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DETERMINATION OF METHYL- AND ETHYLMERCURY IN RAT BLOOD AND TISSUE SAMPLES BY CAPILLARY GAS CHROMATOGRAPHY WITH ELECTRON-CAPTURE DETECTION

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SUMMARY

A precise and accurate method has been developed for the determination of either methyl- or ethylmercury in the blood and tissue of rats using capillary gas chromatography with electron-capture detection. The biological sample was spiked with an internal standard (methyl- or ethylmercury chloride) and after treatment with sodium thiosulphate and cupric bromide the alkylmercurials were extracted into benzene as their bromide derivatives and analysed on an OV-275 glass capillary column. The sensitivity and selectivity of the method enabled determinations to be made on small volumes of blood and tissue homogenates. The method has been applied to a pharmacokinetic study in rats dosed orally with 8 mg/kg mercury as methylmercury chloride or ethylmercury chloride.

INTRODUCTION

The analysis of medical reports of patients in the two ethylmercury [1, 2] and the very much larger-scale methylmercury poisoning epidemics [3] have indicated that both alkylmercurials can cause severe damage in the sensory part of the nervous system but only ethylmercury can cause renal damage. The difference in the renotoxicity of the two alkylmercurials has been attributed to the faster renal accumulation and/or decomposition of ethylmercury [4, 5] but there may also be differences in their kinetic behaviour.

To study the kinetics of these two alkylmercurials in rats a method of analysis was required which would allow methylmercury and ethylmercury to be determined at low concentration levels in small blood and tissue samples.

Numerous methods have been described in the literature for the determina-

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tion of organic mercury compounds in environmental and biological samples using the technique of gas chromatography (GC) with packed columns and electron-capture detection [6—12]. However, organic mercury halides exhibit poor GC properties on packed columns, and adsorption and decomposition of the sample can readily occur particularly at low concentration levels [13]. Dumarey et al. [14] have shown that these chromatographic problems can be overcome by the use of a capillary column.

In the assay described in this paper we have successfully used high-resolution capillary GC with electron-capture detection to determine methyl- and ethylmercury in rat blood, brain, liver and kidney.

EXPERIMENTAL

Standards and reagents

Methylmercuric chloride and ethylmercuric chloride were purchased from Pierce and Warriner U.K. (Chester, U.K.). Stock standard solutions of these reference compounds prepared in 0.05 *M* sodium carbonate at a concentration of 500 μg/ml were stored in the dark at 0°C. Working standards were prepared daily in 0.05 *M* sodium carbonate at concentrations of 1.25 and 0.25 μg/ml. Radiolabelled methylmercuric chloride ($CH_3{}^{203}HgCl$), obtained from Amersham International (Amersham, U.K.), was dissolved in saline and also stored in the dark at 0°C until used.

All reagents, unless otherwise stated, were obtained from BDH (Poole, U.K.) and were of laboratory-reagent grade. Benzene was of chromatography grade. Solutions of 0.01 *M* sodium thiosulphate and 0.5 *M* cupric bromide were extracted twice with equal volumes of benzene before use to remove potential interfering electron-capture contaminants.

Animal studies

Male and female LAC Porton derived Wistar rats (initial body weight 180—213 g; 8—10 weeks old) were dosed with either methylmercuric chloride or ethylmercuric chloride in a volume of 2.0 ml/kg glycerol formal (Fluorochem, Glossop, U.K.) by gastric gavage. Animals were maintained on MRC 41B diet during the experiments with food and water available ad libitum. Blood samples collected in heparinized tubes after tail-vein cannulation or decapitation were diluted from 1:100 up to 1:500 with distilled water. Tissues were weighed and then homogenized in distilled water with an Ultra-Turrax homogenizer. Brain homogenates were diluted 1:25 or 1:50 and both liver and kidney 1:500. All samples were then stored at −20°C prior to analysis.

Sample preparation

To a 200-μl aliquot of diluted rat blood or tissue homogenate contained in a Dreyer tube (60 × 7 mm, Gallenkamp, London, U.K.) 20 μl of the internal standard solution (1.25 μg/ml) were added. The sample was vortex-mixed for 30 s with 50 μl added 0.01 *M* sodium thiosulphate and again with 200 μl added benzene. After centrifugation for 2 min at 15 600 *g* using an Eppendorf high-speed centrifuge, the benzene layer was removed and discarded. To the aqueous layer 50 μl of 0.5 *M* cupric bromide were added and mixed for 30 s.

The solution was then vortex-mixed with 200 μl benzene and centrifuged at 15 600 *g* for 3 min. The benzene layer was finally transferred with a Pasteur pipette into a 1-ml Wheaton V mini-vial for analysis by GC.

Gas chromatography

The gas chromatograph used in this work was a Carlo Erba Fractovap 4160 equipped with a ^{63}Ni electron-capture detector. Analyses were carried out on a 20 m × 0.3 mm glass capillary coated with the polar silicone phase OV-275 (film thickness 0.25 μm). Prior to static coating with the stationary phase the Pyrex glass capillary was leached with 18% hydrochloric acid according to the procedure of Grob and Muller [15] and the walls of the tubing then coated with sodium chloride particles as described by De Nijs et al. [16]. The capillary column had an efficiency of 2100 theoretical plates per metre. Samples were introduced by cold on-column injection into a 1 m × 0.32 mm deactivated fused-silica capillary which was attached to the column by means of a polyimide seal [17]. Helium was used as carrier gas with a linear flow-rate of 3 ml/min. The column was maintained at 80° C for 1 min, then programmed at 40° C/min to 190° C. The electron-capture detector was operated at 300° C in a constant-current mode with a pulse width of 5 μs. Nitrogen was employed as purge gas at a flow-rate of 40 ml/min.

Quantitation of the alkylmercury was made by reference to a standard calibration curve constructed with each batch of samples analysed. The standard curve for the determination of methylmercury was obtained from the analysis of 200-μl aliquots of blank diluted rat blood or tissue homogenate to which had been added 25 ng of the internal standard ethylmercury chloride and various amounts of methylmercury chloride (12.5—50 ng). Peak-height ratio of methylmercury to internal standard was plotted against concentration of methylmercury chloride added. Similar calibration curves were established for the determination of ethylmercury over the same concentration range and using 25 ng added methylmercury chloride as the internal standard.

RESULTS AND DISCUSSION

Attempts were initially made to analyse methyl- and ethylmercurials using a fused-silica capillary column coated with the non-polar stationary phase OV-1 or SE-52 or with the medium-polar phase OV-1701 or Carbowax 20M. Each of these columns exhibited high efficiencies and good peak shapes for the alkylmercury halides when the amounts injected were greater than 10 ng. However, at lower amounts the efficiency and peak shapes deteriorated and column adsorption occurred. Priming the column with repeated injections of microgram amounts of the compounds improved the chromatography when picogram amounts were subsequently injected but the beneficial effects of this treatment were only temporary and the precision and accuracy of the estimation for biological samples was still unsatisfactory,

The glass capillary column coated with the polar cyanophenylsilicone phase OV-275 used in this study allowed the organomercurials to be analysed at the picogram level with good efficiency and with no evidence of absorption or degradation of the sample. Priming the column prior to the analyses was also found to be unnecessary.

The fused-silica capillary which provided the connection between the glass analytical column and the injector of the gas chromatograph not only protected the main column from damage due to contamination or solvent overload, but also acted as a retention gap preventing the broadening, distortion and splitting of peaks which can occur with cold on-column injection [18]. Of the three possible methods of introducing liquid samples into the capillary column, split, splitless and on-column, only the latter method gave the necessary accuracy and precision of estimation.

The extraction procedure used was based on the method described by Cappon and Smith [11]. The alkali digestion of the biological sample and the treatment of the sample matrix with urea, copper sulfate and hydrochloric acid as employed by these authors to aid the initial extraction of the alkylmercury chlorides into benzene was found to be an unnecessary step when analysing blood and tissue samples containing microgram per gram amounts of the alkylmercurials. Sodium thiosulphate was added directly to the diluted biological sample and this served to complex the organic mercury [19] retaining it in the aqueous phase while allowing many of the electron-capturing endogenous components to be removed by benzene extraction. The addition of cupric ions in the form of cupric bromide liberated the methyl- and ethylmercury from their thiosulphate complexes as the corresponding alkylmercury bromides which were then extracted into benzene.

The overall recovery of methylmercury from the blood and tissue was determined by spiking blank rat blood and brain homogenates with $CH_3{}^{203}HgCl$ before dilution. The recovery of the labelled organic mercury in the final benzene extract was from blood 93.06 ± 2.82% (n = 6) and from brain 96.55 ± 2.11% (n = 6).

A representative chromatogram from the analysis of an extract of blood from a rat dosed with methylmercury chloride is illustrated in Fig. 1. The

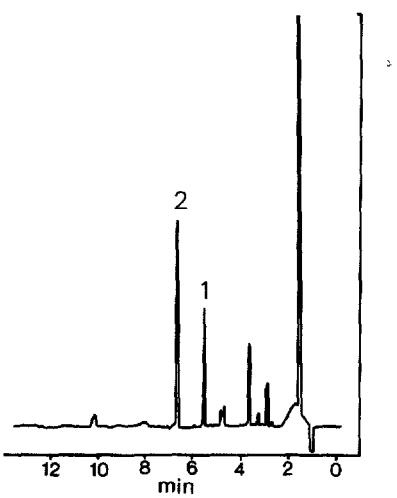

Fig. 1. Chromatogram from the analysis of a blood sample for methylmercury from a rat 6 h after dosing with 8.0 mg/kg mercury as methylmercury chloride. The ethylmercury peak is derived from the ethylmercury chloride added to the sample as internal standard. For GC conditions see text. Peaks: 1 = methylmercury; 2 = ethylmercury.

TABLE I

RECOVERY OF METHYLMERCURY AND ETHYLMERCURY IN SPIKED BLOOD AND BRAIN SAMPLES

In all cases, $n = 6$.

Compound	Concentration added (ng/ml)	Concentration found (mean ± S.D.) (ng/ml)	Recovery (mean ± S.D.) (%)
Blood			
Methylmercury chloride	20	19.48 ± 1.09	97.38 ± 5.44
	50	51.50 ± 1.05	103.00 ± 2.10
Ethylmercury chloride	20	21.11 ± 0.78	105.55 ± 3.92
	50	49.57 ± 1.26	99.14 ± 2.51
Brain			
Methylmercury chloride	20	19.58 ± 0.80	97.92 ± 4.01
	50	52.58 ± 0.86	105.17 ± 1.72
Ethylmercury chloride	20	20.79 ± 0.80	104.01 ± 4.03
	50	51.89 ± 1.19	103.88 ± 2.15

retention times of methylmercury bromide and ethylmercury bromide were 5.5 and 6.6 min, respectively. Similar tracings were obtained from tissue samples for the analysis of methylmercury and from blood and tissue samples for the analysis of ethylmercury. No endogenous or background peaks were observed in blank rat blood or tissue extracts which interfered with the analyses of either alkylmercurial. Goolvard and Smith [12] used ethylmercury chloride as an internal standard and that methylmercury chloride can be used as an internal with electron-capture detection. We have also found this to be a suitable internal stanard and that methylmercury chloride can be used as an internal standard for the analysis of ethylmercury. Calibration curves for methylmercury and ethylmercury passed through the origin and were linear up to 200 ng/ml of the diluted sample. The curves typically had correlation coefficients of 0.995.

The accuracy and precision of the method were determined from replicate recovery experiments of methylmercury chloride and ethylmercury chloride added to the blood and brain homogenates from untreated rats. The calculated recoveries from six replicate analyses at concentration levels of 20 and 50 ng/ml of diluted sample are presented in Table I.

Serial blood and brain samples were analysed immediately after collection and then after storage for four days at 0°C. No significant differences in the results were obtained for either methylmercury or ethylmercury estimations. Samples containing ethylmercury were, however, found to be unstable when kept at room temperature for more than one day.

A number of blood and homogenised brain samples were analysed for total and inorganic mercury by the selective atomic absorption method of Magos [20] modified to prevent decomposition of ethylmercury [5]. The organic mercury estimated as the difference between the total and inorganic results was then compared with the organic mercury levels as determined by the described GC method. Fig. 2 shows such a comparison of ethylmercury

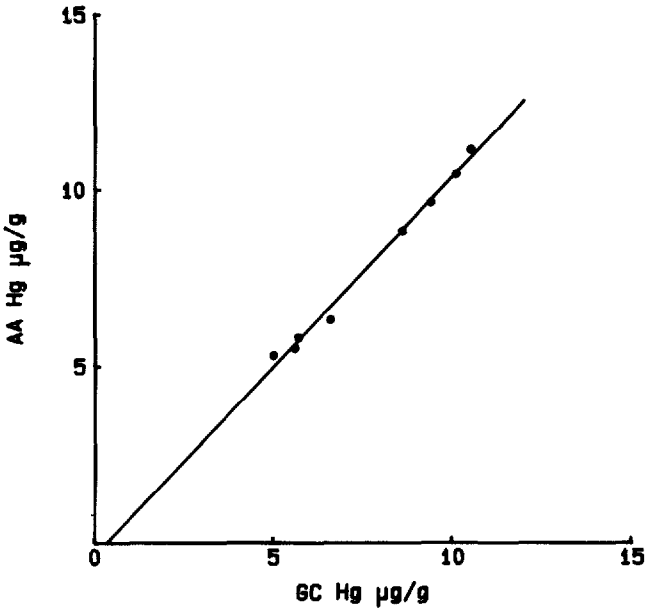

Fig. 2. Comparison of analytical results obtained by estimating ethylmercury in rat brain by atomic absorption (AA) and by GC with electron-capture detection. The results are expressed in μg/g mercury.

estimates in brain samples from rats dosed with ethylmercury chloride. The regression line was $y = 1.0720x - 0.39$ and the correlation coefficient 0.996. Similar correlations were obtained from comparative estimates of ethylmercury in blood and methylmercury in blood and brain samples.

Application

Groups of four rats were given a single oral dose of 8 mg/kg mercury as methylmercury chloride or ethylmercury chloride and 10-μl blood samples were taken from a tail vein by cannula 30 min after dosage and at hourly intervals up to 6 h. Further groups of four rats were also given the same oral dose of the alkylmercury chlorides and at 12, 24, 48, 72, 96 and 120 h after dosage were killed; blood, brain, liver and kidney samples were collected. The mean blood concentration—time profiles for the two alkylmercurials are shown in Fig. 3.

In conclusion the described method allows for the sensitive and reliable quantitation of the organic mercurials in small volumes of rat blood and tissue homogenates with good precision and accuracy and with minimal amount of sample work-up. The use of a capillary column overcomes many of the problems previously encountered with the GC estimation of alkylmercurials using packed columns, namely adsorption and decomposition of the sample, and poor column efficiency with peak tailing.

The method was confirmed to be applicable for assessing the pharmacokinetic parameters of methylmercury and ethylmercury in the rat. The results of this study will be the subject of a future publication.

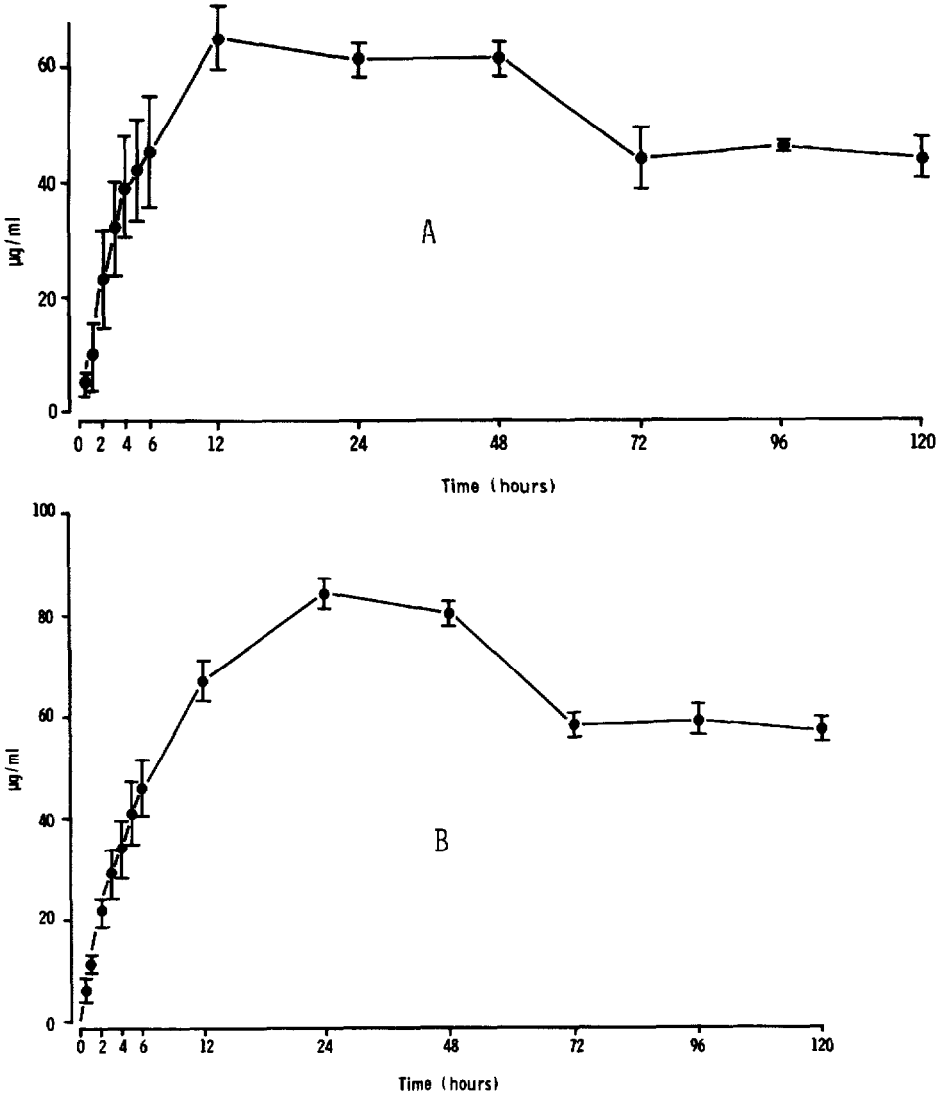

Fig. 3. Blood concentration—time profiles for methylmercury chloride (A) and ethylmercury chloride (B) in rats given a single oral dose of the alkylmercurials. Each point is the mean ± S.E.M. of the estimations from four animals.

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