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# Improved quantitative ion chromatography of industrial sugars: removal of interfering amino acids

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**Abstract**

The effect of interfering amino acids on the accurate quantitation of sugars in sugar industry samples, using ion chromatography with integrated pulsed amperometric detection (IC-IPAD), was investigated. Industrial sugar samples included beet syrup, juice and molasses samples. Using sugar and amino acid standard mixtures, and industrial sugar samples, proline was found to co-elute with fructose and serine with sucrose, and both co-elutions were "additive", which caused both fructose and sucrose to be overestimated. Conversely, the co-elution of arginine with myo-inositol, and glutamine with glucose had a marked "suppressive" effect on sugar quantitation and both myo-inositol and glucose were underestimated. Various methods were investigated for their efficiency to remove interfering amino acids, but not remove sugars: prefiltering of dilute samples through cation exchange syringe filters proved the most efficient method. Using sugar and amino acid standard mixtures, within experimental error, the cation exchange filter restored the mean concentrations of myo-inositol, glucose and sucrose to expected values, and standard deviations for all sugars were reduced markedly. Removal of interfering amino acids in a beet juice from a sweet water tank caused increases in mean concentrations for myo-inositol (+4.2%) and particularly for glucose (+62.5%), and a decrease for fructose (−7.3%). 

## 1. Introduction

Ion chromatography with integrated pulsed amperometric detection IC-IPAD (also known as high performance anion-exchange chromatography HPAE-PAD) is a liquid chromatography technique that is gaining wide acceptance as the preferred technique for the analysis of sucrose, glucose and fructose in sugar industry samples. Such samples include juices, syrups and molasses from cane and beet sugar factories/refineries (see Eggleston & Clarke, 1997). The technique is direct (unlike gas chromatography, no derivatization is required), highly sensitive, generally accurate and specific, and compatible with gradient elution techniques. Separation on anion exchange columns is achieved by exploiting the weakly acidic properties of sugars in alkaline eluent. Sugars are detected by measuring the electrical current generated by their oxidation at the surface of a gold electrode. IC- IPAD is presently being applied to a variety of routine monitoring and research applications, and official methods for glucose, fructose and sucrose analyses in molasses (AOAC, 1997; ICUMSA, 1995) and raw and white sugar (ICUMSA, 1998) samples, based on this technique, have also now been approved.

However, ion chromatograms of industrial sugar samples often exhibit extra, interfering small peaks very close to the analyte sugars, particularly glucose, fructose and sucrose, and many of these "interfering peaks" are amino acids. LaCourse and Johnson (1991) reported that amino acids, amino alcohols, amino glycosides and some sulfur compounds are detected by IPAD. Workers at Dionex Corporation (Anon., 1996) have reported that amino acids, especially hydrophobic amino acids such as lysine, are "known foulants of the gold electrode" used in the amperometric detection of monosaccharides. Furthermore, Singleton, Grimm, and Sanders (1996) observed that amino acids interfered with the IPAD analyses of peanut sugars, although their effect on the statistical quantitation of the peanut sugars was not reported. As sucrose often occurs at much higher concentrations in industry samples than glucose and fructose, a further dilution and, therefore, separate chromatography run is required to accurately quantify sucrose. This further dilution usually removes the interfering amino acid peaks. However, in the less dilute samples for the quantitation of glucose and fructose, the interfering amino acids are still present, reducing the quantitative accuracy. This has caused some sugar

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industry workers (G. Rearick, pers. comm.) not to use IC-IPAD to analyze for glucose and fructose, when they occur at low levels in beet juices, syrups and molasses.

This investigation was undertaken to determine the interference of amino acids in the IC-IPAD quantitative analysis of sucrose, glucose, fructose and myo-inositol in sugar industry samples. Interfering peaks must be removed for accurate sugar quantitation results. Consequently, methods for the effective removal of amino acids from industrial sugar samples were also investigated, and are reported here.

## 2. Materials and methods

### 2.1. Chemicals and reagents

HPLC grade sodium hydroxide (50% solution) was obtained from Fisher Scientific. Millipore water (resistance of 18 MΩ) was used to prepare eluents and samples. Prepared eluents were sparged with high purity helium to remove carbonate. All sugar and amino acid standards were analytical grade. Fructose, raffinose, stachyose and serine were obtained from Aldrich, sucrose, glucosamine-HCl, arginine and proline from Sigma, lactose from Fisher Scientific, glucose from J.T. Baker, myo-inositol from Calbiochem-Behring and glutamine from General Biochemicals.

### 2.2. IC-IPAD analysis of sugars

Diluted industrial samples were filtered through a 0.45 μm filter. The IC-IPAD equipment used was a Dionex (Sunnyvale, CA) BioLC instrument. Sugars were separated on Dionex CarboPac PA-1 guard (25 × 4mm) and analytical (250 × 4mm) columns, at a flow rate of 1.0 ml/min at ambient temperature (~25°C). Column eluent conditions were: 16 mM NaOH isocratic (inject; 0.0–2.0 min), a gradient of 16–160 mM NaOH (2.0–35.0 min), followed by isocratic 200 mM NaOH (35.1–40.0 min), and return to 16 mM NaOH (40.0–49.0 min) to re-equilibriate the column with the initial eluent prior to the next sample injection. Sugars (25 μl injections) were detected using integrated pulsed amperometric detection (IPAD). The Dionex PED-2 detector was equipped with Au working and Ag/AgCl reference electrodes, operating with the following working electrode pulse potentials and durations: $E_1 = +0.05$V ($t_0 = 0.00$s), $E_2 = 0.05$V ($t_1 = 0.42$s), $E_3 = +0.75$V ($t_3 = 0.43$s), $E_4 = +0.75$V ($t_4 = 0.60$s), $E_5 = -0.60$V ($t_5 = 0.61$s), $E_6 = -0.60$V ($t_6 = 0.96$s). The duration of the IPAD integration interval was set at 0.2–0.4 s. The electrode assembly and columns were lagged with Rubatex™ pipe insulation material. Using a Spectra-Physics SP8880 autosampler refrigerated at 4°C, and Dionex PeakNet 4.30 chromatography software, runs were accumulated of reference standards and multiple samples, with a blank being first run to stabilize column and system performance. The standards were myo-inositol, glucose, fructose, sucrose, raffinose and stachyose, with glucosamine-HCl or lactose as internal standards. Six different levels of the standards were run first, and standard curves were generated [sucrose ranged from 0.025 to 0.525 μg (equivalent to 1 to 21 ppm), myo-inositol, glucose and fructose from 0.025 to 0.15 μg [(1 to 6 ppm)] to test linearity in multiple runs and generate area response factors. Ten replicates of each sample were run, and every two samples were sandwiched between check standards. When samples were pre-filtered through a cation-exchange syringe filter, the filter was reconditioned after every two samples, and it was necessary to use lactose as the internal standard. Response factors were generated for each of the carbohydrates, using an internal standard calibration method and adjusted with check standards (Eggleston, 1998).

### 2.3. Industrial sugar dilutions

- Mixed juice: 0.02 g of mixed juice (15.1 °Brix) was diluted in 10 g of water (18 MΩ)
- Beet molasses: 10 g of molasses (79.4 °Brix) was diluted 1000 fold.
- Beet final evaporator effect syrup: 1 g of syrup (64.7 °Brix) was dissolved in 24 g of water (18 MΩ). 1.75 ml of this solution was then pipetted into a 10 ml volumetric flask and diluted.
- Sweet water (SW) tank juice: 1 g of syrup (16.1 °Brix) was dissolved in 24 g of water (18 MΩ). 7 ml of this solution was then pipetted into a 10 ml volumetric flask and diluted.
- Sweet water tank juice with milk of lime (MOL) added: 1 g of syrup (21.4 °Brix) was dissolved in 24 g of water (18 MΩ). 6 ml of this solution was then pipetted into a 10 ml volumetric flask and diluted.

### 2.4. °Brix

°Brix of industrial sugar samples was measured, at least in triplicate, using a Leica Abbe Mark II Refractometer with a crosshair reticule.

### 2.5. Removal of interfering amino acids

#### 2.5.1. Weak cation exchange resin

Weight diluted samples were first filtered through a 0.45 μm filter, then through Supelco Amberlite™ IRC-50 weak cation exchange resin, in the hydrogen form.

The hydrated resin was added to a vertical column to form a bed (~14 $cm^3$), which was kept hydrated. The sample was added to the top of the column, and the first few drops of filtrate were discarded. The subsequent filtrate was analyzed using IC-IPAD.

*2.5.2. Solid phase reversed phase cartridge*

Weight diluted samples were were first filtered through a 0.45 μm filter, then through Dionex OnGuard® reversed phase cartridges, and the subsequent filtrate was analyzed using IC-IPAD.

*2.5.3. Solid phase cation exchange filter*

Weight diluted samples were first filtered through a 0.45 μm filter, then filtered through Phenomenex STAR-ION® strong cation exchange syringe filters (25 mm diameter), in the hydrogen form. Preparation of these solid phase filters was based on the method of Singleton et al. (1996) with modifications. The filter was first washed with methanol (10 ml), then rinsed with 18 MΩ water (10 ml), and a finally washed with dilute (0.01 N) hydrochloric acid (~8 ml) until the effluent reached pH 2–3. Sample was applied and the first drops of the filtrate were discarded and the subsequent filtrate was analyzed using IC-IPAD. After each sample use, the syringe filter was reconditioned.

## 3. Results and discussion

### *3.1. IC-IPAD detection of amino acids*

From the work of Singleton et al. (1996) on the IC-IPAD detection of amino acids and peanut sugars, the amino acids arginine, glutamine, proline and serine were identified as amino acids that could potentially interfere with the identification and quantitation of simple sugars in industrial sugar samples. Fig. 1 illustrates a sample of sugar standards spiked with 16 ppm each of arginine, glutamine, proline and serine. Each amino acid gave a different IC-IPAD area response. Arginine co-eluted with myo-inositol, glutamine with glucose, proline with fructose, and serine co-eluted on the leading edge of the sucrose peak, although complete co-elution sometimes occurs depending on the eluent conditions. These co-elution profiles have also been noted with isocratic IC-IPAD methods. For proline and serine the co-elution was "additive", that is, the peak area response increased with the amount of amino acid and/or sugar added. However, the co-elution of arginine with myo-inositol, and glutamine with glucose was not additive and, as illustrated in Fig. 1, caused a marked reduction in peak area response and, in the case of glucose, altered the peak shape. This "suppressive" effect of arginine and glutamine on myo-inositol and glucose, respectively,

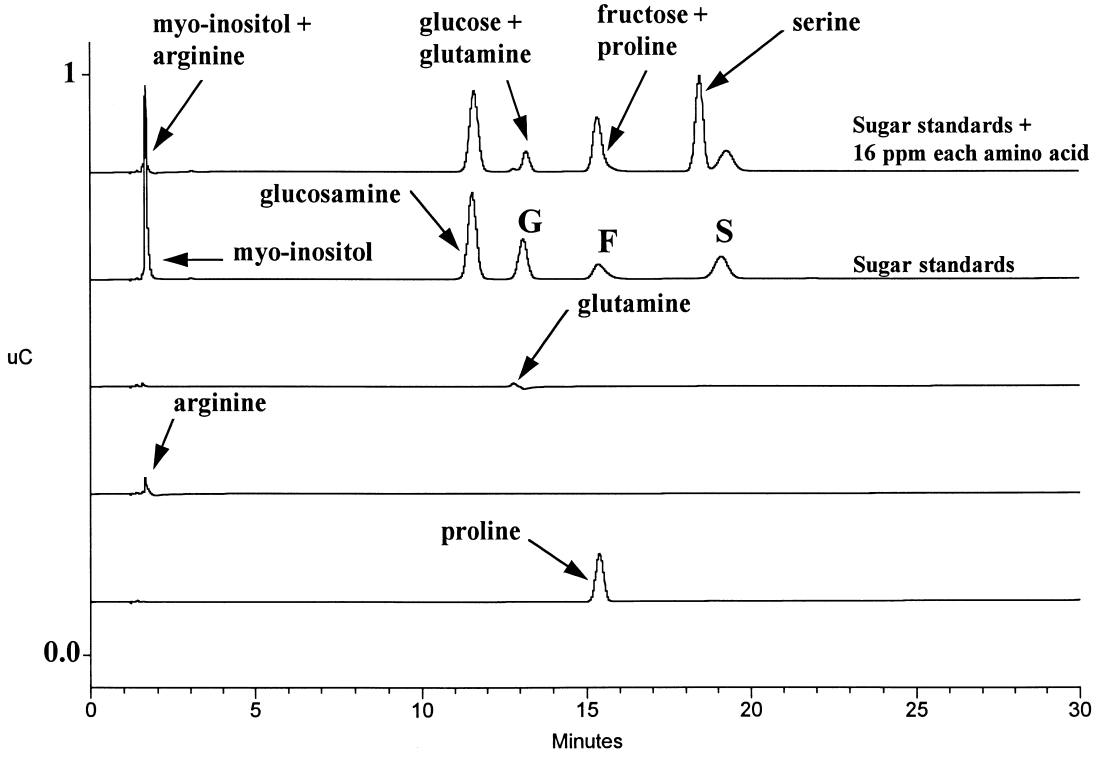



Fig. 1. IC-IPAD profiles of sugar standards (G = glucose, F = fructose, S = sucrose) and interfering amino acids (amino acids at 16 ppm levels).

Table 1
Effect of amino acids on the quantitation of analyte sugars by IC-IPAD

| Sample | Analyte sugars[a] | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Myo-inositol | | | | Glucose | | | | Fructose | | | | Sucrose | | | |
| | Resolution ($R$)[b] | Expected value (μg) | Actual mean value (μg) | SD $\times 10^{-3}$ | Resolution ($R$)[b] | Expected value (μg) | Actual mean value (μg) | SD $\times 10^{-3}$ | Resolution ($R$)[b] | Expected value (μg) | Actual mean value (μg) | SD $\times 10^{-3}$ | Resolution ($R$)[b] | Expected value (μg) | Actual mean value (μg) | SD $\times 10^{-3}$ |
| Sugar standards | 10.90 | 0.100 | 0.099 | 1.3 | 2.01 | 0.100 | 0.100 | 1.3 | 2.29 | 0.100 | 0.100 | 0.6 | 1.73 | 0.325 | 0.324 | 4.2 |
| Sugar standards + amino acids[c] | 36.43[d] | 0.100 | 0.045 | 3.9 | 0.83 | 0.100 | 0.030 | 16 | 3.03[e] | 0.100 | 0.367 | 46 | 0.7 | 0.325 | 0.967[f] | 45 |

[a] $N = 10$.
[b] Resolution was calculated as the difference in retention of adjacent peaks divided by their average peak width.
[c] Sugar standards were spiked with 16 ppm each of arginine, glutamine, proline and serine (see Fig. 1).
[d] There was no separation of the myo-inositol and arginine peaks, therefore, resolution from the nearest peak glutamine was calculated.
[e] There was no separation of the fructose and proline peaks, therefore, resolution from the nearest peak glucose was calculated.
[f] Serine co-eluted directly on the sucrose peak.

may be because these amino acids' oxidation products at the gold electrode inhibit the detector response for the sugar or, because there was a chemical interaction between the two compounds. These phenomena are highly reproducible. The glutamine/glucose phenomenon has serious consequences for the accurate IC-IPAD quantitation of glucose in industrial sugar samples which contain glutamine, as the glucose concentration will be significantly underestimated. Conversely, the presence of proline and serine will cause an overestimation of fructose and sucrose, respectively. These results particularly effect the sugarbeet industry, because amino acids are more abundant in sugarbeet than sugarcane. Furthermore, of the individual amino acids in the sugarbeet, glutamine is present in the largest quantity (Schneider, Reinefield, & Zenker, 1960). Glutamine is present in the beet tuber and is converted to pyroglutamic acid under alkaline conditions in the beet factory processes (McGinnis, 1982), and can occur with glutamine up to 4.17% in beet molasses (Schneider et al.). IC-IPAD runs under alkaline eluent conditions, therefore, it may have been pyroglutamic acid which reacts with glucose at the gold working electrode.

### 3.2. Effect of interfering amino acids on quantitation of sugars

The effect of amino acids on the statistically valid quantitation of sugars has not been reported well. Consequently, an experiment was conducted to measure the effect of arginine, glutamine, proline and serine on sugar quantitation, by spiking known sugar standards with 16 ppm each of the amino acids, and the results are listed in Table 1. All the amino acids had a considerable effect on sugar quantitation. The effect of arginine was to suppress quantitation of myo-inositol by ~50%, and glutamine suppressed glucose by ~70%. Conversely, proline and serine caused up to approximately three-fold increases in the fructose and sucrose concentrations, respectively. Moreover, for all the sugars, the standard deviation increased markedly with the addition of the amino acids and may explain, at least in part, the difficulties encountered in obtaining accurate IC-IPAD quantitation data for such sugars in multiple industrial sugar samples (Schaffler, Morel du Boil, & Walford, 1996; Eggleston, 1998).

### 3.3. Removal of interfering amino acids

One of the major criteria for a method to remove interfering amino acids from industrial sugar samples, was the non-removal of any sugars. Other criteria included that the method was easy to operate, was not too time consuming and was economical. Consequently, the first test of a method was to ensure that no sugars were removed or affected. Fig. 2 illustrates the effect of filtering a mixed juice sample from a sugarcane factory, through a weak cation exchange resin or a reversed phase cartridge. Although the weak cation exchange resin was recommended for selective adsorption of positively charged amino acids and peptides, the resin obviously induced the acid degradation (inversion) of

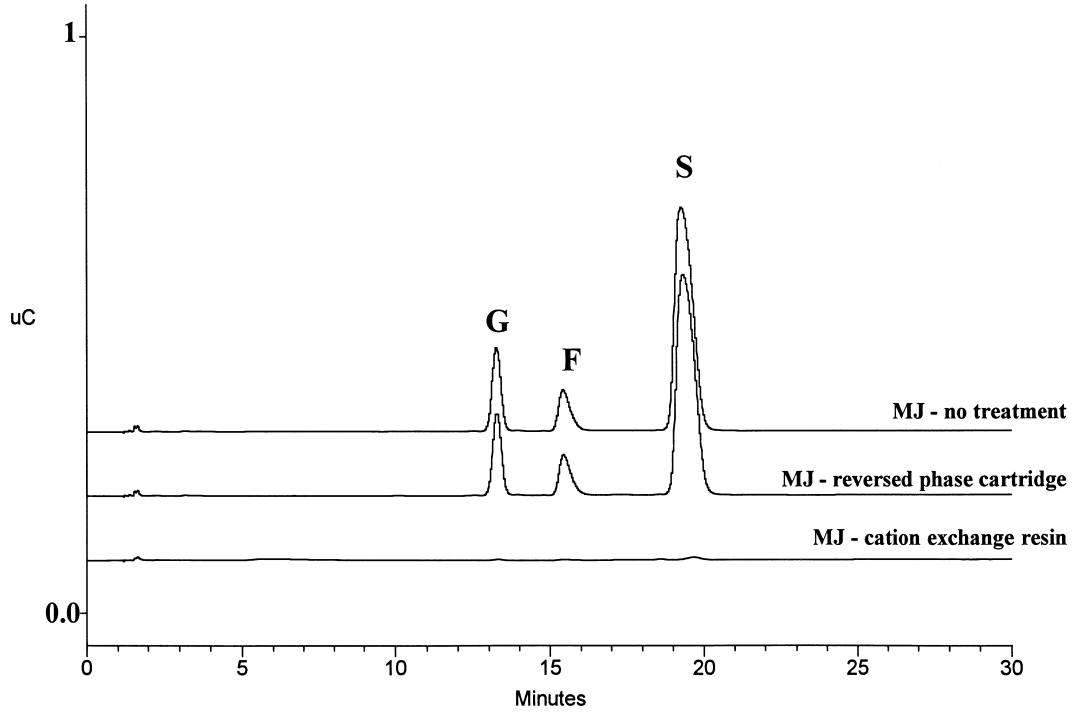



Fig. 2. Effects of filtering mixed juices (MJ) through a weak cation exchange resin, and a reserved phase cartridge.

sucrose to occur and is, therefore, not a viable method. The resin most likely had too many cation exchange active sites, which induced the acid degradation of the sucrose. In comparison, the reverse phase cartridge did not remove or affect any sugars (Fig. 2) but, as shown in Fig. 3, it also had no effect in removing amino acids from a mixture of sugar and amino acid standards.

Excellent results were obtained by filtering sugar and amino acid standards, through a cation exchange syringe filter. Fig. 4 illustrates the effect of filtering sugar standards, spiked or non-spiked with amino acids,

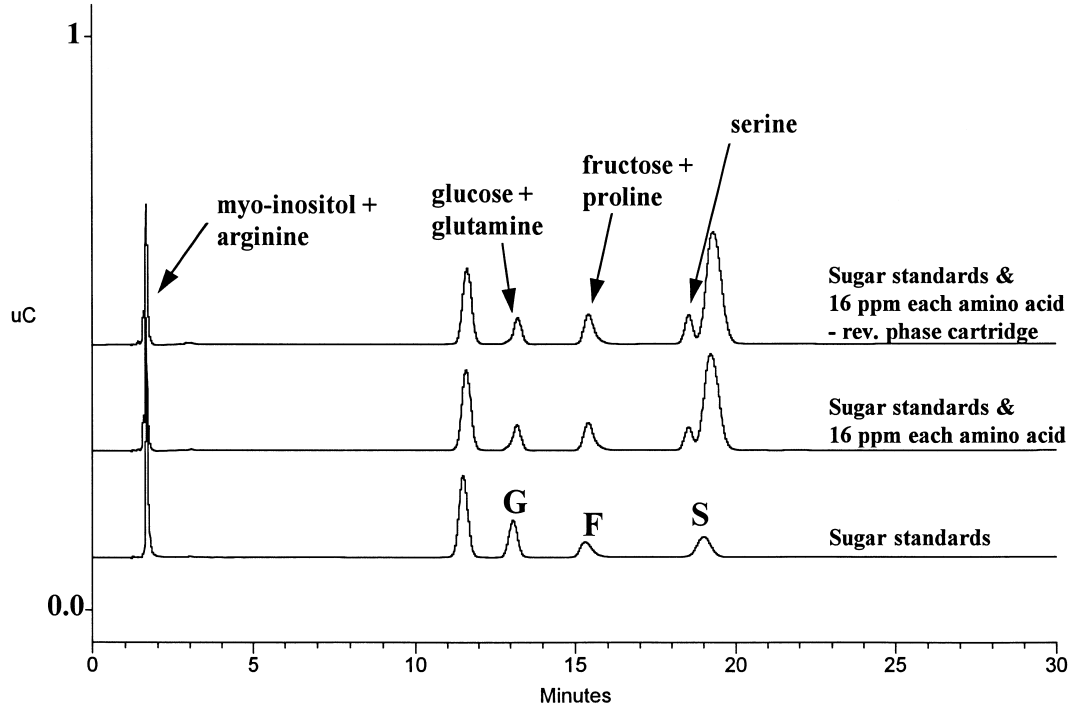



Fig. 3. Non-removal of amino acids using a reversed phase cartridge.

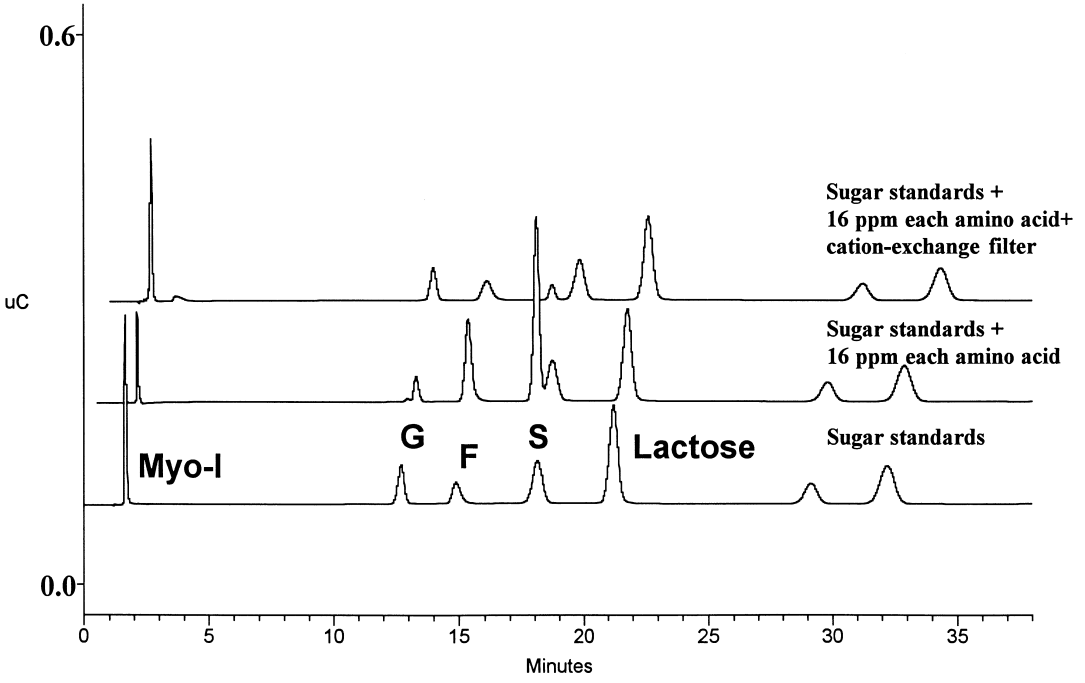



Fig. 4. Effect of cation exchange syringe filter treatment on sugar and amino acid standard mixtures.

through a cation exchange syringe filter. Amino acids were effectively removed without the removal of sugars, and this is reinforced by the quantitative statistical results shown in Table 2. Within experimental error, the use of the cation exchange filter restored the mean concentration values of myo-inositol, glucose and sucrose, to expected values. For fructose, the calculated mean value was still a little higher than the expected value, but considerably less than in the presence of the amino-acid. The use of slightly >0.1 N HCl to condition the filter will most likely remove all the proline. Furthermore, the 16 ppm level of spiked proline, represents the upper level of its occurrence in IC-IPAD diluted industrial samples (McGinnis, 1982; Schiweck, Jeanter-De Beukelaer, & Vogel, 1992), and lesser amounts would be expected to be completely removed. The standard deviations for all the sugars were improved by filtering the samples through a cation exchange filter (Table 2) and, for myo-inositol and glucose the standard deviations were similar to when sugar standards alone were run (compare Table 1). The cation exchange syringe filter most likely performed better than the cation exchange resin because it has markedly less cation exchange active sites. Furthermore, when a pure, dilute sucrose solution (15 ppm) was filtered through a cation exchange syringe filter, no significant inversion occurred.

Fig. 5 illustrates the effect of using a cation exchange syringe filter to remove amino acids from various industrial sugar samples. The industrial samples included a syrup from a final evaporator effect, and a molasses sample, both from different sugarbeet factories. The other industrial samples included a juice from a sugarbeet factory's sweet water tank, before and after milk of lime was added. For all the samples, the length of the IC-IPAD chromatogram between retention times 10.0 to 19.0 min, was clearly "cleaned-up" of interfering peaks, including amino acids, when the sample was treated with the cation exchange filter. The fructose peak, became more symmetrical and, therefore, more pure and easier to quantitate accurately. Furthermore, the cation exchange filter treatment cleaned up the glucose peaks and increased the peak areas, expecially in the juice from the sweet water tank. This was to be expected if glutamine was removed by the filter.

A further experiment was conducted to measure the effect of treatment with a cation exchange filter on the statistical quantitation of myo-inositol, glucose and fructose in the juice from the sweet water tank, and results are listed in Table 3. Increases in mean concentrations were observed for myo-inositol (+4.2%) and particularly for glucose (+62.5%), in the samples that had been treated with the cation exchange filter. This was expected because of the "suppressive" effect of arginine and glutamine on myo-inositol and glucose, respectively. The mean concentration value for fructose decreased slightly (−7.3%). Again this was to be expected because proline has an "additive" effect on fructose. The statistical deviation, especially for glucose and fructose, decreased when amino acids had been removed by the cation exchange filter, which is in agreement with the previous results using sugar and amino acid standard mixtures.

The use of the on-line Dionex Amino Trap™ column, which was placed before the guard column, was found to have no effect on the removal of amino acids from the industrial sugar samples. This may not be unexpected as this trap column was developed for monosaccharide analyses on the CarboPac PA10 column, using very dilute NaOH eluents (Anon., 1996). Moreover, Singleton et al. (1996) investigated the use of on-line cation exchange columns to remove amino acids from peanut extracts, but observed that the accumulation of

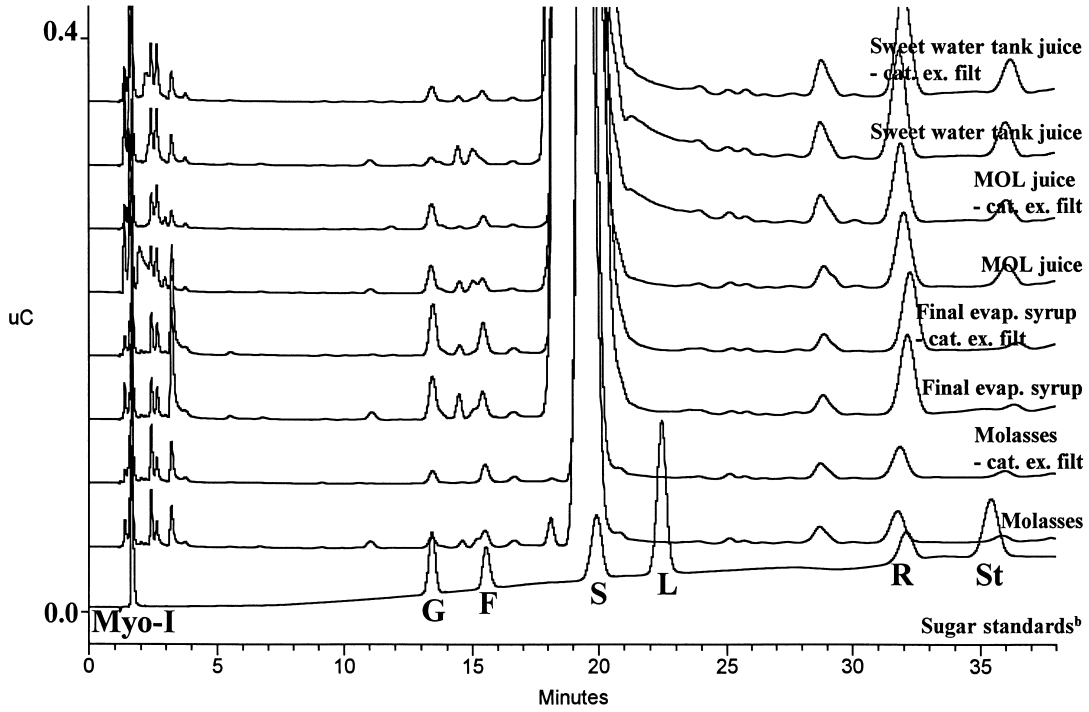
Table 2
The effect of cation exchange filter treatment on the quantitation of sugars spiked with amino acids

| Sample | Analyte sugars[a] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Myo-inositol | | | Glucose | | | Fructose | | | Sucrose | | |
| | Expected value (μg) | Actual mean value (μg) | SD $\times 10^{-3}$ | Expected value (μg) | Actual mean value (μg) | SD $\times 10^{-3}$ | Expected value (μg) | Actual mean value (μg) | SD $\times 10^{-3}$ | Expected value (μg) | Actual mean value (μg) | SD $\times 10^{-3}$ |
| Sugar standards + amino acids[b] | 0.100 | 0.0365 | 2.8 | 0.100 | 0.0536 | 2.2 | 0.100 | 0.324 | 12.6 | 0.325 | 0.493[c] | 372[c] |
| Sugar standards + amino acids: cation exchange filter treatment | 0.100 | 0.099 | 1.8 | 0.100 | 0.095 | 2.2 | 0.100 | 0.123 | 10.6 | 0.325 | 0.331 | 9.5 |

[a] $N = 10$.

[b] Sugar standards were spiked with 16 ppm each of arginine, glutamine, proline and serine.

[c] Temperature changes caused slight shifts in retention time across the run, and progressive separation of serine from sucrose. This contributed to the quantitative variation.




[a] Dilutions are stated in the experimental section, [b] Myo-I=Myo-inositol, G=glucose, F=fructose, S=sucrose, L=lactose (internal standard), R=raffinose and St=stachyose

Fig. 5. Effect of cation exchange syringe filter treatment on glucose and fructose quantitation in diluted [a]industrial sugar samples from sugarbeet factories.

Table 3
The effect of cation exchange syringe filter treatment on the quantitation of sugars in a sweet water tank juice from a sugarbeet factory[a]

| Sample | Analyte sugars[b] | | | | | |
|---|---|---|---|---|---|---|
| | Myo-inositol | | Glucose | | Fructose | |
| | Mean value (μg) | SD $\times 10^{-3}$ | Mean value (μg) | SD $\times 10^{-3}$ | Mean value (μg) | SD $\times 10^{-3}$ |
| Sweet water tank juice | 0.048 | 4.8 | 0.024 | 2.5 | 0.041 | 5.8 |
| Sweet water tank juice: cation exchange filter treatment | 0.050 | 3.7 | 0.039 | 2.0 | 0.038 | 1.7 |

[a] See Fig. 5.
[b] $N=10$.

amino acids on the column led to a deterioration in column performance.

## 4. Conclusions

The effect of interfering amino acids on the accurate quantitation of sugars, by ion chromatography with integrated pulsed amperometric detection (IC-IPAD), in sugar industry samples was reported. Using sugar and amino acid standard mixtures, and industrial sugar samples, proline was found to co-elute with fructose and serine with sucrose, and both co-elutions were "additive", which caused both fructose and sucrose to be overestimated. Conversely, the co-elution of arginine with myo-inositol, and glutamine with glucose had a marked "suppressive" effect on sugar quantitation and both myo-inositol and glucose were underestimated. Prefiltering of dilute industrial sugar samples through cation exchange syringe filters was the most efficient method to remove interfering amino acids. Using sugar and amino acid standard mixtures, within experimental error, the cation exchange filter restored the mean concentrations of myo-inositol, glucose and sucrose to expected values, and standard deviations for all sugars were reduced markedly. Removal of interfering amino acids in a beet juice from a sweet water tank caused an increase in the mean concentrations for myo-inositol (+4.2%) and particularly for glucose (+62.5%), and a decrease for fructose (−7.3%).

The cation exchange syringe filters are easy to operate like the 0.45 μm syringe filters presently being used to remove relatively large particles from industrial sugar samples, before IC-IPAD use. The cation exchange syringe filters are also economical to use because they are easily reconditioned for multiple use, and can be used in less than 7 min.

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## References

Anon. (1996). Installation instructions and troubleshooting guide for the AMINOTRAP column. *Dionex Corporation publication*, p.1.

AOAC (1997). Sugars in cane and beet final molasses. Ion chromatographic method. AOAC official method 996.04, first action 1996. *Official Methods of Analysis 1995*, third revision 16th edn, chapter 44, pp. 17A–17C.

Eggleston, G., & Clarke, M. A. (1997). Applications of HPAE-PAD in the Sugar Industry. *Seminars Food. Anal., 2*, 119–127.

Eggleston, G. (1998) Improved quantification of sucrose, glucose and fructose in multiple industrial sugar samples using ion chromatography. *Proc. 1998 Sugar Proc. Res. Conf.*, Savannah, in press.

ICUMSA (1995). Analysis of sugars in molasses by high performance ion chromatography. *Proc. 21st session of the 1994 International Commission for Uniform Methods of Sugar Analysis*, pp. 294–315.

ICUMSA (1998). Analysis of glucose and fructose in raw and white sugars by HPAEC. *Proc. 22nd session of the 1998 International Commission for Uniform Methods of Sugar Analysis*, in press.

LaCourse, W. R., & Johnson, D. C. (1991). Optimization of waveforms for pulsed amperometric detection of carbohydrates following separation by liquid chromagraphy. *Carbohydr. Res., 215*, 159–178.

McGinnis, R. A. (1982). The sugarbeet and chemistry. In R. A. McGinnis (Ed.), *Beet-Sugar Technology* (3rd ed., p. 51). Colorado: Beet Sugar Development Foundation.

Schaffler, K. J., Morel du Boil, P. J., & Walford, S. N. (1996). HPAEC: some precautions required for the reliable analysis of carbohydrates. *Proc. S. Afr. Sug. Technol. Ass., 70*, 241–250.

Schiweck, H., Jeanter-De Beukelaer, C., & Vogel, M. (1992). The behaviour of nitrogen containing nonsugars of beet during the sugar recovery process. *Proc. 1992 Brit. Sug. Tech. Conf.*, Eastbourne, UK, pp. 1–21.

Schneider, F., Reinefield, E., & Zenker, B. (1960). Group separation of the components of beet olasses by means of ion-exchange. *Zucker Beihefte, 4*, 11–18.

Singleton, J. A., Grimm, D. T., & Sanders, T. H. (1996). Interference of amino acids in pulsed amperometric detection of peanut sugars. *Peanut Science, 23*, 61–65.