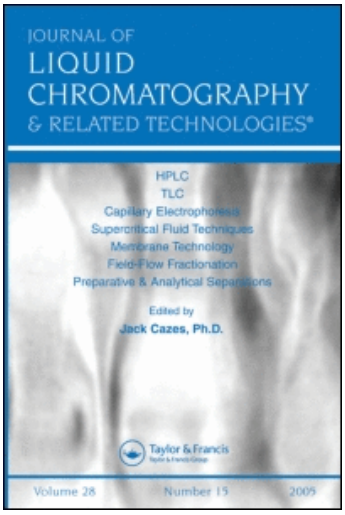

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Radin, L. , Pronzato, C. , Casareto, L. and Calegari, L.(1994) 'Tartaric Acid in Wines May Be Useful for Preventing Renal Calculi: Rapid Determination by HPLC', Journal of Liquid Chromatography & Related Technologies, 17: 10, 2231 — 2246
To link to this Article: DOI: 10.1080/10826079408013543
URL: http://dx.doi.org/10.1080/10826079408013543

JOURNAL OF LIQUID CHROMATOGRAPHY, 17(10), 2231-2246 (1994)

TARTARIC ACID IN WINES MAY BE USEFUL FOR PREVENTING RENAL CALCULI: RAPID DETERMINATION BY HPLC

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ABSTRACT

HPLC has been applied to the analysis of wine samples for the rapid determination of tartaric acid. Separation of carboxylic acids was performed on a LiChrosorb RP-18 column using a mixture of water, methanol and 0.05 M phosphoric acid (69:1:30) as mobile phase. Column eluates were monitored by UV absorbance at 210 nm. Tartaric, malic, lactic, acetic and tannic acids were revealed in the HPLC profile. Tartaric acid eluted as a well-resolved peak at 3.3 min. Mean recovery of known amounts of added tartrate ranges between 94 and 106%. The concentrations of tartaric acid in different European wines have been determined. The presence of tartaric acid can be related to the chemistry of nephrolithiasis: a high amount of this acid in the diet may be useful in preventing the recurrence of calcium oxalate stones in urine. The results of HPLC analyses appear to be interesting for the choice of a wine suitable for the diet of patients suffering from renal calculi.

INTRODUCTION

Wines contain numerous compounds which belong to different classes: alcohols, carboxylic acids, aldheydes and esters. The organoleptic properties of the various types of wines are affected by the carboxylic acid composition. The quality control of wines requires precise determinations of individual carboxylic acids during fermentation and aging.

The main acidic components in wine are tartaric, malic and citric acids (1). Tartaric acid is the major acid which regulates wine acidity. Tartaric acid undergoes degradation by lactic bacteria (such as Lactobacillus brevis) to lactic and acetic acids, with a concomitant increase in volatile acidity.

Most of ingested tartrate is metabolized to bicarbonate by various bacterial species in the colon (2), while only 20% of the dietary tartrate is excreted unchanged in urine (3). Tartrate is a strong chelating agent and may inhibit crystallization of calcium oxalate in whole urine (4). It was pointed out that the alkalinization of urine caused by tartrate feeding might be useful in preventing the recurrence of calcium oxalate stones (5).

Clinical results demonstrated the dependence of tartrate excretion on the composition of diet, as urinary excretion of tartrate was significantly less in subjects on a vegetarian diet than in subjects on mixed Mediterranean diets (3). Other authors reported that the incidence of renal calculi was strikingly low in South India, where one regular constituent of the diet is tamarind, which is very rich in potassium bitartrate and tartaric acid (5). One of

the major exogenous sources of tartrate is wine: due to wine intake, the amount of tartrate excreted can overcome the value of 1 mmol/24 hours (3).

The conventional methods used for the determination of tartrate, such as colorimetry and gas-chromatography, are all quite complex, time consuming and present several drawbacks (6-8). HPLC has been applied to the analysis of carboxylic acids. The methods commonly used have included ion-exchange and ion-exclusion separation (9,10), solvophobic chromatography, ion-pair chromatography and reversed-phase separation of derivatized products. The derivatives most frequently examined have been differently substituted phenacyl, naphthacyl, p-nitrophenyl and p-nitrobenzyl esters (11-13).

The aim of this study was to apply HPLC to the rapid determination of tartaric acid in wines. The amounts of tartaric acid in different European wines have been calculated in order to reveal which wines represent the best defence against recurrent nephrolithiasis.

MATERIALS

Chemicals.

Standard solutions of the investigated acids (tartaric, malic, lactic, acetic, citric, succinic, and tannic) were prepared from analytical-reagent grade chemicals (Merck, Darmstadt, Germany) by dissolving known amounts of compound in distilled water.

The solvents used for the HPLC analyses were of HPLC grade and water was deionized using a Milli-Q system (Millipore, Bedford, MA, U.S.A.). The eluents were filtered through 0.45-μm membrane filters (Millipore) prior to use.

METHODS

HPLC analysis.

Samples of 20 μL of wine filtered through 0.22 μm filters (Bio-Rad Laboratories, Richmond, CA, U.S.A.) were injected into the chromatograph. Analyses were carried out using a Merck-Hitachi liquid chromatograph (Tokyo, Japan), equipped with a model 7125 Rheodyne injector (Cotati, CA, U.S.A.), with a spectrophotometer, and with a model 4290 Varian integrator (Walnut Creek, CA, U.S.A.). Separation of carboxylic acids was performed on a LiChrosorb RP-18 column (25 cm x 4.6 mm) purchased from Merck (Darmstadt, Germany), using a mixture of water - methanol - 0.05 M phosphoric acid (69:1:30) as mobile phase, at a flow-rate of 0.8 mL/min. All chromatographic separations were performed at 22 ± 1°C. Column eluates were monitored by UV absorbance at 210 nm.

Method-validation.

Quantitation of the investigated acids was performed by external standard calibration. The concentration of the analyte was determined by comparing the peak area with that of a known standard.

The standard solutions of tartaric acid used for calibration were prepared by dissolving the required weight of compound in distilled water. A calibration graph was obtained by analyzing aliquots of working standard solutions containing scalar amounts of tartrate from 0.3 to 3.0 g/L.

The detection limit for tartaric acid was estimated in aqueous samples by injecting successively lower concentrations until a signal-to-noise ratio of 3:1 was obtained.

The recoveries of tartaric acid were determined by adding known amounts of tartrate to six wine samples and by comparing the found and calculated amounts of tartrate after spiking.

The precision of the method was evaluated by calculating within-run and between-run coefficients of variation at four different concentrations of tartrate.

RESULTS

A mixture of major acids (tartaric, malic, lactic, acetic, and tannic) present in wines was used as a standard solution for optimizing the chromatographic conditions. The elution behaviour of each acid was investigated at various pH values of eluent. The retention times decreased with increasing pH. Lactic and acetic acids were not completely separated at pH > 3. Separation of each acid was examined at different concentrations of methanol in the mobile phase. The best resolution of all the acids was obtained by using a methanol concentration of 1%. Each acid was identified by comparison with the retention times of single pure

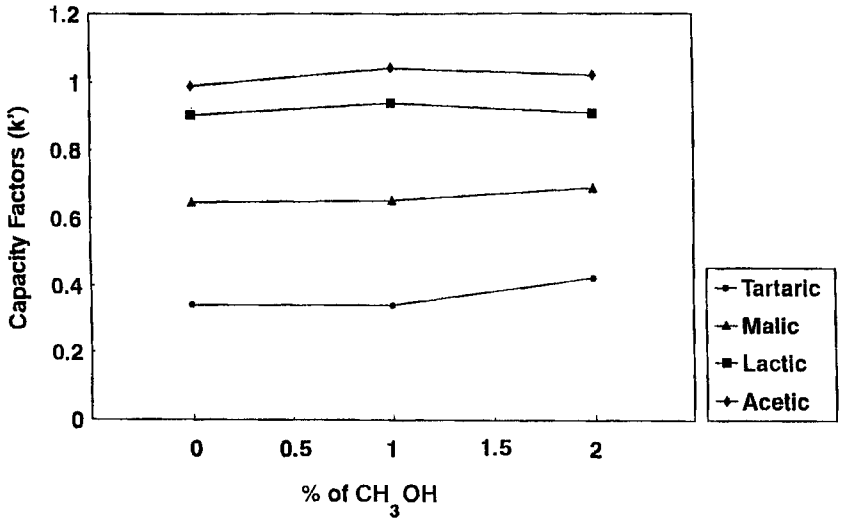

FIGURE 1.
Effect of methanol concentration on the capacity factor of carboxylic acids.
Column: LiChrosorb RP-18 (250 x 4 mm i.d.); mobile phase: water - 0.05 M phosphoric acid (70:30); flow-rate: 0.8 mL/min; temperature: 22°C.

compounds. Relationships between capacity factors and methanol concentrations, for carboxylic acids, are presented in Fig. 1. Results of these experiments have shown that a mobile phase containing 1% methanol at pH 2.5 was suitable for a correct separation of tartaric acid.

Other acids, such as succinic and citric, included in the standard mixture were not completely separated also varying pH and methanol concentration, however, their presence in wines is not important for organoleptic properties.

HPLC profile of a standard mixture of carboxylic acids is shown in Fig. 2. The total analysis time was 18 min,

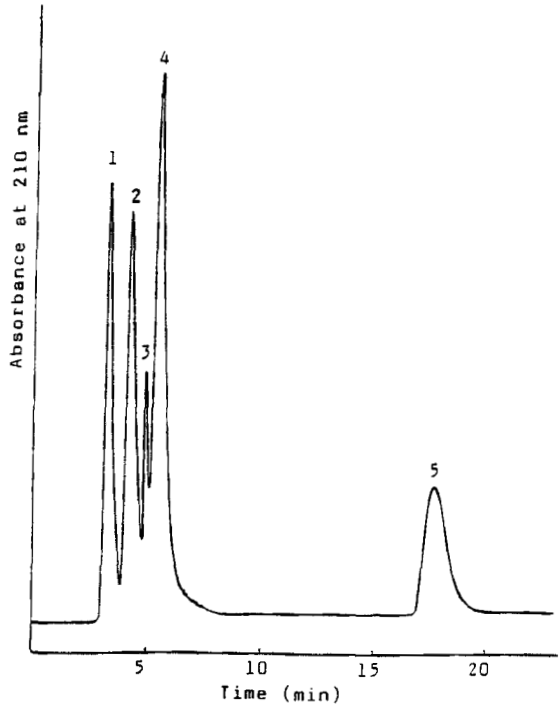

FIGURE 2.
HPLC profile of a standard mixture of carboxylic acids. Column: LiChrosorb RP-18 (250 x 4 mm i.d.); mobile phase: water - methanol - 0.05 M phosphoric acid (69:1:30); flow-rate: 0.8 mL/min; temperature: 22°C. Peaks: 1 = tartaric acid; 2 = malic acid; 3 = lactic acid; 4 = acetic acid; 5 = tannic acid.

while the elution of tartaric acid was very quickly at 3.3 min before the other acids.

HPLC patterns of a sample of Rossese di Finale Ligure (Italy) and Beaujolais (France) wines are shown in Figs. 3 and 4, respectively.

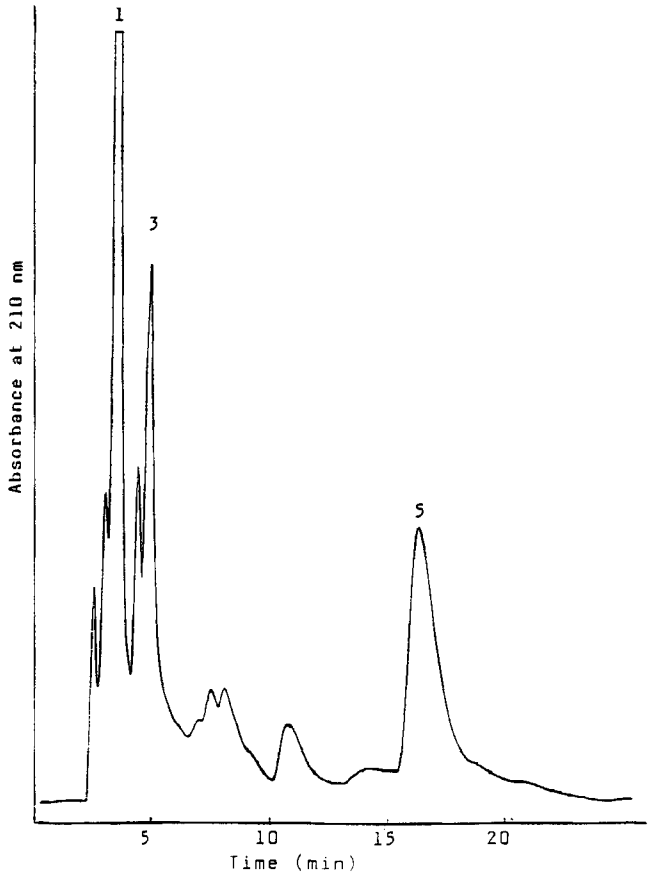

FIGURE 3.
HPLC profile of a sample of Rossese di Finale Ligure wine. Chromatographic conditions as in Fig. 2. Peaks: 1 = tartaric acid; 3 = lactic acid; 5 = tannic acid.

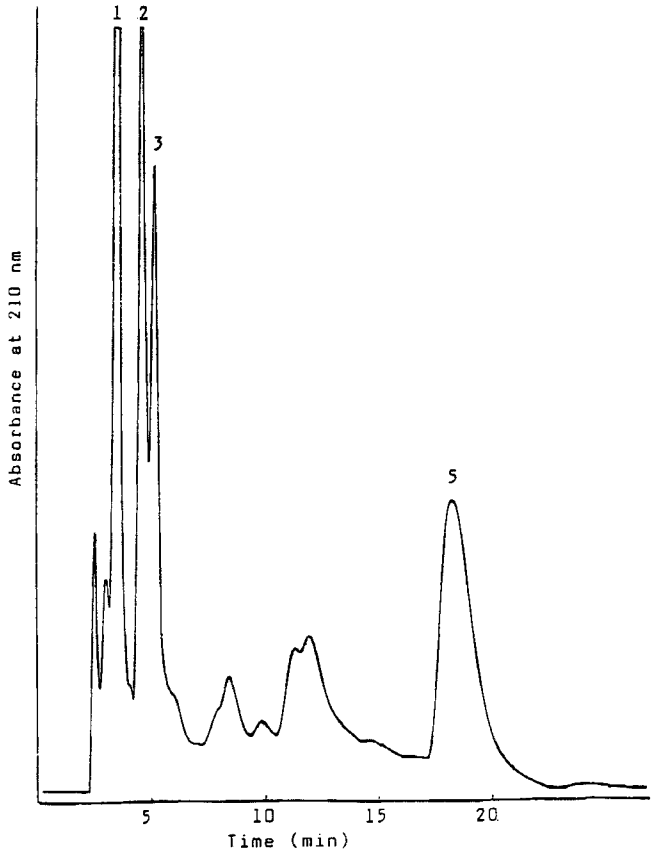

FIGURE 4.
HPLC profile of a sample of Beaujolais wine.
Chromatographic conditions as in Fig. 2. Peaks: 1 = tartaric acid; 2 = malic acid; 3 = lactic acid; 5 = tannic acid.

Quantitative determination.

The calibration graph relating tartrate peak area to concentration was produced by sequential dilution of a stock solution in the range 0.3 - 3.0 g/L. The response of the

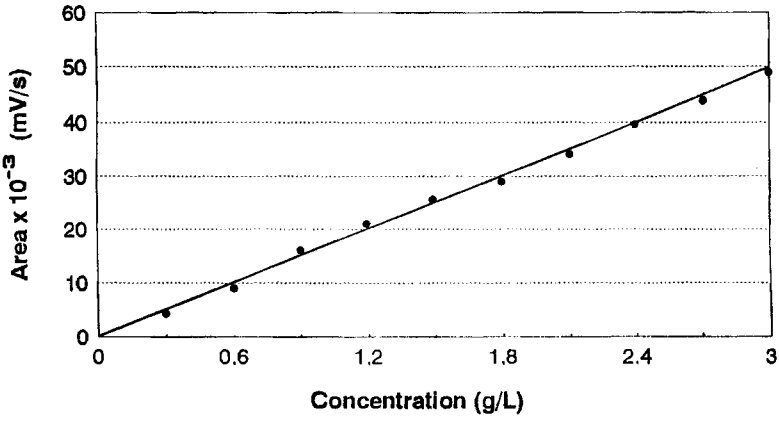

FIGURE 5.
Calibration graph for the determination of tartaric acid eluted according to the method described.

detector was linear in the tested range and linear regression analysis yielded $y = 16.33\ x + 0.31$ with a correlation coefficient of 0.9989 (Fig. 5).

The detection limit for tartrate, defined as signal-to-noise ratio of 3:1, was 12 µg/mL.

Recovery experiments were carried out by adding known amounts of tartaric acid to aliquots of two different wines. Tartaric acid was spiked at six different concentrations for Clos Reginu and Poggese wines. Typical recoveries, determined by comparing the found and calculated amounts of tartrate after spiking, are reported in Table 1. Recoveries ranging from 100 to 103% were obtained for Poggese wine. Tartaric acid concentrations (± S.D.; nine independent measurements) in Clos Reginu and Poggese wines were 1.19 (± 0.13) g/L and 2.71 (± 0.07) g/L, respectively.

Table 1. Recovery of tartrate from wine.

Amount in wine (g/L)	Amount added (g/L)	Amount calculated (g/L)	Amount found (g/L)	Recovery ± SD (mean) (%)
1.191	0.2	1.391	1.323	95.1 ± 4.6
1.191	0.6	1.791	1.682	93.9 ± 4.4
1.191	1.2	2.391	2.543	106.4 ± 5.5
2.709	0.9	3.609	3.732	103.4 ± 5.3
2.709	1.8	4.509	4.578	101.5 ± 5.7
2.709	2.7	5.409	5.412	100.1 ± 5.1

Recovery = (Amount found / amount calculated) x 100.

Of the common acidic components in wine, none have retention characteristics such that they could interfere with the tartrate peak. However, sometimes a peak of unknown identity occurs, which is only partially resolved from the tartrate peak (Fig. 3). In order to evaluate the extent of background interference, it is advisable to use a diode-array detector. Comparison of spectra, obtained at the leading edge, apex and trailing edge of the tartrate peak, can afford a purity check.

To evaluate the precision of the method, within-run and between-run coefficients of variation were calculated at four different concentrations of tartaric acid. The within-run precision, performed by repeated injections (n = 9) of aliquots of the same wine sample with 1.50 g/L of

Table 2. Precision of the assay for tartrate in wine.

Nominal concentration (g/L)	Concentration found (mean ± SD)	C.V. (%)
Within-run variation		
0.30	0.31 ± 0.02	6.45
0.60	0.62 ± 0.03	4.84
1.50	1.54 ± 0.07	4.55
3.00	3.04 ± 0.12	3.95
Between-run variation		
0.30	0.31 ± 0.01	3.23
0.60	0.61 ± 0.03	4.92
1.50	1.52 ± 0.06	3.95
3.00	3.03 ± 0.24	7.92

tartrate, was 4.55%. The between-run precision, obtained from analyses of the same sample repeated on five subsequent days, was 3.95%. The variations for spiked samples are presented in Table 2.

The concentrations of the major carboxylic acids determined by HPLC analysis of different European wines are reported in Table 3. Tartaric acid exhibits values ranging from 0.72 g/L (Liebfraumilch) to 2.88 g/L (Rossese). The wines which contain the highest amounts of tartaric acid (more than 2 g/L) are: Rossese di Finale Ligure, Poggese, Cinque Terre, Cortese del Piemonte, and Beaujolais.

TABLE 3

Determination of Major Carboxylic Acids in Wines by HPLC

Wine	Acid Concentration (g/L)				
	Tartaric	Malic	Lactic	Acetic	Tannic
Beaujolais (F)	2.21	1.74	1.19	-	0.42
Chablis (F)	1.35	0.24	2.61	-	0.01
Cinque Terre (I)	2.61	0.81	1.22	-	-
Clos Reginu (F)	1.19	0.88	2.85	-	0.31
Cortese (I)	2.56	0.56	1.86	-	0.02
Dolcetto (I)	1.86	-	-	0.86	0.32
Liebfraumilch (D)	0.72	1.91	2.84	-	0.01
Malaga (E)	0.97	1.27	0.91	-	0.06
Moscato (I)	0.89	2.41	-	0.73	-
Nebbiolo(I)	1.44	-	1.22	-	0.25
Pigato (I)	1.32	1.05	0.47	0.25	-
Poggese (I)	2.71	0.91	-	-	-
Rosé (GR)	1.78	0.55	3.12		0.14
Rossese (I)	2.88	-	1.34	-	0.37
Santorini (GR)	1.52	0.73	0.61	-	-
Sherry (E)	1.96	0.93	3.95		0.03
Vermentino (I)	1.91	-	2.66	0.21	-

DISCUSSION

Some single acids present in wines can be determined by standard methods (14,15). Tartaric acid concentration can be calculated by the Blouin-Rebelein method based on the reaction with ammonium metavanadate (15) or by gas-chromatographic methods (7,8). Higher values obtained by the colorimetric method with respect to the chromatographic method were assigned to a specific redox interaction of metavanadate ion with the vicinal diol moiety present in tartaric acid and in several other wine components (1).

A simple ion chromatographic method was proposed for the determination of tartrate concentration in urine (3). However, one disadvantage of the method was interference of sulfates, so that elimination of sulfates was a necessary pre-treatment step prior to the chromatographic separation. Under the operating conditions proposed in this paper no problem exists, because absorbance of sulfates at 210 nm is negligible.

Although the main aim of this study was the determination of the tartaric acid concentration in wines for the reasons of medical interest mentioned above, the HPLC analysis described permits determination of other carboxylic acids important to study the organoleptic properties of a wine. In fact, as the choice of a wine is affected also by the organoleptic properties, these can be related to the relative amount of malic, lactic and tannic acids. The best characteristics for a wine are given by a lower quantity of malic acid together with a higher concentration of lactic acid; tannic acid must be present in a moderate amount (16). With regard to this, the most appreciated wines result those presenting a good ratio tartaric acid / malic acid. Since personal choices based on aspect, bouquet and taste, have to be taken into account, it is interesting to note that high amounts of tartaric acid seem to predominate in white rather than red wines. The amount of tannic acid confirms that this acid is present in good quantity in red wines.

The results of HPLC analyses are useful, when the composition of the diet has to be assessed, for choosing a wine with a high amount of tartaric acid, in order to prevent the formation of calcium stones.

The adaptation of reversed-phase HPLC to the determination of tartrate in biological fluids should be evaluated to investigate tartrate metabolism, e.g., the potential role of tartrate as an inhibitor of crystallization in calcium nephrolithiasis.

ACKNOWLEDGEMENTS

We thank Dr. Martino Marangella (Mauriziano Umberto I Hospital, Turin) for helpful suggestions.

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Received: July 16, 1993
Accepted: December 15, 1993