CHAPTER I

INTRODUCTION

1.1 Problem Definition

Mercury is an element that can exist in the environment in both inorganic and organic forms. Both forms are toxic, but it is the organic form that exhibits extreme biological toxicity. In human, mercury poisoning can cause injury to the central nervous system and renal damage. Mercury contamination arises mainly from its industrial uses. Liquid mercury is mostly used as a mobile cathode in the chloroalkali industry. Organomercury compounds are used in agriculture as fungicide and pesticide and for pharmaceutical purposes. However, there have been several accidents from misuse and spillage of mercury compounds such as in Iraq (1971-1972)(1) and well-known mercury accident have occurred in Minamata Bay in Japan (1950-1970). In the case of Iraq, the consumption of seed sprayed with fungicidal methylmercury compounds resulted in 459 fatalities and 6530 were hospitalised. In this case, the seed had been variously treated with methyl, ethyl, and phenylmercury. In Japan, methylmercury from a vinyl chloride-acetic acid plant entered the Minamata Bay in the effluent, it was taken up in fish food chain and consumed by the local populations whose staple food was fish. Although mercury compounds were used for more than 50 years, little is known about the behaviour of organomercury compounds in the environment. So species analysis is necessary that can be help in the understanding of the behaviour of mercury compounds, in assessments of toxicity and in developing strategies for decontamination. Understanding the environmental issues is underpinned by the need for precise and sensitive techniques that are able to accurately quantify mercury at the low concentration in which it frequently occurs. The most frequently used analytical approach is the gas chromatographic separation. However, these methods are inconsistent and have several drawbacks which seem to be good enough

reasons to search for alternative methods of carrying out mercury compounds analysis on a more reliable basis.

1.2 Literature Review

Mercury is known to exist in several forms in the natural environment. Of great interest are the organic mercury compounds, which are highly toxic. They can be released into the environment by emissions or are produced by micro-organisms. In order to investigate the transport, bioavailability, and toxicity of the different forms; there is also the necessity to quantitative identify mercury in both its organic and inorganic forms in various media, such as fish, biological tissue, water, urine, human blood, sediment, and natural gas condensate. There have been several techniques for determination of mercury ranging form titrimetry with dithizone reported by Gage (2) and Thin Layer Chromatography (TLC) by Westoo (3) to Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (4-5), and including spectrometric techniques of absorption and fluorescence, associated, in most instances, with an earlier stage of chromatographic separation—High Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC)—allowing for speciation.

The cold-vapour atomic absorption spectrometry (CV-AAS) (6-27) method is the accepted method for determination of ultra-trace concentration of mercury in solution. The method is based on the chemical reduction of mercuric ions to element mercury with reducing agent. The element mercury is then swept out of solution with a carrier gas into an absorption tube where the atomic absorption at 253.7 nm is measured. Inorganic mercury was selectively determined when using stannous chloride as a mild reductant. Total mercury was determined in two ways.

I) All forms of mercury were converted to inorganic mercury by oxidative pretreatment with permanganate (6), bromide/bromate reagent (7), potassium peroxodisulfate (8), potassium persulfate (9), or photo-oxidation (10).

II) All forms of mercury were reduced to vapour mercury by strong reducing agent, e.g., $NaBH_4$ (10-12) or $CdCl_2$-$SnCl_2$ (13-15) solution.

Then organomercury concentrations were computed as the difference between the values obtained from the two analyses. There have been many improvement of CVAAS method including the developed classical CVAAS method to flow injection CVAAS (8-11, 16), the developed method to the analysis in organised media by Sanz-Medel (16) and Curtius (11). The improvement of sample preparation from liquid-liquid extraction with cysteine complex from Westoo method (3, 18-19) to chelating resin preconcentration by Minagawa (15) and Polito (20). The used of amalgamation with noble metal have been developed by Bloom (21), Dybdahl (22), Hoste (23), Roberts (24), McIntosh (25) and Takahashi (26). Recently, the detection limit of CVAAS method is low as sub-nanogram levels. However, this method does not permit determination of specific form of organic mercury.

Gas chromatography with electron capture detection (ECD) has also been widely employed in identifying individual organomercury (27-29) but the use of extremely pure solvent is necessary, together with tedious clean-up procedures, to avoid co-elution of electron-capturing species with the organomercury compounds. An alternative procedure in which the electron capture detector is replaced by atomic emission spectrometry (AES) (30-38), atomic absorption spectrometry (AAS) (39-40), atomic fluorescence spectrometry (AFS) (41-43), and mass spectrometry (44-46). However, the organomercury chloride must be converted to more volatile bromide derivatives (28) and iodide derivatives (46).

A variety of stationary phases has been recommended (46-48) but all of these columns have exhibited the disadvantage, which is caused by organomercury halides. The organomercury halides affect the chromatographic performance due to the polar nature of the Hg-Cl bond, resulting in peak tailing and degradation of the species due to thermal instability on the column, as well as column degradation. In order to by-pass this problem, the derivatization techniques, ethylation with sodium tetraethylborate (34-35, 39-41, 45, 49) or butylated with butylmagnesium bromide (33), are used to convert the monoalkylmercurials to dialkyl derivatives prior to analysis. However, these techniques usually aim at methylmercury and some organomercury compounds cannot be analysed, e.g. ethylmercury, if ethylation is performed.

Most of gas chromatographic methods require extensive extraction and clean-up steps, and employ a variety of instrumental conditions. A simplified and rapid method for all sample types is highly desirable. Liquid chromatography (LC) allows a simpler sample treatment and has been shown able to separate a great variety of organic mercury compounds. The use of HPLC offers several advantages. The separation of the mercury compounds is performed at ambient temperature; hence decomposition reactions during the chromatographic run are unlikely. It is also possible to determine less volatile or non-volatile species, which is usually a problem in GC determinations.

The speciations of mercury compounds by using high performance liquid chromatographic techniques have been reported as followed.

Holak (50) determined methylmercury in fish by high performance liquid chromatography with atomic absorption. Methylmercury was isolated from sample by chloroform elution from a diatomaceous earth-hydrochloric acid column, and then extracted into a small volume of sodium thiosulfate solution. This solution was injected on the reversed phase column and eluted with methanol-ammonium acetate solution buffer, pH 5.5 (3:2) containing 0.01% 2-mercaptoethanol. The detection limit of methylmercury was 0.6 ng.

Fujita and Takabatake (51) used liquid chromatography couples with cold vapour atomic absorption spectrometry for determination of mercury compounds. The eluents of HPLC were a mixed solution (70:30:1) of acetonitrile, water, and dimethylsulfonate for mercury alkane thiolate, and 0.2M cysteine in 0.02M acetic acid (pH 2.2) for alkyl and inorganic mercury compounds, respectively. Thus, CVAAS works as a continuous detector for mercury in the effluent from HPLC. When stannous chloride is used as a reductant, mercury alkane thiolates were monitored. For the detection of inorganic, methyl, and ethylmercury compounds, sodium borohydride was used.

Krull and co-worker (52) used inductively coupled plasma (ICP) emission spectrometer as a high performance liquid chromatographic detector for the determination of mercury compounds. Four mercury compounds; inorganic mercury, methylmercury, ethylmercury, and dimethylmercury, were separated on reversed phase column with a mobile phase consisting of 0.06 M ammonium acetate and 0.005% v/v 2-mercaptoethanol with a gradient from 15 to 70% acetonitrile within 22 minutes. An aqueous solution of sodium tetrahydroborate(III) in sodium hydroxide solution and a solution of hydrochloric acid served as reagents for the post-column reaction system. Detection limits ranged from 32-62 ppb of mercury for four mercury compounds.

Langseth (53) determined organic and inorganic mercury compounds in human urine, tap water, and tomatoes. The mercury compounds were extracted into toluene or chloroform with dithizone. The mercury chelates were separate by reversed phase high performance liquid chromatography and were detected at 475 nm. Complete resolution was obtained between methyl, ethyl, phenyl, and inorganic mercury with a mobile phase of THF/methanol (2:1) with 0.05 M acetate buffer pH 4 (62:38), containing 50 μM EDTA.

Mckee and Evan (54) studied the speciation of inorganic, methyl, ethyl, and phenyl mercury by high performance liquid chromatography with reductive amperometric electrochemical detection. The mobile phases were mixtures of acetonitrile-water (10:90 to 60:40 m/m), methanol-water (20:80 to 70:30 m/m) including 0.01% v/v 2-mercaptoethanol buffered to pH 5.5 with ultrapure acetic acid and ammonia solutions for separation of mercury compounds on reversed phase column. The electrode potential (-0.800 V vs. Ag/AgCl) utilised for detection was the optimum for the simultaneous determination of all species. The detection limit of inorganic, methyl, ethyl, and phenylmercury were 1.8, 1.9, 1.7, and 0.8 ppb, respectively.

Bushee (55) determined mercury species by liquid chromatography-inductively coupled plasma-mass spectrometry. The separation was performed on C_{18} column with a mobile phase consisting of 0.06 M ammonium acetate, 3% acetonitrile and 0.005%

v/v 2-mercaptoethanol buffered to pH 6.8. A solution of 0.5% sodium tetrahydroborate(III) in 0.25M sodium hydroxide and a solution of 1.2 M hydrochloric acid were used for post-column mercury cold vapour generation. Methyl, ethyl, and inorganic mercury were fully resolved and the separation was completed in less than 18 minutes. The detection limit for methyl, ethyl, and inorganic mercury were 7, 16, and 16 ppb, respectively. The method was applied to the determination of methylmercury in as NBS RM-50 Albacore tuna sample and to the determination of ethylmercury in contact lens solutions.

Evans and Mckee (56) used a combination of reductive electrochemical detection with reversed-phase liquid chromatography to determine inorganic and three organomercury compounds in spiked water samples. The chromatographic analytes were performed in 60% methanol containing 0.01% v/v 2-mercaptoethanol buffered to pH 5.5 with acetic acid and ammonia solution. Inorganic, methyl, ethyl, and phenylmercury were eluted within 6 minutes and detection limits were 1.8, 1.9, 1.7, and 0.8 ppb, respectively.

Gaston Wu (57) designed a quartz reaction vessel to interface HPLC and cold vapour AA for mercury speciation in aqueous samples. Inorganic, methyl, and ethylmercury can be separated within 10 minutes on reversed phase column. The eluent used constitutes 0.1M potassium bromide (which counteracts the ionic characteristic of mercury species) water and acetonitrile 35:65%. The detection limit without preconcentration for the three mercury species were 0.094, 0.085, and 0.124 ppm, respectively. Limits of detection were reduced to 0.78, 0.78, and 0.42 ppb, with the use of preconcentration technique. This method was applied to analysing mercury compounds in synthetic tap and river water samples.

Houk, Shum, and Pang (58) speciated mercury and lead compounds in human urine. Various cationic species of mercury (inorganic, methyl, ethyl, and phenylmercury) and lead (inorganic, trimethyl, triethyllead) were separated as ion pairs by reversed phase liquid chromatography and detected by inductively coupled plasma mass spectrometer. A solution containing 5 mM ammonium pentanesulfonate in 20:80

v/v acetonitrile -water served as a mobile phase. Detection limits were 6-18 pg for the four mercury compound and 0.2 pg for all lead compound.

Wilken, Hintelmann, and Hempel (59) separated nine organomercury compounds: methyl, ethyl, phenyl, methoxyethyl, ethoxyethyl, benzoic, and tolyl mercury by high performance liquid chromatography with UV detection. The nine compounds were successfully separated on reversed phase column by gradient elution with a methanol-water mixture ranging from 30 to 50% v/v. The detection limits for the various compounds were in the range 7.0-95.1 ppb. The extraction of organomercury compound from spiked soils were studied and provided recoveries in the range 53-81%.

Mentasi and co-worker (60) determined mercury compounds simultaneously using high performance liquid chromatography coupled to cold vapour atomic absorption spectrometry. Methyl, ethyl, phenyl, and inorganic mercury were separated on reversed phase column and eluted with an acetonitrile-water (58:42 v/v) containing 0.5 mM ammonium pyrrolidine chloride (APDC) buffered to pH 5.5. The detection limit was 0.8 ng of inorganic mercury and was in the range 5-30 ng for three organic forms. The method was applied to the determination of synthetic mixtures and natural samples of tap water.

Jiang and Huang (61) used inductively coupled plasma mass spectrometer as a detector for the liquid chromatographic determination of mercury compounds. Three mercury compounds (methyl, ethyl, and inorganic mercury) were fully resolved and the separation was completed in less than 15 min. on reversed phase column. The mixture of 3% v/v methanol-1.5% v/v acetonitrile-0.1% v/v 2-mercaptoethanol containing 0.06 M ammonium acetate was served as mobile phase. The detection limits of three compounds were in the range 70-160 pg of mercury.

Whang and Wang (62) used revered phase liquid chromatographic method for the determination of inorganic mercury and organomercury in aqueous solution. Methyl, ethyl, phenyl, and inorganic mercury can be separated in less than 9 minutes

with an eluent of methanol-10 mM sodium acetate buffer (80:20 pH 6.2) containing 0.1 mM 2-mercaptobenzothiazole. The UV detection was carried out at 285 nm. Detection limit ranged from 0.3 to 0.5 ng of Hg. Interference due to metal ions can be eliminated by inclusion of a low concentration of EDTA.

Wilken and Hintelmann (63) separated eight organomercury compounds by high performance liquid chromatography with mixtures of methanol-water (30:70 to 50:50) buffered with ammonium acetate and modified by 2-mercaptoethanol 0.1 mM as mobile phase. Methyl, methyoxyethyl, p-mercuribenzoic acid, ethyl, ethoxyethyl, phenyl, nitromersol, and tolylmercury were separated completely less than 22 minutes. The mercury compounds were detected by cold vapour atomic fluorescence spectrometer (CV-AFS) and detection limit of all species was at the 2.5 ng level.

Cela and co-worker (64) used reversed-phase high performance liquid chromatography for methylmercury determination in marine sample. The mercury compounds, inorganic, methyl, and phenylmercury were treated precolumn with sodium diethyldithiocarbamate as a chelating agent, and then separated with a mixture of methanol-water 70:30 v/v containing 100 μM EDTA. The detection limit of the procedure was 0.2 ng of methylmercury.

Robecke, Cammann, and Bettmer (65) used 2-mercaptoethanol and methyl thioglycolate for the high performance liquid chromatographic separation of ionic lead and mercury compounds with subsequent UV detection. For the first time on-column derivatisation with 2-mercaptoethanol was used for the separation of lead compound. Gradient condition methanol-0.2 citric acid pH 6.7 (20:80 to 55:45 v/v) containing 0.02% 2-mercaptoethanol was optimised for separation. The use of second complexing agent with methanol-0.1 M citric acid pH 5.8 (40:60 v/v) containing 0.02% methyl thioglycolate made it possible to determine all analytes, organolead and organomercury compounds, simultaneously.

Scholer and Falter (66) used high performance liquid chromatography with cold vapour atomic absorption detector for determination of mercury compounds.

Methyl, ethyl, phenyl, and inorganic mercury were separated on C_{18} column using acetonitrile-water (65:35 v/v) buffered with ammonium acetate-acetic acid at pH 5.5 containing 0.5 mM sodium pyrrolidinedithiocarbamate (SPDC) as mobile phase. A solution of 1% sodium borohydride adjusted to pH 13 with 1M sodium hydroxide was used for reduction and UV-irradiation lamp was used for the on-line destruction of the organomercury compounds. The limit of detection for the four tested compounds were the same at 80 pg absolute.

Uden and Ho (67) used tetrabutylammonium bromide as ion pair reagent and sodium chloride in a methanol-water mixture as mobile phase for inorganic and organomercury speciation. UV and three electrodes direct current argon plasma specific element were employed as detector. Inorganic mercury and benzylmercury showed much greater UV response then other mercurials. The detection limits of UV detection were in the range of 0.2-8.0 ng for inorganic, methyl, ethyl, phenyl, and benzylmercury.

Sanz-Medel and co-worker (68) used high performance liquid chromatography coupled to cold vapour atomic absorption spectrometry detection for the speciation of inorganic and methylmercury. The mercury species can be separated within 8 minutes by using a vesicular mobile phase of didodecyldimethylammonium bromide in water containing 0.005% v/v of 2-mercaptoethanol, 5% acetonitrile and buffered with ammonium acetate at pH 5. Detection limits of 0.1-0.2 ppb of mercury were achieved after off-line preconcentration of the aqueous samples using C_{18} Sep-pack cartridges modified with 2-mercaptoethanol solutions. This approach has been applied to the speciation of inorganic mercury and methylmercury in spiked seawater and human urine. Recoveries were obtained ranged between 91-103% for both species.

Trombini and Fabbri (69) determined inorganic mercury(II) in natural waters. The derivatisation of inorganic mercury to diphenylethylmercury with simultaneous extraction into dichloromethane was proposed. The organomercury derivative was analysed by HPLC using UV detection. All analytes were performed at room temperature under isocratic condition using an acetonitrile-water 60:40 v/v mobile

phase and detected at 265 nm. The detection limit was 0.1 ng. The effects of potential interfering agents were studied.

Sarzanini and co-worker (70) studied the determination of methyl, ethyl, and inorganic mercury by using ion chromatographic techniques interfaced with cold vapour atomic absorption spectrometry for detection. The mercury species were separated as cysteine complexes. The complexes were positively charged and the main mechanism expected for the chromatographic separation was cation exchange. The effects of ionic strength, pH, and ligand concentration were evaluated in order to achieve the maximum selectivity, sensitivity, reproducibility, and resolution. The detection limits evaluated on 100.00 mL samples were 2, 10, and 4 ng for inorganic, methyl, and ethylmercury, respectively.

Broekaert and Schickling (71) used high performance liquid chromatography coupled with cold vapour atomic absorption spectrometry for the speciation of mercury species. Inorganic, methyl, phenyl, and diphenylmercury were separated by reversed phase column using gradient elution with mixture of acetonitrile-aqueous potassium bromide solution (0.1 M) (35:65-100:0) used as mobile phase. Mercury compounds were oxidised with potassium dichromate solution, reduced to metallic mercury by a treatment with sodium borohydride solution, and then detected at 253.7 nm. Under optimised condition the detection limits for the four mercury compounds were 9, 9, 8, and 14 ng for inorganic, methyl, phenyl, and diphenylmercury, respectively. The method was applied to the speciation of mercury in two gas condensates and did not require use of any solvent extraction or chemical derivatization steps. In gas condensates, inorganic mercury was found present at the 100 ng/mL level.

Jiang, Wan, and Chen (72) determined inorganic, methyl, and ethylmercury in water by liquid chromatography-inductively coupled plasma mass spectrometry (LC-ICP-MS). The ionic mercury compounds were separated by reversed phase LC with 0.5% m/v L-cysteine solution as the ion pairing reagent and the mobile phase. Effluent from the LC column was delivered to the vapour generation system and ICP-MS

system for mercury determination. The limits of detection for various mercury species were in the range 0.03-0.11 ppb based on peak height. The method was applied to the determination of mercury compounds in open seawater reference material NASS-4 and tap water sample.

1.3 Hypothesis

The high performance liquid chromatography (HPLC) is a simple and an effective method for the speciation of inorganic and organomercury. The mercury compounds can be separated by reversed phase, normal phase, and ion-exchange techniques. Reversed phase liquid chromatography has emerged as the most popular technique because it is usually highly efficient, simple, reproducible, and able to simultaneously analyse a range of species that are either closely related or widely different. The reversed phase technique is suited to separations of non-polar and moderately polar species. Additionally, polar and ionic species have been separated by using secondary chemical equilibria liquid chromatography (SCE-LC) such as acid-base, ion-pairing, metal complexes, and solute-micelle. From the literature reviews, the most separation mechanism of ionic mercury compounds on reversed phase column was performed by *in situ* complexation with complexing agent containing sulfur atom. The term of *in situ* complexation chromatography has proved to be particularly amenable to the determination of trace metal ions. The metal species are formed *in situ* by a complexing agent that has been added to the eluent. The metal species are injected directly into the mobile phase and following complexation are separated by reversed phase. 2-Mercaptoethanol is widely used as a complexing agent for speciation of mercury compounds (50, 52, 54-56, 61, 63). The other mechanism is the ion-pair chromatography. This was adapted mainly from the limitations of ion-exchange chromatography and from the difficulty in handling certain samples by the other method. In this system, mobile phase consists of an aqueous buffer plus an organic solvent and an added ion-pairing agent with oppositely charge to the sample molecule. The sample can be separated on the reversed phase column with the interaction of solute, ion-pairing agent, and the non-polar stationary phase. The speciation of

mercury compounds by the ion-pair chromatography was performed with both anion pairing (58) and cation pairing (67). The complexation chromatography is generally used for speciation of mercury species but this mobile phase contains relative high percentages of organic solvents. In ion-pair chromatography, aqueous is the most proportion of mobile phase. From the disadvantage about the high organic solvent in complexing chromatography, the reducing of organic content was performed by added ion-pairing agent in mobile phase. Furthermore, the selectivity was promoted by the competition between complex formation and the ion-pair formation of analyte to give more resolution. The lower sensitivity of spectrometric detection for ion-pairing chromatography was also increased by higher absorption complex molecule. Thus, the mixed mode of reversed phase separation, complexation and ion-pair, was introduced to reduce the organic solvent content in mobile phase, to increase the selectivity of the separation, and to increase the sensitivity of spectrometric detection from the use of only one mode of separation, complexation or ion-pair chromatography.

1.4 The Purpose of the Study

This work use high performance liquid chromatography with photodiode array detector for the speciation of inorganic mercury, methylmercury, and phenylmercury. The effective method with high resolution, selectivity, sensitivity, rapid, and simple is the aim of this work. Two mobile phase system are studied for the speciation of mercury compounds.

A. The mixed anion-pairing-2-mercaptoethanol system.

B. The mixed cation-pairing-2-mercaptoethanol system.

The various parameters affecting the separation and sensitivity of mercury compounds are studied to determine the optimum conditions. The various parameters in each system are:

1. The pH of mobile phase.
2. The complexing agent (2-mercaptoethanol) concentration.
3. The ion-pairing agent concentration.
4. The composition of methanol in mobile phase.
5. The mobile phase flow rate.
6. The injection volume.

Linearity, calibration curve, detection limit, and precision of mercury compounds in each system are studied. In addition, the retention mechanism of mercury compounds are also proposed.