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Short communication

Determination of carboxylic acids and inorganic anions in wines by ion-exchange chromatography

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Abstract

A simple and rapid ion-exchange chromatographic method with conductimetric detection for the determination of the main carboxylic acids (acetic, lactic, succinic, malic, citric and tartaric acids) and inorganic anions (chloride, nitrate and sulphate) in wines is described. Separation was optimized using the modified simplex method. The mobile phase is 0.975 m*M* phthalic acid at pH 4.15. The chromatographic system is kept at 39°C. The method does not require derivatization or extraction of the sample. The procedure only includes filtration (0.45 μm) and passage through a Sep-Pak C_{18} cartridge. The linearity, sensitivity, recovery (>98%) and reproducibility (>99%) were studied for each acid. Detection limits ranging from 4.7 to 0.31 mg/l were obtained for citric acid and chloride, respectively. Comparison of the results with those obtained by spectrophotometric, GC, potentiometric and enzymatic assays showed that the values were similar. The ion-exchange chromatographic–conductimetric detection method permits the analysis of sweet wines without interference from sugars, and simplifies a type of determination which by other methods is complex or inaccurate.

Keywords: Wine; Food analysis; Carboxylic acids; Inorganic anions

1. Introduction

The nature and concentration of the carboxylic acids and inorganic anions in wine are important in enology because of their effects on organoleptic properties and as indicators of fraudulent practices, alterations and possible adulterations [1,2].

The disadvantages inherent in the traditional methods for determining carboxylic acids and inorganic anions in wine can be overcome by using HPLC techniques [3,4]. The presence of sugar in wine complicates the determination of carboxylic acids in the samples. In reversed-phase HPLC the formation of phenacyl, naphthacyl, *p*-nitrophenyl and *p*-nitrobenzyl esters in wine samples produces a large number of intermediate peaks due to the formation of secondary products [5,6]. Of all the esterifiers, the best one for these acids is phenacyl bromide [7,8]. Since direct injection brings with it problems of co-elutions, prior separation of the sugars, polyphenols and anthocyanins in a system consisting of two columns in series has been

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proposed [9]. Moreover, inorganic anions cannot be determined simultaneously.

When ion-exclusion chromatography with a refractometric and spectrophotometric (UV) detector is used, carboxylic acids and sugars can be determined simultaneously [10]. However, co-elutions occur [11] that can only be overcome by prior separation [12] or using either mathematical algorithms [13] or different chromatographic conditions and detection methods for the acids and sugars [14]. Calull et al. [15] optimized the chromatographic conditions for separating the carboxylic acids, sugars, glycerol and ethanol in wine and obtained good separations except for glucose, malic acid and fructose. In ion-exclusion chromatography, the use of a conductimetric detector offers low sensitivity for sugar, and therefore, there is little interference with carboxylic acid determination, but inorganic anions cannot be measured [16,17].

Nevertheless, excellent separations can be obtained with ion-exchange chromatography because of the different affinities of the ions for the stationary phase. Moroever, the inorganic anions and those coming from weak acids, the existence of which depends on the pH, can be separated and determined in a single stage and with minimal handling of the sample [18]. Ding et al. [19] and Yu et al. [20], for example, determined inorganic anions and a small number of organic acids simultaneously in dry wines and obtained good results. These separations can also be done using a gradient in Dionex columns with suppressed conductivity [21,22]. Ion-exchange chromatography, on the other hand, with conductimetric detection can be selective and does not co-elute or detect neutral compounds, such as carbohydrates, that interfere in complex matrices. The method is therefore very useful, especially for sweet wines, where the sugar content is high.

In the present study, an ion-exchange column and conductimetric detector were used to separate and detect the carboxylic acids and inorganic anions present in wine samples. Different aromatic organic acids were tested as eluents. Separation was optimized using a modified simplex method. The procedure proved to be useful in determining these compounds in wines and in sweet wines with a high sugar content.

Experimental

2.1. Apparatus

We employed a Shimadzu ion chromatograph consisting of a basic module (HIC-6A) equipped with a manual valve injector (20-μl sample loop) and single-piston pump (LP-6A), temperature-controlled oven for column and detector (CTO-6AS), ionic conductivity detector (CDD-6A) and recorder–integrator (Chromatopac C-R6A) for signal processing. pH measurements were performed using a Crison Digilab 517 pH meter.

2.2. Stationary phase

A Shimpack IC-AI column (100 × 4.6 mm I.D.) filled with quaternary ammonium polymethacrylate (particle size 12.5 μm; mass 0.92 g dry resin) was used. This was a low-capacity organic polymer column (0.050 mequiv./g), capable of supporting maximum pressures of 25 kg/cm^2, with an operating temperature of up to 50°C, and a wide operating pH range (2–12).

2.3. Mobile phase

The eluents most frequently cited in the literature are aromatic organic acids, and the most important factor for the separation is the charge of the anion. In general, the greater the charge, the more rapid is the elution. Benzoic acid, the three dicarboxylic acids (phthalic, isophthalic and terephthalic), two of the tricarboxlic acids (1,2,4- and 1,3,5-benzenetricarboxylic) and a series of hydroxylated derivatives (salicyclic, 3-hydroxybenzoic, 4-hydroxybenzoic and gallic) were also compared.

2.4. Sample treatment

The samples of wine studied were Bierzo region (samples 1 and 2), Guarantee of vintage Rioja (sample 3), Guarantee of vintage Valencia

(samples 4, 5, 6, 7 and 8) and Turis Muscatel from the Valentino wine growing region, Valencian community (sample 9).

Prior to chromatographic injection, any interference must be eliminated by means of solid-phase extraction. The wine samples, following 1:5 and 1:2 dilutions with water, pass through 100-mg solid-phase extraction cartridges (Analytichem International, Varian Division). Subsequently, 20 μl filtered through a 0.45-μm filter (Micro Separations) are injected onto the chromatographic system.

3. Results and discussion

3.1. Optimization of eluent

Taking as the starting point for each eluent the concentration 1 m*M*, pH 4 (intermediate between the log *K* of the acids of the samples and the eluents), we obtained chromatograms the morphology of which depended on the number of carboxylic groups in the eluent. This can be explained by the differences in the strength of the eluent: the monocarboxylic acids produce a slow elution (the sulphate peak elutes after ca. 70 min), the tricarboxylic acids produce a rapid elution (all in under 3 min) and the dicarboxylic acids give an intermediate elution (15 min for sulphate) with perfectly acceptable results. In an attempt to improve the elution of the mono- and tricarboxylic acids, the concentration of the former was gradually increased (in order to increase the strength of the eluent), while that of the latter was decreased. In the first case, this led to an improvement in the chromatogram, whereas in the case of the tricarboxylic acids, although there was a notable improvement in the resolution, there was also a drastic decrease in sensitivity owing to the inability of the eluent to buffer the problem acids, which occur mainly in molecular form.

Taking 3-hydroxybenzoic and phthalic acids as the most promising of the eluents, and with the aim of studying the optimum working conditions, we used the modified simplex method [23]. The chromatographic response function used, *CRF*, is similar to those described [24–28]:

$$CRF = \sum R_i + L + \alpha(t_S - t_M) + \beta(t_1 - t_I)$$

where R_i is Kaiser's resolution [29,30] for each pair of adjacent peaks, including that of the system, L is the number of peaks of the sample ions and t_S and t_M are the retention times of the system peak and of the last peak of the detected components of the sample, respectively. Given that the system peak is always much wider than the others, it should be the last to elute and be separated from the previous peak by a reasonable period of time. It is for this reason that the difference $t_S - t_M$ is set within a maximum time limit of 3 mins; t_1 and t_I are the retention times of the first and the injection peak, and the difference between them is limited to a maximum of 1 min. The ponderation factors used were $\alpha = \beta = 0.1$.

The variables to be taken into account along with the intervals tested are the pH (2.5–6.5), the concentration (0.2–5 m*M*) and the temperature (35–50°C).

The simplex evolution, applied to the two eluents mentioned, leads to the optimum conditions shown in Table 1. That phthalic acid gives a globally wider chromatographic separation can

Table 1
Optimum conditions determined by modified simplex method

Eluent	*C* (m*M*)[a]	pH	*T* (°C)	*CRF*[b]
3-Hydroxybenzoic acid	4.95	3.17	44	17.14
Phthalic acid	0.975	4.15	39	19.90

[a] Concentration of the eluent.
[b] Chromatographic response function.

be deduced from its larger response value. As a consequence, phthalic acid, under optimum working conditions, was chosen for use in the analysis of genuine samples.

3.2. Recovery results for the sample pretreatment

The most suitable phase was C_{18}, the least polar of all those tried, since the recovery is high (>98%) and the reproducibility good (>99%) in both dilutions for all the species tested. The capacity of the cartridge is sufficient to fix the interferential components present in 1 ml of problem sample.

3.3. Qualitative analysis

A standard solution (carboxylic acids and inorganic anions) is prepared for qualitative purpose. The presence of up to 10% of ethanol in these solutions affects neither the retention times nor the sensitivity of the components. The elution order is as follows: injection peak (t_R = 0.8 min), lactic (t_R = 2.2 min), fluoride (t_R = 2.3 min), phosphate (t_R = 2.3 min), succinic (t_R = 2.7 min), citromalic (t_R = 3.5 min), chloride (t_R = 3.6 min), pyruvic (t_R = 3.9 min), malic (t_R = 4.4 min), nitrite (t_R = 4.6 min), nitrate (t_R = 6.2 min), citric (t_R = 7.8 min), tartaric (t_R = 8.5 min), oxalic (t_R = 9.5 min), fumaric (t_R = 12.8 min), sulphate (t_R = 13.8 min) and system peak (t_R = 20.2 min).

In some cases, such as with lactic acid, the solutions should be recently prepared, because with time bacteria which affect the composition may be produced. Moreover, in the case of lactic acid, the solution should be prepared from a base of sodium lactate, given that the lactic acid forces the bimolecular esterification with the formation of lactides (non-ionic forms undetectable conductimetrically).

Of all the anions tested, the only ones detected in genuine samples were acetate, lactate, succinate, chloride, malate, nitrate, citrate, tartrate and sulphate anions. Fig. 1 shows the chromatogram for sample 1. In the first zone, the peaks are very close together, and in order to study the overlapping of peaks which appears in some samples, the resolution of this zone was improved by connecting two equal columns in series. Moreover, in these cases there was a breakdown in the signal for the lactic acid (sample 5) and another in the case of the succinic acid (sample 6), which clearly demonstrates the existence in each of unidentified components and permits a correct identification of the acids studied.

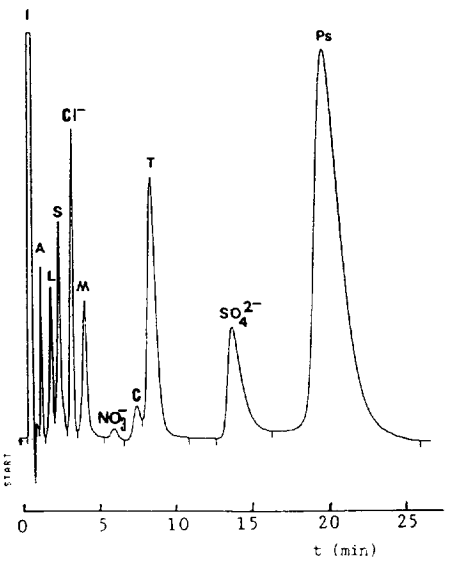

Fig. 1. Chromatogram of sample 1. I = injection peak; A = acetate; L = lactate; S = succinate; M = malate; C = citrate; T = tartrate; Ps = system peak. Chromatographic conditions as in Table 2.

3.4. Quantitative analysis

For quantification, we compared the methods of calibration lines and standard addition, and found that the two provide similar results, there being, therefore, no matrix effect. Standard addition does, however, produce an advance and a disproportionate increase in the system peak, owing to the increase in the total concentration of components in the sample. This leads to partial overlapping with the sulphate peak and a deformation which produces a faulty integration of this anion. This problem does not occur with calibration lines, which at the same time work out quicker if there are multiple samples to be

Table 2
Analytical parameters for organic and inorganic anions by ion-exchange chromatography

Sample ion	Sensitivity (μV s l/mg)	Intercept (μV s)	Background (μV s)	Detection limit (mg/l)
Acetate	156.1 ± 0.7	−130 ± 40	50	0.89
Lactate	139.7 ± 0.5	−270 ± 190	99	2.0
Succinate	159 ± 2	−1000 ± 300	107	1.9
Chloride	1266 ± 9	200 ± 400	143	0.31
Malate	232 ± 2	−1500 ± 300	159	1.9
Nitrate	716 ± 3	190 ± 90	181	0.70
Citrate	1754 ± 2	−4100 ± 200	297	4.7
Tartrate	448.6 ± 1.2	−3900 ± 500	363	2.2
Sulphate	715 ± 19	−6000 ± 2000	484	1.9

Conditions: Eluent, 0.975 m*M* phthalic acid (pH 4.15); flow-rate, 1.5 ml/min at 39°C; detection, ionic conductivity.

analysed, for which reasons this was the method chosen.

Table 2 shows the different analytical parameters. The sensitivities of the organic acids are between 140 and 450 μV s l/mg for lactic and tartaric acid, respectively. The greater sensitivity of the inorganic acids is also clearly shown. Both the sensitivities and the intercepts of the calibration lines are given, together with the standard deviations calculated for the 95% confidence level. Intercepts and larger standard deviations occur with wider peaks.

The detection limit (established as 2.78 times the background noise for five injections with a 95% level of reliability) was determined from the background noise obtained by working with a 1 μS/cm gain. It is clear that for similar sensitivities (such as nitrate and sulphate), the detection limits are larger where the width of the peak is greater. On the other hand, when the organic ions are compared with the inorganic ions, it can be seen that the latter have noticeably smaller detection limits since their sensitivity is greater, as is the case with chloride and malate. With nearby background noise, chloride is ca. six times more sensitive and thus its detection limit is six times less.

By interpolation of the calibration lines, the concentrations in the samples was calculated, taking into account the standard deviations of the slope and the intercept. In this way, the standard deviations and limits of reliability were estimated. Of the nine wines tested, only those considered representative are included in Table 3; the values obtained for the others were similar. These values are comparable to those obtained with the alternative methods tried, as can be seen in Table 3, including a wine with a high content of sugars (sample 9, Muscatel). For this sweet wine sample, the analysis by ion chromatography was carried out in the same way as for table wines. The chromatogram registered, shown in Fig. 2, demonstrates that this technique is suitable for sweet wines, and that the peaks obtained are well defined and quantifiable.

4. Conclusion

The proposed method was used to analyze white table wines and showed that with the prior treatment of the sample that has been established, there is no loss of efficiency on the part of the column even after several hundred injections.

The results obtained are perfectly valid, and there is the added advantage of the speed of the method, since all of the components can be quantified in a single chromatogram. This method therefore constitutes an important alternative for the simultaneous determination of organic acids and inorganic anions in wines.

In the case of sweet wines, where GC after

Table 3
Determination (mg/l) of organic acids and inorganic anions in wines (average concentration of three replicates)

Acid or anion	Sample											
	1		3		5		6		8		9	
	IEC	Altr.	IEC	Altr.	IEC	Altr.	IEC	Altr.	IEC	Altr.	IEC	Altr.
Acetic[a]	500 ± 2	494 ± 9	491 ± 2	490 ± 8	483 ± 2	486 ± 9	270 ± 2	281 ± 4	315 ± 2	325 ± 7	152 ± 2	150 ± 3
Lactic[a]	501 ± 11	490 ± 10	245 ± 11	239 ± 6	419 ± 11	407 ± 10	471 ± 11	458 ± 10	1220 ± 10	1212 ± 15	155 ± 11	155 ± 4
Succinic[b]	626 ± 14	640 ± 12	386 ± 14	381 ± 13	650 ± 14	614 ± 12	477 ± 14	507 ± 11	463 ± 14	439 ± 13	217 ± 15	–
Malic[b]	741 ± 10	760 ± 12	1070 ± 10	1000 ± 12	779 ± 9	760 ± 9	245 ± 10	230 ± 9	445 ± 10	450 ± 9	1390 ± 11	–
Citric[a]	438 ± 4	438 ± 6	252 ± 5	245 ± 5	404 ± 4	380 ± 6	832 ± 7	821 ± 8	102 ± 5	107 ± 4	720 ± 6	710 ± 8
Tartaric[c]	1280 ± 7	1310 ± 9	2748 ± 8	2650 ± 9	1813 ± 7	1860 ± 10	1969 ± 7	2040 ± 10	1841 ± 7	1880 ± 7	1730 ± 7	1680 ± 20
Chloride[d]	15.5 ± 1.7	15.0 ± 1.0	45.7 ± 2.0	46.7 ± 1.0	46.8 ± 2.0	45.4 ± 2.1	32.6 ± 2.0	33.6 ± 1.8	73.4 ± 1.9	71.7 ± 2.0	61.0 ± 1.9	62.9 ± 2.0
Nitrate[e]	12.3 ± 0.6	12.1 ± 0.6	8.1 ± 0.5	8.0 ± 0.5	11.1 ± 0.6	10.9 ± 0.5	7.0 ± 0.5	6.8 ± 0.4	11.2 ± 0.6	11.2 ± 0.5	4.2 ± 0.5	4.1 ± 0.4
Sulphate[f]	650 ± 20	634 ± 17	524 ± 19	502 ± 17	682 ± 20	688 ± 14	387 ± 20	375 ± 14	727 ± 20	718 ± 18	720 ± 20	748 ± 19

IEC = Ion-exchange chromatography. Chromatographic conditions as in Table 2. Altr. = alternative methods.
[a] Enzymatic [31].
[b] GC [32].
[c] GC [32] (samples 1–8), spectrophometric method [33] (sample 9) for sweet wines.
[d] Potentiometric titration with Ag^+.
[e] Spectrophotometric [2].
[f] Spectrophotometric titration [34].

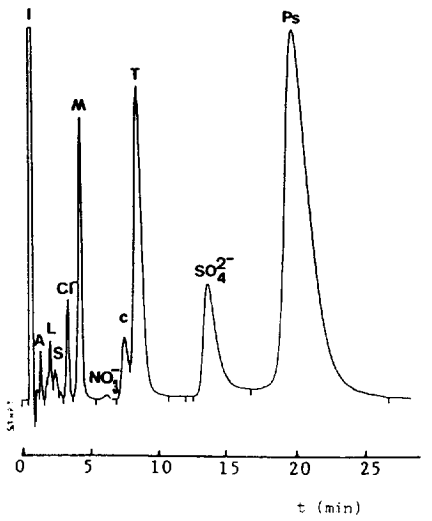

Fig. 2. Chromatogram of Muscatel sample. I = injection peak; A = acetate; L = lactate; S = succinate; M = malate; C = citrate; T = tartrate; Ps = system peak. Chromatographic conditions as in Table 2.

silylation of the sample presents serious difficulties owing to the abundance of sugars and determination by chemical methods can be tedious, ion-exchange chromatography with conductimetric detection produced satisfactory results. The technique used here therefore provides new options for the analysis of these wines.

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