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The Development and Validation of a Methodology for the Analysis of Polyaromatic Hydrocarbons in Air Particulates (Indoor and Outdoor)

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The Development and Validation of a Methodology for the Analysis of Polyaromatic Hydrocarbons in Air Particulates (Indoor and Outdoor).

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Abstract.

PAH form a large class of ubiquitous pollutants mainly of anthropogenic origin. Several PAH found in air particulates have been identified as carcinogens and mutagens. Sixteen of these PAH have been listed by the USEPA as priority pollutants. This research is concerned with the development and optimisation of a method for the analysis of these sixteen PAH in air particulates. Soxhlet and ultrasonic extraction procedures were investigated to optimise the recoveries from spiked glass fibre filters with a standard 1ppm mixture of the 16 PAH. Standard mixtures of the 16 PAH were analysed by HPLC with fluorescence detection with approximate linearity obtained (R^2 0.9920 – 0.9998). For the purposes of quantitation, UV detection of 1ppm response factors were used. The ultrasonic extraction procedure (sonication for 1 hour in 3 x 50ml acetonitrile) yielded the best recoveries. Solid phase extraction (SPE) was used as the sample cleanup procedure. Spiked standard samples were used to optimise retention on the sorbent and identify a suitable wash solvent and elution solvent.

Environmental samples were taken in indoor (public houses) and outdoor locations. Sampling focused on two particle size fractions, PM10 and PM4.5 (particulate matter with aerodynamic diameters of 10μm (inhalable fraction) and 4.5μm (respirable fraction)). The particulate matter was trapped on glass fibre filters by use of a calibrated constant flow air meter (rate=1.9l/min) and extracted ultrasonically. The extract volume was reduced to ca. 5mls and cleaned up by the optimised SPE procedure. Separation and analysis of the PAH was achieved by HPLC with fluorescence detection. Individual PAH were identified by comparison with standard retention times and 10ppb response factors, or 100ppb response factors for dibenz(ah)anthracene and benzo(ghi)perylene as detection at the 10ppb concentration level could not be obtained for these two compounds.

Indoor samples showed mainly a 4-6 ringed PAH profile, typically with higher levels of PAH concentration detected in the $PM_{4.5}$ fraction than the PM_{10} fraction. The ambient samples showed 2-3 and 4-6 ringed PAH profiles at the congested roadway sampling location, with the higher levels being seen in the $PM_{4.5}$ fraction. The other sampling locations (less dense traffic volumes) showed a 2-3 ringed PAH profile, with higher concentration levels found in the PM_{10} fraction. Samples were attained from the Baseline Study on PM_{10} carried out in Dublin City (1996) by Dublin Corporation, and analysed using this method. The same types and levels of PAH found in the Baseline Study's analysis of the PM_{10} fraction for PAH were detected using the method developed in this research.

Chapter 1

Introduction.

1.0 Introduction to PAH.

Polyaromatic hydrocarbons (PAH) by definition, contain only carbon and hydrogen and are the product of any combustion involving fossil fuels (1). Research into the emissions and the environmental behaviour of PAH has tended to focus on a few of the great number of PAH compounds emitted by combustion processes and from other sources. The United States Environmental Protection Agency (USEPA, 1983) have identified sixteen PAH as "Priority Pollutants". In general terms, these pollutants have become the standard suite of compounds involved in many environmental studies of PAH. The sixteen PAH and their structures are identified in table 1.1

Ambient air particulates, in addition to gas species, are important components in the assessment of air quality (1). This chapter aims to provide a catalogue of the primary sources of PAH to the environment. Emissions to air will be the primary focus, as the ubiquity of these compounds in the biosphere is largely a result of their formation and release during the incomplete combustion of coal, petrol and wood (2). Thus, although a part of the PAH in the atmosphere arises from natural combustion such as forest fires and volcanoes, emissions from anthropogenic activities predominate (1) and are detailed in section 1.1 along with two important non-combustion sources. The size distribution of air particulates is also examined in section 1.3. The justification for the measurement of particulate pollution is discussed in section 1.5. Environmental pollutants can pose major health problems for mankind. Toxicological and epidemiological studies of particulate pollution are discussed in section 1.7.

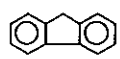
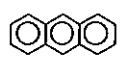
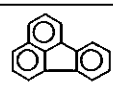
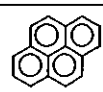
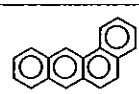
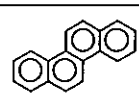
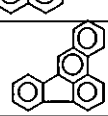
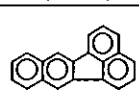
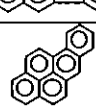
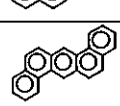
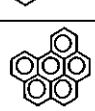
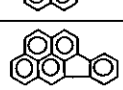
COMPOUND	ABBREVIATION	STRUCTURE
Naphthalene	(Naph.)	
Acenaphthene	(Acen.)	
Acenaphthylene	(Aceny.)	
Fluorene	(F.)	
Phenanthrene	(Phen.)	
Anthracene	(Anth.)	
Fluoranthene	(Fl.)	
Pyrene	(Py.)	
Benzo(a)anthracene	(B(a)Anth.)	
Chrysene	(Chrys.)	
Benzo(b)fluoranthene	(B(b)Fl.)	
Benzo(k)fluoranthene	(B(k)Fl.)	
Benzo(a)pyrene	(B(a)Py.)	
Dibenz(a,h)anthracene	(Dibenz.)	
Benzo(g,h,I)perylene	(Benzo.)	
Indeno(1,2,3-cd)pyrene	(Indeno.)	

Table 1.1 The 16 PAH listed by the USEPA as priority pollutants.

1.1 Major Primary Emission Sources of PAH.

Combustion sources are thought to account for over 90% of the environmental concentrations of PAH (1). The combined effect of fuel type and combustion conditions on the emissions of total and individual PAH is not fully characterised, but mechanisms at work in their formation are discussed in section 1.1.1. Non-combustion processes such as the production and use of creosote and coal tar (and indeed the remediation of sites contaminated with these), though poorly quantified, are potentially very significant primary and secondary sources. Creosote is a complex chemical mixture, comprising in the region of two hundred different compounds, of which ~85% of the quantified organic compounds are PAH. Creosote is a distillate of coal tar, which is a by-product of some coke production processes, and is used as a wood preserver domestically and industrially. The study of mixtures or "profiles" of a range of PAH can be instructive in identifying sources, particularly when this information is married to information on parameters such as particle size.

1.1.1 Thermal Reactions – Types and Significance

Two mechanisms are thought to result in PAH being emitted from combustion processes:

- "Pyrosynthesis" of PAH from aliphatic hydrocarbon and aromatic species:
- "Survival" of PAH in the starter fuel.

The synthesis of PAH is driven by a recurring addition process involving C_2 and C_4 radicals, leading to the formation of C_6-C_2 and C_6-C_4 radicals that are the building blocks of the "parent" PAH (3). These high-energy radicals can be generated by the partial cracking of aliphatic species and/or complex organic molecules with the synthesis of the parent PAH proceeding rapidly via the combination of these radicals (1).

The survival of PAH from the starter fuel is related to the temperature of combustion. However it is extremely difficult to study owing to the heterogeneity of most fuels, variations in local temperature and flame mixing conditions. The percentage of fuel PAH surviving is reported as ranging from 0.04% (benzo(a)pyrene) to 0.87% (fluorene) (4). The majority of PAH in diesel exhaust emissions will result from PAH in the fuel surviving the combustion process. The figures for benzo(a)pyrene suggest that only ~20% of the emitted PAH result from pyrosynthesis or combustion of lubricating oils (5). Combustion within petrol engines appears to result in a high proportion of the fuel PAH being destroyed however. A report by Westerholm et al. (6) states that up to 95% of the fuel PAH content is decomposed during combustion, with >50% of emitted PAH being formed during the combustion process (1).

The emission of PAH during open burning of plant material (i.e. wood, straw and leaves) would seem to be primarily a function of incomplete combustion of the fuel and is linked to the emission of particles (6). In a recent study utilising different sets of biomass, less vigorous burning conditions yielded higher emission levels of PAH bound particulates, which can in part be related to lower combustion efficiencies (6). Combustion of wood produced a greater proportion of total PAH in the gaseous phase than did the straw and cereal burns. This is thought to be a result of the higher combustion temperatures (and hence greater efficiencies) experienced by the dense wood fuels.

These observations provide an insight into the complex interaction of fuel type and combustion conditions in determining the total amount and range of individual PAH emitted, with the "synthesis" and "survival" mechanisms contributing to a varying degree depending on these two interacting variables. PAH emitted from combustion sources are formed during the combustion processes from smaller aliphatic molecules or the remnants of larger aromatic species, or alternatively survive the process. The extent to which any of these mechanisms are responsible for the emissions of PAH can be determined by complex interactions

between combustion conditions and fuel type, although in general, greater combustion efficiency leads to lower emission of PAH.

1.1.2 Combustion Processes.

PAH emissions to the atmosphere are dominated by combustion processes. Anthropogenic sources outweigh the contribution from natural sources, although isolated natural combustion events such as forest fires can have a profound effect upon air quality (7). Table 1.2 is a compilation of data from several publications and illustrates the range of combustion sources as well as the variability in emissions for each source characterised. It can be seen that variation within and between nations for a chosen source category is large (compare columns (a), (b) and (c) for example). A proportion of the discrepancies can be attributed to the range of sampling and analysis techniques employed by different workers. In general, mobile sources dominate in urban and suburban areas, i.e. petrol/diesel fuelled vehicles, aeroplanes, while stationary sources are thought to account for around 90% of total emissions, i.e. industrial sources, power and heat generation, residential heating, incineration and open fires (1).

The emissions from power generation utilising fossil fuels, perhaps surprisingly, appears to be a relatively minor source (<1% of total emissions) alongside the contribution from industrial, residential and mobile sources. There is good agreement between the data sets for this sector in table 1.2, indicating that this is one of the better-characterised sources.

"Residential heating" provides a large part of the total emissions detailed in table 1.2 (1,3,8,9). "Clean air" legislation (1987 Clean Air Act in Ireland) and a shift away from coal as a domestic fuel in many industrialised nations has done much to reduce the emissions in this sector in recent decades (8). Combustion of wood is conceivably the most important domestic fuel globally and can generate very high concentrations of PAH (8). Total PAH concentrations of 3000$\mu g/m^3$ and

concentrations of 60μg/m^3 for benzo(a)pyrene alone have been measured in flue emissions from small residential stoves (8).

"Mobile " and "open/uncontrolled" sources, together with domestic combustion processes outlined above, are likely to provide the majority of human exposure to atmospheric PAH, since humans congregate in urban environments characterised by mobile sources and inefficient burning of domestic fuels and garden waste (7). The emissions from mobile sources are a function not only of fuel type , but also of the age, maintenance and capacity of the engine, the vehicle model and whether the vehicle engine has been warmed up (10). In general, a low fuel:air ratio (i.e. a leaner mixture) will result in more complete combustion and so lower emissions of PAH (10) so that PAH emissions are greatest when the engine is cold, accelerating, decelerating or cruising at high speeds as the fuel:air ratio is highest during these stages (1).

1.1.3 Miscellaneous Sources.

Anthropogenic sources under this heading include crematoria, volatilisation from fuel spills, tobacco smoke, urban run-off, the application of sewage sludge to land and small scale non-ferrous metallurgical industries (11). Urban run-off containing PAH from vehicular exhaust particulates, road dust and tyre and road surface abrasion has been cited as a significant input of PAH to sediments in urbanised coastal areas (11). In smokers' homes, tobacco smoke contributes more than 87% of the total PAH in indoor air (12).

Estimated atmospheric emissions of total PAH by source type (te/yr).					
Source type	USA		Norway	UK	
	(a)	(b)	(c)	(d)	(e)
Residential heating					
Coal & wood	3939	450	48	604	368
Oil & gas	17	930	15	-	-
Subtotal	3956	1380	63	604	368
Industrial processes					
Coke Manuf.	632	2490	43	0.8	69
Aluminium plants	-	1000	160	-	100
Other	10	7	-	18.2	33.1
Subtotal	640	3497	203	19	202
Open burning					
Coal refuse fires	29	100	-	-	-
Agricultural fires	1190	400	2	6	7
Forest fires	1478	600	5	-	8
Other	1328	-	-	-	23
Subtotal	4025	1100	7	6	38
Power generation					
Power plants	13	1	-	6	5
Industrial boilers	75	400	1	-	-
Subtotal	88	401	1	6	5
Mobile sources					
Petrol engines	2161	2100	13	-	-
Diesel engines	105	70	7	80	260
Subtotal	2266	2170	20	80	260
Incineration					
Municipal	-	-	-	-	0.4
Commercial	56	50	1	0.06	0.07
Subtotal	56	50	1	0.06	0.47
Total	**11031**	**8598**	**294**	**715.2**	**873.6**

Table 1.2 Estimated atmospheric emissions of total PAH by source type (te/yr) (a)data from Peters et al (9), (b) and (c) data from Rahmdahl at al. (8),(d) data from Wild et al (1) and (e) data from APARG, 1995 (3).

1.2 Particulate Matter.

Airborne particulate matter may be classified as both primary, i.e. emitted as such into the atmosphere, or secondary, i.e. formed in the atmosphere from chemical reactions of gaseous precursors such as sulphur and nitrogen oxides (13). The two kinds of particulate matter show different seasonal patterns and a different dependence upon the control of sources (see section 1.5.1 for more detailed discussion).

Particulate matter is certainly the most complex of the air pollutants to understand and may ultimately prove one of the most difficult to control because of its multiplicity of sources. However, by targeting major sources of primary emissions, such as road traffic, it should be feasible to make progress (13).

1.2 Size Distribution.

Atmospheric aerosols show evidence of the several categories of sources in their size distribution and typically, three major components are recognisable (13).

The first (nucleation mode) is attributed to the nucleation processes. This process arises when molecules of an involatile substance are present in concentrations that far exceed the saturation vapour pressure; they have a strong tendency to condense. If a molecule encounters other molecules of the same substance it may combine to form a condensation nucleus-a very small particle. The molecule may meet a nucleus that already exists, or a particle of another substance, and condense causing growth in size. This nucleation mode may contain very large numbers of particles of ~10nm in diameter. Due to the small size of each particle the mass in this component is often a small fraction of the total aerosol mass concentration.

The second component is often called the accumulation mode. Particles roughly in the size range 0.05-2μm diameter are long-lived in the atmosphere. Coagulation and condensation leads to growth of particles in the nucleation mode into this region, and often a significant fraction of the aerosol mass accumulates in this region.

Finally, the coarsest particle peak in the atmosphere comprises particles of ~10 μm extending to about 100 μm. in diameter. These are shorter-lived, very variable according to local conditions and are likely to travel distances typically of meters to hundreds of kilometers according to size and wind speed.

1.4 PAH in other sample matrices.

Although this study is concerned with the analysis of PAH containing particulate matter in air, it is worth mentioning the other sample matrices in which PAH are being monitored and assessed in environmental laboratories. Water, soil, marine sediment and food samples are also widely researched for the analysis of PAH (14).

- ***Water.***

Determining PAH in water samples is difficult for several reasons, mainly because of their low solubility in water which means they tend to adsorb on the walls and surfaces with which they come into contact (14). Also light, residual chlorine and biodegradation can change their concentration; thus introducing considerable losses during sampling and storage (14). Due to the usual low levels in real samples, a preconcentration step prior to analysis is of great importance. The USEPA Method 610 describes the analysis of the 16 "priority PAH pollutants" in wastewater.

- ***Soil.***

Highly contaminated land samples, containing large concentrations of PAH are analysed routinely in a lot of environmental laboratories. Soil samples are often regarded as one of the worst environmental matrices to extract as they can contain a large variety of pollutants as well as the analytes of interest. Environmental analysts are turning increasingly to microwave methods for the extraction of PAH from highly contaminated land samples (15).

- ***Marine Sediment.***

PAH levels in open lake or marine surface waters are generally low, they are readily adsorbed and accumulated by sediments and particulate matter and μg/g-ng/g levels of PAH have been reported in many sediment samples (16).

- ***Food.***

Numerous papers have been published about PAH found in smoked and thermally treated foods as a result of pyrolysis or incomplete combustion of organic matter (17). The introduction of the "Ames test" in 1975 (18), provided a rapid method of isolating potential carcinogens in food on the basis of their mutagenic activity. The overlap of mutagenicity and carcinogenicity, although controversial, is now widely accepted (19). The assessment of mutagenic activity in cooked food requires tedious extraction work in order to isolate and quantify the chemicals responsible at the ng level.

1.5 Measurement of Particulate Pollution.

Particulate pollution has been measured for a variety of purposes to a variety of standards. Particulate pollution is mainly measured with an environmental health issue in mind, but its measurement in the work place as an occupational hygiene issue, is becoming more frequent (20). The division between these two standards of research is not clear-cut. This section aims to provide a background to

particulate pollution with respect to its major sources, typical chemical composition and lifetime in ambient air before sedimentation/transportation.

1.5.1 Major Sources of Particulate Pollution.

The atmosphere contains significant concentration of particles, varying in number concentration between about 10^2 cm^{-3} for background or remote locations up to about 10^5 cm^{-3} or greater for urban and more populated areas (21). These particles may have diameters spanning 5 orders of magnitude varying from 0.002 to ~ 100μm. The main determinant of the behaviour of an atmospheric particle is its size and its chemical composition. Size is usually expressed in terms of its "aerodynamic diameter" which refers to unit density spherical particles with the same aerodynamic properties, such as the falling speed. Airborne particles originate from a wide variety of sources. Significant natural sources of PM_{10} particles (particulate matter with an aerodynamic diameter of 10μm) include re-suspension of soil material in rural areas, volcanic activity, sea spray, forest fires and reactions between natural gaseous emissions (21).

As already mentioned in section 1.2, particles are generally classified in two categories. They may be either primary- these are emitted directly from primary sources such as industrial sources- power stations, cement factories, combustion processes and motor vehicles; or may be formed from secondary sources- particles formed within the atmosphere from condensation of vapours, or as a result of chemical reaction processes (21). Many industrial processes and the burning of carboniferous fuels produce PM_{10} particulates.

Combustion which is a major source of particulates is a complex process which produces distinct particles in a number of different ways including vaporisation followed by condensation to yield particles in a particular size range (22). Motor vehicles represent a major source of PM_{10} in urban air (22). Transportation source emissions occur in two main categories:

(1) Vehicle related particles from tyre, clutch and brake wear and

(2) Particles generated from fuel combustion.

Diesel vehicles are of particular concern as they emit approximately 30 to 70 times more particulates than gasoline fuelled vehicles equipped with catalytic converters and burning unleaded fuel (22). Particles emitted by vehicles include unburnt hydrocarbons, oxygenated hydrocarbons, PAH and inorganic species such as sulphur dioxide, nitrogen dioxide and sulphuric acid.

Significant concentrations of potentially harmful substances can be present in the interior of vehicles (23). The main sources of PAH and elemental carbon (EC) inside a car is likely to be combustion emissions (23). Fromme et al. (23) carried out a study (in Berlin, Germany) to quantify PAH and EC particulate (inhalable fraction) emissions inside a car and a subway train. Twice in summer 1995 and winter 1996, PAH and diesel motor emission (estimated as elemental carbon) were determined in the interior of a car (a 2-year-old VW Golf with a three way catalytic converter) and in the passenger compartment of a subway train. On each sampling day (in total 16 daily measurements in the car and 16 in the subway) the substances were determined in the breathing zone of the passengers from 07:00 h to 16:00 h under different meteorological conditions (winter- and summertime). The car followed the route of the subway from the western Berlin borough of Spandau to the south-eastern borough of Neukolln, and back. The sampling represented a realistic exposure model for driving in a high traffic and polluted urban area. The electric subway train (also 2 years in use) connected the same parts of Berlin (31-km underground). The mean values obtained during the two measurement periods (summer/winter) inside the car were 1.0 and 3.2ng/m^3 for benzo(a)pyrene, 10.2 and 28.7ng/m^3 for total-measured PAH, 14.1 and 8.2μg/m^3 for EC. In the subway the levels were quantified at 0.7 and 4.0ng/m^3 for benzo(a)pyrene, 30.2 and 67.5ng/m^3 for total PAH, 109 and 6.9μg/m^3 for EC. A comparison between subway and car exposures shows significantly higher

concentrations of PAH in the subway train, which can be explained by relatively high concentrations of the 2-3 ringed PAH in the subway. So far a satisfactory explanation has not been found (23), but one source might be the wooden railway ties that were formally preserved with tar based products.

1.5.2 Typical Chemical Composition and "Fingerprinting" of Primary Sources.

The study of mixtures of PAH from different sources is driven by the search for specific "marker" compounds that can facilitate the apportionment of sources, enabling identification of the major sources of PAH at a given location. If the PAH emissions from a source are well characterised then it is hoped that the contribution of that source to the total PAH concentration in the environmental sample can be determined. For example, Duval and Friedlander (24) identified the following source "profiles" from PAH data in Los Angeles:

- ***Coal Combustion***- anthracene, phenanthrene, fluoranthene, pyrene and benzo(a)anthracene.
- ***Coke Production***- anthracene, phenanthrene, benzo(a)pyrene and benzo(ghi)perylene.
- ***Incineration***- phenanthrene, fluoranthene and especially pyrene.
- ***Wood Combustion***- anthracene, phenanthrene, fluoranthene and pyrene.
- ***Oil Burning***- fluoranthene and pyrene.
- ***Petrol-Powered Cars***-fluoranthene, pyrene and especially benzo(ghi)perylene.
- ***Diesel-Powered Vehicles***-phenanthrene, fluoranthene and anthracene.

A study to examine the chemical composition of PM_{10} was carried out by Bagnoli et al. (25) in Leghorn, Italy. The objective of the study was to obtain knowledge about the chemical constituents of PM_{10} in the city of Leghorn. The PAH content of the PM_{10} fraction was examined. Also the contents of various heavy metals from anthropic sources were determined. Two sites, characterised by different settings,

were chosen in the city. Both sites were marked by intense motor traffic. The sampling regime at site A (garden overlooking a large very busy square) was:

- PAH sampling period: March to April (17 days)
- PM_{10} and heavy metals (Pb, Cr, Cu, V, Cd, Mn, Fe, and Ni) sampling period: March to November 1997 (40 days).

The sampling regime at site B (a garden between two buildings, overlooking a busy street) was:

- PM_{10} and heavy metals (as above) first sampling period: June to July (7 days)
- PM_{10} and heavy metals (as above) second sampling period: October to November (10 days).

The sampling campaigns were conducted during different periods of the year (spring and autumn) and characterisation by different meteorological conditions. The maximum and minimum values and the standard deviation of PM_{10} found at site A were 107$\mu g/m^3$, 19$\mu g/m^3$, and $\pm$16.6, respectively. The corresponding values for site B were 79$\mu g/m^3$, 18$\mu g/m^3$, and $\pm$16.7, respectively. Comparing these results, the study found that the smallest values (registered during rainy days) are quite similar to each other as are the standard deviations, whereas the maximum values showed a significant difference. Furthermore, the mean value of PM_{10} obtained at site A (48.8$\mu g/m^3$) was higher than that obtained at site B (33$\mu g/m^3$). These two values are lower than the quality target value of 60$\mu g/m^3$ established by Italian law (November 25, 1994) effective until the end of 1998, while the former is higher than the quality target value of 40$\mu g/m^3$ that will be in force beginning January 1,1999.

The PAH present in the atmosphere in the vapour phase were not considered in this study as these compounds are predominantly characterised by lower molecular weights and are of lesser toxicological importance. Only the

concentration of PAH in the particulate matter collected was evaluated at site A. This site in addition to being affected by the urban road traffic looked more interesting because of the site's closeness to the industrial area of the city. The PAH mean concentrations at this site corresponding to the period from March 18 to April 17,1994 are shown in table 1.3.

PAH COMPOUND (Ref Table 1.1)	MEAN CONC. (ng/m^3)	STD. DEV.
Fl.	0.60	1.05
Py.	1.25	1.88
Chrys.	0.47	0.40
B(b)Fl.	0.97	0.56
B(k)Fl.	1.50	1.02
B(a)Py.	0.75	0.47
Dibenz.	0.30	0.65
Benzo.	2.40	1.14
Indeno.	1.05	0.57

Table 1.3. PAH Mean Concentrations and Standard Deviations at site A (25).

Heavy metals that are more representative tracers of anthropic sources of pollution or telluric dusts were considered in this study (25). Lead is a tracer of pollution produced by petroleum-engined vehicles. Vanadium and nickel are tracers of pollution from oil combustion. Cadmium, chromium and manganese are tracers of pollution from various kinds of industries. Iron, however, is known to be a component of telluric dusts and, because there is a shipping industry in Leghorn, the study cannot exclude an anthropic contribution to the presence of iron in the PM_{10} fraction. The distribution of heavy metals concentrations at the two sampling sites appeared to be the result of the superposition of different contributions from the urban traffic and domestic heating systems, from industrial combustion

processes of mineral oils and manufacturing carried out at the dockyard, and finally the telluric composition.

1.5.3 Particulate Behaviour in Ambient Air.

The composition of atmospheric particles is influenced by a balance between sources, chemical transformations in the atmosphere, long-range transport effects and removal processes (22). Particles with a relatively long atmospheric lifetime and no significant localised sources (e.g. sulphate in fine particles) show quite high spatial uniformity, and those with short residence times and/or localised sources (e.g. quarry dust) show strong spatial concentration gradients (22). Thus no generalised statements on the degree of uniformity of atmospheric particles are possible. Undoubtedly there are substantial temporal and spatial variabilities in atmospheric particle loadings, and appreciable spatial variations in mean composition. Nonetheless, there are common chemical components which appear at relatively similar concentrations throughout the developed world (22), and within the UK airborne particle concentrations and composition are not expected to vary greatly from one location to another of the same type (e.g. urban) elsewhere in the country (22).

Even where size selection is used in sampling procedures, it has been found that the effects of high relative humidity can produce inconsistent results. Particles, especially those with a high sulphate content, will grow when exposed to high relative humidity thus changing their size distribution, a phenomenon investigated in detail by Keeler et al (26). Given that night-time humidities are higher than daytime, and that most public exposure occurs indoors under conditions of low relative humidity it seems likely that the "fine" fraction (particles smaller than about 2μm diameter) which arise mainly from condensation of hot vapours and chemically-driven gas to particle conversion process, referred to in this study as $PM_{2.5}$, will frequently be underestimated by outdoor sampling (26).

A relationship study between PM_{10} and $PM_{2.5}$ in indoor (I) and outdoor (O) air was carried out by Monn et al. (27). In this study among 17 homes in Switzerland, the relationship between indoor and outdoor levels of PM_{10} and $PM_{2.5}$ was investigated. In 10 homes, the inhabitants also participated in conducting personal measurements. All homes were naturally ventilated. In homes without any indoor sources and where human activity was low, PM_{10} I/O ratios amounted to approximately 0.7. Of the indoor sources, smoking had the highest influence on I/O ratios (>1.8). In homes not containing any apparent source, "human activity" was an important factor accounting for high indoor levels. However, this factor is difficult to quantify (27).

1.6 Air Quality Standards.

Health standards for fine particulate pollution measured directly as mass are a more recent phenomenon. Considerable epidemiological research over the last 2 decades has led to the introduction of PM_{10} standards in the United States. Standards on an international level are being revised again in light of ongoing epidemiological research indicating the particular importance of fine (~2.5 μm) particles.

The USEPA issued revised particulate matter standards in July 1997 (20). They announced new standards for particulate matter under the national ambient air quality standards (NAAQS). After reviewing hundreds of peer-reviewed scientific studies, the USEPA has determined that these changes are necessary to protect public health and the environment. The USEPA revised the primary (health-based) particulate matter standards by adding a new annual $PM_{2.5}$ standard set at 15μg/m^3 and a new 24-hour $PM_{2.5}$ standard set at 65μg/m^3. The current annual PM_{10} standard of 50μg/m^3 was retained and the PM_{10} 24-hour standard of 150μg/m^3 was adjusted by changing the form of the standard (USEPA, 1997) (20).

Standards for respirable air sampling are based on curves for particle penetration into the respiratory system (22). This is illustrated graphically in figure 1.1.

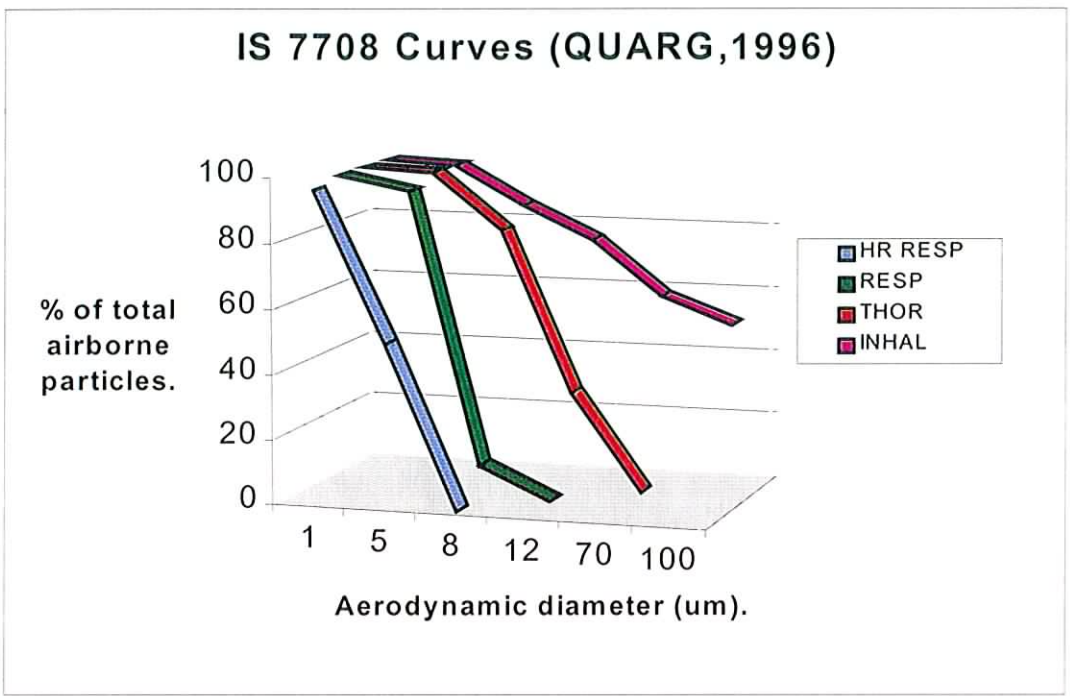


Figure 1.1. IS 7708 curves defining the inhalable (INHAL), thoracic (THOR), respirable (RESP) and high-risk respirable (HR RESP) fractions of aerosol (QUARG, 1996), (22).

1.6.1 Black Smoke.

Black smoke measurements rely on the collection of particles onto a filter and the subsequent estimation of their weight by means of an estimated calibration between reflectance and mass. The sampling inlet used for black smoke measurements has a 50% upper cut-off for particles of aerodynamic diameter of about 4.5μm (28). However, the use of light reflectance to calculate mass concentrations in the air are based on a relationship identified from urban air polluted primarily by coal smoke (28). Attempts to establish a relationship between black smoke and PM_{10} readings have found that the relationship varies seasonally, being stronger in the winter than in the summer (22). This is because secondary

particles formed in sunlight are lighter in colour and thus are not measured as effectively by the black smoke method as primary particles (22).

1.6.2 World Health Organisation Standards.

The World Health Organisation (W.H.O) Regional Office for Europe issued air quality guidelines in 1987 which set a total suspended particulates guideline of 120$\mu g/m^3$ and a thoracic particles (~PM_{10}) guideline of 70$\mu g/m^3$ (29). The guideline value for PAH particulates set by the W.H.O. was zero, i.e. no safe level (29).

Over the past few years the air quality guidelines have been reviewed. In the case of particulate pollution, the Working Group on "Classical" Air Pollutants, due to lack of any identifiable threshold for ill-health effects from particles, decided not to issue any guidelines, (W.H.O., Copenhagen 1996) (30). Instead, the working group made out a table of acute health effects of differing PM_{10} concentrations, reproduced in table1.4.

1.6.3 E.U. standards.

The only existing E.U. standard is contained in the 1980 "Directive on air quality limit values and guide values for sulphur dioxide and suspended particulates" (31). This specifies the Black Smoke method and a total particulates method. It is implemented into Irish law by S.I. No. 244 of 1987.

The "Directive on ambient air quality assessment and management, 1996" (32) specifies fine particulate matter as one of the pollutants "to be taken into consideration in the assessment and management of atmospheric quality". It is intended to be an umbrella directive to which "daughter directives" dealing with specific pollutants or pollution parameters will be added. The European Commission's Technical Working Group on Particles, set up in 1995 (33) has

produced a draft directive, which is being currently studied by member states. The ENDS Report (1997a) says that the working group has recommended a 24-hour limit of 50μg/m^3, and an annual mean level of 20μg/m^3. The working group has also recommended that $PM_{2.5}$ should be measured with the intention of substituting a $PM_{2.5}$ standard for the PM_{10} standard at the first revision of the standard.

Health effect indicator	100μg/m^3		200μg/m^3	
	no. of cases	% increase	no. of cases	% increase
Mortality	8	10	16	20
Hospital admissions for respiratory conditions.	12	20	24	40
Person-days with bronchodilator use among asthmatics.	21,000	70	42,000	140
Person-days with symptom exacerbation among asthmatics.	15,000	50	30,000	100

Table 1.4. Estimates of the number and percentage increase of health effects in a population of 1 million over a three-day period characterised by average PM_{10} concentrations of 100μg/m^3 and 200μg/m^3 (W.H.O., Copenhagen, 1996) (24).

1.6.4 Emergency Levels.

A recent article in Chemistry in Britain (34) on emergency levels of particulate pollution states that PM_{10} concentrations sometimes exceed the $500\mu g/m^3$ "emergency" U.S. level defined by the USEPA. On the 23 September 1997, an API (Air Pollution Index) of 839 was reported in Kuching, Sarawak, Malaysia, the worst day of the haze episode of 1997. This value of the Malaysian API, which differs from the USEPA's Pollutant Standard Index (PSI), corresponds to a PM_{10} concentration of $939\mu g/m^3$ (24-h average), or almost $1mg/m^3$. This may be compared with the total smoke concentration of ca. $1.5mg/m^3$ measured during the infamous London smog of 1952-the worst ever recorded case of air pollution-which resulted in over 4000 deaths (34). No deaths have yet been attributed to the haze in Malaysia, Singapore or Brunei, but scientists are actively investigating mortality statistics (34).

1.6.5 A Baseline Study on the Concentrations of Volatile Organic Compounds and PM_{10} in Dublin City.

Particulate pollution has acute and chronic health effects associated with it (21). TMS Environmental Ltd in conjunction with Dublin Corporation Environmental Health Officers Services, carried out a baseline study for the EPA on the concentrations of volatile organic compounds and PM_{10} in Dublin City (21) over a 13-month period (from January 1st 1996 through January 31st 1997). The main objectives of the PM_{10} measuring programme were to:

- Establish ambient mass concentrations of particulate matter with a diameter less than $10\mu m$ (PM_{10}) at a number of sites in Dublin City and at background sites.

- Determine relationships between PM_{10} mass concentration with that inferred using the standard black smoke method at the same sites and in the laboratory using smoke and diesel particulates.

- Determine chemical composition of PM_{10} for site locations in Dublin City

- Examine daily and seasonal (if any) variability of the PM_{10} at the sites.

- Make recommendations with regard to the necessity or otherwise for a longer term monitoring program.

The main findings from the PM_{10} monitoring survey were as follows:

- Ambient PM_{10} mass concentrations have been established for the first time at 6 sites in Dublin City. This involved taking 1200 averaged PM_{10} (over 24 hour periods) measurements using a USEPA approved standard gravimetric method, and 200 days of continuous PM_{10} measurements every 30 minutes (comprising 9,600 individual PM_{10} measurements in total) using a USEPA equivalent PM_{10} method (21).

- The 2 inner city sites of College Green and Merchants Quay recorded the highest PM_{10} annual average mass concentration values of 44 and 40$\mu g/m^3$. Further data analysis showed that on 86 out of 275 measurement days (31% of occasions) in 1996, PM_{10} levels at the College Green site were greater than 50$\mu g/m^3$ (the USEPA maximum annual averaged recommended level). PM_{10} levels greater than 50$\mu g/m^3$ occurred 20% of the time at the Merchants Quay site, and 11% of the time at the Ashtown Grove residential site, which is some 150m. from a main traffic artery. It is evident that the inner city sites experience a considerably high number of incidences of PM_{10} levels greater than 50$\mu g/m^3$ (the USEPA standard level) (21).

- Good agreement between PM_{10} mass concentrations and mass concentration, inferred using the black smoke method, was obtained at the College Green site, the most polluted of the Dublin City sites. However for the non- city centre sited, varying site specific relations between the mass concentrations using the two methods was found. Estimates of black smoke method cannot act as a surrogate for PM_{10} except for locations with high pollution levels – which are likely to contain predominance of carbonaceous black aerosol (21).

- A seasonal investigation of PM_{10} concentrations showed that the averaged winter (October-March) levels exceeded summer (April-September) levels by between 40 to 50%, for all of the relevant sites (21).

- An investigation of the diurnal variation in PM_{10} levels showed that the PM_{10} concentrations increases substantially during the early morning with maximum concentrations occurring between about 08:00 and 10:00. This is consistent with traffic related pollutants for which elevated emissions occur during the period of peak commuter traffic. Close agreement was observed between the weekly averaged PM_{10} values and the car density (number of cars counted ½ hour interval on the middle day of that week) at the inner city site of College Green. However, additional parallel measurements of traffic density and PM_{10} concentration are desirable to assess the seasonal variation of PM_{10} levels with traffic numbers (21).

This report also recommends that detailed work of the major chemical species of PM_{10} (and $PM_{2.5}$) is required in order to apportion specific sources of the particulate matter. An important requirement would be to quantify the relative contributions from primary and secondary sources of particulate matter. The report acknowledges that there is scope for performing tracer studies through the use of controlled additives in vehicular diesel fuel for example.

Another recommendation is that at least one inner city site be equipped with an array of standard meteorological instruments in order to help determine relationships between PM levels and meteorological parameters.

Some of the filter samples were analysed for PAH in the baseline study, the results of which are given in chapter 4 section 4.13. Also some of the samples from this study were acquired and analysed for PAH using the method developed with this research, these results are given in chapter 4 section 4.14.

1.6.6 PM_{10} Levels in other Major Cities.

A profile analysis of PAH in airborne particulate matter of the area of Thessaloniki, Northern Greece was carried out during October '93 – December '94 by Samara et al. (35). Airborne particulate matter (APM) was collected from the centre of Thessaloniki (sampling site 1) and from two residential communities at the interface of the Thessaloniki industrial area (sampling sites 2 and 3). Oil refining, petrochemical, fertilizer, non-ferrous metal smelting, iron and steel manufacturing, truck and auto painting and metal recovery facilities are located within 1-2km around site 2. Whilst electrolytic MnO_2 production, anodized Al, scrap metal incineration, tyre production, lubricating oil recovery and non-ferrous metal smelting works are operating within 1-2km of sampling site 3. Both fine ($PM_{2.5}$) and coarse (PM_{10}) particulate fractions were collected in this sampling regime along with total suspended particles (TSP), the results of which are given in table 1.5.

As already mentioned in section 1.6.4 the annual PM_{10} mass concentration in College Green and Merchants Quay, Dublin were 44$\mu g/m^3$ and 40$\mu g/m^3$ in 1996 (21). The PM_{10} concentration for the period October '93 to December '94 was found to be 60±26$\mu g/m^3$ in Thessaloniki City centre, higher than the two Dublin City centre sampling sites (35). The sampling period was longer (by 2 months) in the Thessaloniki sampling regime than the Baseline Study carried out in Dublin in

1996. The higher PM_{10} annual mass concentration in Thessaloniki could be attributed to the closeness of the city centre sampling site to the industrial area or the local prevailing meteorological conditions.

	City Centre (1)		Site 2	Site 3	Site 1	Site 2
	$PM_{2.5}$ n=40	PM_{10} n=40	TSP (n=28)	TSP (n=27)	TSP (n=35)	TSP (n=35)
APM ($\mu g/m^{3)}$	116±31	60±26	288±91	323±122	256±94	283±191
ΣPAH (ng/m^3)	290±278	28±19	620±648	866±896	-	-
ΣPAH (%)	0.029	0.003	0.063	0.087	0.019	0.016

Table 1.5 Summary Data (means ±SD) of Airborne Particulate Matter Concentrations from the Area of Thessaloniki in 1994 (35).

The next section (1.7) discusses health effects of particulate pollution with toxicological and epidemiological studies. Both these studies are used to assess health effects of pollutants in research centres.

1.7 Health Effects of Particulate Pollution.

Studies of the health effects of particulate pollution have approached the question from two sides: toxicology and epidemiology.

1.7.1 Toxicological studies.

Studies of particulate intake into the respiratory tract have shown that mechanisms for transferring the dose are various (36). Deposited material is cleared by

mucociliary clearance and alveolar macrophages, which take the particles they have absorbed to the gastrointestinal tract. Some particles may travel through the mucous layer and the cilia and become attached to the respiratory wall. Particles are also cleared by the macrophages into lymph nodes (where they remain, with potential immune system effects). Other particles may dissolve and be carried into the blood and thereby dispersed around the body. Thus the potential health effects go far beyond the respiratory system.

Considering the complex mixture, which is particulate pollution and the presence of other pollutants often with synergistic effects, the picture is one of considerable complexity. It is not only the chemical composition of the particulate pollution that is of significance. Consistent epidemiological results from studies of PM_{10} pollution from different sources have led some commentators to conclude that the size of the particles is a far more important factor, and QUARG (22) suggested there may be a "non-specific effect of particles". Their suggestion is that this may relate to the carriage of material on the particle surfaces.

Sun et al (37) summarised the effects of particle-associated organic compounds, many of them identified carcinogens. In this context the size of the particles is of clear importance. The smaller the particles, the greater the surface/mass ratio, and therefore the greater the surface area on which other compounds can be carried. Changes in measured mass concentrations may represent much greater changes in particle number and surface area. COMEAP (21) put it as follows: "The mass of a 1μm particle of unit density is equivalent to the mass of one thousand 0.1μm particles. However, the surface area of one thousand 0.1μm particles is 10 times greater than the single 1μm particle. Consequently, a 10% increase in PM_{10} could represent a very large percentage increase of both the number of particles and the surface area of particles, and hence toxicant, presented to the bronchial, pulmonary, and alveolar regions of the lung. The increased surface area would also greatly facilitate the rapid dissolution of any sorbed or soluble material. With the increased surface area to volume ratio of submicron particles and their known

entry into and retention within the pulmonary region of the human lung, they present a potential and, as yet, undefined toxicological hazard."

The question of overloading the pulmonary alveolar macrophages has received considerable attention (36). The model of the macrophage clearance mechanisms failing due to overload is of potential significance in the case of those exposed to high levels of urban pollution (36).

Toxicological studies have established the carcinogenity of many of the components of diesel and petrol fumes (38). The International Agency for Research on Cancer (1993) has concluded that diesel emissions are "probably" and petrol emissions "possibly" carcinogenic to humans' (39). Much of the evidence is contained in epidemiological studies referred to by Whitelegg (1993), (38) and Godlee (1991), (39).

The mutagenic activity of organic chemical airborne particles was investigated by Villalobous-Pietrini et al. (40). Total suspended particles (TSP) and PM_{10} were collected in Mexico City (1995). Higher mutagenic activity was found in PM_{10} than in TSP. The largest frequency of mutations was from the Downtown samples, indicating the importance of vehicle emissions (40).

Tyre dust is a specific, serious problem. Williams et al. (41) found "a large number of respirable tyre fragments " in urban air. These arise from tyre wear, particularly of radial tyres, which "create a finer more respirable dust" (42). Williams et al. concluded "Given the adjuvant and sensitizing effects of latex, these airborne particles could contribute, through direct and indirect mechanisms to the increase in both latex sensitization and asthma. The impact of these particles should be considered in the issue of morbidity and mortality rates associated with respiratory diseases and air pollution." Montague (42) suggested that "part of the cause for recent increases in asthma may be the shift from bias ply tyres to radial tyres". (To this should be added, of course, the increase in motor traffic.)

1.7.2 Epidemiological Studies.

Epidemiological studies relate exposure (or dose) of a toxicant with a health effect (whether death, a sign of ill health or a change such as a decrease in lung function).

In the case of most air pollution studies, particularly those carried out in a non-occupational context, ambient air concentrations are used as a substitute for actual exposure information. Whitelegg (38) has pointed out the difficulties of epidemiological investigation of individual pollutants where "potential synergistic effects are so numerous and unknown that we cannot begin to estimate the impact of different pollutants and different levels in the presence or absence of other pollutants."

A substantial body of epidemiological research has consistently indicated a correlation between exposure to particulate pollution (particularly PM_{10}) and indices of ill health, particularly mortality rates (43). The main stream of epidemiological studies referred to below has found positive associations between ambient particulate pollution concentrations and overall mortality and with mortality from lung cancer and cardiopulmonary disease (44). Other studies (38) have found associations with other cancers including associations between traffic levels and leukaemia, lung and colon cancer, between petrol exhaust and rectal cancer, diesel exhaust and colon cancer, and between car ownership and leukaemia (45).

Many individual PAH have been tested for carcinogenity in various animals by different routes of application (oral, subcutaneous, epicutaneous, intratracheal and intrapulmonary) and resulted in both benign and malignant tumors (46). In-vitro experiments with tissue and cell cultures as well as with sub-cellular fractions, but also in microbial test systems, clearly demonstrated the cell-transforming properties of PAH, indicating mutagenic and carcinogenic potential (46). Moreover, the carcinogenic risk potential for humans of PAH may be deduced from

a variety of occupational studies e.g. coke plant workers (47). Sir Percival Pott reported the formation of the comparatively rare scrotum cancer in chimney sweeps some 200 years ago (48). The disease is supposed to be caused by a permanent exposure to coal, tar and soot (48). More recent studies in 1988 by Gustavsson (49) have confirmed these findings and evidenced high incidences of oesophagus, bladder and lung tumors in chimney sweeps.

Table 1.6 shows the percentage contribution of PAH and benzo(a)pyrene to the carcinogenic potency of various matrices using two biological test models (46).

	PERCENTAGE OF EFFECT (%)			
	Mouse, epicutaneous		Rat, intrapulmonary	
	PAH**	B(a)Py	PAH**	B(a)Py
Used motor oil	70	18		
Weight % *	1.1	0.02		
Vehicle exhaust	85	6	81	2.4
Weight %	2.5	0.04	2.8	0.05
Flue gas of coal-fired furnaces	90	11	>90	1.4
Weight %	22.7	0.1	29	0.11
Diesel exhaust condensate (organic extract)			80	4
Weight %			0.9	0.01
Cigarette smoke, sidestream			75	0.17
Weight %			3.5	0.0004

Table 1.6 Percentage contribution of PAH and benzo(a)pyrene to the carcinogenity potency of various matrices using two biological test models (46)

*weight % = percentage of PAH and B(a)Py by weight related to the original material.

** PAH with 4 and more rings.

From table 1.6 it can be seen that benzo(a)pyrene seldom contributes by more than 10% to the total carcinogenic potential of environmental matrices.

Table 1.7 shows the carcinogenic potencies of various PAH relative to benzo(a)pyrene (=1.00) [$CP_{Rel.\ B(a)Py}$]. The test model used was an intrapulmonary injection into female Osborne-Mendel rat (46).

	$CP_{REL.\ B(A)PY}$
Benzo(a)pyrene	1.00
Dibenz(a,l)pyrene	>>2.00
Dibenz(a,h)anthracene	1.91
Anthracene	0.19
Cyclopenta(cd)pyrene	0.15
Benzo(b)fluoranthene	0.11
Indeno(1,2,3-cd)pyrene	0.08
Benzo(k)fluoranthene	0.03
Benzo(j)fluoranthene	0.03
Chrysene	0.03
Benzo(b)naphtho(2,1-d)thiophene	0.02
Benz(a)anthracene	0.01
Fluoranthene	0.00

Table 1.7 Carcinogenic potencies of various PAH relative to benzo(a)pyrene (46).

1.8 Aim Of This Research.

The danger to health associated with PAH particulate pollution have been discussed and to an extent quantified in section 1.6. It is therefore essential to be able to quantify these compounds in air. A baseline study on the concentration of volatile organic compounds and PM_{10} in Dublin city (1997) includes some PAH quantification in air particulates (21) (see chapter 4, section 4.13). This is the most recent study on PAH analysis carried out in Dublin city. One of the main conclusions in the final report was that further work will be required to establish atmospheric concentrations of individual and total PAH levels in Dublin (50).

The primary aim of this work was to develop and validate a quick, efficient and accurate method for analysis of PAH in air particulates (particle size 10μm and 4.5μm). The method is essentially two stages, sample extraction and sample cleanup before analysis by HPLC with fluorescence detection. Sample extraction techniques were examined by spiking filters with standard concentrations of PAH. Soxhlet extraction and ultrasonic extraction procedures were investigated. The most efficient extraction method was identified, and the PAH recoveries optimised by varying the extraction conditions. The sample cleanup procedure used was solid phase extraction. Sample retention on the sorbent was investigated by spiking with standard concentrations of PAH. With the retention optimised, identification of suitable wash and elution solvents followed. All the method development analysis was quantified by HPLC with UV detection.

The optimised and validated method was then employed to quantify PAH in selected environmental samples. Environmental samples were taken at indoor and outdoor locations. The indoor air sampled was in public houses, the outdoor locations were areas of traffic congested roadways and background (urban) sites. Tobacco smoke (12) and petrol/diesel fuelled vehicles (4) are some of the main sources of PAH emissions and the sample sites were chosen with this in mind. The particulate bound PAH in the environmental samples were quantified using

HPLC with fluorescence detection. Due to the low levels expected, i.e. ppb, fluorescence detection was used because of its low detection limits.

Samples were obtained from the Baseline Study, and analysed using the method developed in this research. With the quick and efficient method developed in this work, the monitoring of PAH levels in Dublin city is feasible, at a relatively low cost.

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Chapter 2

Experimental.

2.0 Introduction to Various Approaches to PAH Extraction and Analysis.

The complete analytical procedure for the for identifying and quantifying of compounds usually starts with some kind of planning for strategies of sampling and ends up with results, preferably presented with statistical consideration. This forms the analytical chain and it is certainly true that "a chain is not stronger than its weakest link". Some frequently applied links in this chain can be described by the following scheme:

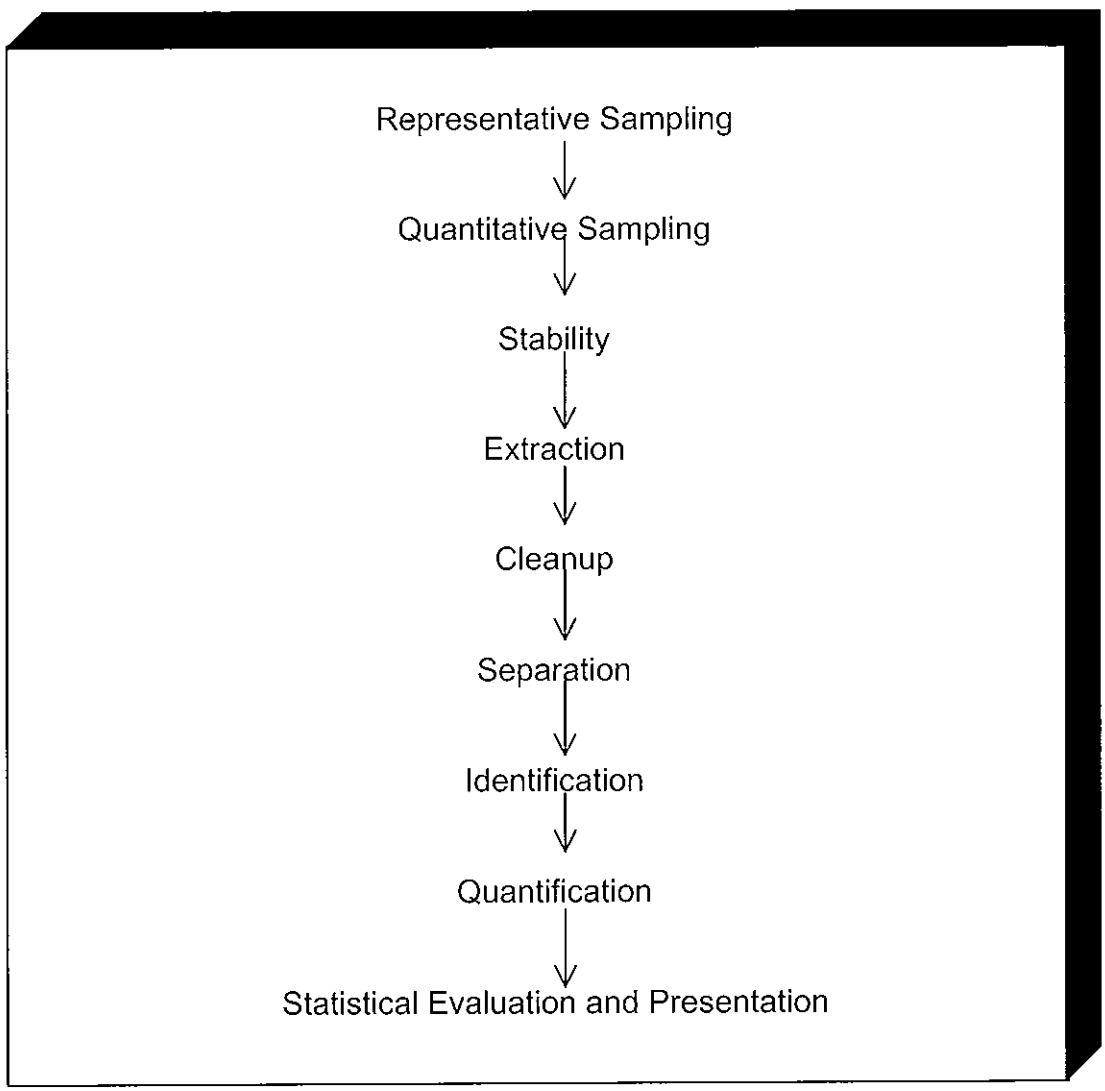

Figure 2.1 Typical Analytical Scheme.

The necessity of efficient extraction techniques, yielding high and stable recoveries, is a prerequisite for any serious attempt to quantify compounds in any procedure involving extraction. Low yields indicates that large quantities of analyte are still trapped in the sample matrix. Evaluation of extraction efficiency and rate are procedures of vital importance. These parameters are very much a product of both extraction method and type of sample. In many instances spiked samples are used for the determination of the extraction rate for particular compounds. However, artificially added compounds (spikes) will not usually be adsorbed in the same way as compounds already present in the sample. It has been shown that the extraction rate of sample spikes can be 10 times higher than the rates of the environmental PAH (1). When recoveries of more than 90% for spiked samples were registered, only 25-80% recoveries were achieved for the native PAH at the same extraction conditions. The necessity of sample cleanup on the other hand is more a question of the complexity of the extract and the selection of separation and detection techniques. The selection of the extraction and pre-concentration method is pratically based on some kind of optimisation of speed, efficiency, cost, and to some extent environmental concern.

2.1 PAH Standard Solutions for Method Development.

A PAH standard mixture (Supelco) for USEPA method 610 (Lot no. LA – 63573) was used to make the standard solutions necessary for method development. This standard contained 200 μg/l each (200ppm) of the 16 PAH of interest in methanol (1ml.). This mixture (1ml) was diluted with methanol (Sigma, HPLC grade) in a 10ml volumetric flask. The resultant 20ppm solution (A) was used to make 1ppm (0.5mls. solution B in 10mls methanol (solution 1)), 0.1ppm(1ml. solution 1 in 10mls. methanol (solution C)) and 0.01ppm(1ml solution 2 in 10mls. methanol (solution D)) solutions.
Each volumetric flask used was silanated (see section 4.4.1).

2.2 Standard Solution Runs by HPLC for Method Development.

For the purposes of method development standard solution 1 was analysed by HPLC (Varian model 9012) with Varian Star chromatography software. The detection systems used were UV (Waters model 441) and fluorescence (Varian model 9075). The conditions (HPLC 1) were as follows (2);

HPLC 1

Column: EnviroSep – PP (Phenomenex).

Dimensions: 125 x 4.6mm.

Pump: Ternary gradient, Varian model 9012.

Flow rate: 2.0 ml/min.

Sample injection volume: 20μl.

Sampling valve: Rhenodyne 7115 (20μl).

Temperature: Ambient.

Mobile phase: A:Water (HPLC grade).

B:Acetonitrile (HPLC grade).

Gradient elution:

Time (min)	%B
0	50
5	50
30	100
34	100

Detection:

UV fixed wavelength (254nm), Waters model 441.

Fluorescence programmable wavelength, Varian model 9075.

The wavelength program was optimised for each PAH, and is shown in table 2.1.

Time (mins)	0	9.6	11.7	19.6	27.2
Excitation (nm.)	250	240	265	290	300
Emission (nm)	345	425	380	430	500

Table 2.1 Optimised PAH Fluorescence Wavelength Program.

Another column was also used in this research (ambient environmental samples, chapter 4 section 4.11). Analysis conditions with this column will be referred to as HPLC 2 conditions where applicable. HPLC 2 conditions are as follows:

HPLC 2

Column: Hypersil ODS (Shandon)

Dimensions: 100 x 4.6mm

Flow rate: 2.0 ml/min

Sample injection: volume: 20μl

Temperature: Ambient

Mobile phase: A:Water (HPLC grade)

B:Acetonitrile (HPLC grade)

Gradient elution:

Time (min)	%B
0	50
5	50
25	100
30	100

Detection:

UV @ 254nm.

Fluorescence programmed wavelength change, optimised for each PAH (see table 2.2)

Time (mins)	0	9.6	17.2	24.6	30.0
Excitation (nm.)	250	240	265	290	300
Emission (nm)	345	425	380	430	500

Table 2.2 Optimised PAH Fluorescence Wavelength Program.

See Appendix A for the standard chromatograms under HPLC 1 and HPLC 2 conditions.

2.2.1 Fluorescence Detector Response.

PAH standard solutions (B), (C) and (D) were run on the HPLC under HPLC 1 and HPLC 2 conditions. Each standard solution was run 5 times with fluorescence detection. These calibration curves showed reasonable linearity (see chapter 3, section 3.7). For the purposes of quantification 10ppb response factors for each PAH were used, except for dibenz(ah)anthracene and benzo(ghi)perylene where 100ppb response factors were used as they were not detectable at the 10ppb concentration range, (see chapter 3, section 3.7).

See Appendix A for the calibration curves.

2.3 Soxhlet Extraction Method Development.

Materials:

PAH standard solution (B).
Glass fibre filters (Sigma No. 25), 25mm. diameter.
Stainless steel scissors and tweezers.
Heating mantle (Buchi)
Soxhlet extraction glassware.
Beakers, conical flasks, vials.
Cyclohexane, dichloromethane, methanol (Sigma, HPLC grade).
Rotary evaporator (Buchi)
Syringe filters (Ministart SRP 25, Sartorius, product no. 17576 K, 0.45μm)

A glass fibre filter was spiked with 1ml. standard solution (B) and solvent allowed to evaporate (see method development and validation section 3.3.3 for spiking procedure). The filter was cut into 3 strips using a stainless steel tweezers and scissors and placed in a glass soxhlet extraction thimble.

Cyclohexane (250mls) (3) and dichloromethane (250mls) (4) were both investigated as extraction solvents, each being tried at 8 and 12-hour extraction periods. The heat setting was such that gave an extraction cycle of 1 cycle every 4 minutes. Each extraction solvent and extraction period was repeated 3 times. The extract volume (250mls) was reduced to ca. 5mls via rotary evaporation. This extract volume of ca. 5mls was syringe filtered into a glass vial and reduced to dryness with a gentle stream of nitrogen gas, and reconstituted with methanol (1ml). This sample was analysed by HPLC under HPLC 1 conditions. The % recovery of PAH was determined by comparison with 1ppm standard responses (UV) of each of the 16 PAH.

2.4 Ultrasonic Extraction Method Development.

Materials:

Base bath (dilute potassium hydroxide)

Acid bath (dilute sulphuric acid)

Deionised water

Silanising agent (Sigma, dimethyldichlorosilane)

Toluene, methanol, acetonitrile (Sigma, HPLC grade)

Conical flasks

Beakers

Pasteur pipettes

PAH standard solution (B)

Glass fibre filters (Sigma no. 25) 25mm. diameter

Ultrasonic bath (Sonomatic, Langford Ultrasonics)

Rotary evaporator (Buchi)

2.4.1 Silanation of Glassware.

Each piece of glassware to be silanated was firstly left steeping in a base bath (KOH) overnight, and then in an acid bath (H_2SO_4) overnight. The glassware was then rinsed with deionised water and dried in an oven (100°C) for 1 hour.

Each vessel was coated with the silanation agent via pasteur pipette. The vessel was then rinsed twice with toluene and three times with methanol, until no foam remained in the rinses. The glassware was left to air dry overnight.

Each piece of glassware used in the ultrasonic extraction method development and the procedures subsequently was silanated.

2.4.2 Ultrasonic Extraction Procedure.

A glass fibre filter spiked with 1ml. standard solution (B) was cut in three, each piece placed in acetonitrile (50mls) and ultrasonically extracted for 15, 30, 60, 120 and 180mins (5). The extract volumes from each extraction time period were combined and reduced to ca. 5mls. via rotary evaporator. This extract volume was then reduced to dryness by flushing with a gentle stream of nitrogen gas, and reconstituted in methanol (1ml).

This sample was analysed by HPLC (under HPLC 1 conditions). The PAH recovered were quantified by the responses of standard solution (B) using UV detection. See chapter 3, section 3.4.6 for results.

2.4.3 Influence of Extraction Treatments on the Recovery.

A glass fibre filter spiked with 1ml standard solution (B) was sonicated for 60mins in 150mls acetonitrile. Another two spiked filters were cut in two and three and sonicated in 2 x 75mls and 3 x 50mls acetonitrile for 60mins.

Each extract volume was reduced as before and analysed by HPLC (HPLC 1 conditions) and quantified as before, see chapter 3, section 3.4.6 for results.

2.5 Solid Phase Extraction.

Materials:

Solid phase extraction tubes (Supelco, styrene/divinylbenzene copolymer resin, 500mgs, tube volume 6mls)

Vac-elute 10 port vacuum manifold (Varian)

Water pump (Buchi)

PAH standard solution (B) (in acetonitrile)

Acetonitrile, methanol, hexane, diisopropylether, toluene, benzene (Sigma, HPLC grade)

Syringe filters (Sartorious, polypropylene housing, cellulose acetate filters)

All glassware used was silanated.

2.5.1 Calibration of Vac-Elute Flowrate.

Acetonitrile (2mls) was drawn through the SPE tube at a pressure reading 135mm mercury. The acetonitrile was drawn through the resin in 48 seconds, giving a flow rate of 2.5ml/min. The pressure was adjusted to 81mm mercury, and 2mls acetonitrile was drawn through the resin in 79 seconds, giving a flow rate of 1.5ml/min. This was repeated with a pressure reading of 54mm mercury, whereby it took 121 seconds for 2mls acetonitrile to be drawn through the resin, giving a flow rate of 1.0ml/min.

2.5.2 Optimisation of Retention of Standards.

A SPE tube was solvated with acetonitrile (4mls), flowrate 1.5ml/min. A 1ml aliquot of PAH standard solution (B) was added via a pasteur pipette. The sample fraction collected was reduced to dryness with nitrogen gas, reconstituted in acetonitrile and analysed under HPLC 1 conditions. See chapter 3, section 3.5.5 for reselts. The same procedure was carried out at a flow-rate of 1.0ml/min, which proved to give the optimum retention conditions.

2.5.3 Washing Stage.

Methanol (2ml) was added to the SPE tube, (previously conditioned and sample applied) (6), at a flow-rate of 1.0ml/min. The resultant fraction was collected and analysed by HPLC as before in order to detect any PAH that may have eluted.

A drying stage followed the washing stage. This was achieved by drawing air through the cartridge for 10mins. at a flow-rate of 1.0ml/min.

2.5.4 Optimisation of Elution.

Hexane (2mls) was added to the SPE tube after the drying stage. The resultant eluant was reduced to dryness with nitrogen gas, reconstituted in acetonitrile (1ml) and analysed by HPLC as before. This procedure was carried out with diisopropylether, toluene and benzene, each solvent being replicated 3 times.

As the results were not satisfactory the drying stage was increased to 20mins. at a rate of 1.0ml/min. Toluene was then tried again as the elution solvent, with satisfactory results, this was replicated 3 times. See chapter 3, section 3.5.7.

2.6 Complete Method Reproducibilities.

Run-to-run reproducibilities of the complete method were carried out.
A glass fibre filter spiked with 1ml of standard solution (B) was cut into three and sonicated in 3 x 50mls acetonitrile for 60mins. These extract volumes were combined and reduced to ca. 5mls via rotary evaporation. This volume was syringe filtered and reduced to dryness with a gentle stream of nitrogen gas, then reconstituted with 1ml acetonitrile followed by analysis under

HPLC 1 conditions. This was carried out three times on one day to establish run-to-run reproducibility. The procedure was carried out over three days, to establish day-to-day reproducibility of the complete method. See chapter 3 section 3.6.

2.7 Environmental samples.

A previously weighed glass fibre filter was carefully placed via a tweezers in the sampling head (particle size 4.5μm or 10μm) of the air sampler, (sampling equipment details described in chapter 4, section 4.6). The air sampler was placed at the sampling site (see chapter 4, sections 4.9 and 4.11) for the period of time required.

After sampling the filter was, conditioned for 1 hour at room temperature. The filter was then analysed by the method described in chapter 3 figure 3.17 with fluorescence detection. The PAH detected were identified by their retention times and quantified with responses factors of standard solution (D) (10ppb).

An environmental sample was analysed by GC-MS subsequent to its HPLC analysis. This was another method of unambiguous identification of the individual PAH peaks assigned from the HPLC analysis method.

The GC-MS (Hewlett Packard) conditions were as follows (7):

Column: SPB-5 capillary, 30m. x 0.25mm ID, 0.25μm film
Oven: 100°C (4min) to 310°C at 8°C/min., hold
Carrier: Helium, 20cm/sec.

PAH standard solution (C) was run on the GC. The mass spectra for each PAH were obtained and compared to the mass spectra of the environmental samples. (See section 4.15).

2.8 References.

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Chapter 3

Method Development and Validation.

3.1 Analytical Method Validation.

Method validation is carried out to ensure that an analytical method is accurate, specific, reproducible and rugged over the specified range in which an analyte will be analysed. Method validation provides an assurance of reliability during normal use. Sometimes method validation is referred to as "the process of providing documented evidence that the method does what it is intended to do"(1). There is no single prescribed validation procedure for a method, different performance characteristics and evaluation procedures have to be addressed. The exact validation procedure adopted depends on the end purpose of the method i.e. the validation procedure is "customised". This research was concerned with developing a method for the analysis of PAH in urban particulates. The next section gives a brief overview of the method to be validated.

3.2 Outline of the Method to be Validated.

PAH are monitored in the air by use of an air sampler. The PAH are trapped on the glass fibre filters from a known volume of air drawn through the sampling head of the air sampler. The filter is then extracted for the PAH in an extraction solvent (stage 1) (see section 3.4.5 and 3.4.6 for method development of extraction procedure). Volume reduction via rotary evaporation and sample clean up by use of solid phase extraction then takes place (stage 2) (see section 3.5.2 for method development of solid phase extraction procedure). The method of analysis is HPLC with gradient elution, (see chapter 2, section 2.2), and detection by UV (254 nm.) and fluorescence spectrometry (programmable wavelength change with time).

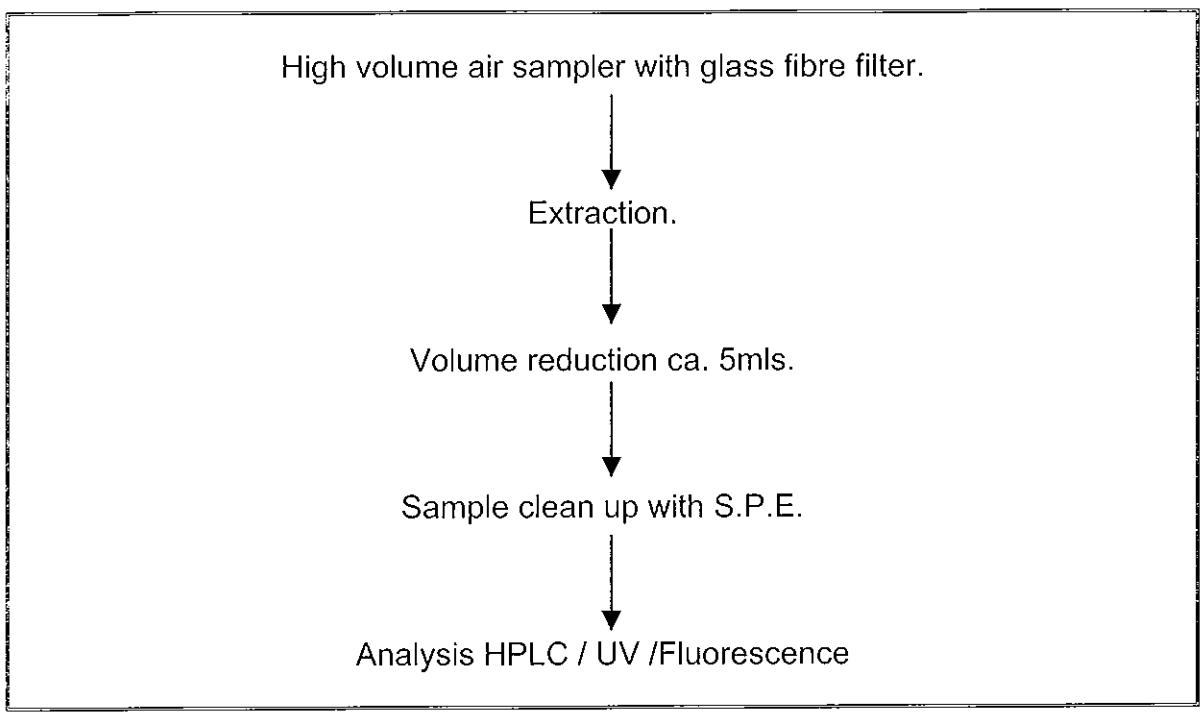


Figure 3.1: Outline of the analytical method to be validated.

Each initial investigation was sourced from the related literature, but modified and adapted for this research. Before any analytical method can be put in use certain performance characteristics must be considered.

3.3 Performance Characteristics.

Performance characteristics evaluate the method to be validated. Each of these will be discussed in the next sections.

3.3.1 Accuracy and Precision:

The accuracy of an assay method is defined as the closeness of the assay result obtained by the assay method. Statisticians usually refer to systematic error as bias. Accuracy is the parameter that measures the exactness of an assay

method. In practice, the accuracy of an assay method is usually determined based on data obtained from experiments. The precision of an assay method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple sampling of a homogeneous sample. Standard deviation (SD) and/or coefficient of variation (CV) or relative standard deviation (RSD) in percentage are generally used to represent the precision of an assay method.

Precision is an assay parameter that measures the degree of repeatability of the assay method from the same population under a normal operating circumstance. It can be used to measure the variation between two repeated assays within the homogeneous preparations (i.e. same lot or same day). Assays in context of precision are independent analyses of samples that have been carried through the complete analytical procedure from sample preparation to final test result. The SD and CV can also be estimated from the recovery amounts of the recovery study.

In practice, for the evaluation of accuracy and precision of an assay method, the assay method is considered validated if its accuracy and precision are within acceptable limits. For example, for accuracy, if the bias is not significant from zero and is within a specified (δ %) of input at each level with 95% assurance, the accuracy of the assay method is considered validated. For precision, if the total variation gives a total CV less than a specified Δ%, the precision of the assay method is considered validated. The bias of an assay method is the difference between the average recovered amount and the actual amount added. Where the method has no bias, accuracy becomes equal to precision. Good precision is necessary for good accuracy, however, good precision can never be used as an indication of good accuracy.

The USEPA Method 610 requires that the repeatability be ±10% (2). Recovery values and relative standard deviations are obtained for three replicate analyses of each compound in order to establish the run-to-run reproducibility of the overall method. Duplicate analyses are carried out on three different days in order to determinate the day-to-day reproducibility of the method (3).

3.3.2 Limit of Detection (LOD) and Quantitation.

The limit of detection is defined as the lowest concentration of an analyte in a sample that can be detected, but not necessarily quantitated, under the experimental conditions specified. It is a parameter of limit tests because the limit of detection merely substantiates that analyte concentration is above or below a certain level. The LOD is usually expressed as the concentration of analyte in the sample at a specified signal-to-noise ratio, usually two- or three-to-one. The LOD can also be calculated based on the standard deviation (SD) of the response and the slope (S) of the calibration curve at levels approximating LOD according to the formula,

$$\mathbf{LOD = 3.3\ (SD/S).}$$

The SD of the response can be determined based on the SD of the blank, on the residual SD of the regression line, or the SD of the y intercepts of the regression lines.

The limit of quantitation (LOQ) is the lowest concentration of analyte in the sample that can be determined with acceptable precision and accuracy under the experimental conditions specified. It is a parameter of quantitative assays for low levels of compounds in sample matrices. Like LOD, LOQ is expressed as a concentration, and the precision and accuracy of measurement are also reported (1). Sometimes a signal-to-noise ratio of 10:1 is used to determine the LOQ (1).

This signal-to-noise ratio is a good rule of thumb, but one should remember that the determination of LOQ is a compromise between the concentration and the required precision and accuracy. That is, as the LOQ concentration level decreases, the precision decreases. If better precision is required, a higher concentration must be reported for LOQ (1). Whereas the establishment of LOD and LOQ is not always a requirement for method validation, it is essential for trace environmental analysis.

The USEPA Method 610 (PAH analysis for wastewater) lists LOD's for the 16 PAH. Table 3.1 shows the LOD's for this USEPA method and the quoted LOD's of the fluorescence detector (Varian, Model 9050) (4). It is interesting to note, but perhaps not surprising, that the manufacturers LOD's are significantly lower than the USEPA limits.

3.3.3 Linearity and Range

The linearity of an assay method is defined as the ability to elicit assay results, such as peak area absorptivity, that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range (1). This parameter can also to be used to determine the effect of a nonzero intercept on assay calibration, which assumes a calibration line passing through the origin. The simple linear regression analysis is usually employed to evaluate the linearity over a given range (1).

The range of an assay method is the interval between the upper and lower levels (inclusive) of analyte that have been determined with accuracy, precision, and linearity using the method as written. The range is usually expressed in the same unit as assay results obtained by the assay method. Hence the range of the assay method is verified by the fact that the assay method provides

acceptable accuracy, precision and linearity not only to samples within the range but also to samples at the extremes of the range.

3.3.4 Selectivity and Ruggedness.

The selectivity (or specificity) is usually referred to as the ability of an assay method to measure the analyte accurately and specifically in the presence of components that may be expected to be present in the sample matrix (1). Selectivity is often expressed as the difference in assay results obtained by the analysis of samples containing added impurities. Hence selectivity is a measure of the degree of interference in analysis of complex sample matrix. An acceptable assay method should be free of any significant interference by substances known to be present in the sample.

Ruggedness is a measure of reproducibility of assay results under normal expected operating conditions, from day to day, analyst to analyst or laboratory to laboratory (1). Hence the reproducibility is sometimes referred to as the variation among assay results obtained from different environmental settings, such as different days, laboratories or analysts.

Compound	USEPA(μg/l)	Fluor. (μg/l)
Naph.	1.8	0.27*
Aceny.	2.3	0.27*
Acen.	1.8	0.31*
F.	0.21	0.02*
Phen.	0.64	0.003
Anth.	0.66	0.012
Fl.	0.21	0.028
Py.	0.27	0.083
B(a)Anth.	0.013	0.0011
Chrys.	0.15	0.0021
B(b)Fl.	0.18	0.00075
B(k)Fl.	0.17	0.00012
B(a)Py.	0.23	0.00079
Dibenz	0.03	0.04
Benzo	0.076	0.016
Indeno.	0.043	0.0018

Table 3.1. Limits of Detection of the 16 PAH for the USEPA method and the Varian fluorescence detector (4).

* indicates detection by UV

3.4 Method Development Stage 1 –Extraction

There are a number of extraction procedures that can be employed to recover airborne particulate PAH from the glass fibre filter after sampling. Some of the extraction methods in the literature did not include the smaller 2-3 ringed PAH, naphthalene, acenaphthylene, acenaphthene and fluorene. This research was also not as concerned with these PAH, as their potency as carcinogens is not as great as the remaining PAH on the USEPA method 610 list.

The next section discusses the main types of extraction procedures commonly used for PAH isolation e.g., pure solvent extraction, microwave extraction, soxhlet extraction and ultrasonic extraction. This research concentrated on the soxhlet and ultrasonic extraction procedures. As the soxhlet extraction results will show different extraction solvents and extraction times were investigated with little success in recoveries of standard concentrations of the PAH. The ultrasonic extraction procedure was successful for recovery of standard concentrations of the PAH and was investigated further to optimise the recoveries.

3.4.1 Pure Solvent Extraction:

Pure solvent extraction is referred to as an extraction of sample with solvent by heating or agitating in one particular vessel. This is a simple and least instrumentally demanding of the extraction methods mentioned in this chapter. A typical extraction involves mixing the sample with a volume of an extraction solvent, followed by an equilibration period of a few days, then passing the extract through a water -removing column followed by concentration and analysis (5). This extraction method yields recoveries in the same range as that of soxhlet. This technique, although it is simple and low in cost, has a number of general drawbacks. The solutes will maintain some equilibrium between the sample and the solvent. Unless this equilibrium strongly favours the solvent, a certain portion of the PAH will adhere to the sample. As the PAH are not removed from the

sample matrix, degradation and decomposition of compounds are possible. Large quantities of solvent are required for extraction and the method is very time consuming.

3.4.2 Microwave Extraction:

Microwave extraction has been used for many years for the digestion of environmental and biological samples in inorganic analysis. The use of microwave energy to aid organic extraction was first achieved using conventional household appliances in the late 1980's (6). Only recently have commercial microwave systems become available which are specifically designed for organic analysis and this has renewed interest in the technique as a genuine alternative to conventional solvent extraction. With microwave assisted extraction rapid heating of the solvent occurs at a temperature above its normal boiling point (in closed vessel extraction), this allows the extraction of samples in minutes as opposed to hours. The commercial instruments have built-in safety procedures, which are required when using organic solvents at high temperatures and pressures. While this extraction technique is quick, and low on solvent use, unfortunately access to this type of instrumentation was not available for this work.

3.4.3 Soxhlet Extraction:

As a development of ordinary solvent extraction, soxhlet extraction was introduced by Franz von Soxhlet, Professor of Agricultural Chemistry in Munich, 1879-1913 (5). Soxhlet extraction is performed in a relatively inexpensive all-glass device, forcing heated distilled solvent to pass through the sample over a number of cycles. The yield is dependent on the solvation power of the solvent, the morphology of the sample and the number of extraction cycles used. Soxhlet extraction is still one of the most efficient methods in use but is considered a comparatively slow technique. Thus, it is regarded as the main time consuming step in an analytical scheme (sampling not included).

3.4.4 Ultrasonic Extraction:

Acoustic vibrations with frequencies above 20kHz, i.e. above the range capable of being detected by the human ear, are called ultrasonic vibrations. These vibrations can be used to generate cavitation in solvents. Small bubbles of solvent vapour are produced and on collapsing, a shock wave results which can be used in order to mechanically remove particles and chemical compounds from adhesive surfaces and sites. If this is used in conjunction with a solvent in which the compounds to be extracted are highly soluble, the rate of desorption of the compounds from the sample matrix is highly accelerated. One of the main advantages of this extraction technique over soxhlet extraction is that the extraction time is considerably faster.

The next section traces the method development of the soxhlet extraction procedure. Spiking filters with known amounts of standard PAH assessed extraction efficiency. Naphthalene, acenaphthylene, acenaphthene and fluorene were not detected using soxhlet extraction.

3.4.5 Method Development Using the Soxhlet Extraction Procedure.

Soxhlet extraction recovery of PAH from a wet sediment was carried out by Lee et al. (7) with different extraction solvent systems. Recoveries are given in table 3.2, with cyclohexane giving the best recoveries. Lee et al. did not include naphthalene, acenaphthylene, acenaphthene or fluorene in their study due to their volatility compared to the other PAH of interest. The investigation of dichloromethane as an extraction solvent was studied as it is the EPA

Compound	Soxhlet extraction recoveries ($\mu g/g^{-1}$), with different extraction solvents				
	Standard spike concentration ($\mu g/g^{-1}$)	Cyclohexane 8 hours	Hexane 8 hours	Hexane/Acetone (41:59) 8 hours	Benzene/Methanol (1:1) 8 hours
Phen	16.57	14.7	15.9	15.9	16.0
Anth	3.92	1.2	1.3	1.3	1.7
Fl	23.45	23.3	22.7	22.2	21.5
Py	18.42	15.8	16.8	17.2	15.1
B(a)anth	8.41	8.2	8.0	8.5	8.3
Chrys	13.7	8.2	7.5	8.4	8.3
B(b)Fl	8.08	8.3	7.8	9.0	7.9
B(k)Fl	5.58	5.2	4.0	4.2	4.4
B(a)Py	6.58	5.1	4.9	5.0	4.8
Dibenz(ah)	3.62	1.5	2.9	5.2	5.2
Benzo(ghi)	7.32	4.8	1.3	1.4	0.8
Indeno(123)	5.10	5.3	2.9	4.3	2.6

Table 3.2. Literature soxhlet extraction recoveries (7) of PAH in sediment. The soxhlet extractions were carried out with different extraction solvents.

recommended solvent for the determination of benzo(a)pyrene and other PAH in ambient air (8). No spiked standard recoveries are quoted in this paper. Spiking is a troubleshooting technique whereby a known amount of standard analyte is introduced to the procedure under investigation for the purposes of assessing the recovery efficiency of the procedure.

A glass fibre filter (Sigma Aldrich, No. 25) 25mm. diameter was spiked with a standard concentration, 1ml of 1ppm., of the sixteen PAH of interest (Supelco, USEPA 610 PAH mix , Lot no.-LA-63573). The spiking procedure was 10 x 100μl aliquots of the PAH standard, delivered via a calibrated micro syringe (Hamilton 100μl. syringe). As each aliquot was added (two at a time) the solvent (methanol) was allowed to evaporate. When the ten aliquots were added, the filter was cut into three strips using a tweezers and a stainless steel scissors in order for the filter to fit into the soxhlet extraction chamber.

Different extraction solvents and times were used to investigate which of these variables would give optimum recoveries. Cyclohexane, 250mls (7) and dichloromethane, 250mls (8) were both investigated with extraction times of 8 and 12 hours. These times were chosen, as they are typical of the times required for efficient extraction recovery (5). The extract volume was reduced via rotary evaporation to ca. 5mls, reduced to dryness by gentle flushing with N_2 gas and reconstituted with 1ml methanol and analysed by HPLC (see experimental, section 4.2, for conditions).

.

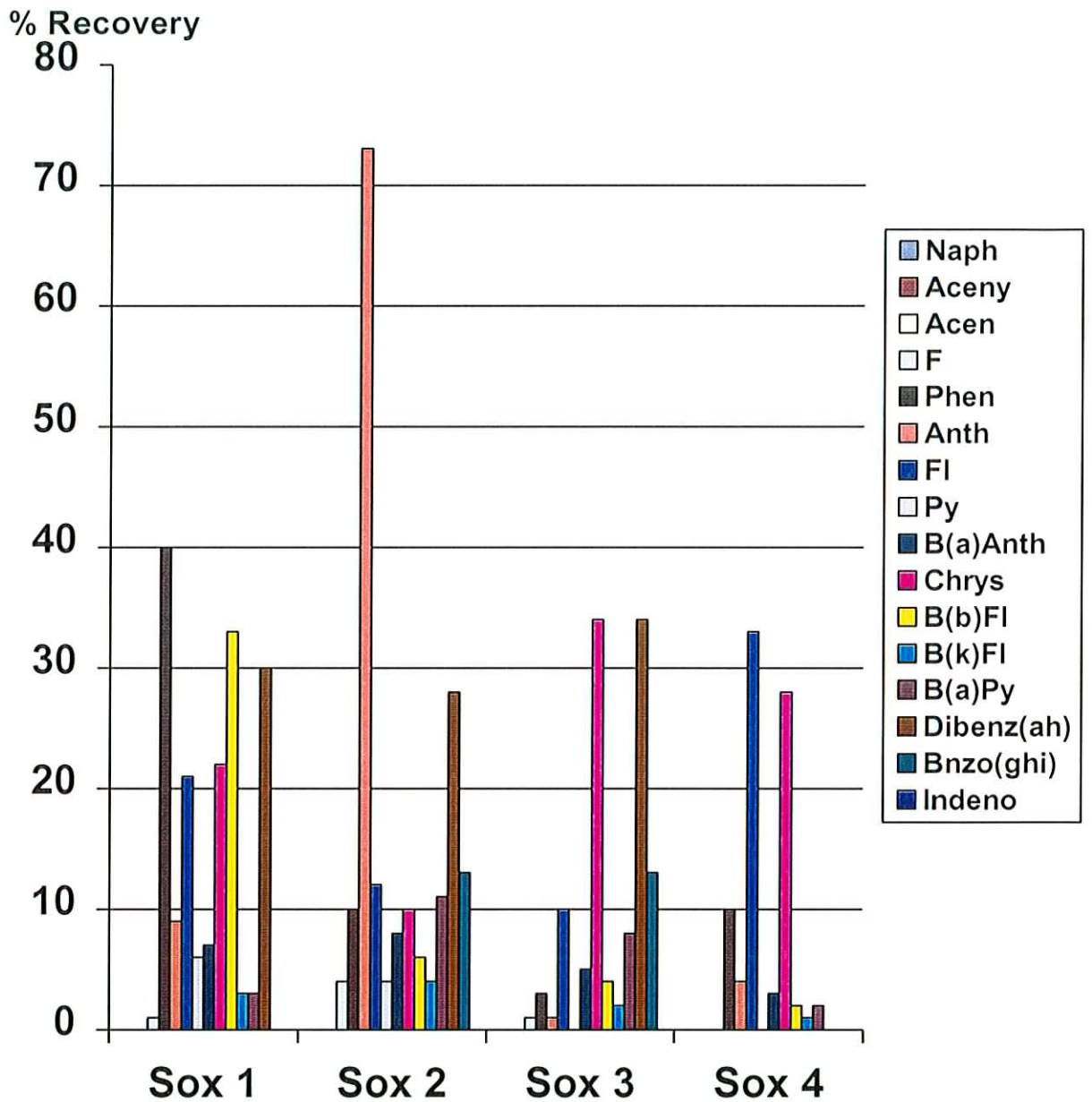
Compound	Soxhlet Extraction Recoveries (%) and RSD(%) (n=3)			
	Cyclohexane 8 hours	Cyclohexane 12 hours	Dichloromethane 8 hours	Dichloromethane 12 hours
Naph	nd	nd	nd	nd
Aceny	nd	nd	nd	nd
Acen	nd	nd	nd	nd
F	1 ±31%	4 ±72%	1 ±58%	1 ±0%*
Phen	39 ±66%	10 ±5%	3 ±0%*	10 ±0%*
Anth	8 ±40%	73 ±84%	1 ±25%	4 ±91%
Fl	21 ±75%	12 ±69%	10.±0%	33 ±89%
Py	6 ±84%	4 ±66%	0.3 ±0%	0.3 ±20%
B(a)anth	7 ±78%	8 ±68%	5 ±87%	3 ±75%
Chrys	22 ±75%	10 ±86%	34 ±41%	28 ±93%
B(b)Fl	33 ±82%	6 ±52%	4.±69%	2 ±0%*
B(k)Fl	3 ±77%	4.±67%	2.±56%	0.6 ±6%
B(a)Py	3 ±51%	11.±0%*	8.±32%	2 ±0%*
Dibenz(ah)	30.±23%	28 ±0%*	34 ±24%	nd
Benzo(ghi)	nd	13 ±0%*	13 ±29%	nd
Indeno(123)	nd	nd	nd	nd

Table 3.3. Soxhlet Extraction Recoveries (%), with different extraction solvents and different extraction times, with RSD(%) (n=3).

* A 0%RSD indicates that compound was only recovered in one of the three replicates.

The results of the recoveries with different extraction solvents and times are shown in table 3.3, given as % average recoveries for three extractions.
The recoveries were low and non-reproducible for both cyclohexane and dichloromethane at the times of 8 and 12 hours investigated. Each solvent and extraction time was replicated 3 times. The % RSD's were very high, up to 90% for some compounds. Although soxhlet extraction is widely used it has a number of disadvantages in that it is very time consuming and yields large extract volumes.

Figure 3.2. Average Soxhlet Extraction Recoveries with Different Extraction Solvents and Times, n=3.



Sox 1 = cyclohexane 8 hours
Sox 2 = cyclohexane 12 hours
Sox 3 = acetonitrile 8 hours
Sox 4 = acetonitrile 12 hours

Another factor that should be taken into account is the affinity of PAH to adhere to glass. Silanation (see experimental) of the glassware is a technique which coats the inside of a glass vessel with a silanation agent occupying the active sites of the glass and blocking adhesion of the PAH to the glass. The soxhlet apparatus is not easily silanated due to design, i.e parts of the extraction chamber are not very accessible. Due to the poor recoveries of the PAH with soxhlet extraction, another method of extraction was investigated, namely ultrasonic extraction (8). The next section traces the method development of the ultrasonic extraction procedure. As with the soxhlet extraction, ultrasonic extraction efficiency was assessed by spiking a filter with known amounts of standard PAH.

3.4.6 Method Development of the Ultrasonic Extraction Procedure

This silanation process was carried out on all the glassware associated with the ultrasonic extraction method, and all procedures following that. Naphthalene. acenaphthylene, acenaphthene and fluorene were not detected in the recovery analysis.

Manoli et al. (9) used acetonitrile as the extraction solvent as it combined high recovery rates for most PAH with its simplicity in use. Having selected the extraction technique and the solvent an attempt was made to optimise the conditions to achieve the highest efficiency with the smallest amount of solvent and extraction time. For this purpose the recoveries of PAH from spiked filters were determined using different extraction durations. The results of which are shown in table 3.4 and graphically shown in figures 3.3, 3.4, 3.5 and 3.6.

Highest recovery rates for almost all PAH were achieved, within 15 mins, see table 3.4. A slight decrease with the duration of sonication was apparent in some cases.

Compound	Extraction Duration				
	15 mins. Recovery(%)	30 mins. Recovery(%)	60 mins Recovery(%)	120 mins Recovery(%)	180 mins. Recovery(%)
Naph	nd	nd	nd	nd	nd
Aceny	nd	nd	nd	nd	nd
Acen	nd	nd	nd	nd	nd
F	40.4	38.2	36.4	32.0	68.2
Phen	71.7	64.2	69.6	69.1	71.9
Anth	76.6	74.2	72.5	70.0	73.0
Fl	82.2	77.7	80.2	76.5	80.7
Py	81.8	77.8	78.5	80.5	80.0
B(a)Anth	80.3	81.2	82.6	83.0	82.0
Chrys	96.0	92.6	97.2	98.7	98.5
B(b)Fl	86.8	88.0	86.0	84.8	85.2
B(k)Fl	101.8	97.5	110.5	109.2	104.2
B(a)Py	89.2	87.6	86.2	85.1	84.8
Dibenz	77.4	76.8	76.0	74.2	73.2
Benzo	77.5	76.1	75.6	74.9	75.2
Indeno	60.2	93.2	92.0	58.6	59.2

Table 3.4. Recoveries (%), from 1ppm loaded filter of each compound from different ultrasonic extraction duration's.

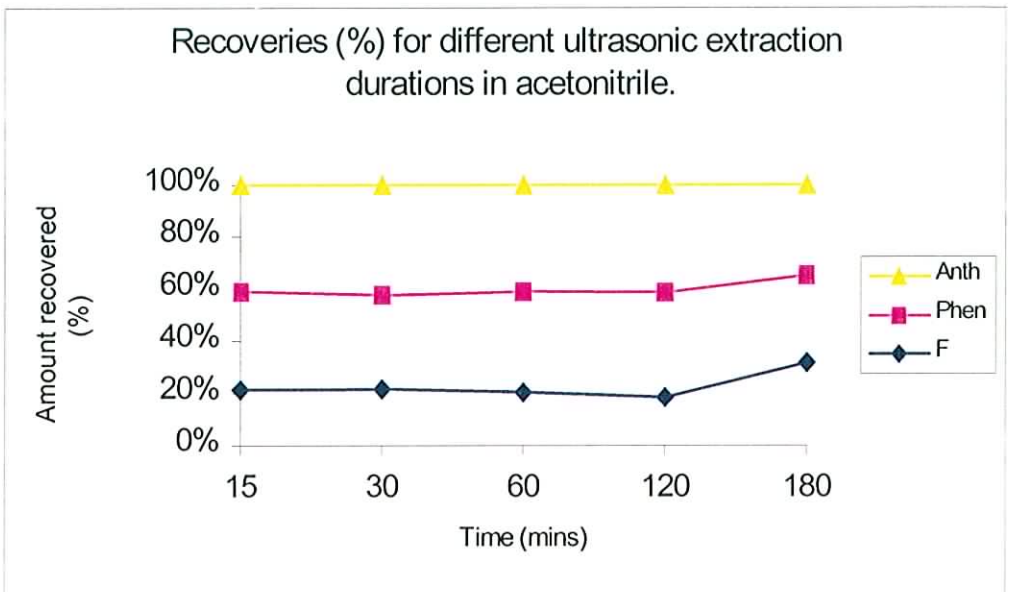


Figure 3.3

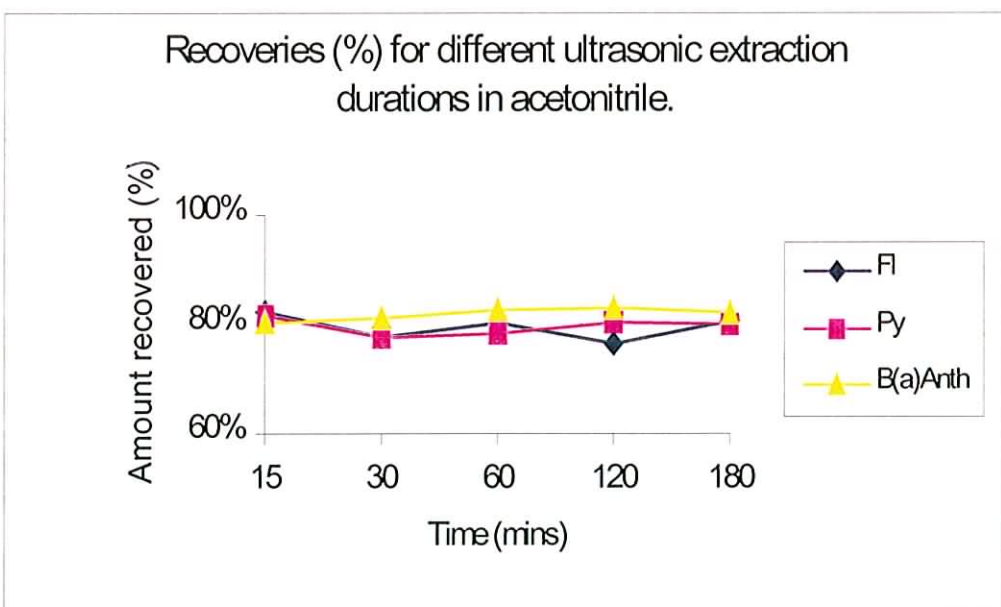


Figure 3.4

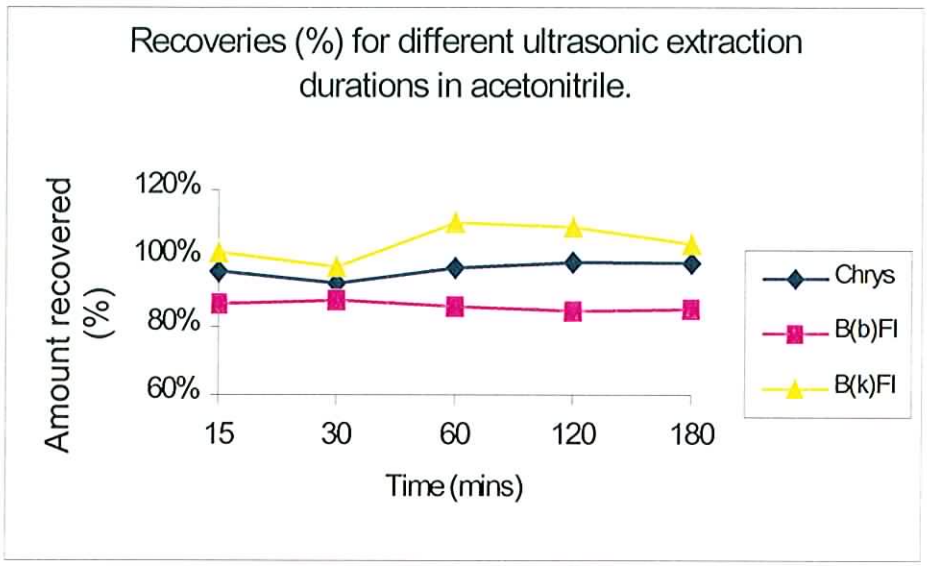


Figure 3.5

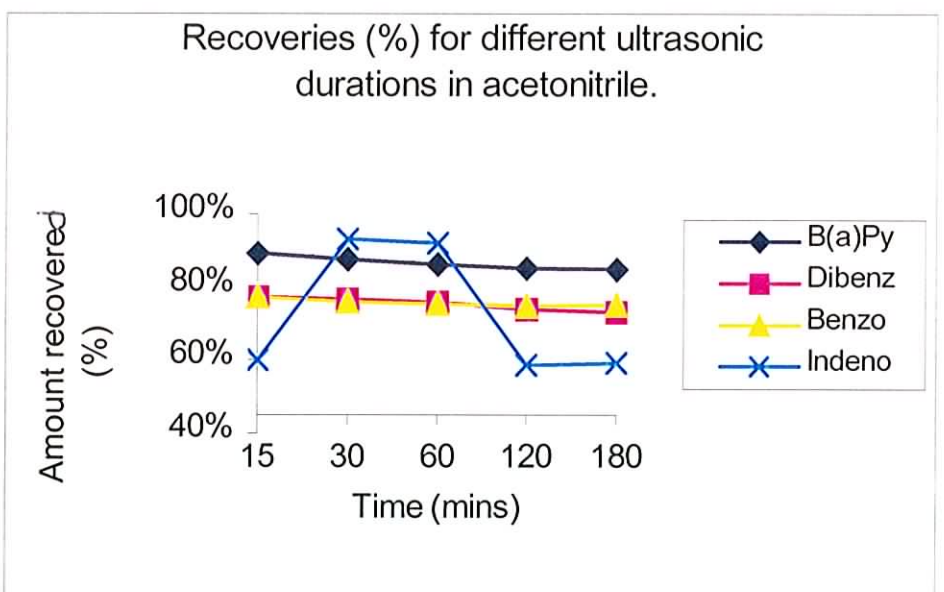


Figure 3.6

An exception was observed for indeno(123-cd)pyrene and fluorene that showed highest extraction rates after 30 and 180mins of sonication, respectively.

The extraction efficiency was examined with respect to the number of the extraction treatments. Experiments were performed with a total of 150mls. acetonitrile where the filter was treated whole (1 x 150mls), cut in two (2 x 75mls), and cut in three (3 x 50mls) respectively. The total extraction time was 60mins. The results are illustrated in table 3.5, and graphically displayed in figures 3.7, 3.8, 3.9 and 3.10.

There is very little difference between the extraction treatments, except that the amount of fluorene recovered is greatest, 41% with the 3 x 50ml treatment, compared to 20% (1 x 150ml treatment) and 28% (2 x 75ml treatment). This procedure was investigated for its repeatability, the results of which are illustrated in table 3.6.

The recovery rates of the detected PAH are acceptable, as the USEPA quote analytical recoveries of 50-150% (2). The repeatability (run-to-run) is also acceptable for all the detected PAH (RSD between 2 and 6%). Taking these factors into account ultrasonic extraction for 1 hour in 3 x 50ml acetonitrile was adopted as the more efficient extraction procedure due to its efficient and repeatable recovery rates.

Reported recoveries for the USEPA Method 610 are shown in table 3.7. These recoveries are high and the standard deviations are all below 10%, with one exception, benzo(b)fluoranthene (recovery 97%), which was 12.9%, compared with 87±3% recovery for benzo(b)fluoranthene with the ultrasonic extraction method (see table 3.6). It should be noted that the USEPA Method 610 recoveries of the PAH are from a waste water sample and they are representative of the overall method.

Compound	Influence of extraction treatments on the recovery		
	1 x 150 mls Recovery (%)	2 x 75 mls. Recovery (%)	3 x 50 mls. Recovery (%)
Naph	nd	nd	nd
Aceny	nd	nd	nd
Acen	nd	nd	nd
F	20	28	41
Phen	68	70	73
Anth	63	61	70
Fl	83	83	85
Py	87	87	86
B(a)anth	96	95	97
Chrys	97	97	98
B(b)Fl	83	82	86
B(k)Fl	99	98	102
B(a)Py	76	79	81
Dibenz(ah)	70	70	76
Benzo(ghi)	73	73	75
Indeno(123)	86	86	92

Table 3.5. Influence of extraction number on the recovery of PAH from glass fibre filters (sonication 60 mins.), recoveries (%) are from a loaded filter of 1ppm of each PAH

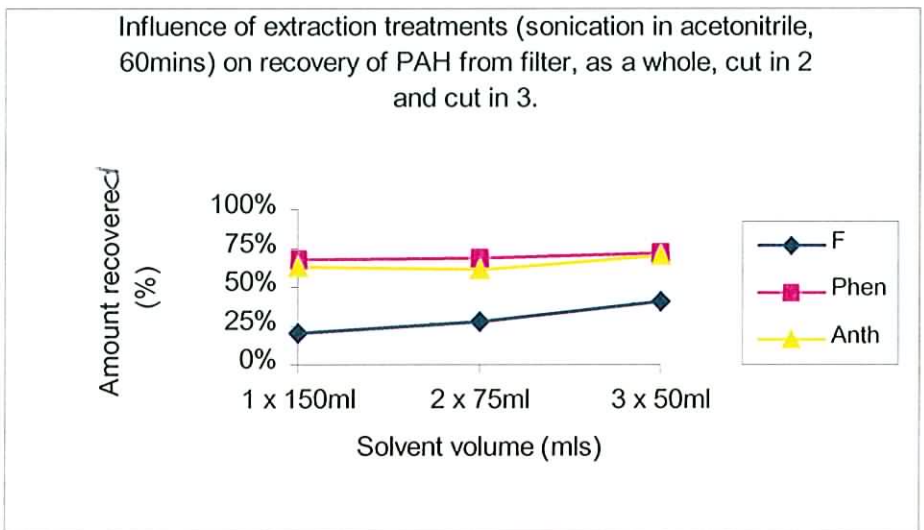


Figure 3.7.

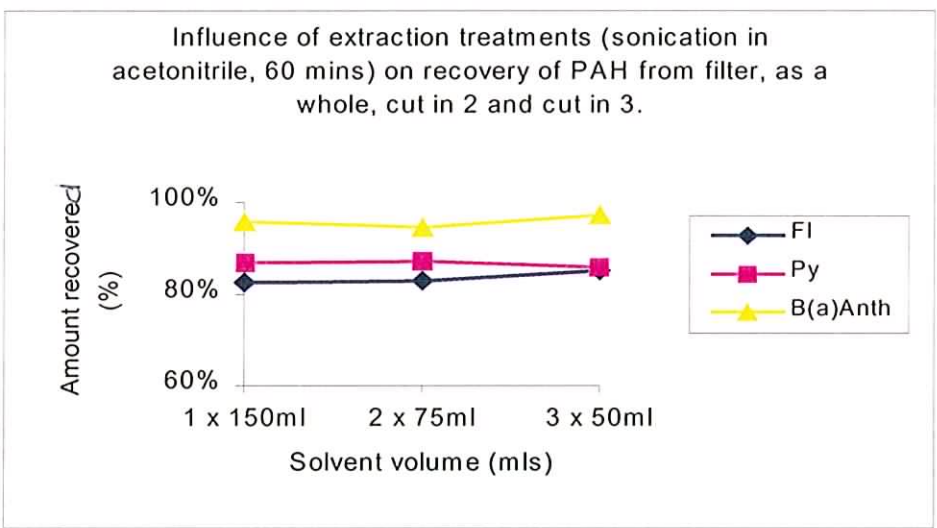


Figure 3.8.

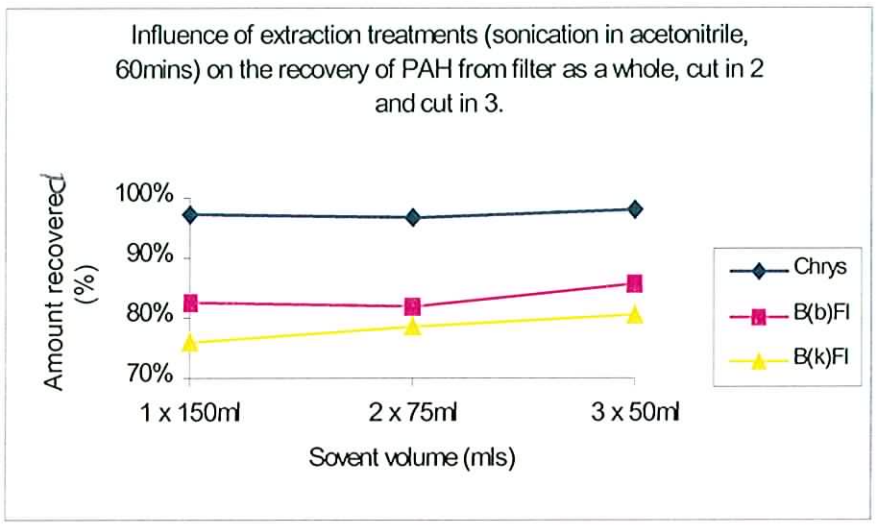


Figure 3.9.

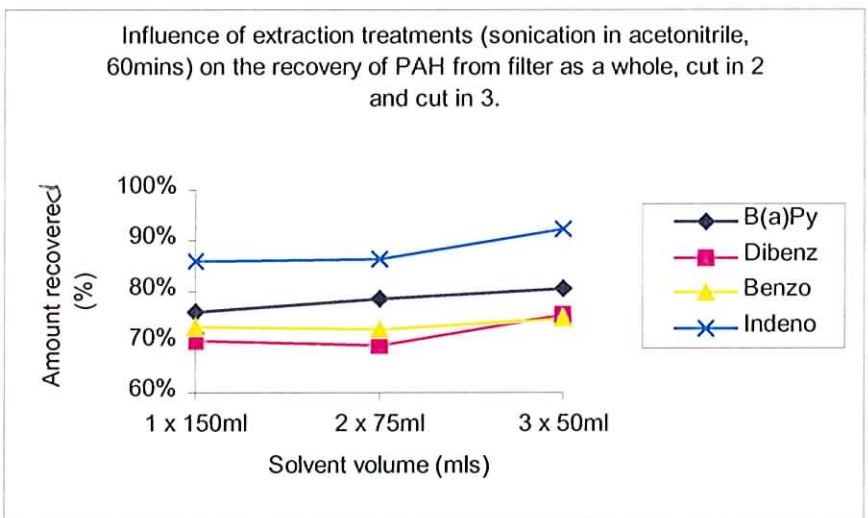
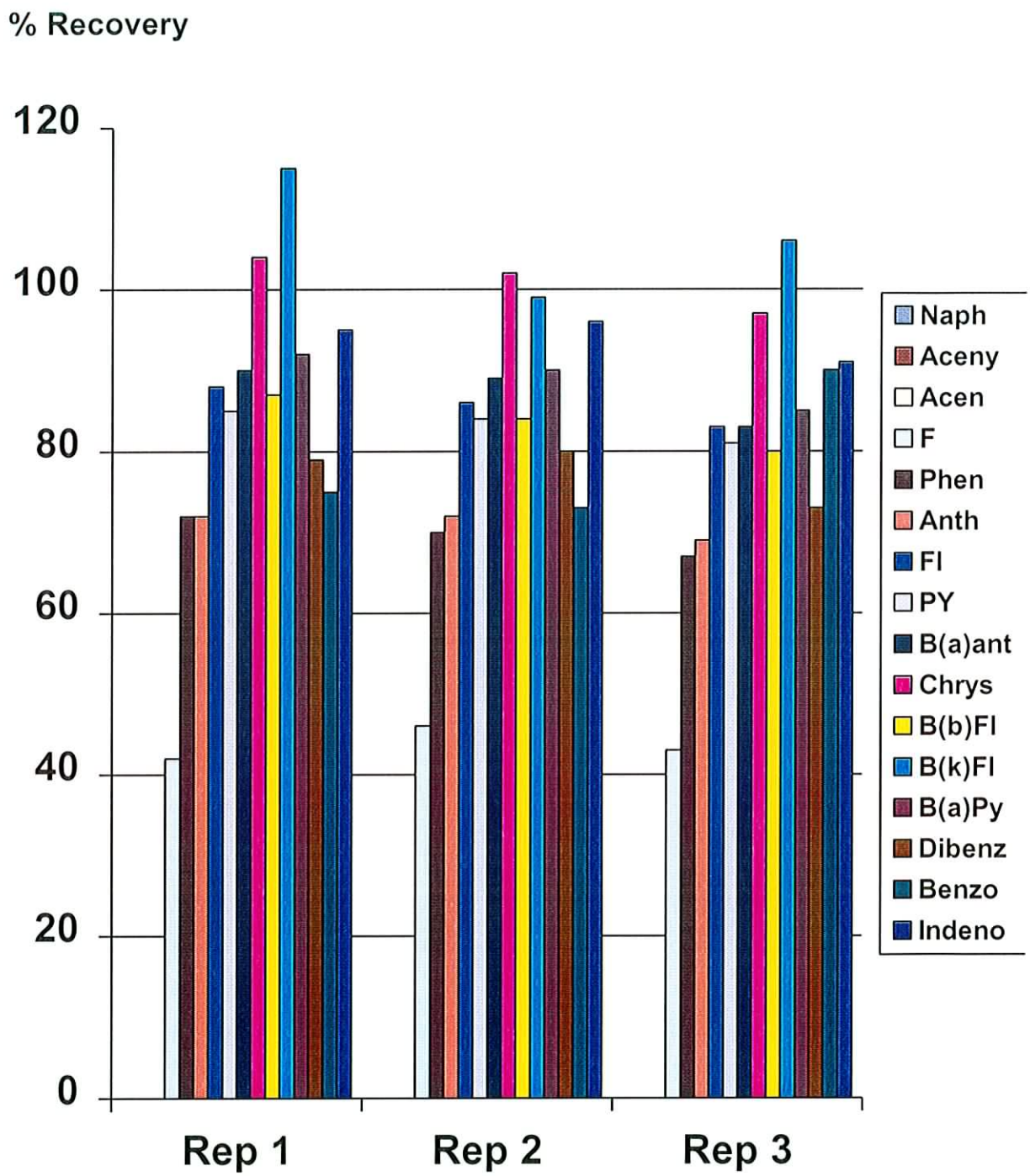


Figure 3.10.

Compound	Ultrasonic Extraction Recoveries (%) and Average Recoveries ± RSD(%).			
	Acetonitrile 1 hour	Acetonitrile 1 hour	Acetonitrile 1 hour	Average recovery ±RDS
Naph	nd	nd	nd	nd
Aceny	nd	nd	nd	nd
Acen	nd	nd	nd	nd
F	42	46	43	44 ±4%
Phen	72	70	67	70 ±3%
Anth	72	72	69	71 ±2%
Fl	88	86	83	86 ±2%
Py	85	84	81	83 ±2%
B(a)anth	90	89	83	87 ±3%
Chrys	104	102	97	101 ±3%
B(b)Fl	87	84	80	84 ±3%
B(k)Fl	115	99	106	107 ±6%
B(a)Py	92	90	85	89 ±3%
Dibenz(ah)	79	80	73	77 ±4%
Benzo(ghi)	75	73	90	79 ±9%
Indeno(123)	95	96	91	94 ±2%

Table 3.6. Ultrasonic extraction replicate recoveries (%) of PAH from spiked filter (1ppm) in acetonitrile (3 x 50mls) for 60mins.

Figure 3.11. Ultrasonic Recovery Replications of PAH from Spiked Filter, in Acetonitrile for 60mins.



Rep 1 = replicate 1

Compound	Average % Recovery	Standard deviation (%)	Spike range (μg/L)	Number of analyses	Matrix types
Naph	78	8.3	20-70	24	4
Aceny	93	6.4	250-450	24	4
Acen	88	5.7	11.6-25	24	4
F	90	7.9	6.1-23	24	4
Phen	98	8.4	3.8-5.0	24	4
Anth	93	6.3	7.9-11.3	24	4
Fl	116	9.7	0.3-2.2	24	4
Py	96	8.5	2.3-6.9	24	4
B(a)Anth	89	6.9	0.64-0.66	24	4
Chrys	88	9.0	2.0-6.8	24	4
B(b)Fl	97	12.9	0.24-0.30	24	4
B(K)Fl	94	9.5	0.14-6.2	24	4
B(a)Py	94	7.4	0.21-0.30	24	4
Dibenz	87	5.8	0.4-1.7	24	4
Benzo	86	7.3	0.42-3.4	24	4
Indeno	94	6.4	0.96-1.4	24	4

Table 3.7. USEPA complete method recoveries for 16 PAH in waste water (2).

3.5 Method Development Stage 2-Sample Clean Up.

The cleanup of an environmental sample before analysis by HPLC is very important. A dirty sample could destroy the functionality of the HPLC column, as well as causing detector fouling and blockages. Environmental sample matrices can be complex and inconsistent and require a good cleanup procedure to ensure detection of the analyte of interest with no loss of material.

Of the traditional sample preparation approaches, liquid-liquid extraction (LLE) is still widely used. It cleans up the sample as well as concentrating it. LLE is a labor intensive and often very slow technique. It requires large volumes of solvents, and the sample transfer steps and possible emulsion formation can result in sample loss, which affects analytical accuracy. Solid phase extraction (SPE) is a cleanup technique that is growing in popularity, especially because of its convenient ease of use and ready availability of SPE cartridges. The SPE cartridges used for most cleanup applications are C_{18} bonded silica phases. Cartridges with specially made phases can be bought for specialised applications.

SPE technology was introduced during the mid 1970's as an alternative to liquid-liquid extraction method of removal of matrix interferences and analyte concentration before analysis (10). SPE can be used as a combined sampling and cleanup method. The principle is to introduce a solvent with solutes that are to be analysed into a column with a packing material (sorbent). If the equilibrium of compounds, e.g. PAH, is strongly oriented towards the stationary phase, the solutes will be retained quantitatively by the solid phase. An initial separation will therefore be made, as all non-retained compounds will elute from the column. Further cleanup treatment with other solvents can be carried out in the same way. Finally, the compounds to be analysed are desorbed and eluted from the column by a solvent with higher solubility properties.

SPE has a number of advantages as a sample preparation cleanup technique. It has lower solvent reagent and apparatus costs; greater recoveries as a result of

fewer sample transfers, minimal evaporation, smaller volumes, and superior selectivity towards compounds of interest; and greater accuracy because there is less cross-contamination. The method is faster because it has fewer operational steps, batch processing can be utilised; it features a wide choice of available stationary phases for excellent selectivity; and it prolongs column lifetime because particulate matter and strongly retained compounds can be removed.

3.5.1 Solid Phase Extraction (SPE).

All SPE Cartridges are used in a similar manner. Figure 3.12 shows how SPE is performed if the compound of interest is to be isolated and interferences passed through the cartridge. Firstly, the cartridge must be conditioned ("solvated") with solvent (step 1). Failure to carry out this step usually results in nonreproducible sorbent behaviour. After conditioning the sample is passed through the column (step 2). At this point, the value of the sample solution flow rate is very important because sorption kinetics can be slow for some mechanisms, the result of which is lowered sample retention (11). The next step is to wash the cartridge with a solvent capable of desorbing interferences without desorbing the compound of interest (step 3). The final step (step 4) is elution of the compound of interest by a strong elution solvent. As this solvent is a strong nonpolar solvent for the compound of interest (PAH are nonpolar) bound on the cartridge column, it probably will be an unsuitable solvent for the HPLC column, which would require a more polar solvent to elute the analyte of interest by reverse phase chromatography. This solvent is reduced to dryness under a gentle stream of N_2 gas and the analyte reconstituted in a suitable solvent for HPLC analysis.

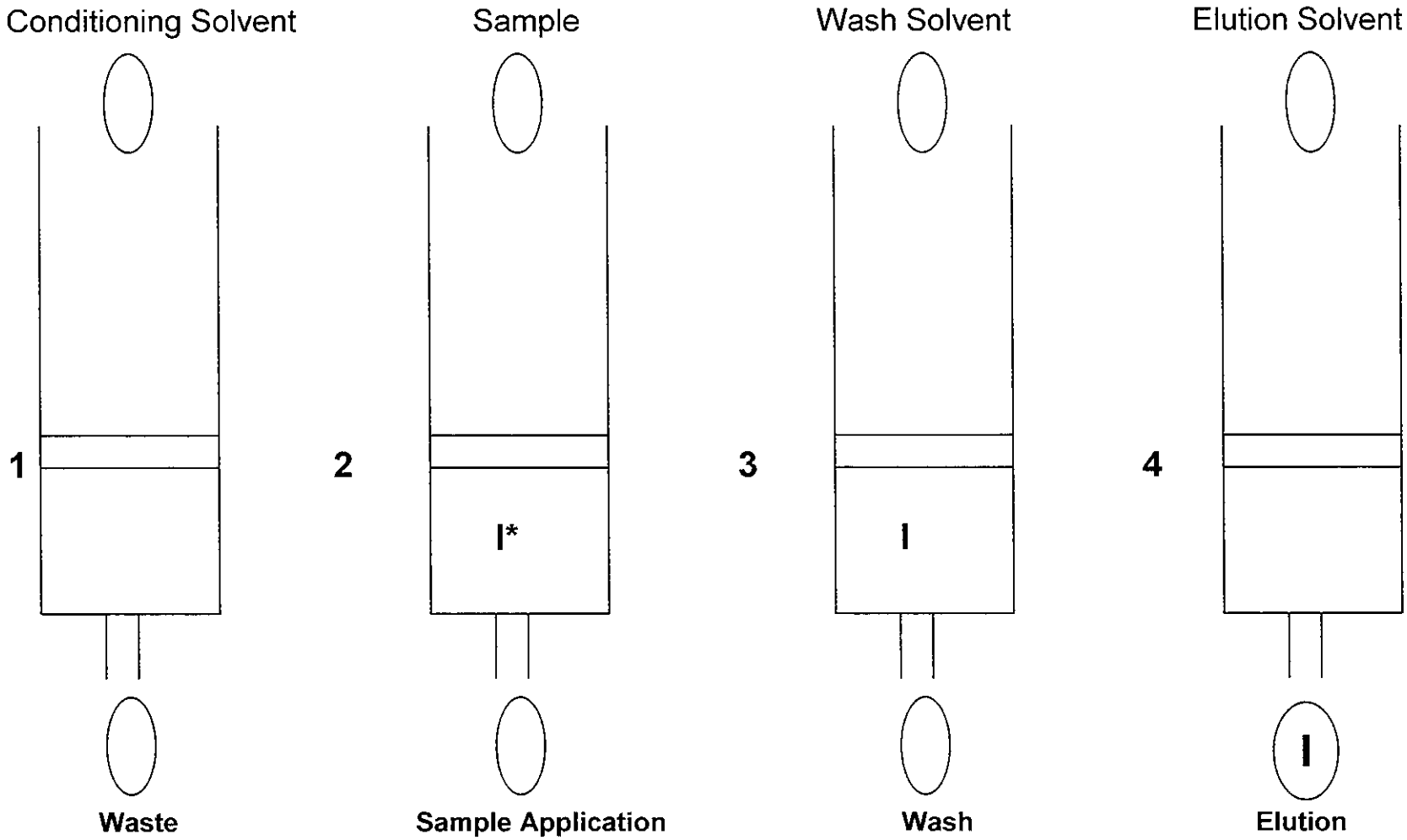


Figure 3.12 Steps in the operation of an SPE separation.
*** = sorption** **I = compound of interest**

The apparatus used for SPE is very simple. The principal component is a vacuum manifold with a custom cover in which the multiple cartridge columns can be accommodated (twelve ports in total). Inside the vacuum chamber is a removable stainless steel rack that is used to hold test tubes that the effluents will be collected in. A vacuum (through a water pump) is applied to pull sample or wash solvent through the cartridges. A flow control valve and pressure gauge are incorporated to allow better control of solvent flow rate. A side arm vacuum flask is connected between the vacuum manifold and the vacuum source. It serves to collect rinses and wash solvent. For this work a Vac-Elute vacuum manifold (Varian) was used.

3.5.2 Method development of the Solid Phase Extraction Procedure.

Development of a SPE method can be a lengthy process in which many solutions are generated for analysis. Prior to the practical work, however, choice of mechanisms and sorbents for the particular extraction problem must be made.

Method development involves three major steps:

1. Selection of extraction process and sorbent.

2. Sorbent testing

3. Method verification.

3.5.3 Selection of Extraction Mechanism and Sorbent.

PAH are nonpolar compounds, so a nonpolar extraction mechanism analogous to "reverse phase" chromatography was selected. Compounds with nonpolar functional groups (aromatic rings) can be extracted from more polar solutions using nonpolar solvents. A styrene / divinylbenzene co-polymer resin (Supelco, 500mgs, 6ml volume) was the sorbent selected (12). This resin is suited to the cleanup of environmental samples and the isolation of PAH.

The most probable interactive properties for undesired major constituents of the matrix must be considered. Properties shared by these components and the isolate indicate mechanisms that should be eliminated as the first step in extraction, because these interferences will compete with the isolate for the sorbent. These components are eliminated in a wash stage after the sample addition. Washing with a relatively polar solvent (methanol) will remove any polar compounds from the sorbent ensuring the nonpolar isolate of the sample is left behind on the sorbent.

Table 3.8 shows characteristic isolates, retention and elution solvents for nonpolar interactions in SPE.

Type of Interaction Solvents	Characteristic Isolates	Retention Solvents	Elution
Nonpolar	Compounds containing alkyl, aromatic, alicyclic or other functional groups with significant hydrocarbon structure. Most isolates exhibit nonpolar interactions	Water, organic buffers of low ionic strength (0.1M) Combinations (water/ methanol, water/ acetonitrile)	Organic solvents (methanol/aceto-nitrile,ethylacetete /THF, dichloro methane, hexane other nonpolar

Table 3.8 Characteristic isolates, retention and elution solvents for nonpolar interactions in SPE.

Many factors affect the choice of sorbent:

- Final solvent required from the extraction
- Isolate concentration desired in the final extract
- Isolate purity required

3.5.4 Sorbent Testing.

Selected sorbents must be tested for retention, elution, capacity and selectivity relative to the application.

There are three steps involved in this process:

1. Optimising the retention of standards.
2. Identification of wash solvents.
3. Optimising the elution of standards.

In order to optimise the retention of standards, columns must be prepared to receive the standard samples. They must be solvated by passing one to two column volumes of an appropriate solvent through them, wetting the sorbent and allowing its functional groups to interact with the solutions to be applied.

Solvation is a wetting of the sorbent creating an environment suitable to isolate retention. Solvation is necessary before the sorbent will react reproducibly with isolates.

Once solvated, the sorbent should not be allowed to become desolvated by excessive drying, specifically before sample application, or the sorbent has to be resolvated. The solvating solvent is usually that with which the sample to be analysed is constituted in, in this case acetonitrile. The column was solvated with one column volume (6 mls) of acetonitrile, before the addition of the standard sample.

3.5.5 Optimising the Retention of Standards.

After solvation of the column, the standard (1 ml., 1ppm.of each PAH) was added via a pasteur pipette. The sample is forced through under pressure. Initially a vacuum pressure of –7.5 Bar, giving a solution flow rate of 1.5 mls/min, was tried.

Absence of the isolate in the resultant fraction of the sample addition solvent (acetonitrile) indicates adequate retention of the standards. Table 3.9 shows the results obtained at this pressure, the fraction was analysed by HPLC (see experimental section 2.2 for conditions) where all the compounds were not retained. Therefore it was decided to lower the pressure to –5 Bar (giving a solution flow rate of 1ml/min) and the resultant fraction was analysed by HPLC, see table 3.10. At this rate of 1ml/min no isolate was detected in the fraction tested indicating 100% retention of each analyte by the sorbent.

As retention of the standard PAH have been achieved the next step is, identification of a suitable wash solvent.

3.5.6 Investigation of Wash Solvent

As PAH are nonpolar compounds, the polar contaminants are the ones easiest removed from the sample matrix. These contaminants are removed so as not to interfere with the analysis of the isolates of interest. Methanol was investigated as a wash solvent as it is polar, and would remove any polar contaminants, and when the resultant fraction was analysed (HPLC) all the PAH were absent.

A drying stage followed this washing stage, the flow rate of the air being drawn through remaining at 1 ml/min for 10 minutes. The drying stage is required because the wash solvent and the elution solvents to be investigated are

Compound	Sample addition (1ppm) (Rate = 1.5ml/min). n=3		
	Replicate 1 (ppm)	Replicate 2 (ppm)	Replicate 3 (ppm)
Naph	nd	nd	nd
Aceny	nd	nd	nd
Acen	nd	nd	nd
F	nd	nd	0.245
Phen	nd	0.022	nd
Anth	0.015	0.102	nd
Fl	nd	0.105	nd
Py	0.102	0.044	0.499
B(a)anth	nd	0.061	nd
Chrys	nd	0.069	nd
B(b)Fl	nd	0.024	nd
B(k)Fl	nd	0.581	0.282
B(a)Py	nd	nd	nd
Dibenz(ah)	nd	nd	nd
Benzo(ghi)	nd	nd	nd
Indeno(123)	nd	nd	nd

Table 3.9. Optimising sample retention on SPE cartridge. Sample (1ppm of each PAH) addition onto the SPE cartridge at a flow rate of 1.5ml/min.

Compound	Sample addition (1ppm) (Rate = 1.0ml/min). n=3		
	Replicate 1 (ppm)	Replicate 2 (ppm)	Replicate 3 (ppm)
Naph	nd	nd	nd
Aceny	nd	nd	nd
Acen	nd	nd	nd
F	nd	nd	nd
Phen	nd	nd	nd
Anth	nd	nd	nd
Fl	nd	nd	nd
Py	nd	nd	nd
B(a)anth	nd	nd	nd
Chrys	nd	nd	nd
B(b)Fl	nd	nd	nd
B(k)Fl	nd	nd	nd
B(a)Py	nd	nd	nd
Dibenz(ah)	nd	nd	nd
Benzo(ghi)	nd	nd	nd
Indeno(123)	nd	nd	nd

Table 3.10. Optimising sample retention on SPE cartridge. Sample (1ppm of each PAH) addition onto the SPE cartridge at a flow rate of 1.0ml/min.

immiscible, so removal of the solvent would optimise the removal of the analyte by an elution solvent.

The final stage in the sorbent testing process is the optimisation of the elution of the standards.

3.5.7 Optimising the Elution of Standards.

One bed volume, the volume the sorbent occupies (2 mls), of the elution solvent was used to try and optimise elution of the isolate (13). As nonpolar solvents are most likely to succeed in the elution process hexane was investigated first. The other elution solvents investigated were diisopropylether, toluene, and benzene.

Each solvent was tested in replicates of three, and reduced to dryness with N_2 and reconstituted in methanol for analysis by HPLC. The results of the recoveries are shown in table 3.11 and figure 3.13.

Benzene gave the best recoveries but due to the carcinogenic nature of this as an extraction solvent (14), another less harmful extraction solvent would be preferable to work with. The School of Chemistry 's also have a policy of avoiding working with such solvents where possible. It was decided to look at the drying stage again, as perhaps there is some residual methanol left on the sorbent. This would effect the efficiency of the nonpolar elution solvent needed to remove the PAH, as it would be in a relatively polar environment. The addition of a nonpolar solvent to the sorbent after a polar solvent is an extreme change in phase. Due to this complete drying of the sorbent was tried and then addition of the nonpolar elution solvent. The drying stage was increased to 20 minutes at the same flow rate of 1 ml/min. The recoveries of PAH using diisopropylether and hexane were poor so it was decided to investigate the use of toluene as the elution solvent after this

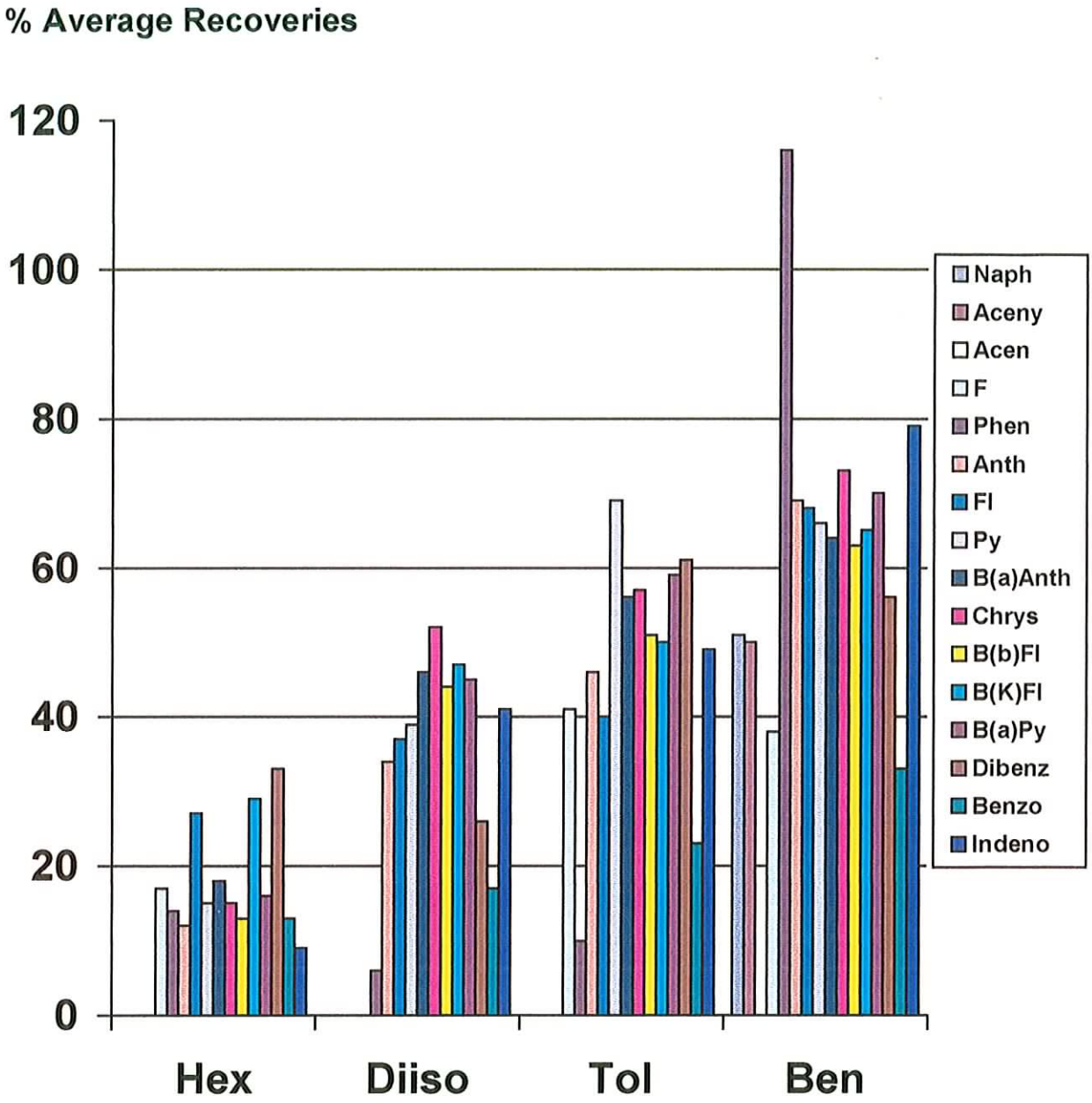
increased drying stage. The recovery results using toluene are shown in table 3.12 and on figure 3.14.

Recoveries with toluene as the elution solvent proved to be acceptable, the USEPA Method 610 quotes analytical recoveries of 50 – 150% as being acceptable. The reproducibility is acceptable also, only naphthalene and fluorene have %RSD >10%, (28% and 13%). The first four PAH; naphthalene, acenaphthylene, acenaphthene, and fluorene are difficult to recover by this method.

Compound	Recovery and RSD(%) with different elution solvents			
	Hexane n=3	Diisopropylether n=3	Toluene n=3	Benzene n=3
Naph	---	---	---	51 ±5%
Aceny	---	---	---	50 ±39%
Acen	---	---	---	---
F	17 ±46%	---	41 ±33%	38 ±5%
Phen	14 ±56%	6 ±28%	10 ±58%	116 ±17%
Anth	12 ±44%	34 ±13%	46 ±49%	69 ±12%
Fl	27 ±66%	37 ±7%	40 ±2%	68 ±24%
Py	15 ±12%	39 ±15%	69 ±8%	66 ±24%
B(a)anth	18 ±15%	46 ±8%	56 ±14%	64 ±9%
Chrys	15 ±31%	52 ±4%	57 ±27%	73 ±14%
B(b)Fl	13 ±30%	44 ±10%	51 ±23%	63 ±21%
B(k)Fl	29 ±21%	47 ±13%	50 ±15%	65 ±21%
B(a)Py	16 ±31%	45 ±14%	59 ±10%	70 ±23%
Dibenz(ah)	33 ±53%	26 ±38%	61 ±7%	56 ±51%
Benzo(ghi)	13 ±59%	17 ±17%	23 ±28%	33 ±34%
Indeno(123)	9 ±40%	41 ±18%	49 ±21%	79 ±19%

Table 3.11. Recoveries of standards with different elution solvents (n=3) at a flow rate of 1.0ml/min.

Figure 3.13. Average Recoveries (%) of the Different Elution Solvents Investigated in the S.P.E. Procedure.

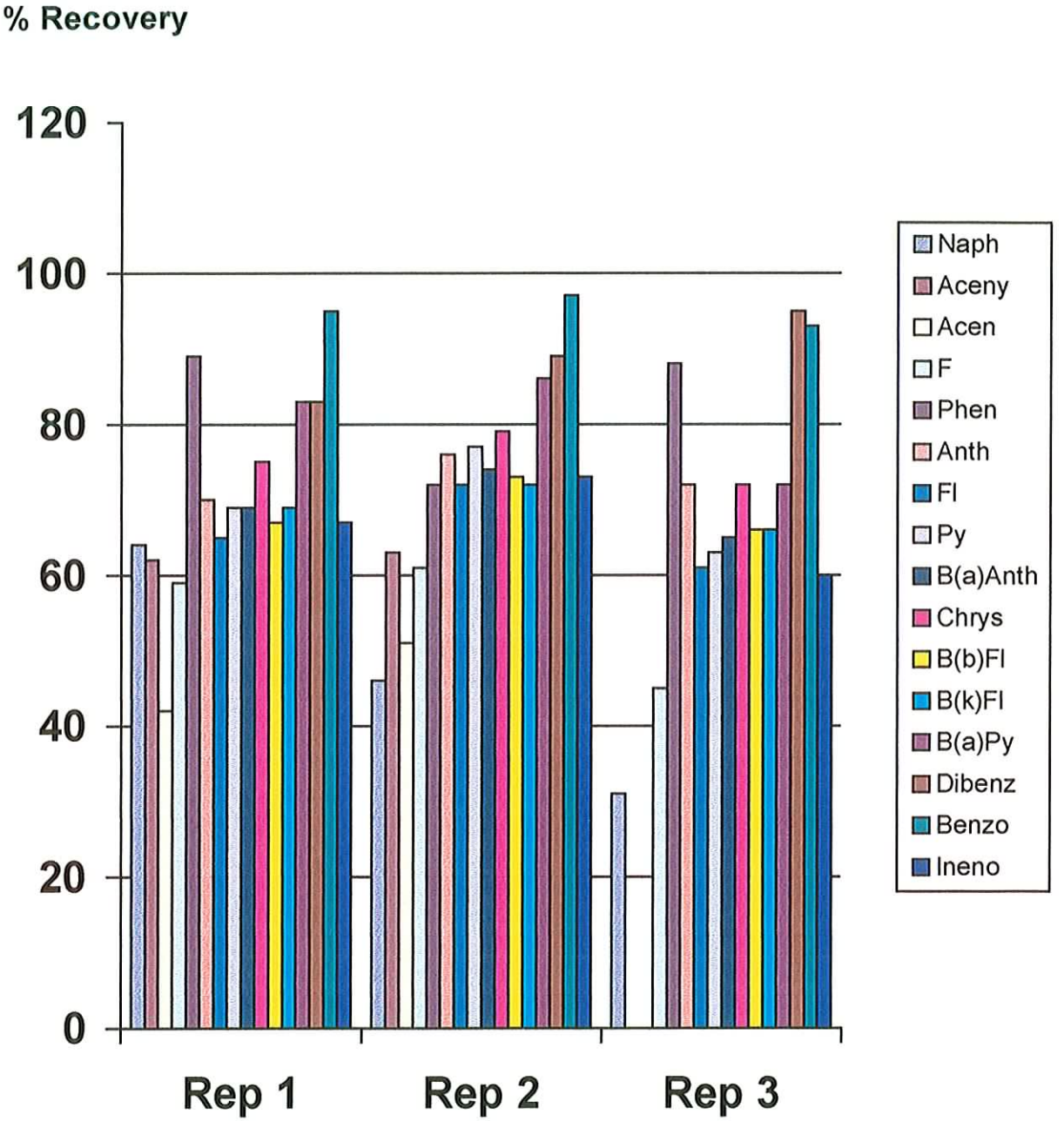


Hex = Hexane, Diiso = Diisopropylether, Tol = Toluene, Ben = Benzene.

Compound	Recovery (%) and RSD(%) with toluene as elution solvent, after increased drying time.			
	Toluene Replicate 1	Toluene Replicate 2	Toluene Replicate 3	Average recovery ± RSD.
Naph	64	46	31	47 ±28%
Aceny	62	63	60	62 ±2%
Acen	42	51	52	48 ±9%
F	59	61	45	55 ±13%
Phen	89	72	88	83 ±9%
Anth	70	76	72	73 ±4%
Fl	65	72	61	66 ±7%
Py	69	77	63	70 ±9%
B(a)anth	69	74	65	69 ±5%
Chrys	75	79	72	75 ±4%
B(b)Fl	67	73	66	68 ±4%
B(k)Fl	69	72	66	69 ±4%
B(a)Py	83	86	72	80 ±8%
Dibenz(ah)	83	89	95	89 ±6%
Benzo(ghi)	95	97	93	95 ±2%
Indeno(123)	67	73	60	67 ±8%

Table 3.12. Recoveries of standard PAH (1ppm) with toluene as elution solvent on the SPE cartridge at a flow rate of 1.0ml/min.

Figure 3.14. Recoveries of Standard PAH Mix (1ppm) with Toluene as Elution Solvent at a Flow Rate of 1.0ml/min.



Rep 1 = Replicate 1.

3.6 Day-to-Day and Run-to-Run Reproducibilities.

As the extraction and clean up optimisation of each step gave satisfactory results, the total procedure was tested for the standard solution recoveries. The recovery values and the %RSD's obtained for three replicate analyses of the standard mix solution were obtained in order to establish run-to-run reproducibility of the overall method. Duplicate analyses were carried out on three different days in order to determine the day-to-day reproducibility of the described method. These results are shown in table 3.13 and figure 3.15.

The losses of PAH analyte concentration can occur at many stages in the method. The method development was first optimised in stages, i.e. extraction (section3.4) and sample clean up (section 3.5). The recoveries from the ultrasonic extraction of spiked concentrations of standard PAH ranged from 44 ±4% recovery for fluorene to 107 ±6% recovery for benzo(k)fluoranthene (see table 3.6). The recoveries from the sample clean up by solid phase extraction ranged from recoveries of 48 ±9% for acenaphthene to 95 ±2% for benzo(ghi)perylene (see table 3.12). Losses inevitably occur at all stages of an analytical methodology. When dealing with environmental samples, it is very important to try and reduce any losses where possible. The silanation of the glassware used during all the procedures of this method is one such way of reducing losses of PAH analyte.

3.6.1 Applicability to "Real" Samples.

Acceptable recoveries and good repeatability (day-to-day) with low RSD (between 5 and 8%) were obtained, showing suitability of the method for the analysis of PAH. This along with the high sensitivity (10 ppb for most of the 16 PAH of interest, with RSD's between 2-11% (see table 3.14)), i.e. ppb concentrations, show the optimised method's applicability to "real", environmental samples. SPE is also a very suitable clean up procedure for environmental samples, mainly due to the selectivity of the sorbents for the analytes of interest. The sorbent used in this

method was styrene/divinylbenzene, which is very selective towards PAH in environmental samples (11).

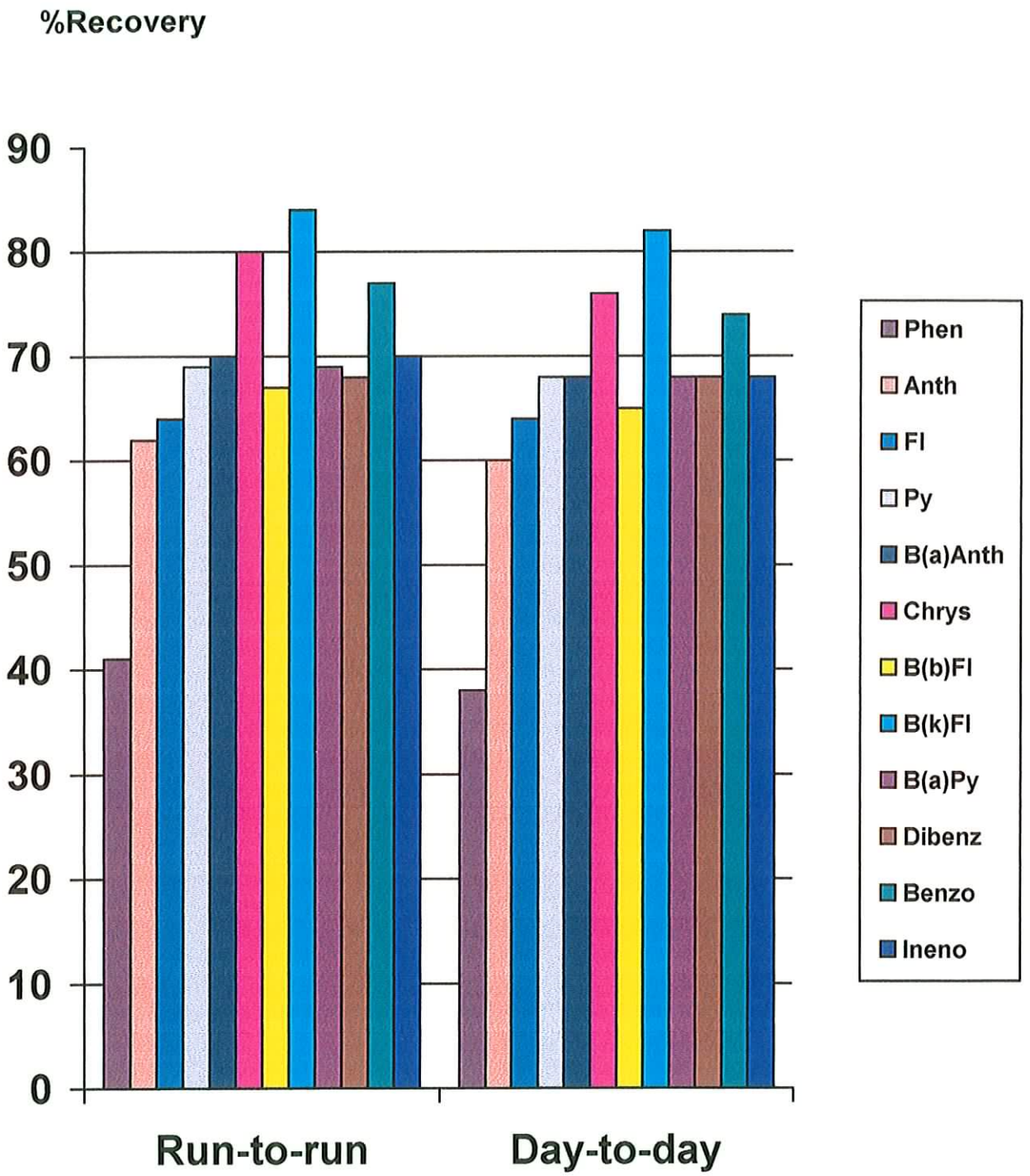
3.7 Fluorescence Detector Response.

Standard solutions of the 16 PAH mixture were analysed by HPLC. Two columns were used in this research. They were Enviro-Sep –PP (Phenomenex) 125mm x 4.6mm and Hypersil ODS –PAH (Shandon) 100mm x 4.6mm. The concentrations analysed were 1ppm, 0.1ppm and 0.01ppm. Each solution was run five times on each column, and the average peak area and %RSD calculated. Approximate linearity was obtained up to 1ppm. The linear regression values (R^2) and equations of the line (forced through (0,0)) for each PAH are shown in table 3.14 and the calibration plots are shown in Appendix A. The calibration plots were not used for quantification purposes, as the 10ppb range is much closer to the expected concentrations in "real" samples. PAH detected in "real" samples were quantified using 10ppb response factors, see table 3.15.

Compound	Average recoveries (%) and % RSD of the complete method, run-to-run and day-to-day repeatabilities n=3	
	Run-to run n=3	Day-to-day n=3
Naph	nd	nd
Aceny	nd	nd
Acen	nd	nd
F	nd	nd
Phen	41 ±4%	38 ±7%
Anth	62 ±4%	60 ±6%
Fl	64 ±6%	64 ±8%
Py	69 ±4%	68 ±5%
B(a)anth	70 ±3%	68 ±5%
Chrys	80 ±4%	76 ±7%
B(b)Fl	67 ±5%	65 ±8%
B(k)Fl	84 ±5%	82 ±6%
B(a)Py	69 ±6%	68 ±8%
Dibenz(ah)	68 ±4%	68 ±6%
Benzo(ghi)	77 ±5%	74 ±8%
Indeno(123)	70 ±5%	68 ±7%

Table 3.13 Run-to-run and day-to-day repeatabilities of a complete method run.

Figure 3.15. Run to Run and Day to Day Recoveries (%) of the Standard PAH mix (1ppm) from the Complete Method.



Compound	Linear regression values of the calibration curves (through (0,0)) of each PAH analysed by the Envi-Sep PP 125 x 4.6mm and Hypersil ODS 100mm x 4.6mm columns.			
	R^2 Envi-Sep PP		R^2 Hypersil ODS	
Naph	0.9920	y=208838x	0.9958	y=199526x
Aceny	nd		nd	
Acen	0.9984	y=558879x	0.9997	y=698389x
F	0.9978	y=1429458x	0.9960	y=36717x
Phen	0.9991	y=2104292x	0.9746	y=43235x
Anth	0.9928	y=5033169x	0.9984	y=1854064x
Fl	0.9957	y=1296905x	0.9964	y=334818x
Py	0.9978	y=1098886x	0.9997	y=348929x
B(a)anth	0.9923	y=1251632x	0.9987	y=491364x
Chrys	0.9955	y=3369156x	0.9958	y=1178178x
B(b)Fl	0.9973	y=70937x	0.9986	y=585631x
B(k)Fl	0.9969	y=6787612x	0.9848	y=2080147x
B(a)Py	0.9965	y=3228626x	0.9943	y=1148429x
Dibenz(ah)	0.9987	y=1652790x	0.9979	y=484050x
Benzo(ghi)	0.9932	y=1434421x	0.9947	y=394960x
Indeno(123)	0.9977	y=219818x	0.9627	y=67416x

Table 3.14. Linear regression values of the calibration curves (through (0,0)) for each PAH run on the Envi-Sep PP and Hypersil ODS HPLC columns..

Compound	Average response factors and %RSD (n=5) of the two columns used for 10ppb standard PAH,	
	Envi-Sep PP 125mm x 4.6mm	Hypersil ODS 100mm x 4.6mm
	10 ppb n=5	10 ppb n=5
Naph	nd	nd
Aceny	nd	nd
Acen	nd	nd
F	4342 ±9%	2863 ±7%
Phen	14971 ±6%	1022 ±9%
Anth	15221 ±2%	25113 ±6%
Fl	2490 ±11%	3205 ±6%
Py	1805 ±2%	2789 ±7%
B(a)anth	21077 ±4%	4785 ±8%
Chrys	4274 ±2%	75604 ±7%
B(b)Fl	3467 ±2%	6267 ±6%
B(k)Fl	34337 ±2%	25106 ±6%
B(a)Py	8651 ±4%	12875 ±5%
Dibenz(ah)	*214441 ±7%	*65114 ±4%
Benzo(ghi)	*261725 ±3%	*61086 ±6%
Indeno(123)	986 ±2%	1086 ±6%

Table 3.15 Average response factors (fluorescence detection) for 10ppb concentrations of the standard PAH mixture.

* 100ppb response factors.

3.8 Summation

A method has now been developed and partially validated for the analysis of PAH in air particulates in the range 4.5-10μm. The most efficient method for extracting the standard PAH from the filter was found to be ultrasonic extraction. Sonication for 1 hour in 3 x 50mls. acetonitrile gave the best recoveries. This extract volume was then reduced to ca. 5mls by rotary evaporation. This extract sample volume was cleaned up by SPE. The SPE cartridge was conditioned with acetonitrile (6mls). Sample addition followed at a flow rate of 1ml/min. The sample was washed with methanol (2mls) at a flow rate of 1ml/min. A drying stage followed for 20mins at a flow rate of 1ml/min. Elution of the PAH was optimised with toluene (2mls) as the solvent. This fraction was reduced to dryness by gently flushing with N_2 gas, and then reconstituted in acetonitrile (1ml). Rrecoveries for all PAH were in the range 41 to 84% with one PAH (phenanthrene) falling outside the acceptable USEPA range (50-150%). The sample was then ready to be quantified by HPLC (see section 2.2 for conditions).

This method was then applied to environmental samples. The next chapter lists the air sampling locations, the sampling period and the particle size fraction sampled. Results of each location are shown and quantified using the calibration plots already shown. Figure 3.17 shows the complete validated method for the analysis of PAH in air particulates established in this work.

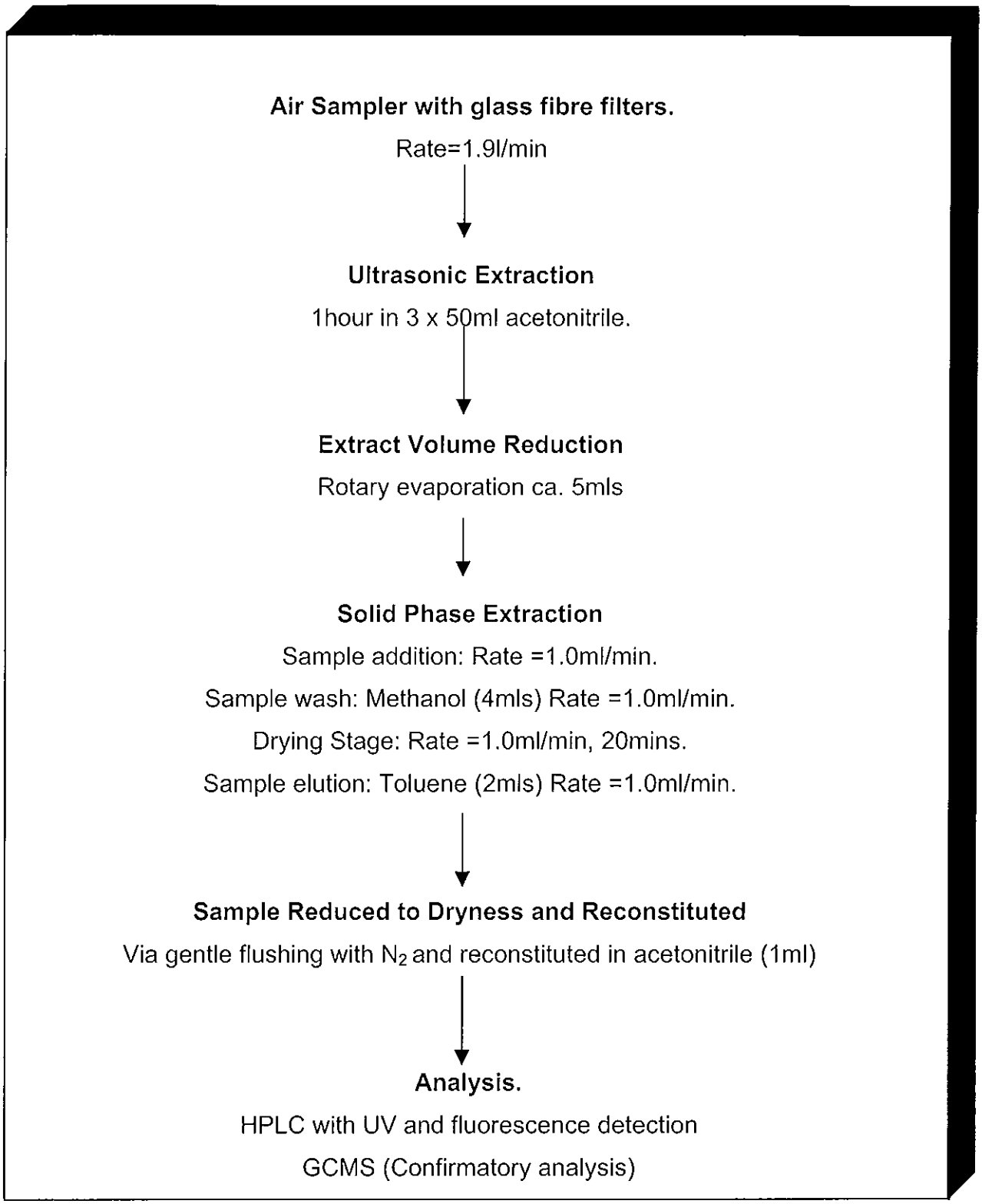


Figure 3.17. Outline of complete validated method for the analysis of PAH in air particulates.

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Chapter 4

Results of Environmental Samples.

4.1 Methodological Aspects of Measuring PAH in the Urban Atmosphere.

PAH are considered among the most important of environmental pollutants. As already mentioned they are produced by incomplete combustion of organic material such as fossil fuels and emitted into the environment from different mobile and stationary sources (see introduction section 1.1.2). PAH can reach the human body by direct contact, inhalation of polluted air or ingestion of contaminated food and water. There are several monitoring methods for PAH, which involve the use of different collection media (1) i.e. filters for particulate and sorbents (tenax, XAD Resin, PUF plugs etc.) for vapour phase PAH compounds. The relationship between particle size and PAH content in the ambient aerosol may be determined using cascade impactors (1). Most of the PAH occur in the particle range of respirable size (i.e.1-5μm). During sampling, extraction and clean-up procedures, significant losses may result due to sampling artifacts, chemical reactions, vapour phase evaporative losses of collected PAH, irreversible adsorption on collecting material, column packing and in particular during the solvent concentration steps. In most cases, the identification and quantitative assessment of individual PAH are possible only after complete separation.

4.2 Mode of Exposure.

Human exposure to PAH can occur through several environmental pathways (e.g. water, soil) due to their numerous sources. However, the occurrence of PAH in urban air has caused particular concern because of the continuous nature of the exposure and the size of the population at risk (1). The urban atmosphere is a very complex and dynamic system containing a large variety of interacting chemical species in both the gas and particulate phases. PAH compounds can reach the human body by four different modes of exposure:

1. Direct inhalation of polluted air.
2. Direct inhalation of tobacco.
3. Ingestion of contaminated and processed food and water.
4. Dermal contact.

4.3 Measurement of PAH in the Environment.

The monitoring methods for PAH may be classified into two categories. Sampling methods for occupational exposures generally involve the use of integrated sampling techniques for collection of materials with filters for particular compounds (PAH) and adsorbents or impingers for vapour forms of PAH over extended periods. These techniques involve sampling rates up to 300 l/min. and are generally called low volume sampling techniques. Passive sampling methods may also be successfully employed in monitoring occupational levels. This technique is usually applied to specific vapour phase PAH compounds.

Sampling methods for ambient exposures, (integrative sampling techniques) are used frequently and given filter media and sorbents are most commonly used. Cryogenic trapping and impingers have received very little use. In these techniques, the sampling rates range between 300-500 l/min. for sampling duration of 24 hours and they are generally called high volume sampling techniques. Quasimeme (EU Measurement and Testing) methods can be employed for ambient monitoring but high cost and narrow range of target compounds (PAH) monitored at any time restrict the widespread application of this technique. There is great concern over the general lack of standardised methods of sampling and analysis for PAH. To ensure feasible analytical control, the standardisation of sampling and analytical procedures is highly desirable. The sampling of PAH either in the gaseous or particulate phase has been performed most frequently by active sampling methods. Particles with aerodynamic equivalent diameters less than about 10μm have been of particular concern because of the possible health

implications. Sampling of PAH with respect to particle size distribution has been performed extensively with cascade impactors.

4.4 Selection of Filters/Collection Media.

Several types of filter materials have been used for sampling of PAH from ambient atmosphere. Among these are glass fibre, cellulose, organic membrane and silver membranes. The choice of filter materials depends on the sampling situation and the analytical techniques, used to determine the collected PAH (2). Due to their good mechanical strength and relatively low costs, glass fibre filters have been most commonly used for high volume sampling (3). Polytetrafluoroethylene (PTFE or Teflon) membrane filters and PTFE coated glass fibre filters are also preferred for PAH sampling owing to their chemical inertness and low impurity levels (4). More recently solid sorbents or impregnated filters and cryogenic traps have been used behind the filter to capture vapour phase components. Glass fibre filters have been most widely used in high volume sampling (5).

Recently, owing to low-pressure drop and ease of handling, polyurethane foam (PUF) plugs have been increasingly used for the collection of organic compounds and have been reported to be an effective means of trapping volatile PAH, particularly with high volume samplers (HSV) (6). The determination of atmospheric PAH is a difficult task as concentrations of these compounds are generally low and many of them are unstable and volatile.

The sampling method, using glass fibre filters instead of PUF filters, may have resulted in a loss of the low molecular weight PAH (e.g. naphthalene. acenaphthene, acenaphthylene). This was previously seen in the method development stage, and these PAH will therefore not be considered in this analytical scheme.

4.5 Sampling Artifacts, Reactions of Atmospheric Pollutants with Collected Material and Vapour Phase Losses of Collected PAH.

Two types of loss processes, namely volatilisation and chemical reactions are of importance when sampling air borne PAH on filter materials either by filtration or impaction. The losses due to volatilisation of higher molecular weight PAH appear to be much smaller than those of the lower molecular weight compounds. The possibility of PAH loss by photochemical and/or chemical reaction on the filter deposited particles with gaseous/air pollutants under varying meteorological conditions has been studied (7). In addition, the observed negative relationship with sunshine hours, ozone and SO_2 seems to give a good indication of the possible losses of PAH by photochemical and/or chemical reactions on the filter deposited particles.

Ambient temperature and NO_2 concentration seem to be the most significant variables (8). Diesel exhaust particles, less than 0.5μm aerodynamic diameter collected on glass fibre filters and subsequently exposed to 1.5ppm NO_2 (as well as ozone (O_3) free air) for up to 4 hours showed loss of PAH (e.g. phenanthrene, anthracene and pyrenes) due to volatilisation (8). However, these as well as less volatile PAH (e.g. benzo(a)pyrene, benzo(ghi)perylene) showed considerable reactivity to O_3 (8). The conversions ranged from 47-100% in 4 hours under the experimental conditions used. Under similar conditions half lives of the order of 0.5 to 1 hours were found for most PAH with molecular weight ranging from 226 to 276 (8). During the extraction and clean up procedures some losses may result from handling, incomplete extraction (see chapter 3, section 3.4.6), irreversible adsorption on column chromatography packings and during solvent concentration steps.

Comparison between a determination of a PAH concentration using different sampling techniques has been previously reported by a number of authors (9-11). In general, concentrations of certain PAH determined using low volume samplers

were consistently higher than those collected by high volume filtration (9-11). Similarly samples collected over an extended period indicated lower concentrations. A large number of studies have clearly demonstrated that considerable evaporative losses of PAH especially for two, three and four ringed compounds, may occur during prolonged sampling.

4.6 Sampling Equipment.

The sampling procedure employed in this work was carried out using the SKC Cyclone units for inhalable dust fraction (10 μm aerodynamic diameter) and respirable dust fraction (4.5 μm aerodynamic diameter) in conjunction with a pump (SKC pump no. 244-43XE). The flow rate was calibrated to 1.9 l/min (representative of the human breathing rate). The cyclone operates by taking in air into a vortex. The larger particles fly out of the air stream tangentially under centrifugal force, falling to a "grit pot" at the base of the sampler. The smaller particles are carried with the airstream back up to be collected on the underside of a filter.

Ness (12) describes the advantages of cyclones as including insensitivity to orientation, a feature of importance in such a highly mobile sampling situation. She quotes an "overall precision" of "about 2%". In this case the cyclone was attached via a plastic tube to a pump (SKC pump no. 244-43XE), which has a built in flowmeter (set at 1.9l/min). For the purposes of this study the pump was run off the electricity mains to ensure an even and accurate flow rate for the duration of the sampling period.

4.7 Sampling Procedure.

• Data log.

Dates, times and sampling duration were recorded for each sample included in this study. The flow rate was checked at the start and end of each sampling period. In all cases the flow rate remained unchanged. In the case of indoor samples a general description of the surroundings was recorded. For ambient samples a brief record of the weather during the sampling period was kept.

• Weighing procedures.

Following some initial weighing of blank glass fibre filters, i.e. the same filter weighed at different intervals, it became clear that random error in the weighing procedure is substantial due to the small sample size. In order to eliminate error due to variations in the tare weight of the balance, the weighing procedure was adjusted to include recording the tare of the balance before and after each sample. The tare weight reading after the sample was removed was then subtracted from the sample reading taken.

• Sample Conditioning.

For accurate weight determination it is recommended (13) that the filters should be conditioned by being placed in individual, labelled, clean cassettes, and with the cassette lids slightly ajar in the balance room for an hour before each weighing. This ensures that any moisture in the filters comes into equilibrium with the balance room atmosphere. After sampling the conditioning procedure should be repeated prior to re-weighing.

4.8 Constituents of Tobacco Smoke.

Bar-workers are subjected to environmental tobacco smoke (ETS) throughout their working day and a survey found that they were the occupational group at highest risk from lung cancer (14). The topic of smoking in public houses was also discussed in an article entitled "Smoking – will publicans' profits go up in smoke?"(14). According to government figures in Ireland, the percentage of smokers to non-smokers is 27% to 73%(15). Thus there is a pressure to introduce no smoking areas in pubs. However the practicality of this needs to be examined as the economic aspect of the no smoking debate is inescapable. Approximately 15% of tobacco sales in Ireland go through pubs. This translates into ca. £124 million of tobacco purchased across the pub counter every year.

Cigarette smoke is an aerosol composed of volatile agents in the vapour phase and of semi-volatiles in the particulate phase. The 400-500mg of the mainstream smoke (MS) emerging from the mouthpiece of a cigarette contain about 10^9 particles per millilitre ranging in diameter from 0.1 to 1.0μm (mean diameter 0.25μm) (16). About 95% of the weight of MS of a non-filter cigarette is comprised of 400-500 individual gaseous compounds. The remainder of the smoke weight is given by more than 3,500 individual components in the particulate phase (16). Table 4.1 lists some of the major constituents of the particulate matter of the MS of non-filter cigarettes.

Compound	μg/Cigarette
Naphthalene	2-4
Phenanthrenes	0.2-0.4*
Anthracenes	0.05-0.1*
Fluorenes	0.6-1.0*
Pyrenes	0.3-0.5*
Fluoranthenes	0.3-0.45*
Carcinogenic PAH	0.1-0.25

Table 4.1.Some of the major constituents of the particulate matter of the MS of nonfilter cigarettes (16).

* Estimate.

Large-scale fractionation studies of cigarette "tar", combined with bioassays, first led to the identification of carcinogenic PAH. Table 4.2 shows the assignments of the identified PAH in tobacco and tobacco smoke components in respect to their carcinogenity in laboratory animals and in humans (International Agency for Research on Cancer (IARC), 1987, 1995).

Compound	In processed tobacco (per/g)	In MS (per/cigarette)	IARC evaluation evidence of carcinogenity.	
			In lab.Animals	In Humans
B(a)anth	0.1-90ng	20-70ng	Sufficient	Probable
B(b)Fl		4-22ng	Sufficient	
B(k)Fl		6-12ng	Sufficient	
B(a)Py		20-40ng	Sufficient	
Dibenz		4ng	Sufficient	
Indeno		4-20ng	Sufficient	

Table 4.2. PAH carcinogens in tobacco and cigarette smoke (IARC 1987,1995)

The health risk from inhaling tobacco smoke is not limited to the smoker but also includes those exposed to "passive smoke". Passive smoking, or exposure to environmental tobacco smoke (ETS), has always been an irritation to non-smokers. It is defined as "the involuntary intake of smoke by a person other than the actual smoker"(17). The obvious way in which this happens is when non-smokers share the same room or confined space as smokers. This makes it almost impossible to avoid inhaling some tobacco smoke with each breath.

The National Cancer Institute published findings of the smoke yields from cigarettes made from reconstituted tobacco (RT). Table 4.3 shows the amounts of benzo(a)anthracene and benzo(a)pyrene in cigarettes made from reconstituted tobacco from stems or tobacco blend and regular cigarettes (18).

	RT STEMS ONLY	RT BLEND	CONTROL REGULAR CIGS
B(a)anth (ng/per cigarette)	13.1	9.8	46.3
B(a)Py (ng/per cigarette)	8.9	7.4	27.8
Ratio	1:1.47	1:1.32	1:1.67

Table 4.3.Smoke yields from cigarettes made from reconstituted tobacco, from stems/butts or tobacco blend and regular cigarettes (National Cancer Institute (1976)) (18).

The Chemical Agents Regulations together with the 1997 Code of Practice (which replaces the 1994 Code of Practice) provides a comprehensive list of chemicals that have appropriate "Occupational Exposure Limits" (OEL's). These OEL's refer to "the maximum permissible concentration, of a chemical agent in the workplace to which workers may be exposed, in relation to a 8 hour or a 15 minute reference period" (19). The concentration of the chemical agent in air is expressed as ppm,

mg/m^3 or fibres per millilitre as appropriate. The 8 hour reference period relates to the procedure whereby the occupational exposures in any 24 hour period are treated as equivalent to a single uniform exposure for 8 hours (the 8 hour time weighted average (TWA) exposure). The TWA may be expressed mathematically by:

$$(C_1 T_1 + C_2 T_2 + \ldots\ldots\ldots\ldots + C_n T_n)/8$$

where $C_1 \ldots\ldots\ldots C_n$ are the occupational exposures and $T_1 \ldots\ldots\ldots T_n$ are the associated exposure times in any 24 hour period.

The 15 minute reference period means the short term exposure reference period and is the sampling period used for assessing compliance with the associated exposure limit. Table 4.4 gives the OEL's for some of the chemicals associated with this research, from the 1997 Code of Practice (19).

Substance	**OEL (8 hour reference period**		**OEL (15 minute reference period)**		
	ppm	mg/m^3	ppm	mg/m^3	Notes
Naphthalene	10	50	15	75	-
Benz(a)anthracene	-	-	-	-	C_2
Benzo(b)fluoranthene	-	-	-	-	C_2
Benzo(a)pyrene	-	-	-	-	C_2
Dusts non-specific					
total inhalable	-	10	-	-	-
respirable	-	5	-	-	-

Table 4.4. PAH and dust OEL's for the 8 hour and 15 minute reference periods (19).

C_2 refers to substances which should be regarded as if they are carcinogenic for man (Category 2 carcinogens) to which the Safety, Health and Welfare at Work (Carcinogens) Regulations, 1993 (S.I. No. 80 of 1993) applies.

Category 2 status infers that there is no safe exposure limit for the compounds. Many of the substances listed in the 1997 Code of Practice are present in environmental tobacco smoke, yet passive smoking is not specifically referred to in any of the relevant safety and health legislation.

4.9 Results of Indoor Air Samples.

The indoor samples were taken in two public houses, in Dublin. The criteria for choosing public houses is the fact that tobacco smoke is a source of particulate bound PAH (section 4.8) and public houses have a reputation of being "smokey". Quantifying the PAH content in pubs has a serious occupational hygiene aspect to it. If the PAH content is frequently high then the patrons and bar staff are "at risk" to disease and illness caused by PAH (see chapter 1 section 1.7). These particular public houses have a very busy local trade. Pub 1 samples were taken at the counter of the bar section. The pump was placed 1.1m from the ground, which is representative of a persons breathing zone. The particle sizes, 10μm and 4.5μm were sampled for different sampling durations (12 and 8 hours) at a flow rate of 1.9l/min.

Pub 2 samples were taken at the counter in the lounge section of the other public house location. The pump was placed 1.1m from the ground. The same particle sizes were sampled here at the same specified sampling durations as before. Again the flow rate here was 1.9l/min. Pub 2 had an extractor fan system installed during the sampling regime. The extractor fans were located above the bar counter area, and these results are included in the study. The air sampler being

located at the counter in both pubs, means that perhaps the air samples taken were more representative to the bar staff than the patrons.

After sampling the filter is conditioned in accordance with the protocol outlined in section 4.7, and the sample is prepared by the previously described method for analysis by HPLC. The samples were taken to evaluate the application of the method developed in this research to environmental samples. The area responses of the individual PAH identified (on the basis of retention times), are quantified by use of 10ppb response factors on the specified column. Dibenz(ah)anthracene and benzo(ghi)perylene were quantified using 100ppb response factors, (see experimental section 2.2 for conditions). The chromatograms are shown in the Appendix B.

Pub 1 Sample A

Compound	Sample resp. (arb. units)	Conc. (ppb.)	Conc. (ng./m^3)
F	nd	-	-
Phen	nd	-	-
Anth	35247	23.16	25.39
Fl	nd	-	-
Py	3002	16.63	18.23
B(a)Anth	35201	16.70	18.31
Chrys	nd	-	-
B(b)Fl	1068	3.08	3.38
B(k)Fl	54923	15.99	17.54
B(a)Py	6020	6.96	7.63
Dibenz	nd	-	-
Benzo	nd	-	-
Indeno	nd	-	-

Pub1 Sample A Conditions

Date: 12/6/98 (Saturday)

Air sampler flow rate: 1.9l./min.

Sampling period: 8 hours (16:30 – 00:30)

Total volume air sampled: 912 litres/0.912m^3

Particle size sampled: 4.5μm.

Analysis: HPLC 1 conditions.

Pub 1 Sample B

Compound	Sample resp. (arb. units)	Conc. (ppb.)	Conc. (ng./m^3)
F	nd	-	-
Phen	nd	-	-
Anth	21313	14.00	10.23
Fl	nd	-	-
Py	7312	40.51	29.61
B(a)Anth	35346	16.77	12.26
Chrys	4557	10.66	7.79
B(b)Fl	4107	11.85	8.66
B(k)Fl	208378	60.68	44.36
B(a)Py	45938	53.10	38.81
Dibenz	nd	-	-
Benzo	nd	-	-
Indeno	nd	-	-

Pub 1 Sample B conditions

Date: 19/6/98 (Saturday)

Air sampler flow rate: 1.9l./min.

Sampling period: 12 hours (12:30 – 00:30)

Total volume air sampled: 1368 litres./1.368m^3

Particle size sampled: 10.0μm.

Analysis: HPLC 1 conditions

Pub 1 Sample C

Compound	Sample resp. (arb. units)	Conc. (ppb.)	Conc. (ng./m^3)
F	nd	-	-
Phen	nd	-	-
Anth	nd	-	-
Fl	nd	-	-
Py	3555	19.69	21.59
B(a)Anth	20084	9.53	10.45
Chrys	14458	33.83	37.09
B(b)Fl	29204	84.23	92.36
B(k)Fl	173267	50.46	55.33
B(a)Py	7009	8.10	8.88
Dibenz	nd	-	-
Benzo	nd	-	-
Indeno	nd	-	-

Pub1 Sample C conditions

Date: 13/6/98 (Sunday)

Air sampler flow rate: 1.9l./min.

Sampling period: 8 hours (16:30 – 00:30)

Total volume air sampled: 912 litres./0.912m^3

Particle size sampled: 4.5μm.

Analysis: HPLC 1 conditions.

Pub1 Sample D

Compound	Sample resp. (arb. units)	Conc. (ppb.)	Conc. (ng./m^3)
F	nd	-	
Phen	nd	-	-
Anth	nd	-	-
Fl	nd	-	-
Py	4039	22.37	16.35
B(a)Anth	19783	9.39	6.86
Chrys	8548	20.00	14.62
B(b)Fl	33739	97.31	71.13
B(k)Fl	205055	59.72	43.65
B(a)Py	8083	9.34	6.83
Dibenz	nd	-	-
Benzo	nd	-	-
Indeno	nd	-	-

Pub 1 Sample D Conditions

Date: 20/6/98 (Sunday)

Air sampler flow rate: 1.9l./min.

Sampling period: 12 hours (12:30 – 00:30)

Total volume air sampled: 1368 litres./1.368m^3

Particle size sampled: 10.0μm.

Analysis: HPLC 1 conditions.

Pub 2 Sample A

Compound	Sample resp. (arb. units)	Conc. (ppb.)	Conc. (ng./m^3)
F	nd	-	-
Phen	nd	-	-
Anth	nd	-	-
Fl	5381	21.61	23.69
Py	7803	43.22	47.40
B(a)Anth	4395	2.08	2.29
Chrys	nd	-	-
B(b)Fl	4103	11.83	12.97
B(k)Fl	33786	9.84	10.79
B(a)Py	2799	3.23	3.55
Dibenz	nd	-	-
Benzo	nd	-	-
Indeno	nd	-	-

Pub 2 Sample A Conditions

Date: 11/7/98 (Saturday)

Air sampler flow rate: 1.9l./min.

Sampling period: 8 hours (16:30 – 00:30)

Total volume air sampled: 912 litres./0.912m^3

Particle size sampled: 4.5μm.

Analysis: HPLC 1 conditions.

Pub 2 Sample B

Compound	Sample resp. (arb. units)	Conc. (ppb.)	Conc. (ng./m^3)
F	nd	-	-
Phen	nd	-	-
Anth	nd	-	-
Fl	3733	14.99	10.95
Py	6082	33.69	24.63
B(a)Anth	4857	2.30	1.68
Chrys	nd	-	-
B(b)Fl	3980	11.48	8.39
B(k)Fl	43303	12.61	9.22
B(a)Py	2537	2.93	2.14
Dibenz	nd	-	-
Benzo	nd	-	-
Indeno	nd	-	-

Pub 2 Sample B conditions

Date: 18/7/98 (Saturday)

Air sampler flow rate: 1.9l./min.

Sampling period: 12 hours (12:30 – 00:30)

Total volume air sampled: 1368 litres./1.368m^3

Particle size sampled: 10.0μm.

Analysis: HPLC 1 conditions.

Pub 2 Sample C

Compound	Sample resp. (arb. units)	Conc. (ppb.)	Conc. (ng./m^3)
F	nd	-	-
Phen	nd	-	-
Anth	60850	39.98	43.84
Fl	nd	-	-
Py	nd	-	-
B(a)Anth	9228	4.38	4.80
Chrys	3401	7.96	8.72
B(b)Fl	25230	7.28	7.98
B(k)Fl	5632	1.64	1.79
B(a)Py	nd	-	-
Dibenz	nd	-	-
Benzo	nd	-	-
Indeno	nd	-	-

Pub 2 Sample C conditions

Date: 12/6/98 (Sunday)

Air sampler flow rate: 1.9l./min.

Sampling period: 8 hours (16:30 – 00:30)

Total volume air sampled: 912 litres..0.912m^3

Particle size sampled: 4.5μm.

Analysis: HPLC 1 conditions.

Compound	Pub2 Sample D*		
	Sample resp. (arb. units)	Conc. (ppb.)	Conc. (ng./m^3)
F	nd	-	-
Phen	nd	-	-
Anth	nd	-	-
Fl	nd	-	-
Py	6833	37.86	27.67
B(a)Anth	108374	51.42	37.58
Chrys	4907	11.48	8.39
B(b)Fl	13392	38.62	28.23
B(k)Fl	75121	136.77	99.98
B(a)Py	92974	107.47	78.56
Dibenz	nd	-	-
Benzo	nd	-	-
Indeno	nd	-	-

*This sample was taken with the extractor fan system in operation.

Pub 2 Sample D conditions

Date: 19/7/98 (Sunday)

Air sampler flow rate: 1.9l./min.

Sampling period: 12 hours (12:30 – 00:30)

Total volume air sampled: 1368 litres./1.368m^3

Particle size sampled: 10.0μm.

Analysis: HPLC 1 conditions.

4.10 Analysis of Indoor Air Results.

From the data in tables 4.1 and 4.2 it can be seen that certain PAH, as expected are detected in locations of high tobacco smoke. The results from Pub 1 indicate carcinogenic PAH were detected. Pub 1 samples A and B were taken on two Saturday nights, perhaps one of the busiest nights in the vintners trade. Sample A represents the 4.5μm particle fraction (respirable dust getting into the inner recesses of the lungs), over an 8-hour period at 1.9 l/min. The reason 8 hours was chosen as a sampling period is its representation as an average working day of many bar staff, and bar staff are exposed for the duration of their shift. All these compounds are constituents of "environmental tobacco smoke", which also has been classified by the EPA as a known human carcinogen. Sample B was sampled over a 12-hour period and represents the 10μm (dust fraction that travels relatively far into the lungs) particle size. Pub 1 Samples C and D were taken on two Sundays, another busy and crowded day in a public house. Sample C represents the 4.5μm particle size for 8 hours at 1.9l/min. Sample D represents the 10μm particle size for 12-hours at 1.9 l/min.

Pub 2 Samples A and B were sampled on two Saturdays. Sample A represents the 4.5μm particle size for 8 hours and sample B represents the 10μm particle size for 12-hours both at 1.9 l/min. The results from these samples were similar in the concentrations of the compounds detected. In both samples A and B, fluoranthene was detected which was not detected in any of the Pub 1 samples.
Pub 2 Sample C represents the 4.5μm particle size for 8 hours at 1.9 l/min on a Sunday. Pub 2 Sample D represents 10μm particle size for 12 hours at 1.9 l/min. The concentrations of the PAH detected here were among the highest of all the samples taken in both public houses. The concentration of benzo(a)pyrene was 78.56ng/m^3 the highest of all the samples taken. Benzo(k)fluoranthene was quantified at 99.98ng/m^3, benzo(a)anthracene was 37.58ng/m^3. This sample was taken in the same position as the others in pub 2 but there was an extractor fan system in operation. The extractor system was in place above the bar. The air

sampler was positioned at the bar also. The extractor system functioned by drawing the air towards it, hence the air sampler was right in the path of this air.

The ratio of benzo(a)pyrene to benzo(a)anthracene in pub 2 sample D was 1:2.09. This ratio found in the sample is a reasonably good match to the findings of the National Cancer Institute (table 4.3). This sample is perhaps not very representative of the air in a pub as it was sampled in a concentrated stream of indoor (pub) air. However the bar staff are directly in the path of this air stream, so while this sample is not representative of exposures of the patrons of a pub it is to the bar staff.

4.11 Results of Ambient Air Samples.

The mutagenic activity of PAH on living tissue has been well documented, but research has suggested that nitrated and oxygenated derivatives of PAH may exhibit equal or greater mutagenic activity than PAH themselves (20, 21). Oxidation of PAH can occur during the combustion process or while resident in the atmosphere. Due to the rapid increase in the number of vehicles in use, especially in urban areas, engine emissions have become suspected culprits for some of the health effects observed in urban populations (22). Both petrol and diesel fuelled vehicles produce PAH and nitro-PAH (23). Diesel exhaust gases tend to contain higher concentrations of carcinogenic nitro-PAH and low toxicity 2,3 and 4 ring PAH (24). Petrol engine exhaust gases tend to produce higher concentrations of the 5 and 6 ring PAH (benzo(a)pyrene, benzo(ghi)perylene, indeno(123)pyrene, dibenz (ah)anthracene) which are more carcinogenic than the 2 and 3 ring PAH (25).

The criteria for the selection of the three sites for PAH monitoring in particulate matter was the fact that traffic is the main contributor to particulate matter in the air, (see chapter 1, section1.2) and petrol/diesel fuelled vehicles are sources of PAH (see chapter 1, section 1.1). The ambient samples were taken at three different

locations in Dublin namely, Newlands Cross in Tallaght, Lower Rathmines Road and Cedarwood Road in Glasnevin. Newlands Cross is one of the most heavily trafficked junctions in Dublin City. Both commercial and commuter vehicles use the route in heavy volumes, inferring both petrol and diesel vehicles. Peak times (rush hours) have especially heavy traffic volumes. The samples taken at Newlands Cross were at 24, 12 and 8-hour (continuous) sampling durations all at a flow rate of 1.9l/min. The particulate matter with 10μm and 4.5μm diameters were sampled. The air sampler was about 2m. from the ground. The Lower Rathmines Road is approximately 2 miles from Dublin city centre, and is one of the main arteries into the city from the southside, the traffic volume here is heavy at peak times but not in the same volume as Newlands Cross. Both commercial and commuter vehicles use the route with frequency. The Lower Rathmines Road samples were at 7 hour sampling durations at a flow rate of 10.0l/min. The particulate matter with 10μm and 4.5μm diameters were sampled. The air sampler was about 4m from the ground. Cedarwood Road is a suburban site and can be used as a background site as it is remote from main traffic routes, relatively isolated from domestic and point sources of air pollution and within the city boundaries. The Cedarwood Road samples were taken with the same parameters as the Lower Rathmines Road samples.

After sampling the filter is conditioned in accordance with the protocol outlined in section 4.7, and the sample is prepared with the prescribed and validated method for analysis by the HPLC. The area responses of the individual PAH identified (on the basis of retention times), are quantified by the use of 10ppb response factors. As with the pub samples dibenz(ah)anthracene and benzo(ghi)perylene were quantified with 100ppb response factors on the specified column (see experimental section 2.2 for conditions). The chromatograms are shown in Appendix C.

Compound	Ambient Sample 1		
	Sample resp. (arb. units)	Conc. (ppb.)	Conc. (ng./m^3)
F	96019	335.78	122.25
Phen	nd	-	-
Anth	24723	9.84	3.59
Fl	8751	27.30	9.98
Py	19768	70.87	25.91
B(a)Anth	23658	49.44	18.07
Chrys	13655	1.81	0.66
B(b)Fl	66206	105.64	38.61
B(k)Fl	176639	70.36	25.72
B(a)Py	108727	84.45	30.87
Dibenz	24225	3.72	1.35
Benzo	nd	-	-
Indeno	nd	-	-
Total			277.34ng/m^3

Ambient Sample 1 Conditions

Location: Newlands Cross, Tallaght Date: 13 and 14/3/98 (Friday/Saturday)

Air sampler flow rate: 1.9l./min. Sampling period: 24 hours (15:00 – 15:00)

Total volume air sampled: 2736litres/2.736m^3 Height of sampler from ground: ~2m.

Particle size sampled: 10 μm.

Analysis: HPLC 2 conditions.

Weather Conditions; Wind strength: 18.3 knots

Wind direction: South-east

Precipitation: Trace

Air temp. 2.1°C.

Ambient Sample 2

Compound	Sample resp. (arb. units)	Conc. (ppb.)	Conc. (ng./m^3)
F	1296	4.53	3.30
Phen	6367	62.29	45.54
Anth	19318	7.69	5.62
Fl	12523	39.07	28.56
Py	37341	133.88	97.94
B(a)Anth	18174	37.98	27.76
Chrys	86439	11.43	8.36
B(b)Fl	39968	63.76	46.62
B(k)Fl	179448	71.48	52.24
B(a)Py	179448	139.38	101.88
Dibenz	33399	5.13	3.75
Benzo	43292	7.09	5.18
Indeno	1744	16.06	11.74
Total			438.49ng/m^3

Ambient Sample 2 Conditions

Location: Newlands Cross, Tallaght.

Air sampler flow rate: 1.9l./min

Total volume air sampled: 1368 litres/1.368m^3

Particle size sampled: 10μm.

Analysis: HPLC 2 conditions.

Weather Conditions: Wind Speed:14.9 knots
Wind direction: North-east
Precipitation: Rainfall-trace;
Air temp. 3.3°C.

Date: 20/3/98 (Friday)

Sampling period: 12 hours (07:00 – 19:00)

Height of sampler from ground: ~2m.

Ambient Sample 3

Compound	Sample resp. (arb. units)	Conc. (ppb.)	Conc. (ng./m^3)
F	nd	-	-
Phen	1514	14.81	10.83
Anth	nd	-	-
Fl	10417	32.50	23.75
Py	34526	123.79	90.49
B(a)Anth	10060	16.05	11.73
Chrys	Nd	-	-
B(b)Fl	19065	30.42	22.24
B(k)Fl	194891	77.62	56.74
B(a)Py	89005	69.13	50.53
Dibenz	37008	5.68	4.15
Benzo	23365	3.82	2.79
Indeno	nd	-	-
Total			273.25ng/m^3

Ambient Sample 3 Conditions

Location : Newlands Cross, Tallaght. Date: 21/3/98 (Saturday)

Air sampler flow rate: 1.9l./min. Sampling period: 12 hours (07:00 – 19:00)

Total volume air sampled: 1368 litres/1.368m^3 Height of sampler from ground: ~2m.

Particle size sampled: 4.5 μm.

Analysis: HPLC 2 conditions.

Weather Conditions: Wind speed: 11.6 knots

Wind direction: North-east

Precipitation: 0.2mm

Air temp. 4.8°C.

Ambient Sample 4

Compound	Sample resp. (arb. units)	Conc. (ppb.)	Conc. (ng./m^3)
F	nd	-	-
Phen	nd	-	-
Anth	3870	2.54	2.79
Fl	nd	-	-
Py	9752	54.03	59.24
B(a)Anth	13864	6.58	7.21
Chrys	2797	6.54	7.18
B(b)Fl	2633	7.59	8.33
B(k)Fl	33052	9.63	10.55
B(a)Py	14777	17.08	18.73
Dibenz	nd	-	-
Benzo	nd	-	-
Indeno	nd	-	-
Total			114.03ng/m^3

Ambient Sample 4 Conditions

Location: Newlands Cross, Tallaght.
Date: 22/3/98 (Sunday)
Air sampler flow rate: 1.9l./min.
Sampling period: 8 hours (11:00–19:00)
Total volume air sampled: 912 litres/0.912m^3
Height of sampler from ground: ~2m.
Particle size sampled: 4.5 μm.
Analysis: HPLC 1 conditions.
Weather Conditions: Wind speed: 7.3 knots
Wind direction: Variable
Precipitation: 0.1mm
Air temp: 5.8°C.

Ambient Sample 5

Compound	Sample resp. (arb. units)	Conc. (ppb.)	Conc. (ng./m^3)
F	136163	313.59	74.66
Phen	492468	328.95	78.32
Anth	60028	39.44	9.39
Fl	nd	-	-
Py	2748	15.22	3.63
B(a)Anth	nd	-	-
Chrys	nd	-	-
B(b)Fl	nd	-	-
B(k)Fl	nd	-	-
B(a)Py	nd	-	-
Dibenz	nd	-	-
Benzo	nd	-	-
Indeno	nd	-	-
Total			166ng/m^3

Ambient Sample 5 Conditions

Location: Lower Rathmines Road

Air sampler flow rate: 10.0l./min.

Total volume air sampled: 4200litres/4.2m^3

Particle size sampled: 10 μm.

Analysis: HPLC 1 conditions.

Weather Conditions: Wind speed: 9.5 knots
Wind direction: Variable
Precipitation: Trace
Air temp 14.3°C.

Date: 17/9/98 (Thursday)

Sampling period: 7 hours (13:00–20:00)

Height of sampler from ground: ~4m

Ambient Sample 6

Compound	Sample resp. (arb. units)	Conc. (ppb.)	Conc. (ng./m^3)
F	76428	51.05	12.15
Phen	114178	76.27	18.16
Anth	49904	32.79	7.81
Fl	nd	-	-
Py	nd	-	-
B(a)Anth	nd	-	-
Chrys	nd	-	-
B(b)Fl	nd	-	-
B(k)Fl	nd	-	-
B(a)Py	nd	-	-
Dibenz	nd	-	-
Benzo	nd	-	-
Indeno	nd	-	-
Total			38.12ng/m^3

Ambient Sample 6 Conditions

Location: Lower Rathmines Road

Air sampler flow rate: 10.0l./min.

Total volume air sampled: 4200 litres/4.2m^3

Particle size sampled: 4.5 μm.

Analysis: HPLC 1 conditions.

Weather Conditions: Wind speed: 13.8 knots
Wind direction: Variable
Precipitation: Trace
Air temp: 13.6°C

Date: 18/9/98 (Friday)

Sampling period: 7 hours (13:00–20:00)

Height of sampler from ground: ~4m,

Ambient Sample 7

Compound	Sample resp. (arb. units)	Conc. (ppb.)	Conc. (ng./m^3)
F	98159	226.07	53.82
Phen	121696	81.29	19.35
Anth	69712	45.79	10.91
Fl	nd	-	-
Py	nd	-	-
B(a)Anth	nd	-	-
Chrys	nd	-	-
B(b)Fl	nd	-	-
B(k)Fl	nd	-	-
B(a)Py	nd	-	-
Dibenz	nd	-	-
Benzo	nd	-	-
Indeno	nd	-	-
Total			84.08ng/m^3

Ambient Sample 7 Conditions

Location: Glasnevin (Control sample)

Air sampler flow rate: 10.0l./min.

Total volume air sampled: 4200 litres/4.2m^3

Particle size sampled: 4.5 μm.

Analysis: HPLC 1 conditions.

Weather Conditions: Wind speed: 14.3 knots
Wind direction: East-north-east
Precipitation: Trace
Air temp: 11.1°C.

Date: 21/9/98 (Monday)

Sampling period: 7 hours (13:00–20:00)

Height of sampler from ground: ~4m

Ambient Sample 8

Compound	Sample resp. (arb. units)	Conc. (ppb.)	Conc. (ng./m^3)
F	167751	386.34	91.98
Phen	108772	72.65	17.29
Anth	51436	33.79	8.04
Fl	nd	-	-
Py	nd	-	-
B(a)Anth	nd	-	-
Chrys	nd	-	-
B(b)Fl	nd	-	-
B(k)Fl	nd	-	-
B(a)Py	nd	-	-
Dibenz	nd	-	-
Benzo	nd	-	-
Indeno	nd	-	-
Total			117.31ng/m^3

Ambient Sample 8 Conditions

Location; Glasnevin (Control Sample)

Air sampler flow rate: 10.0l./min.

Total volume air sampled: 4200 litres/4.2m^3

Particle size sampled: 10μm.

Analysis: HPLC 1 conditions.

Weather Conditions: Wind speed: 9.8 knots
Wind direction: Variable
Precipitation: Trace
Air temp:10.6°C.

Date: 22/9/98 (Tuesday)

Sampling period: 7 hours (13:00–20:00)

Height of sampler from ground: ~4m

4.12 Analysis of Ambient Results.

Ambient sample 1 was sampled for 24 hours at Newlands Cross. The 24-hour sampling duration was used initially to see if any PAH would be detected, i.e. a "dirty" sample. The inhalable dust fraction 10μm was sampled at a rate of 1.9l/min. Both the 2-3 ringed PAH and the 4-6 ringed PAH were detected. This would be indicative of both petrol and diesel engine emissions (25). Ambient samples 2 and 3 were sampled for 12-hour durations at a rate of 1.9l/min. Both these samples were taken between the hours of 07:00-19:00. This period includes the morning and evening "rush hours", where the traffic volume is quite dense. Ambient sample 2 sampled the inhalable dust 10μm fraction. Relatively high concentrations of PAH were detected in this sample. A worrying result in this sample was the concentration of benzo(a)pyrene detected, 101.88ng/m^3, but perhaps this is a once off level and not a typical representation of the levels usually found, if any. Ambient sample 3 sampled the respirable dust 4.5μm fraction. The concentration of benzo(a)pyrene was found to be 50.53ng/m^3, half the concentration of that detected in sample 2. Ambient sample 2 was taken on a Friday, a busy working day and a day when a lot of people leave Dublin for the weekend, Newlands Cross being one of the routes out of the Dublin. Whereas sample 3 was taken on a Saturday, which would not be as commercially busy a day as Friday. Both samples 2 and 3 have profiles of the 2-3 and 4-6 ringed PAH, i.e. petrol and diesel engine emissions. The 2-3 ringed PAH are predominantly produced from diesel-fuelled bus, taxi, van and car usage. The 4-6 ringed PAH are produced from petrol fuelled vehicles, which forms the majority of rush hour traffic.

Ambient sample 4 was taken at Newlands Cross for an 8-hour duration at a rate of 1.9l/min. The respirable dust 4.5μm fraction was sampled. The 4-6 ringed PAH were mainly detected in this sample. Benzo(a)pyrene was also detected. This 4-6 ringed PAH profile is typical of petrol fuelled vehicles, which are mainly commuter vehicles.

Ambient samples 5 and 6 were taken at the Lower Rathmines Road. The sampling rate was 10.0l/min for these samples, both for 7-hour durations. Sample 5 sampled the 10μm particle size and sample 6 sampled the 4.5μm particle size. Both these samples have a 2-3 ringed PAH profile; fluorene, phenanthrene and anthracene. This profile being typical of diesel fuelled vehicles. The Lower Rathmines Road is frequented by a lot of commercial delivery trucks/vans/buses, and would account for the diesel PAH profiles.

The ambient samples 7 and 8 were taken in a residential area, both for 7 hours at 10.0l/min. These samples also showed a 2-3 ringed PAH profile; fluorene, phenanthrene and anthracene. Sample 7 sampled the 10μm fraction and sample 8 sampled the 4.5μm fraction. Both samples showed similar concentration profiles. This PAH profile could be characteristic of a domestic heating, a stationary diesel source(discussed in chapter 1, section 1.1.2), as this is a quite area with a small traffic volume flow.

As already mentioned in chapter 1, section 1.6.5 a baseline study on the concentration of PM_{10} and volatile organic compounds in Dublin city was carried out by TMS Environmental Ltd/Dublin Corporation Environmental Health Officers Services for the EPA. The results of some of the samples for PAH are given in the next section.

4.13 PAH Results in the Baseline Study.

PTFE filters were used as filter media in this study in order to facilitate organic compositional analysis of PM_{10} particulates (26). The filters were collected after exposure for a 24-hour interval, with typical air volumes of 24-25 m^3 per day (26). The analytical procedures used for the PAH determination in this study were adapted from USEPA Method TO-19: Determination of PAH in Ambient Air Using High-Volume Sampling with GCMS or HPLC Analysis (27). The method involves shredding the filters and extracting them in pesticide grade dichloromethane for periods of 24-72 hours in a Soxhlet apparatus. The solvent is then concentrated in a Kuderna Danish concentrator to ca. 1ml. final volume. The samples are cleaned up prior to analysis using conventional clean-up procedures and analysed using GCMS. The quantitative analysis results of PAH concentrations are shown in table 4.5.

The samples from batch numbers B1, B2, B3 and B4-B5 were taken at College Street, with air volumes of 487.027 m^3, 516.162 m^3, 522.941 m^3 and 539.395 m^3 respectively (26). These samples show the highest concentrations of PAH in the study. The samples from batches B8, B9-11 were taken at Rathmines Road, with air volumes 476.051 m^3 and 2022.572 m^3 respectively. The samples from batches B14, B15-18 were taken at the Phoenix Park with air volumes 483.924 m^3 and 2145.100 m^3 respectively (26). One should note that each filter analysed was cut in half, weighed; half of each of the filters was extracted for organic compositional analysis and half was retained for inorganic analysis.

PAH	Concentration of PAH in PM_{10} expressed as airborne concentration, ng/m^3.							
	B1	B2	B3	B4-5	B8	B9-11	B14	B15-19
Naph.	<2.05	<1.94	<1.91	<0.93	<2.10	<0.49	<2.07	11.3
Aceny.	24.1	<1.94	<1.91	<0.93	<2.10	251.3	17.0	<0.47
Acen.	<2.05	<1.94	<1.91	<0.93	<2.10	<0.49	<2.07	<0.47
F.	<2.05	<1.94	<1.91	<0.93	<2.10	<0.49	<2.07	<0.47
Phen.	<2.05	<1.94	<1.91	<0.93	<2.10	4.38	23.8	<0.47
Anth.	<2.05	<1.94	<1.91	<0.93	<2.10	<0.49	<2.07	<0.47
Fl.	<2.05	20.9	<1.91	18.3	<2.10	11.99	21.3	<0.47
Py.	<2.05	20.6	<1.91	19.9	<2.10	8.54	24.1	11.8
B(a)anth.	<10.1	<9.7	<9.6	<4.64	<10.5	<2.45	<10.35	<2.35
Chrys.	<10.1	<9.7	<9.6	<4.64	<10.5	<2.45	<10.35	<2.35
B(b)Fl +B(k)Fl	<10.1	<9.7	<9.6	<4.64	<10.5	<2.45	<10.35	<2.35
B(a)Py + Dibenz	<10.1	<9.7	<9.6	<4.64	<10.5	<2.45	<10.35	<2.35
Benzo + Indeno	<10.1	<9.7	<9.6	<4.64	<10.5	<2.45	<10.35	<2.35

Table 4.5. Results of the Baseline study in Dublin City, showing quantitative analysis of PAH in PM_{10} filter samples (27)

Monitoring dates:

B1 28/9/1996-26/10/1996.
B2 27/10/1996-17/11/1996.
B3 18/11/1996-8/12/1996.
B4-5 9/12/1996-28/1/1997.
B8 24/9/1996-19/10/1996.
B9-11 20/10/1996-20/12/1996.
B14 24/9/1996-20/10/1996.
B15-18 20/10/1996-19/1/1997.

4.14 Results of Baseline Study Samples when Applied to the Method Developed in this Research.

Some of the filters from this study were kept in storage for reference or further analysis purposes. The Air Pollution and Noise Unit of the Dublin Corporation were kind enough to give some of these samples to this research for the purposes of PAH analysis and quantification using the method developed in this research. The results of the PAH quantification and details of the sampling conditions are given in this section. The chromatograms are shown in Appendix D.

Baseline sample 1

Compound	Sample resp. (arb. units)	Conc. (ppb.)	Conc. (ng./m^3)
F	8127	18.72	0.77
Phen	21058	14.06	0.58
Anth	111611	73.32	3.00
Fl	-	-	-
Py	9092	50.37	2.06
B(a)Anth	-	-	-
Chrys	11221	26.25	1.07
B(b)Fl	8607	24.82	1.0.2
B(k)Fl	32194	9.38	0.38
B(a)Py	9467	10.94	0.45
Dibenz	-	-	-
Benzo	-	-	-
Indeno	-	-	-
Total			9.33ng/m^3

Baseline Sample 1 Conditions

Location: Merchants Quay.

Total volume air sampled: 24.434 m^3

Distance of sampler from roadway: ~5m.

PM_{10} Concentration: 77μg/m^3

Weather Conditions: Wind speed: 8.2 knots
Wind direction: Variable
Precipitation: 2.26mm
Air temp: 10.1°C.

Date: 8/4/97

Sampling duration: 10.5 hours.

Particle size sampled: 10μm.

Analysis: HPLC 1 conditions.

Baseline sample 2

Compound	Sample resp. (arb. units)	Conc. (ppb.)	Conc. (ng./m^3)
F	67017	154.34	6.37
Phen	53161	35.50	1.46
Anth	336945	221.36	9.13
Fl	11327	45.49	1.88
Py	27355	151.55	6.25
B(a)Anth	40201	19.07	0.78
Chrys	23805	55.69	2.29
B(b)Fl	22115	36.78	2.63
B(k)Fl	64233	18.71	0.77
B(a)Py	26913	31.10	1.28
Dibenz	-	-	-
Benzo	-	-	-
Indeno	4802	48.70	2.01
Total			34.85ng/m

Baseline Sample 2 Conditions

Location: Merchants Quay

Total volume air sampled: 24.229 m^3

Distance of sampler from roadway; ~5m.

PM_{10} Concentration: 22μg/m^3

Weather Conditions: Wind speed: 10.6 knots
Wind direction: Variable
Precipitation: 5.6mm
Air temp: 7.3°C.

Date: :28/4/97

Sampling duration: 10.5 hours.

Particle size sampled: 10μm.

Analysis: HPLC 1 conditions.

Baseline sample 3

Compound	Sample resp. (arb. units)	Conc. (ppb.)	Conc. (ng./m^3)
F	24484	56.39	2.28
Phen	77844	51.99	2.10
Anth	163431	107.37	4.34
Fl	-	-	-
Py	21051	116.62	4.72
B(a)Anth	15179	7.20	0.29
Chrys	15349	35.91	1.45
B(b)Fl	12585	36.29	1.46
B(k)Fl	41201	11.99	0.48
B(a)Py	18868	21.81	0.88
Dibenz	-	-	-
Benzo	-	-	-
Indeno	-	-	-
Total			18.00ng/m^3

Baseline Sample 3 Conditions

Location: Merchants Quay.

Total volume air sampled: 24.710 m^3

Distance of sampler from roadway: ~5m

PM_{10}Concentration: 76μg/m^3

Weather Conditions: Wind speed: 6.8 knots
Wind direction: Variable
Precipitation: 3.9mm
Air temp: 6.4°C.

Date: 30/4/97

Sampling duration: 10.5 hours.

Particle size sampled: 10μm.

Analysis: HPLC 1 conditions.

Baseline sample 4

Compound	Sample resp. (arb. units)	Conc. (ppb.)	Conc. (ng./m^3)
F	-	-	-
Phen	-	-	-
Anth	-	-	-
Fl	-	-	-
Py	30707	170.12	6.89
B(a)Anth	4364	2.07	0.08
Chrys	11096	25.96	1.05
B(b)Fl	4414	12.73	0.52
B(k)Fl	14922	4.34	0.17
B(a)Py	3665	4.23	0.18
Dibenz	-	-	-
Benzo	-	-	-
Indeno	-	-	-
Total			8.86ng/m^3

Baseline Sample 4 Conditions

Location: Merchants Quay.

Total volume air sampled: 24.687 m^3

Distance of sampler from roadway: ~5m

PM_{10} Concentration: 41μg/m^3

Weather Conditions: Wind speed: 4.6 knots
Wind direction: Variable
Precipitation: 14.8mm
Air temp. 6.7°C.

Date: 1/5/97

Sampling duration 10.5 hours

Particle size sampled: 10μm.

Analysis: HPLC 1 conditions.

4.14.1 Analysis of the Baseline Sample Results when Applied to the Method Developed in this Research.

All the baseline samples analysed using the method developed in this research were taken at the Merchants Quay site. The measurement period was extended for the Merchants Quay site in the Baseline Study in order to obtain a representative coverage of the site. The extended period measurements are reported for the period October '96 to April '97 (26). This site only came on stream during October '96, so all the measurements relate to wintertime, it is expected that this average may decrease when summer data is included (26).

Baseline samples 1, 2 and 3 quantify both the 2-3 ringed and 4-6 ringed PAH, which arise from diesel and petrol fuelled vehicles (25). The levels of individual PAH quantified using the validated method are relatively low in comparison with some of the ambient sample results. Baseline sample 1 shows diesel and petrol PAH profiles, astone would expect from this busy roadway site. The PM_{10} concentration measured on the 8/4/97 was 77$\mu g/m^3$ (over a 10.5-hour period), the PM_{10} 24-hour standard is 150$\mu g/m^3$ (particulate pollution standards already discussed in chapter 1, section 1.6).

The baseline study represents the first compositional analysis of PM_{10} in Ireland and one of the few studies internationally on organic composition of PM_{10} (26). The results were encouraging and the project will form a sound basis for extending the scope of work to a more comprehensive study of organic composition of PM_{10} and associated PAH levels. With a more comprehensive series of results and improved detection limits, conclusions may also be drawn about the main source contributors to PM_{10} in Dublin City. The PAH concentrations of the baseline samples (26) are lower than the PAH concentrations in the ambient environmental samples taken in this work. This may be accounted for in the different sampling techniques used in the baseline study and in this work. The baseline study's PM_{10} monitoring equipment was a Partisol Model 2000 Air Sampler. The Partisol

sampler is a USEPA reference method for the measurement of PM_{10} mass concentrations (26). The sampler is a low flow gravimetric PM_{10} sampler, which can be operated as a stand-alone unit or combined with three additional satellite PM_{10} sampling heads and filter units. Air is drawn in through the PM_{10} inlet at a flow rate of 16.7l/per hour. The particles were collected using a 47mm diameter glass fibre filters. The air sampler used in this work was SKC Cyclone units (PM_{10} or $PM_{4.5}$) attached via plastic tubing to an SKC Pump (no. 244-43XE) which has a built in flow-meter (set at flow rate of 1.9l/min). The particles were collected using 25mm diameter glass fibre filters. The sampling height and distance from the source could be factors in the different levels found. Other factors such as the meteorological conditions may account for the higher levels of PAH concentrations found in this work. The samples in this work were taken with trace precipitation, where as the baseline air samples all have precipitation recorded during the sampling period.

The levels of PAH found in the baseline samples analysed using the method developed in this research were in keeping with the levels shown in table 4.5. The PAH levels in table 4.5 were sampled between Jan'96 and Jan'97, where as the samples analysed in this research were taken after this period, even so, levels are quite similar.

Most of the PAH detected in the baseline samples and analysed in this research were also found in the Baseline Study, giving more creditibility to the method developed in this research.

4.15 Comparison of Types and Levels of PAH found in Indoor and Ambient and Baseline Samples.

The types of PAH detected in pub 1 and 2 samples were mainly the 4-6 ringed, which as already mentioned are the more potent carcinogens of the USEPA 16 priority pollutants. Anthracene, fluoranthene and pyrene were the only 2-3 ringed PAH found in the pub samples. The types and levels of particulate bound PAH detected in both pubs samples were similar, this infers that there is no real difference in PAH profiles in both the bar and lounge areas of a pub. Table 4.6 represents a typical PAH profile in PM_{10} and $PM_{4.5}$ fractions established in this research.

PAH	Pub 1 Sample C $PM_{4.5}$ Fraction (ng/m^3)	Pub 2 Sample PM_{10} Fraction (ng/m^3)
Fl.	-	10.95
Py.	21.59	24.63
B(a)Anth.	10.45	1.68
Chrys.	37.09	8.39
B(b)Fl.	92.36	9.22
B(k)Fl.	55.33	2.14
B(a)Py.	8.88	-

Table 4.6 A Typical Profile of Particulate Bound (PM_{10} and $PM_{4.5}$) found in Tobacco Smoke.

The concentration levels of the 4-6 ringed PAH are a lot higher in the $PM_{4.5}$ size fraction than in the PM_{10} size fraction, indicating they have a susceptibility for the respirable dust fraction. This highlights the occupational hazard of bar staff, and shows that they are a high risk category for potentially fatal illnesses or diseases related to tobacco smoke constituents.

Table 4.7 shows a typical particulate bound PAH profile for ambient air samples at a traffic congested roadway (Newlands Cross). Diesel and Petrol vehicle emissions are the main contribution sources to the 2-3 and 4-6 ringed PAH levels detected.

PAH	Ambient Sample 3 $PM_{4.5}$ Fraction (ng/m^3)	Ambient Sample 1 PM_{10} Fraction (ng/m^3)
F.	-	122.25
Phen.	10.83	-
Anth.	-	2.59
Fl.	23.75	9.98
Py.	90.49	25.91
B(a)Anth.	11.73	18.07
Chrys.	-	0.66
B(b)Fl.	22.24	38.61
B(k)Fl.	56.74	25.72
B(a)Py.	50.53	30.87
Dibenz.	4.15	1.35
Benzo.	2.79	-
Indeno.	-	-

Table 4.7 A Typical Profile of Particulate Bound (PM_{10} and $PM_{4.5}$) found in Ambient Air Samples at a Traffic Congested Roadway.

More of 2-3 ringed PAH compounds are more seen in the PM_{10} fraction than the $PM_{4.5}$ fraction. The levels of the 4-6 ringed particulate bound PAH are higher in the $PM_{4.5}$ fraction, as seen in the indoor samples. The relatively large concentration of benzo(a)pyrene in the $PM_{4.5}$ fraction in comparison to the PM_{10} fraction is alarming, in that smaller respirable fraction penetrates and lodges into the alveoli of the lungs (14).

Table 4.8 shows a particulate bound PAH profile for the PM_{10} fraction of an ambient air sample taken at a traffic congested roadway in the Baseline Study. As with the ambient air sample taken in this research both 2-3 and 4-6 ringed PAH compounds are seen.

PAH	Baseline Sample 2 $PM_{4.5}$ Fraction (ng/m^3)
F.	6.37
Phen.	1.46
Anth.	9.13
Fl.	1.88
Py.	6.25
B(a)Anth.	0.78
Chrys.	2.29
B(b)Fl.	2.63
B(k)Fl.	0.77
B(a)Py.	-
Dibenz.	-
Benzo.	-
Indeno.	2.01

Table 4.8 A Typical Profile of Particulate Bound (PM_{10}) found in Ambient Air Samples taken in the Baseline Study.

The levels of the PAH in the PM_{10} fraction in are lower than the ambient PAH levels in the PM_{10} fraction profile shown in table 4.7, this is attributed to the different sampling procedures employed in the Baseline Study and in this research.

4.16 Analysis of Environmental Samples by GCMS.

One of the recommendations made in the Baseline Study was the possibility of improving sensitivity towards the compounds of interest with analysis by HPLC with fluorescence detection, as opposed to the GCMS analysis used (27). The developed method in this work has shown that analysis by HPLC with fluorescence detection is very sensitive and specific to PAH, with low limits of detection. Some of the samples taken in this work were also analysed by GCMS for further confirmatory identification of the individual PAH quantified by the HPLC with fluorescence detection analysis (see chapter 2, section 2.7 for conditions). The next section shows the results of the GCMS analysis.

GC is probably the most common technique used for the analysis of mixtures of aromatic hydrocarbons present in environmental samples due to the high separation efficiency and potential low detection limits associated with this method (28). However, separation efficiency and sensitivity are strongly related to optimum chromatographic conditions and one must determine the best conditions for the analysis. Several factors should be considered when optimising chromatographic parameters. Examples include solvent effects, injection parameters (speed, liner size, sample size, temperature), and column stationary phase (28). Although packed liquid crystal columns offered the advantage of high selectivity to PAH isomers, their use was severely limited due to their thermal instability and narrow range of molecular mass separation capability. However, these limitations have been overcome with the advent of capillary liquid crystal columns. Ultimately, the use of packed column GC for the determination of PAH generally became obsolete as highly efficient capillary columns with thermally stable stationary phases became available. Capillary GC columns provide excellent PAH separations with low background interference and long stability times, both of which facilitate accurate quantitation of PAH. One of the most widely used stationary phases in environmental analyses consists of a phenyl-methylpolysiloxane polymer (~5% phenyl). Columns prepared with this phase

exhibit high efficiency for PAH, furthering the accuracy of PAH quantification. Capillary GC has traditionally been used for the determination of PAH containing up to 24 carbon atoms. However, the development of stationary phases stable at elevated temperatures (e.g., above 300°C) has facilitated the determination of large PAH compounds (molecular mass >300) in environmental samples (28).

GCMS is often more accurate than GCFID for the quantification of PAH because interferences from co-eluting compounds are minimised by the selective nature of the detector. GCMS is frequently used to characterise PAH in environmental samples. Prior to using GCMS for the measurement of PAH in environmental samples, the samples are usually solvent extracted, concentrated and cleaned-up using solid phase extraction to remove potential interfering polar constituents. In addition, calibration solutions are typically processed and analysed alongside the samples to generate individual MS response factors relative to the internal standards for quantification purposes (28). The library search facility on the GCMS software package is a fast and efficient tool in determining the identification of the resultant peaks on the gas chromatograph. The software gives a % probability of an MS identity match from its MS library of compounds.

4.16.1 Confirmatory Identification of PAH in the Environmental Sample.

An air sample was taken in Pub 1, (Pub 1 Sample E) and the particulate bound PAH analysed with the method developed in this work. This sample was also analysed by GCMS for the purpose of further unambiguous identification (see Experimental, section 2.7 for conditions). The results of the confirmatory identification are given in this section.

Compound	Pub1 Sample E		
	Sample resp. (arb. units)	Conc. (ppb.)	Conc. (ng./m^3)
F	nd	-	-
Phen	nd	-	-
Anth	nd	-	-
Fl	nd	-	-
Py	20099	111.35	122.09
B(a)Anth	6931	3.29	3.61
Chrys	nd	-	-
B(b)Fl	4884	14.09	15.45
B(k)Fl	23234	6.77	7.42
B(a)Py	152974	17.87	19.59
Dibenz	nd	-	-
Benzo	nd	-	-
Indeno	nd	-	-

Pub 1 Sample E.

Date: 20/6/98 (Saturday).

Sampling period: 8 hours (15:30 – 23:30).

Air sampler flow rate: 1.9l./min.

Total volume air sampled: 912 litres./0.912m^3.

Particle size sampled: 10.0μm.

Analysis: HPLC 1 conditions.

The sample was then analysed by GCMS. First a standard mixture of PAH was run on the GCMS, the individual PAH and their retention times are given in table 4.9. The results of the air sample (Pub 1 Sample E) are given in table 4.10. See Appendix D for chromatograms.

PAH Analyte	Molecular Mass	Retention Time (mins)
F.	166	8.02
Anth.	178	9.53
Phen.	178	9.61
Fl.	202	11.44
Py.	202	11.79
B(a)Anth.	228	13.73
Chrys.	228	13.79
B(b)Fl.	252	15.82
B(k)Fl.	252	15.88
B(a)Py.	252	16.62
Indeno.	276	20.34

Table 4.9. Standard Retention Times of PAH Mixture Run on GCMS

PAH Analyte	Retention Time (mins)	% Probability Match by MS Library Search
Anth.	9.53	58
Py.	11.79	87
B(a)Anth.	13.84	92
B(b)Fl.	15.83	90
B(k)Fl.	15.88	90
B(a)Py.	16.62	96

Table 4.10. Results of Environmental Sample Run on GCMS.

From the GCMS results in table 4.10, one can see that the individual PAH compounds identified by HPLC with fluorescence detection are also identified by GCMS analysis. This shows the method developed in this research to be efficient and accurate in the analysis of particulate bound PAH in environmental samples.

4.17 Summation.

The indoor real samples showed a predominantly 4-6 ringed PAH profile for both the PM_{10} and the $PM_{4.5}$ dust fractions, but higher levels of these particulate bound PAH were found in the smaller, respirable fraction. This is cause for concern among bar staff as these people are continuously exposed to such types and levels of PAH as part of their working day.

The ambient real samples exhibited a 2-3 ringed as well as a 4-6 ringed particulate bound PAH profile for the Newlands Cross air samples. The levels of PAH were again higher in the $PM_{4.5}$ dust fraction than in the PM_{10} dust fraction. The Rathmines and Glasnevin air samples showed only a 2-3 ringed PAH profile, attributed to diesel fuelled vehicles and domestic heating. The PM_{10} dust fraction showed slightly higher levels of these PAH than the $PM_{4.5}$ fraction.

The Baseline Study samples analysed in this research showed both 2-3 and 4-6 ringed particulate bound PAH. Although the levels were lower than those found in the actual Baseline Study Final Report (26), the types of PAH detected were the same, giving creditibility to the method developed in this research.

The ambient types and levels of PAH detected in this research are profiles expected in urban areas. A study carried out in an urban area of Berlin, Germany also detected similar types and levels of particulate bound (inhalable fraction) PAH (29).

4.18 References.

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Conclusion.

A quick and efficient method for the analysis of the 16 PAH, listed by the USEPA as "priority pollutants", in both indoor and ambient air particulates has been developed and partially validated.

The applicability of the method to "real" samples was seen in chapter 4, where it was applied to both indoor (pubs) and ambient air samples. The indoor air samples showed mainly a 4-6 ringed PAH profile, indicative of the more potent carcinogens on the USEPA list. The source of the PAH were tobacco smoke. The levels of these more potent 4-6 ringed particulate bound PAH quantified indicate that continuous or frequent exposure to such chemicals could have adverse health effects. While the patrons of the pub are at risk of illness related to inhalable (PM_{10}) and respirable ($PM_{4.5}$) PAH bound particulates, the people most at risk are the bar staff. This was highlighted in the levels of PAH quantified in Pub 2 Sample D, where the air sampler was in the path of the air been drawn towards the bar by an extraction fan system.

The ambient samples, taken at 3 locations, showed both 2-3 and 4-6 ringed PAH profiles. The first location, Newlands Cross in Tallaght, showed both the 2-3 and 4-6 ringed PAH profiles. These profiles could be attributed to the heavy traffic volume utilising this route. The 2-3 ringed PAH profiles are representative of diesel fuelled vehicles, whereas the 4-6 ringed PAH profiles are associated with petrol fuelled vehicles. The Rathmines and Glasnevin sampling sites showed 2-3 ringed PAH profiles. These profiles can be attributed to diesel fuelled vehicles as well as combustion of domestic heating fuel.

The Baseline Study samples acquired form Dublin Corporation and applied to the method developed in this research, quantified PAH levels at Merchants Quay,

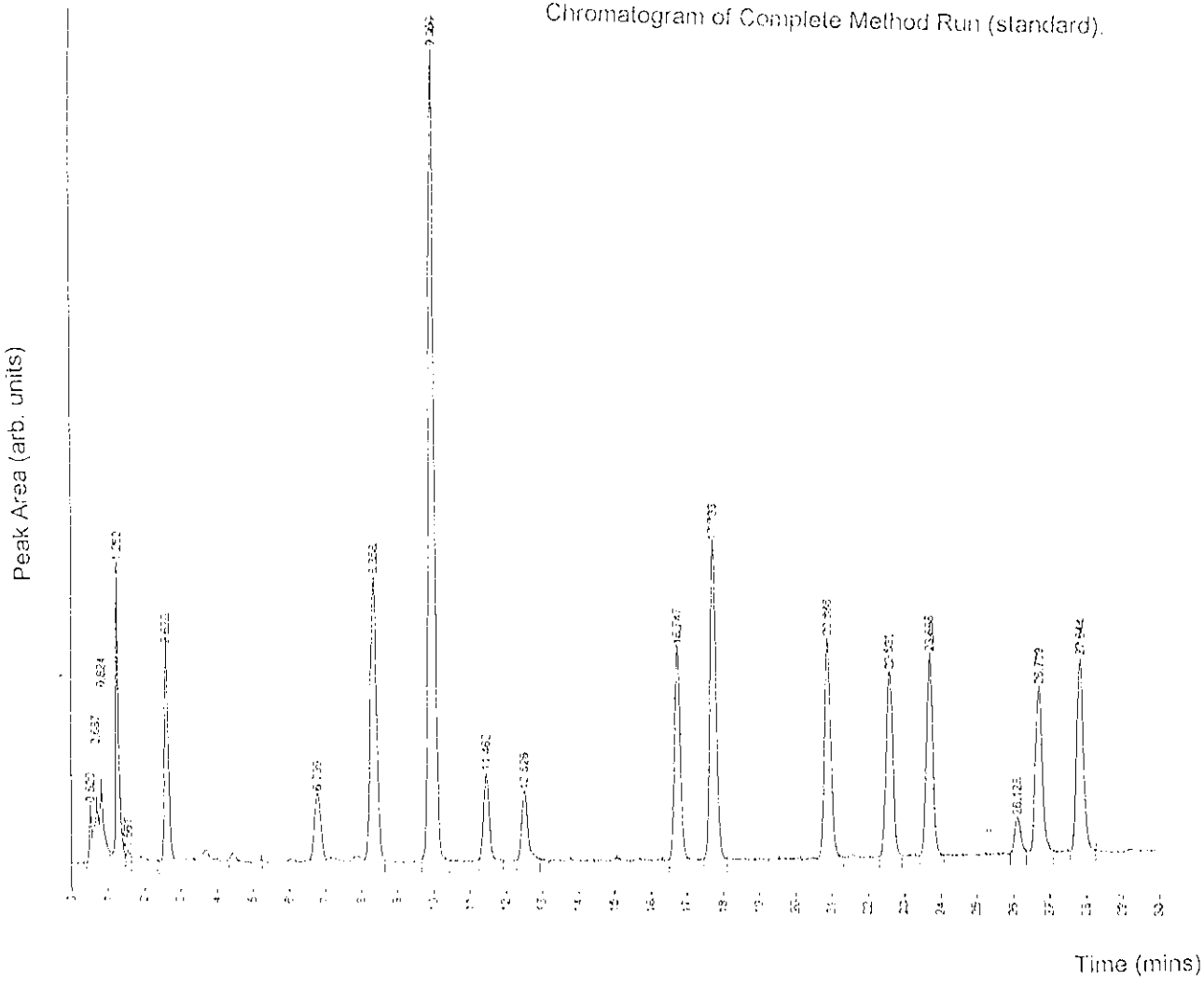
Dublin. The analysis of filters from the Baseline Study gave results in the same range as those previously obtained by GCMS analysis.

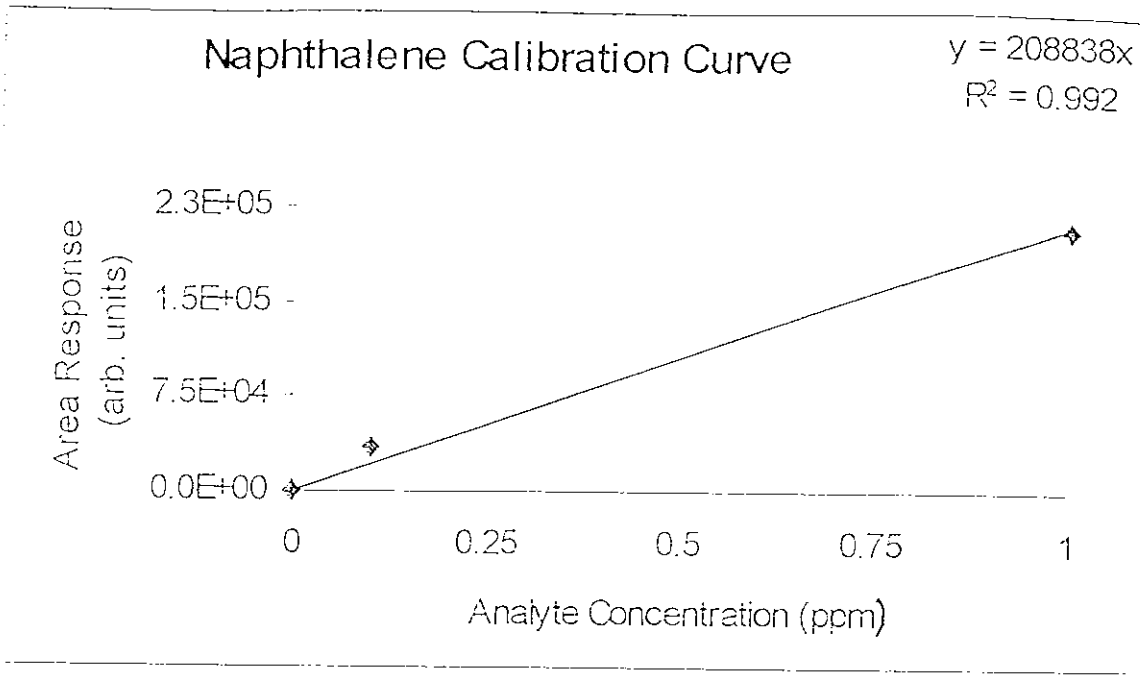
One of the recommendations of this study was that a survey on PAH levels in Dublin city should be carried out. The method developed in this research is a quick and relatively inexpensive procedure for the detection of PAH in air particulates.

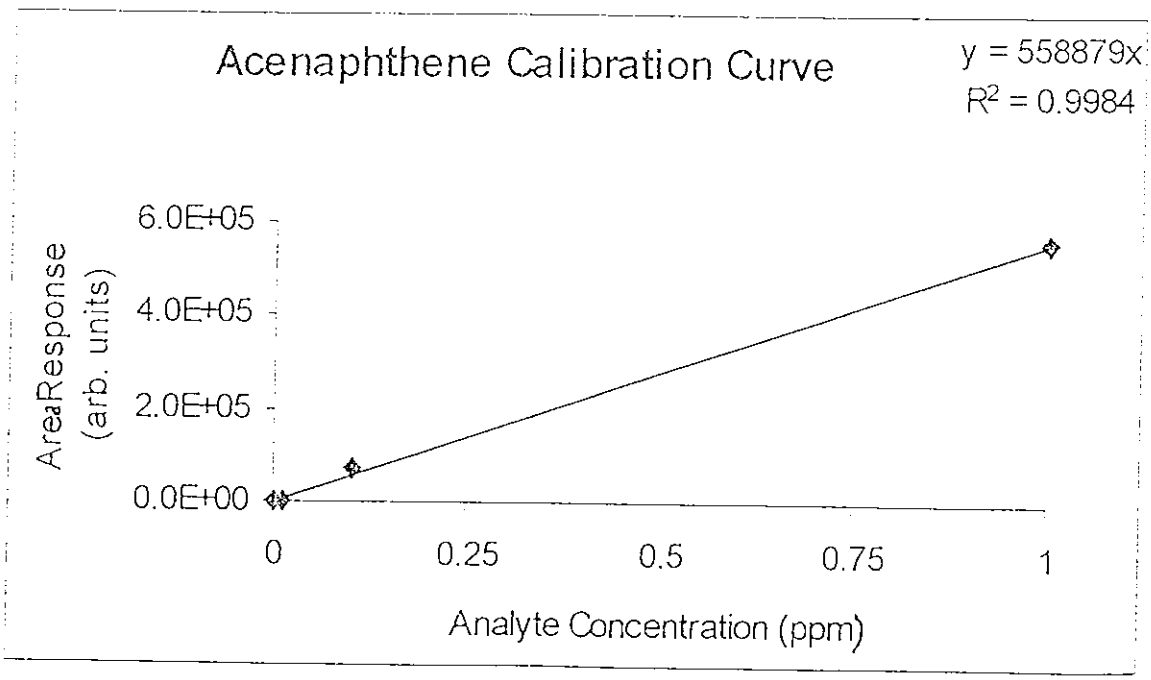
Appendix A.

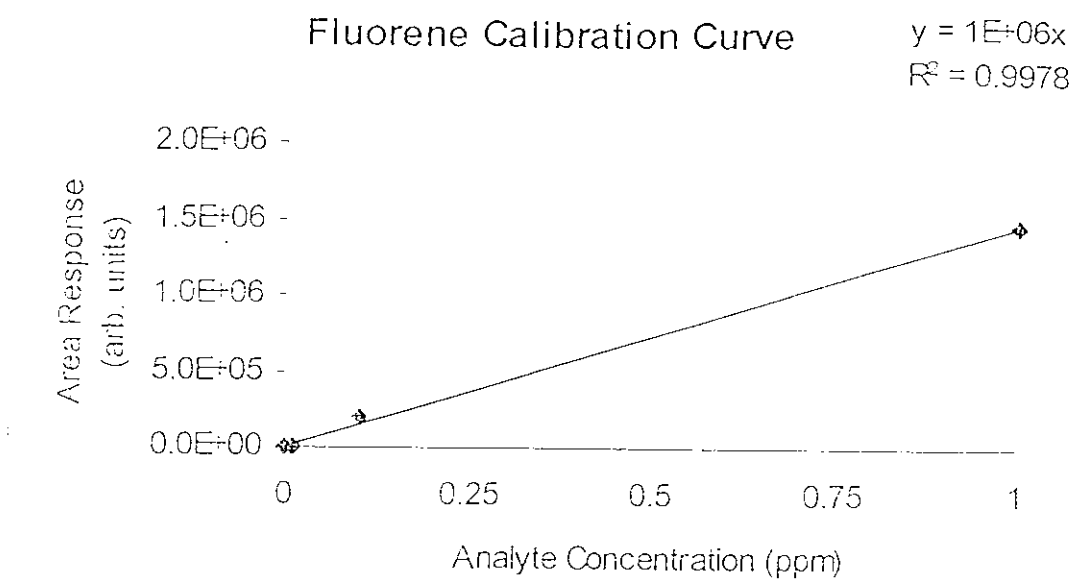
16 PAH Standard Mixture (HPLC 1 conditions).

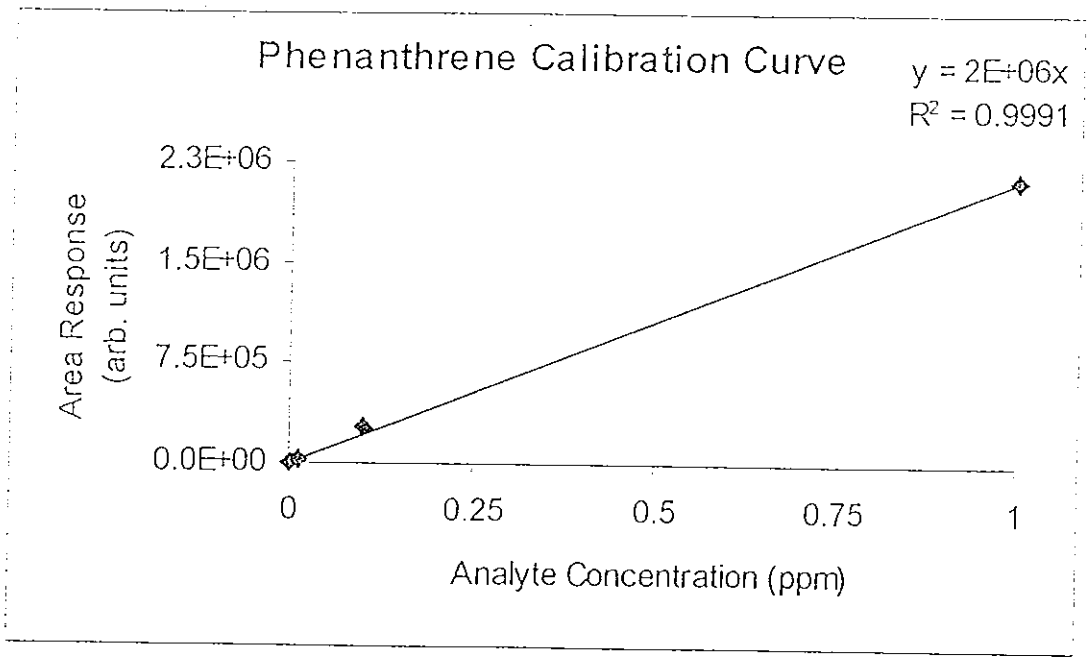
16 PAH Standard Mixture (HPLC 2 conditions).

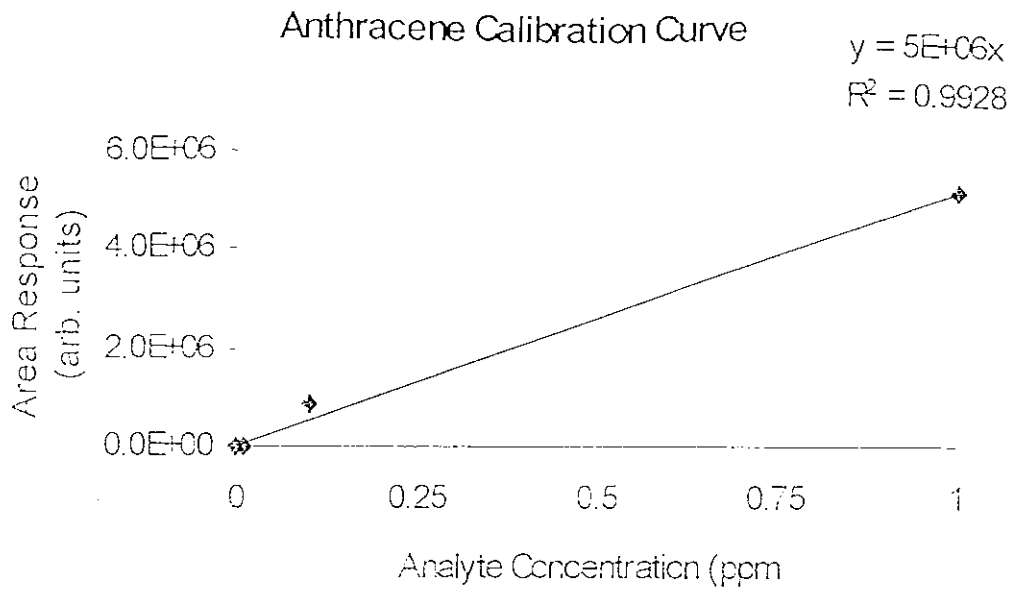

Chromatogram of Complete Method Run (standard).
Peak Area (arb. units)
Time (mins)

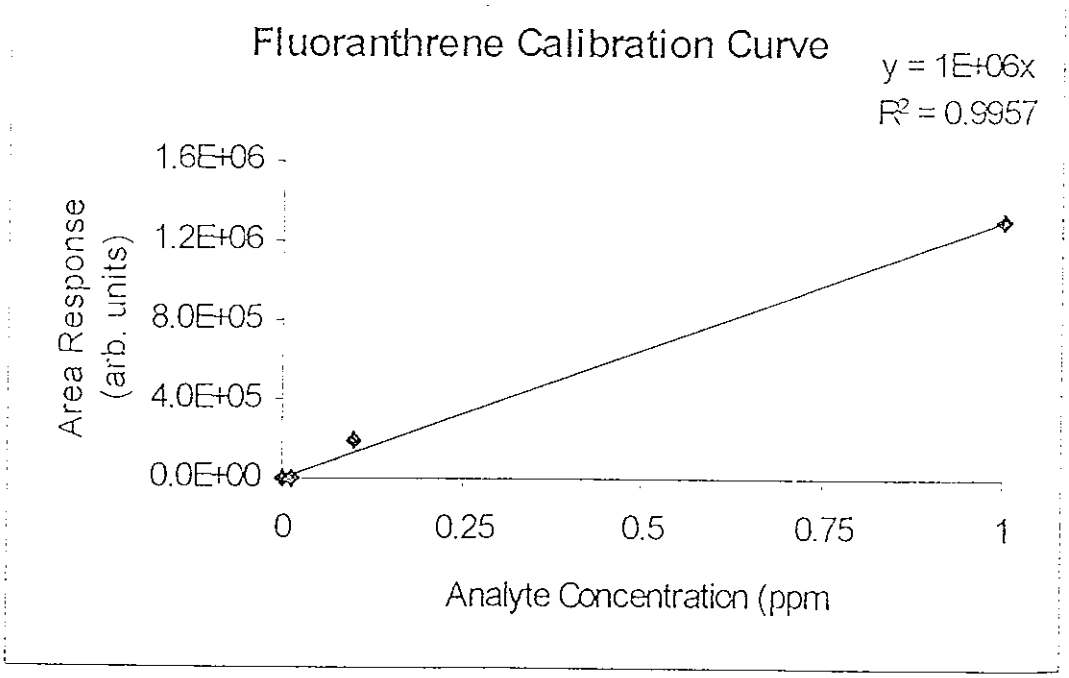

Naphthalene Calibration Curve
y = 208838x
R² = 0.992
Area Response (arb. units)
2.3E+05
1.5E+05
7.5E+04
0.0E+00
0
0.25
0.5
0.75
1
Analyte Concentration (ppm)

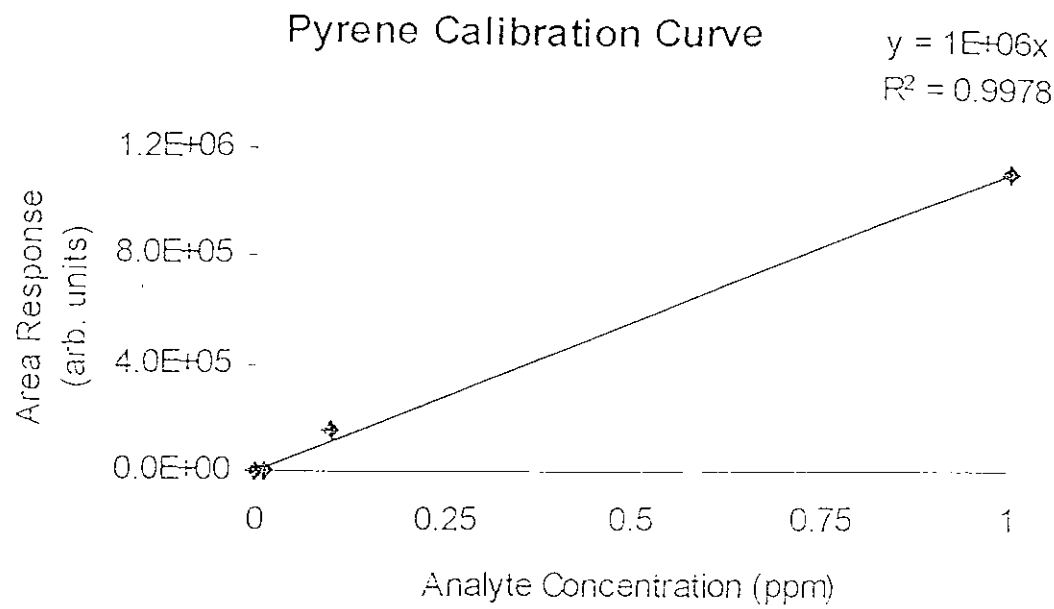

Acenaphthene Calibration Curve
y = 558879x
R² = 0.9984
Area Response (arb. units)
6.0E+05
4.0E+05
2.0E+05
0.0E+00
0
0.25
0.5
0.75
1
Analyte Concentration (ppm)

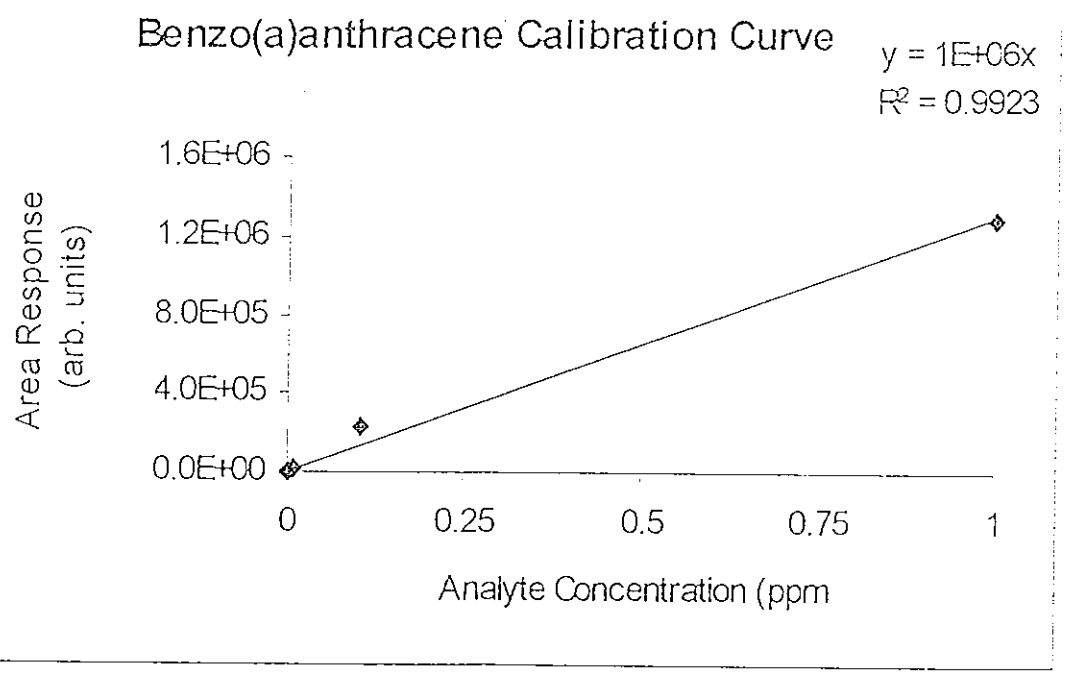

Fluorene Calibration Curve
y = 1E+06x
R² = 0.9978
2.0E+06
1.5E+06
1.0E+06
5.0E+05
0.0E+00
Area Response (arb. units)
0
0.25
0.5
0.75
1
Analyte Concentration (ppm)

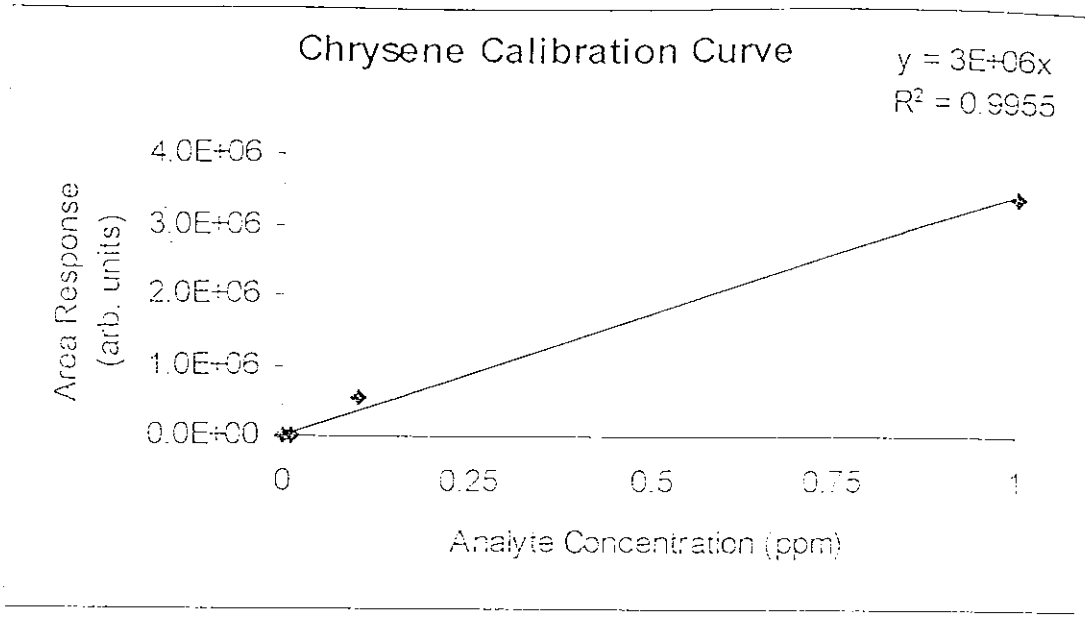

Phenanthrene Calibration Curve
y = 2E+06x
R² = 0.9991
2.3E+06
1.5E+06
7.5E+05
0.0E+00
Area Response (arb. units)
0
0.25
0.5
0.75
1
Analyte Concentration (ppm)

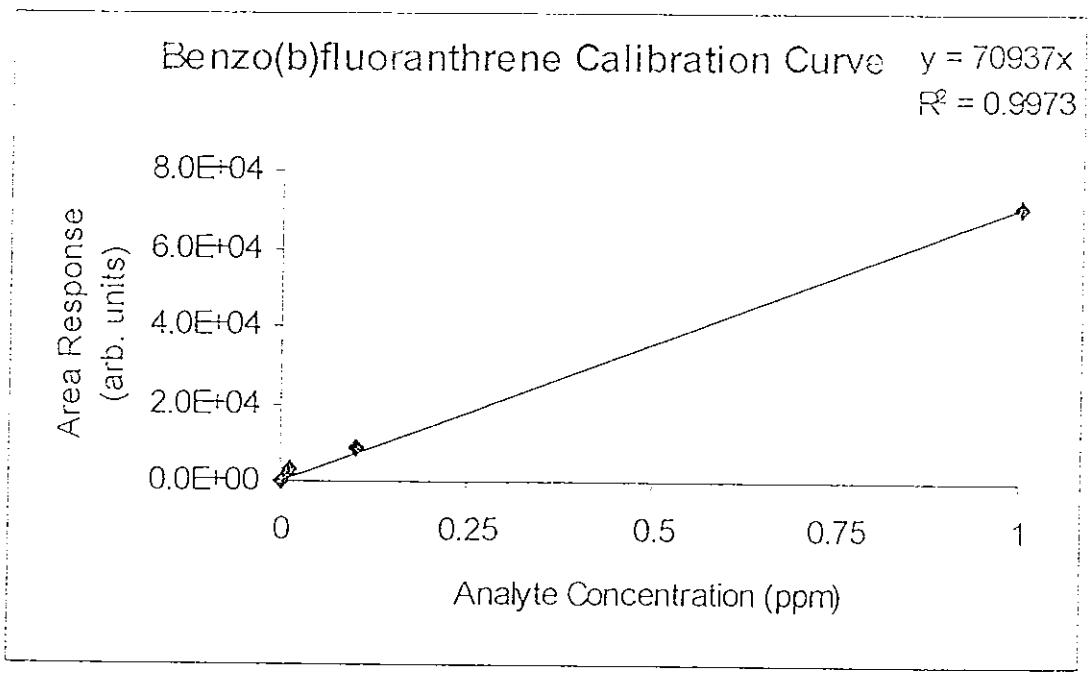

Anthracene Calibration Curve
y = 5E+06x
R² = 0.9928
6.0E+06
4.0E+06
2.0E+06
0.0E+00
Area Response (arb. units)
0
0.25
0.5
0.75
1
Analyte Concentration (ppm

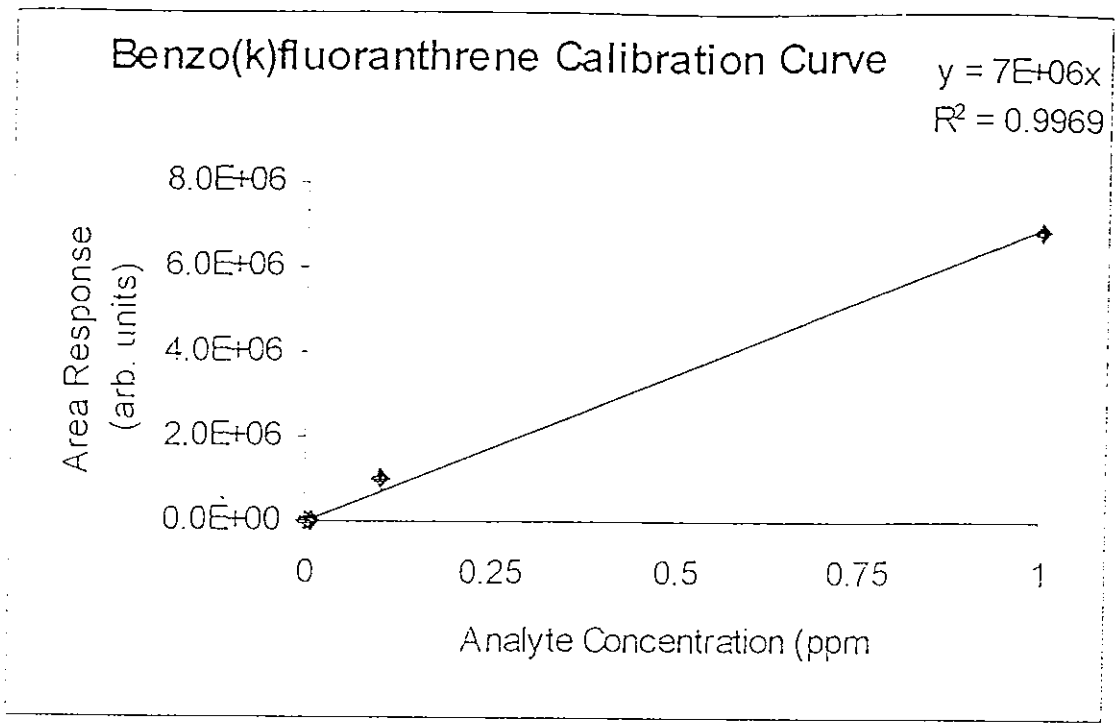

Fluoranthrene Calibration Curve
y = 1E+06x
R² = 0.9957
1.6E+06
1.2E+06
8.0E+05
4.0E+05
0.0E+00
Area Response (arb. units)
0
0.25
0.5
0.75
1
Analyte Concentration (ppm

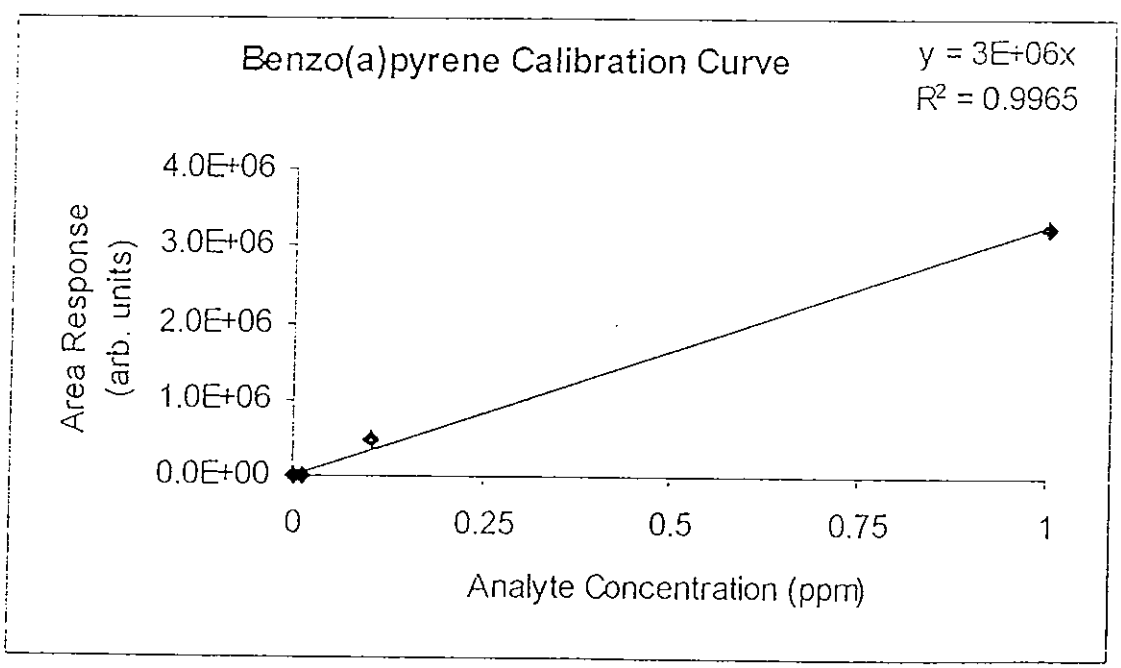

Pyrene Calibration Curve
y = 1E+06x
R² = 0.9978
1.2E+06
8.0E+05
4.0E+05
0.0E+00
Area Response (arb. units)
0
0.25
0.5
0.75
1
Analyte Concentration (ppm)

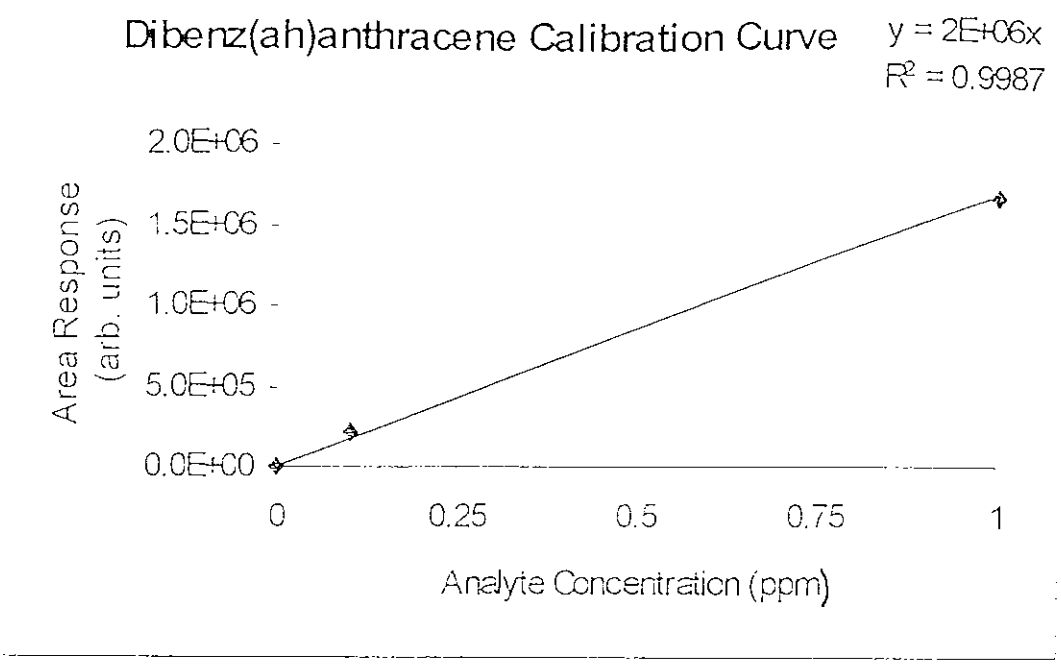

Benzo(a)anthracene Calibration Curve
y = 1E+06x
R² = 0.9923
1.6E+06
1.2E+06
8.0E+05
4.0E+05
0.0E+00
Area Response (arb. units)
0
0.25
0.5
0.75
1
Analyte Concentration (ppm

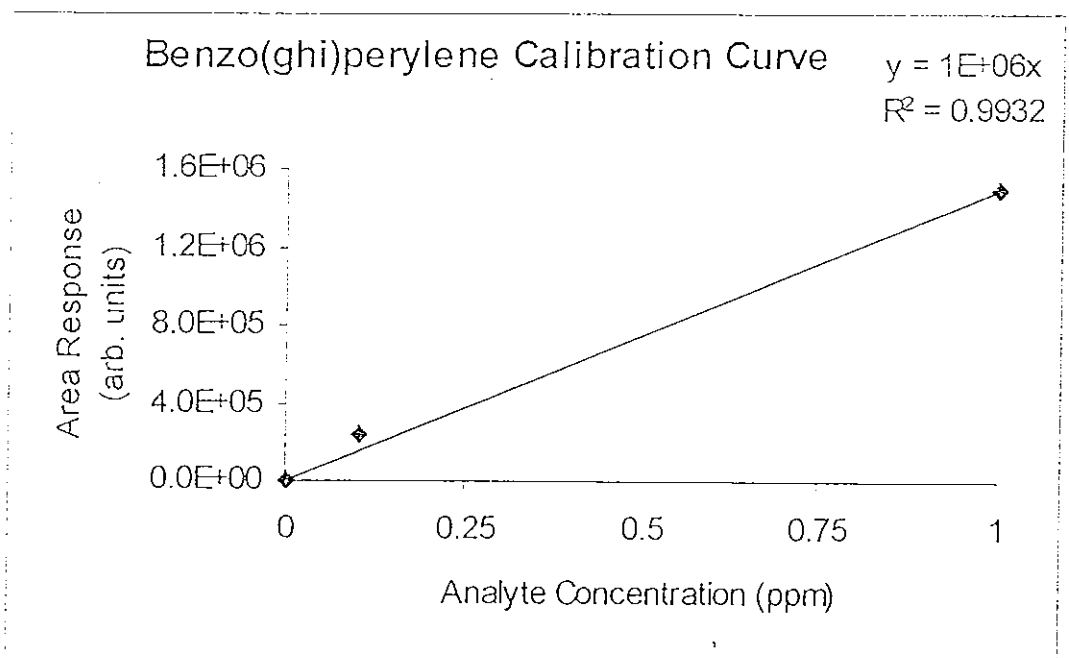

Chrysene Calibration Curve
y = 3E+06x
R² = 0.9955
4.0E+06
3.0E+06
2.0E+06
1.0E+06
0.0E+00
Area Response (arb. units)
0
0.25
0.5
0.75
1
Analyte Concentration (ppm)

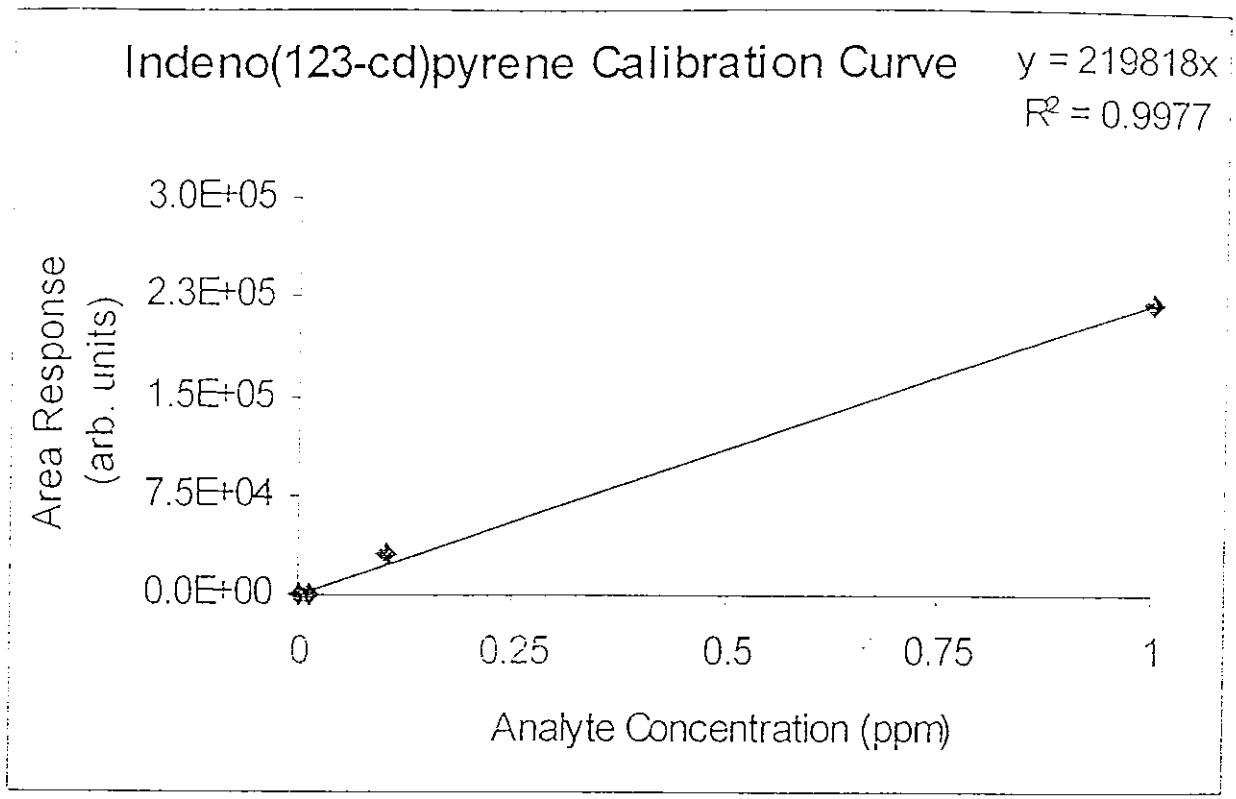

Benzo(b)fluoranthrene Calibration Curve
y = 70937x
R² = 0.9973
8.0E+04
6.0E+04
4.0E+04
2.0E+04
0.0E+00
Area Response (arb. units)
0
0.25
0.5
0.75
1
Analyte Concentration (ppm)


Benzo(k)fluoranthrene Calibration Curve
y = 7E+06x
R² = 0.9969
8.0E+06
6.0E+06
4.0E+06
2.0E+06
0.0E+00
Area Response (arb. units)
0
0.25
0.5
0.75
1
Analyte Concentration (ppm


Benzo(a)pyrene Calibration Curve
y = 3E+06x
R² = 0.9965
4.0E+06
3.0E+06
2.0E+06
1.0E+06
0.0E+00
Area Response (arb. units)
0
0.25
0.5
0.75
1
Analyte Concentration (ppm)


Dibenz(ah)anthracene Calibration Curve
y = 2E+06x
R² = 0.9987
2.0E+06
1.5E+06
1.0E+06
5.0E+05
0.0E+00
Area Response (arb. units)
0
0.25
0.5
0.75
1
Analyte Concentration (ppm)

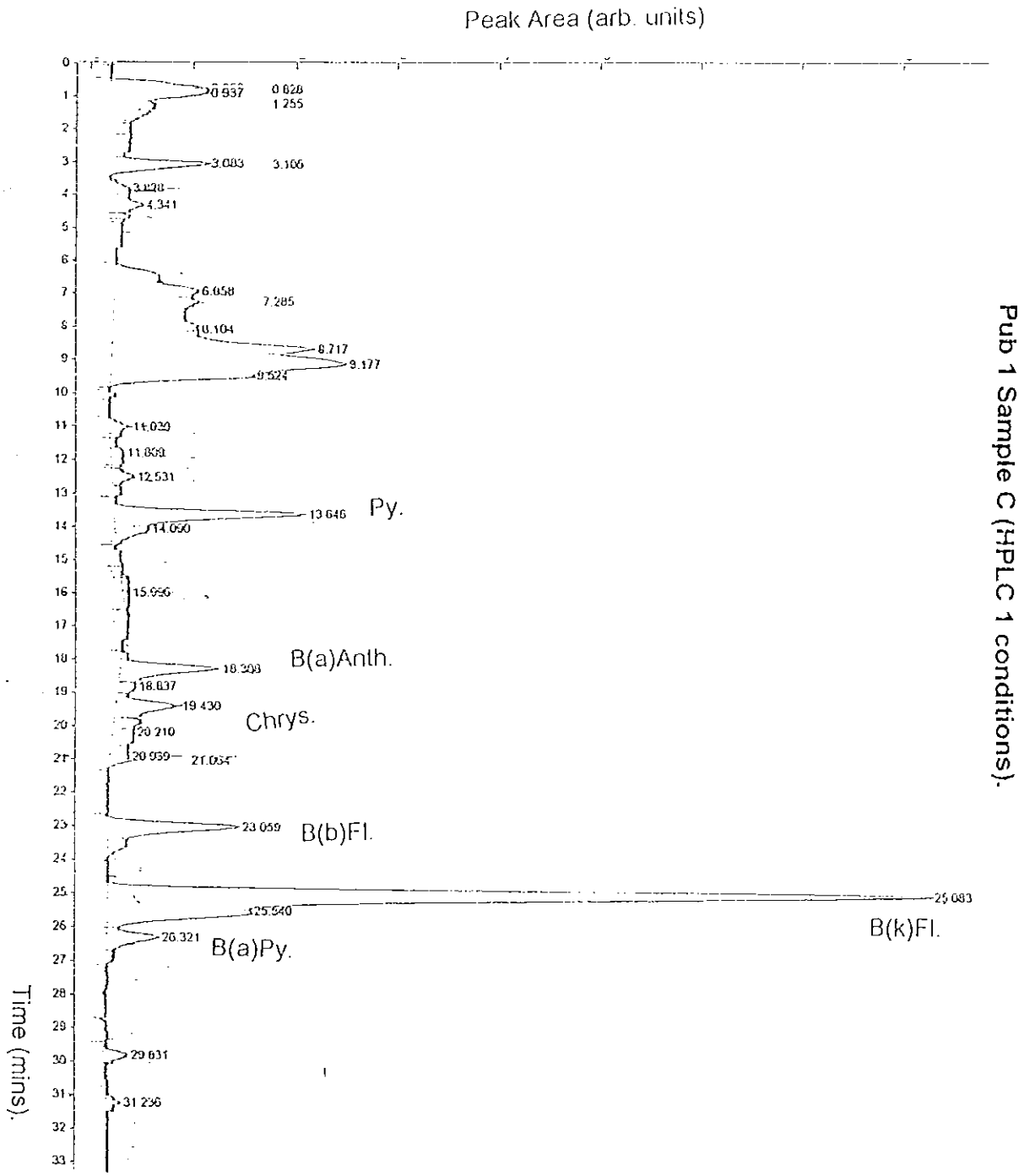

Benzo(ghi)perylene Calibration Curve
y = 1E+06x
R² = 0.9932
1.6E+06
1.2E+06
8.0E+05
4.0E+05
0.0E+00
Area Response (arb. units)
0
0.25
0.5
0.75
1
Analyte Concentration (ppm)

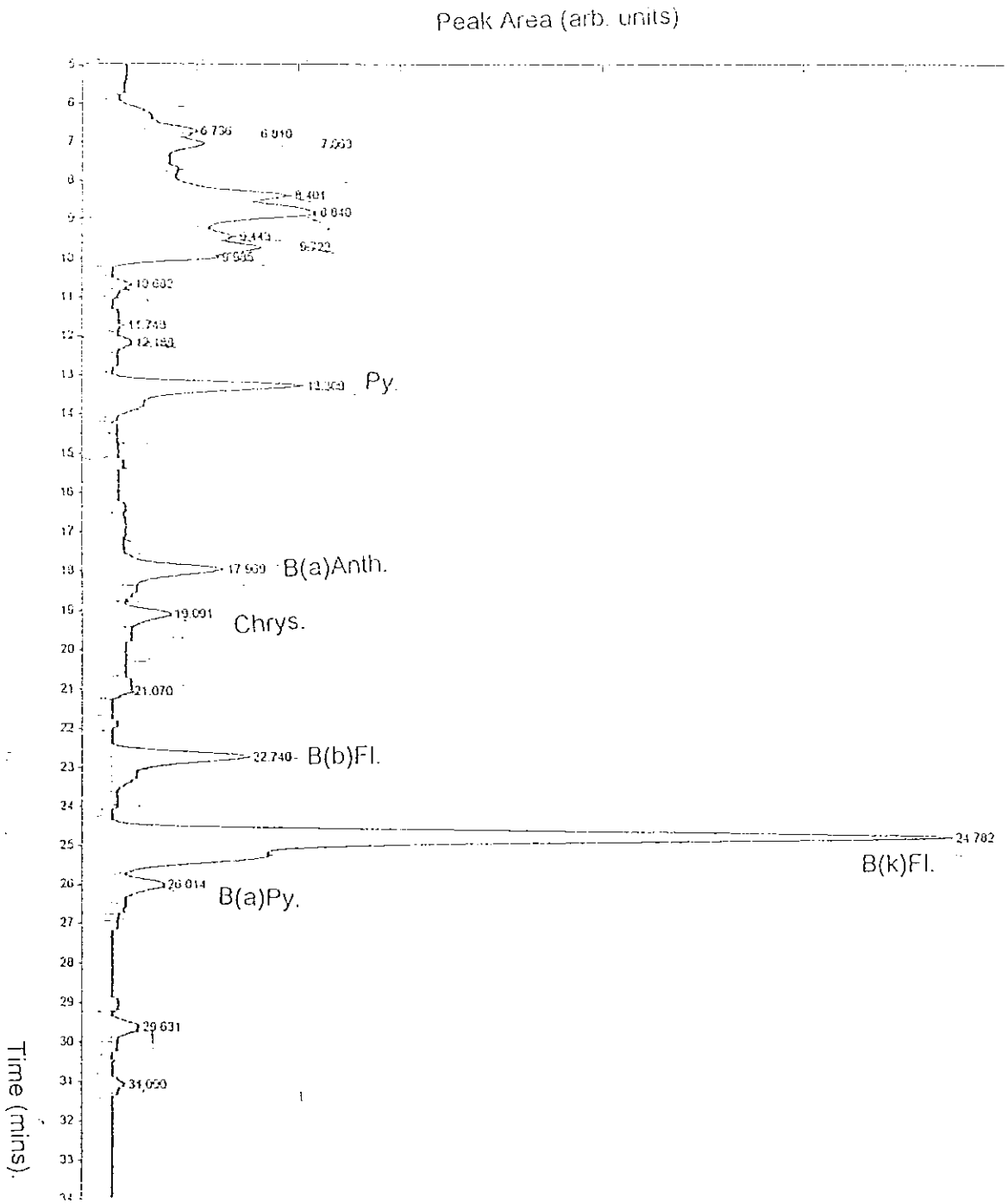

Indeno(123-cd)pyrene Calibration Curve
y = 219818x
R2 = 0.9977
3.0E+05
2.3E+05
1.5E+05
7.5E+04
0.0E+00
Area Response (arb. units)
0
0.25
0.5
0.75
1
Analyte Concentration (ppm)

Appendix B.

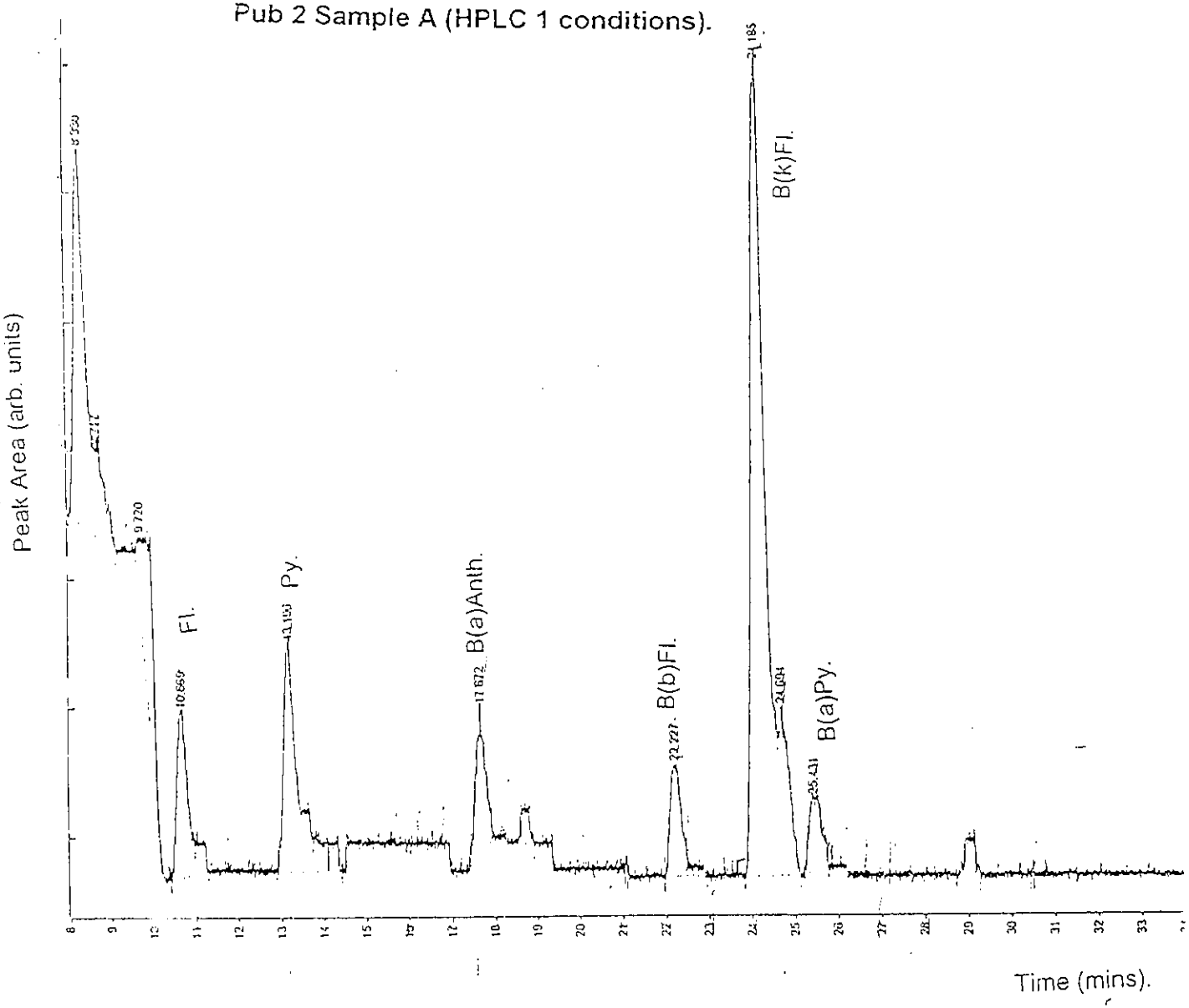
Pub 1 Sample A. (HPLC 1 conditions).

Peak Area (arb. units)
Time (mins).
0.938
1.534
2.954
4.140
4.474
5.450
6.229
6.583
6.977
7.672
8.359
8.863
9.374
10.643
Anth.
12.031
13.223
Py.
14.030
16.050
16.770
17.918
B(a)Anth.
19.025
Chrys.
22.047
B(b)Fl.
24.672
B(k)Fl.
25.934
B(a)Py.
29.542


Peak Area (arb. units)
Time (mins).
0.937
1.255
3.083
3.106
4.341
6.058
7.285
10.104
8.717
9.177
9.524
11.039
11.838
12.531
13.646
Py.
14.050
15.956
18.309
B(a)Anth.
18.837
19.430
Chrys.
20.210
20.939
21.054
23.059
B(b)Fl.
25.083
25.540
B(k)Fl.
26.321
B(a)Py.
29.631
31.296
Pub 1 Sample C (HPLC 1 conditions).


Peak Area (arb. units)
Time (mins).
6.736
6.910
7.503
8.401
8.840
9.443
9.522
9.935
10.630
11.749
12.153
13.369
Py.
17.609
B(a)Anth.
19.091
Chrys.
21.070
22.740
B(b)Fl.
24.782
B(k)Fl.
26.014
B(a)Py.
29.631
34.050

Pub 2 Sample A (HPLC 1 conditions).



Pub 2 Sample B (HPLC 1 conditions).

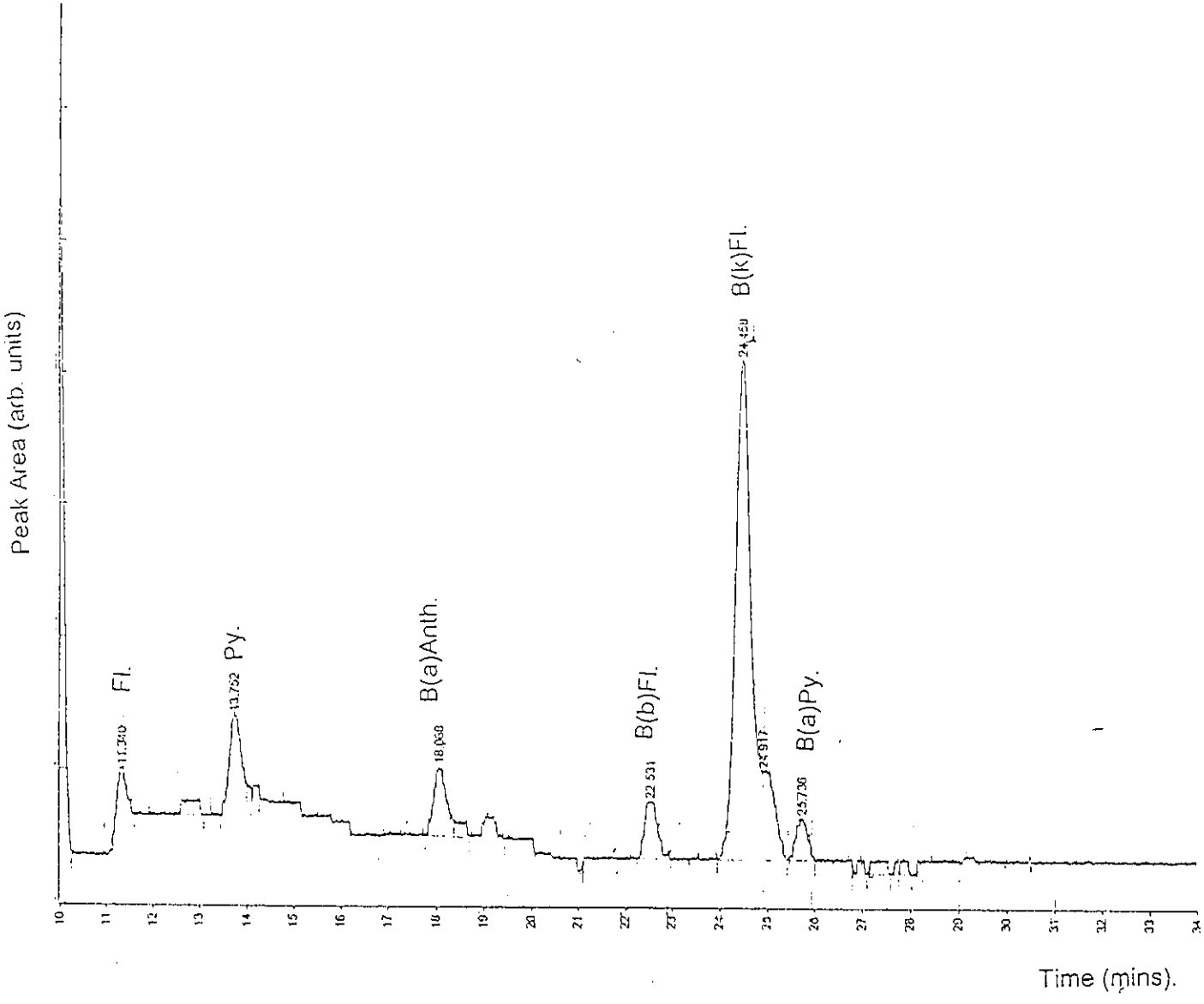
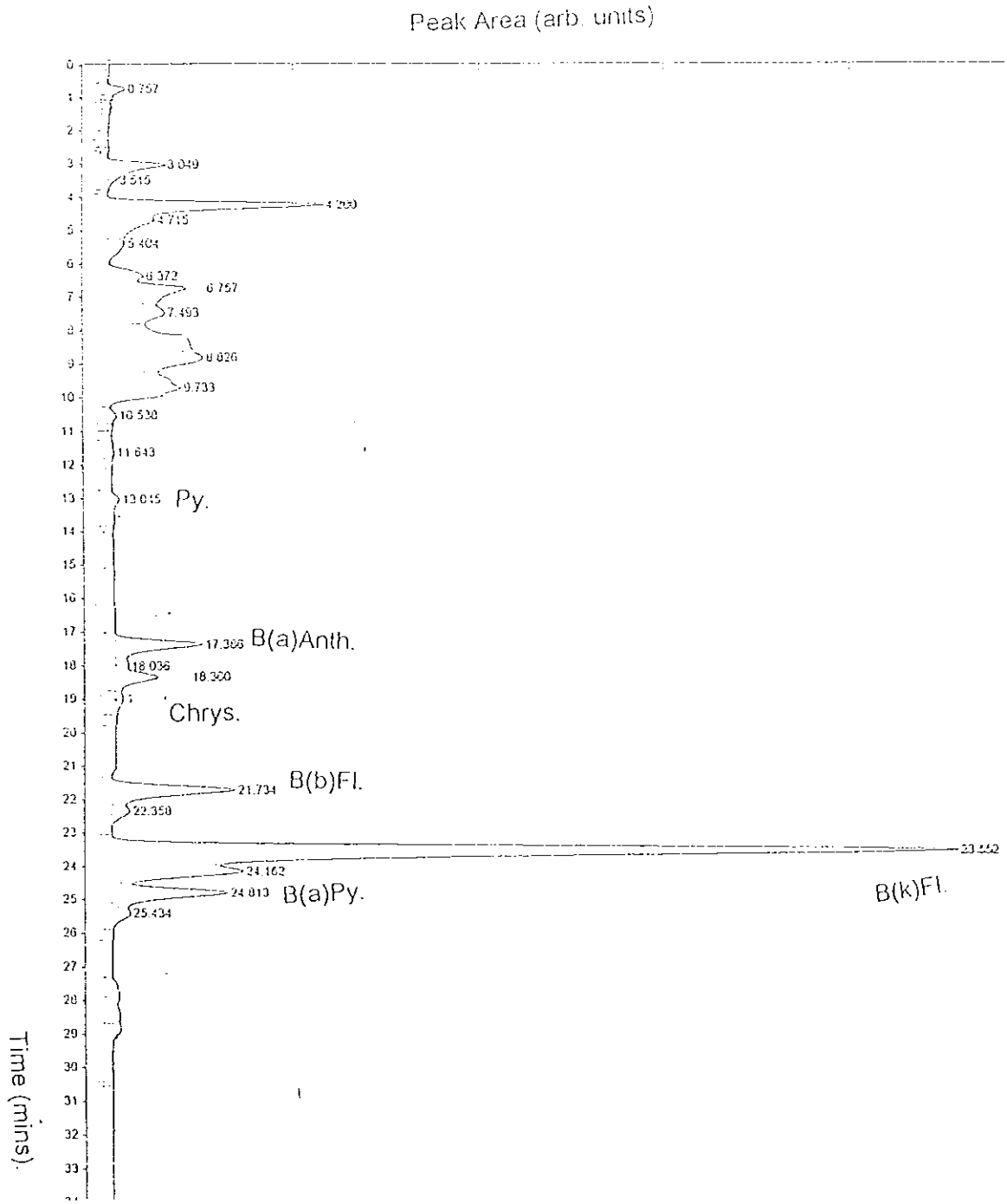


Fig 2 Sample C (HPLC 1 conditions).



Pub 2 Sample D (HPLC 1 conditions).

Appendix C.

Peak Area (arb. units)
Time (mins)
Ambient Sample 1 (HPLC 2 conditions).
0.767
1.058
1.947
1.606
2.198
2.876
3.027
3.336
4.191
F.
4.855
6.149
6.674
7.401
7.512
6.317
Anth.
8.972
Fl.
9.626
10.610
Py.
11.045
12.239
12.465
13.535
B(a)Anth.
14.427
15.207
Chrys.
16.142
B(b)Fl.
19.703
20.657
B(k)Fl.
B(a)Py.
22.691
23.595
Dibenz.
24.385
26.535
27.423
28.237
28.917

Peak Area (arb. units)

Time (mins)

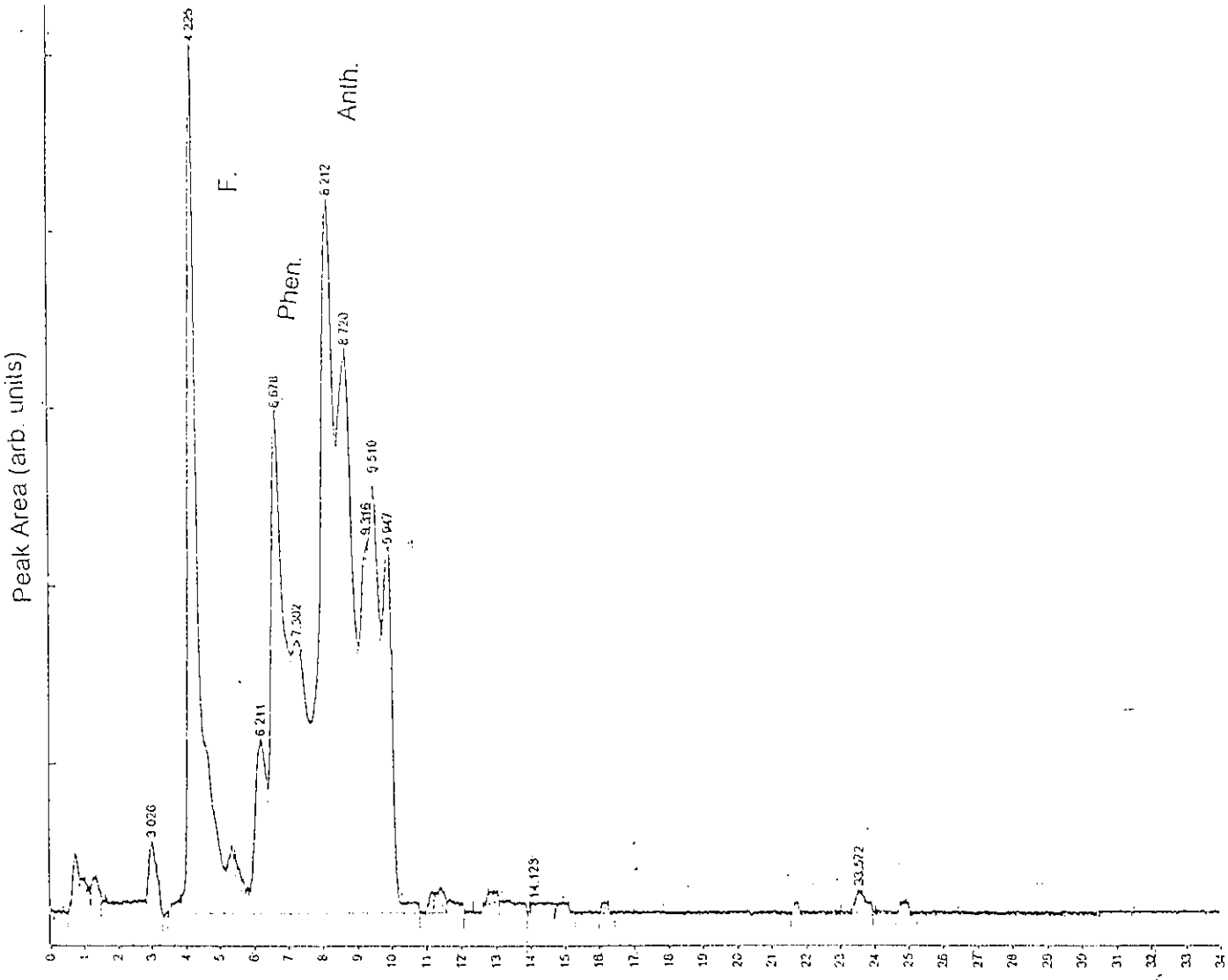
Ambient Sample 2 (HPLC 2 conditions).

Ambient Sample 3 (HPLC 2 conditions).

Ambient Sample 4 (HPLC 2 conditions).

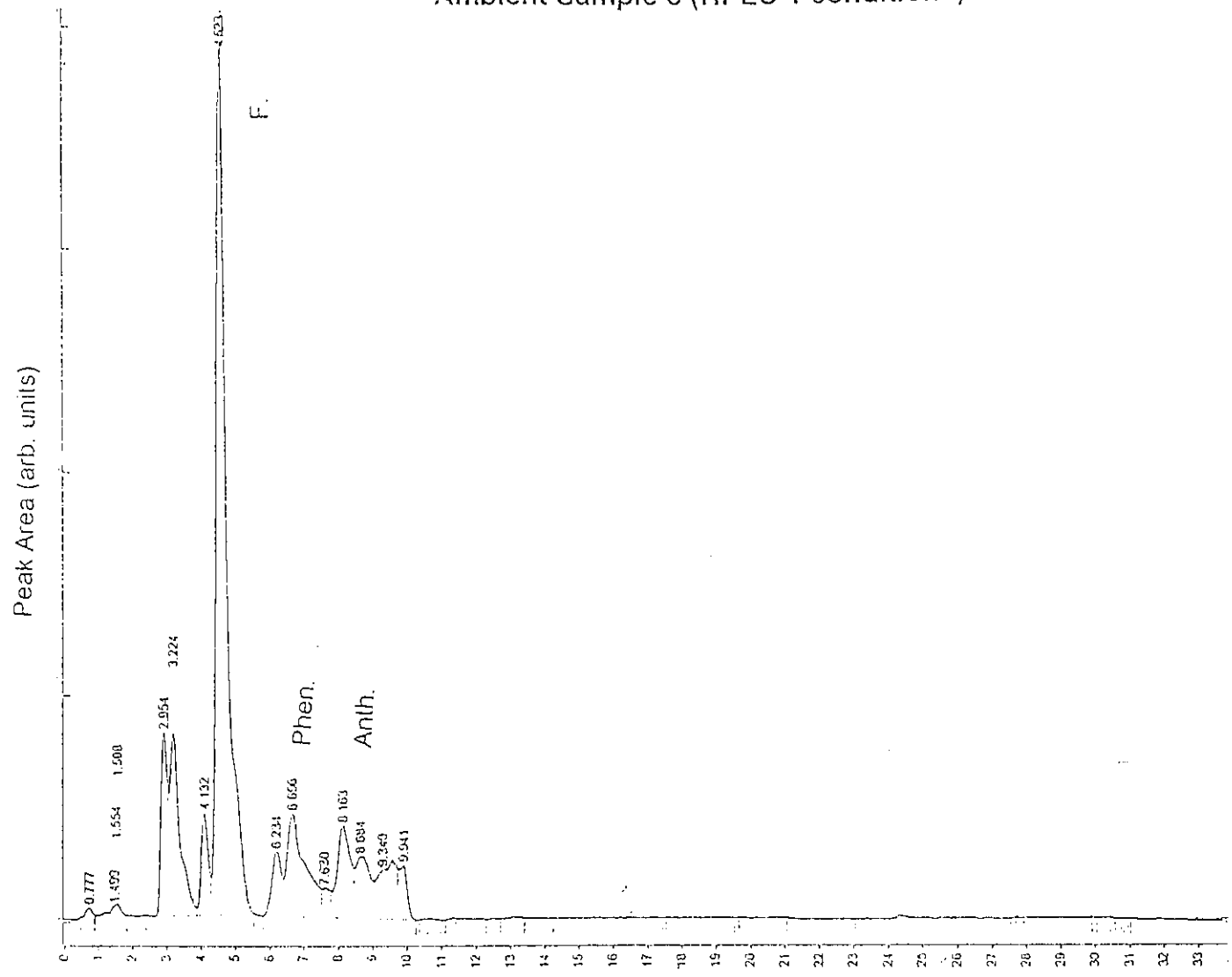
Ambient Sample 5 (HPLC 1 conditions).

Ambient Sample 6 (HPLC 1 conditions).



Ambient Sample 7 (HPLC 1 conditions).

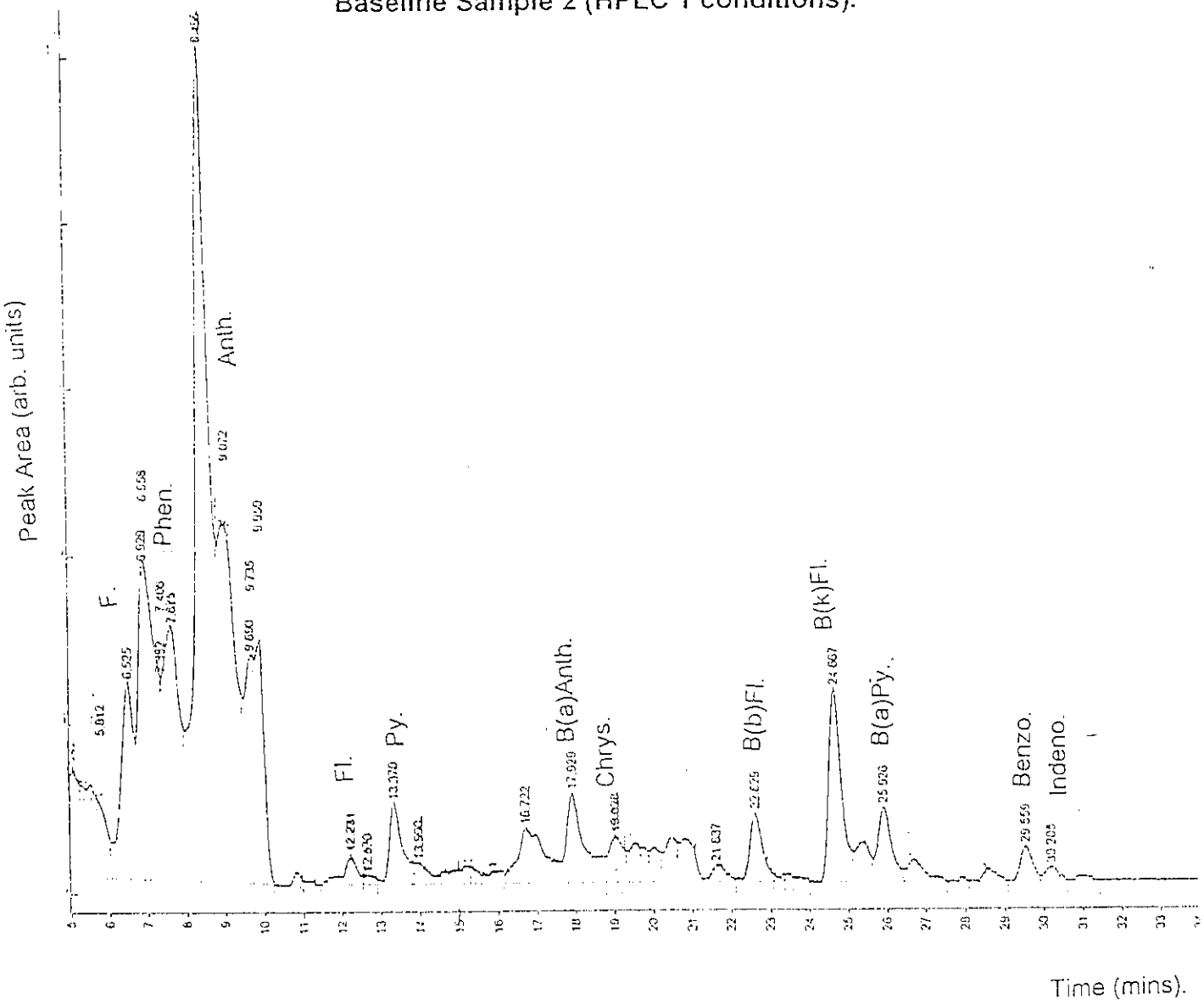
Ambient Sample 8 (HPLC 1 conditions).



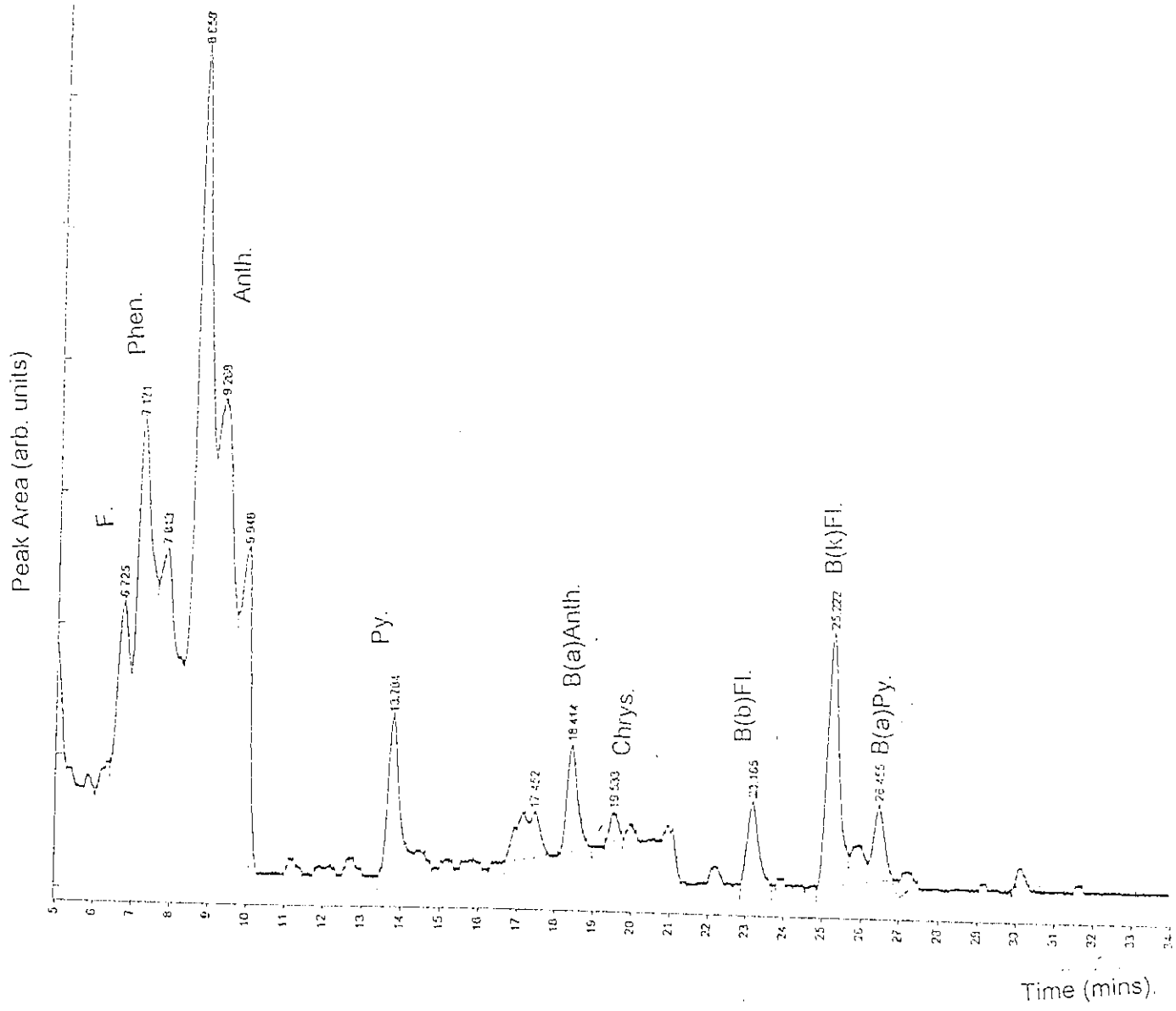
Appendix D.

Baseline Sample 1 (HPLC 1 conditions).

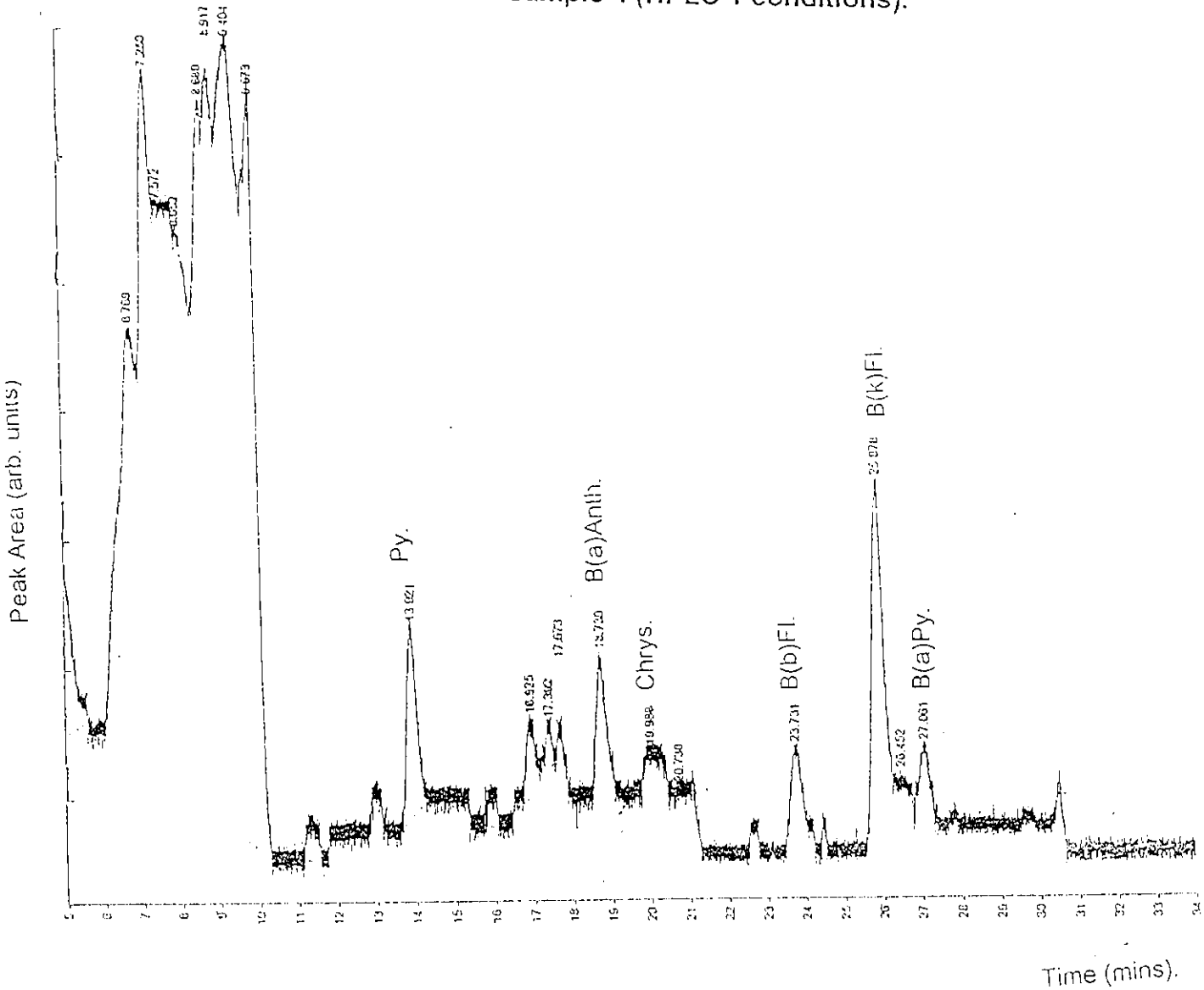
Baseline Sample 2 (HPLC 1 conditions).



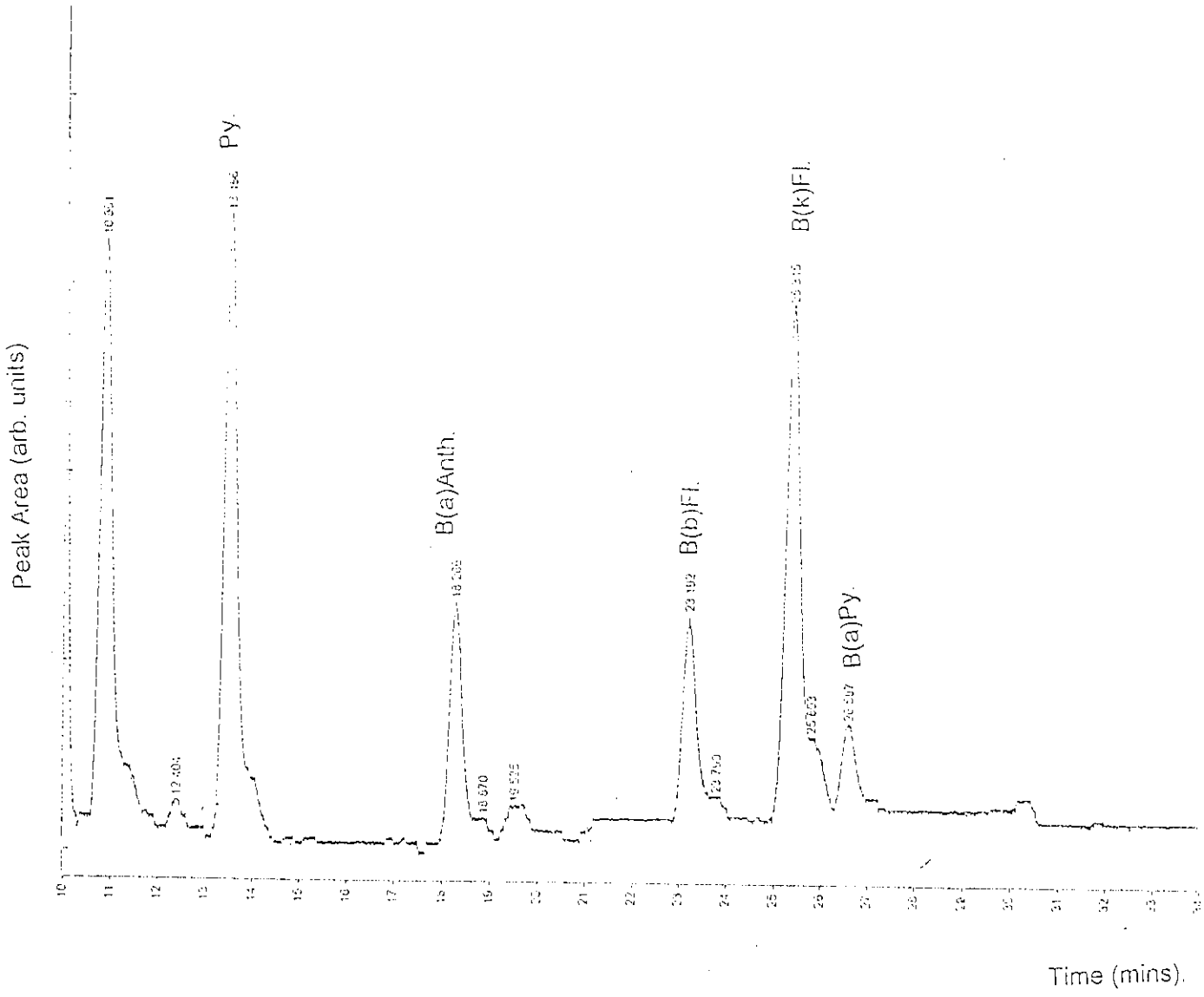
Baseline Sample 3 (HPLC 1 conditions).



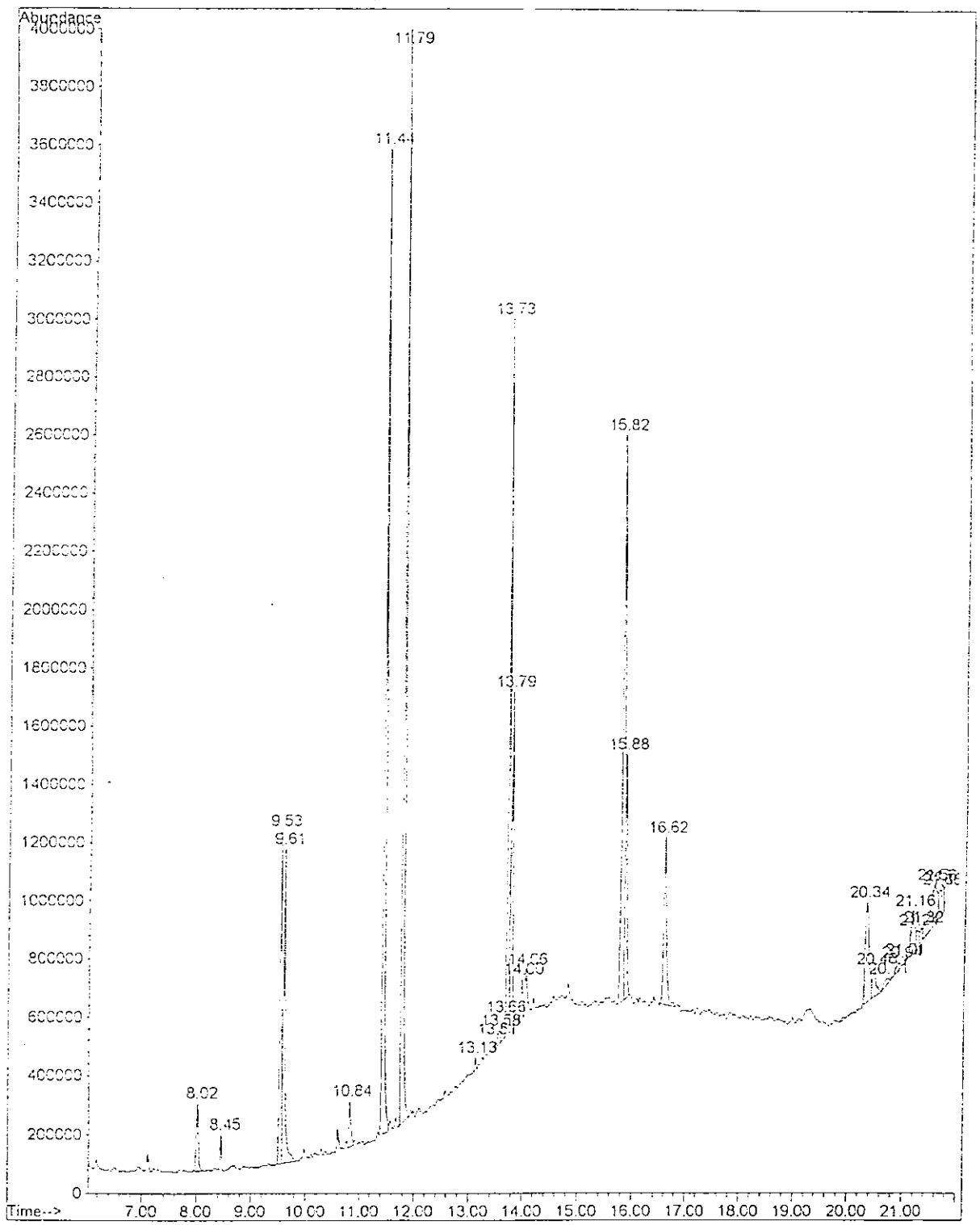
Baseline Sample 4 (HPLC 1 conditions).

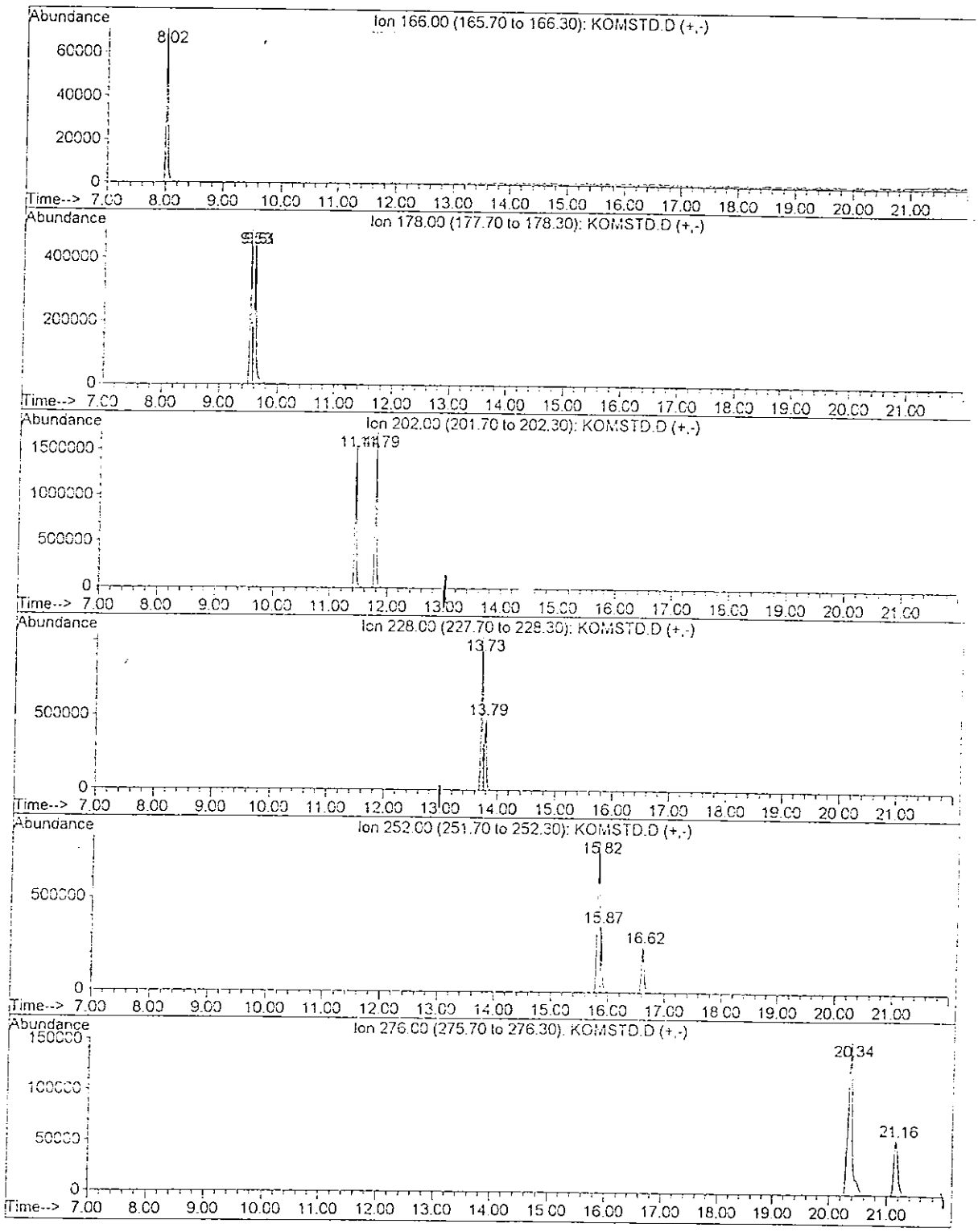


Pub 1 Sample E (HPLC 1 conditions).

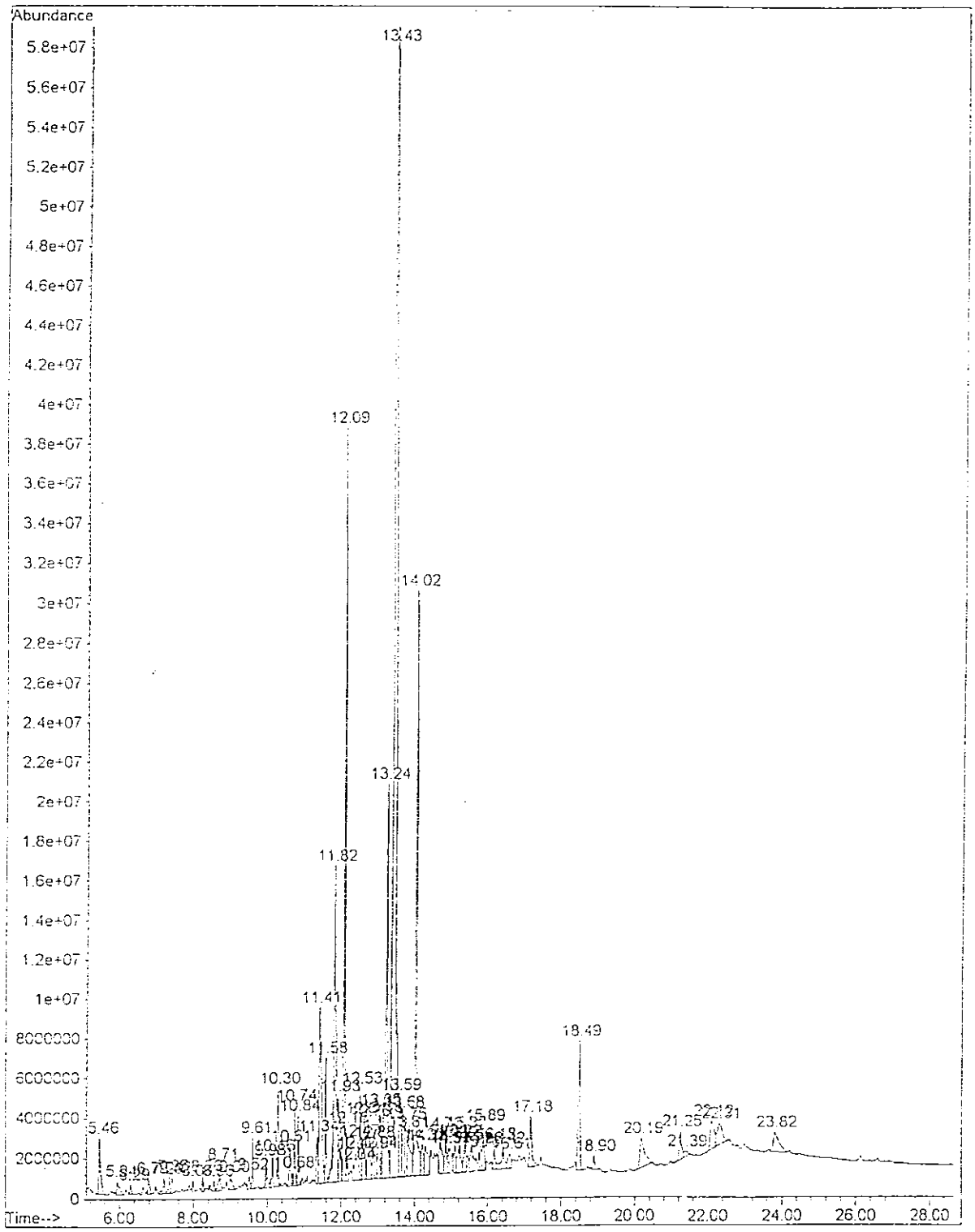


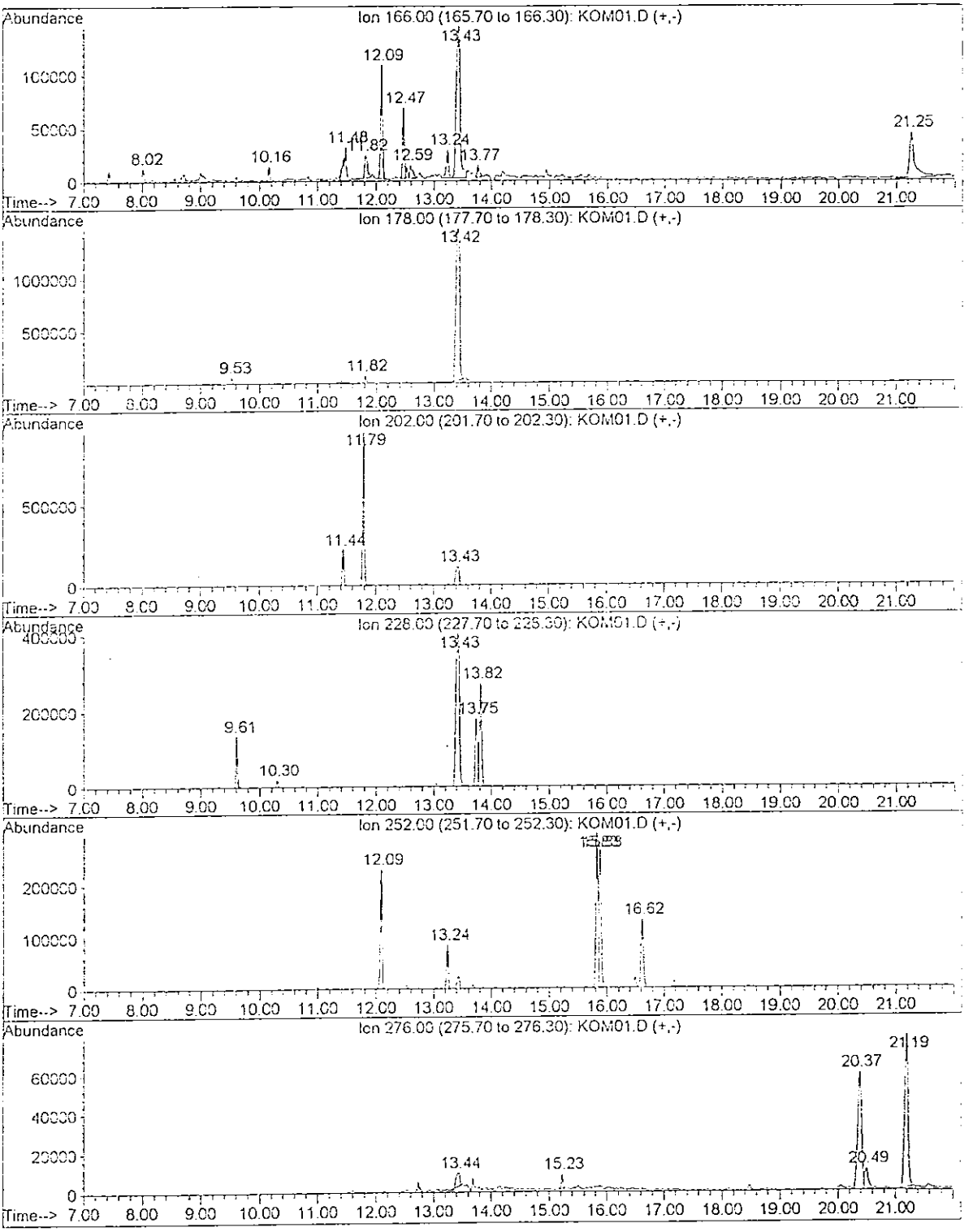
GC PAH Standard.




Abundance
Ion 166.00 (165.70 to 166.30): KOMSTD.D (+,-)
8.02
60000
40000
20000
0
Time--> 7.00 8.00 9.00 10.00 11.00 12.00 13.00 14.00 15.00 16.00 17.00 18.00 19.00 20.00 21.00
Abundance
Ion 178.00 (177.70 to 178.30): KOMSTD.D (+,-)
400000
200000
0
Time--> 7.00 8.00 9.00 10.00 11.00 12.00 13.00 14.00 15.00 16.00 17.00 18.00 19.00 20.00 21.00
Abundance
Ion 202.00 (201.70 to 202.30): KOMSTD.D (+,-)
11.79
1500000
1000000
500000
0
Time--> 7.00 8.00 9.00 10.00 11.00 12.00 13.00 14.00 15.00 16.00 17.00 18.00 19.00 20.00 21.00
Abundance
Ion 228.00 (227.70 to 228.30): KOMSTD.D (+,-)
13.73
13.79
500000
0
Time--> 7.00 8.00 9.00 10.00 11.00 12.00 13.00 14.00 15.00 16.00 17.00 18.00 19.00 20.00 21.00
Abundance
Ion 252.00 (251.70 to 252.30): KOMSTD.D (+,-)
15.82
15.87
16.62
500000
0
Time--> 7.00 8.00 9.00 10.00 11.00 12.00 13.00 14.00 15.00 16.00 17.00 18.00 19.00 20.00 21.00
Abundance
Ion 276.00 (275.70 to 276.30): KOMSTD.D (+,-)
150000
100000
50000
0
20.34
21.16
Time--> 7.00 8.00 9.00 10.00 11.00 12.00 13.00 14.00 15.00 16.00 17.00 18.00 19.00 20.00 21.00

GC Environmental Sample.




Abundance
Ion 166.00 (165.70 to 166.30): KOM01.D (+,-)
13.43
12.09
12.47
21.25
11.48
11.82
13.24
12.59
13.77
8.02
10.16
100000
50000
0
Time--> 7.00 8.00 9.00 10.00 11.00 12.00 13.00 14.00 15.00 16.00 17.00 18.00 19.00 20.00 21.00
Abundance
Ion 178.00 (177.70 to 178.30): KOM01.D (+,-)
13.42
1000000
500000
9.53
11.82
Time--> 7.00 8.00 9.00 10.00 11.00 12.00 13.00 14.00 15.00 16.00 17.00 18.00 19.00 20.00 21.00
Abundance
Ion 202.00 (201.70 to 202.30): KOM01.D (+,-)
11.79
500000
11.44
13.43
0
Time--> 7.00 8.00 9.00 10.00 11.00 12.00 13.00 14.00 15.00 16.00 17.00 18.00 19.00 20.00 21.00
Abundance
Ion 228.00 (227.70 to 228.30): KOM01.D (+,-)
400000
13.43
13.82
13.75
200000
9.61
10.30
0
Time--> 7.00 8.00 9.00 10.00 11.00 12.00 13.00 14.00 15.00 16.00 17.00 18.00 19.00 20.00 21.00
Abundance
Ion 252.00 (251.70 to 252.30): KOM01.D (+,-)
12.09
200000
16.62
100000
13.24
0
Time--> 7.00 8.00 9.00 10.00 11.00 12.00 13.00 14.00 15.00 16.00 17.00 18.00 19.00 20.00 21.00
Abundance
Ion 276.00 (275.70 to 276.30): KOM01.D (+,-)
21.19
20.37
60000
40000
20000
13.44
15.23
20.49
0
Time--> 7.00 8.00 9.00 10.00 11.00 12.00 13.00 14.00 15.00 16.00 17.00 18.00 19.00 20.00 21.00