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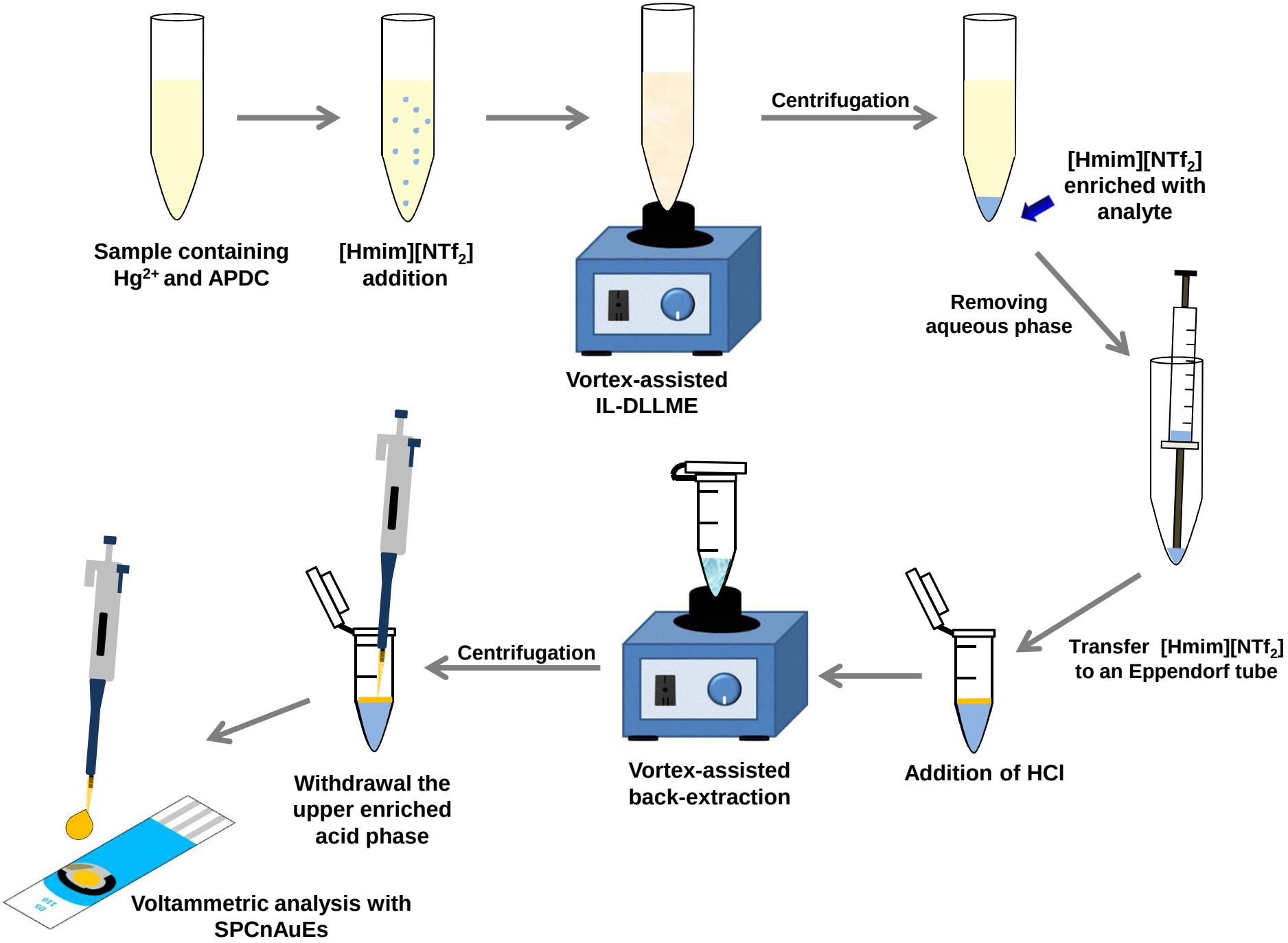
Sample containing $Hg^{2+}$ and APDC
[Hmim][$NTf_2$] addition
Vortex-assisted IL-DLLME
Centrifugation
[Hmim][$NTf_2$] enriched with analyte
Removing aqueous phase
Transfer [Hmim][$NTf_2$] to an Eppendorf tube
Addition of HCl
Vortex-assisted back-extraction
Centrifugation
Withdrawal the upper enriched acid phase
Voltammetric analysis with SPCnAuEs

# Mercury determination in urine samples by gold nanostructured screen-printed carbon electrodes after vortex-assisted ionic liquid dispersive liquid-liquid microextraction

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## Abstract

A novel approach is presented to determine mercury in urine samples, employing vortex-assisted ionic liquid dispersive liquid-liquid microextraction and microvolume back-extraction to prepare samples, and screen-printed electrodes modified with gold nanoparticles for voltammetric analysis. Mercury was extracted directly from non-digested urine samples in a water-immiscible ionic liquid, being back-extracted into an acidic aqueous solution. Subsequently, it was determined using gold nanoparticle-modified screen-printed electrodes. Under optimized microextraction conditions, standard addition calibration was applied to urine samples containing 5, 10 and 15 μg $L^{-1}$ of mercury. Standard addition calibration curves using standards between 0 and 20 μg $L^{-1}$ gave a high level of linearity with correlation coefficients ranging from 0.990 to 0.999 (N=5). The limit of detection was empirical and statistically evaluated, obtaining values that ranged from 0.5 to 1.5 μg $L^{-1}$, and from 1.1 to 1.3 μg $L^{-1}$, respectively, which

are significantly lower than the threshold level established by the World Health Organization for normal mercury content in urine (i.e., 10-20 µg $L^{-1}$). A certified reference material (REC-8848/Level II) was analyzed to assess method accuracy finding 87% and 3 µg $L^{-1}$ as the recovery (trueness) and standard deviation values, respectively. Finally, the method was used to analyze spiked urine samples, obtaining good agreement between spiked and found concentrations (recovery ranged from 97 to 100%).

**Keywords:** vortex-assisted dispersive liquid-liquid microextraction, ionic liquid, mercury, screen-printed electrodes, urine samples.

## 1. Introduction

Mercury is a highly toxic element whose adverse health effects depend on several factors such as chemical form, route of exposure, dose and personal features [1]. Inhalation exposure mainly corresponds to elemental mercury (i.e., $Hg^0$) due to its high vapor pressure. Occupational exposure to $Hg^0$ vapors occurs in mining and fossil-fuel processing activities, manufacture of amalgams, manipulation of mercury-containing fungicides, waste incineration or chloralkaly plants. $Hg^0$ is oxidized to $Hg^{2+}$ in most body tissues and can be retained and accumulated, especially in the brain and kidneys. Oral intake is the main source of inorganic mercury (i.e., $Hg^{2+}$), although its absorption from gastrointestinal tract occurs only to a limited extent [2]. Cutaneous absorption has been proposed as another less significant route of exposure, since dermal penetration of $Hg^{2+}$ can occur through use of skin-lightening cosmetic products containing mercuric salts. Once in the body, $Hg^{2+}$ accumulates mainly in the

kidneys. Methylmecury (i.e., $MeHg^{+}$) is the most toxic and frequent form of organic mercury. $MeHg^{+}$ exposure mainly occurs through a diet high in fish and marine mammals. In contrast to $Hg^{2+}$, $MeHg^{+}$ is rapidly and extensively absorbed through the gastrointestinal tract and accumulates predominantly in the brain [2].

Urine and blood have been broadly employed for risk assessment of mercury exposure and health risk prevention. Mercury content in urine generally reflects recent exposure to inorganic and/or elemental mercury. However, $Hg^{2+}$ accumulates in the kidneys and is slowly excreted through urine, therefore, urinary mercury can also reflect long-term exposure in the past [2]. $MeHg^{+}$ is mostly eliminated by demethylation and excretion in the feces and it is not typically found in urine [1]. Urinary mercury levels are normally expected to be lower than 10-20 μg $L^{-1}$ in an unexposed population.

Different publications report mercury determination in urine using cold-vapor atomic absorption [3] or fluorescence [4] spectrometry, electrothermal absorption spectrometry [5], UV-Vis spectrophotometry [6], inductively coupled plasma atomic emission [7] or mass [8] spectrometry. Besides spectrometric techniques, electrochemical techniques have also been proposed [9–13]. Electrochemistry offers sensitivity, simplicity, rapid response and inexpensive instrumentation with miniaturization and portable options. In this respect, screen-printed electrodes (SPEs) [14] have gained widespread interest. SPEs are size-reduced devices designed to analyze low-volume samples, which also allow de-centralized testing. In addition, SPEs are mass-produced at a low cost and are thus disposable. In this work, screen-printed carbon electrodes modified with gold nanoparticles (SPCnAuEs) have been employed as

electrochemical transducers for mercury determination. Gold nanoparticles exploit the properties of gold as a high affinity material for mercury, with the advantages of including nanosized particles, such as high active surface area, enhanced mass transport and signal to noise ratio [15]. In addition, mercury undergoes a process named underpotential deposition (UPD) on gold electrodes. The presence of gold promotes the adsorption of mercury atoms on the surface once the ionic metal is reduced, forming an amalgam (Au–Hg). This adsorption is usually limited to a monolayer. Due to the strong interaction between gold substrate and reduced mercury, the deposition of mercury is favored energetically and takes place at a less negative potential than the reversible Nernst potential for bulk deposition.

Due to the complexity of biological samples, including urine, sample preparation is necessary prior to electrochemical analysis. To date, the electrochemical methods proposed to determine mercury in urine samples employ initial digestion steps to decompose organic matter, which generally involve wet acid digestion [9–13]. However, these procedures constitute a risk for mercury loss and thus careful manipulation is essential to avoid analyte evaporation. In this work, dispersive liquid-liquid microextraction (DLLME) is presented as a valuable alternative for sample preparation. DLLME is a miniaturized liquid-phase extraction technique whose major advantages include: speed and ease of use, low cost, low sample volume, extremely low solvent consumption, reduced generation of wastes, high enrichment factors and affordability. Classical DLLME is based on the dispersion in tiny droplets of a water-immiscible solvent into the aqueous sample with the aid of a disperser agent [16]. Other formats of DLLME are based on vortex agitation [17],

ultrasound energy [18], temperature changes [19], metathesis reactions [20] or air-assisted methodology [21]. The cloudy solution formed presents a great contact surface area between the donor and acceptor phases, thus enhancing extraction efficiency. In addition to conventional organic solvents, ionic liquids (ILs) have been employed as extractant phase in DLLME (i.e., IL-DLLME) due to their remarkable properties, such as low vapor pressure, good extractability of organic and inorganic compounds, non-flammability and adjustable hydrophobicity [22].

The purpose of this work is to present a novel method for mercury determination in urine samples, combining vortex-assisted IL-DLLME with electrochemical detection by SPCnAuEs. Mercury complexes with ammonium pyrrolidinedithiocarbamate (APDC) are directly extracted from non-digested urine samples into a water-immiscible IL using vortex agitation. Then, mercury is back-extracted into 10 µL of an acidic aqueous solution, which is finally analyzed by anodic stripping voltammetry. The proposed method is based on a previous work [23], in which mercury was determined in water samples, where some changes related with the microextraction techniques are proposed. In the previous work, mercury was extracted from water samples using an in-situ ionic liquid formation dispersive liquid-liquid microextraction [23]. This microextraction technique was not suitable for urine samples since the formation of a precipitate in the extractant phase formed in-situ hindered its retrieval. Hence, vortex-assisted IL-DLLME was adopted in order to overcome the problem. On the other hand, ultrasound-assisted back-extraction [23] has been replaced by vortex agitation in this work to assist back-extraction of mercury to the final aqueous phase, leading to shorter extraction time.

The present method synergistically combines the advantages of an environmentally friendly miniaturized sample-preparation protocol with speed, low cost, high sensitivity and selectivity of the electrochemical detection with SPCnAuEs. To our knowledge, this is the first report of an analytical method in which SPEs are employed to determine mercury in urine samples. The aforementioned method was evaluated in order to demonstrate its applicability to the analysis of real urine samples.

## 2. Experimental part

### 2.1. Reagents and samples

A stock standard solution of $Hg^{2+}$ (1000 mg $L^{-1}$ in 2% $HNO_3$) was obtained from High-purity Standards (Charleston, SC, USA). Working solutions were prepared by proper dilution of this stock standard. IL 1-hexyl-3-methylimidazolium bis[(trifluoromethyl)sulfonyl]imide ([Hmim][$NTf_2$]) (99%) was purchased from Iolitec (Heilbronn, Germany). The chelating agent APDC (~ 99%) was supplied by Sigma-Aldrich (St. Louis, MO, USA). A solution of 2 mg $mL^{-1}$ of the chelating agent was prepared by dissolving APDC in ultrapure water. Fuming HCl (37%) was supplied by Merck (Madrid, Spain) and used to prepare HCl solution (4 M) in ultrapure water. Reactive grade NaOH (≥ 97%, pellets) was from ACS Scharlau (Barcelona, Spain) and used to prepare NaOH solution (0.5 M). Reactive grade NaCl was also from ACS Scharlau. The ultrapure water (resistivity of 18.2 MΩcm at 25℃) employed for preparing all solutions was obtained with a Millipore Direct System Q5$^{TM}$ purification system from Ibérica S.A. (Madrid, Spain).

Standard $Au^{3+}$ tetrachloro complex (1.000 ± 0.002 g of $AuCl_4^-$ in 500 mL of 1.0 M HCl) was purchased from Merck. Solutions of 1 mM $AuCl_4^-$ were prepared by suitable dilution of this standard solution in 0.1 M HCl.

Urine samples from healthy human volunteers, unexposed to mercury, were collected in sterilized containers. Urine samples were filtered before use and stored at 4 ºC. Preliminary analysis using this method revealed that mercury levels were undetectable in all donated urine samples.

The certified reference material "urine control lyophilized for trace elements" REC-8848/Level II was purchased from LGC Standards (Barcelona, Spain).

### 2.2. Apparatus and electrodes

A vortex mixer from Heidolph (Swabach, Germany) was used to assist IL-DLLME and microvolume back-extraction. A centrifuge from Selecta (Barcelona, Spain) was used for phases separation after each extraction.

A Multi Autolab/M101 Potentiostat/Galvanostat from Metrohm Autolab B.V. (Ultrecht, The Netherlands) controlled by NOVA software version 1.10 was used for electrochemical experiments.

Screen-printed carbon electrodes (SPCEs) (ref. DRP-110) with three-electrode configuration were purchased from DropSens (Oviedo, Spain). The working electrode, with a disk-shaped of 4 mm of diameter, and the counter electrode were made of a carbon ink whereas the pseudo-reference electrode was made of silver. Specific connectors obtained from DropSens (ref. DRP-DSC) were used to connect SPCEs to the potentiostat.

### 2.3. Vortex-assisted IL-DLLME and microvolume back-extraction

For vortex-assisted IL-DLLME, 10 mL of aqueous standards or urine samples were placed in a test tube and mixed with 40 µL of APDC solution (2 mg $mL^{-1}$) for analyte complexation. Then, 0.06 g of the water-immiscible IL used as extractant phase (i.e., [Hmim][$NTf_2$]) were added and the mixture was vortexed for 3 min. Next, phases were separated by centrifugation for 10 min at 4000 rpm. The upper aqueous phase was carefully removed with a glass pipette and the IL phase deposited in the bottom of the test tube was transferred to a 0.5 mL Eppendorf tube for microvolume back-extraction. Since direct measurements on the IL were not suitable, back-extraction was necessary for voltammetric analysis [23]. Once the IL was transferred to the Eppendor tube, 10 µL of 4 M HCl aqueous solution were added and the mixture was shaken using a vortex mixer for 9 min. After centrifugation for 2 min at 4000 rpm, the upper acidic aqueous phase enriched with the analyte was retrieved for final analysis by square-wave anodic stripping voltammetry (SWASV) using SPCnAuEs.

### 2.4. Electrochemical analysis

Gold nanoparticles were generated over SPCEs according to previous publications [23, 24]. Briefly, 40 µL of a 1 mM $AuCl_4^-$ solution in 0.1 M HCl were dropped over the electrode surface and subjected to a constant current of -100 µA for 180 s. After gold deposition, the electrode surface was generously rinsed with ultrapure water and dried at room temperature before use. The characterization of SPCnAuEs and the study of the electrochemical behavior of

mercury were carried out in a previous publication [24] and no further discussion will be included in the present work.

For the voltammetric analysis of mercury, 5 µL of the upper acidic aqueous phase, obtained after microvolume back-extraction, were mixed with 37 µL of 0.5 M NaOH to obtain a suitable electrolytic medium [23]. A volume of 40 µL of this solution was dropped onto SPCnAuEs for SWASV experiments. Under optimized conditions [23, 24], mercury was preconcentrated by applying a constant potential of +0.3 V for 240 s. Thereafter, the potential was recorded between +0.3 V and +0.55 V at a frequency of 80 Hz, amplitude of 30 mV and step potential of 4 mV. An anodic peak corresponding to the reoxidation of mercury appears at approximately +0.42 V and the height of this peak was employed to quantify the analyte. All experiments were carried out at room temperature and SPCnAuEs were discarded after a single use.

## 3. Results and discussion

### 3.1. Optimization of sample preparation

Variables affecting the proposed methodology were the amount of chelating agent, sample pH, volume of 4 M HCl acceptor solution during back-extraction, ionic strength, sample volume, IL amount, vortex-assisted IL-DLLME extraction time and back-extraction time. Among them, the amount of chelating agent, sample pH and volume of 4 M HCl acceptor solution were thoroughly evaluated in a previous publication [23], and the results on their behavior are applicable to the present work. The optimum values obtained for the amount of chelating agent and volume of 4 M HCl solution (i.e., 40 µL of 2 mg $mL^{-1}$ solution and 10 µL, respectively) were considered acceptable. Before each

analysis, urine sample pH was measured and ranged between 5.5 and 6.7. Thus, pH adjustment was considered unnecessary in accordance with the previous publication [23].

The other five variables affecting the proposed method (i.e., ionic strength, sample volume, IL amount, vortex-assisted IL-DLLME extraction time and back-extraction time) were investigated and optimized with a one-at-a-time strategy. The experiments were carried out in triplicate, employing 10 µg $L^{-1}$ of $Hg^{2+}$ aqueous standard solutions. The height of the anodic peak corresponding to the reoxidation of mercury was used as analyte signal to evaluate the overall extraction efficiency under different conditions.

### 3.1.1. Effect of ionic strength

Considering the high salt content in urine samples compared to water samples [23,25], the effect of ionic strength was evaluated through the addition of different amounts of NaCl to $Hg^{2+}$ aqueous standard solutions (i.e., 0, 10 and 35% w/v of NaCl). The results (not included) showed no differences between NaCl free and salty solutions, and therefore, ionic strength adjustment of urine samples was unnecessary.

### 3.1.2. Effect of sample volume

The effect of sample volume was examined by testing 4, 6, 8 and 10 mL of the $Hg^{2+}$ solution. The results are shown in Figure 1. As expected, higher sample volume led to a higher response since greater sample volume implies a greater amount of analyte. Volumes above 10 mL were not considered due to the limited availability of biological samples such as urine. A volume of 10 mL was finally employed in subsequent experiments.

### 3.1.3. Effect of IL amount

In order to study the effect of IL amount on the performance of the proposed sample preparation protocol, different quantities of [Hmim][$NTf_2$] were tested (i.e., 0.02, 0.04, 0.06, 0.08 and 0.10 g). As shown in Figure 2, and considering the error bars, non-significant effects were observed when the amount of IL was increased between 0.02 and 0.10 g. However, considering the importance of waste reduction and the ease of IL droplet manipulation, a compromise value of 0.06 g of IL was finally chosen for following experiments.

### 3.1.4. Effect of vortex-assisted IL-DLLME extraction time

Extraction time is an important variable in microextraction techniques. This is because extraction involves transferring the analyte from the sample solution to the extractant phase, which is time-dependent. Extraction time in vortex-assisted IL-DLLME is generally considered as the vortex agitation time, therefore, this variable was evaluated to obtain the best extraction performance. Times between 0.5 and 5 min were examined. As shown in Figure 3, analyte signal increased as extraction time increased during the first 3 min, whereas longer extraction times did not significantly affect extraction efficiency. Consequently, 3 min of vortex agitation was selected as optimum value for further experiments.

### 3.1.5. Effect of back-extraction time

The influence of microvolume back-extraction time was also evaluated considering vortex agitation times between 2 and 10 min. According to Figure 4, extraction efficiency increased with time until 9 min. Then, the signal remained constant. Therefore, the optimum back-extraction time was considered to be 9 min.

### 3.2. Stability of urine samples

Biological samples are known to degrade over time after collection. Therefore, it is important to establish proper preservation treatments or storage conditions to ensure the validity of analysis.

A fresh urine sample obtained from a healthy human volunteer was spiked with 10 µg $L^{-1}$ of $Hg^{2+}$ and divided into two portions. One portion was stored in the refrigerator at 4 ºC whereas the other was kept at room temperature (i.e., 21 ºC). In order to determine their stability, $Hg^{2+}$ was extracted from each urine portion on successive days using the proposed method. As apparent in Figure 5, urine samples need to be stored at low temperature if the analysis is not carried out on the day of collection. In addition, samples stored at 4ºC need to be analyzed within the first two days as $Hg^{2+}$ determination is greatly affected by longer storage times.

### 3.3. Analytical figures of merit

Different matrix effects were found when analyzing different urine samples (Fig. S1), therefore, standard addition calibration was employed to evaluate quality analytical parameters under optimized conditions. To this end, three calibration curves were constructed using standards of five concentration levels from 0 to 20 µg $L^{-1}$. Good linearity was obtained with correlation coefficient values (r) ranging from 0.990 to 0.999. The Student's t-test was applied to assess the linearity [26] showing values ranging from 12.2 (r=0.990; N=5) to 35.8 (r=0.999; N=5), thus rejecting the null hypothesis of non-linear correlation for a 5% significance level and 3 degrees of freedom ($t_{0.05,3}$=3.18).

The sensitivity of the instrumental measurements estimated by the slope of standard addition calibration curves ranged from (0.89 ± 0.08) to (1.26 ± 0.07) μA $\mu g^{-1}$ L. The enrichment factor (EF) of the proposed procedure was studied in three different urine samples. Since complexity matrix of urine hinders $Hg^{2+}$ direct determination by SPCnAuEs, the EF was evaluated by the following strategy. On one hand, the proposed procedure was applied to three non-doped urine samples and the final aqueous extracts were spiked at 15 μg $L^{-1}$ of $Hg^{2+}$ just before the electrochemical measurements. On the other hand, the proposed procedure was applied to the urine samples spiked at 15 μg $L^{-1}$ level. Then, the signals obtained in both procedures were compared obtaining EFs between 20 and 31.

The limit of detection (LOD) was determined empirically, measuring progressively more diluted concentrations of the analyte. Thereby, three different urine samples were spiked with progressively lower $Hg^{2+}$ amounts and analyzed using the proposed method. The LOD was the lowest concentration whose signal could be clearly distinguished from blank. Figure S2 (Supplementary material) shows the voltammograms employed to establish the LOD in different urine samples. The LOD values ranged between 0.5 and 1.5 μg $L^{-1}$, which is significantly lower than the threshold level established by the World Health Organization (WHO) for the normal mercury content in urine (i.e., 10-20 μg $L^{-1}$). Additionally, LOD and limit of quantification (LOQ) were evaluated using the blank signals and their standard deviations. Values between 1.1 and 1.3 μg $L^{-1}$ were obtained for the LOD, which confirm the results obtained by the empirical approach. LOQ ranged between 1.9 and 2.4 μg $L^{-1}$.

The accuracy of the method was evaluated by analyzing a certified urine sample (REC-8848/Level II) containing 17.3 ± 4.3 µg $L^{-1}$ of $Hg^{2+}$. The higher concentration of metals in this sample compared with common concentrations found in urine samples, resulted in a non-detectable amount of analyte in the final acidic aqueous extract. Consequently, the amount of APDC was increased to 0.02 g to provide the excess of chelating agent necessary to efficiently complex and extract metals, including $Hg^{2+}$. In addition, the presence of higher amount of APDC complexes, with $Hg^{2+}$ and other co-existing metals, implies the necessity of using stronger acid conditions for a complete metal release during back-extraction. Therefore larger volume and higher concentration of the HCl acceptor aqueous solution (i.e., 20 µL and 6 M, respectively) were required. Employing the above-described modifications, the certified urine sample was analyzed using the standard addition method, obtaining a recovery (i.e., trueness) of 87%, and a precision expressed as the standard deviation estimated using regression line [26] of 3 µg $L^{-1}$. These results confirm that the method is able to determine Hg at trace levels in urine samples. It should be emphasized that, despite the slight modification to the method when analyzing the reference material, the concentration of metals in this sample was significantly higher than the usual concentration found in human urine samples, for which the applicability of the originally proposed method has been demonstrated (See Section 3.4.).

### 3.4. Analysis of urine samples

The proposed method was applied to determine $Hg^{2+}$ in real urine samples taken from healthy volunteers. Samples were initially analyzed and

their $Hg^{2+}$ content was found to be below the LOD of the present method. Consequently, three different urine samples were spiked adding different known amounts of $Hg^{2+}$, which ranged from 5 to 15 µg $L^{-1}$, and analyzed using standard addition calibration. To prepare the standard addition calibration, five aliquots of 9800 µL of each spiked urine sample were placed in test tubes and subsequently spiked with 0, 50, 100, 150 and 200 µL of a 1 mg $L^{-1}$ $Hg^{2+}$ aqueous solution, to which 200, 150, 100, 50 and 0 µL of ultrapure water were added, respectively, in order to reach an equal final volume (10 mL). After that, the microextraction procedure was applied and final aqueous extracts were electrochemically analyzed. Figure 6 shows the voltammograms obtained from urine sample spiked at 5 µg $L^{-1}$. Analyte concentration was calculated by extrapolation, giving the results in Table 1. According to these results, there were non-significant differences between the concentrations added and those found in the three urine samples, with relative recoveries ranging between 97 and 100%. Therefore, non-significant matrix effects were found with the proposed methodology.

### 3.5. Comparison with other methods

Characteristics of previously reported electrochemical methods for $Hg^{2+}$ determination in urine samples are summarized in Table 2 for comparative purposes. The LOD, obtained using both methodologies (i.e., empirical and statistically), is equal or even better than those obtained in previous publications [9–13] and it is low enough to satisfy the threshold level established by the WHO for normal mercury concentrations in urine. In addition, the present approach affords special advantages. Firstly, the proposed sample preparation

method avoids acid digestion, thus representing a safer and more environmentally-friendly procedure, which avoids the use of concentrated acids. By avoiding acid digestion, the proposed sample preparation protocol does not require time-consuming cooling steps and reduces the risk of mercury losses. Secondly, to our knowledge, this is the first time that SPEs have been employed to determine mercury in urine samples. SPEs are inexpensive devices which can be disposed after a single use. Thus, memory effects and tedious cleaning steps of the electrode surface between measurements are avoided. In addition, SPEs are easy to handle and their modification with gold nanoparticles is simple and, moreover, stripping experiments are rapid.

## 4. Conclusions

SPCnAuEs have been successfully combined with vortex-assisted IL-DLLME and microvolume back-extraction methodologies to determine mercury in non-digested urine samples. This combination synergistically exploits the advantages of environmentally friendly miniaturized sample preparation with the rapid, inexpensive, sensitive and selective determination of mercury with SPCnAuEs. The applicability of the proposed method has been successfully demonstrated in urine samples, reaching a LOD low enough to satisfy the threshold limit established by the WHO for normal mercury content in human urine. Thus, this method is proposed for rapid assessment of mercury contained in the urine of workers exposed to a contaminated space, among other possible applications.

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**Figure captions**

**Fig. 1** Effect of sample volume. Extraction conditions: Amount of chelating agent, 40 µL of 2 mg $mL^{-1}$ solution; IL amount, 0.06 g; vortex-assisted IL-DLLME extraction time, 3 min; volume of 4 M HCl acceptor solution during back-extraction, 10 µL; back-extraction time, 9 min.

**Fig. 2** Effect of IL amount. Extraction conditions: Sample volume, 10 mL; amount of chelating agent, 40 µL of 2 mg $mL^{-1}$ solution; vortex-assisted IL-DLLME extraction time, 3 min; volume of 4 M HCl acceptor solution during back-extraction, 10 µL; back-extraction time, 9 min.

**Fig. 3** Effect of vortex-assisted IL-DLLME extraction time. Extraction conditions: Sample volume, 10 mL; amount of chelating agent, 40 µL of 2 mg $mL^{-1}$ solution; IL amount, 0.06 g; volume of 4 M HCl acceptor solution during back-extraction, 10 µL; back-extraction time, 9 min.

**Fig. 4** Effect of back-extraction time. Extraction conditions: Sample volume, 10 mL; amount of chelating agent, 40 µL of 2 mg $mL^{-1}$ solution; IL amount, 0.06 g; vortex-assisted IL-DLLME extraction time, 3 min; volume of 4 M HCl acceptor solution during back-extraction, 10 µL.

**Fig. 5** Influence of the urine storage temperature on $Hg^{2+}$ determination.

**Fig. 6** Square-wave voltammograms obtained during the analysis of a spiked urine sample at 5 µg $L^{-1}$ by the standard addition method. Legend shows calibration standards concentration.

**Table 1.** Determination of $Hg^{2+}$ in spiked human urine samples.

| Added concentration ($\mu g\ L^{-1}$) | Found concentration ± SD* ($\mu g\ L^{-1}$) | Relative recoveries (CV) |
|---|---|---|
| 5 | 5.0 ± 0.9 | 100 (18) |
| 10 | 9.7 ± 0.9 | 97 (9) |
| 15 | 15 ± 2 | 100 (13) |

*SD: standard deviation of x-value estimated using regression line [26].

**Table 2.** Comparison of different electrochemical methods for $Hg^{2+}$ determination in urine samples.

| Electrode | Sample pretreatment | Electroanalytical technique (deposition time) | LOD | Ref. |
|---|---|---|---|---|
| MBX-EIGE | Acid digestion | FI-DPASV (10 min) | 0.38 µg $L^{-1}$* | [9] |
| Au-RDE | Acid digestion | SWASV (2 min) | 0.8 µg $L^{-1}$ | [10] |
| DTDO modified GCE | Acid digestion | DPASV (25 min) | 6 µg $L^{-1}$* | [11] |
| EDTA-CPME | Acid digestion | SWASV (10 min) | 0.1 µg $L^{-1}$* | [12] |
| Gold film electrode | US-assisted acid digestion | ASCP (5 min) | 0.3 µg $L^{-1}$ | [13] |
| SPCnAuEs | Vortex-assisted IL-DLLME and back-extraction | SWASV (4 min) | 0.5-1.5 µg $L^{-1}$ | This work |

* LOD for aqueous standard solutions.

MBX, 2-mercaptobenzoxazole; EIGE, epoxy impregnated graphite electrode; FI, flow-injection; DPASV, differential pulse anodic stripping voltammetry; Au-RDE, gold rotating disk electrode; DTDO, 1,8-bis(dodecylthio)-3,6-dioxaoctane; GCE, glassy carbon electrode; EDTA, ethylenediaminetetraacetic acid; CPME, conducting polymer modified electrode; US, ultrasounds; ASCP, anodic stripping chronopotentiometry.

Fig. 1

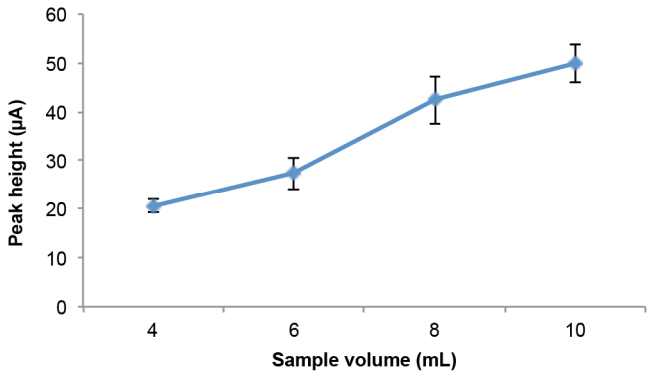


Fig. 2

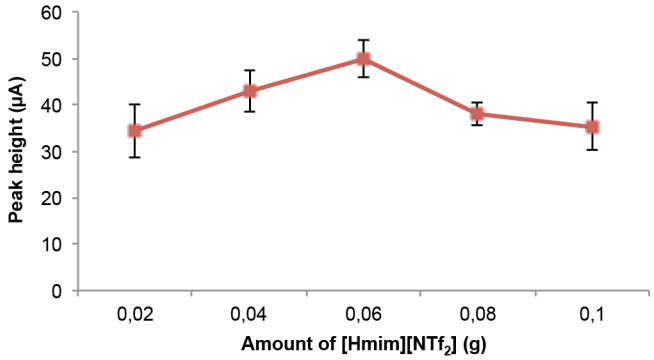


Fig. 3

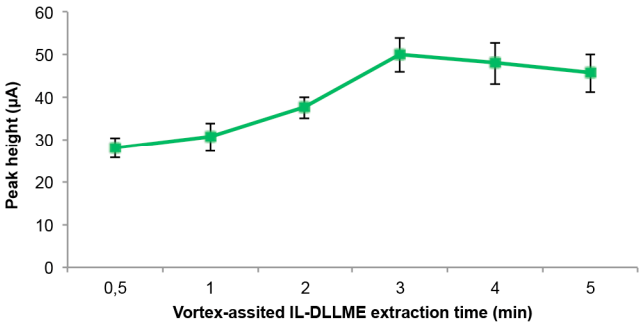


Fig. 4

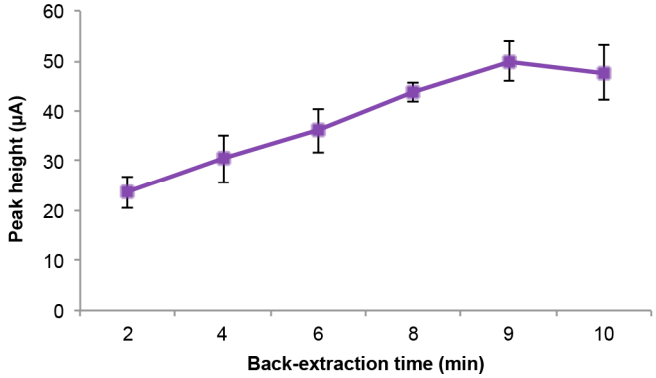


Fig. 5

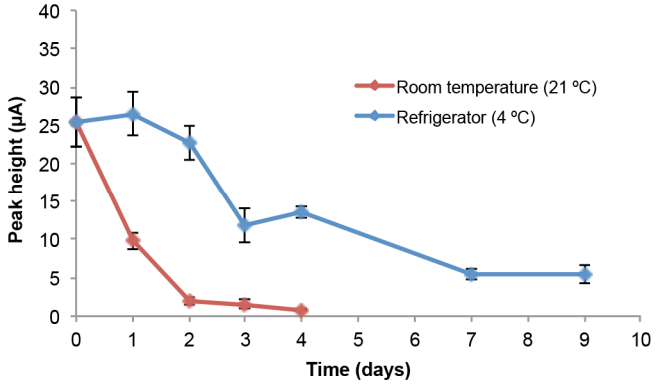
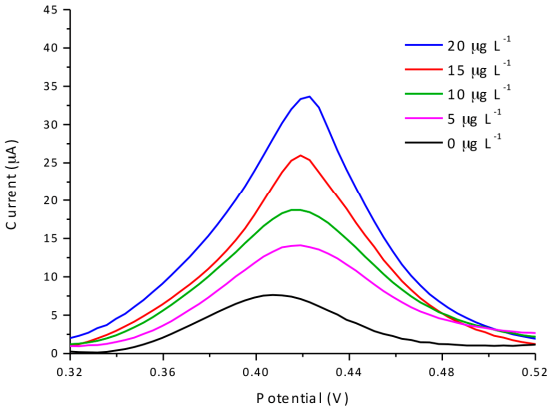


Fig. 6



**Highlights**

Mercury determination in non-digested urine samples.

Vortex-assisted DLLME and back-extraction as sample preparation methodologies.

SPEs are employed for the first time for mercury determination in urine samples.

Limit of detection lower than threshold level for normal content of mercury in urine.