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Simultaneous determination of inorganic mercury and methylmercury compounds in natural waters

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Abstract The purpose of the present work was to develop a simple, rapid, sensitive and accurate method for the simultaneous determination of inorganic mercury (Hg^{2+}) and monomethylmercury compounds (MeHg) in natural water samples at the pg L^{-1} level. The method is based on the simultaneous extraction of MeHg and Hg^{2+} dithizonates into an organic solvent (toluene) after acidification of about 300 mL of a water sample, followed by back extraction into an aqueous solution of Na_2S, removal of H_2S by purging with N_2, subsequent ethylation with sodium tetraethylborate, room temperature precollection on Tenax, isothermal gas chromatographic separation (GC), pyrolysis and cold vapour atomic fluorescence spectrometric detection (CV AFS) of mercury. The limit of detection calculated on the basis of three times the standard deviation of the blank was about 0.006 ng L^{-1} for MeHg and 0.06 ng L^{-1} for Hg^{2+} when 300 mL of water was analysed. The repeatability of the results was about 5% for MeHg and 10% for Hg^{2+}. Recoveries were 90–110% for both species.

Keywords Mercury · Methylmercury · Speciation · Water

Introduction

Mercury is one of the most hazardous contaminants that may be present in the aquatic environment. It exists in a large number of different chemical and physical forms with a wide range of properties, and its ecological and toxicological effects are strongly dependent on the chemical form present. Inorganic mercury species may be transformed by biotic and/or abiotic processes to much more toxic organic, methylated forms, such as methylmercury. The accumulation of methylmercury in biota and its biomagnification in aquatic food chains are of particular concern due to its extreme toxicity and its ability to bioaccumulate in fish tissues [1, 2].

For toxicological and biogeochemical studies the speciation of mercury is very important. The total concentration of the element is of little value without knowledge of its chemical forms. Owing to the importance of speciation information much effort has been made in recent decades in the development of analytical techniques for different forms of mercury.

Due to the very low concentration levels of mercury and its organic compounds in water samples, accurate analyses are still a major problem. But in the studies mentioned above the availability of accurate, sensitive and precise analytical methods for the determination of total mercury and its compounds at picogram levels in natural water samples is of crucial importance.

Analytical techniques mostly employed for Hg-T determination in natural waters at picogram levels are based on CV AAS, inductively coupled plasma mass spectrometry (ICP-MS), plasma atomic emission spectrometry (AES) and CV AFS detection, after decomposition of all mercury species into Hg^{2+}. After a digestion step reduction of the sample with $SnCl_2$ or $NaBH_4$ is usually employed [1].

For speciation analysis a succession of analytical stages is required. The main steps to speciate mercury, particularly inorganic and methylmercury are extraction, preconcentration, separation and specific detection. Liquid-liquid, gas-liquid and solid-phase extraction are employed for preconcentration. Coupled techniques including separation by gas chromatography (packed, capillary or multicapillary) or liquid chromatography and detection by an electron capture detector (ECD), AAS, AFS, ICP-AES, microwave induced plasma atomic emission spectrometry (MIP-AES) or ICP MS are used [1, 3, 4, 5]. Before gas chromatographic separation the derivatization of Hg species

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into volatile derivates is usually employed. The derivatization can be carried out by using a Grignard reagent [6], by hydride generation with $NaBH_4$ [7] or by the most often used ethylation with sodium tetraethylborate ($NaBEt_4$) [8, 9]. An alternative is propylation with $NaBPr_4$, which seems to be free from some interferences during derivatization [10].

The aim of this work was to investigate the analytical potential of simultaneous extraction of MeHg and Hg^{2+} dithizonates to achieve a rapid, sensitive, reliable and accurate method for the simultaneous determination of MeHg and Hg^{2+} in natural waters, in which the sum of MeHg and Hg^{2+} gives the total mercury content. The method was initially developed by Akagi and Nishimura [11] for the separate determination of Hg-T and MeHg in water samples. The method has proven to be suitable and comparable with other methods [12] but it requires larger volumes of water samples (up to 6 L) and is therefore impractical for routine work. The newly developed method differs in the second part of the analytical procedure, that is in the stage of measurement and detection, while the isolation step stays unchanged. After the formation of Hg dithizonathes, instead of acid digestion, $SnCl_2$ reduction and CV AAS detection for Hg-T and GC ECD detection for MeHg, ethylation, room temperature precollection, isotermal GC and CVAFS detection for simultaneous determination of MeHg and Hg^{2+} was employed. The accuracy of MeHg and indirectly Hg-T measurements was checked by comparison with the results obtained by different, independent analytical techniques for MeHg and Hg-T.

Experimental

Reagents

Sodium tetraethylborate solution (1% *w/v*) was prepared from $NaBEt_4$ (Strem Chemicals, Newburyport MA, USA) in Milli-Q water containing 1% (*w/v*) KOH (analytical grade, Merck, Darmstadt, Germany). 2 M potassium acetate buffer was prepared from potassium acetate (extra pure, Merck) and acetic acid (Suprapur, Merck) in Milli-Q water. HCl (1 M) solution was prepared from HCl (30%, Suprapur, Merck) in Milli-Q water. $SnCl_2$ solution (5% *w/v*) was prepared from $SnCl_2 \cdot 2\,H_2O$ (analytical grade, Merck) in Milli-Q water containing 3 M H_2SO_4 (Suprapur, Merck). H_2SO_4 solution (10 M) was prepared from H_2SO_4 (Suprapur, Merck) in Milli-Q water. BrCl solution was prepared by dissolving 1.1 g $KBrO_3$ (analytical grade, Merck) and 1.5 g KBr (analytical grade, Merck) in 20 mL Milli-Q water and 80 mL conc. HCl. $KMnO_4$ solution (0.5% *w/v*) was prepared from $KMnO_4$ (analytical grade, max. 0.00005% Hg, Merck) in Milli-Q water. CH_2Cl_2 (SupraSolv) was obtained from Merck. NaOH solution (10 M) was prepared from NaOH (extra pure, Merck) in Milli-Q water. $NH_2OH{\cdot}HCl$ solution (10% *w/v*) was prepared from $NH_2OH{\cdot}HCl$ (analytical grade, Merck) in Milli-Q water. EDTA solution (10% *w/v*) was prepared from EDTA(4 Na)·2 H_2O (analytical grade, Merck) in Milli-Q water. Dithizone solution (0.01% *w/v*) was prepared from dithizon (analytical grade, Merck) in toluene (analytical grade, Merck). Na_2S solution (5 μg mL^{-1}) was prepared daily from Na_2S (analytical grade, Merck) in 0.1 M NaOH and ethanol (min. 99.5% solvent for scintillation grade) in a (1:1) ratio. Tenax (polymer based on 2,6-diphenyl-*p*-phenylene oxide, 20/35 mesh) was purchased from Alltech, Deerfield IL, USA. OV-3 15% was obtained from Sigma-Aldrich (Deisenhofen, Germany). Milli-Q deionised water (>18 MΩcm, Millipore, Bedford MA, USA).

Standard solutions

Standard stock solutions of Hg^{2+} and MeHg were prepared by dissolving $HgCl_2$ (Merck) in 0.1 M HCl and CH_3HgCl (Merck) in isopropanol, respectively. Working standard solutions were prepared by appropriate dilution of standard stock solutions in Milli-Q water. We used 1 ng mL^{-1} working standard solutions for MeHg (as Hg) and Hg^{2+} for calibration of the CVAFS system, and a 10 ng mL^{-1} Hg^{2+} working standard solution for CVAAS measurements. These were prepared daily and stored in the dark at 4 °C.

Cleaning procedure

Due to the very low concentration of Hg-T and MeHg in natural water it is essential to avoid any possible contamination prior to and during sample processing. One of the most common sources of contamination is laboratory ware. Extreme precautions must be taken in cleaning procedures. All glass and Teflon materials need to be cleaned very carefully. All vessels were left to soak in a soap (Micro-90, Bioblock, Illkirch Cedex, France) solution overnight. They were thoroughly rinsed first with tap water then with Milli-Q water. The vessels were placed in 50% (*v/v*) concentrated HNO_3 solution and heated at 60 °C for 2 days. After being thoroughly rinsed with Milli-Q water, vials were transferred to 10% (*v/v*) concentrated HCl solution and left to soak for a further day at room temperature. They were thoroughly rinsed again with Milli-Q water, filled with 1% HCl and stored in polyethylene plastic bags. Vials were emptied just before use for sample processing.

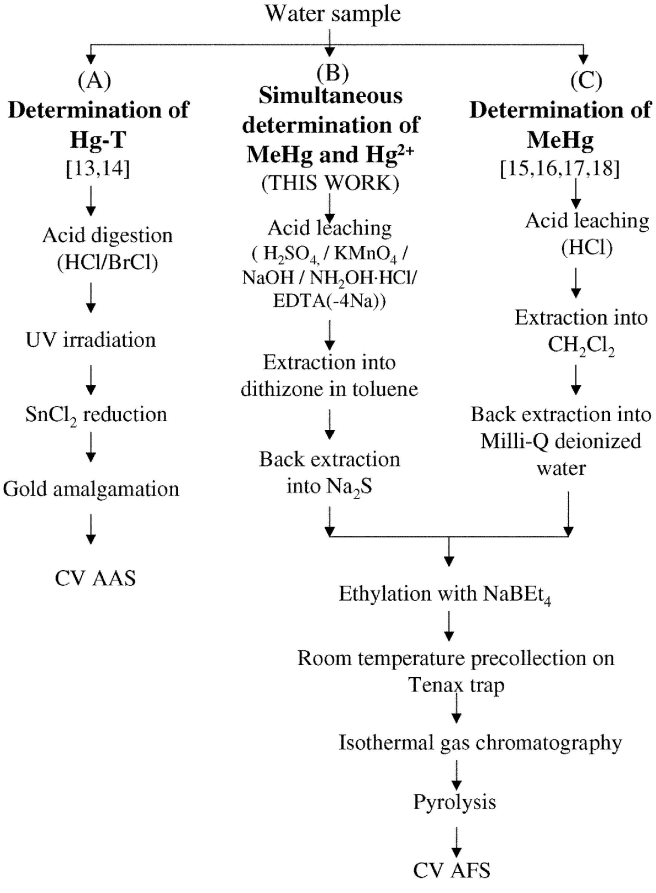

Fig. 1 Analytical procedures for the determination of Hg-T and MeHg in water samples

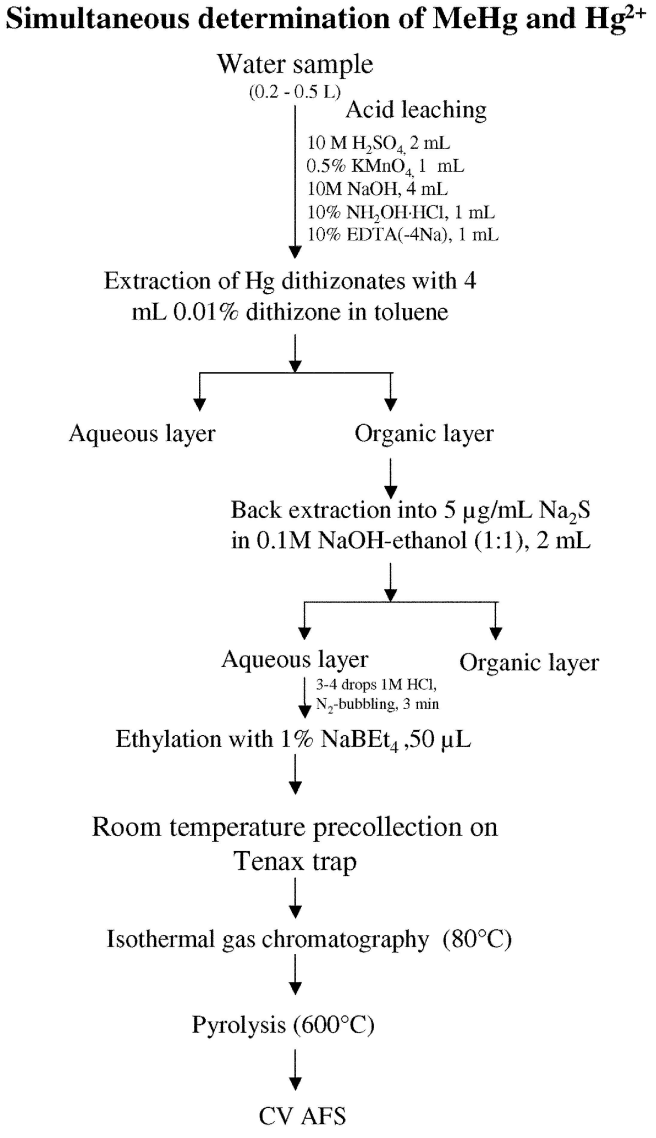

Fig. 2 Detailed schematic presentation of the method used for the simultaneous determination of MeHg and Hg^{2+} in water samples

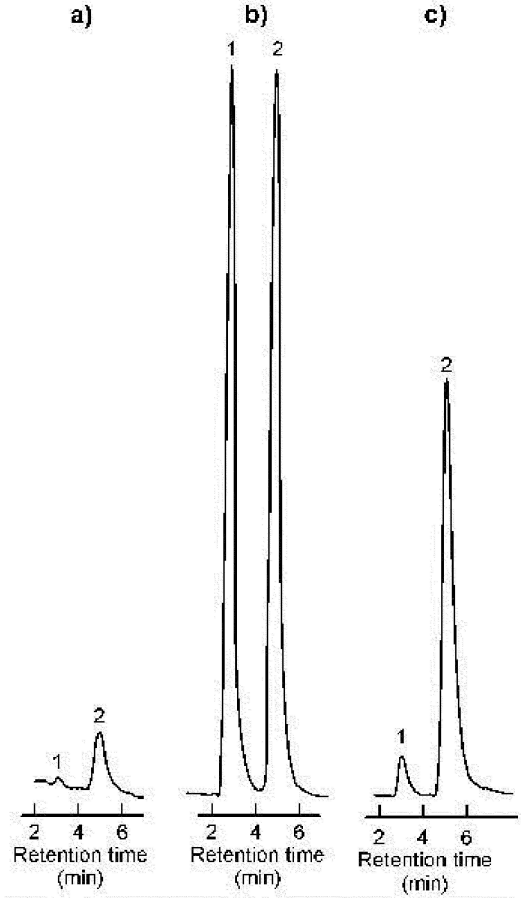

Fig. 3a–c Typical chromatograms obtained in the simultaneous determination of MeHg and Hg^{2+} in water. **a** Reagent blank, **b** standards: 100 pg MeHg (as Hg) and 100 pg of Hg^{2+}, **c** sample: 4.1 pg MeHg (as Hg) and 53.6 pg of Hg^{2+}. Peaks: *1* $CH_3HgCH_2CH_3$, *2* $(CH_3CH_2)_2Hg$

Analytical methods

Various approaches for determination of Hg-T and MeHg were used as presented in Fig. 1. Some of the methods (A and C) have been previously optimised [13, 14, 15, 16, 17, 18] and they were used as control methods to compare the results obtained by the newly developed method for simultaneous determination of MeHg and Hg^{2+} (B) in water samples. A detailed schematic presentation of the method for the simultaneous determination of MeHg and Hg^{2+} is presented in Fig. 2.

Simultaneous determination of MeHg and Hg^{2+}

Approximately 300 mL of water sample were placed into a carefully precleaned 1 L glass separating funnel. A 2 mL aliquot of 10 M H_2SO_4 and 1 mL of 0.5% $KMnO_4$ solution were added to the sample, gently mixed and allowed to stand for 5 min. A 4 mL portion of 10 M NaOH and 1 mL of 10% $NH_2OH{\cdot}HCl$ solution were then added to the treated sample and allowed to stand for 20 min. After addition of 1 mL of 10% EDTA solution, the simultaneous extraction of Hg^{2+} and CH_3Hg^+ dithizonates with 4 mL of purified 0.01% dithizone in toluene was performed. The sample was shaken vigorously for 8 min and left at least 1 h in the dark until the organic and aqueous phases were separated. The aqueous layer was discarded. A 3 mL extract of the toluene layer was transferred to a 10 mL glass vial and washed twice with 1 mL of 1 M NaOH. A 2 mL aliquot of 5µg mL^{-1} Na_2S aqueous solution was added to the vial and the sample was shaken for 15 min. After this step, the sample was centrifuged for 5 min at 1200 rpm and the toluene layer was discarded. The aqueous Na_2S phase was washed with 1 mL of toluene and acidified with three drops of 1 M HCl. The excess of sodium sulfide was removed as H_2S by purging the sample with N_2 at a flow rate of 50 mL min^{-1} for 3 min. An aliquot of diluted sample was transferred to a Teflon reaction vessel, buffered to pH 4.9 with 200 µL of acetate buffer as required for the ethylation process and 50 µL of 1% of $NaBEt_4$ solution was added. The vessel was immediately closed, and the mixture allowed to react without bubbling for 15 min. Ethylated MeHg as ethylmethylmercury and Hg^{2+} as diethylmercury was purged onto a Tenax trap for 15 min with Hg-free nitrogen and thermally desorbed (200 °C) onto an isothermal GC column at 80 °C. Under a flow of argon the eluted Hg species were converted into Hg^0 by pyrolytic decomposition at 600 °C and then detected by a cold vapour atomic fluorescence detector (Brooks Rand Ltd., Model 2, USA).

In Fig. 3 some typical chromatograms obtained in the simultaneous determination of MeHg and Hg^{2+} in water are presented, in which the first peak at 3 min corresponds to MeHg (as ethylmethylmercury) and the second peak at 5 min belongs to Hg^{2+} (as diethylmercury).

Determination of Hg-T

Approximately 200 mL of water sample was placed in a precleaned 250 mL Teflon bottle. The sample was acidified with 1 mL of concentrated HCl. The oxidation of all organic forms of mercury into inorganic mercury was achieved by adding 2 mL of BrCl solution and by additional exposure of the sample to UV irradiation for 3 h (300 W UV lamp). Just before measurement the sample was prereduced with 125 µL of 10% $NH_2OH{\cdot}HCl$ solution to remove the excess bromine, which might otherwise cause problems during analysis. An aliquot (50–200 mL) of the digested sample was added to the reduction vessel containing 10 mL 5% $SnCl_2$ solution. After reduction of Hg^{2+} to elemental mercury with $SnCl_2$, Hg^0 was swept from the solution by aeration with N_2 and concentrated on a gold trap. The collected Hg was thermally desorbed from the gold trap by heating the trap to 600 °C and measured on a Mercury Monitor™, LDC/Milton Roy, USA instrument by cold vapour atomic absorption spectrophotometry. The limit of detection calculated on the basis of three times the standard deviation of blanks was 0.1 ng L^{-1}. Recoveries were quantitative and no recovery corrections were necessary. The repeatability and reproducibility of the method was 5 and 10%, respectively [13, 14].

Determination of MeHg

Approximately 70 mL of water sample was put in a precleaned 125 mL Teflon bottle. Concentrated HCl (5 mL) and 30 mL of CH_2Cl_2 were added to each bottle and the samples were shaken overnight. The upper aqueous layer was discarded. Milli-Q water (40 mL) was added to the remaining organic layer. The bottle was placed in a water bath at about 80 °C to evaporate the CH_2Cl_2. Once the CH_2Cl_2 was visibly evaporated, the sample was purged for 5 min with Hg-free nitrogen to quantitatively remove the CH_2Cl_2. The ethylation and measurement procedure described above for the simultaneous determination of MeHg and Hg^{2+} followed. The limit of detection calculated on the basis of three times the standard deviation of blanks was about 0.01 ng L^{-1} when 70 mL of water sample was analysed. Recoveries for MeHg in water samples were about 75–85%. In each batch of samples analysed the recovery was checked by spiking about 10% of samples with a known amount of MeHg in aqueous solution. All results were then corrected for the recovery factor. The repeatability of the procedure was 5% and reproducibility was 7% [15, 16, 17, 18].

Results and discussion

The performance of the method for the simultaneous determination of MeHg and Hg^{2+} was tested on different types of natural water: seawater, fresh water, fresh well and rain water. Unfiltered and filtered water were examined. Filtration of water samples was performed immediately after sampling through ~0.75 µm Whatman GF/C glass filter.

A comparison of MeHg and Hg-T results for unfiltered and filtered water samples is presented in Table 1. The results for MeHg obtained by solvent extraction and by simultaneous determination of MeHg and Hg^{2+} show excellent agreement for all examined unfiltered and filtered water samples.

For Hg-T good agreement between CV AAS acid digestion and simultaneous determination of MeHg and Hg^{2+} for all examined unfiltered water was obtained, except for fresh well water. The concentration of Hg-T found in unfiltered fresh well water was much lower for the newly developed method, in which the sum of MeHg and Hg^{2+} corresponds to the Hg-T value. The comparison of results for Hg-T in filtered water shows excellent agreement for seawater, but again lower results for fresh well water by simultaneous determination were observed. Slightly lower Hg-T values for other analysed filtered waters were also observed. Data with significant deviation are highlighted in Table 1. Evidently, in these cases the newly developed method yields lower recoveries for Hg^{2+}, which could be either due to insufficient extraction of Hg^{2+} and/or degradation or loses and/or the presence of volatile Hg species.

To find out the reason for the disagreement in Hg-T, additional experiments were performed. Various fresh well waters were examined, since the largest disagreement was obtained for this kind of samples. Fresh well waters were sampled at various locations in Slovenia (Karavanke, Alps, Gorjanci).

Firstly, the influence of potentially present volatile Hg species was checked. Immediately after sampling, 0.5 L of unfiltered water sample was put into a glass bubbler and volatile mercury species were purged onto gold-coated sand for 10 min with Hg-free argon. After collection, the gold trap was immediately transferred to a double amalgamation CV AFS analyzer system for quantification. The analytical procedure used has been discussed in detail elsewhere [19]. The results for dissolved gaseous mercury obtained for all samples analysed were below the LOD (<5 pg L^{-1}).

Additionally, to improve the recoveries for Hg^{2+} the first step of the analytical procedure was investigated. As

Table 1 Comparison of results for unfiltered and filtered water samples obtained by different methods. Results are given in ng L^{-1}

Sample		Simultaneous determination of MeHg and Hg^{2+}			Acid digestion CV AAS	Solvent extraction CV AFS
		MeHg	Hg^{2+}	Hg-T[a]	Hg-T	MeHg
Unfiltered	Seawater	0.130; 0.140	2.1; 1.8	2.23; 1.94	2.4; 2.3	0.125
	Rain	0.071; 0.092	2.0; 1.7	2.07; 1.79	2.0; 2.1	0.065
	Fresh water	0.200; 0.179	1.67; 1.55	1.87; 1.73	1.8; 2.2	0.185
	Well water	0.025; 0.035	13.7; 12.1	13.7[c]; 12.1[c]	33[c]; 35[c]	0.030
	Tap water	< LOD[b]	< LOD	< LOD	< LOD	< LOD
Filtered	Seawater	0.104; 0.095	0.715; 0.923	0.819; 1.01	0.87	0.106
	Rain	0.020; 0.015	0.370; 0.250	0.390[c]; 0.265[c]	0.450[c]	0.025
	Fresh water	0.095; 0.088	0.638; 0.745	0.735[c]; 0.833[c]	1.39[c]	0.080
	Well water	0.025	7.21	7.23[c]	10.5[c]	0.030

[a]Calculated [Hg-T] = [MeHg] + [Hg^{2+}]. [b]LOD limit of detection. [c]Disagreement due to insufficient extraction of Hg^{2+}.

Table 2 Influence of extended time of shaking on MeHg and Hg^{2+}, and Hg-T values in well waters. Results are given in ng L^{-1}

Sample		4 min Simultaneous determination of MeHg and Hg^{2+}			8 min Simultaneous determination of MeHg and Hg^{2+}			Acid digestion CV AAS	Solvent extraction CV AFS
		MeHg	Hg^{2+}	Hg-T[a]	MeHg	Hg^{2+}	Hg-T[a]	Hg-T	MeHg
Unfiltered	I	0.105	10.5	10.6	0.095	15.1	15.2	16.2	0.084
		0.120	11.2	11.3	0.110	16.7	16.8	15.9	
	II	0.095	4.7	5.65	0.090	6.80	6.90	7.05	0.073
		0.085	3.9	4.75	0.075	7.42	7.50	7.22	
	III	0.045	1.85	1.90	0.030	2.20	2.23	2.00	0.052
		0.030	1.97	2.00				2.15	
	IV	0.040	0.77	0.82	0.050	1.01	1.06	0.92	0.031
					0.038	0.85	0.89	0.85	
	V	< LOD[b]	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
	Seawater	0.080	2.02	2.10	0.095	1.96	2.05	1.85	0.092
					0.075	1.78	1.86	1.93	
Filtered	I	0.053	12.2	12.3	0.075	13.8	13.9	14.5	0.063
		0.065	13.4	13.5	0.058	15.2	15.3	14.7	
	II	0.035	5.92	5.96	0.040	6.16	6.20	6.80	0.042
		0.050	4.77	4.82	0.030	7.10	7.13	6.55	
	III	0.041	1.69	1.73	0.059	1.82	1.88	1.79	0.050
								1.69	
	IV	–[c]	–	–	0.035	0.79	0.84	0.69	0.025
		–	–	–	0.020	0.63	0.65	0.78	
	Seawater	0.045	0.48	0.52	0.054	0.57	0.62	0.55	0.065
		0.055	0.67	0.72	0.068	0.48	0.55	0.50	

[a]Calculated [Hg-T] = [MeHg] + [Hg^{2+}]. [b]LOD limit of detection. [c]– sample lost.

Table 3 Influence of increased amount of dithizone in toluene on MeHg in Hg^{2+} and Hg-T values in well waters. Results are given in ng L^{-1}

Sample	4 mL Simultaneous determination of MeHg and Hg^{2+}			6 mL Simultaneous determination of MeHg and Hg^{2+}			Acid digestion CV AAS	Solvent extraction CV AFS
	MeHg	Hg^{2+}	Hg-T[a]	MeHg	Hg^{2+}	Hg-T[a]	Hg-T	MeHg
I	0.105	10.5	10.6	0.095	12.4	12.5	16.2	0.084
	0.120	11.2	11.3	0.067	10.2	10.3	15.9	
II	0.095	4.7	5.65	0.100	5.20	5.30	7.05	0.073
	0.085	3.9	4.75	0.082	6.14	6.22	7.22	

[a]Calculated [Hg-T] = [MeHg] + [Hg^{2+}].

was reported by Akagi and Nishimura [11], appropriate pretreatment of water samples prior to dithizone extraction is necessary. Selection of pretreatment reagents, their concentration and the pretreatment time was found to be of a great importance. Since these factors were already optimised [11], we focused on the stage of formation of Hg dithizonates. The shaking time of the samples in the separating funnel and the amount of dithizone in toluene used for extraction were tested.

Initially, the influence of an extended time of shaking was examined. The results obtained for unfiltered and filtered water are presented in Table 2. It was shown that the initially applied 4 min of shaking gives complete extraction of Hg^{2+} for some examined samples, but not for all. The disagreement for Hg-T values obtained by simultaneous determination and acid digestion is more pronounced for samples with higher Hg-T content. Therefore, we extended the shaking time of the sample in the separating funnel from 4 to 8 min. As evident, the extended time of shaking significantly affected the Hg^{2+} data. An improvement of Hg^{2+} recoveries obtained in the simultaneous determination was shown, but at the same time no decomposition of MeHg was observed.

To check the potential influence of the amount of dithizone used for extraction, 6 mL of 0.01% dithizone in toluene instead of the initially used 4 mL was tested. Due to the good results obtained by an extended time of shaking just described this experiment was performed only on unfiltered water samples with the greatest Hg-T disagreement. The results are given in Table 3. Evidently, increasing the amount of dithizone in toluene did not lead to any improvement of Hg^{2+} recovery. The results obtained are similar to those obtained with 4 min of shaking the samples in separating funnels after addition of 4 mL of dithizone for extraction.

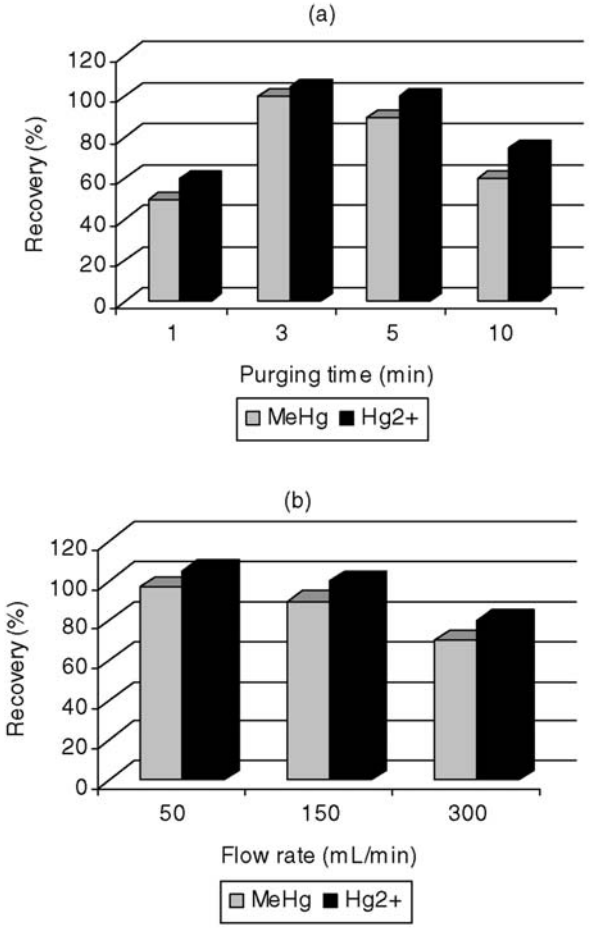

Fig. 4a,b Optimisation of N_2-purging conditions for the simultaneous determination of MeHg and Hg^{2+}. Influence of purging time (**a**) and purging flow rate (**b**)

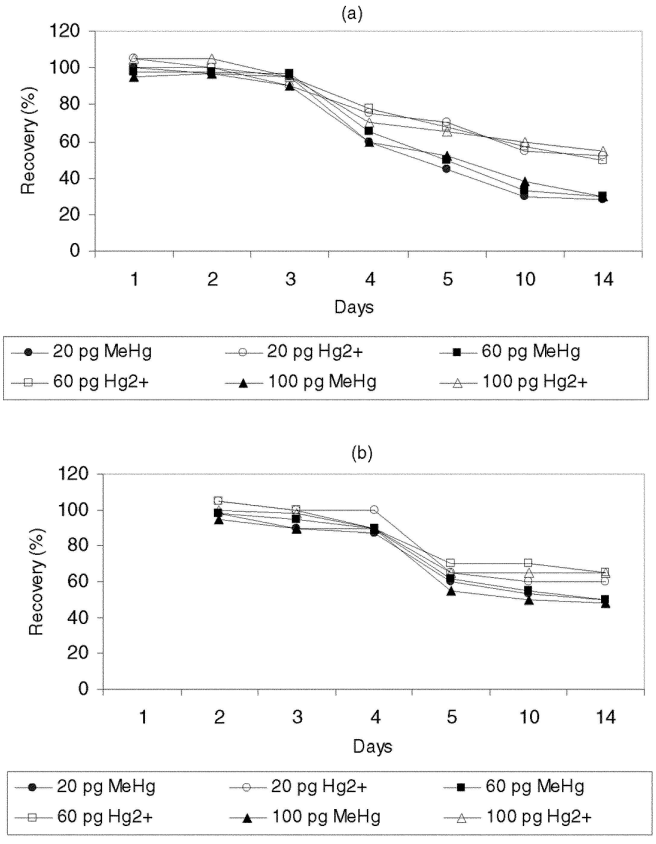

Fig. 5a,b Stability of MeHg and Hg^{2+} in aqueous Na_2S solutions stored at different conditions: refrigerator (**a**) and deep freezer (**b**)

In summary, the comparison of results for MeHg obtained by solvent extraction and by the simultaneous determination of MeHg and Hg^{2+} show excellent agreement regardless of the time of shaking used. But sufficient time of shaking is crucial for quantitative extraction of Hg^{2+} by the method of simultaneous determination. To ensure the complete extraction of Hg^{2+} and indirectly an accurate Hg-T value for various types of water samples, 8 min of vigorous shaking and the use 4 mL of dithizone in toluene is recommended.

The use of this method reduces the time and amount of water sample needed for analysis. Both Hg species are determined from the same aliquot of the sample, so we get the information about MeHg and indirectly Hg-T after one extraction and measurement. About 15 samples can be processed during a day.

One of the most crucial steps of the analytical procedure developed is the removal of H_2S by purging the aqueous Na_2S solution. H_2S needs to be removed due to its well-known influence on the derivatization step [20]. Nitrogen was used as a purging gas. Potential loss of MeHg and Hg^{2+} during the purging step was examined. Some parameters affecting the results were investigated. Various purging times (1, 3, 5, 10 min) and purging gas flow rates (50, 150, 300 mL min^{-1}) were tested. Spiked Milli-Q water was used as the test water sample.

Aqueous Na_2S solutions were spiked with 100 pg of MeHg and 100 pg of Hg^{2+} standard solution. Each solution was slightly acidified with 1 M HCl and nitrogen gas at a flow rate of 50 mL min^{-1} was bubbled through. Figure 4a shows that 1 min of purging is not sufficient, since the recovery of both species is too low. Ten minutes of purging time is evidently too long. The recovery is low probably due to losses of mercury species during the N_2 bubbling. It was shown that the optimal purging time is 3–5 min. The recovery for MeHg is between 90–100% and 100–105% for Hg^{2+}. In further work, 3 min of purging was used because of the higher recovery for MeHg. Results obtained after optimisation of the purging gas flow rate are presented in Fig. 4b. It was shown that the optimal purging gas flow rate is 50 mL min^{-1}. At higher flow rates lower recoveries were observed.

The stability of MeHg and Hg^{2+} in aqueous Na_2S solution under different conditions was also tested. Aqueous Na_2S solutions were spiked with various amounts of MeHg (20, 60, 100 pg) and Hg^{2+} (20, 60, 100 pg) standard solution. The solutions were stored in a refrigerator at 4 °C and in a deep freezer (–20 °C) for a period of 1–14 days. Figure 5a shows the stability of MeHg and Hg^{2+} in aqueous Na_2S solution stored in a refrigerator; MeHg and Hg^{2+}

Table 4 Recoveries for MeHg and Hg^{2+}

Sample	Recovery (%)			
	50 pg MeHg	50 pg Hg^{2+}	100 pg MeHg	100 pg Hg^{2+}
Milli-Q deionised water	97±5	105±10	99±4	102±7
Seawater	95±4	100± 9	102±4	110±6

are stable for 2–3 days. After that, the recovery of both species, especially for MeHg, rapidly decreases. There is no significant difference in behaviour between solutions spiked with various amounts MeHg and Hg^{2+}. Results obtained after storage of the samples in a deep freezer are presented in Fig. 5b. The stability of MeHg and Hg^{2+} is slightly better in the first two days. A decrease of recoveries appeared on the third day and after the fifth day remained unchanged. Therefore, the final measurement/detection step should be performed within 3 days. In both tests MeHg is less stable with time than Hg^{2+}.

The limit of detection (LOD) in this study is defined as three times the standard deviation of the blank. Since the limit of detection is mainly affected by the repeatability of blank values, several blanks were run in each set of measurements. The blank measurements represented at least 10% of all determination. Blanks values for MeHg and Hg^{2+} should be as low as possible, but higher blank values for Hg^{2+} were sometimes observed. Irreproducible blanks were mostly related to contaminated glassware. The LOD calculated on the basis of three times the standard deviation of the blank varied from 0.004–0.008 ng L^{-1} for MeHg and from 0.06–0.10 ng L^{-1} for Hg^{2+} when 300 mL of water was analysed.

Recovery tests were carried out by spiking a water sample before extraction with known amounts of aqueous MeHg and Hg^{2+} standard solution (50 pg and 100 pg of MeHg and Hg^{2+}). The results for Milli-Q water and seawater are presented in Table 4. Evidently, good recoveries for both species were observed, ranging between 95–100% for MeHg and 100–110% for Hg^{2+}. The repeatability of the method was about 5% for MeHg and 10% for Hg^{2+}.

Conclusion

The method developed is a suitable and appropriate method for the simultaneous determination of MeHg and indirectly Hg-T for various types of real water samples (seawater, fresh water, rain, fresh well water). However, special attention should be paid to the stage of formation of Hg dithizonates to obtain quantitative recoveries. The use of this method reduces the time and amount of water sample needed for analysis. Both Hg species are determined from the same aliquot of the sample therefore eliminating any analytical error as a result of inhomogeneity of the sample.

Further investigations will be focused on the optimisation and implementation of the method for the simultaneous determination of MeHg and Hg^{2+} in natural pore water samples. The method developed would be an ideal way of determining MeHg and indirectly Hg-T in such water samples, in which only small amounts of sample are available.

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