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Determination of Arsenic in Honey, Propolis, Pollen and Honey Bees by Microwave Digestion and Hydride Generation Flame Atomic Absorption

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Abstract

The toxic properties of arsenic are well known. Honey has been widely used for monitoring this element. The present work reports a novel method for the determination of arsenic in honey, bees, pollen, and propolis, based on the coupling of microwave digestion and hydride generation. Method development included the quantitative reduction of arsenic(V) to arsenic(III), the acid employed for dilution, and the complete removal of the gases following digestion. The method performance was satisfactory with recoveries between 83% and 111% and corresponding relative standard deviations between 3.1% and 24%. Among the thirty two samples of honey, propolis, pollen and honey bees analyzed, arsenic was detected in four out of six propolis samples at the method limit of detection (0.4 μg g^{-1}). The results indicate that propolis may be an efficient indicator for arsenic.

Keywords: bio-indicators; hive products; hydride generation - atomic absorption spectrometry (HG-AAS); toxic elements

INTRODUCTION

Arsenic (As) has been associated with several adverse environmental and human health effects such as carcinogenicity and skin lesions, with developmental effects, cardiovascular disease, neurotoxicity, and diabetes (International Agency for Research on Cancer 2012; World Health Organization 2012). Because of its toxic properties, monitoring of arsenic in environmental and food samples is essential in order to perform reliable risk assessments and take appropriate actions for the protection of the environment and human health (European Food Safety Authority 2014).

For this scope, honey bees and their products, such as honey and pollen, have been proposed as indicators of environmental contamination related to arsenic among other xenobiotics (Balayiannis and Balayiannis 2008; Bargańska, Ślebioda, and Namieśnik 2016). Monitoring of arsenic levels in honey is important not only for the indication of environmental contamination, but also because of the potential human dietary exposure. In addition, the determination of arsenic in honey and propolis is significant since another potential route of human exposure to this element is through the use of pharmaceutical and cosmetic products containing honey or propolis. These apicultural products are used in medicine and cosmetics because of their antimicrobial, antioxidant, anti-inflammatory, and antitumor properties (Burdock 1998; Kalogeropoulos et al. 2009; Tsiapara et al. 2009; Melliou and Chinou 2011; Burlando and Cornara 2013).

Arsenic has been determined in honey from several countries (Jamoussi, Zafaouf, and Hassine 1995; Caroli et al. 1999; Pisani, Protano, and Riccobono 2008; Bilandžic et al. 2011; Vieira et al. 2012; Ru, Feng, and He 2013; Bilandžić et al. 2014; Conti et al. 2014; Czipa,

Andrási, and Kovács 2015), whilst limited literature data are available regarding the determination of arsenic in propolis (Roman, Madras-Majewska, and Popiela-Pleban 2011; Bonvehí and Bermejo 2013; Matin, Kargar, and Buyukisik 2016), pollen (Roman 2009; Morgano et al. 2010), and honey bees (Van der Steen, de Kraker, and Grotenhuis 2011; Badiou-Bénéteau et al. 2013; Matin, Kargar, and Buyukisik 2016). The analytical techniques used for the determination of arsenic in these matrices primarily involve inductively coupled plasma – mass spectrometry (Caroli et al. 1999; Pisani, Protano, and Riccobono 2008; Roman 2009; Badiou-Bénéteau et al. 2013; Conti et al. 2014; Czipa, Andrási, and Kovács 2015), inductively coupled plasma – optical emission spectrometry (Roman 2009; Morgano et al. 2010; Van der Steen, de Kraker, and Grotenhuis 2011; Roman, Madras-Majewska, and Popiela-Pleban 2011; Bonvehí and Bermejo 2013), and graphite furnace atomic absorption spectrometry (Bilandžic et al. 2011; Bilandžić et al. 2014; Matin, Kargar, and Buyukisik 2016).

Apart from the aforementioned analytical techniques, the conversion of arsenic to volatile derivatives using hydride generation coupled with an element selective detector has been widely used (Anthemidis and Kalogiouri 2013). In this technique, arsenic compounds react most commonly with sodium tetrahydroborate in acidic medium in order to produce volatile derivatives such as arsine. The volatile hydride is transferred with a carrier gas to an atomizer and detector. Hydride generation has been used for the determination of arsenic in several matrices, including seafood products (Muñoz, Vélez, and Montoro 1999) and soft drinks (El-Hadri, Morales-Rubio, and Guardia 2007), but rarely employed for the determination of arsenic in honey. The reported applications concern coupling with atomic fluorescence spectrometry (Ru, Feng, and He 2013) and inductively coupled plasma – optical emission spectrometry

(Jamoussi, Zafaouf, and Hassine 1995), following microwave digestion, as well as coupling with atomic absorption spectrometry without digestion of the sample (Vieira et al. 2012).

To the best of our knowledge, hydride generation has not been used for the determination of arsenic in samples of honey bees, pollen, and propolis. In addition, although apiculture is a traditional activity in Greece and apicultural products are produced and consumed at a large national scale, no information is available on the quality of the Greek apicultural products with regards their concentrations of arsenic.

The goal of this study was the development of a reliable analytical method in order to investigate the possible contamination of honey bees and apicultural products of Greek origin with arsenic. In particular, hydride generation flame atomic absorption in combination with the efficient microwave-assisted digestion technique with closed vessels under pressure was developed and validated for the determination of arsenic in honey, propolis, pollen, and honey bees. The validated analytical method was applied to thirty two samples collected from the northern and western part of Greece.

EXPERIMENTAL

Reagents and Chemicals

Certified arsenic standard solution of 1000 g L^{-1} in 1 M hydrochloric acid, metal-free water, metal-free HCl (30%), and metal-free HNO_3 (65%) were purchased from Fisher Scientific (UK). H_2O_2 was obtained from Carlo Erba (France), $NaBH_4$ (99%) from Acros Organics (USA), NaOH (98%) from Panreac Quimica (Spain), KI (99.5%) and ascorbic acid (99.7%) from Merck

(Germany). A stock solution of 10 μg mL^{-1} arsenic was prepared in 1M HCl and was used for the preparation of intermediate standard solutions (10, 25, and 50 ng mL^{-1}).

Sampling

Four bee samples (honey, propolis, pollen, and honey bees) from thirteen apiaries, located in the northern and western part of Greece, were collected between spring 2013 and August 2014, from rural, industrialized areas and agricultural areas near mines. Samples were stored at -15 °C in their original container until analysis. Nine samples of honey, 6 samples of propolis, 12 samples of pollen, and 5 samples of honey bees were analyzed for arsenic. The location of the sampling areas along with the industrialized activities is illustrated in **Figure 1**.

Sample Preparation

The sample preparation consisted of microwave digestion and the reduction of As(V). For the honey bee samples, drying at 100 °C for 48 hours preceded microwave digestion. Depending on the matrix, an aliquot of the samples (0.1 to 1 g) was treated with 5 mL metal-free HNO_3 (65%) and 2.5 mL H_2O_2 (30%). Special care was taken in order not to have the sample attach to the walls of the vessel. The samples were digested in a microwave oven (CEM Mars, MD 9132, USA) in Omni XP-1500 tubes. The temperature program was as follows: 0 to 15 min ramp to 210 °C and hold for 15 min at 210 °C. After digestion, tubes were allowed to cool, the pressure was carefully released, and the yellow/brown gases were let to escape under sonication, until complete decolorization was achieved. After the removal of the gases, the samples were quantitatively diluted to 25 mL with water. Every microwave digestion cycle consisted of eight samples, two blanks of 5 mL HNO_3 (65%) and 2.5 mL H_2O_2 (30%), and two fortified samples.

After digestion, the reduction of As (V) to As (III) followed (British Standard Institution 2012): a 5 mL aliquot from the 25 mL sample solution was transferred to a plastic container, 2.5 mL of the reduction solution (2.5% w/v KI and 2.5% w/v ascorbic acid) and 2.5 mL of concentrated HCl were added and the mixture was allowed to react for 60 minutes at room temperature. 15 mL of HCl 1 M were then added and the solution was left for another 60 minutes before measurement by hydride generation – flame atomic absorption spectrometry.

Analysis

An atomic absorption spectrometer (Shimadzu AA-6500F), equipped with a self-reversal correction hollow cathode lamp (Hamamatsu Photonics, Japan) was operated at 193.7 nm and at 35 to 500 mA. A hydride generator was used for the determination of arsenic. The carrier liquid was an aqueous solution of 5 M HCl and the reducing agent was 0.4% $NaBH_4$ in 0.5% NaOH. The volatile hydride was transferred with argon gas to the quartz tube, which was heated by an air-acetylene flame.

Validation

For the validation of the method, calibration curves were generated by measuring standard solutions of arsenic prepared according the same procedure followed for the samples, using 5 mL of intermediate standard solutions instead of 5 mL of the digested sample. Linear regression analysis was performed using the absorbance plotted against analyte concentration at 2, 5, and 10 ng mL^{-1}**.**

The instrumental limit of detection was defined to be $3 \times S_{y/x}$, where $S_{y/x}$ is the standard error of the predicted y-value for each x in the regression. For each of the four matrices, the

method limit of detection was calculated based on the instrumental limit of detection, the mass of sample, and the dilution factor.

For the assessment of the accuracy and the precision, the method was used to analyze honey, pollen, propolis and honey bees that were fortified with arsenic at appropriate levels (**Table 1**). Analysis of three replicates of the fortified samples was conducted for the repeatability test. The recovery was calculated by subtracting the concentration measured in the untreated sample from that measured in the fortified sample and then dividing by the fortified concentration.

RESULTS AND DISCUSSION

Method optimization

The following critical points were identified for the determination of arsenic with microwave digestion and hydride generation atomic absorbance. The first critical point was the acid used for the dilution of the measured solution. Experiments were conducted with arsenic standard solutions prepared in 0.1 M HNO_3 and in 1 M HCl from 2 to 60 ng mL^{-1} without a reduction step. The absorbance obtained with 1 M HCl was at least double in comparison to the absorbance obtained with 0.1 M HNO_3. The interference by NO_3^- in the absorbance signal of arsenic has been reported in the literature where it was attributed to the consumption of $NaBH_4$ by the nitrate in acidic solution (Castillo et al. 1986).

The second critical point identified was the complete removal of the gases following microwave digestion of the sample. It was observed that if not all the gases were removed with complete decolorization of the samples, no arsenic signal was obtained. It is assumed that the

produced gases after the digestion of organic matter interfere with the efficient and quantitative reduction of As(V) to As(III) in the following step.

The third critical point was the reduction step because of the difference in signals between As(III) and As(V). Castillo et al. (1986) reported that the valence state of arsenic in the sample should be controlled by reduction or oxidation, and match the calibration standard solutions. The reduction procedure with KI–ascorbic acid was efficient for honey, pollen, propolis, and honey bees with satisfactory recovery and repeatability (**Table 1**).

Method Performance

The instrumental linearity in 1 M HCl was satisfactory for concentrations up to 10 ng mL^{-1} but was impaired above 20 ng mL^{-1}. Similar observations regarding the correlation between absorbance and arsenic concentration were reported by Vieira et al. (2012). Acceptable linearity was obtained for concentrations between 2 and 10 ng mL^{-1}. The calibration relationship was $y = 0.0075x + 0.0128$ with a correlation coefficient of 0.994 and standard error of 0.0021. The instrumental limit of detection was 0.8 ng mL^{-1}. The method limits of detection depended on the matrix. The method limit of detection for honey was 0.1 μg g^{-1}, for pollen 0.2 μg g^{-1}, for propolis 0.4 μg g^{-1}, and for honey bees 1 μg g^{-1} of dry weight (dw). Although the technique is a sensitive with an instrumental limit of detection of 0.8 ng mL^{-1}, the corresponding limits of detection in the samples are higher because of the high dilution factor. Nevertheless, it is noted that since there are no regulated limits for the concentration of arsenic in honey, there is not a specific requirement for the limit of detection from a regulatory point of view. Furthermore, the method limits of detection of the present study are comparable to the maximum limits established in the European Union Regulation 2015/1006 for the maximum levels of inorganic

arsenic in food, which range from 0.1 μg g^{-1} for rice destined for the production of food for infants and young children up to 0.3 μg g^{-1} for rice products such as waffles and crackers.

The mean recovery (%) and relative standard deviation (%) of the method in honey, propolis, pollen, and honey bees for three replicates at two fortification levels are summarized in **Table 1**. These data show that the accuracy and the precision of the method are satisfactory.

Determination of Arsenic in Bee S*amples*

Arsenic was detected at the limit of detection (0.4 μg g^{-1}) in four out of six samples of propolis. These results are in agreement with a study conducted in Poland where an average arsenic concentration of 0.66 μg g^{-1} was reported (Roman, Madras-Majewska, and Popiela-Pleban 2011). In addition, the concentrations of arsenic in propolis from an industrial district in Izmir were from 0.019 to 0.578 μg g^{-1} (Matin, Kargar, and Buyukisik 2016). Lower concentrations of arsenic were determined in propolis from south Spain, with values between 0.075 to 0.13 μg g^{-1} (Bonvehí and Bermejo 2013).

The arsenic concentrations were below 0.1 μg g^{-1} in the honey. The concentrations of arsenic in the literature from various countries were between 0.0005 to 0.276 μg g^{-1} (Jamoussi, Zafaouf, and Hassine 1995; Caroli et al. 1999; Pisani, Protano, and Riccobono 2008; Bilandžic et al. 2011; Roman, Madras-Majewska, and Popiela-Pleban 2011; Vieira et al. 2012; Ru, Feng, and He 2013; Bilandžić et al. 2014; Conti et al. 2014; Czipa, Andrási, and Kovács 2015).

Arsenic was not detected in honey bees in this study. Although the method limit of detection (1 μg g^{-1}) in honey bees is relatively high, the results are indicative and comparable with the limited available data in the literature. The reported arsenic concentrations in honey

bees from the Netherlands were between 0.67 and 0.83 μg g^{-1} dry matter (Van der Steen, de Kraker, and Grotenhuis 2011), and up to 4.56 ng g^{-1} in honey bees from an industrial district in Izmir (Matin, Kargar, and Buyukisik 2016), whereas much higher concentrations, 312 to 498 μg g^{-1}, were reported in honey bees from Reunion Island (Badiou-Bénéteau et al. 2013).

Arsenic was below 0.2 μg g^{-1} in the pollen analyzed in this study. Quantification at lower concentrations has been performed in pollen from Poland, 0.060 to 0.093 μg g^{-1} dry matter (Roman 2009) and in Brazilian pollen, 0.01 to 1.38 μg g^{-1} (Morgano et al. 2010)

Arsenic was detected only in propolis in this study. Although honey bees, flower pollen, and honey are considered to be suitable indicators for trace and toxic elements (Bargańska, Ślebioda, and Namieśnik 2016), the results of this study indicate that propolis may also serve as an indicator for arsenic. This assumption is also supported by the conclusions of Roman, Madras-Majewska, and Popiela-Pleban (2011) who indicated that propolis is more contaminated by toxic elements, including arsenic, than honey.

CONCLUSIONS

The present work describes a significant and novel application of the coupling of microwave digestion and hydride generation for the determination of arsenic in honey, pollen, propolis and honey bees. The method was applied to thirty two samples of honey, propolis, pollen and honey bees. Arsenic was detected in four out of six samples of propolis (0.40 μg g^{-1}), indicating that this material may serve as an efficient indicator for arsenic. The method is suitable for monitoring of arsenic in environmental materials and food to perform reliable risk assessments.

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Table 1. Mean recovery (%) and relative standard deviation (%) in honey, propolis, pollen, and honey bees (n = 3)

	Honey		Propolis		Pollen		Honey bees	
Fortification level (μg g^{-1})	0.2	0.6	0.5	2.5	2.5	5.0	2.5	6.2
Recovery (%)	104	83	99	97	107	97	111	96
Relative standard deviation (%)	17.6	8.2	13.7	5.7	5.6	3.1	24	23

Figure 1. Sampling locations in northern and western Greece.