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Improvements and application of a modified gas chromatography atomic fluorescence spectroscopy method for routine determination of methylmercury in biota samples

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ABSTRACT

Improvements to the application of a combined solid-phase microextraction followed by gas chromatography coupled to pyrolysis and atomic fluorescence spectrometry method (SPME–GC–AFS) for methylmercury (MeHg) determination in biota samples are presented. Our new method includes improvements in the methodology of determination and the quantification technique. A shaker instead of a stirrer was used, in order to reduce the possibility of sample contamination and to simplify cleaning procedures. Then, optimal rotation frequency and shaking time were settled at 800 rpm and 10 min, respectively. Moreover, the GC–AFS system was equipped with a valve and an argon heater to eliminate the effect of the decrease in analytical signal caused by the moisture released from SPME fiber. For its determination, MeHg was first extracted from biota samples with a 25% KOH solution (3h) and then it was quantified by two methods, a conventional double standard addition method (AC) and a modified matrix-matched calibration (MQ) which is two times faster than the AC method. Both procedures were successfully tested with certified reference materials, and applied for the first time to the determination of MeHg in muscle samples of goosander (*Mergus merganser*) and liver samples of white-tailed eagle (*Haliaeetus albicilla*) with values ranging from 1.19 to 3.84 mg/kg dry weight (dw), and from 0.69 to 6.23 mg kg^{-1} dw, respectively.

1. Introduction

Organometallic compounds of mercury are some of the most toxic substances in aquatic systems. Methylmercury (MeHg) is the most common and toxic form of organomercury compounds, and has a dangerous tendency to bioaccumulate in aquatic food chains [1,2]. Determination of total mercury (THg) in birds' tissues [3–6] has been more often performed than the determination of MeHg [7–9]. Nevertheless, it is clear that the determination of MeHg is almost mandatory for risk assessment, since MeHg data facilitates a more complete understanding of toxic effects and risk to biota. The most frequently used separation technique for MeHg determination in environmental samples is gas chromatography (GC). In most cases the gas chromatograph is coupled to atomic emission spectrometry (AES) [10], atomic fluorescence spectrometry (AFS) [11] or mass spectrometry (MS) [12,13]. AFS enables the determination of mercury at the pg level in environmental samples. To determine MeHg using a GC–AFS method, the conversion of mercury to volatile and non-polar alkyl derivatives is required [14–16]. The most common derivatisation methods are ethylation of $MeHg^+$ and Hg^{2+} ions in the water phase by sodium tetraethylborate ($NaBEt_4$) [17,18], phenylation with $NaBPh_4$ [19] and propylation with $NaBPr_4$ [20]. Then, the MeHg derivative is extracted from the headspace with a SPME fiber, being the 100 μm polydimethylsiloxane (PDMS) the most common type of fiber employed for MeHg determination in environmental samples [21–23]. In this regard, the most common derivatisation procedures used for mercury have been reviewed [24,25]. Finally, volatile mercury derivatives are easily separable by gas chromatography [26–28], and after GC separation, MeHgEt is decomposed by means of a pyrolyzer at 800 °C and determined with a AFS detector.

The main aim of this paper was to improve the above-mentioned methodology of MeHg determination in biota samples, mainly focused on the quantification technique and the method of determination. Firstly, determination of MeHg in biological samples with the SPME–GC–AFS method requires releasing of MeHg from the sample into solution by means of alkaline digestion with

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KOH at 60 °C. Unfortunately, this method produces some matrix effects that have been reported and overcome by standard-addition calibration [18]. The standard-addition (AC) method enables one to minimize the influence of the matrix, but the method is time-consuming. On the other hand, owing to instability of the detector signal over a longer period of time, the typical and much faster external calibration (EC) method cannot be used with an AFS detector. The ageing of the detector lamp, and the electronic signal-level correction, change the signal-level in time, especially after turning off/on the detector. An internal standard method helps to correct the signal fluctuations, but complicates the analytical procedure, increases the total error and the cost of the determination.

To speed up the determination of MeHg while maintaining the accuracy and precision of standard-addition methods, and taking into account the limitations of the AFS detector, a modified matrix-matched method of quantification (MQ) was applied.

The correctness of determinations in the MQ method is controlled by calibration-line correlation R^2 and Certified Reference Material (CRM). This method permits to perform twice the amount of daily determinations of MeHg in biota samples.

To make the determination simpler and to reduce the possibility of sample contamination, the stirrer (used at the stage of derivatisation and SPME extraction) was replaced with a shaker. To apply the shaker in the SPME–GC–AFS method, a special holder for the fiber was constructed and successfully tested.

To eliminate the decrease of peak signal, caused by moisture condensing on the optics of AFS detector, the solutions proposed by Yang et al. [29] for a Tenax trap were successfully applied to the procedure with PDMS fiber. The main improvement was the argon heater-module that accelerates the detector-drying process and speeds up the determination process.

Finally, the improved methodology was successfully applied to the determination of MeHg in several unique biota samples.

2. Experimental

2.1. Instrumentation

Determination of MeHg was performed using the system shown in Fig. 1. The system consists of a gas chromatograph (Hewlett Packard 5890 I) – Fig. 1a, equipped with a 15 m, 0.53 mm ID column – Rtx-1 Restek. The outlet of the GC was coupled to an atomic fluorescence detector Tekran 2500 (Fig. 1b) via a home-made 20 cm-long pyrolysis unit (Fig. 1c).

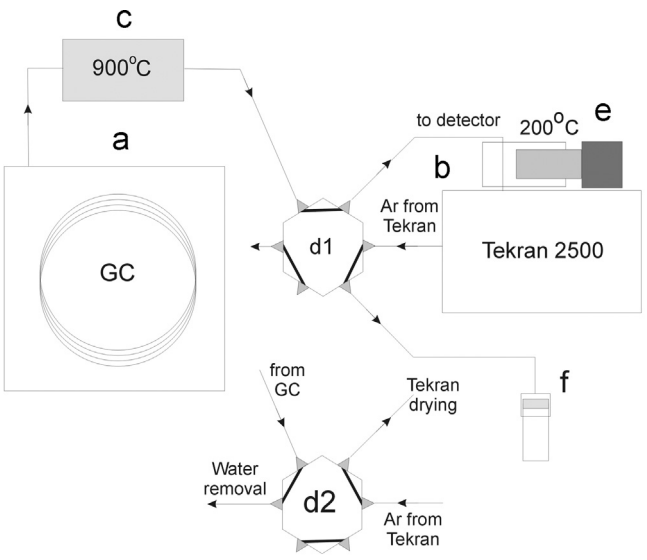

Fig. 1. System for determination of methylmercury: gas chromatograph (a), Tekran 2500 detector (b), pyrolyser unit (c), valve – measure mode (d1), valve – drying and moisture removing mode (d2), argon heater (e), and argon cap (f).

In order to avoid the decrease of peak signal, caused by moisture condensation into a Tekran detector, the modified system for Tenax-GC–AFS system [29] was enhanced and applied to the SPME–GC–AFS system. A 4-way valve (Fig. 1; d1,d2) was added to the typical GC–AFS determination system. The valve enables one to remove moisture at the beginning of the chromatographic separation, and to dry the detector between experimental runs. To shorten the time of a single determination, an argon-heating device (Fig. 1e) was additionally added. This modification increases the efficiency of the detector drying. According to our knowledge this was the first application of a moisture-removal procedure to the SPME–GC–AFS system.

To protect $NaBEt_4$ solution against oxygen during the MeHg measurements, and to extend its time of use, an argon hood (Fig. 1f) connected to the Tekran 2500 argon line was applied. During derivatisation and SPME extraction, a stirrer with a special home-made SPME setup was substituted by a shaker (OS 2 basic, Labart). An Automated Mercury Analyzer MA-2 (Nippon Corporation) was used for the total mercury determination.

2.2. Reagents and standards

All reagents and standards were of an analytical grade or higher. Deionised water was purified by using the HLP5 (Hydrolab, Poland) system. The derivatisation agent, sodium tetraethylborate ($NaBEt_4$, 97%, 1 g), was obtained from Acros Organics (Geel, Belgium). Air-tight microsyringes (100 μL, Hamilton) were used to introduce a 1% solution of derivatisation agent to the vial. Working MeHg solutions (in the range 1–40 ng/mL) were prepared by diluting a certified methylmercury standard (1000 mg kg^{-1}, Alfa Aesar, Karlsruhe, Germany).

Solid-phase microextraction fibers were purchased from Supelco (Bellefonte, PA, USA). Fibers with a 100 μm polydimethylosiloxane (PDMS) coating were used in all experiments. Argon 5.0 has been used as a carrier gas and for Tekran drying. A 25% (w/v) aqueous potassium hydroxide solution (Baker, Deventer, Holland) was used for MeHg extraction from goosander muscle samples.

Literature data [30] suggest that the concentration of MeHg standard solutions may change in time. The real concentration of MeHg in working standard solutions was controlled systematically according to the following procedure. In the MeHg standard, the THg concentration was determined with MA-2 mercury analyzer, the concentration of Hg^{2+} with a double standard-addition method, whereas the concentration of MeHg was the difference between both concentrations.

2.3. Procedure

2.3.1. Preparation and preservation of 1% $NaBEt_4$ solution

The fresh $NaBEt_4$ solution is oxygen-sensitive, and therefore should be prepared daily. To reduce the time spent on reagent preparation, a new procedure for its preparation and protection was developed. A few 20 mL glass vials (cleaned at 600 °C) were weighed and placed in a glove-box filled with argon. About 0.03 g of $NaBEt_4$ powder was added to each vial. Then vials were weighted again to calculate the amount of $NaBEt_4$ in each of the vials. Vials were sealed by septum and stored in a dark bottle at room temperature in an argon atmosphere until needed. In order to prepare a 1% $NaBEt_4$ solution, an appropriate volume of deionised and deoxygenated water was added into the vial through the septum by using a Hamilton microsyringe.