The Determination of Trace Organic Micro-Pollutants by Particle Beam Liquid Chromatography Mass Spectrometry

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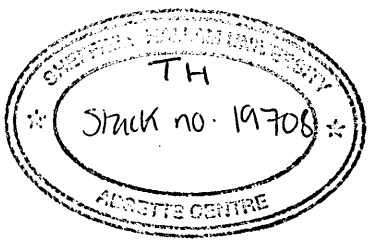
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Abstract

Liquid Chromatography/ Mass Spectrometry (LC/MS) is used to interface the separating power of LC with the sensitivity and specificity of MS for the determination of trace levels of organic compounds in a variety of matrices. The technique is finding increasing application in the field of environmental and pharmaceutical analysis. Particle Beam LC/MS (PB/LC/MS) uses a particle beam interface to connect the LC to the MS. This interface design has the advantage of being able to produce "classical" electron impact (EI) spectra which can then be searched against commercial MS libraries.

The aim of this work was to apply PB/LC/MS to a range of new problems in environmental analysis and evaluate the usefulness of this technique. PB/LC/MS was used to determine compounds that cannot easily be analysed by more conventional techniques such as gas chromatography with mass spectrometry (GC/MS) or liquid chromatography with UV/vis detection (LC/UV). For example, some polycyclic aromatic hydrocarbons (PAH) are too involatile to analyse by GC/MS, some commonly prepared isocyanate derivatives cannot be accurately identified by LC/UV and some classes of pesticides are thermally labile and so cannot be determined by GC/MS.

The work presented in this thesis examines the factors affecting the sensitivity and performance of PB/LC/MS and comparisons are made with other analytical methods. Compound classes examined are polycyclic aromatic hydrocarbons (PAH), pesticides and isocyanate derivatives in a variety of environmental matrices. Methods for improving the sensitivity of PB/LC/MS are investigated and the results of these experiments used to compare the different models are used to explain PB/MS behaviour. Conclusions regarding the accuracy of these models are then made. The ability of PB/MS to provide useful EI MS for identification purposes in complex environmental matrices is also investigated.

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1.1 Background

The need for environmental analysis has increased dramatically since the turn of century. Pollution, arising from industrial processes is now a major concern in most industrialized nations and is regulated by various organizations and laws [1-3]. As a result of these concerns the analytical chemist is required to determine a wide array of compounds (e.g. pesticides, PAH, isocyanates, etc.) produced by various industries (e.g. agriculture, petro-chemical, plastics, etc.). Environmental samples are often in complex matrices, such as water (drinking and surface), air, soils and sludges and food (vegetation and animal tissue). In addition, the analyst is required to develop lower detection limits, better sensitivity and selectivity and lower cost per sample methods. These constraints mean that the analyst must be constantly improving the techniques of analysis and looking for new methods of analysis.

The author is employed by HSL which is an agency of HSE. The HSE (Health and Safety Executive) has the responsibility under various acts of Parliament, for the protection of the workforce, the public and the environment from hazards arising due to workplace related activity. HSL (Health and Safety Laboratory) provides reactive and pro-active scientific support to other divisions within HSE. Part of this support function is the evaluation of new and improved techniques for the analysis of environmental samples.

The Organic Measurement section of HSL (OM) analyses for a variety of organic compounds in a variety of matrices. Classes of compounds commonly measured are volatile organic compounds (VOCs), pharmaceuticals, industrially used materials (e.g. isocyanates) and pesticides. Matrices commonly encountered are air samples

(usually adsorbed onto Tenax or glass fibre filters or similar), water, soil, vegetation and swabs (dermal monitoring). Samples may be trace (e.g. pesticide oversprays on vegetation), concentrates (e.g. confirmation of composition) or taken in response to an incident (e.g. analysis of fallout after a chemical plant fire). To deal with this diverse workload the section has a variety of analytical techniques available. These include gas chromatography (GC) with mass spectrometry (MS), nitrogen-phosphorus (NP), electron capture (ECD) and flame ionisation (FI) detectors and liquid chromatography (LC) with diode array ultra-violet (UV/DAD), fluorescence (F) and electrochemical detectors (EC). The section also has a capillary electrophoresis/diode array analytical system and for sample preparation gel permeation chromatography (GPC), solid phase extraction (SPE) and automated thermal desorption (ATD) systems are available.

1.2 Introduction to LC/MS

Of the techniques listed above the most commonly used is probably GC with MS detection. GC has good separating power but requires that the compounds to be analysed are volatile or can be made so by derivatization. MS is the preferred detector because, as part of the ionisation process characteristic fragmentation patterns of ions ("fingerprints") are produced. These fragmentation patterns are the added dimension that MS offers over other detectors. For example to achieve a similar level of identification by GC with electron capture detection analysis on two columns of different polarity would be required (dual column system) [4]. This is why MS is the detector of first choice in forensic analysis and for the analysis of complex mixtures in complex matrices. MS and GC are discussed at more length in Rose [5],

Willard et al.[6] and Lawson and Todd[7]. As mentioned previously GC is only suitable for compounds that are volatile or can be made so. GC also cannot be used for thermally labile compounds e.g. certain carbamate pesticides decompose on heating. It has been estimated that 90% of organic analytes are not amenable to or are difficult to analyse by GC[8]. Betowski et al.[9] describe the use of thermospray and particle beam liquid chromatography mass spectrometry (PB/LC/MS) for characterising unknown highly polar or ionic organics in contaminated ground water. These authors note that in this type of environmental sample over 95% of the total organic content can be due to this type of compound, which cannot easily be determined by GC/MS.

LC does not suffer from volatility limitations and so the coupling of LC and MS offers exciting possibilities. Polar, ionic, involatile, thermally labile and high molecular weight analytes are readily chromatographed by LC. LC is often used to separate analytes amenable to GC, simply because sample extraction and clean up can be more straightforward for LC or because the LC method is quicker, less complicated or more convenient. LC is also useful where degradation or metabolism of the original pollutant has occurred producing several products.

A definition of chromatography, applicable to GC and LC is a separation method which depends for its efficiency on the equilibrium distribution of the solute molecules between two phases. In LC the stationary phase is the column packing and the mobile phase is a suitable liquid. The extent of interaction between the solute molecules and the molecules of each phase is determined by the physical and chemical properties of the solute in a given environment. Therefore, by correct

choice of mobile phase composition and stationary phase a complex mixture of components may be separated out into its constituent parts. The most commonly used form of LC is called reverse phase LC (RPLC) and uses a non-polar stationary phase (commonly C18 or C8) and a polar mobile phase e.g. acetonitrile/water. As mentioned above the wide variety of combinations of mobile phase compositions and stationary phase chemistries available mean that LC can be used for a far wider range of compounds than GC. The technical aspects affecting the use of LC analysis are discussed elsewhere [6,10-13] and I shall now concentrate specifically on LC/MS.

The requirements for an ideal LC/MS system have been considered by Mellon [14] and are summarised in table 1.1 :-

Table 1.1 *Requirements of an Ideal LC/MS system*

Mass Spectrometer Operation	Liquid Chromatograph Operation	Interface Performance
Maintenance of a good vacuum.	No solvent or buffer restrictions.	High sample to solvent enrichment.
Wide range of ionisation techniques.	Gradient elution should be possible.	High transfer efficiency of analyte.
Retention of full MS sensitivity.	Flow rates from <1ul/min to 2ml/min should be available.	Vaporisation of involatile samples needed.
Quantitative working desirable.	Others detectors should be simultaneously available.	No degradation of analyte peak shape should occur.

The obstacles to achieving an ideal LC/MS system have also be considered by Mellon [14] and can be summarised thus :-

Table 1.2 *Obstacles to an Ideal LC/MS system*

Vapour flow is much greater than in GC/MS e.g. 1ml/min of water becomes aprox. 1250 ml/min of vapour. A typical MS pumping system will only maintain high vacuum if the gas flow into the ion source remains below 10ml/min. Conducting liquids in the magnetic sector can lead to electrical arcing due to high potentials.
The analyte is typically about 50-200 times less concentrated in the solvent compared to the concentration of analyte in a GC carrier gas.
Samples are frequently involatile or thermally labile.
Many routine LC separations specify the use of involatile organic buffers.

The most difficult of these obstacles are the maintenance of the mass spectrometer vacuum in the presence of a liquid flow and problems caused by sample involatility. The interface between the LC and MS can either minimise or increase these obstacles and so is of major importance.

A brief history of LC/MS interfaces is given by Mellon [14] along with a consideration of the strengths and weaknesses of each interface. These are summarised below :-

Table 1.3 *Approximate Assessment of Advantages and Disadvantages of Various LC/MS Interfaces*

(Increasing number of stars indicate better performance, brackets indicate a range of responses)

LC/MS type	Limit of Detection	LC flow range	Solvent Types	Semi-Volatile Samples	Involatile\ Thermally labile samples	Molecular Mass Range
DLI	**	*	***	****	**	**
Belt	**	*****	*****	*****	*	*(*)
TSP	**(**)	*****	****	*****	***(*)	***(*)
LC/FAB	**(**)	*	***	****	*****	****
PB	**	***(*)	****	*****	*(*)	*(*)
ESI	****(*)	*	**(*)	****	*****	*****

Abbreviations

DLI - Direct Liquid Introduction

Belt - Moving Belt Interface

TSP - Thermospray

LC/FAB - Liquid Chromatography-Fast Atom Bombardment

PB - Particle Beam

ESI - ElectroSpray Ionisation

The relative merits of each interface have been considered by Mellon [13] , Niessen and Tinke [15] , Voyksner and Keever [16], Garcia and Barcelo [17] and Slobodnik et al. [18]. Of the interfaces mentioned above the DLI and moving belt have largely been superceded by other types of interface [17,18] . The use of LC/FAB for environmental analysis is relatively less common than the interfaces discussed below [14,17].

Thermospray (TSP) has been widely used for a variety of environmental and forensic samples , such as pesticides [19], explosives [20], drugs of abuse [21] and bio-medical applications [22]. Although quite a successful interface design TSP is being replaced by the newer atmospheric pressure ionisation (API) techniques such as ESI and ionspray (IS) [15,16,23].

API is the newest and most rapidly developing interface design. Bruins [24] considered the use of atmospheric pressure ionisation (API /MS) for various compounds and stated "in recent years the API source has been transformed from an exotic research instrument into a standard tool for problems in (bio)chemistry, pharmacy and bio-technology". Wachs et al [25], Hutton [26], Lawson et al. [27], Bajic et al. [28] Doerge et al.[29,30], Thomson [31] and Hirabayashi et al. [32] have also considered API/LC/MS for use in environmental monitoring. These authors looked at the determination of alkyl sulfates, pesticides, steroids, plasticizers, growth enhancers, antibiotics and other environmental contaminants in a variety of complex matrices, such as, food, milk and animal tissue. The instrumental and theoretical aspects of API have been discussed and compared with other interfaces by a variety of authors [14,15,16,25,31,32] and are therefore not covered here.

The remaining type of interface mentioned above is the particle beam (PB). This was the type of interface used in the majority of the work presented below. The applications and design of this type of interface are discussed in the next section.

1.3 Particle Beam LC/MS

The major difference between of PB/LC/MS and the other interface types mentioned above (except MB) is that it yields classical, library searchable, electron ionization (EI) spectra. The other types of ionization (APCI, ESI and TSP) are "soft" ionization techniques and typically give $[M+H]^+$ or solvent adducts with little fragmentation. The EI spectrum is usually obtained at 70eV and these standard conditions mean that comparisons can be made easily between user obtained and commercial MS libraries. This is important for forensic or identification purposes. Behymer et al. [33] considered this EI capability to be a distinct advantage of PB/LC/MS over thermospray LC/MS for the identification of unknowns in environmental analysis. Jones et al. [8] have used TSP and PB interfaces in an interlaboratory study on pesticides (carbamates and phenyl ureas) and found the two techniques to be complementary. The PB interface also allows use of conventional chemical ionization [18] (CI) but this was not used in this study and so will not be discussed further.

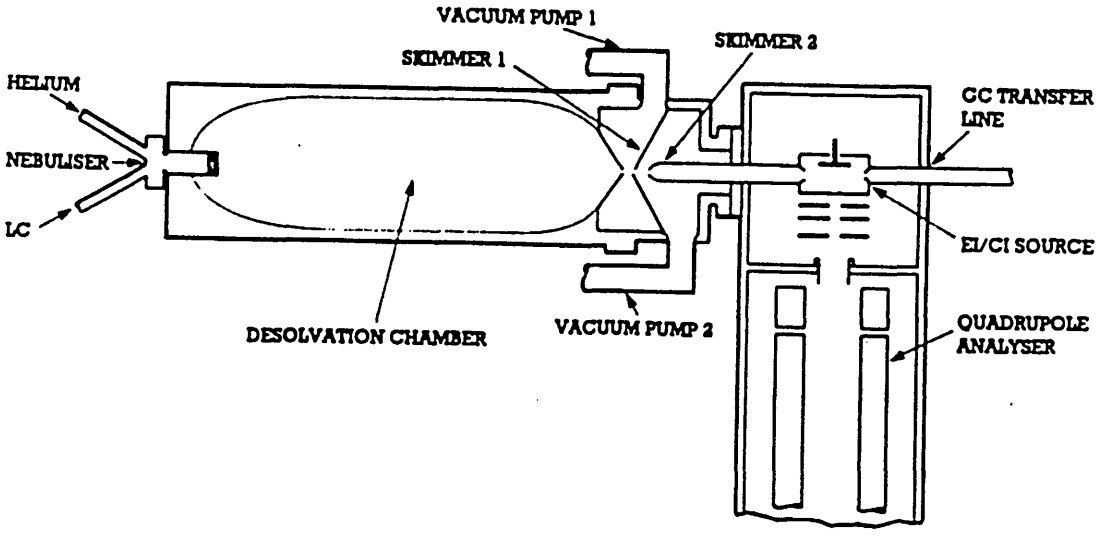
The PB interface has been used for a variety of analytes in various matrices. A library search (April 1998, databases searched: Analytical Abstracts CD issued March 1998, NIOSHTIC, MHIDAS and HSELINE, search terms "particle beam"+"LC") gave 99 references. Analytes featured were organic pollutants, "unknowns", PAHs, vitamins, quinolines and related compounds, isocyanates,

protein extracts, spices and other plant extracts, dyestuffs, drugs and antibiotics and pesticides. Matrices featured were water (drinking, raw, sewer), concentrates and industrial mixes, soils and sludges, coal tar and diesel oil, airborne particulates, fruit,vegetables and other foodstuffs and biological fluids and tissues. Some references (for the non-pesticide and PAH applications) are given at the end of this study. [34-42]

The PB interface used in this study is derived from the MAGIC (**M**onodisperse **A**erosol **G**enerator for **I**nterfacing **C**hromatography) LC/MS interface originally designed by Willoughby and Browner [43,44]. A typical design is shown schematically overleaf (fig. 1.1).

The principles of operation of PB/LC/MS are quite simple and have been examined by Willoughby and Browner [43,44], Creaser and Stygall [45], Mellon [14], Niessen and Tinke [15], Voyksner and Keever [16], Garcia and Barcelo [17] and Slobodnik et al. [18]. These authors also compared PB with the other interfaces mentioned above. In general, the benefits of PB can be summarised as the ability to produce EI spectra, ease of use and compatibility with normal LC conditions. The particle beam interface (PB) can cope with LC flows in the range of 0.1 to 1.0 ml/min. and was originally designed for use with quadrupole MS.

Figure 1.1 *Diagram of Particle Beam-Mass Spectrometry Interface*



The interface can be divided into two sections, the aerosol generator and the momentum separator.In the aerosol generation step the eluent stream from the LC is converted into an aerosol via a nebulizer. This can be achieved by a gas flow, concentric or at right angles to the eluent stream. Heat has also been used with and without a gas flow, as has an ultrasonic nebulizer. Some designs also use grids in combination with the gas flow to aid aerosol formation. The various different nebulizer designs have been compared by Creaser and Stygall [45]. The MAGIC interface originally used by Willoughby and Browner [43] was found to produce a fairly monodisperse aerosol (15μm diameter,± 20%) but the more commonly used concurrent or crossflow pneumatic interfaces give polydisperse aerosols [15,45,46] . The gas flow, usually helium (He), in addition to assisting with vaporization of the LC eluent also prevents coagulation of the droplets in the desolvation chamber which

would decrease analyte transfer [43]. The solvent droplets vaporize producing a mixture of vapour, solid particles and helium. The desolvation chamber is heated to assist this process and to compensate for the heat lost in vaporizing the LC eluent. The analyte rich droplets are desolvated leaving low volatility residual particles. The desolvated aerosol then enters the momentum separator.

The mixture of vapour, desolvated analyte particles and helium enters the first low pressure rotary pumped region through a narrow nozzle. This process accelerates the gas/particle mixture forming a supersonic jet containing a centrally directed beam of particles. The lighter solvent vapour and helium undergo rapid radial expansion and are skimmed and pumped away by a system of two nozzles at the end of the desolvation chamber. The heavier analyte particles have a high momentum and form a linear "beam" which travels to the ion source of the MS where they are ionized in the normal manner. An enrichment factor of 10^4-10^5 for the PB interface relative to the solvent has been quoted [18].

Various studies have been carried out aimed at enhancing PB/LC/MS sensitivity. Factors that have been examined are nebulizer design [45,18], nebulizer temperature [33,34,87] desolvation chamber temperature [34,45,48,49], helium pressure [34,49] eluent flow rate [16] and skimmer design [45,49,50] . All the above factors were found to have significant effects on PB/LC/MS sensitivity. The magnitude of effect varies for each factor, combination of factors and instrument type and the optimum settings are different for each application. In an early study on PB, Blakley et al.[51] used laser vaporization to minimise contact between the analyte and instrument surfaces but no further work seems to have been carried out on this approach. Several authors

have reported on the use of PB linked to other types of MS (i.e. not quadrupoles) such as ion-trap and magnetic sector instruments [18,52-,55]. Creaser and Stygall [53] reported that a quadrupole instrument was more susceptible to interference from the mobile phase than ion-trap or sector instruments. However, in contrast, Bier et al.[54] and Kleintop et al.[55] reported problems in linking a PB interface to an ion-trap detector due to residual solvent ions. Such solvent ions caused loss of sensitivity due to space charging and "self CI" effects. Each of these groups added a third skimmer stage to the momentum separator to reduce these problems.

Several issues have been identified with have restricted the widespread use of PB-MS. These can be summarised as mobile phase limitations, low sensitivity, poor reproducibility and response linearity . Apffel and Perry [56] investigated the limitations of PB/LC/MS and identified linearity at low levels and limited sensitivity as the major issues.

Mobile phase problems have been mentioned by numerous groups [33,34,43.47,54,56] . The most common difficulties are varying response for varying mobile phase composition and low tolerance for involatile buffers (such as phosphate). The latter problem can, to some extent, be avoided by the use of buffers such as acetate. The first problem has implications for the use of gradient elution LC. These authors found that as water content in the LC mobile phase increased sensitivity decreased. This is presumably due to decreased transfer through the interface because of lower efficiency of desolvated particle formation and a "cooling" effect of the water vapour in the MS source. Also, the size of aerosol produced by the nebulizer was found to be dependant on mobile phase composition and nebulizer design. Wilkes et al.[47]

found that a 100% aqueous aerosol gave rise to larger particles (5μm) than an acetonitrile based aerosol (~2μm). These larger particles are more prone to coalescence, collisions with the desolvation chamber walls and "gravitational loss" than the smaller particles and so are less efficiently transferred through the interface. Ligon and Dorn [48] measured the source pressure for a variety of aerosol types. They found that the source pressure increased for increasing water content, suggesting that appreciable amounts of water vapour was entering the source. Cappiello and co-workers [57-59] have reported on the design and evaluation of a modified PB interface for use with capillary LC. This interface gave better performance and was more sensitive than a conventional PB interface for high water content mobile phases and gave a constant response during acetonitrile/water gradient elution [57] using caffeine as test analyte. Cappiello also states that the small particle size aerosol produced was better ionized in the MS source. The low flow rates used with capillary LC (~1μl/min.) also allowed the use of involatile buffer systems, such as phosphate, without causing skimmer blocking.

Low sensitivity relative to other techniques, has been mentioned previously [15,16,18,19,23,56]. Bier et al. [54] have commented on analyte precipitating out on the skimmer assembly during analysis. Low molecular weight compounds are often pumped to waste with the solvent vapour. The lowest useable mass varies for each instrument type but is typically in the region of 150-250 amu. The problem is due to the low analyte transfer efficiency of the interface. Slobodnik et al. [18] give a range of 0.5-1% for typical transfer efficiency. However, Ligon and Dorn [48] using a modified PB interface (hybrid ultrasonic/pneumatic nebulizer, in-chamber heater for desolvation chamber and three stage momentum separator) measured a transfer

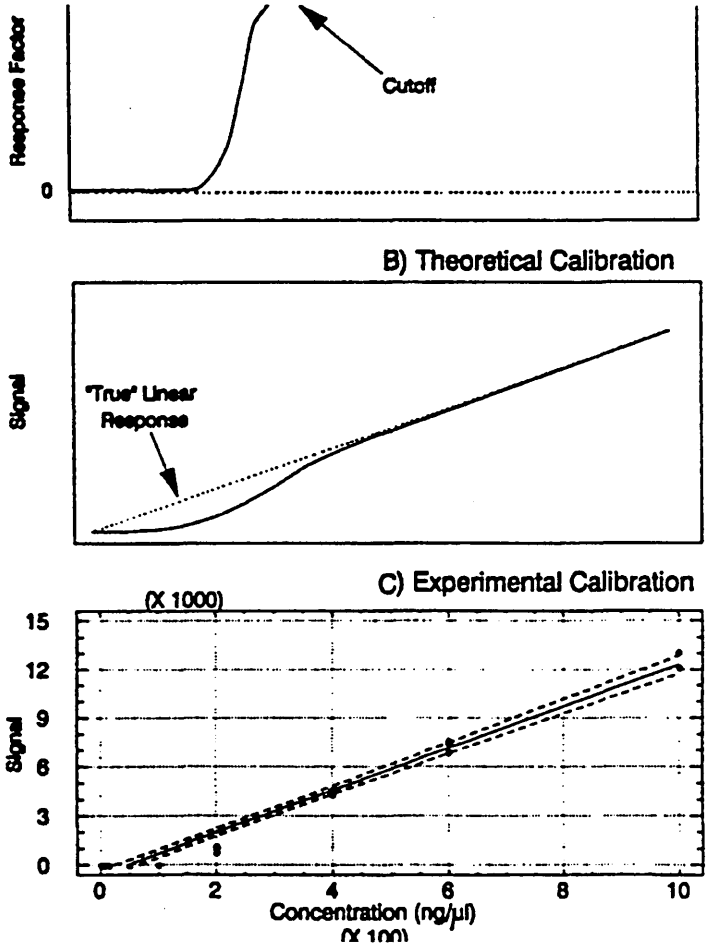
efficiency of 12% for cholesterol (relative to probe MS measurement). Generally, more modern interface designs have improved the sensitivity of PB [50,56]. Related to this improvement in instrument design has been an improvement in instrument reproducibility [8,43,49,50,54]. Early versions of the PB interface were prone to large day-to-day variations but this problem has been reduced in more modern designs. Jones et al.[8] and Ho et al.[49] used PB in multi-laboratory studies and found the technique to give good quantitative results with low RSDs for sustituted benzidine compounds. Jones et al. [8] note the performance of the PB was similar to that of GC/MS. Voyksner and Keever [16] describe the modern PB interface as "rather rugged and user friendly".

Limited linearity at low analyte levels was first mentioned by Bellar et al [60] and has been investigated by Apffel and Perry [56], Ho et al. [429] and Doerge et al. [61] Non-linear calibration curves have also been reported by many other authors [16,17,18,19,45,59]. Bellar et al. [60] noted enhanced positive ion abundances for polar compounds (substituted benzidines, phenyl urea herbicides, carbaryl and caffeine) when ammonium acetate was added to the mobile phase and in the presence of co-eluting "carrier" compounds (benzidines and deuterated analogs). However, these additive and "carrier" effects, although frequently reported, have been found to be very variable in magnitude and sometimes totally absent [62]. Ho et al.[49] extended the work of Bellar et al. [69] using the same set of compounds. Apffel and Perry [56] examined the effect of sample type and mobile phase additives on linear behaviour (24 probe analytes and 10 additives).They also examined the "carrier effect" by injecting co-eluting compounds (p-phenylene diamine with cortisol as carrier). Brown and Draper [63] also noted carrier and matrix effects.

Several authors have pointed out that these carrier effects pose problems in the use of PB for quantitative analysis [49,56,60]. In an attempt to account for these carrier and matrix effects these authors have suggested the use of isotopically labelled co-eluting compounds as internal standards for quantification . Brown and Draper [63] and Doerge et al. [61] describe such isotope dilution experiments and report calibration curves showing excellent linearity. Unfortunately, this approach is not be possible for "unknown" compounds, if no suitable isotopically labelled compound is available and for samples derived from dissimilar matrices. Improved sensitivity was also obtained by pre-saturating the nebulizer gas with organic solvent (via a bubbler) at 4 ^{o}C [64].

Apffel and Perry [56] proposed a model to explain these effects based on the hypothesis that the PB interface has a particle size cut off. Below this size cut off small particles are pumped away in the momentum separator (skimmers) and above the size cut off larger particles are are transfered quantitively into the MS source. These authors acknowledge the use of this "abrupt high pass filter" model is an over simplification and that various assumptions are made in its use. However a comparison of real and predicted results shows quite good agreement, see figure 1.2 :-

Figure 1.2 *Theoretical Model for Non-Linear Behaviour in LC/PB/MS* [56]



The "abrupt high pass filter" model proposes that the addition of a mobile phase modifier or carrier has the effect of increasing the overall concentration of material in each droplet and consequently increases the resulting desolvated particle size. This is due to the formation of "ion-molecular aggregates" between the analyte and additive/carrier [56,60]. These heavier particles should be better transported through the PB/LC/MS interface. This improvement depends on the ability of the additive/carrier and the analyte to interact in such a way that neither is evaporated or pumped away by the system. Apffel and Perry, commenting on their study [56], state "there is

currently no magic bullet additive which leads to linear behaviour under all conditions". Additives commonly used are ammonium acetate and ammonium oxalate and it was assumed that these polar compounds chemically interact via ionic or charge transfer mechanisms. For this reason non-polar compounds such as PAH may be expected to be only minimally enhanced by these additives. The presence of a co-eluting compound may also increase desolvated droplet size, leading to more effective transport and an increased signal. Apffel and Perry [56] and Bellar et al. [60] found that the magnitude of carrier effect depended on the chemical nature of the analyte and carrier and so suggested that a chemical interaction rather than a simple physical process. Miles et al. [65] as part of a major U.S. survey which looked at ground water contamination by pesticides investigated the use of PB/MS and considered the "carrier effect". These workers found the carrier effect observed in their experiments was compound specific and concluded that PB transmission was "an inherent physical property of the major component". It has therefore been proposed that the carrier effect and additive effect are due to the same phenomena which was consistent with the "abrupt high-pass filter" model caused by a particle size effect on analyte transmission.

Ho et al.[49] gave support to this model in studies on benzidines and deuterated benzidines (benzidine/D^8 benzidine, 3,3' dichlorobenzidine and deuterated analog and caffeine and deuterated caffeine). The calibration curve for D^8 benzidine was obtained in the presence of an initially large but steadily declining excess of co-eluting native benzidine. The response for D^8 benzidine increased up to ~15ng injected and then began to decline. The point of descent of the signal corresponded to a total mass of deuterated and native benzidine of ~70ng. These results

suggested that as the masses of the particles were reduced by reducing the amount of co-eluting native benzidine the transmission of the D^8 benzidine was decreased. From these experiments the "abrupt high-pass filter" cut-off can be estimated as ~70ng for this instrument and conditions. Additional experiments by these workers gave linear calibrations for D^8 benzidine and native benzidine when the total mass of deuterated and native benzidine was kept at 250ng and the amount of native benzidene was varied from 0 to 250ng. Support for the "abrupt high-pass filter" explanation of additive effects has also been provided by work by Doerge et al. [61], Bellar et al. [60].

Several groups have published results which call the increased particle size explanation of the "abrupt high-pass filter" model, additive and carrier effects into question.

Doerge et al. [61] found that ammonium acetate and co-eluting (3-$^{13}C_1$) caffeine gave an increased response for (^{12}C) caffeine, whereas GC/MS of similar isotope mixes and PB/LC/MS of co-eluting (1,3,7 $^{13}C_3$) caffeine gave no increase. These workers suggested that spectral overlap was necessary for an observed "carrier effect" and concluded that the non-linearity observed at low levels was due to the MS detector not the PB interface. However, carrier effects have been observed noted for a variety of interface types [16,17,18,19,45,59], MS types [45,52,54,55], for co-eluting compounds with no spectral overlap [48] and by numerous groups in different situations [8,16-19,45,49,50,46,59,60] suggesting that a real "carrier effect" phenomenon exists. Wilkes et al.[47] characterized the particle size distribution and transmission efficiency of a PB interface. Addition of ammonium acetate to the buffer improved transmission

efficiency around threefold but did not increase particle size (polydisperse aerosol, centred at ~0.1μm diameter). This effect was observed without the PB interface momentum separator attached indicating that additive enhancement by ammonium acetate is not solely momentum based. These authors noted an approx. 15 fold increase in the number of smaller particles (< 0.04μm diameter) being transmitted with ammonium acetate in the mobile phase and suggested that improved transmission of these small droplets was the reason for the observed signal increase not increasing droplet size. Wilkes et al. state "many of the phenomena which determine analyte transmission efficiency in the LC/PB/MS system seem to arise in the aerosol transport sub-system, upstream of the momentum separator". This theory is supported by the work of Cappiello and co-workers [57-59] who have reported on the use of a modified PB interface with capillary LC. These workers found increased sensitivity for this interface relative to a conventional PB interface despite the very small aerosol sizes produced (average diameter < 0.05μm).

Aerosol formation and behaviour have also been considered by Fuchs and Sutugin, Zebel, Whitby and Lui [66] and Hinds [67]. Romay et al. [68] listed the mechanisms leading to particle loss as inertial impaction (wall collisions, esp. for large, fast moving particles), gravitational settling (esp. for large, slow moving particles) diffusion and turbulent deposition (for small particles) and electrostatic. These authors showed that electrostatic effects could reduce sampling efficiency for isokinetic sampling. Browner et al. [69] developed a model for the transport of aerosols in inductively coupled plasma (ICP) nebulizer spray chambers. These authors also considered aerosol generation and loss mechanisms. Loss mechanisms were identified as gravitional, impaction, turbulence induced and centrifugal losses. Browner et al.

concluded "the processes which act to modify aerosols under analytical conditions are complex and only partially explicable with the present state of knowledge". This remains the situation today.

Wilkes et al.[47] suggested that static charging during aerosol vaporization may be adversely affecting analyte transmission. They stated that "extreme charging of residuals might exaggerate transverse dispersion or deflect the particles as they approach the mass spectrometer ion source". The smaller particles would presumably be more strongly affected, leading to collisions with the desolvation chamber walls and lower transmission through the interface. The additive effect described above would presumably be due ammonium acetate neutralizing the static charge. Electrostatic contributions to analyte loss have also been mentioned by Creaser and Stygall[45] and Kambhampati et al. [70] . Studies on charging of aerosols via a corona discharge and the effect of this on transmission through a sampling tube have been carried out by Romay et al. [68] Bellar et al. [71] reported increased response for PB/MS when using a glow discharge in the absence of ammonium acetate. No additional effect was seen when ammonium acetate was added. These authors concluded that improved transfer efficiencies due to "neutralization of charged particles and/or the formation of large particles are viable hypotheses". Wilkes et al. [72,73] looked at the effect of a corona discharge device inserted upstream of the nebulizer in a PB interface. This device was used to neutralise static aerosol charging. Similar results to those obtained by Bellar et al. [71] were observed, i.e. signal enhancement with ammonium acetate or corona discharge but no extra enhancement with both. The use of a corona discharge device has also been mentioned by Carroll et al. [74]

If an electrostatic effect is occurring for PB analysis then some of the factors operating will be similar to those observed in electrospray MS. These factors have been discussed elsewhere [6,14-17,22,24,25]. Ikonomou et al. [75] discussed the mechanisms of droplet formation in ESI. They considered the possibilty of electrical double layer formation in a droplet leading to electrophoretic separation of ions and electrochemical reactions at the ESI nebulizer metal capillary wall. They conclude that low potentials (~2V) would be sufficient for these reactions. Ikonomou et al. [75] also give the following equation governing the onset of droplet formation in ESI;

$$E_{on} = (2\alpha \cos \theta_o / \epsilon_o . r_c)^{½}$$

where;

E_{on} is the field strength at the capillary tip required for the onset of electrospray behaviour

α is the surface tension of the liquid

θ_o is the half angle of the liquid cone (Taylor) at the capillary tip

ϵ_o is the permitivity of vacuum

r_c is the inner radius of the capillary

It can be seen that surface tension is a factor in the above equation. Fuchs and Sutugin [66] note that the surface tension of a liquid decreases as electrostatic charge increases. This effect is used in electrostatic atomizers and is due to the build up of repelling forces between opposite charges at the liquid surface. Such an effect occurring in the PB interface would lead to increased droplet formation and so may increase sensitivity.

Loeb has considered the phenomena of electrostatic charging in detail [76] with particular emphasis on spray electrification. The results of this work are too lengthy and complex to discuss in detail here but the following remarks may be made. Loeb observed that static charging was much more pronounced for aqueous systems. He found that liquid flow through a tube or spraying through a nozzle gave rise to static charging. Loeb also found that solute effects on static charging were variable and depended on the chemical nature of the solute. Some solutes increased surface tension of the liquid drop whereas some decreased it and this change in surface tension had an effect on the static charging of the drop.

In conclusion, PB is a useful technique for interfacing LC and MS especially for the determination of "unknown" or polar organic environmental pollutants. Mobile phase composition problems still exist for some interface types. Non linearity of response, especially at low analyte levels has been noticed for many PB applications. Additive, matrix and carrier effects of varying magnitude have also been described by many groups. These effects have implications for quantifive analysis using PB. This behaviour seems to be consistent with the "abrupt high pass filter" model proposed by Apffell and Perry [56]. However, the factors explaining this model are still not clear. Particle size effects, inherent detector non-linearity and electrostatic effects have all been suggested as causes for the non-linear behaviour of the particle beam interface.

2.1 LLE, Soxhlet Extraction and ASE

Instrumental aspects of PB/LC/MS have been discussed in the previous chapter; equally important for environmental samples is the preparation of the sample prior to analysis. This typically involves sampling, extraction and clean-up procedures. Sampling is a complex field and has been discussed elsewhere [77] . The aim of the extraction and clean-up procedures is to recover the analytes of interest from the matrix, minimise or remove interferants and to remove substances that could cause damage or impair the functioning of the instruments used.

Several methods of extraction exist of which the most commonly used are probably solvent extraction (Soxhlet or similar) and liquid-liquid extraction (LLE). The physical processes underlying these techniques are discussed elsewhere [78-80]. In Soxhlet extraction the solvent is refluxed and freshly distilled solvent is constantly passed over the analyte. This solvent is also at elevated temperature, i.e. just below the solvent boiling point. This is normally an advantage due increased solvating efficiency but can cause problems for thermally labile analytes. In these cases a low boiling point solvent may be used. Soxhlet extraction may be considered as the standard method of extraction for soils and other solids.

LLE is the classical "separating funnel" method of extraction. It has been used as the extraction step in a variety of "standard" methods e.g. United States Environmental Protection Agency (US EPA) methods 625 and 553 [81,82] and Health and Safety Laboratory, method IAC2 [83]. Advantages of this technique are its ease of use, ubiquity and the lack of specialised equipment or instrumentation required.

Disadvantages are that the solvents used may be hazardous e.g. hexane and dichloromethane, large quantities of solvent are often used (<100 mls) and clean up and concentration steps are often necessary.

Accelerated Solvent Extraction (ASE) is a relatively modern technique using organic solvents at elevated temperatures and pressures (~50-200°C and ~7-20 MPa). Popp et al. [84] have reported on the ASE of chlorinated pesticides, PAH and dioxins in solid wastes. In this study it was found that 10-20 minute ASE gave similar or better recoveries than 20 hr. Soxhlet extraction for these compounds in soil (pesticides), pine tree bark, slurry from copper smelting (Thiessenschlamm) and contaminated soil (PAH) and fly-ash (dioxins). This technique therefore shows promise as a more rapid substitute for Soxhlet extraction. Kenny and Olesnik [85] compared extraction of PAHs in lignite coal fly ash using ASE, Soxhlet and SFE and found differences in the behaviour for low (<166), medium (178-252) and high RMM PAHs (>252).

2.2 Ultrasound and Microwave Extraction

Ultrasound (sonication) is another commonly encountered extraction technique. This technique bombards the sample with ultrasound (high frequency sound waves) adding energy to the solvent, so increasing its solvating power. This technique is often used as an alternative or in comparison with Soxhlet extraction . A similar technique which involves "energising" a solvent is microwave assisted extraction. Onuska and Terry [86] have reported on the extraction of pesticides, principally chlorinated ones such as lindane, aldrin etc., from sediments using a microwave generator. These authors compared the technique with sonication, Soxhlet and

convection heat extraction techniques. They found that 5 x 30s bursts of microwaves generally gave similar recoveries to around 8 hours of Soxhlet extraction and several hours of sonication. Microwave extraction would therefore appear to offer considerable time savings over these other two methods. Extraction efficiency was found to depend on sediment moisture and type of organic matter present. These authors did not see any degradation or destruction of the pesticides in this study, presumbably due to the relatively short microwave bursts involved.

2.3 Supercritical Fluid Extraction

Supercritical fluid extraction (SFE) is a relatively new extraction technique offering certain advantages over classical extraction methods. Very often these classical methods are time consuming and use large quantities of environmentally unfriendly solvents. The technical and theoretical aspects of SFE have been discussed elsewhere [87-89]. Supercritical fluids have properties between those of a liquid and a gas depending on the temperature, pressure and composition of the supercritical fluid. These properties allow liquid like solvating power and good mass transfer properties. The density of a supercritical fluid is typically 100 to 1000 x that of a gas and hence molecular interactions are stronger than those in gases leading to the properties described above. Unlike liquids supercritical fluids have zero surface tension and so can easily penetrate the sample matrix. The most commonly used supercritical fluid is CO_2. This is relatively safe, non-toxic and non-corrosive but is also non-polar. This means it does not have the high solvating power of an ideal SFE solvent. These considerations have been dealt with at more length by Reikkola and Manninen [90] who also discussed the use of a polar additive e.g. methanol to

enhance SFE efficiency. This is common practice in SFE but Howard et al. [91] highlighted difficulties with this approach, namely introduction of the modifier to the matrix and possible contamination of the SFE system by the modifier. If the modifier is added to the matrix i.e. to the extraction thimble then time must be allowed for the modifier to interact with the matrix which may significantly increase analysis time and so comprise one of SFE advantages. If the modifier is added to the supercritical fluid then contamination of the system is possible. These authors used the more polar trifluoromethane as a supercritical fluid and compared its extraction efficiency with that of CO_2 and 5% methanol modified CO_2. This study looked at the SFE, from clays, of sulphonyl urea herbicides (chlorsulfuron and sulfometuron) and PAH (phenanthrene, pyrene, 1-1' binaphthyl, benzo(c)phenanthrene, benzo (a) anthracene and chrysene) and found trifluromethane to be superior to CO_2 but inferior to methanol modified CO_2. Methanol modified CO_2 has been used to extract PAH from soils [92,93], urban air particulates and petroleum waste sludge [94] and sulphonyl urea herbicides in water [95]. Reikkola and Manninen [90], Howard and Taylor [95] and Meyer et al. [94] looked at linking SFE with solid phase extraction (SPE). SPE was used to trap the analyte and SFE to recover the analyte from the trapping medium. Studies comparing SFE with sonication, microwave assisted extraction and Soxhlet extraction have been carried out [94,96,]. Generally SFE gives comparable or better recoveries in a shorter time than Soxhlet and sonication extraction. Llompart et al. [99] found the efficiency of SFE for the extraction of phenolic compounds in soil was very heavily dependant on the organic content of the soil, presumably due to analyte absorption.

In summary LLE and Soxhlet extraction continue to be the most popular type of extraction technique used. Newer techniques however such as sonication, microwave, ASE or SFE, offer the potential for more rapid extraction with no loss of recovery. It is expected that these techniques will increase in use in the future.

2.4 Sample Clean-up

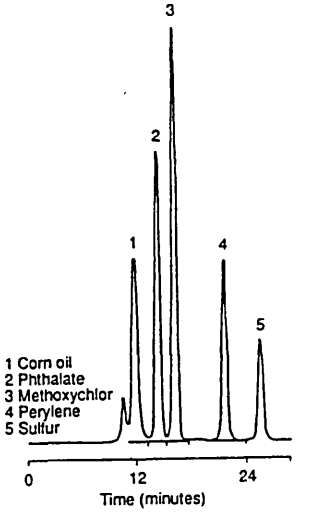
After extraction of the sample some further preparation step is usually necessary i.e. concentration and/or clean-up. The most common and simplest concentration step is drying down in which excess solvent is removed from the sample. This can be achieved by passing a stream of dry nitrogen gas over the sample at either ambient or elevated temperature or by the use of a rotary evaporator (reduced pressure) or similar.

2.5 Gel Permeation Chromatography

One commonly used clean-up procedure is gel permeation chromatography (GPC) also known as size exclusion chromatography (SEC). This technique forms the basis of a standard clean-up method [83] for pesticide contaminated vegetation extracts used by the HSL. GPC may be regarded as a specialised form of LC and separates compounds according to their ability to interact with a variety of gel type packings. Smaller compounds can interact with surface irregularities in these gels and so are retained whereas larger molecules cannot and so are unretained. In this way a separation dependent on relative molecular size (and so usually mass) is achieved with large compounds eluting before small compounds. This technique is described

elsewhere [97] . A GPC trace showing the relative retention of various compounds of varying molecular mass is shown in fig. 2.1 :-

Figure 2.1 *Calibration Standard for Gel Permeation Chromatography System*



Sample Calibration Chromatogram

2.6 Solid Phase Extraction

A technique that covers both extraction and clean-up is solid phase extraction (SPE). SPE devices are typically a syringe barrel filled with an LC type packing giving a short LC column. Other types of SPE device exist e.g. disks.The basic theories and principles underlying SPE are the same as those for LC. A variety of packing types are available and some of these are summarised in fig. 2.1 along with a few notes on the main uses of each packing type :-

Table 2.1 *Packings for SPE and Main Uses for Each Packing*

Packing Material	Surface Character	Suggested Applications
C18	Hydrophobic non-polar Bonded Silica phase	Isolation of hydrophobic species from aqueous solutions e.g. trace organics, pharmaceuticals, pesticides and polynuclear aromatic hydrocarbons.
Silica	Hydrophilic polar neutral phase	Isolation of low to moderate polarity species from non-aqueous solutions e.g. pesticides, fat soluble vitamins, plant pigments and natural products.
Florisil	Hydrophilic polar weakly basic phase	Isolation of low to moderate polarity species from non-aqueous solutions e.g. pesticides in feeds and food, polychlorinated biphenyls in oils and samples containing a large amount of fat or lipid.
Alumina A	Hydrophilic Polar Acidic phase	Isolation of hydrophilic species in non-aqueous solutions e.g. feed additives, caffeine in cola and antibiotics
Alumina N	Hydrophilic polar neutral phase	Isolation of hydrophilic species in non-aqueous solutions e.g. herbicides, petroleum and synthetic organic compounds
Alumina B	Hydrophilic polar Basic Phase	Isolation of hydrophilic species in non-aqueous solutions e.g. pesticides, priority pollutants (EPA) and steroids.
Aminopropyl	Hydrophilic moderately polar slightly basic phase	Low capacity weak anion exchanger, used for phenols and phenolic pigments, drugs and petroleum fractionation.
Cyanopropyl	Hydrophobic moderately non polar neutral phase	Analytes in aqueous or organic solvents e.g. drugs and metabolites in biological fluids, pesticides and hydrophobic peptides.
Diol	Hydrophobic moderately non polar neutral phase	Analytes in aqueous or organic solvents e.g. trace elements from water and antibiotics in cosmetics.
Cation Exchange	Hydrophilic polar acidic phase	Isolation of cationic analytes in aqueous or non-aqueos aolutions e.g. enzymes, weakly basic proteins and synthetic organics.

Anion Exchange	Hydrophilic polar basic phase	Isolation of anionic analytes in aqueous or non-aqueous solutions e.g. phenolic compounds and acid pigments in wine.

Adapted from Ref. 98, p22

The production of improved packings by chemical modification has been discussed by Sun and Fritz [99] . These authors found relatively minor chemical modifications such as insertion of acetyl or hydroxymethyl groups into a porous divinylbenzidine resin gave much increased extraction for polar organics, such as phenols, from aqueous samples.

SPE can operate in two modes, either as a filter or as a retention/enrichment device. In the filter mode the analyte(s) are not retained and the whole of the eluent is collected. Examples are the use of silica gel SPE for the clean-up of PAH or pesticide containing samples [98]. Here the non-polar analyte is unretained by the polar silica gel and will pass through the cartridge. Polar contaminants and potential interferants will be retained on the cartridge which can then be discarded. The eluent may then concentrated as described above or subjected to some other sample preparation.

In the retention/enrichment mode the analyte is retained on the cartridge whilst the eluent and unretained compounds can be discarded. The analyte is then washed off the cartridge using a suitable solvent or progression of solvents. An example of this has been reported by Chang et al. [100] In this work polychlorinated dibenzo-p-dioxins (PCDDs) were retained on a non-polar C18 cartridge. Polar interferants and lipids

were unretained and are discarded with the eluent. The C18 cartridge was then washed with hexane to flush off the PCDDs. This mode of operation offers the capacity to pass large volumes of the analyte containing extract through the cartridge thereby achieving considerable concentration. this is particularly useful for trace levels of contaminant e.g. pesticides in drinking water and forms the basis of numerous methods e.g. Eichelberger et al. [101] . The use of SPE applied to PAH will be discussed later however an idea of the potential of the technique can be gathered by considering the variety of compounds and matrices to which it has been applied, ranging from pesticides, pharmaceuticals, organics and bio-molecules in soils, water, biological fluids, vegetation and others [98,102,103,104] .

Loconto [105] has reviewed SPE research and considered theoretical parameters in the use of SPE. This author also looked at the effectiveness of SPE for various EPA "priority pollutants" . Recoveries were found to vary widely depending on compound re-inforcing the point that whilst SPE may not be a universal extraction procedure it is probably the most flexible technique currently available.

The advantages of SPE may be summed up as follows :- relatively low cost, speed of use, ease of use, ready availability and widespread applicability. SPE does not require the large initial investment that other techniques do e.g. SFE, GPC or microwave extraction. SPE cartridges are designed as single use devices so decreasing the problem of cross contamination or memory effects. They are supplied ready to use by several commercial companies and are considerably easier to use than other widely accepted techniques. For example in comparison to GPC there is no need for lengthy equilibration, a wider range of solvents can be used

(and more environmentally friendly ones, GPC commonly uses dichloromethane) , SPE is relatively uneffected by temperature and is usually quicker. The reference mentioned above [98] gives an idea of SPE versatility and Loconto [105] has pointed out that approximately 70% of all sample matrices are non-solid greatly limiting the scope of SFE. In conclusion it can be stated that SPE offers unrivalled potential for sample extraction and clean-up.

Chapter 3

Analysis of Polycyclic Aromatic Hydrocarbons

3.1 Introduction to the Analysis of Polycyclic Aromatic Hydrocarbons

Polycyclic Aromatic Hydrocarbons (PAH) and their derivatives are toxic compounds which are widespread throughout the environment. They are routinely monitored in drinking and surface waters, airborne particulates e.g. exhaust fumes, sediments and food. Their mutagenic activity has been well documented and the mutagenicity of the nitro and oxy derivatives is suspected to be higher than that of the parent compounds [106-111].

Many methods have been published for the analysis of PAH. EPA-610 [112] uses LLE and GC/flame ionisation detection or LC/UV for the detection of sixteen specified PAH ("the EPA-16") in drinking water. These PAH have become the "industry standard" indicators for PAH and are commonly determined, see fig. 3.1. EPA-625 [82] is a method for the determination of various compounds, some of which are in the "EPA-16", by GC/SPE in drinking and raw waters. EPA Compendium method T0-13 measures the "EPA-16" in ambient air using GC/FID, GC/MS and LC/PFD [113]. A different approach is taken in MDHS 68 [114,115] this method deals with the cyclohexane soluble fraction of coal tar pitches. These are complex mixtures whose composition varies with the source material and process conditions. MDHS 68 attempts to provide an overall measure of the toxicity of the coal tar pitch by relatively simple procedures, such as gravimetry and ultrasonic extraction in cyclohexane.

Analysis of nitro-PAH by GC has been described by Campbell and Lee [116] and Selstrom et al. [117]. Campbell and Lee directly analysed nitro-PAH from diesel

particulates whereas Selstrom et al. reduced the nitro-PAH to amino-PAH with sodium borohydride and copper (II) chloride. These were then subsequently analysed by GC/negative ion chemical ionisation MS. Selstrom et al. also described a cleanup procedure for complex nitro-PAH containing samples. The use of LC/fluorescence to analyse for the amino-PAH derivatives has been described by Gibson [118]. This author analysed for nitro-PAH in wood smoke and diesel particulates. The potential of LC/MS for the analysis of nitro-PAH has been discussed by Bosch [119] who stated that this technique was "potentially useful in many areas of endeavour".

The use of GC for the determination of PAH, from a variety of sources and in various matrices, has been extensively reviewed by Wang and Fingas [120]. LC has been widely used for the determination of PAH. It is commonly used with LC/programmed fluorescence detection (PFD) which offers the possibility of sensitive and selective detection by setting up timetabled changes to excitation and emission wavelengths characteristic of a particular PAH. LC/PFD methods with various fluorescence timetables and detector settings have been described by Gibson [118] Marriott et al. [121], Hansen et al. [122], Lopez-Garcia et al. [123], Kayali et al. [124] and Noël et al. [125]. The use of programmed wavelength UV detection has been described by Dridi et al. [126].

PAH have been found in drinking and surface waters [127-129], airborne particulates and diesel fumes [130,131], pitch [114,125,132], petroleum products [120,133], creosote [134], soils and sludges [82,135] fly-ash [85], oil-spill related materials [1120,136,137] and tarmac [138]. Due to the variety of complex matrices in which PAH are found extraction and clean up steps are usually necessary. The use of SFE has been described by Burford et al. [92], Tena

et al. [93] , Meyer et al. [94] and Kanagasabapathy [139] . The use of ASE has been described by Popp et al. [84] and Kenny and Olesnik [85]. SPE is often used for clean up of PAH contaminated material: this approach has been described by Akhlaq [140] and Smith et al. [141] . Various types of SPE cartridge have been used, the most commonly used are probably Si gel (filter mode, as described in Chapter 2 [112,132,133]) or C18 (retention mode as described in Chapter 2 [102,140,142]). Garrigues and Bellocq [143] have reported on the use of florisil SPE clean up. The use of combined SPE clean up procedures has been reported by Luks-Betelj [144] and Levine et al. [131] . A comparison of Soxhlet, SFE and microwave extractions has been carried out by Dean et al. [145] . These authors found that SFE and microwave extraction gave similar recoveries, which were approximately double those obtained by Soxhlet extraction. Similar results were found by Tena et al. [93]. Hale and Aneiro [134] reviewed extraction and clean up procedures for coal tar and creosote constituents in aqueous samples. These authors concluded that the optimum combination of extraction and clean up technique depended on both target analyte and matrix.

The complex nature of PAH samples and the variety of matrices in which they are found means that modelling and statistical approaches can be useful. Scobbie et al. [115] have described the use of fingerprinting to try to identify PAH by the type of process producing them. Different types of process e.g. aluminium smelting, high temperature and low temperature coke ovens and high and low temperature tar distillation plants were studied. Distinctive "fingerprints" or distribution patterns for the amounts of EPA-16 pesticides were found for each process. The PAH were classified by ring size i.e. two to six rings. This type of approach was considered for

the data presented in this thesis but was not carried out as it is considered that the "x,y" plots were adequately showing the trends in the data, see Chapter 4.

The LC behaviour of PAH has been considered by several workers. Improved separation of the "EPA-16" (SRM-1647A) using sub-ambient temperatures has been reported by Gatzfeld-Huegesen [146] and Ooms [147]. Ooms [147] also gives a brief account of the theory of temperature effects in LC. Chen et al. [148] found that isothermal LC gave a good separation on a polymeric ODS phase whereas a temperature gradient gave optimum separation for a monomeric ODS phase. Similar work has been carried out by Jinno et al. [149] who concentrated on large (>6 ring) systems . These authors found that polymeric phases gave better separation than monomeric phases. They also found an inverse temperature effect on resolution and interpreted these results as being due to temperature controlled rearrangements of the polymeric stationary phase surface structure. At low temperatures, these authors state, the bonded phase has a folded, linked orientation which breaks up as temperature increase to give a less linked "bristle" orientation. For the monomeric stationary phase, the distance between the ODS chains is assumed to be greater than the polymeric phase. This means that the monomer cannot adopt a "folded" form. The binding of the analyte and the stationary phase is assumed by this model to be a surface interaction, so a less linked surface structure would have less area available for bonding than the "folded" structure leading to less interaction and poorer LC resolution.

Sander and Wise [150,151] have also extensively studied the LC behaviour of PAH. These authors have proposed the "slot model" to predict the retention of PAH on

polymeric and monomeric stationary phases. This model says that the ordering within a bonded phase may be considered as "slots" in a layer. The model is concerned with surface bonding only and so is essentially 2D. The surface is considered to be made up of a mixture of slot widths and solute penetration into the slots will result in retention. Long, narrow solutes (i.e. those with high length to breadth (L/B) ratios) will fit a greater proportion of these slots than "square" (low L/B ratios) of the same RMM. A similar argument is put forward for the interaction of planar and non-planar solutes of similar (L/B) ratio with the surface. Non-planar solutes will interact with less of the surface than planar ones and so will be less retained. These authors therefore consider (L/B) ratio to be a good predictor of retention behaviour for PAH. Schabron et al. [152] have also proposed an empirically derived ratio model for the prediction of solute behaviour during LC. Their model is; F = (nos. of double bonds) + (nos. of primary and secondary carbons) - 0.5 (nos. of non-aromatic rings). A plot of log k' versus F (where k' is the capacity factor, a measure of solute retention) gave a reasonably linear relationship with a correlation coefficient of 0.98.

Sander and Wise also found a temperature effect in their work. They reasoned that as temperature increased, the bonded phase adopted a more "open" or "monomeric-like" surface structure. At decreased temperature the surface structure becomes more rigid , or "liquid crystalline" giving "polymeric-like" behaviour. These authors state that the temperature effect is " a universal effect that is not specific to a particular stationary phase type or brand". This explanation of the temperature dependence of LC of PAH is similar to those proposed by Chen et al. [148] and Jinno et

al. [149] . All three sets of workers consider the temperature effect to be due to a temperature dependant ordering of the stationary phase surface.

In the work presented in this thesis the "EPA-16" were used as test compounds, to optimise LC/PB/MS performance and to examine the effect of various factors, such as, interface and source temperature and the addition of modifiers to the mobile phase. The range of masses covered by the "EPA-16" (128-278) allows the "high pass filter" (see above) model of Apffel and Perry [56] to be investigated. These results were then compared with those obtained by LC/F, LC/PFD, GC/MS and LC/UV. Finally the methods developed in the first parts of the study were applied to the quantifiication of PAH in refractory brick dust extracts, (see Chapter 4). In this part of the work, the relative merits of sonication and Soxhlet extraction were examined for a range of extracting solvents and SPE and GPC clean up procedures were compared.

3.2 Experimental

3.2.1 Chemicals

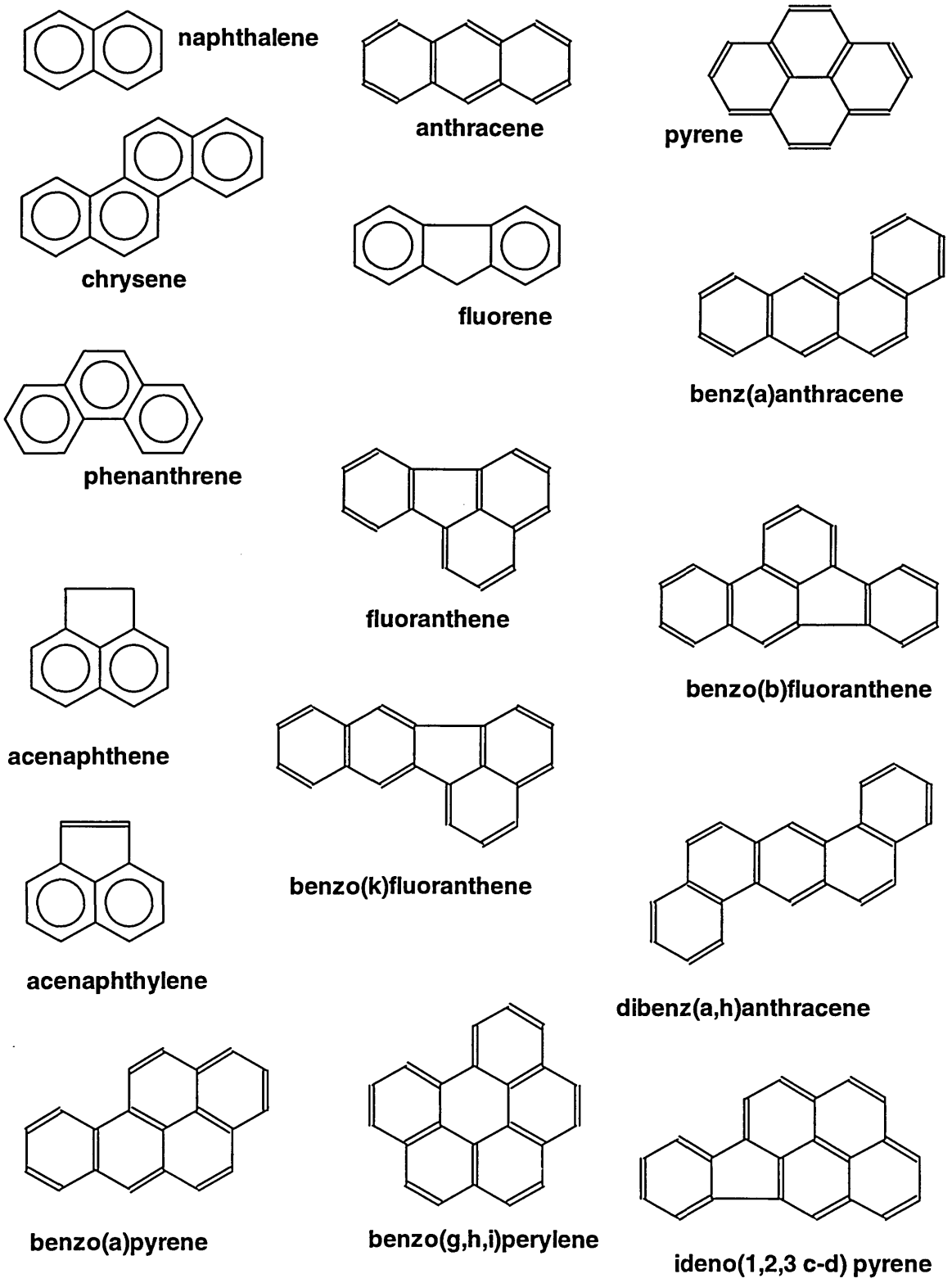
Test mix "EPA-16" (0.1 mg/ml per component, in methanol) was supplied by Alltech, West Chester, Illinois, USA. Coronene and D^{12} perylene were supplied by Aldrich Chemical Co., Gillingham, Dorset, U.K. LC solvents were of "HPLC grade" or better and were obtained from either Fisons Scientific Equipment, Loughborough, England

or Rathburns, Walkerburn, Scotland. Ammonium oxalate, ammonium acetate, tetrabutyl ammonium hydroxide and 1-octane sulphonic acid and other buffers were obtained from Aldrich Chemical Co.; Gillingham, Dorset, U.K. Refractory brick dusts were supplied by Dysons Refractory, Sheffield, U.K.

Table 3.1 *Molecular Masses of the EPA-16*

Compound	Molecular mass	Compound	Molecular mass
napthalene	128	benzo(a)anthracene	228
acenanaphthylene	152	chrysene	228
acenanaphthene	154	benzo(b)fluoranthene	252
fluorene	166	benzo(k)fluoranthene	252
phenanthrene	178	benzo(a)pyrene	252
anthracene	178	dibenzo(a,h)anthracene	278
fluoranthene	202	benzo(g,h,i)perylene	276
pyrene	202	ideno(1,2,3 c-d)pyrene	276

Figure 3.1. *Structures of EPA-16 PAH*



3.2.2 Instrumentation and Equipment

LC systems used (HSL, Sheffield) were either Hewlett-Packard HP 1090 LC system with HP 1046 fluorescence detector and HP diode array detector or Waters Millennium LC system with 717 autosampler, Waters 470 fluorescence detector and Waters 996 diode array detector. Other LC systems were also used, consisting of various makes of LC pump (Jasco, Waters etc.), according to availability at Sheffield Hallam University. The pumps were linked to a VG Trio 1 MS via a VG LINC Particle Beam interface and associated vacuum pumps. LC columns used for the PAH work were either a Hewlett-Packard Hypersil Green (PAH) (100 x 4.6 mm) or Phenomenex Envirosep PP (125 x 2 mm).

Solid phase extraction cartridges were Waters Sep-pak C18 or Waters Sep-pak silica gel columns.

The gel permeation chromatography system used consisted of a Waters 510 pump, Waters 717+ autosampler, Waters fraction collector and Waters Envirogel GPC columns (19 x 300 mm and 19 x 150 mm) with a Perkin-Elmer LC135 DAD used for peak identification as necessary.

A Zymark Turbovap sample concentrator was used for blowing down the extracts.

3.3 Practical

3.3.1 Optimization of PB/LC/MS

Initial experiments involved familiarisation with the PB/LC/MS system. Optimisation of the MS signal was carried out by varying a number of instrumental parameters. A series of optimization experiments were carried out, in which the effect of nebuliser grid position, nebuliser helium pressure and nebuliser mesh size were investigated. The mobile phase flow rate used in these experiments was 0.3ml/min. to 0.5 ml/min.

3.3.2 Effect of PB/LC/MS Interface Temperature

The optimum PB conditions described in section 3.3.1 were applied to the PB/LC/MS system with the flow set to 0.5 ml/min. These conditions were then used to assess the effect of interface temperature on PB/LC/MS sensitivity for the "EPA-16". The interface temperature was varied from 20 to 110 C and the response for an injection of 100ng per component was measured.

3.3.3 Effect of Source Temperature on Peak Shape

For this series of experiments the Phenomenex Envirosep PP (125 x 2 mm) column was used. The mobile phase flow rate was 0.3ml/min.with 0.2 ml/min. post column addition of acetonitrile. The interface temperature was set at 75 °C, with a helium pressure of 20 psi and the MS source temperature varied as required. For each run,

20 µl of 8 µg/ml "EPA-16" mix was injected. The MS was operated in SIM mode as described above.

3.3.4 Effect of Post Column Addition of Organic Modifiers

The addition of organic modifiers, postcolumn, to the eluent stream has been reported to increase PB/LC/MS sensitivity. This effect has been found to be variable in magnitude, or entirely absent in some cases, as reported above. To try to clarify the situation, this phenomenon was investigated by using a second LC pump, connecting via a T-piece after the LC column to deliver the additive into the eluent stream. A short loop of LC pipe was then connected to allow the additive and LC column eluent to mix and the combined flow was sent to the PB/MS interface.

The effect of post column addition of 0.2ml/min. acetonitrile only, 0.2ml/min. acetonitrile containing 0.01M ammonium acetate and 0.2ml/min. acetonitrile containing 0.01M ammonium oxalate was investigated for an injection of 100ng/component. A flow rate of 0.3 ml/min. was used for the LC. Other instrumental conditions were: interface temperature set at 70 °C, helium pressure of 20 psi and the MS source temperature set at 250 °C.

3.3.5 Investigation of the Use of Micelle forming Additives in PB/LC/MS

For this series of experiments both the Phenomenex Envirosep PP (125 x 2 mm) and the Hewlett-Packard Hypersil Green (PAH) (100 x 4.6 mm) columns were used.

A flow rate of 0.3 ml/min. with 0.2 ml/min. post column addition was used for the LC. Other instrumental conditions were: interface temperature set at 70 °C, helium pressure of 20 psi and the MS source temperature set at 250 °C.

3.3.6 Detection Limits for EPA-16 by LC/PFD, LC/DAD, PB/LC/MS and GC/MS

LC/F, LC/PFD and LC/UV (diode array detector DAD) methods were developed for the detection of the "EPA-16". The conditions finally used are summarised below :-

LC/DAD

UV 254nm

column Hypersil Green (PAH) (100 x 4.6 mm)

flow rate 0.5 ml/min. (0.3 ml/min. also used)

gradient elution :-

% acetonitrile	50	50	100	100	50
time(min.)	0	5	25	55	60

balance of mobile phase is water.

LC/PFD columns, flow rate and solvent programme as above

fluorescence time table (Initial work)

time	0	21	46
Excitation λ_{ex}	290	290	290
Emission λ_{em}	320	420	500

fluorescence time table (brickdust work and LOD)

(column Hypersil Green (PAH) (100 x 4.6 mm))

time	0	19	20	22.5	24	25	26	31	32	33
Excitation λ_{ex}	290	280	280	320	280	255	280	280	280	280
Emission λ_{em}	400	330	420	390	425	380	425	460	500	425

Flow rates etc. for the PB/LC/MS work are described in the relevant portions of the results given below.

GC/MS

Hewlett-Packard HP 5972 Mass Selective Detector/HP 5890 series II GC and autosampler, column 30m x 0.25mm HP5-MS 0.25µm film thickness

The GC conditions used were :-

Instrument HP 5972 Mass Selective Detector/ HP 5890 series II GC and autosampler

column 30m x 0.25mm HP5-MS 0.25µm film thickness

injector temp. 250 °C,

oven programme:

40 to 260C at 7.5 °C/min. then to 280 °C at 10 °C/min.

(0 min. hold at 40 °C, 13.67 min. hold at 260 °C, 15 min. hold at 280 °C)

Scan and SIM modes were used.

Scan conditions were; range 50 - 350 a.m.u., dwell time 1s

The ions monitored in SIM were m/z 128, 152, 166, 178, 202, 228, 252, 276 and 278.

3.3.7 Investigation of Recoveries Following SPE of EPA-16

SPE is commonly used for PAH clean up and a variety of SPE procedures have been described above. To investigate further a series of experiments was carried out to determine recoveries from solutions, spiked with the EPA-16, from silica gel and C18 SPE cartridges (Waters SPE cartridges, 1g of sorbent per cartridge). The following procedures were used :-

C18 SPE procedure (enrichment mode)

Condition cartridge with 12ml methanol.

Flush cartridge with 12ml methanol.

Load on sample (in methanol).

Elute with 5ml of water (discard this fraction).

Elute with 5ml of ethyl acetate (collect this fraction).

Elute with 5ml hexane (collect this fraction).

Dry down and resuspend fractions in methanol.

Analyse (LC/DAD).

Si gel SPE procedure (filter mode)

Condition cartridge with 12ml ethyl acetate.

Load sample (in methanol).

Elute with 5ml of methanol (collect this fraction).

Elute with 5ml of hexane (collect this fraction).

Dry down and resuspend fractions in methanol.

Analyse (LC/DAD).

3.4 Results and Discussion

3.4.1 Optimization of PB/LC/MS

The initial part of this work consisted of familiarisation with the PB/LC/MS system. Optimization of the MS signal was carried out by varying a number of instrumental parameters. Ion repeller voltage (SIR) and dynode voltage (DM) appeared to have the greatest effect on signal intensity. Other parameters were varied but had little or no effect. A schematic diagram of the MS source and quadrupole was shown in (fig.1.1). A list of commonly used instrumental settings is given below:-

List of Commonly PB/MS Used Settings

SIR 12V SEC 150μA SF1 30V SF3 180V QL 12.0 QH 12.0

DM 250V SEE 70eV SF2 7V SF4 19V QIE 0.6V QIR 0.0 mV/a.m.u.

SIR source ion repeller voltage , SEC source electrode current

SF1-4 scanning focus , QL quadrupole low voltage ,QH quadrupole high voltage

DM dynode multiplier voltage , SEE source electron energy

QIE quadrupole ion energy voltage, QIR quadrupole ion energy ramp

Optimization was usually carried out by focusing on one ion and maximizing the signal for that ion without an in-line column. This technique allows the analyte,

commonly caffeine (m/z 194) or the target analyte(s) , to be detected rapidly i.e. around thirty seconds after injection. A separate set of experiments optimized the nebuliser position which was also found to have a marked effect on signal strength; a finding previously reported by other workers as mentioned above [18,33,34,43,45,48-50]. It was found that different LC mobile phases had different optimum nebuliser positions and that different nebuliser mesh sizes also had different optimum nebuliser positions. In addition, the mobile phase composition and nebuliser mesh size was also found to affect the optimum helium pressure. As has been noted by Huang and Garza [153] each one of the instrumental factors mentioned above can have an effect on the optimum position of the others. For this reason these workers used a statistical experimental design (response surface modelling) to optimise instrumental conditions. This option was not available for the work described here so a "one at a time" optimization process was used.

Other work carried out in this phase of the project involved loop injections of caffeine and the EPA-16 mix to determine the reproducibility of the system. This was found to be acceptable (~ 5% R.S.D for six replicate injections). The relative sensitivity of the system in scan mode and selected ion mode was also investigated. Selected ion mode was found to be the most sensitive and this mode was used in the following experiments. The ions monitored were, m/z 128, 152, 166, 178, 202, 228, 252, 276 and 278.

3.4.2 Effect of PB/LC/MS Interface Temperature

The results of these experiments are shown in Table 3.2 and Figure 3.2 :-

Table 3.2. *Effect of Interface Temperature on PB/LC/MS of the EPA-16*

Temp. °C	fla	pyr	b(a)a	chr	b(b)f	b(k)f	b(a)p	d(a)a	b(g)p	i(1)p
20	ND	ND	ND	ND	POOR	POOR	POOR	29	22	24
40	POOR	POOR	105	201	87	60	57	30	40	51
55	POOR	POOR	210	171	144	93	52	27	33	53
70	0.4	0.8	233	173	102	70	96	30	34	58
85	0.1	0.1	32	45	56	50	29	19	22	30
110	0.1	0.1	12	34	44	39	32	22	26	27

Notes

100ng per component injected.

Units are (counts/10^6)

ND = not detected, POOR = poor peak shape(not integrated)

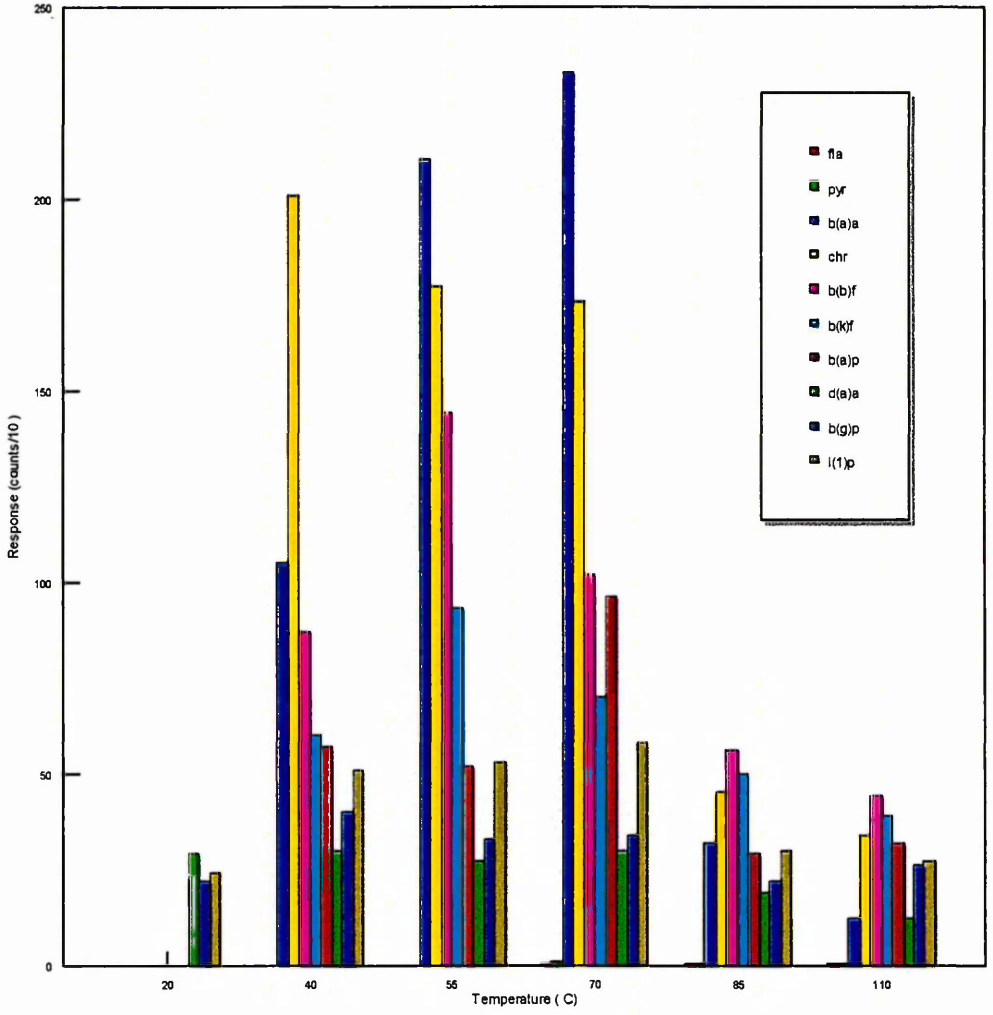
LC column used was the Hewlett-Packard Hypersil Green (PAH) (100 x 4.6 mm)

Key

fla	fluoranthene	pyr	pyrene
b(a)a	benzo(a)anthracene	chr	chrysene
b(b)f	benzo(b)fluoranthene	b(k)f	benzo(k)fluoranthene
b(a)p	benzo(a)pyrene	d(a)a	dibenzo(a,h)anthracene
b(g)p	benzo(g,h,i)perylene	i(1)p	ideno(1,2,3 c-d)pyrene

Figure 3.2. *Effect of Interface Temperature on PB/LC/MS of the EPA-16*

(for Experimental details see Section 3.3.2)



The results for the interface experiments suggested an optimum interface temperature for PAH analysis of between 55 to 70°C on this system. The interface temperature decided upon was 70 °C; this interface temperature was also used in the later experiments unless otherwise stated. These results also showed that the

lowest interface temperature (20 °C) gave very poor results, probably due to inefficient vaporisation. Generally the higher molecular mass PAH (> 275) studied were less affected by interface temperature, probably due to the higher melting points of these compounds. The marked decline in response between 70 and 85 °C of the mid mass PAH (>202 <275) studied is surprising. It is possible that a lower temperature e.g. 55 °C may be optimum for these mid mass PAH but 70°C seemed to give better results for the lower PAH.

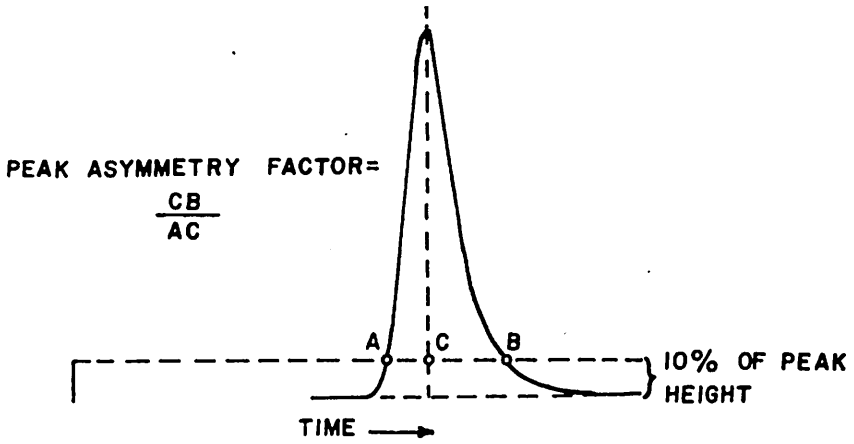
These results for the effect of interface temperature are in agreement with previous work which found this factor to be of importance [34,45,48,49]. The increased interface temperature aids removal of solvent vapour via increased vaporisation and may aid aerosol formation. The other factor to note from this initial PAH LC/PB/MS work is that the lighter compounds in the EPA-16 mix i.e. naphthalene, acenaphthylene, acenaphthene and fluorene were not detected. The compounds at slightly higher molecular mass, phenanthrene, anthracene, fluoranthene and pyrene are detectable by PB/LC/MS but with very poor detection limits.

3.4.3 Effect of Source Temperature on Peak Shape

It has been reported previously [136,154,155] that the peak shape of PAH (esp. the higher RMM ones) when determined by LC/PB/MS is dependant on MS source temperature. A series of experiments was carried out to investigate this effect. A measure of peak shape is the peak asymmetry factor (AF). This is defined as shown in Figure 3.3 and is the ratio of the back portion of a peak divided by the front

portion [10,11,156,157]. For a well packed LC column giving acceptable chromatography the asymmetry factor should be between 0.9 and 1.4.

Figure 3.3 *Definition of Peak Asymmetry Factor*



Determination of peak asymmetry factor (AF).

The results of these experiments are shown in Table 3.3 and Figure 3.4.

Table 3.3. *Effect of MS Source Temperature on Peak Asymmetry Factor for PAH Analysis by LC/PB/MS*

Compound	164 °C	206 °C	250 °C	297 °C
fluoranthene	2	1	ND	ND
pyrene	2	1.8	ND	ND
benzo(a) anthracene	3	1.3	1	1
chrysene	1.5	1.4	1.3	1
benzo(b) fluoranthene	2	1.5	1.5	1.3
benzo(k) fluoranthene	2	1.8	1.4	1.3
benzo(a)pyrene	3	2.3	1.6	1.2
dibenzo(a,h) anthracene	6.2	3.8	2	1.2
benzo(g,h,i) perylene	2.7	1.6	1.6	1
ideno(1,2,3 c-d) pyrene	2	2	1.5	1.3

ND = not detected.

Values obtained from measurements made on expanded SIM traces for an injection of 160ng per component (EPA-16 mix).

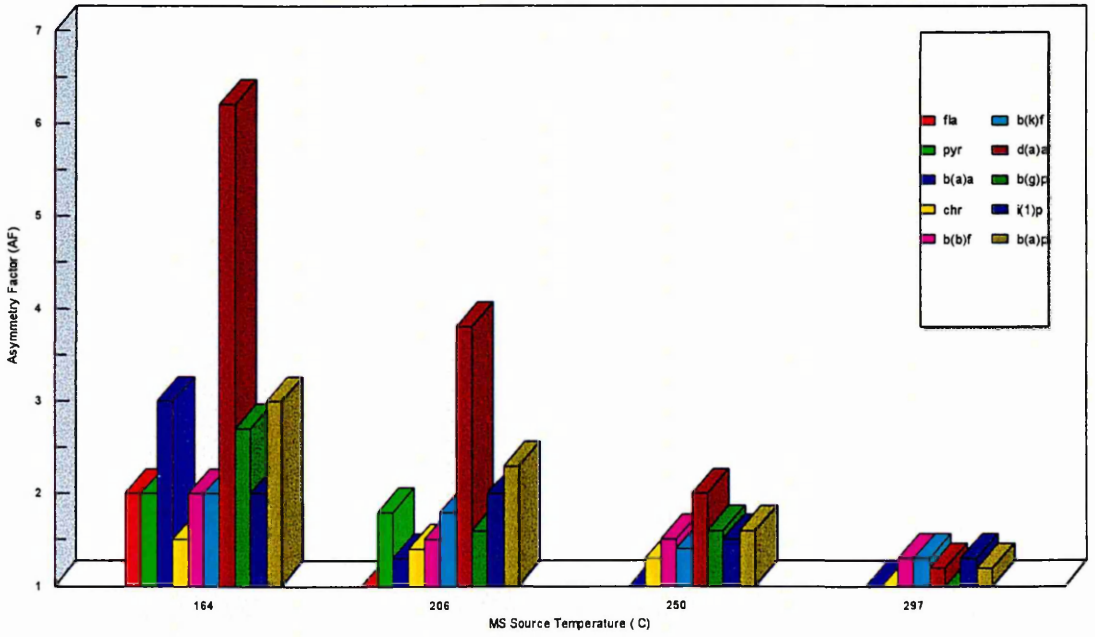
Source temperature varied slightly (< 5 °C per run), temperature values given are the midpoints of the observed source temperature ranges.

Key

fla	fluoranthene
pyr	pyrene
b(a)a	benzo(a)anthracene
chr	chrysene
b(b)f	benzo(b)fluoranthene
b(k)f	benzo(k)fluoranthene
b(a)p	benzo(a)pyrene
d(a)a	dibenzo(a,h)anthracene
b(g)p	benzo(g,h,i)perylene
i(1)p	ideno(1,2,3 c-d)pyrene

Figure 3.4. *Effect of MS Source Temperature on Peak Asymmetry Factor for PAH Analysis by LC/PB/MS*

(for Experimental details see Section 3.3.3)



It can be seen that at lower source temperatures, peak tailing, as shown by AF factors > 1.5, is increased. This effect is especially noticeable for the higher RMM PAH and is due to inefficient vaporization in the MS source. Doerge et al. [136] commented on this effect in their work. These authors found that PB/MS sensitivity for PAH with four rings or fewer (e.g. anthracene and pyrene) was not dependant on source temperature whereas five ring PAH systems gave maximum sensitivity at 300 °C. Pace and Betowski [155] suggested a source temperature of 350 °C may have been insufficient to fully vaporize the large PAH (>6 ring systems, RMMs of 300-450) used in their study. Slobodnik et al. [154] suggested that the higher PAH examined in their work (RMMs >300) required a source temperature of 300 to 400 °C to fully vaporize.

3.4.4 Effect of Post Column Addition of Organic Modifiers

The results of these experiments are shown below in Table 3.4 and Figure 3.5 :-

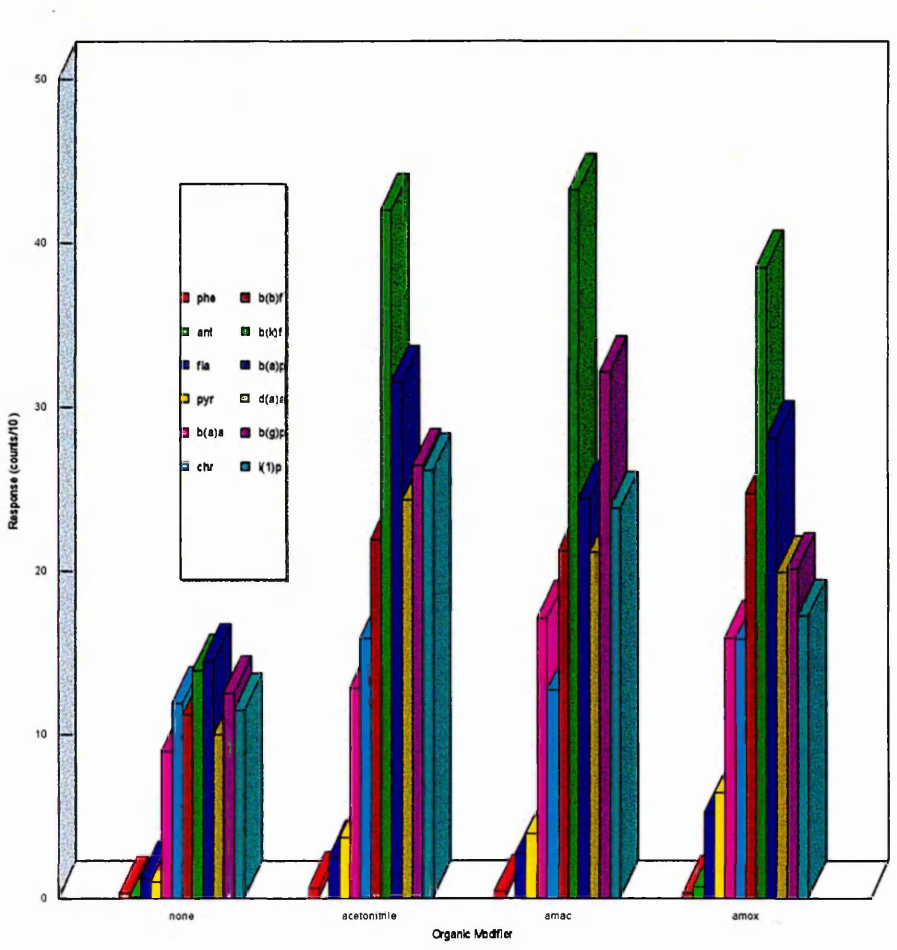
Table 3.4. *Effect of Post Column Addition (pca) of Organic Modifiers*

Compound	no pca	pca of 0.2 ml/min acetonitrile only	pca of 0.2 ml/min 10mM ammonium acetate	pca of 0.2 ml/min 2mM ammonium oxalate
phenanthrene	0.3	0.6	0.4	0.3
anthracene	0.1	ND	ND	0.7
fluoranthene	1.2	2.5	2.7	5.2
pyrene	1	3.7	3.9	6.4
benzo(a) anthracene	9.0	12.8	17.1	15.9
chrysene	11.9	15.9	12.7	15.8
benzo(b) fluoranthene	11.2	21.9	21.2	24.7
benzo(k) fluoranthene	13.9	42	43.2	38.5
benzo(a)pyrene	14.5	31.5	24.4	28.1
dibenzo(a,h) anthracene	10	24.3	21.1	19.9
benzo(g,h,i) perylene	12.5	26.4	32.1	20.1
ideno(1,2,3 c-d) pyrene	11.5	26.1	23.8	17.2

Units are (counts/10^6),ND = not detected, LC column used was the Phenomenex Envirosep PP (125 x 2 mm).

Figure 3.5. *Effect of Post Column Addition (pca) of Organic Modifiers*

(for Experimental details see Section 3.3.4)



The additive experiments reported above showed that post column addition of acetonitrile increased sensitivity but the other additives had no major effect. Further experiments using higher concentrations of ammonium acetate in methanol (at 0.1M) as additive also showed no effect. As the "additive effect" is believed to be due to an interaction between the analyte and additive, leading to increased particle size and so better transfer through the PB interface, it was decided to see if highly polar compounds, such as LC ion-pairing agents, gave the effect. The ion-pairing

agents tetrabutyl ammonium hydroxide (10mM in 80/20 acetonitrile/water) and 1-octane sulphonic acid (25mM in 80/20 acetonitrile/water) were added post-column as described previously. Again no increase in PB response for the PAH was seen and the interface skimmers very rapidly became blocked and required cleaning (after two or three runs).

These findings are in disagreement with those of Singh et al. [158] who found a positive additive effect with ammonium acetate. The reason for this difference may be that Singh et al. did not carry out an acetonitrile only post column addition and so are mistaking the increase in sensitivity caused by decreasing the water content of the eluent stream with an additive effect. Apffel and Perry [56] did see some additive effects but these were not uniform over the compounds studied and no single additive was markedly superior to the others examined. These authors stated "*Although certain combinations of probes and additives show improved linear response, no single additive appears to completely alleviate the non-linear behaviour as has been suggested by the earlier work*". This is in agreement with the results presented here where the effect of the additives studied was minimal. It is not clear how these polar additives are meant to interact with the non-polar PAH, so increasing particle size , which is the proposed explanation for the "carrier effect" given by Apffel and Perry. Alternatively the "additive effect" may be due to improving aerosol formation in some manner as discussed above. In conclusion it can be said that no additive "magic formula" that will increase LC/PB/MS sensitivity for PAH has been found.

It is interesting to note that although post-column addition of acetonitrile is a diluting of the eluent stream an increased signal is observed. This is presumably because the acetonitrile aids transfer through the interface perhaps by improving aerosol formation. A major drawback of post column addition of buffers found in this study is that the additives used e.g. ammonium acetate, ammonium oxalate, tetrabutyl ammonium hydroxide and 1-octane sulphonic acid were found to block up the small holes in the skimmer nozzles more rapidly than occurred without them. This required the skimmers to be cleaned, which is a time consuming process involving disconnecting the LC, cooling the source and interface, cleaning the skimmers (~5 minute sonication in methanol), reassembling the PB interface and pumping back down to vacuum. The post column addition of acetonitrile only was not found to have this drawback.

3.4.5 Investigation of the Use of Micelle forming Additives in PB/LC/MS

Detergents, such as Triton X-100, when in solution above the critical micelle concentration, form micelles. It was thought that, being relatively large in comparison to other particles in the PB interface desolvation chamber, micelles should be efficiently transported into the MS. This should also increase the transport of any compounds inside the micelle. As PAH are lipophilic they would be expected to preferentially concentrate in the lipophilic micelle instead of the relatively polar mobile phase. Experiments were carried out in which micelle forming additives were added post column to the eluent stream using the system described above. Preliminary experiments found, no or very small increases in PB/LC/MS response for the determination of PAH. In addition, the addition of micelle-forming additives

caused greatly increased blocking of the interface skimmers with the associated drawbacks described previously. For this reason, addition of micelle forming additives was not investigated further. The use of a micelle forming additive (Brij -35 polyoxy ethylene lauryl ether) to assist in the analysis of PAH by PB/LC/MS has been mentioned previously by Slobodnik et al. [154] . These workers looked at the use of Brij-35 micelles to decrease PAH losses through adsorption on to LC tubing and the PB interface. They added water post column so reducing the Brij-35 concentration below the critical micelle concentration and therefore the possibility of increased transport due to micelle formation was not examined.

3.4.6 Detection Limits for EPA-16 by LC/PFD, LC/DAD, PB/LC/MS and GC/MS

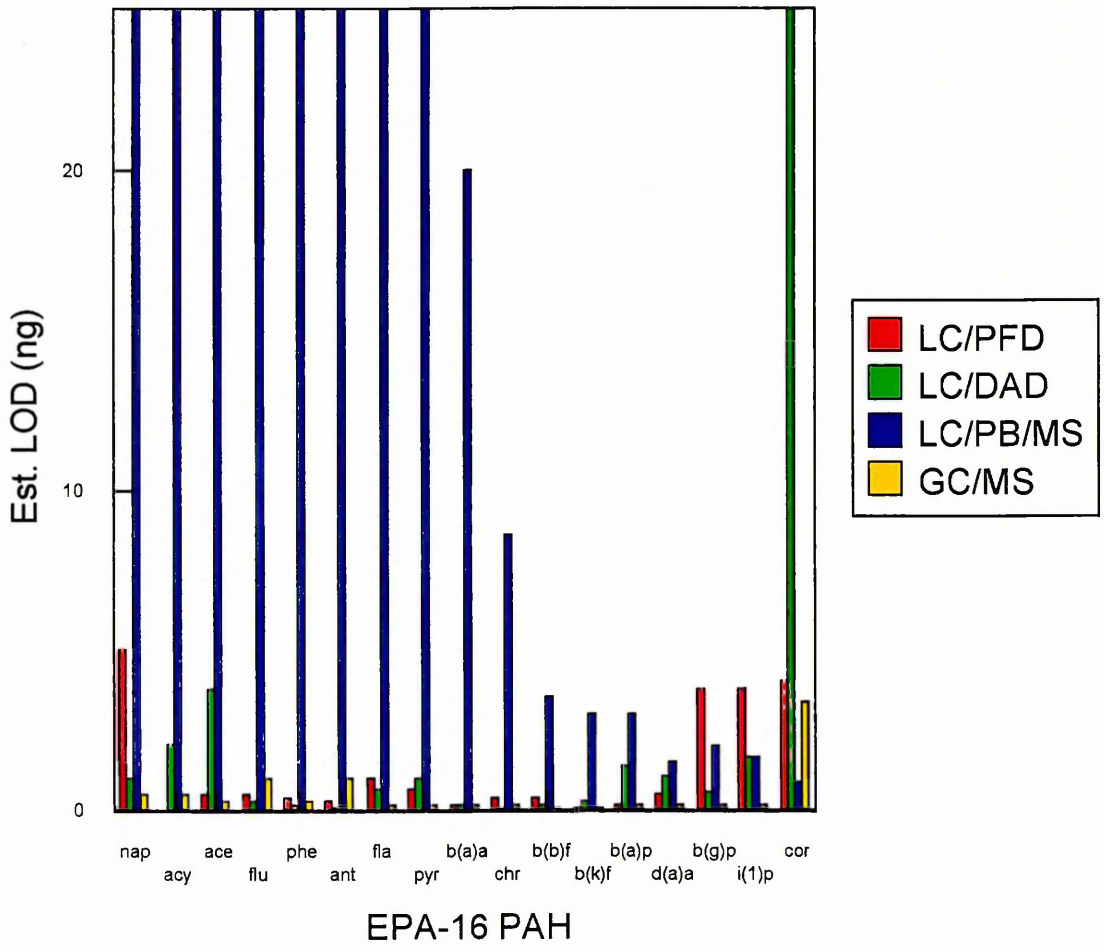
The "EPA-16", as mentioned previously, make a good class of test compounds for PB/LC/MS due to their range of molecular masses. A list of these compounds and their masses was given above in Table 3.1. Instrument conditions were as given in the experimental section. Detection limits for the test mix "EPA-16" were found using the LC systems described previously in order to compare them with PB/LC/MS detection limits. Detection limits for the GC/MS of this mixture were also obtained and the MS of each peak recorded. The estimated limits of detection, determined as described above for the various techniques used are shown in table 3.5 and Figure 3.6. These figures are based on 3 times the signal to noise ratio for at least three replicate injections: 10µl of a 0.5 µg/ml "EPA-16" solution plus coronene for diode-array and fluorescence results, 5 µl of an 8 µg/ml "EPA-16" solution plus coronene for PB/LC/MS results and 2µl of a 0.5 µg/ml "EPA-16" solution plus coronene for GC/MS results.-

Table 3.5. *Detection Limits for EPA-16 (ng on column)*

Compound	LC/PFD	LC/DAD (UV_{255nm})	PB/LC/MS (SIM)	GC/MS (scan)
naphthalene	5	1.5	ND >1000	0.5
acenaphthylene	not detected	2.1	ND >1000	0.5
acenaphthene	0.5	3.8	ND >1000	0.3
fluorene	0.5	0.3	ND >1000	1
phenanthrene	0.4	0.2	ND > 600	0.3
anthracene	0.3	0.1	600	1
fluoranthene	1	0.7	180	0.2
pyrene	0.7	1	60	0.2
benzo(a) anthracene	0.2	0.2	20	0.2
chrysene	0.4	0.1	8.6	0.2
benzo(b) fluoranthene	0.4	0.2	3.6	0.1
benzo(k) fluoranthene	0.1	0.3	3	0.1
benzo(a) pyrene	0.2	1.4	3	0.2
dibenzo(a,h) anthracene	0.5	1.1	1.5	0.2
benzo(g,h,i) perylene	3.8	0.6	2	0.5
indeno(1,2,3 c-d) pyrene	3.8	1.7	1.7	0.2
Coronene	4.1	43.7	0.9	3.4

Figure 3.6. *Detection Limits for EPA-16 (ng on column)*

(for Experimental details see Section 3.3.6)



These limits of detection could be improved by the use of larger injection volumes (e.g. 100 µl) or use of narrow or micro-bore columns for LC [159] or use of SIM mode for GC/MS (Slobodnik et al. [154] have suggested several orders of magnitude improvement for GC/SIM/MS). Optimization of the UV and PFD wavelengths used could also dramatically improve the limits of detection in some cases. For example, using 302nm for the UV determination of coronene would improve the detection limit by about 60x. This was not carried out for this series of experiments due to the

limited processing power of the computer running the LC system (see below). On-column injection has also been reported to give better results for the heavier PAH (RMM >252) by GC [160]. Bemgard et al. [161] have described the use of high temperature GC (up to 400°C) using GC columns with special coatings. These workers looked at PAH with RMMs from 352 to 426 derived from coal tar and carbon black. However the results presented above are adequate for comparison purposes. As can be seen from the results in Table 3.5 PB/LC/MS cannot be used for the lighter members of the "EPA-16" i.e. up to relative molecular mass (RMM)166 (fluorene). This finding has also been reported by Slobodnik et al. [154], Brown et al. [162], Anacleto et al. [163], Singh et al. [158]. From RMM 178 to 228 (phenanthrene to chrysene) detection by PB/LC/MS is possible but the detection limits are poor in comparison to the other techniques.

From RMM 252 to 278 (benzo(b)fluoranthene to dibenzo(a,h)anthracene) the detection limits for PB/LC/MS become more comparable with the other LC based techniques. GC/MS was found to be the most sensitive technique, even in scan mode, but the run time required to separate the higher RMM components and the peak shapes obtained for GC analysis became less satisfactory. Coronene (RMM 300) was also examined as an example of a heavier PAH and again the detection limit by PB/LC/MS was comparable with other techniques. It is expected that PB/LC/MS should be a more attractive option for these heavier compounds as they will not be amenable to GC/MS.LODs reported by other workers, for the analysis of PAH using LC and various detectors, are as follows: Dridi et al. [126] found LODs of 0.2 - 10 ng (b(a)a - ace) for PAH in airborne diesel exhaust using programmed UV

detection. Nirmaier et al. [128] found LODs of 1 - 12 ng (b(g)p -flu) by UV and 0.2 - 1.8 (b(a)p - flu) by EC detection for the determination of PAH in water.

Using PFD the following LODs have been found; 0.8 - 26 ng (b(a)a - chr) by Lopez-Garcia et al. [123] (PAH in col washings), 5 - 80 ng/filter (ant - nap) by Hansen et al. [122] (PAH in smokehouses), 2 - 70 ng (b(a)a - phe) by Noël et al. [125] (PAH in pitch). LODs reported previously using PB/MS detection (SIM mode) are as follows; Slobodnik et al. [154] 3 - 4000 ng/l (pyr - b(g)p in tap water), Bonfanti et al. [164] 20 - 100 ng/g ("PAH"), Pace and Betowski [155] 0.15 - 0.6 ng (IDL for PAH with RMM 300-332) and 2 - 4 ng (IDL for PAH > 352 RMM) and Singh et al. [158] 0.2 ng (chr), 1 ng (d(a)a), 0.5 ng (b(g)p) and 2ng (coronene).

It can be seen that other workers have obtained a wide range of LODs and that these are dependent on detector, matrix type and extraction/clean up procedure. Particularly interesting are the wide variations in LODs recorded for the same compound e.g. b(g)p. Also noticeable was that several LC/PB/MS papers dealing with PAH do not attempt to quantify them but report qualitative data only (see later). The UV and PFD results obtained in the present study are in agreement with the literature values, the PB/MS results are slightly worse than the IDLs quoted by Pace and Betowski [155].

3.4.7 Investigation of Recoveries Following SPE of EPA-16

The results of this work are shown in Table 3.6 and Figure 3.7 :-

Table 3.6 *Recovery Experiments for Spiked SPE Cartridges*

SPE Cartridge	COMPOUND							
	b(a)a	chr	b(b)f	b(k)f	b(a)p	d(a)a	b(g)p	i(1)p
silica gel								
% RECOVERY	48	83	86	81	76	116	86	84
+/- 1 S.D.	26	12	13	26	19	23	29	12
% VARIANCE	54	14	15	32	25	20	34	14
C18								
%RECOVERY	91	97	100	94	87	101	101	92
+/- 1 S.D.	11	9	7	7	5	5	17	7
% VARIANCE	12	9	7	7	6	5	17	8

Cartridges were spiked with 50ul of 100 µg/ml EPA-16 mix.

Each result shown is the result of three spikes (n=3)

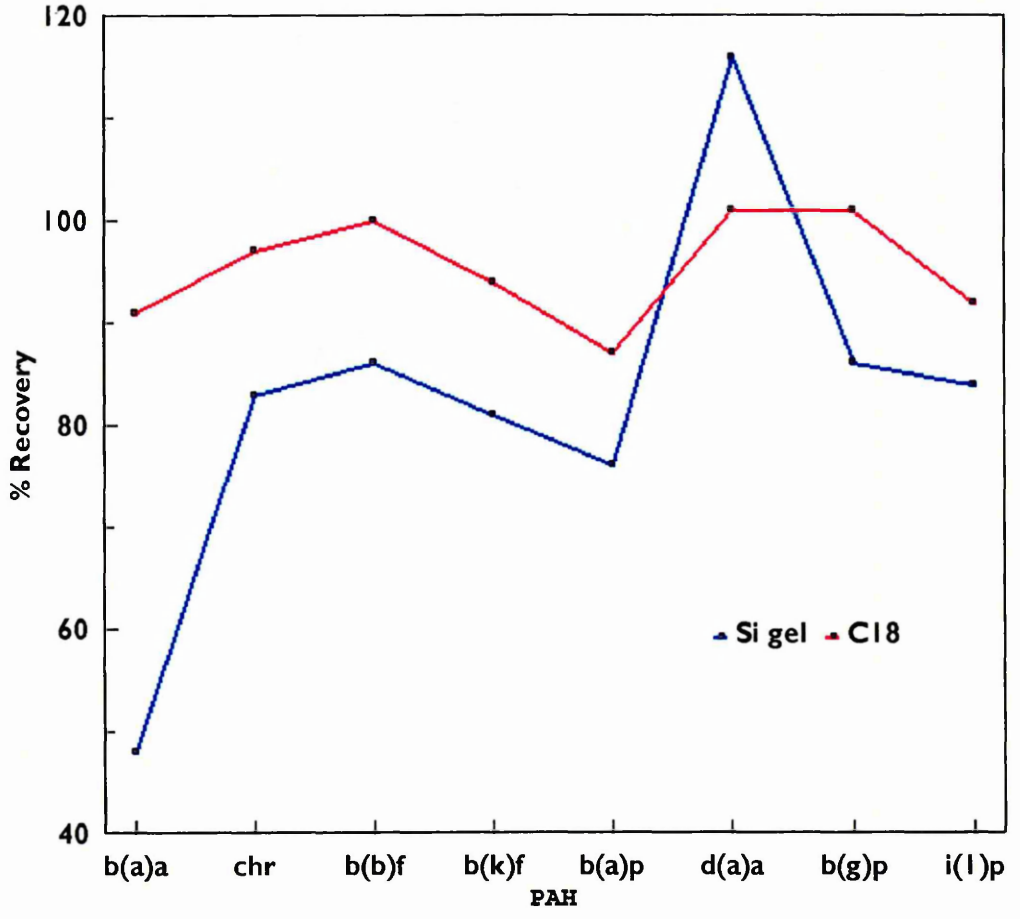
% variance is {1 S.D. (standard deviation) / % recovery }

The Hewlett-Packard Hypersil Green (PAH) (100 x 4.6 mm) column was used in these experiments.

Abbreviations as listed on p68 , i.e. b(a)a = benzo(a)anthracene etc.

Figure 3.7 *Recovery Experiments for Spiked SPE Cartridges*

(for Experimental details see Section 3.3.7)



From the results shown in Table 3.6 and Figure 3.7 it can be seen that C18 SPE cartridges appear to give better recoveries and better reproducibilities than Silica gel SPE cartridges. For both types no recovery was observed for compounds with an RMM less than b(a)a (i.e <228). A similar effect was observed with GPC as the cleanup method and it is believed that this is due to the drying down procedure used to concentrate the samples (e.g. concentration by blowing down with

nitrogen). The use of SPE, strategies for SPE cleanup of PAH and various examples of the use of SPE for PAH analysis were given in Section 3.3.7 [101,111,130,132,139,141-143].

Chapter 4

Analysis of PAH in Brick Dust

4.0 Introduction

The experimental conditions obtained from the work described in Chapter 3 were applied to the analysis of PAH in environmental samples. Brick dust samples from refractory bricks used for lining chimneys were analysed for PAH in the soot and tar that had collected on the bricks during chimney use. Qualitative work, using LC/PB/MS and GC/MS in scan mode, and quantitative work using DAD, PFD, LC/PB/MS(SIM) and GC/MS (scan mode) was carried out. The compounds quantified were the "EPA-16" due to the easy availability of standards for this set of PAH.

4.1 Experimental

Experimental conditions used were those described in Chapter 3, Sections 3.3.1 to 3.3.7. Three different extracting solvents (cyclohexane, methanol and dichloromethane) and two different extraction procedures (sonication (25ml of solvent) and Soxhlet (250 ml of solvent)) were used to assess the relative merits of each. For clean up SPE, using silica gel (filter mode) and C18 (enrichment mode) were used for the LC samples. This is similar to EPA-625, which is a method for the determination of various compounds using GC/solid phase extraction (SPE) in drinking and raw waters. To try to decrease the losses of volatile PAH, an involatile "keeper" was used as suggested by Matthiessen [165]. The compound used was iso-octanol (1ml) added at the drying down stage (see Fig. 4.1).

Selected extracts were then run on the LC/PB/MS and GC/MS systems in scan mode to assess the relative merits of these two systems for qualitative work and to enable comparison of both with DAD (library matching). For the LC/PB/MS work scan and SIM modes were used, with the SIM ions monitored being; (m/z) 128,152,166,178,202,228,252,276, 278, 300,302,326 and 348. SIM mode was used for the quantitative work.

The LC conditions used for this work were: Hypersil Green (PAH) (100 x 4.6 mm), flow rate 0.3 ml/min mobile phase (gradient as section 3.3.6) with 0.2 ml/min. post column addition of acetonitrile for LC/PB/MS or flow rate 0.5 ml/min. for LC/DAD and LC/PFD. GC conditions as described in section 3.3.6.

The quantitative GC/MS samples were all cleaned up using GPC. GPC was used to try to prevent contamination of the GC column by co-extracting compounds in the brickdust and by brickdust particulate. The equipment, theory and use of GPC has been described previously [97] . Before GPC was used for the cleanup of the samples a standard of the EPA-16 PAH mix (1ml injection of Alltech 5µg/ml per component EPA-16 standard) was injected into the GPC system. This provided information on the retention time of the PAH peak and what fractions of the GPC eluent to collect. The resuspended GPC fraction was then analysed by GC/MS and the results compared with the original solution.

The following recoveries, relative to a portion of the standard which had not been treated by GPC, were obtained for the GPC cleanup, drying down of fractions and resuspension (5ml of dichloromethane) of the PAH standard; nap, ace, ace, acy, flu,

phe ant all gave 0% recovery, fla 62%, pyr 65%, b(a)a 110%, chr 122%, b(a)p 121%, b(k)f 114%, b(b)f 97%, b(g)p 125%, d(a)a 136% and i(1)p 109%. The lighter PAH i.e. RMM < 178 are totally lost in the drying down process, the PAH with RMM 202 have low recoveries, the PAH with RMM >202 give acceptable recoveries. The main focus of the quantitative work was the large RMM PAH and so it was therefore decided that GPC would be used to clean up the samples. The use of GPC for the clean up of PAH in urban air particulate (Taipai City, Taipai)) and SRM-1649 (supplied by NIST) has been described by Wu and Chang [166].

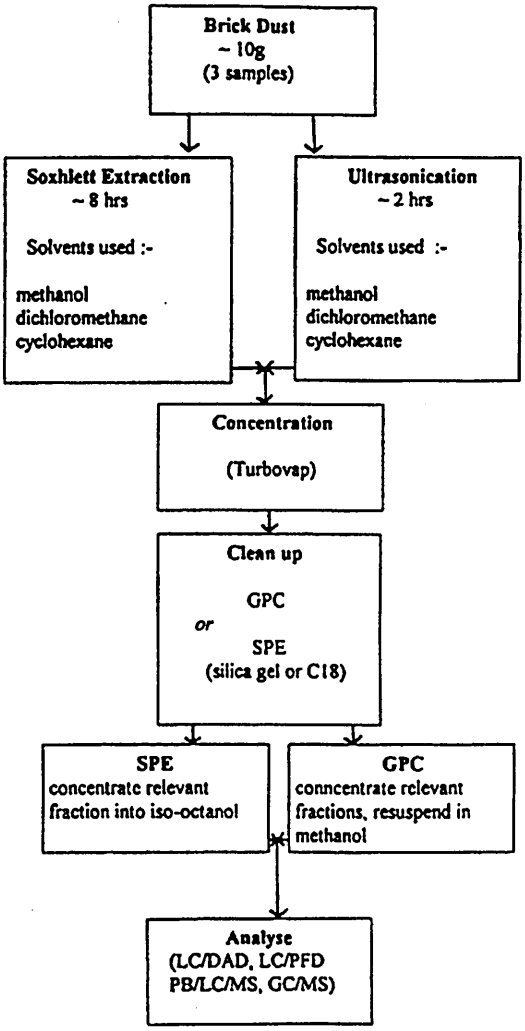
The samples were then analysed using a variety of methods, i.e. LC/DAD, LC/PFD, LC/PB/MS (selected extracts) and GC/MS (selected extracts). A UV method using 255nm for the analytical wavelength was set up and the PFD conditions were as described in section 3.3.6. The PFD timetable is similar to that of Noël et al. [125].

An attempt to use a timetabled UV method, with specified wavelengths for each PAH was unsuccessful due to the limited memory of the computer controlling the LC system. The attempt to save several hours of DAD data and then extract and process this data at several wavelengths caused the system to crash several times and so was abandoned. However, in theory this would be a way to increase selectivity and sensitivity of the UV method.

Such an approach has been successfully used by Dridi et al.[126]. These workers used a UV program with 7 wavelength changes. The LC/PB/MS samples had d^{12}-perylene (RMM 264) added as an internal standard, after to the clean up procedure, this was used to try to compensate for losses due to the PB interface.

Due to the number of analytes determined no spiking or recovery experiments were carried out. Burford et al. [92] have commented on the limitations of spiking experiments for complex environmental matrices.

Figure 4.1. *Analysis of Brickdust Extracts*



4.2 Results

The results and some example chromatograms for each detector type are presented in Tables 4.1.1 to 4.3.4 and figures 4.2 to 4.4. Some examples of the graphs used to deduce the relationships between extraction method, extraction solvent and detector type, for the three brickdust extracts, are shown in figures 4.5 to 4.13.

Key to Figures

PAH

nap	naphthalene	acy	acenaphthylene
ace	acenaphthene	flu	fluorene
phe	phenanthrene	ant	anthracene
fla	fluoranthene	pyr	pyrene
b(a)a	benzo(a)anthracene	chr	chrysene
b(b)f	benzo(b)fluoranthene	b(k)f	benzo(k)fluoranthene
b(a)p	benzo(a)pyrene	d(a)a	dibenzo(a,h)anthracene
b(g)p	benzo(g,h,i)perylene	i(1)p	ideno(1,2,3 c-d)pyrene

Extraction solvents

d	dichloromethane	ch	cyclohexane
m	methanol		

Detectors

F	fluorescence (PFD)	UV	UV/vis at 255 nm
GC	GC/MS scan mode	PB	PB/LC/MS

Extraction Procedure

son	sonication	sox	Soxhlet

Table 4.1.1 Data for the Analysis of Brickdust # 9898 (Fluorescence)

PAH	F /ch/ son	F /m/ son	F /d/ son	F /ch/ sox	F /m/ sox	F /d/ sox
nap	ND	ND	ND	ND	ND	ND
acy	ND	ND	ND	ND	ND	ND
ace	ND	ND	ND	ND	ND	ND
flu	ND	ND	ND	ND	ND	ND
phe	6	ND	ND	ND	ND	ND
ant	1	ND	ND	ND	ND	4500
fla	142	115	114	ND	1436	0.13 $x10^5$
pyr	157	122	136	ND	188	141
baa	56	70	76	ND	ND	ND
chr	50	36	47	ND	145	87
bbf	6	40	55	ND	ND	ND
bkf	8	20	26	100	ND	ND
bap	26	35	44	102	18	5
daa	ND	ND	ND	ND	ND	5
bgp	ND	ND	ND	ND	ND	ND
i1p	ND	4	ND	ND	ND	ND

results are ng PAH per sample (10g of brickdust)

Table 4.1.2 Data for the Analysis of Brickdust # 9898 (UV)

PAH	UV /ch/ son	UV /m/ son	UV /d son	UV /ch/ sox	UV /m/ sox	UV /d/ sox
nap	ND	ND	ND	ND	ND	ND
acy	ND	ND	ND	ND	ND	ND
ace	ND	ND	ND	ND	ND	ND
flu	ND	ND	ND	ND	ND	ND
phe	144	153	234	1287	9108	378
ant	ND	ND	ND	1170	6786	ND
fla	322	721	714	2317	0.16 $x10^5$	ND
pyr	1816	920	1258	2538	0.19 $x10^5$	0.34 $x10^5$
baa	722	667	704	1677	0.12 $x10^5$	0.42 $x10^5$
chr	563	607	640	2114	0.15 $x10^5$	5074
bbf	1398	1006	152	4412	0.36 $x10^5$	6558
bkf	150	252	286	ND	0.12 $x10^5$	17848
bap	936	2012	2384	8824	0.71 $x10^5$	1.32 $x10^5$
daa	ND	238	ND	1092	4928	ND
bgp	88	128	88	872	9624	0.22 $x10^5$
i1p	96	64	320	2920	4684	4600

results are ng PAH per sample (10g of brickdust)

Table 4.1.3 Data for the Analysis of Brickdust # 9898 (PB)

PAH	PB /d/ sox
nap	ND
acy	ND
ace	ND
flu	ND
phe	1
ant	1
fla	1
pyr	1
baa	0.17 $x10^5$
chr	1762
bbf	2030
bkf	ND
bap	1
daa	9399
bgp	482
i1p	1685

results are ng PAH per sample (10g of brickdust)

Table 4.1.4 Data for the Analysis of Brickdust # 9898 (GC)

PAH	GC /ch/ sox	GC /m/ sox	GC /d/ sox	GC /d/ son	GC /m/ son	GC /ch/ son
nap	ND	ND	ND	ND	ND	ND
acy	ND	ND	ND	ND	ND	ND
ace	ND	ND	ND	ND	ND	ND
flu	ND	ND	ND	ND	ND	ND
phe	ND	ND	ND	ND	ND	ND
ant	ND	ND	ND	ND	ND	ND
fla	3000	0.39 $x10^5$	250	250	0.11 $x10^5$	6750
pyr	2750	0.33 $x10^5$	2750	250	0.17 $x10^5$	6000
baa	1750	0.38 $x10^5$	2250	750	0.15 $x10^5$	4750
chr	2500	1.10 $x10^5$	3250	2500	0.43 $x10^5$	5250
bbf	2750	0.53 $x10^5$	5500	3500	0.46 $x10^5$	5250
bkf	5500	0.31 $x10^5$	8000	4500	0.29 $x10^5$	7250
bap	0.13 $x10^5$	1.65 $x10^5$	0.21 $x10^5$	9500	0.70 $x10^5$	0.17 $x10^5$
daa	3000	0.96 $x10^5$	0.11 $x10^5$	5000	0.11 $x10^5$	0.24 $x10^5$
bgp	1750	13500	1750	3000	0.11 $x10^5$	500
i1p	1250	31000	3500	2750	8750	1250

results are ng PAH per sample (10g of brickdust)

Table 4.2.1 Data for the Analysis of Brickdust # 10026 (Fluorescence)

PAH	F /ch/ son	F /m/ son	F /d/ son	F /ch/ sox	F /m/ sox	F /d/ sox
nap	ND	ND	ND	ND	ND	ND
acy	ND	ND	ND	ND	ND	ND
ace	ND	ND	ND	ND	ND	ND
flu	ND	ND	ND	ND	ND	ND
phe	135	ND	ND	ND	ND	ND
ant	ND	ND	ND	ND	ND	1650
fla	90	181	6	ND	43	57
pyr	23	31	ND	ND	8	1
baa	13	176	15	ND	ND	ND
chr	536	696	310	ND	145	145
bbf	6	11	3	ND	113	113
bkf	1	2	1	38	93	93
bap	2	3	2	31	100	100
daa	ND	ND	ND	23	8	8
bgp	ND	ND	ND	ND	ND	ND
i1p	ND	ND	ND	ND	ND	ND

results are ng PAH per sample (10g of brickdust)

Table 4.2.2 Data for the Analysis of Brickdust # 10026 (UV)

PAH	UV /ch/ son	UV /m/ son	UV /d/ son	UV /ch/ sox	UV /m/ sox	UV /d/ sox
nap	ND	ND	ND	ND	ND	ND
acy	ND	ND	ND	90	ND	120
ace	ND	ND	ND	37	ND	ND
flu	ND	ND	ND	ND	ND	ND
phe	423	873	558	219	3591	1791
ant	ND	390	3	ND	1924	1248
fla	ND	ND	ND	ND	6286	3997
pyr	ND	ND	ND	118	2122	3336
baa	ND	ND	1071	94	ND	ND
chr	421	598	598	205	2643	2948
bbf	ND	ND	ND	360	ND	ND
bkf	ND	ND	ND	384	2252	2252
bap	ND	136	ND	ND	ND	ND
daa	ND	1071	ND	91	1316	2289
bgp	32	40	ND	152	1504	2616
i1p	ND	24	ND	228	2184	4696

results are ng PAH per sample (10g of brickdust)

Table 4.2.3 Data for the Analysis of Brickdust # 10026 (PB)

PAH	PB /d/ son
nap	ND
acy	ND
ace	ND
flu	ND
phe	ND
ant	ND
fla	ND
pyr	1
baa	1
chr	828
bbf	589
bkf	668
bap	1
daa	ND
bgp	500
i1p	228

results are ng PAH per sample (10g of brickdust)

Table 4.2.4 Data for the Analysis of Brickdust # 9898 (GC)

PAH	GC /d/ son	GC /d/ sox
nap	ND	ND
acy	ND	ND
ace	ND	ND
flu	ND	ND
phe	ND	ND
ant	ND	ND
fla	ND	ND
pyr	ND	250
baa	ND	1750
chr	3250	7250
bbf	1500	2750
bkf	3500	ND
bap	8000	8000
daa	8250	1250
bgp	1000	2750
i1p	3500	9250

results are ng PAH per sample (10g of brickdust)

Table 4.3.1 Data for the Analysis of Brickdust # 10441 (Fluorescence)

PAH	F /ch/ son	F /d/ son	F /m/ son	F /ch/ sox	F /d/ sox	F /m/ sox
nap	ND	ND	ND	ND	ND	ND
acy	ND	ND	ND	ND	ND	ND
ace	ND	ND	ND	ND	ND	ND
flu	ND	ND	ND	ND	ND	ND
phe	3	ND	ND	ND	1750	70
ant	1	ND	ND	ND	ND	ND
fla	113	619	941	2111	2714	1576
pyr	128	97	719	349	401	302
baa	56	822	370	3800	5704	4732
chr	57	1600	245	1922	5591	3335
bbf	99	78	343	10	247	142
bkf	30	20	138	23	92	47
bap	55	24	201	23	131	68
daa	ND	ND	ND	ND	ND	ND
bgp	ND	ND	ND	ND	ND	ND
i1p	ND	20	ND	ND	147	56

results are ng PAH per sample (10g of brickdust)

Table 4.3.2 Data for the Analysis of Brickdust # 10441 (UV)

PAH	UV /ch/ son	UV /d/ son	UV /m/ son	UV /ch/ sox	UV /d/ sox	UV /m/ sox
nap	ND	ND	ND	ND	ND	ND
acy	ND	ND	ND	ND	ND	ND
ace	ND	ND	ND	ND	ND	ND
flu	ND	ND	ND	ND	ND	ND
phe	495	54	1296	873	2556	4230
ant	ND	ND	364	754	ND	364
fla	756	812	2093	2674	3675	6076
pyr	574	896	2522	2782	3498	6428
baa	287	366	1135	859	1550	2511
chr	305	438	1120	785	2014	2741
bbf	766	1036	2862	1398	3248	6338
bkf	58	210	1226	150	948	1314
bap	1532	2072	1180	2796	6496	$1.27 X10^4$
daa	ND	ND	161	63	182	287
bgp	140	160	640	248	1096	1264
i1p	332	96	2032	352	3616	3592

results are ng PAH per sample (10g of brickdust)

Table 4.3.3 Data for the Analysis of Brickdust # 10441 (PB)

PAH	PB /m/ son	PB /d/ sox	PB /m/ sox	PB /ch/ sox	PB /d/ son
nap	ND	ND	ND	ND	ND
acy	ND	ND	ND	ND	ND
ace	ND	ND	ND	ND	ND
flu	ND	ND	ND	ND	ND
phe	1	1	1	1	1
ant	1	1	1	1	1
fla	1	1	1	1	1
pyr	1	1	1	1	1
baa	5055	3664	$2.36 X10^4$	305	1949
chr	1350	1469	5592	1717	1090
bbf	1	1	517	1	949
bkf	1	208	278	ND	ND
bap	1	ND	$2.58 X10^4$	1	767
daa	ND	ND	320	1	189
bgp	208	548	2701	1440	1003
i1p	304	ND	4391	2936	1440

results are ng PAH per sample (10g of brickdust)

Table 4.3.4 Data for the Analysis of Brickdust # 10441 (GC)

PAH	GC /d/ son	GC /m/ son
nap	ND	ND
acy	ND	ND
ace	ND	ND
flu	ND	ND
phe	ND	ND
ant	ND	ND
fla	1250	750
pyr	1250	750
baa	2750	1500
chr	9250	5250
bbf	7250	7250
bkf	$1.98 X10^4$	3750
bap	$3.23X10^4$	$3.60 X10^4$
daa	3250	3250
bgp	2500	2500
i1p	2250	2200

results are ng PAH per sample (10g of brickdust)

Figure 4.2 Example Chromatograms of EPA-16 Standard using LC/PFD, LC/DAD LC/PB/MS and GC/MS

(For Experimental details see Section 4.2)

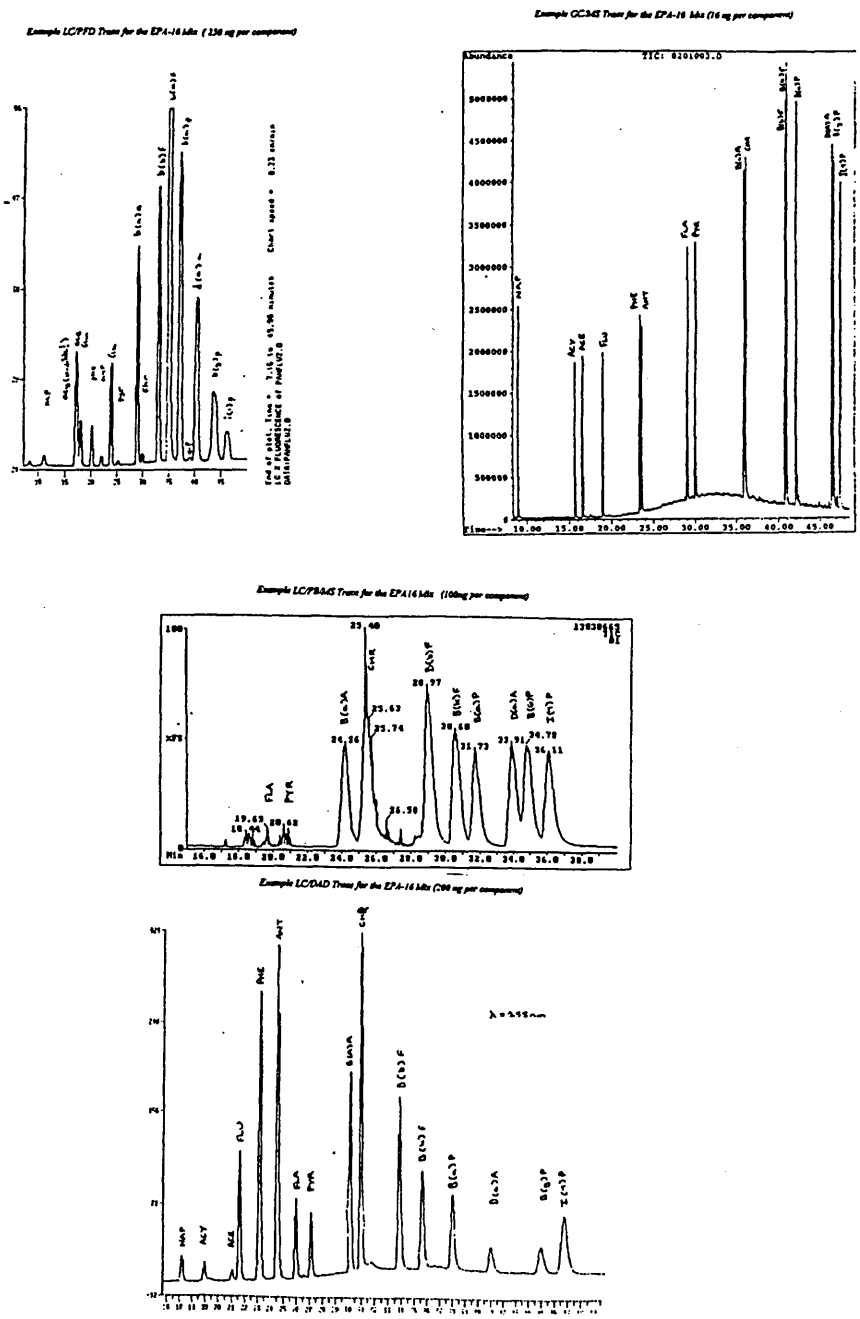


Figure 4.3. Example Chromatograms of Brickdust #10026 by LC/PFD, LC/DAD LC/PB/MS and GC/MS

(For Experimental details see Section 4.2)

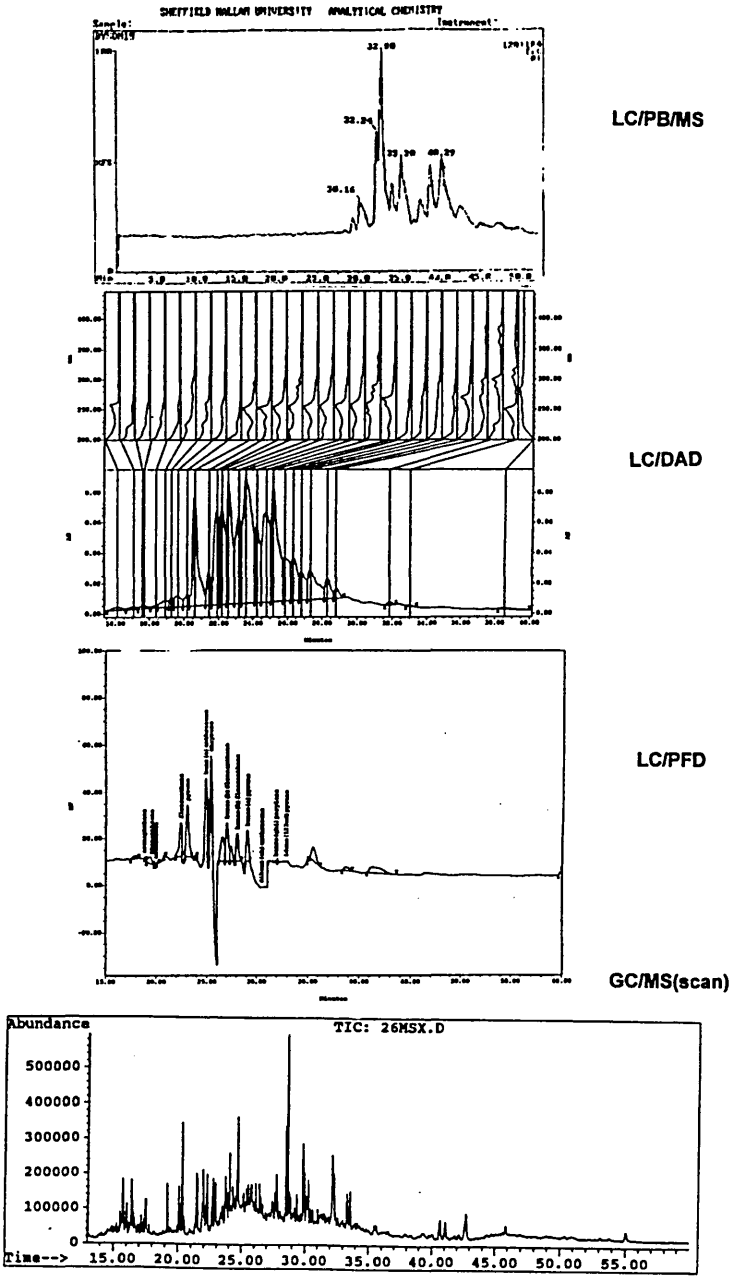
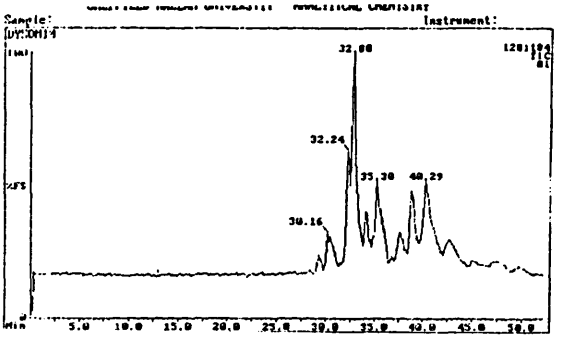


Figure 4.4. Example Chromatogram and Ion Chromatograms for Brickdust #10026, dichloromethane extract, sonication extraction using LC/PB/MS

(For Experimental details see Section 4.2)



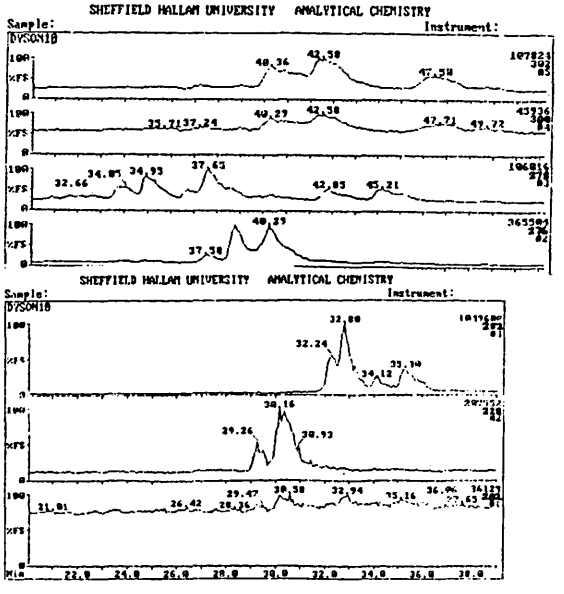


Figure 4.5 PAH in Brickdust # 10026, Effect of the three extraction solvents (dichloromethane, methanol and cyclohexane) Soxhlet extraction UV Detection (255nm)

(for Experimental see Section 4.2)

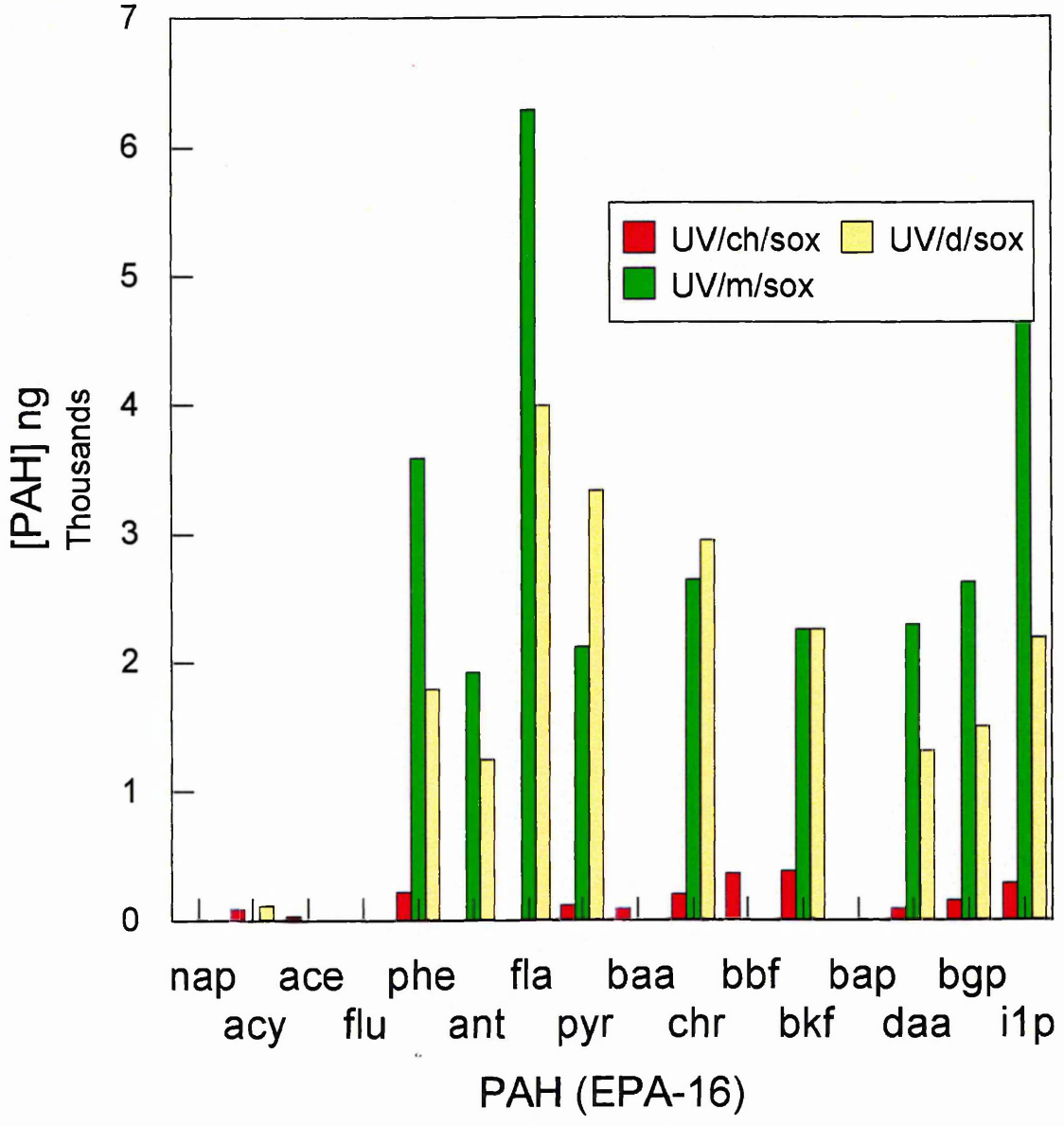


Figure 4.6 PAH in Brickdust # 10026, Effect of the three extraction solvents (dichloromethane, methanol and cyclohexane) Soxhlet extraction Programmed Fluorescence Detection

(for Experimental see Section 4.2)

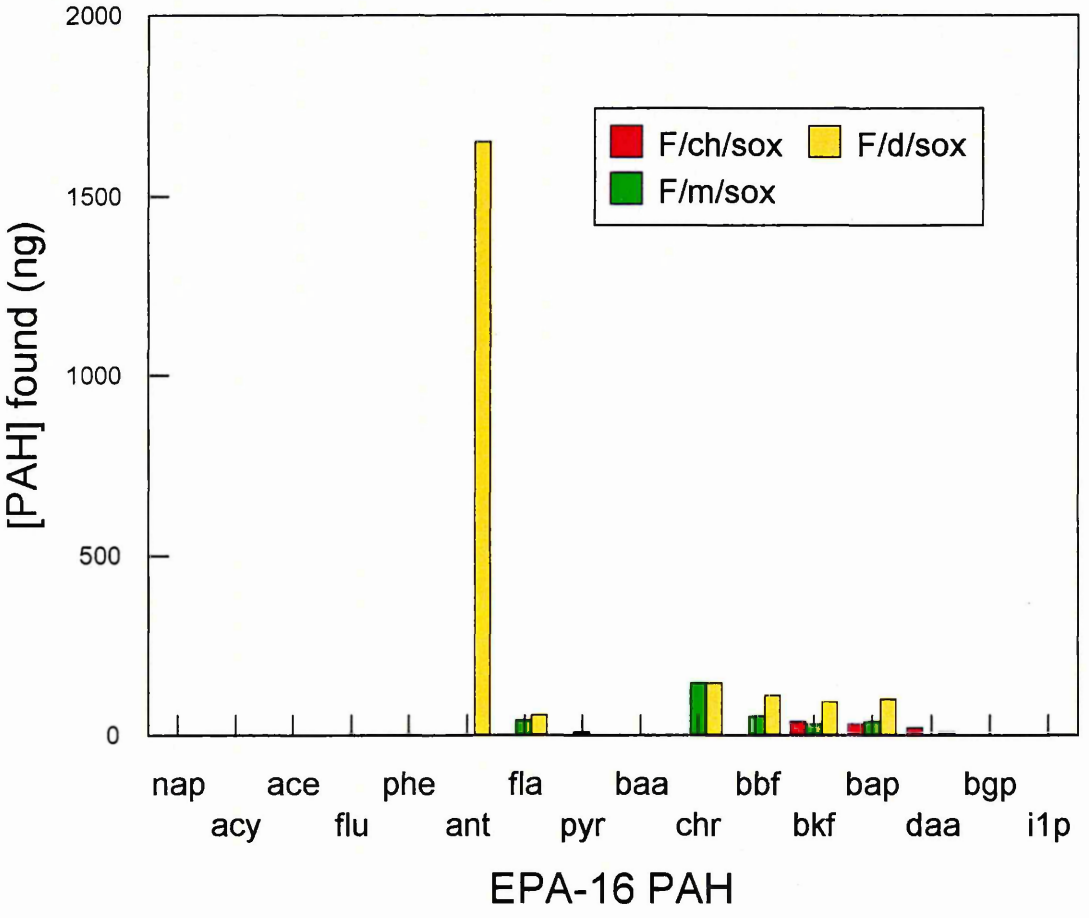


Figure 4.7 PAH in Brickdust # 10441, Effect of the three extraction solvents (dichloromethane, methanol and cyclohexane)

Sonication extraction, UV Detection (255nm)

(for Experimental see Section 4.2)

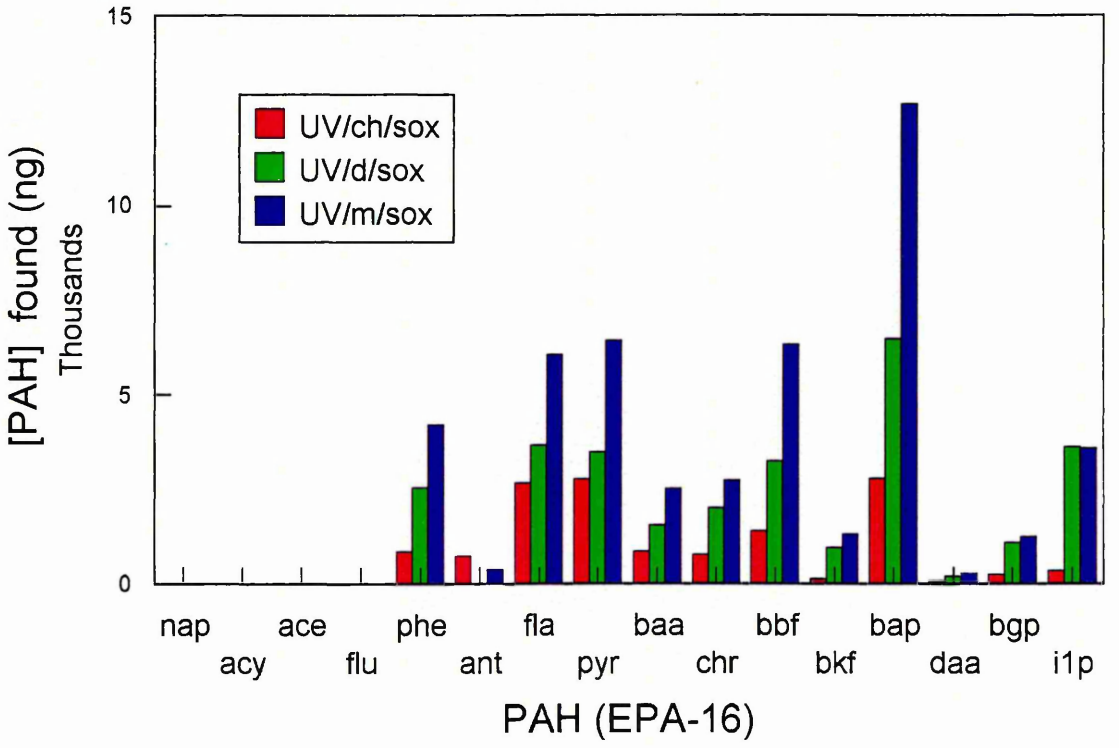


Figure 4.8 PAH in Brickdust # 10441, Effect of the three extraction solvents (dichloromethane, methanol and cyclohexane) Sonication extraction,Programmed Fluorescence Detection) (for Experimental see Section 4.2)

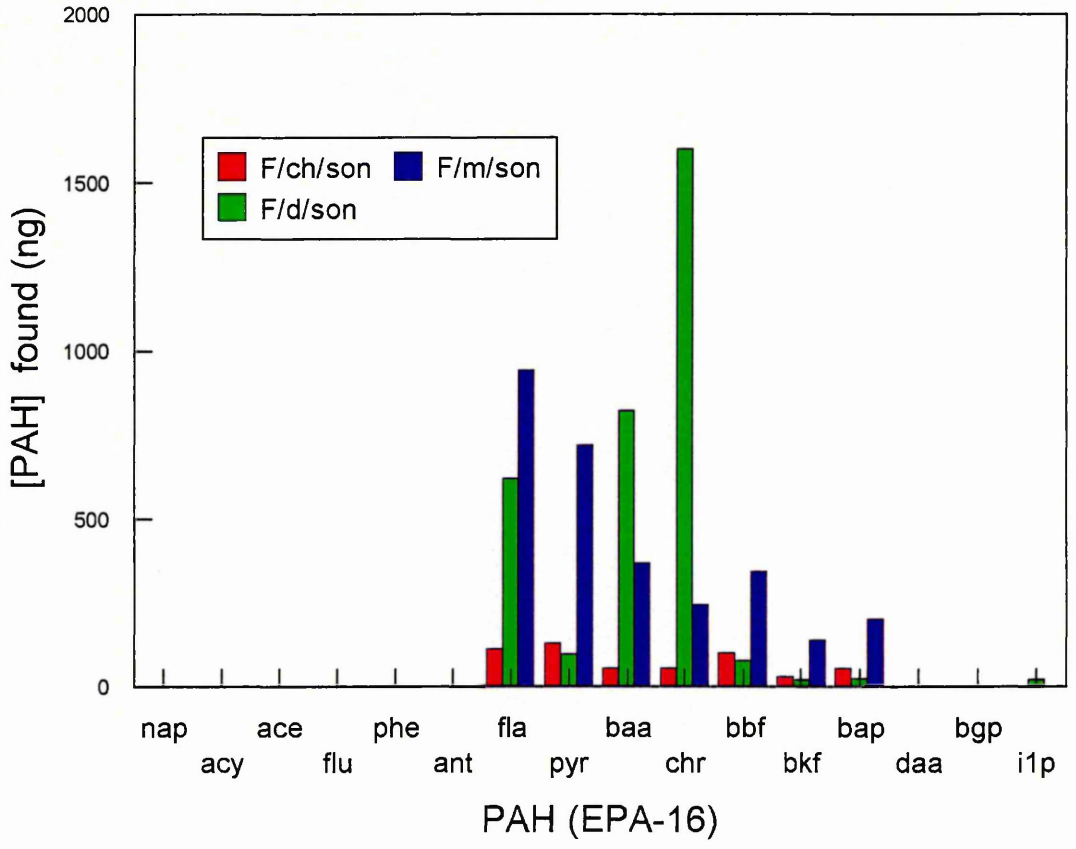
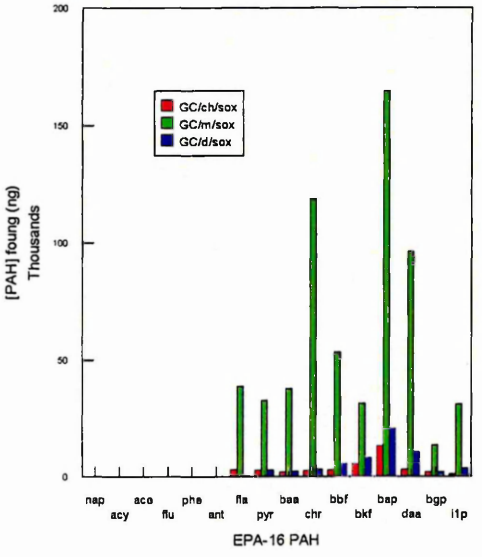


Figure 4.9 PAH in Brickdust # 9898, Effect of the three extraction solvents (dichloromethane, methanol and cyclohexane) Soxhlet and Sonication extraction,GC/MS Detection)

(for Experimental see Section 4.2)



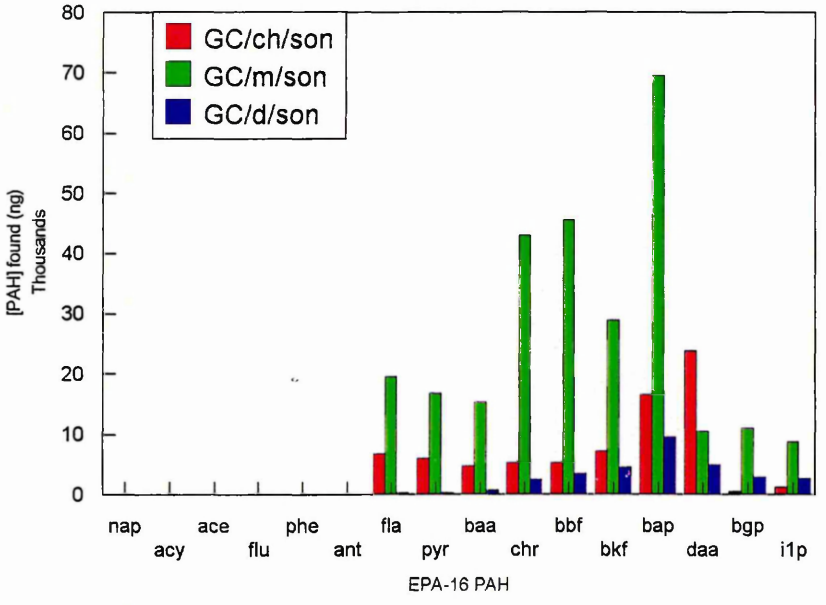


Figure 4.10 PAH in Brickdust # 9898, Effect of the four detector types for a selected extract, dichloromethane solvent, Soxhlet extraction (for Experimental see Section 4.2)

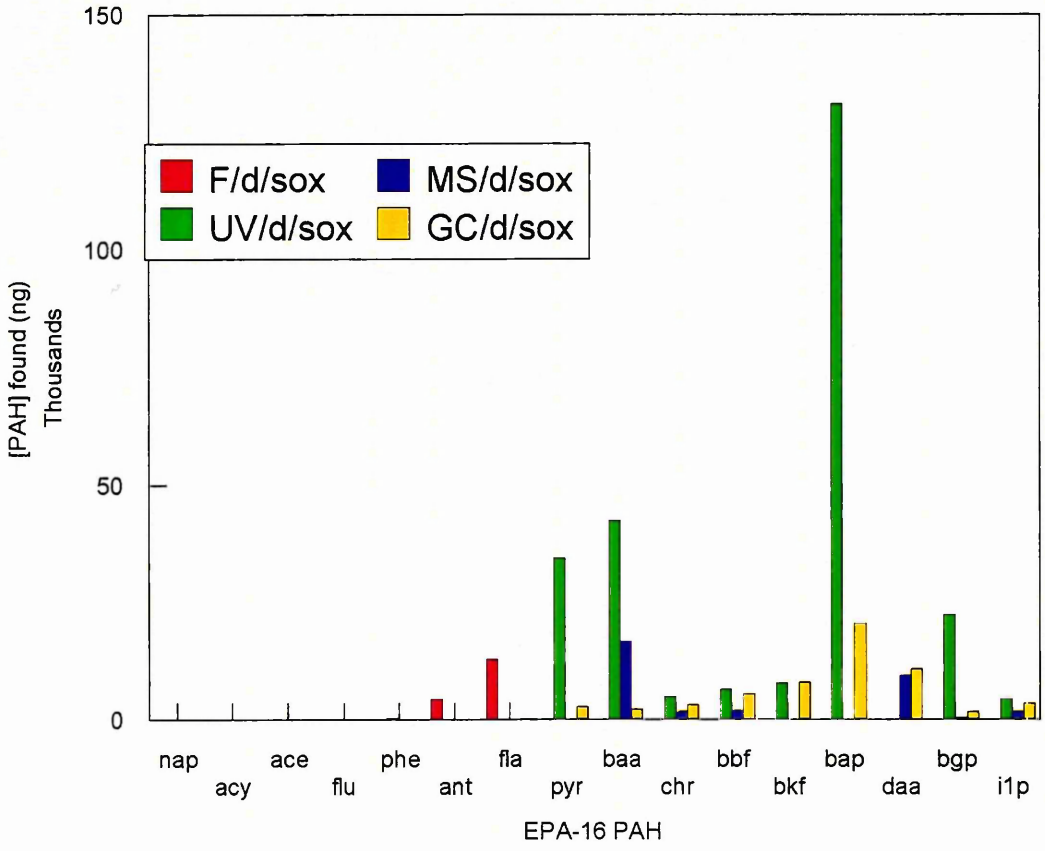


Figure 4.11 PAH in Brickdust # 10441, LC/PB/MS data, various extraction solvents and extraction procedures

(for Experimental see Section 4.2)

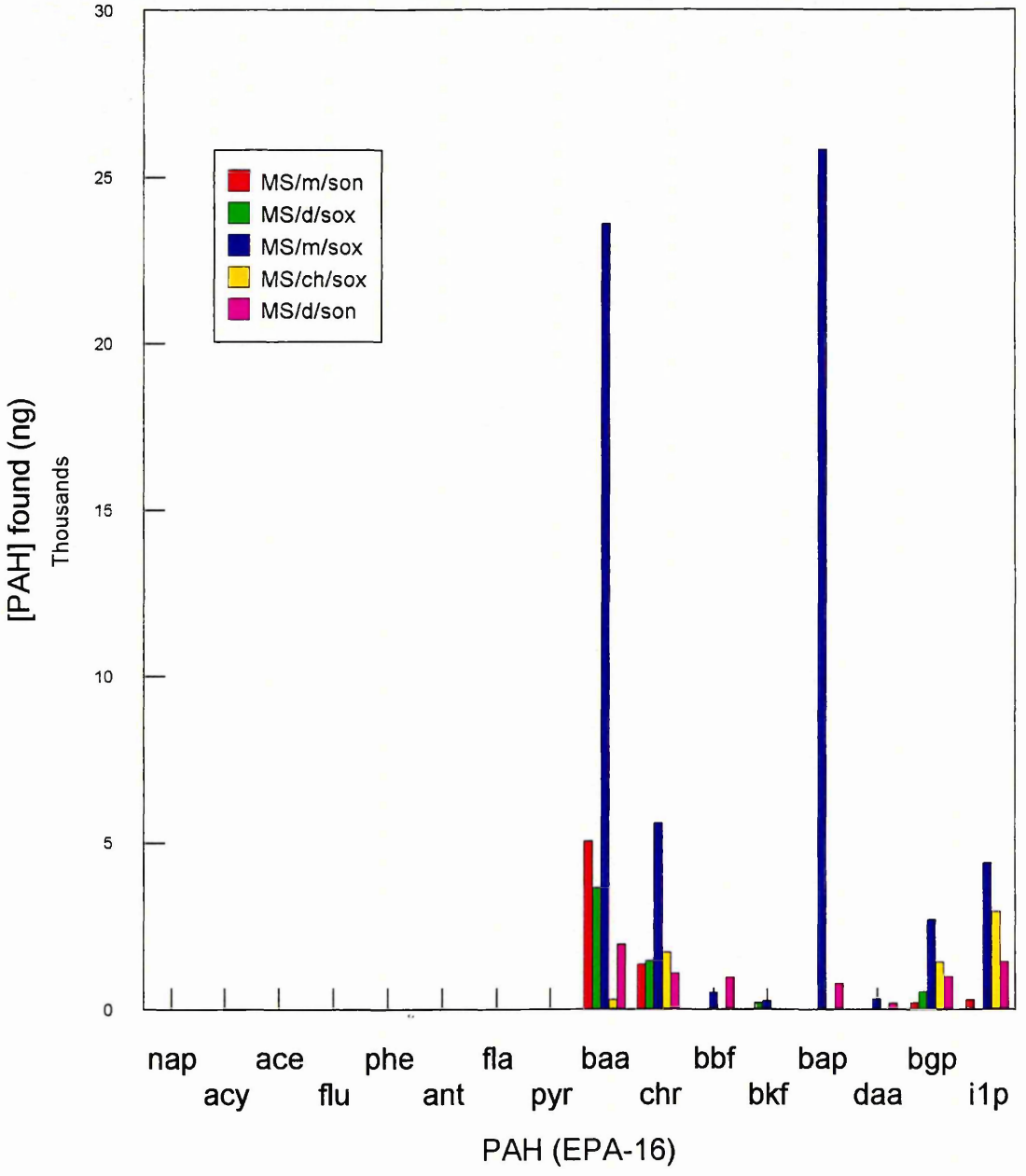


Figure 4.12 PAH in Brickdust # 10441, Effect of Detector type for a selected extract (methanol solvent, sonication extraction)

(for Experimental see Section 4.2)

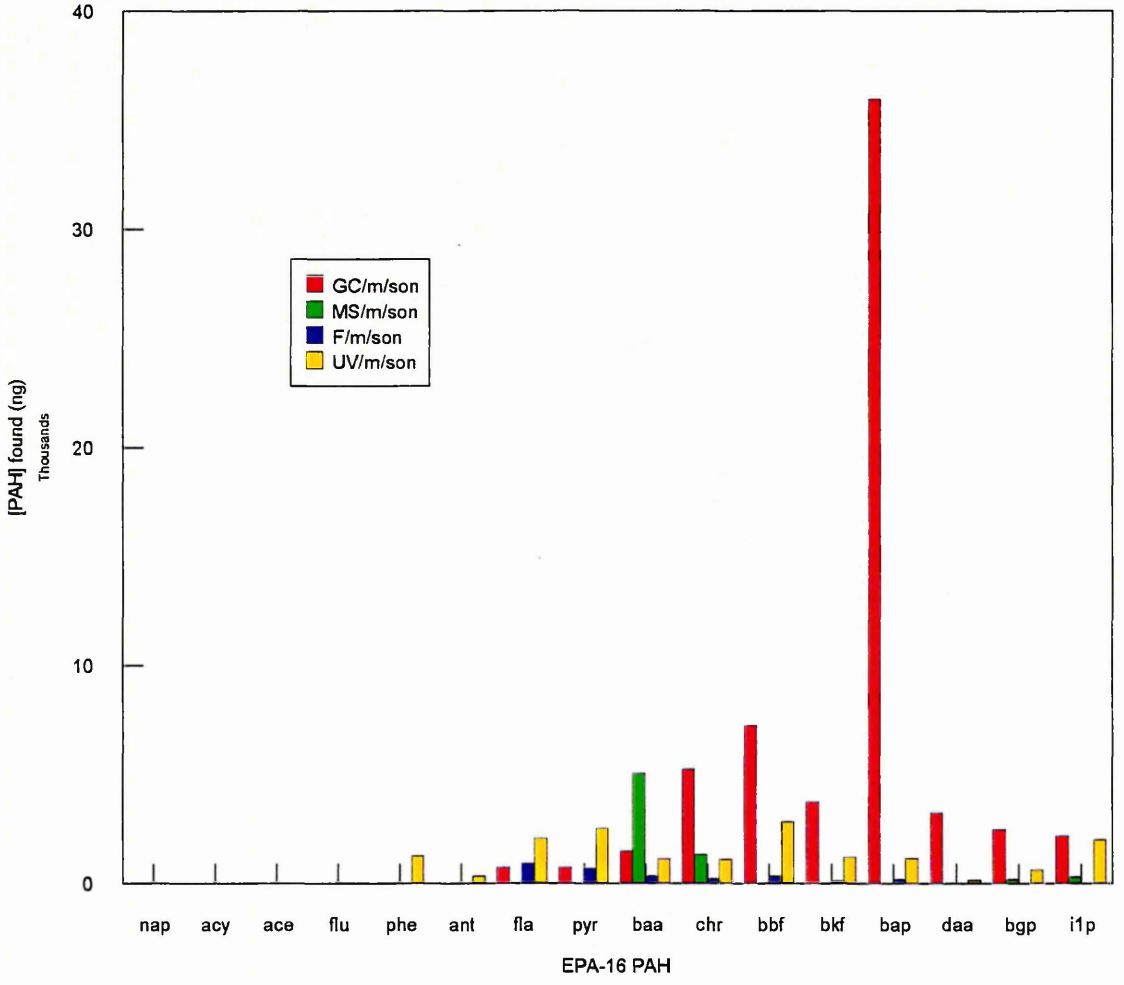
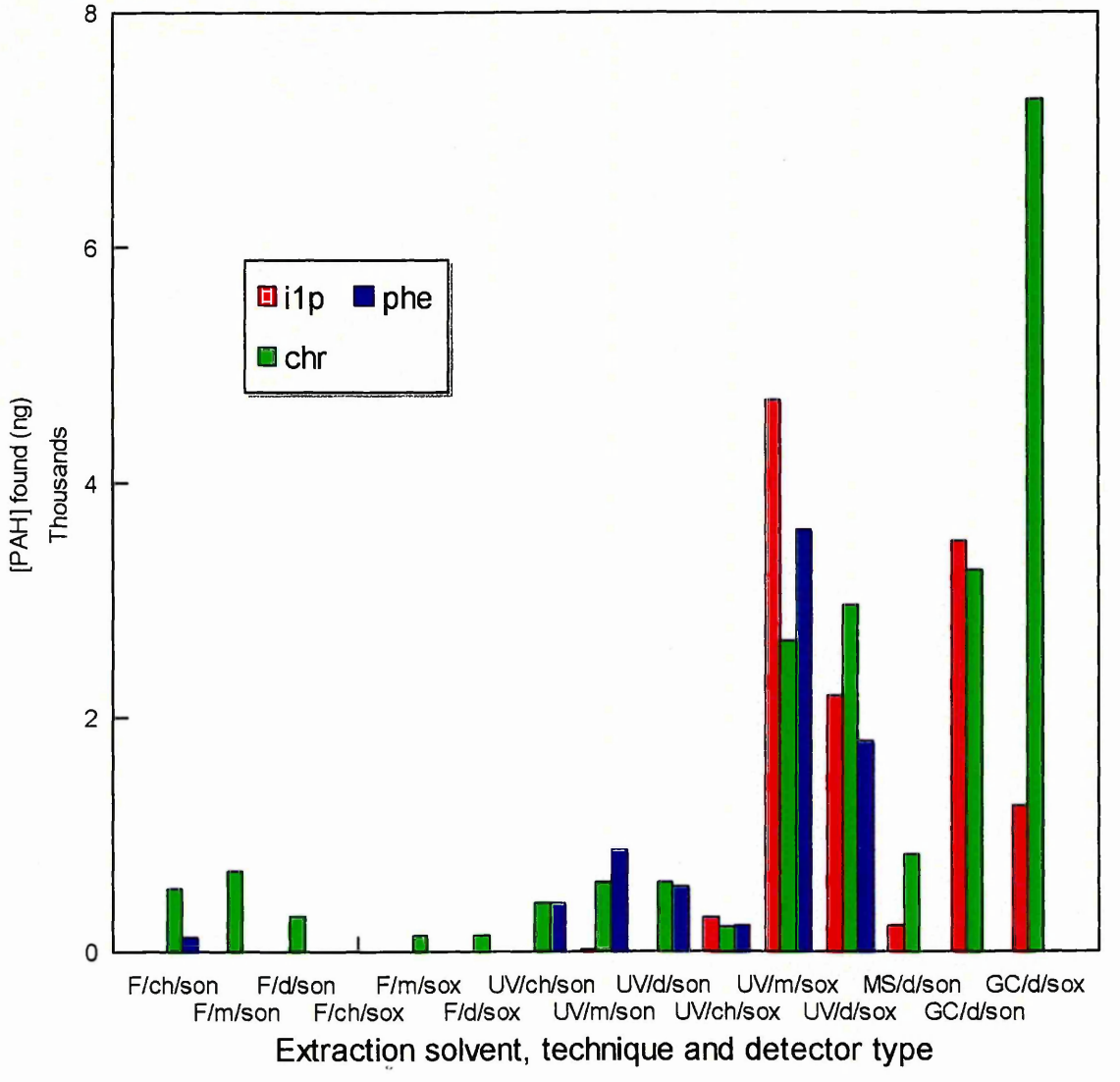


Figure 4.13 PAH in Brickdust # 0026 Using LC/PFD, LC/DAD, LC/PB/MS GC/MS, three extraction solvents (dichloromethane, methanol and cyclohexane) and sonication or Soxhlet extraction, selected analytes (for Experimental see Section 4.2)



4.3 Discussion of Brickdust Results - Quantitative work

The results for the extraction of PAH in brickdusts were consistent within each detector type but variable between the detector types (see Figs. 4.2 to 4.13 and Tables 4.1.1 to 4.3.4). Brickdust #9898 contained a greater number and larger concentrations of PAH than #10441 which, in turn, contained more than #10026. The four techniques studied gave results that were consistent within each detector type i.e Soxhlet extraction gave higher recoveries than sonication and cyclohexane as extracting solvent gave lower recoveries than methanol or dichloromethane for all four detector types. Comparison of results between detector types showed wide variations. The reasons for this between detector variability are discussed below.

For the DAD results, the following conclusions may be drawn. The calibration curves obtained for the 16 PAH quantified ("EPA-16") generally showed excellent linearity. This is in contrast to the LC/PB/MS work as reported below. For all three brickdusts the Soxhlet extracted samples gave larger recoveries than the sonication extracted samples. Dichloromethane and methanol were better extraction solvents than cyclohexane. The LC/DAD runs for all extracts were particularly badly affected by co-eluting peaks. This lead to difficulties with integration of the PAH peaks due to lack of baseline resolution and affected the quantification of these peaks by UV. These co-eluting peaks also make identification of the later eluting PAH (e.g. RMMs >276) more difficult. This is discussed more fully below.

In addition, variations in retention time over the sample sequence were noticed. This took the PAH peaks out of the retention time window for that compound (as entered

in the method) and so peaks were not recognised as PAH peaks or peaks were incorrectly identified. This meant that time consuming re-analysis and eventually manual peak by peak identification (library matching done on the second run) of the peaks in each chromatogram was required.

As the LC method for PAH used a gradient (as described above) it was thought that this might be due to an insufficient equilibrium time between runs. The usual "rule of thumb" for equilibration time is that required for 2 to 3 column volumes of solvent to pass through the column [10f]. This gives an equilibration volume, for a 100 x 4.6 mm O.D. column, of 3 (10 x $(0.23)^2$ x 3.142) or ~ 5.1 cm^3. This corresponds, at a flow rate of 1ml/min., to an equilibration time of ~ 5 mins. An equilibration time of 10 mins. was used in the first run of the brickdust samples. A second run was carried out using a longer equilibration time of 15 mins. The results quoted above are for the second run. However, the retention time drift over the sequence was still observed.

The effect of ambient temperature fluctuations, even in an air conditioned, temperature controlled laboratory have been commented on by Pace and Betowski [155]. These authors noted that maintenance of constant column temperature was important for reproducible chromatography. An ambient temperature change resulted in retention time decrease by ~3% over 4 hours. This is similar to the retention time drift found in these runs. The laboratory temperatures at HSL are controlled at ~21 °C during the day and switched to 16 °C overnight (after 7p.m). At the time these DAD runs were carried out there were no column ovens fitted to the Waters LC system at HSL used for these experiments and so it is possible that temperature fluctuations, rather than column equilibration was the cause of the

retention time drifting. Dridi et al. [126] used a UV programme with 7 wavelength changes for the determination of PAH in airborne diesel particulates. These authors also noted problems with drift and said that for wavelength programming good chromatographic resolution and no co-eluting interferences was required. These authors also said that there was a practical limit on the number of wavelength changes possible during a run and that this was dependant on the stability of the LC system used. These comments are also applicable to the PFD work reported below.

For the second run of these samples, each run was an hour long plus an equilibration time of 15 mins. The sequence was a large one with calibration and check samples (run every ten samples to try to follow any detector drift) as well as the samples and took in excess of a day to run. The problems encountered above could have been reduced by including several surrogate standards as "retention time markers". Another way of eleviating the problems described above would have been a less ambitious experimental design, i.e. looking at one brickdust only or using less extraction solvents or methods.

The PFD results were qualitatively similar to the DAD results. As with the DAD results, the calibrations obtained for the "EPA-16" (except the non-fluorescent acy) were linear. The effects of extraction solvent and extraction type were not as marked as in the DAD results. The PFD programme used gave less noisy runs than LC/DAD and also gave lower amounts of PAH detected. This was presumably due to the more selective nature of the fluorescence detector and the lack of interferences from co-eluting peaks mentioned for DAD analysis. These co-eluting peaks could be contributing to the larger values for PAH concentration seen in the DAD samples.

However, this selectivity also means that the PFD runs lack many peaks that are seen in the UV. Typically, the fluorescence traces contain far fewer peaks than the DAD runs and are dominated by the seven peaks from fla to b(a)p. For example, the number of peaks detected in the UV runs for the brickdust samples varied from 20 to 52 and for the fluorescence samples from 10 to 29. The earlier eluting peaks are not seen by fluorescence. The peaks for nap, acy, ace and flu were not seen by any technique and as these PAH are quite volatile were presumably lost in the blowing down process despite the use of isooctanol as a keeper. The peaks for phe and ant were seen only in some fluorescence runs and at low concentrations. The later eluting peaks d(a)a, b(g)p and i(1)p were not seen in any samples. It is assumed this is because the retention time drifting discussed for the UV work had carried the peaks outside the excitation/emission envelope of the timetable programme. This further emphasises the need for good temperature control, "marker" compounds (where available) and adequate column equilibration times.

LC/DAD analysis would therefore appear to be more useful than PFD as a screening procedure for uncharacterised samples. However, the better selectivity of the PFD method means it is better suited for the analysis of "target" or "indicator" compounds. Due to the complexity of naturally occurring PAH samples several regulatory bodies have adopted the "target" compound approach. The aim is not to quantify all PAH, or even just the most toxic ones, but to try to model the total PAH content by looking at selected compounds. The "EPA-16" is an example of this approach. Another example is the German DIN standard for PAH in water [140], DIN 38407/8 which uses the six PAH fla, b(b)f, b(k)f, b(a)p, b(g)p and i(1)p. This DIN standard forms the basis of an European Union (EU) method for PAH in water (limit

250 ng carbon/l water) and a World Health Organisation (WHO) method (limit 200 ng/l water) [137, 127,165] . The need for stable retention times imposes a practical limit on the number of excitation and emission wavelength changes that are desirable in a PFD method. For this reason the methods of Marriott et al. [121] and Hansen et al. [122] with nine and eleven wavelength shifts respectively would require a very stable system and environment.

Kayali et al. [124] described the use of a wavelength timetable with 9 wavelength changes for the analysis of PAH in urban particulate. These authors found that this program performed effectively for standards but encountered problems with slow detector response, lack of resolution and retention drift for real samples. These findings are confirmed by the results of this study. Marriott et al. [121] state that the wavelength program needs to be developed for each system under study i.e. LC and detector. They found that simply "plugging in" values obtained from the literature or on other instruments will not always lead to maximum sensitivity. These workers concluded that the wavelength program decided upon is a compromise between abundance of analyte, separation, relative sensitivity, ease of fitting a wavelength change in to the elution profile and relative importance of each analyte. The requirement for a stable system suggests that PFD with multiple, i.e. >6 wavelength changes, will be of more use in relatively simple matrices (e.g. air particulate [121,122,123,124]) than complex ones (e.g. sludges [125,138]). Noël et al [125] compared GC/MS, LC/PB/MS and LC/PFD for the analysis of PAH in pitch samples destined for use in the aluminium industry (Alcan). They recommended PFD as the best technique for these samples because LC/PB/MS could not be used for the lighter PAH and GC/MS had poor resolution, non-linear calibration curves for b(g)p and i(1)p and a

higher limit of quantification than LC/PFD. These authors also give useful information on the quality control (QC) and quality assurance (QA) procedures carried out to ensure long term reproducibility of the PAH analysis system that they used. The QC and QA procedures are rather complicated involving preparation of a fresh 5- point calibration for every run, duplicate analyses, drift samples at specified intervals, analyses at two dilution levels, and rigorous data reviewing procedures to ensure the system is operating within specification. This complexity underlines the difficult nature of determining PAH in this type of complex matrix.

LC/PB/MS was not found to be suitable for the analysis of PAH with relative molecular masses (RMMs) less than ~220; this is presumably due to the low mass filter effect discussed above and a loss of light PAH due to the drying down steps in the sample work up procedure. The MS results were qualitatively similar to the DAD and PFD results. For the early eluting compounds, nap, acy, ace, and flu nothing was seen. For the mid-eluting compounds, phe, ant, fla and pyr small peaks were seen but these could not be accurately quantified. For the peaks b(a)a, chr, b(b)f, b(k)f, b(a)p, d(a)a, b(g)p and i(1)p the MS results obtained were usually smaller than the UV/DAD results but larger than the PFD. This is largely due to the wavelength timetable problem reported earlier for the PFD results. The MS results appear to be getting more similar to the UV results as RMM increases. This may be due to better transfer of the analyte through the PB/MS interface or less interference from co-eluting compounds for the UV results for the higher RMM, later eluting compounds. Similar effects of extraction method and extraction solvent were seen for the MS results as were seen for the PFD and DAD results. Namely, less extraction for cyclohexane compared to methanol and dichloromethane and less

extraction for sonication compared to Soxhlet extraction. Guerin [167] found sonication and Soxhlet extraction to give similar recoveries for the EPA-16 PAH from contaminated clay soils. This finding is not in agreement with work reported here but these workers used sonication and Soxhlet extraction for 8 hours each.

The use of LC/PB/MS for quantification has several problems associated with it. Some of these problems were encountered in the brickdust work reported here. In addition to the low mass filter effect noted above, the calibration curves obtained for several of the PAH were distinctly curved . This has been reported previously by Doerge et al. [61,136], Brown and Draper [63], Apffel and Perry [56], Pace and Betowski [155] and Anacleto et al. [163]. The reasons for this non-linearity, possible causes and techniques to ameliorate its effect have been discussed earlier. The curves appeared to become more linear as RMM increased. This would be consistent with the low mass filter effect proposed by Apffel and Perry [56]. Anacleto et al. [163] compared PB, moving belt interface and APCI for the analysis of PAH in a coal tar reference material and concluded that APCI was the best technique because of lower detection limits and a bigger linear dynamic range.

Several other workers have used LC/PB/MS for the determination of PAH in environmental samples. Gremm and Frimmel [168] looked at PAH metabolites in water by LC/PB/MS and GC/MS. These authors found that the identification of these metabolites was difficult by LC/PB/MS due to poor library matching and the possible numbers of isomers for the larger PAH. Several of the suspected metabolites could not be seen by GC/MS due to thermal decomposition. Mao et al. [133] found the use of LC/UV and LC/PB/MS to be useful complementary techniques for the determination

of benzoquinolines and acridines in diesel oil. Slobodnik et al. [154] found that LC/PB/MS was useful for the identification of PAH compounds in water and soil compounds when used in scan mode. Bonfanti et al. [164] reported similar findings for the LC/PB/MS of nitro-PAH as did Doerge et al. [61,136] who looked at PAH in soils and sediments from the Exxon Valdez oil spill.

The GC/MS results obtained for the selected extracts analysed were qualitatively similar to those obtained by the other three techniques studied. For the brickdust extracts studied the Soxhlet extracted samples gave larger recoveries than the sonication extracted samples. Dichloromethane and methanol were better extraction solvents than cyclohexane. The samples analysed by GC/MS gave no peaks for the lighter PAH i.e. RMM < 178 (nap, ace, acy, flu, phe, ant) this is due to the GPC process used for cleanup rather than a problem with the GC/MS. Generally, GC/MS gave slightly higher amounts of PAH found for the later PAH in comparison to the PB/MS and UV results. Where detected, the PAH results by fluorescence were usually lower than the other techniques.

4.4 Discussion of Brickdust Results - Qualitative work

The scan MS of a PAH is rather simple, i.e. very little fragmentation pattern (see Fig. 33). Typically a large molecular ion is seen with a smaller set of associated peaks corresponding to loss of hydrogens (up to 3) and occasionally loss of C_2H_2. This gives characteristic clusters in the spectra. In addition, less intense doubly charged ions are also present. In the LC/PB/MS experiments reported here , these ($M^{2+\cdot}$) ions increased in size with increasing RMM of the PAH. Similar observations were made by Slobodnik et al. [154]. Pace and Betowski [155] noted a similar trend in their work and said that it was due to the second ionization potential decreasing as the size of the PAH increased. This would make formation of the doubly charged ion more likely following interaction with the 70eV electrons produced in the EI source.

For the samples analysed qualitatively by LC/PB/MS the following observations can be made. Several PAH or PAH derivatives with RMM greater than 278 were observed. Peaks with (m/z) ratios of 300, 302, 328,326 and 348 were detected in most of the samples and peaks with (m/z) of 298, 350 and 352 were detected in some samples. In addition, several peaks with (m/z) of 252, 276 and 278 were detected suggesting the presence of isomers. Characterisation of the unknown compounds for one of the brickdust extracts (#9898 dcm, Soxhlet extraction) was attempted. The peaks were library matched against the MS library on the LC/PB/MS system. The following identifications were made by the system (see overleaf);

7,8,9,10-tetrahydrobenzo (a) pyrene (RMM 256)

3,4-dihydro benzo (g,h,i) perylene (RMM 278)

coronene (RMM 300)

1,1'- acenaphthylidene (RMM 302)

azadibenzopyrene (RMM 303)

dibenzo (fg,op) naphthacene (RMM 302)

1H-1 benzoxepino 2,3,4- i,j- isoquinoline (RMM 326)

phenanthro 3,4-c phenanthrene (RMM 328)

5,12:1',2':6,11:1",2"-dibenzodibenzo-a,e-cyclooctene (RMM 352)

However none of these library search identifications (except coronene, 1,1'-acenaphthylidene , azadibenzopyrene and 1H-1 benzoxepino 2,3,4-i,j-isoquinoline) were particularly good when inspected by the analyst. The presence of co-eluting compounds and MS background from the matrix limits the usefulness of library searching by adding to the MS displayed. This problem can be overcome to some extent by manipulating the MS data (i.e. background and co-eluting peak subtraction) but altering the MS in this way must be done carefully or identification errors could be caused by the subtractions. Several other problems exist for the identification of the higher RMM compounds in the brickdust extracts. Several peaks with (m/z) of 276, 278, 300 302 and 326 were detected suggesting the presence of more than one isomer. The number of possible isomers makes correct identification difficult. The MS spectra of several of the isomers is very similar and so does not assist in identification. In addition, the lack of reference compounds also makes identification difficult and for that reason only tentative identifications could be made. This finding confirms the work of Pace and Betowski [155] who have

carried out studies on PAH in the range RMM 300-450 using LC/PB/MS. Peaden et al. [169] looked at the identification of PAH with RMM >300 and had similar problems with identification of the PAH detected. The likelihood of breakdown and oxidation products is also a complicating factor. Better LC resolution would be an advantage for the identification of these heavier compounds. This could be achieved by using a specially designed stationary phase or altering the LC gradient to optimise separation for the later eluting compounds. This last suggestion would have a knock on effect on the earlier eluting PAH so the relative importance of these compounds to the analyst would need to be taken into account.

Many PAH or PAH derived compounds were detected in all the brickdust extracts studied by GC/MS in scan mode. Brickdust extract # 9898, dcm, Soxhlet extraction was studied in detail and the following compounds gave good matches against the NBS32K and Wiley186 libraries installed on the GC/MS system:

EPA-16 PAH (except nap, ant, b(a)a

anthraquinone (RMM 208)

xanthene

perylene (RMM 252)

benzo(e)pyrene (RMM 252)

benzo(b)chrysene (RMM 278)

triphenylene

coronene (RMM 300)

1,2:4,5 dibenzpyrene (RMM 302)

substituted anthracenes (e.g. methyl anthracenes)

substituted pyrenes

substituted chrysenes

substituted triphenylenes

substituted thiophenes

carbazole and substituted carbazoles

substituted furans

The GC/MS results gave good identifications for many peaks that were not identified by DAD or LC/PB/MS. It was also noticeable that GC/MS detected many low mass compounds (RMMs < 200) that were not detected by the other techniques. This may be due to better sensitivity by GC/MS or due to the better library search facility available on the GC/MS (two large libraries). Several classes of compounds containing hetero atoms were detected e.g. carbazoles (N), thiophenes (S) and furans (O). These are presumably combustion or oxidation products of PAH and associated compounds. GC/MS was also detecting compounds with RMMs up to ~302 e.g. 1,2:3,4 dibenzpyrene. GC/MS did not seem to be detecting PAH with masses greater than 302. The unambiguous identification of the compounds listed above is made more complicated by the factors described above for LC/PB/MS analysis i.e. number of isomers, similarity of MS for isomers, simple MS spectra of PAH, contribution of background and co-eluting peaks to MS and limitation of library.

Similar work to that described above was carried out for the DAD work. The UV spectra for the peaks obtained from brickdust sample (#9898 dcm, Soxhlet extraction) were matched against the user created libraries ("EPA-16" PAH and "PAH" library (EPA-16 plus 12 other PAH, kindly supplied by Mr. Andrew Simpson,

HSL). Of the 43 compounds detected in this sample several gave possible matches for PAH compounds. EPA-16 PAH phe and pyr to i(1)p were detected. In addition, tentative identifications for triphenylene (RMM 228), perylene (RMM252), benzo (b) chrysene (RMM 278), picene (RMM 278) and coronene (RMM 300) were made. The points made concerning the difficulty of differentiating between isomers is even more acute for DAD work. For example, the isomers, dibenzo (a,h) anthracene, dibenzo (a,c) anthracene, dibenzo (a,j) anthracene all have very similar UV spectra. Also valid are the comments made above about co-eluting compounds effecting the UV spectra. Using the "peak purity" software supplied with the LC system used in this work it was confirmed that several of the UV peaks had a co-eluting component. The main drawback of the library search facility in UV/DAD analysis is the requirement for the library to be user created i.e. the UV spectra are quite machine specific. This requirement means that building up a UV library takes a lot of operator time and obviously spectra can only be matched against ones entered into the library. Good library matches can sometimes be achieved using spectra obtained on other LC systems, for example, the library supplied by Mr. Andrew Simpson was created on a different type of LC to the one used in the experiments reported above. However, the use of libraries created on other instruments does add another potential source of error in identification.

The techniques studied for qualitative analysis of the brickdust extracts may be compared. The most useful technique for compounds with RMMs < 303 was definitely GC/MS. This technique detected far more compounds than the other two and the data obtained was easier to library match and manipulate than that produced by LC/DAD or LC/PB/MS. GC/MS in SIM mode is also more sensitive than

the other techniques. If the compounds of interest were the "EPA-16" or similar low RMM PAH then GC/MS would be the favoured technique for qualitative work. A comparison of LC/MS and GC/MS runs in scan mode for the late eluting compounds shows that LC system is possibly detecting some high RMM PAH not seen by the GC system (i.e RMMs >302) . The use of PB/LC/MS for the analysis of these heavier compounds, e.g. RMM 326, would appear to be favoured over GC/MS due to the low volatility of these compounds and hence their poor peak shapes and long retention times by GC. The LC/DAD system was not able to characterise all the components due to co-eluting interferences and the limited UV libraries available. However, LC/DAD does provide useful confirmation to the other techniques.

As mentioned above other authors have looked specifically at the analysis of large PAH. Peaden et al. [169] looked at PAH with RMMs >300 from carbon black using fluorescence detection. These authors commented on the problems of accurately identifying the PAH when faced with the large numbers of potential isomers. The problem becomes more acute as the RMM and number of rings increases. Biggs and Fetzer [170] reviewed the techniques available for the analysis of large PAH (7 rings) and advocated a sequential extraction process, using solvents of increasing strength, to fully extract these larger PAH. Pace and Betowski [155] found that LC/PB/MS gave good linearity for RMMs 300-352 and poorer linearity for >352. These authors attributed this to decreasing volatility as RMM increases. Pace and Betowski also stated that current LC stationary phases were not adequate for the resolution of these large PAH and that work on novel stationary phases was required.

4.5 Conclusions

As mentioned at the beginning of this thesis, the coupling of LC and MS offers great possibilities for the analysis of those compounds not amenable to GC analysis. The major drawbacks with the present LC/PB/MS systems are their lack of sensitivity, low mass cut off and non-linear behaviour at low analyte concentrations. This work has studied the use of various additives in an attempt to increase sensitivity and improve linearity. None of the additives studied was found to markedly improve the LC/PB/MS sensitivity for PAH. The effect of various instrumental factors on LC/PB/MS performance has also been studied. Instrumental factors such as interface temperature, helium pressure, source temperature etc. were all found to affect sensitivity.

Studies on extraction and clean-up methods for these brick dust extracts showed that Soxhlet was more effective than sonication (although Soxhlet extraction was carried out over a longer period) and that methanol and dichloromethane were more efficient extracting solvents than cyclohexane. SPE and GPC were studied as cleanup methods. GPC was found to be adequate for the cleanup of PAH with RMMs > 202. C-18 SPE was found to give higher recoveries and less variation than Silica gel for PAH spiked cartridges. Both GPC and SPE suffered from the same disadvantage, as used in this work, of requiring concentration prior to analysis as there was evidence that this step was losing the lighter PAH. This was despite the use of isooctanol as an involatile keeper.

A few conclusions may be drawn regarding analytical technique. The estimated detection limits of the different techniques have been discussed above. However it must be noted that these were obtained from the analysis of standard mixes and for real samples interferants would cause problems at low PAH levels.

Work on environmental samples (brick dusts) showed differences in the concentration and number of components for the three brickdusts studied (#9898, #10441 and #10026). Brickdust #9898 was shown to contain more PAH than brickdust #10441, which contained more PAH than brickdust #10026. These experiments also compared a variety of extraction and detection techniques. Four systems were studied (LC/PFD, LC/DAD, LC/PB/MS and GC/MS) for quantitative and qualitative analysis of the brickdust extracts. For the quantitative work, LC/DAD gave noisy chromatograms that made identification difficult and suffered from co-eluting compounds that effected quantification. LC/PFD appeared to be not detecting the majority of peaks in the chromatogram and quantification was badly affected by retention drift. The limitations of GC/MS for the analysis of involatile compounds i.e. RMM > 303, have been discussed above. Of the four techniques studied for the quantitative determination of PAH in brickdust, DAD and GC/MS analysis appear to most suitable for PAH with RMMs less than 303. For compound with RMMs in excess of 303 LC/PB/MS becomes a more attractive option. The limitations of LC/PB/MS for quantitative work are summarised below. PFD was found to be most suited to a "target analyte" approach.

LC/PB/MS was not found to be suitable for lower range PAH due to the low mass cut off effect (< 180-220) noted earlier. It was found that LC/PB/MS approached the

sensitivity of other techniques (gas chromatography/mass spectrometry (GC/MS) and liquid chromatography with diode array and fluorescence detection) as the RMM of the PAH increased. For PAH with RMMs greater than 310, LC/PB/MS appeared to be the most favoured technique. It is to be expected that PAH above RMM 302 will be difficult to analyse by GC due to low volatility. Quantification using LC/PB/MS was affected by the non-linear calibration curves also reported by other workers. The curves appeared to become more linear at high RMM. The effect of co-eluting compounds, especially for the highly complex matrices likely to be encountered with environmental samples, also will effect quantification due to the "carrier effect" as noted by other workers [46,56,61]. These "carrier effect" problems could be overcome by the use of matrix matching for the standards or isotope dilution (as described earlier [61,63]. However, isotopically labelled co-eluting compounds are expensive, especially if several are required e.g. "EPA-16", and will not be available for "unknown" and environmental samples. Such an approach would be useful for the "target analyte" approach e.g. DIN 38407/8.

For the lighter compounds, e.g. RMM < 303 GC/MS was clearly the most favoured technique for the qualitative analysis and identification of the brickdust extracts. LC/DAD was found to be a useful complementary technique limited by the UV libraries available. LC/PB/MS was useful for the identification of compounds with RMM > 302 which are not easily analyzed by GC.

A few other comments may be made regarding the work presented here. It is thought that a system routinely used for analysis of PAH would need to be less variable than the one used in these studies. Another source of variability in this work

was that the DAD and PFD results were run on one LC system and the LC/PB/MS results were run on a different system at a different time. The GC/MS samples were also run at a different time to the other techniques. Storage of the PAH could therefore have introduced variation for the "between detector" results. Another way of trying to decrease the between detector variability seen in these experiments would be a more complex system of internal standards.

Chapter 5

Analysis of Pesticides by Particle Beam LC/MS

5.0 Introduction

Pesticides are defined as "substances, preparations or organisms used or prepared for destroying pests [171]. Pesticide use is widespread. A European Union survey [172] found that in 1988, in the U.K., twenty nine different pesticides amounting to over 7,500 tonnes in total were used. Pesticides used in less than 50 tonne amounts, substances formally not classed as pesticides e.g. biocides and timber treatments, were not included in these figures. Other EU countries showed similar levels of use. Pesticides are the cause of many concerns on environmental and health grounds (See Fig. 5.1 overleaf). From these examples it can be seen that pesticides are hazardous chemicals and it is necessary to control their impact on users, the public and the environment [173,174] .

The determination of pesticide residues in a variety of matrices (vegetation, food, air, soil, water etc.) can therefore be required for legal purposes e.g. to support enforcement action, demonstrate compliance with environmental limits, minimum residue levels, formulation composition or for occupational exposure purposes [175-181] . A vast amount of previous work has been carried out on the determination of pesticides in the matrices described above. Sherma [182] reviewed over 400 papers on pesticide analysis and sample preparation published between 1st Dec. 1988 and 1st Dec. 1990 and concluded that GC and SPE were the most commonly used techniques. Multi-residue methods, for screening purposes, have been described by many workers, e.g. [183-190]. These methods are also largely GC based with SPE extraction.

Figure 5.1. Recent Examples of Pesticide Related News Items

Key

1. 16/9/99, The Guardian

A MAFF survey finds unacceptable levels of pesticides in vegetables on sale in supermarkets

2. 24/1/99, The Sunday Telegraph

Judge orders permethrin to be used to treat flat for dry rot against owners' wishes

3. 7/11/95, Daily Mirror

Carbaryl, used in shampoos used to treat head lice, is linked to cancer

4. 19/5/97, Chemistry and Industry

A Royal Society for the Protection of Birds report links over use of pesticides with bird decline

5. 7/8/97, Chemistry and Industry

A Medical Research Council report suggests certain pesticides which act as oestrogen mimics are linked to cancer and male infertility.

6. Daily Telegraph

A report of compensation award for a child born without eyes after his mother was allegedly contaminated with the pesticide benomyl.

7. 5/5/95, Sunday Telegraph

Farmers vow to fight a Government decision not to ban organophosphorus sheep dips.

8. Daily Telegraph

Synthetic pyrethroid pesticides, introduced as a substitute for OPs, are less toxic to humans but far more toxic to fish.

9. 4/10/93 Chemistry and Industry

Debate on the decision by the EU to consider relaxing limits on the allowed levels of pesticide residues in drinking water.

However some pesticides are not amenable to analysis by conventional GC methods. They may be either thermally unstable (e.g. phenyl urea herbicides and carbamates [191,192]) or require pre-GC derivatization (e.g. phenoxy acetic acid herbicides). In addition, as mentioned earlier, LC methods can often require a less extensive clean up procedure than GC methods. Conventional LC detectors, such as diode array or fluorescence may also have problems associated with them. For example some otherwise LC amenable pesticides have no UV chromophore e.g. benzalkonium chloride [193,194] .

Liquid Chromatography-Mass Spectrometry (LC-MS) is a very powerful analytical tool of use for forensic, environmental and occupational hygiene analysis. Many workers have looked at the use of LC/MS for the determination of pesticides. Several early papers on LC/MS analysis of pesticides used direct liquid introduction (DLI) and the moving belt (MB) interface [195-199]. These workers found that these interfaces gave useful information but suffered from sensitivity limitations. DLI gave CI type spectra with little fragmentation and MB interfaces had problems with memory effects due to adsorption on to the belt. Voyksner and Cairns [199] compared DLI, MB and thermospray (TSP) interfaces and found the (at that time) more modern TSP interface to give the best performance.

TSP was for many years the most popular interface for LC/MS work. Volmer et al. [200] described the use of TSP for the multi-residue determination of 128 pesticides. TSP has also been used for the determination of phenylurea and carbamate pesticides by Jones and Moore [201] . Several workers examined the use of additives for TSP analysis [202-206]. These workers found that there were problems with thermally labile

carbamates due to thermally assisted hydrolysis prior to ionization. Ammonium formate and ammonium acetate were found to be be complementary buffer systems. Barcelo [206] examined the use of TSP in positive ion and negative ion mode for the analysis of various organophosphate pesticides. He found positive ion mode to give approximately three times better sensitivity than negative ion mode. Although TSP provided a useful technique for the determination of "difficult" pesticides it has now largely been superceded by the more modern atmospheric pressure ionization (API) techniques. Limitations of TSP have been mentioned earlier. In summary they are fluctuating ion signals, thermal degradation and the lack of structurally significant fragment ions [33,207] .

API techniques are probably the most commonly used LC/MS methods at the present time. Several workers have compared API interfaces with other LC/MS interfacing techniques. Molina et al. [208] looked at ESI for the analysis of organo-phosphorus pesticides. These workers noted that some OP pesticides, for example trichlorforon, have been found to suffer thermal degradation using TSP analysis due to the high probe tip and gas phase temperatures commonly used in TSP. In addition TSP was not sensitive enough to meet the EU drinking water limit for pesticides (<0.1 µg/l). These authors found that ESI gave ~100x better sensitivity than TSP. ESI also gave mass spectra which contained more fragmentation and so more information than was found be TSP. Volmer and Levsen [209] looked at the use of LC/MS as part of the National Survey of Pesticides in Ground Water carried out by the United States Environmental Protection Agency in the mid 1990's. These workers looked at over 80 nitrogen and phosphorus pesticides using various LC/MS techniques, such as, APCI, ESI, TSP, fast atom bombardment, collisional activated

desorption and ^{252}Cf plasma desorption. They reported that "the combination of different ionization techniques such as APCI, CAD, FAB and TSP is useful for the elucidation of fragmentation mechanisms in TSP/MS". Pleasance et al. [210] used LC/MS with atmospheric pressure ionization techniques e.g. APCI and ESI, to look at N-methyl carbamate pesticides and compared these interfaces with TSP and PB. These pesticides cannot be analysed directly by GC due to their thermal lability. The most commonly used LC technique for carbamates involves fluorescence detection with post column hydrolysis and derivatization [211]. This method can require specialized equipment, specific to this analysis, to be bought and is time consuming and complicated. Pleasance et al. [210] found that for the N-methyl carbamates APCI was the best technique. APCI gave improved sensitivity over the other interfaces and also gave abundant fragment ions for structural confirmation. These authors concluded that ESI would be more suited to even more thermally labile compounds and to polar analytes. They stated that "it is the range of analytes covered by these two readily interchangeable interfaces that makes the API system so attractive". Several other groups have used API LC/MS for the determination of pesticides in serum [212], mushrooms [213], food [214], industrial effluents [215] and waters [216,217,218]. This selection of applications gives some idea of the versatility of API techniques.

Several papers have been written comparing API and PB techniques. Pleasance et al. [210] concluded that APCI was >150 x more sensitive than PB for the 17 pesticides included in their study. These authors also concluded that APCI sensitivity was less affected than PB sensitivity by chemical differences in analyte structure . Increasing the potential on the sampling cone in APCI gave voltage dependent collisional induced dissociation which formed diagnostic fragment ions so mimicking to some

extent the EI spectra supplied by PB. Ferrer and Barcelo [219] and Barcelo et al. [220] compared APCI, ESI and PB and reached similar conclusions to those given above. Ferrer and Barcelo [219] stated that APCI was "the most universal technique for environmental analysis due to high sensitivity, the possibility of detecting a broad range of analytes and the useful structural information obtained by fragmentation". Doerge and Bajic [217] also found similar sensitivity gains to those reported above when comparing APCI and PB.

Despite the numerous drawbacks of PB discussed above this technique has been widely used for the analysis of pesticides. A method using LC/PB/MS for the determination of nitrogen-containing pesticides has been produced by Bellar et al. [221]. The main advantage of PB over other LC/MS interfaces is the ability to generate standard EI spectra and then use commercially available GC/MS libraries for library matching and the identification of unknowns. Ferrer and Barcelo [219] stated that with "PB a better identification of unknown compounds can be obtained". A similar conclusion was reached by Kim et al. [222] who stated "because of the legal implications of environmental analytical data, the analyte confirmation provided by full scan EI-MS detection is an important aspect of an environmental method". Aguilar et al. [223] considered that EI was useful for the identification of breakdown products or other non-target compounds. Some examples of the use of PB for the analysis of pesticides are paraquat and diquat in water [70], rotenone in water [224], organo-phosphorus, phenyl ureas, triazines and chlorophenoxyacids in soil and water [223,225,226,65].

The work described below looks at the use of PB for the identification of pesticides in concentrates and environmentally derived samples. The pesticide groups examined were those that cannot be analysed by GC/MS due to thermal lability, such as phenyl ureas and carbamates, or require derivitization prior to GC analysis, such as phenoxyacetic acid herbicides. Preliminary work looked at the effect of source temperature and interface temperature. The use of LC/PB/MS for the unambiguous identification of pesticides in environmental samples was investigated. Further work looked at the affect of post-column addition of organic modifiers on PB sensitivity.

5.1 Experimental

5.1.1 Chemicals

Solvents used (acetonitrile, methanol, ethanol, propanol, butanol, pentanol) were of LC grade or better (from Rathburns, Walkerburn, U.K. or Fisher Scientific, Loughborough, Leics., U.K.). Water was purified in-house using an Elgastat UHQ II sytem (Elga Ltd., High Wycombe, Bucks., U.K.) and buffers were prepared using this. Ammonium acetate was supplied by Fisons, Loughborough, Leics. U.K., tetrabutyl ammonium chloride, tetramethyl ammonium hydroxide and octane sulphonic acid were supplied by Aldrich Chemical Co., Gillingham, U.K. Pesticide standards (phenyl ureas, phenoxy acid herbicides, bromoxynil and ioxynil) and were purchased from Promochem, Welwyn Garden City, Herts. U.K. and Qm_x, Thaxted, Essex, U.K.. Pesticide-contaminated (linuron) vegetation and soil samples

and concentrate samples, "Doublet" and "Seritox 50", were originally submitted for forensic analysis by HSE Inspectors.

5.1.2 Instrumentation

The PB/LC/MS system consisted of a VG Trio 1 MS and a VG LINC PB interface (VG Analytical, Altrincham, U.K.). Various LC pumps (Waters 510 supplied by Waters , Watford, Herts. U.K. or Jasco LG-980-02, supplied by JEOL (U.K.) Ltd., U.K.) were connected to this system. Injections were made manually through a 7125 Rheodyne valve (Alltech, Carnforth, Lancs. U.K.). Probe Electron Impact MS was carried out on a VG Micromass 7070S (VG Analytical, Altrincham, U.K.).

Other LC work was carried out on the Hewlett-Packard HP1090 with diode array detection (HP, Stockport, Cheshire, U.K.) and Waters Millennium 2010 with fluorescence and diode-array detectors (Waters , Watford, Herts. U.K.) systems available at the HSL .

LC columns used were 25 x 0.46 cm S5 ODS2 (PhaseSep, Deeside, Clwyd, U.K.) for the vegetation and soil extracts, 15 x 0.46 cm S3 ODS2 (PhaseSep, Deeside, Clwyd, U.K.) for the concentrates and additive experiments, 15 x 0.46 cm S3 ODS2 and 12.5 x 0.46 cm S3 ODS2 (Thames Chromatography, Maidenhead, Berks. U.K.) for the phenyl urea herbicide work and a 15 x 0.1 cm S3 ODS2 (PhaseSep, Deeside, Clwyd, U.K.) for the microbore column work.

Clean up of the environmental pesticide samples was carried out using a Waters gel permeation chromatography (GPC) system and associated fraction collector (Waters, Watford, Herts., U.K.) .

5.1.3 Development of an LC method for Seven Phenyl Urea Herbicides

An LC method for the determination of seven phenyl urea herbicides was developed. Final conditions were; column 3µ ODS2 PhaseSep Spherisorb 12.5 x 0.46 cm and 1cm guard, mobile phase 45% acetonitrile, 45% 0.01M acetic acid, 10% methanol and UV detection at 240 nm [227]. The seven phenyl urea mix was also analysed using a 1mm microbore LC column to see if this would increase sensitivity. The flow rate used was 50µl/min.

5.1.4 LC/PB/MS of Phenoxy Acid Herbicides

Initial LC work carried out at HSL developed a suitable LC method for the phenoxy acid herbicides mecoprop, potassium MCPA, dicamba and dichlorprop. LC conditions were; mobile phase 45% acetonitrile, 45% 10mM acetic acid and 10% methanol, run isocratically, column 15 x 0.46 cm S3 ODS2, PhaseSep, Deeside, Clwyd, U.K. The flow rate was 1ml/min. with diode array detection.

The LC/PB/MS work used flow rates of 0.3 ml/min. mobile phase (45% acetonitrile, 45% 10mM acetic acid and 10% methanol, run isocratically) + 0.2 ml/min. of a "t-pieced" in "post column". Operating conditions for the LC-PB-MS system were:

interface temp. 60 °C, nebulizer helium pressure 30 p.s.i., electron energy 70eV and source temp. 220 °C.

5.1.5 Effect of Interface and Source Temperature for Selected Pesticides

The system was optimized in the manner described for the PAH work. These settings were then used to assess the affect of interface temperature on PB/LC/MS sensitivity for the pesticide carbaryl. The LC flow was set to 0.3 ml/min., with a mobile phase of 50/50 methanol and acetonitrile and 0.2 ml/min. acetonitrile post column. The interface temperature was varied from 25 °C to 80 °C and the response for an injection of 1.57 μg of carbaryl was measured using m/z = 144 .
A similar set of runs were carried out to examine the affect of source temperature on PB/LC/MS sensitivity. The source temperature was set at 200 °C, 220 °C and 250 °C and the test compounds were the seven phenyl urea herbicides analysed using the LC conditions described above. The seven pesticides were at concentrations between 15 and 50 μg/ml and 50 μl was injected for each run.

5.1.6 PB/LC/MS of Phenyl Urea Herbicides, Pesticide Concentrates and Environmental Samples containing Pesticides

Pesticide samples were worked up following standard operating procedures for this type of sample [83] . Phenyl Urea herbicides were analysed using conditions previously described [227]. The mobile phase used for these experiments was 45% acetonitrile, 45% 10mM acetic acid and 10% methanol, run isocratically. For the phenoxy acid

herbicides the same LC conditions were used. Flow rates for the PB experiments were 0.3ml/min mobile phase + 0.2 ml/min. post-column methanol. For the LC/DAD experiments flow rates of 1 or 0.3 ml/min. were used. Typical operating conditions for the LC-PB-MS system were: interface temp. 60-70 °C, nebulizer helium pressure 20-30 p.s.i., electron energy 70eV and source temp. 200-220 °C. MS analysis in both scan and SIM modes were carried out, using ions specific to the pesticides under analysis (determined from the scan runs).

5.1.7 Investigation of the Post-Column Addition of Additives as a Means of Increasing PB/LC/MS Sensitivity for Pesticides

Post column addition of additive experiments used the following flow rates of 0.3 ml/min. mobile phase (45% acetonitrile, 45% 10mM acetic acid and 10% methanol, run isocratically for isoproturon and fenuron and 50%, 50% acetonitrile; methanol for carbaryl) + 0.2 ml/min. of additive containing acetonitrile/water (80%/20%) "t-pieced" in "post column". The additives used were ammonium acetate, tetrabutyl ammonium chloride, tetramethyl ammonium hydroxide and octane sulphonic acid at concentrations of approx. 1-6 g/l depending on solubility. Repeat injections, on a 15 x 0.46 cm S3 ODS2 LC column, PhaseSep, Deeside, Clwyd, U.K., of 10 or 50ul were made for this series of experiments. The test compounds used were carbaryl, fenuron and isoproturon. The response for injections of 1.57 µg of carbaryl was measured using (m/z) = 144 and for the fenuron and isoproturon experiments scan mode was used and the total ion currents integrated. Operating conditions for the LC-PB-MS system were: interface temp. 70 °C, nebulizer helium pressure 20 p.s.i., electron energy 70eV and source temp. 200 °C.

5.1.8 Investigation of the Post Column Addition of Alcohols as a Means of Increasing PB/LC/MS Sensitivity

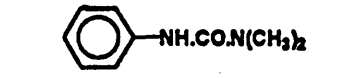
Post column addition of alcohol experiments used flow rates of 0.3 ml/min. mobile phase (45% acetonitrile, 45% 10mM acetic acid and 10% methanol, run isocratically) + 0.2 ml/min. of alcohol "t-pieced" in "post column". Repeat loop injections (1cm guard column only in-line, i.e. no analytical column) of 25ul were made for this series of experiments. Operating conditions for the LC-PB-MS system were: interface temp. 70 °C, nebulizer helium pressure 20 p.s.i., electron energy 70eV and source temp. 200 °C. The test compounds used were monuron, neburon and fenuron.

5.2 *Results and Discussion*

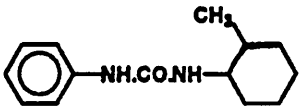
5.2.1 Development of an LC method for Seven Phenyl Urea Herbicides

An LC method was developed for the determination of seven phenyl urea herbicides (fenuron, monuron, isoproturon, diuron, linuron, siduron and neburon, Fig. 5.2) using diode array or particle beam/MS detection. The chromatograms shown below (Fig. 5.3) are for the seven phenyl urea mix using LC/DAD and LC/MS systems. Estimated limits of detection (based on 3x S/N (signal to noise ratio)) were found to be approx. 1 µg/ml for DAD and approx. 1-20 µg/ml for LC/PB/MS (scan mode, depending on compound). Full EI spectra were obtained for the seven phenyl ureas under study. Monitoring in SIM mode will reduce the quoted MS detection limits.

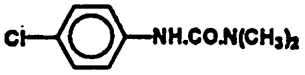
Figure 5.2. *Structures of the Seven Phenyl Urea Herbicides used*



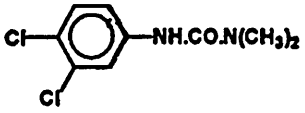
Fenuron



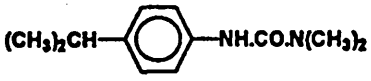
Siduron



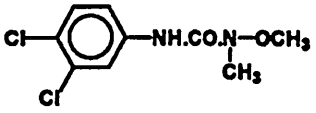
Monuron



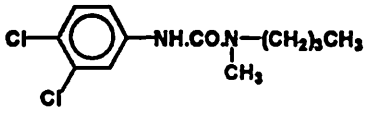
Diuron



Isoproturon



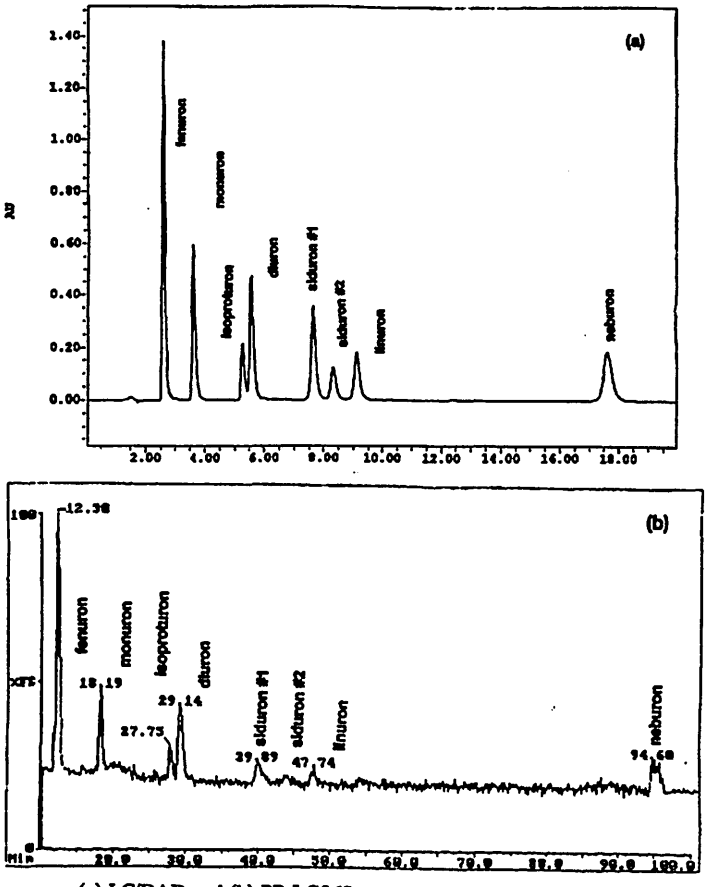
Linuron



Neburon

Figure 5.3. *Chromatograms of Seven Phenyl Urea mix by LC/DAD and LC/PB/MS*

(for Experimental details see Sections 5.1.3 and 5.1.6)



(a) LC/DAD and (b) PB-LC/MS

The microbore column work was unsuccessful. For the LC/DAD experiments poorer chromatography (tailing peaks) and higher limits of detection were found using this column. For PB/MS work, no signal was obtained with a 50 µl flow rate. This is presumably because this small volume of liquid sticks to the PB/MS interface, is inadequately aerosolized or is evaporated by the nebulizing gas. With 0.2 ml/min.

post column acetonitrile some peaks were observed but again these were broad and inferior to those obtained using a normal bore LC column.

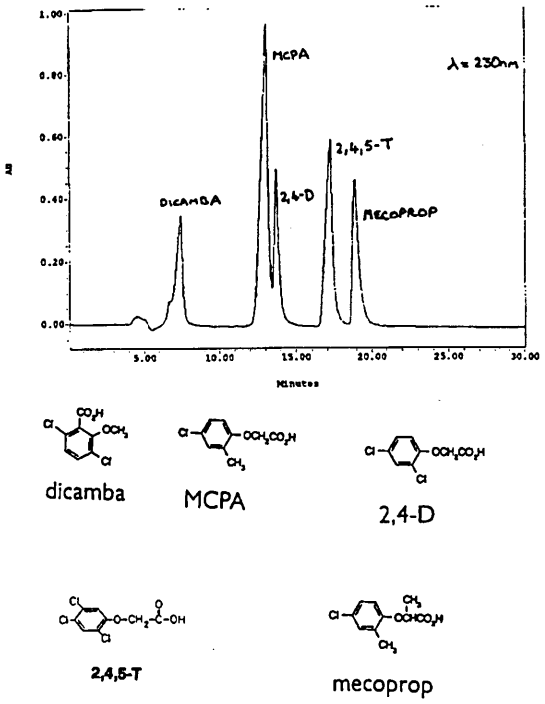
In conclusion, an LC method was developed for seven phenyl urea herbicides and a suitably modified version of this method was successfully applied to LC/PB/MS.

5.2.2 LC/PB/MS of Phenoxy Acid Herbicides

An LC method was developed for selected phenoxy acid herbicides. An example chromatogram is shown below, Fig 5.4.

Figure 5.4. *LC of Phenoxy Acid Herbicides*

(for Experimental details see Section 5.1.4)



These conditions were then used to look at PB/MS of selected phenoxy acid herbicides, using the flow rates described earlier. For this work very poor peak shape (tailing) and low sensitivity was obtained. Attempts to improve this sensitivity by addition of additive (octane sulphonic acid) were unsuccessful and led to frequent blocking of the interface skimmers.

Phenoxy acid herbicides have previously been analysed by GC/MS after derivatization. Allender [228] described the preparation of pentafluorobenzyl derivatives with pentafluorobenzyl bromide for spray drift contaminated vegetation. Phenoxy acid analysis using GC/MS after derivatization using trimethylsilyldiazomethane has been described by Rimmer et al. [229]. Munch and Pawlecki-Vonderheide [230] reported on a base-promoted esterification procedure for GC/MS which was stated to have advantages over the two derivatization techniques mentioned above.

PB/MS analysis of phenoxy acid herbicides has been described by several groups. Kim et al. [222] examined phenoxy acid herbicides in soil and water samples and compared LC/DAD with LC/PB/MS. These authors concluded that PB/MS was able to give unambiguous identification when in EI mode. However, these authors found a distinct memory effect in the mass spectra obtained when analysing these compounds with PB/MS due to previously eluting compounds. Bruner et al. [226] looked at a modification to U.S. E.P.A. 515.1 using SPE extraction instead of LLE. These workers found that SPE gave higher and more precise (lower standard deviations) recoveries than LLE. This work also compared GC/ECD (after derivatization), LC/UV and LC/PB/MS and found that GC/ECD was the most sensitive method, followed by LC/PB/MS and finally LC/UV. Memory effects in the

MS of phenoxy acid herbicides were also encountered by Betowski et al. [21]. These authors found a broadening of the ion profiles for some ions indicating a longer residence time in the ion source. It was postulated that such ions were formed due to the ionization of thermal degradation products of the herbicides. The generation of these ions was found to be dependent on ion source temperature, ion source cleanliness and analyte concentration. Cappiello et al. [232] and Cappiello and Famiglini [233] have also reported the presence of thermal degradation products when analysing these herbicides by LC/PB/MS. These workers described the use of a Teflon source target and found that this modification drastically improved the mass spectra obtained. The Teflon source target modification was also found to be effective for a micro-litre flow rate capillary LC/PB/MS system [233,234]. Detection limits of 1 ppb or less were found for this system.

In conclusion, the attempt to use PB/MS for the determination of phenoxy acid herbicides was unsuccessful. Previous work has also highlighted problems in using PB/MS for these compounds.

5.2.3 Effect of Interface and Source Temperature for Selected Pesticides

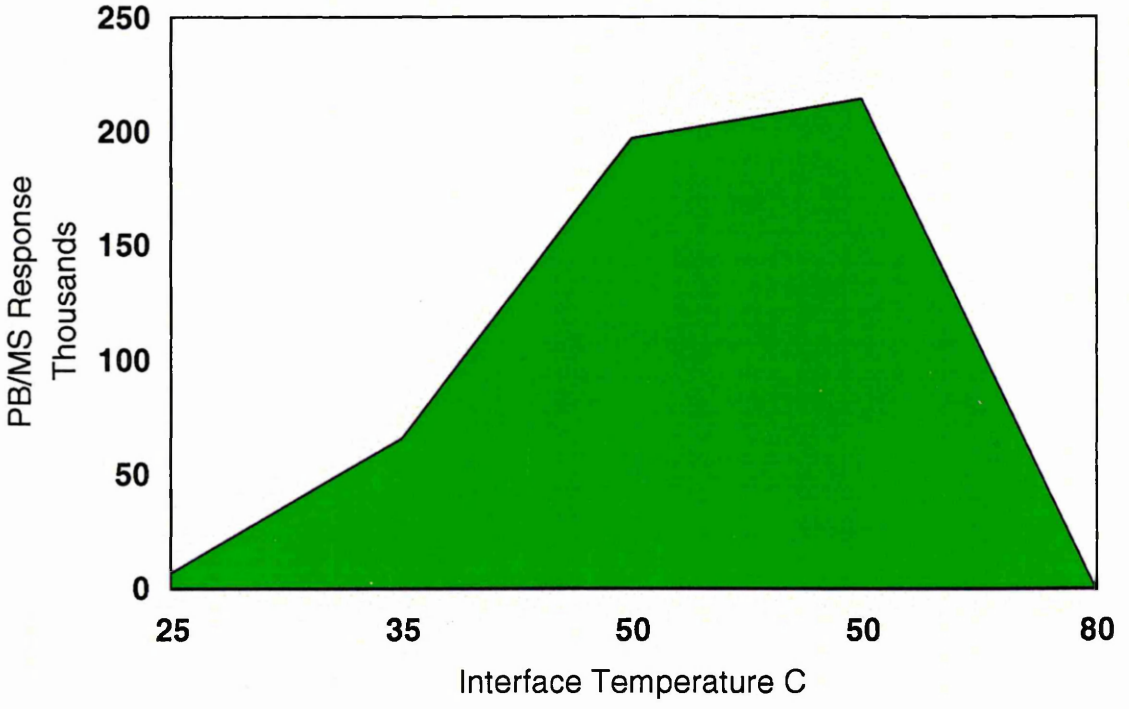
The effect of interface temperature for the PB/MS sensitivity for carbaryl was investigated using the conditions described above. The results are given below in table 5.1 and Fig. 5.5.

Table 5.1. *The Effect of PB Interface Temperature for Carbaryl*

Interface Temp. °C	MS response (m/z)=144
25	6,363
35	65,830
50	197,030
50 (second run)	214,420
80	ND < 5000

Figure 5.5. *The Effect of PB Interface Temperature for Carbaryl*

(for Experimental details see Section 5.1.5)



From these results it can be seen that too high an interface temperature drastically reduces the sensitivity of PB for carbaryl. It is assumed that this is due to the carbaryl decomposing in the interface. Presumably the analyte is present in the ion source for a shorter period of time compared to that spent in the PB interface and so does not decompose under the much higher ion source temperature. Too low an

interface temperature also gives low PB/MS response presumably because of insufficient volatilization in the interface.

Other workers have also looked at the analysis of carbamates by PB/MS. Honing et al. [235] looked at PB/chemical ionization/MS of carbamates. They found that ion source pressure and ion source temperature affected PB/MS sensitivity and found variable responses in their study. Slobodnik et al. [236] compared GC/MS and LC/PB/MS and obtained identical MS spectra for the two techniques under EI conditions. These workers concluded that LC/PB/MS required further work to improve limits of detection. An LC method for the determination of phenyl urea herbicides in milk has been described by Brandsteterovea et al. [237] . In addition to the work mentioned above, LC/APCI/MS has also been used for the determination of these pesticides by Jeannot and Sauvard [238] and Yarita et al.[239].

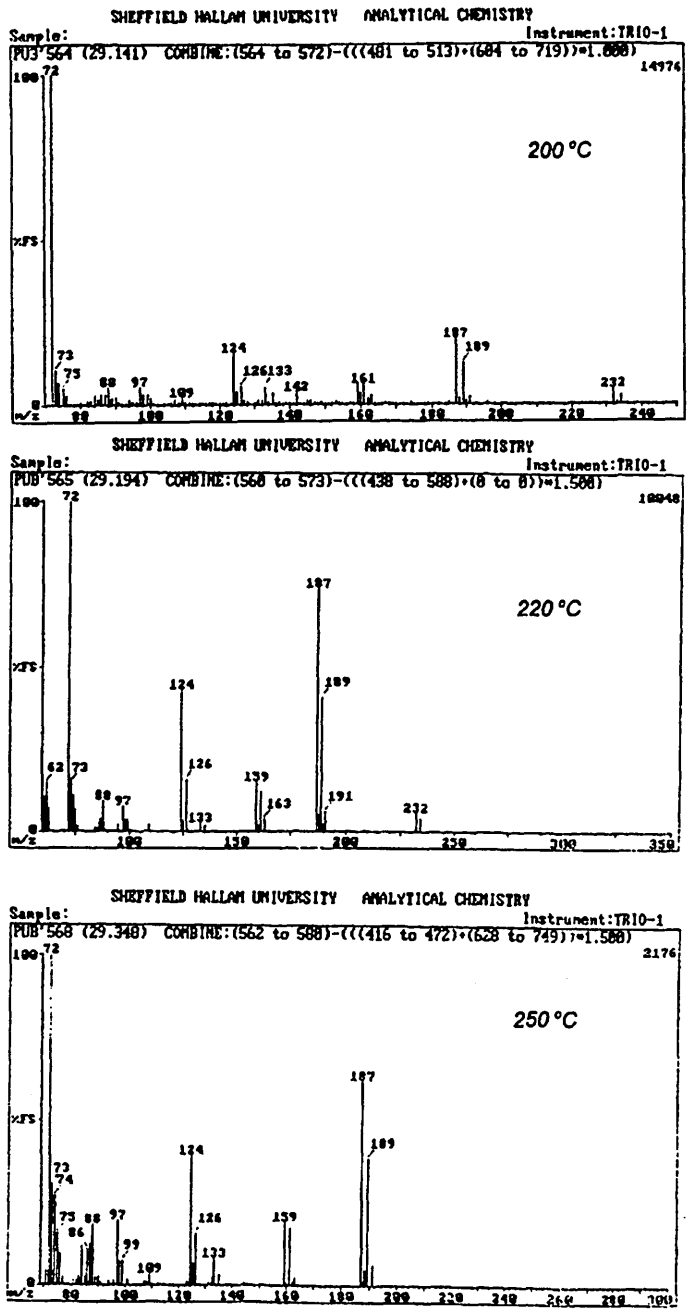
Other workers have also noted the decomposition of carbamates. Honing et al. [240] reported on carbamate decomposition during TSP analysis. Okumura et al. [241] have also described the decomposition of carbamates by GC/MS. These authors reported on a derivatization method for GC/MS, using diazomethane to produce a methyl derivitive, which avoided this problem. Newsome et al. [214] and Kawasaki et al. [212] have reported on the use of LC/APCI/MS for the analysis of carbamates. These workers found that this API technique did not suffer from thermal degradation. Another approach to avoiding thermal decomposition has been described by Dagan and Amirav [242] who described the use of fast, very fast and ultra fast GC/MS for the determination of a variety of compounds including carbamates. These authors described the use of a modified GC system and very short GC columns to give

retention times of 5 to 100 seconds. This extremely rapid analysis limits the opportunity for thermal decomposition.

The effect of source temperature was also examined for the seven phenyl urea herbicide mix. The source temperature was altered from 200 to 250 °C. The higher source temperature appeared to give more peaks presumably due to greater fragmentation in the ion source. The highest source temperature also gave a reduced response and smaller or reduced molecular ions for the seven phenyl urea herbicides. An example of the results obtained is shown for Diuron, see figure 5.6.

Figure 5.6. *Effect of Source Temperature on the EI Mass Spectrum of Diuron*

(for Experimental details see Section 5.1.5)



5.2.4 PB/LC/MS of Pesticide Concentrates and Environmental Samples containing Pesticides

Following the initial examination of standards, it was decided to attempt to use LC/PB/MS on vegetation contaminated by linuron spray drift and spiked soil samples. Experimental details are given in Section 5.1.6. The vegetation sample was a sample taken during enforcement action by HSE Inspectors and was run in scan and SIM mode. The soil sample was a spiked sample (approx. 100 µg/kg) and was also run in scan and SIM mode. For the soil and vegetation extracts, extracted ion chromatograms were necessary to clearly observe the linuron peak from the background, even after GPC clean up. Ions monitored in SIM mode, for linuron, were (m/z) 248 (M^+), 187, 161, 160. The chromatograms and MS obtained are shown overleaf, figs 5.7 and 5.8.

Figure 5.7. *Chromatograms and Mass Spectrum for Spray Drift Contaminated (Linuron) Vegetation Sample*

(for Experimental details see Section 5.1.6)

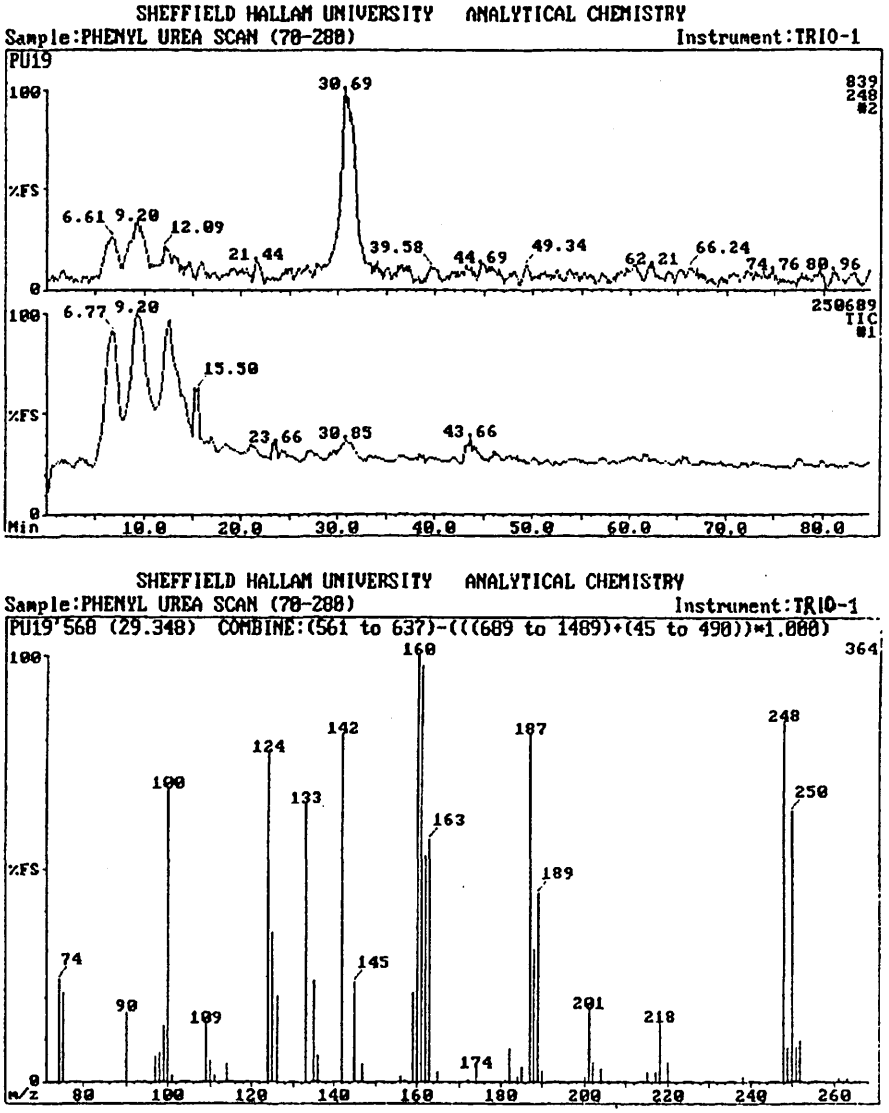
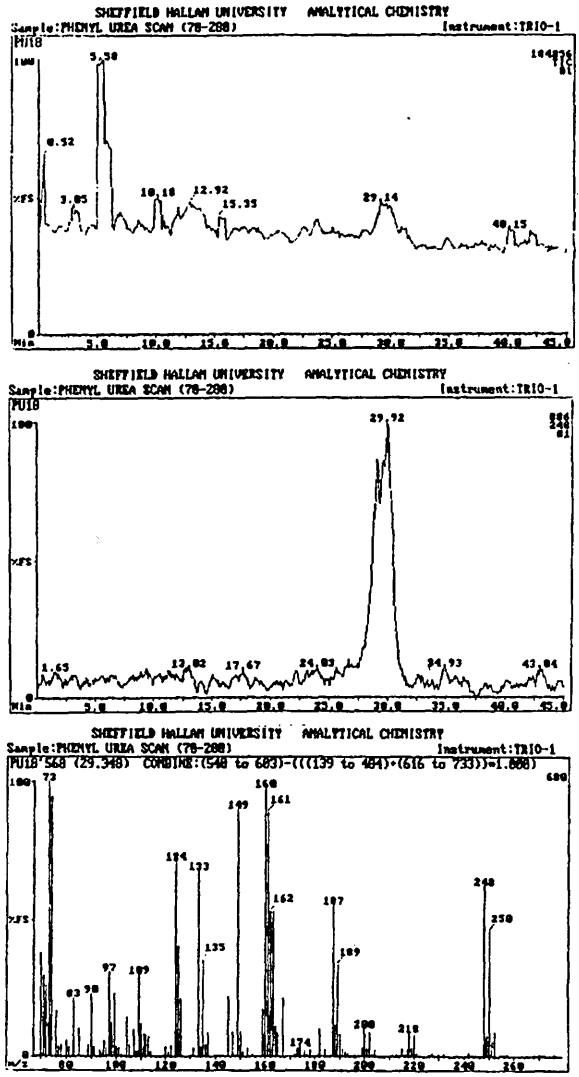


Figure 5.8. *Chromatogram and Mass Spectrum for Linuron Spiked Soil*

(for Experimental details see Section 5.1.6)



These findings are similar to those reported earlier by Miles et al. and Bagheri et al. [65,243]. These authors looked at pesticides in ground, drinking and surface waters by a variety of techniques, such as, GC/MS and PB/MS. Bagheri et al. [243] found that PB/MS was especially useful for the identification of "unknowns", e.g. degradation products and other environmental pollutants. Miles et al. [65] looked at 126 polar water soluble pesticides in ground water. They found that only 25 of these pesticides were readily analysed by GC/MS. These workers looked at PB/MS for the other 101 pesticides and found it to be acceptable for 44. This work also found linear and non-linear calibrations by LC/PB/MS and a "compound dependent carrier effect". These authors concluded that PB/MS was a useful addition to the available MS techniques for the identification of pollutants.

In conclusion, the results presented here show that LC/PB/MS is able to separate a target analyte from a complex matrix and provide unambiguous identification by EI/MS.

Similar LC work was carried out on two pesticide concentrates, using the LC conditions described in Section 5.1.6, taken by HSE inspectors during enforcement action. One was called "Doublet" with active ingredients of isoproturon, bromoxynil and ioxynil. The other was "Seritox 50" with active ingredients of MCPA and dichlorprop (source, 1999 version of "Pesticides" Pub. MAFF and HSE). The PB/MS results obtained for these concentrates are shown in figs 5.9 and 5.10.

Figure 5.9. *Chromatogram and Mass Spectra for "Seritox 50" Concentrate*

(for Experimental details see Section 5.1.6)

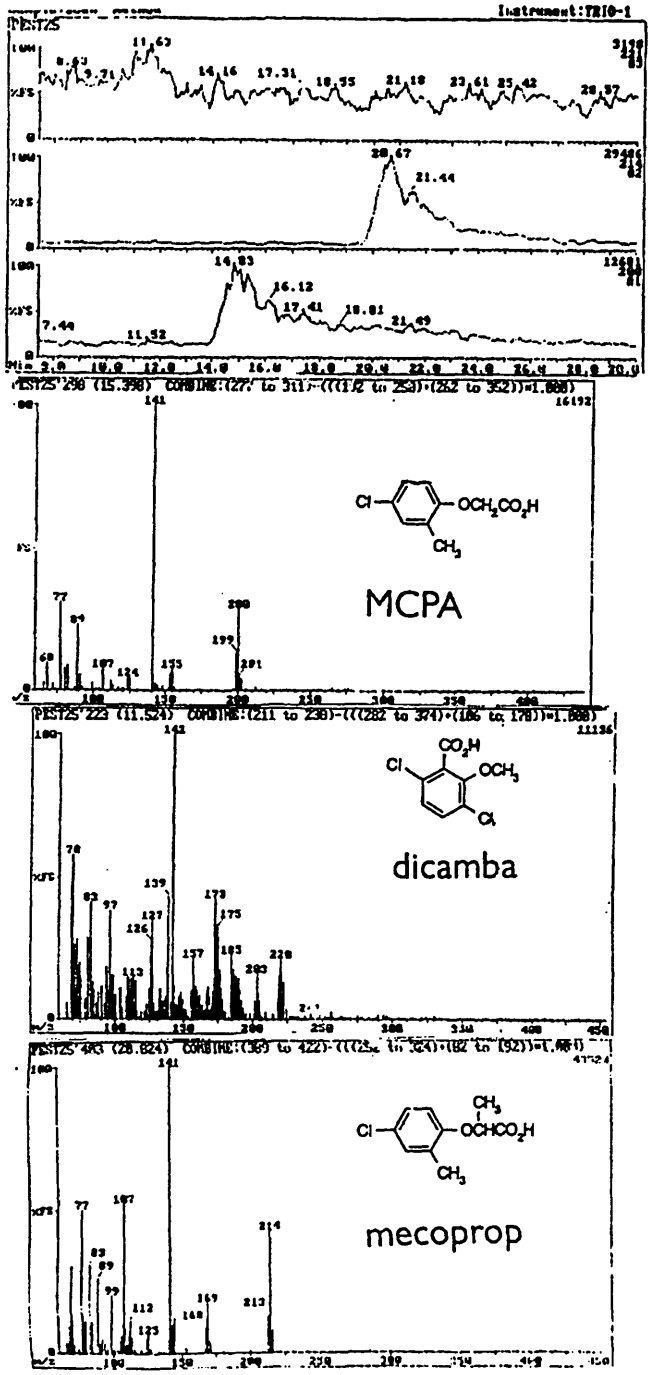
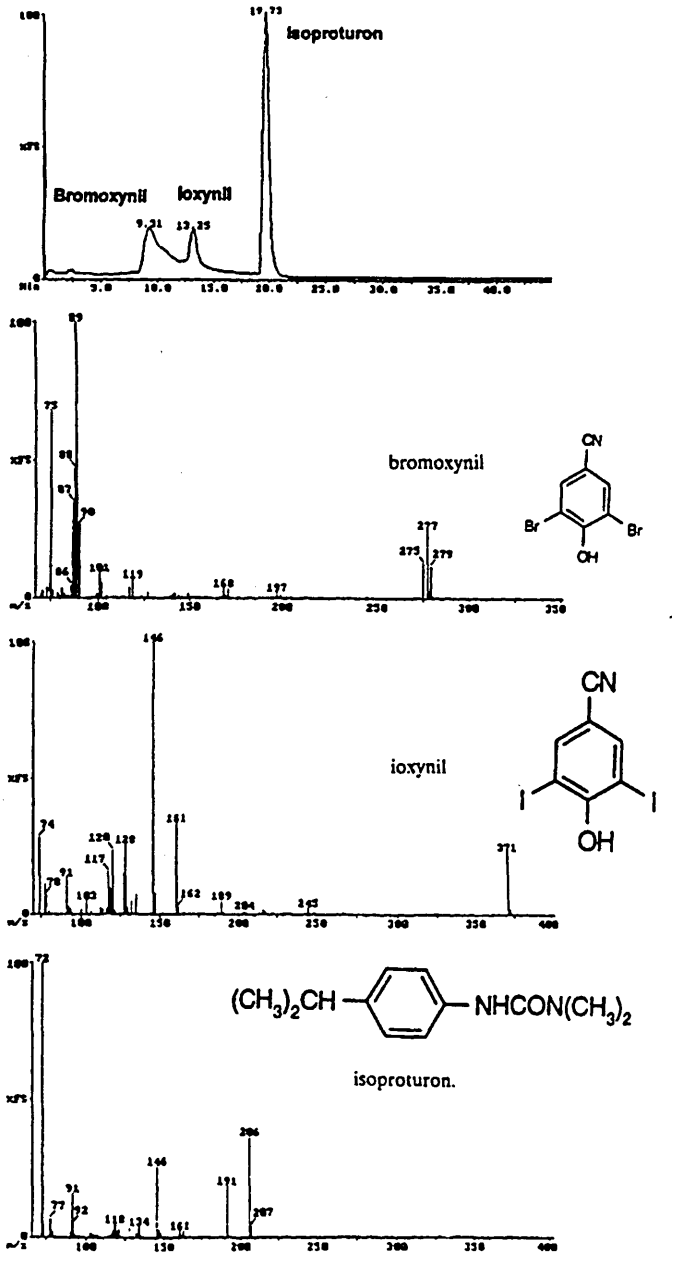


Figure 5.10. *Chromatogram and Mass Spectra for "Doublet" Concentrate*

(for Experimental details see Section 5.1.6)



For the "Doublet" concentrate these results are of note as no derivatization steps were necessary for bromoxynil or ioxynil and isoproturon is known to be unsuitable for GC analysis. LC/DAD gave spectra which matched with a user-created DAD library. LC/PB/MS work, in scan mode, gave classical EI spectra which were matched against pesticide standards. SIM mode was used to provide more sensitivity and better selectivity for the three compounds of interest. The ions monitored in SIM mode were (m/z) 146, 161, 371 (ioxynil), 275, 277, 279 (bromoxynil) and 146,191, 206 (isoproturon). The MS of ioxynil is rather simple and the ions chosen for SIM were the molecular ion ($M^{+\cdot}$) and the two major fragment ions. For bromoxynil, the ions chosen are a characteristic 1:2:1 triplet due to the two Br atoms in the molecule. For isoproturon, the ions chosen were the molecular ion and the two largest fragment ions above (m/z) 100. SIM monitoring using ions below (m/z) 100 is usually avoided, when possible, to decrease the risk of interferences.

For the "Seritox 50" concentrate poor PB/MS results were obtained. This is in keeping with the work described above. Low sensitivity in scan mode was found. However, the mass spectra obtained did show that dicamba (RMM 221.0), mecoprop (RMM 214.6) and MCPA (RMM 200.6) were present. In the 1999 version of "Pesticides" (Pub. MAFF and HSE) Seritox 50 has a stated formulation of MCPA and dichlorprop (RMM 235.1). Dichlorprop was not identified in the concentrate and dicamba and mecoprop were identified by PB/MS and LC/DAD. These results suggest that the concentrate seized was not actually Seritox 50.

"Confirmation of composition" for a pesticide concentrate sample i.e. an unambiguous identification of the pesticides present, is a common forensic requirement. Here the ability of particle beam/MS to give classical electron ionization mass spectra that can be matched against commercially available reference libraries is an advantage over other LC/MS interfaces such as thermospray, electrospray and atmospheric pressure chemical ionization that give little or no fragmentation data. For the vegetation and soil extracts, described earlier, the great selectivity of SIM was essential in filtering out interferants. Again, the ability of PB/MS to give library-searchable EI spectra was a decided advantage over other MS interfaces. LC/PB/MS therefore shows promise for "confirmation of composition" analysis, where unambiguous identification is necessary and for determination of low levels of target compounds in complex matrices.

5.2.5 Investigation of the Post-Column Addition of Additives as a Means of Increasing PB/LC/MS sensitivity for Pesticides

Previous work has highlighted two major problems with PB/LC/MS. These are non-linear response at low levels and poor sensitivity for low molecular mass analytes. Discussion of these problems and their possible causes has been given above. Also discussed above, several authors have suggested the use of additives as "carrier" compounds to increase PB sensitivity and linearity. The use of a variety of additives was investigated for the PB/MS analysis of several pesticides.

Experiments were carried out using the LC ion pairing reagents (octane sulphonic acid, tetramethyl ammonium bromide and tetrabutyl ammonium chloride) and

ammonium acetate (at approx. 1 to 6g/l depending on solubility in 80/20 acetonitrile/water). Also the effect of post column addition of methanol or acetonitrile only was examined. The conditions used have been described above in section 5.1.7. Test compounds used were fenuron, isoproturon and carbaryl. The results obtained for these experiments are given below in tables 5.2, 5.3, 5.4 and fig. 5.11.

Table 5.2. *The Effect of Post Column Addition of Additives on PB/MS Sensitivity for Carbaryl*

Additive Used	PB/MS response (counts)
none	47,896
methanol	183,500
acetonitrile	67,556
tetrabutyl ammonium chloride (3g/l)	blocked skimmer (x2)

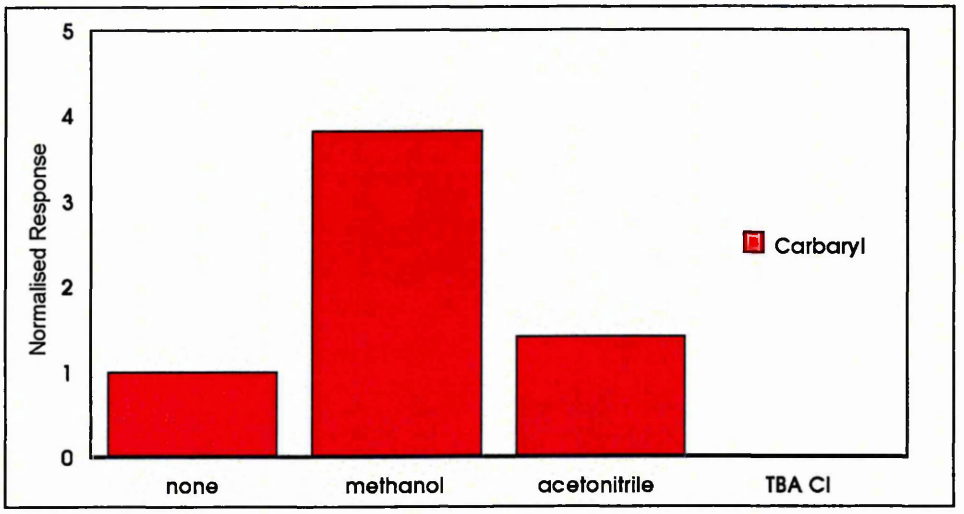
Table 5.3. *Effect of Post Column Addition of Additives on PB/MS Sensitivity for Isoproturon*

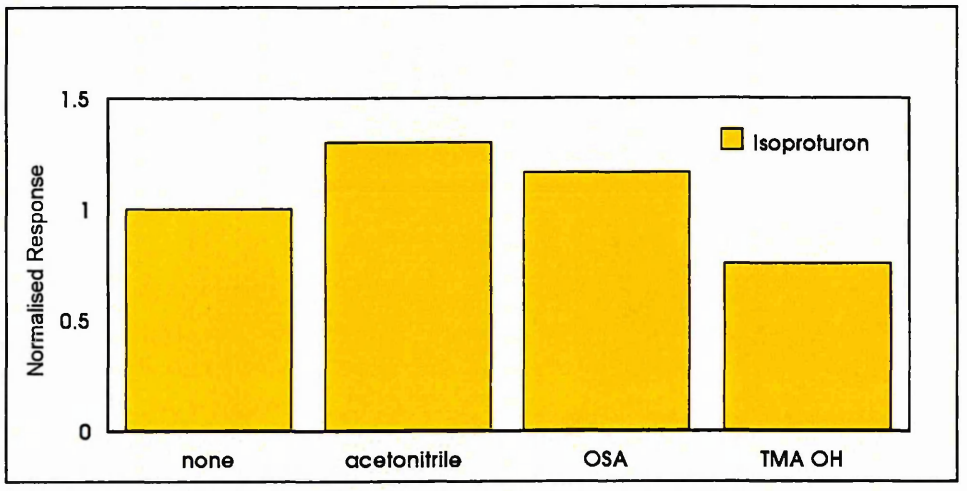
Additive Used	PB/MS response (counts)
none	14,439,836
acetonitrile	18,818,864
octane sulphonic acid (6g/l)	16,884,924
tetramethyl ammonium hydroxide (5g/l)	11,045,393

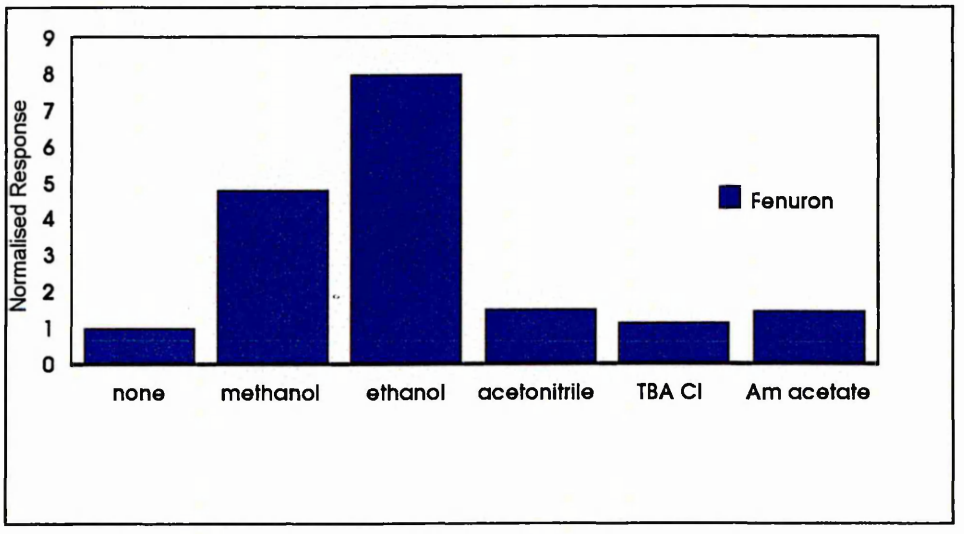
Table 5.4. *Effect of Post Column Addition of Additives on PB/MS Sensitivity for Fenuron*

Additive Used	PB/MS response (10^{-6} x counts)
none	5.595 (%RSD 15, n=4)
methanol	26.683 (%RSD 12, n=4)
ethanol	44.543 (%RSD 9, n=4)
acetonitrile	8.328 (%RSD 7, n=4)
tetrabutyl ammonium chloride (3g/l)	6.312 (%RSD 18, n=3)
ammonium acetate (1g/l)	8.022 (%RSD 57, n=3)blocking skimmer

Figure 5.11. *Effect of the Post Column Addition of Additives on PB/MS Sensitivity for Fenuron, Carbaryl and Isoproturon*







Post column addition of additives such as mobile phase buffers or ion pair reagents, e.g. tetrabutyl ammonium chloride, tetramethyl ammonium hydroxide, ammonium acetate and octane sulphonic acid proved to be unsuccessful. Although small increases in PB sensitivity, over no addition, were observed; these were negated by frequent and repeated blocking of the PB skimmers and the ensuing need to cool, vent and clean the interface. The blocking of the skimmers was caused by the dissolved additive precipitating out over the PB interface skimmer cone. Even relatively volatile buffers, such as ammonium acetate, left a thick, viscous covering over the interface after around half an hour of post column addition. Slight increases in PB/MS sensitivity were seen for the post column addition of acetonitrile for all three compounds. For fenuron the addition of alcohols seemed to have a much larger effect on PB/MS sensitivity and this was investigated further in the experiments reported below. One problem encountered in all the above experiments was large variability in the repeat injections. This is shown by the high %RSD figures given for the fenuron results.

These results provide no support for the "carrier effect". It is possible that the conditions used in this set of experiments were not correct for an observed carrier effect, because as discussed above, this effect has been found to be extremely variable and dependent on a host of instrumental, analytical and analyte conditions. Razzazi-Fazelli and Schmid [46] concluded that PB sensitivity was dependent on nozzle and skimmer design, Helium flow rate, interface temperature, LC flow rate, LC mobile phase composition and source temperature and that the optimum combination of these factors was different for each analyte and each make of PB. From this it follows that there will be no single combination of settings that will

provide optimum PB performance. The results reported here suggest that the formation of large "ion- molecule aggregates", as predicted by the "abrupt high pass filter" model, is also not occuring to any appreciable extent. Both these findings are suprising considering the polar nature of the additives used and the relatively polar nature of the target compounds.

5.2.6 Investigation of the Post Column Addition of Alcohols as a Means of Increasing PB-LC-MS sensitivity

The post-column addition of alcohols was investigated in an attempt to improve PB/MS response. Three phenyl urea herbicides (fenuron, monuron and neburon) were used as target compounds. The results obtained for fenuron have been given above (table 5.4 and fig. 5.11) . Those obtained for monuron and neburon are summarised in tables 5.5, 5.6 and in fig. 5.12. The original set of experiments was carried out fenuron using post column addition of both solvents and additives (see fig. 5.11). It was noticed for fenuron that ethanol gave the best result. The work with monuron reproduced these findings. For neburon a larger number of alcohols were investigated as post column additives to obtain a better picture of the effect of alkyl chain length on PB/MS response (see fig. 5.12).

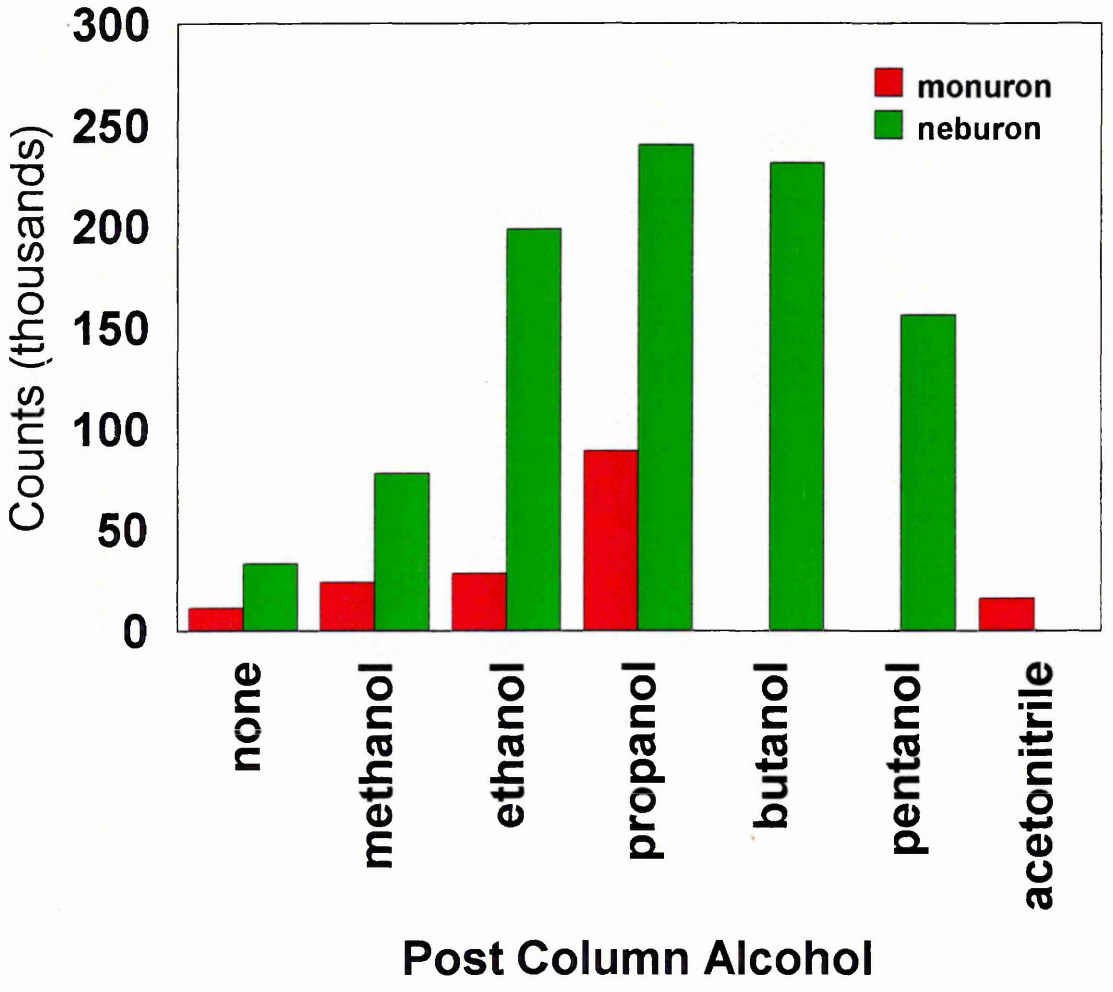
Table 5.5. *Effect of Post Column Addition of Additives on PB/MS Sensitivity for Neburon*

Additive Used	PB/MS response (10^{-5} x counts)
none	0.33 (%RSD 15, n=4)
methanol	0.78 (%RSD 14, n=3)
ethanol	1.99 (n=2)
propanol	2.40 (%RSD 12, n=4)
butanol	2.31 (%RSD 9, n=5)
pentanol	1.56 (%RSD 9, n=5)

Table 5.6. *Effect of Post Column Addition of Additives on PB/MS Sensitivity for Monuron*

Additive Used	PB/MS response (10^{-5} x counts)
none	0.11 (%RSD 15, n=4)
methanol	0.23 (%RSD 16, n=3)
ethanol	0.27 (%RSD 12, n=5)
propanol	0.89 (%RSD 9, n=3)
acetonitrile	0.16 (%RSD 8, n=4)

Figure 5.12. *Effect of Post Column Addition of Alcohols on PB/MS Sensitivity for Monuron and Neburon*



Significance testing on the neburon results (two tailed t-test) showed PB response with post column butanol significantly different (higher in all cases) than that with pentanol, ethanol, methanol and no post column (P= 0.05). The PB response obtained for propanol was similar to that for butanol (i.e. not significantly different).

Significance testing on the monuron and fenuron results gave similar results. For these compounds post column addition of any alcohol or acetonitrile was found to give significantly different results from no post column addition (two tailed t-test P=0.01).

For all of these compounds the maximum increase in sensitivity was of the same order of magnitude (i.e. fenuron 8x, monuron 8x, neburon 7x w.r.t no post column addition) and a similar curves were seen. Generally, PB/MS response increased for the series; no addition, methanol, ethanol, propanol. Propanol and butanol gave similar responses for neburon. Experiments with pentanol showed a slightly lower response than ethanol.

It is suggested the addition of alcohols post-column is improving sample transfer through the interface by aiding aerosol formation in the expansion chamber. The mechanism for this effect will be rather complex and possible factors are considered below.

One possible factor is related to the volatilities of the alcohols used. As volatility decreases (b.p. °C, methanol 64.9, ethanol 78.5, propanol 97.1, butanol 117.3 pentanol 137.3); the particles formed in the aerosol will be less liable to evaporation. This means that they will be less likely to pumped away with the solvent vapour and so, possibly, more likely to pass through the PB interface and enter the MS. However, Voyksner et al. [244] have reported that the sensitivity of PB/MS, for various pharmaceuticals, was greatest when using a solvent with a low heat capacity, i.e. methanol > acetonitrile > isopropanol > water. This order is in agreement with work

reported above. This is presumably due to increased vaporization for the more volatile solvents. This finding suggests that normal phase chromatography i.e. with a non-polar and usually volatile LC mobile phase, would give good PB/MS sensitivity. A more stable aerosol should also be favourable for the formation of large aerosol particles [66] which according to the increased particle size explanation of the "abrupt high pass filter" model should be more effective in passing through the PB interface [56]. However these larger particles are also more prone to losses due to gravitational, centrifugal and impaction processes [66].

Balancing this effect will be the consideration that, for a given interface temperature, very involatile additives may not be aerosolized effectively by the nebulizer due to increased viscosity. This will reduce the amount of material in the vapour phase of the aerosol. Increased viscosity will also decrease evaporation of particles deposited on the side of the heated expansion chamber. Also, the less volatile the alcohol becomes, the more "oily" it will be. There will therefore be a problem of miscibility with the aqueous component of the LC mobile phase which may lead to incomplete aerosol formation and partioning of the analyte and any additive into the aqueous component of the aerosol.

Another effect of adding alcohols post column to the LC mobile phase will be to reduce the surface tension of the droplets. It is suggested that a decrease in surface tension would facilitate the droplet breaking down, i.e. as the surface tension forces at the liquid drop interface decrease the droplet is more likely to lose its integrity and fragment into smaller droplets.These smaller droplets would have a greater surface area to volume ratio than the original large droplet and, in the

absence of any electrostatic charging mechanism, would therefore have a smaller charge density at the surface of the droplet. This reduced surface charge would presumably result in a decreased loss of particles through electrostatic process. In addition, smaller particles are less prone to losses due to gravitational settling [66].

The processes occuring in particle beams have been considered before. Mallina et al. [245,246] presented a theoretical model which considered the factors governing particle growth in a high speed PB inlet. They considered that nucleation due to homogeneous particles, a process called agglomeration, had a negligible effect on particle growth. However in a heterogeneous aerosol the size of condensation nucleus was an important factor governing particle growth. These authors found that large particles (> 40 μm) were extremely prone to loss due to collisions with the interface wall. The theoretical model used in this work predicted an optimum particle size "window" for transmission through the PB interface. This optimum size would vary according to instrument type and experimental conditions in use. Particles larger than this optimum range are lost due to gravity (wall collisions) and particles smaller than this range are lost due to electrostatic forces or are pumped away by the system. These authors concluded that the factors governing particle growth in a particle beam were complicated.

Li and Koropchak [247] carried out an investigation into particle sizes in PB with reference to the ammonium acetate "additive effect". These workers found no increase in particle size for the addition of ammonium acetate to the LC mobile phase, thereby supporting the findings of Wilkes et al. [47]. Li and Koropchak found that ammonium acetate, in some cases, decreased the particle sizes measured.

Particle sizes were measured using a scanning mobility particle sizer. The effect of ammonium acetate was found to be variable and dependent on analyte type and desolvation method. However, ammonium acetate did increase PB sensitivity in this study. These authors looked at the shapes of the droplets produced using a high resolution transmission electron microscope. They found that ammonium acetate produced irregular shaped particles compared with the roughly spherical particles produced with no ammonium acetate. Li and Koropchak concluded that the ammonium acetate was increasing PB transmission by three separate mechanisms. These were; by decreasing the size of the particles formed leading to less gravitional loss; by neutralising charged particles leading to less electrostatic loss; and by increasing analyte solubility by forming complexes with analyte.

This work supports the findings of the post column addition of alcohols experiments outlined above. As suggested above, the smaller particle sizes promoted by addition of alcohol and the resulting lowering of surface tension at the droplet surface would also lead to decreased gravitional and electrostatic loss. The surface tension explanation can also be applied to the additive and carrier effects noted by several groups. It has been stated that the surface tension of liquid decreases as a solute accumulates at the surface [248]. This decreased surface tension would lead to decreased losses due to the processes described previously for the post column addition of alcohols. However, published data on the addition of organic compounds to organic solvents shows that sometimes surface tension decreases, stays the same or increases with increasing solute concentration [249]. This is discussed in more detail by Loeb [76]. In contrast, the surface tensions of the alcohols in water mixes quoted in this source [249] always decrease as the concentration of alcohol

increases. This variable behaviour for organic solutes in organic solvents may also explain the variability observed for the additive and carrier effects. Finally, the suggestion that smaller particles give increased PB transmission is consistent with the findings of Cappiello et al. [233] who have reported increased PB sensitivity when using a capillary scale interface which generates very small particle sized aerosols (mean droplet area, (from electron micrographs) conventional interface ~8-10 square microns, capillary scale interface 2-3 square microns) .

In summary, based on the work reported above it is believed that the increases in PB sensitivity observed for the post column addition of alcohols, and by other workers on addition of mobile phase additives and carrier compounds, are due to a variety of factors. A major one of these factors is a lowering of the surface tension of the droplets produced on nebulization. This leads to smaller and less electrically charged particles which are less prone to gravitational and electrostatic losses. This explanation is at odds with the explanation of the "abrupt high pass filter" model proposed by Apffel and Perry [56]. These workers proposed an increased particle size as the explanation for increased PB sensitivity with additives and carrier compounds.

5.3 Conclusions

LC/PB/MS offers some advantages over GC analysis and over other LC/MS interfaces and LC detectors for pesticide analysis. Particularly useful is the ability to generate EI spectra for confirmation and identification purposes. The selectivity of MS detection is also necessary for samples in complex environmental matrices. The problem of low sensitivity of PB interfaces still needs to be solved. Methods for

increasing PB sensitivity were examined. No increase on addition of mobile phase additives was found but post column addition of alcohols was found to be beneficial. It is proposed that this is chiefly due to the heat capacity of the alcohol and a surface tension effect which decreases particle size and so reduces electrostatic and gravitional losses and not due to an increase in particle size which is the usual explanation of the "abrupt high pass filter model".

Chapter 6

Analysis of Isocyanates by Particle Beam LC/MS

6.0 Introduction

Isocyanates are highly reactive molecules widely used in industry, for example, in paints, polyurethane foams, coatings, plastics and adhesives [250,251]. They are known respiratory sensitizers and are the commonest cause of occupational asthma [252,253]. For this reason the Health and Safety Executive (HSE) has set a long term occupational exposure limit (8 hr Time Weighted Average reference period) [254] of 20 $\mu g/m^3$ (as isocyanate (NCO) group) for workplace air.

As the isocyanate function is so reactive, analysis in the workplace is commonly carried out by trapping the isocyanate with a derivatization reagent to produce a stable derivative. Numerous analytical methods have been proposed for isocyanates. HPLC with fluorescence detection, after derivatization by a variety of reagents, has been reported by Schultz and Salthammer [255] and Gifford et al.[256], Wu et al.[257] and Rando et al.[258]. Schultz and Salthammer[255] derivatized the isocyanate with1-(2-pyridyl)piperazine (2PP). This reagent is also used in OSHA method #47 [259]. Gifford et al.[256] reported on the use of several acenaphthene containing compound as derivatization reagents. Wu et al.[257] described the use of tryptamine and Rando et al.[258] on the use of 9-methylamino-methylanthracene (MAMA) as derivatization reagents for isocyanates.

A method for the colorimetric determination of isocyanates has been described by Rando and Hammad [260]. This method is based on diazotization of the isocyanate with naphthylethylenediamine and is commonly known as the "Marcali" method. A more recent UV method has been proposed by Streicher et al. [261]. This method uses

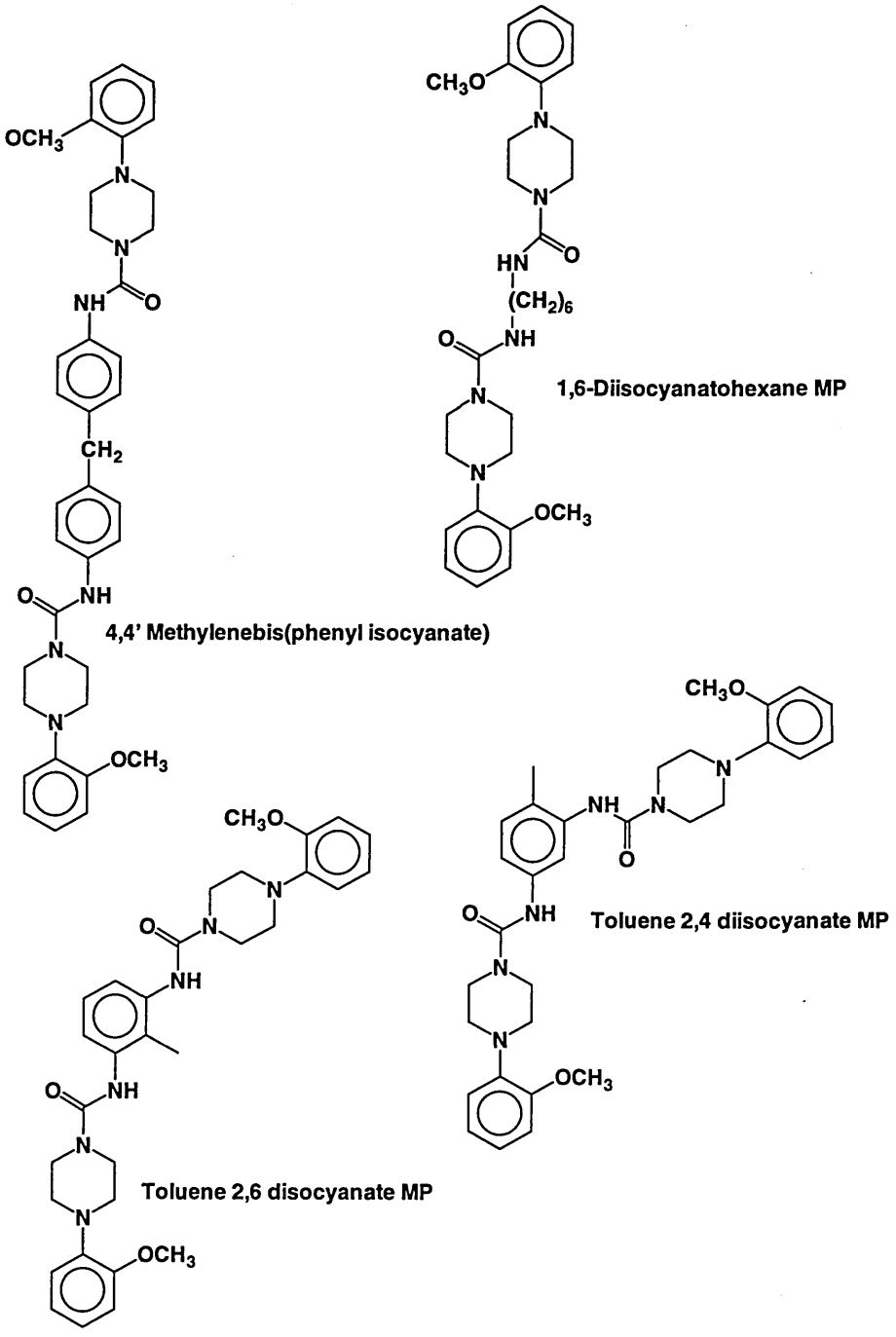
a specifically synthesized derivatization reagent, 1-(9-anthracenylmethyl)piperazine to trap the isocyanate. This method has been evaluated in a workplace environment by Rudzinski et al. who looked at airborne monomeric and oligomeric isocyanate aerosols produced during spray painting of aircraft [262]. These workers found this method to give comparable results with other commonly used methods.

A different approach has been described by Hughes [263]. This method used propan-1-ol to react with the isocyanate to produce a urethane. The urethane is then hydrolysed to regenerate the alcohol which is then measured. This approach has several potential advantages for the determination of the total isocyanate content, particularly for the monomeric and polymeric mixtures of isocyanate used in industry.

All of the methods currently used are adequate for the determination of isocyanate monomers but have problems with polymeric or oligomeric isocyanates, particularly when sampling from the aerosol phase. Unfortunately isocyanate oligomers and polymers are the most commonly used in industry as they are less volatile than the monomers and so pose less of a vapour hazard.

The U.K. method for isocyanate determination is MDHS 25/3 [264]. This method traps the isocyanate with 1(2-methoxyphenyl)piperazine reagent (MP) to form the urea derivative. The structures of the MP urea derivatives, for the most commonly used NCOs, are shown in Fig. 6.1.

Figure 6.1. *Structures of most Commonly used Monomeric NCO-MP derivatives*

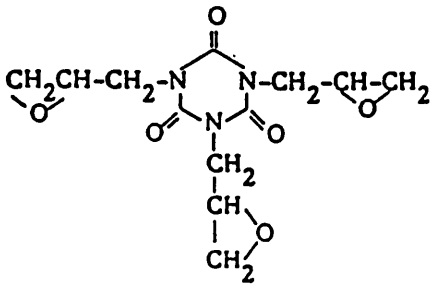


The urea derivative is too involatile to determine by GC and so is analysed by LC with electro-chemical (EC) and UV detection. In an earlier version of this method (MDHS 25/2), isocyanates were identified by a response ratio approach. In this approach the isocyanate derivative peak is measured by the two detectors

mentioned above, the two peaks are integrated and the peak areas are calculated for the two detectors. The peak area for the EC detector is divided by the peak area for a given UV wavelength and the response ratio calculated ("EC/UV" ratio). This ratio is then divided by the similar ratio derived from the relevant isocyanate monomer derivative and the result compared with an empirically derived range of acceptable values. Recently several questions have been raised regarding the suitability of this method for isocyanate pre-polymers [265-268]. Particularly, the correct identification of isocyanate derived peaks has emerged as an issue with these workers suggesting that the response ratio method used to identify isocyanate derived peaks may be suspect. Although the most recent version of MDHS 25 (25/3) has largely answered these criticisms, the use of LC/MS to correctly identify isocyanate derived peaks is a promising approach and has been investigated in the work reported in this thesis.

Also reported in this thesis is some preliminary PB/LC/MS work carried out on the compound triglycidyl isocyanurate (TGIC). This compound is commonly used as a binder in paints and has been shown to cause ill health. It is currently measured using LC with UV detection [269] . However the UV spectrum of TGIC is not particularly unique as TGIC lacks a UV chromophore (see Fig. 6.2) and so it was decided to see if PB/LC/MS would provide a better means of detection.

Figure 6.2. *Structure of Triglycidyl Isocyanurate*



6.1 Experimental

6.1.1 Chemicals

Solvents used (acetonitrile, methanol, ethanol, propanol, butanol, pentanol) were of LC grade or better (from Rathburns, Walkerburn, U.K. or Fisher Scientific, Loughborough, Leics., U.K.). Water was purified in-house using an Elgastat UHQ II system (Elga Ltd., High Wycombe, Bucks., U.K.) and buffers were prepared using this. Ammonium acetate was supplied by Fisons, Loughborough, Leics. U.K.. Acetic acid, sodium acetate, 1-(2-methoxyphenyl)piperazine (MP), phenyl isocyanate (PI), 1,6-Diisocyanatohexane (HDI), 4-4' methylene bis(phenyl isocyanate) (MDI) toluene

2,6-diisocyanate and toluene 2,4-diisocyanate (TDI) and triglycidyl isocyanaurate (TGIC) were supplied by Aldrich Chemical Co., Gillingham, Dorset, U.K.. Diisocyanate and monoisocyanate MP derivatives were prepared at the Health and Safety Laboratory (HSL) from the relevant monomers and from samples submitted by HSE Inspectors during routine occupational hygiene monitoring or enforcement action. The TGIC based coating examined was a sample submitted by HSE Inspectors during routine occupational hygiene monitoring.

6.1.2 Instrumentation

The PB/LC/MS system used in this work consisted of a VG Trio 1 MS and a VG LINC PB interface (VG Analytical, Altrincham, U.K.). Various LC pumps (Waters 510 supplied by Waters , Watford, Herts. U.K. or Jasco LG-980-02, supplied by JEOL (U.K.) Ltd., U.K.) were connected to this system. Injections were made manually through a 7125 Rheodyne valve (Alltech, Carnforth, Lancs. U.K.). Probe Electron Impact MS was carried out, by Mrs. J. Hague at Sheffield Hallam University, on the VG Micromass 7070S (VG Analytical, Altrincham, U.K.).

The LC/ES/MS system used in this work was that available at the University of Sheffield. This consisted of a Fisons VG Platform MS with a VG ES interface. The LC pump used with this system was a Jasco LG-980-02 (JEOL (U.K.) Ltd., U.K.). The flow was split through a ~100:1 flow splitter (homemade). Injections were made manually through a 7125 Rheodyne valve (Alltech, Carnforth, Lancs. U.K.).

Other LC work was carried out on the Hewlett-Packard HP1090 (HP, Stockport, Cheshire, U.K.) and Waters Millennium 2010 systems available at the HSL . Fluorescence and Diode-array detectors were also used.

LC columns used were 15 x 0.46 cm S3 ODS2 and 25 x 0.46 cm S3 ODS2 (Thames Chromatography, Maidenhead, Berks. U.K.) or 10 x 0.46 cm S5 ODS2 (PhaseSep, Clwyd, Deeside, U.K.).

6.2 Practical

6.2.1 Determination of Isocyanate MP Derivitives Using LC/PB/MS

Diisocyanate derivatives were prepared following MDHS 25/3 . The mobile phase used for the LC/DAD experiments was 60% acetonitrile/40% 60mM sodium acetate buffer, flow rates of 0.3 and 1ml/min. were used. For the PB experiments the mobile phase described above was found to cause unacceptably frequent skimmer blocking and so 50mM ammonium acetate was used in the buffer instead of the sodium acetate. The flow rate used was 0.3 ml/min. The LC/PB/MS system operating conditions finally used were: interface temp. 70 °C, nebulizer helium pressure 20 p.s.i., electron energy 70eV and source temp. 200 °C. Loop injections of the MP derivatives were made to obtain the EI/MS.

6.2.2 Determination of Isocyanate MP Derivitives Using Electrospray Ionisation/MS

Loop injections were made using a flow rate of 10 and 20 µl/min. The LC column used was a 10 x 0.46 cm S5 ODS2 (PhaseSep, Clwyd, Deeside, U.K.), with a flow rate of 1ml/min. of which 10µl (100:1 split) entered the MS interface. The ESI nebulizer gas was 2 p.s.i. (N_2) and a drying gas pressure of 250 p.s.i. (N_2). The cone voltage was set at +5kV. The mobile phase used for these experiments was 60% acetonitrile/40% 50mM ammonium acetate buffer.

6.2.3 Investigation of the Post Column Addition of Alcohols as a Means of Increasing LC/PB/MS sensitivity

Post column addition of alcohol experiments used flow rates of 0.3 ml/min. mobile phase (45% acetonitrile, 45% 10mM acetic acid and 10% methanol, run isocratically) + 0.2 ml/min. of alcohol "t-pieced" in "post column". Repeat loop injections (1cm guard column only in-line, i.e. no analytical column) of 25ul were made for this series of experiments Operating conditions for the LC/PB/MS system were: interface temp. 70 °C, nebulizer helium pressure 20 p.s.i., electron energy 70eV and source temp. 200 °C.

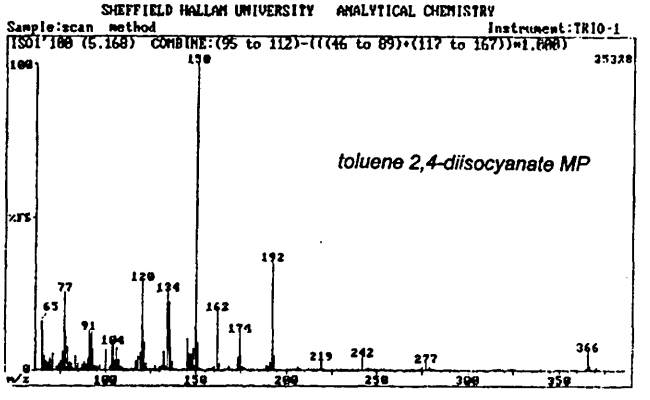
6.3 Results and Discussion

6.3.1 Determination of Isocyanate MP Derivitives Using LC/PB/MS

LC/PB/MS was carried out on diisocyanate 1-(2-methoxyphenyl)piperazine (MP) derivatives prepared from monomers and industrial pre-polymer samples. For the diisocyanate monomer MP derivatives studied (toluene 2,6-diisocyanate and toluene 2,4-diisocyanate (both TDI isomers)) (Fig 6.3), a peak at m/z 366 corresponding to the fragment TDI-$(MP)_1$ was seen for both isomers, but no molecular ion was observed (RMM TDI-$(MP)_2$ 558). It was thought that this was due to the diisocyanate-MP derivative decomposing, either in the interace or in the MS. Various experiments were carried out to try to obtain a molecular ion for the TDI-MP derivatives; these are described below. For the other commonly used isocyanate MP derivatives; 1,6-Diisocyanatohexane (HDI) and 4,4'-methylene bis(phenyl isocyanate) (MDI), no molecular ions were observed. A peak at m/z 360, corresponding to HDI-MP_1 was observed but for the MDI-MP derivative only peaks relating to the derivatizing agent were seen (Fig. 6.4).

Figure 6.3. *LC/PB/Mass Spectra of toluene 2,4-diisocyanate MP and toluene 2,6-diisocyanate MP derivatives*

(for Experimental details see Section 6.2.1)



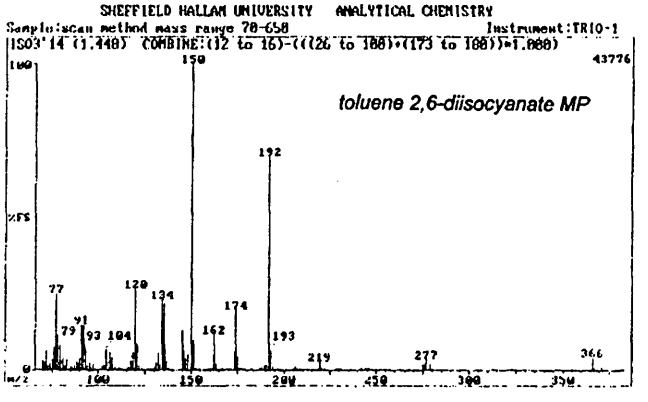
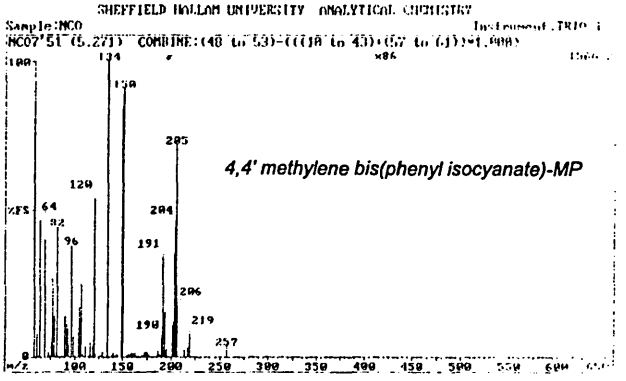
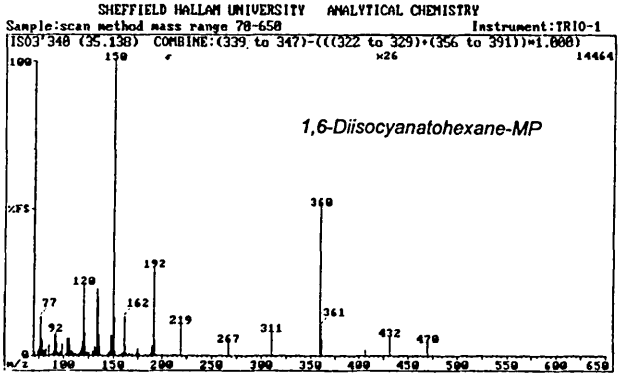


Figure 6.4. *PB/EI Mass Spectra of 4,4' methylene bis(phenyl isocyanate)-MP and 1,6-Diisocyanatohexane-MP derivatives*

(for Experimental details see Section 6.2.1)

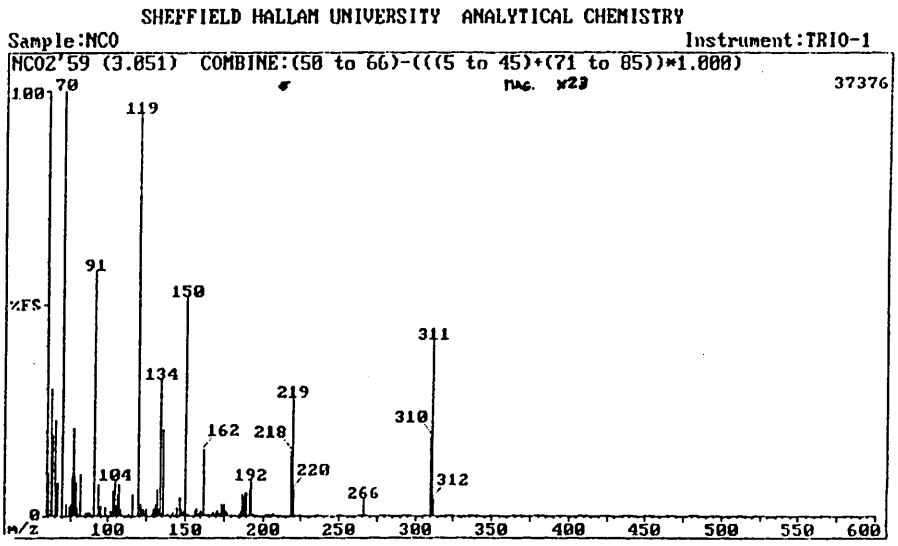




Varying the interface temperature from 70 to 20 °C had no effect on the fragmentation patterns observed for the TDI-MP derivatives, but markedly decreased sensitivity; no molecular ion was observed. The source temperature was varied from 250 to 150 °C but again no molecular ion was observed. Altering the source electron energy from 70 to 25 eV altered the fragmentation pattern but did not result in a molecular ion being observed. These results suggest the diisocyanate derivatives are very easily decomposed in the PB/MS system, essentially losing one MP group. Interestingly, for the MP derivative of a mono-isocyanate, phenyl isocyanate, a small molecular ion (RMM 311) was observed on the LC/PB/MS system used in this study (Fig 6.5).

Figure 6.5. *PB/MS of Phenyl isocyanate-MP derivative*

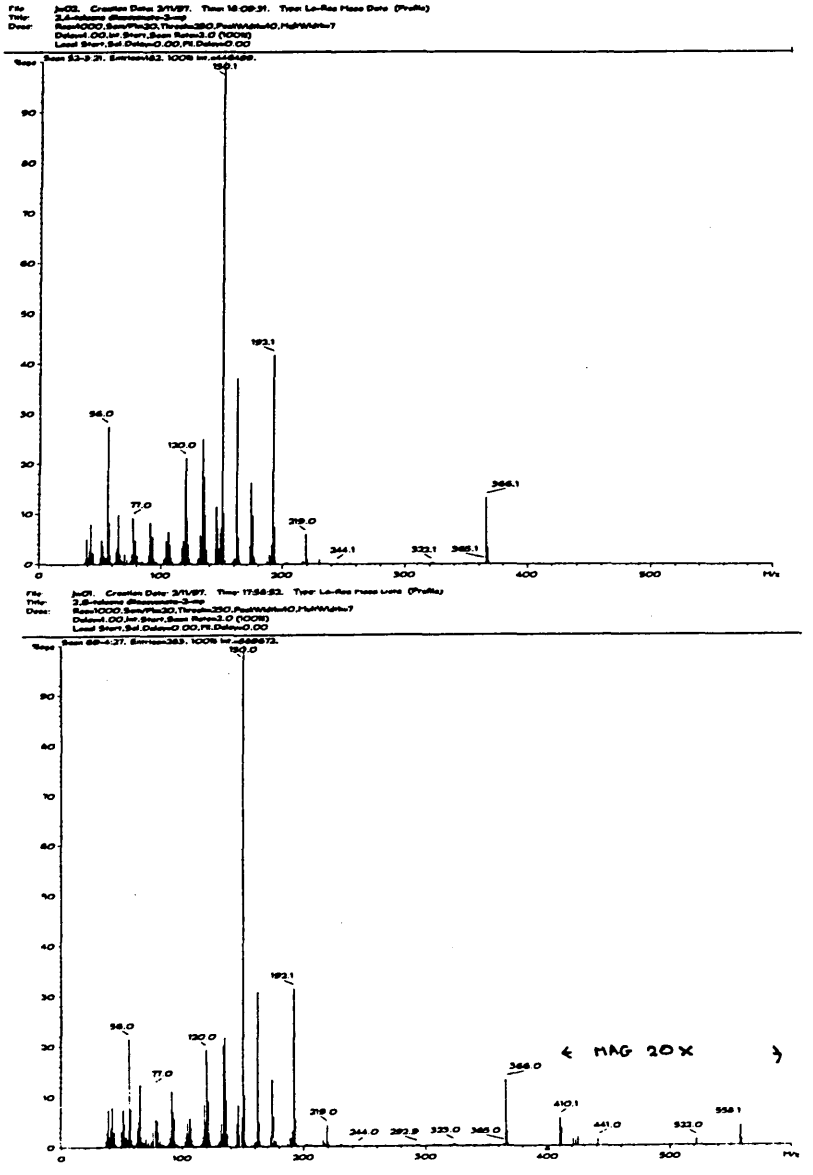
(for Experimental details see Section 6.2.1)



Finally, both TDI isomers were examined by probe MS and their EI mass spectrum obtained. The MS obtained for the 2,4 isomer (Fig. 6.6) was the same as that observed using PB-MS, in that no molecular ion (m/z) 558 was seen. For the 2,6 isomer a very small molecular ion was observed (see Fig. 6.6) . This was not seen with PB/MS.

Figure 6.6. *Probe/MS of toluene 2,4-diisocyanate MP (upper) and toluene 2,6-diisocyanate MP (lower) derivatives*

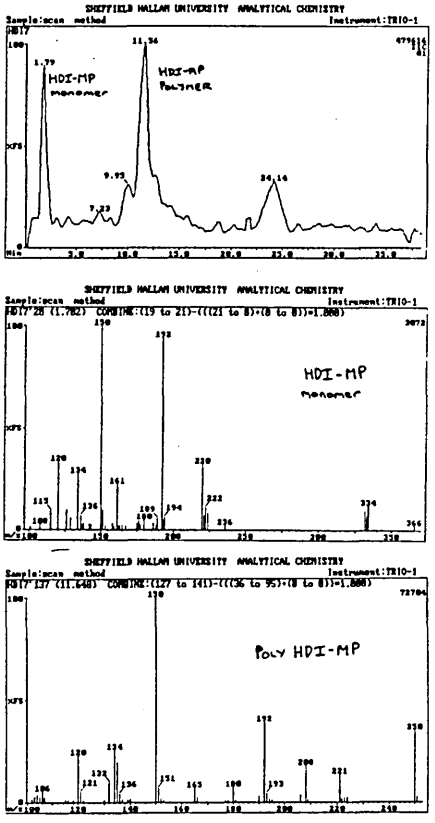
(for Experimental details see Section 6.2.1)



The PB/MS of the diisocyanate derivatives (oligomers and pre-polymers) were dominated by peaks derived from the derivatizing reagent (1-(2-methoxyphenyl)piperazine, (MP) RMM 192). However, peaks were observed that correspond to isocyanate-containing fragments (eg TDI RMM 174 and TDI-(MP) (m/z) 366 (one MP group). The monomer molecular ion , TDI-$(MP)_2$ (m/z) 558 was not seen (Fig. 6.7). Similar results were obtained for the MDI and HDI based polymers.

Figure 6.7. *PB/MS of a Polymeric TDI based Adhesive*

(for Experimental details see Section 6.2.1)



These spectra were dominated by the peaks at m/z 150 and 192 with occasional higher mass fragments being seen for some of the polymeric NCO species. These results suggest that PB/MS may be of use for confirmation that a peak is isocyanate derived. The particular fragment ions observed could also provide information on which isocyanate monomer is the parent compound. This would be useful for occupational hygiene monitoring when pre-polymers are being analysed. Finally, it may be possible to carry out quantitative work using the peaks derived from the derivatizing agent as these should be present for all isocyanate containing compounds. Further work is required to see if this approach will be useful.

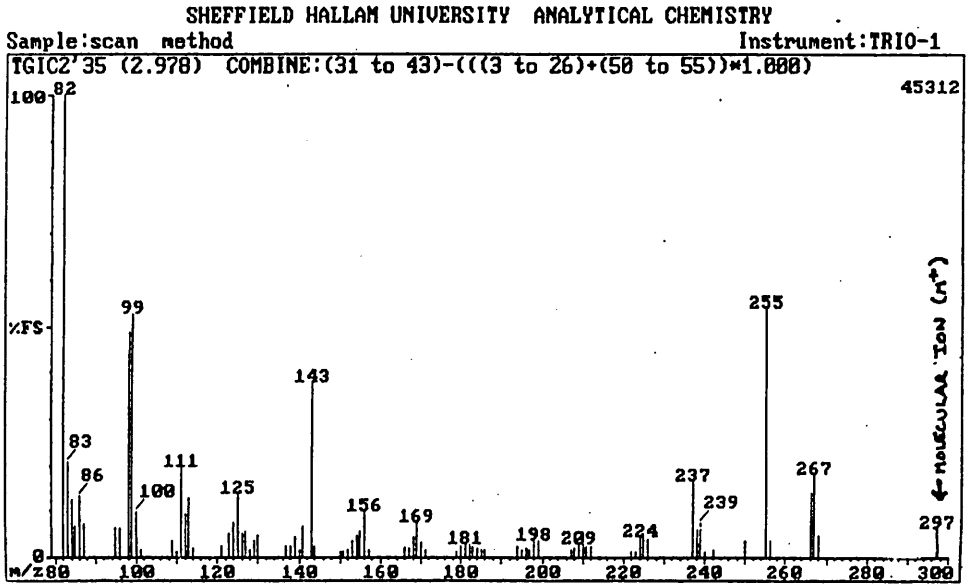
A recent literature search found no references to ESI/MS or PB/MS work on NCO-MP derivatives other than the paper by this author (White et al. Rapid Communications in Mass Spectrometry, (1997), Vol.11, p618-623) which records some of the work reported here. However some work has been published on other polymeric compounds. Jones et al. [270] looked at styrene oligomers by LC/PB/MS. These workers found that PB gave a similar response to UV for polymers up to an 18-mer (RMM ~2000). These workers also looked at the different transport efficiencies for the styrene oligomers and found transport to decrease by approx. 3x as the RMM increased from a 14-mer to an 18-mer. More noticable was the fact that a higher degree of fragmentation was seen as the RMM of the oligomer increased. This is in agreement with the pre-polymer and polymeric NCO work described above. Similar work has been reported by Murphy et al. [271] for poly(methyl methacrylate- butyl acrylate) co-polymers. For these compounds it was found that the fragment ions produced were proportional to the co-monomers present and were quantitatively related to the copolymer composition. This finding suggests that

PB/MS may be of quantitative use for the determination of NCOs. Yu et al. [272] compared LC/API/MS and LC/PB/MS and found the two techniques to be complementary, with PB giving classical EI spectra and APCI giving molecular weight information.

The TGIC work gave a molecular ion at m/z 297 as well as various fragment ions (Fig. 6.8). An extract from a TGIC based industrial powder coating gave a similar MS. This work suggests that PB/MS may be of use for TGIC determination but more work would be required to develop a validated analytical method.

Figure 6.8. *PB/MS of Triglycidyl Isocyanurate*

(for Experimental details see Section 6.2.1)

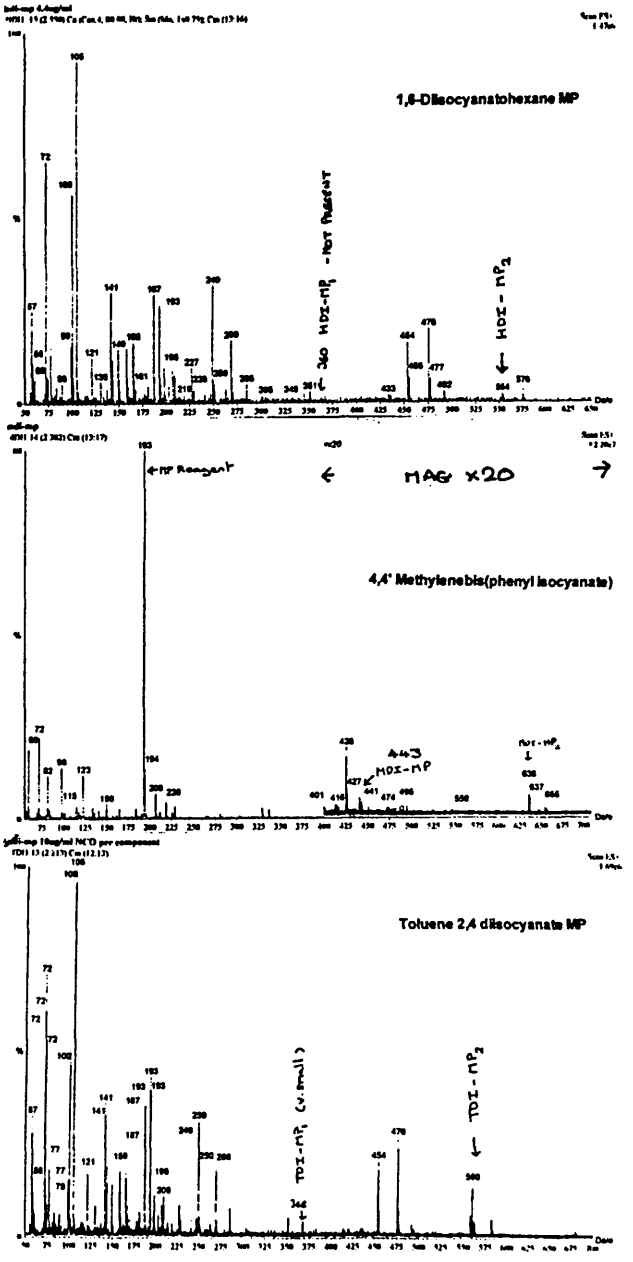


6.3.2 Determination of Isocyanate MP Derivatives Using Electrospray Ionisation/MS

Loop injections were carried out on the monomeric NCO derivatives (HDI, MDI and the two TDI isomers) to obtain the ES/MS of these compounds. The spectra obtained are shown overleaf (Fig. 6.9).

Figure 6.9. *ESI/MS of monomeric NCO-MP derivatives*

(for Experimental details see Section 6.2.2)



These spectra are similar to those obtained by PB and Probe MS in that the major peaks come from the MP reagent. However, the ES/MS do show peaks corresponding to the molecular ion, MP_2 (and smaller MP_1) fragments and their Na^+ adducts $(M+23)^+$, for the monomer derivatives. This is presumably due to the fact that ES is a softer ionization technique than EI and so less fragmentation of the parent molecule occurs. Both TDI derivatives gave similar mass spectra. MP derivitized polymeric NCOs were run, using the LC conditions described above, and these experiments gave similar spectra to those observed for the monomers. Again, more work is required to see if ES/MS can be developed into a practical working method for polymeric NCOs.

No other work on the ESI/MS of NCO-MP derivatives has been found. However, Skarping et al. have produced several papers on the use of API/MS for NCO-dibutylamine derivatives [273-277]. These workers found that ESI/MS and APCI/MS were helpful for identification and qualitative purposes but have presented no quantitative data for the polymeric NCOs in their work. These workers found considerable fragmentation for the NCO polymers which was useful for characterization purposes.

6.3.3 Investigation of the Post Column Addition of Alcohols as a Means of Increasing LC/PB/MS Sensitivity

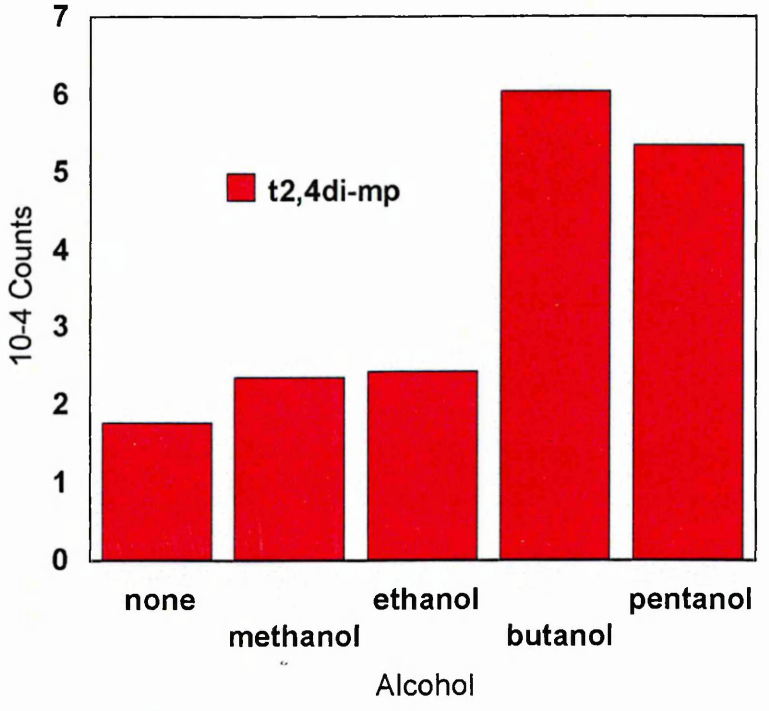
The post-column addition of alcohols was investigated in an attempt to improve PB/MS response. Post-column addition experiments were carried out using loop injections of toluene 2,4-diisocyanate-MP (TDI-MP). The results of this work are shown in Table 6.1 and Fig 6.10.

Table 6.1. *Effect of Post Column Addition of Alcohols on PB/MS Sensitivity for toluene 2,4-diisocyanate-MP*

Alcohol	10^{-4}Counts	% RSD	n
none	1.76	21	4
methanol	2.35	29	4
ethanol	2.43	19	5
butanol	6.05	26	6
pentanol	5.34	24	5

Figure 6.10. *Effect of Post Column Addition of Alcohols on PB/MS Sensitivity for toluene 2,4-diisocyanate-MP*

(for Experimental details see Section 6.2.3)

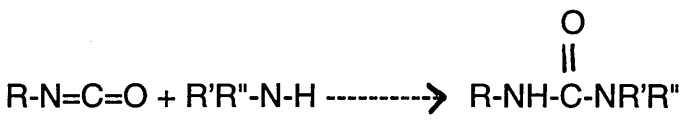


For this compound the same shape of curve as that mentioned for the pesticide work reported in section 5.2.6 was seen i.e. butanol > ethanol > methanol > nothing and butanol > pentanol. The magnitude of the increase was however smaller than

that seen for the pesticide work (butanol 3x no post column addition). Significance testing of the butanol and ethanol results showed that these results were significantly different (two tailed t-test) at P=0.01.

6.4 Conclusions

As discussed in Section 6.0, the monitoring of isocyanates is commonly carried out using a trapping reaction to produce a derivative. At present the most frequenty used trapping reactions for isocyanates rely on the reaction of the NCO group with an amine, usually a piperazine compound [255-261,264] or other secondary amine [273-277] (e.g. dibutylamine).This reaction can be presented schematically as;



This reaction has been discussed previously [251,278-281]. It is worth noting that most of the derivatization reagents currently used for isocyanate monitoring were not developed specifically for mass spectrometric detection.

LC/PB/MS and LC/ESI/MS of isocyanate-MP derivatives was carried out and found to be potentially useful for identification purposes. More work is required to fully exploit these techniques. Post column addition of alcohols was found to increase PB/MS sensitivity in a manner similar to that described earlier for pesticides.

7.0 Conclusions and Suggestions for Future Work

The use of PB/LC/MS was examined for a variety of matrix and analyte types. The PAH work compared PB/MS with LC/PFD, LC/DAD and GC/MS for the determination of PAH in brickdust. In general, PB/MS becomes a more attractive analytical technique as the PAH RMM increases. None of the four techniques used was entirely satisfactory and PB/MS was found to be a useful complementary technique. Future work in this area could include the analysis of PAH extracted from different matrix types or from different processes, i.e. in foundry fumes. The aim of this work would be to further examine the benefits of PB/LC/MS for high RMM PAH.

The pesticide work showed that PB/MS is of use when unambiguous indentification of pesticide is required i.e. forensic work. The use of an LC based technique is an advantage for thermally labile pesticides such as phenyl urea herbicides and carbamates which can not be easily analysed by GC/MS. Studies on post column addition of solute additives, e.g. ammonium acetate, to the LC mobile phase found no beneficial effect on PB/MS sensitivity. This finding was supported by the PAH and isocyanate work. However, post column addition of solvents, e.g. alcohols, was found to have a beneficial effect on PB/MS sensitivity. Further work on the "carrier" effect and the use of post column addition of alcohols is suggested. An examination of post column addition of more volatile solvents and a more detailed attempt to identify the effect of post column addition of alcohols and other solvents would also be of interest .

The results of the post column addition work suggest that the increased particle size explanation of the "high band pass filter" model is incorrect. This model states that the effect of additives is to increase particle size in the PB interface desolvation chamber and that these larger particles are better transported through the interface leading to an increase in PB/MS sensitivity. An alternative explanation, based on a decrease in particle size which leads to decreased gravitational and coagulation losses in the PB desolvation chamber and so to increased transfer was suggested.

The isocyanate derivative work looked at the use of PB and ESI MS for the determination of these compounds. The results of this work suggest that both techniques are of interest for these compounds but more work is required to fully develop this approach. Of particular interest is the ability of these techniques to correctly identify and quantify derivative peaks from high RMM isocyanate oligomers and pre-polymers.

In conclusion, although the use of PB/MS has decreased since the introduction of LC/API/MS the original advantage of PB i.e. the ability to generate classical EI spectra remains. The capability to carry out library searches for unknown compounds is a requirement for many types of analysis e.g. environmental and occupational hygiene monitoring. Many of the compounds of interest in these types of sample are not easily amenable to GC analysis leaving LC as the technique of choice. The development of an improved PB interface with better linearity, robustness and mass range, or the development of experimental procedures that will achieve these goals is still a task worth pursuing.

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Particle Beam Liquid Chromatography/Mass Spectrometry Analysis of Hazardous Agricultural and Industrial Chemicals†

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Particle-beam liquid chromatography/mass spectrometry (PB-LC/MS) has been used for the determination of several classes of compounds that are not easily analysed using gas chromatography. The compounds studied were either of low volatility (isocyanate derivatives), thermally unstable (phenylurea herbicides) or highly polar (phenoxyacetic acid herbicides). PB-LC/MS was found to be suitable for the analysis of these compounds and gave library searchable electron impact mass spectra for a range of samples in various matrices (soil, vegetation, filter (air) and concentrate). An advantage of PB-LC/MS, compared with GC/MS, was that the extracts could be run immediately on the LC system (no pre or post work-up derivitization necessary). The post-column addition of alcohols and their effect on PB-LC/MS response was also investigated. An increase in response was found for the series, no additives < methanol < ethanol < propanol = butanol, but pentanol showed a decreased response compared with butanol.

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Liquid chromatography (LC) is the most versatile separation technique available. It can be used for compounds of low volatility and low thermal stability and with a wide range of polarities. Liquid chromatography/mass spectrometry (LC/MS) combines LC with mass spectrometry to produce a very powerful analytical tool. Some particular areas of use for LC/MS are forensic, environmental and occupational hygiene analysis. These areas require high sensitivity and high selectivity, e.g. the analysis of pesticide residues from spray drift on vegetation. One of the simplest LC/MS interfaces is the particle beam (PB) interface. This type of interface was used in the work described in this paper. The PB interface has been described elsewhere.[1,2]

Pesticides are hazardous chemicals[3] and it is necessary to control their impact on users, the public and the environment. The determination of pesticide residues in a variety of matrices (vegetation, air, soil, water etc.) is therefore needed to support enforcement action or for occupational exposure purposes. Some pesticides are not amenable to analysis by conventional gas chromatography (GC) methods. They may be either thermally unstable (e.g. phenylurea herbicides) or require derivatization before GC analysis (e.g. phenoxyacetic acid herbicides).

Isocyanates are highly reactive molecules that are widely used in industry; they are known respiratory sensitizers causing occupational asthma.[4,5] For this reason the Health and Safety Executive (HSE) has set a long term occupational exposure limit (8 h Time-Weighted Average reference period (TWA))[6] of 20 g/m^3 (as isocyanate (NCO) group) for workplace air. As the isocyanate is so reactive, analysis is commonly carried out by trapping the isocyanate to produce a stable derivative. The method most commonly used in the UK is to trap the isocyanate with 1(2-methyoxyphenyl)piperazine reagent to form the urea derivative.[7] The urea derivative is too involatile to determine by GC and so is analysed by LC with electro-chemical and UV detection. Recently several questions have been raised regarding the suitability of this method for isocyanate pre-polymers.[8–10] Particularly, the correct identification of isocyanate-derived peaks has emerged as an issue. PB-LC/MS has been used in an attempt to clarify these problems.

EXPERIMENTAL

Reagents

Solvents used (acetonitrile, methanol, ethanol, propanol, butanol and pentanol) were of LC grade or better (from Rathburns, Walkerburn, UK or Fisher Scientific, Loughborough, Leics., UK). Water was purified in-house using an Elgastat UHQ II system (Elga Ltd., High Wycombe, Bucks, UK) and buffers were prepared using this product. Ammonium acetate was supplied by Fisons (Loughborough, Leics. UK). Acetic acid, sodium acetate, 1-(2-methoxyphenyl)piperazine (MP), phenyl isocyanate, tolylene, 2,6-diisocyanate and tolylene 2,4-diisocyanate (TDI) were supplied by Aldrich Chemical Co. Gillingham, Dorset, UK). Diisocyanate MP derivatives were prepared from monomers and from samples submitted by Health and Safety Executive (HSE) Inspectors during routine occupational hygiene monitoring. Pesticide standards (phenylureas, bromoxynil and ioxynil) and diisocyanate monomers were purchased from Promochem (Welwyn Garden City, Herts, UK). Pesticide-contaminated vegetation and soil samples and concentrate sample were originally submitted for forensic analysis by HSE Inspectors.

Instrumentation

The PB-LC/MS system used in this work consisted of a

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CCC 0951–4198/97/060618–06 $17.50

VG Trio 1 mass spectrometer and a VG LINC PB interface (VG Analytical, Altrincham, UK). Various LC pumps (Waters 510 supplied by Waters Watford, Herts, UK or Jasco LG-980-02, supplied by JEOL (UK) Ltd. UK) were connected to this system. Injections were made manually through a Rheodyne 7125 valve (Altech, Carnforth, Lancs, UK). Solids probe electron ionization mass spectrometry was carried out using a VG Analytical 70705 instrument. Other LC work was carried out on Hewlett-Packard HP 1090 (Hewlett-Packard, Stockport, Cheshire, UK) and Waters Millennium 2010 systems. Fluorescence and diode-array detectors (DAD) were used.

LC columns used were 25 × 0.46 cm S5 ODS2 (PhaseSep, Deeside, Clwyd, UK) for the vegetation and soil extracts, 15 × 0.46 cm S3 ODS2 (PhaseSep) for the concentrate, and 15 × 0.46 cm S3 ODS2 and 25 × 0.46 cm S3 ODS2 (Thames Chromatography, Maidenhead, Berks, UK) for the diisocyanate derivative and phenylurea herbicide work.

Clean up of the environmental pesticide samples was carried out using a Waters gel permeation chromatography (GPC) system and associated fraction collector.

Procedures

PB-LC/MS phenylurea herbicides, pesticide concentrate and environmental samples containing pesticides. Pesticide samples were worked up following standard operating procedures for this type of sample.[11,12] Phenylurea herbicides were analysed using conditions previously described.[13] The mobile phase used for these experiments was 45% acetonitrile, 45% 10 mM acetic acid and 10% methanol, run isocratically. Flow rates for the PB experiments were 0.3 mL/min mobile phase +0.2 mL/min post-column methanol. For the LC/DAD experiments flow rates of 1 or 0.3 mL/min were used. Typical operating conditions for the PB-LC/MS system were: interface temperature 70 °C, nebulizer helium pressure 20 p.s.i., electron energy 70 eV, and source temperature 200–250 °C.

Post-column addition of alcohols as a means of increasing PB-LC/MS sensitivity. Experiments on the post-column addition of alcohols used flow rates of 0.3 mL/min mobile phase (45% acetonitrile, 45% 10 mM acetic acid and 10% methanol, run isocratically) +0.2 mL/min of alcohol introduced via a T-piece situated after the column. Repeat loop injections (1 cm guard column only in-line, i.e. no analytical column) of 25 uL were made for this series of experiments. Operating conditions for the PB-LC/MS system were: interface temperature 70 °C, nebulizer helium pressure 20 p.s.i.,

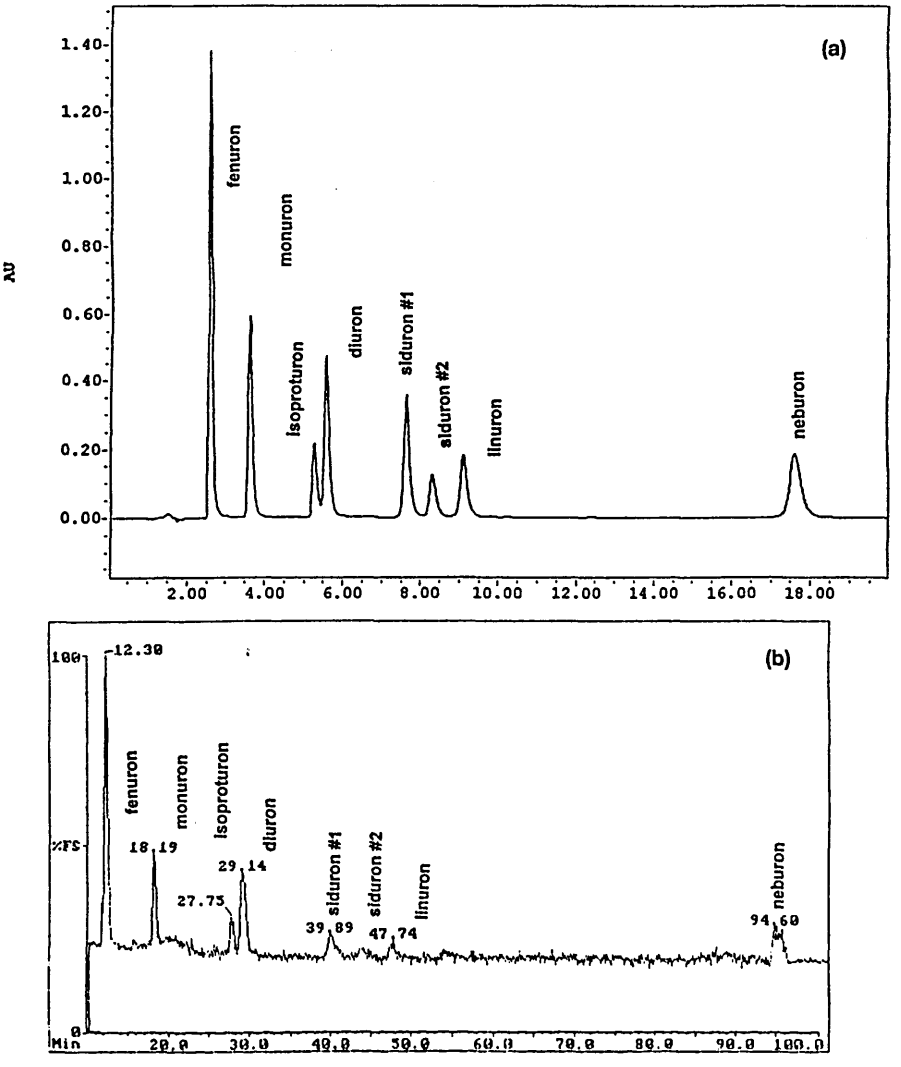

Figure 1. (a) LC/DAD and (b) PB-LC/MS chromatograms of seven phenylurea mix.

electron energy 70 eV, and source temperature 200 °C.

Determination of isocyanate MP derivatives using PB-LC/MS. Diisocyanate derivatives were prepared following Reference[7]. The mobile phase used for the LC/DAD experiments with 60% acetonitrile/40% 60 mM sodium acetate buffer; flow rates of 0.3 and 1 mL/min were used. For the PB experiments the mobile phase described above was found to cause unacceptably frequent skimmer blocking and so 50 mM ammonium acetate was used in the buffer instead of the sodium acetate. The flow rate used was 0.3 mL/min. The PB-LC/MS system operating conditions finally used were: interface temperature 70 °C, nebulizer helium pressure 20 p.s.i., electron energy 70 eV, and source temperature 200 °C.

RESULTS AND DISCUSSION

PB-LC/MS of phenylurea herbicides, pesticide concentrate, and environmental samples containing pesticides

An LC method was developed for the determination of seven phenylurea herbicides (fenuron, monuron, isoproturon, diuron, linuron, siduron and neburon) using diode array or particle beam LC/MS detection. The chromatograms shown (Fig. 1) are for the seven phenylurea mix using LC/DAD and LC/MS systems. Estimated limits of detection (based on 3 × S/N were found to be approx. 1 μm/mL for DAD and approx. 1–20 μg/mL for PB-LC/MS (scan mode, analyte dependent). Monitoring in the selected-ion monitoring (SIM) mode improves these MS detection limits.

PB-LC/MS was also used on linuron-contaminated vegetation and spiked soil samples. For the soil and vegetation extracts, SIM was necessary to visualize the linuron peak in the background, even after GPC clean up. Ions monitored in SIM mode were *m/z* 248 (M^+), 187, 161 and 160. These findings are similar to those reported earlier by Miles *et al.* and Bagheri *et al.*[14,15]

Similar LC work was carried out on a pesticide concentrate, taken by HSE inspectors during enforcement action. The PB-LC/MS results obtained for this concentrate are shown in Fig. 2. These results are of note as no derivatization step was necessary for bromoxynil or ioxynil and in addition, isoproturon is known to be unsuitable for GC analysis. LC/DAD gave spectra which matched with a user-created DAD library. PB-LC/MS, in scan mode, gave classical EI spectra which were matched against pesticide standards. SIM mode was used to provide more sensitivity and better selectivity for the three compounds of interest. The ions monitored in SIM mode were *m/z* 146, 161 and 371 for ioxynil; 275, 277 and 279 for bromoxynil; and 146, 191 and 206 for isoproturon. The spectrum of ioxynil is rather simple and the ions chosen are the molecular ion and the next most intense ions. For bromoxynil, the ions chosen constitute a characteristic 1:2:1 triplet due to the two Br atoms in the molecule. For isoproturon, the ions chosen are the molecular ion and the next two most intense ions above *m/z* 100. SIM monitoring using ions below *m/z* 100 is avoided, where possible, to decrease the risk of interference.

Confirmation of composition for a pesticide concentrate sample, i.e. an unambiguous identification of the pesticides present, is a common forensic requirement. Here, the ability of particle beam LC/MS to give classical electron impact spectra is an advantage over other LC/MS interfaces such as thermospray, electrospray and atmospheric pressure chemical ionization that give little or no fragmentation data. For the vegetation and soil extracts, described earlier, the selectivity of SIM was essential in filtering out interfering materials and the ability of PB-LC/MS to give library-searchable EI spectra was a decided advantage over other MS interfaces. PB-LC/MS therefore shows

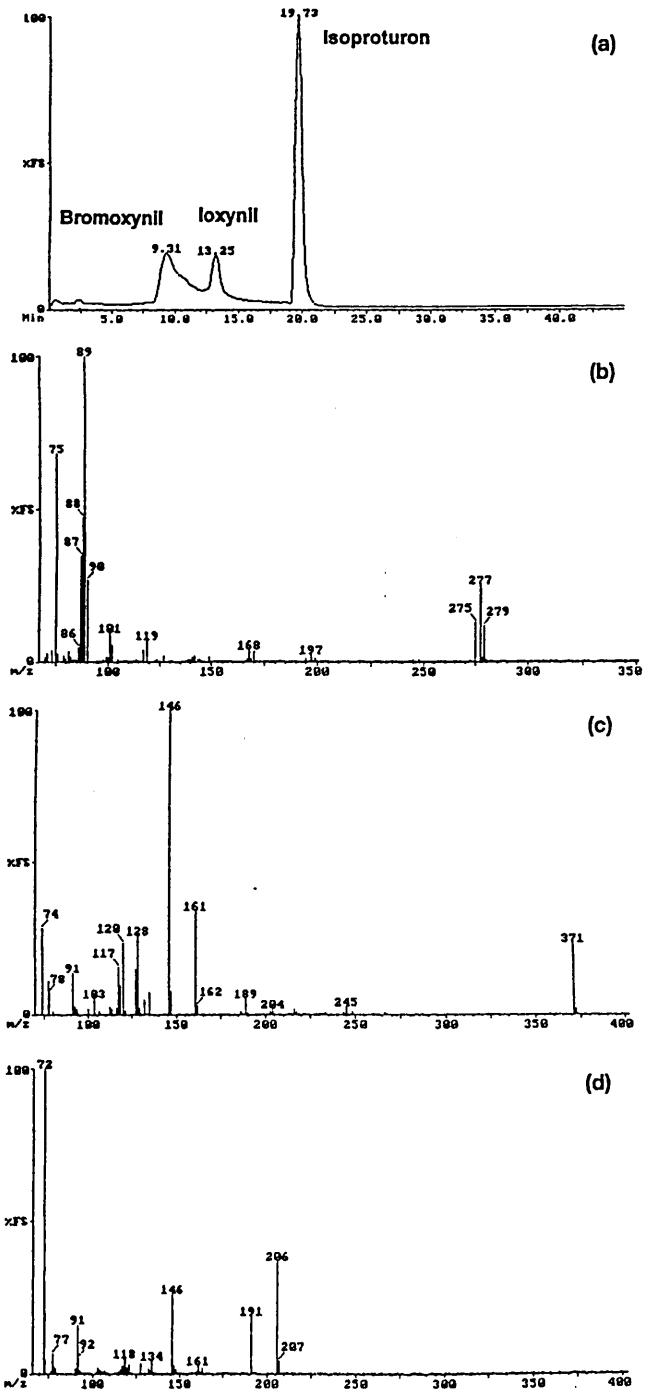

Figure 2. (a) PB-LC/MS chromatogram of pesticide concentrate (SIM mode – see text for details) and individual spectra; (b) ioxynil, (c) bromoxynil and (d) isoproturon.

promise for confirmation of composition analysis, where unambiguous identification is necessary as well as for determination of low levels of target compounds in complex matrices.

Post-column addition of alcohols as a means of increasing PB-LC/MS sensitivity

Previous work has highlighted two major problems with PB-LC/MS. These are non-linear response at low levels and poor sensitivity for low molecular mass analytes. Discussion of these problems and their possible causes have been published elsewhere.[16–18] The post-column addition of alcohols was investigated in an attempt to improve PB-LC/MS response. Various phenylurea herbicides (fenuron, monuron and neburon) were used as target compounds. The results obtained for neburon are summarized in Fig. 3.

Significance testing on these results (two tailed *t*-test) showedthat the response with post-column addition of butanol was significantly different (higher) than that with addition of pentanol, ethanol, methanol or no additive ($P = 0.10$ or less). The response obtained for addition of propanol was similar to that for butanol (not significantly different). Similar statistics were obtained from the monuron and fenuron experiments. For all of these compounds, the maximum increase in sensitivity was of the same order of magnitude (i.e. fenuron × 8, monuron × 8, neburon × 7 with respect to (no post-column addition) and curves similar to Fig. 3 were seen. Generally, the response increased for the series; no additive, methanol, ethanol and propanol. Propanol and butanol gave similar responses. Experiments with pentanol showed a slightly lower response than with ethanol.

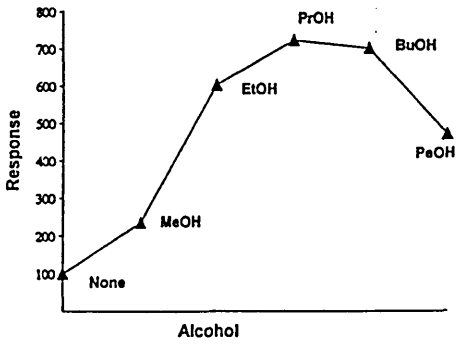

Figure 3. Effect of post-column addition of alcohols (x) on PB response (y).

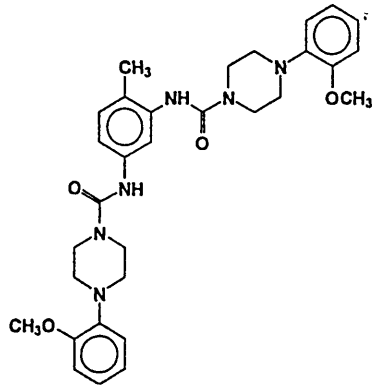

Figure 4. Structure of tolylene-2,4-diisocyanate [-(2-methoxyphenyl)piperazine derivative]

Post-column addition experiments were also carried out with tolylene 2,4-diisocyanate. For this compound, the same shape of curve was seen, i.e. the responses were butanol > ethanol > methanol > nothing and butanol > pentanol. The magnitude of the increase was however small the response with butanol was 3 times that without an additive.

It is suggested that the addition of alcohols post-column is improving sample transfer through the interface by aiding aerosol formation in the expansion chamber. The mechanism for this effect will be rather complex. One factor will be related to the volatilities of the alcohols used. As volatility decreases methanol to pentanol); the particles formed in the aerosol will be less liable to evaporation. Such an aerosol will be favourable for the formation of large aerosol particles which are more effective in passing through the PB interface and into the mass spectrometer.[1,19] Balancing this effect will be the consideration that, for a given interface temperature, very involatile additives may not be vaporized effectively. Also, the less volatile the alcohol, the more hydrophobic it becomes. There will therefore be a problem of miscibility with the aqueous component of the LC mobile phase which may lead to incomplete aerosol formation and partioning of the analyte into the aqueous component of the aerosol.

Experiments carried out using LC ion-pairing reagents (octane sulphonic acid, tetramethyl ammonium bromide) and ammonium acetate (all at approx. 50 mM in 50:50 ethanol + water) as post-column additives proved to be unsuccessful. Although small increases in PB sensitivity were observed; these were negated by frequent blocking of the PB skimmers and the consequent need to vent and clean the interface. These results suggest that electrostatic effects do not play a major part in aerosol formation.

Determination of isocyanate derivatives using PB-LC/MS

PB-LC/MS analyses of diisocyanate 1-(2-methoxyphenyl)piperazine (MP) derivatives prepared from monomer and an industrial pre-polymer sample were carried out. For the MP derivatives of the diisocyanate monomers (tolylene 2,6-diisocyanate and tolylene 2,4-diisocyanate; both TDI isomers studied (Fig. 4), no molecular ion was observed (mol. wt. TDI-$(MP)_2$ = 558). It was thought that this difficulty was due to the diisocyanate-MP derivative decomposing, either in the PB interface or in the mass spectrometer. Various experiments were then undertaken to try and obtain a molecular ion for the TDI-MP derivatives; these are described below.

Varying the interface temperature from 70 °C to 20 °C had no effect on the fragmentation patterns observed but markedly decreased sensitivity, no molecular ion was observed. The source temperature was varied from 250 °C to 150 °C but, again, no molecular ion was observed. Altering the ionization electron energy from 70 eV to 25 eV affected the fragmentation pattern but did not result in a molecular ion being observed. These results suggest the diisocyanate derivatives are very easily decomposed in the PB-LC/MS

for the MP derivative of a mono-isocyanate, phenyl isocyanate, a molecular ion (*m/z* 311) has been observed on the PB-LC/MS system used in this study.

Finally, both isomers were introduced directly into the ion source using a solids probe and their EI spectra recorded. The spectrum obtained for the 2,4 isomer (Fig. 5) was the same as that observed by PC-LC/MS, isomer, a very small molecular ion was seen (Fig. 5); this was not seen by PB-LC/MS.

The spectra of the diisocyanate derivatives (TDI and pre-polymer) were dominated by peaks derived from the derivatizing reagent (1-(2-methoxyphenyl)piperazine, mol. wt. 192). However, peaks were observed that correspond to isocyanate containing fragments, e.g.

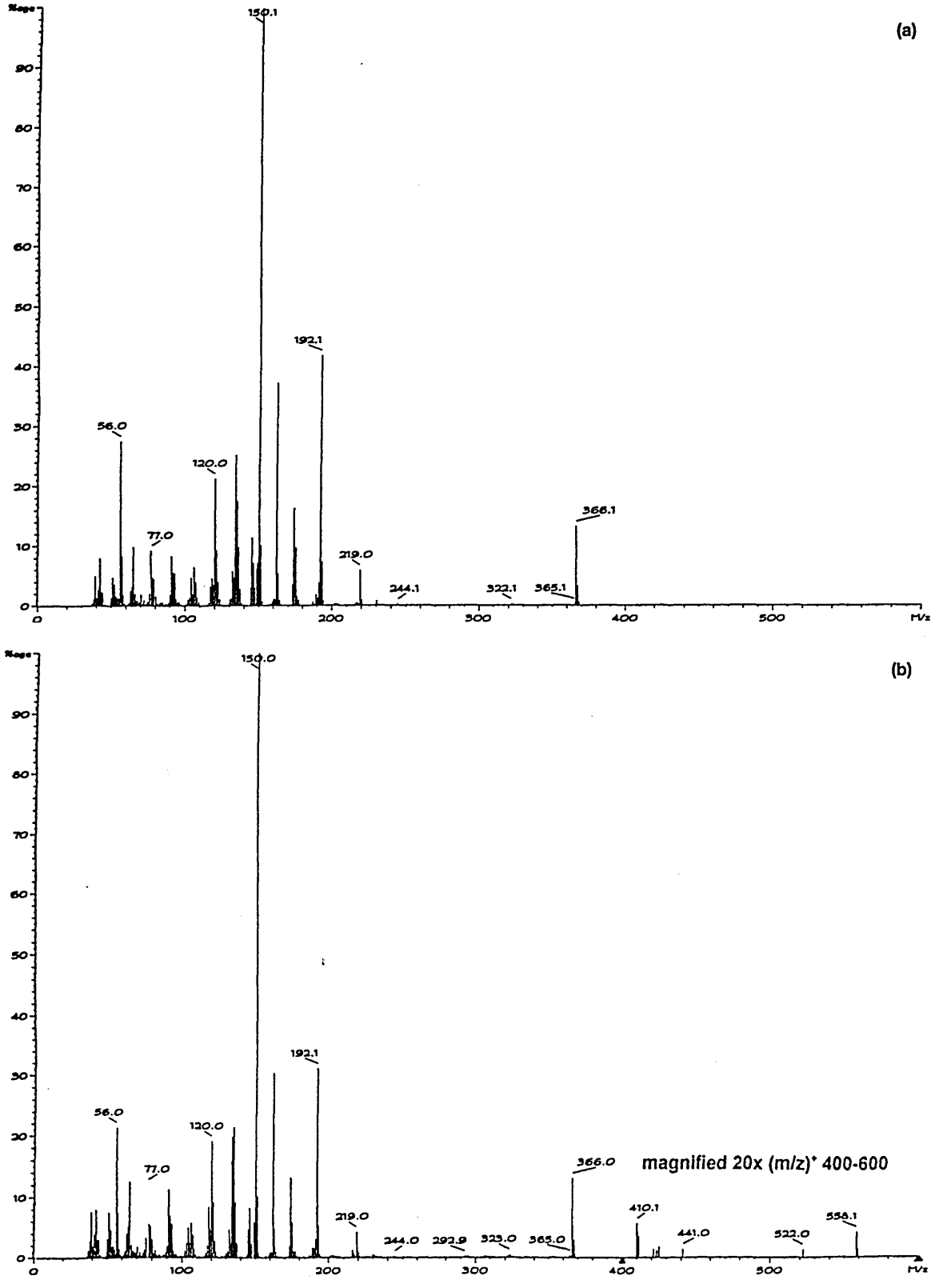

Figure 5. Standard (probe) EI spectra of (a) toluene 2,4- and (b) toluene 2,6-diisocyanate.

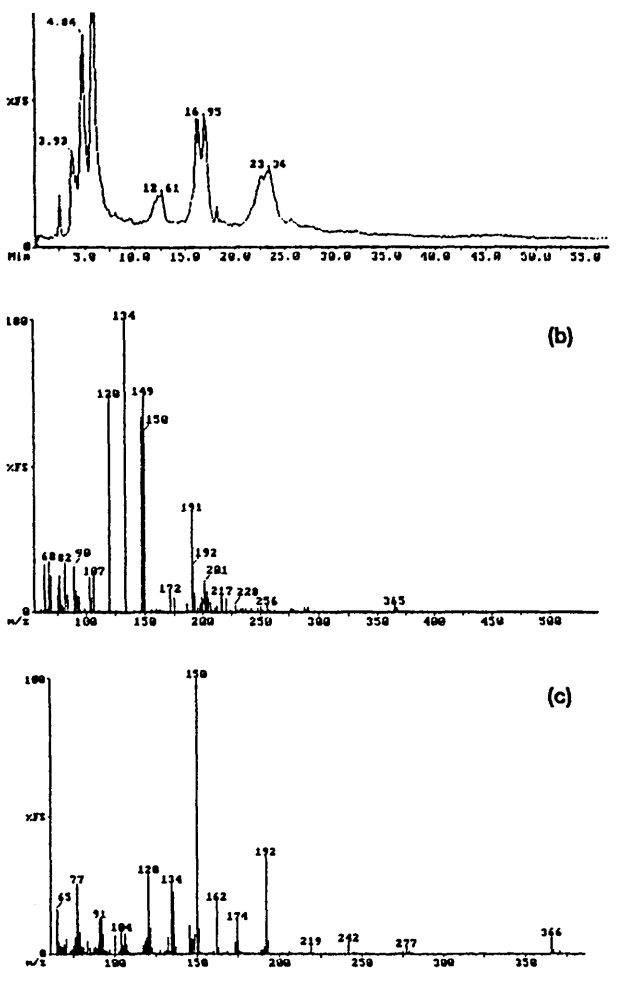

Figure 6. (a) Chromatogram of TDI-based isocyanate pre-polymer MP derivative and (b) spectrum of one of the isocyanate-derived peaks. (c) The spectrum of an MP derivative of TDI is shown for comparison.

TDI (mol. wt. 174) and TDI-(MP) (*m/z* 366 corresponding to one MP group). The molecular ion, TDI-$(MP)_2$ (*m/z* 558), is absent (Fig. 5).

These results suggest that PB-MS may be of use for confirmation that a peak is isocyanate derived. The particular fragment ions observed could also provide information on which isocyanate monomer is the parent compound. This would be useful for occupational hygiene monitoring, it may be possible to carry out quantitative work using the peaks derived from the derivatizing agent as these should be present for all isocyanate containing compounds. Further work is required to see if this approach will be useful.

CONCLUSION

PB-LC/MS offers considerable advantages over GC analysis and over other LC/MS interfaces and LC detectors for certain compounds. Particularly useful is the ability to generate EI spectra for confirmation and identification purposes. The selectivity of mass spectrometic detection is also necessary for samples in complex environmental matrices. The problem of the low sensitivity of PB interfaces still remains to be solved.

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Liquid chromatography/mass spectrometry in environmental analysis

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Introduction

The selectivity afforded by the use of mass spectrometry as a detection method for liquid chromatography has proved attractive in many areas of analysis. Its widespread adoption in environmental applications has, however, been hampered by the plethora of interface types and the deserved reputation of earlier interfaces for unreliability and poor sensitivity. However, the advent of more robust interfaces in the 1980s and 90s has led to several groups applying LC/MS techniques in the environmental area.

In this article rather than attempt an exhaustive review of LC/MS techniques, brief descriptions of the current "state of the art" will be offered and specific application areas examined. The techniques to be covered are Thermospray (TSP), Particle Beam (PB), Atmospheric Pressure Chemical Ionisation (APCI) and Electrospray/IonSpray (ES/ISP). Applications in the analysis of polycyclic aromatic hydrocarbons, surfactants, dyestuffs and pesticides will be described. For a complete description of the various interface types the interested reader is referred to books by Ardrey[1] and Yergey *et al.*[2]

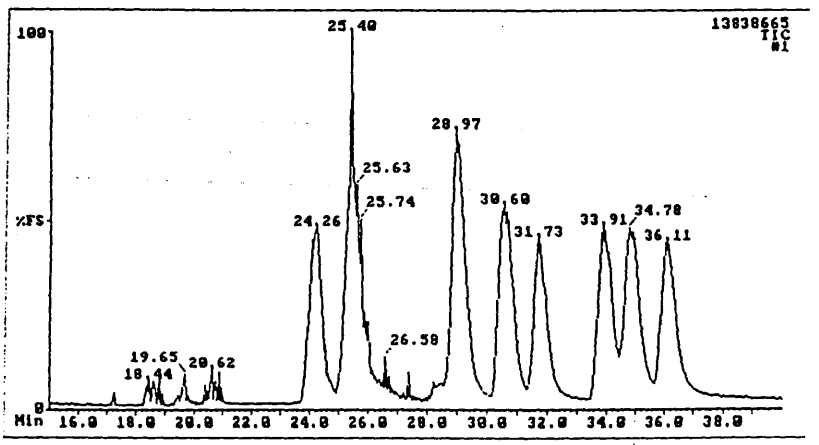


Figure 2. Determination of 16 PAHs by particle beam LC/MS (selected ion monitoring mode) (100 ng per component on column).

Polycyclic aromatic hydrocarbons

Determination of polycyclic aromatic hydrocarbons (PAHs) in a range of matrices is required owing to their carcinogenic properties. Several groups have applied the particle beam technique (Figure 1) to the analysis of (PAHs).[3,4,5]

The particle beam technique is intrinsically the simplest of the available interfaces. Eluant from the HPLC column is nebulised and sprayed into a heated desolvation chamber. The mixed nebulising gas, mobile phase and sample stream enters the dual stage momentum separator where the majority of the mobile phase and nebulising gas is removed. This results in a stream of "snowballs" of sample clustered with a few solvent molecules entering a

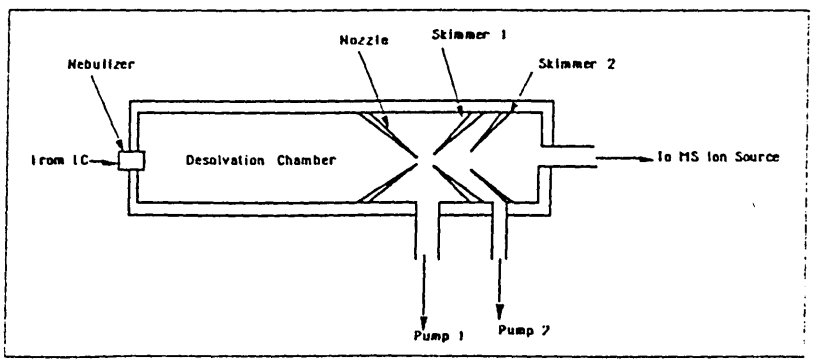
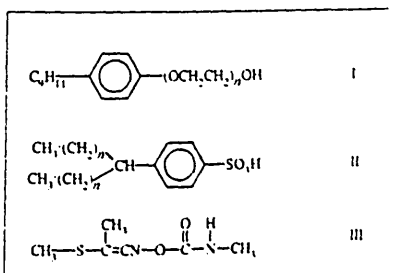


Figure 1. Schematic representation of a particle beam LC/MS interface.



Structures.

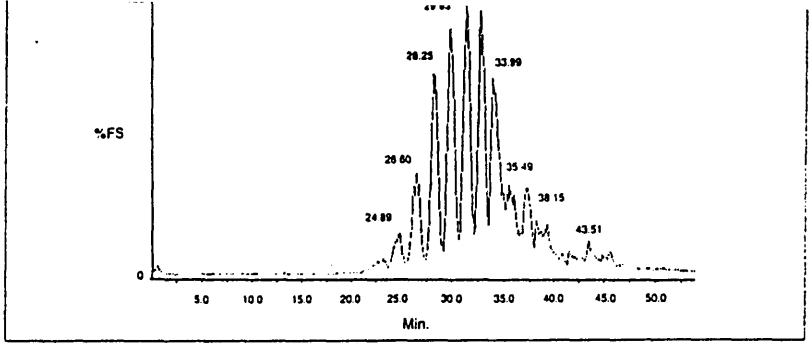


Figure 3(a). Analysis of a commercial nonyl phenol ethoxylate type non-ionic surfactant by particle beam LC/MS.

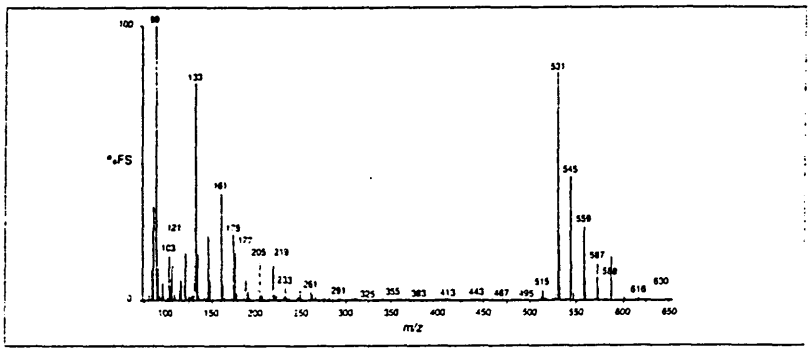


Figure 3(b). Mass spectrum of peak at t_r = 31.4 min. from the above chromatogram (identified as the nine ethylene oxide unit containing oligomer).

conventional electron impact (EI) or chemical ionisation (CI) ion source.

The data shown in Figure 2 come from the analysis of the standard US EPA mixture of 16 PAHs using PB/LC/MS in the selected ion monitoring (SIM) mode. In this analysis we have attempted to minimise the reduction in sensitivity of the PB/LC/MS technique with increasing aqueous ontent in the mobile phase, by adding 0.2 ml min^{-1} of acetonitrile post column. In this data where each component was injected at the 100 ng level on column, the sensitivity of, for example, benz(α)anthracene (t_r = 24.3 min.) which normally elutes at 25% aqueous content is increased by a factor of three. However, as can be clearly seen the relative sensitivity of PB/LC/MS for PAHs still dramatically increases with increasing RMM. The earlier eluting compounds, e.g. acenapthene (RMM 154), are not detectable at these levels and the relative sensitivity for chrysene (RMM 202, t_r = 20.62 min.) is only around 10% of that of benz(β)fluoranthene (t_r = 28.97 min., RMM 252). In general the limits of detection achievable with this technique do not approach those of the normally used fluorescence detection methods for the smaller compounds although they are comparable above RMM 252.

Surfactants

The determination of surfactants in drinking, surface and groundwater is currently a very "live" topic of analysis. It has been shown[6] that non-ionic surfactants of the alkylphenol ethoxylate dation products exhibit weak oesterogenic properties and may be implicated in the increase in male infertility in the western world.

The analysis of non-ionic surfactants of this type is made complex by the fact that they are formulated as structures such as Structure I with a distribution of ethoxylate chain length. The length of the alkyl chain may also vary. Analysis may be carried out by spectrophotometric or LC methods employing conventional detectors. However, neither of these techniques offers unambiguous identification of surfactant type and chain length. Hence LC/MS has been investigated as a possible method of analysis.

Particle beam LC/MS has been used in the determination of non-ionic surfactants in surface[3] and drinking water.[7] Clark *et al.*[7] determined alkylphenol ethoxylates in drinking water after continuous liquid/liquid extraction of 500 litres of finished water. The resulting extract was then analysed by PB/LC/MS and detection limits of ng l^{-1} were obtained. Figure 3(a) shows the total ion chromatogram obtained from the analysis of a standard NP9 nonylphenol ethoxylate carried out in our own laboratory, by PB/LC/MS. Figure 3(b) shows the mass spectrum obtained from the peak eluting at t_r = 31.4 min. The EI mass spectrum obtained shows both a molecular ion and structurally significant fragmentation allowing this peak to be assigned to be a nine ethylene oxide unit containing nonylphenol ethoxylate.

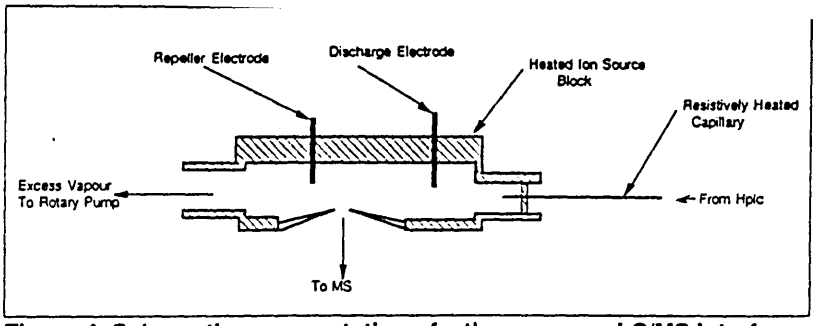


Figure 4. Schematic representation of a thermospray LC/MS interface.

The thermospray interface (Figure 4) has also been successfully applied to the analysis of surfactants. In thermospray LC/MS the HPLC eluant is sprayed through a resistively heated capillary into the heated thermospray ion source. The droplets so formed then shrink under the action of heat from the ion source block. When the droplets have shrunk such that the coloumbic repulsion between charged species in the droplet exceeds the surface tension of the droplet, ions are ejected into the gas phase. This process is known as ion evaporation and is facilitated by the addition of a volatile electrolyte (often ammonium acetate) to the mobile phase or post column. The ammonium actetate may also serve as a source of NH_4^+ ions for conventional chemical ionisation processes to occur in the gas phase. Thermospray spectra generally show reduced fragmentation compared to EI spectra and are "CI like" showing either protonated molecular species $(M + H)^+$ or adduct ions $(M + NH_4)^+$ in positive ion mode.

Papers describing the use of thermospray in the analysis of surfactants have appeared from Evans *et al.*[8] and Schroder.[9] Evans showed that the thermospray mass spectra of linear primary alcohol ethoxylates are characterised by intense $(M + NH_4)^+$ ions, with structural information. They established limits of detection in the low nanogram region for each species analysed. The thermospray method was applied to the analysis of surface water and sewage effluent samples by using solid phase extraction as a method of sample preparation. The method was validated for concentrations of individual alcohol ethoxylates in the range 0.06 to 2.17 ppb by spiking 1 litre samples.

Schroder[9] examined the concentrations of a range of surfactants in sewage treatment plants by thermospray LC/MS using flow injection mode and a tandem mass spectrometer. In flow injection mode the sample is injected into the mobile phase which is pumped into the interface without passing through a column. The resulting mass spectrum can be regarded as a "survey" of components present. This technique is obviously best suited to "soft ionisation" techniques such as thermospray which yield molecular species in abundance. Figure 5 shows the mass spectrum obtained from a sample of waste water treatment plant inffluent by this technique. In this positive ion spectrum ions corresponding

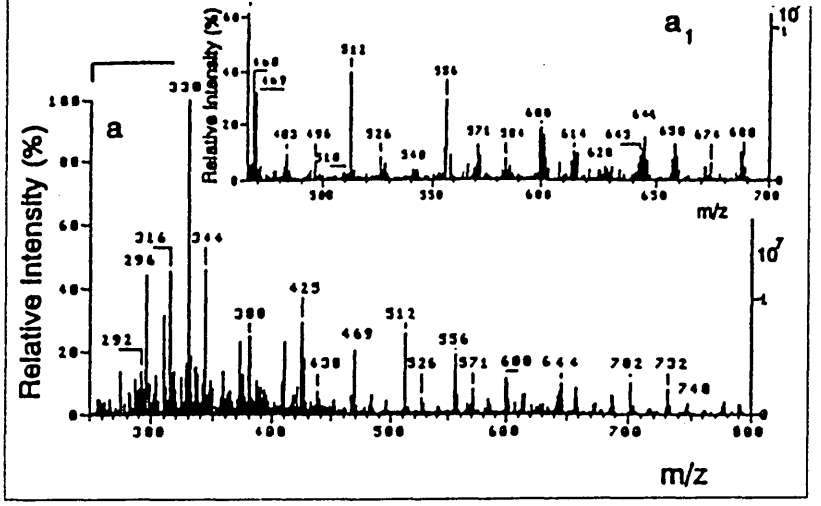
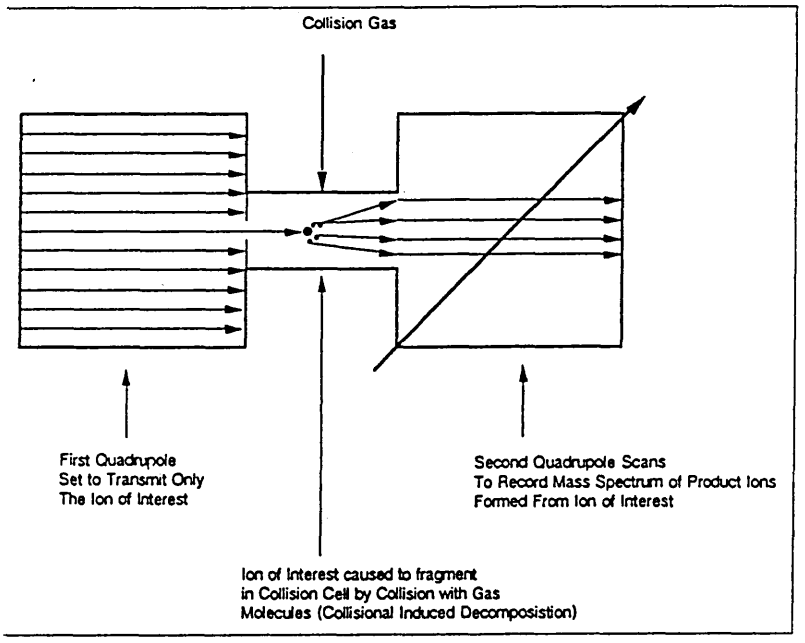


Figure 5. Thermospray "survey scan" obtained in flow injection mode from a waste water extract. Note the series of ions at *m/z* 316, 330, 344 corresponding to anionic surfactants and the series 44 daltons apart beginning at *m/z* 468 corresponding to non-ionics. (Reproduced from Reference 9 by Permission of Elsevier Science BV).



igure 6. Schematic representation of a triple quadrupole mass spec-rometer, showing the experimental set up for obtaining product ion cans.

o the presence of a series of linear kyl benzene sulphonates (Structure) (an anionic surfactant formulation) n be observed at *m/z* 316, 330 and 44. Also the series of ions 44 daltons art beginning at *m/z* 468 may be signed to surfactant and represent (M $NH_4)^+$ ions from the oligomers of a olyethylene glycol ether type non-nic surfactant.

Confirmation of this assignment was tained by acquiring product ion ectra from some of these ions using e tandem mass spectrometer. This chnique is shown diagrammatically in Figure 6, as it is performed on a triple quadrupole mass spectrometer, the type of tandem instrument used in this work. The first quadrupole mass filter is set to transmit the ion of interest (in this case the $(M + NH_4)^+$ ion occurring at *m/z* 468) into the collision cell, where collisions with a gas cause it to fragment. The "product ions" thus formed are then separated by mass to charge ratio in the second quadrupole mass filter and a mass spectrum recorded. Figure 7 shows the product ion spectrum record from the *m/z* 468 ion. As can be seen sufficient structural

along with the other experiments available on tandem instruments is often used to generate additional structural information.

Dyestuffs

Straub *et al.*[10] have produced an interesting paper comparing the performance of three different LC/MS techniques for the analysis of azo dyes. They compared results obtained from the use of thermospray and particle beam from those obtained from electrospray. In the electrospray technique the (Figure 8) eluant from the LC column is passed through a capillary held at around 5 kV. This high potential creates a fine spray of charged droplets. These droplets are broken up either by collisions with a "curtain gas" or by heat or a combination of the two. Ionisation then proceeds from the smaller droplets via ion evaporation as previously described for thermospray. This technique is more often applied in the analysis of high molecular weight biopolymers although Henion *et al.*[11] have also used electrospray MS, interfaced to capillary electrophoresis and in a later paper[12] with liquid chromatography in conjunction with a high flow rate thermal nebuliser system, to analyse dyestuffs.

A comparison of the type of spectra obtained is shown in Figures 9(a, b and c). The expected differences in fragmentation between thermospray and particle beam in electron impact mode, are observed. Figure 9(c) also exhibits the phenomenon of the generation of multiply charged ions commonly seen in electrospray mass spectra. In this case the dye Acid Orange 10 which contains two sodium sulphonate groups exhibits dominant peaks arising from doubly negatively charged ions, when analysed by electrospray ionisation mass spectrometry in negative ion mode.

Pesticides

Each of the techniques described above has been applied to the analysis of pesticides. Two US EPA methods have been published describing the use of LC/MS for this application, Method 8321A describes the use of thermospray LC/MS for the analysis of chlorinated phenoxy acid herbicides and Method 553 the use of particle beam in the analysis of nitrogen containing compounds.

A paper by Pleasance *et al.*[13] provides an interesting comparison between the interfaces already described and the

more recently developed technique of Atmospheric Pressure Chemical Ionisation (APCI). In this paper a comparison between the techniques for the analysis of N-methyl carbamate pesticides is presented.

In the APCI interface (Figure 10), pneumatic nebulisation is employed to convert the mobile phase into droplets. These are then carried by a sheath gas through a heated tube where vaporisation of the solvent and analyte takes place. The solvent and sample vapour now flows towards the ion formation region where a corona discharge initiates chemical ionisation at atmospheric pressure. In this APCI technique the vaporised mobile phase is used as the reagent gas, unlike conventional CI where reagent gas is introduced into the ion source.

A comparison of spectra obtained for methomyl (Structure III) is shown in Figure 11, and is indicative of the type of spectra that are obtained from each of the techniques. The "ion spray" (electrospray with a nebuliser) spectrum Figure 11(a) is dominated by the $(M + H)^+$ ion at *m/z* 163. In order to increase the amount of structurally significant fragmentation it was necessary to use either collisionally induced decomposition in the source [Figure 11(b)] or an MS/MS product ion scan as previously described [Figure 11(c)]. The APCI spectrum [Figure 11(d)] again shows an $(M + H)^+$ ion but in this case appreciable fragmentation is observed. The particle beam spectra are conventional EI and CI spectra. In Figure 11(g) the thermospray spectrum is dominated by intense $(M + NH_4)^+$ and $(M + H)^+$ ions with little fragmentation. This data is very typical of the type of spectra generated by each of the techniques.

Table 1 (adapted from Reference 13) shows the absolute limits of detection for a methyl carbamate pesticides obtained by Pleasance *et al.* The trends shown again provide typical relative values. Taking, as an example, methomyl and scaling the sensitivity obtained to the particle beam EI results, we arrive at approximate values for the relative sensitivities of APCI (4250) > ISP (635) > TSP (90) > PB(EI) (1). These figures amply illustrate the reasons for the current interest in the APCI technique, the sensitivity obtainable with this technique is often a couple of orders of magnitude greater than that for either thermospray or particle beam.

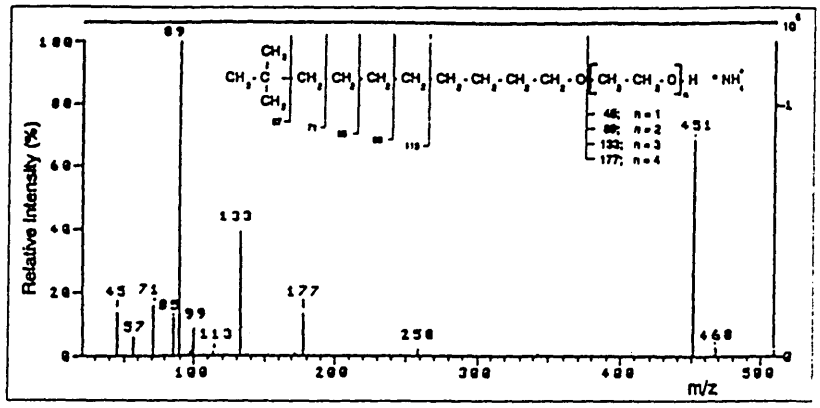


Figure 7. Product ion spectrum from the peak at *m/z* 468 in Figure 5. The structurally significant fragmentation induced in this type of experiment allows unambiguous identification of this ion as arising from a polyethylene glycol type non-ionic surfactant. (Reproduced from Reference 9 by Permission of Elsevier Science Publishers BV).

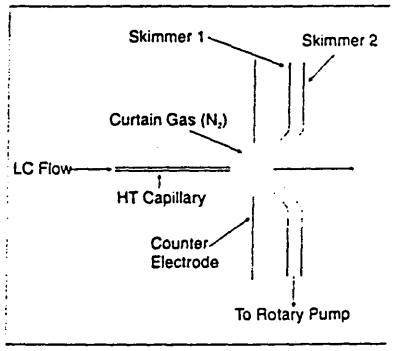


Figure 8. Schematic representation of an electrospray LC/MS interface. (Note in the "ion spray" version of this technique the creation of a spray of droplets from the LC eluant is assisted by the use of a pneumatic nebuliser.)

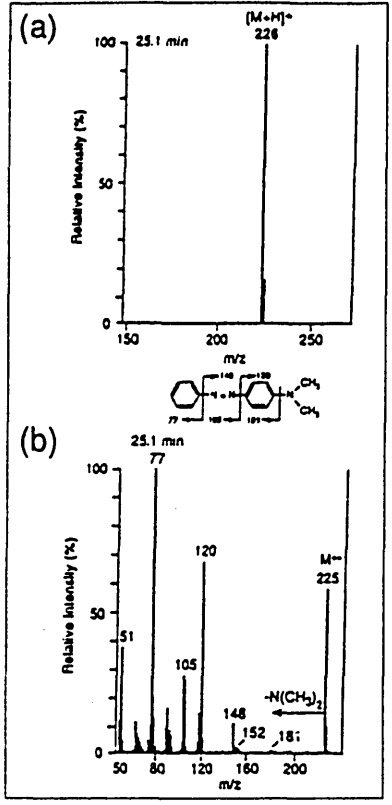


Table 1. Comparison of limits of detection obtained for methomyl by different LC/MS techniques. Adapted from Reference 13 by Permission of Elsevier Science Inc., copyright 1992 The American Society for Mass Spectrometry.

Compound name	Ion Spray	APCI	Particle Beam (EI)	Thermospray
Methomyl	0.40	0.06	254	2.8
Aldicarb	1.5	0.07	>500	0.9
Aldicarb sulphoxide	1.0	0.18	152	2.5
Aldicarb sulphone	1.8	0.1	79	1.1
Carbaryl	1.0	0.05	10	0.8
Methiocarb	0.6	0.07	19	0.8
Carbofuran	0.3	0.05	55	0.8
3-hydroxy-carbofuran	1.0	0.07	7	1.1

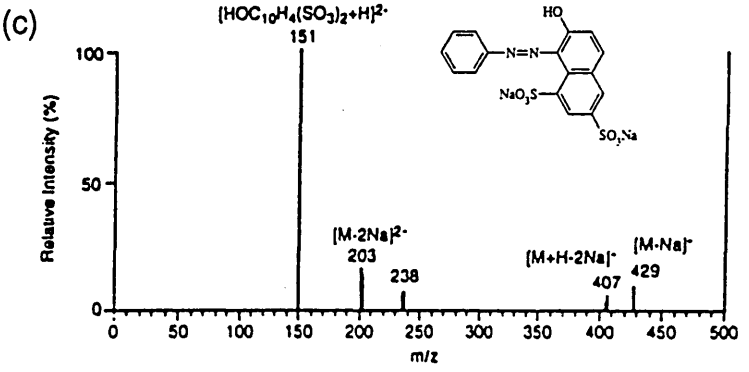


Figure 9. Comparison of mass spectra obtained from dyestuffs. (a) Thermospray positive ion mass spectrum of Solvent Yellow 2. (b) Particle Beam (EI) mass spectrum of Solvent Yellow 2. (c) Electrospray negative ion mass spectrum of Acid Orange 10. Adatpted from Reference 10 with permission of Elsevier Science Publishers BV.

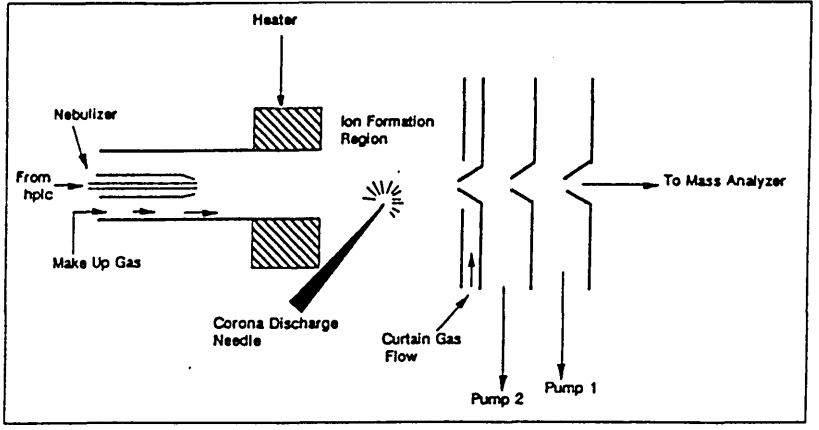


Figure 10. Schematic representation of an atmospheric pressure chemical ionisation (APCI) LC/MS interface.

Conclusions

Liquid chromatography mass spectrometry is a technique that has offered a great deal of promise in environmental analysis for a number of years. It is now starting to deliver. Developments in interface technology have led to the current situation where a choice of reliable techniques is on offer. Each has found useful areas of application. The analytical scientist entering this area for the first time still has to make choices, e.g. the trade off between sensitivity and structural information, when choosing to use particle beam or APCI. However, there is now enough information in the literature for these to be informed decisions rather than "inject and hope".

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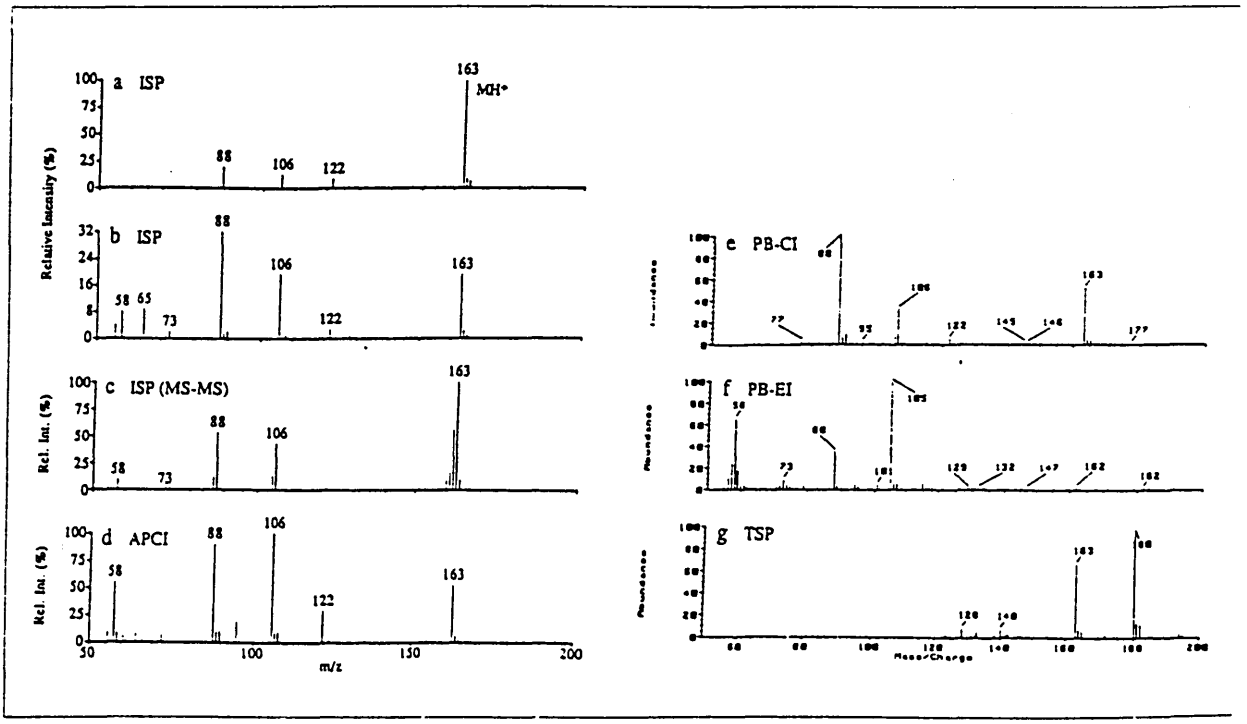

Figure 11. Comparison of mass spectra obtained from methomyl (RMM = 162). (a) Ion spray, (b) ion spray with "in source" collisional induced decomposition, (c) ion spray MS/MS (product ion scan), (d) APCI, (e) particle beam (CI), (f) particle beam (EI), (g) thermospray. Reproduced by Permission of Elsevier Science Inc. from Reference 13, copyright 1992 The American Society for Mass Spectrometry.