Multielement Chromatographic Profiling of Environmental Pollution

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Abstract

This thesis describes the evaluation of the first commercially available Hewlett Packard gas chromatograph - microwave induced plasma - atomic emission detector (GC-MIP-AED) for application to qualitative and quantitative analysis. The technique was applied to a range of environmentally significant samples.

A general introduction to the development of the technique and its suitability for environmental applications is discussed and a number of typical applications are reviewed.

A fundamental study of the capabilities of the GC-MIP-AED was undertaken. It became apparent that the manufacturers pre-programmed recipes for the different elemental channels required optimising to eliminate ghost emission signals from other sources such as carbon molecular emission. After the optimisation of these elemental recipes for C, S, N, O, Pb and Cl had been achieved the ability of the instrument to perform multielement heteroatom profiling of a range of pollutants with a high degree of selectivity was established. Samples included oils, leaded and unleaded petrols, and coal pyrolyzates. A critical assessment of the instruments capabilities with respect to these applications and other operational issues is also described.

The instruments ability to perform quantitative analysis was then studied highlighting a number of problem areas, such as, variable repeatability and limits of detection, relating to the automatic injection facilities. When the appropriate injection liner and injection technique were used, the limit of detection on all the elemental channels monitored was greatly improved.

The study was then extended to utilise the GC-MIP-AED for the multielemental analysis of organomercury, organolead and organotin compounds. The instrumental conditions were optimised for each element individually with respect to instrumental operating parameters, repeatability, limit of detection, linearity of response, and also the chosen extraction and derivatisation technique. All parameters were then optimised for the analysis of organomercury, organolead and organotin compounds within a single chromatographic injection for environmental applications including sediment, fish tissue and water samples.

The overall objective of this thesis was to identify and evaluate the multielement capabilities of the analytical technique for qualitative and quantitative application to samples of environmental relevance, in particular simultaneous multielement organometallic speciation.

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1.0 Introduction

1.1 Environmental Pollution - The Need for Chemical Speciation

In order to consider the effects of environmental pollution, it must first be defined. One broadly applicable definition states that pollution is "*too much of something in the wrong place*" [1]. This definition can easily be applied to the chemical industry as any chemical found in high enough concentrations is a pollutant. Once defined it is then possible to identify problem areas by quantifying specific pollutants and then monitoring the effect both the chemical pollutant and its degradation products have on the surrounding ecosystem. Only then can a system be devised to try and control the extent of environmental contamination.

In recent years, analytical atomic spectroscopy has been used in order to obtain total elemental information on environmental pollutants. Currently there has been an increased demand for elemental speciation, as different chemical species, in particular organometallic species, exhibit variable degrees of toxicity to living organisms within their ecosystem.

One of the most promising approaches to speciation has been the use of gas chromatography (GC), for the separation of volatile organic pollutants, coupled with an appropriate detector. This technique while being routine for organics is much less so for organometallics, in particular for the analysis of more than one metal on the same detector. The technique of gas chromatography [2] has

been widely covered by numerous authors and will not be covered in this thesis.

1.2 GC Detectors for Environmental Analysis

The detectors may be classified into two categories, although some overlap may be observed,:-

1) Universal

2) Selective

Universal detectors respond to almost any component eluting from a GC column, whereas selective detectors are more specific and respond to components with certain properties.

1.2.1 The Role of Universal Detectors in the Analysis of Environmental Pollutants

The almost universal response to organic compounds exhibited by a number of detectors, for example, the flame ionisation detector (FID) [3, 4] or the thermal conductivity detector (TCD) [3], can be of great value for fingerprinting or characterising samples consisting of complex mixtures such as products of the petroleum industry. The FID will give a response for any material that will produce ions in an air / hydrogen flame. The TCD will give a response for any material with a thermal conductivity different from the carrier gas used. The FID chromatogram obtained from the analysis of an oil sample provides a characteristic fingerprint for that oil sample, possibly allowing for differentiation between oils from different sources [5-7]. This fingerprint could be of use if an environmental situation such as an oil spillage occurred. Tentative identification

of the source involved would be possible, after making due allowance for the effect of weathering. (Further information would be required for a conclusive identification.)

Alternatively, this universal response may be counter-productive as poor chromatographic separation of complex materials may be observed. If, for example, it was necessary to identify and quantify a component such as tetra-ethyl lead in a petroleum sample using an FID or TCD, all the components in the sample would have to be completely separated, requiring an extended analysis period. This extent of separation is not always necessary when a degree of selectivity is observed by a detector. There is also a lack of sensitivity exhibited by the FID and especially by the TCD hence a response is sometimes not observed for components present at trace levels.

Universal detectors do however have a part to play in routine analysis. Once one or more components have been identified, for example using a specialised detector such as a mass spectrometer, a batch of samples may be analysed for the selected components, using retention times on an FID, leaving the mass spectrometer available for research purposes. Although an authentic standard may be used to help identify analytes by FID, this is not conclusive, as a response observed in a sample at a similar retention time may be due to a compound with similar retention behaviour to the standard on the analytical column used. Also, authentic standards are not very widely available.

In general, although the universal detector has its part to play within the field of environmental analysis, the lack of sensitivity and unselective response limits its use to a large extent. Also the only information provided is retention time and hence retention index. In this respect a selective detector such as a spectrometric detector would prove more valuable as a greater degree of information can be obtained. For example, the mass spectrum of the component (mass spectrometer), structural information (infrared spectrometer) or elemental information (atomic spectrometric detector).

1.2.2 The Role of Selective Detectors for the Analysis of Environmental Pollutants

Selective detectors respond to the different properties of components within a sample and can distinguish between particular structures or elements with a high degree of certainty. A number of selective detectors have been used for environmental analysis. These include the electron capture detector (ECD), the flame photometric detector (FPD), and the nitrogen phosphorus detector (NPD) also known as a thermionic detector, which have been reviewed by Sevcík [3] and also Hill and McMinn [4]. Although each of these detectors exhibit much greater selectivity for certain components thereby simplifying the chromatogram obtained, they are not truly specific. For example, the electron capture detector responds to organohalogens, organomercury species, organolead species and other species capable of capturing low energy electrons to form negatively charged ions, whereas the NPD is selective for organic compounds containing nitrogen and phosphorus. It is not always possible to confirm which species gave a response on an ECD or NPD without confirmatory analysis such as

provided by a spectrometric detector (i.e. a mass spectrometer, infrared spectrometer or atomic spectrometer).

A mass spectrometer [4, 8] would appear to be an almost ideal detector for environmental analysis due to the relatively high sensitivity and the large quantity of qualitative information provided, such as the mass and fragmentation pattern of the components. However a number of drawbacks exist. For example some isomers give similar mass spectral data, fragmentation spectra are often instrument dependant and difficult to interpret and excessive fragmentation may result in the loss of the ionised molecular species allowing for a degree of ambiguity in the identification of components, hence additional information is often required. Traditionally, the mass spectrometer also requires the complete separation of all the components requiring extended analysis periods, as for the FID, although this requirement is now somewhat less important with the advent of MS-MS techniques.

A range of detectors based upon atomic spectroscopy have also been used for environmental analysis [4, 9-12] which can be categorised into atomic absorption, atomic fluorescence and atomic emission of which undoubtedly atomic emission is the most important for its simultaneous multielement capabilities.

A number of theoretical features show its applicability to environmental analysis. These include:-

1) A high degree of sensitivity (pg / s) and a large linear dynamic range.
2) The ability to provide elemental information for unknown components with a high degree of selectivity
3) The ability to act as a "universal" detector for organic compounds by monitoring the carbon emission wavelengths
4) The ability to monitor more than one element simultaneously
5) The ability, at least in principle, to obtain elemental composition data, leading to empirical formulae approximations and compound independent calibration

In theory, atomic emission spectroscopy elemental response should be independent of the molecular structure. Hence it should be possible to determine the elemental composition of a particular molecule. This indicates that any compound of known molecular structure containing a specific element may be used to quantify an unknown compound for that particular element. This is termed compound independent calibration.

These features give a distinct advantage over other detectors previously mentioned. For example, the fingerprinting of each element in a given sample such as petroleum allows not only for the carbon chromatogram to be obtained but also for chromatograms of sulphur, oxygen and nitrogen etc. showing a distinct advantage over the FID as a large amount of additional information is

provided for a single analysis. Hence if this type of detector was to be used in the earlier example discussed in section 1.2.1 for the identification of the source of an oil spillage, the combined information of the elemental fingerprints would be almost conclusive.

It is also possible to identify components such as tetra-ethyl lead in petroleum, with a high degree of certainty by monitoring the lead emission wavelength and obtaining elemental verification from spectral data, which, in conjunction with retention time / index, would eliminate any ambiguity which may arise when using an authentic standard.

1.3 The Historical Development of an Atomic Emission Detection System for Gas Chromatography

In atomic emission spectroscopy, samples are vaporised at very high temperatures in a flame or plasma, which break down most compounds into atoms. Some of the atoms are promoted to excited electronic states by collision with other atoms. These atoms emit a characteristic radiation at specific wavelengths for each element when they return to their ground state. The radiation is dispersed using either a prism or a diffraction grating and the intensity of the emitted radiation is measured by a sensor.

The development of various plasma emission sources such as:-

1) The microwave induced plasma (MIP)
2) The inductively coupled plasma (ICP)
3) The direct current plasma (DCP)

opened the door for the development of emission detection systems for GC. These systems have been critically appraised by Ebdon *et al* [9], Harrison *et al* [10], Uden [11, 13], Krull [12], Hill *et al* [14] and more recently Croslyn *et al* [15], with applications given for each type of system.

Plasma emission sources are able to produce numerous emission lines because of their high excitation temperatures. When inert gas plasmas are used in conjunction with high resolution monochromators a high degree of selectivity and sensitivity can be achieved.

The most popular excitation source for GC atomic emission detection is the MIP due to the relative ease of coupling the techniques. This source has hence been extensively reviewed by a number of authors [4, 10-12, 15-18] and a detailed review of the development of the cavity design has been reported by Webster [19].

The technique was first reported by McCormack *et al* [20] in 1965, when argon gas was used to create a plasma under reduced pressure for the analysis of organohalogens. Since then a number of laboratories experimented with in-house built atomic emission detectors for GC. For example, Bache *et al* [21] who applied such a system to the determination of organic bromine, chlorine, iodine, phosphorus and sulphur compounds.

The technique initially developed quite slowly until a significant breakthrough occurred in 1977, when Beenakker [22-24] developed a cavity capable of containing a helium plasma at atmospheric pressure, while retaining comparable selectivity and sensitivity to those exhibited by reduced pressure helium plasmas. This greatly simplified the interface between the GC and the detector, as the eluent of a GC column is normally at atmospheric pressure.

Although prior to 1977 it had been possible to sustain an argon plasma both at reduced pressure and atmospheric pressure, it had been found that a helium plasma was preferable. (Until the development of this cavity it had only been possible to maintain a helium plasma under reduced pressure.) The fragmentation and excitation processes within a helium discharge gave much stronger elemental emission lines enabling the detection of both organic and organometallic compounds, even though the helium discharge is maintained at a lower temperature than an argon plasma [22]. At this stage in the detector's development it had only been possible to detect organometallic compounds from their atomic emission lines with an argon discharge, leaving the non-metallic elements such as the halogens and nitrogen, sulphur etc., to be determined from their diatomic emission bands.

Since the development of the Beenakker TM_{010} cavity a number of other cavity designs based upon similar principles have been developed, four of which have been reviewed by Forbes *et al* [25]. One cavity in particular developed by Quimby and Sullivan [26] which is based upon a modification of the re-entrant

cavity and has been adapted for interfacing with a GC, has shown great promise. It is this cavity coupled with a photo diode array spectrometer which has been used in the first commercially available GC-MIP atomic emission detector (GC-MIP-AED) from Hewlett Packard - see section 1.5. In 1995 Hewlett Packard launched their second generation GC-MIP-AED (HP G2350A) [27], however this thesis will be restricted to the first commercially available instrument (HP 5921A).

1.4 Environmental Applications of GC-MIP-AED

A GC-MIP-AED exhibits the capability to provide valuable information on a very diverse range of samples, such as pharmaceutical compounds / metabolites [28-29] and polymer additives [30-33], although its major application to date appears to be in the field of environmental analysis. A comprehensive review of applications was performed in 1986 by Ebdon *et al* [9] who present 95 references for the analysis of a diverse range of environmental and biological samples. Since the completion of the laboratory stage of this research programme a further review of environmental applications has been published by Pedersen-Bjergaard *et al* [34].

Since the Hewlett Packard GC-MIP-AED became commercially available in 1989 an increase in applications has been observed, particularly for organic components by this technique. A number of applications have also been reported for organometallic components and in 1994 an application published by Lui *et al* [35] exploited the instrument's multielement capabilities in this field.

The references found in tables 1-4 are concerned only with applications relevant to the field of environmental analysis that had not been reported in the first review by Ebdon *et al* [9]. These applications have been categorised by sample matrix the majority of which are primarily for the elemental analysis of non-organometallic compounds.

Four categories of sample matrices have been defined:-

Table 1	:	Petrochemical industry products
Table 2	:	Water
Table 3	:	Sediments / Sludges / Coal
Table 4	:	Biological species - (a) Plants
		(b)Animals

1.4.1 Petrochemical Industry Products

Of the four categories table 1 stands out in that the analysis is performed on a sample product as opposed to the actual environmental pollutant. Sixteen references are given for the analysis of a combination of gasolines, diesels, oils and petroleum feed stocks, all of which exhibit grave environmental consequences upon combustion or spillage.

In petrochemical products, especially gasolines, the analysis and characterisation of organosulphur containing species is of great interest. Not only do they poison catalysts within the manufacturing process, they can also lead to the failure of automobile catalytic converters and result in excessive SO_2 emissions upon combustion in power stations or motor vehicle engines. A

critical review of different detectors used for the quantitative determination of organosulphur compounds in C_1-C_{13} petrochemical products has been presented by Albro *et al* [36]. The GC-MIP-AED is reported to offer high sensitivity, satisfactory linearity with no interference from quenching effects as seen when using the ECD or FPD, while simultaneously providing the carbon chromatogram comparable to that produced by the FID and confirming the presence of sulphur via the atomic emission spectrum.

Organolead containing components specifically found in gasoline additives are also of environmental concern [37], although leaded gasoline is currently being phased out and replaced with methyl tertiary butyl ether (MTBE). The major source of environmental contamination is from motor vehicle exhaust fumes as approximately 1% of organolead compounds in gasoline leave the exhaust unchanged. Although the majority of these compounds are unstable, breaking down to give inorganic lead, which is less toxic than its organic counterpart, organolead components also exist in the environment and hence cannot be ignored. The ability of the GC-MIP-AED to selectively monitor organolead components allows the determination of organolead species present in gasoline in a short analysis time, as complete chromatographic separation is not critical as with other detectors such as MS, FID and IR.

Table 1. Petrochemical Sample Analysis

SAMPLE MATRIX	COMPONENT OF INTEREST	ELEMENTS MONITORED	COMMENTS	REF.
Unleaded gasoline, Aviation gasolines, Kerosene, Jet fuel and Diesel. Water from ground water wells.	Carbon fingerprints, organosulphur and organolead compounds	C H S Pb Wavelengths not detailed.	Illustrates the use of multielemental capabilities to allow differentiation between a wide range of intact petroleum products, and also after a degree of evaporation. An in-house data handling system was used to allow multitasking data acquisition and analysis to be performed simultaneously.	6
Petroleum feed stock	$C_1 \rightarrow C_{13}$ hydrocarbons	C (193 nm) S (181 nm)	GC equipped with a prefractionator to selectively allow $C_1 \rightarrow C_{13}$ compounds on to the column	36
Gasoline	Organosulphur components	C (193 nm) S (181 nm)	Shows the application of fingerprinting	38
Petroleum	100-400°F boiling point range	C (248 nm) O (777 nm)	Evaluation is given on the applicability of the GC-MIP-AED for oxygen analysis	39
Gasoline	Organolead in leaded petrol	C (193 nm) Pb (405 nm)	Screening for petroleum additives	40
Gasoline / diesel fuel	(a) Oxygenates in gasoline (b) Organosulphur compounds	O (777 nm) C (193 nm) C (193 nm) S (181 nm)	General discussion on the use of the GC-MIP-AED for a number of applications	41
Naphtha	45-210°C boiling point range	C (193 nm) S (181 nm) N (174 nm) O (777 nm)	Sample characterisation	42
Petrochemical precursors	Pyrolysates of kerogen samples	C (193 nm) N (174 nm) S (181 nm) O (777 nm) P (186 nm) S (181 nm)	Organophophorus and organoselenium compounds were only seen after an alkylating reagent had been added to the sample. The sample was introduced by pyrolysis.	43
Crude oil	Metalloporphyrins present in Californian heavy crude	Ni (301 nm) V (292 nm) Fe (302 nm) C (193 nm) H (486 nm) S (181 nm)	A comparison of crude oils from a number of different countries is presented	44
Light hydrocarbon feed stock	Organochlorides	C (496 nm) Cl (479 nm)	Compounds are identified as containing chlorine	45
Gasoline	Organosulphur and organomercury components	C (193 nm) S (181 nm) Hg (184 nm)	A general discussion of applications and a comparison with FPD and FID is presented	46

SAMPLE MATRIX	COMPONENT OF INTEREST	ELEMENTS MONITORED	COMMENTS	REF.
Lubricating oil	Oil additives	C (193 nm) H (486 nm) Zn (213 nm) S (181 nm) P (178 nm) Cl (479 nm) O (777 nm)	Illustrates the use of spectral confirmation . Zinc has a secondary emission line which interferes with chlorine at 479 nm. The only way to differentiate between the elements is by monitoring the emission data.	47
Unleaded gasoline	Carbon fingerprints, organosulphur and oxygenates.	C (343 nm) S (361 nm) O (777 nm)	The sulphur second order wavelength was used and also the CO molecular emission line for C. The CO molecular emission line exhibits a greater dynamic range for C and the secondary sulphur emission line exhibits greater selectivity over carbon due to increased spectral resolution. Optimising the hydrogen reagent gas was found to prevent loss in sensitivity from the deposition of carbon particles in the discharge tube.	48
Hydrocarbon samples	Oxygenates	O (308.9 nm)	Oxygenates were determined using an argon plasma at the OH molecular emission band. Different plasma gases and emission wavelengths, torch designs and gas flow rates were compared.	49
Gasoline	Oxygenates	O (777 nm)	A Beenakker TM_{010} cavity was used and both axial and lateral viewing positions were compared.	50
Low molecular weight sulphur gases	SO_2, COS, H_2S	S (182 nm)	The analysis was performed using just the S (182.0 nm) line rather than a sum of the three lines at 180.7, 182.0 and 182.6 nm as in the HP GC-MIP-AED.	51

1.4.2 Water Analysis

Pollutants of primary concern found in water systems are halogenated components such as pesticides, insecticides or herbicides and their degradation products, although as can be seen from table 2 other components are also of interest. The application of organochlorine pesticides is restricted in most countries. However the use of modern triazines within the agricultural community, for example, is widespread hence the determination of these

pesticides and their degradation products which may leach into aquatic environments due to their relatively high degree of solubility, is of great importance.

The GC-MIP-AED has the ability to differentiate between heteroatoms while retaining a high degree of sensitivity, giving a clear advantage over, for example, the ECD and NPD currently used for pesticide analysis. Another advantage is the ability to provide a means of identification from complex environmental samples without the need for complex extraction or clean up procedures which are necessary when using these other detectors. Additionally it provides complementary information to that provided by mass spectrometry.

The identification of organometallics in the aquatic environment is also of great importance due to the high toxicity of many organometallic species. The use of the GC-MIP-AED to determine organolead species in complex samples has already been mentioned and may also be applied to many other organometallic compounds containing elements such as beryllium, selenium, mercury and tin as the metallic component.

Table 2. Water Analysis

SAMPLE MATRIX	COMPONENT OF INTEREST	ELEMENTS MONITORED	COMMENTS	REF.
Snow from Greenland	Organolead compounds	Pb	Organolead species are extracted as dithiocarbamates with pentane or hexane and derivatised with Grignards reagent. Pre concentration occurred in the injection liner.	37

SAMPLE MATRIX	COMPONENT OF INTEREST	ELEMENTS MONITORED	COMMENTS	REF.
Surface water	Triazines	C (193 nm) N (174 nm) S (181 nm) Cl (479 nm)	A real surface water sample was spiked with standards. Liquid/liquid extraction into dichloromethane (DCM) is performed. Illustrates the selectivity of the GC-MIP-AED	46
Industrial waste water	Components extractable into dichloromethane at pH 7	C (193 nm) O (777 nm) N (174 nm) S (181 nm)	The paper attempts to identify the extractable components via GC-MIP-AED, GC-MS, GC-FTIR as no single technique can conclusively identify the components	52
Waste water from a nickel refinery	Extractable organohalogens	C (248 nm) H (486 nm) Cl (479 nm) Br (478 nm)	Molecular formula determination is attempted using external compound independent reference standards	53
Natural water	Organoberyllium	Be (313 nm)	Sample was extracted as acetyl acetone complex and analysed. Problems were observed due to the dissociation of the complex in the analytical column	54
Volatiles from the ocean surface layer	Di-methyl selenide	Se (196 nm)	Volatiles were expelled with a flow of He, dried and collected in a trap immersed in liquid nitrogen.	55
Sea water	Alkylmercury species	Hg (253 nm)	A 500 fold preconcentration step is performed using a benzene / cysteine extraction. Analysed as the halide derivative	56
Surface water	Pesticides were spiked in the water at 1 ppb	C S P Cl	The pesticides were preconcentrated on an extraction disc before being eluted with ethyl acetate and analysed.	57
River water and bottled mineral water	Organoselenium compounds (Se^{IV} and Se^{VI})	Se (196 nm)	Se^{IV} was determined in the presence of Se^{VI}, and Se^{VI} was determined by reduction to Se^{IV}.	58
Surface water and industrial waste water	Pesticides and other heteroatom containing organic compounds.	Cl (479 nm) P (178 nm) P (186 nm) S (181 nm) N (174 nm) Br I F (690 nm)	The multielement capabilities of the instrument are exploited by screening samples for the presence of heteroatoms as an aid to compound identification by GC MS.	59
Artificial rain water	Tri-methyl lead	Pb (406 nm)	The extraction / derivatisation of samples are optimised and an artificial rainwater was analysed as part of a project to produce a certified reference material for rain water.	60
Harbour water	Organotin and organolead compounds	Sn Pb	The effects of matrix and reagent impurities are investigated for different extraction/ derivatisation procedures performed to allow for the analysis of organometallic compounds	61

SAMPLE MATRIX	COMPONENT OF INTEREST	ELEMENTS MONITORED	COMMENTS	REF.
Drinking water	Trihalomethanes	Cl (481 nm) Br (471 nm) I (206 nm)	A GC interfaced with a TM_{010} cavity and echelle grating monochromator and photomultiplier is used with purge and trap apparatus. The optimisation of the system is discussed and the selectivity and sensitivity of the system is compared to that of a GC-ECD and GC-HECD.	62
Waste water effluent	Tri-ethyl lead chloride and tri-methyl lead chloride	Pb (283 nm)	The organolead compounds were extracted into benzene and butylated with n-butyl magnesium bromide. Tetra-butyl lead was added as an internal standard. The sample was preconcentrated into a tenax trap and the solvent removed. The sample was analysed using an adapted inlet port.	63
Rain water	Methylated and ethylated organolead compounds	Pb (406 nm)	A complete evaluation of operating conditions for the analysis of organolead containing species is given. The organolead species were extracted using NaDDTC and EDTA into hexane and alkylated prior to analysis.	64
Natural waters	Methylated, ethylated and inorganic mercury	Hg (254 nm)	Mercury species within water samples including sea water were preconcentrated on a dithiocarbamate resin loaded microcolumn and eluted using thiourea. The mercury species were extracted into toluene and butylated prior to analysis. This technique was not suitable for the analysis of humic rich waters.	65
Tap water	Organotin species	Sn (303 nm) Sn (284 nm)	Organotin species were extracted into hexane using ascorbic acid and tropolone and either phenylated or ethylated by Grignard reaction.	66
River water	Organotin species	Sn (303 nm)	A comprehensive optimisation study of a semi automated flow-injection sample preparation for the analysis of organotin species is presented.	67
Natural water sample	Organotin species	Sn (303 nm)	Organotin species are extracted into pentane as DDTC complexes. The pentane is evaporated and the residue pentylated prior to analysis. The accuracy of the instrumental method was confirmed by GC-AAS.	68

1.4.3 The Analysis of Sediments, Sludges and Coals

A large number of organic pollutants may be found in sediment and sludge samples, adsorbed onto the surface of particulate matter. As in water analysis the GC-MIP-AED does not require complex extraction procedures and extensive sample clean up as for the ECD, FPD and NPD. Element specific chromatograms for the elements most commonly found in hazardous waste can be obtained such as organohalogens, organosulphur and organonitrogen compounds as well as organometallic species providing elemental composition information. The characterisation of sedimentary samples was reviewed in 1982 by Larter *et al* [69] and also in 1987 by Philp and Gilbert [70].

Coal analysis is slightly different to sediment analysis as it is the combustion products that are given off on burning that are of environmental concern. As in petrochemical feed stocks, organosulphur compounds and inorganic sulphur species are given off on combustion of some types of coal which, when emitted into the atmosphere, can react to form acid rain. The GC-MIP-AED equipped with an appropriate inlet device, such as a pyrolysis unit, can be used for screening purposes because different types of coal produce different organosulphur compounds on burning, thereby coals which emit large amounts of organosulphur components may be identified.

Table 3. Sediments, Sludge and Coal Analysis

SAMPLE MATRIX	COMPONENT OF INTEREST	ELEMENTS MONITORED	COMMENTS	REF.
Coal	Coal (Liquid distillates)	C (193 nm) O (777 nm)	The sample was used primarily to evaluate the capability of the system for the analysis of a complex mixture without complex pretreatment	39
Sediment extract from a hazardous waste dump	Extractable components	C (193 nm) Cl (478 nm) S (181 nm) N (174 nm)	A fingerprint for each element is given. Full identification of each component is attempted using additional information obtained from ECD, NPD, PID, MS and FTIR	41
Coal	Volatile Pyrolyzates of coal	C (193 nm) S (181 nm) O (777 nm)	Coal samples from around the USA were analysed and quantified. The results were compared with alternative techniques	43
Marine sediment	Alkyl tin species extractable into hexane	Sn (303 nm)	Speciation of organotin pollutants and degradation products. May allow for the pollution source to be identified	45
Sludge sample from near a nickel refinery	Analysis of Soxhlet extractable halogenated compounds	C (248 nm) H (486 nm) Cl (479 nm) Br (478 nm)	Elemental ratios were determined in an attempt to obtain empirical formulae for components present	53
Road dust	Tri-methyl lead	Pb (406 nm)	The road dust was extracted by shaking with EDTA and NaDDTC followed by derivatisation with $NaBEt_4$. A comparison of shaking and ultrasonic extraction is given.	60
Riverbank sediment from abandoned chemical dump site	Components extractable into isooctane	C (496 nm) H (486 nm) Cl (479 nm) Br (478 nm) F (686 nm) O (777 nm) S (181 nm) P (178 nm) N (174 nm)	A number of hyphenated techniques were used to analyse the sample, including GC-MS, GC-IR and GC-MIP-AED to confirm component identity	71
Riverbank sediment from abandoned chemical dump site	Components extractable into isooctane	C (496 nm) H (486 nm) Cl (479 nm) Br (478 nm) F (686 nm) O (777 nm) S (181 nm) P (178 nm) N (174 nm)	A critical appraisal is given on the use of other detectors for the analysis of this sample, including the ECD, NPD, FPD.	72
Top soil	Organotin species	Sn (271 nm) C (248 nm) H (656 nm)	The soil samples were extracted using super critical fluid extraction with carbon dioxide. The extract was pentylated using a Grignard technique and analysed. Optimisation of instrumental parameters is detailed.	73

SAMPLE MATRIX	COMPONENT OF INTEREST	ELEMENTS MONITORED	COMMENTS	REF.
Top soil and marine sediments	Organotin species	Sn (271 nm) C (248 nm)	A comparison of two super critical fluid extraction techniques is discussed (one collecting material in a solvent and the second by desorption on an octadecyl bonded silica sorbant trap).	74
Harbour sediment	Organotins extractable into hexane with 0.5% Tropolone at pH 1.5	Sn (303 nm) C (496 nm) H (486 nm)	Optimisation for the analysis of organotins is discussed with experimental conditions	75

1.4.4 The Analysis of Biological Samples

Again the specificity of the GC-MIP-AED allows its application to the complex sample matrices obtained from both plants and animals. In plants the primary pollutants found are pesticide residues and their degradation products. Here the ability to differentiate between heteroatoms shows its superiority over other detectors.

In animals, pesticide residues are also of concern as are organometallic species, due to bioaccumulation effects. Toxic substances such as organomercury species are accumulated in aquatic animals, such as fish, and then migrate along ecological food chains. The effects of such pollutants may have grave consequences hence having the ability to detect these substances in complex matrices is invaluable.

Table 4a. Plant Analysis

SAMPLE MATRIX	COMPONENT OF INTEREST	ELEMENTS MONITORED	COMMENTS	REF.
Onion extract spiked with nine pesticides	Pesticide residues	Cl (479 nm) F (686 nm) P (178 nm)	The chromatograms obtained were easily able to distinguish between peaks, whereas a chromatogram from an ECD detector of the same sample proved extremely difficult to interpret	45

SAMPLE MATRIX	COMPONENT OF INTEREST	ELEMENTS MONITORED	COMMENTS	REF.
Wine	Organolead compounds	Sn Pb	The effects of matrix and reagent impurities are investigated for different extraction/ derivatisation procedures performed to allow for the analysis of organometallic compounds	61
Seaweed sample	Methyl arsenic species, extracted and derivatised	As (229 nm) S (545 nm)	Three derivatisation procedures were carried out using mono-methyl arsonic acid (MMAA), di-methyl arsenic (DMAA) and thioglycolic acid methyl ester (TMG)	76
An apple spiked with pesticides	Pesticide residues	C (193 nm) S (181 nm) N (174 nm) P (178 nm) H (486 nm)	A discussion as to the importance of the GC-MIP-AED is given in comparison to a number of other detectors including ECD, FPD, NPD.	77
Plant extracts	Pesticide residues	Cl (479 nm) F (686 nm) P (178 nm) C (193 nm) S (181 nm) N (174 nm)	A comparison of different detectors is given for the analysis of each sample, which included:- Alfalfa, Almonds, Broccoli, Carrot, Cauliflower, Onion, Lettuce, Orange, Strawberry, Zucchini	78
Plant extracts	Pesticide residues	Br Cl I P S	One of the first applications of an MIP detector published	79
Orange essence and celery	Pesticide residues	P (254 nm) I (206 nm)	This analysis utilises a very early GC-MIP-AED. Instrumental aspects are critically discussed and a comparison is detailed for the analysis of parathion in celery.	80
A range of plant foodstuffs (fruit and vegetables)	Pesticide residues	Cl (479 nm) S (181 nm) P (178 nm) N (174 nm) C (193 nm) F (690 nm)	A comparison of a number of GC detectors is given for the application of pesticide analysis in food stuffs. Detectors include : FPD/ ECD/ NPD/ MIP-AED/ MS.	81
Plant sap and wine	Organolead species	Pb (261 nm) Pb (406 nm)	A comparison is given of GC-MIP-AED with GC-QF-AAS and GC-GF-AAS. Particular attention is paid to factors affecting the accuracy of the analysis.	82
Wine, lees, burbs and grapes	Organolead species	Pb	Wine samples were obtained from a number of locations, including Southern France, California, Australia and Argentina. Organolead compounds were found in old vintages of French wine possibly of atmospheric origin from exhaust emissions.	83

Table 4b. Animal Analysis

SAMPLE MATRIX	COMPONENT OF INTEREST	ELEMENTS MONITORED	COMMENTS	REF.
Spiked methanolic extract of bird stomach oil deposits from Antarctica	Pesticide residues	C (248 nm) H (486 nm) Cl (479 nm)	Molecular formula determination of the components was attempted	53
Fish flesh	Alkyl tin species extracted in 0.5% tropolone in hexane	Sn (303 nm) C (193 nm) H (486 nm)	Optimisation of conditions for the analysis of organotin components.	75
Animal products / flesh	Pesticide residues	Cl (479 nm) S (181 nm) P (178 nm)	One of the first publications on the use of MIP detectors for GC	79
Dolphin liver	Di-methyl selenide	Se (204 nm)	The di-methyl selenide was collected on molecular sieve by heating before extracting into methanol prior to GC analysis	84
Stomach oil deposits from birds living in Antarctica, spiked with pesticides	Pesticide residues extractable into methanol	C (496 nm) H (486 nm) Cl (479 nm)	Application of the GC-MIP-AED to complex samples	85
Freeze dried tuna fish, dog fish muscle and mussels	Extractable methyl mercury iodide	Hg (254 nm)	Sample introduction was by headspace injection. Problems were observed with the extraction procedure. H_2SO_4 was used as a cleaving agent instead of iodoacetic acid as it increased the extraction efficiency.	86
Fresh swordfish, shark, shrimp and oysters. Canned clams and tuna	Methyl mercury chloride	Hg (254 nm)	The analysis was performed by ECD and also by MIP as the chloride derivative. Packed columns were used following pretreatment with mercury chloride. Comparable results were obtained for both techniques	87
Mussel reference material	Alkylmercury iodide derivatives	Hg (254 nm)	A detailed study of a number of columns used for alkyl mercury analysis is given. Sample introduction was performed using head space injection	88

SAMPLE MATRIX	COMPONENT OF INTEREST	ELEMENTS MONITORED	COMMENTS	REF.
The liver of a mouse intoxicated with methyl mercury	Alkyl mercury iodide derivatives	Hg (254 nm)	The sample was introduced by head space injection which had been shown to reduce column degradation. A study of halide derivatives was presented to evaluate which derivative gave the highest vapour concentration	89
Fish extract (flounder, mussel and tuna fish)	Methyl mercury chloride and methyl mercury cyanide	No emission wavelength specified	An intercomparison of methyl mercury determination for a number of laboratories is presented using a number of techniques including GC-MIP-AED.	90
Human blood	Butyl methyl mercury, butyl ethyl mercury and di-butyl mercury.	Hg (254 nm)	Species of interest are extracted using sodium diethyldithiocarbamate before butylation via a Grignards derivative	91

1.4.5 Summary

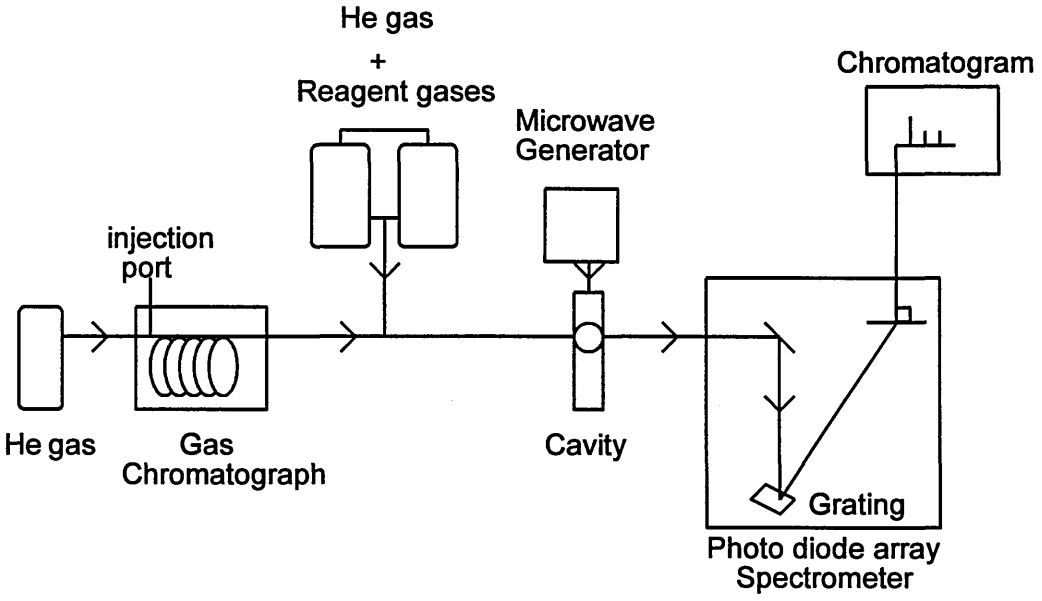
In summary it would appear that the GC-MIP-AED has a large part to play providing valuable information on environmental samples. It can provide corroboratory information to that from the ECD, FPD and NPD and complementary information to GC-MS and GC-FTIR systems, without suffering from chromatographic separatory problems because of the high elemental specificity exhibited. The reported ability of the GC-MIP-AED to provide qualitative and quantitative information for both organic and organometallic components at trace levels suggests the detector may be of great value in the field of environmental chemistry. Prior to 1994 the majority of organometallic analysis was performed on each metallic species in isolation, hence did not fully utilise the detection systems capabilities to perform multielemental analysis as shown for heteroatoms in organic compounds. Since 1994 a number of applications to speciate a number of organometallics simultaneously have been reported.

1.5 The Hewlett Packard GC-MIP-AED

1.5.1 Instrumental Development

The Hewlett Packard GC-MIP-AED consists of a gas chromatograph, to enable analyte separation, coupled to a re-entrant microwave cavity, which sustains the plasma source, and an optical spectrometer to measure the atomic emission of the elements being analysed. A schematic diagram of the GC-MIP-AED is shown in figure 1, and a diagram of the Hewlett Packard GC-MIP-AED instrumentation is shown in figure 2.

Figure 1. Schematic Diagram of the Hewlett Packard GC-MIP-AED

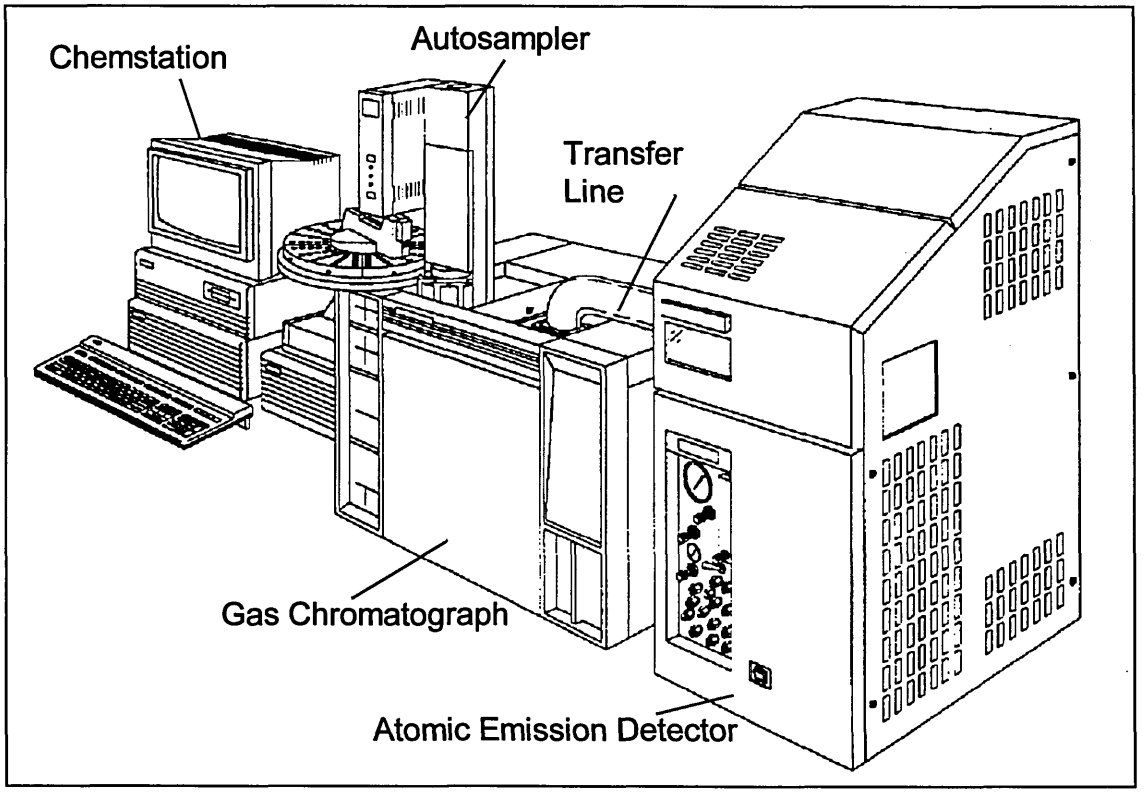


The gas chromatograph can utilise either capillary or packed columns in order to separate a mixture of analytes. A capillary column may easily be coupled to the cavity through a heated transfer line. However if packed column separation

is required this would have to be connected to a capillary guard column to allow for coupling to the cavity. The end of the capillary column is inserted directly into the cavity, via the heated transfer line, to allow the end of the column to be positioned approximately 10 mm from the plasma.

The cavity design is a re-entrant cavity which is based on the TM_{010} cavity described by Beenakker [22-24]. A plasma of helium gas is generated at atmospheric pressure by means of a magnetron which supplies the microwave radiation to the cavity. A basic description of the principle operating procedure has been given in a number of papers [41, 46, 92, 93].

Figure 2. Diagram of the Hewlett Packard GC-MIP-AED Instrumentation



A sample is introduced into the injection port (split / splitless), where it becomes vaporised before being carried through an analytical column in a flow of helium

carrier gas. The split / splitless inlet allows for two injection techniques. Splitless injection allows all of the injected sample to enter the analytical column and as such is the most applicable sample introduction technique for trace component environmental analysis. Split injection allows only a specified percentage of the sample to enter the analytical column. Other inlet techniques may also be used, such as, on-column and packed port as well as thermal desorption, head space injection and supercritical fluid coupling to the gas chromatograph.

Separation of the components in the sample takes place before they enter the discharge tube where subsequent atomisation and excitation takes place. The emitted radiation, at wavelengths characteristic of the elements and molecules concerned, pass through a window into the spectrometer where it is dispersed by a diffraction grating and the signals are monitored by a photo diode array (PDA). A portion of the spectrum is simultaneously monitored over a range of about 20 to 40 nm between 165-780 nm, allowing for the detection of a number of elements depending upon the location of the PDA in the focal plane. The spectral information obtained is transferred to a computer where it is stored for future data manipulation, elemental identification and chromatographic integration.

A number of features specific to this system and aimed at improving the selectivity, sensitivity and also reducing the amount of general maintenance have been reviewed by Quimby and Sullivan in a number of papers [26, 94-95] and are also discussed in a number of books [10-12]. These features include:

1) A water cooling system.

2) A solvent venting system.

3) A reagent gas introduction system.

4) A photo diode array detector incorporating real time background correction.

1.5.2 The Water Cooling System

In the past, for the majority of GC-MIP-AED systems, problems were always encountered with the quartz or ceramic tube used to contain the plasma [9, 96-100]. The extreme temperature generated by the plasma greatly weakened the tube causing it to break, and to require replacement approximately every seven days depending upon the system. Problems were also observed with the deposition of material on the inner surface of the discharge tube walls, again reducing the life time of the discharge tube [101]. Replacing the discharge tube is a major maintenance procedure which results in a large amount of instrument down time.

This was taken into account by Quimby and Sullivan who adapted the re-entrant cavity mentioned earlier in section 1.3. The cavity incorporated a water cooling system for the quartz discharge tube which contained the plasma [102]. Initially air was suggested as the coolant but it was found that the conduction of heat from the discharge tube material to the air was too slow to give sufficient cooling. A number of other materials were also considered, for example, hydrocarbons, freons, oils etc; but these also had drawbacks. If the coolant flow was interrupted then a carbon coating was observed on the outside of the

discharge tube. This had the adverse effect of shielding the energy produced by the cavity from the discharge tube and reducing the sensitivity of the detector. None of these problems occurred when water was used as the coolant. It was found necessary to electrically ground the flow path of the water to prevent the flow behaving like an electrical conductor and transmitting the microwave power away from the cavity preventing the formation of a microwave plasma.

The water cooling system also greatly reduced the erosion of the discharge tube wall and improved the chromatographic signal, as the tailing observed for the signal of some elements such as S and P was reduced. As a result of this modification, the discharge tube required replacing approximately every twenty eight days thereby reducing instrument down time.

1.5.3 The Solvent Venting Mechanism

Another major adaptation was the incorporation of a solvent venting mechanism which prevents the solvent from entering the plasma over a predetermined time period, usually at the beginning of the chromatogram. The problem of solvent entering the plasma was identified early in the development of the GC-MIP-AED by both McCormack *et al* [20] and Bache and Lisk [103]. As the solvent enters the plasma it may cause instability or even extinguish the helium discharge. Carbon deposits may also form on the inner surface of the discharge tube reducing sensitivity levels. Initially this was overcome by either reducing the amount of sample injected onto the analytical column or by delaying the ignition of the plasma. Neither of these two options were ideal. By

reducing the amount of sample injected, the sensitivity of the instrument was limited and delaying the ignition of the plasma may allow volatile unretained analytes to pass through the cavity undetected.

The solvent venting mechanism used in the Hewlett Packard GC-MIP-AED has been discussed and illustrated by Quimby and Sullivan [26, 104] and is shown in figures 3a and 3b. The solvent venting process is controlled by a back pressure regulator (BPR) and a switching valve. The helium gas from the column flow is supplemented by helium make up gas. A portion of this make up gas is used to purge the column connection to the cavity. The remaining make up gas plus the column effluent enters the plasma and combines with the spectrometer window purge gas when the solvent venting is off as shown in figure 3a. When the switching valve is adjusted to allow solvent venting to take place, the back pressure regulator draws the spectrometer window purge gas backwards through the plasma preventing the column effluent from entering the plasma over a predetermined time interval as shown in figure 3b.

As an additional feature the spectrometer window purge gas also prevents the back diffusion of air into the plasma, allowing the plasma make up gas to be operated at low flow rates while remaining stable. This is a major advantage for the analysis of some elements, such as, nitrogen, sulphur, bromine and chlorine as it was found that this improved the limit of detection, giving rise to greater sensitivity [26].

Figure 3a. Solvent Vent Off - Normal Mode of Operation.

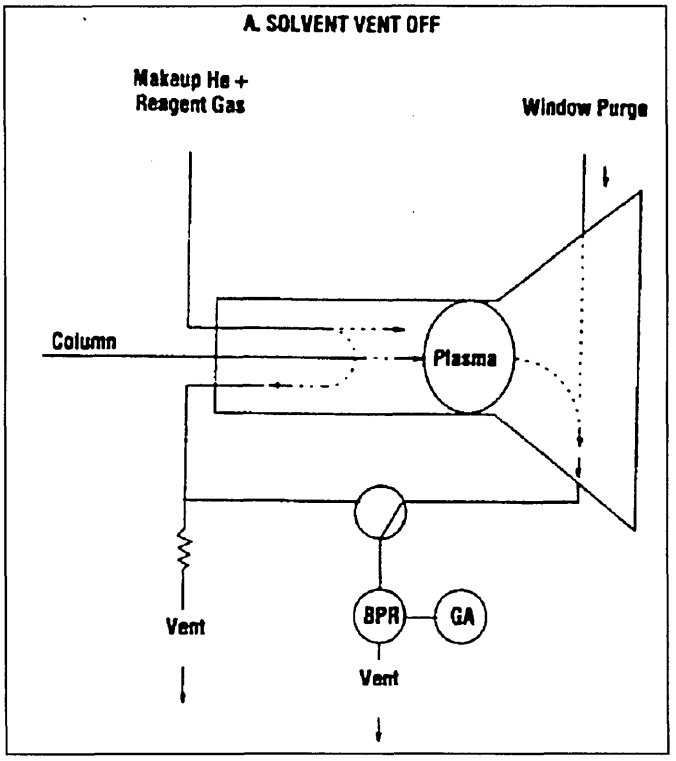
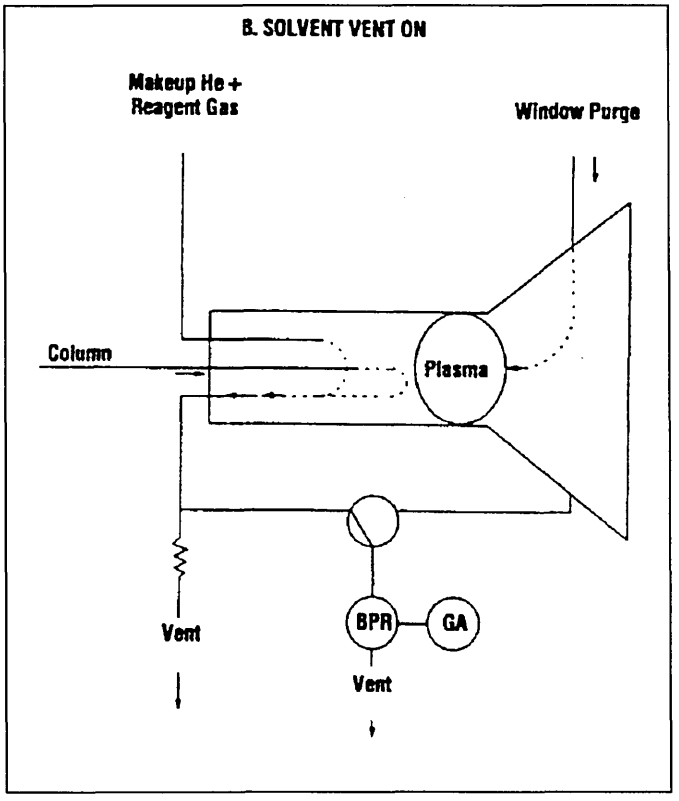


Figure 3b. Solvent Vent On.



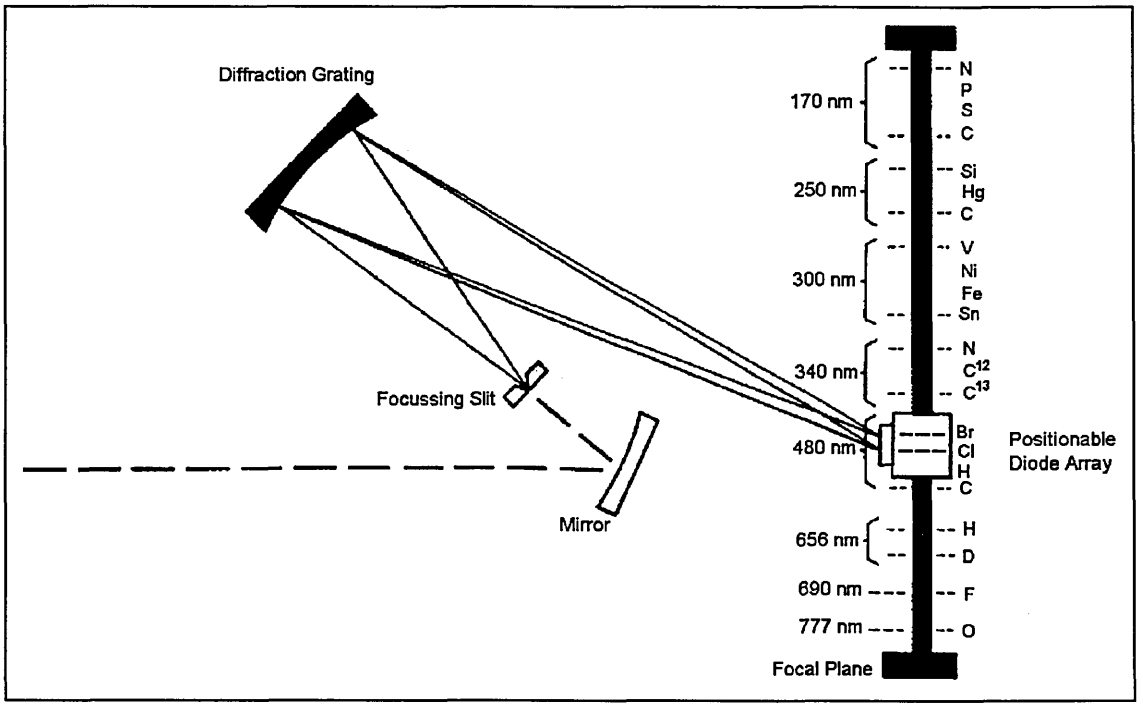
1.5.4 The Reagent Gas System

A computer controlled gas flow system allows reagent gases, also known as scavenger gases, to be blended with the make up gas. Different combinations of reagent gases are used for different elements [22, 26, 96, 105] - see appendix 1 for a list of elements with their respective wavelengths and reagent gases. The reagent gases used in the Hewlett Packard GC-MIP-AED are oxygen, hydrogen and 10% methane in nitrogen, and these can be varied independently and switched on or off in any combination. The reagent gases are added to keep the sample volatile, which helps to reduce carbon deposition, or to prevent oxide formation which may occur due to interactions between the sample components and the discharge tube wall [101]. Oxygen is the most effective reagent gas for removing carbon deposits so is often the best choice, but elements which form involatile oxides, such as tin or lead, need a different choice of reagent gas, such as hydrogen. When used in the correct combination the reagent gases can also help to further reduce tailing of the chromatographic signal observed for some elements such as sulphur, nitrogen, oxygen and hydrogen. For a number of elements, such as, phosphorus, tin and fluorine, when variation of the reagent gases did not improve the chromatographic signal it was found that, by increasing the plasma make up gas flow rate, a reduction in peak tailing was achieved [26]. This is due to the fact that a number of elements react with the oxygen in the walls of the silica discharge tube. By increasing the rate of the make up gas flow some atoms in elemental form travel far enough through the discharge tube to emit light into the spectrometer.

1.5.5 The Use of a Photodiode Array in Atomic Emission Detection

After the emitted radiation travels through the spectrometer window, it is focused through a slit, firstly onto a mirror before being separated using a concave holographic grating into its constituent wavelengths. The signal is then detected using a photo diode array (PDA). Ideally a PDA capable of covering the entire spectrum is required, but at the time this work was completed the technology was not commercially available without compromising selectivity and sensitivity, as the vast amount of data produced could not be acquired or stored by current computer systems. In this system a PDA is used only to monitor a small segment (between 20 and 40 nm) of the spectrum at a time but has the capacity to monitor the entire spectrum (165 - 780 nm), by multiple injections, in segments by moving along a flat focal plane hence allowing a high degree of selectivity and sensitivity to be achieved, see figure 4.

Figure 4. Schematic Diagram of the Photodiode Array Spectrometer

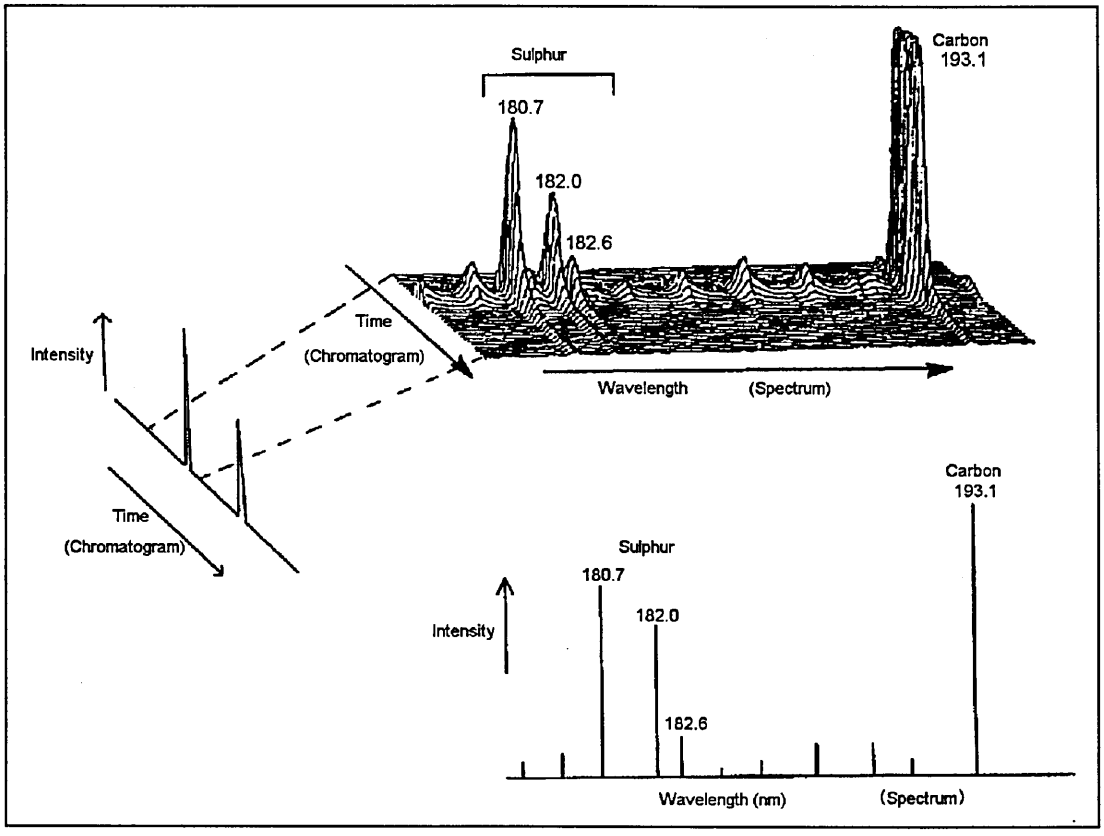


The PDA is composed of 211 detecting elements called pixels all responding simultaneously. Each pixel consists of a photodiode and a capacitor. When the emitted radiation is focused onto a pixel, photoelectrons are created which discharge the capacitor. The degree of discharge is proportional to photocurrent created. The emission intensity is determined by measuring the degree of capacitor discharge. The capacitor is recharged after each measurement. A large number of measurements are taken every second over each segment with the data being transfer to a computer. This capacity for simultaneous measurement shows an advantage of this instrument over scanning instruments as it is possible to take background measurements at exactly the same time as signal measurements. This allows for accurate background correction, which is the subtraction of undesirable emission signals such as molecular emission, which may occur in close proximity to the atomic emission signal of interest hence reducing the selectivity of that element. The process by which signal measurements and background corrections are made have been discussed by Sullivan and Quimby [11, 94-95].

The greatest advantage of this detector is its ability to verify elemental identity by the acquisition of numerous "snapshots" throughout the accumulation of each chromatogram. A "snapshot" is the portion of the optical spectrum recorded by the 211 pixels, every time a signal measurement is taken, therefore at any point in a chromatogram it is possible to obtain this portion of the spectrum, allowing verification from the characteristic emission lines for a specific element. This is illustrated in figure 5 for an organosulphur compound.

It is the ability to record these snapshots of data that is the basis for the entire detection process. The chromatographic signal recorded for each element is the result of a "recipe" created from the elemental emission lines for that particular element. Each "recipe" contains a matched filter to detect atomic emission for a specific element, for example, sulphur and also a matched filter for background correction which detects interfering background emission during a chromatogram. The matched filters used to record the sulphur chromatogram and the background signal are shown in figure 6.

Figure 5. The Ability of the GC-MIP-AED to Confirm Elemental Data from a Chromatographic Signal



The matched filters used by the GC-MIP-AED are software algorithms which are part of the data handling system. In the case of organosulphur compounds

the data signal from six pixels is recorded. Each signal is multiplied by a different weighting to produce a matched filter. Similarly a number of pixels, each with a different weighting, record the signal for background correction. The resulting signal from each matched filter is collected and stored.

Figure 7 shows the typical output of the sulphur element filter and the background filter. By subtracting a specific amount of the background signal the unwanted responses of interfering molecular emission can be suppressed. This specific amount is known as the background amount. If the background amount subtracted is too small then not all the interfering emission is removed. If too much background amount is subtracted then negative interfering peaks can occur. As the signal for each filter is stored separately the background amount can be optimised after the chromatogram has been acquired using the data processing software to obtain the best elemental selectivity possible. After adequate background subtraction, the chromatogram resulting from figure 7 would contain only one peak for an organosulphur compound eluting at 2.1 minutes.

One of the main advantages of this detector is its ability to produce "real time" multipoint background correction. This is "real time" in the sense that the background measurement is recorded at exactly the same time as the signal measurement and multipoint because a large number of pixels are used to record the background as a matched filter.

Figure 6. **Sulphur Matched Filter for (A) the Signal used to Produce the Chromatogram and (B) the Signal used for Background Correction**

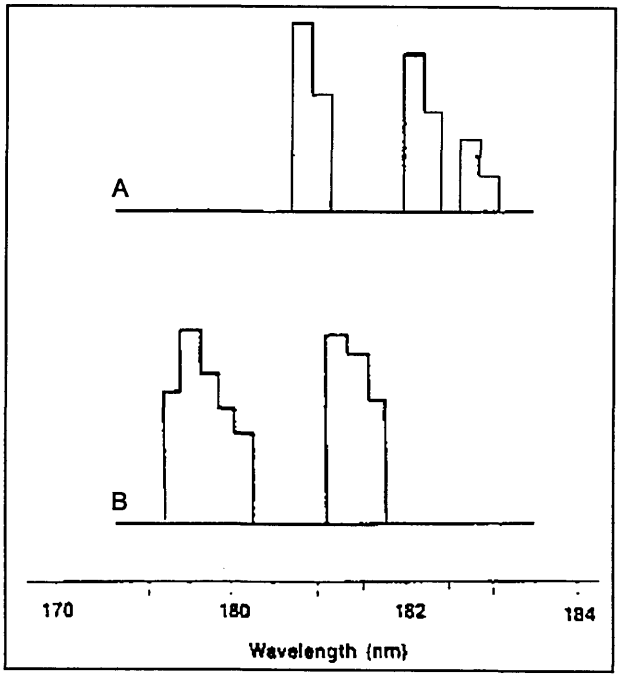
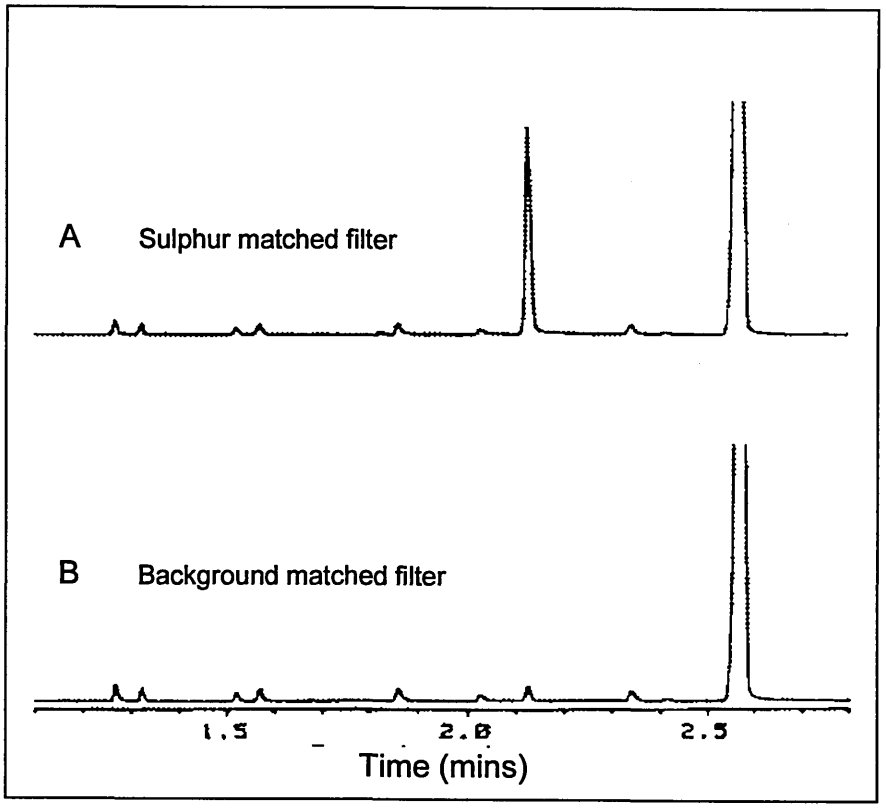


Figure 7. **Signals used to Create a Chromatogram for Organosulphur Compounds.**



The photodiode array also has the capability to use ultra violet (UV) and visible order sorting filters. For example, if a high wavelength line is being monitored, such as hydrogen at 468 nm, a UV filter will help remove interferences due to second or third order emission from other elements. Carbon emission at 193 nm is the primary emission line for GC-MIP-AED. If no filters are used and a number of wavelengths are monitored, for example, between 380 nm and 390 nm, a signal for carbon in the second order is observed at 386 nm. When a UV filter is applied to prevent emission from wavelengths below 300 nm reaching the photodiode array the signal at 386 nm due to carbon emission is no longer observed. The UV filter blocks light emitted below 280 nm so is generally applied for elements being monitored with wavelengths between 300 nm and 500 nm. Elements which have emission spectra above 500 nm generally require the use of a visible light filter. However if a number of elements are analysed simultaneously using wavelengths of say 271 nm and 303 nm the UV filter could not be applied as any emission at 271 nm would be filtered out. Hence the use of such filters is dependant upon the elements for which simultaneous analysis is required and also the degree of interference by second and third order emission.

In summary it is the ability to record three dimensional data and apply real time multipoint background correction that shows the GC-MIP-AED's superiority over other GC detectors such as the GC-FID or GC-ECD and similarity to GC-MS/GC-FTIR.

1.6 The Aims of the Research Project.

Initially the aim of this research was to gain familiarity with the Hewlett Packard GC-MIP-AED, which was purchased by Sheffield Hallam University in 1993. This involved the qualitative analysis of a range of environmentally significant samples and also a critical assessment of the instrumentation.

Secondly the capability of the instrument to provide quantitative information using simple laboratory prepared standards was assessed for the elements carbon, sulphur and nitrogen at 193 nm, 181 nm and 174 nm respectively in terms of:-

1) Precision
2) Limit of detection
3) Linearity
4) Selectivity

Finally when the instrument's capabilities had been assessed, the instrument was used to study trace organometallic compounds and their degradation products found in the environment, with particular attention being given to organomercury, lead and tin compounds with the objective of exploiting the simultaneous multielement capabilities of the GC-MIP-AED.

2.0 Experimental

2.1 Reagents and Materials

Oil Samples	Simon Petroleum Technology Ltd; Gwynedd
Coal samples	University of Leeds
2-Picoline standard 2-Picoline reaction mixture	Sheffield Hallam University
Haloxyfop (butyl ester derivative)	Dow Elanco, Oxfordshire
Leaded and unleaded petrol	ESSO garage, Sheffield
Commercially available Hewlett Packard test mix	Contains nitrobenzene, n-tridecane, 4-fluoroanisole, tert-butyldisulphide, 1,2,4-trichlorobenzene, tetraethyl orthosilicate, n-decane (perdeuterated), n-dodecane, n-tetradecane, 1-bromohexane, n-octane (isooctane as solvent)
Certified reference materials DORM-1 and PACS-1	Laboratory of the Government Chemist, The Office of Reference Materials
Water samples	Bottled mineral water, River Don water and a water sample provided by Analytical and Environmental Services, Tyne and Wear.

2.2 Analytical Instrumentation

2.2.1 GC-MIP-AED

Automatic sampler	HP 7673
Gas chromatograph	HP 5890 Series II with electronic pressure control
Detector	HP 5921A

Controlled by a HP ChemStation (Pascal based)

2.2.2 GC-MS

Gas chromatograph	HP 5890 Series I without electronic pressure control - Manual injection
Detector	VG Trio-1

Controlled by a computer controlled Dos based integration system

2.3 Analysis of Oil Samples

The oil samples were dissolved in 1 ml of dichloromethane prior to analysis.

GC Parameters

Split ratio :	Splitless (60 second purge delay)
Carrier gas flow rate :	1 ml / min helium
Constant flow :	On
Injection volume :	1 µl
Injection liner :	Single restriction liner
Injection temperature :	310°C
Column :	HP 5 (25 m x 0.32 mm I.D. x 0.5 µm film thickness)

Oven program : 120°C (1 min); 6°C / min to 210°C; 3°C / min to 300°C (10 min); 10°C / min to 325°C (20 min)

AED Parameters

Transfer line temperature : 330°C

Cavity temperature : 330°C

Ferrule purge vent flow : 30 ml / min

Makeup flow : 60 ml / min

Solvent vent : 2-6 min

Spectrometer purge flow : 2 l / min nitrogen

Elements analysed :

Element	Wavelength (nm)	Reagent gases
Carbon	193 nm	O_2, H_2
Sulphur	181 nm	O_2, H_2

FPD Instrumental Parameters

GC-FPD analysis performed at Simon Laboratories

Column : DB-5 fused silica 5% di-phenyl 95% di-methylsiloxane (12 m x 0.32 mm I.D. x 0.25 µm film)

Oven program : 80°C (1 min), 6°C / min to 300°C (10 min)

2.4 Analysis of Coal Pyrolyzate Samples

The pyrolysis sample introduction system was supplied by the University of Leeds. A 4 mg (approx.) lump of coal was introduced into the pyrolysis unit.

Pyrolysis Unit Parameters

Pyrolysis unit temperature : 850°C

Pyrolysis unit pressure : 10 psi helium

GC Parameters

Split ratio :	Splitless (60 second purge delay)
Carrier gas flow rate :	4.75 ml / min helium
Constant flow :	Off (pressure maintained by pyrolysis unit)
Injection liner :	Universal straight through liner
Injection temperature :	250°C
Column :	Supelcowax (10 m x 0.32 mm I.D. x 0.25 µm film thickness)
Oven program :	40°C (10 min); 20°C / min to 120°C (6 min); 20°C / min to 200°C (6 min); 20°C / min to 280°C (6 min)

AED Parameters

Transfer line temperature :	330°C
Cavity temperature :	330°C
Ferrule purge vent flow :	30 ml / min
Makeup flow :	60 ml / min
Solvent vent :	Off
Spectrometer purge flow :	2 l / min nitrogen

Elements analysed :

Element	Wavelength (nm)	Reagent gases
Carbon	193 nm	O_2, H_2
Sulphur	181 nm	O_2, H_2
Nitrogen	174 nm	O_2, H_2

2.5 Analysis of the Degradation Products of 2-Picoline

A sample of 2-picoline starting material and 2-picoline after it had undergone oxidation under a typical Fenton chemical reaction were provided for analysis by a colleague. Dichloromethane was used as a solvent.

GC Parameters

Split ratio :	Splitless (60 second purge delay)
Carrier gas flow rate :	1 ml / min helium
Constant flow :	On
Injection volume :	1 µl
Injection liner :	Single restriction liner
Injection temperature :	250°C
Column :	Supelcowax (10 m x 0.32 mm I.D. x 0.25 µm film thickness)
Oven program :	40°C; 10°C / min to 250°C (10 min)

AED Parameters

Transfer line temperature : 260°C

Cavity temperature :	260°C
Ferrule purge vent flow :	30 ml / min
Makeup flow :	60 ml / min
Solvent vent :	Off
Spectrometer purge flow :	2 l / min nitrogen

Elements analysed :

Element	Wavelength (nm)	Reagent gases
Carbon	193 nm	O_2, H_2
Nitrogen	174 nm	O_2, H_2
Oxygen	777 nm	AUX (10% methane in nitrogen), H_2

2.6 Analysis of Haloxyfop - Butyl Ester Derivative

A 1% solution of Haloxyfop-butyl ester in dichloromethane was prepared.

GC Parameters

Split ratio :	50:1
Carrier gas flow rate :	2 ml / min helium
Constant flow :	On
Injection volume :	1 µl
Injection liner :	Single restriction liner
Injection temperature :	330°C
Column :	HP 5 (25 m x 0.32 mm I.D. x 0.5 µm film thickness)
Oven program :	140°C (1 min); 10°C / min to 315°C (5 min)

AED Parameters

Transfer line temperature : 330°C

Cavity temperature : 330°C

Ferrule purge vent flow : 30 ml / min

Makeup flow : 60 ml / min

Solvent vent : 2-4 min

Spectrometer purge flow : 2 l / min nitrogen

Elements analysed :

Element	Wavelength (nm)	Reagent gases
Carbon	193 nm	O_2, H_2
Nitrogen	174 nm	O_2, H_2
Chlorine	479 nm	O_2
Fluorine	690 nm	H_2
Oxygen	777 nm	AUX (10% methane in nitrogen), H_2

2.7 Analysis of Leaded and Unleaded Petrol Samples

The petrol samples were injected neat.

GC Parameters

Split ratio : 100:1

Carrier gas flow rate : 1 ml / min helium

Constant flow : On

Injection volume : 1 µl

Injection liner : Single restriction liner

Injection temperature : 250°C

Column : HP 1 (25 m x 0.32 mm I.D. x 0.17 µm film thickness)

Oven program : 35°C (5 min); 10°C / min to 300°C (5 min)

AED Parameters

Transfer line temperature : 300°C

Cavity temperature : 300°C

Ferrule purge vent flow : 30 ml / min

Makeup flow : 60 ml / min

Solvent vent : 0-2 min

Spectrometer purge flow : 2 l / min nitrogen

Elements analysed :

Element	Wavelength (nm)	Reagent gases
Carbon	193 nm	O_2, H_2
Sulphur	181 nm	O_2, H_2
Carbon	496 nm	O_2
Chlorine	479 nm	O_2
Bromine	478 nm	O_2
Lead	406 nm	O_2, H_2, Fast flow
Oxygen	777 nm	AUX (10% methane in nitrogen), H_2

2.8 Analysis of Octane Thiol Standards in Hexane

A 10,000 ng / µl stock standard solution of octane thiol in hexane was prepared. From this stock a range of dilutions were prepared. (500 ng / µl - 1 pg / µl)

GC Parameters

Split ratio :	Splitless (60 second purge delay)
Carrier gas flow rate :	1 ml / min helium
Constant flow :	On
Injection volume :	1 µl
Injection liner :	Assorted liners were used as detailed in chapter 3
Injection temperature :	250°C
Column :	HP 1 (25 m x 0.32 mm I.D. x 0.17 µm film thickness)
Oven program :	35°C (5 min); 10°C / min to 280°C

AED Parameters

Transfer line temperature :	300°C
Cavity temperature :	300°C
Ferrule purge vent flow :	30 ml / min
Makeup flow :	60 ml / min
Solvent vent :	0-2 min
Spectrometer purge flow :	2 l / min nitrogen

Elements analysed :

Element	Wavelength (nm)	Reagent gases
Carbon	193 nm	O_2, H_2
Sulphur	181 nm	O_2, H_2

2.9 Analysis of Commercially Available Hewlett Packard Test Mix

The test mix was analysed as received.

GC Parameters

Split ratio : 36:1

Carrier gas flow rate : 2.8 ml / min helium

Constant flow : On

Injection volume : 1 µl

Injection liner : Assorted liners were used as detailed in chapter 3.

Injection temperature : 250°C

Column : HP 1 (25 m x 0.32 mm I.D. x 0.17 µm film thickness)

Oven program : 60°C; 30°C / min to 180°C

AED Parameters

Transfer line temperature : 250°C

Cavity temperature : 250°C

Ferrule purge vent flow : 30 ml / min

Makeup flow : 60 ml / min

Solvent vent : 0-2 min

Spectrometer purge flow : 2 l / min nitrogen

Elements analysed :

Element	Wavelength (nm)	Reagent gases
Carbon	193 nm	O_2, H_2
Nitrogen	174 nm	O_2, H_2
Sulphur	181 nm	O_2, H_2

2.10 Analysis of Organometallic Species

A 1000 ng / µl stock solution of each organometallic species was prepared in either toluene or methanol and were stored at 4°C in the dark. These stock solutions were diluted to give a range of solutions between 0.1 pg / µl and 1 ng / µl. The stock solutions prepared in methanol were used to prepare aqueous solutions. All organometallic sample preparation procedures were performed in silanised glassware to prevent losses due to adsorption on to the surface of the glass.

A deviation from the standard software recipe was made for the simultaneous multielemental analysis of organolead, organotin and organomercury compounds. A cavity gas flow rate of 290 ml / min was observed to be required, particularly for the analysis of organolead and organotin compounds. The increase in the flow rate was found to have no adverse effects on organomercury quantitation. This requirement will be discussed further in chapters 5 and 6. This is an increase from the software standard fast flow option of an additional 90 ml / min.

To enable the identification of each organometallic species by GC-MS, a 1000 ng / µl stock solution was prepared in toluene, derivatised and analysed.

2.10.1 GC-MIP-AED Conditions

Column 1

GC Parameters

Split ratio :	Splitless injection (40 second purge delay)
Carrier gas flow rate :	2.3 ml / min helium
Constant flow :	On
Injection volume :	1 µl
Injection liner :	Single restriction liner.
Injection temperature :	250°C
Column :	HP 1 (30 m x 0.32 mm I.D. x 0.17 µm film thickness)
Oven program :	50°C (3 min); 5°C / min to 190°C, 40°C / min to 300°C (2.0 min)

Column 2

GC Parameters

Split ratio :	Splitless injection (40 second purge delay)
Carrier gas flow rate :	2.3 ml / min helium
Constant flow :	On
Injection volume :	1 µl
Injection liner :	Single restriction liner.

Injection temperature : 250°C

Column : RTX 1 (30 m x 0.32 mm I.D. x 0.5 µm film thickness) plus 5 m inert fused silica guard column.

Oven program : 50°C (7.2 min); 10°C / min to 250°C, 30°C / min to 300°C (1.0 min)

AED Parameters

Transfer line temperature : 300°C

Cavity temperature : 300°C

Ferrule purge vent flow : 30 ml / min

Makeup flow : 290 ml / min

Solvent vent : 0-5.0 min (Column 1)

0-7.5 min (Column 2)

Spectrometer purge flow : 2 l / min nitrogen

Elements analysed :

Element	Wavelength (nm)	Reagent gases
Mercury	254 nm	O_2, H_2
Lead	261 nm	O_2, H_2
Tin	271 nm	O_2, H_2

NB If organomercury species was monitored in isolation then the AED Make Up flow was reduced to 60 ml / min.

2.10.2 GC-MS Conditions

Column 1

GC Parameters

Split ratio :	Splitless injection (40 second purge delay)
Carrier gas flow rate :	2.3 ml / min helium
Constant pressure :	On
Injection volume :	1 µl
Injection liner :	Single restriction liner.
Injection temperature :	250°C
Column :	HP 1 (30 m x 0.32 mm I.D. x 0.17 µm film thickness)
Oven program :	50°C (3 min); 5°C / min to 190°C, 40°C / min to 300°C (2.0 min)

MS Parameters

MS Mode:	Scan
Ionisation mode:	Electron ionisation (+)
Solvent delay:	3.0 min
Scanning control	Start mass - 35, End mass - 500
Scan time	0.9 min
Interscan time	0.1 min

Column 2

GC Parameters

Split ratio :	Splitless injection (40 second purge delay)
Carrier gas flow rate :	2.3 ml / min helium
Constant pressure :	On
Injection volume :	1 µl
Injection liner :	Single restriction liner.
Injection temperature :	250°C
Column :	RTX 1 (30 m x 0.32 mm I.D. x 0.5 µm film thickness) plus 5 m inert fused silica guard column.
Oven program :	50°C (7.2 min); 10°C / min to 250°C, 30°C / min to 300°C (1.0 min)

MS Parameters

MS Mode:	Scanning
Ionisation mode:	Negative chemical ionisation (NCI) using isobutane
Solvent delay:	8.0 min
Scanning control	Start mass - 60, End mass - 700
Scan time	0.9 min
Interscan time	0.1 min

MS Mode:	Selected ion monitoring
Ionisation mode:	Electron ionisation
Solvent delay:	8.0 min

Interchannel delay: 0.02 min

Mass value monitored:

8.0-20.5 min	200, 202, 207, 208, 236, 237, 286, 288, 342, 344
20.5-30.0 min	119, 121, 289, 291, 303, 305, 317, 319, 331, 333

A comparison between GC-MS and GC-MIP-AED was achieved by the determination of retention indices. Although the same analytical columns and oven programs were used on both instruments, a direct comparison of retention times was not possible as the GC-MIP-AED was operated using constant flow whereas the GC-MS was operated using constant pressure.

2.10.3 Extraction Procedures

a) Aqueous Samples

35 ml of aqueous sample was added to a 100 ml volumetric flask by pipette. 2 ml of chelation reagent was added and the flask stoppered and shaken for 10 min on a mechanical shaker. 3.5 ml of toluene was added by pipette and the flask stoppered and shaken for a further 10 min. The toluene layer was collected for derivatisation.

b) Sediment Certified Reference Material Analysis

Approximately 0.5 g of sediment was accurately weighed into a centrifuge tube. 5 ml of Milli Q water and 5 ml of Aristar HCl was added by pipette and the tube sealed. The tubes were shaken on a mechanical shaker for 30 min. The pH

was then adjusted to 8.5 using the conc ammonia solution and narrow range indicator paper. Then 2 ml of chelation reagent was added, the tube sealed, and shaken on a mechanical shaker for 30 min. Next 5 ml of toluene was added by pipette, the tubes resealed, and shaken for a further 30 min. The samples were then centrifuged at 18,000 rpm for 30 min and the toluene layer collected for derivatisation. A further 5 ml of toluene was added by pipette to the aqueous phase and the tubes shaken for 30 min. The tubes were then centrifuged for a further 30 min and the toluene layer added to the first layer. The procedure was followed for a third time to give approximately 15 ml of toluene extract.

c) Dog Fish Muscle (fish tissue) Certified Reference Material Analysis

Approximately 0.5 g of fish tissue was accurately weighed into a centrifuge tube. 5 ml of Milli Q water and 5 ml of Aristar HCl was added by pipette and the tube sealed. The tubes were shaken on a mechanical shaker for 30 min. The pH was then adjusted to 8.5 using the conc ammonia solution and narrow range indicator paper. Then 2 ml of chelation reagent was added, the tube sealed, and shaken on a mechanical shaker for 30 min. Next 5 ml of toluene was added by pipette, the tubes resealed, and shaken for a further 30 min. The samples were then centrifuged at 18,000 rpm for 30 min and the toluene layer collected for derivatisation. A further 5 ml of toluene was added by pipette to the aqueous phase and the tubes shaken for 30 min. The tubes were then centrifuged for a further 30 min and the toluene layer added to the first layer.

Ammonia / Citrate Buffer (pH 8.5)

20.6 g of citric acid was dissolved in 950 ml Milli Q H_2O. The pH was adjusted to 8.5 with concentrated ammonia and the solution made up to 1 l with Milli Q H_2O.

Chelation Reagent - Prepared just prior to Analysis. (25 ml)

2.94 g Sodium diethyldithiocarbamate ($NaDDTC.3H_2O$) was dissolved in the buffer solution and made up to 25 ml. This was purified by shaking the reagent with 2 x 5 ml extractions for 10 min with toluene which removed any organometallics from the reagent. The toluene layers were then discarded.

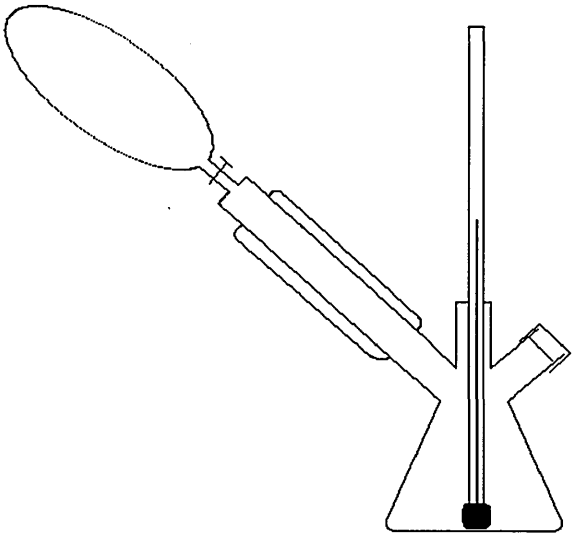
2.10.4 Derivatisation Procedure

2 ml of the toluene layer from the extraction procedure, or 2 ml of standard was accurately pipetted into a 25 ml conical bottomed 3 necked flask, with a thermometer and condenser attached in 2 necks and a rubber suba-seal in the third - See figure 8.

The apparatus was placed under nitrogen and 1 ml of 2.0 M Grignard reagent was injected (butylation - butyl magnesium chloride in tetrahydrofuran / pentylation - pentyl magnesium bromide in diethylether). The reaction flask was placed in an ice bath and then left for 30 min before the addition of 3 ml of either 0.5 M sulphuric acid or 20 % ammonium chloride solution. This was added slowly to prevent sudden temperature change (the temperature was kept

below 20 °C at all times to reduce losses). The organic layer was collected, dried over magnesium sulphate, filtered under vacuum and analysed (1 µl injection).

Figure 8. Apparatus used for the Derivatisation Procedure



Quantification was achieved by comparison with an external standard and recoveries determined against certified reference materials.

Chapter 3

Preliminary Practical Experience

with the Hewlett Packard

GC-MIP-AED

3.0 Preliminary Practical Experience with the Hewlett Packard GC-MIP-AED

3.1 Introduction

In this chapter a basic overview of the instrument is presented. The theoretical abilities of the instrument are tested both qualitatively and quantitatively for a number of elements. The selectivity of the instrument is demonstrated using real samples and the precision, linear dynamic range and limit of detection for carbon, sulphur and nitrogen is demonstrated using simple laboratory prepared standards. A number of practical instrumental difficulties are also highlighted.

3.2 Qualitative Analysis

In order to gain familiarity with the GC-MIP-AED system and assess its suitability for environmental applications, a number of samples of environmental relevance were analysed qualitatively. In this section the selectivity of the detector was evaluated i.e. the extent of molecular emission breaking through onto the channel of interest and causing a signal to be detected.

The samples analysed included:-

1)	Oils		(C, S)
2)	Coal Pyrolyzates		(C, S, N)
3)	Degradation products of 2-picoline		(C, O, N)
4)	Pesticides		(C, F, Cl, N, O)
5)	Petrols	a) Leaded	(C, O, Pb, Cl, Br, S)
		b) Unleaded	(C, O, Pb, Cl, Br, S)

In general satisfactory results were obtained although a number of major practical difficulties were encountered with the instrumentation - see section 3.4

3.2.1 Oil Analysis

A number of oil samples in dichloromethane were submitted for quantitative sulphur analysis by Simon Laboratories, with the main compounds of interest being a series of alkylbenzothiophenes. These compounds are found predominantly in sulphur rich kerogens and have been previously studied in detail by Sinninghe Damsté *et al* [106].

Each sample was provided with the corresponding FPD chromatogram, with the peaks of interest identified, and chromatographic conditions.

Although only the sulphur chromatogram was necessary, the multielement capabilities of the instrument were used by monitoring both the carbon and the sulphur emission lines at 193 nm and 181 nm simultaneously as 193 nm and 181 nm are both within the range of the photodiode array.

As only one standard could be provided, it was essential that the retention behaviour of the sample was maintained to allow for identification of the relevant peaks from the FPD chromatogram. Initially the same column and oven program was used but poor chromatographic separation was observed. In order to improve the chromatography the initial oven temperature was elevated

from 80°C to 120°C and the temperature ramp was reduced from 6°C / min to 3 °C / min when the components of interest were eluting. These changes did not effect the relative retention behaviour of the alkylbenzothiophenes as the same column stationary phase was used.

Figure 9a shows the FPD chromatogram for a representative sample and figure 9b shows the GC-MIP-AED chromatograms for carbon and sulphur obtained from the same sample.

All the peaks obtained on the sulphur channel were confirmed from spectral data and are identified on each chromatogram as described in section 1.5.5.(see appendix 2 for an example of a sulphur snapshot).

A high degree of selectivity was observed on the sulphur channel with no apparent breakthrough of molecular emission from carbon. The major difference between the FPD and the GC-MIP-AED chromatograms appears to be a higher level of noise on the GC-MIP-AED chromatogram, which could be attributed to different sample concentrations or split ratio.

Figure 9a. FPD Chromatogram Obtained from an Oil Sample.

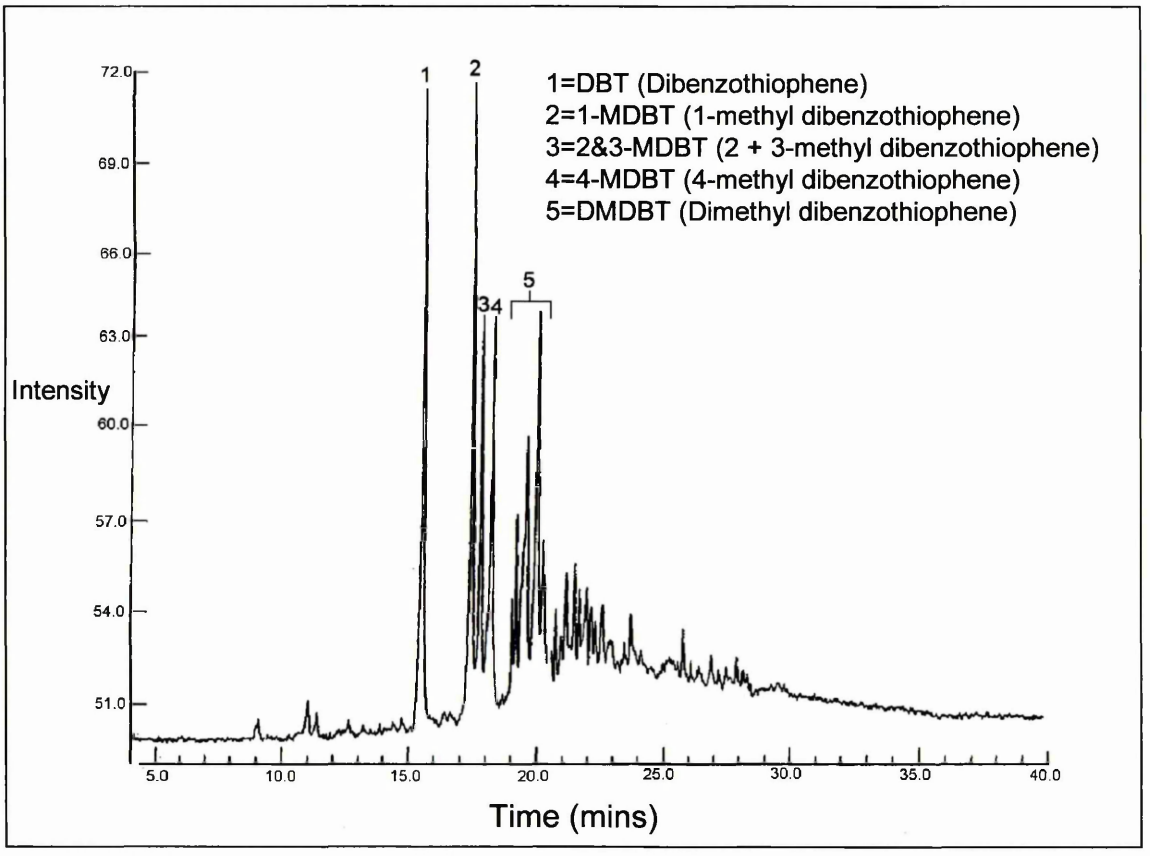
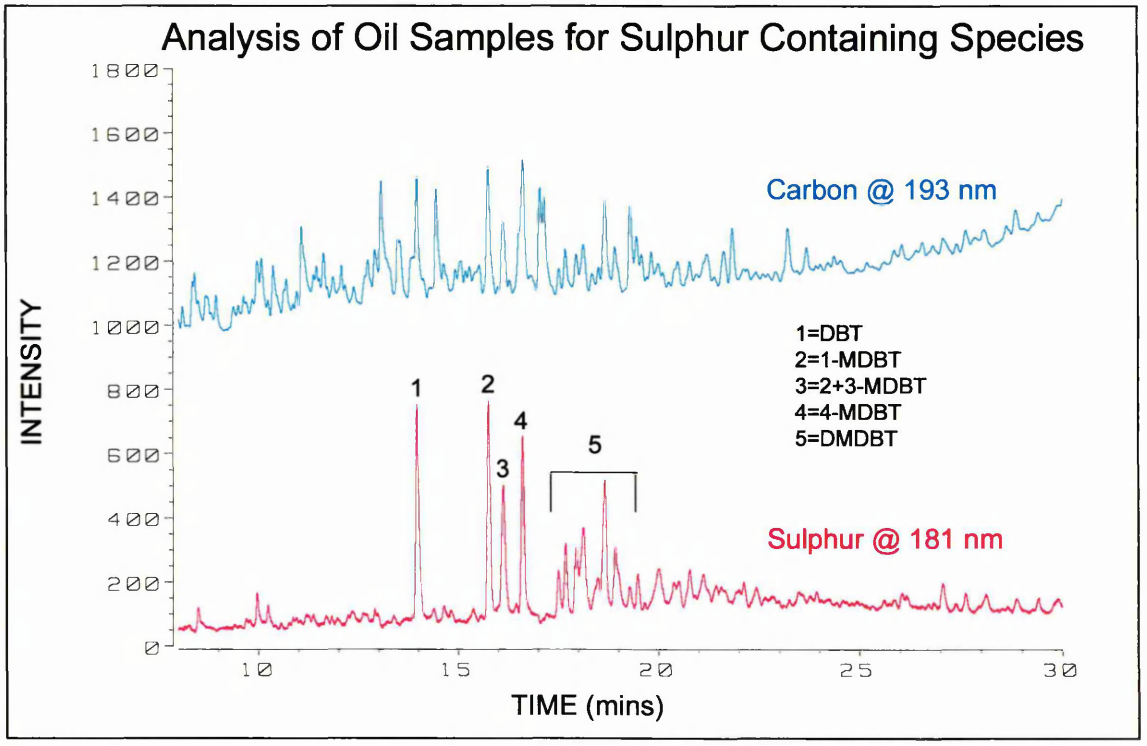


Figure 9b. GC-MIP-AED Chromatogram for the same Oil Sample



3.2.2 Analysis of Coal Pyrolyzates

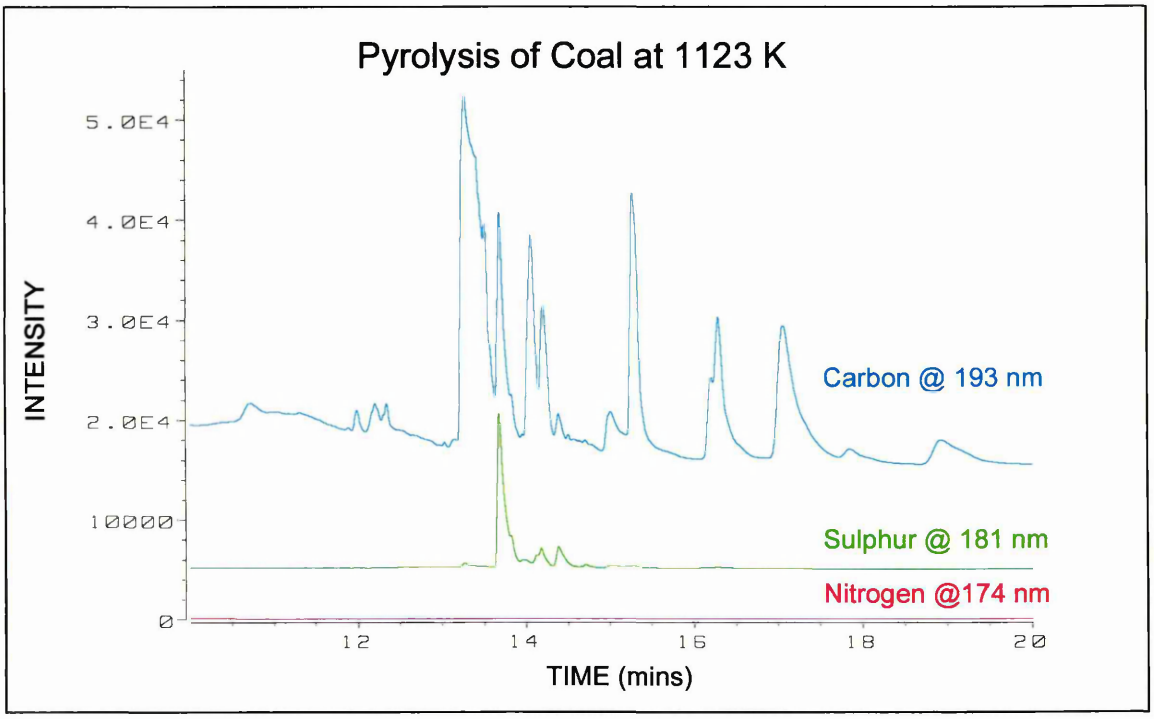
GC has been previously used for the analysis of coal products [107-108]. The use of pyrolysis as a sample introduction technique for GC has previously been applied to a number of sample matrices such as coals and sediments [43], kerogens [106] and polymers [30-33], and provides an effective means of investigating insoluble organic fractions with minimal sample pre-treatment.

The coal samples and the pyrolysis equipment were loaned from Professor K. Bartle at the University of Leeds. 4 mg of a coal sample was introduced into the pyrolyser which was held at 850°C and 10 psi. Any volatile components became volatilised and were swept onto the head of the column. The pyrolyzates were monitored for carbon, sulphur and nitrogen emission simultaneously at 193 nm, 181 nm and 174 nm.

Figure 10 shows the chromatograms of carbon, sulphur and nitrogen obtained as preliminary data for a typical sample. This particular data shows very poor peak shapes and band broadening, possibly due to column overload. The application was not progressed further as the pyrolysis unit was only available for a short period of time. A high degree of selectivity is observed for sulphur showing no signals due to molecular emission. The sample showed three bands of sulphur containing components, possibly dialkyl thioether and thiophene containing compounds, at t_R = 13.7, 14.2 and 14.4 mins, all of which were confirmed as sulphur containing species from spectral data. A typical snapshot for sulphur at 181 nm is shown in appendix 2. No nitrogen containing

species were observed which was unusual as nitrogen containing compounds were expected, such as, aromatic nitriles, pyridines and pyrroles.

Figure 10. GC-MIP-AED Chromatogram of Coal Pyrolyzates showing the Carbon, Sulphur and Nitrogen Channels Monitored Simultaneously.



3.2.3 Analysis of the Degradation Products of 2-Picoline

Wet air oxidation in conjunction with Fenton chemistry is currently undergoing research for its applicability to the disposal of waste chemicals. Wet air oxidation is a waste treatment process intended to break down organic molecules in aqueous solution to simple biodegradable species at elevated temperature and pressures. Fenton reagent is a mixture of acidified aqueous hydrogen peroxide and a ferrous salt. This mixture can produce hydroxyl radicals which help to catalyse the oxidation process.

A typical Fenton chemistry reaction was performed in the laboratory using $CuSO_4$ in addition to $FeSO_4$ and H_2O_2 on 2-picoline and the reaction products extracted before the sample was submitted [109]. Elemental characterisation was performed using the GC-MIP-AED for the elements carbon, nitrogen and oxygen at 193 nm, 174 nm and 777 nm. The carbon and nitrogen chromatograms were obtained simultaneously while the oxygen emission chromatogram was obtained from a further injection.

Figure 11a shows the carbon, oxygen and nitrogen emission chromatograms for the 2-picoline starting material and figure 11b shows the corresponding emission chromatograms following the oxidation process.

It can be seen that a large number of less volatile more complex compounds have been formed during the reaction. Four predominant species containing both oxygen and nitrogen are observed at t_R = 6.1, 12.2, 13.2 and 18.2 mins, while in contrast a group of 5 components eluting between 13.9 and 15.2 mins contain only nitrogen. Most surprising however is the appearance of a number of components, for example, at t_R = 15.5 mins which do not contain nitrogen. Examples of O and N snapshots used for elemental confirmation are given in appendix 2.

Figure 11a. GC-MIP-AED Chromatogram for 2-Picoline

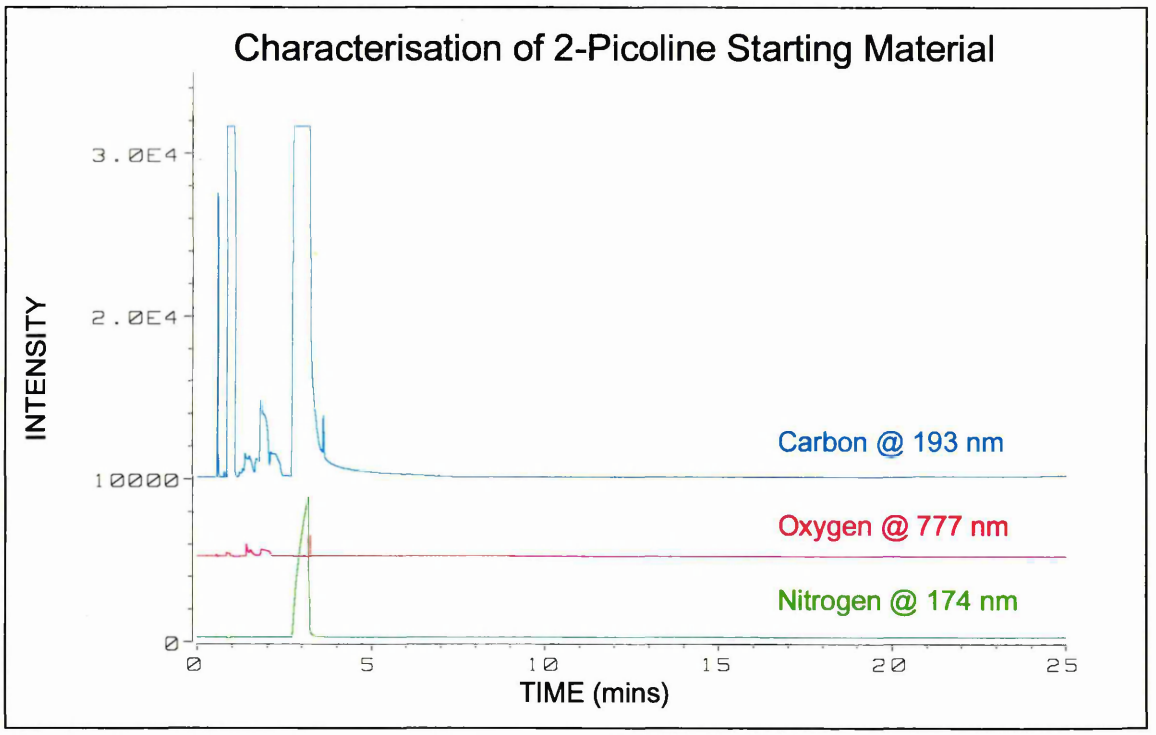
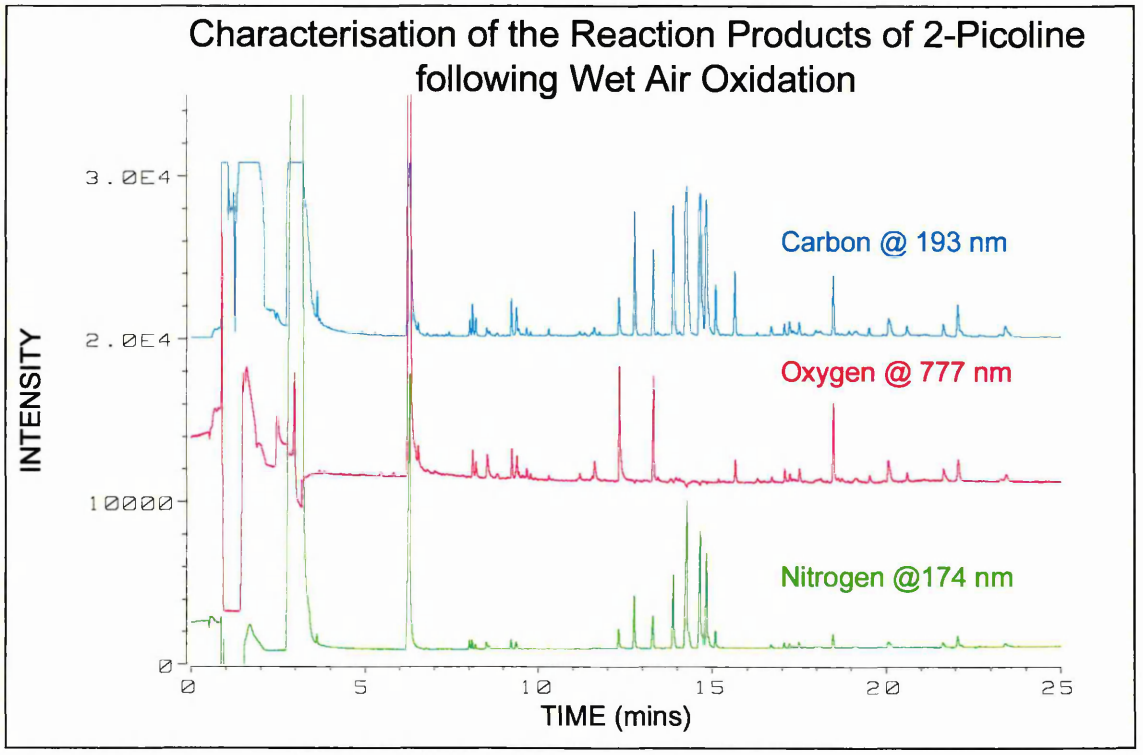
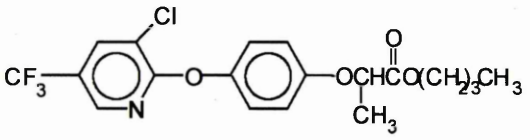


Figure 11b. GC-MIP-AED Chromatogram for the Reaction Products of 2-Picoline following a Wet Air Oxidation Process



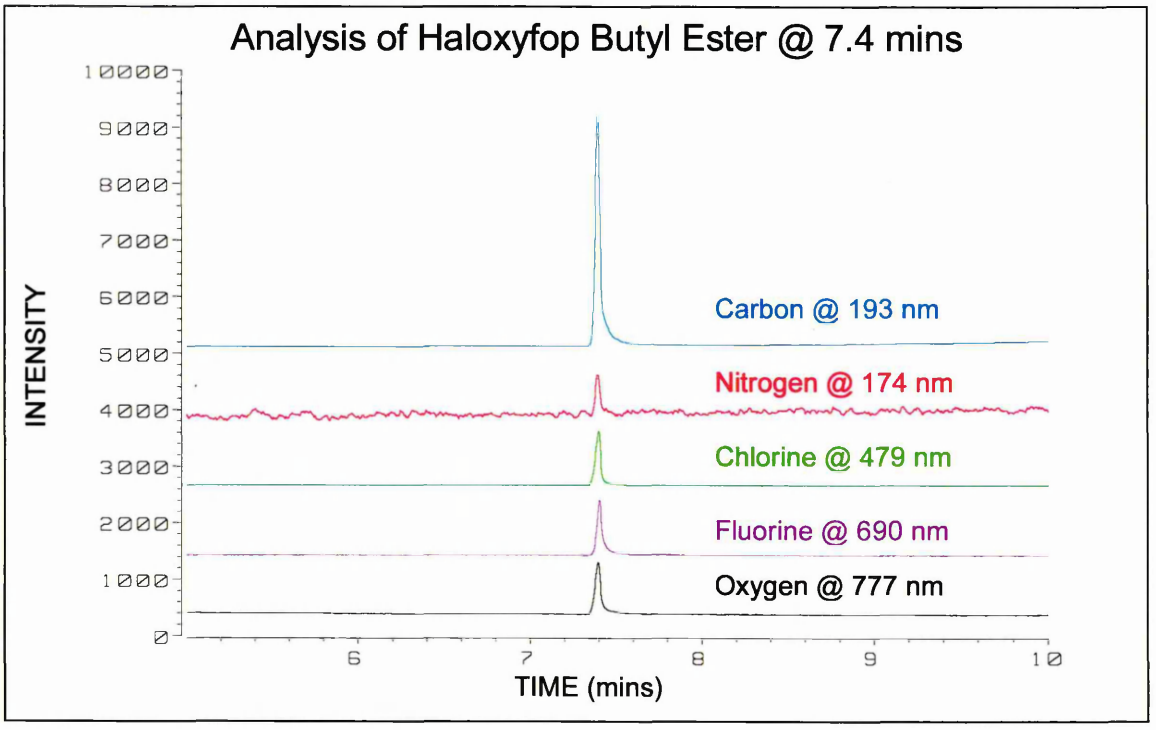
3.2.4 Pesticide Analysis

A butyl ester derivative of the pesticide Haloxyfop ($C_{19}H_{19}F_3ClNO_4$) was provided by Dow Elanco, Oxfordshire.



In this instance a number of elemental emission wavelengths were monitored which required 4 injections. The elemental channels monitored include carbon (193 nm) and nitrogen (174 nm), both of which could be monitored with one injection, and chlorine (479 nm), fluorine (690 nm) and oxygen (777 nm), each of which required separate injections. See figure 12 for the elemental emission chromatograms.

Figure 12. GC-MIP-AED Chromatogram for Haloxyfop Butyl Ester



A snapshot was taken for the signal on each channel giving elemental confirmation. A typical snapshot for each of the elements can be found in appendix 2.

3.2.5 Analysis of Leaded and Unleaded Petrol

Leaded and unleaded petrol were the most complex samples to be studied in terms of the number of elements monitored. An attempt to characterise the samples by producing a fingerprint chromatogram for a number of elements for each type of petrol, including carbon, sulphur, oxygen, bromine, chlorine and lead, was made.

Figure 13a(i) and figure 13a(ii) shows the chromatograms of leaded petrol and unleaded petrol for carbon, sulphur and oxygen at 193 nm, 181 nm and 777 nm.

Figure 13b shows a direct comparison of the sulphur containing species for leaded and unleaded petrol at 181 nm, with figure 13c showing a direct comparison of the oxygen containing species for leaded and unleaded petrol at 777 nm.

Figures 13a(i) and 13a(ii) highlight the ability of the GC-MIP-AED to analyse complex samples. A number of organosulphur compounds are evident in both petrols, whereas oxygenates are predominant in unleaded petrol.

Figure 13a(i). GC-MIP-AED Chromatogram of Leaded Petrol for Carbon, Sulphur and Oxygen

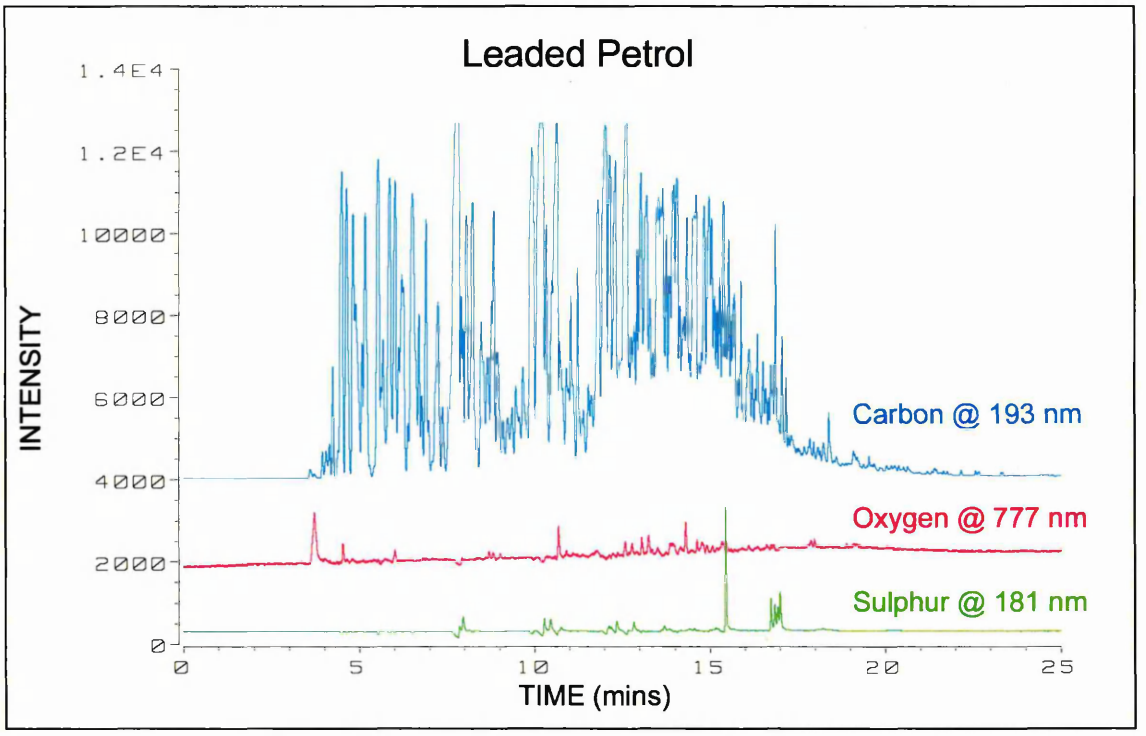


Figure 13a(ii). GC-MIP-AED Chromatogram of Unleaded Petrol for Carbon, Sulphur and Oxygen

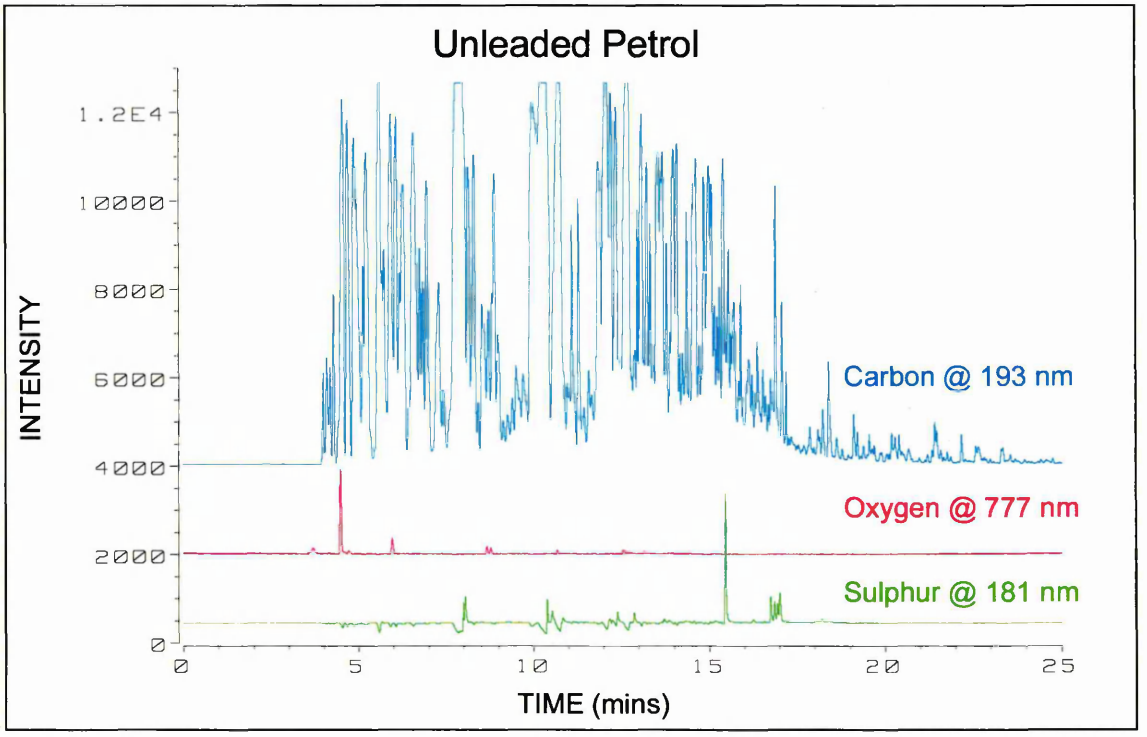


Figure 13b. GC-MIP-AED Chromatogram showing a comparison of Sulphur containing Species for Leaded and Unleaded Petrol

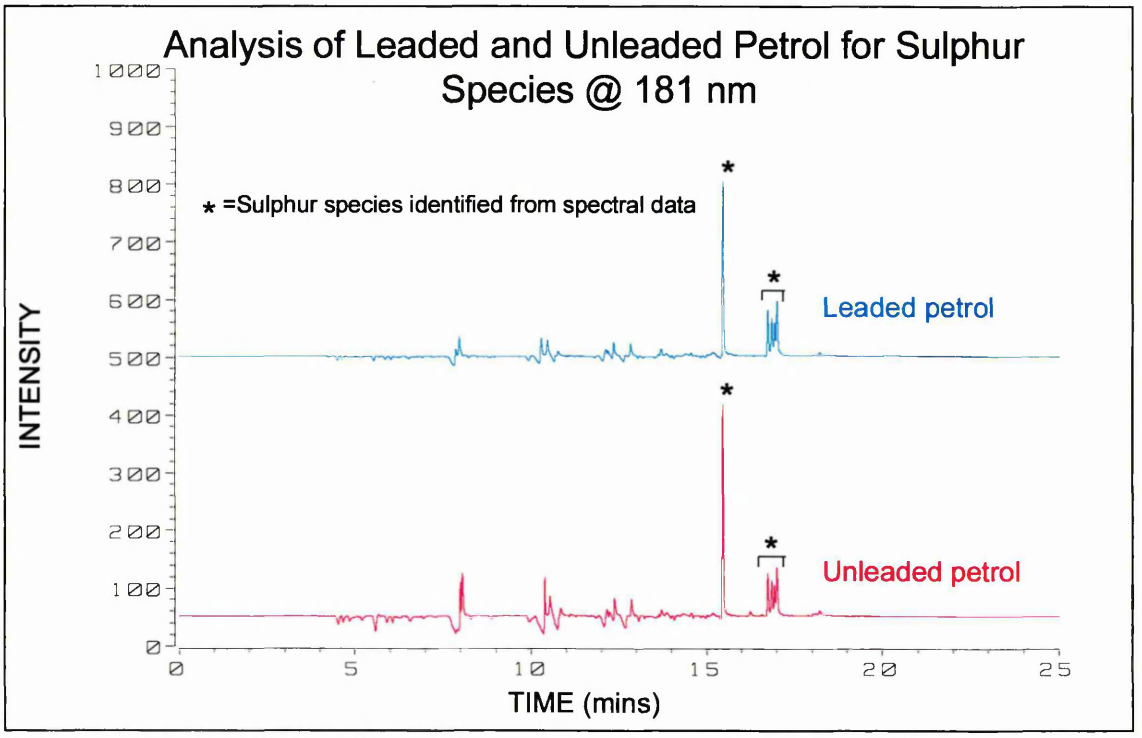
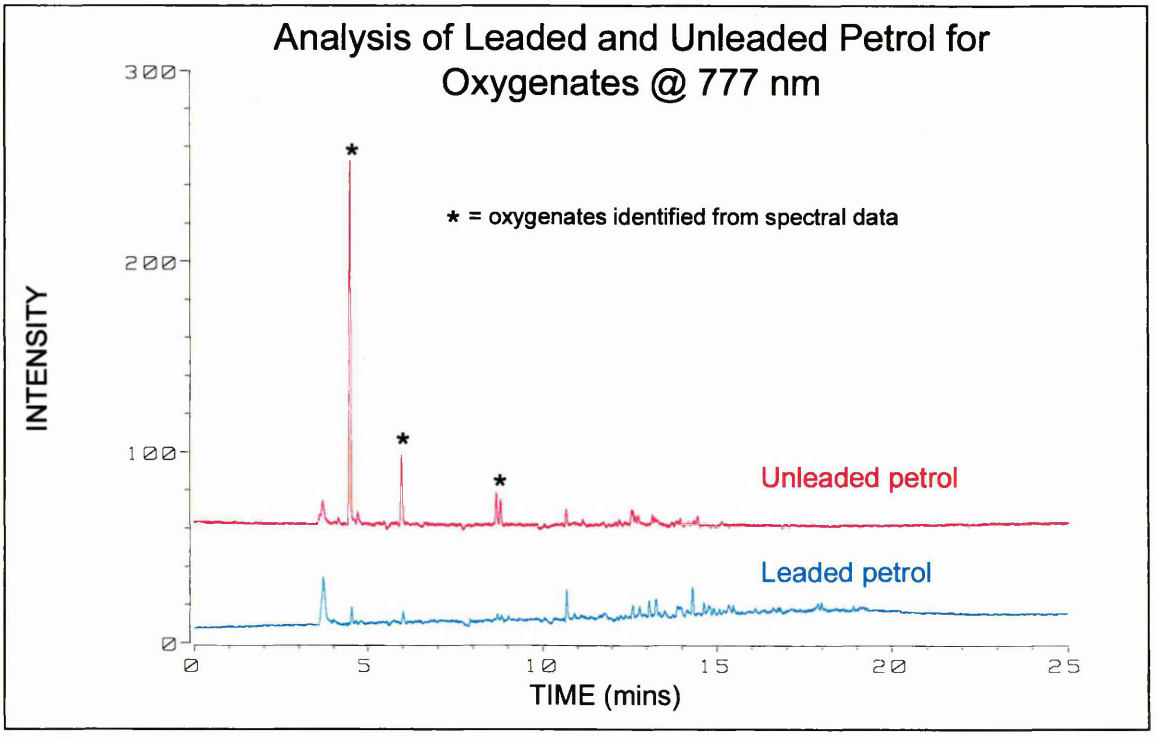


Figure 13c. GC-MIP-AED Chromatogram showing a comparison of Oxygen containing Species for Leaded and Unleaded Petrol



The comparison of leaded and unleaded petrol for organosulphur components shown in figure 13b shows that the same species are present. The components were confirmed at t_R = 15.5 mins and also between 16.6 and 17.2 mins. A number of other signals were also observed at retention times below 15.5 mins but did not give the characteristic sulphur snapshot.

The signals observed are most likely due to carbon molecular emission due to the high carbon content of the sample at these low retention times, hence for this type of sample a poor degree of selectivity is observed for organosulphur components. It was attempted to suppress the molecular emission signal but this only resulted in the formation of large negative peaks.

The problems encountered with the selectivity of the sulphur channel for this sample matrix prompted a study of a synthetic petrol mixture containing a number of n-alkanes ranging between C_6-C_{17}, mixed xylenes and octane thiol as a sulphur additive. The experiments showed that the molecular emission breakthrough on the sulphur channel was specific to the petrol as similar breakthrough was not observed in the synthetic petrol mixture.

A comparison of the oxygenates present in both petrols (figure 13c) showed that although a number of oxygenates were observed and confirmed at t_R = 4.5, 6.0, 8.7 and 8.8 min for unleaded petrol, none were observed for leaded petrol. See appendix 2 for a typical snapshot obtained for oxygenates. A number of small signals were observed for the leaded petrol which were also thought to be

due to matrix effects. The different chromatograms were expected as oxygenates are added to unleaded petrol to increase the octane value of the fuel which is not necessary for leaded petrol.

Figure 13d(i) and figure 13d(ii) show the chromatograms for leaded and unleaded petrol for carbon, chlorine and bromine at 496 nm, 479 nm and 478 nm.

From figure 13d(i) it can be seen that leaded petrol contains both an organobromine (t_R = 2.0 min) component, possibly ethylene di-bromide, and an organochlorine (t_R = 1.5 min) component, possibly ethylene di-chloride. Elemental confirmation was obtained for each component from spectral data, although additional information is required for conclusive identification. Examples of these snapshots can be found in appendix 2. An organochlorine component was observed for the unleaded petrol at t_R = 1.5 min as in the leaded petrol but no corresponding organobromine component was observed.

Figure 13e(i) and figure 13e(ii) show the chromatograms of leaded and unleaded petrol for carbon and lead at 496 nm and 406 nm respectively. From figure 13e(i) it is evident that a number of organolead components are present in the leaded petrol at t_R = 2.8, 4.3, 7.0-8.0, 9.4, 10.0 and 13.0 min, each of which were confirmed from the spectral data. A typical example of a lead emission snapshot is shown in appendix 2. As expected no organolead components were observed in the unleaded petrol (figure 13e(ii)).

Figure 13d(i). GC-MIP-AED Chromatogram of Leaded Petrol for Carbon, Chlorine and Bromine

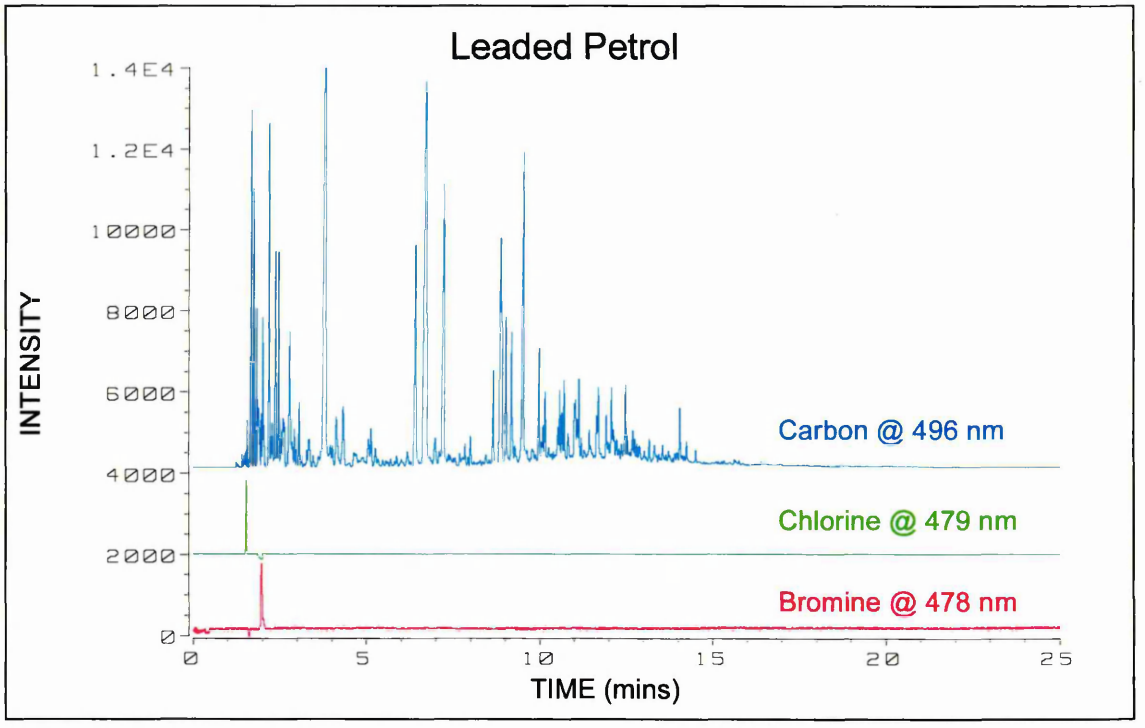


Figure 13d(ii). GC-MIP-AED Chromatogram of Unleaded Petrol for Carbon, Chlorine and Bromine

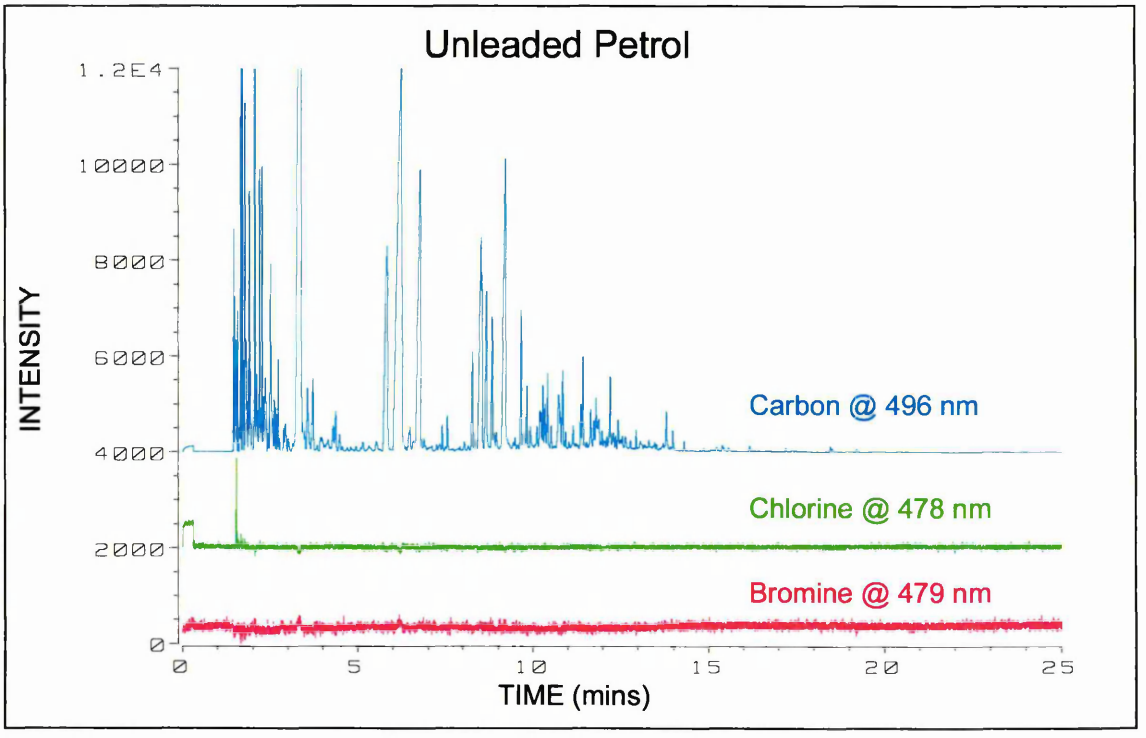


Figure 13e(i). GC-MIP-AED Chromatogram of Leaded Petrol for Carbon and Lead

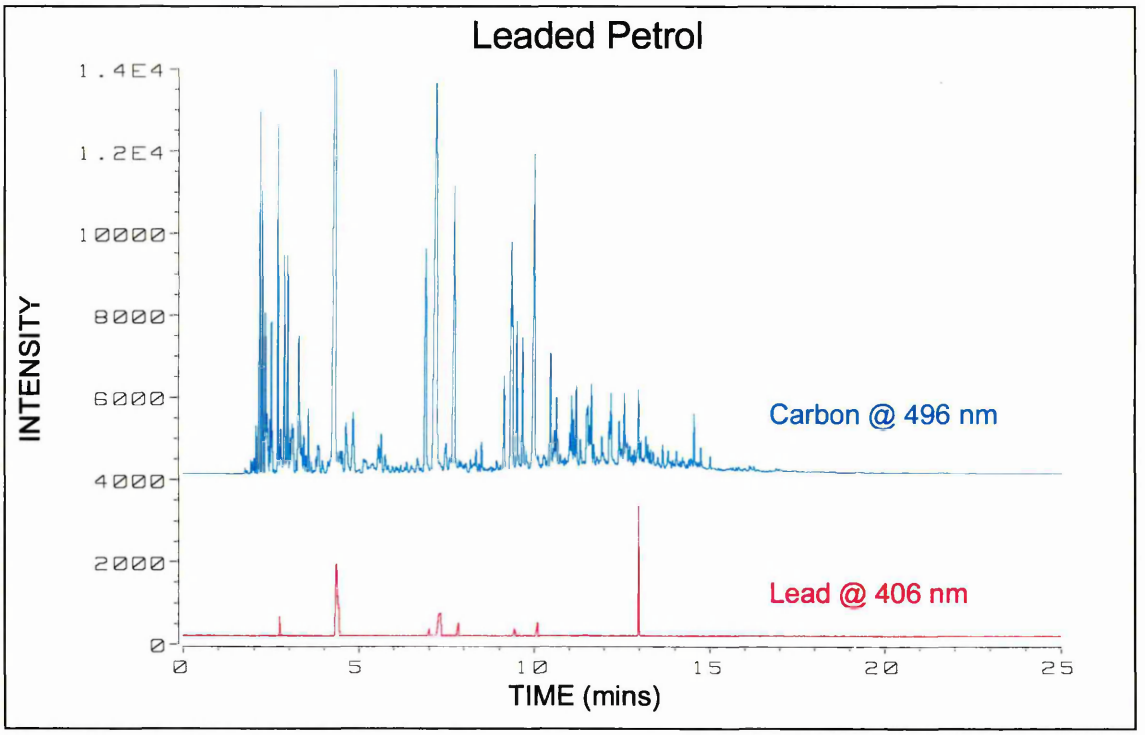
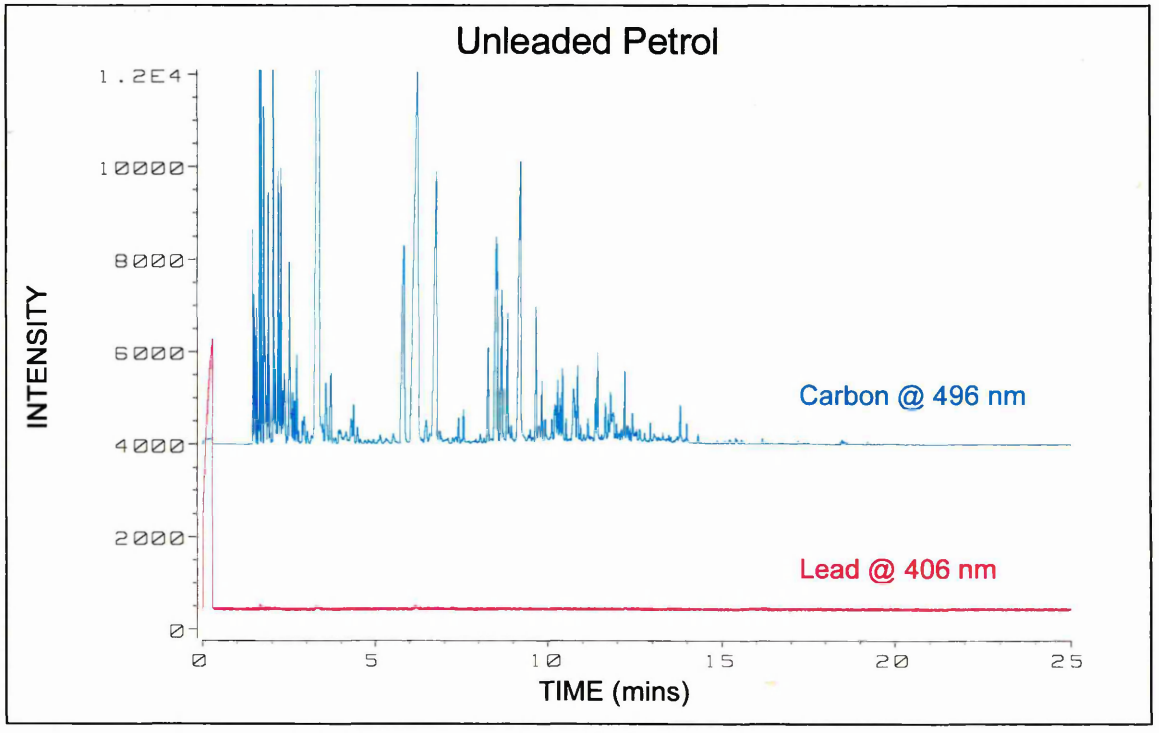


Figure 13e(ii). GC-MIP-AED Chromatogram of Unleaded Petrol for Carbon and Lead



Overall, the GC-MIP-AED has proved itself to be very useful for showing the presence of heteroatoms in the range of environmental samples discussed. The degree of selectivity observed for the majority of elements was very high with the exception of organosulphur components in a complex hydrocarbon matrix. However the GC-MIP-AED does not provide structural information to aid identification, for example, it was not possible to conclusively identify the organohalogen compounds in petrol. In this respect although elemental information was obtained, the use of the GC-MIP-AED is limited in comparison to the GC-MS.

3.3 Quantitative Analysis

A range of simple experiments were performed in order to determine the precision (repeatability), linear dynamic range and the limit of detection for carbon, sulphur and nitrogen. All quantitative analysis was performed using external standard techniques.

Linear Dynamic Range: The number of orders of magnitude over which a linear calibration graph can be obtained for a range of concentrations.

Limit of Detection: The smallest mass of a standard giving a signal to noise ratio of 3:1

Precision (Repeatability): Repeat analysis of the sample with the mean and %RSD (% Relative Standard Deviation) being calculated. The lower the %RSD the greater the degree of precision.

$$[\ \%RSD = \frac{\text{Standard Deviation}}{\text{Mean}} \times 100]$$

A number of experiments were performed using simple laboratory prepared standards and also the commercially available Hewlett Packard test mix, using a variety of injection port liners by both manual and automatic injection (high and low speed) in either split or splitless configuration, to evaluate the applicability of the GC-MIP-AED to quantitative analysis.

Initially, octane thiol standards ($CH_3(CH_2)_7SH$) in hexane were analysed using a single restriction liner by high speed automatic injection in splitless mode. A linear range was observed between 1 ng / µl and 100 ng / µl i.e. giving a linear dynamic range of $1x10^2$. Below 1 ng / µl when a ten fold dilution of the 1 ng / µl solution (100 pg / µl) was analysed only a three fold reduction in the signal intensity was observed for both sulphur and carbon. As signal intensity is theoretically directly proportional to concentration a ten fold decrease in the signal intensity was expected. This restricted the linear range with respect to the manufacturers stated range of $2x10^4$. Poor precision was also observed between replicate injections of the same sample (%RSD of 3-12%).

In later experiments alternative standards (tert-butyl disulphide and nitrobenzene) were used as octane thiol was found to degrade and form a dimer. The rate of dimer formation was greater at lower concentrations hence causing the concentration of the monomer to be unstable.

However the initial experiments highlighted a number of issues which required further study. These included:-

1) Precision (Repeatability)
2) Linear range
3) Limit of detection

3.3.1 Precision (Repeatability)

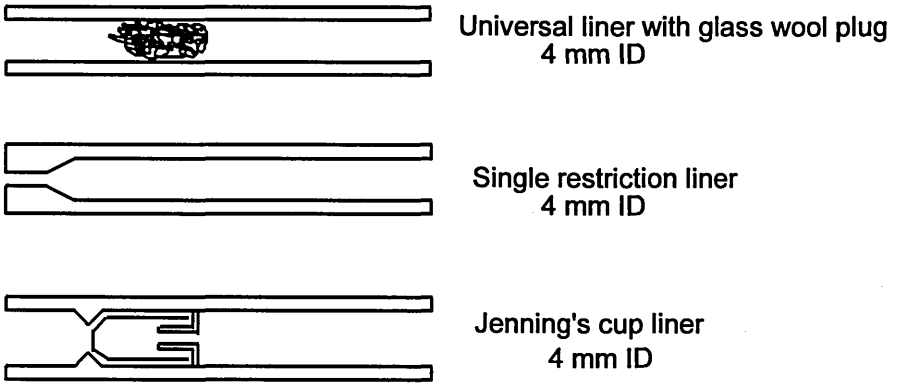
A range of repeatability studies were performed using a number of different standard concentrations for each liner (see figure 14 for liners used) and mode of injection (split / splitless, high speed/low speed/manual injection). A commercially available test mix was also used, with results being gathered for the nitrobenzene and tert-butyl disulphide analytes. The results are summarised below. (%RSD's are based on between 5-10 injections of the same solution.)

Split Configuration

It was observed that for a split injection the universal liner containing a glass wool plug was the only liner to give acceptable %RSD's by high speed

automatic injection (%RSD ≅ 1%) and manual injection (%RSD ≅2%). The Jennings cup liner gave a %RSD of ≅4% for both manual and high speed automatic injection, with the single restriction liner showing the worst precision by high speed automatic injection with a %RSD between 10-20% although by manual injection for this liner in split mode it was possible to obtain an average %RSD of ≅3%. It was however possible to obtain repeatable results using a single restriction liner by low speed automatic injection when a %RSD of ≅1% was achieved. The absolute integration areas for the same sample but using the different liners showed large differences, as did the areas obtained for the same liner but using different methods of sample introduction.

Figure 14. A Schematic Diagram of the Universal, Jenning's Cup and Single Restriction Liner



Splitless Configuration

The results obtained using the universal liner by high speed automatic injection gave a % RSD of between 4-10%, with the results for low speed automatic injection giving a similar %RSD but with the signal intensity reduced by a factor

of 2. The best precision was obtained using a single restriction liner with a small glass wool plug which gave a %RSD of ≅2% by high speed automatic injection. For low speed injection a similar %RSD was achieved but the signal intensity was reduced by a factor of 3. For manual injection a %RSD of ≅3% was achieved. Again the absolute integration areas differed greatly depending upon the liner and mode of sample introduction. Only the universal liner and single restriction liner where used in splitless mode as the Jennings cup liner is not recommended for splitless injection.

From these experiments it may be concluded that for samples such as nitrobenzene and tert-butyl disulphide a suitable liner for split injection is the universal liner containing a glass wool plug for high speed automatic injection, whereas if the sample requires the use of a single restriction liner due to sample instability, analysis by split injection must be in low speed configuration if the automatic injector is used. Manual injection may be used in conjunction with any of the above mentioned liners. The most suitable injection liner for splitless operation appears to be the single restriction liner by high speed automatic injection, with manual injection as an acceptable alternative using the same liner.

3.3.2 Linear Range

The restriction observed at low concentrations for octane thiol appeared to be sample specific as further investigations performed using nitrobenzene and

tert-butyl disulphide generated a linear dynamic range comparable with the manufacturers specifications.

3.3.3 Limit of Detection

Initially problems were encountered achieving acceptable limits of detection in line with the manufacturers specifications. This was overcome by using an appropriate liner and mode of injection for the sample to be analysed as described in section 3.3.1. The liners specified give the largest signal to noise ratio, hence the best limit of detection.

In conclusion it appears that although manual injection is largely acceptable for either split or splitless injection with any liner, problems arise when performing automatic injections. The type of liner used has a large effect on the signal intensity, linearity and the precision achieved, and as such the mode of injection and the liner used must be given due consideration for each sample type.

3.4 Instrumental Difficulties

The first commercially available GC-MIP-AED from Hewlett Packard showed a number of weaknesses related to the hardware over a period of time. The three major problems encountered include:-

a) The design of the water cooling system for the cavity

b) The software supplied

c) The reliability of the Auto injector

3.4.1 The Design of the Water Cooling System for the Cavity

The ability to cool the discharge tube during operation has been shown to be a major advantage, as it increases the discharge tubes lifetime, as previously discussed in section 1.5.2. Prior to this facility it had been reported that when using the Beenakker type cavity the discharge tube walls became visibly etched within 8-12 hours of operation [26, 102]. The degree of erosion was found to correlate directly with observed tailing on certain elements, for example, sulphur [26]. As the degree of etching increased so did the degree of tailing on the chromatographic peak. In the water cooled system the re-entrant cavity adapted by Sullivan and Quimby showed only very slight erosion after a 28 day operating period.

The need for cooling the discharge tube during operation cannot be disputed however a number of problems have arisen using the water cooling system described.

If, for whatever reason, the discharge tube should shatter during operation, water is allowed into the discharge tube. This has major repercussions as the water and any quartz fragments are carried, by the carrier gas, through the gas lines and solenoid valves, leaving the gas lines wet and open to microbial growth which can cause blockages. More importantly any quartz fragments reaching the solenoid valves could block the valve so it could no longer be opened or closed. No cut off switch has been incorporated in the instrument to

stop the pump if this happens so water is pumped from the system until it runs dry. On a number of occasions after such an occurrence the pump has been irreversibly damaged necessitating replacement.

If a sensor was incorporated within the reservoir, switching off the pump when the water dropped to a certain level, the extent of the damage to the system would be minimised as excess water would be prevented from reaching the cavity and damage to the pump would be prevented. Improvements to the water cooling system are suggested in figure 15a.

The installation of air filters on all gas lines leaving the cavity would protect the solenoid valves from the quartz fragments, with a form of reservoir fitted into the gas lines close to the cavity to capture any water. Improvements to the cavity design are suggested in figure 15b.

3.4.2 The Software

The Hewlett Packard GC-MIP-AED system which has been evaluated during this study was operated using a PASCAL Chem Station which is unable to perform more than one task simultaneously, i.e. if a chromatogram was being acquired it would not be possible to edit previously acquired data. The adaptation of the software to a multitasking form of operation such as Windows would be a considerable improvement. The recent release of the Hewlett Packard second generation AED has incorporated this change and is currently operated using windows NT4.

Figure 15a. A Cross Sectional View of the Water Cooling System Indicating the Suggested Position of a Sensor

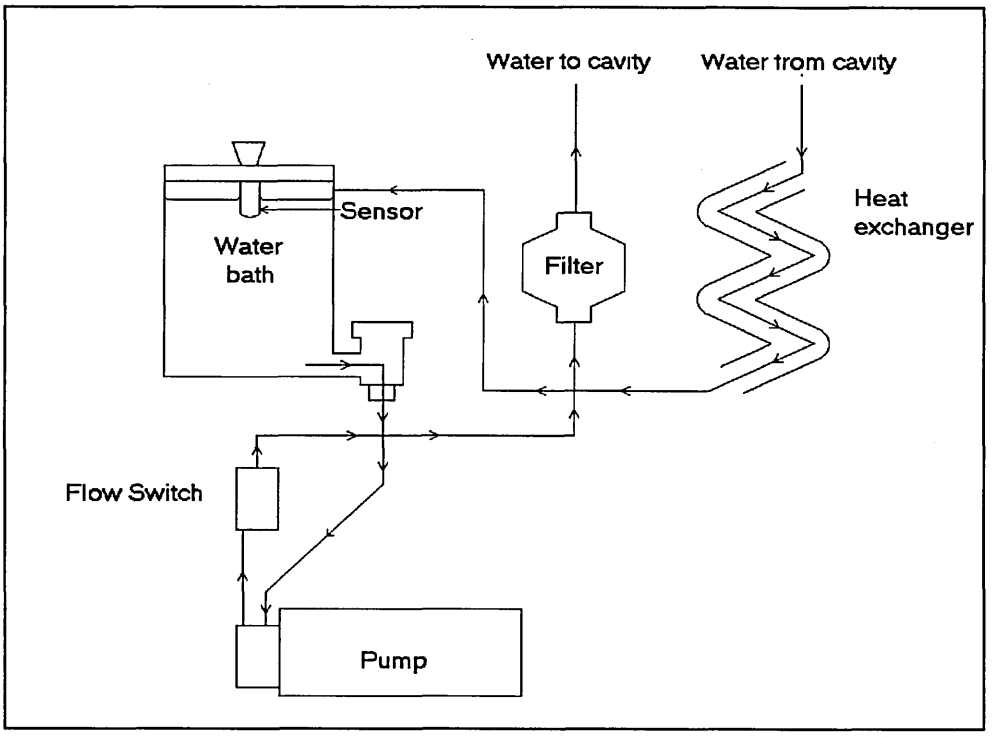
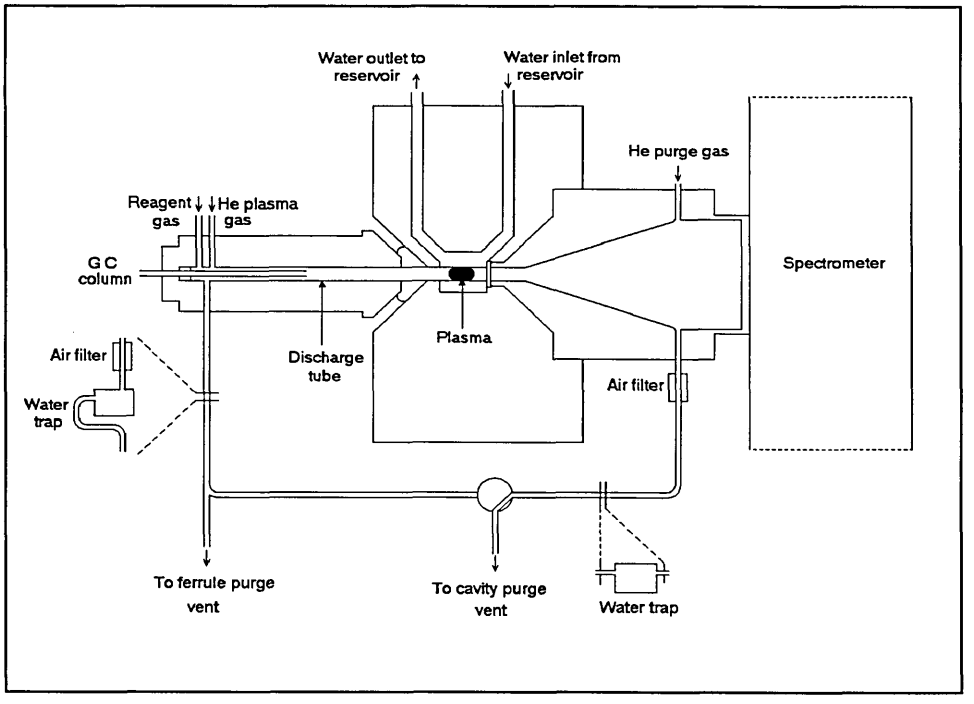


Figure 15b. A Cross Sectional View of the Cavity Indicating the Suggested Positions of Water Traps and Air Filter



Problems were also experienced within the software recipes themselves. The cavity gas flow rate has limited software control. The software recipe system relies on the cavity gas flow rate being set at 60 ml / min prior to plasma ignition. A software controlled switch allows for an increase in gas flow by an additional 140 ml / min for elements requiring higher cavity gas flow rates while retaining the ability to return to 60 ml / min flow rate for the next automatic injection. However this switch only operates under an either / or system. The cavity gas flow rate had to be manually increased to 290 ml / min for the analysis of organolead and organotin compounds, restricting automation of the instrument (see chapters 5 and 6 for details). Manual intervention is required before the system can be used at a flow of 60 ml / min. This issue could be removed by introducing comprehensive electronic gas flow control for the cavity gas flow system, including control for the scavenger gases used.

In the original Pascal based Chem Station the data storage capacity was of concern. The size of the data files produced with spectral data, often exceeded 10 megabytes for a half hour run, hence the system was adapted by Hewlett Packard to delete the spectral data after each chromatographic run. This removes the possibility of obtaining spectral data confirmation at a later date and also restricts the use of the auto sampler facility for use only with samples of known elemental composition.

More flexibility would have been achieved by incorporating a larger hard disc (at the time of study the disc size was only 40 megabytes) and from the

addition of an optical disk drive to store the data obtained giving a larger capacity than the flexible disc drive (1.25 megabyte storage capacity). These issues have been taken into consideration by Hewlett Packard in their second generation AED.

3.4.3 The Auto Injector Unit

A number of problems occurred using the high speed injection system for sample introduction. Irreproducible results were initially obtained on a number of occasions, pointing towards a problem with this mode of sample introduction. This has been discussed in section 3.3.1.

Chapter 4

Organomercury Quantitation

4.0 Organomercury Quantitation

4.1 Introduction

Mercury exists at trace levels in various forms in the environment. This can be attributed to both natural emissions and also the industrial use of mercury and its derivatives. A variety of natural processes, such as volcanic activity, degassing of the earth's crust and weathering of rocks and sediments, are responsible for transporting the majority of mercury compounds within our environment.

Mercury compounds have been used within the chemical industry in both its metallic form and also as a variety of organomercury derivatives. In the past the major use of mercury has been in the chloro-alkali industry, where liquid mercury was used as a mobile cathode in the electrolysis of brine to produce chlorine and sodium hydroxide. Organomercury species have also been used in the agricultural, pharmaceutical, polymer, paints and adhesives industries, however these products have been banned or greatly reduced due to the toxicity of the mercury species and concerns over the effects of mercury entering the environment. Mercury is also released into the atmosphere in vapour form during the combustion of coal, for example, from power stations.

The industrial emission of mercury compounds is of much greater concern than that of naturally occurring emissions, as industrial usage concentrates the emission in a specific area, increasing the exposure of local inhabitants to mercury.

In the early 1950's no specific hazards were known to exist to animals or people who were not directly exposed to mercury. Later in the decade, in Sweden, it was noted that some species of birds were declining in numbers and that the deaths increased when cereal crops treated with organomercury compounds were sown. High levels of total mercury were also found in fish, which was attributed to the effluent containing mercury compounds produced by pulp and paper mills.

In Japan (1953-1975) the release of mercury compounds formed from catalysts used in the production of acetaldehyde and vinyl chloride in Minamata bay resulted in the poisoning of a large number of the local population and over 100 deaths. Here it was thought that natural processes converted some of the mercury compounds in the effluent to methyl mercury compounds. These compounds accumulated in the fish, which were in turn caught and eaten by the people living in the surrounding area. This poisoning became known as "Minamata Disease".

Other incidents also occurred in Iraq, Pakistan, Guatemala and the USA during the early 1970's where poisoning occurred due to the consumption of food substances containing high concentrations of organomercury compounds.

It has been shown that organomercury compounds exhibit a much higher toxicity than inorganic forms of mercury. Organomercury species when inhaled,

ingested or absorbed via the skin in sufficient quantities lead rapidly to nervous system disorders due to the solubility of mercury species in lipids. They have the tendency to enter into the blood stream and can cross the blood : brain barrier and also the placental membrane where they may cause irreversible damage to the developing foetus. Elimination of methyl mercury compounds is slow, exhibiting a half-life of 60-70 days in man compared with inorganic mercury compounds, which have a half-life of 3-4 days [110a].

Microbial action in natural waters and sediments have been shown to convert various forms of naturally occurring and anthropological inorganic mercury to methyl mercury, the primary organomercury pollutant in the environment. This process may be enzymatic or non-enzymatic. One route that has been shown to form methyl mercury is by reaction with a methyl cobalamin derivative (a derivative of vitamin B_{12}) and occurs both in anaerobic conditions and also in living organisms. Methylation by this route is thought to be by carbanion transfer. The methyl mercury is absorbed and concentrated by aquatic organisms and passed along ecological food chains by a process known as bioamplification. The pollutant becomes progressively more concentrated at each level of the food chain. As humans are in general at the top of the food chain, high concentrations of methyl mercury may be found in humans with a largely seafood diet. The chemical pathways for the methylation and the demethylation of mercury have been previously reviewed [110a, 111-112].

It can be seen that from the above discussion that there is a need for rapid, specific and sensitive analytical techniques which can distinguish between the chemical forms of organic and inorganic mercury compounds and allow for reliable quantitation.

4.2 Review of Analytical Techniques for Organomercury Speciation

4.2.1 Sample Preparation - Extraction and Derivatisation Techniques

Prior to quantitation, a suitable extraction procedure must be performed in order to extract the species of interest from animal / plant tissue, sediments, water or the atmosphere [110a]. A pre-concentration step may also be incorporated due to the low concentrations of the pollutants. Puk and Weber [113] and more recently Clevenger *et al* [114] have presented a general review of analytical methods used for the determination of inorganic and methyl mercury compounds.

In the early 1960's total mercury could be quantified at trace levels (pg / µl), however this was deemed to be unsatisfactory as total mercury did not give any information about the chemical form of mercury. Westöö developed one of the first chromatographic methods on the quantitation of methyl mercury compounds. This method was applied to fish tissue and also to egg white [115].

Westöö's method was based on the extraction of methyl mercury compounds from homogenised sample by acidification with hydrochloric acid to form methyl mercury chloride, which was extracted into benzene. The benzene was distilled

off to remove volatile sulphur containing compounds and to preconcentrate the extract in a heptane - acetic acid solution. The methyl mercury chloride was then back extracted into an ammonium hydroxide solution as the methyl mercury hydroxide species. The aqueous extract was again acidified and re-extracted into benzene as methyl mercury chloride prior to analysis by GC-ECD. The back extraction was essential in order to clean up the sample and prevent any interference from coextracted analytes due to the non-specific nature of the ECD detector. Approximately 70% recoveries were consistently achieved with losses being due to unfavourable partition coefficients during the extraction procedures. This extraction procedure was also performed by Bache and Lisk in 1971 where recoveries of approximately 70% were also achieved [116].

Problems were however encountered when applying this method to other food substances, for example, liver. Methyl mercury compounds are known to form a strong bond to thiol-groups. The mercury / sulphur bond was reported to reform at high pH, such as in the back extraction stage of the method, in preference to the hydroxide species. Hence any methyl mercury bonded to the thiol group of non-volatile compounds, not removed in the distillation stage, would return to the organic layer resulting in poor recoveries.

In 1967 [117] Westöö addressed these problems by replacing the back extraction into alkali with either a cysteine acetate solution or a combination of cysteine acetate and mercuric chloride solution. Here the cysteine component

is acting as a chelating agent by exploiting the affinity of methyl mercury for thiol groups. The mercuric chloride ions were added to bind with the free thiol groups formed within the sample from which the methyl mercury ions had been liberated. This simplified the extraction procedure by eliminating the need for the distillation step and gave recoveries of 98% ± 3% (n=10) [118]. Westöö's method of extraction and purification is still the basis for many extraction techniques reported today, however toluene is now used as the extraction solvent due to concerns over the toxicity of benzene. For example, Carro *et al* in 1994 [119] applied the method to the analysis of methyl mercury in estuarine sediment samples. The method was adapted to incorporate additional toluene extractions from the aqueous cysteine solution prior to analysis by GC-ECD. Recoveries of 67% were reported from the sediment.

A more recent variation of Westöö's method, developed by Horvat *et al* in 1990 [120], replaced the aqueous cysteine back extraction with cysteine impregnated paper. Interfering compounds were washed off with toluene and methyl mercury species eluted with sulphuric acid, containing potassium bromide to form the methyl mercury bromide species. The halide was extracted into benzene prior to analysis.

A variety of sample types have been analysed based on Westöö's extraction procedure, incorporating the optimisation of one or more parameters. These include:

1. An initial sample digestion procedure using sodium hydroxide and a cysteine solution, used prior to Westöö's method of extraction, to reduce matrix effects [121-124].

   Protein and lipid materials were found to breakdown during alkaline digestion to give a more homogeneous sample. It was found necessary to have a cysteine solution present to complex the organic mercury and prevent cleavage of the carbon mercury bond in order to achieve high recoveries. Harms [121] studied three digest procedures, one omitting the sodium hydroxide digest, one with a sodium hydroxide digest and a third incorporating a sodium hydroxide and cysteine solution digest, and applied them to certified reference materials. Recoveries of 60%, 71% and 98% respectively were achieved highlighting the need for the cysteine chelation reagent. However the digested sample solutions were found to degrade on standing as the cysteine was gradually oxidised in strong alkaline solution, allowing carbon mercury bonds to slowly break down [123]. This was shown to be controlled to an extent by storage at -20°C [124].

2. Replacing mercuric chloride with copper sulphate and potassium bromide during the back extraction of methyl mercury ions into a cysteine solution. The methyl mercury ions are then re extracted into toluene as the bromide derivative. This method has been used for the extraction of human hair [125].

Mercuric chloride, as used by Westöö [118], has been reported to decompose di-methyl mercury to mono-methyl mercury species however this was not observed when the addition of mercuric chloride was replaced by cupric ions [110a].

3. Replacing the cysteine acetate back extraction with a solution of sodium thiosulphate, followed by the addition of cupric bromide to form the methyl mercury bromide compound, for example, in the extraction of methyl mercury compounds in fish tissue [126].

   In this extraction procedure the thiosulphate ions act as the chelate reagent to bind methyl mercury ions. Cupric ions are added to allow for a more complete liberation of organic mercury from its thiosulphate complex by preferentially binding to the thiol groups.

An alternative extraction method was developed by Hight *et al* [127] in 1983, which has been applied to the quantitation of methyl mercury in fish and shellfish. The Food and Drug Administration in the USA adopted this method as the first official method for the determination of methyl mercury compounds. A homogenised seafood sample was washed three times with acetone and then benzene to remove organic interferences. Methyl mercury was released from the tissue with hydrochloric acid and extracted three times into benzene as methyl mercury chloride. The extracts were combined prior to analysis by GC-

ECD. In this method the extraction procedure is simplified by the initial clean up of the sample, and eliminates the need for further extractions into the aqueous phase and back extraction into solvent. Average recoveries of between 99% and 120% were achieved in a collaborative study.

Hight *et al* updated this method in 1987 [87] by substituting toluene as a replacement solvent for benzene and optimising the extraction parameters. Quantitation was achieved by GC-ECD and GC-MIP-AED. Average recoveries of 100.5% were achieved.

In 1993, Lorenzo *et al* [128] applied this method to various fish and shellfish samples using GC-ECD to achieve quantitation. A certified reference material was analysed to evaluate the method, however for the reference material recoveries of between 70% and 85% were obtained. For other fish tissue, which had been spiked, recoveries of between 98% and 117% were achieved.

An alternative to these multistage extraction procedures has also been used for the speciation of methyl mercury compounds. Decadt *et al* [89] released organomercury compounds from biological material using iodoacetic acid as both a cleaving agent and a derivatisation reagent. However, non-selective cleavage of protein metal bonds in the sample resulted in the liberation of additional iodide ions, which were oxidised to iodine. The presence of this iodine led to photochemical degradation of methyl mercury iodide. This side reaction was suppressed by the addition of sodium thiosulphate solution to

react with any iodide ions formed, preventing the formation of iodine. Methyl mercury iodide was quantified by headspace injection of the aqueous solution.

This technique was further investigated by Lansens *et al* [86, 88, 129], who found that iodoacetic acid was not strong enough to cleave all methyl mercury from biological tissue and observed that the addition of sodium thiosulphate to standard preparations resulted in a decrease in analyte signal. By the addition of optimal quantities of only sulphuric acid and iodoacetic acid, quantitation could be achieved by standard addition techniques and the addition of sodium thiosulphate was not required, eliminating a possible error which may cause an analytical bias to reported methyl mercury concentrations.

In 1989 Lee *et al* [130] reported an extraction procedure for the determination of methyl mercury in natural waters. The methyl mercury species was preconcentrated from water samples by chelation onto a sulph-hydryl cotton fibre adsorbent, exploiting the affinity of methyl mercury ions for sulphur species. Dilute hydrochloric acid was used to elute methyl mercury from the adsorbent and the methyl mercury was extracted as the chloride into benzene. Recoveries of 95.8 ± 9.3% (n=8) were achieved.

A similar procedure was adopted by Chai *et al* [131] to human hair samples taken from fishermen, post and antenatal women and newborn babies.

In the above extraction procedures quantitation was achieved as the halide derivative of methyl mercury by packed column GC separation. A wide variety of packed column stationary phases have been used however poor and often variable response was observed due to interactions with, or decomposition on, the column. Column tailing and poor column efficiency were also observed. These effects were studied by O'Reilly [132] who recommended a column conditioning procedure with mercury (II) chloride solution. This greatly increased the peak height, improved the peak shape and gave an increased number of theoretical plates. However the improvements were only temporary and a decline in sensitivity was observed over a period of 4-5 hr requiring frequent recalibration. The column could however be reconditioned overnight by further injections of mercuric chloride solution and reducing the column temperature. This degradation of the column was reported to be greatly reduced using headspace injection as the technique of sample introduction, as opposed to split / spiltless injection for the analysis of the halide derivative [86, 89].

A number of papers report the use of capillary columns, however conditioning was still required with mercuric chloride solution to prevent decomposition. Tailing and memory effects have also been observed for methyl mercury halide derivatives [88]. It has also been reported that although conditioning of capillary columns with mercuric chloride blocks any active sites on the analytical column, it also destroys the bonded stationary phase reducing the lifetime of the column [128]. In 1991 Bulska *et al* [133] also found and addressed the problems

associated with interactions of organomercury halides with bonded phase fused silica capillary columns.

A simplified extraction procedure was adopted in contrast to the analytical extraction procedures described earlier. The problem of column degradation and the need for regular conditioning was addressed by suggesting an alternative to the halide derivative. The method was applied to fish tissue. Hydrochloric acid was used to leach the methyl mercury species from the sample material. The solution was neutralised and buffered at pH 9. The methyl mercury compounds were chelated with an aqueous solution of sodium diethyldithiocarbamate and extracted into toluene. The methyl mercury complexes were then derivatised to form the di-alkyl species, in this case butylated, using a Grignard reagent and quantified as butyl methyl mercury. This derivatisation procedure excludes the use of ECD detection, as the halide moiety that enables a response to be observed by electron capture is no longer present. Detection and quantitation was achieved by GC-MIP-AED [133].

The analysis of the di-alkyl species did not show any of the problems associated with the analysis of the organomercury halide and the extraction method using a non-specific chelation reagent followed by butylation could in theory be applied to the general determination of metallic and organometallic species, providing an opportunity for multielemental analysis.

This technique has also been applied to whole blood samples [91] and has been adapted for mercury speciation in natural waters, incorporating an additional preconcentration stage using dithiocarbamate resin microcolumns [65].

Fischer *et al* [134] also applied an alkylation procedure to determine methyl mercury in fish tissue. Methanolic potassium hydroxide was used to digest the fish tissue, followed by aqueous phase ethylation by derivatisation with sodium tetraethyl borate. A cryogenic trapping technique was then used to trap the di-alkyl mercury species onto a packed GC column prior to quantification. This procedure has a drawback in that inorganic mercury will also be subject to ethylation, hence precluding the simultaneous determination of ethyl mercury species.

In order to overcome this problem sodium tetra-phenyl borate was used as an alternative derviatisation reagent by Mena et al [135] for the analysis of organomercury species in water samples. The organomercury species were extracted and preconcentrated onto a sulph-hydryl cotton microcolumn incorporated in a flow injection system. The retained mercury species were eluted from the column with hydrochloric acid before being subjected to aqueous phase phenylation. Quantification was achieved by GC-MIP-AED. Recoveries of 90-94% were achieved for spiked water samples containing methyl, ethyl and inorganic mercury. This initial extraction and preconcentration procedure utilizing sulph-hydryl cotton is similar to that described previously by

Lee *et al* [130] and Chai *et al* [131] for quantitation of methyl mercury chloride as the halide derivative.

4.2.2 Gas Chromatographic Detection of Organomercury Species

A number of detectors have been employed in the determination of organomercury species using gas chromatography. The most commonly reported techniques include:

1) ECD (Electron Capture Detector)
2) MS (Mass Spectrometry)
3) AFS (Cold Vapour - Atomic Fluorescence Spectrometry)
4) AAS (Cold Vapour / Electrothermal decomposition- Atomic Absorption Spectrometry)
5) MIP-AED (Microwave Induced Plasma - Atomic Emission Detector)

The type of detector used depends upon the derivative of the organomercury species formed. The ECD detector is one of the most commonly used detectors for the analysis of organomercury halide species [115, 117-119, 121-122, 125-128], however this derivative has been shown to interact with analytical columns which require extensive conditioning procedures as previously described. The ECD detector is classed as a selective detector but is not truly specific, and as such extensive clean up of samples is required prior to analysis to eliminate interference from coextracted analytes.

The mass spectrometry (MS) detector has been used for the analysis of methyl mercury halide species [136] and also alkylated methyl mercury species [137]. Again similar problems were associated with the analysis of methyl mercury halide species as described previously. A high degree of sensitivity was achieved by operating the MS detector in selected ion monitoring mode after initial identification. However in this operating mode conclusive identification is not possible as in scanning mode, and some of the observed signal may be due to inteferences in the sample matrix which fragment to give the same mass ion or incomplete separation of analytes, effecting analyte quantitation.

Atomic spectroscopy techniques undoubtedly offer the best detection capabilities for the analysis of organomercury species, and for organometallic compounds in general. Unambiguous identification and quantification can be achieved without interferences caused by co-eluting compounds which may lead to unrealistically high results. The use of AFS for the determination of organomercury species has been reviewed by Morita *et al* [138]. GC - AFS has been used for a number of studies by Bloom *et al* [139-142] and is based on the thermal decomposition of organomercury species as they elute from the analytical column, followed by cold vapour atomic fluorescence detection.

GC - AAS has been reviewed by Rapsomanikis [10]. Problems associated with nebulization and atomisation are avoided by chemical reduction of the organomercury species prior to detection by cold vapour atomic absorption [143]. Alternatively GC coupled with a quartz or graphite furnace -AAS systems

may be used removing the need for the reduction / pyrolysis of the organomercury species prior to entering the AAS detector [134, 144-146].

GC-MIP-AED techniques have also been used extensively for the analysis of organomercury species [10, 65, 86-91, 110a, 116, 129, 133, 135]. This technique while being comparable to AAS and AFS for this particular application is a much more diverse technique due to the multielemental capabilities that it exhibits. This allows for the quantitation and speciation of a number of organometallic species within a single chromatographic run, which is not possible for AAS and limited for AFS.

Alternative techniques for the quantitation of organomercury species include GC -LSS (Liquid Scintillation Spectrometry) [123] for the analysis of biological materials and GC with atmospheric pressure active nitrogen detection [147] for the analysis of fish; water, urine and sediments are also reported in the literature.

HPLC chromatographic techniques coupled with UV [148-149] and atomic spectroscopy detection [150-152] have also been successfully used for the speciation of organomercury compounds but this separation technique will not be discussed in this thesis.

4.2.3 Summary

To summarise the techniques employed to extract, derivatise and quantitate methyl mercury are extensive and diverse. However during the initiation of this study the analysis of organomercury compounds was performed mainly as an isolated technique. The opportunity existed to develop an analytical procedure for the multielemental analysis of organometallic compounds by adapting the principles of previously reported analytical techniques for application to the Hewlett Packard GC-MIP-AED.

4.3 Application of the GC-MIP-AED for the Determination of Organomercury Species.

The objectives of this program of research was to utilise the GC-MIP-AED, initially concentrating on organomercury speciation, while maintaining the possibility of widening the scope of the technique to incorporate the simultaneous analysis of additional organometallic species. Specifically, the multielemental analysis of organomercury, organotin and organolead species within a single chromatographic injection for environmental samples.

The extraction and derivatisation procedure described by Bulska *et al* [91] was adopted as a starting point due to the potential of the extraction and derivatisation technique to be applied to multielemental organometallic analysis. The derivatisation technique described was for the butylation of the organomercury species after extraction using the non-specific chelation reagent sodium diethyldithiocarbamate.

The technique was also extended in this study to use pentylation as the derivatisation procedure with the future outlook of applying the technique to butyl tin compounds which could not be speciated using a butylation procedure. Both the butylation and pentylation procedures were evaluated as although favourable results had previously been published for the butylation technique, alternative instrumentation was to be used for detection. In order to show equivalence of the Hewlett Packard GC-MIP-AED in this field, repeatable analytical results were deemed necessary. The possibility of structurally independent atomic emission was also pursued for organomercury compounds to identify opportunities for compound independent calibration.

GC-MS was used to aid identification of the organometallic derivatives and also as a comparative quantitative technique.

The Hewlett Packard GC-MIP-AED allows for organomercury detection at 254 nm and also at 185 nm within the standard recipe software package. The analysis was performed at 254 nm to allow for the simultaneous detection of mercury, lead and tin organometallic compounds on the PDA detector in order to meet the objectives of this program of study.

4.3.1 Identification and Confirmation of Organomercury Species

In order to assess the GC-MIP-AED's applicability for organometallic speciation, a preliminary investigation was performed under the conditions

specified in chapter 2. Pentylated and butylated methyl mercury chloride standards were prepared using Grignard's reagent and analysed qualitatively by capillary GC-MIP-AED (column 2) and GC-MS (column 1).

Sample introduction was achieved by high-speed automatic injection and the GC injection port was configured for splitless operation using a single restriction liner containing a small glass wool plug. Solvent focusing was achieved by holding the oven temperature at 50°C in order to obtain good peak shape. This configuration had been shown to give the best precision in preliminary investigations for simple organic compounds (see chapter 3), and has been evaluated for this application later in this chapter.

Figures 16a and 16b show a high degree of specificity for both the butylated and pentylated mercury species respectively. Inorganic mercury was present as a significant contaminant however the alkylation procedure was able to differentiate between the organic and inorganic mercury species. No interference was observed due to carbon breakthrough, and a high degree of selectivity was exhibited between the alkylated organomercury compounds. The chromatogram obtained for carbon following alkylation is extremely complex, and highlights the selectivity of the technique for mercury over carbon (figure 16c shows a typical carbon profile following pentylation). The mercury emission was confirmed from spectral data. A typical snapshot for mercury at 254 nm is shown in appendix 2.

Figure 16a. GC-MIP-AED Chromatogram showing Butylated Methyl Mercury.

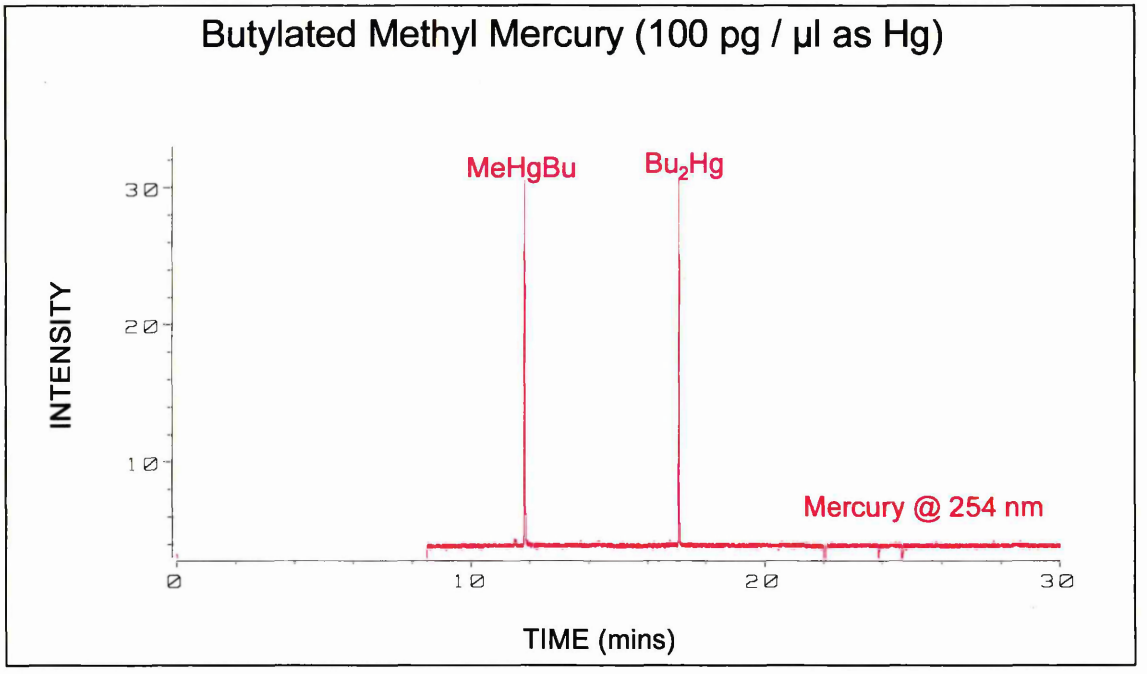


Figure 16b. GC-MIP-AED Chromatogram showing Pentylated Methyl Mercury.

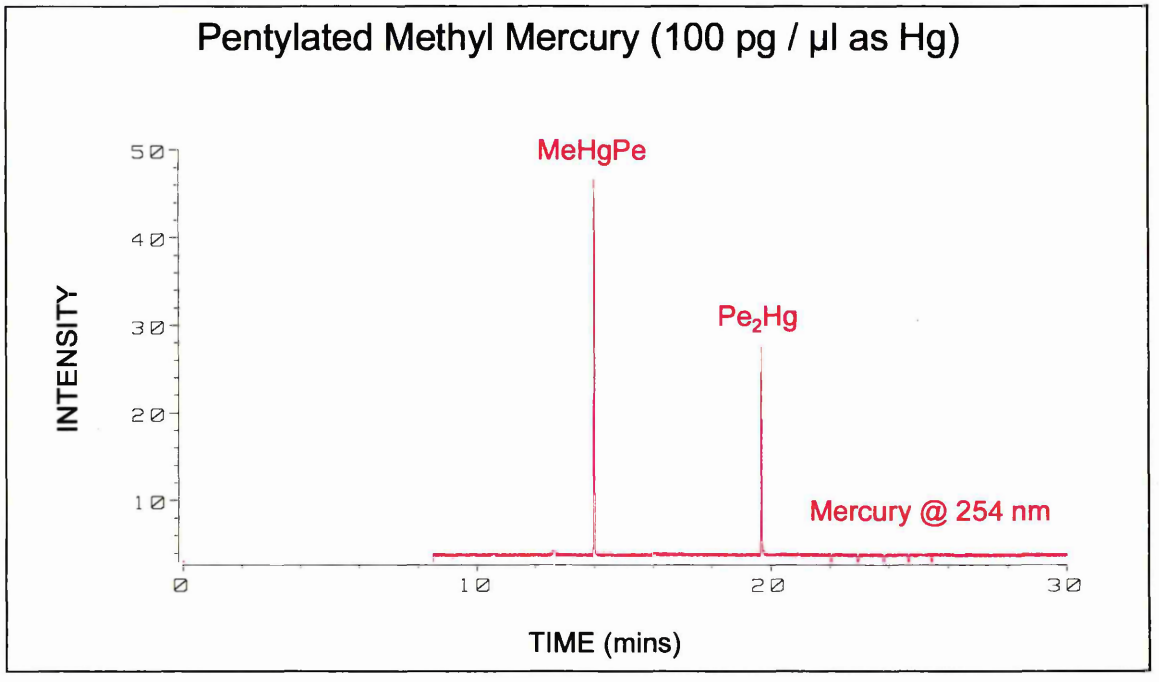
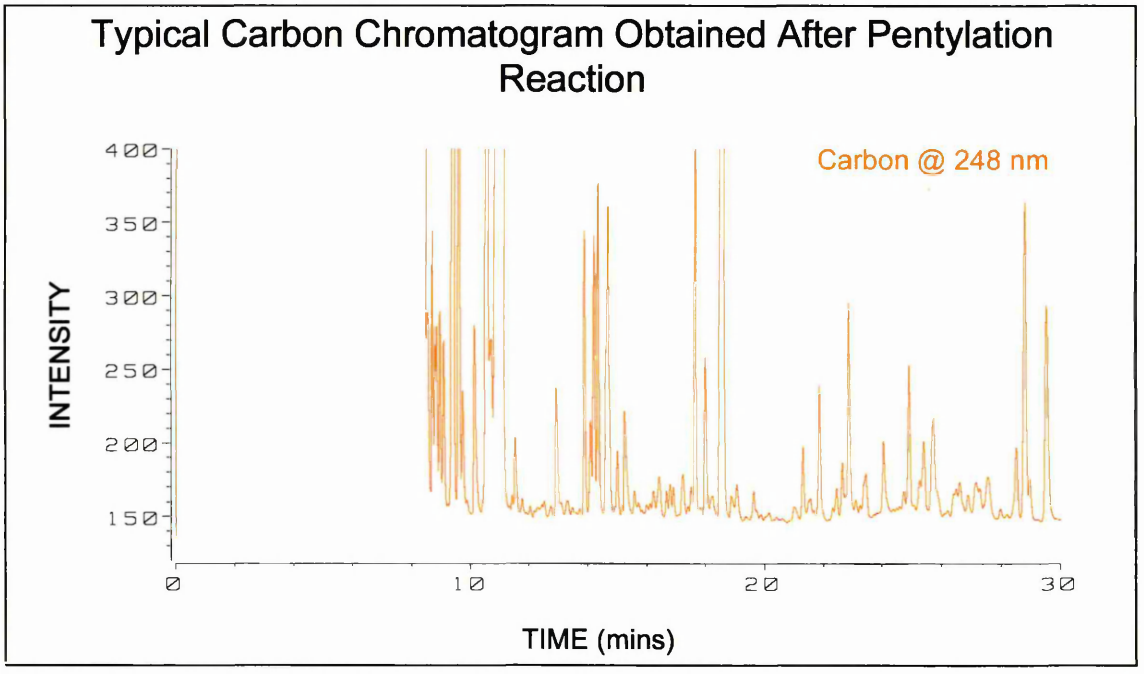


Figure 16c. GC-MIP-AED Chromatogram showing the Carbon Chromatogram obtained after Pentylation of Methyl Mercury Chloride.



The identification of each organomercury species was confirmed by GC-MS analysis. The different retention times between the GC-MIP-AED and GC-MS were accounted for by retention index (see table 5). The complexity of the carbon chromatograph obtained by the GC-MIP-AED indicated that stronger concentrations of the organomercury species would be required in order to give a large detector response by GC-MS, hence 1000 ng / µl solutions were prepared and analysed.

Table 5. Retention Index Comparison between GC-MIP-AED and GC-MS for Organomercury Species

Organomercury Species	Retention Index	
	GC-MIP-AED (column 2)	GC-MS (column 1)
MeHgBu	979	1000
MeHgPe	1096	1079
Pe_2Hg	1471	1445

Table 6. The Abundance and Mass of Naturally Occurring Isotopes of Mercury

Isotope	Accurate mass	Abundance
Hg^{196}	195.965825	0.146
Hg^{198}	197.966745	10.02
Hg^{199}	198.968222	16.84
Hg^{200}	199.968328	23.13
Hg^{201}	200.970290	13.22
Hg^{202}	201.970625	29.80
Hg^{204}	203.973471	6.85

The EI mass spectra obtained are shown in figures 17a-c. There are seven naturally occurring isotopes of mercury ranging from mass 196 to 204 as shown in table 6 with mercury^{202} being the most abundant. Hence the mass spectral data of mercury compounds have a distinctive isotope pattern.

The mass spectral data for butyl methyl mercury gives the ionised molecular species at 274 mass units. Fragments are also seen at 57 mass units from the butyl side chain and at 41 mass units indicating the loss of a methyl group from the butyl chain. At 202 mass units a range of isotopes are seen indicating the presence of mercury. The butyl chain fragment is lost from the ionised molecular species giving the ionic methyl mercury fragment at 217 mass units. In addition a cluster of peaks are seen at 245 mass units. This could be due to either the loss of the methyl side chain and a methyl group from the butyl chain, or the loss of an ethyl group from the butyl side chain.

Figure 17a. Electron Ionisation Mass Spectrum of Peak Eluting at t_R=4.3 min in the GC-MS Analysis of a Solution containing Butylated Mercury Species. This Peak was Identified as Butyl Methyl Mercury and these Data used to Confirm GC-MIP-AED Results.

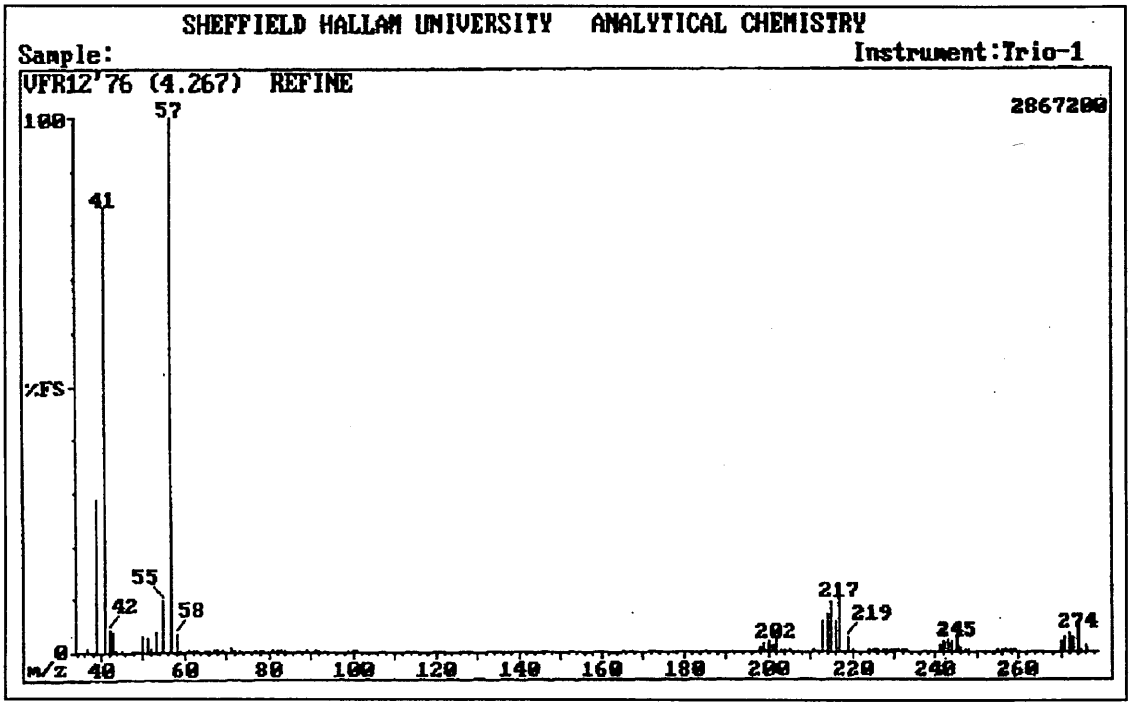


Figure 17b. Electron Ionisation Mass Spectrum of Peak Eluting at t_R=6.4 min in the GC-MS Analysis of a Solution containing Pentylated Mercury Species. This Peak was Identified as Pentyl Methyl Mercury and these Data used to Confirm GC-MIP-AED Results.

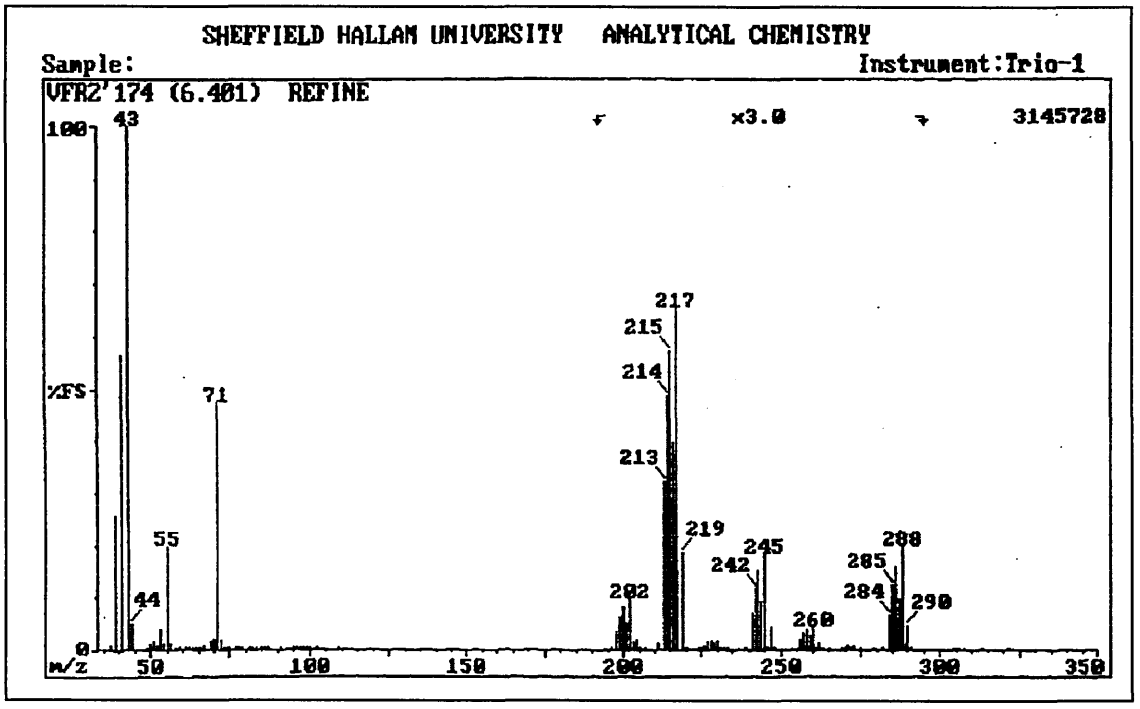
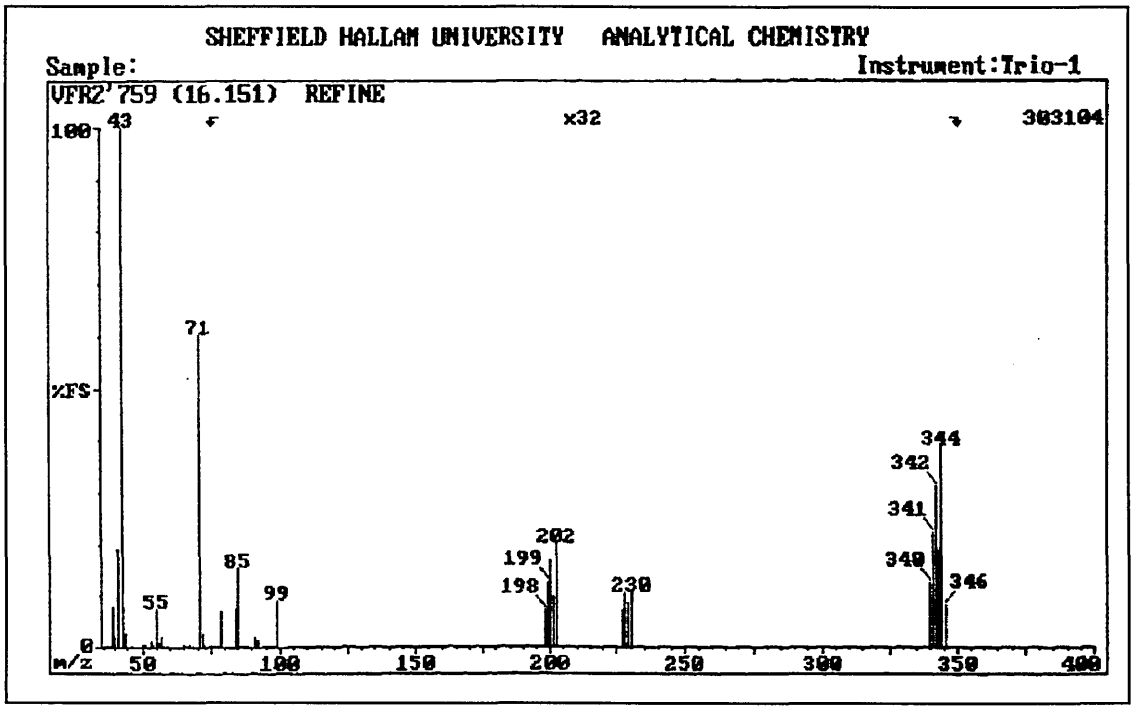


Figure 17c. Electron Ionisation Mass Spectrum of Peak Eluting at t_R=16.2 min in the GC-MS Analysis of a Solution containing Pentylated Mercury Species. This Peak was Identified as Pentyl Methyl Mercury and these Data used to Confirm GC-MIP-AED Results.



Similarly for pentyl methyl mercury the mercury isotopes are seen at 202 mass units indicating the presence of mercury and the ionised molecular species is observed at 288 mass units. The pentyl chain fragment is lost from the ionised molecular species at 71 mass units. This is seen to further fragment to loose both a methyl group and an ethyl group indicated by fragments at 55 and 43 mass units. Ionic methyl mercury is again seen at 217 mass units. Fragments containing mercury are also seen at 245 mass units, possibly due to either the loss of the methyl side chain and an ethyl group from the pentyl side chain or a propyl group from the pentyl side chain, and 260 mass units due to either the loss of an ethyl group from the pentyl side chain or the methyl side chain and a methyl group from the pentyl side chain.

Di-pentyl mercury exhibits less fragmentation than seen for the two methyl mercury species. The ionised molecular species is seen at 344 mass units and the isotope pattern is shown for mercury at 202 mass units. Fragments are also seen around 230 mass units indicating the loss of either a butyl group from both of the pentyl side chains or the loss of one pentyl side chain and a propyl group from the remaining side chain. This is supported by the presence of a fragment at 71 mass units indicting the loss of a pentyl side chain and further fragments due to the loss of a methyl group and an ethyl group, at 55 and 43 mass units. Fragments are also observed at 85 and 99 mass units, possibly from the re-combination of a methyl or ethyl fragment to a pentyl side chain. These two fragments are however very small and have been subjected to a magnification factor of 32.

The mass spectrum was not obtained for di-butyl mercury as only a very weak response was observed on the total ion chromatogram.

4.3.2 Optimisation of GC-MIP-AED Control Parameters

A number of parameters were varied in order to optimise the GC-MIP-AED for organomercury analysis. These included:

1 Heated zones including injection port temperature, transfer line temperature and microwave cavity temperature.

2 Gas flow rates including carrier gas flow rate and cavity make up gas flow rate.

3 Injection port liner and injection technique

4.3.2.1 Effect of Injection Port, Transfer Line and Microwave Cavity Temperature Variations.

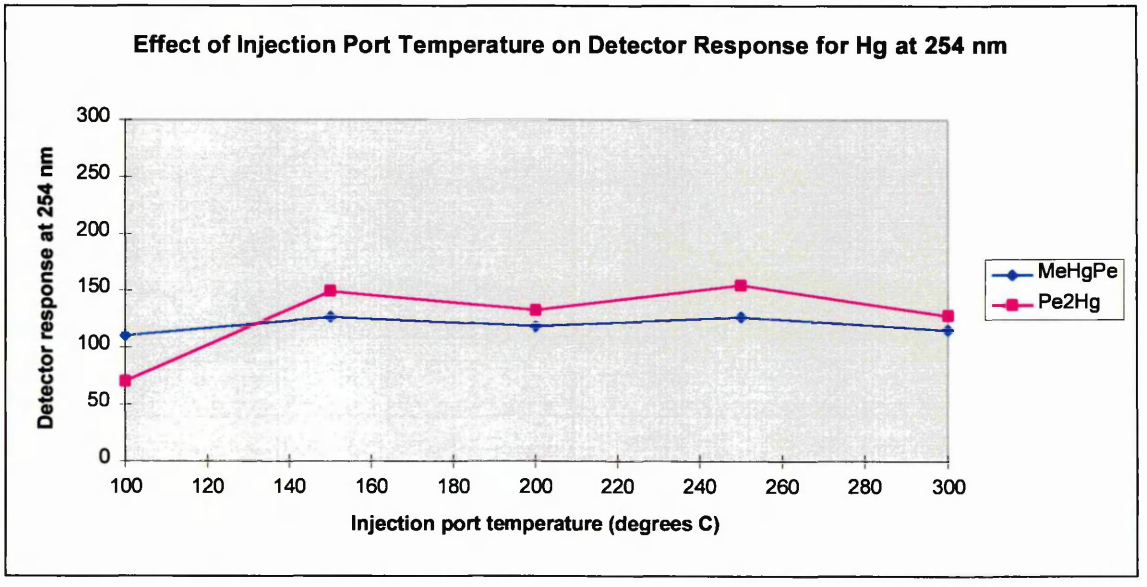
A 10 pg / µl standard of methyl mercury chloride was alkylated and analysed under the conditions specified in chapter 2 using column 2 and a cavity make up gas flow of 290 ml / min. The injection port, transfer line and microwave cavity temperature were varied from 100°C to 300°C.

Figure 18 shows the effect of injection port temperature on the detector signal obtained for pentyl methyl mercury at 254 nm. Pentylated methyl mercury gave a constant response over the full temperature range evaluated. Di-pentyl mercury initially gave a low response at lower temperatures then became

constant over the range 150°C to 300°C. The same profile was observed for the butylated derivative. This suggested that under the conditions stated alkylated organomercury compounds appeared to be stable up to 300°C and not subject to thermal decomposition or adsorption onto the injection port liner. An injection port temperature of 250°C was chosen for future work as it would allow for the volatilisation of high boiling point compounds, hence widening the scope of the analytical procedure.

Variations in the temperature of the transfer line and microwave cavity temperature showed no noticeable effect in the short term, however due to the risk of condensation a transfer line and microwave cavity temperature of 300°C was used. This was in line with the oven program which also had a top temperature of 300°C.

Figure 18. Effect of Injection Port Temperature on Detector Response for Organomercury Species.



4.3.2.2 Effect of Variation of Carrier Gas and Cavity Make Up Gas Flow Rates.

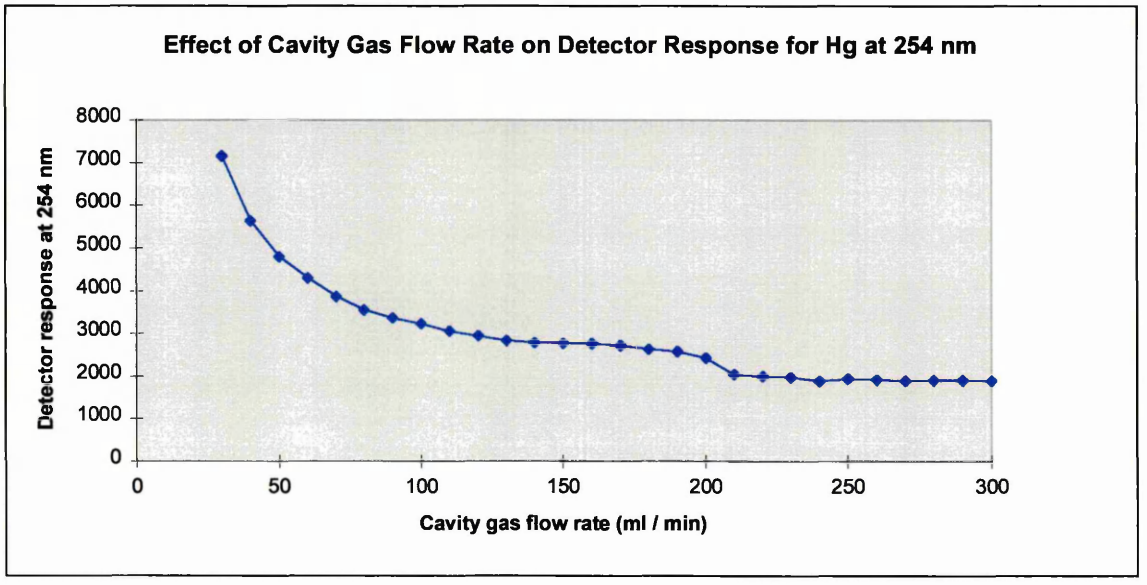
In order to achieve adequate separation and good peak shape the optimum carrier gas flow rate was determined to be 2.3 ml / min. Increasing the flow rate reduced the retention time of the methyl mercury species, thereby reducing the separation between the analyte and solvent peak. The requirement to vent the solvent when using the GC-MIP-AED may lead to the loss of this species due to co-elution with the solvent. Increasing the column flow also caused the peak shape to become broader and reduced in height. A reduction in the gas flow rate led to increased analytical run times and also a decrease in the analytical response.

The effect of cavity gas flow rate was studied for both butylated and pentylated methyl mercury species. (Cavity gas is composed of helium make up gas and reagent scavenger gases.) The same trend was observed for both species. Figure 19 shows a graph of cavity gas flow rate against signal response. The cavity gas flow rate was measured at the cavity vent.

The software recipe for organomercury speciation specifies two cavity gas flow rates, one at 60 ml / min and a second incorporating a fast flow mechanism, which when activated provides an additional flow of 140 ml / min. Figure 19 shows that the highest sensitivity for mercury is observed at the 60 ml / min flow rate. The noise level remained constant throughout the study hence the lower the cavity gas flow rate the lower the limit of detection of the technique.

Operating at faster flows will decrease the limit of detection by a factor of 2, however the higher flow rate is recommended for organotin and organolead detection. In practise cavity gas flow rates of 290 ml / min were found to be necessary as described in chapter 5.

Figure 19. The Effect of Cavity Gas Flow Rate on Detector Response for Hg at 254 nm



Hydrogen and oxygen are also required for the detection of organomercury compounds as reagent scavenger gases. The flow rates used throughout the study were set at the manufacturers recommended level of 20 ml / min.

4.3.2.3 Injection Port Liner and Injection Technique

Two injection port liners, a universal liner (without glass wool) and a single restriction liner (with a small glass wool plug at the base of the liner), were evaluated for the analysis of butyl methyl mercury (10 ng / µl as Hg) by splitless injection. The results were compared with those achieved for the quantitation of nitrobenzene and tert-butyl disulphide as discussed in chapter 3. The GC-MIP-

AED parameters used are detailed in chapter 2. Column 1 was used for this study with a cavity gas flow rate of 60 ml / min.

For high speed automatic injection the universal liner and single restriction liner gave a % RSD of 3.5 and 0.8 respectively with similar absolute peak areas (n=5).

For low speed automatic injection the universal liner and single restriction liner gave a % RSD of 4.7 and 3.6 respectively (n=5). Again similar absolute peak areas were observed however the absolute peak area for low speed injection was reduced by a factor of 2 when compared with high speed automatic injection.

These results follow the same trend as described in chapter 3. A single restriction liner with high speed automatic injection was selected as the most appropriate injection port liner and injection technique for the analysis of analytes by GC-MIP-AED using splitless injection and was used for the remainder of this research programme.

4.3.3 Evaluation of the Derivatisation Technique.

The derivatisation technique as described in chapter 2 was evaluated using column 1 with respect to the following parameters for laboratory prepared standards for both butyl and pentyl methyl mercury derivatives:

1 Reaction length

2 Linearity

3 Limit of detection

4 Precision (short term repeatability and long term reproducibility)

The butylation procedure had previously been reported by Bulska *et al* [91]. The pentylation procedure was developed as the most appropriate alkylation procedure for the analysis of multielemental organometallic species in environmental samples by GC-MIP-AED. Propylation was considered as an alternative to pentylation, however due the requirement to vent the solvent away from the plasma and the shortened retention times that would be achieved by propylation, this was not pursued.

Reaction Mechanism

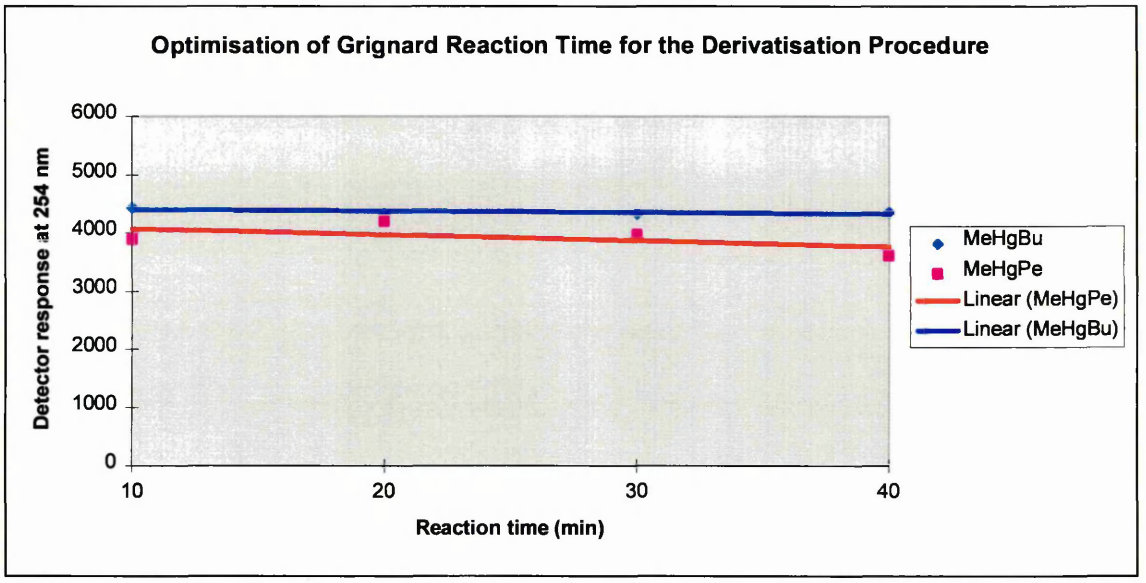
$$R_1\text{—}MgCl + MeHg^+Cl^- \longrightarrow R_1\text{—}Hg\text{—}Me + MgCl_2$$

R_1 = Butyl or Pentyl chain

The effect of different reaction times in the presence of the Grignard reagent was studied and the optimum reaction time determined for MeHgBu and MeHgPe formation. The effect of reaction time is shown in figure 20.

Figure 20 shows that for the alkylation of both mercury species the Grignard reaction has gone to completion after 10 min and no appreciable change in response was observed for extended contact with excess Grignard reagent.

Figure 20. Optimisation of Grignard Reaction Time for Derivatisation.



The linearity of the analytical technique was determined for the alkylated (derivatised) standard ranging from 1 - 1000 pg / µl. A linear response was observed for both the butylation and pentylation procedure. Typical data for MeHgPe species is given in table 7 and a typical calibration graph is shown in figure 21. The correlation coefficient was calculated to be 0.9997 over 3 orders of magnitude for both MeHgPe and MeHgBu. The limit of detection was demonstrated to be 1 pg / µl as Hg for both MeHgPe and MeHgBu with a cavity gas flow rate of 60 ml / min. At 290 ml / min cavity gas flow rate the limit of detection for both species was demonstrated to be 2 pg / µl as Hg. The derivatisation and analysis was demonstrated to be repeatable and reproducible for mercury quantitation throughout this study when using silanised glassware.

Table 7. Linearity of MeHgPe Derivative by GC-MIP-AED

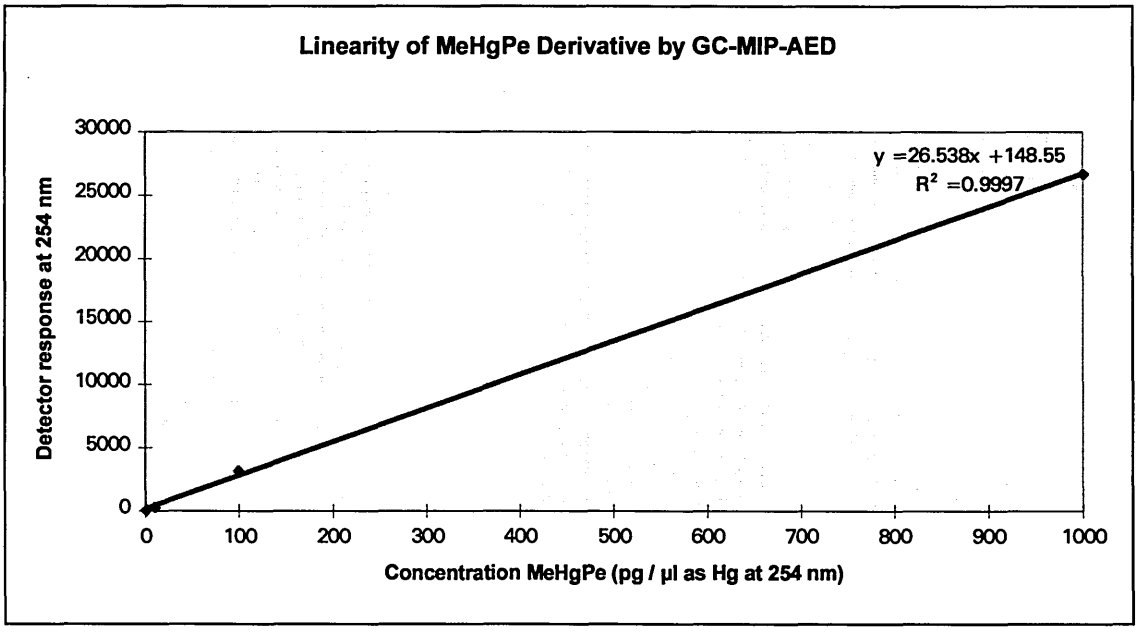
Concentration (pg / pl)	Area 1	Area 2	Mean	Standard deviation	% RSD
1 (1st prep)	27.32	25.54	26.43	1.3	4.8
1 (2nd prep)	35.3	31.38	33.34	2.8	8.3
1 (3rd prep)	26.74	26.59	26.67	0.1	0.4
1 (mean)			***28.8***		***13.1***
10 (1st prep)	267.85	264.27	266.06	2.5	1.0
10 (2nd prep)	256.21	256.98	256.60	0.5	0.2
10 (3rd prep)	264.79	272.87	268.83	5.7	2.1
10 (mean)			***263.8***		***2.4***
100 (1st prep)	3016.8	3033.2	3025.0	11.6	0.4
100 (2nd prep)	3176.5	3202.3	3189.4	18.2	0.6
100 (3rd prep)	3185.6	3164.8	3175.2	14.7	0.5
100 (mean)			***3129.9***		***2.6***
1000 (1st prep)	26241	26238	26239.5	2.1	<0.1
1000 (2nd prep)	27315	27273	27294	29.7	0.1
1000 (3rd prep)	26505	26363	26434	100.4	0.4
1000 (mean)			***26655.8***		***1.9***

The equation for the trend line for MeHgPe was determined as y=27.748x+154.76 and for MeHgBu as y=26.538x+148.55. These equations are very similar and indicate that mercury may exhibit a degree of compound independent emission. Extensive research into compound independent calibration has taken place for the Hewlett Packard GC-MIP-AED, primarily for organic compounds containing heteroatoms, but has been limited for organometallic compounds.

The determination of calibration graphs for MeHgBu and MeHgPe however took place at different time periods during this study. In order to confirm the structural independence, additional calibration graphs were compiled over the

same time period and instrumental conditions for Bu_2Hg and Pe_2Hg, with the aim of using Bu_2Hg as an internal calibration standard for organomercury determination. The calibration graphs are shown in figure 22.

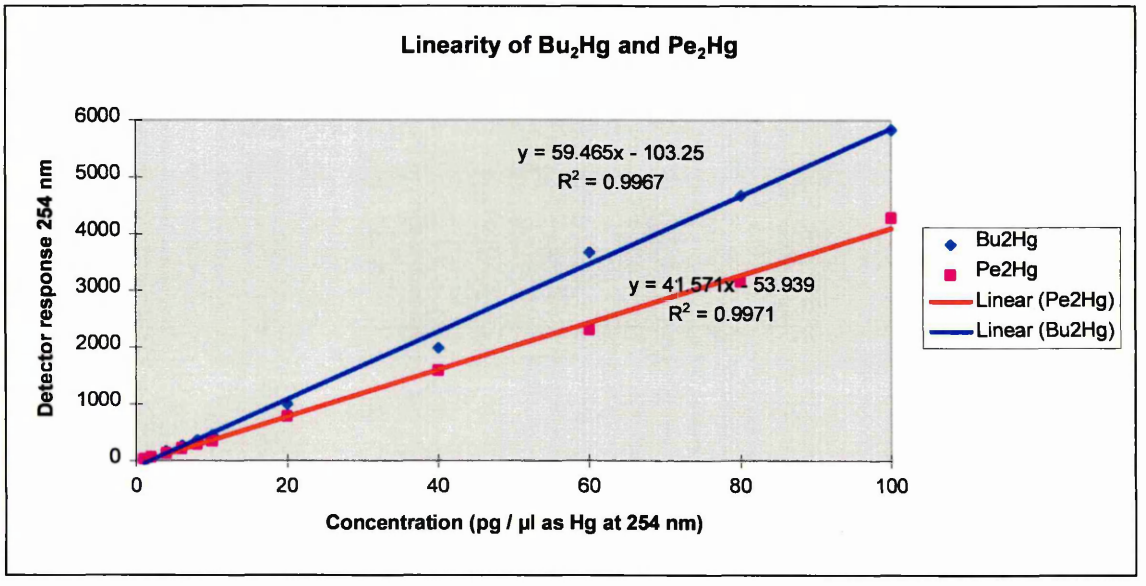
Figure 21. Linearity of MeHgPe Derivative by GC-MIP-AED



For the emission of mercury to be considered independent of structure a graph of response against concentration (as Hg) would give the same slope and y-intercept. In figure 22, if the mercury emission was independent of structure then the graphs of Pe_2Hg, Bu_2Hg and also MeHgBu and MeHgPe should all be superimposable. This was found not to be the case in practise. The correlation coefficients gave an acceptable degree of linearity, however the slopes and intercepts differ for the organomercury compounds indicating a degree of structural dependency. This precludes the use of Bu_2Hg as an internal calibration standard for compound independent calibration. The observed structural dependency may be due to incomplete atomisation or molecular

recombination within the plasma, which has been reported for a number of organic compounds. For example, the ^{13}CO molecular emission line at 171 nm is used to determine the response for labelled compounds. Similar side reactions within the plasma were also observed for organotin and organolead compounds and the effect will be discussed in more detail in chapters 5 and 6.

Figure 22. Linearity of Bu_2Hg and Pe_2Hg



Linearity, linear dynamic range and limit of detection were also determined for GC-MS selected ion monitoring detection over the range 1-1000 pg / µl as Hg for pentyl methyl mercury. The ions monitored are listed in chapter 2. This technique gave a limit of detection of 100 pg / µl as Hg hence only producing a 2 point calibration graph. A linear response was seen for mass 200, however the response seen at mass 286 and 288 for the 100 pg / µl standard was only a factor of 5 less than the response for the 1000 pg / µl standard. At mass unit 202 a response was observed for the 1000 pg / µl standard only. The GC-MS relies on complete separation of all peaks within a chromatogram and by

monitoring selected mass ions the possibility of co-elution of a number of compounds is possible. If co-eluting compounds also fragment to give the mass ion of interest this would contribute to the given response signal for a particular compound causing a non linear response. By obtaining a response signal for a given element, for example mercury, by atomic emission detection, it is unlikely that any co-eluting compounds also contain the same element hence they will not contribute to the analytical signal.

In summary the derivatisation procedure and detector response for GC-MIP-AED appear to be robust, linear and precise for the qualification and quantification of organomercury species. The limit of detection and linear dynamic range were found to be adequate for application to environmental samples. The results obtained for GC-MS-SIM showed that the technique lacked the sensitivity of the GC-MIP-AED and did not appear to show consistent linearity over the range of mass ions monitored. This shows the superiority of the GC-MIP-AED over the GC-MS for routine quantitative analysis and also the combination of the two techniques give complementary evidence for compound identification.

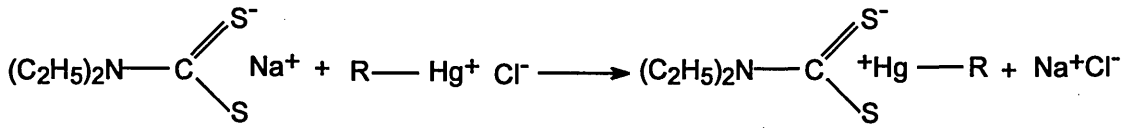
4.3.4 Evaluation of the Extraction Procedure

Solvent extraction systems have been extensively used in the field of analytical chemistry. However, simple organic / aqueous phase solvent extraction were found to give poor recoveries for the extraction of metal and organometal salts due to the ionic nature of these compounds. In order for metal extraction to

take place into organic solvent with high efficiency, an uncharged species should be formed, for example, by the formation of a neutral chelate complex. The formation of a specific chelate complex can be dependant on a number of variables, particularly pH. In the past the role of chelate extraction was to enable the specific extraction of a particular element for quantitation by a none specific quantitative procedure, for example, by titration. The introduction of specific detection techniques has lead to a change in application of this separation procedure to allow for non specific extraction of ionic species. For this particular application the pH is adjusted to 8.5 to allow for multielemental extraction as a diethyldithiocarbamate chelate complex into toluene.

The extraction technique as described in chapter 2 was evaluated using laboratory prepared aqueous samples. The technique was based on the procedure described by Bulska *et al* [91]. Initially a commercially available borate buffer was used to adjust the pH of the solution as described by Bulska *et al*, however high levels of inorganic mercury were found in the buffer used. To avoid any contamination from extraction reagents the chelation reagent and the buffer were prepared as a single solution and purified before use by extraction with toluene. This preparation was as described by Lobinski *et al* [153] for the analysis of organolead compounds and removed any organometallic and inorganic metal species.

Chelate Extraction Mechanism for Organomercury Chloride Salts

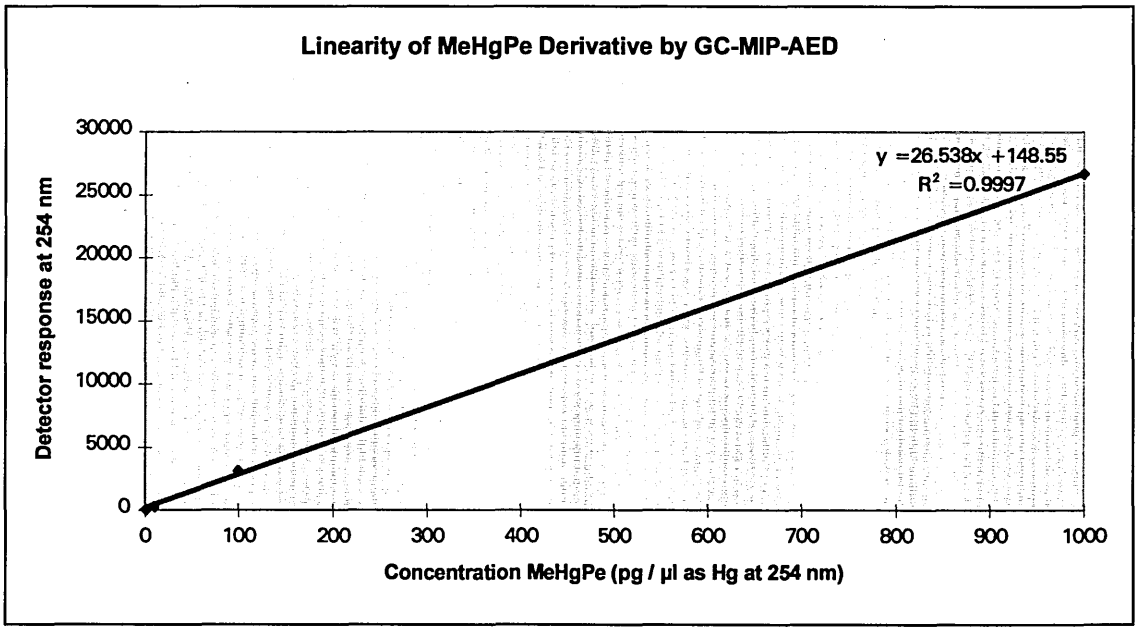


Each extraction was performed in duplicate incorporating a ten fold preconcentration and derivatised together with an appropriate MeHgCl standard in toluene of the expected final concentration. Both butylation and pentylation derivatisation procedures were evaluated and similar results were observed for both alkylation procedures. For the purpose of this thesis the discussion will be limited to the pentylation procedure as the equivalence of the derivatisation technique has already been discussed with respect to linearity. Typical results for aqueous extraction followed by pentylation for aqueous solutions ranging from 0.1 – 100 pg / μl are shown in figure 23. % Recoveries were calculated for the aqueous extracts against standards and are shown in table 8.

Table 8. Recoveries for the Aqueous Extraction Technique

Concentration (pg / μl)	Actual concentration (pg / μl)	% Recovery	%RSD
0.1	0.109688	109.69	4.8
1	1.004954	100.5	2.9
10	9.501587	95.02	3.6
100	109.5953	109.6	3.5

Figure 23. Linearity of Aqueous Extracts of MeHgCl followed by Pentylation



A linear response was observed for the aqueous extraction procedure. The correlation coefficient for the aqueous extraction followed by pentylation was r^2=0.9998. The recoveries of the extraction procedure with respect to standards were also high for the laboratory prepared aqueous samples. The limit of detection for the extraction procedure followed by derivatisation as described was observed to be 0.1 pg / µl for an aqueous solution at 60 ml / min cavity gas flow rate. This detection limit may be further reduced by incorporating a higher preconcentration factor.

In summary the extraction procedure gives high recoveries for low concentrations of methyl mercury species as found in environmental samples. A linear response was also observed indicating that the presence of the chelate complex does not appear to effect the alkylation procedure.

4.4 Summary

The extraction and butylation procedure described by Bulska *et al* [91] was successfully replicated for methyl mercury species giving acceptable results using the commercially available GC-MIP-AED. The technique was then customised for this study incorporating pentylation as the alkylation procedure. At the time of study this was a novel approach for the determination of organomercury species and a review of the literature reported only butylation via Grignard reaction. This chapter has critically evaluated the extraction, derivatisation and detection procedure, which in principle is suitable for application to environmental samples. The GC-MIP-AED method has been shown to be linear, precise and repeatable, with a high degree of accuracy and a limit of detection of 1 pg / µl prior to preconcentration at 60 ml / min cavity gas flow rate. Although the GC-MS was unable to compete with the limit of detection and degree of linearity of the GC-MIP-AED, the two techniques provide complementary evidence relating to the identification of a particular species.

Lui *et al* [35], performed a similar study using pentylated organomercury compounds during the same time period as this research project with similar objectives, however a number of differences were observed. Lui *et al* reported that reproducible separation and response where not achievable using a split / splitless inlet. Their study moved to use a cool on-column injector. The reasons for the difference in results are unclear as details of operation of the split /

splitless injection port, together with mode of injection, liner and injection port temperature are not given. The importance of tailoring these parameters for quantitative analysis by external standard has already been identified in chapter 3 in order to achieve an acceptable level of precision. Lui *et al* however found that split / splitless injection performed well for organolead and organotin compounds. This effect was corroborated in this current study and will be discussed in chapters 5 and 6. Different analytical column stationary phases were also used for the two studies.

The GC-MIP-AED has been shown to give a compound dependant response for similar molecular structures containing mercury suggesting that compound independent calibration is not possible for mercury containing species. Webster [19] has extensively reviewed this topic in the literature and studied the possibility of compound independent calibration. The results reported were variable, for example, in the analysis of a group of phenols elemental response was found to be independent of molecular structure for chlorine, but structurally related for carbon and oxygen. A group of chloroanisoles however showed no such structural dependence which was attributed to the polar nature of these phenolic compounds. This structural dependence has been linked to the variation of breakdown pathway of the molecule within the plasma and the formation of stable oxygen containing molecules. More recently Stevens and Borgerding [154] have suggested that column flow rate and sample injection mode may also effect the ability of the GC-MIP-AED to perform compound independent calibration.

The effect of possible matrix interferences from real samples will be assessed in chapter 7 where the technique is applied to a number of real samples, including water, fish tissue and sediment.

Chapter 5

Organolead Quantitation

5.0 Organolead Quantitation

5.1 Introduction

Lead exists in various forms in the environment. The major anthropological uses of lead today include the production of lead-acid batteries, as a fuel additive, in building materials and armaments. Over the past century a high proportion of lead has been consumed within the automotive industry in the production of organolead compounds, which are used as antiknock agents in gasoline and aviation fuels. This particular use is considered to be the major anthropological source of organolead compounds and has led to wide distribution of this heavy metal within the urban environment [155]. Other sources of lead in the environment include agricultural sprays (lead arsenate), lead smelters, cable insulation, anticorrosion products, ceramic glasses, radiation shields and power stations (lead in coal). The possibility of environmental alkylation has been reported and must also be considered [110b].

Diet is the major source of inorganic lead in man. The most frequent cause of lead poisoning occurs through absorption of lead through the gastrointestinal tract. Drinking water is perhaps the most common source of lead within the diet particularly in areas where lead plumbing is still in current use. It has also been shown that absorption of lead can occur through the skin and the respiratory tract, particularly with organolead compounds [156].

Organolead compounds exhibit a much greater toxicity to man than inorganic forms of lead due to their increased solubility in lipids. Tetra-alkyl lead is broken down in the liver to form tri-alkyl lead salts which are the most toxic species of organolead compounds known to man. Tri-alkyl lead compounds are known to inhibit oxidative phosphorylation which primarily effects the central nervous system causing the death of cells within the brain tissue [157]. Dealkylation can occur in the liver by oxidative processes, followed by excretion in urine [156].

The sources and uses of organolead compounds and their industrial manufacture has been reviewed by Hewitt and Harrison [110b]. The current trend has been for the reduction of organolead additives in gasoline world-wide, which has been achieved by legislative action due to concerns over the toxicity of these compounds. Organolead compounds are released into the environment mainly from motor vehicles, as in general approximately 1% of their content in gasoline leaves the exhaust unchanged [37]. This figure may fluctuate depending upon engine type. The fate, behaviour, toxicity and transport of these organolead compounds and their degradation products in the environment has been reviewed by Rhue *et al* [155]. Jarvie *et al* also studied the decomposition of tetra-alkyl lead compounds in aqueous systems due to sunlight and the presence of iron and copper ions [158].

The localised pollution caused by organolead compounds from gasoline additives is thought to be increasing the overall global lead concentration due to the high volatility of these compounds. Lobinski *et al* [37] developed an

approach, originally pioneered by Patterson *et al* and Boutron *et al*, which proposed that lead concentration levels in successive layers of Greenland snow and ice may hold the key to lead concentration mapping over the past centuries, to identify whether an actual increase in global lead concentration can be attributed to natural or anthropological sources. This proposal is based on the assumption that the concentration of lead in successive layers of precisely dated snow and ice are proportional to those existing in the polar troposphere at the time of precipitation. Results from a survey of Greenland and Antarctica snow and ice revealed a general increase in total lead concentration through the ages especially in the last century. By applying sensitive analytical speciation techniques it may be possible to identify conclusively the source of the increased lead concentrations.

The highly toxic nature of organolead compounds highlight the need for rapid, highly sensitive, accurate and precise, analytical speciation techniques for localised and global pollution quantification.

5.2 Review of Analytical Techniques for Organolead Speciation

5.2.1 Sample Preparation - Extraction and Derivatisation Techniques

Prior to quantitation, a suitable extraction procedure must be performed in order to extract the species of interest from animal / plant tissue, sediments, water or the atmosphere [10, 110b, 159], as described earlier for organomercury compounds. A pre-concentration step may also be incorporated due to the low concentrations of the pollutants.

A review of the literature for the speciation of organolead compounds indicated a general trend towards Grignard derivatisation coupled with a variety of extraction techniques however a general overview of typical extraction and derivatisation procedures will be discussed. The extraction and derivatisation technique employed is dependant upon the particular species of interest, for example, if only tetra-alkyl lead species are of interest then the extraction procedure is greatly simplified and derivatisation is not necessary. If ionic alkyl lead salts are the species of interest in addition to tetra-alkyl lead species then the analytical extraction and derivatisation procedures become more demanding.

Brunetto *et al* [160] studied the analysis of tetra-alkyl lead compounds in gasoline. In this instance no extraction or derivatisation procedures were required. Quantitation was achieved by GC-AAS.

Nielsen *et al* [161] developed a method for the analysis of tetra-methyl lead (TML) and tetra-ethyl lead (TEL) in air samples. Adsorption tubes (Porapak packing) containing known amounts of deuteriated TML and TEL were used to sample a variety of localised atmospheres. Any tetra-alkyl lead compounds were collected on the tube packing material. Prior to analysis the tetra-alkyl lead compounds were thermally desorbed from the sampling tube and collected on a different packing material before vaporisation and detection by GC-MS-

SIM. The research concluded that in 1980, 5-10% of total airborne lead content in European cities is due to tetra-alkyl lead compounds.

Nerin *et al* [162-163] progressed this method for the determination of tetra-alkyl lead compounds for sampling in air by monitoring their adsorption behaviour on different packing materials using a synthetic air mix containing a known concentration of alkyl lead standard. In this instance the packing material was extracted three times with hexane in an ultrasonic bath, the hexane reduced under a stream of nitrogen and diluted to 5 ml. Quantitation was achieved by GC-MS-SIM. Porapak and Tenax packing material gave recoveries of 92 ± 8 % and 96 ± 5 % respectively for TEL and a combination of the two packing materials is recommended. The adsorption cartridges were found to be stable for at least 45 days when stored in the dark at 4°C.

These methods are specific to air monitoring and concentrate on the more volatile tetra-alkyl lead species, hence precluding the simultaneous quantitation of ionic alkyl lead salts. In order to achieve this objective by GC a method of derivatisation to form a volatile tetra-alkylated species is essential.

The speciation of organolead compounds in general follows a similar concept to that described for organomercury compounds in chapter 4. First a digestion procedure is employed in order to release any bound compounds, when necessary, depending upon sample matrix. The organolead compounds are then separated, and in some cases preconcentrated in an extraction procedure,

followed by a derivatisation procedure to render any ionic alkyl lead species to their tetra-alkylated form.

Each of these analytical stages will be considered individually as any digestion procedure could in theory be coupled with any extraction procedure, which in turn may also be coupled to an appropriate derivatisation procedure.

For sample matrices, such as fish tissue and sediment, a number of pre-extraction and / or digestion procedures have been employed. These include, alkaline digestion [164-165], enzymatic digestion [157, 166-168] and supercritical fluid extraction [35, 169]. Enzymatic and alkaline digestion procedures are typically applied to organic tissue samples such as fish tissue where the sample matrix is broken down to enable the organolead compounds to be extracted. For example, Chau *et al* [165] used a tetra-methyl ammonium hydroxide solution, held at 60°C for 2 hrs, to dissolve fish tissue prior to extraction. Forsyth *et al* [157, 166-168] used a mixture of lipase and protease, buffered to pH 8.5 and incubated at 37°C for up to 48 hrs, to digest fish tissue and egg samples. Ethanol was added to suppress bacterial growth during the incubation period.

Supercritical fluid extraction (SFE) is a technique which has also been applied to extract ionic organolead compounds from sediments. Supercritical fluid has a high density which gives it high solvation properties and the ability of the fluid to diffuse through the sample and extract analytes suggest it to be a suitable

extraction technique for this sample matrix. Johansson *et al* [169] used carbon dioxide containing methanol as a modifier, under appropriate temperature and pressure conditions as a supercritical fluid. Methanol was found to be the most appropriate modifier for this application due to the polar nature of the ionic alkyl lead salts and was also used as a collecting solvent. The technique unfortunately could not simultaneously extract tetra-alkyl lead compounds. For this particular application SFE is acting as a sample pre-treatment to remove any matrix effects prior to extraction from methanol into a non-polar solvent followed by derivatisation.

The extraction techniques for the analysis of organolead compounds are generally based on liquid liquid extraction either directly into solvent or with the aid of a chelation reagent to aid the extraction of ionic compounds by the formation of a neutral complex. A variety of solvents have been used for this application including hexane, benzene, pentane and tetrahydrofuran in conjunction with either sodium diethyldithiocarbamate [164-165] or dithizone [157, 166-168], buffered to a specific pH, as metal chelation reagents. It has also been reported that tetra-alkyl lead compounds have a tendency to adsorb on to the surface of the sample collection vessel, which must be taken into account in the extraction procedure in order to achieve meaningful analytical results [170].

Estes *et al* [63] employed a simple liquid liquid extraction procedure from aqueous samples into benzene at neutral pH. Sodium chloride was added to

increase the partition coefficient of tri-alkyl lead salts into the organic benzene layer and also to aid separation of the two liquids.

Lobinski *et al* [64, 82, 153, 171-173] used sodium diethyldithiocarbamate buffered to pH 8 with a citric acid / ammonia buffer system to extract organolead species into hexane from a variety of samples including water, wine, plant sap and snow. EDTA was added to the extraction system to reduce the transfer of inorganic lead into the hexane solvent layer. Specifically designed glassware was used for one particular extraction procedure to allow for a preconcentration factor of approximately 400 [153]. Low recoveries of organolead compounds were reported in the presence of inorganic lead, which was addressed by the use of EDTA to remove this interference. The effect of EDTA addition has been described in detail by Chakraborti *et al* [174].

This general procedure was also reported in a number of publications, with a number of variations, including preconcentration factor, a reduction of the final sample extract by evaporation [175], and solvent exchange prior to derivatisation [174, 176-179]. The pH of extraction was found to be critical for high recoveries for the simultaneous extraction of organolead species [174].

An alternative extraction procedure was reported by Hewitt *et al* [180] using solid phase extraction on ion exchange resin for the preconcentration of ionic organolead compounds prior to extraction.

The primary derivatisation procedure following extraction was reported to be alkylation by Grignard reaction in all of the above examples. A variety of alkylation reagents have been used for this purpose resulting in the formation of the propyl [64, 82, 153, 171-173], butyl [64, 166-168] or more recently pentyl [35, 157] derivatives. The derivatisation procedure was as previously described for organomercury Grignard alkylation in chapter 4.

Forsyth [181] and Estes *et al* [63] have reported an additional clean up / preconcentration stage by applying a purge and trap technique to organolead compounds after undergoing alkylation.

Lobinski *et al* [153] also reported additional preconcentration of the derivatised sample. In this case preconcentration was achieved on-line in a packed injection port liner using a temperature programmed injection port.

Witte *et al* [60] reported an alternative alkylation procedure for organolead speciation, based on aqueous phase ethylation by sodium tetraethylborate followed by extraction into hexane. This particular technique precludes the simultaneous analysis of ethyl lead compounds. It may however be possible to substitute sodium tetraethylborate for sodium tetraphenylborate. More recently Heisterkamp and Adams [182] reported a procedure for the aqueous phase butylation of organolead species, using tetra-butylammonium tetra-butylborate, which would also enable the speciation of ethyl lead compounds.

Quevauviller *et al* [183-184] published reports on interlaboratory studies based on a variety of the extraction and derivatisation procedures previously described, also using a range of analytical detection systems.

5.2.2 Gas Chromatographic Detection of Organolead Species

A number of detectors have been used in gas chromatography, the most commonly reported techniques include:

1) MS (Mass Spectrometry)
2) AAS (Flame / Electrothermal decomposition Atomic Absorption Spectrometry)
3) MIP-AED (Microwave Induced Plasma - Atomic Emission Detector)

GC coupled with ECD [157] and FID [185-186] have also been reported however their application is limited due to the non-specific nature and lack of sensitivity of these detectors.

GC-MS is also limited as complete separation of all analytes, which leads to extended chromatographic run times, is required. Adequate sensitivity can be achieved in selected ion monitoring mode, however as discussed for organomercury if complete separation of all analytes is not achieved then some of the observed signal may be due to interferences which fragment to give the same mass ion, thereby effecting quantitation.

As for organomercury, atomic spectroscopy offers the best detection capabilities for organolead compounds and has been reviewed by Radojevic (Harrison and Rapsomanikis) [10]. GC-AAS appears to be the most extensively used detection technique using a lead discharge lamp with a deuterium lamp used for background correction. GC-AAS coupling has been achieved in a number of ways. Early systems introduced the GC eluent directly into the AAS nebuliser or burner head and atomisation was achieved using an air / acetylene flame. More recently graphite furnace and custom built electrothermal detector cells have been used. Ebdon *et al* [187] reviewed a number of different detector cells including the conventional flame technique. The best results were achieved with a flame heated ceramic detector tube made of recrystallised alumina used in conjunction with hydrogen make up gas.

Chakraborti *et al* [174] used a specially developed GC-AAS interface using an open silica cell, normally used with a mercury hydride system, suspended in an air / acetylene flame. This system was developed as the tetra-alkylated lead species were reported to condense inside the inner gas flow entrances of a system based on graphite furnace atomisation. Hydrogen make up gas was again reported to be necessary for atomisation to occur. This was attributed to the higher temperature which is achieved when hydrogen gas burns. The system was applied to the determination of ionic alkyl lead salts in water. Chelate extraction with sodium diethyldithiocarbamate followed by butylation with Grignard reagent was employed prior to analysis. Recoveries of between 90-108% were achieved.

The difficulties associated with the use of Graphite furnace were also experienced by Nygren [176] and soot formation in the furnace with injection volumes exceeding 1 µl was observed. In this case hydrogen make up gas was not specified. However the reported technique, again based on chelate extraction with sodium diethyldithiocarbamate and Grignard butylation, for the quantification of tri-methyl lead salts in human blood gave recoveries of between 89-99%.

Forsyth and Marshall [188] studied the effect of hydrogen make up gas on the atomisation process of organolead compounds. They proposed that the hydrogen make up gas atomised to form hydrogen radicals which were thought to be involved in the formation of lead hydride, facilitating the atomisation process. In the absence of hydrogen make up gas, lead was deposited on the walls of the atomisation furnace. These deposits were reported to be removed when hydrogen was reintroduced to the furnace.

GC-AAS is limited in that simultaneous multielemental analysis is not possible due to the different atomisation requirements and elemental lamps needed for detection. Here atomic emission spectroscopy holds the advantage. GC-MIP-AED has more recently been used for the analysis of organolead compounds due to the development of commercially available instrumentation and has detection limits suitable for environmental applications.

Lobinski *et al* [64, 82, 153, 171-173] has evaluated the use of the GC-MIP-AED for the analysis of organolead compounds in a range of different sample materials. Optimum transfer line and cavity temperatures of 300°C and a cavity gas flow rate of 300 ml / min were reported. Hydrogen make up gas was also found to be critical as by increasing the concentration of hydrogen in the cavity gas flow mixture an increase in signal was reported [64].

5.2.3 Summary

To summarise the techniques employed to extract, derivatise and quantitate organolead compounds are extensive and diverse. Again during the initiation of this study the analysis of organolead compounds was performed mainly as an isolated technique. The opportunity existed to develop an analytical procedure for the multielemental analysis of organometallic compounds by adapting the principles of previously reported analytical techniques for application to the Hewlett Packard GC-MIP-AED.

5.3 Application of the GC-MIP-AED for the Determination of Organolead Species.

The objectives of this program of research was to utilise the GC-MIP-AED, initially concentrating on organomercury speciation, while maintaining the possibility of widening the scope of the technique to incorporate the simultaneous analysis of additional organometallic species. Specifically, the multielemental analysis of organomercury, organotin and organolead species within a single chromatographic injection for environmental samples.

The extraction and derivatisation procedure described in chapter 4 for the analysis of organomercury species was extended to the analysis of organolead compounds. Pentylation was used as the derivatisation procedure as although from a review of the literature the most popular alkylation techniques were propylation and butylation, pentylation was deemed to be the most appropriate alkylation procedure for simultaneous multielemental analysis.

GC-MS was used to aid identification of the organometallic derivatives and also as a comparative quantitative technique.

The Hewlett Packard GC-MIP-AED allows for detection of organoleads by use of the emission line for lead at 261 nm and also at 406 nm within the standard recipe software package. The analysis was performed at 261 nm to allow for the simultaneous detection of mercury, lead and tin organometallic compounds on the PDA detector in order to meet the objectives of this program of study.

5.3.1 Identification and Confirmation of Organolead Species

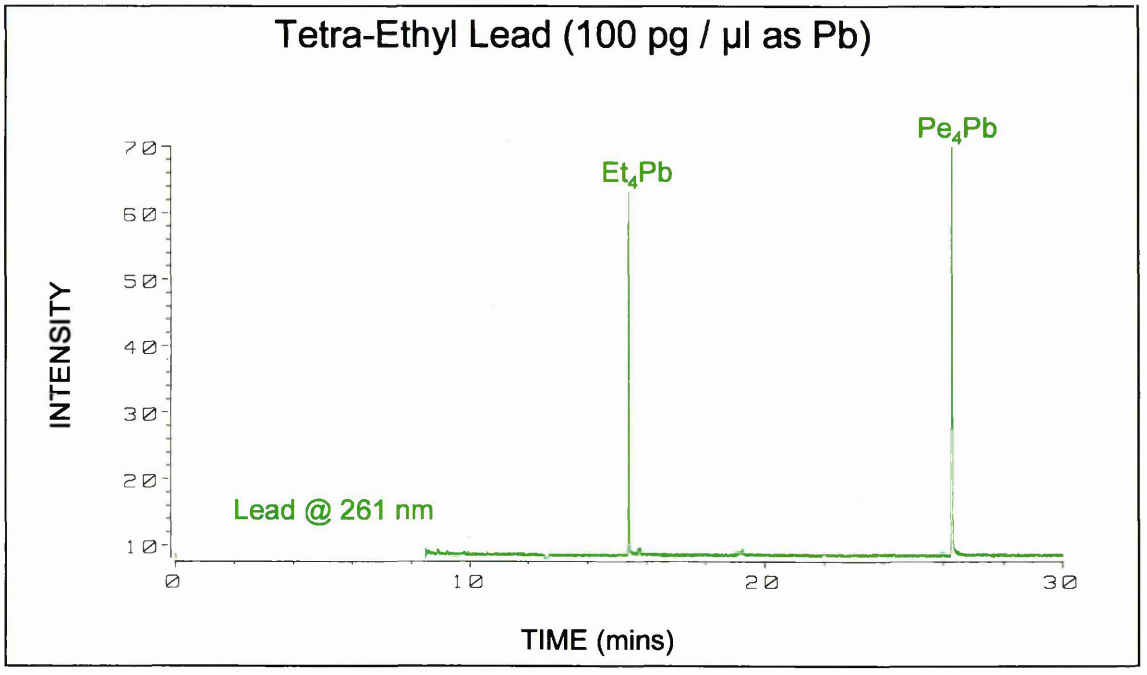
In order to assess the GC-MIP-AED's applicability for organometallic speciation, a preliminary investigation was performed under the conditions specified in chapter 2. Commercially available tetra-ethyl lead was subjected to the pentylation procedure described in chapter 2 and analysed qualitatively by capillary GC-MIP-AED (column 2) and GC-MS (column 1). Tetra-methyl lead was also analysed under the same conditions however the analyte eluted

during the solvent venting period. As such the analysis of this compound was not pursued.

Sample introduction was performed as previously described in chapter 4 for organomercury analysis.

Figure 24 shows a high degree of selectivity and specificity for both the tetra-ethyl lead species and inorganic lead (present as an impurity). No interference was observed from carbon breakthrough. The lead emission line spectrum was confirmed from spectral data. A typical snapshot for lead emission at 261 nm is shown in appendix 2.

Figure 24. GC-MIP-AED Chromatogram showing Tetra-Ethyl Lead.



The identification of tetra-ethyl lead was confirmed by GC-MS analysis. The different retention times between the GC-MIP-AED and GC-MS were

accounted for by retention index (see table 9). A 1000 ng / µl solution was prepared and analysed by GC-MS.

Table 9. Retention Index Comparison between GC-MIP-AED and GC-MS for Organolead Species

Organolead Species	Retention Index	
	GC-MIP-AED (column 2)	GC-MS (column 1)
TEL	1173	1151

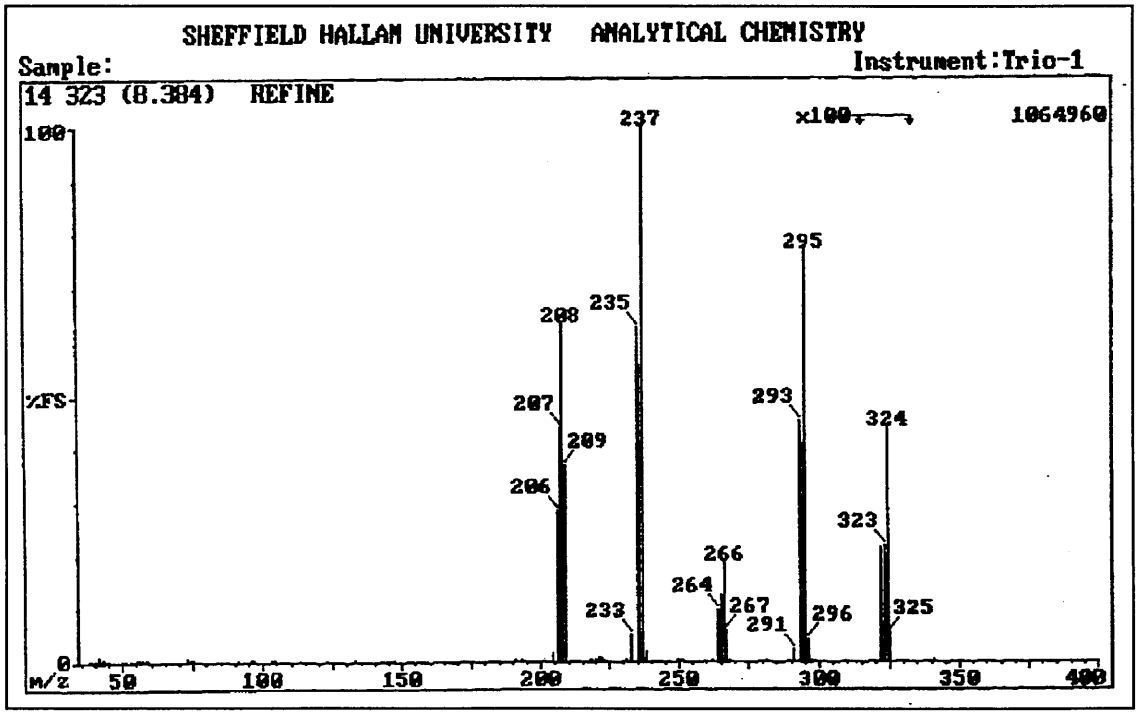
The EI mass spectrum obtained is shown in figure 25. There are four naturally occurring isotopes of lead ranging from mass 204 to 208 as shown in table 10 with lead^{208} being the most abundant. Hence the mass spectral data of lead compounds have a distinctive isotope pattern as seen for organomercury compounds.

Table 10. The Abundance and Mass of Naturally Occurring Isotopes of Lead

Isotope	Accurate mass	Abundance
Pb^{204}	203.973078	1.37
Pb^{206}	205.974435	25.15
Pb^{207}	206.975884	21.11
Pb^{208}	207.976649	52.38

The mass spectral data for tetra-ethyl lead gives the ionised molecular species at 325 (M+1) mass units. At 208 mass units a range of isotopes are seen indicating the presence of lead. Ethyl chain fragments are lost from the ionised molecular species giving molecular fragments at 295, 266 and 237 mass units corresponding to Et_3Pb^+, Et_2Pb^{2+} and $EtPb^{3+}$ respectively.

Figure 25. Electron Ionisation Mass Spectrum of Peak Eluting at t_R=8.4 min in the GC-MS Analysis of a Solution containing Pentylated Lead Species. This Peak was Identified as Tetra-Ethyl Lead and these Data used to Confirm GC-MIP-AED Results.

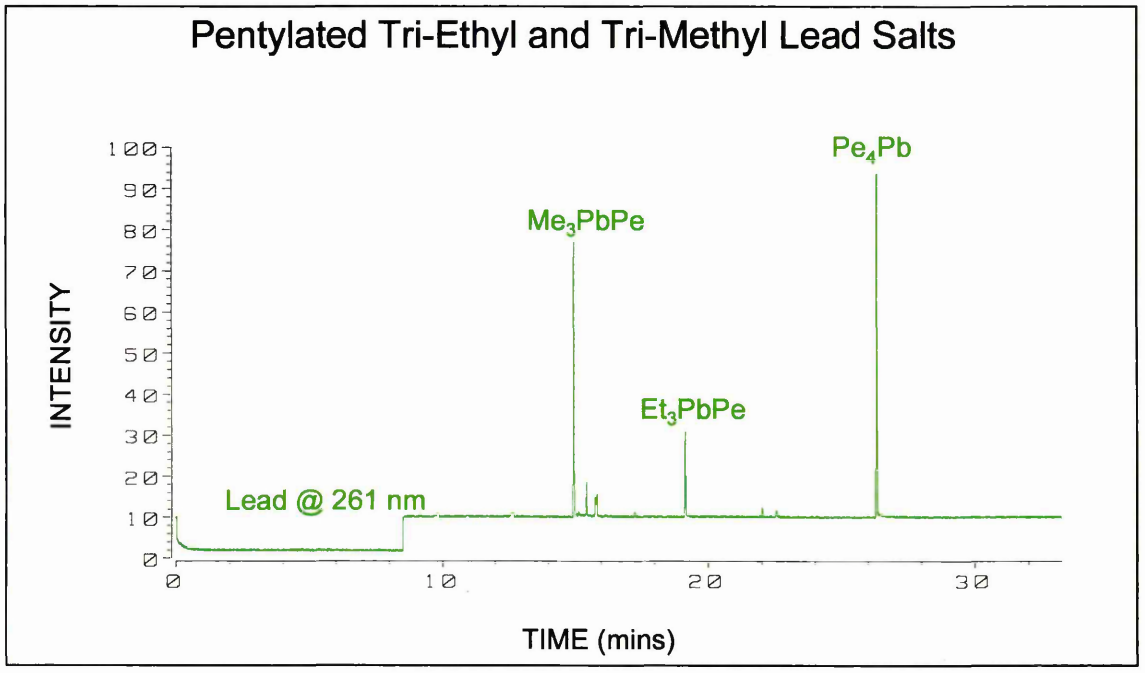


The mass spectrum was not obtained for tetra-pentyl lead as only a very weak response was observed on the total ion chromatogram.

Methylated and ethylated organolead salts were not available as standards commercially. However an old aqueous solution containing tri-methyl and tri-ethyl lead salts became available towards the end of the research period. Analysis performed on this aqueous sample was limited to a qualitative nature by GC-MIP-AED and the analytes identified by retention index. The

chromatogram obtained is shown in figure 26. The presence of lead was confirmed by elemental snapshot data only, as insufficient sample was available to achieve the concentration necessary to confirm the identification of the species by GC-MS.

Figure 26. GC-MIP-AED Chromatogram showing Tri-Ethyl and Tri-Methyl Pentylated Lead Salts.



5.3.2 Optimisation of GC-MIP-AED Control Parameters

A number of parameters were varied in order to optimise the GC-MIP-AED for organolead analysis. These included:

1 Heated zones including injection port temperature, transfer line temperature and microwave cavity temperature.

2 Cavity make up gas flow rate.

3 Linearity of response

A single restriction liner with high speed automatic injection was used for the analysis of tetra-ethyl lead by GC-MIP-AED using splitless injection based on the results previously observed in chapters 3 and 4 for the analysis of nitrobenzene, tert-butyl disulphide and alkylated organomercury compounds.

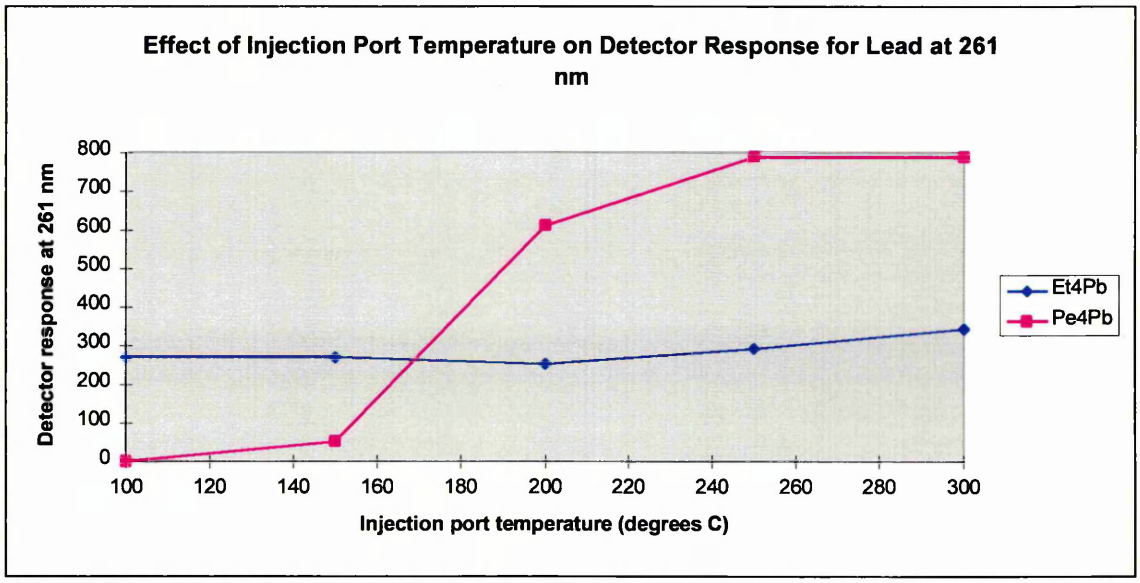
The optimum carrier gas flow rate determined for organomercury compounds at 2.3 ml / min was used and gave a good peak shape.

5.3.2.1 Effect of Injection Port, Transfer Line and Microwave Cavity Temperature Variations.

A 10 pg / µl standard of tetra-ethyl lead was pentylated and analysed under the conditions specified in chapter 2 using column 2 and a cavity make up gas flow of 290 ml / min. The injection port, transfer line and microwave cavity temperature were varied from 100°C to 300°C, as for organomercury analysis.

Figure 27 shows the effect of injection port temperature on the detector signal obtained for tetra-ethyl lead at 261 nm. Tetra-ethyl lead maintained a constant signal over the temperature range 100-300°C. Tetra-pentyl lead however required an injection port temperature of 250-300°C to achieve a constant response. This suggested that under the conditions stated tetra-alkylated lead compounds appeared to be stable up to 300°C and not subject to thermal decomposition or adsorption onto the injection port liner. An injection port temperature of 250°C was adopted in line with requirements identified for the analysis of organomercury compounds.

Figure 27. Effect of Injection Port Temperature on Detector Response for Organolead Species.



Variations in the temperature of the transfer line and microwave cavity temperature again showed no noticeable effect in the short term as for the analysis of organomercury compounds, however due to the risk of condensation a transfer line and microwave cavity temperature of 300°C was used. This was in line with the oven program which also had a top temperature of 300°C.

5.3.2.2 Effect of Variation of Cavity Make Up Gas Flow Rates.

The effect of cavity gas flow rate was studied for tetra-ethyl lead. Figures 28a and b show graphs of cavity gas flow rate against signal response for both lead and carbon, and carbon only. The cavity gas flow rate was measured at the cavity vent.

Figure 28a. The Effect of Cavity Gas Flow Rate on Detector Response for Pb at 261 nm and C at 248 nm

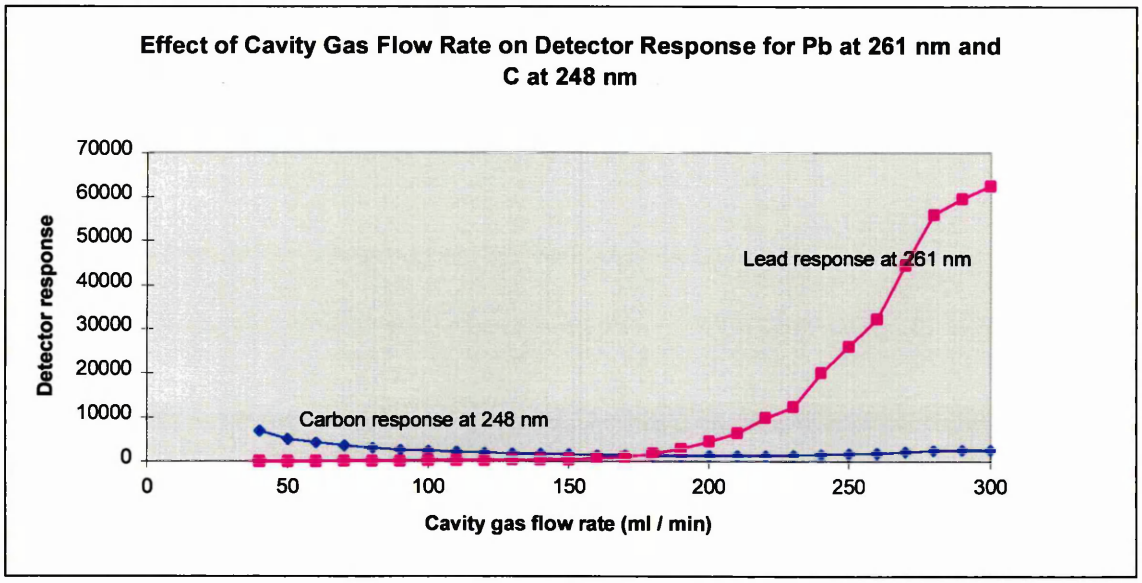
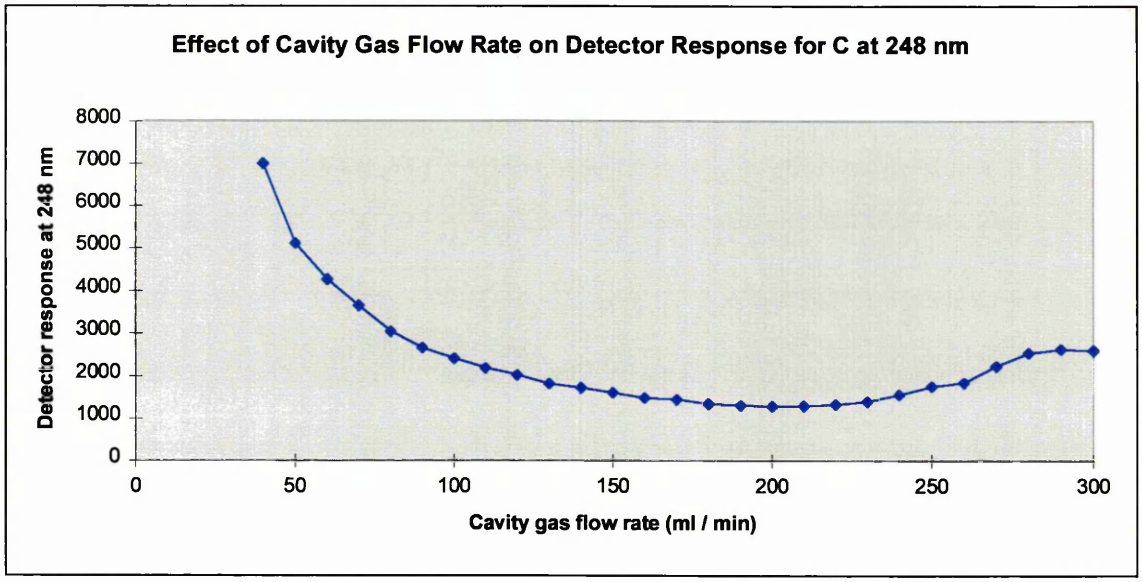


Figure 28b. The Effect of Cavity Gas Flow Rate on Detector Response for C at 248 nm



The software recipe for organolead speciation specifies a cavity gas flow rate of 60 ml / min with fast flow, which when activated provides an additional flow of 140 ml / min. Figure 28a shows that the highest sensitivity for lead is observed at the 290-300 ml / min flow rate, 90-100 ml / min higher than the specified level. The noise level remained constant throughout the study. Operating at the manufacturer's recommended flow rate will decrease the limit of detection by a factor of 10. In practise cavity gas flow rates of 290 ml / min were found to be necessary as lower flow rates also had implications for the linearity of response for lead compounds, which will be discussed later in this chapter.

Hydrogen and oxygen are also required for the detection of organolead compounds as reagent scavenger gases. The flow rates used throughout the study were set at the manufacturers recommended level of 20 ml / min.

The profile for lead atomisation is very different from organomercury compounds, which gave a response similar to carbon. The response is somewhat unusual in that increasing the cavity gas flow rate leads to a decrease of analyte residence time in the plasma. This should cause a dilution effect on the signal response as exhibited by carbon and mercury. For tetra-alkyl lead the opposite was observed, indicating that at lower cavity gas flow rates incomplete atomisation or molecular recombination of the analyte occurs. This effect has implications for compound independent calibration which has been previously discussed in chapter 4.

The response for carbon differs slightly from that of mercury as a minimum is seen in detector response at a cavity gas flow rate of approximately 200 ml / min, followed by a gradual increase in detector response to 280 ml / min where a constant response is observed.

5.3.2.3 Effect of Cavity Make Up Gas Flow Rate on the Linearity of Response for Tetra-Ethyl Lead

The linearity of the detectors response for carbon and lead emission was investigated for tetra-ethyl lead, over a range of concentrations. For this exercise different dilutions of tetra-ethyl lead were prepared and analysed without undergoing the derivatisation procedure. The cavity gas flow rate was varied and the effect of response for both carbon and lead monitored at 248 nm and 261 nm respectively. The results are shown in tables 11 -14, with linearity determined by response factor. The response factor was calculated as mean response divided by concentration and in order to show linearity the response factor should remain constant.

The results obtained using the manufacturers recommended cavity gas flow rate (table 11) shows a high degree of linearity for carbon response for tetra-ethyl lead, however a very different response is observed for lead. At this cavity gas flow rate, although the response is linear from 100-1000 pg / µl a sharp increase in response factor is observed at higher concentrations. This is unusual as at higher concentrations a decrease in response factor is expected,

indicating saturation of the analytical detector for that particular analyte. The limit of detection is also high for environmental applications.

Table 11. Analysis of Tetra-Ethyl Lead at 200 ml / min (Manufacturers Specified Cavity Gas Flow Rate).

Carbon Channel at 248 nm

Concentration (pg / µl)	Area A	Area B	Mean	R.F.
100	28.19	26.67	27.43	0.27430
200	65.36	60.96	63.16	0.31580
400	129.13	117.43	123.28	0.30820
600	190.61	188.85	189.73	0.31622
800	253.33	262.27	257.80	0.32225
1000	338.46	330.17	334.32	0.33432
2000	646.80	657.55	652.18	0.32609
4000	1330.50	1317.40	1323.95	0.33099
6000	2046.90	2066.80	2056.85	0.34281
8000	2733.40	2724.70	2729.05	0.34113
10000	3475.10	3505.30	3490.20	0.34902
100000	39257.00	39195.00	39226.00	0.39226

Lead Channel at 261 nm

Concentration (pg / µl)	Area A	Area B	Mean	R.F.
100	0	14.42	14.42	0.14420
200	23.90	26.19	25.05	0.12523
400	46.73	50.11	48.42	0.12105
600	79.62	71.21	75.42	0.12569
800	112.75	110.28	111.52	0.13939
1000	145.11	143.37	144.24	0.14424
2000	337.21	332.99	335.10	0.16755
4000	857.25	873.14	865.20	0.21630
6000	1740.40	1730.10	1735.25	0.28921
8000	2825.50	2823.60	2824.55	0.35307
10000	4290.90	4331.00	4310.95	0.43110
100000	100394.00	102651.00	101522.50	1.01523

Table 12. Analysis of Tetra-Ethyl Lead at 250 ml / min.

Carbon Channel at 248 nm

Concentration (pg / µl)	Area A	Area B	Mean	R.F.
20	0	0	0	0
40	0	0	0	0
60	22.49	0	22.49	0.37483
80	21.29	19.79	20.54	0.25675
100	45.69	31.93	38.81	0.38810
200	69.88	67.15	68.52	0.34258
400	156.55	166.10	161.33	0.40331
600	199.89	199.79	199.84	0.33307
800	283.13	288.45	285.79	0.35724
1000	380.62	371.32	375.97	0.37597
10000	3866.60	3807.40	3837.00	0.38370
100000	27799.00	27086.00	27442.50	0.27443

Lead Channel at 261 nm

Concentration (pg / µl)	Area A	Area B	Mean	R.F.
20	0	77.52	77.52	3.87600
40	153.25	141.30	147.28	3.68188
60	255.31	224.49	239.90	3.99833
80	297.02	335.14	316.08	3.95100
100	472.47	494.60	483.54	4.83535
200	907.72	989.61	948.67	4.74333
400	2403.60	2391.20	2397.40	5.99350
600	3331.70	3360.50	3346.10	5.57683
800	4781.80	4849.90	4815.85	6.01981
1000	6414.70	6173.50	6294.10	6.29410
10000	55395.00	56573.00	55984.00	5.59840
100000	189485.00	191890.00	190687.50	1.90688

Table 13. Analysis of Tetra-Ethyl Lead at 270 ml / min.

Carbon Channel at 248 nm

Concentration (pg / µl)	Area A	Area B	Mean	R.F.
20	0	0	0	0
40	27.90	17.00	22.45	0.56125
60	43.06	34.08	38.57	0.64283
80	46.25	50.01	48.13	0.60163
100	58.68	60.01	59.35	0.59345
200	103.91	110.64	107.28	0.53638
400	240.36	250.51	245.44	0.61359
600	340.73	361.17	328.45	0.54742
800	446.84	438.98	442.91	0.55364
1000	568.23	571.71	569.97	0.56997
10000	4234.00	4242.10	4238.05	0.42381
100000	26272.00	25665.00	25968.50	0.25969

Lead Channel at 261 nm

Concentration (pg / µl)	Area A	Area B	Mean	R.F.
20	0	135.65	135.65	6.78250
40	317.94	326.74	322.34	8.05850
60	626.67	630.19	628.43	10.47383
80	875.31	921.46	898.39	11.22981
100	1209.20	1264.10	1236.65	12.36650
200	2501.70	2573.40	2537.55	12.68775
400	5651.80	5668.50	5660.15	14.15038
600	7879.60	7683.30	7781.45	12.96908
800	10549.00	10568.00	10558.50	13.19813
1000	13205.00	13356.00	13280.50	13.28050
10000	72009.00	73160.00	72584.50	7.25845
100000	199501.00	198111.00	198806.00	1.98806

Table 14. Analysis of Tetra-Ethyl Lead at 290 ml / min.

Carbon Channel at 248 nm

Concentration (pg / µl)	Area A	Area B	Mean	R.F.
20	30.79	0	30.79	1.53950
40	39.70	42.16	40.93	1.02325
60	71.58	64.67	68.13	1.13542
80	71.62	80.77	76.20	0.95244
100	101.16	96.16	98.66	0.98660
200	188.76	190.30	189.53	0.94765
400	356.71	360.53	358.62	0.89655
600	495.69	481.33	488.51	0.81418
800	656.43	671.99	664.21	0.83026
1000	829.87	780.97	805.42	0.80542
10000	4422.90	4405.20	4414.05	0.44141
100000	26749.00	25267.00	26008.00	0.26008

Lead Channel at 261 nm

Concentration (pg / µl)	Area A	Area B	Mean	R.F.
20	444.74	424.53	434.64	21.73175
40	910.28	903.20	906.74	22.66850
60	1537.10	165.10	1551.10	25.85167
80	2056.40	2091.20	2073.80	25.92250
100	2675.80	2652.40	2664.10	26.64100
200	5015.80	5093.40	5054.60	25.27300
400	9691.00	9769.10	9730.05	24.32513
600	13082.00	13049.00	13065.50	21.77583
800	16760.00	16789.00	16774.50	20.96813
1000	19833.00	19864.00	19848.50	19.84850
10000	73866.00	73446.00	73656.00	7.36560
100000	198703.00	208430.00	203566.50	2.03567

The effect of cavity gas flow rate on detector response for lead indicated that a lower limit of detection could be achieved at higher cavity gas flow rates. The effect of increasing the cavity gas flow rate on linearity was further investigated at 250, 270 and 290 ml / min. Flow rates exceeding 290 ml / min could not be achieved without increasing the pressure within the cavity.

As previously stated, an increase in the cavity gas flow rate should lead to a dilution effect of that element due to a decreased resonance time for that element in the plasma giving rise to a decreased emission signal. This effect should lead to a shift in the linear dynamic range and a decrease in the response factor.

The response observed for carbon at the different flow rates shows a general increase in response factor as the flow rate increases from 200 - 290 ml / min. This effect corroborates the profile observed for the response of carbon at increasing cavity gas flow rates in figure 28b. However the increase in cavity gas flow rate does not show a shift in linear dynamic range as hypothesised. An apparent effect of detector saturation is observed at high concentrations when subjected to an increase in cavity gas flow rate, reducing the linear dynamic range. This may be attributed to reduced elemental residence time in the plasma or decreased plasma temperatures from the increased gas flow, leading to incomplete atomisation rather than saturation. Alternatively it may be due to the recombination of carbon to form simple molecular compounds,

hence shifting the emission signal. This effect has been discussed in relation to compound independent calibration in chapter 4.

The effect on the linearity of lead is more dramatic. An increase in the detection limit for lead is shown by the increase in response factor from 0.14 at 200 ml / min to 24.0 at 290 ml / min. The degree of linearity is also increased at the lower concentrations of lead, which is shown by the stability of the response factor at 290 ml / min cavity gas flow rate. This study for the linearity of lead also corroborates the lead emission profile shown in figure 28a.

The actual limit of detection for the analysis of organolead compounds will be calculated later in this chapter, allowing for the dilution effects of the derivatisation procedure.

On completion of this particular study a white deposit was observed on the surface of the discharge tube in the area that contained the plasma. It was suspected that this solid was lead oxide. It was hoped to obtain a scanning electron micrograph of both the clean and contaminated surface. Unfortunately the quartz discharge tube was extremely weak after use and attempts made to obtain a cross section of the surface area failed.

In order to confirm the linearity of lead emission and to assertion the effect of old / new discharge tubes, the linearity study was repeated with a new discharge tube. Standards of tetra-ethyl lead were prepared at 20, 40, 60, 80

and 100 pg / µl and analysed as described in chapter 2 for analytical column 2. The cavity gas flow rate was set to 290 ml / min. The results are shown in table 15.

The results show a similar degree of linearity, however an increase in the response factor is observed from 24 to 33. This increase in response when a new discharge tube is used has been reported by Webster [19] for heteroatoms.

Again on completion of the study a white solid deposit was noted on the inside surface of the discharge tube in approximately the same position as the plasma. The formation of lead oxide has been previously reported for the quantitation of organolead compounds by GC-FID [185], GC-SF-AAS [188] and GC-MIP-AED [172]. Low levels of oxygen and hydrogen gas were added in line with the manufacturers recommendations. It may be possible to prevent the formation of lead oxide by optimising the ratios of these gases within the cavity make up gas, however automatic gas flow control to enable this study to be performed was not available on the commercial instrument. The scavenger gas flows for use with the Hewlett Packard GC-MIP-AED are set by cylinder head pressure. This study may be considered for future work but was not progressed further within this research project.

Table 15. Analysis of Tetra-Ethyl Lead at 290 ml / min using a New Discharge Tube.

Carbon Channel at 248 nm

Conc (pg / µl)	Area A	Area B	Area C	Area D	Area E	Mean	%RSD	R.F.
20	31.35	27.60	30.94	30.46	21.27	28.32	14.86	1.41620
40	49.68	55.37	48.54	47.20	39.92	48.14	11.53	1.20355
60	76.85	64.89	75.07	75.26	68.70	72.15	7.10	1.20257
80	101.09	101.26	100.06	97.82	101.03	100.25	1.43	1.25315
100	121.22	117.78	138.94	129.13	124.45	126.30	6.50	1.26304
20	27.82	23.18	28.29	31.43	28.79	27.90	10.71	1.39510
100	123.25	125.36	118.48	123.37	137.01	125.49	5.51	1.25494
40	59.14	58.24	54.35	57.73	54.68	56.83	3.83	1.42070
80	101.32	96.00	97.04	103.18	100.78	99.66	3.04	1.24580
60	71.25	72.38	78.79	76.22	85.13	76.75	7.25	1.27923

Lead Channel at 261 nm

Conc (pg / µl)	Area A	Area B	Area C	Area D	Area E	Mean	%RSD	R.F.
20	1005.00	838.05	803.13	732.27	701.79	816.05	14.55	40.80240
40	1286.50	1259.10	1230.90	1232.20	1243.90	1250.52	1.85	31.26300
60	1823.00	1793.20	1818.30	1840.10	1840.10	1822.94	1.06	30.38233
80	2472.00	2496.10	2543.70	2535.80	2571.30	2523.78	1.57	31.54725
100	3119.80	3162.00	3183.80	3169.60	3235.60	3174.16	1.32	31.74160
20	791.76	785.69	795.20	784.21	776.15	786.60	0.93	39.33010
100	3231.60	3240.30	3281.00	3276.80	3297.40	3265.42	0.86	32.65420
40	1491.70	1482.50	1487.40	1436.80	1423.50	1464.38	2.17	36.60950
80	2536.60	2513.80	2448.70	2463.30	2400.70	2472.62	2.18	30.90775
60	1792.80	1817.80	1921.00	1957.80	2049.20	1907.72	5.50	31.79533

5.3.3 Evaluation of the Derivatisation Technique.

The derivatisation technique as described in chapter 2 was evaluated with respect to linearity, limit of detection and precision using laboratory prepared standards for tetra-ethyl lead. Although derivatisation is not required for this particular analyte the study was performed to ensure the procedure did not cause molecular degradation.

The linearity of the analytical technique was determined for derivatised standards ranging from 1 - 1000 pg / µl. A linear response was observed between 1 - 100 pg / µl. Typical data for tetra-ethyl lead is given in table 16 and a typical calibration graph is shown in figure 29. The correlation coefficient was calculated to be 1.0000 over 2 orders of magnitude. The limit of detection was demonstrated to be 1 pg / µl as Pb with a cavity gas flow rate of 290 ml / min. The derivatisation procedure was shown to have no detrimental effect on the quantitation of tetra-ethyl lead when using silanised glassware and no additional organolead species were confirmed by snapshot data. The equation for the trend line for tetra-ethyl lead was determined as $y=27.312x+15.611$.

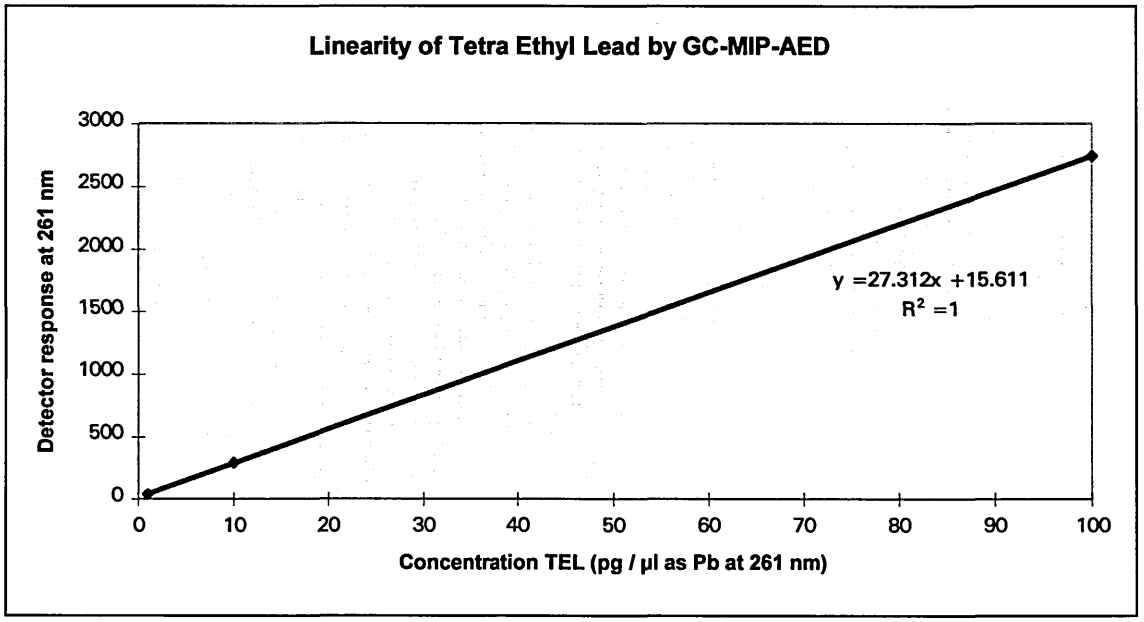
Linearity, linear dynamic range and limit of detection were also determined for GC-MS selected ion monitoring detection over the range 1-1000 pg / µl as Pb for tetra-ethyl lead. The ions monitored are listed in chapter 2. This technique gave a limit of detection of 10 pg / µl as Pb. A linear response was seen for mass 208 with a linear dynamic range of 10-1000 pg / µl as Pb and a correlation coefficient of 0.9999, however the response seen at mass 207, 236 and 237 was non-linear. The difference between the two analytical detectors has been

discussed for a similar context in chapter 4 for the analysis of methyl mercury species.

Table 16. Linearity of Tetra-Ethyl Lead by GC-MIP-AED after Derivatisation.

Concentration (pg / µl)	Area 1	Area 2	Mean	Standard deviation	% RSD
1 (1st prep)	48.22	39.68	43.95	6.04	13.7
1 (2nd prep)	35.18	37.97	36.58	1.97	5.4
1 (3rd prep)	33.64	37.60	35.62	2.80	7.9
1 (mean)	***39.01***	***38.42***	***38.72***	***3.60***	***9.0***
10 (1st prep)	299.91	281.80	290.86	12.81	4.4
10 (2nd prep)	269.71	284.28	277.00	10.30	3.7
10 (3rd prep)	315.35	309.12	312.24	4.41	1.4
10 (mean)	***294.99***	***291.73***	***293.36***	***9.17***	***3.18***
100 (1st prep)	3173.4	2678.0	2925.7	350.30	12.0
100 (2nd prep)	2773.6	2770.0	2771.8	2.55	0.09
100 (3rd prep)	2600.6	2482.8	2541.7	83.30	3.3
100 (mean)	***2849.2***	***2643.6***	***2746.4***	***145.38***	***5.1***
1000 (1st prep)	16187.00	15746.00	15966.50	311.83	2.0
1000 (2nd prep)	16327.00	16205.00	16266.00	86.27	0.5
1000 (3rd prep)	15411.00	16550.00	15980.50	805.39	5.0
1000 (mean)	***15975***	***16167***	***16071***	***401.17***	***2.5***

Figure 29. Linearity of Tetra-Ethyl Lead after Derivatisation by GC-MIP-AED



In summary the derivatisation procedure does not appear to cause degradation of tetra-ethyl lead and detector response for lead emission by GC-MIP-AED appears to be robust, linear and precise. The limit of detection and linear dynamic range were found to be adequate for application to environmental samples. The technique also showed a high degree of precision for multiple injections of the same solution and also multiple solution preparations indicated by the % RSD's exhibited in tables 15 and 16. The results obtained for GC-MS SIM showed that the technique lacked the sensitivity of the GC-MIP-AED by and order of magnitude and did not appear to show consistent linearity for all mass ions monitored.

With hindsight the length of time tetra-ethyl lead was exposed to the derivatisation reagent would have been of interest to show no degradation of this species over time. However all derivatisation reactions were quenched after 30 mins of exposure to the derivatisation reagent throughout the study.

5.3.4 Evaluation of the Extraction Procedure

The extraction technique as described in chapter 2 was evaluated using laboratory prepared aqueous samples. The background to the technique is described in detail in chapter 4 for the extraction of methyl mercury species, and is similar to the technique reported by Lobinski *et al* [153] for the extraction of organolead compounds.

As tetra-ethyl lead is a neutral compound the presence of sodium diethyldithiocarbamate was not necessary, however the extraction was

performed to show that the procedure did not cause degradation of the organolead species.

Each extraction incorporated a ten fold preconcentration and was subjected to the pentylation procedure together with a standard prepared in toluene of the expected final concentration. % Recoveries were calculated for the aqueous extracts against standards and are shown in table 17.

Table 17. Recoveries for the Aqueous Extraction Technique

Concentration (pg / μl)	Calculated concentration (pg / μl)	% Recovery	%RSD n=2
20	20.55	102.7	0.11
40	34.60	86.5	21.65
60	62.50	104.2	0.94
80	81.37	101.71	1.87
100	96.33	96.33	0.57

The correlation coefficient for the aqueous extraction followed by pentylation was r^2=0.9883 and the equation for the trend line was y=0.9917x-0.429. The recoveries of the extraction procedure with respect to standards were high for the laboratory prepared aqueous samples. The limit of detection for the extraction procedure followed by derivatisation as described was observed to be 0.1 pg / μl for an aqueous solution. As for organomercury compounds the detection limit may be further reduced by increasing the preconcentration factor during extraction.

In summary the extraction procedure gives high recoveries for low concentrations of tetra-ethyl lead such as found in environmental samples. A linear response was also observed indicating that the presence of the chelate

reagent, and the alkylation procedure does not appear to cause transalkylation or degradation of the tetra-alkylated organolead species.

5.4 Summary

The technique used in chapter 4 for the extraction and pentylation of methyl mercury was successfully applied to tetra-ethyl lead, without causing degradation or transalkylation based on laboratory prepared standards. Acceptable levels of linearity were achieved only by deviating from the manufacturers recommended flow rates. It would not be possible to revert back to a flow of 60 ml / min without operator intervention, as the cavity gas flow rate must be adjusted manually. The implications of this drawback are discussed in chapter 3.

With this minor modification the method has been shown to be linear, precise and repeatable, with a high degree of accuracy and a limit of detection of 1 pg / µl prior to preconcentration. The absolute response for a particular concentration was shown to be dependant upon the discharge tube, with a higher response being observed for a newly installed tube, hence day to day reproducibility must be monitored with care. The method unfortunately was not studied for ionic alkyl lead salts in a quantitative capacity, due to none availability of suitable standards during the study period.

During the application of the technique to organolead analysis a white solid was found to form on the surface of the discharge tube. This may be linked to the variation of the breakdown pathway of the molecule in the plasma and the formation of lead oxide. The use of tetra-alkyl lead compounds in petrol relies

on the formation of lead oxide to prevent detonation of the petrol - air mix caused by peroxy radicals and to facilitate smooth combustion [110b]. A similar pathway may be followed for the atomisation of tetra-ethyl lead at slow cavity gas flow rates, causing the formation of lead oxide prior to atomisation, hence reducing the intensity of the lead emission signal. Forsyth *et al* [188] also observed the formation of white deposits on the surface of the silica tube used for the atomisation of organolead compounds by GC-SF-AAS. These deposits were quickly removed by the addition of hydrogen gas to the furnace. Hydrogen and oxygen scavenger gases were used in this application, however optimisation of the ratios of these gases within the cavity make up gas may prove to be an area for further study, when automated flow control can be achieved on the commercial GC-MIP-AED system.

The effects of possible matrix interferences from real samples will be assessed in chapter 7 where the technique of multielemental, simultaneous, organometallic analysis will be applied to a number of real samples.

Chapter 6

Organotin Quantitation

6.0 Organotin Quantitation

6.1 Introduction

Tin exists in various forms within our environment today. The industrial manufacture of organotin compounds has increased rapidly since the 1950's. The major uses of organotin compounds are as biocides, fungicides and PVC stabilisers. These applications have led to various forms of organotin compounds entering the environment. Methylation of tin compounds under environmental conditions has also been reported and reviewed [1].

Organotin compounds have been used in:

1) Agriculture as fungicides and biocides
2) Antifouling paints as biocides for marine applications
3) Wood preservatives as fungicides
4) PVC as heat and light stabilisers
5) Scratch resistant glass by coating the surface with SnO_2 (formed by the decomposition of organotin compounds on a hot glass surface)
6) Catalysts for the production of polymeric material

Their specific uses for these applications have been reviewed by Blunden and Chapman [110c].

Organotin compounds enter the environment either by direct application, leaching or weathering and land fill disposal, where degradation of the compounds occur primarily by UV light. There are many different organotin

compounds each exhibiting varying degrees of toxicity to specific inhabitants within an ecosystem.

The toxicology of organotin compounds has been review by Blunden and Chapman [110c] and shows a general trend. Maximum toxicity has been reported for tetra and tri-alkylated tin compounds with reduced toxicity for di and mono-alkyl tins. The chain length of the alkylated group also has a toxicological significance, with methyl to butyl alkyl chains exhibiting higher degrees of toxicity than longer side chains.

The main causes of acute organotin toxicity occur from the disruption of ion transport systems within an organism, interference in mitochondrial action (as for organolead toxicity) and in the inhibition of ATP synthesis [1]. Tri-methyl tin compounds have also been found to cause selective and irreversible neurological damage in the brain [110c]. Di-organotin compounds also interfere with mitochondrial processes, however the mode of action differs from that of tri-organotin compounds as they appear to combine with enzymes or coenzymes. Mono-organotin, inorganic tin salts and elemental tin are not reported to have any serious toxicological effects.

The use of tri-butyl tin compounds in marine antifouling paints is now controlled and has been banned in a number of countries, due to the toxic effects of this particular compound on aquatic organisms [189].

The highly toxic nature of organotin compounds and their wide spread use within our environment today, highlight the need for rapid, highly sensitive, accurate and precise, analytical speciation techniques for quantification.

6.2 Review of Analytical Techniques for Organotin Speciation

6.2.1 Sample Preparation - Extraction and Derivatisation Techniques

Prior to quantitation, a suitable extraction procedure must be performed in order to extract the species of interest from animal / plant tissue, sediments, water or the atmosphere [10, 110c], as for organomercury and organolead compounds. A pre-concentration step may also be incorporated due to the low concentrations of the pollutants. A number of reviews on the analysis of organotin compounds have been published since the completion of the experimental stage of this research programme [190-192].

The speciation of organotin compounds in general follows a similar concept to that described for organomercury and organolead compounds in chapters 4 and 5. First a digestion procedure is employed in order to release any bound compounds, when necessary, depending upon the sample matrix. The organotin compounds are then separated, and in some cases preconcentrated in an extraction procedure, followed by a derivatisation procedure to render any ionic alkyl tin species to their volatile tetra-alkylated form.

Each of these analytical stages will be considered individually, as any digestion procedure could in theory be coupled with any extraction procedure, which in turn may also be coupled to an appropriate derivatisation procedure.

For sample matrices, such as fish tissue and sediment, a number of pre-extraction and / or digestion procedures have been employed in a similar manner to organomercury and organolead compounds. These include, acid digestion [189, 193-207], alkaline digestion [189, 199, 207-210], enzymatic digestion [207, 211], soxhlet extraction [206] and supercritical fluid extraction [35, 73-74, 212]. A number of different acid combinations have been used to release organotin compounds from sediments and biological samples. These include sulphuric acid [193], acetic acid [68, 194-196], a methanolic solution of hydrochloric acid [189, 197-198], hydrochloric acid [199-203], hydrochloric acid and ascorbic acid [66, 204], hydrobromic acid [205] and nitric acid [206]. Alkaline digestion reagents have included methanolic sodium hydroxide solution [189, 199, 209], tetramethylammonium hydroxide [207-208] and ethanolic potassium hydroxide solution [210]. Enzymatic digestion procedures [207, 211] are typically applied to organic tissue samples, such as, fish tissue where the sample matrix is broken down to enable the organotin compounds to be extracted.

Supercritical fluid extraction (SFE) is a technique which has also been applied to extract organotin compounds from sediments and the principles have been discussed in chapter 5 for organolead compounds. Lui *et al* [35, 73-74] used

carbon dioxide containing methanol as a modifier, under appropriate temperature and pressure conditions as a supercritical fluid to extract organotin compounds from sediments which had been pre-treated with either solid sodium diethyldithiocarbamate [73], sodium chloride [73] or diethylammonium diethyldithiocarbamate [35, 74, 212] as a chelation reagent to form a neutral complex. The organotin species were collected in methylene chloride solvent or on C_{18} bonded silica. For this application SFE is acting as a sample pre-treatment and extraction technique to remove any matrix effects prior to solvent exchange to a non-polar solvent, followed by derivatisation. An interlaboratory study using this procedure for the speciation of organotin compounds has been reported by Lui *et al* [212].

More recently a microwave assisted digestion procedure has been reported [213-215], in conjunction with either acetic acid [214-215] or TMAH [213, 215].

The extraction techniques for the analysis of organotin compounds are generally based on liquid liquid extraction, following digestion, either directly into solvent [197, 203, 210] or with the aid of a chelation reagent to extract ionic compounds by the formation of a neutral complex. A variety of solvents have been used for this application including hexane, benzene, pentane, dichloromethane and diethylether, in conjunction with either sodium diethyldithiocarbamate [68, 206, 216-217], tropolone [66, 199, 201-202, 204-205, 207-208] or dithizone [211], buffered to a specific pH, as the metal chelates.

Solid phase extraction has also been reported for the extraction of ionic alkyl tin compounds using a range of cartridge stationary phases, such as, C_{18}, C_8, C_2 and phenyl [193, 218].

The derivatisation procedure following extraction was in general alkylation by either Grignard reaction, hydride generation [194-196, 198, 200-201, 209], or aqueous phase ethylation using sodium tetraethylborate reagent [67, 189, 207].

A variety of alkylation reagents have been used for Grignard derivatisation resulting in the formation of the methyl [199, 202, 204, 206, 211], ethyl [66, 208], propyl [210], butyl [219] or pentyl [35, 68, 73-74, 193, 197, 205, 216-218, 220] derivatives. The derivatisation procedure was as previously described for organomercury Grignard alkylation in chapter 4.

These general procedures have been reported in a number of publications, with a number of variations, including preconcentration factor, a reduction of the final sample extract before or after derivatisation by evaporation [199, 204, 211, 220] and solvent exchange prior to derivatisation [68, 193, 197, 216].

Astruc *et al* [194], reported a procedure for the determination of tri-butyl tin species in marine sediments. The procedure involved acetic acid leaching, followed by aqueous phase hydride generation using sodium borohydride, to form the volatile tri-butyl tin hydride. The derivatised species was collected in a

cold trap prior to analysis by GC-QF-AAS. In this example only a digestion and derivatisation procedure are required. This technique is simple with only a few sample pretreatment steps, however the use of hydride generation has been reported to give poor recoveries for mono-butyl tin and both mono and di-phenyl tin species [193].

Cai *et al* [189] compared two digestion procedures for the analysis of butyl tin compounds in river sediment. Methanolic hydrochloric acid and methanolic sodium hydroxide solutions were used to leach the butyl tin compounds in separate procedures. The pH was adjusted to 4.1 and the organotin species were then derivatised in the aqueous phase using sodium tetraethylborate. The volatile derivatised compounds were again collected in a cold trap prior to analysis by GC-QF-AAS.

Ceulemans *et al* [207] also used aqueous phase ethylation for the analysis of organotin compounds in marine biomaterials. Three digestion procedures were compared. Two simple procedures were performed using tetramethylammonium hydroxide digestion and enzymatic digestion, followed by direct aqueous phase ethylation. The derivatised species were then collected by extraction into hexane and passed through an alumina column to clean up the extract prior to analysis by GC-MIP-AED. The third procedure involved more handling steps. The organotin compounds were leached from the tissue with hydrochloric acid solution, followed by chelation extraction with tropolone in hexane using ultrasound. The extract was evaporated to dryness

and the residue re-dissolved in hexane. This was then subjected to aqueous phase ethylation by the addition of sodium tetraethylborate in a buffered solution at pH 5. The hexane layer was subjected to a clean up procedure using an alumina column before analysis by GC-MIP-AED.

Forsyth *et al* [211] applied a number of extraction and derivatisation procedures to the determination of organotin compounds in marine food products. The procedures employed involved enzymatic hydrolysis followed by liquid liquid extraction using either dithizone or tropolone as the chelation reagent. The organotins were butylated or methylated using Grignards reagents prior to analysis by GC-AAS and species confirmation by GC-MS.

Lobinski *et al* [68] reported the analysis of organotin compounds in water and sediments. Acetic acid was used to leach the bound organotin compounds from the sediment. The pH was adjusted to 5 and the organotin compounds extracted into hexane or pentane using sodium diethyldithiocarbamate as a chelation reagent. The solvent was evaporated and exchanged for octane prior to pentylation with Grignards reagent. Quantification was achieved by GC-MIP-AED.

The method employed by Lobinski *et al* is similar to the extraction technique employed in chapters 4 and 5 for the analysis of organomercury and organolead compounds, however the pH of extraction is lower at a pH of only 5 in comparison to 8.5, as used in this programme of study.

6.2.2 Gas Chromatographic Detection of Organotin Species

A number of detectors have been used in gas chromatography, the most commonly reported techniques include:

1) FPD (Flame Photometric Detector)
2) MS (Mass Spectrometry)
3) AAS (Flame / Electrothermal decomposition Atomic Absorption Spectrometry)
4) MIP-AED (Microwave Induced Plasma - Atomic Emission Detector)

GC coupled with ECD [200] has also been reported however this application is limited due to the non-specific nature and lack of sensitivity of this detector.

GC-MS is also limited as complete separation of all analytes, which leads to extended chromatographic run times, is required. Adequate sensitivity can be achieved in selected ion monitoring mode, however as discussed for organomercury if complete separation of all analytes is not achieved then some of the observed signal may be due to interferences which fragment to give the same mass ion.

As for organomercury and organolead, atomic spectroscopy is the most widely used detection technique for organotin compounds and has been reviewed by Donard and Pinel (Harrison and Rapsomanikis) [10]. Flame photometric detection has been widely reported for the quantitation of organotin compounds

[197, 205, 209-210, 218, 220], however the technique lacks the specificity of atomic absorption and emission spectroscopy. It is also reported to suffer from possible interferences from carbon dioxide, hydrocarbons and sulphur containing species.

Graphite and quartz furnace atomic absorption spectroscopy have also been widely used [189, 194-196, 198, 204, 208, 211, 216, 219]. As for the analysis of organolead compounds, hydrogen reagent gas is required to help the atomisation process. GC-AAS is limited, however, in that simultaneous multielemental analysis is not possible due to the different atomisation requirements and elemental lamps needed for detection. Here atomic emission spectroscopy holds the advantage. GC-MIP-AED has more recently been used for the analysis of organotin compounds due to the development of commercially available instrumentation and has detection limits suitable for environmental applications [35, 66-68, 73-74, 203, 207].

6.2.3 Summary

To summarise the techniques employed to extract, derivatise and quantitate organotin compounds are extensive and diverse. Again during the initiation of this study the analysis of organotin compounds was performed mainly as an isolated technique. The opportunity existed to develop an analytical procedure for the multielemental analysis of organometallic compounds by adapting the principles of previously reported analytical techniques for application to the Hewlett Packard GC-MIP-AED.

6.3 Application of the GC-MIP-AED for the Determination of Organotin Species.

The objectives of the programme of research was to utilise the GC-MIP-AED initially concentrating on organomercury speciation, while maintaining the possibility to widen the scope of the technique to incorporate the simultaneous analysis of additional organometallic species. Specifically, the multielemental analysis of organomercury, organotin and organolead species within a single chromatographic injection for environmental samples.

The extraction and derivatisation procedure described in chapter 4 for the analysis of organomercury species was extended to the analysis of organotin compounds. Pentylation was used as the derivatisation procedure and is the most applicable alkylation technique from a review of the literature. Pentylation was also deemed to be the most appropriate alkylation procedure for simultaneous multielemental analysis.

GC-MS was used to aid identification of the organometallic derivatives and also as a comparative quantitative technique.

The Hewlett Packard GC-MIP-AED allows for organotin detection at 271 nm and also at 303 nm within the standard recipe software package. The analysis was performed at 271 nm to allow for the simultaneous detection of mercury,

lead and tin organometallic compounds on the PDA detector in order to meet the objectives of this programme of study.

6.3.1 Identification and Confirmation of Organotin Species

In order to assess the GC-MIP-AED's applicability for organometallic speciation, a preliminary investigation was performed under the conditions specified in chapter 2. Commercially available butyl tin species were subjected to the pentylation procedure described in chapter 2 and analysed qualitatively by capillary GC-MIP-AED (column 2) and GC-MS (column 1 and 2).

Sample introduction was performed as previously described in chapter 4 for organomercury analysis.

Figure 30 shows a high degree of selectivity and specificity for the pentylated butyl tin species and inorganic tin (present as an impurity). No interference was observed due to carbon breakthrough. The tin emission was confirmed from spectral data. A typical snapshot for tin emission at 271 nm is shown in appendix 2.

The identification of each pentylated butyl tin species was confirmed by GC-MS analysis. The different retention times between the GC-MIP-AED and GC-MS were accounted for by retention index - see table 18. A 1000 ng / µl solution was prepared and analysed by GC-MS by electron impact ionisation (EI) and

also by negative ion chemical ionisation (NCI) using isobutane gas since no ionised molecular species was observed in EI.

Figure 30. GC-MIP-AED Chromatogram showing Pentylated Organotin Species.

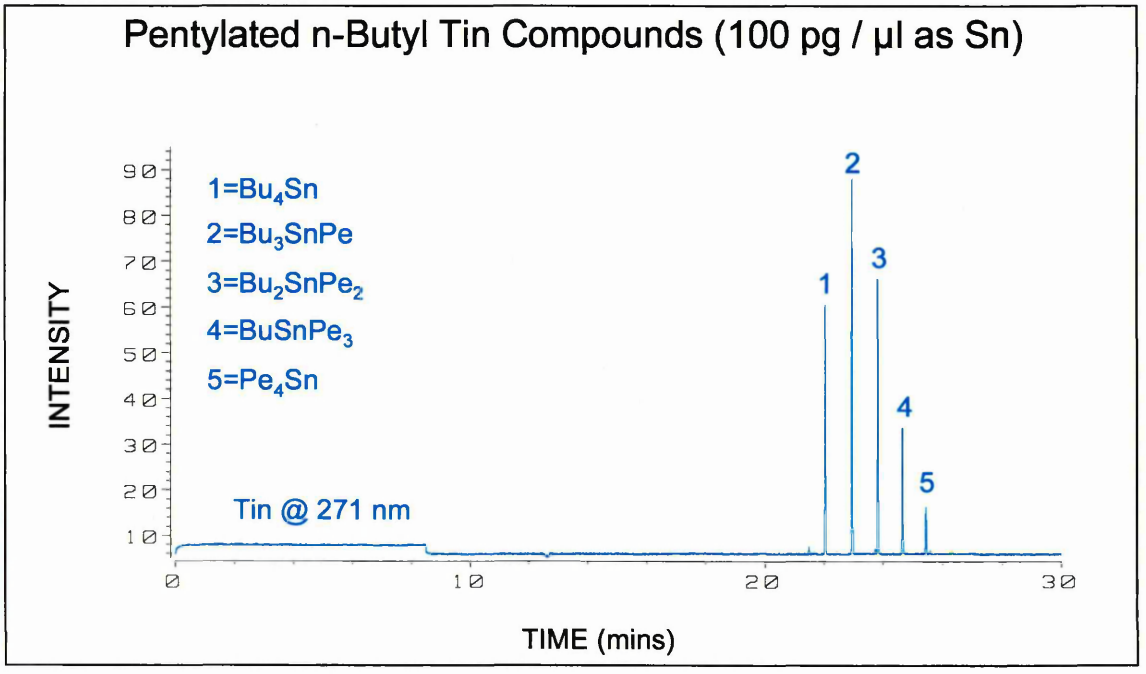


Table 18. Retention Index Comparison between GC-MIP-AED and GC-MS for Organotin Species

Organotin Species	Retention Index		
	GC-MIP-AED (column 2)	GC-MS (EI) (column 1)	GC-MS (NCI) (column 2)
Bu_4Sn	1679	1685	1651
Bu_3SnPe	1764	1766	1746
Bu_2SnPe_2	1850	1856	1829
$BuSnPe_3$	1934	1941	1908

The EI and NCI mass spectra obtained are shown in figures 31-34. There are ten naturally occurring isotopes of tin ranging from mass 112 to 124 as shown in table 19 with tin^{120} being the most abundant. Hence the mass spectral data of

tin compounds have a distinctive isotope pattern as seen for organomercury and organolead compounds.

Table 19. The Abundance and Mass of Naturally Occurring Isotopes of Tin

Isotope	Accurate mass	Abundance
Sn^{112}	111.9051	0.95
Sn^{114}	113.9039	0.65
Sn^{115}	114.90335	0.34
Sn^{116}	115.90219	14.24
Sn^{117}	116.90310	7.57
Sn^{118}	117.90205	24.01
Sn^{119}	118.90315	8.58
Sn^{120}	119.90220	32.97
Sn^{122}	121.90346	4.71
Sn^{124}	123.90524	5.98

Electron ionisation caused excessive fragmentation for the analysis of all butyl tin species resulting in the loss of the ionised molecular species in all cases. However the fragmentation pattern observed was consistent with the appropriate species. In order to confirm the identification, negative chemical ionisation using isobutane gas was used to obtain the ionised molecular species. The two ionisation techniques are detailed elsewhere and will not be discussed in this thesis [8].

Figure 31a shows the EI mass spectrum for tetra-butyl tin. At 121 mass units a range of isotopes are seen indicating the presence of tin. Butyl chain fragments are lost from the ionised molecular species, shown by molecular fragments at 291, 235 and 177 corresponding to Bu_3Sn^+, Bu_2Sn^{2+} and $BuSn^{3+}$ respectively. Fragments are also observed at 57 mass units corresponding to a butyl side

chain fragment. Figure 31b shows the NCI mass spectrum for tetra-butyl tin. In this ionisation mode the ionised molecular species is present at 349 mass units.

Figure 31a. Electron Ionisation Mass Spectrum of Peak Eluting at t_R=21.8 min in the GC-MS Analysis of a Solution containing Pentylated n-Butyl Tin Species. This Peak was Identified as Tetra-Butyl Tin and these Data used to Confirm GC-MIP-AED Results.

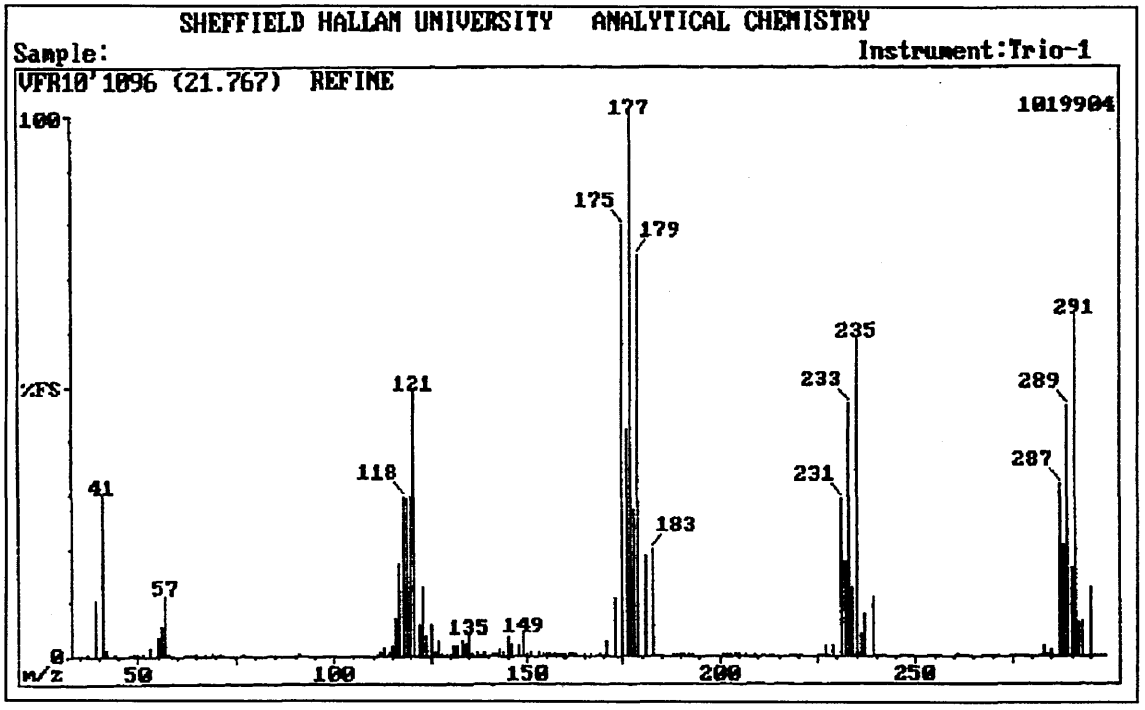


Figure 31b. Negative Chemical Ionisation Mass Spectrum of Peak Eluting at t_R=21.9 min in the GC-MS Analysis of a Solution containing Pentylated n-Butyl Tin Species. This Peak was Identified as Tetra-Butyl Tin and these Data used to Confirm GC-MIP-AED Results.

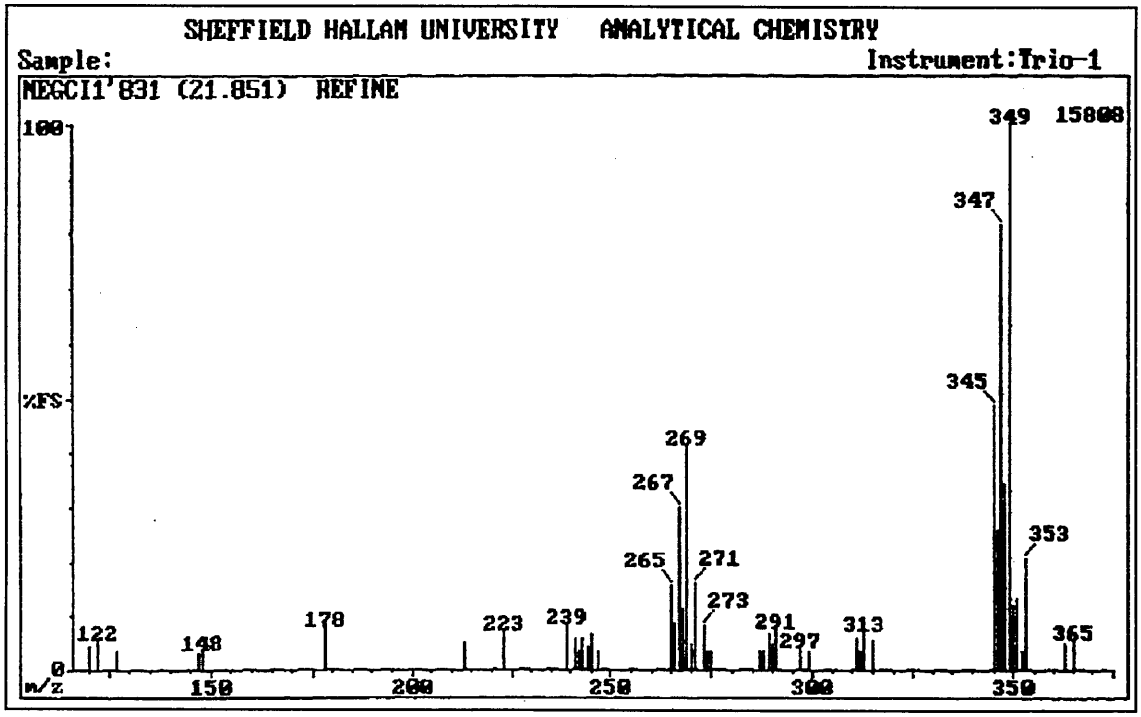


Figure 32a. Electron Ionisation Mass Spectrum of Peak Eluting at t_R=23.5 min in the GC-MS Analysis of a Solution containing Pentylated n-Butyl Tin Species. This Peak was Identified as Pentylated Tri-Butyl Tin and these Data used to Confirm GC-MIP-AED Results.

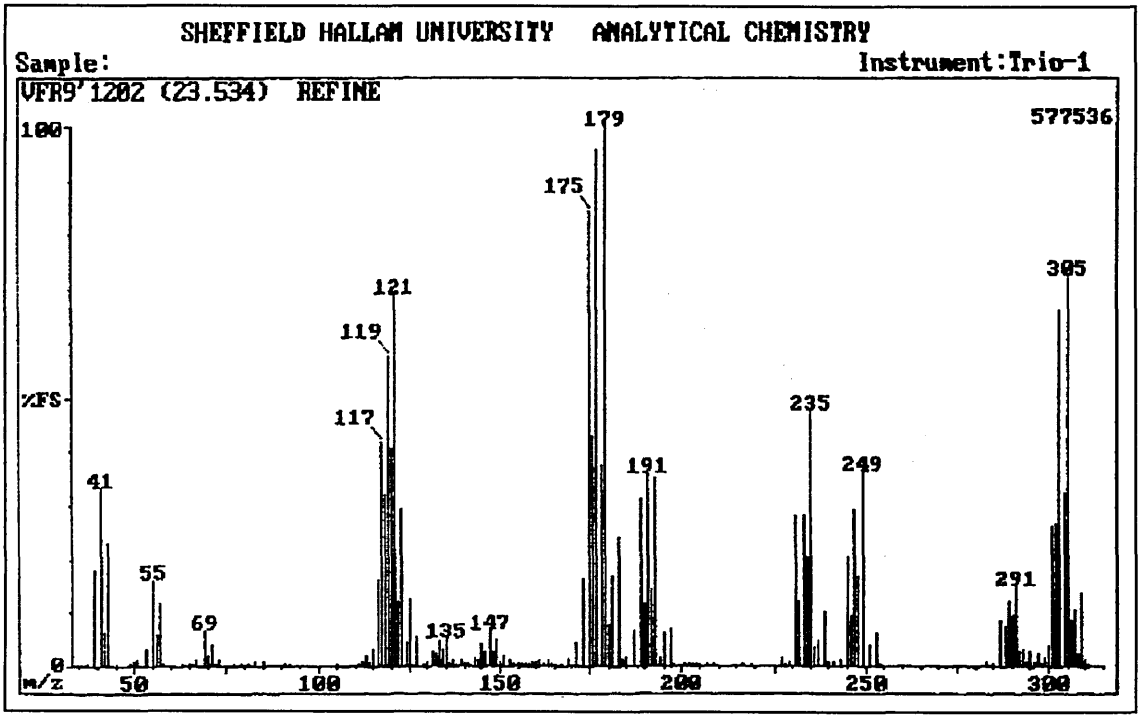


Figure 32b. Negative Chemical Ionisation Mass Spectrum of Peak Eluting at t_R=22.9 min in the GC-MS Analysis of a Solution containing Pentylated n-Butyl Tin Species. This Peak was Identified as Pentylated Tri-Butyl Tin and these Data used to Confirm GC-MIP-AED Results.

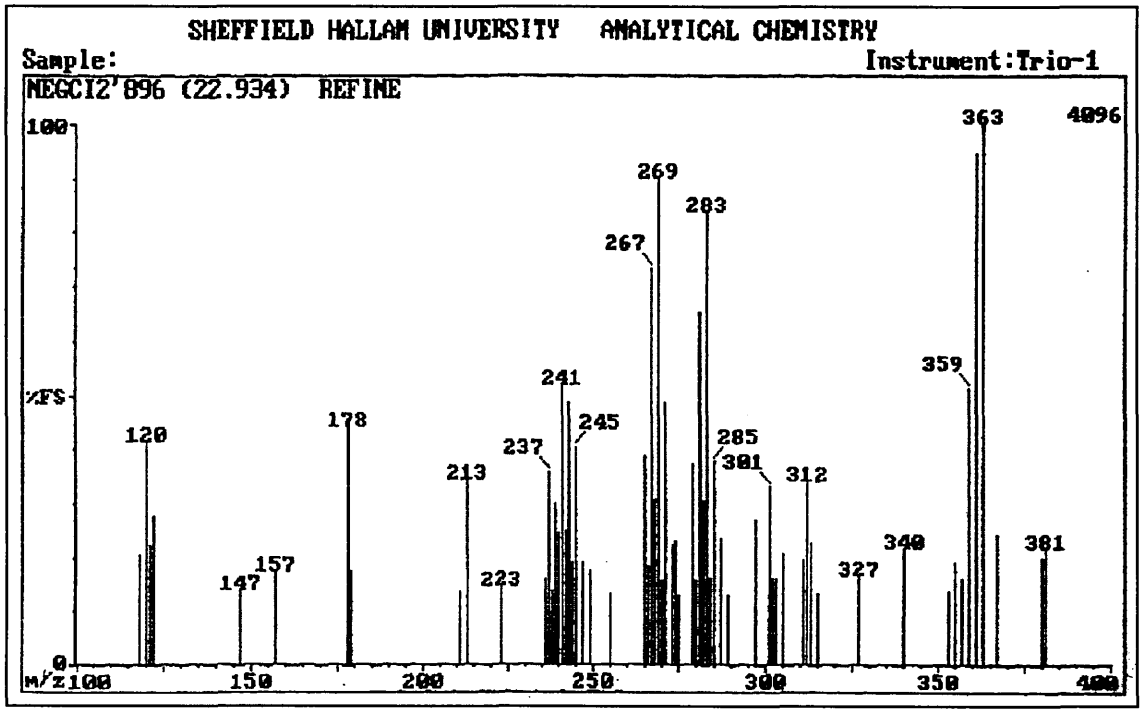


Figure 33a. Electron Ionisation Mass Spectrum of Peak Eluting at t_R=25.3 min in the GC-MS Analysis of a Solution containing Pentylated n-Butyl Tin Species. This Peak was Identified as Pentylated Di-Butyl Tin and these Data used to Confirm GC-MIP-AED Results.

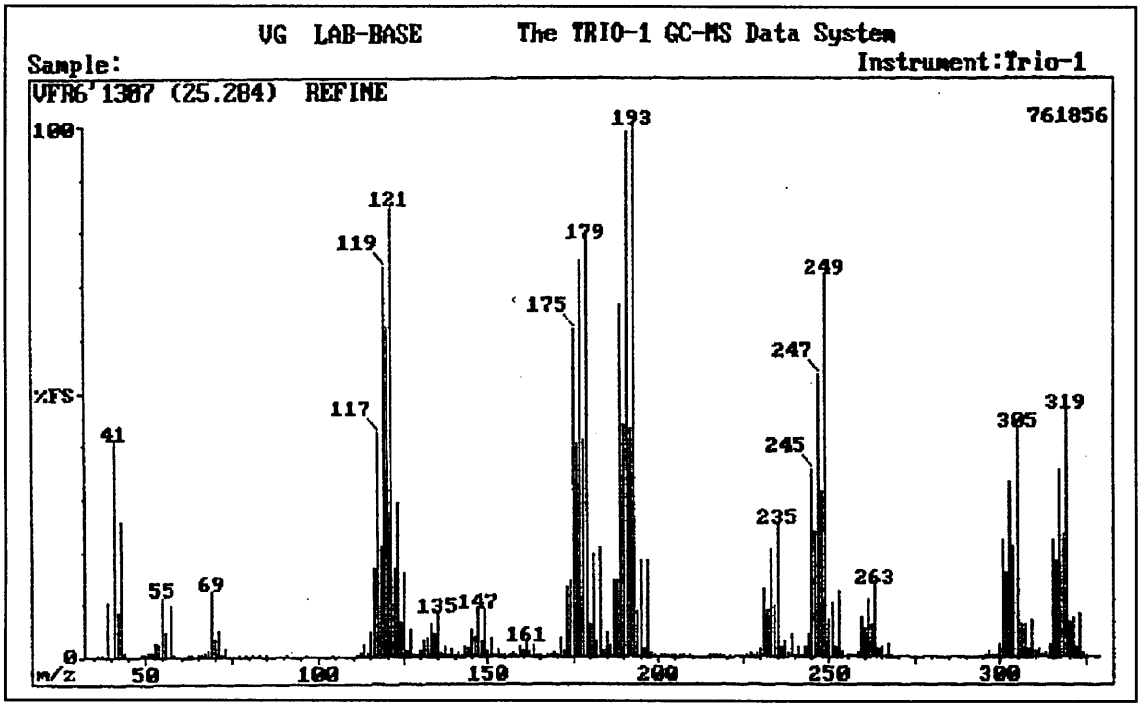


Figure 33b. Negative Chemical Ionisation Mass Spectrum of Peak Eluting at t_R=23.9 min in the GC-MS Analysis of a Solution containing Pentylated n-Butyl Tin Species. This Peak was Identified as Pentylated Di-Butyl Tin and these Data used to Confirm GC-MIP-AED Results.

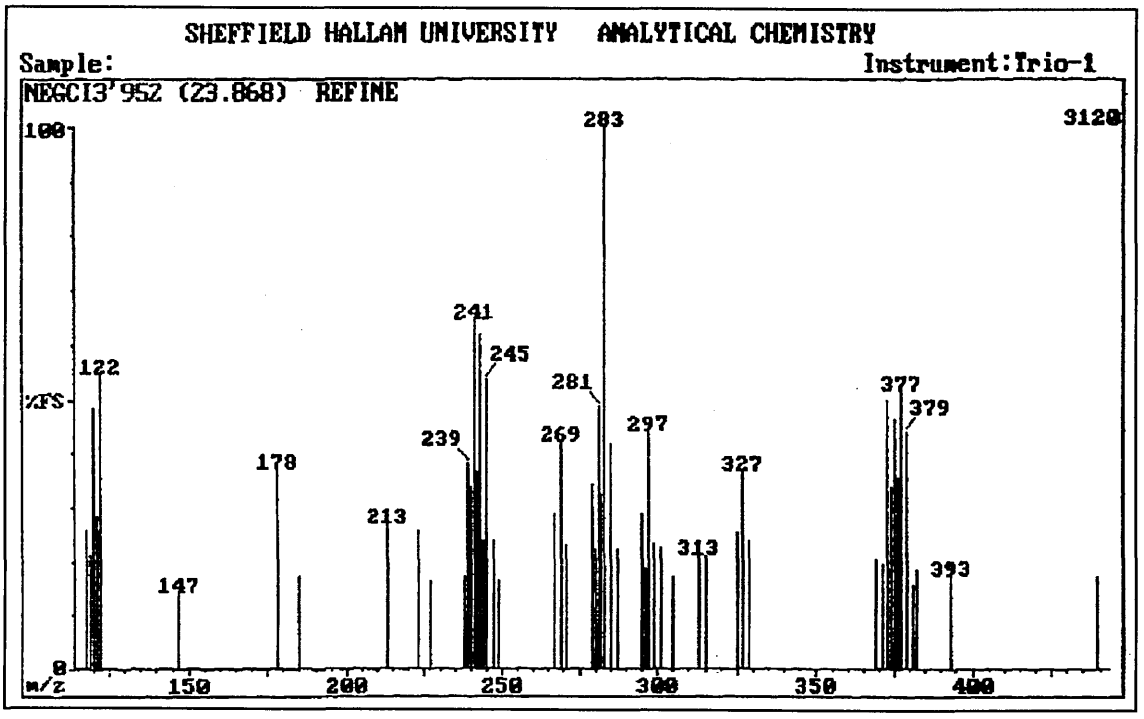


Figure 34a. Electron Ionisation Mass Spectrum of Peak Eluting at t_R=27.0 min in the GC-MS Analysis of a Solution containing Pentylated n-Butyl Tin Species. This Peak was Identified as Pentylated Mono-Butyl Tin and these Data used to Confirm GC-MIP-AED Results.

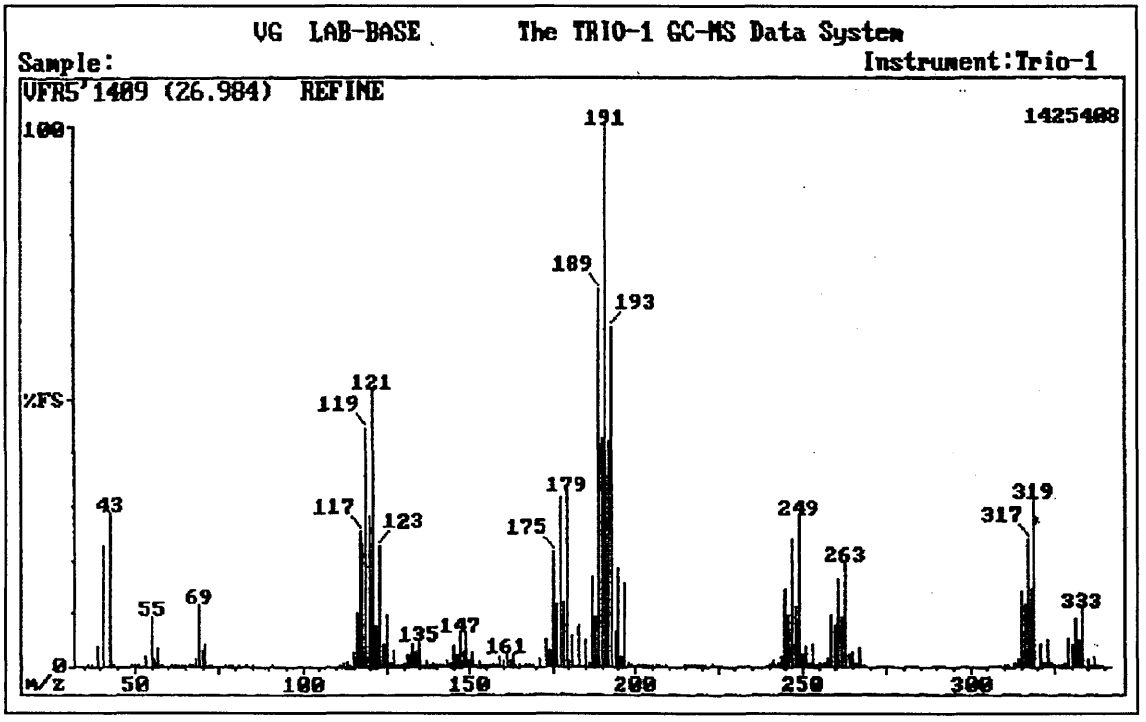


Figure 34b. Negative Chemical Ionisation Mass Spectrum of Peak Eluting at t_R=24.7 min in the GC-MS Analysis of a Solution containing Pentylated n-Butyl Tin Species. This Peak was Identified as Pentylated Mono-Butyl Tin and these Data used to Confirm GC-MIP-AED Results.

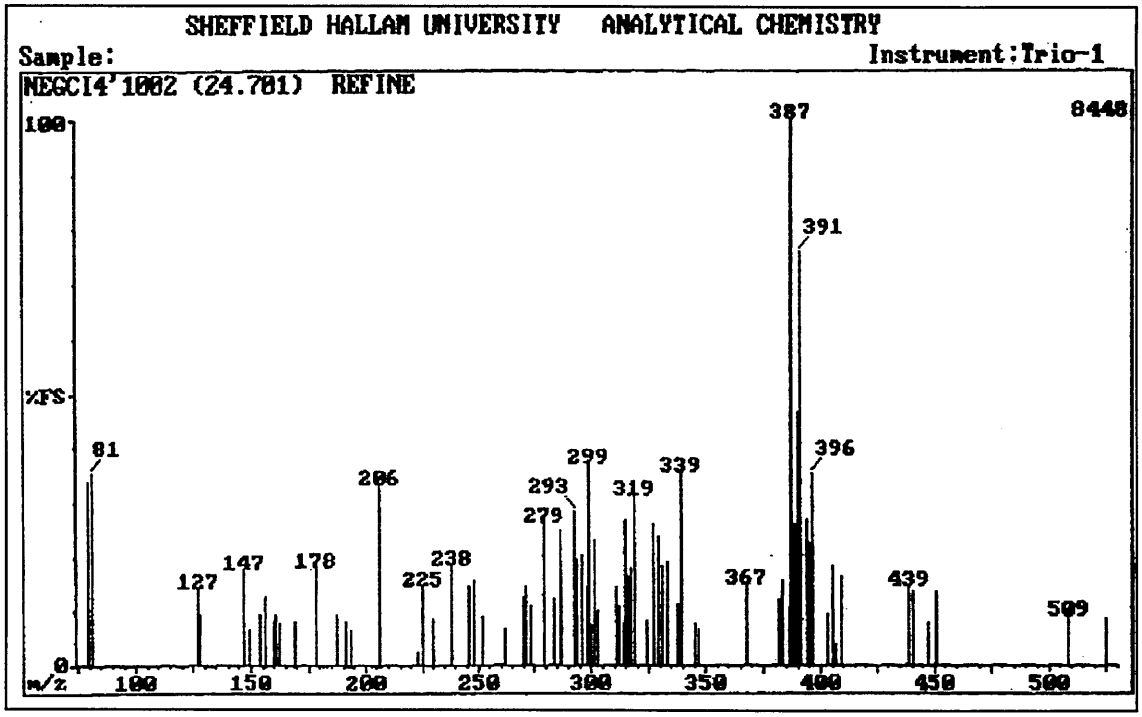


Figure 32a shows the EI mass spectrum for pentylated tri-butyl tin. Again at 121 mass units the typical isotope pattern is observed for tin. The fragmentation pattern is more complex than that for tetra-butyl tin. Six major fragments are observed at 179, 191, 235, 249, 291 and 305 mass units. The breakdown pathway observed for tetra-butyl tin indicated the loss of whole side chains as opposed to fragmentation of the side chains. If the same breakdown pathway is assumed for pentylated tri-butyl tin the fragments are most likely to

be $BuSn^{3+}$, $PeSn^{3+}$, Bu_2Sn^{2+}, $BuPeSn^{2+}$, Bu_3Sn^{+} and Bu_2PeSn^{+} respectively. Fragments are also observed at 55 and 69 mass units corresponding to butyl and pentyl chain fragments. At 135 and 147 minor fragments are observed showing the same isotope pattern as tin and may be due to the addition of a methyl or ethyl group formed from the fragmentation of either a butyl or pentyl side chain.

Figure 32b shows the NCI mass spectrum of pentylated tri-butyl tin. The ionised molecular species is observed at 363 mass units.

Figure 33a shows the EI mass spectrum for pentylated di-butyl tin. Again at 121 mass units the typical isotope pattern is observed for tin. The fragmentation pattern shows 7 major fragments at 179, 193, 235, 249, 263, 305 and 319 mass units. Assuming the major fragmentation pathway is due to the loss of whole side chains as opposed to fragmentation of the side chains, the fragments are most likely to be $BuSn^{3+}$, $PeSn^{3+}$, Bu_2Sn^{2+}, $BuPeSn^{2+}$, Pe_2Sn^{2+}, Bu_2PeSn^{+} and $BuPe_2Sn^{+}$ respectively. Fragments are also observed at 55 and 69 mass units corresponding to butyl and pentyl chain fragments. At 135, 147 and 161 minor fragments are observed showing the same isotope pattern as tin and may be due to the addition of methyl, ethyl or propyl groups formed from the fragmentation of either a butyl or pentyl side chain.

Figure 33b shows the NCI mass spectrum of pentylated di-butyl tin. The ionised molecular species is observed at 377 mass units.

Figure 34a shows the EI mass spectrum for pentylated mono-butyl tin. Again at 121 mass units the typical isotope pattern is observed for tin. The fragmentation pattern shows 6 major fragments at 179, 191, 249, 263, 319 and 333 mass units. Again assuming the major fragmentation pathway is due to the loss of whole side chains as opposed to fragmentation of the side chains, the fragments are most likely to be $BuSn^{3+}$, $PeSn^{3+}$, $BuPeSn^{2+}$, Pe_2Sn^{2+}, $BuPe_2Sn^{+}$ and Pe_3Sn^{+} respectively. Fragments are also observed at 55 and 69 mass units corresponding to butyl and pentyl chain fragments. At 135, 147 and 161 minor fragments are observed showing the same isotope pattern as tin and may be due to the addition of methyl, ethyl or propyl groups formed from the fragmentation of either a butyl or pentyl side chain.

Figure 34b shows the NCI mass spectrum of pentylated mono-butyl tin. The ionised molecular species is observed at 387 mass units (M-1).

The mass spectrum was not obtained for tetra-pentyl tin as only a very weak response was observed on the total ion chromatogram.

6.3.2 Optimisation of GC-MIP-AED Control Parameters

A number of parameters were varied in order to optimise the GC-MIP-AED for organotin analysis. These included:

1 Heated zones including injection port temperature, transfer line temperature and microwave cavity temperature.

2 Cavity make up gas flow rate.

3 Linearity of response

A single restriction liner with high speed automatic injection was used for the analysis of pentylated butyl tin species by GC-MIP-AED using splitless injection based on the results previously observed in chapters 3 and 4 for the analysis of nitrobenzene, tert-butyl disulphide and alkylated organomercury compounds.

The optimum carrier gas flow rate determined for organomercury compounds at 2.3 ml / min was used and gave a good peak shape for the organotin species.

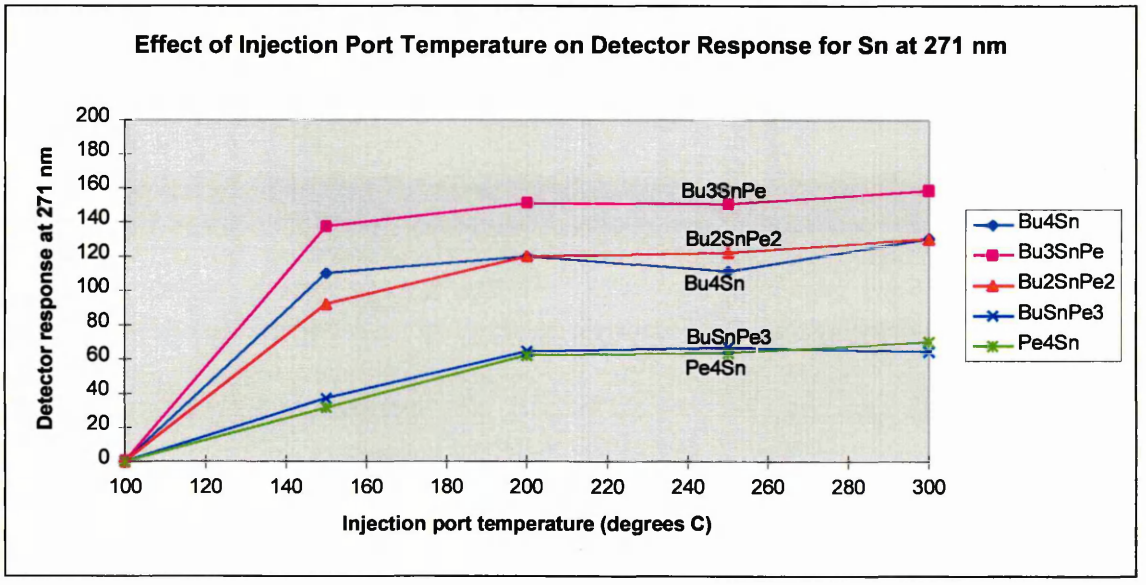
6.3.2.1 Effect of Injection Port, Transfer Line and Microwave Cavity Temperature Variations.

A 10 pg / µl mixed standard of pentylated butyl tin compounds where analysed under the conditions specified in chapter 2 using column 2 and a cavity make up gas flow rate of 290 ml / min. The injection port, transfer line and microwave cavity temperature were varied from 100°C to 300°C, as for organomercury and organolead analysis.

Figure 35 shows the effect of injection port temperature on the detector signal obtained for pentylated butyl tin species. All species maintained a constant signal over the temperature range 200-300°C. This suggests that under the conditions stated pentylated butyl tin species appear to be stable up to 300°C and are not subject to thermal decomposition or adsorption on to the injection port liner. An injection port temperature of 250°C was adopted in line with

requirements identified for the analysis of organomercury and organolead compounds.

Figure 35. Effect of Injection Port Temperature on Detector Response for Organotin Species.



Variations in the temperature of the transfer line and microwave cavity temperature again showed no noticeable effect in the short term as for the analysis of organomercury and organolead compounds, however due to the risk of condensation a transfer line and microwave cavity temperature of 300°C was used. This was in line with the oven program which also had a top temperature of 300°C.

6.3.2.2 Effect of Variation of Cavity Make Up Gas Flow Rates.

The effect of cavity gas flow rate was studied for tetra-butyl tin. Figures 36a and b show graphs of cavity gas flow rate against signal response for both tin and

carbon, and carbon only. The cavity gas flow rate was measured at the cavity vent.

Figure 36a. The Effect of Cavity Gas Flow Rate on Detector Response for Sn at 271 nm and C at 248 nm

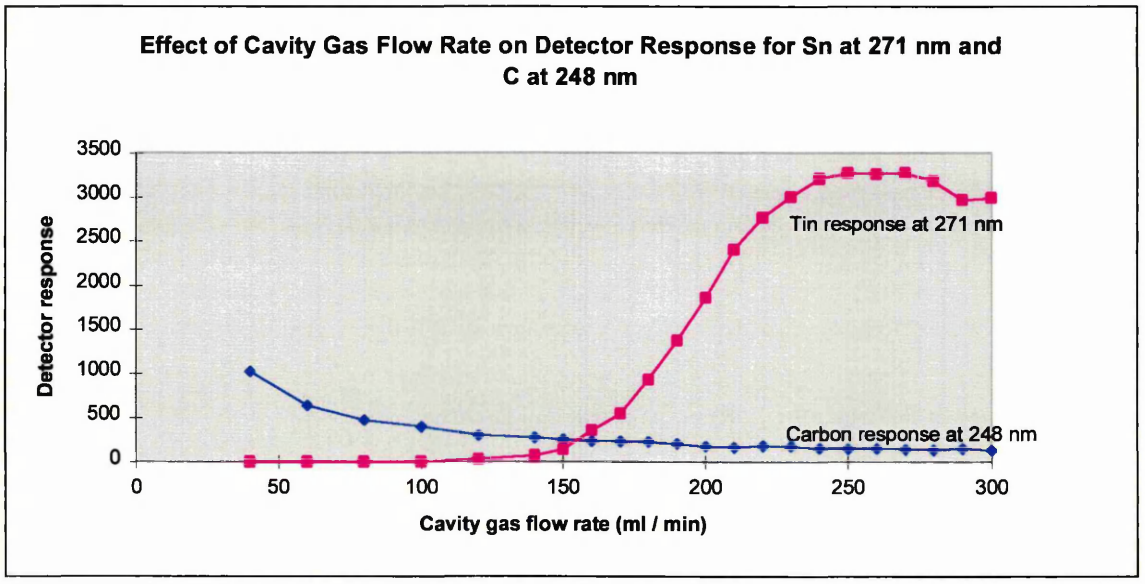
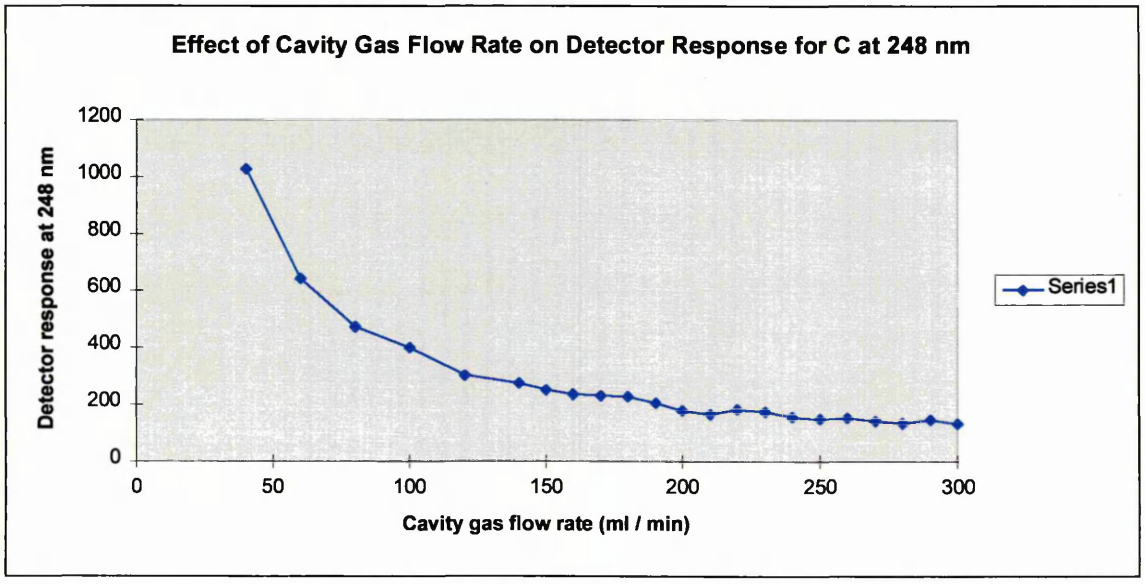


Figure 36b. The Effect of Cavity Gas Flow Rate on Detector Response for C at 248 nm



The software recipe for organotin speciation specifies a cavity gas flow rate of 60 ml / min with fast flow, which when activated provides an additional flow of 140 ml / min. Figure 36a shows that the highest sensitivity for tin is observed at 240-280 ml / min flow rate, 40-80 ml / min higher than the specified level. The noise level remained constant throughout the study. Operating at the manufacturer's recommended flow rate will decrease the limit of detection by approximately 40 %. In practise cavity gas flow rates of 290 ml / min were found to be necessary as lower flow rates also had implications for the linearity of response for lead compounds, which were discussed in chapter 5.

Hydrogen and oxygen are also required for the detection of organotin compounds as reagent scavenger gases. The flow rates used throughout the study were set at the manufacturers recommended level of 20 ml / min.

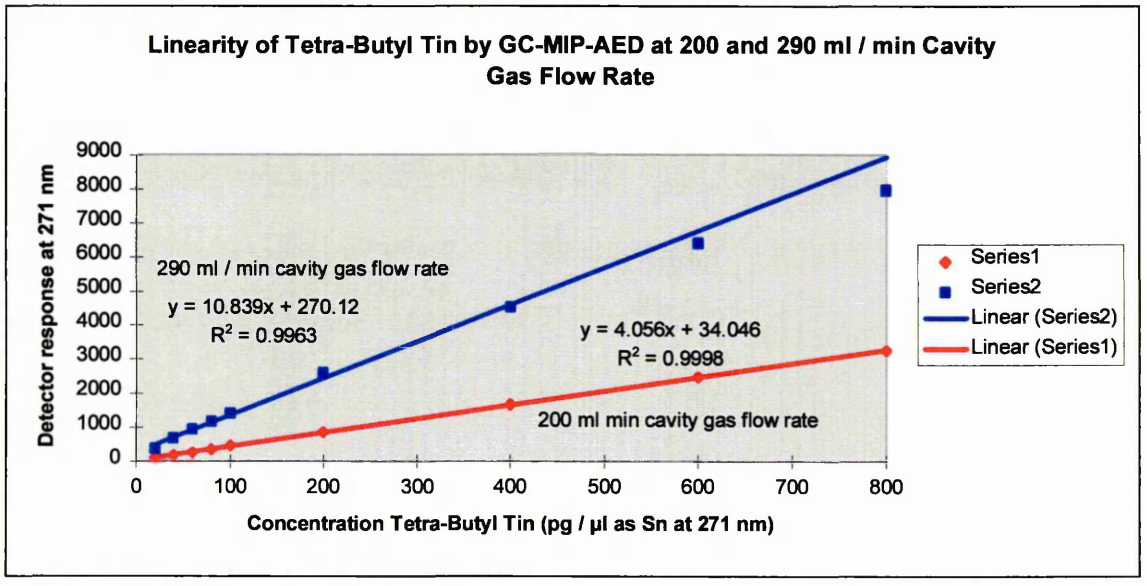
The profile for tin atomisation is similar to the profile obtained for lead atomisation, both of which were very different from organomercury compounds, which gave a response similar to carbon. The response is somewhat unusual in that increasing the cavity gas flow rate leads to a decrease of analyte residence time in the plasma. This should cause a dilution effect on the signal response as exhibited by carbon and mercury. For tetra-butyl tin the opposite was observed in common with tetra-ethyl lead, indicating that at lower cavity gas flow rates incomplete atomisation or molecular recombination of the analyte occurs. This effect has implications for compound independent calibration which has been previously discussed in chapter 4.

The response for carbon in figure 36 b is similar to that of mercury unlike the carbon response for tetra-ethyl lead where a minimum is seen in detector response.

6.3.2.3 Effect of Cavity Make Up Gas Flow Rate on the Linearity of Response for Tetra-Butyl Tin

The linearity of response was investigated for tin using a range of dilutions of tetra-butyl tin without undergoing the derivatisation procedure, as for tetra-ethyl lead. The solutions were analysed at the manufacturers recommended cavity gas flow rate of 200 ml / min and also at 290 ml / min which had been found to be necessary for tetra-ethyl lead analysis. The results are shown in figure 37.

Figure 37. Linearity of Tetra-Butyl Tin by GC-MIP-AED at 200 ml / min and 290 ml / min Cavity Gas Flow Rate



At the manufacturers recommended cavity gas flow rate of 200 ml / min a high degree of linearity is observed with a correlation coefficient of 0.9998 from 20-800 pg / µl. At 290 ml / min flow rate although a linear response is observed the linear range is much smaller, from 20-400 pg / µl, with a correlation coefficient of 0.9963. This may be attributed to saturation of the detector at a response of approximately 4500 area units, as the response seen at 400 pg / µl at 290 ml / min gas flow and the response at 800 pg / µl at 200 ml / min are both within this range.

The linearity of response for tin differs from that of lead although the general profile of response against cavity gas flow rate was similar. Tin is slightly less likely to form refractory oxides than lead due to its higher position in the group 4 metals in the periodic table, hence tin is more susceptible to the atomisation process. This trend for the thermodynamics of oxide formation was reported by Craig and Brinckman [110d].

However, on completion of this study a faint white deposit was observed on the surface of the discharge tube in the area that contained the plasma, as seen for tetra-ethyl lead. It was suspected that this solid was tin oxide, as tin has also been reported as being prone to form refractory oxides [101]. Again it was hoped to obtain a scanning electron micrograph of both the clean and contaminated surface. Unfortunately the quartz discharge tube was extremely weak after use and attempts made to obtain a cross section of the surface area failed, as reported in chapter 5 for tetra-ethyl lead.

Low levels of oxygen and hydrogen gas were added in line with the manufacturers recommendations. The effects of these scavenger gases has been studied by Besner *et al* [101] for use with microwave induced plasma emission. It may be possible to prevent the formation of both tin and lead oxides by optimising the ratios of these gases within the cavity make up gas, however automatic gas flow control to enable this study to be performed was not available on the commercial instrument. As for the determination of tetra-ethyl lead, this study may be considered for future work but was not progressed further within this research project.

6.3.3 Evaluation of the Derivatisation Technique.

The derivatisation technique as described in chapter 2 was evaluated with respect to the following parameters using laboratory prepared standards for pentylated butyl tin species:

1 Reaction length

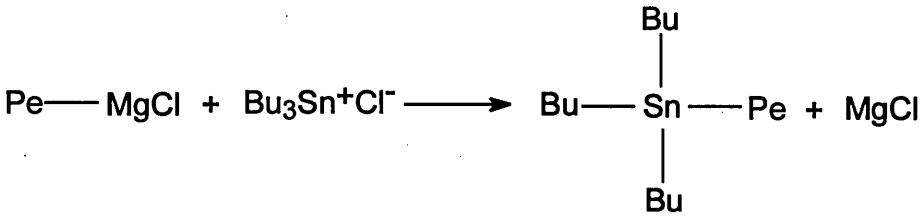
2 Linearity

3 Limit of detection

4 Precision

Although derivatisation is not required for tetra-butyl tin the species was included in the study to ensure the molecule was unaffected by the procedure.

Reaction mechanism for tri-butyl tin chloride derivatisation



A similar reaction mechanism is followed for di and mono-butyl tin species to form a tetra-alkylated neutral complex. The effect of different reaction times in the presence of the Grignard reagent was studied and the optimum reaction time determined for organotin pentylation. The effect of reaction time is shown in figure 38.

Figure 38 shows that for the alkylation of butyl tin species the Grignard reaction has gone to completion after 30 min and no appreciable change in response was observed for extended contact with excess Grignard reagent. Figure 38 also suggests that transalkylation reactions are not taking place during the derivatisation period. Each butyl tin species was subjected to pentylation individually and again no significant evidence was observed for transalklylation processes or degradation for any species.

The linearity of the pentylation procedure was determined by derivatising mixed butyl tin standards ranging from 20 - 100 pg / µl. Linearity was determined at a cavity gas flow rate of 290 ml / min and the correlation coefficient, trend line equation and limit of detection for each butyl tin species was calculated. The results are shown in figure 39 and table 20.

Figure 38. Optimisation of Grignard Reaction Time for the Derivatisation of Butyl Tin Species

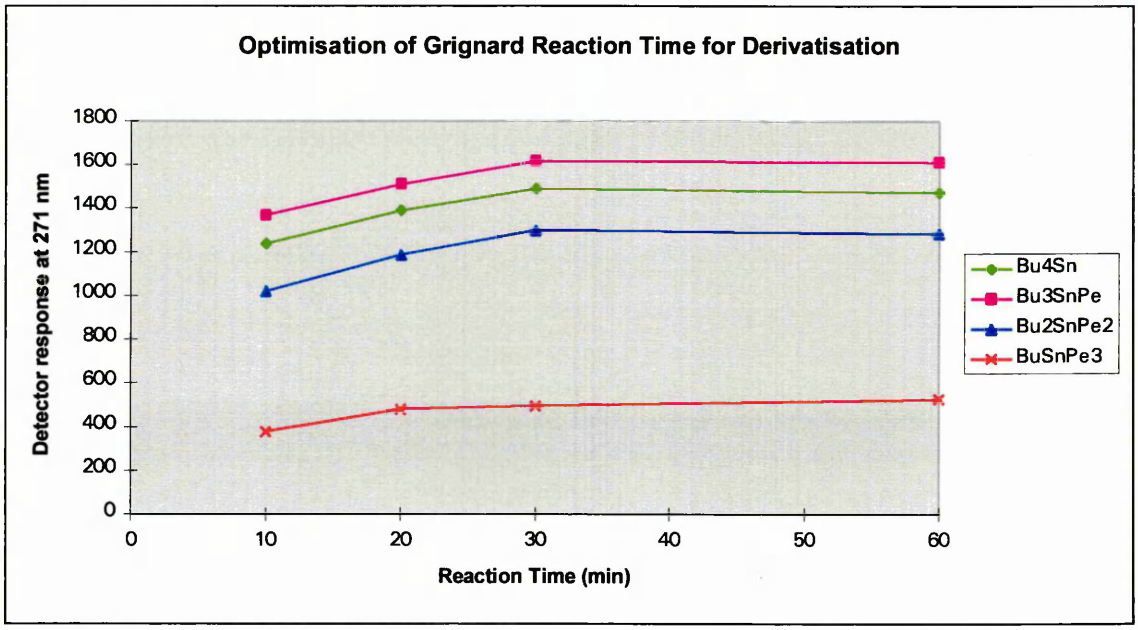
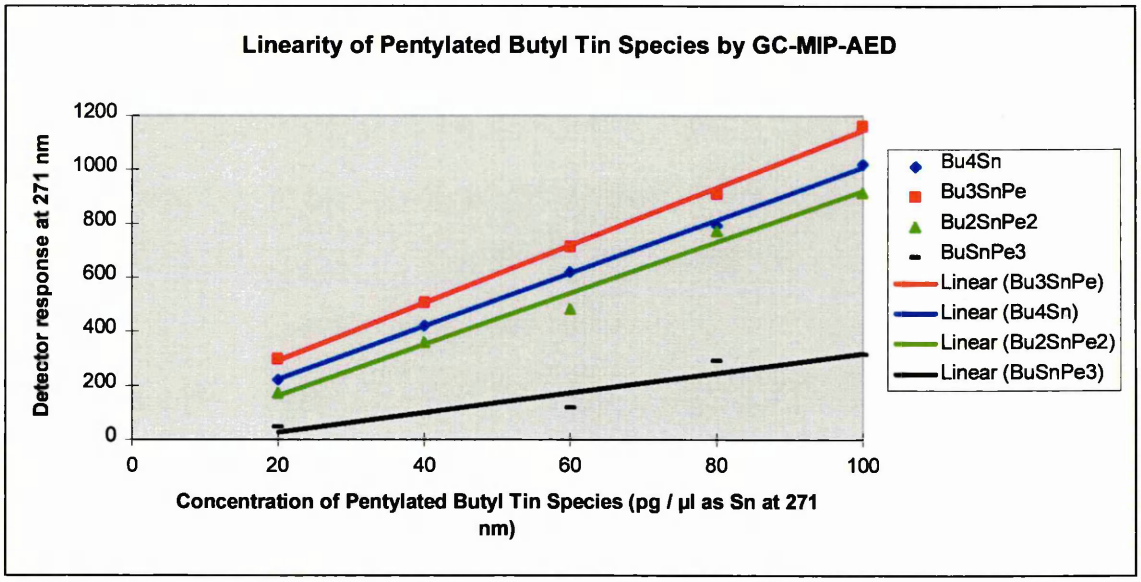


Figure 39. Linearity of Pentylated Butyl Tin Derivatives by GC-MIP-AED



The derivatisation procedure was shown to have no detrimental effect on the quantitation of tetra-butyl tin when using silanised glassware and no additional organotin species were confirmed by snapshot data.

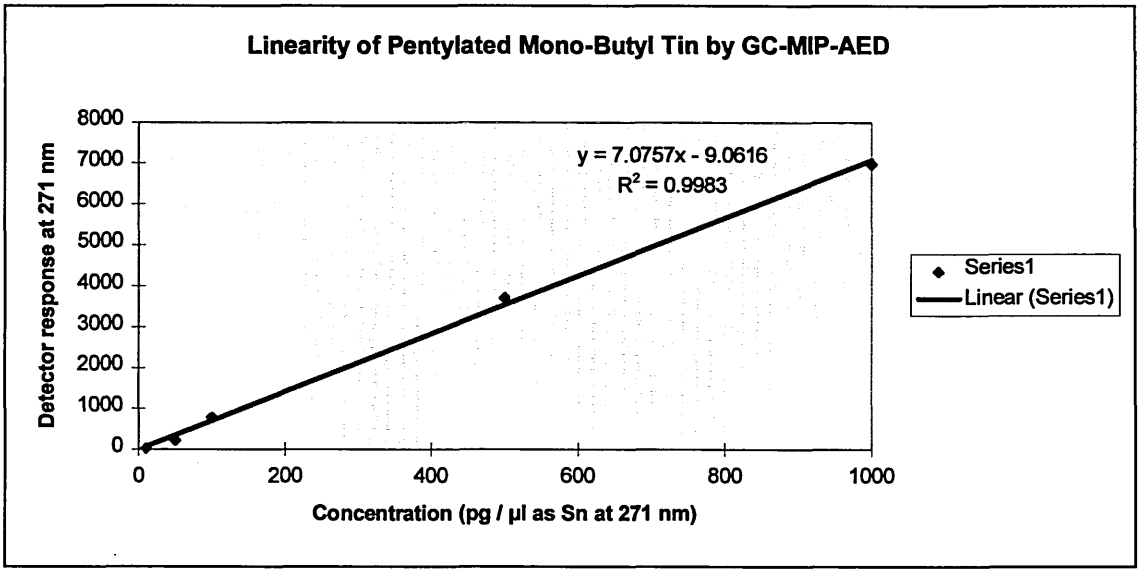
Table 20. Correlation Coefficient, Trend line Equation and Limit of Detection for Pentylated Butyl Tin Species shown in Figure 39

Butyl Tin Species	Correlation Coefficient	Trend line Equation	Calculated Limit of Detection (pg / µl)
Bu_4Sn	0.9987	y=10.658x+78	2.7
Bu_3SnPe	0.9983	y=9.8115x+26.447	2.0
Bu_2SnPe_2	0.9858	y=9.4676x-27.892	3.5
$BuSnPe_3$	0.907	y=3.6571x-46.171	13.0

A high degree of linearity was observed for Bu_4Sn and Bu_3SnPe. A degree of scatter was observed for Bu_2SnPe_2 and particularly for $BuSnPe_3$, shown by the reduction in the value of the correlation coefficient.

$BuSnPe_3$ was derivatised over a wider range of concentrations in isolation to try to ascertain the cause of the scatter. A new discharge tube was used for the study. The results are shown in figure 40.

Figure 40. Linearity of Pentylated Mono-Butyl Tin by GC-MIP-AED



Over the extended range of concentrations analysed for $BuSnPe_3$ the degree of linearity increased to give a correlation coefficient of 0.9983. A degree of scatter is still evident between 10-100 pg / µl. The equation for the trend line has changed slightly. This change may be attributed to the change of discharge tube as seen for the analysis of tetra-ethyl lead, or to incomplete atomisation within the plasma.

The variation in the trend line equations shown in table 20 indicate that the pentylated butyl tin species give a compound dependant response by GC-MIP-AED, as described for organomercury species. For the emission of tin to be considered independent of structure a graph of response against concentration (as Sn) would give the same slope and y-intercept. It can be seen from figure 39 that this is not the case.

Linearity, linear dynamic range and limit of detection were also determined for GC-MS selected ion monitoring detection over the range 1-1000 pg / µl as Sn for pentylated butyl tin species. The ions monitored are listed in chapter 2. This technique gave a limit of detection of 10 pg / µl as Sn for Bu_4Sn, Bu_3SnPe and Bu_2SnPe_2 and 100 pg / µl as Sn for $BuSnPe_3$. A linear response was seen for mass 121 for Bu_4Sn and Bu_3SnPe, however the response seen at mass 119, 289, 291, 303 and 305 was non-linear for these species. A linear response was observed for Bu_2SnPe_2 at mass 317 and $BuSnPe_3$ at mass 119. Again other mass ions monitored for these species at 119, 121 and 319 for Bu_2SnPe_2, and 121, 331 and 333 for $BuSnPe_3$, gave a non-linear response. The difference

between the two analytical detectors has been discussed for a similar context in chapter 4 for the analysis of methyl pentyl mercury.

In summary the derivatisation procedure does not appear to cause the degradation of tetra-butyl tin and detector response for tin emission, by GC-MIP-AED, for butyl tin species in general appears to be robust, linear and precise. The limit of detection and linear dynamic range were found to be adequate for application to environmental samples. The results obtained for GC-MS SIM showed that the technique lacked the sensitivity of the GC-MIP-AED by an order of magnitude and did not appear to show consistent linearity over the range of mass ions monitored.

6.3.4 Evaluation of the Extraction Procedure

The extraction technique as described in chapter 2 was evaluated using laboratory prepared aqueous samples. The background to the technique is described in detail in chapter 4 for the extraction of methyl mercury species, and is similar to the technique reported by Lobinski *et al* [153] for the extraction of organolead compounds.

As tetra-butyl tin is a neutral compound the presence of sodium diethyldithiocarbamate was not necessary, however the extraction was performed to show that the procedure did not cause degradation of the organotin species.

Each extraction incorporated a ten fold preconcentration and was subjected to the pentylation procedure together with a standard prepared in toluene of the expected final concentration. % Recoveries were calculated for the aqueous extracts against standards and are shown in tables 21-24.

The recoveries of the extraction procedure with respect to standards were high for the laboratory prepared aqueous samples. The limit of detection for the extraction procedure followed by derivatisation as described, was observed to be between 0.2-1.3 pg / μl, depending upon the butyl tin species, for an aqueous solution. As for organomercury and organolead compounds the detection limit may be further reduced by increasing the preconcentration factor during extraction.

In summary the extraction procedure gives high recoveries for low concentrations of tetra-ethyl lead such as found in environmental samples, although the results were observed to be more variable for Bu_2SnPe_2 and $BuSnPe_3$. A linear response was also observed for all pentylated butyl tin species indicating that the presence of the chelate reagent, and the alkylation procedure does not appear to cause transalkylation or degradation of the tetraalkylated organotin species.

Table 21. Recoveries of Bu_4Sn Aqueous Extraction Technique

Concentration (pg / µl)	Calculated concentration (pg / µl)	% Recovery	%RSD n=2
20	20.25	101.3	0.63
40	38.29	95.7	5.57
60	62.59	104.3	1.77
80	80.17	100.2	0.21
100	96.41	96.4	0.55

Table 22. Recoveries of Bu_3SnPe Aqueous Extraction Technique

Concentration (pg / µl)	Calculated concentration (pg / µl)	% Recovery	%RSD n=2
20	19.82	99.1	0.39
40	38.40	96.0	3.59
60	61.89	103.1	1.01
80	78.54	98.2	0.71
100	94.30	94.3	0.43

Table 23. Recoveries of Bu_2SnPe_2 Aqueous Extraction Technique

Concentration (pg / µl)	Calculated concentration (pg / µl)	% Recovery	%RSD n=2
20	22.39	111.9	1.53
40	39.03	97.6	3.54
60	58.60	97.7	0.91
80	90.23	112.8	0.98
100	98.20	98.2	0.11

Table 24. Recoveries of $BuSnPe_3$ Aqueous Extraction Technique

Concentration (pg / µl)	Calculated concentration (pg / µl)	% Recovery	%RSD n=2
20	21.97	109.8	4.85
40	36.74	116.5	1.29
60	69.91	91.8	0.66
80	91.82	114.8	0.89
100	96.15	96.2	0.01

6.4 Summary

The technique used in chapters 4 and 5 for the extraction and pentylation of methyl mercury and tetra-ethyl lead was successfully applied to pentylated butyl tin species, without causing degradation or transalkylation based on laboratory prepared standards. Acceptable levels of linearity were achieved for the manufacturers recommended flow rate and a cavity gas flow rate of 290 ml / min required for the simultaneous quantitation of organolead compounds.

The GC-MIP-AED has been shown to give a compound dependant response for similar molecular structures containing tin, as seen for organomercury compounds in chapter 4.

During the application of the technique to organotin compounds, a faint white solid was found to form on the surface of the discharge tube. This could be linked to the variation of the breakdown pathway of the molecule in the plasma and the formation of tin oxide. A similar pathway may be followed as for the atomisation of tetra-ethyl lead, causing the formation of tin oxide prior to atomisation, hence reducing the intensity of the tin emission signal. Besner *et al* [101] also observed the formation of tin oxide during the atomisation of organotin compounds by GC-MIP-AED. Hydrogen and oxygen scavenger gases were used in the application, however variation of the ratios of these gases within the cavity make up gas may prove to control this problem. This is an area for further study, when automated flow control can be achieved on the commercial GC-MIP-AED system.

The effects of possible matrix interferences from real samples will be assessed in chapter 7 where the technique of multielemental, simultaneous, organometallic analysis will be applied to a number of real samples.

Chapter 7

Organometallic Multielemental Analysis by GC-MIP-AED

7.0 Organometallic Multielement Analysis by GC-MIP-AED

7.1 Introduction

In order to achieve multielement organometallic speciation by GC-MIP-AED a suitable digestion / extraction / derivatisation procedure is required. In chapters 4, 5 and 6 a variety of procedures have been discussed for the extraction of organomercury, organolead and organotin compounds individually, and a number of these procedures may be adapted for multielement speciation. The technique evaluated in this research programme is described in chapter 2, involving acid digestion, non-specific chelation extraction into toluene to allow for the extraction of both polar and non-polar organometallic compounds, followed by Grignard pentylation. The technique was optimised in chapters 4, 5 and 6 and applied to certified reference materials to evaluate the validity of the analytical procedure for organometallic speciation.

Certified reference materials (CRM's) are widely used in the field of analytical chemistry today to ensure the quality, accuracy and validation of analytical data. A CRM is defined by the Laboratory of the Government Chemist as "*A reference material one or more of whose property values are certified by a technically valid procedure, accompanied by or traceable to a certificate or other documentation which is issued by a certifying body*". They serve a number of purposes in both research and industry which include:

1) Independent calibration and verification of analytical quantification procedures

2) Laboratory quality control and quality assurance

3) The development and validation of new analytical procedures

To date there are a large number of CRM producers world-wide including:

1) The Laboratory of the Government Chemist (LGC) - UK

2) Community Bureau of Reference (BCR) - European Community

3) National Institute of Standards and Technology (NIST) - USA

4) Laboratoire National d'Essais (LNE) - France

5) National Research Council (NRC) - Canada

6) National Research Centre for Certified Reference Materials (NRCCRM) - China

7) National Institute for Environmental Studies (NIES) - Japan

Typical certification approaches have included:

1) The analysis of a material within a single laboratory by two or more independent validated analytical procedures.

2) Interlaboratory comparisons which involve the analysis of a material by either the same analytical technique or a number of analytical techniques at a number of laboratories.

The second approach is probably the most appropriate technique as a range of independent methods are used to characterise a particular material within a

number of laboratories, avoiding any bias associated with a particular method or laboratory.

In order to evaluate the extraction, derivatisation and quantification procedure for the simultaneous multielemental speciation of organometallic compounds by GC-MIP-AED, the techniques previously discussed in this thesis were applied to two certified reference materials; a sediment and a fish tissue.

7.2 Optimisation of Experimental Parameters

The objectives of this program of research was to utilise the GC-MIP-AED for the multielemental analysis of organomercury, organotin and organolead species for environmental samples within a single chromatographic injection.

Chapters 4, 5 and 6 have identified the optimum operating conditions in order to achieve this objective. These conditions are stated in chapter 2. The extraction and derivatisation procedures described were found to give a linear response for all organometallic species studied for multielement laboratory prepared standards.

The effect of variations in injection port temperature using a split / splitless injection port were examined for the organometallic species studied. The results indicated the derivatised species were stable up to an operating temperature of 300°C and were not subject to thermal decomposition or adsorption on to the injection port liner. The least volatile species studied after

derivatisation were the butyl tin compounds which required an injection port temperature of at least 200°C. Hence an injection port temperature of 250°C was used for the multielement organometallic study.

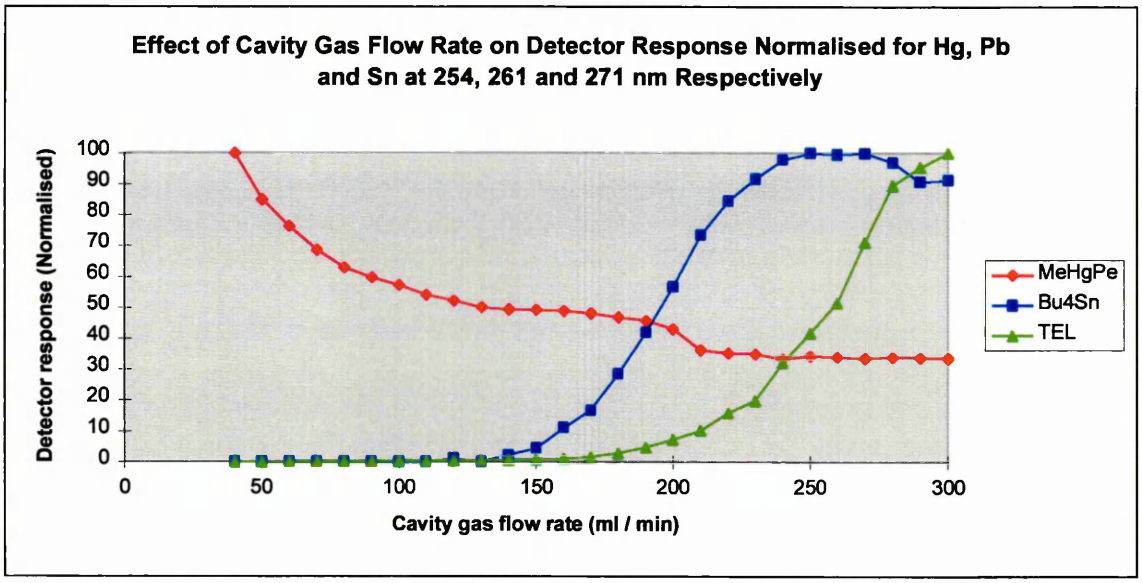
A single restriction liner was used with high speed automatic injection as previously described, with a carrier gas flow rate of 2.3 ml / min, which had been identified to give high precision, and good analyte peak shape. A temperature of 300°C for the transfer line and microwave cavity was also applied for this multielement organometallic study to prevent condensation.

For the analysis of all three organometallic species within a single chromatographic injection compromises were required. A cavity gas flow rate of 290 ml / min was required to ensure linearity of response for organolead compounds. This high flow rate is a deviation from the manufacturers recommended flow rate by an increase of 90 ml /min. The effect of this increased flow rate on normalised response for all three organometallic species is shown in figure 41.

Figure 41 highlights the differences between the three organometallic species. At low cavity gas flow rates an optimum response is observed for organomercury species, however no response is observed for organolead or organotin compounds. Both tin and lead organometallic species gave an optimum response at approximately 290 ml / min. Operating at high cavity gas

flow rates will require a compromise for organomercury sensitivity by a factor of 2.

Figure 41. The Effect of Cavity Gas Flow Rate on Detector Response for Hg, Pb and Sn at 254, 261 and 271 nm Respectively.



Optimisation of the derivatisation technique indicated no adverse reaction to extended contact with the pentylation reagent. For complete derivatisation to occur a derivatisation period of at least 30 min was required for organotin compounds.

A linear response was observed for all three organometallic species, however a compound dependant response was observed for the analysis of standards of organomercury and organotin compounds. This indicated that calibration of each individual species would be required for quantification.

Extraction of organometallic compounds from aqueous systems was shown to give high recoveries using sodium diethyldithiocarbamate as a metal chelation reagent and toluene as the extraction solvent.

7.3 Application of Multielemental Analysis to Environmental Samples

Certified reference materials for the analysis of methyl mercury (DORM-1), butyl tin species (PACS-1) and a number of aqueous samples were analysed under the conditions specified in chapter 2. DORM-1 is a reference material of dogfish muscle and PACS-1 is a marine sediment reference material, both of which were obtained from the National Research Council, Canada.

A number of aqueous samples were also analysed, including bottled mineral water, water from the River Don and water samples provided by Analytical and Environmental Services, Tyne and Wear.

The extraction and derivatisation procedure described in chapter 4 for the analysis of organomercury species was applied for the analysis of organomercury, organolead and organotin compounds under the conditions described in Chapter 2. However the dogfish muscle and marine sediment were first subjected to an acid leaching pre-treatment step to release any bound organomercury, organolead and organotin compounds. The acidic solution was then neutralised using concentrated ammonia and buffered to pH 8.5 prior to extraction into toluene with the aid of sodium diethyldithiocarbamate as a chelation reagent. Calibration was achieved by external standard quantitation

techniques using laboratory prepared standards prepared in toluene which were subjected to the pentylation procedure.

7.3.1 Quantification of DORM-1 Reference Material

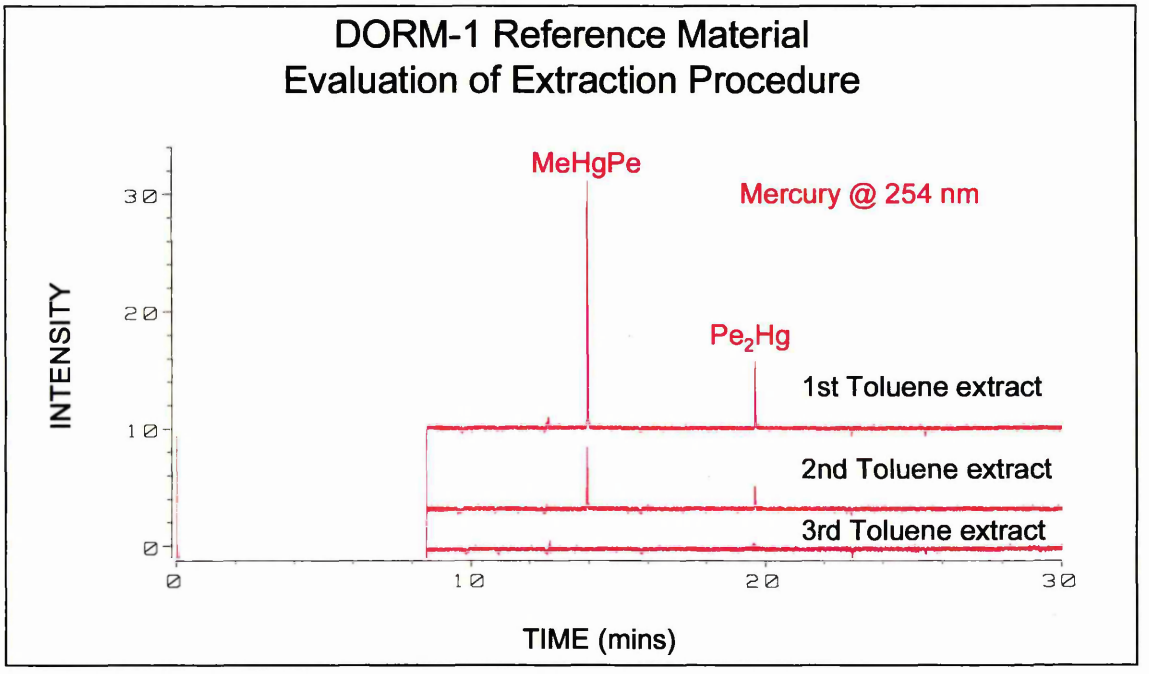
A hydrochloric acid digestion procedure was developed in order to leach the organometallic compounds from the fish tissue. From a review of the literature in chapters 4, 5 and 6 this technique appears to be a common approach for liberating bound organometallic compounds from both sediments and fish tissue.

The study initially concentrated on achieving high recoveries with respect to the certified value of methyl mercury during the development of the sample digestion and extraction procedure.

A range of concentrations of acid strength were evaluated (5%, 25%, 50% and 66% v / v), specifically for the analysis of methyl mercury, as a certified concentration was available. This enabled the recoveries of the digestion procedure to be evaluated. Only a single toluene extraction was performed throughout this initial study. The optimum recovery was achieved using an acid concentration of 50% v / v were a mean recovery of 71% was achieved with respect to the certified value. Acid concentrations at 5%, 25% and 66% gave mean recoveries of 55%, 66% and 60% respectively.

This initial digestion, extraction and derivatisation procedure still left 29% of methyl mercury unaccounted for. The extraction procedure was then optimised to evaluate the effectiveness of a single extraction. The toluene extraction step was repeated three times with the individual extracts derivatised and analysed. The results are shown in figure 42.

Figure 42. The Effect of Multiple Toluene Extractions for the Determination of Methyl Mercury in DORM-1



This indicated that a single extraction was not efficiently removing the total liberated methyl mercury from the fish tissue. The extraction procedure was adapted to incorporated two toluene extractions, as detailed in chapter 2.

The amended extraction procedure was applied to the quantification of methyl mercury in DORM-1 (as Hg). The results are shown in table 25. Standard

solutions were prepared at 25, 37.5 and 50 pg / µl as Hg. A linear response was observed for the standards with a calibration coefficient of 0.9897 and a trend line of y=14.992x + 15.367. Quantitation was achieved by external standard calibration techniques using the standard with the closest area response to that of the samples, in this case 37.5 pg / µl. All results for DORM-1 were within the calibration linear range. The recoveries were calculated with respect to the certified value of 0.731 ± 0.060 mg / kg methyl mercury as Hg. The concentration (mg / kg as Hg) was calculated using the following equation:

$$\text{Conc. (mg/kg as Hg)} = \frac{\text{Area}_{\text{sample}}}{\text{Area}_{\text{standard}}} \times \frac{\text{std conc(mg/ml as Hg)}}{\text{Mass (mg)}_{\text{sample}} \Big/ {}^{(a)}\text{extraction volume (ml)}} \times 1000 \times 1000$$

(a) This is the volume of toluene (ml) used in the extraction of the sample.

Table 25. Quantification of Methyl Mercury in DORM-1 Certified Reference Material as Hg

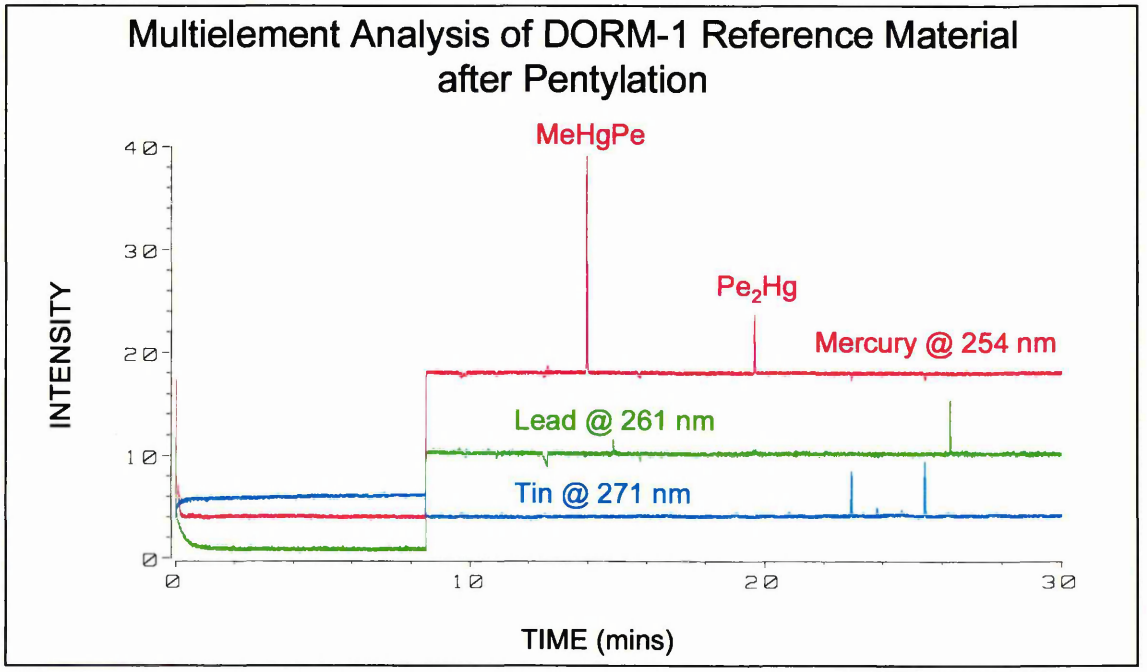
Analytical Solution	Area A	Area B	Mean (n=2)	Mass of sample (g)	Conc. (mg/kg as Hg)	Recovery (%)
25 pg / µl (standard)	401.71	400.96	401.2			
37.5 pg / µl (standard)	546.17	564.90	555.5			
50 pg / µl (standard)	780.91	771.13	776.0			
DORM-1 A	483.03	487.47	485.3	0.49286	0.665	91.4
DORM-1 B	509.55	523.04	516.3	0.49581	0.703	96.2
DORM-1 C	537.13	537.40	537.3	0.49391	0.734	100.4
DORM-1 D	502.25	482.35	492.3	0.49479	0.672	91.9
DORM-1 E	496.91	520.90	508.9	0.49498	0.694	94.9

The %RSD for the 5 determinations was calculated to be 4.0, and samples B-E were within the certified tolerance range. Sample A was outside the given tolerance range by 0.006 mg / kg as Hg.

Once recoveries within the certified range were achieved the samples were analysed for organomercury, organolead and organotin compounds simultaneously.

The sample was found to contain organotin compounds which were identified by retention time as tri and di-butyl tin species at t_R=23.1 and 23.9 min respectively. Inorganic mercury, tin and lead was also found to be present at t_R=19.6, 25.2 and 26.3 min respectively. A typical chromatogram is shown in figure 43.

Figure 43. Multielement Analysis of DORM-1 Certified Reference Material after Pentylation



The mean tri and di-butyl lead concentration for DORM-1 (n=5) was calculated as 0.284 and 0.048 mg / kg as Sn respectively. These results were not confirmed by spiked recoveries due to lack of time. This is however an opportunity for further work.

A small response at t_R=14.5 min was observed on the lead channel however the presence of lead was not confirmed by snapshot data. This peak was thought to be due to carbon breakthrough emission.

7.3.2 Quantification of PACS-1 Reference Material

The procedure developed for the quantification of methyl mercury in DORM-1 was applied to the multielemental analysis of the sediment reference material PACS-1.

The study concentrated on achieving high recoveries with respect to the certified value of tri, di and mono-butyl tin species for the application of the digestion, extraction and derivatisation procedure to sediments.

Initially the acid digestion of 50% v / v was applied to release any bound organometallic compounds. Two extractions were performed with 5 ml of toluene and the extracts derivatised individually to identify the efficiency of the extraction procedure. The recoveries were calculated with respect to the certified values of 1.27 ± 0.22, 1.16 ± 0.18 and 0.28 ± 0.17 mg / kg as Sn for tri, di and mono-butyl tin species respectively. The results are shown in table 26.

Table 26. Recoveries of Butyl Tin Species following Two Extractions

	Recovery of Bu_3Sn^+ (% as Sn)	Recovery of Bu_2Sn^{2+} (% as Sn)	Recovery of $BuSn^{3+}$ (% as Sn)
1st extraction	46	42	11
2nd extraction	22	34	11

This indicated that the extraction efficiency for two extractions from sediment was not sufficient to recover the butyl tin species at the certified values.

The digestion period was extended from 30 min to 24 and 48 hr prior to extraction, however this did not show any increase in the recoveries with respect to the certified value.

Two variations on the extraction procedure were then applied. The first variation increased the sodium diethyldithiocarbamate concentration by a factor of 5 with two toluene extractions, and the second variation used the same concentration of chelation reagent but three toluene extractions.

The extraction procedures were applied to the quantification of butyl tin species in PACS-1 (as Sn). The results are shown in table 27a-c. Mixed standard solutions were prepared at 50 and 75 pg / µl (as Sn) for tri and di-butyl tin and 5 and 25 pg / µl (as Sn) for mono-butyl tin.

Table 27a. Quantification of Tri-Butyl Tin in PACS-1 Certified Reference Material as Sn

Analytical Solution	Area A	Area B	Mean (n=2)	Mass of sample (g)	Conc. (mg/kg as Sn)	Recovery (%)
50 pg / µl (standard)	405.73	423.91				
50 pg / µl (standard)	564.25	546.32	485.1 (16.9% RSD)			
75 pg / µl (standard)	685.29	709.39				
75 pg / µl (standard)	795.19	782.46	743.1 (7.3% RSD)			
PACS-1 A	549.92	561.85	555.9	0.49385	1.160	91.3
PACS-1 B	604.00	594.43	599.2	0.50234	1.229	96.8
PACS-1 C	386.51	400.57	393.5	0.50886	1.196	94.2
PACS-1 D	339.79	337.47	338.6	0.50131	1.044	82.2

Table 27b. Quantification of Di-Butyl Tin in PACS-1 Certified Reference Material as Sn

Analytical Solution	Area A	Area B	Mean (n=2)	Mass of sample (g)	Conc. (mg/kg as Sn)	Recovery (%)
50 pg / µl (standard)	476.52	471.65				
50 pg / µl (standard)	547.62	546.32	510.5 (8.3% RSD)			
75 pg / µl (standard)	727.85	748.04				
75 pg / µl (standard)	795.19	812.75	771.0 (5.1% RSD)			
PACS-1 A	340.40	349.94	345.2	0.49385	0.685	59.1
PACS-1 B	399.39	392.72	396.1	0.50234	0.772	66.6
PACS-1 C	284.50	307.50	296.0	0.50886	0.855	73.7
PACS-1 D	275.37	270.98	273.2	0.50131	0.801	69.1

Table 27c. Quantification of Mono-Butyl Tin in PACS-1 Certified Reference Material as Sn

Analytical Solution	Area A	Area B	Mean (n=2)	Mass of sample (g)	Conc. (mg/kg as Sn)	Recovery (%)
5 pg / µl (standard)	65.77	62.51				
5 pg / µl (standard)	78.84	78.03	71.3 (11.7% RSD)			
25 pg / µl (standard)	191.43	203.50				
25 pg / µl (standard)	187.14	192.50	193.6 (3.6% RSD)			
PACS-1 A	153.68	165.37	159.5	0.49385	0.417	148.9
PACS-1 B	221.98	201.82	211.9	0.50234	0.545	194.6
PACS-1 C	116.00	114.03	115.0	0.50886	0.438	156.4
PACS-1 D	102.16	99.29	100.7	0.50131	0.389	138.9

Quantitation was achieved by external standard calibration techniques using the standard with the closest area response to that of the samples. All results for PACS-1 were within the calibration linear range. The recoveries were calculated with respect to the certified values (as Sn) detailed earlier. The concentration (mg / kg as Sn) was calculated using the following equation:

$$\text{Conc. (mg/kg as Sn)} = \frac{\text{Area}_{\text{sample}}}{\text{Area}_{\text{standard}}} \times \frac{\text{std conc(mg/ml as Sn)}}{\text{Mass (mg)}_{\text{sample}} \Big/ {}^{(a)}\text{extraction volume (ml)}} \times 1000 \times 1000$$

(a) This is the volume of toluene (ml) used in the extraction of the sample.

The % recoveries for the two extraction procedures indicated that both procedures give similar results. The results obtained for tri-butyl tin were within

the tolerance levels of the certified concentration, except for sample PACS-1 D, were the value was 0.006 mg / kg below the lower tolerance level. The results obtained for di-butyl tin were all below the lower tolerance level by up to 30 mg / kg. The results obtained for mono-butyl tin were within the tolerance levels of the certified concentration, excluding sample PACS-1 B which was 0.095 mg / kg above the upper tolerance level.

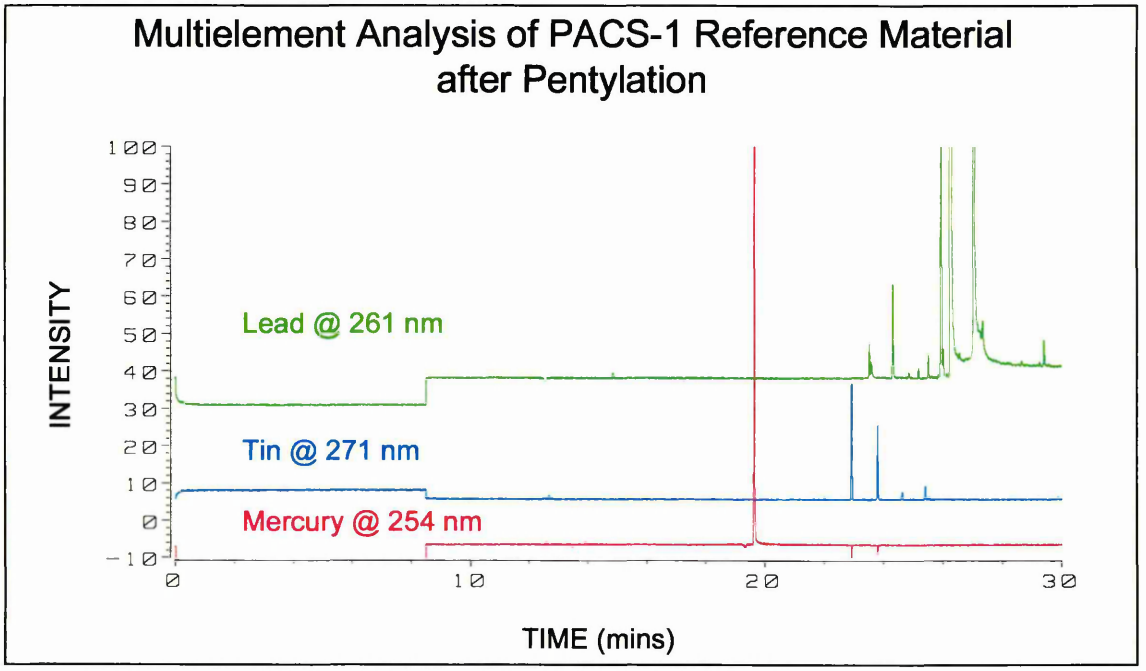
The samples were analysed qualitatively for organomercury, organolead and organotin compounds simultaneously and a chromatogram is shown in figure 44. This chromatogram was obtained immediately after a new discharge tube had been installed in the GC-MIP-AED.

The chromatogram shows that inorganic mercury is present in the reference material, however no response is observed for any additional organomercury compounds. Tri, di, mono and inorganic tin species were observed as expected. The chromatogram obtained for the lead channel was however unusual. A number of peaks were observed between 24-28 min, possibly associated with inorganic lead.

Any subsequent injection of either derivatised sample or even a solvent blank caused carryover on the lead channel as seen in figure 45. This was traced to contamination in the discharge tube. The certified level of inorganic lead in the sediment was reported as 404 ± 20 mg / kg as Pb. This is much higher than seen in the DORM-1 reference material were an inorganic lead concentration of

0.40 ± 0.12 mg / kg as Pb was reported. The response observed for lead may be a contributory factor towards the variable agreement with the certified butyl tin concentrations.

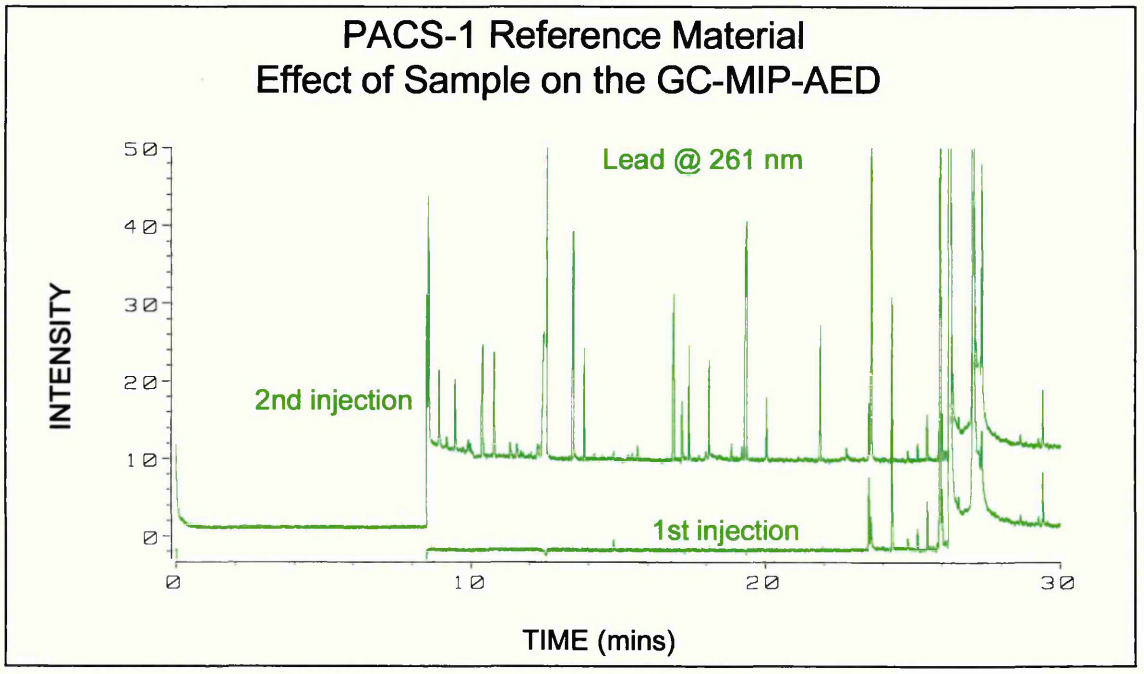
Figure 44. Multielement Analysis of PACS-1 Reference Material after Pentylation



The digestion, extraction and derivatisation procedure for the determination of organometallic compounds in this sediment sample require re-evaluation and further work. One possible route may be to selectively extract the organometallic compounds in the presence of inorganic metals. This could be achieved by the appropriate choice of chelation reagents and pH, for example, by using EDTA as a chelation reagent to selectively chelate inorganic metal ions and retain them in the aqueous layer during extraction. Higher recoveries may also be achieved by adopting a different digestion technique to release the organometallic compounds from the sediment, such as, alkaline or microwave

assisted digestion or supercritical fluid extraction. These avenues were not explored due to time and instrumentation constraints.

Figure 45. The Effect of PACS-1 Reference Material derivatised extract on the GC-MIP-AED



7.3.3 Multielement Profiling of Aqueous Solutions.

This particular application highlights the qualitative aspect of GC-MIP-AED for elemental screening of aqueous samples. Water samples from the River Don, a bottled mineral water sample and a sample from Analytical and Environmental Services, Tyne and Wear were screened for organometallic compounds. The samples were extracted in triplicate as described in chapter 2 for the extraction of aqueous samples. The sample from Analytical and Environmental Services was screened for organomercury, organolead and organotin compounds. The samples of River Don water and bottled mineral water were screened for

organomercury and organotin compounds only. A sample spiked with methyl mercury and n-butyl tin species was also analysed to aid identification. The results of the unspiked and spiked samples are shown in figures 46-48 a-b.

The water sample provided by Analytical and Environmental Services, Tyne and Wear, showed the presence of tri-butyl tin and inorganic mercury. The sample taken from the River Don indicated the presence of inorganic mercury only. The bottled mineral water sample indicated the presence of inorganic mercury and also a unknown volatile organotin compound at t_R=6.5 min. The presence of tin was confirmed from elemental snapshot data. All other peaks were below the limit of detection based on a signal to noise ratio of 3:1.

The unknown peak observed in the bottled mineral water may have originated from organotin stabilisers incorporated into the plastic packaging. The organotin compound having leached out of the plastic over time.

7.4 Summary

To summarise the GC-MIP-AED was successfully used to perform the simultaneous multielement speciation of organomercury, organolead and organotin compounds within a single chromatographic run in sediment and fish tissue CRM's and a number of aqueous samples.

In order to achieve this objective a number of parameters were optimised, deviating from the manufacturers recommended operating conditions.

Figure 46a. Elemental Profile of a Water Sample provided by Analytical and Environmental Services, Tyne and Wear (Unspiked)

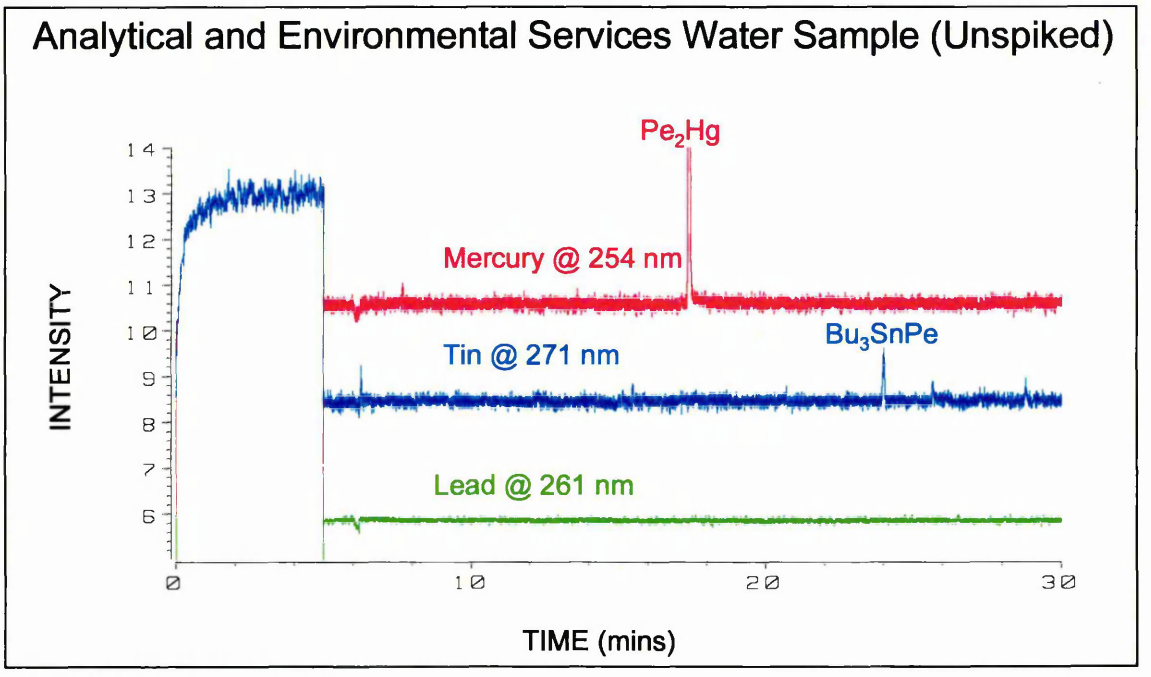


Figure 46b. Elemental Profile of a Water Sample provided by Analytical and Environmental Services, Tyne and Wear (Spiked)

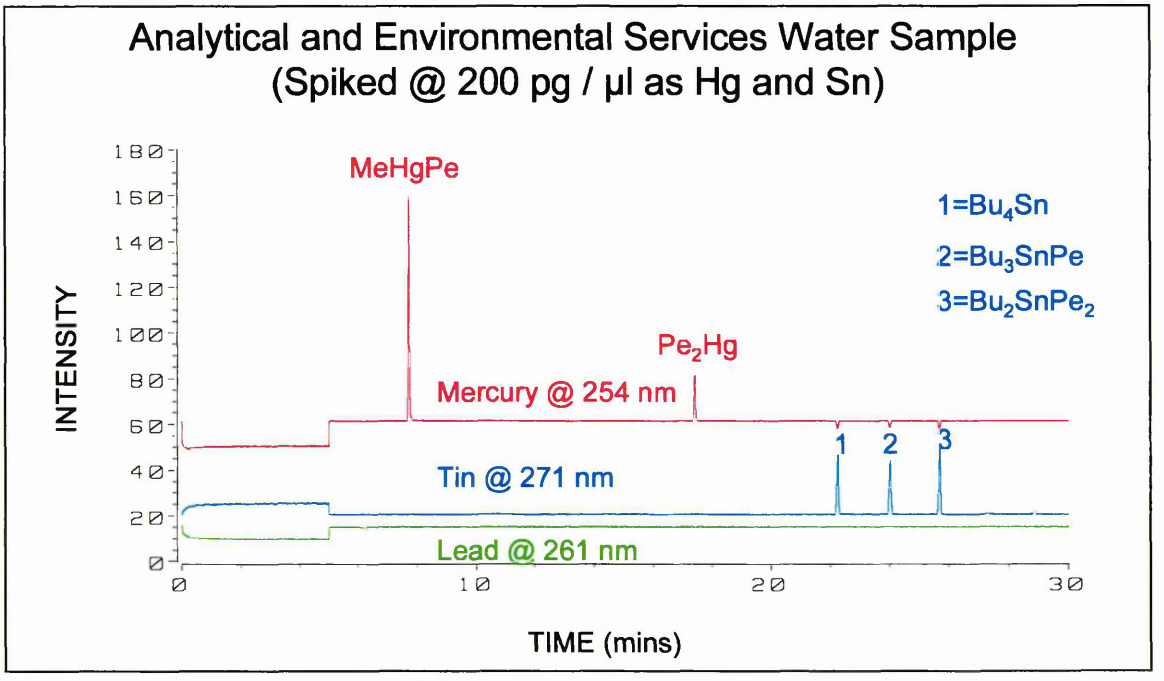


Figure 47a. Elemental Profile of a Water Sample from the River Don (Unspiked)

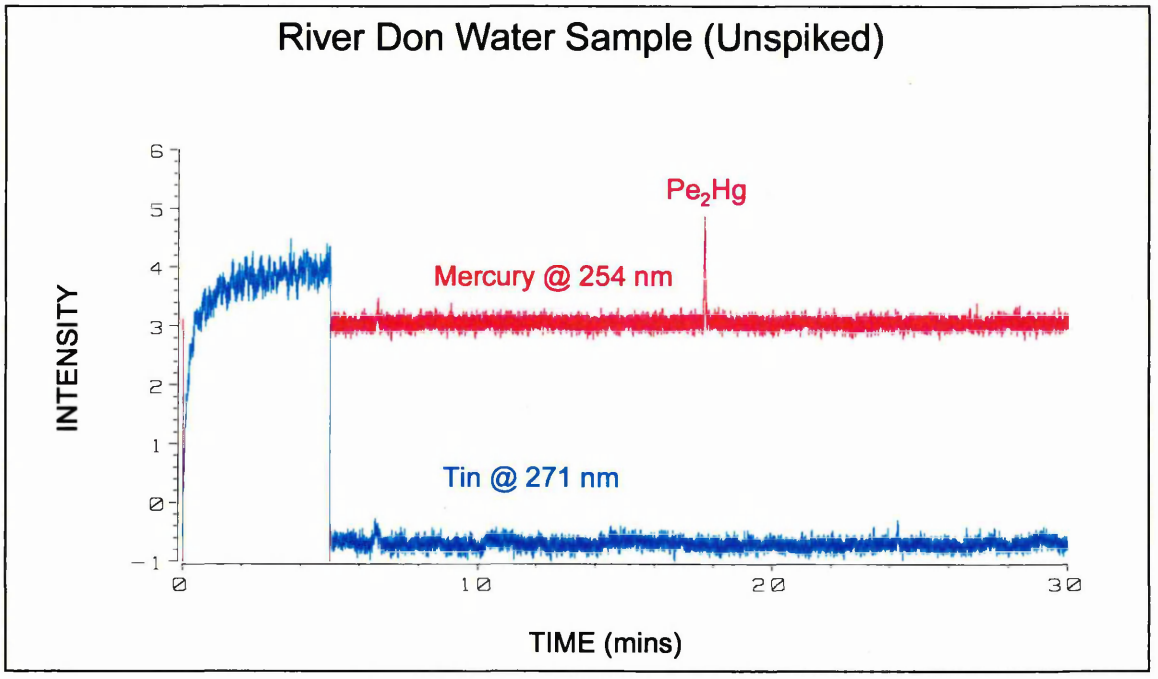


Figure 47b. Elemental Profile of a Water Sample from the River Don (Spiked)

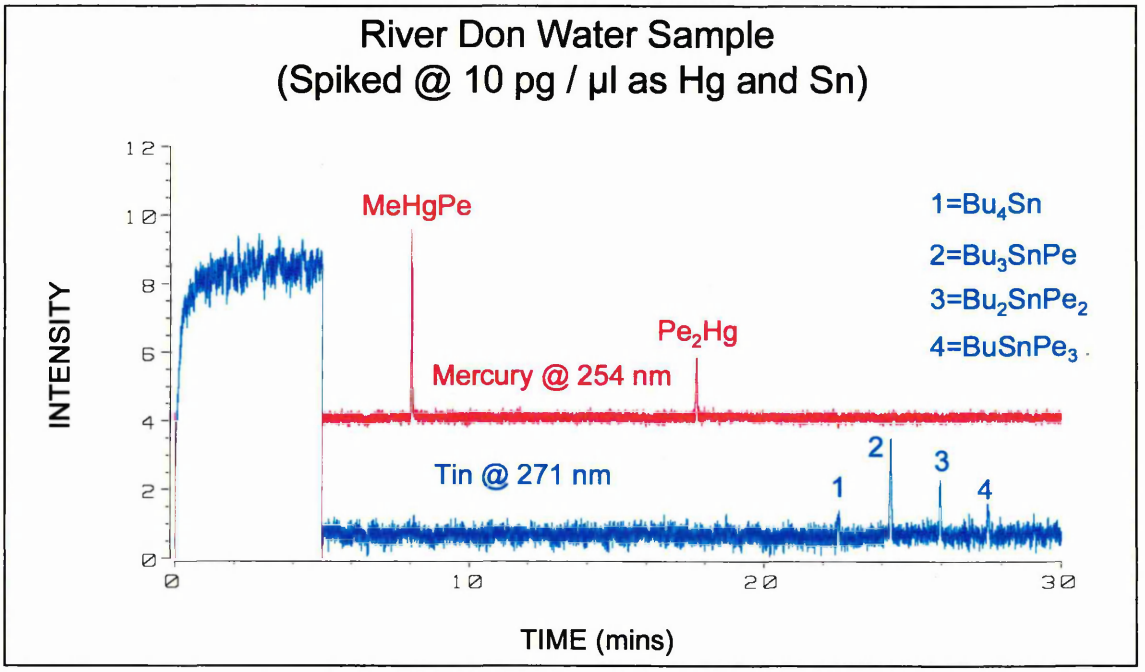


Figure 48a. Elemental Profile of a Bottled Mineral Water Sample (Unspiked)

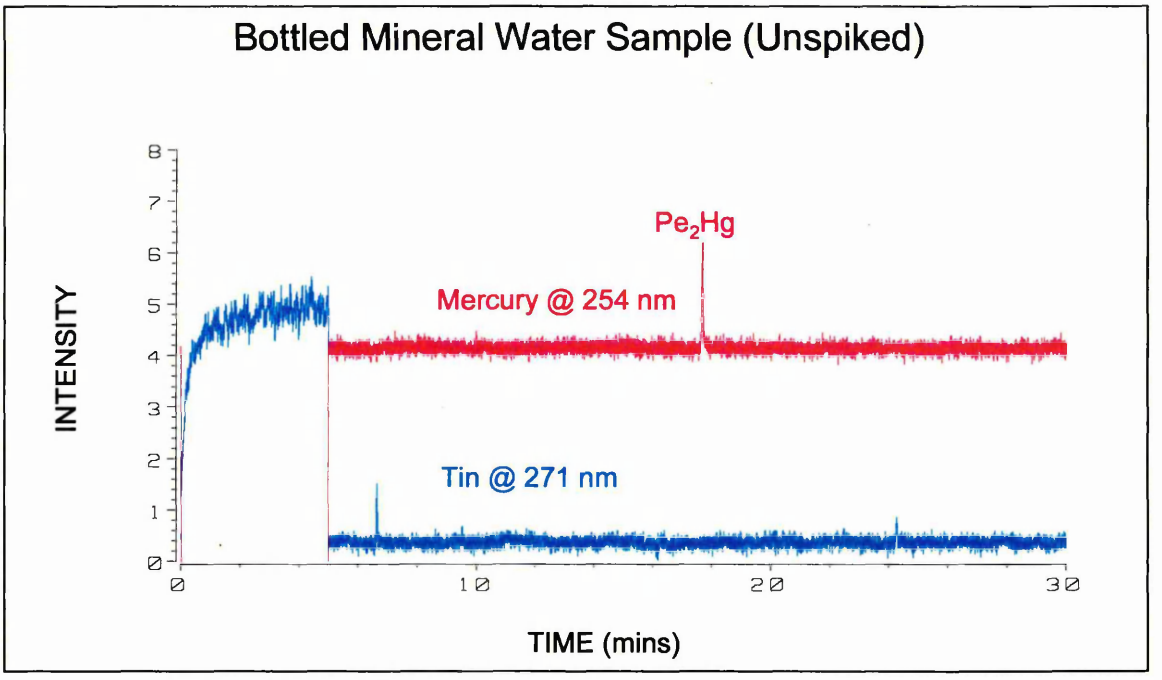
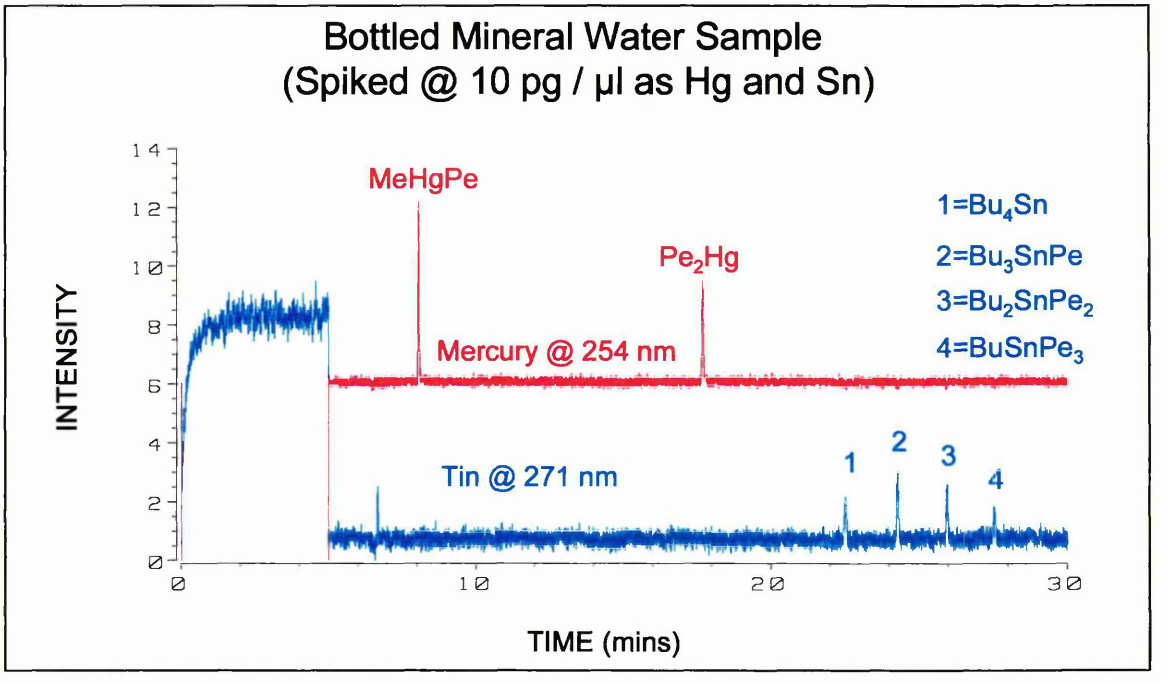


Figure 48b. Elemental Profile of a Bottled Mineral Water Sample (Spiked)



The analytical procedure applied for the determination of methyl mercury in fish tissue gave good recoveries, within the certified tolerance levels of the CRM. Problems were however encountered when the same technique was applied to the determination of butyl tin species in sediment. This may be due to the high inorganic lead content in the sample, as inorganic metal species will be coextracted with organometallic compounds using the extraction technique specified in chapter 2. Due to time restrictions alternative digestion and extraction techniques were not pursued within this programme of research however a number of alternative digestion, extraction and alkylation procedures may be applied, as previously described in chapters 4-6.

The application of the procedure for the elemental profiling of water samples highlights the high selectivity of the GC-MIP-AED for the metals hence the technique could be used on a routine basis to screen for, and identify organometallic contaminants prior to quantification.

During the final stages of the laboratory study for this programme of research a number of publications on the multielement analysis of organomercury, organolead and organotin compounds were published in the literature for GC-MIP-AED [35, 221-222] and GC-ICP-MS [223-224].

Moens *et al* [223] and De Smaele *et al* [224] used an "in house" built GC-ICP-MS for the analysis of organomercury, organolead and organotin compounds in surface waters and sediment samples. The organometallic compounds were

derivatised *in situ* by aqueous phase ethylation and where simultaneously adsorbed onto a polydimethylsiloxane fiber. The volatile species were then thermally desorbed in the GC injection port. This technique was applied to the quantitation of butyl tin compounds in PACS-1 CRM. The results obtained were 1.24, 1.05 and 0.43 mg / kg as Sn for tri, di and mono-butyl tin species respectively.

Lui *et al* [35] used GC-MIP-AED for the simultaneous determination of organomercury, organolead and organotin compounds in soil and sediment extracts. Supercritical carbon dioxide modified with 5% methanol was used to extract the organometallic compounds from the sample matrix, after the addition of diethylammonium diethyldithiocarbamate to form the metal chelate. The extract was then pentylated in n-octane with pentyl magnesium bromide prior to quantitation by on column injection GC-MIP-AED.

Minganti *et al* [221] have compared a number of extraction and derivatisation techniques including aqueous phase ethylation and phenylation, and chelate extraction followed by Grignard pentylation. Quantitation was achieved by GC-MIP-AED. The procedures were applied to laboratory prepared standards and also biological tissue.

Ceulemans and Adams [222] used purge and trap GC-MIP-AED for the determination of methylated species of mercury, lead and tin in water by aqueous phase ethylation of the organometallic species.

These applications differ from the analytical procedures described in this thesis for extraction / derivatisation and sample introduction procedures however the results with respect to instrumental operating parameters for the GC-MIP-AED corroborate the work reported in this thesis for simultaneous multielement quantitation.

Chapter 8

Conclusions and Further Work

8.0 Conclusions and Further Work

The aim of this programme of research was to evaluate the use of GC-MIP-AED for application to environmentally significant samples.

The GC-MIP-AED has proved itself to be a powerful analytical technique for qualitative elemental profiling of environmental samples for both heteroatoms and organometallic species due to its simultaneous multielement capabilities. After the optimisation of the pre-programmed elemental recipes for the GC-MIP-AED a high degree of selectivity was established. This is highlighted in chapters 3 and 7 for a range of applications, including elemental heteroatom profiling of leaded and unleaded petrol and organometallic profiling of river and bottled mineral water samples. It has also been shown that the technique is comparable to the GC-FPD for the analysis of alkylbenzothiophenes, and provides complementary information to GC-MS as seen in chapters 4, 5 and 6 for the identification of organometallic species.

The instruments ability to perform quantitative analysis was shown to be dependant on injection technique as seen for the quantitation of tert-butyl disulphide and nitrobenzene in chapter 3, and also on the cavity gas flow rate as seen in the linearity study of tetra-ethyl lead in chapter 5.

An in depth study established that the repeatability and limit of detection of the instrument was dependant upon injection technique, injection port liner used and cavity gas flow rate. The use of an inappropriate injection port liner and

injection speed led to %RSD's of up to 20% hence the appropriate choice of liner and sample introduction for a particular application was found to be essential.

The effect of cavity gas flow rate is highlighted in chapters 4-7 for the determination of organometallic compounds. Inappropriate choice of flow rate was found to effect the atomisation processes within the plasma for particular elements. This parameter was found to be crucial for the quantitation of organolead species. By increasing the cavity gas flow rate and reducing the residence time of tetra-ethyl lead in the discharge tube an increase in emission signal was observed. The formation of a white deposit on the walls of the discharge tube, thought to be lead oxide, support the theory that molecular recombination is taking place within the plasma.

The response of the GC-MIP-AED for organotin and organomercury compounds was found to be dependant upon the nature of the parent molecule, supported by variations in the slope and intercepts of calibration graphs for n-butyl tin species and also alkylated mercury compounds. This refutes the claim that quantitation of elemental composition within an unknown compound can be achieved using a calibration compound of known elemental composition. It also throws doubt on the instruments theoretical capability to determine the empirical formula of an unknown compound.

The application of the GC-MIP-AED for the simultaneous multielement quantitation of organomercury, organotin and organolead compounds was shown to be possible however the detection limit for organomercury species was compromised by a factor of two. This could be overcome by monitoring for organomercury compounds individually in a second injection. An analytical procedure was developed for the digestion, extraction and derivatisation of organometallic compounds. The extraction and derivatisation procedure was shown to give high recoveries for laboratory prepared aqueous samples. The recoveries observed for certified reference materials were variable. High recoveries were observed for the determination of methyl mercury in DORM-1 and tri-butyl tin in PACS-1. Results for di and mono-butyl tin in PACS-1 showed a bias away from the certified values.

The work in this thesis may be continued by exploiting the GC-MIP-AED's simultaneous multielement capabilities and extending it to additional organometallic compounds, such as organic selenium and arsenic compounds.

The fundamental study of the instruments capabilities for the simultaneous multielement analysis of organomercury, organolead and organotin compounds presented in this thesis may also be applied to future studies of the environmental effects of organometallic compounds within a specific ecosystem.

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Appendix 1

Atomic Emission Detection

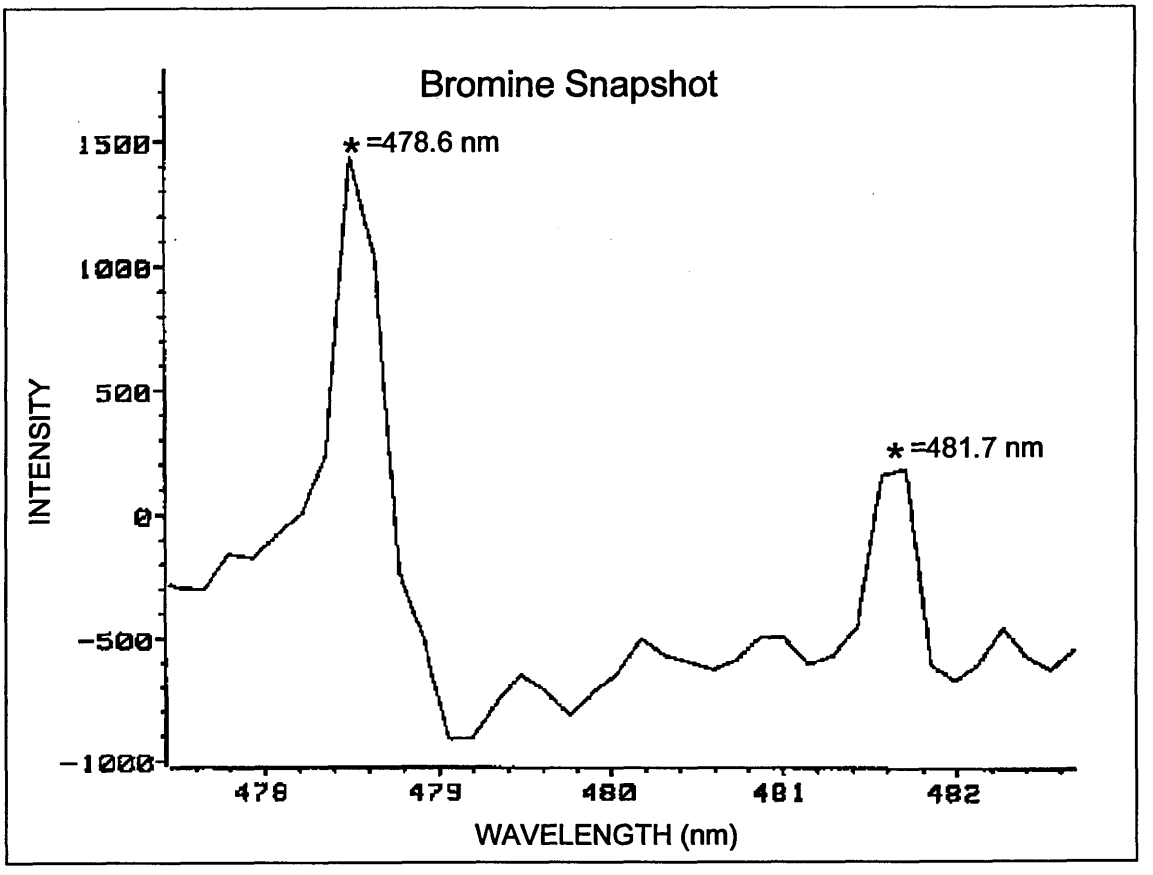
Wavelengths and Reagent Gas

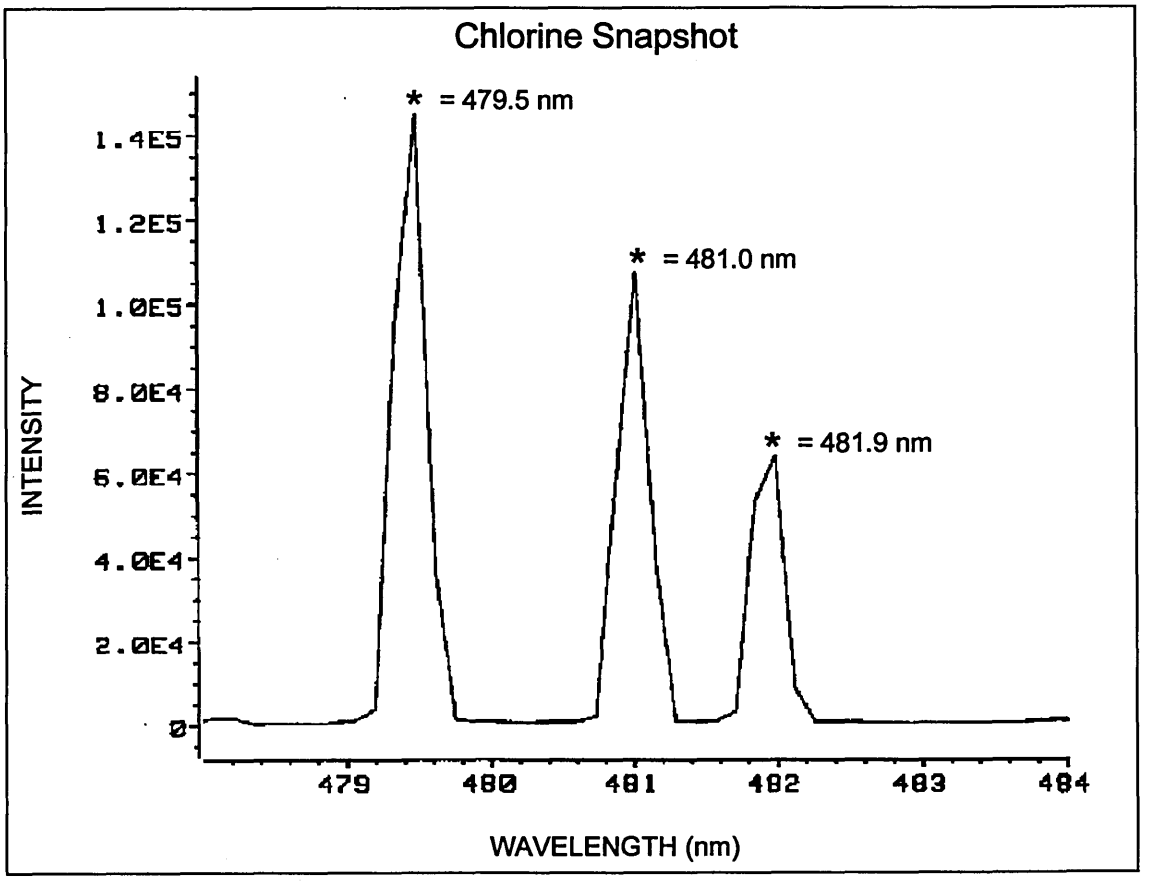
Requirements

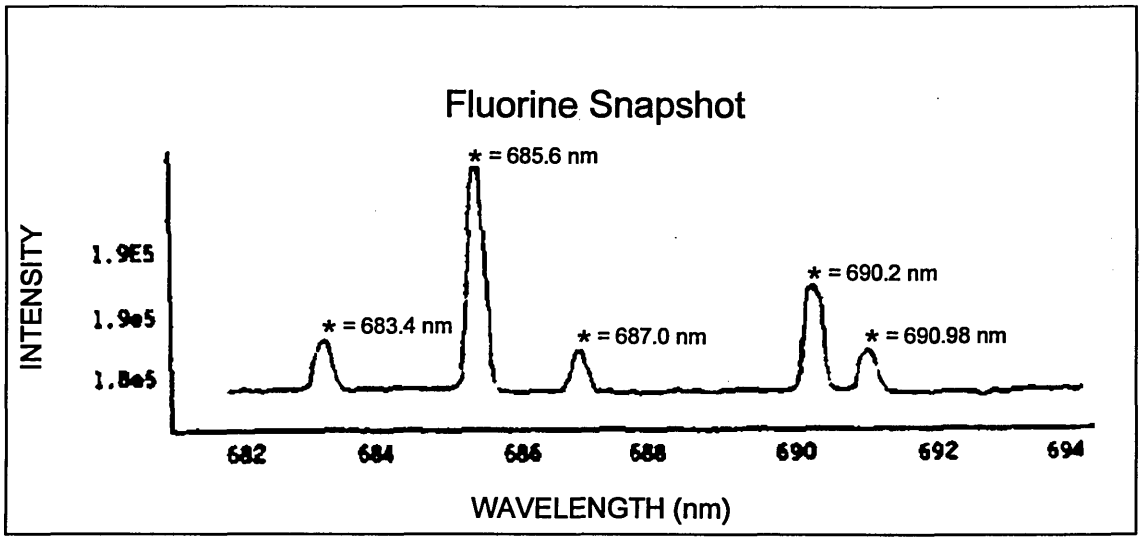
ELEMENT	WAVELENGTH (nm)	REAGENT GASES
Antimony	217.581	H_2 Fast flow
Arsenic	189.042	H_2 Fast flow
Bromine	478.578	O_2
Carbon	179.393	O_2 H_2
	193.031	H_2 Fast flow
	193.031	O_2 H_2
	247.857	O_2 H_2
	247.857	O_2 H_2 Fast flow
	341.712	O_2 H_2
	342.574	O_2 H_2
	495.724	O_2
Chlorine	480.192	O_2
Cobalt	345.351	O_2 H_2 Fast flow
Copper	324.754	O_2 H_2 Fast flow
Deuterium	656.039	O_2 Fast flow
(as OH)	307.452	O_2
Fluorine	690.466	H_2
Germanium	265.131	O_2 H_2 Fast flow
Hydrogen	486.133	O_2
	656.302	O_2 Fast flow
(as OH)	308.431	O_2
Iodine	182.966	O_2 H_2
	206.156	O_2 H_2
Iron	302.064	O_2 H_2 Fast flow
Lead	261.418	O_2 H_2 Fast flow
	405.780	O_2 H_2 Fast flow
Mercury	184.870	O_2 H_2
	253.652	O_2 H_2
	253.652	O_2 H_2 Fast flow
Nickel	301.200	O_2 H_2 Fast flow
Nitrogen	174.200	O_2 H_2
	348.424	O_2 H_2
(isotope 15)	420.168	O_2 H_2 AUX
Oxygen	777.302	H_2 AUX
Phosphorus	178.079	H_2 Fast flow
	185.917	H_2 Fast flow
Selenium	196.090	H_2 Fast flow
Silicon	251.418	O_2 H_2 Fast flow
Sulphur	181.379	O_2 H_2
	181.379	H_2 Fast flow
Tin	303.419	O_2 H_2 Fast flow
	270.651	O_2 H_2 Fast flow
Vanadium	292.402	O_2 H_2 Fast flow
Zinc	206.174	H_2 Fast flow

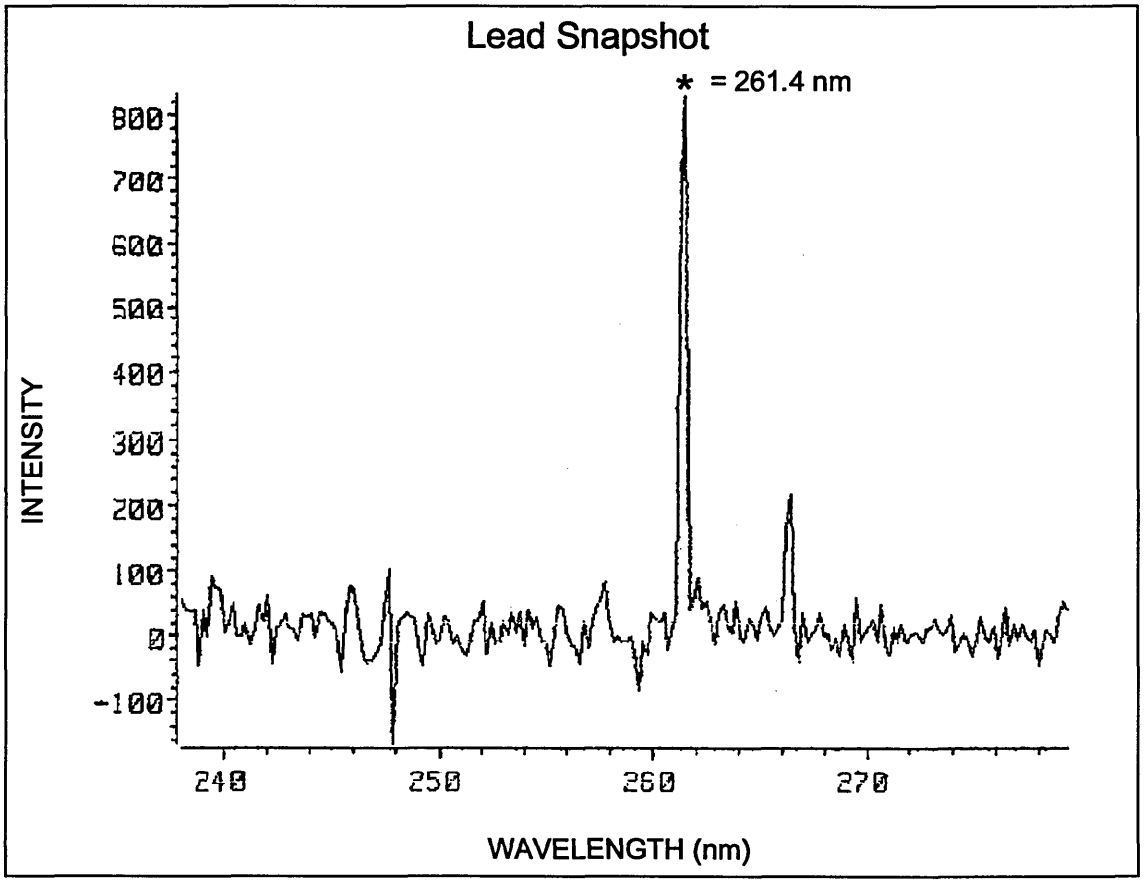
Appendix 2

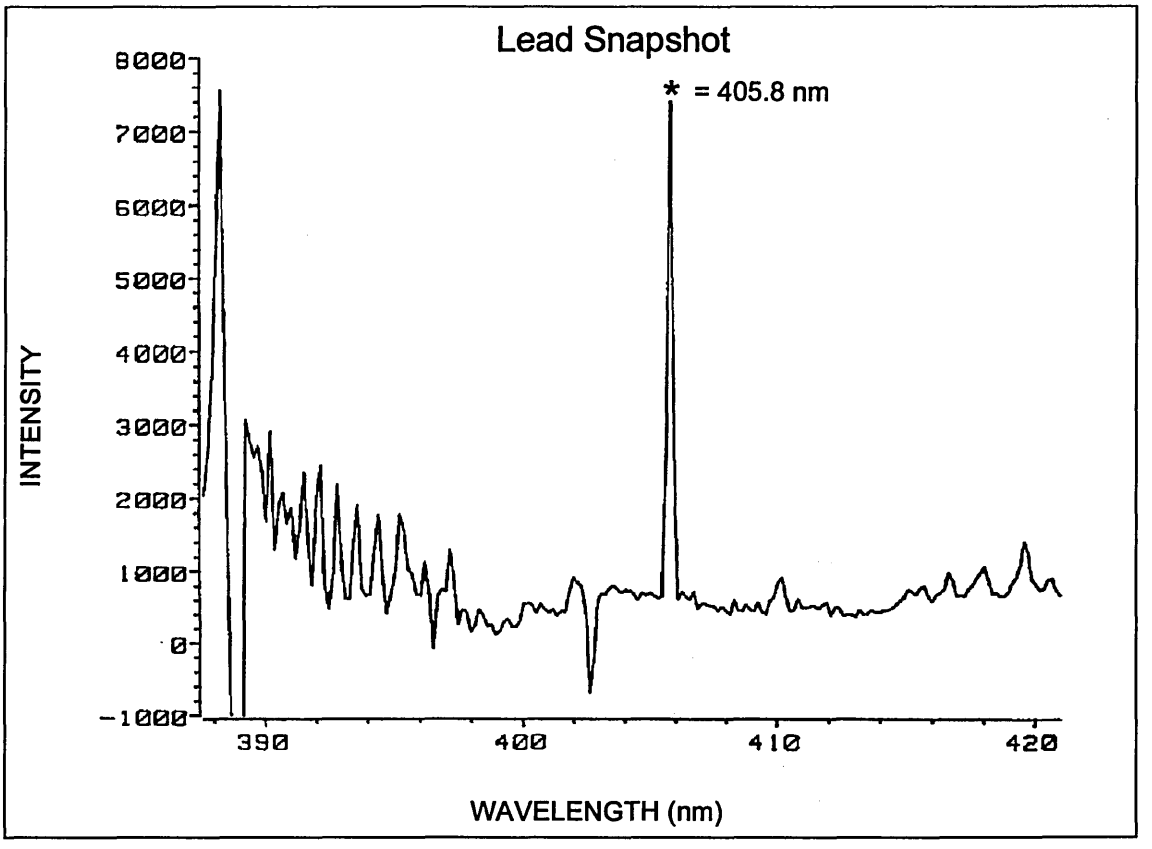
Atomic Emission Snaphot Profiles

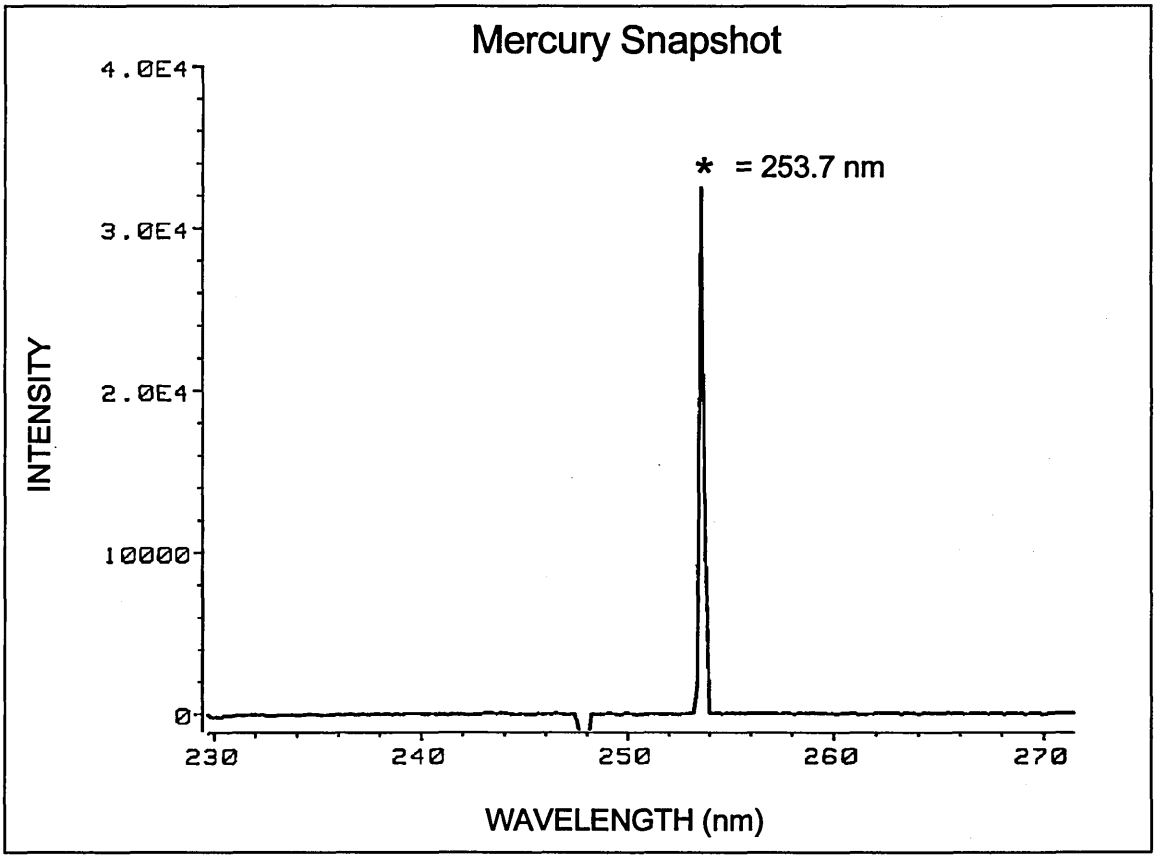

Bromine Snapshot
* =478.6 nm
* =481.7 nm
INTENSITY
1500
1000
500
0
-500
-1000
478
479
480
481
482
WAVELENGTH (nm)

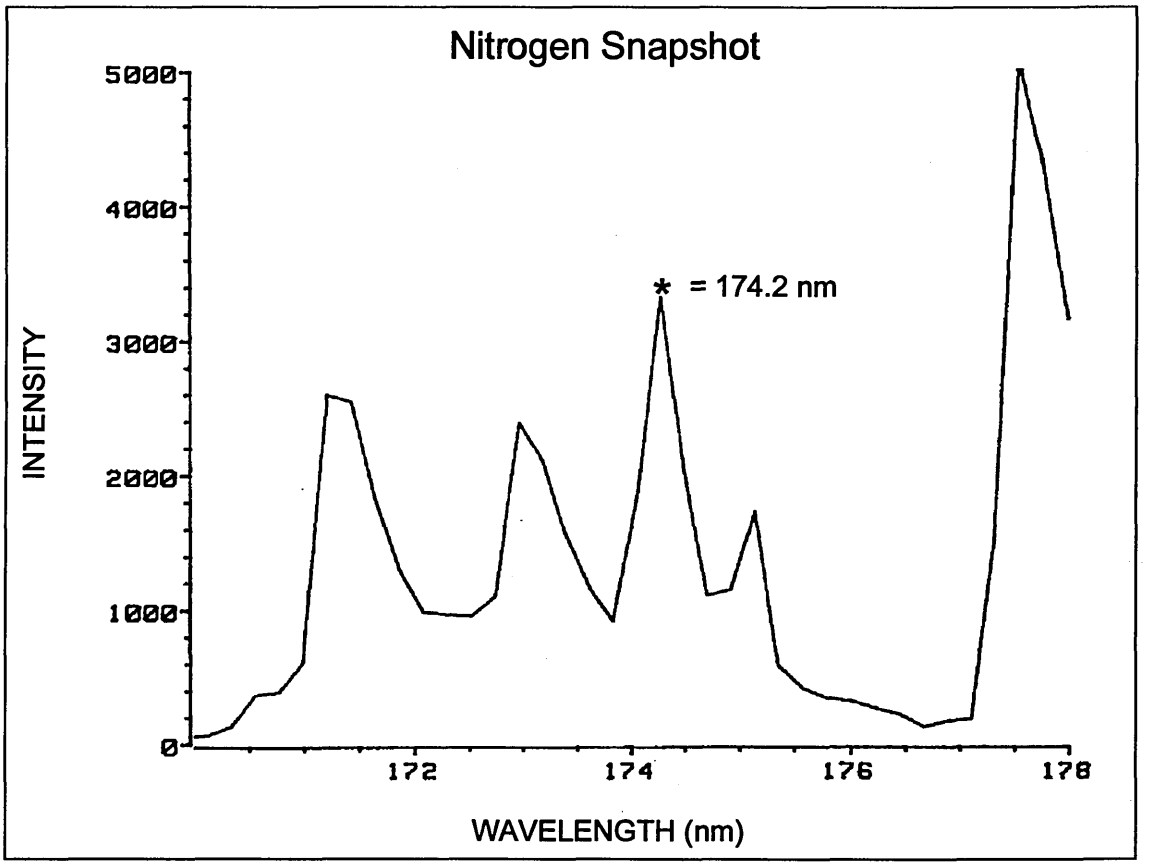

Chlorine Snapshot
* = 479.5 nm
* = 481.0 nm
* = 481.9 nm
INTENSITY
1.4E5
1.2E5
1.0E5
8.0E4
6.0E4
4.0E4
2.0E4
0
479
480
481
482
483
484
WAVELENGTH (nm)

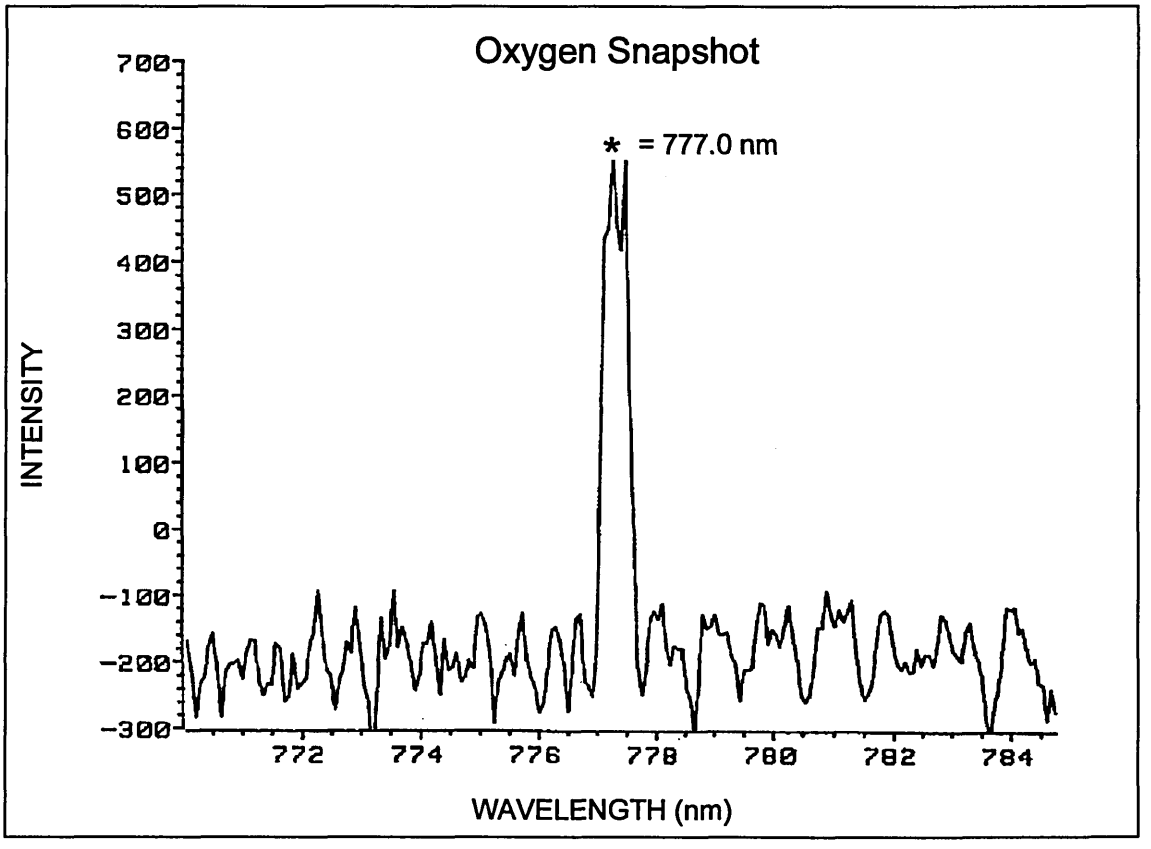

Fluorine Snapshot
* = 685.6 nm
* = 683.4 nm
* = 687.0 nm
* = 690.2 nm
* = 690.98 nm
INTENSITY
1.9E5
1.9e5
1.8e5
682
684
686
688
690
692
694
WAVELENGTH (nm)

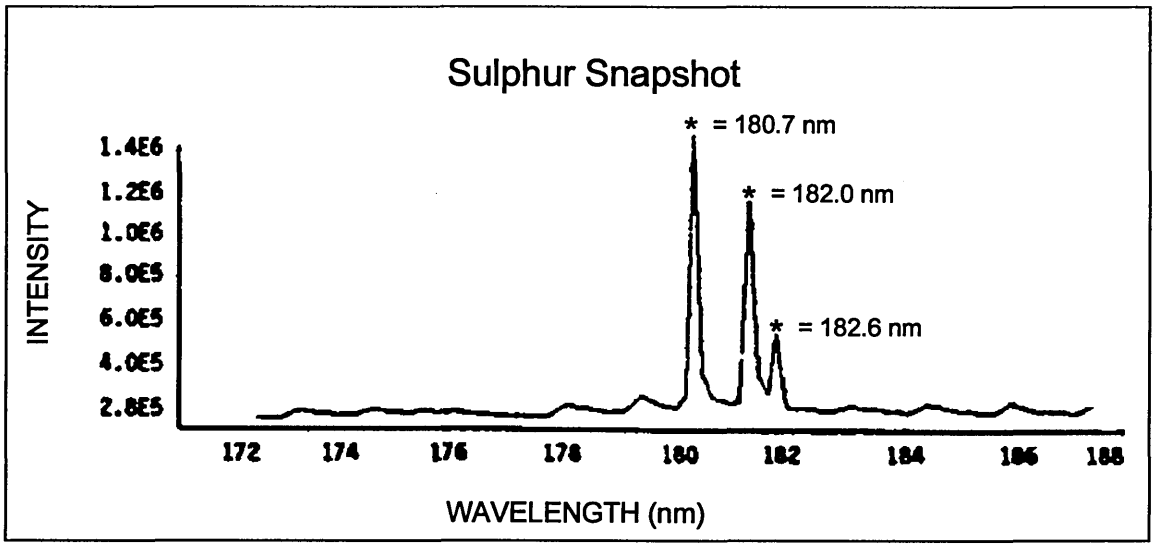

Lead Snapshot
* = 261.4 nm
INTENSITY
800
700
600
500
400
300
200
100
0
-100
240
250
260
270
WAVELENGTH (nm)

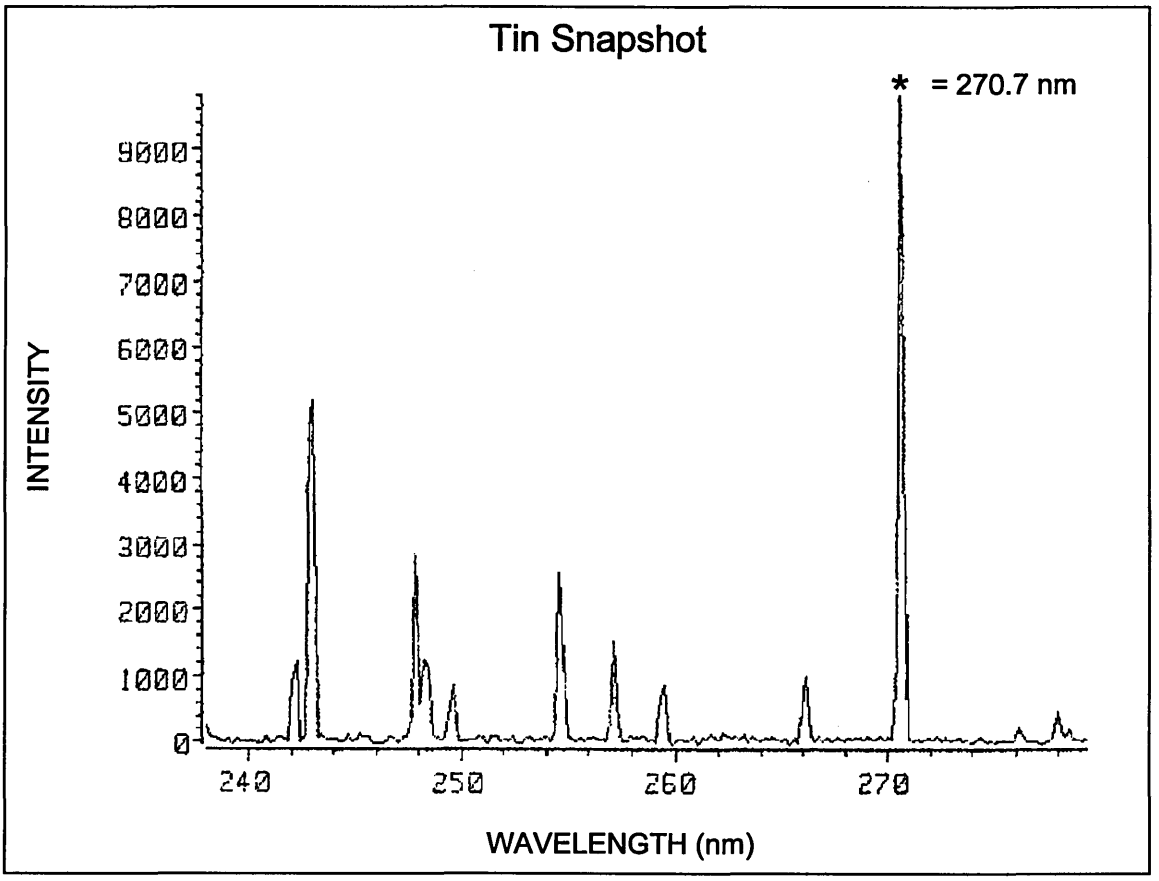

Lead Snapshot
* = 405.8 nm
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
INTENSITY
390
400
410
420
WAVELENGTH (nm)


Mercury Snapshot
* = 253.7 nm
4.0E4
3.0E4
2.0E4
10000
0
INTENSITY
230
240
250
260
270
WAVELENGTH (nm)


Nitrogen Snapshot
5000
4000
3000
2000
1000
0
INTENSITY
* = 174.2 nm
172
174
176
178
WAVELENGTH (nm)


Oxygen Snapshot
700
600
500
400
300
200
100
0
-100
-200
-300
INTENSITY
* = 777.0 nm
772
774
776
778
780
782
784
WAVELENGTH (nm)


Sulphur Snapshot
* = 180.7 nm
* = 182.0 nm
* = 182.6 nm
INTENSITY
1.4E6
1.2E6
1.0E6
8.0E5
6.0E5
4.0E5
2.8E5
172
174
176
178
180
182
184
186
188
WAVELENGTH (nm)


Tin Snapshot
* = 270.7 nm
INTENSITY
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
240
250
260
270
WAVELENGTH (nm)

Publications and Conference Participation

PUBLICATIONS

"Element Selective Detection using Capillary Chromatography with Atomic Emission Detection"

Cooke, M., Leathard D.A., Webster C and **Rogerson V**.

Journal of High Resolution Chromatography, 1993, **16**, 660

"Environmental Contamination from Lead, Copper and Cadmium. Determination using the Woodlouse as a Bioaccumulator and Potentiometric Stripping Analysis for Measurement"

Crowther D, **Rogerson V** and Cooke M

Analytical Communications 1996, **33**, 93

COURSES

June 1993 Restek's Capillary Chromatography Seminar

July 1998 Advanced Course in Analytical Validation and Regulatory Issues

CONFERENCE PARTICIPATION DETAILS

1. Fifteenth International Symposium on Capillary Chromatography, Palazzo dei Congressi, Riva del Garda Italy. 24-27th May 1993. - **Poster**

"Capillary GC-AES in Environmental Analysis"

M. Cooke, D.A. Leathard, C. Webster and **V. Rogerson.**

2. R&D Topics Meeting, Bradford, 13-14th July 1993. - **Poster 1**

" The Analysis of Cu, Cd and Pb in Woodlice by Stripping Potentiometry using the Radiometer Trace Lab System"

V. Rogerson, D. Crowther and M. Cooke

3. Euroanalysis VIII, Edinburgh, UK., 5-11th September 1993 - **Poster 2**

" The Analysis of Cu, Cd and Pb in Woodlice by Stripping Potentiometry using the Radiometer Trace Lab System"

V. Rogerson, D. Crowther and M. Cooke

4. 1994 RSC Annual Chemical Congress, University of Liverpool, 12-15th April 1994

5. R&D Topics Meeting, University of Hertfordshire. 18-19th July 1994 - **Poster 3**

"Multielement Chromatographic Profiling of Environmental Pollution."

V. Rogerson, D.A. Leathard and P.Gardiner

6. EnviroMan 1994, St. Albans, 28-30th June 1994. **Oral Presentation - M. Cooke**

"Detection of Organometallic Compounds in Natural Waters by Capillary GC-Atomic Emission Detection"

M. Cooke, D.A. Leathard and **V. Rogerson**

7. Sixteenth International Symposium on Capillary Chromatography, Palazzo dei Congressi, Riva del Garda Italy. 26-30th September 1994. - **Poster 4**

"Multielement Chromatographic Profiling of Environmental Pollution."

V. Rogerson, D.A. Leathard and P.Gardiner

8. RSC Autumn Meeting and Pre-Doctoral Chemistry Symposium, University of Glasgow, 6-9th September 1994 - **Oral Presentation 1**

"Multielement Chromatographic Profiling of Environmental Pollution."

V. Rogerson, D.A. Leathard and P. Gardiner

9. Young Scientists Meeting, Royal Society of Chemistry North West Region, UMIST, 15th November 1994 - **Oral Presentation 2**

"Multielement Chromatographic Profiling of Environmental Pollution."

V. Rogerson, D.A. Leathard and P. Gardiner

10. European Winter Conference on Plasma Spectrochemistry 1995, Cambridge, 9th January 1995 - **Poster 5**

"Multielement Chromatographic Profiling of Environmental Pollution."

V. Rogerson, D.A. Leathard and P. Gardiner

Element-Selective Detection using Capillary Gas Chromatography with Atomic Emission Detection

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Key Words:

Atomic emission detection
Capillary GC
Environmental analysis
Element selective detection

Summary

Capillary GC coupled to an atomic emission detector (AED) provides a powerful new hyphenated technique for the separation and characterization of complex mixtures and compounds. The AED provides simultaneous and truly specific multi-element detection. The specificity of detection reduces the need for the complex sample pretreatment procedures which are necessary to reduce the interference from co-eluted substances which is experienced with detectors such as the FID and the ECD. A range of environmentally significant problems has been studied, including PCB analysis, the characterization of the reaction products of a novel waste treatment process, and the profiling of sulfur-containing species formed by the pyrolysis of various types of coal.

1 Introduction

The microwave-induced plasma atomic emission detector is but one form of the general technique of GC–atomic emission spectroscopy which has been extensively reviewed [1].

McCormack et al. [2] first demonstrated the technique of GC with an atomic emission detector (AED) in 1965. Since then the technique has developed slowly, primarily for technical reasons, but a key development occurred in 1976 when *Beenakker* [3] invented a cavity able to sustain a microwave-induced helium plasma at atmospheric pressure. Subsequent developments centered on improving plasma stability, optical technology, automation, and software. The incorporation of a photodiode array (PDA) [4] provides extremely high selectivity while maintaining sensitivities comparable with those of other GC detectors [5].

The capillary gas chromatograph is interfaced directly to a discharge cavity. A magnetron supplies microwave radiation to the cavity, inducing a helium plasma which is contained within a quartz discharge tube. The excess heat produced by the plasma is dissipated *via* a water cooling system to prolong the life of the discharge tube. As the eluent enters the plasma, the separated components are atomized and the outer shell electrons excited, promoting them to a higher energy level. When electrons decay back down to ground level they emit radiation at wavelengths characteristic of the elements concerned. The dispersed radiation is monitored by a PDA covering part of the range between 165–780 nm, enabling the simultaneous detection of a number of elements depending upon the location of the PDA in the focal plane. The complex spectral information is transferred to a computer where it is stored for future data manipulation, element identification, and chromatographic integration.

The simultaneous monitoring of carbon, nitrogen, and sulfur is useful for drug screening and characterization, as is the ability to monitor oxygen, chlorine, bromine, fluorine, arsenic [6], and metals such as mercury [7,8] for environmental applications. GC-AED is, in principle, able to provide elemental ratios giving information on empirical formulas [9]. In this respect the AED provides information complementary to that furnished by the mass spectrometer and hence provides confirmatory identification data.

Three applications showing the use of the GC-AED have been studied—PCB analysis, the characterization of the reaction products of the wet air oxidation of 2-picoline, and the profiling of sulfur-containing species formed by the pyrolysis of coal.

2 Experimental

The GC-AED (Hewlett-Packard, Avondale, Pennsylvania, USA) consisted of an HP5890 capillary chromatograph interfaced to an HP5921A atomic emission detector and coupled to a ChemStation. Carrier (1 ml min^{-1}), make-up, and reagent gases were as recommended by the manufacturer. PCB standards were donated by the original manufacturers.

2.1 Sample Preparation and Analysis

2.1.1 PCBs

Sewage sludge was extracted with dichloromethane (20 mL) for 2 h in a Soxhlet apparatus and the extract reduced to *ca* 1 mL under a stream of dry nitrogen. 1 µL of the extract was injected splitless (60 s purge delay) on to a 25 m × 0.32 mm i.d. column coated with a 0.17 µm film of polydimethylsiloxane HP-1 (Hewlett-Packard). The column was programmed from 60 to 150 °C at 10 °min^{-1} and then to 300 °C at 5 °min^{-1}. Carbon, chlorine, and hydrogen were monitored at 496, 479, and 486 nm, respectively.

2.1.2 Oxidation of 2-Picoline

In a typical *Fenton* chemistry reaction [10] a mixture of dilute sulfuric acid, iron sulfate, and copper sulfate was refluxed under nitrogen and 2-picoline was added, followed by excess hydrogen peroxide. The reaction products were extracted with dichloromethane (4 × 15 mL) and reduced to *ca* 1 mL under a stream of dry nitrogen. 1 µL of the extract was injected splitless (60 s purge delay) on to a 10 m × 0.32 mm i.d. column coated with a 0.25 µm immobilized film of the polar polyethylene glycol Supelcowax (Supelco). The column was programmed at 10 °min^{-1} from 40 to 250 °C which was held for 10 min. Carbon, nitrogen, and oxygen were monitored at 193, 174, and 777 nm, respectively.

2.1.3 Coal Pyrolysis

Coal samples (0.5 mg) were pyrolyzed at 1123 K and transferred to the head of the polyethylene glycol column (Section 2.1.2) *via* a splitter (split ratio 100:1). The column was held at 40 °C for 10 min

after pyrolysis, programmed at 20 °min^{-1} to 120 °C, which was held for 6 min, then programmed at 20 °min^{-1} to 200 °C, which was held for 6 min, and finally programmed at 20 °min^{-1} to 280 °C, which was held for 6 min. Carbon, nitrogen, and sulfur were monitored at 193, 174, and 181 nm, respectively.

3 Results and Discussion

PCB analysis of environmental samples is complicated both by the presence of other chlorine-containing species in the extracts (*e.g.* organochlorine pesticide residues) and by the non-specificity of the traditional detector (ECD). The chlorine selectivity of the AED (479.0 nm) was used to analyze a standard PCB (Aroclor 1254, 10 µg mL^{-1} in hexane), giving the characteristic profile (not shown), and a sewage sludge extract spiked with Aroclor 1254 (10 µg mL^{-1}) (**Figure 1**). Analysis of the sludge extract revealed the absence of PCB, hence the spiked sample was analyzed to show the sensitivity of the instrument. Under these conditions the implied limit of detection for chlorine is equivalent to approximately 1 ng total PCB. The use of the snapshot facility (the emission spectrum for the peak of interest) confirmed the presence of chlorine (peaks at 479.0, 461.0, and 481.8 nm) in each component.

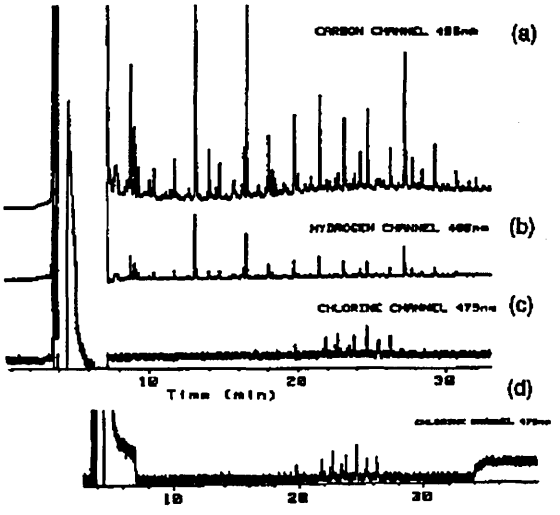

Figure 1
Analysis of sewage sludge extract spiked with Aroclor 1254; GC-AED responses from (a) carbon, (b) hydrogen, and (c) chlorine channels; chlorine trace (d) is that obtained from Aroclor 1254 standard.

Wet air oxidation is a technique which offers great promise for the disposal of waste chemicals. The process is intended to degrade molecules to simple biodegradable species such as ammonia, acetic acid, and methanol but little is known of the mechanism by which this occurs. Preliminary studies using 2-picoline (a typical nitrogen-containing heteroaromatic compound) as a model compound have been performed in an attempt to elucidate the pathway of the reaction process (**Figure 2**).

The chromatogram of the reaction mixture shows that a large number of less volatile, more complex compounds has been formed during the reaction. Three species containing both oxygen and nitrogen are present at t_R = 6.1, 12.1, and 13.2 min. In contrast there

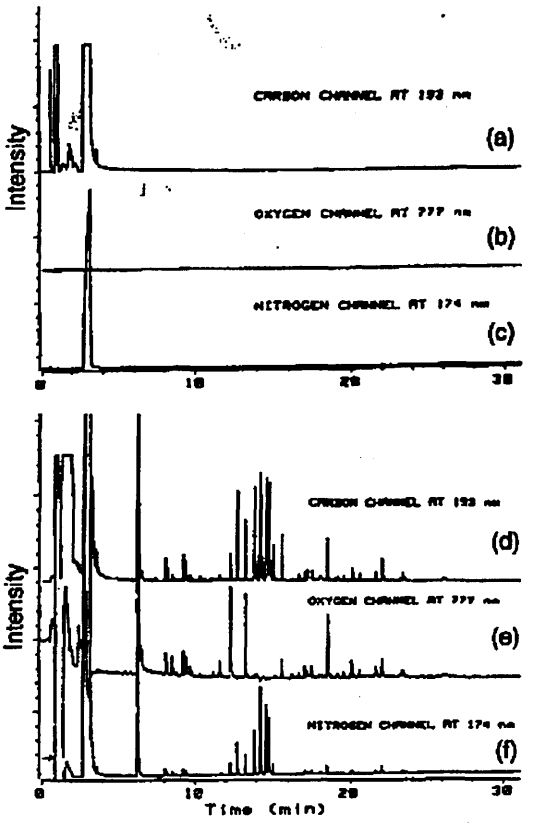

Figure 2
Characterization of the products from wet air oxidation of 2-picoline; (a), (b), and (c), are carbon, hydrogen, and chlorine channel signals from starting material; (d), (e), and (f), are carbon, hydrogen, and chlorine channel signals from analysis of reaction products.

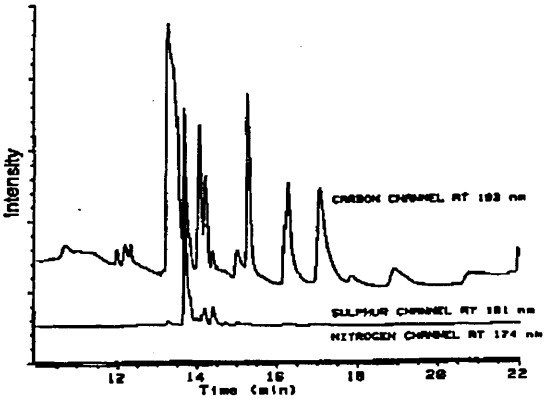

Figure 3
Characterization of the products resulting from the pyrolysis of coal at 1123 K: GC-AED responses from carbon, sulfur, and nitrogen channels.

is a group of five components which contain no oxygen eluting between 13.95 and 15.2 min. Most surprising is the appearance of several components, for example that at t_R = 15.5 min, which contain oxygen, carbon, and hydrogen but no nitrogen. It should be noted that separate injections are required for the oxygen-selective response and the carbon-and nitrogen-selective responses; the two selective chromatograms are then merged.

The combustion of sulfur-containing fossil fuels produces sulfur dioxide which is emitted to the atmosphere where it reacts to form acid rain. Characterizing fossil fuels by examining the organosulfur compounds produced on heating is a way of assessing their potential for producing sulfur dioxide when burnt. To simulate burning, a small amount of the coal is pyrolyzed and the carbon, sulfur, and nitrogen wavelengths monitored. As is apparent from **Figure 3** relatively few sulfur-containing compounds were produced; they were relatively non-polar and of low molecular weight. There was no evidence of the production of the larger polycyclic aromatic sulfur compounds of the type often found when crude oil is pyrolyzed. No nitrogen-containing compounds were observed.

4 Conclusion

GC-AED is a powerful technique for profiling heteroatom-containing compounds in complex matrices; it also requires minimal sample pretreatment. The information produced is complementary to that produced by GC–MS and hence the two techniques can be combined to achieve full characterization of complex mixtures.

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Environmental Contamination from Lead, Copper and Cadmium—Determination Using the Woodlouse as Bioaccumulator and Potentiometric Stripping Analysis for Measurement

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A simple and rapid method for investigating the extent of bioavailable heavy metal contamination is reported. The woodlouse, which grazes on plant debris, is used as a bioaccumulator of lead, copper and cadmium and a rapid multi-element determination is performed on woodlouse digest by stripping potentiometry. The results show large changes in the distribution of these elements between geographical sites, in line with usage of the areas.

Environmental contamination by heavy metals is a widespread problem, with sources of pollution arising from natural causes (*e.g.*, ore-rich areas) and, more importantly, from industrial activities both past and present. The principal cause for concern is not the total metal loading in a soil, because some soils may bind these elements tightly, but the concentrations of pollutants that are readily available to the biosphere and which may migrate up the food chain. It is therefore sensible, in studies of heavy metal pollution, to use biological material from the contaminated site to assess the amount of utilizable pollutant. Plant materials have been used for this purpose in the past, as have earthworms and snails. Recent reports[1–3] have indicated that the woodlouse is a remarkably good accumulator of heavy metals, both common species being able to store heavy metal ions safely in a specialized organ. Because the woodlouse grazes on fallen plant debris it should be an excellent natural sampling system for heavy metals in the environment.

Laboratory determination of low concentrations of metals is generally the province of atomic spectroscopy. For multi-element determination the most convenient technique by far is inductively coupled plasma optical emission spectroscopy (ICP-OES); unfortunately, the limits of detection of this technique are not good enough for the determination of low-ppb levels of cadmium, copper and (especially) lead. ICP-MS provides better detection limits, but the equipment is not yet widespread and is very expensive. Graphite furnace AAS gives good detection limits but is generally limited to single-element determination and still involves a substantial capital cost.

Electrochemical techniques offer alternative methods of determination. Anodic stripping voltammetry (ASV), which involves electrochemical preconcentration at a mercury electrode, followed by differential-pulse stripping, is capable of determining easily reducible metals at low-ppb levels, but is a relatively slow technique. Potentiometric stripping analysis[4] (PSA) is a more modern and more rapid technique, which uses an electrochemical concentration step in the same way as ASV, but on stripping the electrode potential is measured as a function of time at a constant stripping rate. Measurements are more rapid and, for many elements, determination is possible in the presence of atmospheric oxygen, unlike ASV, where anoxic conditions must be employed. Sub-ppb limits of detection are possible for reducible, amalgam-forming metals.

Sample preparation for either atomic spectroscopy or electrochemistry is similar in that the sample must be fully digested and acidified to prevent precipitation of hydroxides, but the PSA approach also requires that the sample contain no large amounts of redox-active reagents. If nitric or perchloric acids have been used in sample digestion then these must be boiled off or reduced prior to determination.

Experimental

Woodlice were collected from disparate sites, some where contamination was known or suspected, and some where low levels were expected. The samples were transported to the laboratory, separated from large debris, washed, sieved to remove small particle debris and plunged into liquid nitrogen. The carcases were then oven-dried at 105 °C to constant mass (approximately 3 h), weighed and ground to a powder. Aliquots (10 ml) of concentrated nitric acid (Aristar grade, Merck Ltd, Poole, Dorset) were added to 0.15 g samples of dried woodlouse powder, and the mixture gently boiled for 2 h with a loose cover to digest organic matter. Excess nitric acid was then displaced by the addition of 5 ml of concentrated hydrochloric acid and boiling continued for 1 h to displace NO_2 (removal of excess oxidant is crucial to successful determination by PSA), following which the cover was removed and the liquid boiled down to 2 ml. Water (20 ml) was then added and the pH adjusted to 2.0 with sodium hydroxide, following which the sample was filtered through a 0.2 μm pore cellulose nitrate disc and made up to 100 ml with water. All glassware was kept in 10% nitric acid to minimize contamination and carryover. All of the water used was purified by distillation, then passage through a Milli-Q system (Millipore Ltd., Watford, Hertfordshire, UK). Blanks were subjected to the same procedure as the samples and blank results were subtracted from the sample data. No anomalously high blank readings were noted.

A Tracelab PSA system (Radiometer Ltd., Crawley, Sussex) with a mercury-coated glassy carbon working electrode, calomel reference electrode and platinum counter electrode was controlled by Tracelab TAP-2 software (version 3.0) running on a 386-SX computer (Viglen Ltd., Alperton, Middlesex). The electrode was manually re-polished every day with the polishing powder supplied by the manufacturer and re-plated using the pre-programmed plating routine (8.0 min at −0.9 V in 80 mg l^{-1} Hg^{2+}) for every set of replicates. The accumulation time before stripping was varied to suit the concentration of element present, and stripping occurred through passive oxidation by 80 mg l^{-1} Hg^{2+} in solution. Calibrations were made by standard additions.

Comparative measurements on the same samples were obtained using a Thermo-Jarrell Ash ICAP-9000 ICP-OES spectrometer.

Results and Discussion

Contaminated and Uncontaminated Land

Samples of woodlice were obtained from a rural site with no known history of contamination (open moorland at Lockerbie, Scotland), a site adjacent to the closed Capper Pass smelter on Humberside and from the ruins of a smelter flue in a long disused lead mine at Charterhouse, Somerset.

Three replicate digests were carried out on each sample of dried woodlouse powder, then the PSA and ICP-OES determinations were performed on each digest. The results (Table 1) show the expected high levels of lead at Charterhouse and of copper at Capper Pass. Zinc levels at Capper Pass (results not shown) were approximately three times the levels at Lockerbie.

Results from the two techniques were generally in good agreement, with the exception of low level lead measurements. In these, the ICP-OES system was near its detection limit and the PSA system was likely to be the more accurate since lead easily amalgamates with mercury, resulting in a high sensitivity for lead by this technique. For simple solutions of lead, copper and cadmium salts (diluted commercial atomic spectroscopy standard solutions) in water, ten replicate measurements with the PSA equipment showed relative standard deviations at 100 ppb of 1.3, 2.5 and 2.0%, respectively. The precision observed with the digested samples was poorer, suggesting some matrix effects despite the digestion and subsequent oxidant displacement. This might account for the disagreement between PSA and ICP results for copper in the Capper Pass sample, although all three replicates gave higher results by PSA. Intermetallic complex formation, particularly with zinc, can also affect copper data by PSA, although the deposition potential of −0.6 V used for copper measurements should result in minimal zinc accumulation.

Table 1 Copper, lead and cadmium determinations from woodlice at one uncontaminated and two contaminated locations. Three replicate digests were made for each sample. Results are in $\mu g\ g^{-1}$ of each element in dried whole woodlice. Errors are ± one standard deviation

		Copper		Lead		Cadmium	
		PSA	ICP	PSA	ICP	PSA	ICP
Lockerbie	Mean	290	296	97	40	19	13
	±	17	18	5	5	3	1
Capper Pass	Mean	850	582	13	6	55	58
	±	180	30	2	1	9	3
Charterhouse	Mean	89	94	1277	1030	53	43
	±	37	8	127	57	1	4

Table 2 Copper, lead and cadmium determinations from woodlice in domestic gardens by PSA. Three replicate digests were made for each sample. Results are in $\mu g\ g^{-1}$ of each element in dried whole woodlice. Errors are ± one standard deviation

		Copper	Lead	Cadmium
Sheffield	Mean	297	58	7
	±	34	6	2
Somerset	Mean	242	9	3
	±	5	3	1
Wawne	mean	303	28	5
	±	44	3	2

Domestic Gardens

Woodlice were also obtained from a suburban garden outside Bath, Somerset, an urban garden in Sheffield and a garden in Wawne (rural, approximately 10 miles NE of the Capper Pass smelter in Humberside). Data from these samples (Table 2) show, as expected, low levels of cadmium in each. Lead was highest in the urban garden in Sheffield, as might be expected from the level of motor traffic, lower in the Wawne sample and in the Somerset sample was almost down to the levels seen in the Lockerbie samples. Levels of copper were similar in all three, being two to three times the levels from the Lockerbie site.

Conclusions

The results presented here demonstrate that PSA, a low-cost analytical technique, is capable of rapidly determining lead, copper and cadmium at ppb levels in environmental samples, with sample preparation times only slightly longer than for atomic spectroscopy. In agreement with Hopkin *et al.*,[1,2] the woodlouse seems a useful marker of bioavailable heavy metals in the environment, being widespread geographically and clearly capable of accumulating these elements to average whole-body levels in excess of 1 mg g^{-1} of dry matter. The combined approach described here should prove useful in studies of pollutant contour mapping.

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