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The analysis of organic mercury compounds using liquid chromatography with on-line atomic fluorescence spectrometric detection

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A new method for the analysis of organic mercury compounds is reported. The organomercurials are separated by high-performance liquid chromatography (HPLC). The compounds are converted to mercury(0) in a continuous-flow system by means of an oxidizing and a subsequent reducing solution. The elemental mercury generated is swept into the cell of an atomic fluorescence spectrometer (AFS) by a stream of argon. The compositions of the oxidizing solution, which contains peroxodisulphate and copper(II) in dilute sulphuric acid, and the reducing solution, which contains alkaline tin(II) chloride, were optimized, as were the gas–liquid separator (GLS), the condensing system and the geometry of the reaction coils. The method is applied to extracts of certified reference material (CRM) and to river sediments. High concentrations of methylmercury were found in the sediment samples. At one location, the presence of ethylmercury is derived from the sample chromatogram.

Keywords: Liquid chromatography (HPLC), atomic fluorescence spectrometry (AFS), methylmercury, ethylmercury, organomercury, sediment

INTRODUCTION

Mercury is known to exist in several forms in the natural environment. Of great interest are the organic mercury compounds which are highly toxic. They can be released into the environment by anthropogenic emissions or are produced by micro-organisms which can convert inorganic mercury to methylmercury. In order to investigate the transport, bioavailability and toxicity of the different forms it is necessary to determine the exact physico-chemical species of mercury in environmental samples.

In environmental matrices such as sediments, soils or water samples, usually just a fraction of the total mercury is in an organic form. This requires careful and non-destructive isolation of the compounds of interest from the matrix followed by a powerful separation of the different organic species. Because of the low concentrations usually involved, a very sensitive detection system is needed to conclude the analysis.

The most widely used technique for the determination of individual mercury species involves gas chromatography (GC) with detection by electron capture (ECD)[1,2], emission spectrometry (MIP)[3] or atomic absorption (AAS).[4] However, without derivatization of the organomercurials, problems with the chromatographic behaviour of the monoalkylmercurials have been reported.[5] In order to by-pass this GC problem, some authors have developed derivatization techniques and have converted the monoalkylmercurials to dialkyl derivatives prior to analysis.[6–9] However, these techniques usually aim at methylmercury and some organomercury compounds cannot be analysed, e.g. ethylmercury, if ethylation is performed.

Liquid chromatography (LC) allows a simpler sample treatment and has been shown to be able to separate a great variety of organic mercury compounds.[10] However, the common detectors in LC have often shown a poor sensitivity towards organomercurials. Recently, detection by inductively coupled plasma atomic emission spectrometry (ICP-AES),[11] atomic absorption spectrometry (AAS)[12] and electrochemical devices[13] have been reported.

This paper describes the coupling of an HPLC system and an atomic fluorescence spectrometer (AFS) using a continuous-flow reducing interface for the determination of organic mercury compounds in sediments. To our knowledge, this is the first HPLC-AFS system for the analysis of organomercurials reported in the literature. The method combines the element specificity of the

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atomic fluorescence detection with its unique sensitivity and has been applied to certified reference material (CRM) and to river sediments neighbouring an old plant where organomercurials have been produced.

EXPERIMENTAL

Reagents

The organomercury stock solutions (RHgCl; R = CH_3, C_2H_5, $CH_3OC_2H_4$, $C_2H_5OC_2H_4$, C_6H_5, $CH_3C_6H_4$, $HOOCC_6H_4$ and nitromersol) were prepared by dissolving the compounds in water or methanol as previously described.[10] Standard solutions (usually 0.04 mg dm^{-3}) were prepared by diluting the stock solutions with water. Chromatographic solvents were HPLC grade; all other chemicals were analytical grade. The oxidizing and the reducing solutions were freshly prepared as needed, and the reducing solution was further purified from mercury by bubbling with nitrogen for two hours. The citrate buffer consisted of citric acid (21 g dm^{-3}) and sodium hydroxide (8 g dm^{-3}) and was adjusted to pH 2 with hydrochloric acid (0.1 mol dm^{-3}). The dithizone extractant (0.25 mmol dm^{-3}) was prepared in chloroform from a stock solution. The destruction of the dithizone–mercury complexes was achieved using a 1:1 (w/v) mixture of 5% sodium nitrite and an acid solution consisting of hydrochloric acid (0.01 mol dm^{-3}), sulphuric acid (0.01 mol dm^{-3}) and sodium chloride (0.1 mol dm^{-3}). The two solutions were mixed immediately before use.

Instrumentation

An HPLC system was interfaced to an atomic fluorescence spectrometer (AFS) using a continuous-flow system and a gas–liquid separator (GLS) and was used throughout the analyses. The solutions used in the oxidation/reduction interface and the operating parameters are summarized in Table 1.

HPLC system

A Beckman model 126 pump module equipped with a Beckman model 501 autosampler running a 200 mm × 3 mm i.d. column with 10 mm × 3 mm i.d. guard column packed with Chromspher RP/18 material (3 μm, Chrompack) was used for the analysis of organomercury compounds. The mobile phase consisted of mixtures of methanol/water (30:70–50:50) buffered with ammonium acetate (20 mmol dm^{-3}) and modified by 2-mercaptoethanol (0.1 mmol dm^{-3}). The mobile phase flow rate was 0.5 cm^3 min^{-1}.

Table 1 Instrumental and operating conditions

Parameter/Component	Specification
Flow rate of HPLC	0.5 cm^3 min^{-1}
Flow rate of ox. solution	0.3 cm^3 min^{-1}
Flow rate of red. solution	0.4 cm^3 min^{-1}
Flow rate of argon	50 cm^3 min^{-1}
Oxidizing solution	0.25 mol dm^{-3} sulphuric acid
	0.008 mol dm^{-3} copper sulphate
	2.5% potassium peroxodisulphate
Reducing solution	1.5% tin(II) chloride
	1.2 mol dm^{-3} sodium hydroxide
Oxidizing coil	100 mm × 0.3 mm i.d. (7 mm^3)
Reducing coil	500 mm × 0.3 mm i.d. (35 mm^3)

Atomic fluorescence spectrometer

A Brooks Rand AFS instrument was used throughout this work. The flowmeter was disconnected and placed in front of the whole system. The AFS was fixed to the GLS and the analogue output was connected to a PE Nelson 3000 chromatography data system.

Continuous-flow reducing system

The apparatus for the continuous generation of elemental mercury is shown in Fig. 1.

The oxidizing and the reducing solutions were fed into the system by a peristaltic pump (Gilson, model Minipuls 3). The mixing joints (Valco) and all connecting capillaries (PTFE) were of 0.25 mm i.d. The reaction coils (PTFE, 0.3 mm i.d.) were of the knotted-coil type. The gas–liquid separator (40 mm × 10 mm i.d.) was constructed from quartz. The gas inlet and outlet were of 1 mm i.d. and the outlet for the liquid (3 mm i.d.) was positioned approximately 10 mm above the bottom of the tube. The condenser had the same dimensions but without a drainage, and was cooled by a dry ice ethanol mixture. The elemental mercury generated in this system was swept into the AFS by a stream of argon (50 cm^3 min^{-1}).

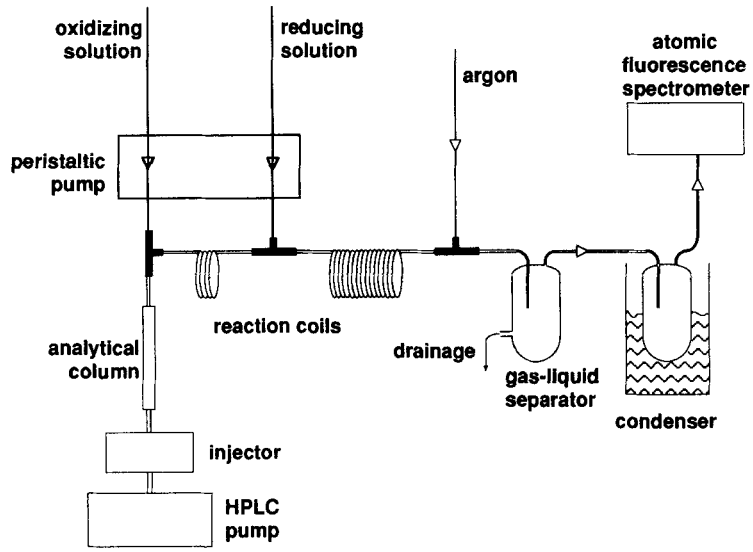

Figure 1 The HPLC/AFS system coupled with the continuous-flow mercury generation interface for the separation and detection of organic mercury compounds (for details see text).

Optimization procedure

The sequential simplex method was used to optimize the oxidizing and reducing conditions of the continuous-flow system. This method allows the simultaneous optimization of several parameters using a minimum number of experiments and avoiding factorial design experiments. The original method[14] was used with a modified computer program[15] which increased its efficiency.

Extraction procedure

A 5 cm^3 portion of the citrate buffer were added to 5 g of sediment sample. The slurry was extracted with 10 cm^3 dithizone in chloroform for 15 min. The sample was filtered off and the organic phase was transferred to a test-tube. The dithizone–mercury complexes were destroyed by shaking with 1 cm^3 of a nitrite/acid mixture until the colour of the solution changed from green to yellow. With this procedure the organomercury compounds remain in the organic phase, whereas the inorganic mercury (Hg^{2+}) is extracted into the aqueous phase as the chloro complex. The chloroform was washed with 1 cm^3 of water and transferred to another test-tube. Finally, the organomercurials were back-extracted into an aqueous phase with 1 cm^3 of sodium thiosulphate solution (1 mmol dm^{-3}) buffered with ammonium acetate (0.05 mol dm^{-3}). If necessary, 0.5 cm^3 of the thiosulphate solution was evaporated to dryness in a gentle stream of nitrogen and the organic mercury compounds were redissolved in 50 mm^3 of water without any losses of the organomercury compounds.

The marine samples (~0.3 g of CRM) were digested first with 5 cm^3 20% w/v tetramethylammonium hydroxide at 60 °C for 4 h until the tissue had completely dissolved to a brown solution. After the solution had cooled it was acidified by adding 5 cm^3 HCl (6 mol dm^{-3}). This solution was extracted with dithizone, and the procedure described above was then followed.

RESULTS AND DISCUSSION

Gas–liquid separator (GLS) and condensing system

The liquid stream and the argon gas are premixed in a mixing joint and enter the GLS. The liquid is drained to waste, and the argon loaded with elemental mercury proceeds to AFS measurement. Since the mercury fluorescence is known to be quenched by polyatomic molecules such as N_2, O_2, H_2 and especially H_2O (Eqn [1]),[16] water vapour must be removed before the argon is swept into the fluorescence cell to enhance the sensitivity.

$$Hg(^3P_1) \rightarrow Hg(^1S_0) + h\nu\,(253\ nm) \qquad [1]$$

Table 2 Limits of the parameters varied during the simplex optimization

Parameter	Lower limit	Upper limit
Potassium peroxodisulphate	0	5% w/v
Copper sulphate	0	0.015 mol dm^{-3}
Sulphuric acid	0	1 mol dm^{-3}
Tin(II) chloride	0.1% w/v	7% w/v
Sodium hydroxide	0.5 mol dm^{-3}	10 mol dm^{-3}
Flow rate of ox. solution	0.060 cm^3 min^{-1}	0.425 cm^3 min^{-1}
Flow rate of red. solution	0.075 cm^3 min^{-1}	0.600 cm^3 min^{-1}

Several systems were tested. Water removal by means of a glass-fibre filter was not very effective. Drying with magnesium perchlorate was better, but after a short time the capacity of the perchlorate was exceeded. Good results were obtained by passing the gas stream through concentrated sulphuric acid. The water vapour was separated well but this system was very delicate to handle. After some hours of operation a slight foaming of the acid could not be avoided and we ran the risk of the acid entering the fluorescence cell. However, in the end, a condensing system was the separator of choice. Ice with sodium chloride (0 to 5°C) and dry ice with ethanol (−65 to −70 °C) were tested as coolants. As expected, the dry ice trapped the water more efficiently. Surprisingly, the mercury vapour was not condensed though the cooling temperature was beyond the melting point of metallic mercury (−39 °C). Therefore a condenser cooled with dry ice in ethanol was used in all further experiments in order to remove water vapour from the argon stream.

Generation of elemental mercury

In the literature, numerous methods have been reported for the reduction of mercury compounds to the element. A common reagent used is

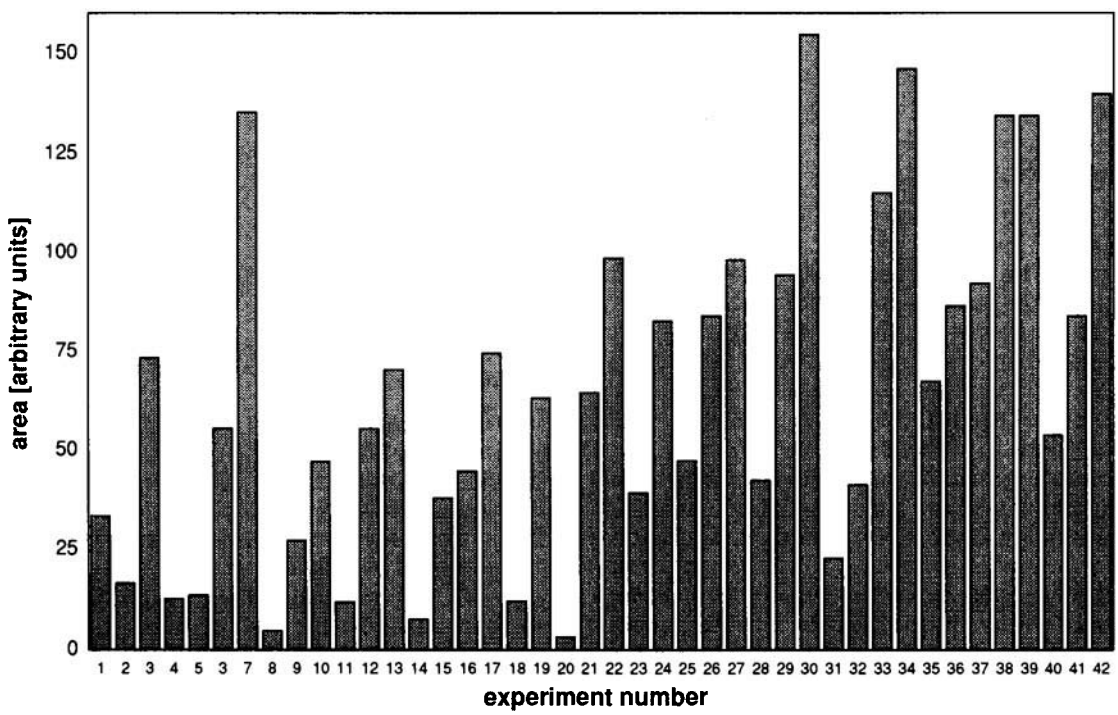

Figure 2 Development of the sequential simplex method.

sodium borohydride in alkaline[17] solution. However, with borohydride not only metallic mercury but also hydrogen is generated. In our tests the use of borohydride both in alkaline and in acidic solution showed very poor sensitivity. We think this is due to the quenching effect of hydrogen on the mercury fluorescence.

The other reducing reagent used is tin(II) chloride either in acidic[18] or in alkaline solution[19,20] and some authors have reported the use of a catalytic or an oxidizing reagent to accelerate the reduction of organic mercury compounds.[21,22] Our preliminary experiments confirmed that the stronger reducing power of tin(II) chloride in alkaline medium, and a preoxidation step with potassium peroxodisulphate and copper(II) as a catalyst, were necessary to obtain a quantitative on-line reduction of the organic mercury compounds in the HPLC effluent.

The experimental conditions were optimized by a computer program based on a simplex algorithm. The response to be maximized was the area of the fluorescence signal. The optimization was carried out with methylmercury chloride (0.04 mg dm^{-3}, injected volume 25 mm^3). The parameters under investigation were the concentration of tin(II) chloride, sodium hydroxide, copper(II) sulphate, potassium peroxodisulphate and sulphuric acid and the flow rate of the oxidizing and reducing solutions. The upper and the lower limits of the range where the parameters were varied are listed in Table 2.

Six parameters were optimized, so seven initial experiments with randomly chosen values of the parameters had to be performed to start the simplex program. After 35 consecutive experiments the simplex algorithm stopped; this means the six best results were within the standard deviation of the maximum response. The experimental conditions of the most sensitive run (No. 30) were chosen for all further work and are listed in Table 1. The development of the simplex optimization is shown in Fig. 2.

Naturally, the simplex approach does not indicate relations between the parameters changed. However, from the individual experiments, some conclusions can be drawn. Peroxodisulphate, as well as copper(II), was necessary in the oxidizing solution to obtain good signals (no $K_2S_2O_8$: Nos 5, 8, 18, 20; no Cu^{2+}: Nos 4, 5). The H_2SO_4 concentration alone had no significant effect on the sensitivity. The only restriction is that the overall pH in the final reducing solution has to be in the alkaline range (in run No. 11, the final solution was acidic). Low tin(II) concentrations (0.1–0.3 in Nos 2, 8, 14, 18, 20) are responsible for poor sensitivity, too.

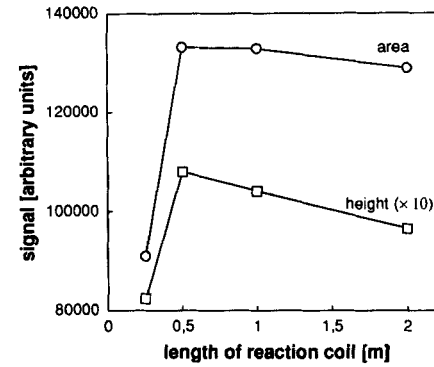

Figure 3 Effect of the length of the reducing reaction coil on the fluorescence signal.

Geometry of the reaction coils

Preliminary experiments showed that the tubes of the knotted-coil type were superior to coils of the conventional loop type regarding the peak shape produced in the chromatograms. This is due to the more efficient mixing of the reagents in the knotted coils and a reduced lateral diffusion of the analyte in the reagent stream which avoids undesired dilution. The continuous-flow oxidizing–reducing system under investigation here allows only a limited reaction time for generation of a maximum of elemental mercury. Thus, different lengths of the oxidizing and the reducing reaction tubes were tested. The coils were hand-

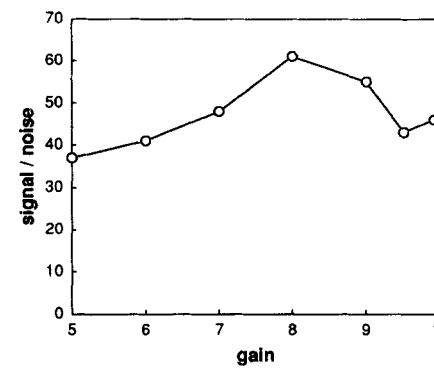

Figure 4 Effect of the gain adjustment on the signal-to-noise ratio.

knitted from PTFE tubing and the effect of the length of the reducing coil on the peak area and height is shown in Fig. 3.

The reaction time resulting from a coil length shorter than 50 cm was insufficient for a complete reduction. Longer coils had no significant influence on the peak areas, so all the organic mercury was reduced and measured in the fluorescence cell. With longer tubes the peak heights decreased, resulting in broad peaks. This is caused by diffusion in the longer reaction coils. Thus a length of 50 cm was found to be the optimum. The length of the oxidizing reaction tube was varied from 10 to 50 cm and no changes in peak heights or areas were noticed. This dimension was not critical and the shortest tube possible (10 cm) was chosen for further work.

Signal-to-noise ratio

Because the gain of the AFS instrument is not linear, the optimum adjustment for the highest sensitivity had to be established experimentally. The gain was varied from 5.0 to 9.9 (instrumental range: 0.0–10.0). The area of an injected methylmercury solution (0.01 mg dm^{-3}, injected volume 25 mm^3) was measured and the baseline noise was determined. The ratio is plotted against the gain adjustment and the results are shown in Fig. 4. The maximum sensitivity could be obtained at an adjustment of 8.0 and this was therefore chosen for further analyses.

Separation of organic mercury compounds

Recently, we have optimized the chromatographic conditions for the separation of different organic mercury compounds.[10] In order to adopt these conditions we tested several methanol concentrations in the mobile phase of the HPLC to see its effect on the fluorescence detection. In the range investigated (30–50% methanol) the fluorescence signals of the organic mercury compounds were not affected. Figure 5 illustrates the separation of eight different organomercurials with cold-vapour atomic fluorescence detection by means of a gradient run. All species were at the 2.5 ng level. The methanol content was changed after 5 min from 30 to 50% within one minute. The analysis was completed in less than 22 min.

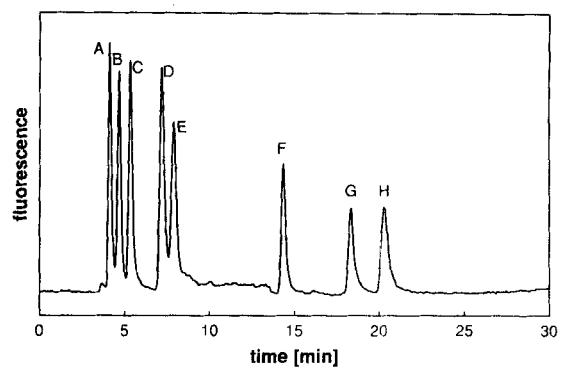

Figure 5 Gradient separation of eight organic mercury compounds by HPLC with on-line detection by AFS after their reduction to elemental mercury. Compounds are methylmercury (A, 4.12 min), methoxyethylmercury (B, 4.65 min), *p*-mercuribenzoic acid (C, 5.32 min), ethylmercury (D, 7.17 min), ethoxyethylmercury (E, 7.88 min), phenylmercury (F, 14.32 min), nitromersol (G, 18.35 min) and tolylmercury (H, 20.30 min).

Figures of merit

The calibration graph for methylmercury was linear in the range tested (0.05–10 ng) and identical to that for ethylmercury.

The extraction efficiency of the proposed method was evaluated with spiked sediment samples. The recoveries of CH_3HgCl, C_2H_5HgCl, $CH_3OC_2H_4HgCl$ and $C_2H_5OC_2H_4HgCl$ were 80%; C_6H_5HgCl, $CH_3C_6H_4HgCl$ and nitromersol were recovered from the sediment with 50% efficiency.[23] Mercury benzoate was only 15% extractable. A paper discussing the details of the optimized extraction procedure is in preparation.

Because of the uniform response of the HPLC/AFS coupling to various organomercury species, the detection limits for different compounds are almost the same and the observed decreasing sensitivity to later-eluting compounds is only as a result of their broader peak shapes. The limit of detection (LOD) for methylmercury ($S/N = 3$) was calculated to be about 0.02 ng of mercury (in absolute terms) or 0.8 μg dm^{-3} for 25 mm^3 injected volume. The LOD for ethylmercury, methoxyethylmercury, ethoxyethylmercury and mercuribenzoic acid is also 0.02 ng of mercury. Only the later-eluting aromatic species have an LOD of 0.035 ng mercury. Therefore, detection limits of 0.1 for methylmercury, ethylmercury, methoxyethylmercury, ethoxyethylmercury and 0.3 μg Hg per kg sediment (dw) for phenylmercury, tolylmercury and nitromersol can be

Table 3 Analyses of marine reference material and sediment samples

Sample	Methylmercury (ng g^{-1})	Certified (ng g^{-1})	Ethylmercury (ng g^{-1})
DORM-1	695 ± 53	731 ± 60	n.d.
LUTS-1	9.9 ± 0.5	9.4 ± 0.6	n.d.
River sediments			
At 1 km,[a] top layer	16 ± 1.5	—	8.7 ± 1.9
15 cm	27 ± 7.0	—	13 ± 2.6
50 cm	36 ± 8.0	—	8.3 ± 1.6
At 3 km, top layer	5.0 ± 1.5	—	n.d.
At 5 km, top layer	6.7 ± 1.5	—	n.d.
30 cm	6.0 ± 1.5	—	n.d.

[a] The distances of the sediment samples are given downstream of the plant drainage.

derived. The relative standard deviation for the measurement at the 20 μg level was below 6% ($n = 5$ repeated injections).

Analyses of certified reference material

In order to check the method, the optimized procedure was used to analyse the certified materials DORM-1 (dogfish muscle) and LUTS-1 (non-defatted lobster hepatopancreas). The samples were treated as described in the experimental section. The results obtained are given in Table 3 and are in good agreement with the certified values. In contrast to findings in other marine reference materials,[24] no ethylmercury could be detected in the samples analysed.

Analyses of sediment samples

Sediment samples from a small river, adjacent to a plant which until recently produced organic mercury compounds, were analysed as described in the experimental section. The concentrations of organic mercury compounds found in these sediments are listed in Table 3. The samples showed elevated levels of methylmercury, with the highest concentrations at the location closest to the drainage of the plant. The contamination increased with depth, indicating that the pollution was very persistent. Figure 6 illustrates the chromatogram of a sediment sample. The unusual peak at 6.67 min indicating the presence of ethylmercury in the river sediment is interesting.

The shift in retention time from 7.17 min (standard solution) to 6.67 min (sample solution) is due to a slight change of the methanol content in the mobile phase. There is much evidence that this peak is probably ethylmercury. The identity of ethylmercury was verified by analysing a sediment sample spiked with ethylmercury. This sample showed the same retention time of 6.67 min for ethylmercury.

This is one of the rare findings of ethylmercury in natural samples documented in the literature.[25–29] No other organic mercury compounds except methyl- and ethylmercury were detected in the sediments. Ethylmercury could only be found at this point; further downstream its concentration was below the detection limit of 0.1 μg kg^{-1}. We never detected a peak corresponding to ethylmercury at other locations, which are not in contact with potential point sources of ethylmercury.

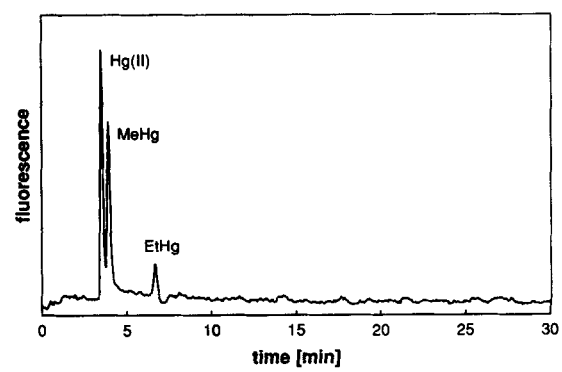

Figure 6 Chromatogram of a sediment sample containing methylmercury (4.08 min), ethylmercury (6.67 min) and traces of mercury(II) (3.52 min).

CONCLUSION

The HPLC/AFS coupling is a new and powerful method for the determination of methylmercury and other organomercury compounds. The detector is easily interfaced with the chromatographic equipment. All coupling parts are commercially available; only the gas–liquid separator is laboratory-constructed.

The importance and the application of the proposed method is summarized as follows:

(1) The sample preparation is easily carried out. Complex matrices such as soils, sediments and fish can be analysed without any special clean-up steps because of the specificity of the AFS detector. Until now, no false positive peaks have been observed. The HPLC column is one year old. Only the guard column was replaced from time to time.

(2) The conventional GC methods are troublesome when analysing monoalkylmercurials. Other GC techniques require time-consuming derivatization steps.

(3) The extraction of the organomercurials is achieved under mild conditions. Because of dithizone (a strong mercury complexing agent) the sediment has to be acidified with a citrate buffer to pH 2, only. All tested organomercury compounds remained in their chemical form during the extraction steps. No chemical conversions or transalkylations have been observed during spiking experiments under the conditions described.

(4) By the HPLC/AFS method ethylmercury can be detected beyond doubt. This is not possible with ethylation techniques.

The major difficulty in applying the HPLC/AFS method is that the handling of the system is tedious; in particular, the condensing system must be controlled during operation. However, efforts are under way to improve the water removal and to overcome this drawback. Only monoalkyl- or monoarylmercury compounds can be separated with the method described; dimethylmercury, for example, cannot be analysed.

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