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Comparison of gas chromatographic hyphenated techniques for mercury speciation analysis

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ABSTRACT

In this study, we evaluate advantages and disadvantages of three hyphenated techniques for mercury speciation analysis in different sample matrices using gas chromatography (GC) with mass spectrometry (GC–MS), inductively coupled plasma mass spectrometry (GC–ICP-MS) and pyrolysis atomic fluorescence (GC-pyro-AFS) detection. Aqueous ethylation with $NaBEt_4$ was required in all cases. All systems were validated with respect to precision, with repeatability and reproducibility <5% RSD, confirmed by the Snedecor *F*-test. All methods proved to be robust according to a Plackett–Burnham design for 7 factors and 15 experiments, and calculations were carried out using the procedures described by Youden and Steiner. In order to evaluate accuracy, certified reference materials (DORM-2 and DOLT-3) were analyzed after closed-vessel microwave extraction with tetramethylammonium hydroxide (TMAH). No statistically significant differences were found to the certified values ($p = 0.05$). The suitability for water samples analysis with different organic matter and chloride contents was evaluated by recovery experiments in synthetic spiked waters. Absolute detection and quantification limits were in the range of 2–6 pg for GC-pyro-AFS, 1–4 pg for GC–MS, with 0.05–0.21 pg for GC–ICP-MS showing the best limits of detection for the three systems employed. However, all systems are sufficiently sensitive for mercury speciation in environmental samples, with GC–MS and GC–ICP-MS offering isotope analysis capabilities for the use of species-specific isotope dilution analysis, and GC-pyro-AFS being the most cost effective alternative.

1. Introduction

Mercury is a non-essential trace element which shows high toxicity [1]. It exists in different molecular forms with specific biogeochemical transformation and ecotoxicity [2]. Although all forms of mercury are poisonous, alkylmercury compounds are of special concern because of their easy penetration through biological membranes, efficient bio-accumulation, high volatility and long-term elimination from tissues [3,4]. Monomethylmercury (MeHg) is the most commonly occurring organo-mercury compound and one of the most toxic, and it is recognized as a major environmental pollution issue and health hazard for humans [5]. MeHg is biomagnified through the trophic chain and, as a result, the average proportion of MeHg over total Hg in fish tissues can be up to 95% [6]. Contaminated seafood is the major route of exposure for humans to MeHg.

The World Health Organization (WHO) recommends an adult intake less than 0.3 mg of total mercury per person per week with not more than 0.2 mg of methylmercury [7]. The European Union (EU) has recently included Hg and its compounds in the list of priority pollutants (Decision 2455/2001/EC amending the Water Framework Directive 2000/60/EC). Additionally, the EU has established 0.5 $\mu g\,g^{-1}$ (wet weight) as maximum level of Hg in different foodstuffs (Commission Regulation EC-78/2005 amending regulation CE-466/2001). Consequently, routine analysis of mercury species, and more precisely MeHg, has become an analytical problem of first order.

Several methods for determining the concentration of inorganic mercury and organomercury species have been developed, as discussed in previous monographs and reviews [2,8,9]. For the sample preparation step, each kind of sample matrix presents very different analytical problems. The analysis of solid samples, such as sediments or biological tissues, requires an extraction step before the detection to separate the analytes from the matrix. Special attention should be given to species conservation (species decomposition, artefact methylation) during the extraction/digestion procedures. For aqueous samples, the main problem is that concen-

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Table 1
Optimum GC, interface and detector operating conditions for the different hyphenated systems.

GC-pyro-AFS		GC–MS		GC–ICP-MS	
Gas chromatograph		***Gas chromatograph***		***Gas chromatograph***	
Column	TRB-5	Column	DB-5MS	Column	MXT-1
	30 m × 0.25 mm × 0.25 μm		30 m × 0.25 mm × 0.25 μm		30 m × 0.53 mm × 1.0μm
Injector type	Splitless	Injector type	Splitless	Injector type	Splitless
Injection volume	1 μL	Injection volume	1 μL	Injection volume	1 μL
Injector temperature	300 °C	Injector temperature	250 °C	Injector temperature	200 °C
Temperature programme	40 °C (2 min), 40 °C/min up to 200 °C (2 min)	Temperature programme	40 °C (1 min), 15 °C/min up to 90 °C, 50 °C/min up to 200 °C (4 min)	Temperature programme	50 °C (1 min), 40 °C/min up to 200 °C (1 min)
He carrier gas flow	3 mL min^{-1}	He carrier gas flow	1 mL min^{-1}	He carrier gas flow	16 mL min^{-1}
Pyrolyser		***Interface***		***Transfer line***	
Pyrolysis temperature	800 °C	Interface temperature	280 °C	Transfer line temperature	170 °C
AFS		***MS***		***ICP-MS***	
Make-up gas flow	150 mL min^{-1}	Ionisation current	300 μA	Forward power (W)	1350 W
Sheath gas flow	300 mL min^{-1}	Ionisation energy	70 eV	Plasma gas flow	15 L min^{-1}
AFS gain	1000	Source temperature	250 °C	Auxiliary gas flow	0.7 L min^{-1}
Filter factor	16	Dwell time	60 ms	Nebulizer	0.6 L min^{-1}
		m/z	246 for EtMeHg 260 for Et_2Hg	Dwell time	30 ms for ^{200}Hg; ^{202}Hg 10 ms for ^{203}Tl; ^{205}Tl

tration levels of dissolved mercury species in the environment are very low. Thus, in natural unpolluted waters, concentrations of total dissolved mercury are between 0.2 and 15 ng L^{-1}, most often below 5 ng L^{-1} [10] and dissolved MeHg is usually only 1% of the total [11]. Additionally, mercury in these kinds of samples is present in the form of different complexes with chloride and organic ligands, especially those containing thiol groups [12].

Trace metal speciation analysis is most commonly achieved by the coupling of a separation technique (usually gas or liquid chromatography) with an element selective detector, e.g. atomic spectroscopy or mass spectrometry, with or without a previous preconcentration step [8,13]. The principal advantage of gas chromatography (GC) is the quantitative transfer of analytes from the chromatographic column to the detector without sample nebulisation, which considerably improves the limits of detection with respect to liquid chromatography (LC). A drawback of the GC is the need for derivatization of the ionic mercury species to obtain volatile forms but a very well accepted strategy is the formation of peralkylated volatile compounds by ethylation [9]. After separation by GC, suitable detection techniques for mercury species are atomic fluorescence (AFS) [14,15] and inductively coupled plasma mass spectrometry (ICP-MS) [16,17]. Both techniques are extremely sensitive and selective detectors for mercury. And, more recently, GC coupled to mass spectrometry (GC–MS) with electron ionisation has been introduced as an attractive alternative since is cheaper and widespread in routine testing laboratories [18,19].

Therefore, there are a number of different systems for mercury speciation at research level but the key question is the transfer to laboratories devoted to routine analysis because more and more analytical laboratories demand worldwide speciation methodologies capable of analyzing such compounds. Thus, it is necessary to transfer ready-to-use analytical methods from research centres to laboratories devoted to routine analysis covering the widest possible range of sample matrices. Speciation procedures provide the most complete information but they involve many steps in sample pre-treatment which reduce the procedure robustness and make them time consuming. Moreover, they use very critical instrumental couplings that affect method reliability and compromise their application in routine laboratories. In this context, three GC systems employing GC coupled to different detectors (GC-pyro-AFS, GC–ICP-MS and GC–MS) currently used for mercury speciation analysis were evaluated in terms of linearity, precision, accuracy, specificity, linear dynamic range, limits of detection and quantification, and robustness. Robustness testing is gaining interest and becoming increasingly more important to meet the strict guidelines set by the regulatory authorities [20–22], however few robustness tests on GC methods were found in the literature [23–26] and none at all for hyphenated systems. Biological materials and spiked water samples, with different salinity and organic matter content, have been used to evaluate the methods' applicability. Additionally, other secondary but not less important factors, such as cost, time of analysis or possible interferences, are also discussed.

2. Materials and methods

2.1. Instrumentation

Operating conditions and instrumentation are listed in Table 1.

2.1.1. GC-pyro-AFS

A gas chromatograph (Varian 3900, Varian Ibérica, Spain) was coupled to an AFS detector (Millenium Merlin, P. S. Analytical, United Kingdom) via a pyrolysis unit. The instrumental configuration is described elsewhere [15]. The chromatograph was provided with a non-polar capillary column (TRB-5,

30 m × 0.25 mm × 0.25 μm, Teknokroma, Barcelona, Spain) and helium was used as carrier gas. The eluted mercury species were transferred to the detector through a pyrolysis oven which converts the different mercury species to atomic mercury vapour. The pyrolysis unit is provided with a temperature control module. The analytical column ending was linked to a deactivated silica capillary tube (50 cm length) through a deactivated universal press-tight connector (Teknokroma, Sant Cugat del Valles, Spain). This connection was used to preserve the life of the column as well as to interface the GC and detection system. After the pyrolysis unit, a T-connection (1/16 in.) was placed in the deactivated silica capillary tube to allow additional make-up gas flow to improve the transport efficiency. Argon was used as make-up and also as sheath gas for the AFS detector. Finally, data were acquired using the Speciation Application Millenium Systems Software (P. S. Analytical, United Kingdom) and processed by Microcal Origin 5.0 (Microcal Software, Inc., Northampton, MA, USA).

2.1.2. GC–MS

A commercial GC–MS system consisting of an Agilent 5890 GC equipped with a DB-5MS capillary column (J&W Scientific, Folson, USA (5% phenyl-95% dimethyl polysiloxane, 30 m × 0.25 mm × 0.25 μm)) linked to an Agilent 5975 MS detector was used. Samples were injected (1 μL) in the splitless mode and He was used as carrier gas at 1 mL min^{-1}. The selected *masses* to be monitored were *m*/*z* 246 for EtMeHg and *m*/*z* 260 for Et_2Hg.

2.1.3. GC–ICP-MS

A gas chromatograph (Varian 3900) equipped with a capillary column (30 m, 0.53 mm i.d., 1 μm, Crossbond 100% dimethyl polysiloxane) (Restek, USA) was coupled to a Thermo Electron Model XSeries II Inductively Coupled Plasma Mass Spectrometer [17]. The outlet from the GC column was connected to the torch of the ICP-MS using a heated (170 °C) transfer line. The use of a special three legged X Series ICP-MS torch allows the simultaneous connection of a nebulizer/impact bead spray chamber for the introduction of liquids simultaneous to the GC input. The instrumental configuration allows the continuous aspiration of tuning or internal standard solutions, here thallium in a concentration of 10 μg L^{-1}, whilst operating in the GC–ICP-MS mode. GC separation parameters were optimized in order to obtain symmetrical peaks and to minimize peak integration errors. The raw data of the transient isotope signals for the different mercury species were further processed using the XSeries PlasmaLab Software.

2.1.4. Closed-vessel microwave oven

A laboratory microwave system (Ethos Plus; Milestone, Monroe, CT, USA), equipped with temperature and pressure feedback control was used in this study. This device is accurate in sensing temperature within ±2.0 °C of set temperature, and automatically adjusts the microwave field output power. This device is prepared for extracting ten samples simultaneously. The high pressure closed digestion vessels used for extraction are made of high purity TFM (a thermally resistant form of Teflon) and have a capacity of 100 mL.

2.2. Reagents and standards

Stock standard solutions of 1000 μg mL^{-1} of Hg^{2+} and MeHg were prepared by dissolving mercury (II) chloride (Panreac) in 5% HNO_3 (Merck) and methylmercury chloride (Strem Chemicals) in methanol, respectively. All stock solutions were stored in amber glass bottles in a cold room at 4 °C. Working standards were prepared daily by proper dilution with ultrapure water.

For the sample extraction methanolic tetramethylammonium hydroxide (25%, w/w) was obtained from Sigma–Aldrich (Steinheim, Germany). Sodium tetraethylborate 98% was purchased from Strem Chemicals (Bischheim, France). Humic acid, sodium salt, was obtained from Sigma–Aldrich (Steinheim, Germany). Hexane (Merck) was used as organic solvent. All chemicals were of analytical-reagent grade.

Ultrapure water (18.2 MΩ cm) was obtained from an Elga Purelab Ultra Analytic water purification system.

Helium C-50 was used as a carrier gas and Argon C-50 was used as a make-up and sheath gas at the transfer line and the AFS detector, respectively (Carburos Metálicos, Spain).

2.3. Certified reference materials and water samples

The certified reference materials used were DORM-2 (dogfish muscle) and DOLT-3 (dogfish liver) from National Measurement Standard of Research Council of Canada (NRCC). The certified reference materials were used as provided.

Synthetic water samples, containing different environmental levels of humic acid (HA) (0.1 and 8 mg L^{-1}) and 0‰ or 35‰ NaCl matrices, were prepared in Ultrapure water to simulate different types of water samples. The samples were spiked prior ethylation with standard solutions of inorganic mercury and MeHg at different concentration levels. Samples were stored 3 days in a refrigerator (4 °C) for equilibration. Hg was determined using the three analytical techniques described previously. The measurements for each procedure were performed in duplicate.

2.4. Procedures

All samples were analyzed by triplicate before and after a standard. In this work we use the "standard-sample-standard" bracketing technique. Thus, the samples were quantified according to the response factors obtained for standards. Blanks were analyzed regularly between samples and standards.

2.4.1. Biological samples

Mercury extraction for speciation analysis in biological samples was carried out with 0.2 g of sample and 2.0 mL of tetramethylammonium hydroxide (TMAH) following a previously optimized procedure [27]. The final volume was adjusted to 10 mL with ultrapure water for microwave requirements. A clear solution was obtained after microwave irradiation. Then the vessels were cooled down to room temperature, made-up to a known volume and stored in the cold room until analyzed. Blanks were prepared along with the samples in each batch.

Volumes of 2 mL of the alkaline extracts were used for derivatization. The pH was adjusted to 3.9 using concentrated acetic acid and 5 mL of 0.1 M acetic acid–sodium acetate buffer. Then, 5 mL of sodium tetraethylborate (0.3%, w/v) and 2 mL of hexane were added and the mixture was manually shaken for 5 min. The sample was centrifuged for 5 min at 600 × *g*. The organic layer was transferred to a glass vial and stored at 4 °C.

Finally, portions of the hexane extracts (100 μL) were accurately transferred to fresh 1.1 mL screw cap vials. An aliquot of 1 μL was analyzed by the GC coupled system.

2.4.2. Water samples

Volumes of 2 mL of water samples were used for derivatization. The pH was adjusted to 3.9 using concentrated acetic acid and 5 mL of 0.1 M acetic acid–sodium acetate buffer. Then, hexane and sodium tetraethylborate were used as organic solvent and derivatizating reagent, respectively, in the derivatization process. Finally, an aliquot of 1 μL of hexane extract was analyzed by the GC coupled system.

Table 2
Linearity ($n = 5$), LOD and LOQ of mercury species by GC-pyro-AFS, GC–MS and GC–ICP-MS.

	GC-pyro-AFS		GC–MS		GC–ICP-MS	
	MeHg	Hg^{2+}	MeHg	Hg^{2+}	MeHg	Hg^{2+}
Equation[a]	$y = 0.705C - 2.043$	$y = 1.108C - 0.138$	$y = 0.011C - 0.037$	$y = 0.017C - 0.075$	$y = 0.18 \times 10^8 C + 0.22 \times 10^7$	$y = 0.27 \times 10^8 C + 0.52 \times 10^7$
SD slope[b]	0.006	0.010	0.048	0.025	0.32×10^6	0.55×10^6
SD intercept[b]	0.585	0.933	2.244	1.159	0.17×10^7	0.28×10^7
R^2	0.9997	0.9997	0.996	0.997	0.9984	0.9979
LODs (pg)	1.8	1.0	1.2	0.7	0.06	0.05
LOQs (pg)	5.9	3.4	3.9	3.0	0.21	0.15

[a] Relative peak area (y, no units) versus concentration (C) in $\mu g\,L^{-1}$.
[b] Standard deviation.

Table 3
Variables selected as factors and values chosen as levels for robustness test of GC-pyro-AFS, GC–MS and GC–ICP-MS.

Factor	GC-pyro-AFS	GC–MS	GC–ICP-MS
A	Carrier gas flow: 2.5, 3.0, 3.5 mL min^{-1}	Carrier gas flow: 0.8, 1.0, 1.2 mL min^{-1}	Initial temperature: 48, 50, 52 °C
B	Make up gas flow: 1.25, 1.5, 1.75 mL min^{-1}	Time of splitless: 0.8, 1.0, 1.2 min	Initial time: 0.8, 1.0, 1.2 min
C	Initial temperature: 38, 40, 42 °C	Initial temperature: 35, 40, 45 °C	Injector temperature: 180, 200, 220 °C
D	Injector temperature: 280, 300, 320 °C	Injector temperature: 240, 250, 260 °C	Injected volumen:0.8, 1.0, 1.2 μL
E	Injection volume: 0.8, 1.0, 1.2 μL	Injection speed: 280, 300, 320 μL min^{-1}	Carrier gas flow: 13.5, 16.0, 18.5 mL min^{-1}
F	Pyroliser temperature: 750, 800, 850 °C	Detector temperature: 270, 280, 290 °C	Make up gas flow: 190, 200, 210 mL min^{-1}
G	Final oven temperature: 230, 250, 270 °C	Final oven temperature: 270, 280, 290 °C	Transfer line temperature: 150, 170, 190 °C

3. Results and discussion

3.1. Analytical figures of merit

In this study two home-made interface coupled systems, GC-pyro-AFS and GC–ICP-MS, and a standard GC–MS instrument with electron impact ionisation have been used. The instrumental working conditions are shown in Table 1. The first step was the evaluation and comparison of the systems in terms of classic analytical parameters.

Limits of detection and quantification (LOD and LOQ, respectively) were estimated in accordance to the base line. The base line noise was evaluated by recording the detector response over a period about 10 times the peak width. The LOD and LOQ were obtained as the sample concentration that caused a peak with a height 3-fold or 10-fold the base line noise level, respectively. Absolute detection limits, calculated as the mass of Hg required to obtain a net signal equivalent to three times the standard deviation of the background noise next to the chromatographic peak, are in the pg range for all the evaluated systems (Table 2). Procedural detection limits for solid samples depend on the mass of initial sample, volume of hexane for extraction, injection volume and reagent blanks, but they are better than 9.0 ng/g. Blanks were processed regularly between samples and standards and no memory effects were found.

Linearity was checked in a range from 5 to 200 $\mu g\,L^{-1}$ of the species for GC-pyro-AFS and GC–MS and in a range from 0.25 to 10 $\mu g\,L^{-1}$ for GC–ICP-MS. The equations and regression coef-

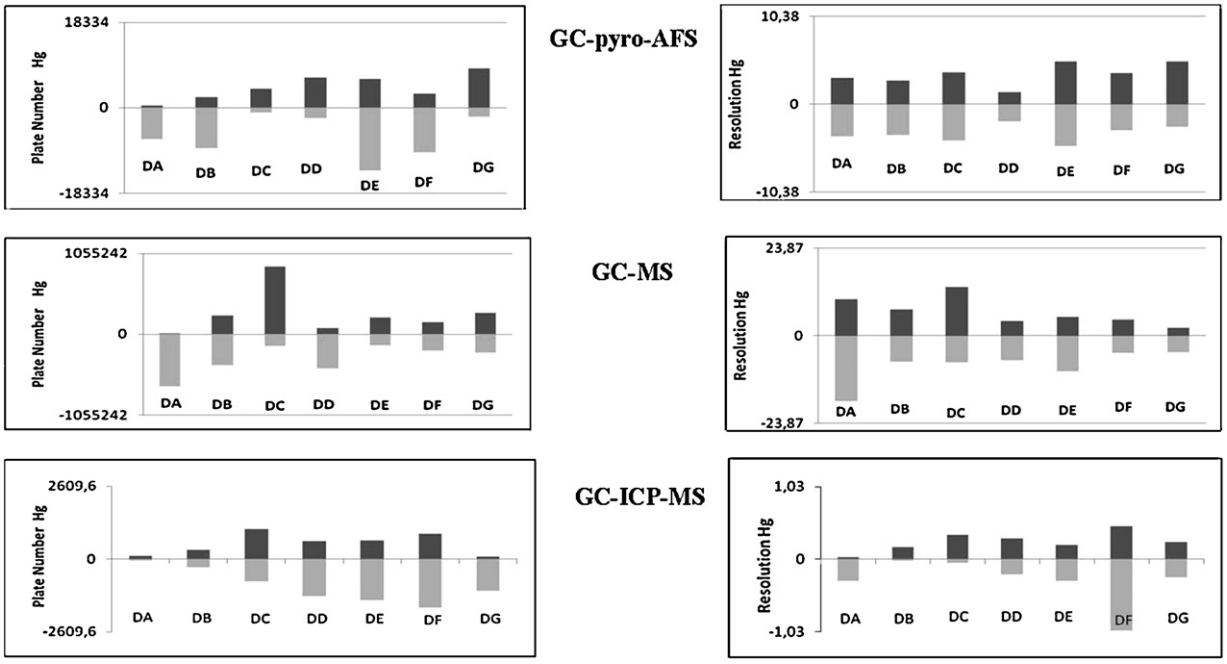

Fig. 1. Variation effects on Hg^{2+} plate number and resolution of the seven operating factors using GC-pyro-AFS, GC–MS and GC–ICP-MS.

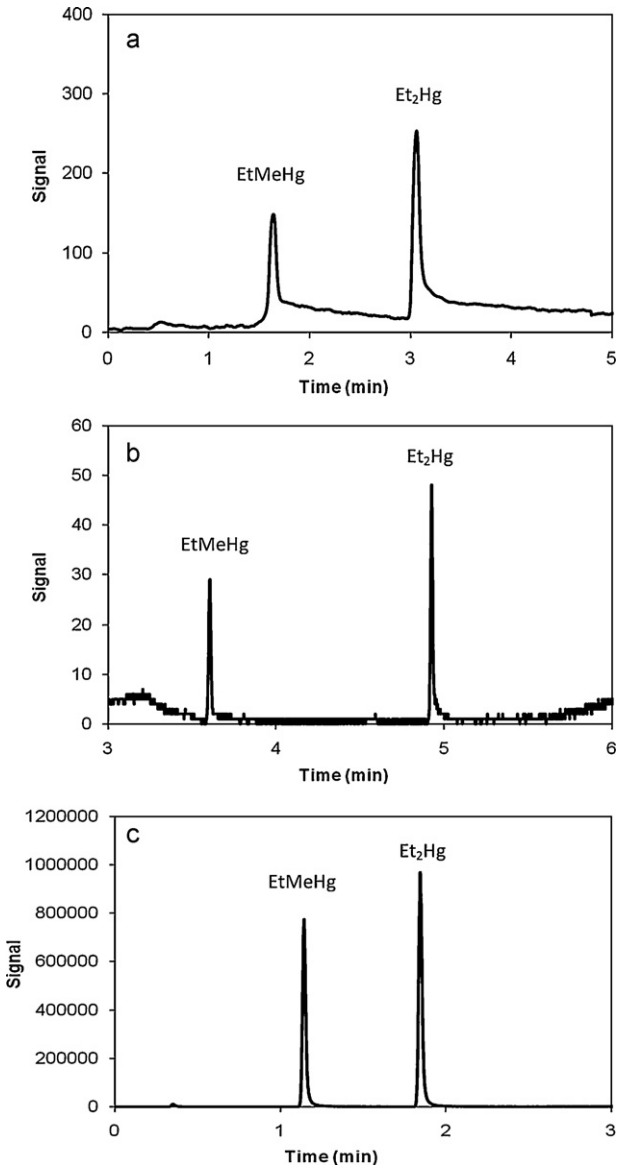

Fig. 2. Chromatograms obtained by GC-pyro-AFS (a), GC–MS (b) and GC–ICP-MS (c) after closed-vessel microwave-assisted extraction of DOLT-3.

ficients are summarized in Table 2. In all cases, the calibration curves showed an excellent linear relationship between areas and concentrations with r^2 values > 0.99 for all systems in the concentration ranges applied. Intercepts were found not different from zero according to Student's test "*t*" ($p = 0.05$), proving that no blank offset was obtained.

The precision of all systems is expressed in terms of relative standard deviation (RSD, %). The repeatability was assessed by running a series of 10 replicates of a standard solution containing 50 μg L^{-1} of the species for GC-pyro-AFS and GC–MS analysis and 5 μg L^{-1} of the species for GC–ICP-MS. The results showed that the standard deviation of the areas for MeHg and Hg^{2+} were below 5% in all three systems.

The reproducibility over time of the three systems was evaluated by separating 10 replicates of a stock solution prepared as explained above, in 2 consecutive days and comparing the standard deviations (below 5% in both days for each compound) of the peak areas of each compound. For this purpose, the Snedecor test "*F*" for two tails was used and, as a result, no significant differences between the series for both days were found ($p = 0.05$).

Table 4
Analysis of mercury species (expressed in μg g^{-1} as Hg) in biological certified reference materials by different hyphenated techniques.

CRM	Certified value		GC-pyro-AFS		GC–MS		GC–ICP-MS	
	MeHg	Hg^{2+}	MeHg	Hg^{2+}	MeHg	Hg^{2+}	MeHg	Hg^{2+}
DOLT-3	1.70	1.67[a]	1.71 ± 0.12 ($n = 7$)	1.71 ± 0.06 ($n = 7$)	1.72 ± 0.05 ($n = 8$)	1.64 ± 0.08 ($n = 8$)	1.62 ± 0.21 ($n = 5$)	1.76 ± 0.21 ($n = 5$)
DORM-2	4.47 ± 0.32	0.17[a]	4.54 ± 0.32 ($n = 6$)	0.17 ± 0.04 ($n = 6$)	4.51 ± 0.22 ($n = 8$)	0.174 ± 0.008 ($n = 8$)	[b]	[b]

[a] Proposed value.
[b] Not available.

Table 5
Recoveries (%) obtained for mercury species in synthetic water samples by different hyphenated techniques.

Sample matrix	GC-pyro-AFS		GC–MS		GC–ICP-MS	
	MeHg	Hg^{2+}	MeHg	Hg^{2+}	MeHg	Hg^{2+}
Milli-Q water	102.0 ± 1.2	100.6 ± 4.6	97.7 ± 8.7	90.3 ± 7.9	107.3 ± 2.3	98.9 ± 0.6
1 mg L^{-1} HA/ultrapure water	108.9 ± 6.6	97.2 ± 0.3	97.0 ± 4.0	97.1 ± 4.3	94.5 ± 2.0	99.1 ± 0.3
8 mg L^{-1} HA/ultrapure water	118.5 ± 3.5	92.2 ± 0.3	96.8 ± 7.6	105.1 ± 3.6	103.4 ± 0.5	100.7 ± 1.2
35‰ NaCl	68.8 ± 6.7	76.2 ± 4.9	57.1 ± 6.2	71.2 ± 2.6	67.4 ± 0.6	76.7 ± 1.4
1 mg L^{-1} HA/35‰ NaCl	59.5 ± 1.3	73.9 ± 5.7	41.6 ± 3.9	68.8 ± 1.5	49.2 ± 0.4	83.1 ± 0.3
8 mg L^{-1} HA/35‰ NaCl	56.1 ± 1.6	76.7 ± 5.3	42.3 ± 4.0	62.8 ± 5.0	49.3 ± 0.7	82.4 ± 0.6

Uncertainties are expressed as standard deviation for two measurements.

The separation of both mercury species was reached in less than five minutes for all three systems, with GC–ICP-MS providing elution of both species with baseline separation in less than two minutes. Differences in chromatographic performance between the systems is mainly due to the GC setup itself, e.g. normal bore column in GC–MS and GC-pyro-AFS versus wide-bore column in GC–ICP-MS with high carrier gas flow. Another factor is the species transfer between the GC and the respective detector, which in the case of the laboratory made GC-pyro-AFS is a major reason for peak broadening.

3.2. Robustness

The United States Pharmacopoeia (USP) definition of robustness is: "The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage" [28]. Robustness testing is gaining interest and becoming increasingly more important to meet the authorities' strict regulations [21,22]. However, robustness tests on GC methods are only scarcely found in the literature. Thus, in this work the robustness was tested in order to study the influence of slight changes in factors affecting the separation conditions as is peak resolution, plate number, and peak area or relative peak area of the analytes. For this study, a Plackett–Burman fractional factorial model based on an experimental design of 7 factors and 15 experiments ($n = 15$) has been used [29]. The selected factors for each technique and the variations studied around the optimal value (underlined) are shown in Table 3.

The mean effect of each variable is the average difference between observations at the extreme levels and those at the optimal level. Mean effects and standard errors (DA, DB, DC, etc.) were calculated using the procedures described by Youden and Steiner [30]. The values of the variations of the seven factors on all the responses evaluated for the three systems GC-pyro-AFS, GC–MS and GC–ICP-MS were always within the range calculated using the Youden and Steiner statistical model [30]. This means that all three methods are robust. As an example, in Fig. 1 the variations of the factors on the plate number and resolution of Hg^{2+} using GC-pyro-AFS, GC–MS and GC–ICP-MS are shown. Similar results were obtained for peak resolution, plate number, peak areas and also relative peak areas in the case of MeHg for any of the three systems.

3.3. Analysis of CRMs

The analytical performance of the systems was evaluated by the analysis of two biological certified reference materials (DOLT-3 and DORM-2). A previously optimized closed-vessel microwave-assisted extraction procedure with TMAH as extractant was used (see Section 2.4). Then the derivatization was carried out at pH 3.9 with $NaBEt_4$.

Typical chromatograms obtained for MeHg and Hg^{2+} in fish tissues using aqueous ethylation are shown in Fig. 2. No GC–ICP-MS data were obtained for DORM-2, as the reference material ran out in the course of this work and could not be sourced again. The concentrations obtained for the reference materials are given in Table 4 showing excellent agreement with the certified values for inorganic and methylmercury compounds, and recoveries ranging from 95 to 101%. The three average results were compared by using Student's "*t*" test, and no statistically significant differences were found in either case ($p = 0.05$).

3.4. Application to spiked water samples

Mercury is present in the environment in form of different complexes with inorganic and organic ligands [12,31]. For inorganic ligands, the most important are the hydroxo-complexes and chloro-complexes for freshwater and marine systems, respectively. Organic ligands containing thiol groups (SH), and especially humic acids (HA) are very important complexing agents influencing the biogeochemical cycle of Hg. Therefore, to perform an evaluation of the methods for environmental matrices, synthetic water matrices with different salinities and HA contents were spiked with both mercury species in concentrations of 5 μg/L and 10 μg/L for MeHg and Hg^{2+}, respectively, in the GC-pyro-AFS and GC–MS systems, and 1 μg/L and 4 μg/L MeHg and Hg^{2+}, for GC–ICP-MS analysis. Tests with high levels of NaCl were chosen to simulate sea-water conditions. The recovery for the determination of mercury species under these conditions is presented in Table 5, showing that both species are quantitatively determined despite high humic acid concentration in the absence of high salinity (no NaCl added). However, the recoveries were below 70% and 83% for MeHg and Hg^{2+}, respectively, in the presence of 35‰ NaCl, mimicking sea water conditions. This behaviour backs the fact that Cl^- interferes in the derivatization of mercury species [32,33] and as a conse-

Table 6
Comparison of different mercury speciation hyphenated systems.

Factor	GC-pyro-AFS	GC–MS	GC–ICP-MS
Sensitivity	Good	Good	Excellent
Selectivity	Excellent	Good	Excellent
Commercially available instrumentation	Poor	Excellent	Poor
Cost of analysis	Good	Good	Poor
Simplicity of operation	Poor	Excellent	Poor
Isotope dilution analysis	Not possible	Good	Excellent
Multielemental analysis	Not possible	Good	Excellent

quence, the recoveries cannot be quantitative. In contrast to other authors' observations, we did not find significant conversion of mercury species to elemental mercury. Most probably, the recovery is compromised by non-quantitative ethylation rather than species conversion and loss. Here, species specific isotope spikes may be employed; however this is beyond the scope of this paper.

4. Conclusion

All evaluated techniques showed sufficient sensitivity and selectivity for the speciation of mercury in environmental samples in a variety of matrices. According to the findings of the robustness test, all varieties of instrumentation is considered to be robust, which is encouraging with regards to application on a routine basis. A comparison of is shown in Table 6. GC–MS shows enough sensitivity with regards to detection and quantification limits of both species. The linear dynamic range is sufficient for the low analyte concentrations found in environmental samples, particularly biological tissues. The main advantage is that there are commercially available instruments supported by a certified post-sale service. However, the elution at the analytes' retention time of organic compounds producing interfering molecular ions may pose important sources of error. To overcome these problems, several approaches can be adopted. The need for a clean-up step at the end of the sample preparation procedure to minimize the occurrence of co-eluting organic compounds would be mandatory, particularly when the analysis of complicated matrices is carried out. Thus, it represents a real alternative only when species-specific isotope dilution analysis is carried out. In this case, also different mathematical equations are used for correction [18,34].

The GC–ICP-MS and GC-pyro-AFS benefit from a high degree of element specificity, are relatively free from interferences and show low detection and quantification limits with a satisfactory linear dynamic range. The best performance is obtained with GC–ICP-MS due to its simultaneous multielemental capabilities and the possibility for highly accurate species-specific isotope dilution analysis. Their main inconveniences are the high instrumental and operational costs, also it is a quite sophisticated system and qualified personal is required, with GC-pyro-AFS being comparatively lower in cost and simpler to operate. However, the main problem for routine laboratory analysis application of GC–ICP-MS and GC-pyro-AFS systems is that, in general, they are home-made hyphenated systems and there are not generally available instruments, only two GC–ICP-MS and one GC-pyro-AFS interface are commercially available from instrument manufacturers. Therefore, it is necessary to develop cheaper and widespread instrumentation to really implement these systems in routine testing laboratories. This necessity is underpinned by recent developments of commercial systems for automated speciation analysis.

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