METHOD VALIDATION

Determination of trace and essential elements in honey by quadrupole-inductively coupled plasma-mass spectrometry

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Abstract

Honey is a nutritionally valuable food product of animal origin with a very complex chemical composition. The composition of honey depends on species of melliferous plants and the pollution of the area they were grown. It can accumulate toxic metals, which may present hazards to human health. The aim of the study was to develop a simultaneous method for determination of (aluminium (Al), arsenic (As), barium (Ba), beryllium (Be), cadmium (Cd), cobalt (Co), copper (Cu), chromium (Cr), lead (Pb), manganese (Mn), nickel (Ni), selenium (Se), silver (Ag), thallium (Tl), vanadium (V) and zinc (Zn) in honey by inductively coupled plasma mass spectrometry (ICP-MS) after microwave digestion of samples with nitric and hydrochloric acids and hydrogen peroxide solution. The results of quality controls confirmed that the described method is reliable for routine analysis of chemical elements in honey.

Keywords

- Determination
- Essential elements
- Honey
- Method development
- QICP-MS
- Trace elements

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Introduction

Honey is primarily a carbohydrate product, but it contains some organic and inorganic substances as well as trace elements. High concentrations of toxic elements in honey may result from environmental pollution and could pose a risk to the health of bees and to consumers of honey. Exposure to toxic elements could be minimized by regular control of food. Commission Regulation (EC) No 1881/2006 of 19 December 2006 established limits for lead, cadmium and mercury in foodstuffs [EU, 2006]. Based on this, the method of determining trace and essential elements in honey was developed.

This paper describes a simple method for determination of sixteen elements (aluminium (Al), arsenic (As), barium (Ba), beryllium (Be), cadmium (Cd), cobalt (Co), copper (Cu), chromium (Cr), lead (Pb), manganese (Mn), nickel (Ni), selenium (Se), silver (Ag), thallium (Tl), vanadium (V) and zinc (Zn)) in honey using inductively coupled plasma-mass spectrometry (ICP-MS). The analyses were performed after sample mineralization according to a microwave digestion procedure.

Equipment, reagents and certified materials

An inductively coupled plasma-mass spectrometer (ICP-MS 7700x, Agilent Technologies) equipped with a concentric nebulizer, cyclonic spray chamber, a quartz torch, and an octopole reaction system was used for all measurements. The ICP-MS was optimized daily to reach manufacturer-recommended sensitivity and stability. The standard sample introduction system was used with a glass concentric nebulizer, quartz spray chamber, and quartz torch (2.5 mm internal diameter injector). An Agilent ASX-520 autosampler system was used to deliver the samples. Standard Ni-cones were used. The operating conditions are shown in Table 1.

A Speedwave 4 (Berghof, Eningen, Germany) microwave digestion system equipped with DAK-100 high-pressure vessels made of TFM™-PTFE (pressure range: 0-100 bar) was used for sample digestion.

TABLE 1 / ICP-MS operating conditions.

Agilent Technologies 7700x			
ORS mode	No gas	He*	HEHe**
Spray chamber temperature (ºC)		2	
Nebulizer	Concentric		
RF Power (W)	1450 - 1550 W (optimized daily)		
Plasma (Ar) gas flow	15.0 L/min		
Carrier (Ar) gas flow	1.0 - 1.05 L/min (optimized daily)		
He flow rate	-	-5 mL/min	10 mL/min
Sampling depth	10 mm		
Ext 1 lens	0 V		
Ext 2 lens	-185 V		
Replicates	3		
Isotopes	^{9}Be, ^{27}Al, ^{51}V, ^{52}Cr, ^{55}Mn, ^{59}Co, ^{60}Ni, ^{63}Cu, ^{66}Zn, ^{75}As, ^{80}Se, ^{107,109}Ag, ^{111}Cd, ^{135,137}Ba, ^{205}Tl, ^{208}Pb		
Internal standard	^{45}Sc, ^{89}Y, ^{103}Rh, ^{115}In, ^{159}Tb, ^{165}Ho		
Correction equation	^{208}Pb=^{206}Pb+^{207}Pb		

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All solutions were prepared using Milli-Q® water (18 MΩ·cm-1) and analytical grade reagents: nitric acid HNO3 (Suprapur®, Merck, Darmstadt, Germany), and hydrogen peroxide (POCH, Poland). To prevent contamination, all glassware and plasticware were acid-washed before use. All vessels were soaked in HNO3 (10% v/v) for at least 12 hours, rinsed with distilled water, soaked for 24 hours in Milli-Q water and dried.

An ICP multi-element standard stock solution at 100 µg/mL, VAR-CAL-2 (Inorganic Ventures, USA) of Al, Ag, As, Ba, Be, Cd, Co, Cr3+, Cu, Mn, Ni, Pb, Se, Tl, Th, U, V and Zn was used to prepare the external calibration curve for each element (from 0.5 to 250 µg/L).

A multi-element stock solution (6020 ISS, 10 µg/mL) containing Bi, Li, Ho, In, Rh, Sc, Tb and Y (Inorganic Ventures, USA) was chosen as the internal standard. The tuning solution for ICP-MS 7500 cs (Agilent Technologies, USA), containing 1 µg/L of Ce, Co and Y was used to carry out daily optimisation of the mass analyser. Certified reference materials (CRMs) (SRM 1643e – trace elements in water (NIST, USA), SRM 2976 – mussel tissue, and ERM-BD150 – skimmed milk powder (IRMM, Belgium)) were used to check the accuracy of the method. High-purity argon (99.9995%) and high-purity helium (99.9995%) were supplied by Messer.

Procedure

For sample preparation, we used a procedure described by other authors [Batista *et al.*, 2012; Chudzinska and Baralkiewicz, 2011], modified as follows. Honey samples (0.3-1.0 g) were weighed into a digestion vessel. Reagent blanks were prepared by addition of Milli-Q™ water in place of the sample. For each digestion batch (8 vessels), one reagent blank was prepared to monitor the background concentration of studied elements. A volume of 5 mL of concentrated HNO_3 and 2 mL of non-stabilized 30% H_2O_2 solution was added to each digestion vessel.

Samples were left for approximately 1 h (pre-digestion) then sealed, placed in the microwave, and digested using the program described in Table 2. Afterwards they were cooled, transferred quantitatively into acid-washed polypropylene tubes, diluted to the final volume (30 mL) with Milli-Q™ water, and stored at 6±4°C in the dark until analysis. Reagent blanks were prepared under the same conditions.

TABLE 2 / Microwave digestion program.

Step	Temperature (ºC)	Pressure	Rise Time (min)	Time (min)	Power (%)
1	175	50	20	10	60
2	230	50	15	15	90
3	100	40	1	10	60
4	50	0	1	5	0

Analyses were carried out by ICP-MS in five-fold diluted samples, according to the conditions summarized in Table 1. The internal standard was delivered on line by the sample introduction system. Calibration curves were assigned using standards prepared by diluting multi-element solution. All analysed samples and standards were diluted with 5% HNO_3/1% HCl. No matrix matching of the standards to the samples was necessary.

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Validation

The presented method was validated according to the requirements of ISO/IEC 17025 [2005] and following guidance from the Eurachem Guide [Eurachem, 2014].

The following validation parameters were calculated: linearity, limits of detection (LOD) and quantification (LOQ), precision, and accuracy. The LOD and LOQ were calculated as a 3 or 10 times SD from results of blank matrix measurements (n=10), respectively, with a low content of the analyte. Precision was assessed using the coefficient of variation (CV with 3 independent series of 6 replicates), and accuracy was evaluated by analyte recovery. Uncertainty was estimated based on results of the validation study according to the Eurachem Guide.

Results

An effective decomposition of organic sample matrix and optimization procedures made it possible to avoid interference problems and matrix-induced ionization effects during the analysis of chemical elements in samples of food of animal origin. The LOQ was established by the determination of spiked blank samples (n=20) at the following levels: Be - 1.1, Al – 2.7, V – 1.0, Cr – 0.9, Mn – 1.1, Co – 0.8, Ni – 1.1, Cu – 1.5, Zn – 2.5, As – 0.9, Se – 1.1, Ag – 0.9, Cd – 0.8, Ba – 0.9, Tl – 1.0, and Pb – 1.3 (µg/kg).

The linearity of the calibration curves were considered satisfactory in the range 0-250 µg/L and the obtained values of determination coefficient were $r2 \geq 0.999$ (Figure 1).

FIGURE 1 / Typical calibration curves obtained with the method.
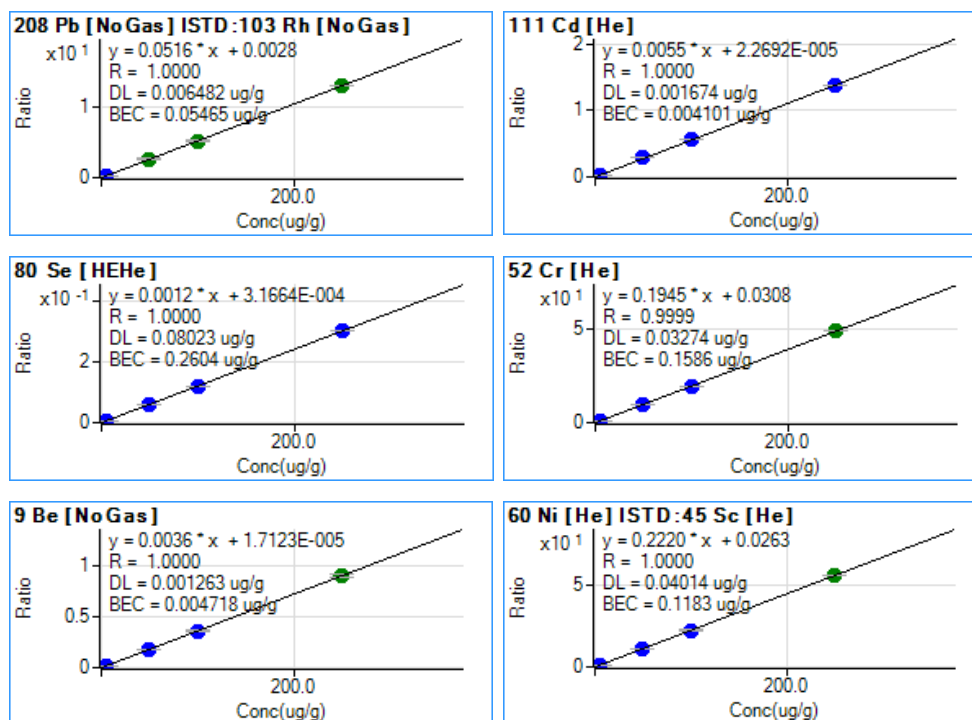
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TABLE 3 / Process of validation determination of Cd, Pb in honey.

		Cd		Pb	
Linearity	y=ax+b	y=1,004x-0.9017		y=1.004x-0.9017	
	r	1.000		1.000	
Method LOD (µg/kg)		0.58		0.75	
Method LOQ (µg/kg)		0.79		1.29	
Concentration (µg/kg)		0.50*	19.9**	0.50*	102.0**
Repeatability	X	0.57	22.8	0.53	119.43
	s_r	0.07	1.41	0.02	8.28
	CV (%)	12.9	6.2	3.4	6.9
Within-laboratory repea-tability	X	0.58	23.4	0.53	123.2
	s_r	0.04	1.56	0.02	9.12
	CV (%)	5.8	6.7	2.9	7.4
Recovery (%)		116.9	117.4	105.2	120.8
Uncertainty	$u_c(y)$	0.07	0.03	0.02	0.79
	k	2	2	2	2
	U	0.50 ± 0.04	19.9 ± 0.05	0.50 ± 0.04	102.0 ± 1.6

*Sample fortified at 0.50 µg/kg

**19th PT on Honey, EURL-CEFAO ISS Rome

TABLE 4 / Recoveries in selected CRMs.

CRM	Value	Mn	Se	Cd	Pb	Cu	Zn	V	Co
SRM 1643e (µg/L)	Estimated	40.2	12.0	6.5	19.24	24.7	74.4	38.7	26.8
	Reference	38.97 ± 0.59	11.97 ± 0.14	6.568 ± 0.073	19.63 ± 0.21	22.76 ± 0.31	78.5 ± 2.2	37.86 ± 0.59	27.06 ± 0.31
	Recovery (%)	103.2	100.3	99.0	98.0	108.5	94.8	102.2	99.0
ERM-BD150 (mg/kg)	Estimated	0.283	0.160	0.011	0.022	1.15	37.11	-	-
	Reference	0.289 ± 0.018	0.188 ± 0.014	0.011 ± 0.0029	0.019 ± 0.004	1.08 ± 0.06	44.8 ± 2.0		
	Recovery (%)	98.0	85.3	95.0	115.7	106.2	82.8	-	-
SRM 2979 (mg/kg)	Estimated	-	1.894	0.931	1.224	4.15	142.8	-	-
	Reference	-	1.800 ± 0.15	0.82 ± 0.16	1.190 ± 0.18	4.02 ± 0.33	137 ± 13		
	Recovery (%)	-	105.2	113.5	102.9	103.1	104.2	-	-

CRM	Value	As	Ag	Cr	Ba	Tl	Be	Al	Ni
SRM 1643e (µg/L)	Estimated	59.6	1.0	20.1	572.1	7.1	13.8	156.4	61.5
	Reference	60.45 ± 0.72	1.062 ± 0.075	20.40 ± 0.24	544.2 ± 5.8	7.445 ± 0.096	13.98 ± 0.7	141.8 ± 8.6	62.4 ± 0.69
	Recovery (%)	98.6	94.2	98.5	105.1	95.4	98.7	110.3	98.6
SRM 2979 (mg/kg)	Estimated	14.46	0.012	0.434	-	-	-	144.	0.82
	Reference	13.30 ± 1.8	0.011 ± 0.005	0.500 ± 0.16				134 ± 34	0.93 ± 0.12
	Recovery (%)	108.7	91.7	86.8	-	-	-	108.2	113.4

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The accuracy and precision of the presented method (Table 3) were confirmed by the analysis of available CRMs and participation in proficiency tests.

Recoveries of investigated elements in CRMs are summarized in Table 4.

As a Polish National Reference Laboratory (NRL) for Heavy Metals in Food of Animal Origin and Feed, it is our duty to participate regularly in proficiency testing schemes organized by European Union Reference Laboratories (EURL-CEFAO Rome, Italy and EURL-IRMM Geel, Belgium). The results of selected proficiency tests are presented in Table 5. It is worth mentioning that our laboratory periodically organizes proficiency tests for regional veterinary laboratories dealing with the analysis of toxic elements in food of animal origin and feed.

TABLE 5 / Results of proficiency tests.

Proficiency Test	Element	Reference value (mg/kg)	Estimated value (mg/kg)	z-score σpEURL	z-score σpHorwitz
19th PT on Honey EURL-CEFAO ISS Roma	Pb	0.102	0.127	2.1	1.1
	Cd	0.0199	0.0251	2.0	1.2
IMEP-118: Determination of total As, Pb, Hg, Sn and iAs in canned food EURL-IRMM Geel	As	0.1299	0.130	0.35	0.64
	Pb	0.1043	0.104	0.63	1.27
	Cd	0.1307	0.131	0.02	0.05
22nd PT on Powdered Infant Formula EURL-CEFAO ISS Roma	Pb	0.034	0.0343	0.1	0.0
	Cd	0.0120	0.00867	-1.9	-1.3

Conclusion

This paper describes a method for the determination of trace and essential elements in food of animal origin including Cd and Pb concentrations in honey using ICP-MS. The validation parameters obtained in the validation process indicate that the proposed method presents satisfactory sensitivity, selectivity, precision, and accuracy. The results of quality controls proved that the procedure described is reliable for routine analysis of honey and other animal origin samples.

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