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Sugar-rich Food: Determination of Inorganic Anions by Ionic Chromatography

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ABSTRACT

A method for the determination of Cl^-, HPO_4^{2-} *and* SO_4^{2-} *by ionic chromatography in a sugar-rich model system is proposed. This system consists of a 65° Brix glucose–fructose mixture with a low content of anions. The method is tested at two concentration levels: (A) from 20 to 60 ppm and (B) from 0·5 to 3 ppm.*

The method shows a good linearity at the studied concentrations. There is no matrix effect and the reproducibility is acceptable. This technique is successfully applied to real samples of solid mixtures, such as cane sugar and dietetic vegetable sugar, and liquid mixtures, e.g. honey, lemon syrup, condensed milk, sulphited grape must, rectified concentrated grape must, liquid caramel, concentrated pear and apple juice. Organic anions such as malate, tartrate and oxalate are determined, as well as sulphites (found in some samples).

INTRODUCTION

The number of applications of HPLC in the food field is continually increasing as the technique permits the resolution of complex mixtures of anions and cations in a few minutes (Cox *et al.*, 1985; Gjerde & Fritz, 1987).

HPLC has been applied to the determination of major anions, Cl^-, NO_3^-, SO_4^{2-} and HPO_4^{2-}, in samples such as surface and underground waters (Iskandarini & Pletrzyk, 1982; Darimont *et al.*, 1983; Jay & Judd, 1985),

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Food Chemistry 0308-8146/89/$03·50

wines (Powel *et al.*, 1986), food (Edwards, 1983; Kim *et al.*, 1987), meat (Eek & Ferrer, 1985), beer (Jancar *et al.*, 1984; Knudson & Sieberd, 1984), milk (Brunet-Clopes, 1987), sugars (Edwards, 1983) and bread (Gicowa *et al.*, 1982). A bibliographic review carried out for this work in the FSTA shows that in the last five years only 37 papers based on this technique were published in this field; this is a low yield taking into account the great possibilities offered by HPLC. All the above-mentioned applications were carried out on aqueous and 'clean' extracts, and the previous sample treatment had removed interfering substances.

The determination of inorganic anions in food containing them in low concentration is becoming more necessary (EEC, 1985), as stricter legal and technical norms are already being established for quality control. Also new or improved products are being introduced, replacing traditional types and requiring characterisation. The analysis of anions in these products has the disadvantage that, due to the matrix effect, most of the classical analytical methods cannot be applied or should be preceded by complicated pretreatments, which then lead to worse reproducibility. Among the products of greatest interest are sugar-rich foodstuffs (+60° Brix) such as rectified concentrated must (RCM), honey and syrups.

The purpose of this work is to develop a new method that allows for the determination of the main inorganic anions (Cl^-, HPO_4^{2-} and SO_4^{2-}) in samples with high contents of sugars and low in anions requiring the least sample treatment.

MATERIALS AND METHODS

Apparatus

A Dionex 2000i/sp ionic chromatograph, with a conductivity detector and Spectra-Physics sp 4290 integrator, equipped with a HPIC AS4A analytical column, an HPIC AG4A guard column and an AMMS-1 anion micromembrane suppressor column. A 50-μl sample loop was used.

Reagents

All reagents employed were Merck reagent grade. Water used to prepare the solutions was obtained from a Milli-Q Waters equipment.

Glucose–fructose solution

A 65° Brix solution was prepared containing 32·5% (w/w) of glucose and 32·5% (w/w) of fructose, the rest being water. This solution was the matrix for the anions' standard solution.

Anion solution

For each of the studied anions, solutions of 1000 mg/litre were prepared from their sodium salts (NaCl, $Na_2HPO_4 . 12 H_2O$ and Na_2SO_4).

Anion standard solution

Two solutions containing different concentrations of anions were prepared. Both had the glucose–fructose solution as matrix. Standard A contained 23 ppm of Cl^-, 20 ppm of HPO_4^{2-} and 62 pp, of SO_4^{2-}. Standard B contained 3 ppm of Cl^-, 0·5 ppm of HPO_4^{2-} and 2 ppm of SO_4^{2-}.

Eluent: aqueous solution of $Na_2CO_3/NaHCO_3$, 1·8/1·7 mM.
Regenerating agent: H_2SO_4, 1·25 mM (aqueous solution).

Samples

Spanish sulphited grape must and rectified concentrated grape musts (RCM) (from different geographical sites), concentrated pear and apple juice from Lérida (Spain), and commercial liquid and solid samples: condensed milk, caramel liquid, honey, lemon syrups, cane and dietetic vegetable sugar (DVS) (fructose/lactose 99·3%, saccharin 0·7%).

RESULTS AND DISCUSSION

The separation of major anions, Cl^-, HPO_4^{2-} and SO_4^{2-}, in a sugar-rich medium was carried out by dilution of the model system in aqueous medium. The optimisation of variables took place according to the univariate method (Massart *et al.*, 1978). Figure 1 shows a typical chromatogram obtained with a sample having a low content of anions.

To study the dilution effect, samples of different concentrations were chromatographed. The concentrations are expressed as sample weight/total solution weight. The results obtained with the Standard A (Fig. 2A) show that, for a 10-μS sensitivity, the best results are obtained with a 1:15 sample dilution in water. The high content of organic matter in the model does not interfere with the chloride determination, even in the samples with higher content of anions. For a 30-μS sensitivity, the best peaks are obtained in the chromatograms with the 1:10 dilution in water. Greater dilutions for each sensitivity have the advantage of less column pollution, but the obtained signals are low, especially for phosphates. Chlorides, as well as sulphates, can be perfectly quantified.

If the studied system is the Standard B (Fig. 2B), it can be seen that, for the

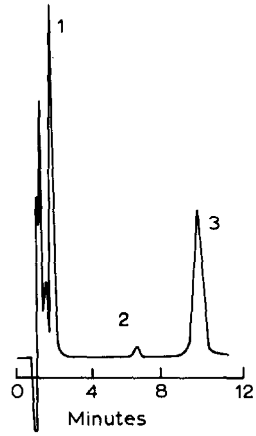

Fig. 1. Chromatogram of the Standard B diluted 1:5 at 3 μS. (1) Cl^-, 3 ppm; (2) HPO_4^{2-}, 0·5 ppm; (3) SO_4^{2-}, 2 ppm.

1:5 dilution and 10-μS sensitivity, the phosphate signal disappears. For this reason a compromise is achieved by working at higher sensitivity and maximum dilution. The optimum results for the three anions were obtained with a 1:5 dilution and 3-μS sensitivity. Lower dilutions are not recommendable because the noise increases too much.

In conclusion, for the Standard System A a 1:25 dilution factor and a 10-μS sensitivity are established. On the other hand, for the Standard System B, the optimum sensitivity is 3 μS, 1:5 being the dilution factor.

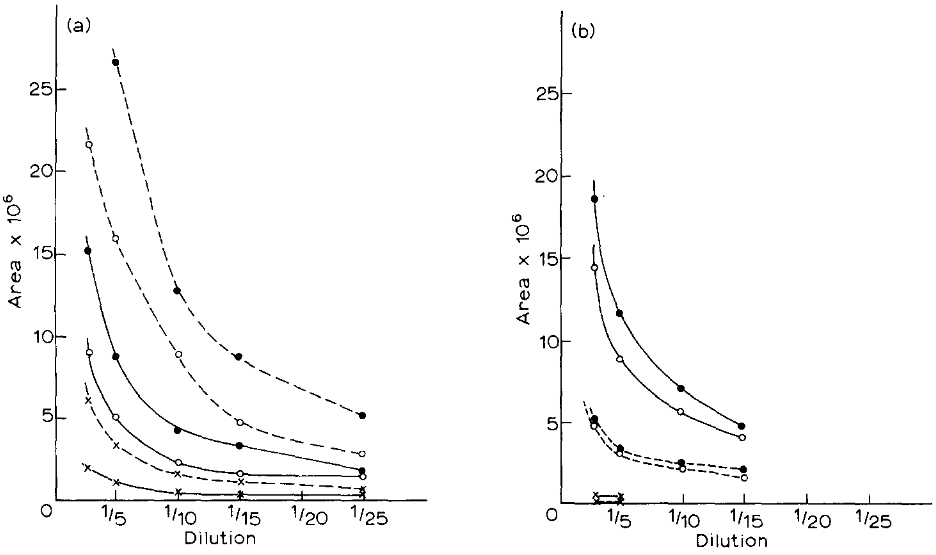

Fig. 2. (a) Effect of dilution and sensitivity on the relative ratios of the peak areas of each component for the Standard A. (b) The same relationship for the Standard B. ○, Cl^-; ●, SO_4^{2-}; ×, HPO_4^{2-}. ——, 10 μS; – – –, 30 μS.

The detection limits for the studied anions under optimum conditions were: (a) For a 3-μS sensitivity: Cl^-, 0·15 ppm; HPO_4^{2-}, 0·2 ppm; SO_4^{2-}, 0·1 ppm. (b) For a 10-μS sensitivity: Cl^-, 0·5 ppm; HPO_4^{2-}, 0·4 ppm; SO_4^{2-}, 0·2 ppm. Peak threshold (ratio signal/noise) was 10 000 in all cases.

The dissolution of samples in water or in the chromatographic eluent itself does not affect the retention times or the peak areas. Neither is there any interference with the chloride peak.

The flow effect was studied by changing the pump pressure; when it increases, the retention times go down, which is obvious. Owing to the relative difference between retention times, the best results were obtained with flow rates ranging between 1·5 ml/min (750 psi) and 2·25 ml/min (1100 psi). These results show that, with the maximum flow rate, the total elution time is 7·64 min, which allows for a high sampling frequency with a good separation, even in the case of the most diluted samples (Standard B).

Therefore a pump pressure of 1000 psi was taken as optimum, which means that a complete analysis can be carried out in 10 min.

Matrix effect

Due to the fact that the samples for analysis have a high content of sugars (65° Brix) and a relatively low content of anions, the matrix effect can be important, as dilution with water cannot be excessive. Because of that the influence of sugar concentration on the determination of the anions was evaluated.

TABLE 1
Comparison of the Matrix Effect of the Working Media: Sugar and Aqueous at Different Concentration Levels of Anions

Anion	*Sample type A** (*3° Brix*)				*Sample type B*** (*13·5° Brix*)			
	Range (*ppm*)	*a*	*b*	*r*	*Range* (*ppm*)	*a*	*b*	*r*
Glucose/fructose medium								
Cl^-	0·5–4	$1{\cdot}5 \times 10^{6}$	$3{\cdot}22 \times 10^{6}$	0·998	0·15–1·3	$2{\cdot}82 \times 10^{6}$	$1{\cdot}15 \times 10^{7}$	0·990
HPO_4^{2-}	0·4–4	$-1{\cdot}3 \times 10^{5}$	$1{\cdot}03 \times 10^{6}$	0·998	0·20–1·0	$-2{\cdot}45 \times 10^{5}$	$2{\cdot}91 \times 10^{6}$	0·990
SO_4^{2-}	0·2–4	$3{\cdot}0 \times 10^{4}$	$2{\cdot}53 \times 10^{6}$	0·999	0·10–2·0	$5{\cdot}96 \times 10^{5}$	$7{\cdot}91 \times 10^{6}$	0·990
Aqueous medium								
Cl^-	0·5–4	$1{\cdot}8 \times 10^{6}$	$3{\cdot}71 \times 10^{6}$	0·998	0·15–1·3	$1{\cdot}29 \times 10^{6}$	$1{\cdot}30 \times 10^{7}$	0·999
HPO_4^{2-}	0·4–4	$-7{\cdot}5 \times 10^{4}$	$1{\cdot}00 \times 10^{6}$	0·998	0·20–1·0	$-4{\cdot}8 \times 10^{5}$	$2{\cdot}69 \times 10^{6}$	0·999
SO_4^{2-}	0·2–4	$3{\cdot}8 \times 10^{4}$	$2{\cdot}68 \times 10^{6}$	0·995	0·10–2·0	$-1{\cdot}0 \times 10^{5}$	$9{\cdot}14 \times 10^{6}$	0·999

* Dilution 1:25, sensitivity 10 μS.

** Dilution 1:5, sensitivity 3 μS.

a, Intercept. *b*, Slope. *r*, Correlation coefficient. Range in ppm.

TABLE 2
Values of Retention Times Depending on the Medium[a]

Anion	*Matrix*					
	G/F		*Water*		*RCM*	
	3 μS	*10 μS*	*3 μS*	*10 μS*	*3 μS*	*10 μS*
Cl^-	2·0	2·0	2·0	2·1	2·0	2·0
HPO_4^{2-}	5·9	5·8	5·8	5·9	5·9	5·7
SO_4^{2-}	8·7	8·7	8·6	8·5	8·8	8·5

[a] Average values of four determinations expressed in minutes.

The results of the study of the matrix effect were obtained by analysing samples with a high and low content of anions (Standards A and B) dissolved in water or in a glucose/fructose medium, running the calibration graphs with their corresponding linear regression for each anion.

Table 1 shows the work ranges and the data of the curves obtained with both matrices: the Standards A and B, and deionised water.

The values of the slopes indicate that there is no matrix effect for any of the studied anions in either standard (A or B), which, after being diluted, had 3° and 13·5° Brix, respectively.

The retention times for the different anions do not vary with the medium in which they have been determined (Table 2).

The results obtained when the determinations of real RCM samples are carried out by the standard addition are nearly the same (Table 3), which shows that these anions act in the same way, no matter what the matrix is.

TABLE 3
Results of the Evaluation of the Matrix Effect in Real Samples (Medium RCM)

Anion	*High levels**				*Low levels***			
	Range (ppm)	*a*	*b*	*r*	*Range (ppm)*	*a*	*b*	*r*
Cl^-	0·5–4	2·0 × 10^{6}	3·63 × 10^{6}	0·986	0·15–1·3	5·7 × 10^{6}	1·19 × 10^{7}	0·999
HPO_4^{2-}	0·4–4	−3·1 × 10^{4}	0·99 × 10^{6}	0·998	0·20–1·0	1·9 × 10^{5}	2·97 × 10^{6}	0·996
SO_4^{2-}	0·2–4	8·9 × 10^{4}	2·57 × 10^{6}	0·999	0·10–2·0	2·2 × 10^{6}	8·88 × 10^{6}	0·999

a, Intercept. *b*, Slope. *r*, Correlation coefficient.
Dilutions: *, 1:25; **, 1:5.
Sensitivity: *, 10 μS; **, 3 μS.

As a consequence of the above, it can be concluded that, for the study of mixtures with high contents of sugars, solutions for calibration prepared in water are suitable for the determination of anions in a sugar-rich medium.

The recoveries in the model medium, as well as in RCM real samples or in water, are very acceptable values, both for the 3-μS and the 10-μS sensitivity. The results are shown in Table 4 and range from 93·8% to 102%.

Eleven samples of Standard B at 3 μS in glucose/fructose were analysed to test the reproducibility. RSD values of 2·85%, 4·08% and 2·40% were obtained for Cl^-, HPO_4^{2-} and SO_4^{2-}, respectively. The precision was obtained from 20 analytical repetitions of the same sample (Standards B) at a 3-μS sensitivity. RSD values of 1·6%, 2·9% and 1·9% were obtained for Cl^-, HPO_4^{2-} and SO_4^{2-}, respectively.

This method was applied to real samples. The results are given in Table 5.

Figure 3 shows a typical chromatogram of the samples. It can be seen that, before the Cl^- signal, there are several peaks corresponding to natural compounds of organic origin; at 2 min Cl^- appears, at 5·8 min HPO_4^{2-} and at 8·7 min SO_4^{2-}. Under such conditions the analysis is complete in about 10 min.

The identification of the components was carried out by comparison with the retention times of the model system and by internal addition to the mixtures themselves.

The results obtained indicate that, for each mixture, a determined dilution and sensitivity are required, depending on the anion content. The retention times for any of the studied anions do not vary according to the analysed product. The variation of the retention time is due mainly to instrumental effect.

TABLE 4
Recoveries in the Different Media at Two Levels of Sensitivity

Anion	*Range (ppm)*	*Medium*		
		Glucose/fructose	*Water*	*RCM*
Recovery at 3 μS (%)				
Cl^-	0·4–1·3	101·6	99·6	101
HPO_4^{2-}	0·4–1·0	102·2	92·9	99
SO_4^{2-}	0·5–2·0	93·8	100·0	101·8
Recovery at 10 μS (%)				
Cl^-	0·7–4·0	98·8	99·2	94·0
HPO_4^{2-}	0·5–4·0	95·8	95·4	99·5
SO_4^{2-}	0·6–4·0	102·0	99·0	96·7

TABLE 5
Results of Application to Real Samples[a]

Product	*° Brix*	*Dilution (w/w)*	*Sensitivity (μS)*	*Anions*			
				Cl^-	HPO_4^{2-}	SO_4^{2-}	*Others*
Sulphited must	19·5	1/50	30	19·0	174	761	Tartrate 6 900
							Nitrates 5
							HSO_3^- 761
							Malate 3 200
RCM	64·2	1/5	3	3·1	0·3	1·4	
Cane sugar	—	1/5	10	11·5	—	13·5	Nitrates 4
							Malate 9
							Oxalate 7
DVS	—	1/5	10	3·5	3·7	7·6	Nitrates 2
Condensed milk	77·5	1/200	30	5 992·0	1 945·0	108	Nitrates 58
Lemon syrup	63·0	1/50	10	202·7	—	125	Nitrates 6
Liquid caramel	78·4	1/200	10	3 938·0	20·0	299	Nitrates 18
Conc. apple juice	71·3	1/200	30	139·5	802·0	1 395	Nitrates 470
							Malate 57 540
Conc. pear juice	70·6	1/200	30	107·3	1 760·0	1 534	Nitrates 170
							Malate 29 212
Honey	82·7	1/100	10	833·0	229·0	38	Malate 746
							Oxalate 235

[a] Average results of three determinations expressed in ppm (mg/kg).

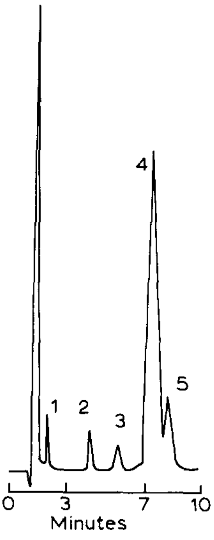

Fig. 3. Chromatogram of concentrated apple juice (dilution 1:200 and sensitivity 30 μS). (1) Cl^-, 0·7 ppm; (2) NO_3^-, 2·3 ppm; (3) HPO_4^{2-}, 4·0 ppm; (4) malate, 287·7 ppm; (5) SO_4^{2-}, 7·0 ppm.

The reproducibility in real samples was evaluated by means of 11 samples of RCM; the RSD values obtained were, respectively, 3·01%, 3·82% and 2·27% for Cl^-, HPO_4^{2-} and SO_4^{2-}.

It can be seen that the purest product is the RCM, followed by DVS, cane sugar and lemon syrup. The products with the highest content of anions are the pear and apple concentrated juices, and the sulphited must.

Apart from Cl^-, HPO_4^{2-} and SO_4^{2-}, considerable amounts of tartrate, malate and oxalate have been detected, the content of the first of these being very high in sulphited musts and those of the other two being also high in apple and pear concentrated juices. Oxalate has been detected in honey and cane sugar in concentrations of 235 and 7 ppm, respectively. Seven hundred and sixty-one ppm of sulphur dioxide have been found in sulphited musts as well as nitrates in all samples (except RCM and honey), in concentrations ranging from 2·1 ppm (DVS) to 470 ppm (concentrated apple juice).

Condensed milk is especially high in chlorides and phosphates, coming from the original raw material. It is also remarkable in its high content of nitrates, no trace of which is found in fresh milk; they possibly appear because of the sugars that are added during the manufacture of the product.

CONCLUSIONS

The method described is suitable for studying mixtures at a wide range of concentrations, taking always into account that dilution and sensitivity should previously be adjusted. There is no matrix effect in the glucose/fructose system; consequently the calibration can be carried out in water.

This technique also allows for the identification and determination of anions other than the three mentioned, especially nitrates and organic acids. The sampling frequency is 6/h, which is very satisfactory for a 7-anion simultaneous determination.

This method has, for the studied working conditions, a very good linearity, precision, recovery and reproducibility, which is adequate for application to the analysis of real samples, especially in the food field. It challenges other methods because neither sample pretreatment nor titrated reagents nor multiple detectors are required.

Finally, as there are neither official norms nor adequate analytical methods in existence, this work may be a valuable contribution in this field.

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