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GAS CHROMATOGRAPHIC DETERMINATION OF TRACE AMOUNTS OF INORGANIC MERCURY

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SUMMARY

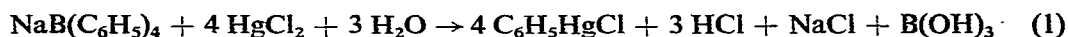
A procedure for the gas chromatographic (GC) determination of trace amounts of inorganic mercury is described. Hg^{2+} is converted into diphenylmercury (DPM) with tetraphenylborate and the DPM is extracted with toluene. The derivatization yield was measured radiochemically and by GC and was found to be between 95 and 100% for amounts of Hg^{2+} in the range 50 ng–10 μg , and was not affected by K^+ and Cl^- ions or matrix effects due to decomposed tissue. At the nanogram level, derivatization at a low pH is advisable, which may be restricted by the instability of the reagent in acidic medium.

DPM was determined by GC on specially pre-treated columns with OV-17 or ethylene glycol succinate as the liquid phase. The detection limit was 45 pg of Hg^{2+} (absolute) using a tritium electron-capture detector.

INTRODUCTION

Owing to its selectivity, gas chromatography (GC) has become a very valuable technique during the past 15 years, even in inorganic trace analysis. Usually, inorganic ions must be derivatized to organic compounds so as to be sufficiently volatile for GC separation and to be sensitively detectable by a common GC detector.

For mercury, some derivatization procedures have already been developed and tested at the microgram and nanogram levels^{1-4,11}. Phenylation of mercury(II) chloride or nitrate with sodium tetraphenylborate proved to be the easiest and most elegant route to a derivative with the required properties⁵. Early applications of this reaction in the analysis of organoboron compounds were reported by Wittig *et al.*⁶. On the gram scale, with a five-fold excess of mercury(II) chloride, phenylmercury(II) chloride (PMC) is formed according to the reaction



If, however, reaction 1 is used to derivatize mercury(II) chloride at the microgram level, an excess of tetraphenylborate is required. In this case, PMC is analogously

phenylated in a second step to diphenylmercury (DPM). If the stoichiometric excess of the reagent,

$$\lambda = \frac{4 m_{\text{NaB}\phi_4}}{m_{\text{HgCl}_2}}$$

is greater than 4, only DPM is found as the reaction product. The reaction consequently proceeds as follows:



Corresponding results have been obtained with mercury(II) nitrate as substrate.

DPM can be extracted quantitatively from aqueous media with toluene, and can be determined by reaction GC using chlorosilanized quartz-wool as a "reaction zone"⁷. In this paper, the development and application of this procedure for the GC determination of nanogram amounts of Hg^{2+} is described. The recovery of the derivatization/extraction steps and its dependence on the amount of Hg^{2+} , on the excess of tetraphenylborate and on the concentration of possible interfering ions such as Cl^- , K^+ and H^+ , were investigated radiochemically using $^{203}\text{HgCl}_2$ as the source material. Radioactivity was determined by liquid scintillation counting (LSC). Under optimized conditions and in decomposition solutions the recoveries were measured by GC.

EXPERIMENTAL

Apparatus

GC column preparation. The GC of organomercury compounds is difficult as it is influenced by adsorption and reaction effects on the columns. This necessitates very accurate pre-treatment of the carrier and columns, including the following steps:

(1) Drying the carrier at 100° in a stream of dry nitrogen (in a fluidized bed, if possible).

(2) Silanization of carrier and columns. Only Kieselguhr carriers previously silanized can be used. It is advisable additionally to silanize these carriers with a 20% solution of dimethyl- or diphenyldichlorosilane in benzene. The glass column is also filled with this solution. After standing overnight, the materials are washed repeatedly with acetone.

(3) Impregnating the carrier.

(4) Sieving the impregnated carrier to re-adjust the original grain-size distribution.

(5) Filling and conditioning the column and plugging the ends of the column with chlorosilanized quartz-wool. Before starting the measurements, DPM must be injected repeatedly until the peak sizes remain constant.

This procedure introduces a certain amount of Si-Cl groups into the GC system. Earlier investigations⁷ indicated that DPM is converted into PMC by reaction with these groups. This reaction is an important condition for the successful GC analysis of DPM.

Gas chromatographic conditions. A Packard Series 7700 gas chromatograph

with a tritium electron-capture detector (ECD) was used. The carrier gas was nitrogen at a flow-rate of 70–100 ml·min⁻¹. The columns contained 3% OV-17 silicone oil (at 200°) or 3% ethyleneglycol succinate (EGS) (at 190°), both on Gas-Chrom Q.

Liquid scintillation counting. A Packard Tri Carb 3375 liquid scintillation spectrometer was used at 4.2% gain and a channel width of 12–240 keV. The scintillators were (1) 3.92 g of diphenyloxazole (PPO) + 0.08 g of *p*-bis-(*o*-methylstyryl)-benzene (bis-MSB) in 1000 ml of toluene and (2) Unisolve (Koch-Light, Coinbrook, Great Britain).

Radiochemicals

The stock solution for the radiochemical experiments contained ²⁰³HgCl₂ with an initial specific molar activity of 4.67·10¹² sec⁻¹ mole⁻¹ (0.126 kCi·mole⁻¹) (Amersham-Buchler, Braunschweig, G.F.R.). For the tracer experiments this solution was diluted 1:1000 with 0.1 *N* hydrochloric acid and a few drops of chlorine water were added so as to prevent the formation of mercury(I) chloride. Its mercury concentration was 8.42 μg·ml⁻¹.

For calibration purposes, a toluene solution of labelled DPM was made by phenylating a known amount of ²⁰³HgCl₂, extracting the DPM with toluene and measuring the remainder in the aqueous phase against another known amount of ²⁰³HgCl₂. The labelled DPM concentration was 12.20 μg·ml⁻¹.

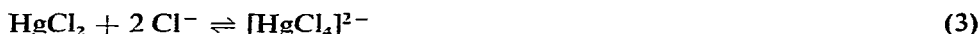
Standard solutions

Mercury(II) chloride. A 76.6-mg amount of mercury(II) chloride (p.a.) was dissolved in double-distilled water in a 100-ml flask; 1 ml of this solution corresponded to 1 mg of DPM.

Sodium tetraphenylborate. A 718-mg amount of sodium tetraphenylborate was dissolved in double-distilled water in a 10-ml flask; 1 ml of this solution corresponded to 10⁴ ml of the ²⁰³HgCl₂ tracer solution as above in the sense of eqn. 2. This solution was diluted four times in succession in the ratio 1:10.

K⁺. A 212-mg amount of potassium nitrate (p.a.) was dissolved in double-distilled water in a 10-ml flask; 1 ml of this solution was equivalent to 1 ml of the above sodium tetraphenylborate solution. This solution was diluted four times in succession in the ratio 1:10.

Cl⁻. A 491-mg amount of sodium chloride (p.a.) was dissolved in double-distilled water in a 10-ml flask; 1 ml of this solution was equivalent to 10⁴ ml of the above ²⁰³HgCl₂ tracer solution in the sense of the equation



This solution was diluted four times in succession in the ratio 1:10.

Decomposition solution

To investigate the matrix effect of ions other than the above present in solutions of decomposed tissue material, a mercury-free decomposition solution was prepared by dry-ashing 30 g of liver, evaporating the combustion residue with concentrated nitric acid and diluting to 100 ml with water.

Recovery with pure solutions

General procedure for radiochemical measurements. The solutions of which the compositions are described below were shaken for 5 min with 1.00 ml of toluene. Unless otherwise stated, the solutions were neutral. The organic layer was removed and dried over sodium sulphate. Aliquots of 0.50 ml of this solution were mixed in a counting vial with 10 ml of PPO-bis-MSB scintillator and measured by LSC. Normally the scintillation counter was calibrated with 0.50 ml of labelled DPM solution as above.

Dependence on amount of mercury. Amounts of $^{203}\text{Hg}^{2+}$ between 10.5 and 0.17 μg and a 10^4 -fold stoichiometric excess of tetraphenylborate each were processed following the general procedure.

Dependence on stoichiometric excess of tetraphenylborate. Amounts of 4.21 μg of $^{203}\text{Hg}^{2+}$ were each derivatized with increasing amounts of sodium tetraphenylborate, the molar ratio $2 m_{\text{NaB}\phi_4} : m_{\text{Hg}^{2+}}$ ranging from 10^0 to 10^4 in decadic steps. The above procedure was then carried out.

Influence of K^+ ions. To 8.42 μg of $^{203}\text{Hg}^{2+}$ and 72 mg of sodium tetraphenylborate were added increasing amounts of potassium nitrate, the molar ratio $m_{\text{K}^+} : m_{\text{B}\phi_4^-}$ ranging from 10^{-4} to 10^0 in decadic steps. The above procedure was then carried out.

Influence of Cl^- ions. To 8.42 μg of $^{203}\text{Hg}^{2+}$ and 40 mg of sodium tetraphenylborate were added increasing amounts of sodium chloride, the molar ratio $m_{\text{Cl}^-} : 2m_{\text{Hg}^{2+}}$ ranging from 10^0 to 10^4 in decadic steps. The above procedure was then carried out.

Dependence on pH. The pH values of solutions containing 8.42 μg of $^{203}\text{Hg}^{2+}$ and 72 mg of sodium tetraphenylborate were adjusted to 1.0, 2.0, 3.2, 5.1, 7.0, 7.65 and 8.0 with hydrochloric acid and/or sodium acetate using a glass electrode. The above procedure was then carried out.

Dependence on time. Five batches of 0.85 μg of Hg^{2+} in 0.1 *N* hydrochloric acid and 10 mg of sodium tetraphenylborate were shaken with 1.00 ml of toluene by hand or on a shaking machine for 15, 30, 60, 120 and 300 sec.

Recovery determined by gas chromatography

The results of the above experiments, which are discussed below, showed that the best recoveries were obtained under the following conditions: (1) mercury(II) chloride is made to react with tetraphenylborate in a 0.1 *N* acidic medium; and (2) the mixture is extracted with toluene by shaking for 30 sec.

It was necessary to verify these results by GC. Amounts of mercury(II) chloride between 0.766 and 38.3 μg (\equiv 0.566–28.3 μg of Hg^{2+}) were processed following the above procedure. The blank value was recorded using 1 ml of 0.1 *N* hydrochloric acid. The extracts were dried over sodium sulphate and gas chromatographed on a 3% OV-17 column at 190°. Finally, the results were checked with mercury(II) nitrate instead of mercury(II) chloride as the substrate: amounts of mercury(II) nitrate between 0.326 and 16.25 μg (\equiv 0.20–10.0 μg of Hg^{2+}) in 0.1 *N* nitric acid were made to react in the same way, the blank value being recorded using 1 ml of 0.1 *N* nitric acid.

Recovery in solutions of decomposed tissue

As interferences due to ions present in solutions of decomposed biological material cannot be predicted completely or studied separately, recoveries were also measured in a decomposition solution as described above. In 5-ml aliquots of this solution (corresponding to 1.5 g of liver), the acid content was partially neutralized to pH 1-2 with sodium carbonate that had been demercurized by heating at 500° overnight. Amounts of mercury between 57 ng and 5.66 μg and 1 ml of a 0.01% solution of sodium tetraphenylborate were added to each batch, followed by extraction with 1.00 ml of toluene by shaking manually for 1 min. The extracts were dried and chromatographed on an EGS or OV-17 column.

Statistical analysis

The results were analyzed statistically for homogeneity in accordance with the Nalimov test⁸. The correlation was tested by calculating the correlation coefficient (r) and applying the null hypothesis to it^{9,10}. Whenever groups of data were to be combined, their homogeneity was tested by the χ^2 -test and analysis of variance. In the event of non-homogeneity, the "wild" result was eliminated by means of the Duncan test. Mean values were compared by means of the t -test.

RESULTS AND DISCUSSION

Gas chromatography

A typical gas chromatogram is shown in Fig. 1. On an accurately made column as specified above, a detection limit (peak height = $3 \times$ noise) of 45 pg of Hg^{2+} (absolute) as DPM was obtained. The calibration graph is linear between 0.1 and 5.0 ng of DPM (Fig. 2). With larger amounts it is concave to the abscissa owing to overloading of the detector and column. The detector sensitivity is $1.2 \text{ C}\cdot\text{g}^{-1}$ in this range.

Derivatization in pure solutions

The mean values and standard deviations ($\bar{x}_i \pm s_i$) of the homogeneous data of the radiochemical measurements are given in Table I, together with the correlation coefficients (r_i). All r_i values are not significantly different from zero, which means that effects of the possible influences hitherto investigated are not likely to occur within the ranges considered. Only the parameter "time" had an effect, insofar as the recoveries remained constant only after 30-sec shaking at pH 1. It is particularly surprising that the potassium ions exert no effect; no difference was observed between a reaction with water-soluble sodium tetraphenylborate and a reaction with insoluble potassium tetraphenylborate.

The standard deviations in Table I are homogeneous, whereas the variation between the groups is larger than the variation within the groups. The Duncan test eliminates the value $104.3 \pm 2.4\%$, which is evidently a calibration error, and the data are then homogeneous. By combining the recoveries the result is $\bar{x} \pm s = 95.4 \pm 3.8\%$; $n = 26$.

Although statistical analysis revealed no pH dependence of the derivatization yield, adsorption losses were frequently encountered in neutral media, especially when working with amounts of mercury of less than 1 μg . This effect was not evident

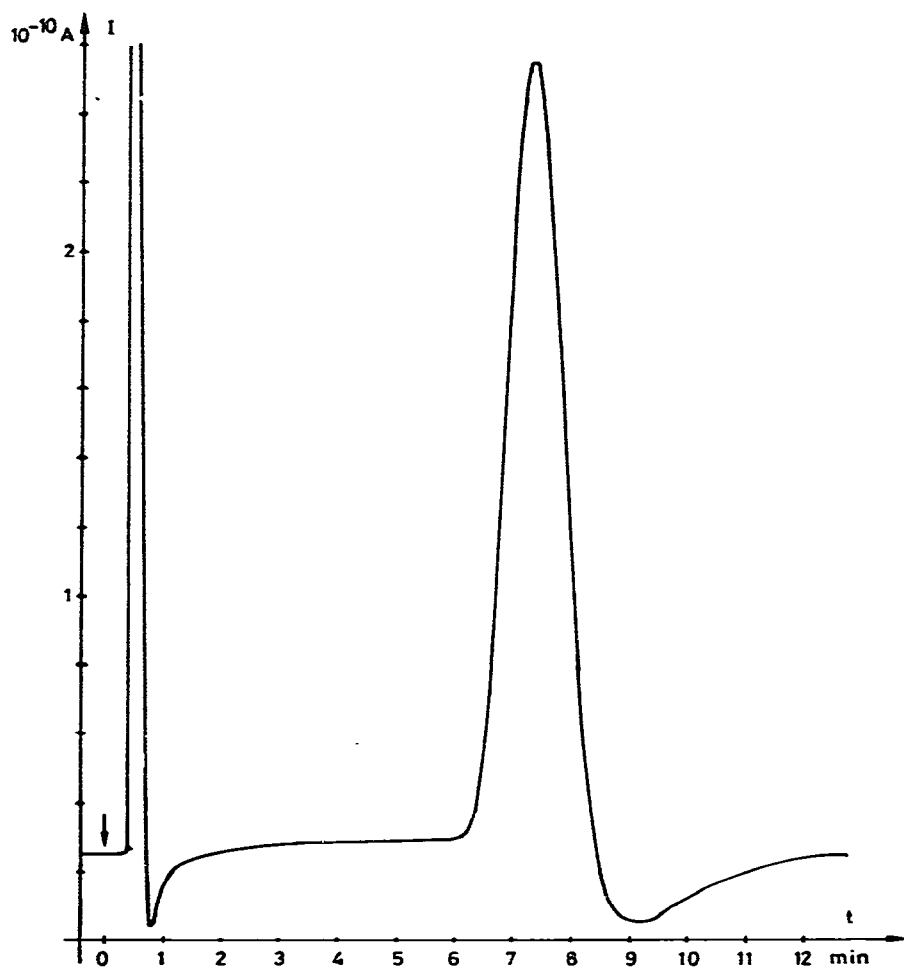


Fig. 1. Gas chromatogram of 10 ng of diphenylmercury. Column, 3% EGS on Gas-Chrom Q; column temperature, 185°; carrier gas, nitrogen; flow-rate, 65 ml·min⁻¹; detector, tritium ECD at 150 V; volume injected, 1 μl (toluene).

TABLE I
DEPENDENCE OF RECOVERY ON DIFFERENT PARAMETERS

Parameter	$m_{Hg^{2+}}$	$\frac{2 m_{NaB\Phi_4}}{m_{Hg^{2+}}}$	$\frac{m_{K^+}}{m_{B\Phi_4^-}}$	$\frac{m_{Cl^-}}{2 m_{Hg^{2+}}}$	pH
Range	10.5–0.17 μg	10 ⁰ –10 ⁴	10 ⁻⁴ –10 ⁻¹	10 ⁰ –10 ⁴	1.0–8.0
Recovery, $\bar{x}_i \pm s_i$ (%)	95.3 ± 3.3	96.9 ± 0.6	97.9 ± 2.4	104.3 ± 2.4	93.3 ± 4.9
Number of data	9	4	4	5	9
Correlation coefficient (r_i)	-0.12	0.75	-0.09	0.13	-0.35

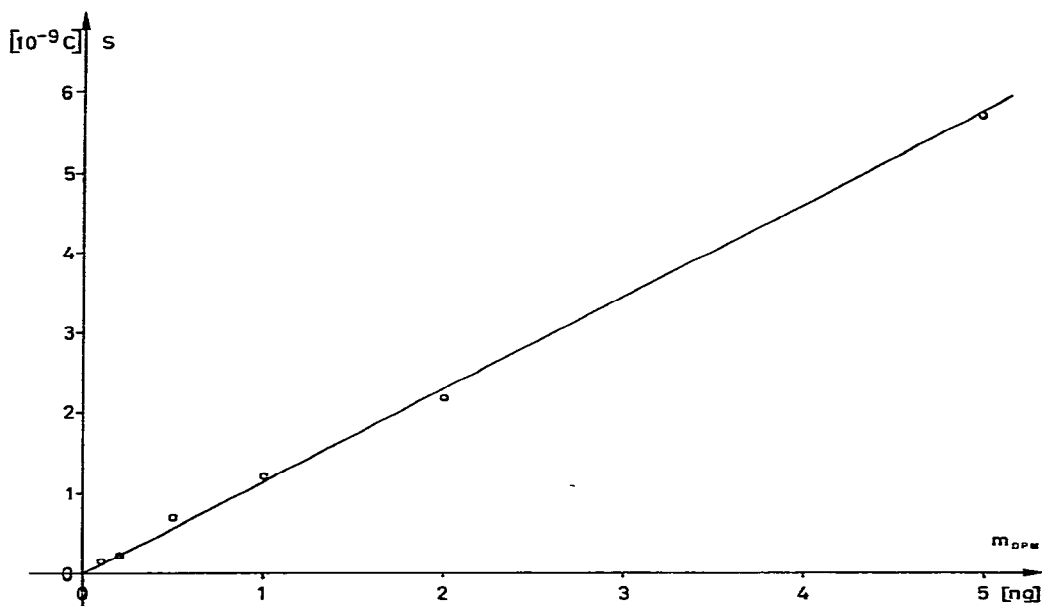


Fig. 2. Calibration graph for diphenylmercury. Column, 3% EGS on Gas-Chrom Q; column temperature, 185°; carrier gas, nitrogen; flow-rate, 65 ml·min⁻¹; detector, tritium ECD at 50 V; volume injected, 1 μ l (toluene).

at pH 1 even at the nanogram level, so that the use of the lowest possible pH is recommended. This is restricted by the instability of tetraphenylborate in acidic media, which is discussed below in more detail. On determining the recovery by GC under the optimal conditions we obtained $\bar{x} \pm s = 97.1 \pm 1.5\%$; $n = 5$; $r = 0.59$, when mercury(II) chloride was used as the starting material. The value $s = 3.8\%$ is different from $s = 1.5\%$ at a level of $P = 95\%$. The mean values of 97.1 and 95.4% are not significantly different. [With mercury(II) nitrate as the starting material the result was $\bar{x} \pm s = 104.5 \pm 6.7\%$; $n = 3$; $r = -0.33$.] Hence GC corroborated the results obtained by LSC.

Simulation of matrix effect

In decomposition solutions, the yields were determined down to the detection limit of the method and the results are given in Table II. Their distribution is random

TABLE II
RECOVERY IN SOLUTIONS OF DECOMPOSED TISSUE

Parameter	Value							
m_{HgCl_2} (μ g)	5.66	2.83	1.13	0.566	0.283	0.113	0.057	
Recovery, $\bar{x}_t \pm s_t$ (%)	98.6 \pm 2.9	98.8 \pm 5.1	96.4 \pm 5.0	102.2 \pm 3.7	97.1 \pm 3.8	101.1 \pm 5.1	103.0 \pm 8.2	
Number of data	3	4	5	8	4	4	8	

and homogeneous. The common mean and the mean standard deviation are $\bar{x} \pm s = 100.2 \pm 5.6\%$; $n = 36$; $r = -0.33$. Evidently the matrix effect has no adverse influence.

The blank value problem

All of the glass vessels used were cleaned with chromosulfuric acid or by heating with concentrated nitric acid. All of the chemicals were of analytical-reagent grade. Thus no blank value originating from any mercury residue was observed. However, because of the instability of tetraphenylborate in acidic media, a number of foreign peaks occurred in the gas chromatograms. On some of the columns used, DPM (*i.e.*, PMC) could not be separated satisfactorily from these peaks, which caused problems as the interfering peaks simulated up to $0.2 \mu\text{g}$ of Hg^{2+} per millilitre of toluene. If a more appropriate column is not available, this apparent blank value can be avoided empirically by taking the following into account: (a) during derivatization the pH should not be substantially lower than 2; (b) the shaking time should not exceed 1 min; after this time the organic layer should be removed; (c) 1 ml of 0.01 % sodium tetraphenylborate solution will suffice in every instance; the solution should be freshly prepared. It is advisable to check these possible interferences prior to starting an analysis.

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