Determination of Methylmercury in Biological Samples by Semiautomated Headspace Analysis

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Gas–liquid distribution coefficients of methylmercury halides dissolved in water or benzene have been assessed by using a headspace injection system coupled to a gas chromatograph–microwave-induced plasma system. Vapor concentrations decreased in the following order: CH_3HgI > CH_3HgBr > CH_3HgCl. To transform all methylmercury compounds present in a biological sample into methylmercury iodide, iodoacetic acid (IAc) is used as the liberating agent. Methylmercury iodide degradation is prevented by the addition of sodium thiosulfate (0.05 M). The results obtained with internal standard addition are not influenced by other volatile compounds including other mercury compounds. The method has been applied to a series of biological samples. The detection limit of methylmercury in biological samples is 1.5 ng/mL of homogenate. Contrary to direct on-column injection, no column performance degradation was observed when using a headspace sampler after more than 50 analyses.

Methylmercury compounds belong to the most dangerous substances in the environment. They are highly toxic, even more than inorganic mercury compounds, and are often found concentrated at the end of the food web. This has led to a considerable effort in the development of reliable, precise, and sensitive analytical methods specific for the determination of methylmercury in biological samples. Nowadays, the most widely used methods are all based on the isolation procedure of Gage (*1*), which involves liberation of the alkylmercury moiety from the sample by acidification, isolation by multiple extraction with benzene or toluene, and subsequent analysis by gas chromatography with electron capture detection (GC/ECD). A variety of stationary phases has been recommended but all of these columns have exhibited one or more of the following disadvantages (*2–5*): (a) poor and often variable response to methylmercury chloride, (b) moderate to very severe tailing, and (c) poor column efficiency that can then lead to problems with interferences. Hence, time-consuming and laborious column conditioning procedures are necessary (*2, 5–10*). The beneficial effects of the treatment are only temporary, since the presence of high molecular weight compounds in the sample often leads to "column performance" degradation (*2*). Another major disadvantage of the GC/ECD method is that halogen-bearing compounds, coextracted with the methylmercury, can interfere because of the nonspecificity of the EC detector (*11*). The nonspecificity of the EC detector for methylmercury has been overcome with the introduction of a microwave-induced-plasma emission detector system (MIP) (*11–13*). The essential problem of "column performance" degradation, however, remained unsolved. Zelenko et al. (*14*) developed a new isolation procedure by taking advantage of the volatility of methylmercury cyanide and its selective captation on cysteine-impregnated paper. The final steps involved benzene extraction and GC/ECD. Although good results were obtained, complete volatilization of produced MeHgCN is very time-consuming and a serious restraint on the applicability of the method. In this paper, a simple methylmercury extraction procedure from biological samples is presented using iodoacetic acid as the liberating agent. Direct headspace sampling (HS) and subsequent GC–MIP analysis suppressed the need of further sample cleanup while "column performance" degradation was no longer a problem.

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EXPERIMENTAL SECTION

Apparatus. For the construction of our GC–MIP system, we took the concept described by McCormack et al. (*12*) as a basis. This analytical system consists of a F&M-700 gas chromatograph, a heated four-way valve for solvent ventilation (Valco GC-T), a heated transfer tube, and a detector system. The heated transfer tube (150 °C) is connected to a quartz capillary (2 mm i.d.) that is centered in a $^1/_4$-wave Evenson-type cavity (Electro Medical Supplies, Model 214 L). The microwave generator (Electro Medical Supplies Microtron 200) is operated at 75 W, providing a maximum signal-to-noise ratio. Emission measurements are carried out with a Perkin-Elmer AAS-403 at 253.7 nm. The GC–MIP system is coupled to a semiautomated headspace sampler (Perkin-Elmer, HS-6) (*15, 16*). GC analyses are carried out by using a 1-m (3 mm i.d.) Teflon column packed with 10% (w/w) AT-1000 (Alltech Associates) on Chromosorb WAW 80/100 mesh and argon as carrier gas. Methylmercury concentrations are determined on the basis of recorded peak heights.

Reagents. All chemicals are of analytical reagent grade. Stock solutions of 1 g/L methylmercury chloride are prepared every 6 months. Analytical standard solutions are prepared daily.

Instrumental Conditions. The headspace sample vials (HS-6) were carefully cleaned with 0.1 M sodium thiosulfate (*17*), thoroughly rinsed with very pure water (Millipore, Milli-Q), and baked at 450 °C overnight. Once the vials had cooled, they were filled with 2 mL of a standard solution of 1 mg/L CH_3HgCl in water and closed with butyl rubber septa coated with Teflon. It was pointed out that butyl and silicone rubber septa did absorb considerable amounts of gaseous methylmercury at 50 °C (up to 50%). On the other hand butyl or silicone rubber septa coated with Al or Teflon showed no affinity for this compound, which made their use preferable. For sample volumes up to 2 mL, no liquid sample volume effect was observed on the delivery of the gas phase sample quantity during injection. The minimum thermostating time needed to reach gas–liquid equilibrium was 5 min at the maximum thermostating temperature (80 °C). Injection time was limited to three possibilities: 5, 15, or 60 s. A sufficient sample quantity was delivered after 15 s with minimal perturbation of the GC-separation efficiency. The pressure in the headspace vials equals 1.3×10^5 Pa; the time for pressure stabilization is 1 min; the gas chromatographic column temperature is 150 °C; and the carrier gas flow amounts to 100 mL/min. Very little variation in HETP was observed for carrier gas flow rates between 64 and 120 mL/min.

Under the above described instrumental conditions, CH_3HgI and C_2H_5HgI elute after 5.1 and 9.8 min, respectively. A typical chromatogram is given in Figure 1. The detection limit measured with the HS-GC–MIP system and calculated from the peak height, using 2 mL of a 10 ng/mL aqueous standard solution of methylmercury iodide, was 0.05 ng/mL. This detection limit is defined as the signal level corresponding to twice the standard deviation of background emission at the analytical wavelength. The directly measured detection limit (GC–MIP system) equaled 0.6 ng/mL.

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Table I. Detection Limits

GC–MIP				GC/ECD
this work	ref 18	ref 10	ref 19	ref 20
3 pg	16 pg	0.36 pg	–	1 pg
–	–	0.02 pg/s	1 pg/s	–

This detection limit is listed in Table I, together with the detection limits obtained previously with Ar (*18*) and He (*10, 19*) microwave plasmas at atmospheric pressure and with a typical GC/ECD system (*20*). The Ar plasma was also produced in a $^1/_4$-wave Evenson-type cavity, while the He plasmas were produced in a Beenakker (TM_{010}) resonant cavity. It appears from Table I that lower detection limits for methylmercury were obtained with the He plasmas. However, the conclusion that the kind of plasma or cavity solely determines the detection limit seems to us a little bit premature. Mulligan et al. (*21*) compared with the same apparatus several microwave cavities, including the TM_{010} and the $^1/_4$-wave Evenson cavity, for the simultaneous determination of As, Ge, Sb, and Sn. Despite the better fragmentation and excitation characteristics of the He plasma vs. the Ar plasma, similar detection limits and dynamic ranges were observed. An undeniable advantage of the TM_{010} cavity is, however, that it can be viewed axially. Deposition of materials on the discharge tube walls can occur and result in a gradual attenuation of sample response with time. Although this process was rather slow, we changed the quartz tube every 4 months as a preventive measure.

RESULTS AND DISCUSSION

Vapor–Liquid Phase Partitioning of Methylmercury.

Distribution Constants. The primary step in the determination of methylmercury in environmental samples involves the liberation of this compound from the sample. Most procedures (*1, 2, 6–11, 13, 14, 22–28*) involve the formation of a water-soluble adduct of methylmercury and a halide by acidification of the aqueous sample homogenate (pH <1), often followed by extraction of the liberated MeHgX (X = Cl, Br, I) with benzene to improve the GC determination. We tested therefore the gas–liquid behavior of MeHgCl dissolved in each of these two common solvents, over a temperature range of 30–80 °C. The results are shown in Table II. The relative standard deviation was always less than 5%. It is obvious that the headspace analysis above an aqueous solution provides a vapor-phase enrichment of at least a factor of 2 compared to the analysis above a benzene solution. The dimensionless distribution constant of MeHgCl at 25 °C (1.64×10^{-5}), obtained by extrapolation of our experimental results, agrees well with that found by Iverfeldt and Lindqvist ($1.9 \times 10^{-5} \pm 2 \times 10^{-5}$) (*28*). In addition, the gas–liquid distribution results shown in Table II also allow for the calculation of benzene/water distribution constants for MeHgCl. For example at 25 °C, we obtain a K value of 4.7 or an extraction efficiency of 83%, which falls in the range cited in the literature (*10, 11, 29*). Since aromatic/water distribution constants of methylmercury iodide and bromide are still higher than for the chloride compound (*30*), aqueous solutions of the former compounds will also provide higher vapor concentrations. The influence of the anion on the gas–liquid partition of MeHgX dissolved in water is shown in Table III. Since vapor concentrations decrease in the order MeHgI > MeHgBr > MeHgCl over the whole temperature range, we decided to investigate a procedure involving the transformation of all methylmercury compounds present in the biological sample into methylmercury iodide. However, for the standardization of the analytical method, it is interesting to note that by varying the temperature or the nature of the anion, the extraction efficiency of methylmercury from the sample matrix as well as possible contaminations or interference during the analysis can effectively be verified.

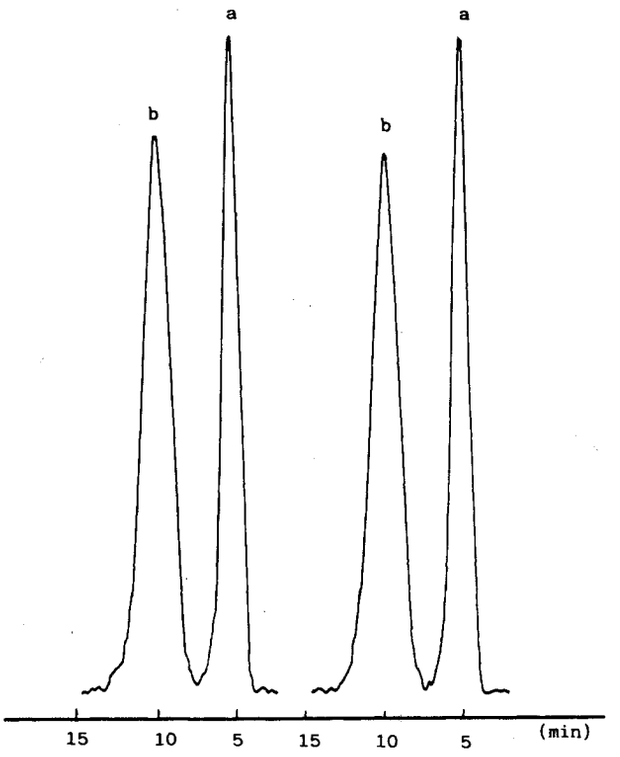

Figure 1. Gas chromatogram of methylmercury iodide (a) and ethylmercury iodide (b).

Effect of pH and Anion Concentration. The study of the distribution of a particular compound requires the knowledge of its speciation in the different phases. The predominant methylmercury compound in aqueous solution is mainly dependent on the pH and the anion concentration. By use of the equilibrium constants of Schwarzenbach (*31*), it can be shown that below pH 5 almost all methylmercury (98%) is in the undissociated halide form, even at low salt concentrations. Above pH 6 for the chloride complex, pH 7 for the bromide complex, and pH 10 for the iodide complex, MeHgOH(aq) progressively appears. Due to its lower vapor tension (*32*), a decrease of the methylmercury concentration in the vapor phase will be observed. Analogous to inorganic mercury(II) halides, methylmercury compounds show a

Table II. Gas–Liquid Distribution Constants of MeHgCl above Water (1 M HCl) and Benzene (H) and Benzene–Water (Liquid–Liquid) Distribution Constants of MeHgCl (K)

		distribution constants							
		20 °C[a]	25 °C[a]	30 °C	40 °C	50 °C	60 °C	70 °C	80 °C
10^5H[b]	water	1.07	1.64	2.47 ± 0.05	5.39 ± 0.10	11.2 ± 0.5	22.3 ± 0.9	42.7 ± 0.6	78.8 ± 1.5
	benzene	0.21	0.35	0.58 ± 0.03	1.49 ± 0.06	3.61 ± 0.11	8.32 ± 0.19	18.2 ± 0.3	38.3 ± 0.9
K[c]		5.1	4.7	4.3	3.6	3.1	2.7	2.3	2.1

[a] Extrapolated values obtained by the least-squares method assuming that the temperature dependence obeys the Clausius–Clapeyron equation: $\log H = C - \Delta H/(2.303RT)$, with C the constant, ΔH the latent heat of vaporization, R the gas constant, and T the temperature. [b] $H = C_{gas}/C_{solvent}$ (dimensionless). [c] $K = C_{benzene}/C_{water}$.

Table III. Gas–Liquid Distribution Constants of MeHgX above Aqueous Media

	10^4H			
compd	50 °C	60 °C	70 °C	80 °C
MeHgCl	1.12 ± 0.05	2.23 ± 0.09	4.27 ± 0.06	7.88 ± 0.15
MeHgBr	2.98 ± 0.07	6.35 ± 0.24	12.9 ± 0.6	25.4 ± 0.8
MeHgI	17.7 ± 0.6	29.2 ± 0.4	46.8 ± 0.7	72.9 ± 1.8

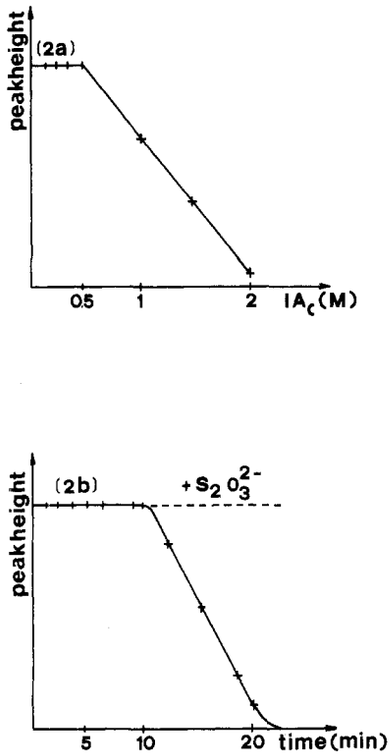

Figure 2. (a) Effect of iodoacetic concentration on the detection signal. (equilibration time is 5 min) and (b) effect of equilibration time on the detection signal (iodoacetic concentration is 0.1 M).

tendency to form complex ions in the presence of excess halide ions. It is clear therefore that the anion concentration can have a strong influence on the volatility of the methylmercury compound. This effect is most pronounced for the iodide form

$$MeHgI + I^- = MeHgI_2^- \quad (1)$$

where $K \simeq 2$ (*30*). One can calculate that for a 1 M hydroiodic acid concentration, which is absolutely essential to cleave all bound methylmercury from environmental samples, especially proteinaceous samples (*20*), only about one-third of the methylmercury will be in the volatile nonionic form. However, this side reaction (eq 1) can almost completely be avoided by the use of iodoacetic acid as the cleaving reagent. In that case, the iodide concentration can be kept so small that the former equilibrium becomes negligible due to the following mechanism:

$$RS\text{–}HgMe + ICH_2COOH \rightarrow RS\text{–}CH_2COOH + MeHgI \quad (2)$$

Nevertheless, the vapor-phase concentration of MeHgI is also dependent on the iodoacetic acid concentration as shown in Figure 2a. Other metal–protein bonds will also be attacked, resulting in a further liberation of iodide ions, which can be oxidized to iodine

$$4I^- + O_2 + 4H^+ = 2I_2 + 2H_2O \quad (3)$$

and as reported by Talmi (*32*) and Zepp (*33*) can lead to a photochemical degradation of MeHgI

$$MeHgI + I_2 = MeI + HgI_2(s) \quad (4)$$

Table IV. Analysis of Methylmercury in the Liver of a Mouse[a]

	direct calibration		int std add method	
t, °C	Br	I	Br	I
60	194 ± 7	113 ± 5	84 ± 3	82 ± 2
70	153 ± 2	96 ± 5	81 ± 2	82 ± 2
80	136 ± 4	89 ± 4	86 ± 2	82 ± 2

[a] Results expressed in ng of CH_3Hg/g of homogenate.

The addition of a small amount of sodium thiosulfate (0.05 M) prevents this photochemical degradation, as shown in Figure 2b

$$2S_2O_3^{2-} + I_2 = S_4O_6^{2-} + 2I^- \quad (5)$$

while the side reaction formulated by eq 1 is still suppressed.

Analysis of Methylmercury in Biological Samples. *Accuracy of Method.* To date no reference material of methylmercury in biological samples exists. Therefore, the liver of a mouse intoxicated with methylmercury was subjected to two different extraction procedures. The liver was first lyophilized, then homogenized in 20 mL of water. Next, one of the following treatments was carried out on a 0.5-mL portion of the aqueous sample homogenate added to 0.5 mL of H_2O or 0.5 mL of an appropriate methylmercury standard solution: (a) addition of 0.05 mL concentrated sulfuric acid and 180 mg of potassium bromide resulting in the liberation of methylmercury bromide or (b) addition of 18.5 mg of iodoacetic acid and 12.5 mg of sodium thiosulfate resulting in the liberation of methylmercury iodide. Immediately after the addition of the reagents, the vessel was closed with the septum and placed in one of the headspace compartments for 5 min at one of three selected thermostating temperatures (60, 70, and 80 °C). After a pressurization time of 1 min, the sample was injected. Results were obtained with either an internal standard addition method or a direct or external calibration method. For a given anion and temperature, reproducible data were obtained with both methods. However, for the six temperature–anion combinations investigated, the direct calibration method yielded a broad range of results: 89–194 ng of CH_3Hg/g of homogenate (Table IV). This is most probably due to the sample matrix composition. Consequently, this calibration method was abandoned. Considering the results obtained with the internal standard addition method, we presume that (a) there is no influence by the presence of other volatile compounds in the sample, since no temperature effect on the results is observed, and (b) all methylmercury has been effectively liberated from the sample, since extraction with different acids at different temperatures yields the same results. The question remains whether interferences from other mercury compounds are excluded as well. Headspace sampling of a standard solution of 10 mg/L HgI_2, which is about 2 orders of magnitude higher than the mercury concentration found in the liver, did not result in a measurable signal. This is in agreement with calculations based upon the vapor pressure of this compound in the considered temperature range (*34*). Interferences from other organomercury compounds such as ethylmercury or phenylmercury are not to be feared, since they elute at longer retention times than methylmercury. With the iodoacetic extraction procedure, a detection limit of 1.5 ng/mL is achievable, since the relative standard deviation at the 10 ng/mL level equals 7.5%. The analysis of one sample, including homogenization, lasts about 25 min.

Applications of Method. Two hundred birds found dead in Belgium between 1970 and 1980 and belonging to thirty species were analyzed for total mercury contamination (*35*).

Table V. Determinations of Methylmercury in Organs of Birds of Prey with HS-GC-MIP[a]

no.	organ, species	tot Hg, μg/g	CH_3Hg, μg/g	% CH_3Hg
1	liver, guillemot	2.26 ± 0.01	1.89 ± 0.07	84
2	liver, buzzard	0.93 ± 0.07	0.93 ± 0.06	100
3	liver, buzzard	1.50 ± 0.01	1.46 ± 0.01	97
4	liver, common gull	0.59 ± 0.02	0.50 ± 0.04	85
5	liver, common gull	3.02 ± 0.09	2.60 ± 0.08	86
6	liver, guillemot	1.87 ± 0.01	1.84 ± 0.06	98
7	kidney, guillemot	1.97 ± 0.07	1.52 ± 0.01	77
8	kidney, buzzard	1.62 ± 0.08	0.72 ± 0.01	44
9	kidney, buzzard	1.90 ± 0.06	1.05 ± 0.01	55
10	kidney, guillemot	2.00 ± 0.14	1.57 ± 0.05	79
11	kidney, guillemot	1.60 ± 0.08	1.53 ± 0.11	96

[a] Headspace gas chromatography–microwave induced plasma.

Liver, kidney, and in some cases muscle and heart were kept deep-frozen (−20 °C). From this stock of organs we received a number of large samples, livers and kidneys of birds of prey, to determine the ratio of methylmercury to total mercury. After the samples were thawed, they were chopped in small pieces. Two representative aliquots were weighed and analyzed for total mercury after mineralization and digestion of the sample. The mineralization and digestion methods are described in detail elsewhere (*36*). Moreover, two other aliquots of the biological tissue were weighed and blended with a known mass of water to produce a fine suspension. One-half milliliter of that suspension was then subjected to the iodoacetic acid extraction procedure, described in the previous section "Accuracy of Method". The concentration of total mercury and the ratios of methylmercury to total mercury are shown in Table V. The total mercury concentrations corroborate the earlier reported results (*35*). Further, it appears from Table V that in most cases a relatively high percentage of methylmercury is obtained. As a comparison, the percentages of methylmercury in fish reported by Westöo (*37*) and Huckabee et al. (*22*) amounted to 93 ± 15 and 93 ± 2.6%, respectively. The mean percentage of methylmercury in liver (92%) is substantially higher than in kidney (70%), probably as a result of the different metabolic function of these organs.

To conclude this paper, one of the major advantages of our headspace method is that no particular column conditioning procedures are required before or during a series of analyses. No sign of "column performance" degradation was observed.

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Registry No. MeHgI, 143-36-2; MeHgBr, 506-83-2; MeHgCl, 115-09-3; H_2O, 7732-18-5; C_6H_6, 71-43-2.

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