Journal of Chromatography, 642 (1993) 329–349
Elsevier Science Publishers B.V., Amsterdam

CHROMSYMP. 2744

Review

Determination of polycyclic aromatic hydrocarbons by liquid chromatography

Stephen A. Wise*, Lane C. Sander and Willie E. May

Organic Analytical Research Division, National Institute of Standards and Technology, Gaithersburg, MD 20899 (USA)

ABSTRACT

Reversed-phase liquid chromatography (LC) using fluorescence detection is a powerful analytical technique for the measurement of polycyclic aromatic hydrocarbons (PAHs) in environmental samples. The National Institute of Standards and Technology (NIST) has been involved in the development of LC methods for the measurement of PAHs since the mid-1970's particularly for the development of standard reference materials (SRMs) for PAH measurements in environmental samples. The NIST experience in the use of LC for the determination of PAHs in environmental samples is summarized in this paper including: selection of the appropriate column, approaches to analyzing complex PAH mixtures, and the accurate quantitation of PAHs in environmental samples.

CONTENTS

1. INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are widespread environmental contaminants resulting from emissions from a variety of sources including: industrial combustion and discharge of fossil fuels, residential heating (both fossil fuels and wood burning), and motor vehicle exhaust. Because of their mutagenic and carcinogenic properties, PAHs have been measured in a variety of environmental

* Corresponding author.

0021-9673/93/$24.00

matrices including air, water, soil (sediment), and tissue samples. PAHs are usually present in environmental samples as extremely complex mixtures; these mixtures contain many isomeric structures and alkylated isomers which vary greatly in relative concentrations of the individual components and in carcinogenic and/or mutagenic properties.

Since its inception in the early 1970's, high-performance liquid chromatography (LC) has been used for the separation of PAHs. In 1971, Schmit *et al.* [1] first described the separation of PAHs using a chemically bonded octadecylsilane (C_{18}) stationary phase. Since Schmit's report, reversed-phase (RP) LC on chemically bonded C_{18} phases has become the most popular LC mode for the separation of PAHs [2–5]. The popularity of RP-LC for PAH separations is due, in part, to the excellent selectivity of this technique for the separation of PAH isomers. The complex mixtures of PAHs encountered in environmental samples contain numerous isomeric structures. Even when using high resolution open tubular column gas chromatography (GC), a number of isomeric PAHs are still difficult to separate on conventional methylpolysiloxane stationary phases, *e.g.*, chrysene and triphenylene; benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, and benzo[*k*]fluoranthene; and dibenz[*a,c*]anthracene and dibenz[*a,h*]anthracene. Ultraviolet (UV) absorption and fluorescence spectroscopy provide extremely sensitive and, more important, selective detection for PAHs in LC. Finally, LC provides a useful fractionation technique for the isolation of PAHs for subsequent analysis by other chromatographic and spectroscopic techniques. Because of the excellent separation and detection selectivity of RP-LC, this technique has been specified as the method of choice by the U.S. Environmental Protection Agency (EPA) for the analyses of aqueous effluents for the determination of PAHs [6]. The structures of the 16 PAHs on the EPA priority pollutant list are shown in Fig. 1.

At the National Institute of Standards and Technology (NIST), we have been involved in the development and use of LC methods for the measurement of PAHs since the mid-1970's. These efforts have been part of both environmental monitoring programs and the development of standard reference materials (SRMs) for the measurement PAHs in environmental samples. This paper is a review article summarizing the NIST experience in the use of LC for the determination of PAHs in environmental samples including: selection of the appropriate column (*i.e.*, selectivity), approaches to analyzing complex PAH mixtures (*e.g.*, isolation of PAHs, selective detection and multidimensional LC), and accurate quantitation of PAHs in environmental samples particularly as applied to the measurement of PAHs in SRMs.

2. SELECTION OF THE APPROPRIATE LC COLUMN

2.1. Differences in selectivity

RP-LC on C_{18} stationary phases has been shown to provide excellent separations of PAHs. However, not all C_{18} stationary phases provide the same selectivity (*i.e.*, relative separation) for PAHs. In the early 1980's several studies [7–12] compared different commercial C_{18} columns from various manufacturers for the separation of PAHs with particular emphasis on the separation of the 16 PAHs identified by the EPA. These studies found that even though all of the different columns were "generically" C_{18} phases, some provided significantly enhanced selectivity for the separation of the 16 PAHs on EPA's priority pollutant list. During these early studies, it became evident that such investigations were somewhat limited because the exact details concerning the silica substrate and the bonded-phase syntheses were difficult to obtain from the LC column manufacturers. As a result of this limitation, investigations were initiated at NIST to understand more fully the influence of factors such as bonded-phase type, silica substrate characteristics, alkyl chain length, and C_{18} ligand density on selectivity of PAH separations in RP-LC. The results of these investigations have been published in several papers [13–19] and summarized in several review articles [20–22].

One of the most important findings of these investigations was that the separation of the 16 priority pollutant PAHs was greatly influenced by the type of synthesis used to prepare the bonded C_{18} phase. Bonded phases that are prepared using silane modification procedures can be classified as either monomeric or polymeric phases depending on the reagents and reaction conditions used for the bonded phase synthesis. The vast majority of C_{18} phases

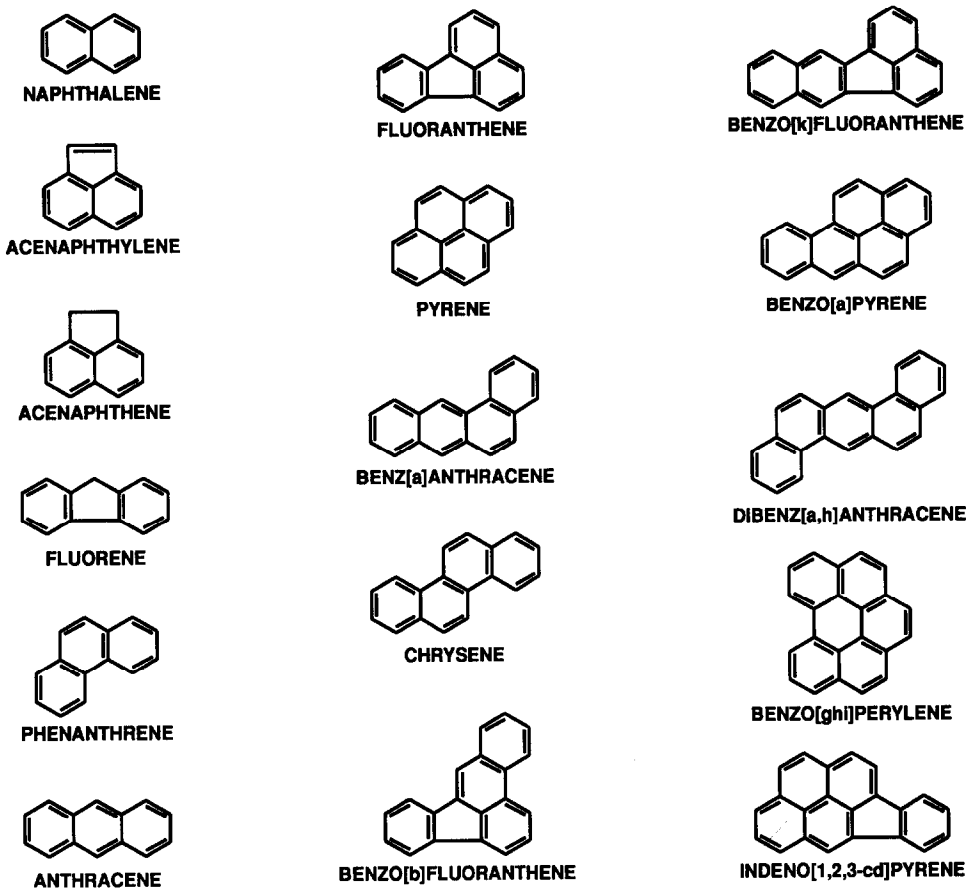

Fig. 1. Structures of the 16 PAHs identified as priority pollutants by the U.S. Environmental Protection Agency and contained in standard reference material (SRM) 1647.

are prepared by reaction of monofunctional silanes (*e.g.*, monochlorosilanes) with silica to form "monomeric" bond linkages. Polymeric phases are prepared using trifunctional silanes in the presence of water which results in cross-linking to form silane polymers on the silica surface. The resulting phase is conceptually not as well-defined as a monomeric phase; however, the chromatographic selectivity characteristics of polymeric C_{18} phases for PAH separations are distinct from those of monomeric C_{18} phases. The difference in the separation of the 16 priority pollutant PAHs on a monomeric and a polymeric C_{18} phase is illustrated in Fig. 2. Separation of all 16 PAHs is achieved on the polymeric C_{18} phase. However, on the monomeric C_{18} phase, the four-ring isomers chrysene and benz[*a*]anthracene are unresolved, and the six-ring isomers benzo[*ghi*]-perylene and indeno[1,2,3-*cd*]pyrene, the five-ring isomers benzo[*k*]fluoranthene and benzo[*b*]fluoranthene, and fluorene and acenaphthene are only partially resolved.

2.2. Classification of phase selectivity

A simle empirical test has been developed to assess the selectivity of C_{18} stationary phases for the separation of PAHs [18,21]. The test is based on the relative retention of three carefully selected PAH solutes as shown in Fig. 3. The retention of benzo[*a*]-pyrene (BaP), relative to 1,2:3,4:5,6:7,8-tetrabenzo-naphthalene (TBN, alternate name dibenzo[*g,p*]-chrysene) and phenanthro[3,4-*c*]phenanthrene (Ph-

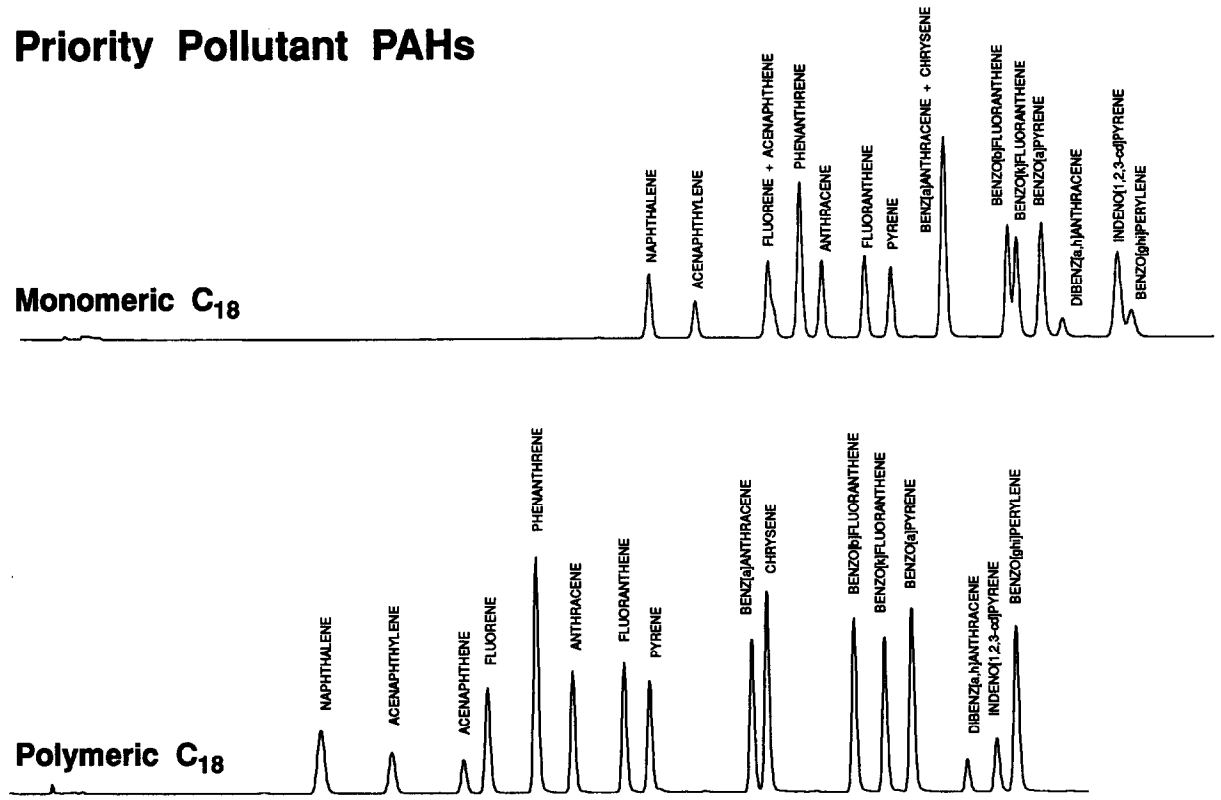

Fig. 2. Comparison of the separation of the 16 priority pollutant PAHs on a monomeric (Zorbax ODS) and a polymeric (Vydac 201TP) C_{18} stationary phase. Chromatographic conditions: mobile phase isocratic at 50% acetonitrile in water for 4 min, then linear gradient to 100% acetonitrile in 15 min at 2 ml/min; UV detection at 254 nm.

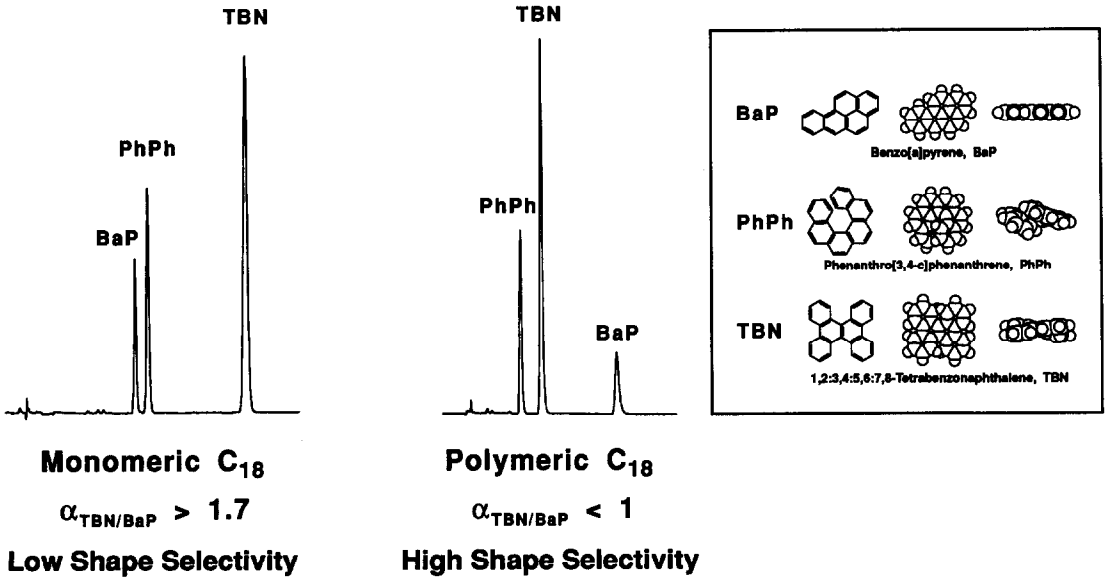

Fig. 3. Separation of SRM 869, Column Selectivity Test Mixture for Liquid Chromatography, on a polymeric and a monomeric C_{18} stationary phase. Structures of the three components in the mixture are illustrated in the box. Chromatographic conditions: mobile phase isocratic at 85% acetonitrile in water at 2 ml/min; UV detection at 254 nm.

Ph), provides a sensitive measure of the "polymeric" or "monomeric" character of the stationary phase. As shown in Fig. 3, the elution order of these three solutes on phases prepared with monomeric surface modification is BaP < PhPh < TBN, whereas phases prepared with polymeric surface modification give the elution order of PhPh < TBN < BaP. This test mixture is available from NIST as SRM 869, Column Selectivity Test Mixture for Liquid Chromatography (see discussion below for SRMs).

A quantitative measure of the phase selectivity can be calculated to allow relative comparisons among different C_{18} phases. The selectivity factor $\alpha_{TBN/BaP}$ (defined as k'_{TBN}/k'_{BaP}) has been shown to correlate with the retention behavior of PAHs and the bonded phase type [18,21]. A classification scheme has been adopted based on the measurement of $\alpha_{TBN/BaP}$ values for experimental and commercial C_{18} phases. Values of $\alpha_{TBN/BaP} \leqslant 1$ indicate polymeric C_{18} phases, and values of $\alpha_{TBN/BaP} \geqslant 1.7$ indicate monomeric C_{18} phases. For values $1 < \alpha_{TBN/BaP} < 1.7$, the bonded phase synthesis is less certain and may indicate a densely loaded monomeric phase or light polymerization with di- or trifunctional reagents. A listing of over 40 commercial C_{18} columns, grouped according to this classification scheme, is provided in Table 1. For the commercial columns the $\alpha_{TBN/BaP}$ values range from 0.56 to 2.18 with the majority of the columns classified as monomeric phases. Values of $\alpha_{TBN/BaP}$ as low as 0.38 have been obtained on heavily loaded experimental polymeric C_{18} phases.

The separation of the 16 priority pollutant PAHs on four C_{18} columns with different $\alpha_{TBN/BaP}$ values is shown in Fig. 4. Generally, only those columns with $\alpha_{TBN/BaP}$ values between *ca.* 0.6 and *ca.* 0.9 will provide complete resolution of the 16 EPA priority pollutants [21]. Separations of all 16 can also be achieved on columns with a $\alpha_{TBN/BaP} \leqslant 0.4$ but the elution order of dibenz[*a*,*h*]anthracene and benzo[*ghi*]perylene is reversed on the heavily loaded polymeric phase (see Fig. 4). Separation of all 16 components is generally not possible for $\alpha_{TBN/BaP} > 0.9$. As indicated in Table 1, only a small number of columns have the appropriate selectivity for the separation of the 16 PAHs. Several columns listed in Table 1 that are classified as having polymeric-like selectivity are specifically marketed by the manufacturer for the separation of the 16 priority pollutant PAHs (*e.g.*, Hypersil Green PAH, Spherisorb PAH,

TABLE 1

SELECTIVITY CLASSIFICATION ($\alpha_{TBN/BaP}$) FOR VARIOUS COMMERCIAL C_{18} COLUMNS

Column	$\alpha_{TBN/BaP}$
Polymeric phases	
Bakerbond C_{18} Wide-Pore	0.56
Hypersil Green PAH	0.58
Phenomenex Envirosep PP	0.58
Chromspher PAH	0.59
BioRad RP 318	0.59
Supelcosil LC-PAH	0.63
Vydac 201TP	0.74
Spherisorb PAH	0.82
Erbasil C_{18} H	0.91
Intermediate phases	
ES Industries BF-C_{18}	1.04
LiChrospher 100 RP-18	1.11
Bakerbond C_{18}	1.27
Erbasil C_{18} M	1.28
LiChrospher 60 RP-select B	1.36
Partisil 5 ODS-2	1.40
Partisil 5 ODS	1.48
Spherisorb ODS-1	1.50
Zorbax RX C_{18}	1.50
Brownlee ODS 5A	1.51
Sepralyte C_{18}	1.61
Spherisorb ODS-2	1.68
Monomeric phases	
Erbasil C_{18} L	1.76
Pecospher 5 Cr C_{18}	1.76
Partisphere C_{18}	1.79
Zorbax ODS	1.80
Serva C_{18}	1.84
Partisil 5 ODS-3	1.93
Hypersil ODS (HP)	1.94
Microsorb C_{18}	1.95
J&W Accuphase ODS 2	1.96
Novapak C_{18}	1.97
Ultrasphere ODS	1.98
Capcell C_{18} SG120Å	1.99
Supelcosil LC-18	2.00
IBM ODS	2.00
Brownlee Spheri 5 RP-18	2.02
ODS Hypersil	2.04
Cosmosil C_{18}-P	2.04
Ultracarb 5 C_{18} (20%)	2.05
J&W Accuphase ODS	2.07
YMC 120 Å "A"	2.08
Ultracarb 5 C_{18} (30%)	2.10
Adsorbosphere C_{18} HS	2.10
Supelcosil LC-18-DB	2.18

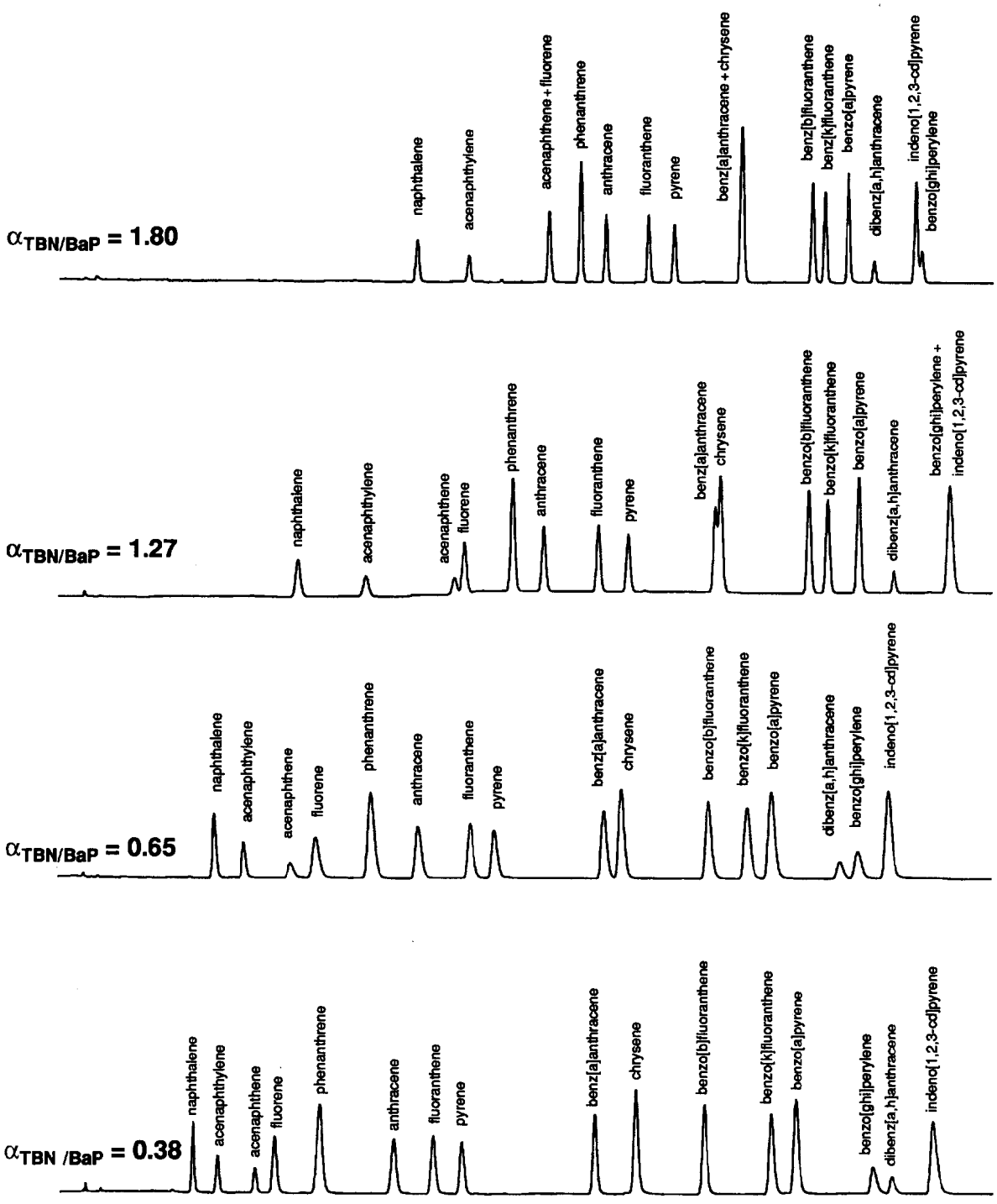

Fig. 4. Separation of SRM 1647 on four different C_{18} columns with different $\alpha_{TBN/BaP}$ values. Chromatographic conditions: columns, Zorbax ODS ($\alpha_{TBN/BaP} = 1.80$), Bakerbond C_{18} (120 Å pore size) ($\alpha_{TBN/BaP} = 1.27$), Vydac 201TP ($\alpha_{TBN/BaP} = 0.65$), and Vydac 201TP (experimental high load) ($\alpha_{TBN/BaP} = 0.38$); mobile phase linear gradient from 40% acetonitrile in water to 100% acetonitrile in 30 min at 2 ml/min; UV detection at 254 nm (from ref. 21).

Chromspher PAH, and Supelcosil-LC PAH)*. However, several of these columns are actually the same bonded phase material repackaged by a different supplier (*e.g.*, Supelcosil-LC PAH and BioRad RP 318 are repackaged Vydac 201TP material). Most manufacturers of polymeric C_{18} phases typically select production batches of material that have the selectivity characteristics necessary to separate the 16 priority pollutant PAHs. However, the $\alpha_{TBN/BaP}$ value for polymeric C_{18} phases from different production lots from the same manufacturer may vary from 0.5 to 0.9; thus, the user may find unexpected selectivity differences that will require slight modifications of the LC method.

In addition to studies at NIST, SRM 869 has found use in a number of studies to characterize stationary phase selectivity [23,24], and several column manufacturers routinely use this mixture to monitor the quality control of the production of their C_{18} phases [25–27]. However, at present no LC column manufacturers routinely report the $\alpha_{TBN/BaP}$ value for each column production lot of stationary phase material. Analysts involved in the determination of PAHs by LC should be aware of the different selectivity characteristics of C_{18} phases and should determine the $\alpha_{TBN/BaP}$ value for each polymeric C_{18} phase used in their laboratory to access its selectivity characteristics prior to use. Polymeric phases with different selectivity characteristics are often useful for specific PAH separation applications. For example, Wise *et al.* [16,28,29] reported the use of a heavily loaded polymeric C_{18} phase ($\alpha_{TBN/BaP} = 0.46$) for the separation PAH isomers of molecular mass 278 and 302.

2.3. Selectivity in RP-LC of PAHs

Although the separation of the 16 EPA priority pollutant PAHs was the goal of some of the early studies on selectivity, the polymeric C_{18} phases were found to exhibit unique selectivity for the separation of PAH isomers. As mentioned previously, the separation of isomers is vital for the determination of PAHs since environmental PAH mixtures contain numerous isomeric structures. Several studies have examined the enhanced selectivity of polymeric *vs.* monomeric C_{18} phases for the separation of PAH isomers in general [11] and specifically for isomers of molecular mass 278 [16,29] and 302 [28,29] and methyl-substituted isomers [11,19,30]. Since PAH isomers differ only in the relative positioning of the aromatic rings or the substitution position of a methyl group, *i.e.*, the shape of the PAH, the enhanced selectivity of PAH separations is often referred to as the shape recognition ability of the stationary phase. The relationship between the PAH solute shape and retention in RP-LC on a polymeric C_{18} phase was first reported by Wise *et al.* [11]. In this study Wise *et al.* [11] defined the shape of the PAH as the length-to-breath ratio (L/B) of the box drawn about the molecule such that the maximum L/B value is produced. Retention of PAH isomers was observed to increase with increasing L/B values, *i.e.*, the more "rod-like" solutes eluted later than the more compact solutes as illustrated in Fig. 5 for the separation of the 278 molecular mass PAH isomers. In a later study, the similarity of LC retention on polymeric C_{18} phases and GC retention on liquid crystalline stationary phases was demonstrated for the same groups of PAH isomers [31]. The planarity of PAH solutes also affects their relative retention on C_{18} phases particularly on polymeric phases. In fact, the differences in the relative retention of planar *vs.* non-planar PAHs is the basis for the selectivity test mixture (SRM 869) discussed above. As shown in Fig. 3, BaP is a planar structure whereas PhPh and TBN are non-planar structures. On polymeric C_{18} phases non-planar PAHs elute early relative to planar PAHs [16,19]. To help visualize the combined effects of PAH shape (L/B) and non-planarity on retention on polymeric C_{18} phases, an empirical model was developed known as the "slot model" [16]. The retention of PAHs in reversed-phase LC on C_{18} phases is discussed in detail in a recent review [22].

Detailed investigations have been reported on the factors that affect PAH selectivity, as measured by the $\alpha_{TBN/BaP}$ value, including phase type [14], substrate pore size [15], alkyl phase length [17], phase coverage/density [13,16], and temperature [32]. These

* Certain commercial equipment, instruments, or materials are identified in this report to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are the best available for the purpose.

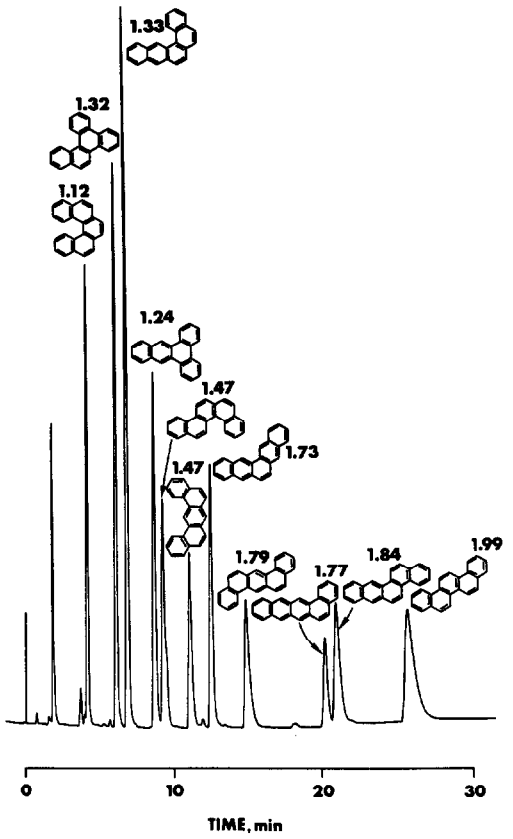

Fig. 5. Separation of eleven PAH isomers of molecular mass 278 on a polymeric C_{18} phase. Numbers above each peak correspond to length-to-breadth values for each isomer. Chromatographic conditions: Vydac 201TP column (experimental high loading); mobile phase linear gradient from 85% acetonitrile in water to 100% acetonitrile in 15 min at 1.5 ml/min; UV detection at 254 nm (from ref. 16).

results have been summarized recently in a review [21]. In general, selectivity for the separation of PAHs increases with decreasing $\alpha_{TBN/BaP}$ values. Furthermore, separations often can be reproduced under different conditions (*i.e.*, different combinations of phase type, alkyl chain length, and column temperature) as long as the $\alpha_{TBN/BaP}$ value is held constant. In practice, parameters such as phase type, ligand density, or alkyl chain length can only be varied by changing the column. However, column temperature is an important parameter that can be used to readily modify selectivity for PAH separations. Generally in LC analyses temperature is considered only for potentially increasing the column efficiency by increasing the temperature. The effect of temperature on phase selectivity (as monitored by the $\alpha_{TBN/BaP}$ value) for temperatures from −20°C to 100°C is illustrated in Fig. 6 for both monomeric and polymeric C_{18} phases. Changes in shape selectivity are relatively uniform for the polymeric C_{18} phase, whereas for the monomeric phase the selectivity is nearly constant above 25°C with significant changes at subambient temperatures. The selectivity *vs.* temperature plots in Fig. 6 offer useful information for the separation of PAHs by RP-LC. First, separations of PAHs using polymeric C_{18} phases are very sensitive to temperature variations which may result in changes in selectivity and not just variations in absolute retention time as would generally be observed with changes in temperature. As a result temperature control for PAH separations on a polymeric C_{18} phase is more important than for other LC analyses. Secondly, polymeric C_{18} phases generally should not be used at elevated temperature since any increases in column efficiency are greatly outweighed by a significant reduction in selectivity. Finally, a monomeric C_{18} phase will have "polymeric-like" selectivity at temperatures near 0°C and below. The separation of all 16 of the priority pollutant PAHs on a monomeric C_{18} has been demonstrated at −8°C [32]. Subambient temperatures can also be used with polymeric C_{18} phases to achieve enhanced selectivity characteristics that are not possible with available polymeric phases at ambient temperatures. This enhanced capability was demonstrated for the separation of all six methylchrysene isomers, which previously had not been separated by LC [32].

3. APPROACHES FOR THE DETERMINATION OF PAHs

Even though separation of the 16 priority pollutant PAHs is easily achieved by RP-LC on the appropriate C_{18} column (see Fig. 2), natural mixtures of PAHs from environmental sample extracts are extremely complex due to numerous isomeric structures including alkyl-substituted isomers. Thus, the analysis of these PAH mixtures requires the use of selective detection and/or the use of "multidimensional" LC procedures to accurately measure individual PAHs.

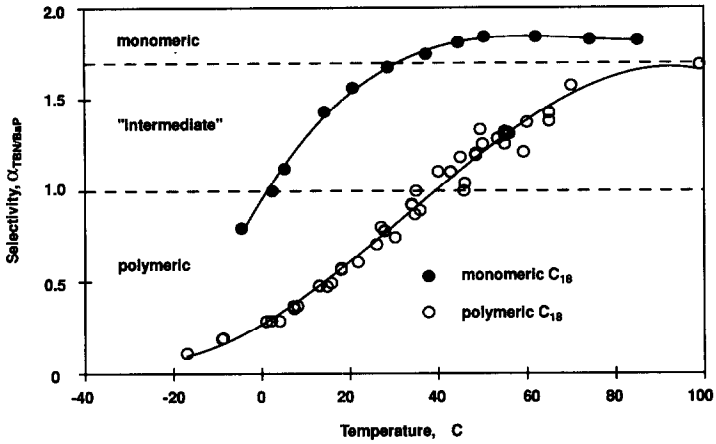

Fig. 6. Shape selectivity plotted as a function of temperature for a monomeric (Zorbax ODS) and a polymeric (Vydac 201TP) C_{18} phase (from ref. 32).

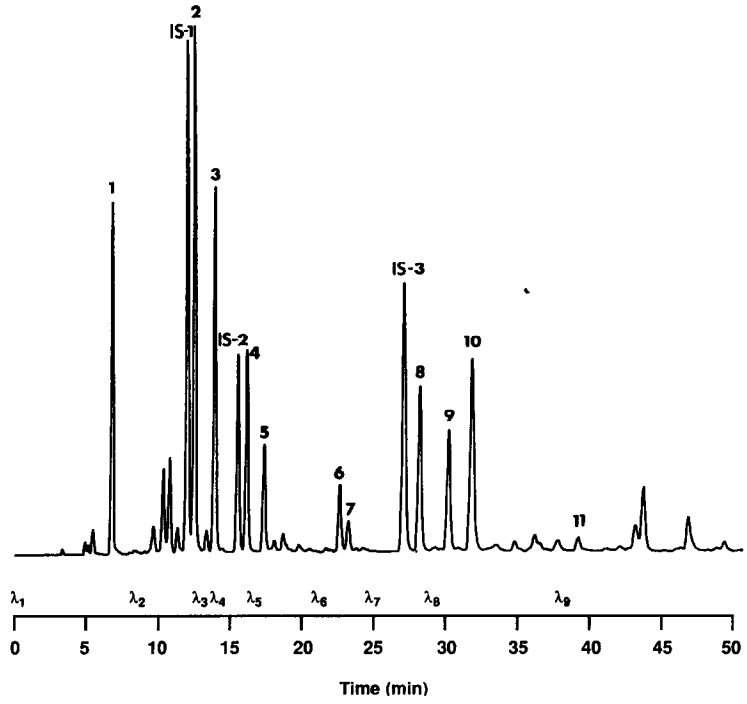

Fig. 7. RP-LC analysis of a coal tar sample (SRM 1597) using wavelength programmed fluorescence detection. Fluorescence conditions (excitation λ/emission λ in nm): λ_1 (280/340), λ_2 (249/380), λ_3 (250/442), λ_4 (285/450), λ_5 (333/390), λ_6 (285/385), λ_7 (406/440), λ_8 (296/405), and λ_9 (300/500). Peak identifications: 1 = naphthalene, IS-1 = [$^2H_{10}$]phenanthrene, 2 = phenanthrene, 3 = anthracene, IS-2 = [$^2H_{10}$]fluoranthene, 4 = fluoranthene, 5 = pyrene, 6 = benz[*a*]anthracene, 7 = chrysene, IS-3 = [$^2H_{12}$]perylene, 8 = perylene, 9 = benzo[*k*]fluoranthene, 10 = benzo[*a*]pyrene, and 11 = indeno[1,2,3,-*cd*]pyrene. Chromatographic conditions: Vydac 201TP column; mobile phase linear gradient from 50% acetonitrile in water to 100% acetonitrile in 50 min at 1.5 ml/min (from ref. 37).

3.1. Selective detection

UV absorption and fluorescence detection are the most widely used LC detectors for the measurement of PAHs. UV detection provides a nearly "universal" detector for PAHs; however, for quantitation in complex environmental PAH mixtures, the fluorescence detector offers far more sensitivity and, more importantly, selectivity than UV detection. By selection of the appropriate excitation and emission wavelengths, a high degree of specificity can be achieved. The analysis of a coal tar extract using RP-LC with fluorescence detection is illustrated in Fig. 7. The excitation and emisson wavelengths were changed during the chromatographic run (*i.e.*, wavelength programming) to achieve optimal sensitivity and/or selectivity for individual PAHs. The excitation and emission wavelengths currently used at NIST for the analysis of environmental samples are summarized in Table 2. Fluorescence excitation and emission spectra for a number of PAH standards have been published [33–35] and these spectra can be used as the basis for selection of optimal wavelength conditions for the PAHs measured. RP-LC with fluorescence wavelength programmed detection has been used at NIST to measure PAHs in several sample types including oil [36], coal tar [37], air and diesel particulate samples [38], marine sediment [39], and mussel tissue [40]. Three perdeuterated PAHs ([$^2H_{10}$]phenanthrene, [$^2H_{10}$]fluoranthene, and [$^2H_{12}$]perylene) were used as the internal standards for quantification of the PAHs in the coal tar sample shown in Fig. 7. Perdeuterated PAHs are excellent internals standards for RP-LC separations of PAHs since they elute immediately prior to the non-deuterated PAH (generally with baseline resolution) and they have nearly the same fluorescence characteristics as the non-deuterated PAHs [36,38]. Recently the method illustrated in Fig. 7 has been modified to include [$^2H_8$]naphthalene and [$^2H_{12}$]-benzo[*a*]pyrene for a total of five internals standards from two to five aromatic rings as shown in Table 2.

TABLE 2

FLUORESCENCE WAVELENGTH PROGRAMMING CONDITIONS FOR THE LC DETERMINATION OF SELECTED PAHs

Wavelength change	Excitation wavelength (nm)	Emission wavelength (nm)	PAHs determined
1	280	340	[$^2H_8$]Naphthalene (IS)[a]
			Naphthalene
2	249	362	[$^2H_{10}$]Phenanthrene (IS)
			Phenanthrene
3	250	400	Anthracene
4	285	450	[$^2H_{10}$]Fluoranthene (IS)
			Fluoranthene
5	333	390	Pyrene
6	285	385	Benz[*a*]anthracene
7	260	360	Chrysene
8[b]	406	440	[$^2H_{12}$]Perylene (IS)
			Perylene
8a	295	425	Benzo[*b*]fluoranthene
9	296	405	Benzo[*k*]fluoranthene
			[$^2H_{12}$]Benzo[*a*]pyrene (IS)
			Benzo[*a*]pyrene
			Dibenz[*a,h*]anthracene
			Benzo[*ghi*]perylene
10	300	500	Indeno[1,2,3,-*cd*]pyrene

[a] IS = internal standard.

[b] Wavelength conditions for 8 and 8a are used during separate chromatographic runs due to coelution of perylene and benzo[*b*]fluoranthene.

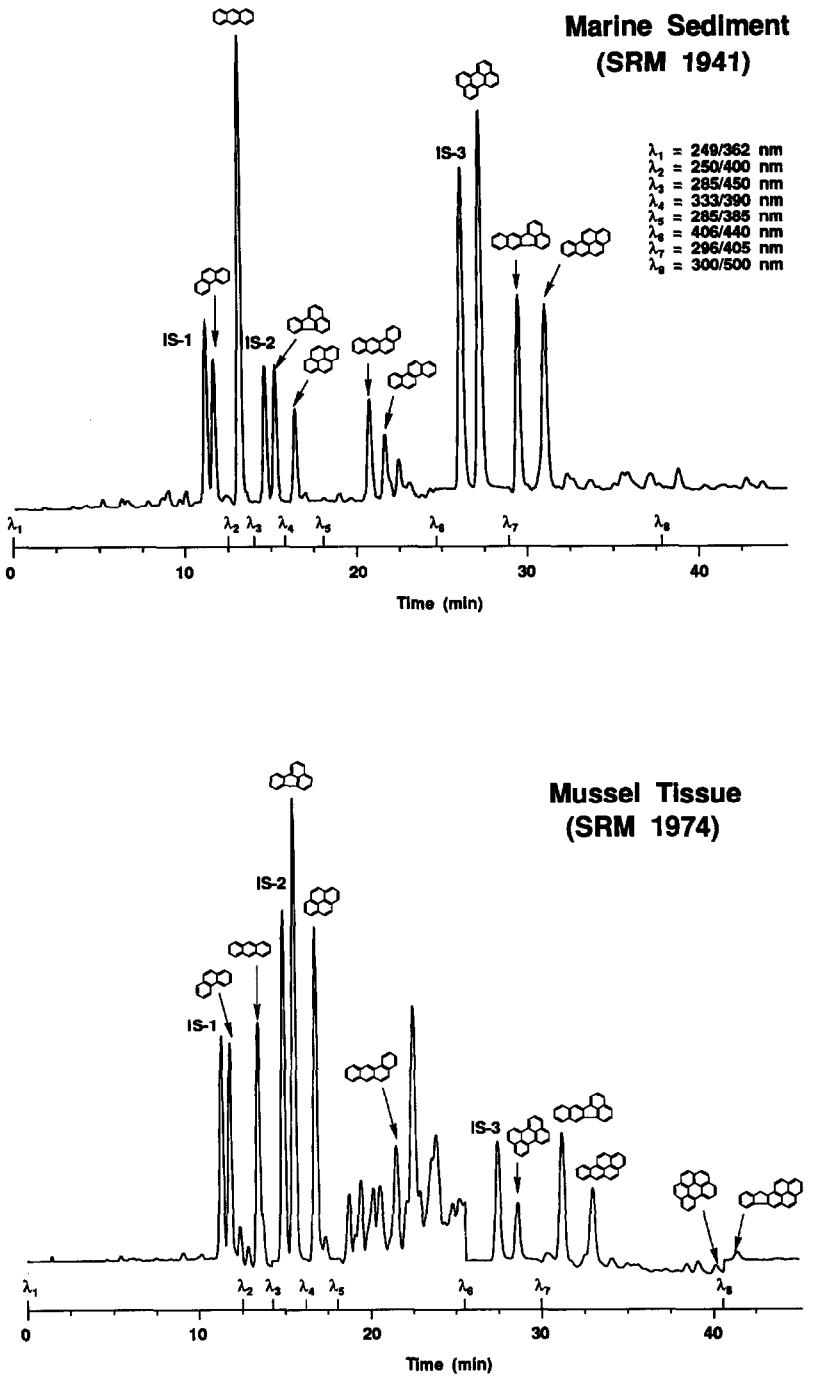

Fig. 8. RP-LC analysis of marine sediment (SRM 1941) and mussel tissue (SRM 1974) using wavelength programmed fluorescence detection. Chromatographic conditions: Vydac 201TP column; mobile phase linear gradient from 50% acetonitrile in water to 100% acetonitrile in 50 min at 1.5 ml/min.

For the coal tar sample in Fig. 7, a wavelength change was not performed between benz[*a*]anthracene and chrysene. However, by using a column with the appropriate selectivity, sufficient separation can be achieved to allow a wavelength change. Generally, temperature control must be maintained to achieve the retention time reproducibility required to make wavelength changes between closely eluting chromatographic peaks.

Even though the coal tar extract shown in Fig. 7 is a complex mixture of PAHs, it could be analyzed directly by LC without any additional cleanup or isolation of the PAH fraction. However, extracts of environmental samples such as sediment and tissue generally require additional cleanup or isolation steps to obtain accurate results for the measurement of individual PAHs. However, because of the selectivity of fluorescence detection, environmental extracts can often be analyzed by LC with less sample cleanup than would be required for GC analyses [38]. The analyses of marine sediment and tissue samples using RP-LC with wavelength programmed fluorescence detection are shown in Fig. 8 [39,40]. These sample extracts were cleaned up using a normal-phase solid phase extraction (SPE) procedure to remove the polar compounds (see below).

3.2. Multidimensional LC approach

A second approach to the measurement of PAHs in complex mixtures is the use of multidimensional chromatographic procedures. At NIST we have routinely used a multidimensional LC approach to isolate the PAH fraction or groups of PAH isomers prior to analysis by RP-LC with fluorescence or UV detection or by gas chromatographic techniques. This multidimensional procedure consists of a normal-phase LC separation of the environmental extract or PAH mixture on an aminopropylsilane stationary phase. In 1979 Wise *et al.* [41] demonstrated that the normal-phase elution of PAHs from the aminopropylsilane column was based on the number of aromatic carbon atoms in the PAH, *i.e.*, isomeric PAHs elute as a group and alkyl-substituted PAHs elute very near the unsubstituted parent PAHs. Thus, the aminopropylsilane column can be used to isolate a fraction containing only isomeric PAHs and alkyl-substituted isomers. The normal-phase LC retention characteristics for over 80 PAHs on the aminopropylsilane column have been reported [7,40]. This normal-phase LC procedure has been used as the first step in multidimensional chromatographic procedures for (i) cleanup and isolation of the total PAH fraction from environmental samples, (ii) isolation of selected isomeric PAHs in complex mixtures, and (iii) detailed characterization of PAH mixtures.

3.2.1. Cleanup and isolation of total PAH fraction

The normal-phase LC cleanup and isolation of the total PAH fraction is routinely used at NIST as the sample preparation procedure prior to analysis by GC with flame ionization detection (FID) and mass spectrometric (MS) detection or by RP-LC with fluorescence detection. For GC–FID analysis complete isolation of the PAH fraction is required (*i.e.*, using an LC separation) to eliminate the aliphatic hydrocarbon and polar interferences. The same cleanup/isolation procedure is generally applied for GC–MS analyses, even though MS provides sufficient detection specificity to allow minimal cleanup, to minimize loss of chromatographic efficiency when using on-column injection techniques for open tubular GC columns. However, a simple SPE procedure using the same aminopropylsilane phase can be used to remove the compounds more polar than PAHs. The SPE procedure does not remove the aliphatic hydrocarbons from the PAH fraction; however, these compounds do not interfere in the RP-LC with fluorescence detection procedure or in the selective GC–MS procedure because of the selectivity of the detector. This approach was used for the cleanup of the sediment and mussel tissue samples shown in Fig. 8.

3.2.2. Isolation of selected isomeric PAHs

The second application of the multidimensional LC procedure is for the isolation and measurement of selected isomeric PAHs in complex samples. This approach is useful in two ways: (i) to isolate an isomeric fraction from a complex mixture such as an oil matrix to measure the major PAH components and (ii) to isolate an isomeric PAH fraction to enrich the concentration of minor PAH isomers not easily measured in the total PAH fraction. The first approach is illustrated in Fig. 9 for the determination of pyrene and fluoranthene in a shale oil sample. The pyrene–fluoranthene isomer fraction was iso-

lated from the shale oil by injection of the diluted shale oil onto the aminopropylsilane column and collecting the fraction corresponding to the elution of pyrene and fluoranthene. This fraction was then analyzed by RP-LC with UV and fluorescence detection. The necessity of the fluorescence detection to achieve accurate quantitation of the pyrene and fluoranthene is illustrated by the comparison of the chromatograms with UV and fluorescence detection (Fig. 9). This application also illustrates the suitability of the perdeuterated PAHs as internal standards for the complete multidimensional LC procedure, *i.e.*, on the aminopropylsilane column the perdeuterated PAHs elute slightly after the

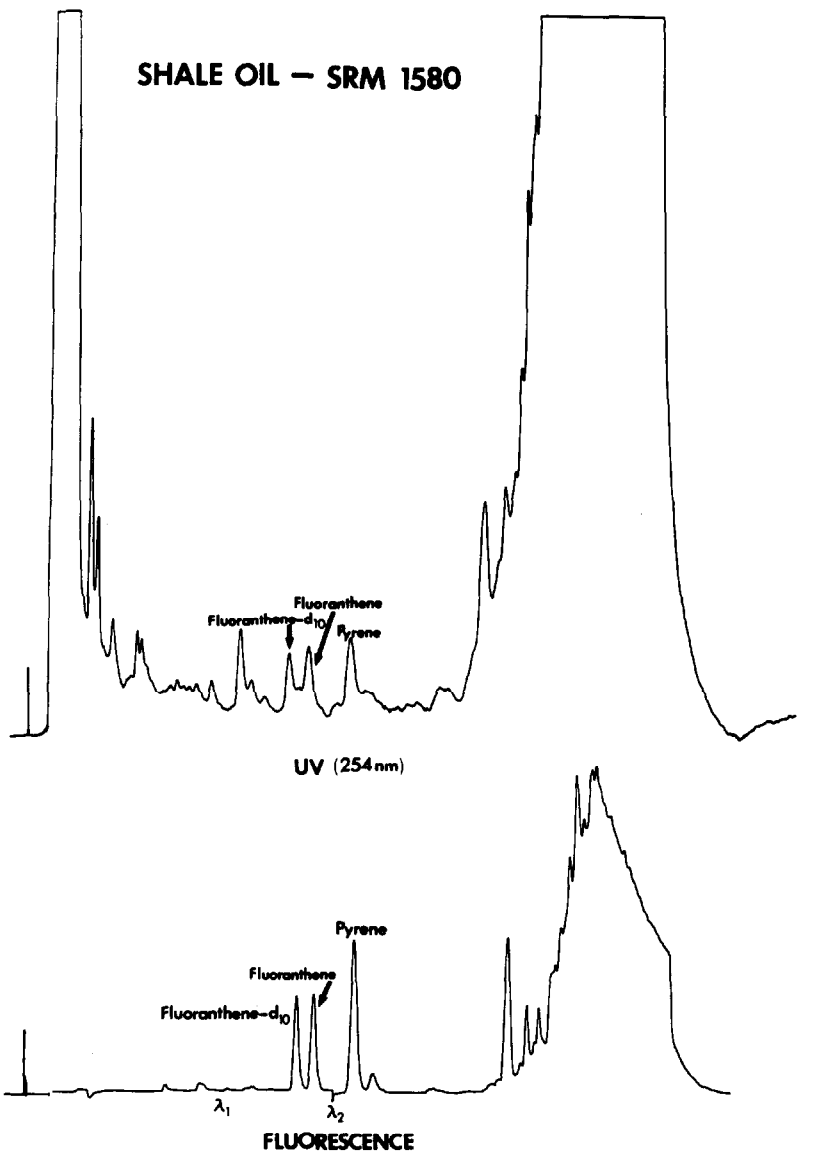

Fig. 9. RP-LC analysis of the fluoranthene–pyrene fraction (16 aromatic carbon atoms) isolated from a shale oil sample (SRM 1580). Fluorescence conditions (excitation λ/emission λ in nm): λ_1 (285/450) and λ_2 (335/385). Chromatographic conditions: Vydac 201TP column; mobile phase isocratic at 45% acetonitrile in water until fluoranthene eluted, then linear gradient to 100% acetonitrile in 5 min at 1.5 ml/min (from ref. 36).

non-deuterated PAHs but still within the isomeric group. This multidimensional LC approach was used to measure PAHs in the shale oil and petroleum crude oil SRMs [36,42,43] (see discussion below) including a novel on-line approach [44].

Another application of the multidimensional LC procedure for the isolation and measurement of isomeric PAHs is to enrich the concentration of minor PAHs components that are not easily measured in the total PAH fraction. This approach is illustrated by the determination of triphenylene and benzo[*ghi*]perylene in the coal tar and marine sediment samples shown in Figs. 7 and 8. Using the LC–fluorescence approach for the analysis of the coal tar and PAH fraction from the marine sediment extracts, it was not possible to obtain accurate data for triphenylene or benzo[*ghi*]perylene because of low detection sensitivity and/or selectivity (see Figs. 7 and 8). To obtain accurate LC measurements for these two compounds, the normal-phase LC fractionation procedure was employed to isolate separate fractions for the four-ring PAH isomers (18 aromatic carbons: triphenylene, chrysene, and benz[*a*]anthracene) and the six-ring isomers (22 aromatic carbons: benzo[*ghi*]perylene, indeno[1,2,3-*cd*]pyrene, and anthanthrene). Prior to sample extraction, perdeuterated PAHs were added as internal standards to represent each isomer group, *e.g.*, [$^2H_{12}$]triphenylene and [$^2H_{12}$]benz[*a*]anthracene were added for the four-ring isomer fraction and [$^2H_{14}$]benzo[*ghi*]perylene was added for the six-ring fraction. The normal-phase LC separation of an air particulate extract is shown in Fig. 10. The four-ring and six-ring PAH fractions were collected, concentrated, and analyzed by RP-LC–fluorescence as illustrated in Fig. 11 for a sediment sample. Chrysene and triphenylene are generally not quantified individually by GC analysis since they coelute on conventional stationary phases, and their concentrations are reported as a combined value. Using the multidimensional LC procedure, results for triphenylene and chrysene were determined in the sediment sample as shown in Table 3. The two LC results in Table 3 for chrysene (Direct and Fraction) are in good agreement (425 ± 42 and 473 ± 5 ng/g). The GC–FID and GC–MS results for chrysene–triphenylene are 577 ± 12 and 702 ± 16, respectively, (mean of 639 ng/g) compared to the sum of LC results for triphenylene (192 ng/g) and chrysene (mean of 449 ng/g) which is 641 ng/g. This same approach was also used to measure chrysene and triphenylene in air particulate material [38] and a coal tar extract [37].

The measurement of benzo[*ghi*]perylene (and often indeno[1,2,3-*cd*]pyrene) in the total PAH fraction is generally difficult (even though their concentrations are similar to other major PAHs), due to low fluorescence sensitivity and selectivity for these PAHs. By using the normal-phase LC fractionation procedure, these two isomers are enriched sufficiently to provide good measurements. The RP-LC–fluorescence analysis of the six-ring PAH fraction from the marine sediment SRM is shown in Fig. 11. The results of these analyses are summarized and compared in Table 3. Using this approach, the LC results for benzo[*ghi*]perylene (504 ± 7) were in good agreement with the GC–FID results (478 ± 14 ng/g) and within about 12% of the GC–MS measurements (567 ± 26 ng/g). For indeno[1,2,3-*cd*]pyrene all four results agreed within 3%, *i.e.*, 572 ± 28 ng/g (GC–FID), 573 ± 20 ng/g (LC Direct), 575 ± 8 ng/g (LC Fraction), and 559 ± 19 ng/g (GC–MS). Anthanthrene, which has not been mea-

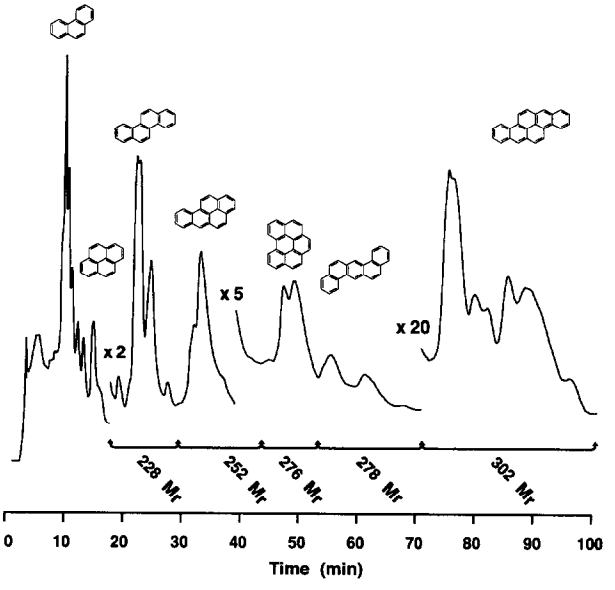

Fig. 10. Normal-phase LC fractionation of air particulate extract (SRM 1648) to isolate the isomeric PAH groups. Chromatographic conditions: μBondapak NH_2 semipreparative column; mobile phase of 2% methylene chloride in hexane at 5 ml/min; UV detection at 254 nm.

TABLE 3

SUMMARY OF ANALYTICAL RESULTS (ng/g DRY WEIGHT) FOR THE DETERMINATION OF PAHs IN SRM 1941, ORGANICS IN MARINE SEDIMENT

Compound	GC–FID	GC–MS	LC–FL (Direct)	LC–FL (Fraction)	Certified value[a]
Phenanthrene	597 (4)[b]	603 (10)	531 (12)		577 ± 59
Anthracene	202 (6)	228 (12)	174 (8)		202 ± 42
Fluoranthene	1116 (20)	1401 (41)	1135 (10)		1220 ± 240
Pyrene	1008 (16)	1238 (18)	989 (34)		1080 ± 200
Benz[*a*]anthracene	538 (12)	599 (14)	516 (7)	521 (11)[d]	550 ± 78
Chrysene	577 (12)[c]	702 (16)[c]	425 (42)	473 (5)[d]	
Triphenylene				192 (3)[d]	
Benzo[*b*]fluoranthene	635 (17)	864 (28)	839 (14)	843	780 ± 190
Benzo[*j*]fluoranthene	351 (14)				
Benzo[*k*]fluoranthene	439 (19)	857 (25)[f]	456 (6)[e] 441 (8)[e]	443 (16)	444 ± 49
Benzo[*e*]pyrene	472 (25)	672 (24)			
Benzo[*a*]pyrene	566 (12)	754 (49)	674 (12)	690 (25)	670 ± 130
Perylene	415 (8)	437 (27)	411 (6)	426 (5)	442 ± 33
Benzo[*ghi*]perylene	478 (14)	566 (26)		504 (7)	516 ± 83
Indeno[1,2,3-*cd*]pyrene	572 (28)	559 (19)	573 (20)	575 (8)	569 ± 40

[a] The certified values are weighted means of results from two or more analytical techniques. Each uncertainty is obtained from a 95% prediction interval plus an allowance for systematic error among the methods used. The allowance for systematic error is equal to the greatest difference between the weighted mean (certified value) and the component means for the analytical methods used. In the absence of systematic error, the resulting uncertainty limits will cover the concentration of approximately 95% of samples of this SRM having a minimum sample size of approximately 5 g.

[b] Uncertainties (values in parentheses) for GC–FID, LC–FL, and GC–MS measurements are ± one standard deviation of a single measurement; for GC–FID measurements, twelve samples analyzed in triplicate; for LC measurements, three samples analyzed in triplicate; for GC–MS measurements, four samples analyzed in duplicate.

[c] Value is for chrysene and triphenylene.

[d] Determined using [$^2H_{12}$]triphenylene as internal standard.

[e] Benzo[*k*]fluoranthene was determined at different times, *i.e.*, during initial analyses of total PAH fraction and during benzo[*b*]fluoranthene analyses.

[f] Value is for benzo[*k*]fluoranthene and benzo[*j*]fluoranthene.

sured previously at NIST in total PAH fractions, is readily quantified in the six-ring fraction and has been measured in a new marine sediment SRM using this approach.

The measurement of dibenz[*a,h*]anthracene and other five-ring catacondensed PAH isomers (molecular mass 278) is another example of the application of the multidimensional LC technique to measure minor level PAHs. Dibenz[*a,h*]anthracene, which is listed as one of the EPA priority pollutant PAHs, is often quantified based on either GC or LC measurements. However, for GC measurements on a stationary typically used for PAH analyses (*e.g.*, 5% phenyl-substituted methylpolysiloxane), dibenz[*a,c*]-anthracene coelutes with dibenz[*a,h*]anthracene. RP-LC measurements of dibenz[*a,h*]anthracene are highly questionable since the resolution of isomers is dependent on the column used (see discussion above), and the fluorescence selectivity for dibenz-[*a,h*]anthracene is generally not sufficient to preclude the possibility of interference from coeluting PAHs in a complex mixture [43]. To quantify dibenz[*a,h*]anthracene and five other 278 molecular mass isomers in several environmental matrix SRMs, the normal-phase LC procedure was used to isolate the dibenz[*a,h*]anthracene isomer fraction as shown in Fig. 10. The dibenz[*a,h*]anthracene fraction was then analyzed by RP-LC–fluorescence as shown in

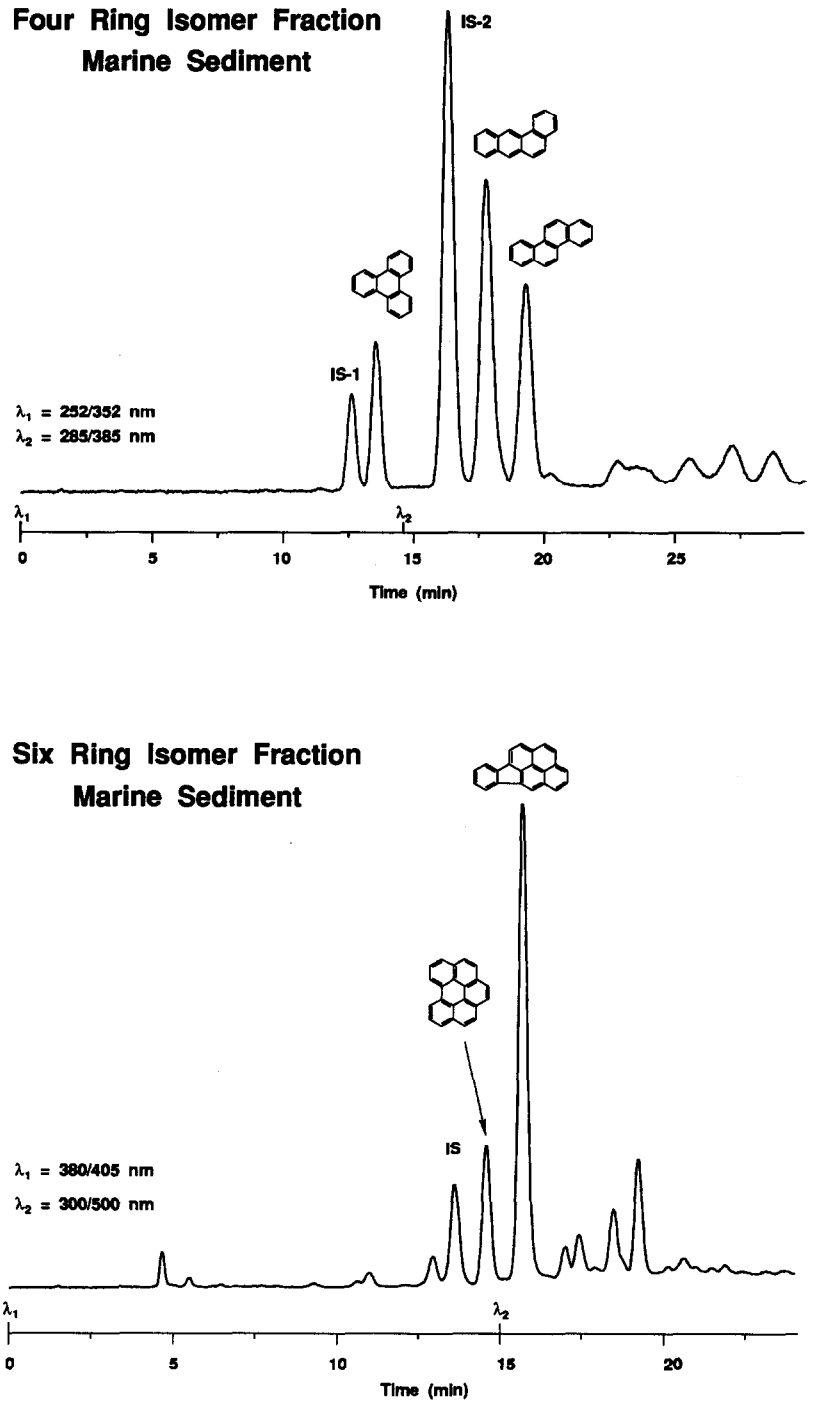

Fig. 11. RP-LC analysis of the 228 and 276 molecular mass fractions isolated from the marine sediment (SRM 1941) extract. Chromatographic conditions: Vydac 201TP column; mobile phase (four ring fraction): isocratic at 60% acetonitrile in water for 15 min, then linear gradient to 100% acetonitrile in 5 min at 1.5 ml/min; mobile phase (six ring fraction): isocratic at 80% acetonitrile in water for 5 min, then linear gradient to 100% acetonitrile in 15 min at 1.5 ml/min; fluorescence detection.

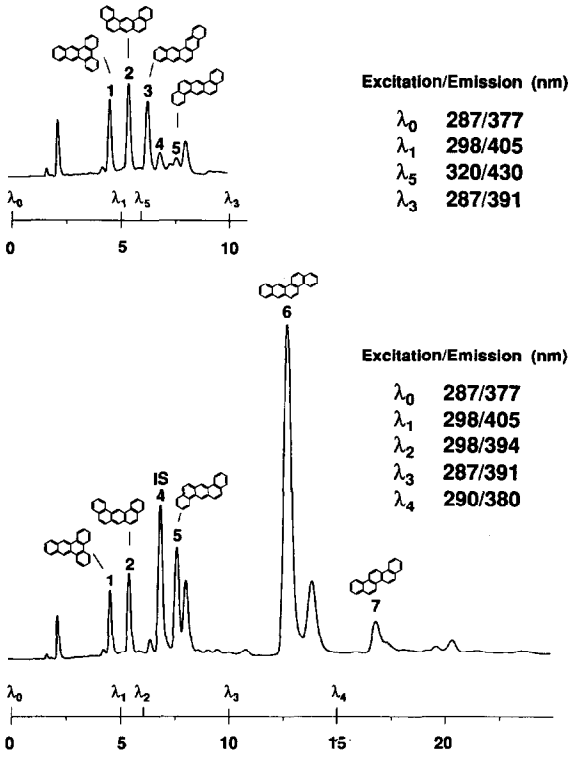

Fig. 12. RP-LC analysis of 278 molecular mass fraction isolated from a coal tar extract (SRM 1597). Peak identifications: 1 = dibenz[*a*,*c*]anthracene, 2 = dibenz[*a*,*j*]anthracene, 3 = pentaphene, 4 IS (internal standard) = [$^2H_{14}$]dibenz[*a*,*h*]anthracene, 5 = dibenz[*a*,*h*]anthracene, 6 = benzo[*b*]chrysene, 7 = picene. Chromatographic conditions: Vydac 201TP column ($\alpha_{TBN/BaP}$ = 0.46); mobile phase isocratic at 90% acetonitrile in water for 10 min and then a linear gradient to 100% acetonitrile in 2 min at 1.5 ml/min; column temperature of 32°C (from ref. 29).

Fig. 12 for the coal tar extract (SRM 1597). The same multidimensional LC approach was used to measure individual isomers of molecular mass 302 (dibenzopyrene–fluoranthenes); the RP-LC analysis of the dibenzopyrene–fluoranthene fraction is shown in Fig. 13. Using this multidimensional procedure, the concentrations of six PAH isomers of molecular mass 278 and nine PAH isomers of molecular mass 302 were determined in four natural matrix SRMs [29].

3.2.3. Detailed characterization of PAH mixtures

The third application of the normal-phase LC procedure is to isolate isomer fractions for analysis by GC–MS and RP-LC–fluorescence to provide detailed characterization of the PAH mixture. This approach has been used to provide qualitative information on over 180 PAHs in two air particulate material SRMs [45].

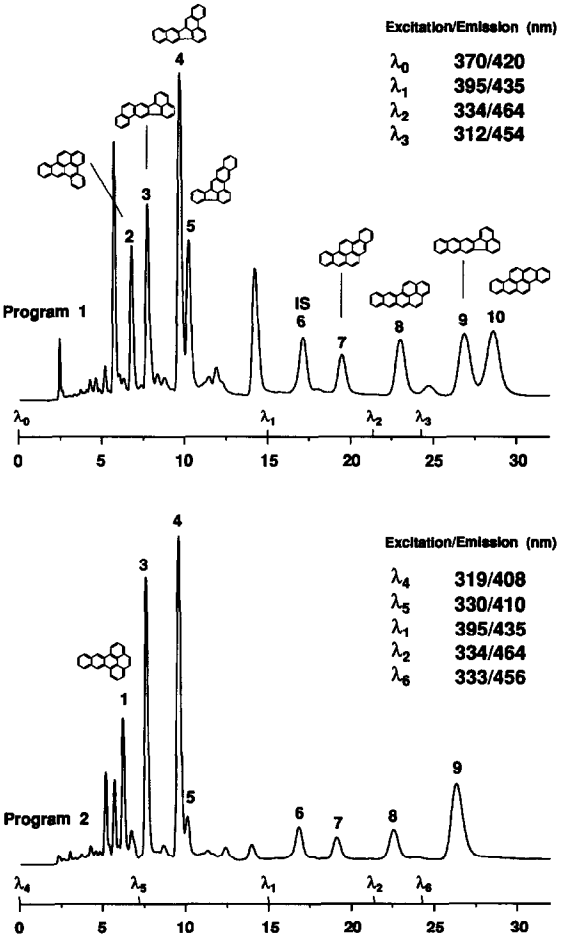

Fig. 13. RP-LC analysis of 302 molecular mass fraction isolated from coal tar extract (SRM 1597). Peak identifications: 1 = naphtho[2,3-*e*]pyrene, 2 = dibenzo[*a*,*e*]pyrene, 3 = naphtho[1,2-*k*]fluoranthene, 4 = dibenzo[*b*,*k*]fluoranthene, 5 = naphtho[2,3-*b*]fluoranthene, 6 IS (internal standard) = [$^2H_{14}$]dibenzo[*a*,*i*]pyrene, 7 = dibenzo[*a*,*i*]pyrene, 8 = naphtho[2,3-*a*]pyrene, 9 = naphtho[2,3-*k*]fluoranthene, 10 = dibenzo[*a*,*h*]pyrene. Chromatographic conditions: Vydac 201TP column ($\alpha_{TBN/BaP}$ = 0.46); mobile phase isocratic at 100% acetonitrile at 1.5 ml/min; column temperature of 29°C (from ref. 29).

TABLE 4

NIST SRMs FOR THE DETERMINATION OF PAHs

PANH = polycyclic aromatic nitrogen heterocycles; PAQ = polycyclic aromatic quinones; PASH = polycyclic aromatic sulphur heterocycles.

SRM No.	Title	Date issued	Certified constituents	Non-certified constituents	Literature references
Performance standard					
869	Column selectivity test mixture for liquid chromatography (PAHs)	1990			18, 21
Calibration solutions					
1491	Aromatic hydrocarbons in hexane–toluene	1989	PAHs (23)	PAHs (1)	
1644	Generator columns for PAHs	1981	PAHs (3)		50,51
1647c	Priority pollutant PAHs (in acetonitrile)	1993	PAHs (16)		
2260	Aromatic hydrocarbons in toluene (nominal concentration 60 μg/ml)	1991	PAHs (23)	PAHs (1)	
Natural matrix materials					
1580	Organics in shale oil	1980	PAHs (5); phenols (3) PANH (1)	Phenols (6) PANH (1)	36, 42, 43
1582	Petroleum crude oil	1984	PAHs (5); PASH (1)	PAHs (5) Phenols (2); PANH (1)	36, 43
1597	Complex mixture of PAHs from coal tar	1987	PAHs (12)	PAHs/PACs (18)	37
1648	Urban particulate matter	1978	Trace elements (9)	Trace elements (25) PAHs (13)	45
1649	Urban dust/organics	1982	PAHs (5)	PAHs (9)	40, 45
1650	Diesel particulate material	1985	PAHs (5); Nitro-PAHs (1)	PAHs (6); Nitro-PAHs (3); PAQ (1)	43
1939	Polychlorinated biphenyls (congeners) in river sediment	1990	PCBs (3)	PCBs (14); pesticides (5); PAHs (5)	49
1941	Organics in marine sediment	1989	PAHs (11)	PAHs (24); pesticides (7); PCBs (15); trace elements (32)	39
1974	Organics in mussel tissue (*Mytilus edulis*)	1990	PAHs (9)	PAHs (19); pesticides (9); PCBs (13); trace elements (36)	40
1975	Diesel particulate extract (in preparation)	1993	PAHs; nitro-PAHs		

4. SRMs FOR THE DETERMINATION OF PAHs

To accurately identify and quantify individual PAHs in complex environmental samples, the analyst must use analytical procedures that have been validated as to their accuracy. To assist in validating the accuracy of analytical methods for the determination of PAHs, NIST has developed a number of PAH related SRMs [46–49]. Reference materials are used primarily for the following purposes: (i) to calibrate the measurement system, (ii) to validate the reliability and precision of a new analytical method, and (iii) to provide quality control of routine analyses by analyzing the SRM at appropriate, regular time intervals. The NIST SRMs for PAH measurements are summarized in Table 4. These SRMs represent two different levels of analytical difficulty: (i) simple calibration solutions containing a number of PAH analytes and (ii) natural matrix materials. A third category, performance standards such as SRM 869, has been described above. The calibration solutions are useful for several purposes including: (i) calibration of chromatographic instrumentation for retention times and detector response factors for quantitation, (ii) spiking or fortifying samples, (iii) analyte recovery studies, and (iv) determining method response factors. The natural matrix materials, which are similar to the actual

environmental samples analyzed both in analyte concentrations and potential matrix interferences, can be used to validate the complete analytical procedure including extraction, isolation/cleanup procedures, and the final chromatographic separation and quantification. Thus, the natural matrix SRMs are generally more suitable for use in the validation of new analytical procedures and for routine quality control purposes.

The most popular of the organic environmental SRMs is SRM 1647, an acetonitrile solution of the 16 PAHs on the EPA's priority pollutant list (see Fig. 1). This SRM was prepared at the request of EPA in support of EPA Method 610, which specifies the use of RP-LC with fluorescence detection for the determination of PAHs [6]. SRM 1647 has found widespread use as a calibration solution to determine retention times and detector response factors in LC. Because of the popularity of SRM 1647, it has been reissued three times since first issued in 1981 and is now available as SRM 1647c. Since SRM 1647 is used primarily for calibration of RP-LC instrumentation and spiking of aqueous-based matrices (*i.e.*, because of the acetonitrile solvent), two similar solutions (SRMs 1491 and 2260) have been prepared in hexane and/or toluene to provide a solvent more compatible with GC and normal-phase LC analyses and for spiking into non-aqueous matrices. In addition to the 16 PAHs included in SRM 1647, SRMs 1491 and 2260 contain eight additional PAH analytes, which were included in these SRMs specifically to meet the needs of a national marine pollution monitoring program in the U.S. SRMs 1491 and 2260 contain the same 24 analytes but at concentrations that differ by approximately a factor of 10.

Since 1980, seven natural matrix SRMs have been issued with certified concentrations of PAHs and other polycyclic aromatic compounds (PACs): SRMs 1580, 1582, 1597, 1649, 1650, 1941, and 1974. SRM 1648, Urban Particulate Matter, was issued in 1978 and certified for inorganic constituents; however, data have been reported in the literature for PAH concentrations in this material [45]. Summaries of the concentrations of PAHs in these SRMs have been published [46–49]. These SRMs represent several matrix types, relative PAH concentrations, and sources of the PAH (*i.e.*, petrogenic or pyrolytic). SRMs 1580 and 1582 are representative of oil matrices with petrogenic PAHs (*i.e.*, formed from low-temperature processes) which have high levels of alkyl-substituted PAHs relative to the unsubstituted PAHs. These two materials have been described in more detail elsewhere [37,42]. SRMs 1648 and 1649 are two air particulate samples that were collected in the mid-1970's in St. Louis MO, USA and Washington, DC, USA, respectively; the PAHs mixtures on these materials are representative of pyrolytic sources. An extensive characterization and comparison of the PAH content of these two air particulate SRMs have been reported in the literature [45]. The diesel particulate sample (SRM 1650) is representative of heavy duty diesel emissions in the early 1980's. SRM 1597, which is a complex natural pyrolytic mixture of PAHs from a coke oven tar, has the most extensive quantitative characterization for PAHs.

The most recent SRM matrices for PAH measurements are sediment and mussel tissue. SRM 1941 is a marine sediment that was collected in the Baltimore Harbor (MD, USA) with PAH concentrations of 500–1300 ng/g [39]. SRM 1939, which is certified for polychlorinated biphenyl (PCB) congeners, is a river sediment collected from the Hudson River (NY, USA) and is representative of sediment with high levels of PCB congeners and chlorinated pesticides (100–7000 ng/g and 60–550 ng/g, respectively), but low levels of PAHs (50–200 ng/g) [48]. To meet the need for a natural matrix marine tissue reference material for organic contaminants, NIST issued SRM 1974, "Organics in Mussel Tissue (*Mytilus edulis*)" which was prepared from mussels collected in Boston Harbor (MA, USA) [40]. The mussel tissue was cryogenically homogenized and the SRM is provided as a frozen powder-like homogenate, thereby providing a matrix similar to the sample matrices typically encountered in marine tissue analyses. The PAH concentrations of these marine matrix SRMs have been summarized recently [47,48].

The natural matrix SRMs in Table 4 are also useful for methods development for PAHs that have not been certified or measured at NIST. In this instance SRMs are homogeneous natural environmental matrices that are readily available to other laboratories for comparison of analytical results. As laboratories involved in environmental pollution monatoring expand the number of PAHs measured due to emphasis on toxicity/mutagenicity, pollution

TABLE 5

SUMMARY OF ANALYTICAL RESULTS (ng/g DRY WEIGHT) FOR THE DETERMINATION OF PAHs IN SRM 1974, ORGANICS IN MUSSEL TISSUE (*Mytilus edulis*)

Compound	LC–FL[a]	GC–MS[a]	Certified[b]
Phenanthrene	44.6 (2.7)	45.3 (7.3)	45 ± 11
Anthracene	5.97 (0.52)	6.14 (0.72)	6.1 ± 1.7
Fluoranthene	289 (10)	255 (21)	272 ± 47
Pyrene	294 (10)	259 (12)	276 ± 30
Perylene	8.56 (0.35)	8.5 (1.7)	8.5 ± 2.4
Benzo[*b*]fluoranthene	55.9 (2.2)	48.7 (5.2)	52.3 ± 9.4
Benzo[*a*]pyrene	20.1 (2.3)	17.1 (2.2)	18.6 ± 3.8
Benzo[*ghi*]perylene	19.6 (1.4)	20.3 (2.3)	20.0 ± 2.3
Indeno[1,2,3-*cd*]pyrene	15.6 (1.4)	13.6 (1.4)	14.6 ± 2.7

[a] Uncertainties (values in parentheses) are one standard deviation of a single measurement treating all measurements as statistically independent and identically distributed; LC–FL results are from analyses of six samples and GC–MS results are from analyses of twelve samples.

[b] The certified values are equally weighted means of results from two analytical techniques. The uncertainty is obtained from a 95% prediction interval plus an allowance for systematic error between the methods used. In the absence of systematic error, the resulting uncertainty limits will cover the concentration of approximately 95% of samples of this SRM having a minimum sample size of 15 g (wet weight).

source identification, etc., publishd results of analyses of these SRMs by other laboratories will provide a valuable database for comparison within the scientific community.

4.1. Comparison of LC vs. GC–MS

At NIST the certification of environmental matrix SRMs is based on the use of two or more "independent" analytical methods. The required independent analytical procedures include different extraction and cleanup/isolation procedures as well as separation and detection techniques. For the measurement of PAHs in all of the natural matrix materials in Table 4, RP-LC using fluorescence detection was used as one of the analytical techniques. The LC approaches described above for the determination of PAHs were developed primarily for use in the certification of these materials. GC–MS has been used as the second technique for the certification analyses of the majority of these SRMs. As a result of the certification measurements for these SRMs, we have several sets of data comparing LC–fluorescence and GC–MS for the determination of PAHs. In 1990 we reported an extensive comparison of LC–fluorescence and GC–MS measurements for PAHs in three natural matrix SRMs (SRMs 1580, 1582, and 1650) and in extracts of the air particulate SRM (1648) and diesel particulate SRM (1650) [43]. A similar comparison can be made for the results of the analyses of the two most recent natural matrix SRMs, the marine sediment and the mussel tissue, as shown in Tables 3 and 5, respectively. The LC–fluorescence and GC–MS results for SRM 1941 and 1974 were generally in good agreement. For the most recent SRM measurements (see Table 5), differences in the mean values for the two techniques were 1–4% for phenanthrene, anthracene, perylene, and benzo[*ghi*]perylene; 12% for fluoranthene and pyrene; and 13–15% for benzo[*a*]pyrene, benzo[*b*]fluoranthene and indeno[1,2,3-*cd*]pyrene.

5. CONCLUSIONS

RP-LC with fluorescence detection is an excellent analytical technique for the measurement of PAHs in environmental matrices. In using RP-LC the analysts must be aware of selectivity differences among C_{18} columns from various manufacturers and make effective use of these differences for a specific separation need. Results obtained by using

LC–fluorescence are comparable to those obtained from GC–MS; however, LC–fluorescence has the advantage of being able to measure some PAH isomers that can not be quantified easily by GC–MS. A number of environmental matrix SRMs are available for use in validating analytical procedures for the determination of PAHs.

REFERENCES

1 J. A. Schmit, R. A. Henry, R. C. Williams and J. F. Dieckman, *J. Chromatogr. Sci.*, 9 (1971) 645.
2 S. A. Wise, in A. Bjørseth (Editor), *Handbook of Polycyclic Aromatic Hydrocarbons*, Vol. I, Marcel Dekker, New York, 1983, Ch. 5, p. 183.
3 S. A. Wise, in A. Bjørseth and T. Ramdahl (Editors), *Handbook of Polycyclic Aromatic Hydrocarbons —Emission Sources and Recent Progress in Analytical Chemistry*, Vol. II, Marcel Dekker, New York, 1985, Ch. 5, p. 113.
4 J. C. Fetzer, in T. Vo-Dinh (Editor), *Chemical Analysis of Polycyclic Aromatic Compounds*, Wiley, New York, 1989, Ch. 5, p. 59.
5 K. D. Bartle, M. L. Lee and S. A. Wise, *Chem. Soc. Rev.*, 10 (1981) 113.
6 *EPA Test Method, Polynuclear Aromatic Hydrocarbons —Method 610*, U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, OH, July 1982.
7 S. A. Wise, W. J. Bonnett and W. E. May, in A. Bjørseth and A. J. Dennis (Editors), *Polynuclear Aromatic Hydrocarbons: Chemistry and Biological Effects*, Battelle Press, Columbus, OH, 1980, p. 791.
8 K. Ogan and E. Katz, *J. Chromatogr.*, 188 (1980) 115.
9 E. Katz and K. Ogan, *J. Liq. Chromatogr.*, 3 (1980) 1151.
10 A. Colmsjö and J. C. MacDonald, *Chromatographia*, 13 (1980) 350.
11 S. A. Wise, W. J. Bonnett, F. R. Guenther and W. E. May, *J. Chromatogr. Sci.*, 19 (1981) 457.
12 R. Amos, *J. Chromatogr.*, 204 (1981) 469.
13 S. A. Wise and W. E. May, *Anal. Chem.*, 55 (1983) 1479.
14 L. C. Sander and S. A. Wise, *Anal. Chem.*, 56 (1984) 504.
15 L. C. Sander and S. A. Wise, *J. Chromatogr.*, 316 (1984) 163.
16 S. A. Wise and L. C. Sander, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 8 (1985) 248.
17 L. C. Sander and S. A. Wise, *Anal. Chem.*, 59 (1987) 2309.
18 L. C. Sander and S. A. Wise, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 11 (1988) 383.
19 S. A. Wise, L. C. Sander, R. Lapouyade and P. Garrigues, *J. Chromatogr.*, 514 (1990) 111.
20 L. C. Sander and S. A. Wise, *Adv. Chromatogr.*, 25 (1986) 139.
21 L. C. Sander and S. A. Wise, *LC · GC*, 8 (5) (1990) 378.
22 L. C. Sander and S. A. Wise, *J. Chromatogr.*, in press.
23 K. B. Sentell and J. G. Dorsey, *J. Chromatogr.*, 461 (1989) 193.
24 S. R. Cole and J. G. Dorsey, *J. Chromatogr.*, 635 (1993) 177.
25 W. Campbell, *The Separations Group*, Hesperia, CA, 1992, personal communication.
26 D. Baschke, M. Jendziezyck, D. Youngs and M. Henry, presented at the *12th Int. Symp. Polynuclear Aromatic Hydrocarbons Symposium, September 1989*, Abstract Book, p. 120.
27 M. P. Henry, *J. Chromatogr.*, 544 (1991) 413.
28 S. A. Wise, B. A. Benner, Jr., G. D. Byrd, H. Liu and A. Colmsjö, *Anal. Chem.*, 60 (1988) 630.
29 S. A. Wise, A. Deissler and L. C. Sander, *J. Polycyclic Aromatic Compounds*, in press.
30 P. Garrigues, M. Radke, O. Druez, H. Willsch and J. Bellocq, *J. Chromatogr.*, 473 (1989) 207.
31 S. A. Wise, L. C. Sander, H.-Ch. K. Chang, K. E. Markides and M. L. Lee, *Chromatographia*, 25 (1988) 473.
32 L. C. Sander and S. A. Wise, *Anal. Chem.*, 61 (1989) 1749.
33 W. Karcher, R. J. Fordham, J. J. Dubois, P. G. J. M. Glaude and J. A. M. Ligthart (Editors), *Spectral Atlas of Polycyclic Aromatic Compounds*, Vol. I, Reidel, Dordrecht, 1983.
34 W. Karcher (Editor), *Spectral Atlas of Polycyclic Aromatic Compounds*, Vol. II, Kluwer Academic Publishers, Dordrecht, 1988.
35 W. Karcher, J. Devillers, Ph. Garrigues and J. Jacob (Editors), *Spectral Atlas of Polycyclic Aromatic Compounds*, Vol. III, Kluwer Academic Publishers, Dordrecht, 1991.
36 W. F. Kline, S. A. Wise and W. E. May, *J. Liq. Chromatogr.*, 8 (1985) 223.
37 S. A. Wise, B. A. Benner, Jr., G. D. Byrd, S. N. Chesler, R. E. Rebbert and M. M. Schantz, *Anal. Chem.*, 60 (1988) 887.
38 W. E. May and S. A. Wise, *Anal. Chem.*, 56 (1984) 225.
39 M. M. Schantz, B. A. Benner, Jr., S. N. Chesler, B. J. Koster, K. E. Hehn, S. F. Stone, W. R. Kelly, R. Zeisler and S. A. Wise, *Fresenius' J. Anal. Chem.*, 338 (1990) 501.
40 S. A. Wise, B. A. Benner, Jr., R. G. Christensen, B. J. Koster, J. Kurz, M. M. Schantz and R. Zeisler, *Environ. Sci. Technol.*, 25 (1991) 1695.
41 S. A. Wise, S. N. Chesler, H. S. Hertz, L. R. Hilpert and W. E. May, *Anal. Chem.*, 49 (1977) 2306.
42 H. S. Hertz, J. M. Brown, S. N. Chesler, F. R. Guenther, L. R. Hilpert, W. E. May and S. A. Wise, *Anal. Chem.*, 52 (1980) 1650.
43 S. A. Wise, L. R. Hilpert, G. D. Byrd and W. E. May, *J. Polycyclic Aromatic Compounds*, 1 (1990) 81.
44 W. J. Sonnefeld, W. H. Zoller, W. E. May and S. A. Wise, *Anal. Chem.*, 54 (1982) 723.
45 S. A. Wise, B. A. Benner, Jr., S. N. Chesler, L. R. Hilpert, C. R. Vogt and W. E. May, *Anal. Chem.*, 58 (1986) 3067.
46 S. A. Wise, L. R. Hilpert, R. E. Rebbert, L. C. Sander, M. M. Schantz, S. N. Chesler and W. E. May, *Fresenius' Z. Anal. Chem.*, 332 (1988) 573.
47 S. A. Wise, M. M. Schantz, R. M. Parris, R. E. Rebbert, B. A. Benner, Jr. and T. E. Gills, *Analusis*, 20 (1992) M57.
48 S. A. Wise, M. M. Schantz, B. A. Benner, Jr., R. M. Parris, R. E. Rebbert, L. C. Sander, B. J. Koster, S. N. Chesler and W. E. May, *Fresenius' J. Anal. Chem.*, 345 (1993) 325.
49 R. E. Rebbert, S. N. Chesler, F. R. Guenther, B. J. Koster, R. M. Parris, M. M. Schantz and S. A. Wise, *Fresenius' J. Anal. Chem.*, 342 (1992) 30.
50 W. E. May, S. P. Wasik and D. H. Freeman, *Anal. Chem.*, 50 (1978) 175.
51 R. A. Velapoldi, P. A. White, W. E. May and K. R. Eberhardt, *Anal. Chem.*, 55 (1983) 1896.