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Ion Exchange and Ion Exclusion Chromatographic Characterization of Wines Using Conductivity Detection

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ABSTRACT

High performance ion chromatography system employing ion exchange and ion exclusion modes was used for the separation and quantitative analysis of tartarate, malate, citrate, lactate, chloride, nitrate, phosphate, sulfate, lithium, sodium, potassium, magnesium, and calcium in 15 wines originated worldwide. All anions were measured by means of conductivity detection. Analytical figures of merit were assessed and reported for all analyzed ions. In addition, the classic physicochemical properties of wines, i.e. color, acidity and density, were determined. Correlations between measured variables were evaluated and discussed. The proposed procedure is exceptionally simple and requires only regular instrumentation; nevertheless, it provides possibility for fast, straightforward and sensitive quantification of aforementioned ions. Sample preparation required only filtration and dilution where necessary.

Key words: wine, organic acids, ionic profile, ion-exclusion chromatography, ion exchange chromatography

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INTRODUCTION

The mineral and metal content of wines has been extensively studied in terms of geographical differentiation, organoleptic and diabetic characteristics[1-3] and fingerprinting possibilities[4-6]. Less attention has been paid to the analysis of wine ionic content[7-9] despite the fact that inorganic and organic ions in wines have profound effect on taste and nutritional value. Furthermore, potential classification of provenance and differentiation based on the wine ionic content has not been fully recognized although such an approach is utilized for fruit juices where organic acids profile is regarded as a dependable tool for tracing juice origin and possible adulteration[10].

Ionic characterization of wines can be useful in preventing health threat associated with high level of nitrites or nitrates, which can be reduced to nitrites directly in wine or in gastric system. Further reaction of nitrite with nitrosable amines can form nitrosoamines - potential mutagen precursors and carcinogens[11,12]. Sulfur oxide ionic compounds can also cause severe reactions, especially in asthmatics[13].

Organic acids contribute significantly to the alcoholic beverage taste and aroma. For example, the sour and sharp flavors of cider are ascribed to lactic acid and total acidity respectively. Increase in malic acid concentration decreases sweet taste and scented flavors of the beverage. The citric acid possesses sweet-and-sour sensory notes, whereas lactic acid can be associated with smell of raw meat[14].

Since ancient times attempts were made to prevent defilement of wine, ancient Greek and Roman laws prohibited adulteration or addition of impurities to wine[13]. In this term, ionic content analysis can provide valuable information concerning illegal changes, since distinct alteration in the ions pattern of wine and fruit juices, accompanied by a change in their concentrations, will demonstrate adulteration. Ionic profiles provide equally unique characteristics of wines. From these 'fingerprints' conclusions about possible adulteration or recognition of the products can be obtained[10]. Furthermore, complex wine characterization can help trace mislabeling. Mislabeling is not always associated with adulteration and thus, despite appropriate ionic profiles, it is still a serious fault associated with sale and distribution of the products. Concerning the above, many misbranded products would be legal but they lack consumer appeal of the mislabeled products[15].

Conductivity detection is a 'workhorse' in ion chromatographic analysis of inorganic ions. However, in case of organic acids, usually UV detection provides simplicity of use and decent analytical performance. The last detection technique, however, can not be directly applied to the analysis of inorganic ions. Since the characterization of all ionic species in wine is concerned, two detec-

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tors can be applied or one that hopefully can provide required sensitivity and dynamic range.

In the present work, inorganic anions (chloride, nitrate, phosphate, sulfate) and cations (lithium, sodium, potassium, magnesium, calcium), as well as organic anions (tartarate, malate, citrate, lactate) were determined in 15 wine samples. All measurements were performed using the same system setup with a conductivity detector except for the column and the guard column. Three different columns were employed in the described study that is, ion exclusion, cation exchange and anion exchange. Quality of the measurements was checked through spiking experiments and performing recovery tests. Analytical figures of merit were assessed to evaluate the applicability of the HPIC system for the analysis of broad range of ions in wines. Correlations among ions concentrations, and ion concentrations and physicochemical properties of wines were evaluated and discussed regarding processes occurring in wine.

MATERIAL AND METHODS

I. *Equipment*

Details of the HPIC system employed in this study are shown in Table 1. A spectrophotometer Metertek SP-850 (Metertech Inc., Taiwan) was used for the color assessment of wines. Acidity of wines was measured by a digital pH-meter PM1 (Polmed, Poland). For degassing and filtration purposes, an ultrasonic bath Ultrasons-H (Selecta, Spain) and a vacuum filtration device (Supelco, USA) were applied, respectively.

II. *Chemicals*

De-ionized water (18.3 MΩ cm resistivity) obtained from a Barnstead (Dubuque, IA, USA) EASYpure^{TM} (model D7033) water system was used for preparation of all solutions and eluents. HPLC grade methyl alcohol (99.93% pure; Aldrich, USA), *p*-hydroxybenzoic acid (99% pure; Aldrich), ethylenediamine (99% pure; Aldrich), nitric acid (extra pure; Merck, Germany), sulfuric acid (instra-analyzed reagent; J.T. Baker, USA) were employed for the preparation of the eluents.

Working standard solutions of Li^+, K^+, Cl^-, NO_3^-, HPO_4^{2-}, and SO_4^{2-} ions were prepared from ion chromatography stock standard solutions (1000 mg L^{-1}; Fluka, Switzerland) by appropriate dilutions. In case of other ions, 1000 mg L^{-1} standard solutions were obtained by dissolution of precisely weighed citric acid (>99.5%, Aldrich, USA), DL-malic acid (>99%, Fluka, Switzerland), DL-tartaric acid, DL-lactic acid sodium salt (analytical grade POCh, Poland) and sodium chloride (spectral grade, POCh, Poland).

Standard buffer solutions of pH 1.0, 4.0, 9.0, used for calibration of the pH-meter, were purchased from POCh.

Table 1. Components of the HPIC system

Solvent pump	Knauer WellChrom Mini-Star K-500
Column thermostat	Knauer Jetstream 2
6-port valve	Knauer A0258
Data acquisition unit	Knauer WellChrom v7566
Software	Knauer EuroChrom 2000 version 1.2
Conductivity detector	Alltech 550
Anion exchange column with guard column	Hamilton PRP–X100 (4.1 × 150 mm) Hamilton PRP–X100 (2.3 × 25 mm)
Cation exchange column with guard column	Hamilton PRP–X200 (4.1 × 150 mm) Hamilton PRP–X200 (2.3 × 25 mm)
Ion exclusion column with guard column	Hamilton PRP–X300 (4.1 × 150 mm) Hamilton PRP–X300 (2.3 × 25 mm)

III. *Eluents and Conditions*

An isocratic elution mode by non-suppressed ion chromatography was employed. Identification of each ion was performed by injection of solutions containing a single ion. Three columns and four types of eluents were employed:

- Inorganic anions: Hamilton PRP-X100; 4.0 mmol L^{-1} *p*-hydroxybenzoic acid in methyl alcohol-water solution (1:40, v/v), pH 8.5.
- Organic anions: Hamilton PRP-X300; 0.5 mmol L^{-1} sulfuric acid in water.
- Monovalent cations: Hamilton PRP-X200; 4.0 mmol L^{-1} nitric acid in methyl alcohol-water solution (3:7, v/v).
- Divalent cations: Hamilton PRP-X200; 1.5 mmol L^{-1} ethylenediamine in water, pH 6.0.

Eluents were prepared and filtered at 0.45 µm. The instrument was calibrated with standards of concentrations corresponding to 1.0, 2.0, 5.0, 10 and 20 mg L^{-1} in the case of inorganic and organic anions and 0.5, 1.0, 2.0, 5.0, 10 mg L^{-1} in the case of cations.

A time constant for all measurements was of 0.5 sec and a measurement range was set to 0-100 µS.

IV. *Samples*

Eleven red wine samples and four white wine samples were analyzed in this study. Among them, six originated from Plovdiv (Bulgaria) with different grape varieties: Blanc de Blanc, Cabernet Sauvignon, Chardonnay, Merlot, Muscat, Sauvignon Blanc. The remaining analyzed wines were produced from Cabernet Sauvignon, with three wines originated from the same vineyard in Valle Centarl (Chile) and were produced in three successive years 2002-2004. Additional wine samples were from Languedoc (France), Primorski region (Slove-

nia), Santa Helena (CA, USA), and Riverland (Australia). The exact description of wines under investigation together with associated numbers and physical properties are given Table 2. The sample wine numbers were used consistently throughout.

V. *Procedures and Analysis*

Wine samples were filtered through 0.45 µm nylon 66 membranes and if necessary diluted. The acidity of wines was measured directly after calibration of the pH-meter. According to the standard procedure[16], the color of red wines was measured after ten fold dilution of the wine samples with a buffer of the same pH as the respective wine. The white wines color measurements were made without any dilution. All the ion concentrations were measured using HPIC system with the conductivity detection.

RESULTS AND DISCUSSION

I. *Physicochemical Properties*

Acidity, density and color of analyzed wines are given in Table 3. The acidity of wines under investigation ranged from 3.04 to 3.55. The most acidic wine is semi-dry white Muscat wine from Plovdiv (Bulgaria), while Cabernet Sauvignon from Valle Central (Chile) has the lowest acidity among analyzed wines. It can be seen that wines from the Plovdiv region (samples 1 to 6) have the lowest pH. Comparing the semi-dry and dry wines,

no significant difference in acidity can be observed as classification of wines into sweet, semi-sweet, semi dry and dry is based only on the sugar content. Changes in wine pH are usually attributed to wine aging during which crystals of tartaric acid precipitate from solution. Considering the concentration of that acid and its pK_a, it is the most important contributor to the wine acidity.

The density was very consistent and is in the typical range from 0.980 to 0.990 g mL^{-1} for that kind of alcoholic beverage.

The most distinct color belongs to white wine from the Blanc de Blanc grape variety. It is a unique grape variety growing in Bulgaria and known for its intensive yellow color. No serious discrepancies in color were noted in other wines.

II. *Analytical Performance and Analysis*

(I) *Organic Acids*

Quantification of low molecular weight organic acids present in wines and other food samples is typically made by high performance liquid chromatography or capillary electrophoresis. Both techniques predominantly utilize UV detection for that purpose[9,17]. However, low molar extinction coefficients of those acids chromophores connected with lack of more sensitive detection alternatives as fluorescence or electrochemical activity lead to the extensive use of derivatization techniques devoted to the obtainment of compounds that can be detected with higher sensitivity[14]. Moreover, UV detection cannot be

Table 2. Analyzed wines samples

No.	Name	Region	Country	Year	Grape variety	Dryness
1	Sophia	Plovdiv	Bulgaria	2003	Sauvignon Blanc	Dry
2	Sophia	Plovdiv	Bulgaria	2002	Chardonnay	Dry
3	Sophia	Plovdiv	Bulgaria	2002	Blanc de Blanc	Semi-dry
4	Sophia	Plovdiv	Bulgaria	2002	Muscat	Semi-dry
5	Sophia	Plovdiv	Bulgaria	2002	Merlot	Dry
6	Sophia	Plovdiv	Bulgaria	2003	Cabernet Sauvignon	Dry
7	Riverland Angoves	Riverland	Australia	2001	Cabernet Sauvignon	Dry
8	Shutter Home	St. Helena	USA	2002	Cabernet Sauvignon	Dry
9	Vin de Pays d'Oc	Languedoc	France	2002	Cabernet Sauvignon	Dry
10	Slovin	Primorski	Slovenia	2003	Cabernet Sauvignon	Dry
11	Frontera	Valle Central	Chille	2003	Cabernet Sauvignon	Dry
12	Casillero del Diablo	Valle Central	Chille	2003	Cabernet Sauvignon	Dry
13	Baron Pilars	Valle Central	Chille	2002	Cabernet Sauvignon	Dry
14	Baron Pilars	Valle Central	Chille	2003	Cabernet Sauvignon	Dry
15	Baron Pilars	Valle Central	Chille	2004	Cabernet Sauvignon	Dry

Table 3. Physicochemical properties of the analyzed wines

No.	Acidity pH	Density g mL^{-1}	Color			
			A$_{420}$	A$_{520}$	A$_{420}$/A$_{520}$	A$_{420}$ + A$_{520}$
1	3.15	0.985	0.125	–	–	–
2	3.08	0.983	0.118	–	–	–
3	3.18	0.987	0.419	–	–	–
4	3.04	0.985	0.102	–	–	–
5	3.30	0.988	0.370	0.696	0.532	1.066
6	3.10	0.983	0.208	0.433	0.48	0.641
7	3.29	0.980	0.475	0.647	0.734	1.122
8	3.33	0.988	0.350	0.604	0.579	0.954
9	3.40	0.985	0.339	0.464	0.730	0.803
10	3.46	0.982	0.322	0.469	0.687	0.791
11	3.45	0.988	0.550	1.018	0.450	1.569
12	3.50	0.987	0.604	1.031	0.586	1.635
13	3.55	0.980	0.266	0.262	1.015	0.528
14	3.48	0.982	0.360	0.582	0.619	0.942
15	3.34	0.990	0.391	0.556	0.703	0.947

A$_{420}$, A$_{520}$: absorbance measured at 420 and 520 nm, respectively

Table 4. Analytical characteristics of the determined ions

| Ion	Retention time (min)	Limit of detection (mg L^{-1})	Upper limit of dynamic range (mg L^{-1})
Ion exclusion			
Tartarate	1.66	0.29	35
Malate	2.15	0.17	40
Citrate	2.57	0.21	50
Lactate	3.32	1.40	110
Anion exchange			
Chloride	2.60	0.005	360
Nitrate	5.21	0.22	170
Phosphate	8.69	0.42	260
Sulfate	12.23	0.13	210
Cation exchange of monovalent ions			
Lithium	2.94	0.002	18
Sodium	3.33	0.006	10
Potassium	4.68	0.046	210
Cation exchange of divalent ions			
Magnesium	3.24	0.13	45
Calcium	4.19	0.18	80
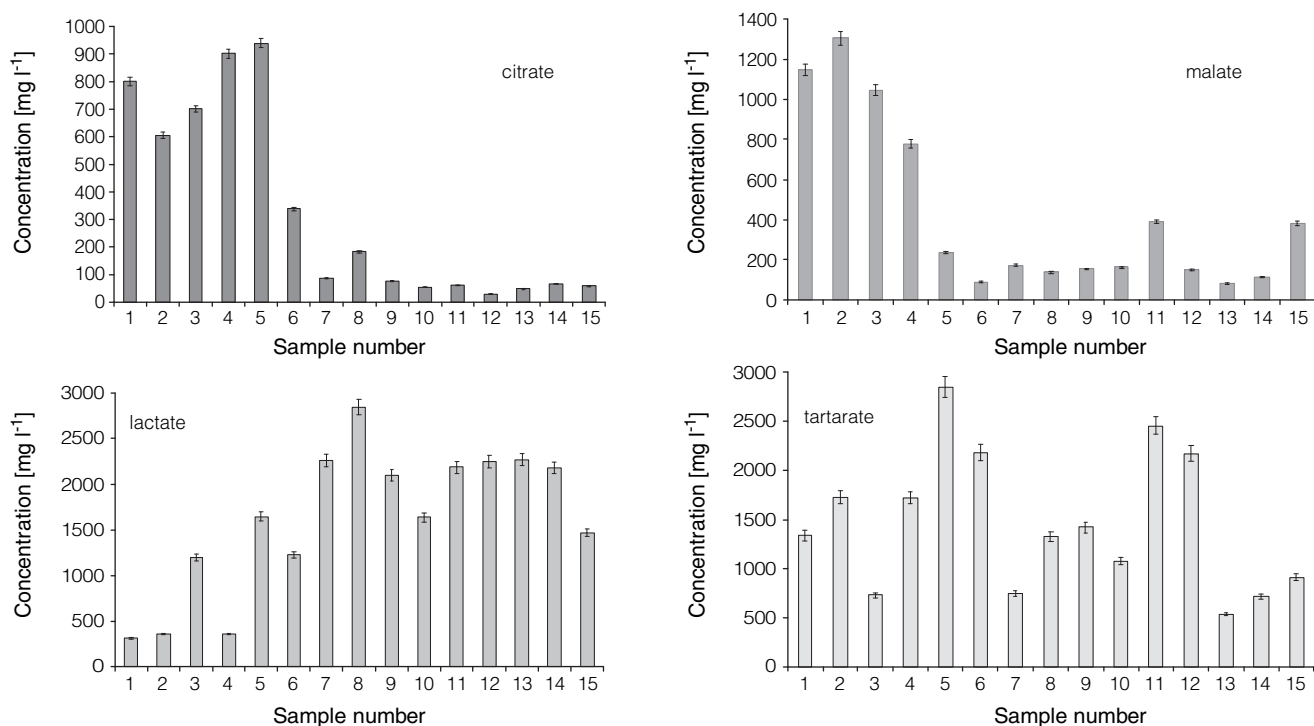
Figure 1. Content of organic anions in analyzed wines.

easily applied to inorganic ions, whereas conductivity detection can be a flexible alternative providing reasonably low detection limits and useful dynamic ranges for both inorganic and organic ions.

In the present study, a HPIC system with conductivity detection was used for determination of different organic ions and inorganic ionic constituents of wine. The analytical figures of merit assessed for employed system are reported in Table 4. Detection limits were evaluated by measuring ten successive signals of ions in solutions containing traces of investigated ions. These figures were calculated as concentrations equivalent to the signals three folded of standard deviations of the measured ions peaks. Unfortunately, analytical figures of merit are rarely available in previous publications. So far, use of conductivity detector in determination of inorganic and organic ions was reported only once in an analysis applying mixed-bed ion exchange mode[18]. Referring to the available data, the detection limits of this method were well comparable with the results obtained for systems employing different detectors, e.g., capillary electrophoresis with UV detection and phtalic acid as background electrolyte where detection limits of 1.47, 2.30, 1.56 mg L^{-1} were reported for tartarate, malate and lactate, respectively[9]. Whereas, tartarate detection limit for HPLC with UV detector where reported[19] as 0.19 mg L^{-1}. Also, in case of HPLC with refractive index detection, narrower dynamic ranges for citrate, tartarate, malate, lactate and (as can be evaluated) lower detection limits were reported[20].

Determined concentrations of citrate, tartarate, malate, lactate are depicted in Figure 1. The results are the mean values of three independent replicates, and the error bars denote one standard deviation. For all organic anions except tartarate, the concentrations among wines vary by one order of magnitude or more. In the exception of the ion, its concentration is more consistent in all wines and changes from 533 to 2850 mg L^{-1}. The white wines under discussion possess the highest content of malate and the lowest content of lactate, probably due to the shorter malo-lactic fermentation.

(II) *Inorganic Ions*

Conductometric detection in analysis of inorganic ions is a mellow, dependable method with well established analytical performance[21]. Table 4 shows that analytical figures of merit for inorganic anions (chloride, nitrate, phosphate, sulfate) and cations (lithium, sodium, potassium, magnesium, calcium) lie within the range of the reported data for that kind of system.

The inorganic ionic content (average value of three measurements and one standard deviation as error bars) of wines is presented in Figures 2 and 3. Nitrate concentrations in all analyzed wines did not exceed 20 mg L^{-1}, while the chloride content differed significantly from 65 to 738 mg L^{-1}, reaching 1650 mg L^{-1} in one case. Relatively constant amount of phosphate was present in all wines and ranged from 271 to 681 mg L^{-1}. This relatively constant value can be caused by the fact that microorganisms conducting fermentation need that element, and
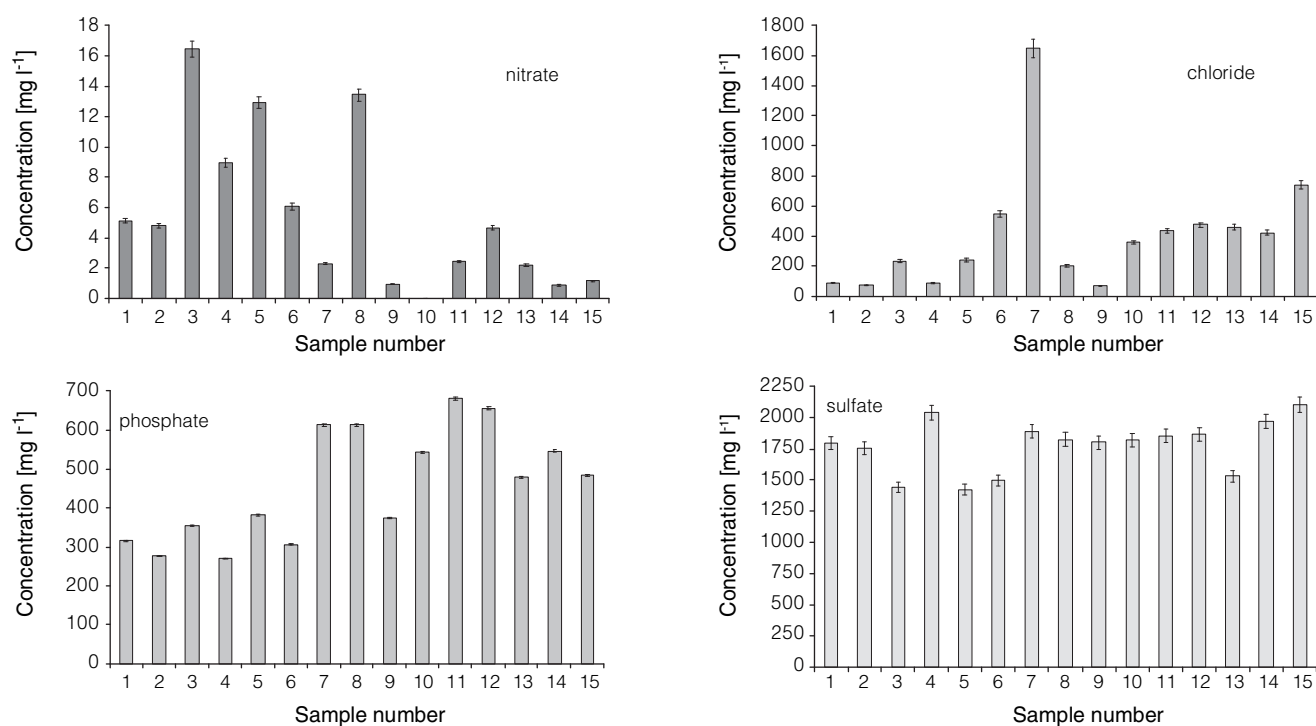
Figure 2. Content of inorganic anions in analyzed wines.

Table 5. Statistically significant correlations found in analyzed wines

| Variables	Correlation coefficient	Variables	Correlation coefficient
K^+ and pH	0.9161	Malate and Li^+	-0.7072
PO_4^{3-} and Li^+	0.8806	Malate and citrate	0.6778
Lactate and PO_4^{3-}	0.8231	Malate and K^+	-0.6922
Citrate and K^+	-0.8235	Malate and pH	-0.6861
Lactate and Li^+	0.8215	Li^+ and pH	0.6616
Malate and lactate	-0.8156	Citrate and NO_3^-	0.6615
Lactate and pH	0.7994	Li^+ and K^+	0.6604
Malate and Mg^{2+}	-0.7912	Cl^- and Na^+	0.6263
Lactate and K+	0.7865	Cl^- and Ca^{2+}	0.6138
Citrate and pH	-0.7634	Cl^- and Mg^{2+}	0.6078
PO_4^{3-} and pH	0.7671	Malate and PO_4^{3-}	-0.5960
Citrate and PO_4^{3-}	-0.7474	Citrate and Ca^{2+}	-0.5883
Citrate and Mg^{2+}	-0.7582	K^+ and Mg^{2+}	0.5615
Citrate and lactate	-0.7452	Mg^{2+} and pH	0.5563
Cl- and Li^+	0.7426	K^+ and Ca^{2+}	0.5456
Citrate and Li^+	-0.7406	PO_4^{3-} and Ca^{2+}	0.5447
PO_4^{3-} and Mg^{2+}	0.7345	SO_4^{2-} and Ca^{2+}	0.5361
Li^+ and Mg^{2+}	0.7298	Mg^{2+} and Ca^{2+}	0.5327
PO_4^{3-} and K^+	0.7231	Li^+ and Ca^{2+}	0.5238
Lactate and Mg^{2+}	0.7112	NO_3^- and K^+	-0.5115
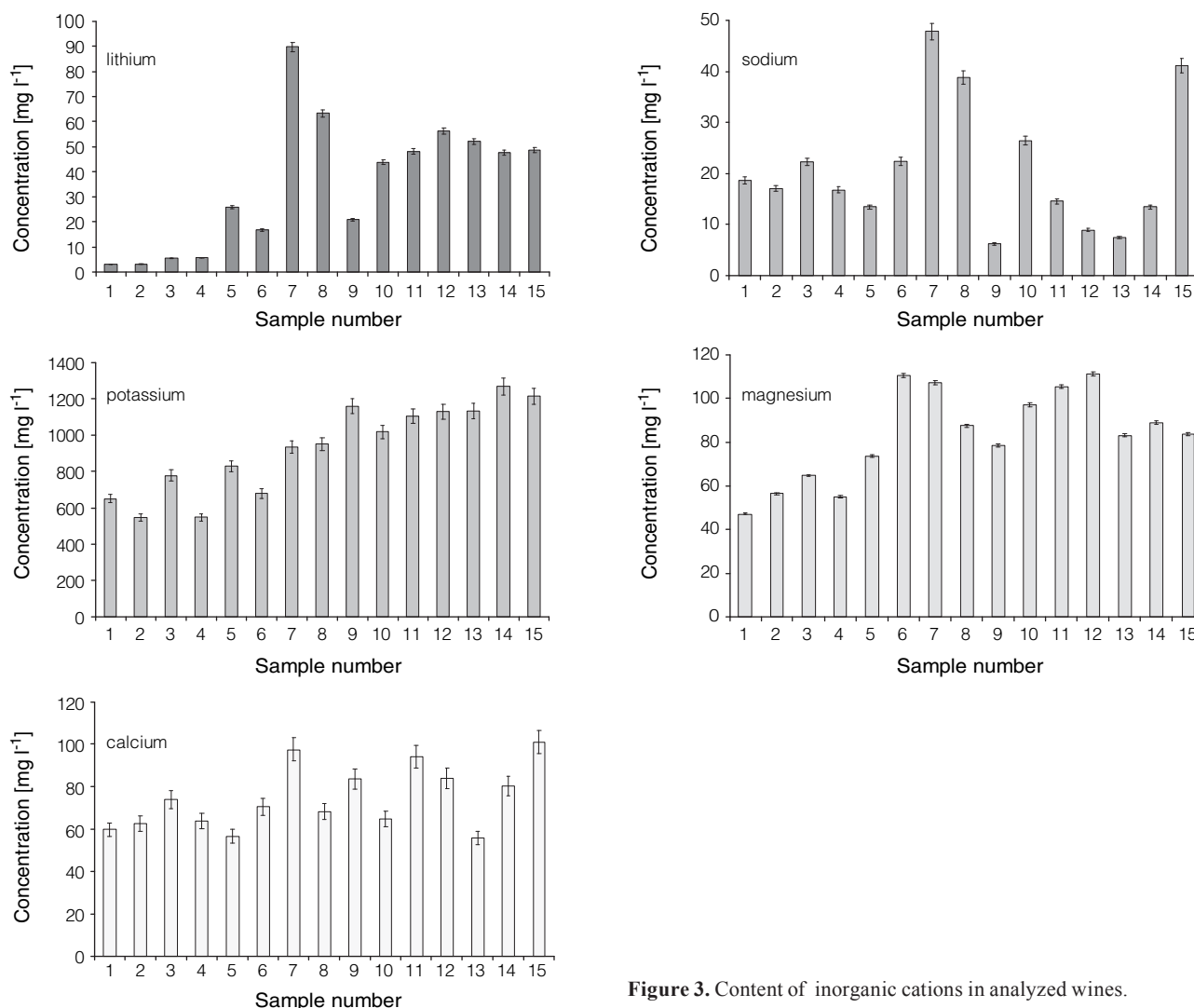
Figure 3. Content of inorganic cations in analyzed wines.

therefore phosphate salts are added to wines to assure proper fermentation[22]. Similarly constant but extensively larger amounts of sulfates (1.42 to 2.10 g L^{-1}) were observed in all wines. In this case, high presence of those ions can be attributed to 'sulfiting' - one of the steps in wine production[11]. For inorganic cations, the concentrations between 3 and 100 mg L^{-1} were found in all wines with one exception being potassium which concentration was much higher, i.e., from 545 to 1270 mg L^{-1}.

III. *Accuracy and Precision*

To verify quality of the obtained results, the recovery test was performed using one selected wine. For that purpose, the standard solution containing organic and inorganic ions was added to the wine samples in quantity adequate to approximately double the measured ion concentrations in the final solutions. Then, the solutions were appropriately diluted and filtered. Next, the samples were measured and the recoveries were calculated for measured ions. Obtained recoveries were in the ranged from 95.8% for lactate to 109.3% for malate, what prove suitable accuracy of the performed measurements.

In terms of relative standard deviation, uncertainty of the results was conveniently small for all ions. It reached a maximum of 5.6% for Ca^{2+}, while for all other inorganic species the precision was better than 3.6%. Measurements of tartarate ion showed the lowest precision among organic ions (3.7%). Better precision occurred in case of lactate (3.0%) and malate (2.6%) measurements, whereas the best precision was observed for citrate (1.8%).

IV. *Correlations Study*

After collection of the data, Pearson's correlation coefficients were computed for all 15 analyzed wines in order to estimate possible relationships between various wine variables. Correlations statistically relevant[23] at the significance level of 0.05 are given in Table 5. Among these correlations, a negative relationship between
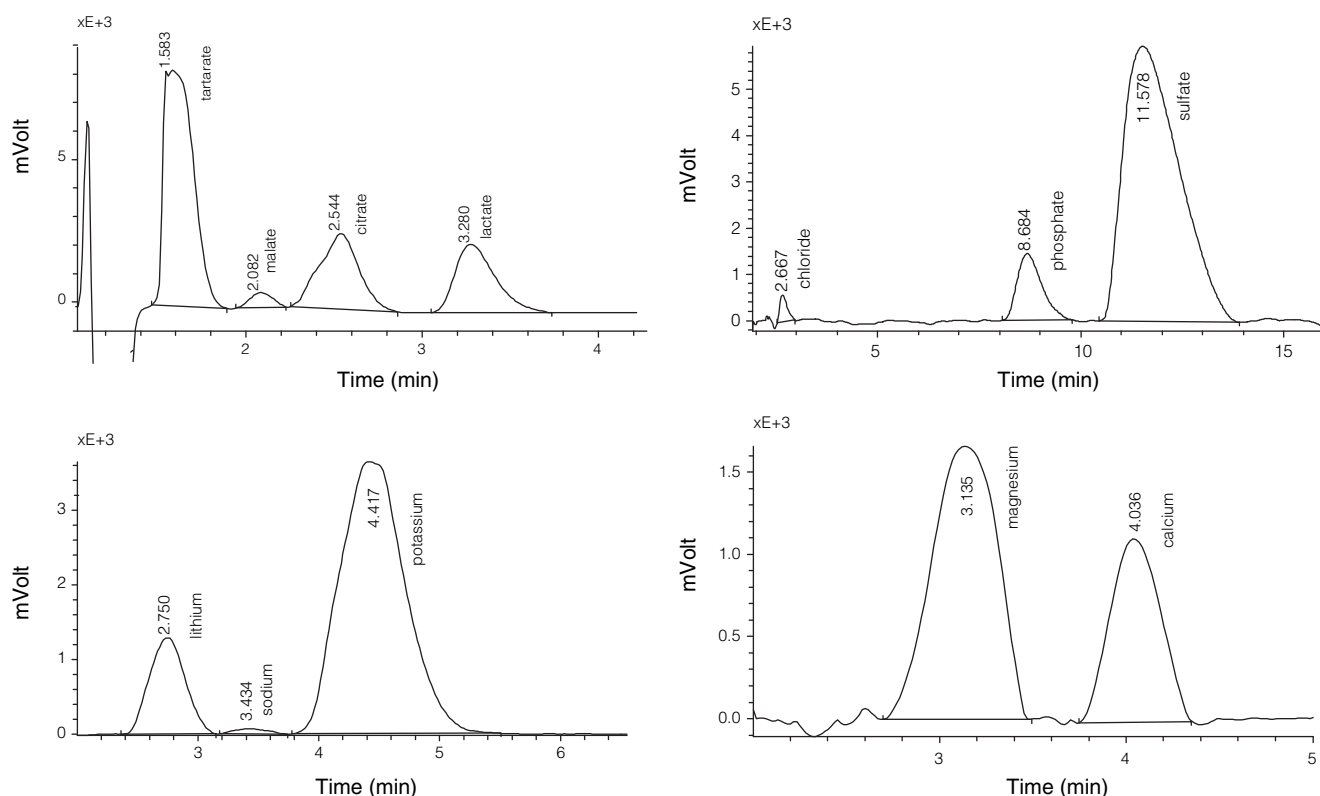
Figure 4. Typical chromatograms obtained in wine analysis. Measured concentrations in mg L^{-1} were tartarate 1740; malate 792; citrate 925; lactate 418; chloride 98.0; phosphate 275; sulfate 2092; lithium 6.07; sodium 18.4; potassium 560; magnesium 57.2; calcium 63.6.

lactate and malate can be easily explained by malo-lactic fermentation[11]. Also easily foreseeable is a negative correlation between concentrations of citrate and malate (originated predominantly from respective acids) and pH. Since there is a strong negative relationship between malic acid (pK$_{a1}$ 3.40) and lactic acid (pK$_a$ 3.86), a positive correlation between lactate concentration and pH is of no surprise. However, not all correlations found can be easily explained, i.e., a strong positive correlation between potassium concentration and pH. To the best of our knowledge, this relationship cannot be explained on the basis of known processes. Since the samples originated from various parts of the world, climatic or environmental conditions cannot be regarded as decisive. Noteworthy, the increase in citrate concentration corresponds to the decrease in the mineral content of wines – a negative correlation between the citrate concentration and the concentrations of K$^+$, PO$_4^{3-}$, Mg^{2+}, Li$^+$ and Ca^{2+}. The aforementioned observations needs to be proven on larger data sets and if confirmed, further studies can explain mechanisms behind those relations.

lactate, chloride, nitrate, phosphate, sulfate, lithium, sodium, potassium, magnesium, and calcium (Figure 4). Providing suitable sensitivity and practical dynamic ranges, it can be an alternative for the UV detection in wine analysis. The employment of the same, simple chromatographic setup with three different columns was found to be sufficient for the quantification of above-mentioned ions in all wine samples. Moreover, the good accuracy and high precision of the measurements are clear benefits of using the described HPIC system. Application of the system allows straightforward quantifications of thirteen ions, thus provides useful information about malo-lactic fermentation and concentrations of ions having impact on human health. The results of analysis of such a wide range of ions in wines are reported for the first time.

Statistical analysis of the obtained results concerning relationship between ions present in wines is in agreement with current knowledge (malo-lactic fermentation, correlation between organic acids concentrations and wine acidity).

CONCLUSIONS

In this study, conductivity detection has proven its applicability to the analysis of tartarate, malate, citrate,

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