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Artículo Científico

Speciation analysis of organomercurial compounds in Fish Tissue by capillary gas chromatography coupled to microwave-induced plasma atomic emission detection

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Resumen:

El presente trabajo describe una metodología para la determinación de especies de mercurio en tejido de peces mediante Cromatografía de Gases utilizando como detección la Espectrometría de Emisión Óptica con Plasma Inducido por Microondas (GC-MIP-OES) utilizando un surfatron como cavidad resonante. Las especies de mercurio fueron extraídas cuantitativamente por lixiviación con ultrasonido utilizando una mezcla ácido-tolueno. Los límites de detección obtenidos fueron de 5 y 9 pg para metilmercurio (MeHg) y etilmercurio (EtHg), respectivamente. Los cromatogramas fueron obtenidos en 1,5 min. Las concentraciones de MeHg con GC-MIP-OES fueron similares a las obtenidas para mercurio orgánico mediante el método de reducción selectiva y análisis por espectrometría de absorción atómica con vapor frío (CV-AAS).

Palabras clave: Especies organomercuriales; análisis de especiacion; tejido de peces; Cromatografía capilar de gases, Espectrometría de emisión con plasmas de microondas, cavidad resonante de surfatron

Abstract

This paper describes a novel approach for analysis of mercury speciation in fish using gas chromatography coupled with microwave-induced plasma optical emission spectrometry (GC-MIP-OES) in surfatron resonant cavity. Sample treatment was based on quantitative leaching of mercury species from fish tissue with ultrasound-assisted acid-toluene extraction. The extracted mercury species analyzed with GC-MIP-OES attained detection limits of 5 and 9 pg for methylmercury (MeHg) and ethylmercury (EtHg), respectively. A complete chromatogram could be completed in 1.5 min. MeHg values obtained with GC-MIP-OES were matched with organic mercury values obtained with selective reduction cold vapour- atomic absorption spectrometry (CV-AAS).

Keywords: Organomercurial compounds; speciation analysis; fish tissue; capillary gas chromatography; microwave-induced plasma emission spectrometry; surfatron resonance cavity.

Introduction

Mercury from the environment is one of the most toxic elements for humans. Organomercury compounds present greater toxicity than inorganic ones. Methylmercury (MeHg) is the most toxic substance in aquatic ecosystems, because of bioaccumulation and biomagnification in the aquatic food chain[1,2]. Dietary intake of contaminated fish and seafood is the main route to Hg exposure in human populations. It has been reported that the relative contents of MeHg in fish muscle tissue may reach up to 100% of its total Hg[3-8]. MeHg from fish consumption is considered a dominant source of mercury exposure in no occupationally-exposed populations. Blood and urine mercury concentrations were positively correlated with fish consumption[9,10]. On the other hand, studies performed on environmental and human samples from places where mining activities had ceased approximately 15-20 years before revealed Hg concentrations in fish and humans similar to those measured during the Gold Rush[11].

Several techniques have been proposed for mercury speciation in biological and environmental samples. In general, coupled-analysis techniques are applied for speciation. Gas chromatography (GC) is preferred for volatile or easily formed volatile derivative species. Thus,

GC is the most popular separation technique for mercury and organomercury compound speciation, while detection is carried out by atomic spectrometric, such as atomic absorption spectrometry (AAS), atomic fluorescence spectrometry (AFS), electrothermal atomization atomic absorption spectrometry (ETAAS), mass spectrometry (MS), inductively-coupled plasma mass spectrometry (ICP-MS), inductively-coupled plasma atomic emission spectrometry (ICP-OES), and microwave-induced plasma atomic emission spectrometry (MIP-OES)[12-14]. GC–MIP-OES is a viable alternative for mercury speciation. The fundamental aspects of microwave-induced plasmas (MIPs) and their physical properties and applications are reviewed by Rosenkranz[15] and Broekaert[16]. Several types of plasma cavities have been coupled with GC. The most commonly used for this purpose are the Beenakker cavity as a laboratory assembled system coupling technique[17-21] or the commercial atomic emission detector[22-31]. After evaluating the application of commercial GC-MIP-OES instrumentation, Sanz et al.[28] concluded that the relatively low price of required instrumentation, commercial availability of hyphenation between chromatograph and detector, and simplicity of sample preparation, confirm the suitability of this technique for routine mercury speciation analysis. The Evenson-type cavity[5,32,33] has also been used with good results. However, surfatron-type cavity coupled with GC has only been used for halogenated compound determination[34]. Siemens[35] made a comparative study of Beenakker and Surfatron cavities, and found similar detection limits. Rosenkranz and Bettmer[15] discussed the advantages of surfatron over the Beenakker cavity. Mainly they emphasized that the surfatron is easier to tune and to operate than the TM_{010} cavity. We obtained a very low limit of detection (LOD) in total mercury determination by MIP-OES in surfatron resonant cavity with cold vapour generation[36]. Use of MIP in surfatron cavity for mercury speciation analysis appears, is still unreported. Given the low LOD found by us in total Hg determination by MIP with surfatron cavity[36], it seemed important to study its potential as a detector for mercury speciation analysis.

Our research intended to develop a simple and rapid method for methylmercury and ethylmercury speciation in fish tissue samples involving organomercurial compound extraction with ultrasound-assisted organic solvents and later capillary gas GC-MIP-OES in surfatron resonant cavity as the detection technique. Instrumental variables and conditions for mercury speciation were optimized.

2. Experimental

2.1. Reagents

All reagents used were of analytical-reagent grade unless otherwise started. Methylmercury chloride (MeHgCl 99.8%) and chloride acid (HCl) were purchased from Merck (Darmstadt Germany), ethylmercury chloride (EtHgCl) from Alfa Aestar (Ward Hill, MA, USA), and chloroform and benzene from Fisher Scientific Co. (USA). Toluene and dichloromethane were obtained from Riedel-deHaen (Seelze, Germany) and tetrahydrofuran; from Baker Analyzed (Phillipsburg, NJ, USA). Stock solutions of 1000 mg l^{-1} (as $MeHg^{+}$ and $EtHg^{+}$) were prepared dissolving the appropriate amounts of reagent in toluene. Working standard solutions were prepared daily diluting stock in toluene. A 1% solution of mercury chloride in toluene was used as the column conditioning solution. Helium (99.9999%) (BOC GAS, Venezuela) was used as carrier gas. Stannous chloride (2% w/v) used as a reducing agent was prepared by dissolving the appropriate mass of stannous chloride dehydrate ($SnCl_2$) (Merck, Darmstadt, Germany) in 2M HCl. Sodium borohydride ($NaBH_4$) from Riedel-deHaen (Seelze, Germany) 0.5% w/v was prepared fresh daily by dissolving the solid in 0.2% NaOH solution. Inorganic mercury (Hg^{2+}) stock standard solution (100 mg l^{-1}) was prepared from mercury chloride ($HgCl_2$), (Merck, Darmstadt Germany). For the sample digestion procedures, 10 M potassium hydroxide (KOH) and 10% w/v sodium chloride (NaCl) Baker Analyzed (Phillipsburg, NJ, USA) were prepared.

2.2. Instrumentation

A Hewlett-Packard 5730A gas chromatograph (GC) (Hewlett-Packard, Wilmington, DE, USA) was coupled to helium microwave-induced plasma (MIP). Instrument configuration is schematically illustrated in Fig. 1 and optimal working conditions are shown in Table 1. The microwave plasma was generated in a laboratory-built Surfatron cavity described earlier[34,36]. The Surfatron is fed by an Electromedical Supplies (Oxfordshire England) model Microtron 200 Mark III (0-200 W, 2450±24 MHz) microwave generator. Helium gas is used to induce and support the discharge. A Brook model 5878 (USA) flowmeter was used for plasma gas flow rate controlled. The chromatographer was provided with two non-polar capillary columns (HP-1, 12m×0.25mm×250µm, and HP-5, 30m×0.25mm×250µm, USA) and helium was used as carrier gas.

The eluted mercury species were transferred to the detector through an interface. Fig. 2 shows a drawing of the interface used, which includes a quartz T tube. The interface was kept at 215ºC with a heating resistance to avoid product condensation. The end of the analytical column was inserted into the T, horizontally to the plasma tube, up to 2 mm short of the start of plasma, while helium gas was introduced through the lower vertical extreme. Plasma was observed axially using a Jarrel-Ash model 82-000 (U.S.A.) spectrometer. Data acquisition was

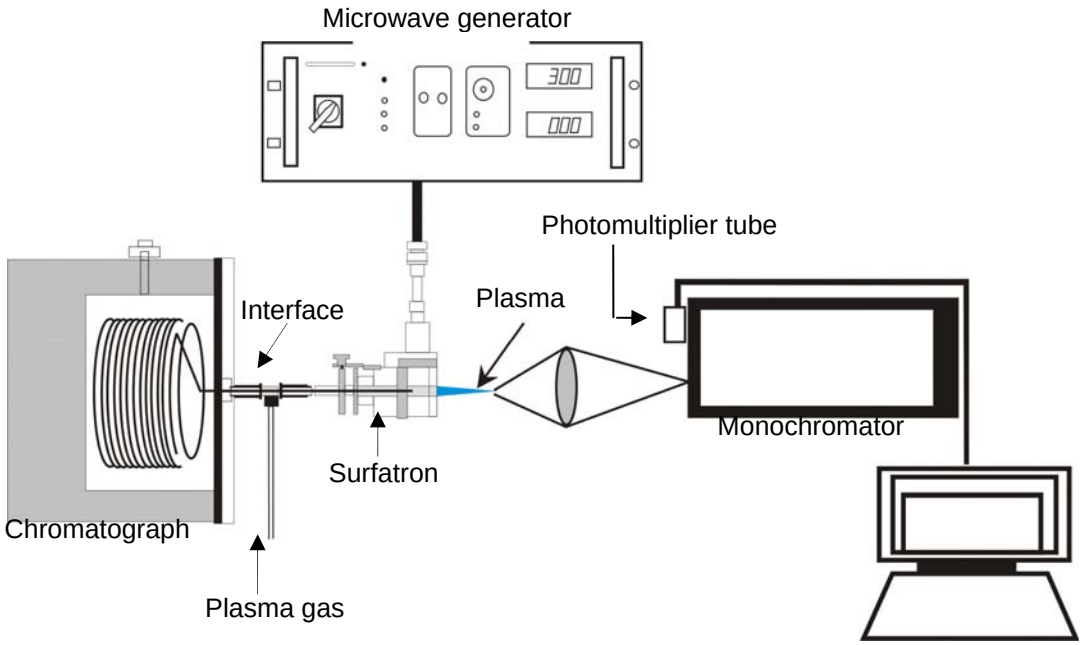

Figure 1. Diagram of the CG-MIP-OES system

performed using the EZChrom Chromatography Data System version 6.8 Scientific Software, Inc. (USA). A Hewlett-Packard model HP-6890 gas chromatograph-mass spectrometer (GC-MS) and a Mass Selective Detector 5973 with a HP-5 column were used for preliminary studies to examine separation of methyl- and ethyl mercury. Working conditions for this separation were as follows: injection volume, 0.2 µl; mode of injection, split (1/100); temperature at point of injection, 210°C; He column flow, 1 ml/min; the program temperature used, 150°C (5 min). A Perkin-Elmer flow injection mercury system (FIMS) Model 100 (Überlingen, Germany), equipped with a flow injection analysis system (FIAS) was used for all total and inorganic determinations[37]. Two ultrasonic bath systems were used for sample preparation: A Cole Palmer model 8894 (U.S.A.) and an Eppendorf (Hamburg, Germany), model 580.

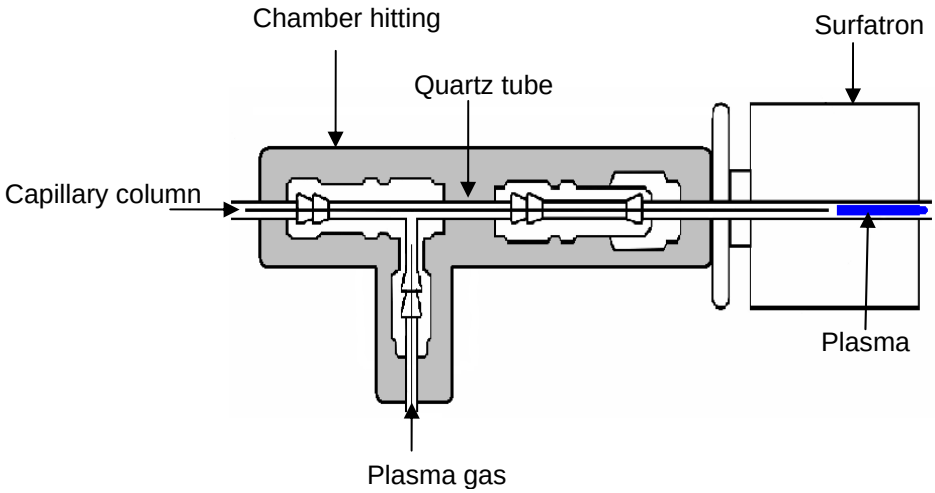

Figure 2. GC-MIP-OES interface

Table 1. Working conditions (GC-MIP-OES)

Gas Chromatography	Microwave-Induced Plasma-Optical Emission Spectroscopy
Column HP-5MS	Wavelength: 253.65 nm
Carrier gas: Helium	Helium flow at cavity: 50 ml min^{-1}
Carrier gas flow: 1.4 ml min^{-1}	Plasma power: 85 W
Split ratio: 2/10	Incident power: <1%
Injection port temperature: 250ºC	
Oven Temperature: 250°C	
Injection volume: 0.4 µl	

2.3 Analytical procedure

2.3.1 Procedure A (for speciation analysis by GC-MIP-OES)

Sample extraction was performed with a procedure similar to that described by the Association of Official Analytical Chemistry (AOAC)[38]. About 0.5 g of the fish tissue previously homogenised, was accurately weighed in a vial and 0.4 ml of 6 M HCl and 1.6 ml of toluene were added. The mixture was crushed with a glass rod and 0.2 ml of 6 M HCl was added. The slurry was sonicated in an ultrasonic bath at 60ºC until the organic and aqueous layers were separated (30 minutes). The organic phase was finally removed and kept at 5ºC until analysis. Four tenth microliters of organic extract were injected into the injection port of the chromatograph on split mode and analyzed by GC-MIP OES under the experimental conditions shown in Table 1. Blanks were prepared in parallel.

2.3.2 Procedure B (for selective determination of inorganic and total mercury by CVAAS)

This method was optimized in a previous study[2,6,37], about 1 g of the fish tissue was accurately weighed in a digestion tube, and 5 ml of 10 M KOH and 2 ml of 10% (w/v) NaCl were added. The centrifuge tube was then stoppered and was heating at 60ºC in one ultrasonic bath during 30 min. Them H_2O was added until a weight of 10 g., finally centrifuged at 3000 rpm for 10 min. After, the supernatant was transferred into a vial. An aliquot of 1 ml was transferred into a centrifuge tube, and 1 ml of 10% (w/v) NaCl and 5 ml of 7.2 M $HN0_3$ were added. Them H_2O was added until a weight of 10 g., finally centrifuged at 3000 rpm for 10 min. and the supernatant was taken for to determination of the inorganic mercury by CV-AAS using $SnCl_2$ as reducing agent. For total mercury determination the samples following, the same procedure described plus 0.5 ml of 1% w/v $K_2Cr_2O_7$ was added with the acid. For the total mercury determination by CV-AAS, $NaBH_4$ was used as reducing agents. Blanks were prepared in parallel.

2.3.3 Fish samples

Five samples of fish from Bolívar State, in southern Venezuela were analyzed. The samples were transported to the laboratory in an ice compartment. The samples were homogenised, frozen and kept at -20ºC until use.

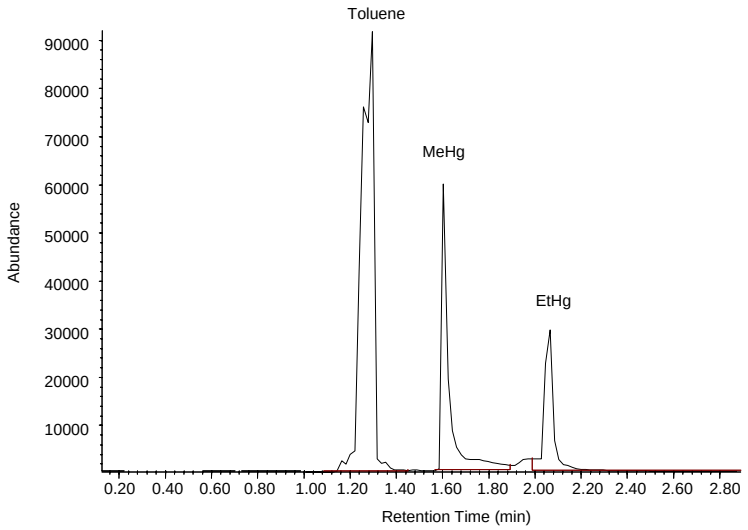

Figure 3. Chromatogram of 200 mg l^{-1} of species MeHg and EtHg obtained by GC-MS

3. Results and discussion

3.1 Preliminary studies

The chromatograph-mass spectrometer (GC-MS) technique was used for determining retention times of MeHg and EtHg. Retention times values obtained were 1.65 and 2.05 minutes for MeHg and EtHg, respectively. The chromatogram obtained displaying a good separation of MeHg and EtHg is shown in Fig. 3.

3.2. Study of the instrumental parameters of the GC-MIP-OES system

Generator power was optimized to the level it gave the best emission signal, and the top peak area obtained was at 85 W. Similarly, several solvents were tried to choose the least disturbing for plasma operating conditions. Toluene turned out to be the most adequate. In addition, mercury species show a high solubility in that solvent and it is the most frequently used.

Interface heating, as shown in Fig. 4, generated an increase in the chromatographic peak areas and a drop off in their retention times. Temperatures between 200 and 220°C were evaluated and the best signal was found at 215°C. Below that temperature, the chromatographic peak widens, probably due to mercury species condensation during interface transfer, which is most evident when heating is not applied. The temperature of 215°C was chosen for the next essays. Fig. 5 shows chromatograms of a fish sample, the blank and standards of MeHg and EtHg in toluene using the best experimental conditions found in this work. A good separation was achieved for MeHg and EtHg.

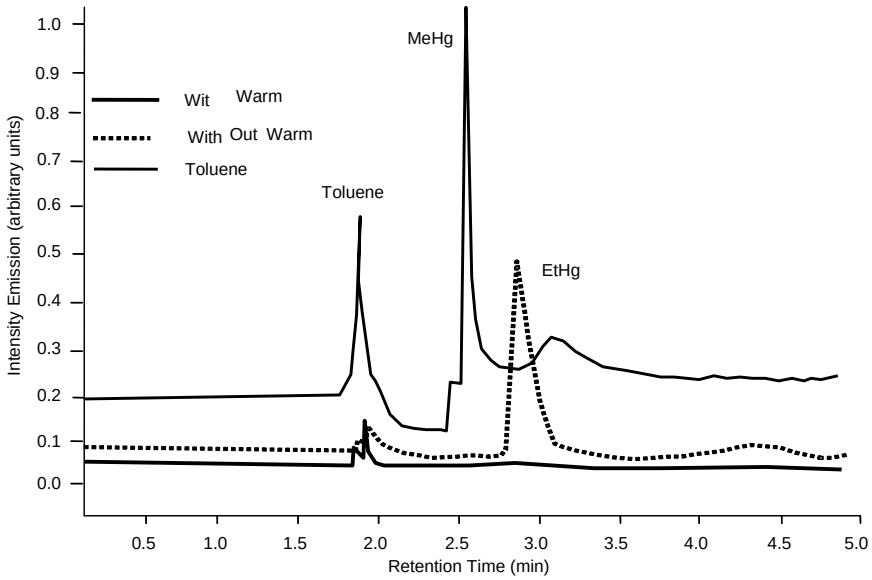

Figure 4. Chromatogram for 0.4 µl of a standard of 40 µg l^{-1} of **MeHg** and 160 µg l^{-1} of **EtHg** in toluene

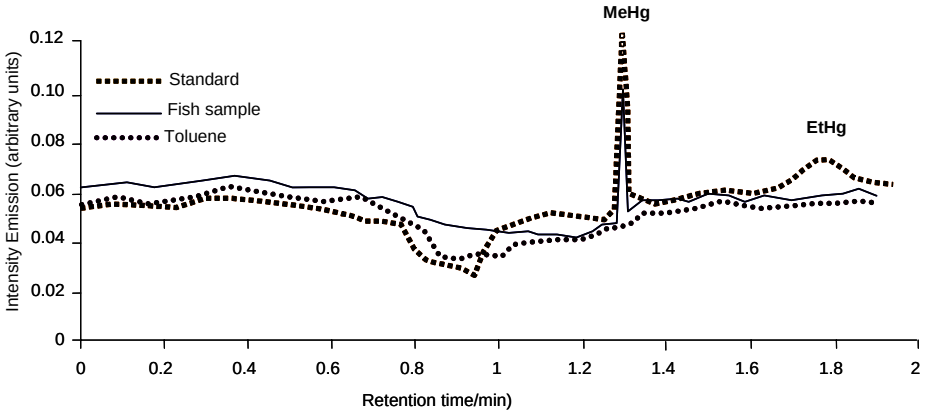

Figure 5. Chromatograms 0.4 µl of fish sample, blank and standard of 0.4 µg ml^{-1} MeHg and 1 µg ml^{-1} EtHg chloride in toluene.

3.3 Analytical figures of merit

Table 2 shows characteristics of calibration curves. Clearly both curves exhibit an R^2 close to 1, implying a good linear correlation between signal and concentration for both species.

Table 2. Characteristics of calibration curves.

Compounds	Equation of straight	R^2	Concentration Interval µg l^{-1}
MeHg	Y = 19,604X+1694,3	0,9913	70 – 510
EtHg	Y = 6,9833X+3108,5	0,9952	200 - 630

Limits of detection (LOD) and limits of determination calculated as three times and ten times, respectively, the standard deviation of ten blank measurements are shown in Table 3, where the LOD value of MeHg is lower than that of EtHg, which was predictable as the method was more sensitive for MeHg, as indicated by the calibration curve slopes for both species. Slope value for MeHg is approximately three times that of EtHg (Table 2).

Table 3. Limits of detection and limits of quantification for MeHg and EtHg

Organic species	LOD (µg/L)	LOD (pg)	LOQ (µg/L)
MeHg	53	5	75
EtHg	178	9	322

LOD: limit of detection; LOQ: limit of quantification

LODs obtained by derivatization are found to be of the same order than those reported using the Beenakker cavity as the laboratory coupling technique (Table 4). As seen in Table 4, the best LODs reported were obtained with the commercial coupling.

Reproducibility of the method was verified by calculating mean deviation (S = 20), standard deviation (S = 25), quotient of variation (%CV = 6.25) and confidence interval at 95% (L.C. 95% = 379-417) for nine determinations of the same sample with $\alpha = 0.025$ and a mean of 398 ng g^{-1} of MeHg.

Table 4. Hyphenated techniques with element specific detection for mercury speciation.

Mercury compounds	Cavity type	Limit of detection	Reference
CH_3Hg^+ $CH_3CH_2Hg^+$	GC-MIP-AES Beenakker derivatization	0.8 pg of Hg [a] 12.7 pg of Hg [a]	18
CH_3Hg^+	GC-MIP-AES Beenakker derivatization	0.4 pg of Hg ($3\sigma_b$)[b]	19
CH_3Hg^+	GC-MIP-AES Beenakker	4.4 ng g^{-1} of Hg ($3\sigma_b$)	20
$(CH_3)_2Hg^+$ CH_3Hg^+ Hg^{+2}	CCT[c]-MIP-AES Beenakker derivatization	150 pg ($3\sigma_b$) 24 pg ($3\sigma_b$) 32 pg ($3\sigma_b$)	21
CH_3Hg^+	GC-MIP-AES (MIP/AED HP 5921A)	1.2 pg (S/N[d]=3)	22
CH_3Hg^+	GC-MIP-AES (MIP/AED HP 5921A)	0.8 pg of Hg (S/N=3:1)	23
CH_3Hg^+Cl	GC-MIP-AES (MIP/AED HP 5921A) derivatization	0.6 and 2.5 pg of Hg ($3\sigma_b$)	24
CH_3Hg^+ derivatization	SPMD[e]-GC-MIP-AES (MIP/AED HP 5921A)	0.12 µg l^{-1} of Hg ($3\sigma_b$)	26
CH_3Hg^+ derivatization	SPMD-GC-MIP-AES (MIP/AED HP 5921A) derivatization	0.1 µg l^{-1}	27
CH_3Hg^+ derivatization $(CH_3)_2Hg^+$ Hg^{+2}	GC-MIP-AES (MIP/AED HP 2350)	3 pg (S/N=3) 0.5 pg 15 pg	28
CH_3Hg^+ derivatization	GC-MIP-AES (MIP/AED HP 2350)	3 pg ($3\sigma_b$)	29
CH_3Hg^+	GC-MIP-AES (MIP/AED Agilent Technologies G2350A)	640 pg ($3\sigma_b$)	30
CH_3Hg^+	GC-MIP-AES Evenson	20 ng g^{-1} ($2\sigma_b$)	32
CH_3Hg^+	GC-MIP Evenson	0.5 µg l^{-1} ($2\sigma_b$)	33
CH_3Hg^+ $(CH_3)_2Hg^+$	GC-MIP- OES Surfatron	5 pg 9 pg	Our results

[a]Detection limit as mass of analyte (as Hg) giving a peak height equal to the peak-to-peak baseline noise. [b]Standard deviation of the background. [c]Capillary cold trap. [d]Signal/noise. [e]Solid-phase microextraction procedure.

Table 5 shows a comparison of the organic mercury content determined by CV-AAS with selective reduction and the content of the species of MeHg determined by GC MIP-OES with the surfatron cavity proposed. In four of the five samples, the value of the concentration of MeHg determined by the GC-MIP-OES in surfatron cavity method statistically matched (95% confidence) the total values organic mercury previously determined by CV-AAS. These results were verified by a significance contrast test between the means of total mercury and MeHg for equal variances. These results demonstrate the accuracy of the proposed method.

Table 5. Concentration organic Hg and MeHg by selective reduction CV-AAS and GC-MIP-OES

Sample	CV-AAS Total Hg (ng g^{-1})	*% CV*	GC-MIP-OES MeHg (ng g^{-1})	*% CV*
Guri-8	688 ± 110	16	720 ± 50	7
Tuna fish	415 ± 58	11	392 ± 28	7
Guri-5	504 ± 20	4	427 ± 23	6
Guri-6	585 ± 31	7	578 ± 36	5
Castellón	1254 ± 122	11	1300 ± 60	5

3.4 Application to fish samples

The method was applied to determine MeHg and EtHg content in five samples of fish tissue from Bolívar State, in southern Venezuela. Results, shown in Table 6, indicate that concentrations of MeHg are within the same range as reported by other researchers[5,21]. It was impossible to determine the concentration of EtHg in the samples, as this species is under the limit of quantification. Results agree with other researchers' findings, who have reported that 95% of total mercury from samples of fish tissue is in the form of MeHg[1,5,21,39].

Table 6. Results of speciation analysis of fish tissue samples by GC-MIP-OES

Sample	MeHg (ng g^{-1})	*% CV*	EtHg (ng g^{-1})*
Guri-8	720 ± 50	7	<320
Tuna fish	392 ± 28	7	<320
Guri-5	427 ± 23	6	<320
Guri-6	578 ± 36	5	<320
Castellón	1300 ± 60	5	<320

* Non detectable in a 0.5-g sample

4. Conclusions

The proposed method allows for direct separation and determination of MeHg and EtHg, replacing the tedious methods of species derivatization.

The limits of detection found for MeHg and EtHg were 5 and 9 pg, respectively.

With this procedure, once the sample treatment has been performed, more than 60 samples may be analyzed every day. It requires no large amount of sample for analysis.

Reproducibility of the method expressed as %RSD was 6%, which is acceptable with complex systems coupling two techniques, and at the low concentrations shown in biological samples.

Values of concentration of MeHg and organic Hg determined by different techniques show no statistically significant differences may be an indication of the reliability of the proposed method.

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