

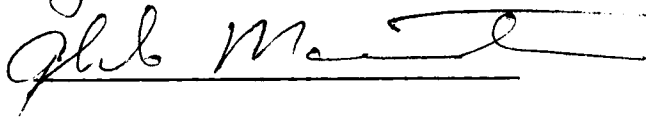
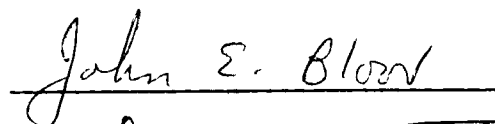
To the Graduate Council:

I am submitting herewith a dissertation written by Sumaria Indra Mohan-Neill entitled "Normal-Phase High Performance Liquid Chromatography and Characterization of Polycyclic Aromatic Hydrocarbon Fraction by Matrix-Isolation Fluorometry." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Chemistry.

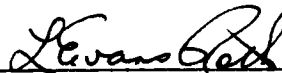


Earl L. Wehry, Major Professor

We have read this dissertation
and recommend its acceptance:



Accepted for the Council:



Vice Chancellor
Graduate Studies and Research

NORMAL-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY AND
CHARACTERIZATION OF POLYCYCLIC AROMATIC HYDROCARBON
FRACTION BY MATRIX-ISOLATION FLUOROMETRY

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Sumaria Indra Mohan-Neill

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3051750

DEDICATION

This small measure of what I have accomplished is dwarfed by the dimension of those I would like to dedicate it to.

To the memory of my sister, Cynty:

Some enter life and create a stir;

Few leave and create a vacuum.

She left me the priceless legacy of having her as a sister.

To the memory of my father, who taught me quiet wisdom.

To my mother, who loved unselfishly and taught me gentle strength.

To my husband, whose love and faith in us has weathered my ups and downs.

To the rest of my family, for their love and trust.

Ultimately, to God, from whom I receive all things.

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For typing this thesis, I would like to thank Mr. G. McGhee.

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ABSTRACT

High performance liquid chromatography (HPLC) was employed for normal-phase separation of a complex sample (a shale oil) into fractions containing major classes of compounds. A polar bonded-phase column (Partisil-10 PAC) facilitated the separation. Gradient elution was employed, with n-heptane and dichloromethane comprising the mobile phase. The total elution time was approximately thirty minutes. Room-temperature solution fluorometry, HPLC and gas chromatography were utilized to identify the various fractions of the three-component mixture which was used as a model for the shale oil separation. The polynuclear aromatic hydrocarbon (PAH) fraction of the shale oil was analyzed by matrix-isolation fluorometry. Most of the work used nitrogen matrices. Benzo(b)fluorene, chrysene, pyrene, benz(a)anthracene and benzo(a)pyrene were identified in the PAH fraction. A quantitative analysis revealed 27 ± 3 ppm BaP in the shale oil sample. These results compared favorably with the results reported by the National Bureau of Standards (NBS).

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CHAPTER I

INTRODUCTION

A. BACKGROUND

Energy and the environment encompass the major concerns of the Eighties, the rest of this century and probably the future of this planet. Those concerns rise above all the political frenzy and economic gluttony and they demand responsible and informed decision making. International politics and economics have forced the United States (U.S.) to enter an era of energy alternatives. A politically volatile and "not-so-naive" Middle East has catalyzed the search for alternative energy sources in the U.S. Part of the current solution appears to be the exploitation of the large reserves of coal and shale oil in the continental U.S. However, this godsend is paradoxical in character. On one hand, use of the "synthetic" fuels derived from coal and shale may alleviate the energy crunch but, on the other hand, it poses some severe environmental and health hazards. Polynuclear aromatic hydrocarbons (PAHs) are the carcinogens commonly linked with coal liquids and shale oil. This thesis will briefly survey the PAH problem and then elaborate on two analytical techniques, High Performance Liquid Chromatography (HPLC) and low temperature Matrix-Isolation Fluorometry (MIF), which were employed for PAH analysis.

B. THE PAH PROBLEM

The human population is being exposed to PAH carcinogens at an accelerated rate as a consequence of industrial growth.¹ Principal

sources of ambient PAHs are heat and power generation, refuse burning and coke production. These combustion processes contribute significantly to the PAH problem. In addition to PAHs in the ambient air, exposure also occurs from sources of PAHs in the indoor environment, including industrial contamination and smoking. Cigarettes, in general, represent the most important overall source of exposure among smokers. However, side-stream cigarette smoke may result in high exposures for nonsmokers in the indoor environment.² Total exposure to PAHs for any individual, using benzo(a)pyrene (BaP) as an index compound, is a function of a number of factors including occupations, smoking habits, activity patterns and residence.³

BaP is a chemical carcinogen to animals.⁴ It is a universal air pollutant that is produced by incomplete combustion, and it is estimated that 2,000 tons of BaP are released into the air over the U.S. each year!⁵ The initial observation of chemical carcinogenesis goes back to about 200 years ago when Percival Pott gave an excellent description of scrotal cancer and its relationship to chimney sweeping.⁶ It was not until 100 years later that it was established that coal tar contained the agent causing this type of cancer. Then 30 to 40 years ago, BaP was recognized as one of the carcinogens in coal tar.⁷ Today, some epidemiologists consider that 70-90% of human cancers, excluding skin cancers, are caused by environmental chemicals.⁸

Although many PAHs are considered to be carcinogenic, it appears that most require metabolic transformation to exhibit carcinogenic activity.⁹ Elucidation of the metabolic profile of PAHs has significantly progressed with the advent of HPLC. This technique has allowed some workers to isolate isomers which could not be separated by other

forms of chromatography, and has allowed unequivocal determination of at least one epoxide, a metabolic intermediate in the formation of dihydrodiols.¹⁰ Some of the epoxides produced are more carcinogenic than the parent hydrocarbons.¹¹

Energy and the environment will continue to confront society with some of the greatest challenges to biological survival. Considering that more coal and shale oil will be used in the future might give bleak prognosis for the incidence of carcinomas. In this respect Stemmer⁷ makes a most unsettling observation. He suggests that serious thought should be given to the fact that it took 200 years to eliminate scrotal cancer not because a cure was developed, but because the occupation of chimney sweeps ceased to be.

What does this society want, and how do society's wants create problems? Fouts¹² believes that society wants the worst carcinogenic offenders to be identified and for exposures to be minimized without massive disruptions in lifestyle. In the case of PAH carcinogens, he claims that an impasse has almost been reached. Certainly a major source of chemicals is related to life-style, to society's use of energy, its industries and methods of dispensing of wastes. He argues that countries like Sweden have been able to maintain equivalent or better standards of living compared to the U.S., with reduced PAH exposure. Preventative steps short of reducing exposure, e.g., drugs for inactivation of hydrocarbons, are not likely to succeed because they may further complicate a complex problem. Thus, it appears that modification of lifestyle here would be the long-term solution. In the meantime, the urgent need for identifying and analyzing sources of

chemical carcinogens cannot be over-emphasized, hence the need for analytical methodology to accomplish the determination of these compounds.

C. ANALYTICAL METHODOLOGY

The ultimate test of any analytical technique for environmental study is its successful application to "real" environmental samples. This is no less true for PAH analysis. Thus, it is necessary to consider the various methods available for PAH determination. Stroupe gave a relatively comprehensive comparison of the various methods, in an attempt to identify the "best" one.¹³ He concluded that low-temperature fluorometry had the most overall advantages for PAH determination. At that time (1977), he pointed out that MIF had a detection limit of 10^{-11} g or less¹⁴ compared with 10^{-9} g for gas chromatography-mass spectrometry (GC-MS) and modern liquid chromatography (LC or HPLC), using UV absorption detection.¹⁵ It should be noted from Table I¹⁶ that electrochemical and fluorescence detectors for HPLC can now detect less than a picogram of a favorable sample. He also argued that LC yielded only retention data for identification, poor resolution of isomers and no structural information.¹³ This is now only partly true; for example, isomers of PAH metabolites have recently been separated¹⁰ by HPLC. Moreover, if a detection method is used which provides information related to molecular structure, other data in addition to retention volumes can be obtained from HPLC experiments.

The many virtues of MIF have already been lauded by Stroupe¹³ and Bilotta.¹⁷ However, Stroupe dismissed LC because of what he described

TABLE I
TYPICAL SPECIFICATIONS FOR MOST-USED LC DETECTORS

Parameter (units)	UV (Absorbance)	RI ^a (RI units)	Radioactivity	Electrochemical (μ A)	Infrared (Absorbance)	Fluorometer	Conductivity (μ Mho)
Type	Selective	General	Selective	Selective	Selective	Selective	Selective
Useful with gradients	Yes	No	Yes	No	Yes	Yes	No
Upper limit of linear dynamic range	2-3	10^{-3}	N.A. ^b	2×10^{-5}	1	N.A.	1000
Linear range (max)	10^5	10^4	Large	10^6	10^4	$\sim 10^3$	2×10^4
Sensitivity at $\pm 1\%$ noise, full-scale	0.002	2×10^{-6}	N.A.	2×10^{-9}	0.01	0.005	0.05
Sensitivity to favorable sample	2×10^{-10} g/ml	1×10^{-7} g/ml	1^4 cpm	10^{-12} g/ml	10^{-6} g/ml	10^{-11} g/ml	10^{-8} g/ml
Inherent flow sensitivity ^c	No	No	No	Yes	No	No	Yes
Temperature sensitivity	Low	10^{-4}° C	Negligible	1.5° /C	Low	Low	2° /C

^aRefractive Index.

^bN.A., not available.

^cBecause of sensitivity to temperature changes, some detectors appear to be flow sensitive.

as "extensive time-consuming separations to isolate one or two PAHs in a chromatographic fraction so that identification and quantification of PAHs can be accomplished without interference from overlapping excitation and emission bands of the other PAHs."¹³ This has its element of truth; however, one must consider that there are many other classes of compounds beside PAHs in a real sample. Generally, crude oil samples can be divided into four chemical groups: (i) asphaltenes (hexane insolubles), (ii) alkanes and alkenes (aliphatics), (iii) aromatics, and (iv) polar (oxygen- and nitrogen-containing) compounds.

"Group separation" using normal-phase HPLC would quickly separate a real sample into individual fractions, each of which presumably contain compounds having the same major functional groups, thus negating the time argument. If one considers the time requirements for preparation of a MIF deposit for each experiment, less than an hour for normal-phase HPLC separation for a real sample should not create any prohibitive hardship. Initially, additional time may be required to develop the optimum conditions for separation, but once the procedure has been established, routine separation should take considerably less effort than the MIF experiment.

D. THE ADVENT OF HPLC

If statisticians are to be believed, the recent growth of HPLC has been the most spectacular development in analytical chemistry and has not yet abated because its vast potential for application remains to be fully exploited.¹⁸ The specific application for this thesis required a procedure to separate environmental samples, such as shale

oil, into fractions containing the major classes of compounds. Chemically bonded phases are the most widely used and proven stationary phases¹⁹ for this type of application. The great versatility of chemically bonded phases has recently prompted numerous review articles and papers.²⁰⁻³⁰

Chromatography, in all its forms, is a method of separation of components of a mixture which depends on the relative affinities of the various solute molecules for the stationary phase and the mobile phase. The chromatographic methods are classified according to the type of stationary and mobile phase used, the state in which they are present and by the different mechanisms which control the separation.

HPLC is but an extension of classical chromatographic separation techniques. Compared with classical chromatography, HPLC encompasses smaller stationary phase particles (10 μ m in diameter) of narrow size distribution and higher mobile phase flow rates (1-5 ml/min) achieved by pumping under pressure.

While liquid chromatography was proposed in an early paper of Martin and Synge,³¹ its development as a useful analytical tool did not come until the mid-1960's, when several workers who had advanced the development of GC turned their attention to the use of liquid chromatography. Using lessons learned in GC, the technology required to produce high speed, high efficiency liquid chromatography columns was developed.³²

Tremendous changes in HPLC technology have occurred within the last five years and practical applications of HPLC seem to be increasing geometrically. The ubiquitous detector problem has largely been

solved with the advent of UV absorption spectrophotometers operating down to 190 nm, making possible the sensitive detection of almost any type of compound.³³ Table 1 (p 5) lists the typical specifications of the seven most-used HPLC detectors. In addition, Snyder and Kirkland have prepared a listing of about two dozen less common L.C. detectors.³⁴

As previously indicated, electrochemical and fluorescence detectors can detect less than a picogram of certain specific sample components.¹⁶ Increased use of these detectors, plus off-line and on-line derivatization, have pushed detection problems further into the background, even for trace analyses of complex samples such as blood, food and soil. Recent developments in microprocessor-controlled instrumentation have greatly improved equipment performance, to permit more convenient, versatile and precise L.C. separations.³⁵

Only in the past four years has the tremendous potential of small-particle, reverse-phase L.C. been exploited, particularly when augmented with gradient elution and special methods, such as ion-pair formation.^{36,37} As a result, previously difficult or impossible separations of such compounds as polar dyes or basic drugs and their metabolites are now performed more or less routinely. The exponential improvement in column packings that occurred between 1968 and 1973 also greatly contributed to the development of practical L.C. techniques. There is a continuing emphasis by manufacturers on reliability and reproducibility of both packing materials and pre-packed columns. Problem areas such as band tailing, trace analysis, preparative separation, and so on, have also received considerable attention during the last five years, and these problems and applications can now be approached in a relatively systematic fashion.³⁸

Bonded-phase columns were originally developed to eliminate the disadvantages of liquid-liquid chromatography columns based on mechanically-held stationary liquids. Compared with classical columns, the main advantage of bonded-phase columns is that they are quite stable, because the stationary phase is chemically bonded to the support and cannot be easily removed or lost during use. Therefore pre-saturation of the two phases is not required. Bonded-phase columns are normally preferred, if for no other reason than greater convenience. Additionally, choices of bonded-phase packings and mobile phases that can be used with these packings are sufficiently flexible and diverse to permit most separations to be achieved by this method. Polar bonded-phase packings are used for normal-phase separation in much the same manner as adsorbents for liquid-solid chromatography, e.g., silica. Samples with moderate to strong polarity are usually well separated on the polar bonded-phase packings.¹⁹

The mechanism for retention onto most bonded-phase columns has not been definitely established.³⁹ The stability of the bonded-phase packings is largely determined by the silica support. Consequently, most can be used within a pH range of 2-8.5.³⁹ Solvent strength and selectivity have been discussed by Snyder and Kirkland.⁴⁰

There are several class separation schemes for hydrocarbon fuels which have been reported in the literature.⁴¹⁻⁴⁶ These separations are generally time-consuming and inefficient because they rely on conventional gravity feed columns packed with either silica or alumina, or on solvent/solvent extraction. A separation scheme based on HPLC on florisil and 32-63 μm silica have been proposed by DiSanzo and

co-workers.⁴⁷ Although this method proved to be more efficient than earlier procedures, it was still time-consuming. Other HPLC procedures have been reported by Suatoni and co-workers,^{48,49} and Dark and co-workers.⁵⁰

Probably the most significant and similar class separation scheme for a shale oil sample compared to the one developed in this thesis was reported by Crowley and co-workers.⁵¹ They used a 20- μ m cyano bonded-phase column, and achieved separation between aliphatic, aromatic and polar fractions within thirty minutes. The aliphatic compounds were unretained, the aromatics were slightly retained and the polar species were held more strongly.

E. MATRIX-ISOLATION FLUOROMETRY (MIF)

Staggering demands are imposed upon analytical methodology by many present-day samples. One major demand is the need for development of techniques for identification and reliable quantitation of individual trace organic compounds in complex samples. For example, a "synthetic fuel" sample, such as a shale oil or coal liquid, may contain hundreds of different organic compounds, some of which may be carcinogenic. A sensible way to proceed with the analysis of such a complex sample begins with the application of modern separation procedures. However, if the separation is accomplished in a realistic time, the fractions themselves will still be relatively complex mixtures. Hence, even when it is coupled with separation procedures, the ultimate analysis should produce reliable qualitative and quantitative results for mixtures of considerable complexity.

Optical spectroscopy (UV-visible absorption or luminescence and IR absorption) has been applied widely to the analysis of organic samples. Compared to the substantial improvements in the performance of spectroscopic instrumentation (particularly for infrared absorption and molecular fluorescence), considerably less attention has been devoted to improvements in the quality of the spectroscopic sample itself. Wehry and Mamantov have reviewed analytical applications of matrix-isolation spectroscopy.⁵² They present persuasive arguments for matrix isolation (MI) as a sampling technique for spectroscopic analysis of appropriate samples.

The technique of matrix isolation was originally developed^{53,54} in the mid-1950's as a technique for obtaining infrared and electron spin resonance spectra of transient species.⁵⁵⁻⁵⁸ The following is a brief summary of the fundamentals of matrix isolation. Small closed-cycle cryostats⁵⁶ are employed. The cryostat allows the MI deposit to be made and sustained at cryogenic temperatures 11-15 K. A nearly "ideal" spectroscopic matrix⁵² such as nitrogen, argon or n-heptane is deposited in large excess (typically a factor of 10^4 - 10^8 on a mole basis) with sample constituents on a surface (window) which exhibits high thermal conductivity and suitable optical properties. Essentially, the desired goal is the formation of a matrix-isolation deposit with the sample well dispersed in the solid matrix (ideally, all sample molecules have only matrix molecules as near neighbors). More details on MI sample preparation are given in Chapter II of this thesis and in the references previously cited. Detailed descriptions of the practice of MI spectroscopy can be found in books by Meyer⁵⁹ and Hallam,⁵⁸ as well as various papers⁶⁰⁻⁶³ and theses.^{13,17,64-66}

The principal analytical advantage of molecular fluorescence spectrometry is its extremely high sensitivity. However, besides high sensitivity, two other features (high resolution and reliable quantitation) are essential for the analysis of complex mixtures. Conventional fluorescence spectrometry in room-temperature solution offers high sensitivity but poor spectral resolution, leading to unreliable identification and quantitation of constituents of a mixture. Fluorescence quenching caused by molecular collisions and long-range energy transfer can produce significant error in fluorometric analysis of complex samples by room-temperature fluorometry. Collisions of solute molecules in solution may be partially overcome by dilution, but the concentration may then be too low for determination of trace sample constituents.

The analytical characteristics of matrix-isolation fluorometry have been discussed in detail elsewhere.^{13,52} Use of an internal standard makes "quantitative transfer" from the Knudsen cell an insignificant consideration. MIF calibration curves that are linear and parallel over four decades in concentration have been reported.⁵² Also, the calibration line for chrysene in a mixture is virtually superimposable upon that obtained for pure chrysene using benzo(b)-fluorene as an internal standard. This behavior was observed even when each compound was present in microgram quantities.⁵² These results confirm the expectation that fluorescence quenching due to molecular collisions and long-range energy transfer can be suppressed adequately by MI, and that the internal standard procedure is satisfactory for quantitation in both pure compounds and mixtures. In addition, MIF

calibration curves which are linear for five decades in concentration have been observed, with relative standard deviations for individual results typically falling in the 4-7 % range.⁶² In contrast, linearity over extended concentration ranges and precision may be difficult to achieve in frozen-solution matrices⁶⁷ because of the possibility of aggregation of solute molecules (or even formation of solute micro-crystallites) as a liquid solution is frozen. The resolution for MIF can be further enhanced by the use of organic "Shpol'skii solvents" as matrices⁶³ or by the use of selective laser excitation of "site-selected" molecules in conventional MI media.^{66,68} By use of these procedures, the resolution attainable by MIF is rendered comparable to Shpol'skii frozen solutions.

F. ANALYTICAL PROCEDURE

The analytical procedure followed in this thesis was two-fold. Firstly, it was concerned with the development of a procedure for separating environmental samples, such as synthetic fuels, into fractions containing major classes of compounds. Secondly, matrix-isolation fluorometry was employed for analysis of the fraction containing the PAHs. Initially, the analysis was qualitative and then one component, BaP, was chosen for quantitative analysis.

Normal-phase HPLC was employed for development of a separation procedure. A polar bonded-phase column (Partisil-10 PAC) was utilized for normal phase group separation. The primary factors considered when developing the HPLC procedure were reliability and reproducibility of column performance (indicated by the retention data), sample size effects and mobile phase selection.

A model procedure was developed using a three-component mixture, containing a PAH (BaP), a hydroxy compound (phenol) and a nitrogen heterocycle (dibenz[a,j]acridine). This procedure was duplicated for the separation of a "real" shale oil sample. Based on retention data from the three-component mixture, it was expected that the major fractions of the shale oil sample contained PAHs, hydroxy compounds and nitrogen heterocyclic compounds, respectively. GC analysis and fluorescence spectra were used to verify the separation procedure.

A brief comparison of the advantages of matrix-isolation fluorometry and room-temperature solution fluorometry was carried out. The shale oil PAH fraction was analyzed by matrix-isolation fluorometry. After initial qualitative analysis, BaP was quantified in the PAH fraction. The primary factors considered in matrix-isolation fluorometric analysis were sample size and choice of matrix.

The goals of reasonable group separation by HPLC and then reliable quantitation of a sample constituent by matrix-isolation fluorometry were accomplished. Synthetic mixtures were initial guides in the development of procedures for analysis.

CHAPTER II

EXPERIMENTAL DETAILS

A. MATERIALS

1. Solvents

"Distilled in Glass" isooctane, dichloromethane, methanol and n-heptane, obtained from Burdick and Jackson Laboratories (B&J), Incorporated, Muskegon, Michigan, were used as mobile phases for high performance liquid chromatography (HPLC). "Distilled in Glass" n-hexane from B&J, and toluene from Fisher Scientific Company (F.S. Co.), Fair Lawn, New Jersey, were used as elution solvents for preparative-scale adsorption chromatography. Acetone (Certified A.C.S. "Spectroanalyzed") obtained from F.S. Co. and hexane ("Distilled in Glass") from B&J were used for cleaning purposes for HPLC and matrix isolation fluorometry (MIF). "Distilled in Glass" n-heptane from B&J was used for preparing solutions of samples. The preceding solvents were used without further purification.

2. Matrices for Matrix-Isolation Fluorometry (MIF)

"Ultra high purity" argon and nitrogen gases (99.9995 %) were obtained from Matheson Gas Products, Lynhurst, New Jersey. N-hexane and n-heptane ("Distilled in Glass") obtained from B&J were used for organic matrices.

3. Samples

"Real" Sample: a shale oil produced by in situ combustion of shale from the Mahogany Zone of the Colorado Green River Formation

was obtained from the National Bureau of Standards (NBS), Washington, D.C. (Dr. Willie E. May).

Table II lists the compounds used as standards for the HPLC and MIF experiments. The compounds were used as described, without further purification.

B. EQUIPMENT

1. HPLC

The high performance liquid chromatograph is actually a combination of units (from various sources) which were assembled to build the instrument. Figure 1 is a schematic of the instrument. The following units were utilized:

(a) Spectrophotometer, Model 100-40, was obtained from Hitachi Ltd., Tokyo, Japan, (Figures 2 and 3).

(b) Variable Wavelength Detector, Model 155, was obtained from Altex Scientific Inc., Berkeley, California.

(c) Solvent Metering Pump, Model 110, was obtained from Altex Scientific Inc.

(d) Microprocessor, Controller/Programmer, Model 420, was also obtained from Altex Scientific Inc.

(e) Strip Chart Recorder, Omnigraphic Series 3000, was obtained from Houston Instrument Corp., Austin, Texas.

(f) Analytical Column, "Partisil-10 PAC" (bonded cyano-amino type polar phase), was obtained from Whatman, Inc., Clifton, New Jersey.

(g) Guard Column, "CO: Pell PAC" (cyano-amino groups chemically bonded to 30-38 μ m glass beads) was also obtained from Whatman, Inc.

TABLE II
COMPOUND SOURCES AND PURITY

COMPOUND	SOURCE	PURITY
Benzo(a)pyrene	B. Pauric, Philadelphia, Pa.	Zone Refined
Pyrene	B. Pauric, Philadelphia, Pa.	Zone Refined
Anthracene	B. Pauric, Philadelphia, Pa.	Zone Refined
Fluorene	Duke Standards, Palo Alto, Ca.	Zone Refined
Benzo(b)fluorene	K&K Laboratories, Plainview, N.Y.	Zone Refined
Toluene	Fisher Scientific Company	Zone Refined
Napthalene	Fisher Scientific Company	"Purified"
Dibenz(a,j)acridine	Aldrich Chemical Co., Milwaukee, Wis.	"97 %"
Phenol	J.J. Baker Chemical Co., Phillipsburg, N.J.	ACS reagent grade

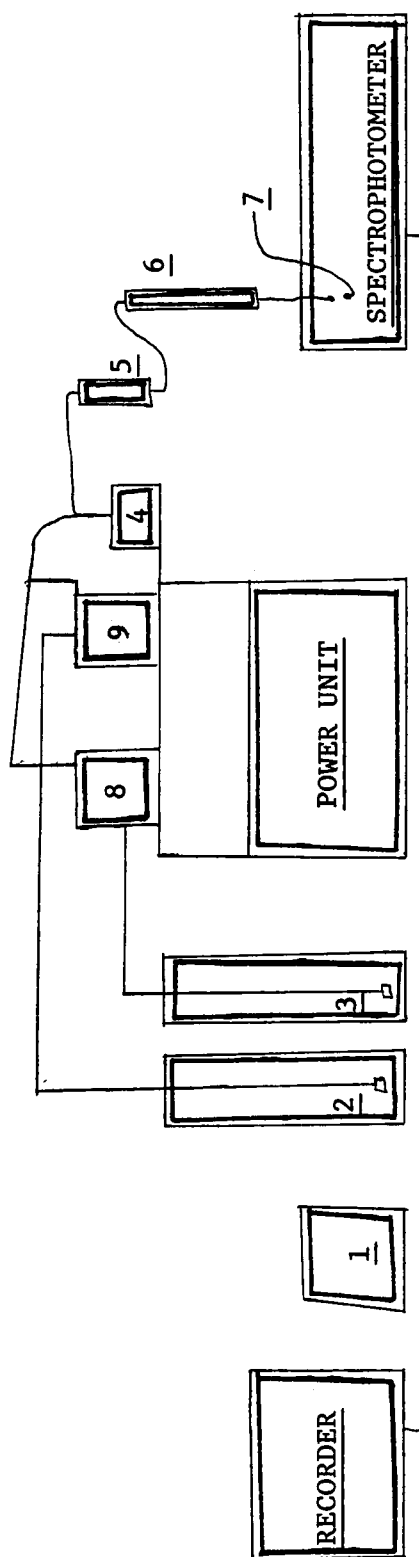


Figure 1. Block Diagram of HPLC Instrument.

1. Microprocessor, Controller/Programmer
2. Solvent Reservoir A
3. Solvent Reservoir B
4. Sample Injection System
5. Guard Column
6. Analytical Column
7. Sample Out of Detector
8. Pump B
9. Pump A

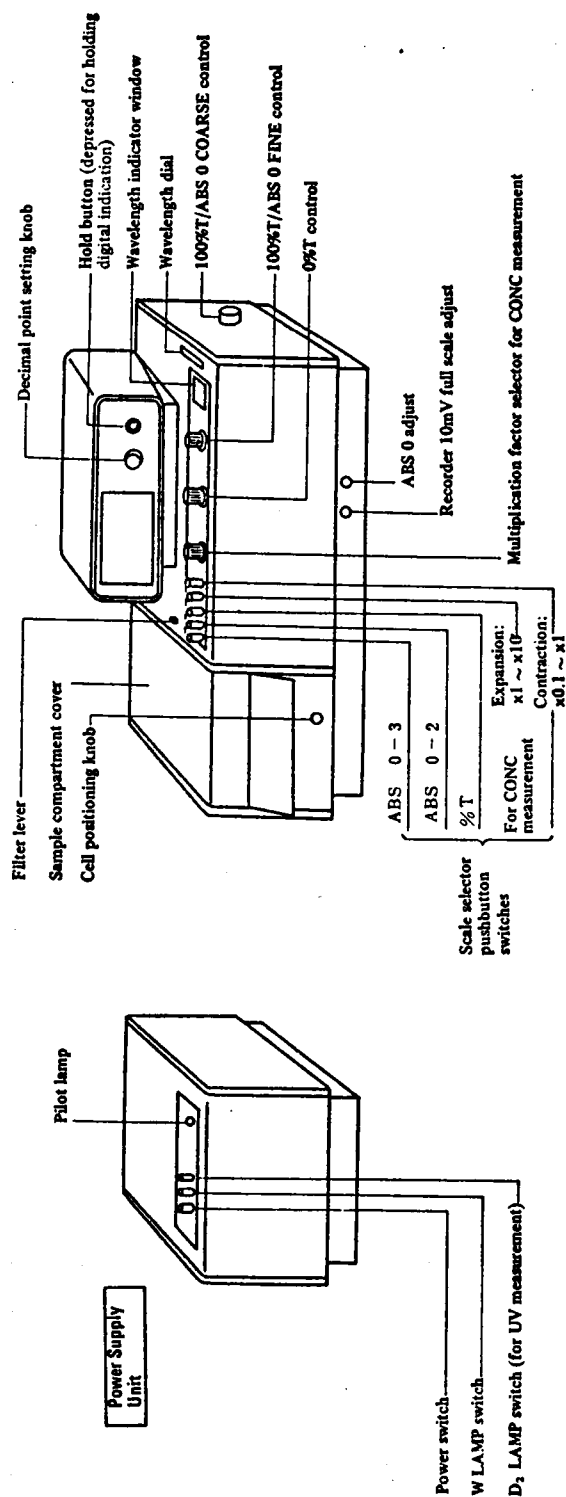


Figure 2. Diagram of uv-visible spectrometric detector for liquid chromatograph.

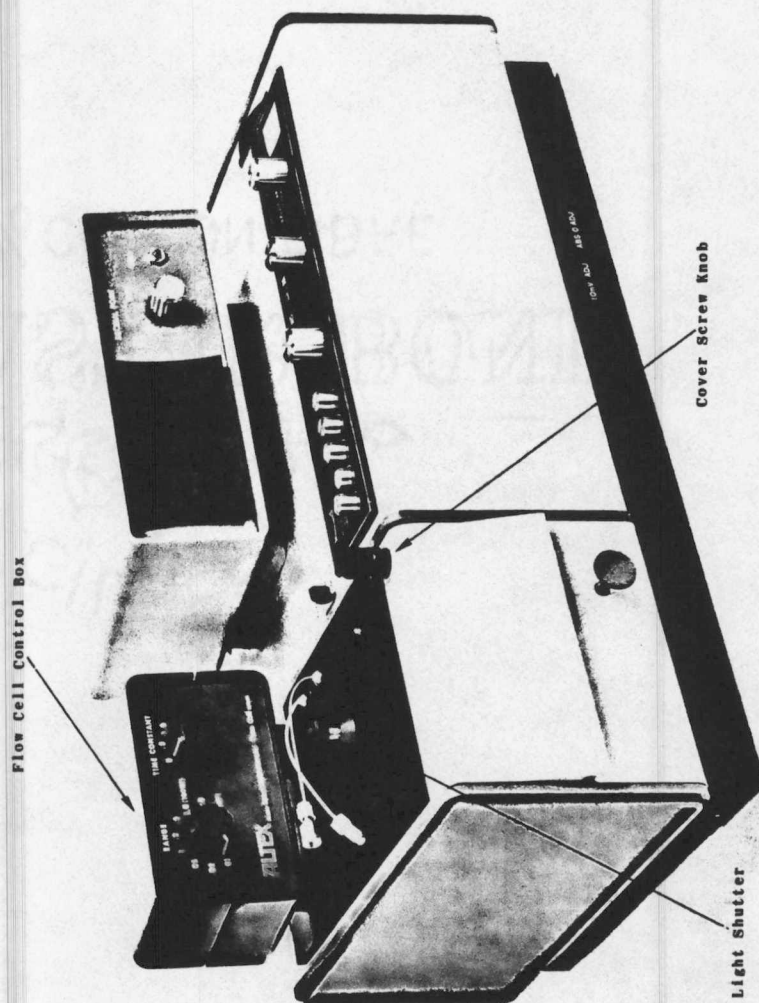


Figure 3. Model 155-40 Spectrophotometer.

2. Matrix-Isolation-Fluorometry (MIF)

The equipment needed for matrix-isolation fluorometry has been described in great detail by both Stroupe¹³ and Bilotta.¹⁷ The equipment can be divided into two major components based on use, the MIF sample preparation and the MIF detection systems:

(a) MIF Sample Preparation System: Cryostats are employed for this function.

(1) Model 21 "Cryodyne" was obtained from CTI Cryogenics, Waltham, Mass.

(2) Model 20 "Cryodyne" was obtained from CTI Cryogenics, Waltham, Mass.

The vacuum line used in the preparation of matrix-isolation samples is illustrated in Figure 4. The vacuum pump is employed to evacuate the line. The devices indicated by CT. A and CT. B are glass traps which are attached to the line. When the traps are immersed in dewars containing liquid nitrogen, they protect the vacuum pump, and also prevent pump oil from getting into the line (oil fluoresces). The traps indicated by CT. A and CT. B also trap organic matrix gases which would otherwise enter the vacuum pump and increase the vapor pressure of the contents, thus reducing the efficiency of the pump. The trap denoted CT. A is connected to the line with Cajon unions, which makes removal of the trap easy. The trap is removed for cleaning after organic matrix vapors have collected in it. Details concerning cleaning the trap will be discussed later.

The remaining portion of the vacuum line consists of the matrix reservoir and deposition line. The matrix gas reservoir stores the

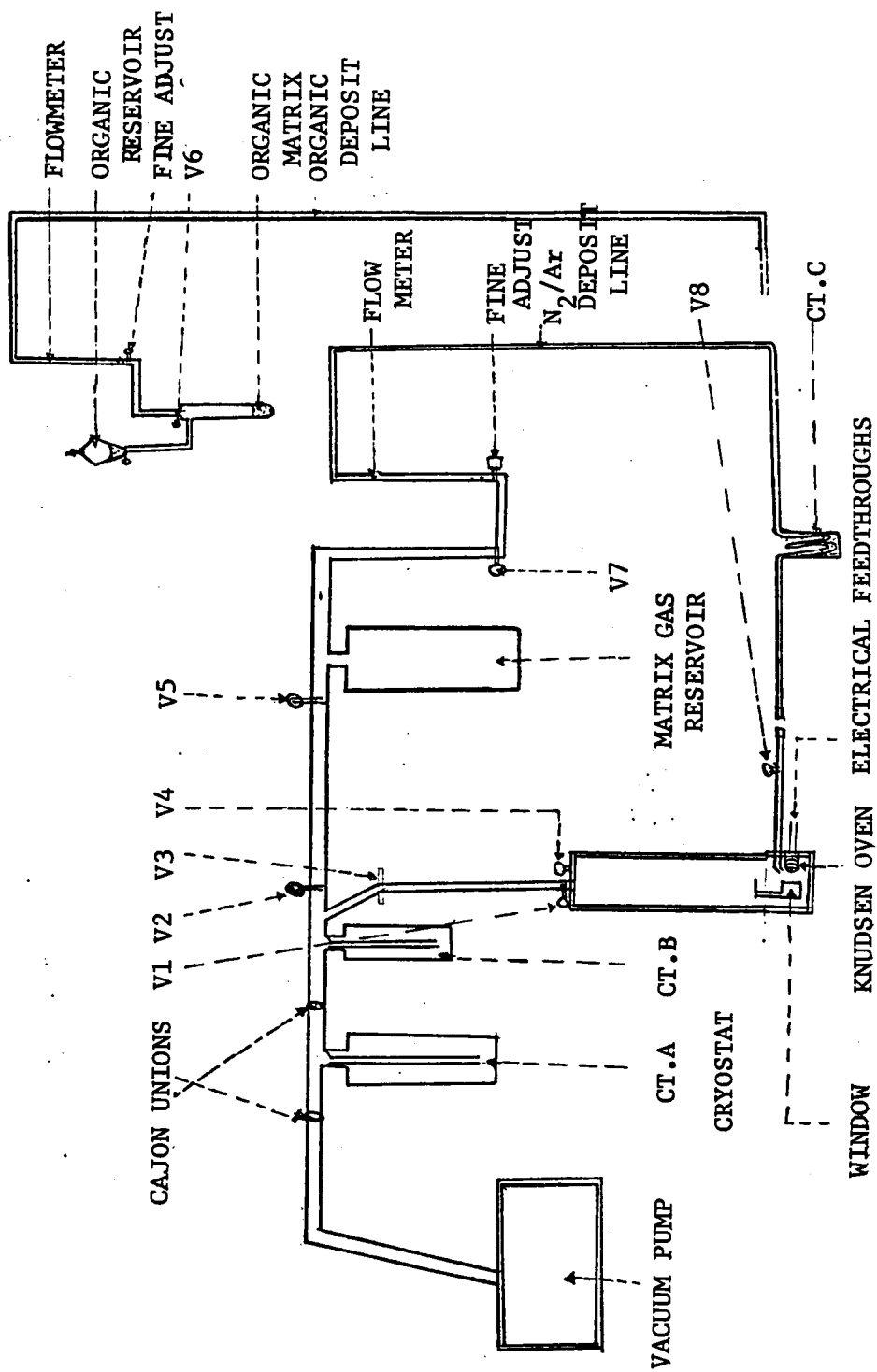


Figure 4. Matrix-Isolation Sample Preparation Assembly

CT. - Cold Trap, V. Valve

matrix gas (e.g., argon or nitrogen). The reservoir is attached to the deposition line which has a rough adjustment valve, V7, to provide coarse control of the flow of matrix gas to the deposition line. A fine adjustment valve regulates the specific flow rate of matrix through the line. The flowmeter indicates the flow rate of matrix gas. Trap CT. C is used to trap any nonvolatile impurities in the matrix gas, to prevent them from being deposited.

When an organic matrix is used, the organic liquid is stored in a glass test tube-like reservoir. The organic reservoir, which is connected to the organic deposition line, has valves and a flowmeter for regulating the flow of organic matrix vapor. There is no cold trap on the organic deposition line, because such a trap would cause premature condensation of the organic vapor in it.

(b) MIF Detection:

(1) A high-resolution spectrofluorometer is employed for this function. The primary units are as follows:

(a) Excitation Source (a 2500-watt mercury-xenon lamp, Model 929B, Canrad Hanovia, Newark, New Jersey) powered by a Schoeffel LPS 400 Lamp power supply.

(b) An Excitation Monochromator (high intensity grating monochromator, GM-250) Schoeffel Instrument Corporation.

(c) Sample chamber with focusing and collection optics.

(d) An Emission Monochromator (Spex Model 1702 0.75m grating spectrometer) Spex Industries Inc., Metuchen, New Jersey.

(e) A photoelectric detection system (photon counting method) consisting of an RCA 8850 photomultiplier tube and seven Ortec

modules: 9201 photomultiplier base, 456 high voltage power supply, 9301 pre-amplifier-discriminator, 9315 photon counter, 9325 digital-to-analog converter, and 401A power bin.

(c) A strip chart recorder (Leeds & Northrup Speedomax W 10" recorder).

Schematic diagrams of this spectrofluorometer can be found in previous theses.^{13,17}

C. ANALYTICAL METHODS

1. High Performance Liquid Chromatography (HPLC)

The analytical column is the heart of the liquid chromatograph because it is the most vital link in the separation of the sample. Therefore, maximum care must be exercised in order to maintain optimum efficiency of the column. As previously indicated, a Partisil-10 PAC cyano-amino bonded phase column was employed for this work. Thus, it was extremely important that polar solvents should not be left in the column after use, because they would probably react with the polar bonded-phase groups of the stationary phase. The final consequence would be degradation of the column due to chemical changes or destruction of the original bonded phase by the polar solvents.

After connecting a new column, it was necessary to condition or equilibrate it with mobile phase before injecting a sample. This was accomplished by letting n-heptane flow through the column at 1 ml/min for one hour. This was equivalent to 30 column volumes of mobile phase for the standard Partisil 4.6 mm OD x 25 cm long column. The detector was set at high sensitivity and a steady baseline indicated that the

column was in equilibrium. This was a fairly good indicator, but a more certain assurance would be to also check k' (capacity factor) values of injected sample compounds over a period of several hours. If there is no change in the value of k' , one can be more certain of column equilibration.

The new column was accompanied by the manufacturer's Quality Assurance (QA) test result. Basically, the QA test result is a chromatogram of a standard mixture which was separated under specified conditions. An example of a standard mixture such as a six-component mixture containing toluene, naphthalene, fluorene, anthracene, pyrene and benzo(a)pyrene can be used. One can use n-heptane as the mobile phase and a flow rate of 1.0 ml/min for elution of the various components. A chromatogram displaying the individual peaks and retention times is obtained.

One could try to duplicate the manufacturer's QA test exactly or perform one that is similar. The main consideration is for the user to continuously use the same standard test after the first testing.

After column equilibration, the column was tested as previously described. The results of QA test result were comparable to those obtained by the manufacturer. There are no specific time limits for retesting the column. Therefore, the frequency of retesting will depend on several other factors, including hours of column use, types of sample (polarity), types of mobile phase (polarity), sample size and volume of the mobile phase. An accumulation of these factors may contribute to reduced column efficiency.

It is then left up to the user's knowledge and intuition to

determine whether column performance has decreased. Highly irreproducible results are usually good indicators of column deterioration. If one suspects deactivation of the column, the QA test should be redone. If the result does not compare well with the result obtained when the column was initially tested, the column will need to be regenerated as subsequently described.

The label "guard column" aptly describes the function of the pre-column. It was packed with "CO: Pell PAC" (p. 16) and gave reasonable protection to the analytical column from impurities. Periodic replacement of the packing in the precolumn may greatly reduce the frequency of column deactivation. However, after prolonged use, even the analytical column would probably deteriorate due to the accumulation of impurities from solvent and sample. With continuous use, several liters of solvent passed through the column per week. Thus, the polar impurity problem would build in a gradual, cumulative process.

Deterioration of the analytical column can be corrected by deactivation and then reactivation. The deactivation and clean-up involved flushing out the column with progressively more polar solvents. When making mobile phase changes, each mobile phase must be soluble in the preceding mobile phase. The increasing polarity of the solvent should result in the elution of polar impurities. The solvents should be used in the following order: n-heptane (first), 1,1,1-trichloroethane, ethyl acetate, acetone, ethanol and water (last). Only reagent (or higher) grade solvents should be used for the deactivation and clean-up stages. Reactivation involves the same list of solvents; in this case,

the solvents should be spectral grade or better. During reactivation, the solvents listed are used in the reverse order.

The solvents used for reactivation must be cleaned and dried before use. For solvent clean-up and drying, three silica gel adsorption columns are utilized. The columns are arranged in sequence and the solvents are arranged in sequence and the solvents are separated on them by frontal analysis.¹⁸ As the solvent goes through the three columns, it is cleaned progressively, thus leaving smaller quantities of impurities on the final column compared to the first. The first column is then removed and a new column replaces it at the end of the sequence (so, the second becomes the first, the third the second and the new column becomes the third). Then more solvent could be dried and purified on the new sequence of columns. Details of stringent purification procedures have been described by Riddick and Bunger.⁶⁹

The Altex Model 110 Solvent Metering Pump is especially designed for modern liquid chromatographic applications. The Altex Model 110 manual has a thorough and comprehensive coverage on installation, operation and maintenance of the unit. However, there are some very important precautions which should be noted here. Solvents which are reactive with the materials of the Model 110 wetted surfaces (i.e., type 316 stainless steel, Kel-F and sapphire) should not be pumped through it. For example, halide ions should never be used, and aqueous solutions should be replaced with methanol or similar organic solvents when the pump is not being used.

It is recommended that solvents be filtered and degassed prior

to usage. Unfiltered solvents, depending on grade, may cause check-valve leakage. The solvent inlet filter blocks particles greater than 5 microns in diameter, and the filter should be used at all times. The solvent inlet filter should always be maintained in solvent, in order to prevent air bubbles from entering the system. When air bubbles enter, the detector response becomes very erratic, and the pumps need to be primed.

If, despite precautions, it is necessary to prime the pump, the following procedure should be followed:

(a) The power to Model 110 is turned off and the intake line is disconnected at the solvent port.

(b) The solvent reservoir is lifted above the level of the port, so that solvent flows through the line by gravitational force.

(c) A waste beaker is used to collect excess solvent, and the intake line is reconnected to the port.

(d) The power is turned on.

(e) Solvent is pumped through the system.

Sometimes the solvent inlet filter may become clogged and will require cleaning. Again, the power is turned off and the intake line is disconnected at the port. Depending on how dirty the filter is, it may be cleaned simply by backflushing with solvent from a syringe. More stringent cleaning would involve initial washing of the filter with nitric acid first. Then, the filter should be rinsed by backflushing with solvent. If those two steps do not succeed, the Teflon tube from the filter should be removed and the filter should then be placed in a oven at 600°F. The high temperature should vaporize any

volatile compounds and decompose organic compounds that would block the filter. After the filter is removed from the oven, it should be backflushed with solvent and the pumps should be primed as previously described.

The HPLC instrument is modular in design. There are two panels on the wall which have the remote switches that control the power supply to the various units of the instrument. In addition, the individual units have localized switches located on the respective components.

The pre-operational check involved turning on all the remote switches of the instrument. The individual units were not activated until their specific switches located on the respective components were turned on. Many of these switches were pushbuttons which were OFF in the released position (up) and ON when depressed.

The POWER switch was depressed and then the switches for the W and D₂ lamps were depressed. The D₂ lamp usually came on about ten seconds after the switch was depressed. In case it did not come on after ten seconds, the D₂ lamp switch was released and depressed again after twenty to thirty seconds. At least fifteen minutes was allowed for warmup after depressing the power switch.

On the main unit, the % T switch was depressed. Then the appropriate Exciter Lamp was selected; in this work the D₂ lamp was employed. The operating wavelength was chosen by turning the wavelength dial, always from shorter to longer wavelengths. The 100 % T coarse control was adjusted to obtain a reading of about 100.0 on the digital panel meter.

The Model 100-40 could be used for three different measurements: concentration, transmittance and absorbance measurements. The ABS 0-2 range was adequate for this work. After depressing the ABS 0-2 switch, the ABS 0 COARSE control was turned to obtain a reading of about 0.000 on the digital panel meter. Then, the ABS 0 FINE control was used to adjust the reading to exactly 0.000 on the digital panel meter. Then, the strip chart recorder was turned on.

There were two pumps A, B (Figure 1, p 18) connected to two solvent reservoirs. Solvent was pumped at flow rates (1-5 ml/min) and solvent gradients (e.g., start with 0 % B in A+B and increase to 5 % B; A and B are elution solvents) were dictated by the user via the Model 420 Microprocessor, Controller/Programmer. The mobile phase was eluted through a guard column, then an analytical column and next into the sample compartment for detection and out again for disposal or collection. The column was flushed with solvent in this way until a steady zero baseline was observed.

The sample (10-25 μ l) of interest was loaded into the sample injection system via a microliter syringe. It was then actually injected by changing the dial from LOAD to INJECT. It was then carried by the eluting solvent through the guard and analytical columns at rates defined by the retention times of the various sample constituents under those operating conditions. One was able to detect the elution of the various components by monitoring the change in absorbance on the digital panel meter and on the chart recorder. The chromatograms revealed such information as retention times, elution volumes and relative absorbance of the components. As indicated previously, one

had the choice of either collecting the fractions after they left the detector for further analysis or letting the flow into a waste container for disposal.

2. Matrix-Isolation Fluorometry (MIF)

a. Sample Preparation. Figure 4 (p 22) illustrates the major components used for MIF sample preparation. The Knudsen cell was washed several times with "Certified A.C.S. Spectroanalyzed" acetone in order to eliminate any contaminants. The window was also cleaned in acetone, while the oven assembly and the head were cleaned with boiling hexane.

While the sample was injected into the Knudsen cell with a microliter syringe, part of the line was evacuated. With V1, V2, and V4 closed and V3 open (Figure 4, p 22) and with liquid nitrogen used to cool traps CT. A and CT. B, the line was evacuated to the shroud of the cryostat. A stream of air was used to vaporize the solvent from the sample in the Knudsen cell.

After the cell was snugly fitted into the Knudsen oven assembly in the head, the head was mounted on the cryostat in order to be connected to the cryocooler and vacuum shroud and head (sample chamber). The matrix gas deposition line was then connected to the head. There were some additional details at this point in the procedure if an organic matrix was to be used, which will be discussed later. A copper gasket was used at the point where the deposition line connected to the line leading to the head, in order to ensure a good seal. A liquid nitrogen cold trap was placed at CT. C to trap any impurities in the deposition line. V7 was always closed until the deposition

commenced. The fine adjustment valve was opened somewhat in order to evacuate the deposit line up to the coarse valve V7.

When the pressure on the vacuum shroud was less than or equal to fifty microns of mercury ($P \leq 5 \times 10^{-5}$ torr), the compressor of the cryostat was turned on. A digital temperature indicator/controller was connected to the shroud in order to monitor the temperature during cryopumping. Under ideal conditions, about 15-20 minutes was required to pump down to a pressure of less than 5×10^{-5} torr and an additional 30-40 minutes to cool down to 13-15 K. Then, the valves to the shroud, V1 and V4, were closed. The electrical feed-throughs were connected to two variable transformers, operated in series, which were used to power the nichrome wire heater and to finely adjust the oven temperature. A voltmeter was also used in order to measure the voltage applied to the oven when vaporizing the sample. The deposition time varied with the complexity of the sample being analyzed, ranging from 30 minutes to over an hour.

When an organic matrix was used instead of nitrogen or argon, a freeze-thaw cycle was employed to remove oxygen from the organic solvent. As described before, the deposition line was first connected to the head. The test tube containing the organic matrix was immersed in a liquid nitrogen cold trap. This action caused the organic vapor to freeze, and any oxygen was evacuated in the normal fashion. About 15 minutes before the deposition was started, the cold trap was removed and the organic matrix was allowed to thaw out. The purified matrix vapor was then free of oxygen which would have tended to quench fluorescence of many sample constituents.

When heptane was used as the matrix, an annealing process was carried out after the deposit was completed. The temperature was usually around 13 K after deposition. The temperature controller was then set at 12 W heating power and 150 K. The compressor on the cryostat was turned off. About 5 minutes was required for the temperature to reach 145 K. The compressor was then turned on again. The sample was left under those annealing conditions for 5 minutes. The temperature remained at 145.1 ± 0.1 K during annealing. The heater was then turned off and the temperature was set to 0 K. The temperature began decreasing, and when it reached about 50 K, the valves to the shroud were opened to the vacuum pump, and the shroud was allowed to pump out while it was cooling down. About 35 minutes was usually required to reach cryogenic temperature and vacuum pressure. Thus, after the deposit was made, an additional 45 minutes was required to anneal the heptane deposit and to return to the original conditions.

b. MIF Detection. A relatively rigorous treatment of the experimental considerations has previously been described by Stroupe.¹³ Thus, a skeletal outline should suffice here. Photon counting was used for processing the signal produced by the photomultiplier. After sample preparation was completed, the cryostat was placed on a stand at the fluorometer. The window was positioned so that it would be in the optimum range of the optical paths necessary for fluorescence detection. The excitation wavelength (e.g. 292 nm for BaP excitation) and counting dwell time (500–600 msec) were chosen and the initial emission wavelength (320 nm) was set. The emission slits and shutters on the monochromators were opened and the chart recorder was turned on. The emission monochromator scan and recorder drives were activated and the spectrum was recorded.

After the spectrum was acquired, the cryostat was removed from the fluorometer and the compressor was turned off. The cryostat was connected to the vacuum line as shown in Figure 4 (p 22). The digital temperature controller/indicator was set at 12 W heating power and 299 K, to accelerate elimination of the deposit through the vacuum system. At this point, an additional chore surfaced when an organic matrix such as heptane was used; in that case, the matrix was trapped in CT. A, which necessitated additional cleaning. The Cajon unions facilitated easy removal of this trap, which was placed in an oven at about 80°C, causing rapid vaporization of the matrix. The trap was then rinsed in acetone and dried in the oven or with a stream of air and subsequently returned to the vacuum line.

CHAPTER III

RESULTS AND DISCUSSION

A. DEVELOPMENT AND VERIFICATION OF AN HPLC PROCEDURE

The objectives of the chromatographic work were four-fold:

- (1) To test the reproducibility and reliability of retention data for a polar bonded-phase column.
- (2) To develop a separation procedure which would facilitate class separation of a three-component synthetic mixture.
- (3) To verify the HPLC procedure developed in (2) by identification of the compounds contained in the various fractions.
- (4) To duplicate the separation procedure used for a three-component mixture in order to obtain similar class separation of a real environmental sample (in this case, a shale oil sample).

Preliminary experiments involved the analysis of standard compounds in order to become more familiar with the operation of the HPLC instrument. Standard compounds and mixtures were analyzed to test the reliability and reproducibility of the "Partisil-10 PAC" cyano-amino bonded-phase column which was employed for this work.

Table III is a comparison of the retention times (t_R) for six aromatic hydrocarbons. The data were obtained from four runs of each compound. The first HPLC run analyzed the pure stock solution of each individual compound. The second run analyzed 10 μ l of a mixture of the six aromatic hydrocarbons, and the third and fourth runs were duplicates of 25 μ l aliquot portions of the six-component mixture. Figure 5 is a typical chromatogram obtained for normal-phase separation of the six-component mixture.

TABLE III
REPRODUCIBILITY OF RETENTION DATA FOR AROMATIC HYDROCARBONS^a

COMPOUND	PURE SOLUTION	10 μ l MIXTURE	25 μ l MIXTURE	25 μ l MIXTURE	MEAN	SD	RSD
I. Toluene	5.1	5.4	5.4	5.6	5.4	0.21	3.8 %
II. Napthalene	6.9	6.9	6.9	7.3	7.0	0.20	2.9 %
III. Fluorene	7.6	8.3	8.3	8.9	8.3	0.53	6.4 %
IV. Anthracene	8.2	9.8	9.8	10.5	9.6	0.97	10. %
V. Pyrene	10.5	11.7	11.7	12.7	11.7	0.90	7.7 %
VI. Benzo(a)pyrene	>10.5	16.5	15.1	20.0	-	-	-

^aAll entries in minutes.

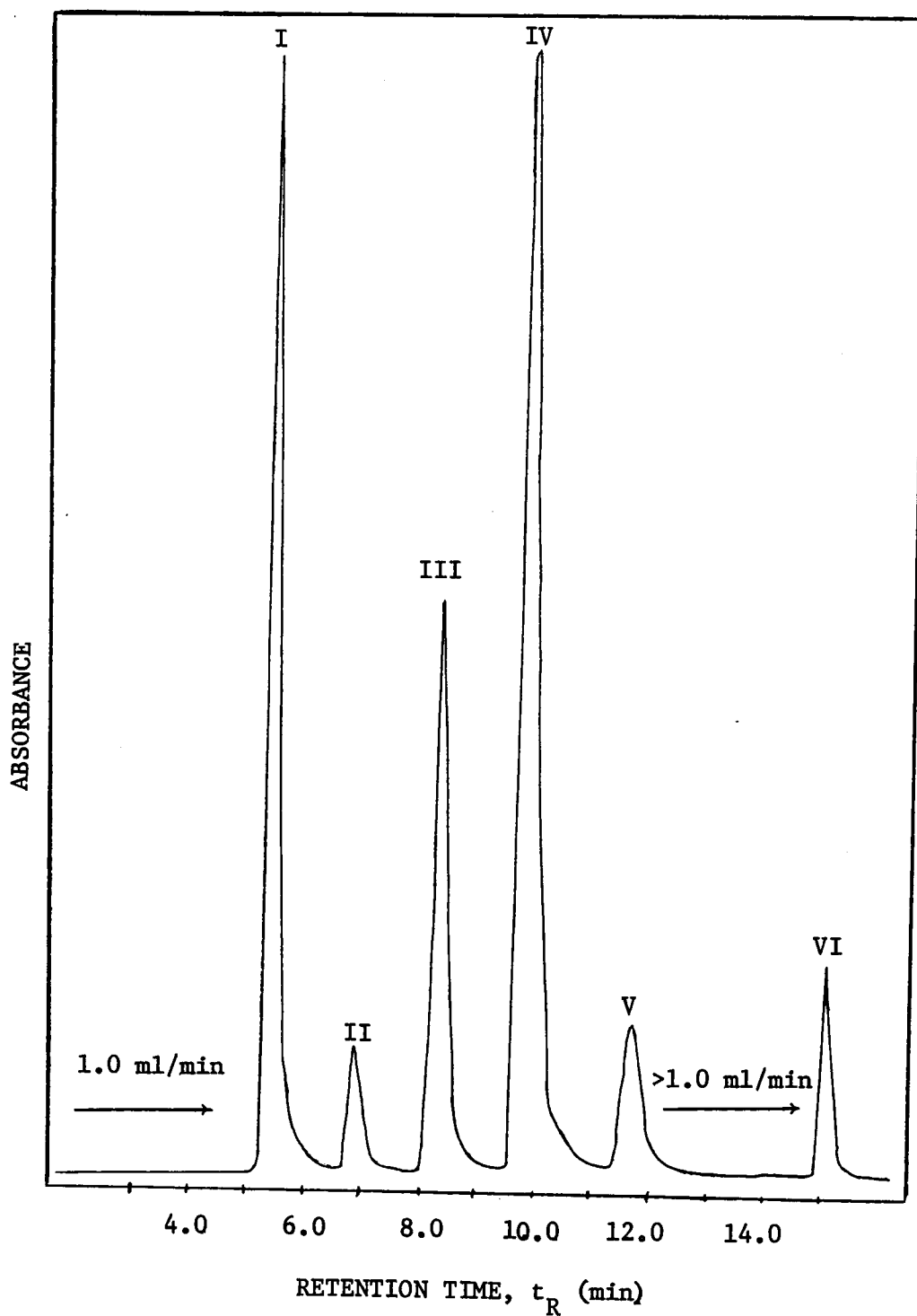


Figure 5. HPLC chromatogram for normal-phase separation of a six-component mixture of aromatic hydrocarbons.

I - Toluene, II - Napthalene, III - Fluorene, IV - Anthracene,
V - Pyrene and VI - Benzo(a)pyrene (BaP)

The mobile phase used in the experiments summarized in Table III (p 36) was n-heptane, and the flow rate was 1.0 ml/min for the first five components. The greater variation in t_R for BaP was due to the fact that the flow rate was increased non-uniformly after the first five components were eluted.

Several factors could influence the reproducibility of retention data. Theoretically, increases in t_R could be a consequence of any of the following factors: lower flow rate, lower column temperature, increasing column activity (i.e. system is not equilibrated), and improper gradient setting. Theoretically, decreases in t_R could be the result of higher flow rate, higher column temperature, decreasing column activity (i.e. deactivation by strongly retained impurities), improper gradient setting, loss of stationary phase or column deactivation and column overloading.

It was observed that retention times obtained on the same day were virtually identical for a given compound, whereas the reproducibility of retention times measured on different days varied somewhat. The flowrate was fixed (1.0 ml/min), but it was possible that there were small variations in flow rate from one day to the next. Temperature variations, also, may have been a probable determining factor in the variations of t_R .

The other factors, which were previously listed, were eliminated as probable causes for variations in t_R , for various reasons. For example, since the mobile phase was n-heptane (i.e. there was no gradient elution), there was virtually no probability of having improper gradient settings. Care was taken to ensure column

equilibration, the sample size was not excessive and use of a bonded-phase eliminated the possibility of loss of stationary phase. Also, since the column was relatively new and no polar samples had been injected onto it, it appeared unlikely that the column had been deactivated.

The normal-phase "group or class separation" of a three-component mixture was studied next. The mixture contained a polycyclic aromatic hydrocarbon, benzo(a)pyrene (BaP), a hydroxy compound, phenol, and a nitrogen heterocyclic compound, dibenz(a,j)acridine. The model HPLC procedure developed here was designed to serve as a guide for the separation of a real sample into three major fractions, each containing the respective class of compounds.

The choice of mobile phase was crucial to successful separation. For normal-phase separation using bonded-phase columns, typically, the solvent mixtures which can be used consist of a hydrocarbon solvent, such as n-hexane or n-heptane, plus small amounts of solvents such as dichloromethane (CH_2Cl_2). In order to elute very polar solutes, solvents such as 1-chlorobutane or dichloromethane could be used with polar modifiers such as methanol.

This work utilized n-heptane and dichloromethane as solvents. The procedure developed for the separation of the three-component mixture employed a mixture of 5 % dichloromethane and 95 % n-heptane for elution of the first fraction. Then, through gradient elution, the mobile phase was changed to 100 % dichloromethane over a time interval of ten minutes, in order to elute the more polar components of the mixture (Figure 6). The chromatogram illustrates where the mobile phase changes were made.

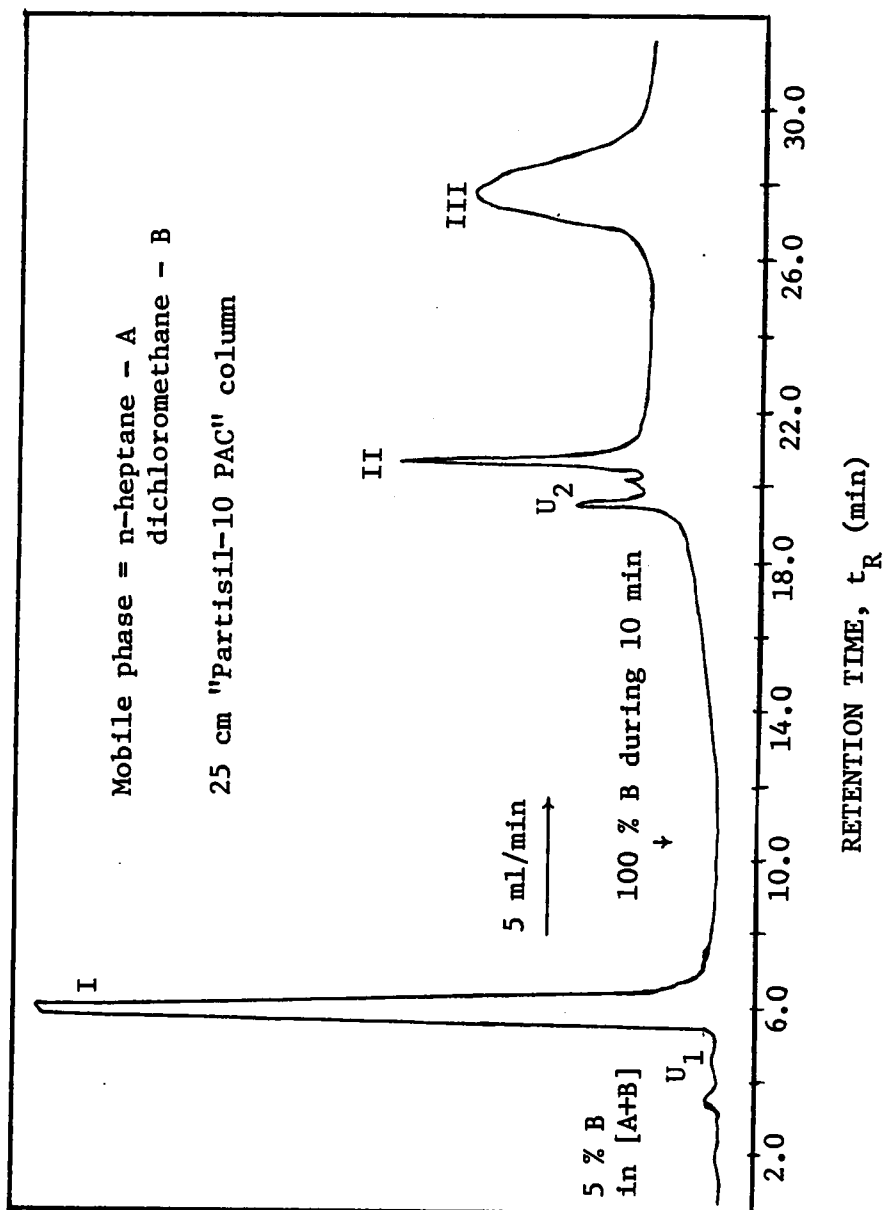


Figure 6. HPLC Chromatogram of a three-component mixture (normal-phase separation).

I - BaP, II - Phenol, III - dibenz(a,j)acridine,
 U_1 - unidentified impurity in BaP, U_2 - unidentified impurities in phenol.

Stock solutions of BaP, phenol and dibenz(a,j)acridine were run individually under the same conditions as the mixtures. Based on HPLC retention data, the peak indicated by I in Figure 6 (p 40) was identified as BaP, and U_1 was an unidentified impurity in the BaP solution. The peak indicated by II was phenol, and the two small peaks indicated by U_2 were unidentified impurities in the phenol sample. The peak indicated by III was dibenz(a,j)acridine.

In addition to the above mentioned HPLC data, room-temperature solution fluorometry and gas chromatography (GC) were employed to verify the identifications of the major peaks observed in the HPLC procedure.

Figure 7 compares the room-temperature solution fluorescence spectra of a BaP sample and the first liquid chromatographic fraction of the three-component mixture. The obvious similarity of the spectra confirmed the presence of BaP in the first HPLC fraction. At room temperature, no detectable fluorescence was observed in the second and third HPLC fractions of the three-component mixture.

GC analyses were performed using a Hewlett-Packard 5880 A gas chromatograph and terminal. The "OV101" column used was twenty inches in length and constituted Chromosorb W High Performance (WHP) support. Helium was used as the carrier gas and a thermal conductivity detector was employed.

The first temperature program had an initial temperature of 70°C for three minutes. Then, the temperature was increased at the rate of 25°C/min for eight minutes. The final temperature, 270°C, was maintained for three minutes before terminating the experiment.

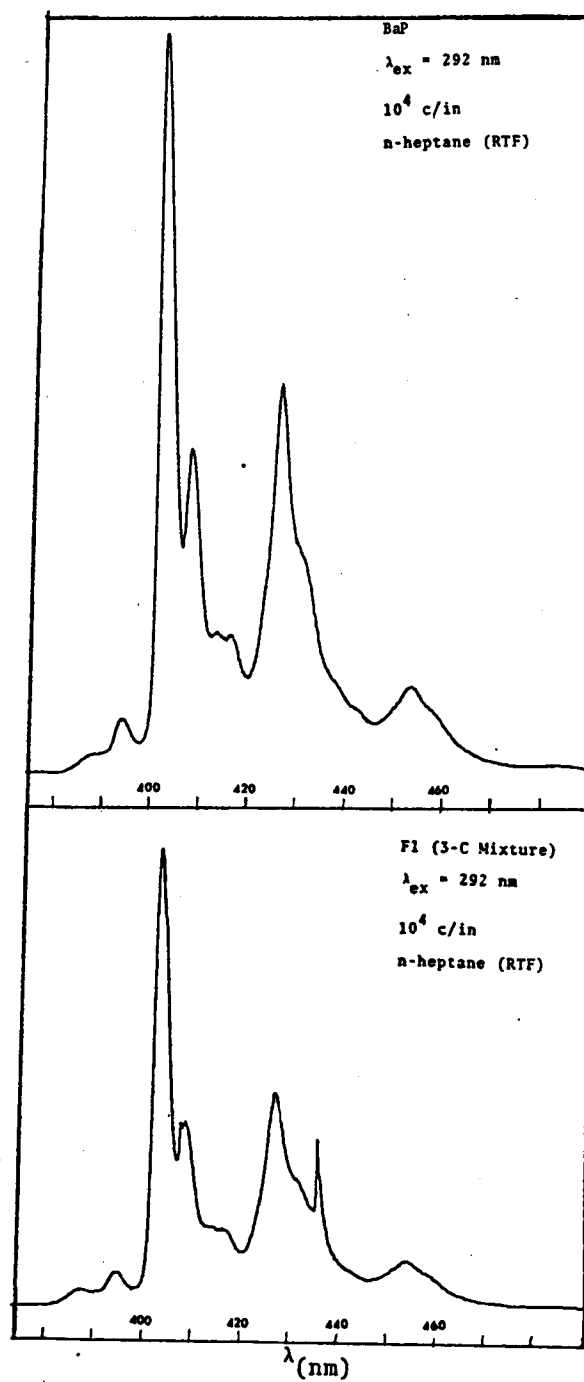


Figure 7. Comparison of room temperature solution fluorescence spectra of BaP and the first fraction of the three-component synthetic mixture.

Figure 8 is a gas chromatogram of a mixture of phenol, BaP and dibenz(a,j)acridine. Concentrations ranged from 10^1 to 10^2 $\mu\text{g/ml}$. Based on the GC retention data for individual components, it was concluded that the peak labelled A represented phenol, B represented BaP and C represented dibenz(a,j)acridine.

Figure 9 compares the gas chromatograms of pure phenol and the second HPLC fraction of the three-component mixture. The retention times correspond, indicating the presence of phenol in the second HPLC fraction. Figure 10 compares the gas chromatograms of pure dibenz(a,j)acridine and the third HPLC fraction. A second temperature program was used for matching these two samples. The second temperature program had an initial temperature of 260°C for one minute. Then, the temperature was increased at the rate of 5°C/min for two minutes. The final temperature, 270°C , was maintained for one minute before terminating the experiment. The comparison of the GC retention times of the third HPLC fraction and dibenz(a,j)acridine (Figure 10) indicated that dibenz(a,j)acridine was the major constituent of the third HPLC fraction.

The procedure used for HPLC separation of a three-component mixture was then duplicated for separation of a shale oil sample (Figure 11). Based on the data and arguments which verified the three-component separation, it was presumed that PAHs should be contained predominantly in the first fraction, hydroxy compounds predominantly in the second and nitrogen heterocyclic compounds predominantly in the third HPLC fraction of the shale oil. Further analysis of the first or so-called PAH fraction did confirm the presence of PAHs (including BaP)

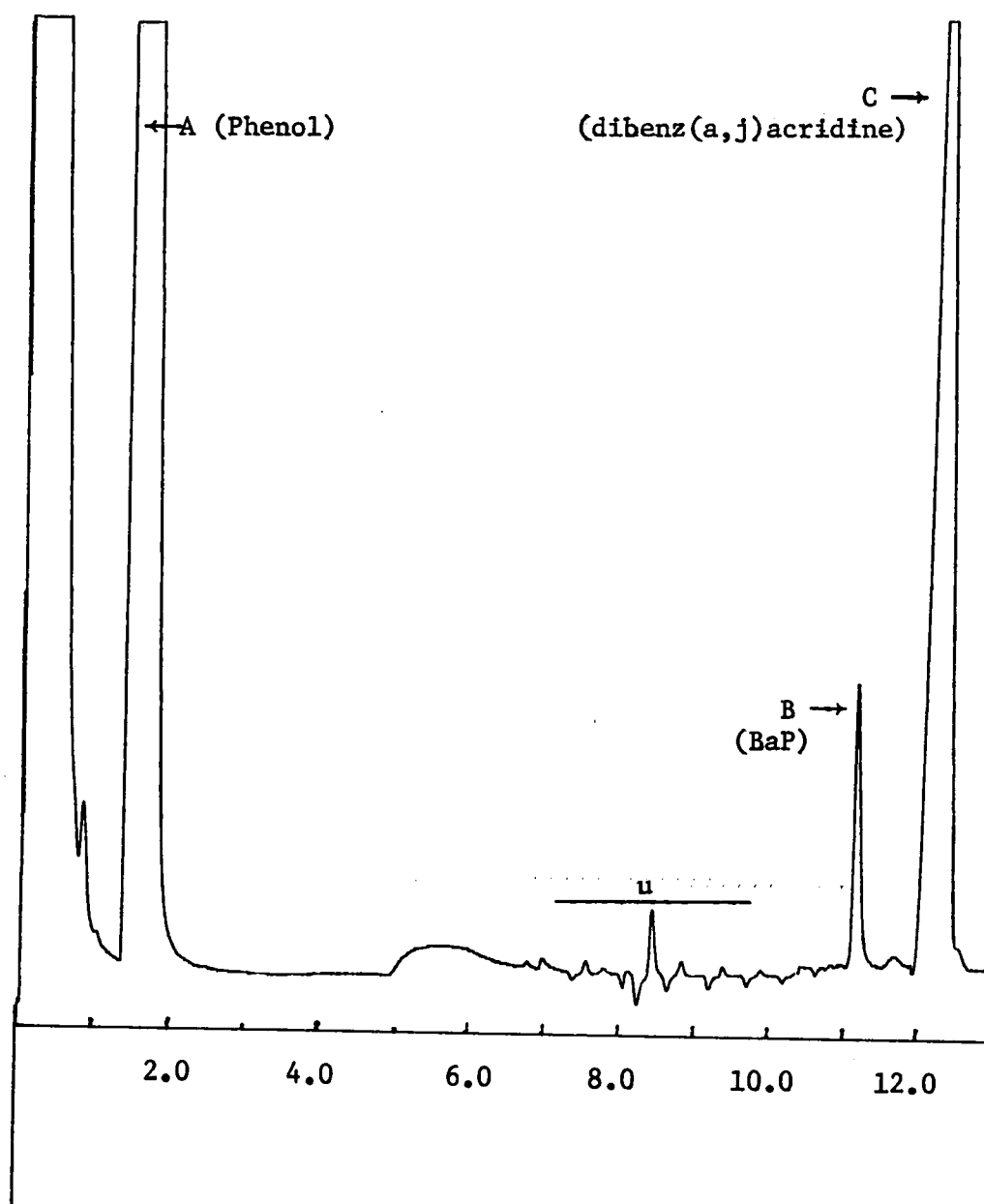


Figure 8. Gas chromatogram of a mixture of phenol, BaP and dibenz(a,j)acridine in dichloromethane and n-heptane.

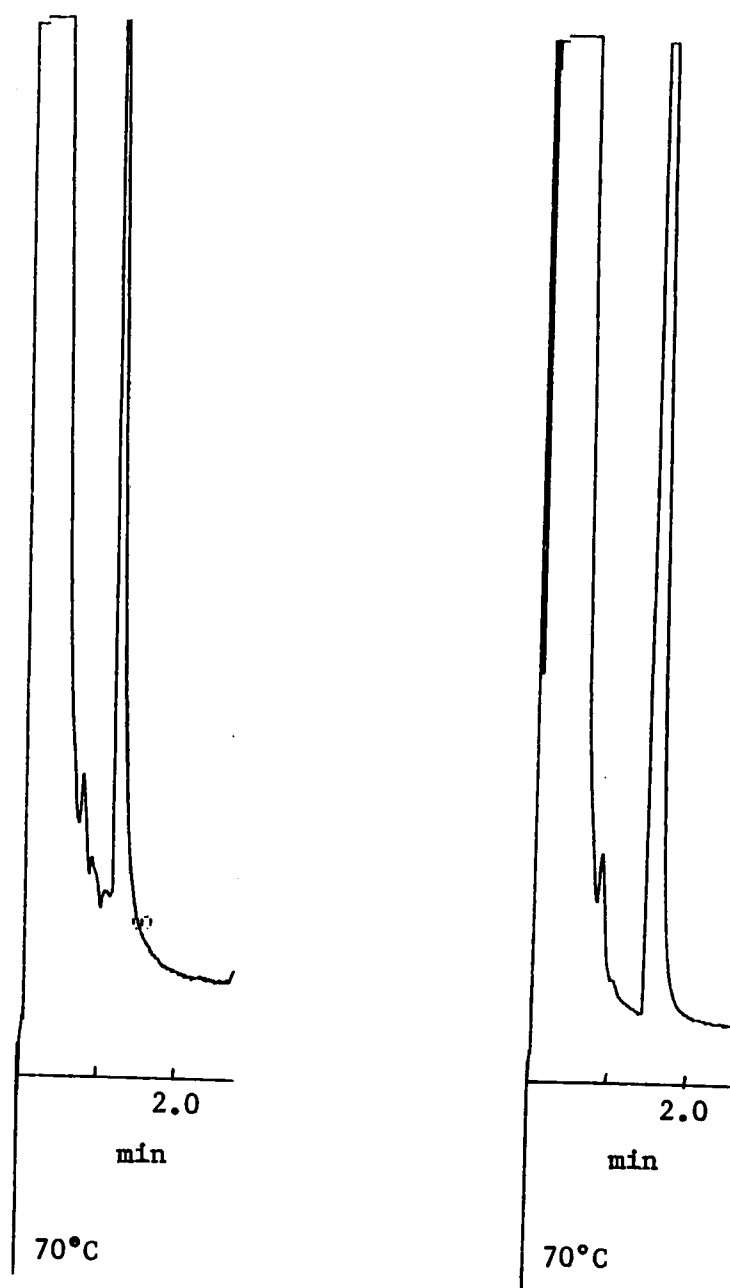


Figure 9. Comparison of gas chromatograms of the second HPLC fraction of the three-component mixture (left) and phenol (right).

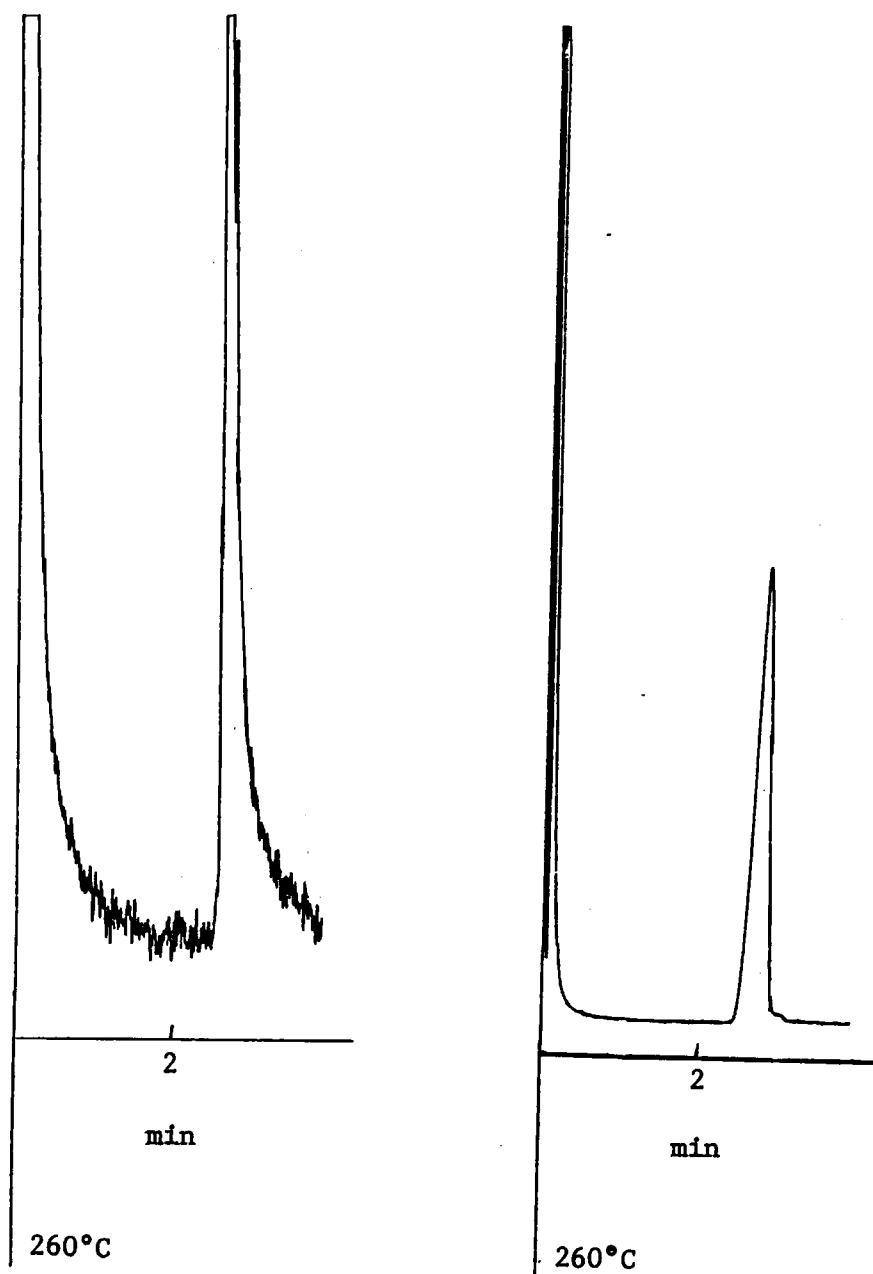


Figure 10. Comparison of gas chromatograms of the third HPLC fraction of the three-component mixture (left) and dibenz(a,j)acridine (right).

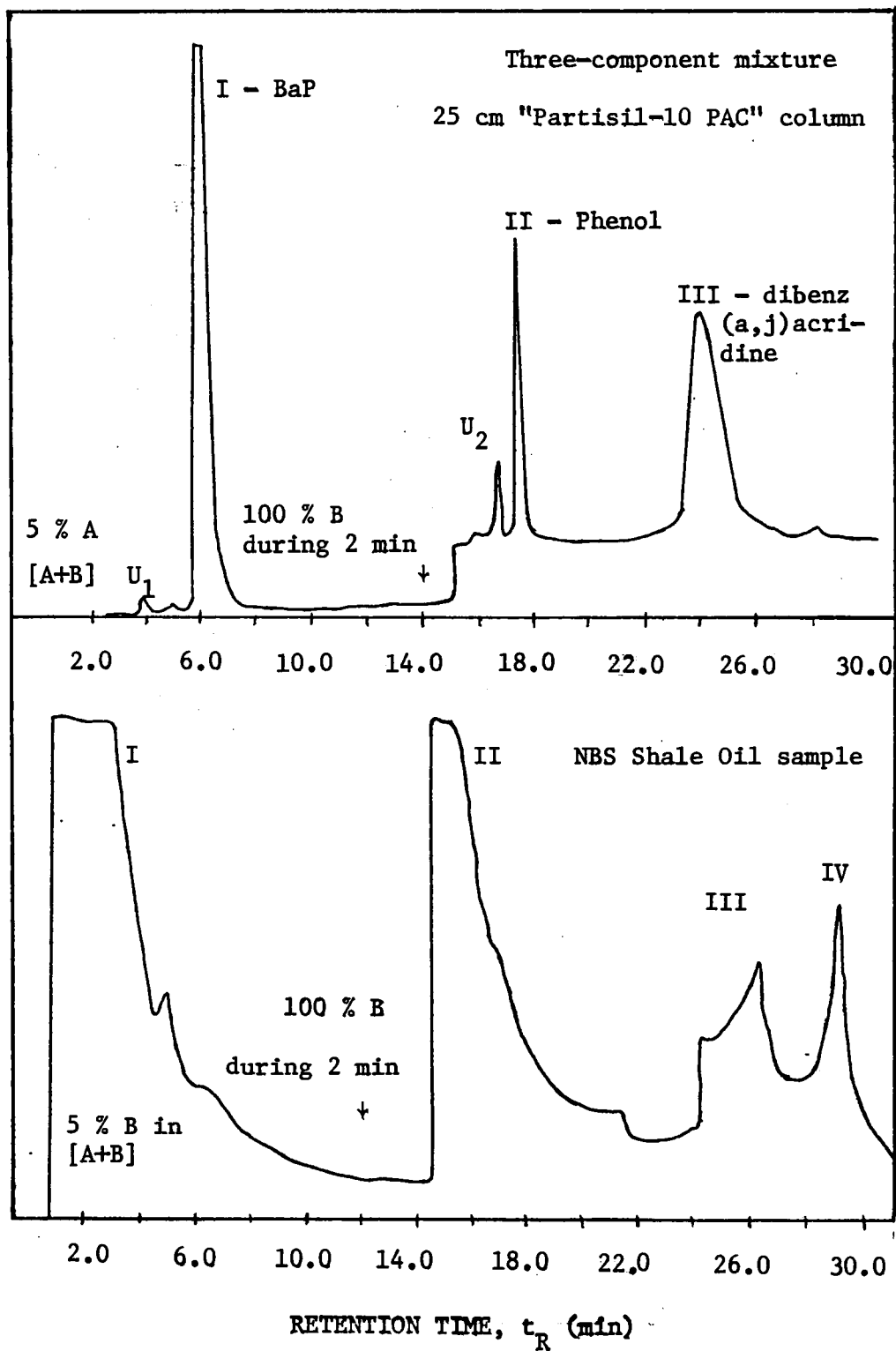


Figure 11. Comparison of HPLC chromatograms of a three-component mixture (top) and a shale oil sample (bottom), (normal-phase class separation).

Mobile Phase: A = n-heptane, B = dichloromethane.

in the first fraction (matrix-isolation fluorescence spectra reveal this, as discussed subsequently, p. 49).

If one were to compare the two chromatograms in Figure 11 (p 47), the overall separation trend would correspond reasonably well, except for a few features. The first two major peaks of the shale oil chromatogram are broader and shifted towards shorter retention time values, as compared with those of the simple three-component mixture. The last two peaks are also spread out over a longer time interval. The broader peaks are a consequence of the complexity of the shale sample. Each peak does not represent a single compound but a large number of compounds with small differences in retention time. The shift towards shorter retention time is due in part to the sample size effects. An increase in sample size may lead to column overloading, which results in shorter retention times. The overall elution time was about thirty minutes. Intermediate fractions were collected between major peaks in order to reduce "carry over" contamination of the preceding fraction.

B. FLUORESCENCE (ROOM-TEMPERATURE AND MATRIX-ISOLATION FLUOROMETRY)

The fluorescence spectrometric portion of this work had three major goals:

- (1) Verification of the previously described HPLC procedure by identification of the major groups of compounds contained in the various fractions. The results of these studies have already been presented (see Figure 7, p 42).

- (2) A brief comparison of room-temperature solution and matrix-isolation fluorometry for analysis of a multicomponent sample.

(3) Qualitative analysis of a multicomponent chromatographic fraction, which contained the PAH group of compounds from a shale oil sample.

(4) Quantitative analysis of BaP in the PAH fraction of this shale oil sample.

Figure 12 compares the matrix-isolation and room-temperature solution fluorescence spectra of the first HPLC fraction of a shale oil sample. The room-temperature spectrum (Figure 12a) reveals a broad continuous band with negligible spectral information. On the other hand, matrix-isolation spectra (Figure 12b,c) reveal spectral information lacking in Figure 12a. The presence of PAHs such as BaP, chrysene(C), pyrene(P) and benzo(b)fluorene (BbF) is evident from the matrix-isolation fluorescence spectra of the first HPLC fraction of the shale oil.

The main advantage of room-temperature solution fluorometry is the relative ease with which the measurements can be made. However, matrix-isolation fluorometry is superior to room-temperature fluorometry in spectral resolution and overall spectral information. Figure 12 underscores the utility of matrix-isolation fluorometry compared with room-temperature solution fluorometry for multicomponent analysis of trace fluorescent species in complex samples.

Four materials (argon, n-hexane, n-heptane and nitrogen) were explored for their suitability as matrices in this work. Figure 13 compares the spectra of the first HPLC fraction of shale oil which was diluted by a factor of 200 [N_{200} FR1] obtained in argon and n-heptane matrices, respectively. The dramatic difference between the spectra

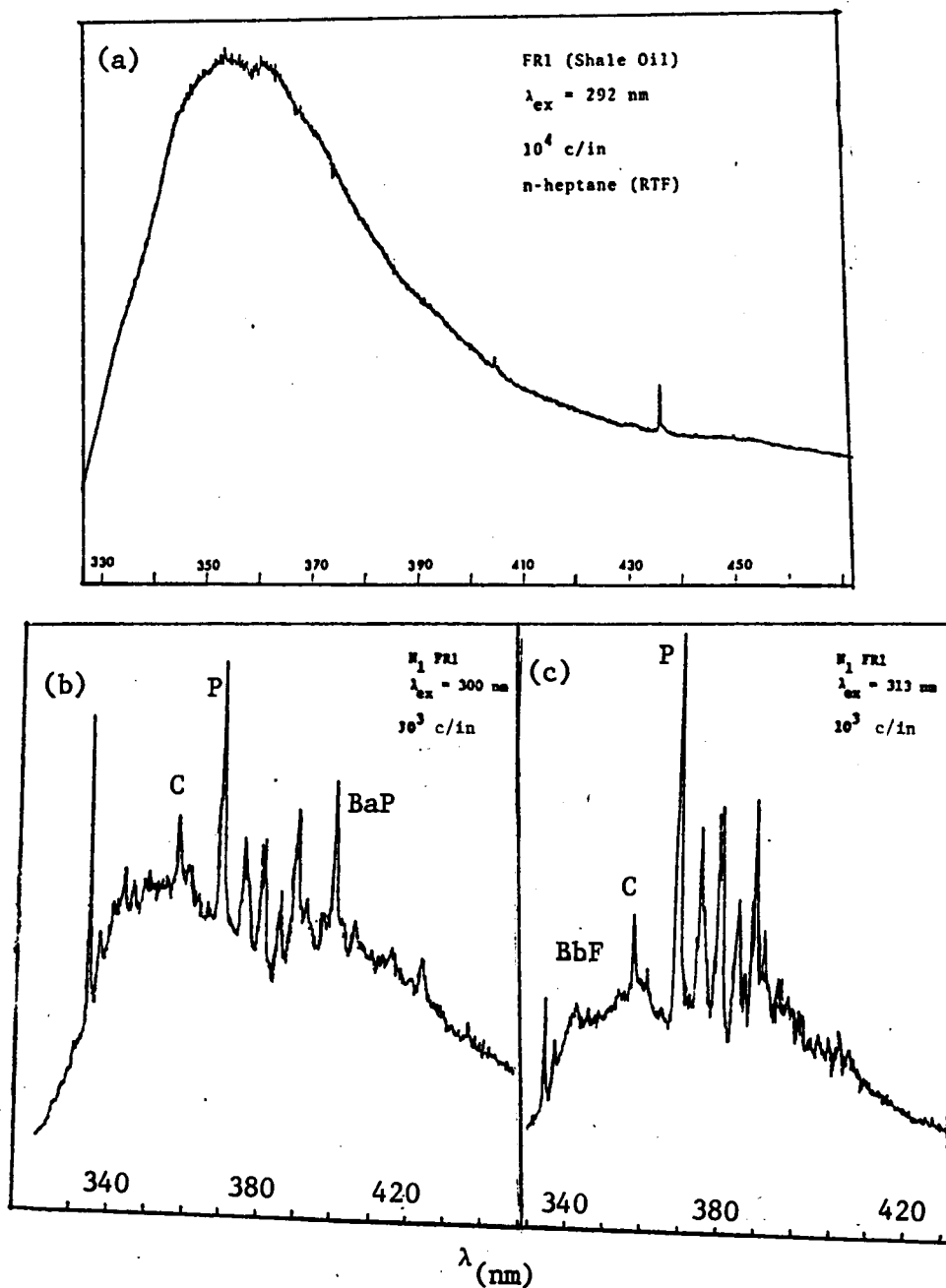


Figure 12. Comparison of spectra of PAH shale oil fraction by room-temperature solution fluorometry (a), and matrix-isolation fluorometry (b,c).

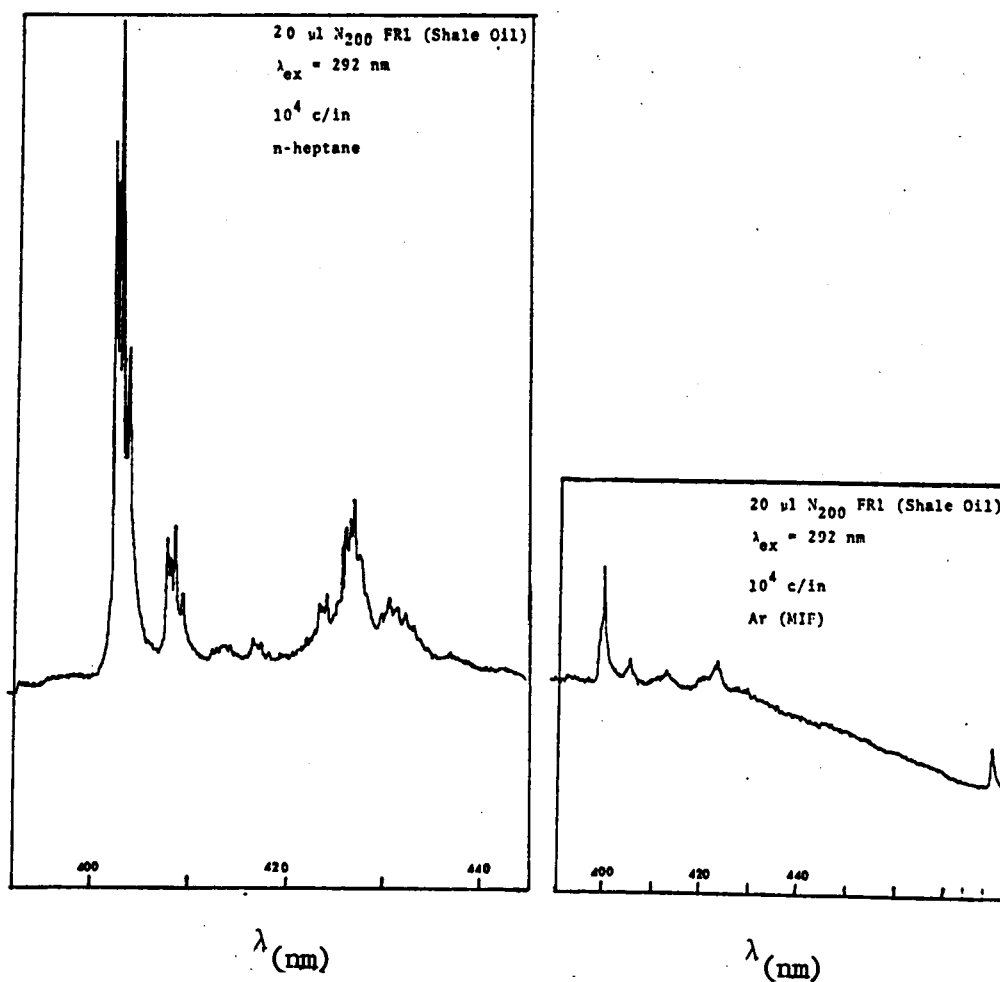


Figure 13. Comparison of fluorescence spectra of 20 μ l PAH fraction (N₂₀₀ dilution), of shale oil sample in n-heptane (left) and argon (right) matrices.

obtained in these two different matrices for the same sample is evident. The spectrum in the n-heptane matrix is highly resolved, has more fine structure and exhibits a larger fluorescence intensity, compared with the spectrum obtained in an argon matrix. For these reasons argon was eliminated as a matrix material.

Matrix-isolation fluorescence spectra were obtained of standard BaP and BbF samples with n-hexane as the matrix. BbF appeared to have a relatively higher fluorescence quantum yield than BaP in n-hexane, whereas BaP tended to have relatively higher fluorescence quantum yields than BbF in nitrogen and n-heptane matrices.

Figure 14 also demonstrates how fluorescence spectra obtained in n-hexane matrices were altered when the annealing conditions were varied. The spectrum of the mixture observed prior to annealing contained a contribution from pyrene (a contaminant); the intensity of the pyrene band increased significantly when the deposit was annealed. The annealing process involved heating the sample to 145 K. A malfunctioning Temperature Controller and Indicator made it impossible to duplicate precisely the annealing conditions. Therefore, it was considered unwise to use any alkane matrix (for which annealing was required) for quantitative work.

The elimination of n-hexane, n-heptane and argon as matrix materials for quantitative work led to the use of nitrogen, which was considered to be the most suitable matrix material for matrix-isolation fluorescence of polynuclear hydrocarbons for this work. Its use facilitated the acquisition of good spectra (well-resolved, intense peaks with relatively small bandwidths), and it did not require

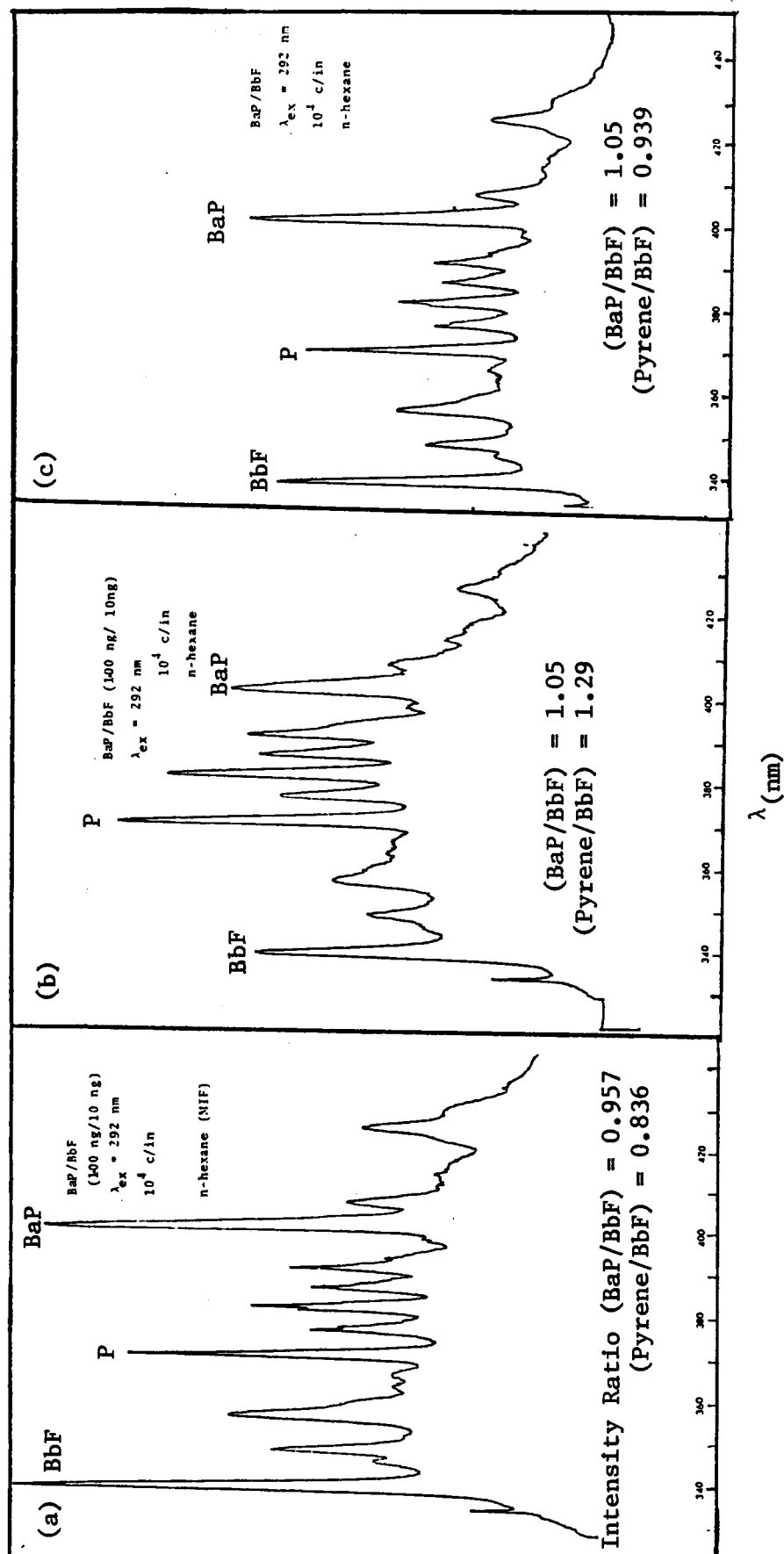


Figure 14. Comparison of fluorescence spectra of BaP and BbF in n-hexane matrix, showing the effect of annealing in the intensity ratio of peaks. (a) unannealed (b) annealed at 145 K (c) another deposit annealed at 145 K.

annealing. Therefore, unless otherwise indicated, the remaining spectra were obtained from deposits using nitrogen as matrix material.

One of the potential problems in the analysis of a very complex sample is the possibility of aggregation and spectral overlap due to the many compounds present. If the sample size is too large, aggregation, which works against the idea of matrix isolation, will occur. If guest molecules come into contact with each other, spectral broadening and fluorescence quenching can occur.⁵⁹ To avoid aggregation effects while at the same time producing signals sufficiently large to be detected, it was necessary to determine the optimum sample size empirically. In this work, a dilution series was used to determine the appropriate sample size of the PAH fraction of the shale oil. It was decided that, because BaP was a major carcinogen and had a large fluorescence quantum yield, it would be appropriate to carry out a quantitative analysis of it in the PAH fraction of the shale oil sample. Thus, the dilution series was designed to determine the appropriate dilution of the shale oil fraction at which the BaP fluorescence was well-resolved and had reasonably intense peaks. BbF was chosen to be the internal standard because it fulfilled the following conditions:

- (1) It sublimed at approximately the same temperature as the sample.

- (2) It had no emission band overlap with sample constituents at the concentrations of sample used in this work.

- (3) Its emission occurred at shorter wavelength than the sample, so that fluorescence quenching and long-range transfer from the analyte to BbF were inhibited.

(4) Its emission did not occur in the absorption range of sample constituents of interest. BbF has the additional advantage of being a good fluorescer, so that only small amounts were required, thus minimizing the possibility of energy transfer in the "spiked" sample.

The dilution series consisted of N_1 , N_{10} , N_{25} , N_{50} , N_{100} , N_{200} (the subscript indicates the factor by which the PAH fraction of the shale oil sample was diluted). The N_{50} dilution was considered optimal because it fulfilled the required conditions, which are briefly summarized:

- (1) BaP exhibited a fairly intense peak at $\lambda_{ex} = 292$ nm.
- (2) Negligible BbF fluorescence at $\lambda_{ex} = 300$ nm (in the absence of addition of BbF).
- (3) Reduced background and spectral interferences compared to more concentrated samples (e.g. N_1).

Table IV lists the PAHs which were revealed in the matrix-isolation spectra of the dilution series [Figures 15-21]. Figure 21 illustrates the reasons why the N_{50} dilution was chosen for quantitative analysis.

Data were obtained for four calibration curves for BaP quantitation, using pure BaP and BbF as an internal standard. Table V indicates the reproducibility of the data obtained with respect to their respective slopes and correlation coefficients. Table V also reports the results for BaP quantitation in a shale oil sample (PAH fraction).

The PAH fraction of the shale oil (10 μ l of N_{50} dilution) was deposited with 10 ng BbF. Figure 22 shows the fluorescence spectrum

TABLE IV

RESULTS OF QUALITATIVE ANALYSIS OF A SHALE OIL SAMPLE (PAH FRACTION)

COMPOUND (PAH)	ABBREVIATION USED IN FIGURES
Benzo(a)pyrene	BaP
Benzo(b)fluorene	BbF
Chrysene	C
Pyrene	P
Benz(a)anthracene	BaA

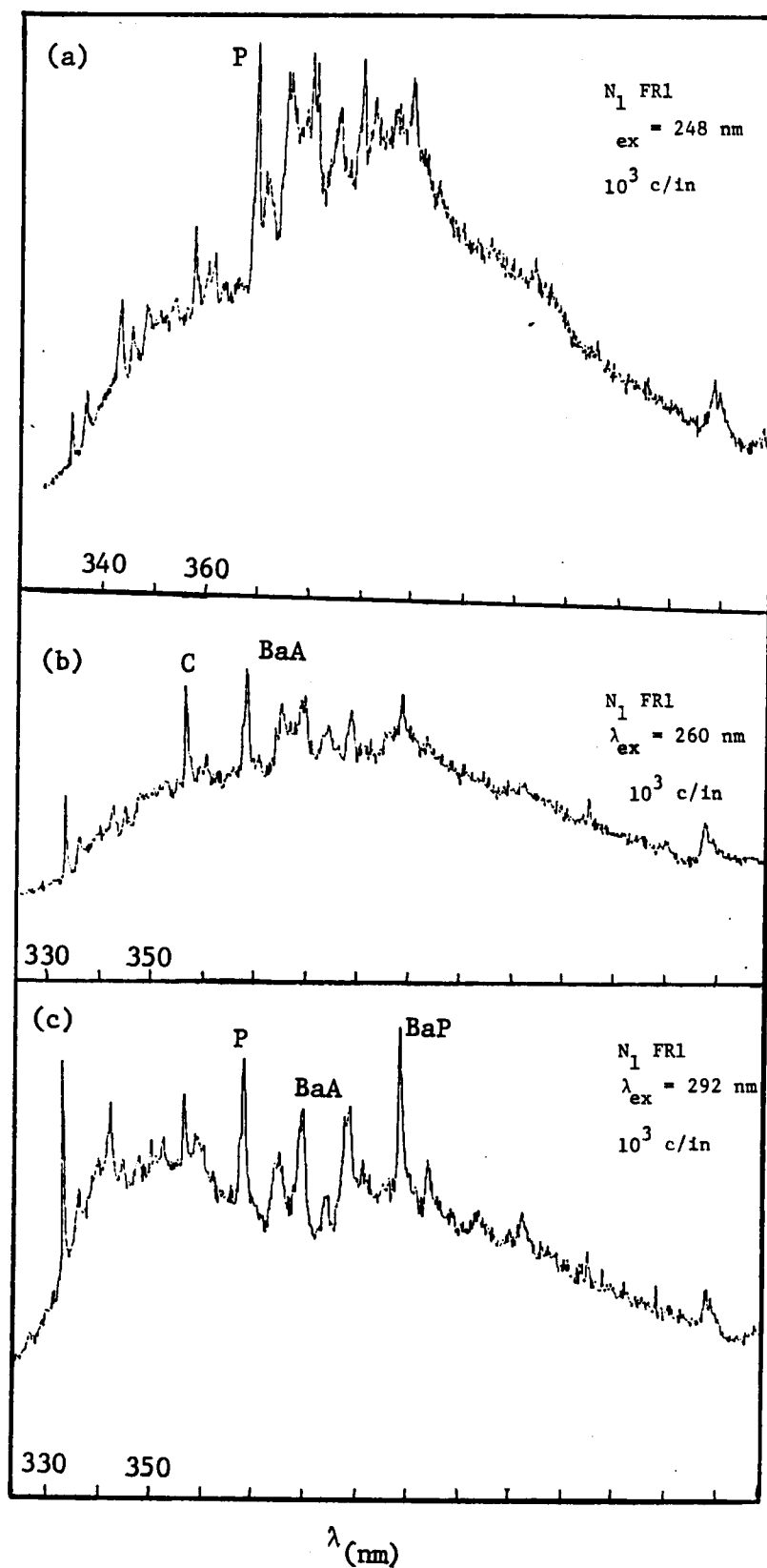


Figure 15. Fluorescence spectra of the HPLC, PAH fraction of shale oil (undiluted), (a) $\lambda_{ex} = 248$ nm, (b) $\lambda_{ex} = 260$ nm (c) $\lambda_{ex} = 292$ nm.

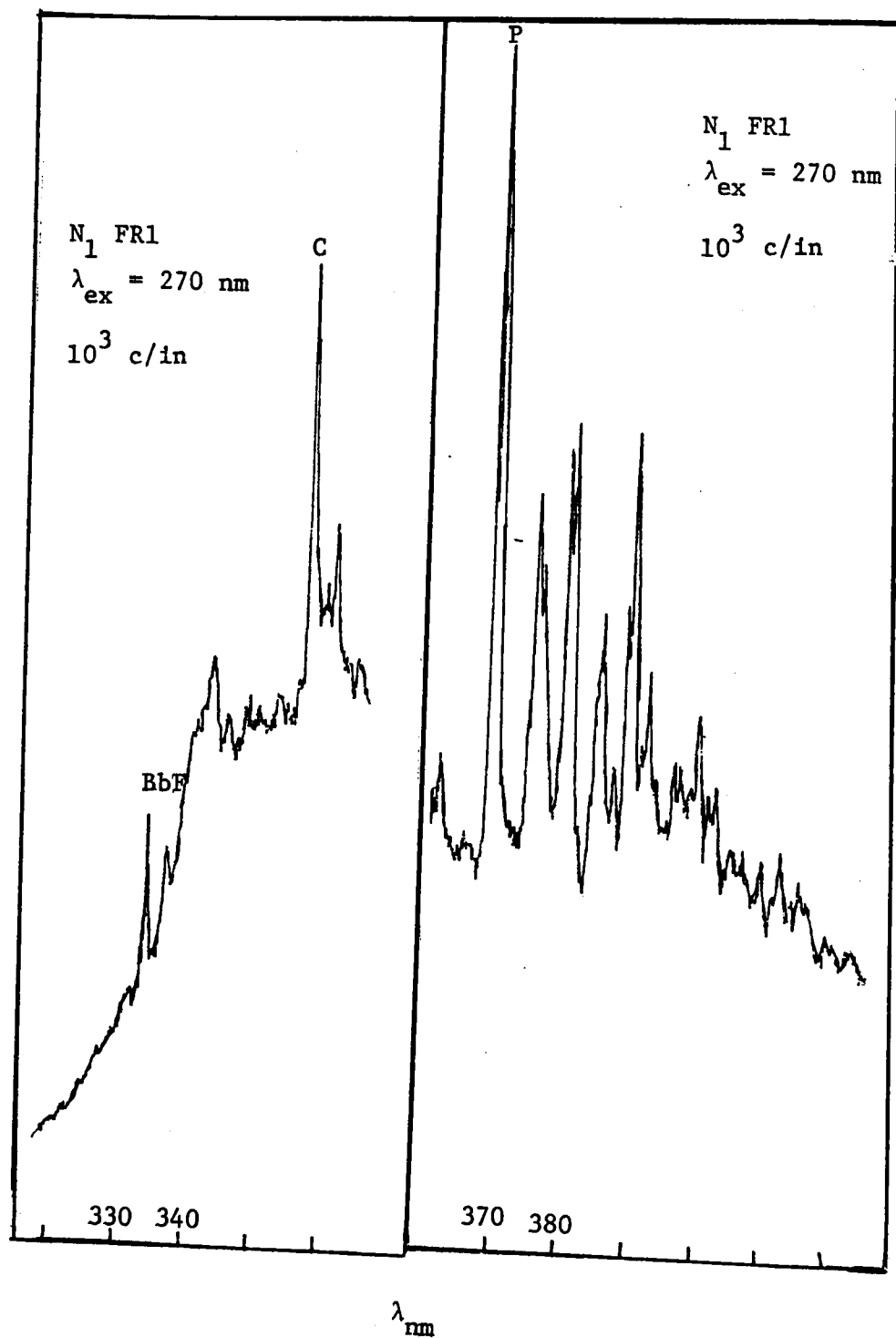


Figure 16. Fluorescence spectrum of undiluted PAH fraction of shale oil, $\lambda_{ex} = 270$ nm.

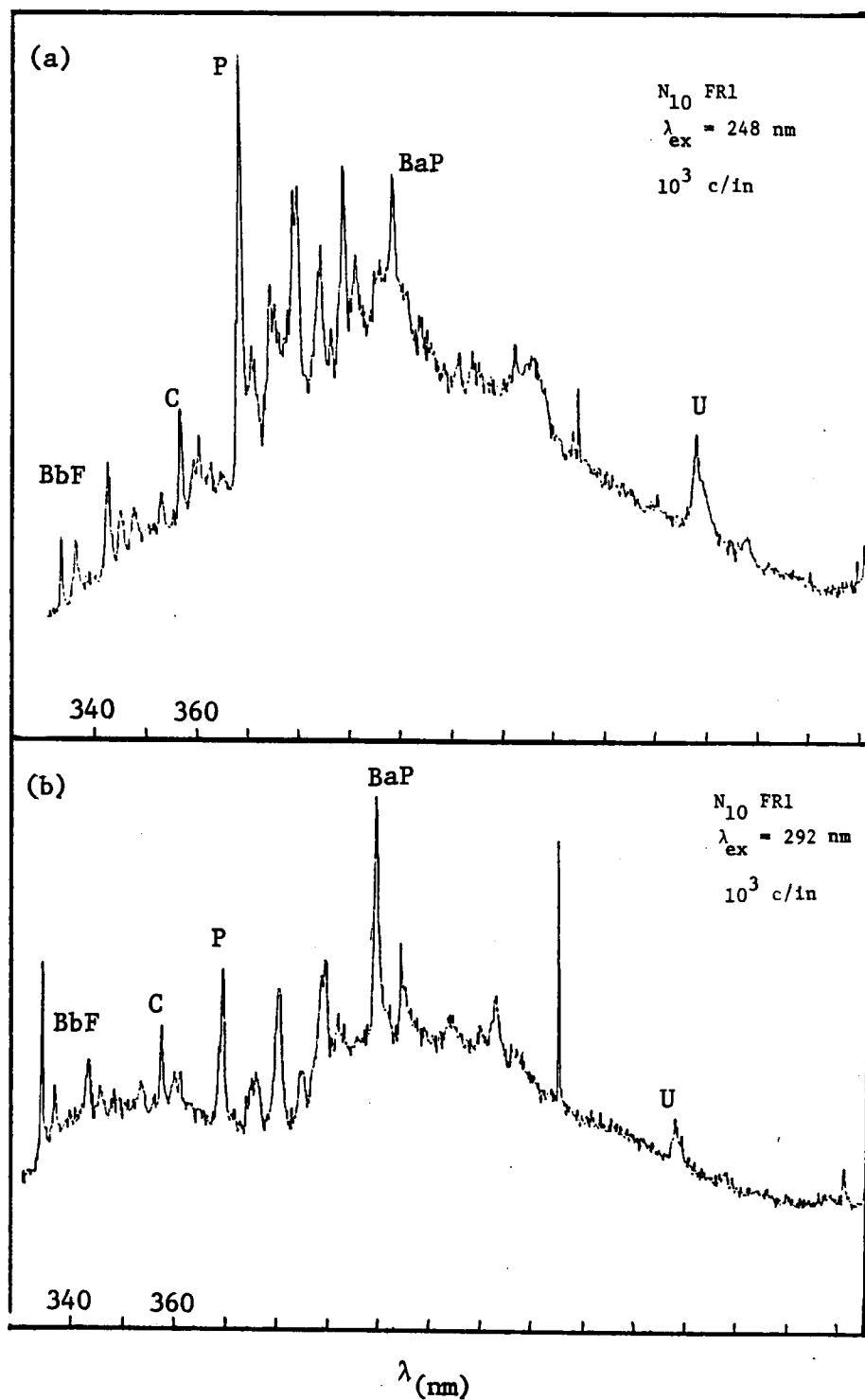


Figure 17. Fluorescence spectra of diluted PAH fraction of shale oil sample (dilution fraction is 10), (a) $\lambda_{ex} = 248$ nm, (b) $\lambda_{ex} = 292$ nm.

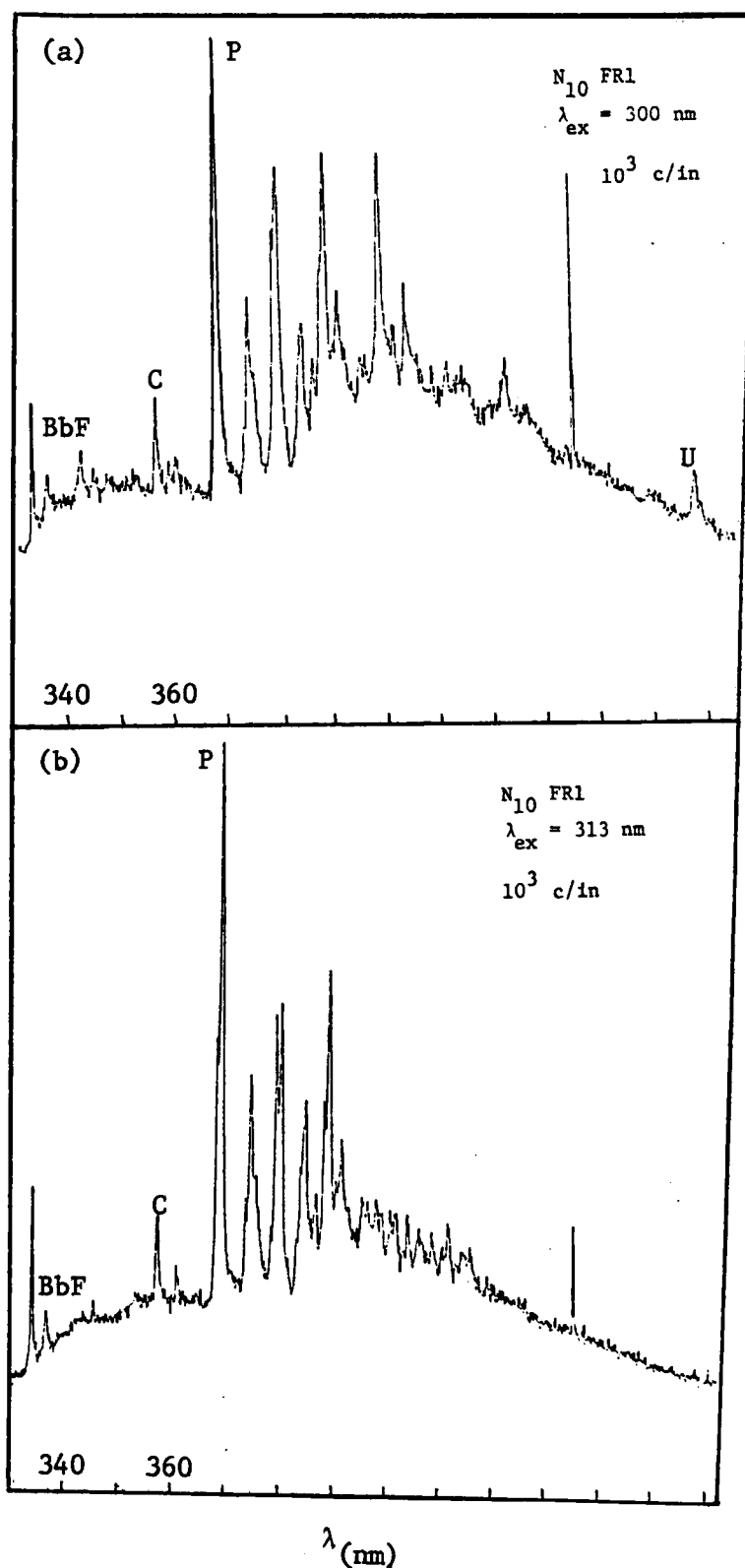


Figure 18. Fluorescence spectra of diluted PAH fraction of shale oil sample (dilution fraction is 10), (a) $\lambda_{ex} = 300 \text{ nm}$, (b) $\lambda_{ex} = 313 \text{ nm}$.

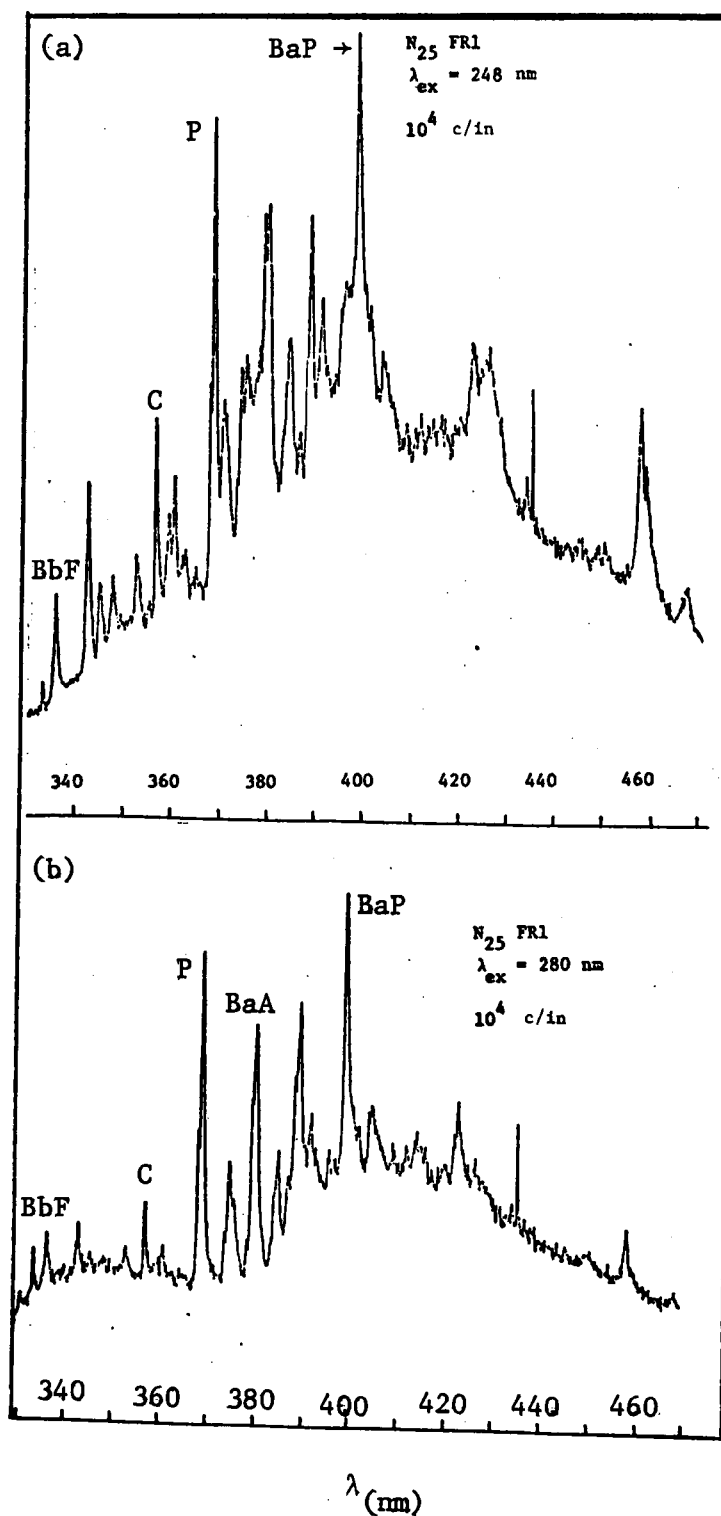


Figure 19. Fluorescence spectra of diluted PAH fraction of shale oil sample (dilution fraction is 25), (a) $\lambda_{ex} = 248$ nm, (b) $\lambda_{ex} = 280$ nm.

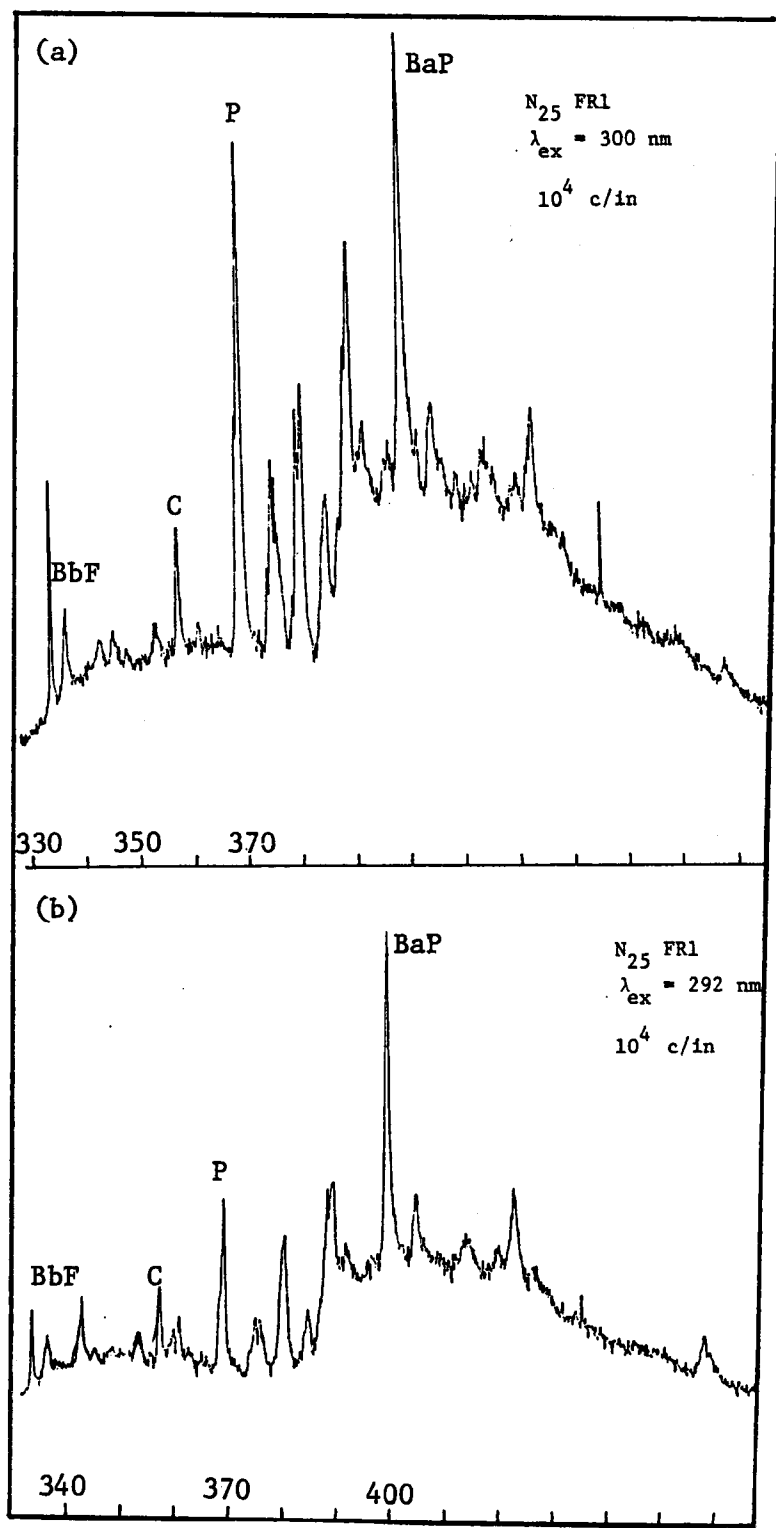


Figure 20. Fluorescence spectra of diluted PAH fraction of shale oil sample (dilution fraction is 25), (a) $\lambda_{\text{ex}} = 300 \text{ nm}$, (b) $\lambda_{\text{ex}} = 292 \text{ nm}$.

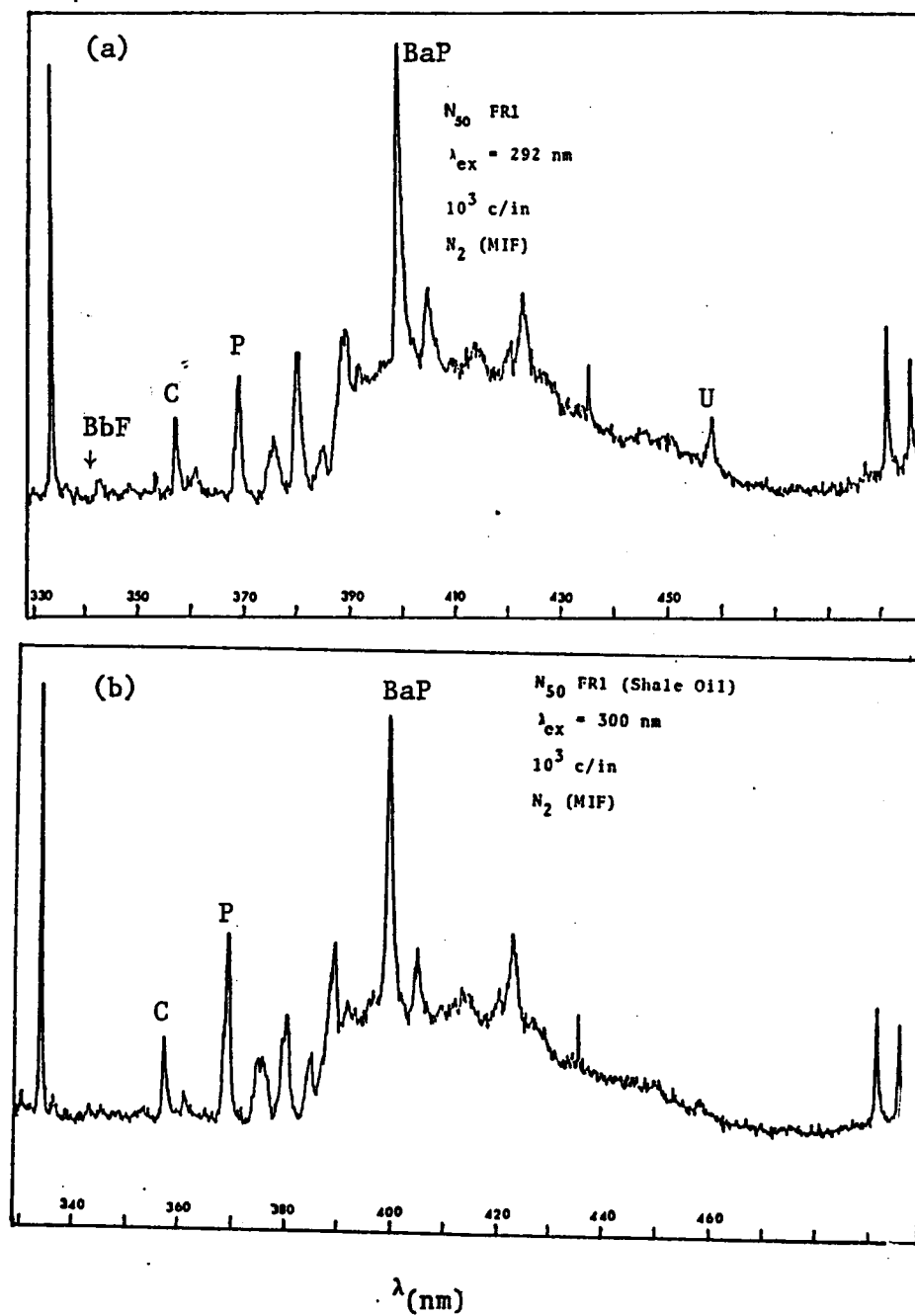


Figure 21. Fluorescence spectra of shale oil PAH fraction (N₅₀ dilution), (a) $\lambda_{ex} = 292$ nm, (b) $\lambda_{ex} = 300$ nm.

TABLE V
REPRODUCIBILITY OF RESULTS FOR BaP QUANTITATION BY MATRIX-ISOLATION FLUOROMETRY

CURVE	SLOPE	CORRELATION COEFFICIENT	BaP IN SHALE OIL SAMPLE (ppm)
I	0.783	0.987	24
II	0.787	0.990	25
III	0.804	0.992	28
IV	0.776	0.993	30
Mean	0.786	0.991	26.8
Standard Deviation	0.012	0.0026	2.75
RSD	1.51 %	0.26 %	10.3 %

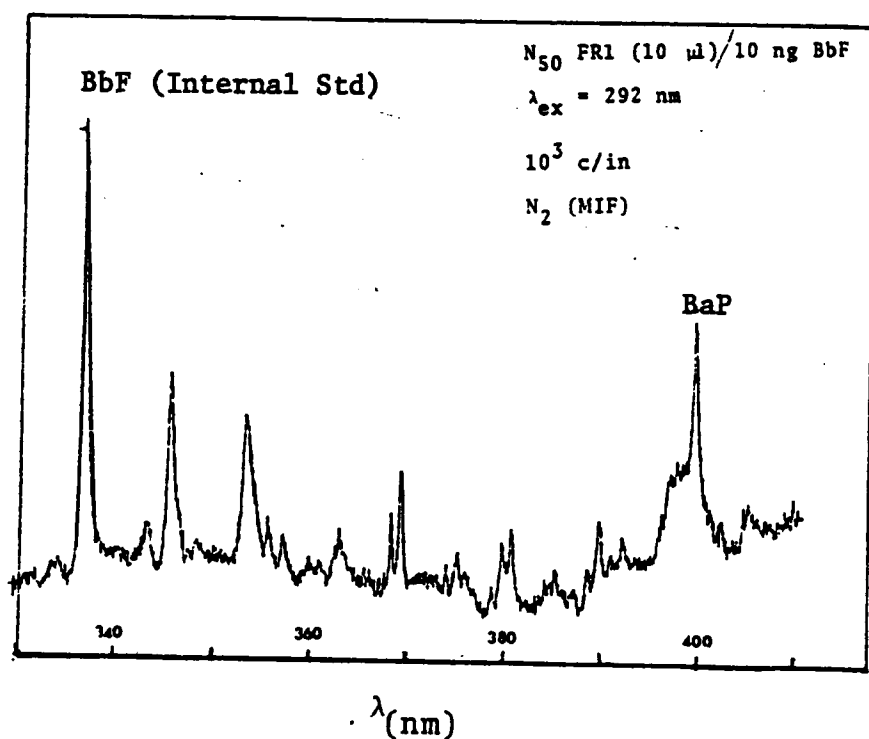


Figure 22. Fluorescence spectrum of 10 μl shale oil PAH fraction (N_{50} dilution) "spiked" with 10 ng BbF, internal standard.

of the 'spiked' PAH fraction. Table VI illustrates the results of BaP quantitation for the shale oil obtained in this work compared to those reported by the National Bureau of Standards (NBS). The degree of agreement of the present results with those reported by NBS is quite encouraging.

C. SUMMARY

High performance liquid chromatography and matrix-isolation fluorometry are viable analytical techniques for the separation and analysis of real samples. There are innumerable HPLC applications in the literature; this application was by no means the most elegant, but it appeared to accomplish its design, which was normal-phase class separation of a shale oil sample. Compared to some other class separation schemes for petroleum which have been reported in the literature,⁴¹⁻⁴⁶ HPLC provided a method for a useful and rapid separation scheme. The procedure which was devised required negligible sample preparation; it was rapid and efficient and it required a minimal number of steps. It was also reproducible.

Room-temperature solution fluorometry, HPLC and GC data were used to verify the separation procedure. The HPLC fraction which contained the PAHs was analyzed by matrix-isolation fluorometry, which provided extremely sensitive detection of fluorescent species.

Fast class separation by HPLC, followed by sensitive detection of specific sample components by matrix-isolation fluorometry, provide a very powerful tool for the analysis of specific PAHs in complex samples such as shale oils.

TABLE VI

COMPARISON OF RESULTS OF BaP QUANTITATION OF A SHALE OIL SAMPLE REPORTED BY NBS AND THIS WORK

METHOD OF ANALYSIS	BaP IN SHALE OIL
LC, "HPLC extraction" (NBS)	20 $\pm$ 3 ppm
GC-MS, "HPLC extraction" (NBS)	21 $\pm$ 5 ppm
GC-MS, acid/base extraction (NBS)	24 $\pm$ 2 ppm
Matrix-isolation fluorometry, HPLC separation	27 $\pm$ 3 ppm

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LIST OF REFERENCES

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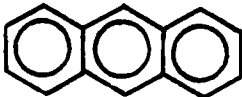
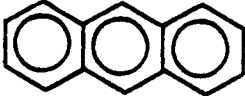
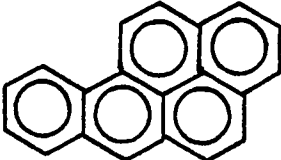
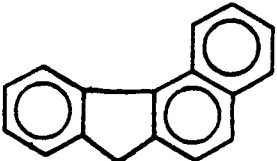
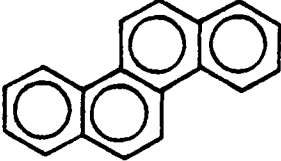
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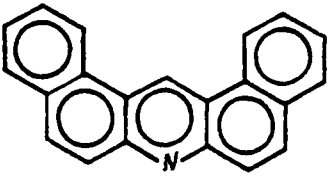
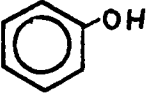
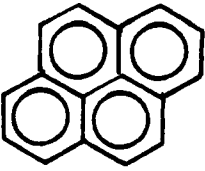
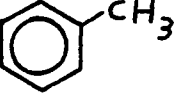
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APPENDIX

APPENDIX

STRUCTURE OF COMPOUNDS

COMPOUND	STRUCTURE
Anthracene	
Benz(a)anthracene	
Benzo(a)pyrene	
Benzo(b)fluorene	
Chrysene	

COMPOUND	STRUCTURE
Dibenz(a,j)acridine	
Fluorene	
Naphthalene	
Phenol	
Pyrene	
Toluene	

VITA

Sumaria Indra Mohan was born in Princes Town, Trinidad; she was the granddaughter of East Indian immigrants to that country. She graduated from high school (St. Stephen's) in 1973 with Cambridge G.C.E. "O" and "A" Level certificates. She then interned as a science teacher for one year at Open Bible High School, San Fernando. In 1974 she entered Morgan State University in Baltimore. During semester breaks she was employed by Blue Ridge Assembly in Asheville, N.C. (1975), Smith Kline & French in Philadelphia (1976) and Mobay Chemical in Pittsburgh (1977). She was elected to college honor societies and graduated in May of 1977 with a Bachelor of Science degree in Biology.

In the fall of 1977 she accepted a teaching assistantship at The University of Tennessee, Knoxville, and began studies in chemistry. Her research was supported by grants from the Electric Power Research Institute and the National Science Foundation. The Master of Science degree in chemistry was awarded in June of 1981.

The author was a student affiliate of the American Chemical Society and a member of the Association of Chemistry Graduate Students.

She married Paul H. Neill of Glenside, Pa., on December 20, 1980.