

Analytical, Nutritional and Clinical Methods

Direct determination of copper, lead and cadmium in aniseed spirits by electrothermal atomic absorption spectrometry

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Abstract

Direct determination of copper, lead and cadmium by electrothermal atomic absorption spectrometry in aniseed spirits was proposed. The methods were validated by studying quality parameters such as trueness, precision, linearity and sensitivity. Recoveries ranging between 96% and 104% were obtained from spiked samples at several concentration levels. Repeatability was less than 5% and intermediate precision was less than 8%. The procedure is fast and shows limits of detection of 0.6, 0.7 and 0.04 $\mu\text{g l}^{-1}$ for Cu, Pb and Cd, respectively. Samples were just diluted in a water/ethanol/nitric acid mixture. Cu was present at higher concentrations, with values in the range 6–473 $\mu\text{g l}^{-1}$. Minor levels of Pb and Cd were present with concentrations of less than 6 and 1.4 $\mu\text{g l}^{-1}$, respectively.

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1. Introduction

The determination of heavy metals in environmental, biological and food samples has drawn significant attention due to the toxic and nutritional effects of these elements or their compounds (González, Martínez, & Aguilar, 1998; Goyer & Clarkson, 2001). Copper is widely distributed in nature and is an essential trace element for humans (Goyer & Clarkson, 2001; Turnlund, 1998). Cu functions as a cofactor and is required for structural and catalytic properties of a variety of important enzymes, including cytochrome *c* oxidase, tyrosinase, *p*-hydroxyphenylpyruvate hydrolase and dopamine β hydroxylase (Gaetke & Chow, 2003). Nonetheless, Cu shows some toxicological effects. Food, beverages and drinking water are potential sources of excess exposure. Daily intake of Cu in adults varies between 0.9 and

2.2 mg. The amount of Cu ingested is relatively low, and most humans are able to control excess amounts of Cu in the body by either decreased absorption or increased excretion. Ingestion of drinking water with concentrations higher than 3 mg l^{-1} can produce gastrointestinal symptoms (Pizarrro et al., 1999). Exposure to elevated concentrations of Cu primarily affects the liver and is typically manifested by the development of liver cirrhosis, episodes of hemolysis and damage to renal tubules, brain and other organs (Goyer & Clarkson, 2001; Winge & Mehra, 1990). Pb and Cd can be accumulated in biological systems becoming potential contaminants along the alimentary chain. These elements produce harmful effects on the human health, affecting to several organ systems, such as nervous, gastrointestinal, reproductive and skeletal, and biochemical activities (Goyer & Clarkson, 2001; Hsu & Guo, 2002; Kim, 2004).

Aniseed spirits are produced by the distillation of pressed fermented grapes, dregs and other fermented saccharate raw materials, flavoured with aniseed-type

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plants, mainly star anise, green anise or fennel (Council Regulation, 1989). They are elaborated in many countries with different denominations like sambuca in Italy, ouzo in Greece, pastis in France, anis in Spain. Production of this kind of beverages normally is a batch process using bronze or copper pot still. Part of the metal content present in these beverages is attributed to the production process and another to the flavouring plants and combined use of metallic pot stills (Moutsatsou, Chalarakis, & Zarangas, 2003). As a consequence some heavy metals such as copper, lead and cadmium can be present in aniseed drinks (Fernández, 1988; Moutsatsou et al., 2003). These elements are usually present at low levels in most alcoholic beverages (Kaufman, 1998; Mena, Cabrera, Lorenzo, & López, 1996; Moutsatsou et al., 2003) and sensitive analytical techniques are required for their determination. Several authors have determined copper in wine, beer and other alcoholic beverages by using flame atomic absorption spectrometry (FAAS) (Cameán, Moreno, López-Artiguez, Repetto, & González, 2001; Frias, Conde, Rodríguez-Bencomo, García-Montelongo, & Pérez-Trujillo, 2003; González et al., 1998; Soufleros, Mygdalia, & Natskoulis, 2004), inductively coupled plasma optical emission spectrometry (ICP-OES) (Alcázar, Pablos, Martín, & González, 2002; Jos, Moreno, González, López-Artiguez, & Cameán, 2004; Matsushige & Oliveira, 1993), total reflection X-ray spectrometry (Galani-Nikolakaki, Kallithrakas-Kontos, & Katsanos, 2002), voltammetry and ion chromatography (Buldini, Cavalli, & Sharma, 1999). Lead has been determined by flow injection hydride generation AAS (Mena, Cabrera, Lorenzo, & López, 1997), ICP mass spectrometry (ICP-MS) (Almeida & Vasconcelos, 2003). Voltammetry and ion chromatography (Buldini et al., 1999) have also been used for determining lead and cadmium. Electrothermal atomic absorption spectrometry (ET-AAS) is suitable and widely used for the determination of lead and cadmium in wines (Almeida & Vasconcelos, 2003; Buldini et al., 1999; Freschi, Dakuzaku, Moraes, Nóbrega, & Gomes Neto, 2001; Galani-Nikolakaki et al., 2002; Kim, 2004; Kristl, Veber, & Slekovec, 2002) and other alcoholic beverages like brandy (Cameán et al., 2001) and cava (Jos et al., 2004). Several authors (Butcher & Sneddon, 1998; Freschi et al., 2001; Welz & Sperling, 1999) have proposed ET-AAS for direct determination of lead and cadmium in various matrices. This is particularly advantageous because a minimum sample preparation is required leading to a saving of time and reducing the possibilities of cross-contamination.

This work reports simple and fast methods for the direct determination of copper, lead and cadmium in aniseed spirits by using ET-AAS. Since there are almost no data in the literature dealing with ET-AAS determination of these metals in such samples, our aim was to find suitable chemical modifiers and furnace conditions to suppress the interferences associated to the elevated organic content present in the sample matrices.

2. Materials and methods

2.1. Apparatus

Measurements were carried out using a Perkin–Elmer 1100B atomic absorption spectrometer with deuterium background correction equipped with a HGA-400 graphite furnace atomiser and an AS-40 autosampler. Instrumental and graphite furnace conditions are summarized in Table 1.

2.2. Reagents and solutions

Chemicals were of ultrapure grade, and ultrapure water (18.2 MΩ cm), obtained from a Milli-Q water purification system (Millipore, Bedford, MA), was used throughout. Stock standard solutions (1 g l⁻¹) of copper, cadmium and lead were supplied by Merck (Merck, Darmstadt, Germany). Working solutions were obtained by appropriate dilution of the standard ones. Pd(NO₃)₂ (SCP Science, NY, USA), 20 g l⁻¹ in Pd, and Mg(NO₃)₂ (Merck), 4 g l⁻¹ in Mg, stock solutions were used to prepare chemical modifiers. Nitric acid 69% (Merck) and absolute ethanol (Panreac, Barcelona, Spain) were also used for

Table 1
Graphite furnace conditions^a for the determination of Cu, Pb and Cd in aniseed spirits by ET-AAS

Operating conditions	Cu	Pb	Cd
Lamp	HCL	HCL	EDL
Wavelength (nm)	324.8	283.3	228.8
Slit width (nm)	0.7	0.7	0.7
Injection volume (μl)	20	20	20
D ₂ background correction	On	On	On

Graphite furnace-temperature programs

	Step	Temperature (°C)	Ramp (s)	Hold (s)	Ar flow rate (ml min ⁻¹)
Cu	Drying 1	80	5	15	300
	Drying 2	110	10	20	300
	Pyrolysis	1300	10	15	300
	Atomisation	2300	0	3	0 (READ)
	Cleaning	2500	2	3	300
Pb	Drying 1	80	5	15	300
	Drying 2	110	10	20	300
	Pyrolysis	900	10	15	300
	Atomisation	1800	0	3	0 (READ)
	Cleaning	2500	2	3	300
Cd	Drying 1	80	5	15	300
	Drying 2	110	10	20	300
	Pyrolysis	700	10	30	300
	Atomisation	1500	0	3	0 (READ)
	Cleaning	2500	2	3	300

HCL, hollow cathode lamp; EDL, electrodeless discharge lamp.

^a Pyrocoated graphite tubes with L'vov platforms, integrated absorbance measurement.

diluting the samples. Argon N50, with 99.999% purity, was used as sheath gas for the atomiser and to purge internally. All glassware were kept in 10% nitric acid for at least 48 h and rinsed with ultrapure water before use.

2.3. Samples

Samples of aniseed spirits were obtained from liquor retailers and markets. All previous experiments were carried out using a control sample prepared by mixing the content of several bottles. Due to the high organic content in these beverages and to prevent the formation of emulsions, samples were diluted with a water/ethanol/nitric acid (58:40:2) mixture after the addition of the appropriate amount of chemical modifier. Therefore, samples were diluted by a factor 1:10 when they were injected into the graphite furnace.

3. Results and discussion

3.1. Optimization of the furnace conditions

3.1.1. Drying

As it was mentioned in the previous section, samples were diluted in a water/ethanol/nitric acid solution. Due to the high content in ethanol of the diluted samples, a double drying step was necessary. In the first one, the temperature was set at 80 °C to assure a slowly alcohol evaporation. In the second step, a 110 °C temperature was set in order to evaporate the water present in the sample. Several experiments were carried out to establish the optimum ramp and hold times to assure the complete dryness of

the samples before the pyrolysis step. The corresponding values for the determination of each element are shown in Table 1.

3.1.2. Pyrolysis

Considering the high content of organic matter in the samples and to obtain a complete mineralization, the conditions used in the pyrolysis step were optimised. Mineralization curves were obtained for each studied element. Due to the low content of cadmium and lead in the samples, the study for these elements was carried out using a spiked control sample, with a final content of 3, 5 and 2 $\mu\text{g l}^{-1}$ of Cu, Pb and Cd, respectively. Pyrolysis temperature was changed setting the atomisation temperature according to the specifications of the manufacturer of the instrument (Perkin–Elmer, 1984), 2300, 1800 and 1600 °C for Cu, Pb and Cd, respectively. Figs. 1–3 show the obtained curves in presence of several matrix modifiers, namely $\text{Mg}(\text{NO}_3)_2$, $\text{Pd}(\text{NO}_3)_2$ and a mixture of $\text{Mg}(\text{NO}_3)_2$ and $\text{Pd}(\text{NO}_3)_2$. Adequate volumes of the solutions of these compounds were added to obtain final concentrations of 43 mg l^{-1} of $\text{Pd}(\text{NO}_3)_2$ and 25 mg l^{-1} of $\text{Mg}(\text{NO}_3)_2$.

In the case of Cu, a constant signal was obtained in the interval 1000–1400 °C. At higher temperatures the analytical signal decreases. In order to obtain a good mineralization of the sample in the cited temperature interval, the pyrolysis temperature was set at 1300 °C. At a first glance, no significant differences were observed between the curves obtained with the different modifiers tested.

Fig. 2 shows the influence of the pyrolysis temperature on Pb signal. With no chemical modifier and using magnesium nitrate the signal decreased continuously, since the

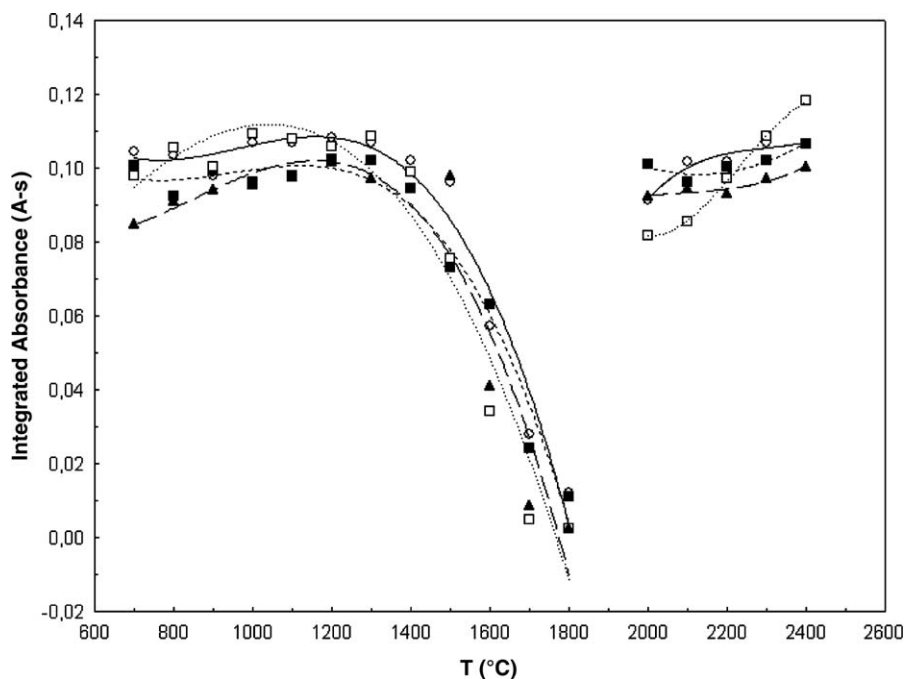


Fig. 1. Influence of pyrolysis (left) and atomisation (right) temperatures for Cu determination. 10% (v/v) aniseed drink with 3 $\mu\text{g l}^{-1}$ of Cu. Matrix modifier: magnesium nitrate, 25 mg l^{-1} , (■); palladium nitrate, 43 mg l^{-1} (□); Pd–Mg nitrates, 43–25 mg l^{-1} , respectively (▲); no matrix modifier (○).

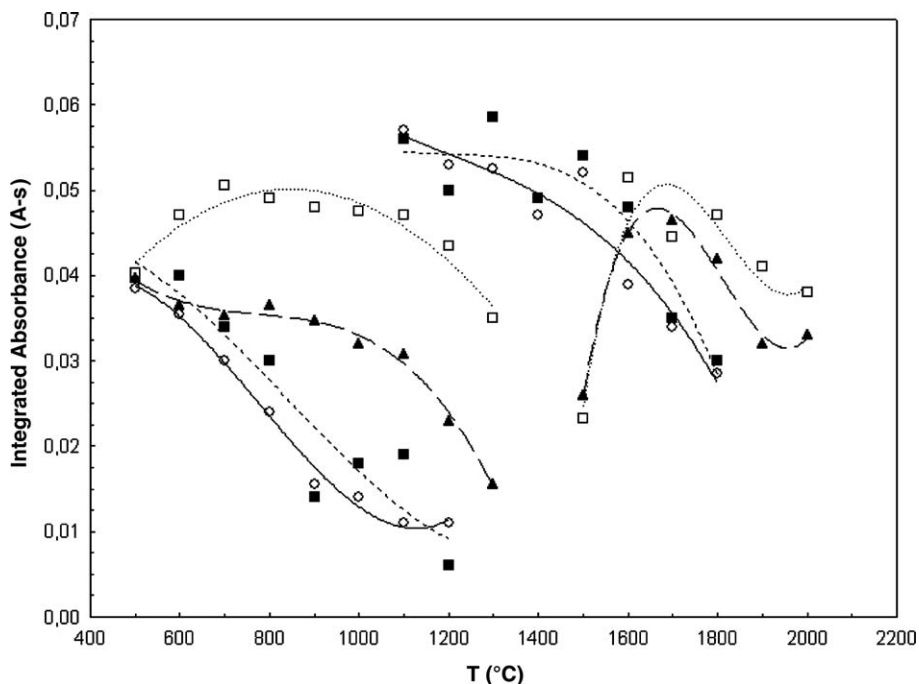


Fig. 2. Influence of pyrolysis (left) and atomisation (right) temperatures for Pb determination. 10% (v/v) aniseed drink spiked with $5 \mu\text{g l}^{-1}$ of Pb. Matrix modifier: magnesium nitrate, 25 mg l^{-1} , (■); palladium nitrate, 43 mg l^{-1} (□); Pd–Mg nitrates, $43\text{--}25 \text{ mg l}^{-1}$, respectively (▲); no matrix modifier (○).

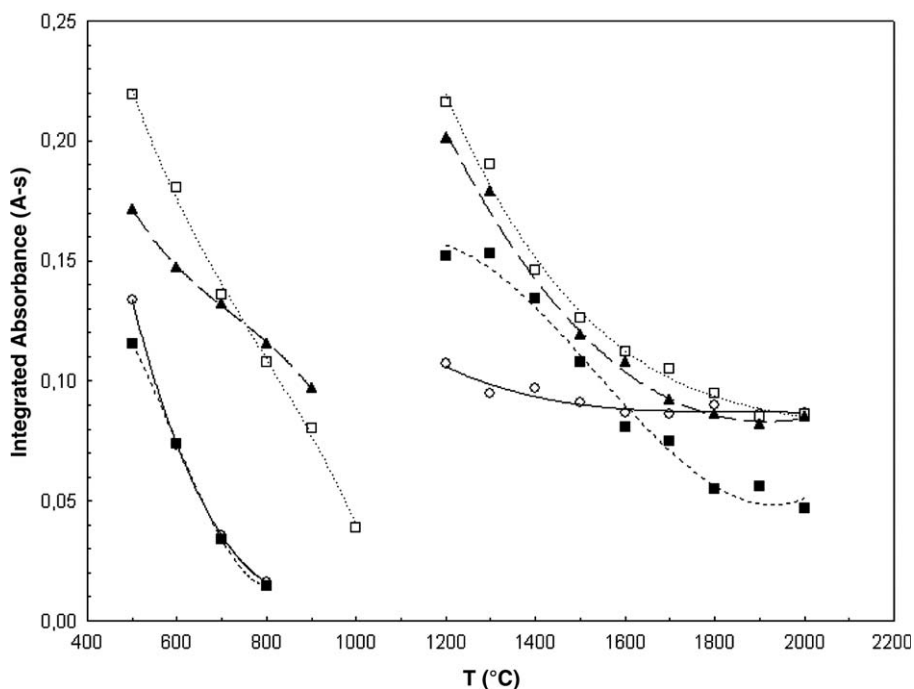


Fig. 3. Influence of pyrolysis (left) and atomisation (right) temperatures for Cd determination. 10% (v/v) aniseed drink spiked with $2 \mu\text{g l}^{-1}$ of Cd. Matrix modifier: magnesium nitrate, 25 mg l^{-1} , (■); palladium nitrate, 43 mg l^{-1} (□); Pd–Mg nitrates, $43\text{--}25 \text{ mg l}^{-1}$, respectively (▲); no matrix modifier (○).

first pyrolysis temperature studied which was $500 \text{ }^{\circ}\text{C}$. For palladium modifier a constant signal was obtained between the interval 700 and $1100 \text{ }^{\circ}\text{C}$. When using Pd–Mg modifier, at $1100 \text{ }^{\circ}\text{C}$ the analytical signal begins to decrease. A temperature of $900 \text{ }^{\circ}\text{C}$ was selected as pyrolysis temperature to avoid possible losses of analyte.

It can be observed in Fig. 3 that the absorbance of Cd was higher when palladium nitrate and palladium magnesium nitrates were used as chemical modifiers. The analytical signal of Cd showed a continuous decreasing when pyrolysis temperature was raised, but the background signal was lower at $700 \text{ }^{\circ}\text{C}$ with a hold time of 30 s (Fig. 4).

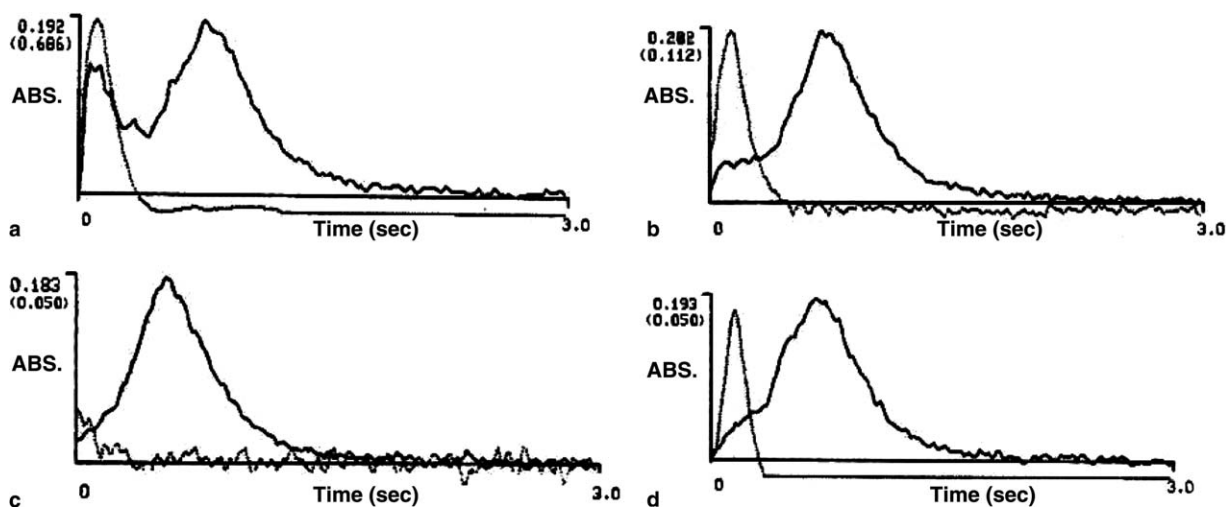


Fig. 4. Influence of temperature and hold time of pyrolysis on the peak profile of Cd. 10% (v/v) aniseed drink spiked with $2 \mu\text{g l}^{-1}$ of Cd. Pyrolysis temperature ($^{\circ}\text{C}$)/hold time (s): (a) 500/30, (b) 600/30, (c) 700/30 and (d) 700/15. Atomisation temperature, 1600°C . Matrix modifier: Pd (43 mg l^{-1}) and Mg (25 mg l^{-1}) nitrates. Scale of the background signal (dotted line) in brackets.

High background signals lead to an overestimation of the peak area, then, in order to avoid this effect, the optimum pyrolysis temperature and hold time were fixed at 700°C and 30 s, respectively.

Under these conditions the presence of carbonaceous residues after several firings were minimized.

3.1.3. Atomisation

Once the optimum conditions for the pyrolysis step were fixed, the atomisation curves for Cu, Pb and Cd were obtained. Figs. 1–3 show the atomisation curves for Cu, Pb and Cd, respectively. For Cu the pyrolysis temperature used for all the experiments was 1300°C . In the case of Pb, the pyrolysis temperatures used for atomisation studies were: 600°C for the curves obtained with Mg as modifier and without modifier, 1100°C for Pd and 900°C for Pd–Mg modifiers. For Cd the pyrolysis temperatures were 500°C for Mg modifier or without modifier, and 700°C for Pd and Pd–Mg modifiers. The atomisation temperatures were set by taking into account the highest peak areas, the narrowness of absorbance profiles and the repeatability of the measurements. For Cu, the highest signals were obtained between 2300 and 2400°C . Considering the absorbance profiles (Fig. 5) and signal repeatability, the atomisation temperature was fixed at 2300°C . In the case of Pb, very bad repeatability was obtained when palladium was not used as chemical modifier. Consequently, in presence of this chemical modifier and taking into account the peak shape (Fig. 5) the atomisation temperature for Pb was set at 1800°C . For Cd determination higher signals and narrower peaks (Fig. 5) were also obtained in presence of palladium and at 1500°C .

3.1.4. Chemical modifiers

Due to the role of magnesium and palladium nitrates as universal chemical modifiers (Schlemmer & Welz, 1986;

Welz, Schlemmer, & Mudakavi, 1992), they were considered for the determination of Cu, Pb and Cd. The influence of different amounts of modifier was tested. Fig. 6 shows the plot of the analytical signal against the amount of modifier. In the case of Cu, there is no almost influence of the magnesium nitrate concentration on the signal. The highest value was obtained with a palladium concentration of 30 mg l^{-1} . The analytical signals obtained for lead and cadmium determination were very low when palladium was not present. The maximum signal of Pb was obtained with 30 mg l^{-1} of palladium and 10 mg l^{-1} of magnesium nitrate. A similar behaviour is observed for Cd, but, in this case, the optimum response was obtained at 40 mg l^{-1} of magnesium nitrate and palladium.

3.2. Analytical performance

3.2.1. Matrix effect and calibration

In order to check the presence of matrix effect, external and standard addition calibrations were performed. To avoid differences in the behaviour of the analyte present in the sample and the added one, samples prepared for standard addition measurements were spiked 24 h before the analysis. This fact allows interactions between analytes and the components of the sample matrix. The obtained results are present in Table 2. Student *t*-test was applied to compare the slopes (Miller & Miller, 2000). For Cu and Pb, at a 95.0% confidence level, there is no significant difference between the slopes of the external and addition standard calibration lines. Then it can be concluded that the matrix effect is not significant and the quantification of Cu and Pb in aniseed samples can be carried out by using external calibration. On the other hand, significant difference was detected between external and standard addition slopes for Cd. Therefore, in this case there is significant matrix effect and, consequently, quantification was

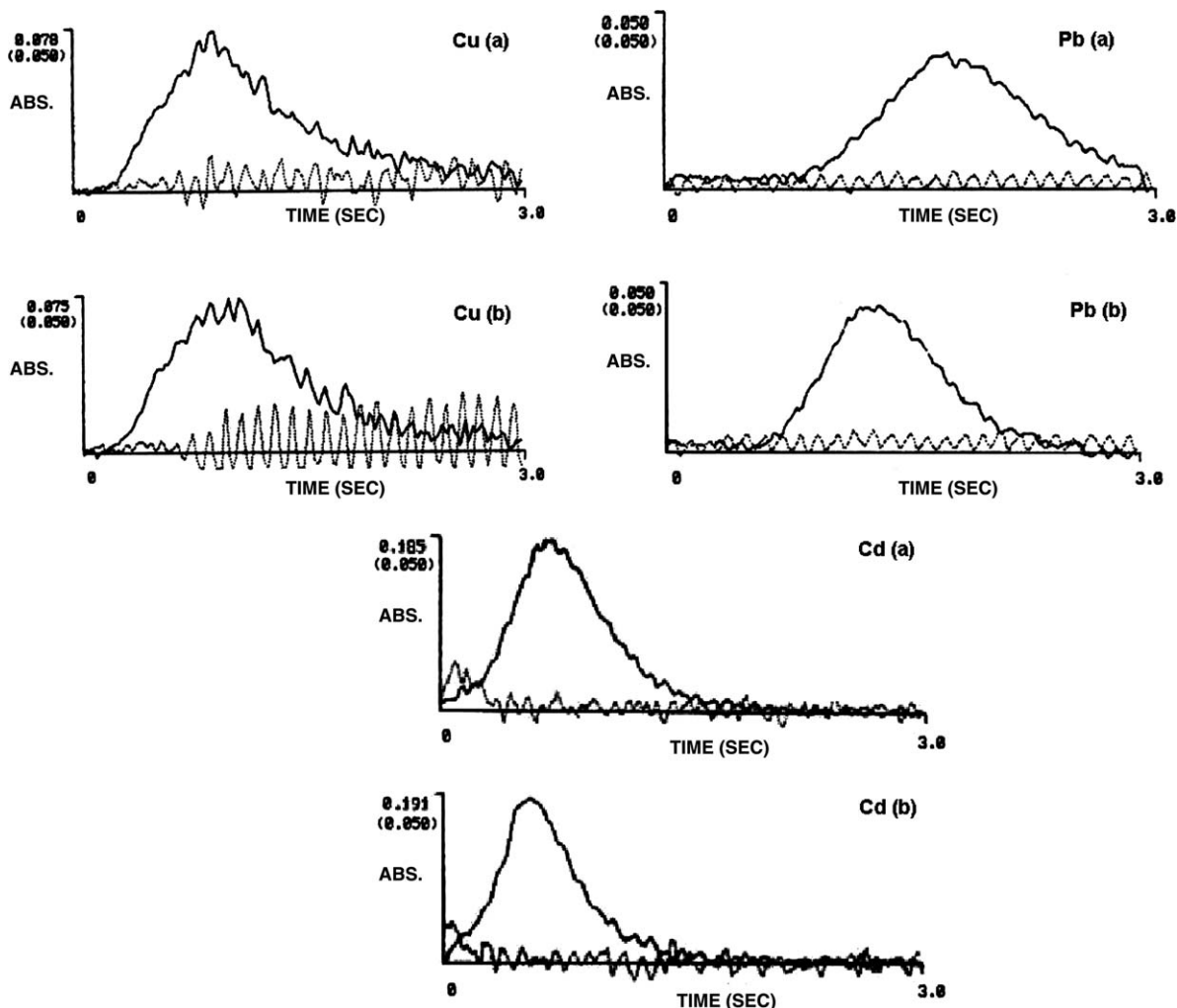


Fig. 5. Absorption peak profile of Cu, Pb and Cd. Atomisation temperature ($^{\circ}\text{C}$): Cu (a) 2300, Cu (b) 2400; Pb (a) 1700, Pb (b) 1800; Cd (a) 1400, Cd (b) 1500. 10% (v/v) aniseed drink with 3, 5 and $2\ \mu\text{g l}^{-1}$ of Cu, Pb and Cd, respectively. Matrix modifier: Pd ($43\ \text{mg l}^{-1}$) and Mg ($25\ \text{mg l}^{-1}$) nitrates. Scale of the background signal (dotted line) in brackets.

performed by using the standard addition method. Linear range and linearity (Rodríguez, Campaña, & Sendra, 1996) were also studied. The intervals of linear range are presented in Table 2. Linearity, calculated as $(1 - s_b/b)100$, was 97.7% in all cases.

3.2.2. Trueness

Taking into account that there is not CRM available for this kind of samples, recovery assays were carried out for trueness study purposes (González, Herrador, & Asuero, 1999; Maroto, Boqué, Riu, & Rius, 2001). A control sample was fortified at i levels ($i = 5$) of concentration and was analysed in j replicates ($j = 5$). Fortification to obtain concentrations in sample between the interval $10\text{--}50\ \mu\text{g l}^{-1}$ for Cu and Pb and $2\text{--}10\ \mu\text{g l}^{-1}$ in the case of Cd was performed. Once the elemental recovery for each ij th 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. One-way ANOVA was performed to find out possible bias contribution due to between levels variance. In order to

detect the level that introduced significant differences, a Tukey-HSD test was applied (Rafter, Abell, & Braselton, 2002). After ANOVA calculations were performed, significant bias was detected due to fortification levels 1 and 2 (10 and $20\ \mu\text{g l}^{-1}$) for Pb analysis and level 1 ($2\ \mu\text{g l}^{-1}$) in the case of Cd. No significant bias was detected for Cu.

The uncertainty for each \bar{R}_i value, $u(\bar{R}_i)$, was computed as the standard deviation of the j replication measurements. The uncertainty corresponding to \bar{R} consists of two contributions, a combined uncertainty coming from the calculation of \bar{R} and the pure repetition uncertainty (Jurado, Alcázar, Pablos, Martín, & González, 2004). Finally, uncertainty (u) was converted into the expanded one (U) multiplying by a coverage factor $k = 2$ (EURACHEM/CITAC Guide, 2000).

Recoveries of each fortification level, \bar{R}_i , and the global one, \bar{R} , with their expanded uncertainties for the studied elements are shown in Table 3. As can be seen, the confidence interval for each level and the global one include the 100% for Cu, Pb and Cd. Accordingly, and taking into

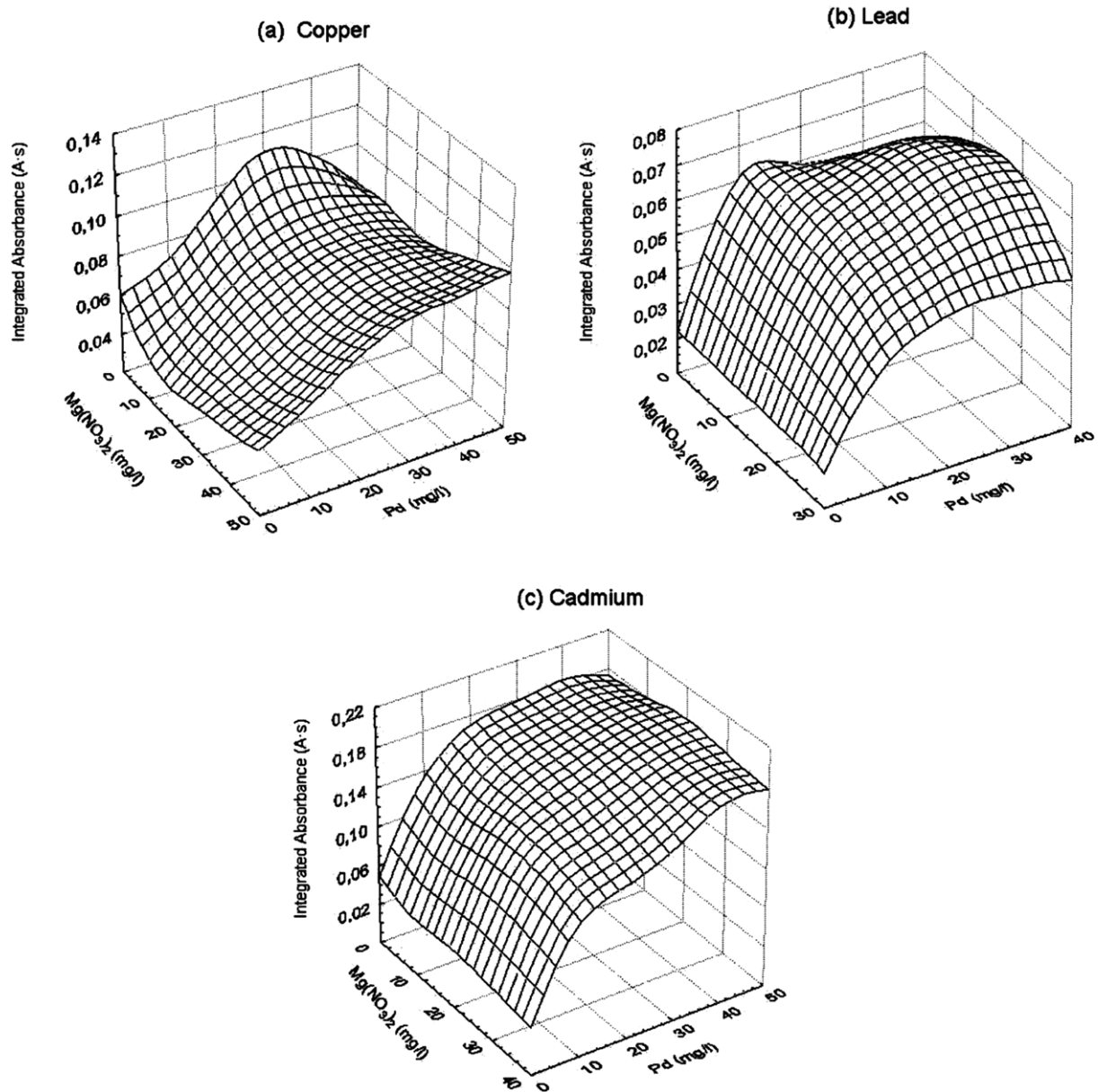


Fig. 6. Influence of the amount of matrix modifier. 10% (v/v) aniseed drinks with 3, 5 and 2 $\mu\text{g l}^{-1}$ of Cu, Pb and Cd, respectively. See furnace conditions in Table 1.

Table 2
External and standard addition calibration parameters and linear range

Element	External	Standard addition	Linear range ($\mu\text{g l}^{-1}$)
Cu	$b = 0.012 \pm 0.004$ $a = -0.001 \pm 0.003$ $r = 0.9993$	$b = 0.012 \pm 0.003$ $a = 0.040 \pm 0.006$ $r = 0.9990$	2.1–10
Pb	$b = 0.007 \pm 0.001$ $a = 0.000 \pm 0.003$ $r = 0.9993$	$b = 0.007 \pm 0.001$ $a = 0.004 \pm 0.002$ $r = 0.9984$	2.3–10
Cd	$b = 0.11 \pm 0.02$ $a = -0.003 \pm 0.004$ $r = 0.9993$	$b = 0.08 \pm 0.01$ $a = 0.005 \pm 0.002$ $r = 0.9990$	0.13–1

b , slope; a , intercept; r , correlation coefficient.

Table 3
Recoveries^a of Cu, Pb and Cd in aniseed spirits

Level	Cu	Pb	Cd
1	104 \pm 13	–	–
2	104 \pm 14	–	99 \pm 9
3	96 \pm 9	100 \pm 14	98 \pm 4
4	102 \pm 5	102 \pm 15	101 \pm 3
5	100 \pm 6	100 \pm 5	100 \pm 3
Global	101 \pm 5	101 \pm 7	101 \pm 3

^a in % \pm expanded uncertainty.

account the ANOVA results, trueness can be assured over all the studied range for Cu, from 30 to 50 $\mu\text{g l}^{-1}$ for Pb and 4 to 10 $\mu\text{g l}^{-1}$ for Cd.

Table 4
Precision and sensitivity parameters

Element	RSD _{repeat} (%)	RSD _{ip} (%)	LOD ($\mu\text{g l}^{-1}$)	LOQ ($\mu\text{g l}^{-1}$)	m_0 (pg)
Cu	2.85	5.96	0.6	2.1	8.4 ± 0.2
Pb	3.95	4.23	0.7	2.3	13.2 ± 0.3
Cd	5.08	8.41	0.04	0.13	0.89 ± 0.03

RSD_{repeat}, repeatability; RSD_{ip}, intermediate precision; LOD, limit of detection; LOQ, limit of quantification; m_0 , characteristic mass.

3.2.3. Precision

Repeatability and intermediate precision were evaluated for the three studied elements. Repeatability was obtained by analysing 10 times a control sample in a short period of time, without changing any operational condition. The relative standard deviation in repeatability conditions (RSD_{repeat}) was calculated. On the other hand, by analysing the same control sample in intermediate precision conditions, the relative standard deviation (RSD_{ip}) was evaluated. The results obtained for each element are shown in Table 4. As can be seen, in all cases the RSD of repeatability and intermediate precision are under the value recommended by the AOAC (21–30%) for concentration levels present in the analysed samples (AOAC, 1993).

3.2.4. Sensitivity

Limit of detection (LOD) and limit of quantification (LOQ) were calculated as $3S_{\text{blank}}/b$ and $10S_{\text{blank}}/b$, respectively (Miller & Miller, 2000). S_{blank} is the standard deviation obtained from 10 measurements of the blank and b is the slope of the calibration line used for the element quantification. Blank was prepared by mixing diluting solution and matrix modifier. On the other hand, the characteristic mass (m_0) was evaluated as:

$$m_0 = \frac{0.0044CV}{A_{ic} - A_{ib}}$$

where C is the analyte concentration in a standard, V is the injected volume, A_{ic} is the absorbance of the standard and A_{ib} is the absorbance of the blank. A characteristic mass was calculated at each concentration level in the external calibration curve and an average value was obtained (Ure, Butler, L'vov, Rubeska, & Sturgeon, 1992). Limit of detection and quantification, as well as the characteristic mass, calculated for the three studied elements, are shown in Table 4. The obtained characteristic masses were consistent with the manufacturer's specifications (Perkin-Elmer, 1984). Considering the LOD values and the dilution factor (1:10) of the samples, the minimum detectable amounts in sample were 6.0, 7.0 and $0.4 \mu\text{g l}^{-1}$ for Cu, Pb and Cd, respectively.

3.3. Determination of copper, lead and cadmium in aniseed spirits

The content of Cu, Pb and Cd was determined in 26 samples of commercial aniseed spirits by using the pro-

Table 5
Concentrations^a ($\mu\text{g l}^{-1}$) of Cu, Pb and Cd in aniseed spirits

Sample	Cu	Pb	Cd
1	n.d.	n.d.	n.d.
2	27.4 ± 0.9	n.d.	n.d.
3	13 ± 2	n.d.	n.d.
4	147 ± 7	n.d.	n.d.
5	56 ± 1	n.d.	n.d.
6	16 ± 1	n.d.	n.d.
7	108 ± 3	n.d.	n.d.
8	52 ± 2	n.d.	n.d.
9	8.8 ± 0.8	n.d.	n.d.
10	437 ± 6	n.d.	n.d.
11	380 ± 8	n.d.	n.d.
12	36.6 ± 0.7	6 ± 2	n.d.
13	173 ± 4	n.d.	0.8 ± 0.4
14	n.d.	n.d.	n.d.
15	n.d.	n.d.	n.d.
16	81 ± 2	n.d.	n.d.
17	37.4 ± 0.9	n.d.	n.d.
18	30 ± 6	n.d.	0.6 ± 0.4
19	22 ± 1	n.d.	0.4 ± 0.2
20	6 ± 1	n.d.	n.d.
21	n.d.	n.d.	n.d.
22	9.9 ± 0.5	n.d.	n.d.
23	32 ± 2	n.d.	n.d.
24	12 ± 2	n.d.	n.d.
25	58 ± 1	n.d.	1.4 ± 0.4
26	45.1 ± 0.7	4 ± 1	n.d.

n.d., not detected.

^a Average of triplicate determinations \pm standard deviation.

posed ET-AAS methods. Table 5 shows the obtained results. According to the IUPAC recommendations (Inczédy, Lengyel, & Ure, 1997), values under the limit of detection have also been included. Cu was present in almost all the analysed samples with concentrations ranging between 6 and $473 \mu\text{g l}^{-1}$ and an average value of $81.3 \mu\text{g l}^{-1}$. Pb was detected in two samples with values lower than $6 \mu\text{g l}^{-1}$. Concentration of Cd ranged between 0.4 and $1.4 \mu\text{g l}^{-1}$ with an average value of $0.8 \mu\text{g l}^{-1}$ in the four samples in which it was present. The remaining samples do not have detectable amounts of Cd.

References

- Alcázar, A., Pablos, F., Martín, M. J., & González, A. G. (2002). Multivariate characterisation of beers according to their mineral content. *Talanta*, *57*, 45–52.
- Almeida, C. M. R., & Vasconcelos, M. T. S. D. (2003). Lead contamination in Portuguese red wines from the douro region: from the vineyard to the final product. *Journal of Agricultural and Food Chemistry*, *51*, 3012–3023.
- AOAC Peer verified methods program (1993). *Manual on policies and procedures*. Arlington, VA, USA.
- Buldini, P. L., Cavalli, S., & Sharma, J. L. (1999). Determination of transition metals in wine by ICP, DPASV-DPCSV and ZGFAAS coupled with UV photolysis. *Journal of Agricultural and Food Chemistry*, *47*, 1993–1998.
- Butcher, J., & Sneddon, J. (1998). A practical guide to graphite furnace atomic absorption spectrometry. In J. D. Winefordner (Ed.), *Chemical analysis: a series of monographs on analytical chemistry and its applications* (149). New York: Wiley-Interscience.

- Cameán, A. M., Moreno, I., López-Artiguez, M., Repetto, M., & González, A. G. (2001). Differentiation of Spanish brandies according to their metal content. *Talanta*, *54*, 53–59.
- Council Regulation (ECC) No. 1576/89 of 29 May 1989. Official Journal L160, 12/06/1989, pp. 0001–0017.
- EURACHEM/CITAC Guide (2000). *Quantifying uncertainty in analytical measurement* (2nd ed.). Budapest: EURACHEM.
- Fernández, C. (1988). The importance of metallic elements in wine. A literature survey. *Zeitschrift für Lebensmittel-Untersuchung und-Forschung*, *186*, 295–300.
- Freschi, G. P. G., Dakuzaku, C. S., Moraes, M., Nóbrega, J. A., & Gomes Neto, J. A. (2001). Simultaneous determination of cadmium and lead in wine by electrothermal atomic absorption spectrometry. *Spectrochimica Acta Part B*, *56*, 1987–1993.
- Frias, S., Conde, J. E., Rodríguez-Bencomo, J. J., García-Montelongo, F., & Pérez-Trujillo, J. P. (2003). Classification of commercial wines from the Canary Islands (Spain) by chemometric techniques using metallic contents. *Talanta*, *59*, 335–344.
- Gaetke, L. M., & Chow, C. K. (2003). Copper toxicity, oxidative stress, and antioxidant nutrients. *Toxicology*, *189*, 147–163.
- Galani-Nikolakaki, S., Kallithrakas-Kontos, N., & Katsanos, A. A. (2002). Trace element analysis of Cretan wines and wine products. *Science of the Total Environment*, *285*, 155–163.
- González, A. G., Herrador, M. A., & Asuero, A. G. (1999). Intra-laboratory testing of method accuracy from recovery assays. *Talanta*, *48*, 729–736.
- González, M. J., Martínez, M. C., & Aguilar, M. V. Z. (1998). Determination of Fe, Cu, Zn, Mn and Pb in D.O.C. Méntrida wines. *Zeitschrift für Lebensmittel-Untersuchung und-Forschung*, *186*, 325–329.
- Goyer, R. A., & Clarkson, T. W. (2001). Toxic effects of metals. In C. D. Klaassen (Ed.), *Casarett and Doull's Toxicology* (6th ed., pp. 811–867). New York: McGraw-Hill.
- Hsu, P. C., & Guo, Y. L. (2002). Antioxidant nutrients and lead toxicity. *Toxicology*, *180*, 33–44.
- Inczédy, J., Lengyel, T., Ure, A. M. (1997). *Compendium of analytical nomenclature. Definitive rules. Section 18.4.3.7 Detection and quantification capabilities* (3rd ed.) IUPAC. Available from www.iupac.org.
- Jos, A., Moreno, I., González, A. G., López-Artiguez, M., & Cameán, A. M. (2004). Study of the mineral profile of Catalonian “brut” cava using atomic spectrometric methods. *European Food Research and Technology*, *218*, 448–451.
- Jurado, J. M., Alcázar, A., Pablos, F., Martín, M. J., & González, A. G. (2004). Determination of Zn, B, Fe, Mg, Ca, Na and Si in anisette samples by inductively coupled plasma atomic emission spectrometry. *Talanta*, *63*, 297–302.
- Kaufman, A. (1998). Lead in wine. *Food Additives and Contaminants*, *15*, 437–445.
- Kim, M. (2004). Determination of lead and cadmium in wines by graphite furnace atomic absorption spectrometry. *Food Additives and Contaminants*, *21*, 154–157.
- Kristl, J., Veber, M., & Slekovec, M. (2002). The application of ETAAS to the determination of Cr, Pb and Cd in samples taken during different stages of the winemaking process. *Analytical and Bioanalytical Chemistry*, *373*, 200–204.
- Maroto, A., Boqué, R., Riu, J., & Rius, F. X. (2001). Measurement uncertainty in analytical methods in which trueness is assessed from recovery assays. *Analytica Chimica Acta*, *440*, 171–184.
- Matsushige, I., & Oliveira, E. (1993). Determination of trace elements in Brazilian beers by ICP-AES. *Food Chemistry*, *47*, 205–207.
- Mena, C., Cabrera, C., Lorenzo, M. L., & López, M. C. (1996). Cadmium levels in wines, beers and other alcoholic beverages: possible sources of contamination. *Science of the Total Environment*, *181*(3), 201–208.
- Mena, C. M., Cabrera, C., Lorenzo, M. L., & López, M. C. (1997). Determination of lead contamination in Spanish wines and other alcoholic beverages by flow injection atomic absorption spectrometry. *Journal of Agricultural and Food Chemistry*, *45*, 1812–1815.
- Miller, J. N., & Miller, J. C. (2000). *Statistics and chemometrics for analytical chemistry* (4th ed.). London: Prentice Hall.
- Moutsatsou, A., Chalarakis, E., & Zarangas, G. (2003). Influence of raw materials and distillation equipment on the heavy metal content of waste from an alcoholic anis-type beverage. *Journal of Hazardous Materials*, *B96*, 53–64.
- Perkin-Elmer (1984). *Analytical techniques for graphite furnace AAS*. Veberlingen: Bodenseewerck Perkin-Elmer GmbH.
- Pizarro, F., Olivares, M., Uauy, R., Contreras, P., Rebelo, A., & Gidi, V. (1999). Acute gastrointestinal effects of graded levels of copper in drinking water. *Environmental Health Perspectives*, *107*, 117–121.
- Rafter, J. A., Abell, M. L., & Braselton, J. P. (2002). Multiple comparison methods for means. *SIAM Review*, *44*, 259–278.
- Rodríguez, L. C., Campaña, A. M. G., & Sendra, J. M. B. (1996). Statistical estimation of linear calibration range. *Analytical Letters*, *29*, 1231–1239.
- Schlemmer, G., & Welz, B. (1986). Palladium and magnesium nitrates, a more universal modifier for graphite-furnace atomic-absorption spectrometry. *Spectrochimica Acta Part B*, *41B*, 1157–1165.
- Soufleros, E. H., Mygdalia, A. S., & Natskoulis, P. (2004). Characterization and safety evaluation of the traditional Greek fruit distillate “Mouro” by flavour compounds and mineral analysis. *Food Chemistry*, *86*, 625–636.
- Turnlund, J. R. (1998). Human whole-body copper metabolism. *American Journal of Clinical Nutrition*, *67*, 960S–964S.
- Ure, T. A. M., Butler, L. R. P., L'vov, B. V., Rubeska, I., & Sturgeon, R. (1992). Nomenclature, symbols, units and their usage in spectrochemical analysis-XII. Terms related to electrothermal atomization. (IUPAC recommendations). *Pure and Applied Chemistry*, *64*, 253–259.
- Welz, B., Schlemmer, G., & Mudakavi, J. R. (1992). Palladium nitrate magnesium nitrate modifier for electrothermal atomic absorption spectrometry. Performance for the determination of 21 elements. *Journal of Analytical Atomic Spectrometry*, *7*, 1257–1271.
- Welz, B., & Sperling, M. (1999). *Atomic absorption spectrometry*. Wiley-VCH: Weinheim.
- Winge, D. R., & Mehra, R. K. (1990). Host defences against copper toxicity. *International Review of Experimental Pathology*, *31*, 47–83.