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Determination of volatile and non-volatile organic acids in technical sugar solutions by ion-exclusion chromatography

CARLA ALBERTA ACCORSI* and GABRIELLA BLO
Department of Chemistry, University of Ferrara, Via L. Borsari 46, 44100 Ferrara (Italy)
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ABSTRACT

Volatile and non-volatile organic acids present in sugar process juices were separated by ion-exclusion chromatography on an HPICE-AS1 cation-exchange column (H^+ form) using both dilute hydrochloric acid and 2-propanol–water solutions of tridecafluoroheptanoic acid. Coupled with an anion-exchange micromembrane suppressor, a conductivity detector made it possible to reveal citric, tartaric, gluconic, malic, lactic, succinic, glycolic, formic, acetic and pyrrolidonecarboxylic acid in the presence of inorganic acids and non-ionic organic matter. In sugar process juices subjected to alkaline and/or oxidative treatment, increasing concentrations of the major acid components, *i.e.*, acetic, formic and lactic acid, can be measured. The analysis is performed in the presence of sucrose and a simple clean-up by rapid batch treatment with a cation exchanger (H^+ form) is sufficient to remove proteins and cations. The multiple standard addition procedure is used for calibration.

INTRODUCTION

Organic acids can be successfully determined in sugar process juices by anion-exchange high-performance liquid chromatography (HPLC) using either a differential refractometer or a UV detector if, and only if, the acid fraction is isolated by an appropriate but tedious pretreatment of the sample [1–4].

The problems related to co-elution and quantification in the analysis of cane sugar juices are overcome by using a dual-column system in which each column is equilibrated at two temperatures [3]. Interference of sucrose with the acid elution profile is observed with a refractive index detector and the results for lactic, formic and acetic acid are found to have a high relative standard deviation (R.S.D.) [1]. Interference from inorganic anions (nitrates) is also evident [5].

In all chromatographic applications [HPLC and gas chromatography (GC)], analysis of the previously isolated acid fraction requires treatment with cation- and anion-exchange resins with subsequent recovery by elution from the anion exchanger. Preconcentration by vacuum evaporation of the eluate is always necessary. The volatile acids are removed to different extents, rendering their accurate determination impossible [1–4,6–8]. In this paper, a method is described for the determination of volatile and non-volatile organic acids in sugar process juices. The method, employ-

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ing ion-exclusion chromatography, does not require any previous isolation of the acid fraction.

Ion-exclusion chromatography on a high-capacity cation-exchange resin, using either water or a dilute acid as eluent, is a valuable technique for separating weakly acidic compounds [9–12]. Ionized solutes are excluded from the exchanger matrix by the Donnan potential and eluted faster than non-ionic compounds, retained by partition through Van der Waals or other forces. Weakly ionized compounds are eluted according to their pK_1 value and hydrophobic character. Size exclusion dominates the separation mechanism for polycarboxylic acids.

A conductivity detector, the response of which is enhanced by an anion-exchange micromembrane suppressor, offers a sensitivity and selectivity higher than those of the UV and refractive index detectors currently employed in HPLC for the matrices considered here.

EXPERIMENTAL

Analyses were performed by using a Dionex (Sunnyvale, CA, USA) Qic ion chromatograph equipped with a conductivity detector and a 50-μ sample loop. The separation column contained Dionex HPICE-AS1, a totally sulphonated cation-exchange resin with polystyrene–divinylbenzene as support material (degree of cross-linking 9%, capacity 5 mequiv./g). The eluents were dilute hydrochloric acid or solutions of tridecafluoroheptanoic acid (TDFHA). A Dionex anion-exchange micromembrane suppressor for ion-exclusion chromatography (AMMS-ICE) was inserted between the column and the detector in order to enhance the detection of organic acids in standard solutions and sugar juice samples. The regenerant was 5 m*M* tetrabutylammonium hydroxide (TBA), flowing at 2 ml/min for the continuous regeneration of the cation-exchange sites within the suppressor. The exchange between H^+ and TBA^+ reduced the background conductivity of the acidic eluent generated by hydrochloric acid or TDFHA in 2-propanol–water to values of about 100 and 40 μS, respectively. All determinations were performed at room temperature. The eluent flow-rate was always 0.8 ml/min (column pressure 62 bar).

All the reagents were of analytical-reagent grade. The water used in eluent preparation and sample dilution was obtained from a Milli-Q water purification system (Millipore–Waters, Milford, MA, USA).

Standard and sample treatment

Concentrated standard solutions in water were prepared by dissolving weighed amounts of the acids or their salts. The sugar juice samples and standards were diluted with water or eluent, when TDFHA was used, in order to prevent system peak interferences. To prevent the contamination of the analytical column with proteins and to avoid cation-exchange processes that could modify the elution profiles of the acids, both the standard solutions and suitably diluted samples were batch treated with a strong cationic exchanger (H^+ form), IR-120 (exchange capacity 1.8 mequiv./ml). To avoid dilution effects, the exchanger was first liberated from the excess of water by filtration. Subsequently, an amount corresponding to 2 ml of wet product was added to 100 ml of the solution to be analysed. The solution was passed through a Millipore 0.45-μm membrane filter before injection.

RESULTS AND DISCUSSION

Influence of the eluent

Various concentrations of hydrochloric acid (0.5–2 m*M*) were tested as the first eluent. Good resolution was achieved for the main acids involved in the oxidative process studied (formic, lactic and acetic) only with a 2 m*M* concentration of hydrochloric acid. As a relatively high background conductivity was obtained by using hydrochloric acid, a second eluent was considered, *viz.*, TDFHA in 2-propanol–water solutions, which provides lower background conductivity values. The 2-propanol, added to eluent to solubilize the TDFHA, has no significant effect on the acid retention. A 5% concentration of 2-propanol in water was used, as this does not endanger the analytical column but is sufficient to solubilize the TDFHA.

In Fig. 1 the capacity factor k' [$k' = (t_R - t_0)/t_0$] is plotted against eluent concentration. The dead time, t_0, is the retention time for dissociated inorganic species. With increasing TDFHA concentration in the eluent, the pH decreases from 3.7 (0.1 m*M* TDFHA) to 2.4 (2 m*M* TDFHA). The ionized acid molecule fraction together with the capacity factor varies with the pH according to the various pK_1 values of the acids (Table I). With concentrations of TDFHA between 0.1 and 0.5 m*M*, formic, malic and pyrrolidonecarboxylic acid show a greater increase in k', as the respective pK_1 values fall within this range and the variation of the dissociated/undissociated species ratio is maximum. At pH 3.7 the dissociated fraction has the highest relative value and the elution is principally affected by Donnan exclusion. When the degree of association increases because of a decrease in pH, the electrostatic repulsion forces progressively diminish and other effects start to influence the elution process, such as adsorption by the matrix and steric exclusion. The dominant role of these effects is reflected, for example, in the elution of lactic and formic acid.

Although not involved in the oxidative reaction, PCA cannot be ignored because it is the most prominent acid in sugar beet process juices. This acid demonstrates peculiar behaviour: its retention time at low pH is higher even than that of acetic acid. This behaviour is probably determined by the interaction between the fixed negative charges on the matrix ($-SO_3^-$) and the positive charge of the polar form of amide group, resulting from the partial double bond character of the C–N bond. Further, in an acidic environment, as revealed by NMR spectra, the O-protonated

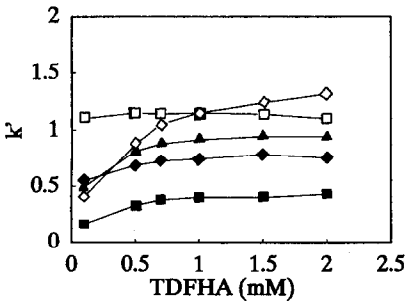

Fig. 1. Plots of capacity factors (k') *vs.* TDFHA concentration. Conditions: column, HPICE-AS1; eluent, TDFHA in 5% 2-propanol–water; flow-rate; 0.8 ml/min. ■ = Malic acid; ◆ = lactic acid; ▲ = formic acid; □ = acetic acid; ◇ = pyrrolidonecarboxylic acid (PCA).

TABLE I

CAPACITY FACTORS (k') AND pK_1 VALUES FOR DIFFERENT ACIDS

Column: HPICE-AS1; eluent, 2 mM TDFHA in 5% 2-propanol–water; flow-rate, 0.8 ml/min.

Acid	k'	pK_1	Acid	k'	pK_1
Oxalic	0.00	1.04	Succinic	0.75	4.00
Citric	0.23	2.87	Glycolic	0.82	3.63
Tartaric	0.30	2.82	Formic	0.95	3.55
Gluconic	0.36	3.56	Acetic	1.10	4.56
Malic	0.42	3.20	PCA	1.32	3.13
Lactic	0.75	3.66			

form is more frequent than the N-protonated form. The electrostatic interaction is positive in this instance and the elution process is consequently slowed.

In order to establish the optimum separation conditions, the selectivity coefficients for possible acid pairs were determined. In the window diagram in Fig. 2 [13], where selectivity is plotted against TDFHA concentration, the acids under study provided well separated peaks at three different TDFHA concentrations, 0.3, 0.65 and 2 mM. As small concentration variations around 0.3 and/or 0.65 mM are sufficient to modify the elution sequence, 2 mM TDFHA solution was chosen as the eluent. This concentration provided both optimum selectivity and equally well separated peaks.

We also studied the retention of other organic acids, generally present in the sugar beet process juice samples in negligible amounts. Their k' values in standard solutions, reported in Table I, together with the corresponding pK_1 values, show how the main acids involved in the present study can be determined. Possible co-elutions refer to the gluconic–malic, and succinic–lactic pairs. Fortunately, the amount of succinic acid normally found in technical sugar juices is negligible. In fact, this acid is used as an external standard in GC determinations (via trimethylsilyl derivatives) in this type of sample. Gluconic acid also is present in small amounts and provides a lower conductimetric response.

Fig. 3 shows the chromatogram obtained from the analysis of a technical sugar juice sample suitably diluted and treated as described above.

The identities of the peaks were confirmed by eluting samples to which the

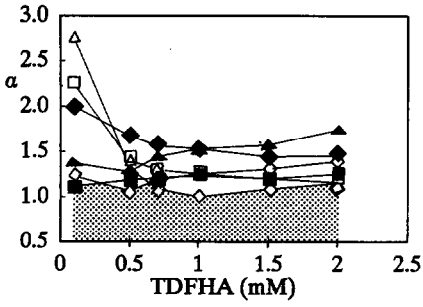

Fig. 2. Window diagram for organic acid pairs. Conditions as in Fig. 1. ▲ = Lactic–PCA; ◆ = lactic–acetic; ◇ = formic–PCA; ■ = lactic–formic; □ = formic–acetic; △ = acetic–PCA.

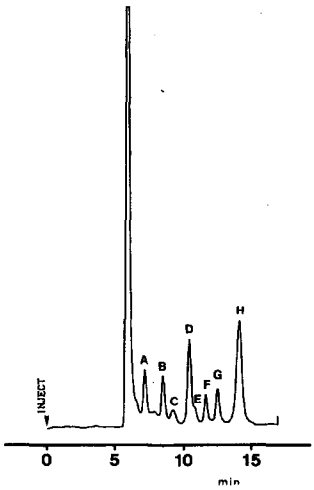

Fig. 3. Typical chromatogram of acids in a sugar beet juice sample. Conditions: column, HPICE-AS1; eluent, 2 m*M* TDFHA in 5% 2-propanol–water; flow-rate, 0.8 ml/min. A = Citric acid; B = malic acid; C = not identified; D = lactic acid; E = glycolic acid; F = formic acid; G = acetic acid; H = PCA.

various acids were added stepwise. This was necessary because of small but not negligible variations of the retention time during the measurements. All the peaks were identified except one, which appeared in all the technical solutions analysed and which lay between the malic and lactic acid peaks. Because of the Donnan exclusion, the inorganic anions elute together with the column void volume. The presence of citric acid, in addition to malic acid, is confirmed among the polycarboxylic acids with selectivity factors that conform to the pK_1 values, whereas oxalic acid, strongly excluded by the Donnan effect, co-elutes with the inorganic anions.

Because of the high lactic acid concentrations in the samples, the peaks of lactic and glycolic acid also overlap. The latter elutes as shoulder peak but it does not interfere because its concentration in the samples is generally low.

Quantitative analysis

Calibration graphs with correlation coefficients higher than 0.999 were obtained for all the pure standard acids in the concentration range 0–120 ppm (injection volume 50 μl). Quantitative analysis was performed by measuring peak heights, which proved better than peak areas. The repeatability of the analysis as relative standard deviation (R.S.D.) was calculated from five replicate analyses. The R.D.S.s for malic, lactic, formic and acetic acid and PCA were 2.5, 3.4, 3.2, 4.0 and 1.1%, respectively.

Significant differences between the slopes of the regression line calculated in recovery experiments on different juice samples and that of the calibration line for pure standards were obtained (confidence interval 95%) [14], thus confirming the expected matrix effect. Considering this effect and that the aim of this work was to determine concentration variations, the multiple standard addition procedure [15] was employed for quantitative analysis.

TABLE II

EXTRAPOLATED AMOUNTS OF DIFFERENT ACIDS IN SUGAR BEET PROCESS JUICES AND RELATIVE STANDARD DEVIATIONS

Malic acid		Formic acid		Lactic acid		Acetic acid		PCA	
Amount (ppm)	R.S.D. (%)	Amount (ppm)	R.S.D. (%)	Amount (ppm)	R.S.D. (%)	Amount (ppm)	R.S.D. (%)	Amount (ppm)	R.S.D. (%)
5.8	3.1	3.8	7.6	16.1	8.1	5.0	5.8	11.1	9.0
7.1	1.4	4.0	3.0	35.1	1.0	7.3	1.0	64.8	3.0
9.1	2.0	5.0	3.2	46.9	2.9	7.6	3.4	81.4	4.5
16.4	2.3	6.5	2.3	62.6	5.4	13.8	1.2	95.8	4.4
36.8	1.8	9.5	3.0	80.5	1.0	17.0	1.0	100.7	1.1

For calibration, five different known amounts ranging from 5 to 50 ppm (10 to 100 ppm for PCA and lactic acid) of each analyte were added to the samples under the condition of constant volume. Regression analysis yielded a linear plot with correlation coefficients higher than 0.999 in all instances.

The differences between the slopes of the regression lines from different samples (thin juices, thick juices, molasses) were also significant. The presence of different amounts of sucrose, the main component in these solutions, alters their physical properties, *i.e.*, viscosity and dielectric constant, and consequently the intensity of the detector signal.

The amounts of organic acids in the tested samples were determined by using the calculated regression coefficients [16]. The R.S.D.s of the extrapolated concentration values for five juice samples (0.5–1.0 g per 100 ml) are reported in Table II.

The PCA response factor is relatively low in comparison with the other acids determined, so the determination of PCA, of which the concentration is normally greater than that of the other acids, does not require excessive dilution of the samples.

CONCLUSION

Ion-exclusion chromatography offers a rapid method for determining oxy acids and organic acids in sugar process juices. The procedure described here, with the multiple standard addition method for calibration, is faster than other techniques such as HPLC and GC. No preliminary separation is required and the only pretreatment consists in the addition of a cation exchanger in the H^+ form to the solution before injection to protect the column life. The main advantage is the possibility of simultaneously determining volatile and non-volatile products. In particular cases, *e.g.*, when one is studying the behaviour of solutions having a high monosaccharide content, possible interference between glycolic and lactic acid must be carefully taken into consideration. Such situations are commonly encountered when processing sugar cane juices but are not usual in beet sugar factories.

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