

Supplementary Material

An Analytical Method for the Biomonitoring of Mercury in Bees and Beehive Products by Cold Vapor Atomic Fluorescence Spectrometry

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Table S1 - Summary of the analytical characteristics of the proposed method and comparison with some previous methods published during the last decade (2010-2020).

Reference	Sample matrix	Sample preparation	Total sample digestion time	Technique	LOD	LOQ	Accuracy (R%)	Precision (CV%)	Dynamic range (Log)
This study	Bees, honey, beeswax, honeydew, pollen, propolis, and royal jelly	0.02 g bee, pollen, propolis and royal jelly, 0.05 g beeswax and honey, or 0.1 g honeydew + 0.5 mL HCl, 0.2 mL HNO ₃ and 0.1 mL H ₂ O ₂ were digested in a water bath (95 °C, 30 min) → volume completed to 5 mL deionized water.	About 30 min for 120 samples or more.	CV-AFS	0.5 µg kg ⁻¹ (for a sample mass of 0.1 g)–3 µg kg ⁻¹ (for a sample mass of 0.02 g)	1 µg kg ⁻¹ (for a sample mass of 0.1 g)–5 µg kg ⁻¹ (for a sample mass of 0.02 g)	86 (honey, 0.05 g)–117% (royal jelly, 0.02 g)	<10%	1.9
[1]	Bees, honey, and pollen	20–200 mg bee, 20–100 mg pollen or 50–200 mg honey + 0.2 mL HNO ₃ followed by 0.1 mL H ₂ O ₂ in a digestion block (80 °C, 2 h total); samples were made up to a final volume of 6 mL.	2h	ICP-MS	14, and 4 µg kg ⁻¹ for a honey sample mass of 0.05 and 0.2 g, respectively	-	96 (B)–129% (As) for trace elements and 91 (Pr)–112% (La) for rare earth elements	<15%	-
[2]	Bees, honey, pollen, and propolis	For the bee samples drying at 100 °C for 48 h preceded microwave digestion. 1g honey, and pollen, 0.25 g propolis, or 0.1 g bees dried weight (at 100 °C for 48 h) + 5 mL HNO ₃ and 2.5 mL H ₂ O ₂ were digested in the microwave oven (30 min) → volume completed to 25 mL	About 30 min	CV-AAS	50 µg kg ⁻¹ for honey, 60 µg kg ⁻¹ for pollen, 300 µg kg ⁻¹ for propolis and 800 µg kg ⁻¹ dw for bees	-	64 (propolis)–129% (honey)	<13%	0.6
[3]	Honey	0.1–2 g + 0.5 mL HNO ₃ and 0.5 mL H ₂ O ₂ → the mixture allowed to stand for 12 h → heated to 100 °C in a metal block for 3 h → volume brought to 25 mL by adding 0.5 mol L ⁻¹ HCl.	15 h	Cloud point extraction (CPE), CV-ICP-OES or ICP-OES	2 µg kg ⁻¹ (CPE CV-ICP OES)–300 µg kg ⁻¹ (ICP-OES)	-	R% close to 100% when up to 2.0 g of honey were subjected to sonication or up to 1.0 g of honey was decomposed with acid.	-	0.5 (ICP-OES)–1.5 (CPE CV-ICP OES)

[4]	Honey	0.5 g + 3 mL HNO ₃ and 4 mL H ₂ O ₂ were digested in the microwave oven (1400 W, 30 min) → volume completed to 50 mL high-purity water	30 min	CV-AFS	0.15 µg kg ⁻¹	-	99%	1%	-
[5]	Honey	0.5 g sample + 4 mL HNO ₃ and 2 mL H ₂ O ₂ were digested in the microwave oven (1400 W, 30 min) → volume completed to 50 mL double deionised water	30 min	AMA	0.1 µg kg ⁻¹	-	99%	-	3.7
[6]	Honey	For CV-AAS analysis: dilution in an acidic solution without heating. In the case of DMA, sample preparation was not necessary.	-	DMA or CV-AAS	1 or 30 µg kg ⁻¹ using DMA or CV-AAS, respectively	2.5 or 60 µg kg ⁻¹ using DMA or CV-AAS, respectively	CV-AAS: 98.7–102.4%; DMA: 95.6–99.1%	CV-AAS: 3.5–5.7%; DMA: 2.7–3.4%	1.3 or 1.5 for DMA or CV-AAS, respectively
[7]	Honey	1 g sample + 12 mL HNO ₃ and 3 mL H ₂ O ₂ were digested in the microwave oven	-	GF-AAS with a flow injection analysis system	-	-	91–113%	<10%	-
[8]	Bees, honey, and pollen	No sample pretreatment	-	AMA	0.5 µg kg ⁻¹	-	-	-	-
[9]	Bees	bee were dried in a moisture analyzer to constant weight at 105 °C and ground → were digested in the microwave oven (45 min) → volume completed to 50 mL	45 min	ICP-OES	1 mg kg ⁻¹	-	-	-	-
[10]	Honey, and pollen	0.5 g honey, and 0.3 g pollen + 7 mL HNO ₃ and 2 mL H ₂ O ₂ were digested in the microwave oven (from 180 to 240 °C, 40 min) → volume completed to 50 mL with deionized water.	40 min	ICP-MS	-	-	-	-	-
[11]	Beeswax, and honey	0.25 g beeswax + 5 mL HNO ₃ and 1 mL H ₂ O ₂ or 2 g honey + 5 mL HNO ₃ were pre-digested at room temperature for 30 min, then digested using microwave oven (20 min)	50 min	High-Resolution ICP-OES	0.1 or 0.3 µg kg ⁻¹ for honey or beeswax, respectively	0.4 or 9 µg kg ⁻¹ for honey or beeswax, respectively	-	-	-

[12]	Pollen	0.65-0.70 g + 10 mL HNO ₃ and 3 mL H ₂ O ₂ were digested in the microwave oven → volume completed to 25 mL with a 5% HCl (v/v).	120 min	Hydride Generator coupled with ICP-OES	0.4 µg L ⁻¹	2 µg L ⁻¹	79–123%	6.4%	-
[13]	Propolis	~0.1 g; no sample pre-treatment	-	AMA-254	-	2 µg kg ⁻¹	98%	<5.1%	2
[14]	Honey, and propolis	2 g of lyophilized samples + 5 mL HNO ₃ were heated at 50 °C for 2 h and then at 110 °C for 18 h; in the next step 5 mL H ₂ O ₂ was added to the digested sample and was heated for 6 h → volume completed to 25 mL.	26 h	GF-AAS	-	-	-	-	-
[15]	Bees	0.7 g of lyophilized sample + 7 ml HNO ₃ and 1.5 mL H ₂ O ₂ were digested	-	DMA	-	10 µg kg ⁻¹	-	-	-
[16]	Bees	~0.5 g of dried sample + 7 ml HNO ₃ and 2 mL H ₂ O ₂ were digested in the microwave oven → volume completed to 25 mL with deionized water.	30 min	ICP-MS	10 µg kg ⁻¹	-	94–108%	-	-

Table S2. Recovery and precision data for Hg in bees and beehive products (n = 3) by water bath digestion (95 °C, 30 min or 60 min).

Matrix	mass (g)	Intermediate level spike (0.2 µg L ⁻¹)				High level spike (1 µg L ⁻¹)			
		30 min		60 min		30 min		60 min	
		R%	CV%	R%	CV%	R%	CV%	R%	CV%
Honey	0.05	116	9.3	119	8.1	96	7.8	91	0.6
	0.1	104	14	115	4.8	96	3.0	92	4.0
	0.2	79	9.4	116	19	82	3.7	54	28
	1	48	52	71	9.7	82	8.9	18	2.8
Honeydew	0.05	121	6.5	113	4.1	101	3.5	94	4.9
	0.1	113	10	113	2.5	91	8.4	90	9.0
	0.2	107	21	114	2.8	82	1.5	92	2.6
	1	134	9.2	91	11	81	2.6	16	1.6
Pollen	0.02	90	3.7	110	5.9	95	3.6	84	1.4
	0.05	99	6.2	110	6.4	93	5.5	92	3.9
	0.1	116	5.7	113	4.6	91	5.7	91	5.5
	0.2	92	2.3	108	8.0	91	0.9	100	7.3
Propolis	0.02	98	8.6	109	9.3	91	2.5	80	3.9
	0.05	131	4.0	153	21	80	4.3	80	1.0
	0.1	161	15	145	19	55	26	74	37
	0.2	228	9.2	144	56	131	45	99	41
Beeswax	0.02	97	11	116	1.1	99	5.7	95	3.6
	0.05	111	8.5	112	0.7	99	2.0	93	6.1
	0.1	101	0.8	117	1.3	102	9.2	82	4.2
	0.2	110	13	108	0.5	76	39	89	5.0
Royal Jelly	0.02	108	4.4	111	5.8	110	0.9	95	2.0
	0.05	110	2.4	96	2.7	110	0.8	86	3.0
	0.1	100	2.8	87	6.0	101	3.2	89	7.7
	0.2	102	6.7	88	0.8	105	2.2	88	4.4
Bees	0.02	97	4.5	92	9.9	91	10	86	1.1
	0.05	99	4.1	105	8.9	97	9.8	80	0.1
	0.1	82	8.7	100	9.0	93	5.1	85	3.5
	0.2	108	7.8	99	0.7	98	7.0	89	3.6

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