

On-line preconcentration and determination of mercury in biological and environmental samples by cold vapor-atomic absorption spectrometry

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Abstract

An on-line procedure for the determination of traces of total mercury in environmental and biological samples is described. The present methodology combines cold vapor generation associated to atomic absorption spectrometry (CV-AAS) with preconcentration of the analyte on a minicolumn packed with activated carbon. The retained analyte was quantitatively eluted from the minicolumn with nitric acid. After that, volatile specie of mercury was generated by merging the acidified sample and sodium tetrahydroborate(III) in a continuous flow system. The gaseous analyte was subsequently introduced via a stream of Ar carrier into the atomizer device. Optimizations of both, preconcentration and mercury volatile specie generation variables were carried out using two level full factorial design (2^3) with 3 replicates of the central point. Considering a sample consumption of 25 mL, an enrichment factor of 13-fold was obtained. The detection limit (3σ) was 10 ng L^{-1} and the precision (relative standard deviation) was 3.1% ($n = 10$) at the $5 \text{ } \mu\text{g L}^{-1}$ level. The calibration curve using the preconcentration system for mercury was linear with a correlation coefficient of 0.9995 at levels near the detection limit up to at least $1000 \text{ } \mu\text{g L}^{-1}$. Satisfactory results were obtained for the analysis of mercury in tap water and hair samples.

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1. Introduction

The toxic effects of mercury are well known [1]. Mercury may enter a human body by inhalation of mercury vapor (mainly in the form of Hg^0); drinking water (mainly as inorganic mercury, Hg^{2+}), and/or by the consumption of fish and fish products (mainly as methylmercury, CH_3Hg^+) in the diet [2,3]. The content of mercury in hair may represent the cumulative exposure from the occupational environment and/or daily diet. Although the levels of total mercury in hair for a normal person are in the range of $0.4\text{--}6.0 \text{ } \mu\text{g g}^{-1}$, a concentration greater than $50 \text{ } \mu\text{g g}^{-1}$ is considered to be toxic [1]. In adults, the ratio of mercury in hair to that in blood has been modeled with hair containing approximately 250 times more Hg [4]. Hair analysis is the preferred biomarker of exposure because, in addition to correlating with levels in the brain [5], hair samples are easily obtained making

it a practical biomonitoring tool for large populations [6], and it is less invasive compared to blood collection [4].

Being drinking water one of the routes of incorporation of mercury into the human body, its determination in this type of samples becomes very important [7]. The total mercury concentration estimated in waters ranging from 0.006 to $3 \text{ } \mu\text{g L}^{-1}$, the upper limit for total mercury concentration in drinking water recommended by the European being $1 \text{ } \mu\text{g L}^{-1}$ [7,8].

Because of the extremely low levels of mercury in various matrices, high sensitivity and sophistication in the analytical procedures are required for its determination, and the most commonly used ones are cold vapor atomic absorption spectrometry (CV-AAS) [3,9–11]; cold vapor atomic fluorescence spectrometry (CV-AFS) [12–16], inductively coupled plasma optical emission spectrometry (ICP-OES) [7,17], and inductively coupled plasma mass spectrometry (ICP-MS) [18–20].

Mercury cold vapor generation associated to atomic absorption spectrometer (Hg-CV-AAS) detection is one of the most powerful analytical methods for the determination of mercury. However, in order to circumvent the limitation of low mercury

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concentration in environmental and biological samples, different methodologies involving preconcentration procedures are often needed before Hg-CV-AAS [21]. In this sense, activated carbon (AC) has been widely used for many purposes both in laboratory and industrial settings, due to its ability to adsorb organic and inorganic mercury compounds [22–24]. Since its introduction in analytical chemistry, enrichment of trace metals using AC has been favorably performed with very high concentration factors in different matrices [21]. The mechanism of sorption on AC is still under investigation and the adsorption of metals on this material can be explained using Langmuir and Freundlich equations. The major advantage of these models is their simplicity. However, the models fail to describe accurately the adsorption equilibrium under varying conditions, such as pH and ionic strength. Adsorption equilibrium studies have revealed that the pH is the dominant parameter controlling the adsorption [25]. The pH of the medium controls the sorption limit of the metal ions from aqueous solutions to the solid phase, and consequently determining values of metal uptake capacities and distribution coefficients [26].

Generally, sorption of dissolved metal ions on AC can be improved in the presence of chelating or precipitating agents. There are only few papers reported in the literature in which activated carbon has been used to preconcentrate mercury complexes. In general, the most successful solid phase extractors for Hg(II) are those immobilized basically, sulfur and nitrogen or phosphorous containing compounds [26]. Amongst these compounds are dithizone and dithiocarbamate derivatives loaded to activated carbon for selective extraction and preconcentration of Hg(II) ions. In this sense, Starvin and Prasada Rao [27] have used 1-(2-thiazolylazo)-2-naphthol (TAN) functionalized activated carbon for the removal and recovery of Hg(II) from brine sludge and cell house effluents. The adsorption capacity of TAN-functionalized AC material was found to be 2.24 mg g^{-1} of the adsorbent. TAN functionalized AC selectively separates Hg(II) when present together with several alkali, alkaline earth, transition and heavy metal ions; and the recovery was found to be >99.8% indicating the quantitative removal of Hg(II). Also, Peraniemi and Ahlgren [28] have reported a procedure in which activated carbon was modified with zirconium (ZrC) for the preconcentration of Hg and its determination by energy dispersive X-ray fluorescence (EDXRF). A detection limit of 0.058 mg L^{-1} was achieved. In this work, the cross-interference of the analyte was investigated. In this sense, it was found that mercury, unlike other ions, did not adsorb quantitatively onto the ZrC, but the adsorption was increased linearly with Hg(II) concentration and this made the mercury determinations possible. Chloride ion increased the adsorption of Hg(II) slightly, possibly due to the formation of Hg_2Cl_2 on carbon.

On the other hand, pyrrolidine dithiocarbamate, dithizone, and 8-hydroxyquinoline [29–31] have also been used as chemical reagents for the complexation and enrichment of Hg(II) on activated carbon. The detection limits achieved with the last two above-mentioned procedures were 10 and 0.12 ng mL^{-1} , respectively.

In this work, an on-line procedure for the preconcentration and determination of mercury at low concentration levels by

cold vapor-atomic absorption spectrometric determination using a conical mini column packed with AC is proposed. Mercury was retained by sorption on activated carbon in the absence of a complexing reagent. The pH adjustment of the solution suffices to retain mercury ion. In this sense and taking into account that the optimum pH value (1.5) was attained by adjusting with a hydrochloric acid solution, the medium was rich in chloride. Hg(II) ions were present as neutral HgCl_2 and chloroanionic HgCl_3^{1-} and HgCl_4^{2-} complexes. At this working pH, the binding of Hg(II) chloroanionic species to protonated positive sites at AC through electrostatic attraction was greatly favored [32].

On the other hand, the optimization of analytical procedures by multivariate techniques [33] is faster, more economical and effective than the traditional “one-at-a-time”. The multivariate optimization makes possible to understand circumstances that are not explained by the traditional approach, for example, the interactions between the factors that influence the analytical response. These advantages have been reported in the literature [34].

In this paper, factorial design was applied for optimization of the preconcentration and mercury cold vapor generation variables for mercury determination in environmental and biological samples. It was based on solid-phase extraction of mercury onto activated carbon in absence of a complexing reagent. The on-line preconcentration method was coupled to CV-AAS, in which a quartz T-tube was used. The vapor released was introduced into the tube by means of carrier gas flow of argon.

2. Experimental

2.1. Instrumentation

The measurements were performed with a Shimadzu Model AA-6800 atomic absorption spectrometer (Tokyo, Japan), equipped with a deuterium background corrector. Mercury hollow cathode lamp (Hamamatsu Photonics K.K., Japan) was employed as the radiation source. A spectral slit width of 0.1 nm was used to isolate the 253.7 nm resonance line. The flow injection system used is shown in Fig. 1. A Minipuls 3 peristaltic pump [Gilson (Villiers-Le-Bell, France)] was used. The sample injection was achieved using a Rheodyne (Cotati, CA, USA) Model 50, injection valve. The hydride unit used was a PS Analytical LTD hydride generator and the flow speeds of the reagents were controlled by a Watson-Marlow 303X peristaltic pump. The 1400- μL sample loop was prepared using PTFE tubing. A home-made conical minicolumn (40 mm length, 4.5 mm internal upper-diameter and 1.5 mm internal lower-diameter) was used as the activated carbon holder. A homemade quartz tube (0.8 cm internal diameter; 15 cm length) was employed for the mercury vapor determination. Pump tubes, Tygon® type (Ismatec, Cole-Parmer Instrument Company, Niles, IL, USA) were employed to propel the sample, reagent, and eluent.

2.2. Reagents

The activated carbon (Merck, Darmstadt, Germany, 50–70 mesh) was used after pretreatment with acid (activated carbon

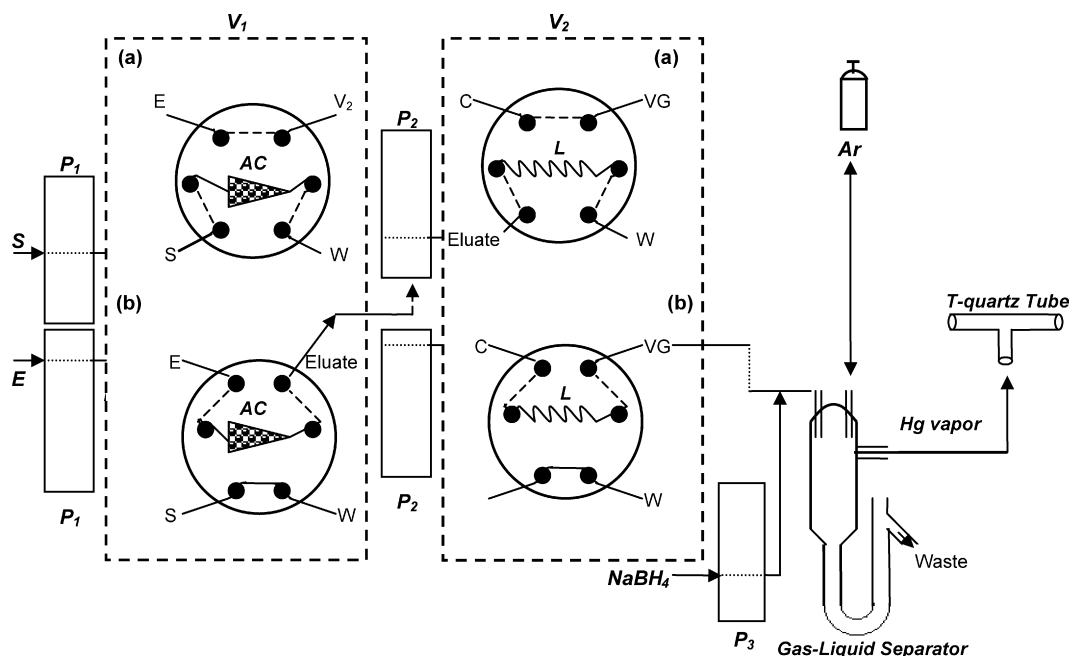


Fig. 1. Schematic diagram of the instrumental setup. S: sample (flow rate: 15.0 mL min^{-1}); E: eluent (flow rate: 5.0 mL min^{-1}); NaBH_4 : solution (flow rate 3.0 mL min^{-1}); L: loop ($1400 \mu\text{L}$); C: carrier (flow rate: 8 mL min^{-1}); VG: vapor generator; W: waste; AC: activated carbon column; Ar: argon (flow rate: 700 mL min^{-1}); P_1 , P_2 , P_3 , peristaltic pumps; V_1 and V_2 , injection valves, valve positions: (a) sample loading; (b) injection.

was heated to 60°C with 10% (v/v) hydrochloric acid for 30 min and then with 10% (v/v) nitric acid for 20 min and finally washed with deionized water until neutral pH was reached).

A stock inorganic mercury standard (1000 mg L^{-1}) was prepared from mercury (II) chloride (Merck, Darmstadt, Germany) and made up to 1000 mL with ultrapure water.

The mercury solution's pH was adjusted with hydrochloric acid solution. The hydrochloric acid was Suprapure (Merck, Darmstadt, Germany). Hydrochloric acid solutions were prepared by direct dilution with ultrapure water from the concentrated suprapure solution.

Sodium tetrahydroborate reagent was freshly prepared daily by dissolving the appropriate amount of NaBH_4 (Merck, Darmstadt, Germany) in 0.5% (m/v) sodium hydroxide solution (Merck, Darmstadt, Germany). After dissolution of the reagents, the solution was filtered through a Whatman No. 42 filter paper to remove undissolved solids.

Nitric acid (Merck, Darmstadt, Germany) was of ultrapure reagent grade. Nitric acid was prepared by direct dilution with ultrapure water from the concentrated solution.

Welding argon from Praxair (Buenos Aires, Argentina) was found to be sufficiently pure for Hg determination.

All the solutions containing the potentially interfering ions were prepared by adding appropriate amounts of stock solutions made from Merck Titrisol or their chloride salts in 0.3 mol L^{-1} hydrochloric acid.

Ultrapure water ($18 \text{ M}\Omega \text{ cm}$) was obtained from an EASY pure RF (Barnstead, Dubuque, IA, USA).

All solvents and reagents were of analytical-reagent grade or better, and the presence of mercury was not detected in the working range.

2.3. Column preparation

The conical minicolumn was prepared by replacing 100 mg of activated carbon into an empty column using the dry packing method. To avoid loss of activated carbon when the sample solution passes through the conical minicolumn, a small amount of quartz wool was placed at both sides of the conical minicolumn. The column was then connected to a peristaltic pump with PTFE tubing to form the preconcentration system.

2.4. Sample pretreatment

To obtain the water sample, tap water was allowed to run for 20 min and approximately 2500 mL of tap water was collected. The water samples were filtered through $0.45\text{-}\mu\text{m}$ pore-sized membrane filters immediately after sampling, and were adjusted to pH 1.5 with hydrochloric acid solution. The sample was collected immediately before use. All the instruments used were previously washed with 10% (v/v) HNO_3 water solution and then with ultrapure water.

On the other hand, hair samples were obtained using the following standardized cutting and washing procedure [35]: the hair was first cut into approximately 0.3-cm pieces and mixed to allow a representative sub-sampling of the hair specimen. After cutting, each sample was washed four times with a 1:200 (v/v) dilution of Triton X-100. The samples were then rinsed with acetone and allowed to drain. This was followed by three rinses with ultrapure water and two rinses with acetone. The samples were then dried in an oven at $40 \pm 5^\circ\text{C}$. To carry out the digestion of the samples, 0.5 g of washed hair samples, accurately weighed, were used and placed in a refluxing digestion system.

Then, 5 mL of concentrated nitric acid was added and the samples were digested. A clear solution was obtained. Then, the clear solutions were heated until incipient dryness in a hot plate. The sample pH was adjusted with hydrochloric acid and the solution was diluted with water to 25 mL. Following that procedure, losses of mercury, which can be important in a digestion system without reflux, were avoided. The sample pretreatment takes approximately 50 min. However, it must be taken into account that it is possible to simultaneously treat as many samples as can be placed in the digestion system.

2.5. Operational procedure

25 mL sample (prepared such as is indicated in Section 2.4) buffered to pH 1.5 was loaded on the minicolumn packed with activated carbon (AC) at a flow rate of 15 mL min⁻¹ with valve V₁ in load position (a). Then, valve V₁ was switched to the injection position (b) and the retained metal was eluted with 50% (v/v) nitric acid (at 5.0 mL min⁻¹ flow rate) into the 1400- μ L loop (L), with the V₂ valve in load position (a); and injected into the 50% (v/v) HNO₃ carrier solution (C) through the V₂ valve (b). The acidified sample solution (at 8 mL min⁻¹ flow rate) was merged with the reductant (2% (w/v) NaBH₄ solution, at 3.0 mL min⁻¹ flow rate) into the vapor generator in a continuous flow system using a peristaltic pump. The volatile mercury compound generated was separated from the solution by means of a U-tube separator and swept by Ar (at 700 mL min⁻¹ flow rate) into the quartz cell. The FI system used is shown in Fig. 1.

3. Results and discussion

3.1. Optimization strategy

A two level full factorial design with 2³ = 8 runs plus three replicates of a central point, was carried out for both, variables controlling preconcentration and vapor generation processes.

3.1.1. Parameters controlling the preconcentration procedure

The optimization step of the proposed procedure was performed using a full factorial design (2³) with (3) replicates of the central point, involving the variables: pH, loading flow rate (LFR), and eluent concentration (EC) and the recovery (R%) signal as analytical response (the experimental data were processed by using the Statistic computer program) [36]. Minimum and maximum levels of each factor were chosen based on previous experiments. These levels are shown in Table 1. Besides, Table 2 shows the experimental design matrix and the analyti-

Table 1
Factors and levels used in factorial design performed for the preconcentration variables

Variable	Low (-)	Central point (0)	High (+)
pH	1.0	1.5	3.0
Load flow rate (LFR) (mL min ⁻¹)	10.0	15.0	20.0
Eluent concentration (EC) (%)	30.0	50.0	70.0

Table 2

Design matrix and results of the factorial design performed for the preconcentration variables

Experiment	pH	LFR	EC	R (%)
1	+	+	+	88.3
2	+	+	-	89.1
3	+	-	+	88.6
4	+	-	-	89.4
5	-	+	+	87.9
6	-	+	-	87.0
7	-	-	+	87.8
8	-	-	-	88.6
9	0	0	0	90.0
10	0	0	0	89.5
11	0	0	0	88.7

Table 3

Analysis of data given in Table 2

Factor	SS ^a	d.f. ^b	MS ^c	F-ratio	P-level ^d
pH	2.101250	1	2.101250	4.886628	0.157633
LFR	0.551250	1	0.551250	1.281977	0.375011
EC	0.281250	1	0.281250	0.654070	0.503572
pH-LFR	0.101250	1	0.101250	0.235465	0.675452
pH-EC	0.361250	1	0.361250	0.840116	0.456122
LFR-EC	0.361250	1	0.361250	0.840116	0.456122
pH-LFR-EC	0.361250	1	0.361250	0.840116	0.456122
Error	0.860000	2	0.430000		
Total	7.441818	10			

^a Sum of squares.

^b Degrees of freedom.

^c Mean squares.

^d Probability level.

cal signal. The results of this study, considering the analysis of variance (ANOVA) at 95% confidence level (Table 3) and the Pareto chart (Fig. 2) demonstrate that in the studied levels, all these variables and their interactions are not statistically significant. In this way, the pH recommended is 1.5 considering that it is the central point of the pH interval tested. It guarantees the robustness of the system for this variable; the chosen loading flow rate was 15 mL min⁻¹ in order to obtain a better throughput sample, and the eluent concentration selected was 50% (v/v) nitric acid.

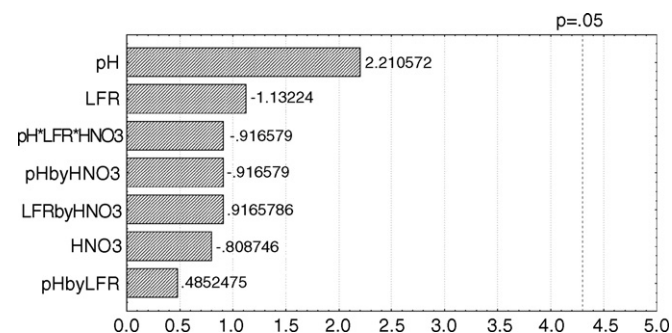


Fig. 2. Pareto chart of standardized effects for variables in the mercury preconcentration.

Table 4
Factors and levels used in factorial design performed for the mercury vapor generation variables

Variable	Low (–)	Central point (0)	High (+)
NaBH ₄ (% m/v)	1.0	2.0	3.0
Argon flow rate (Ar) (mL min ⁻¹)	500.0	700.0	1000.0
Eluent flow rate (EFR) (mL min ⁻¹)	2.0	4.0	5.0

3.1.2. Parameters controlling the cold vapor generation

The flow rate at which the analyte is eluted from the AC strongly influences the analytical performance—preconcentration factor and throughput sample of the whole procedure. In this sense, the optimum value for this parameter was carefully studied. Besides, the effect of NaBH₄ dissolved in 0.5% (w/v) NaOH to generate the mercury vapor was evaluated. On the other hand, argon was used as a carrier gas and it was responsible for the transport of mercury vapor from the gas–liquid separator to the T-quartz tube. The influence of argon flow rate on the absorbance of mercury was investigated.

To optimize the parameters controlling the mercury cold vapor generation, a full factorial design (2³) with (3) replicates of the central point, involving the variables: sodium tetrahydroborate concentration (NaBH₄), argon flow rate (Ar), and eluent flow rate (EFR); and preconcentration factor as analytical response was evaluated. Minimum and maximum levels of each factor based on preliminary experiments were chosen. These levels are shown in Table 4.

Table 5 shows the experimental design matrix and the analytical signal. The results of this study, considering the analysis of variance (ANOVA) at 95% confidence level (Table 6) and the Pareto chart (Fig. 3) demonstrates that in the studied levels NaBH₄, Ar, EFR, and their interactions are not statistically significant. Considering the results showed in the Pareto chart, the NaBH₄ recommended is 2.0% (w/v) considering that it is the central point of the NaBH₄ interval tested. It guarantees the robustness of the system for this variable. The chosen argon flow rate was 700 mL min⁻¹. Finally, a 5 mL min⁻¹ EFR was selected in order to obtain the best throughput sample for the optimized conditions.

Due to the optimum EFR (5 mL min⁻¹) was not compatible with the sample flow rate (8 mL min⁻¹) needed for the

Table 5
Design matrix and results of the factorial design performed for the mercury vapor generation variables

Experiment	NaBH ₄	Ar	EFR	Preconcentration factor
1	+	+	+	13.0
2	+	+	–	13.2
3	+	–	+	12.9
4	+	–	–	13.0
5	–	+	+	12.7
6	–	+	–	12.5
7	–	–	+	12.5
8	–	–	–	12.8
9	0	0	0	12.9
10	0	0	0	13.2
11	0	0	0	13.1

Table 6
Analysis of data given in Table 5

Factor	SS ^a	d.f. ^b	MS ^c	F-ratio	P-level ^d
NaBH ₄	0.020000	1	0.020000	0.85714	0.452277
Ar	0.005000	1	0.005000	0.21429	0.688915
EFR	0.320000	1	0.320000	13.71429	0.065801
NaBH ₄ –Ar	0.020000	1	0.020000	0.85714	0.452277
NaBH ₄ –EFR	0.005000	1	0.005000	0.21429	0.688915
Ar–EFR	0.020000	1	0.020000	0.85714	0.452277
NaBH ₄ –Ar–EFR	0.045000	1	0.045000	1.92857	0.299351
Error	0.046667	2	0.023333		
Total	0.0609091	10			

^a Sum of squares.

^b Degrees of freedom.

^c Mean squares.

^d Probability level.

cold vapor generation system, a 1400 μL loop was used. The implementation of this loop permitted to reach the optimum preconcentration factor for this system without compromise the releasing of mercury from the minicolumn packed with AC.

3.2. Interferences

The effects of representative potential interfering species at the concentration levels at which they may occur in the sample studied were tested. Thus, Cu(II), Zn(II), Pb(II), Co(II), Mn(II) and Fe(III) could be tolerated up to at least 2000 μg L⁻¹. Commonly encountered matrix components such as alkali and alkaline earth elements are not retained on activated carbon.

3.3. Method validation

The proposed method of digestion following cold vapor-atomic absorption spectrometric determination was applied to the quantification of trace amounts of mercury in hair samples. Due to the fact that we do not have a reference material of hair with a certified value for Hg, and the recovery test is considered as a validation method [37]; we used it in order to demonstrate the validity of our method. 10 portions of 0.5 g of the hair sample were collected. All the samples were digested

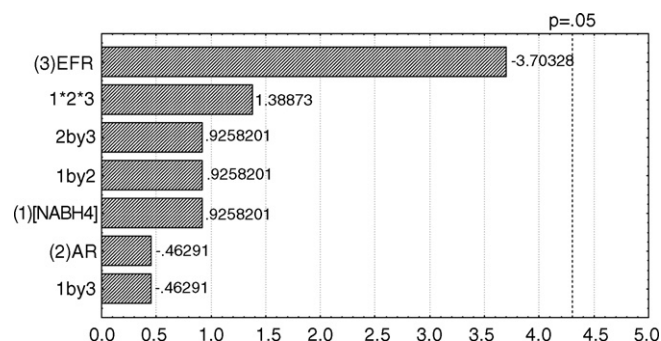


Fig. 3. Pareto chart of standardized effects for variables in the mercury vapor generation.

following the proposed procedure. The proposed method was applied to six portions and the average quantity of mercury obtained was taken as a base value. Then increasing quantities of Hg were added to the other aliquots of sample and the analyte was determined by the same method. The results obtained are reported in Table 7. Additionally, the proposed method was applied to a standard reference material, QC METAL LL3 mercury in water, with a mercury content of $6.48 \pm 0.51 \mu\text{g L}^{-1}$. Using the proposed method, the content of mercury determined in this CRM was $6.46 \pm 0.21 \mu\text{g L}^{-1}$ (95% confidence interval; $n = 6$).

3.4. Analytical performance

The overall time required for preconcentration of 25 mL of solution (1.67 min, at a flow rate of 15 mL min^{-1}); washing (0.20 min, at a flow rate of 2 mL min^{-1}); eluting (approx. 0.3 min, at a flow rate of 5.0 mL min^{-1}); volatile mercury generation (approx. 0.2 min, at a flow rate of 8.0 mL min^{-1}); and conditioning (0.3 min, at flow rate of 2.0 mL min^{-1}) was about 2.7 min. Thus, the throughput was about 22 samples per hour. A total enrichment factor of approximately 13-fold for a sample volume of 25 mL was obtained with respect to the mercury determination by CV-AAS without preconcentration.

Due to the association of the on-line preconcentration procedure with the mercury vapor generation, a detection limit value (calculated as the amount of Hg required to yield a net peak that was equal to three times the standard deviation of the background signal (3σ)) of 10 ng L^{-1} was obtained. The precision for 10 replicate determinations at the $5 \mu\text{g L}^{-1}$ Hg level was 3.1% relative standard deviation, calculated from the peak heights obtained.

3.5. Application to hair and water samples

Finally, the proposed methodology was applied to hair samples. The average mercury level found in the samples under study was 210 ng g^{-1} (Table 7). The mean mercury concentration obtained is in good agreement with those results reported by other authors [1,4]. In addition, the proposed methodology was applied to the determination of mercury in tap water samples. The results obtained are reported in Table 8. The mercury concentration levels found in the sample under study are in agreement with those values reported by Wuilloud and co-workers [3,7].

Table 7
Recovery study for digestion/preconcentration and determination of mercury in hair samples (95% confidence interval; $n = 6$)

Aliquots	Base value (ng g^{-1})	Hg added (ng g^{-1})	Hg found (ng g^{-1})	Recovery (%) ^a
1–6	–	–	210.0 ± 6.6	–
7	210.0	100.0	307.0	97.0
8	210.0	150.0	358.9	99.3
9	210.0	200.0	410.0	100.0
10	210.0	250.0	459.0	99.6

^a $100 \times [(\text{found} - \text{base})/\text{added}]$.

Table 8
Concentration of mercury in tap water samples (95% confidence interval; $n = 6$)

Sample	Hg concentration ($\mu\text{g L}^{-1}$)
A (1st week)	0.61 ± 0.04
B (2nd week)	0.57 ± 0.05
C (3rd week)	0.60 ± 0.04
D (4th week)	0.53 ± 0.05

The tap water samples were collected at different times.

4. Conclusion

The proposed preconcentration methodology was completely automated and this fact increased the speed of the preconcentration and analysis process. The preconcentration procedure coupled to CV-AAS allowed to obtain a better detection limit, with respect to the mercury determination by CV-AAS without preconcentration. The studies performed indicate that the determination shows good reproducibility and accuracy. In addition, all the variables and their effects were evaluated using a full factorial design approach, which permitted a simpler and faster optimization of the preconcentration/mercury vapor generation parameters.

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