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Comparative studies of methylmercury determination in biological and environmental samples

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Some parameters affecting the accuracy of various approaches to methylmercury (MeHg) determination in biological and environmental samples were studied. Different isolation techniques (ion-exchange, extraction, volatilization, distillation) and final measurement via cold vapour atomic absorption spectroscopy (CV AA) or gas chromatography (GC) were compared. Results obtained by the various isolation techniques are comparable for almost all biological and environmental samples, except for soils and some sediments, where disagreement between the results obtained by GC and CV AA was found. In order to resolve these problems, a new separation technique based on distillation of MeHg from the sample followed either by CV AA or GC was developed. The new method results in very good recovery and reproducibility (95 ± 2%) for all samples examined (fish, mussel, shrimp, blood, hair, algae, sediment, etc.), is specific for MeHg and provides for its differentiation from other species by an indirect CV AA determination.

Gas-chromatographic measurement of the isolated MeHg using different packings and conditioning of the columns is also discussed. The distillation method with GC detection is advantageous in producing cleaner chromatograms and in prolonging the life-time of the packing and the intervals between reconditioning.

Keywords: Methylmercury, analysis, biological tissue, environmental samples

INTRODUCTION

Studies of the ecological cycle of mercury and its organic compounds and their health effects in man, especially at low concentration levels, are still of great interest. The most frequently found organic mercury compound is methylmercury (MeHg), which is formed naturally by methylation of inorganic mercury. Concentrations of mercury and MeHg in environmental and biological samples are relatively low, except in exposed or industrial areas. In order to define the range of natural levels of this element and its organic compounds, sensitive and accurate analytical procedures must be available.

For total mercury, standard reference materials are certainly an important aid in quality control of analytical results. They are available for total mercury in a range of biological and environmental matrices. In the absence of any certified reference material for MeHg, however, accuracy of analytical procedures has to be assured by other means of analytical quality control, including interlaboratory comparisons, standard addition experiments, radiotracer methods and comparison of the results obtained by different analytical techniques. Aiming to validate analytical procedures for methylmercury determination, we compared results obtained by methods developed in two laboratories participating in an extended interlaboratory cooperation scheme. Using a combination of methodological experience for MeHg determination from both laboratories, we developed a new isolation technique based on distillation of MeHg from biological and environmental samples followed by cold vapour atomic absorption spectrometry (CV AA) or gas chromatography (GC) as the final measurement.

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In the present work the results of a collaborative intercomparison is reported and the new technique for MeHg is compared with existing methods.

EXPERIMENTAL

Determination of total mercury

The principles of methods for determination of total mercury by CV AA and neutron activation analysis (NAA) are presented in Scheme 1, together with their primary references.

Determination of methylmercury

Different approaches to the separation and determination of MeHg in various biological and environmental samples were compared and their principles are presented in Scheme 2. Most of the methods, except distillation, are in routine use and have already been described elsewhere.[5–11]

The separation of MeHg from inorganic Hg by distillation and its determination by CV AA and GC are presented below.

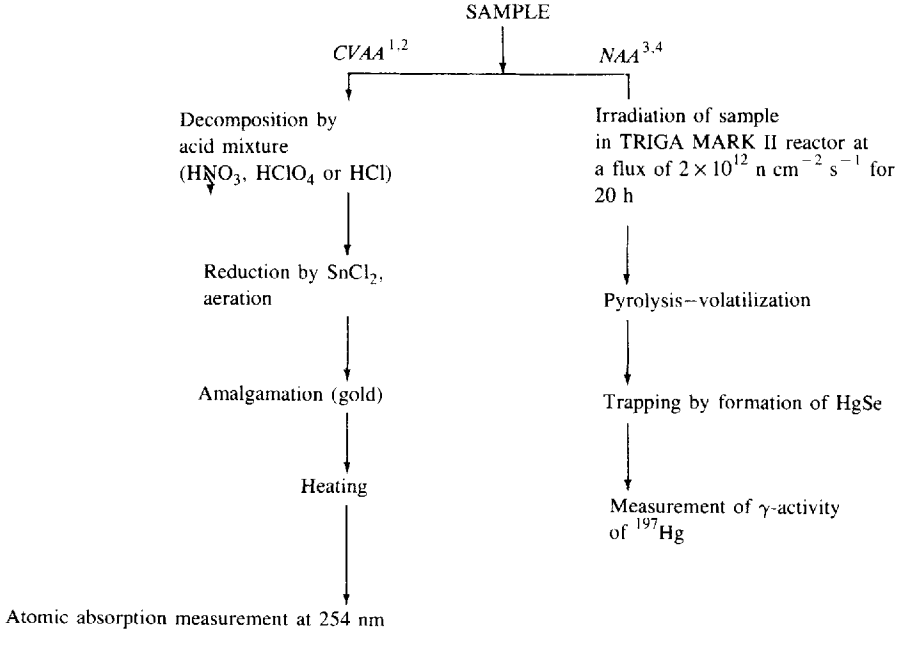
Distillation

The apparatus for distillation of MeHg is shown in Fig. 1. To a definite quantity of homogenized sample (up to 3 g of dry and up to 5 g of fresh (wet) sample) in glass tube 1, 1 cm^3 of 2 mol dm^{-3} H_2SO_4 and 1 cm^3 of 10 % NaCl were added. The mixture was diluted to 10 cm^3 with distilled water. Distillation was started immediately after addition of the reagents at an air flow of 14 d^3 h^{-1} and a heating block temperature of 150 °C. The distillate was collected in glass tube 4, which was cooled with water at room temperature. Distillation was finished in approximately 1.5 h (depending on the quantity and type of sample) when 8.5 cm^3 of distillate had been collected. The aqueous distillate must be stored in the dark to avoid loss of MeHg by decomposition. The above procedure gave optimum recoveries.

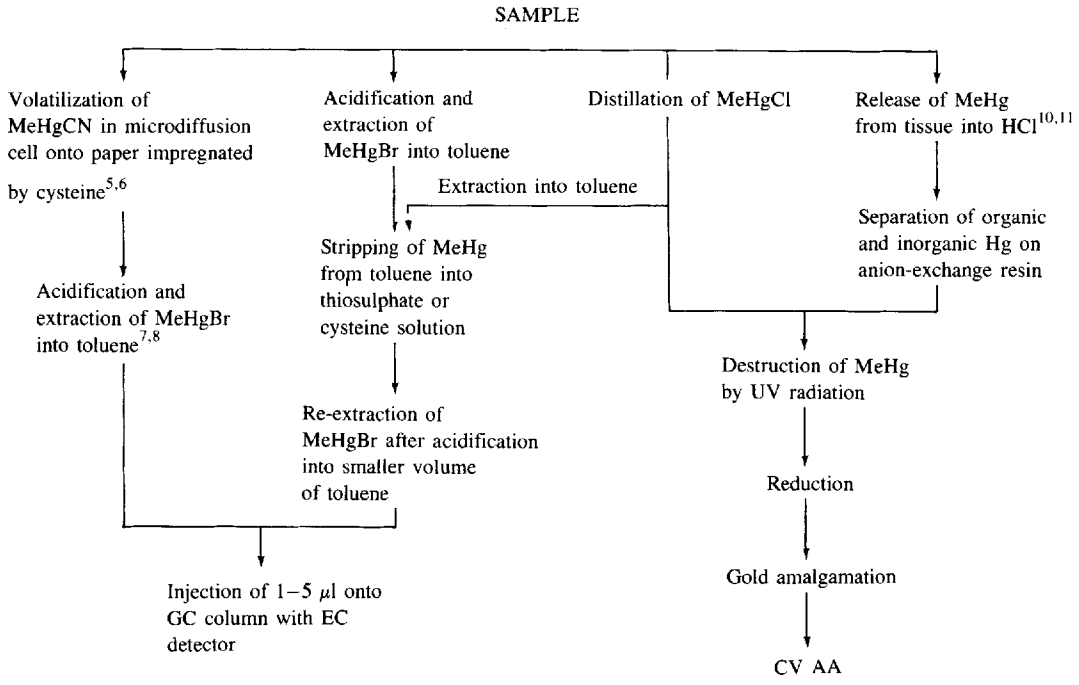
Determination of MeHg by CV AA

To an aliquot of aqueous distillate (Fig. 1), 1 cm^3 of conc. hydrochloric acid was added to prevent adsorption of mercury on the glass wall. An aliquot was checked for any inorganic mercury present by injection into the reduction–aeration cell followed by CV

Scheme 1 Methods for determination of total mercury.



Scheme 2 Alternative approaches to the separation and determination of methylmercury



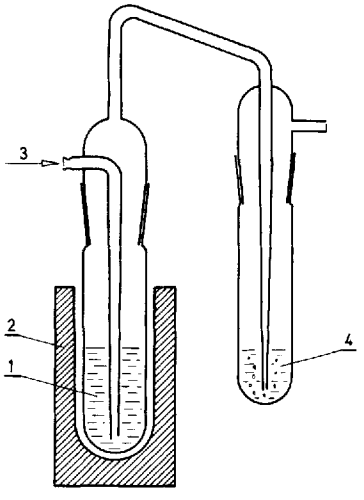


Figure 1 Apparatus for distillation of MeHg from the sample. 1. sample with reagents and water, 2. heating device, 150 °C. 3. flow of N_2 or air, 4. distillate

AA (Scheme 1). MeHg was decomposed to inorganic mercury(II) either by ultraviolet (UV) irradiation (150 W mercury lamp) or by digestion with an equal volume of a 3:1 conc. nitric acid/perchloric acid mixture. It is advisable to use UV irradiation because, in the clean and colourless distillate solution, decomposition is rapid (10–20 min), and no additional reagents which would contribute to the analytical blank are needed.

Mercury was determined as mercury vapour by an extremely sensitive CV AA method using reduction and preconcentration on a gold trap, as previously described.[10,11]

Determination of MeHg by GC

To separate MeHg from the distillate, a modified Westöö extraction procedure was used.[7] To a (1–8 cm^3) aliquot of the distillate, 1 cm^3 of potassium bromide/sulphuric acid solution (4 mmol

dm^{-3} KBr : 2 mol dm^{-3} H_2SO_4, 1:1) was added and MeHgBr was then extracted twice with 1 cm^3 toluene, followed by stripping of MeHgBr from the combined toluene phases into 1 cm^3 of cysteine solution (1%, L-+-cysteine: hydrochloride in 20% sodium citrate). The cysteine phase was quantitatively separated in a conical test tube. After addition of 1 cm^3 potassium bromide/sulphuric acid solution to the separated cysteine phase, MeHgBr was re-extracted into benzene (0.2–1.0 cm^3, depending on the expected MeHg concentration). Each extraction step was equilibrated for 5 min and followed by 5 min centrifugation at 3000 rpm.

Conditioning procedure for GC columns

The gas chromatograph used was a Hewlett–Packard Model 5890, equipped with ^{63}Ni electron capture detection. Injector and detector temperatures were maintained at 190 and 280 °C, respectively. Carrier gas (nitrogen) flow rate was 50 cm^3 min^{-1} for a glass column 2 mm i.d. and 1.6 m in length.

Three commercially prepared packings were used: GP 5 % DEGS-PS on Supelcoport (100–120 mesh), 5 % Carbowax R 20M on Supelcoport (100–120 mesh) and 5 % PEGS on Diatomite 'C' 100–120 mesh. All analyses were performed isothermally; the column temperature depended on the packing used (145–170 °C) and was adjusted to obtain satisfactory sensitivity and stable response (for 20 pg MeHg with ± 3 % reproducibility), and an appropriate peak shape with minimum tailing.

Conditioning of a new column was done by repeated injection of mercuric chloride ($HgCl_2$) in benzene (saturated solution, at column temperature of 140 °C). Later, when the column is used for routine injections, a decrease in peak height and increased tailing is usually eventually observed, so that passivation of the column by $HgCl_2$ injection is frequently also needed, depending on the temperature of the column and the type of sample injection.

RESULTS AND DISCUSSION

Comparison of the results for total mercury in various samples obtained by the methods presented in Scheme 1 in the laboratories involved in the interlaboratory studies showed good agreement (Table 1). The advantages of CV AA are numerous, as it is more rapid, inexpensive, suitable for routine analysis, and in combination with a preconcentration stage using a gold trap, also achieves very low detection limits for aqueous, liquid and solid samples. NAA is nevertheless valuable as a complementary reference technique.

The advantages and drawbacks of different approaches for isolation and final measurement of MeHg are presented in Table 2. The selected method has to

Table 1 Intercomparison of the results for total mercury in KFA ESB Reference materials (μg Hg kg^{-1} dry weight) and some other samples

	Lab 1[a]	Lab 2[b]	
Sample	CV AA	CV AA	NAA
Mussel (ESB, Muschell standard F, 1981)	187 ± 5 (8)[c]	170 ± 7 (3)	158 ± 7 (6)
Poplar (ESB, Pappel–blätter standard II, 1981)	48.4 ± 0.7 (8)	45.9 ± 2.0 (3)	45.4 ± 2.1 (6)
Algae (ESB, Algenstandard II, 1981)	87.2 ± 1.0 (6)	84.2 ± 2.9 (3)	86.1 ± 3.7 (6)
Sludge (SOE-M-081)	5800 ± 187	5111 ± 106 (3)	4930 ± 110 (2)
Sludge (Düs-M-108)	4100 ± 160	3441 ± 130 (3)	3150 ± 255 (2)
Algae fresh (KFA, 93)	10.3 ± 0.2	4.19 ± 0.27 (3)	3.67 ± 0.27 (2)

[a] Lab 1, Nuclear Research Centre, Jülich, FRG. [b] Lab 2, Institute 'Jozef Stefan', Ljubljana, Yugoslavia. [c] ± The standard deviation; the number in parentheses is the number of independent determinations.

be either specific for MeHg or it has to provide for its differentiation from other species by an indirect method. A method which promises to fulfil sufficiently all these requirements is based on distillation of MeHg from the sample followed by GC or CV AA as the final measurement. Results obtained by the distillation method are discussed below.

The present distillation method differs from that described by Nagase *et al.*[9] in using different reagents to release MeHg from the sample, different apparatus for distillation and a different decomposition method for MeHg; its applicability not only for sediments but for various biological and environmental samples was also tested. Actually, the use of sulphuric acid and

Table 2 Advantages and drawbacks of different approaches to determination of methylmercury

Method	Advantages	Drawbacks	References
Volatilization	Direct determination; simple and selective, suitable for routine work.	Applicable mainly to biological and not to environmental samples. Decrease in recovery with higher amounts of sample.	5,6
Extraction	Direct determination; application to biological and environmental samples.	Problems with emulsion formation during extraction steps. Low recovery with some samples (water, sediment).	8,11
Anion-exchange	Extremely sensitive; simple.	Indirect determination.	10
Distillation	Fast, simple, selective; applicable to biological and environmental samples.	Need further development.	9

Table 3 Results for MeHg obtained by distillation/CV AA method using different reagents

Sample	Reagent	n[a]	Result (ng Hg g)	Recovery[b] (%)
Mussel, 166–11, KFA, fresh	HCl, NH_4Cl	15	6.1 ± 0.2	80 ± 3
	H_2SO_4, NaCl	3	7.4 ± 0.3	98 ± 4
ESB Fish standard I, 1981	HCl, NH_4Cl	4	187 ± 4	74 ± 2
	H_2SO_4, NaCl	11	249 ± 6	98 ± 3
	H_2SO_4, NaBr	3	234 ± 8	92 ± 3
Algae, 166–11, KFA, fresh	HCl, NH_4Cl	2	0.61 ± 0.05	84 ± 8
	H_2SO_4, NaCl	2	0.82 ± 0.01	112 ± 1
MeHgCl standard solution[c]	HCl, NH_4Cl	3	—	80 ± 3
	H_2SO_4, NaCl	4	—	94 ± 2
	H_2SO_4, NaBr	2	—	92 ± 2
EtHgCl standard solution[c]	HCl, NH_4Cl	2	—	78 ± 4
	H_2SO_4, NaCl	3	—	83 ± 6
	H_2SO_4, NaBr	2	—	20 ± 1

[a] n, Number of determinations; error quoted is standard deviation. [b] recovery is calculated from the mean value of the ion-exchange/CV AA determination (see Table 4). [c] Different amounts of MeHgCl and EtHgCl were taken (from 24 to 210 ng).

sodium chloride instead of hydrochloric acid and sodium chloride in order to release MeHg quantitatively from the sample in a volatilization technique[5,6] gave us the idea of checking different reagents to separate MeHg as chloride or bromide; the results are given in Table 3. It is evident that using sulphuric acid and sodium chloride resulted in good recovery (95 ± 2 %) for all samples examined and also for methyl and ethylmercury standard solutions. Use of other reagents resulted in lower recoveries, mainly because of decomposition of MeHg/EtHg in glass tube 1 (Fig. 1). The use of sulphuric and sodium chloride results in a nearly linear release of MeHg from the sample, which is evident from the recovery curves of MeHg (Fig. 2) from a fish standard (ESB KFA fish standard I), whereas use of hydrochloric acid and sodium chloride results in lower recoveries. Comparison of recovery curves obtained by the use of various volume ratios of sulphuric acid and sodium chloride provided the optimum ratio.

Phenylmercury does not distil because it decomposes in test tube 1 (Fig. 1). Inorganic mercury(II) does not distil even when present in a 1000-fold excess and therefore does not affect the final CV AA determination.

Comparison of the results for MeHg obtained by ion-exchange and distillation followed by CV AA shows good agreement for almost all samples, except soils

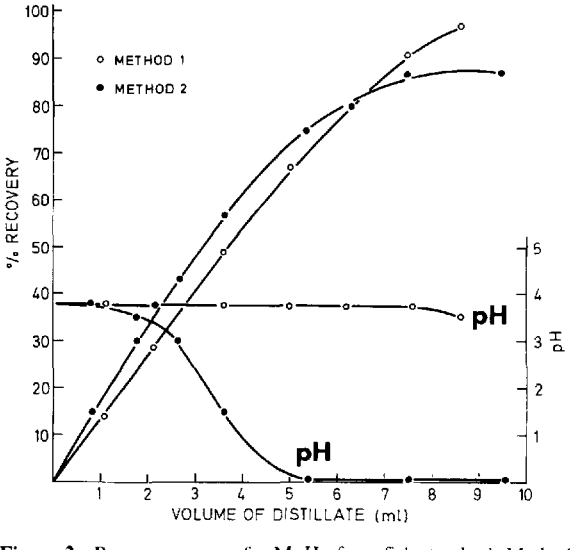


Figure 2 Recovery curves for MgHg from fish standard. Method 1, H_2SO_4, NaCl. Method 2, HCl, NaCl. pH, pH of distillate fractions.

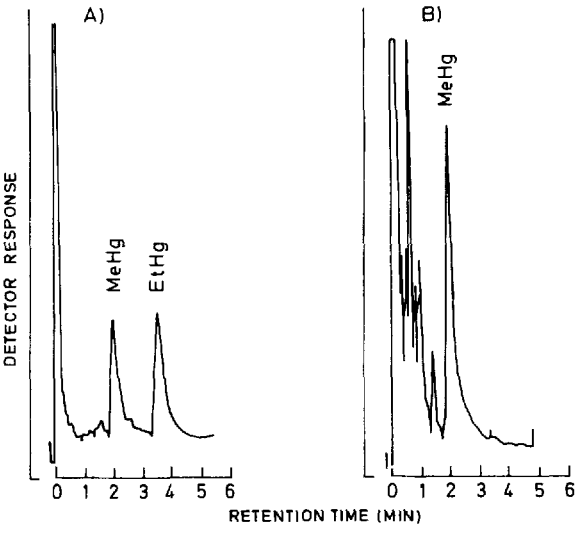


Figure 3 Typical chromatogram; for (A) 1 μl injection of a standard solution of 0.02 $\mu g\ cm^{-3}$ methylmercury and ethylmercury in benzene; and (B) 1 μl from 0.5 cm^3 of final benzene extract (using modified Westöö method) from fresh mussel sample with a concentration of 7 ng MeHg g^{-1}. Column: 5 % PEGS on Diatomite 'C', operated at 145 °C and 60 $cm^3\ min^{-1}$ carrier gas-flow rate.

and some sediments (Tables 4, 5). Therefore, more comparisons were done using direct GC determination, which requires a relatively time-consuming extraction clean-up procedure, but as MeHg is measured directly (not after conversion to inorganic Hg), it is intrinsically a more reliable approach. (Actually, using an electron capture detector even GC is not really a direct measure of MeHg as in fact the halide (bromide or chloride) is measured, and therefore the use of a microwave emission spectrometric detector would be advantageous.[12])

The main advantage of the distillation/extraction isolation of MeHg over extraction of MeHg directly from the sample, is in its better recovery (93 ± 4 %), mainly due to avoidance of difficulties associated with emulsion formation during the extraction steps, and in obtaining clean chromatograms from the final benzene extract. Therefore treatment of the GC column with mercury(II) chloride–benzene solution is not very often needed, as it is known that active sites, as well as impurities in the sample adsorbed or bonded to column material, cause decomposition and poor chromatography of organic mercury compounds.[13] The absolute detection limit for a standard solution is about 5 pg of MeHgCl and EtHgCl. The operational

Table 4 Comparison of results for MeHg (given as μg Hg kg^{-1}) obtained by two different isolation techniques followed by CV AA determination

Sample	Ion-exchange[a]	Distillation[a]
Mussel 166–11 KFA, fresh	7.5 ± 0.3 (8)	7.4 ± 0.3 (3)
ESB Fish standard I, 1981	253 ± 4 (4)	249 ± 6 (11)
Algae 166–11 KFA, fresh	0.73 ± 0.07 (2)	0.82 ± 0.01 (2)
Soil 3.2	14.3 ± 3.2 (6)	0.27 ± 0.05 (4)

[a] ± Standard deviation; the number in parentheses is the number of determinations.

limit of quantitation for the new distillation/extraction procedure (5 g fresh sample, 0.5 cm^3 final benzene extract, 1 μl injection) is about 0.2 μg MeHg kg^{-1}.

In Figs 3, 4 and 5 some chromatograms obtained by three different column packings and different isolation techniques for various samples are given, illustrating the improvement achieved by the distillation/extraction approach.

All column packings were used successfuly. A newly prepared column showed more variable time stability (peak height/area, retention time) than an older one. After conditioning mercury(II) chloride treatment (Figs 5B, C), peak height is twice as great as before, with smaller tailing. It was found that with repeated use the peak height/area tends to decrease slowly and tailing increases; for PEGS at a column temperature of 145 °C passivation of the column was needed in 4–5 days, whereas for DEGS at 170 °C, it was required after 1–2 days. No differences of peak height and retention time between MeHgCl and MeHgBr injection were found, as was also proved by other authors using GC MS.[14] For some samples, particularly those that are apparently very low in MeHg, there is always a suspicion that the peak may be due to some other component in the extract. To provide firm evidence for the presence of MeHgCl or EtHgCl in a sample, its re-extraction from the final benzene extract into

Table 5 A comparison of the results obtained by different approaches to MeHg determination in various samples, expressed as μg Hg kg^{-1}

Sample	Volatilization GC	Extraction GC	Distillation CV AA[a]	Distillation GC	Ion-exchange CV AA[a]	Total Hg CV AA/NAA
KFA mussel, fresh, 538520	—	12.4 ± 0.8	11.0 ± 0.3	10.4 ± 0.9	11.6 ± 0.7	32.4 ± 1.83
IEAE mussel tissue,[b] MA-M-2/OC	—	MeHg 60.3 ± 4.1 EtHg 727 ± 42	732 ± 24	MeHg 74 ± 5 EtHg 710 ± 40	643 ± 5	837 ± 31
IAEA fish homogenate, MA-A-2/OC	317 ± 21	312 ± 17	309 ± 13	318 ± 15	300 ± 9	445 ± 28
KFA algae, fresh, 538511	—	0.25 ± 0.03	0.30 ± 0.06	0.40 ± 0.09	0.35 ± 0.05	6.1 ± 1.2
NBS oyster tissue, 1566, 1979	—	14.9 ± 0.7	16.3 ± 0.8	—	—	48.8 ± 3.1
NBS oyster tissue, 1566a, 1985	—	9.8 ± 1.0	88 ± 1.1	9.6 ± 1.0	—	62.1 ± 5.1
Human placentae	5.1 ± 0.3	4.9 ± 0.5	4.1 ± 0.7	4.0 ± 1.2	3.2 ± 0.2	13.3 ± 0.5
Hair 1	161 ± 10	156 ± 8	147 ± 16	152 ± 13	165 ± 13	241 ± 12
Hair 2	688 ± 51	620 ± 43	573 ± 64	619 ± 42	720 ± 28	11369 ± 517
Sediment 1[c]	—	—	0.45 ± 0.10	0.30 ± 0.08	0.32 ± 0.10	673 ± 24
Sediment 2[c]	—	0.84 ± 0.13	1.15 ± 0.21	1.04 ± 0.18	2.74 ± 0.15	7340 ± 112
Sediment 3[c]	—	0.29 ± 0.7	0.58 ± 0.13	0.41 ± 0.10	4.99 ± 0.61	723 ± 74
Soil 3.2	—	< 0.2	0.27 ± 0.05	< 0.2	14.3 ± 3.2	

Results are expressed as the mean value ± S.D. of at least three or more independent determinations. [a] Total organic mercury [b] Ethyl mercury was spiked during the preparation of the standard reference material. [c] Sediments were obtained from Kastela Bay, Central Adriatic.

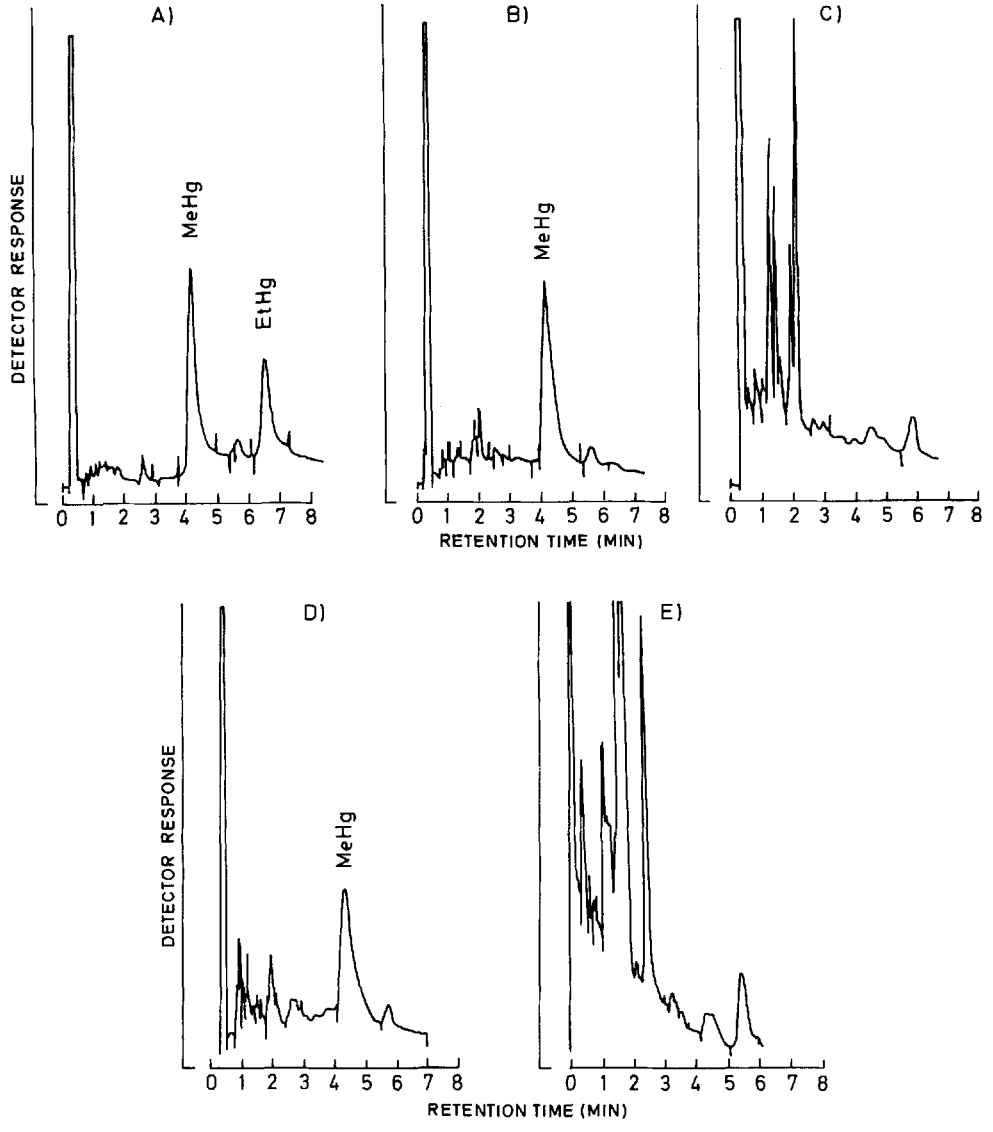


Figure 4 (A) Chromatogram for 1 μl standard solution of 0.023 μg MeHg cm^{-3}. (B,D) Chromatograms for 1 μl from 0.5 cm^3 of the final benzene extract (using distillation/extraction procedure) for KFA fresh mussel and algae, respectively; (C,E) chromatograms for 5 μl injection of the same benzene, *after* extraction of MeHg into aqueous cysteine solution. Column: 5 % Carbowax 10M on Supelcoport (100–120 mesh) operated at 170 °C and 60 cm^3 min^{-1} carrier gas-flow rate.

aqueous cysteine solution must result in the disappearance of the MeHg or EtHg peak, as shown in Fig. 4 (C,E).

Table 5 shows that the results obtained by different approaches for MeHg determination (presented in Scheme 2) are in good agreement for all biological materials tested, except that significantly higher values for MeHg in some fresh sediments and soil were obtained by ion exchange/CV AA in comparison with the other techniques. This is probably due to the non-specific separation of organic and inorganic mercury and/or the indirect measurement of MeHg in such environmental samples by CV AA. Additionally, the results in Table 5 confirm the advantages and

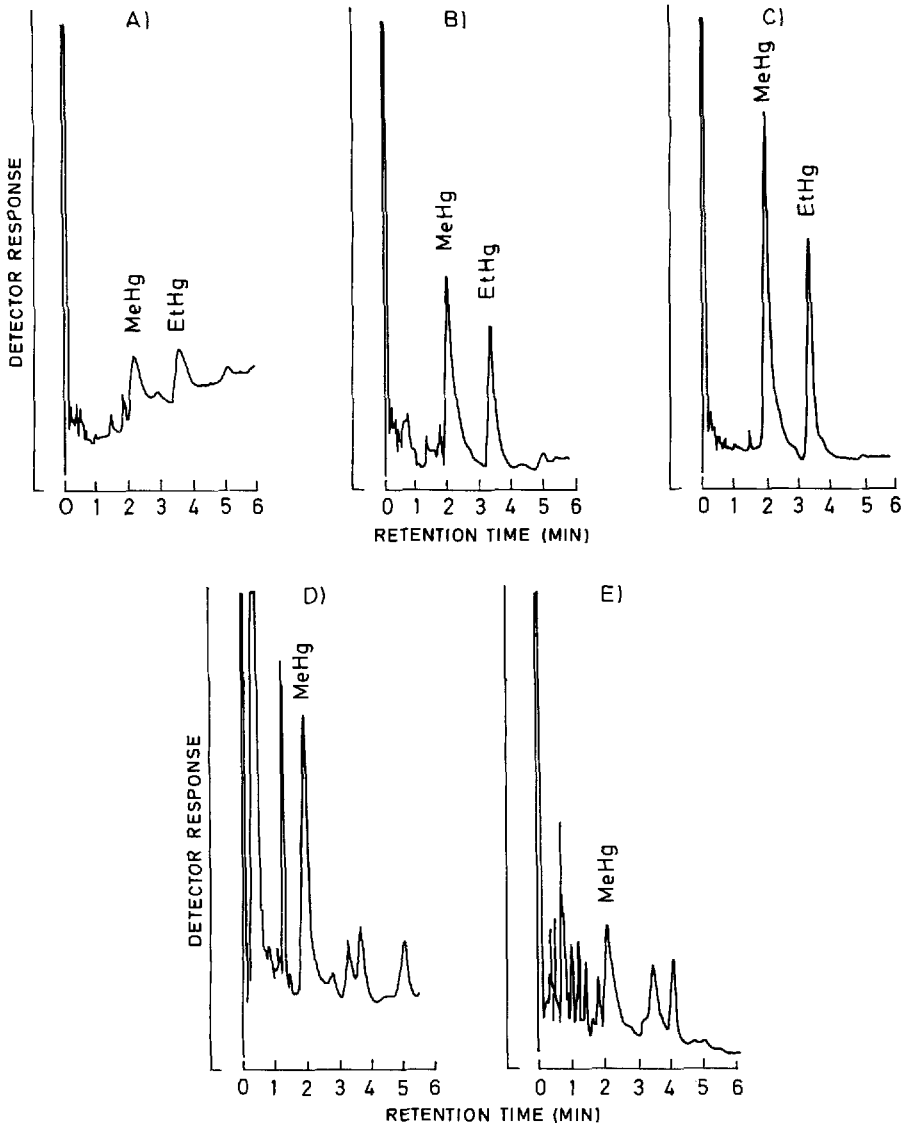


Figure 5 Chromatogram for 1 μl standard of 0.023 μg MeHg cm^{-3} and 0.020 μg EtHg cm^{-3} (A) Chromatogram on a new column (B) Chromatogram obtained 2 h after mercuric chloride treatment. (C) Chromatogram obtained on an older column after mercuric chloride treatment. (D,E) Chromatograms for 1 μl from 0.5 cm^3 of the final benzene extract (using distillation/extraction and modified Westöö method, respectively) for NBS oyster tissue 1566. Column: GP 5 % DEGS-PS Supelcoport (100–120 mesh) operated at 170 °C and 60 cm^3 min^{-1} carrier gas-flow rate.

drawbacks presented in Table 2. The good agreement of the results obtained by distillation followed by CV AA or by GC for all materials tested confirms the specific separation of MeHg (and EtHg if present), which provides for its differentiation from other species by an indirect CV AA determination.

It can also be concluded that before starting a large series of measurements, especially for a system in which varying ratios of inorganic to organic mercury are to be expected, it is advisable to cross-check results using two or more different analytical methods.

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REFERENCES

1. Horvat, M, Zvonaric, T and Stegnar, P *Vestn. Slov. Kem. Drus.*, 1986, 33(4): 475
2. May, K and Stoeppler, M *Fresenius Z. Anal. Chem.*, 1984, 317: 248
3. Kosta, L and Byrne, A R *Talanta*, 1969, 16: 1297
4. Byrne, A R and Kosta, L *Talanta*, 1974, 21: 115
5. Zelenko, V and Kosta, L *Talanta*, 1973, 20: 115
6. Gvardjancic, I, Kosta, L and Zelenko, V *Zh. Anal. Khim.*, 1978, 32: 812
7. Westöö, G *Acta Chim. Scand.*, 1968, 22: 2277
8. Dermelj, M, Horvat, M, Byrne, A R and Stegnar, P *Chemosphere*, 1987, 16(4): 877
9. Nagase, H, Ose, Y, Sato, T and Ishikawa, T *Intern. Environ. Anal. Chem.* 1980, 7: 287
10. May, K, Stoeppler and M, Reisinger, K *Toxicol. Environ. Chem.*, 1987, 13: 153
11. May, K and Stoeppler, M *Fresenius Z. Anal. Chem.*, 1984, 317: 248
12. Talmi, Y *Anal. Chim. Act*, 1975, 74: 117
13. O'Reilly, I E *J. Chromatogr.*, 1982, 238: 433
14. Baughman, G L, Carter, M H, Wolf, N L and Zepp, R G *J. Chromatogr.*, 1973, 75: 471