Influence of organic solvents in the mobile phase on the determination of carboxylic acids and inorganic anions in grape juice by ion chromatography

P. Masson*

[a]Station d'Agronomie, Centre de Recherches INRA de Bordeaux, B.P. 81, 33883 Villenave d'Ornon Cedex, France

Abstract

Investigations have been initiated to develop a sufficiently good separation of both major and minor organic acids and inorganic anions present in grape musts using a Dionex As11 column, a sodium hydroxide gradient elution and a suppressed conductivity detection. Separation was complicated in aqueous mobile phase by co-elutions and selectivity was optimized using organic modifiers that alter ion-exchange selectivity for hydrophobic ions. In this study, the influence of three different solvents (methanol, ethanol and acetonitrile) on the efficiency of column was compared. The best separation of all ions in a synthetic solution was achieved with an eluent containing 13% (v/v) methanol and 13% (v/v) ethanol in water, the run during only 20 min. This method was next applied to grape juices with success and has shown sensitivity and reproducibility. Moreover, sample preparation was a simple 20-fold dilution with 0.45 μm filtration and direct injection without prior sample clean-up. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Mobile phase composition; Fruit juices; Food analysis; Carboxylic acids; Inorganic anions.

1. Introduction

The nature and concentration of the carboxylic acids and inorganic anions present in grape juices are of considerable importance for the development and the overall quality of wine [1,2]. Tartaric and malic acids are commonly the acids of interest [3] but other acids are also present as well as inorganic anions such as chloride, sulfate and phosphate. However, a better knowledge of the carboxylic acids and inorganic anions profile is required to take maximum advantage of the technical improvement of culture methods. Fast and accurate measurement methods would be then extremely helpful in agronomy studies and quality control of vine.

Many papers have been published on acids determination in beverages. Various analytical methods such as enzymatic and liquid chromatography (HPLC) procedures have been developed with this aim. Commonly used enzymatic techniques are time-consuming and use large amounts of reagents, which make them expensive. The speed and selectivity of liquid chromatography methods render them more useful for the analyses of juices [4–10]. Nevertheless, for reversed-phase chromatography, the detection techniques (refraction index or UV) lack specificity for carboxylic acids and are very sensitive to many interfering compounds such as sugars and phenolic compounds. Preventive sample clean-up procedures are often required [4,5,7,9] with solid-phase extraction cartridges. Consequently, the method can be expensive and time-consuming. Moreover, inorganic anions cannot be quantified either with

*Corresponding author.

enzymatic methods, or with reversed-phase chromatography.

Under these conditions, ion chromatography (IC) was certainly the technique of choice to separate and quantify, not only the main organic acid present in grape juices, but also the inorganic anions. Therefore, IC using suppressed conductivity presents highly specific and sensitive detection, which minimizes interference and sample clean-up. Simple and automated methods could be developed [6,8,10]. The purpose of this investigation was then to develop a specific separation program allowing the measurement of carboxylic acids and inorganic anions in grape juices without any previous purification procedures on an AS11 Dionex ion-exchange column. This column was especially carried out for the determination of organic acids and can run with sodium hydroxide gradient elution. It is a nice option to maximize the resolving power of ion chromatography but, to date, there is very little literature on its applications. However, matrices can be very complex and analytes of interest may be present only at trace levels. Co-elutions can occur and a sufficiently good separation of species must firstly be achieved even when some ions are present in large excess.

Separation improvement is possible to manipulate the chemical composition of eluents [11] especially through addition of organic solvents in aqueous phase. Organic solvents play an important role in ion chromatography and several examples are present in the literature [12–15] to describe their utility. Current research in this field is directed towards a fundamental understanding of how changes in the eluent affect the separation process. The degree of hydration of the ions in the bulk eluent is a major factor in ion-exchange selectivity. Adding solvent changes the degree of hydration of the ions in solution and the ion-exchange sites in the polymer. Consequently, the degree of solvation and ability of an ion to remove the surrounding solvent molecules would alter the affinity that ion has for the ion-exchange sites. So, ions that are highly hydrated tend to have shorter retention times than ions of lower hydration [13]. Changing the retention characteristics of the column packing toward the analyte permits the analyst to alter retention and resolution to optimize the separation. Solvents also reduce hydrophobic interactions and maintain organic analyte solubility [10].

A wide variety of the organic acids found in food products have been resolved in IC with methanol [15] but methanol is a very dangerous solvent. Others organic solvents as acetonitrile or ethanol are less dangerous and may be envisaged as an alternative. They have been tested on column conceived for hydrophobic anionic analytes (Dionex Omni-Pac PAX-100) [12] but their response on the Dionex AS11 column is poorly known. So, this paper describes a comparison of selectivity effects for this specific column resulting in the use of methanol, ethanol or acetonitrile in the mobile phase. For each solvent, the chromatographic conditions will be achieved for the carboxylic acids and inorganic anions commonly encountered in juice samples on a synthetic solution. Then, the best separations will be tested on fresh grape juice samples.

2. Experimental

2.1. Instrumentation

The high-performance liquid chromatograph used was a Dionex (Sunnyvale, CA, USA) Model DX 500 IC equipped with a GP 40 gradient pump, an ED 40 electrochemical detector and an AS 40 automated sampler. The chemical separation was carry out on an AS11 analytical column and an AG11 pre-column. The detector was preceded by an ASRS II anion self-regenerating suppressor. This cation exchange membrane in the H+ form is used to suppress the background conductivity of the eluents. The Anion Self-Regenerating Suppressor was used in the auto-suppression recycle mode for the upper chromatogram which uses an aqueous eluent (containing no solvent) and in auto-suppression external water mode to generate the others chromatogram that use an eluent containing solvent. Table 1 summarized details of the operating parameters.

2.2. Eluents

All reagents were analytical grade. Water purified (18 MΩ) using an ELGA (Bucks, UK) UHQ water purification system was used for all eluents. Ternary gradient elution with 2 ml/min flow rate was used with the following mobile phases. Eluent A: Water or water–organic solvent mixture; eluent B: 100 m*M*

Table 1
Operating parameters for the ion chromatography system[a]

Sample loop volume	25 μl
Separator column	Dionex AS 11 (250×4 mm I.D.)
Guard column	Dionex AG 11 (50×4 mm I.D.)
Eluent	Water or water–organic modifier/0.5 mM NaOH/100 mM NaOH
Eluent flow-rate	2 ml/min
Pump pressure (water)	1050 p.s.i.
Background conductivity (water)	0.8 μS
Pump pressure (methanol)	1500 p.s.i.
Background conductivity (methanol)	2.5 μS
Pump pressure (ethanol)	1550 p.s.i.
Background conductivity (ethanol)	2.0 μS
Pump pressure (acetonitrile)	1600 p.s.i.
Background conductivity (acetonitrile)	1.8 μS
Pump pressure (methanol–ethanol)	1550 p.s.i.
Background conductivity (methanol–ethanol)	3.0 μS

[a] 1 p.s.i.=6894.76 Pa.

Table 2
Gradient profile[a]

Time (min)	Flow (ml/min)	A (%)	B (%)	C (%)	Curve	Comments
Column equilibration						
Initial	2.0	50	50	0	5	
4.60	2.0	50	50	0	5	Sampling
7.00	2.0	50	50	0	5	Injection
Analysis						
0.00	2.0	50	50	0	5	Start reading
1.00	2.0	50	50	0	5	
13.00	2.0	50	42	8	4	
18.00	2.0	50	15	35	5	
20.00	2.0	50	15	35	5	End reading

[a] Eluent A: Water or water–organic modifier; eluent B: 0.5 mM NaOH; eluent C: 100 mM NaOH.

NaOH in water; eluent C: 0.5 mM NaOH in water. Eluents were prepared daily, degassed and pressurized by argon grade C. The gradient profile is given in Table 2.

2.3. Samples

A standard solution which contains mixture of carboxylic acids and inorganic anions (summarized in Table 3) is prepared for qualitative purpose and for solute peaks identification. The efficiency of the solvents was tested on this synthetic solution. Grape juice was obtained by crushing the berries using a mixer followed by centrifugation. The resulting juices were diluted (1:20) with 18 MΩ purified

Table 3
Efficiency of method for each solvent

Compound	Concentration (mg/l)	Resolution					Detection limit (μg/l)	Reproducibility, RSD (%)
		Water	CH$_3$CN 30%	MeOH 28%	EtOH 22%	EtOH/MeOH 26%		
Chloride	10						5	2.49
Nitrate	20						19	2.34
Succinic acid	20	0.2	0.9	1.2	1.1	1.2	122	1.18
Malic acid	20	2.5	2.2	2.9	2.3	2.7	94	1.44
Tartaric acid	20	3.2	2.0	2.3	2.3	2.2	80	1.90
Ketoglutaric acid	20	0.0	1.5	1.1	1.1	1.2	151	1.75
Fumaric acid	20	2.6	1.6	1.3	1.4	1.7	76	0.94
Sulfate ion	15	0.2	1.4	1.3	1.5	1.3	21	1.73
Oxalic acid	20						86	2.23
Phosphate	15						41	2.23
Citric acid	20						164	2.14

water, filtered at 0.45 μm and directly injected into the HPLC. The instruments were calibrated with blanks and three mixed standards prepared by dissolving the organic acids and the corresponding sodium salts of mineral anions in 18 MΩ purified water (5, 10 and 25 mg/l Cl^-, 10, 20 and 50 mg/l NO_3^-; 7.5, 15 and 37.5 mg/l PO_4^{3-}; 7.5, 15 and 37.5 mg/l SO_4^{2-}, and 10, 20 and 50 mg/l of each organic acid). With the exception of aqueous eluent, the linearity of the standards was satisfactory for each species ($r^2 \geq 0.990$) over these concentration ranges with peak area evaluation.

3. Results and discussion

Table 3 summarizes the efficiency of method tested with each solvent in this study based on resolution of peaks when co-elutions occurred.

3.1. Gradient less organic solvent

Fig. 1 shows the chromatogram obtained for the synthetic mixture using the gradient profile given in Table 2. Gradient elution with NaOH permitted to optimize the best selectivity for anions within a reasonable elution time. In fact, the separation was achieved in 18 min.

Results showed that chloride, nitrate, phosphate, citric acid and tartaric acid — which is generally the major constituent of total organic acid contents in grape juices — were well resolved. The other acids and sulfate anions were most resistant to quantitative determinations. That can be seen on the second chromatogram, which is an enlargement of portion corresponding to the separation of divalent ions. α-Ketoglutarate ions co-elute with sulfate ions and the two peaks overlap completely. Similarly, the separations between succinic and malic acids and fumaric and oxalic acids were not achieved and presented large peaks. Co-elutions of the compounds occurred probably owing to their nearly equal ionic radii in the hydrated form.

3.2. Gradient with organic solvents

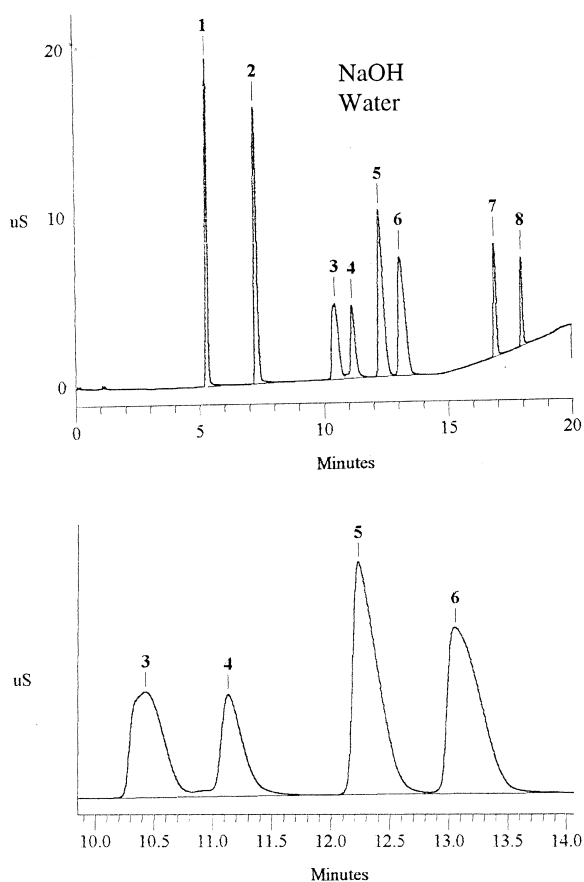Investigations were conducted to obtain a sufficiently good chromatographic separation of each

Fig. 1. Ion chromatogram of standard solution. Eluent=NaOH gradient in water. Peaks: 1=chloride, 2=nitrate, 3=succinate+malate, 4=tartarate, 5=sulfate+α-ketoglutarate, 6=oxalate+fumarate, 7=phosphate, 8=citrate.

unresolved pair of peaks by optimization of solvent ratio in the neutral eluent A. The best results are shown in Figs. 2–4 for methanol (28%, v/v), ethanol (22%, v/v) and acetonitrile (30%, v/v) respectively. Compared with aqueous mobile phase (Fig. 1), some organic acids appeared on the chromatograms.

A longer retention time results with addition of methanol, yielding better resolution of closely eluting ions (Fig. 2). Adding methanol, the highly hydrated hydroxide ions would tend to lose waters of hydration less readily than the stationary phase or the other ions in solution. That tends to decrease the selectivity of the ion-exchange sites for the hydroxide eluent ions causing an increasing of retention for other ions [12]. Nevertheless, the resolutions
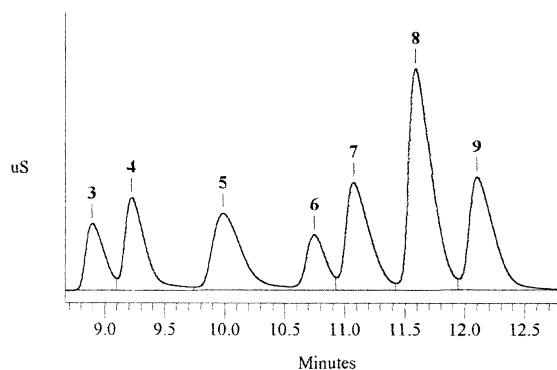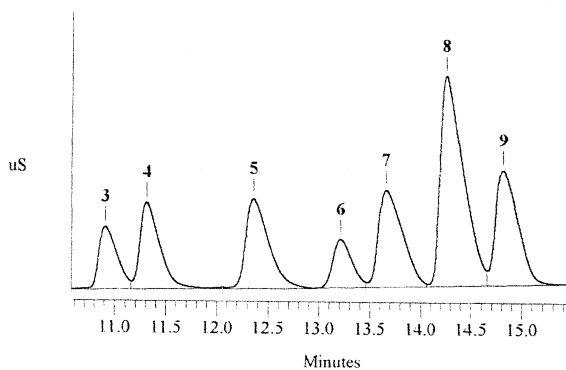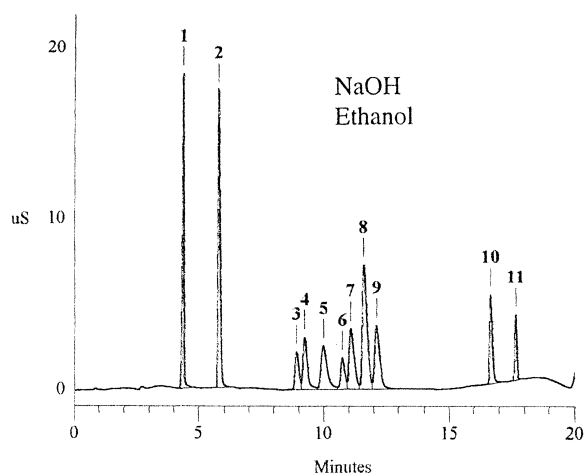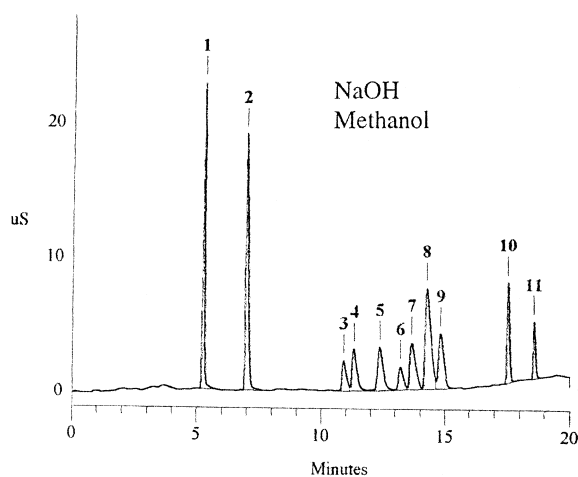
Fig. 2. Ion chromatogram of standard solution. Eluent=NaOH gradient in water–methanol (72:28, v/v). Peaks: 1=chloride, 2= nitrate, 3=succinate, 4=malate, 5=tartarate, 6=α-ketoglutarate, 7=fumarate, 8=sulfate, 9=oxalate, 10=phosphate, 11=citrate.

Fig. 3. Ion chromatogram of standard solution. Eluent=NaOH gradient in water–ethanol (78:22, v/v). Peaks: 1=chloride, 2= nitrate, 3=succinate, 4=malate, 5=tartarate, 6=α-ketoglutarate, 7=fumarate, 8=sulfate, 9=oxalate, 10=phosphate, 11=citrate.

between α-ketoglutarate, fumarate and sulfate ions were just sufficient. Problems can occur if one is present in large excess compared to the others. Conversely, malic and succinic acids were well separated.

Fig. 3 depicts a typical chromatogram obtained with ethanol. The improvement of the elution efficiency was achieved with 22% of solvent. Ethanol has similar effects of methanol but gives a shorter run time. This can be explained in terms of solvation and dielectric constant effects. The dielectric constant is significantly lower than methanol. Therefore, a solution of ethanol in water would be less polar and affects less than an equivalent methanolic solution the degree of solvation and the ability of ions to

exchange [13]. However, all ions showed good separation individually except α-ketoglutaric and fumaric acid.

In Fig. 4, it can be seen that the addition of acetonitrile decreases the retention times, especially for the monovalent and divalent ions. The time required for complete separation of the anions is less than 18 min. Stillian and Pohl had observed this phenomenon on a Dionex Omni-pac PAX-100 column [12]. They hypothesize that the peculiar ion-exchange phase formed by a latex with low cross-linking is probably swollen to a greater extent in an acetonitrile–water mixture than in water alone. The result of this swelling is fewer ion-exchange sites per unit volume of latex. Acetonitrile had physical
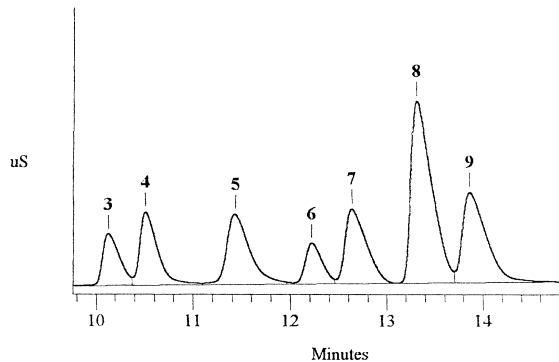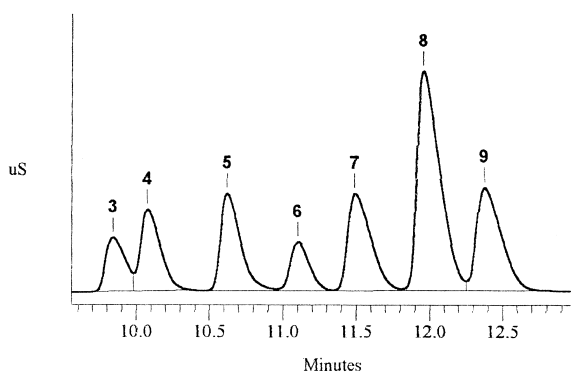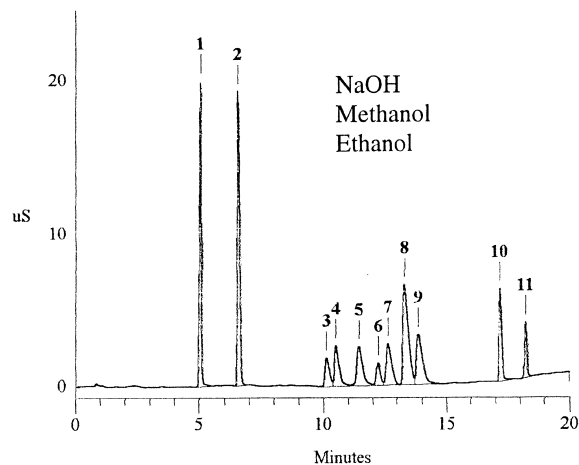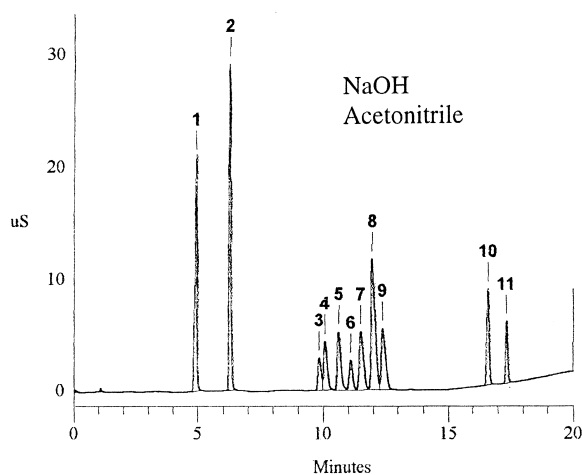
Fig. 4. Ion chromatogram of standard solution. Eluent=NaOH gradient in water–acetonitrile (70:30, v/v). Peaks: 1=chloride, 2=nitrate, 3=succinate, 4=malate, 5=tartarate, 6=α-ketoglutarate, 7=fumarate, 8=sulfate, 9=oxalate, 10=phosphate, 11=citrate.

Fig. 5. Ion chromatogram of standard solution. Eluent=NaOH gradient in water–methanol–ethanol (74:13:13, v/v/v). Peaks: 1=chloride, 2=nitrate, 3=succinate, 4=malate, 5=tartarate, 6= α-ketoglutarate, 7=fumarate, 8=sulfate, 9=oxalate, 10= phosphate, 11=citrate.

properties close to methanol and probably causes a change in hydration of ion-like methanol but this effect is over-ridden by the swelling effect. However, almost all of anions were very well resolved. The main problem was the separation between succinic and malic acids.

The chromatogram presented in Fig. 5 show the typical chromatogram obtained with an eluent constituted by water, methanol and ethanol. This type of ternary mixture had been used by Saccani et al. on a Dionex Omni-Pac PAX-100 column [6]. The best elution efficiency has been performed with 13% of both organic solvents. With this way, all anions, organic and inorganic, showed clear individual sepa-

ration ($R_s \geq 1.2$). This solution appeared as the best for simultaneous analysis of organic and mineral anions. In comparison with the methanol eluent, recommended by the manufacturer, the gain was small but real (Table 3).

3.3. Application to grape juices

The chromatogram presented in Fig. 6 shows the profile of organic and inorganic anions for grape juice without organic solvent in eluent. Sample was diluted twenty times. This dilution ratio allows good sensitivity of the majority of compounds but the analysis is complicated by the quality of separation.
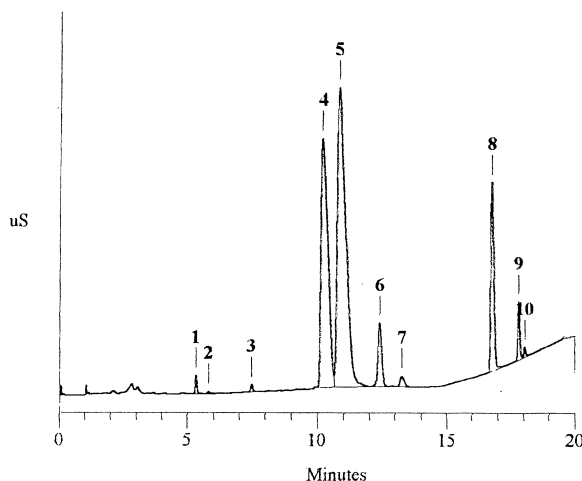
Fig. 6. Ion chromatogram of grape must sample. Eluent=NaOH gradient in water. Peaks: 1=chloride, 2=unknown, 3=nitrate, 4=succinate+malate, 5=tartarate, 6=sulfate, 7=fumarate+ oxalate, 8=phosphate, 9=citrate, 10=isocitrate.

Solute concentrations showed great variability and thus affect the detection of the components. Tartaric and malic acids were the major acids. Inorganic anions (chloride, sulfate and phosphate) and oxalic and citric acids are also present in smaller quantities. Conversely, the identification and determination of fumaric and succinic acids in grape musts is impossible because co-elutions occurred between malate and succinate ions and fumarate and oxalate ions. These results were close to those obtained with the synthetic solution. The use of aqueous eluent only can be available if tartaric and malic acids are the compounds of interest as in several agronomic studies and if succinic acid is present at trace level. Higher dilution (above 1/100, v/v) of the sample allows acceptable quantification of the two main acids but the concentrations of others species cannot be quantified in the same injection at such low concentrations.

Figs. 7 and 8 show the chromatograms of grape juice with water–acetonitrile and water–methanol– ethanol mobile phases respectively. Adding organic solvent, especially methanol and ethanol together, any interference occurred on the separation. Comparative with aqueous eluent, fumaric and succinic acids appeared, but in trace amounts. The ternary water–methanol–ethanol mixture offered the best agreement despite the complexity of the matrices and
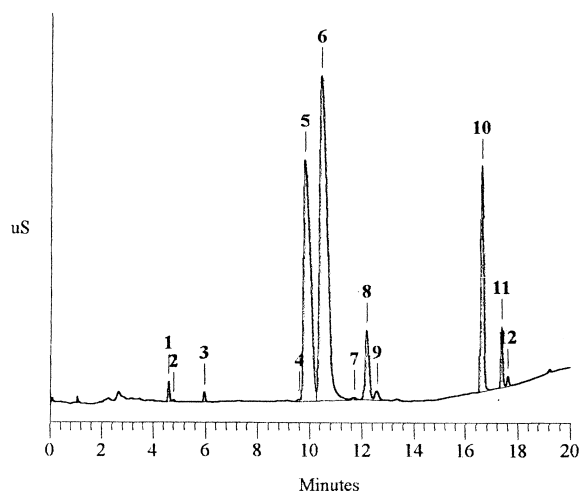

Fig. 7. Ion chromatogram of grape must sample. Eluent=NaOH gradient in water–acetonitrile (70/30, v/v). Peaks: 1=chloride, 2=unknown, 3=nitrate, 4=succinate, 5=malate, 6=tartarate, 7= fumarate, 8=sulfate, 9=oxalate, 10=phosphate, 11=citrate, 12= isocitrate.

the considerable quantitative imbalances between the various anions. The same good results were obtained on other samples, fruit juices and plant saps (data not shown). This was in accordance with the previous investigation on the synthetic solution. With acetoni-
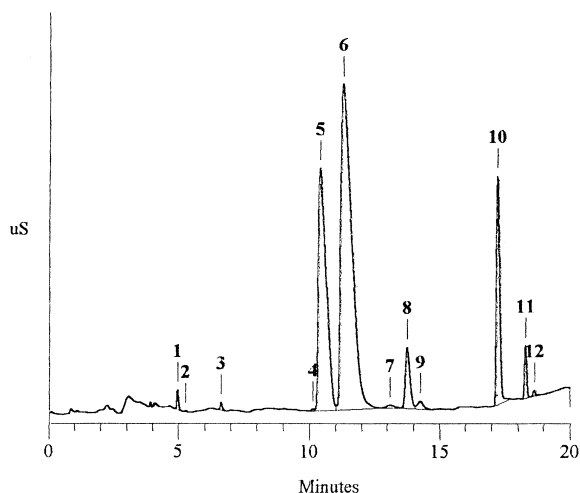

Fig. 8. Ion chromatogram of grape must sample. Eluent=NaOH gradient in water–methanol–ethanol (74/13/13, v/v/v). Peaks: 1=chloride, 2=unknown, 3=nitrate, 4=succinate, 5=malate, 6= tartarate, 7=fumarate, 8=sulfate, 9=oxalate, 10=phosphate, 11= citrate, 12=isocitrate.

trile, the separation succinic/malic acids was poorly resolved as with synthetic solution. Acetonitrile can be used when succinic acid is only present at trace levels and is not a compound of interest.

It can be seen in Table 3 that the ion chromatography method considered with a NaOH gradient in methanol–ethanol allows quantification of the ions with good sensitivity and reproducibility. The limit of detection, L_D, was defined as three times the standard deviation of the background signal that was determined by measuring ten replicate samples of purified water. Reproducibility was assessed as the relative standard deviation from triplicate analyses of anions in the standard mixture. Evaluation of the chromatograms was performed by measuring the peak area.

4. Conclusions

The analysis of anions in grape juices may be complicated by the presence of various levels of constituents in the sample matrix that affects the quality of separation. Co-elution between anions species peaks occurred and ion chromatography can give poor resolution even with a specifically designed column for organic acids analysis. The results of this investigation have shown how organic acids and inorganic anions can be separated effectively in ion chromatography with no sample preparation by the ability to control the ion-exchange selectivity with the addition of methanol, ethanol or acetonitrile. The choice of organic solvent was an important parameter of the separation because solvating power and hydrophobicity of the solvent can influence the retention mechanism and allows separating co-eluting species.

The best overall separation, giving the profile of all the organic acids and inorganic anions, was achieved with a water–methanol–ethanol mixture as neutral eluent. The proposed analytical method showed high sensitivity and reproducibility and allows quantification of the main organic and inorganic anions with a single analysis without prior sample clean-up. The juices should be only diluted (1/20, v/v). This method is certainly reliable for routine quality control but it is not limited to the fruit juice analysis and would also be an ideal research tool for organic and inorganic anions analyses in many complex matrices.

References

[1] J. Riberau-Gayon, E. Peinaud, Traité d'Ampélologie — Science et Techniques de la Vigne, Vol. 1 — Biologie de la Vigne, Dunod, Bordas, Paris, 1971.
[2] J. Blouin, Techniques d'Analyses des Moûts et des Vins, Ed. Dujardin-Salleron, Paris, 1992.
[3] G.W.A. Fowles, J. Wine Res. 3 (1992) 25.
[4] M. Callul, R.M. Marcé, F. Borrull, J. Chromatogr. A 590 (1992) 215.
[5] A. Billingsley, M. Parker, P. Bowden, A.J. Buglass, Analusis 24 (1996) 29.
[6] G. Saccani, S. Gherardi, A. Trifiro, C. Soresi Bordini, M. Calza, C. Freddi, J. Chromatogr. A 706 (1995) 395.
[7] C. Mongay, A. Pastor, C. Olmos, J. Chromatogr. A 736 (1996) 351.
[8] S.A. Kupina, C.A. Pohl, J.L. Gannotti, Am. J. Enol. Vitic. 42 (1991) 1.
[9] J.J. Hunter, J.H. Visser, O.T. DeVilliers, Am. J. Enol. Vitic. 42 (1991) 237.
[10] A. Henshall, Cereal Foods World 42 (1997) 414.
[11] P. Hajos, L. Nagy, J. Chromatogr. B 717 (1998) 27.
[12] J.R. Stillian, C.A. Pohl, J. Chromatogr. 499 (1990) 249.
[13] S. Rabbin, J. Stillian, J. Chromatogr. A 671 (1994) 63.
[14] C.A. Pohl, J.R. Stillin, P.E. Jackson, J. Chromatogr. A 789 (1997) 29.
[15] Dionex Application Note, Dionex Corporation, Sunnyvale, CA, 1995.