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Spectrophotometric determination of mercury using vortex-assisted liquid–liquid microextraction

Tatiana ANTOLOVÁ[1], Serhii ZARUBA[2], Jana ŠANDREJOVÁ[1,*], Lívia KOCÚROVÁ[1], Andriy B. VISHNIKIN[2], Yaroslav BAZEĽ[1], Vasil ANDRUCH[1], Ioseph S. BALOGH[3]

[1]Department of Analytical Chemistry, Pavol Jozef Šafárik University, Košice, Slovakia

[2]Department of Analytical Chemistry, Faculty of Chemistry, Oles Honchar Dnipropetrovsk National University, Dnipropetrovsk, Ukraine

[3]Department of Chemistry, University of Nyíregyháza, Nyíregyháza, Hungary

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Abstract: A novel method for the determination of mercury(II) is suggested. The procedure is based on the formation of an ion associate between the bromide complex of Hg(II) and Astrazon Red 6B dye and vortex-assisted liquid–liquid microextraction of the ion associate formed, with subsequent spectrophotometric detection. The variables that affect the procedure, such as pH, the concentration of ligand and dye, the type and volume of extraction solvent, and the rate and time of vortex mixing, were optimized. Under optimum conditions (pH 2.0, 0.01 mol L^{-1} KBr, 2×10^{-4} mol L^{-1} AR6B, 50 μL of the extraction mixture toluene:dichlorethane, 4:1, v:v, vortex mixing for 100 s at 1600 rpm) the linear range was 8 to 200 μg L^{-1} Hg(II), with the limit of detection at 1.5 μg L^{-1}. The method was applied to the determination of mercury in water samples.

Key words: Vortex-assisted liquid–liquid microextraction, mercury, spectrophotometry, green analytical chemistry, water samples

1. Introduction

Due to the high impact of mercury compounds on the environment as well as human health, the development of methods for the determination of mercury in a variety of samples is still crucial.[1] This is why a range of methodologies have recently been published. Here we mention only selected methodologies: differential pulse polarography,[2] isotope-dilution inductively coupled plasma mass spectrometry (ICP–MS),[3] the kinetic spectrophotometric method,[4] flow injection–green chemical vapor generation–atomic fluorescence spectrometry (AFS),[5] chemiluminescence quenching,[6] and high–performance liquid chromatography–inductively coupled plasma mass spectrometry (HPLC–ICP–MS).[7] One can also find review articles devoted to the determination of mercury.[1,8–10]

A requirement today is that newly developed methods meet the requirements of green analytical chemistry. For this reason, a great many articles devoted to dispersive liquid–liquid microextraction and dispersive liquid–phase microextraction as well as their modalities for the determination of both organic and inorganic analytes have recently published.[11–14]

Several solvent microextraction methods for mercury determination have been reported. These include

*Correspondence: jana.skrlikova@googlemail.com

dispersive liquid–liquid microextraction (DLLME),[15,16] ionic liquid–based dispersive liquid–liquid microextraction (IL–DLLME),[17] dispersive liquid–liquid microextraction based on solidification of floating organic drop (DLLME–SFO),[18] surfactant–assisted dispersive liquid–liquid microextraction based on the solidification of the floating organic drop (SA–DLLME–SFO),[19] one-step displacement dispersive liquid–liquid microextraction (D–DLLME),[20] dispersive liquid-phase microextraction (DLPME),[21] task-specific ionic liquid-based ultrasound-assisted dispersive liquid-phase microextraction (UA–IL–DLPME),[22] and ionic liquid-based vortex assisted liquid–liquid microextraction (IL–VALLME)[23] coupled with a variety of spectrometric detection techniques, such as graphite furnace atomic absorption spectrometry (GFAAS),[18–20] flame atomic absorption spectrometry (FAAS),[21] cold vapor atomic absorption spectrometry (CV–AAS),[15,22] flow injection-hydride generation/cold vapor atomic absorption spectroscopy (FI–HG/CV–AAS),[17] cold vapor atomic fluorescence spectroscopic detection (CV–AFS),[23] and inductively coupled plasma atomic emission spectrometry (ICP–AES).[16]

Besides the above-mentioned spectrometric detection techniques, the combination of DLLME,[24,25] IL–DLLME,[26–28] VALLME,[29] and IL–VALLME[30] with various chromatographic techniques, such as HPLC–ICP–MS,[24,26] HPLC–UV,[27] HPLC–CV–AFS,[29,30] HPLC–HG–AFS,[28] and GC–FID,[25] has also been described. The combination of solvent microextraction for mercury determination with other techniques, such as capillary electrophoresis,[31,32] electrochemical detections,[33–35] and corona discharge ionization ion mobility spectrometry,[36] occurs to a lesser extent.

Despite the fact that plenty of the solvent microextraction procedures for determination of mercury have been reported, the number of procedures coupled with UV–Vis detection is limited. This can be considered a great shame, at least in our opinion, due mainly to the lower instrumental cost of spectrophotometry compared with other techniques. Therefore, the aim of this work was to develop a liquid-phase microextraction procedure for mercury determination coupled with UV–Vis detection. The determination is based on the formation of ion associate between the bromide complex of Hg(II) and Astrazon Red 6B dye reagent (Figure 1) and vortex-assisted liquid–liquid microextraction of the ion associate formed. In our opinion, the reaction chemistry (the formation of the complex and ion associate as well as its extraction) may be expressed by the following equations:

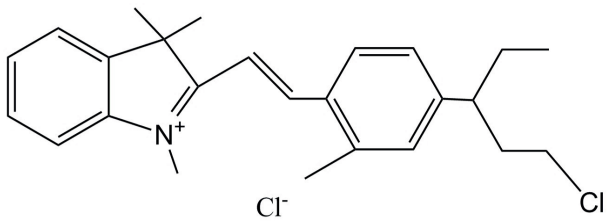

Figure 1. Chemical structure of the Astrazon Red 6B dye reagent.

$$Hg^{2+}_{(aq)} + 3Br^{-}_{(aq)} \rightarrow [HgBr_3]^{-}_{(aq)}$$

$$[HgBr_3]^{-}_{(aq)} + R^{+}_{(aq)} \rightarrow [HgBr_3]^{-}\ R^{+}_{(aq)}$$

$$[HgBr_3]^{-}\ R^{+}_{(aq)} + nS_{(org)} \rightarrow [HgBr_3]^{-}\ R^{+} \times nS_{(org)},$$

where S means the mixture of organic solvents, R^+ the dye reagent, and (aq) means the aqueous phase and (org) the organic phase.

The method was applied to the determination of mercury in spiked water samples.

2. Results and discussion

2.1. Effect of chemical variables

Firstly, the effect of chemical variables was studied in following order: pH, concentration of bromide anion, and dye reagent. The univariate optimization method, in which the concentration of one component was altered while the concentrations of the other components were kept constant, was applied to investigate the effect of the chemical variables. The following concentration collections were studied: pH 0–7.0, KBr 0.002–0.014 mol L^{-1}, AR6B 2.0×10^{-5}–3.0×10^{-4} mol L^{-1}, at constant concentration of mercury(II), 5.0×10^{-7} mol L^{-1}. Based on the results obtained (Figure 2), the following conditions were chosen as optimum: pH 2.0, 0.01 mol L^{-1} KBr, and 2×10^{-4} mol L^{-1} AR6B. We should note that the required pH of the aqueous phase was achieved by the addition of a solution of H_2SO_4 (for pH range 0–3.0) or by using HOAc–NH_4OH buffer solution prepared by mixing equimolar (1 mol L^{-1}) solutions of acetic acid and ammonium hydroxide in various volume ratios (for other pHs). Hydrochloric acid is not suitable for sample acidity adjustment due to the conditions appropriate for competing reaction and formation of chloride complexes.

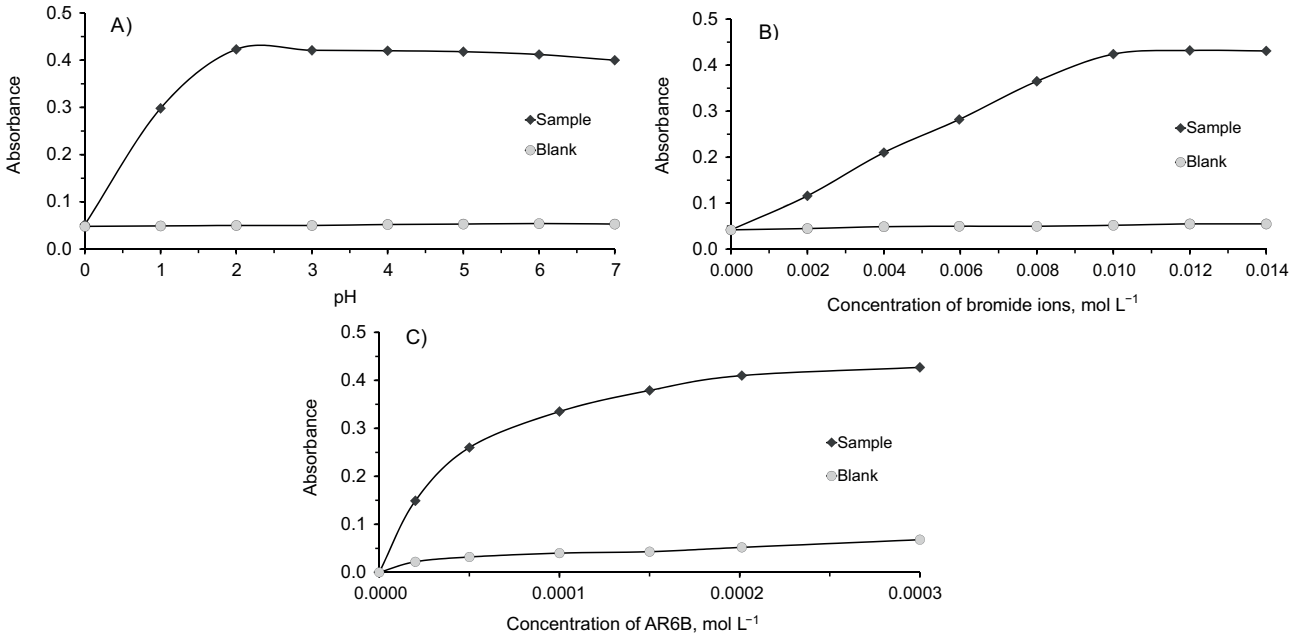

Figure 2. Effects of chemical variables. Conditions: 5.0×10^{-7} mol L^{-1} Hg(II); 200 μL mixture of toluene and dichlorethane, 4:1 v/v; vortex mixing, 1600 rpm, 100 s; centrifugation, 2000 rpm, 2 min A) Effect of pH (0.01 mol L^{-1} KBr, 2.0×10^{-4} mol L^{-1} AR6B); B) Effect of bromide ions (pH 2.0, 2.0×10^{-4} mol L^{-1} AR6B); C) Effect of AR6B (pH 2.0, 0.01 mol L^{-1} KBr).

2.2. Effect of organic solvents

In the second step, the influence of the type and volume of organic solvent was investigated. Selection of a suitable solvent is an important step in the development of new microextraction procedures. Various organic solvents, such as benzene, toluene, chlorobenzene, nitrobenzene, acetophenone, chloroform, dichloroethane, butyl acetate, and amyl acetate, were studied in preliminary experiments. Several of them are characterized by low extraction efficiency, such as benzene, toluene, butyl acetate, and amyl acetate. On the other hand,

others, such as chlorobenzene, nitrobenzene, acetophenone, chloroform, and dichloroethane have good extraction efficiency but are inappropriate due to high absorbance of the blank test. Therefore, mixtures of solvents were also investigated. The best results were obtained in the case of the mixture of toluene and dichlorethane. Thus, various ratios of these solvents were studied (Figure 3), and based on the results obtained a 4:1 volume ratio of toluene and dichlorethane was chosen for further experiments.

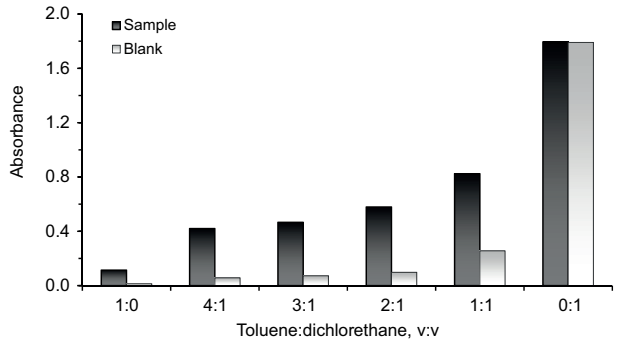

Figure 3. Effect of toluene–dichlorethane ratio. Conditions: 5.0×10^{-7} mol L^{-1} Hg(II); pH 2.0, 0.01 mol L^{-1} KBr, 2.0×10^{-4} mol L^{-1} AR6B; 200 μL mixture of toluene and dichlorethane, vortex mixing, 1600 rpm, 100 s; centrifugation, 2000 rpm, 2 min.

To investigate the effect of volume of the extraction solvent mixture, experiments involving different volumes of the toluene:dichlorethane mixture 4:1 (v/v) were performed under the previously optimized chemical conditions. In general, decreasing the volume of organic solvent leads to an increase in the enrichment factor; however, this also leads to a decrease in the volume of separated organic phase and consequently complicates handling during the extraction phase withdrawal and measurement steps.[37] Therefore, due to easy handling, 200-μL mixtures of extraction solvents were used for optimization of the chemical parameters. However, due to an increase in the sensitivity for calibration of the method, a volume of 50 μL was used.

2.3. Effect of vortex mixing

Finally, the effect of the vortex mixing rate and time was examined. The influence of a vortex agitator on the 'quality' of the formed emulsion and consequently on the effectiveness of the microextraction was discussed in detail.[38] The formation of the fine droplets of extraction solvent in aqueous phase under vortex mixing conditions leads to an increase in the extraction efficiency and consequently to a reduction in extraction time. Therefore, two series of experiments were performed in which the influence of vortex mixing rate and time were studied under the previously optimized chemical conditions. The influence of the vortex mixing rate was investigated in the range of 0–3200 rpm (Figure 4A). Next, the influence of the vortex mixing time was studied in the range of 0–180 s (Figure 4B). Based on the results obtained, a vortex mixing rate of 1600 rpm and 100 s of vortex extraction time were selected for further experiments.

2.4. Figures of merit

Under optimum experimental conditions, a calibration plot was constructed from five data points. A linear analytical response was obtained in the range 8–200 μg L^{-1} of mercury with the regression equation $A = -0.007 + 0.007 \times C$ (where A means the absorbance and C the concentration of Hg(II) in μg L^{-1}) and with a correlation coefficient of 0.9999. The limit of detection (LOD), calculated as $3 \times s_b / b$ (where s_b is the standard

deviation of the 10 blanks and b is the slope of the calibration graph), was found to be 1.5 μg L^{-1} Hg(II). The enrichment factor of mercury for a 5-mL sample was 7.3.

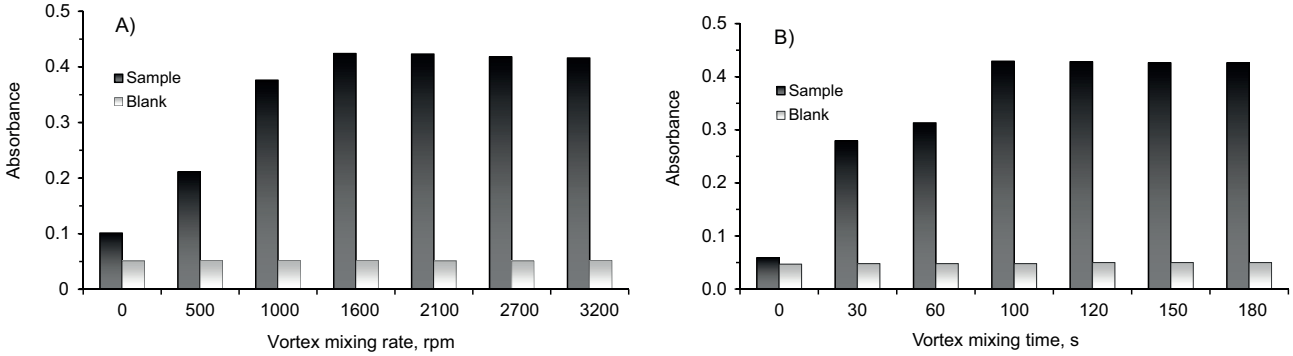

Figure 4. Effect of vortex mixing. Conditions: 5.0 × 10^{-7} mol L^{-1} Hg(II); pH 2.0, 0.01 mol L^{-1} KBr, 2.0 × 10^{-4} mol L^{-1} AR6B; 200 μL mixture of toluene and dichlorethane, 4:1 v/v; centrifugation, 2000 rpm, 2 min A) Effect of vortex mixing rate (100 s); B) Effect of vortex mixing time (1600 rpm).

Precision and accuracy were evaluated for five replicate determinations at three different concentration levels of mercury(II) (16 and 64 μg L^{-1}) over 2 days during a single week (Table 1). The relative standard deviations and recoveries were in the range 2.5%–5.0% and 96.9%–106.3%, respectively.

Table 1. Intraday and interday precision and accuracy data for the determination of mercury (n = 5).

Taken (μg L^{-1})	Intraday			Interday		
	Determined (μg L^{-1})	RSD (%)	R (%)	Determined (μg L^{-1})	RSD (%)	R (%)
16	16 ± 1	5.0	100.0	17 ± 1	4.7	106.3
32	31 ± 1	2.6	96.9	32 ± 2	5.0	100.0
64	64 ± 0	2.5	100.0	63 ± 2	2.6	98.4

The effect of some interfering ions on the determination of Hg(II) was examined. A ratio of Hg:interferent that resulted in an error not exceeding ±5% was taken as the tolerable amount of each ion. Most of the examined ions (Ni^{2+}, Fe^{2+}, Fe^{3+}, Cr^{3+}, Ca^{2+}, Mg^{2+}, Co^{2+}, Zn^{2+}) did not interfere with the determination of mercury at more than a 5000-fold excess; Cd^{2+}, Hg^{+}, Ga^{3+}, and In^{3+} did not interfere at more than a 1000-fold excess and Pb^{2+} did not disturb determination at more than a 100-fold excess.

2.5. Comparison with other methods

Despite the fact that several solvent microextraction procedures for the determination of mercury have been reported, only a few of them have been coupled with UV–Vis detection (Table 2). Gharehbaghi et al.[39] described a method based on the complexation of Hg cations by 4,4'–bis(dimethylamino)thiobenzophenone (TMK) in the presence of sodium dodecyl sulfate as the antisticking agent, followed by extraction of Hg–TMK complex by 1-hexyl-3-methylimmidazolium bis(trifluormethylsulfonyl)imid ionic liquid as the extraction solvent dissolved in acetone as the disperser solvent with subsequent spectrophotometric detection at 575 nm. Lemos et al.[40] developed a method for the determination of mercury in water samples after DLLME preconcentration in the form of a complex with 2-(2-benzothiazolylazo)-p-cresol. The spectrophotometric detection at 650

nm is performed on a triacetylcellulose membrane. Niazi et al.[41] reported simultaneous spectrophotometric determination of copper and mercury developed by DLLME preconcentration and orthogonal signal correction-partial least squares (OSC–PLS).

Table 2. Comparison of the developed method with other microextraction methods for UV–Vis determination of mercury in water samples.

Method	Sample	Remarks	Linear range and LOD	Ref.
IL–DLLME	Water (mineral, river)	Selected conditions: sample; 500 µL buffer (pH 3.8, 1 mol L^{-1}); 250 µL $NaNO_3$ 10% (w/v); 250 µL SDS 1% (w/v); 120 µL 4,4'-bis(dimethylamino)thiobenzophenone (TMK) (2×10^{-4} mol L^{-1}); doubly distilled water up to 10.00 mL total volume; 500 µL acetone containing 60 mg [Hmim][Tf_2N]; centrifuged, 5000 rpm, 6 min. Measurement: removing the whole aqueous solution; the extraction phase diluted with 350 µL of ethanol (85%); transferred to a 500 µL cell.	LR: 12–140 µg L^{-1} LOD: 3.9 µg L^{-1}	39
DLLME	Water (drinking, sea, river)	Selected conditions: 10 mL sample; pH 9.5; 50 µL carbon tetrachloride; 50 µL 2–(2–benzothiazolylazo)–*p*–cresol (BTAC) 2 mL ethanol; centrifuged, 5000 rpm, 2 min. Measurement: 5 µL residue; membrane; solvent evaporated; spherical part placed in front of spectrophotometer beam.	LR: 11.1–200 µg L^{-1} LOD: 3.3 µg L^{-1}	40
DLLME	Water (tap, mineral, river, waste)	Selected conditions: 10 mL sample; potassium nitrate (5%); 1 mL dithizone (1.6×10^{-4} mol L^{-1}); pH 3.4; 800 µL acetonitrile and 200 µL carbon tetrachloride; centrifuged, 3000 rpm, 5 min. Measurement: removing the aqueous phase; transferring to 100 µL cell; absorbance measured at 400–700 nm; orthogonal signal correction–partial least squares (OSC–PLS) multivariate calibration.	LR: 10–300 ng mL^{-1} LOD: 2.8 ng mL^{-1}	41
VALLME	Water (tap, thermal, waste)	Selected conditions: 5 mL sample; pH 2.0; 0.01 mol L^{-1} KBr, 2.0×10^{-4} mol L^{-1} AR6B; 50 µL tolune:dichlorethane (4:1; v:v). Measurement: 5 mm, 5 µL microvolume cell.	LR: 8–200 µg L^{-1} LOD: 1.5 µg L^{-1}	This work

In comparison with other reported methods, our method has a comparable linear range and detection limit. However, our procedure does not require the use of a dispersive solvent, in contrast to 500 μL of acetone,[39] 2 mL of ethanol,[40] and 800 μL acetonitrile,[41] and it does not require dilution of the sedimented phase[39] or evaporation of the residue on triacetylcellulose membrane.[40]

2.6. Analytical application

To demonstrate the practicability of the method, some water samples were spiked with various concentrations of mercury and analyzed according to the suggested procedure using calibration plot or by method of standard additions. The obtained results are given in Table 3.

We have suggested a vortex-assisted liquid–liquid microextraction procedure for spectrophotometric determination of mercury based on the formation of an ion associate with Astrazon Red 6B dye in the presence

of bromide ions as ligand. The method is simple, low cost, and environmentally friendly due to the small amount of extraction solvents used. Moreover, using spectrophotometry as a detection system results in a low operational cost compared with other detection techniques. The method was applied to the determination of mercury in water samples.

Table 3. Determination of mercury in water samples (n = 5).

Sample	Added (μg L^{-1})	Found (μg L^{-1})	RSD (%)	R (%)
Tap water*	0	<LOQ	–	–
	200	198 ± 12	4.9	98.5
	602	580 ± 9	1.2	96.3
Thermal water*‡	0	<LOQ	–	–
	401	382 ± 7	1.5	95.3
Wastewater**‡	0	<LOQ	–	–
	802	844 ± 16	1.5	105.2
*5-times dilution and **10-times dilution; ‡determination by method of standard additions (3 additions).				

3. Experimental

3.1. Reagents

All reagents used were of analytical grade. Distilled water was used throughout the work. A stock solution of mercury containing 1 × 10^{-2} mol L^{-1} Hg(II) was prepared by dissolving of $Hg(NO_3)_2 \times 1/2H_2O$ in 100 mL of water. A 1 × 10^{-5} mol L^{-1} working solution of Hg(II) was prepared by appropriate dilution of the stock solution with water. A 0.1 mol L^{-1} aqueous solution of KBr was used to set the appropriate concentration of ligand. A 1 × 10^{-3} mol L^{-1} aqueous solution of Astrazon Red 6B dye (AR6B) was prepared by dissolving of AR6B in 2 mL of methanol and subsequent dilution with water up to a volume of 100 mL.

3.2. Apparatus

A Lightwave II UV–Vis spectrophotometer (Biochrom, UK) equipped with a matched cell of 5-mm path length was used for absorbance measurements. A VM–3000 MD vortex mixer (Medline Scientific, UK) was used to assist the extraction process. The dispersion was disrupted by centrifugation using a CN–2060 centrifuge (MRC, Israel). The pH values of the solutions were measured using an ORION $720A^+$ pH meter with a glass electrode.

3.3. General procedure

A 5-mL volume of aqueous sample or standard solution containing from 8 to 200 μg L^{-1} mercury as well as all the necessary reagents in appropriate concentrations (0.5 mL of 0.05 mol L^{-1} H_2SO_4, 0.5 mL of 0.1 mol L^{-1} KBr, and 1.0 mL of 0.001 mol L^{-1} AR6B) were placed into 15-mL polyethylene test tubes and mixed thoroughly. Next, a 50-μL portion of the toluene:dichlorethane 4:1 (v:v) mixture was added, and the content of the tubes was shaken using the vortex for 100 s at 1600 rpm. After centrifugation, 2 min at 2000 rpm, about 45 μL of the organic phase was floated on the surface of the aqueous solution. Finally, the necessary volume of extractant was separated using a microsyringe and transferred into an ultramicro cell (5 μL) with 5-mm path length for absorbance measurement at 550 nm.

3.4. Sampling and sample pretreatment

Tap water samples were taken in our laboratory and analyzed immediately after collection by the suggested procedure without filtration or any other treatment. Thermal water sample was taken in thermal spa. The certified reference water sample (SPS-WW2 Wastewater) was diluted appropriately with double distilled water prior to VALLME.

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