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Method Article

Methylmercury determination in freshwater biota and sediments: Static headspace GC-MS compared to direct mercury analyzer

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A B S T R A C T

We developed and compared two analytical methods for determination of MeHg in freshwater biota and sediments, by: I) simplified static headspace GC-MS using internal standard (IS) isotope dilution quantification, after microwave acid digestion and aqueous phase $NaBEt_4$ ethylation; II) Automated Mercury Analyzer, after double toluene extraction followed by back-extraction with L-cystein. The performance was evaluated by analysis of certified reference materials. For biota, mean recovery was 100 ± 2% and relative standard deviation (RSD) ≤ 6.8% for method I, and mean recovery was 98 ± 7% and RSD ≤13% for method II. For sediments, recovery of 94.5% and RSD of 8.8% were obtained with method I, and recovery of 90.3% and RSD of 9.4% with method II. Limits of detection (LOD) were 0.7 μg kg^{-1} and 6 μg kg^{-1}, respectively. Both techniques were tested for MeHg analysis in freshwater invertebrates, fish and sediments, covering a large range of MeHg values (1.9–670 μg kg^{-1} d.w.).

• Both protocols proved to be suitable for MeHg analysis in complex environmental matrices, even if, for method II, interferences in the extraction phase and limited sensitivity may hinder sediment analysis.

• Passing-Bablock regression revealed a slight disproportion between methods, with line slope = 1.058 (95% CI ranging from 1.001 to 1.090).

A R T I C L E I N F O
Method name: Methylmercury in freshwater biota and sediments by GC-MS or automatic mercury analyzer
Keywords: Organometallic compounds, Methylmercury determination, Benthic invertebrates, Sediments
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Specifications table

Subject Area	Environmental science
More specific subject area	Methylmercury analysis
Method name	Methylmercury in freshwater biota and sediments by GC-MS or automatic mercury analyzer
Name and reference of original methods	Determination of MeHg in biological tissues with GC-MS using isotope dilution: [1] Determination of MeHg in biological tissues and sediments with direct mercury analysis: [2,3]
Resource availability	A calculation sheet is provided to support quantification

Background

We developed and compared two methods for the analysis of methylmercury (MeHg) in freshwater biota and sediment samples, using two different analytical systems: I) static headspace and gas chromatography with single quadrupole mass spectrometry detection, and II) Automated Mercury Analyzer after double liquid-liquid extraction.

GC-MS method was derived by the original method by Cavalheiro et al. [1] by applying some procedural modifications. Preliminary tests were carried out using tetramethylammonium hydroxide (TMAH) as an extractant, but it was discarded mainly for the formation of emulsions. Thus, HCl for sample digestion was preferred, also because in the literature it is considered as more suitable for sediments [4,5]. The derivatization with sodium tetraethylborate ($NaBEt_4$) before the static headspace was performed directly in aqueous solution instead of in the solvent (*n*-hexane) after liquid-liquid extraction to limit the extraction of matrix interferences, and thus to decrease the LOD. To optimize the aqueous ethylation, pH was restored to 5.5 [6] before the addition of the derivatizing agent. For what concerns quantification, isotope dilution was used because it was shown to improve repeatability of results then other quantification methods [7].

For method II, we used the protocol by Calderón et al. [3]. This method aims at the determination of the sum of all organic forms of mercury, after solvent extraction. As regards biota, MeHg is largely the dominant form of organomercury, thus the results can be considered as a measure of MeHg, with good approximation [8,9]. As regards sediments, other organic forms of Hg, such as EthHg and PhHg, may be present, generally deriving from anthropogenic sources [8], even if with low concentrations in comparison with MeHg [10]. Thus, the application of this technique to sediments may account for the sum of all organomercury compounds. However, previous studies referred to this technique as analysis of MeHg in sediments [2]. This technique has been used for analysis of marine sediments and biota [2,11–13], but to our knowledge, testing on freshwater sediments and organisms is not reported in the literature. The direct mercury analyzer is used here also for determination of total mercury (THg) in samples according to US-EPA [14].

Sample collection and pretreatment

Both analytical methods were assessed using the following certified reference materials: SRM-2974a *Mytilus edulis* tissue (National Institute of Standards and Technologies - NIST, USA), SRM-1946 Lake Superior fish tissue (NIST, USA), BCR-CRM414 plankton powder (Institute for Reference Materials and Measurements - IRMM, European Commission, Belgium) and ERM-CC580 estuarine sediment (IRMM, European Commission).

The performance of both methods was tested by analyzing samples of biota and sediments collected in Lake Maggiore basin (Northern Italy). The ecological quality of the lake and its main tributaries is constantly monitored by the International Commission for the Protection of the Italian-Swiss Waters (CIPAIS) (www.cipais.org) [15,16]. Fish and sediments collected in the western part of the lake (Pallanza Basin) and benthic invertebrates (insects, crustaceans) and sediments collected in the Toce River, a tributary of Lake Maggiore, flowing into the Pallanza Basin, were analyzed. Both ecosystems are characterized by a residual mercury contamination due to past activity of a mercury cell chlor-alkali plant located along the Toce River [17–19]. Moreover, fish collected in another

Table 1
Working parameters of GC-MS system for MeHg analysis.

Gas chromatography	
Injector type	Split/splitless
Injection mode	Split (1:10)
Injection temperature (°C)	110
Volume (mL)	1.2
Oven initial temperature (°C)	50
Ramp (°C min^{-1})	25
Final temperature (°C)	250
Old (min)	4
Column	Supelco SLB-5ms 30 m X 0.25 mm X 0.25 µm
Carrier gas	Helium 6.0
Flow (mL min^{-1})	1.1
Static headspace	
Incubation time (min)	12
Syringe temperature (°C)	90
Sample volume (mL)	20
Mass spectrometry	
Acquisition mode	SIM
Ionization	EI, 70 eV
Transfer Line temperature (°C)	175
Polarity	+
Ions (m/z)	215, 216, 244, 245

tributary of Lake Maggiore, the Boesio River, which flows through a heavily anthropized watershed [15,16], were also analyzed.

For invertebrates, whole bodies were analyzed. For fish, the caudal fillet was separated and analyzed. All samples were freeze-dried before analysis. Biological tissues were homogenized with a ball mill. Sediments were sieved and the fraction < 63 µm mesh-size was analyzed.

Method I: GC-MS

GC-MS analysis was carried out using Thermo Fisher GC-MS system (respectively Focus GC and DSQ™ II single quadrupole) equipped with TriPlus RSH™ autosampler (Thermo Fisher Scientific, Rodano, Milan, Italy) able to static headspace technique. Instrumental conditions are given in Table 1.

Quantification results were obtained by the internal standard method considering $[CH_3\text{-}Hg]^+$ and $[CH_3\text{-}Hg\text{-}CH_2CH_3]^+$ ions in the MS spectra, corresponding to the *m/z* 215 and 244 (native $Me^{200}Hg$) and 216 and 245 ($Me^{201}Hg$) (Table 1, Spreadsheet 1). Due to the isotopic pattern of mercury, there is a cross contribution between the signals of the $Me^{201}Hg$-enriched internal standard and natural MeHg. The Full Scan MS spectra of the $Me^{201}Hg$-enriched internal standard show the main mass at 216 *m/z* but also a smaller mass at 215 *m/z* is present (due to standard impurity), and both can be also found in the native MeHg MS spectra. Thus, the contributions need to be separated to get an accurate determination of MeHg in the samples. The cross-signal contributions were experimentally verified injecting $Me^{201}Hg$-enriched internal standard and native MeHg standard solution at different concentrations and then calculating the average ratio of common ions used for the quantification. Considering x = contribution of $Me^{201}Hg$ by internal standard in MeHg (ratio *m/z* 215 + 244/216 + 245 = 0.13 ± 0.03) and y = contribution of MeHg in $Me^{201}Hg$-enriched (ratio *m/z* 216 + 245/215 + 244 = 0.72 ± 0.05), the corrected peak area can be obtained from:

$$\begin{cases} MeHg_{corr} = MeHg_{obs} - x\left(Me^{201}Hg_{corr}\right) \\ Me^{201}Hg_{corr} = Me^{201}Hg_{obs} - y(MeHg_{corr}) \end{cases} \quad (1)$$

where $MeHg_{corr}$ and $Me^{201}Hg_{corr}$ are the areas corrected by cross contribution (native and internal standard, respectively) whereas $MeHg_{obs}$ and $Me^{201}Hg_{obs}$ are the observed areas in the acquired chromatogram. Thus, final areas used for calibration and quantification were calculated as follows:

$$Me^{201}Hg_{corr} = \frac{\left[Me^{201}Hg_{obs} - yMeHg_{obs}\right]}{[1 - xy]} \quad (2)$$

$$MeHg_{corr} = \frac{\left[MeHg_{obs} - xMe^{201}Hg_{obs}\right]}{[1 - xy]} \quad (3)$$

A proper IS amount was added to each sample considering the predicted natural MeHg concentration in the sample, basing on the literature (i.e., percent MeHg on THg according to e.g. [11,20–22]), to avoid a MeHg/IS signal ratio too large or too small (Spreadsheet 1).

Reagents, standards and calibration

Potassium hydroxide and hydrochloric acid were purchased from Fluka (Darmstadt, Germany), methanol pesticide grade, sodium acetate, acetic acid methylmercury chloride, and sodium tetraethylborate 97% from Sigma-Aldrich (Darmstadt, Germany).

All materials needed for the procedure (vessels, centrifuge tubes, etc.) were soaked into a clean diluted HNO_3 10% (v/v) bath for 24 h, then they were rinsed with ultrapure water and dried into a clean drying oven.

A stock standard solution was prepared by dissolving methylmercury chloride salt in a solution made with methanol and hydrochloric acid 18% with a proportion of 30/70% v/v. An internal standard solution was prepared by diluting 1 mL of a Hg-enriched monomethylmercury solution (^{201}Hg, 5.49 ± 0.04 µg g^{-1}, 96.5 %, ISC Science, Oviedo, Asturias, Spain) in 99 mL of ultrapure water 1% HCl. $NaBEt_4$ 1% was obtained by dissolving 100 mg of sodium tetraethylborate 97% in 10 mL of ultrapure water. Calibration standard solutions was prepared in the range 0.03–2 µg MeHg and a six-point calibration curve was used for quantification.

Procedure

For analysis, 0.25–0.5 g d.w. of biological samples and 0.5–1 g d.w. of sediment samples were used. Samples were microwave digested at 70 °C for 3 min after adding the IS ^{201}Hg solution and 3 mL of 1 M HCl. Digested samples were then centrifugated (10 min at 5000 rpm) or filtered (CA 0.4 µm), transferred to the autosampler vials, and mixed with 10 mL of buffer solution (sodium acetate/acetic acid 1 M) and 3 mL of 1 M KOH to reach a pH value of 5.5. For ethylation, 1 mL of $NaBEt_4$ 1% was added immediately before vial crimping. The samples were then incubated for 12 min at 90 °C, and 1.2 mL of the headspace was injected in the GC-MS.

The procedure is summarized in Fig. 1. Spreadsheet 1 can be used for quantification.

Method II direct mercury analyzer

The method involves a double liquid-liquid extraction, first with toluene and then with a cysteine solution, and is based on the affinity of MeHg for the thiol group of cysteine. Detection was carried out using the automatic Hg analyzer AMA-254 (FKV srl, Bergamo, Italy). The instrument detection limit (LOD) is 0.01 ng Hg, the working range is 0.05 to 600 ng Hg.

Reagents, standards and calibration

Sodium acetate anhydrous was achieved from BDH (Darmstadt, Germany), L-cysteine hydrochloride monohydrate, potassium dichromate and hydrochloric acid from Sigma-Aldrich (Darmstadt, Germany),

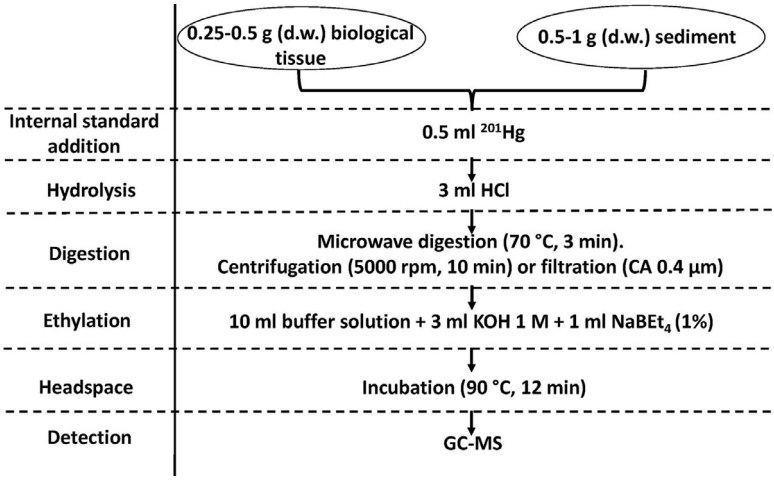

Fig. 1. Procedure for MeHg analysis using GC-MS (method I).

sodium sulfate and toluene from Fluka (Darmstadt, Germany), hydrobromic acid 48% from Panreac (Castellar del Vallès, Spain). All materials needed for the procedure were previously soaked into a clean diluted HNO_3 10% (v/v) bath for 24 h, then rinsed with ultrapure water and dried into a clean drying oven.

L-cysteine solution (1%) was obtained by mixing 12.5 g of sodium acetate anhydrous, 1 g of L-cysteine hydrochloride monohydrate and 0.8 g of sodium sulfate in 100 mL of ultrapure water.

A stock solution of 10 mg Hg L^{-1} was prepared by dilution of a standard solution of mercury (1000 mg Hg L^{-1}, Scharlab S.L., Barcelona, Spain) with potassium dichromate 1 % and hydrochloric acid 1:1 v/v. From this stock solution a 500 µg Hg L^{-1} solution was prepared by dilution. Both solutions were stored in the dark at 4 °C.

Calibration curve of the method was obtained between 1 and 75 µg Hg L^{-1} using calibration solutions prepared by dilution of the 500 µg Hg L^{-1} stock solution with the L-cysteine solution (1%).

The concentration values obtained after analysis of the sample extract (expressed as µg Hg L^{-1}) were converted into concentration in the initial solid sample (expressed as µg MeHg kg^{-1}) using the following formula:

$$MeHg = \frac{6 * C}{w} * f \tag{4}$$

where MeHg is the concentration of methylmercury in the sample (µg MeHg kg^{-1}), C is the concentration of mercury into the extract (µg Hg L^{-1}), 6 is the volume of L-cysteine solution (1%) (mL), w is the weight of the sample (g), f is the ratio between molecular weight of MeHg and Hg (1.075), in order to convert the value expressed as Hg to MeHg (Spreadsheet 1).

Procedure

MeHg analysis was carried out following the protocol by Calderón et al. [3] (Fig. 2).

Ten mL of hydrobromic acid was added to 0.15–0.2 g d.w. of the biological or sediment sample in a vial and manually shaken. Then 20 mL of toluene were added. The solution was mixed with a Vortex for 2 min and centrifuged for 10 min at 5000 rpm. Approximately 15 mL of toluene phase (i.e., the supernatant) were recovered and transferred into a second vial, where 6 mL of L-cysteine solution were added. An additional volume of 15 mL of toluene was added to the remaining sample in the first vial and the solution was shaken and centrifuged as described above. The toluene phase was

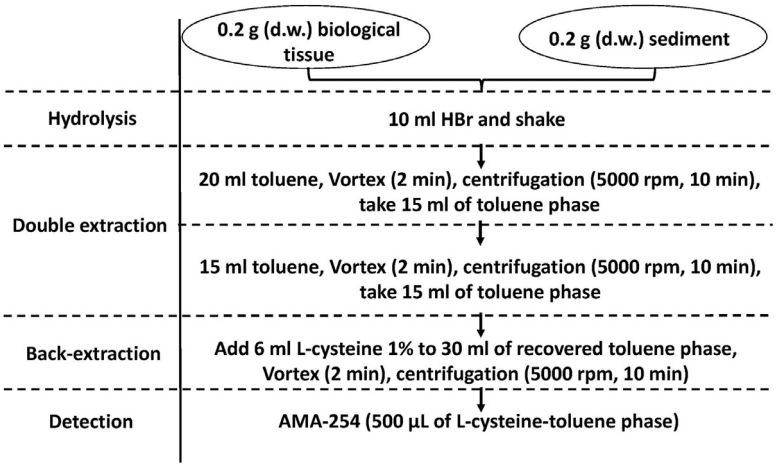

Fig. 2. Procedure for MeHg analysis using a Direct Mercury Analyzer (method II).

recovered and added into the second vial. Finally, the second vial (i.e., the toluene phase in L-cysteine solution) was centrifuged and 500 µL of supernatant was analyzed using AMA-254.

The procedure is summarized in Fig. 2. Spreadsheet 1 can be used for quantification.

Validation of the methods

For the GC-MS method, procedural blanks were evaluated by performing the procedure without a sample every six-eight analysis. For method II, for each cycle of analysis, the L-cysteine solution was analyzed in triplicate as a blank before the samples, and re-analyzed after 6–8 sample analyses to assess the absence of a memory effect (which was not observed). For both methods, the method blank showed concentration below the estimated LOD.

To calculate the limit of detection (LOD) and the limit of quantification (LOQ) of the methods, two different approaches were used. For method I, LOD and LOQ were estimated using the signal-to-noise ratio of 3 and 5, respectively. Signal-to-noise ratios were calculated using sample chromatograms, where the baseline signal is uniform for the type of sample considered, confirming that the matrix effect does not affect the detection limits for headspace analysis. For method II, LOD was calculated as mean absorbance of the L-cysteine solution (1%) + 3 times the standard deviation and LOQ as mean absorbance of the L-cysteine solution (1%) + 10 times the standard deviation. Using Eq. (4) and considering a sample weight of 0.2 g, values can be calculated as µg kg^{-1}. For method I, calculated LOD was 0.7 µg kg^{-1} and LOQ 1.4 µg kg^{-1} of MeHg (Table 2). For method II, resulting LOD was 6 µg kg^{-1} and LOQ 11 µg kg^{-1} (Table 2).

Repeatability was estimated as percent relative standard deviation (RSD), i.e., percent ratio of the standard deviation to the mean, while precision was estimated by analysis of certified reference materials and it was calculated as percent recovery (R), i.e., percent ratio of measured value to certified value. To evaluate these parameters, certified biota and sediment certified reference materials were analyzed (Table 2).

For what concerns biological materials, method I showed good agreement with certified values, with recovery ranging between 97.8 and 102.5% and a mean RSD of 5.4%. For method II recovery ranged between 90.8 and 103.4%, while average RDS was 8.4%.

For what concerns sediments, analysis of the certified material ERM-CC580 (certified value: 75 ± 4 µg MeHg kg^{-1}) allowed to obtain an average of 70.9 ± 6.2 µg MeHg kg^{-1} for method I, with a mean recovery of 94.5% (RSD = 8.8%) and 67.7 ± 6.4 µg MeHg kg^{-1} for method II (the

Table 2
Values of MeHg (μg kg^{-1}) in biological and sediment certified reference materials (mean ± standard deviation) obtained with methods I and II. *n* = number of analyses, R = mean percent recovery in comparison to certified value, RSD = percent relative standard deviation, * = MeHg value is referred to wet weight (in the other cases to dry weight). Limits of Detection (LOD) and of Quantification (LOQ) for both methods are provided (see text for explanations).

Certified reference material		Certified reference value	Method I: GC-MS				Method II: AMA-254			
		MeHg μg kg^{-1}	n.	MeHg μg kg^{-1}	R %	RSD %	n.	MeHg μg kg^{-1}	R %	RSD %
Biota	SRM-2974a	69.06 ± 0.81	6	70.7 ± 4.8	102.5	6.8	6	69.4 ± 10.4	100.6	13.0
	SRM-1946	394 ± 15*	6	388.8 ± 22.9*	98.7	5.9	6	357.9 ± 5.8*	90.8	1.6
	BCR-CRM414	200 ± 10	6	195.5 ± 6.6	97.8	3.4	6	206.7 ± 22.2	103.4	10.7
Sediment	ERM-CC580	75 ± 4	6	70.9 ± 6.2 LOD = 0.7 μg kg^{-1} LOQ = 1.4 μg kg^{-1}	94.5	8.8	6	67.7 ± 6.4 LOD = 6 μg kg^{-1} LOQ = 11 μg kg^{-1}	90.3	9.4

GC chromatogram showed no other organomercury forms in the sample), with a recovery of 90.3% (RSD = 9.4%).

Testing of the methods on biological and sediment samples

Both methods were evaluated for analysis of biological samples collected in the field (Table 3). In particular, the performance was tested by analyzing organisms belonging to different taxonomic groups (i.e., with a different matrix), with different levels of THg and, potentially, with different MeHg:THg ratio [20,23,24], i.e., freshwater benthic invertebrates (insects, crustaceans) and fishes. Both methods successfully determined MeHg (the GC chromatogram showed no other organomercury forms in the samples) and accuracy showed RSD $\leq$ 6.7% for method I and $\leq$ 13.2% for method II.

Sediment samples were successfully analysed in GC-MS over a range of 1.9–26 µg MeHg kg^{-1}. Average RDS was 7.5%. As expected for freshwater sediments [23,25,26], MeHg concentrations represented $\leq$ 1.5% of total Hg (Table 3). For this reason, most sediment analyses carried out with AMA-254 (using 0.2 g d.w. of the sediment sample) resulted as < LOD (Table 2).

In fact, the latter method does not seem to be always effective for freshwater sediments, as in many cases the results may be <LOD. To overcome this limitation by increasing MeHg concentrations, some extraction tests were carried out using increasing amounts of the sediment sample "Pallanza littoral" (26 µg kg^{-1} MeHg according to method I analysis, Table 2): 0.4 g, 0.6 g and 0.8 d.w.. However, the values obtained remained below the LOD. Thus, the extraction is likely to be only partial, probably because HBr may not be strong enough to separate organomercury from strong ligands, such as organic matter. Maggi et al. [2] used HCl instead of HBr for the analysis of marine sediments. By using HCl, we observed the development of a strong effervescence reaction, probably due to the release of CO_2 from carbonate dissolution, which probably affected the extraction phase. Again, values for this sample remained below the LOD.

Method II showed also some other limitations. In particular, for some fish samples, after the addition of toluene the formation of an emulsion was observed, that limited the recovery capacity of L-cysteine. This drawback was highlighted also by Watanabe et al. [27] and Maggi et al. [2].

Comparison between methods

The comparison between methods was carried out considering the available analyses of both certified materials and natural samples (n = 20). Spearman's rank correlation coefficient was calculated. Then, the non-parametric regression of Passing-Bablok [28] was calculated. Passing-Bablok model requires the least number of assumptions. Extreme values (outliers) can be included, imprecision in both methods is allowed and it is not required that the error be normally distributed nor constant along the range of concentrations. This regression analysis allows to evaluate the presence of a systematic constant error (intercept) or proportional error (slope). To demonstrate the absence of a systematic error, the 95% confidence intervals must include 0 for the intercept and 1 for the slope. Then, Bland and Altman graph can be used to calculate the limits of agreement between methods, basing on the differences between methods [29]. Normality of differences was tested with D'Agostino-Pearson test. Analyses were carried out with MedCalc 19.3 (MedCalc Software Ltd, Ostend, Belgium).

Spearman's correlation coefficient resulted 0.988 (p < 0.001). The non-parametric Passing-Bablok regression showed that the confidence intervals of the intercept include the value 0, proving the absence of constant systematic errors, while the slope of the line is slightly higher than 1 (even if the lower limit of the confidence interval is 1.0010), thus indicating a slight disproportion between the two methods (Fig. 3). Bland and Altman dispersion plot (Fig. 4) shows that the values measured with AMA-254 were slightly lower compared to those determined with GC-MS method. Average percent bias is -7.36% (95% CI: -11.6 to -3.0%), and the agreement interval falls between -25.7 and 11.0% (Fig. 4b), i.e., from -39.8 to 19.1 µg kg^{-1} (Fig. 4a). The highest disagreement may be bound to the lowest concentrations (< 200 µg kg^{-1}), since percent difference between methods seems to become lower with increasing concentration (Fig. 4b).

Table 3
Values of MeHg in biological and sediment samples obtained with methods I and II: n = number of analyses, measured values of MeHg as mean ± standard deviation, RSD = percent relative standard deviation, THg = total mercury (n = 3), N.D. = not determined, i.e., the method failed determining MeHg in the sample (see text for explanations).

	Sample	Sampling site and year	Method I: GC-MS			Method II: AMA-254			THg
			n	MeHg μg kg^{-1} d.w.	RSD %	n	MeHg μg kg^{-1} d.w.	RSD %	μg kg^{-1} d.w.
Biota	Fish *Squalius cephalus*, aged 1-2 years	Boesio River mouth, 2017	5	207 ± 13	6.3	5	183 ± 24	13.2	179 ± 5
	Fish *Squalius cephalus*, aged 7-10 years	Boesio River mouth, 2017	5	670 ± 30	4.4	5	621 ± 49	7.9	844 ± 1
	Fish *Alosa fallax lacustris*, aged 3-4 years	Lake, Pallanza Basin, 2016	5	585 ± 27	4.6	5	553 ± 45	8.2	585 ± 8
	Crustacea Gammaridae	Toce River[a], 2018	5	98 ± 7	6.7	5	96 ± 1	0.6	125 ± 1
	Ephemeroptera Heptageniidae	Toce River[a], 2018	5	56 ± 4	6.4	5	52 ± 4	7.3	75 ± 5
	Trichoptera Limnephilidae	Toce River[a], 2018	5	62 ± 3	5.1	5	63 ± 8	12.1	93 ± 4
	Plecoptera *Perla*	Toce River[a], 2018	5	119 ± 4	3.4	5	108 ± 13	11.8	127 ± 3
Sediment	Toce River	Toce River mouth, 2017	4	3.3 ± 0.2	6.3	4	<LOD	-	214 ± 7
	Toce River	Toce River, Prata, 2013	4	1.9 ± 0.3	14.7	4	<LOD	-	50 ± 4
	Lake Maggiore, Pallanza Basin	Pallanza sublittoral, 2013	4	6.1 ± 0.7	10.7	4	<LOD	-	3207 ± 13
	Lake Maggiore, Pallanza Basin	Pallanza littoral, 2013	4	26 ± 0.7	3.6	4	N.D.	-	4072 ± 148
	Lake Maggiore, Pallanza Basin	South of Isola Bella, 2013	4	6.8 ± 0.1	2.2	4	<LOD	-	6276 ± 167

[a] invertebrate samples were collected downstream the chlor-alkali plant located at Pieve Vergonte (VB, Northern Italy).

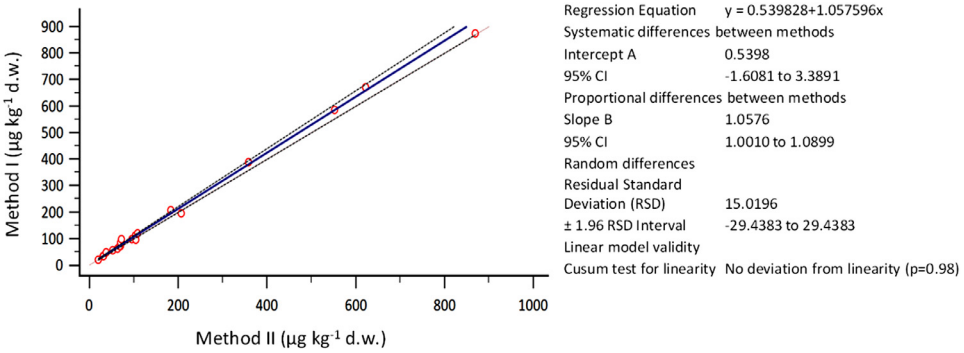

Fig. 3. Non-parametric passing-bablok regression between methods. Regression equation and parameters of agreement between methods are also reported.

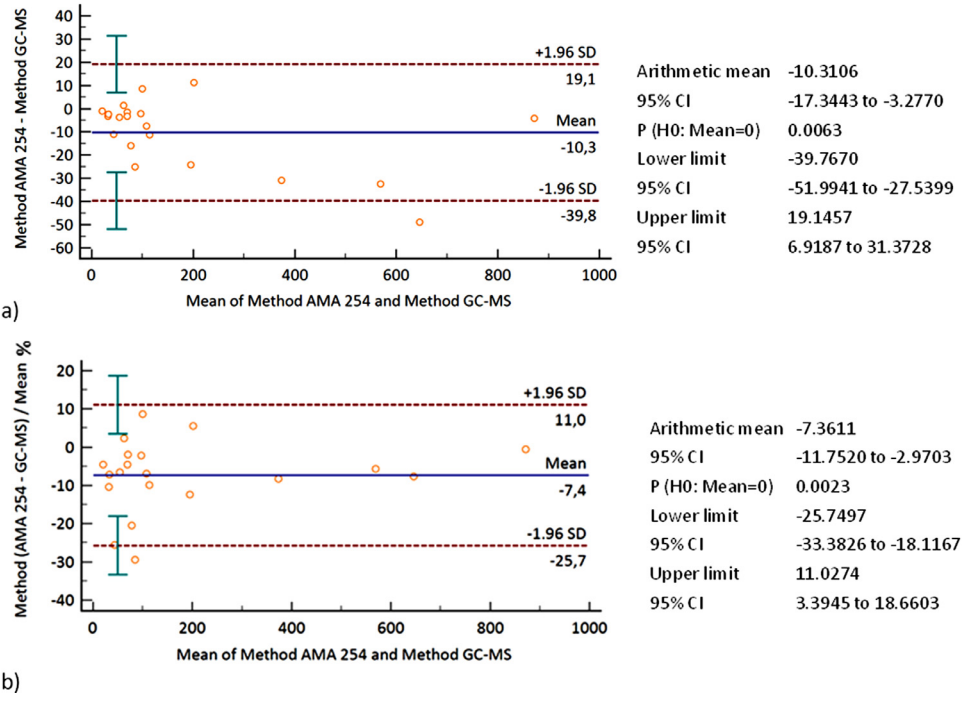

Fig. 4. Bland-altman plot of differences between methods: (a) as units (µg kg^{-1}), (b) as percentage. Parameters on intervals of agreement are also reported.

This slight disproportion is also confirmed by the recoveries obtained with analysis of the certified reference materials SRM-1946 and ERM-CC580, which were lower for method II in comparison to method I (-8.0 and -4.4%, respectively) (Table 2).

We did not deepen the reasons of this disproportion between methods. Possibly, the extraction of the organic complex methylmercury-cysteine from the extraction solution performed in method II may not be complete, despite the double extraction. Furthermore, liquid-liquid extraction efficiency could be limited by the generation of emulsions, especially in organic matrices [2,27]. Poor recovery may also lower LOD in method II.

However, both approaches showed good precision according to recovery in certificate reference materials, and good accuracy according to RDS values, proving to be suitable for MeHg analysis in complex environmental matrices. As expected, method I showed higher sensitivity than method II and thus it is suitable for analysis of MeHg also in sediment samples. Method II can be used to extract all organic forms of mercury. However, given that MeHg in organisms generally represent the totality of organomercury compounds, method II can be used as rapid and cost-effective technique for MeHg analysis in aquatic organisms.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.mex.2021.101581.

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