

The optimization of the methods for Cu, Zn and Pb content determination in Romanian wines by AAS after dry or microwave mineralization

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Abstract

The optimal conditions for determination of Cu and Zn in wine samples (red and white wines) by flame atomic absorption spectrometry after dry mineralization and of Pb by electrothermal atomic absorption spectrometry after microwave mineralization are presented in this paper. Working linear ranges are given: for Cu, 0.8-8mg/l, for Zn, 0.2-1mg/l and for Pb, 2-20µg/l. Found detection limits are 0.2mg/l, 0.13mg/l and 0.02µg/l for Cu, Zn and Pb, respectively. The trueness of the method has been verified through the determination of the recovery rate of the three metals added to the samples before mineralization. Global recoveries for three levels of concentration for all elements, 83.60%, 84.26% and 67.31% for Cu, Zn and Pb, register in the theoretical limits. Precision was evaluated through repeatability on the wines. The results obtained are under the values recommended for concentration levels present in the analyzed samples.

Keywords: copper, zinc, lead, atomic absorption spectrometry, wine

Introduction

The determination of heavy metals in environmental, biological and food samples has drawn significant attention due both to the nutritional and toxic effects of these elements or their compounds [1,2,3,4,5]. Some of heavy metals such copper and zinc are in very small quantities essential for life. Beyond the limits of each specific forms of life, these metals become toxic. These metals can be gained by animals and people through drinking water, air, food.

Copper is widely distributed in nature and is an essential trace element for humans. It functions as a cofactor and is required for structural and catalytic properties of a variety of important enzymes, including cytochrome c oxidase, tyrosinase, p-hydroxy-phenilpyruvate hydrolase and dopamine β hydroxylase. Food, beverages and drinking water are potential sources of excess exposure [3].

Zinc is one of the most important mineral elements for the human body due to it's involving in a series of physiological systems, like the metabolism of nuclear acids, protein synthesis, the metabolism of carbohydrates and many important contributions in the biochemical reactions. It is the component of many metal-enzymes. The deficiency of Zn manifest through loss of food appetite, loss of weight, osseous deformations, skin injury, growth of infection availability, etc [6]. Marginal zinc deficiency and suboptimal zinc status have been recognized in many population groups in both less developed and industrialized countries. Although the assessment of zinc status is a complicated task, supplementation or fortification with zinc has been associated with increased linear growth, reduction in diarrhoeal disease, enhanced immune function and improved pregnancy outcome. Even if the

cause of suboptimal zinc status in some cases may be inadequate dietary intake of zinc, inhibitors of zinc absorption are likely the most common causative factor [7].

Lead, unlike copper and zinc is a toxic metal exclusively. Its effects, on people being disastrous even in very small quantities. Lead can be accumulated in biological systems becoming potential contaminants mainly along the alimentary chain [3].

Wine has been widely consumed beverage in the world for centuries with very obvious commercial value and social importance. Numerous studies have shown that the moderate consumption of wine, especially red wines, improves good health and longevity [8,9].

The analysis of trace metals in wine is of great for quality and authenticity control of wine, metals' bioavailability, and toxicity. The presence of these elements can influence the wine making process or can change the taste and quality of the final product. The content of metals in wine can be attributed to natural sources (air, soil, oenological treatments) and to contamination during the wine-making process [10].

High residual copper can contribute to an enhanced rate of oxidative spoilage, which ultimately results in the browning of the wine. The phenomena called "browning", particularly in white wines, constitutes one of the principal oenological problems for wine producers and some metal ions (Cu, Fe, Mn) are the activators of this process. In organoleptic terms, this phenomenon translates into a process of continuous oxidation, a loss of aromatic freshness and, in the final stages, the appearance of precipitates of condensed phenolic material in the bottled wine. To minimize the incidence of these problems, it is generally recommended to maintain copper concentration below 0.3-0.5mg/L [10]. Lead contamination of wine is a problem dating back to ancient times. It has been estimated that 70% of the Pb intake by humans comes from food and drinks, and wine is the alcoholic beverage that shows the highest level of lead. An excess of ingested lead may present a real health hazard affecting both the nervous system and the biosynthesis of haemoglobin. In response to this health concern, the threshold limit value of Pb in wine has been reduced progressively by the OIV, and is at present 200µg/L [10]. Minor metals (Cu, Fe, Mn, Zn) and some trace metals are favourable for yeasts as they are required for prosthetic metallo-enzyme activation [11]. In wines, Cu and Zn are minor metals present at concentrations in the range 0.1-10µg/ml. Pb, trace metal, is in the range 0.1-100ng/ml or lower [11].

The official methods for the determination of metals in wine recommended by the OIV and the American Society of Oenologists are essentially based on atomic absorption spectrometry (AAS). Alkali metals and alkali earth metals as well as many of the transition metals like Mn, Cu, Fe or Zn are all atomized with good efficiency using a common air/acetylene flame (FAAS) with typical detection limits in the sub-ppm range. Electrothermal atomic absorption spectrometry (ETAAS) is mainly used for trace metals determination in wine samples. However, during the direct analysis of complex matrixes, the non-specific background absorption from interfering chemical species often occurs and could cause serious problems. To minimize the unspecific absorption, the application of a continuum deuterium source or Zeeman Effect as the background correction system is recommended. The interferences depend mainly on the details of the applied analytical conditions for measurement, and a careful optimization of the temperature programs for the particular elements is needed. It has been observed that large amount of fumes developed during the pyrolysis step and accumulation of carbonaceous residue after several firings which strongly affected performance [10]. For the diminution of this disadvantage, wine samples have initially been liable to a pre-treatment. So, regarding the determination of Cu and Zn, wine samples have been mineralized using the dry method and for the determination of Pb element, the microwave method.

The aim of this article is to present the optimization of the analytical methods applied for the determination of Cu, Zn and Pb contents in wine samples. In addition, some aspects regarding validation of the methods are discussed below.

Materials and methods

Instrument

Measurements were carried out using a Zeenit700 Analytic Jena atomic absorption spectrometer with deuterium and Zeeman background correction equipped with acetylene/air flame and graphite furnace (centre fixed platform tubes) as atomizers and, with AS52 S and MPE60 auto-samplers for flame and furnace, respectively. The atomization cell was purged with argon. The instrument was calibrated by the Romanian Metrology National Institute. Instrumental and flame/graphite furnace conditions are summarized in Table 1.

For the microwave digestion it was used a Milestone ETHOS PRO microwave digester.

Table 1 - Spectrometer conditions for the determination of Cu, Zn and Pb in wine

Operating conditions	Cu	Zn	Pb		
Lamp	HCL	HCL	HCL		
Wavelength (nm)	324.8	213.9	283.3		
Slit width (nm)	1.2	0.5	0.8		
Lamp current (mA)	3.0	4.0	4.0		
Background correction	D ₂	D ₂	Zeeman		
Mode	Double beam	Double beam	Single beam		
Technique	Flame	Flame	Graphite tube/Platform		
Flame conditions					
Cu	Flame: C ₂ H ₂ /air Fuel flow: 40l/h Burner type: 50mm		Burner height: 7mm Nebulizer rate: 5.0ml/min		
Zn	Flame: C ₂ H ₂ /air Fuel flow: 40l/h Burner type: 50mm		Burner height: 5mm Nebulizer rate: 7.0ml/min		
Graphite furnace temperature programs					
Pb	Temperature (°C)	Rate (°C/s)	Hold (s)	Time (s)	Air flow rate
Drying 1	95	20	10	13.8	Max
Drying 2	105	1	10	20	Max
Drying 3	120	1	10	25	Max
Pyrolysis	500	250	10	11.5	Max
AZ (auto zero)	500	0	5	5	Stop (read)
Atomize	1500	1300	4	4.8	Stop (read)
Cleanout	2400	500	4	5.6	Max

Reagents and solutions

Chemicals were of ultrapure grade, and ultrapure water (18.2MΩcm), obtained from a Milli-Q water purification system (Millipore) was used throughout. Stock standard solutions (1g/l) of copper, zinc and lead were supplied by Merck. Working solutions were obtained by

appropriate dilution of the standard ones. Hydrochloric acid, nitric acid and hydrogen peroxide (Merck) were also used for digestion and diluting the samples. All glassware were kept in 10% nitric acid for at least 48 h and rinsed with ultrapure water before use.

Samples

Samples of wines were obtained from markets. It has been analyzed four wine types, two red and two white wines. All previous experiments were carried out using a control samples prepared by mixing the content of several bottles.

Analytical procedure

Dry mineralization

50 ml wine sample has been calcinated at 450⁰C and brought down at a 10 ml volume, with HNO₃ 0.1mol/l. The method was made according to SR EN 14082:2003[12]. The content of Zn and Cu were determined from the mineralized product.

Wet mineralization

Wine samples (4ml) have been added with pipette in PTF recipients and treated with 6ml HNO₃ 65%. The recipients have been left in the niche for the organic matrix of the wine to stabilize, approximately 10 minutes it where added 2 ml H₂O₂ 30%. Covered recipients have been introduced into the microwave furnace and mineralized according to table 2. After the digestion recipient content is quantitatively transferred into a 25 ml carboy and brought down to sign with ultra pure water. From these mineralization was determined Pb element. The standards used in calibration have been prepared in HNO₃ 3.47 mole/l for the same acid concentration as the samples. All subsequent dilutions have been prepared with HNO₃ 3.47 mole/l.

Table 2 – The program of the microwave furnace

Stage	Time (minutes)	Temperature (⁰ C)	Microwave power (W)
1	10	150	600
2	20	150	750
3	15 – cooling	0	0

Results and Discussions

1. Optimization

The furnace program for lead was optimized according to the pyrolysis and atomization curves, for microwave digested wine samples, using centre fixed platform tubes without modifier. For the determination of Cu and Zn, the air/acetylene flame was optimized for calcinated wine samples. Optimal parameters obtained after these studies for both, flame and furnace, are presented in table 1.

2. Analytical performances

Calibration

Linear range and linearity for each element were studied. Linear regression analysis and its statistical results were presented in table 3. For assignation of linear domain have been

applied two statistic tests: dispersion homogeneity test and linearity test [14,15]. Linearity, calculated as $(1-S_b/b) \times 100$ [3], was 98.56% for Cu, 98.39% for Pb and 98.33% for Zn.

Table 3. Linear regression analysis and its statistics

Element	Data		
Cu	$b=0.0876 \pm 0.004$ DL=0.2035mg/l	$a=0.0031 \pm 0.0189$ DML=0.6783mg/l	$r=0.9997$ Linear range: .88mg/l
Zn	$b=0.3619 \pm 0.0192$ DL=0.1298mg/l	$a=0.0114 \pm 0.01205$ DML=0.4326mg/l	$r=0.9996$ Linear range: 0.2-1mg/l
Pb	$b=0.0049 \pm 0.00025$ DL=0.0168 μ g/l	$a=-0.0003 \pm 0.00156$ DML=0.0561 μ g/l	$r=0.9996$ Linear range: 2-20 μ g/l

b, slope; a, intercept; r, correlation coefficient; S_a , standard deviation of the intercept; S_b , standard deviation of the slope; DL, detection limit; DML, determination limit.

Limit of detection and limit of determination

Limit of detection and of determination of the methods have been set according to OIV recommended technique [13]. The two limits were based on values of the standard deviation of the intercept (S_a) and they were deduced of mathematical expressions: $DL=(3*S_a)/b$ and $DML=(10*S_a)/b$. The obtained results are presented in the table 3.

Trueness

Taking into account that we have not CRM available for this kind of samples, recovery assays were carried out for trueness study purpose [3, 18, 19]. A red wine sample was fortified, before mineralization, at i levels ($i=3$) of concentration and was analysed in j replicates ($j=3$). Fortification to obtain concentrations in sample between the interval 1-3mg/l for Cu, 0.5-1.5mg/l for Zn and 4-10 μ g/l in the case of Pb was performed. Once the elemental recovery for each sample (R_{ij}) was obtained, the average recovery for each level (\bar{R}_i) and the global recovery for each element (\bar{R}) were calculated. It has been observed that the recoveries in all cases were in range recommended by the literature of the speciality [16] (table 4-6). One-way ANOVA was performed to find out possible bias contribution due to between levels variance. After ANOVA calculations were performed, no significant bias was detected for all three elements.

Table 4 - Statistical results obtained by application of ANOVA test to recovery for Cu in wine By FAAS

ANOVA: single factor							
SUMMARY							
Groups (addition)	Recovery (%)			Count	Sum	Average	Variance
A (1mg/L)	81.09	84.56	80.63	3	246.28	82.09	4.62
B (2mg/L)	83.23	85.03	84.99	3	253.25	84.42	1.06
C (3mg/L)	86.78	82.27	83.78	3	252.83	84.28	5.27
Overall mean							83.60
ANOVA							
Source of Variation	SS	df	MS	F-calculated	F-critical		
Between Groups	10.19	2	5.1	1.40	5.143		
Within Groups	21.88	6	3.65				
Total	32.07	8					

Table 5 - Statistical results obtained by application of ANOVA test to recovery for Zn in wine by FAAS

ANOVA: single factor SUMMARY							
Groups (addition)	Recovery (%)			Count	Sum	Average	Variance
A (0.5mg/L)	81.78	86.42	84.55	3	252.75	84.25	5.45
B (1.0mg/L)	85.66	83.14	80.98	3	249.78	83.26	5.49
C (1.5mg/L)	84.62	83.79	87.40	3	255.81	85.27	3.57
Overall mean							84.26
ANOVA							
Source of Variation	SS	df	MS	F-calculated	F-critical		
Between Groups	6.06	2	3.03	0.63	5.143		
Within Groups	29.02	6	4.84				
Total	35.08	8					

Table 6 - Statistical results obtained by application of ANOVA test to recovery for Pb in wine by ETAAS

ANOVA: single factor SUMMARY							
Groups (addition)	Recovery (%)			Count	Sum	Average	Variance
A (2µg/L)	76.93	67.21	58.86	3	203	67.67	81.79
B (4µg/L)	64.22	62.31	70.62	3	197.15	65.72	18.94
C (6µg/L)	68.00	59.40	78.24	3	205.64	68.55	88.96
Overall mean							67.31
ANOVA							
Source of Variation	SS	df	MS	F-calculated	F-critical		
Between Groups	12.59	2	6.29	0.1	5.143		
Within Groups	379.38	6	63.23				
Total	391.97	8					

Precision in determination of copper, zinc and lead in wines

Precision of the AAS methods was evaluated through the determination of Cu, Zn and Pb contents in four samples of commercial wines. For this, each sample was analyzed six times and the relative standard deviation in repeatability conditions was calculated. As can be seen, the results obtained don't exceed maximum acceptable limits by OIV [17] for studied metals. On the other hand, $RSD_{\text{repeatability}}$ values are under the ones recommended by the Horwitz equation and by AOAC for concentration levels present in the analyzed samples [16] (table 7).

Table 7 – Concentration^(*) of Cu, Zn and Pb in wine samples

Sample	Cu		Zn		Pb	
	Conc.(mg/L)	RSD _r ,%	Conc.(mg/L)	RSD _r ,%	Conc.(mg/L)	RSD _r ,%
1 Fetească Neagră	0.243±0.018	7.41	0.334±0.001	0.30	0.054±0.005	9.26
2 Bordeaux	0.980±0.014	1.43	0.545±0.005	0.92	0.067±0.002	2.99
3 Riesling Italian	0.272±0.013	4.78	0.175±0.010	5.71	0.066±0.010	15.15
4 Fetească regală albă	0.324±0.026	8.02	0.181±0.012	6.63	0.094±0.008	8.51

^(*) It was made the average of six determinations ± standard deviation

Conclusions

The methods presented here regarding determination of Cu and Zn contents from wine by FAAS method after five times pre-concentration after thermal calcinations, and of Pb content by ETAAS method after a microwave mineralization, were optimized and then, validated.

Dry or wet mineralization of wine samples and Zeeman adjustment applied in Pb determination reduce the errors caused by the interferences present in the complex matrix of wine.

The obtained performance parameters as linearity, detection and determination limits, trueness, followed as recovery and, repeatability of the four wine samples investigated, respect the theoretical values provided by specialty literature for these analytical methods permitting in this way their validation.

These methods can be applied successfully in analyses of Cu, Zn and Pb present in wine.

References

1. DUMITRESCU, V., AVRAMESCU, A., AVRAMESCU, M., Rev. Chim. (Bucuresti), **48**, nr.12, 1997, p.965.
2. SERDARU, M., PASCU, L., VLADESCU, L., Rev. Chim. (Bucuresti), **55**, nr.12, 2004, p.935.
3. JURADO, J.M., MARTIN, M.J., PABLOS, F., MOREDA-PINEIRO, A., BERMEJO-BARRERA, P., Food Chemistry, **101**, 2007, p.1296.
4. VLADESCU, L., PASCU, L., Rev. Chim. (Bucuresti), **56**, nr.4, 2005, p.331.
5. VLADESCU, L., SERDARU, M., PASCU, L., ROPOTA, M., Rev. Chim. (Bucuresti), **56**, nr. 6, 2005, p.585.
6. SCRIPCARIU, M.D., Teza de doctorat, Bucuresti, 2007, p.29.
7. BO LÖNNERDAL, Dietary Factors Influencing Zinc Absorption, Journal of Nutrition, **130**, 2000, p.1378S-1383S.
8. GOLDBERG, D. M., BROMBERG, I. L., Health effects of moderate alcohol consumption: a paradigmatic risk factor, Clinica Chimica Acta, **246**, 1996, p.14.
9. GRONBAEK, M., DEIS, M., SORENSEN, T. I., BECKER, U., SCHNOHR, P., JENSEN, P., Mortality associated with moderate intakes of wine, beer or spirits, British Medical Journal, **10**, 1995, p.1165.
10. PYRZYNSKA, K., Critical Reviews in Analytical Chemistry, **34**, 2004, p.69.
11. PAWEL, P., Trends in Analytical Chemistry, **26**, 9, 2007, p.941.
12. SR EN 14082:2003, Produse alimentare. Determinarea microelementelor. Determinare plumb, cadmiu, zinc, cupru, fier si crom prin spectrometrie de absorbtie atomica (AAS) dupa calcinare.
13. Compendium of International Methods of Analysis – OIV, **2**, 2007, MA-F-AS1-12-GUIVAL, p. 16.
14. SR ISO 8466-1, Calitatea apei – Etalonarea si evaluarea metodelor de analiza si estimarea caracteristicilor de performanta – Partea 1: Evaluarea statistica a functiei liniare de etalonare.
15. SR ISO 8466-2, Calitatea apei – Etalonarea si evaluarea metodelor de analiza si estimarea caracteristicilor de performanta – Partea 2: Strategia de etalonare pentru functii de etalonare de gradul doi.
16. TANASE, I., GH., PANA, A., RADU, G., L., BULEANDRA, M., Validarea metodelor analitice-Principii teoretice si studii de caz, Editura Printech, Bucuresti, 2007, p.199.
17. Compendium of International Methods of Analysis – OIV, **2**, 2007, MA-E-C1-01-LIMMAX, p. 1-3.
18. GOMEZ, M.R., CERUTTI, S., SOMBRA, L. L., SILVA, M. F., MARTÍNEZ, L. D., Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association, **45**(6), 2007, p. 1060-4.
19. LARA, R., CERUTTI, S., SALONIA, J. A., OLSINA, R. A., MARTINEZ, L. D., Food and Chemical Toxicology, **43**, 2005, p. 293-297.