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Comparison of palladium–magnesium nitrate and ammonium dihydrogenphosphate modifiers for lead determination in honey by electrothermal atomic absorption spectrometry

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Abstract

The aim of the present work was to develop and optimize two procedures for the determination of Pb content in honeys by electrothermal atomic absorption spectrometry without any sample pretreatment. Palladium–magnesium nitrate and ammonium dihydrogenphosphate were used as chemical modifiers. Honey was diluted in water, and hydrogen peroxide, nitric acid, and Triton X-100 were added to minimize the matrix effect. RSD (lower than 10%) and the analytical recovery (98–101%) were acceptable for both methods. Pd–$Mg(NO_3)_2$ was the method selected for further direct Pb determinations in honey samples because it presented the best limit of detection (LOD = 1.6 ng g^{-1}). Moreover, this method allowed Pb determination using a calibration graph, instead of an addition graph, which is an important advantage. The direct proposed methods have been applied to the determination of Pb content in representative honey samples from Galicia (NW Spain). The lead concentrations found in the analyzed samples were in the range 1.71–75.0 ng g^{-1}.

Keywords: ETAAS; Lead; Honey; Chemical modification; Palladium–magnesium nitrate; Ammonium dihydrogenphosphate

1. Introduction

Levels of heavy metals in the environment have recently increased as a consequence of human and industrial activity. Thus, a study of environmental pollution in a specific zone, involves the evaluation of heavy metals in different matrices, such as water, soil, vegetables and sediments: Among the heavy metals, Pb has been widely studied because the continuous assimilation of small quantities of this element causes toxic effects. Lead is released into the atmosphere from industrial sources (power plants, manufacturing operations, recycling efforts), peeling or flaking of Pb-based paint and automobile emissions from leaded fuels (Baird, 1998). Pb is still detected in dust and it has been incorporated in the trophic chain even though lead emissions from cars have recently decreased.

In recent studies, honey has been proposed as an environmental marker in order to evaluate trace element levels over a wide area where beehives are placed (Caroli et al., 2000; Fodor & Molnar, 1993; Uren, Serifoglu, & Sarikahya, 1998). Honeybees may forage in a variety of environments. Hence they effectively sample their surroundings for the constituents in or on forage plants, soil and atmosphere of a specific area. Honey can be an accumulative indicator of the level of soil, plant and air pollution. In this sense, interest in the development of

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accurate and sensitive methods for Pb determination in honey is double: in the first place, the availability of these methods allows the use of the Pb levels in honey as a suitable environmental pollution indicator and, secondly, it allows the analytical control of this heavy metal in a widely consumed product.

Anodic stripping voltammetry (Sanna, Pilo, Piu, Tapparo, & Seeber, 2000) and potentiometric stripping analysis (Yingjian, Faramaz, & Rolf, 1995) have been used to determine trace amounts of lead in honey. In recent years, inductively coupled plasma atomic emission spectrometry (ICP-AES) (Caroli, Forte, Iamiceli, & Galopi, 1999), high resolution inductively coupled plasma mass spectrometry (HR-ICP-MS) (Caroli et al., 1999) and electrothermal vapourization-inductively coupled plasma mass spectrometry (ETV-ICP-MS) (Bettinelli, Baroni, Spezia, & Terni, 2000) have also been employed in Pb analysis. The advantage of these techniques is their multielemental character, extended dynamic concentration ranges and high sensitivity. Electrothermal atomic absorption spectrometry (ETAAS) has been used (Stein & Umland, 1987; Viñas, López-García, Lanzón, & Hernández-Córdoba, 1997) because it combines relative simplicity, cheaper cost, low sample volume requirements and a low detection limit. It seems that this sequential technique is no longer attractive in comparison with ICP. However, all of these features have been responsible for its widespread use for the determination of trace amounts of lead in environmental and biological samples.

In previous papers concerning the determination of Pb by ETAAS, spectral and non-spectral interferences, depending on the presence of concomitants and on the high volatility of this metal, were reported (Weltz & Sperling, 1999). Most of these interferences are successfully eliminated by employing the appropriate stabilized temperature platform furnace (STPF) conditions (Slavin, Carnrick, Manning, & Pruszkowska, 1983; Tahvonen & Kumpulainen, 1994); however, the use of chemical modifiers, in order to reduce or remove the matrix interferences is also recommended. Viñas et al. (1997, 2000) proposed the use of $NH_4H_2PO_4$ as a chemical modifier for lead analysis in honey samples and baby food. This modifier forms stable lead phosphate and removes halides, allowing the use of higher pyrolysis temperatures (Schlemmer & Radzick, 1999). Palladium has been widely employed as a chemical modifier in food lead ETAAS analysis, alone or mixed with other reagents. Pd assures good thermal stabilization by forming very stable compounds between lead and palladium (Shan & Wang, 1985). When it is used as reduced palladium (Bin & Zhe-Ming, 1996), it forms a stable intermetallic solid solution with the analyte (Yang, Ni, Zhuang, Xu, & Jiang, 1992). Pd has been also applied, in combination with magnesium nitrate, for the determination of other metals in honey (Rodríguez et al., 2003), since it acts as an ashing aid and it improves the similarity of the peak shapes in several compounds studied. Miller-Ihli and Greene (1993) and Miller-Ihli (1994) have studied this modifier, together with magnesium or magnesium in combination with phosphate, for a sugar sample after acid digestion, choosing the magnesium nitrate as better. Palladium magnesium nitrate and dihydrogenphosphate have been recommended in recent years for Pb determination in different food samples, as chemical modifiers (Rodríguez et al., 2003; Stalikas, Pilidis, & Karayannis, 1996), and for this reason they have been studied in this work. However, it is important to note that this is the first comparative study performed in a honey matrix with the aim of selecting the best chemical modifier and its optimum working conditions.

It is well known that metal analysis in this matrix has traditionally been carried out after a sample pretreatment consisting of a sample digestion or sample charring and later acid dilution (Miller-Ihli, 1994) but sample pretreatment has been demonstrated to have large effects on Pb determination (Chmilenko & Baklanova, 1998; Uren et al., 1998). For this reason, in this paper, the optimisation of two methods of lead determination in honeys without any sample pretreatment by ETAAS has been carried out.

Experimental design has been used in order to optimize both methods. Plackett–Burman designs were applied as a screening method to evaluate the most significant factors with few experiments (Morgan, 1991). The optimum conditions for the significant parameters have been obtained using a central composite design that starts with a two-level factorial and some centre-points (Massart et al., 1998).

2. Materials and methods

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 Varian hollow cathode lamp operating at 217 and 283.3 nm, with a current intensity of 5 mA. The bandwidth was 1 nm in all cases. Argon was employed as inert gas at 3 $l\,min^{-1}$. Pyrolytic graphite-coated graphite tubes with platform atomization were employed.

2.2. Reagents

Stock standard solution of lead (1 $g\,l^{-1}$) was obtained from Panreac, (Barcelona, Spain) and diluted as necessary to obtain working standard solutions. Ammonium dihydrogenphosphate was obtained from Fluka, (Swit-

zerland). Nitric acid (65% w/v) Suprapur reagent was obtained from Merck (Darmstadt, Germany), while hydrogen peroxide (33% w/v) was purchased from Panreac (Barcelona, Spain) and Triton X-100 from Merck (Darmstadt, Germany). Palladium was obtained from Fluka (Switzerland). The palladium modifier working solution (2000 mg l^{-1}) was prepared by diluting the appropriate amount of a 10 g l^{-1} stock solution with ultrapure water. Magnesium nitrate was purchased from Fluka (Switzerland). The working solution (2000 mg l^{-1}) was prepared by diluting an appropriate amount of a 10 g l^{-1} stock solution with ultrapure water. Ammonium dihydrogenphosphate was obtained from Fluka (Switzerland). The working solution (1%) was prepared diluting the appropriate amount in ultrapure water. 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 rinsing 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. Honey samples

Thirty representative honey samples from Galicia (NW Spain), with guaranteed origin, and processed using the traditional procedures in the producing region, were provided by the Galician Association of Beekeepers (APLA). All the samples examined were honeys of random (mixed) floral type. Samples were collected in glass bottles and stored in the dark at 3–4 °C prior to analysis. Three replicates of each sample were measured in duplicate.

2.4. Sample preparations

The determination of Pb by ETAAS was carried out directly on the sample diluted with ultrapure water in two of the methods. For the two methods, one gramme of honey was transferred to a 5 ml volumetric flask and made up with ultrapure water to the mark. To prevent the activity of anaerobic microorganisms, the working sample solution was prepared daily.

2.5. Analytical procedures

2.5.1. Palladium–magnesium nitrate method

About 500 μl diluted honey (prepared as described in Section 2.4) was mixed in the autosampler cup with 125 μl of HNO_3 (10%), 10 μl Triton X-100 (1%), 150 μl hydrogen peroxide (33 % w/v) and made up to 1000 μl with ultrapure water. 12 μl of this solution was introduced in the graphite tube together with 9 μl of Pd solution (2000 μg ml^{-1}) and 9 μl of $Mg(NO_3)_2$ solution (2000 g ml^{-1}) and were subjected to AAS under the optimized conditions (see Table 1).

Table 1
Furnace heating programmes

Step	Parameter	Method	
		Pd–Mg $(NO_3)_2$	$NH_4H_2PO_4$
Dry	Temperature (°C)	105	105
	Ramp (s)	10	5
	Hold (s)	40	25
Ash	Temperature (°C)	950	700
	Ramp (s)	10	25
	Hold (s)	40	30
Atomization	Temperature (°C)	1850	1300
	Ramp (s)	0.7	0.3
	Hold (s)	3	3
Clean	Temperature (°C)	2400	2600
	Ramp (s)	2	2
	Hold (s)	0	0

2.5.2. Ammonium dihydrogenphosphate method

About 250 μl diluted honey sample (see Section 2.4) was put into autosampler cup, together with 10 μl Triton X-100 (1% v/v), 300 μl hydrogen peroxide (33 % w/v), 85 μl of HNO_3 (10% w/v) and 100 μl ammonium dihydrogenphosphate (1% w/v) and made up to 1000 μl with ultrapure water. 30 μl of this solution was placed inside the graphite tube and subjected to AAS under the optimized conditions.

For all these cases, the background-corrected peak area due to the analyte was obtained, applying the heating programmes described in Section 3, and summarized in Table 1. Standard addition procedure was performed for the ammonium dihydrogenphosphate method. For the palladium–magnesium nitrate method, a calibration graph was used.

3. Results and discussion

3.1. General

Experimental design has been used in order to optimize both methods. Plackett–Burman fractional factorial designs were used as a screening approach with the aim of establishing the significant factors influencing the determination. Design central composite 2^3 + star was performed to evaluate the response surfaces.

The quantities of modifier, as well as the used programme of temperatures, were optimized for the two methods. When the sample is introduced without a previous treatment, accumulation of carbonaceous residues can occur inside the atomizer and this could increase the background absorption due to molecular species or light

scattering from particulate matter. In order to minimize this effect, the addition of hydrogen peroxide and nitric acid has been evaluated and their influence in the sample signal was studied because they are effective for preventing the formation of these residues inside the tube. In all cases, the use of mild conditions was preferred in order to prevent premature deterioration of the pyrolytic atomizer.

Viscosity may influence the repeatability of the signal. Thus the addition of Triton X-100 have been studied in order to avoid this problem by modification of the physical properties of the sample solution.

The different background signal absorptions for the 217.0 and 283.3 nm Pb lines (due to light scattering by solid particles and/or to molecular absorption) have been studied by different authors (Tahvonen & Kumpulainen, 1994). In the present case, the two wavelengths were evaluated in order to select the optimum one. For all the studied methods, the highest analyte signal and the lowest background were obtained at 217.0 nm. Therefore, all measurements were performed at this wavelength.

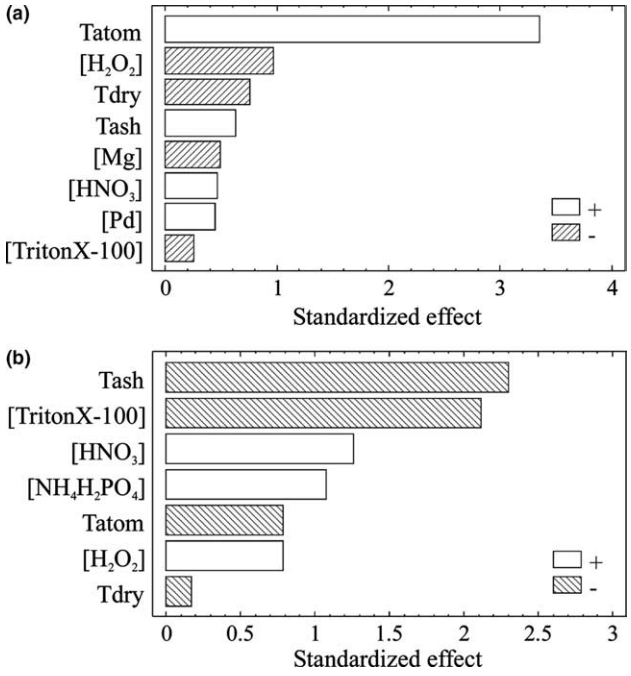

Fig. 1. Standardized Pareto chart. (a) Pd–$Mg(NO_3)_2$ method. (b) $NH_4H_2PO_4$ method. (Tatom: atomization temperature, Tash: ash temperature, Tdry: drying temperature.)

3.2. Pd–$Mg(NO_3)_2$ method optimization

In order to perform a preliminary factor screening, a Plackett–Burman design was carried out in a single block to study the effect of eight factors in 13 runs. Table 2 shows the factors evaluated and their high and low levels. Maximum and minimum levels were chosen according to our previous experience, taking into account the values reported in the bibliography and considering that an adequate strategy for a first screening involves working with a wide interval. The order of the experiments has been fully randomized to protect against the effect of lurking variables.

As can be seen in Fig. 1(a), atomization temperature (Tatom) had a major influence on the signal response. The effects of this factor, together with the concentration of hydrogen peroxide and the ash temperature, have been studied by means of a central composite design 2^3 + star, in 16 runs, since they are statistically the most influential factors. Fig. 2(a) shows the response surfaces for ash and atomization temperatures. The signal of lead increased slightly when temperatures increased, reaching a maximum at 950 °C. For the atomization temperature, the signal increase is more significant, and in this case it allowed a maximum at 1850 °C. These atomization and ash temperatures are slightly lower than those reported for other samples. However, they allowed the removal of the volatile matrix during the ash stage and the complete atomization of lead. The addition of hydrogen peroxide increased the absorbance signal slightly; the optimum concentration observed was 5%. The signal is badly affected by the drying temperature. Therefore, a temperature of 105 °C was selected as appropriate.

In spite of their minor significance in the screening (Fig. 1(a)), palladium, magnesium nitrate and nitric acid concentrations were also optimized by means of a central composite 2^3 + star design for response surfaces, in order to assure optimum modifier concentrations. The response surface obtained is shown on Fig. 2(b). Optimum concentrations were 2000 mg l^{-1} for both palladium and magnesium nitrate. The effect of palladium on the response signal was slight, and a signal decrease for palladium concentrations above 2000 mg l^{-1} could be observed. The analyte signal increased with the nitric acid concentration, reaching a maximum for 1.25% HNO_3. Addition of Triton X-100 was carried out in or-

Table 2
Factors evaluated for the four developed methods. High and low values.

Factor	Pd–$Mg(NO_3)_2$		$NH_4H_2PO_4$	
	Low	High	Low	High
[H_2O_2] (%)	0.3	9.9	0.3	9.9
[HNO_3] (%)	0.1	2.0	0.1	2.0
[Triton X-100] (%)	0.01	0.1	0.01	0.1
[$NH_4H_2PO_4$] (%)	–	–	0.01	0.5
[Pd] (mg l^{-1})	500	2000	–	–
[$Mg(NO_3)_2$] (mg l^{-1})	500	2000	–	–
Dry temperature (°C)	100	200	100	200
Ash temperature (°C)	400	900	600	1200
Atomization temperature (°C)	1100	2000	1300	2000

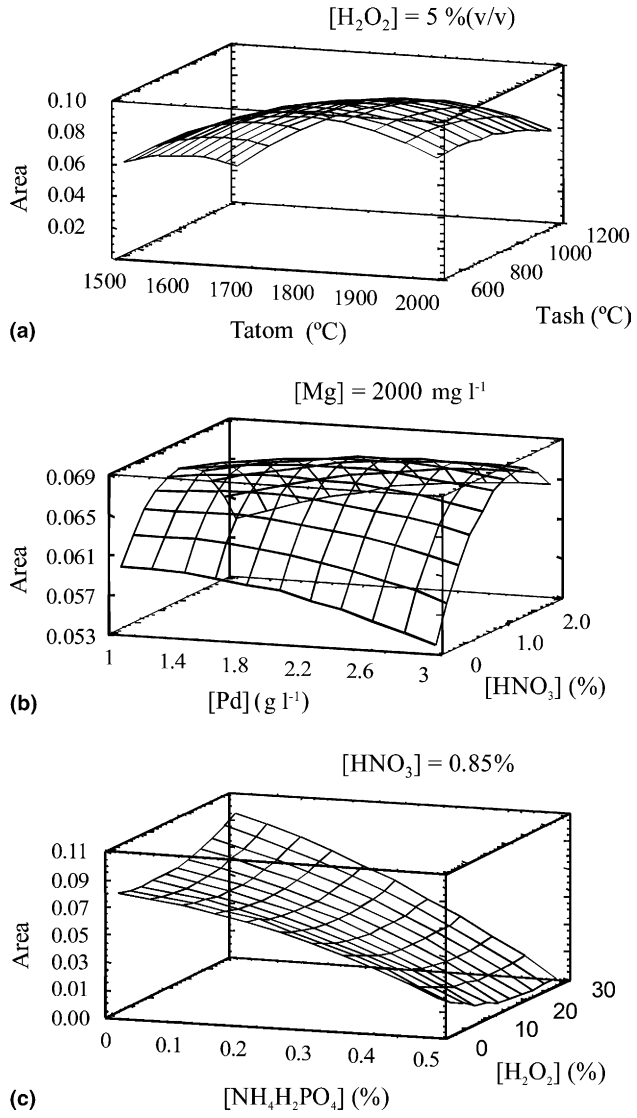

Fig. 2. (a) Effect of ash and atomization temperature on Pb area response. (b) Effect of Pd and HNO_3 concentrations on area response. (c) Effect of $NH_4H_2PO_4$ concentration and H_2O_2 on integrated area.

der to achieve the appropriate sample dispensation. Since this addition negatively affected the analyte signal, the concentration used was the minimum quantity producing an adequate honey sample dispensation to assure the signal repeatability: (0.01%).

Under these optimum conditions, ramps and hold times were studied. High hold drying and ash times proved to be necessary in order to assure the complete drying of the sample and to remove the honey matrix before atomization. Atomization ramp time was short, because it is advisable to rapidly heat the furnace up to the selected optimum selected temperature so that a difference between tube and platform can be achieved. The optimum furnace operation conditions for this method are given in Table 1.

3.3. $NH_4H_2PO_4$ method optimization

In order to perform a factor screening to evaluate factor influence on response signal, a Plackett–Burman design was carried out. Table 2 shows the evaluated factors and their high and low levels. Seven factors were studied in 13 experiments in a totally randomized single block. As can be seen in Fig. 1(b), the most significant factors were ash temperature, Triton X-100 and nitric acid concentrations. These three factors were optimized by means of a central composite 2^3+ start design in 16 runs. By means of this design, a signal decay for ash temperatures above 700 °C was checked. Thus, this value was chosen as optimum. In relation to the nitric acid concentration, it was also observed that 0.85% nitric acid offered the best absorbance signal; higher nitric acid concentrations produced a decrease of the response signal. It was also observed that higher additions of Triton X-100 produced a signal decrease. Thus, the smallest quantity of Triton X-100 producing an appropriate sample dispensation was used (0.01%).

Dihydrogenphosphate and hydrogen peroxide concentrations were optimized by means of response surface. As can be seen in Fig. 2(c), dihydrogenphosphate negatively affected the result by drastically increasing the background signal. The smallest concentration necessary to allow the lead stabilization in the ash stage was 0.1%. The optimum peroxide hydrogen concentration was 9.9%. It was experimentally proven that higher concentrations do not improve the absorbance signal. However, the use of these severe conditions significantly shortened the graphite tube lifetime.

The last step was a central composite design applied in order to find the adequate values for drying hold, as well as drying and mineralization ramps. The optimum times for holds in drying and ash steps were 25 and 30 s, respectively. The optimum selected value for ash ramp was 25 s. Short drying ramp times produced better results. In this case, 5 s were used as drying ramp time. The value chosen as appropriate for atomization temperature was the one obtained in the factor screening: 1300 °C, later checked as the optimum. The selected furnace operation conditions are summarized in Table 1.

3.4. Analytical characteristics

3.4.1. Calibration

Instrumental calibration was performed in the 0.0–6.0 $\mu g\,l^{-1}$ range (at concentration levels of 2.0, 4.0, 6.0 $\mu g\,l^{-1}$) in water solution or in honey matrix. Table 3 shows the equations obtained for calibration and standard addition graphs. The slope comparison of calibration curves for $NH_4H_2PO_4$ modifier (t-test for a confidence level of 95%) was indicative of a slight but significant matrix effect. For this reason, and in order to obtain accurate results, the samples were analysed

Table 3
Blank absorbances and equations obtained for calibration and standard addition graphs for the two developed methods

Method	Calibration	Addition	Blank absorbance
Pd–$Mg(NO_3)_2$	$A = 0.011[Pb] + 0.013$	$A = 0.012[Pb] + 0.023$	0.013
$NH_4H_2PO_4$	$A = 0.009[Pb] + 0.004$	$A = 0.019[Pb] + 0.026$	0.004

in standard addition mode when this matrix modifier was used. However, when the Pd–$Mg(NO_3)_2$ modifier was employed, the comparison of slopes for calibration and standard addition graphs indicated absence of a significant matrix effect. Therefore, direct calibration with standard solutions was applied in honey analysis using this modifier.

3.4.2. Sensitivity

Characteristic results for the developed methods, based on the integrated absorbance, are given in Table 4. Detection limits (LOD) were calculated on the basis of 3 *SD*/*m*, where *m* is the slope of the addition graph and *SD* the standard deviation of 10 consecutive measurements of blank solutions. The results obtained for LOD and the limits of quantification, LOQ (defined as 10 *SD*/*m*) are also presented in Table 4. The best detection limit was obtained for the Pd–$Mg(NO_3)_2$ method.

In an ETAAS method proposed by other authors (Viñas et al., 1997), in which ammonium dihydrogenphosphate modifier was also used, the LOD reported (1.4 ng ml^{-1}, using a 4% w/v solution of honey) was worse than the one obtained in the present work. Perhaps, the LOD improvement obtained may be related to the use of a lower dry temperature, avoiding analyte losses before the atomization step. Also, the optimization performed using the experimental design ensured the best determination conditions. Furthermore, these optimum conditions avoided carbonaceous residues in the graphite tube after the atomization process, increasing the tube's useful lifetime. Considering that the honey is constituted, for the most part, of glucose and fructose, the method proposed by Miller-Ihli and Greene (1993) for lead determination in sugar presents a better LOD than our method, but this method has not been applied to honey samples. Moreover, in this work, the use of severe conditions (temperatures and oxygen as modifier) caused a fast deterioration of the atomizer.

3.4.3. Precision

The within-run precision was studied using honey solutions spiked with lead (0.0, 2.0, 4.0 and 6.0 μg l^{-1}). The relative standard deviation, for 10 determinations of each solution, was below 10% for both methods. The coefficients of variation for the four concentration levels assayed by the three methods are showed in Table 4. In all cases, an acceptable precision was obtained. The repeatability of analytical signal obtained is similar for the two direct methods with both modifiers through the atomizer lifetime.

3.4.4. Accuracy

No honey reference material with certified Pb concentration is currently available. However Caroli et al. (1999, 2000) investigated the feasibility of producing and certifying a certified reference material for the determination of heavy metals in honey (Forte, D'Ilio, & Caroli, 2001). Thus, the accuracy of the proposed methods was measured by evaluating the recovery on a real sample spiked with 2.0, 4.0, 6.0 μg l^{-1} of Pb. The recoveries were in the range 99.1%–101% for $NH_4H_2PO_4$ and 99.0%–101% for the Pd–$Mg(NO_3)_2$ methods. The results showed an acceptable accuracy for the proposed methods.

3.5. Application

The applicability of the developed methods for Pb determination at the levels present in Galician honeys was demonstrated. Nine replicates of a honey sample from Galicia were analysed using the two proposed methods and the obtained lead contents showed a high level of agreement. An *F*-test for standard deviation and *T*-test for mean comparison of these replicates proved that there are no significant differences between them (at the 95.0% confidence level), the results provided by the two methods are comparable. Nevertheless, the Pd–Mg method was chosen to analyze the samples because a better LOD is presented and it allows the use of calibration graphs to carry out the analyses.

It is important to accurately know the concentration of lead in honey since it allows us to evaluate the quality of this product and perhaps identify environmental pollution. Since the evaluation of the effects of lead on

Table 4
Analytical parameters for the Pb determination by the two proposed methods

Method	m_o (pg)	LOD (ng g^{-1})	LOQ (ng g^{-1})	RSD[a](%)
Pd–$Mg(NO_3)_2$	6.3	1.6	5.5	6.3; 6.1; 3.9; 3.6
$NH_4H_2PO_4$	8.6	3.2	10.8	8.7; 6.7; 6.2; 9.1

[a] At the four concentration levels assayed (0, 2, 4 and 6 μg l^{-1}).

Table 5
Pb concentrations in the Galician honey samples analyzed

Sample code	City of origin	[Pb] ± SD (ng g^{-1}) Pd–Mg Nitrate method
M-P0200	Vigo (Pontevedra)	7.60 ± 0.78
M-P0201	Mos (Pontevedra)	11.6 ± 0.97
M-P0202	Mos (Pontevedra)	6.94 ± 0.57
M-P0203	Mos (Pontevedra)	<LOD
M-P0204	Mos (Pontevedra)	5.78 ± 0.40
M-P0300	Mos (Pontevedra)	22.3 ± 0.67
M-P0301	Vigo (Pontevedra)	12.9 ± 1.03
M-P0302	Vigo (Pontevedra)	75.0 ± 2.27
M-P0303	Tomiño (Pontevedra)	15.2 ± 1.31
M-P0304	Porriño (Pontevedra)	4.54 ± 0.00
M-P0305	Aradas (Pontevedra)	9.47 ± 0.66
M-P0306	Redondela (Pontevedra)	5.30 ± 0.50
M-C0301	As Pontes (A Coruña)	7.58 ± 0.66
M-L0201	Fonsagrada (Lugo)	3.82 ± 0.34
M-L0202	Cervantes (Lugo)	2.31 ± 0.23
M-L0203	Teixeiro (Lugo)	1.71 ± 0.13
M-L0204	Vicedo (Lugo)	12.8 ± 0.80
M-L0205	Cervantes (Lugo)	<LOD
M-L0206	Guitiriz (Lugo)	9.94 ± 0.00
M-L0210	Fonsagrada (Lugo)	4.33 ± 0.45
M-L0214	Sober (Lugo)	6.39 ± 0.44
M-L0215	Outeiro de Rey (Lugo)	6.39 ± 0.51
M-L0315	Lugo (Lugo)	5.75 ± 0.52
M-L03016	Lugo (Lugo)	10.7 ± 0.96
M-L0317	Vilalba (Lugo)	4.63 ± 0.00
M-L0318	A Pastoriza (Lugo)	7.72 ± 0.77
M-L0319	Lugo (Lugo)	18.4 ± 0.34
M-O0301	Luintra (Ourense)	8.33 ± 0.79
M-O0302	Muiños (Ourense)	16.7 ± 0.66
M-O0303	Baltar (Ourense)	9.45 ± 0.66

human health involves exposure from all sources, any increase in lead from non-food sources will decrease the amount that can be tolerated from foods (25 μg g^{-1}, provisional tolerable intake (463. Lead, WHO Food additives Series 13)). Therefore, it is important to study the lead content in Galician honeys and, this is the first study of heavy metals, in this region, referred to honey. Lead determination was carried out for 30 representative honey samples from Galicia (NW Spain). Lead was found in the analysed samples at concentrations between 1.7 and 75.0 ng g^{-1} (see Table 5). This is a lower range that those published by other authors and other regions. For example, for Italian honeys, the concentration is between 3.2 and 186 ng g^{-1} (Bettinelli et al., 2000; Caroli et al., 1999; Caroli et al., 2000; Sanna et al., 2000). For Turkish samples, Uren et al. (1998) report concentrations between 32.9 and 55.2 ng g^{-1} and for honeys from Pomerania (Poland), Przybylowski and Wilczynska (2001) report 250–700 μg g^{-1}. However, Celechovská and Vorlová (2001), in Czech honeys, find values up to 1000 ng g^{-1}. It is quite difficult to compare results because of the different botanical species investigated and, compositional geochemistry of soil and possible environmental contamination. Neither has the Codex Alimentarius (Revised Codex Standard for honey, 2001) established maximum values for heavy metals in honey.

After dividing the thirty samples into two groups (industrialized and not industrialized areas, 12 and 16, respectively), significant difference between means ($p = 0.05$) were not found, except for an outlier, that is M-P0302. This higher value is explained because the beehive was located in Vigo, where the traffic load is quite heavy. In order to reach a definitive conclusion about the influence of the area on the total content of metals, it is probably necessary to analyze more honey samples and to quantify more metals in them.

4. Conclusion

Two methods for direct Pb determination in honey samples without any sample pre-treatment or pre-concentration step, by electrothermal atomic absorption spectrometry were optimized using palladium–magnesium nitrate, and ammonium dihydrogenphosphate as matrix modifiers. The Pd–$Mg(NO_3)_2$ method is the procedure selected for further direct Pb determinations in honey samples. This procedure presented a better detection limit than others reported in the literature. Moreover, this method allowed Pb determination using a calibration graph, instead of an addition graph, which is an important advantage.

The proposed methods have been applied to the determination of Pb content in representative honey samples from Galicia (NW Spain), with comparable results between samples from different areas. Lead concentration was, in all cases, lower than 75.0 ng g^{-1}.

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