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Determination of Methylmercury in Human Hair by Capillary GC with Electron Capture Detection

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An analytical procedure was developed for the determination of methylmercury in human hair using capillary gas chromatography with electron capture detection (GC–ECD). Hair samples are digested in alkaline solution/toluene in an ultrasonic bath at about 50 °C. After cooling and treatment with hydrochloric acid (6 mol l^{-1}) and a saturated solution of copper sulphate, the organic phase is extracted with a cysteine solution. Methylmercury is back-extracted in toluene by adding copper sulphate and potassium bromide and analysed by GC–ECD using a DB17 capillary column. The practical detection limit of the method for methylmercury is 50 ng g^{-1} using 100 mg of hair sample. Column performances and injection reproducibility have been evaluated. Results on 13 samples of human hair are presented and related to the total mercury concentration. The method, consisting of the optimization of similar analytical procedures through improvements in the various steps, allows for sensitive and reliable quantitation of methylmercury in hair with good precision and accuracy.

Keywords: Methylmercury, human hair, capillary GC–electron capture detection

INTRODUCTION

Mercury has a widespread environmental distribution originating both from natural (natural degassing of the Earth's crust, leaching from rocks) and industrial sources (production of caustic soda and chlorine, the electrical industry, seed dressing, biocides, extraction of gold, etc.). Deposition of atmospheric mercury contributes to its global distribution in the environment. Even if the exact contribution of each source is indeterminable, both natural and anthropogenic sources contributions vary in the range 40–60%;[1] for example, Nriagu[2] estimated that natural sources made a 41% contribution to the total emission to the atmosphere in 1983. Emissions from anthropogenic sources generate the greatest risks because they are localized or released in confined areas. Inorganic mercury is generally predominant in the environment, but the formation of the much more toxic methylmercury by methylation of the inorganic species via abiotic[3] or biotic processes[4,5] has been well demonstrated. From a toxicological and public health point of view, methylmercury is the best known compound.

Excluding occupational exposure, the consumption of fish and other seafood is the major pathway through which methylmercury enters the human body. Starting from the mercury contamination of the Minamata Bay and Agano River in Niigata (Japan) and the evidence of the effects on the exposed population,[6,7] public and scientific concern about methylmercury has increased considerably.

Because of the relatively long biological half-time of 39–70 days (average approximately 50 days), methylmercury may accumulate in the human body following consumption of fish; and hair is one of the main accumulation sites of mercury. Mercury half-times in hair are similar to those in blood, but have a wider variation (35–100 days, average 65 days).[1] Therefore, hair can be used to monitor methylmercury contamination of the human body. The concentration of total mercury and methylmercury in seafood and/or in human hair in Italy,[8,9] in the equatorial Pacific,[10,11] in the USA,[12,13] in Greenland[14] and in other countries,[15–18] has been reported.

Analytical methods have generally relied on the use of packed-column gas chromatography with electron capture detection (GC–ECD)[19–21] because of its ease of application in routine analyses, even where alternative methods exist.[22] Unfortunately, alkylmercury compounds create problems in chromatographic analyses; adsorption or decomposition of the compounds can readily occur, particularly at low concentration

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levels. Priming the columns with repeated injections of high levels of a mercury compound prior to the analysis is often used to improve the chromatographic performance, especially for biological samples. Even though several methods have been published,[23–25] use of capillary columns for the determination of organomercury compounds is still limited. Several difficulties were shown concerning the reproducibility of analytical data. This was probably due to interactions between mercury compounds and injector and column surfaces at high temperatures, generally related to insufficient deactivation.[26]

The current study was carried out to evaluate the suitability of a medium-bore (0.53 mm) DB17 column. A stationary-phase film thickness of 0.5 μm was chosen in order to verify whether it is actually necessary to use columns with thicker coatings as suggested in the literature.[24,25]

EXPERIMENTAL

Apparatus

Analyses were performed on a Varian 3500 gas chromatograph equipped with a ^{63}Ni ECD and a Perkin–Elmer 1020 integrator. Analyses were carried out on a 30 m × 0.53 mm capillary column with the intermediate polarity stationary phase DB17 (film thickness 0.5 μm; J&W Scientific). The chromatographic operating conditions were the following.

Oven temperature programme	100 °C × 1 min 5 °C min^{-1} to 140 °C 140 °C × 4 min
Injector (on-column) temperature	140 °C
Detector (ECD) temperature	240 °C
Carrier gas	Helium (8 ml min^{-1})
Make-up gas	Nitrogen (30 ml min^{-1})

Sonication (alkaline digestion) of samples was performed with a Bransonic 52 ultrasonic bath.

Centrifugation operations were performed with a Sorvall T6000B Centrifuge (Du Pont).

Gamma spectrometry analyses were performed using high-purity germanium detectors with a relative efficiency of about 20% and resolution (FWHM) of 1.9 keV at the 1332 keV peak and the computer program Omnigam was used for the analysis of the gamma spectra. The detectors, electronic components and computer program were purchased from Eg&G ORTEC (100 Midland Rd, Oak Ridge, TN 27831-0895, USA).

Reagents

Methylmercury chloride (MeHgCl) and ethylmercury chloride (EtHgCl) were purchased from JM Alfa Products, Karlsruhe, Germany; L-cysteine was purchased from Aldrich, Steinheim, Germany; sodium chloride, anhydrous sodium sulphate, copper sulphate, sodium hydroxide, potassium bromide, mercury chloride ($HgCl_2$), hydrochloric acid (all RPE) and toluene RS for pesticide residue analysis were purchased from Carlo Erba, Milan, Italy.

Anhydrous sodium sulphate was pre-treated in an oven at 600 °C overnight and stored in a closed glass bottle.

Working solutions

The following solutions were prepared.

Cysteine solution (1%). A 500 mg portion of L-cysteine was weighed into a 50-ml volumetric flask and brought up to volume with distilled water. This solution had to be prepared daily.

Sodium hydroxide solution (45%). Sodium hydroxide (22.5 g) was dissolved carefully in 50 ml of distilled water.

Sodium chloride solution (10%). Sodium chloride (10 g) was dissolved in 100 ml of distilled water.

Saturated copper sulphate solution. Sufficient copper sulphate crystals were added to 100 ml of distilled water so that some remained in the bottom of the flask without dissolving.

Potassium bromide solution (4 mol l^{-1}). After 47 g of potassium bromide had been weighed into a 100 ml volumetric flask the volume was made up with distilled water.

Hydrochloric acid solution (1:1). Concentrated hydrochloric acid (50 ml) was added carefully to 40 ml of distilled water in a 100-ml volumetric flask, and brought up to volume with distilled water.

It was necessary to purify all the above working solutions by toluene extraction before use. The extraction should be repeated at least twice.

Mercuric chloride column treatment solution. $HgCl_2$ (0.1 g) was dissolved in 100 ml toluene.

Calibrant solutions

Stock solutions in toluene

Solution A (Hg, 1 mg ml^{-1}). A portion of

125.2 mg of MeHgCl was weighed into a 100-ml volumetric flask and brought up to volume with toluene. These solutions were stable for at least six months at 4 °C in the dark and were renewed every three months.

Solution B (Hg, 1 mg ml^{-1}). A portion of 132.1 mg of EtHgCl was weighed into a 100-ml volumetric flask and brought up to volume with toluene. These solutions were stable for at least six months at 4 °C in the dark and were renewed every three months.

Solution A1 (Hg 10 μg ml^{-1}). Using a graduated pipette, 1 ml of solution A was transferred into a 100-ml volumetric flask and brought to volume with toluene. These solutions were stable for at least three months at 4 °C in the dark and were renewed every month.

Solution B1 (Hg, 10 μg ml^{-1}). Using a graduated pipette, 1 ml of solution B was transferred into a 100-ml volumetric flask and brought to volume with toluene. These solutions were stable for at least three months at 4 °C in the dark and were renewed every month.

Solutions for chromatography

The methylmercury working calibrant solutions for chromatography (10, 50, 100, 200 and 400 ng Hg ml^{-1}) were obtained by successive dilution of solution A1 in volumetric flasks. An adequate volume of solution B1 was added to each calibrant solution in order to obtain the appropriate concentration of ethylmercury (estimated on the basis of the expected concentration of methylmercury in the samples; generally 100 ng Hg ml^{-1}), used as internal standard. These solutions, stored at 4 °C in the dark, were renewed weekly.

Reference materials

The BCR CRM397 'Trace elements in human hair' reference material was used as the control for the precision and accuracy of the analytical method, although only an 'indicative value' is known for methylmercury.[27]

The NIST 1566 oyster tissue and IAEA MA-A-1, copepod homogenate reference materials were also used as the control for the total mercury analysis by neutron activation.

Sample preparation

Hair samples were washed according to the IAEA protocol once in acetone, three times in doubly-distilled water, and once in acetone. Hair samples were finely chopped in very small sections (1–2 mm) and mixed to ensure homogeneity.

Analytical procedure

Total mercury

Neutron activation analysis was used for the determination of total mercury. Hair samples were enclosed in pure quartz vials and irradiated in the 1 MW Triga rector at the ENEA Casaccia Research Centre for about 14 h in a thermal flux of approximately 2.6×10^{12} n cm^{-2} s^{-1}. Reference materials (NIST, BCR and IAEA) were also irradiated during each run. The continuous rotation of the irradiation facility ensures a uniform neutron flux for all the samples. After an appropriate cooling time the samples were transferred into polyethylene containers and measured by gamma spectrometry.

Methylmercury

About 100 mg of sample was accurately weighed and transferred to a 20-ml conical-bottom Pyrex vial with a Teflon-lined screw cap. Then 2 ml of 45% NaOH, 1 ml of 10% NaCl, 2 ml of toluene and EtHgCl (as internal standard) were added consecutively. The vial was tightly closed and put in an ultrasonic bath at about 50 °C until the sample had dissolved completely (about 1 h). After cooling, 2 ml of toluene and 3.5 ml of HCl (6 mol l^{-1}) were carefully added (it is important to verify that the aqueous phase is acid; otherwise more acid must be added). A saturated solution of $CuSO_4$ (2 ml) was added and the vial was shaken vigorously for 5 min. The phases were separated by centrifugation at 1500 rpm for 10 min. The supernatant organic phase was transferred with a Pasteur pipette into a 12-ml vial closed with a Teflon-lined screw cap. The extraction was repeated twice with 2-ml aliquots of toluene. Particular care should be used in the separation step to avoid the collection of even a minimum amount of the aqueous phase which could interfere with the successive steps. It can be useful to filter the collected organic phases on anhydrous sodium sulphate (0.5 g in a Pasteur pipette) in order to remove any residual water.

A back-extraction was performed to eliminate electron-capturing species co-eluting with the organomercury compounds. A 1% cysteine solution (2 ml) was added to the vial containing the toluene extract. The vial was shaken vigorously for 3 min. After the complete separation of the two phases, the aqueous phase was transferred into a 10-ml vial closed with a Teflon-lined screw cap, avoiding collection of the organic phase. This step was repeated twice and the aqueous phases were collected together. Then 2 ml of toluene and

1 ml of saturated $CuSO_4$ solution were added to the aqueous solution and the vial was shaken moderately. KBr solution (4 mol l^{-1}; 1 ml) was added and the solution was shaken vigorously for 3 min. After the separation of the phases, 2 µl of toluene phase was injected in the gas chromatograph.

RESULTS AND DISCUSSION

The column used in this study allowed the detection of methylmercury down to levels of 2 pg injected (corresponding to a concentration of 20 ng g^{-1} in hair, using the described procedure and 100 mg of hair), with no evidence of adsorption or degradation of the sample. The common practice of GC column conditioning by mercury salt injection was found to be still necessary, to ensure a proper deactivation, because after a few injections in the untreated column the peaks tended to broaden and to be irreproducible. Other authors have observed[25] that conditioning with mercury chloride deteriorates stationary phases very quickly, making the columns useless. In this study we conditioned the column by injecting 5 µl of mercury chloride treatment solution three times at 20-min intervals at 140 °C. Large, broad peaks appeared and after 2 h the baseline was steady. The column was left at 120 °C overnight; on the next working day it was equilibrated at 100 °C for 1 h and several injections of toluene were performed in order to verify the baseline in regular chromatographic runs. When the baseline was steady the calibrant solutions and sample extract were injected. During three weeks the column maintained satisfactory chromatographic performance, e.g. giving symmetrical peak shapes and high reproducibility of retention times. Sometimes, after repeated injections of sample extracts at low methylmercury concentration levels, large peaks appeared at a column temperature of 140 °C. In these cases the column was maintained at 140 °C for 30 min and two or more injections of toluene were performed until chromatograms appeared free of undesired peaks. An injector temperature of 140 °C was selected because a higher frequency of the inconvenience was observed at lower temperature (120 °C), and reactivation of the injector section occurred at higher temperatures ($\geq$ 150 °C).

Calibration curves for the quantitation of methylmercury were linear for injected amounts from 20 pg up to 800 pg and typically showed correlation coefficients >0.995, but not passing through the origin. It is necessary to use different calibration curves for contents ranging from 50 to 1000 ng g^{-1} and from 1000 to 10000 ng g^{-1}, respectively; furthermore, it is better to use a smaller sample for contents >10000 ng g^{-1}. Figures 1(a,b,c) shows the chromatograms of calibrant

Table 1 Analytical results of nine repeated injections of mercury solutions

Standard solution[a]		Peak area			Peak height		
		MeHg	EtHg	MeHg/EtHg	MeHg	EtHg	MeHg/EtHg
10/100	Mean	1196	13846	0.087	155.8	2044	0.076
	S.D.	51	257	0.003	13	156	0.002
	CV (%)	4.2	1.9	3.4	8.3	7.6	2.2
50/100	Mean	4227	12414	0.341	744	2274	0.327
	S.D.	68	364	0.008	24	46	0.014
	CV (%)	1.6	2.9	2.4	3.3	2.0	4.3
100/100	Mean	8078	12354	0.653	1534	2383	0.645
	S.D.	310	523	0.006	20	99	0.024
	CV (%)	3.8	4.2	0.9	1.3	4.2	3.7
200/100	Mean	15662	12586	1.245	2710	2409	1.119
	S.D.	333	240	0.013	144	53	0.059
	CV (%)	2.1	1.9	1.0	5.3	2.2	5.3
400/100	Mean	29499	12381	2.3833	4838	2417	2.012
	S.D.	856	461	0.031	278	164	0.211
	CV (%)	2.9	3.7	1.3	5.1	6.8	10.5

[a] Ratio between ng (as Hg) of MeHg and ng (as Hg) of EtHg in 1 ml of toluene, e.g. 10/100 means 10 ng (as Hg) of MeHg + 100 ng (as Hg) of EtHg in 1 ml of toluene.

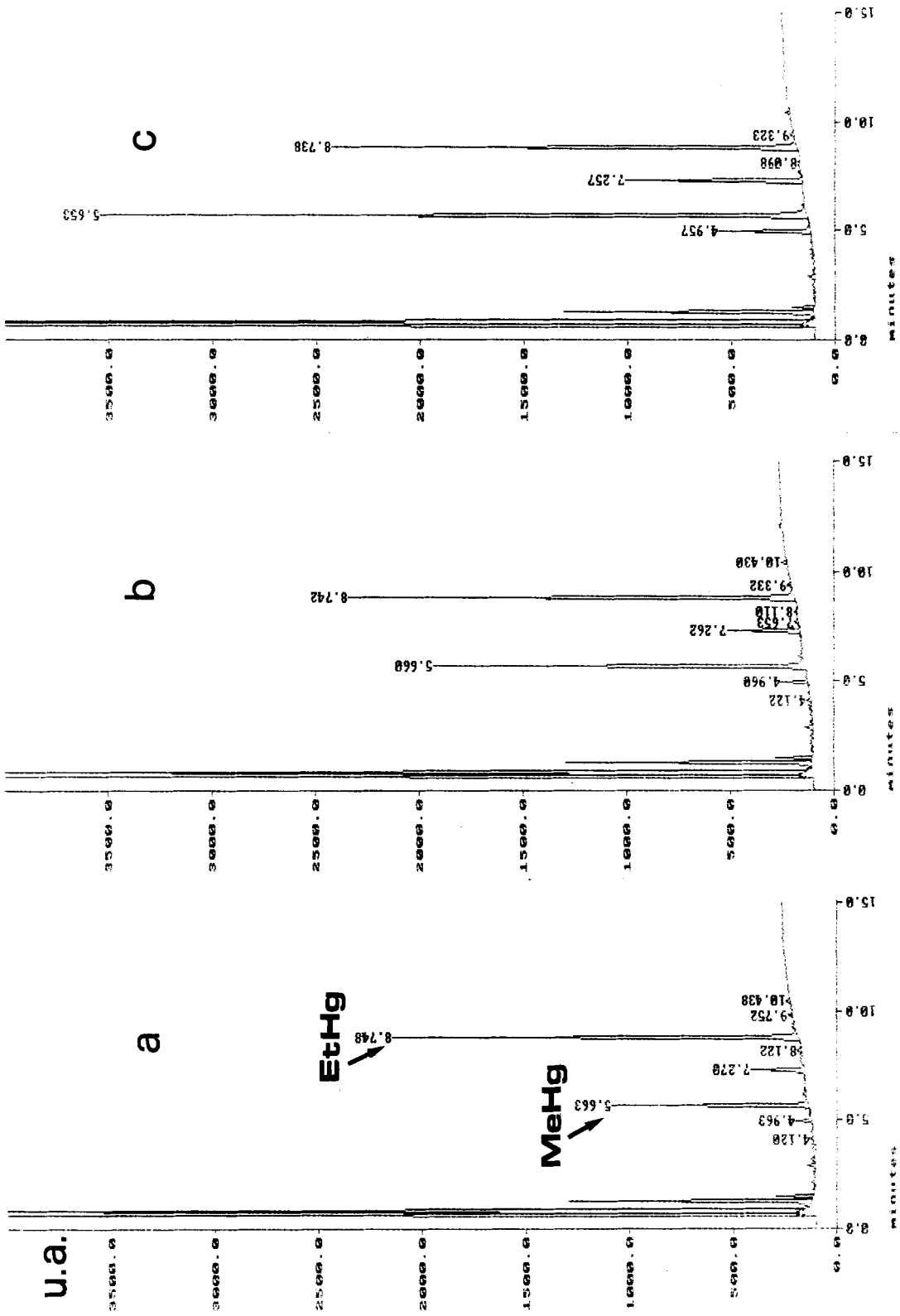

Figure 1 Chromatograms of calibrant solutions at (a) 100 μg Hg l^{-1}, (b) 200 μg Hg l^{-1} and (c) 400 μg Hg l^{-1}.

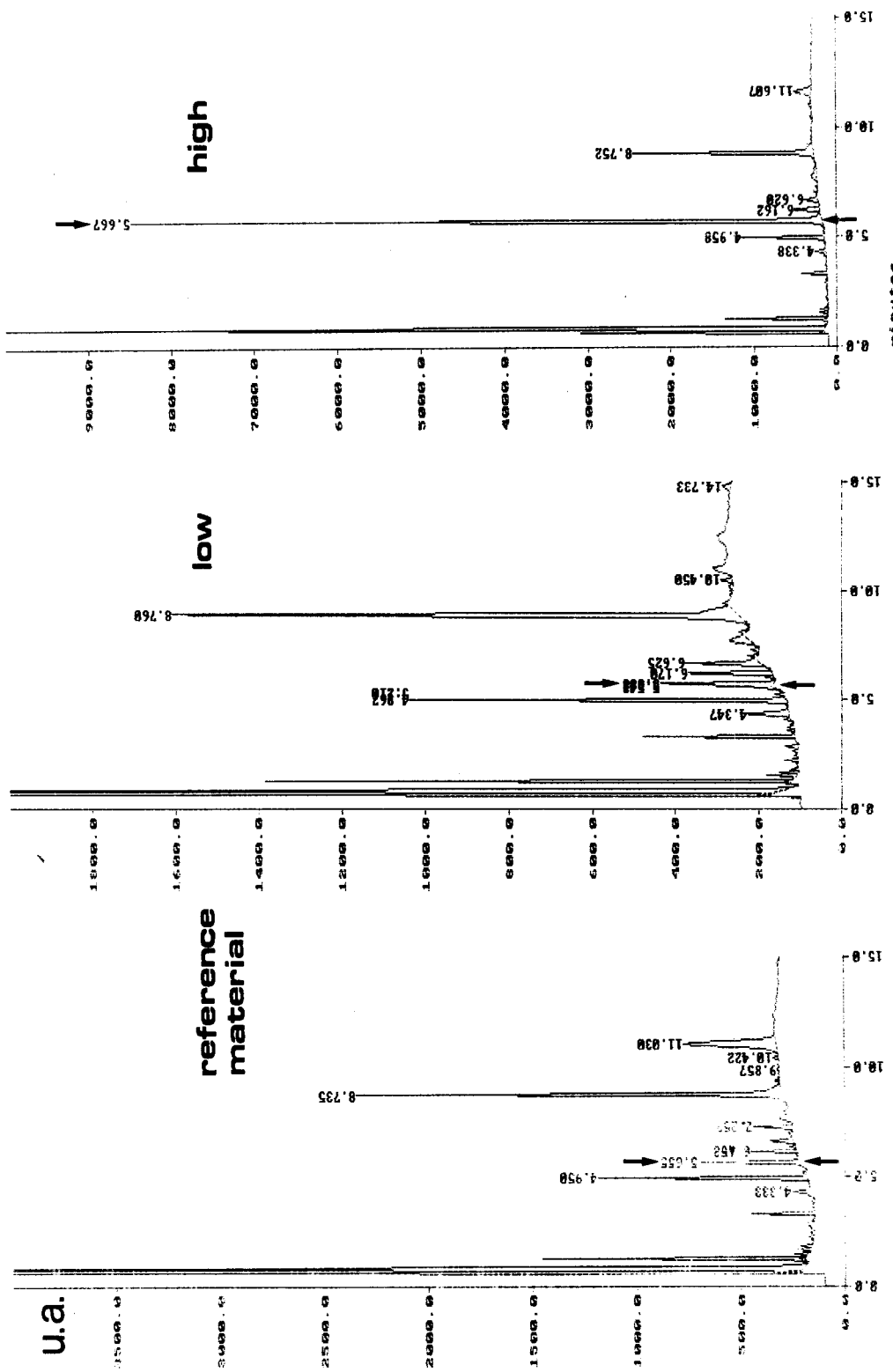

Figure 2 Chromatograms of (a) BCR CRM 397 Reference Material, and hair samples with (b) relatively low and (c) very high methylmercury concentrations.

Table 2 Total mercury and methylmercury concentration (as ng Hg g^{-1}) in hair samples and BCR CRM397 reference material

Sample	Total Hg	Certified	MeHg	(I)[a]
BCR 397	12100	12300 ± 500	950 ± 130[b]	870 ± 40
Hair H1	27500	—	26700 ± 1640[c]	—
Hair L1	610	—	580 ± 27[c]	—

[a] Informative value, individually determined by some laboratories during the course of the certification.[27]
[b] Six replicates.
[c] Four replicates.

solutions at concentration levels of 100, 200 and 400 μg l^{-1} respectively, corresponding to 200, 400 and 800 pg of methylmercury injected.

The reproducibility of injection and detector responses was evaluated using five calibrant solutions and the results are reported in Table 1.

Ethylmercury was used as internal standard, according to a reference method,[28] however doubts have been expressed on the specificity of the determination, in terms of clear separation of ethylmercury from interfering peaks from toluene.[24] In our experience these problems were not shown and only the methylmercury peak was sometimes affected by minor interferences. Absolute recovery of ethylmercury in hair sample analyses is very good (≥90%) and we think that, due to the indubitable chemical similarity to methylmercury, it is still the best choice as surrogate (recovery) compound. An internal (injection) standard could be added to compensate more easily for final volume and injection volume variability. The choice of such a compound is obviously dependent on the chromatographic system adopted and we are now addressing this problem as a further improvement to the method.

In order to check the reproducibility of the analytical method, the BCR CRM 397 Reference Material[27,29] (the 'indicative value' for methylmercury may be used only to give an indication of the accuracy of the method) and two hair samples, representating relatively low and very high methylmercury concentrations, were selected. Chromatograms obtained from these samples are reported in Fig. 2(a,b,c, respectively).

As can be seen, in all cases, even for the sample at a low concentration level, the methylmercury peak was easily quantitated because of the absence of significant interferences. Reference material analysis was repeated six times, while the analysis of the other two samples was replicated four times. The results are reported in Table 2. It has been suggested that, because of the very low percentage of mercury present as methylmercury in BCR CRM 397 reference material, the sample was probably contaminated with inorganic mercury during its preparation.[30] The high level of inorganic mercury was explained alternatively by the proximity of the sampling area to highly contaminated areas. Of the daily intake of total mercury from fish and fish products, 80% is methylmercury[1] and it is well known that the percentage of methylmercury in hair from fish-eating humans is generally more than 80% of total mercury.[11]

Results on the two hair samples showed good precision and sufficient accuracy with respect to the 'informative value' of the reference material.

Other 13 hair samples were analysed for the determination of total mercury and methylmercury concentration and the results are reported in Table 3. These hair samples were collected from adult individuals belonging to population groups living in seaside towns in the south, centre and north of Italy and having a high fish consumption. It can be observed that the results are in good agreement, even if in some cases the methylmercury concentration was higher than the total mercury concentration. This is probably due to an insufficient homogeneity of samples and to the relatively high standard deviation of the methods used for the total mercury determination (>15%), calculated by repeated analyses on reference materials.

Table 3 Total mercury and methylmercury concentration as μg Hg g^{-1}) in 13 hair samples[a]

Sample	Total Hg	MeHg
F46	27.5	25.5
F25	9.9	8.2
B2	12.6	16.8
B16	6.7	5.0
M6	19.9	15.5
M7	14.1	10.9
M1	5.7	5.8
M8	7.0	6.5
F11	12.4	17.2
R6	7.2	9.5
B3	12.5	18.9
F4	8.8	7.9
F25	9.9	10.8

[a] Results are means of two replicate analyses.

CONCLUSION

The proposed method allows for the sensitive and reliable quantitation of methylmercury in hair with good precision and accuracy. The capillary column used in this study seems to overcome many of the problems previously encountered with the determination of methylmercury confirming that the use of capillary GC represents a clear improvement in comparison with packed GC columns as demonstrated in interlaboratory studies organized by BCR.[31] Further work, especially on column deactivation, is still needed however.

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