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A New Procedure for the Speciation of Mercury in Water Based on the Transformation of Mercury(II) and Methylmercury(II) into Stable Acetylides Followed by HPLC Analysis

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Conversion of mercury(II) and methylmercury(II) species dissolved in water into di(phenylethynyl)mercury and methyl(phenylethynyl) mercury takes place in satisfactory yield under alkaline conditions by stirring the aqueous solution with phenylacetylene at room temperature. Mercury speciation is simply obtained by HPLC analysis of the two organometallic species. The presence of heavy metals such as copper(II), zinc(II), cadmium(II) and lead(II) in concentrations 10 000 times higher than mercury is tolerated, while little interference is displayed by humic acids and cysteine. Seawater samples can also be analysed following a properly adapted procedure.

Keywords: mercury; methylmercury; mercury acetylides; speciation; HPLC

INTRODUCTION

Current standards of environmental analysis require the identification of the chemical forms of metals in polluted samples owing to the different toxicological and ecotoxicological features associated with each species. Concern regarding anthropogenic and natural contamination by mercury[1] continues the stimulation of the development of new techniques of analysis. A review of the most effective procedures for the speciation of mercury has recently appeared.[2]

In a project aimed at studying the harbour of Ravenna, we recently focused our attention on, among other methods, derivatization procedures which convert Hg(II) and $CH_3Hg(II)$ species into fully alkylated organometallic derivatives suitable for chromatographic analysis. The most successful alkylating agent is sodium tetraethylborate;[3] it reacts in water with Hg(II) and $CH_3Hg(II)$ to give diethylmercury and ethyl(methyl)mercury, which are analysed by GC with atomic absorption (AA) or atomic fluorescence (AF) detection.[4,5] The sensitivity is high, the absolute detection limits being about 0.6 pg for $CH_3Hg(II)$ and 1.3 pg for Hg(II),and the overall methodology is rapid and precise. Drawbacks are represented (i) by the strict control of pH in order to minimize reagent loss by hydrolysis, (ii) by the cost and the kinetic instability of the reagent to air oxidation and to moisture, and (iii) by the properly designed instrumental apparatus required.

Adopting the same strategy, we recently proposed a new procedure for the analysis of inorganic Hg(II) in water which involves transformation of Hg(II) into di(phenylethynyl)mercury upon reaction with phenylacetylene in alkaline medium, followed by extraction in dichloromethane (CH_2Cl_2) and HPLC analysis using UV detection.[6] The process is based on the reactivity of monosubstituted alkynes with Hg(II) salts (Scheme 1).

The η^2 complex **A** (Scheme 1) is in equilibrium with the η^1 complex **B** and HCl; under acidic conditions **A** prevails and the electron-poor ligand easily undergoes a regioselective nucleophilic addition of water leading to a vinyl alcohol and hence to a methyl ketone. On the other hand, in alkaline medium the equilibrium is displaced towards **B**, and, if an excess of alkyne is present, the dialkynylated product **C** can be isolated.

While we were carrying out this preliminary study we observed that CH_3HgCl dissolved in alkaline solution is similarly trapped by phenylacetylene and converted into methyl(phenylethynyl)mercury in good yield.

Here we wish to report a modified version of our original methodology for the determination of inorganic mercury;[6] the new procedure allows

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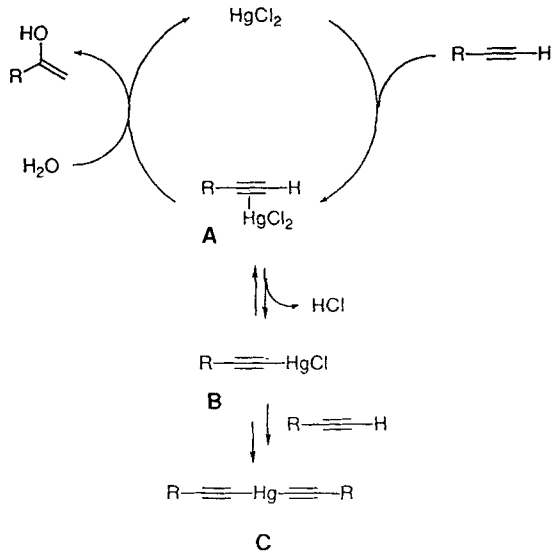
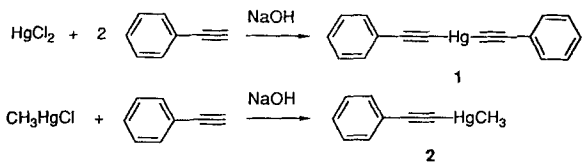


Scheme 1

us to simultaneously determine inorganic Hg(II) and $CH_3Hg(II)$ in an aqueous solution by performing a single derivatization reaction, as shown in Scheme 2.



Scheme 2

RESULTS AND DISCUSSION

Hereafter, concentrations of mercury species including the organometallic derivatives **1** and **2** are given as mg l^{-1} or μg l^{-1} of mercury.

Derivatization procedure

A simple, straightforward derivatization reaction is carried out by using standard glassware and cheap and stable chemicals without special precautions. To the aqueous solution containing $HgCl_2$ and CH_3HgCl are added NaCl, NaOH and phenylacetylene, then the reaction mixture is stirred at room temperature. Among other variables, we fixed the temperature at 20–25 °C; in fact raising the reaction temperature to 40–50 °C does not appreciably increase the reaction rate. The excess of phenylacetylene is not critical; a huge excess amount of alkyne in any case is to be avoided since it makes the work-up step more complex. We focused our attention on the concentration of NaOH and NaCl, and on the reaction time, and we carried out a simplex optimization[7] of these three parameters using $HgCl_2$ and CH_3HgCl solutions in deionized water at the concentration of each species of 20 μg l^{-1}.

After stirring the reaction mixture for 45 min at 20 °C, a crucial work-up procedure allows us to transfer **1** and **2** into acetonitrile and to increase up to 100 times the original concentration of mercury species. Work-up involves extraction of the organomercury derivatives **1** and **2** with n-hexane followed by a solid-phase extraction on silica gel by eluting the hexane phases through a short-path column. At this stage most of the excess of phenylacetylene is washed off. Elution with a small volume of acetonitrile quantitatively extracts adsorbed **1** and **2** and the eluate is directly analysed by HPLC. We wish to stress that the solid-phase extraction technique replaces the evaporation of hexane and excess phenylacetylene under vacuum, which causes significant losses of methyl(phenylethynyl)mercury **2**. The operational limit of quantification when 50 ml of aqueous sample is analysed according to the typical procedure reported in the Experimental section (2 ml final acetonitrile solution, 20 μl injection) has been found to be about 2 μg l^{-1} for both $HgCl_2$ and CH_3HgCl.

Chromatographic analysis

Authentic di(phenylethynyl)mercury **1** and methyl(phenylethynyl)mercury **2** samples were prepared and their HPLC and UV properties examined. Peaks of **1** (retention time $t_R = 18.3$ min) and **2** ($t_R = 6.9$ min) are completely separated and symmetric using a C-18 reversed-phase column and eluting with acetonitrile–water (45:55) for 10 min, then linearly changing to acetonitrile–water (60:40) within 12 min. Detection was set at 250 nm (λ_{max} of **2**) for 10 min, then shifted to 265 nm (λ_{max} of **1**). The eluent flow was 1 ml min^{-1}.

Calibration graphs, based on peak areas, are linear over 0.05–100 mg l^{-1} for **1** with a correlation coefficient of 0.99981, and over 0.1–50 mg l^{-1} for **2** with a correlation coefficient of 0.99997. Detection limits, expressed as the absolute amount of analyte injected giving a signal three times higher than the signal noise, were found to

be 0.2 ng for **1** and 0.5 ng for **2**, and they reflect the different values of the extinction coefficients (see the Experimental section). Absorptions are centered in the 250–270 nm region and correspond to ligand-centered π–π^* transitions, as shown by the value of ε(**1**) which is twice as high as ε(**2**).

Figure 1 shows a typical chromatogram referring to the derivatization of a solution of $HgCl_2$ and CH_3HgCl (20 $\mu g\,l^{-1}$ of each). The results obtained in the analysis of solutions at various concentrations are reported in Table 1.

Effect of typical interfering agents

Here we present the preliminary results on possible interfering species in this method of mercury speciation. At first we studied the effect of the presence of copper(II), zinc(II), cadmium(II) and lead(II) at concentration of 20 $mg\,l^{-1}$ and we observed no effect on mercury determination in solutions containing 20 and 2 $\mu g\,l^{-1}$ of $HgCl_2$ and CH_3HgCl (Table 2). On the other hand, partial inferference was displayed by humic acids and cysteine (Table 3).

Analysis of seawater samples spiked with $HgCl_2$ and CH_3HgCl

When seawater samples (50 ml) collected in the Adriatic sea (Marina di Ravenna) and spiked with $HgCl_2$ and CH_3HgCl (1 μg for each species), are analysed by the typical procedure reported in the Experimental section, with the only difference being that the addition of NaCl was omitted, results are quite disappointing, recoveries of Hg species being less than 25%. We believe that colloidal alkaline-earth hydroxides formed in the required alkaline conditions and/or natural organic complexing agents could affect the derivatization and/or the extraction step. As concerns the derivatization procedure, we replaced NaOH with ammonia (we added 12 ml of 1 M NH_3/NH_4Cl buffer at pH 9.5 to 50 ml of the seawater sample), so avoiding formation of precipitates, and we added $CuCl_2 \cdot 2H_2O$ (0.20 g) as a

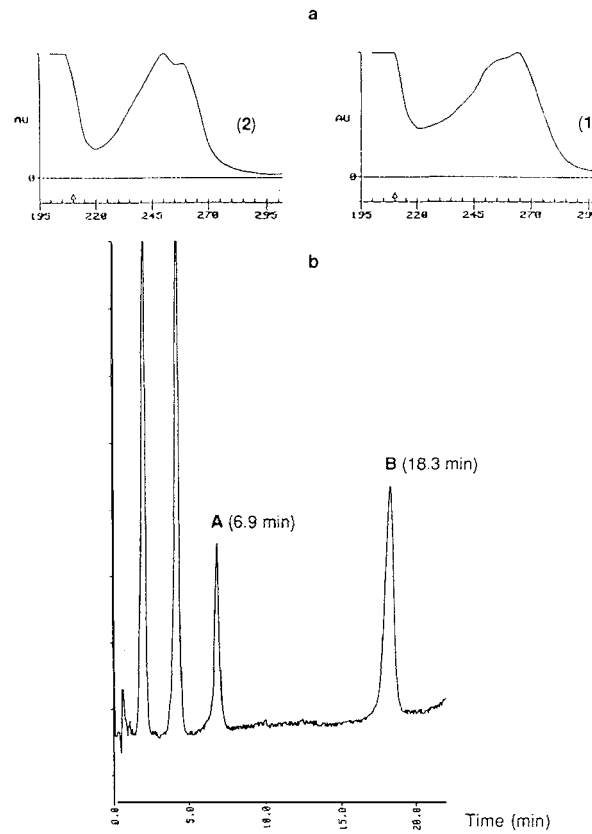


Figure 1 (a) Diode array spectra for di(phenylethynyl)mercury (**1**) and methyl(phenylethynyl)mercury (**2**) corresponding to chromatographic peaks B (t_R = 18.3 min) and A (t_R = 6.9 min), respectively. (b) UV absorption chromatogram showing **1** and **2** obtained after derivatization of a mixture of $HgCl_2$ (20 $\mu g\,l^{-1}$) and CH_3HgCl (20 $\mu g\,l^{-1}$).

Table 1 Analysis of $HgCl_2$ and CH_3HgCl in deionized water[a]

Run	Concn (μg Hg l^{-1})	Found ($\mu g\,l^{-1}$)[b]	Recovery (%)
1	Hg(II): 200	182 ± 13	91
	CH_3Hg(II): 200	162 ± 12	81
2	Hg(II): 20	17.1 ± 1.6	86
	CH_3Hg(II): 20	17.0 ± 1.7	85
3[c]	Hg(II): 2	1.61 ± 0.18	81
	CH_3Hg(II): 2	1.54 ± 0.17	77

[a] Working solution, 50 ml; [NaOH] = 1 M; [NaCl] = 0.3 M.
[b] Mean values ± standard deviations; n = 4.
[c] Working solution, 200 ml.

Table 2 Analysis of $HgCl_2$ and CH_3HgCl in deionized water containing Cu(II), Zn(II), Cd(II) and Pb(II)[a]

Run	Concn ($\mu g\ Hg\ l^{-1}$)	Found ($\mu g\ l^{-1}$)[b]	Recovery (%)
1[c]	Hg(II): 20	17.0 ± 1.5	85
	CH_3Hg(II): 20	16.8 ± 1.6	84
2[d]	Hg(II): 2	1.56 ± 0.19	78
	CH_3Hg(II): 2	1.50 ± 0.17	75

[a] Concentration of each metal, $20\ mg\ l^{-1}$; [NaOH] = 1 M; [NaCl] = 0.3 M.
[b] Mean values ± standard deviations, $n = 3$.
[c] Working solution, 50 ml.
[d] Working solution, 200 ml.

promoter of mercury release from the binding sites of possible complexing agents. After the normal work-up (hexane extraction, solid-phase extraction of **1** and **2** on silica and elution with acetonitrile) we obtained in nine replicate analyses the following results: $HgCl_2$ $14.0\ \mu g\ l^{-1}$ ± 17% (expected $20\ \mu g\ l^{-1}$, recovery 70%); CH_3HgCl $12.1\ \mu g\ l^{-1}$ ± 12% (expected $20\ \mu g\ l^{-1}$, recovery 60%). To control the efficiency of the extraction with hexane, we re-extracted the aqueous layer with 3 ml pentane–CH_2Cl_2 (2:1, v/v), the organic phase was now too polar to carry out the solid-phase extraction step and so solvents were removed under reduced pressure at room temperature and the residue was diluted with acetonitrile and analysed by HPLC. No traces of **2** were observed, while variable amounts of **1** were present, and, in particular higher amounts of inorganic mercury were detected when the usual work-up gave the lowest recoveries. In this way the final overall recovery of inorganic mercury was 83% (expected $20\ \mu g\ l^{-1}$, found $16.7\ \mu g\ l^{-1}$ ± 11%) with a significant reduction of the standard deviation. Even though the procedure can be further improved, we wish to emphasize that these preliminary results obtained in seawater are similar to those obtained in deionized water (see Table 1).

Table 3 Analysis of $HgCl_2$ ($20\ \mu g\ l^{-1}$) and CH_3HgCl ($20\ \mu g\ l^{-1}$) in deionized water containing humic acids or cysteine[a]

Run	Interfering agent ($mg\ l^{-1}$)	Found ($\mu g\ l^{-1}$)[b]	Recovery (%)
1	Humic acid (10)	Hg(II): 14.0 ± 1.3	70
		CH_3Hg(II): 14.6 ± 1.5	73
2	Cysteine (0.02)	Hg(II): 15.3 ± 1.2	77
		CH_3Hg(II): 16.2 ± 2.1	81
3	Cysteine (0.2)	Hg(II): 14.0 ± 1.2	70
		CH_3Hg(II): 13.4 ± 1.0	67
4	Cysteine (2)	Hg(II): 8.7 ± 1.5	44
		CH_3Hg(II): 11.5 ± 2.0	58

[a] Working solution, 50 ml; [NaOH] = 1 M; [NaCl] = 0.3 M.
[b] Mean values ± standard deviations, $n = 3$.

CONCLUSIONS

A simple and inexpensive procedure for the speciation of mercury in water is proposed. It is based on the classical reactivity of mercury(II) towards 1-alkynes which is currently exploited in industrial chemistry for the synthesis of carbonyl compounds (e.g. synthesis of acetaldehyde from acetylene), but which so far, to our knowledge, has never been applied for analytical purposes. The rate constants of the reactions of Hg(II) and CH_3Hg species in water with phenylacetylene are exceptionally high since the process takes place in 40 min at the ppb (10^{-9}) level under heterogeneous conditions.

The results obtained with working solutions in deionized water containing tyical interfering agents, as well as the results obtained using seawater samples under slightly modified conditions, are promising.

A limitation is the concentration detection limit of the overall procedure (0.1 μg per litre of sample) which makes our procedure useless for samples of open seawater,[8] where natural levels of mercury lie around $0.01–0.03\ \mu g\ l^{-1}$. Improvements are under study; they involve the use of particular alkynes so as to exploit different detection techniques and element-specific methods such as AA or AF spectrometry coupled with gas chromatography or HPLC.

Moreover, in order to evaluate properly the scope of our procedure, our efforts are now focused on the application of this methodology to biological and environmental samples such as tissues and sediments collected in a severely contaminated area such as the Ravenna harbour and connected wetlands.[9]

EXPERIMENTAL

Equipment

The HPLC system was a Perkin–Elmer instrument consisting of a model 250 binary pump, a 235C diode array UV detector, and a PE Nelson

1020 Personal Integrator. A reversed-phase column (Hypersil ODS, 150 mm × 0.46 mm i.d.: 5 μm-diameter particles) was used. Samples were loaded on to a Rheodyne 7125 NS model with a 20 μL loop.

Chemicals

Deionized water was used for the preparation of all the solutions. Mercury chloride was purchased from Aldrich and methylmercury chloride from Johnson–Matthey GmbH. Stock solutions of mercury chloride (1000 mg l^{-1}) were prepared by dissolving 0.1354 g of $HgCl_2$ in about 70 ml water containing 1.5 ml HNO_3, and diluted to 100 ml with water. Stock methylmercury solutions (100 mg l^{-1}) were prepared by dissolving 0.0251 g of CH_3HgCl in 3 ml of methanol and diluting to 200 ml with water. More dilute working standard solutions were freshly prepared by the appropriate dilution with water. Standard solutions of copper(II), zinc(II), cadmium(II) and lead(II) (1000 mg l^{-1} of each) were purchased from BDH. Humic acids (C 50.8%, ash 1.7%) were extracted from a sample of soil.[10]

Phenylacetylene

Phenylacetylene (Janssen, 96%) was used after filtration on a short-path column of silica gel for concentrations of mercury ≥ 20 μg l^{-1}. For more dilute solutions, phenylacetylene has to be 99% pure since the main impurity, styrene, affects the HPLC analysis of **2**. To this purpose we modified a previous general synthesis of terminal alkynes[11] as follows.

To a solution of CBr_4 (7.96 g, 24 mmol) in CH_2Cl_2 (15 ml) cooled at 0 °C is slowly added triphenylphosphine (12.59 g, 48 mmol) under efficient stirring. After 10 min benzaldehyde (2.0 ml, 20 mmol) is added dropwise and the reaction mixture is stirred for 20 min at 0 °C. Cyclohexane (100 ml) is added, the solid residue is filtered off over Celite and 1,1-dibromo-2-phenylethene (4.79 g, 91%) is purified by flash-chromatography ion silica gel eluting with cyclohexane. MS: *m/z* (%) 262 (23, M^+), 264 (46, M^+), 266 (23, M^+), 181 (34), 102 (100), 71 (42).

To a solution of 1,1-dibromo-2-phenylethene (4.71 g, 18 mmol) in anhydrous tetrahydrofuran (THF) (20 ml) cooled at −78 °C is slowly added t-BuOK (2.02 g, 18 mmol). The reaction mixture is stirred at −78 °C. After 1 h GC–MS analysis revealed 95% conversion of 1,1-dibromo-2-phenylethene into 1-bromo-2-phenylacetylene. MS: *m/z* (%) 180 (100, M^+), 182 (100, M^+), 101 (51), 75 (33), 61 (7).

To the same solution 2.5 *M butyllithium in* hexane (7.2 ml, 18 mmol) is added at −78 °C, the reaction mixture is stirred for 1 h at −78 °C and quenched with phosphate buffer (pH 7, 5 ml). Extraction with ether and distillation afford phenylacetylene (1.61 g, 74%) which was found to be 99% pure by HPLC and GC analysis.

Di(phenylethynyl)mercury (1)

The following modified literature procedure[12] was adopted to synthesize a sample of **1**. To a solution of $HgCl_2$ (0.27 g, 1 mmol) and NaCl (1.46 g, 25 mmol) in water (90 ml) are added 2 M NaOH (10 ml) and a solution of phenylacetylene (0.25 g, 2.5 mmol) in CH_2Cl_2 (2 ml). The heterogeneous reaction mixture is efficiently stirred for 1 h at room temperature. After extraction with CH_2Cl_2 (2 × 5 ml), the collected organic phases are dried over Na_2SO_4 and **1** is purified by flash chromatography on silica gel using cyclohexane–ether (99:1) (0.30 g, 74% yield), m.p. 124–125 °C (ethanol), lit.[12] m.p. 124–125 °C. R_f 0.45 (cyclohexane–ethyl acetate, 99:1). UV (acetonitrile): $\varepsilon = 47\,800$ at $\lambda_{max} = 264$ nm. IR (KBr): 2080 cm^{-1}. ^1H NMR (300 MHz): δ ($CDCl_3$) 7.31–7.33 (m,6H), 7.48–7.50 (m, 4H). ^{13}C NMR (75 MHz): δ ($CDCl_3$) 106.0 (C—Hg), 120.6 (C), 122.3 (C), 128.3 (CH), 128.6 (CH), 132.3 (CH). MS: *m/z* (%) 404 (24, M^+ relative to ^{202}Hg), 202 (100, $[PhCC]_2$), 176 (6), 161 (2), 150 (7), 102 (46), 88 (9), 75 (35). Analysis: Found: C, 47.6, H 2.6; Calcd: C, 47.7, H 2.5%.

Methyl(phenylethynyl)mercury (2)

To a solution of CH_3HgCl (0.50 g, 2 mmol) and NaCl (1.46 g, 25 mmol) in water (90 ml) are added 2 M NaOH (10 ml) and a solution of phenylacetylene (0.25 g, 2.5 mmol) in CH_2Cl_2 (2 ml). The heterogeneous reaction mixture is efficiently stirred for 1 h at room temperature. After extraction with CH_2Cl_2 (2 × 5 ml), the collected organic phases are dried over Na_2SO_4 and **2** is purified by flash chromatography on silica gel using cyclohexane–ether (98:2) (0.40 g, 63% yield), m.p. 46–47 °C (benzene), lit.[13] m.p. 43–44 °C. R_f 0.47 (cyclohexane). UV (acetonitrile): $\varepsilon = 22\,500$ at $\lambda_{max} = 250$ nm. IR (KBr): 2140 cm^{-1}. ^1H NMR (300 MHz): δ ($CDCl_3$) 0.70 (s, 3H), 0.70 (d, $J = 147.9$ Hz, 3H relative to ^{199}Hg), 7.3 (m, 3H), 7.5 (m, 2H). ^{13}C NMR (75 MHz): δ ($CDCl_3$) 7.0 (CH_3), 105.6 (C—Hg), 123.2 (C),

127.9 (CH), 128.2 (CH), 132.1 (CH), 142.8 (C). MS: *m/z* (%) 318 (34, M^+ relative to ^{202}Hg), 303 (28), 217 (5), 202 (9), 115 (16), 101 (100), 75 (60), 63 (5). Analysis: Found: C 34.2, H 2.4; Calcd: C 34.1, H 2.55%.

Stock solutions of **1** and **2** (100–200 mg l^{-1}) in acetonitrile were stored at 4 °C for at least four months without appreciable decomposition according to HPLC analysis. More dilute solutions of **1** and **2** used for calibration and for recording UV spectra were freshly prepared by the appropriate dilution of stock solutions.

Analysis of $HgCl_2$ (20 μg l^{-1}) and CH_3HgCl (20 μg l^{-1}) in deionized water: typical procedure

To the working solution prepared by pouring $HgCl_2$ (1 ml of 1 mg l^{-1} standard solution) and CH_3HgCl (1 ml of 1 mg l^{-1} standard solution) in deionized water (48 ml) are added in turn NaCl (0.89 g), NaOH (2 g) and phenylacetylene (50 μl). The reaction mixture is vigorously stirred at 20 °C for 45 min and extracted with n-hexane (2 × 5 ml). Solid-phase extraction of **1** and **2** on silica gel is performed by passing the collected organic phases through a short-path column fitted with silica (0.5 g, Merck, 230–400 mesh); silica is then rinsed with pentane (10 ml) to wash away most of the excess phenylacetylene. Organomercury derivatives **1** and **2** are extracted by eluting with acetonitrile (2 ml) and analyzed directly by HPLC. The overall chromatographic processing of **1** to **2** must be carried out as quickly as possible using a positive nitrogen pressure to avoid drying the silica at any time.

For the interference studies, the foreign metal is added to the solution of mercury derivatives before the addition of NaCl and NaOH. In the case of humic acids and cysteine, the interfering species are pre-equilibrated with $HgCl_2$ and CH_3HgCl for 1 h with stirring before carrying out the derivatization process.

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