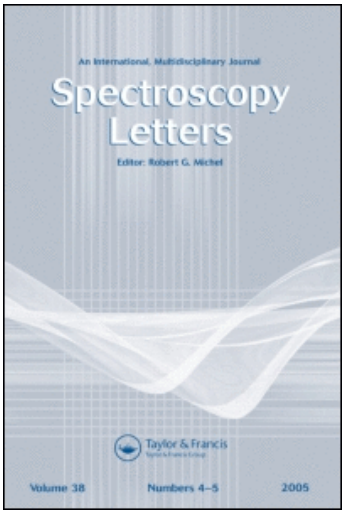


Spectroscopy Letters
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597299

Speciation of Mercury Compounds in Biological Samples Using GC-AA

J. W. Robinson[a]; J. C. Wu[a]
[a] Louisiana State University, Baton Rouge, Louisiana, USA

To cite this Article Robinson, J. W. and Wu, J. C.(1985) 'Speciation of Mercury Compounds in Biological Samples Using GC-AA', Spectroscopy Letters, 18: 1, 47 — 69
To link to this Article: DOI: 10.1080/00387018509438134
URL: http://dx.doi.org/10.1080/00387018509438134

SPECTROSCOPY LETTERS, 18(1), 47-69 (1985)

SPECIATION OF MERCURY COMPOUNDS IN BIOLOGICAL SAMPLES USING GC-AA

J. W. Robinson and J. C. Wu
Louisiana State University
Baton Rouge, Louisiana 70803

ABSTRACT

An interfaced GC-thermal atomizer AA system has been developed and used for the speciation and determination of volatile mercury compounds in biological fluids, perspiration and urine.

The interfaced system is highly selective to mercury compounds and has high sensitivity. No sample preparation was necessary, avoiding losses during pretreatment procedures such as the cold vapor technique.

The levels of inorganic mercury found in urine were from 0.0 to 3.3 ppm, and 0.0 to 0.6 ppm for sweat. Total mercury ranged from 0.6 to 3.9 ppm in urine and 0.2 to 1.0 ppm in sweat.

The results also indicated the presence of unidentified non-volatile mercury in the samples. However, inorganic mercury seemed to be the major form of excretion of mercury from human bodies.

1. INTRODUCTION

The physiological, toxicological and chemical properties of mercury depend on both its concentration and chemical form. The chemical form of mercury in the human body controls its retention,

 0038-7010/85/1801-0047$3.50/0

transport and excretion (1). It is necessary to identify mercury compounds in biological system before their metabolic pathways can be thoroughly studied, understood and controlled.

There has been a number of methods developed for mercury speciation. Many chromatographic techniques, such as thin-layer chromatography, paper chromatography, gas chromatography, liquid chromatography, ion exchange chromatography, gel filtration, and others, have been applied to the speciation of mercury compounds (2).

Total mercury and methylmercury in hairs have been determined with isotope dilution after digestion (3). Isotope exchange has been used to determine inorganic mercury in the presence of organic mercury in biological samples (4).

Selective adsorption followed by AAS has been utilized to speciate volatile mercury compounds and elemental mercury vapor in air samples (5).

Cold Vapor - AAS methods have been used by some researchers for mercury speciation in solutions (6), (7), (8). These methods involve changes in the reducing conditions to allow different forms of mercury to be released from solutions before the determination with AAS.

However, gas chromatography with an electron capture detector (GC-ECD) was the most commonly used method for mercury speciation in biological and environmental samples (9-12). This method does not distinquish mercury from many compounds and involves a series of extractions followed by the GC-ECD determination.

These techniques inevitably require elaborate sample pretreatment such as digestion, separation, extraction and concentration. Minimal sample handling and chemical pretreatment are important in

trace element analysis in order to avoid contamination of the samples, loss of the analytes and changes of chemical natures of the species present.

In this study, a graphite furnace atomizer was directly interfaced with a gas chromatographic system for mercury speciation of biological urine and sweat samples. Sensitivity of the system was about 10^{-10}g Hg.

Samples were directly injected into the system after collection without chemical pretreatment. After separation on the GC column the AAS detector was able to detect volatile mercury compounds without interferences from other components in the sample.

For comparative purposes the total mercury concentration was determined using a quartz T graphite AAS which was heated with a R. F. coil (13) (14).

2. EXPERIMENTAL

A. Equipment

1. GC-AA (Fig. 1)
   a) Hollow cathode lamp (designed and built in this lab)
   b) HCL power supply
   c) Pressure gauge for demountable HCL
   d) Microtek GC-2000-R gas chromatograph
   e) Mechanical chopper
   f) Graphite atomizer (designed and built in this lab)
   g) Varian-Techtron type M1 (SI-RO-SPEC) monochromator
   h) Hamamatsu Model R-106 photomultiplier
   i) Hewlett Packard Model 6516-A high voltage power supply
   j) Heathkit photometric readout amplifier Model EV-703-31
   k) Sargent Model XK recorder with variable range attachment power to atomic absorption detector

2. a) Barnes demountable hollow cathode lamp system

   b) Jarrel-Ash mechanical chopper from a Model 82-360-AAS

   c) Quartz T atomizer made of Quartz Scientific Inc. clear fused quartz tubing

   d) Jarrel-Ash Model 82-020 monochromator

   e) Hamamatsu R-106-UH photomultiplier

   f) Princeton Applied Research Model 126 lock-in, with Model 184 photometric preamplifier

   g) Beckman Model 1005, 10-inch potentiometric strip chart recorder

   h) Hewlett Packard Model 6516-A high voltage power supply

   i) Lepel high frequency induction heating unit Model T-5-3-KC-E-SW

   j) Drummon 1-5 microliter microdispenser

3. TLC and UV

   a) Whatman KC18 reversed-phase analytical TLC precoated plates

   b) Microcaps disposable pipettes

   c) Beckman DBG spectrophotometer

4. Chemicals and Reagents

   a) Mercuric chloride-MCB, Inc.

   b) MeHgCl-Alfa Products

   c) EtHgCl-Alfa Products

   d) Acetonitrile -J. T. Baker

   e) Dithizone-Fisher Scientific

   f) Chloroform-MCB, Inc.

   g) NaBr-Baker and Adamson

   h) 80/100 mesh Chromosorb W. AW. DMCS column support-Tek Lab

   i) Diethylene Glycol Succinate column substrate-Tek Lab

   j) Graphite rods-Ultra Carbon

3. ANALYTICAL PROCEDURES

A. Operating Conditions:

1. GC-AA

   a) Column: 1/8" diameter Teflon column 24" long, packed with 5% DEGS on Chromosorb W. AW. DMCS

   b) Carrier Gas: Argon (70 ml/min.)

   c) Column Temperature: 170°C

   d) Injection Port Temperature: 210°C

   e) Transfer Line Temperature: 180°C

   f) Hollow Cathode Lamp Current: 3 mA, He filler gas

   g) High Voltage on PM Tube: 350 volts

   h) Slit Width: 25 microns at 253.7 nm line, 0.8 A spetral slit width

   i) Atomizer Temperature: 1500°C

2. Quartz T AA

   a) Hollow Cathode Lamp Current: 3 mA, He filler gas

   b) Carbon Bed Temperature: 1450°C

   c) Atomizer Purge Gas: purge gas was supplied to the atomizer at 270 ml/min. to provide a positive pressure system and prevent the entrance of ambient air into the atomizer

   d) Light Path Temperature: 900°C

   e) Slit Width: 25 microns at 253.7 nm line, 0.8 A spectral slit width

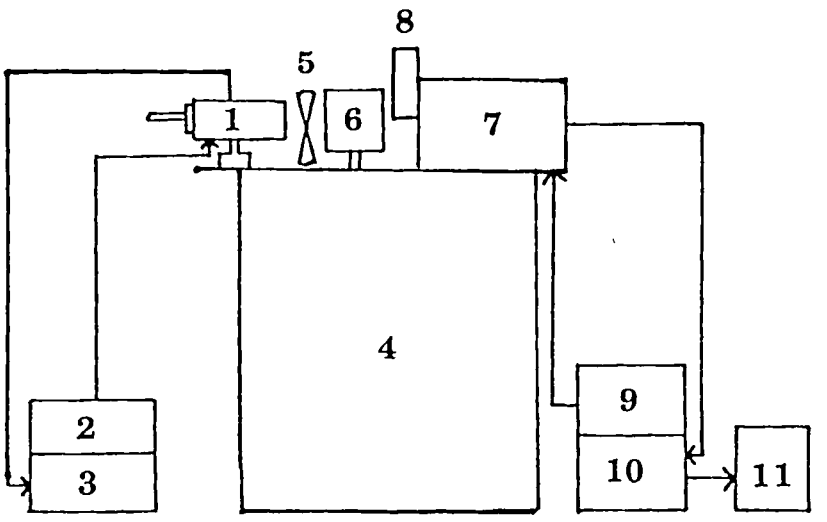
   f) High Voltage on PM Tube: 500 volts

   g) Cell Pumping Rate: 250 ml/min.

   h) Amplifier: ACVM mode, 200 mv sensitivity, 100 ms time constant

   i) Recorder: 100 mv, linear mode

B. Modification of graphite atomizer: An AA graphite T was developed in this group for the determination of lead in gasoline (15).



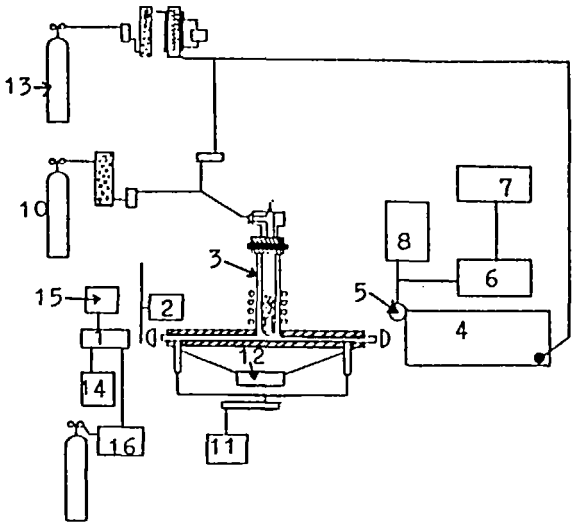
1. HOLLOW CATHODE LAMP
2. HCL POWER SUPPLY
3. PRESSURE GAUGE FOR DEMOUNTABLE HCL
4. MICROTEK GC-2000-R GAS CHROMATOGRAPH
5. MECHANICAL CHOPPER
6. GRAPHITE ATOMIZER
7. MONOCHROMATOR
8. PHOTOMULTIPLIER
9. POWER SUPPLY FOR PMT
10. AMPLIFIER
11. RECORDER

FIGURE 1 SCHEMATIC DIAGRAM OF GC-AA

This detector left a gap between the interfacing transfer line from GC column and the atomizer due to the temperature difference. This caused sample losses and reduced the sensitivity of the system.

For the analysis of Hg in a biological fluid, a more sensitive system was required. A modified graphite atomizer (Fig. 3) was machined from carbon rods providing a direct connection between the transfer line and the graphite detector. This improved the sensitivity.

DIAGRAM OF QUARTZ T AA



1. HOLLOW CATHODE LAMP
2. MECHANICAL CHOPPER
3. QUARTZ T ATOMIZER
4. MONOCHROMATOR
5. PHOTOMULTIPLIER
6. AMPLIFIER
7. RECORDER
8. POWER SUPPLY
9. R. F. COIL
10. PURGING GAS FOR ATOMIZER
11. CELL PUMP
12. LIGHT PATH HEATER
13. PURGING GAS FOR MONOCHROMATOR (FOR VACUUM UV LINE)
14. POWER SUPPLY FOR HCL
15. PRESSURE GAUGE FOR HCL
16. PRESSURE REGULATOR

FIGURE 2.

C. Calibration of mercury: A series of standard solutions of mercuric chloride with concentrations 0.1, 0.25, 0.5, 1.0, 2.5 and 5.0 ppm was injected into the system. The corresponding peak heights provided a calibration curve.

Calibration curves for methyl mercury were made from 0.1, 0.25, 0.5, 1.0, 2.5 and 5.0 ppm MeHgCl solutions.

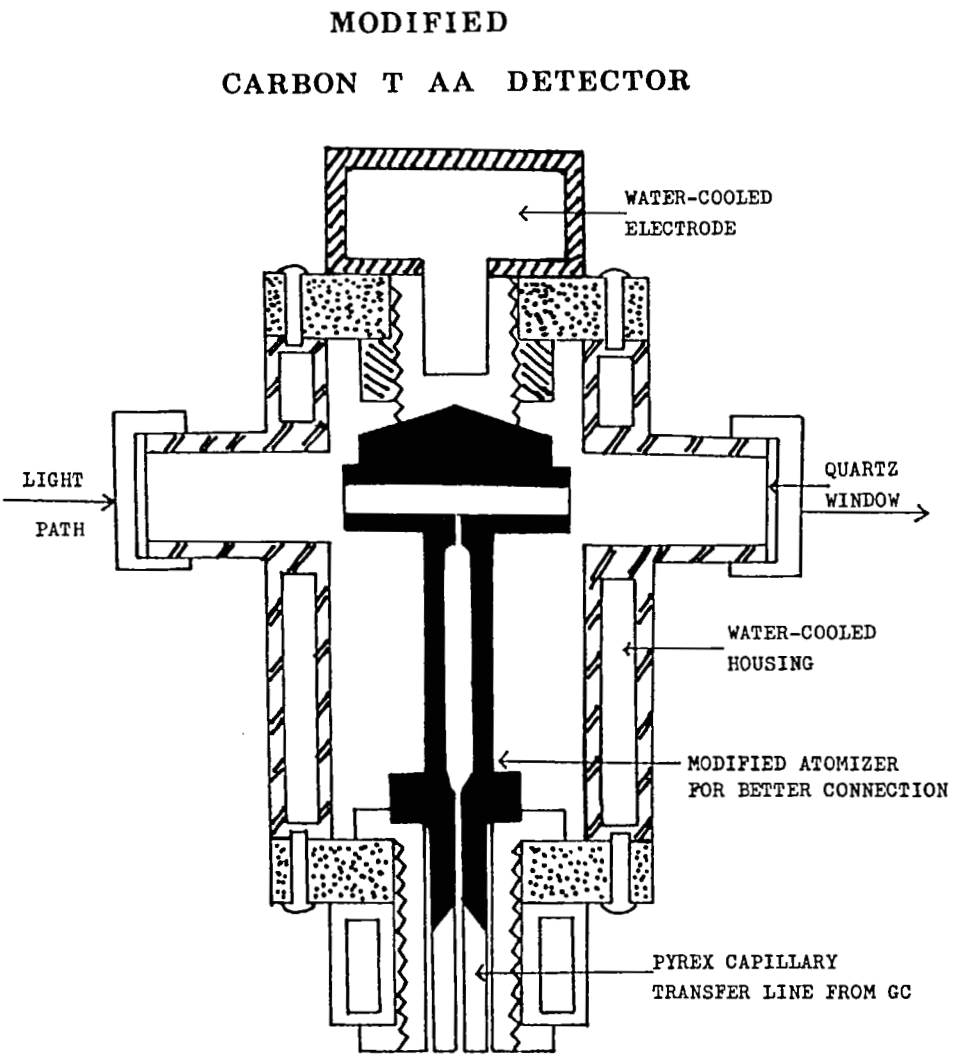


FIGURE 3 MODIFIED CARBON T DETECTOR OF GC-AA

Standard solutions containing 0.1, 0.25, 0.5, 2.5 and 5.0 ppm of mercury were introduced into the quartz T graphite AA using a 2 microliter Drummond microdispenser to obtain the calibration curve.

The GC column was short to reduce the retention time. Peak heights were then used to prepare calibration curves both for inorganic mercury (Fig. 4) and organic mercury (Fig. 5) although peak areas are probably more accurate. Calibration curve was also made for total mercury from quartz T AA determination (Fig. 6).

D. Chemical impurity determination by Thin Layer Chromatography (TLC): TLC was used to confirm and determine trace impurity of inorganic mercury in MeHgCl standard.

Saturated MeHgCl solutions were spotted on reverse phase KC18 TLC plates (5 x 10 cm). The plates were developed by a solvent mixture, 55% acetonitrile and 43% 1.0 M NaBr aqueous solution. Rf values were determined; spots for mercuric chloride and MeHgCl were scratched off the plates and their concentrations were determined with dithizone spectrophotometric method (16) (17).

E. Sample collection and analysis: Sweat and urine sample collection procedures were the same as those described earlier (13)(14).

Urine samples were collected from volunteers who were members of the university population. Subjects samples were not occupationally exposed to mercury.

Sweat samples were collected under supervision in a sauna at the LSU Field House. Subjects sampled had undergone physical exercise programs and then showered before entering the sauna. Sweat samples were collected only after profuse sweating. Skin contaminants were avoided. Sweat drops were caught from the nose or fore-

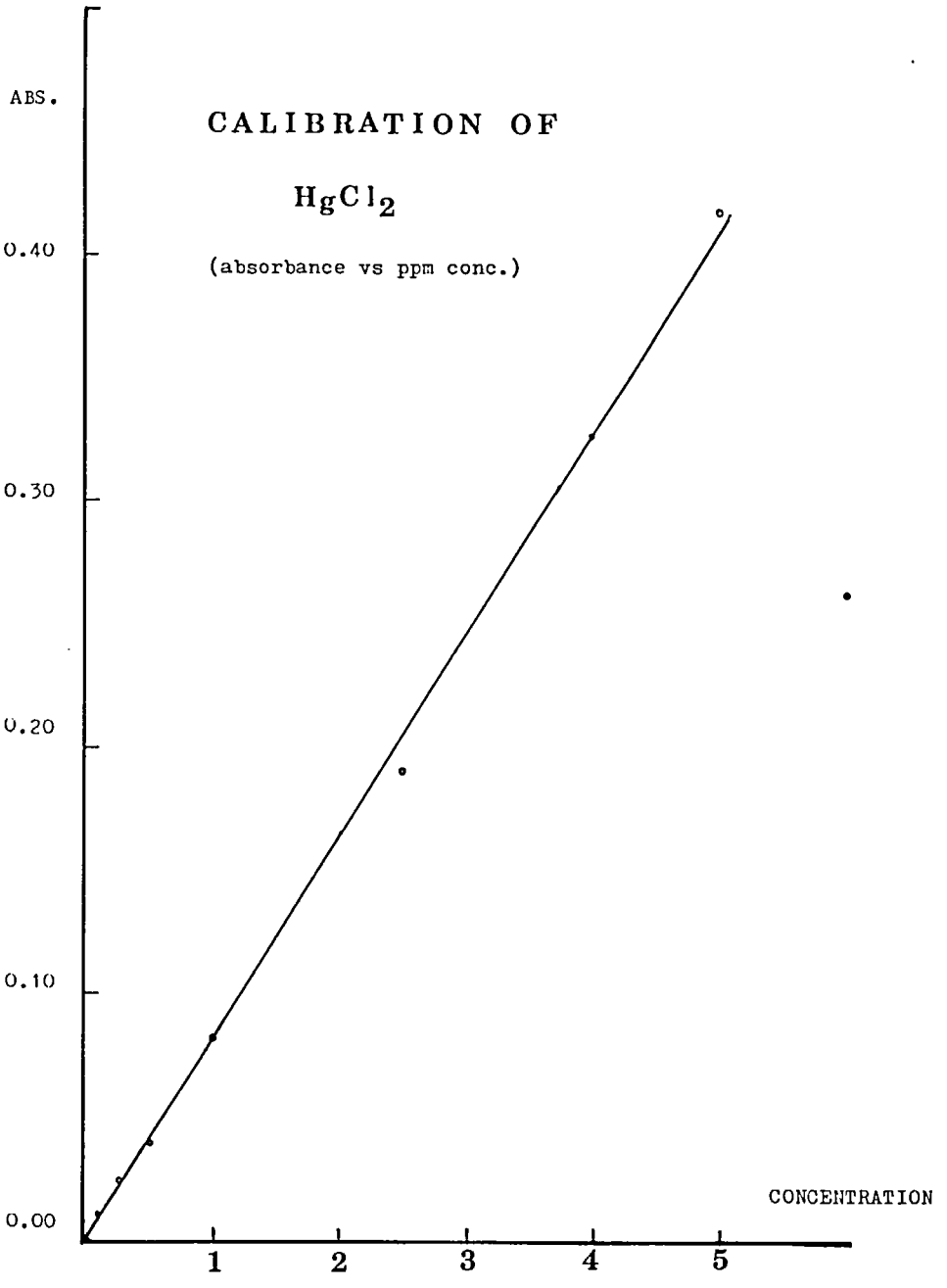


FIGURE 4

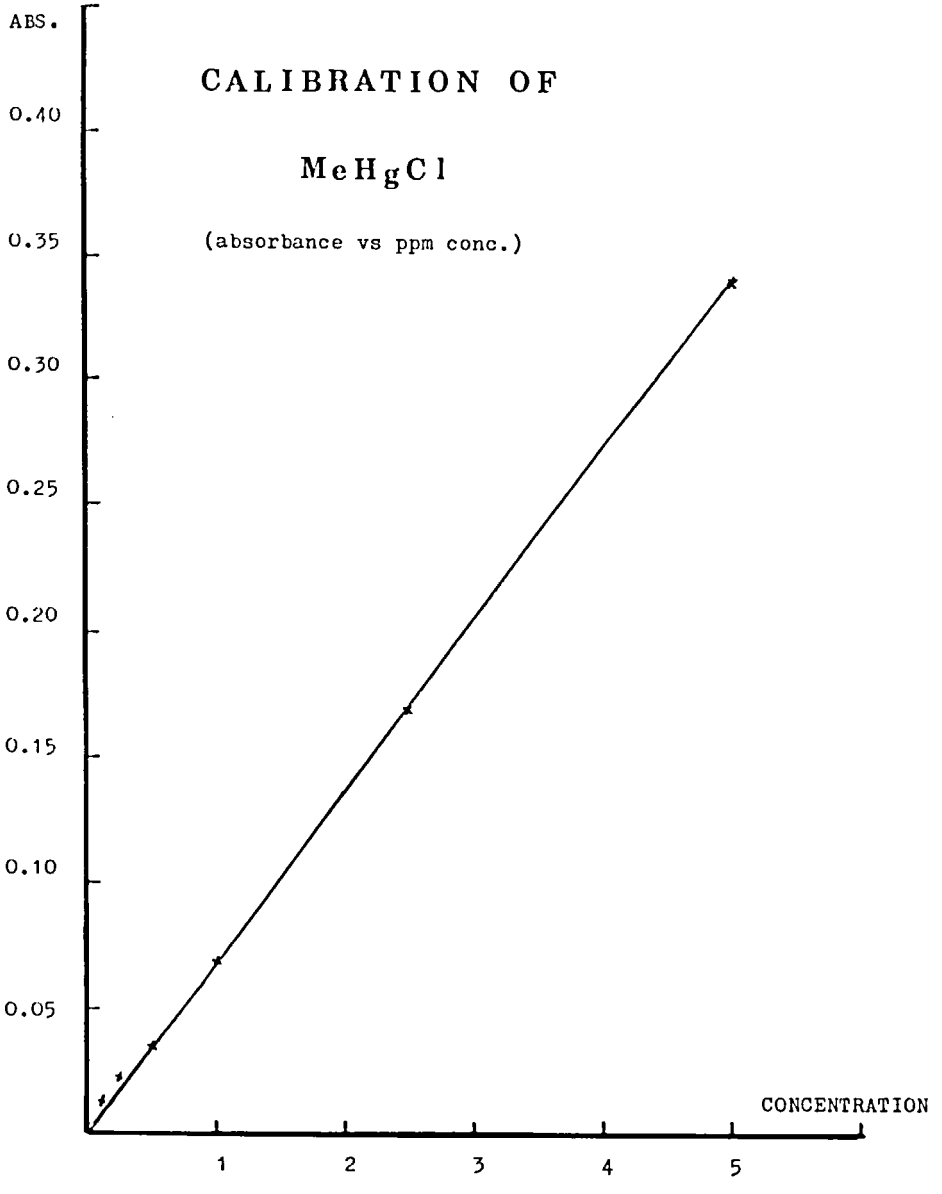


FIGURE 5

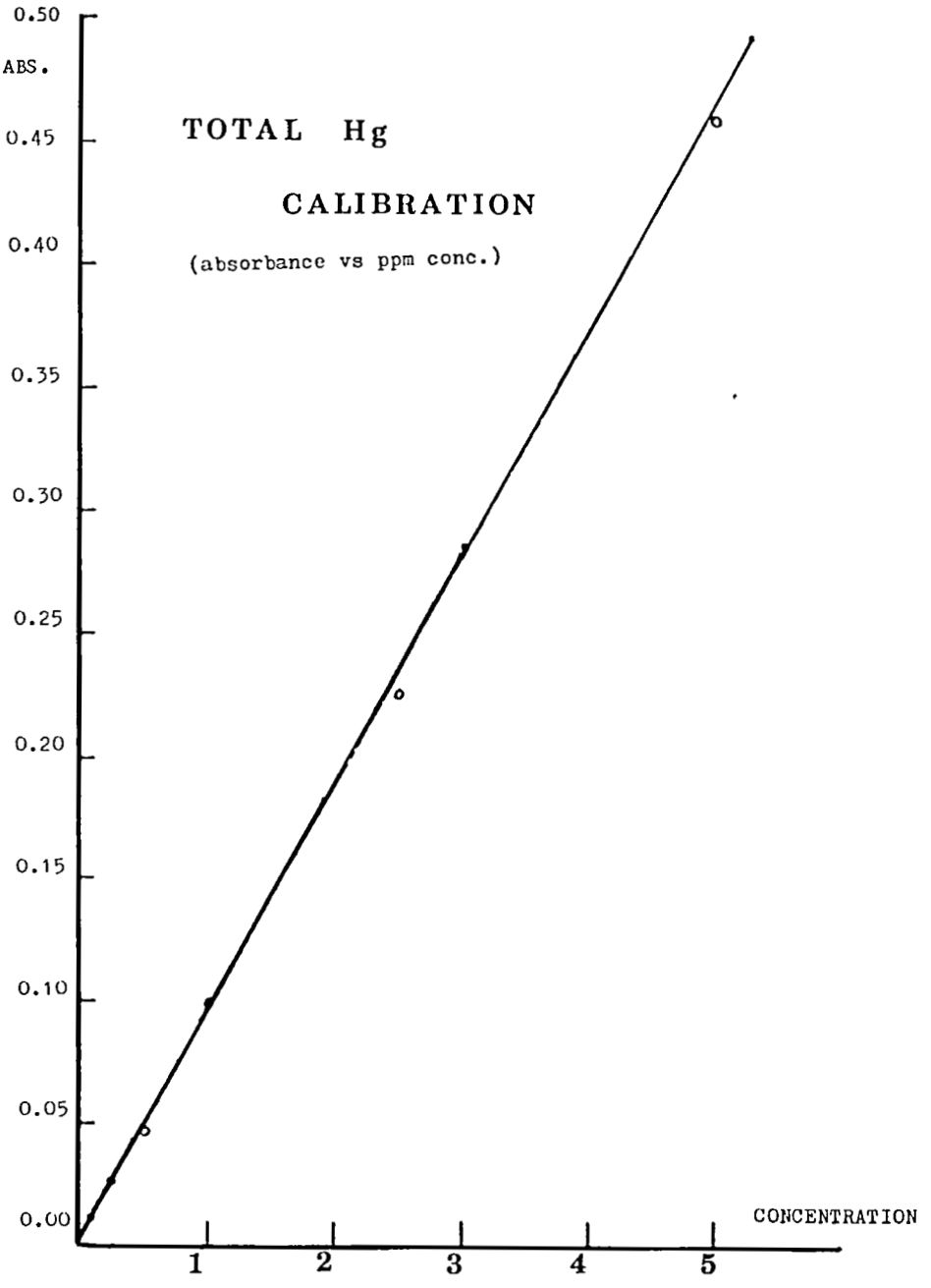


FIGURE 6

head using cleaned polyethylene vials; no contact was made between the vial and skin of sampled subjects during sample collection.

All the polyethylene vials used were cleaned in nitric acid and rinsed with distilled deionized water between use.

All samples were injected directly into the system without any other pretreatments and within three hours after collection to avoid losses, contamination and decomposition.

4. RESULTS AND DISCUSSION

A. Sensitivity improvement: The sensitivity normally obtained with a standard commercial carbon atomizer is 4.9×10^{-10}g (1% absorption). Using an earlier design, sensitivity of 1.5×10^{-9}g was obtained for lead, and 5.0×10^{-8}g for mercury (15). The average sensitivity obtained from this design (Fig. 4) was 3.0×10^{-10}g for mercury (1% absorption). The sensitivity decreased with the lifetime of the atomizer, (24 hours continuous running time).

B. Retention times of mercury: Mercuric chloride, methylmercuric chloride, ethylmercuric chloride, mixture of mercuric chloride, methylmercuric chloride as well as ethylmercuric chloride were injected separately into this GC-AA system. From the chromatograms, the retention times were obtained.

Chromatograms obtained for solutions of mercuric chloride, MeHgCl and mixtures are shown as Fig. 7, 8, 9 and 10. Separation can be improved by using a longer column and lower oven temperature for qualitative purposes. Retention times are shown in Table 1.

C. TLC Results: One of the limitations of GC is the decomposition of thermally labile compounds in the column. A minor peak, with the same retention time as mercuric chloride, on MeHgCl chromatogram proved to be an impurity in the standard by reverse phase TLC

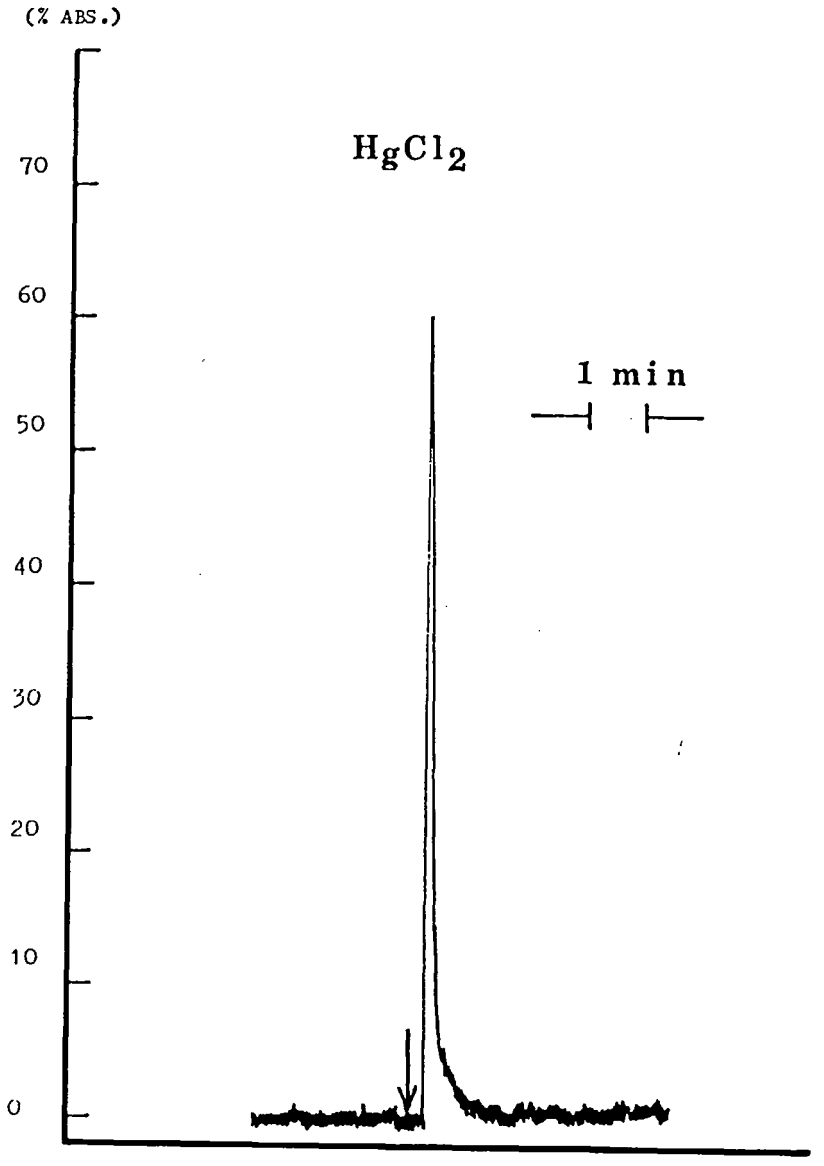


FIGURE 7 CHROMATOGRAM OF MERCURIC CHLORIDE BY GC-AA

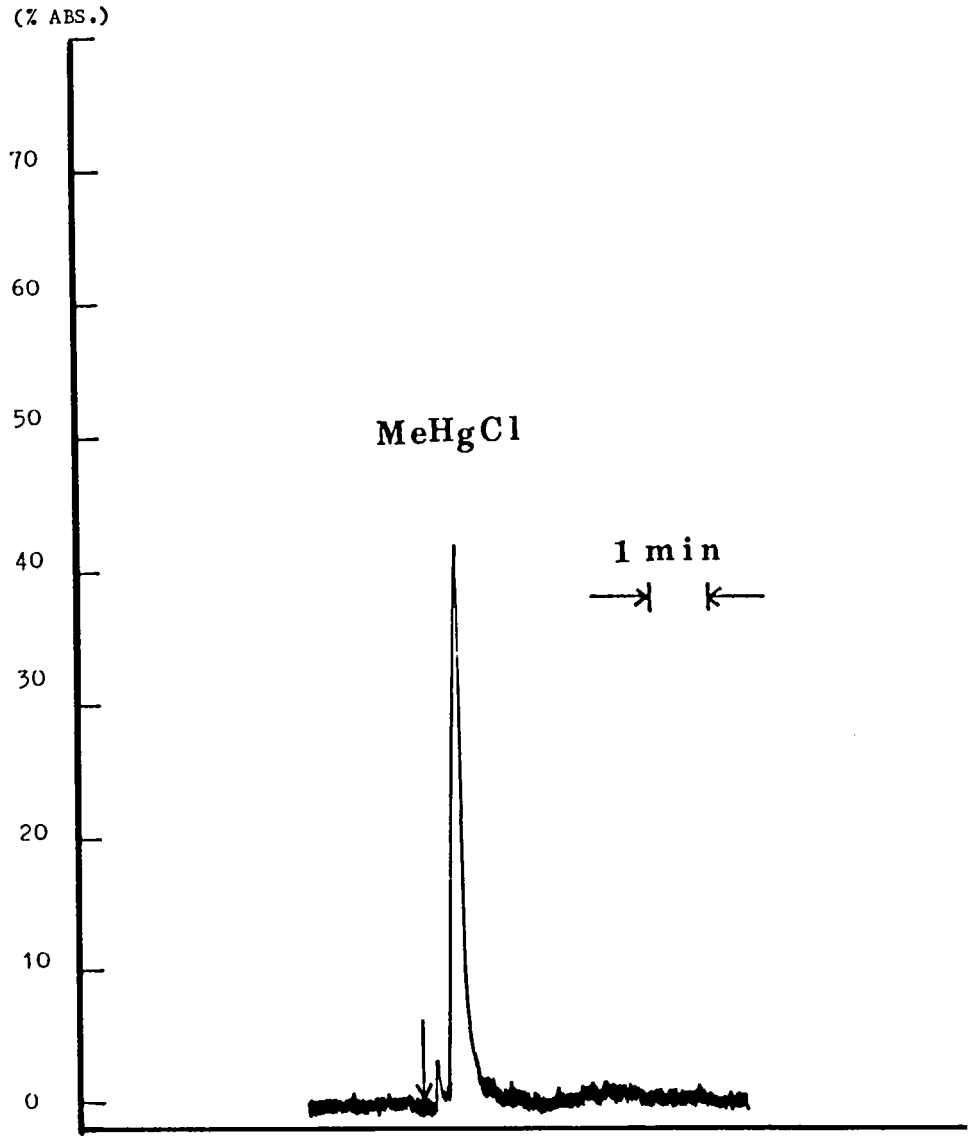


FIGURE 8 CHROMATOGRAM OF METHYLMERCURIC CHLORIDE BY GC-AA

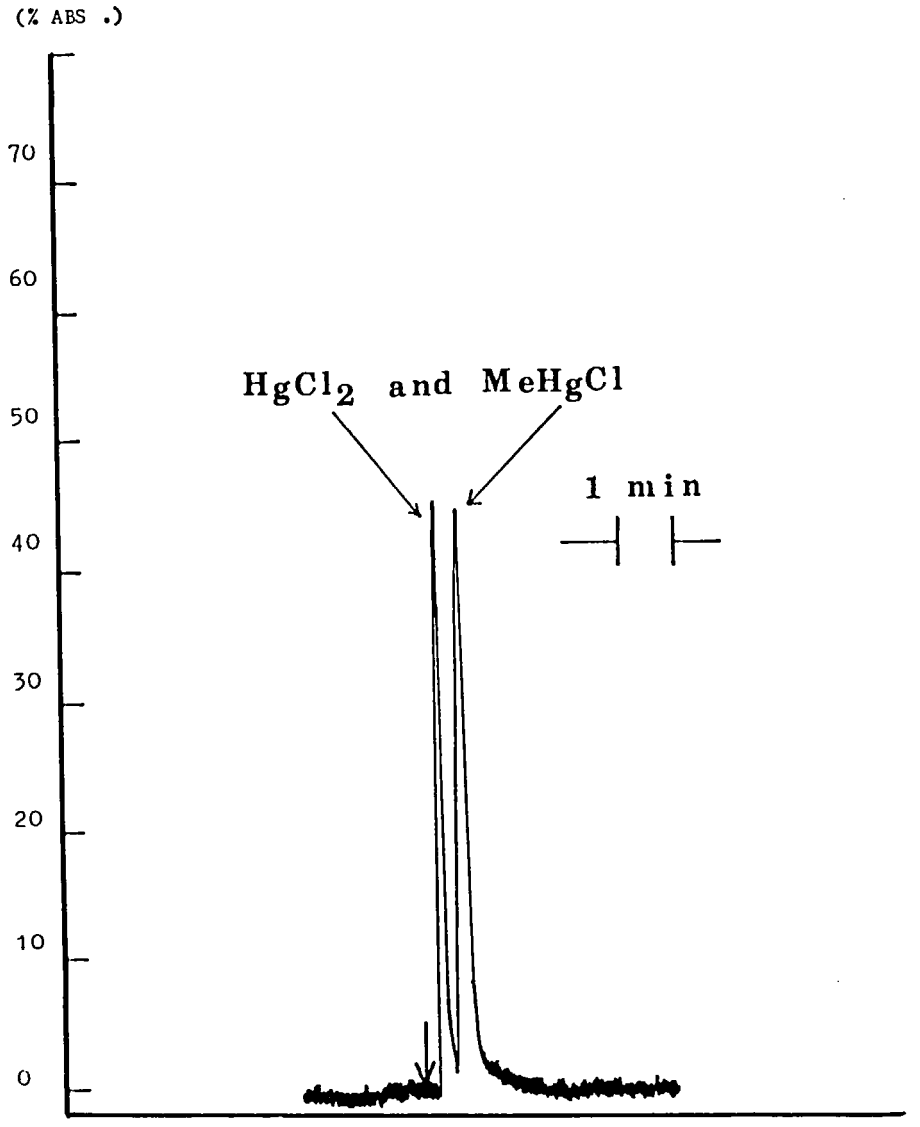


FIGURE 9 CHROMATOGRAM OF MERCURIC CHLORIDE AND METHYLMERCURIC CHLORIDE BY GC-AA

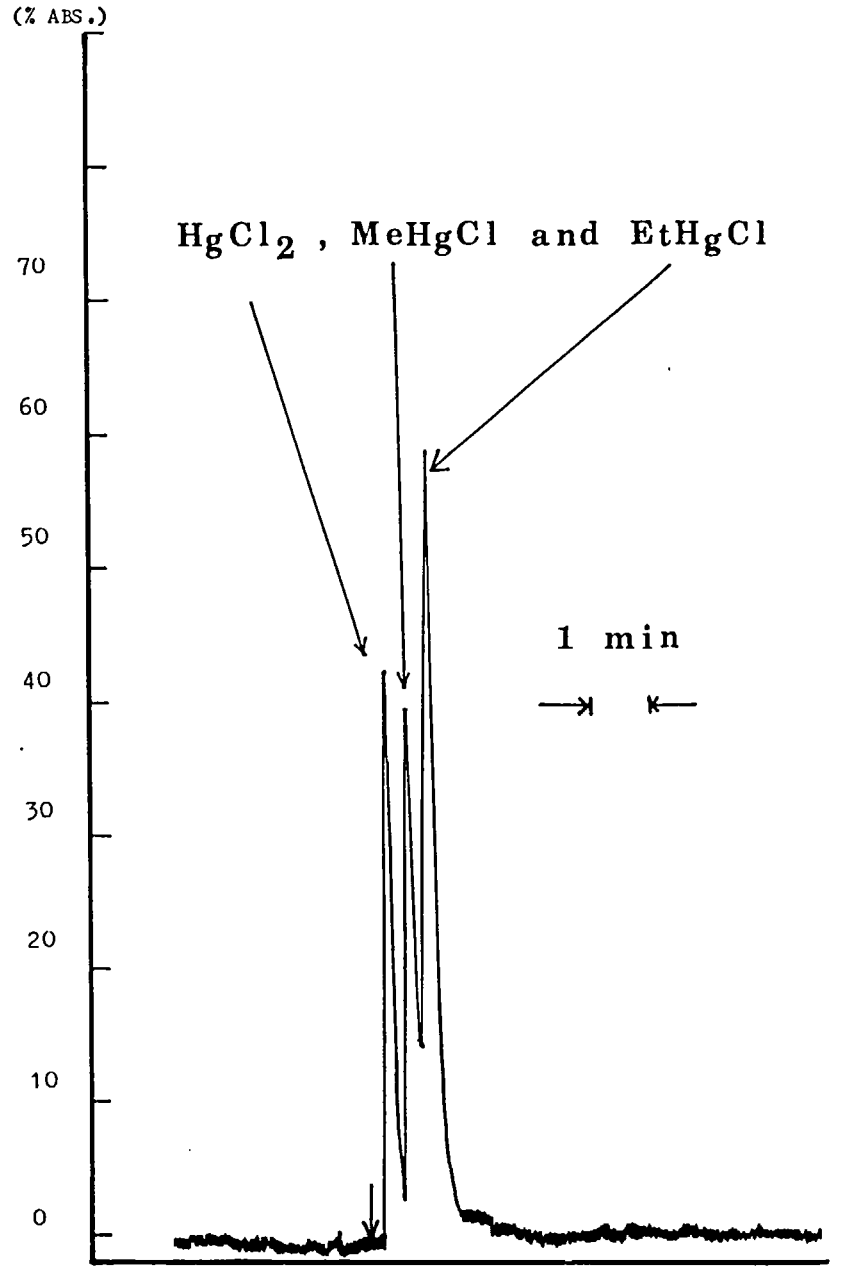


FIGURE 10 CHROMATOGRAM OF MERCURIC CHLORIDE, METHYLMERCURIC CHLORIDE AND ETHYLMERCURIC CHLORIDE BY GC-AA

TABLE I

MERCURY COMPOUNDS	$HgCl_2$	MeHgCl	EtHgCl
RETENTION TIMES (SEC)	12	42	60

TLC OF MeHgCl STANDARD

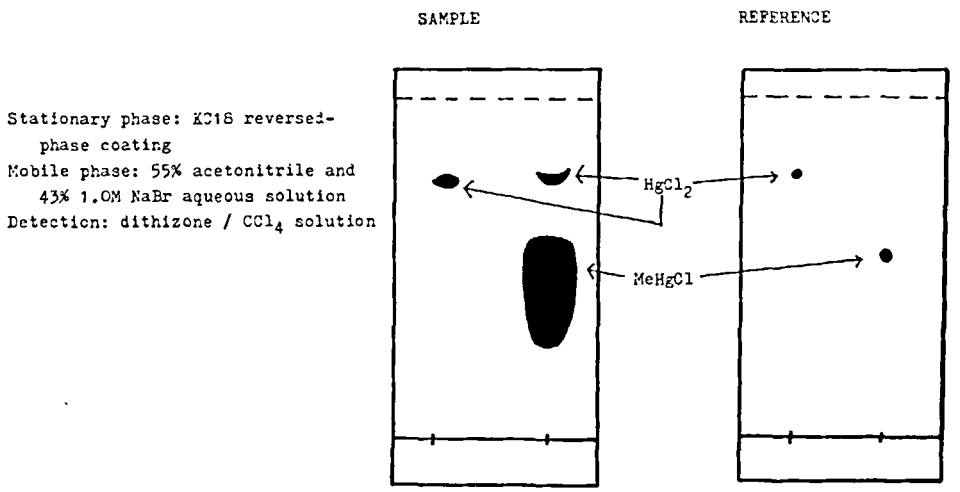


FIGURE 11 TLC OF METHYLMERCURIC CHLORIDE STANDARD

and spectrophotometric method using dithizone (Fig. 11). There was 13.36% Hg by weight in the form of mercuric chloride in the MeHgCl standard.

D. Mercury in urine and sweat samples: Inorganic mercury was the major chemical form found in the samples analyzed. Wide fluctuations existed among samples taken (1) from different people, (2) from the same person on different days and (3)between urine and sweat

TABLE II MERCURY IN URINE

Inorganic mercury determined by GC-AA.

Total mercury determined by Quartz T AA.

URINE ALIQUOT #	PPM INORGANIC MERCURY	PPM TOTAL MERCURY
1	1.8	2.5
2	1.0	2.0
3	2.2	3.0
4	0.0	1.8
5	3.3	3.9
6	0.5	1.2
7	2.6	3.2
8	0.9	1.5
9	3.1	3.7
10	0.3	0.6
11	1.8	2.8
12	1.2	2.0
13	0.8	1.5
14	2.5	3.4
15	0.0	1.0

sample from the same person on the same day. The collected data for urine aliquots are presented in Table 2. Those for sweat samples are in Table 3 and 4.

The results also indicated the presence of unidentified non-volatile mercury in the samples. However, inorganic mercury seemed to be the major form of excretion of mercury from human bodies.

TABLE III MERCURY IN SWEAT

Inorganic mercury determined by GC-AA.

Total mercury determined by Quartz T AA

SWEAT ALIQUOT #	PPM INORGANIC MERCURY	PPM TOTAL MERCURY
1	0.2	0.4
2	0.0	0.2
3	0.5	0.8
4	0.4	0.6
5	0.6	0.8
6	0.2	0.5
7	0.1	0.3
8	0.5	0.7
9	0.6	1.0
10	0.3	0.5

There appeared to be no correlation between the results, i.e. high or low levels in urine did not seem to correspond to high or low levels in sweat. However the sets were too small to be statistically significant.

5. CONCLUSIONS

A. Analysis speed: The sample could be analyzed within ten minutes after collection with almost no pretreatment. This greatly reduced the sample loss and contamination, gave more reliable results and decreased the time for analysis. Direct analysis with minimal sample handling and pretreatment is one of the goals of trace element analysis.

B. Sensitivity: The sensitivity for Hg by AAS is relatively poor compared to other elements. The improved design of the atomizer

TABLE 4

MERCURY CONCENTRATIONS (PPM) IN URINE AND SWEAT FROM SUBJECTS TAKEN ON THE SAME DAY

	URINE Hg		SWEAT Hg	
SAMPLE NO.	INORG.	TOTAL	INORG.	TOTAL
		SUBJECT 1		
1	2.0	3.0	1.8	2.2
2	1.4	2.0	0.3	0.8
3	0.8	1.5	0.0	0.3
4	1.8	2.8	0.6	1.0
5	0.9	1.5	0.1	0.3
6	0.5	1.2	0.0	0.2
7	1.8	2.5	0.5	0.8
		SUBJECT 2		
1	1.2	2.5	0.2	1.2
2	0.5	0.9	0.0	0.4
3	0.7	1.2	0.6	1.0
4	0.4	1.2	0.2	0.5
5	0.0	1.0	0.0	0.4
6	0.3	0.6	0.5	0.7
7	2.5	3.4	0.4	0.6

offered a marginally better sensitivity. One reason was the relatively high temperature maintained in this system which causes physical and chemical background absorption to be minimized. Another reason may be due to the "filter effect" of the column which removes most interferences before the detected species reached the atomizer. A better sensitivity required no sample preconcentration.

C. Selectivity: Using AA as a detector coupled with a chromatographic technique for analyzing metal species in biological samples avoided other interferences present in the samples. The chromatograms obtained were much simpler than those from common nonselective GC detectors. This also minimized some of the sample pretreatment work, such as extraction, to remove interferences.

D. Simultaneous determination of both organic and inorganic mercury: Many mercury compounds are volatile. Nevertheless, few detectors can be used to analyze both organic and inorganic mercury at the same time. Most require isolation of specific chemical form or trnasformation into a certain chemical form before the final detection. This system offered a method to determine both organic mercury and inorganic mercury simultaneously.

E. Memory effect: Peaks referred to as "memory" or "ghost" peaks are common to aqueous-injection gas chromatography. The major portion of the memory peak results from hold up in the injection port (18). On column injection is used to minimize the accumulation of sample in the injection port. Several aliquots of water injection are usually necessary to clean the system between sample injections.

F. Column life time: Direct injection of biological samples into the GC column does poison the column. A filter or precolumn was used in this study. The column was usually re-packed after fifty sample injections.

6. REFERENCES

1. Piotrowski, J. K., Inskip, M. J., Marc Report 1981, 24.
2. Fishbein, L., Chromatog. Rev. 1970, 13, 83.
3. Kanda, Y, Suzuki, N., Anal. Chem. 1980, 52, 1672.

4. Norseth, T., Clarkson, T. W., Biochem. Pharmacol. 1970, 19, 1774.

5. Braman, R., Johnson, D., Envir. Sci. Tech. 1974, 8, 996.

6. Magos, L., Analyst 1971, 96, 847.

7. Matusiak, W., Cefola, M., Dal Cortivo, L., Umberger, C., Anal. Biochem. 1964, 8, 463.

8. Goulden, P. D., Anthony, D. H. J., Anal. Chim. Acta 1980, 120, 129.

9. Berman, E., "Toxic metals and their analysis", Heyden: London, 1980

10. Westoo, G., Acta Chem. Scand. 1966, 20, 2131.

11. Westoo, G., Acta Chem. Scand. 1967, 21, 1790.

12. Westoo, G., Acta Chem. Scand. 1968, 22, 2277.

13. Robinson, J. W., Skelly, E. M., Spect. Letters 1983, 16, 117.

14. Robinson, J. W., Skelly, E. M., Spect. Letters 1983, 16, 133.

15. Kiessel, E., Dissert. LSU 1980.

16. Boothe, E. D., Dissert. LSU 1984.

17. Kiwan, A. M., Fouda, M. F., Anal. Chim. Acta 1968, 40, 517.

Received: 08/08/84
Accepted: 10/22/84