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Ion chromatographic and voltammetric determination of heavy and transition metals in honey

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Abstract

Heavy metals in honey are of interest not only for quality control, but can be used also as an environmental indicator. In the present work, in order to minimize sample pre-treatment, the interference by organic constituents of the matrix is overcome by using oxidative UV photolysis. The matrix degrades in less than 1 h, while most common metallic impurities, like iron, copper, nickel, zinc, lead, cadmium and cobalt, remain unaffected by UV radiation, with the exception of manganese. After UV photolysis, the resulting solution is directly analyzed by ion chromatography and differential pulse anodic or cathodic stripping voltammetry. In absence of official standards, the results obtained by these techniques on spiked matrix-matched blank solutions and original and spiked real samples are compared with those of the well established electrothermal atomic absorption spectrometry and they are found in good agreement. The proposed techniques show satisfactory sensitivity, detection limits and standard deviation for heavy and transition metals determination in honey. In addition, both ion chromatography and pulsed voltammetries permit multielement analyses which can be fully automated.

Keywords: Electrothermal atomic absorption spectrometry; Honey; Ion chromatography; Pulsed voltammetry; Trace metals; UV photolysis

1. Introduction

From early times honey was considered a delicious food, but it is also the result of a bio-accumulative process useful for collecting information about the environment within the bees' forage area. Honeybees' accretions are related to air, water and soil; they go from flower to flower, touch branches and leaves, drink water from pools and their hairy bodies collect aerosol particles. Bees are estimated to forage on plants growing in a relatively large area of more than 7 km^2 (Bromenshenk & Carlson, 1985; Celli, 1984). If it is assumed that any hive includes at least 1000 worker-bees and that each of them forage on one thousand flowers per day, the honey produced daily can be considered the outcome of at least one million interactions. In this way, the forage area is effectively sampled for trace elements and the concentration in honey of heavy and transition metals reflects levels in the forage area (Barišić et al., 1999; Hoefel, 1985; Jones, 1987; Kump, Necemer, & Ŝnajder, 1996; Leita, Muhlbachova, Cesco, Barbattini, & Mondini, 1996).

The mineral content of honey, usually calculated to be 0.17% although this can vary within a wide range (White, 1979), is recognized as an environmental indicator at least since 1984, when Crane published the first data on metals content in honey collected near or far from highways.

In addition, the determination of heavy and transition metals in honey is of interest for quality control when considering it as food. High levels of metals are undesirable because of their known or supposed toxicity so that, for instance, a limit of 0.215 mg kg^{-1} for lead was

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proposed by Otto and Jekat (1977) and actually 1 mg kg^{-1} is a limit in some countries. The amounts of heavy and transition metals in honey are usually so small that even 100 g eaten daily would not contribute appreciably to dietary requirements. Iron, only, comes closest to doing so in dark honey because 100 g of it contains about 5% of the daily iron requirement. An environment rich in one or more of these metals is unlikely to enhance the content in honey by an amount large enough to alter the picture.

Considered as an analytical sample, honey is one of the most complex mixtures of carbohydrates produced in nature. Glucose and fructose are the major components (65–75% of total soluble solids) and other oligosaccharides are present with small quantities of proteins, aminoacids and vitamins. In addition honey contains 15–20% of water (e.g. Clarke, 1995). Metals determination in sugar-rich foodstuffs ($+60^{\circ}$ Brix) has been a challenging analytical task because of the interference due to the matrix. Its effect is very important, as dilution may reduce concentrations below the limit of detection. So, sample pre-treatment is usually required to destroy the organic matrix and/or to extract the metal ions bound in organic complexes. Obviously, the selection of the procedure must take into account the analytes of interest, the sample matrix and the time requirements of the analytical technique considered.

Several techniques were proposed to determine metallic impurities in honey, but in most cases a matrix mineralization is required. In 1979, Scheubeck, Nielsen and Iwantscheff made a comparison of spectrophotometry, atomic absorption spectrometry and X-ray fluorescence spectrometry, after honey decomposition. The detection limit proved to be the most important limiting factor of these techniques because it is not possible to reach the p.p.b. range, as also recently confirmed by Kump et al. (1996).

On the other hand neutron activation analysis (e.g Iskander, 1995; Sevimli, Bayulgen, & Varinlioglu, 1992), that provides the lowest detection limits, is very expensive and difficult to manage so that it seems not possible to make routine analysis in this way.

The most established techniques for heavy metal determinations in honey are electrothermal atomic absorption spectrometry (ETAAS) (but which requires sequential analysis) and ICP–AES, which usually involve similar sample preparation techniques such as acid dilution, as reported by Vinas, Lopez-Garcia, Lanzon, and Hernandez-Cordoba (1997) or Voget and Baudisch (1983), extraction of diethyldithiocarbamate complexes in methylisobutylketone (e.g. Stein & Umland, 1986) or wet digestion (e.g. Chung & Tsai, 1992). The most relevant disadvantage of these techniques is the mineralization stage necessary to avoid the problems related to the high amount of organic compounds introduced into the atomizer. Currently, the most accepted approach to mineralization seems to be the microwave oven digestion that permits reduced reagent amounts, temperature and time required for sample treatment as reported by Fodor and Molnar (1993) and Jamoussi, Zafaouf, and Ben Hassine (1995). The digestion of organic matrices is delicate and its progress must be strictly controlled. In addition, the non-volatile residues, remaining after the treatment, are a problem for the analytical techniques that are sensitive to organic interference as described by Reid, Greenfield, and Edmonds (1995).

Also differential pulse anodic stripping voltammetry (DPASV) was taken into account by Li, Wahdat, and Neeb (1995), after sample acid dilution, but it is somewhat difficult to remove a broad interfering peak, probably due to the adsorption of one or more organic compounds present in honey.

Dealing with the honey mineral content analysis, ion chromatography (IC) was used in 1989 by Perez Cerrada, Herrero-Villen and Maguieira to determine inorganic anions and in 1996 by Kim and Rhee for the determination of alkali and alkaline earth metals only, without considering it for the determination of heavy and transition metals, even if the use of this technique in food analysis is continuously increasing (e.g. Buldini, Cavalli, and Trifiró, 1997). In addition, a recent paper by Cardellicchio, Cavalli, Ragone, and Riviello (1999) introduced the use of gradient elution and a different post-column reagent in order to evaluate increases in selectivity and sensitivity.

Among the wide range of the analytical techniques considered, the last two are very attractive for trace analysis owing to the low cost of the apparatus, ease of operation and satisfactory sensitivity coupled with the advantage of simultaneous determinations.

The purpose of the present paper is to describe a method that allows for the determination of the main metals in a matrix with high contents of sugars and low in metals, requiring the least sample treatment. Honey is oxidized by UV photolysis and iron, copper, nickel, zinc, lead, cadmium, and cobalt are simultaneously determined by IC or DPASV–differential pulse cathodic stripping voltammentry (DPCSV). As there are not official standards, the results obtained by the proposed techniques on spiked matrix-matched blank solutions and original and spiked real samples are compared with those of the well-established ETAAS and they are found in good agreement.

2. Experimental

2.1. Reagents and standards

Sodium chloride, sodium hydrogen carbonate, sodium nitrate, oxalic acid, 2-dimethylaminoethanol, 4-(2-pyridylazo)-resorcinol monosodium salt (PAR) were

chromatographic grade (Novachimica, Milan, Italy). Hydrogen peroxide (30% m/m), ammonium hydroxide (30%), and hydrochloric acid (37%) were Erbatron electronic grade (Carlo Erba Reagenti, Milan, Italy). Ammonium acetate, ammonium dihydrogen phosphate, magnesium nitrate, dimethylglyoxime and ethyl alcohol (94-96%) were ACS reagent grade. Ammonium acetate (2 M; pH = 5.5) was chelation grade (Dionex, Sunnyvale, CA, USA). Suprapur nitric acid (65%; Merck KGaA, Darmstadt, Germany) was used for microwave oven digestion. Ultrapure water with conductivity < 0.1 μS (DI water) was obtained from a Milli-Q (Millipore, Bedford, MA, USA) deionization system. Working standards were prepared daily by diluting Carlo Erba Reagenti Normex atomic absorption standards (1.000 g l^{-1}).

To perform experiments on a matrix-matched blank, a 65° Brix solution was prepared containing 32.5% (w/w) of glucose and 32.5% (w/w) of fructose in DI water. Quartz test tubes and all glassware were cleaned by refluxing in hot and concentrated nitric acid, then carefully washed with DI water and finally dried with filtered air in a clean atmosphere. Details of cleaning procedures and apparatus are reported in the standard texts. Normal precautions for trace analysis were observed throughout. Manipulations were done on a laminar-flow clean bench to avoid accidental contamination.

2.2. Voltammetric complexing solution

For the determination of nickel (II) and cobalt (II), a 43 m*M* dimethylglyoxime solution was prepared in ethyl alcohol (94–96%).

2.3. Instrumentation

For ETAAS analyses honey samples were digested in a microwave oven model MLS 1200 (Milestone, Sorisole, Italy). For pulsed voltammetric and IC analyses, honey samples were subjected to UV photolysis in a 705 UV digester (Metrohm, Herisau, Switzerland) equipped with a high-pressure 500 W lamp. The temperature of the sample was maintained at about 85 ± 5°C with the help of the combined air/water cooling system built into the digester.

Chromatographic analyses were performed on a model DX-300 ion chromatograph (Dionex, Sunnyvale, CA, USA) which included one gradient pump AGP, a Post-column Pneumatic Controller for post-column reagent addition, and a DSA UV-Vis multiple wavelength detector operating at 530 nm. Traces of metals in the chromatographic system were removed by flushing all flow paths, pump and columns with 0.2 M oxalic acid for 2 h at 1 ml min^{-1}, followed by rinsing with deionized water.

All measurements were made at room temperature. In all cases, injection of the sample was done at least in triplicate. All the samples were filtered through a 0.2-μm filter before injection. Chromatographic conditions are summarized in Table 1. Data manipulation and the operation of all the components in the system were controlled by PeakNet (Dionex, Sunnyvale, CA, USA) chromatographic software interfaced via an ACI-2 Advanced Computer Interface to a Pentium-based computer (Siemens PC Systems, Augsburg, Germany).

Voltammetric measurements were performed on a model 646 VA processor (Metrohm, Herisau, Switzerland) equipped with a 647 VA stand, a 675 VA sample changer, a 677 drive unit and Dosimat 665 automatic addition burettes. A conventional three-electrode arrangement consisting of a multi-mode electrode working electrode, an Ag/AgCl [3 M KNO_3] reference electrode and 6.5-cm long platinum wire auxiliary electrode was used. All the voltammetric conditions are listed in Table 2.

Table 1
Ion chromatographic conditions

Elution order		Cu, Cd, Ni, Zn, Co, Mn, Fe, Pb	
Column		IonPac CG5A + CS5A	
Eluents		(A)	(B)
		28 mM $(COOH)_2$	28 mM $(COOH)_2$
		45 mM NaCl	45 mM NaCl
		116 mM $NaNO_3$	265 mM $NaNO_3$
		40 mM HCl	40 mM HCl
Gradient program	Time (min)	%A	%B
	0	100	0
	9	0	100
Eluent flow rate		1.0 ml min^{-1}	
Injection volume		150 μl	
Detection		Visible absorbance (530 nm)	
Post-column reagent (PCR)		0.4 mM PAR	
		1 M $(CH_3)_2NCH_2CH_2OH$	
		0.5 M NH_4OH	
		0.3 M $NaHCO_3$ (pH 10.4)	
PCR flow rate		0.6 ml min^{-1}	

Table 2
Voltammetric conditions

Electrode type	MME operated as HMDE, Hg drop size 0.60 mm^2
Electrode stirring speed (during deposition time)	1920 rpm
Zn (II), Cd (II), Pb(II), Cu (II)	Deposition (at −1.450 V) 120 s, quiet time before potential scan 20 s, DPASV from −1.450 to −0.100 V, pulse amplitude 60 mV, potential scan rate 10 mV/s
Ni (II), Co (II)	Deposition (at −1.100 V) 35 s, quiet time before potential scan 30 s, DPCSV from −1.100 to −1.450 V, pulse amplitude −40 mV, potential scan rate 10 mV/s

For comparison purposes a Zeeman 3030 (Perkin-Elmer, Beaconsfield, USA) atomic absorption spectrometer, equipped with an HGA 400 (Perkin-Elmer, Beaconsfield, USA) furnace module, was used. A pyro/platform tube in an argon stream was used, with a slit of 0.7 nm (for copper, cadmium, lead and zinc) or 0.2 nm (for iron, cobalt and nickel), at the resonance lines reported in Table 3. Lead only was analyzed in an uncoated tube. The results were obtained by computerized graphic evaluation of the standard addition method, taking into account the matrix modifier dilution when required.

2.4. Procedure

Honey samples are both commercially available and directly collected by private beekeepers from a variety of sites and they were stored in the dark in tightly closed plastic bottles in order to prevent metals contamination. To decrease viscosity and obtain homogeneous samples, the pots were lightly heated in a water bath to liquefy the honey and only acid-rinsed glass rods were used to transfer the samples from the original vessels to the photolysis quartz tubes.

Honey (0.5–1.0 g) was weighed in a quartz test tube and 3 ml of DI water added. The solution was sonicated for 5 min in order to completely dissolve the sample, then 1 ml of concentrated hydrogen peroxide (warning: suitable precautions must be adopted to avoid burns when working with this chemical!) was added and UV photolysis performed. After 15 min of oxidation, 10 μl of 1 M nitric acid was added to the test tube.

At the end of the UV photolysis time, which varies from 30 to 60 min depending from honey characteristics, the pH of the solution is adjusted to 5–6, by adding 50 μl of 2 M ammonium acetate, before making up to a final volume of 5 ml with DI water. The solution was directly analyzed by IC. For DPASV–DPCSV analysis, at the end of the UV photolysis period the sample solution was simply diluted to 10 ml with DI water and transferred to the voltammetric cell. The equipment is programmed for: (1) a starting addition of 300 μl of 2 M ammonium acetate before the determination of zinc, lead, cadmium and copper; and (2) a following addition of 300 μl of 5 M ammonia/ammonium acetate buffer (pH 9.5) and of 50 μl of 43 mM dimethylglyoxime solution before the successive determination of nickel and cobalt.

For comparison purposes, 0.5–1.0 g of honey was added with 3 ml of concentrated nitric acid and microwave digested using 30% power (360 W) for 2 min, 50% power (600 W) for another 2 min and 90% power (1000 W) for the last minute. The digests were cooled in an ice water bath and 2 ml of concentrated nitric acid was added. The mineralization was completed by a second working cycle of the microwave digestion system. After, the digestion vessel was cooled to room temperature and the resulting solution was diluted to 5 ml with DI water. Each Zeeman electrothermal atomic absorption spectroscopic analysis calls for 20 μl of solution and 10 μl of the matrix modifier was added if necessary, according to Table 3.

Table 3
Zeeman graphite furnace atomic absorption spectrometric conditions

Element	Wavelength (nm)	Temperature (°C)			Matrix modifier[a]
		Drying[b]	Ashing[c]	Atomization[d]	
Zn	213.9	110	700	1800	A
Cd	228.8	110	900	1600	B
Ni	232.0	110	1400	2500	C
Co	242.5	110	1400	2500	C
Fe	248.3	110	1400	2400	C
Pb	283.3	110	900	1800	B
Cu	324.8	110	900	2000	-

[a] A, 6 μg $Mg(NO_3)_2$; B, 200 μg $NH_4H_2PO_4$ + 10 μg $Mg(NO_3)_2$; C, 50 μg $Mg(NO_3)_2$.
[b] 50 s ramp, 30 s hold.
[c] 60 s ramp, 40 s hold.
[d] 10 s hold.

3. Results and discussion

The use of honey as a biological indicator or its quality control for heavy and transition metals contamination entails the analysis of a great number of samples. The selection of the sample pre-treatment procedure must take into account the analytes of interest, the matrix characteristics and the time requirements of the analytical technique considered.

Different procedures have been experienced in order to minimize sample manipulation. For instance, the addition of activated charcoal or fumed silica, after the honey dissolution, in order to absorb organic species was shown to remove not only organics but also some metal (probably bound by organics) content, giving low and poor reproducible results. Among modern sample pre-treatment techniques, oxidative UV photolysis was proved to be simple and effective, because of minimal reagent requirement and very low blank values that minimize interferences.

3.1. UV photolysis

For making the proposed method amenable for the widest range of applicability, samples of honey of different origin and variable composition were chosen and the effect of UV radiation on various cations has been investigated in detail. Matrix matching 65° Brix blank solution as well as honey samples have been spiked with varying amounts of heavy and transition metal ions and subjected to UV photolysis for 3 h prior to performing instrumental analyses as reported in the procedure. The

results obtained with independent sample pre-treatments and techniques showed that cadmium (II), cobalt (II), copper (II), iron (III), lead (II), nickel (II) and zinc (II) are not affected by UV photolysis and the recovery of these species is between 97 and 103% in matrix matching blank solution as well as in honey samples. Manganese (II) cannot be completely recovered because of its oxidation due to the influence of UV radiation. The UV photolysis time was found strictly dependent from the sample composition and the amount of hydrogen peroxide added.

The purpose of using hydrogen peroxide in the course of photolysis is to supply free OH• radicals which accelerate the decomposition of organic components present in honey and in addition, the decomposition products of this reagent are water and oxygen that do not interfere in the subsequent analysis. Hence, depending upon the analytical requirements and the type of sample to be analyzed, the best compromise can be made between the photolysis time and the amount of hydrogen peroxide added.

3.2. pH effect

The IC or pulsed voltammetric analysis of metals in honey is dependent on the pH. After UV photolysis, the sample pH generally rises to about 9 and metals present in the solution can get hydrolyzed/precipitated and losses may take place. It is therefore necessary to lower the pH to ensure that all the metals present remain in soluble ionic form. If 10 μl of 1 M nitric acid is added, as proposed in the procedure, the pH is lowered to about 2, so that all the metals present remain in unbound/free form and insoluble oxides formation is prevented. When performing metals determination by IC, via complexation with eluent and anionic or cationic exchange followed by a post-column derivatization technique and absorbance detection, the best pH range of the sample solution is between 5 and 6, in order to permit the forming of the complexes with the eluent. It is therefore advisable that the solution after UV photolysis is buffered in the required pH range. The addition of 50 μl of 2 M ammonium acetate to the sample was found to be quite effective.

If pulsed voltammetries are used, the same pH range was found effective for the analysis of copper, cadmium, lead and zinc only, while the analysis of nickel and cobalt dimethylglyoximates is better performed at pH 9.5. In this case the procedure consists of two steps at different pH: the DPASV determination of zinc, cadmium, lead and copper is performed at pH 5.5 (acetate buffer) and the DPCSV determination of nickel and cobalt as dimethylglyoxime complex follows at pH 9.5 (ammonia–ammonium acetate buffer).

3.3. Ion chromatography

A gradient elution, based on oxalic and hydrochloric acids with nitrate anion eluents, is used. This chromatographic procedure was developed for the separation of nine metals on an IonPac CS5A column in the same run and, in particular, to perform lead and iron determination in the same run (Cardellicchio et al., 1999).

Despite of the improvement in cadmium sensitivity due to the post-column reagent 5-Br-PADAP, as suggested by Haitao, Shifen, Yan, Shenyang, and Riviello (1998), PAR is preferred in order to have good sensitivity for lead with a minimum loss in cadmium sensitivity.

Alkaline metals do not interfere in the determination, while calcium and magnesium react with post-column reagent and elute in the chromatogram, but the sensitivity is 100–500 times lower than that of transition metals in the proposed column operating conditions.

Typical chromatograms of honey samples, obtained with gradient elution, are shown in Fig. 1. In this figure an honey sample collected in open country is compared with another collected in a city. An advantage of the ion chromatographic approach is that the instrumentation is cheap and widespread and the same system with minimal modification can be used for the determination of the other ionic components (i.e. alkaline, alkaline-earths and inorganic or organic anions; e.g. Buldini et al., 1997).

3.4. Pulsed voltammetries

The proposed procedure permits the simultaneous and completely automatic analyses of a wide range of metals by microprocessor controlled voltammetry. A typical voltammogram of a honey sample is shown in Fig. 2. In the same figure it is shown that data processing for copper and zinc DPASV peak areas is not subjected to any interference. On the contrary, cadmium and lead DPASV peaks, as well as nickel and cobalt DPCSV ones, have been evaluated by taking into consideration only the front half of the first peak (cadmium or nickel) and the rear half of the second one (lead or cobalt), to avoid any interference that might occur in the presence of a very large excess (>1000 fold) of one element over the other.

More than 40 elements were tested for possible interfering effects as reported by Buldini, Ferri, and Nobili (1991). Interferences were found in the presence of a large excess of bismuth (III; on copper), titanium (IV; on copper, cadmium and lead), indium (III; on cadmium), chromium (VI; on lead), molybdenum (VI; on lead) and tungsten (VI; on zinc), but their occurrence in large excess over the species subject to interference is unusual in honey.

A particular characteristic of the voltammetric techniques is that the pre-concentration step takes place directly into the voltammetric cell, without risk of sample contamination; on the other hand the electrochemical techniques usually require more skill.

Some results obtained on different varieties of honey are summarized in Table 4. The same table evidences

the good agreement between the results obtained with IC and DPASV–DPCSV, after oxidative UV photolysis of the sample, and those obtained with ETAAS, after microwave oven digestion of the sample.

In Table 5 the concentration ranges in which calibration curves arelinear, with correlation coefficients greather than 0.995, are shown. In the absence of standards, they are determined by spiking a matrix matching 65° Brix blank solution with various amounts of the different cations, subjecting them to oxidative UV photolysis and then analyzing them by means of the proposed procedures. In the same table the detection limits, calculated according to Long and Winefordner (1983), are also shown.

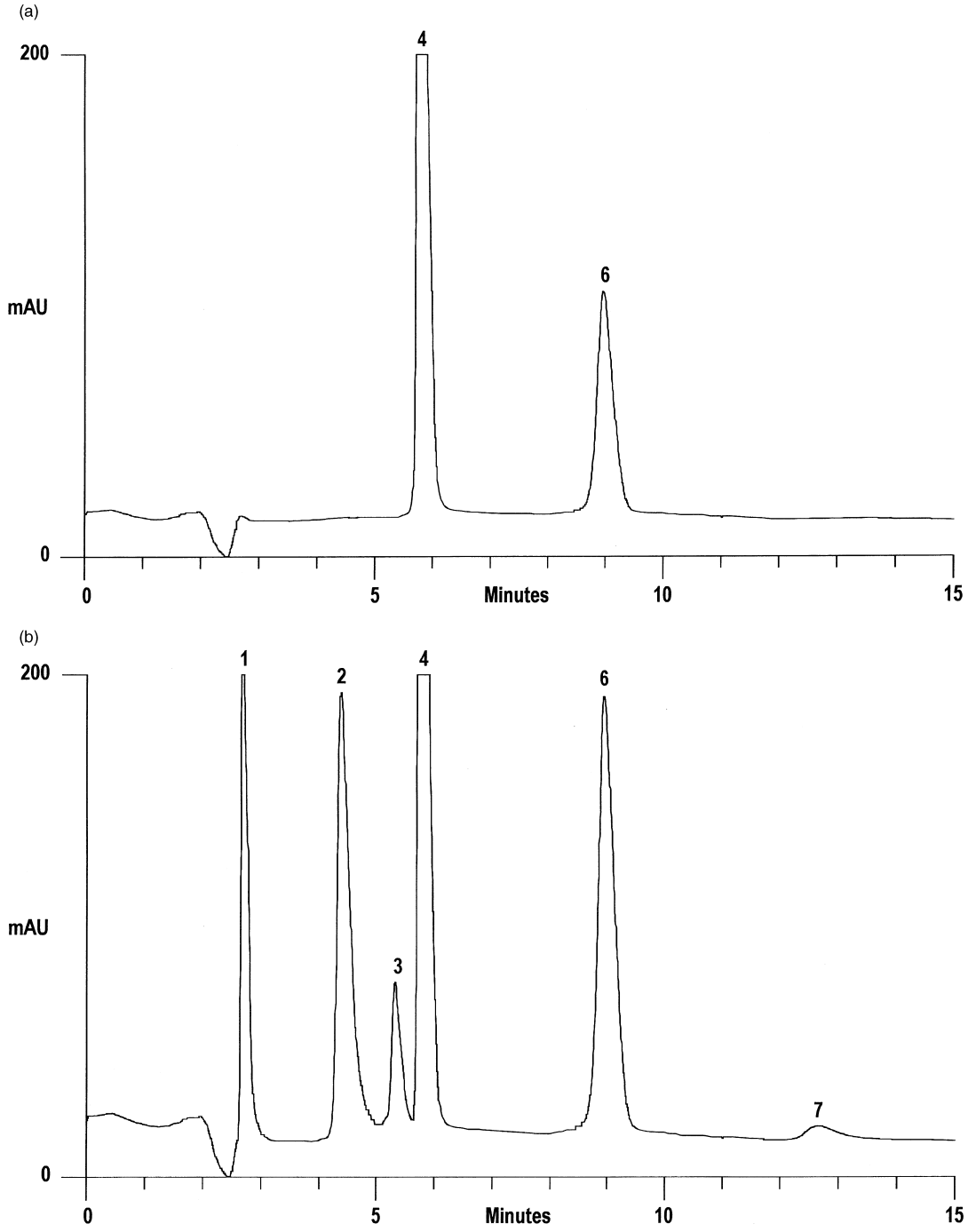

Fig. 1. Chromatogram of honey samples analyzed as described in Section 2. Chromatographic conditions as in Table 1. (a) Cations present in a honey sample collected in open country (*Mixed flower, Lizzano)*; peaks: 4, Zn^{2+} (2800 μg/Kg); 6, Fe^{3+} (630 μg/Kg). (b) Cations present in a honey sample collected in a city (*Mixed flower, Bologna*); peaks: 1, Cu^{2+} (900 μg/Kg); 2, Cd^{2+} (308 μg/Kg); 3, Ni^{2+} (400 μg/Kg); 4, Zn^{2+} (3200 μg/Kg); 6, Fe^{3+} (1250 μg/Kg); 7, Pb^{2+} (620 μg/Kg).

Table 4
Results obtained on different varieties of honey

Element	Analytical technique	Sample pre-treatment	Honey type: flower and geographical origin													
			Mixed flower, Punjab (India)		Mixed flower, U.P. (India)		Mixed flower, Ashok (India)		Mixed flower, Kashmir (India)		Mixed flower, Lizzano (Italy)		Mixed flower, Bologna (Italy)		Chestnut, Varese (Italy)	
			μg kg^{-1}	s[a]	μg kg^{-1}	s[a]	μg kg^{-1}	s[a]	μg kg^{-1}	s[a]	μg kg^{-1}	s[a]	μg kg^{-1}	s[a]	μg kg^{-1}	s[a]
Cd	IC	UV photolysis	< 125		< 125		< 125		550	±11	< 125		308	±7	< 125	
	DPASV	UV photolysis	< 50		< 50		< 50		545	±6	< 50		310	±4	< 50	
	ETAAS	MOD[b]	< 50		< 50		< 50		550	±10	< 50		305	±6	< 50	
Co	IC	UV photolysis	< 50		< 50		< 50		< 50		< 50		< 50		< 50	
	DPCSV	UV photolysis	< 100		< 100		< 100		< 100		< 100		< 100		< 100	
	ETAAS	MOD[b]	< 250		< 250		< 250		< 250		< 250		< 250		< 250	
Cu	IC	UV photolysis	1200	±25	1080	±20	2800	±60	1980	±40	< 25		900	±20	50	±3
	DPASV	UV photolysis	1220	±34	1070	±30	2750	±53	2000	±50	< 100		870	±35	< 100	
	ETAAS	MOD[b]	1200	±25	1065	±25	2750	±50	1950	±55	< 50		890	±20	< 50	
Fe	IC	UV photolysis	28000	±400	8000	±80	25000	±250	3550	±50	630	±8	1250	±18	1750	±20
	ETAAS	MOD[b]	28350	±500	7950	±100	24750	±390	3590	±50	650	±10	1265	±25	1750	±25
Ni	IC	UV photolysis	375	±18	< 100		400	±13	650	±18	< 100		400	±18	< 100	
	DPCSV	UV photolysis	380	±8	< 50		390	±8	640	±12	< 50		390	±10	< 50	
	ETAAS	MOD[b]	380	±10	< 50		400	±10	650	±15	< 50		385	±10	< 50	
Pb	IC	UV photolysis	< 50		680	±15	710	±20	925	±20	< 50		620	±15	< 50	
	DPASV	UV photolysis	< 100		660	±20	700	±25	910	±35	< 100		615	±20	< 100	
	ETAAS	MOD[b]	< 50		670	±15	695	±15	920	±25	< 50		620	±15	< 50	
Zn	IC	UV photolysis	4700	±50	1077	±12	11000	±100	29000	±200	2775	±25	3200	±30	2650	±20
	DPASV	UV photolysis	4670	±90	1070	±25	11200	±150	28900	±300	2760	±40	3220	±50	2665	±35
	ETAAS	MOD[b]	4725	±50	1080	±12	11100	±100	29000	±200	2800	±30	3205	±35	2640	±30

[a] $n = 10$.
[b] Microwave oven digestion.

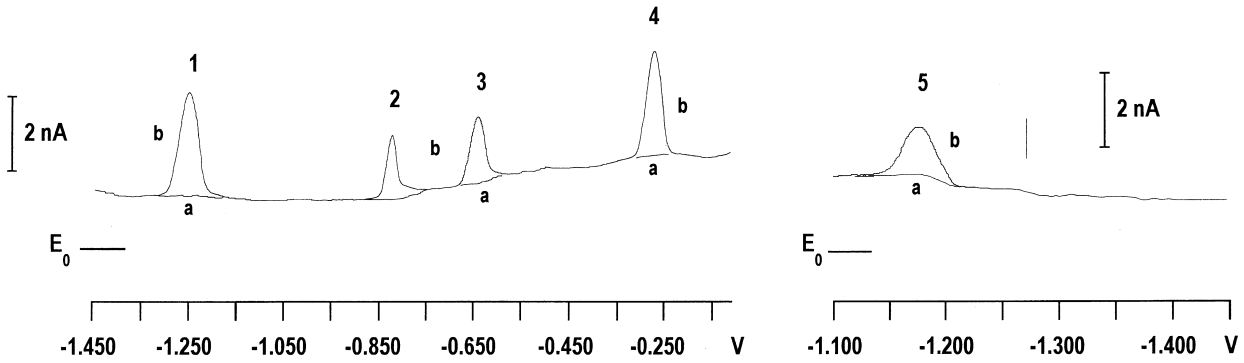

Fig. 2. Voltammogram of cations present in a honey sample collected in a city (*Mixed flower, Bologna*) and analyzed as described in Section 2. Voltammetric conditions as in Table 2. DPASV peaks in ammonium acetate buffer (pH 5.5): 1, Zn^{2+} E_p-1.250 V (3220 μg/Kg); 2, Cd^{2+} E_p-0.810 V (310 μg/Kg); 3, Pb^{2+} E_p-0.640 V (615 μg/Kg); 4, Cu^{2+} E_p-0.270 V (870 μg/Kg). DPCSV peaks in ammonia–ammonium acetate buffer (pH 9.5); 5, Ni^{2+} E_p-1.175 V (390 μg/Kg); Co^{2+} E_p-1.275 V, not found. (a) Reagents blank, (b) honey sample.

Table 5
Detection limits and concentration ranges

Element	Technique	Detection limit[a] (μg l^{-1})	Range (μg l^{-1})
Ion chromatography (injection volume 150 μl)			
Lead(II)		40	50–50000
Cadmium(II)		15	50–50000
Iron(III)		2	20–20000
Copper(II)		3	10–15000
Nickel(II)		4	10–15000
Zinc(II)		3	20–20000
Cobalt(II)		2	20–20000
Voltammetry			
Copper(II)	DPASV	10	20–10000
Cadmium(II)	DPASV	5	10–10000
Lead(II)	DPASV	10	20–10000
Zinc(II)	DPASV	20	40–10000
Nickel(II)	DPCSV	5	10–10000
Cobalt(II)	DPCSV	10	20–5000

[a] Calculated according to IUPAC guidelines (Long & Winefordner, 1983).

4. Conclusions

Both IC and pulsed voltammetries are shown to be suitable for the determination of metallic impurities in honey even though sample pre-treatment is needed. Oxidative UV photolysis was found to be superior compared to microwave oven digestion, because of minimal reagent requirement and very low blank values that minimize interferences. ETAAS was found to be more time consuming, not permitting multiple analyses and its short linearity range requires multiple dilution steps, especially when analyzing unknown samples of different origin.

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References

Barišić, D., Vertaénik, A., Bromenshenk, J. J., Kezić, N., Lulić, S., Hus, M., Kraljević, P., Šimpraga, M., & Seletković, Z. (1999). Radionuclides and selected elements in soil and honey from Gorski Kotar, Croatia. *Apidologie, 30*, 277–287.

Bromenshenk, J. J., & Carlson, S. R. (1985). Pollution monitoring of Pugget Sound with honey bees. *Science, 227*, 632–634.

Buldini, P. L., Cavalli, S., & Trifiró, A. (1997). State-of-the-art ion chromatographic determination of inorganic ions in food. *Journal of Chromatography A, 789*, 529–548.

Buldini, P. L., Ferri, D., & Nobili, D. (1991). Determination of transition metals in natural waters by microprocessor-controlled voltammetry in comparison with graphite furnace atomic absorption spectrometry. *Electroanalysis, 3*, 559–566.

Cardellicchio, N., Cavalli, S., Ragone, P., & Riviello, J. M. (1999). New strategies for determination of transition metals by complexation ion-exchange chromatography and post column reaction. *Journal of Chromatography A, 847*, 251–259.

Celli, G. (1984). L'Ape come insetto test della salute di un territorio. *Apicolt. Mod, 75*, 133–140.

Chung, S. W., & Tsai, W. C. (1992). A.A.S. Determination of heavy metals in foodstuffs using a simple digester. *At. Spectr, 13*, 185–189.

Clarke M. A. (1995). Bulk Sweeteners. In A. Townshend, *Encyclopedia of Analytical Science* (Vol. 9, p. 509). London: Academic Press.

Crane, E. (1984). Bee, honey and pollen as indicators of metals in the environment. *Bee World, 65*(1), 47–49.

Fodor, P., & Molnar, E. (1993). Honey as an environmental indicator: effect of sample preparation on trace element determination by ICP-AES. *Mikrochim. Acta, 112*, 113–118.

Haitao, L., Shifen, M., Yan, Y., Shenyang, T., & Riviello, J. M. (1998). On-line pretreatment and determination of Pb, Cu and Cd at the μg l^{-1} level in drinking water by chelation ion chromatography. *Journal of Chromatography A, 800*, 247–255.

Hoefel, I. (1985). Heavy metals in bees and bee products. *Apidologie, 16*, 196–197.

Iskander, F. Y. (1995). Trace and minor elements in four commercial honey brands. *Journal of Radioanalytical and Nuclear Chemistry Letters, 201*, 401–408.

Jamoussi, B., Zafaouf, M., & Ben Hassine, B. (1995). Hydride generation-condensation system with an inductively coupled argon plasma polychromator for simultaneous determination of As, Sb, Se, Pb, Hg, Sn in honey. *Intern. J. Environm. Anal. Chem., 61*, 249–256.

Jones, K. C. (1987). Honey as an Indicator of heavy metal contamination. *Water, Air and Soil Pollution, 33*, 179–189.

Kim, E. S., & Rhee, C. O. (1996). Comparison of quality attributes of Korean native-bee honey and foreign-bee honey by K/Na ratio. *J. Korean Soc. Food Sc. Nutrit, 25*, 672–679.

Kump, P., Necemer, M., & Šnajder, J. (1996). Determination of trace elements in bee honey, pollen and tissue by total reflection and radioisotope X-ray fluorescence spectrometry. *Spectrochim. Acta B, 51*, 499–507.

Leita, L., Muhlbachova, G., Cesco, S., Barbattini, R., & Mondini, C. (1996). Investigation of the use of honey bees and honey bee products to assess heavy metals contamination. *Environmental Monitoring and Assessment, 43*, 1–9.

Li, Y., Wahdat, F., & Neeb, R. (1995). Digestion-free determination of heavy metals (Pb, Cd, Cu) in honey using ASDPV and potentiometric stripping analysis. *Fresenius' Z. Anal. Chem., 351*, 678–682.

Long, G. L., & Winefordner, J. D. (1983). Limit of detection — a closer look at the IUPAC definition. *Analytical Chemistry, 55*, 712A–724A.

Otto, K., & Jekat, F. (1977). Experimental investigations on the contamination of a food (honey) with residues of lead, zinc and cadmium. *Ernaehrungs Umschau, 24*(4), 107–109.

Perez Cerrada, M., Herrero-Villen, M. A., & Maguieira, A. (1989). Sugar-rich food — determination of inorganic anions by ion chromatography. *Food Chemistry, 34*, 285–294.

Reid, H. J., Greenfield, S., & Edmonds, T. E. (1995). Investigation of decomposition products of microwave digestion of food samples. *Analyst (London), 120*, 1543–1548.

Scheubeck, E., Nielsen, A., & Iwantscheff, G. (1979). Schnellverfahren fuer den aufschluss von groesseren mengen an biomaterial zur analytischen erfassung von schwermetallspuren. *Fresenius' Z. Anal. Chem., 294*, 398- 401.

Sevimli, H., Bayulgen, N., & Varinlioglu, A. (1992). Determination of trace elements in honey by I.N.A.A. in Turkey. *Journal of Radioanalytical and Nuclear Chemistry Letters, 165*, 319–325.

Stein, K., & Umland, F. (1986). Spurenbestimmung von blei, cadmium und mangan in honingen und zuckern. *Fresenius' Z. Anal. Chem., 323*, 176–177.

Viñas, P., Lopez-Garcia, I., Lanzon, M., & Hernandez-Cordoba, M. (1997). Direct determination of Pb, Cd, Zn and Cu in honey by electrothermal atomic absorption spectrometry using hydrogen peroxide as a matrix modifier. *Journal of Agricultural and Food Chemistry, 45*, 3952–3956.

Voget, M., & Baudisch, H. (1983). Schnelle und direkte bleibestimmung in honey. *Z. Lebensm. Unters. Forsch, 176*, 360–362.

White Jr. J. W. (1979) Composition of Honey. In E. Crane, *Honey: A Comprehensive Survey*, chapter 5, pp. 157–206. London: Heinemann—International Bee Research Association.