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Automatic Flow-Batch System for Cold Vapor Atomic Absorption Spectroscopy Determination of Mercury in Honey from Argentina Using Online Sample Treatment

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ABSTRACT: An automatic flow-batch system that includes two borosilicate glass chambers to perform sample digestion and cold vapor atomic absorption spectroscopy determination of mercury in honey samples was designed. The sample digestion was performed by using a low-cost halogen lamp to obtain the optimum temperature. Optimization of the digestion procedure was done using a Box−Behnken experimental design. A linear response was observed from 2.30 to 11.20 μg Hg L^{-1}. The relative standard deviation was 3.20% (n = 11, 6.81 μg Hg L^{-1}), the sample throughput was 4 sample h^{-1}, and the detection limit was 0.68 μg Hg L^{-1}. The obtained results with the flow-batch method are in good agreement with those obtained with the reference method. The flow-batch system is simple, allows the use of both chambers simultaneously, is seen as a promising methodology for achieving green chemistry goals, and is a good proposal to improving the quality control of honey.

KEYWORDS: *flow-batch, automatization, honey, mercury*

INTRODUCTION

Honey is a natural food produced by honey bees (*Apis mellifera*), with important nutritional properties and therapeutic applications. This bee product is mainly composed of sugars, water, and other minor constituents that include organic acids, amino acids, aliphatic acid salts, minerals, vitamins, lipids, proteins, pollen grains, and flavouring components.[1,2]

Argentina is one of the major producers of bee honey, with only 5% destined to domestic consumption; it is also the leading global exporter of high-quality honey, characterized by a delicate flavor and aroma. Between 50 and 60% of the production in Argentina comes from the Province of Buenos Aires. According to Código Alimetario Argentino, honey is a sweet viscous substance elaborated by honey bees from nectar that they collect, transform, and combine with their own specific substances. Then, they store it in honeycombs, where it matures.[3]

Bees and their products may serve as biomarkers of environmental pollution in their area of flight, and such contamination may be related to the geographical and botanical origin.[4] Honey may contain potentially toxic metals that may come from industrial and urban areas, motor traffic, incorrect manipulation during processing, agrochemicals, and pesticides. Among toxic metals, mercury deserves special attention for its ability to accumulate in highly toxic forms in the food chain, in aquatic ecosystems, and in the body and transform into organic mercury (methylmercury).[5] The determination of mercury levels in food is invaluable to assess mercury exposure risks from food consumption. The technique often used for its determination in these samples is cold vapor atomic absorption spectrometry (CV-AAS).[6,7]

The established AOAC official method for mercury determination in food is the flameless atomic absorption spectrophotometric method.[8] This determination involves several steps. In the first stage, acid digestion of the sample is performed. For this purpose, sulfuric and nitric acids and sodium molybdate solution were added to the sample and heated for 1 h. After the sample was cooled, a solution of nitric acid and perchloric acid was added, and heating was continued for 20 min. Finally, it was made up to 100 mL with water. In a second step, the mercury determination was carried out. An aliquot of the digested was treated with nitric acid, sulfuric acid, and reducing solution (hydroxylamine sulfate and chloride stannous in acidic medium), and the signal was recorded al 253.7 nm.

Taking into account its chemical composition, honey is considered a complex matrix. For this reason, to determine mercury, an adequate sample digestion is necessary to avoid sample interferences and loss of mercury due to its volatilization or an incomplete digestion. Commonly, the sample digestion involves heating with dilute acids, concentrated acids, different acid mixtures, and other oxidizing or complexing agents. Among inorganic acids, we can mention hydrochloridric, nitric, perchloric, and sulfuric acids.[9] The use of mixtures of acids (nitric acid, hydrochloric acid, and sulfuric acid) increases the efficiency of the dissolution process. Hydrogen peroxide, potassium permanganate, potassium chlorate, sodium chlorate, and anions of organic acids such as citrates and tartrates can be used as oxidizing or complexing agents.[10] Nowadays, digestion processes have gradually evolved to focus the efforts on obtaining higher acceleration,

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simplification, miniaturization, and automation of the operations involved.

There are many experimental factors that influence sample preparation; therefore, they must be optimized. The application of chemometric tools to the optimization of analytical methods presents the advantage of reducing the number of experiments. It also allows the development of mathematical models to evaluate the factor effects as well as to evaluate the interaction effects between them under study and the effects of interaction between them. The Box–Behnken design is one of the most efficient experimental design methods[11,12] and has been applied to the optimization of chemical factors in food analysis,[13] food technologies processes,[14] microbiological studies,[15,16] and pharmaceutical analysis,[17,18] among others. Within our knowledge, in the literature, there is no information about variables optimization in the sample treatment employing this experimental design in honey samples.

Flow-batch methodology (FB) is an important alternative to manual analytical methods that may include sample digestion and analyte determination steps. This methodology combines the intrinsic favorable features of the flow, batch, and multicommutation techniques and can be coupled to conventional analytical instruments.[19] Therefore, they can be considered as a multipurpose analytical accessory. These systems are characterized by the use of a dilution/mixing/reaction chamber, containing a magnetic stirring bar and three-way solenoids valves fully computer-controlled. During the past decade, these systems have been used for many determinations including fluorescent determination with online extraction,[19] titrations,[20,21] preparation of calibration solutions,[22] screening analysis,[23] nephelometric,[24] turbidimetric[25] and chemiluminescence determinations,[17] chemometric-assisted method,[26] extraction procedures,[27] and online matching of pH.[28] The developments of new methods in the context of green analytical chemistry are promising. Flow-batch systems allow high sampling frequencies, low cost per analysis, less consumption of reagent and sample, and less chemical waste than classical methods, principles considered in green analytical chemistry.

Therefore, in this paper, an automatic flow-batch system for mercury determination in honey samples was proposed. For this purpose, the system contains two laboratory-made glass-connected chambers: a sample treatment chamber (STC) and a mixing chamber (MC). The STC was designed to improve the efficiency of the heating of the sample and the kinetics of the reaction. To generate the appropriate digestion temperature, a low-cost halogen lamp was placed inside the chamber. The advantages of STC over the conventional hot-plate digestion methods include a significant decrease in digestion time and energy consumption. The other chamber is used to generate the mercury vapor for subsequent spectrophotometric determination employing the technique of CV-AAS. The inclusion of a STC together with the MC highlights the advantages (low contamination, consumption, manipulation of reagent and sample, and easy to handle) and versatility (multitask characteristic) of the FB systems. In the proposed method, the stage of digestion takes place in 16 min unlike the AOAC method, which employs approximately 2 h. To optimize the digestion stage, a Box–Behnken experimental design was used. The proposed method was validated by comparing the obtained results with those generated by the reference method (AOAC) when both were applied to real samples.

MATERIALS AND METHODS

Reagents and Solutions. All reagents were of analytical grade. To prepare the solutions, ultra pure water (18 MΩ) was used. Mercury standard solution (100 mg L^{-1}) was prepared by dissolving 7.0 mg of mercury(I) nitrate (Merck) in 5 mL of 65% nitric acid (w/v) (Merck) and made up to 50.0 mL with water. This solution was stabilized by adding a few drops of potassium permanganate solution [5.0% (w/v)]. Mercury working solution (0.1 mg L^{-1}) was prepared by appropriate dilution of the standard solution. Stannous chloride 15% (w/v) was prepared daily by dissolving 17.8 g of stannous chloride dehydrate (Mallinckrodt) in 50 mL of hydrochloric acid (Merck) and made up to 100 mL with water. Thirty percent (v/v) hydrogen peroxide solution (Cicarelli) was used.

Different commercial samples purchased in Province of Buenos Aires, Argentina, were analyzed. Samples were kept in a cool dry place until analysis.

Optimization of Digestion Procedure. In the present work, a three-level three-factor Box–Behnken experimental design was applied to investigate and validate parameters affecting the digestion process of analyzed samples. The studied factors were as follows: time of digestion (X_1), nitric acid (X_2), and hydrogen peroxide (X_3) volumes. The interval of the allowed values for these factors was deduced from the preliminary tests. The levels corresponding to each factor are shown in the Table 1. Fifteen experiments that included three central point replicates were carried out. The evaluated response (R) was the recovery percentage of mercury corresponding to spiked honey samples. The optimization criterion was the best percentage recovery (i.e., nearest to 100%).

Table 1. Levels Corresponding to the Studied Variables

variable	low (−)	middle (0)	high (+)
X_1: digestion time (min)	10.0	15.0	20.0
X_2: nitric acid (mL)	6.0	8.0	10.0
X_3: hydrogen peroxide (mL)	4.0	6.0	8.0

Flow-Batch System. Mercury determination was performed using a Mercury analyzer SMT Seefelder Messtechnik model, Hg Monitor 3000 (CV-AAS). Solutions were propelled by an eight-channel Gilson Minipuls-3 M312 peristaltic pump.

The flow-batch system was composed of two laboratory-made glass chambers. In the first chamber (STC), the digestion procedure was carried out, and the second one was used as the MC. Hanna Instruments magnetic stirrers (model HI 190M) were placed underneath the STC and MC. Nine three-way solenoids valves (model 137 161T031, Nresearch) allowing the admission and removal of fluids used in the chambers were used as follows: V_{NA}, nitric acid; V_{HP}, hydrogen peroxide; V_{W1}, water; V_{W2}, water; V_R, reducing agent; V_1, digested sample/mercury working solution/air; and V_2, mercury working solution/air. Valves V_{Ws1} and V_{Ws2} were used to evacuate the liquids of the chambers. Tygon pumping tubes of different internal diameter were used.

The STC was composed of a borosilicate glass flask with a 35 mL internal volume and a device that holds a 24 V halogen lamp to obtain the digestion temperature (Figure 1a). The device was built with borosilicate glass to protect the lamp from the produced gas during digestion and allowed the output of them. In this way, the digestion step was achieved in less time than in conventional heating and with minor energy consumption.

MCs of polytetrafluoroethylene (PTFE) and borosilicate glass were tested. Whereas PTFE MC results in incomplete vapor generation of mercury, borosilicate glass was selected to design this chamber. The MC offers the following features: 3.0 cm internal diameter, 15.0 cm height, and internal conical cavities that allow the complete diffusion of mercury from the liquid phase into the gas phase and consists of two parts, which fit together one inside the other (Figure 1b). The top part consists of two cylindrical glass tubes held together at one end, covered by a plastic cap with three holes, which allow the entry of

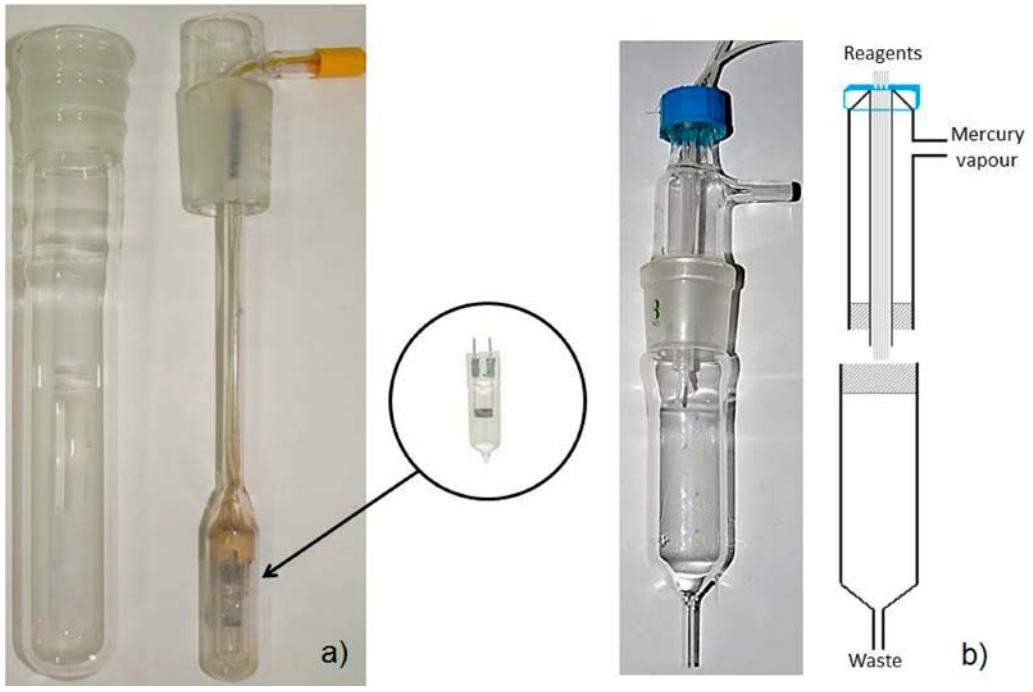

Figure 1. (a) STC and (b) MC.

reagents. The external cylinder has a lateral outlet to allow the mercury vapor to reach the detector. The bottom of the chamber performs the generation of mercury vapor. The waste is evacuated through a hole at the base. To eliminate human exposure to mercury fumes, an extractor hood and a fume removal system containing activated carbon as a chemical sorbent were used.

A computer supplied with a laboratory-made parallel interface was used to an automatic handling of the valves through a computer program developed in a Labview 5.1 graphic language (National Instruments, Austin, TX).

Procedure. A schematic diagram of the proposed FB system is shown in Figure 2. Before starting the analysis, the channels had to be filled with the respective solutions.

The honey sample was placed in the STC, and the nitric acid valve (V_{NA}) and hydrogen peroxide valve (V_{HP}) were switched on during t_{NA} and t_{HP}, respectively. Then, the glass device was introduced in the flask, and the lamp was switched on by applying a voltage through a power supply, to achieve the optimum values of digestion temperature.

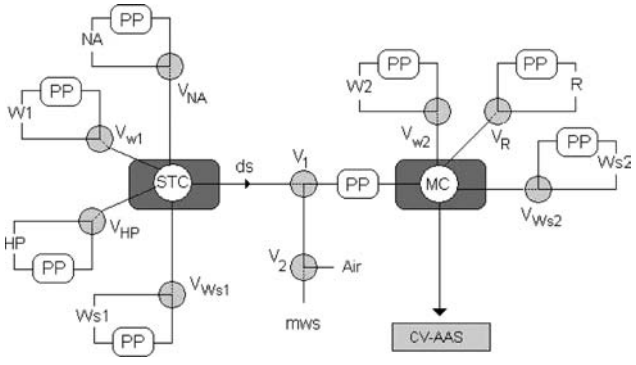

Figure 2. Flow-batch system to determine mercury concentration in honey. PP, peristaltic pump; V, solenoid valves: V_{NA}, nitric acid; V_{HP}, hydrogen peroxide; V_{W1}, water; V_{W2}, water; V_R, reducing agent; V_1, ds/mws/air; V_2, mws/air; NA, nitric acid; HP, hydrogen peroxide; ds, digested sample; R, reducing agent; mws, mercury working solution; W1 and W2, water; and Ws1 and Ws2, waste.

This step was carried out during 16 min with magnetic stirring. During this time, the brown-yellow fumes of NO_2 could be observed. Then, valve V_{w1} was switched on during t_{w1} so the final volume of the digestion mixture was 20 mL.

After the digestion step, valve V_1 was switched on during t_{V1} s, and a certain volume of the digested sample (ds) enters the MC. Afterward, valve V_{W2} was switched on for t_{W2} promoting the aspiration of water toward the MC. Finally, valve V_R was switched on (t_R), and the reducing agent was added so as to generate mercury vapor. The absorbance was read at 253.7 nm. The magnetic stirrer was always activated during the insertion of the fluids aliquots into the MC to ensure a good homogenization of the solutions and improve the analytical sensitivity.

The standard solutions were prepared by using the same procedure in the MC. However, time interval of valves V_2 and V_{w2} increased and decreased, respectively, while V1 was switched off. The standard solution and water valves are activated sequentially during t_2 and t_{w2}. Time intervals of valves V_R remain fixed.

Two cleaning steps may be carried out with water between each recorder for the two chambers. For this purpose, valves V_{W1} and V_{W2} were switched on during 10 s. To empty both chambers, V_{Ws1} and V_{Ws2} were turned on.

RESULTS AND DISCUSSION

Optimization of the Flow-Batch Variables. The flow-batch system was optimized, and the optimum values were selected as a compromise between sensitivity and reproducibility of the analytical signals. Table 2 shows the optimum values for the different flow rates and the switching time intervals of the solenoid valves.

Optimization of Digestion Procedure. At first, the Box–Behnken design allowed usto calculate the effects that produce changes on the variables and their possible interactions. Figure 3 shows the Pareto graphic where the estimated effects and their interactions are detailed. As can be seen, the X_1 factor and the interaction $X_1{*}X_2$ are not significant at the 95% confidence level.

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Table 2. Optimum Values for Flow Batch Parameters[a]

	q_{NA}	q_{W1}	q_{HP}	q_{W2}	q_R	q_{ds}	q_{mws}	q_{Ws1}	q_{Ws2}
flow rate (mL min^{-1})	18.0	18.0	18.0	4.38	18.0	4.38	4.38	18.0	18.0
valve switching time intervals (s)	V_{NA}	V_{W1}	V_{HP}	V_{W2}	V_R	V_1	$V_1 - V_2$	V_{Ws1}	V_{Ws2}
mercury stock solution				6.0−2.0	7.0		1.0−5.0		10
samples	30	33	23	5.0−4.0	7.0	7.0	2.0−3.0	10	10

[a]NA, nitric acid; HP, hydrogen peroxide; R, reducing agent; ds, digested sample; mws, mercury working solution; W1 and W2, water; and Ws1 and Ws2, waste.

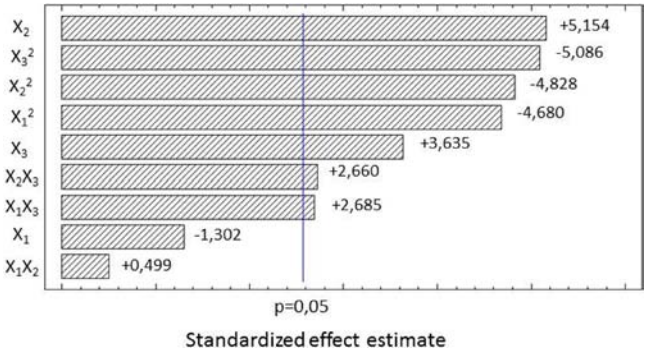

Figure 3. Pareto chart showing the standardized effect of independent variables and their interaction.

The responses to each experiment corresponding to the applied experimental design were fitted to the following second order polynomial model:

$$R = \underset{(\pm 1.35)}{-6.35X_1^2} - \underset{(\pm 1.35)}{6.55X_2^2} - \underset{(\pm 1.35)}{6.9X_3^2} + \underset{(\pm 0.9)}{4.75X_2} + \underset{(\pm 0.9)}{3.35X_3} + \underset{(\pm 1.3)}{3.5X_1{*}X_3} + \underset{(\pm 1.3)}{3.55X_2{*}X_3} + \underset{(\pm 1.5)}{92.7}$$

where R is the dependent variable and X_1, X_2, and X_3 are the independent variables as mentioned previously. In this model, nonsignificant effects have been removed. An analysis of variance (ANOVA) was carried out to evaluate the quality of fit of the polynomial model. For the proposed quadratic model, there was no evidence of significant lack of fit at the 95% level: the MS_{lof}/MS_{pe} ratio was 1.12, less than the $F_{3,17}$ critical value of 2.70. The MS_R/MS_r ratio was 18.3, larger than the 95% $F_{6,23}$ critical value of 2.53, indicating a significant regression. The corresponding surface responses (Figure 4) show that the optimum values for X_1, X_2, and X_3 are inside of the experimental region. Therefore, a digestion time of 16 min and volumes of 8.90 and 6.80 mL of nitric acid (65%) and hydrogen peroxide (30%), respectively, were considered as optimum for the digestion procedure of analyzed samples.

Optimization of the Reducing Agent. Stannous chloride in acidic medium is the most commonly used reducing agent to promote mercury vapor.[29,30] To select its appropriate concentration, an univariate method was used. The influence of the concentration of stannous chloride was studied in the range from 5 to 20% (w/v) with a mercury standard solution of 5 μg Hg L^{-1}. The results showed that concentrations of 5 and 10% (w/v) displaced incompletely the mercury present in the sample. Furthermore, no differences were observed in the signal when concentrations of 15 and 20% (w/v) were used. The optimum value was 15% (w/v).

Analytical Parameters. The calibration curve was linear for mercury, in the concentration range from 2.30 to 11.20 μg L^{-1}, corresponding to 36.4−177.1 μg kg^{-1} when an appropriate amount of sample has been digested. The calibration curve was

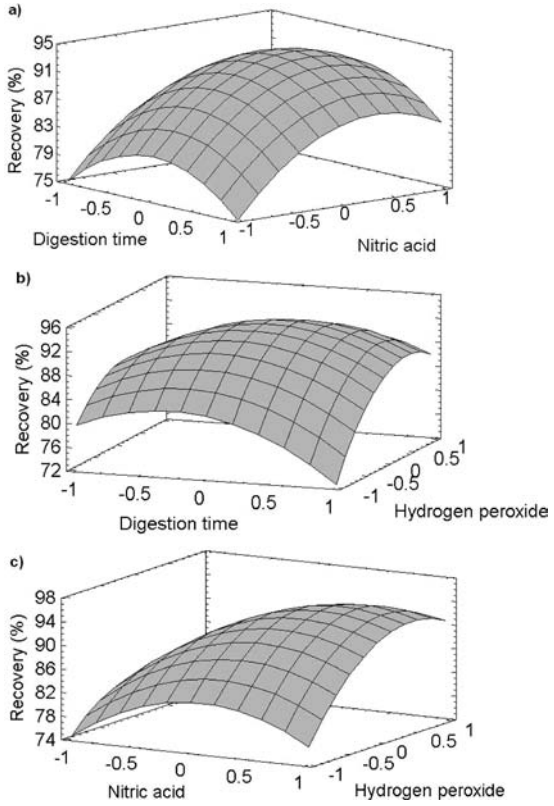

Figure 4. Response surface diagrams showing the effect of the mutual interactions between two independent variables (other variables were held at their respective center level). (a) Hydrogen peroxide = 0. (b) Nitric acid = 0. (c) Digestion time = 0.

$y = (9.68 \times 10^{-4} \pm 1.82 \times 10^{-5})x - (2.81 \times 10^{-4} \pm 1.36 \times 10^{-4})$, where y is the absorbance and x is the concentration of mercury μg L^{-1}. The precision was expressed as percentage of the relative standard deviation of replicate measurements, and it was calculated by using standard solutions. The obtained value was 3.20% (n = 11, 6.81 μg L^{-1}). The detection limit, estimated as three times $S_{y/x}$/slope,[31] was 0.68 μg L^{-1}. The sample throughput was 4 h^{-1}.

Application to Real Samples. The developed method was applied to the determination of mercury in honey samples, using the optimum experimental conditions. The mercury content was negligible in the analyzed honey samples. Therefore, aliquots of the mercury working solution were added to the honey samples. To validate the flow-batch proposed procedure, the AOAC method was used. By this way, an acidic digestion was performed to the spiked samples, and then, the mercury content was determined by employing a cold vapor atomic absorption spectrophotometer Coleman MAS 50D (253.7 nm).

The results shown in Table 3 are expressed as μg Hg kg^{-1} of honey sample and revealed a good agreement between both

Table 3. Analysis of Real Samples

		Hg(II) found (μg kg^{-1}) ± s		recovery (%)	
sample	Hg(II) added (μg kg^{-1})	proposed method[a]	reference method[a]	proposed method[a]	reference method[a]
1	107.8	112.6 ± 4.6	107.5 ± 1.5	104.4	99.8
2	107.8	113.5 ± 4.8	106.4 ± 4.2	105.3	98.7
	71.5	69.3 ± 1.8	71.1 ± 0.2	96.9	99.4
3	107.8	111.6 ± 3.3	102.5 ± 0.7	103.5	95.1
	71.5	68.5 ± 1.7	70.1 ± 0.3	95.8	98.0
4	107.8	105.9 ± 1.6	109.6 ± 5.9	98.2	101.6
	71.5	69.3 ± 0.4	72.1 ± 0.3	96.9	100.8
5	107.8	105.6 ± 4.8	103.0 ± 4.9	97.9	95.5
	71.5	74.6 ± 5.5	72.6 ± 0.2	104.4	101.5

[a]The samples were analyzed in triplicate.

methods. Additionally, the application of a paired Student's t test [31] confirmed that there is no statistical differences (t estimated = 0.93, t tabulated = 2.31, n = 8, and α = 0.05) between the results obtained by both procedures.

Therefore, it can be concluded that the proposed automatic flow-batch system is shown to be suitable for the determination of mercury in honey from Province of Buenos Aires, Argentina, and includes online sample digestion. This system is composed of two chambers, which allow sample treatment simultaneously with the preparation of working solutions. The sample treatment was carried out in a borosilicate glass chamber, which contains a device that holds a low-cost halogen lamp to obtain the optimum temperature. In this way, it is possible to reduce the digestion time of approximately 2 h (AOAC method) to 16 min. This digestion chamber for the sample treatment is an innovation and highlights the versatility of flow-batch systems. The generated mercury vapor in the MC was determined at 253.7 nm employing cold vapor atomic absorption spectroscopy.

The results obtained using a Box–Behnken design revealed that the response surface method was suitable to optimize the experimental variables that affect the digestion of honey samples by the proposed method. One of the major features of this system is that takes into account some principles of the green chemistry, such as the decrease in reagent consumption, amount of sample, waste volume (environmentally friendly), and lower energy consumption.

The honey samples were analyzed, and the obtained results were validated using the AOAC method, showing good agreement between them. This automated system facilitates the analytical determination of mercury in honey samples and can be implemented in routine laboratories

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Notes

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