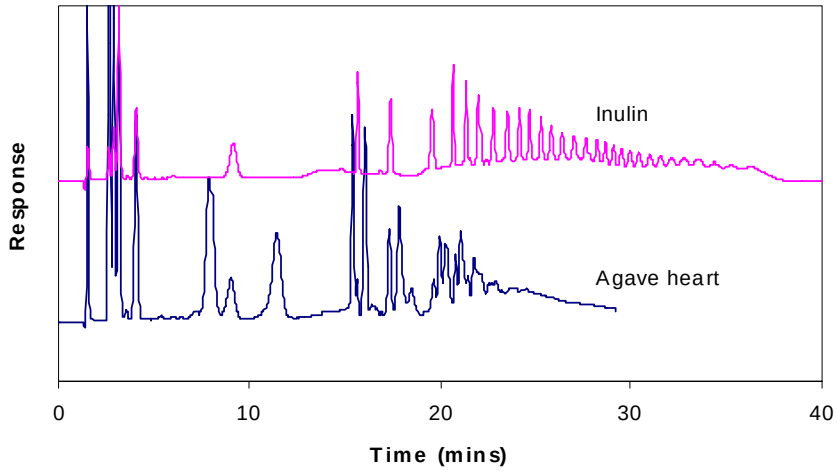

Figure 12. Gradient elution of polysaccharides in inulin (upper) and agave heart extract (lower).

Conclusion

Analytical chromatography has been used in the South African sugar industry over the last 30 years as both a research tool and for process control. Most forms of modern chromatography have been applied to problem solving in various areas of the sugar process and these applications have been reviewed and discussed.

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