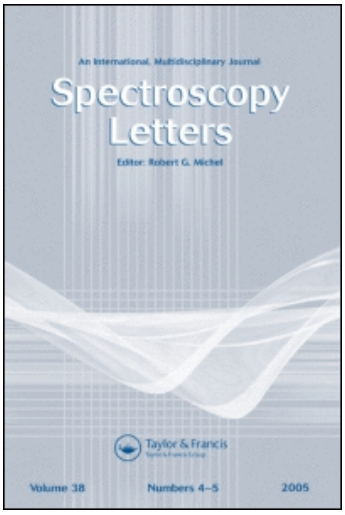

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Interfacing HPLC and Cold-Vapor AA with On-Line Preconcentration for Mercury Speciation

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INTERFACING HPLC AND COLD-VAPOR AA WITH ON-LINE PRECONCENTRATION FOR MERCURY SPECIATION

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ABSTRACT

A quartz reaction vessel is designed and constructed to interface HPLC and cold-vapor AA for mercury speciation in aqueous samples. Inorganic mercury, methyl mercuric and ethyl mercuric compounds can be separated effectively within 10 minutes. An on-line preconcentration unit is used to reduce the limit of detection (LOD) before the sample is introduced into the reversed-phase HPLC. Without preconcentration, the detection limits for the three mercury species are 0.094, 0.085 and 0.124 ppm, respectively. Limits of detection are reduced to 0.78, 0.78 and 0.42 ppb, with the use of preconcentration technique. The feasibility of this method is tested by analyzing mercury compounds in synthetic tap and river water samples.

INTRODUCTION

The toxicity and environmental fate of mercury is well known to be highly depending on its chemical forms [1][2]. Inorganic mercury can be biomethylated in the environment to form methyl mercuric compounds which can be easily bioaccumulated and shows significant biomagnification [3][4]. The factor of bioaccumulation in aquatic species can be as high as ten thousand times [5]. The estimated half-life of methyl mecury in human is 75 days, and that for the inorganic mercury is around 42 days [6]. The target organs for mercury species in human are central nerve system, kidney and liver. However, 98% of methyl mercuric species in human is known to be retained in the brain [7]. Teratogenic effects of methylmercury have been well documented [3][6]. Pronounced toxic symptoms is observable as the concentration of methylmercury in human blood exceeds 0.2 ug/g [2]. The human body tolerance of mercury is estimated to be around 80 mg, and 5.0 ug/g of methylmercuric compounds in brain is lethal [3].

Determination of total mercury in different environmental samples can be achieved with colorimetry, neutron avtivation analysis, isotope dilution, atomic absorption spectrometry, atomic fluorescence spectroscopy, and inductively coupled plasma [8][9][10]. Speciation of mercury compounds can be accomplished with tedious sample pretreatment followed by chromatographic separation and detection with a typical detector such as ECD [11][12]. Recent advances enable one to interface chromatographic unit with specfic detectors, such as dc plasma, microwave induced plasma, graphite furnace AA, or inductively coupled plasma, for mercury speciation work with less pretreatment and better accuracy

[13][14][15][16]. There are still strong needs for the commercial availability of instruments for metal speciation studies.

An interfacing reaction vessel is developed and constructed for the combination of a regular HPLC system, with on-line preconcentration as described earlier [17], and cold-vapor AA for mercury speciation in this study. The cost for the manufacture of this interface is around US$ 300.00, and can be easily manipulated and adapted to a regular flame AA. Although cold-vapor technique is normally applied to inorganic mercury analysis, organic mercury compounds can be converted to inorganic forms followed by subsequent analysis. Several parameters which affect the performance of this method are evaluated.

EXPERIMENTAL

1. REAGENTS

Reagent water is obtained by purifying distilled water with Milli-RO 60 water purification system and Milli-Q reagent water system. Both are products of Millipore.

Stock solutions of mercuric compounds are prepared by dissolving mercuric chloride, methylmercuric chloride and ethylmercuric chloride in minimum amount of methanol, an E. Merck product, and dilution with water. Analytical grade mercuric chloride is a product of E. Merck with 99.5% purity. Methyl mercuric chloride is from Tokyo Kaisei Kogyo Co. with purity over 98%. Ethylmercuric chloride is also an E. Merck product with purity better than 98%.

The eluent used for the chromatographic separation is an aqueous mixture of acetonitrile from J. T. Baker (HPLC grade) and potassium bromide from E. Merck of 99.5% purity.

The reagents for the cold-vapor AA system are analytical grade nitric acid from J. T. Baker and sodium borohydride (purity over 96%) from E. Merck. A solution of potassium perchromate, a Kanto Chemical Co. product with 99.5% purity, is utilized to transform organic mercury species prior to the cold-vapor process. The perchromate solution is prepared by dissolving potassium perchromate into 20% nitric acid solution; and sodium borohydride solution is freshly prepared daily by its dissolution into 0.05 N aqueous sodium hydroxide and kept at 5° C until use.

All standards and samples injected into the chromatographic system are subjected to membrane filtration; HVLP membranes from Millipore with pore size of 0.45 um are applied.

II. EQUIPMENT

The complete instrumental system consists of four major parts: a. the on-line preconcentration unit as described earlier [17], b. an HPLC system in which reversed-phase mode is used, c. the cold-vapor AA, and d. an air pump and a series of sorbent (charcoal) and trapping solution (perchromate) for retaining mercury vapor.

The chromatographic system contains a Kratos Spectroflow 400 solvent delivery pump, Rheodyne 7125 and 7000 valves with 100 ul and 1000ul loops for injection and preconcentration.

The separation of mercury species is accomplished with a 25 cm C-18 column equipped with a 5 cm guard column from Macherey-Nagel Co. The volume of the injection loop is 100 ul. A separate pump from IRICA, Japan (Model 871) is used for sample delivery at the preconcen-

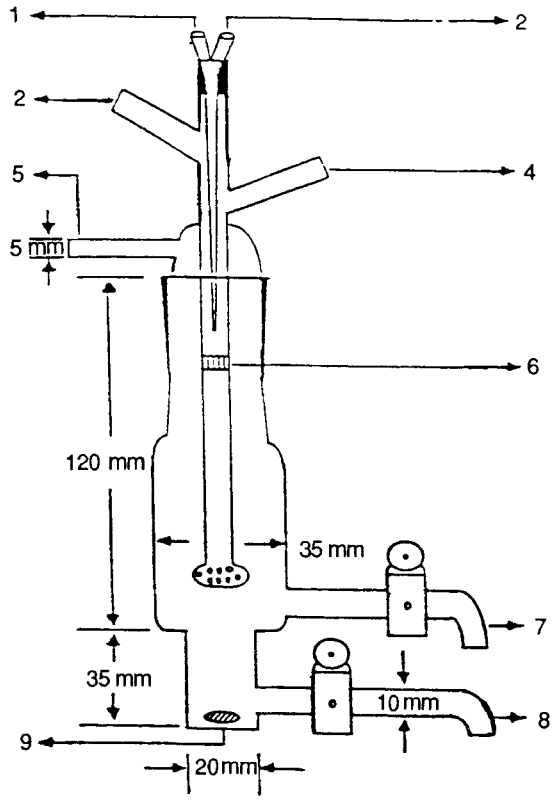


Fig. 1. HPLC-AA interface for mercury speciation.
1. effluent from HPLC. 2. inlet of perchromate.
3. inlet of air. 4. inlet of reducing agent.
5. exit for Hg vapor. 6. porous frit disc.
7. outlet for waste. 8. outlet for waste.
9. magnetic stirrer.

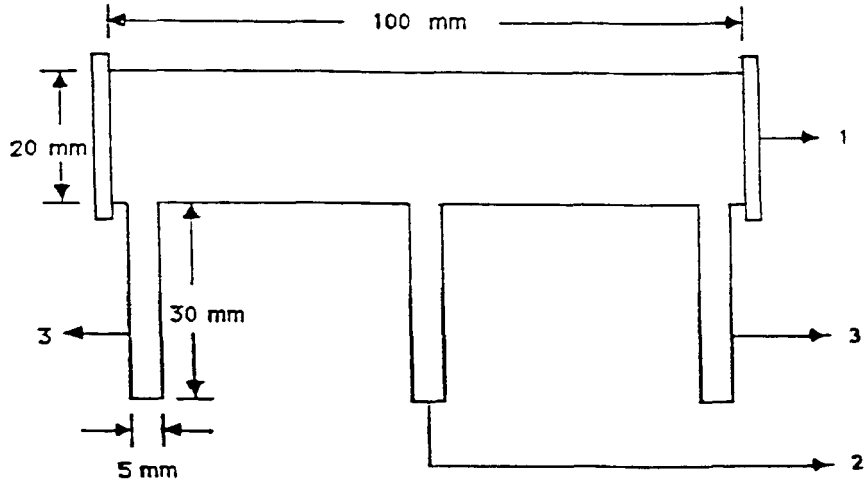


Fig. 2. Quartz absorption cell for mercury.
1. quartz window for light path.
2. inlet of mercury vapor.
3. outlet of mercury vapor.

tration stage in which a second 5 cm C-18 column is applied for concentration purpose, and samples are injected through the 1000 ul loop.

Preliminary HPLC separation is investigated with UV detection. A Shimadzu UV-Visible Spectrometer (UV-160) is used for the selection of optimum detecting wavelength and the detection of mercury species in a preliminary study is achieved with an ERC-7211 UV detector from Japan.

The schematic of the interfacing reaction vessel, as shown in figure 1, is designed and sent for construction at Instrumental Center of National Science Council, Taiwan, R. O. C. The material used for this vessel is quartz with Teflon stopcocks. The eluted mercury species react with acidic perchromate solution followed by subsequent reaction with freshly prepared sodium borohydride solution. A magnetic stirrer is placed at the bottom of the vessel to facilitate the reaction.

Figure 2 illustrates the cold-vapor quartz cell which is electrically heated with heating wire to prevent condensation of moisture. The heating temperature is maintained at 150° C. The complete instrumental set-up used in this study is shown in figure 3, which includes a Perkin-Elmer 2280 AA spectrometer and an SKC air sampler pump (model 224-PCXR7).

III. PROCEDURE

Preliminary study on the separation of mercury species is carried out with the aid of thin layer chromatgraphy (TLC) and HPLC with an UV

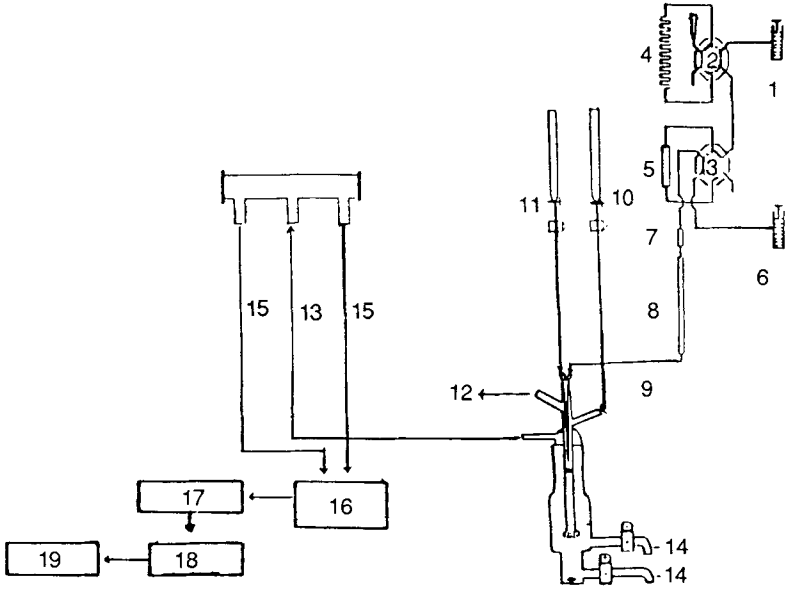


Fig. 3. Schematic of the HPLC-CVAAS with on-line preconcentration for Hg speciation.
1. preconcentration pump.
2. Rheodyne 7000 valve. 3. Rheodyne 7125 valve.
4. injection loop. 5. concentration column.
6. HPLC pump. 7. guard column.
8. analytical column. 9. inlet of effluent from HPLC.
10. reducing solution.
11. perchromate solution.
12. inlet of air. 13. Hg vapor transfer line.
14. outlet of waste. 15. outlet of Hg vapor.
16. trapping solution. 17. desiccant.
18. charcoal sorbent. 19. air pump.

detector. The optimum detecting wavelength is selected by scannig standard solutions with an UV spectrometer.

Parameters affecting chromatographic resolution, such as mobile phase composition and flow rate, are examined using the cold-vapor technique and standard solutions of fixed concentrations.

After the chromatographic resolution is optimized, parameters influencing cold-vapor detection, such as perchromate concentration and flow rate, sodium borohydride concentration and flow rate, pumping rate of mercury vapor through the quartz cell, flow rate for sample preconcentration and KBr concentration needed at the concentration stage, are studied and optimized.

Synthetic samples prepared from mercury free tap and river water are utilized after membrane flitration to evaluate the performance of the developed method.

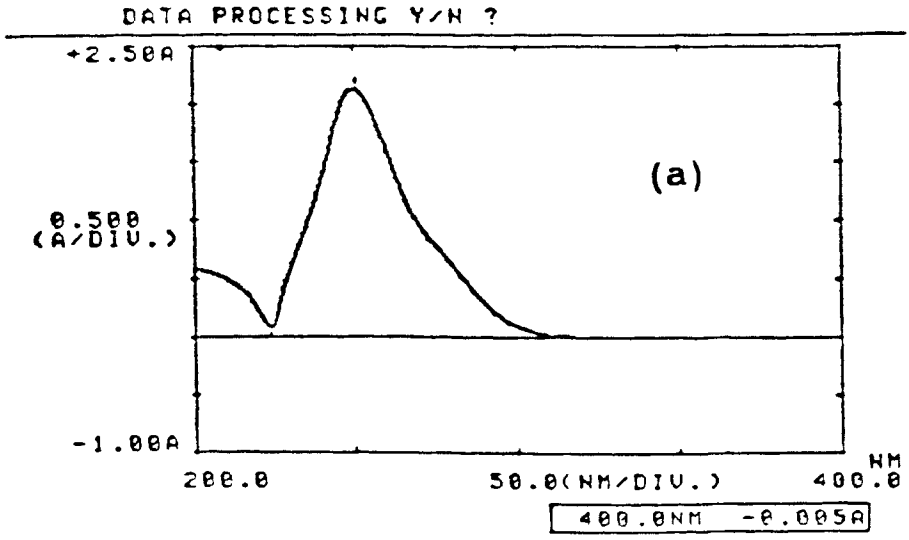


Fig. 4. UV spectra for (a). mercuric chloride, (b). methylmercuric chloride, (c). ethylmercuric chloride.

IV. RESULTS AND DISCUSSION

A. PRELIMINARY HPLC-UV STUDY

Figure 4 illustrates the UV spectra of the three mercury species; the wavelength for UV detection is selected to be 250.0 nm. The chromatogram obtained with HPLC-UV at optimized separating condition (to be disscussed later) is shown in figure 5. The fact that the use of UV detection suffers low sensitivity and interference is obvious. However, result demostrates satisfactory resolution of these species.

B. MOBILE PHASE AND FLOW RATE

The eluent used constitutes potassium bromide, which counteracts the ionic characteristic of mercury species, water and acetonitrile. The effect of mobile phase composition on the chromatographic resolution is tabulated in table 1. A mixture of acetonitrile 35%/water 65%/KBr 0.1M is chosen for this study. The effect of KBr concentration in cold-vapor detection is investigated and 0.1 M serves to give optimum detection. A separate study shows 1.00 ml/min of eluent flow gives just sufficient resolution. The result is also shown in table 1.

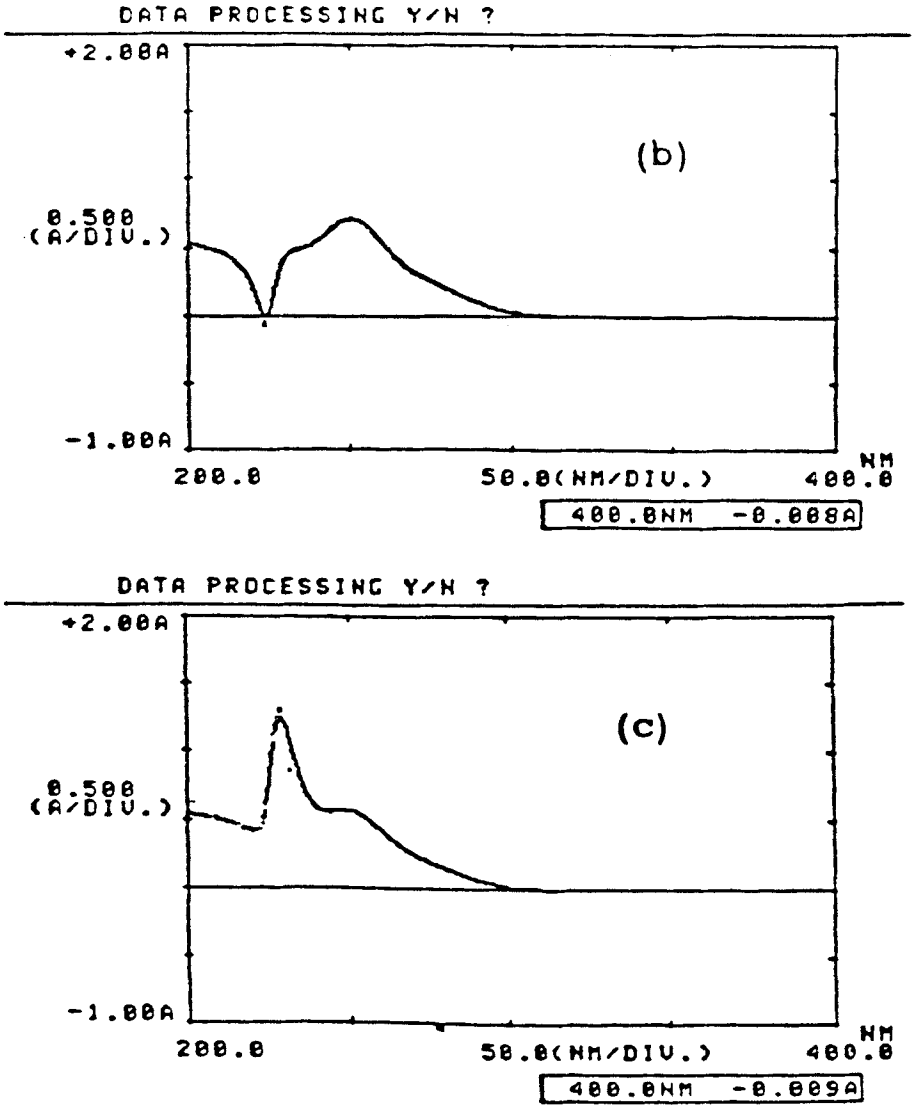


Fig. 4 Continued

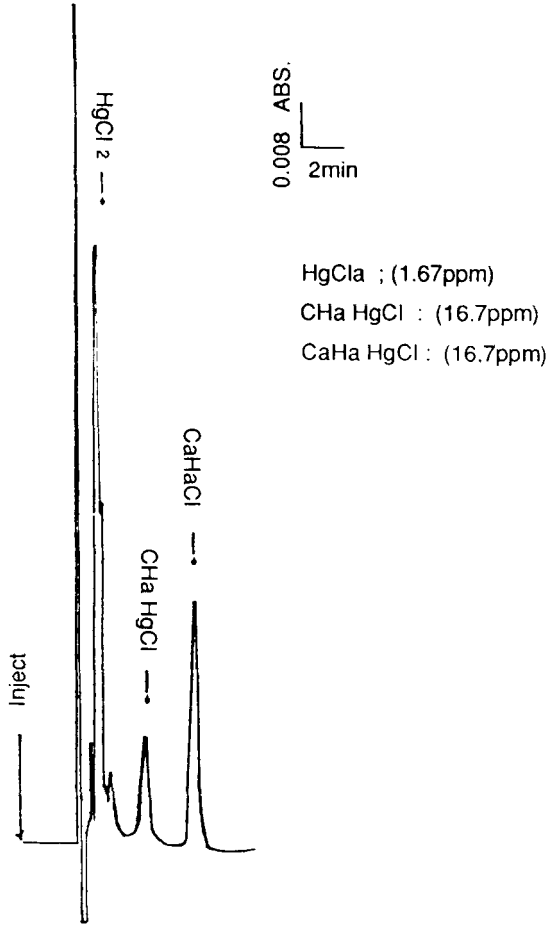


Fig. 5. HPLC chromatogram of mercury species using UV detector.

C. PERCHROMATE CONCENTRATION AND FLOW RATE

Perchromate is applied to convert organic mercury species into inorganic mercuric form and facilitates the subsequent reaction with sodium borohydride. The quantity of perchromate should be sufficient for the convertion of organic mercury and its flow must be accommodated by the reaction vessel. Both concentration and flow rate for perchromate are studied and optimized at 0.5% and 1.20 ml/min. If the quantity of

Table 1. The effect of mobile phase composition on the chromatographic resolution.

	Resolution	
Moble phase	$HgCl_2$-CH_3HgCl	CH_3HgCl-C_6H_5HgCl
CH_3CN55%/H_2O45%/KBr0.1M	0.89	0.48
CH_3CN50%/H_2O50%/KBr0.1M	1.09	0.65
CH_3CN45%/H_2O55%/KBr0.1M	1.19	0.73
CH_3CN40%/H_2O60%/KBr0.1M	1.45	1.18
CH_3CN35%/H_2O65%/KBr0.1M	1.56	1.46
CH_3CN30%/H_2O70%/KBr0.1M	1.70	2.01

flow rate : 1.0 ml/min

perchromate is too high, its reaction with subsequent reagent, sodium borohydride, will affect the cold-vapor reaction.

D. SODIUM BOROHYDRIDE CONCENTRATION AND FLOW RATE

Figure 6 explains the effect of $NaBH_4$ concentration and flow rate on the detection of mercury species. The concentration 0.5% is chosen along with the flow rate 2.2 ml/min. Although the selected conditions don't seem to be the most pertinent for ethylmercuric species, sufficient sensitivity is obtainable for other interested components. The result indicates higher degree of ease to reduce inorganic mercury than organic ones.

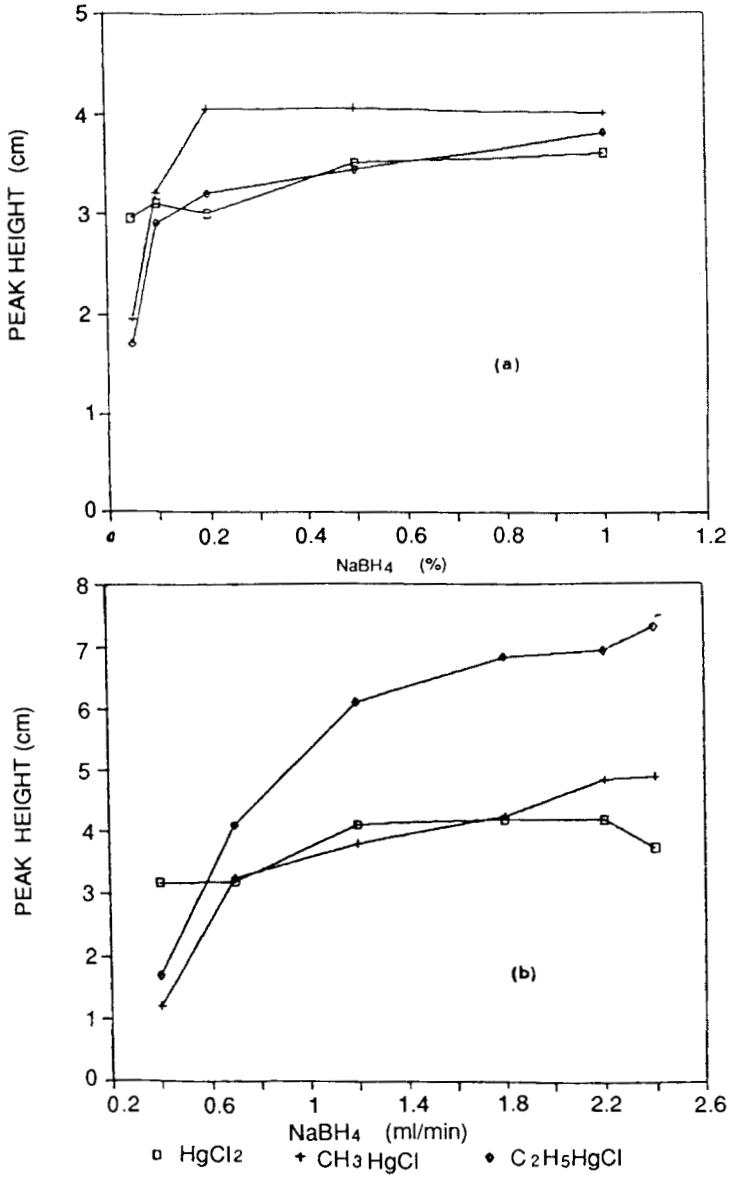


Fig. 6. Effects of (a). sodium borohydride concentration and (b). flow rate on signal heights.

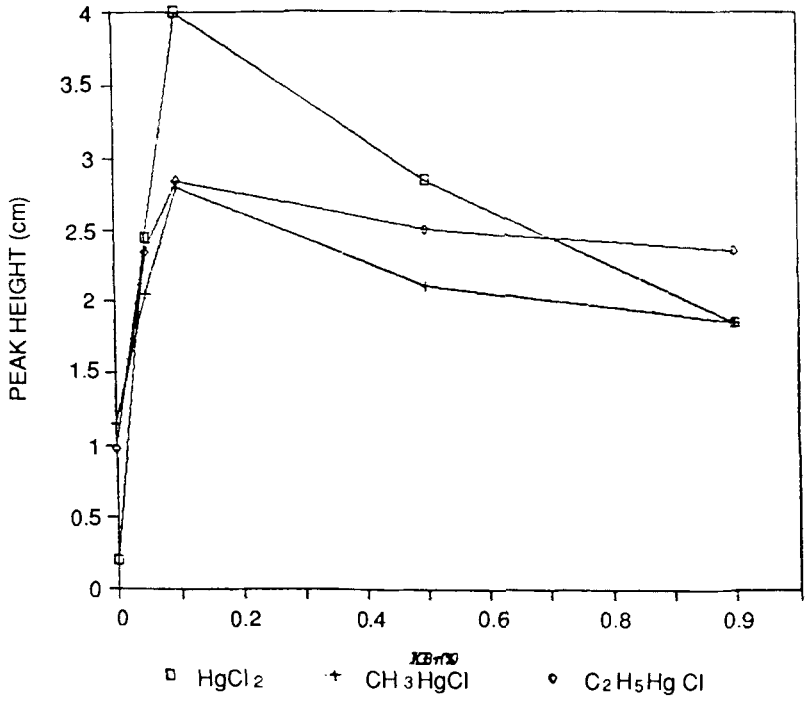


Fig. 7. Relationship between peak height and the KBr concentration at preconcentration step.

E. COLD-VAPOR PUMPING FLOW RATE AND KBr CONCENTRATION AT PRECONCENTRATION STAGE

The generated elemental mercury vapor is introduced into the quartz absorption cell with the application of an air pump used for the sampling of environmental air samples. An optimum pumping flow rate is evaluated and set at 0.60 L/min. Changing the pumping flow either way affects the senistivity of detection due to insufficient sample drawn into the cell or dilution of sample by withdrawing too much air into the absorption cell.

It is necessary to spike potassium bromide into the sample at the preconcentration step to assure the retention of mercury species by the C-18 preconcentration column. The added KBr is believed to counteract the possible ionic characteristic of mercury analyte. Figure 7 shows a typical study of the effect of KBr concentration on the detection of

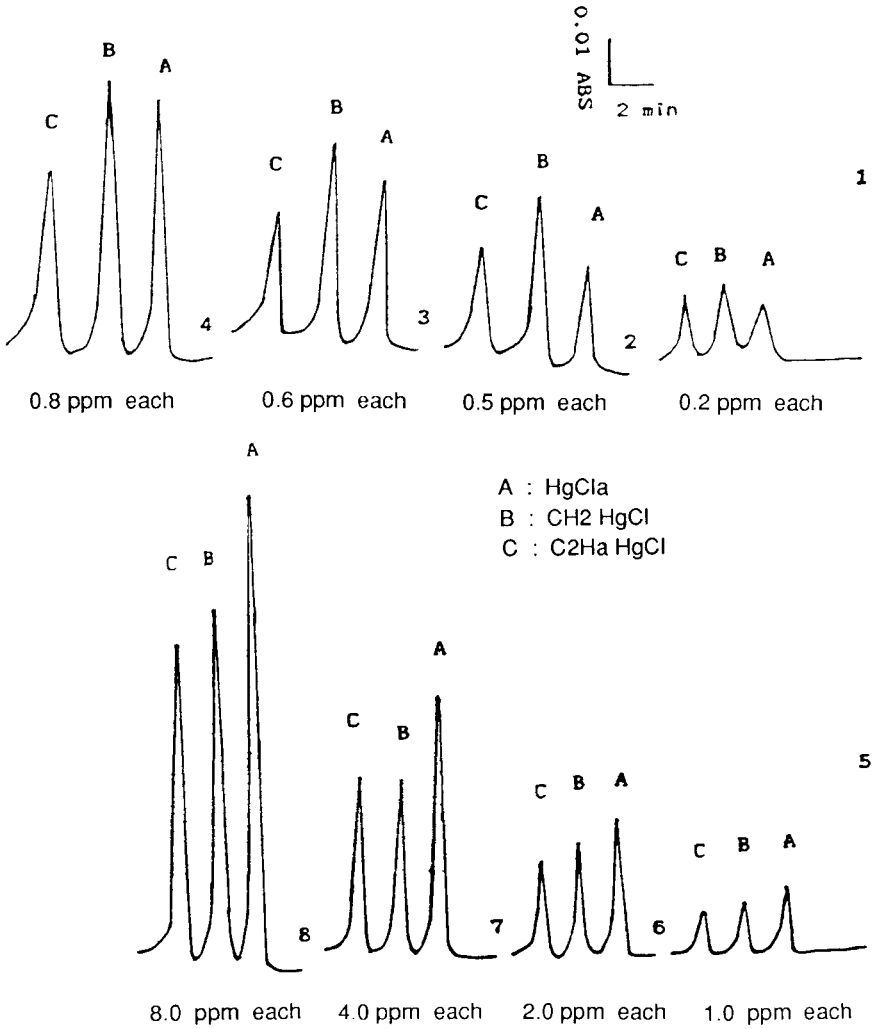
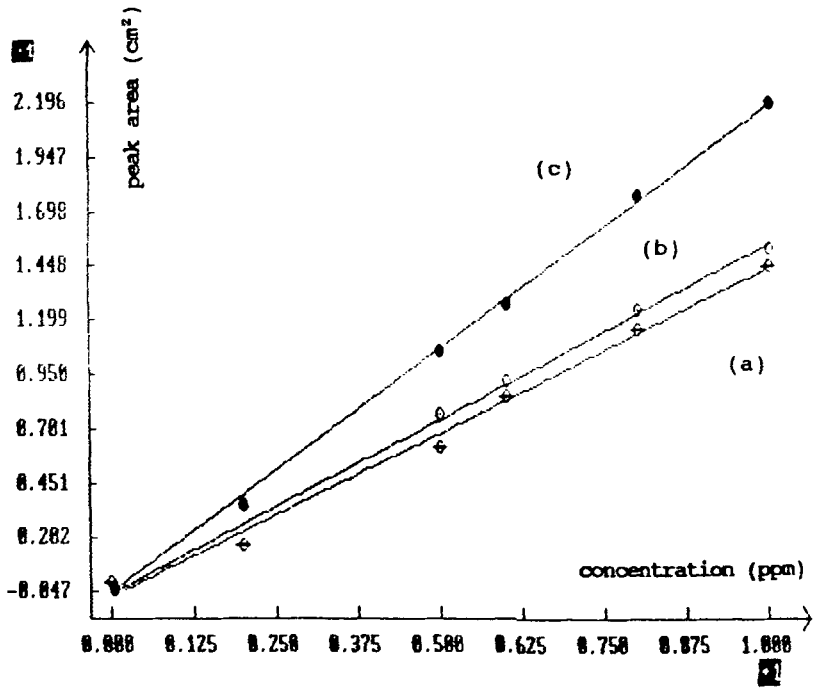


Fig. 8. Chromatograms of mercury species at different concentrations using HPLC-CVAAS without preconcentration. (Recorder range 1.0 mv: 1 - 4, 10.0 mv: 5 - 8.)



The polynomials for calibration lines are:
$Y = -7.13777e^{-5} + 1.50654*X^1$ for $HgCl_2$,
$Y = -4.68935e^{-5} + 2.23413*X^1$ for CH_3HgCl and
$Y = -5.66925e^{-5} + 1.60199*X^1$ for C_2H_5HgCl,
with correlation coefficients 0.9956, 0.999 and 0.9954, respectively.

Fig. 9. Calibration lines for a. inorganic mercury, b. methyl mercuric chloride, and c. ethylmercuric chloride without preconcentration.

interested mercury species (12.0 ppb each). The selected KBr concentration for preconcentration is 8.4×10^{-3} M with the concentration pump flow rate set at 0.80 ml/min.

F. CALIBRATION AND SYNTHETIC SAMPLE ANALYSIS

Figure 8 shows eight chromatograms at different concentrations obtained at two recorder ranges, 1.0 mv and 10.0 mv, for the studied mercury compounds using the developed method without any preconcen-

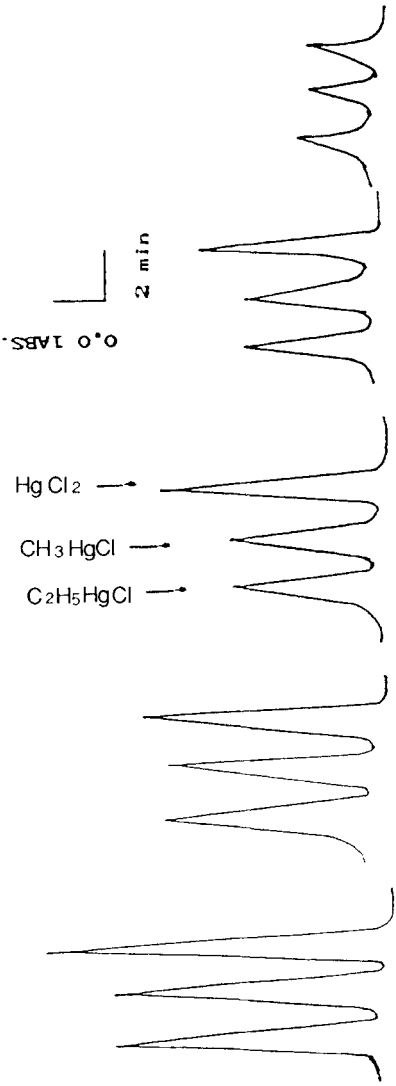
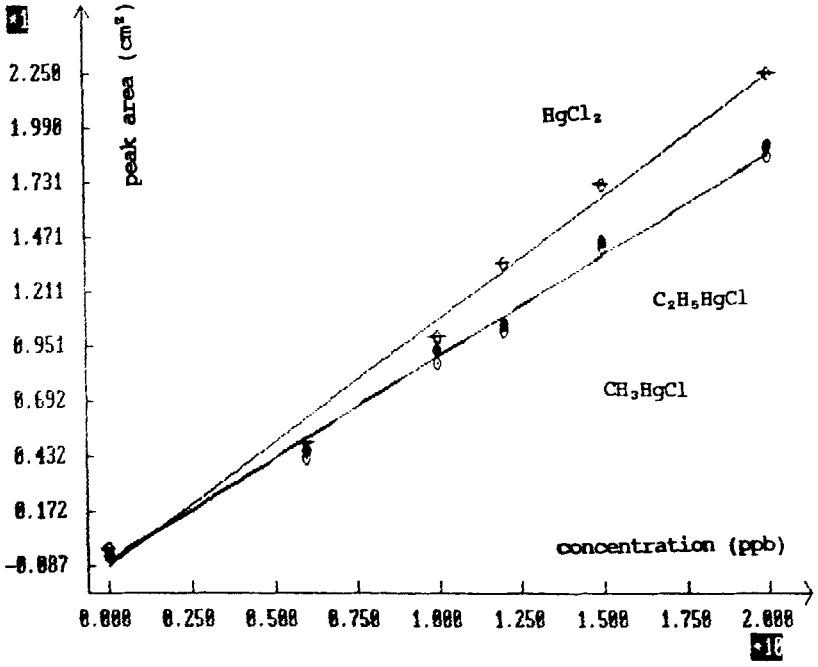


Fig. 10. Chromatograms of three mercury compounds at different concentrations using HPLC-CVAAS with on-line preconcentration.



The polynomials for calibration lines are:
$Y = -8.72717e^{-2} + 0.116708*X^1$ for $HgCl_2$,
$Y = -5.66502e^{-2} + 9.61889e^{-2}*X^1$ for CH_3HgCl and
$Y = -6.77221e^{-2} + 9.68624e^{-2}*X^1$ for C_2H_5HgCl,
with correlation coefficients 0.9952, 0.9956 and 0.9964, respectively.

Fig. 11. Calibration lines for a. inorganic mercury, b. methylmercuric chloride, and c. ethylmercuric chloride with on-line preconcentration.

tration. Figure 9 illustrates the calibration lines thus obtained. Without the aid of preconcentration unit, systhetic samples containing 0.200 ppm of each mercury component, prepared from membrane filtered and mercury free river water (pH 7.5), are subjected to multiple analysis. The detection limits, defined as three times of the standard deviations, are 0.094, 0.085 and 0.124 ppm, respectively for inorganic mercury, methylmercuric and ethylmercuric species.

With the application of preconcentration technique, limits of detection are enhanced. Figure 10 shows the chromatograms obtained by injections of standards at ppb levels into the HPLC-cold-vapor AA system

Table 2. Reproducibility and detection limits for mercury species in synthetic tap water samples using HPLC-CVAAS with on-line preconcentration.

Compound	$HgCl_2$	CH_3HgCl	C_2H_5HgCl
Standard Concentration	6.00	6.00	6.00
Measured results	4.67	4.09	5.19
	4.87	4.38	4.89
	5.20	4.18	5.23
	5.07	4.66	5.10
	4.59	4.00	4.98
Mean	4.88	4.26	5.08
S.D.	0.26	0.26	0.14
D.L.	0.78	0.78	0.42

all units : ppb

with on-line preconcentration. Sample volume introduced is 5.60 ml with multiple injection. Figure 11 indicates the calibration lines thus obtained. Synthetic samples containing these mercury species (6.00 ppb each), prepared from membrane-filtered mercury free tap water, are subjected to multiple injection. Table 2 shows the results. The calculated standard deviations range from 2.8% to 6.10%. The detection limits are 0.78, 0.78 and 0.42 ppb, respectively for inorganic mercury, methyl mercuric and ethylmercuric compounds. The larger decrease in limit of detection for ethylmercuric species may be attributed to better preconcentration efficiency by the C-18 packing material for this compound.

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