ORIGINAL ARTICLE

Improvements on a total mercury determination method in human hair using graphite-furnace atomic absorption spectrophotometry detection

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Key words

Total Mercury • Human Hair • 2,3-Dimercaptopropane-1-Sulfonate (DMPS) • Ammonium Pyrrolidine Dithiocarbamate (APDC) • Graphite Furnace Atomic Absorption Spectrophotometry (GF-AAS)

Summary

In this work, a methodology for the determination of total mercury in human hair is presented. This methodology is an improvement of a previous technique which has been reported by Chen et al. in 2002. This previous work was based on an acid digestion, C_{18} cartridge clean-up, a 2,3-dimercaptopropane-1-sulfonate complexing agent, solid phase extraction and a graphite furnace atomic absorption spectrophotometric determination. In the present study, the complexing agent has been replaced by the ammonium pyrrolidine dithiocarbamate followed by a liquid-liquid extraction and the clean-up has been avoided in order to obtain a less expensive and less time consuming methodology.

Introduction

Mercury is a naturally occurring element that is widespread in the environment [1-3]. Human activity can release some of that mercury, increasing the amount, which is currently cycling in the atmosphere, soils, lakes, streams and oceans [4, 5].
In the 1950s, it was established that emissions of mercury to the environment could have serious effects on human health [6-9]. It has to be pointed out that environmental cycling of mercury is extremely complicated involving a variety of physical and chemical processes that affect its toxicity and mobility. Critical species in this cycling are elemental Hg vaporous (Hg^0), a common form in air, and the methylated forms due to their bioaccumulation capacity and toxicity. Generally, humans uptake mercury in two ways: as methylmercury from fish consumption, or by breathing vaporous mercury (Hg^0) emitted from various sources such as metallic mercury, dental amalgams, and ambient air [10]. Many industrialized countries have issued cautionary advisories about dental amalgam restorations and eating fish caught in some of their waterways because of the presence of mercury [11]. In spite of persistent efforts by the industrialized countries over the last decades to reduce mercury uses and releases, its decline is not yet reflected in the mercury levels [12, 13].
Recent studies demonstrate that the population had increased their exposure to mercury [14, 15]. In order to assess human mercury exposure, many studies have been carried out analyzing different human body fluids such as blood [16, 17], urine [16] or tissues such as hair [16]. Hair has proven to be a suitable indicator for monitoring of human exposure to mercury reflecting mercury levels as well as individual intake. A convincing relationship between the content of mercury in hair versus its content in blood has been reported in several studies. The hair-to-blood ratio in humans has been estimated as approximately 250:1 expressed as ng Hg/g hair to ng Hg/mL blood [16, 18, 19].
Different methodologies are available for the analysis of total Hg in human hair. In this way, in a recent interlaboratory report, 15 different digestion schemes and 5 different detection techniques were reported [20]. They evaluated different digestion techniques at high temperature, with a wide range of strong acids (HCl, HNO_3 and H_2SO_4), a strong base (NaOH) and oxidants such as H_2O_2, $KMnO_4$ and $K_2S_2O_8$. Also several detection techniques have been used for total Hg analysis such as combustion atomic absorption spectrophotometry (combustion-AAS) [21], cold vapor atomic fluorescence spectrophotometry (CV-AFS) [20], CV-AAS [22], graphite furnace atomic absorption spectrophotometry (GF-AAS) [23], cold vapor inductively coupled plasma atomic emission spectrophotometry (CV-ICP-AES) [24], inductively coupled plasma mass spectrometry (ICP-MS) [25], neutron activation analysis (NAA) [26] and particle induced X-ray emission (PIXE) [27].
The aim of the present work consists in improving an earlier reported methodology, which was based on the following procedure. Human hair was digested using a microwave assisted nitric acid digestion; then, after a C_{18} solid phase extraction (SPE) cleanup, mercury was complexed using 2,3-dimercaptopropane-1-sulfonate (DMPS) and preconcentrated into two C_{18} SPE cartridges disposed in series. Eluted with methanol the determination was carried out with a GF-AAS [23]. In order to make it faster, more economic, more accurate and suitable for routine analysis, special care has been taken in simplifying and reducing the analytical steps of the reported procedure.
Ammonium pyrrolidine dithiocarbamate (APDC), a chelating agent, has been widely used for the solvent

extraction of different metals in environmental samples; usually the preferred solvent being methylisobutyl ketone (MIBK). To the best of our knowledge, only three studies were performed, for mercury analysis, using APDC as chelating agent. Li and coworkers developed, a novel methodology for the determination of trace mercury in environmental and food samples by online coupling of flow injection with the displacement sorption preconcentration in a knotted reactor to electrothermal atomic absorption spectrometry (ETAAS) [28]. In this case the Hg-PDC displaced previously formed Cu-PDC complexes. In the same way, Karadjova et al. (2004) described an accurate, simple and precise method for total mercury determination in wines by APDC and liquid/liquid extraction of inorganic and organic mercury as a preconcentration procedure prior to their determination by electrothermal atomic absorption spectrometry (ET-AAS) [29]. Finally, a recent study [24] describes a method to determine total Hg in human hair by its complexation with APDC, coupled to an automatic sequential dispersing on-line SPE procedure and a cold vapor generation inductively coupled plasma atomic emission spectrometry (CV-ICP-AES) determination.
In the present work, the reported methodology from Chen and coworkers will be improved by avoiding the SPE cleanup and preconcentrations and by using APDC as Hg complexing agent.

Experimental

Reagents and materials

Ammonium pyrrolidine dithiocarbamate (APDC) 99%, mercury dichloride ($HgCl_2$, 99.9995%), methyl-isobutylketone (MIBK) and nitric acid (67%) for trace analysis were purchased from Carlo Erba Reagenti (Milan, Italy). Stock standards were prepared at 1000 mg/L (as Hg) in ultrapure water. The chelating reagent solution, 1% w/v APDC, was prepared daily by dissolving the appropriate amount of APDC in ultrapure water. All standards were stored at 4 °C, and working solutions were prepared weekly by diluting the stock solutions with ultrapure water. A buffer solution at pH 4.5 was prepared by mixing sodium acetate (0.2 M) and acetic acid (0.2 M). A certified human hair reference material from the Shanghai Institute of Nuclear Research, China (GBW09101 containing 2.16 + 0.21 μg/g of total Hg) was purchased.

Apparatus

A Perkin Elmer (Norwalk, CT, USA) Model 1100B atomic absorption spectrometer equipped with a deuterium lamp as a background corrector and a HGA-700 graphite furnace atomizer was used for the atomic absorption measurement of mercury at 253.7 nm with a slit width of 0.2 nm (high). The source of radiation was a hollow cathode Hg lamp (Hg lumina™ lamp, Perkin Elmer) operated at 6 mA. A Centrifuge Model 4236 (A.L.C., Milan, Italy) was also used in order to improve organic/aqueous phase separation. Optimum GF-AAS working conditions are summarized in Table I.

Sample preparation and acid digestion

In this study, sample preparation and digestion were conducted as reported in a previous work [30] for the determination of methylmercury in human hair. Hair samples, collected from local sources, were finely cut and placed in a 100 mL beaker and ultrasonically washed in a 0.1% Triton X-100 solution for 20 min. After vigorous rinsing with a large volume of deionized water, hair samples were rinsed with acetone and air-dried.
An amount of finely cut hair (40 mg) was placed in a 5 mL Pyrex tube and then acid solution was added (700 μL of HNO_3 5M). The tube was capped non-tightly and then heated at 100 °C for 120 minutes using an electronically-controlled heating plate Ikamag® RCT Basic with an electronic contact thermometer Ikatron® ETS-D4 fuzzy which was provided by Ika (Staufen, Germany). Digestions were stored, protected from light, at 4 °C.

Extraction procedure

After the digest was cooled down to room temperature, 4 mL of NaOH (1 M) were added in a 5 mL Pyrex tube and 1 mL of acetate buffer solution in order to obtain a pH of 4.5. Then, 200 μL of the 1% APDC solution and 500 μL of methyl-isobutylketone (MIBK) were added. After a vigorous mixing, the solution was separated by centrifugation at 2700 rpm for 20 min. Finally, a 20 μL aliquot of the supernatant organic phase containing the Hg-APDC complex was directly introduced into a graphite tube and atomized.

Results and discussion

The goal of the present work consisted in improving an earlier methodology reported by Chen and coworkers which used nitric acid digestion, SPE cleanup, DMPS complexation, SPE preconcentration and GF-AAS determination to the analysis of total mercury in human hair [23], but it has shown to be expensive and time consuming as for example 3 SPE cartridges were needed for each individual analysis. Therefore, it is expected that reducing its length and improving its cost-effectiveness, the methodology will

Tab. I. Graphite furnace working conditions for the total mercury determination.

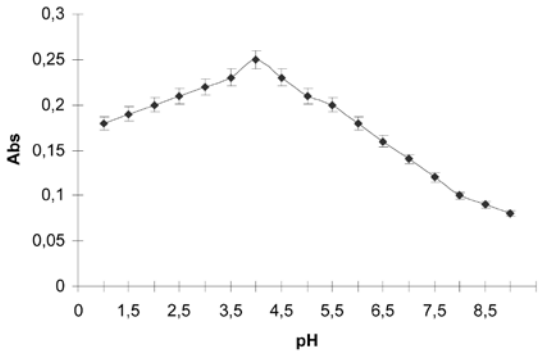
Step	Temperature (°C)	Ramp rate (°C/s)	Hold time (s)	Argon flow rate (mL min^{-1})
Drying	120	20	15	300
Pyrolysis	250	15	20	300
Atomisation	1600	1	4	0
Cleaning	2000	4	4	300

be more suitable for routine and epidemiological studies. For this reason, special care has been taken in avoiding the SPE cleanup and preconcentration steps.
Sample digestion was carried out using nitric acid as reported but, compared to the previous work, this technique permitted to avoid the use of concentrated nitric acid. Even if this digestion method is more time consuming (120 min *vs.* 10 min), as the digested samples are stable over long time periods [30] and several samples could be digested at the same time, this step does not limit the sample throughput of the methodology. In summary, optimum digestion conditions were an acid digestion using 40 mg of hair with 700 μL of 5 M HNO_3 at 100 °C for 120 min.

Extraction procedure

The ammonium pyrrolidine dithiocarbamate (APDC) is a selective chelating agent, which has already been used for the solvent extraction of Hg [24, 28, 29]. A liquid-liquid extraction using APDC/MIBK has already been shown to be able to quantitatively extract the different mercury species in wine [29]. In this work, this system was used for the mercury extraction in human hair. For this reason, several parameters such as pH, APDC and MIBK concentrations and volume have been optimized working with real samples obtained from local sources.
In order to optimize the extraction pH, the levels of the Hg-dithiocarbamate complexing agent at different values of pH have been evaluated. The effect of the pH was studied in the range 1-9 by adjusting it with diluted nitric acid or sodium hydroxide solutions. As can be seen in Figure 1, the Hg extraction yield depended on the pH values obtaining a maximum between pH 3 and 5.
Different volumes (25-500 μL) of a chelating reagent solution (1% w/v APDC) were tested in order to evaluate the influence of this parameter. The results of the APDC volume effect on the Hg-dithiocarbamate complexation are reported in Figure 2. As can be seen in this Figure, absorbance reaches a plateau at APDC volumes higher than 150 μL. Consequently, a higher volume of APDC is required as we should work with an excess of this reagent but, qualitatively, an increase in GF-AAS interferences is observed for the higher volumes. For this reason, a volume of 200 μL of APDC was established to carry out the complexation of the Hg. It should be pointed out, that the amount of APDC needed is related to the mercury concentration of the sample, and at higher Hg concentrations higher amounts of APDC are needed. With the described proportions, concentrations of Hg up to 5 μg/g with an overall recovery higher than 90% could be analyzed. If higher concentrations were found the analysis should be repeated using 20 mg of hair instead of 40 mg reducing in this way the amount of APDC needed.
Also the effect of MIBK volume on the Hg-PDC complex extraction was evaluated. The effect of MIBK was tested in the range 0.1-2.0 mL and the results show, as expected, the dilution effect of an increase of the MIBK volume if the aliquot introduced in the graphite tube is constant (20 μL). In fact, when we increase the MIBK volume, we are decreasing the concentration factor achieved during the liquid-liquid extraction step. Furthermore, an increase of this aliquot is not possible as it causes an increase in GF-AAS interferences. Therefore,

Fig. 1. Hg-dithiocarbamate complexation yields depending on the pH.



the MIBK volume was chosen as the minimum volume (maximum concentration factor), which can be easily handled.
In summary, the chosen extraction conditions were at pH 4,5 using 200 μL of APDC (1% m/v) and extracting the Hg-PDC complex with 0,5 mL of MIBK.

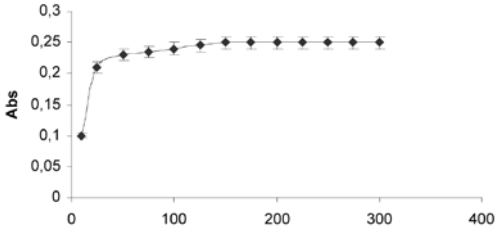
Sample analysis

In order to evaluate the developed methodology real samples (N = 15) have been analyzed together with a human hair reference material from the Shanghai Institute of Nuclear Research, China (GBW09101 containing 2.16 + 0.21 μg/g of total Hg).
Extraction recoveries were calculated using reference material analysis with obtained yields of 97 ± 4% (range 92-102) (N = 19). Technique detection limit (blank level more three times its standard deviation) was of 50 ng/g and the quantification limit was 170 ng/g evaluated as the lowest analyzed concentration on the calibration plot. Quantification of hair samples was carried out by external calibration with a $R^2 > 0.99$. Encountered total Hg concentrations ranged between 289 ng/g and 3.33 μg/g with a median value of 421 ng/g.

Conclusion

In this paper a methodology for the determination of total Hg in human hair has been developed based on a previous reported technique [23]. The presented technique consisted on a nitric acid digestion, pH adjustment (i.e. 3.5-4.5), an APDC complexation, a MIBK solvent extraction and GF-AAS determination. A fast, precise and economic technique has been obtained by improving the earlier

Fig. 2. Effect of APDC volume (1% m/v APDC in ultrapure water) on the Hg-dithiocarbamate complexation recovery.



reported methodology in order to make it suitable for routine analysis. For this reason, special care has been taken in avoiding the SPE cleanup and the two SPE in series preconcentration. Extraction parameters for the APDC/MIBK such as pH reagent and solvent volumes have been optimized. The resulting technique has been evaluated by analyzing 15 real samples and a reference certified material. The technique presented a median recovery of 97 ± 4% and a limit of detection of 50 ng/g of total Hg with an RSD < 5%. This developed methodology can represent a useful tool in order to implement epidemiological studies of a contaminant, which is especially relevant for human health due to its toxicity and ubiquity.

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