

## Direct and Combined Methods for the Determination of Chromium, Copper, and Nickel in Honey by Electrothermal Atomic Absorption Spectroscopy

J. C. RODRÍGUEZ GARCÍA, J. BARCIELA GARCÍA, C. HERRERO LATORRE,\*  
S. GARCÍA MARTÍN, AND R. M. PEÑA CRECENTE

Departamento de Química Analítica, Nutrición y Bromatología, Facultad de Ciencias, Universidad de Santiago de Compostela, Campus de Lugo, Avenida Alfonso X El Sabio s/n, 27002 Lugo, Spain

In the present work, direct methods for the determination of chromium, copper, and nickel in honey by electrothermal atomic absorption spectroscopy were developed using experimental design as an optimization tool. Once the optimum conditions for the individual methods were established, a direct method for the combined determination of the three elements was optimized using the response surface tool. Palladium was used as chemical modifier in all cases. Honey was diluted in water, hydrogen peroxide, and nitric acid. Triton X-100 was added to minimize the matrix effect and the viscosity of the sample. The RSD (better than 10%) and the analytical recovery (98–103%) were acceptable for all of the developed methods. Calibration graphs were used in the four methods to determine the concentration of the analytes in the sample. The detection limits of the combined method (0.21, 0.35, and 0.37  $\mu\text{g L}^{-1}$  for Cr, Cu, and Ni, respectively) were similar to those obtained for the individual methods (LOD = 0.17, 0.21, 0.33  $\mu\text{g L}^{-1}$  for Cr, Cu, and Ni, respectively). The direct-combined proposed method has been applied to the determination of chromium, copper, and nickel content in representative honey samples from Galicia (northwestern Spain). The concentrations found in the analyzed samples were in the range of (5.75  $\pm$  0.64)–(26.4  $\pm$  0.38) ng g<sup>-1</sup> of Cr, (79  $\pm$  7.8)–(2049  $\pm$  80) ng g<sup>-1</sup> of Cu, and (12.6  $\pm$  1.36)–(172  $\pm$  6.88) ng g<sup>-1</sup> of Ni.

**KEYWORDS:** ETAAS; chromium; copper; nickel; combined determination; honey; chemical modification; palladium

### 1. INTRODUCTION

Nickel, chromium, and copper are heavy metals emitted to the atmosphere from industrial sources: mining production, ore refining, chemical manufacture, coal-fired power plants, and combustion of fossil fuels. Metal particles may be spread on the earth, or they can fall with rain drops after reactions (1). Nickel, chromium(VI), and copper are toxic at high concentrations; however, traces of Cu and Cr(III) (2) are essential for human life. The functions of Ni in the organism are not well understood, and it has not been established as an essential element in human beings (3).

Knowledge of environmental pollution requires that the polluting substances be monitored. However, detection and measurement of certain pollutants in the environment are not evident. In the past few years, several methods (4) with appropriate analytical characteristics and acceptably low cost and based on the use of bioindicators (such as algae, lichens, and pine needles) have been proposed. Since 1935, many studies have considered beehive products to be adequate environmental

markers for certain pollutants such as heavy metals. Honey is a good biological indicator as long as heavy metals present in the atmosphere are translocated from the environment to the flower's nectar and finally to honey (5). Therefore, honey analysis is considered to be an appropriate measure of the surroundings in which the hive is located. Being a widely consumed product, information records about metal content in honey are relevant to ensure its quality (6). In addition, the combination of trace and metal content data of honey with modern statistical data evaluation techniques is a good approach to detect geographical and botanical origins of the product (7). For all of these reasons, simple, cheap, and exact methods for determining heavy metals in honey are widely demanded by producers, consumers, and regulatory bodies.

Electrothermal atomic absorption spectroscopy (ETAAS) is one of the most useful and powerful analytical techniques for the determination of trace and ultratrace elements in different samples. Other techniques such as inductively coupled plasma atomic emission spectroscopy (ICP-AES) or inductively coupled plasma mass spectrometry (ICP-MS) are usually employed for multielemental honey analysis (8–10). However, these two techniques also present inconveniences. Honey sample analysis

\* Corresponding author (telephone +34-982 285 900, ext. 24064; fax +34-982 285 872; e-mail cherrero@lugo.usc.es).

by ICP needs a previous sample treatment (11); in addition, ICP-MS has a high detection power (comparable to the ETAAS), the MS detector is rather complex and expensive, which limits the widespread use of ICP-MS for routine analytical works (4), and it involves other instrumental difficulties (for example, the isobaric interferences affecting the Cr signals) (12). It seems that ETAAS is no longer attractive in comparison with ICP, but this technique is still being used because it combines fast analysis time, relative simplicity, cheaper cost, low sample volume requirements, and low detection limits (13). All of these features have been responsible for its widespread use for the determination of trace amounts of heavy metals in biological samples such as honey.

In the present paper, a combined method for the determination of three elements has been carried out after the optimization of three determination ETAAS methods for the determination of chromium, nickel, and copper in honey without any sample pretreatment. This procedure implies the direct sample introduction into the graphite tube (avoiding the sample manipulation and saving time of analysis) and the use of nitric acid and hydrogen peroxide (14) to help the sample mineralization.

Experimental design has been used to optimize the methods proposed. Plackett–Burman designs were applied as a screening method to evaluate the most significant factors with few experiments (15). The optimum conditions for the significant parameters have been obtained using a central composite design that starts with a two-level factorial and some centerpoints (16). Once the optimum conditions for each metal were found, the common conditions were obtained by studying the individual response surfaces in order to develop a combined method. Also in this case, the experimental design applied offered a fast and appropriate tool to carry out the optimization of this combined method. The combined method shortened considerably the time of analysis because the sample was prepared at the same time to measure the three metals.

## 2. EXPERIMENTAL PROCEDURES

**2.1. Instrumentation.** An atomic absorption spectrometer, Varian-SpectrAA-600, with Zeeman correction equipped with a Varian GTA-100 electrothermal atomizer linked to an automatic sample dispenser was used for this work. Measurements were performed using a multielemental Varian hollow cathode lamp (current intensity = 10 mA), operating at 359.7, 327.4, and 232.0 nm for Cr, Cu, and Ni, respectively. The bandwidths were 0.2, 0.5, and 0.2 nm, respectively. Argon was employed as inert gas at 3 L min<sup>-1</sup>. Pyrolytic graphite-coated tubes were used. A Varian forked platform was studied. A domestic microwave oven was used for the digestion procedure.

**2.2. Reagents.** Stock standard solutions of Cr, Ni, and Cu (1.000 ± 0.002 g L<sup>-1</sup>) were obtained from Panreac (Barcelona, Spain) and diluted as necessary to obtain working standard solutions.

Nitric acid (65% w/v) Suprapur reagent was obtained from Merck (Darmstadt, Germany).

Hydrogen peroxide (33% w/v) was purchased from Panreac.

Triton X-100 was from Merck.

Palladium was obtained from Fluka (Buchs, Switzerland). The palladium modifier working solution (2000 mg L<sup>-1</sup>) was prepared by diluting the appropriate amount of a 10 g L<sup>-1</sup> stock solution with ultrapure water.

Magnesium nitrate was purchased from Fluka. The working solution (2000 mg L<sup>-1</sup>) was prepared by diluting the appropriate amount of a 10 g L<sup>-1</sup> stock solution with ultrapure water.

High-purity water used was provided by a Milli-Q deionizing system (Millipore, Bedford, MA).

All sample containers, autosampler cups, and all other material employed were washed with nitric acid 10% v/v for 24 h before rinsing with copious amounts of ultrapure water and shaking dry prior to use.

The cleaning solution employed to wash the sampling capillary contained 0.7% (w/v) HNO<sub>3</sub> and 0.2% (v/v) Triton X-100.

**2.3. Statistical Software.** Experimental design and surface and contour plots were carried out using Statgraphics (5.0 plus) software package.

**2.4. Honey Samples.** Eighteen representative honey samples from Galicia (northwestern Spain) with guaranteed origin and processed using the traditional procedures in the producing region were provided by Galician Associations of Beekeepers (APLA). All of the samples examined were honeys of random (mixed) floral type, coming from an area considered to be unpolluted and far from large industrial plants. Samples were collected in glass bottles and stored in the dark at 3–4 °C until analysis. Three replicates of each sample were measured in duplicate.

**2.5. Sample Preparation.** The determination of chromium, copper, and nickel by ETAAS was carried out directly on the sample diluted with ultrapure water. One gram of honey was quantitatively transferred to a 5 mL volumetric flask and made up to the mark with ultrapure water. The sample working solution was daily prepared.

**2.6. Analytical Procedures.** **2.6.1. Copper Determination.** One hundred microliters of diluted honey was mixed in the autosampler cup with 20 μL of Triton X-100 (1%), 65 μL of hydrogen peroxide (33% w/v), and 65 μL of HNO<sub>3</sub> (10%) and made up to 1000 μL with ultrapure water. Twelve microliters of this solution was introduced in the graphite tube together with 18 μL of Pd solution (1000 μg mL<sup>-1</sup>).

**2.6.2. Chromium Determination.** Five hundred microliters of diluted honey (prepared as described in section 2.5) was mixed in the autosampler cup with 20 μL of Triton X-100 (1%), 300 μL of hydrogen peroxide (33% w/v), and 50 μL of HNO<sub>3</sub> (10%) and made up to 1000 μL with ultrapure water. Twelve microliters of this solution was introduced in the graphite tube together with 18 μL of Pd solution (750 μg mL<sup>-1</sup>).

**2.6.3. Nickel Determination.** Five hundred microliters of diluted honey was mixed in the autosampler cup with 20 μL of Triton X-100 (1%) and 160 μL of hydrogen peroxide (33% w/v) and made up to 1000 μL with ultrapure water. Twelve microliters of this solution was introduced in the graphite tube together with 18 μL of Pd solution (1220 μg mL<sup>-1</sup>).

**2.6.4. Combined Method (Determination of Chromium, Copper, and Nickel).** Five hundred microliters of diluted honey (prepared as described in section 2.5) was mixed in the autosampler cup with 20 μL of Triton X-100 (1%), 260 μL of hydrogen peroxide (33% w/v), and 65 μL of HNO<sub>3</sub> (10%) and made up to 1000 μL with ultrapure water. Twelve microliters of this solution was introduced in the graphite tube together with 18 μL of Pd solution (800 μg mL<sup>-1</sup>).

In all of the methods, the mixtures obtained were introduced into the graphite furnace and subjected to AAS under the optimized conditions listed in **Table 1**.

**2.6.5. Digestion Procedure.** The results obtained for the Cr, Cu, and Ni determinations in honey employing the direct and combined methods described above were compared with those achieved when the honey was previously digested using microwave energy. In this case, 0.6 g of honey was accurately weighed, transferred to a 30 mL PTFE vessel, and mixed with 1 mL of HNO<sub>3</sub> (2.5% w/v).

The closed vessel was subjected to (600 W) microwave energy during 40 s. After cooling, 425 μL of H<sub>2</sub>O<sub>2</sub> (30% w/v) and 575 μL of Milli-Q water were added. A new microwave heating procedure under the same conditions was carried out (600 W and 40 s). The digested sample was diluted to 25 mL with ultrapure water and analyzed using the ETAAS method.

## 3. RESULTS AND DISCUSSION

**3.1. Optimization of the Direct Methods.** To select the best graphite furnace for the determination of the three metals, preliminary tests have been performed using graphite furnaces with and without platform. Chromium measurements cannot be fully performed under stabilized temperature platform furnace (STPF) conditions. The use of a carbon L'vov platform is circumvented due to the refractor character of Cr; however,

**Table 1.** Optimum Furnace Conditions and Reagent Concentrations for Direct and Combined Chromium, Copper, and Nickel Determination in Honey Samples

Furnace Parameters					
step	parameter	method			
		Cu	Cr	Ni	combined
dry	temp (°C)	105	105	105	105
	ramp (s)	10	10	15	10
	hold (s)	20	30	20	20
ash	temp (°C)	900	1265	1200	900
	ramp (s)	10	10	10	10
	hold (s)	15	40	10	15
atomization	temp (°C)	2500	2700	2620	2700
	ramp (s)	0.7	0.7	0.8	0.9
	hold (s)	2.5	2.5	2.5	2.5
clean	temp (°C)	2600	2700	2700	2700
	ramp (s)	2	2	2	2
	hold (s)	0	0	0	0

Reagent Concentrations					
		method			
		Cu	Cr	Ni	combined
HNO <sub>3</sub> (%)		0.66	0.50	0	0.66
H <sub>2</sub> O <sub>2</sub> (%)		6.50	30.0	16	26
Triton X-100 (%)		0.02	0.02	0.02	0.02
Pd (mg L <sup>-1</sup> )		2000	1500	2448	1600
Mg (mg L <sup>-1</sup> )		0	0	0	0

Nunes et al. (17) and Canuto et al. (18) have proposed an atomization wall for this metal. Filgueiras et al. (19) used tubes without platform for Ni and Cr. Silva et al. (20) employed a wall atomization for Cu determination in coal. In the present work, previous experiments indicated that the best analyte signals were obtained when the analyses were carried out using a graphite furnace without platform (see **Figure 1**).

Because honey presented a high viscosity, the sample was introduced in the graphite tube with Triton X-100. The addition of this reagent favored the drop fall, improving the repeatability of the analytical signals. The addition of nitric acid and hydrogen peroxide has a great influence in the ashing stage efficiency. Taking into account that the sample was introduced in the atomizer without any previous treatment, these two compounds were added to prevent the formation of carbonaceous residues inside the tube. In all cases, the use of soft conditions was preferred to preserve the pyrolytic atomizer from premature deterioration.

Certain authors do not recommend the use of chemical modification for the measurement of the considered metals in certain samples. Prohaska et al. (21) determined Cu and Cr in blood; Meereveli et al. (4) analyzed Cu and Ni in algae matrix, and Cabrera et al. (3) measured the same metals in vegetables, in all cases without any modifier. Also, for the Cr determination in serum, Nunes et al. (17) proved that when modifier was used, the peak obtained was irregular and noisy and the method presented lower sensitivity than without modifier. However, for the Cr determination other authors used chemical modifiers (either in the sample pretreatment or directly introduced into the atomizer) that would promote the formation of Cr<sub>2</sub>O<sub>3</sub> (22). Therefore, different modifiers were assayed: Pd (23, 24), Mg-(NO<sub>3</sub>)<sub>2</sub> (4, 25, 26), Eu, Ni(NO<sub>3</sub>)<sub>2</sub> (22), and permanent modifiers such as Ru (18) or Ir (recommended for Ni and Cr determination) (27, 28). Cu is an element successfully determined by ETAAS without modification. Viñas et al. (29) measured Cu in honey without chemical modifiers. However, Grotti et al.

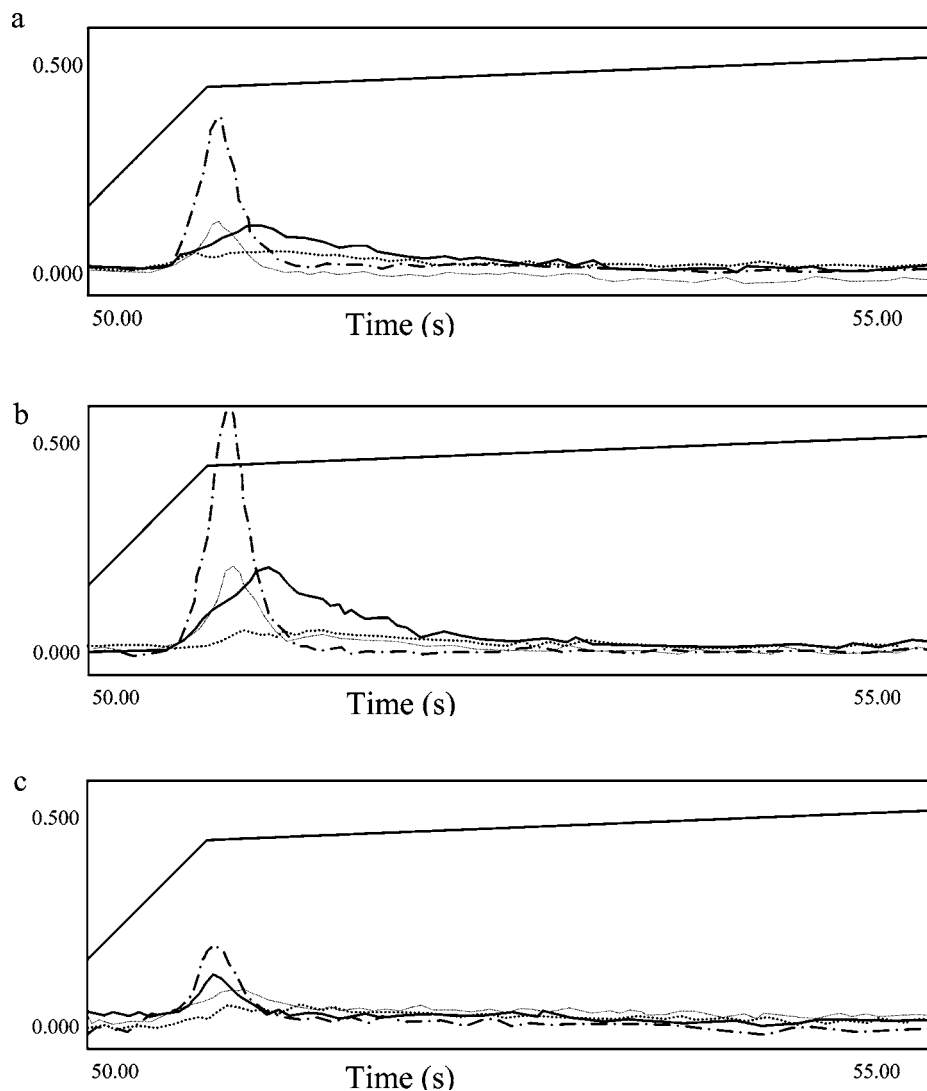
(30) analyzed Cu employing oxalic acid as well as Pd(NO<sub>3</sub>)–Mg(NO<sub>3</sub>)<sub>2</sub> as modifiers. In the case of Ni determination, ammonium phosphate (31, 32) magnesium nitrate (33), and palladium (23, 24, 34, 35) have been intended as chemical modifiers. In the present paper, the selection of the employed modifiers has been made by keeping in mind their universal character (which is appropriate for a combined multielemental method such as the one developed in this work). Thus, palladium and magnesium nitrate were studied as modifiers in the direct and combined methods for Cr, Cu, and Ni determination.

Experimental design was used to optimize all methods. First, Plackett–Burman fractional factorial design was applied as a screening approach with the aim of establishing the statistically significant factors affecting the Cu, Ni, and Cr signals. Second, a central composite design was performed to evaluate the response surfaces. The optimum conditions were established in this way for the direct and combined methods. Maximum and minimum levels for the factors assayed were chosen according to our previous experiences and by taking into account the values reported in the literature. In all cases, the initial strategy for the first screening was to work with wide condition intervals. For each method, the factors assayed as well as their high and low values are summarized in **Table 2**.

**3.1.1. Copper Direct Method Optimization.** A Plackett–Burman design was carried out with the purpose of finding those factors of greater statistical significance. Nine factors have been studied in 13 randomized runs. As can be seen in the Pareto chart (**Figure 2a**), nitric acid and Triton X-100 concentrations as well as dry and mineralization temperatures were the most significant factors affecting the Cu signal. The effect of these significant factors was studied by applying a central composite design 2<sup>4</sup>+ star, in 26 runs. In **Figure 2b**, the response surfaces for nitric acid and Triton X-100 concentrations are presented. The Cu signal increases with the two reagents, up to optimum values of 0.66% for the nitric acid and 0.02% for the Triton X-100 concentrations. For higher concentrations the response signal started a diminution. For dry temperature, the signal diminished progressively for high temperatures; therefore, the lowest dry temperature was chosen (105 °C). The absorbance signal increased slightly when ash temperatures increased, reaching a maximum for 900 °C, which was selected as the optimum.

In relation with the chemical modifiers, the previous screening study illustrated the influence of palladium in the analytical signal and demonstrated that magnesium nitrate addition was not advisable for the Cu determination (see **Figure 2a**). Palladium modifier concentrations were studied, and the optimum value obtained was 2000 mg L<sup>-1</sup> (injecting 9 μL). The Cu signal enlarged considerably up to 1200 mg L<sup>-1</sup> and enlarged slightly up to the optimum. From the optimum to 2400 mg L<sup>-1</sup>, a weak diminution was observed. For values over this concentration, the negative effect was clearly evident.

**3.1.2. Chromium Direct Method Optimization.** A two-level Plackett–Burmann design was used to perform the preliminary factor screening. Eight factors were evaluated in 13 randomized runs. Triton X-100 concentration was not introduced in the screening because it has been proved in previous experiences and in other published works (6, 36) that its behavior is always similar. For this reason the same optimum concentration (0.02% for Cu) was used in all of the developed methods. The results for the factors producing the main effects are presented in the Pareto chart (*P* = 95%). As can be seen in **Figure 3a**, the atomization temperature and hydrogen peroxide concentration were the factors statistically significant in Cr determination. A



**Figure 1.** Chromium (a), copper (b), and nickel (c) peaks and background profiles using graphite tubes with platform (—, ···) and without platform (---, -).

**Table 2.** Factors Evaluated for the Four Developed Methods

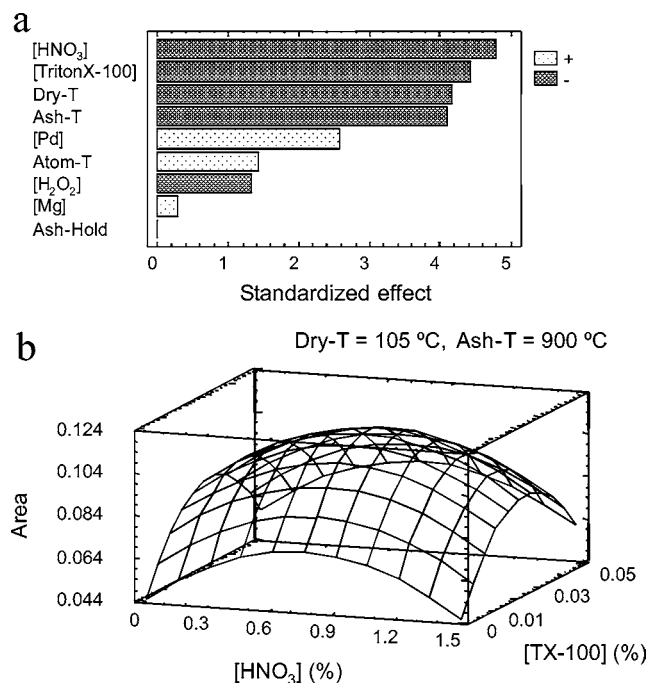
factor	method					
	Cr		Ni		Cu	
	low	high	low	high	low	high
[H <sub>2</sub> O <sub>2</sub> ] (%)	0	20	0	30	5	30
[HNO <sub>3</sub> ] (%)	0.5	2.0	0	2.0	0.5	2.0
[Triton X-100] (%)					0.01	0.05
[Pd] (mg L <sup>-1</sup> )	0	3000	0	3000	0	2000
[Mg(NO <sub>3</sub> ) <sub>2</sub> ] (mg L <sup>-1</sup> )	0	3000	0	3000	0	1000
dry temp (°C)	105	300	105	200	105	300
ash temp (°C)	1100	1900	500	1600	500	1300
atomization temp (°C)	2300	2700	2200	2700	1800	2500
ash hold	10	40	10	40	10	40

central composite design 2<sup>3</sup>+ star, in 16 runs, was carried out to study the effect of the atomization temperature and hydrogen peroxide and nitric acid concentrations for the chromium signal using the response surface obtained. The response signal increases continuously when the atomization temperature and hydrogen peroxide concentration increase to the extreme values of 2700 °C and 30%, respectively (see **Figure 3b**). Higher values were not assayed due to the severe atomizer deterioration produced for these strong conditions. Despite their minor significance in the screening step, nitric acid concentration was

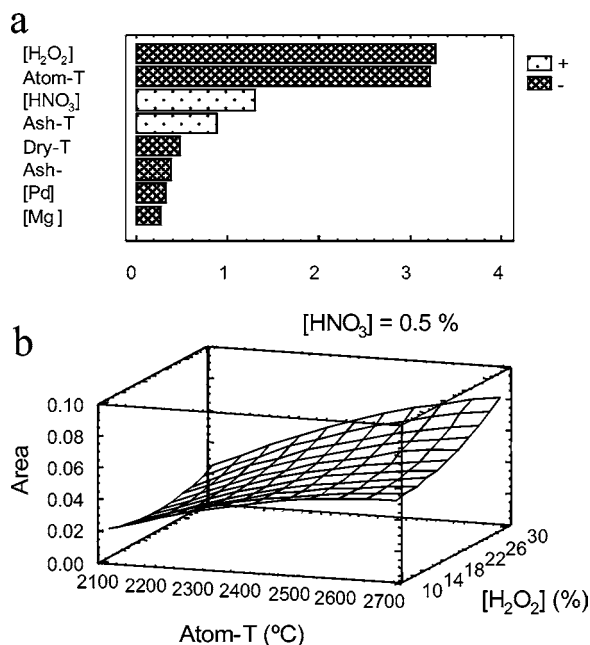
also optimized in this study. The optimum concentration was 0.50%; the Cr signal increased slightly until this value and then diminished slightly.

The effect of modifiers (Pd and Mg) was also studied. The Cr signal increases progressively up to palladium concentrations of 1500 mg L<sup>-1</sup>; higher concentrations produced a signal diminution. According to the screening step, magnesium concentration was one of the less influential factors in the Cr response. On the other hand, the addition of Mg produced high absorbance blank values. For these reasons, as in the case of Cu determination, magnesium was not used as modifier because it does not show any advantage.

**3.1.3. Nickel Direct Method Optimization.** The Pareto chart (**Figure 4a**) constructed for the Ni direct method showed that dry, ash, and atomization temperatures as well as hydrogen peroxide and palladium concentrations were the most important factors affecting Ni response. These factors were optimized by means of a central composite design 2<sup>5</sup>-1+ star, in 28 runs. In **Figure 4b**, the response surfaces for dry and atomization temperatures are presented. As can be seen, the Ni signal decreased slightly when dry temperatures increased. A dry temperature in the range of 105–130 °C has been chosen as optimum. For the atomization temperature, a signal enlargement



**Figure 2.** (a) Standardized Pareto chart for copper direct method; (b) effect of Triton X-100 and HNO<sub>3</sub> concentrations on Cu absorbance area.

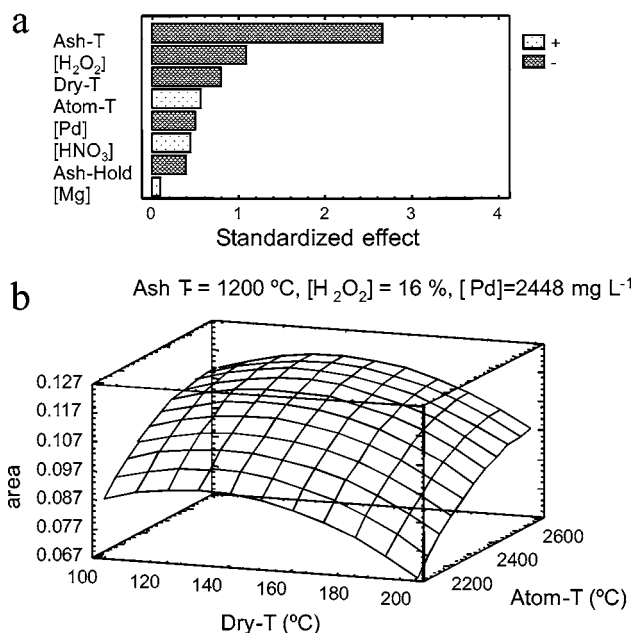


**Figure 3.** (a) Standardized Pareto chart for chromium direct method; (b) effect of hydrogen peroxide and atomization temperature on Cr area response.

was observed when the temperature increased. The maximum signal was achieved at 2620 °C.

For Ni determination, it was also established that the addition of magnesium as modifier does not improve the sensibility (see **Figure 4a**). However, the use of palladium produced a signal augmentation (more remarkable for low than for high concentrations). The optimum Pd concentration was established as 2448 mg L<sup>-1</sup>; however, in the range of 1000–3000 mg L<sup>-1</sup>, the signal variation observed was not very important.

The optimum furnace conditions and reagents concentrations for each method developed are summarized in **Table 1**.



**Figure 4.** (a) Standardized Pareto chart for nickel direct method; (b) effect of dry temperature and atomization temperature on Ni integrated area.

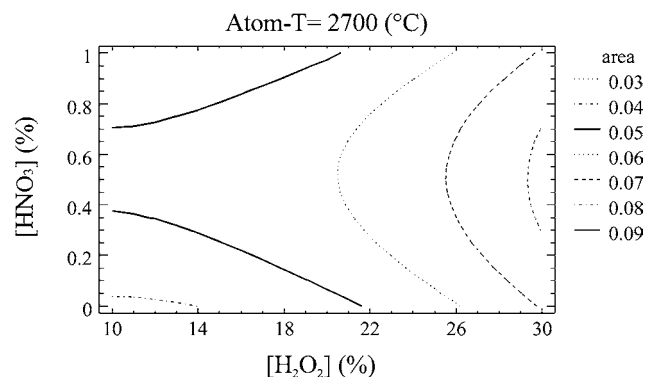
**3.2. Combined Method Optimization.** In the above explained direct methods certain variables were significant for one metal but not for the others. In addition, different optimal values for the studied features were obtained from the experimental designs applied in section 3.1. In this section (on the basis of the response surfaces obtained and their contour response plots) a selection of the values for these variables that could be used for the three methods is performed. This selection was carried out to obtain the better conditions to be applied in the combined determination of Cu, Cr, and Ni.

The hydrogen peroxide addition was demonstrated to be relevant for Cr determination ([H<sub>2</sub>O<sub>2</sub>] 30% as optimum). However, it was found to be less important for the Cu method. In the case of the Ni method, although the optimum H<sub>2</sub>O<sub>2</sub> concentration was 16%, there is not a significant signal variation (>10%) if a H<sub>2</sub>O<sub>2</sub> concentration equal to 26% is used. This is an equilibrium value causing no significant loss in the Cr, Cu, and Ni absorbance.

Nitric acid was a significant variable for Cu analysis with an optimal value of 0.66% (see section 3.1.1); for the Cr method according to the response surface obtained, the nitric acid concentration could be modified in the range of 0.3–0.70% without influence in the absorbance signal (see the contour surface plot in **Figure 5**); and, for nickel the effect observed was minimum. Therefore, 0.66% of nitric acid has been chosen as the best value for the three metals. The effect of the addition of Triton X-100 to the honey sample has been studied in this work and in other previous studies (6, 36). In all cases, the optimum Triton X-100 concentration was always 0.02%. This amount has been used as the selected concentration for the three metals in direct and combined methods.

In the three individually developed methods the effect of drying temperature was negative; when the drying temperature increases, there is a loss of sensibility as response surface graphs show. Thus, the low temperature (105 °C) has been chosen as the most favorable for the combined method.

The mineralization temperature is a significant feature. Optimum values according to the central design performed were 900 °C for Cu, 1265 °C for Cr, and 1200 °C for Ni. The mineralization temperature selected for the combined procedure



**Figure 5.** Plot of estimate response surface for the Cr absorbance according to the  $\text{HNO}_3$  and  $\text{H}_2\text{O}_2$  concentrations.

was 900 °C. This temperature has no significant influence in the signal of Cr and Ni according to the obtained response graphs. However, ashing temperatures >900 °C produced an important diminution of the Cu signal. The atomization temperature is the critical feature to build a common method; in this case the three metals studied atomize at high temperatures: 2500 °C is enough for Cu and 2620 °C for Ni. The atomization temperature selected for the combined method was 2700 °C (as for the chromium). It was proved that when this temperature is used, a loss of signal for Cu and Ni does not take place.

In the three direct methods previously optimized, the addition of certain quantities of palladium as chemical modifier is found to be adequate. For the combined determination, a concentration of 1600  $\text{mgL}^{-1}$  of palladium has been chosen, because this is the value included in the optimum intervals of each individual case.

The choice of these common conditions allows the measure of the three metals simultaneously, without losing much sensibility. The concentrations of reagents and the furnace parameters are listed in **Table 1**.

**3.3. Analytical Characteristics.** **3.3.1. Calibration.** Instrumental calibration was performed at different ranges for the direct methods (0–1.5  $\mu\text{g}$  of Cr  $\text{L}^{-1}$ , 0–16  $\mu\text{g}$  of Cu  $\text{L}^{-1}$ , and 0–8  $\mu\text{g}$  of Ni  $\text{L}^{-1}$ ) in aqueous solution and honey matrix. In the case of the combined procedure, the calibration was carried out in water and honey matrix in the range of 0–6  $\mu\text{g}$   $\text{L}^{-1}$  for Cr and Ni. For Cu the calibration range was between 0 and 20  $\mu\text{g}$   $\text{L}^{-1}$ . These intervals have been selected according to the concentrations found in honey samples. The equations of the calibration and standard addition graphs for all developed methods are shown in **Table 3**. From the comparison of calibration and standard addition graph slopes for the individual and combined methods, the absence of a significant matrix effect was pointed out in all cases. Therefore, direct calibration with aqueous standard solutions was applied for the measurement of the studied metals in honey.

**3.3.2. Detection and Quantification Capabilities.** Detection limits (LOD) were calculated on the basis of  $3\text{SD}/m$ , where  $m$  is the slope of the addition graph and SD the standard deviation

**Table 4.** Characteristic Mass, Detection Limit, Quantification Limit, and Relative Standard Deviation Obtained for the Four Developed Methods

method	element	$m_0$ (pg)	LOD ( $\mu\text{g L}^{-1}$ )	LOQ ( $\mu\text{g L}^{-1}$ )	RDS <sup>a</sup> (%)
individual	Cr	2.3	0.17	0.58	9.0; 8.8; 8.4; 8.3
	Cu	12.3	0.21	0.68	7.0; 6.6; 5.2; 4.8
	Ni	12.2	0.33	1.07	9.9; 9.8; 8.8; 6.0
combined	Cr	3.3	0.21	0.73	9.1; 3.2; 3.1; 1.1
	Cu	12.2	0.35	1.18	4.4; 1.8; 2.5; 2.6
	Ni	12.8	0.37	1.20	9.9; 8.9; 5.4; 3.5

<sup>a</sup> At the four concentration levels assayed.

**Table 5.** Recovery for the Individual and Combined Methods for Chromium, Copper, and Nickel Determination

element	individual		combined	
	recovery (%)	range ( $\mu\text{g L}^{-1}$ )	recovery (%)	range ( $\mu\text{g L}^{-1}$ )
Cr	98–101	0.0–3.0	98–103	0.0–6.0
Cu	99–103	0.0–16.0	98–102	0.0–20.0
Ni	98–100	0.0–8.0	99–101	0.0–6.0

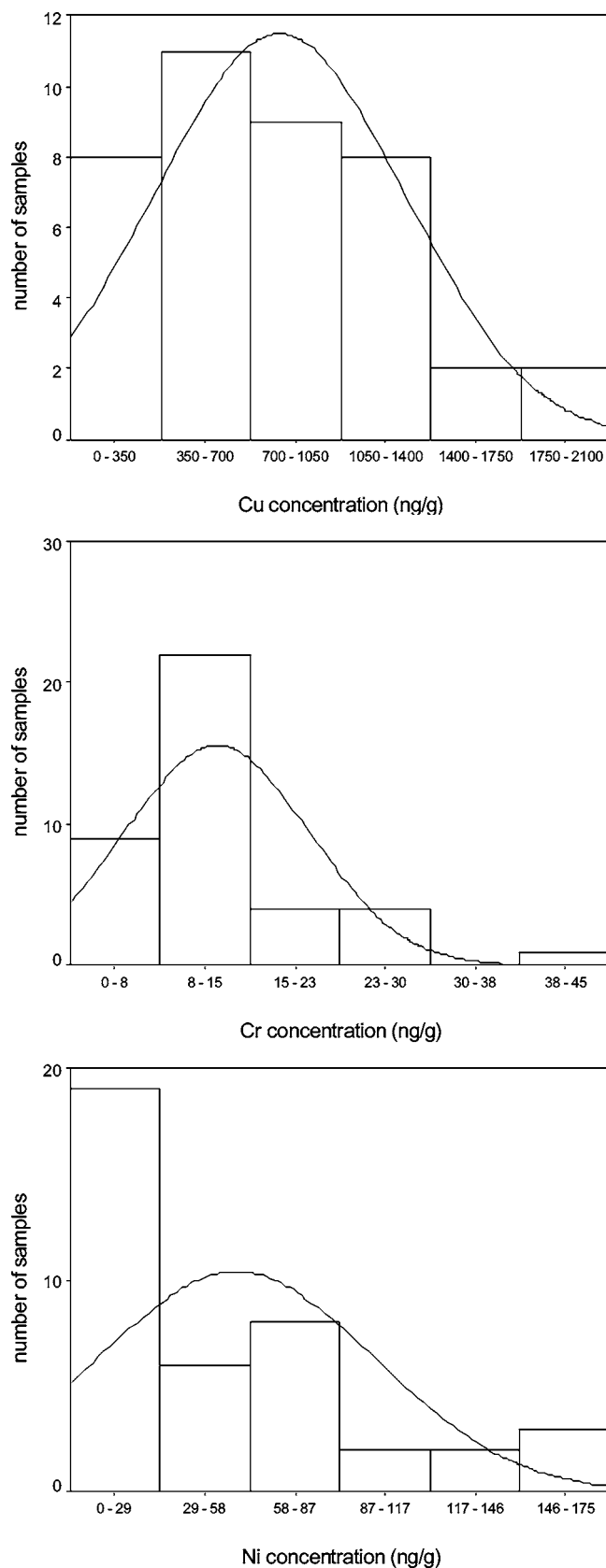
of 10 consecutive measurements of blank solutions. Quantification limits (LOQ) were calculated as  $10\text{SD}/m$ . The results obtained for LOD, LOQ, and characteristic masses (based on the integrated absorbance) are given in **Table 4**. In different ETAAS methods for Cu determination in honey (without chemical modification) proposed by other authors (29) the LOD reported (1.4  $\mu\text{g L}^{-1}$ ) was worse than that obtained in the present work. Also, Chmilenko et al. (37) obtained a poorer LOD (0.013  $\text{mg kg}^{-1}$ ) for copper determination in sugars. With respect to Cr determination, the characteristic mass reported by Burguera et al. (22) for the measurement of this element in urine samples is comparable to that obtained in the present work [2.2 and 3.3 pg using Eu or  $\text{Mg}(\text{NO}_3)_2$  as modifier, respectively]. However, the LOD they report is better (0.02–0.04  $\mu\text{g L}^{-1}$ ). The LOD for the Ni determination obtained in this work (0.33  $\mu\text{g L}^{-1}$ ) is slightly better than that reported by Man-Chiang et al. (13) in a multielemental ETAAS method for As, Mn, Co, and Ni in urine (0.35  $\mu\text{g L}^{-1}$ ). For the three metals studied in a sediment matrix Filgueiras et al. (19) reported worse detection limits than those obtained in the present paper.

As can be seen in **Table 4**, the best detection limits were obtained for the direct methods in comparison with the combined procedure, as was expected. Nevertheless, the limits obtained for the combined method are not very different from those achieved in the direct methods (except for Cu). The combined procedure reached adequate sensibility to quantify the studied metals at the levels present in honey samples.

**3.3.3. Precision.** The within-run precision was studied using honey solutions spiked with Cr (0.0, 1.0, 2.0, and 3.0  $\mu\text{g L}^{-1}$ ), Cu (0.0, 4.0, 8.0, and 16.0  $\mu\text{g L}^{-1}$ ), and Ni (0.0, 2.0, 4.0, and 8.0  $\mu\text{g L}^{-1}$ ). For the combined method the solutions were spiked with concentrations of 0.0, 2.0, 4.0, and 6.0  $\mu\text{g L}^{-1}$  for Cr and

**Table 3.** Calibration and Standard Addition Equations Obtained for Chromium, Copper, and Nickel (Micrograms per Liter) Determination

method	calibration	addition
individual	$A = 0.024(\pm 7.2 \times 10^{-4})[\text{Cr}] + 0.062(\pm 2.7 \times 10^{-4})$	$A = 0.025(\pm 8.0 \times 10^{-4})[\text{Cr}] + 0.108(\pm 9.8 \times 10^{-4})$
	$A = 0.006(\pm 5.9 \times 10^{-5})[\text{Cu}] + 0.015(\pm 5.4 \times 10^{-4})$	$A = 0.006(\pm 1.0 \times 10^{-4})[\text{Cu}] + 0.047(\pm 9.4 \times 10^{-4})$
	$A = 0.042(\pm 4.9 \times 10^{-5})[\text{Ni}] + 0.026(\pm 2.3 \times 10^{-4})$	$A = 0.039(\pm 1.5 \times 10^{-4})[\text{Ni}] + 0.044(\pm 6.8 \times 10^{-4})$
combined	$A = 0.034(\pm 4.1 \times 10^{-4})[\text{Cr}] + 0.074(\pm 1.5 \times 10^{-3})$	$A = 0.035(\pm 8.5 \times 10^{-4})[\text{Cr}] + 0.088(\pm 3.1 \times 10^{-3})$
	$A = 0.071(\pm 8.2 \times 10^{-5})[\text{Cu}] + 0.014(\pm 9.4 \times 10^{-4})$	$A = 0.068(\pm 2.3 \times 10^{-4})[\text{Cu}] + 0.050(\pm 3.1 \times 10^{-3})$
	$A = 0.041(\pm 1.8 \times 10^{-4})[\text{Ni}] + 0.019(\pm 1.7 \times 10^{-3})$	$A = 0.040(\pm 1.6 \times 10^{-4})[\text{Ni}] + 0.031(\pm 1.5 \times 10^{-3})$



**Figure 6.** Copper, chromium, and nickel content distribution in the Galician honeys analyzed.

Ni and 0.0, 5.0, 10.0, 20.0, for Cu (calibration ranges previously used). The relative standard deviation for 10 determinations of each solution was below 10% for all developed methods. The coefficients of variation for the four concentration levels assayed

with the different methods are shown in **Table 4**. In all cases an acceptable precision was obtained.

**3.3.4. Accuracy.** Even though Caroli et al. (8, 38) investigated the possibility of producing a Certified Reference Material for heavy metal determination in honey, at the present no honey CRM for Cr, Cu, and Ni is available. Thus, the accuracy of the proposed methods was evaluated by measuring the recovery on a real sample spiked with the three metals. The range used in each case was selected by taking into account the concentration found in real samples. The ranges and the recoveries achieved are shown in **Table 5**. The results confirmed an acceptable accuracy for the proposed methods with recovery ranging between 98 and 103%.

The results obtained in honey samples for Cu, Cr, and Ni by applying both the direct and combined methods were compared among them and also with those achieved when the sample was previously digested using microwave energy. Ten replicates of a Galician honey sample were analyzed by employing the direct and the combined methods as well as the digestion method described in section 2.6. The *t* test for mean comparison and the *F* test for variance comparison showed no significant differences among the developed methods at a 95% confidence level. The results provided by direct, combined, and digestion methods were comparable.

**3.4. Application.** Forty Galician honey samples were analyzed in triplicate using the combined method developed in this work. The content distribution range found for Cr, Cu, and Ni in the Galician honeys analyzed can be seen in **Figure 6**. Chromium content varied in the range from  $5.75 \pm 0.64$  to  $26.4 \pm 0.38$  ng g<sup>-1</sup>. The levels of this metal reported for Devillers et al. (39) in French honeys varied from 80 to 360 ng g<sup>-1</sup>, a higher range than found in Galician honeys. The copper concentrations for the samples studied were between  $79 \pm 7.8$  and  $2049 \pm 80$  ng g<sup>-1</sup>. These reported data indicate that the Cu inferior levels were lower than those reported by Üren et al. (40) for Turkish honeys (350–1240 ng g<sup>-1</sup>). Cu concentrations between 60 and 1320 ng g<sup>-1</sup> for other Spanish honeys (29) and between 60 and 1710 ng g<sup>-1</sup> in French honeys (39) were also reported. Although the higher obtained levels were greater than the levels reported in the literature, they correspond to only four samples. The nickel content observed was involved in the range from  $12.6 \pm 1.36$  to  $172 \pm 6.88$  ng g<sup>-1</sup>, lower than the levels reported for French samples (90–340 ng g<sup>-1</sup>). For the three metals studied, diverse authors (8–10, 38) have reported slightly lower values for Italian honeys.

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