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Analytical Methods

Determination of Cr and Ni in *Orujo* spirit samples by ETAAS using different chemical modifiers

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Abstract

Several analytical methods are proposed for chromium and nickel determination in *Orujo* spirit samples using ETAAS. Permanent chemical modifiers such as W, Ir, Ru, W–Ir and W–Ru were comparatively studied in relation to the common chemical modifier employed, $Pd(NO_3)_2$–$Mg(NO_3)_2$. Taking into account the analytical performance, the method based on the use of Ru as a permanent modifier was selected for further direct Cr determinations in *Orujo* samples. In the case of Ni, after comparison among the different methods developed, a method with no modifier which allows the direct interpolation in calibration graphs was chosen. Detection limits of $0.13\ \mu g\ L^{-1}$ and $0.30\ \mu g\ L^{-1}$ were obtained for Cr and Ni, respectively. For all methods developed, recoveries (ranged 98.6–102%) and precision (RSD < 10%) were acceptable. The selected methods were applied for the determination of the Cr and Ni contents in 80 representative *Orujo* Galician samples. The Cr concentrations ranged from <LOD and $14.61\ \mu g\ L^{-1}$ and Ni concentrations ranged <LOD and $34.17\ \mu g\ L^{-1}$.

Keywords: Chromium determination; Nickel determination; ETAAS; Matrix modifier; *Orujo*; Alcoholic distillate spirits

1. Introduction

Galicia constitutes an important winegrowing region located in the Northwest of Spain. *Orujo* is a traditional alcoholic distillate from vinacce (grape skins, seeds and stalks) elaborated, after alcoholic fermentation, in cooper or steel hand-made distillation devices called *alquitar*a and *alambique*. In 1989, the European Union published the general Regulations (EEC, 1989) for the definition, denomination and production of spirits. Galicia was included as the only region with the possibility of obtaining a geographic denomination for *Orujo*, in the same category as French *Marcs*, Italian *Grappas*, Portuguese *Bagaçeiras* and Greek *Tsipouros*. From the establishment, in 1993 (DOG 193, 1993), of the certified brand of origin (CBO) "Denominación Geográfica: *Orujo* de Galicia" (BOE 194, 2005), interest in the study of the complete chemical composition of *Orujo*, mainly its metallic composition, increased continuously due to the producers aim for quality; since, economically speaking, *Orujo* production is an important complementary activity to the elaboration of wine. Metals are principally incorporated into the product from soil and water, from fertilization and pesticide use in phytosanitary treatments (Li, Van Loon, & Barefoot, 1993), and also from distillation apparatus when the traditional elaboration process was carried out in *alquitara* or *alambique* devices.

Chromium is an essential nutrient with significant functions in carbohydrate metabolism aiding in the sugar degradation in the blood, lipid (mainly cholesterol) and nucleic metabolisms (Anderson & Kozlowsky, 1985). The main sources of Cr in the environment include industrial and urban sewages, waste chromates from electroplating baths,

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corrosion inhibitors from heat exchange systems, and trade effluents from tanning process (Alvarez-Cabal, Wrobel, Marchante, & Sanz-Medel, 1994). For human health, high Cr levels are dangerous, particularly as Cr(VI). Hexavalent chromium is associated with some fatal pathogenesis such as lung and gastrointestinal cancer (Burguera et al., 1999). Nickel is a moderately toxic element in high concentrations (compared with other transition metals), and its deficient state has yet to be described in humans (Cempel & Nikel, 2006). The presence of this metal in elevated levels causes a skin disorder known as "nickel-eczema" (Cristensen, Kristiansen, Nielsen, Menne, & Byrialsen, 1999). Ni is mainly used as an alloying metal, along with chromium and others, in the production of stainless and heat-resisting steels. Stainless steel is widely used for food processing and storage (Dugo, La Pera, Lo Turco, Di Bella, & Salvo, 2004), and it has been found to be the main contamination source of these metals in food and beverages (Cempel & Nikel, 2006; Kristl, Veber, & Slekovec, 2002) due to the possible dissolution of Cr and Ni from stainless steel. Therefore, because consumption represents an important route of chromium and nickel intake (García, Cabrera, Sánchez, Lorenzo, & López, 1999) it is necessary develop sensible analytical procedures for its determination in food and beverages.

The determination of Cr and Ni in beverages could be carried out by various analytical techniques that yield enough sensitivity such as: inductively coupling plasma mass spectrometry (ICP-MS), electrothermal atomic absorption spectrometry (ETAAS) and electrochemical techniques (Bas, 2006; Dugo et al., 2004). Among them, E-TAAS (widely applied in food, environmental, geological and industrial samples) is probably the most common technique used for low level concentrations of both metals in beverages due to its availability, simplicity, high sensitivity and specificity, speed of analysis, and minimum sample preparation. However, in the analysis of complex samples, the non-specific background absorption caused by interfering species of the sample matrix often occurs. This undesirable effect can be reduced by the use of effective instrumental background correction and by the application of suitable modifiers (Welz & Sperling, 1999). In general, the use of modifiers allows high pyrolysis temperatures, thereby reducing or eliminating volatilization and vapour phase interferences and minimizing background signals. The employment of the mixture of $Pd(NO_3)_2 + Mg(NO_3)_2$ as a universal modifier greatly facilitates interference-free determinations, especially for volatile elements. Among other chemical modifiers reported for ETAAS, the use of permanent modifiers has reached an important application in the past few years. Graphite tubes and platforms are coated with carbide-forming elements (W, Zr, Nb, Mo and Ta), pre-reduced noble metals (Pd, Rh, Ru, Pt, Ir) or their combinations, as permanent chemical modifiers (Acar, 2004; Alves-Lelis, Gonzalves-Magalhaes, Aparecida Rocha, & Borba da Silva, 2002; Meeravali & Kumar, 2001; Vassileva, Baeten, & Hoenig, 2001). These modification systems have several advantages over conventional modifiers. First, since it is not necessary to deliver the modifier solution for each determination, simpler and faster heating programs can be used. Second, since chemical modifier blanks often do not need to be employed (because the volatile impurities of the modifier solutions were eliminated during graphite tube treatment), better detection limits are obtained. Third, permanent chemical modifier improves a catalytic effect on the partial or total matrix destruction that allows ETAAS's use without previous digestion of samples turning it a sequential digesters/analyzer, what is only obtained in this technique or by ETV-ICP-MS (Magalhaes, Nunes, Giacomelli, & Borba da Silva, 2003). In addition, the use of permanent chemical modifiers increases, in most cases, the long-term stability of the signal and permits a reduction in the number of recalibrations during the routine analysis. (Almeida Pereira, Amorim, & Borba da Silva, 2006; Alves-Lelis et al., 2002; Barbosa, Simiao de Souza, Santos, & Krug, 2004).

Taking all this into account, the objective of this study was develop and optimize different analytical methods for the determination of Cr and Ni in *Orujo* spirits by ETAAS using different modifiers. Moreover, the selected determination procedures were applied to measure the Cr and Ni content in representative Galician *Orujo* samples. In all cases, the optimization of the different analytical ETAAS methods has been carried out with an experimental design because the use of this chemometrical procedure offered a fast and appropriate tool to study the different factors that influence the analytical signal.

2. Materials and methods

2.1. Instrumentation

An atomic absorption spectrometer, Varian-SpectrAA-600 (Varian Inc., Palo Alto, CA, USA) with Zeeman correction, equipped with a Varian GTA-100 electrothermal atomizer linked to an automatic sample dispenser was used for this study. Measurements were performed using two Varian hollow cathode lamps, for Cr operating at 259.7 nm, and for Ni operating a 232.0 nm, with a current intensity of 10 mA in both cases. The bandwidth used was 0.5 nm in all cases. Argon was employed as the inert gas at a flow equal to $3\ L\ min^{-1}$. Pyrolitic graphite-coated tubes without atomization platform were used.

2.2. Reagents

Stock standard solutions of chromium and nickel ($1.0\ g\ L^{-1}$) were obtained from Panreac (Barcelona, Spain) and diluted as necessary to obtain the working standard solutions. Palladium and magnesium nitrate was obtained from Fluka (Switzerland). The working solutions were prepared by diluting the appropriate amount of a $10\ g\ L^{-1}$ stock solution. Permanent modifier solutions ($1.0\ g\ L^{-1}$) of W, and Ir were prepared from 179.4 mg

$Na_2WO_4 \cdot 2H_2O$ powder (Sigma–Aldrich, Germany) dissolved in ultrapure water and 62.7 mg $(NH_4)_3IrCl_6 \cdot H_2O$ powder (Sigma–Aldrich, Germany) dissolved in a 37% HCl solution, respectively, and diluted in both cases to 25 mL with water. Ru permanent modifier solution (1.0 g L^{-1}) was purchased from Fluka (Switzerland). Nitric acid (65% w/v) Suprapur reagent and Triton X-100 were obtained from Merck (Darmstadt, Germany). High-purity water was provided by a Milli-Q deionizing system (Millipore, Bedford, MA, USA).

All sampler containers, autosampler cups and other materials were washed with nitric acid 10% v/v for 24 h before rising with copious amount of ultrapure water and shaking dry prior to use. The cleaning solution employed to wash the sampling capillary contained 0.7% (w/v) HNO_3 and 0.2% (v/v) Triton X-100.

2.3. Statistical software

All experimental designs employed (Plackett–Burman and factorial central composite designs) as well as surface and contour plots were carried out using the statistical software package *Statgraphics 5.1 Plus*.

2.4. Orujo spirit samples

Eighty representative *Orujo* alcoholic distillates from Galicia (NW Spain) were analyzed. All samples studied were from Galician guaranteed origin including both brands: with and without Certified Brand of Origin (CBO) "Denominación Geográfica *Orujo* de Galicia". Thirty one of these samples were manufactured using traditional procedures in the producing region, the remaining forty nine were industrial and commercial samples obtained from local stores. For each case, the sample information available included the method of elaboration. Three kinds of samples were analyzed: samples elaborated with traditional devices either (1) *Alambique* or (2) *Alquitara* and (3) samples elaborated in refineries with industrial distillation elaboration procedures.

2.5. Analytical procedures

2.5.1. Chromium determination

2.5.1.1. Palladium nitrate method. Eight hundred microliters of *Orujo* spirit were made up to 1000 μL with ultrapure water. Twelve microliters of this solution were introduced in the graphite tube together with 18 μL of Pd solution (3000 mg L^{-1}) and were subjected to ETAAS under the optimized conditions (see Table 1A).

2.5.1.2. Permanent chemical modifier methods. Eight hundred microliters of *Orujo* spirit were made up to 1000 μL with ultrapure water. Twenty microliters of this solution were placed inside the graphite tube and subjected to ETAAS under the optimized conditions (see Table 1A). In this study, the quantities used for permanent modifiers were: 250 μg W, 200 μg Ru, 200 μg Ir, 250 μg W+200 μg Ru, and 250 μg W+200 μg Ir. The treatment of the graphite tubes has been described in detail in previous studies (Acar, 2004; Lima, Barbosa, & Krug, 1999; Lima, Brasil, & Santos, 2003; Queiroz, Rocha, Knapp, & Krug, 2002).

2.5.2. Nickel determination

2.5.2.1. Method with and without modifier. Eight hundred microliters of *Orujo* spirit were made up to 1000 μL with ultrapure water. Twenty microliters of this solution were placed inside the graphite tube and subjected to ETAAS under the optimized conditions (see Table 1B).

3. Results and discussion

An experimental design was used to optimize all the proposed methods in two steps, screening and optimization. In addition, an univariate study of ash and atomization temperatures was carried out. The first step (screening) was

Table 1A
Furnace heating programs for ETAAS determination of chromium using the different chemical modifiers studied

Step	Parameter	Modifier					
		$Pd(NO_3)_2$	W	Ir	Ru	W–Ru	W–Ir
Dry	Temperature (°C)	100	100	100	100	100	100
	Ramp (s)	25	30	30	22	19	35
	Hold (s)	26	7	28	6	6	40
Ash	Temperature (°C)	1500	750	830	1300	1100	1300
	Ramp (s)	25	30	30	5	17	5
	Hold (s)	5	22	15	15	31	29
Atomization	Temperature (°C)	2500	2550	2700	2500	2700	2500
	Ramp (s)	0	0	0	0	0	0
	Hold (s)	3	3	3	3	3	3
Clean	Temperature (°C)	2700	2700	2700	2700	2700	2700
	Ramp (s)	1	1	1	1	1	1
	Hold (s)	3	3	3	3	3	3

Table 1B
Furnace heating programs for ETAAS determination of nickel without modifier

Step	Temperature (°C)	Ramp (s)	Hold (s)
Dry	100	5	5
Ash	1300	40	5
Atomization	2600	0	3
Clean	2700	1	3

applied in order to discover what factors significantly affected the analytical signal; for this aim a Plackett–Burman fractional factorial design was used. Once the significant factors influencing the signal were identified, the second step (optimization) was carried out to obtain the optimum conditions for these factors by applying different central composite designs (according to the number of significant factors) and by constructing the appropriate response surfaces. In addition, a comparative evaluation of the stabilization effect for the different chemical modifiers assayed was carried out by means of ash and atomization curves.

According to a previous research (Barbosa et al., 2004) (in which certain authors suggest that the use of platform is inappropriate for analytes that require high-atomization temperatures) and our experience (Rodríguez García, Barciela García, Herrero Latorre, García Martín, & Peña Crecente, 2005), it has been demonstrated that the Cr and Ni atomization in graphite surfaces from the wall of the atomizer is more efficient than from L'vov platform. Preliminary experiments employing graphite tubes with platforms conducted to peaks with long tails, as the result of a delayed atomization. For these reasons, the method optimizations were developed, in all cases, using graphite tube without platform.

3.1. Chromium determination: optimization of ETAAS conditions

3.1.1. Palladium nitrate method optimization

The influence of the different factors in Cr determination by ETAAS was checked using a Plackett–Burman design. It was carried out in a single block to study the effect of nine factors in 14 runs. The factors evaluated and their high and low levels have been summarized in Table 2. They were chosen according to our previous experience and by taking into account the values reported in other investigations (Canuto, Siebald, de Lima, & Borba Silva, 2003; Rodríguez García et al., 2005). The order of the experiments has been fully randomized to protect against the effect of uncontrolled variables. The factors that presented a higher influence in the response were ash and atomization temperatures, palladium concentration and ash ramp time. In order to optimize these four parameters, a central composite design 2^4+ star, in 26 randomized runs was carried out. Ash temperature was studied in the range 800–1900 °C, it was observed that the Cr signal increased with temperature up to 1040 °C, for higher values the signal fell abruptly. The atomization temperature range studied was between 2000 °C and 2700 °C; as can be seen in Fig. 1A, the analytical signal increased to achieve a maximum for 2500 °C; higher temperatures produced a small decrease in the absorbance. For these reasons, the optimum ash and atomization temperatures selected were 800 °C and 2500 °C, respectively (see Fig. 1A). In relation to the chemical modifiers employed, the previous screening design demonstrated two facts: the key role effect of palladium in the Cr analytical signal and that magnesium nitrate addition had no influence on the Cr determination. The optimum palladium concentration selected was 3000 mg L^{-1}. Higher palladium concentrations were studied using a univariate strategy and the results obtained demonstrated that, for Pd levels upper to 3000 mg L^{-1}, the analytical signal did not improved.

As the last stage of optimization, a univariate study for ash and atomization temperatures was carried out to choose the most favourable conditions according to the peak shape and sensitivity. In Fig. 2A, the ash temperature effect in the Cr signal in alcoholic matrix is presented for all modifiers studied and also in the absence of a modifier. When the palladium modifier was used, the signal remained constant up to an ash temperature of 1500 °C (for this temperature, the analytical signal was reduced in a quantity less than 10%, with respect to the optimum value obtained using the central composite design). Thus, the ash temper-

Table 2
Factors evaluated in the ETAAS Cr, and Ni determination method using $Pd(NO_3)_2$–$Mg(NO_3)_2$ and factors evaluated for Cr using permanent chemical modifiers, and for Ni using permanent modifiers and no modifier. High and low levels

Factor	Code	$Pd(NO_3)_2$-$Mg(NO_3)_2$		Cr permanent modifiers		Ni modifiers	
		Low	High	Low	High	Low	High
$[Pd(NO_3)_2]$ (mg L^{-1})	[Pd]	0	3000	–	–	–	–
$[Mg(NO_3)_2]$ (mg L^{-1})	[Mg]	0	3000	–	–	–	–
Dry temperature (°C)	Dry	70	300	–	–	–	–
Ash temperature (°C)	Ash	400	1500	200	1200	500	1700
Atomization temperature (°C)	Atom	2000	2700	2200	2700	2100	2700
Dry Ramp (s)	Dry Ramp	5	25	5	35	5	40
Dry Hold (s)	Dry Hold	5	30	5	40	5	50
Ash Ramp (s)	Ash Ramp	5	25	5	35	5	50
Ash Hold (s)	Ash Hold	5	30	5	40	5	50

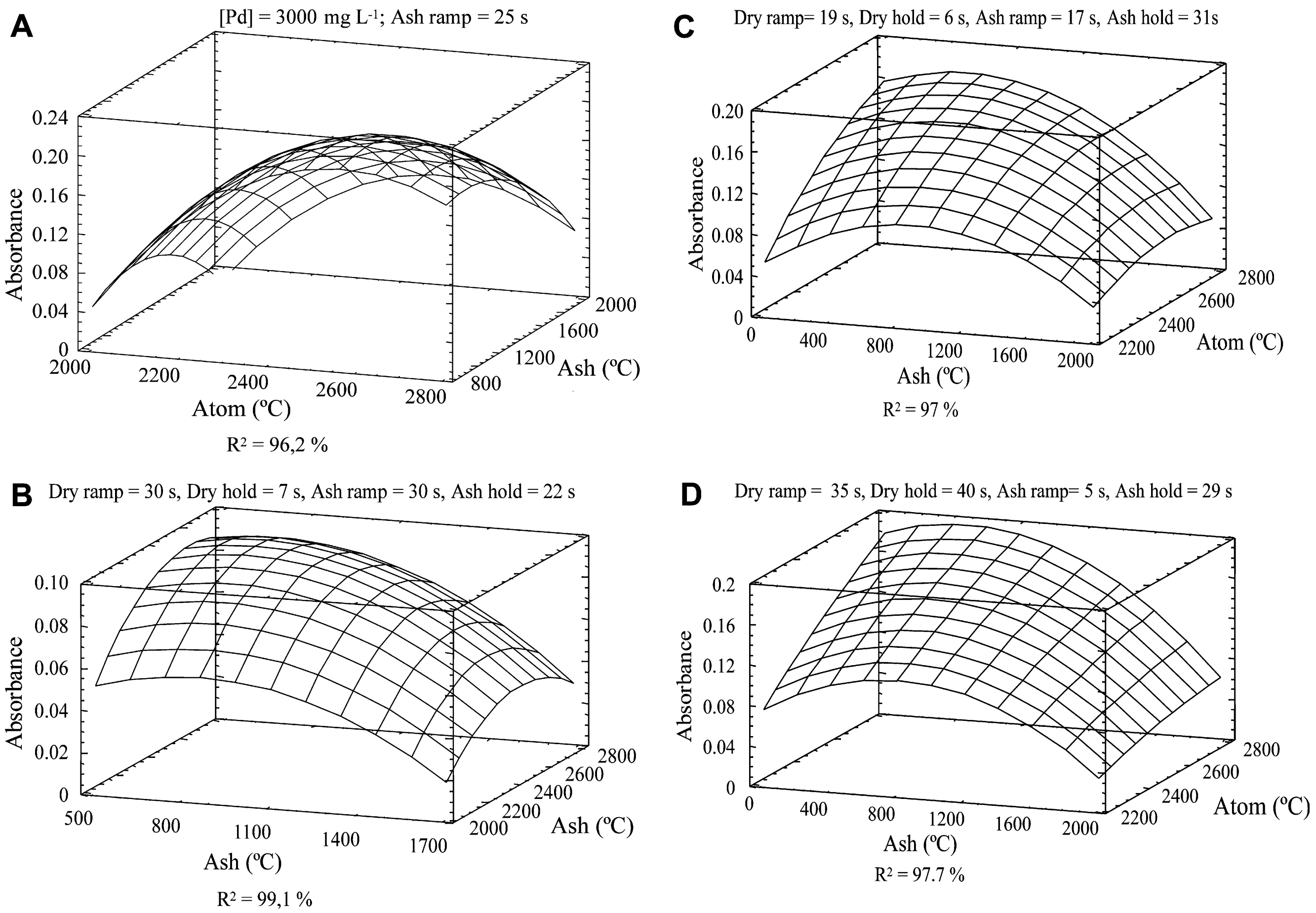

Fig. 1. Response surfaces for the effect of ash and atomization temperatures on Cr absorbance. Chemical modifiers: (A) Pd; (B) W; (C) W–Ru and (D) W–Ir.

ature of 1500 °C was selected as the optimum value. Dry temperature and ramp and hold times in ash and dry stages were studied using the screening design and later on, they were checked with a univariate study. Dry temperature was similar that obtained in previous studies by the determination of cadmium and lead in the same *Orujo* matrix (Vilar, Barciela, García-Martín, Peña, & Herrero, 2007; Vilar Fariñas, Barciela García, García Martín, Peña Crecente, & Herrero Latorre, 2007). The optimum temperature program is summarized in Table 1A.

3.1.2. Permanent modifier methods optimization

Chromium, as a refractory element, tends to form stable chromium-carbides. The use of graphite tubes impregnated with metals has been demonstrated the reduction of carbide formation (Bermejo-Barrera, Moreda-Piñeiro, Moreda-Piñeiro, & Bermejo-Barrera, 1998); for this reason, five different methods with diverse permanent modifiers or their mixtures (W, Ru, Ir, W–Ru, W–Ir) were developed for Cr determination in *Orujo* samples. For all the procedures, six factors were evaluated and optimized (see Table 2) by a central composite 2^{6-1}+ star design. The modifier masses employed for each case were selected as per previous research, because their effect on the response signal has been thoroughly studied (Acar, 2004; Lima et al., 2003; Queiroz et al., 2002). For the five methods examined, the optimum temperature programs were obtained by means of a central composite 2^{6-1}+ star design, in 46 randomized experiments. In Table 2, the six factors evaluated and their high and low levels have been detailed. Based on the 2^{6-1}+ star central composite design developed, the response surface for ash and atomization temperatures obtained for W, W–Ru and W–Ir modifiers is shown in Figs. 1B, C and D, respectively. In all cases, a significant interaction effect between both temperatures was observed. For the W, W–Ru and W–Ir methods, the analytical signal increased up to the optimum value and decreased for higher values; this effect was clearest for the highest values of atomization temperature. For the Ir and Ru methods the opposite was observed; when ash temperature went up, the signal diminished and this effect was higher for high values of atomization temperature. The optimum ash temperatures were normally lower than 1000 °C, but when univariate studies were developed, it was shown that, in all of the cases, the values may be extended at temperatures higher than 1300 °C, without losing more than 10% of the analytical signal (see Fig. 2A). Higher ash temperatures help to eliminate matrix effect; thus, the chosen optimum ash temperatures were those

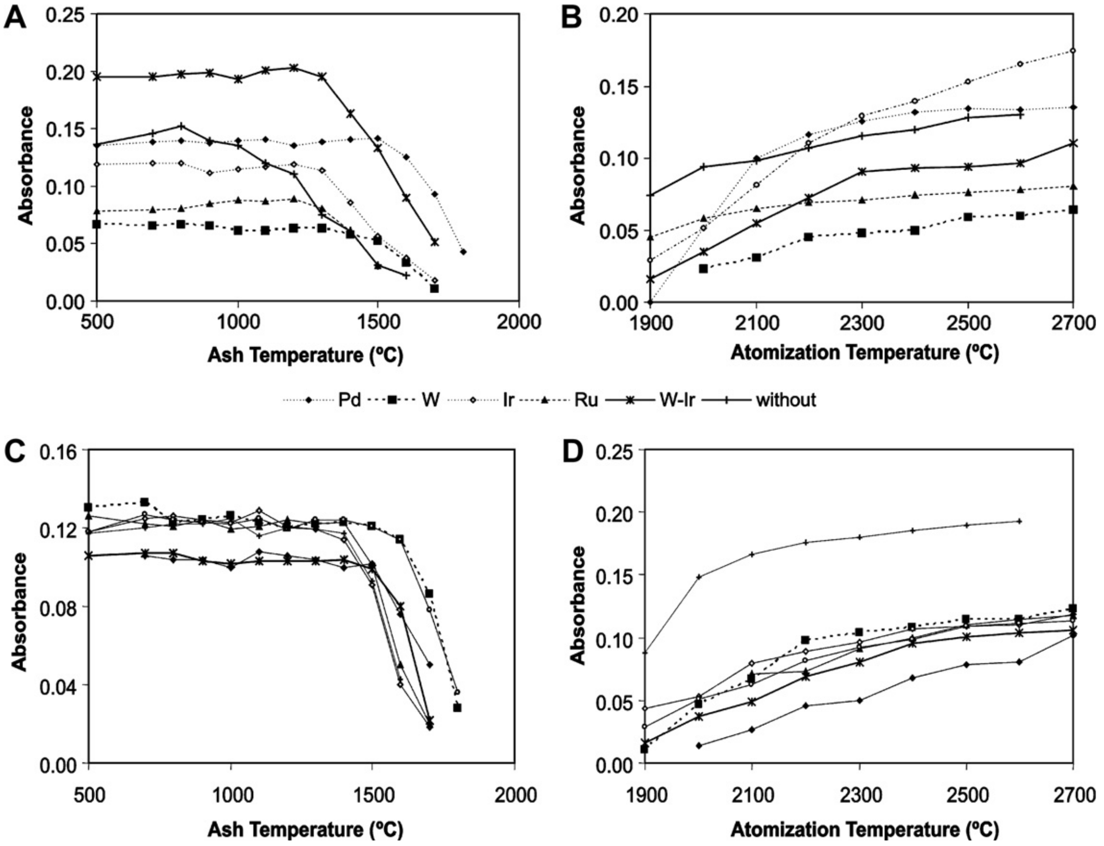

Fig. 2. Ash (A) and atomization (B) temperature curves for Cr determination in *Orujo* samples with and without the different modifiers assayed. Ash (C) and atomization (D) temperature curves for Ni determination with and without the different modifiers studied.

obtained with the univariate study, and they are presented in Table 1A. In all the cases, a higher ash temperature was reached using permanent modifiers in comparison with the ash temperature obtained (800 °C) in the absence of a modifier, which is indicative for the stabilization effect of these modifiers. For all cases, the optimum atomization temperatures (see Figs. 1B–D) were 2700 °C except for W, 2550 °C. These values can be corrected by employing the atomization curves in Fig. 2B. The atomization temperature may be decreased to 2500 °C for Ru and W–Ir methods, this will extend the tube life and preserve the modifier layer. Ramp and hold dry times, and ramp and hold ash times were optimized using the above mentioned central composite design. The high-atomization temperatures obtained for Cr could affect the thermal stability of the permanent chemical modifiers and life-time of the graphite tube. However, in this work, it has been demonstrated a good operation of the atomizer during about 400 injections. The optimum established furnace operation conditions for all the methods studied, based on permanent modifiers, have been listed in Table 1A.

3.2. Nickel determination: optimization of ETAAS conditions

The optimization strategy used for Ni determination was the same one employed in the case of chromium: the use of experimental design in two steps (screening and optimization) followed by a univariate study of ash and atomization temperatures. Therefore, a Pd-method and different permanent modifier methods were developed for ETAAS Ni determination. Palladium method was optimized first, by using a Plackett–Burman design so as to know the more significant factors. Second, a 2^4+ star design was carried out in order to evaluate the effect of palladium, ash and atomization temperature, and dry ramp time. The results obtained showed a very small optimum palladium concentration (4 mg L^{-1}) and optimum ash and atomization temperatures of 1200 °C and 2700 °C, respectively.

The appropriate conditions of the Ni determination method with permanent modifiers and without modifier were optimized using a central composite 2^{6-1}+ star design, in 46 randomized experiments. In Table 2, the six factors evaluated and their high and low levels have been detailed. In all cases, the atomization temperature had a positive effect on the Ni absorbance, which continuously increased the analytical signal. The optimum atomization temperature was 2600 °C. The study of the ash temperature showed that for temperatures higher than 1200 °C, the analytical signal decreased. Dry temperature (100 °C) was selected according to the above mentioned criteria. The remaining factors were optimized using the previously cited central composite design: short dry ramp and dry hold times were necessary. However, a high ash ramp time is recommended to ensure sample mineralization. When the univariate study was carried out (Figs. 2C and D), it was confirmed that no chemical modifier was needed to reach nickel stabilization. The optimum ash temperature in the absence of a modifier is comparable to those reached when different permanent chemical modifiers are used (see Fig. 2C). Even though the use of W–Ir and W–Ru modifiers provided for an improvement in sensitivity as well as a slightly higher ash temperature, the modest enhancement of these analytical characteristics does not merit the use of chemical modifiers. Therefore, for the ETAAS determination of Ni in *Orujo* samples, the recommendation is to work without chemical modifiers, since the sensitivity attained is adequate enough and the method is cheaper. This conclusion is consistent with those noted by several authors who recommended methods with no chemical modification for the measurement of Ni in different matrices: Meeravali and Kumar (2000) in algae matrix; Cabrera, Lloris, Giménez, Olalla, and López (2003) in vegetables, Viñas, Pardo-Martínez, and Hernández-Córdoba (2000) in baby food slurries or Viñas, Campillo, López, and Hernández (1995) in food samples.

The established optimum conditions for the ETAAS determination method of Ni in *Orujo* samples, in the absence of a chemical modifier, are summarized in Table 1B.

3.3. Analytical characteristics

3.3.1. Calibration

Calibration curves for Cr and Ni were constructed following the procedures described in *Analytical procedures* section and by applying the temperature programs given in Tables 1A and B. Instrumental calibration was performed in the range LOQ–7.5 μg L^{-1} for Cr and LOQ–12.0 μg L^{-1} for Ni, in a water solution and in an *Orujo* spirit matrix. In Table 3, the equations obtained for calibration and standard addition graphs for the different methods developed have been presented. For the different Cr determination methods, the comparison of slopes for both graphs was indicative of a significant matrix effect with Ru, W–Ru and W–Ir modifiers. In these cases, Cr determination should be carried out using addition graph. According to several authors (Aceto et al., 2002; Jurado, Martín, Pablos, Moreda-Piñeiro, & Bermejo-Barrera, 2007; Vilar Fariñas et al., 2007), this matrix effect can be corrected with a sample supplementation with a HNO_3 solution. However, the effect of different concentrations of nitric acid on the analytical signal was studied for the diverse methods, and in none case the pointed matrix effect was corrected. Therefore, in all methods developed in the present study, the nitric acid addition for Cr determination in *Orujo* samples is not employed.

For the Ni determination method without modifier, the slopes for calibration and addition curves were statistically comparables, indicating the absence of a significant matrix effect, in the other cases were also comparable except in Ru and W–Ru methods.

3.3.2. Detection limits

Detection limits (LOD) were calculated on the basis of 3 SD/*m* (Currie, 1995), where *m* is the slope of the calibration graph and SD the standard deviation of 10 consecutive measurements of blank solutions. Quantification limits (LOQ) were calculated as 10 SD/*m*. The results obtained for LOD, LOQ and characteristic masses (based on the integrated absorbance) are given in Table 4. From the comparative study of the detection limits for the various methods for Cr determination, it can be concluded that the best sensitivity limit was obtained for the Ru method (0.13 μg L^{-1}). This LOD was better than those obtained for the other permanent modifiers; however it will be necessary to use the addition graph for Cr measurement. Using Pd as chemical modifier, the LOD obtained (0.29 μg L^{-1}) was higher than the one for Ru method (0.13 μg L^{-1}); however, when Pd-method was employed, the absence of matrix effect permitted the use of calibration graph. In contrast with other ETAAS methods for chro-

Table 3
Equations of calibration and standard addition graphs obtained for ETAAS Cr and Ni determination methods using the different modifiers studied

Modifier	Calibration	Standard addition
Chromium		
$Pd(NO_3)_2$	$A = 0.013\ [Cr] + 0.028;\ R^2 = 0.997$	$A = 0.014\ [Cr] + 0.067;\ R^2 = 0.999$
W	$A = 0.010\ [Cr] + 0.001;\ R^2 = 0.998$	$A = 0.011\ [Cr] + 0.039;\ R^2 = 0.997$
Ir	$A = 0.016\ [Cr] + 0.005;\ R^2 = 0.998$	$A = 0.016\ [Cr] + 0.045;\ R^2 = 0.997$
Ru	$A = 0.011\ [Cr];\ R^2 = 0.999$	$A = 0.026\ [Cr] + 0.031;\ R^2 = 0.997$
W–Ir	$A = 0.017\ [Cr] + 0.007;\ R^2 = 0.998$	$A = 0.025\ [Cr] + 0.052;\ R^2 = 0.999$
W–Ru	$A = 0.019\ [Cr] + 0.004;\ R^2 = 0.998$	$A = 0.025\ [Cr] + 0.069;\ R^2 = 0.999$
Nickel		
$Pd(NO_3)_2$	$A = 0.006\ [Ni] + 0.008;\ R^2 = 0.997$	$A = 0.006\ [Ni] + 0.038;\ R^2 = 0.999$
W	$A = 0.008\ [Ni] + 0.006;\ R^2 = 0.998$	$A = 0.008\ [Ni] + 0.021;\ R^2 = 0.998$
Ir	$A = 0.008\ [Ni] + 0.013;\ R^2 = 0.998$	$A = 0.009\ [Ni] + 0.027;\ R^2 = 0.998$
Ru	$A = 0.010\ [Ni] + 0.001;\ R^2 = 0.998$	$A = 0.015\ [Ni] + 0.038;\ R^2 = 0.997$
W–Ir	$A = 0.010\ [Ni] + 0.001;\ R^2 = 0.999$	$A = 0.010\ [Ni] + 0.034;\ R^2 = 0.999$
W–Ru	$A = 0.008\ [Ni] + 0.007;\ R^2 = 0.999$	$A = 0.009\ [Ni] + 0.020;\ R^2 = 0.999$
Without	$A = 0.007\ [Ni] + 0.004;\ R^2 = 0.998$	$A = 0.007\ [Cr] + 0.028;\ R^2 = 0.999$

Table 4
Analytical characteristics for the ETAAS Cr and Ni determination methods using the different chemical modifiers assayed

Modifier	m_0 (pg)	LOD (μg L^{-1})	LOQ (μg L^{-1})	RSD[a] (%)
Chromium				
$Pd(NO_3)_2$	2.05	0.29	0.97	3.9; 4.5; 2.4; 1.0
W	5.13	0.72	2.39	4.2; 1.5; 4.4; 4.4
Ir	3.84	0.48	3.37	7.7; 1.6; 8.7; 6.0
Ru	2.65	0.13	0.42	6.8; 4.5; 1.2; 3.0
W–Ir	4.77	0.34	1.14	7.8; 4.5; 3.3; 3.7
W–Ru	4.58	0.35	1.07	2.9; 1.5; 3.4; 3.2
Nickel				
With no modifier	8.12	0.30	1.01	7.8; 4.3; 2.7; 2.5
W	13.2	0.84	2.79	7.9; 7.7; 8.0; 5.3
Ir	12.4	0.86	2.88	9.9; 8.7; 6.6; 6.0
Ru	13.66	0.42	1.40	4.0; 5.2; 4.7; 3.5
W–Ir	13.31	1.26	4.21	5.8; 8.3; 9.3; 4.9

[a] At the four concentration levels assayed (see the text).

mium determination in other matrices, the procedure for Cr determination based on the use of Ru as a permanent modifier achieved similar LOD: in sugar cane spirits (Canuto et al., 2003) (using Ru as modifier, LOD 2.0 pg) or in potable water, fruit juices and soft drink (using $Mg(NO_3)_2$ as a modifier, 0.1 ng mL^{-1}). With reference to the method developed for Ni determination without modifier, the obtained LOD is better than those achieved in this study for different methods employing permanent modifiers (see Table 4). Moreover, this LOD improved those obtained by other authors using ETAAS in soft drinks (Paiva de Oliveira, Gomez Neto, Nóbrega, & Oliveira, 2005) (1.4 μg L^{-1}) and it is comparable to those obtained with an Adsorptive Stripping Chronopotentiometry method (Dugo et al., 2004) developed for beverages (0.20 μg L^{-1}).

3.3.3. Precision

The within-run precision was studied for all modifiers and metals using separate *Orujo* solutions spiked with different chromium (0.0, 2.5, 5.0 and 7.5 μg L^{-1}) and nickel (0.0, 4.0, 8.0 and 12.0 μg L^{-1}) quantities. The relative standard deviations (RSD) obtained for 10 determinations of each solution were below 10%, in all cases. The repeatability of the analytical signal was comparable for all the modifiers studied. The variation coefficients for the four concentration levels assayed for the different modifiers and the two analytes appear in Table 4. In all the chromium methods, the precision obtained was acceptable. For nickel determination, the methods assayed yielded an appropriate precision (RSD lower than 10%) except for Pd and W–Ru modifiers which presented unacceptable RSD values (higher than 10%). Therefore, the latter two methods were unsuccessful for Ni determination in *Orujo* samples.

3.3.4. Accuracy

Since currently no *Orujo* spirit reference material is available with certified Cr and/or Ni concentrations, the accuracy of the proposed methods was measured a/ by comparison with other analytical methods and b/ by recovery studies. For the first approach, the *Orujo* samples were measured using ICP-AES. Thus, a new method based on this technique was developed taking into account that the high alcohol content of these samples could affect the plasma stability. However, when the ICP-based method was applied, its sensitivity was not enough for the Cr and Ni content in the samples analyzed. The second mode for measure the accuracy was the evaluation of the recovery on real samples spiked with diverse quantities of both analytes (0.0, 2.5, 5.0 and 7.5 μg L^{-1} for Cr, and 0.0, 4.0, 8.0 and 12.0 μg L^{-1} for Ni). The mean recoveries attained for the different procedures were as follows for the different E-TAAS methods used in chromium determination: 100% for $Pd(NO_3)_2$, 98.7% for W, 99.7% for Ir and 100% for Ru, 99.9% for the W–Ir method and 101% for W–Ru. In the case of the ETAAS determination method for Ni without modifier, the result achieved for the mean recovery was 98.6%, which is similar to the values obtained for the W (101%), Ir (102%), Ru (99.0%) and W–Ir methods (100%). Hence, for both metals, these results demonstrated the appropriate accuracy of the methods optimized.

3.4. Application

In order to study the applicability of the methods developed, nine replicates of an *Orujo* sample from Galicia were analyzed in triplicate using the five selected permanent modifiers and the $Pd(NO_3)_2$ method. The *t*-test for mean comparison and the *F*-test for variance comparison showed no significant differences (at a 95% confidence level) in the Cr content measured with the methods developed; therefore, the results provided by the different modifiers studied were statistically comparable. According to the statistical comparability of the results obtained for the different methods, the Cr content was measured in 80 Galician samples with and without CBO in triplicate using the ETAAS–Ru method. This determination procedure, which needs the use of addition graph, was selected due to its high sensitivity (best detection limit and characteristic mass) since the *Orujo* samples presented very low Cr content. For Ni determination, the same statistic strategy was developed for the methods with permanent modifiers (W, Ir, Ru, W–Ir) and for the method without modifier. The results showed that there are no significant differences for the Ni content measured using the different methods. Thus, the method chosen for Ni determination in *Orujo* spirit samples was the one without modifier.

Chromium was found in the analyzed samples at concentrations ranging between <LOD and 14.61 $\mu g\,L^{-1}$. Fifty percent of the samples measured had levels lower than the LOD obtained. This result is indicative of the low Cr content in Galician *Orujo* samples which presented levels lower to those reported by certain authors in *wine* (6.6–90.0 $\mu g\,L^{-1}$) or champagne samples (10.5–36.0 $\mu g\,L^{-1}$) (Cabrera-Vique, Teissedre, Cabanis, & Cabanis, 1997). In other research, Kristl et al. (2002) found Cr contents in wine samples that were similar to the results at hand (5.2–23.1 $\mu g\,L^{-1}$). Lendínez, López, Cabrera, and Lorenzo (1998) reported Cr concentrations in beer of 3.94–30.10 $\mu g\,L^{-1}$ and in cider of 3.85–15.30 $\mu g\,L^{-1}$. In relation to other spirituous beverages, the chromium contents for Galician *Orujo* are only comparable to those obtained by Canuto et al. (2003) in *Cachaça* samples (0.64–1.53 $\mu g\,L^{-1}$), which were still higher than those found in Galician samples.

The Ni contents ranged between <LOD and 34.17 $\mu g\,L^{-1}$. 27.5% of the samples were lower than the LOD obtained and 62% were lower than 3 $\mu g\,L^{-1}$. The values found do not agree with the results shown by Dugo et al. (2004), which detected low amounts of Ni in distillates (<1.0 $\mu g\,L^{-1}$). However, the same authors have reported higher values for soft drinks (12.0–105.0 $\mu g\,L^{-1}$).

An analysis of variance (ANOVA) was applied to the Cr and Ni values in the samples studied in order to find out if there were significant differences between the elaboration methods employed (*alquitara*, *alambique* and industrial elaboration). The *P*-values obtained showed that there are no significant differences between the levels of Ni or Cr present in the three kinds of samples studied.

4. Conclusions

Different ETAAS methods (based on conventional modifiers Pd $(NO_3)_2$ and on permanent modifiers W, Ir, Ru, W–Ru and W–Ir) were developed for chromium determination in *Orujo* spirit samples. It was demonstrated that there exists a matrix effect for some modifiers evaluated. However, in the case of the Pd, W and Ir based methods the absence of a matrix effect allowed for a direct measurement in calibration curves. Of all the methods developed, those based on the Ru permanent modifier presented suitable analytical characteristics and also appropriate sensitivity for Cr determination in low level samples. In the case of nickel, it was demonstrated that the use of any chemical modifiers to achieve a proper method for its determination in *Orujo* samples with good analytical figures is not necessary. Therefore, the ETAAS method based on Ru as a permanent chemical modifier has been chosen as the most suitable procedure for Cr determination, and a method without chemical modification was selected for Ni determination in *Orujo* samples. The usefulness of these methods have been demonstrated by determining the Cr and Ni contents in 80 *Orujo* spirit samples from Galicia with and without Certification Brand of Origin and elaborated with two different distillation devices. The Cr and Ni content range found in these samples was comprised between <LOD and 14.61 $\mu g\,L^{-1}$ and <LOD and 34.17 $\mu g\,L^{-1}$, respectively. In this study, no significant differences as per the distillation device employed (*alquitara*, *alambique* or industrial distillation) for the two analyzed metals were detected. However, a more comprehensive study (with a higher number of *Orujo* spirit samples) should be performed in order to obtain an evaluation of exactly how the system used in the production of *Orujo* influences the metal content found in these alcoholic spirits.

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References

Acar, O. (2004). Electrothermal atomic absorption spectrometric determination of cadmium and lead in environmental, botanic and biological samples by different permanent modifiers. *Journal of Analytical Atomic Spectrometry, 19*, 709–711.

Aceto, M., Abollino, O., Bruzzoniti, M. C., Mentasti, E., Sarzanini, C., & Malandrino, M. (2002). Determination of metals in wine with atomic spectroscopy (Flame-AAS, GF-AAS and ICP-AES), a review. *Food Additives and Contaminants, 19*, 126–133.

Almeida Pereira, L., Amorim, I., & Borba da Silva, J. B. (2006). Determination of cadmium, chromium and lead in marine sediment slurry samples by electrothermal atomic absorption spectrometry using permanent modifiers. *Talanta, 68*, 771–775.

Alvarez-Cabal, E., Wrobel, E., Marchante, J. M., & Sanz-Medel, A. (1994). A. Determination of chromium in biological fluids by electrothermal atomic absorption spectrometry using wall, platform and probe atomization form different graphite surfaces. *Journal of Analytical Atomic Spectrometry, 9*, 117–123.

Alves-Lelis, K. L., Gonzalves-Magalhaes, C., Aparecida Rocha, C., & Borba da Silva, J. B. (2002). Direct determination of Cr and Cu in urine samples by electrothermal atomic absorption spectrometry using ruthenium as permanent modifier. *Analytical and Bioanalytical Chemistry, 374*, 1301–1305.

Anderson, R. A., & Kozlowsky, A. S. (1985). Chromium intake, absorption and excretion of subjects consuming self-selected diets. *The American Journal of Clinical Nutrition, 41*, 1177–1183.

Barbosa, F., Simiao de Souza, S., Santos, D., & Krug, F. J. (2004). Evaluation of electrodeposited tungsten chemical modifier for direct determination of chromium in urine by ETAAS. *Microchemical Journal, 78*, 7–13.

Bas, B. (2006). Refreshable mercury film silver based electrode for determination of chromium (VI) using catalytic adsorptive stripping voltammetry. *Analytica Chimica Acta, 570*, 195–201.

Bermejo-Barrera, P., Moreda-Piñeiro, A., Moreda-Piñeiro, J., & Bermejo-Barrera, A. (1998). Comparative study on the use of Ir, W and Zr-coated graphite tubes for the determination of chromium in slurries of human scalp hair by electrothermal atomic absorption spectrometry. *Fresenius' Journal of Analytical Chemistry, 360*, 712–716.

BOE 194 (2005), Orden APA/2668/2005 de 20 de Julio de 2005. *Boletín Oficial del Estado, 194*, 28516–28519.

Burguera, J. L., Burguera, M., Rondon, C., Rodríguez, L., Carrero, P., Petit de Peña, Y., et al. (1999). Determination of chromium in urine by electrothermal atomic absorption spectrometry using different chemical modifiers. *Journal of Analytical Atomic Spectrometry, 14*(5), 821–825.

Cabrera, C., Lloris, F., Giménez, R., Olalla, M., & López, M. C. (2003). Mineral content in legumes and nuts: Contribution to the Spanish dietary intake. *Science of the Total Environment, 308*(1–3), 1–14.

Cabrera-Vique, C., Teissedre, P. L., Cabanis, M. T., & Cabanis, J. C. (1997). Determination and levels of chromium in French wine and grapes by graphite furnace atomic absorption spectrometry. *Journal of Agricultural and Food Chemistry, 45*, 1808–1811.

Canuto, M. H., Siebald, H. G. L., de Lima, G. M., & Borba Silva, J. B. (2003). Antimony and chromium determination in Brazilian sugar cane spirit, cachaça, by electrothermal atomic absorption spectrometry using matrix matching calibration and ruthenium as permanent modifier. *Journal of Analytical Atomic Spectrometry, 18*, 1404–1406.

Cempel, M., & Nikel, G. (2006). Nickel: A review of its sources and environmental toxicology. *Polish Journal of Environmental Studies, 15*, 375–382.

Commission of the European Communities (2005). Council Regulation (EEC) No 1576/89 of 29 May 1989 laying down general rules on the definition, description and presentation of spirit drinks. *Official Journal of the European Communities, L, 160*, 0001–0017.

Cristensen, J. M., Kristiansen, J., Nielsen, N. H., Menne, T., & Byrialsen, K. (1999). Nickel concentrations in serum and urine of patients with nickel eczema. *Toxicology Letters, 108*(2–3), 185–189.

Currie, L. A. (1995). Nomenclature in evaluation of analytical methods including detection and quantification capabilities. *Pure Applied Chemistry, 67*(10), 1699–1723.

DOG 193 (1993). Orde de 16 de setembro de 1993. *Diario Oficial de Galicia, 193*, 6640–6651.

Dugo, G., La Pera, L., Lo Turco, V., Di Bella, G., & Salvo, F. J. (2004). Determination of Ni(II) in beverages without any sample pre-treatment by adsorptive stripping chronopotentiometry (AdSCP). *Journal of Agricultural and Food Chemistry, 52*, 1829–1834.

García, E. M., Cabrera, C., Sánchez, J., Lorenzo, M. L., & López, M. C. (1999). Chromium levels in potable water, fruit juices and soft drinks: influence on dietary intake. *Science of the Total Environment, 241*, 143–150.

Jurado, J. M., Martín, M. J., Pablos, F., Moreda-Piñeiro, A., & Bermejo-Barrera, P. (2007). Direct determination of copper, lead and cadmium in aniseed spirits by electrothermal atomic absorption spectrometry. *Food Chemistry, 101*, 1296–1304.

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.

Lendínez, E., López, M. C., Cabrera, C., & Lorenzo, M. L. (1998). Determination of chromium in wine and other alcoholic beverages consumed in Spain by electrothermal atomic absorption spectrometry. *Journal of AOAC International, 8*, 1043–1047.

Li, Y., Van Loon, J. C., & Barefoot, R. R. (1993). Preconcentration of trace element in potable liquid by means of a liquid membrane emulsion for flame atomic absorption determination. *Fresenius' Journal of Analytical Chemistry, 345*, 467–470.

Lima, E. C., Barbosa, F., & Krug, F. J. (1999). Tungsten–rhodium permanent chemical modifier for lead determination in sediment slurries by electrothermal atomic absorption spectrometry. *Journal of Analytical Atomic Spectrometry, 14*, 1913–1918.

Lima, E. C., Brasil, J. L., & Santos, A. H. D. P. (2003). Evaluation of Rh, Ir, Ru, W–Rh, W–Ir and W–Ru as permanent modifiers for the determination of lead in ashes, coals, sediments, sludges soils and freshwaters by electrothermal atomic absorption spectrometry. *Analytica Chimica Acta, 484*, 233–244.

Magalhaes, C. G., Nunes, B. R., Giacomelli, M. B., & Borba da Silva, J. B. (2003). Direct determination of bismuth in urine samples by electrothermal atomic absorption spectrometry: Study of chemical modifiers. *Journal of Analytical Atomic Spectrometry, 18*(7), 787–789.

Meeravali, N. N., & Kumar, S. J. (2000). Slurry-sampling with rapid atomization versus microwave digestion with conventional atomization for the determination of copper, manganese and nickel in algae matrix using transverse heated-electrothermal atomic absorption spectrometry. *Analytica Chimica Acta, 404*, 295–302.

Meeravali, N. N., & Kumar, S. J. (2001). The utility of a W–Ir permanent chemical modifier for the determination of Ni and V in emulsified fuel oils and naphta by transverse head electrothermal atomic absorption spectrometer. *Journal of Analytical Atomic Spectrometry, 16*, 527–532.

Paiva de Oliveira, A., Gomez Neto, J. A., Nóbrega, J. A., & Oliveira, P. V. (2005). Electrothermal AAS determination of Ni in soft drinks using Co as the internal standard. *Atomic Spectroscopy, 26*, 158–161.

Queiroz, Z. F., Rocha, F. R. P., Knapp, G., & Krug, F. J. (2002). Flow system with in-line separation/preconcentration coupled to graphite furnace atomic absorption spectrometry with W–Rh permanent modifier for copper determination in seawater. *Analytica Chimica Acta, 463*(2), 275–282.

Rodríguez García, J. C., Barciela García, J., Herrero Latorre, C., García Martín, S., & Peña Crecente, R. M. (2005). Direct and combined methods for the determination of chromium, copper, and nickel in honey by electrothermal atomic absorption spectroscopy. *Journal of Agricultural and Food Chemistry, 53*, 6616–6623.

Vassileva, E., Baeten, H., & Hoenig, M. (2001). Advantages of the Iridium permanent modifier in fast programs applied to trace-element analysis of plant samples by electrothermal atomic absorption spectrometry. *Fresenius ́ Journal of Analytical Chemistry, 369*, 491–495.

Vilar, M., Barciela, J., García-Martín, S., Peña, R. M., & Herrero, C. (2007). Comparison of different permanent chemical modifiers for lead determination in *Orujo* spirits by electrothermal atomic absorption spectrometry. *Talanta, 71*(4), 1629–1636.

Vilar Fariñas, M., Barciela García, J., García Martín, S., Peña Crecente, R. M., & Herrero Latorre, C. (2007). Direct determination of cadmium in *Orujo* spirit samples by electrothermal atomic absorption spectrometry: Comparative study of different chemical modifiers. *Analytica Chimica Acta, 591*, 231–238.

Viñas, P., Campillo, I., López, M., & Hernández, M. (1995). Rapid procedure for cobalt and nickel determination in slurries food samples by electrothermal atomic absorption spectrometry. *Atomic Spectroscopy, 16*(2), 86–89.

Viñas, P., Pardo-Martínez, M., & Hernández-Córdoba, M. (2000). Determination of copper, cobalt, nickel, and manganese in baby food slurries using electrothermal atomic absorption spectrometry. *Journal of Agricultural and Food Chemistry, 48*, 5789–5794.

Welz, B., & Sperling, M. (1999). *Atomic absorption spectrometry* (3rd ed.). Weinheim, Germany: Wiley-VCH.