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Determination of Methylmercury by the Species-specific Isotope Addition Method Using a Newly Developed HPLC–ICP MS Coupling Technique with Ultrasonic Nebulization†

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A novel technique has been developed for the determination of trace amounts of methylmercury in various sample matrices. The newly developed HPLC method makes it possible to separate methylmercury and inorganic mercury with ultrasonic nebulization and detection by ICP MS for different mercury isotopic masses. The isotope-specific detection allows the application of the species-specific isotope addition method for the determination of methylmercury with a correction for artifact formation. The well-known water-vapour distillation method was used in combination with an enriched stable inorganic mercury isotope ($^{200}Hg^{2+}$) for the separation of methylmercury from various matrices. The subsequent determination of $CH_3—^{200}Hg^{+}$ generated from $^{200}Hg^{2+}$ was used in the correction for artifact formation during sample preparation. In comparison with a previously developed HPLC coupling technique with HPF–HHPN (high-performance flow/hydraulic high-pressure nebulization), the stability of the detection procedure was improved considerably. The limit of detection ($S/N = 3$) for methylmercury was calculated to be about 0.015 μg kg^{-1}. © **1998 John Wiley & Sons, Ltd.**

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† Dedicated to Y. K. Chau, from whom we learned 'Speciation'.

INTRODUCTION

The use of ICP MS technology with enriched mercury isotopes ($^{200}Hg^{2+}$, $^{199}Hg^{2+}$) has shown that methylmercury was formed within a short time during sample preparation due to the presence of inorganic mercury,[1–4] particularly when the water-vapour distillation method was applied. In a few cases more than 100% methylmercury was formed, as an artifact. Other sample preparation methods, such as extraction by acids, alkalis or organic solvents, have shown this effect. At present, for certain matrices (e.g. sediment, soil, leaves, spruce needles) there is no sample preparation method available which is free of artifact production. This leads to the suspicion that the analysis of many of the above-mentioned sample types over recent years overestimated the methylmercury content, so contamination levels also were probably overestimated.

The species-specific isotope addition (SSIA) method in combination with the ICP MS technique allows the determination of methylmercury artifact formation during sample preparation and subsequent correction of the results obtained. A great advantage of the method is its independence of the sample preparation procedure; the requirement for artifact correction, however, is the application of the ICP MS technique.[5,6] For this reason reliable ICP MS techniques are necessary for application of the new SSIA method. Therefore we developed a new HPLC–ICP MS technique with on-line nebulization of the HPLC eluent for the separation and determination of isotope-specific methylmercury.

MATERIALS AND METHODS

Standard solutions and chemicals

The stock solutions of methylmercury (100 mg l^{-1})

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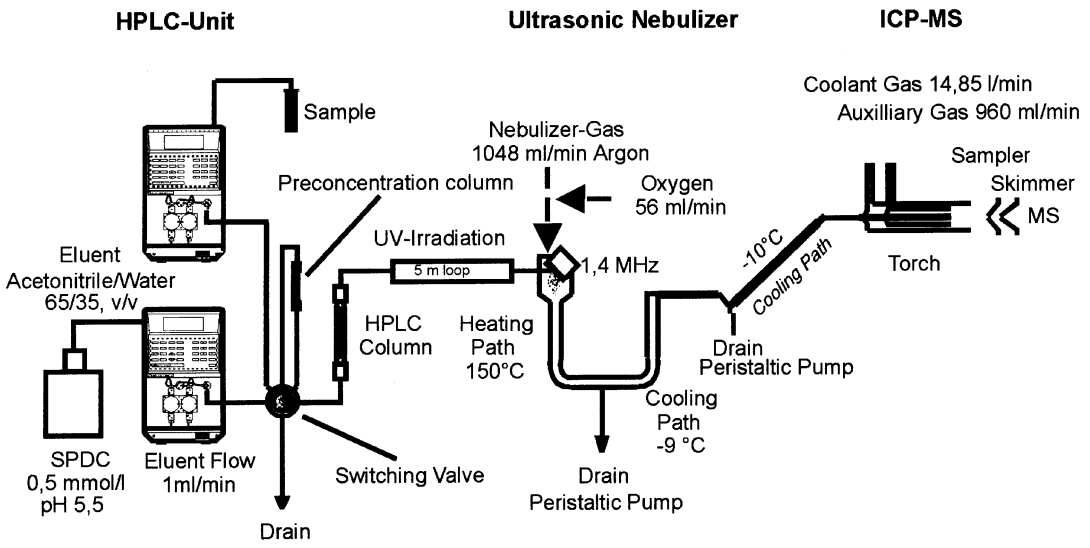

Figure 1 Analytical HPLC system coupled with an ultrasonic nebulizer for determination of methylmercury according the species-specific isotope addition method.

and inorganic mercury (100 mg l^{-1}) were prepared by dissolving the dry compounds in acetonitrile–water (70:30, v/v, HPLC grade), and stored in the dark at 4 °C. The standard solutions were prepared fresh daily by diluting the stock solutions with acetonitrile–water (70:30, v/v; HPLC grade). They were handled in brown bottles, which were washed with nitric acid and deionized water before use. ^{200}HgO was obtained from Chemotrade Chemiehandels-GmbH (Düsseldorf, Germany). The isotope-enriched HgO was dissolved in 1 ml HCl (5 mol l^{-1}) and diluted to 10 ml with twice–distilled water. All the chemicals used, such as sodium pyrrolidinedithiocarbamate (SPDC), ammonium acetate, acetonitrile, sodium chloride, sulphuric acid, hydrochloric acid and acetone, were of analytical grade. Argon 5.0 and nitrogen (research grade) were used. All chemicals were used without further purification.

ICP MS

A Perkin-Elmer/Sciex Elan 5000 was used. The operating parameters after the ICP MS optimization were as follows. ICP: forward power 1100 W, reflected power < 10 W, cooling gas 14.85 l min^{-1}, auxiliary gas 0.960 l min^{-1}, nebulizer gas 1.048 l min^{-1}, oxygen 0.056 l min^{-1}. Mass spectrometer: 2×10^0 mbar, intermediate pressure $< 1 \times 10^{-4}$ mbar, analyzer pressure 5.4×10^{-6} mbar.

Ultrasonic nebulizer and additional cooling path

An ultrasonic nebulizer, UT 6000 AT from CETAC, was used (heating path length 25 cm, heating temperature 150 °C, cooling path length 35 cm, cooling temperature −9 °C; nebulization frequency 1.4 MHz. The ultrasonic nebulizer head was wrapped with a Teflon band to protect it against the acetonitrile from the HPLC eluent. If there is no protection, the head ultrasonic head glue together and the operating life is decreased by about one month. To reduce the HPLC eluent after nebulization, an additional cooling path was used, consisting of a Liebig condenser 30 cm long with 0.8 mm i.d. The temperature of the Liebig condenser was set at −10 °C; it was insulated for the best cooling effect. For the connections between the ultrasonic nebulizer and the additional condenser, and between the ultrasonic nebulizer and the ICP MS, Teflon tubes with 0.8 mm i.d. were used.

HPLC equipment and operating conditions

The HPLC–ICP MS system is shown in Fig. 1. The HPLC pumps were from Thermo Separation Products. For all connections PEEK plastic material was used. An RP C18 column (Hypersil-ODS, 80 mm × 4.6 mm from Grom, 3 μm particle size)

was employed for the separation. For irradiation a 4 W low-pressure UV lamp and hand-knitted PTFE irradiation coil 5 m long (i.d. 0.3 mm low-pressure model) were obtained from ICT. The UV lamp had a length of 15 cm and a diameter of 15 mm. The lamp was placed in a box for eye protection. The operating parameters after the HPLC optimization were as follows: mobile phase acetonitrile-water (65:35, v/v), complexing agent sodium pyrrolidinedithiocarbamate (0.5 mmol l^{-1}), pH buffer ammonium acetate adjusted to pH 4.5 (0.5 mmol l^{-1}), eluent flow rate 1 ml min^{-1}, eluent pressure 90–140 bar, retention time for methylmercury 3.5 min, retention time for inorganic mercury 5 min.

HPLC preconcentration equipment and sample enrichment

For the preconcentration of methylmercury, a 20 mm × 4.6 mm reverse-phase (RP) column with Hypersil ODS material (3 μm) was used. The preconcentration was carried out by an HPLC pump. For sample loading, a Rheodyne injector valve 7010 with a sample loop of 10 ml was used for the experiments. For distribution of the eluent flow between the preconcentration column and the separation procedure, a Rheodyne 7060 switching valve was used. The preconcentration eluent consists of 0.5 mmol l^{-1} ammonium acetate in twice-distilled water. The preconcentration flow was adjusted to 1.3 ml min^{-1}. For the preconcentration procedure a reagent solution of 500 mg l^{-1} sodium pyrrolidinedithiocarbamate in 25 mmol l^{-1} ammonium acetate in twice-distilled water adjusted to pH 5.5 was prepared fresh daily.

Sample material

Sample material was collected and frozen at −30 °C until analysis. For sample homogenization, a ball mill was used. Sediment and soil samples were dried at 40 °C. Leaves and moss were used without drying and the dry weight was determined afterwards.

Total mercury determination

For the application of the SSIA method the determination of total mercury in the sample is required: 1–3 g of the respective sample was mixed with 30 ml aqua regia. Afterwards it was digested in closed Teflon bottles by microwave, within 1.5 h. The samples were diluted to a final volume of 50 ml with twice-distilled water and the total mercury was finally determined by ICP MS with ultrasonic nebulization. Rhodium was used as internal standard. Ultrasonic nebulizer settings: sample flow 1.5 ml min^{-1}, heating path temperature 160 °C, cooling path temperature 4 °C. ICP MS settings: forward power 1000 W, cooling gas 14.95 min^{-1}, auxiliary gas 0.98 l min^{-1}, nebulizer gas 0.96 l min^{-1}. The detection limit was calculated to be 0.25 μg kg^{-1}.

PROCEDURES

Distillation

The distillation procedure was carried out in glass vials. Sample weights were in the range 100–500 mg. Sodium chloride (0.2 ml) solution and twice-distilled water (9.5 ml) were added to the sample. Shortly before the distillation was started, 0.5 ml of 8 mol^{-1} sulphuric acid and 500 ng $^{200}Hg^{2+}$ in 0.1 mol^{-1} HCl (isotope 96.41% enriched) was added. Then the mixture was distilled in an oil bath with a heating temperature of 150 °C over a period of 30 min with a nitrogen stream of 100 ml min^{-1}. About 9 ml of distillate was obtained after the procedure. The samples were stored in the dark and refrigerated at 3–5 °C for a subsequent preconcentration. Samples were not stored longer than 2–3 days, to avoid significant methylmercury losses.

Operation of HPLC–ICP MS equipment

The newly developed equipment was easy to handle; attention was required to only a few critical points.

First the torch, sampler and the high-frequency coil had to be cleaned of residual oxidation products to avoid misfiring and a ring plasma. Unfavourable misfiring could lead to a melt-together of the torch. It was necessary that the ultrasonic nebulizer and the additional cooling path have reached the operating temperature. Operation started without HPLC-eluent flow, and at the beginning there was a low oxygen gas flow of 5 ml min^{-1} for the ignition of the plasma. After ignition the oxygen gas stream was increased to 56 ml min^{-1}. Then the eluent flow was set to 0.05 ml min^{-1} without nebulization. The eluent flow was raised over a period of 10 min to 1 ml min^{-1} so that condensation of the eluent starts in

the cool paths. Afterwards the ultrasonic nebulizer could be switched on for eluent nebulization. The condensed eluent was removed by a peristaltic pump. The additional cooling path was installed with a negative inclination so that the condensed eluent could run down to the connecting tube to drain, as shown in Fig. 1. To turn off the equipment, the eluent and the ultrasonic nebulizer had to be switched off first. The residual eluent in the cooling paths was removed and cleaned for the next equipment start-up. Finally the plasma was switched off.

Preconcentration and measurement

For analysis, 0.4 ml of the complexing agent (500 mg l^{-1} SPDC, buffered at pH 5.5) was added to the distillate and then diluted to a volume of 10 ml for calibration. Afterwards the 10 ml volume was injected into the injection loop for the HPLC-preconcentration step. The preconcentration column was switched into the HPLC-eluent flow using the switching valve. The complexed mercury species were desorbed by the acetonitrile–water mixture and transported to the analytical column. After the separation, the mercury species were oxidized by UV irradiation and nebulized by the ultrasonic nebulizer. The aerosol was transported by the nebulizer gas stream after passing the heating and additional eluent removing path into the plasma for ionization and isotope-specific detection. Mercury at *m/z* 200 and 202 and lead at *m/z* 207 were determined from the traces: The low content of lead (*m/z* 207) was used as an internal standard. The background content of lead (*m/z* 207) in the SPDC spiked in the HPLC-eluent was used for the daily optimization of the ICP MS gas setting for sensitivity. After 20 preconcentration procedures the equipment had to be rinsed, without the column, with acetone for 5 min to remove residual components due to the sample preconcentration.

RESULTS AND DISCUSSION

Capacity of plasma for loading with eluent

The loading of the nebulized eluent into the argon plasma is limited. The aerosol which ultimately reaches the plasma has to be regulated. If there is not enough eluent nebulized, the sensitivity and therefore the detection limits decrease rapidly. If

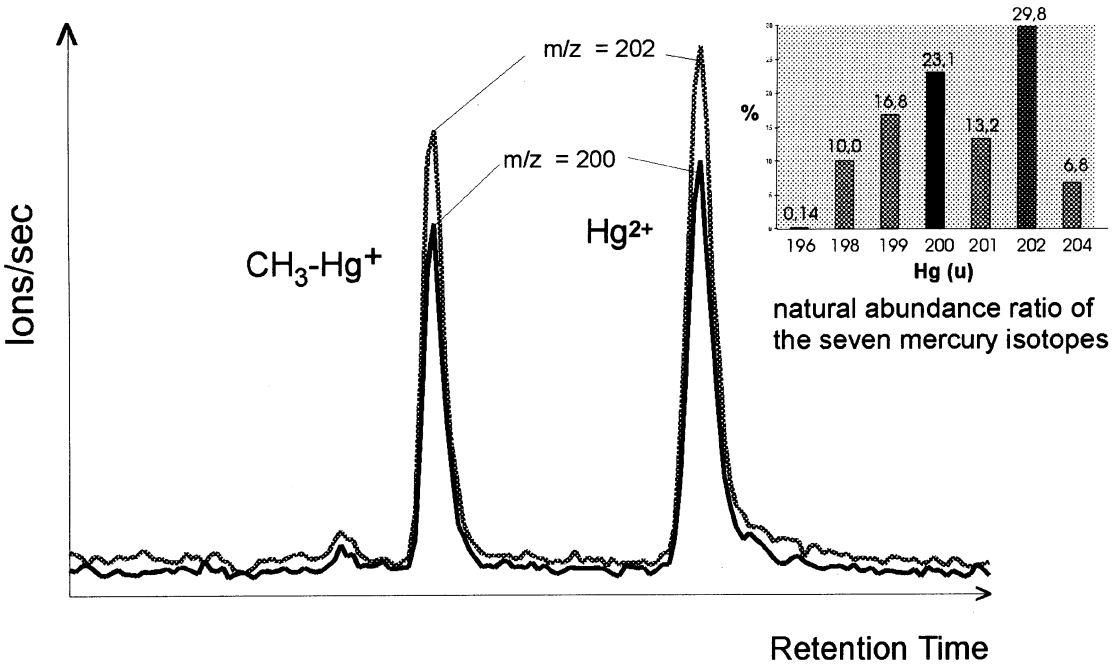

Figure 2 HPLC separation of methylmercury (CH_3Hg^+) and inorganic mercury (Hg^{2+}): mercury mass traces *m/z* 200 and 202, and (inset) the natural abundance ratio of the seven mercury isotopes.

Table 1 Artifact formation potential of various matrices

Matrix	Total Hg ($\mu g\ g^{-1}$)	Spike value $^{200}Hg^{2+}$ ($ng\ g^{-1}$)	MeHg ($\mu g\ g^{-1}$)	MeHg (SSIA) ($\mu g\ g^{-1}$)	Methylation error (%)
Pine needles	68	500	0,46	0,4	13
Spruce needles	73	500	0,61	0,54	11,2
Star moss	145	500	0,49	0,44	10,3
Stone moss	70	500	0,43	0,39	6
Beech leaves	75	500	0,15	0,12	18,7
Forest soil	82	500	0,23	0,20	18
Sediment	688	500	1,56	0,72	54
Raspberry leaves	17	500	0,43	0,43	2
Grass	11	500	0,54	0,54	1,5
Potato	6,2	500	0,05	0,05	0,13
Apple	3,1	500	0,29	0,29	0,1
Banana	4,5	500	0,12	0,12	0,3
Cheese	14	500	1,2	1,2	0,3
Liver pate	56	500	8,6	8,6	0,6
Trout muscle tissue	167	500	167	167	0
Trout liver	211	500	211	211	0
Cockle	152	500	152	152	0
Cuttlefish	156	500	156	156	0

the amount of nebulized aerosol is too high, the plasma conditions and the degree of ionization change. Eventually the plasma is extinguished.

For the optimization experiments the HPLC-eluent flow rate was set to 1 ml min^{-1}. This flow rate was sufficient for a good separation of methylmercury and inorganic mercury within 5 min. The higher proportion of acetonitrile reduced the retention times and the speed of analysis, and resulted in increased plasma loading. Therefore the proportion of acetonitrile in the HPLC eluent was set to 65%.

The regulation of the eluent reaching the plasma was optimized by the additional cooling path. Without this additional cooling, the plasma loading was too high, the measurements were very unstable and the baselines of the detected mass traces varied widely. The plasma required values of more than 10% oxygen of the nebulizer and a forward power of more than 1300 W for sufficient stability. The additional oxygen would oxidize the acetonitrile and the carbon deposit at the sample inlet, but the sample opening would be enlarged unintentionally by oxidation of the sampler material (nickel). If there was not enough oxygen, the sample inlet would be closed by carbon deposition and the sensitivity would decrease rapidly. The temperature of the Liebig condenser was optimized for sensitivity at −10 °C. A change in the temperature (up or down) would lower the sensitivity of detection. The best results were obtained for 5% oxygen and a slight adaptation of the plasma was possible by adjustment of the forward power between 1050 and 1200 W.

Interferences

The seven mercury mass traces were free from interferences; only the mass trace for *m/z* 204 could be influenced by a lead mass. For the measurements

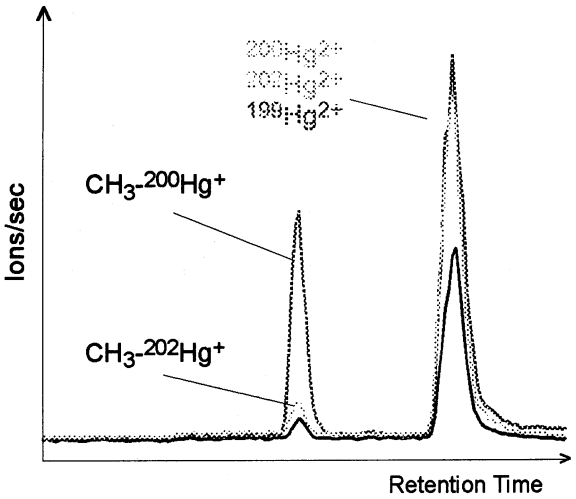

Figure 3 Chromatogram obtained from a sediment sample from the River Rhine. The mass trace of the mercury isotope at *m/z* 200 shows methylmercury formed artifactually during the sample preparation procedure. The unspiked mass trace *m/z* 200 shows that the actual methylmercury content in the sample is very small.

the mercury masses *m/z* 200 and 202 (23.13% and 29.8%, respectively, of the natural abundance ratio) with the highest natural abundance ratio were used for detection (Fig. 2). The background concentrations of metals in the complexing agent showed no influence.

Calculation of the methylmercury content by the SSIA method in different matrices

The methylmercury content in each sample was calculated according to Hintelmann *et al.*[1] By measuring different isotopes it is possible to determine the apparent concentration and the artifact formation factor. The SSIA correction can be carried out with a single measurement. For the calculation the following equation can be used:

$$[CH_3Hg^+] = [CH_3{}^nHg^+]/{}^nA - [Hg_{total}] \times M \quad [1]$$

where

$[CH_3Hg^+]$ = content of CH_3Hg^+;
$[CH_3{}^nHg^+]$ = measured apparent $CH_3{}^nHg^+$ content, based on measurement of non-spiked Hg isotope;
${}^nA$ = natural abundance of the non-spiked Hg isotope;
$[Hg_{total}]$ = total mercury content of the sample;
M = specific methylation (%) of added Hg^{2+} isotopec

This method of calculation was applied for the measurement of various matrices. The equation consists of two parts: the second term on the right-hand side of Eq. [1] is the calculation of the isotope-specific methylation due to inorganic mercury (e.g. $^{200}Hg^{2+}$, enrichment 96.41%) in the specific matrix. It was calculated from the specific methylation factor M of the matrix, obtained from the isotope spiking and the total mercury content of the sample. The artifactual formation of methylmercury is dependent on the matrix type and the total mercury content. The formation of methylmercury increases linearly with the increase in the total mercury content.[1] This term was subtracted from the first term on the right-hand side of Eq [1], the measured apparent methylmercury content of a non-spiked isotope. Table 1 shows the sample type, the total mercury content, the spike value of the enriched mercury isotope $^{200}Hg^{2+}$, the apparent methylmercury content, the methylmercury content after the artifact correction and the corresponding error value. Spruce needles, mosses, leaves, forest soil and in particular sediment samples are prone to artifact formation of methylmercury when the distillation technique is applied. An example is shown in Fig. 3. In soil, leaves, mosses and sediments where the methylmercury fraction is generally small and typically 0.01–0.1% of the total mercury content is methylated artifactually, even a small conversion factor can lead to significant errors. Samples from marine organisms which show high proportions of methylmercury are less problematic. Such samples showed insignificant errors.

Detection limits

The detection limit for methylmercury was 12 pg (as Hg; $S/N = 3$, detected at *m/z* 202, which shows the highest proportion of the natural abundance ratio with 29.8%). The relative standard deviation (RSD) for methylmercury at the 500 pg level was 5%. There was good linearity for the methylmercury determination (regression coefficient $r = 0.9995$) between 50 pg and 900 pg.

CONCLUSIONS

HPLC–ICP MS coupling using ultrasonic nebulization is a new and powerful method for the determination of methylmercury by the species-specific isotope addition method. The easy separation of methylmercury by the watervapour distillation method can be used furthermore for sample preparation. Although this method showed the highest artifact values, the application of the SSIA method offered the possibility of compensating for the errors by spiking enriched mercury isotopes and by simultaneous determination of the artifact potential. In comparison with a previously developed HPLC coupling technique with HPF–HHPN (high-performance flow/hydraulic high-pressure nebulization) the stability of the detection procedure was improved considerably.[6]

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