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Note

Determination of the principal anionic components in wines and soft drinks, by ion interaction reversed-phase high-performance liquid chromatography

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Previous work[1,2] in this laboratory has shown the applicability of ion interaction reversed-phase high-performance chromatography in separations of anionic species. Salicylates of some aliphatic amines were used as ion interaction reagents. Retention and resolution were shown[2] to depend on the alkyl chain length of the ion interaction reagent, the flow-rate, the stationary phase and the method of detection, which leads to the well known[3–8] versatility of the technique.

The aim of this work was to optimize the experimental conditions for the identification, separation and determination of anionic species, both inorganic and carboxylic. The method was applied to the analysis of the major anionic components of some Italian wines and soft drinks.

EXPERIMENTAL

Apparatus

Analyses were performed using a Varian LC 5000 chromatograph, equipped with a Vista 401 data system and a UV-100 spectrophotometric detector. Alternatively, a Wescan 213A conductimetric detector was employed; it was interfaced to the Vista 401 data system with a 1 V exit. For pH measurements a Metrohm 654 pH-meter was used.

Merck Hibar LiChrosorb RP-18, 10 μm (250 × 4 mm I.D.), Merck Hibar LiChrospher RP-18, 10 μm (250 × 4 mm I.D.) and Waters Bondapak C_{18}, 10 μm (150 × 3.9 mm I.D.) columns, equipped with a Merck Hibar LiChrocart LiChrosorb RP-18 guard column (25 × 4 mm I.D.), were used.

Chemicals

Analytical-reagent grade chemicals and ultra-pure water from a Millipore Milli-Q system were used. Heptylamine was from Aldrich and octylamine from Fluka. Salicylic acid and all other reagents were obtained from Carlo Erba.

The solutions of the eluents were prepared by dissolving a weighed amount of the amine in ultra-pure water and adjusting the pH to 6.2 ± 0.4 by addition of salicylic acid. Taking into account the acid formation constants of amines, the com-

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TABLE I

RETENTION TIMES OF SOME ANIONS USING DIFFERENT ION INTERACTION REAGENTS, STATIONARY PHASES AND FLOW-RATES

Estimates of standard deviations are based on at least four determinations.

Anion	*Retention time (min)*					
	0.005 M heptylamine salicylate		*0.005 M octylamine salicylate*			
	Merck LiChrosorb RP-18 (10 μm), 0.5 ml/min	*Waters Bondapak C_{18} (10 μm), 0.5 ml/min*	*Merck LiChrosorb RP-18 (10 μm), 3.0 ml/min*	*Waters Bondapak C_{18} (10 μm), 1.9 ml/min*	*Merck LiChrospher RP-18 (10 μm)*	
					2.0 ml/min	*2.5 ml/min*
Chloride	6.4 ± 0.2	5.2 ± 0.2				
Carbonate	7.1 ± 0.2	5.0 ± 0.2			3.0 ± 0.3	2.1 ± 0.3
Acetate	7.2 ± 0.2	5.2 ± 0.2	1.7 ± 0.3	2.0 ± 0.2	3.0 ± 0.3	
Nitrate	7.1 ± 0.2	5.5 ± 0.2				
Orthophosphate	9.1 ± 0.2	6.2 ± 0.3				
Glycolate	7.4 ± 0.3			2.2 ± 0.3		
Gluconate				2.2 ± 0.3		
Ascorbate	7.7 ± 0.3	5.7 ± 0.2		2.2 ± 0.3		
Lactate	7.9 ± 0.2	5.2 ± 0.3	2.0 ± 0.3	2.2 ± 0.3		
Butyrate			4.3 ± 0.3			
Succinate	12.6 ± 0.2	8.6 ± 0.2	9.3 ± 0.3	9.2 ± 0.3	17.2 ± 0.3	
Malate	13.5 ± 0.4	11.5 ± 0.4	10.4 ± 0.4	10.1 ± 0.3	19.8 ± 0.3	
Glutarate	15.0 ± 0.4	11.9 ± 0.4		9.7 ± 0.3		
Malonate	15.0 ± 0.4	11.9 ± 0.4		10.0 ± 0.4	21.6 ± 0.4	17.4 ± 0.4
Tartrate	15.5 ± 0.5	12.2 ± 0.5	12.7 ± 0.5	12.3 ± 0.5	24.9 ± 0.5	
Maleate	17.8 ± 0.5	12.7 ± 0.4	11.7 ± 0.4	11.2 ± 0.5	22.6 ± 0.5	
Adipate				11.5 ± 0.4	20.9 ± 0.5	17.0 ± 0.4
Citrate	34.0 ± 2.0	15.6 ± 0.5				
System peak	67.0 ± 2.0	62.0 ± 2.0	15.0 ± 0.6	20.4 ± 0.6	24.0 ± 1.0	

position of the eluents so prepared is not exactly stoichiometric. For simplicity, however, they will be referred to henceforth as amine salicylates.

Chromatography

In order to condition the chromatographic system, eluent was allowed to flow through the column until a stable baseline was obtained, generally requiring about 1 h. Eluent solution was prepared freshly every third day.

The reproducibility of the retention times and sensitivity was very good for sequential analyses, but it was poorer for different eluent preparations and column conditionings. The sensitivity and, consequently, the accuracy and precision of determined followed a similar trend. Retention times and quantitation data were evaluated for different preparations.

Between use, the columns were regenerated by passage of water–methanol (1:1); no particular deterioration of the column was observed with respect to its use in other chromatographic techniques.

The samples of wines and beverages to be injected were prepared by filtration through a Nucleopore Syrfil 0.45-μm filter and diluting, when necessary, in ultra-pure water.

RESULTS AND DISCUSSION

Preliminary measurements showed that, in the separation of carboxylic acids, 10-μm C_{18} stationary phases are more suitable than the 5-μm type as the former allow a better separation and generally lower retention times. In this study, different 10-μm stationary phases (with spherical or irregular particles) were compared.

Salicylates of heptylamine and octylamine were used as ion interaction reagents. The relatively low ionic equivalent conductivity and the high molar absorptivity in the UV region ($\varepsilon = 308 \pm 2$ l mol^{-1} cm^{-1} at 254 nm) of salicylate ions allow both conductimetric and spectrophotometric, direct and indirect, detection. The results obtained for a series of anionic species are given in Table I.

The behaviour of the ion interaction reagent and its elution flow agree with previous conclusions[2] that the shorter is the alkyl chain length, the shorter are the retention times and, as the flow-rate increases, the retention decreases.

Further, interactions between the eluate and modified stationary phase depend on the alkyl chain length and modify the elution order (compare, for example, the retention times of tartaric and maleic acids obtained with the two eluents).

Fig. 1 shows the separation of a mixture of acetic, ascorbic, lactic, succinic, malic, glutaric, tartaric and maleic acids under optimized experimental conditions. Owing to the molar absorptivities at 254 nm of ascorbate [$\varepsilon_{254\ nm} = (8.72 \pm 0.04) \cdot 10^3$ l mol^{-1} cm^{-1}] and maleate [$\varepsilon_{254\ nm} = (7.2 \pm 0.2) \cdot 10^2$ l mol^{-1} cm^{-1}], these anions give rise to positive peaks. Hence they can be easily identified and separated from UV-transparent anions, in spite of the poor separation factors. Taking into account the molar absorptivity of salicylate ions at this wavelength, UV-transparent anions appear as negative peaks.

The use of octylamine salicylate, characterized by lower retention, permits the separation of the series of the anions listed in Table I, whereas the use of heptylamine allows the separation of the components with lower retention times to be improved.

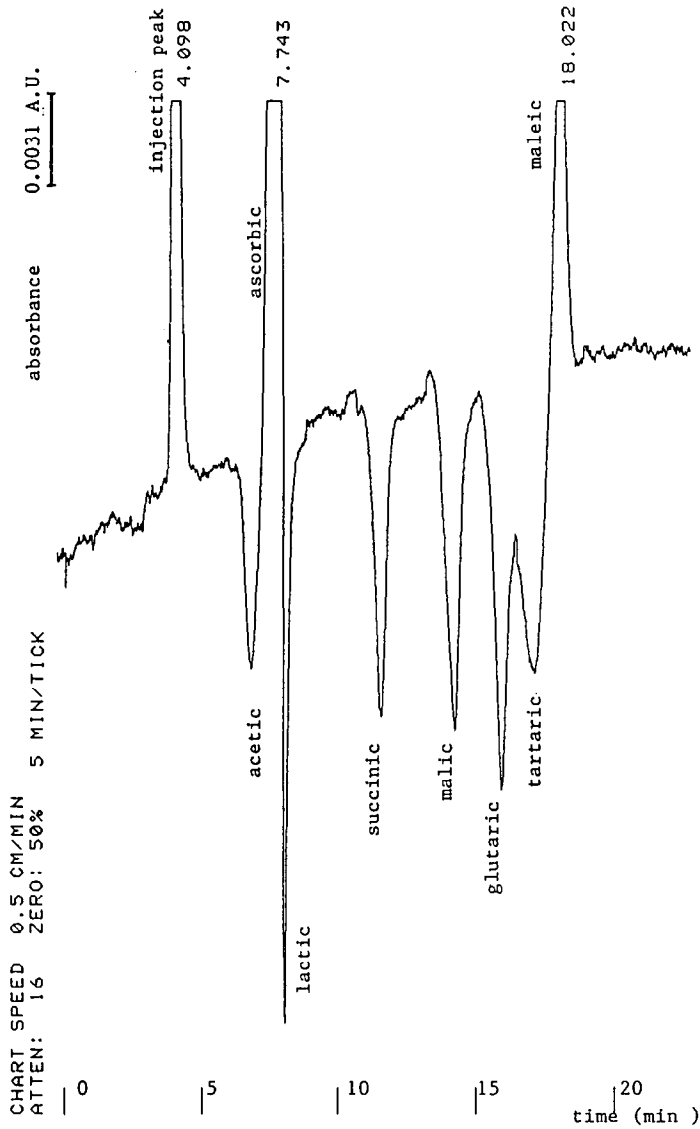

Fig. 1. Separation of a mixture of acetic, ascorbic, lactic, succinic, malic, glutaric, tartaric and maleic acids (50.0 ppm each). Injection volume, 100 μl; column, Merck LiChrosorb RP-18, 10 μm; ion interaction reagent, 0.0050 *M* heptylamine salicylate; flow-rate, 0.5 ml/min; spectrophotometric detection (254 nm).

These observations were applied to the analysis of some Italian wines and soft drinks. The qualitative and quantitative determination of the anionic species is of interest with regard to organoleptic properties and quality control.

Red wines (Barbera delle Langhe 1987, Barbaresco DOCG 1984, Grignolino 1987, Chianti DOCG 1987), and white wines (Pinot Grigio 1987 and boxed Tavernello Trebbiano) were considered. Fig. 2 shows, as an example, the chromatograms for Barbera wine when using (a) 0.0050 *M* octylamine salicylate as the eluent at a flow-

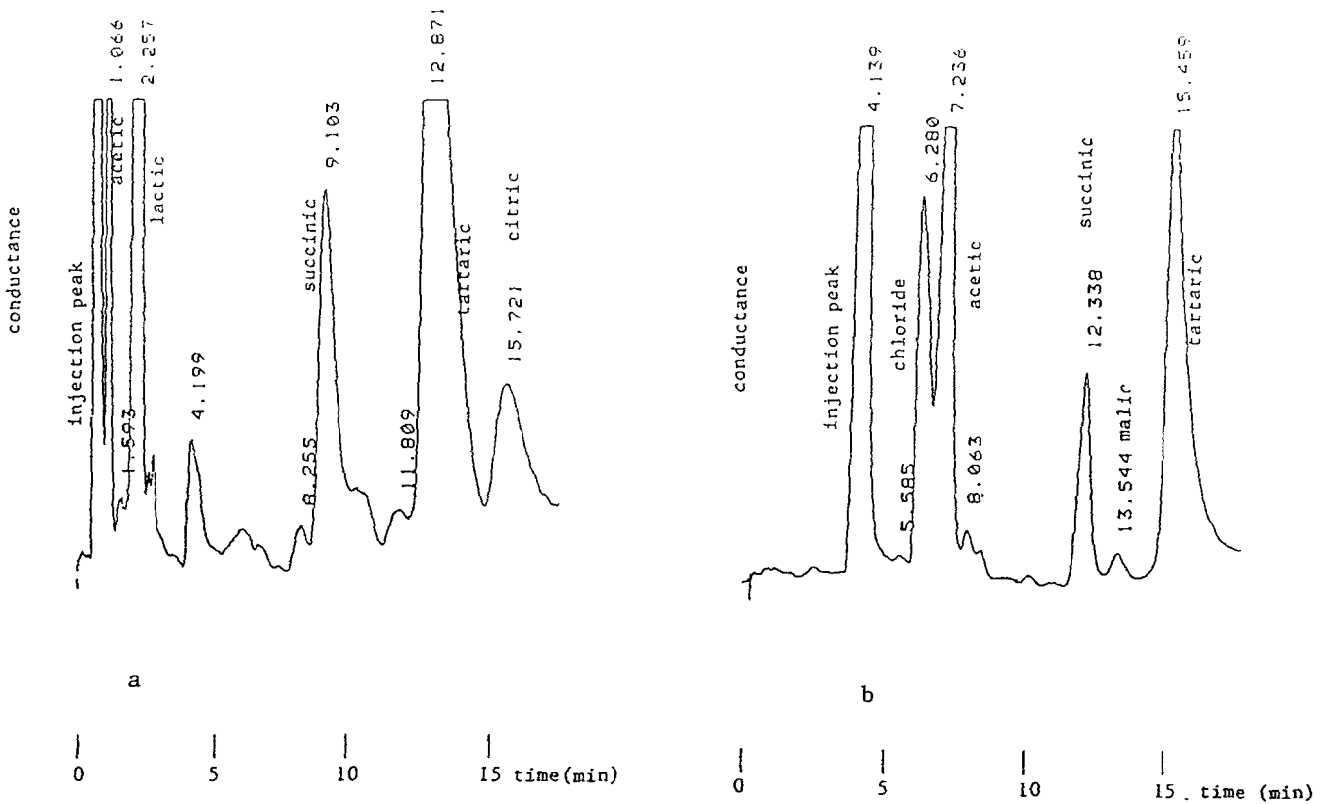

Fig. 2. Analysis of Barbera delle Langhe 1987 wine. Injection volume, 100 μl; column, Waters Bondapak C_{18}, 10 μm; conductimetric detection. (a) Ion interaction reagent, 0.0050 *M* octylamine salicylate; flow-rate, 1.9 ml/min; sample dilution, 1:10 (v/v). (b) *Ion interaction* reagent, 0.0050 *M* heptylamine salicylate; flow-rate, 0.5 ml/min; sample dilution, 1:50 (v/v).

rate of 1.9 ml/min and, for comparison, (b) 0.0050 *M* heptylamine salicylate at a flow-rate of 0.5 ml/min. The sample was diluted 1:10 (v/v) with ultrapure water when using octylamine salicylate and 1:50 (v/v) when using heptylamine salicylate. The sensitivity of the method for the analysis of the of major organic acids in wines is satisfactory.

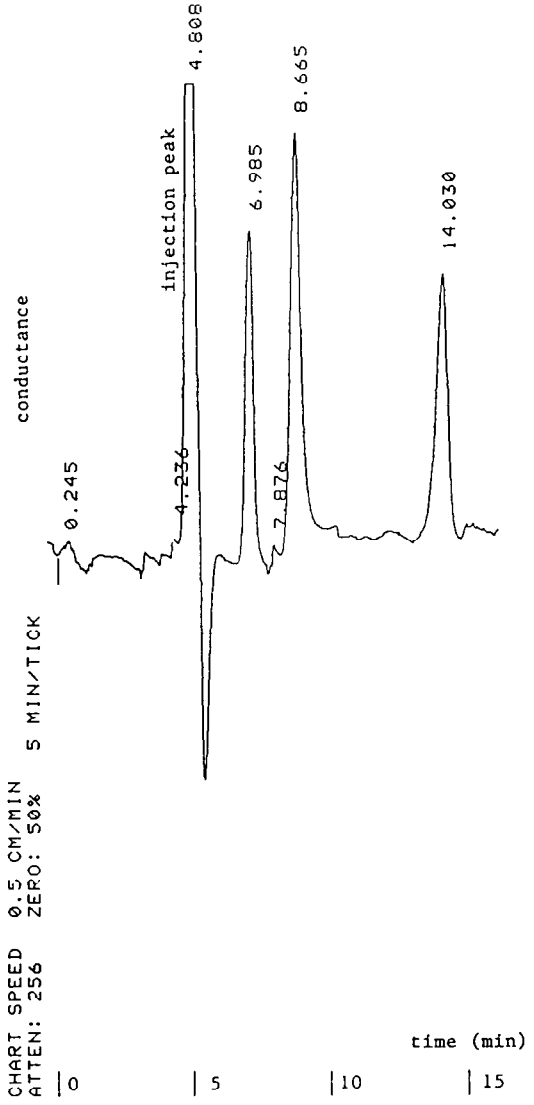

Fig. 3. Chromatogram recorded for a cola drink, diluted 1:15 (v/v). Column, Merck LiChrosorb RP-18, 10 μm; ion interaction reagent, 0.0050 *M* heptylamine salicylate; flow-rate, 0.7 ml/min; injection volume, 100 μl; conductimetric detection. Peaks: 6.985 min = chloride; 8.665 min = orthophosphate; 14.030 min = tartrate.

TABLE II

DETERMINATION OF MAJOR CARBOXYLIC ACIDS IN ITALIAN WINES

Estimates of standard deviations are based on at least three determinations.

Wine	*Acid (g/l)*			
	Succinic	*Malic*	*Tartaric*	*Citric*
Grignolino 1987	0.85 ± 0.08	0.11 ± 0.02	1.93 ± 0.19	0.23 ± 0.07
Barbera delle Langhe 1987	0.60 ± 0.07	traces	2.20 ± 0.20	0.08 ± 0.03
Chianti DOCG 1987	0.79 ± 0.07	0.27 ± 0.04	2.41 ± 0.24	0.09 ± 0.03
Barbaresco DOCG 1984	0.69 ± 0.06	0.59 ± 0.06	2.13 ± 0.11	0.22 ± 0.03
Pinot Grigio 1987	0.40 ± 0.05	1.41 ± 0.14	1.34 ± 0.13	0.06 ± 0.02
Tavernello Trebbiano	0.70 ± 0.07	0.63 ± 0.06	2.42 ± 0.24	0.11 ± 0.02

As mentioned above, the use of heptylamine permits a better separation of the less retained components. As inorganic anions are characterized under these conditions by lower retentions, separations of mixtures of organic and inorganic anions are therefore made possible by the use of this reagent. A typical example (flow-rate 0.7 ml/min, LiChrosorb RP-18 column and conductimetric detector) is the analysis of anions in commercial soft drinks, namely a cola beverage, a fizzy drink and a dietetic drink, previously degassed and diluted 1:15 (v/v). Fig. 3 shows, as an example, a chromatogram of a cola drink.

Preliminary measurements performed on wines and drinks to which known amounts of the investigated acids had been added permitted any matrix effect on the determination of the anions to be excluded. Plots of peak area *versus* standard concentration showed good linearity. For all the wines the concentrations of succinic, malic, tartaric and citric acids were determined (Table II). Table III reports quantitative data for chlorides, orthophosphates, tartrates and citrates in soft drinks. The results for the cola drink agree reasonably well with literature data[9] with respect to the pH of the drink. However, our results show that not only orthophosphoric acid but also citric acid contribute to the acidity.

The advantage of the proposed technique is that it requires no pretreatment or derivatization of the sample, except for a filtration and, sometimes, dilution.

If chromatograms of the sample and of standard solutions are recorded sequentially for the same eluent preparation, a precision below 2% is obtained in quantitative analysis.

TABLE III

DETERMINATION OF ANIONIC SPECIES IN SOFT DRINKS

Estimates of standard deviations are based on at least three determinations.

Soft drink	*Anion (g/l)*			
	Chloride	*Orthophosphate*	*Tartrate*	*Citrate*
Dietetic drink	0.28 ± 0.04	0.12 ± 0.03	–	3.0 ± 0.2
Fizzy drink	0.05 ± 0.02	–	0.17 ± 0.03	1.5 ± 0.2
Cola drink	0.03 ± 0.02	0.27 ± 0.03	0.24 ± 0.04	–

The sensitivity depends on the operating conditions, as can be seen by comparing the chromatograms for Barbera wine obtained using octylamine and heptylamine salicylate (Fig. 2). The chromatograms show similar peak areas, notwithstanding the different dilutions of the samples [1:10 (v/v) for octylamine salicylate and 1:50 (v/v) for heptylamine salicylate]. The interpretation of this difference in sensitivity, related to the alkyl chain length, requires further investigation. It can be concluded that the sensitivity of the proposed method is more than sufficient for the analysis of anionic species in wines and beverages. The precision is comparable to that obtained by other methods that require derivatization reaction[10,11]. The lack of a need for pretreatment is a major advantage, especially in food control analysis.

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