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PERHALOBENZENESULFINATES AS REAGENTS IN THE DETERMINATION OF INORGANIC MERCURY IN VARIOUS MEDIA BY GAS–LIQUID CHROMATOGRAPHY

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SUMMARY

Inorganic mercury and organomercurials present in water, urine and serum are evaluated by gas–liquid chromatography and the use of perhalobenzenesulfinates which react with inorganic mercury to yield a perhalophenyl mercury. Best results are obtained with lithium pentafluorobenzenesulfinate as arylating agent for inorganic mercury. Recovery and precision (percent S.D.) data include: water, 70.5% (6.8); urine, 81.4 (10.5); serum, 51.0 (9.4). Lower detection limit of inorganic mercury, as the pentafluorophenyl analyte, is 20 ng of mercury per milliliter of sample. Optimal chromatographic results were obtained with 10% Dexsil-300 on Anakrom SD, 70–80 mesh, and Durapak Carbowax 400 (low Kováts index) on Porasil F.

INTRODUCTION

Presently, the necessity of evaluating the presence of mercury in all its chemical forms in various biological media is a dilemma facing workers in the area of mercurial toxicology and metabolism. Flameless atomic absorption (FAA) spectrometry, as commonly used, measures total mercury[1] while gas–liquid chromatographic (GLC) techniques pioneered by Westöö[2,3] furnish levels of organomercurials. Evaluation of inorganic mercury is usually taken as the difference between total mercury determined via FAA and organomercurials by GLC analysis. Problems arise when levels by difference of an element are procured by disparate techniques, especially in the case of mercury. Recently, Magos and Clarkson[4] described FAA techniques for the determination of both total and organic mercury. However, this procedure does not permit determination of the nature of the organomercurial.

As part of a study involving development of GLC techniques for quantitative evaluation of the inorganic and organic mercury content of biological samples, we have extensively investigated the utility of the Peters reaction[5] in inorganic mercury analysis, whereby inorganic mercury reacts with an arene sulfinate to yield an aryl

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mercurial via the intermediacy of an arylsulfonyl mercury:

$$Ar\text{–}SO_2M + Hg^{2+} \rightarrow [Ar\text{–}SO_2\text{–}Hg]^{+} \rightarrow SO_2 + Ar\text{–}Hg^{+}$$

Ar = phenyl, tolyl.

M = H, Na, K

In outline, the procedures described in this report involve treatment of a sample containing inorganic mercury(II) with an arene sulfinate under appropriate conditions to form an aryl mercurial. The aryl mercury thus formed, as well as any other organomercurials already present, is then measured by GLC techniques, which are refinements of the Westöö procedure[2–4].

EXPERIMENTAL

Apparatus

A Glowall Model 320 dual-oven gas–liquid chromatograph equipped with an electron capture detector (^{3}H foil) and 18 in. $\times$ $^3/_{16}$ in. glass coil columns were utilized in our studies. Column packings evaluated included 10% Dexsil-300 on Anakrom SD, 70–80 mesh (Analabs, North Haven, Conn.) and Durapak Carbowax 400 (low Kováts index, K') on Porasil F, 80–100 mesh (Waters Ass., Framingham, Mass.). Preparation of the former packing involved the use of a fluidizing apparatus (Applied Science Lab., State College, Pa.). Instrument temperature parameters were: Dexsil-300 packing, column, 200°; flash, 245°; and detector, 210°. Durapak Carbowax 400 packing, column, 195°; flash, 245°; and detector, 210°. Sample agitation for extraction of organomercurials were carried out using a Vortex-Genie mixer.

Analysis vessels for evaluation of inorganic and organic mercury consisted of 1-dram (1.8 g) vials (Fisher Scientific, Pittsburgh, Pa.) with screw caps. Preparation of vials for use consists of an initial soap and water wash, thorough rinsing with deionized water and immersion (24 h) in 50% analytical grade nitric acid. Six successive washings with deionized water are then carried out. The screw caps are prepared for first-time use by manual removal of the cap liner and adhesive, immersion in xylene (24 h), and soap and water rinse. Further treatment consists of soaking in a saturated EDTA salt solution. The caps are vulnerable to corrosion by nitric and other acids and can therefore only be used after treatment with the chelating solution to remove metal contaminants.

Reagents

For procurement of GLC data, authentic samples of pentachloro- and pentafluorophenylmercuric chloride, bis[pentafluorophenylmercury(II)] and its perchlorinated analog were synthesized by published procedures[6,7]. Lithium salts of pentafluorobenzene- and pentachlorobenzenesulfinic acid were synthesized as follows.

Lithium pentafluorobenzenesulfinate. Pentafluorophenyl lithium[8] 0.02 moles, in 100 ml of anhydrous ethyl ether was prepared under nitrogen at −78° using 5.8 g (0.02 moles) of iodopentafluorobenzene (Peninsular Chem Research, Inc., Gainesville, Fla., U.S.A.) and a solution of 0.02 moles of *n*-butyl lithium (Alfa Inorganics, Inc., Beverly, Mass., U.S.A.) in 8.5 ml of *n*-hexane. Anhydrous sulfur dioxide (Matheson, Coleman & Bell, East Rutherford, N.J.) was then bubbled through the rapidly stirred solution at −78° causing the immediate formation of a white precipitate. Sulfur dioxide addition at this temperature was carried out during 20 min whereupon the

vessel was allowed to come to room temperature over a 1-h period while sulfur dioxide bubbling continued at a reduced rate. The mixture was filtered, the collected material washed three times with ethyl ether (50 ml) and dried *in vacuo* to produce 4.23 g (99% yield based on iodopentafluorobenzene consumed) of microcrystalline lithium pentafluorobenzenesulfinate. In the IR spectrum of the lithium salt (KBr pellet) bands are seen characteristic of the pentafluorophenyl group[9] at 1645 and 1522 cm^{-1} and of the sulfinyl group in the 1000–1100 cm^{-1} region[10]. Yield calculated for $C_6F_5LiO_2S$:Li, 2.91%; found: 2.99%.

Lithium pentachlorobenzenesulfinate. The procedure of Rausch *et al.*[11] was utilized to prepare pentachlorophenyllithium (8 mmole) in 300 ml of anhydrous ethyl ether under nitrogen at −10°. Via the procedure described for the perfluorinated analog sulfur dioxide was caused to react with the lithium perchloroaryl. Filtration and washing (three times) of the colorless residue with ethyl ether (50 ml) was followed by drying *in vacuo*, yielding 2.09 g (96% yield based on halohydrocarbon consumed) of white microcrystalline salt. IR (KBr pellet): 690 and 872 cm^{-1} (pentachlorophenyl group)[9] and 1000–1100 cm^{-1} (sulfinyl group). Yield calculated for $C_6Cl_5LiO_2S$: Li, 2.10%; found: 1.53%.

All common reagents employed in these investigations were commercially obtained as reagent grade and employed directly.

Methods

GLC analysis of inorganic mercury using lithium pentafluorobenzenesulfinate in water, serum or urine.

Water. Samples of water (1.0 ml) in 1-dram acid-washed vials are made 2 *N* in perchloric acid (0.2 ml of conc. $HClO_4$). Similarly, water samples containing known added trace amounts of mercury were acidified. To the samples is then added a freshly prepared aqueous solution (0.2 ml, 0.4 *M*) of lithium pentafluorobenzenesulfinate. Sealing of the vials using the cleaned screw caps and PTFE tape is followed by heating in a sand-bath at 80° for 30 min. The vessels are cooled, opened and saturated sodium chloride (0.2 ml) is introduced. Extraction of the pentafluorophenylmercury (chloride) from the aqueous medium into chromatographically clean xylene (2.0 ml) is achieved using vigorous agitation with a Vortex-Genie shaker for 1 min at maximum speed. Centrifugation of the vials is done at 3000 rpm (125 *g*) for 5 min. A portion of the xylene layer (1.0 ml) is removed by pipette and back-extracted in a second vial with 1 ml of a 1:1 mixture of saturated sodium chloride and 0.05 *M* Tris buffer, pH 7.6. After centrifugation, as above, a portion of the organic layer is transferred to a third vial containing anhydrous magnesium sulfate (100 mg). After drying, an aliquot of the layer is injected into the gas chromatograph equipped with the 10% Dexsil-300-packed column (see above). Quantitation is carried out in the usual manner by relating peak height of mercury-containing standard sample minus blank value to peak height of unknown.

Serum or urine. To volumes (1.0 ml) of urine samples in 1-dram acid-washed vials is added dilute aqueous perchloric acid (2 *N*, 0.2 ml) and the samples are set aside for 30 min. In a similar manner is treated a urine sample to which a known trace amount of inorganic mercury is added and first allowed to bind with coordinating groups in urine for 10 min. A blank sample containing no or a known small amount of inorganic mercury is also included.

Serum samples (1.0 ml) along with a blank and a vessel containing serum with added mercury are treated with 0.1 ml of concentrated perchloric acid for 30 min.

Vials containing urine samples are treated with 0.2 ml of the freshly prepared aqueous lithium organosulfinate (0.2 *M*) and heated, as with water, for 30 min in a sand-bath maintained at 80°. The serum samples, after addition of 0.2 ml of 0.6 *M* sulfinate reagent are set aside for 3 h at room temperature.

Further work-up of samples from these media follow essentially the procedure described in water analysis.

If necessary, concomitant analysis of organomercurials already present as well as inorganic mercury in the various media may be achieved using the Dexsil-300 and the Carbowax 400 (low *K'*) packings, methyl-, ethyl- and phenylmercury being determined with the latter packing (see Experimental). The temperatures for methyl- and ethylmercury were: column, 140°; injector, 210°; and detector, 190°. Phenylmercury: column, 175°; injector and detector settings as above. In an instrument allowing dual-column capability temperature programming on the Dexsil-300 column is possible.

RESULTS AND DISCUSSION

Synthesis and properties of perhalobenzenesulfinates

The Peters reaction, although not particularly well known, has been an excellent route to the synthesis of monoarylmercury compounds for a number of years. Arylation of inorganic mercury proceeds cleanly to the monoaryl product. Substrates in this reaction, to date, have required extended reaction time and elevated temperatures to achieve acceptable yields of arylmercurials.

Initial consideration of the analytical utility of the Peters reaction in inorganic mercury analysis, whereby the inorganic mercury in a sample is treated with an arenesulfinate and the generated arylmercury isolated and measured by GLC, pointed to the desirability of modifying the nature of the organic moiety in an arenesulfinate of interest such that reaction (arylation) with inorganic mercury would proceed very rapidly and at mild temperature conditions. Furthermore, the resulting mercurial, as well as arenesulfinate reactant, should be sufficiently stable to conditions likely to be necessary in analysis of any medium for inorganic mercury content, particularly high acidity.

In this regard, the perhalogenated analogs of benzenesulfinic acid were desirable candidates as reagents in view of likely reaction mechanisms for the Peters transformation. An added attractive feature of these halogenated derivatives was the known sensitivity of halogen groups to the electron capture detector (ECD), suggesting enhanced detectability of the resulting organomercurial in a gas chromatograph equipped with an ECD.

We have selected a novel route to obtain a high yield, using a simple synthesis of perhaloarene sulfinates (as the lithium salts) whereby mixed perhalobenzenes are converted to the corresponding lithium aryl via transmetalation with butyllithium followed by ring sulfination using sulfur dioxide. The lithium sulfinates precipitate in analytically acceptible purity without further work-up required. These salts are moderately stable when stored in amber bottles and kept dry. In solution, organic sulfinates undergo an oxidation–reduction reaction whereby two units of sulfinate are

converted to one unit of sulfenic and one unit of sulfonic acid[12]. This process is slower for aryl sulfinates and we have found that the perfluorinated sulfinate may be used for several days in solution, while the perchloro analog is stable for about one day.

At room temperature, the lithium salts in neutral or acidic medium rapidly react with one equivalent of inorganic mercury to produce, under evolution of sulfur dioxide, the corresponding perhalophenylmercury in acceptable yield. The mercury products are isolated as their chlorides by agitation of the precipitates with sodium chloride.

The perhalophenylmercury compounds as chlorides were characterized and were identical in all respects to authentic samples of pentafluorophenyl-[6] and pentachlorophenylmercury[7] chloride.

The use of perhalosulfinate reactant in greater than 1:1 stoichiometry relative to mercury led to isolation of mixtures of monoarylmercury and the corresponding bis[pentahalophenylmercury(II)], the latter compounds being characterized by comparison with authentic samples[6,7]. As the relative amount of sulfinate was increased, the amount of bis(mercurial) isolated was increased.

Under circumstances of actual analytical use, where trace levels of inorganic mercury in a given sample would be treated with sulfinates in great excess, it was considered likely that one would encounter the bis(mercurial) as the actual mercury-containing analyte. Furthermore, bis(perhalophenylmercury), unlike other bis(organomercury) compounds, is not cleaved by usual acids[6] and hence would survive any analysis scheme for inorganic mercury necessarily requiring strong acid reagents.

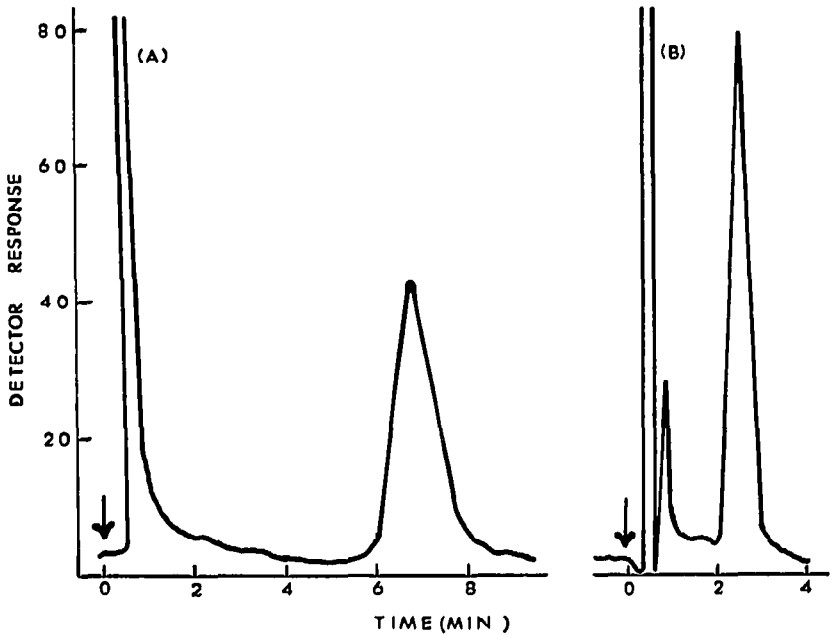
The ring halogen content of the bis (mercurials) of interest in this study, however, still permit detectability in a gas chromatograph equipped with an ECD almost to the degree seen with monoarylmercury halides. Alternatively, one may convert bis[pentahalophenylmercury(II)] to the monoarylmercury halide by cleavage with inorganic mercuric halide.

Analysis of inorganic mercury in various media using perhalobenzenesulfinates

Evaluation of a variety of column packings for use with pentachloro- and pentafluorophenylmercury chloride has been carried out and best results were achieved by the use of Dexsil-300, a new packing phase having a carborane–silicone skeleton, for measurement of pentafluorophenylmercury chloride and bis[pentafluorophenylmercury(II)] while pentachlorophenylmercury chloride was best chromatographed using a packing having a chemically-bonded phase, *e.g.* Durapak Carbowax 400 (low K'). It was not possible to satisfactorily chromatograph bis[pentachlorophenylmercury(II)] using a wide variety of packings.

Solutions of the perhalophenylmercury chlorides in benzene or xylene show with time loss in levels of the monomercurial and formation of the bis(mercurial), arising from a probable bimolecular rearrangement equilibrium in which two units of the former interact to furnish one unit each of mercuric chloride and the bis(mercurial). This process can be effectively retarded by storage of analytical solutions over several crystals of mercuric chloride.

In Fig. 1 are depicted chromatographic data of pentafluorophenylmercury chloride (Fig. 1A) and the bis analog (Fig. 1B) using 10% Dexsil-300 as stationary phase. The monomercurial elutes first from this packing and it should be noted that detector responses for equivalent mercury-containing analytes in the two chemical



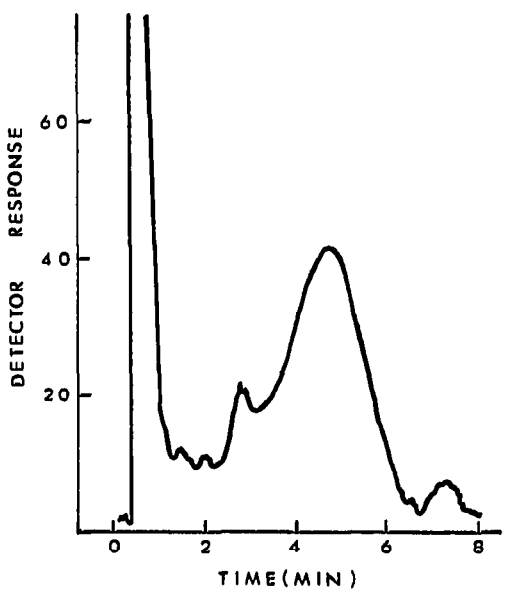


Fig. 1. Chromatograms of (A) standard solution of bis[pentafluorophenylmercury(II)] in benzene and (B) standard solution of pentafluorophenylmercury chloride in benzene. Packing: 10% Dexsil-300 on Anakrom SD, 70/80. Temperatures: column, 200°; injector: 245°; detector, 210°.
Fig. 2. Chromatogram of a standard solution of pentachlorophenylmercury chloride in benzene. Packing: Durapak Carbowax 400 (low K') on Porasil F. Temperatures: column, 195°; injector, 245°; detector, 210°.

forms are not greatly different. Chromatograms obtained with pentachlorophenylmercury chloride are shown in Fig. 2. The perchloro analog gives rather broad peaks even under optimal chromatographic conditions and invariably solutions also show the presence of a contaminant which may arise from slow cleavage of the carbon–mercury bond.

The Westöö procedure[2,3] in its various refinements for GLC evaluation of organomercury, chiefly methylmercury, in a wide variety of media is increasingly being employed for organomercury analysis. This technique, however, calls for the use of relatively large sample sizes and corresponding reagent amounts and glassware size. We have revised the overall assay to employ small sample sizes (1.0 ml), small reagent volumes and correspondingly small vessels consisting of 1-dram vials as well as highly efficient extraction steps possible with such small sampling vessels.

Samples of water, urine or serum are treated with perchloric acid. This treatment is followed by addition of the perhaloarylating reagent to convert inorganic mercury to the corresponding bis[perhalophenylmercury] and subsequent manipulation of the sample for isolation of the bis(mercurial) in a form suitable for GLC analysis.

Studies on the ease of arylation of trace levels of inorganic mercury in strongly acidic media showed that arylation proceeds at a satisfactory recovery yield in the case of lithium pentafluorobenzenesulfinate while reduced yields are obtained with the perchloro analog.

The difficulty in obtaining good chromatograms with the perchlorophenylmercurials as well as a decrease in arylation rate of inorganic mercury in strong acid, a condition necessary in a reasonable procedure for mercury analysis, has led us to carry out most of our analytical studies using the perfluorinated reagent.

Conditions for optimal liberation of inorganic mercury in water, urine and serum samples from binding groups, necessary for arylation, have been studied in detail. Samples are rendered acidic to various final normalities, depending on the nature of the medium, using perchloric acid. Further enhancement of inorganic mercury release from matrix binding by use of oxidants has also been attempted as was the use of bivalent ions added in excess, such as copper(II) and cadmium(II) to afford binding site competition with mercury. Oxidants such as dichromate and permanganate in acidic solution destroy the arylating capacity of the reagents, likely via oxidation of the sulfinate group to the sulfonate structure, the latter compounds having no demonstrable arylation reactivity towards inorganic mercury. In our hands, the use of excess cadmium ion was without effect relative to data obtained in recovery of mercury from media where the ion is omitted, while copper(II) ion appears to actually reduce the recovery.

A related problem deals with the tightness of binding in various samples of added mercury where mineralization is not subsequently carried out as a reflection of the coordination picture in samples where mercury association with matrix occurs *in vivo*. Inorganic mercury added to serum or urine, in our study, is allowed to bind with available groups for a minimum time of 10 min prior to any manipulation of the sample. This time period is indirectly and arbitrarily arrived at by relating binding of inorganic mercury with a time-variable study we have carried out using methylmercury. Samples are treated with methylmercury and sodium chloride addition and extraction is carried out at various times after methylmercury addition. The sodium

chloride solution does not degrade the matrix nor does it successfully compete with likely binders present such as sulfhydryl groups at the chloride level employed. Decrease in amount of methylmercury extracted as a function of time is taken as a measure of uptake of mercury by the medium. It was found that in urine and serum methylmercury uptake is complete in about 1 min. Since inorganic mercury contains more potential binding sites (depending on the nature of ligands available to the metal) than methylmercury, the binding time was extended ten-fold. While such an approach is but a crude simulation of binding as occurring *in vivo* one can assume that inorganic mercury as well as other added mercurials are not circulating unbound in the matrix, such a situation likely leading to erroneous recovery study data.

Chromatographic data obtained in the analysis of inorganic mercury in water using lithium pentafluorobenzenesulfinate are presented in Fig. 3. No trace of reagent or other contaminant is observed in the chromatogram (Fig. 3B), which is identical to an authentic bis[pentafluorophenyl mercury(II)] chromatogram in terms of retention times, packing, temperature and flow dependence. The response to the addition of inorganic mercuric chloride is also characteristic: a new peak due to the monomercury chloride is observed and the peak due to the bis(mercurial) is suppressed.

Unlike the Westöö procedure, where liberated organomercurial is removed from the original extraction layer, our purification steps involve retention of the generated organomercurial in the original organic layer (xylene) and removal of any of the water-soluble reagent extracted into the organic layer. Impurities arising from the matrix and remaining in the organic layer do not appear to be eluted in the region

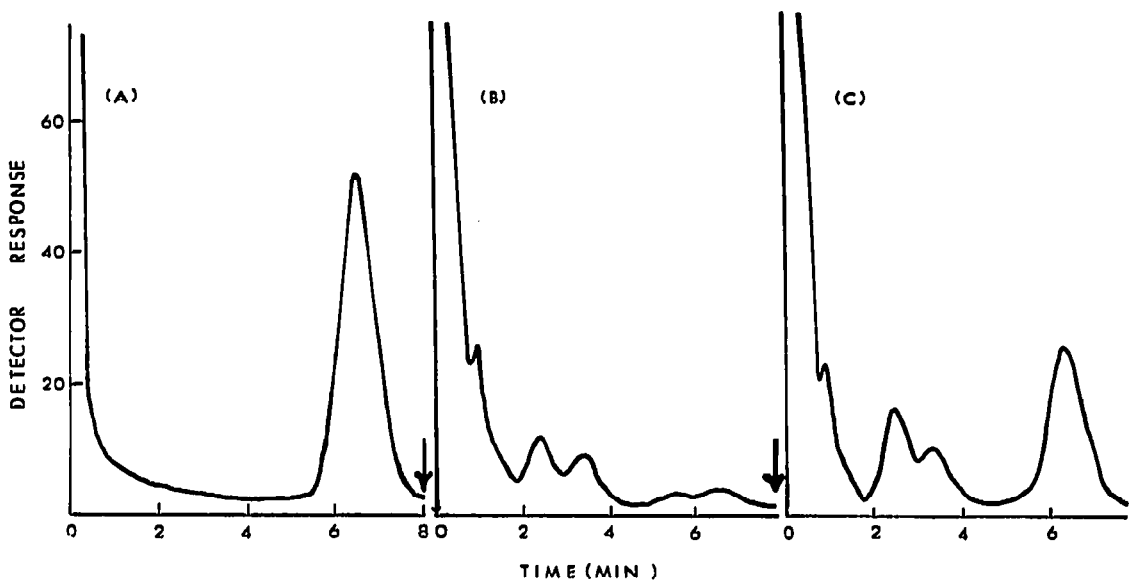


Fig. 3. Chromatograms from measurement of inorganic mercury(II) in water using lithium pentafluorobenzenesulfinate reagent. (A) Standard solution of bis[pentafluorophenylmercury(II)] in xylene; (B) blank water sample treated with reagent and carried throughout analysis; (C) water sample with added inorganic mercury(II) and carried through analysis. Conditions as in Fig. 1.

where the arylmercury emerges. As extracting solvent, xylene appears to be superior to benzene or toluene in that minimal removal of sulfinic acid reagent from the highly acidic aqueous sample occurs. The latter organic solvents yield chromatograms, when used in the assay scheme, containing trace impurities which complicate clean isolation of the mercury peak.

Levels of inorganic mercury in water evaluated by this procedure may be detected and quantitated in the range of 50 ppb* and the lower detection limit, as commonly defined, is 20 ppb. Such sensitivities are less than would be necessary in assessing levels of inorganic mercury in uncontaminated or slightly mercury-contaminated environmental water sources and we have broadened the procedure in the case of water to include use of mercury (inorganic and organic)-selective resins in tandem with GLC analysis. By this means, the extremely low levels of mercury isolated from large volumes of water samples may be collected in amounts permitting quantitation. Results of these studies will be presented elsewhere.

TABLE I

RECOVERY DATA—INORGANIC Hg ADDED TO VARIOUS MEDIA (LITHIUM PENTAFLUOROBENZENESULFINATE)

Medium	*Amount Hg added* (*μg Hg/ml*)	*Recovery* (%)	*S.D.* (±%)
Water	1.6	70.5	6.8
Urine	1.6	81.4	10.5
Serum	1.6	51.0	9.4

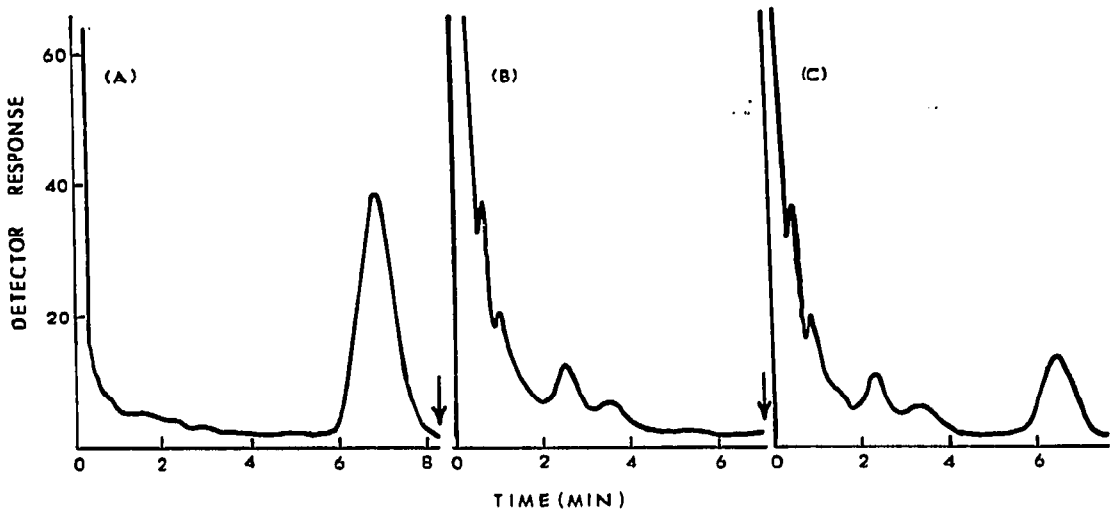
In Table I are included inorganic mercury recovery data for added inorganic mercury in water along with the accompanying precision (percent S.D.) value.

Some procedural variation is necessary in analyzing samples of urine or serum for inorganic mercury content. Exposure of serum to elevated temperature was found to give rise to the appearance of chromatographic interferents and optimal and contaminant-free results may be achieved by extended treatment (3 h) of acidified serum samples with the arylating reagent at room temperature. Furthermore, the amount of acid necessary for water, to achieve recoveries comparable to that using serum or urine, is greater than in the latter cases. Possibly, the absence of binding groups in our water samples (deionized) may not prevent some reduction of the bivalent inorganic mercury to lower oxidation states of the element, these states being unreactive to the reagent. Such reduction would be ameliorated by increase of the amount of an oxidizing acid, such as perchloric acid.

Chromatograms depicted in Figs. 4 and 5 were obtained with urine and serum samples, respectively, employing added inorganic mercury. As in the case with water, they are relatively free of impurities in the region where the bis(mercurial) emerges.

Recovery of added inorganic mercury (Table I) is lower in the case of serum than in either of the other two media studied owing to either reduction of mercury(II) to mercury(0) or incomplete liberation from the binding matrix. Working calibration curves are linear over the range of added inorganic mercury.

* Throughout this article the American billion (10^9) is meant.



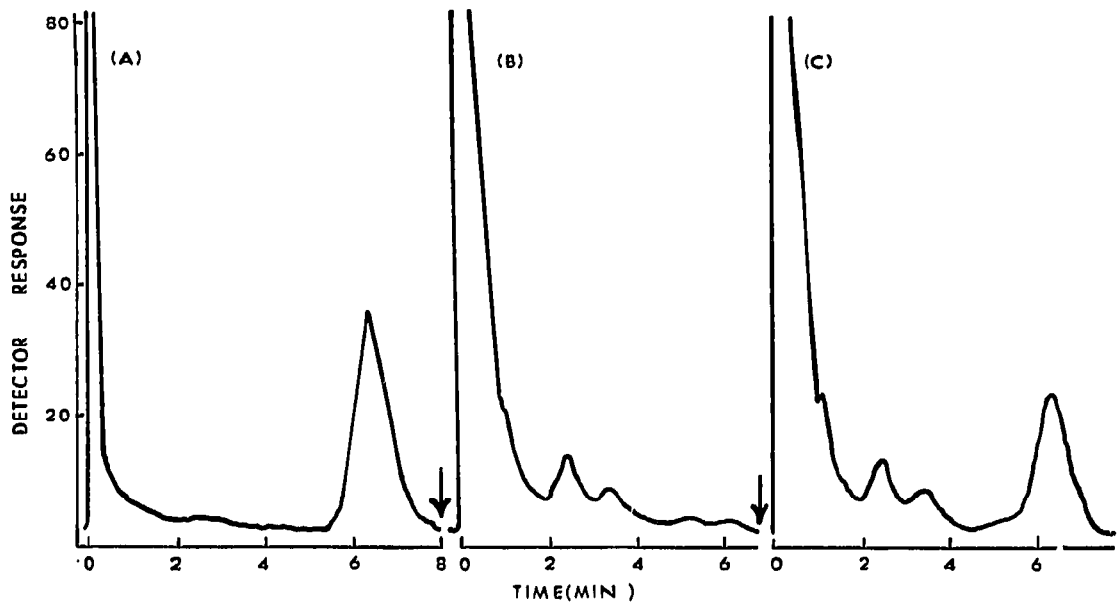


Fig. 4. Chromatograms of measurement of inorganic mercury(II) in urine using the lithium pentafluorobenzenesulfinate reagent. (A) Standard solution of bis[pentafluorophenylmercury(II)] in xylene; (B) blank urine sample carried through the analysis; (C) urine sample containing inorganic mercury(II) incubated for 10 min and carried through the analysis. Conditions as in Fig. 1.

Fig. 5. Chromatograms of measurement of inorganic mercury(II) in human serum using the lithium pentafluorobenzenesulfinate reagent. (A) Standard solution of bis[pentafluorophenylmercury(II)] in xylene; (B) blank serum sample carried through the analysis; (C) serum sample containing inorganic mercury(II) incubated for 10 min and carried through the analysis. Conditions as in Fig. 1.

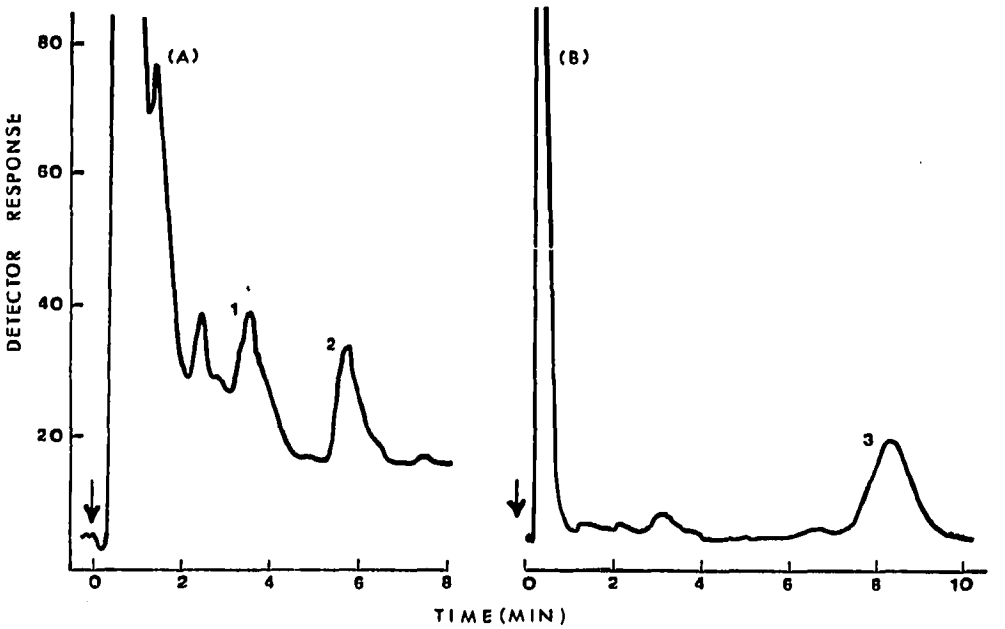


Fig. 6. Chromatograms from measurement of (1) methyl-, (2) ethyl-, and (3) inorganic mercury in urine using the lithium pentafluorobenzenesulfinate reagent. (A) Packing: Durapak Carbowax 400 (low K'). Temperatures: column, 140°; injector, 210°; detector, 190°. (B) Conditions as in Fig. 1.

Measurement of other mercurials in the presence of inorganic mercury may be carried out in several ways. Sequential analysis of the sample at various column temperatures may be carried out, methyl- and ethylmercury eluting rather rapidly while phenyl- and pentafluorophenylmercury are chromatographed at a higher temperature setting. Alternatively, the two packings, Duropak Carbowax 400 and Dexsil-300 may be used. The use of an instrument equipped with dual-column capability would, however, permit simultaneous analysis under conditions of optimal programming range and rate.

Chromatograms obtained from a urine sample containing methyl-, ethyl- and inorganic mercury carried through the analysis are depicted in Fig. 6, methyl- and ethylmercury eluting cleanly from Durapak Carbowax 400 (low K') and the bis(mercurial) chromatographed as before.

At the present time, attempts at inorganic mercury analysis in tissue homogenates and other biological media, not discussed in this report, have initially furnished indifferent results. Low recoveries of inorganic mercury have been observed and further refinements of the procedure, presently under study, are necessary.

Comparison studies for the evaluation of inorganic mercury in water, urine and blood using activation analysis and FAA spectrometry are presently in progress.

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