Short Communication

DETERMINATION OF LEAD IN CROATIAN WINES BY GRAPHITE FURNACE ATOMIC ABSORPTION SPECTROMETRY

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A method has been developed for direct determination of lead in wine by graphite furnace atomic absorption spectrometry (GFAAS) with Zeeman-effect background correction. The thermal behaviour of Pb during pyrolysis and atomisation stages was investigated without matrix modifier and in the presence of $Pd(NO_3)_2$, $Pd(NO_3)_2 + Mg(NO_3)_2$ x $6H_2O$, and $NH_4H_2PO_4 + Mg(NO_3)_2$ x $6H_2O$ as matrix modifiers. A simple 1:1 dilution of wine samples with $Pd(NO_3)_2$ as a matrix modifier proved optimal for accurate determination of Pb in wine. Mean recoveries were 106 % for red and 114 % for white wine, and the detection limit was 3 µg L^{-1}. Within-run precision of measurements for red and white wine was 2.1 % and 1.8 %, respectively. The proposed method was applied for analysis of 23 Croatian wines. Median Pb concentrations were 33 µg L^{-1}, range (16 to 49) µg L^{-1} in commercially available wines and 46 µg L^{-1}, range (14 to 559) µg L^{-1} in home-made wines. There were no statistically significant differences (P<0.05) in Pb concentration between commercial and home-made wines or between red and white wines.

KEY WORDS: *analytical validation, commercial wines, GFAAS, home-made wines*

Lead (Pb) in wine may originate from natural and anthropogenic sources. Before the ban in the 1990s, the major source of Pb in wine was the atmospheric deposition of lead from leaded gasoline (1). Atmospheric contribution is now much smaller. Today instead, among the most significant sources are lead-based pesticides (2). Other factors that determine the metal concentration in wine are the capacity of grapes to take up mineral substances, type of vineyard soil, steps in the production cycle (fermentation reactions, addition of compounds with various functions), wine processing equipment, conservation, and bottling. A significant contributor to Pb contamination is the use of brass components in wine-making, such as pumps, valves, faucets, and piping (3, 4).

The maximum acceptable limit for Pb in wine set by the International Organization of Vine and Wine (IOVW) is 0.15 mg L^{-1} (5). The national legislation in Croatia, however, allows the maximum level of 0.2 mg L^{-1} (6).

Lead concentration in wines deserves special consideration because of its toxicity and cumulative character. Consumption of alcoholic beverages, especially wines, can influence the concentration of Pb in blood (7-10). Long-term consumption of wine with high Pb levels could lead to chronic poisoning. Even at low levels, Pb is associated with hypertension, cardiovascular disease, kidney dysfunction, impaired bone synthesis, impaired sperm production and osteoporosis (11).

Because of toxicity concerns, it is important to measure Pb concentration in wine. Methods described so far include atomic absorption spectrometry (3, 12, 13), inductively coupled plasma optical emission

26

Tariba B, et al. GFAAS DETERMINATION OF LEAD IN WINE
Arh Hig Rada Toksikol 2011;62:25-31

spectrometry (14), inductively coupled plasma mass spectrometry (15, 16), energy dispersive X-ray fluorescence (17), potentiometric stripping analysis, and differential pulse anodic stripping voltammetry (13, 18). The most common among them for determining Pb at trace levels is graphite furnace atomic absorption spectrometry (GFAAS) (14, 19-22), a selective, simple, and highly sensitive method, adequate for direct determination in various matrices.

This study reports a simple and fast method for direct quantitative determination of trace Pb in wines using GFAAS. The method was validated and applied for analysis of 23 wines obtained from six Croatian viticultural areas.

MATERIALS AND METHODS

Instrumentation

We used a Perkin-Elmer® 5100 atomic absorption spectrometer (Norwalk, CO, USA) with Zeeman-effect background correction, an end-heated graphite tube atomiser (HGA-600, Perkin-Elmer Instruments, Norwalk, CO, USA), and an AS-60 furnace autosampler (Perkin-Elmer Instruments, Norwalk, CO, USA). For atomisers, we used graphite tubes with standard L'vov pyrolytic platforms. To purge the graphite tubes we used argon gas (purity >99.999 %) with an internal flow rate of 300 mL min^{-1}. The measurement mode was integrated absorbance. Perkin-Elmer electrodeless discharge lamp (EDL) was used to determine Pb at the analytical wavelength recommended by the manufacturer (283.3 nm, spectral bandpass 0.7 nm). Graphite furnace heating programme used for the determination of Pb is shown in Table 1.

Reagents and solutions

Pyrolysis and atomisation curves were established in the absence of a chemical modifier and in the presence of $Pd(NO_3)_2$, $Pd(NO_3)_2 + Mg(NO_3)_2$ x $6H_2O$ and $NH_4H_2PO_4 + Mg(NO_3)_2$ x $6H_2O$ as chemical modifiers. The 1 g L^{-1} $Pd(NO_3)_2$ solution, 1 g L^{-1} $Pd(NO_3)_2 + 0.6$ g L^{-1} $Mg(NO_3)_2$ x $6H_2O$ and 40 g L^{-1} $NH_4H_2PO_4 + 2$ g L^{-1} $Mg(NO_3)_2$ x $6H_2O$ solutions were prepared from modifier stock solutions [(10.0±0.2) g L^{-1} $Pd(NO_3)_2$ in 15 % (v/v) HNO_3, Merck, Darmstadt, Germany; 10 % $NH_4H_2PO_4$, Perkin Elmer Co., Norwalk, CO, USA] and $Mg(NO_3)_2$ x $6H_2O$ (BDH Chemicals Ltd, Poole, England) with bi-distilled water.

Standard solutions of Pb (10, 40, 75, 100, 175 and 250) µg L^{-1} were prepared daily from a 1000 mg L^{-1} Pb standard solution (Merck, Darmstadt, Germany) with 12 % (v/v) ethanol (Kemika, Zagreb, Croatia) to achieve alcohol concentration similar to that of a typical wine sample. One red and one white Croatian wine were purchased at a local supermarket. Wines investigated included grape varieties Plavac mali (red) and Riesling (white). Wine samples and standard solutions were diluted (1:1) prior to analysis by mixing 500 µL of wine/standard solution with 300 µL of 1 % HNO_3 and 200 µL of 0.05 % Triton X-100 (BDH Chemicals Ltd, Poole, England).

Wine samples

Eight commercially available and fifteen home-made wines were obtained from six Croatian viticultural areas (Dalmatia, Hrvatsko Primorje, Istria, Prigorje, Zagorje and Slavonia) and analysed according to the developed analytical procedure. The results are presented as means of four measurements. Since the data were not normally distributed, the

Table 1 *Graphite furnace heating programme for determination of lead in wine*

Step	Temp / °C	Ramp / s	Hold / s	Internal flow / mL min^{-1}
Drying	110	1	30	300
Drying	140	10	25	300
Pyrolysis	1100	1	30	300
Cooling-down	20	1	15	300
Atomisation	2000	0	5	0
Cleaning	2600	1	5	300

significance of the difference in Pb concentrations between the groups of commercial and home-made wines and white and red wines was calculated by using the Mann-Whitney U-test. Results were considered to be significant at the 5 % critical level (P<0.05).

RESULTS AND DISCUSSION

Optimisation

Compared to aqueous solutions, wine is a fairly complex matrix due to its organic content, especially ethanol, which influences sample transport to atomisation devices because of changes in density and surface tension (23). Matrix-matched calibration makes it possible to prepare standard solutions as similar as possible to samples (14, 20, 24-26).

Optimisation of the furnace programme was based on the pyrolysis and atomisation curves (Figure 1) for a standard solution of Pb (20 µg L^{-1}) containing 12 % ethanol in order to simulate wine matrix, and diluted samples (1:1) of one red and one white wine. The effect of pyrolysis and atomisation temperature on sample absorbance was tested in the absence and in the presence of three modifiers. The results indicate a similar thermal behaviour of Pb in the standard solution and in wine samples. Modifiers showed a stabilising effect on Pb in the standard solution as well as in wine samples. Pd modifier and Mg/Pd modifier ensured stability of Pb to up to 1200 °C, while phosphate/Mg modifier was associated with a loss in sensitivity over 900 °C. In addition, matrix modifiers enhanced the integrated absorbance signal in red wine analysis, for which the optimal pyrolysis temperature was 1100 °C and optimal modifiers Pd and Mg/Pd. During optimisation of atomisation temperature, we observed a slightly better sensitivity with the Pd than with the Mg/Pd modifier at pyrolysis temperature of 1100 °C. Maximum sensitivity was obtained when atomisation temperature was 2000 °C. Based on these results, we selected 1 g L^{-1} Pd as the optimum modifier for determination of Pb in wines, with the pyrolysis temperature of 1100 °C and the atomisation temperature of 2000 °C.

Method validation

The linear calibration was obtained using six standard solutions prepared in 12 % (v/v) ethanol in the range of 10 µg L^{-1} to 250 µg L^{-1} Pb. Relative standard deviations of five replicate samples for each
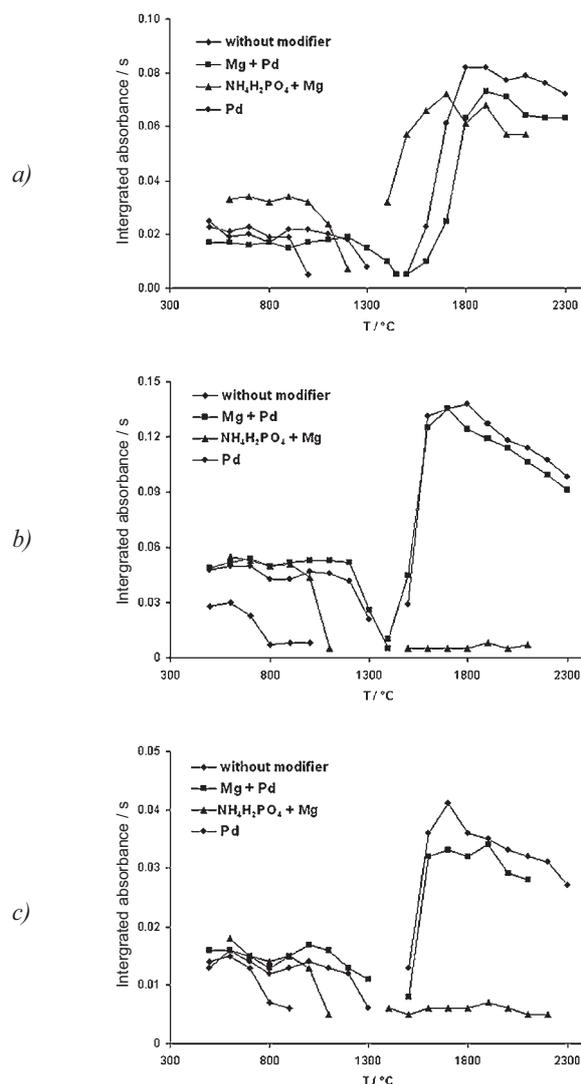

Figure 1 *Pyrolysis and atomisation curves in the absence and in the presence of different modifiers for: a) standard solution (20 µg L^{-1}); b) red wine sample (dilution 1:1); c) white wine sample (dilution 1:1)*

standard solution were lower than 3 %. The correlation coefficient (R), obtained by linear fitting to the calibration points, was better than 0.9995. Accuracy was evaluated by standard additions to determine recovery of the spiked analyte. Spike recovery tests were carried out with one red and one white commercially available Croatian wine, because no certified reference material was available. Wine samples were spiked with five concentration levels of Pb, and added spike concentration did not exceed 30 µg L^{-1} for the red wine sample (Merlot), and 40 µg L^{-1} for the white wine sample (Riesling). Adding an appropriate volume of standard solution of Pb to commercially available Croatian wines resulted in an

Table 2 *Lead concentrations in analysed wines*

Area	Grape variety	Wine type	Vintage	$\gamma(Pb)^a$ / µg L^{-1}
Commercial				
Ston	Plavac mali	Dry red	2007	48±2
Pelješac	Plavac mali	Semi-dry red	2008	16±2
Istria	Merlot	Dry red	2008	29±2
Istria	Malvasia	Dry white	2008	49±1
Istria	Malvasia	Dry white	2009	40±1
Đakovo	Riesling	Dry white	2007	22±1
Kutjevo	Riesling	Dry white	2008	21±2
Srijem	Chardonnay	Dry white	2007	36±1
				33 (16 to 49)^b
Home made				
Kaštel Kambelovac	Plavac mali	Dry red	2009	43±1
Kaštel Lukšić	Crljenak	Dry red	2009	46±2
Kaštel Lukšić	Babić	Dry red	2009	58±1
Vrgorac	Vranac	Dry red	2009	68±2
Istria	Merlot + Vranac	Dry red	2009	259±3
Istria	Merlot	Dry red	2009	82±1
Rab	Plavac mali	Dry red	2009	559±3
Istria	Refosco	Dry red	2009	31±2
Istria	Mix	Dry red	2009	36±2
Istria	Malvasia	Dry white	2009	51±1
Rab	Mix	Dry white	2009	276±2
Kalnik	Semillon	Dry white	2009	14±2
Trema	Riesling + Silvaner	Dry white	2009	17±1
Donje Križevčine	Riesling + Kraljevina	Dry white	2009	16±1
Varaždin	Mix	Dry white	2009	19±0.5
				46 (14 to 559)^b

^{a}*Mean±standard deviation*
^{b}*Median value and range*

average recovery of 106 % for red, and 114 % for white wine. The sensitivity of the calibration curves for the spiked samples (sensitivity $_{white\,wine}$=0.0042; sensitivity $_{red\,wine}$=0.0038) was similar to the sensitivity of the calibration curve for the matrix-matched standard solutions, indicating that there were no matrix effects and the selectivity of the method was satisfactory. The characteristic masses of the standard solutions (m_0=11 pg) were within the 6 % of the recommended value for Pb ($m_{recomended}$=12 pg) (27). Measurements of ten different replicates of one red and one white wine sample (measured in triplicates) gave relative standard deviations of 2.1 % and 1.8 %, respectively, indicating high within-run precision.

The detection limit (3 µg L^{-1}) was calculated as three times the standard deviation of 25 consecutive measurements of blank (3σ). The quantification limit (9 µg L^{-1}) was calculated as ten times the standard deviation of 25 consecutive measurements of the blank (10σ).

Analysis of wine samples

Eight commercially available and fifteen home-made wines were analysed according to the developed analytical procedure (Table 2). The median Pb concentration in commercially available Croatian wines was 33 µg L^{-1} (range from 16 µg L^{-1} to 49 µg L^{-1}), which is far below the upper permissible limits (5, 6). In home-made wines Pb concentration ranged from 14 µg L^{-1} to 559 µg L^{-1}, and the median was 46 µg L^{-1}. Concentration above the permitted level established in Croatia of 200 µg L^{-1} was found in three

home-made wines. Regardless the wider range of concentrations in home-made wines, we found no significant differences (P<0.05) in Pb concentrations between commercial and home-made wines or between red and white wines.

Although wine is relatively widely consumed drink in Croatia (28), there are limited data on Pb concentrations in Croatian wines. Our results are generally in agreement with the results obtained by Banović et al. (12), where Pb concentration ranged from 67 µg L^{-1} to 355 µg L^{-1} (median 190 µg L^{-1}). Vinković-Vrček et al. (16) found lower concentrations of Pb in conventionally and organically produced red and white Croatian wines; mean concentrations ranged from 1.15 µg L^{-1} to 7.23 µg L^{-1}. In contrast, Pb in Croatian wines investigated by Šeruga et al. (13) ranged from 4.39 µg L^{-1} to 35.52 µg L^{-1} (mean 9.50 µg L^{-1}). Considering the findings from other parts of Europe, our results are similar to those obtained for 45 Cretan wines by Galani-Nikolakaki et al. (24), where Pb ranged from 18 µg L^{-1} to 421 µg L^{-1} (median 64 µg L^{-1}). Somewhat similar concentrations of Pb were found in wines from Serbia (29, 30) and Germany (31). Especially high values of Pb, up to 1125 µg L^{-1}, were found in wines widely consumed in Spain (32), but also in wine samples from a Czech viticulture region (from 10.9 to 1253 µg L^{-1}; mean 67.1 µg L^{-1}) (33). Lower Pb concentrations than in our study were found in Brazilian (21), Uruguayan (3), and Portuguese wines (34).

CONCLUSION

In the GFAAS method we developed to determine Pb in wine no sample preparation was required, save for the dilution step. This enables direct determination with little chance of contamination. Our results and recovery values of spiked samples indicate that a simple 1:1 dilution of the samples was sufficient to accurately determine Pb in Croatian wines. Although obviously not enough samples were investigated in this work to allow general conclusions, our findings appear to be in agreement with values reported by other investigators.

Even though 13 % of the wine samples tested exceeded the maximum permissible limit established by the International Organization of Vine and Wine and by Croatian law, consumption of such wines does not pose any imminent toxicological risk to moderate wine consumers (i.e. if they consume one or two glasses of wine per day).

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Sažetak

ODREĐIVANJE OLOVA U HRVATSKIM VINIMA ELEKTROTERMIČKOM ATOMSKOM APSORPCIJSKOM SPEKTROMETRIJOM

Razvijena je metoda za izravno određivanje olova u vinu primjenom elektrotermičke atomske apsorpcijske spektrometrije (ETAAS) uz Zeemanovu korekciju nespecifične apsorpcije. Termalno ponašanje Pb za vrijeme pirolize i atomizacije uzorka ispitano je bez primjene modifikatora matrice te u prisutnosti modifikatora $Pd(NO_3)_2$, $Pd(NO_3)_2 + Mg(NO_3)_2$ x $6H_2O$ i $NH_4H_2PO_4 + Mg(NO_3)_2$ x $6H_2O$. Pokazalo se kako je jednostavno razrjeđenje uzoraka (1:1), uz $Pd(NO_3)_2$ kao modifikator, optimalno za točno određivanje Pb u vinima. Srednje vrijednosti analitičkih povrata bile su 106 % za crno i 114 % za bijelo vino, dok je granica detekcije mjerenja iznosila 3 μg L^{-1}. Preciznost mjerenja unutar istog dana iznosila je 2,1 % za crno vino te 1,8 % za bijelo vino. Metoda je primijenjena za analizu 23-ju uzoraka vina hrvatskog podrijetla. Koncentracije Pb bile su u rasponu od 16 μg L^{-1} do 49 μg L^{-1} (medijan 33 μg L^{-1}) u komercijalnim vinima te od 14 μg L^{-1} do 559 μg L^{-1} (medijan 46 μg L^{-1}) u vinima domaće proizvodnje. Nije pronađena statistički značajna razlika između koncentracije Pb u komercijalnim i domaćim vinima, kao ni razlika između crnih i bijelih vina.

KLJUČNE RIJEČI: *analitičko vrednovanje, domaća vina, ETAAS, komercijalna vina*

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