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Comparative study of microwave-induced plasma atomic emission spectrometry and atomic fluorescence spectrometry as gas-chromatographic detectors for the determination of methylmercury in biological samples

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In order to attain a lower detection limit with the HS GC MIP analytical method (Head-Space Gas Chromatography with Microwave-Induced Plasma detection) recently developed for the analysis of methylmercury in biological samples, the quarter-wave Evenson-type cavity used until now was replaced by a TM_{010} Beenakker-type cavity, which was used with both argon and helium as carrier gas. With an argon plasma, an eightfold increase in detection limit was gained compared with the argon plasma sustained by the Evenson cavity, while only a four-fold increase was gained with the helium plasma. In a second step of the study, the MIP detector was replaced by an AFS (atomic fluorescence) detector (CVAFS Model-2, Brooks Rand Ltd, Seattle, USA). With this AFS detector a detection limit of 1 ng methyl mercury per g biological tissue could be reached; i.e. measurements were 40 times more sensitive than those using the Evenson cavity. This detector has some other advantages compared with MIP detection: it is less expensive and easier to manipulate, while the same precision and accuracy are obtained. The use of AFS as detector in the head-space gas chromatographic system is therefore an important improvement for the analysis of methylmercury in biological samples.

Keywords: Methylmercury, analysis, microwave-induced plasma atomic emission spectrometry (AES), atomic fluorescence spectrometry (AFS), gas-chromatographic (GC) detectors

INTRODUCTION

Methylmercury is one of the most dangerous pollutants that can be encountered in the environment. Fish especially tend to concentrate mercury in their tissues and analyses have shown that most of this mercury is in the form of methylmercury,[1–5] despite the lack of obvious significant methylmercury inputs to natural aquatic systems. The methylmercury concentrations in natural waters and sediments are very low ($pg\,kg^{-1}$ level).[2,6] Compared with this, the methylmercury content in fish is mostly found at the $\mu g\,kg^{-1}$ (viz. $ng\,g^{-1}$) level, often the upper $\mu g\,kg^{-1}$ level and sometimes even the $mg\,kg^{-1}$ level.[2,6] Concentration factors of 10^5 to 10^7 are commonly observed.[6–8]

The methods used in most laboratories to analyze methylmercury in fish and other aquatic organisms are based on the method developed by Westöo.[1,9–16] In these methods, the methylmercury is determined by gas chromatography with electron capture detection or microwave-induced plasma atomic emission spectrometry detection. However, these methods have several disadvantages. Prior to injection of the sample onto the GC column, elaborate and time-consuming extractions have to be carried out to

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obtain a clean-up of the sample. The GC separation itself is also very cumbersome. A variety of stationary phases has been recommended but all of these columns have exhibited one or more of the following disadvantages:[17] (a) poor and often variable response to methylmercury chloride; (b) moderate to very severe tailing; and (c) poor column efficiency that can then lead to interferences. Hence, time-consuming and laborious column-conditioning procedures are necessary. The beneficial effects of the treatment are only temporary, since the presence of high-molecular-weight compounds in the sample often leads to 'column performance degradation'. Moreover, if the electron capture detector is used, halogen-bearing compounds coextracted with the methylmercury can interfere because of the nonspecificity of the EC detector.

Very recently, an important improvement of the Westöo method was developed in this laboratory.[18,19] In this method, the methylmercury is cleaved from the biological tissue by sulfuric acid and by addition of iodoacetic acid is converted to the volatile iodide form. These reaction steps take place in a closed head-space vial. Immediately after this extraction step, the liberated methylmercury iodide is head-space-injected into a gas chromatograph and detected by microwave-induced plasma detection (HS GC MIP). No clean-up of the sample has to be done. The chromatographic column requires no special conditioning procedures and, because of the use of head-space injection, no column performance degradation is observed. The method was proved to be fast, accurate, precise and sensitive (detection limit 0.4 $\mu g\ dm^{-3}$ or 20 $ng\ g^{-1}$ biological sample when 50 mg of tissue is taken).

In a recent study, an attempt was made to improve further the detection limit of the HS GC MIP method by using a wide-bore thick-film fused-silica open tubular (FSOT) column instead of a packed column.[20] However, it was demonstrated that the use of an FSOT column gives only a small decrease in detection limit compared with a packed colum. Then, we focused on the detector part of the HS GC MIP system and tried some configurations other than the one so far used. Until now, the plasma was sustained in a quarter-wave Evenson-type cavity using argon as plasma support gas. In this work, we evaluated the use of a TM_{010} Beenakker cavity to sustain the plasma, and used it with both argon and helium as plasma support gas. We also evaluated the use of an atomic fluorescence spectrometry (AFS) detector and compared the performance of the four detector systems for methylmercury measurement.

EXPERIMENTAL

The HS GC system

The HS GC system consists of a modified HS-6 semi-automated head-space sampler[21] (Perkin–Elmer) mounted on an Intersmat 120 gas chromatograph. GC analyses are carried out by using a 1 m × 3 mm i.d. PTFE column packed with 10% (w/w) AT-1000 (Alltech) on Chromosorb W AW (80–100 mesh) with argon or helium as carrier gas at flow-rates of 100 $cm^3\ min^{-1}$ (for MIP detection) and 40 $cm^3\ min^{-1}$ (for AFS detection). The outlet of the GC column is connected to a heated four-way valve for solvent ventilation (Valco GC-T). From there a heated transfer tube (180 °C) is connected, guiding the sample to the detector system. The configuration of this transfer tube depends on the particular detection system.

To analyze methylmercury standard solutions, 2 cm^3 of the standard solution is placed in the head-space sample vials (HS-6) and 30 mg of iodoacetic acid is added. To analyze biological samples, 50 mg of the biological sample is placed in the headspace sample vials (HS-6), then 2 cm^3 of the CH_3HgCl standard solution (the standard addition method is used), 30 mg of iodoacetic acid and 0.60 cm^3 sulfuric acid are added, in this order. The vials are closed with a PTFE-coated butyl rubber septum, shaken vigorously and thermostated for 4.5 min at 80 °C. The pressurization time and injection time are 30 and 15 s, respectively, when a carrier gas flow rate of 100 $cm^3\ min^{-1}$ is used, and 60 and 15 s respectively, at a flow rate of 40 $cm^3\ min^{-1}$. The injector block is maintained at 120 °C and the GC oven at 160 °C.

MIP detection systems

Until this study, all measurements were carried out using a quarter-wave Evenson cavity.[18,19,22] The argon plasma was sustained in a 2 mm i.d. quartz capillary that was centred in the cavity (Electro Medical Supplies, Model 214L). The cavity was connected via a 50 Ω coaxial cable to the microwave generator (Electro Medical Supplies, Microtron 200) which was operated at 75 W, providing an optimum signal to noise ratio; the reflected power was tuned to 0 W.

When a Beenakker cavity was to be used, the argon and helium plasma were sustained in a

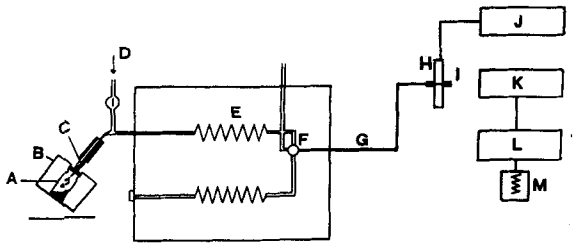

Figure 1 Scheme of the HS GC MIP system for methylmercury analysis: A, sample vial; B, thermostating room; C, sampling needle; D, carrier gas supply; E, GC column; F, four-way valve; G, heated transfer tube; H, resonance cavity; I, quartz capillary; J, microwave generator; K, monochromator; L, photomultiplier; M, recorder.

3 mm i.d. quartz tube, 9 cm in length, placed in a modified TM_{010} Beenakker-type cavity (Scientific Equipment Services, Bancroft, Milton Keynes, UK). The cavity was connected via a 50 Ω coaxial cable to the microwave generator that is operated at 65 W for the argon plasma and at 125 W for the helium plasma. The minimum reflected powers are 1–2 W and 15–20 W, respectively.

For both cavities, the outlet of the four-way valve was connected to the plasma tube by means of a teflon tube (1.6 mm i.d., 50 cm length) enclosed in a 5 mm i.d. insulated copper tube that was heated to 180 °C with a heating tape.

Emission measurements are made with an MPD 850 spectrometer (Applied Chromatography Systems Ltd), incorporating a ¾ m Rowland circle monochromator with six phototubes and associated slits at 253.7 nm. A reciprocal linear UV dispersion of 0.695 nm mm^{-1} is achieved using a 960 grooves mm^{-1} holographic grating fitted into a Paschen–Runge mounting.

A schematic representation of the HS GC MIP system is given in Fig. 1.

AFS detection system

The Cold Vapour Atomic Fluorescence Spectrometer (CVAFS Model-2 Brooks Rand Ltd, Seattle, USA) consist of a 4 W low-pressure mercury vapor lamp, emitting predominantly at 254 nm, a 10 mm square quartz fluorescence cell and a UV–visible general-purpose photomultiplier, shielded from stray light with a 253.7 nm interference filter.

The GC outlet was connected to a thermal decomposition tube via Teflon tubing 1.6 mm i.d., 50 cm length) enclosed in a 5 mm i.d. insulated copper tube that was heated to 180 °C with a heating tape. The decomposition tube consisted of a 9.5 mm o.d. × 6 mm i.d. quartz tube, 20 cm in length, with the central 7 cm packed with quartz wool. The tube is electrically heated with a winding of Nichrome resistance wire to 600 °C. The outlet of the decomposition tube is connected to the fluorescence cell of the CVAFS detector by means of a 1.6 mm i.d. × 30 cm Teflon tubing. The thermal breakdown of the methylmercury compounds to mercury atoms (Hg^0) is necessary for making them detectable by CVAFS.

A scheme of the HS GC AFS system is given in Fig. 2.

Reagents and gases

All chemicals were of analytical reagent grade and the sulfuric acid of suprapur quality (Merck). Analytical standard solutions of methylmercury chloride (concentrations ranging from 0.1 to 100 ng cm^{-3}) were prepared daily from a stock solution of 10 μg cm^{-3}, which was stored in a refigerator.[23] All solutions were made in distilled, deionized water, obtained with a Milli-Q apparatus (Millipore).

The helium and argon carrier gases (Oxhydrique) are further purified from oxygen (O_2) by an oxytrap (Alltech).

RESULTS AND DISCUSSION

In an attempt to attain a lower detection limit with the HS GC MIP system, the quarter-wave Evenson-type cavity used so far was replaced by a

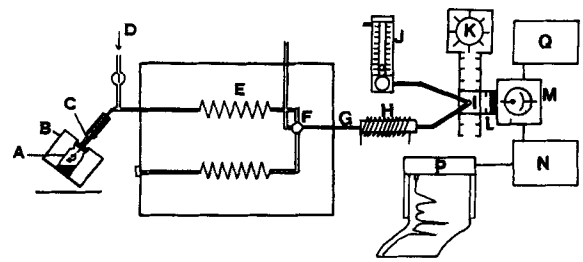

Figure 2 Scheme of the HS GC AFS system for methylmercury analysis: A, sample vial; B, thermostating room; C, sampling needle; D, carrier gas supply; E, GC column; F, four-way valve; G, heated transfer tube; H, pyrolysis tube; I, fluorescence cell; J, flowmeter; K, UV-lamp; L, interference filter; M, PM tube; N, current-to-voltage converter; P, recorder; Q, power supply.

TM_{010} Beenakker-type cavity.[24–27] An undeniable advantage of this cavity is that the plasma can be viewed axially, as deposition of materials on the discharge tube walls can occur. In addition, this is a cavity with an improved transfer of electrical energy so that a helium plasma can be obtained at atmospheric pressure. However, despite the better fragmentation and excitation characteristics of the helium plasma versus the argon plasma, similar detection limits have been reported for arsenic (As), germanium (Ge), antimony (Sb) and tin (Sn).[28] Some authors have used the Beenakker cavity with helium for the determination of methylmercury.[14,15] However, the Beenakker cavity was not used with argon and both cavities have never been compared in a direct study for methylmercury determination.

In a first step, argon was used as carrier gas and thus plasma support gas. With this carrier gas, the plasma could very easily be ignited and a very stable plasma was obtained. Firstly, the effects of the plasma tube internal diameter (0.5, 1, 2 and 3 mm i.d.) were tested; the position of the plasma tube before the entrance slit of the monochromator and the microwave power supply were carefully evaluated. The best signal-to-noise ratio was obtained with an i.d. of 3 mm, while the position of the tube before the entrance slit had only a very slight effect on the detection limit. There was no marked effect of the microwave power supply (in the range 55–85 W) on the detection limit and the reflected power could be easily tuned to ≤ 2 W. A detection limit of 0.1 $\mu g\,dm^{-3}$ could be reached (defined as the signal level corresponding to twice the standard deviation of the background signal), which is eight times better than the detection limit obtained with the Evenson cavity. With the Evenson cavity, a detection limit of 0.4 $\mu g\,dm^{-3}$ was reached in previous studies, using a Perkin–Elmer AAS-403 for the emission measurements.[18,19] It was shown, however, that the best detection limit that could be attained using the MPD-850 detector was twice as high as the detection limit attained when using the AAS-403.[20] The reproducibility (relative standard deviation on the methylmercury peak heights obtained in six replicate measurements) was typically 2–3%, the calibration curve yielded a correlation coefficient $R = 0.9988$ (five concentrations, each concentration injected three times to give a total of 15 injections). These results are comparable with those obtained with the Evenson cavity.

In a second step, the use of helium was evaluated. With this gas, it was rather difficult to ignite the plasma. A high forward microwave power had to be set on the generator and even then a plasma with a small volume was formed. The argon plasma extended 3 cm out of the cavity at both sides, while a helium plasma was only formed in the cavity itself. The plasma extinguished if the microwave power was set lower than 100 W. There was no marked effect of the power on the detection limit (range 100–175 W) and the reflected power could not be tuned lower than typically 15–20 W. In contrast with argon, the position of the plasma tube before the entrance slit of the monochromator was very critical here. This is possibly due to the small volume of the plasma. The best detection limit attainable with helium was 0.2 $\mu g\,dm^{-3}$, or twice as high as when argon was used. The noise level obtained with the helium plasma was approximately two times lower than with the argon plasma, but the intensity of the mercury emission was lower. This can be due to the small volume of the plasma: the residence time of the analyte decreases if the plasma length decreases.[29] Moreover, self-absorption mechanisms and condensation phenomena can take place in the colder parts of the discharge tube. The reproducibility and linearity of the calibration curve were as good as with argon (RSD = 2–3%, $R = 0.9996$).

In a third step, we used another type of detector, an atomic fluorescence spectrometry detector (AFS), although the principle of this detector is the same as that of the MIP detector, namely atomic emission spectrometry. The CVAFS Model-2 is a highly sensitive detector for the determination of mercury atoms (Hg^0) in the gas phase.[30,31]

With this detector, it is very important to use a carrier gas of a very high purity, since sensitivity varies dramatically with inertness, due to quenching of the excited mercury atoms by collisions with polyatomic species. High-purity argon or helium, alternatively argon or helium of a lower grade which was then passed through an oxygen/water removal trap, should be used. With the CVAFS Model-2, mercury sensitivity decreases with increasing flow rate due to sample dilution. Lower flow settings will yield lower detection limits, but at the cost of greater peak broadening. For the GC separation, however, higher flow settings will give lower detection limits (we use 100 $cm^3\,min^{-1}$ with MIP detection). So, when the AFS is used as GC detector, a compromise in flow rate should be searched for. The effect of the carrier gas flow rate on the methylmercury detec-

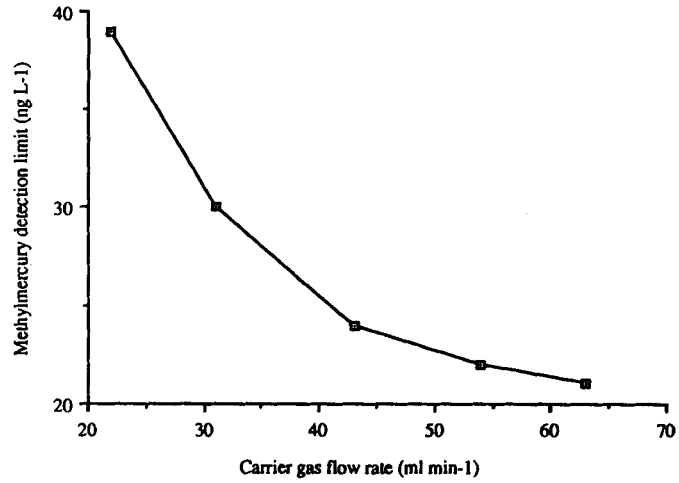

Figure 3 Effect of the carrier gas flow rate on the methylmercury detection limit (HS GC AFS system).

tion limit is shown in Fig. 3. For flow rates above 50 $cm^3 min^{-1}$ the detector stability decreased, resulting in a decrease in the reproducibility of the methylmercury measurements. Therefore, 40 $cm^3 min^{-1}$ was used in all measurements.

In contrast to the use of an MIP detector, which acts at the same time as a fragmentation and excitation source, all mercury species must be converted to Hg^0 prior to detection by AFS. Therefore, the species eluting from the GC column are guided through a pyrolytic decomposition cell into the AFS detector. In Fig. 4 the effect of the temperature of this atomization unit on the methylmercury signal is given. To ensure a complete atomization, 600 °C was taken as pyrolysis temperature.

At the flow rate and pyrolysis temperature as determined above, an RSD of typically 3–4% was obtained. The linearity of the calibration curve was as good or even better than with the MIP detector ($R = 0.9999$). A detection limit of 0.02 $\mu g\ dm^{-3}$ could be reached, which is 40 times better than the lowest detection limit reached with the HS GC MIP system using an Evenson cavity. A comparison of the detection limits reached with the four detector systems evaluated in this study is given in Table 1.

With each of the detectors evaluated here, we

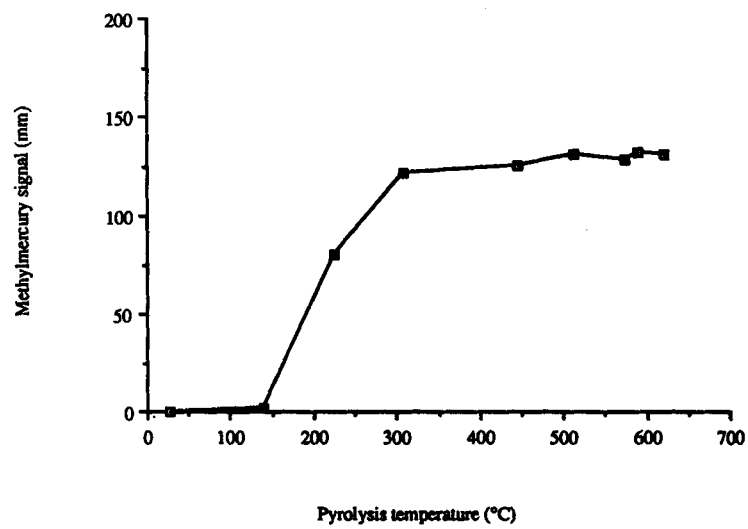

Figure 4 Effect of the pyrolysis temperature on the methylmercury signal (HS GC AFS system).

Table 1 Detection limits reached with the different analytical systems

Analytical system	Detection limit	
	Solution ($\mu g\ CH_3HgCl\ dm^{-3}$)	Biol. samples ($ng\ CH_3HgCl\ g^{-1}$)
HS GC MIP, quarter-wave Evenson, Ar	0.8	40
HS GC MIP, Beenakker, Ar	0.1	5
HS GC MIP, Beenakker, He	0.2	13
HS GC AFS, Ar (and He)	0.02	1

analyzed the same biological sample, in this case a mussel sample (*Mytilus edulis*). The results are given in Table 2. This sample was analyzed before by ten laboratories in an intercalibration exercise organized by the Community Bureau of Reference (BCR) on the determination of methylmercury in biological tissues. As can be seen in the table, very good overlapping results are found with the four systems, proving the accuracy of each of the four systems.

By the use of AFS, the detection limit of the methylmercury head-space analysis method could be lowered to $1\ ng\ g^{-1}$. The detector has some other advantages: it is less expensive than an MIP detector and easier to manipulate. The same precision and accuracy are obtained. Moreover, as with the MIP detector and in contrast to the ECD, interferences are not to be feared since the AFS is a mercury-specific detector. The use of AFS as detector in the head-space gas-chromatographic method is therefore an important improvement for the analysis of methylmercury in biological samples.

Table 2 Comparison of the results obtained with the different analytical systems for the methylmercury content in the mussel sample

Analytical system	Methylmercury content found ($ng\ CH_3HgCl\ g^{-1}$): mean ± SD
HS GC MIP, quarter-wave Evenson, Ar	157 ± 15
HS GC MIP, Beenakker, Ar	164 ± 11
HS GC MIP, Beenakker, He	153 ± 16
HS GC AFS, Ar (and He)	159 ± 9
BCR intercalibration exercise:	
Mean of 10 labs, mean of mean values	148 ± 39
Mean of 10 labs, mean of individual values	142 ± 25

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