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Determination of Pb and Cd in Macedonian Wines by Electrothermal Atomic Absorption Spectrometry (ETAAS)

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Abstract In this study, electrothermal atomic absorption spectrometry (ETAAS) was used for determination of lead and cadmium in Macedonian white wines. Wine samples, without prior purification, were directly injected into the ETAAS system, using matrix modifiers for Pb and Cd. The standard addition method was used for quantitative analysis of Pb and Cd content. Reliability of the method was verified by determination of selected validation characteristics. The results of the measurements indicated satisfactory precision and accuracy, confirming that the method is accurate and convenient for quantitative analysis. The lead and cadmium concentrations were ranged between 2–28.5 and 0.4–5.69 μg/L, respectively. The levels were below the maximal allowed concentration in wine and compare well with those reported for similar wines from other parts of the world.

Keywords Lead · Cadmium · Wines · Electrothermal atomic absorption spectrometry

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Introduction

Wine is a very complex natural product consisting of organic and inorganic compounds that have a great influence on wine quality. Determination of wine composition and following the concentration of various wine compounds at all stages of winemaking allows control of the technological process and obtaining wines with certain taste, bouquet, flavor, colour and astringency (Stafilov and Karadjova 2009). Mineral composition of wines is an important factor that influences its quality as well as nutritional value. In fact, determination of the elemental composition of wines is very important from toxicological point of view, not only since it could contain harmful elements, such as Pb, As and Cd, but also, from nutritional point of view, since wine contains essential elements for the human organism, such as Ca, Cr, Co, K, Se and Zn (Grindlay et al. 2011; Ivanova-Petropulos et al. 2013). Elements can be considered as indicators for wine origin since they are neither metabolized nor modified during the technological process of winemaking (Kallithraka et al. 2001). Their origin in wine can be influenced by various factors, starting from the vineyards (soil, fertilization practices, climate changes, vine variety, etc.) to the end of fermentation and vinification (addition of yeasts, maceration, content of proteins, fining agents (i.e. bentonite)).

Heavy metals, such as lead and cadmium, are naturally present in the environment at low concentrations. These elements are toxic for humans at low doses. As Pb and Cd are usually present at low levels in different foods and beverages,

their concentration has to be controlled. For their accurate determination, sensitive techniques are required, such as flame atomic absorption spectrometry, atomic fluorescence spectrometry, inductively coupled plasma mass spectrometry, inductively coupled plasma optical emission spectrometry, electroanalysis or neutron activation analysis (Matsushige and Oliveira 1993; Perez-Jordan et al. 1999; Harrington et al. 1999; Stafilov and Karadjova 2009; Ivanova-Petropulos et al. 2013).

Atomic absorption spectroscopy (AAS) is a technique of choice for food authentication, and for wine analysis too, suitable for direct determination of trace elements in wine (Harnly et al. 1979; Kim 2004; Stafilov and Karadjova 2009). Electrothermal atomic absorption spectroscopy (ETAAS) technique is not suitable for fast multielement analysis, but this technique offers high sensitivity and selectivity for determination of low levels of metals, and therefore, it is suitable and widely used for determination of Pb and Cd at low levels (Edel et al. 1995; Cvetković et al. 2006; Banović et al. 2009; Tariba et al. 2011). Furthermore, this technique allows direct determination of Pb and Cd in wine samples, which is advantageous for routine analyses, saving time and requiring minimum sample preparation (Bruno et al. 1994).

Wine is one of the most important products in the Republic of Macedonia. Wine characterization of Macedonian wines has been performed, including determination of phenolic and aroma compounds in commercial wines, as well as wines produced by different winemaking procedures (Ivanova et al. (2009, 2011a, 2011b, 2011c, 2012, 2013)). But, til now, low number of papers is dealing with determination of heavy metals in wines (Ivanova-Petropulos et al. 2013), especially for Cd and Pb which have been separately determined in Macedonian wines and published in separate papers in 2006 and 2007 (Cvetković et al. 2006; Karadjova et al. 2007). Moreover, a few air pollution studies in Macedonia using a moss biomonitoring technique have been performed (Barandovski et al. 2013), indicating a high content of Cu, Zn, Cd and Pb in mosses near the Skopje, Veles and Radoviš cities, as well as in the eastern part of the country. A lead and zinc smelter plant was operational in the city of Veles by May 2003 affecting the high levels of toxic elements. The high density of road traffic in the region of Skopje and the presence of a central heating station and an oil refinery in the vicinity of the city contribute to the high presence of these elements in the Skopje region. High values of Cd, Pb and Zn were noticed in the eastern parts of Macedonia because of the operation of three lead and zinc mines (Sasa, Toranica and Zletovo) as well as one open pit copper mine on the southeast, near the city of Radoviš. Reported median values for Cd and Pb in the mosses samples were higher compared to the reported results for other European countries and Norway (Barandovski et al. 2013). Based on these published data as well as on the fact that from 2006/2007 there are no additional studies performed on lead and cadmium determination in wines, we considered that determination of Pb and Cd levels in wine would be very important in order to have recent results for these toxic elements that would be of great importance for the winemaking industry in Macedonia. Moreover, long-term consumption of wine with high Pb and Cd levels could lead to chronic poisoning, which is another important fact for determination of their concentration in wine.

Therefore, the aims of this work were (i) to report a very simple and fast method for determination of Pb and Cd in wine and (ii) to analyze and present a joint results for the content of Pb and Cd in white wines from different varieties, such as Smederevka, Chardonnay, Riesling and Sauvignon Blanc, from two different regions in Macedonia (Tikveš region, the largest and most important wine region, and Skopje region) in order to determine their content and nutrition value. In addition, applied ETAAS method was optimized and several validation parameters were determined.

Materials and Methods

Reagents and Materials

Nitric acid (67 %, Analpure®, Analytika. Spol. s r.o. Praha, Czech Republic) was used in solutions of 1 and 0.1 % as a floating medium and for dilution of standards (Cd, Pb) and wine samples, respectively. Standard solutions of metals (Lead AA Standard, Cadmium AA Standard 1000 μg/mL, ULTRA Scientific, Analytical Solutions, USA) were used for analysis.

Determination requires matrix modifiers: 0.1 % H_3PO_4 (for Cd analysis) and palladium modifier (Merck, Germany) enriched with Mg solution (Certipur, Merck, Germany) for Pb analysis. Preparation of working solutions, standards and samples was performed with pipettes and volumetric flasks of class A.

Wine Samples

A total of seven white wines from different *Vitis vinifera* L. grape varieties from vintage 2012 were collected directly from the commercial wineries located in Tikveš wine region (two Smederevka wines, two Chardonnay wines and one Riesling wine) and Skopje wine region (one Chardonnay and one Sauvignon Blanc). Wines sampled directly from the corresponding tanks were the targeted wine samples and were kept for 8 months. A volume of 0.5 L of each wine was taken for analysis in glass bottles. Analyses were performed in triplicate.

Grapes from the Tikveš region (Smederevka, Chardonnay and Riesling) were grown in two different territories: Negotino (1) and Disan (2). Smederevka, Riesling and

Chardonnay grapes from Negotino were collected from 10, 8 and 7-year-old vineyards with area of 8, 4 and 5 ha, respectively. Smederevka and Riesling grapes from Disan were collected from 10-year-old vineyards, with areas of 8 and 3 ha, respectively. Concerning the grapes from Skopje region, Chardonnay was grown at 1 ha, 8-year-old vineyard and Sauvignon Blanc at 1 ha, 10-year-old vineyard. The distance between the rows was 2.8 m and the distance between the vines was 1.2 m.

Wine samples were kept in a cooling room at 4 °C before analysis.

The standard addition method was used for checking the accuracy of the method. Therefore, samples were spiked directly before the analysis with a mixture of Cd and Pb standards in amounts of 20 μg Pb and 1 μg Cd in addition "1" and 40 μg Pb and 2 μg Cd in addition "2".

Instrumentation

Atomic absorption spectrometer AA240Z Varian (Mulgrave Virginia, Australia) with the Zeeman background correction was used. The electrothermal technique (ETAAS) was suitable for determination of total Cd and Pb contents in wine samples. Cadmium and lead hollow cathode lamps were operated at the wavelengths of 228.8 and 283.3 nm, respectively. Atomizing environment was graphite furnace tube (coated partitioned tube with rings for Varian) heated to 2100 and 1800 °C when lead and cadmium were determined. The floated solution was HNO_3 (1 %).

Both methods used were optimized and subjected for validation. The limits of detection (LOD) were 0.20 μg/L (for Cd) and 2.8 μg/L (for Pb) and limits of quantitation (LOQ) were 0.40 and 5.6 μg/L for Cd and Pb, respectively.

Statistical Analysis

Statistical analysis, including one-way ANOVA with Student–Newman Keuls test, was performed using the XLSTAT software, Version 2012.6.09, Copyright Addinsoft 1995–2012, on the data for Pb and Cd in order to interpret their content in the wines.

Results and Discussion

Flame Atomic Absorption Spectrometry and ETAAS Methods

In this study, both techniques, flame atomic absorption spectrometry (FAAS) and ETAAS techniques, were tested for analysis of Pb and Cd in the wines. In fact, flame atomic absorption is a very common technique for detecting metals and metalloids in different samples, including wine too (Freschi et al. 2001; Stafilov and Karadjova 2009; Kinaree and Chanthai 2013). It is very reliable and simple to use. This technique is based on the fact that metals absorb light at specific wavelengths. The samples are decomposed into ions by heating them to quite high temperatures, and then, the absorption or emission of ultraviolet of visible radiation of these atoms is measured. For each element, a specific cathode lamp is used. For example, if a Pb hollow cathode lamp is used, it will be possible to determine the amount of lead in a sample. It is not possible to analyze Pb and other elements at the same time, but they can be analyzed in series. For most of these elements, a Beer's law relationship will hold between approximately 0.5 and 15–20 ppm, which means that FAAS will not be able to determine the concentration of an analyte that is below or above this range. In our study, it was noticed that the concentration of Pb and Cd in wines was not in the range of FAAS technique and the work was extended by applying the second available technique for metal analysis, the ETAAS as more appropriate for determination of Pb and Cd in the wine samples. This technique is not suitable for fast multielement analysis (it can analyze only one element in one run), but offers high sensitivity and selectivity for determination of low levels of metals.

Development of Method for Purification and Preparation of the Wine Samples

Wine is a complex matrix, containing high ethanol content and other organic compounds, and therefore, sample pretreatments, such as dilution, extraction ion exchange, precipitation or heating the samples (digestion) with HNO_3, $HClO_4$ and H_2SO_4 or mixtures of these acids, are commonly used methods (Castiñeira Gómez et al. 2004; Gonzalvez et al. 2008; Álvarez et al. 2007). In order to abbreviate and speed up the analysis, determination of some elements in wine by ETAAS can be performed without sample preparation or simple dilution can be applied. In this regard, in order to avoid the matrix interferences, it is necessary to use matrix modifier. In our research work, purification of the wine samples was not applied. Wines were directly injected in the ETAAS system, using matrix modifiers for Pb and Cd. The obtained results were satisfied, obtaining peaks with good shapes, without any interference.

Determination of Method Parameters

For direct ETAAS determination of Pb and Cd in wine, optimal parameters (temperature programme, modifiers, calibration) should be obtained. Therefore, in order to develop a convenient, simple, accurate and fast method for determination of Pb and Cd, several investigations were performed, obtaining the best results: temperatures of 2100

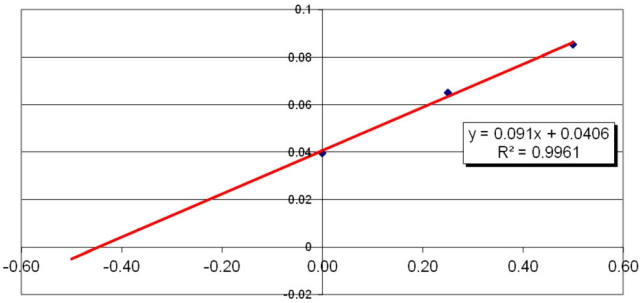

Fig. 1 Results for intercept, slope and R^2 of the calibration curve. Ivanova-Petropulos, Jakabová, Nedelkovski, Pavlík, Balážová, Hegedűs

and 1800 °C for lead and cadmium, respectively; HNO_3 (1 %) as a floated solution; modifiers H_3PO_4 (0.1 %) for Cd analysis and palladium modifier with Mg solution for Pb analysis.

The standard addition method was used for quantitative analysis of Cd and Pb content (Hegedűs et al. 2005). Slope, intercept and correlation coefficient (0.9961) were calculated and the results are presented in Fig. 1.

The accuracy and precision of the method were checked using a standard addition method. The results of the measurements are presented in Table 1, indicating satisfactory precision and accuracy. Wine samples were spiked with two appropriate volumes of standard solutions of Pb and Cd and satisfactory results were obtained confirming that the method is accurate and convenient for quantitative analysis.

Elemental Characterization of Wines

Few white wines from different varieties and different wine regions, such as Smederevka, Riesling and Chardonnay from Tikveš region and Chardonnay and Sauvignon Blanc from Skopje region, were analyzed for Pb and Cd, applying the optimized procedure described above. The results are presented in Table 2.

Table 2 Results for Pb and Cd content in the analyzed wines

Wine	Wine region	Pb [μg/L]	Cd [μg/L]
Smederevka-1	Tikveš	28.5	0.71
Smederevka-2	Tikveš	9.08	1.07
Riesling-1	Tikveš	24.7	1.43
Riesling-2	Tikveš	2.01	0.40
Chardonnay-1	Tikveš	18.6a	1.50b
Chardonnay	Skopje	18.6a	1.46b
Sauvignon Blanc	Skopje	n.d.	5.69

n.d. not detected, *1* Negotino, *2* Disan

Same letters (a and b) in the columnsindicate the values that are not significantly different ($p>0.05$), determined with one-way ANOVA, Student–Newman Keuls test

In general, all wines presented low concentration of Pb and Cd, lower than the maximal allowed concentration in wine (200 and 100 μg/L, respectively) according to the Macedonian food safety regulation (Official Gazette of the Republic of Macedonia 2005). Obtained contents correspond to the maximal allowed threshold recommended by the L'Organisation Internationale de la Vigne et la Vin (OIV 2011).

The content of lead (Pb) in wines ranged from 2 to 28.5 μg/L. Pb was not detected in one wine, Sauvignon Blanc from Skopje region. The highest content of Pb was present in Smederevka-1 wine (28.5 μg/L) and Riesling-1 (24.7 μg/L), and lowest in Riesling-2 wine (2.01 μg/L). The results obtained are in a good agreement with those previously reported for Macedonian wines (Karadjova et al. 2007) and Brazilian wines (Freschi et al. 2001). In addition, Macedonian wines presented lower amounts of Pb (on average 16.9 μg/L) compared to data for Croatian wines reported by Tariba et al. (2011) (on average 33 μg/L) and Banović et al. (2009) (on average 197 μg/L), as well as lower compared to Serbian wines (Ražić et al. 2007), Ethiopian white wines (Woldemariam and Chandravanshi 2011) and wines from the Korean market (Kim 2004). In fact, Pb is a toxic heavy metal with widespread industrial uses and no nutritional benefits are

Table 1 Results of lead and cadmium analysis for checking the accuracy and precision of the method by standard addition method

Sample of wine	Standard addition of Pb [μg/L]	Determined Pb [μg/L]	Standard addition of Pb [μg/L]	Determined Pb [μg/L]	Standard addition of Cd [μg/L]	Determined Cd [μg/L]	Standard addition of Cd [μg/L]	Determined Cd [μg/L]
1	20.0	20.0	40.0	40.0	1.00	1.00	2.00	1.99
2	20.0	20.3	40.0	39.9	1.00	1.29	2.00	1.85
3	20.0	18.1	40.0	40.9	1.00	0.91	2.00	2.04
4	20.0	24.2	40.0	37.9	1.00	0.81	2.00	2.09
5	20.0	26.2	40.0	36.9	1.00	1.17	2.00	1.92
Average		21.7		39.1		1.04		1.98
sd		3.33		1.67		0.19		0.10
v %		15.3		4.26		18.7		4.90

sd standard deviation, *v %* variation coefficient

known for it. Lead contamination of wine arises from numerous sources, including natural and anthropogenic. Atmospheric lead from industrial pollution or leaded gasoline can contaminate wine through deposition on vine plants. Moreover, significant source of lead are lead-based pesticides (Handson 1984). In the present study, Smederevka-1 and Riesling-1 wines that showed the highest content of Pb were produced from grapes grown at vineyards located near a road with high density of traffic in the Tikveš region that could be a reason for the higher level of Pb in both wines, but also, application of pesticides on vine plants could be a reason for the increased level of Pb in the wines. In addition, water could also be a source of lead contamination of wine. Surface water sources can be contaminated through runoff (drainage) or atmospheric deposition. In addition, lead contamination of wine can arise from winemaking practices and packaging (Stockley et al. 2003). The use of brass components in winemaking, such as pumps, valves, faucets and piping, could be a significant contributor to Pb contamination (Tariba et al. 2011).

Concerning the presence of Cd, its content ranged from 0.40 to 1.5 μg/L. Only Sauvignon Blanc from Skopje region presented the highest concentration of this element (5.69 μg/L). Two Chardonnay wines, one from Tikveš region (Chardonnay-1) and the other from Skopje region (Chardonnay), presented similar contents of Cd (1.50 and 1.46 μg/L) which were not statistically different ($p>0.05$). The results are in a good agreement with those reported by Cvetković et al. for Macedonian white wines (2006), similar to results for Slovenian wines (Kristl et al. 2001), Brazilian wines (Freschi et al. 2001) as well as Argentinean wines (Lara et al. 2005). In addition, the mean value of Cd in this study (1.75 μg/L) was slightly lower compared to the results obtained for Spanish white wines (mean 3.44 μg/L) (Mena et al. 1996). In general, cadmium (Cd) content in wine, which is a highly toxic element, depends on both natural and exogenous factors. Natural factors that influence the Cd levels could be soil type and its composition, grape variety, climate, chemical composition and the amounts of products used in a particular vineyard, the vintage period, the amount of rain during this period, etc. Exogenous factors, such as fermentation process, including maceration time, addition of sulfur dioxide, yeasts or different types of contamination, could affect the Cd concentration in wine. Generally, the cadmium content in the analyzed wines has been found to be low and the highest concentration found in Sauvignon Blanc could be due to the use of pesticides or fertilizers which contain salts of this metal.

Conclusion

In this study, white Macedonian wines from two different wine regions, Tikveš and Skopje, were analyzed by electrothermal atomic absorption spectrometry (ETAAS) for determination of heavy metals Pb and Cd. Wines were directly injected into the ETAAS system without previous sample preparation. The accuracy and precision of the method were checked, obtaining satisfactory results and confirming that the method is accurate and convenient for quantitative analysis of the harmful elements Pb and Cd in wines. In general, white wines analyzed in this research study applying ETAAS for direct determination of Pb and Cd did not contain these harmful elements in concentrations above the maximal allowed.

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Conflict of Interest Violeta Ivanova-Petropulos declares that she has no conflict of interest. Silvia Jakabová declares that she has no conflict of interest. Dusko Nedelkovski declares that he has no conflict of interest. Vladimír Pavlík declares that he has no conflict of interest. Želmira Balážová declares that she has no conflict of interest. Ondrej Hegedűs declares that he has no conflict of interest.

Compliance with Ethics Requirements This is an original research article that has neither been published previously nor considered presently for publication elsewhere. All authors named in the manuscript are entitled to the authorship and have approved the final version of the submitted manuscript.

This article does not contain any studies with human or animal subjects.

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