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AN IMPROVED SPECIATION METHOD FOR MERCURY BY GC/CVAFS AFTER AQUEOUS PHASE ETHYLATION AND ROOM TEMPERATURE PRECOLLECTION

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Summary—An improvement has been made in previous mercury speciation methods, which omits the use of liquid nitrogen by modifying the GC conditions In addition, the sometimes observed thermal decomposition of ethylation derivatives in the process of desorbing the compounds to the GC column has been investigated and overcome. The resulting improvements have allowed simultaneous determination of methylmercury (MMHg) and inorganic mercury (Hg(II)) They have also increased the precision of the ethylation reaction and refined the MMHg determination. Optimal conditions for MMHg were confirmed and supplemented, while the parameters for dimethyl mercury (DMHg) and Hg(II) were thoroughly investigated for the first time The absolute detection limits (DL) for 2σ of the reagent blanks as Hg and about 0.6, 0.6 and 1 3 pg for DMHg, MMHg and Hg(II), respectively The improved method is five-fold faster than the original method, allowing up to 80 samples to be analyzed within 8 hr

Considerable progress has been made in understanding the biogeochemical cycle and toxicology of mercury.[1] Today, not only is the toxicity of methyl mercury (MMHg) well recognized, but also that of Hg(II). Recently, Clarkson suggested that Hg(II) is the proximate toxic species upon exposure to Hg^0.[2] Reliable and sensitive speciation methods are lacking, relative to the importance of the issue. Some methods have been published but most are only for MMHg.[3–15] The reason for this is probably that the toxicity of MMHg has historically been better recognized than that of other mercury species.

Current methodologies involve isolating MMHg from its matrix by solvent extraction, or by purging after volatilization by aqueous phase ethylation or hydride generation. The MMHg is then separated by gas chromatography (GC) and detected by one of the following: electron-capture, atomic absorption (AA), atomic emission, atomic fluorescence (AFS), microwave plasma emission, and Fourier transform infrared spectroscopy. Absolute DLs using these methods vary widely, from 1 pg to 150 ng.

Methods based on aqueous phase ethylation with sodium tetraethylborate ($NaEt_4$) exhibit significant advantages. using this technique, ethylation derivatives are isolated from the matrix by purging just prior to GC separation, thus eliminating the need for solvent extraction. Detection limits of 25 and 230 pg, as Hg for MMHg and Hg(II) are reported using conventional GC/AA, and DLs of 4 and 75 pg are reached by cryogenic GC/AA.[14,15] The most sensitive method to date, with a DL of 0.6 pg for MMHg, based on the same technique, is a combination of trapping, precollection, and cold vapor atomic fluorescence spectrometry (CVAFS) described by Bloom.[8] This method is not only extremely sensitive compared to earlier efforts, but also relatively efficient in terms of sample throughput.[15]

Using these methods, the ethylation derivatives are precollected on a liquid nitrogen trap connected on-line to a GC/AA system. Thus, one reaction vessel is connected to one GC/AA system.[15] Using Bloom's method, the ethylation derivatives are precollected on trapping columns at room temperature independent of the GC/CVAFS system. Thus while samples collected on trapping columns are being analyzed by GC/CVAFS, several others can be collected from the reaction vessels. This is one of the benefits of Bloom's method. A disadvantage, however, is the time-consuming collection of the ethylation derivatives onto a two-stage Carbotrap® and

liquid nitrogen trap. Further, although that method reported the potential to perform simultaneous MMHg and Hg(II) measurements, the necessary conditions were never fully investigated. This was in part due to the observation of higher blanks for the ethylation technique compared to more traditional methods ($SnCl_2$ reduction) and in part due to the greater tendancy for the Hg(II) derivative, diethylmercury, to thermally decompose when desorbed from the carbotrap column. The method described in this paper maintains the advantages of Bloom's method, *i.e.*, the use of CVAFS and trapping precollection of organo-Hg compounds at room temperature, but omits the use of liquid nitrogen and addresses these problems.

EXPERIMENTAL

Instrumentation

A Brooks Rand, Ltd. CVAFS-2 was used as a Hg^0 detector. The design and installation of the instrument's progenitor has been described in detail elsewhere.[16] A chromatographic peak integrator (Laboratory Data Control I-4100) was used to quantify signal peaks.

Materials and reagents

Reaction/purge vessel. As illustrated in Fig. 1(a), a 150 ml flat bottom flask with 24/40 tapered fitting and a special 4-way valve sparging-tube-cap assembly served as the reaction vessel. The construction and function of the 4-way valve is described by Bloom.[8]

Trapping columns. A column for the collection of purged organo-Hg species was constructed from quartz tubing as described by Bloom.[8] Either 200 mg of Carbotrap® (Supelco, 754200, 20/40 mesh) or 100 mg of Tenax® (Alltech, 20/35 mesh) was packed into the tubing. Silanized glass wool was used to hold the packing in place. In Figs 1(a) and (b), the construction of the trap and its connection with the purge vessel is illustrated. The layer of glass wool on end A should be thin, just enough to block the grains from falling out. The glass wool on end B should form a tight plug about 2 cm in length. The traps are heated by applying approximately 20 VAC to a coil of 0.8 m made from 22 ga nichrome wire. The actual voltage setting must be adjusted to reach an internal temperature of 200°C within 30 sec. A thermocouple set in a dummy trap is initially used to obtain this setting. To initially remove residual Hg from the filling, traps were heated to about 200°C for 5 min.

GC/CVAFS system. An isothermal GC system was used throughout, while a cryogenic GC system was also used for a comparison study. The latter was described by Bloom and Fitzgerald.[16] The isothermal GC system is illustrated in Fig. 2. The GC column contained packing to a length of 75 cm. Organo-Hg species desorbed from the trapping column were carried by gas passed through the GC column, which was held in a cylindrical oven at 85°C and eluted. If DMHg determination was not required, the oven was heated to 100°C to decrease elution

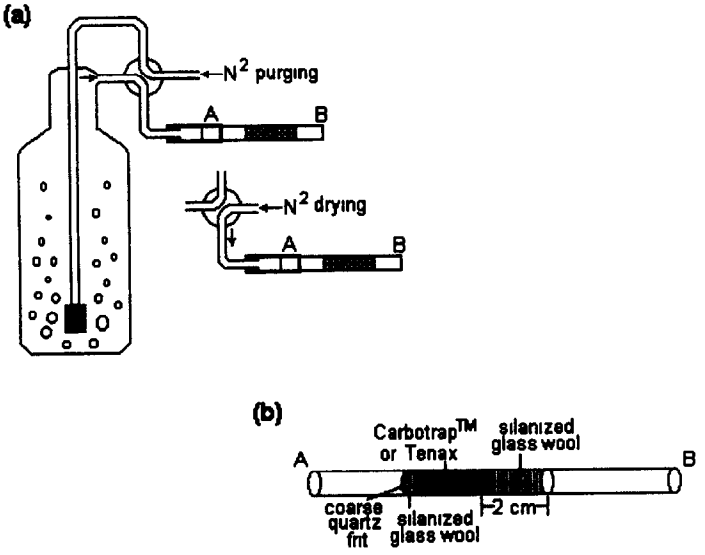

Fig 1. Connection of trapping column with a reaction vessel (a), construction of trapping column (b).

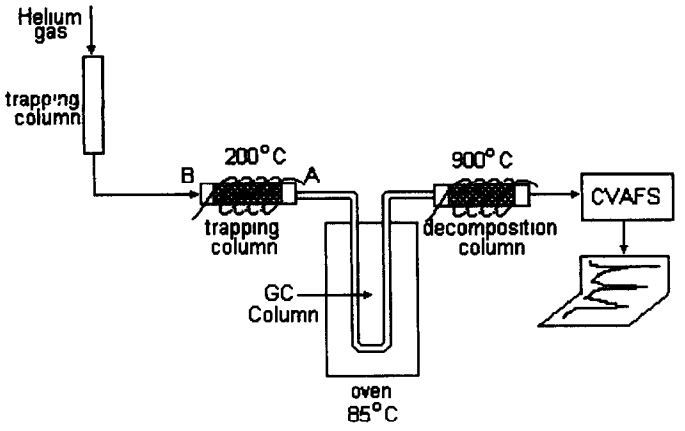

Fig 2 Thermal desorption, isothermal GC separation, pyrolytic decomposition and CVAFS detection system

times. After elution species were decomposed in a thermal decomposition tube at 700–900°C and finally detected by CVAFS.

Standard solutions.

(a) A 1000 mg/l as Hg MMHg standard stock solution was prepared by dissolving 0.1252 g of CH_3HgCl reagent (Johnson Matthey, U.S.A., 95%) in isopropanol to a final volume of 100 ml. From this, a 1 ng/ml working standard solution was prepared by serial dilution with 0.5% HCl in water.

(b) A 1 ng/ml Hg(II) working standard solution was prepared by serial dilution of a 1000 mg/l stock solution (Mallinckrodt, U.S.A., Atomic Absorption, lot H548) with 1% HCl.

(c) A DMHg standard solution was prepared by diluting 5 g reagent grade $(CH_3)_2Hg$ (Johnson Matthey) to 1 l isopropanol. This solution must be standardized. The decomposition column in Fig. 2 was used for the standardization. The left end of the column was connected with the output of the purge vessel in Fig. 1(a) while the right end was connected to a gold trap.[17] A known volume of the solution was added in the vessel, which was closed immediately and purged with N_2 for 20 min. DMHg was passed through the decomposition column at a temperature of 900°C to decompose into Hg^0, which was then collected on the gold trap. Analysis was then conducted by a single stage amalgam method.[17]

1% *Sodium tetraethyl borate solution.* Analytical reagent grade $NaBEt_4$ (Strem, U.S.A.) was used to prepare a 1% solution in DDW containing 2% KOH. The solution is divided into 10 ml vials and frozen until use.[8]

2M *Potassium acetate buffer.* 98 g of analytical reagent grade $KC_2H_3O_2$ (Mallinckrodt Inc., U.S.A.) and 60 ml glacial acetic acid (J. T. Baker) were diluted to 500 ml with DDW.

Procedure

The focus here is on improvement of the method for the speciation of mercury in a pure standard solution only. Procedures for the analysis of sediment, water and biological materials based on this method are presented elsewhere [18–20]

A standard calibration curve of 0, 50, 100 and 200 pg of Hg for DMHg, MMHg, and Hg(II) was added to vessels followed by the procedure described by Bloom. The reaction, purging and drying times, however, were decreased to 15, 12 and 5 min, respectively.[8] After drying, using the isothermal method, the trapping column is removed and connected in-line with GC/CVAFS, as illustrated in Fig. 2. Special attention must be paid to the trapping column orientation, especially when a Carbotrap® column is used. Under a helium flow of 80 ml/min, the trapping column is heated quickly, reaching a peak of approximately 200°C (internal) in 30 sec. Heating is controlled by a timer connected in-line, which is activated at the same time as the integrator or chart recorder. The organo-Hg species are eluted according to their boiling points and passed through the thermal decomposition tube at a temperature of 700–900°C, where they were decomposed into Hg^0 and detected by CVAFS. Fluorescence signals were recorded by integrator or chart recorder, and peak heights were measured manually with a milimeter ruler. Once

the Hg(II) peak returns to baseline, the next trap can be analyzed immediately. For the conditions and operation of the cryogenic GC system, see the article by Bloom [8]

RESULTS AND DISCUSSION

Decomposition of organo-Hg and its elimination

It has been observed that organo-Hg species sometimes decompose during thermal desorption from the Carbotrap® column.[8] This effect is due to a combination of impurities on the collection trap, the temperature and rate of trap heating, and how the trap is physically prepared. The decomposition effect is much more significant for diethylmercury than for methylethylmercury, sometimes making quantification of the former impossible. Only rarely has the phenomenon been observed to compromise the MMHg signal, however.

Illustrated in Fig. 3 are several typical chromatograms. Since they represent analyses performed days to months apart, the GC conditions (gas flow rate, column temperature, *etc.*) were different, resulting in varied retention times As can be seen in Fig. 3(a), the ethylation derivatives of MMHg and Hg(II) totally decomposed to Hg°. In this case, a ground pyrolytic graphite furnace tube that was used previously for GFAAS analysis was used in place of Carbotrap®, which suggests that impure Carbotrap® with refractory carbide of metals can break down all organo-Hg species into Hg°. The chromatogram in Fig. 3(b) shows that the derivative of MMHg partially decomposed and the Hg(II) almost completely decomposed. In Fig. 3(c), some derivatives of MMHg decomposed, while Hg(II) significantly decomposed In Fig. 3(d), the Hg° peak appears small, suggesting no significant decomposition. The chromatograms in Figs 3(b) and (c) were the result of a poor Carbotrap® batch which reproducibly decomposed the organo-Hg species. The chromatogram in Fig. 3(d) was the result of the more typical Carbotrap® material. Conversations with the manufacturer suggest that the tendency to break down organo-Hg compounds may be due to higher levels of tracemetals, such as iron, in the carbon. We have also observed that the poor quality material was of a smaller mean grain size which can promote decomposition due to the higher surface area of heated material. Thus,

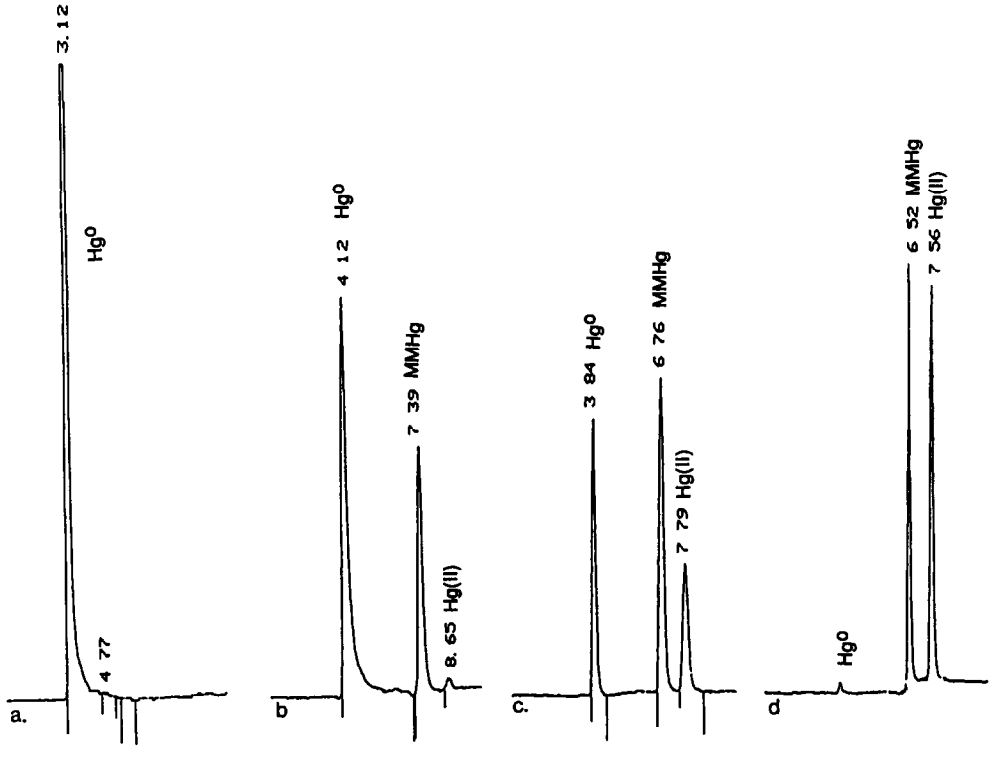

Fig 3 Chromatograms of ethylation derivatives of MMHg and Hg(II) (200 pg, as Hg for each) obtained using different carbon traps The first peak in each chromatogram is the Hg° resulting from breakdown of organo-Hg during the thermal desorption step The second peak is from MMHg and the third peak is from Hg(II)

to reduce the breakdown of organo-Hg on the Carbotrap®, it is critical that the material be from a previously tested high quality lot number. It also should not be crushed into a fine powder, and heating should be carried out quickly and to the lowest temperature which still releases all of the trapped organo-Hg (approximately 350°C wire, or 200°C internal tube temperature, after 30 sec of heating).

The method of packing the columns is also critical. The chromatograms in Figs 3(b) and (c) were obtained by packing two columns with Carbotrap® from the same batch, but each in a different manner. The column which provided the chromatogram for Fig. 3(b) was loosely packed, while the column for Fig. 3(c) was tightly packed as described in this paper. This indicates that appropriate packing can be helpful for reducing the decomposition of organo-Hg. Even Carbotrap® which provides chromatograms such as in Fig. 3(d) will give results more like 3(b) or (c) if too loosely packed. Most likely this is because the organo-Hg species may spread out in a loosely packed column under gas flow, increasing the contact with Carbotrap® Thus, at an elevated temperature decomposition may be promoted. Tight packing can avoid deep penetration of organo-Hg because of the shorter mean free path of the carrier gas. In this case, the organo-Hg species will be mostly trapped out at the entrance of the column rather than distributed over a long distance within the column.

This also explains our emphasis on the orientation of the Carbotrap® column. If the orientation illustrated in Fig. 2 is not followed, the organo-Hg will pass along the entire length of the column during thermal desorption, increasing contact with Carbotrap® and thus increasing the contact time with heated surfaces. The result is severe decomposition, yielding chromatograms like those in Figs 3(b) and (c).

In addition, it was found that when unsilanized quartz tubing and columns are used, decomposition occurs, also yielding chromatograms like those in Figs 3(b) and (c). Since the successful use of Carbotrap® relies excessively on a number of procedural conditions, an alternative absorbent, Tenax-TA®, was compared. Tenax-TA® columns were packed like the Carbotrap® columns, but contained only 80 mg of adsorbant due to its lower density. When Tenax-TA® columns were used, no decomposition of organo-Hg compounds was observed even when the column orientation was reversed.

Table 1 Stability of ethylation derivatives trapped on trapping columns for the determination of mercury species (800 pg as Hg for each)

	Storage time (hr) in which nondegradation is seen		
	Carbotrap™ #754140	Carbotrap™ #754200	Tenax-TA*
MMHg	15	15	144
Hg(II)	8	8	144

*It was no longer observed after 144 hr

Although there appears to be less risk of thermal decomposition using Tenax-TA® columns, packing and orienting as with Carbotrap® columns is still recommended.

Stability of organo-Hg on the trapping column

Two batches of Carbotrap® and Tenax-TA® were tested. All columns used for this experiment were newly packed and loaded with ethylation derivatives of 800 pg of Hg as each MMHg and Hg(II) by aqueous phase ethylation reaction. After collection, they were stored at room temperature and separate samples analyzed after 20 min, 1 hr, 2 hr, .. 1 day, two days, ... until significant decomposition was observed (Table 1). It was found that the organo-Hg species trapped on Tenax-TA® columns were very stable After storage for 6 days, no decomposition was observed Using Carbotrap® columns, the ethylation derivatives of MMHg were more stable than that of Hg(II).

Trapping efficiency and mass of absorbents

To avoid breakthrough of organo-Hg species during purging and to find an appropriate mass of fillings for packing columns, the trapping efficient *vs* mass of absorbents for collection of ethylation derivatives of 800 pg as Hg of each DMHg, MMHg, and Hg(II) was observed. In this experiment, Carbotrap® and Tenax-TA® were used for packing columns with different mass, *i.e.*; packed lengths. The results are illustrated in Fig. 4. It was found that trapping efficiency increases with increasing molecular weight of the ethyl analogs. When trapping columns were packed with either 200 mg of Carbotrap® or 100 mg of Tenax-TA®, no breakthrough of organo-Hg was found.

Usable life of trapping column

Figure 5 illustrates the effect of the age (number of uses) of a Carbotrap® column on the sensitivity (a) and precision (b) presented as relative standard deviation (RSD). The value of each point in the figure is the mean of four

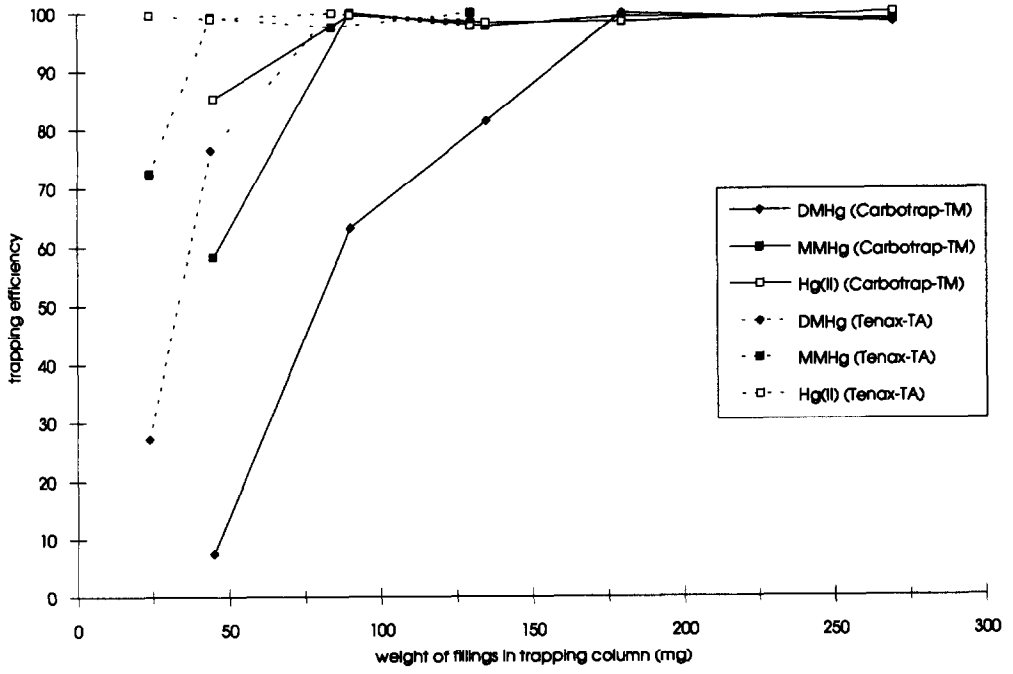

Fig 4 Relationship of filling mass of trapping columns to trapping efficiency for the ethylation derivatives of mercury species (800 pg, as Hg for each)

measurements For the Tenax-TA® column, we found a pattern similar to the Carbotrap® column. After 300 measurements, the sensitivity and precision did not decrease significantly.

Optimization of ethylation conditions

Ethylation reaction parameters which may affect results such as pH, reaction temperature, reaction time, concentration of ethylation reagent ($NaBEt_4$), purge time, and purge gas flow rate, were investigated for DMHg, MMHg and Hg(II). Results are illustrated in Fig. 6. The three species consistently exhibited similar behaviors. Figure 6(a) shows a broad optimum pH range of 3–7 where relative signals over 90% were obtained; 2*M* acetic acid/acetate was

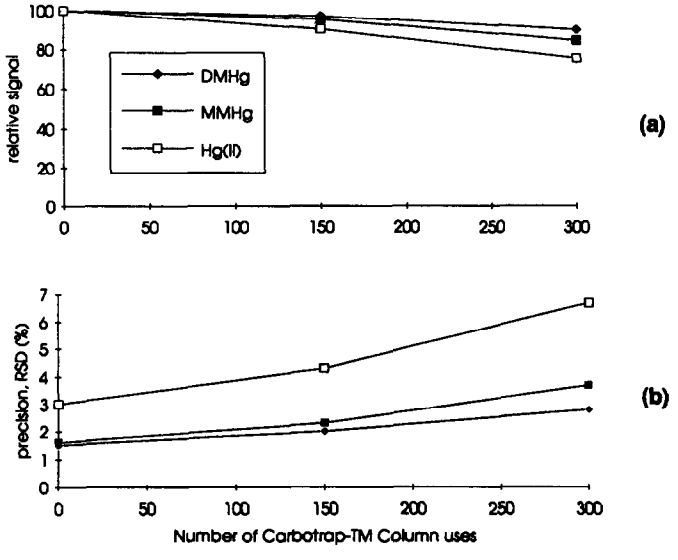

Fig 5 Observation of sensitivity (a) and precision (b) for the determination of mercury species (200 pg, as Hg for each) by ethylation reaction and trapping column collection as a function of columns' age (number of uses).

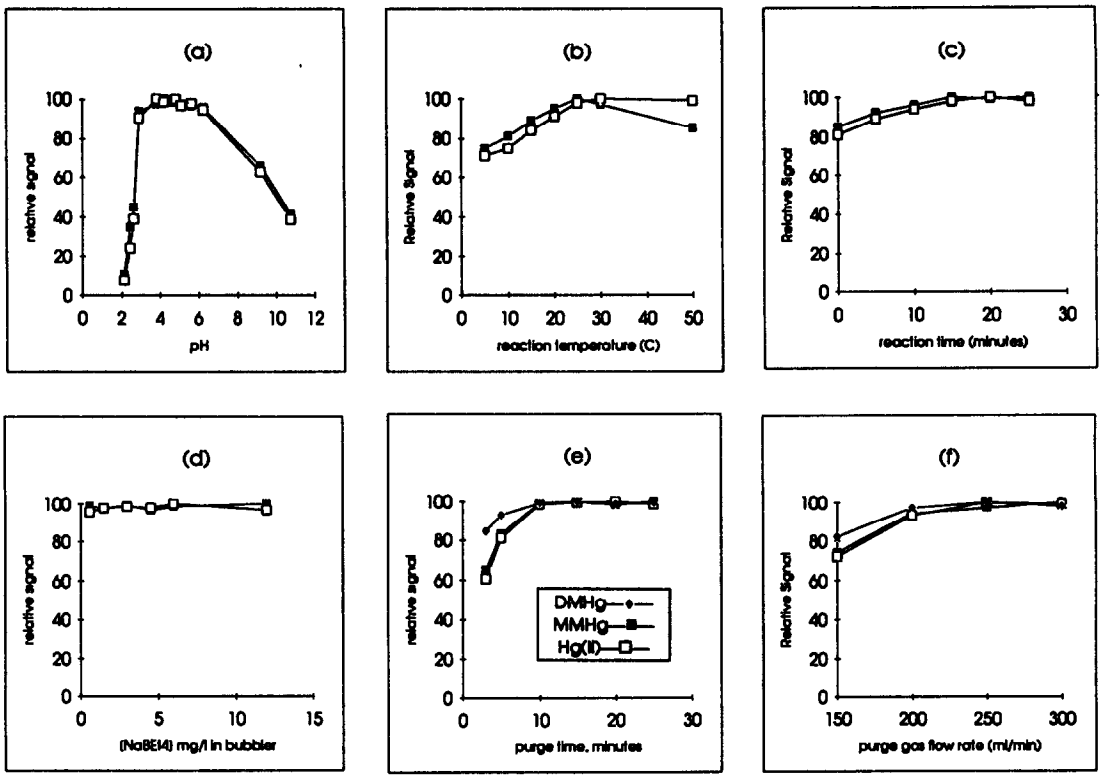

Fig 6 Optimization of ethylation conditions for the determination of mercury species (200 pg, as Hg for each) in DDW Effects of pH (a) reaction temperature (b), reaction time (c), concentration of $NaBEt_4$ (d), purge time (e), and purge rate (f).

chosen because it buffers at a pH of 4.9, which is in the middle range of the acceptable ethylation pH. In Fig. 6(b), based on a reaction time of 15 min, the optimal reaction temperature range was found to be 20–30°C. If the reaction temperature is lower, a longer reaction time is required. Figure 6(d) shows that an ethylation reagent concentration of 0.5 mg/l was strong enough for ethylation of 2 ng as Hg of both MMHg and Hg(II). However, for the direct determination of Hg in complex materials such as KOH digested biological materials, a higher concentration, typically 3 mg/l is required.[17]

Simplification and modification of the method

According to the original method, the organo-Hg was collected on the Carbotrap® first, then transferred to a liquid nitrogen GC column;

Table 2. Gas Chromatographic conditions and detection limits

Parameter	Gas chromatographic method Cryogenic	Isothermal		
Column length	45 cm	45 cm	75 cm	150 cm
Packing	15% OV-3	15% OV-3	15% OV-3	15% OV-3
Carrier gas	Ar or He	Ar or He	Ar or He	Ar or He
Flow (ml/min)	60	60	60	60
Des. temp*/Time	RT†-200°C	RT-200°C	RT-200°C	RT-200°C
GC Temperature	−196 to 180°C	75°C	85°C (100°C)‡	100°C
Peak separation	Excellent	Good	Excellent	Excellent
D.L. (pg Hg)				
DMHg	0.6	0.6	0.6	0.6
MMHg	0.4	0 6	0.6	0.6
Hg(II)	0.6	1 3	0.8	0.8
Anal time (min)§	23	4.5	6 (4.5)	8

*Desorption temperature.

†Room temperature

‡If no requirement of DMHg determination, 100°C can be used to obtain a better peak height sensitivity and speed up the analysis.

§Including time of desorbing from trapping column.

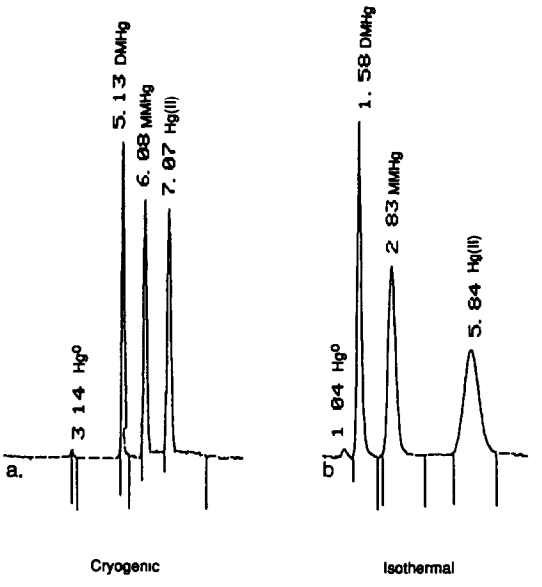

Fig 7 Typical chromatograms of methylation derivatives for the determination of mercury species (100 pg, as Hg for each) obtained by isothermal GC system (a) and cryogenic GC system (b)

hence the phrase "two-stage". Cryogenic GC techniques have been used for some time for collection and consequent separation of volatile derivatives of metal after ethylation reactions or hydride generation reactions.[21-23] Metal hydrides like Se and As are more volatile than the ethylation derivatives of mercury species that the liquid nitrogen GC column is used for. While using Bloom's method, the organo-Hg species were collected carefully on Carbotrap® columns at room temperature. Apparently, the use of liquid nitrogen is not necessary. In this work, using a longer GC column and an appropriate GC column temperature, the same accurate and precise results were obtained. Table 2 lists the GC conditions for both cryogenic and isothermal GC.

Table 3. Comparison of results obtained by cryogenic GC and isothermal GC for the determination of MMHg in distilled and alkaline digested riverine sediment samples

Sample (lab code)	MMHg (ng/g as Hg) Cryogenic	Isothermal
Digested		
2	3.80 ± 0 08	3 78 ± 0 01
5	3 41 ± 0 01	3 22 ± 0 09
7	2 00 ± 0.03	2.10 ± 0 03
9	3 07 ± 0 00	3 15 ± 0 04
Distilled		
1	0 033 ± 0.001	0 030 ± 0.002
10	0 026 ± 0.001	0 026 ± 0 001
16	0 182 ± 0 004	0.205 ± 0.027

Figure 7 illustrates typical chromatograms obtained by isothermal GC and cryogenic GC. As can be seen in Fig. 7(b), the isothermal GC provides an excellent separation. Table 3 lists some analytical results for the determination of MMHg in sediment samples obtained by isothermal GC and cryogenic GC. Samples were prepared both by distillation[19] and alkaline digestion.[8] There is good agreement in the results between the two techniques. Elimination of liquid nitrogen results in a three-fold improvement in analytical speed. By coordinating eight reaction vessels with eight trapping columns to one GC/CVAFS system, about 80 analyses can be performed within 8 hr, including standardization.

Choosing the best desorption temperature is critical to the isothermal method. A higher temperature favors rapid release of the species in a tight "plug", resulting in good peak separation. However, higher temperatures also risk thermal decomposition of the organo-Hg species. Thus, it is best to choose the lowest temperature which gives good separation, reproducibility, and no carryover. We have found that a ramped heating from room temperature to 200°C (interior of trap—approximately 350°C coil temperature) within 30 sec was optimal for both Carbotrap® and Tenax-TA® columns. This temperature is reached when the coil begins to show a faint red glow in a darkened room, just as the timer (30 sec) cuts off the power.

CONCLUSION

The improved method is sensitive, rapid and precise. It has been reliable for mercury determination and speciation of a variety of practice samples, certified reference materials and intercomparison samples.[18-20] Using this improved method, MMHg and Hg(II) were ethylated with $NaEt_4$. The ethylation derivatives were isolated from matrices by purging and precollection on the Carbotrap® or Tenax-TA® trapping column at room temperature, disconnected to GC/CVAFS. The derivatives were then desorbed onto an isothermal GC column and separated. The separated species were decomposed into Hg° and finally detected by CVAFS. About 80 analyses can be performed within an 8 hr period.

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REFERENCES

1 T Suzuki, N Imura and T Clarkson, *Advances in Mercury Toxicology* Plenum Press, New York, 1991
2. T W Clarkson, *The Toxicologist, an Official Publication of the Society of Toxicology, 31st Annual Meeting* **12,** 1992
3 G Westöo *Acta Chem Scand*, 1967, **21,** 1790
4 J E O'Reilly, *J Chromatogr*, 1982, **238,** 443
5 W Holak, *Analyst*, 1982, **107,** 1457
6 P J Craig and P A Morton, *Mar Pollut Bull*, 1983, **14,** 408.
7 D S. Ballantine Jr and W H Zoller, *Anal Chem*, 1984, 1288
8 N S Bloom, *Can J of Fish and Aq Sci*, 1989, **46,** 1131
9 M Horvat and A Byrne, *Talanta*, 1990, **37,** 207
10 S Rapsomanikis and P J Craig, *Anal Chim Acta*, 1991, **248,** 563
11 P Lansens, C Meuleman and W Baeyens, Abstracts, *Int Conf on Mercury as a Global Pollutant*, 31 May–4 June 1992 Monterey, Ca, USA
12. M. Fillipelli, F. Baldi, F E. Brinckman and G J Olson, *Env. Sci. and Technol*, 1992, **26,** 1457
13 S Charbonneau, H. V Tra and P Pichet, Abstracts, *Int Conf. on Mercury as a Global Pollutant* 31 May–4 June, 1992, Monterey, CA, USA
14 R. Fischer, S. Rapsomanikis and M O Andreae Abstracts, *Int. Conf on Mercury as a Global Pollutant*, 31 May–4 June, 1992 Monterey, Ca, USA
15 R Fischer, S Rapsomanikis and M O Andreae, *Anal Chem*, 1993, **65,** 763
16 N S Bloom and W F Fitzgerald, *Anal Chim Acta*, 1988, **208,** 151
17 L Liang and N S Bloom, *JAAS*, 1993, **8,** 591
18 L Liang, N S Bloom and M Horvat, *Simultaneous Determination of Mercury Speciation in Biological Materials by GC/CVAFS after Ethylation and Room Temperature Precollection*, Clin Chem, 1994 (in press)
19 M Horvat, N S Bloom and L Liang, *Anal Chem Acta*, 1993, **281,** 135
20 M Horvat, L Liang and N S Bloom, *Anal Chem Acta*, 1993, **281,** 153
21 L S Cutter, G A Cutter and M L C San Diego-McGlone, *Anal Chem*, 1991, **63,** 1138
22 L S Cutter, *Speciation of Selenium and Arsenic in Natural Waters and Sediment, Vol 1 Selenium Speciation* EPRI, 1986
23 E A Crecelius, N S Bloom, C E Cowan and E A Jenne, *Speciation of Selenium and Arsenic in Natural Waters and Sediment, Vol 2 Arsenic Speciation* EPRI, 1986