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Speciation of Mercury by Chromatography Coupled with Atomic Spectrometry

Armstrong, Helen Elisabeth Louise

Speciation of Mercury by Chromatography Coupled with Atomic Spectrometry

by

Helen Elisabeth Louise Armstrong

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Abstract

Helen Elisabeth Louise Armstrong

Speciation of Mercury by Chromatography Coupled with Atomic Spectrometry

A commercial GC-AFS instrument has been developed and optimised for the speciation of organomercury. This instrument couples a GC oven to a modified atomic fluorescence detector via a ceramic pyrolyser. Organomercury compounds in dichloromethane solvent were directly injected through a Programmable Temperature Vaporiser Injector onto a DB1 Megabore column. Once separated, the compounds eluted from the column and were atomised in the pyrolyser then detected by AFS. The direct injection technique, ceramic pyrolysis design and argon purged detector have improved previous instrument designs by enhancing and maintaining sensitivity. The instrumental limit of detection was determined to be 0.25 pg Hg absolute.

Methods were developed for the extraction of methylmercury from a variety of marine samples. The techniques were validated using mussel homogenate and dogfish liver (IAEA 142, SRM 8044 and DOLT-2) certified reference materials. An interlaboratory comparision exercise was participated in and a method was developed for the detemination of methylmercury in *Fucus* sea plant (IAEA 140). A concentration of 0.63 $\pm$ 0.006 ng g^{-1} was reported. The material is now certified at 0.626 $\pm$0.139 ng g^{-1}. Of all the participating laboratories, this was the closest result to the certified value.

The instrument and methods were also applied to soil and sediment samples. Once again validation was performed with a CRM sediment, IAEA 356. Although this material has been reported to give positive artifact formation when using a steam distillation sample preparation procedure, good agreement and no artifacts were observed upon analysis. A further contaminated land, an uncontaminated soil and sediment sample were also studied. For all the samples studied by GC-AFS total mercury measurements were also made following an appropriate digestion procedure and CV-AFS.

A gas chromatograph was also coupled with ICP-MS and HPLC was coupled to CV-AFS as comparative techniques. Both approaches were optimised and validated with CRM's. The GC-ICP-MS had the advantage of providing additional element information and confirmed the presence of methylmercury bromide in the final mussel homogenate extract. The HPLC approach found to be much less sensitive than the GC techniques and also suffered from vapour generation interferences.

The PTV injector was considered for large volume injection and thermal desorption techniques. Injector breakdown problems were overcome by optimising the conditions and solid phase adsorbent for cold splitless injection. A recovery of 70% was achieved for a 50 μl large volume injection of methylmercury chloride in DCM. This technique indicated the possibility that LVI may in the future offer increased method sensitivity.

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Author's Declaration

At no time during the registration for the Degree of Doctor of Philosophy has the author been registered for any other University award.

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Various training courses were attended including the TCS Associate Development Modules: 1 - Project Handling Issues, 2 - Personal and Teamwork Issues, 3 – Environment (environmental auditing) and 4 - Business Management Skills in addition to training courses run by PS Analytical on Cold Vapour – Atomic Fluorescence Spectrometry and Hydride Generation - Atomic Fluorescence Spectrometry.

Relevant scientific seminars and conferences were regularly attended at which work was often presented; external institutions were visited for consultation purposes and papers prepared for publication.

Louise Armstrong

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PSA Application Notes

Determination of Methylmercury in Mussel Homogenate.

Determination of Total Mercury in Mussel Homogenate.

Determination of Mercury in Contaminated Land.

The Use of the PSA Millenium Merlin for the Determination of Mercury in a Polluted Marine Sediment.

Determination of Methylmercury in Marine Liver Tissue by Gas Chromatography-Atomic Fluorescence Spectrometry.

Determination of Methylmercury in *Fucus* Sea Plant by Gas Chromatography-Atomic Fluorescence Spectrometry.

Determination of Total Mercury in *Fucus* Sea Plant using the Merlin Plus CV-AFS System.

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External Contacts:

Florida International University, Miami, USA – Prof R Jones and Dr Y Cai

University of Eindhoven, Netherlands – Dr H Gerd Janssen

Chapter 1

INTRODUCTION

This thesis outlines a programme of research undertaken to develop novel, reliable and robust methods and instrumentation for the speciation of mercury in a range of sample matrices. Chapter 1 reviews the distribution of mercury in the environment and briefly considers its chemical forms. A review of atomic spectrometric detection methods for mercury was then made, initially considering total mercury measurements followed by a review of separation techniques coupled to atomic spectrometric detection for speciation studies. This review particularly considered sample preparation procedures and problems associated with mercury speciation methods. After completing this literature study the aims and objectives of the research programme were clearly defined.

1.1 Occurrence of Mercury Compounds in the Environment

Mercury is ubiquitous in the environment. It has been present in the earth since the earliest of times. Natural processes such as weathering have aided its distribution throughout the solid, liquid and gaseous phases of Earth, where it has become incorporated into living tissues. In most cases mercury is only present at trace and ultra trace levels, but there are numerous areas throughout the world, such as the Almaden mine in Spain, where highly concentrated deposits can be found. The usefulness of mercury was recognised thousands of years ago and as life and technology have developed, natural reserves have been exploited. In relatively recent time high concentrations of mercury were recognised as being harmful to life. However, it is now known that certain chemical forms are more toxic than others due to their interaction with biochemical processes. In this section, natural and anthropogenic sources of mercury will be discussed and related to its cycle

within the environment. The harmful effects of mercury compounds will also be considered along with modern measures in place to limit exposure.

1.1.1 Natural Sources

Mercury is distributed in all areas of the ecosystem. It can be found naturally in elemental, inorganic and organic forms. Elemental mercury is a silver-white liquid metal that forms amalgams with most other metals except iron. It is sensitive to temperature and pressure and easily volatilises to an atomic vapour. In the earth's crust mercury is commonly found in minerals and ores. Its most common ore is Cinnabar, HgS, although this is estimated to account for only 0.02% of the total mercury concentration within the crust.[1] Natural weathering and volcanic activity release metallic and particulate bound mercury into the atmosphere. Precipitation then re-deposits it onto soils or into watercourses. Leaching from rock and soils is also responsible for the transfer of mercury compounds into water whilst submarine leaching is particularly important in seas and oceans. The most common forms of mercury in salt-water bodies are halo-complexes. Organometallic forms of mercury also occur in nature. In general these are monomethylmercury and dimethylmercury. It is well established that microorganisms can methylate inorganic mercury mainly via the enzyme methylcobalamin. The most predominant reaction in nature involves the carbanion:

$$CH_3CoB_{12} + Hg_2^{+} \xrightarrow[H_2O]{} CH_3Hg^{+} + H_2OCoB_{12}^{+} \qquad [1.1]$$

Where $CoB_{12}^{\cdot}$ = represents cobalamin enzyme

The methylation of mercury via methylcobalamin is most likely to occur in sediments and is enhanced with low pH and high sulphate levels. It is inhibited in the presence of H_2S.

Under suitable conditions, this reaction will proceed to give dimethylmercury but kinetic studies have shown that the addition of the second methyl group occurs much more slowly. Dimethylmercury is a very volatile compound and is known to permeate out of sediments. It has also been shown to undergo decomposition to the more stable monomethylmercury either through the further actions of microorganisms or through photolytic degradation. A number of reviews on the mechanisms of methylation and demethylation have been published [2-7]. These include studies into the role that microbial activity plays in the mercury cycle [8-10].

The uptake and bioconcentration of methylated mercury in the aquatic food chain has been established. A report by Jernelov and Jenssen in 1969 is commonly cited as one of the earliest studies confirming high concentrations of methylmercury in fish [11]. More recent studies have also presented evidence that normal bacterial flora in the gills and guts of fish may also contribute to methylmercury body-burden through in-vitro methylation of inorganic mercury [8], however this is thought to account for only a small amount. Recent reports have suggested similar formation in mammalian livers.

In addition to naturally occurring methylmercury compounds, the formation of ethylmercury in pea plants when exposed to elemental mercury vapour was reported by Fortman, Gay and Wirtz [12] some twenty years ago. No explanation has so far been offered as to the pathway involved although it is generally agreed by the biochemical community that ethylation cannot occur following the same mechanism as methylation [13].

In further studies, wide ranges of mercury compounds have been reported in gas condensates, depending on the origin, temperature and pressure of the condensate [14]. These include Hg^0, Hg^{II}, RHgX and R_2Hg. It can be concluded therefore that as a result of

its inherent reactivity and availability, natural sources and forms of mercury are many-fold and perhaps not yet fully understood.

1.1.2 Anthropogenic Sources

Man has used mercury compounds for thousands of years. Archaeologists have found drawings in the ruins of ancient Egypt and Pakistan featuring Cinnabar, HgS ore, as a red pigment. The Romans also used Cinnabar to decorate their tombs, statues and walls. They were also one of the first civilisations to isolate gold using mercury amalgam. As time and science progressed, a range of both inorganic and organic mercury compounds were developed for use in medicine as they were found to be particularly suitable for controlling bacterial and fungal infections. This application spread to agriculture and, in 1705 Hg_2Cl_2 was first used to preserve wood. By the late 19^{th} century it was also being used extensively to control pests such as worms and maggots along with fungal growths on seed crops [1]. Organomercury compounds were then noted to be more effective at controlling fungal disease than inorganic compounds and so the agricultural application of these compounds increased. This continued well into the 20th century. At the same time medical uses of mercury compounds also increased. They were used to treat a range of conditions from skin disorders to syphilis as well as being administered as teething powders, laxatives and diuretics.

Throughout this century the uses of mercury compounds and therefore the number of man-made sources increased significantly in line with technological advances. Anthropogenic emissions peaked in the nineteen fifties and sixties during the post war boom until the environmental impact and health effects of mercury were appreciated and legislative actions were introduced [15]. Some of the main areas in which mercury compounds have been employed, and in some cases continue to be used today are outlined below:

a The chloroalkali industry uses liquid mercury as a cathode in the electrolysis of brine to produce chlorine and sodium hydroxide. Although most of the mercury is recycled within this process, losses to the atmosphere and within the product chemicals result in significant emissions to the environment, mainly in elemental and inorganic forms.

b Mercury oxide is a common constituent of dry cell batteries and the disposable nature of this product leads to another man-made source, specifically in landfill or incineration wastes. Other electrical or scientific apparatus using mercury include vapour discharge lamps and thermometers. Once again these are sources of elemental and inorganic mercury.

c Mercury compounds are used in the synthesis of plastic compounds or precursors to plastics. Inorganic mercury compounds are often used as catalysts whilst naturally occurring compounds may be present in the constituent hydrocarbons. In addition, some plastics are treated with phenylmercurials to prevent fungal growth, which can destroy cross-linking and inherent strength. These compounds are widely used throughout the plastics industry, from PVC to adhesives.

d Elemental mercury is widely used in dentistry as the main constituent of amalgam fillings. Although small amounts of mercury vapour are released upon the preparation or removal of fillings, by far the most significant source here comes from crematoria chimneys.

e The synthesis of laboratory chemicals and their use is another anthropogenic source of mercury. This is rising in importance today as other uses decline.

f Although recent uses of mercury compounds have decreased dramatically in the past two decades, agriculture has been a notable area where mercury compounds were applied for much of the twentieth century. Many of these applications have

involved organomercurials and despite modern reduction in use, their legacy persists. Alkyl, alkoxy, and arylmercury compounds have all been used in agriculture. In particular these include methyl, ethyl and phenylmercury compounds such as cyano–(methylmercury) guanidine, N-(ethylmercury)-p-toluene sulfonanilide and phenylmercury acetate [15-17].

g Until relatively recently mercury compounds were used as antiseptics and preservatives in soaps, in cosmetics and in antiseptic preparations. They have also been used in ammoniated form as bleaching agents in skin cream, and as contraceptives in a number of countries including Japan [18].

h The existence of mercury in the earth's crust ensures its release when fossil fuels are combusted. The conditions here ensure the release of elemental mercury vapour, ionic mercury and particulate bound mercury to the atmosphere.

i Elemental mercury has traditionally been used in gold prospecting by pouring "quicksilver" onto stones and shale where it amalgamates with any gold present. The mercury is removed by roasting the amalgam causing liquid gold to remain behind whilst the mercury is vaporised to the air. A number of developing countries still use this techniques particularly along the Amazon basin. This process is a significant cause of mercury pollution to the surrounding air, soil, plants, watercourses and therefore the human and animal populations.

j Other minor uses of mercury include munitions, fireworks, pigments, photography [19] and black magic where it is sprinkled in homes to ward away evil spirits [20].

1.1.3 Mercury Cycle

The transformation and transportation of mercury compounds throughout the environment has been outlined in sections 1.1.1 and 1.1.2. A number of attempts have been made to describe and quantify mercury fluxes these are often subjective and date rapidly. Some of the main areas of transformations between Hg^0, Hg^{II} and RHg where R = C_nH_{2n-1} are shown in Figure 1.1.

1.1.4 Toxicity of Mercury Compounds

The harmful effects of heavy metals have been known and exploited for hundreds of years. The first recorded death from mercury poisoning was that of a miner in the fifteenth century [21]. In the nineteenth century a number of poisoners used the harmful properties of heavy metals to dispatch their victims. However, the physical and psychological symptoms of chronic mercury poisoning were clearly recognisable with the exposed person appearing to go mad. It is understandable therefore that the origin of the phrase "mad as a hatter" came from the symptoms shown by hat makers who routinely used mercuric oxides to treat felts. In 1869 organomercurials were first identified as lethal agents. This realisation came from a number of incidents including the deaths of two laboratory technicians at St. Bartholomew's Hospital, London in 1866. In this case the technicians had been studying the structure of dimethylmercury [1, 21-22]. In 1887 in Germany, 0.1-1.0ml of 1.0% diethylmercury solutions were administered as a treatment for syphilis. No one survived more than two injections. Despite these cases, industrial evaluation of organomercurials continued, particularly once their fungicidal properties were recognised.

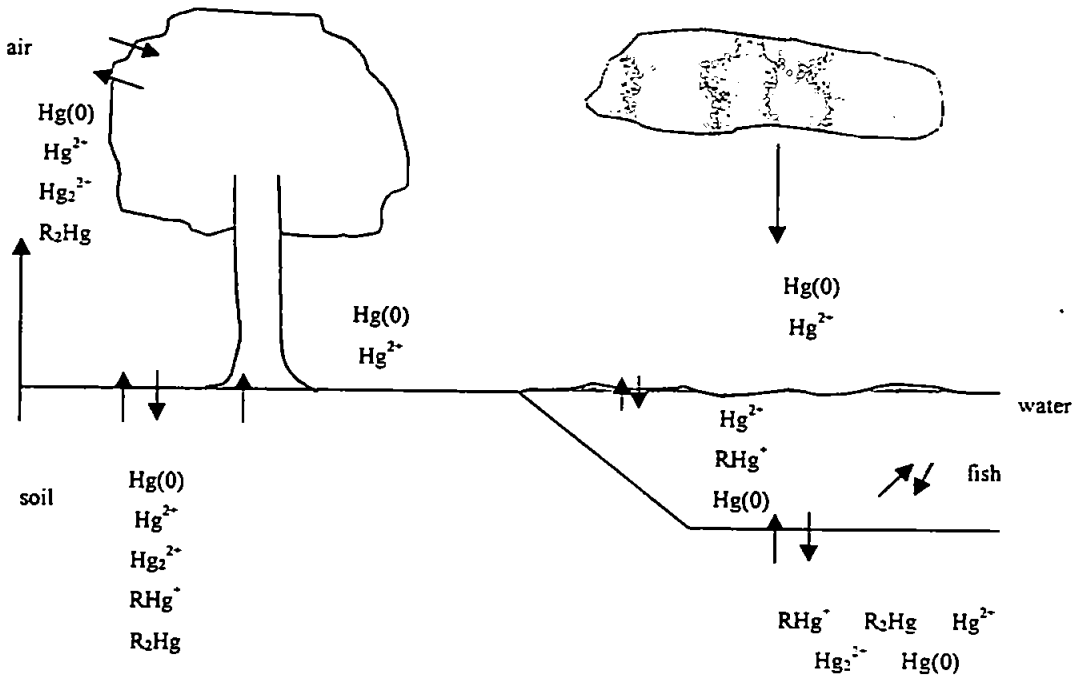

Figure 1.1 Mercury Cycle

In the 1930's, large-scale production of methyl, ethyl and phenylmercury compounds began for the treatment of seed crops. This was after a number of reports by German scientists during the 1920's, which claimed that livestock fed on treated seed, did not suffer any untoward effects [21]. Despite a number of further animal experiments which refuted these findings, such as by Borg in Sweden in 1938, seed treatments continued. As a consequence four people died in England, two in Canada, six in Russia and two in Sweden [22]. It is reported that a book by Hunter in 1940 stated that methyl and ethylmercury compounds were so dangerous that they should never be used again [22]. However organomercury seed treatments continued accompanied by a series of sporadic deaths. The first large-scale poisonings due to agricultural use were in Iraq in 1956 followed by a second incident in 1960 when around 350 people died after eating contaminated seed [21].

The full impact of the toxicity of organomercury compounds and in particular methylmercury was experienced in Minimata Bay, Japan in the late 1950's and 1960's. This incident has been the driving force behind the past forty years of research into mercury speciation and the modern wish to quantify these compounds. In 1956 in Minimata Bay a six year old girl was admitted to hospital suffering from an unidentified disease of the nervous system. This was the first clinical case of "Minimata Disease" to be recognised, although it is now known that the epidemic started as early as 1953. Cats, fish and birds showed many of the initial symptoms but it was not until the first human victims appeared that the severity of the problem was realised. [21, 23-26]. The symptoms of Minimata Disease included lack of co-ordination, blindness, deafness, intelligence deficiency, paralysis and in the most serious of cases coma and death. These effects seemed to build up over a long period of time and in some cases it took years before a victim died. Despite the best efforts of the medical teams no effective treatment was found. After three years of study methylmercury was identified as the cause of this disease [27] although it took many more years to accurately identify the industrial source. The

Chisso Corporation factory, in Minimata Bay, made acetaldehyde for PVC production and used a mercury sulphate catalyst. A side reaction within the reaction tank led to the formation of methylmercury, which was discharged in this form to the Bay. Methylmercury chloride was later identified in sludges taken from the Bay whilst methylmercury sulphide was found in shellfish. The primary diet of this Japanese population was fish. A subsequent outbreak of methylmercury poisoning was also observed further along the coast in Niigata, Japan in the 1960's [15, 21, 23]. In total 54 people died in Minimata Bay and 6 died in Niigata whilst hundreds suffered lasting effects.

Following these major poisoning incidents more detailed records have been kept detailing the effects of mercury compounds. In Sweden in the 1950's bird populations were depleted as a result of feeding on methylmercury treated seed [21, 23]. In New Mexico in 1969 a family died after eating meat from a hog fed on organomercury treated seed. In Canada, fish throughout many of the Great Lakes were found to be contaminated with methylmercury. This was traced to chloroalkali wastes and biomethylation of inorganic mercury. Aboriginal populations at White Dogs and Grassy Narrows are still today being monitored for long term effects [23, 28]. Other incidents in Pakistan, Guatemala, Yugoslavia, Australia, South America and Fiji, either through natural methylation or direct contamination have promoted world-wide measures to reduce human exposure to organomercurials [1, 23-24].

Organomercury compounds are more toxic than inorganic mercury. The order of toxicity, as found by Hempel *et al* in 1995 [29] is:

$$MeHg^{+} > \begin{matrix} MeOEtHg^{+} \\ EtHg^{+} \\ EtOEtHg^{+} \\ PhHg^{+} \end{matrix} > \begin{matrix} Nitromersal \\ TolyHg^{+} \end{matrix} > Hg^{2+} \qquad [1.2]$$

The increased toxicity of RHg compared with inorganic and elemental forms is related to the lipophilic nature of many of these compounds, allowing them to cross the blood brain barrier and bind with sulphydryl sites in the brain. In addition, organomercury compounds are associated with red blood cells and so are easily transported around the body. The half-life of organomercury compounds is averaged to be 70 days compared to 6 days for inorganic mercury. However some organs retain methylmercury for much longer. For example its half-life in the brain is 150 days. Long-term chronic exposure can then lead to a gradual accumulation of organomercury in the body until symptoms appear.

Both Norseth and Clarkson, and Lind *et al* [30] have studied *in-vitro* demethylation of methylmercury. Breakdown reactions have been found to occur in the liver, kidneys and brain leading to the formation of inorganic compounds that are more easily removed from the body. Inorganic mercury leaves the body through the normal urinary and faecal routes. In the case of pregnant and lactating women, organic mercury can be transferred to babies through the placenta and in breast milk, and a number of congenital cases of methylmercury poisoning have been recorded as a result.

In summary, mercury compounds have no known beneficial effects to human life. Organomercury compounds are in general more toxic than inorganic mercury compounds. These factors are particularly significant, as organomercury compounds are known to bioaccumulate in the marine food chain to harmful levels.

Finally, the handling of organomercury compounds presents a significant risk to scientists today. This was reinforced in 1996 when a Canadian chemist of many years experience died from dimethylmercury poisoning after spilling a few drops onto latex gloves whilst preparing standards [31-33]. All the work performed in this research program was therefore carefully assessed and all relevant safety precautions were followed.

1.1.5 Mercury Emissions and Legislative Requirements

As a result of the methylmercury poisoning incidents in Minimata Bay and Niigata, a number of major actions were taken in Japan. The use of organomercury compounds as fungicides in the manufacture of paints was stopped, mercury pesticide use as seed dressings was phased out, the sale and manufacture of mercury contraceptives preparations was halted and restrictions were imposed on trade effluent and water emissions. Other countries that suffered the effects of organomercury poisonings also introduced legislative measures. Sweden and the USA restricted the use of organomercury compounds in agriculture, paper and pulp production and mercury emissions to water and air. Canada took these steps further and closed polluted water bodies to fishing until levels had fallen and declared safe. Most developed countries followed suit and nowadays mercury emissions to air, water and land are routinely monitored and severely restricted. The EEC currently states that the average effluents must not exceed 0.05mg l^{-1} Hg. They have also published a series of quality objectives for fish with maximum permitted levels similar to Canadian and American levels [23]. While most developed countries have seriously restricted mercury use and are monitoring emissions, these only require the determination and monitoring of total mercury. Specific measurement of organomercury compounds would allow more accurate risk assessment and it is possible that such legislation will be introduced in the future.

1.2 Mercury Determination by Atomic Spectrometry

Atomic Spectrometry has revolutionised analytical chemistry since the introduction of the first atomic absorption instrument by Walsh in 1954. Until this time trace metal analysis was commonly performed using gravimetric or complexometric analyses where milligram detection levels were typical. Most mercury analyses are now performed using atomic

spectrometric techniques due to high sensitivity and wide availability of instrumentation, although that there are other non-spectrometric techniques that offer similar capabilities including radiochemical methods such as neutron activation analysis (NAA).

Atomic spectrometric techniques rely upon exciting atoms and measuring the amount of absorption or emission that results at specific wavelengths. Calibrating the techniques with standards allows the determination of atom concentration within a sample. These techniques helped to establish analytical chemistry as a branch of chemistry in its own right by allowing routine measurements of elements at parts per million (mg kg^{-1}) concentrations and below for the first time.

1.2.1 Atomic Absorption Spectrometry

In all atomic spectrometric techniques the sample must be atomised and then held in an excitation source in order for absorption or emission to be measured. In atomic absorption spectrometry (AAS) the atomisation is normally achieved using a flame or furnace with the excitation source provided by a vapour discharge lamp.

Mercury exists as a monoatomic vapour at room temperature so temperature induced atomisation is not required as with other elements. Under standard FAAS procedures, a standard or sample solution is aspirated into a flame which is held within a path of light from a source of the element of interest. A monochromator is utilised to select the wavelength of interest whilst a photocell or photomultiplier tube collects the transmitted light. This arrangement is shown in Figure 1.2. The reduction of inorganic mercury to elemental mercury can be easily achieved in solution with a chemical reducing agent and the resulting Hg^0 can then be purged into the vapour phase presenting a gas for spectroscopic measurement. This cold vapour (CV) generation has become the most

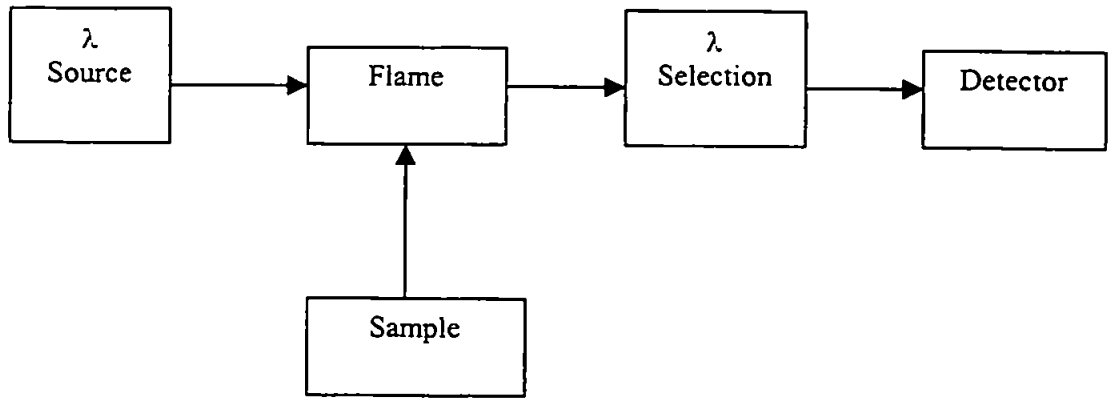

Figure 1.2 Schematic Diagram of Flame Atomic Absorption Spectroscopy (FAAS)

widely used method by which mercury atoms are produced for spectrometric detection because it provides theoretically 100% sample introduction compared to 2-10% for nebulised samples, resulting in substantial enhancement of sensitivity for CV-AAS compared to conventional flame atomic absorption spectrometry (FAAS).

When the CV technique is used, Hg^0 is chemically reduced from Hg^{II} using a reductant such as $SnCl_2$ or $NaBH_4$. The Hg^0 is then sparged from solution in a stream of gas. At this stage the atomic vapour is associated with water and other volatile compounds and this must be dried before analysis in order to avoid interferences. This may be done by passing the gas over a chemical water trap but is now more efficiently achieved using a hygroscopic membrane. As the mercury atoms are already present in the vapour, the flame atomisation source is not required. However the gas must be retained within the light path in order for the absorption to be measured so it is common for a quartz flow cell to be placed on the unlit burner of the FAAS instrument. Other methods have been used with FAAS to increase its sensitivity for mercury. These include pre-concentration using a solvent such as MIBK (Methylisobutylketone) or chloroform after complexation with APDC (Ammonium pyrrolidine dithiocarbamate) or dithizone.

Electrothermal vaporisation – atomic absorption spectrometry (ETV-AAS) is the other main AAS technique. This is also a flameless technique and can be used for either liquid or solid samples. A sample is placed on a platform or in a cup on a retractable rod within an oven. Controlled temperature programming via an electrical current dries and ashes the sample before a final rapid heating stage causes atomisation. The measurement of atomic absorption is effected in the same way as for FAAS, with the light path situated above and

across the sample holder. For mercury, this technique has been reported to be less sensitive than CV-AAS. In one review [34] the limit of detection for mercury using CV-AAS and ETV-AAS were 0.001μg l^{-1} and 0.2μg l^{-1} respectively.

1.2.2 Flame Emission Spectrometry

Atomic emission techniques have also been used for the determination of mercury. In general flame emission spectrometry (FES) has been found to be less sensitive than absorption spectrometry for elements with resonance lines below 270nm. This is because flame temperatures are often insufficient to excite a large population of atoms to the first excited state. In recent years the use of plasma sources with temperatures of a few thousand Kelvin have been used for the study of most elements including mercury. It must be noted that atomic emission techniques can be prone to spectral interferences that are particularly significant in the UV region where many molecular bands are observed. Atomic fluorescence spectrometry, another emission technique is often viewed as complimentary to atomic emission techniques. The use of AFS and Plasma Emission techniques for the determination of mercury will be considered in more detail in sections 1.2.3 and 1.2.4.

1.2.3 Atomic Fluorescence Spectrometry

Fluorescence was first reported (and named) by Wood in 1905 when he observed the re-emission of light from sodium vapour after the absorption of light from a sodium chloride flame. However it was not until the 1960's and work by Wineforder *et al* [35] that the first successful analytical applications of atomic fluorescence were performed.

Atomic Fluorescence Spectrometry (AFS) is an emission technique, which unlike AES is highly sensitive in the ultra violet region and not so sensitive in the visible region due to intense background interferences and quenching. It can only be measured after an initial absorption process and is the result of two or more electronic transitions. There are many types of atomic fluorescence which can occur and which are illustrated in Figure 1.3. Mercury is one element that is commonly measured by AFS due to its absorption and subsequent resonance fluorescence at 253.7nm.

This approach is highly selective and sensitive compared to other atomic spectrometric techniques mainly because the amount of fluorescence generated depends on the intensity of the incident radiation. This means that unlike AAS, increasing the intensity of the radiation source leads to an increase in AFS and therefore an increase in sensitivity. Continuum or broad band light sources are not sufficiently intense for AFS and so in the case of mercury, vapour discharge lamps are often employed. A schematic diagram illustrating the AFS approach is shown in Figure 1.4. This shows many similarities with the arrangement shown in 1.2 for AAS and indeed some of the earliest AFS instruments where combined with atomic absorption spectrometers. Atomic fluorescence spectrometry is traditionally measured at right angles to the incident radiation where it can be clearly separated from both the excitation source and any atomic absorption that may be occurring. It is a simple and selective technique that does not suffer from background and noise interferences to the same extent as AAS or AES. It can however be affected by quenching from gas species within the atom cell and is not as sensitive if used with high temperature flames required for the refractory oxides.

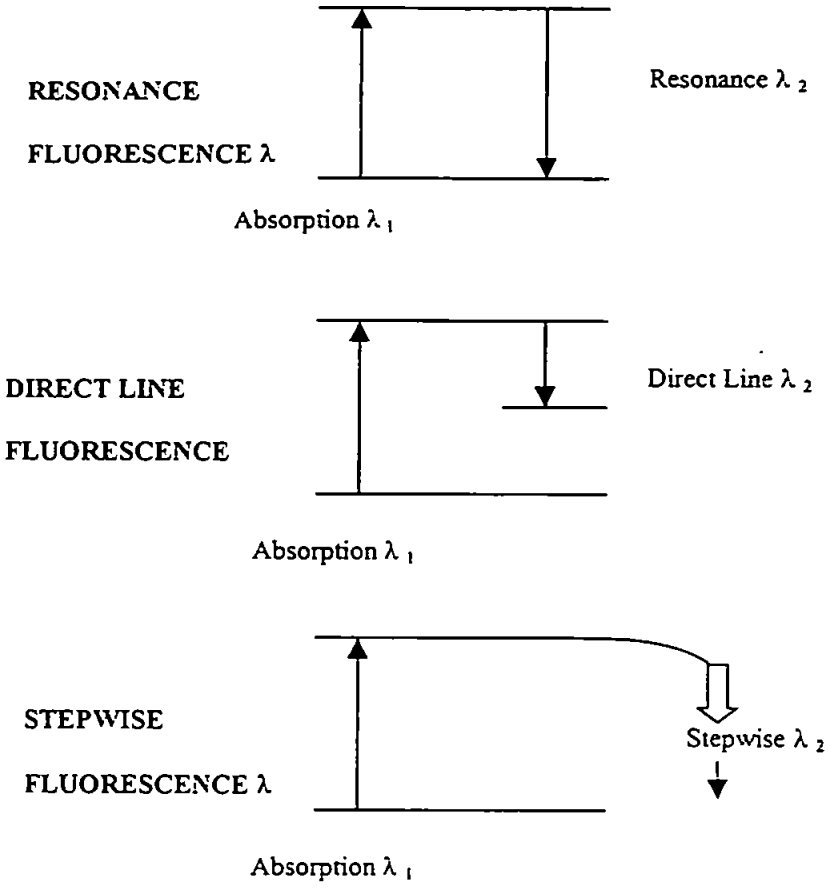

Figure 1.3 Types of Atomic Fluorescence

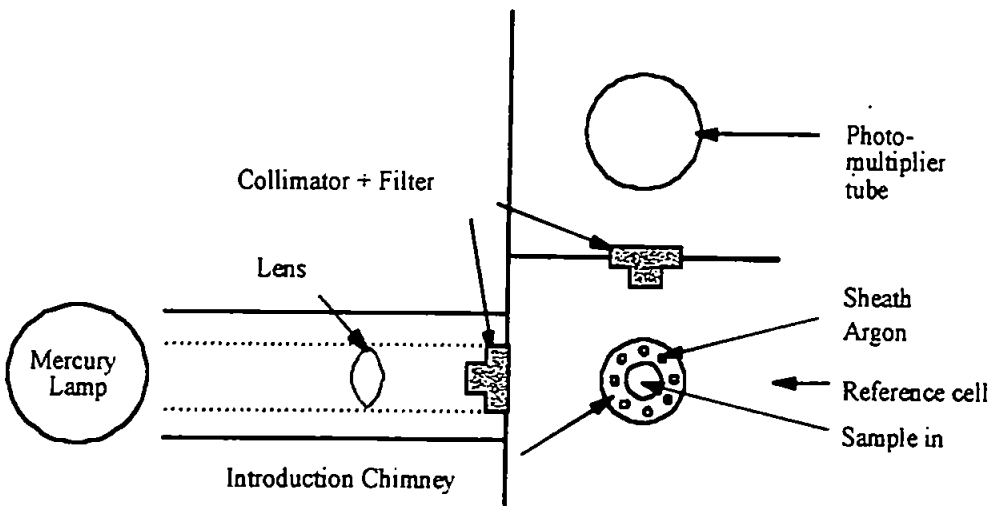

Figure 1.4 Schematic Diagram of CV-AFS Detector

Cold vapour generation is the most common technique combined to AFS for the determination of mercury. It can be generated in two different ways, either chemically as described in Section 1.2.1 or by pyrolysis after preconcentration onto an adsorbent such as gold. In both approaches mercury atoms are transferred as a vapour to the AFS detector in a stream of gas. The choice of this gas is very important in order to avoid quenching and so to exploit the sensitivity of the analytical technique. Air and nitrogen are two gases which have been shown to lead to quenching. Argon is the gas of choice as it has a low quenching cross-section. A commercially available detector, which is based on a design used by Thompson [36], is shown in Figure 1.5. This detector can be coupled to a cold vapour generator to determine mercury. The atomic vapour is introduced to the atom cell in a carrier stream of argon where it is contained within an outer sheath of this gas.

Atomic fluorescence is generated by exciting the atoms with a mercury vapour discharge source and the resultant emission is detected by a photomultiplier tube positioned at right angles to the incident radiation. It must be noted that sample preparation is very important so that all the mercury is oxidised and therefore detected. This is discussed in more detail in Section 1.4. An atomic vapour of mercury can also be produced by pyrolysis and this is most often coupled with gold amalgamation for preconcentration. This technique works by passing a sample gas over a gold trap for a given time or volume where any mercury present forms an amalgam and is retained. Rapid heating of the trap (often made of gold wire, gold/platinum wire or gold sand impregnated on a molecular sieve) in a stream of inert carrier gas releases the mercury from the amalgam and transfers it to the detector. CV-AFS and amalgamation AFS techniques have become very popular over the past decade for mercury determinations with limits of detection quoted at 0.1ng l^{-1}. These methods may be used for the direct analysis of mercury in gas samples or for further preconcentration after cold vapour generation.

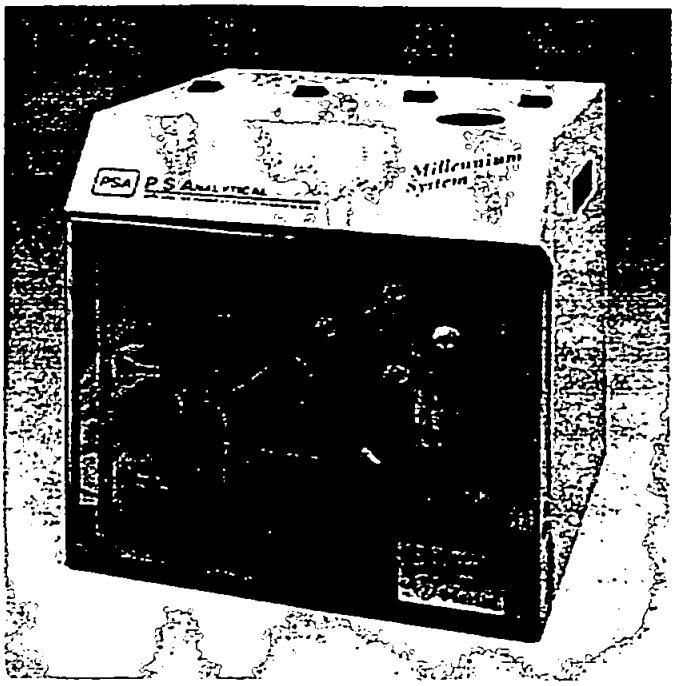

Figure 1.5 Commercially available CV-AFS Detector

(reproduced with permission of PS Analytical Ltd)

1.2.4 Plasma Emission Spectrometry

As described previously, flame emission techniques offer poor sensitivity for mercury due to insufficient energy to promote electrons to the first excited state. The introduction of electrical discharge sources such as arcs, sparks and plasmas, have significantly improved the performance of AES and have consequently led to an expansion in the range of applications possible by atomic emission spectrometry. The most significant advances in AES technology have arisen since the introduction of non-combustion flame-like plasma sources in the 1960's [37]. These plasmas are high temperature neutral gas discharges consisting of approximately equal numbers of positive ions and negative electrons in addition to unionised atoms and molecules.

Plasma sources offer performance and operational advantages over other emission sources as they have sufficient power to atomise and ionise most elements. Liquid and gas samples are easily handled by plasma sources resulting in improved accuracy, sensitivity and precision for a large number of elements. There are three main types of plasmas used as atomic emission sources: Direct Current Plasma (DCP), Inductively Coupled Plasma (ICP) and Microwave Induced Plasma (MIP).

A direct current plasma (DCP) is formed from high velocity argon gas positioned between two carbon anodes and a tungsten cathode, in an inverted Y shape. The sample excitation and observation zone is located between the anodes. The arc is initiated by bringing the electrodes together and then by drawing them apart. Once ignited the plasma is sustained by a low voltage with temperatures in the region of 9000-10000K. The temperature in the excitation region is normally around 6000K. Inductively coupled plasmas (ICP) are the most commonly used plasma sources today. They are flame shaped and are sustained through induction from a high frequency magnetic field. The argon plasma gas passes

through a radio frequency induction coil where it is seeded with free electrons from a Tesla discharge coil. The electrons interact with the magnetic field of the induction coil and gain sufficient energy to ionise the argon gas and any sample present in this stream. Temperatures within this plasma are normally in the range 6000 –10000K. Microwave induced plasmas (MIP) are less complex to operate than DCP's and ICP's as they can be run at lower powers whilst achieving similar results. They are most frequently used for gaseous samples as they often have insufficient enthalpy to desolvate and vaporise aerosols effectively. MIP's may be used with Helium gas that gives them high electron temperatures despite low thermal temperature. This makes them particularly useful for many elements that respond poorly in the argon ICP and DCP's.

Optical emission spectrometers were the first detectors to be coupled to plasma sources. These were originally large dispersive monochromators but these have gradually given way to plane-grating and scanning monochromators, which are smaller, cheaper and offer improved resolution. Sensitivity is limited in AES by spectral interferences resulting from background noise. Narrow band passes with high resolution are used to part compensate for this. One of the major advantages of the modern emission detectors is their multi-element capacity which is a limitation for AAS and AFS techniques where multi-element analyses have never proved very successful. Detection limits for mercury determined by ICP-AES, DCP-AES and FES were quoted to be 1 $\mu g\ l^{-1}$, 75 $\mu g\ l^{-1}$ and 150 $\mu g\ l^{-1}$ respectively [38]. The coupling of ICP and MIP plasmas with mass spectrometers has provided an even more powerful analytical technique for the determination of elements such as mercury which lie in the centre of the periodic table and possess very rich emission spectra. These techniques can reach detection limits of a few parts per trillion, comparable to AFS [38].

1.2.5 Comparison of AAS, AFS and Plasma-MS Techniques for Mercury Determinations

It can be concluded that atomic absorption, fluorescence and emission techniques are all powerful tools for mercury determinations which can all now achieve instrumental detection limits in the part per trillion range (ng l^{-1}). Plasma emission instrumentation however is very expensive both to purchase and to run. AFS and AAS are less expensive techniques with fluorescence taking the lead on sensitivity due to the relationship between intensity and emission and lower number of interferences. It is generally believed that the limiting factor for lower detection levels does not now lie with the technology but with the purity of the reagents used within the analytical methods. All of these atomic spectrometric techniques have been widely applied to a range of total mercury measurements analyses. These are too numerous to consider in detail but a selection of references has been included to illustrate methods [36, 39-90].

1.3 Speciation of Mercury Compounds

The speciation of mercury compounds can be divided into three areas: sample preparation, separation of compounds and detection. In the simplest of cases the first two steps may be combined in the selective extraction of the species of interest. It is more common however to follow an extraction procedure selective for a class of compounds, which are then physically, separated using chromatography. Standard chromatographic detectors do not offer the same specificity and sensitivity as atomic spectrometric detectors so chromatography is often coupled to atomic spectrometry for organometallic speciation studies.

1.3.1 Non Chromatographic Speciation

Cold vapour generation techniques combined with AFS and AAS detectors have been used for the determination of specific forms of mercury after selective extraction procedures. By varying the chemistries used for cold vapour generation determination of either total mercury, inorganic mercury or elemental mercury for a range of samples can be performed. In many cases organic mercury, which is almost always methylmercury, can be simply measured by subtracting the inorganic content from the total concentration of mercury in a sample. Specific procedures will be considered in Section 1.4. Other non chromatographic techniques used for mercury speciation include capillary electrophoresis [91-92] and an electrochemical sensor [93].

1.3.2 Gas Chromatography

1.3.2.1 Separation

Gas chromatography was first used in the 1960's for the determination of organomercury compounds. At this stage columns packed with an inert material were used for the separation of mercury species. As with all chromatographic techniques, the separation of compounds depends on the degree of interaction between the sample constituents carried in a mobile phase, in this case the carrier gas, and the stationary phase. The polarity of the stationary phase is normally chosen so that it is similar to the compounds of interest. The aim is to obtain discrete signals for each eluting compound. Mercury compounds are separated on the basis of their interaction with the stationary phase so temperature programming is often used to increase the rate of elution and to improve peak resolution. Numerous problems were found when using packed columns for the speciation of mercury compounds. These included peak broadening and tailing, peak splitting, compound

degradation and rearrangements including anion interchange on the stationary phase [94-95]. These problems have been reduced by derivatising the compounds to more volatile and less polar species prior to chromatographic separation. The most common derivatisation procedure is ethylation used in conjunction with 40-90cm columns packed with 15% OV-3 (polydiphenyldimethylsiloxane 10%/90%) on Chromosorb W stationary phase [96-111]. There are a number of problems and limitations with the ethylation procedure and these will be considered in more detail in Section 1 5. Other workers have turned to capillary type columns that do not suffer problems to the same extent.. A number of studies comparing columns have concluded that the use of non-polar columns such as dimethylpolysiloxane (BP1, DB1, HP1) and 5% diphenyldimethylsiloxane (BP5, DB5, HP5, AT5) result in good chromatography for methylmercury. However Donais and Uden *et al* [112] found that these were unsuitable for their GC-AES instrument because the methylmercury peak eluted too close to the solvent front. More polar columns such as polyethylene glycol columns (BP 20, DB 20, DBWax) require higher temperatures for elution and suffer peak broadening [113] while poly(14% cyanopropyl-86%-dimethylsioloxane) columns (DB1701, OV1701) also suffer from peak broadening and have been found to lead to compound decomposition [112]. In general, non polar capillary columns are most commonly used with a stationary phase film thickness of at least 1μm, following work by Rubi *et al* [113] that suggested that this parameter was critical for good chromatography. The limitations of capillary columns will also be considered in more detail in Section 1.5.

The efficiency of a GC column is defined by the number of theoretical plates it contains, with the higher the number indicating more separating power. The number of theoretical plates, n, is defined as $16(t_R-t_W)^2$ where t_R is the retention time of a peak and t_W is peak width. This is often more easily measured as $5.54(t_R/w_{1/2})^2$ where $w_{1/2}$ = peak width at half height. This relationship is a simple mathematical formula derived from the van Deemter

equation [1.3], which is a mathematical representation of the processes taking place on a column leading to compound elution.

$$H = A + B/u + C_{stationary}u + C_{mobile}u \quad [1.3]$$

where A represents the uniformity of the path for all molecules, B depends on forwards and backwards diffusions in the carrier, C allows for equilibration and u is the average linear velocity. H or HETP (height equivalent theoretical plates) is also defined as l/n where l = length of the column and n = number of theoretical plates. A typical plot of HETP against carrier flow is shown in Figure 1.6 illustrating the contribution of each van Dempter term to the overall plot.

In organomercury speciation studies using GC a sample is introduced to the column either through an injector or from purge and trap apparatus. In order to protect the column the sample is usually extensively prepared including a compound specific clean-up step in order to remove constituents that may interfere or damage the stationary phase. Common modes of injection for gas chromatography are split, splitless, on-column and direct. In a split injection, a volume of sample is introduced into the injector of the GC through which a flow of carrier gas is passing. A valve arrangement splits the carrier gas so that one part continues to the column whilst the remainder flows to waste. In splitless injection the valve arrangement ensures complete transfer of the carrier flow to the column. Both of these injection types normally use a glass liner filled with some silanised glass wool or solid adsorbent to focus the sample before it reaches the column and to collect debris from septa degradation. For these modes a 1µl injection is common. In on-column injection a special needle and injection valve is used so that the sample can be introduced directly to the column. When capillary (0.32mm id) or Megabore capillary (0.53mm id) columns are used a typical sample volume is 0.5µl. This injection type should only be used for clean samples. Direct injection is most commonly used with packed columns but is finding

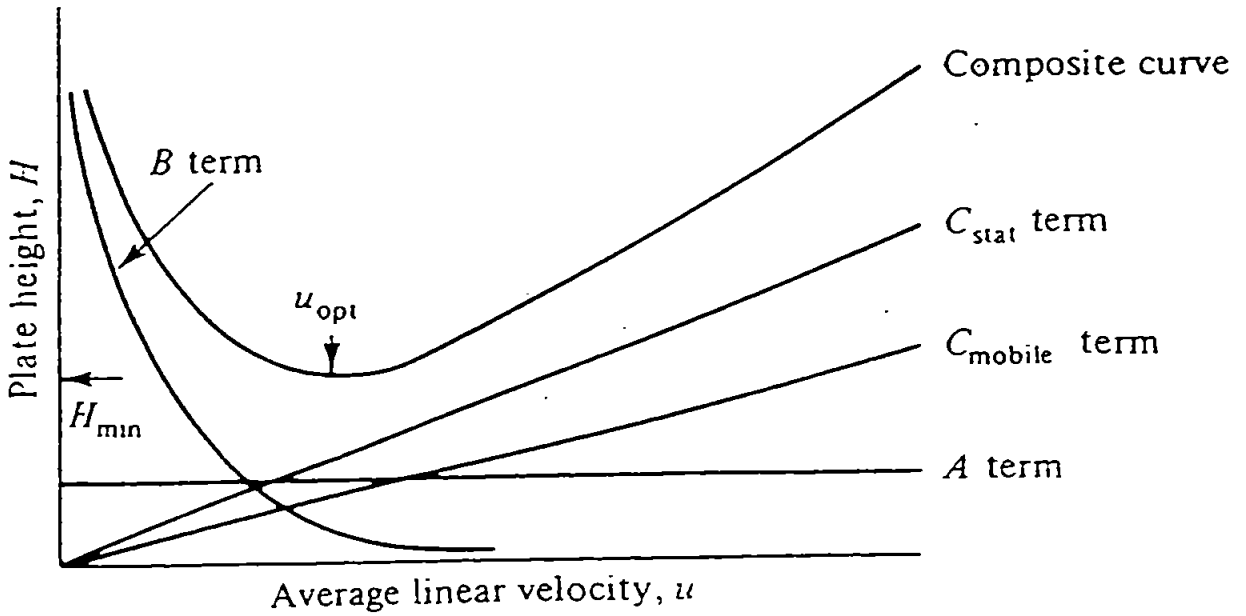

Figure 1.6 HETP against Carrier Gas Flow

(Reproduced from Willard, H., Merritt, L., Dean, J., and Settle, F., Instrumental Methods of Analysis 7th Ed, 1998, Wadsworth Inc.)

increasing application with capillary columns and involves the injection of the sample into an empty glass liner which is directly connected to the column. A carrier gas flow ensures complete transfer onto and through the column.

1.3.2.2 Applications

Some of the earliest and most influential speciation studies of organomercury were by Westöö in the 1960's using gas chromatographic separation with electron capture detection [114-115]. Despite the chromatography problems highlighted by Longbottom in 1973 [94] and the increase in use of atomic spectrometric detection techniques through the 1980's, the use of GC-ECD for organomercury speciation has undergone a resurgence in this decade. All of the papers reviewed [113, 116-120] with the exception of one by Harms [121] described the use of capillary columns and were capable of parts per billion level analysis with the lowest LOD stated by Chiavarini *et al.* as 2pg Hg absolute [121].

By far the most popular approach at the current time is gas chromatography coupled with atomic fluorescence detection (GC-AFS). There are essentially two main approaches, organomercury speciation after compound derivatisation with separation on a packed column and organomercury speciation without derivatisation using capillary columns. Both of these approaches have yielded a number of publications in the past few years [96-105, 122-129] with the former method being more widely recognised.

The sensitivity and specificity of atomic absorption had also led to its use as an element specific detector for organomercury speciation. Most of the papers reviewed here involved packed GC columns [106-107, 130-135] although some use of capillary columns has also been reported [136-138].

Despite the high and almost prohibitive economic costs of atomic emission and plasma detection techniques a number of research groups have coupled these with gas chromatography for mercury speciation studies. The most common approach uses microwave induced plasma sources (MIP) with AES detection, due to the compatibility of the GC column flow of helium which can be used to form the plasma itself. In these capillary columns were mainly used [95, 108-109, 139-147] although the use of packed columns has occasionally been reported [113, 141]. A comparative study by Bulska, Baxter and Frech [95] gave valuable observations on the differences between such columns. The use of MIP-MS techniques for organometallic speciation studies was reviewed by Caruso [148] although no specific applications to mercury were found. Other plasma techniques have also been used as GC detectors for mercury including a more unusual Furnace Atomisation Plasma with an Atomic Emission Detector described by Sturgeon [142]. More typical is the use of inductively coupled plasma sources now more commonly used with mass spectrometric detection and packed columns [110-111, 149]. Other variations including capillary columns and emission detection have also been described [150-151].

Other gas chromatographic detectors have also been reported for these compounds although none have become established. These include GC-MS [152] and GC-FTIR-AAS [153-154].

1.3.3 Liquid Chromatography

1.3.3.1 Separation

In general, 80% of known compounds cannot be analysed by gas chromatography due to insufficient volatility or poor thermal stability however, many of these compounds can be

studied using liquid chromatography which is not limited by these factors. High performance liquid chromatography (HPLC) can be applied to a wide range of samples and has found specific use in organomercury speciation studies. Organomercury speciation is achieved using a reverse phase C18 column with a stationary phase particle size of approximately 3μm. A high pressure pump is used to control the flow of a mobile phase over the column and a sample loop is usually employed to load samples onto the column. The chromatographic separation in HPLC is a result of specific interactions between the mobile and stationary phases. In organomercury speciation applications the mobile phase is typically a methanol/water mixture or acetonitrile/water mixture buffered with ammonium acetate. However, this alone does not allow successful resolution of mercury compounds and its has almost always been found necessary to add a matrix modifier to the mobile phase to improve the chromatography. The most commonly used modifier is mercaptoethanol [155-164] although other complexing agents such as cysteine [156-157, 165] and sodium pyrrolidinedithiocarbamate (SPDC) [156, 166-167] which also form stable inorganic and organic mercury compounds can also be separated by HPLC. Other compounds such as didodecyldimethylammonium bromide (DDAB), [157, 163] and sodium diethyldithiocarbamate (SPDC), hexamethyleneammonium-heaxamethylenedithiocarbamate (HMA-HMDC), have also been reported for this purpose [167]. One of the major advantages of LC techniques over GC techniques is the ability to separate and determine both inorganic and organic mercury compounds.

Ion chromatography in the forms of anionic exchange [168] and cationic exchange chromatography [169] have both been reported for organomercury speciation. These techniques have not become popular due to the tendency of Hg^{II} to form stable neutral complexes that are not separated by these techniques.

1.3.3.2 Applications

Traditional ultra violet detectors have been used for the speciation of organomercury after separation on a C18 column [164-165] although coupling with atomic spectrometric detectors is much more common. In particular atomic absorption detectors have been described [156-158, 167-171] although the small size, sensitivity and low cost of the AFS detector has also led to its use in this way [155, 166]. Once again the high cost of plasma techniques has not prevented its use as an element specific detector with the majority using ICP-MS [159-162, 172] although the coupling of HPLC with MIP-AES has also been described [163].

1.4 Sample Preparation Procedures

The determination of mercury using atomic spectrometric techniques is now well established. Successful applications therefore depend greatly on the sample preparation procedures followed. In this section methods for total, inorganic and organic mercury determinations will be considered.

1.4.1 Determination of Total Mercury

Cold vapour generation may be used for liquid samples. A schematic diagram of a continuous flow CV generator is shown in Figure 1.7. Samples are pre-treated to ensure that all the mercury is present in the +2 oxidation state. When this mixes with the reducing agent in the gas liquid separator Hg^0 is formed which is purged from the solution and carried to the detector. For water and effluent type samples it is common to treat the sample with an oxidising agent such as bromine produced from the oxidation of bromide using acid bromate, prior to analysis. This bromination technique successfully converts

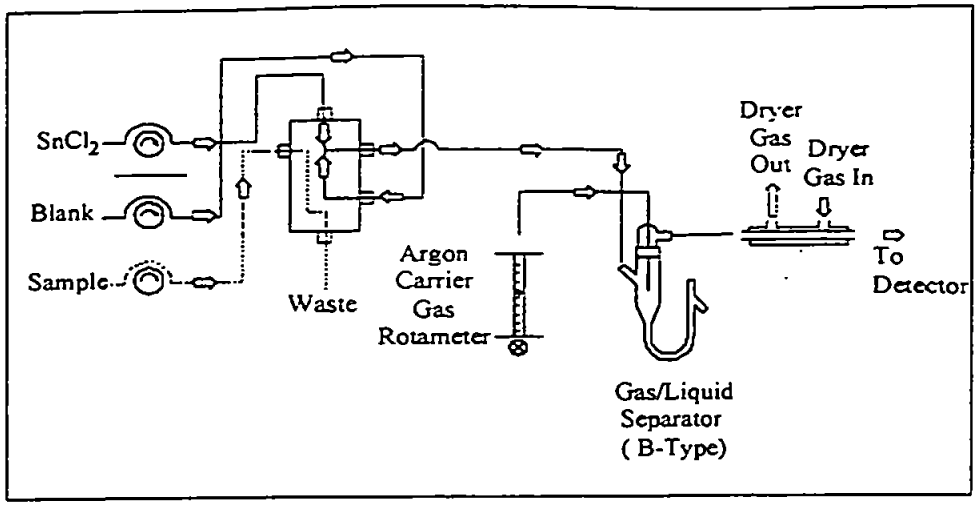

Figure 1.7 Continuous Flow Vapour Generator

Hg^{I} and RHg to the Hg^{II} oxidation state required to give a result for total mercury. This procedure was first suggested by Farey, Nelson and Rolph in 1978 [58] and is now established as a standard procedure. In fact bromination was the recognised sample preparation procedure used in the certification of a sea water reference material, CRM 579 in 1988 [53]. In this exercise both CV-AAS and CV-AFS were used. Other sample preparation procedures have also been used for natural waters. Baxter and Frech compared two methods incorporating nitric acid, sulphuric acid, potassium permanganate and potassium persulphate [61].

Other more complex liquid samples such as trade effluents often require more intense treatment, with stronger oxidising agents such as permanganate or persulphate combined with a mixture of acids, such as the EPA Method 7470 for Mercury in Liquids [60]. A combination of such procedures following the same methodology has been used for on-line mercury determination in urine. Welz *et al* described procedures using nitric acid, potassium dichromate and bromination [75]. Applications to sulphuric acid [173], caustic soda, wastewater and incineration waste have been developed [174].

Solid samples and complex biological fluids such as blood may also be analysed for total mercury after sample pre-treatment. Here it is common to digest the sample using acids either on a heating block or in a microwave field [39-40, 43, 51, 53, 56-57, 62, 65, 69, 71-74, 76, 79-84, 86].

Total mercury is regularly determined in gas and air samples after amalgamation on a gold trap. In one such analyser, the untreated sample is collected by passing over a gold trap, which is subsequently placed in a furnace and heated. Mercury vapour is then swept onto a second trap that is used for calibration. A further heating period releases the mercury to the AFS detector. Calibrations are made using known masses of elemental mercury vapour

calculated from the injection volume and vapour temperature [44]. This and similar amalgamation procedures have been applied to air, breath, flue gas and gas condensate samples [14, 175-178]. A range of chemical traps, such as permanganate solutions, have also been described for gaseous samples [177, 179].

1.4.2 Speciation of Inorganic Mercury

Elemental mercury in solution is already in the atomic form so it may be measured by simply purging a solution with argon gas connected to an AAS or AFS detector. By mixing an aqueous sample with $SnCl_2$, Hg^{2+} will be reduced to Hg^0 that will also be detected by AAS or AFS if purged from solution in the same way. This reducing agent does not react with Hg_2Cl_2. Inorganic Hg^{II} speciation of mercury may be achieved in aqueous solution using a continuous flow vapour generator as previously described. By analysing the same sample with water/water and reductant/water respectively flowing in the reagent streams, against individual calibration curves, the elemental mercury content and the combined Hg^{II} plus elemental content can be determined respectively. The difference between these measurements is the Hg^{II} content of an aqueous sample. These simple procedures are of limited use as they can only be used for liquid samples.

Magos first described methods for the determination of inorganic mercury in undigested biological materials in 1971 based on a variation of the cold vapour generation technique. [180] In summary Magos found that by mixing $SnCl_2$ in alkaline medium with a sample that inorganic mercury alone would be reduced. Re-acidification and reduction using a mixed $SnCl_2/CdCl_2$ reducing agent could then be used to release methylmercury from a sample. Undigested samples were prepared in a mixture of L-cysteine, NaCl and NaOH. This method has been subsequently improved and automated by other researchers [180-

183] and is commonly used to determine inorganic and methylmercury in brain and liver tissues [30].

Recently Bergdahl, Schutz and Hansson described a new method for the automated determination of inorganic mercury in blood after sulphuric acid treatment. This reported that overnight treatment of blood with sulphuric acid alone allowed the determination of inorganic Hg^{II}, while overnight treatment in a mixture of nitric, sulphuric and perchloric acids allowed the determination of total mercury. Cold vapour atomic absorption combined with gold amalgamation preconcentration was used [184].

1.4.3 Speciation of Organomercury

Organomercury concentrations in natural water samples are very low, often in the pg l^{-1} region. Despite the analytical capabilities of the techniques discussed earlier in this chapter, these levels are generally too low to be determined directly. Pre-concentration procedures are normally used to overcome these problems.

Sulphydryl cotton (SCF), a synthetic material which can be prepared following the description by Lee [185] is a solid phase adsorbent selective to organomercury in solution. This has been extensively used by McLeod for flow injection organomercury studies [186-189]. SCF is often packed into microcolumns over which water samples are passed. Inorganic mercury passes over the cotton whilst organomercury is retained. Acidic elution is used to collect the organomercury for analysis [124]. Other preconcentration sorbents have also been reported including dithiocarbamate resins [137, 144]. On-line preconcentration procedures usually exploit the affinity of mercury species to organic reagents with sulphur donor atoms. This means that chelating agents such as diethyldithiocarbamate (DDC), pyrrolidin-1-yldithioformate (APDC) and dithizone (DZ)

have all been applied in this manner [105, 190-193]. Another procedure often used for water samples is purge and trap. This is particularly favoured by groups using ethylation for compound derivatisation. Here the sample is reacted with sodium tetraethylborate and purged. The derivatised volatile organomercury compounds are then trapped onto a solid adsorbent such as Tenax or Carbotrap and determined after desorption [103, 128]. Variations on this method have been reported with some including a further preconcentration step into dichloroethane solvent [98] or following a distillation procedure [98].

The Magos method may also be used for the determination of organomercury in undigested biological samples. This does not provide any specific information about the species involved [180]. However, all the organomercury in mammalian tissues is believed to be methylmercury and if the concentration of organomercury is all that is required, with no speciation information, this can also be calculated as the difference between total mercury and inorganic mercury [30, 194].

The most common methods used for the preparation of solid samples are based on those described by Westöö [114-115]. These have been applied to a wide range of samples including soils, sediments, fish, shellfish, animal and plant tissues and hair samples. These involve an initial solvent extraction originally using benzene, followed by a mercury selective aqueous extraction using L-cysteine with a final extraction into organic solvent for GC analysis. Many variations on these methods have been published [89, 91, 94, 109, 120-123, 125, 136, 138, 139, 164-165, 195]. Benzene has been replaced by toluene and more recently with dichloromethane; L-cysteine and thiosulphate have been used interchangeably and the procedure can be halted at this stage if LC is the chromatographic method of choice. Other chelating reagents and solvents such as dithizone and chloroform have also been used [13].

This procedure and its variations are the most widely used procedures for organomercury speciation studies. However in order to transfer methylmercury into the solvent phase it is usual to lower the pH with acid. This also has the effect of removing particulate bound organomercury into the aqueous phase [15]. Halide ions in the form of bromide or iodide are also normally added at this stage to complex with protonated organomercury compounds and to aid their transfer into the solvent, copper ions also displace organomercury from thiosulphate complexes following an aliphatic electrophillic subsitution reaction (transmetallation with a metal halide) [196]. Alkaline extraction is often used at the beginning of this procedure if the sample is a biological tissue with high fat content, in order to destroy cell walls and so to facilitate the extraction of the organomercury. Unfortunately the acidic nature of this procedure means that any dialkymercury present in the sample is converted to monoalkymercury during the sample preparation procedure.

Other preparation procedures for solid samples include acid leaching [98], alkaline leaching [98, 119], distillation procedures [98, 197-202], sonication [152, 203] and accelerated microwave extraction procedures using open focussed microwaves [116, 132, 134, 136, 140]. A selection of publications comparing such sample preparation procedures primarily within intercomparison exercises is also available [87-89, 195, 201-202, 204-207].

The speciation of organomercury in gaseous samples is less established. Methods have generally been developed to preconcentrate mercury species from a sample, using a variety of solid phase adsorbents such as Tenax and Chromosorb [130, 208]. Gold amalgamation usually involves temperatures that destroy the speciation on release of the mercury, which makes it an impractical technique for these studies.

1.4.4 Preconcentration

The common theme throughout all of the sample preparation procedures discussed in 4.2.2 is pre-concentration. Even the solvent extraction procedures for solid samples incorporate a pre-concentration step. In general this is because natural levels of organomercury in waters, air samples, soil, sediment and plant tissues are low, normally accounting for less than a few ng g^{-1}. Higher levels, up to percent level, are usually only found in the marine food chain as a result of bioconcentration. A range of solid phase adsorbents such as sulphydryl cotton fibre, dithiocarbamate and Tenax were all introduced for organomercury preconcentration.

Another methodology for increasing the sensitivity of chromatographic speciation techniques is to introduce larger samples onto the columns, however this is limited by the column capacity. Large volume injectors have recently been gaining in popularity and application for GC techniques. These rely on injecting a large volume of sample in solvent, up to 100µl, onto a solid adsorbent or column retention gap. The solvent use must have boiling point much lower than the compounds of interest and careful temperature programming is used to vent the solvent whilst trapping the analytes of interest. The trap is then heated to normal injection temperature to elute the pre-concentrated compounds. The use of programmable temperature vaporiser injectors (PTV) for large volume sample introduction has been developed and described by Gerd-Janssen [209-211] with reference to organic compounds. Only one procedure has been described with application to organomercury speciation, using a separately heated packed pre-column within the GC oven but not incorporating the automation of a programmable temperature vaporiser (PTV) injector [146].

1.4.5 Derivatisation Methods for GC

Longbottom described peak tailing, poor resolution and anion interchange leading to variable signals as main problems associated with organomercury speciation using GC columns [94]. Other problems such as compound degradation and column degeneration have also been reported [113]. The choice of column and the elimination of metallic fittings, which provide active sites for degradation could help to limit but not remove these effects. As a result column preconditioning using methylmercury iodide and other compounds such as inorganic mercury chloride has been frequently employed, however, despite these precautions columns still deteriorated with time as they gradually became poisoned. The most significant procedure introduced in an attempt to limit these problems was that of compound derivatisation led by Rapsomanikis [106] and Bloom [97]. Ethylation using sodium tetraethylborate has become the most widely used procedure prior to separation. Here volatile ethyl derivatives of organomercury species are formed which give discrete signals when eluting from appropriate GC columns. However there are a number of limitations with the ethylation procedure most notably its propensity to result in the *in-situ* formation of organomercury artifacts when high inorganic mercury levels are present in the ethylating agent (see section 1.5.2), and also the fact that this procedure will mask any ethylmercury present in the sample. Until recently this latter issue was not perceived to be a problem as it was generally accepted that all organomercury in nature was methylmercury. However reports by Jones [125], Hintelmann [158] and Jernelov [212] have all described ethylmercury in soil and sediments. In addition Donais, Uden *et al.* have also reported the detection of ethylmercury in a CRM mussel tissue [139]. This has led a number of groups to consider butylation [95, 125, 144-147] but this will also mask butyl mercury compounds. To date, this compound has not been reported but it is plausible that it may be found in contaminated sites used for organomercury fungicide synthesis or in gas condensate samples.

1.4.5.1 Artifact Formation

Positive artifact formation is a serious problem that has been found to effect methylmercury measurements when following certain procedures. This is of particular significance at the current time because a certified tuna fish sample, IAEA 350 has recently been withdrawn with doubt over the certified value. The importance of this topic was highlighted in a Standards, Measurements and Testing Workshop on Artifactual Formation in Speciation studies, in Mainz, Germany, May 98 [213].

There are two main sample preparation procedures that have been implicated in artifact formation, both of which have been found to yield high results for methylmercury when performed in the presence of high concentrations of inorganic mercury. This problem was first documented by Bloom and Horvat and related to the ethylation procedure. Batches of sodium tetraethylborate, the ethylating reagent were found to contain high levels of inorganic mercury that resulted in positive methylmercury formation [93]. The other and more significant procedure leading to artifact formation is steam distillation. For some time this was the most popular sample preparation procedure for organomercury speciation which led to its over representation in intercomparison programs and hence the withdrawal of IAEA 350. A number of reports stemming from the 4th Mercury as a Global Pollutant Conference, Hamburg, 1996 have described factors which may influence artifact formation [201, 214-215]. However despite these facts and the withdrawal of IAEA 350, a recent publication by Mackey and Decker describing methylmercury determination in biological samples using this material for method validation was noted [202].

1.5 Aims and Objectives

The aim of the research was to develop robust and reliable methods and instrumentation for organomercury speciation, by coupling chromatography with atomic spectrometric detection. Specific objectives were: to develop a GC-AFS system in conjunction with large volume injection, to develop rapid and reliable sample preparation methods for biological tissues, soils and sediments and to compare different instrumental methods such as GC-AFS, GC-ICP-MS and HPLC-CV-AFS.

Chapter 2

GC-AFS INSTRUMENTATION AND DEVELOPMENT

The gas chromatography – atomic fluorescence instrument was built by coupling commercially available hardware with specially designed components. This chapter considers the purpose of each of these main components and how they were assembled to give a fully automated mercury speciation instrument. Testing of the instrument led to the discovery that some of the components were unreliable when used in this way. As a result, modifications were introduced to complete the instrumental development. The next stage of the work was to optimise the operating conditions in order to produce figures of merit.

2.1 Instrumentation

A gas chromatograph was coupled to an Atomic Fluorescence detector via a pyrolysis unit for the speciation of organomercury compounds (GC-AFS). The instrument comprised five main components arranged as shown in Figure 2.1. Each component is described in detail within this section.

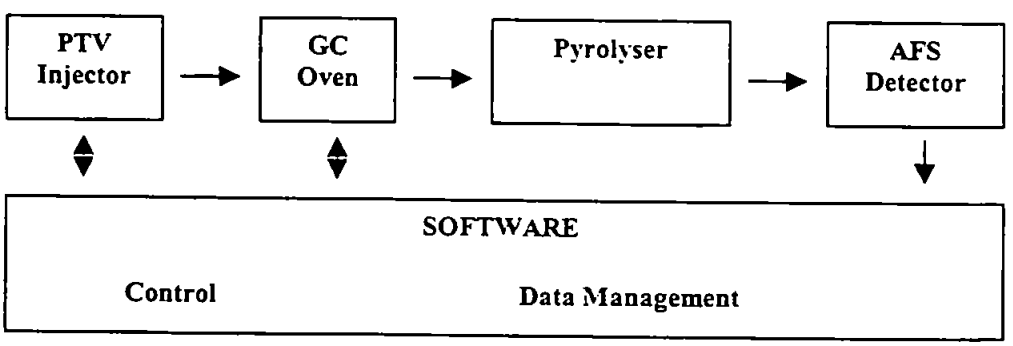

Figure 2.1 Components of a GC-AFS Instrument

2.1.1 Programmable Temperature Vaporiser Injector

The programmable temperature vaporiser (PTV) injector was a commercial unit produced by Ai Cambridge (Optic, Ai Cambridge Ltd., Cambridge, UK) which can be interfaced with most gas chromatographs. It consisted of a free standing control unit and an injector, mounted on the top of the GC oven in place of an existing injector port. The PTV injector allowed the introduction of a sample onto a capillary type column at a controlled temperature normally set just below the boiling point of the solvent. The injector was heated to allow rapid and controlled vaporisation of the sample onto the chromatographic column. This form of injection greatly reduces problems such as sample fractionation within the needle, thermal decomposition within the injector and solvent flashback often encountered with traditional split or splitless operations.

The PTV injector was able to duplicate the injection modes of nearly all common types of capillary and megabore injectors such as split, splitless, direct and on-column injections in addition to applications such as thermal desorption. Temperature programming was performed via an external control panel. It was possible to select up to three temperatures in the range 0-500°C, namely the initial temperature, intermediate or "pause" temperature and the final temperature. A heating rate of 1, 4, 8 or 16°C per second was also chosen along with a delay time of 0, 1, 5 or 10 minutes. The program was initialised either manually or through GLC Control software (GLC Control Version 1.10d, Scientific Software, San Ramon, USA). Rapid cooling of the PTV injector was achieved using a compressed air line directed onto the injector cavity. An exploded diagram of the injector is shown in Figure 2.2.

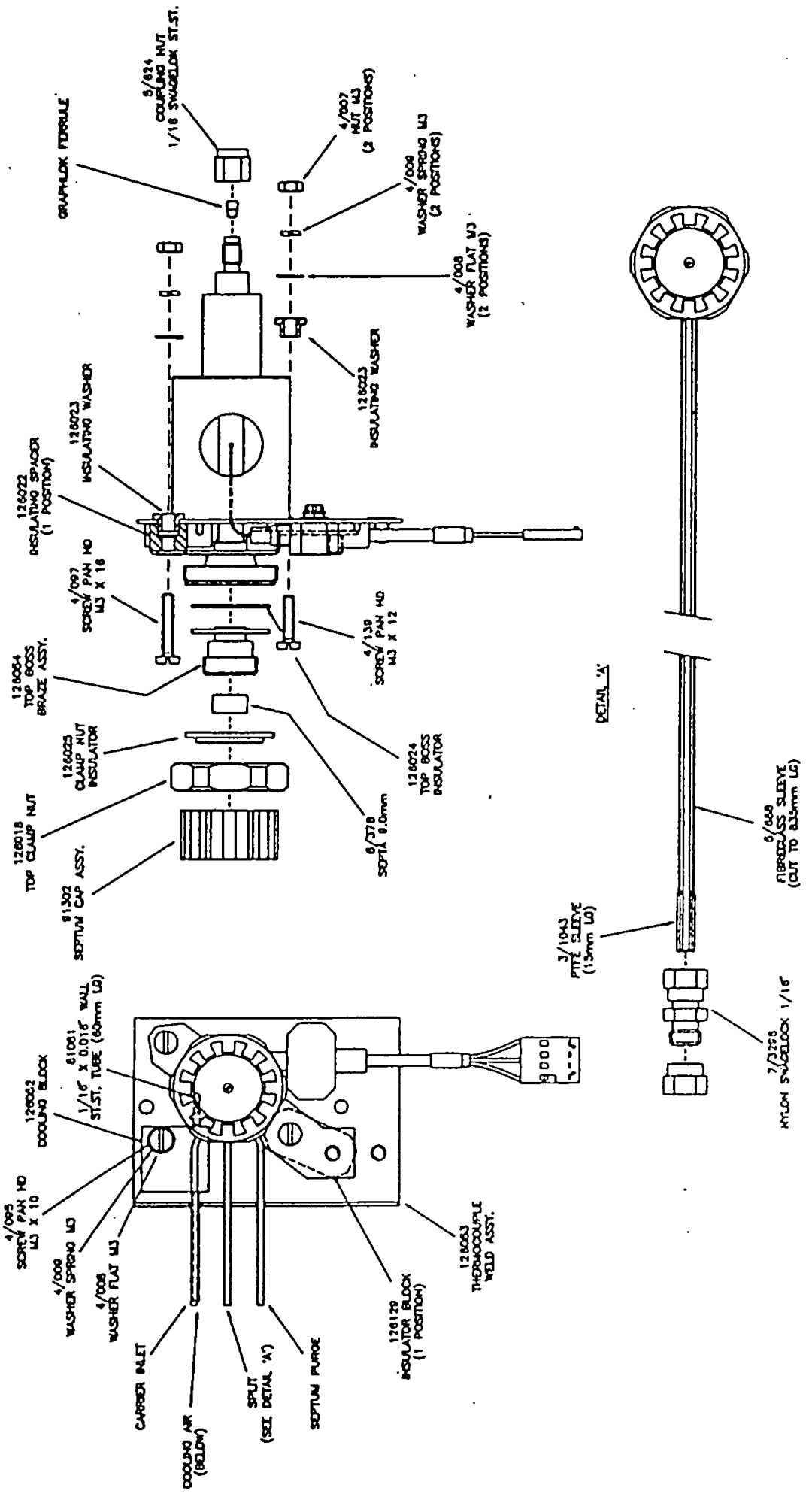

Figure 2.2 Exploded Diagram of PTV Injector

Reproduced with permission of Ai Cambridge Ltd. and ATAS Ltd., Cambridge, UK

2.1.2 Gas Chromatography Oven

The Gas Chromatograph (GC) was a commercial instrument produced by Ai Cambridge (GC94, Ai Cambridge Ltd., Cambridge, UK). The standard injector and detector were removed to allow the installation of the PTV injector and the AFS detector. The instrument consisted of a precision oven for the temperature control of the column, and gas control components for both the column carrier gas and the gases used in the detection system. The oven was controlled by GLC control software which allowed temperature programming with up to four steps.

2.1.3 Pyrolyser

A pyrolysis unit was fitted at the end of the GC column to facilitate the breakdown of organomercury compounds to elemental mercury vapour required for the Atomic Fluorescence detector. The unit was produced by PS Analytical Ltd. (PS Analytical Ltd., Orpington, UK) and comprised a resistively heated coil around a hollow quartz head. A sample transmission tube was positioned passing through the unit and a thermocouple was used to monitor the internal temperature. The quartz head was packaged inside a steel case which itself was placed inside an aluminium heat sink. The unit was controlled by a CAL 9900 autotune PID temperature controller unit (CAL Controls Ltd., Hitchin, UK) in the range 0-900°C.

2.1.4 Atomic Fluorescence Detector (AFS)

The Atomic Fluorescence Spectrometer was a commercial unit manufactured by PS Analytical Ltd. (Merlin, PS Analytical Ltd., Orpington, UK). A schematic diagram of the optics box layout is shown in Figure 2.3.

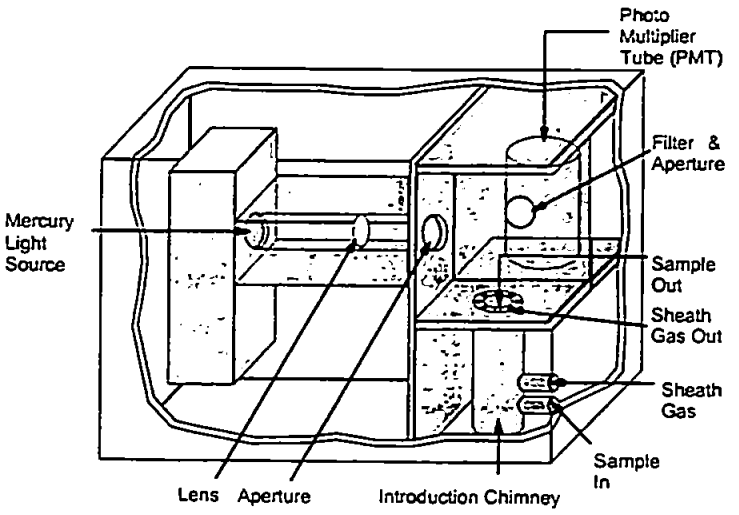

Figure 2.3 Layout of Atomic Fluorescence Detector Optics Box

Reproduced with permission of PS Analytical Ltd., Orpington UK

A gaseous sample of mercury atoms, surrounded in a sheath of argon gas, entered the atom cell via a chimney positioned in its floor. The sheath gas retained the sample within the chamber where it was excited by a mercury vapour discharge lamp at 253.7nm and focused using a lens. Atomic fluorescence was detected by a photomultiplier tube aligned at right angles to the incident radiation. A wavelength range of 254 +/- 10 nm half width was isolated using a Fabry-Perot interference filter. Waste gases escaped from the chamber via an exit hole on the floor of the cell. These passed through an activated charcoal trap which removed mercury before venting to atmosphere. The detector was positioned beneath an extractor fan.

2.1.5 Control Software

GLC Control Software (GLC Control Version 1.10d, Scientific Software Systems, San Ramon, USA) was used to control the oven temperature programmes and injection modes. EZChrom software (EZChrom Version 6.6, Scientific Software Inc, San Ramon, USA) was used to record and integrate the detector output.

2.1.6 Coupling Gas Chromatography to AFS

The PTV injector was fitted into an injector cavity on the top of left-hand side of the GC oven towards the rear (Figure 2.4), and was connected to the free standing control unit via electrical connections. A pressurised air cooling line was connected via the control unit to a cooling jet positioned on the injector and supplied with compressed air at approximately 30psi. Power was supplied through a standard fused power cable. The pyrolyser was fitted in a cavity on the top right hand side of the GC oven (Figure 2.4) suspended from an aluminium heat sink. The thermocouple, placed inside the heated zone was electrically

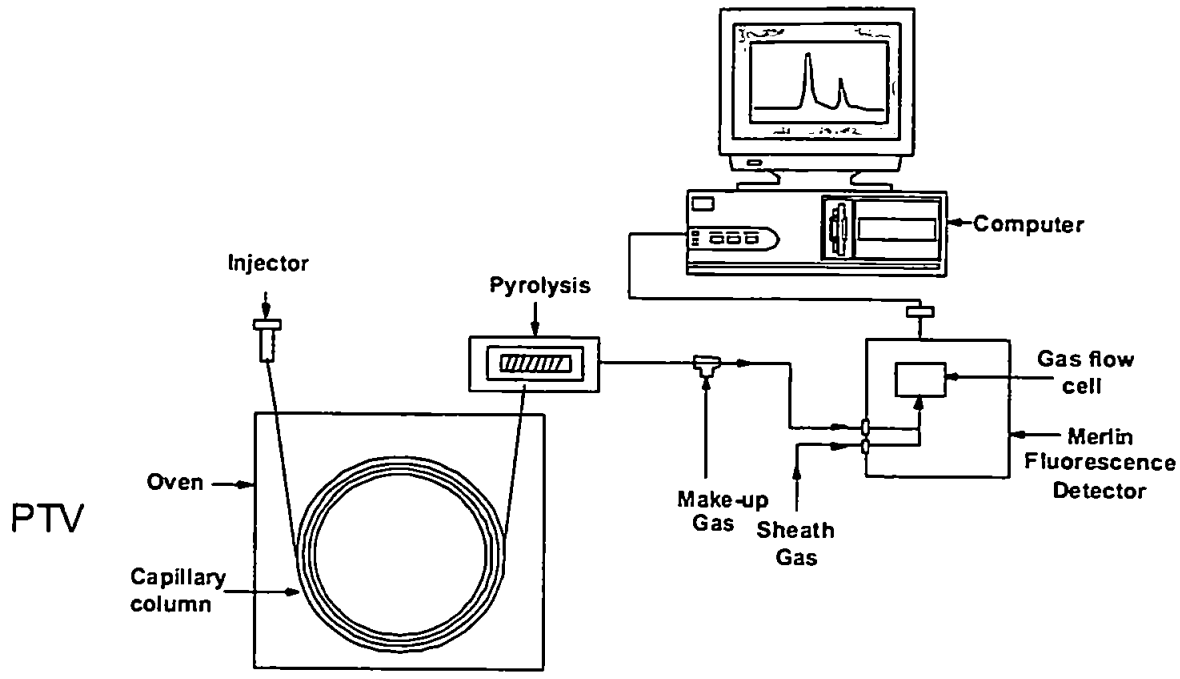

Figure 2.4 Schematic Representation of the Coupled GC-AFS Instrument

connected to the temperature controller, with its display unit installed on the front panel of the GC.

The gas chromatography column was a DB1 Megabore column (J&W Scientific, Folsom, USA) made of dimethylpolysiloxane, 15m x 0.53mm id x 1.5µm film thickness. This was specified for operation between -60°C to 320°C. The column was connected to the base of the PTV injector, into which a glass liner had been fitted using a graphitised (Vespel) ferrule and locking nut. The opposite end of the column was attached to a length of deactivated fused silica (20cm x 0.53mm, Phase Separations Ltd., Deeside, UK) via a glass universal pressfit connector (Phase Separations Ltd.). The deactivated portion of column was fed through the pyrolyser and attached to a length of PTFE tubing (20-30cm, 1/16" od x 0.8mm id, Omnifit Ltd., Cambridge, UK). These were fixed in place using a brass ferrule set (1/16", Swagelock, Ohio, USA) and a locking nut on the top of the pyrolyser.

The AFS detector was positioned on a shelf to the right hand side of the oven, within the GC outer casing above the instrumental circuitry and gas controls. Typical gas flow rates for the standard Merlin AFS detector are 300ml min^{-1} for both sample carrier and sheath, whereas typical Megabore capillary column flow rates are normally much lower at only a few ml min^{-1} with a maximum capacity of around 50 ml min^{-1}, hence it was necessary to introduce a make-up gas to the eluting carrier gas prior to detection. The interface between the GC column and the detector is shown in Figure 2.5. The shortest possible connection lengths were used in order to minimise signal broadening. In addition to the make-up gas, an argon sheath gas was also supplied to the detector via the existing gas controls of the unit. A further length of flexible silicone rubber tubing was used to join the gas exit port (after pressure/flow regulation) to the detector.

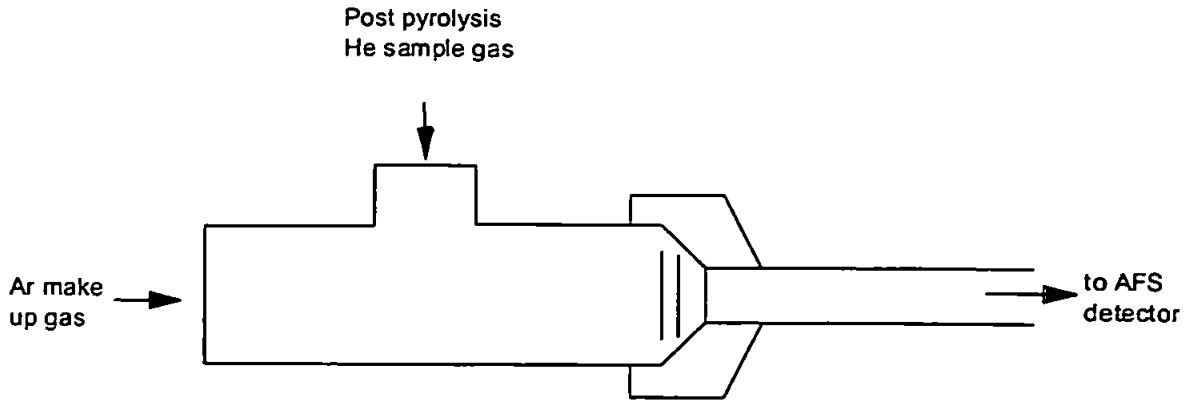

Figure 2.5 Sample Gas Interface to Detector

Plate 2.1 shows a photograph of the commercial GC-AFS instrument which has resulted from this work. The coupling of the individual components has been achieved essentially within the casing of the Ai Cambridge GC94.

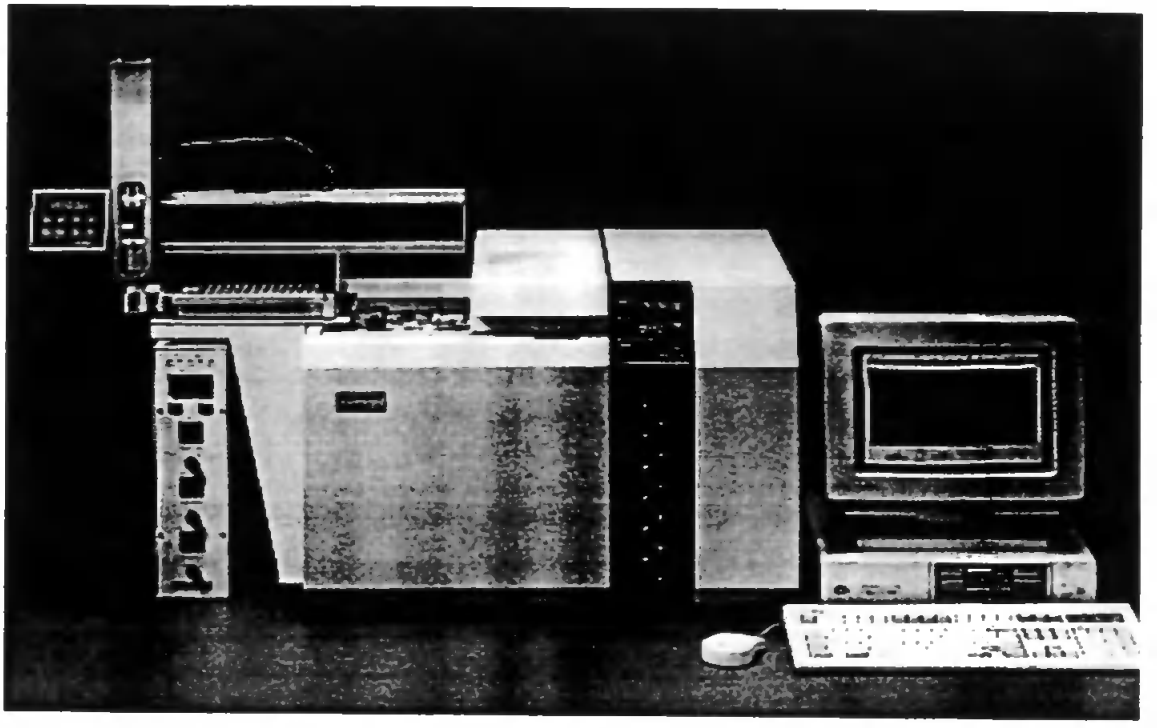

Plate 2.1 Photograph of Commercial GC-AFS Instrument

Reproduced with permission of PS Analytical Ltd.

2.2 Instrument Modification

2.2.1 Detector Purge

During the initial coupling and testing of the instrument it was noted that the sensitivity of the detector degraded over time, resulting in a detector lifetime of approximately three months. The cause of this damage was attributed to the effect of helium gas. It has been reported [216] that helium can diffuse into photomultiplier tubes and other vacuum apparatus such as vapour discharge lamps thereby increasing the gas pressure and causing them to burn out. Helium was the GC carrier gas of choice for organomercury speciation, so it was necessary to identify and implement a way of limiting its effect on the internal detector components. Under the initial working conditions of the GC-AFS instrument, helium was used at a flow rate of 12 ml min^{-1} along with an argon make-up flow of 60 ml min^{-1}. This 1:5 helium:argon ratio proved insufficient dilution to prevent component damage, hence, an argon flow was installed inside the PMT housing of the detector by drilling a hole in the floor and fitting a critical orifice to control flow rate, at a pressure of 30psi. The whole PMT area was then sealed with a silicone rubber sealant.

Figure 2.6 shows plots of the outputs observed with and without argon pressurisation. With argon pressurisation outputs were steady but declined with increasing carrier flow. This was expected because increasing carrier flow means less residence time in the atom cell and therefore less signal, however, a lower signal was observed when the argon was omitted, suggesting that helium caused a degradation in detector sensitivity.

This modification significantly improved the performance and long term sensitivity of the detector within this instrument such that, after the installation of the argon gas purge, no further PMT sensitivity problems were encountered for the duration of this project.

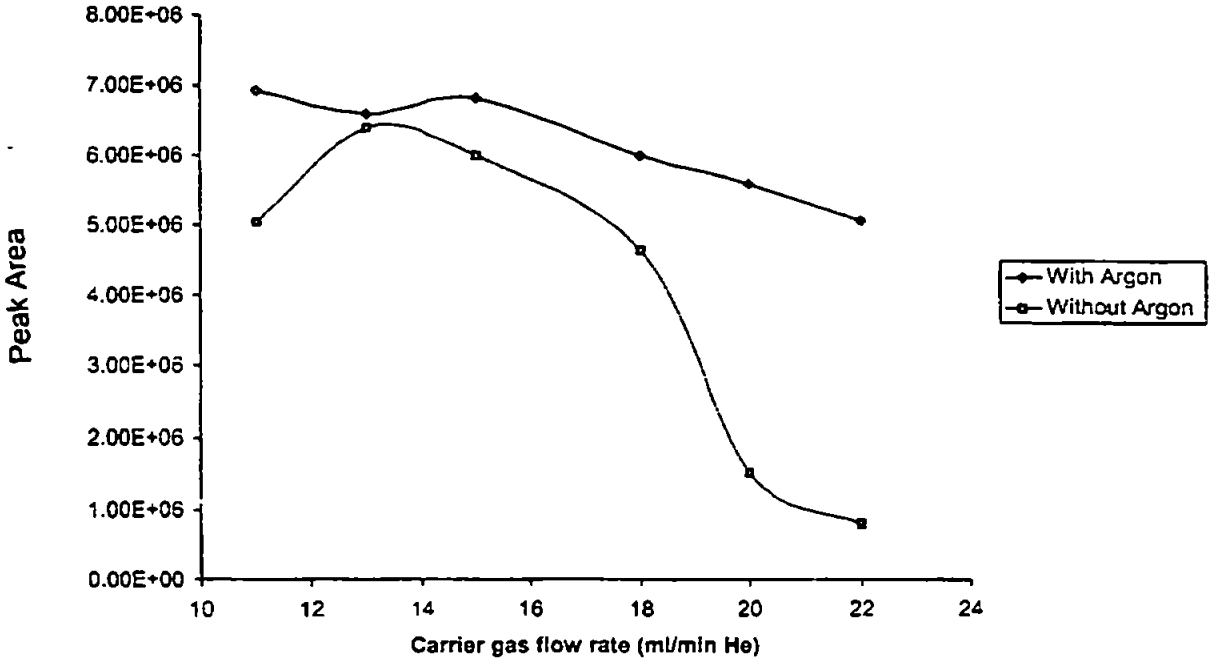

Figure 2.6 **Comparison of Peak Areas for Methylmercury Standard Measured by GC-AFS, With and Without Argon Flow to the PMT**

2.2.2 Pyrolysis Head Design

In the initial design the pyrolysis head was a simple quartz tube mounted vertically with a heating coil wound around it. The effect of gravity led to the formation of hot spots where loops of coil slipped together which in turn led to uneven heating and inaccurate temperature measurement within the pyrolyser. In one instance the thermocouple was found to be situated in a cold spot, monitoring an internal temperature of 440°C which corresponded to a real temperature of 640°C.

In order to avoid the formation of hot spots and to ensure rapid, even pyrolysis of eluting compounds a new pyrolysis head was manufactured with two main modifications. First, the outer surface of the quartz was scored to provide ridges onto which the heating coil could be wound. Second, two internal channels were introduced, equidistant from the heater walls to provide identical channels for the pyrolysis tube and thermocouple thereby ensuring more reproducible and representative temperature measurement compared to the initial design. Figure 2.7 shows the second pyrolysis heater head design.

The modified pyrolysis head provided even heating with accurate temperature control, however this design was found to be very fragile, often cracking after single use at 850°C, which then led to the formation of hot spots when the heater wire slipped out of place on the damaged heater head. In addition, the component was too fragile for transportation so an alternative material, which could be fashioned into the same design, was required.

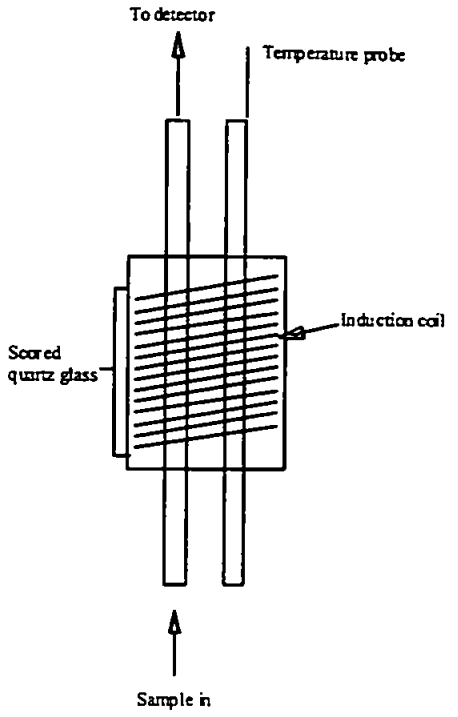

Figure 2.7 Pyrolysis Heater Head Design 2

A machinable ceramic material, capable of withstanding temperatures up to 1300°C was used to manufacture a third pyrolysis head similar to the second design, the only difference being that, instead of attaching two channels inside the quartz shell, the channels were made by machining paths through the solid ceramic block. Fire cement was coated on the outside of the heater wire to secure it in place. A test was made to confirm that the ceramic material did not affect the degree of pyrolysis at a given temperature on a standard of methylmercury. No difference was observed, confirming accurate temperature measurement *i.e.* 500°C inside a quartz head = 500°C inside a ceramic head. Vigorous heating and cooling of ceramic pyrolyser design 3, between 40 to 900°C was performed over a period of days. After this time, no weaknesses were observed. In fact it appeared that the heating coil had burned into the ceramic, forming its own ridges. Continued use over a period of months found that this did not progress with time.

This design was incorporated within the instrument for the remainder of the project, approximately eighteen months. During this time it failed on only one occasion, when uncontrolled heating was applied taking the material above its melting point for 5-10 minutes. In this instance the thermocouple and pyrolysis tube had both been removed from the heater. The effect of this heating was to cause the ceramic to bend and become warped, and it was not possible to re-fit either the thermocouple or pyrolysis tube to the component. The Cal 9900 autotune unit fitted to control and display the pyrolysis temperature, cannot easily be used above 900°C, because manual reprogramming is required each time to take the temperature over this limit and the controller is designed to recognise errors such as short circuits which may lead to uncontrolled heating. Therefore, under normal conditions (up to 900°C), the ceramic head is unlikely to fail.

2.3 Optimisation and Figures of Merit

2.3.1 Preparation of Standards

A range of organomercury standards were prepared from commercially available salts and solutions following strict handling procedures. Table 2.1 lists the standards used during this project.

Much of the work within this project was limited to the monoalkylhalides, which are salts at room temperature, and are consequently easier to handle than the more volatile dialkylmercury liquids. Studies with the latter more dangerous compounds were kept to a minimum, and used only in brief performance studies to assess chromatographic characteristics and injector performance studies (see Chapter 6).

2.3.1.1 Storage

Concentrated organomercury standards were stored in a refrigerator at 4°C. Standards were double bagged and stored under argon within a dessicator in a designated refrigerator.

2.3.1.2 Standard Handling

Organomercury standards were only handled following strict safety procedures. All work was undertaken in a fume cupboard with surrounding personnel having been made aware of what was going on. It was necessary to wear two pairs of protective gloves. Long neoprene or nitrile gloves worn underneath Viton or butyl gloves are recommended. It was noted that latex gloves alone were unsuitable when handling these compounds. A lab coat and safety glasses were also imperative. Organomercury compounds were weighed in

sealed vessels, and spatulas and pipette tips were decontaminated using acidified bromine solutions.

2.3.1.3 Waste Disposal

Organomercury compounds in aqueous solution were brominated to reduce R-Hg to Hg^{II} Normal procedures for inorganic mercury were then followed to dispose of the waste. Solvent based waste was collected in a clearly marked sealed glass bottle and special arrangements were made for its removal and disposal.

In general, all stock solutions were prepared in the same way. First, a portion of the standard of interest (salt or liquid) was dissolved in methanol. Further dilutions in methanol, water or other solvents such as dichloromethane were made as required. As solvents of different densities were often used, standards were always prepared by weight until the final stage where concentrations were converted to volume (µl) to correspond to injections onto the instrument. All standards of organomercury were calculated as mass of mercury.

2.3.2 Initial Operating Conditions

Initial operating conditions used are shown in Table 2.2. The chromatogram resulting from a 1pg injection of mixture of methylmercury chloride (MM), ethylmercury chloride (EM), dimethylmercury (DMM), and diethylmercury (DEM) using these conditions is shown in Figure 2.8. Table 2.3 lists retention time data for each species using the initial operating conditions listed in Table 2.2. The concentration of each compound was undetermined and varied from a few pg μl^{-1} to a several hundred pg μl^{-1}.

Table 2.1 Organomercury standards

Monoalkylhalides	**RHgX**	**Purity (%)**	**Formula weight (g)**	**%Hg**	**Supplier**
Methylmercury chloride	CH_3HgCl	98+	251.1	79.9	Strem*
Methylmercury iodide	CH_3HgI	98+	342.5	58.6	Strem
Ethylmercury chloride	C_2H_5HgCl	98+	265.1	75.7	Alfa**
Dialkylmercury	**R_2Hg**				
Dimethylmercury	$(CH_3)_2Hg$	98	230.7	87.0	Strem
Diethylmercury	$(C_2H_5)_2Hg$	99	258.7	77.5	Strem
Arylmercury compounds	**PhHg-?**				
Phenylmercury chloride	PhHgCl	96	313.2	64.0	Strem
Phenylmercury acetate	$PhHgO_2C_2H_3$	97.5	336.8	59.6	Strem
Diphenylmercury	$(Ph)_2Hg$	96+	354.8	56.5	Strem

* Strem Chemicals UK, Royston, UK

** Alfa, Johnson Matthey plc, Royston, UK

Table 2.2 GC-AFS Initial Operating Conditions

Injector	1µl in DCM splitless injection, isothermal 250°C with glass liner
Column specification	Megabore DB1 (J&W Scientific), 15m x 0.53mm x 1.5µm
Oven temperature	40°C for 1 min, ramp to 115°C at 10°C min^{-1}, ramp to 200°C at 10°C min^{-1} held for 1 min,
Pyrolyser temperature	640°C
Gases	Column 12ml min^{-1} (Helium) Make-up 60ml min^{-1} (Argon) Sheath 300 ml min^{-1} (Argon)
Gain range	As required

Table 2.3 Retention Times of Organomercury Species Under Initial Operating Conditions

Compound	Retention time (min)
Hg(0)	0.225
DMM	1.175
DEM	3.764
MM	4.042
EM	6.833

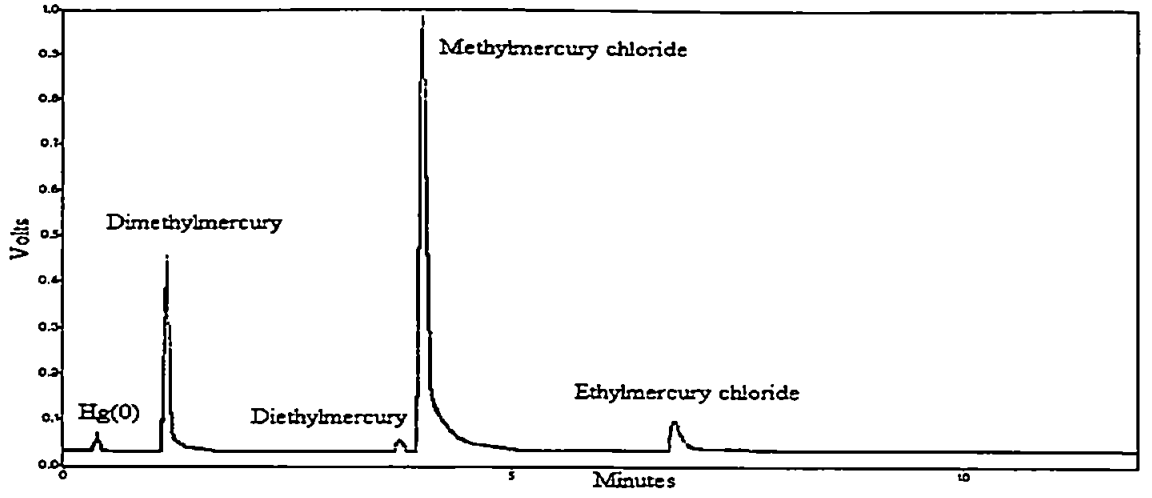

Figure 2.8 **Example of compound separation by GC-AFS: Chromatogram of Mixed Standard containing variable concentrations of Dimethylmercury, Diethylmercury, Methylmercury chloride and Ethylmercury chloride in Dichloromethane solvent**

As can be seen from Figure 2.8, increasing peak broadening was observed with decreasing compound volatility and increasing molecular weight. However, good separation of each species was noted which was a good starting point from which to optimise the analytical conditions.

2.3.3 Optimisation of Analytical Conditions

A range of optimisation experiments were performed to find the most efficient operating conditions for the instrument. These included the injection mode, injector temperature, solvent choice, oven temperature program, pyrolyser temperature, carrier gas, make-up gas flow rate and sheath gas flow rate.

2.3.3.1 Injection mode

The Programmable Temperature Vaporiser (PTV) injector is capable of split, splitless, on-column or direct injection modes in addition to offering the capability for large volume injections and thermal desorption. Initial studies considered the standard modes for 1μl injections of organomercury standards prepared in dichloromethane solvent. In the beginning both split and splitless injection modes were found to lead to some compound degradation, probably occurring on active sites within the injector. Direct injection of the sample into a glass lined cavity, with the column attached at the bottom, was found to be the most efficient mode with no degradation and repeatable results. The degradation problems with the split and splitless arrangements have since been overcome by polishing the internal injector surfaces and preconditioning, however, the direct injection arrangement was selected for this work.

2.3.3.2 Injection Temperature

Optimisation of injection temperature for the direct injection arrangement was performed using the PTV controller. The injection temperature was varied between 50-300°C. The effect of injection temperature on the peak area observed for a 104 pg μl^{-1} standard of methylmercury chloride is shown in Figure 2.9. It was noted that increasing injection temperatures to 300°C, after a chromatographic run performed at a lower isothermal injection temperature, yielded an elemental mercury signal which was not observed when injecting solvent blanks at 300°C. These signals were a result of incomplete sample transfer to the column at lower temperatures. As the column upper working limit was 320°C an isothermal operating temperature of 300°C was selected for quantitative measurements.

2.3.3.3 Solvent Choice

Toluene (99.8% HPLC grade, Aldrich) and 1,2-dichloromethane (99.8% HPLC grade, Aldrich) are the two main solvents used in Westöö type extractions of organomercury compounds. It was decided to consider both of these in addition to a simple n-alkane, hexane (99+% HPLC grade, Aldrich).

Initially blank injections of each solvent were made into the instrument. Dichloromethane and hexane gave clean blanks whereas toluene resulted in a large signal. This was identified as elemental mercury by repeating the injection with the pyrolyser switched off. Attempts to remove the mercury by cleaning the toluene with gold gauze did not help. The concentration of mercury was estimated to be around 1ng μl^{-1}. Toluene was therefore rejected as a possible solvent for this work.

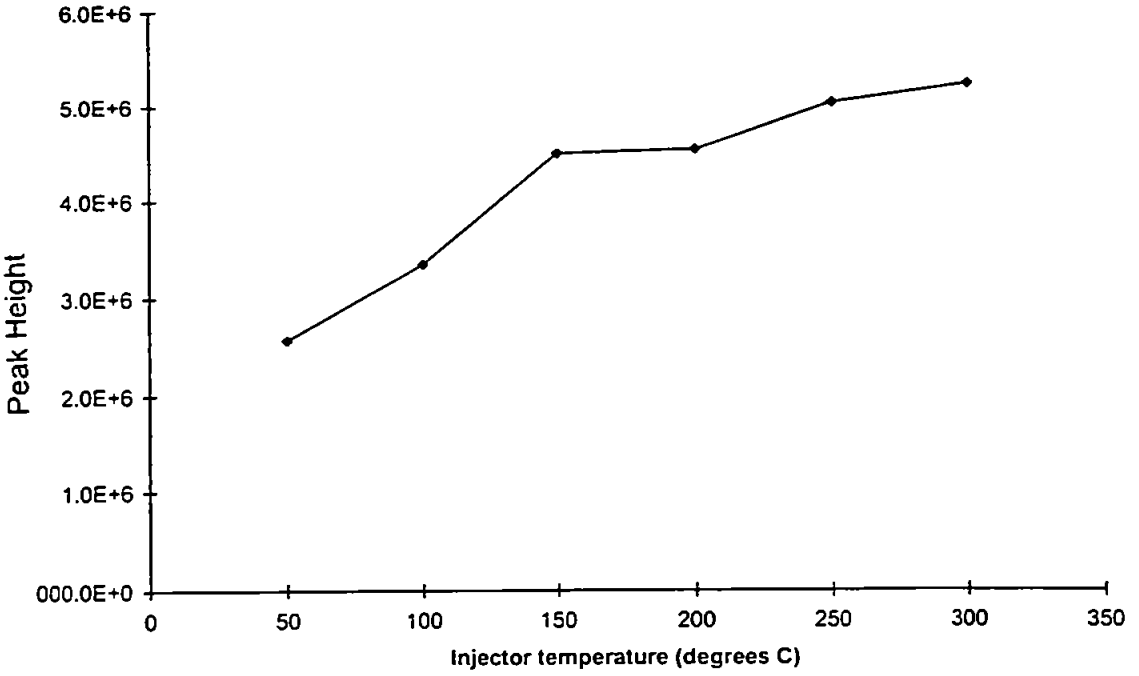

Figure 2.9 **Effect of Injector Temperature on Signal for 104pg Methylmercury Chloride**

The signal for the standard prepared in hexane was much smaller than that observed for dichloromethane. Repeat sample preparation and analysis confirmed that this was a real effect and was concluded to be due to the poor solubility of methylmercury chloride in hexane. Dichloromethane was therefore selected as the solvent of choice.

2.3.3.4 Oven Temperature Programming

The use of an oven temperature programme was necessary to allow the rapid separation of the organomercury species. An increase in oven temperature from 40°C to around 115°C was found to be sufficient to give good separation of the compounds of interest. Varying the ramp rate appeared to increase the separation whilst increasing the chromatographic run time. Table 2.4 compares the retention times of methyl- and ethyl- mercury chloride standards observed for two temperature programmes, both starting at 40°C and ending at 200°C, with respective ramp rates of 10°C/min and 25°C/min. The latter programme was selected for this work as it reduced the overall chromatographic run time whilst retaining good separation of the compounds.

Table 2.4 Effect of Oven Ramp Rate on Retention Time

Ramp rate (°C/min)	Retention time MMC (min)	Retention time EMC (min)
10	4.44	7.30
25	4.01	5.58

2.3.3.5 Carrier Gas

Three carrier gases argon, helium and nitrogen were all considered within the GC-AFS instrument. The effect of flow rate between 5-40 ml min^{-1} on two single compound standards containing 91 pg μl^{-1} methylmercury chloride and 79 pg μl^{-1} ethylmercury as Hg was studied. For both compounds the use of nitrogen as a carrier gas resulted in substantially smaller signals, which was attributed to quenching within the atom cell. Argon and helium both appeared to be good carrier gases resulting in similar sensitivities for the analytes. Figure 2.10 shows van Deemter plots for methylmercury and ethylmercury with both helium and argon carrier gases. In all cases the minima were achieved at 10ml min^{-1}. It was noted that helium was slightly better than argon at this optimal flow, although argon would be the gas of choice if flow rates >15ml min were required. The peak resolution for methyl and ethylmercury chlorides was calculated to be 0.9 for both argon and helium, under these conditions.

2.3.3.6 Pyrolyser Temperature

A mixed standard of methyl- and ethylmercury chlorides (as Hg) was prepared in dichloromethane at a level of 86pg and 66pg μl^{-1} respectively. The effect of pyrolysis temperature on peak areas was studied between 0-900°C with results shown in Figure 2.11. As the pyrolysis temperature was increased, a signal for ethylmercury was observed before the signal for methylmercury indicating that the former compound was more easily thermally decomposed. Quantitative recovery of both compounds was obtained at pyrolysis temperatures >800°C. At a pyrolyser temperature of 900°C a characteristic triplet peak was observed at the front of the chromatogram. This was believed to be due to carbon scatter as a soot like deposit which was in the pyrolysis tube. An optimum pyrolysis temperature range between 800-850°C was chosen.

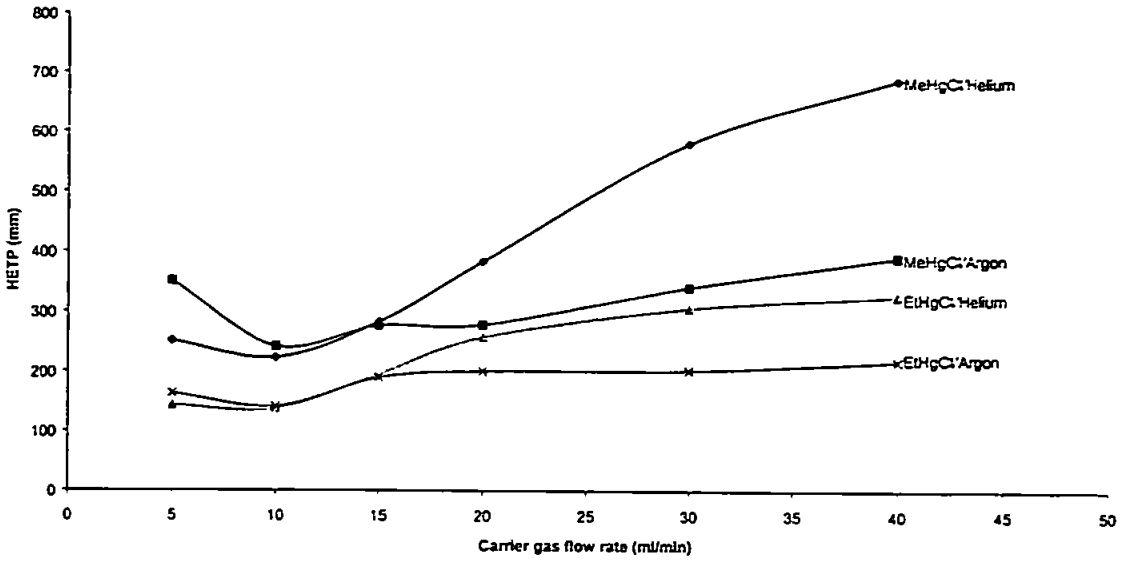

Figure 2.10 Variation of HETP with Carrier Gas

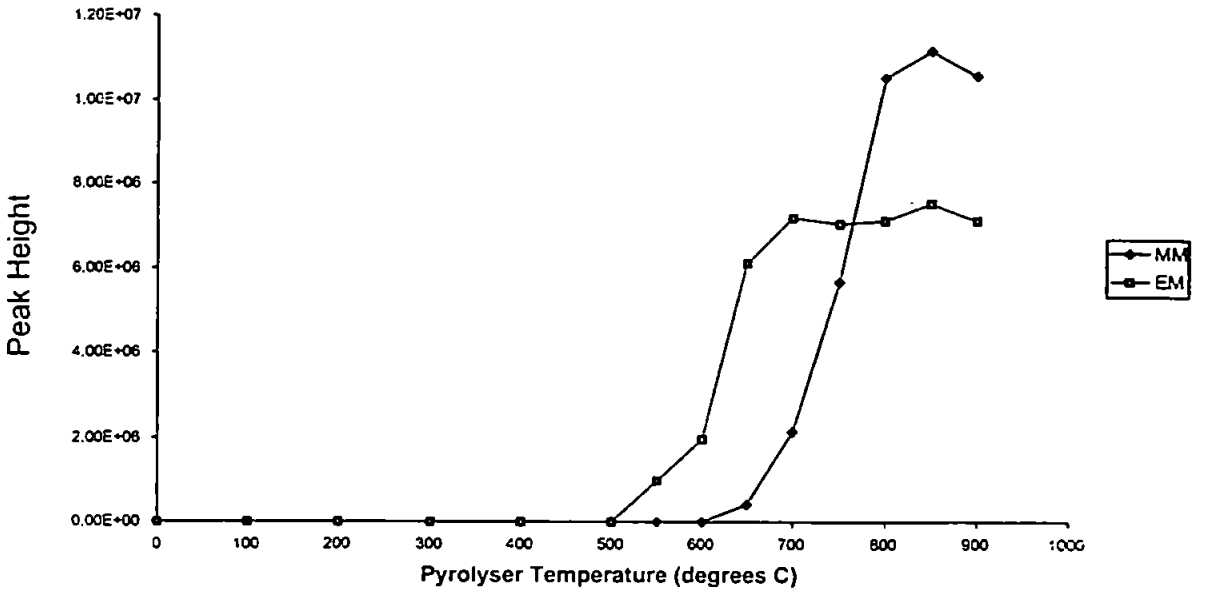

Figure 2.11 Effect of Pyrolysis Temperature on 86pg Methylmercury Chloride and 66pg Ethylmercury Chloride

2.3.3.7 Make-up Gas Flow Rate

Argon gas was mixed with the sample gas as it left the pyrolyser before entering the detector. The maximum possible dilution of the helium with argon was required at this stage in order to minimise the amount of helium reaching the detector. This was measured as 60ml min^{-1}.

2.3.3.8 Sheath Gas Flow Rate

The sheath gas surrounds the sample gas as it enters the detector and prevents it from spreading within the atom cell. Argon was chosen as the sheath gas for mercury determination by AFS. It has been used previously for cold vapour – AFS applications at flows of around 300 ml min^{-1} [48]. For this experiment, a mixed standard of methyl and ethylmercury chlorides (as Hg) of approximately 70pg μl^{-1} as Hg, of each compound, was used. The sheath gas flow rate was varied between 0-450ml min^{-1} and results are shown in Figure 2.12. The optimum sheath gas range was identified between 25-150 ml min^{-1} with a loss of sensitivity at flow rates less than 10 ml min^{-1} and greater than 200 ml min^{-1}. As helium was chosen as the carrier gas for these studies and given the detrimental effects of helium on a standard AFS detector, the highest compromise flow of argon, 150ml min^{-1} was selected.

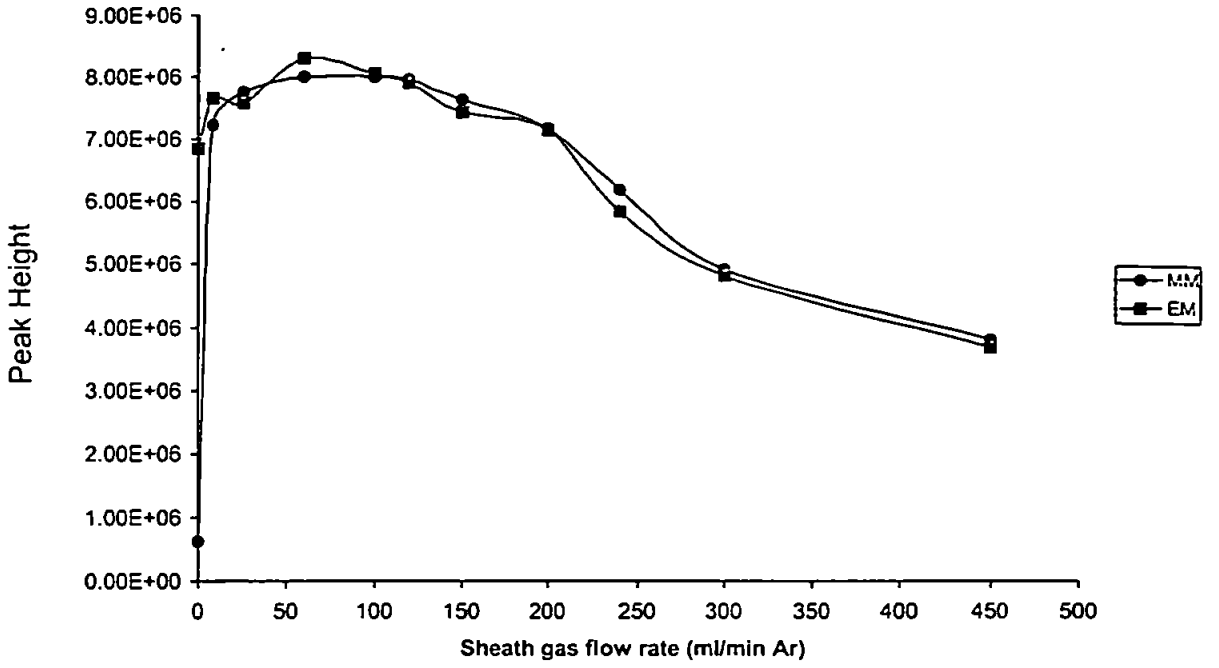

Figure 2.12 Effect of Sheath Gas Flow Rate on 70pg Methylmercury and 70pg Ethylmercury Signals

2.3.4 Analytical Performance

Table 2.5 lists the final operating conditions for the GC-AFS instrument which were used for performance studies and application development.

For all of these studies manual injections were made using a 10µl syringe (Hamilton 701 RN, Phase Separations Ltd., UK). A repeatability test was performed on a mixed standard of methyl and ethylmercury chlorides (as Hg) containing 86pg and 66pg μl^{-1} respectively. The relative standard deviation of injections was MM = 7% and EM = 5% based on ten sequential injections. A chromatogram illustrating the separation between the components of this mixed standard is shown in Figure 2.13.

Table 2.5 Optimised Operating Conditions

Injection	1µl in DCM, 300°C isothermal direct injection
Column	DB1 Megabore (J&W Scientific), 15m x 0.53mm x 1.5µm
Oven Program	40°C held 2min, ramp to 115°C at 25°C/min, held 5 min, ramp to 200°C at 25°C/min, held 0.1min
Pyrolyser Temperature	850°C
Gases	11ml/min He carrier flow 60ml/min Ar make-up flow 150ml/min Ar sheath flow
Range	As required

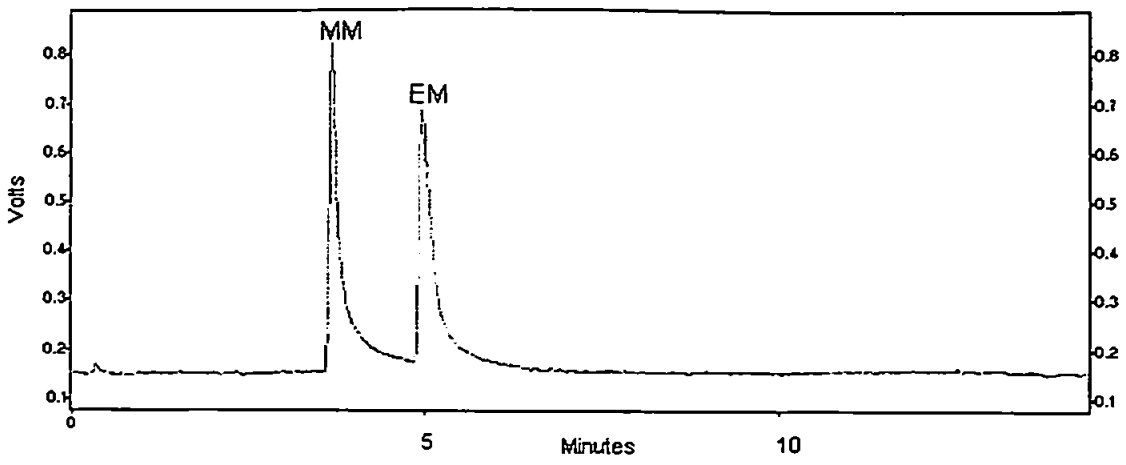

Figure 2.13 Chromatogram of 86pg Methylmercury and 66pg Ethylmercury Mixed Standard

The instrumental limit of detection was established by calibrating the instrument on its maximum amplification range, between 0-10pg methylmercury chloride (as Hg). Ten injections of a low level standard, 0.6pg μl^{-1} methylmercury were made. The limit of detection was determined as $3\sigma_{n-1}/m$, (where m = gradient of calibration curve) and equated to 0.25pg methylmercury as mercury.

The amplification control on the AFS detector allows it to be used over five orders of magnitude. Linear calibrations were obtained between 0-10pg on the most sensitive setting (1000 x 10) up to 0-2ng on range 10 x 2, for 1µl injections.

Increasing concentrations were sometimes noted to give a degree of peak splitting although this did not effect the linearity of the technique when integrated peak areas were used. Calibration curves had R^2 values of 0.9973 and 0.9986 respectively and the same response was observed for equal masses of mercury, regardless if it was in the form of methyl or ethylmercury chloride. These are shown in Figure 2.14.

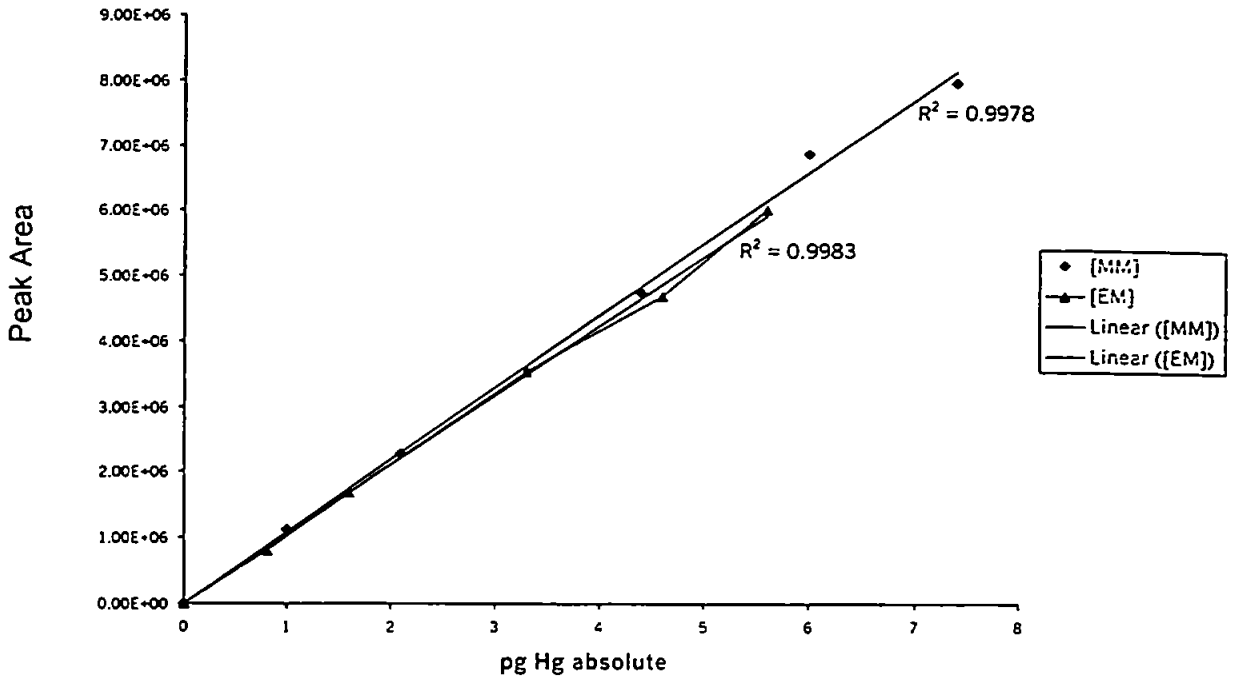

Figure 2.14 Methyl and Ethylmercury Calibration Curves 0-8pg (as Hg)

Chapter 3

DETERMINATION OF ORGANOMERCURY IN BIOLOGICAL SAMPLES

3.1 Introduction

This chapter considers the application of the fully automated GC-AFS instrument for the determination of organomercury in biological samples. For each of the samples studied an extraction procedure was performed in order to extract organomercury, primarily methylmercury, from the matrix. A variation on the Westöö procedure, favoured by Jones *et al* was selected as the starting point. Initial studies were undertaken with two certified reference materials (CRM's): IAEA 142 and NIST SRM 8044, *Mytilus edulis* mussel homogenate. Following this, an international interlaboratory comparison exercise was undertaken for the certification of a *Fucus* sea plant material, IAEA 140. This involved a detailed period of method development. The procedure was evaluated and tested with both organomercury standards and a range of sample matrices, including fresh mussel homogenate, pig liver, pig kidney and cooked rice. As these samples considered relatively low levels of methylmercury *i.e.* <50ng g^{-1}, it was also decided to consider a range of samples containing much higher levels of this species. As a result, a method was developed for the determination of methylmercury in fresh beluga and ringed seal livers, with method validation using a Dogfish liver tissue reference material, DOLT 2. For each of the samples analysed for organomercury content, total mercury determinations were also made, using an appropriate digestion procedure, followed by CV-AFS.

3.2 Experimental

3.2.1 Instrumentation

The Gas Chromatography – Atomic Fluorescence instrument was used to determine organomercury in biological samples under the optimised operating conditions described in Chapter 2. The extraction of organomercury from the sample matrices involved the use of a platform shaker, centrifuges and a vortex mixer. Cold Vapour – Atomic Fluorescence instruments (Merlin Plus and Millennium Merlin CV-AFS systems, PS Analytical Ltd, Orpington, UK) were used to determine total mercury levels after sample digestion. The systems were fully automated (Avalon software, PS Analytical Ltd). Samples were digested on a block digester (Lachat Block Digestor BD-26, Lachat, Milwaukee, USA).

3.2.2 Reagents and Chemicals

Organomercury was determined in biological samples after a selective extraction and clean-up procedure. All reagents were of analytical grade or better (Aldrich Chemical Company, Gillingham, UK or Fisher Chemicals, Hampton, USA), acids were of AristaR grade (BDH Ltd, Poole, UK or Fisher Chemicals, USA) and solutions were made with double de-ionised water (Elga Ltd, High Wycombe, UK or B-pure, Barnstead, USA). In some procedures an alkaline pre-treatment step was performed using KOH (6 mol dm^{-3}) followed by acidification using HCl (50% v/v). The main extraction into dichloromethane solvent (98% +, HPLC grade) was achieved using a 3:1 mixture of acidic KBr (18% m/v in 0.5% v/v H_2SO_4) and $CuSO_4.5H_2O$ (1 mol dm^{-3}). Clean-up of the initial extract was with $Na_2S_2O_3$ (0.01 mol dm^{-3}) before back extraction into solvent. Prior to analysis by GC-AFS the solvent was dried using anhydrous granular Na_2SO_4. Organomercury standards were prepared from methylmercury chloride (98%+, Strem Chemicals UK, Royston, UK) and

ethylmercury chloride salts (98+%, Alfa, Johnson Matthey plc, Royston, UK). Initial stock solutions were prepared by dissolving in methanol (HPLC grade, Aldrich). From these a mixed working stock was prepared by dilution in water. Subsequent standard solutions were freshly prepared by further dilution in water. These were used for both sample spikes and to prepare calibration series for extraction.

For total mercury determinations samples and standards were digested in concentrated HNO_3 (AristaR grade, Merck Ltd, Poole, UK) and H_2O_2 (27.5% in water, Aldrich Chemical Company, Gillingham UK). Standards were prepared by appropriate dilution of a 1000 mg l^{-1} Hg^{2+} standard (SpectrosoL, Merck Ltd, UK). Vapour generation in the CV-AFS system used $SnCl_2$ (2% m/v, ACS grade, Aldrich, UK) in HCl (10% v/v, AnalaR grade, Merck Ltd, UK) reductant and HNO_3 (20% v/v, AristaR grade, Merck Ltd, UK) blank.

3.2.3 Organomercury Extraction Procedure

In order to test the GC-AFS instrument with biological samples, two organomercury extraction procedures were selected based upon methods used by Jones *et al* [124]. Methods 1 and 2 have been shown in Figure 3.1. Both methods were reported to be suitable for fish samples. Method 2 was selected as the main method of choice as this procedure incorporated a strong alkaline digestion step in an attempt to increase extraction efficiency from complex biological tissues. Initial experiments were performed to test both extraction procedures. Validation experiments indicated that larger transfer volumes than described in the procedure might be possible at each extraction stage. As a result, it was decided to vary the methods and to transfer the maximum possible volume between each stage therefore maximising the preconcentration factor. Transfers were made by volume using autopipettes and disposable plastic tips.

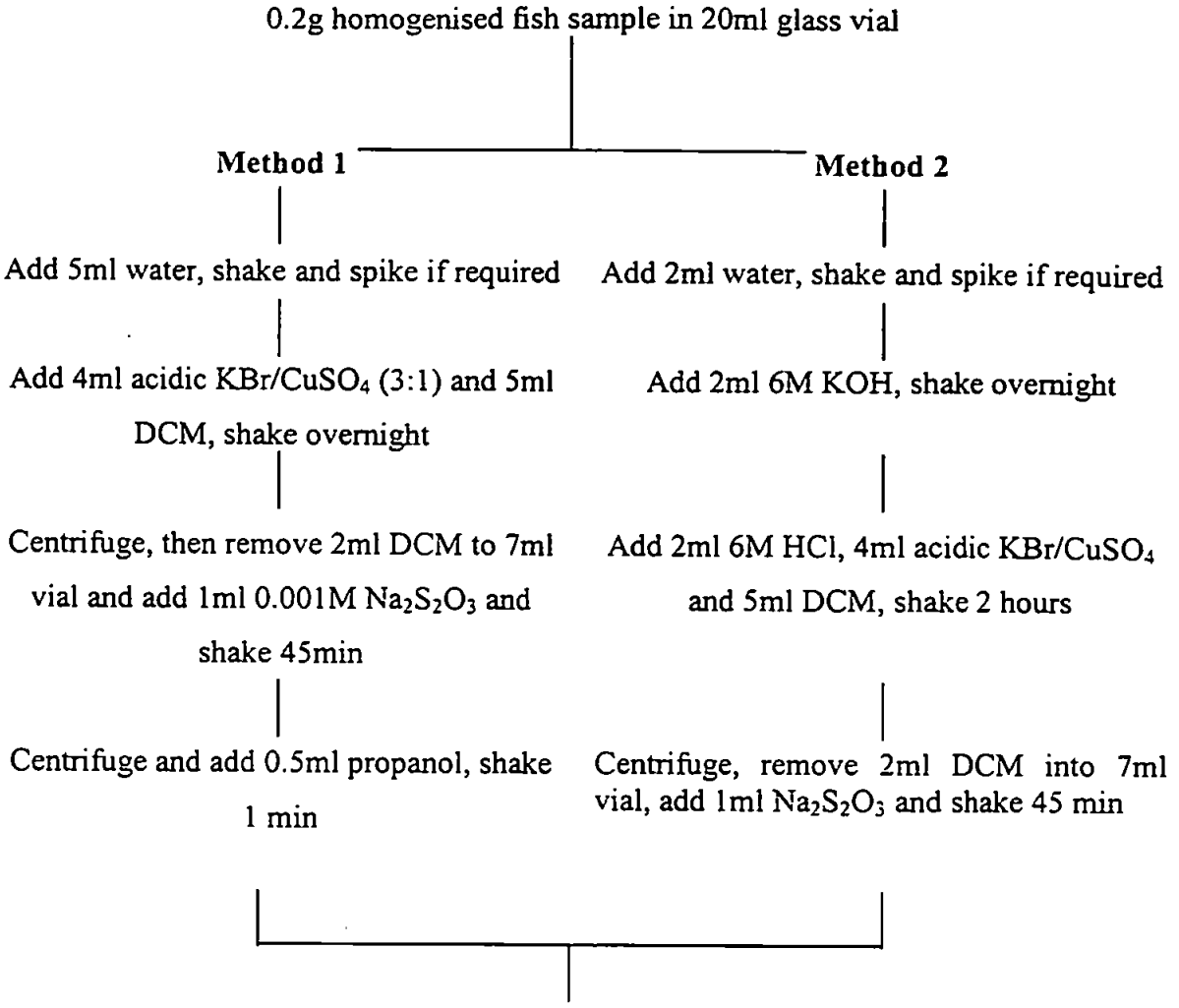

Figure 3.1 Initial Organomercury Extraction Procedures for Fish

The initial centrifugation step described by Jones involved a refrigerated model at 5000 rpm. Tests showed that centrifuging at 3000-3500 rpm in a non-refrigerated model did not appear to have any significant effect upon extraction efficiency. It was also noted in stage 6 of the procedure that vortex mixing would perhaps be more appropriate prior to shaking. These steps of the procedure were therefore reversed. The final volume of dichloromethane (DCM) added at the final extract stage was only 0.15ml. This was increased to 0.3ml for ease of handling. Finally as DCM is very slightly soluble in water and given the incompatibility of water with the GC column, it was important to 'dry' the extraction solvent prior to analysis. This was achieved using a micropipette tip filled with a few 100mg anhydrous sodium sulphate, through which the sample was passed prior to sealing in the sample vial. This step was an improvement on a procedure previously described by Jones, where a few mg of the anhydrous salt was added to the sample vial. The latter method had been observed to give rise to chromatographic problems when small crystals of the salt were invariably injected onto the GC column.

3.2.4 Total Mercury Digestion Procedure

Portions of sample (approximately 0.2000g-1.0000g) were accurately weighed in triplicate and transferred to digestion tubes previously cleaned by heating in HNO_3 (50% v/v). A portion of concentrated HNO_3 (10ml, AristaR grade) was used to aid the transfer of the sample. Two further portions of each material were weighed and spiked with an aliquot of Hg^{2+} standard (100µg l^{-1} in 10% HNO_3). An appropriate calibration series was then prepared by spiking concentrated HNO_3 (10ml) with portions of Hg^{2+} standard (100µg l^{-1} in 10% HNO_3). Three procedural blanks were also prepared. The digestion tubes were heated in a block digester at 180°C for 90min until most of the brown fumes evolved had subsided. The vessels were then removed and allowed to cool for 10-15min before adding portions of H_2O_2 (0.5ml). Once the effervescence had ceased, the tubes were returned to

the digester and heated at 180°C for 30min. After this time the temperature was raised to 200°C and heating continued for a further 30min. Once again the digestion tubes were removed to cool for 10-15min before addition of a second portion of H_2O_2 (0.5ml). A final heating period of 30min at 200°C was then performed. Once cool, the contents of the tubes were transferred to clean volumetric flasks and made up to 50ml with water. The samples and standards were analysed by CV-AFS against 2% m/v $SnCl_2$ in 10% v/v HCl reductant and 20% v/v HNO_3 blank.

3.3 Contamination Problems

Initial extraction procedures, performed at Florida International University, Miami gave completely clean chromatograms for blank control samples passed through both Methods 1 and 2. This confirmed that the chemicals, glassware and handling procedures did not result in background contamination. Upon establishing the facilities to perform such extractions in our own laboratory, signals corresponding to methylmercury were observed in blank control samples. These appeared to remain at a constant level within a procedure but were observed to vary between methods. Experiments traced the contamination source to the initial extraction vial and cap set (20ml borosilicate scintillation bottle and urea cap, Chromacol Ltd, Welwyn Garden City, UK). In an attempt to remove this contamination, acid washing (8M HNO_3 overnight, deionised water overnight) was considered. This did not have any effect on the methylmercury contamination. PTFE disc inserts were obtained and fitted inside the caps during extraction. These were of variable benefit; some vessels gave clean blanks, while some gave rise to methylmercury signals. It was subsequently noted that these discs varied slightly in size, forming a complete seal in some caps and not in others. This led to methylmercury contamination from acid leaching of the caps typically in the order of a few pg μl^{-1}. The level of mercury in the urea based caps was quantified by leaching with concentrated hydrochloric acid, diluting to 10% v/v and

analysing by CV-AFS. An average concentration of 2.4ng of mercury was determined per cap. This equated to 0.88 ng g^{-1}. As a result a range of alternative vial caps were obtained from various suppliers. After evaluation, a polypropylene cap was found which was both acid resistant and did not give rise to methylmercury contamination (Jubb Ltd, Leicester, UK). These caps were subsequently used for all extraction procedures.

Further contamination sources were also identified during method development. In particular cross contamination was encountered when solvent was transferred using an autopipette. It was noted that particular care had to be taken when transferring Dichloromethane as it had a tendency of vortexing within the tip and coming into contact with the pipette shaft. This was overcome by reducing the maximum volume of solvent transferred by autopipette by 25% *i.e.* 150µl instead of 200µl. An initial solvent rinse step was also introduced before sample transfer. This had the effect of eliminating drips and improving the accuracy of transfer. A further serious contamination source was also identified at the final sample preparation stage. If the drier tube contained too much salt, or if sufficient time was not given to allow the solvent to start to pass through the reagent, the liquid level would be high enough to come into contact with the pipette shaft. As a result a wash step was introduced where the pipette shaft was rinsed in double deionised water and dried between samples.

3.4 Initial Studies – Analyses of Sea Plant and Mussel Homogenates

3.4.1 Introduction

The aim of this work was to take part in an international interlaboratory comparison exercise for the certification of methylmercury in *Fucus*, sea plant homogenate. In order to do this it was necessary to first validate the detection techniques along with the sample preparation procedures. This was achieved with mussel homogenate reference materials.

3.4.2 Experimental

3.4.2.1 Materials

Certified reference materials IAEA 142 and NIST SRM 8044 mussel homogenates (*Mytilus edulis*) were provided by the International Atomic Energy Agency, Marine Environment Laboratory, Monaco for method validation. A bottle of IAEA 140 sea plant homogenate (*Fucus*) was supplied for the intercomparison exercise. This sample, collected on the Atlantic coast was in the form of a powder with a particle size < 40μm. It was suggested that the methylmercury content of this sample would be very low, perhaps in the region of a few ng g^{-1}. The moisture content of all of the samples was determined by taking separate portions and drying to constant weight for 48 hours at 105°C.

3.4.2.2 Extraction of Methylmercury from Mussel Homogenate Reference Materials

Organomercury was extracted following a procedure based upon Method 2 described in Section 3.1.3. Ten portions of each reference material, approximately 30 ± 0.5 mg were accurately weighed into glass vials. Four portions were spiked (0.2ml x 5ng ml^{-1} MM +

5ng ml^{-1} EM respectively, prepared in water). Two procedural blanks were also taken through the procedure. The samples were shaken with KOH for two hours and extracted into DCM overnight. The volume of DCM transferred for thiosulphate extraction T1 was approximately 3.8 ml and was recorded for each sample whilst T2 was 0.6ml. A final volume of 0.15ml DCM was prepared for analysis.

3.4.2.3 Digestion of Mussel Homogenate Samples for Total Mercury Determination

The digestion procedure outlined in Section 3.1.4 was used to prepare the mussel homogenate samples. Five portions of each material (approximately 0.2000g) were prepared, two of which were spiked with 50µl and 100µg l^{-1} Hg^{2+} in 10% HNO_3 respectively. Three procedural blanks and a calibration series were also prepared. Samples were diluted to 50ml final volume. The samples and standards were analysed by CV-AFS under the conditions given in Table 3.1.

Table 3.1 Analysis Conditions for CV-AFS

Reductant	2% m/v $SnCl_2$ in 10% v/v HCl, 3.8ml min^{-1}
Blank / Sample	20% v/v HNO_3, 7.9ml min^{-1}
Analysis time	Total = 130s (Delay = 10s, Measure = 60s, Delay = 60s)
Detector range	100 x 4
Autozero	On
Carrier gas (Ar)	300ml min^{-1}
Sheath gas (Ar)	300ml min^{-1}
Drier gas (air)	3l min^{-1}

3.4.2.4 Method Development for the Determination of Methylmercury in IAEA 140 Intercomparison Sample

The procedure followed for the extraction of methylmercury from the mussel homogenates was selected as the starting point for this sample. As the concentration of methylmercury was expected to be low, the initial sample mass was increased to 500mg (Method A). At the same time a second procedure was followed without the alkaline step (Method B). A series of further procedures were then developed based on the observations and results of each method (Method C-H). Table 3.2 summarises the initial pre-treatment/extraction step of Method A-H, which replaced steps 1-4 of the main procedure. Ten portions of each sample were prepared for each method with four being spiked at a concentration of 1000pg MM (as Hg) absolute.

Table 3.2 Sea Plant Sample Preparation Method Development

Method	Initial pretreatment/extraction step
A	**500mg** sample, **2ml** H_2O, 2ml 6M **KOH**, shaken 2 hours. Acidify **4ml** 6N HCl, 4ml acidic KBr/$CuSO_4$ mix, 5ml DCM, shaken overnight
B	**500mg** sample, **5ml** H_2O, **4ml** acidic KBr/$CuSO_4$ mix, 5ml DCM, shaken overnight
C	**300mg** sample, **3ml** H_2O, 2ml 6M **KOH**, shaken 2 hours. Acidify **4ml** 6N HCl, 4ml acidic KBr/$CuSO_4$ mix, 5ml DCM, shaken overnight
D	**300**mg sample, **10ml** H_2O, 4ml acidic KBr/$CuSO_4$ mix, 5ml DCM, shaken overnight
E	**300**mg sample, **2ml** H_2O, **12ml** acidic KBr/$CuSO_4$ mix, 5ml DCM, shaken overnight
F	**300**mg sample, **2ml** H_2O, **4ml** 6N HCl, **10ml** acidic KBr/$CuSO_4$ mix, 5ml DCM, shaken overnight
G	**300**mg sample, **2ml** H_2O, **8ml** 6N HCl, **4ml** acidic KBr/$CuSO_4$ mix, 5ml DCM, **shaken 6 hours**
H	**300**mg sample, **2ml** H_2O, **8ml** 6N HCl, **4ml** acidic KBr/$CuSO_4$ mix, 5ml DCM, **shaken 24 hours**

3.4.2.5 Determination of Total Mercury in IAEA 140

The total mercury content of IAEA 140 sea plant homogenate was determined. As with the mussel homogenates the procedure followed involved the digestion of the sample in nitric acid with hydrogen peroxide prior to CV-AFS determination. The mass of sample taken was approximately 0.1000g, whilst initial heating on the block digester was at 140°C for 120 minutes.

3.4.4 Results and Discussion

3.4.4.1 Determination of Methylmercury in Mussel Homogenates

Figure 3.2 illustrates a chromatogram obtained for one of the IAEA 142 samples. The absolute concentration of mercury here was determined as 12pg against calibration standards. The results for the determination of methylmercury in the mussel homogenates are shown in Table 3.3. These have been corrected for weight dilution, spike recovery and moisture content. Good correlation between measured concentrations and certified values was noted. The high variation in spike recovery for IAEA 142 was due to an outlier in one of the measurements. As only three recoveries were made this could not be eliminated from the results.

Table 3.3 Determination of Methylmercury in *Mytilus edulis* Mussel Homogenates

Certified reference material	Moisture Content (%)	Spike recovery (%)	Corrected [MM] n = 6 (ng g^{-1})	Certified [MM] (ng g^{-1})
NIST SRM 8044	5.0	63 ± 5	26 ± 4	28 ± 2
IAEA 142	7.0	95 ± 20	45 ±7	47 ±4

Good agreement between measured and certified levels were obtained for methylmercury concentrations in NIST SRM 8044 and IAEA 142 mussel homogenates. These results have validated both the extraction procedure and the instrumentation.

3.4.4.2 Determination of Total Mercury in Mussel Homogenates

A linear calibration over the range 0-1 µg l^{-1} was obtained for the digested standards with R^2 = 0.9992. The samples, spiked samples and blanks were measured against this calibration. No mercury was detected in the procedural blanks. Table 3.4 shows the results obtained for the mussel homogenates corrected for weight dilution. Spike recoveries for both samples were good. The final results have been corrected for moisture content measured in the reference materials, NIST SRM 8044 = 5.0% and IAEA 142 = 7.0%. Good agreement was obtained between the concentrations of total mercury measured in the mussel homogenate reference materials and the certified values.

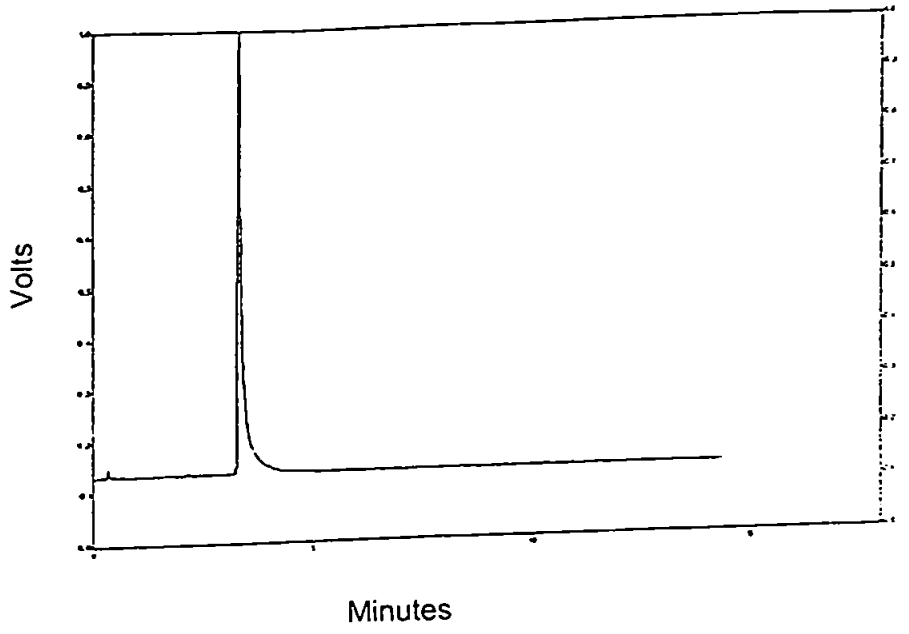

Figure 3.2 Chromatogram of Methylmercury in IAEA 142 Mussel Homogenate

Table 3.4 Determination of Total Mercury in *Mytilus edulis* Mussel Homogenates

Certified reference material	Total [Hg] determined n=3 (ng g^{-1})	Spike recovery (%)	Dry weight corrected Total [Hg] (ng g^{-1})	Certified [Hg] (ng g^{-1})
NIST SRM 8044	56 ± 7	124	59 ± 7	62 ±3
IAEA 142	122 ± 6	97	131 ± 6	126 ± 7

3.4.4.3 Determination of Methylmercury in Sea Plant

The results in Table 3.5 show the concentration of methylmercury determined in IAEA 140 for each method. These results have been corrected for spike recovery and 9.5% moisture content and are based on six replicate and four spike measurements.

Table 3.5 *Fucus* Method Development Results

Method	Spike recovery MM (%)	Corrected MM concentration in sample (ng g^{-1})
A	0	0
B	0	0
C	0	0
D	0	0
E	31	0
F	46	0.7 ± 0.2
G	47	0.33 ± 0.05
H	69.8	0.63 ± 0.06

Each sample was spiked with a mixture of methylmercury and ethylmercury chlorides. The ethylmercury recoveries were not calculated as no ethylmercury was found in the

samples. It was noted that a low ethylmercury recovery of 30% was observed for Method B, approximately 60% for Method E and approximately 90% for subsequent methods.

Methods A and B required500mg portions of sample. With this mass of sample it was very difficult to slurry given the volumes and vessels used. A smaller mass of 300mg was selected for further methods in conjunction with an increase in reagent volume. Methods A and C, alkaline pretreatment methods, resulted in 0% spike recovery. Acidic methods B and D also resulted in 0% methylmercury spike recovery, however a small ethylmercury recovery was seen for Method B when a higher acid strength was used extraction. It was concluded from these procedures that acidic extraction was required for the sea plant sample. This was supported by literature where the release of methylmercury from sediments has been found to be related to pH *i.e.* changing from pH 7.0 to 5.0 doubles the release of methylmercury [24]. Methods E-H involved varying acid concentrations and extraction time. Methylmercury was measured using Method F, however the precision was poor. Method G gave good precision but a lower concentration was measured due to reduced extraction time. Finally Method H gave a similar result to Method F with good precision. Method H was chosen as the method that gave highest recovery. Using this method the concentration of MM in IAEA 140 of 0.63 ± 0.006 ng g^{-1} was confirmed by repeat analyses and the results are shown in Table 3.6.

Table 3.6 Results of Intercomparison Exercise

Certified concentration of MM (as Hg)	0.626 ± 0.139 ng g^{-1}
Concentration of MM (as Hg) determined	0.63 ± 0.006 ng g^{-1}

Figure 3.3 shows the results of the participating laboratories within this exercise. The result for laboratory No76 corresponds with the results reported here. This was in the certification exercise and was in excellent agreement with the certified value. This reference material is now available and currently offers the lowest concentration of methylmercury certified in any such material.

3.4.4.4 Determination of Total Mercury in Sea Plant

A linear calibration between 0-500pg ml^{-1} Hg was obtained with equation y = 0.2385x and R^2 = 0.9999. Figure 3.4 shows one of the signal plots for the sea plant digest. Table 3.7 shows the results obtained based on four portions of sample. Good agreement can be observed between this value and the certified result. No mercury was detected in the procedural blanks.

Table 3.7 Determination of Total Mercury in IAEA 140

Certified concentration of Total Mercury (ng g^{-1})	Concentration found (ng g^{-1}) (n=4)
39 ± 8	38.6 ± 2.7

The total mercury results obtained after nitric acid/peroxide digestion of *Fucus* followed by CV-AFS determination, were in very good agreement with the certified value. The sensitivity of the technique has clearly been demonstrated, as the concentration of mercury in each solution after digestion was only 80pg ml^{-1}

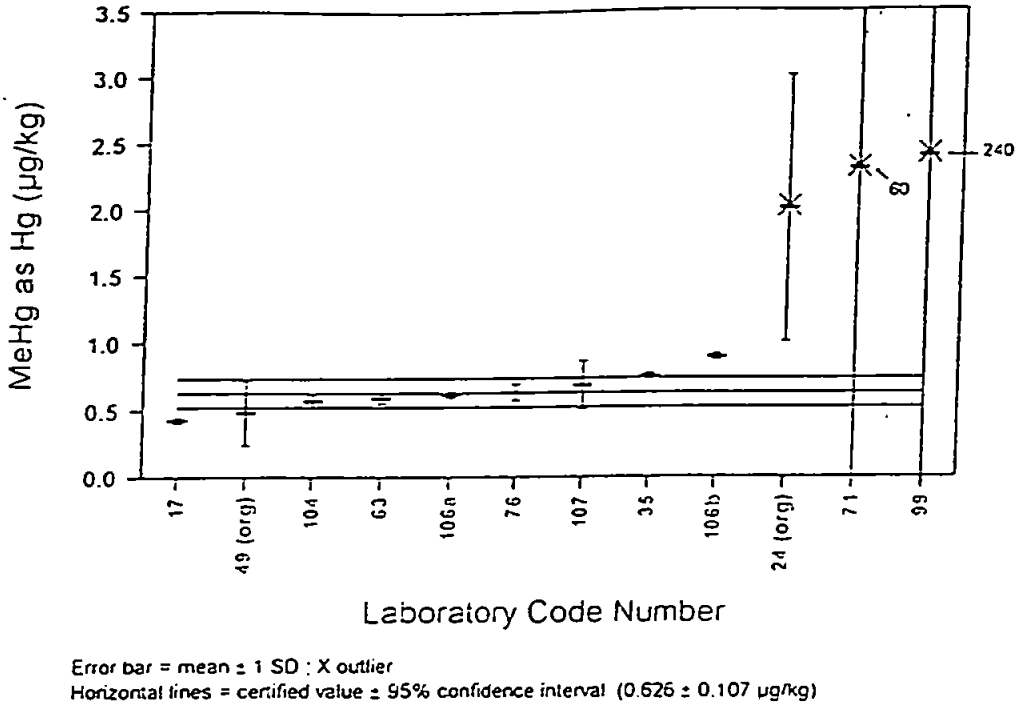

Figure 3.3 Results of IAEA140 Intercomparison Exercise

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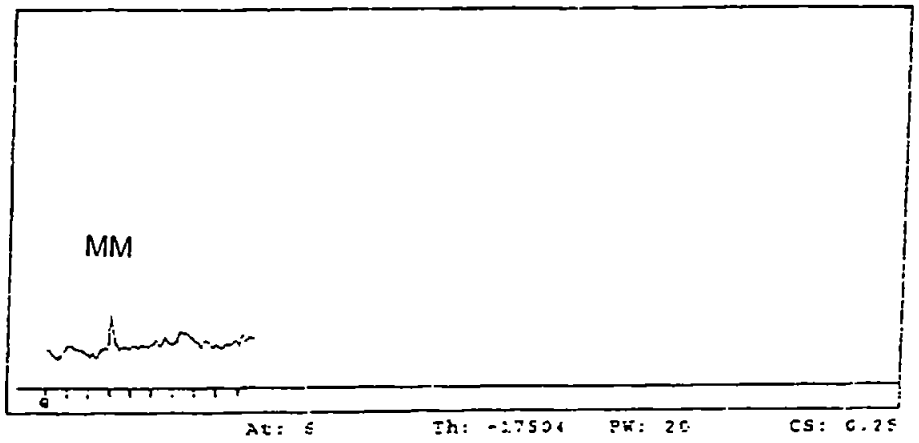


Figure 3.4 Signal Plot of Total Mercury in IAEA 140

3.5 Optimisation of Extraction Procedures

3.5.1 Introduction

Following the validation of the instrumentation and methods, the organomercury extraction procedures were considered for optimisation. The aim was to reduce the time taken to prepare samples and standards for analysis. Initial consideration was given to the stage at which standards could be prepared by comparing the extraction efficiencies of three procedures. As a result of the method development studies of the sea plant homogenate, where extraction time appeared to have a significant effect upon the extraction of methylmercury, an experiment was designed to study the effect of shaking time upon recovery. This was applied to a range of samples: fresh mussels, potting compost, pig kidney, pig liver, uncooked rice, cooked rice, cod liver oil and vegetable oil.

3.5.2 Extraction of Standards

In order to test the efficiency of extracting methylmercury and ethylmercury standards from the aqueous phase into dichloromethane solvent, standards were prepared in water and extracted following step 6 of the methods shown in Figure 3.1. Each standard was analysed five times and the mean areas compared to standards prepared directly in dichloromethane solvent. As AFS is a linear technique it was possible to normalise the mean areas to a concentration of 100pg Hg absolute. The results are shown in Table 3.8. The standards extracted from step 6 were described as partially extracted (PE) whilst the standards prepared directly in dichloromethane were described as non-extracted (NE).

Table 3.8 Comparison of Partially Extracted with Non Extracted Standards

		[Hg](pg)	Mean peak area	RSD(%) n=5	Standardised peak area (100 pg)
PE	MM	101	5643503	7.0	5587627
	EM	101	6007923	8.4	5948439
NE	MM	55	2970844	6.4	5401535
	EM	55	3003934	8.3	5461698

The average RSD for each set of analyses was 7.5%. The difference between PE and NE areas for each species was MM = 3% and EM = 8%. This variation lies within the error associated with injection repeatability previously determined in Chapter 2. It was therefore concluded that there was no significant difference between the aqueous standards extracted into DCM, in the presence of acidic $KBr/CuSO_4$ compared to standards prepared directly in solvent.

A similar experiment was conducted for standards passing through the complete extraction procedure. An overall recovery of 94% was measured for each species. This was explained by small losses at each transfer stage.

These studies confirmed the validity of preparing standards for calibration in water and extracting them through the final stage of the procedure. This was the preferred procedure for three reasons. First stock solutions in water were less volatile than standards prepared in dichloromethane. As dichloromethane easily evaporates into the atmosphere it was decided that a more inert solvent was required in order to contain the toxic organomercurials. Second standards in water were more conveniently disposed of through bromination to inorganic mercury. Thirdly, one stock solution could be used to prepare sample spikes and calibration standards, therefore reducing potential experimental errors.

Finally, calibration series of approximately 0-1ng MM and EM (as Hg) were prepared in water and extracted from step 6 into DCM to test the linearity of this procedure. The results are shown in Table 3.9. Comparing the slopes of both lines confirmed the similar extraction efficiencies of both compounds while R^2 confirmed linearity.

Table 3.9 Extracted Calibration Data

Calibration Range	Equation of Line	Linear Correlation R^2
0-970pg MM	y = 10252x	0.9985
0-1310pg EM	y = 10673x	0.9967

3.6 Optimisation of Extraction Shaking Time

3.6.1 Procedure

A factorial experiment was designed to investigate the effect of extractant shaking times on overall extraction efficiency for a range of real samples. The extraction procedure incorporating the alkaline pre-treatment step was the method of choice as many of the samples contained a high fat content. The samples chosen were fresh mussels, potting compost, pig kidney, pig liver, uncooked rice, cooked rice, cod liver oil and vegetable oil. These samples were selected as they covered most of the sample types of interest.

Shaking times for three of the extraction steps were selected for optimisation, namely KOH, KBr/$CuSO_4$ and DCM, all affecting the efficiency of the initial solvent extraction stage. Two variables, one high and one low, were selected for each step, as shown in Table 3.10.

Table 3.10 Factorial Experiment Design

	Low	High
KOH	2hr	4hr
KBr/$CuSO_4$	30min	2hr
DCM	2hr	16hr

The eight combinations of variables were:

LLL, LLH, LHL, LHH, HHH, HLH, HHL, HLL

In addition to these eight procedures, two further combinations were also included, by-passing the initial alkaline step *i.e.* /LL and /HH. These were performed in order to assess the effect of the KOH pre-treatment on each matrix.

3.6.1.1 Sample Preparation

a) Mussels

Two bags of frozen Scottish mussels (2 x 250g) were purchased and allowed to defrost overnight. The mussels were transferred to an acid rinsed blender (Moulinex Blender 2 Model 531) and liquidised until smooth. The mussel homogenate was then transferred to an acid washed plastic bowl and sealed.

b) Potting Compost

A bag of potting compost containing "sterilised" loam, Sphagnum moss peat, horticultural sand and a blend of fertilisers was purchased. The sample was well shaken before use.

c) & d) Pig Kidney/Liver

Two fresh pig kidneys and a portion of pig liver were purchased. Each sample was blended in the same way as the mussels until smooth, using a clean, acid rinsed liquidiser. The samples were transferred to acid washed plastic containers and sealed.

e) Uncooked Rice

A bag of long grain white rice was purchased. Using the coffee/nut grinder attachment on the blender a portion of the rice was ground to a powder.

f) Cooked Rice

A portion of the dry rice was cooked according to the instructions in a microwave, using an acid washed glass bowl and double deionised water. Once cool, the rice was transferred to an acid washed plastic container.

g) & h) Oil

The cod liver oil and vegetable oil samples did not require further preparation. An initial experiment was undertaken with the fresh mussel homogenate to determine the appropriate sample mass for the procedure. Two masses, 0.5000g and 2.0000g were selected and passed through Method 2. It was noted that the higher mass limited the solvent volume transferable (T1) with typically < 1ml from 5ml being taken to the next stage. Consequently 0.5000g was selected as an appropriate sample mass for this experiment allowing T1 = 3.0-4.0ml.

Twelve portions of each sample were accurately weighed, ten were spiked with a mixed MM + EM standard (1$\mu g\ ml^{-1}$ in water or methanol for the oil samples). Samples were set aside in a refrigerator for 5-7 days to equilibrate. Extraction Method 2, Figure 3.1 was

then followed. The shaking times within the initial step were varied as described. The samples were analysed by GC-AFS against standards.

3.6.2 Results and Discussion

Figure 3.5 shows the trends observed for each of the compounds in the mussel matrix. The results for the all of the samples are shown in Table 3.11.

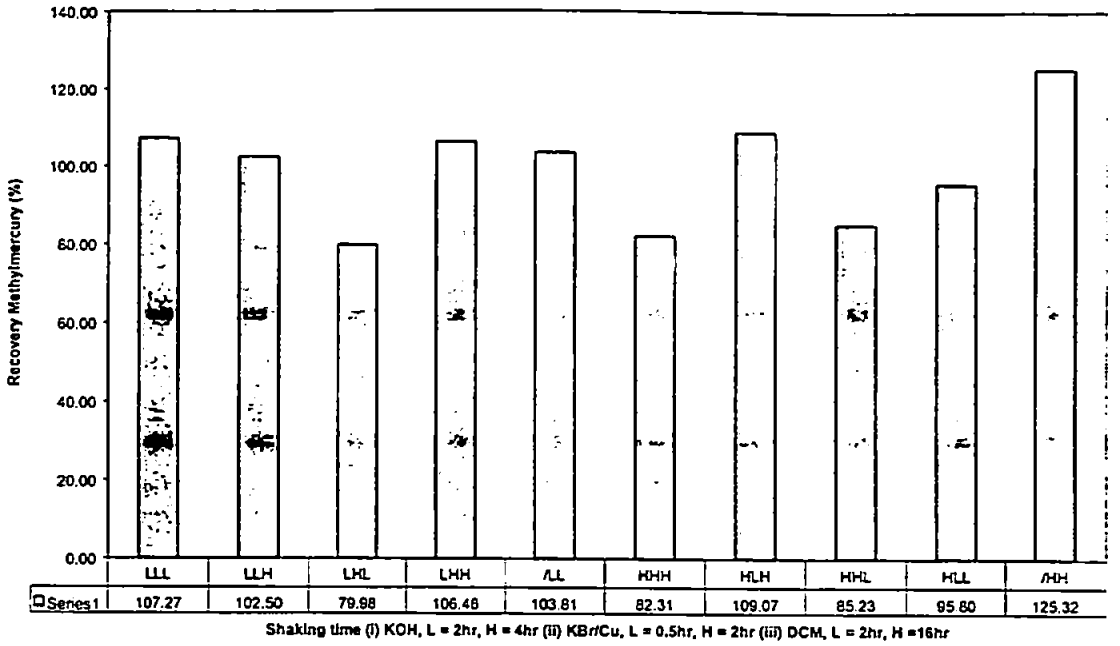
The overall recovery of methylmercury was better than the recovery of ethylmercury. In general it appeared that lower extraction times were better than higher extraction times. A signal corresponding to 22.2ng g^{-1} methylmercury as mercury was observed in the blank mussel extraction and it was noted that the overall recovery of organomercury seemed to improve when the alkaline step was removed. The extraction of liver and kidney tissues without the initial alkaline pre-treatment step resulted in yellow viscous extracts due to the fat content of the samples. These extracts were not analysed and it was concluded that shaking with NaOH prior to extraction was necessary for samples of this nature.

3.6.2.1 Analysis of Variance (ANOVA)

A balanced ANOVA was performed on each set of results except the pig kidney as sample loss meant that there was insufficient data for analysis. This was achieved using a statistical software package (Minitab v.8.2, Microsoft Corporation, USA). The analysis of variance between results indicated that the NULL hypothesis could not be rejected. In other words, there were no significant interactions between these variables.

For the sample matrices studied, these tests showed that there was no specific combination of shaking times which had a marked effect on extraction efficiency. It was noted however

i Methylmercury



ii Ethylmercury

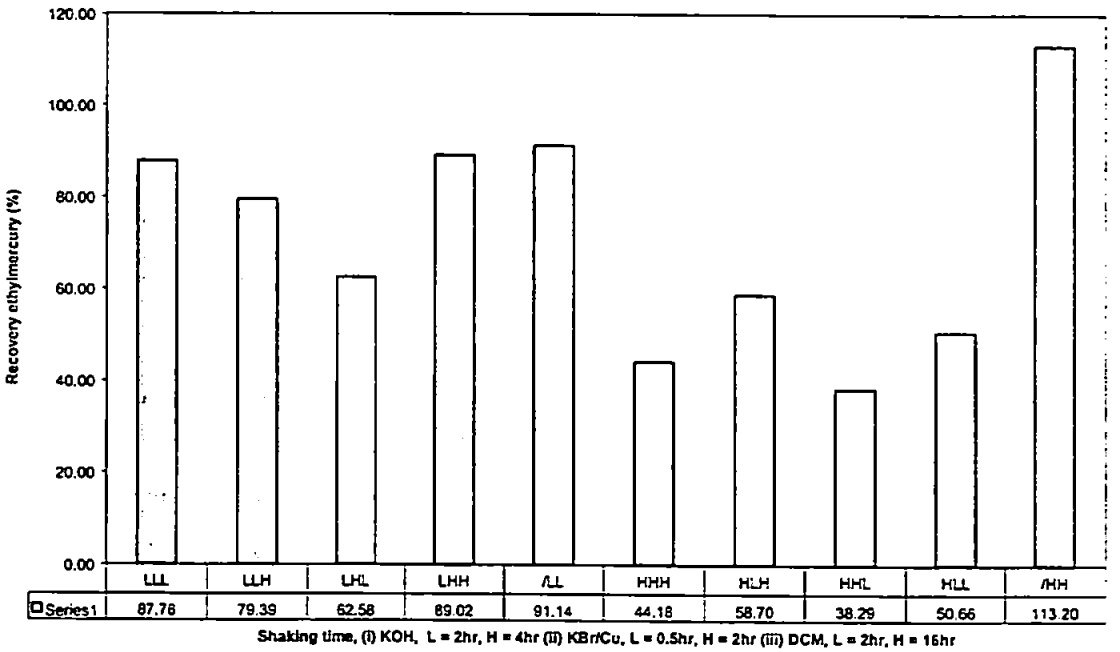


Figure 3.5 – Effect of Shaking Time Combinations on Organomercury Recovery from Spiked Fresh Mussels

Table 3.11 Variation of Organomercury Recovery with Extractant Shaking Time

	Mussel		Potting Compost		Pig Kidney		Pig Liver		Uncooked Rice		Cooked Rice		Cod Liver Oil		Vegetable Oil	
	MM%	EM %	MM%	EM %	MM%	EM %	MM%	EM %	MM%	EM %	MM%	EM %	MM%	EM %	MM%	EM %
LLL	107.3	87.8	37.4	12.3	76.3	56.9	83.9	51.4	130.5	62.7	84.9	84.2	32.0	10.7	71.5	50.8
LLH	102.5	79.4	42.2	16.1	97.4	72.2	86.2	73.5	82.3	51.0	87.6	91.8	45.5	21.3	62.0	40.8
LHL	80.0	62.6	42.2	19.5	65.1	46.2	98.7	59.6	106.9	38.5	88	73.5	44.7	17.5	61.4	32.4
LHH	106.5	89.0	33.9	13.7	96.3	69.8	97.0	101.3	87.9	71.5	70.4	88.8	36.2	12.9	71.3	44.5
HHH	82.3	44.2	31.8	18.2	-	-	78.9	33.8	73.6	46.8	97.1	97.4	35.1	12.9	73.7	55.9
HLH	109.1	58.7	31.5	17.5	71	56.6	73.7	46.7	79.8	58.5	85.6	88.9	39.6	21.4	59.0	46.3
HHL	85.2	38.3	36.7	25.2	74.5	54.1	70.4	46.6	80.1	50.8	108.0	105.9	33.2	13.2	54.1	26.8
HLL	95.8	50.7	29.8	15.8	76.6	56.8	86.5	54.5	99.9	59.8	96.3	88.2	42.4	20.1	62.2	43.3

during these procedures that whilst alkaline pre-treatment, shaking with 50% NaOH was very important for some samples - kidney and liver, other samples - compost, cooked rice and oils suffered a significant decline in recovery. The mussels appeared to favour a slightly acidic extraction although good recoveries of organomercury, especially methylmercury were observed for the alkaline procedure.

3.7 Further Method Validation - Analysis of Marine Liver Samples

3.7.1 Introduction

The aim of this experiment was to determine the total and methylmercury contents of DOLT 2, dogfish liver tissue. This sample presented a completely different challenge to the sea plant homogenate, as its methylmercury concentration was three orders of magnitude greater. Based on the results of the shaking time optimisation experiments described in Section 3.6, it was concluded that an alkaline extraction procedure was necessary. Following method validation, two unknown marine liver samples – beluga and ringed seal were also studied.

3.7.2 Materials

Dogfish liver tissue 2 (DOLT 2) certified for both total mercury and methylmercury was obtained from the National Research Council of Canada. Two fresh marine liver samples, a ringed seal liver (ARVIAT-92-48) and a beluga whale liver (HI-94-06) were provided by the Freshwater Institute, Winnipeg, Canada. These were received packed in dry ice and were frozen until required for analysis. One portion of each fresh liver sample was allowed to thaw overnight. The samples were then homogenised using an acid washed* blender. Each sample was then transferred to an acid washed plastic storage vessel. A portion of

each sample was dried at 105°C in an oven for 72 hours to constant weight. The moisture contents were determined as the percentage weight loss at the end of this period.

*Note: acid washing refers to washing or storing in 50% HNO_3 followed by repeated rinsing in double de-ionised water (n = 5)

3.7.3 Experimental

3.7.3.1 Organomercury Extraction Procedure for Marine Liver Samples

The extraction procedure used for these samples was based upon Method B, Section 3.1. Portions of sample (0.1000-0.5000g) were accurately weighed and spikes of methylmercury chloride (1.0ng μl^{-1} in water, as Hg) were added to test recovery. All samples and procedural blanks were sealed with PTFE tape and stored in a refrigerator for one week to allow the spike to equilibrate with the samples prior to extraction. The volume of KOH and HCl added in the initial stage was increased to 3ml. An initial extraction procedure was performed on eight portions (0.5000g) of both fresh liver and homogenates. From literature [199] it had been established that typical concentrations of methylmercury in dolphin livers were in the region of a few $\mu g\ g^{-1}$ (ppm) therefore, a 10.1 ng μl^{-1} stock solution of methylmercury chloride in water (as mass of mercury) was prepared, and 100µl spikes were added to four portions of each liver.

During the second stage of the procedure *i.e.* extraction into thiosulphate, samples were stored in a refrigerator at 4°C for 2 hours to clarify them. This procedure was an alternative to centrifugation or the addition of propanol which often have been used [136].

3.7.3.2 Determination of Total Mercury in Marine Liver Tissues

Samples were digested in duplicate following the procedure outlined in Section 3.2.4. The reference material was analysed alongside the fresh homogenate samples, so spike recoveries were not necessary. The digests were analysed using CV-AFS (Millennium Merlin, PS Analytical, UK) calibrated in the ranges 0-100 ng g^{-1} Hg and 0-20 ng g^{-1} Hg respectively.

3.7.4 Results and Discussion

3.7.4.1 Determination of Methylmercury in Marine Liver Samples

The results of the initial extraction procedure performed on the fresh liver homogenate are shown in Table 3.12.

Table 3.12 Initial Methylmercury Concentrations Determined in Marine Liver Samples

Sample	Spike recovery (%) n = 4	Corrected methylmercury concentration (ng g^{-1}) n = 4
Ringed seal	47 ± 8	877 ± 15
Beluga	50 ± 4	2775 ± 132

Poor spike recoveries were obtained because the sample mass was large compared to the reagent volume used. When the extraction was repeated with smaller sample masses (Table 3.13) spike recoveries were improved to between 69-86% (Table 3.14) and the result for the analysis of DOLT-2 was within the certified range.

Table 3.13 Optimised Conditions for Marine Liver Extraction Procedure

Sample	Mass (g)	Spike volume (MM as Hg in water)
Dolt 2	0.3000	200µl of 1.0ng μl^{-1}
Ringed Seal ARVIAT-92-06	0.2000	200µl of 1.0ng μl^{-1}
Beluga HI-94-48	0.1000	200µl of 1.0ng μl^{-1}

Table 3.14 Methylmercury Concentrations Determined in Marine Liver Samples

Sample	Water Content (%)	Spike Recovery (%) n=4	MM Determined (as ng g^{-1} Hg)	Certified Concentration (as ng g^{-1} Hg)
Dolt 2	10.6	86 ± 9	671 ± 41	693 ± 53
Ringed Seal	72	74 ± 4	801 ± 62*	-
Beluga	74	69 ± 13+	2830 ± 113*	-

+ n=3

* These results have not been corrected for moisture content as it is more common for organomercury concentrations of this type of sample to be reported as wet weight.

This result validated the newly developed procedure for his sample type. The concentration of methylmercury determined in the fresh liver homogenates was also in good agreement with the results of the initial experiment. By reducing the sample mass taken, the spike recoveries were significantly improved.

3.7.4.2 Determination of Total Mercury

The total mercury concentration determined in each of the marine liver samples is shown in Table 3.15. All of the sample digests were diluted to 100ml with double distilled water prior to analysis. It was necessary to further dilute the ringed seal and beluga digests ten-fold with 20% HNO_3 in order to bring them within the range of the calibration series.

Table 3.15 Determination of Total Mercury in Marine Liver Samples (μg g^{-1})

Dolt 2		Ringed Seal		Beluga	
Total Hg (n=6)	Certified Hg	Initial result (n = 3)	Total Hg (n = 4)	Initial result (n = 3)	Total Hg (n = 4)
1.94 ± 0.06	1.99 ± 0.1	54 ± 3	53 ± 3	106 ± 5	121 ± 14

The final concentration of Hg in DOLT-2 was within the certified range thereby validating the method. These results showed that the methylmercury content of the ringed seal and beluga livers was between 1.5-2.3% of the total mercury content. This is what would be expected in an uncontaminated sample compared to the certified material, which contains 35% organomercury.

3.8 Conclusion

The application of the GC-AFS instrument to biological samples has been established. The initial sample preparation/extraction procedures were investigated, validated and improved for different sample matrices. Contamination problems were identified and eliminated. Ranges of reference materials have been studied covering three matrix types: shellfish, sea plant and marine liver. Good agreement with certified values was observed in each case for both total mercury and methylmercury content. The result for the sea plant sample was submitted as part of an interlaboratory comparison exercise and proved to be the closest result to the certified concentration. IAEA 140 is now a commercially available CRM and is currently the sample with the lowest certified concentration of methylmercury. Other unknown samples analysed in this chapter included fresh marine mammalian livers. The results were in agreement with the expected order of magnitude suggested by literature. Overall the range of samples analysed contained widely differing concentrations of methylmercury, from <1ng g^{-1} to approximately 3000ng g^{-1}.

Chapter 4

DETERMINATION OF ORGANOMERCURY IN SOILS AND SEDIMENTS

This chapter deals with the application of the GC-AFS instrument for the determination of organomercury in soils and sediments. In general, the concentration of methylmercury expected to be found in these types of samples is very low *i.e.* only a few ng g^{-1}. There are currently very few certified reference materials for this type of sample, but those which are available tend to be polluted marine sediments. For this study, IAEA 356 polluted marine sediment, certified for both total and methylmercury was considered for method validation.

Initial studies were undertaken in order to identify which extraction procedure was likely to give the best performance. Following this, a method was developed to determine methylmercury in LGC 6138, a recently available contaminated land reference material. This material, a coal-carbonisation site soil, was only certified for its total mercury content. This was identified as a potentially difficult sample for methylmercury determination due to the high carbon content of the sample and its bonding strength with mercury. Two further natural or non polluted samples were also studied; potting compost and Portuguese estuarine sediment. Total mercury determinations were made for all of the samples using acid digestion CV-AFS. A brief study was undertaken with IAEA 356 considering accelerated microwave extraction in a closed microwave oven in order to reduce sample preparation time.

4.1 Experimental

4.1.1 Instrumentation

The instrumentation and apparatus for the extraction and determination of methyl and ethylmercury in soils and sediments is described in Chapter 3. The 20ml borosilicate vials used in the first extraction stage were fitted with polypropylene caps to prevent contamination as long-term contamination studies had proved that mercury was leaching into extractant solutions from the original caps caused by prolonged contact with acid. Due to the low organomercury concentrations expected in these sample types, it was necessary to find a way of either increasing the pre-concentration factor in the sample preparation stage or increasing the sample volume introduced to the instrument in order to reliably detect the methylmercury. The simplest approach was to increase the volume of sample injected however it was first necessary to check the validity of this. Figure 4.1 shows a calibration by volume of methylmercury chloride in Dichloromethane solvent. This shows a linear relationship between injection volume and mercury signal detected up to 5µl in the direct injection mode under standard optimised operating conditions. Table 4.1 shows the typical GC-AFS operating conditions used for soils and sediments.

Table 4.1 Typical GC-AFS Operating Conditions for Soils and Sediments

Injection volume	3µl
Injector conditions	Direct injection into glass liner at 300°C isothermal
Column	DB-1 approx. 10m x 0.53mm x 1.5µm
Pyrolyser	800°C
He Carrier Gas Flow	5ml min^{-1}
Ar Make-up Gas Flow	60ml min^{-1}
Ar Sheath Gas	150ml min^{-1}
Detector Range	1000 x 10 (dark current offset 2.3)

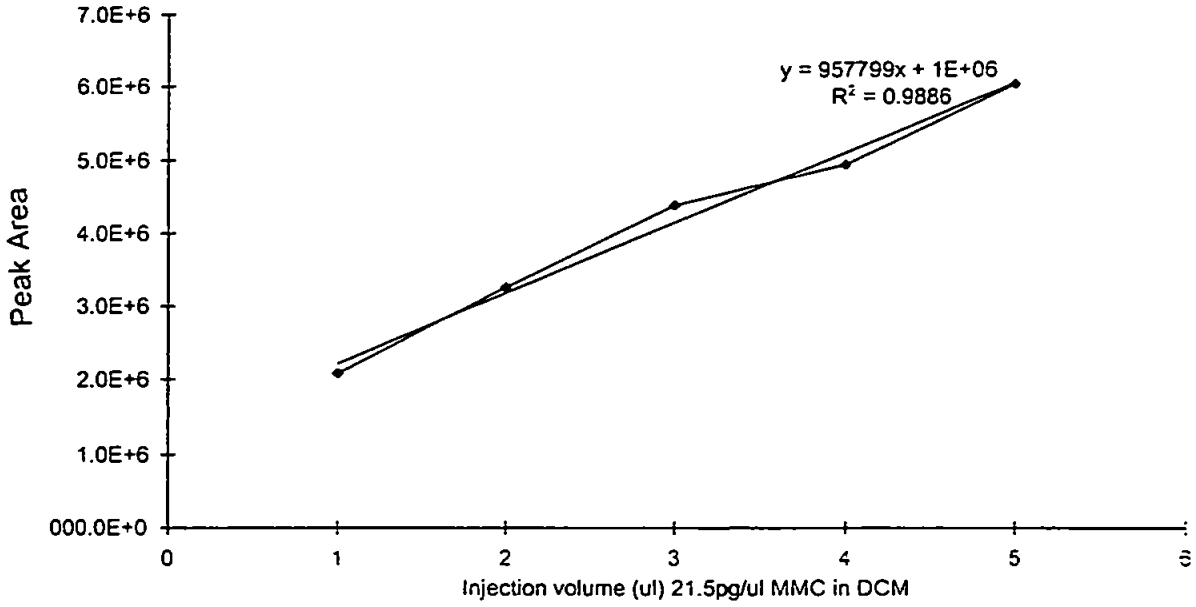


Figure 4.1 Relationship Between Volume of Methylmercury Chloride Injected with Signal Detected using Direct Injection into GC-AFS

A CV-AFS system (Merlin Plus or Millennium Merlin, PS Analytical Ltd, Orpington UK) was used for the determination of total mercury in soils and sediments. Sample and standard digestions were made on a Lachat BD-26 Block Digestor. Typical Operation conditions are shown in Table 4.2.

Table 4.2 CV-AFS Operating Conditions

Settings	Merlin Plus	Millennium Merlin
Delay time	10s	10s
Rise time	30s	n/a
Analysis time	30s	30s
Memory time	60s	60s
Zero	Off	Auto
Gain	100 x 3	100
Pump 1	Polyethylene pump tubing to supply 9 ml min^{-1} and 4.5 ml min^{-1} reagents respectfully	Full – 9ml min^{-1}
Pump 2	n/a	Half – 4.5 ml min^{-1}
Carrier gas - Ar	300ml min^{-1} (manual)	300ml min^{-1} (automatic)
Sheath gas - Ar	300ml min^{-1} (manual)	300ml min^{-1} (automatic)
Dryer gas - Ar	3l min^{-1} (manual)	3l min^{-1} (automatic)

4.1.2 Reagents and Standards

Methyl and ethylmercury chloride standards were prepared (as mass Hg) by dissolving in methanol, followed by subsequent dilution in water. All reagents were of analytical grade or better, prepared in double de-ionised water or HPLC Grade solvent, as required. The reagents employed in the extraction procedure are described in Chapter 3. For total mercury determinations standards were prepared by dilution of a SpectrosoL 1000µg ml^{-1} Hg standard. Aqua regia was prepared by mixing concentrated HCl with concentrated HNO_3 (3:1) mixture.

4.1.3 Soil and Sediment Samples

A range of samples was obtained for this study. IAEA 356 (International Atomic Energy Agency, Monaco) is a polluted marine sediment collected in the Venice Lagoon, Italy and certified for a range of elements including total and methylmercury. A portion of unpolluted estuarine sediment was obtained through the University of Aveiro, Portugal for total and methylmercury analysis. This sediment was taken from the Aveiro Lagoon, at point 2 on the map shown in Figure 4.2. This sampling point was an unpolluted site compared to point 1, an industrial discharge source containing mercury. LGC 6138 is a contaminated coal carbonisation site soil taken from a UK gas works. This material is certified for total mercury, but no speciation data was available. This sample was selected for organomercury analysis as it represented i) a contaminated land sample where many different compounds of mercury could exist and ii) a carbon/coal type matrix which was expected to present challenges in method development. A commercial potting compost comprising 'sterilised' loam Sphagnum moss peat, horticultural sand and a blend of fertilisers was also selected as a non contaminated soil representing "background" mercury levels.

4.1.4 Sample Preparation Procedures for Total and Organomercury Determinations in Soils and Sediments

Figure 4.3 shows two extraction procedures, Method A and Method B, for the extractions of methyl and ethylmercury from soil and sediments samples. These methods differ from those used in Chapter 3 as no alkaline pre-treatment step is included. During the shaking time experiment discussed in Chapter 3 it was noted that alkaline pre-treatment significantly reduced the recovery of methyl and ethyl mercury from compost. Method A is

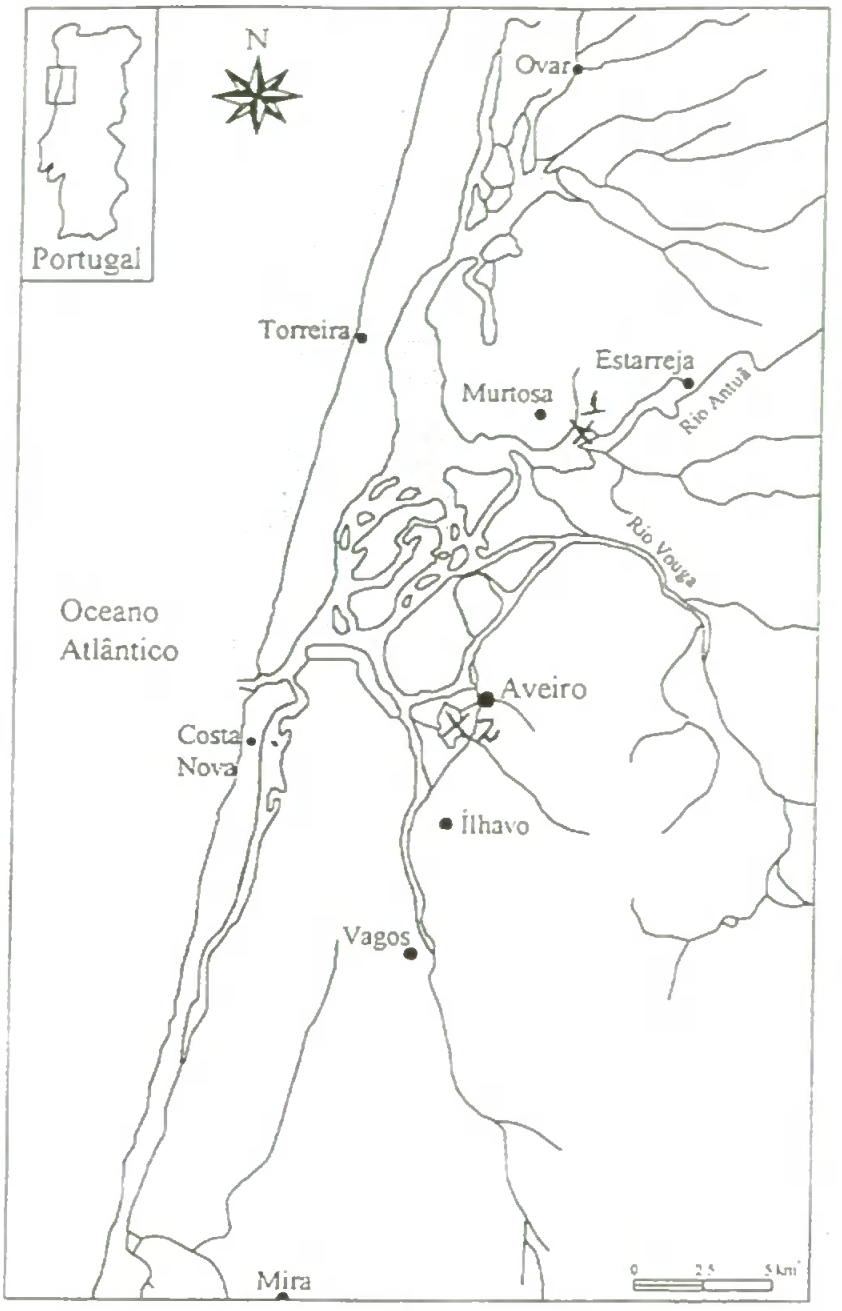


Figure 4.2 Sample Point, Aveiro Lagoon, Portugal

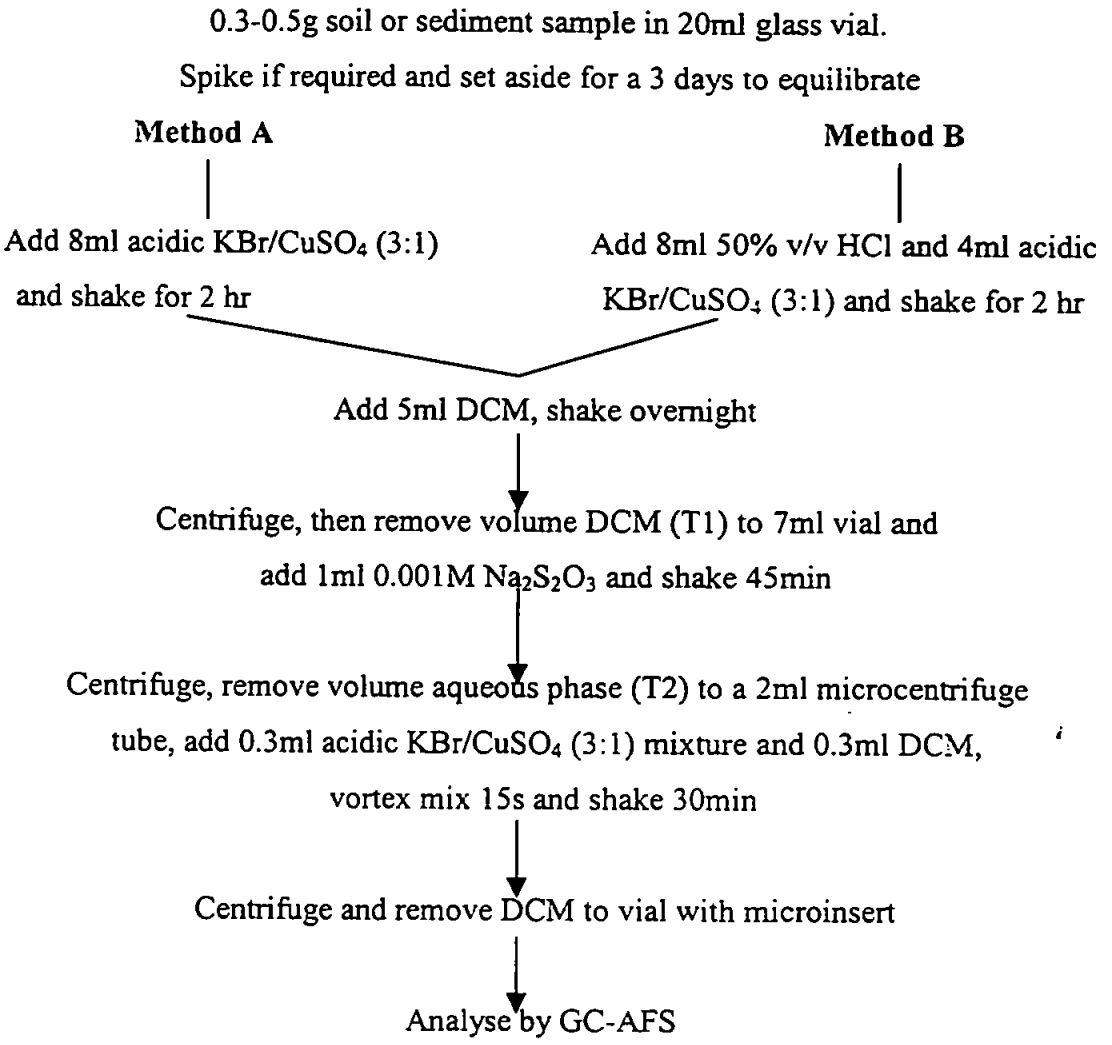


Figure 4.3 Organomercury Extraction Procedures for Soils and Sediments

based on a procedure previously used whereas Method B increases the acidity and volume of the initial extractant mixture. Literature suggests that lowering the pH of the extraction mixture aids the transfer of particulate bound methylmercury into the aqueous phase for extraction [24]. This was observed during the method development for IAEA 140, sea plant homogenate (Chapter 3). An initial experiment was designed to investigate the effect of acid concentration on methylmercury and ethylmercury spike recovery from potting compost in order to optimise the extraction procedure. This optimisation experiment was performed on 0.5g portions of potting compost. To each a volume of 50% v/v HCl was added (0, 2, 4, 6, 8 ml) and the total volume adjusted to 8ml. Each set of conditions was repeated in triplicate. 4ml acidic KBr mixture was added to each and Method A followed from step 2. Following this, portions of each of the samples (n=6 plus 2 spikes) were analysed for organomercury using Methods A or B as described. Figure 4.4 shows the acid digestion procedure of choice for the soil and sediment samples for total mercury determinations.

4.2 Results and Discussion

4.2.1 Optimisation of Acidic Extraction Procedure

The recovery of methyl and ethylmercury from each extractant mixture is shown in Figure 4.5. Varying the acid concentration in the initial mixture had no effect on the recovery of either species. The mean recovery of all samples was 68±2% and 54±5% for methylmercury and ethylmercury respectively (n=15). It was noted that the potting compost was a very light and loose sample, and although the spike had been given considerable time to equilibrate it was unlikely to need harsh treatment in the same way as the sea plant homogenate to release the organomercury compounds.

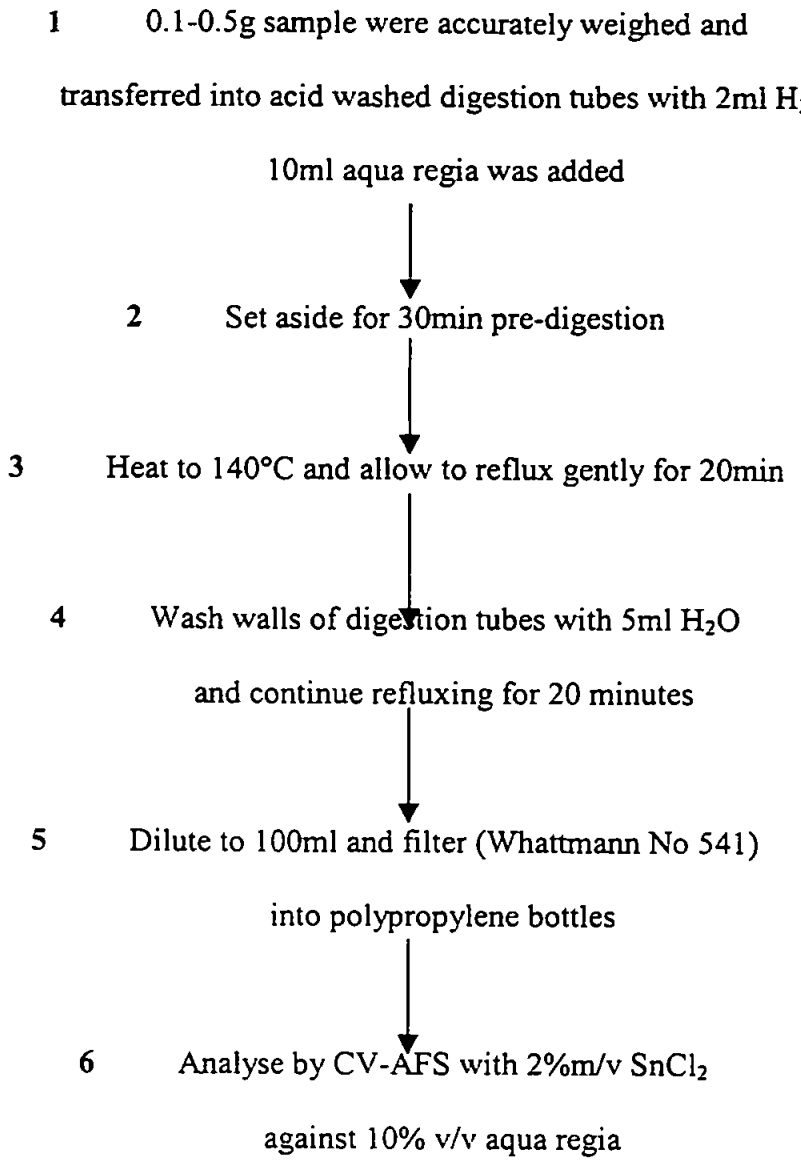


Figure 4.4 Aqua regia Digestion of Soils and Sediments for Total Mercury Analysis

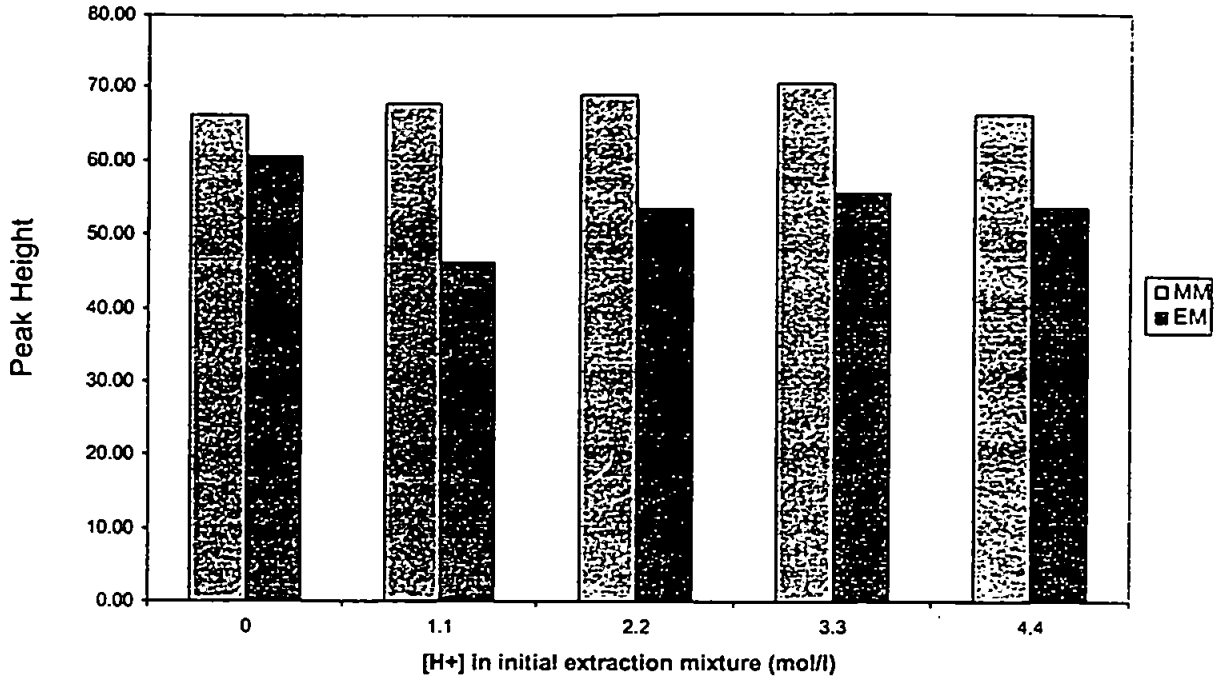


Figure 4.5 Effect of Increasing Extractant Acid Concentration on the Recovery of Organomercury Compounds from Potting Compost

It was concluded that Method A would be the main extraction procedure followed for soils and sediments, and Method B was an alternative procedure incorporating high [H^+] for use with more complex sample matrices if required.

4.2.2 Determination of Total and Organomercury in Soils and Sediments

The water content of each of the four samples of interest was determined by drying to constant weight in an oven at 105°C for 48 hr. Portions of each sample type were extracted following Method A for organomercury determinations and digested using the aqua regia procedure for total mercury determinations. The results obtained are shown in Table 4.3.

Methylmercury was the only form of organomercury determined in each of the samples. IAEA 356 has recently come under scrutiny, especially with regard to steam distillation extraction procedures, which have been found to lead to positive MM artifact formation. The certified value has been re-affirmed by the producers as it was established using an unbiased range of procedures. A single peak corresponding to methylmercury was observed in the chromatogram of each sample analysed, the concentration of which was in good agreement with the certified values. These results confirmed that no artifact formation was occurring within this method. A lower spike recovery was observed for the Portuguese sediment compared to the other samples studied. This was concluded to be due to insufficient reagent volume compared to sample mass, as previously observed during the sea plant method development. Good spike recovery from LGC 6138 indicated that no matrix interference effects were inhibiting methylmercury extraction from this matrix. The spike recovery of 95% for the compost sample was based on only one measurement as the replicate results were unreliable due to sample leakage. This value was slightly higher than those observed for the prior samples but this was within the expected recovery range

(70-100%). The overall MM concentration determined was lower than the other samples, but once again of the order of magnitude expected. Good agreement was obtained between the total mercury concentrations determined in the certified soil and sediment materials and the certified values. Much lower concentrations of total mercury were found in the unpolluted samples as expected.

4.3 An Improved Extraction Procedure for Methylmercury in Sediment

The extraction procedures so far considered for methylmercury determinations in both biological and soil and sediment sample relied on a three step extraction:

(pre-treatment)

1. Acidic KBr/$CuSO_4$/DCM
2. Thiosulphate
3. Acidic KBr/$CuSO_4$/DCM

The first of these steps (1), although found not to be time sensitive for the range of samples considered in the Shaking Time Factorial Experiment (Chapter 3) was in fact found to be extremely time sensitive for the sea plant material. These results indicated that if a complex dry sample was to be studied, overnight extraction was required in order to increase efficiency. A multiple extraction of one sample *i.e.* 3 portions of DCM was considered, but this was rejected due to the increased handling, potential losses and sources of potential error that this presented.

Table 4.3 Determination of Methylmercury and Total Mercury in Soils and Sediments

		Methylmercury		Total Mercury		
Material	Moisture Content (%)	Spike Recovery (%)	Concentration found ($ng\ g^{-1}$)	Certified value ($ng\ g^{-1}$)	Concentration found ($\mu g\ g^{-1}$)	Certified value ($\mu g\ g^{-1}$)
IAEA 356	0.25	80±10	5.21±0.19	5.46±0.39	7024±400	6740-7980
LGC 6138	2.36	75±5	4.04±0.24	-	1270±20	1200±100
Potting Compost	9.3	95	3.23±0.3	-	120±6	-
Portuguese Sediment	41	58±13	2.650.01	-	154±13	-

Literature reports were found, particularly by Donard *et al* [132] describing an accelerated extraction procedure using an open focused microwave. It was decided to apply such a method to a closed microwave system and to test the procedures using IAEA 356.

4.3.1 Instrumentation

A closed microwave system (560W, Remote Microwave System, Floyd Inc, USA) comprising microwave oven, time/power controller and a carousel of PTFE bombs fitted with rupture discs and connected to an overflow reservoir, was used in this study. The PTFE microwave vessels were cleaned in acid prior to use. This involved heating 10ml concentrated AristaR hydrochloric acid for 10min at 30% power (168W). After cooling, this was repeated with water.

4.3.2 Microwave Extraction

Five portions of IAEA 356 (0.3000g) were accurately weighed into the digestion bombs. Two portions were spiked with 70µl of 22.9ng ml^{-1} MM as Hg in water. The samples were set aside for two hours to allow the spike to become incorporated. 10ml 2M HCl was added to each vessel, a rupture disc fitted and closed. The bombs were heated at 10% power (56W) for 3min and allowed to cool for 30min before opening. The contents of each vessel were transferred to 20ml borosilicate scintillation vials. 4ml acidic $KBr/CuSO_4$ mixture was added and the mixture shaken by hand. 5ml DCM was then added followed by 30min extraction on a shaker. The procedure was the continued following the standard extraction Method A extracting into thiosulphate then back into DCM for analysis.

4.3.3 Results and Discussion

Table 4.4 shows the results for the microwave extracted samples. A linear calibration between 0-39pg MM was obtained with $y = 709769x$ and $R^2 = 0.9999$. The spike recovery was determined to be 81 ± 12% (n=2) and the moisture content was 0.25%.

Table 4.4 Summary of Results

Corrected [MM] determined (n=3) (ng g^{-1})	Certified [MM] (ng g^{-1})
5.52 ± 0.005	5.46 ± 0.39

Good agreement was obtained between the certified value and the measurement obtained. This procedure reduced the overall sample preparation time from to a few days to a few hours. No reports have been found describing the use of a closed microwave system in this way.

The results of the analysis of IAEA 356 by both extraction methods are compared in Table 4.6. The microwave procedure was noted to have better precision than the conventional shaking procedure. However, the maximum number of samples that could be prepared in the microwave at the same time, was limited to six. This approach was also found to suffer from severe contamination problems if the microwave vessels had previously been used for the treatment of samples with high mercury levels. A strict and often time-consuming procedure of heating with acid and subsequent extraction into DCM with thiosulphate was required to ensure clean blanks.

Table 4.5 Comparison of Extraction Methods

Certified [MM] (ng g^{-1})	Conventional extraction	Microwave extraction
5.46±0.39	5.21±0.19	5.52±0.005

4.4 Comparison of Total and Organomercury Concentrations Determined

The overall MM and THg contents of all the samples measured are compared in Table 4.5. These results show lower MM ratios in contaminated samples compared to unpolluted samples, possibly explained by a reduction in methylating bacteria in these contaminated soils. Overall, these experiments have indicated reliable methylmercury determinations in the presence of very high total mercury levels, free of any artifact formation. No ethylmercury was found in any of the samples studied.

Table 4.6 Comparison of MM and THg Contents of Each Sample

Sample	[MM] ng g^{-1}	[THg] ng g^{-1}	% MM of THg
IAEA 356	5.21 ± 0.19	7024 ± 400	0.07
LGC 6138	4.04 ± 0.24	1270 ± 20	0.32
Potting Compost	3.23 ± 0.30	120 ± 6	2.69
Portuguese Sediment	2.65 ± 0.01	154 ± 13	1.72

4.5 Conclusion

A range of soil and sediment samples was analysed for total and organomercury. These included reference materials to validate the technique along with unknown samples. Methylmercury was the only organomercury compound to be determined in each sample with concentrations in the range of 0.12mg g^{-1} to 7.02mg g^{-1}. An improved extraction technique was developed which significantly reduced the sample preparation time using a closed microwave system. This approach was found to be very promising however it was limited by the number of samples that could be prepared at the same time, and was found to be prone to contamination. Both sample preparation techniques gave good agreement with the certified value of MM in IAEA356 and did not lead to artifact formation.

Chapter 5

COMPARATIVE TECHNIQUES

Two comparative chromatographic coupled atomic spectrometric techniques were considered for the speciation of methylmercury in certified reference materials. First, a gas chromatography approach was used, along with ICP-MS detection followed by liquid chromatography coupled with CV-AFS. Both of these approaches were studied in detail to optimise experimental conditions prior to performance testing and method validation.

5.1 Determination of Methylmercury in Mussel Homogenate Reference Materials using GC- ICP-MS

The aim of this study was to consider a comparative technique to GC-AFS for the speciation of methylmercury. In this case the gas chromatographic separation was retained and an alternative atomic spectrometric detector was selected. The approach described here was to couple a gas chromatograph with an ICP-MS instrument via a heated transfer line positioned in the torch. Optimisation of the instrumentation was performed in order to obtain maximum sensitivity for methylmercury. IAEA 142 and NIST SRM 8044 mussel homogenate materials were selected for method validation. Samples were prepared following a three stage extraction procedure based on those described in Chapters 3 and 4.

5.1.1 Experimental

5.1.1.1 Instrumentation

An inductively coupled plasma mass spectrometer (PQ2+ VG Elemental, Winsford, Cheshire, UK) was coupled to a gas chromatograph (model HRGC5300, Carlo Erba, Fisons, Crawley, UK) via a heated transfer line. This has been described in detail previously [217-219]. A DB-1 non polar Megabore column (15m x 0.53mm x 1.5µm, J&W Scientific, Folsom, USA) was fitted in the GC oven attached to an on-column injector with mass flow controller. Temperature programming of the oven was manually controlled via a keypad on the instrument. The column was connected to 1m of deactivated fused silica of the same dimensions (Phase Separations, Deeside, UK) via a glass union and wrapped in an electrically heated jacket. The temperature of the transfer line was controlled by a variable DC power supply. A small length of the transfer line was allowed to protrude from the end of the jacket directly into the rear of the ICP torch (H Baumbach and Co, Woodbridge, Suffolk). A make-up gas was introduced into the rear of the torch via a T-joint. Optimum operating conditions of the GC-ICP-MS for methylmercury are shown in Table 1. The ion lenses of the ICP were tuned on the most abundant isotope of mercury at 202 m/z units. Initial tests were performed to establish that all the isotopes of mercury at 198, 199, 200, 202 and 204 m/z units were being observed in their correct relative ratios.

Table 5.1 Operating Conditions for the GC-ICP-MS

GC conditions	
Injector type	On-column
Injection volume	1µl
Column	DB-1 (J&W Scientific), 15m x 0.53mm x 1.5µm
Oven temperature programme	40°C ramped to 200°C at 20°C min^{-1}
Carrier gas	8 ml min^{-1} Helium
Transfer line temperature	220°C
ICP-MS conditions	
Make-up gas	1.06 l min^{-1} Argon
Auxiliary gas	0.8 ml min^{-1} Argon
Cooling gas	16 l min^{-1} Argon
Forward power	1350W
Reflective power	0W
Sampler	Ni, 1.0mm orifice
Skimmer	Ni, 0.7mm orifice
Data acquisition mode	Single ion monitoring m/z 202
Dwell time	150 milliseconds
Acquire time	10 min

5.1.1.2 Materials

Mussel homogenate (*Mytilus edulis*) certified reference materials IAEA 142 and NIST SRM 8044, were provided by the International Atomic Energy Agency, Marine Environment Laboratory, Monaco. The moisture content of both samples was determined by drying portions in an oven at 105°C for 72hrs.

5.1.1.3 Reagents and Standards

Organomercury standards, methylmercury chloride (Strem Chemicals, Inc., Royston, UK) and ethylmercury chloride (Johnson Matthey plc, Reading, UK) were prepared by dissolving in methanol (HPLC grade, Rathburn Chemicals Ltd, Broxburn, UK) prior to dilution in dichloromethane solvent (HPLC grade, Rathburn Chemicals Ltd, Broxburn, UK) or double de-ionised water, as required. All reagents used in the extraction procedure were of analytical grade (Aldrich Chemical Company, Gillingham, UK) and were prepared in double-deionised water (Elga Maxima, Elga Ltd, High Wycombe, UK). Alkaline extraction of the samples was performed using KOH (6 mol dm^{-3}) followed by neutralisation with HCl (6 mol dm^{-3}). An acidified mixture of KBr (18% m/v in 0.5% v/v H_2SO_4) and $CuSO_4.5H_2O$ (1 mol dm^{-3}) in a 3:1 ratio was added to the samples with dichloromethane solvent. The mercury specific clean-up step of the procedure involved $Na_2S_2O_3$ (0.01 mol dm^{-3}).

5.1.1.4 Optimisation of GC-ICP-MS for Methylmercury

A range of parameters were varied in order to optimise the GC-ICP-MS for methylmercury. Standards of methylmercury chloride and ethylmercury chloride in dichloromethane (350 pg μl^{-1}) were prepared for these studies. Portions of standard (1.0µl) were injected onto the column held at 80°C followed by heating to 200°C at 20°C min^{-1}.

The argon make-up gas flow rate, position of transfer line, transfer line temperature and helium carrier gas flow rate were optimised. Linearity over the desired range, repeatability and the instrumental limit of detection were also investigated.

5.1.1.5 Sample Preparation

The methylmercury extraction procedure described for mussel reference materials in Chapter 3 was used for these studies. As techniques such as GC-ICP-MS can suffer from instrumental drift, an internal standard was added to each sample. Previous studies of these reference materials by GC-AFS had shown that ethylmercury was not present, and so this compound was prepared as the internal standard. The concentration of ethylmercury in each 1µl aliquot of sample was calculated as 50pg (as Hg). The GC-ICP-MS instrument was calibrated between 0-180 pg methylmercury as mass of mercury, plotted against the ratio of standard concentration peak area and internal standard peak area. Each of the samples and four procedural blanks were analysed against the calibration, spiked samples were run in triplicate. No signals were observed in the blanks.

5.1.2 Results and Discussion

5.1.2.1 Optimisation

A series of 1.0µl injections of a mixed methylmercury chloride and ethylmercury chloride standard (350pg μl^{-1} of each, as Hg) were made into the GC-ICP-MS system at a range of make-up gas flow rates between 0.9 l min^{-1} and 1.3 l min^{-1}. The ion lenses were tuned to the most abundant isotope of mercury at 202 m/z units and the signals observed. Methylmercury was found to elute with a retention time of 135s, followed by ethylmercury at 200s. Confirmation of peak identity was made with single compound standards. The variation of integrated peak area with make-up gas flow is shown in Figure 5.1. A make-up gas flow rate of 1.07 l min^{-1} Ar was selected as make-up gas flow greater than resulted in a reduced signal.

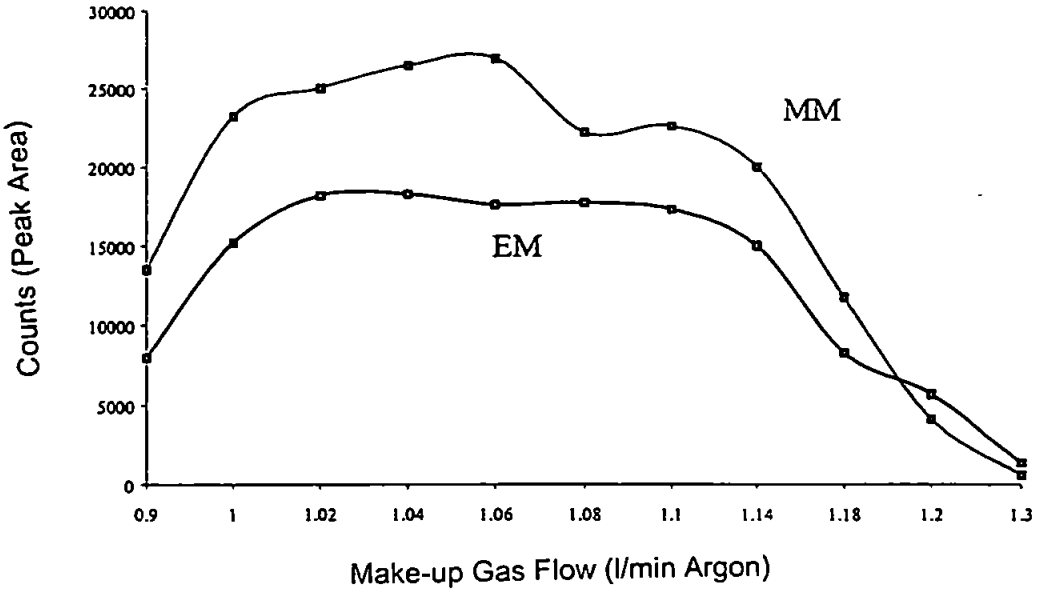


Figure 5.1 Effect of Make-up Gas Flow Rate on Peak Area Signal for 350pg MM and EM

A heated transfer line was required to prevent losses and peak broadening between the GC and the ICP-MS. The temperature setting must by high enough to retain the species in the gaseous phase without causing degradation. The temperature of the transfer line was therefore varied and measured using a thermocouple within the heated jacket, and peak areas for 1.0µl injections of a 350pg μl^{-1} methylmercury chloride standard were measured. As can be seen from Figure 5.2, the transfer line temperature had very little effect on peak area signal. However, at low temperature the peaks were very broad (Figure 5.3b), but as temperature increased, the peaks became narrower with increased peak height (Figure 5.3a). Hence, a temperature of 220°C was selected to ensure complete and rapid transfer of the compound to the ICP-MS.

The use of Megabore columns for the speciation of organo-mercury, with element selective detection by atomic fluorescence spectrometry has been reported with optimum carrier gas flow rates varying between 4 ml min^{-1} and 10-15 ml min^{-1}. In this work the carrier gas flow rate was optimised to provide minimal peak broadening with maximum area. A plot of peak height versus carrier gas flow rate is shown in Figure 5.4. A flow rate of 8.0 ml min^{-1} was chosen for these studies.

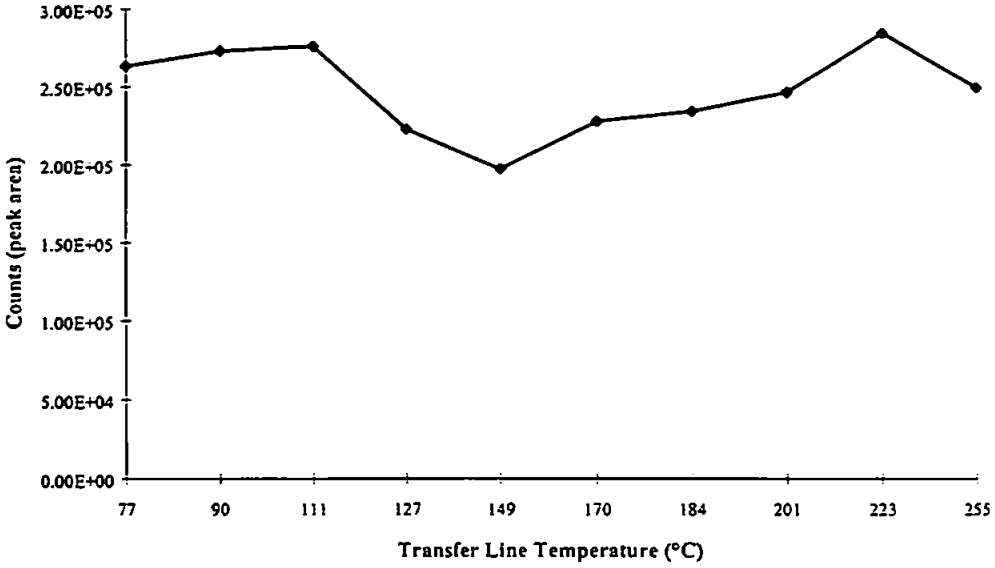
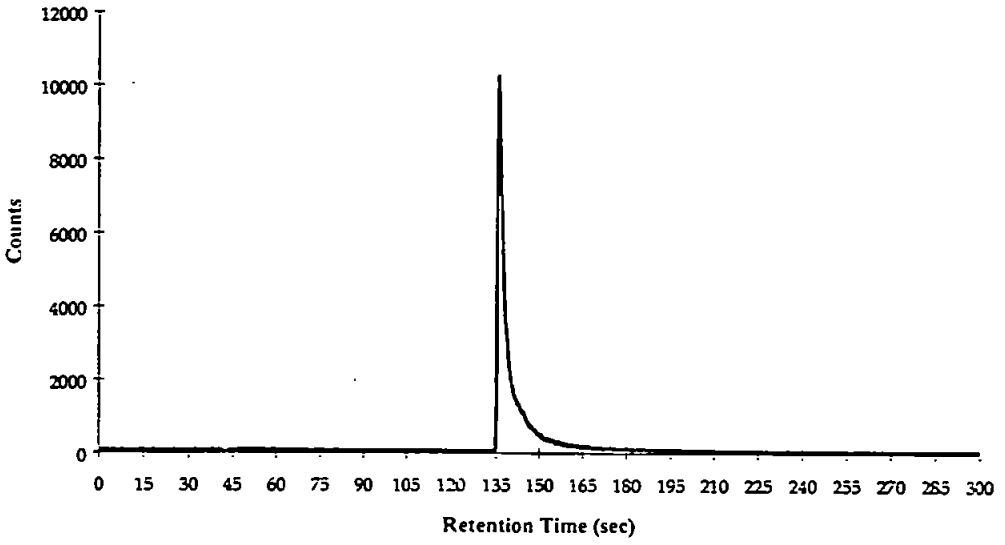


Figure 5.2 Effect of Transfer Line Temperature on Integrated Peak Area Signal for 350pg methylmercury chloride

a)



b)

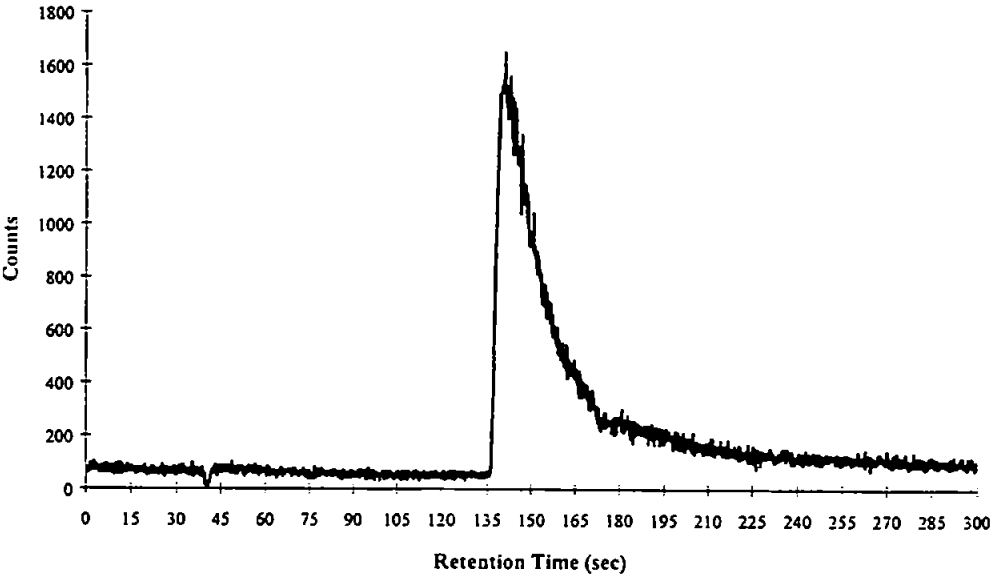


Figure 5.3 Effect of Transfer Line Temperature on Peak Shape for 350pg Injections of Methylmercury chloride: a) 220°C; b) 30°C.

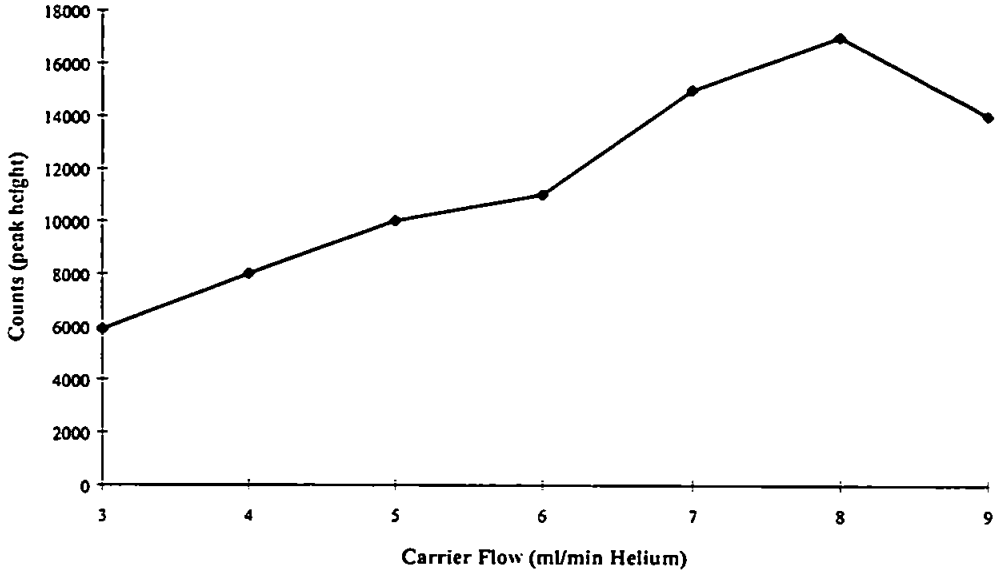


Figure 5.4 **Effect of GC Carrier Gas Flow Rate on Peak Height of 350pg Methylmercury chloride**

5.1.2.2 Figures of Merit

The limit of detection was 0.9pg methylmercury chloride (as Hg), and the calibration was linear up to at least 180pg with R=0.9975. The relative precision of five replicate injections of 1pg methylmercury chloride was 9%. Figure 5.5 shows the chromatogram obtained for a 5pg μl^{-1} standard of methylmercury chloride.

5.1.2.3 Determination of Methylmercury in Mussel Homogenate CRM's

Figure 5.6 shows one of the chromatogram obtained for IAEA 142. The first peak is methylmercury extracted from the mussel homogenate and the second is the internal standard, ethylmercury. The retention time of methylmercury was 127s and ethylmercury, 190s.

Results of spike recoveries and found values for the reference materials are shown in Table 5.2. Low spike recoveries were observed, probably due to insufficient reagent volume for the mass of sample used at the KOH extraction stage. By increasing the ratio of reagents to the dry homogenate sample, an improvement in extraction efficiency should be observed. We have previously obtained spike recoveries of 60-80% for these samples, taking initial masses of 0.2000g with the reagent volumes used here. Only three unspiked portions of IAEA 142 were measured as two samples were lost during the preparation stage. The concentration of methylmercury (as mass of mercury) corrected for spike recovery and water content agreed well with certified values for both IAEA 142 and NIST SRM 8044 (Table 5.2).

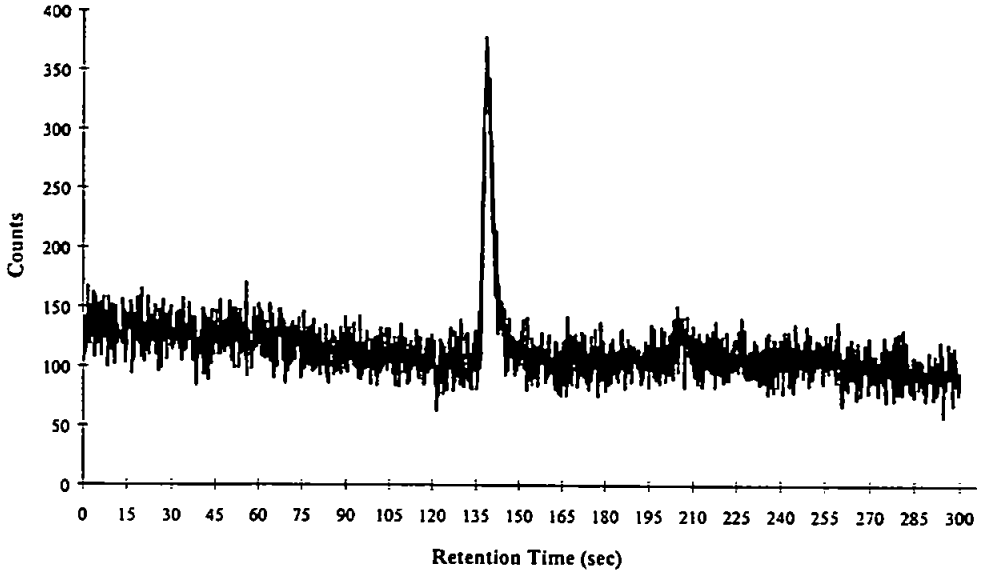


Figure 5.5 Chromatogram of 5pg Methylmercury chloride Standard

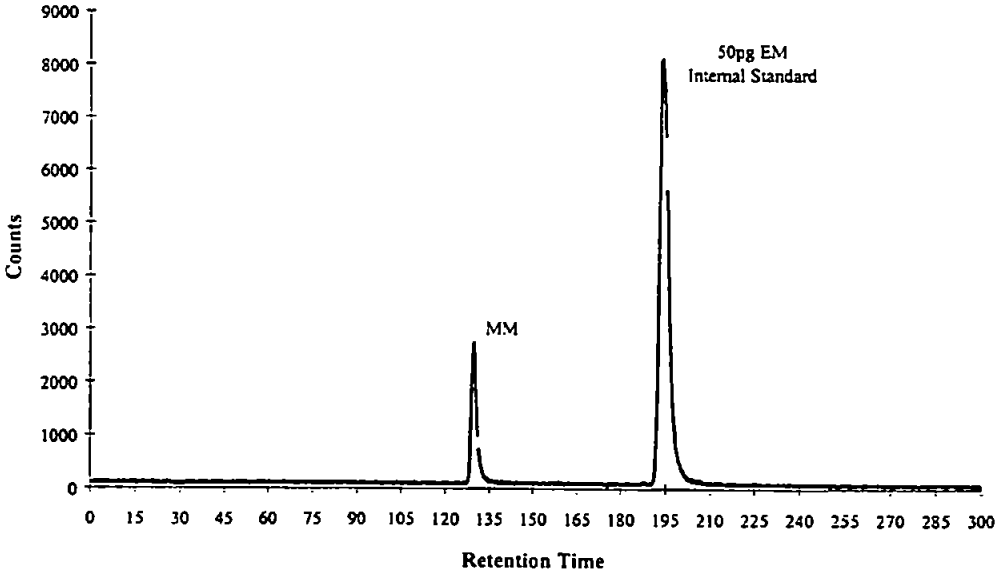


Figure 5.6 Chromatogram of IAEA 142 Sample

Table 5.2 Determination of Methylmercury in *Mytilus edulis* Mussel Homogenate Materials

Reference material	Spike recovery (%, n=6)	Certified concentration (ng g^{-1})	Found concentration[a] (ng g^{-1})
IAEA 142	54 ± 6	47 ± 4	48 ± 9
NIST 8044	47 ± 4	28 ± 2	30 ± 3

[a] corrected for spike recovery and moisture content

5.1.2.4 Simultaneous Halide m/z Scanning of the Mussel Homogenate Extracts

The multi-element capacity of the ICP-MS was used to investigate the halide species associated with extracted methylmercury in these materials. Injections of a 5pg methylmercury chloride standard followed by IAEA 142 and NIST SRM 8044 extracts with added ethylmercury chloride internal standard, were made. The ICP-MS was set to monitor four m/z ratios, ^{79}Br, ^{127}I, ^{202}Hg and ^{35}Cl. It is well known that the halides have varying sensitivity by ICP-MS due to differences in ionisation energy which decreases down the group, so no attempt was made to quantify results.

Figure 5.7 shows multi-element chromatograms for a 5pg MMC standard and IAEA 142 respectively. Due to the low sensitivity of this technique for chloride and high background due to the presence of dichloromethane as the solvent, no chloride peaks could be observed for the standards or extracts.

Two peaks for mercury at m/z 202 were observed in the 5pg MMC standard (Figure 5.7a). The first was identified by retention time as methylmercury and the second was attributed to ethylmercury chloride contamination from a previous high standard. It was evident from the absence of peaks at m/z 127 and 79, that no bromide or iodide was associated with these compounds.

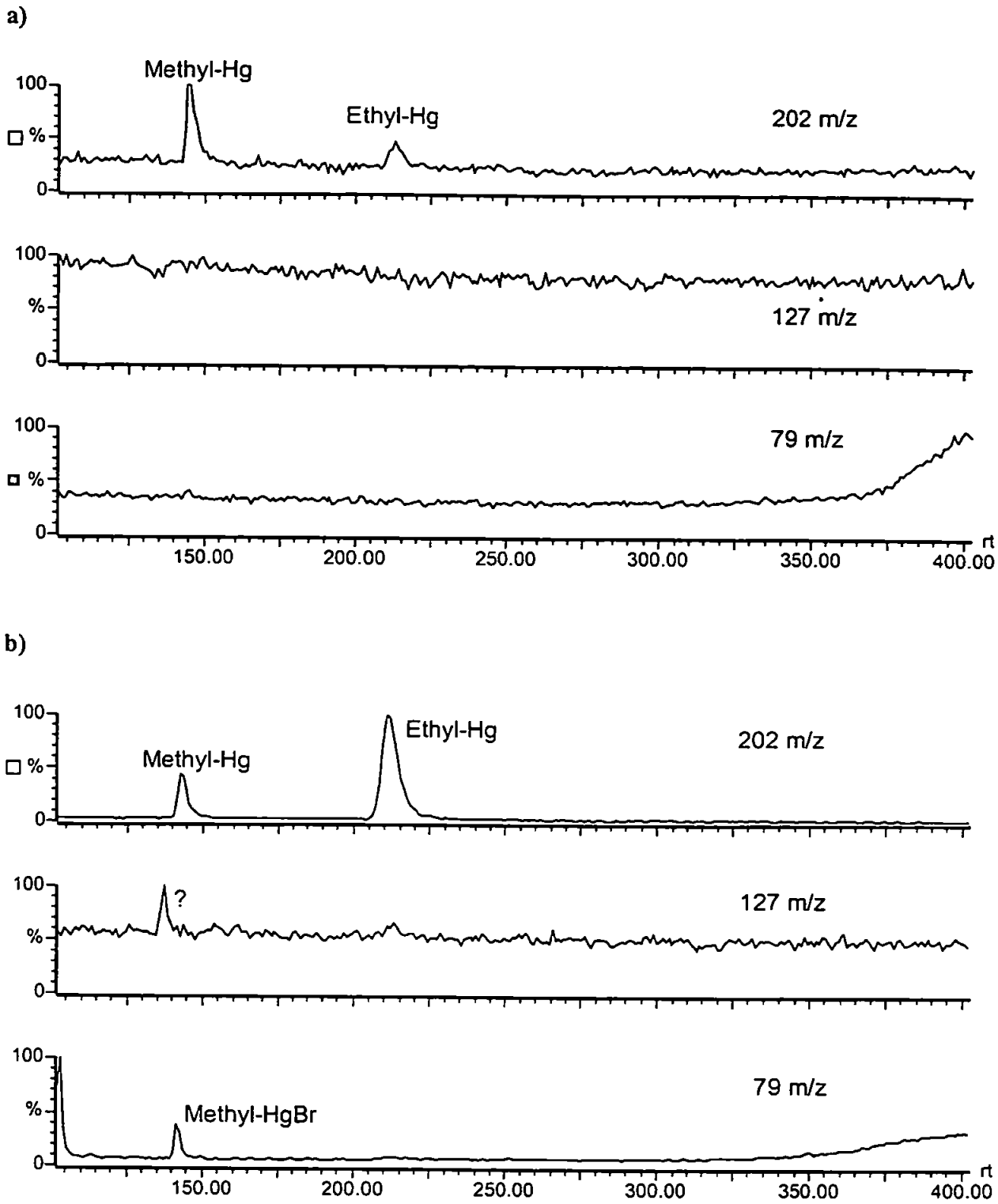


Figure 5.7 **Simultaneous Multi-element Scanning of m/z ^{79}Br, ^{127}I, ^{202}Hg and ^{35}Cl: a) 5pg Methylmercury Chloride Standard; b) IAEA 142 Extract (retention time shown in sec)**

Results observed for both of the mussel extracts were very similar, so only one of the chromatograms has been included here (Figure 5.7b). It was evident from the chromatograms that the methylmercury species eluted at exactly the same retention time as a species containing bromide (*c.f.* the first eluting species observed at m/z/ 202 and 79). However, species containing iodide eluted five seconds earlier (*c.f.* signal at m/z 127). It is generally believed that the Br^- used during extraction complexes with RHg^+ and that it is this form that is present in the final extract. These results suggest very strongly that methylmercury was extracted as a brominated species. Given that no iodide was added during sample preparation it appears that this halide was present in both of the mussel homogenates, however, as the retention time of iodide did not correspond exactly with mercury, it is not possible to conclude that its source was methylmercury iodide. Further work is required to identify the iodide containing compound.

5.1.3 Conclusions

Despite the low spike recoveries obtained for both mussel homogenate samples, good agreement was observed between determined concentrations of methylmercury and the certified values after correction for spike recovery. As the extraction efficiency of this procedure can vary greatly from one sample to the next, it is important to perform spike recovery measurements, even on reference materials. These recovery experiments will only be valid however, if samples are given sufficient time for the spike to become incorporated.

Inductively coupled plasma – mass spectrometry has been shown to be a suitable detection technique for organomercury speciation after separation by gas chromatography. An added advantage of this approach was the use of its multi-element capacity, which allowed the confirmation that methylmercury bromide was present in extracted samples.

5.2 Determination of Methylmercury in Dogfish Liver Tissue by HPLC-UV-CV-AFS

5.2.1 Introduction

An HPLC approach was selected as an alternative chromatographic technique, coupled to a CV-AFS system for organomercury speciation. The initial instrumental set up, methodology and starting conditions were selected from a review of recent publications. A UV photolysis lamp was included in the instrumental arrangement between the HPLC column and the cold vapour generation stage in order to maximise the oxidation stage *i.e.* R-Hg-X oxidation to Hg^{2+}. A range of studies were undertaken to optimise the sensitivity and performance of this technique. Method validation was achieved using DOLT-2 certified reference material.

5.2.2 Experimental

5.2.2.1 Instrumentation

An isocratic HPLC pump (SpectraSYSTEM P1000, Spectra-Physics Analytical, UK) was fitted with a six port rotary injection valve (Rheodyne 7125, Phase Separations Ltd, Deeside, UK) and a reverse phase C_{18} column (3µm ODS2, Phase Separations Ltd). The column was attached to a UV photolysis unit comprising a 350mm x 15W UV immersion lamp (Heraeus TNN 15/35, Heraeus Noblelight Ltd, Cambridge UK), with housing and power control (PS Analytical Ltd) and fitted with a PTFE coil through which the sample was passed. The output was attached to the vapour generation apparatus via a PTFE T-

piece. The gas-liquid separator was in turn connected to the detector (10.023 Merlin, PS Analytical Ltd) via a hygroscopic membrane drier tube (Perma Pure Inc, Toms River, NJ, USA). Figure 5.8 illustrates this instrumental arrangement. Signal monitoring was performed using a 0-1V analogue chart recorder output and subsequently by attaching EZChrom software (Version 6.6, Scientific Software Inc, San Ramon, USA) data acquisition software.

5.2.2.2 Material

Dogfish liver tissue (DOLT-2, National Research Council of Canada, Canada) was selected for method validation. This material has previously been described in Chapter 3. The moisture content of this material was determined by drying a portion to constant weight, in an oven at 105°C for 48 hours.

5.2.2.3 Reagents and Standards

All reagents were of analytical grade or better, prepared in double de-ionised water (Elga Option 3, Elga Ltd, High Wycombe, UK) or HPLC grade solvent. The mobile phase (10:90 or 30:70 methanol/water) was prepared by mixing appropriate volumes of solutes. This was matrix modified by adding the required volume 2- mercaptoethanol (98%, Aldrich Chemical Co.) in a fume cupboard. Buffering was achieved by dissolving ammonium acetate salt in the solution adjusting the pH using acetic acid. Standards of methylmercury chloride (Strem UK) and ethylmercury chloride (Alfa, Johnson Matthey) were prepared by dilution in methanol, followed by further dilution in water, L-cysteine or sodium thiosulphate as required.

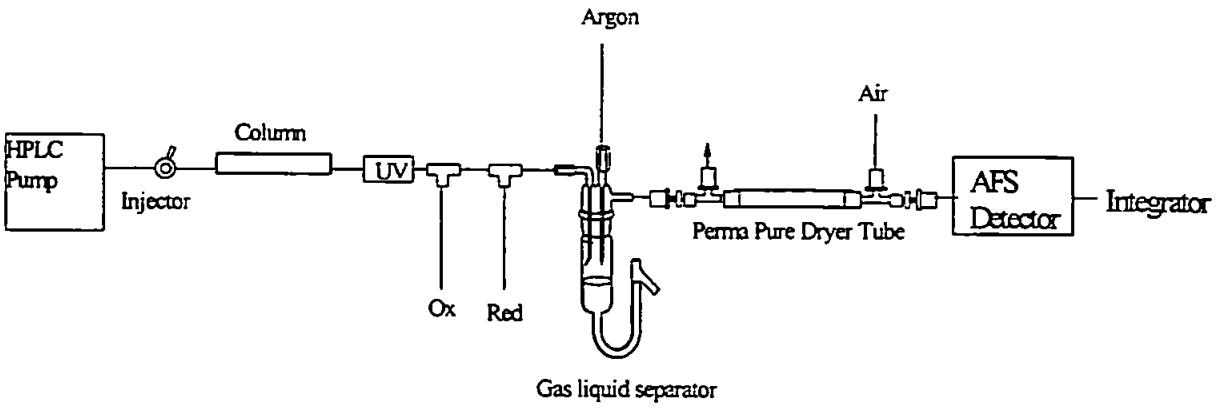


Figure 5.8 HPLC-UV-CV-AFS Instrumental Arrangement

5.2.2.4 Optimisation Studies

Initial sets of experiments were performed to consider the effect of different chemical oxidants, with and without UV photolysis, on the signal generated for single compound standards of MM and EM. These tests were performed using the instrumental arrangement shown in Figure 5.8, with the column removed for simplicity (Flow Injection Analysis arrangement). The operating conditions used are shown in Table 5.3.

Table 5.3 Initial Operating Conditions of FIA-UV-CV-AFS

Mobile Phase	Water
Injection Loop	20µl
Reductant	2% m/v $SnCl_2$ in 10% v/v HCl
Carrier gas	Ar, 250 ml min^{-1}
Sheath gas	Ar, 250 ml min^{-1}
Drier gas	Air, 3l min^{-1}
Range	As required

Once appropriate oxidation conditions had been selected, the column was attached and a range of optimisation studies performed. These included the optimisation of oxidant concentration, flow rate, effect of UV photolysis and UV coil length. Further optimisation studies were undertaken to select the reductant matrix, reductant concentration and flow rate, the mobile phase composition (methanol:water, [2-mercaptoethanol] and pH) and the mobile phase flow rate. Once optimised the performance of the technique was tested.

5.2.2.5 Application Development

Prior to extracting MM from DOLT-2 following a procedure based on those described in Chapters 3 and 4, a series of tests were performed to investigate both possible sample/standard matrices. At the second stage of the extraction procedure, the solvent containing organomercury was extracted into an organomercury specific aqueous phase. Previously, only sodium thiosulphate had been considered in this project. An alternative matrix L-cysteine had also been reported in the original Westöö work. These two possible extractants were compared.

5.2.2.6 Extraction of Methylmercury from DOLT-2 for HPLC-UV-CV-AFS Analysis

The extraction procedure developed for DOLT-2 and described in Chapter 3 formed the basis of this procedure. Initial portions of sample (0.2g) were prepared along with a calibration series. The initial acidic extraction into DCM was performed followed by extraction into L-cysteine 1ml 0.001M. The L-cysteine extracts were analysed by HPLC-CV-AFS.

5.2.3 Results and Discussion

5.2.3.1 Optimisation of Oxidant

The effect of different oxidants on Peak Height signal for a 20 ng absolute MM standard (20 µl of 1µg ml^{-1}) with and without UV photolysis is shown in Figure 5.9. Little difference was observed between acidic bromate/bromide or acidic persulphate (with/without copper catalysis). However, the use of UV photolysis resulted in a marked improvement. It was concluded from these results that a chemical oxidation, coupled with

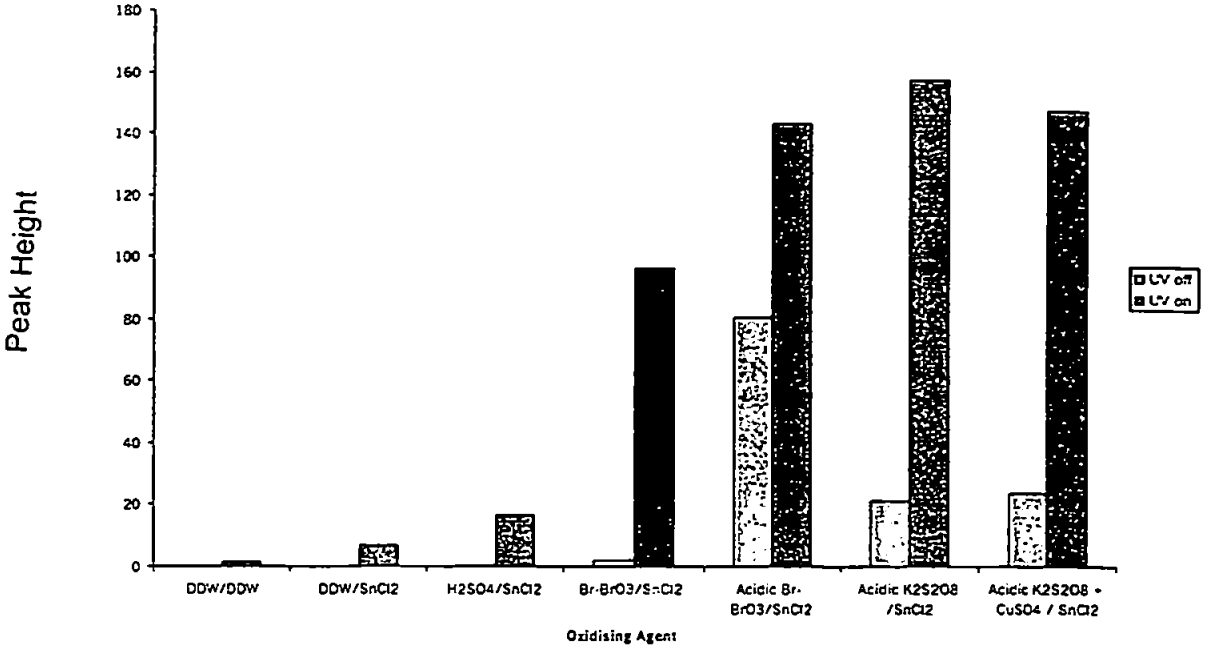


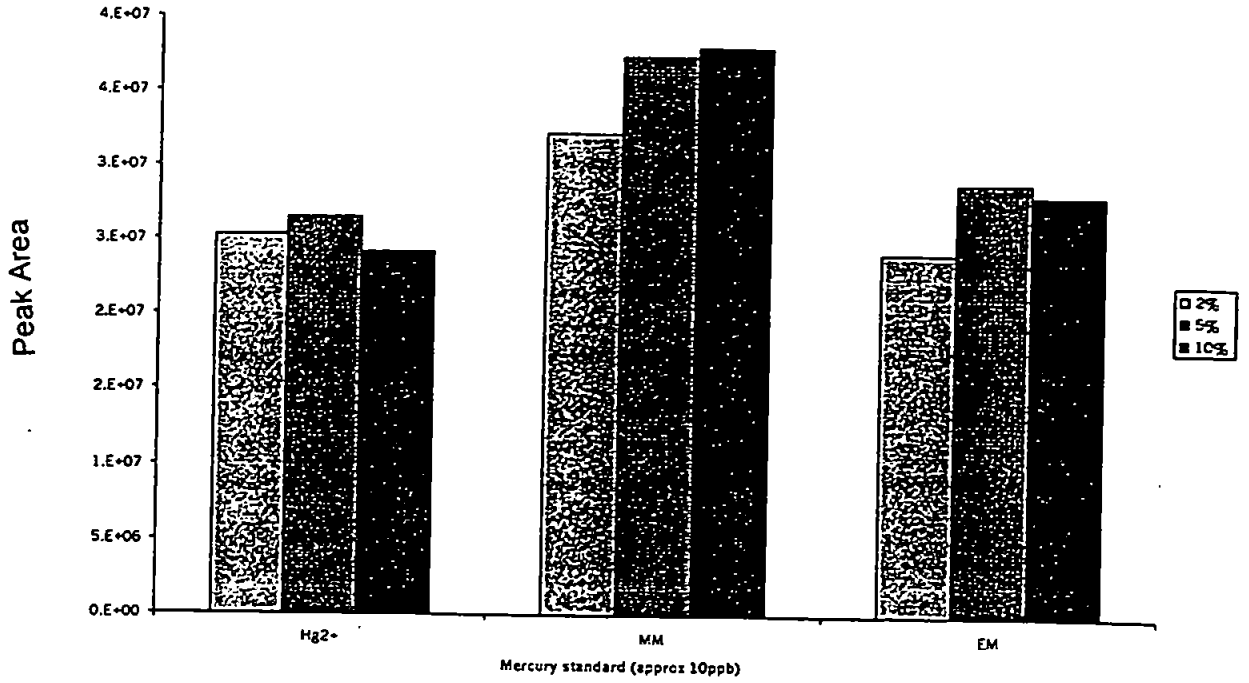
Figure 5.9 Effect of Oxidant, With/Without UV on 20ng MM Peak Height

UV photolysis would provide the best oxidation conditions. Acidified bromate/bromide solution was selected for this study as it presented the most powerful oxidant without UV photolysis. During this study permanganate chemistry was also considered as a possible approach, however, MnO_2 precipitate was formed on exposure to UV light leading to blockages in the sample lines.

The effect of BrO_3^- /Br^- concentration on the signal for 10ng ml^{-1} standards of Hg^{2+}, MM and EM (all as mass Hg) are shown in Figure 5.10. Increased peak area for the organomercury compounds was observed with both 0.005N and 0.01N bromate/bromide oxidant solutions compared to 0.002N, and the MM and EM signals increased with UV photolysis. No significant effects of oxidant concentration or UV status were observed on the Hg^{2+} standard, as expected. A concentration of 0.005N BrO_3^-/Br^- in 5% v/v HCl was selected for this work.

The effect of oxidant flow rate on peak signal for 10ng ml^{-1} standards of MM and EM is shown in Figure 5.11 with and without UV photolysis. It was concluded that, providing UV photolysis was employed, the flow rate of the oxidant was not significant. The lowest rate (2.5ml min^{-1}) conserved reagents but led to a slight increase in retention time whereas the upper rate (9ml min^{-1}) contributed to dilution. As a result, the lower flow was selected for this work.

a)



b)

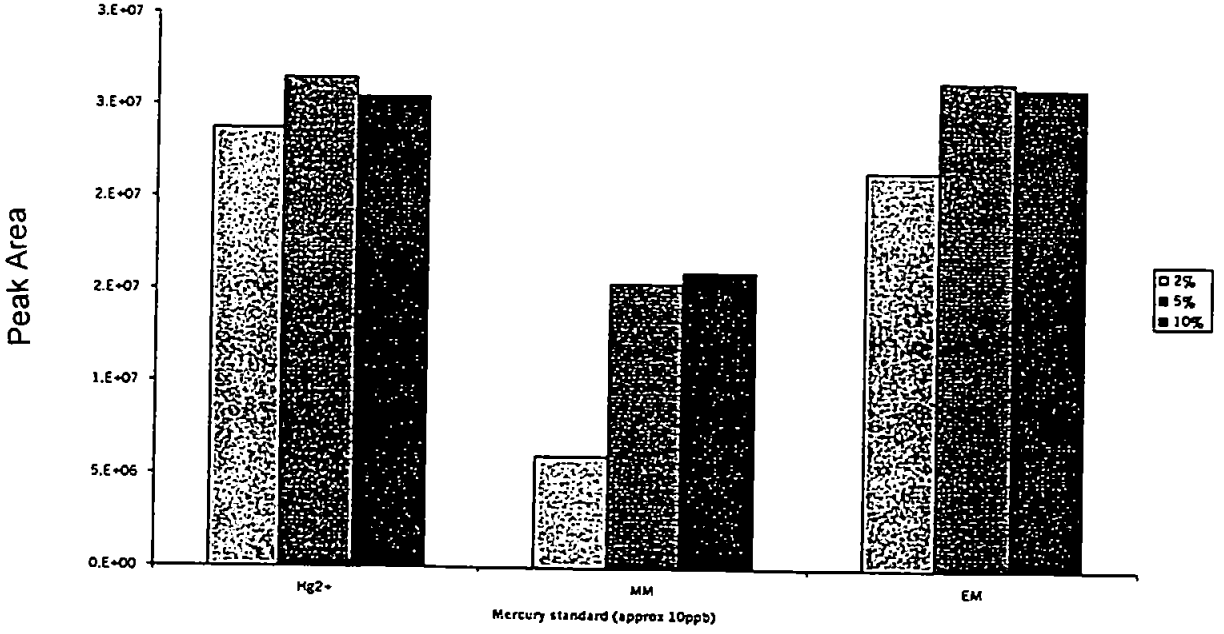


Figure 5.10 **Effect of [0.1N BrO_3^-/Br^-] on Mercury Compound Peak Areas a) With UV b) Without UV**

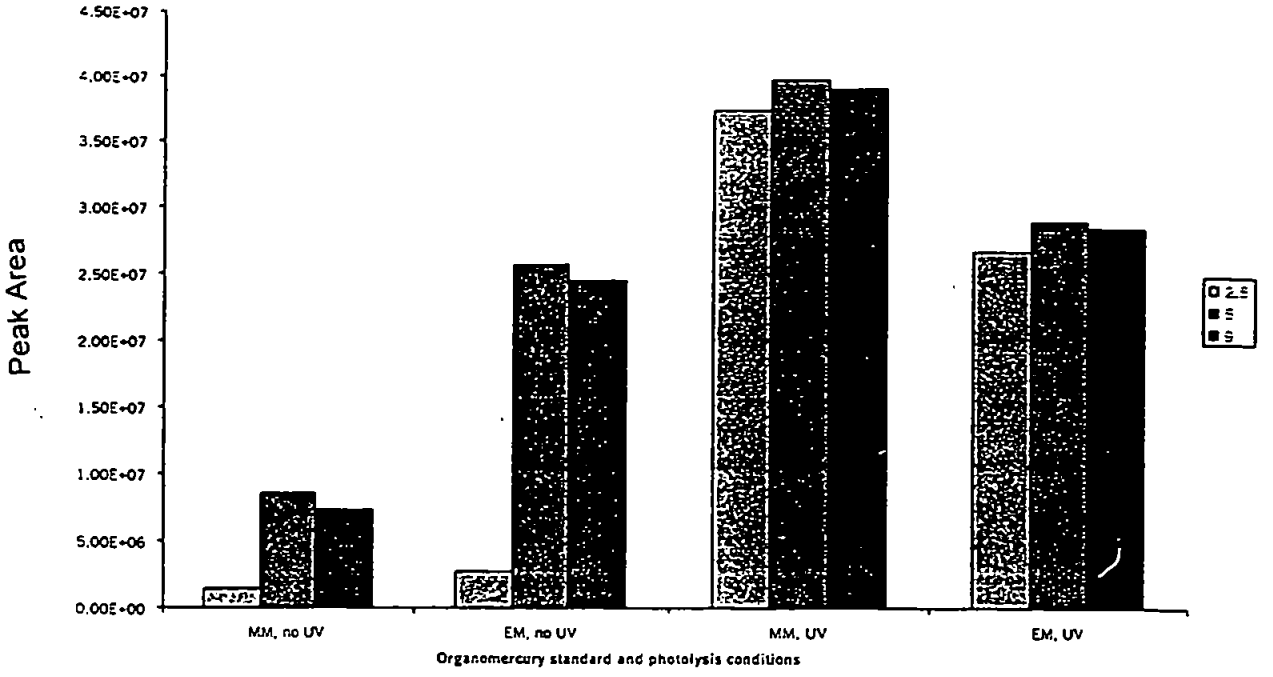


Figure 5.11 Effect of Oxidant Flow Rate on Organomercury Compound Peak Areas (ml min^{-1})

5.2.3.2 Optimisation of UV Photolysis Systems

Initial studies were made using a 15m of polytetrafluoroethylene (PTFE) tubing (0.8mm id) coiled around the UV source. However, a study was performed in order to investigate the effect of coil length and diameter. A quartz flow cell (30cm x 2mm id) was made and positioned alongside the UV lamp. A mixed standard was studied in the system using both these irradiation apparatus. In addition a shorter PTFE coil of narrower bore (5m x 0.5mm id) was also considered. Figure 5.12 illustrates the differences observed. Overall, the peak areas were smaller (62%) for the quartz flow cell, with some broadening caused by the wider bore flow cell. The same peak areas were noted for the 5 and 15m long coils confirming that complete oxidation of both compounds is achieved in the shorter length. In addition, noticeably sharper peak profiles were observed when using the narrower bore/ shorter length tubing. This was selected as optimal for this system.

5.2.3.3 Optimisation of Reductant

$SnCl_2$ was selected as the reductant for these studies rather than $NaBH_4$, which leads to significant water formation as a by-product of the reaction, is water. Two reductants were compared, 2% m/v $SnCl_2$ in 10% v/v HCl and 3% m/v $SnCl_2$ in 20% NaOH. Chromatograms for a mixed MM and EM standard acquired using the two different reductants are shown in Figure 5.13. Acidified $SnCl_2$ resulted in greater peak area signal than alkaline $SnCl_2$ with RSD = 2% (n=3)so the former was chosen.

The effect of the concentration of acidified $SnCl_2$ on peak area is shown in Figure 5.14. Very little effect was observed, though a higher concentration (10% m/v $SnCl_2$) resulted in precipitation of tin and poor precision (ca ±30% RSD). Hence 2% m/v $SnCl_2$ in 10% v/v HCl was chosen as optimal.

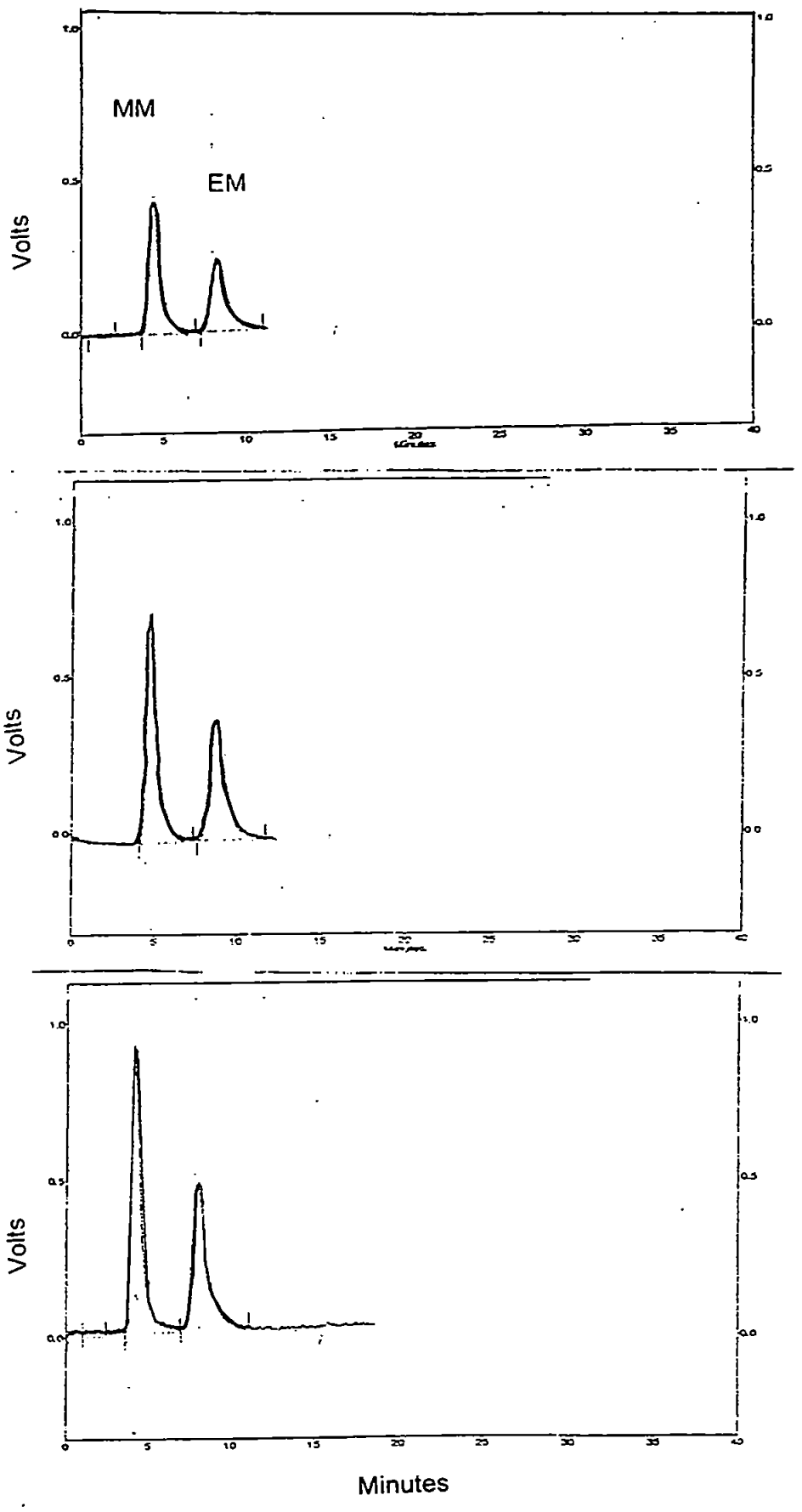


Figure 5.12 Effect of UV Flow Path on Chromatographic Separation

a) 30mm x 2mm Quartz Flow Cell, b) 15m x 0.8mm PTFE Coil, c) 5m x 0.55mm PTFE Coil

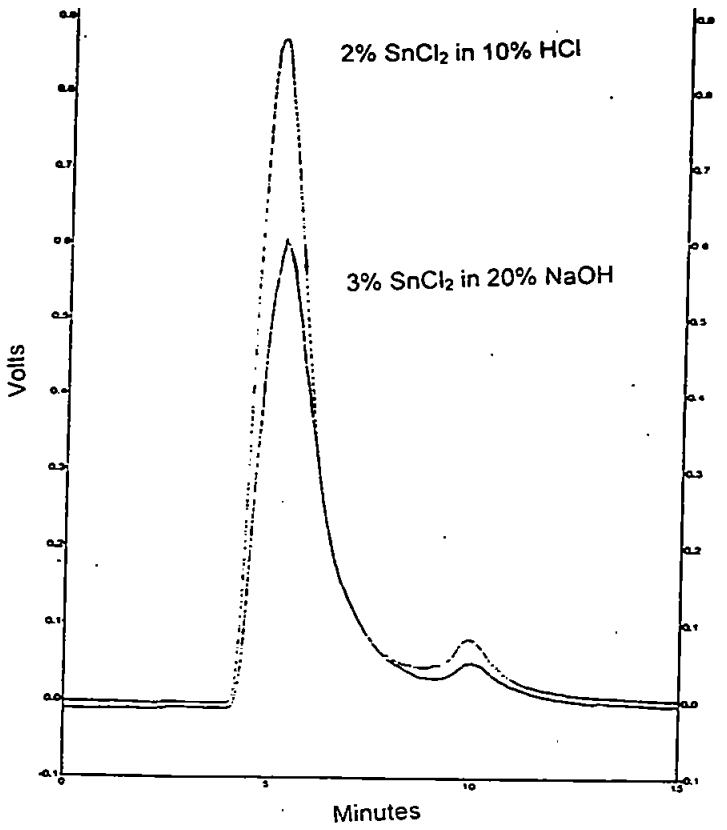


Figure 5.13 Comparison of Acidic and Alkaline $SnCl_2$ Reductants on MM and EM Mixed Standard

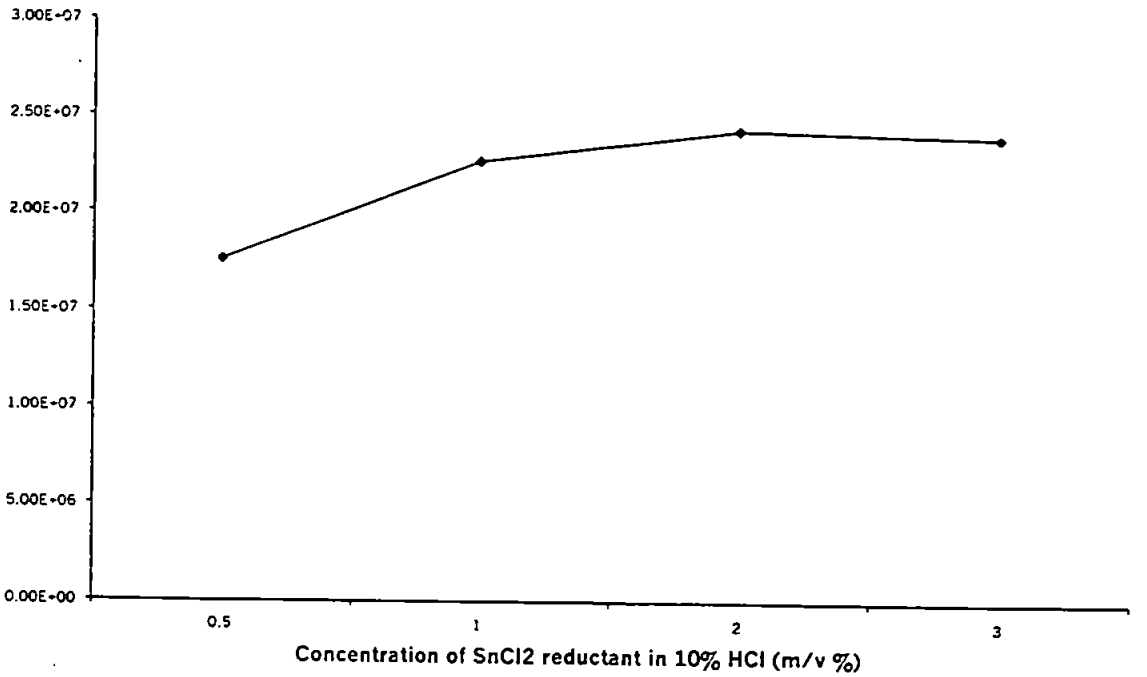


Figure 5.14 Effect of Reductant Concentration on MM Peak Area

The peak area signal for a MM standard was measured in triplicate for three reductant flow rates, supplied by different rated peristaltic pump tubing. Results are shown in Figure 5.15. A decrease in signal with increased flow rate was observed probably due to increased dilution. Hence, the lowest flow rate of 2.5ml min^{-1} was chosen.

5.2.3.4 Optimisation of Mobile Phase

Van Deemter plots of HETP (Height Equivalent Theoretical Plates) against mobile phase flow rate are shown in Figure 5.16 for triplicate measurements of a MM standard with both 10:90 and 30:70 (methanol/water) phase mixtures. The mobile phase also consisted of 50µl l^{-1} 2-mercaptoethanol, 1.54g l^{-1} ammonium acetate, adjusted to pH 5.5 with acetic acid. The flow rate of 0.4ml min^{-1} resulted in the lowest HETP values for both reagent mixtures, with lower values being observed for the 10:90 reagent. This was as expected for this column which is a strong anionic exchange column which elutes compounds with increasing alcohol strength. The optimal practical flow rate was 1.0ml min^{-1}, corresponding to double the value found on the van Deemter plot, in order to reduce the retention times of the compounds of interest. A further study at this flow rate was later undertaken for a mixed MM and EM standard, for both reagent mixtures after the addition of a guard column. The 30:70 mobile phase ratio was found to lead to poor peak resolution with both compounds co-eluting. As a result the 10:90 mixture was selected.

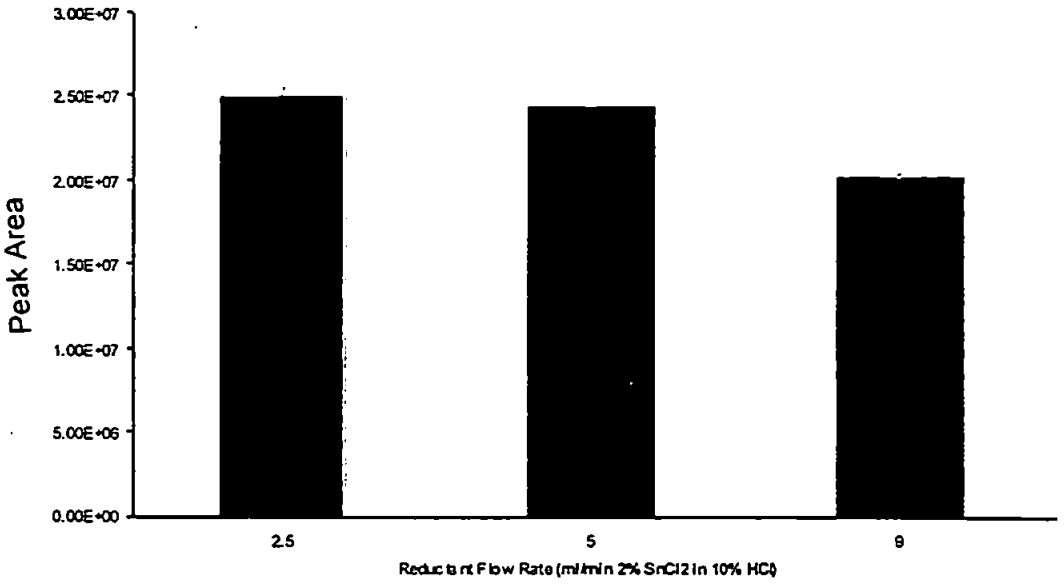


Figure 5.15 Effect of Reductant Flow Rate on MM Peak Area

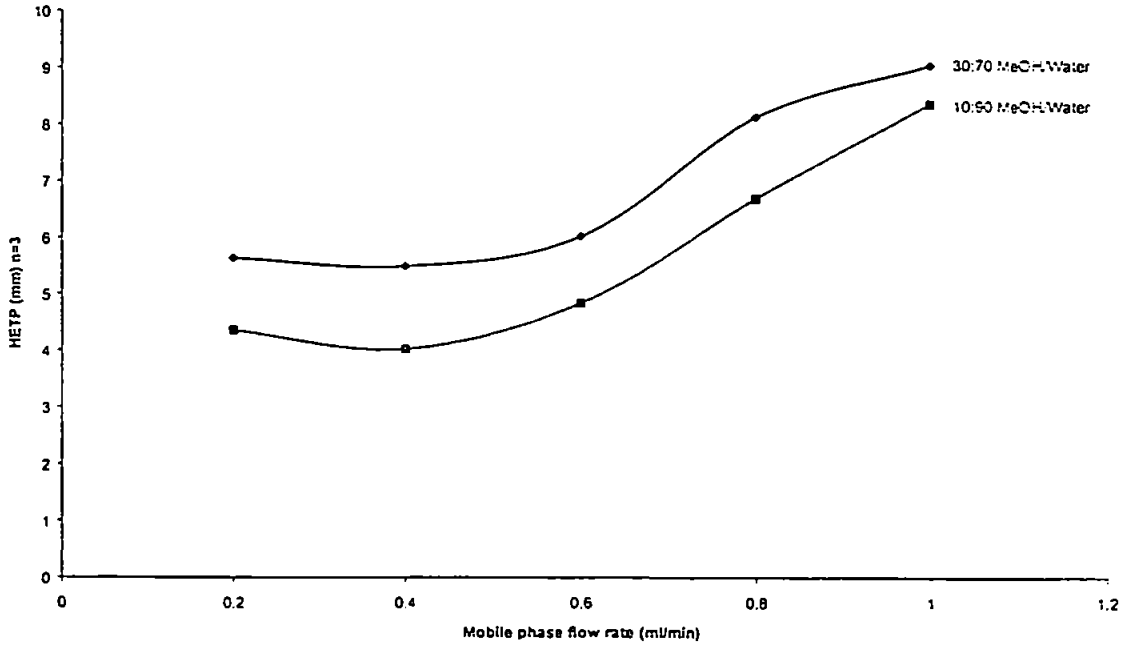


Figure 5.16 van Deemter Plots: HETP against Mobile Phase Flow Rate

Three concentrations of 2-mercaptoethanol, the chosen matrix modifier, (50µl l^{-1}, 100µl l^{-1} and 200µl l^{-1}) and a mobile phase with no mercaptoethanol added were compared over a range of flow rates. Poor chromatography was observed without mercaptoethanol, as shown in Figure 5.17a. The effect of increasing mercaptoethanol concentration on peak areas for a mixed standard of MM and EM is shown in Figure 5.17b-d. These plots indicate that maximum sensitivity was observed at lower flow rates for increasing [mercaptoethanol]. 50µl l^{-1} was selected for this work.

The effect of pH on peak area for MM and EM is shown In Figure 5.18. A range of pH 4.5-7.9 pH units, adjusted with acetic acid was investigated. A slight decrease in signal was observed at the upper end of the range that was also noted to be unstable. Little difference was observed between pH 4.5-6.0 so a value of pH 5.5 was selected.

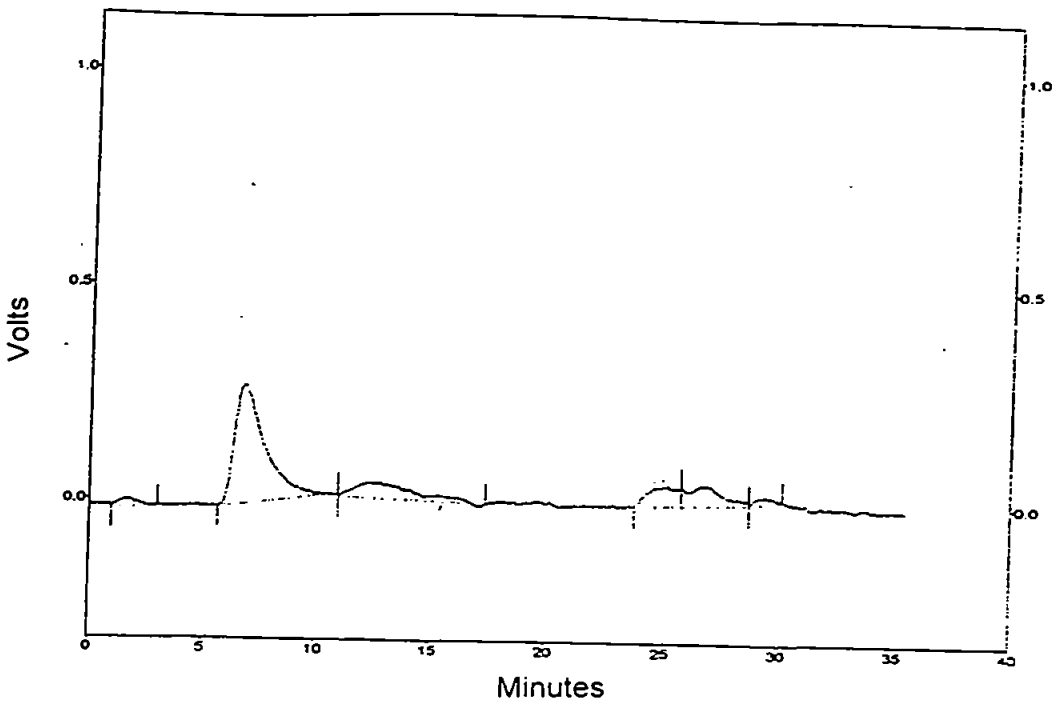
5.2.3.5 Optimised Conditions

The optimised conditions for the HPLC-UV-CV-AFS approach as shown in Table 5.4. Figure 5.19 shows a chromatogram obtained under these conditions.

Table 5.4 Optimised HPLC-UV-CV-AFS Conditions

Mobile Phase	10:90 Methanol/Water, 50µl l^{-1} 2-mercaptoethanol, 1.54g l^{-1} $CH_3CO_2NH_4$, pH 5.5 acetic acid
Injection Loop	200µl
Column	3µm ODS2 C_{18} Phase Separations
Oxidant	5%v/v 0.1N BrO_3^-/Br^- in 10% v/v HCl at 2.5ml min^{-1}
Reductant	2% m/v $SnCl_2$ in 10% v/v HCl at 2.5ml min^{-1}
Carrier gas	Ar, 250 ml min^{-1}
Sheath gas	Ar, 250 ml min^{-1}
Drier gas	Air, 3l min^{-1}
Range	As required

a)



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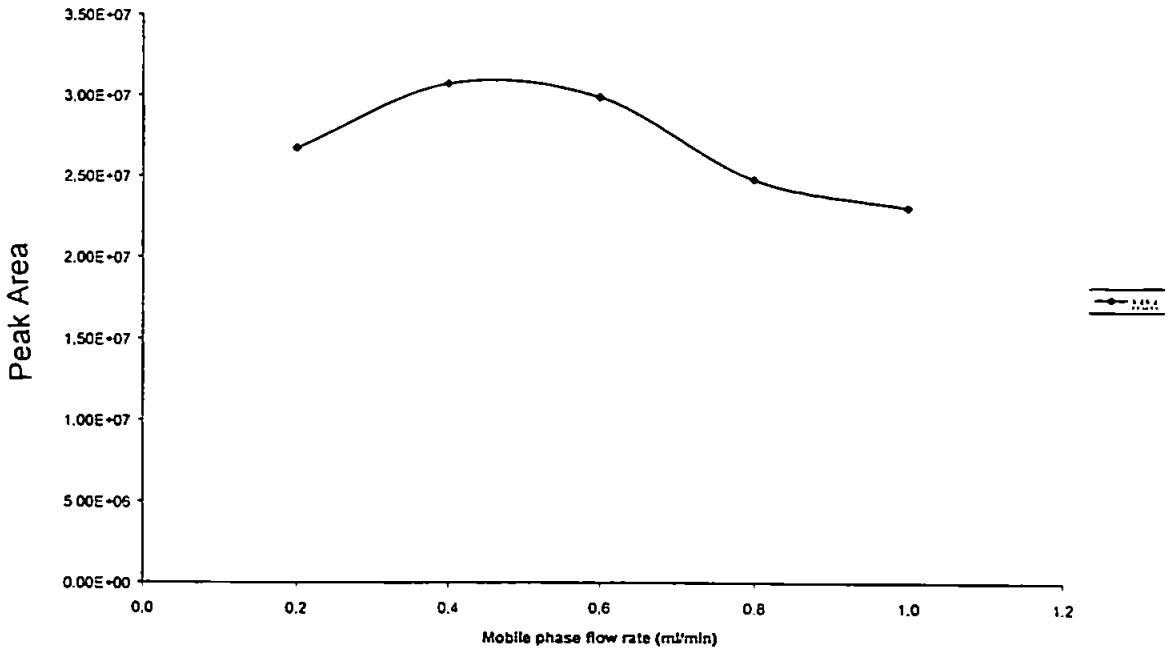
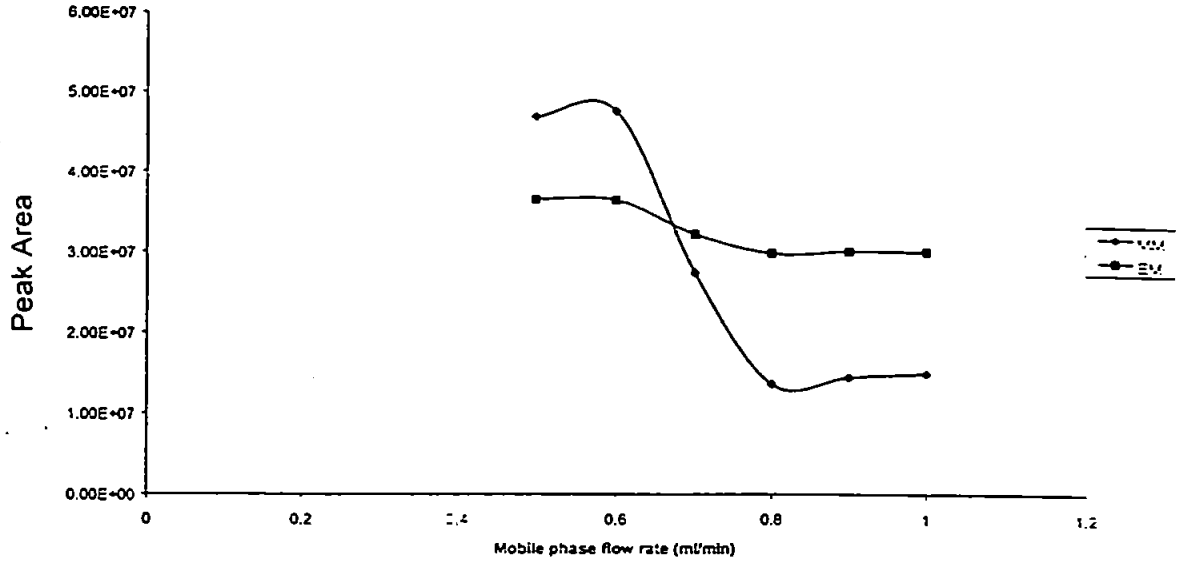


Figure 5.17 **Effect of 2-Mercaptoethanol Matrix Modifier on Organomercury Speciation a) No 2-Mercaptoethanol; b) Effect of Flow Rate on MM Peak Area with 0.005% v/v 2-Mercaptoethanol**

c)



d)

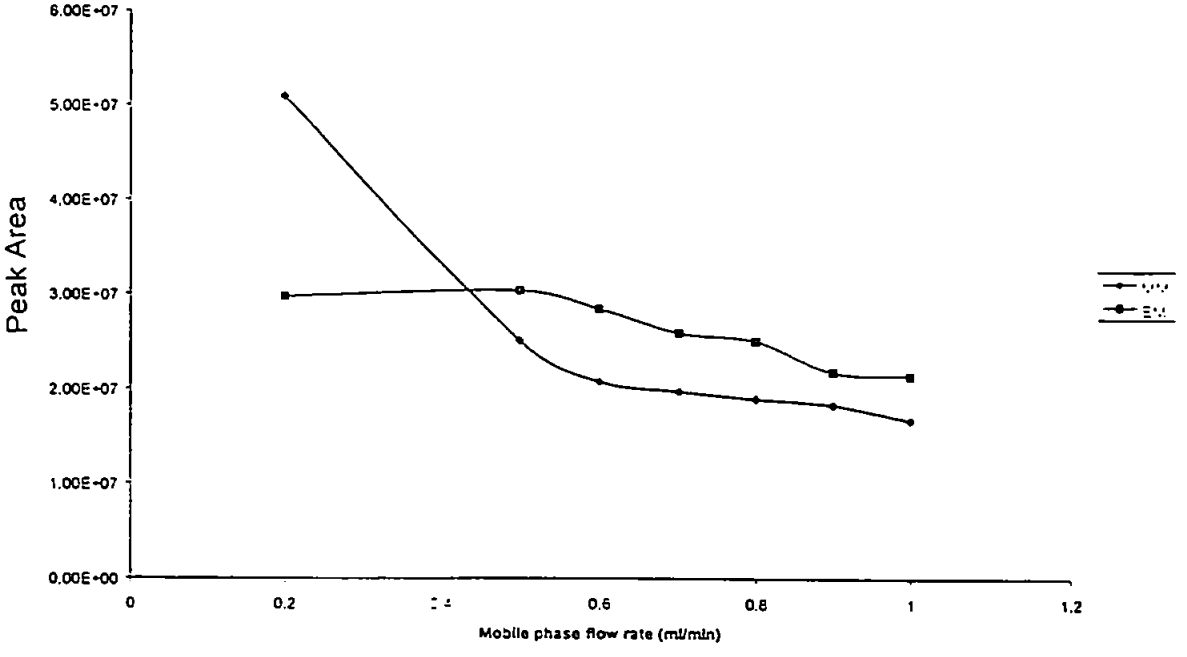


Figure 5.17 **Effect of 2-Mercaptoethanol Matrix Modifier on Organomercury Speciation c) Effect of Flow Rate on Peak Area with 0.01% v/v 2-Mercaptoethanol d) Effect of Flow Rate on Peak Area with 0.02% v/v 2-Mercaptoethanol**

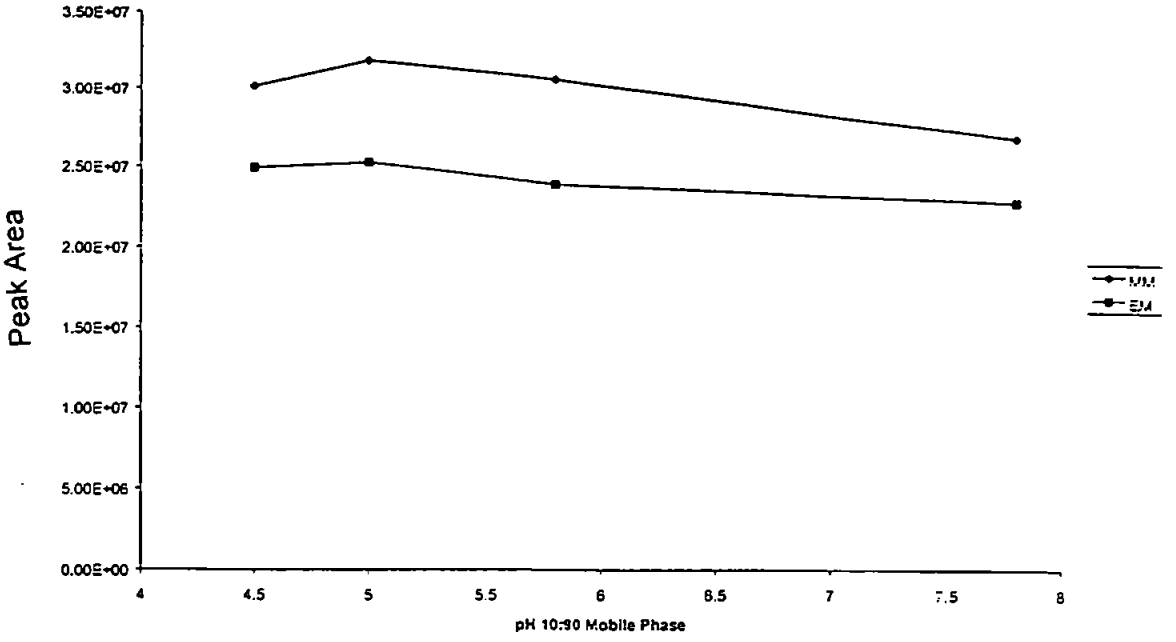


Figure 5.18 Effect of Mobile Phase pH on Organomercury Peak Areas

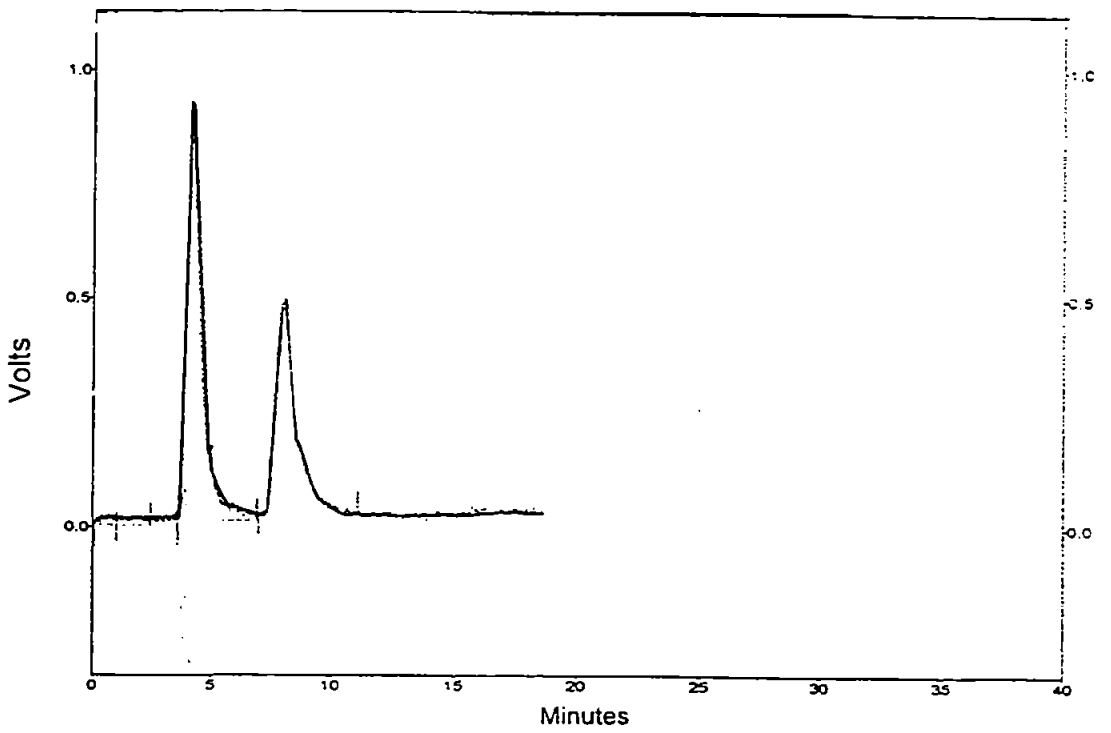


Figure 5.19 Separation of Methyl and Ethylmercury by HPLC-CV-AFS

Standards were prepared in water, 0.001M L-cysteine and 0.01M $Na_2S_2O_3$ and compared. No difference was observed between the peak areas for standards prepared in water or L-cysteine although the retention times of the compounds in the latter matrix were slightly shorter. Thiosulphate, was found to interfere with the vapour generation stage, leading to a negative signal over the period of MM and EM elution. It was also noted that a black precipitate was observed. Hence the standards were prepared in L-cysteine.

Calibrations were performed over three orders of magnitude; 0-10ng ml^{-1}, 0-100ng ml^{-1} and 0-1µg ml^{-1} mixed MM and EM standards in 0.001M l-cysteine (as Hg). Linear plots were obtained with equations and linear correlation co-efficients shown in Table 5.5.

Table 5.5 Calibration Data for MM and EM by HPLC-UV-CV-AFS

Range ng ml^{-1}	Compound	Equation	R^2
0-10	MM	$y = 9 \times 10^6 x$	0.9983
0-10	EM	$y = 9 \times 10^6 x$	0.9996
0-100	MM	$y = 466253x$	0.9984
0-100	EM	$y = 467401x$	0.9997
0-1000	MM	$y = 43918x$	0.9997
0-1000	EM	$y = 43965x$	0.9973

Good linearity was observed over each of these ranges, with equal sensitivity observed for both compounds. The limit of detection (LOD) was determined from eight replicate injections of a 1.8ng ml^{-1} MM and 1.9ng ml^{-1} EM mixed standard as $3\sigma_{n-1}$ against the low range calibration. This was calculated to be 0.25 ng ml^{-1} MM and 0.23ng ml^{-1}EM corresponding to 50pg MM and 46pg EM absolute. The repeatability of injection was calculated as 4.7% and 4.2% RSD respectively.

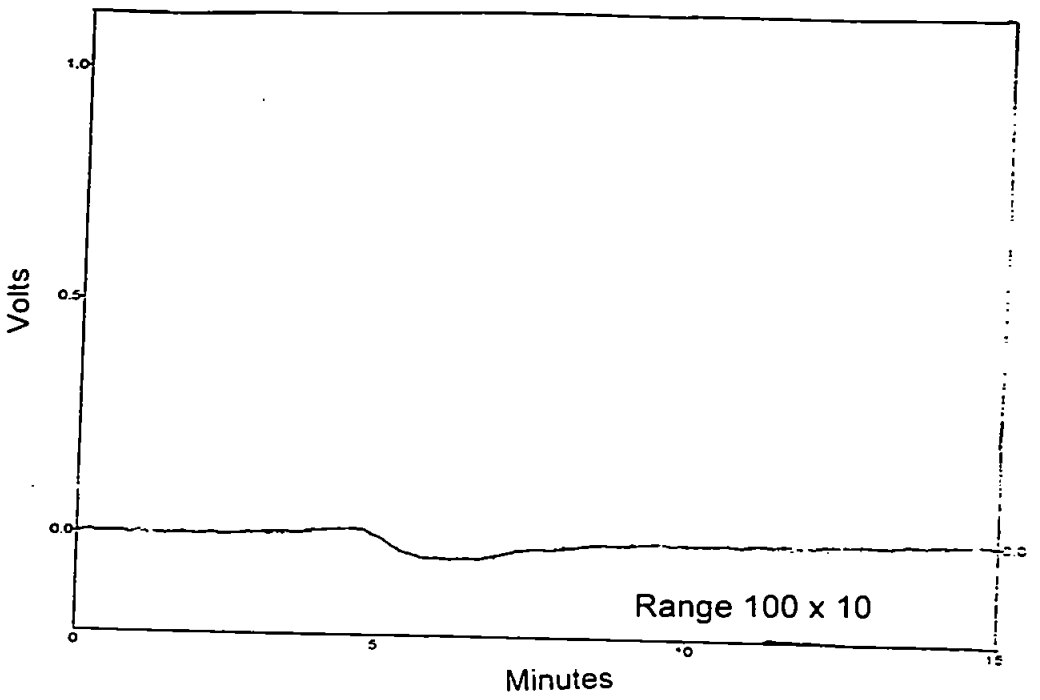
5.2.3.6 Determination of Methylmercury in DOLT-2

An initial procedure was undertaken to compare extracted standards with standards prepared directly in L-cysteine. This revealed a significant problem. For extracted blanks and standards in the range 0-10ng ml^{-1} or 0-100ng ml^{-1}, an unidentified compound was observed to elute as the MM signal reached its optimum, leading to a negative signal as vapour generation was interrupted. The use of alkaline $SnCl_2$ as a reductant was considered at this stage as it has been reported to tolerate high levels of interferences [220], however, although the negative signal was indeed reduced, the sensitivity was also reduced indicating that this approach was not appropriate. The negative signal was much less significant at higher concentrations such as 1 μg ml^{-1} and as a result it was concluded that the vapour generation interference would be insignificant for the DOLT-2 application. Examples of this interference are shown in Figure 5.20. From the results of this test it was possible to directly compare the recovery of non extracted and extracted standards. The recovery calculated from duplicate injection of both MM and EM compounds was 94%, hence, it was necessary to pass the standards through the extraction procedure in order to account for small losses and to allow for the small amount of negative interference. The results for DOLT-2 are shown in Table 5.6.

Table 5.6 Results for the Determination of Methylmercury in DOLT-2

Spike recovery n=3 (%)	Moisture Content (%)	[MM] Determined ng g^{-1}	Certified [MM] ng g^{-1}
90 ± 3	10.6	739 ± 48	693 ± 53

Good agreement was observed between the certified value and the concentration determined. This approach was only possible for high level MM determinations under such conditions.



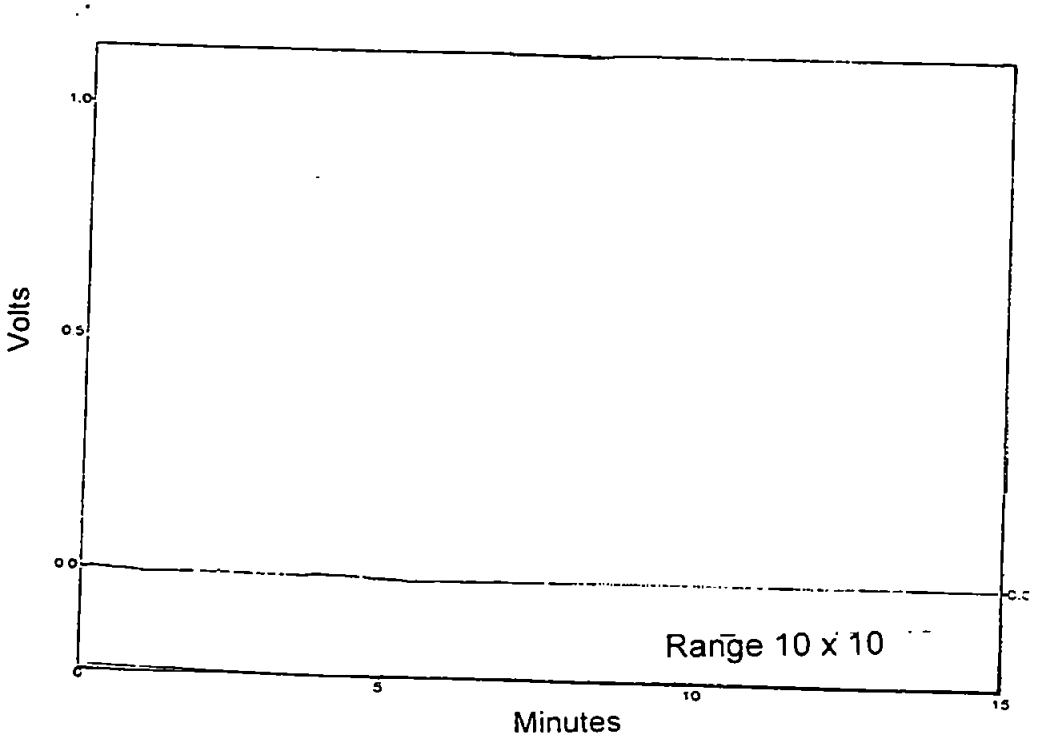


Figure 5.20 Example of Vapour Generation Interference

5.3 Conclusions

Two comparative techniques to the GC-AFS approach have been considered in detail for the determination of methylmercury in certified reference materials. In both cases the instrumental parameters were optimised and performance tested. Although agreement was observed between the certified results and MM concentrations determined in both cases, the overall sensitivity of both techniques were lower than for GC-AFS. The major limitation of the GC-ICP-MS approach was the complicated set-up and expense of using the ICP-MS as an element specific detector. In the case of the HPLC technique, the major drawback was its unsuitability for extracted MM concentrations less than a few hundred ng ml^{-1}. Further studies with an alternative column would be required to identify if the co-eluting interferent was from previous column use or the extraction procedure. Overall, the GC-AFS technique was confirmed as the most sensitive, reliable and most cost effective approach.

Chapter 6

APPLICATION OF A PTV INJECTOR FOR INCREASED GC-AFS SENSITIVITY AND ITS APPLICATION TO MERCURY SPECIATION IN GASES

6.1 Introduction

Within this project the GC-AFS approach has been found to offer sensitivity and reliability for the determination of methylmercury in trace amounts. The limit of detection ($3\sigma_{n-1}$) of methylmercury was found to be 0.25pg as mass of mercury. The limit of determination was therefore 0.5pg ($6\sigma_{n-1}$) which equates as 0.5ng ml^{-1} for a standard $1\mu l^{-1}$ injection. During the instrumental development period all aspects of the GC-AFS configuration were considered in detail to achieve maximum sensitivity from the instrument, with one exception, the injector. The aim of these studies was to investigate the use of a PTV (Programmable Temperature Vaporiser) injector, specifically with regard to its use for large volume injections and thermal desorption. Successful large volume injections could be used to increase instrument sensitivity, which would allow low level samples (sub ng g^{-1}) to be analysed with confidence and could also allow a simpler sample preparation procedure to be developed. Thermal desorption was identified as a means of speciating mercury compounds in air or gas, where the sample would be trapped on a solid adsorbent and the injector temperature controls varied to desorb the species onto the chromatography column.

The use of gas chromatography for the speciation of gaseous volatile organomercury compounds has been established and reviewed in Chapter 1. In particular the ethylation of

organomercury compounds followed by cryotrapping on a solid phase adsorbent before heating to desorb materials onto the GC column. These methods have been found to induce positive artifact formation and can mask natural ethylmercury compounds in samples. A separate report of large volume injection was found for mercury speciation, used with capillary GC-MIP-AES. In this case Hanstrom *et al* [146], used a separately heated 1m packed pre-column onto which butylated organomercury compounds were loaded. At the end of the sampling time, the pre-column was attached in reverse to the main analytical column with oven programming as normal. These methods indicated that gaseous organomercury samples can be preconcentrated onto adsorbents and thermally desorbed onto chromatographic columns for speciation and most recently reports have appeared suggesting that cryotrapping is not required. A specific objective of this project was therefore to attempt to pre-concentrate mercury compounds on solid phase adsorbents followed by desorption into the GC-AFS detector. Initial studies were to be made with gaseous mercury samples (headspace) analysis with later application to large volume injections of both solvent based and gas/air samples.

6.2 Experimental

6.2.1 Injection Manifolds

The Programmable Temperature Vaporiser (PTV) injector (Optic, Atas International, Eindhoven, Netherlands) was selected for this project due to its flexibility and control. The unit allowed for standard split, splitless, on-column or direct injection modes, whilst temperature control enabled the optimisation of injection parameters in order to prevent compound degradation, in addition to its capability for large volume injections (LVI) and thermal desorption. Figure 6.1 indicates the injection configurations for the four main

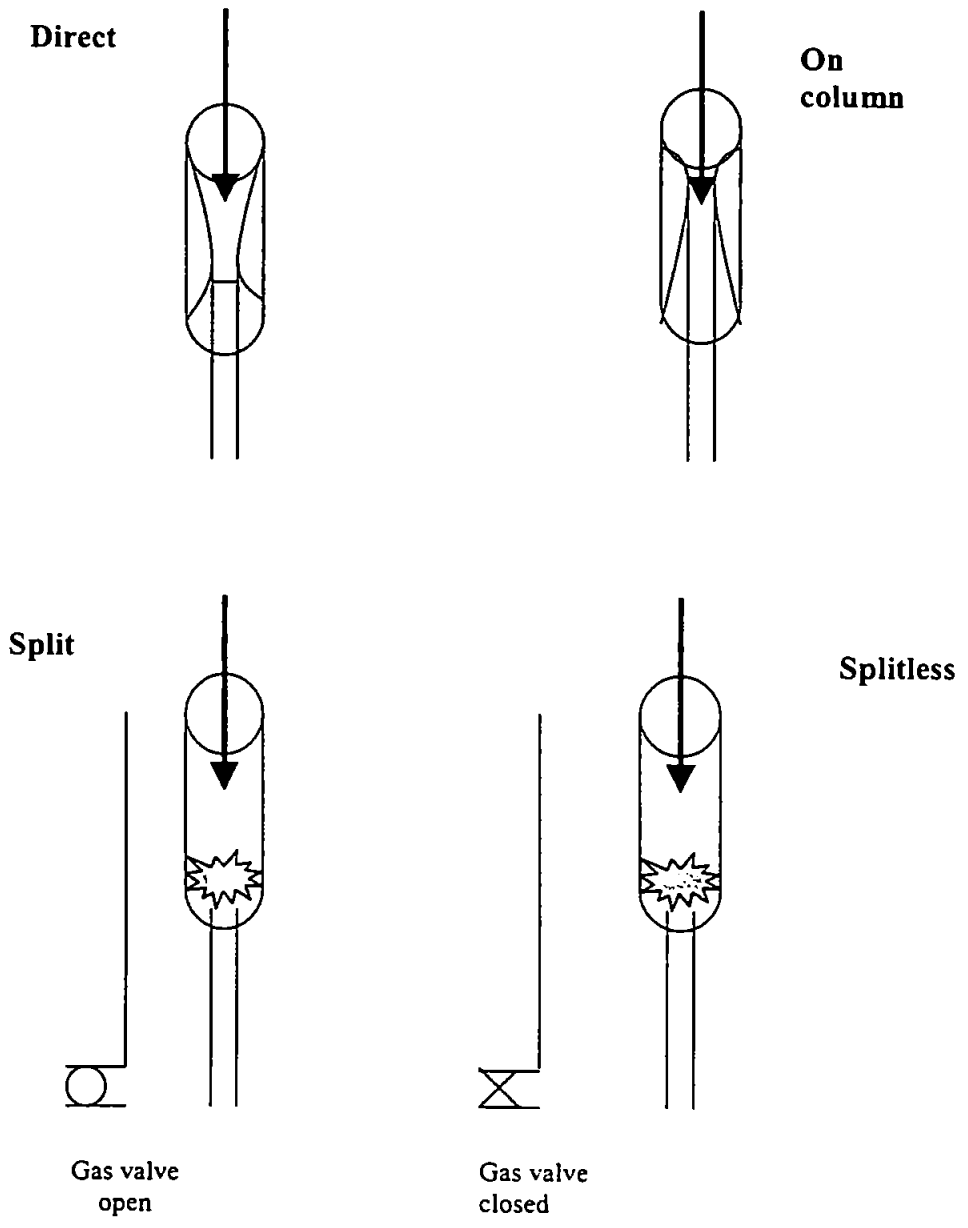


Figure 6.1 Four Injection Modes of PTV

injection modes. The PTV injector allowed temperature programming of up to two steps with variable heating rate and hold times.

A second controllable temperature injection manifold was used during developmental work as the metallic surfaces of the PTV injector were suspected to be the cause of compound degradation. The injection system comprised a 1/4" port hot on-column liner (J &W Scientific, Folsom, USA) which could be packed from one end and was connected directly to the column via a press-fit connection at the other. Helium gas lines were redirected from the GC oven providing a controllable carrier flow over the liner and into the column. Silicone rubber tubing was used for connections up to 250°C. The column was directed through an exit hole in the side wall of the GC oven and the packed liner was wrapped in an electrically heated jacket, controlled via a Cal9900 Autotune Temperature Controller. A PTFE septum injection port with T connection was inserted before the on-column liner and injections were using a gas tight syringe. A modification was made to this arrangement when faster temperature variations were required. The heated jacket was replaced by a pyrolysis unit similar to that used in the GC-AFS instrument. This allowed higher temperatures to be used and required the use of a quartz liner.

6.2.2 Initial Investigation of Injection Modes

In all of the studies made discussed until this point, a direct injection configuration was used in the PTV allowing 1-5µl portions of organomercury solvent extract to be introduced to the column. In order for large volume injections (*e.g.* 50-100µl) and thermal desorption to be used it was necessary to use a splitless arrangement. In this mode a sample was injected onto a trapping material positioned in the injection liner. A stream of carrier gas constantly flowed over the material and was vented to an activated carbon waste trap. Once the injection was complete a valve was switched and the carrier gas redirected

through the column. Initial studies were performed to find optimal conditions for splitless injections avoiding compound degradation.

6.2.3 Headspace Analyses

Calibration and repeatability studies were performed by injecting elemental mercury and dimethylmercury vapours into the GC-AFS to evaluate its applicability to gas phase samples. Known volumes of Hg(0) vapour were injected using a certified gas syringe. These were drawn from a calibration vessel with the temperature varied in ice and noted each time. Weast's [221] data for the saturation concentration of atomic mercury was used to calculate the mass of elemental mercury vapour in each injection. Similarly a calibration vessel was prepared for dimethylmercury by placing a few drops of standard solution in an air tight vessel with septum lid. This was placed in ice and held at 0°C in a fume cupboard. The Antoine equation then used to calculate the partial pressure relationship between the elemental and DMM vapour at 0°C using constants derived by Long and Cattanach as described by Thompson [36].

Antoine equation: $\log_{10}$ pressure = A - (B / C + t°C) [Equation 1]

where A = 7.01688, B = 1342.2 and C = 232

At 0°C pp Hg(0) = 9 x 10^{-6} atm and at 0°C pp DMM = 0.9 atm. This indicated a 10^5 fold difference in volatility. The mass of mercury removed from the DMM calibration vessel was then calculated for each injection correcting Weast's data table for the volatility difference calculated.

6.2.4 Feasibility Study for the Application of Mercury Speciation using Large Volume Injection (LVI) and Thermal Desorption (TD)

A feasibility study was performed to establish the possibility of trapping volatile mercury compounds on a solid phase adsorbent followed by thermal desorption and into the GC-AFS. The alternative injection manifold was used in order to eliminate metallic surfaces from the system and hence to limit compound degradation. Injections of Hg(0) vapour were made into traps packed with silanised wool and silanised wool plus Tenax 60-80 mesh to investigate the repeatability and trapping qualities of the adsorbents. Further studies were undertaken with DMM vapour.

6.2.5 Evaluation and Optimisation of Solid Phase Adsorbents for use in LVI and TD

A range of commonly used solid phase adsorbents were considered for 1µl and 50µl large volume injections of a mixed MM and EM standard in DCM (112pg μl^{-1} MM and EM as Hg respectfully). Injections were made at 40°C with the carrier vented to waste. The carrier flow was then switched over the column followed by temperature programming of the injector to thermally desorb the compounds. Each adsorbent packed liner was subject to pre-conditioning at 350°C with injections of DCM solvent. The adsorbents studied are outlined in Table 6.1 and were all provided by the University of Eindhoven.

Table 6.1 Solid Phase Adsorbents

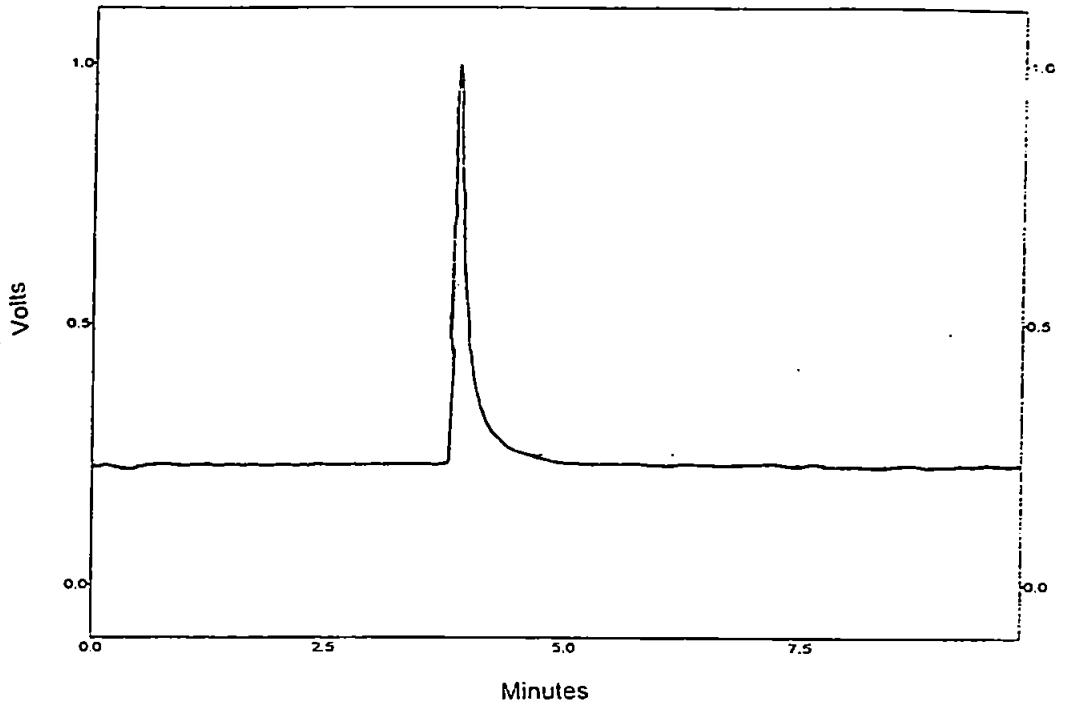
Adsorbent	Description
Carbograph	Graphitised carbon black
Supelcoport	Silica based Diatomite
Porapak Q	Divinylbenzene polymer
Silanised Glass Wool	Silanised Glass Wool
PTFE Wool	Shaved PTFE Rod
Silcoport 5%	Silica based with 5% Me groups

6.3 Results and Discussion

6.3.1 Optimisation of Splitless Injection Technique for Mercury Speciation Studies

Figure 6.2a shows the chromatogram obtained for a 1µl injection of 107pg μl^{-1} MM as Hg under the optimised direct injection conditions. Figure 6.2b shows the chromatogram obtained for the same standard in a splitless mode. The injection liner used here was a conventional glass liner packed with OV101 adsorbent, held in place with silanised glass wool (Phase Separations Ltd, Deeside, UK). The injection temperature and carrier flow remained the same and the splitless vent time was set at 2.0min. The significant peak at the front of Figure 6.2b was identified as elemental mercury by both its retention time compared to elemental Hg vapour and by confirming its presence with the pyrolyser set to ambient temperature. After confirming the presence of this Hg(0) peak with lower injection temperatures in this splitless mode, the most likely cause was identified as active sites on the injector walls. In the splitless arrangement, the carrier gas travels through the injection liner and then spreads throughout the chamber exiting through the split vent and/or column depending on the open pathway. Contact is made with the metallic injector surfaces in a way that is impossible with the direct injection mode. Organomercury compounds have previously been reported by Rubi *et al* to breakdown when in contact with aluminium connections within gas chromatography.

a)



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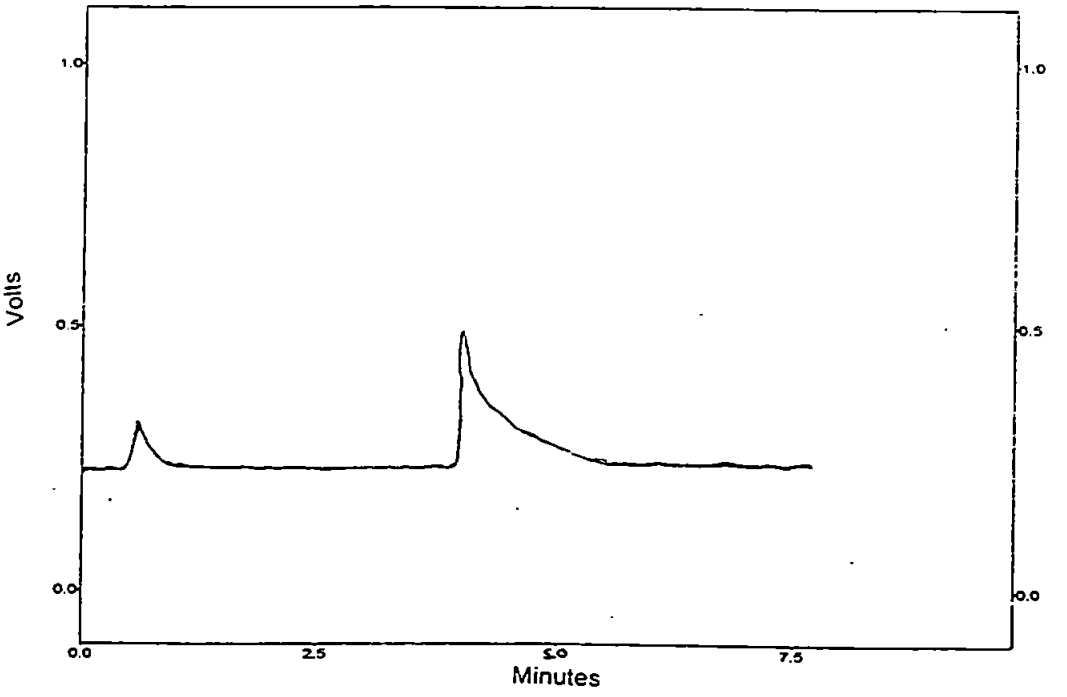


Figure 6.2 Chromatogram of 107pg MM: a) by Direct Injection; b) by Splitless Injection In order to attempt to eliminate the compound degradation problems

experienced with split and splitless modes of injection the injector surfaces were polished. This involved polishing the internal cavity walls of the injector using a rotary probe with cloths impregnated with metal polishing cream. This was repeated over a period of 2-3 hours, rinsing with methanol. At the end of this time, the injector surfaces appeared smooth under a jeweller's eyeglass.

Repeat injections of organomercury standards in dichloromethane showed no improvement. A second injector was fitted to the instrument and the experiments repeated. Once again organomercury compounds were found to degradade.

It was suspected that the adsorbent properties of the OV1 material used might not have been optimal for the compounds being considered. The packing material was removed and a small plug of silanised glass wool replaced in the injection liner. After a period of prolonged pre-conditioning it was found that MM standard in DCM could be injected in the splitless mode without breakdown.

As thermal desorption was the ultimate aim of this work a cold splitless injection technique was evaluated. The conditions used are described in Table 6.2 for repeat injections of a 107pg μl^{-1} standard of MM as Hg in DCM.

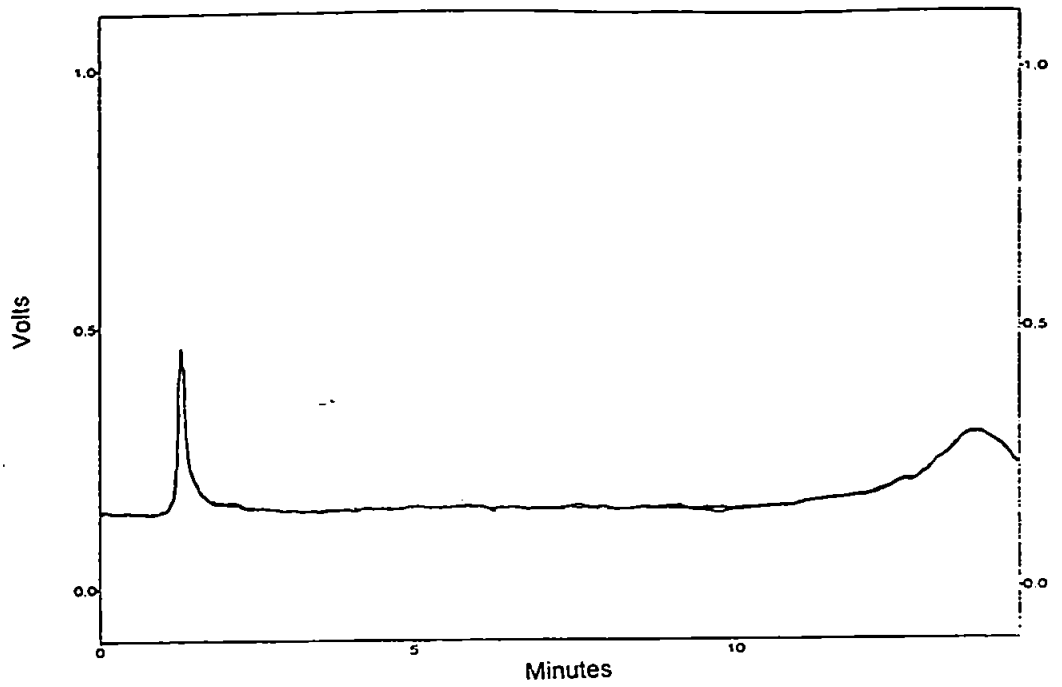
Table 6.2 Cold Splitless Injection Conditions

Injection conditions	1µl splitless, standard glass liner with silanised glass wool.
Injector temperature programme	35°C ramped to 300°C at 4°C min^{-1}, held at 300°C until end of oven programme.
Splitless vent time	1.5min.
Oven temperature programme	35°C held 1min, ramp to 115 at 25°C min^{-1}, held 5min, ramp to 200°C at 25°C min^{-1}, held 2min.

An initial blank run of 1µl DCM solvent gave a chromatogram with two peaks; the first corresponds with the retention time and characteristics of Hg (0) and the second with PhHg or possibly column bleed. This has been shown in Figure 6.3a. These peaks have previously been observed and it is believed that they result from the silanised glass wool in the injector. Subsequent blank analyses gave clean signals as shown in Figure 6.3b.

Repeat injections of the MM standard did not show any breakdown signals although the peaks appeared smaller than expected for the previously used direct injection technique. Subsequent injections gave increasing peak size pointing to conditioning of the system. After eight injections the system appeared to have stabilised. As all of the injections were made from the same injection vial, a set of six new standards was prepared from a common stock solution. This was in order to eliminate the possibility that increased peak size may be due to increasing concentration in the sample vial due to solvent evaporation through the pierced septum. Injections from the six individual sample vials gave similar peak sizes. Table 6.3 shows the repeatability and LOD data for replicate injections

a)



b)

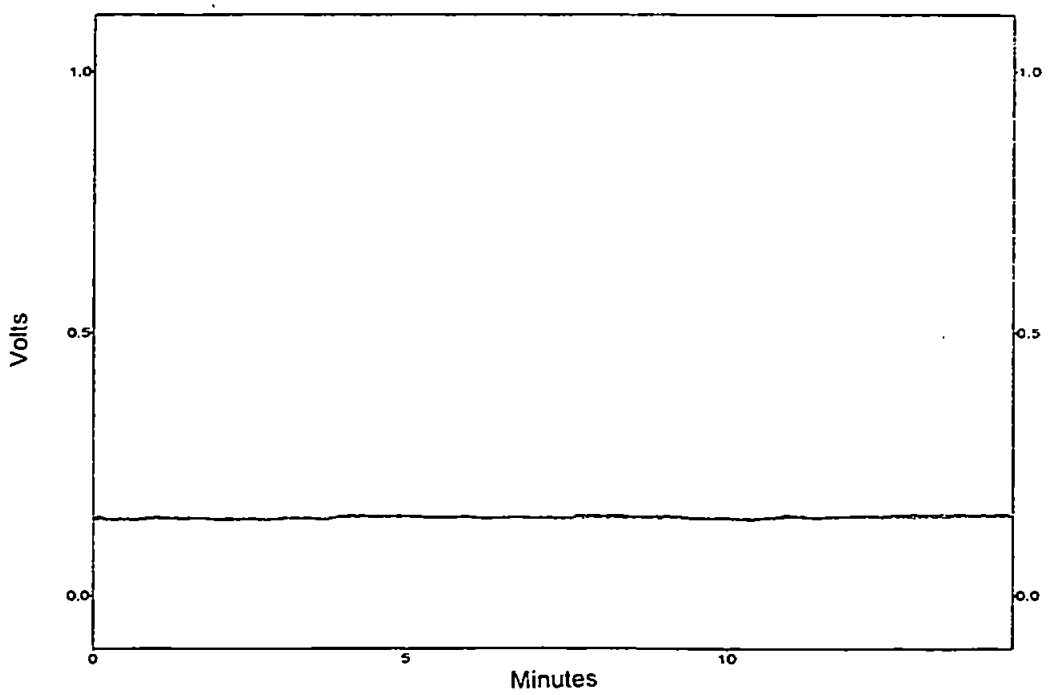


Figure 6.3 Effect of Activating Silanised Glass Wool on Blank Signals: a) Initial DCM blank; b) DCM blank after DCM Conditioning of Wool

Table 6.3 Figures of Merit for Cold Splitless Injections of MM

No. of samples	6
Mean peak area	5363665
Standard deviation	162547
Relative standard deviation	3.0%
Limit of detection as $3\sigma_{n-1}$ calculated against 107pg on range 100 x 10.	9.7pg

It was concluded that these results show similar repeatability and sensitivity to the direct injection techniques after compound conditioning.

Further tests were performed to investigate the effect of both hot splitless and hot split injection mode on the same methylmercury standard. In the case of hot splitless injection, where the carrier gas conditions remained unchanged whilst the injection temperature was held isothermally at 300°C, a small elemental mercury degradation peak was observed. It was concluded that this could be removed by optimising the injector temperature and gas flow conditions, reducing time spent by the compound in the injector. Finally hot split injections were performed by reducing the vent split time to 0.0min. This ensured that the carrier gas was constantly split between the column and waste. The result did not indicate elemental breakdown peak but a did result in a much smaller peak for MMC with some peak splitting.

6.3.2 Headspace Analyses

A linear calibration between 86-430pg Hg(0) was obtained with R^2 = 0.9946. Repeated 12pg injections gave a RSD = 7.9% (n=8) and LOD = 2.9pg. This was on an amplification range of 1000 x 3 (maximum 1000 x 10). Figure 6.4 shows these results. Repeated

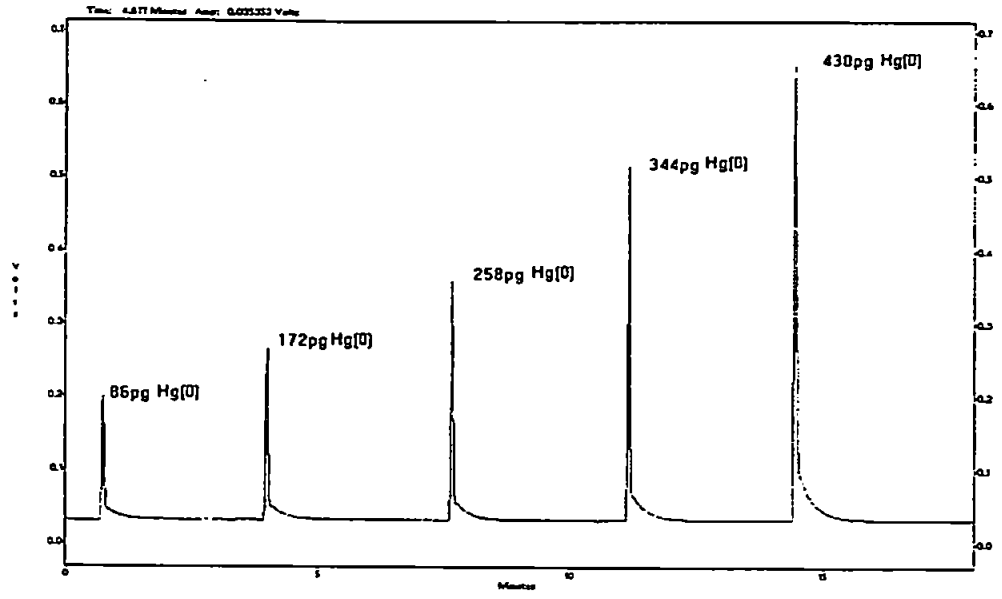
injections of 219ng DMM vapour gave an RSD = 2.4% and LOD = 15.6ng (n=6). This was performed on range 1 x 1. Due to the volatility differences between the compounds this was the smallest known mass of mercury which could be delivered to the system as dimethylmercury. It was therefore not possible to investigate the calibration of this compound. These results clearly showed that the GC-AFS instrument was suitable for the speciation of gas type samples.

6.3.3 Evaluation of Adsorbents for LVI and TD

Initial injections of Hg(0) vapour into the heated jacket arrangement with silanised glass wool packing were noted to be very variable for the same concentration of mercury injected. This was found to be as a result of mercury vapour evaporating from the syringe needle on injection depending on the temperature of the manifold at the point of injection. This was particularly a problem of the heating jacket, which did not present uniform heating throughout. As a result, an alternative controllable heating source provided by a standard horizontal pyrolysis unit was employed. This eliminated the injection repeatability problems by fixing the injection liner and port in one position relative to the heat source.

A series of 10µl Hg(0) vapour injections were made into the injection manifold and the detector alternately to test both the repeatability and transfer efficiency of mercury through the system. The results are shown in Table 6.4.

a)



b)

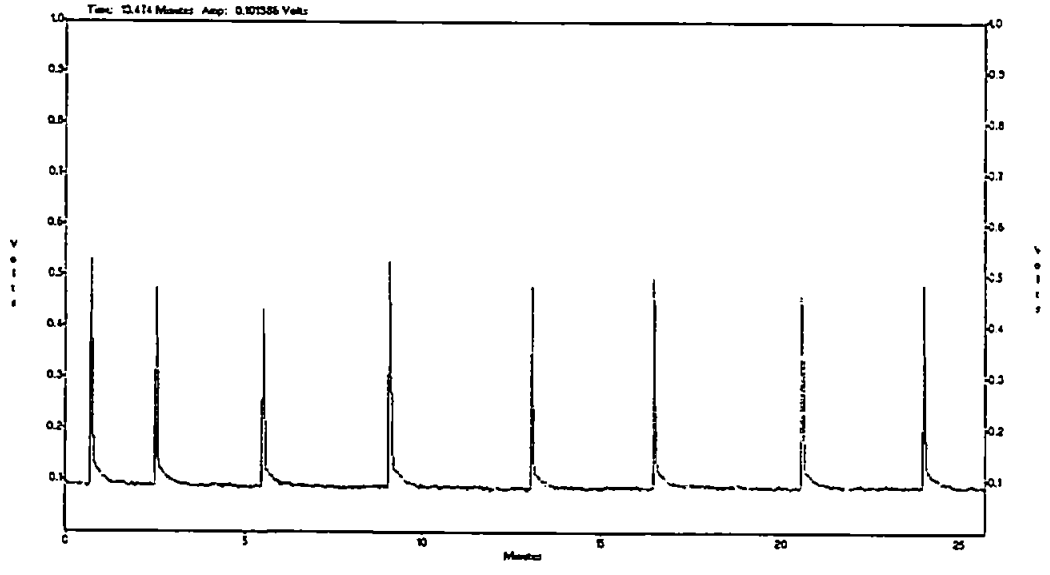


Figure 6.4 **Hg(0) Vapour Analyses: a) Calibration Series (86-430pg); b) Repeatability of 12pg Hg(0) Injections**

Table 6.4 Transfer Efficiency and Repeatability of Alternative Injection Manifold

Injection Point	n	Mean area	RSD (%)
Heated Liner	6	2263582 ± 263467	11.6
Detector	6	3287012 ± 353241	10.7

These results indicated that only 69% recovery of the peak signal was observed for injections through the whole system. Injections were made at 300°C and subsequent heating of the injection system to 350°C and 400°C respectively did not yield further mercury signals. This indicated that mercury was not being retained on the injector components. Although the source of the losses were not discovered, it was concluded that elemental mercury was not retained on silanised glass wool in this system at 300°C. This allowed further studies with Tenax 60-80 mesh adsorbent to be undertaken in the presence of silanised wool, with retention characteristics attributable to the adsorbent alone.

Figure 6.5 shows peaks observed for 10µl Hg(0) injections onto a Tenax 60-80 mesh filled liner in the heating arrangement described above. In this case the injector heater was held at an isothermal temperature of 350°C. These peaks are clearly not the same size as each other and illustrate the broadening characteristics of this adsorbent despite the high isothermal temperature. Similar injections were made with the injector held at 30°C for 5min followed by heating to 200°C in order to investigate the thermal desorption of Hg(0) from Tenax. Figure 6.6a shows the peak observed under these conditions whilst Figure 6.6b shows the difference when the sample was loaded at 40°C. Further injections were made over a range of loading and desorption temperatures. It was concluded from these results that Tenax 60-80 mesh traps Hg(0) and releases it in one broad peak on heating to

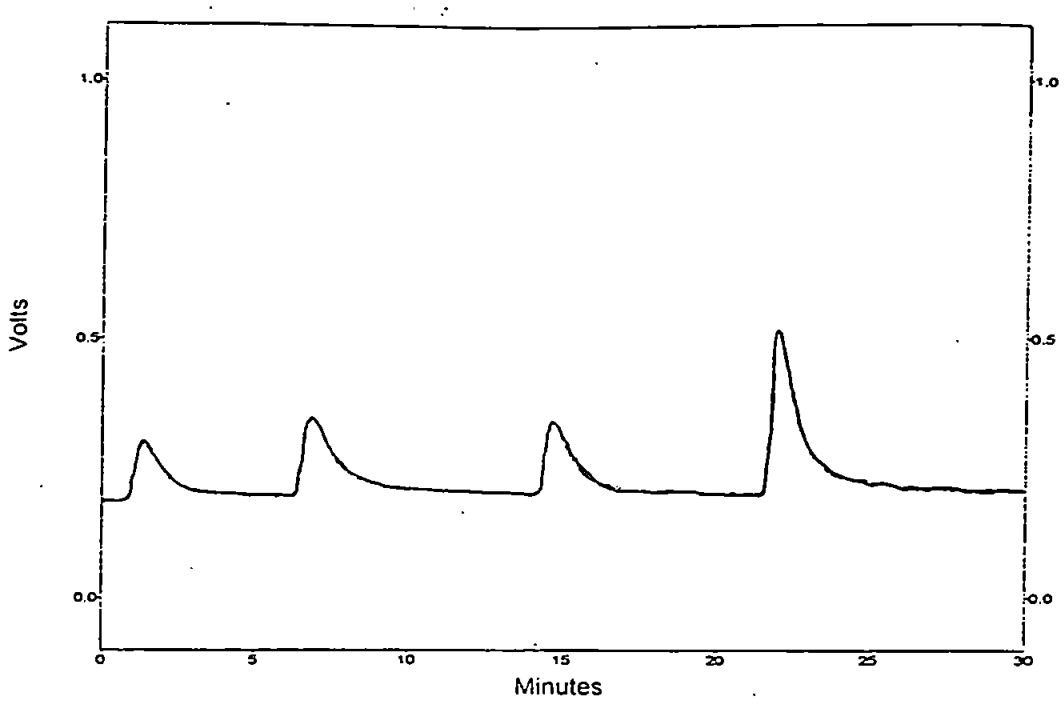
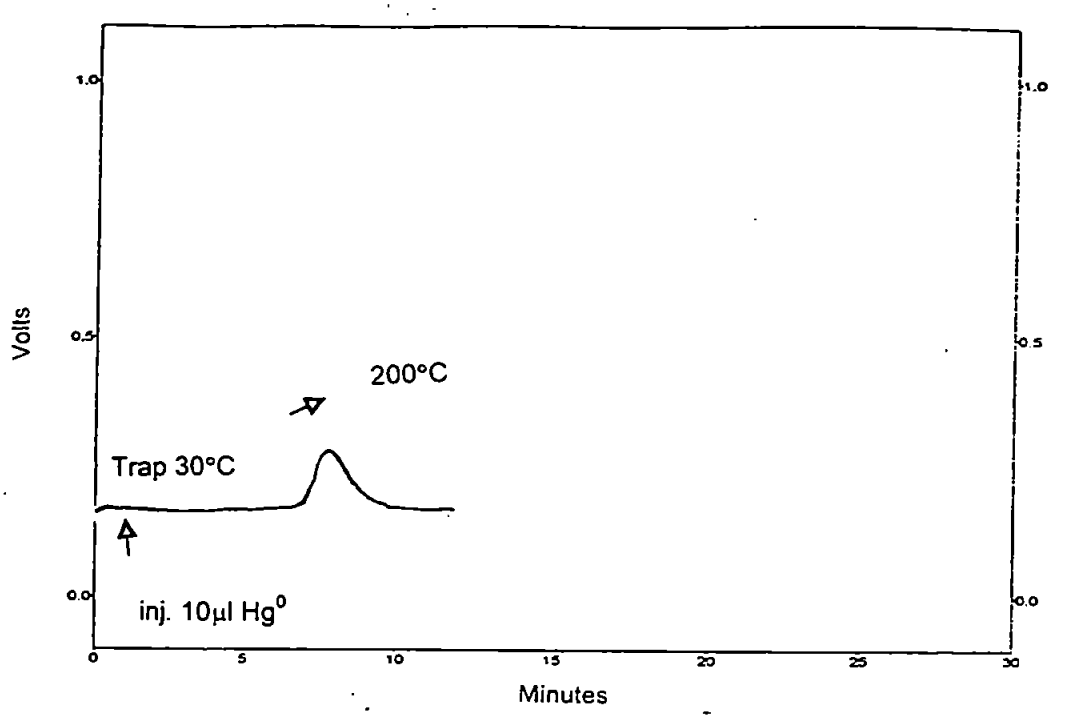


Figure 6.5 Chromatograms of 10µl Replicate Hg(0) Injections onto Tenax Trap

a)



b)

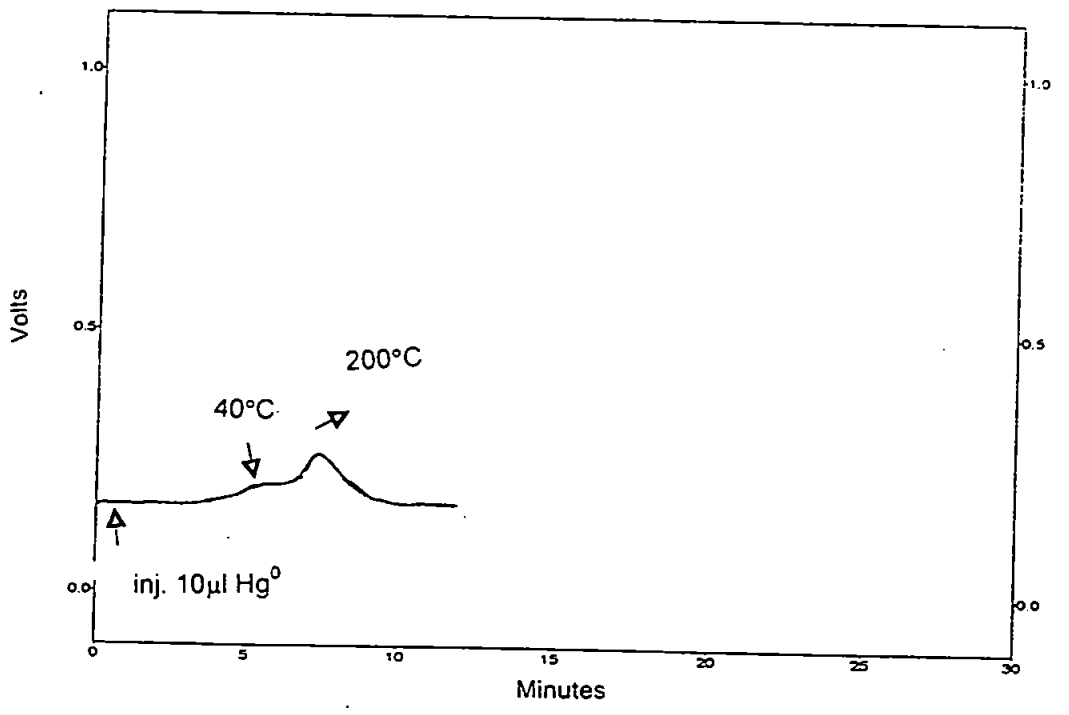


Figure 6.6 **Thermal Desorption of Hg(0) from Tenax Trap: a) Injection at 30°C; b) Injection at 40°C**

at least 200°C. Loading temperatures of <50°C were required to prevent breakthrough with room temperature giving the sharpest peaks.

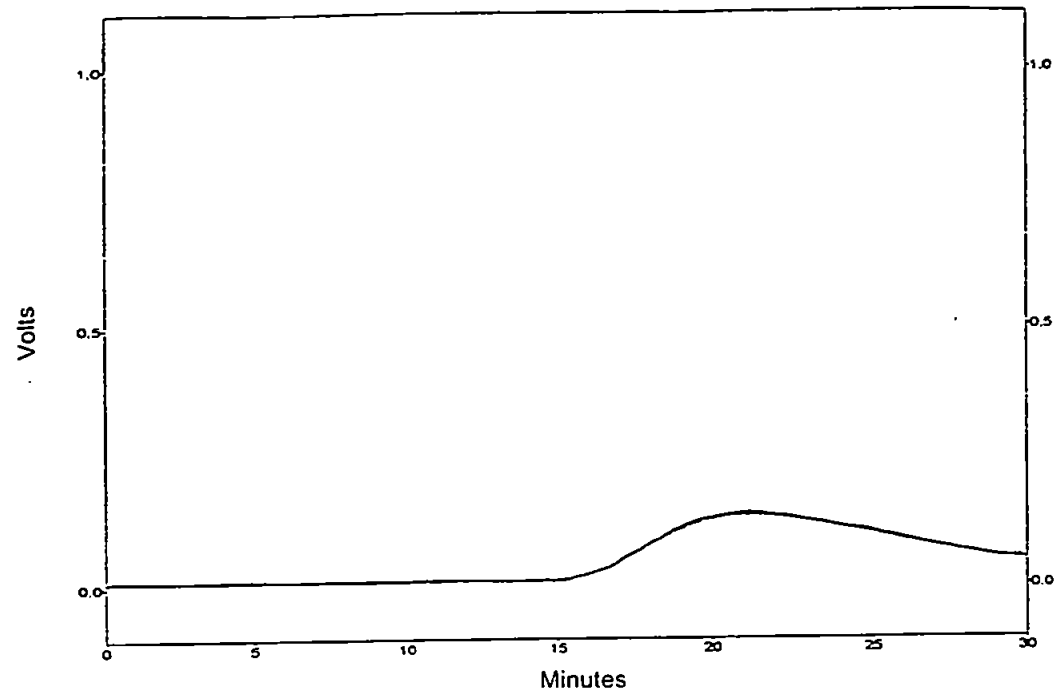
A 4ml injection vial was prepared with 2ml DCM and approximately 10µl DMM. This was sealed and set aside to equilibrate overnight. 10µl headspace injections were made onto the Tenax trap at 300°C with subsequent ramping to 400°C to desorb the compound. Figure 6.7a illustrates the profile observed under these conditions and Figure 6.7b shows the chromatogram obtained with injection onto a hot trap (400°C).

These results indicated that Tenax traps and allowed thermal desorption of DMM vapour without breakdown, at low injection temperatures. However the thermal desorption of this compound was noted to be very slow, probably due to the slow heating rate of the external injector employed. It was concluded that Thermal Desorption should be possible for organomercury compounds without compound degradation and that the PTV injector should once again be considered..

6.3.4 Evaluation and Optimisation of Solid Phase Adsorbents for use in LVI and TD

Figure 6.8a shows the mixed standard under direct injection *i.e.* with no adsorbent present and formed the benchmark for these investigations. The two peaks were identified as methyl and ethyl mercury respectively. A number of adsorbents were considered and the chromatograms obtained have been shown in Figures 6.8b-i respectfully.

a)



b)

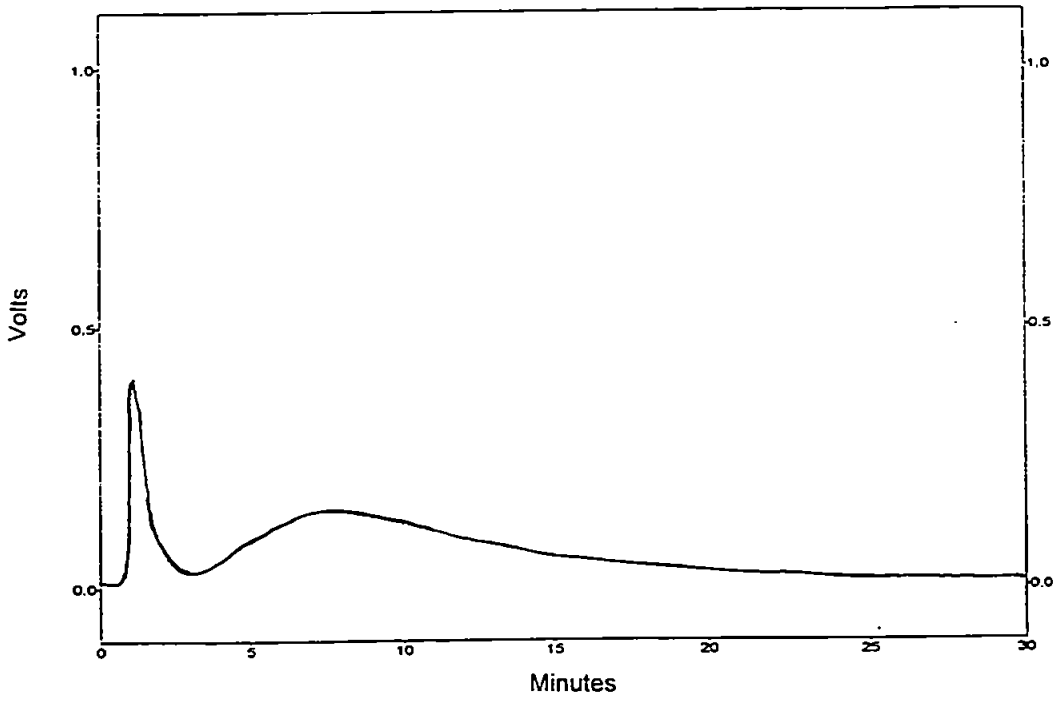


Figure 6.7 DMM on Tenax Trap: a) Thermal Desorption; b) Hot Injection

6.3.4.1 Carbograph

No peaks were observed for any MM or EM compounds in DCM injected. This is a carbon based compound that forms strong covalent bonds with mercury compounds. Carbon traps are often used as scrubbers for mercury wastes and its is therefore not surprising that the organomercury compounds were not released at 350°C.

6.3.4.2 Supelcoport

Two injections were made of the mixed standard, a 1µl injection and a 50µl large volume injection. The profiles obtained for both volumes were almost identical. The two largest peaks were of the analytes of interest, however two additional peaks were observed, elemental mercury breakdown and an unidentified peak at the end of the trace. As both of the main compounds of interest were almost resolved, this packing material was identified as promising.

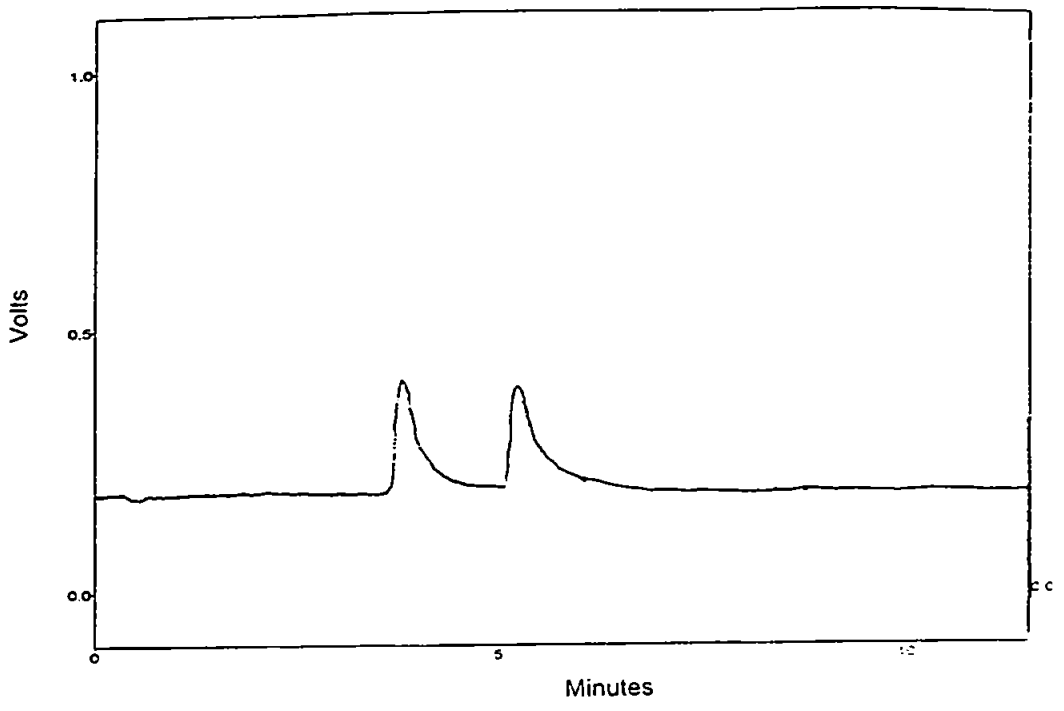
6.3.4.3 Porapak Q

This material was found to be unsuitable for these speciation studies as the two main compounds of interest were lost in the desorption process. Three distinct peaks were noted as increasing levels of mercury were released.

6.3.4.4 Silanised Glass Wool

The large volume injection of the mixed standard onto a column packed with glass wool gave rise to a distinct elemental mercury breakdown peak in addition to the two main compounds of interest. A second peak eluting after the elemental peak was also noted.

a)



b)

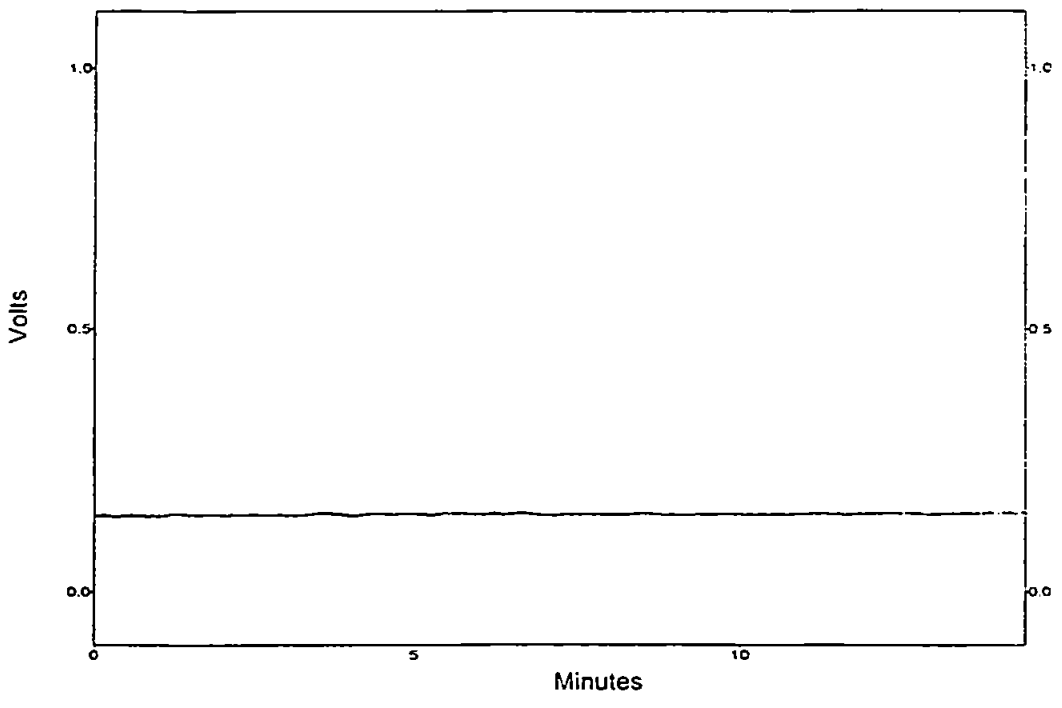


Figure 6.8 a) Direct Injection 1µl 112pg μl^{-1} mixed MM and EM standard; b) 1µl, Carbograph

The sharp profile of this species indicated a volatile compound. Injections of DMM and DEM were made in order to identify the compound however the retention times did not agree. The retention time of the unidentified peak was found to be 3.9 min whilst DMM was 2.9 min and DEM was 4.8min respectfully. It was suggested that the unidentified peak may be a volatile recombination of a methylethylmercury as it eluted exactly halfway between dimethylmercury and diethylmercury. It was not possible to obtain or to prepare this compound to confirm this hypothesis.

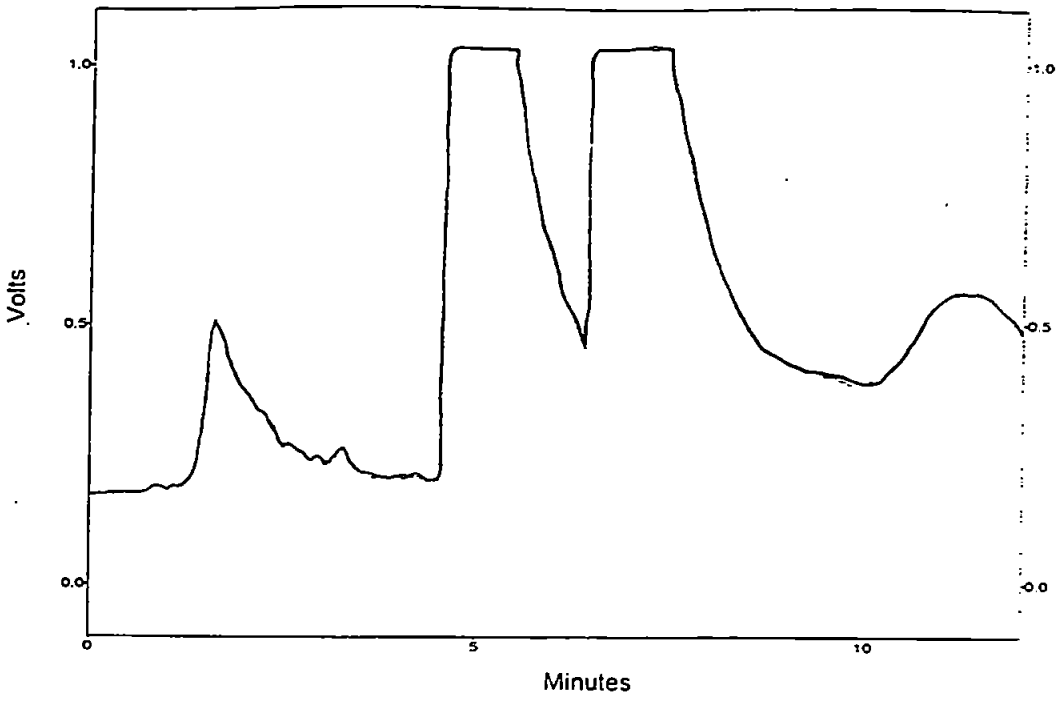
6.3.4.5 PTFE Wool

Once again the PTFE wool gave rise to elemental mercury breakdown, reduced resolution between the species of interest and a very large unidentified fourth mercury peak. This final peak was thought to be due to the release of mercury compounds made thermally labile with the final increase in oven temperature to 400°C. This suggests that a certain portion of the mercury compounds were not transferred under the conditions employed. The reduced peak height of the MM and EM compounds supported this explanation.

6.3.4.6 Silcoport 5%

A series of peaks were observed following the LVI of MM and EM onto this material. The first broad peak was identified as elemental mercury breakdown. Three small sharp peaks were then observed with retention times corresponding to DMM and DEM along with the suspected MEM in between. The two main compounds of interest did not form the main peaks and were poorly resolved. The final unidentifiable peak with a retention time of 13 min was again thought to be due to the release of additional compounds above 400°C.

c)



d)

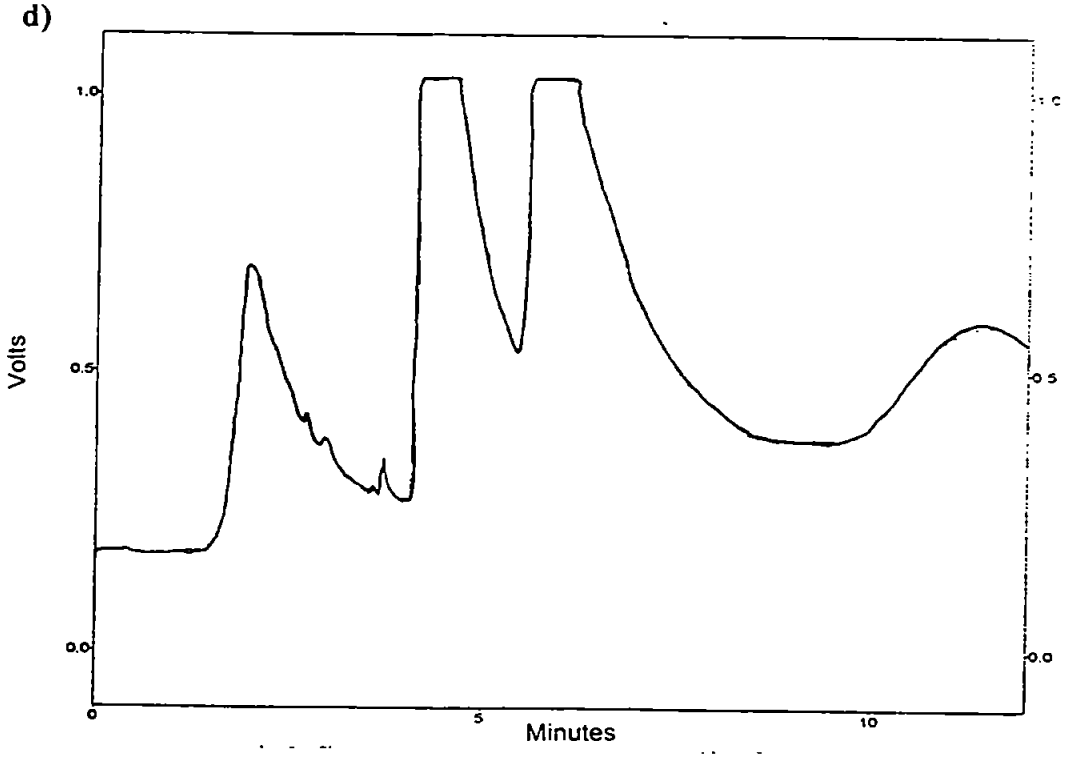
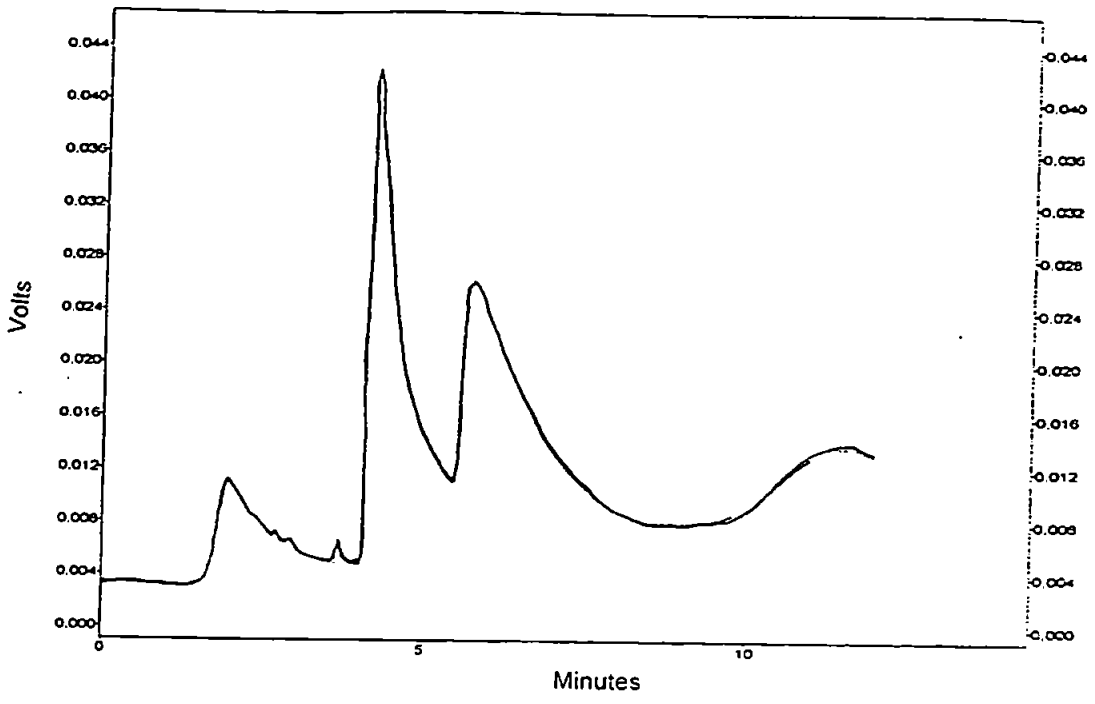


Figure 6.8 c) 1µl, Supelcoport, d) 50µl LVI, Supelcoport

e)



f)

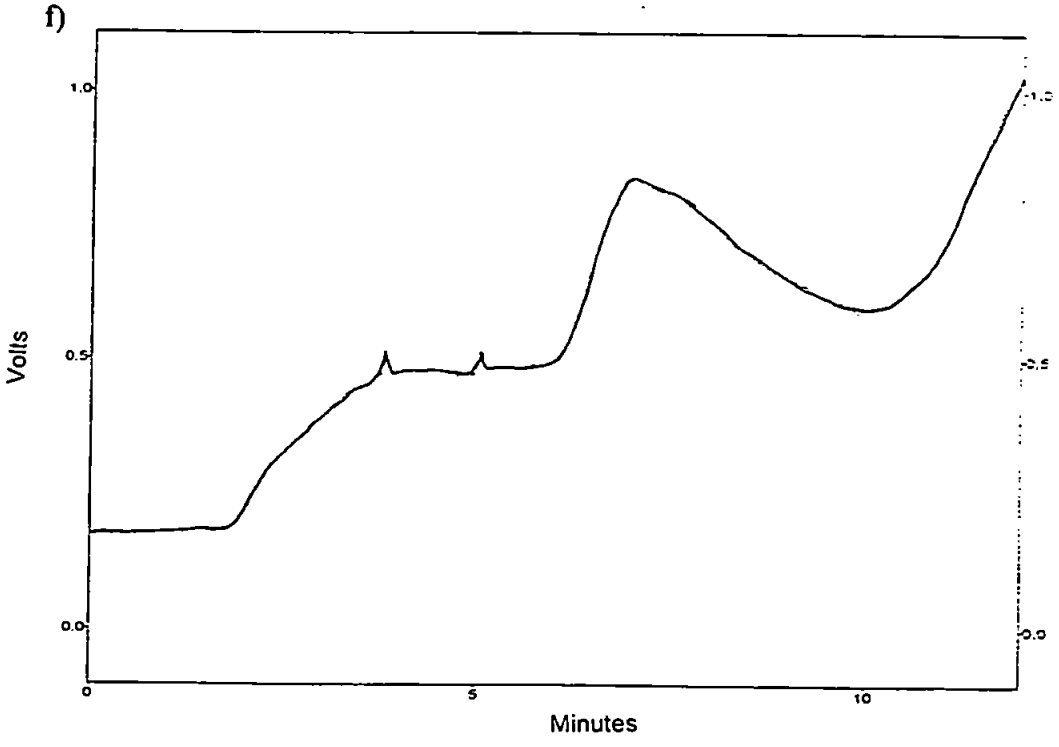
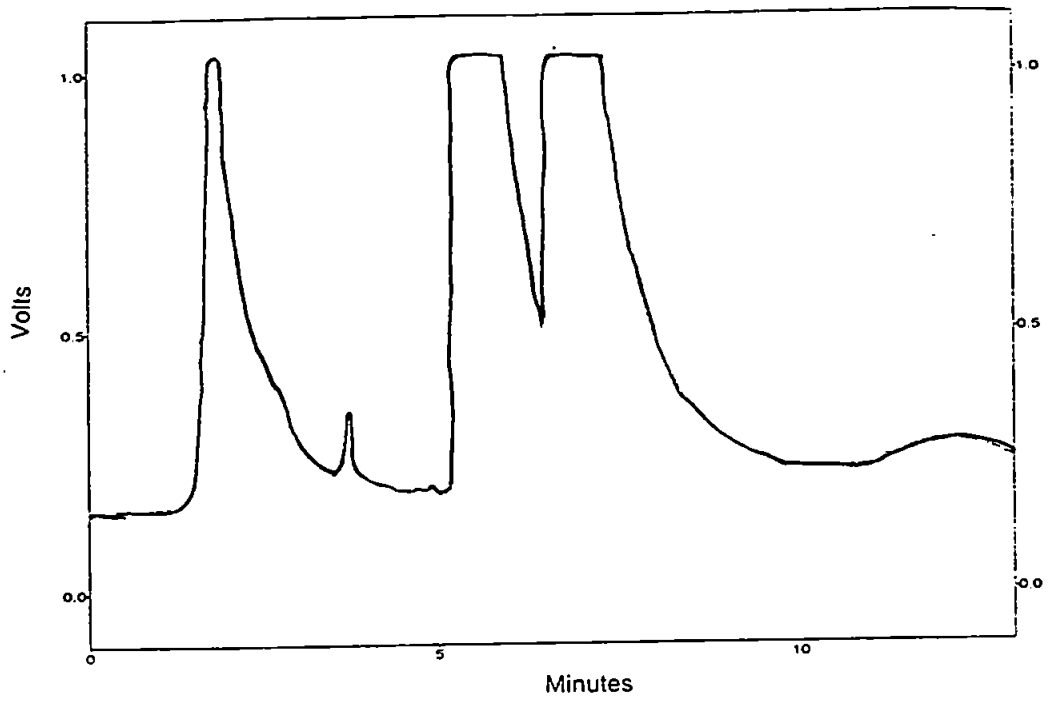


Figure 6.8 e) 1µl, Supelcoport, Re-scaled, f) 50µl LVI, Poropak Q

g)



h)

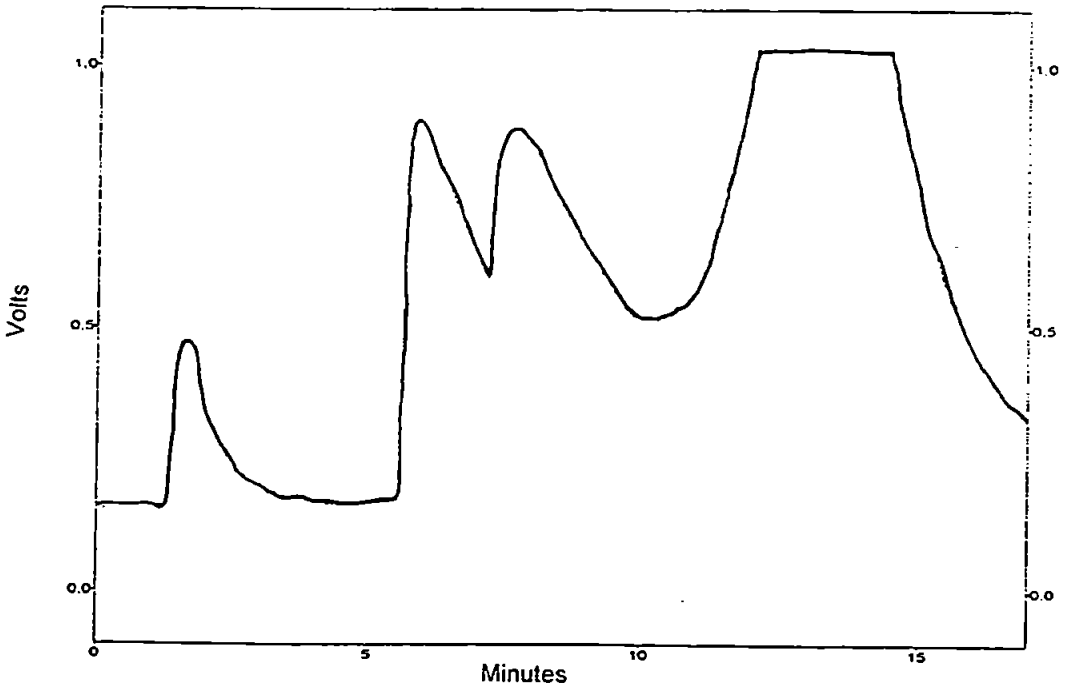


Figure 6.8 g) 50µl LVI, Silanised Glass Wool, h) 50µl LVI, PTFE "Wool"

i)

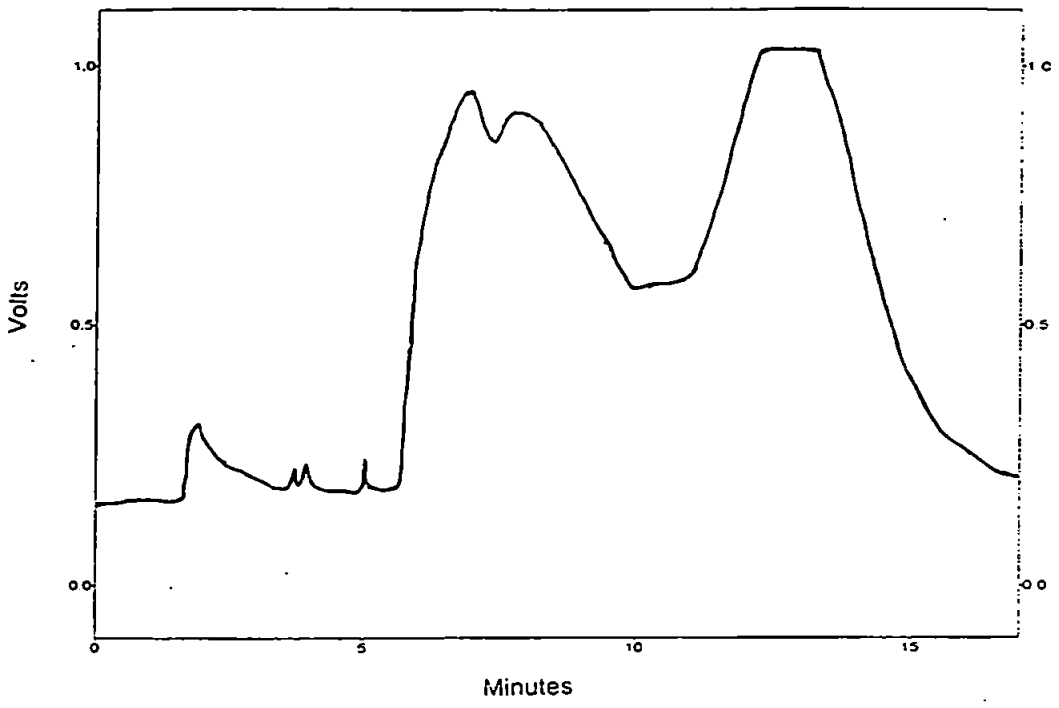


Figure 6.8 i) 50µl LVI, Silcoport 5% Me

These studies were of limited success, confirming breakdown and rearrangement reactions of organomercury compounds for most of the adsorbents considered. It was possible to confirm that carbon based adsorbents were definitely not suitable for these type of compounds due to the strength of the complex formed. The most promising materials appeared to be silanised glass wool and Supelcoport, due to the separation and size of the MM and EM compounds compared to the breakdown peaks. Supelcoport was chosen for the following studies as it presented a higher surface area than the wool and would therefore be most appropriate for LVI applications

6.3.5 Optimisation of LVI and TD using Supelcoport

Following this study an injection liner with a glass frit in the bottom was prepared with Supelcoport and an upper plug of silanised glass wool. Following some remedial actions to restore the performance of the column for a mixed standard of MM and EM in DCM the liner was conditioned during which period the column was disconnected to prevent poisoning. A comparison was made between a 1µl injection of an 86pg μl^{-1} MM and 66pg μl^{-1} EM mixed standard in DCM and a 50µl injection of a 1.7pg μl^{-1} MM and 1.3pg μl^{-1} EM mixed standard in DCM. The 1µl injection was made with an isothermal injector at 300°C whilst the large volume injection was made at 40°C with thermal desorption by heating the injector to the column temperature. The results of both injections are shown in the overlaid chromatograms in Figure 6.9. A small amount of compound degradation was observed for the 1µl hot splitless injection that was not observed for the LVI. The recovery of the compounds was only 51% MM and 46% EM respectively compared to the areas expected. However no compound degradation was observed at this level for the LVI. It was noted however that a small amount of MM peak splitting was apparent for both injections.

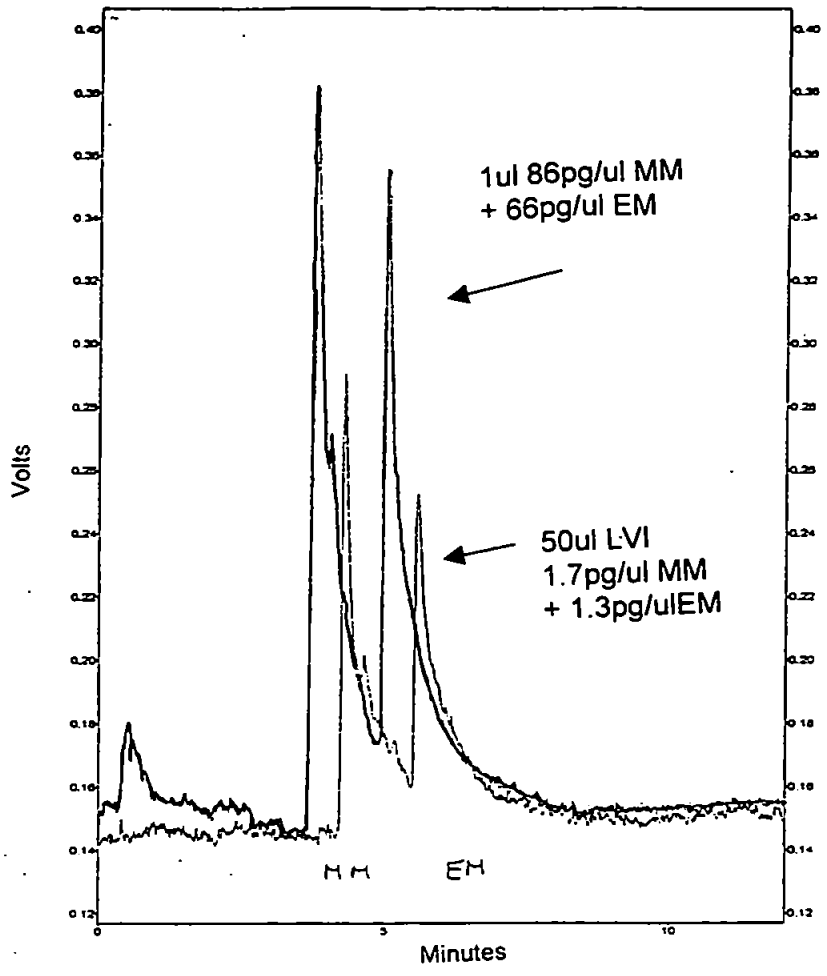


Figure 6.9 Comparison of 1µl Injection Mixed Organomercury Standard with 50µl LVI of 50x Diluted Standard

These results confirmed that LVI and TD was indeed possible for MM and EM standards in DCM without compound degradation. A series of optimisation experiments were then performed to improve the recovery of the compounds studied. The parameters studied were the injector heating rate, the delay before diverting the carrier gas to the column and the helium split gas flow rate. The results are shown in Figures 6.10 a-c.

The fastest heating rate of the PTV unit was found to lead to minimal elemental mercury formation, the delay time to before directing the carrier gas to the column was found to have little effect whilst the most appropriate He flow rate was identified as 100ml min^{-1}.

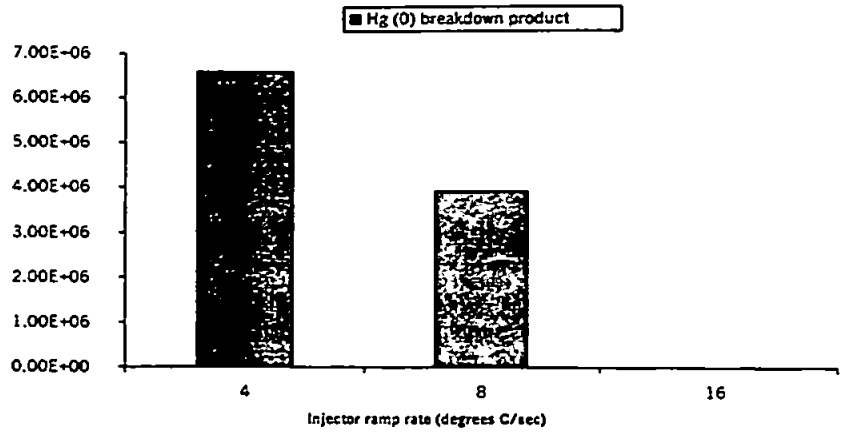
Under these conditions the repeatability and recovery of the two compounds was determined. The results have been shown in Table 6.5

Table 6.5 Figures of Merit of Optimised LVI Technique

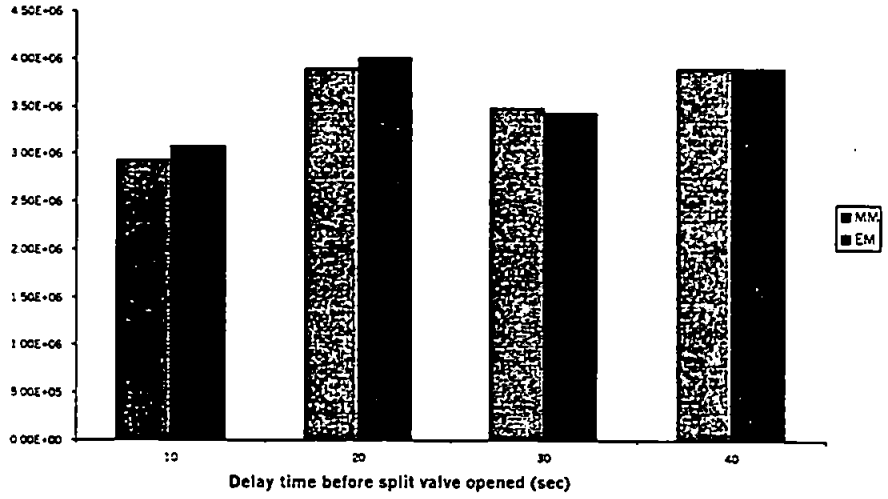
Compound	RSD (n=4) %	Recovery %
50µl MMC	9.3	51
50µl EMC	13.9	75

In conclusion successful large volume injections of MM and EM mixed standards were achieved with no compound degradation observed. However only partial recovery (50-75%) compared to expected peak areas were found. Further work is required to establish the linearity of this approach and therefore to establish its usefulness. LVI will only work for organomercury speciation at very low levels as compound degradation and rearrangements will otherwise become apparent. As a result of these studies, no further work on the air and gas application was undertaken.

a)



b)



c)

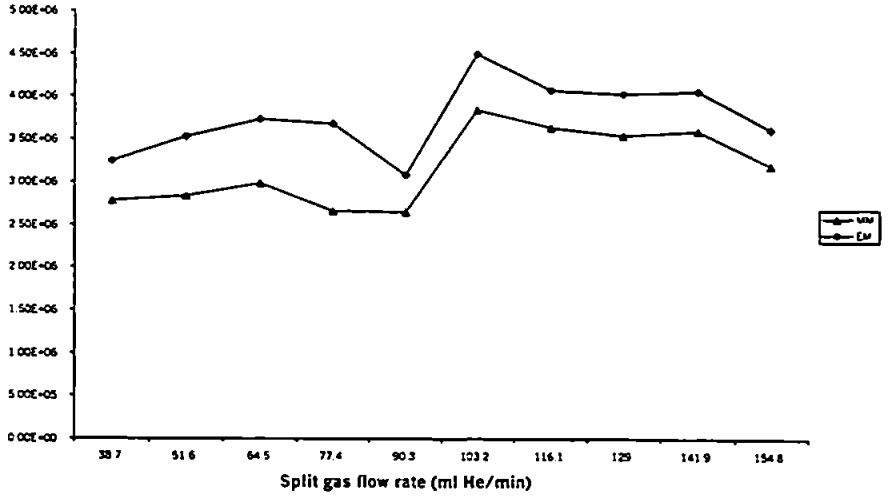


Figure 6.10 Optimisation studies for LVI; a) Injector Heating Rate; b) Delay Time Before Split; c) He Split Flow Rate

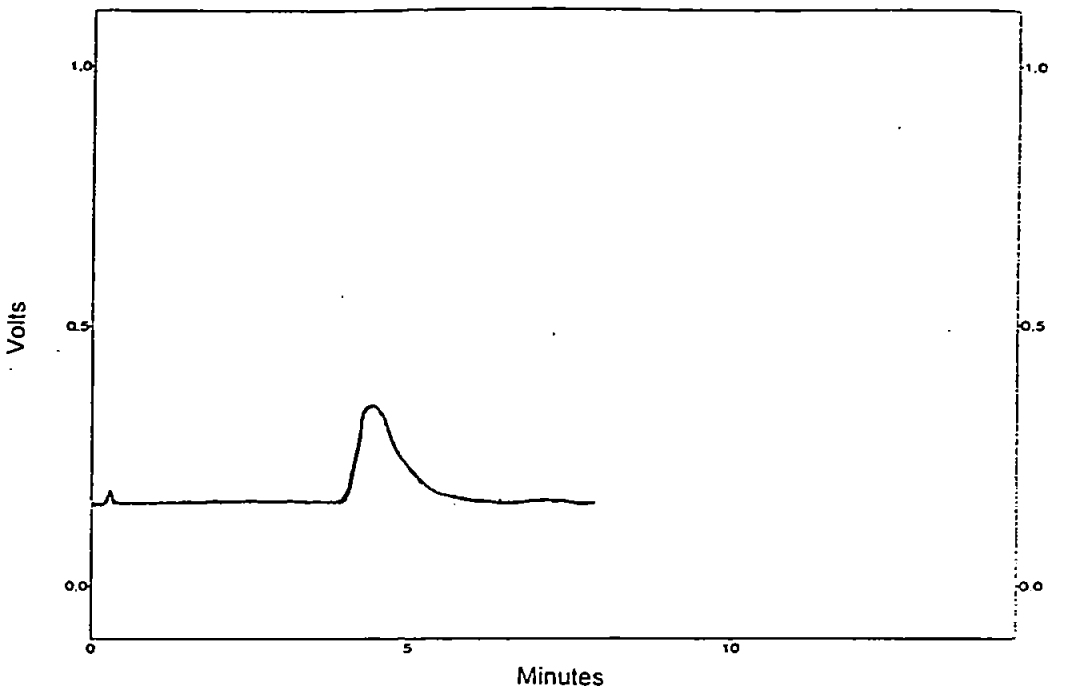
6.3.6 Effect of Thermal Desorption on Column

All of these TD studies severely impaired the performance of the GC column once reconnected for direct injections of organomercury standards in solvent. Figure 6.11a shows an example of a direct injection of 107pg MM. Remedial action such as column trimming or conditioning improved but was not found to restore the chromatography to that previously observed. Figure 6.11b shows the chromatogram obtained for a mixed standard of MM and EM compounds after column trimming. The peak shapes were found to be shorter and broader than those observed for the same conditions in earlier chapters. Despite this, the instrument could still be used in this way as calibrations remained linear. .

6.4 Conclusions

These studies allowed the investigation of injection techniques for the improvement of GC-AFS sensitivity and its application to air and gas speciation. Considerable time was spent in an attempt to find conditions suitable for LVI and TD that would not lead to compound degradation. Conditions for cold splitless injection techniques which allowed the preconcentration of organomercury compounds on packed column liners, followed by successful thermal desorption were identified. However complete compound recovery was not achieved despite optimisation studies. As a result of the thermal desorption work column deterioration was observed throughout. Despite remedial action columns could not be regenerated to previous levels.

a)



b)

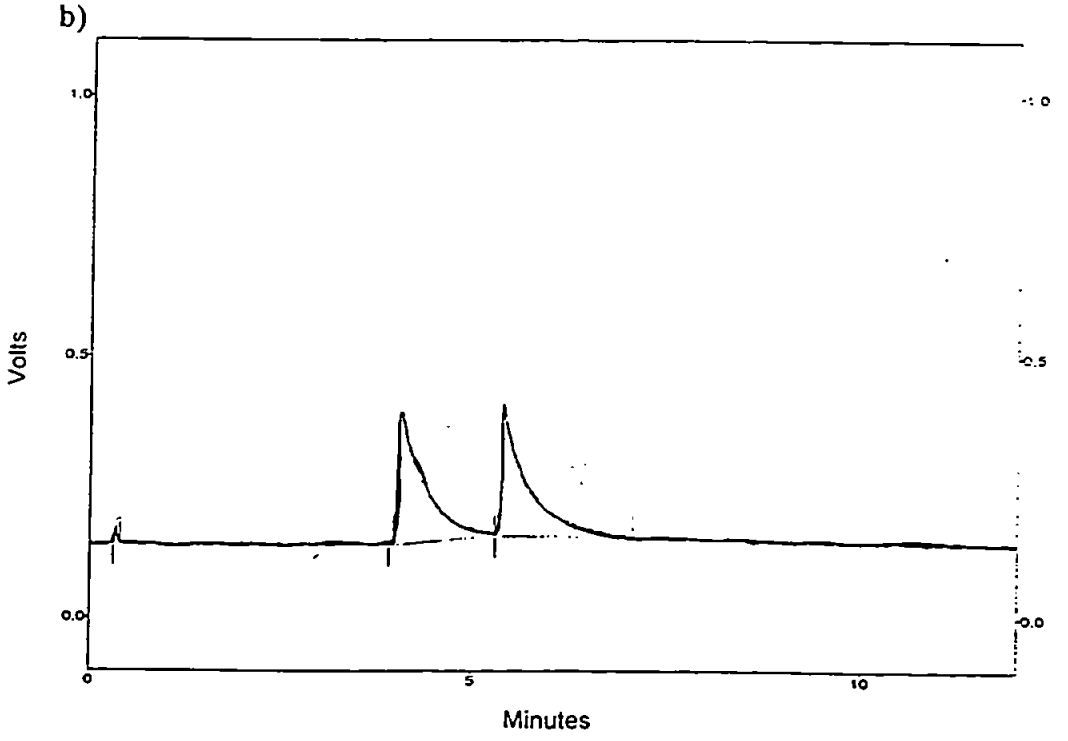


Figure 6.11 Effect of TD/LVI on Column Performance: a) After TD/LVI Use; b) After Remedial Action

In conclusion it may be possible to use LVI for organomercury studies for extracted standards in DCM as repeatable results were obtainable. However the general problems encountered in this work meant that TD applications to air and gas sampling were not possible and would require a more complex means of sampling to ensure i) conditioned adsorbent and ii) removal of water from the sample prior to analysis.

Chapter 7

CONCLUSIONS AND FUTURE WORK

7.1 Conclusion

Prior to this project the technique of gas chromatography coupled to atomic fluorescence spectrometry had been described for the speciation of methylmercury by both Bloom [96-102, 128, 176, 201, 204] and Jones [122-126, 142]. Bloom's work included sample ethylation, preconcentration and cyrogenic trapping followed by thermal desorption. However this was both cumbersome and prone to artifact formation. Jones had reported a GC-AFS instrument with splitless injection of dichloromethane extracts of methylmercury, with application to the analysis of fish and water samples from the Florida Everglades. However some problems had been reported including compound degradation and the reduction of detector sensitivity over time. The initial aim of this project was to develop a commercial instrument for routine analysis of organomercury compounds; overcoming column contamination and breakdown problems. This instrument differed from those previously described in that it involved a new injector system – a Programmable Temperature Vaporiser Injector, capable of direct, on-column, split and splitless modes along with the capability of large volume injection and thermal desorption.

The first main achievement of this project was the development of a robust and reliable instrument with modified injection mode, pyrolyser and detector. The use of a direct injection technique for 1μl volumes of solvent was found to overcome compound degradation on the

injector surfaces, when optimised for column flow and isothermal injection temperature. The use of a ceramic pyrolysis heater overcame the problems previously described for quartz pyrolysis tubes, namely fragility and uneven thermal gradient leading to either ashing or incomplete pyrolysis of the compounds of interest. The modification of the detector to introduce an argon purge removed the sensitivity problems due to the effect of the helium carrier gas on the mercury vapour discharge lamp.

The instrumental operating conditions were optimised and the detection limit determined to be 0.25 pg Hg based upon $3\sigma_{n-1}$ of ten replicate injections of a 0.6pg μl^{-1} standard of Methylmercury chloride (as mass Hg). The instrument was found to give equal response to each compound studied and was linear over the complete ranges studied, up to 2ng Hg absolute. A degree of peak splitting was observed at higher concentrations but this did not effect the linearity of the technique. However high concentrations were found to effect the long-term performance and sensitivity of the chromatographic column.

The optimised instrument was tested using two certified mussel homogenate materials (IAEA 142 and SRM 8044) following an extraction procedure based on that described by Jones. Good agreement was observed between the results obtained and the certified values. An interlaboratory comparision exercise was then undertaken to determine the level of methylmercury in *Fucus* sea plant (IAEA 140). A method was developed to extract the methylmercury from this sample and a result of 0.63 ± 0.006 ng g^{-1} was submitted. This material has since been certified at 0.626 ± 0.139 ng g^{-1}. Further method development was undertaken in an attempt to understand the effect of different steps on the procedure in order to simplify and reduce the work-up involved in the preparation of each sample. A range of marine liver samples were also considered representing higher methylmercury concentrations.

The method was validated using DOLT-2 and fresh beluga whale and ringed seal livers were analysed. These samples showed that this approach was suitable over a wide range of methylmercury contents, from 0.63 to 3000ng g^{-1} Hg. Additional determinations of the total mercury content of each sample was also made using CV-AFS. Good agreement was found for each certified reference material.

The next main application area studied was the determination of methylmercury in soils and sediments. The method developed for the extraction of methylmercury for the *Fucus* sea plant sample was applied here. The IAEA 356 sediment reference material was analysed for both total and methylmercury. This material had recently come under scrutiny due to artifact formation reported when using a steam distillation procedure. Good agreement was found and no artifact formation was observed. The method was also applied to a range of other samples, an uncontaminated Portuguese sediment, a potting compost (uncontaminated soil) and a contaminated land sample LGC6138. Finally a closed microwave extraction procedure was studied to replace the initial extraction step by shaking. This method improved the ease of handling and time taken by this first step and seemed very promising. However the method was prone to background contamination from microwave vessels used to digest higher concentration samples, and in this case was limited by the number of vessels available (*ie* 6). It was not possible within the time frame of this work to pursue this method.

Two comparative techniques were studied, GC-ICP-MS and HPLC-CV-AFS. A gas chromatograph was coupled to the ICP-MS and optimised. IAEA 142 mussel homogenate was analysed and despite low spike recoveries gave good agreement with the certified value. The added advantage of this approach was the multi-element capacity of the detector, which allowed the confirmation of the presence of methylmercury bromide in the final extract. This

study confirmed the suitability of the GC-ICP-MS for organomercury speciation but did not offer a practical solution. The optimisation of the system was crucial and would be required to be repeated each time the instrument was set-up. As this instrument is a very expensive technique, it would not be possible to dedicate it to GC-ICP-MS studies. The HPLC-CV-AFS approach, on the other hand represented the lowest cost option with less sample preparation as the extract was ready for analysis one step earlier. Every parameter of this technique was extensively studied to give the maximum sensitivity possible. DOLT-2 was extracted and analysed using this method and good agreement was observed with the certified value. However it was found that this technique was only suitable for extracts containing > 100ng ml^{-1} Hg and was also prone to vapour generation interferences.

In the final part of this project the PTV injector was considered in detail with a view to further increasing sensitivity through large volume injections and thermal desorption. Initially the split and splitless injection modes of the injector were optimised to overcome compound degradation problems. Conditions and were found to allow cold splitless injections of solvent extracts onto a supelcoport liner packing material followed by thermal desorption into the column. These did not lead to mercury breakdown. However despite lengthy optimisation, complete compound recovery was not achieved. Furthermore the effect of the thermal desorption studies on the chromatographic column was severe. The sensitivity of the column was impaired and methyl and ethylmercury compounds could not be fully resolved due to peak tailing. Despite remedial action, the column could not be regenerated.

7.2 Future Work

There are three main areas of future work using the GC-AFS instrument that I feel could be considered further. These are improved sample preparation techniques including the use of a closed microwave for accelerated extraction, the application of the PTV injector for improved method sensitivity through large volume injections and the application of the technique to water based and gaseous samples.

The initial studies here showed the closed microwave as being a very promising technique for accelerated extraction. There are several reports of the use of open microwaves for this purpose but closed microwaves have not been reported so far. The closed microwave is a much more accessible tool for most laboratories and so I feel that this approach may be more useful in the long-term. In addition this technique has the potential of vastly reducing sample preparation time from days to hours and would therefore be a substantial improvement.

The PTV studies reached a stage where LVI could be used with cold splitless injections to increase method sensitivity. However complete recovery was not achieved. In future work it would be important to establish if despite this loss, if the technique is in fact linear. If so, then the conditions established in this project would form the basis of future developments. After the LVI/TD experiments studied here the column was severely effected. Many different packing materials and conditions were considered over this period and it is possible that the final optimised material and conditions did not lead to these problems. It would important useful to establish if this is the case. The GC-AFS could then be applied to gas and air samples, although this will also present a number of new questions *e.g.* how to sample, pump

or not, sample flow rate, trapping material, drying, desorption, reversing sample liner, sample blanks and calibration procedure.

One further area which was broached within this project but is not reported here was the application of the instrument to the analysis of water based samples, methods studied involved the use of sulphydryl cotton for preconcentration. This material is not commercially available, takes one week to produce and varies in efficacy. Further work may involve searching for alternatives. A further application of this technique could be the determination of organomercury in urine, which may also be relevant to the clinical studies of biochemical methylation and demethylation of mercury.

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Appendix

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ANALYTICA CHIMICA ACTA

Comparison of AFS and ICP-MS detection coupled with gas chromatography for the determination of methylmercury in marine samples

H.E. Louise Armstrong[a], Warren T. Corns[a], Peter B. Stockwell[a], Gavin O'Connor[b], Les Ebdon[b], E. Hywel Evans[b,*]

[a]*PS Analytical Ltd, Arthur House, Crayfields Industrial Estate, Main Road, Orpington, Kent BR5 3HP, UK*
[b]*Department of Environmental Sciences, University of Plymouth, Drake Circus, Plymouth, Devon PL4 8AA, UK*

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Abstract

Inductively coupled plasma mass spectrometry (ICP-MS) and atomic fluorescence spectrometry (AFS) coupled with gas chromatography (GC) have been evaluated as element specific detectors for the determination of methylmercury in marine samples. Detection limits for methylmercury chloride, obtained using ICP-MS and AFS, were 0.9 and 0.25 pg as Hg, respectively. Methylmercury was determined in marine tissue reference materials IAEA 142 and NIST 8044 mussel homogenate, and DOLT-2 dogfish liver by GC–AFS, with found values of 45±7, 26±4, and 671±41 ng g^{-1}, compared with certified values of 47±4, 28±2, and 693±53 ng g^{-1}. The analyses of IAEA 142 and NIST 8044 were repeated using GC–ICP-MS, with found values of 48±9 and 30±3 ng g^{-1}, respectively. Methylmercury was determined in real samples of ringed seal and beluga whale, with found values of 801±62 and 2830±113 ng g^{-1}, respectively.

Keywords: Gas chromatography; Atomic fluorescence spectrometry; Inductively coupled plasma mass spectrometry; Organo-mercury; Marine tissue

1. Introduction

Since the major poisoning incident in Minimata Bay methylmercury has been identified as an extremely toxic pollutant that can accumulate in fish [1], reaching levels toxic to humans [2], primarily due to the lipid solubility of the compound [3]. A number of methods have been described for the identification of methylmercury by separately determining total and organic mercury by selective digestion, and cold-vapour AAS or AFS [4–6], with the presumption that the organic fraction is comprised solely of methylmercury. Methods which selectively extract and identify different organo-mercury compounds are now widely used, often based on the methods developed by Westöö [7,8]. Using these methods, the solvent extracts have traditionally been analysed by gas chromatography with electron capture detection [9,10]. In order to overcome chromatographic problems such as peak tailing, Rapsomanikis and Craig [11] have suggested the use of ethylation to form more volatile

*Corresponding author. Tel.: +44-1752-23-3000; fax: +44-1752-23-3035; e-mail: hevans@plymouth.ac.uk

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compounds which are very easily chromatographed, and Rubi et al. [12] have found that increasing stationary phase thickness minimises. The ethylation approach suffers two major drawbacks. First, high concentrations of inorganic mercury present in the sodium tetraethylborate ethylating agent have been found to lead to positive artifact formation [13]. Second, ethylmercury present in the sample is masked. Ethylmercury has not generally been found in marine animal samples [14], but there are a number of reports of ethylmercury in soil, sediment, both polluted and natural, and plants [15–17], so it would be preferable to avoid ethylation where possible. One alternative is to butylate the sample [16] but this again will mask any butylmercury which may be determined in the future. The most sensible approach therefore would be to eliminate the derivatisation step completely.

Atomic emission techniques have previously been coupled to both gas and liquid chromatography systems to give a range of techniques for organo-metallic speciation with the required sensitivity for speciation studies. Inductively coupled plasma mass spectrometry (ICP-MS), coupled with gas chromatography, has been described for the speciation of mercury, tin and lead compounds [18–21], and with HPLC for mercury and arsenic compounds [22]. Similarly, atomic fluorescence spectrometry (AFS) affords a high degree of element specificity and is relatively free from interferences. This paper compares the performance of a custom-made AFS detector with ICP-MS, for the determination of methylmercury in marine tissue samples.

2. Experimental

2.1. Instrumentation

2.1.1. GC–ICP-MS

An inductively coupled plasma mass spectrometer (PQ2+ VG Elemental, Winsford, Cheshire, UK) was coupled to a gas chromatograph (model HRGC5300, Carlo Erba, Fisons, Crawley, UK) via a heated transfer line. This has been described in detail previously [19–21]. ADB-1 non-polar megabore column (15 m×0.53 mm×1.5 μm, J&W Scientific, Folsom, USA) was connected to 1 m of deactivated fused silica of the same dimensions (Phase Separations, Deeside, UK) via a glass union and wrapped in an electrically heated jacket. The temperature of the transfer line was controlled by a variable DC power supply. A small length of the transfer line was allowed to protrude from the end of the jacket directly into the rear of the ICP torch (H Baumbach, Woodbridge, Suffolk). A make-up gas was introduced into the rear of the torch via a T-joint. Optimum operating conditions of the GC–ICP-MS for methylmercury are shown in Table 1.

Table 1
Operating conditions for GC–ICP-MS

Gas Chromatography	
Injector type	On-column
Injection volume (μl)	1
Column	DB-1 (J&W Scientific), 15 m×0.53 mm×1.5 μm
Temperature programme	40°C ramped to 200°C at 20°C min^{-1}
He carrier gas flow (ml min^{-1})	10
Transfer line temperature (°C)	220 (77–255)[a]
ICP-MS	
Make-up gas (l min^{-1})	1.1 (0.9–1.3)[a]
Auxilliary gas (l min^{-1})	0.8
Cool gas (l min^{-1})	16
Forward power (W)	1350
Sampler	Ni, 1.0 mm orifice
Skimmer	Ni, 0.7 mm orifice
Data acquisition mode	Single ion monitoring 202 m/z
Dwell time (ms)	150
Acquire time (min)	10

[a] Optimised range shown in parenthesis.

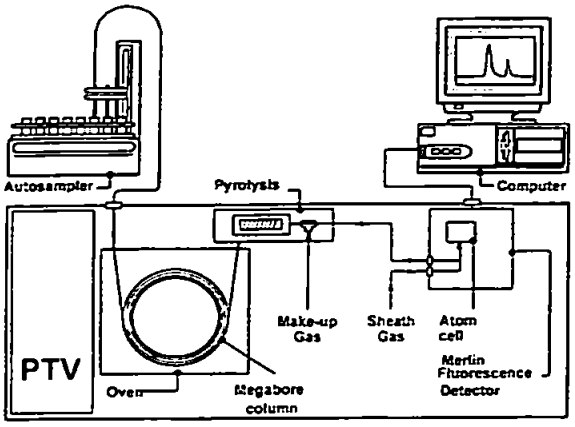

Fig. 1. Schematic diagram of GC–AFS.

The ion lenses of the ICP were tuned on the most abundant isotope of mercury at 202 m/z units. Initial tests were performed to establish that all the isotopes of mercury at 198, 199, 200, 202 and 204 m/z units were being observed in their correct relative ratios.

2.1.2. GC–AFS

A GC–AFS instrument (Mercury Speciation System, PS Analyticals, Orpington, UK) was used. This system comprises a gas chromatograph with programmable temperature vapouriser (PTV) injector (Ai Cambridge, Cambridge, UK), an integral pyrolyser and a modified atomic fluorescence detector (PS Analytical). A schematic diagram of the instrument is shown in Fig. 1. The programmable temperature vapouriser (PTV) injector is capable of split, splitless, on-column or direct injection modes in addition to offering the capability for large volume injections and thermal desorption. The direct injection arrangement was selected for this work. A non-polar megabore column (DB1, 15 m×0.53 mm×1.5 μm, J&W Scientific, Folsom, USA) and helium carrier gas were used. Optimised operating conditions for the GC–AFS instrument are shown in Table 2.

2.2. Reagents and standards

Organo-mercury standards, methylmercury chloride (Strem, Royston, UK) and ethylmercury chloride (Johnson Matthey plc, Reading, UK) were prepared by dissolving in methanol (HPLC grade, Rathburn, Broxburn, Scotland) prior to dilution in dichloromethane solvent (HPLC grade, Rathburn, Broxburn, Scotland) or double de-ionised water, as required. All reagents used in the extraction procedure were of analytical grade (Aldrich, Gillingham, UK) and were prepared in double de-ionised water (Elga Maxima, Elga, High Wycombe, UK). Alkaline extraction of the samples was performed using KOH (6 mol dm^{-3}) followed by neutralisation with HCl (6 mol dm^{-3}). An acidified mixture of KBr (18% m/v in 0.5% v/v H_2SO_4) and $CuSO_4 \cdot 5H_2O$ (1 mol dm^{-3}) in a 3:1 ratio was added to the samples with dichloromethane solvent. The mercury specific clean-up step of the procedure involved $Na_2S_2O_3$ (0.01 mol dm^{-3}).

2.3. Samples

The certified reference materials IAEA 142 mussel homogenate (International Atomic Energy Agency,

Table 2
Operating conditions for GC–AFS

Gas chromatography	
Injector type	PTV used in direct injection mode
Injector temperature (°C)	300
Injection volume (μl)	1
Column	DB-1 (J&W Scientific), 15 m×0.53 mm×1.5 μm
Temperature programme	40°C ramped to 200°C at 20°C min^{-1}
He carrier gas flow (ml min^{-1})	10
Pyrolysis unit temperature (°C)	850
AFS	
Make-up gas (ml min^{-1})	60
Sheath gas (ml min^{-1})	150

Marine Environment Laboratory, Monaco), NIST 8044 mussel homogenate (National Institute of Science and Technology, Gaithersburg, Maryland, USA) and DOLT-2 dogfish liver (National Research Council of Canada) were used for method validation. The moisture content of the reference materials was determined by drying portions in an oven at 105°C for 72 h. In addition, two fresh marine liver samples, a ringed seal liver and a beluga whale liver were also provided by the Freshwater Institute, Winnipeg, Canada. These were received packed in dry ice and were frozen until required for analysis. Fresh liver samples were allowed to thaw and homogenised in a blender prior to extraction. Homogenised samples were stored in acid washed plastic containers.

2.4. Procedure

2.4.1. Sample extraction

The extraction procedure was a variation of the Westöö method and is described in detail elsewhere [7,8]. Between 0.5 g portions of sample were weighed into 20 ml glass scintillation vials with PTFE lined caps. Five samples remained unspiked, and a further five were spiked with 100 μl of 150 pg ml^{-1} methylmercury chloride spike solution, and set aside in a cool, dark place for one month.

Water (2 ml) and KOH (3 ml, 6 mol dm^{-3}) were added to each sample, spiked sample, procedural blanks and standards, and shaken for 2 h (Platform shaker, Gallenkamp, England). Portions of HCl (3 ml, 50% v/v) were added to each vial to neutralise and slightly acidify the contents. Once the effervescence and heat had subsided, acidic KBr/ $CuSO_4$ 3:1 ratio (4 ml) was added. The mixtures were shaken for a few seconds and set aside for 20 min. Dichloromethane solvent (5 ml) was then added to each vessel followed by extraction on a shaker overnight. The samples were centrifuged for 10 min at 2500 rpm (Centaur 2, Sanyo, Japan) and the upper aqueous layer was removed. Known volumes (2.6–3.5 ml) of clean solvent were removed through the central layer of organic matter and transferred to clean 7 ml borosilicate vials. Thiosulphate (1 ml, 0.01 mol dm^{-3}) was then added to each vial followed by shaking for 1 h. Propan-2-ol was added to aid in phase separation, and samples were centrifuged for 10 min at 2500 rpm. Known volumes of the clear upper layer were then transferred to clean 1–2 ml polyethylene vials (0.6–0.8 ml, Fisher Scientific, Loughborough, UK). Further portions of the 3:1 acidic KBr/$CuSO_4$ mixture (0.3 ml) were added to the thiosulphate extracts followed by dichloromethane solvent (0.2 ml) and each vial was vortex mixed for 2 min. The lower solvent layer was removed by micropipette and transferred to a clean 1–2 ml screw cap glass vial, via a Na_2SO_4 drier tube. Finally portions of the dichloromethane extracts (100 μl) were accurately transferred to fresh 1–2 ml screw cap vials and spiked with ethylmercury chloride in dichloromethane as an internal standard (1 μl, 500 pg μl^{-1} as Hg).

3. Results and discussion

3.1. Evaluation of GC–AFS

3.1.1. Optimisation

The AFS instrument was optimised for maximum sensitivity, and the chromatography was also optimised to achieve best resolution. The optimised parameters were injection mode, injector temperature, pyrolyser temperature, carrier gas, make-up gas flow rate and sheath gas flow rate.

Injector temperature. The effect of injection temperature on the peak area observed for a 104 pg μl^{-1} standard of methylmercury chloride is shown in Fig. 2(a). When the injection temperature was increased to 300°C after a chromatographic run an elemental mercury signal was observed, which was not present when injecting solvent blanks at 300°C. This was thought to be the result of incomplete sample transfer to the column at lower temperatures. As the column upper working limit was 320°C an isothermal operating temperature of 300°C was selected for quantitative measurements.

Carrier gas flow. The use of megabore columns for the speciation of organo-mercury, with element selective detection by AFS has been reported with optimum carrier gas flow rates varying between 4 and 15 ml min^{-1}. In this work the carrier gas flow rate was optimised to provide minimal peak broadening with maximum area. Van Deemter plots for methylmercury and ethylmercury with both helium and argon carrier gases are shown in Fig. 2(b). These clearly show that both gases performed similarly at low flow

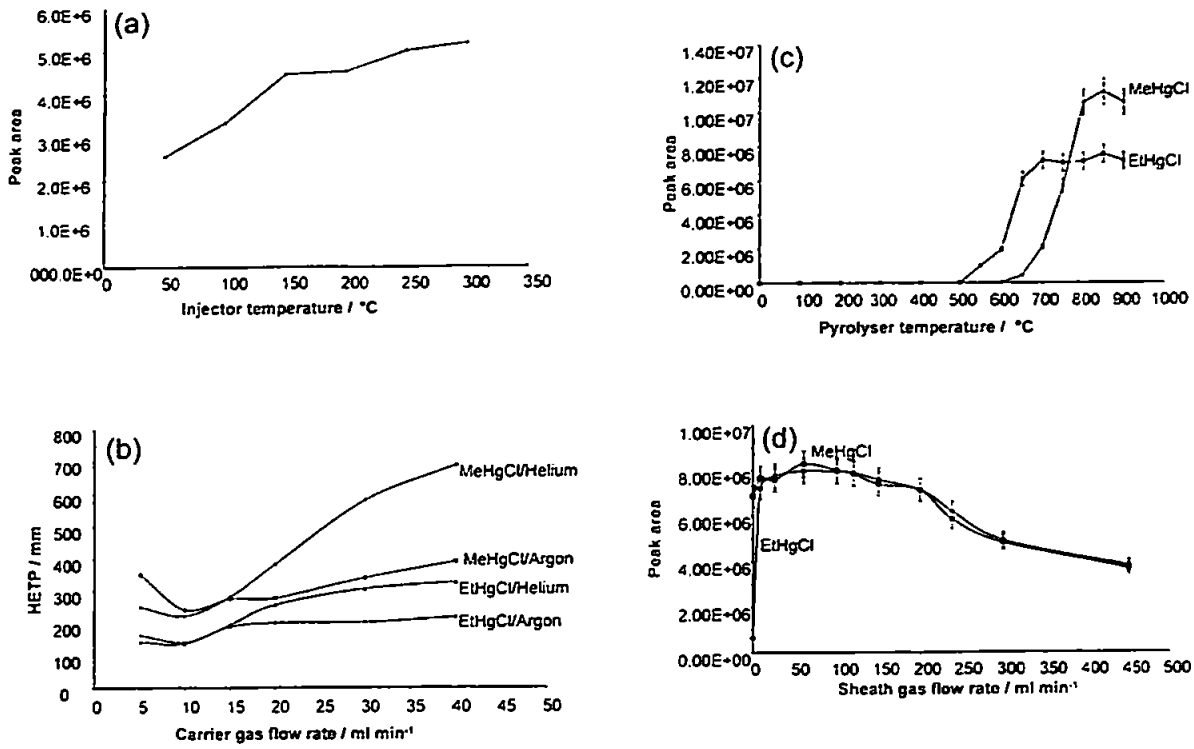

Fig. 2. Optimisation of parameters for GC–AFS. Effect of: (a) injector temperature on signal for 104 pg MMC; (b) carrier gas flow on HETP; .. (c) pyrolysis temperature on peak area signal for 86 pg MMC and 66 pg EMC standards; (d) sheath gas flow rate on peak area signal for 70 pg MMC and 70 pg EMC standards.

rates (<15 ml min^{-1}). In all cases the minima were observed at 10 ml min^{-1}. It was noted that helium was slightly better than argon at this optimal flow, although argon would be the gas of choice if flow rates >15 ml min^{-1} were required because helium degrades the detector. The peak resolution for methyl and ethylmercury chlorides was calculated to be 0.9 for both argon and helium, under these conditions. For both compounds the use of nitrogen as a carrier gas resulted in substantially smaller signals, which was attributed to quenching within the atom cell.

Pyrolyser temperature. The effect of pyrolysis temperature on peak areas observed for methylmercury chloride and ethylmercury chloride is shown in Fig. 2(c). As the pyrolysis temperature was increased a signal for ethylmercury was observed before the signal for methylmercury indicating a more easily thermally decomposed compound. Quantitative recovery of both compounds was obtained at pyrolysis temperatures >800°C. At a pyrolyser temperature of 900°C a characteristic triplet peak was observed at the front of the chromatogram. This was identified as carbon scatter, hence an optimum pyrolysis temperature range between 800°C and 850°C was chosen.

Make-up gas flow rate. Argon gas was mixed with the sample gas as it left the pyrolyser before entering the detector. The maximum possible dilution of the helium with argon was required at this stage in order to minimise the effect of helium on the detector, so an argon flow rate of 60 ml min^{-1} was chosen.

Sheath gas flow rate. The effect of sheath gas on peak area signal for methyl and ethylmercury chlorides is shown in Fig. 2(d). The sheath gas surrounds the sample gas as it enters the detector and prevents it from spreading within the atom cell. Argon was chosen as the sheath gas for mercury determination by AFS because helium reduces the lifetime of the detector, hence, the highest compromise flow of argon of 150 ml min^{-1}, was selected.

3.1.2. Figures of merit

The relative standard deviation of 10 repeat 10 µl injections of methylmercury chloride (86 pg μl^{-1} as Hg) and ethylmercury chloride (66 pg μl^{-1} as Hg) were 7% and 5%, respectively. The limit of detection (3σ) for methylmercury chloride was 0.25 pg as Hg. The amplification control on the AFS detector allows it to be used over five orders of magnitude. Linear calibrations have been obtained between 0 and 10 pg on the most sensitive setting and between 0 and 2 ng on the least sensitive setting. Increasing concentrations sometimes leads to a degree of peak splitting, but this did not effect the linearity of the technique when integrated peak areas were used. Calibration curves had R^2 values of 0.9996 and 0.9977, respectively, and the same response was observed for equal masses of mercury, regardless if it was in the form of methyl- or ethylmercury chloride.

3.1.3. Analysis of certified reference materials

Results of spike recoveries and found values for the reference materials are shown in Table 3. Found values were within the certified ranges after correction for spike recovery. Results for the determination of total mercury are shown in Table 4. As can be seen, almost half of the mercury was present as methylmercury.

3.2. Evaluation of GC–ICP-MS

3.2.1. Optimisation

The make-up gas flow rate, transfer line temperature and carrier gas flow were optimised by injecting 1.0 µl injections of a mixed methylmercury chloride and ethylmercury chloride standard (350 pg μl^{-1} of each, as Hg). The ion lenses were tuned to the most abundant isotope of mercury at 202 m/z units and the peak area signals measured. Optimal values and ranges for the three parameters studied are shown in Table 1. The make-up gas had very little effect over the range studied.

Table 4
Results for the determination of total mercury in certified reference materials by GC–AFS

Reference material	Certified concentration (ng g^{-1})	Found concentration[a] (ng g^{-1})
IAEA 142	126±7	131±6
NIST 8044	62±3	57±7

[a] Corrected for spike recovery and moisture content.

The transfer line temperature had very little effect on peak area signal, however, at low temperature the peaks were very broad (Fig. 3(b)), but as temperature increased, the peaks became narrower with increased peak height (Fig. 3(a)), hence, a temperature of 220°C was selected as optimal.

3.2.2. Figures of merit

The limit of detection (3σ) was determined to be 0.9 pg methylmercury chloride, as Hg, and the calibration was linear up to at least 180 pg with R^2=0.9975. The relative precision of five replicate injections of 1 pg methylmercury chloride was 9%.

3.2.3. Analysis of certified reference materials

Two mussel homogenate CRMs were analysed, namely IAEA 142 and NIST 8044. A chromatogram obtained for IAEA 142 is shown in Fig. 4. The first peak is methylmercury extracted from the mussel homogenate and the second is the internal standard, ethylmercury. Results of spike recoveries and found values for the reference materials are shown in Table 5. The concentration of methylmercury (as mass of mercury) corrected for spike recovery and water content were within the certified ranges for both CRMs.

Table 3
Results for the determination of methylmercury in certified reference materials by GC–AFS

Reference material	Spike recovery (%, n=6)	Certified concentration (ng g^{-1})	Found concentration[a] (ng g^{-1})
IAEA 142	95±20	47±4	45±7
NIST 8044	63±5	28±2	26±4
DOLT-2	86±9	693±53	671±41

[a] Corrected for spike recovery and moisture content.

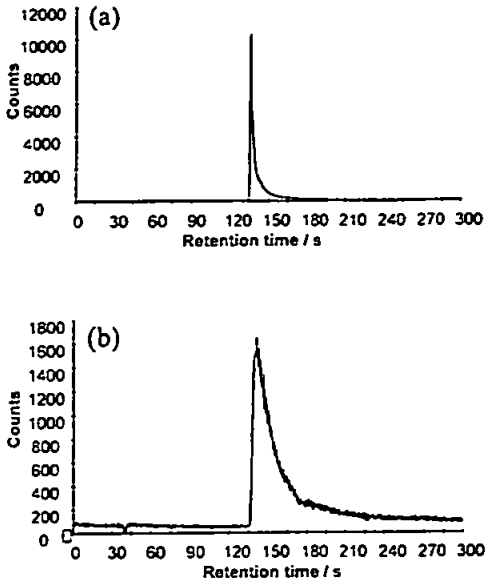

Fig. 3. Effect of transfer line temperature on peak shape for 350 pg injections of methylmercury chloride: (a) 220°C; (b) 30°C, with ICP-MS detection at 202 m/z.

3.2.4. Simultaneous halide m/z scanning of the mussel homogenate extracts

The multi-element capacity of the ICP-MS was used to investigate the halide species associated with extracted methylmercury in these materials. Injections of a 5 pg methylmercury chloride standard followed by IAEA 142 and NIST SRM 8044 extracts with added ethylmercury chloride internal standard, were made. The ICP-MS was set to monitor four masses, namely ^{79}Br, ^{127}I, ^{202}Hg and ^{35}Cl. It is well known that the halides have varying sensitivity by ICP-MS due to differences in ionisation energy which decreases down the group, so no attempt was made to quantify results.

Multi-element chromatograms for a 5 pg MMC standard and IAEA 142 extract are shown in Fig. 5. Due to the low sensitivity of this technique for chloride and high background due to the presence of

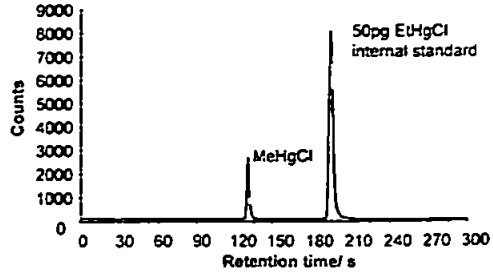

Fig. 4. Chromatogram of mussel homogenate extract, with ICP-MS detection.

dichloromethane as the solvent, no chloride peaks could be observed for the standards or extracts, so the chromatograms obtained for ^{35}Cl are not shown. Two peaks for mercury at 202 m/z were observed in the 5 pg MMC standard (Fig. 5(a)). The first was identified by retention time as methylmercury while the second was attributed to ethylmercury chloride carry-over from a previous high standard. It was evident from the absence of peaks at 127 and 79 m/z, that no bromide or iodide was associated with these compounds. The results observed for both of the mussel extracts were very similar, so only one of the chromatograms has been included here (Fig. 5(b)). It was evident from the chromatograms that the methylmercury species eluted at exactly the same retention time as a species containing bromide (cf. the first eluting species observed at 202 and 79 m/z). However, species containing iodide eluted 5 s earlier (cf. signal at 127 m/z). It is generally believed that the Br^- used during extraction complexes with RHg^+ and that it is this form that is present in the final extract. These results suggest very strongly that methylmercury was extracted as a brominated species. Given that no iodide was added during sample preparation it appears that this halide was present in both of the mussel homogenates, however, as the retention time of iodide did not correspond exactly with mercury, it is not possible to conclude that its source was methyl-

Table 5
Results for the determination of methylmercury in certified reference materials by GC–ICP-MS

Reference material	Spike recovery (%, n=6)	Certified concentration (ng g^{-1})	Found concentration[a] (ng g^{-1})
IAEA 142	54±6	47±4	48±9
NIST 8044	47±4	28±2	30±3

[a]Corrected for spike recovery and moisture content.

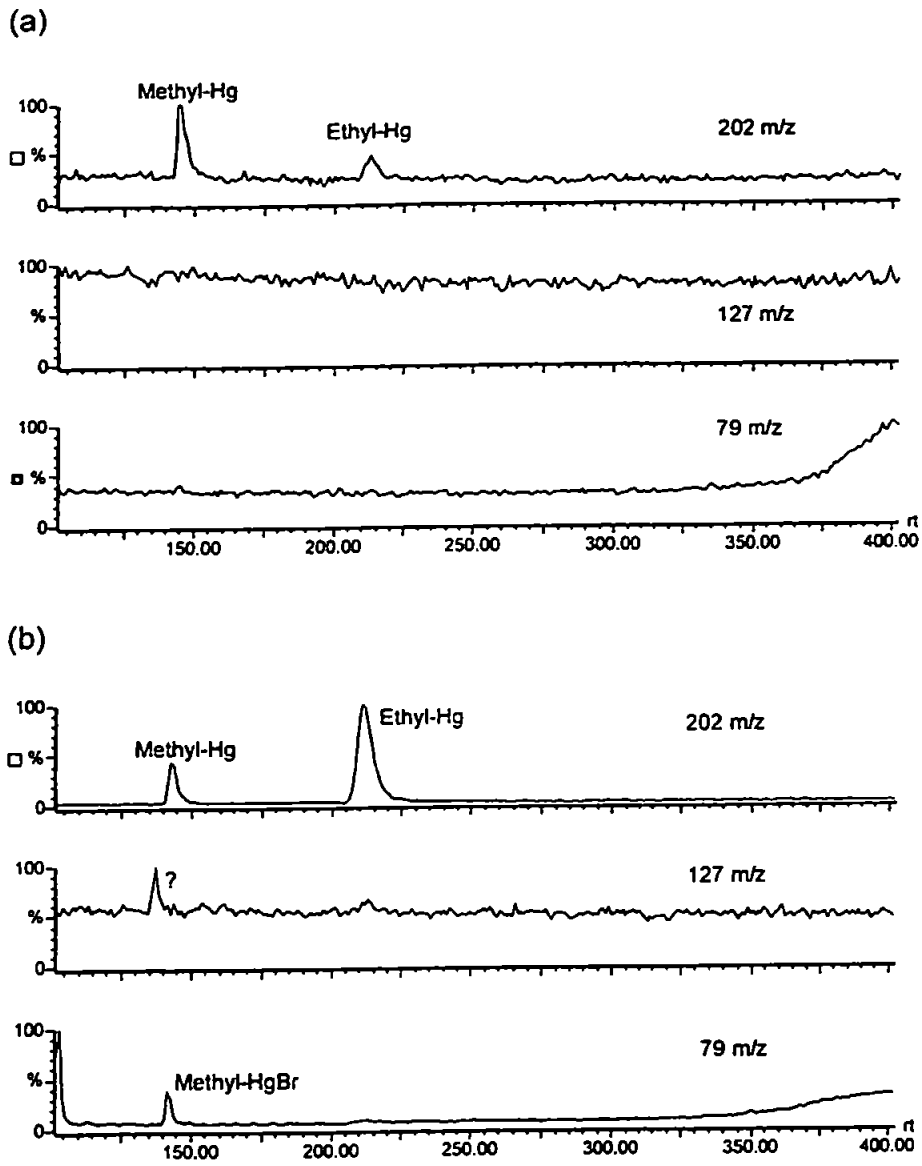

Fig. 5. Simultaneous multi-element scanning of m/z ^{79}Br, ^{127}I, ^{202}Hg and ^{35}Cl : (a) 5 pg methylmercury chloride standard; (b) IAEA 142 extract.

mercury iodide. Further work is required to identify the iodide containing compound.

3.3. Analysis of real samples by GC–AFS

The GC–AFS method had the lowest limit of detection so this was chosen for the analysis of real marine liver samples. There was only one organo-mercury compound in this sample which was identified as methylmercury by co-injection of a standard. The results for the liver samples are listed in Table 6. Two experiments were performed on these samples. The first procedure involved extracting 0.5000 g of each sample to establish the concentration range

Table 6
Determination of methylmercury in fresh marine livers

Sample	Mass of sample extracted (g, n=4)	Spike recovery (%, n=4)	Corrected methylmercury concentration (ng g^{-1}, n=4)
Ringed seal	0.5000	47±8	877±15
Ringed seal	0.2000	74±4	801±62
Beluga	0.5000	50±4	2775±132
Beluga	0.1000	69±13	2830±113

involved (Table 6). By reducing the mass of sample taken in the second procedure, spike recoveries were improved to between 69% and 74%. Moisture contents for beluga whale and ringed seal were 73% and 74%, respectively. The results for these materials have been reported as wet weight in line with common practise.

4. Conclusions

Atomic fluorescence spectrometry and inductively coupled plasma mass spectrometry have both been shown to be suitable detection techniques for organomercury speciation after separation by gas chromatography. Both techniques were extremely sensitive and selective detectors for mercury, present as methylmercury. The advantages of ICP-MS are its multi-element and multi-isotopic capability, whereas AFS has the advantage of comparatively low cost and simple operation. Validation of both techniques was achieved by the analysis of certified reference materials, and GC–AFS was successfully applied to the analysis of real marine tissue samples.

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