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Development of a technique for determining 18 PAHs in small volume water samples

BGS Laboratory Technique Development (E2156S67)

Internal Report IR/06/118

BRITISH GEOLOGICAL SURVEY

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Foreword

This report is the published product of a study by the British Geological Survey (BGS) into the development of a technique for the determination of 18 PAHs in small volume water samples. The 18 PAHs comprised fifteen of the sixteen USEPA priority PAHs together with 1- & 2-methylnaphthalenes and the biogenically significant PAH, perylene. The developed technique involved their sorption, from a 10 ml volume of porewater, through the immersion of a small silicone rubber rod. After a specified period the rod, enriched in PAHs, was withdrawn from the porewater and the PAHs desorbed by submerging in 100 µl of 80% aqueous acetonitrile. An aliquot of the resulting extract was then analysed by means of HPLC coupled with fluorescence detection. Five authentic porewaters had their PAH content successfully analysed demonstrating the efficacy of the technique.

Acknowledgements

The author would like to thank Mr Antoni Milodowski for providing the five small volume porewater samples that were used to illustrate the potential of the developed analytical technique.

Thanks also go to Mr Shaun Reeder for supporting the concept of the work and for making available the corresponding time and funding under the BGS Laboratory Technique Development Programme (MaDCap Project).

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The capability within the BGS Laboratories for the determination of PAHs by HPLC was extended by undertaking a development of technique investigation (MaDCap). As a result, it is now possible to determine 18 PAHs by the sensitive and selective means of HPLC coupled with fluorescence detection. These PAHs comprise 15 of the 16 USEPA PAH (acenaphthylene cannot be determined because it is not sufficiently fluorescent), 1- and 2- methylnaphthalenes together with the biogenically significant PAH, perylene. Subsequent further development of a technique, known as Silicone Rod Sorptive Extraction (SRSE), permitting the extraction and concentration of PAHs from small volume water samples, resulted in its successful application to the determination of the 18 PAHs in five samples of porewater. These porewaters had been obtained by the high pressure squeezing of Oxford and Corallian Clay core material in a heavy-duty pressure apparatus designed at BGS.

Previously, porewater samples have been dispatched from BGS to a contract analytical laboratory where it was found that standard methods for PAH analysis were not suitable for the acquisition of meaningful data from limited sample volumes. Indeed, the determination of PAHs in small volumes of natural waters represents a substantial analytical challenge. This report details the development of a technique to successfully surmount that challenge.

1. Introduction

1.1 GENERAL

The abbreviation PAHs denotes polycyclic aromatic hydrocarbons, which are a class of organic compounds, characterised by two or more fused aromatic rings. Occurring in the environment, they give cause for concern because some display toxic, mutagenic and carcinogenic activity (Menzie *et al*., 1992). In general, low molecular weight two- and three-ringed PAHs have a significant acute toxicity, whereas four- to six-ringed PAHs tend to display a greater carcinogenicity (Witt, 1995).

The presence of PAHs in the environment is the result of a variety of anthropogenic and biogenic activities with incomplete combustion and pyrolysis of fossil fuels serving as the major source (McCready *et al*., 2000). This pyrolitic input may be supplemented by PAHs originating from grass and forest fires. In specific locations there may also be a petrogenic contribution of PAHs from crude oil, coal and various refinery products. Frequently, anthropogenic in origin and commonly arising from run-off, industrial and sewage discharges, spillage, shipping activities *etc.* this source can in some cases though be natural as, for instance, oil seepage from depth. Additionally, but to a lesser extent, petrogenic PAHs in sediments can originate from the diagenesis of natural precursors like terpenes, pigments and steroids.

Many hundreds of PAHs exist in the environment, but the US Environmental Protection Agency (USEPA) has listed sixteen as "Consent Decree" priority pollutants chosen because:

- most information is available on these PAHs
- they are suspected of being more harmful than most other PAHs
- they exhibit harmful effects representative of PAHs
- chance of exposure to these is greater than to other PAHs
- these PAHs had the highest concentrations at hazardous waste sites.

Normally, it is the USEPA 16 PAHs that are selected in the majority of publications that focus upon environmental PAH pollution. However, information arising from knowledge of these does not usually provide sufficient detail on PAH distributions to permit definitive links to be made to specific sources of contamination. Their principle value is in providing an estimate of total and individual PAH concentrations.

1.2 PAH ANALYSIS AT BGS

BGS Laboratories have been successfully employing HPLC (High Performance Liquid Chromatography) with fluorescence detection, for the selective and sensitive quantification of environmental PAHs in waters, soils and sediments, for approximately seven years. The technique has the advantage of offering lower detection limits (typically *ca.* 0.01 µg/kg) than those found for most gas chromatographic systems. Presently, 17 PAHs are determined, *i.e.* 15 of the 16 US EPA (one, acenaphthylene, is not detected because of its low quantum fluorescent yield) and 1- and 2-methylnaphthalene (1-MN & 2-MN). These latter two can yield information on fuel spillages as PAH contamination sources.

Ace…….. Acenaphthene	B[k]F…..… Benzo[k]fluoranthene	Fluor…..… Fluorene
Anth…..... Anthracene	B[ghi]P...… Benzo[g,h,i]perylene	I[123cd]P… Indeno[1,2,3-c,d]pyrene
B[a]A..…. Benz[a]anthracene	Chrys……..Chrysene	Naph…..….Naphthalene
B[a]P..…. Benzo[a]pyrene	DB[ah]A… Dibenz[a,h]anthracene	Phen……..... Phenanthrene
B[b]F..…. Benzo[b]fluoranthene	Fanth……. Fluoranthene	Pyr……. Pyrene

Table 1 Abbreviations for the 15 USEPA PAHs routinely determined.

The effectiveness of the method has, since January 2004, been regularly tested at quarterly intervals by participation in the LGC Contest Proficiency Testing Scheme. Recently, *i.e.* since April 2005, data have also been submitted to Quasimeme (Quality Assurance of Information for Marine Environmental Monitoring).

Because unit costs for PAH analysis at BGS tend to be somewhat higher than those charged by contract analytical laboratories, Organic Geochemistry Section have endeavoured to enhance value by assessing possible origins and source apportionment of PAHs in samples. This has been achieved mainly by examination of relative abundances and isomeric ratios allowing estimates, of petrogenic and pyrogenic character, and of the contribution of natural or anthropogenic inputs (Readman, 2002).

Following the discovery that the PAH, perylene, could be generated naturally from degradation of sedimentary terrestrial and aquatic organic debris (Venkatesan, 1988), it has since become apparent that it is probably the most important diagenetic PAH to be found in sedimentary environments (Baumard *et al*., 1998). Nevertheless, the environmental origins of perylene are complex including both natural and anthropogenic contributions and so only when a high abundance relative to other PAHs is encountered can a natural origin be reliably ascribed. Typically, perylene only constitutes 1% – 4% of the total PAH content of pyrolytically produced PAHs (Wakeham *et al*., 1980). A criterion for differentiating perylene of pyrogenic origin from that arising from diagenetic processes has been formulated, *i.e.* if the concentration of perylene in a given sample is >10% of the total concentration of penta-aromatic isomers (benzofluoranthenes and benzopyrenes) then the provenance is probably diagenesis (Baumard *et al*., 1998). Unfortunately, we are at present unable to determine all the penta-aromatic isomers by HPLC. Nevertheless, if concentrations of perylene are high (>10%) compared with the total unsubstituted PAH content (*i.e*. not including alkylated PAHs) again biogenic diagenesis may be confidently attributed (Bixian *et al*., 2001). A close correlation between the USEPA PAH content and the total unsubstituted PAH content is also demonstrated in this work, such that the former is approximately half the latter (Bixian *et al*., 2001). Accordingly, if perylene concentration were found to be >20% of the USEPA content a strong biogenic contribution to the overall PAH could be inferred.

The addition of perylene to the suite of 17 PAHs, currently determined at BGS, would, it was therefore considered, afford a fuller organic geochemical characterisation of environmental samples.

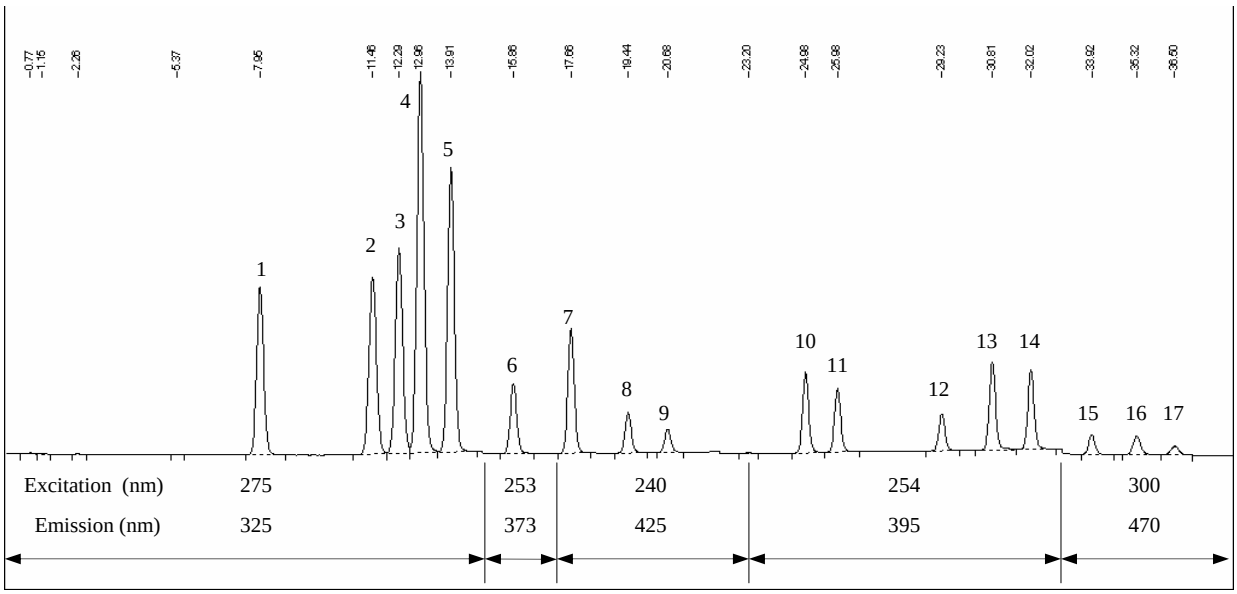
Only one manufacturer (Merck KgaA, Darmstadt, Germany) offering HPLC columns specifically capable of analysing the USEPA PAHs and perylene could be found. Following some preliminary investigations, the requisite HPLC column, *i.e*. LichroCART® 250-4 LiChrospher® PAH, 5 µm, was purchased from VWR International Ltd., Dorset, UK and development of a suitable method was undertaken.

Following development, the method was subsequently successfully applied to the determination of 18 PAHs in small volumes of porewaters.

2. Method Development

2.1 PRELIMINARY INVESTIGATIONS

Before contemplating purchase of the LiChrospher® PAH, the existing HPLC system was first evaluated for its ability to separate perylene from the 17 PAH that were already being routinely determined. To do this a standard solution of the 17 PAH (Supelco - Sigma Aldrich) and a solution of perylene (Acros Chemicals) were run using the usual mobile phase gradient programme used for the 17 PAH analysis (see 1.1a Appendix 1). Figure 1 shows a typical chromatogram obtained for the 17 PAH standard annotated with the excitation/emission wavelength pairs used for peak detection (see 1.2a Appendix 1). Perusal of promotional application notes for the Lichrospher® PAH columns made clear that perylene eluted very shortly after benzo[b]fluoranthene. They also made clear that wavelengths of 350 nm (excitation) and 440 nm (emission) were suitable for the fluorescent detection of perylene.



1. Nap 2. 1-MN 3. 2-MN 4. Ace 5. Fluor 6. Phen 7. Anth 8. Fanth 9. Pyr
10. B[a]A 11. Chrys 12. B[b]F 13. B[k]F 14. B[a]P 15. DB[ah]A 16. B[ghi]P 17. I[123cd]P

Figure 1 Chromatogram of 17 PAH standard using ThermoHypersil® PAH column

Accordingly the wavelength programming of the fluorescence detector was modified by the inclusion of an additional step at 27 mins. in which the excitation wavelength was changed to 350 nm and the emission wavelength became 440 nm. It became apparent that B[b]F and perylene co-eluted (*i.e.* 100 % overlap) with a shared retention time of 29 mins. (see Table 2: Run 1) and so could not be resolved using the system as it stood. On the basis of this experience the acquisition of the Merck PAH column system (Guard & Analytical columns) was considered necessary and the purchase made.

2.2 OPTIMISATION OF THE MERCK COLUMN SYSTEM

Since the analytical column of the Merck column system was of much greater length than the ThermoHypersil column system it was anticipated that considerably different chromatographic behaviour would probably result from its use. So, initially, a UV absorbance detector (Waters 2487) operated at a single wavelength of 254 nm, a wavelength at which all the PAHs absorb,

was substituted for the fluorescence detector to facilitate optimisation of peak resolution. Once this had been achieved then the fluorescence detector was to be reconnected and a suitable wavelength programme formulated for the optimised chromatography.

A variety of mobile phase gradient programmes were investigated (see Table 2) by means of a solution containing the USEPA PAHs, the two methylnaphthalenes and perylene. It was necessary for this solution to be relatively concentrated, compared with the standard 17 PAH solution used normally, because UV detection is much less sensitive than fluorescence detection (see 1.3a Appendix1). It became apparent that the separation of B[b]F and perylene was greater when the concentration of acetonitrile in the mobile phase was high. Thus, when run isocratically at 100% acetonitrile (see Table 2: Run 3) the B[b]F peak and the perylene peak overlapped only very slightly by 3.5%, *i.e*. the ratio of the height of the valley to the average height of the overlapping peaks (Braithwaite & Smith, 1985) and the time separating the peaks was 0.469 mins. It was also discovered that the ThermoHypersil® column behaved similarly under these conditions (see Table 2: Run 4) though the overlap was somewhat greater at 13% and the separation time was only 0.248 mins. But it needs to be borne in mind that the ThermoHypersil® was a much shorter (100 mm) column than the Lichrospher® (250 mm). In fact, it was found that the efficiency (N_{eff}) per unit length of the former was greater than that of the latter, 68,160 plates/m compared to 35,723 plates/m respectively. These efficiencies were calculated using a standard formula (Braithwaite & Smith, 1985), *i.e*.

$$N_{eff} = 1000/L \times 5.54(RT/W_{½})^2$$

where N_{eff} is the efficiency (theoretical plates/m), L is the column length (mm), RT is the retention time (mins) and $W_{½}$ is the peak width at half height (mins) - the peaks for B[a]P on both columns were selected for this examination. The probability is, therefore, that the ThermoHypersil® column would offer superior separations and peak resolution when used in its 250 mm length format and it is strongly recommended that such a column be evaluated.

It might be argued then that an isocratic elution at 100% acetonitrile would offer the optimum chromatography. Unfortunately, maximum resolution of B[b]F and perylene is not the only criterion involved. Other considerations are that:

- the times separating peaks in between which changes are made to the excitation and emission wavelengths of the fluorescence detector, as part of the wavelength programming, need to be as large as possible (so that peaks are not 'lost' because of the retention time shifts that inevitably occur on a day-to-day basis caused by, for example, column ageing, differing daily temperatures *etc*.
- the overall run time should as far as possible be minimised (to increase sample throughput and reduce on solvent usage).
- the peaks of the 18 PAH should all be adequately resolved.

The use of 100% acetonitrile was precluded mainly because of inadequate resolution of early eluting PAHs which were found to be very heavily overlapped, some to the extent of co-elution.

During the early stages of mobile phase gradient optimisation a flowrate of 1 ml/min, typical for the standard 250 mm x 4.6 mm i.d. 5µm particle column format, was employed. Later, it was discovered that separation of 14 USEPA (acenaphthylene and B[b]F missing) and perylene had been reported using HPLC/fluorescence detection with a LichroCART® 250-3 LiChrospher® PAH, 5 µm column (Quiroz *et al*., 2005). Such a column was very similar to the column we were investigating, the only difference being that it had a 3 mm i.d. compared with our 4 mm i.d. Quiroz *et al.* had employed a flowrate of 0.4 ml/min. Scaling up to a 4 mm i.d. column, on the basis that flow is proportional to the column diameter squared (*i.e*. $^{16}/_{9}$ x 0.4 = 0.71), suggested that 0.7 ml/min might be a more efficient flowrate than 1 ml/min for our column.

This was reinforced when applying the same logic to a 4 mm id column given that the optimum flow for a 4.6 mm i.d. column is 1 ml/min (*i.e.* $^{16}/_{21.16}$ x 1.0 = 0.76).

	Gradient Programming			Critical Separation Times (mins.)						Other Criteria		Comments
Run	Initial % ACN	Hold Time mins	Final Time* mins	Fluor/ Phen	Phen/ Anth	Pyr/ B[a]A	Chrys/ B[b]F	B[a]P/ DB [ah]A	B[b]F/ Pery	% Over-lap	Total Run Time mins	TH = ThermoHypersil column used. Flowrates 0.7 ml/min except for runs 1,2,3 & 4
1	50	5	27	1.92	1.78	4.28	3.22	1.90	0.000	100	38	TH Flowrate 1 ml/min
2	60	5	27	2.05	2.14	5.09	3.67	2.93	0.000	100	42	Flowrate 1 ml/min
3	100	na	na	0.24	0.28	0.70	1.68	2.36	0.469	3.5	18	Flowrate 1 ml/min
4	100	na	na	0.16	0.15	0.51	0.99	0.89	0.248	13	10	TH Flowrate 1 ml/min
5	50	3 †	14	1.16	1.19	2.55	2.86	4.06	0.481	12	45	Linear gradient
6	50	3 †	14	1.17	1.22	2.63	2.99	4.43	0.504	11	46	Linear gradient
7	60	3	14	1.41	1.41	2.75	2.83	3.93	0.438	16	43	Linear gradient
8	60	3	14	1.43	1.43	2.78	2.88	4.07	0.449	15	44	Linear gradient
9	60	5	14	1.45	1.37	2.54	2.72	3.73	0.433	16	43	Linear gradient
10	60	3	14	0.89	0.97	2.42	3.04	4.64	0.558	8	43	Convex gradient (3)
11	60	3	14	1.79	1.31	2.57	3.06	4.59	0.519	11	49	Concave gradient (9)
12	60	0	14	1.80	1.37	2.44	2.80	3.98	0.461	15	46	Concave gradient (9)
13	60	0	14	1.82	1.26	2.38	2.77	3.89	0.463	14	46	Concave gradient (10)
14	70	3	14	1.83	1.90	3.71	2.97	4.00	0.384	26	42	Linear gradient
15	70	3	14	1.85	1.91	3.73	3.03	4.15	0.395	24	43	Linear gradient
16	70	3	14	1.34	1.26	2.62	2.94	4.22	0.487	12	40	Convex gradient (3)
17	70	3	14	2.37	3.05	3.35	2.99	4.23	0.400	24	45	Concave gradient (9)
18	65	0	14	2.66	2.34	2.74	2.82	3.95	0.421	17	45	Concave gradient (9)
19	65	0	14	2.67	2.43	2.72	2.75	3.70	0.404	20	44	Concave gradient (9)
20	65	0	14	2.55	2.38	2.52	2.37	2.71	0.327	52	40	Concave gradient (9)
21	65	0	14	2.53	2.37	2.48	2.30	2.40	0.310	54	39	Concave gradient (9)
22	65	0	14	2.59	2.41	2.58	2.46	2.96	0.344	47	41	Concave gradient (9)

* Time at which the linearly increased concentration of acetonitrile reaches 100% (thereafter held at this value).
† Linear increase in acetonitrile concentration from 50% to 60% at 3 minutes.
Experiments with fluorescence detection shown by italicised run numbers, all others with UV detection at 254 nm.
ACN = acetonitrile; Pery = perylene

Table 2 Experiments on mobile phase gradient for the optimum resolution of 18 PAHs.

Increased efficiency was found to result from the use of 0.7 ml/min flow of mobile phase and this manifested itself as reduced overlap between B[b]F and perylene.

The optimisation at 0.7 ml/min took the gradient programme of Quiroz *et al*. as the starting point (see Table 2: Runs 5 & 6). It quickly became clear that this programme allowed insufficient intervals between some of the early eluters, (*i.e.* fluorene, phenanthrene and anthracene), for the necessary programmed wavelength changes to be made, (as shown in the critical separation times in Table 2), such that peak 'loss' due to possible retention time shifts would not occur.

Experiments, in which the starting concentration of acetonitrile was raised from 50% to 60% (see Table 2: Runs 7, 8 & 9), increased the intervals between the peaks for fluorene, phenanthrene and anthracene. This was further enhanced by raising the acetonitrile starting concentration to 70% (see Table 2: Runs 14 & 15) but at the expense of a concomitant increase in the overlap of the B[b]F and perylene peaks.

The effect of using curved rather than linear gradients to reduce the overlap without decreasing the resolution of the early eluters was then investigated. Both concave (where the rate of increase in acetonitrile concentration is slow to begin with and then rises sharply towards the end of the chromatogram) and convex (where the rate of increase in acetonitrile concentration is rapid to begin with and then plateaus towards the end of the chromatogram) were studied (see Figure 2).

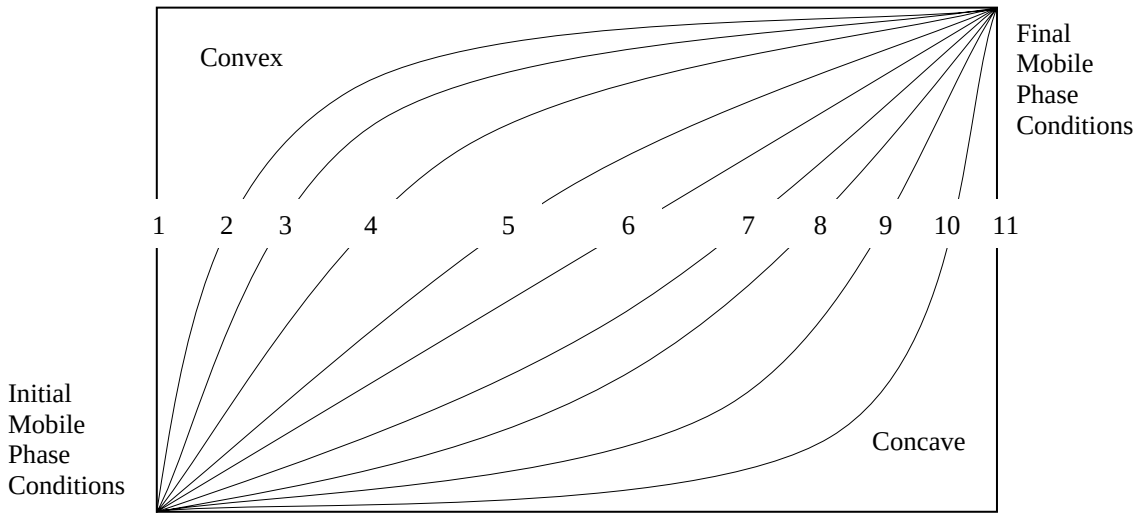
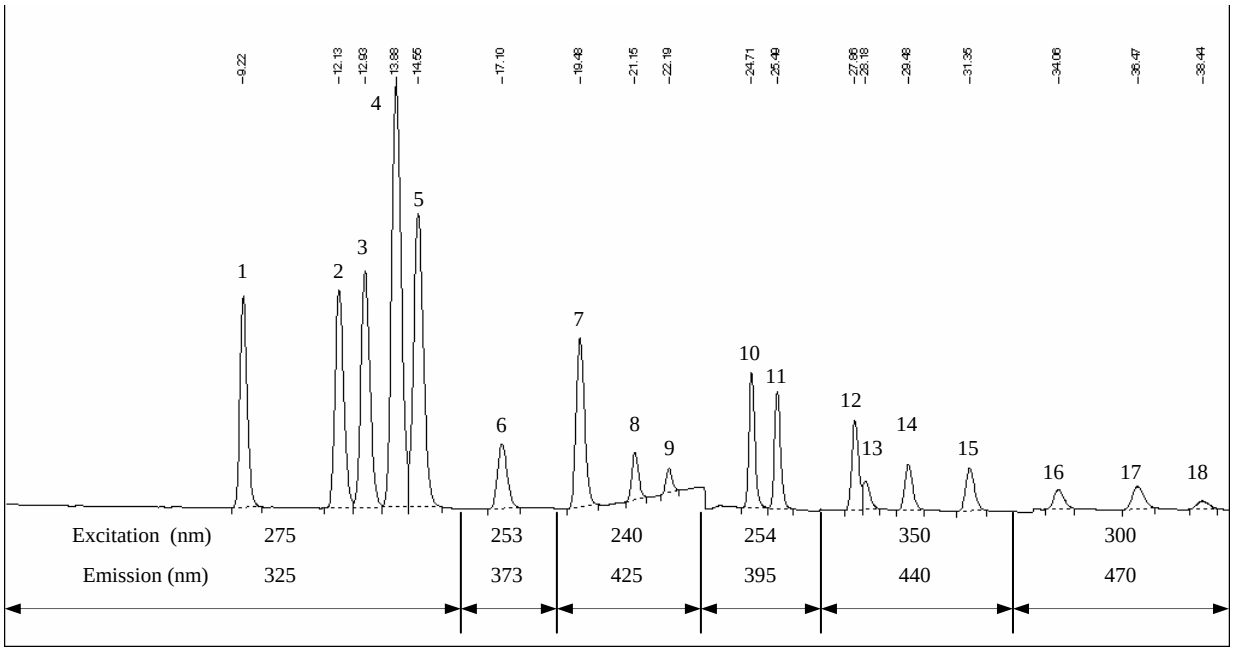


Figure 2 Gradient curve shapes generated by the Waters 600E HPLC pump

It became apparent that using a convex gradient (Table 2: Run 10) produced even smaller intervals between the early eluters (Table 2: Runs 10 & 16), whereas, use of concave gradients increased the intervals (Table 2: Runs 11,12, 13, 17, 18 &19). Use of concave gradient curve 9 in conjunction with an initial mobile phase of 65% acetonitrile appeared to afford optimum chromatography (Table 2: Runs 18 & 19), *i.e.* combining ample intervals between peaks, (where wavelength changes were required to occur), with the best achievable resolution of all peaks and minimisation of run time. The latter property was found to be influenced strongly by the laboratory ambient temperature such that a run under optimum conditions took 37 mins. at *ca.* 25°C increasing to 45 mins. when the laboratory temperature was lower *ca.* 21°C.

Employment of the optimised mobile phase gradient with the fluorescence detector substituted for the UV detector led to some peak broadening (see Figure 3), probably associated with the larger volume of the fluorescence detector flowcell (16 µl) compared with that of the UV detector (10 µl). Thus, comparing runs conducted at the optimised gradient using the UV detector (Table 2: Runs 18 & 19) with those utilising the fluorescence detector (Table 2: Runs 20, 21 & 22) indicated that overlap increased from *ca.* 20% (UV) to *ca.* 50% (Fluorescence). In spite of the reduction in resolution, the peak for perylene remained as an easily discerned and pronounced satellite peak adjoining the B[b]F peak and as such could be quantified with a reasonable degree of precision as was later demonstrated during the calibration associated with the porewater analysis (see 2.2a Appendix 2).



1. Nap 2. 1-MN 3. 2-MN 4. Ace 5. Fluor 6. Phen 7. Anth 8. Fanth 9. Pyr 10. B[a]A 11. Chrys 12. B[b]F 13. Pery 14. B[k]F 15. B[a]P 16. DB[ah]A 17. B[ghi]P 18. I[123cd]P

Figure 3 Chromatogram of 18 PAH standard using Lichrospher® PAH column

Having successfully developed an analytical method for the determination of 18 PAHs, (*i.e.* the 15 fluorescent EPA PAHs, the two methylnaphthalenes and perylene), its application to environmental samples was subsequently evaluated through the analysis of five porewaters.

3. Application of Method to Porewater Analysis

3.1 INTRODUCTION

Small volumes of water represent a substantial challenge for PAH analysis because PAHs are extremely hydrophobic compounds and their solubilities in water are correspondingly very low. Consequently, their concentrations in most aqueous environmental samples are typically at the nanogram/l (parts per trillion) level (*e.g.* King *et al.*, 2005; Persson *et al.*, 2005; Maruya *et al.*, 1996). The LMW (low molecular weight) PAHs are the most soluble, the HMW (high molecular weight) PAHs the least (see Table 3). With larger volumes of water, pre-concentration by a factor of *ca.* 1000 can normally be accomplished to aid in the accurate determination of PAH content.

PAH	Mol. Wt.	Solubility in Pure Water (mg/l) – Various Sources				Probable Solubility	
		from Brown et al., (1999)	from Sverdup et al., (2002)	from Brion et al., (2005)	from El Nemr (2006)	mg/l	µg/l
Naphthalene	128.17	31.0	31.7		30-34	31	31,000
1-Methylnaphthalene	142.20	28.0			28	28	28,000
2-Methylnaphthalene	142.20	24.6			25	25	25,000
Acenaphthene	154.21	3.8	16.1			4	4,000
Fluorene	166.22	1.9	2.0	1.9	1.66-1.98	1.9	1,900
Phenanthrene	178.23	1.10	1.29	0.4-1.6	0.71-1.29	1	1,000
Anthracene	178.23	0.04	0.07		0.03-0.113	0.05	50
Fluoranthene	202.26	0.26	0.26	0.1-0.3	0.206-0.373	0.26	260
Pyrene	202.26	0.13	0.14	0.16	0.013-0.171	0.14	140
Benz[a]anthracene	228.29	0.011	0.014			0.01	10
Chrysene	228.29	0.002	0.002			0.002	2
Benzo[b]fluoranthene	252.32	0.0015	0.0015		0.0012	0.0015	1.5
Perylene	252.32	0.0005	0.0004			0.0005	0.5
Benzo[k]fluoranthene	252.32	0.0008	0.0008	0.0008		0.0008	0.8
Benzo[a]pyrene	252.32	0.004	0.004	0.003	0.004	0.004	4
Dibenz[a,h]anthracene	278.35	0.0005	0.0006			0.0005	0.5
Benzo[g,h,i]perylene	276.34	0.0003		0.0003		0.0003	0.3
Indeno[1,2,3-c,d]pyrene	276.34	0.062 ?	0.0002			0.0002	0.2

Table 3 Solubilities of PAHs in pure water

One way in which this may be achieved is SPE, *i.e.* solid phase extraction (Manoli & Samara, 1996; Kicinski *et al.*, 1989) where a 1 litre volume of water sample can be processed, through a standard manufactured SPE cartridge containing a sorbent. The sorbed PAHs are then desorbed by the passage of a small volume of organic solvent to furnish a 1 ml volume of an extract suitable for HPLC.

However, in certain instances only small, limited volumes of environmental waters are available, *e.g.* porewaters (*ca.* 10 - 20 ml) and then special pre-concentration techniques need to be devised to yield a suitably concentrated extract for PAH analysis.

Previously, some PAH analysis of porewaters had been undertaken in the BGS laboratories employing specialised SPE cartridges (Cave *et al.*, 2004). The 15 USEPA PAH were determined in 15 ml samples using the following technique.

To each porewater for PAH analysis 2-propanol was added to create a 5% v/v solution, to prevent PAH sorbing to surfaces (*i.e*. 50 µl per ml of porewater). The resulting mixture was passed through a pre-conditioned reversed-phase SPE cartridge (Ansys SPEC C18AR) cartridge at *ca*. 2 ml/min. These cartridges have a very small dead volume and so allow the elution of the PAHs with only a small volume (500 µl) of the tetrahydrofuran eluant. A 30 fold pre-concentration maximum could theoretically result, if a 100% recovery were assumed. PAH analysis of this eluate was performed by injection of 5 µl into the HPLC-fluorescence detection system.

However, the recovery of some PAHs from the SPEC cartridges was found to be relatively low (see Table 4). Thus, recovery ranged from *ca.* 70% - 80% for LMW/medium MW PAHs (*i.e.* up to B[b]F), to *ca.* 50% for HMW PAHs - except for DB[ah]A and B[ghi]P (23.5% and 28.7% respectively).

In an attempt to overcome the poor recoveries and to gain a higher pre-concentration a technique known as SRSE (Silicone Rod Sorptive Extraction) was evaluated.

3.2 SRSE (SILICONE ROD SORPTIVE EXTRACTION) EVALUATION

SRSE is a very recent, simplistic and inexpensive development combining sample extraction with pre-concentration and was first applied to PAH in environmental water samples (Popp *et al.*, 2004). Essentially, a small rod of silicone rubber is placed into a sample of aqueous PAHs and shaken. PAHs sorb to the surface of the rod which after a given time is removed from the water and placed into a small vial containing solvent. The PAHs desorb into the solvent and the resulting solution is analysed by HPLC. Following the directions provided in Popp *et al*, 2004, short lengths (10 mm) were cut from a proprietary brand of silicone rod material 1.0 mm dia. (Silastic® - polydimethylsiloxane) purchased from Goodfellow Cambridge, Ltd. A magnifying glass and scalpel blade were utilised to ensure uniformity of the rods during the cutting. The cylindrical surfaces of the cut pieces of rod are able to sorb PAHs from the aqueous phase, because they present an organic layer of methyl groups (together with some phenyl and vinyl groups) at the rod/water interface.

However, the rods require conditioning before use to remove extraneous volatile/semivolatile organic contaminants which interfere with the HPLC (*i.e*. produce spurious peaks in the PAH chromatograms). This was accomplished by heating them at 250°C in a flow of pure nitrogen. The oven of a gas chromatograph proved ideal for this purpose since the oven temperature is very stable and pure nitrogen is normally plumbed in as the make-up gas for the detectors. The rods held in a glass tube within the oven were connected by suitable pipework to the make-up gas supply and the conditioning process was allowed to proceed overnight.

Immediately prior to their use the conditioned rods were rinsed three times in small volumes (10 ml) of a 1:1 mixture of methanol and dichloromethane (see Appendix 2: 2.1).

Optimisation of SRSE

Some experiments were conducted with the aim of maximising the recovery of PAH. Thus, the effect of increasing the ionic strength of the aqueous phase through the addition of sodium chloride was assessed as was the effect of increasing the overall organic content through the addition of methanol. The results of the optimisation the SRSE are presented in Table 4 and recoveries from the optimised SRSE are compared with those from SPE using Ansys SPEC C18AR cartridges.

	SPE	SRSE Optimisation Experiments				Optimised	Optimised
	Recovery %	Recovery %				Recovery %	RSD %
PAH	Ansys SPEC C18AR	No Additions	+ NaCl	+ NaCl + 10% MeOH	+ NaCl + 20% MeOH	No NaCl + 10% MeOH	No NaCl + 10% MeOH
Naphthalene	72.6	33.0	36.8	26.4	17.1	27.7	3.6
1-Methylnaphthalene	nd	54.8	58.4	46.2	31.8	47.2	2.2
2-Methylnaphthalene	nd	55.9	59.2	47.3	32.7	48.2	2.4
Acenaphthene	83.7	62.9	66.1	53.5	37.9	53.4	2.1
Fluorene	82.8	68.2	71.6	58.3	41.7	59.0	2.1
Phenanthrene	83.2	75.0	77.1	71.7	54.0	73.7	1.8
Anthracene	85.1	74.0	75.1	72.8	59.4	77.2	1.8
Fluoranthene	79.1	84.2	84.5	86.4	72.4	86.3	6.2
Pyrene	87.5	79.3	79.8	83.5	68.4	77.4	5.2
Benz[a]anthracene	76.8	89.7	90.5	92.1	87.6	92.9	2.3
Chrysene	69.2	89.5	88.7	91.7	87.2	93.4	4.0
Benzo[b]fluoranthene	69.2	91.0	90.6	91.7	88.5	92.1	2.1
Perylene	nd	80.4	78.0	91.1	86.3	89.2	2.7
Benzo[k]fluoranthene	52.7	89.5	87.5	91.5	89.2	93.2	2.8
Benzo[a]pyrene	51.2	76.5	82.7	89.5	88.3	90.2	2.0
Dibenz[a,h]anthracene	23.5	42.8	31.0	75.0	89.2	92.0	3.3
Benzo[g,h,i]perylene	28.7	73.1	60.1	79.4	86.2	88.2	2.7
Indeno[1,2,3-c,d]pyrene	45.6	78.9	76.4	80.7	85.0	91.1	2.5

Table 4 Optimisation of SRSE and comparison with SPE

For each SRSE experiment 10 ml of pure water was weighed into a 25 ml amber glass bottle. Blanks then had nothing added (No Additions), 0.2 ml saturated aqueous sodium chloride added (+ NaCl expts), 1 ml methanol added (+ 10% MeOH expts), 2 ml methanol added (+ 20% MeOH expts). Spiked samples were prepared in a similar manner to the blanks except that a further 100 µl of a PAH standard solution in acetonitrile was added to each. The composition of the PAH standard corresponds with that of the Fluorescence Detection Soln. as described in the section 'Solutions Employed in Optimisation' (see Appendix 1: 1.3).

Blanks (with or without NaCl and/or MeOH) and PAH spiked samples (with or without NaCl and/or MeOH) each had a conditioned, rinsed and dried silicone rod added and were subjected to the process specified in the Sample Preparation section of Silicone Rod Extraction Method (Appendix 2: 2.1). The method, it will be noted, entails a theoretical 100 fold pre-concentration, *i.e*. assuming 100% recovery is achieved, *cf.* 30 fold theoretically for SPE (see section 3.1).

With reference to Table 4, it was clear that increasing the ionic strength of the sample by the addition of saturated aqueous sodium chloride, had little effect upon recovery. Essentially, the PAHs are already so hydrophobic in a water matrix that their hydrophobicity and hence their affinity for sorption to the surface of the silicone rod is not appreciably enhanced by adding salt. This is an interesting finding because it implies that the ionic strength of a given sample (be it groundwater, seawater, rainwater *etc.*) will have no significant influence on sorption to the rod and so recoveries of PAHs will effectively show no dependence upon this parameter. That said, a slight effect is just about perceptible with the more water soluble, LMW PAHs, which show the least propensity to sorb to relatively polar surfaces, (*e.g*. the glass constituting the amber bottles), undergoing somewhat greater sorption to the rods upon salt addition. Conversely, the extremely hydrophobic, HMW PAHs, which will partition appreciably to the glass, have their affinity for such surfaces enhanced to some extent when ionic strength is increased and

accordingly their partitioning onto the rod is correspondingly somewhat reduced by the addition of salt.

Addition of methanol had the opposite effect since it rendered the aqueous phase less polar and accordingly more attractive particularly to the already slightly water soluble LMW PAHs. Thus, methanol addition tended to reduce the recoveries of LMW PAHs. Conversely, HMW PAHs had their recoveries improved by methanol addition presumably as a consequence of reducing their partition onto the glass surfaces.

Optimised recovery for SRSE (*i.e.* with no added NaCl and 10% added methanol), although affording lower recoveries for the LMW PAHs *cf.* SPE, produced much higher recoveries for the HMW PAHs *cf.* SPE (see Table 4). Since the HMW PAHs, because of their much lower water solubilities, are likely to be far less abundant in natural water samples it was felt that attaining high recoveries for these was of value analytically. It will be noted that even the lower recoveries for the LMW PAHs were consistent as expressed in their percentage relative standard deviations, RSD %.

3.3 SRSE OF POREWATER SAMPLES

The porewaters were obtained from five cores of Oxford and Corallian Clay by means of high pressure, in a heavy-duty squeezing apparatus devised at BGS (Entwisle & Reeder, 1993). Enough of each sample was provided to allow aliquots of 10 ml to undergo the SRSE process.

	PAH Concentration in Porewater (ng/l)					**Limit of Quantification LOQ** (ng/l)
Sample Lab No.	11459-0001	11459-0002	11459-0003	11459-0004	11459-0005	
Naphthalene	58.0	47.5	351	34.0	36.4	32.9
1-Methylnaphthalene	38.1	19.2	562	33.9	17.0	7.2
2-Methylnaphthalene	<LOQ	<LOQ	1370	233.1	85.4	32.3
Acenaphthene	<LOQ	<LOQ	72	19.7	17.9	10.0
Fluorene	<LOQ	8.6	218	34.8	21.1	3.4
Phenanthrene	<LOQ	<LOQ	450	103	49.3	6.3
Anthracene	3.1	1.5	55.2	7.9	2.0	1.1
Fluoranthene	<LOQ	8.6	55.1	21.1	6.5	6.3
Pyrene	<LOQ	<LOQ	34.1	12.3	11.7	5.5
Benz[a]anthracene	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.9
Chrysene	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	2.4
Benzo[b]fluoranthene	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	5.6
Perylene	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	9.4
Benzo[k]fluoranthene	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	2.4
Benzo[a]pyrene	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	3.1
Dibenz[a,h]anthracene	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	15.6
Benzo[g,h,i]perylene	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	8.8
Indeno[1,2,3-c,d]pyrene	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	4.2
TOC	21.6 mg/l	<LOQ	13.3 mg/l	14.2 mg/l	37.9 mg/l	12.0 mg/l

Table 5 Results of Porewaters Analysis

Three point calibrations consisting of a procedural blank, lower concentration PAHs solution and higher concentration PAHs solution for each of the 18 PAHs were undertaken using mixtures with known compositions (see 2.2a Appendix 2). All the resultant calibration curves could be described linearly and all had regression coefficients > 0.992.

Limits of quantification (see Table 5) were based on quantification of peaks in the procedural blanks that had retention times that coincided with those of the PAHs. The standard deviation of the area of these coincident peaks was calculated. The limit of quantification for each PAH was taken to be the average area of the coincident peak in the blank with 3 times the associated standard deviation added – a typical statistical procedure (Popp *et al*., 2004).

The analysis of the porewaters (see Table 5) revealed that it was the LMW PAHs that tended to predominate in the five samples and all contained measurable quantities of naphthalene, 1-methylnaphthalene (2-ringed PAH) and anthracene (3-ringed PAH). Four samples also possessed appreciable concentrations of fluoranthene (4-ringed PAH). For three of the samples, concentrations of PAHs up to pyrene (4-ringed PAH - MW 202.26) could be detected. For benz[a]anthracene (4-ringed - MW 228.29) and PAHs of greater MW, their concentrations were below the limit of quantification in all samples. This is probably due to the increase in hydrophobicity of PAHs as MW rises. The more hydrophobic HMW PAHs would tend to partition to the solid clay matrix in preference to the aqueous porewater phase, greatly reducing their concentrations in the latter.

4. Conclusions

Previously, BGS have submitted porewater samples, similar to the five described in this report, to a contract analytical laboratory. Because such laboratories tend to run a suite of standard analytical procedures for determining PAH in waters they were unable to determine aqueous PAHs in samples of small volume. The method outlined in this report has overcome the shortcomings of the standard procedures in this respect. A method that can successfully undertake to determine 18 PAHs in small volumes of natural waters has been developed. It should be noted, however, that the presence of large amounts of dissolved organic carbon can influence partition of PAHs onto the silicone rods (Popp *et al*., 2004) and consequently, for waters contaminated by dissolved anthropogenic organic compounds (*e.g*. solvents), the method may not be relied upon to produce meaningful data, unless the gross organic chemistry of the water (minus PAHs) may be simulated to permit the construction of a matching procedural blank.

Although the method has been developed specifically for small volumes of natural waters there is no reason, given the low relative standard deviations encountered on the partitions (see Table 4), for not applying it in the when larger volumes of waters are available. Perhaps, its largest drawback is that it does not lend itself to any obvious means of automation.

During development of the method there were indications that a 25 cm long ThermoHypersil® PAH column may offer superior resolution to the 25 cm long Merck Lichrospher® PAH column and assessment of the former is strongly recommended (see section 2.2). Also, retention time alterations caused by fluctuations of the ambient temperature in the laboratory caused problems with fluorescence wavelength pair changes. Such temperature effects could be overcome by the acquisition of a column heater/chiller to provide a controlled and steady column temperature (see section 2.2).

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Most of the references listed below are held in the Library of the British Geological Survey at Keyworth, Nottingham. Copies of the references may be purchased from the Library subject to the current copyright legislation.

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Appendix 1 Analytical Parameters

1.1a HPLC CONDITIONS

Analytical Columns:

Column - 17 PAH:	ThermoHYPERSIL® Green PAH (100 mm x 4.6 mm i.d.) 5µm
Column - 18 PAH:	Merck LichroCART® 250-4 LiChrospher® PAH, 5 µm

Each of these analytical columns was protected by appropriate guard columns inserted between the injector and the head of the column:

Guard Columns -17 PAH:	Injector to Phenomenex C18 SecurityGuard™ (2 mm x 4mm i.d.) to Thermo HYPERSIL® Green PAH (10 mm x 4 mm i.d.)
Guard Columns -18 PAH:	Injector to Phenomenex C18 SecurityGuard™ (2 mm x 4mm i.d.) to LiChrospher® PAH (4 mm x 4 mm i.d.)

HPLC Pump:	Waters 600E Low Pressure Gradient Mixing
HPLC Injector:	Rheodyne 7125 fitted with sample loop of volume 5 µl
Mobile Phase:	Acetonitrile Gradient HPLC Grade (Rathburn) Water (Millipore 18MΩ low TOC)
Mobile Phase Degassing :	X-Act Degassing Unit (Jour Research)

Mobile Phase Gradient Programme - 17 PAH: Flowrate 1 ml/min
Initially 50% acetonitrile maintained for 5 mins. Thereafter, to 27 mins, linear increase of proportion of acetonitrile to 100%. Up to end of run (40 mins) 100% acetonitrile maintained.

Mobile Phase Gradient Programme - 18 PAH: Flowrate 0.7 ml/min (Optimised)
Initially 65% acetonitrile followed by immediate increase of proportion of acetonitrile to 100% by 14 mins employing concave gradient curve 9. Up to end of run (45 mins) 100% acetonitrile maintained.

1.2a FLUORESCENCE DETECTOR SETTINGS

Wavelength Programme - 17 PAH:

Time (mins)	0	14.6	16.6	22.0	33.0
Excitation (nm)	275	253	240	254	300
Emission (nm)	325	373	425	395	470

Wavelength Programme - 18 PAH:

Time (mins)	0	16.4	18.6	23.6	27.1	34.2
Excitation (nm)	275	253	240	254	350	300
Emission (nm)	325	373	425	395	440	470

HPLC Detector: Waters 474 Scanning Fluorescence Detector

Attenuation: 256 Gain: 100 Response time: 5 secs.

1.3a SOLUTIONS EMPLOYED IN OPTIMISATION

UV mg/l range run on Waters 2487 Dual Absorbance Detector - Range 0-1 aufs 254 nm

Fluor µg/l range run on Waters 474 Scanning Fluorescence Detector – Atten. 256 Gain 100

		UV Detection Soln. mg/l (all at 254 nm)	Fluorescence Detection Soln. µg/l	Ex (nm)	Em (nm)
1).	Naphthalene	16.81	337.3	275	325
2).	Acenaphthylene	33.66	668.3	275	325
3).	1-Methylnaphthalene	13.28	283.3	275	325
4).	2-Methylnaphthalene	15.63	333.3	275	325
5).	Acenaphthene	16.73	335.7	275	325
6).	Fluorene	3.36	67.0	275	325
7).	Phenanthrene	1.68	33.7	253	373
8).	Anthracene	1.66	33.4	240	425
9).	Fluoranthene	3.41	67.3	240	425
10).	Pyrene	1.69	33.8	240	425
11).	Benz[a]anthracene	1.65	33.7	254	395
12).	Chrysene	1.70	33.7	254	395
13).	Benzo[b]fluoranthene	3.43	67.3	350	440
14).	Perylene	6.25	33.3	350	440
15).	Benzo[k]fluoranthene	1.70	33.5	350	440
16).	Benzo[a]pyrene	1.67	33.9	350	440
17).	Dibenz[a,h]anthracene	3.39	69.4	300	470
18).	Benzo[g,h,i]perylene	3.40	67.9	300	470
19).	Indeno[1,2,3-c,d]pyrene	1.65	33.5	300	470

Appendix 2 Silicone Rod Sorptive Extractions

2.1a SILICONE ROD EXTRACTION METHOD

Rod Preparation

- Rods cut with scalpel to 1 cm using rule and magnifying (x 20) glass.
- Rods (ca. 50) nitrogen conditioned in GC oven overnight at 250°C.
- Rods placed in amber vial (with PTFE lined cap) that had been placed in the GC oven during the rod conditioning.
- Rods stored in methanol in amber bottle (with PTFE lined cap).
- Required number of rods (up to 10) taken from methanol storage with acetone-cleaned dedicated forceps and placed in 4ml amber vial with 2 ml 1:1 methanol/DCM (Rathburn). PTFE-lined cap put on and vial placed in 50 ml tall-form beaker.
- Beaker and vial put on Buhler shaker for 10 mins (at 300 min^{-1} rotatory).
- Methanol/DCM discarded and rinsing (as per steps 5 and 6) repeated a further two times.
- Rods taken out with forceps and placed on clean tissue to air-dry for 40 mins.

Sample Preparation

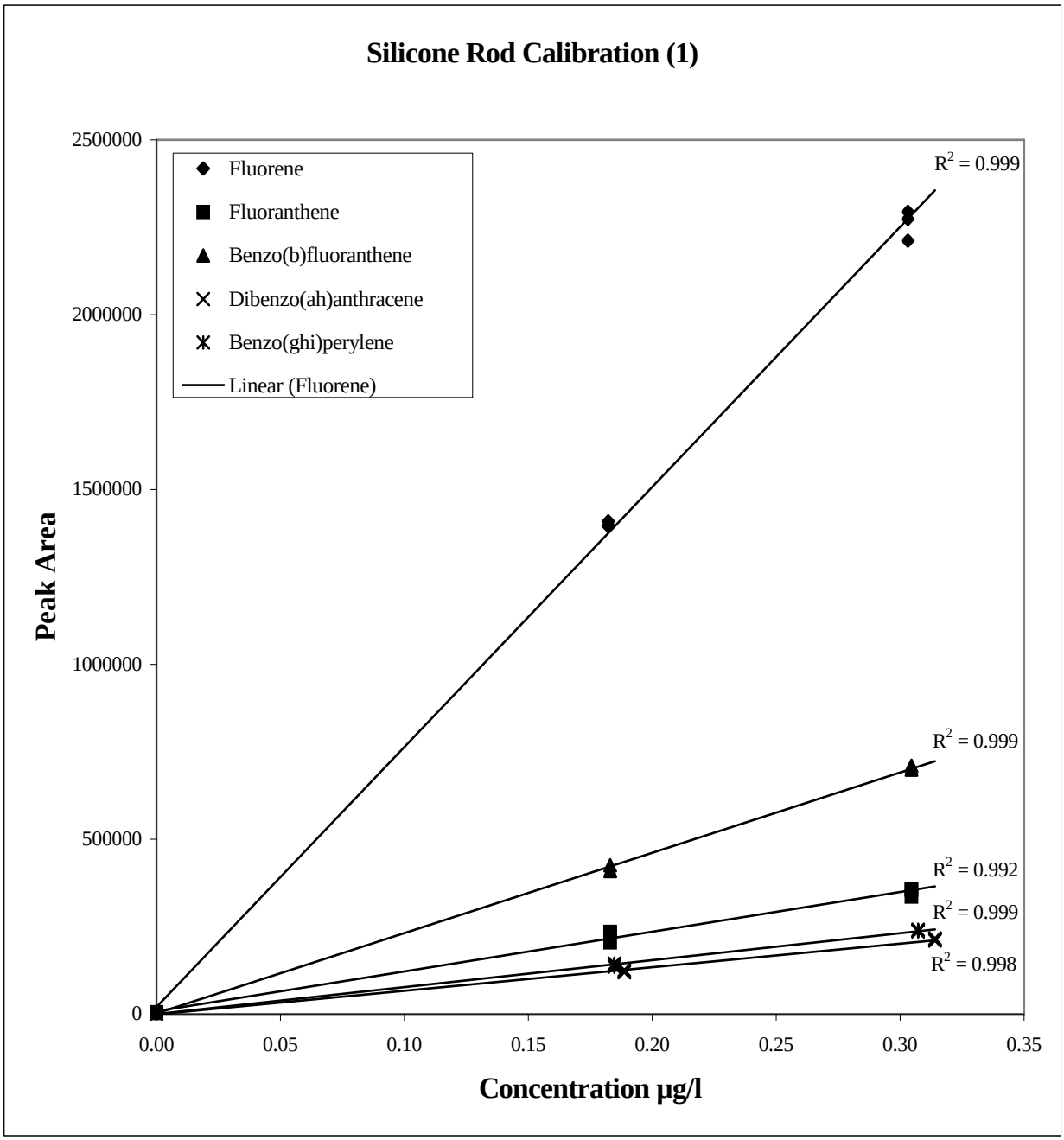
- Take 10 ml water sample (*e.g.* Porewater), 1 ml of methanol and 100 ul of acetonitrile (Raths non-grad).
- Add rod using forceps, cap tightly, shake bottle contents so that rod sinks within the liquid.
- Place on shaker Buhler shaker overnight ca. 17 h (at 300 min^{-1} rotatory) to equilibrate.
- Next day take requisite number of small-volume vial inserts (250 ul) and place into 2 ml glass vials. Into each insert dispense 100 ul of 80% acetonitrile aq. from a dedicated 100 ul GT syringe. Put PTFE-lined screw cap closures on the vials.
- Take bottles from shaker.
- Uncap a bottle and using acetone-cleaned dedicated forceps remove the rod and drop onto clean tissue to absorb excess moisture before rapidly transferring with the forceps (after dabbing dry the tips) into the 100 µl of 80% acetonitrile aq.
- Recap the vial, place in the special plastic vial holder and ultra-sonicate for 15 mins. to allow the organics sorbed from the water sample to desorb into the 80% acetonitrile.
- Use modified seeker needle to remove silicone rod from vial and recap.
- Put used rod to soak in methanol for later clean-up and recycle.
- Take the 100 µl extract into an HPLC syringe and inject into HPLC system.

2.2a CALIBRATION

Three point calibration (PAH std = Fluorescence Detection Soln - see 1.3a Appendix 1)

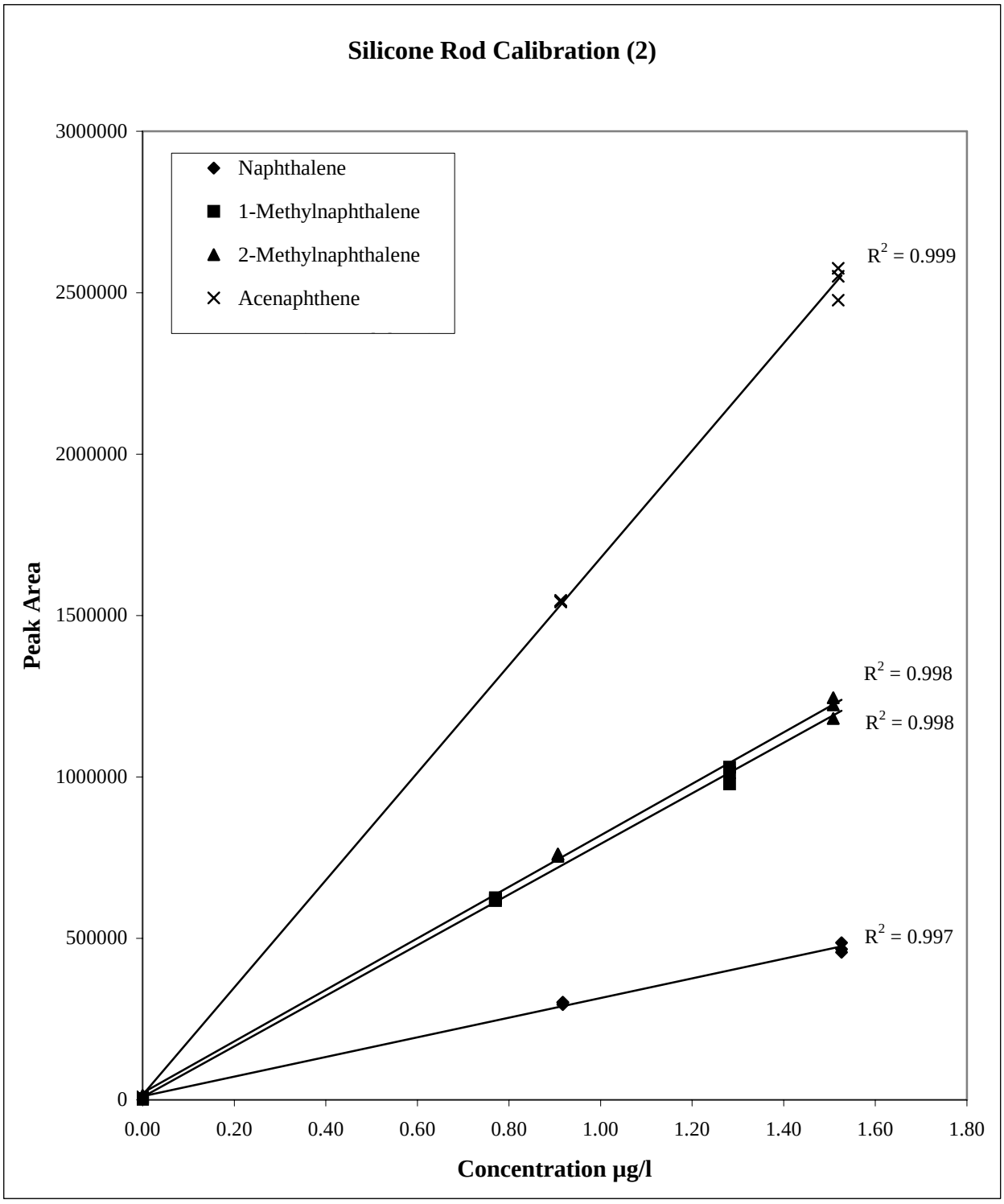
Proc. Blank	10 ml pure water + 1 ml methanol +	100 µl acetonitrile +	No PAH std
Lower Conc.	10 ml pure water + 1 ml methanol +	70 µl acetonitrile +	30 µl PAH std
Higher Conc.	10 ml pure water + 1 ml methanol +	50 µl acetonitrile +	50 µl PAH std

Resultant calibration curves presented overleaf:



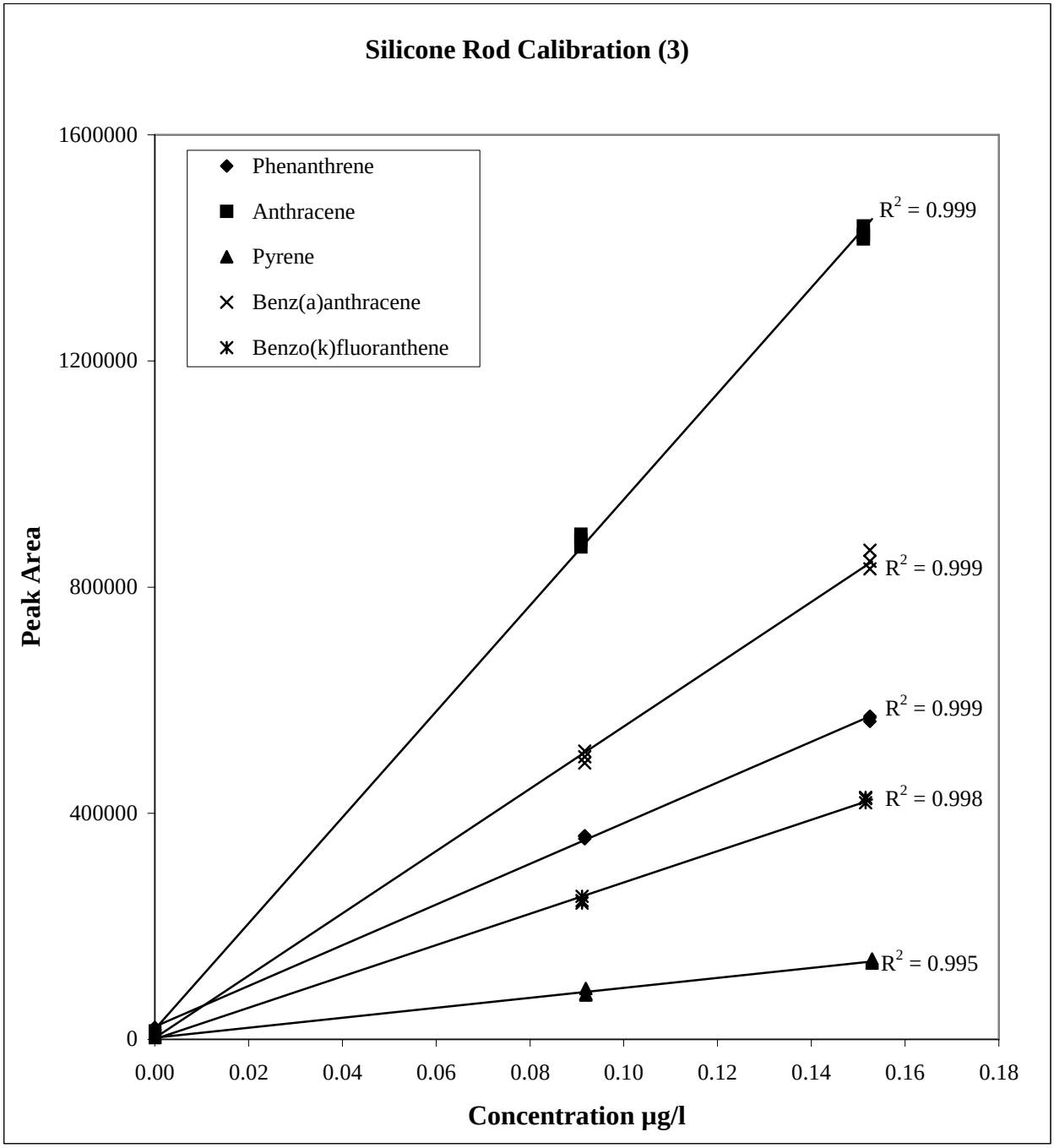
Composition of PAH Standard (in Acetonitrile) used to prepare calibration solutions and the concentrations of PAHs in the Lower and Higher Calibration Solutions

PAH	Conc. of PAH (µg/l) in PAH std	Conc. of PAH (µg/l) in Lower Calibrant	Conc. of PAH (µg/l) in Higher Calibrant
Fluorene	67.0	0.181	0.302
Fluoranthene	67.3	0.182	0.303
Benzo[b] fluoranthene	67.3	0.182	0.303
Dibenzo[a,h]anthracene	69.4	0.188	0.313
Benzo[g,h,i]perylene	67.9	0.184	0.306



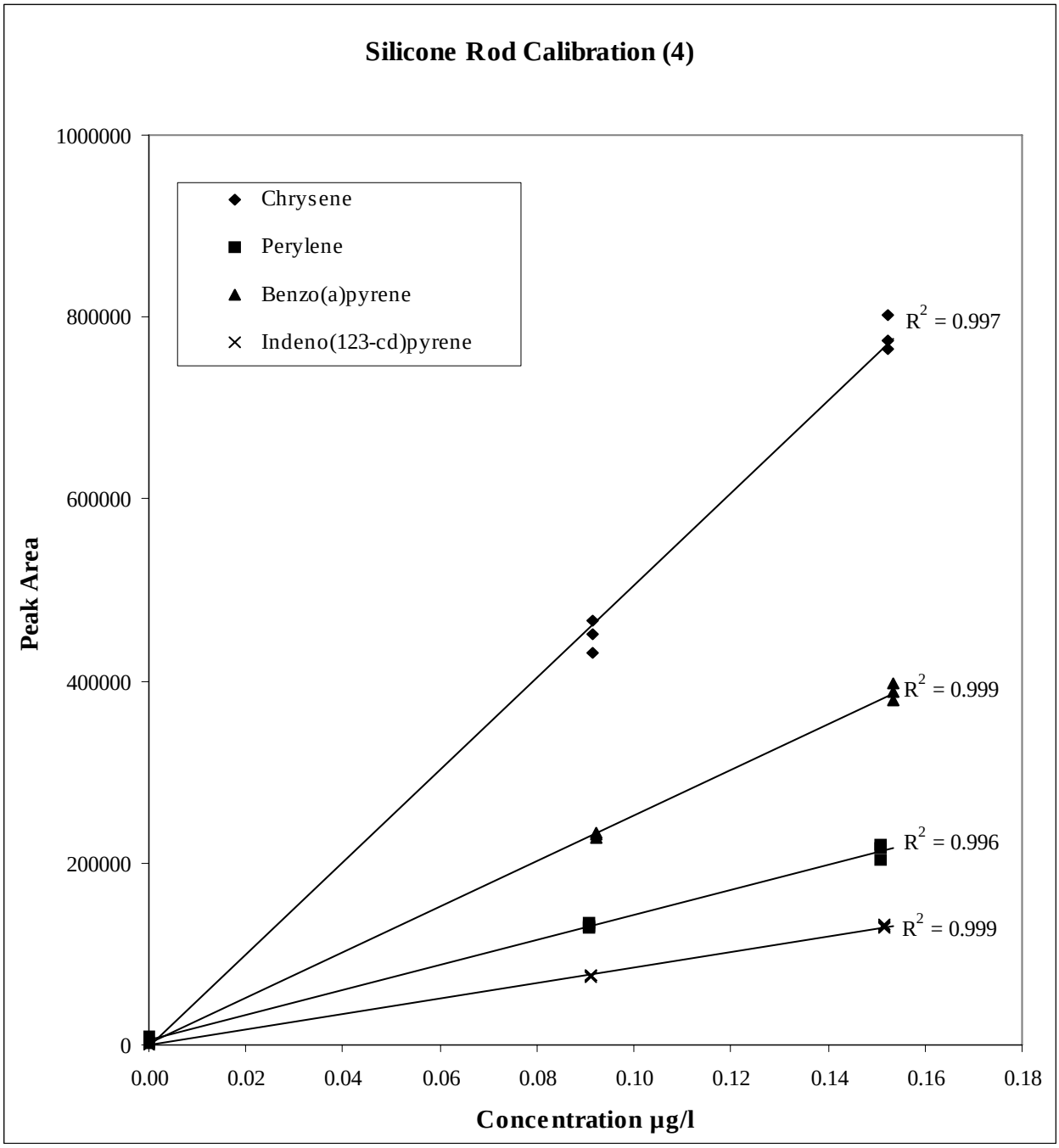
Composition of PAH Standard (in Acetonitrile) used to prepare calibration solutions and the concentrations of PAHs in the Lower and Higher Calibration Solutions

PAH	Conc. of PAH (µg/l) in PAH std	Conc. of PAH (µg/l) in Lower Calibrant	Conc. of PAH (µg/l) in Higher Calibrant
Naphthalene	337.3	0.912	1.519
1-Methylnaphthalene	283.3	0.766	1.276
2-Methylnaphthalene	333.3	0.901	1.501
Acenaphthene	335.7	0.907	1.512



Composition of PAH Standard (in Acetonitrile) used to prepare calibration solutions and the concentrations of PAHs in the Lower and Higher Calibration Solutions

PAH	Conc. of PAH (µg/l) in PAH std	Conc. of PAH (µg/l) in Lower Calibrant	Conc. of PAH (µg/l) in Higher Calibrant
Phenanthrene	33.7	0.0911	0.152
Anthracene	33.4	0.0903	0.150
Pyrene	33.8	0.0914	0.152
Benz[a]anthracene	33.7	0.0911	0.152
Benzo[k]fluoranthene	33.5	0.0905	0.151



Composition of PAH Standard (in Acetonitrile) used to prepare calibration solutions and the concentrations of PAHs in the Lower and Higher Calibration Solutions

PAH	Conc. of PAH (µg/l) in PAH std	Conc. of PAH (µg/l) in Lower Calibrant	Conc. of PAH (µg/l) in Higher Calibrant
Chrysene	33.7	0.0911	0.152
Perylene	33.3	0.0900	0.150
Benzo[a]pyrene	33.9	0.0916	0.153
Indeno[1,2,3-cd]pyrene	33.5	0.0905	0.151