Rapid Determination of Methylmercury in Fish Tissues

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Abstract

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The aim of the present study was to develop a rapid and inexpensive method for the determination of methylmercury in fish tissues based on GC/ECD instrumentation. The new method is based on acidic digestion in hydrochloric acid and subsequent extraction with toluene. Methylmercury is determined by the GC/ECD technique using a DB-608 capillary column. The following parameters of the method were established: detection limit 13 μg/kg, limit of quantification 22 μg/kg, linearity 0.2–200 ng/ml, reproducibility 9.4%, and recovery 90%. The method was developed and verified using CRM 464 reference material and was successfully tested in inter-laboratory comparisons IMEP – 20 "Trace elements in tuna fish" organised by the Joint Research Centre – Institute for Reference Materials and Measurements (Belgium), with the success rate of E_n = 0.43.

Keywords: gas chromatography; ECD; capillary column

Methylmercury (MeHg) is the predominantly occurring form of mercury (up to 100%) in the tissues of a majority of fish species (Porcella 1994; Mason *et al*. 1995; Kannan *et al*. 1998). Its neurotoxicity (Igata 1986) makes it the most toxic form of mercury (WHO 1990). Fishes are the main source of methylmercury intoxication of humans (WHO 1990), and are therefore the main target in monitoring aqueous system contamination for both environmental and public health purposes.

A number of studies on the determination of MeHg have been published. Most of the methods published up to now are based on combinations of a separation techniques (GC, HPLC, electrophoresis) and selective spectrometric (AAS, MIP-AES, AFS, MS, ICP-MS) or voltametric detections.

One of the oldest and most frequently used procedures is based on the combination of GC and a non-selective detector ECD (Cappon & Smith 1977; Hight 1987; Horvat *et al*. 1990; Caricchia *et al*. 1997). The advantage of this instrumentation is its easy availability and the possibility of the direct determination without any need for further derivatisation. The disadvantage of the method is the adsorption of polar MeHg on the active centres of the stationary phase, which is the cause of poor repeatability, changes of retentions times, peak tailing and broadening, reproducibility of retention times, and analyses themselves. To eliminate these effects, columns are conditioned with Hg^{2+} salts (O'Reilly 1982; Hight 1987; AOAC 1992), or capillary columns are used which need much less conditioning or none at all due to their more

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inert nature in comparison with packed columns, (Cappon & Smith 1977; Lorenzo *et al*. 1993; Caricchia *et al*. 1997).

The aim of this study was to develop and verify a rapid and inexpensive method with minimal need for the treatment of the column that will enable to determine MeHg in fish in concentrations below 0.1 mg/kg which, until recently, was the public health limit in the Czech Republic for the mercury concentration in non-predatory fish. In the study, GC/ECD instrumentation was selected for its easy availability.

MATERIAL AND METHODS

Instrumentation. For the determination, a Hewlet Packard 5890 Series II gas chromatograph was used. A capillary column DB 608 (30 m, 0.53 mm) from J&W Scientific and an electron capture detector (ECD) were used. Thw evaluation was made using HP 3365 ChemStation Series II software (Hewlett Packard).

Chemicals, standards and reference materials. The chemicals and materials used included solvents (acetone and toluene) of the grade suitable for the residual trace analysis (Merck, Germany), the standard solution of methylmercury chloride in isooctane (Ehrenstorfer, Germany), anhydrous sodium sulphate of the p.a. grade (Merck), hydrochloric acid of the p.a. grade (Merck), and redistilled water. As the certified reference material, CRM 464 tuna fish (5.50 ± 0.17 mg/kg $MeHg^+$) (IRMM Belgium) was used.

Experimental and calibration solutions. Calibration solutions of methylmercury chloride in toluene in the concentrations of 0.2, 5.0, 10.0, 20.0, 80.0 and 200.0 ng/ml were used. The experimental solution of hydrochloric acid was the 1:1 solution (v/v).

Analytical method. The sample preparation was based on acidic digestion and the extraction with toluene (AOAC 1992) designed for the packed column determination. Volumetric parameters as well as the quantities of the samples and solutions used were modified.

A total of 0.05–0.2 g of homogenised sample weighed on an analytic balance was put into a 10 ml centrifugation test tube, 5 ml acetone was added and the solution was stirred vigorously for 30 s with a glass rod. After centrifugation (3500 rev per min, 5 min), 4 ml acetone were carefully pipetted away without agitating the suspension. The same procedure was repeated 3 times, twice with the addition of 4 ml acetone and once with 4 ml toluene added. When 4 ml of toluene were drawn out and discarded, 0.7 ml hydrochloric acid (at 1:1 dilution (v/v) and washed with toluene) and 4 ml of toluene were added to the test tube. First, only the aqueous phase containing the sample was vigorously stirred with a glass rod to make paste-like consistency (to guarantee a complete digestion of the sample and a maximum yield), and then it was mixed for another 1 min together with the toluene phase. After centrifugation (3500 rev/min, 5 min), 4 ml of the toluene phase were carefully pipetted away (to prevent them from mixing with the aqueous phase), and the toluene phase was then transferred to a stoppered test tube with 2 g of anhydrous sodium sulphate (extraction part 1). 4 ml of toluene were added to the test tube and the extraction was repeated once more. The toluene phase (extraction part 2) was added to the test tube with the extraction part 1. The combined extracts were left for 1 hour in a freezer at 4°C, and then they were used for the MeHg determination on a gas chromatograph. The shelf life of the extract at –23°C was 14 days.

The following heating pattern was used: 2 min 140°C; gradient 4°C/min to 160°C; 2 min at 160°C; injector temperature 240°C, detector temperature 300°C. The samples of the volume of 2 µl were injected on the column. The external standard method was used for the calibration.

RESULTS AND DISCUSSION

Chromatographic elution

With the overall period of the chromatographic elution of 9 min, the MeHg peak retention time was 4.5–4.9 min depending on the condition of the column, i.e. the retention times fell roughly to the middle of the chromatographic elution period. (Figure 1). No interference phenomena were observed and it was therefore possible to use the external standard calibration method. In the present study, the adsorption of methylmercury chloride on the DB-608 column was observed, which is at variance with the report by Caricchia *et al*. (1997) who used an equivalent SPB-608 column for the determination of MeHg in sediment samples after alkaline digestion. The column was conditioned with a standard solution of 300 ng·ml methylmercury chloride until

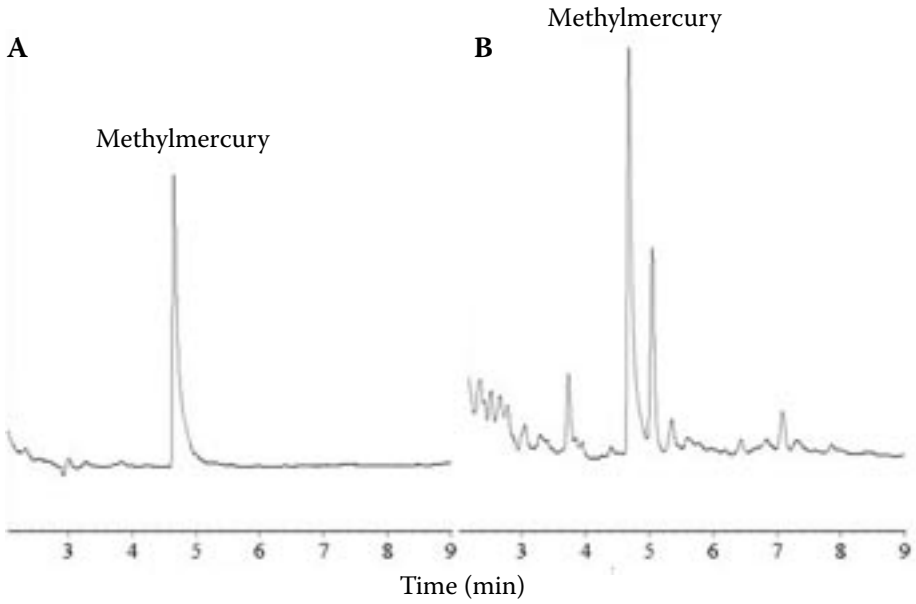

Figure 1. Chromatogram of standard calibration solution. Concentration c = 20 ng/ml; injected volume V = 2.0 µl; retention time t = 4.65 min

the peak area steadied itself in the range of ± 5%. The time necessary for the column conditioning depended on how much time had elapsed from the previous measurement.

Repeatability of results and uncertainty

To study the repeatability, CRM 464 was used as the reference material. Twenty-five determinations, each on a different day, were made over a period of 3 months. The relative standard deviation of the analyses was 9.4%. The injection repeatability was tested using the standard methylmercury chloride solution (c = 20 ng/ml). A total of 20 chromatographic elutions were performed in one day. The relative standard deviation in these measurements was 5.2%. The combined standard uncertainty was calculated from the standard uncertainty type A and the standard uncertainty type B. The standard uncertainty type A (u_A = 0.52 mg/kg) was calculated as the standard deviation from twenty-five determinations of the reference material and was **CHYBI UDAJ**. The standard uncertainty type B (u_B) was the uncertainty of the reference material used u_B = 0.17 mg/kg. The calculated combined standard uncertainty was u_x = 0.54 mg/kg.

Limit of detection and limit of quantification

The limit of detection (LOD) was set as the sum of tree times the standard deviation of the blank and the blank mean value, and the limit of quantification (LOQ) as the sum of nine times the standard deviation of the blank determination and the blank mean value. The mean of the blank determination was 200 ± 30 ng/l (n = 15). In absolute terms, LOD and LOQ are then 0.57 pg and 0.98 pg, respectively. When calculated for the sample weight of 0.2 g, LOD and LOQ were then 13 µg/kg and 22 µg/kg, respectively. These LOD and LOQ values make it possible to determine the MeHg content ofwell below 500 µg/kg, which is in many countries the legal limit for the mercury content in fish for human consumption. Penedo de Pinho *et al*. (2002) achieved the detection limit of 50 µg/kg in fish samples using GC-ECD with different extraction and sample cleaning phases. In comparison to other different common technics based on GC, Qvarnström *et al*. (2003) presented the detection limit of 70 µg/kg for the determination of methylmercury in mouse tissues by GC-ICP-MS. Palmieri and Leonel (2000) presented the detection limit 100 µg/kg in fish samples with GC-MIP-AES.

Recovery: To determine the recovery, CRM 464 was used as the reference material. A total of 15 eterminations were performed over a period of 10 days. The overall recovery was 90 ± 2.5%. The recovery achieved is completely satisfactory.

Linearity: Linearity was tested for the range of concentrations corresponding to mercury levels in fishes. The relationship was linear (Figure 2)

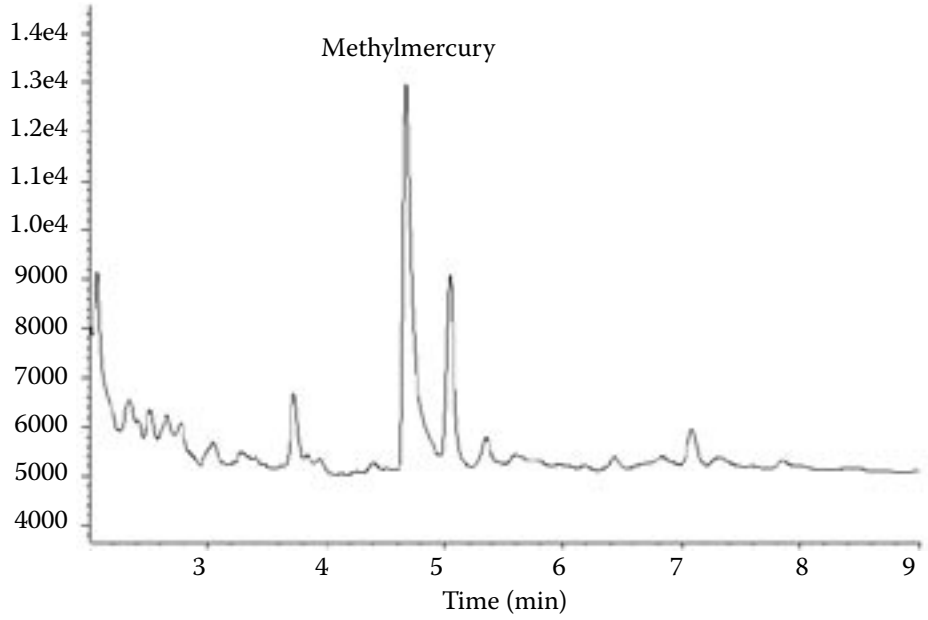

Figure 2. Chromatogram of reference material CRM 464. Weight m = 49 mg; iInjected volume V = 2.0 µl; retention time t = 4.65 min

from 0.2 ng/ml to 200 ng/ml, and the correlation coefficient, r, was 0.999. Given the sample weight of 0.2 g, it equals the range of 0.009–8.889 mg/kg. Fisher-Snedecor tests were used for testing the linear regression. The model selected was significant (α = 0.05).

Inter-laboratory comparison

The method was tested in inter-laboratory comparisons IMEP-20 "Trace elements in tuna fish" organised by the Joint Research Centre – Institute for Reference Materials and Measurements (Belgium). The success rate of the method was expressed as E_n calculated according to ISO/IEC Guide 43-1 (1997).

The following formula was used to calculate E_n:

$$E_n = \frac{x - X_{\text{Ref}}}{\sqrt{u_x^2 + (0.1\,X_{\text{Ref}})^2}}$$

where:

X_{Ref} – certified IMEP value (4.24 mg/kg)
x – value reported by the laboratory (4.58 mg/kg)
u_x – value of combined uncertainty reported by the laboratory (9.9%)
0.1 X_{Ref} – selected performance criterion

Using our method, E_n = 0.43, while $E_n \leq 2$ is satisfactory, E_n = 2–3 is questionable, and $E_n \geq 3$ is unsatisfactory. It follows from the results that

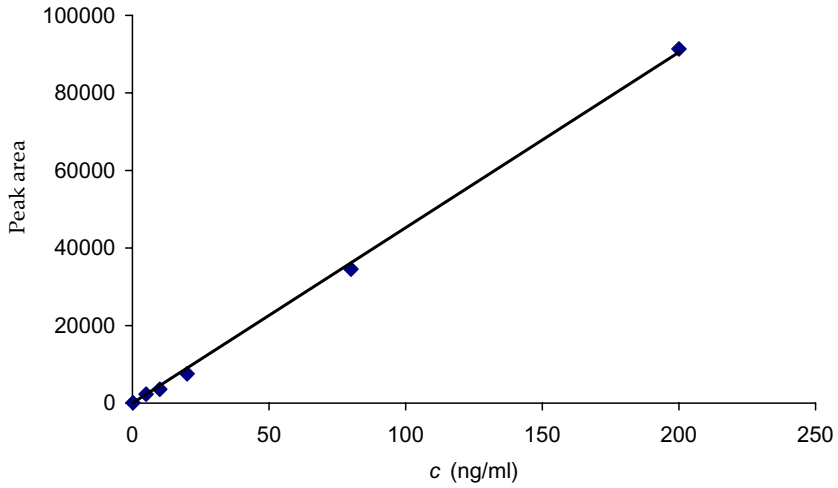

Figure 3. Calibration curve. Concentrations of standard solutions: c = 0.2; 5.0; 20.0; 80.0 and 200.0 ng/ml

our method successfully passed the inter-laboratory test.

Examining field samples

The method was used to test MeHg levels in tissues of fishes from various places in the Czech Republic. Hundreds of samples were analysed and the results will be published.

CONCLUSIONS

Analytical parameters of the method and its verification in the inter-laboratory test showed that the method is suitable for the MeHg determination in fish tissues. Because it relies on the commonly available GC/ECD instrumentation, the method can be used in everyday laboratory practice with minimum requirements for the sample preparation. At the same time, its parameters make this method comparable with a number of much more expensive techniques.

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