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Speciation of organomercurials in biological and environmental samples by gas chromatography with microwave-induced plasma atomic emission detection

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Abstract

The applicability of a commercial microwave-induced plasma atomic emission detector with capillary gas chromatography for mercury speciation in environmental samples was examined. The chromatographic conditions were optimized in order to obtain an adequate resolution of the methylmercury peak vs. interfering carbon signals. Under the proposed operational conditions, the detection limit (signal-to-noise ratio = 3) was 1.2 pg with a linear range of 1–40 ng ml^{-1} (as methylmercury in samples). Certified reference material (DORM-1) was used to evaluate the accuracy. The results of the proposed procedure were compared with those obtained by means of the usual GC method with electron-capture detection.

1. Introduction

The impact of the production and application of mercury and its compounds on the environment has increased in recent decades [1]. Methylmercury is the organomercurial compound most commonly found in aqueous environments. The ecotoxicity of this compound is well known [2] and it has been found in high concentrations in tuna, swordfish, molluscs and sediments [3].

Several highly sensitive methods have been developed for the determination and speciation of mercury in the environment. Gas chromatography with electron-capture detection (GC–ECD) has been the most widely used. Columns packed with different stationary phases [4–8] have been used, but they have all had some drawbacks on application, e.g., a small and variable response to methylmercury, peaks with tails and poor selectivity in the presence of interferences [9]. Recently, various capillary columns with polar and non-polar stationary phases have been evaluated [6,10–12]. The columns having the thickest phase and lowest polarity are the most appropriate for obtaining satisfactory separations and reproducible results.

In addition, other techniques, such as atomic spectroscopy, coupled with GC have been used. In order to improve the separation and detection of mercury compounds, they have been derivatized with sodium tetraethylborate ($NaBEt_4$), sodium tetrahydroborate ($NaBH_4$) and lithium triethylhydroborate ($LiBEt_3H$). Volatile derivatives, separated using GC, have been detected by means of atomic absorption [13–17], atomic

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fluorescence [18], mass [16] or Fourier transform infrared spectrometry [19].

All the procedures proposed for the determination of organomercurial compounds highlight the need for a highly selective and sensitive detection system. Since the introduction of detection using atomic emission spectrometry combined with GC (GC–AED) [20,21], the selectivity in the speciation of organometallic compounds [22,23] has improved enormously. Not only is there high selectivity, but there is also high sensitivity and the possibility of multi-elemental analysis with a wide dynamic range. Plasma detectors that have been used for mercury speciation include the inductively coupled plasma (ICP) [24] and microwave-induced plasma (MIP) [6,25–28] types. Organomercurial compounds were converted into the iodide form [24–26] or derivatized with a Grignard reagent to obtain dialkyl derivatives [27].

In this paper we present the results of a study carried out to check the applicability of GC–AED to the direct determination of methylmercury in environmental samples.

2. Experimental

2.1. Chemicals

Methylmercury chloride (99%) was obtained from Merck (Darmstadt, Germany). A stock standard solution (1.164 mg ml^{-1} in toluene) was used to prepare the working standard solutions by dilution and to spike the samples where necessary. A 1% solution of mercury chloride (99.5%) (Merck) in toluene was used as a column-conditioning solution. Cysteine chlorohydrate (98.5%), 2-propanol, HCl, sodium sulphate (anhydrous, 99%), toluene and acetone were obtained from BDH (Poole, UK) and sodium acetate (99.5%) from Merck. Helium (99.9999%) (Carburos Metálicos, Coruña, Spain) was used as both the carrier gas and reagent gas. Oxygen and hydrogen (99.999%) (Carburos Metálicos) were used as auxiliary gases.

2.2. GC and AED instrumentation

All the experiments were carried out with Hewlett-Packard (Palo Alto, CA, USA) Model 5890 Series II gas chromatographs. For GC–ECD the instruments were equipped with a nickel-63 electron-capture detector using N55 nitrogen as the carrier and make-up gas. Data were acquired by means of a Hewlett-Packard Model 3396A integrator. For GC–AED a Hewlett-Packard Model 5921A microwave-induced plasma atomic emission spectrometer tuned at 185 nm (for mercury) and 193 nm (for carbon) was used. Data acquisition and reprocessing were carried out by means of a Hewlett-Packard Model 3592A Chemstation. The chromatographic columns tested (Hewlett-Packard) and operating conditions are summarized in Table 1.

2.3. Sample preparation

The procedure described by Hight and Corcoran [5] was used for the extraction of methylmercury from marine samples, with modifications as detailed elsewhere [29]. The procedure of Westöö [30] was used for the extraction of sediment samples, with modifications for freeze-dried sediment samples as described recently [31].

3. Results and discussion

3.1. Optimization of GC and detection conditions

The GC conditions were adapted from the parameters previously optimized for GC–ECD [10]. The effect of the injector temperature on the separation of the methylmercury peak was studied. Using the splitless mode, the temperatures tested were 150, 200 and 250°C. The column head pressure ranged between 140 and 160 kPa.

Several oven temperatures were tested with the purpose of separating the methylmercury peak from the front solvent (toluene) and concentrating the sample on the column head. The

Table 1
Optimum GC–AED parameters

Parameter	HP-1 column	HP-5 column
GC parameters		
Dimensions	25 m × 32 μm × 0.17 μm	25 m × 32 μm × 0.17 μm
Injection port	Split–splitless	Split–splitless
Injection port temperature	150°C	200°C
Split mode	Splitless	Splitless
Purge time	20 s	90 s
Splitting ratio	1:10	1:10
Septum purge	2.6 ml min^{-1}	2 ml min^{-1}
Injection volume	1.5 μl	1.5 μl
Column head pressure	140 kPa	140 kPa
Oven initial temperature	90°C	75°C
Ramp rate	–	30°C min^{-1}
Oven final temperature	–	140°C
Interface parameters		
Transfer line	HP-1 column	HP-5 column
Transfer line temperature	160°C	160°C
AED parameters		
Wavelength	185 nm (Hg), 193 nm (C)	185 nm (Hg), 193 nm (C)
Helium make-up flow-rate	60 ml min^{-1}	60 ml min^{-1}
Ferrule purge vent	20 ml min^{-1}	30 ml min^{-1}
Scavenger gases:		
Hydrogen	200 kPa	200 kPa
Oxygen	200 kPa	200 kPa
Helium supply purge	205 kPa	205 kPa
Spectrometer purge flow-rate	2 ml min^{-1} N_2	2 ml min^{-1} N_2
Solvent vent off-time	0.6–1.45 min	0.5–2.2 min
Cavity temperature	250°C	250°C

best separation was obtained for a constant temperature of 90°C with the HP-1 column. With an HP-5 column a good separation was achieved with a constant temperature of 90°C but sensitivity was improved and the analysis time was shortened by using temperature programming. The transfer line temperature must be higher than the oven temperature in order to avoid condensation, which could broaden the peak. This temperature was varied between 150 and 250°C. The optimum conditions are summarized in Table 1.

The need for the chromatographic columns to be conditioned with $HgCl_2$ to obtain satisfactory separations and reproducible results has been sufficiently confirmed [4,10]. The treatment of the HP-1 column consisted of 3–5 injections of 2–3 μl of 1% $HgCl_2$ solution in toluene. The HP-5 column underwent treatments consisting of 2–3 injections of 10 μl of 1% $HgCl_2$ solution. Both columns remained disconnected from the detector while treatment was applied, at a temperature of 90°C in the oven and in the transfer line for 12 h. The column was then reconnected to the detector, the baseline checked and a new calibration started.

The effect of the treatment was observed to be short-lived with the HP-1 column, with the sensitivity diminishing after a few hours of work. With the HP-5 column, the treatments remained efficient for over 1 week. This is to be expected when the thicknesses of the phases in the HP-5 (1.05 μm) and HP-1 (0.17 μm) columns [10] are considered.

3.2. AED optimization

The AED instrument allows the Hg emission signal to be measured at two wavelengths, 185 and 254 nm. The responses obtained for the two wavelengths were similar. However, we chose 185 nm because it allows the presence of Hg to be confirmed in the methylmercury peak by means of the emission spectrum. The equipment prevented us from using the same emission spectrum of Hg at 254 nm.

Solvent vent-off time

In order to prevent the solvent (toluene) from entering the discharge tube at high concentrations, toluene vapour was injected while monitoring the carbon emission line at 193 nm. Under the conditions described above, most of the toluene was eluted between 0.6 and 1.45 min for the HP-1 and between 0.5 and 2.2 min for the HP-5 column.

Effect of make-up gas flow-rate

In accordance with published data [32,33], it is necessary to work with a high helium make-up gas flow-rate in order to have good sensitivity for the detection of organometallic compounds. However, with Hg, a high helium make-up gas flow-rate produces a significant decrease in sensitivity [34]. In order to determine the optimum helium make-up gas flow-rate that allows the maximum sensitivity, a standard solution of 0.010 μg ml^{-1} MeHg was injected using different make-up gas flow-rates. Fig. 1 shows the variation of the peak area with the make-up gas flow-rate. Similarly, differences in the baseline and shape of the methylmercury peak were seen when the flow-rate was changed. The optimum flow-rate was established to be 60 ml min^{-1}.

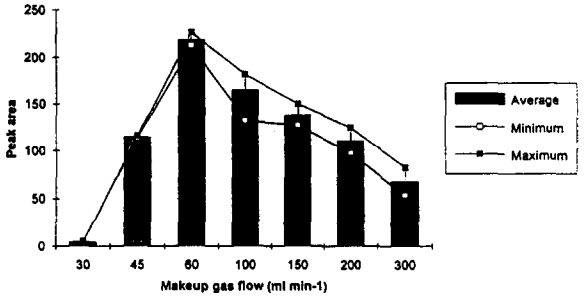

Fig. 1. Influence of the make-up gas flow-rate on the peak area of methylmercury using GC–AED.

3.3. Identification of the methylmercury peak

Although the AED system is highly selective for Hg at 185 nm, the presence of methylmercury must be confirmed by recording the emission spectrum at the peak and comparing it with the emission spectrum of Hg (Fig. 2a and b). Fig. 2a shows a chromatogram with the methylmercury peak (HP-5 column) and Fig. 2b shows the emission spectrum recorded at the apex of the methylmercury peak, showing that this peak contains Hg. The 184.9 and 194.2 nm lines correspond to Hg and the 193 nm line to the carbon background.

When methylmercury standards are injected, as shown in Fig. 2, no other peaks are recorded at 185 nm. However, when extracts of real

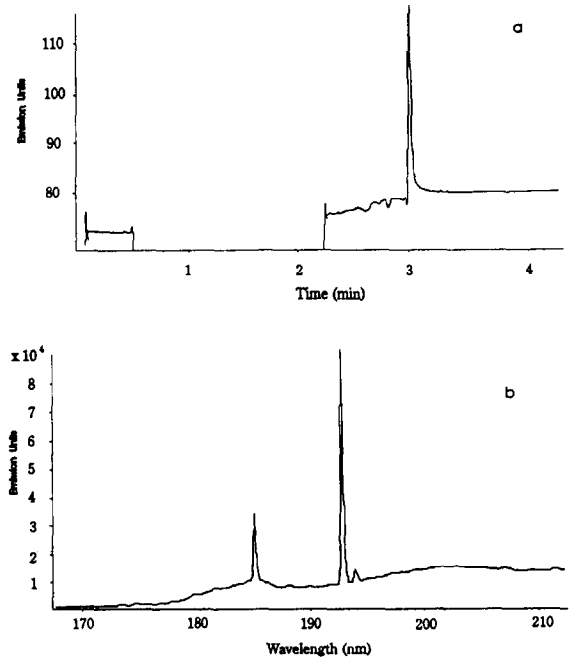

Fig. 2. Identification of the methylmercury peak. (a) Chromatogram for a standard solution in toluene (0.030 μg ml^{-1} MeHg); (b) emission spectrum recorded at the apex of the methylmercury peak.

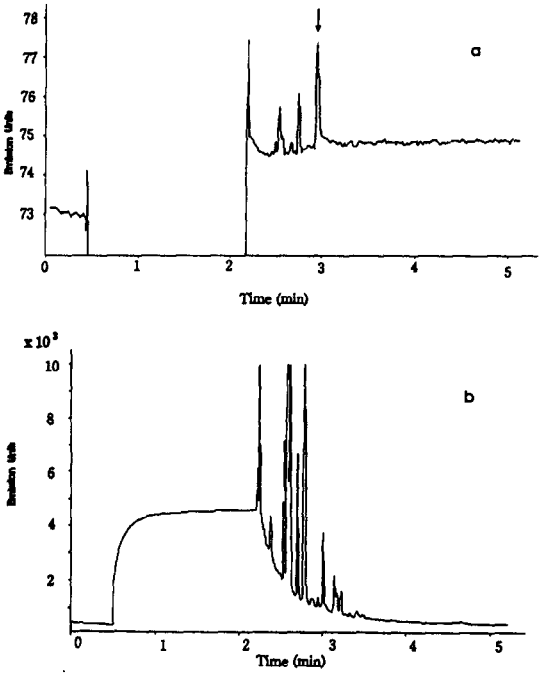

Fig. 3. Chromatograms for a clam sample using the GC–AED system. (a) 185 nm mercury line; (b) 193 nm carbon line.

samples are injected (see Fig. 3), two or more weaker peaks usually appear next to the methylmercury peak, which may lead to errors. The hypothesis that the AED system is highly selective might suggest that these peaks are due to mercury-containing species. Fig. 3b shows a chromatogram for the same sample recorded at the 193-nm carbon line. The presence of high-intensity carbon peaks at the position of the interferencing peaks in the 185-nm chromatogram can be clearly seen. The closeness of both spectral lines and the fact that the instrument cannot completely separate them explains the appearance of the small interfering peaks. In any case, the spectrum shows the absence of mercury in these peaks. Therefore, it is convenient to make a systematic check of the purity of the peaks by recording the spectra.

3.4. Calibration

Working standard solutions of 1, 5, 10, 20, 30 and 40 ng ml^{-1} MeHg were prepared for calibration from a 1.164 mg ml^{-1} MeHg stock standard solution. Each standard solution was injected three times and the results were expressed as peak area.

With the HP-1 column, a good linear response was obtained (correlation coefficient 0.990, R^2 98.10%, standard error of estimate 2.16). The detection limit (signal-to-noise ratio = 3) was 1.5 pg of methylmercury, which corresponds to a concentration of 1 ng ml^{-1} MeHg in the samples. The quantification limit (signal-to-noise ratio = 10) was 4.5 pg of methylmercury, corresponding to a concentration of 3 ng ml^{-1} MeHg in the samples.

The HP-5 column showed excellent linearity of response (correlation coefficient 0.999, R^2 99.88%, standard error of estimate 9.93). The detection limit was 1.2 pg of methylmercury, corresponding to a concentration of 0.8 ng ml^{-1} MeHg in the samples. The quantification limit was 2.6 pg of methylmercury, corresponding to a concentration of 1.7 ng ml^{-1} MeHg in the samples.

Table 2 shows a comparison of published methylmercury and mercury detection limits for different coupled techniques. The detection limit of the proposed method is low, very similar to those of other techniques, and lower than the results obtained with GC–ECD [5,10].

Table 2
Comparison of detection limits reported for the determination of methylmercury

Technique[a]	Detection limit	Ref.
GC–MIP–ICP	3 pg Hg	24
GC–MPD	1 pg Hg	28
GC–AAS	4 pg Hg	17
PT-GC–FTIR	0.15 μg Hg	19
GC–ECD	50 ng g^{-1} MeHg	10
GC–ECD	250 ng g^{-1} Hg	5
HS-GC–MIP	0.5 μg l^{-1} Hg	6
cGC–CVAFD	0.6 pg Hg	35
GC–AED	1.2 pg MeHg (HP-5)	This work
	1.5 pg MeHg (HP-1)	This work

[a] MIP = Microwave induced plasma; ICP = inductively coupled plasma; MPD = microwave plasma detector; AAS = atomic absorption spectrometry; PT = programmed-temperature; HS = headspace; cGC = capillary GC; CVAFD = cold vapor atomic fluorescence detection.

3.5. Precision

In order to evaluate the precision of the HP-5 column, a series of repeated injections ($n = 11$) of 1.5 μl of 30 ng ml^{-1} MeHg standard solution were made. The relative standard derivation (R.S.D.) was 4.4%. During a similar attempt to evaluate the precision of HP-1 column, the signal decreased as the samples were injected. This can be attributed to the rapid decrease in efficiency in the treatment of the column with use, owing to the small phase thickness (0.17 μm).

3.6. Accuracy

Recovery studies were carried out using a certified reference material (DORM-1), supplied by the National Research Council of Canada, with a certified methylmercury content of 0.731 ± 0.060 μg g^{-1} Hg, and with a tuna sample obtained from the Community Bureau of Reference (BCR) during an intercalibration exercise in 1993, spiked with methylmercury.

The results are given in Table 3. The mean recovery for DORM-1 was $88.81 \pm 3.92\%$ and for tuna $84.15 \pm 8.74\%$. When the same recovery study was done using GC–ECD, the mean recovery was $88.3 \pm 2.80\%$ for DORM-1 and $90.5 \pm 4.10\%$ for tuna [36].

3.7. Analysis of environmental samples and comparison with GC–ECD

Figs. 3a, 4 and 5 show the chromatograms obtained after injecting extracts of clam and mussel samples into a GC–ECD system in comparison with the results after injection into the GC–AED system using the conditions proposed here. In both instances the same column (HP-5) was used and we used the chromatographic conditions established as optimum for each system. In general terms, for relatively clean samples, it is possible to make an accurate integration of the methylmercury peak in both systems. However, for the samples such as those in Figs. 4 and 5b, the integration of the methylmercury peak in the GC–ECD system is impossible or highly inaccurate. In both figures the theoretical position of the peak is marked on the chromatograms by an arrow. The selectivity using the

Table 3
Results of the determination of methylmercury in environmental samples by GC–AED (μg g^{-1} MeHg ± standard errors)

Sample[a]	Sample mass (g)	n	MeHg content	MeHg found		Average recovery (%)	
				GC–ECD	GC–AED	GC–ECD	GC–AED
DORM-1	0.5	10	0.785 ± 0.060	0.694 ± 0.028	0.697 ± 0.032	88.30 ± 2.80	88.81 ± 3.92
Tuna muscle 1	0.5	4	Unknown	2.859	1.599	–	–
Tuna muscle 1	0.5	7	Unknown	2.779 ± 0.131	2.581 ± 0.268	90.56 ± 4.10	84.15 ± 8.74
Sediment	0.5	2	Unknown	0.151	0.127	–	–
Cockle	0.5	4	Unknown	–	0.656 ± 0.072	–	–
Clam 1	0.5	4	Unknown	–	1.034 ± 0.108	–	–
Mussel 1	0.5	4	Unknown	0.1402 ± 0.020	0.187 ± 0.054	–	–
Tuna muscle 1	0.5	2	Unknown	3.341	4.176	–	–
Tuna muscle 2	0.5	2	Unknown	5.133	7.112	–	–
*Mussel 2	0.5	4	Unknown	0.741 ± 0.168	0.242 ± 0.027	–	–
*Mussel 3	0.5	2	Unknown	–	0.099	–	–
*Mussel 4	0.5	2	Unknown	–	0.026	–	–
*Mussel 5	0.5	2	Unknown	–	0.555	–	–
*Clam 2	0.5	2	Unknown	–	0.044	–	–
*Clam 3	0.5	2	Unknown	–	0.112	–	–
*Clam 4	0.5	2	Unknown	–	0.111	–	–

[a] Samples marked with asterisks could not be analysed using the GC–ECD system.
[b] 3.0708 μg g^{-1} MeHg added.

Fig. 4. Chromatogram for a clam sample using the GC–ECD system.

GC–ECD system can be improved by means of additional stages of extraction and cleaning of the samples, although clearly errors will increase during these operations. On the other hand, the GC–AED system (Figs. 3a and 5a) allows the reliable quantification of the methylmercury peak without the need to force the chromatographic separation or to apply clean-up of the samples.

Table 3 gives the results for several analyses of marine samples for methylmercury. When the same samples were analysed using both GC–ECD and GC–AED, both sets of results are given. However, several of these samples could not be analysed using the GC–ECD system owing to the presence of peaks that strongly overlapped with that of methylmercury. These samples are marked with asterisks.

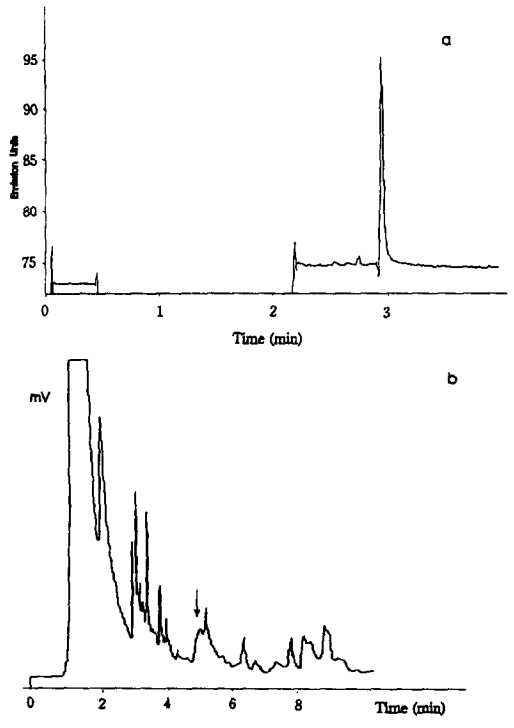

Fig. 5. Chromatograms for a mussel sample using (a) the GC–AED and (b) the GC–ECD system.

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