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Article

Pre-Concentration of Traces of Cadmium, Cobalt, Nickel and Lead in Natural Honeys by Solid Phase Extraction Followed by Their Determination Using Flame Atomic Absorption Spectrometry

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Neste trabalho foi proposto um procedimento de preparo de amostra simples e rápido, para a determinação de traços de Cd, Co, Ni e Pb em méis. O procedimento consiste no preparo da amostra em meio ácido para a retenção dos elementos de interesse em uma resina de troca catiônica fortemente ácida, Dowex 50W×8-400, e a eluição com uma solução de HCl 3,0 mol L^{-1} de HCl, antes da determinação por espectrometria de absorção atômica com chama (FAAS). O método proposto apresentou precisão e exatidão superior a 4,5% e limites de detecção de 0,005 µg g^{-1} (Cd), 0,015 µg g^{-1} (Co, Ni) e 0,073 µg g^{-1} (Pb). A confiabilidade dos resultados obtidos com o preparo de amostra desenvolvido e a determinação por FAAS foi validado com testes de adição e recuperação, além da comparação com os resultados obtidos usando a espectrometria de emissão óptica com plasma acoplado indutivamente, após a digestão por via úmida. O método foi aplicado para a análise de sessenta e nove amostras de méis da região da Baixa Silésia (sudeste da Polônia). Verificou-se que os teores de Cd, Co e Pb estavam abaixo do limite de detecção, enquanto Ni foi determinado em nove amostras de mel no intervalo de concentração de 0,19-0,93 µg g^{-1}.

A simple and fast sample preparation procedure for the determination of traces of Cd, Co, Ni and Pb in honeys was proposed. It included the preparation of acidified sample solutions of honeys, the retention of elements of interest on resin beds of a strongly acidic gel cation exchanger Dowex 50W×8-400, and their elution with a 3.0 mol L^{-1} HCl solution prior to measurements by flame atomic absorption spectrometry (FAAS). The method provided the precision and the accuracy better than 4.5% and detection limits of 0.005 µg g^{-1} (Cd), 0.015 µg g^{-1} (Co, Ni) and 0.073 µg g^{-1} (Pb). The reliability of results obtained with the developed procedure and FAAS was validated by the recovery test and the comparison with results achieved using the wet digestion and inductively coupled plasma optical emission spectrometry. The method was applied for the analysis of sixty nine samples of honeys originated from the Lower Silesia region (southeast Poland). It was found that Cd, Co and Pb were under detection limits, while Ni was determined in nine honeys at the level of 0.19-0.93 µg g^{-1}.

Keywords: honey, trace analysis, solid phase extraction, flame atomic absorption spectrometry

Introduction

The contamination of honey with elements is acknowledged to be a result of various anthropogenic factors, primarily the environmental pollution.[1-4] When bees forage in industrialized and urban areas, they collect the dust and other pollutions and carry them into apiaries. As a result, honey maturing in the hive is exposed to the contamination with certain trace elements, e.g., Al, Cd, Co, Cr, Cu, Mn, Ni, Pb, Pd and Zn, that changes its unique quality and safety.[1-9] Regardless of botanical or geographical origins of honey, this is usually reflected by strong correlations between concentrations of certain contaminating trace elements, i.e., Cd and Cu, Cd and Co, Co and Ni, or Cu and Ni.[3,10] In reference to the content of specified trace elements, beekeeping practices are also relevant to the quality of honey.[1,3-5,8,9,11-14] Accordingly, the contamination of honey with certain trace elements can happen during the harvest, at different stages of the honey processing or the storage. The use of tools and containers made from inappropriate materials, i.e., stainless steel or galvanized steel, brass, aluminum alloys, and/or prohibited

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practices, materials and chemicals is recognized to be the main source of different minor and trace elements. In particular, elements can be released from paints, lacquer coverings, preservatives and impregnates. The corrosion of materials and the release of trace elements are facilitated by the natural honey acidity, i.e., pH of 3.5-4.8.[1,3,13,15]

The presence of Cd, Co, Ni and Pb at concentrations that significantly exceed prescribed health safety standards are undesirable because of their possible toxicity and adverse effects on the human body.[5,16-18] Since the consumption of honey as such or as an ingredient of other foods cannot be associated with any anxiety about the public health, its quality should be controlled and assured. This can be assessed by means of routine analyses carried out by using fast and dependable analytical methods and procedures.[19] Such information on the content of trace elements in honey that constitute a risk to humans is quite relevant to consumers and beekeepers in terms of the food nutritional value and the food safety of this product.

The analysis of different varieties of honey collected from one geographical region on the content of Cd, Co, Ni and Pb may provide the useful information about its quality and additionally give the possibility to assess the state of the cumulative environmental pollution in this region. Unfortunately, the determination of Cd, Co, Ni and Pb in honey with flame atomic absorption spectrometry (FAAS), customarily applied for measurements of major and minor elements in this food product, appears to be difficult due to low concentrations of these elements and possible matrix effects.[1,2,4-6,10-12] As reported in cited works, relatively high samples of honey are initially digested using dry and wet decomposition procedures in order to enrich trace elements of interest and eliminate possible chemical and physical interferences originated from the presence of high concentrations of carbohydrates. In special cases, it is necessary to complex ions of these elements, e.g. Cd, Pb, in digested sample solutions with ammonium pyrrolidine-dithiocarbamate (APDC) and extract them into the organic phase of methyl isobutyl ketone (MIBK) prior to measurements by FAAS.[5]

In the routine analysis of a large number of honey samples, the risk of their contamination owing to long-lasting sample digestion treatments and the addition of oxidative reagents or the loss of determined trace elements through the vaporization of their volatile species formed in conditions of high-temperature dry and wet decompositions is quite high and may distort the interpretation of final results.[19] Relatively long times required for such treatments of honey samples and large amounts of expensive reagents used are also critical and support the search for alternative sample preparation procedures, which would provide a substantial pre-concentration of trace elements in a relatively short period of time without the need for tedious and cumbersome sample matrix decompositions.

Assuming that the simplicity of the sample preparation step is one of the most important criterion evaluating its usefulness for the trace analysis, instead of time-consuming and cumbersome dry or wet decompositions of honeys, a simplified solid phase extraction (SPE) based procedure was developed in the present work for the pre-concentration of traces of Cd, Co, Ni and Pb prior to the determination of their total concentrations by FAAS. Ions of elements of interest were retained from solutions of honey samples dissolved in water and acidified with HNO_3 to a certain concentration, i.e., 0.2 mol L^{-1}, on cation-exchange Dowex 50W×8-400 resin beds. This alternative method of the analysis of honeys was applied for the reliable determination of traces of Cd, Co, Ni and Pb in honeys available in the market and directly collected from apiaries in the Lower Silesia region (southwest of Poland) to assess their food safety and quality.

Experimental

Reagents and solutions

Re-distilled water was used throughout. ACS grade solutions of 30% (m/m) H_2O_2, 37% (m/m) HCl and 65% (m/m) HNO_3 were purchased from J. T. Baker (Deventer, Netherlands). TraceCERT® single-element 1000 mg L^{-1} standard solutions of Cd, Co, Ni and Pb were supplied by Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Other reagents were of analytical grade. Solid $CaCl_2$, KCl, $MgCl_2$ and NaCl from POCH (Gliwice, Poland) were used to prepare 10000 mg L^{-1} single-element standard solutions of alkali and alkaline earth elements. D-fructose (> 98%) and D-glucose (> 98%) in addition to other chemicals, i.e., Na_2CO_3, $NaHCO_3$, $NaKC_4H_4O_6{\cdot}4H_2O$, Na_2SO_4, $CuSO_4{\cdot}5H_2O$, $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, H_2SO_4, $Na_2HAsO_4{\cdot}7H_2O$, were obtained from POCH as well. The latter reagents were used to prepare working solutions used in the spectrophotometric determination of the sum of fructose and glucose by the Somogy-Nelson method according to the description given by BeMiller and Low.[20]

For the optimization study of sorption and desorption properties of cation-exchange and amphoteric resins, 100 mL working standard solutions containing Cd, Co, Ni and Pb, all at 0.2 mg L^{-1}, in addition to fructose and glucose, both at 40 g L^{-1}, were prepared. Concentrations of elements of interest and both monosaccharides in these solutions corresponded to their average contents in 10% (m/v) water solutions of Polish honeys and established on the basis of the data reported by Dobrzanski *et al.*,[21] Bulinski *et al.*,[22] Przybylowski *et al.*,[23] Spodniewska and Romaniuk,[24] and Chudzinska and Baralkiewicz.[25] These working standard solutions were acidified to pH 3.5, 4.0 and 4.5 using a 0.02 mol L^{-1} HCl solution.

Instrumentation and measurements

Concentrations of Cd, Co, Ni and Pb in sample solutions and eluates were measured using a Perkin Elmer (Waltham, MA, USA) single-beam FAAS instrument, model 1100B with a deuterium lamp background corrector. A single-slot 10 cm burner head for the combustion of a C_2H_2-air flame was mounted onto a burner chamber integrated with an end cup and a drain interlock assemblage. A stainless steel nebulizer was used to nebulize solutions, while a flow spoiler was applied to discriminate the size of droplets of the solution aerosol. Operational conditions recommended by the instrument manufacturer were used for the determination of elements of interest (see Table 1). Absorbance readings were carried out using a time-average integration mode; 5 readings were integrated at 0.1 s intervals over a 10 s integration time and averaged.

Table 1. Experimental conditions for FAAS and ICP OES measurements

FAAS	
C_2H_2 flow rate / (L min^{-1})	1.4
Air flow rate / (L min^{-1})	8.0
Analytical wavelength / nm	228.8 (Cd), 240.7 (Co), 232.0 (Ni), 283.3 (Pb)
Slit width / nm	0.7 (Cd, Pb), 0.2 (Co, Ni)
HCL current / mA	5 (Cd), 20 (Co), 15 (Ni), 8 (Pb)
ICP OES	
Forward power / kW	1.0
Observation height (ALC) / mm	12
Ar plasma gas flow rate / (L min^{-1})	13.0
Ar sheath gas flow rate / (L min^{-1})	0.2
Ar carrier gas flow rate / (L min^{-1})	0.3
Sample uptake rate / (mL min^{-1})	1.0
Analytical wavelength / nm	228.8 (Cd I), 228.6 (Co II), 221.6 (Ni II), 220.3 (Pb II)
Integration time per each point / s	0.1
Number of points per peak	8

HCL: hollow cathode lamp; ALC: above load coil.

In addition, the determination of Cd, Co, Ni and Pb concentrations in solutions of digested samples of selected honeys were made using a Jobin Yvon (Jobin Yvon, France) sequential inductively coupled Ar plasma optical emission spectrometer (ICP OES), model JY 38S. The instrument was equipped with a 40.68 MHz RF generator and a 1-m monochromator with two gratings (4320 and 2400 grooves mm^{-1}). A concentric Meinhard-type pneumatic nebulizer mounted into a cyclonic spray chamber was used for the pneumatic nebulization of solutions. Working parameters for ICP OES measurements are given in Table 1.

A Thermo Scientific Spectronic 20D+ digital spectrophotometer (Bremen, Germany) was used in a two step procedure to measure the sum of fructose and glucose using the Somogy-Nelson method as described by BeMiller and Low.[20]

Resins and pre-conditioning of resin beds

Polymeric resins used for SPE were obtained from Sigma-Aldrich. These were the following ion-exchange resins: weakly acidic cation-exchangers Amberlite IRP-69 (matrix: methacrylic-divinylbenzene, particle size: 38-150 µm, capacity: 10 meq g^{-1} by dry weight) and Diaion WT01S (matrix: acrylic polymer, particle size: 100-200 µm, capacity: 3.0 meq mL^{-1} by wetted bed volume), strongly acidic cation-exchangers Dowex 50W×8-400 (matrix: styrene-divinylbenzene (gel), particle size: 38-75 µm, capacity: 1.7 meq mL^{-1} by wetted bed volume) and Dowex HCR-W2 (matrix: styrene-divinylbenzene (gel), particle size: 425-1180 µm), and an amphoteric exchanger Dowex Retardion 11A8 (matrix: styrene-divinylbenzene (gel), particle size: 150-300 µm, capacity 1.2 and 1.1 meq g^{-1} by dry weight for $-COO^-$ and $-N(CH_3)_3^+$ functional groups, respectively). Water slurries of these resins (portions of 1.0 g) were packed into glass SPE columns (10 mm i.d.) equipped with coarse frits and polytetrafluoroethylene (PTFE) stopcocks.

Resin beds formed in SPE columns were conditioned by passing through them 10 mL of a 2.0 mol L^{-1} HCl solution. Afterwards, they were washed with 40 (for Amberlite IRP-69, Dowex 50W×8-400, Dowex HCR-W2, Dowex Retardion 11A8) and 60 mL of water (for Diaion WT01S) to remove an excess of HCl. Column beds of the Dowex Retardion 11A8 resin were additionally rinsed with 10 mL of a 1.0 mol L^{-1} $NH_3{\cdot}H_2O$ solution, followed by 40 mL of water. Conditioning solutions and water were delivered at a flow rate of 4.0 mL min^{-1} using a 4-channel MasterFlex L/S peristaltic pump (Cole-Parmer, Vernon Hill, IL, USA).

Samples and their treatment

Sixty nine honeys were analyzed in this study, including 9 samples commercially available in the market and 60 honeys directly taken from apiaries located in different cities and villages of the Lower Silesia region, the southwest part of Poland (see Figure 1 and Table 2). All honeys were collected in 2012.

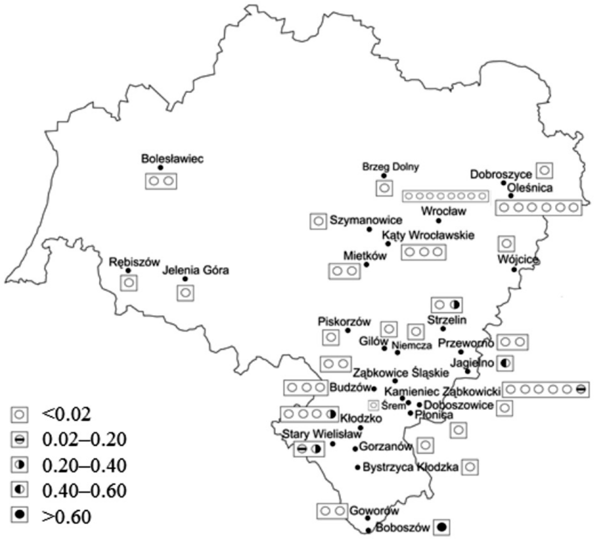

Figure 1. The concentration of Ni (μg g^{-1}) in honeys collected from apiaries located in different cities and villages of the Lower Silesia region.

Three different sample treatments were tested to prepare solutions of honey samples prior to the pre-concentration of Cd, Co, Ni and Pb by the pre-concentration SPE-based procedure. Accordingly, samples of honeys (10.0 g) were dissolved and diluted in water to 100 mL (A), treated with a concentrated HNO_3 solution (1.5 mL *per* 100 mL of a sample solution) and next dissolved and diluted with water to 100 mL (B), and treated with a concentrated HNO_3 solution (1.5 mL *per* 100 mL of a sample solution), diluted with 20 mL of water and heated on a hot plate for about 1 hour, and finally diluted with water to 100 mL (C).

Resulting 10% (m/v) sample solutions of honeys were subjected to the pre-concentration SPE-based procedure. These solutions were passed at a flow rate of 20 mL min^{-1} through resin beds of Dowex 50W×8-400 and then washed with 20 mL of water at the same flow rate. Elements retained were eluted using 5.0 mL of a 3.0 mol L^{-1} HCl solution passed at a flow rate of 2.0 mL min^{-1}. Respective portions of eluates were collected and subjected to the analysis by FAAS on the content of Cd, Co, Ni and Pb against matrix matching standard solutions (containing the appropriate amount of HCl) and solutions of procedural blanks.

Table 2. Honeys collected from the Lower Silesia region

Label (the type and the variety of honey)	Distributor / geographical location
1_m_A, 2_m_R, 3_m_H	PH Barc
4_m_HD	Sadecki Bartnik
5_m_B	CD S. A.
6_m_L, 7_m_B, 8_m_HD, 9_m_M	Huzar Sp. z o. o.
12_a_B	Boboszow
44_a_M, 69_a_H	Boleslawiec
34_a_R	Brzeg Dolny
13_a_B, 45_a_M, 46_a_M	Budzow
47_a_M	Bystrzyca Klodzka
48_a_M	Doboszowice
35_a_R	Dobroszyce
49_a_M, 50_a_M	Gaworow
21_a_L	Gilow
14_a_B	Gorzanow
22_a_L	Jagielno
51_a_M	Jelenia Gora
15_a_B, 23_a_L, 24_a_L, 25_a_L, 43_a_HD, 52_a_M	Kamieniec Zabkowicki
10_a_A, 26_a_L, 53_a_M	Katy Wroclawskie
16_a_B, 17_a_B, 54_a_M, 55_a_M	Klodzko
56_a_M, 57_a_M	Mietkow
58_a_M	Niemcza
18_a_B, 31_a_G, 36_a_R, 37_a_R, 38_a_R, 59_a_M	Olesnica
39_a_R	Pieszyce
60_a_M	Plonica
19_a_B	Rebiszow
28_a_L	Srem
20_a_B, 62_a_M	Stary Wielislaw
27_a_L, 63_a_M	Strzelin
32_a_G	Wojcice
29_a_L, 33_a_G, 41_a_R, 64_a_M, 65_a_M, 66_a_M, 67_a_M, 68_a_M	Wroclaw
40_a_R	Szymanowice
30_a_L, 42_a_R	Zabkowice Slaskie

m: samples taken from the market; a: samples taken from apiaries; A: acacia honey; B: buckwheat honey; G: goldenrod honey; R: rape honey; H: heather honey: HD: honeydew honey; M: multiflower honey.

For the comparison of results, a reference method, including the wet digestion sample preparation and the analysis of resulting solutions by ICP OES, was used. Accordingly, samples of honeys (10.0 g) were treated with 50 mL of a concentrated HNO_3 solution and left overnight under the cover. Afterwards, they were heated on a hot plate to decompose the matrix and reduce the volume of sample aliquots to about 1-2 mL. Then, another portion of a concentrated HNO_3 solution (25 mL) was added and again the volume of sample aliquots was reduced to about 1-2 mL. After cooling, 10 mL of a 30% (m/v) H_2O_2 solution were added to sample residues left and heated to a complete decay of this reagent. Finally, remnants were reconstituted with water and diluted to 25 mL. Resulting 40% (m/v) sample solutions were analyzed by ICP OES on the content of Cd, Co, Ni and Pb against simple standard solutions and respective procedural blanks.

Results and Discussion

Sorption behavior of ion exchange resins

At the outset, sorption properties of studied ion exchange resins, i.e., Amberlite IRP-69, Diaion WT01S, Dowex 50W×8-400, Dowex HCR-W2 and Dowex Retardion 11A8, toward simple ions of Cd, Co, Ni and Pb were evaluated. For that purpose, 100-mL working standard solutions containing Cd, Co, Ni and Pb (pH 3.5, 4.0 and 4.5) were passed at a flow rate of 2.0 mL min^{-1} through SPE columns filled with ion-exchange resins. After loading more than the 4/5 of the volume of these solutions, column effluents (10 mL) were collected and analyzed to determine the concentration of the sum of fructose and glucose not retained by resin beds. Considering concentrations of the sum of both sugars determined in effluents and their concentrations in loaded working standard solutions, the separation efficiency of fructose and glucose was assessed. Ions of Cd, Co, Ni and Pb, retained in these conditions on resin beds, were eluted using 10 mL of a 2.0 mol L^{-1} HCl solution. Eluates were collected and analyzed by FAAS on the content of Cd, Co, Ni and Pb against matrix matching standard solutions (containing HCl at a concentration of 2.0 mol L^{-1}) and respective procedural blanks. Considering concentrations of Cd, Co, Ni and Pb found in eluates and their concentrations in loaded solutions, it was possible to assess the retention efficiency of these elements on given resins versus the pH of solutions. In a similar way, working standard solutions (pH 4.0) were passed at 5.0, 10 and 20 mL min^{-1} to evaluate the effect of the flow rate on the separation efficiency of sugars and the retention efficiency of Cd, Co, Ni and Pb on Dowex 50×8-400 and Dowex HCR-W2 resins.

It was established that regardless of the resin used, concentrations of the sum of fructose and glucose determined in effluents resulted from loading working standard solutions (pH 3.5, 4.0 and 4.5) through resin beds corresponded to their concentrations in these solutions, i.e., 80 g L^{-1}. Accordingly, separations efficiencies of fructose and glucose evaluated in these conditions were changed from 95.3 to 101%. As can be seen from Table 3, only both strongly acidic cation-exchanger gel resins, i.e., Dowex 50W×8-400 and Dowex HCR-W2, were found to yield quantitative retention efficiencies of simple ions of Cd, Co, Ni and Pb. Retention efficiencies of these elements assessed at pH of 3.5, 4.0 and 4.5 were within 97.6-100% (Dowex 50W×8-400) and 95.7-100% (Dowex HCR-W2).

In addition, it was verified that both resins also completely retained Cd, Co, Ni and Pb from working standard solutions (pH 3.5, 4.0 and 4.5) containing 2000 mg L^{-1} of gallic acid or tannic acid. In this case, retention efficiencies of elements were established to change from 96.8 to 100% (Dowex 50W×8-400) and from 96.4 to 100% (Dowex HCR-W2).

The weakly acidic cation-exchanger Amberlite IRP-69 was ascertained to completely retain only Cd and Pb, and partly Co. Another weakly acidic cation-exchanger, i.e.,

Table 3. Retention efficiencies of Cd, Co, Ni and Pb *vs.* the solution pH

	pH	Amberlite IRP-69	Diation WT01S	Dowex 50W×8-400	Dowex HCR-W2	Dowex Retardion 11A8
Cd	3.5	93.3 ± 4.9	6.4 ± 0.7	97.6 ± 2.2	99.7 ± 0.6	61.4 ± 0.5
	4.0	94.2 ± 2.1	7.1 ± 1.4	100 ± 0.1	99.5 ± 0.8	54.5 ± 1.9
	4.5	95.0 ± 5.1	6.2 ± 0.3	100 ± 0.1	100 ± 0.1	65.0 ± 2.0
Co	3.5	94.3 ± 3.3	0.1 ± 0.1	98.0 ± 0.1	95.7 ± 3.7	88.6 ± 8.3
	4.0	93.1 ± 8.3	0.1 ± 0.1	100 ± 0.1	100 ± 0.1	90.5 ± 4.3
	4.5	88.3 ± 0.1	0.1 ± 0.1	100 ± 0.1	100 ± 0.1	99.0 ± 1.4
Ni	3.5	85.1 ± 4.2	14.0 ± 3.3	98.0 ± 0.1	100 ± 0.1	89.4 ± 5.0
	4.0	88.2 ± 0.5	14.2 ± 4.7	100 ± 0.1	100 ± 0.1	89.9 ± 1.6
	4.5	83.6 ± 5.2	10.5 ± 0.5	97.7 ± 1.8	100 ± 0.1	91.5 ± 5.5
Pb	3.5	93.3 ± 0.1	42.2 ± 1.6	100 ± 0.1	100 ± 0.1	86.1 ± 0.1
	4.0	100 ± 0.1	54.1 ± 0.1	100 ± 0.1	100 ± 0.1	87.2 ± 0.1
	4.5	100 ± 0.1	49.5 ± 1.8	99.0 ± 1.8	100 ± 0.1	89.1 ± 5.3

Average values for n = 3 with standard deviations.

Diaion WT01S, was useless; retention efficiencies of Cd and Ni were lower than 15%, while Co was not retained at all. In case of the amphoteric resin Dowex Retardion 11A8, retention efficiencies of elements of interest were lower than 90%, particularly in case of Cd and Pb.

Considering all of this, Dowex 50W×8-400 and Dowex HCR-W2 resins were taken for further experiments. Using working standard solutions of Cd, Co, Ni and Pb (pH 4.0), it was established that these two resins quantitatively retained Cd, Co, Ni and Pb from solutions passed through SPE columns at higher flow rates. Retention efficiencies assessed for elements of interest were within the following ranges: 96.3-100% (Dowex 50W×8-400) and 97.1-100% (Dowex HCR-W2). It was also found that such high flow rates had no effect on the separation efficiency of sugars, which were separated from Cd, Co, Ni and Pb with efficiencies ranged from 98.0 to 100%. In practice, such behavior of both resins was quite convenient since enabled to shorten the time of the sample treatment aimed at pre-concentrating Cd, Co, Ni and Pb ions without a risk of their loss.

Since the particle size of Dowex 50W×8-400 (38-74 µm) is lower and more homogenous than this for Dowex HCR-W2 (425-1180 µm), the first resin was selected for further investigations. It was expected that this resin would provide a more uniform flow of sample solutions through resin beds.

Desorption behavior of Dowex 50W×8-400

Desorption properties of Dowex 50W×8-400 were examined by applying 1.0, 2.0 and 3.0 mol L^{-1} HCl and HNO_3 solutions in the amount of 5.0 and 10 mL. Initially, simple ions of Cd, Co, Ni and Pb were retained on resin beds of Dowex 50W×8-400 by passing 100-mL working standard solutions (pH 4.0) at a flow rate of 20 mL min^{-1}. Then, 5.0 and 10 mL portions of acid solutions were passed at a flow rate of 2.0 mL min^{-1} through resin beds to strip elements of interest. Respective portions of eluates were collected and subjected to the analysis by FAAS on the content of Cd, Co, Ni and Pb versus matrix matching standard solutions and respective procedural blanks. Results of these experiments are given in Table 4.

It was established that none of HNO_3 solutions (in the amount of 5.0 or 10 mL) provided the complete recovery of all four elements of interest. The application of a 1.0 mol L^{-1} HCl solution was either useless. The quantitative recovery was achieved when more concentrated solutions of HCl were used, i.e., 10 mL of a 2.0 mol L^{-1} solution or 5.0 and 10 mL of a 3.0 mol L^{-1} solution. In the latter case, recovery efficiencies of Cd, Co, Ni and Pb were assessed to have changed from 97.3 to 100%.

Effect of alkali and alkaline earth elements

In earlier works[26,27] it was established that Dowex 50W×8 resins are capable of retaining Ca, Na, K and Mg ions, predominant mineral constituents of honey, hence, it was expected that in conditions of the retention and the elution of Cd, Co, Ni and Pb alkali and alkaline earth elements would be concomitantly retained and eluted as well. Therefore, the effect of high concentrations of Ca (30, 60, 90 and 120 mg L^{-1}), K (850, 1700, 2550 and 3400 mg L^{-1}), Mg (15, 30, 45 and 60 mg L^{-1}) and Na (20, 40, 60 and 80 mg L^{-1}), corresponding to their content in 10% (m/v) solutions of different Polish honeys,[28] was examined on the analytical signal of Cd, Co, Ni and Pb. It was found that high concentrations of Ca, K, Mg and Na

Table 4. Recovery efficiencies of Cd, Co, Ni and Pb from Dowex 50W×8-400 obtained with HCl and HNO_3 solutions of different concentrations and volumes

	1.0 mol L^{-1}		2.0 mol L^{-1}		3.0 mol L^{-1}	
	5.0 mL	10 mL	5.0 mL	10 mL	5.0 mL	10 mL
			HCl			
Cd	99.7 ± 0.1	100 ± 0.1	100 ± 0.1	100 ± 0.1	100 ± 0.1	100 ± 0.1
Co	51.8 ± 1.7	70.9 ± 4.6	83.0 ± 0.1	100 ± 0.1	97.6 ± 0.6	100 ± 0.1
Ni	36.1 ± 6.8	94.8 ± 4.3	92.1 ± 0.1	100 ± 0.1	99.4 ± 0.4	98.5 ± 0.7
Pb	83.2 ± 0.1	91.2 ± 6.4	91.6 ± 7.5	100 ± 0.1	97.5 ± 0.7	100 ± 0.1
			HNO_3			
Cd	38.3 ± 5.6	73.7 ± 9.2	87.0 ± 1.3	94.4 ± 0.1	89.9 ± 3.9	93.2 ± 1.3
Co	24.0 ± 7.9	73.6 ± 5.3	89.7 ± 1.1	91.1 ± 9.0	66.1 ± 0.1	84.4 ± 0.1
Ni	40.2 ± 4.2	71.5 ± 5.4	91.8 ± 2.4	88.1 ± 6.2	90.9 ± 1.1	97.0 ± 2.9
Pb	4.6 ± 5.9	29.4 ± 5.9	94.4 ± 5.0	91.5 ± 2.8	91.8 ± 8.5	92.3 ± 0.1

Average values for n = 3 with standard deviations.

do not affect signals of Cd, Co, Ni and Pb measured by FAAS. In relation to absorbances of Cd, Co, Ni and Pb achieved in conditions without alkali and alkaline earth elements, absorbances of these elements measured in solutions with alkali and alkaline earth elements at different concentrations studied were within 95.7-100% for Ca, 97.3-100% for K, 96.4-100% for Mg and 97.1-100% for Na. Therefore, there was no need to separate these elements from Cd, Co, Ni and Pb in a sequential elution procedure.

Treatment of honey samples prior to SPE pre-concentration

The SPE-based procedure of the pre-concentration of Cd, Co, Ni and Pb in honey samples and the separation of these elements from fructose and glucose relied on the passage of 100 mL of 10% (m/v) honey solutions through 1.0-g resin beads of Dowex 50W×8-400. Elements retained were eluted using 5.0 mL of a 3.0 mol L^{-1} HCl solution and measured in eluates by FAAS versus matrix matching standard solutions and respective procedural blanks.

Before the determination of traces of Cd, Co, Ni and Pb by FAAS in honeys collected for this study by the developed SPE-based procedure, different means of the preparation of sample solutions were tested. Samples of selected honeys, i.e., 4_m_HD, 5_m_B, 7_m_B, 8_m_HD, were spiked with known amounts of Cd (0.009 $\mu g\ g^{-1}$), Co and Ni (0.040 $\mu g\ g^{-1}$) and Pb (0.180 $\mu g\ g^{-1}$) and treated in the following way: dissolved and diluted with water (A), poured with concentrated HNO_3 and diluted with water (B), poured with concentrated HNO_3, heated on a hot plate and diluted with water (C). Solutions of unspiked and spiked samples of honeys prepared in this way were subjected to the SPE-based procedure followed by the FAAS analysis in order to evaluate recoveries of added elements.

The best results were obtained when samples were acidified with HNO_3 (average recoveries for all 4 honeys were 100, 99.3, 100 and 100% for Cd, Co, Ni and Pb, respectively) or acidified and heated later on (average recoveries for all 4 honeys were 99.3, 98.6, 100 and 99.9% for Cd, Co, Ni and Pb, respectively). The precision of these results, expressed as relative standard deviations for 3 independent replicates, varied from 1.2 to 4.5%.

Unfortunately, the dissolution of samples only in water was found to provide average recoveries of 92.1, 86.9, 87.5 and 62.4% for Cd, Co, Ni and Pb, respectively. This pointed out that elements of interest could be complexed by matrix components and not retained by the cation-exchange resin used. To deliver the complete release of elements and simplify the whole procedure, it was decided that samples of honeys would be treated with HNO_3 and then diluted with water to prepare respective sample solutions for the SPE-based pre-concentration of Cd, Co, Ni and Pb before FAAS measurements. Using such procedure, detection limits (DLs) of Cd, Co, Ni and Pb were evaluated to be 0.005, 0.015, 0.015 and 0.073 $\mu g\ g^{-1}$ for Cd, Co, Ni and Pb, respectively.

The reliability of results and the analytical application

Due to the lack of a honey certified reference material, the reliability of results obtained using the developed SPE-based pre-concentration/separation procedure has been verified for selected honeys, i.e., 4_m_HD, 5_m_B, 7_m_B, 8_m_HD, with those achieved for the wet digestion of 10.0-g samples of these honeys and the ICP OES analysis of resulting 40% (m/v) sample solutions. As can be seen in Table 5, results achieved with both analytical methods are quite comparable, particularly in case of concentrations of Cd determined in 8_m_HD and concentrations of Ni determined in 4_m_HD and 8_m_HD; these concentrations are statistically indistinguishable according to the *t*-test. DLs of Cd, Co, Ni and Pb assessed for ICP OES, where 40% (m/v) sample solutions of honeys were measured, were also corresponding to those for FAAS preceded by the SPE pre-concentration.

Table 5. The comparison of results of the analysis of selected honeys by FAAS with the developed solid phase extraction (SPE) pre-concentration/separation procedure and ICP OES with the wet digestion (WD) sample preparation procedure

	Sample	FAAS with SPE	ICP OES with WD
Cd	4_m_HD	< 0.005[a]	< 0.004[a]
	5_m_B	< 0.005[a]	< 0.004[a]
	7_m_B	< 0.005[a]	< 0.004[a]
	8_m_HD	< 0.005[a]	< 0.004[a]
Co	4_m_HD	< 0.015[a]	0.013 ± 0.004
	5_m_B	< 0.015[a]	< 0.009[a]
	7_m_B	< 0.015[a]	< 0.009[a]
	8_m_HD	0.029 ± 0.002	0.028 ± 0.001
Ni	4_m_HD	0.389 ± 0.002	0.411 ± 0.034
	5_m_B	< 0.015[a]	0.014 ± 0.006
	7_m_B	< 0.015[a]	0.015 ± 0.005
	8_m_HD	0.273 ± 0.003	0.268 ± 0.009
Pb	4_m_HD	< 0.073[a]	0.059 ± 0.020
	5_m_B	< 0.073[a]	< 0.050[a]
	7_m_B	< 0.073[a]	< 0.050[a]
	8_m_HD	< 0.073[a]	< 0.050[a]

[a] Below the detection limit. Average values for n = 3 with standard deviations. m: samples taken from the market; HD: honeydew honey; B: buckwheat honey.

Finally, the developed sample preparation procedure was applied for the quality control analysis of 69 honeys collected from the Lower Silesia region in reference to concentrations of Cd, Co, Ni and Pb. Despite of relatively low DLs assessed for Cd, Co and Pb using the developed analytical method, it was established that concentrations of these elements in analyzed samples of honey were below 0.005 µg g^{-1} (Cd), 0.015 µg g^{-1} (Co) and 0.073 µg g^{-1} (Pb). This pointed out that honeys originated from the Lower Silesia region are of the high quality and the food safety. Indirectly, it could suggest that the region is free from a serious environmental pollution. Nickel was determined in 9 samples (see Fig. 1) including 1 buckwheat honey (20_a_B: 0.192 ± 0.001 µg g^{-1}), 4 lime honeys (22_a_L: 0.531 ± 0.049 µg g^{-1}, 23_a_L: 0.191 ± 0.002 µg g^{-1}, 27_a_L: 0.239 ± 0.001 µg g^{-1}, 30_a_L: 0.931 ± 0.023 µg g^{-1}), 2 honeydew honeys (4_m_HD: 0.389 ± 0.002 µg g^{-1}, 8_m_HD: 0.273 ± 0.003 µg g^{-1}) and 2 multiflower honeys (54_a_M: 0.245 ± 0.056 µg g^{-1}, 62_a_M: 0.258 ± 0.026 µg g^{-1}). These results correspond well to those reported for other Polish honeys (0.01-1.87 µg g^{-1}).[22,25] However, in some of these honeys the content of Ni was about 2-5 times higher than the acceptable limit of 0.20 µg g^{-1} set for other food products sold in Poland and given in the Regulation of the Minister of Health[29] from 2003.

Conclusions

The developed SPE-based sample preparation procedure of honeys prior to the reliable determination of traces of Cd, Co, Ni and Pb by FAAS is much simpler and faster than overwhelmingly reported in the literature procedures aimed at the complete decomposition of analyzed samples. The total time required to prepare samples of honeys using the procedure is only about 30 minutes and includes the preparation of resin beds and 10% (m/v) sample solutions of honeys, the pre-concentration of elements on SPE columns and their elution before free from physical and chemical interferences measurements by FAAS. However, the described procedure is alternative to long-termed and cumbersome wet or dry ashing sample digestions, which also commonly require the use of large amounts of reagents, increasing the risk of the contamination of samples and the loss of elements of interest. The procedure provides the precision and the accuracy up to 4.5% and reasonably good DLs, comparable to those obtained for ICP OES with the wet digestion. All these features give the real perspective for the application of the developed analytical procedure for the routine analysis of a large number of honeys samples aimed at controlling their quality in terms of the content of such trace elements as Cd, Co, Ni and Pb.

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