METHODS VALIDATION FOR DETERMINATION OF LEAD AND CADMIUM IN WINES BY MEANS OF GRAPHITE FURNACE ATOMIC ABSORPTION SPECTROMETRY

V. Nour, I. Trandafir and M.E. Ionică

University of Craiova, 13 A.I. Cuza St., 200585 Craiova, Romania

E-mail: vionor@yahoo.com

Abstract

An excess of ingested lead may present a real health hazard, affecting both the nervous system and the biosynthesis of hemoglobin. It has been estimated that 70% of the lead intake of humans comes from food and drinks, and wine is the alcoholic beverage that shows the highest level of lead. In response to this health concern, the threshold limit value of lead in wine has been reduced progressively. According to Commission Regulation (EC) No. 1881/2006 wines are subject to maximum limits of 0.20 mg/kg (wet weight) for lead.

Although, depending on the origin, particularly red wines have been found that clearly exceeded these limits with lead levels even higher than 1000 $\mu g/L$, most wines nowadays are within the above limits.

It is well known that cadmium and its compounds are highly toxic even at low concentration levels and may result in bioaccumulative. Food and beverages, especially wine, are the most important sources of Cd human intake.

Cadmium contents in wine may vary within a broad range and it is possible to find very low concentration levels in the order of a few $\mu g/L$. On the other hand, because the levels of cadmium in environmental samples are now mandated, sensitive analytical techniques are required to obtain low detection limits.

There is clearly an increasing demand for rapid, reliable and sensitive methods for the routine determination of lead and cadmium in wine.

Due to the high sensitivity, selectivity, and easy operation, graphite furnace atomic absorption spectrometry (GFAAS) has frequently been used for the determination of cadmium and lead in foods. The aim of this study was to validate GFAAS methods for the determination of lead and cadmium in wines according to Commission Decision 2002/657/EC, UNI CEI EN ISO/IEC 17025/2005 and Commission Regulation (EC) No. 333/2007.

The main parameters evaluated in the validation process were: the detection and the quantification limits (LoD, LoQ), the recovery, the repeatability, the reproducibility, the linearity range and the standard measurement uncertainty. The results obtained for LoD and LoQ were, respectively: Pb, 13.34 and 26.64 $\mu g/kg$; Cd, 2.80 and 5.60 $\mu g/kg$. While for the recovery: Pb, 95.61%; Cd, 93.29%. The expanded standard measurement uncertainty was estimated as follows: Pb, 18.4% and Cd, 13%.

Keywords: method validation, wines, graphite furnace atomic absorption spectrometry, lead, cadmium

1. INTRODUCTION

Wine is a natural product, widely consumed in the world with thousands of years of tradition. The chemical composition of wine is very complex: besides ethanol, sugars and organic acids, wine contains tannins, aromatic and coloring substances and microelements.

Numerous studies have shown that a moderate consumption of wine, especially red, improves good health and longevity when it is combined with a balanced diet. Daily consumption of wine in moderate quantities contributes significantly to the requirements of the human organism for essential elements (B, Co, Mn, Ni, Mo, Se, Zn), even though with elements like As, Pb, Cd which are well known as toxic. Beverages of different kinds have been investigated for their content of Pb, Cd, Ni, Cr, As and Hg [1]. About a ten times higher Pb content was found in wine than in most other beverages, so wine is the most significant source of Pb. Evidently strict analytical control of trace elements levels in wine is important to asses the dietary intake of essential as well as toxic elements for humans.

A plethora of substances and processes can affect the elemental composition of wine during production and packing. Unless exposed to significant airborne pollution grapes accumulate small amounts of toxic metals by translocation from the roots or by direct contact with vineyard sprays. Investigations carried out on

the migration of toxic elements in the system soilgrapevine-grape for polluted regions showed that most of the toxic elements in grapevine are mainly due to the toxic metal containing aerosols falling from the atmosphere [2]. However Orescanin et al. [3] detected V, Cr, Mn, Fe, Ni, Cu, Zn, As and Pb in soil, grape and wine and concluded that the main source of heavy metals in grapes is absorption from the soil. Trace elements are normally absorbed onto the yeast cell and are removed from the final product during the preformentation clarification [4].

Sources of lead in wine were inferred from systematic assay of grapes must and wine during winemaking. It was found that Pb concentration in fermenting must vary during vinification. Lead concentration increased significantly in open-top vessels, in holding bins, and during pressing. Juice and wine stored in concrete or waxed wood have significantly higher concentration of lead compared to juice and wine stored in stainless steel.

Moreover fining with bentonite or filtering with diatomaceous earth contributes further to final Pb concentration, while fermentation, both primary and secondary, removed Pb [5]. In another study measurements of 7000 wines were used to identify possible sources of Pb in wine and these showed that atmospheric–related contamination (leaded gasoline) was not responsible for elevated Pb levels in wine. It was also shown that the presence or absence of tin-lead capsules as well as the stare of tin-lead capsule corrosion had only a very minor influence on the Pb concentration in wine. It was concluded that brass is the main contamination source for elevated Pb content in wine [6].

The main sources of cadmium pollution are the nonferrous metal production, waste incineration, phosphate fertilizer manufacture, wood, coal, oil and gasoline combustion, iron and steel production, industrial Cd applications and nonferrous metals mining. In industrialized areas, Cd in air varies from 1 to 50 ng/m^3 *vs*. 0.1 to 6 ng/m^3 in rural air. Cadmium lands on agricultural soil via air deposition, phosphate fertilizers, sludge application and via liquid effluents and solid wastes from Cd-processing plants. In highly contaminated soils, Cd level

may reach values of up to 800 µg/g, while the least polluted soils contain 0.2 to 0.6 µg/g.

The residence time of Cd in soils is up to 300 years [7].

Widely consumed wine could contribute an important fraction of the dietary intake of Cd. The maximum allowable concentration of Cd in drinking water is 5 µg/L, whereas for wine this concentration limit is 10 µg/L according to Roumanian legislation [8]. Therefore, accurate and reliable control of the whole wine making process is required.

The determination of heavy metals in beverages can be investigated by spectrometric techniques, in both absorption and emission [9, 10] and by electroanalytical techniques [11, 12, 13]. Due to the high sensitivity, selectivity, and easy operation, graphite furnace atomic absorption spectrometry (GFAAS) has frequently been used for the determination of cadmium and lead in foods.

The implementation of a quality system in analytical laboratories, based on 17025, is now a reality. The requirements of this standard deeply modified the organization of the laboratories, whereas it also improved the quality of the analytical results [14].

One of the quality control/quality assurance requirements in residue analysis is method validation. The International Standardisation Organisation (ISO) definition of validation is the confirmation by examination and the provision of objective evidence that the particular requirements for a specific intended use are fulfilled [15]. The EURACHEM Guide defines method validation as the process of establishing the performance characteristics and limitations of a method and the identification of the influences which may change these characteristics and to what extent [16].

Validation studies for quantitative analytical methods typically determine some or all of the following parameters: accuracy, scope, specificity, sensitivity, precision (repeatability and reproducibility), bias, linearity, detection limit, robustness, ruggedness and selectivity.

The aim of this study was to validate GFAAS methods for the determination of lead and cadmium in wines according to Commission

Decision 2002/657/EC [17], UNI CEI EN ISO/IEC 17025/2005 [15], Commission Regulation (EC) No. 333/2007 [18] and by taking into account Commission Regulation (EC) No. 1881/2006 [19].

2. MATERIALS AND METHODS

Apparatus

For sample preparation, microwave digestion was used in a Milestone Microwave Labstation, max. 1200 W, maximum pressure 100 Bar, maximum temperature 200°C, with 75 ml TFM vessels and a 10-hole heating block. For the measurements, a SOLAAR S Atomic Absorption Spectrometer from Thermo Electron Corporation fitted with a GF95 Graphite Furnace equipped with a deuterium background corrector and a GFS97 Furnace Autosampler were used.

Reagents

High-purity water was prepared using a Basic TWF system. Nitric acid (65%, Fluka 84380), hydrochloric acid (37%, Riedel-de-Haën 30721), hydrogen peroxide (30%, Fluka 95302) and ammonium nitrate (Sigma Aldrich A 9642) were of analytical purity. Single element 1000 mg/L stock standard solutions of Cd and Pb in 2% (v/v) HNO_3 (Fluka, 1290454 and 1311730) were utilised in the study. The working solutions were freshly prepared by diluting an appropriate aliquot of the stock solutions using 10% HNO_3 for diluting lead and cadmium solutions. For the study of the interferences, a standard solution of 1000 mg/L Fe was used (Ultra Scientific, H00922).

Sample preparation and digestion

For the microwave digestion, 8 ml of wine sample were pipetted into the TFM vessels, after that 5 ml 65% nitric acid and 2 ml 30% hydrogen peroxide were added. The heating programme is given in table 1. Reagent blanks were included in each series of digestions.

Table 1. Microwave system: digestion programme

Step	1	2	3	4	5	6
Temp. (^{o}C)	80	80	120	120	200	Cooling
Power (W)	200	200	400	400	600	0
Time (min)	5	2	15	2	10	20

The vessel content was transferred to a 50 ml volumetric flask and make up to the mark with ultrapure water. The test solution was properly diluted with ultrapure water in order to attain the calibration range. Magnesium nitrate was used as a matrix modifier.

The quantity of the matrix modifier, the ash and the atomization temperature were established through optimization studies, following the maximum absorbance, using standard solutions of 50 μg/L. It was found necessary to use a relatively slow two step drying stage to ensure good precision. Operating conditions are shown in table 2.

Table 2. Instrumental conditions for lead, cadmium and tin determination by GF-AAS

Analyte	Pb	Cd
Cuvette type	Normal	Normal
Wavelength, nm	217	228.8
Bandpass, nm	0.5	0.5
Lamp Current	90%	50%
Calibration mode	Absorbtion, peak area	Absorbtion, peak area
Modifier	$Mg(NO_3)_2$	$Mg(NO_3)_2$
Background correction	D2	D2
Temperature (^{o}C)	90	90
Hold time (s)	1	1
Ramp time (^{o}C/s)	40	40
Temperature (^{o}C)	120	120
Hold time (s)	20	20
Ramp time (^{o}C/s)	1	1
Temperature (^{o}C)	450	350
Hold time (s)	20	10
Ramp time (^{o}C/s)	50	15
Temperature (^{o}C)	1600	1200
Hold time (s)	3	3
Ramp time (^{o}C/s)	0	0
Temperature (^{o}C)	2500	2500
Hold time (s)	3	3
Ramp time (^{o}C/s)	0	0

3. RESULTS AND DISCUSSION

Several parameters have been taken into account and evaluated for the in-house validation of method in wines, namely: selectivity/specificity, trueness by recovery at three level of concentration, repeatability, reproducibility, instrumental/method detection limits (LoDs) and quantification limits (LoQs), range of linearity, standard measurement

uncertainty. In this study, the definitions and procedure for validation parameters have been applied according to the EU standards for foodstuff [17, 18].

Selectivity/specificity

Both terms describe the extent to which a method uniquely reacts to a selected element. Then, selectivity studies must be performed in order to investigate the effect of potential interferent ions. We investigated the influence of iron as potential interferent for lead by measuring the absorbance of lead in a series of samples with varying concentrations of iron. The concentrations of lead were similar at low concentrations of iron. However, iron tends to decrease the absorbance of lead at high concentration.

Recovery study

Since for Cd no maximum level in wines has been established so far at EU level, the concentrations, to be considered eligible for the additions in the whole validation, were based on the sensitivity of the analytical technique. The three levels of additions were selected on the basis of the criterion indicated in CD 657/2002 as 1, 1.5 and 2 times these eligible concentrations. For lead, instead, CR No. 1881/2006 sets maximum levels in wines; thus, an addition of 0.5, 1.0 and 1.5 the maximum level was chosen. Consequently, six independent aliquots of wines were spiked with the right amount of standard solution of Pb and Cd in three different levels of concentration (µg/kg).

All samples were digested according to the pre-established MW programme and then analysed. The recovery was calculated as value observed divided by value expected.

The results of the recovery study are summarized in table 3. The recovery results fulfil the acceptable validation requirements.

Table 3. Resuts of the recovery study

Analyte	Expected value	Observed value	Recovery (%)
Pb	100 µg/L	95.611	95.61
	175 µg/L	156.651	89.51
	250 µg/L	215.024	86.01
Cd	25 µg/L	23.322	93.29
	50 µg/L	47.701	95.40
	75 µg/L	66.963	89.28

Repeatability

The repeatability was calculated as Horrat value by analysing four independent sets of samples in four different days. *HORRAT* value is the ratio of the repeatability relative standard deviation calculated from the data to the $PRSD_R$:

$$HORRAT(r) = RSD_r / PRSD_R \qquad (1)$$

Predicted relative standard deviation ($PRSD_R$) is calculated from the Horwitz formula:

$$PRSD_R = 2C^{-0.15} \qquad (2)$$

where *C* is expressed as a mass fraction.
Acceptable *HORRAT(r)* values are 0.3 - 1.3.
As for the repeatability, no changes were applied to the analytical conditions; being repeatability the precision under repeatability conditions, as stated in CR No. 333/2007 and CD No. 657/2002. In fact, repeatability conditions refer to those mean conditions where independent test results are obtained with the same method on the same sample in the same laboratory by the same operator using the same equipment in a short interval of time. The results concerning method repeatability are presented in table 4.

$HORRAT_r$ values are within the acceptable limits so we considered the repeatability parameter as validated.

Table 4. HORRAT values from intralaboratory data

Analyte	Average conc. (µg/L)	S_R	$PRSD_R$	RSD_r	$HORRAT_r$
Pb	51.004	4.448	25.034	8.7208	0.348
Cd	16.8396	0.935	29.578	5.5547	0.188

Instrumental/method detection and quantification limits

In order to calculate the instrumental/method limits of detection and quantification (LoDs and LoQs), the standard 3σ and 10σ approach were employed. A number of 20 reagents blanks/digested wine were prepared and analysed. The reagent blanks were used for the calculation of instrumental LoDs and LoQs, while digested wine was used for the

calculation of method LoDs and LoQs. For method LoDs and LoQs, final dilution and weight were taken into account to calculate the final values (table 6).

Requirements for LoD and LoQ are set in Table 5 of CR No. 333/2007 for those elements for which a maximum level has been set. For example, as regards LoQ, it says: "For inorganic tin less than 10 mg kg^{-1}. For other elements less than one fifth of the maximum level in Regulation (EC) No. 1881/2006, except if the maximum level for lead is less than 100 μg kg^{-1}. For the latter, less than two fifth of the maximum level".

LoD and LoQ for the two analytes fulfil the requirements of CR No. 333/2007.

Table 6. Instrumental and methods LoDs and LoQs

Parameters	Pb	Cd
Instrumental LoD	0.61 μg/L	0.16 μg/L
Instrumental LoQ	2.28 μg/L	0.56 μg/L
Method LoD	13.34 μg/L	2.80 μg/L
Method LoQ	26.68 μg/L	5.60 μg/L

Range of linearity and calibration curve

This parameter was evaluated by checking the linear regression coefficient (r^2) of a calibration curve constructed with 5 standard solutions. The linearity of the calibration curve was considered acceptable when $r^2 > 0.999$. In the whole validation, the calibration curve for the measurements was always prepared with at least five points (blank not included), as recommended by CD No. 657/2002. The results are presented in table 7.

Table 7. Range of linearity and r^2 values

Analyte	Range of linearity, μg/L	Correlation coefficients, r^2
Pb	2,5…20	0,9990-0,9996
Cd	1,0….4,0	0,9991-0,9995

Measurement uncertainty

A laboratory has to demonstrate the quality of the results produced and its fitness for purpose by giving a measure of the confidence that can be placed on the result. All possible sources of uncertainty have to be carefully identified. When the major contributions are detected, a good estimate of the measurement uncertainty can be made by concentrating effort on the largest and most significant contributions. Afterwards, the uncertainty components are quantified and the combined uncertainty is calculated [20].

In this study, the following contributions to combined measurement uncertainty were selected: the preparation of the standard solutions (u_f), the standard uncertainty associated to the recovery (u_{rec}) and the within-laboratory reproducibility of the measurements (u_m). Then, the combined uncertainty (u_C) was calculated as the square sum of the three contributes:

$$u_C \, / \, C = \sqrt{u_m^2 + u_{rec}^2 + u_f^2} \qquad (3)$$

While, the expanded measurement uncertainty (U) was:

$$U = u_C(x)k \qquad (4)$$

where k is the coverage factor of 2, which considers a normal distribution of measurements with a 95% confidence level.

The expanded standard measurement uncertainty, expressed as percentage was estimated according to the adopted procedure as follows: Pb, 18.4 % and Cd, 13 %.

4. CONCLUSIONS

The validated methods presented in this paper show to meet the performance criteria and the requirements set in the regulations of the European Union for method validation to be used in official food control. Furthermore, the present methods offer satisfactory detection limits due to the powerful spectrometric analytical technique employed and provide precise and accurate for determination of Pb and Cd in wines.

5. REFERENCES

[1] Pedersen G. A., Mortensen G. K., Larsen E. H., Beverages as a source of toxic trace-element intake. Food Addit. Contam., 1994: 11: 351-363.

[2] Angelova V. R., Ivanov A. S., Braikov D. M., Heavy metals (Pb, Cu, Zn and Cd) in the system soil-grapevine-grape. J. Sci. Food Agric., 1999: 79: 713-721.

[3] Orescanin V., Katunar A., Kutle A., Valkovic V., Heavy metals in soil, grape, and wine. J. Trace Microprobe Tech., 2003: 21: 171-180.

[4] Garrido J., Ayestaran B., Fraile P., Ancin C., Influence of prefermentation clarification on heavy metal lability in Garnacha must and rose wine using differential pulse anodic stripping voltammetry. J. Agric. Food Chem., 1997: 45: 2843-2848.

[5] Stockley C. S., Smith L. H., Tiller K. G., Gulson B. L., Osborn C. D., Lee T. H., Lead in wine: a case study on two varieties at two wineries in South Australia. Aust. J. Grape Wine Res., 2003: 9: 47-55.

[6] Kaufmann A., Lead in wine. Food Addit. Contam., 1998: 15: 437-445.

[7] Cvetkovic J., Arpadjan S., Karadjova I., Stafilov T. Determination of cadmium in wine by electrothermal atomic absorption spectrometry. Acta Pharm., 2006: 56: 69-77.

[8] *** Hotărâre nr. 1.134 din 10 octombrie 2002 pentru aprobarea Normelor metodologice de aplicare a Legii viei şi vinului în sistemul organizării comune a pieţei vitivinicole, nr. 244/2002.

[9] Tahvonen R., Lead and cadmium in beverages consumed in Finland. Food Additives and Contaminants, 1998: 15: 446-450.

[10] Zbinden P., Andrey D., Determination of trace element contaminants in food matrices using a robust, routine analytical method for inductively coupled plasma-mass spectrometry (ICP-MS). Atomic Spectroscopy, 1998: 19: 214-219.

[11] Baldo M.A., Bragato C., Daniele S., Determination of lead and copper in wine by anodic stripping voltammetry with mercury microelectrodes: assessment of influence of sample pretreatment procedures. The Analyst, 1997: 122: 1-5.

[12] Fomintseva E.E., Zakharova E.A., Pikula P., Determination of tin and lead in canned juices and fruit by stripping voltammetry. Journal of Analytical Chemistry, 1997: 52: 590-594.

[13] Mannino S., Simultaneous determination of lead and tin in fruit juices and soft drinks by potentiometric stripping analysis. The Analyst, 1983: 108: 1257-1260.

[14] Feinberg M., Laurentie M., A global approach to method validation and measurement uncertainty. Accreditation and Quality Assurance, 2006: 11: 3-9.

[15] ISO/IEC 17025, General requirements for the competence of testing and calibration laboratories. International Standardisation Organisation, Geneva, Switzerland, 1999.

[16] EURACHEM GUIDE, The fitness for purpose of analytical methods. A laboratory guide to method validation and related topics, 1998, http://www.eurachem.ul.pt/guides/mval.htm

[17] Commission Decision 2002/657/EC of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results.

[18] Commission Regulation (EC) No. 333/2007 of 28 March 2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs.

[19] Commission Regulation (EC) No. 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs.

[20] EURACHEM/CITAC, Guide quantifying uncertainty in analytical measurements, 2nd edn., 2000, http://www.measurementuncertainty.org