EPA-600/4-82-025

DETERMINATION OF POLYNUCLEAR AROMATIC HYDROCARBONS IN INDUSTRIAL AND MUNICIPAL WASTEWATERS

by

Paul E. Strup
Battelle Columbus Laboratories
Columbus, Ohio, 43201

Contract No. 68-03-2624

Project Officer

James E. Longbottom
Physical and Chemical Methods Branch
U.S. Environmental Protection Agency
Cincinnati, Ohio, 45268

ENVIRONMENTAL MONITORING AND SUPPORT LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OHIO 45268

DISCLAIMER

This report has been reviewed by the Environmental Monitoring and Support Laboratory, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the U.S. Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

FOREWARD

Environmental measurements are required to determine the quality of ambient waters and the character of waste effluents. The Environmental Monitoring and Support Laboratory-Cincinnati conducts research to:

- Develop and evaluate techniques to measure the presence and concentration of physical, chemical, and radiological pollutants in water, wastewater, bottom sediments, and solid waste.

- Investigate methods for the concentration, recovery, and identification of viruses, bacteria, and other microbiological organisms in water. Conduct studies to determine the responses of aquatic organisms to water quality.

- Conduct an Agency-wide quality assurance program to assure standardization and quality control of systems for monitoring water and wastewater.

Under provisions of the Clean Water Act, the Environmental Protection Agency is required to promulgate guidelines establishing test procedures for the analysis of pollutants. The Clean Water Act Amendments of 1977 emphasize the control of toxic pollutants and declare the 65 "priority" pollutants and classes of pollutants to be toxic under Section 307(a) of the Act. This report is one of a series that investigate the analytical behavior of selected priority pollutants and suggests a suitable test procedure for their measurement.

Dwight G. Ballinger
Director
Environmental Monitoring and Support Laboratory

ABSTRACT

A method for the determination of 16 polynuclear aromatic hydrocarbons (PAH) in wastewater was developed. This method, based on the use of high performance liquid chromatography with ultraviolet and fluorescence detection, is readily suited for detection of condensed ring aromatics in many types of aqueous samples. Aqueous sources included in this study are flyash wash water, municipal sewage, and industrial effluent samples.

Precision and accuracy of the method were estimated from the results of five wastewater samples spiked at levels between 0.1 and 250 ppb for the various PAH compounds. Recoveries were generally 85% or better from these wastewater sources.

Storage of several spiked wastewater samples for 0 and 7 days at various temperatures, pH and chlorine levels resulted in a matrix of recovery data for the various PAH species. This data indicated that the highest recovery for PAH in wastewater are obtained at a pH level near 7, in the absence of any chlorine with the analysis being completed as soon after collection as possible.

This report was submitted in fulfillment of Contract No. 68-03-2624 by Battelle Columbus Laboratories under the sponsorship of the U.S. Environmental Protection Agency. This report covers a period from November 1, 1977, to March 1, 1979, and work was completed as of March 1, 1979.

CONTENTS

FIGURES

TABLES

SECTION 1

INTRODUCTION

The objective of this program was to establish and verify procedures for the analysis of 16 priority pollutant PAHs in complex aqueous media. Data obtained during this study will be used to aid in the selection of appropriate test procedures which will ultimately be used by the EPA in monitoring water and wastewater pollutants. These procedures should employ simple sample treatment as well as common laboratory instrumental and analytical approaches.

Sixteen polynuclear aromatics were studied in this program with regard to solvent stability, extraction efficiency, and preservation. These 16 PAHs were: acenaphthene; benzo(ghi)perylene; fluorene; phenanthrene; dibenzo(ah)anthracene; indeno(1,2,3-cd)pyrene. These compounds were combined into two groups for simultaneous study, in order to simplify initial analyses.

Further studies were then conducted to evaluate the extraction and analytical procedures that had been developed during this initial phase. These studies were performed using wastewaters obtained from various sources.

SECTION 2

OBJECTIVE

The objective of this study was to develop an optimized analytical method for the determination of 16 priority pollutant PAH in aqueous media and to validate the method on a variety of aqueous effluents.

SECTION 3

TECHNICAL APPROACH

The successful completion of this program involved the fulfillment of certain directives set forth in the contract by EPA. An extensive literature review was first conducted to evaluate the previous work in the area. Subsequent work was directed toward determination and then full evaluation of an appropriate measurement technique, which best satisfied the requirements for sensitivity and selectivity, as well as the considerations of sample cost, i.e. equipment, time, and training, which would be needed for the method. The stability of the PAH compounds in water miscible solvents and their instability in chlorinated and unchlorinated buffered water at different pHs and storage temperatures were studied over the prescribed time periods. Extraction efficiency of two organic solvents was also studied for the standard compounds. The remainder of the program involved the study of the sample preparation and clean-up steps which would be necessary to eliminate sample interferences. The complete method was then applied to several representative wastewater samples and an assessment was made of the precision and accuracy of the complete procedure.

SECTION 4

LITERATURE REVIEW

A literature review was conducted to establish the most attractive approach to the analysis of PAH in water. Although there is a large volume of literature on the subject, for the purposes of this report we will present only a few of the most important references. Our discussions will focus on the methodologies used for preservation, extraction, cleanup, and determination of the PAH's.

Preservation of PAH in water has not apparently been studied in detail in any single investigation. In one report the fate of PAH in water was studied, with special emphasis on the effect of UV light (1). In this study benz(a)anthracene and benzo(a)pyrene were used as model compounds since they are among the most reactive PAH's. The conclusions reached by these authors include:

- The PAH's are primarily adsorbed on particulate in natural water samples.
- The PAH's are degraded to quinones when exposed to UV light above a certain threshold intensity.
- The degradation of PAH by UV light is not significantly affected by pH or ionic strength.

A second study (2) has evaluated the effect of chlorination on PAH stability. This study concluded that PAH's can be degraded by added chlorine, especially at low pH and at elevated temperature. At low temperature and neutral or high pH the effect of chlorination is greatly reduced.

There are several methods reported for extraction of PAH from water including solvent extraction (1-7), polyurethane foams (8), and XAD-2 resin (9). In general good recoveries have been obtained using all of these techniques, primarily because of the extremely hydrophobic nature of the PAH's. Solvents used for extraction include benzene (1), isooctane (3), and methylene chloride (2,4,6,7). At least one report (7) concluded methylene chloride was the solvent of choice because of its good extraction properties and ease of work up.

There have been two methods reported for cleanup of PAH extracts: a) solvent-solvent partition (4,7) and b) adsorption (column or thin layer) chromatography (3,4,8). One study (4) compared the recoveries using these two techniques and found them to be equivalent. The solvent partitioning method involves 1) extraction of the PAH's into isooctane,

partitioning of PAH's into DMSO or nitromethane, and 3) dilution of the extract with water and back extraction into isoctane. Absorption chromatography has been performed using alumina (3), acetylated cellulose (8), and silica gel (4). Silica gel and alumina appear to be the best for this application since they are readily available in pure form and can be produced with uniform activity.

For the determination of PAH's GC/FID (1,2,8,9), GCMS (3,4,6), TLC (7,10), and HPLC (11,12) have been employed. TLC does not appear to have sufficient resolution to separate all sixteen if the priority pollutant PAH's and GCMS is considered to expensive for the purposes of this study. Therefore the candidate methods appear to be GC/FID and HPLC with a fluorescence detector. Although no direct comparisions of these two techniques for PAH analysis were found it appears that HPLC with fluorescence detection is more sensitive than GC/FID whereas GC/FID may be somewhat more readily available. The largest unknown about both techniques is the degree of resolution which can be achieved and whether or not all sixteen PAH's can be resolved. Separation of all sixteen PAH's in a single chromatographic run has not been previously reported. A comparison of some of the characteriztics of these two techniques is given below.

GC	HPLC
• Use of capillary column 20-30 meters will give 50-60K plates	• Packed columns (reverse or normal phase)
• Commercial coatings limited	• Commercial coating limited
• FID-universal detector	• UV or Fluorescence detector selective
• May require extensive sample clean-up	• May require less extensive clean-up
• Sensitivity advantage for lower MW compounds	• Greater sensitivity for selected compounds UV and/or fluorescence
• FID-linear response over wide range of concentrations	• UV-linear response over wide range of concentrations
• Possible one analysis for all PAHs	• May require several fractionations for all PAHs
• PAH identification by retention time only (with ready facility for GC-MS)	• PAH identification by retention time plus UV absorbance or fluorescence
• Automation easy	• Automation more difficult
• Investment low ($10K)	• Investment medium ($30K)
• Sample destroyed	• Sample not destroyed

SECTION 5

EXPERIMENTAL PROCEDURES

ANALYTICAL METHODS EVALUATION

The objective of this phase of the study was to ascertain which of the two chromatographic techniques (GC or HPLC) is most appropriate for the determination of the sixteen priority pollutant PAH's.

Gas Chromatography (GC)

Glass capillary gas chromatography was investigated as an analytical technique for the 16 PAH's. Various glass capillary columns were investigated using the following GC conditions:

Instrument - Hewlett-Packard 5730A
Detector - FID
Detection temperature - 300°C
Injector temperature - 300°C
Injection mode - 2 µl split 10:1
Column temperature - Group A - 2 minutes isothermal at 40°C and then 40° - 260°C at 8°/min.
Group B - 2 minutes isothermal at 80°C and then 80° - 260°C at 8°/min.

A spectra-Physics 4000 chromatographic data system was used to determine peak areas.

Detection limits for each compound were determined, based on a signal to noise ratio of 10:1.

High Performance Liquid Chromatography (HPLC)

Both normal and reversed phase forms of HPLC were investigated for the separation of PAH's. For detection of the PAH's both fluorescence (at various excitation and emission wavelength settings) and UV (fixed at 254 nm) were evaluated.

Stationary phases were packed in 4.6 m.m. I.D. x 25 cm stainless steel columns and were either purchased commerically or slurry packed using conventional techniques.

Solvents were filtered through .22 µM Millipore filters and degassed by boiling for a few minutes prior to use on the HPLC. The chromatographic

apparatus employed was as follows:

Solvent delivery system - Altex 420 microprocessor controlling dual Altex 100A pumps.

Injector - Rheodyne 7120 with a 20 µl loop.
Detectors - Fluorescence - Schoeffel FS970
UV-LDC UV-3 at 254 nm.
Data system - Hewlett Packard Model 3385A.

SOLVENT STABILITY STUDIES

The purpose of this phase of the study was to determine which water miscible organic solvents are best for the preparation of standard solutions of the PAH's for QA/QC purposes.

Solvent stability studies were conducted using DMSO and acetone as the solvents of choice. These solvents were selected because they are both water miscible and readily dissolve PAH.

Four ml of acetone and DMSO solutions of PAHs at a concentration of 20 ppm were sealed in glass ampules. The PAHs were separated into 2 groups to facilitate the separation and quantitative analysis. These groups were: (A) naphthalene, anthracene, pyrene, chrysene, acenaphthene and dibenzo(ah)anthracene; and (B) phenanthrene, fluorene, benz(a)anthracene, fluoranthene, benz(a)pyrene, and acenaphthylene.

Benzo (k+b)fluoranthenes were not included in these studies due to an insufficient quantity of these standards. Because of supplier backlog, indeno(1,2,3-cd)pyrene and benzo(ghi)perylene were not incorporated into these initial groups and were analyzed independently.

The sealed ampules were kept in the dark, at room temperature for 30, 60, and 90 day time periods. Following chromatographic calibration, 3 ampules from each group were opened at the appropriate time interval. Analysis of PAHs in acetone was conducted using capillary GC using the conditions described previously.

Because of solvent tailing, analysis of PAHs in DMSO was performed by HPLC using the following conditions.

Column - 250 x 4.6 mm Spherisorb ODS 5µ particle diameter
Gradient - 50% acetonitrile in water to 100% in Acetonitrile in 50 minutes.
Flow - 1.0 ml/min
Solvent delivery system - Altex 420 microprocessor controlling dual Altex 100A pumps.
Injector - Rheodyne 7120 with a 20 µl loop
Detector - UV-LDC UV-3 at 254 nm

Quantitation for both GC and HPLC was conducted by peak area integration using either a Spectra Physics 4000 or Hewlett-Packard 8085A integrator. Calibration curves were prepared by appropriate dilution of a standard stock solution which were run at suitable sensitivity levels. Peak areas of interest were compared to the calibration curves and reported as percent recovery.

EXTRACTION STUDIES

The purpose of this phase of the study was to determine the extraction efficiencies of the various PAH's under a variety of conditions, in order to select an optimized extraction protocol.

Extraction studies were conducted to evaluate the extraction efficiencies of methylene chloride and 15 percent methylene chloride in hexane for PAH's in water at pHs 2,7, and 10.

One ml of standard solution in acetone was added to 500 ml of appropriately buffered water to yield an approximate concentration of 40 ppb for each of the PAH in a 1000 ml separatory funnel. Certified pH 7 and pH 10 buffers were purchased from VWR. The buffers used are as follows:

pH 2 - NaH_2PO_4 in phosphoric acid, 0.05 M Ionic Strength

pH 7 - sodium and potassium phosphate, 0.05 M Ionic Strength

pH 10 - sodium borate and sodium carbonate, 0.05M Ionic Strength

PAH's were extracted three times using 30 ml portions of the appropriate solvent. The extract was then dried with $MgSO_4$, filtered, and the $MgSO_4$ washed with an additional 30 ml of solvent. The resultant extract was reduced in volume to one ml using micro Kuderna-Danish evaporation. Extractions were done in triplicate for pH 2 and 10 and quadruplicity for pH 7.

Triplicate analysis on each of the above extracts was conducted using a capillary column GC equipped with a Hewlett-Packard 7671A automatic sampler.

Benzo(b)fluoranthene was not included in these studies due to an insufficient quantity of this standard.

PRESERVATION STUDIES

The purpose of this phase of the study was to examine the effects of sample storage under a variety of pH, temperature, and oxidant conditions in order to develop protocols for sampling and sample storage.

Preservation studies were conducted to evaluate the stability of PAH's in water for selected conditions. These conditions were: pH 2, 7, and 10; chlorine 0 and 2 ppm; temperature, ambient and 4°C. The

three pH levels were achieved using the buffers described in the Extraction Section. The two ppm chlorine level was achieved by spiking the sample with 2 ml of a solution containing 1500 ppm of calcium hypochlorite.

One ml of standard PAH solution in acetone was added to 500 ml distilled water, (to achieve a concentration of 40 ppb for each PAH) which had previously been buffered and/or chlorinated in accordance with the above matrix. Each matrix was performed in two groups as in the previous studies with the addition of benzo(k)fluoranthene and indeno(1,2,3-cd)-pyrene to Group A and benzo(ghi)perylene to Group B. All matrix weries were stored in the dark for a period of 7 days; one series at ambient temperature, the other at 4°C. Each condition was run in duplicate.

Following the 7 day storage period, each solution was extracted with methylene chloride as described in the Extraction Studies section. The storage bottle was also rinsed with each 30 ml portion of methylene chloride prior to being used for extraction in the separatory funnel. Each extract was then dried, and reduced in volume as described above. Duplicate injections were made onto a capillary column GC equipped with a Hewlett-Packard 7671A automatic sampler. Quantitation was performed by peak area integration using a Spectra Physics 4000 integrator. Calibrations were performed every 24 hours with periodic calibration checks at four run intervals.

LC CLEAN-UP STUDIES

The purpose of this phase of the study was to evaluate several clean-up techniques in order to determine which technique gives best recoveries for the various PAH's.

Recoveries of PAH's from two LC clean-up procedures were investigated. Each LC separation scheme was performed by applying 0.5 ml of a 200 ppm cyclohexane solution of representative PAH's. The PAH's in solution were chosen on the basis of representative ring number and relative elution volumes. These PAH's were: naphthalene, anthracene, fluoranthene, chrysene, and dibenz(a,h)anthracene.

The alumina LC column was prepared by placing 10 g of alumina (3% H_2O w/w) in a 25 x 1 cm column, and wetting with 15 ml pentane. As the pentane reached the top of the alumina, 3/4" of Na_2SO_4 was added. The column was washed with 20 ml of methylene chloride and then reconditioned with 20 ml of pentane. The PAH extract is then placed on the head of the column and eluted with 25 ml of pentane and 25 ml of methylene chloride. The latter methylene chloride fraction contained the PAH's. The LC scheme is presented in Figure 1.

The silica gel LC column was prepared by slurry packing 10 g of silica gel (Davidson Grade 923, 100-200 mesh) with methylene chloride into a 1 x 25 cm LC column. The column is then washed with 40 ml of pentane. The sample extract is then placed on the head of the silica

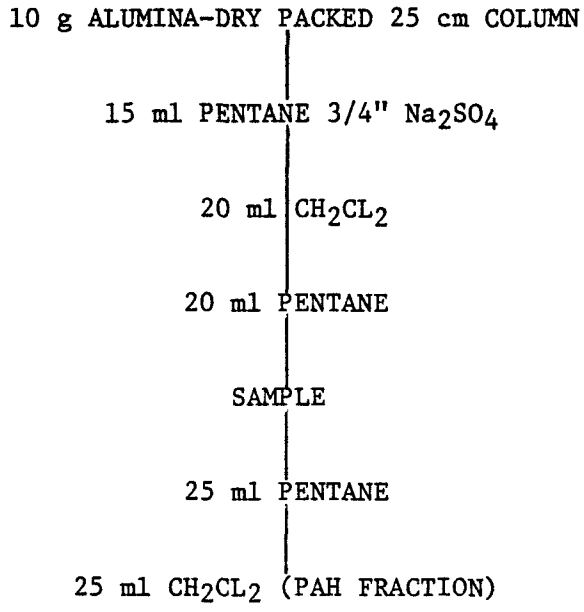


Figure 1. Alumina LC clean-up scheme used for PAH.

gel and eluted with 25 ml of pentane and 25 ml of 40% methylene chloride in pentane. This latter fraction contained the PAH's of interest. This LC scheme is presented in Figure 2. After allowing the first 25 ml of pentane to pass through the LC column, successive 10 ml fractions were collected and analyzed by HPLC using UV detection at 254 nm. No volume reduction was performed since the initial concentration of PNAs was chosen to eliminate this step.

WASTEWATER STUDIES

The purpose of this phase of the study was to evaluate the analytical protocols, developed on the basis of information collected in earlier phases of this study, on actual wastewater samples, and to make modifications as required.

Based on our extraction efficiency studies the following procedure was initially employed for the analysis of actual wastewater samples as supplied by EPA as well as samples collected by our laboratories.

- Extract immediately three 1 liter aliquots of each wastewater using 3 x 60 ml methylene chloride.
- Spike six 1 liter aliquots of each wastewater with PAH compounds prior to extraction, with 3 aliquots being stored for 7 days at 4°C.
- Pour extract through drying column containing sodium sulfate.
- Reduce volume of extract to 1.0 ml using Kuderna - Danish concentrator apparatus.
- Solvent exchange with cyclohexane and perform silica gel clean-up technique (Figure 2).
- Concentrate the PAH fraction to 2-3 ml using Kuderna - Danish concentrator apparatus.
- Solvent exchange with acetonitrile and adjust the extract volume to 1.0 ml.
- Analyze by injecting 5 µl into the HPLC equipped with an HC-ODS column and fluorescence detector at λex 280 and λem >389.

Quantitation was performed by comparing the peak areas for the individual PAH species to those of a standard made up to the concentration representing 100% recovery.

Following discussions with EPA personnel the extraction procedure was modified so that all concentration steps were performed using a

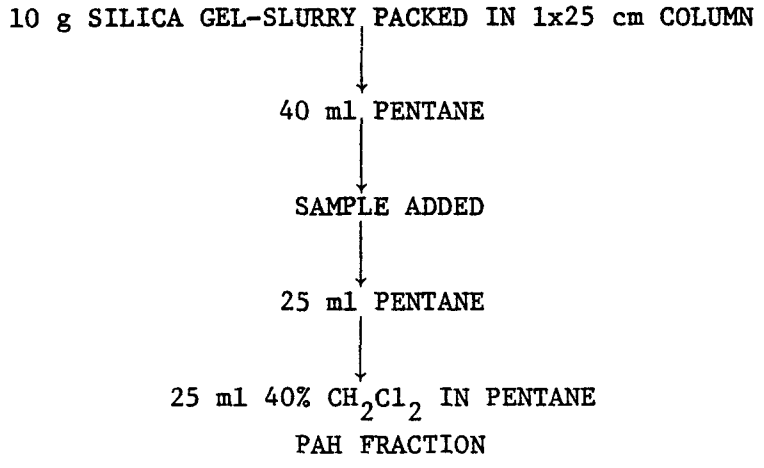


Figure 2. Silica Gel LC clean-up scheme used for PAH.

hot water bath rather than a tube heater. Also a 254 nm UV detector was coupled to the fluorescence detector to achieve lower detection limits for naphthalene and acenaphthylene. The revised procedures are described in detail in Appendix A.

The following water samples were selected for analysis:

- A plastics manufacturing industry (Samples supplied by EPA-Source Unknown).
- Raw municipal sewage water (Columbus, Ohio).
- Treated municipal sewage water prior to chlorination (Columbus, Ohio).
- Flyash Wash from coal fired power plant (Columbus, Ohio).
- Flyash settling pond prior to runoff (Columbus, Ohio).

All wastewater samples were collected by our laboratories except the plastics manufacturing industry wastewater which was supplied by EPA.

Extractions were conducted immediately on three 1 liter aliquots of each wastewater as received. In addition, six 1 liter aliquots were spiked with PAH at a level at least 5 times background prior to extraction, 3 aliquots being stored for 7 days at 4°C.

SECTION 6

RESULTS AND DISCUSSION

ANALYTICAL METHODS EVALUATION

Gas Chromatography

Experimental GC conditions were based on our considerable experience with analysis of PAHs utilizing capillary columns. Although PAH separation in this study was achieved using an SE-30 capillary column, equal resolution has been achieved using capillary columns coated with SP-2100, OV-101, and SE-54. Examples of these separations are shown in Figures 3 and 4, with retention times given in Table 1.

The conditions used for GC analysis are as follows:

30 meter SE-30 glass capillary
FID detector - 300°C
Injector - 200°C
2 µl split injection 10:1 at 40°C Group A: 80°C Group B
Isothermal for 2 min then programmed 8°C/min to 260°C

Instrument detection limits for the various PAHs, using capillary GC, are listed in Table 3. Due to the rather poor peak shape, and resulting poor detection limits and resolution, for the high molecular weight PAHs (e.g. indeno-pyrene) capillary column GC was not pursued further as an analytical approach. However, use of specially coated capillary columns could reduce these problems and for the lower molecular PNAs (e.g. napthalene, anthracene, etc.) GC is probably a very good approach.

High Performance Liquid Chromatography

Normal and reverse phase HPLC were investigated for the separation of the PAHs of interest. There is much in the literature describing the separation of a limited number of PAH using reverse phase HPLC (11,12); however, no references could be located on the separation of all 16 PAH of interest using a single stationary phase.

Initial HPLC reverse phase separations were conducted using a 250 x 4.6 mm Spherisorb ODS 5 µ column using a 75-100% MeOH in water gradient elution in 30 min. This gradient gave acceptable resolution for PAHs in Group A but failed to separate fluorene and phenanthrene in Group B. Although using a longer gradient elution time (i.e. 40 min)

TABLE 1. GC CAPILLARY COLUMN RETENTION TIMES FOR PAH ON 30 METER SE-30

Group A		Group B	
Compound	RT (min)*	Compound	RT (min)*
Napthalene	9.53	Acenaphthylene	9.65
Acenaphthene	14.30	Fluorene	11.51
Anthracene	20.70	Phenanthrene	13.95
Pyrene	21.98	Fluoranthene	17.21
Cyrysene	25.81	Benz(a)anthracene	21.40
Benz(a)fluoranthene	30.93	Benz(a)pyrene	28.37
Indeno(1,2,3-cd)pyrene	43.49	Benz(ghi)perylene	42.10
Dibenz(a,h)anthracene	43.84		

* Elution conditions for Group A and B were different so that retention times cannot be directly compared.

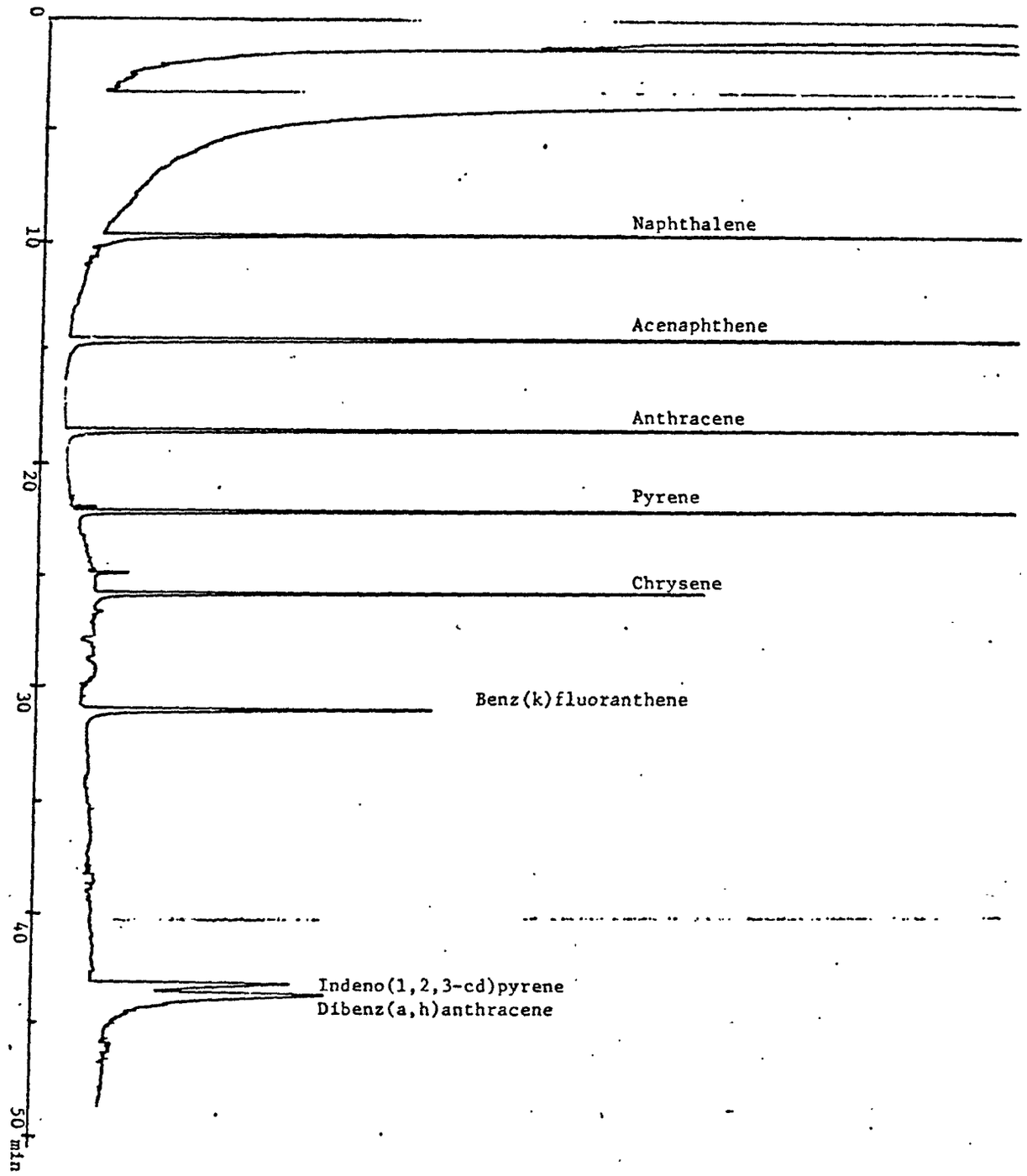


Figure 3. GC capillary column separation of Group A PAH.

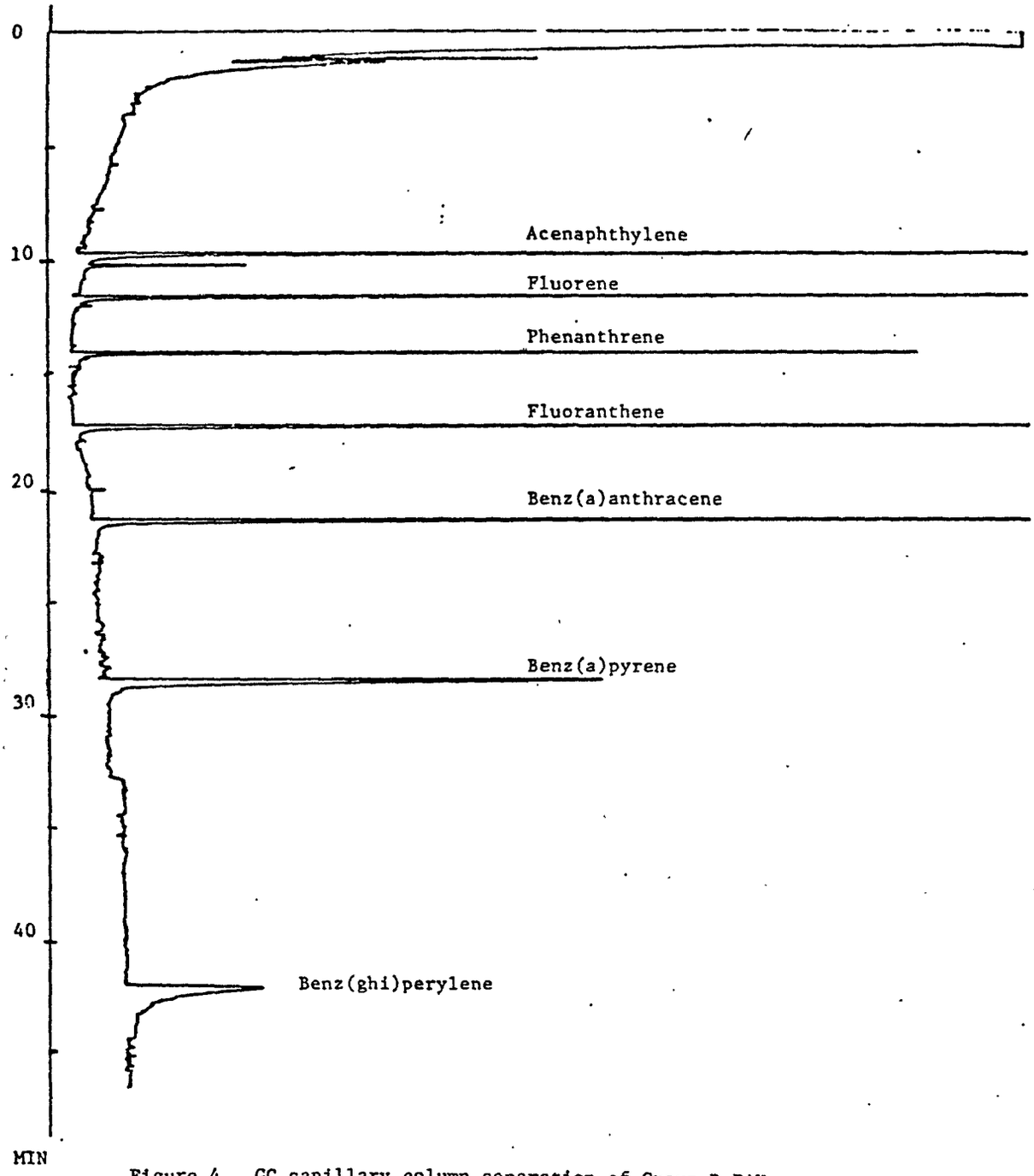


Figure 4. GC capillary column separation of Group B PAH.

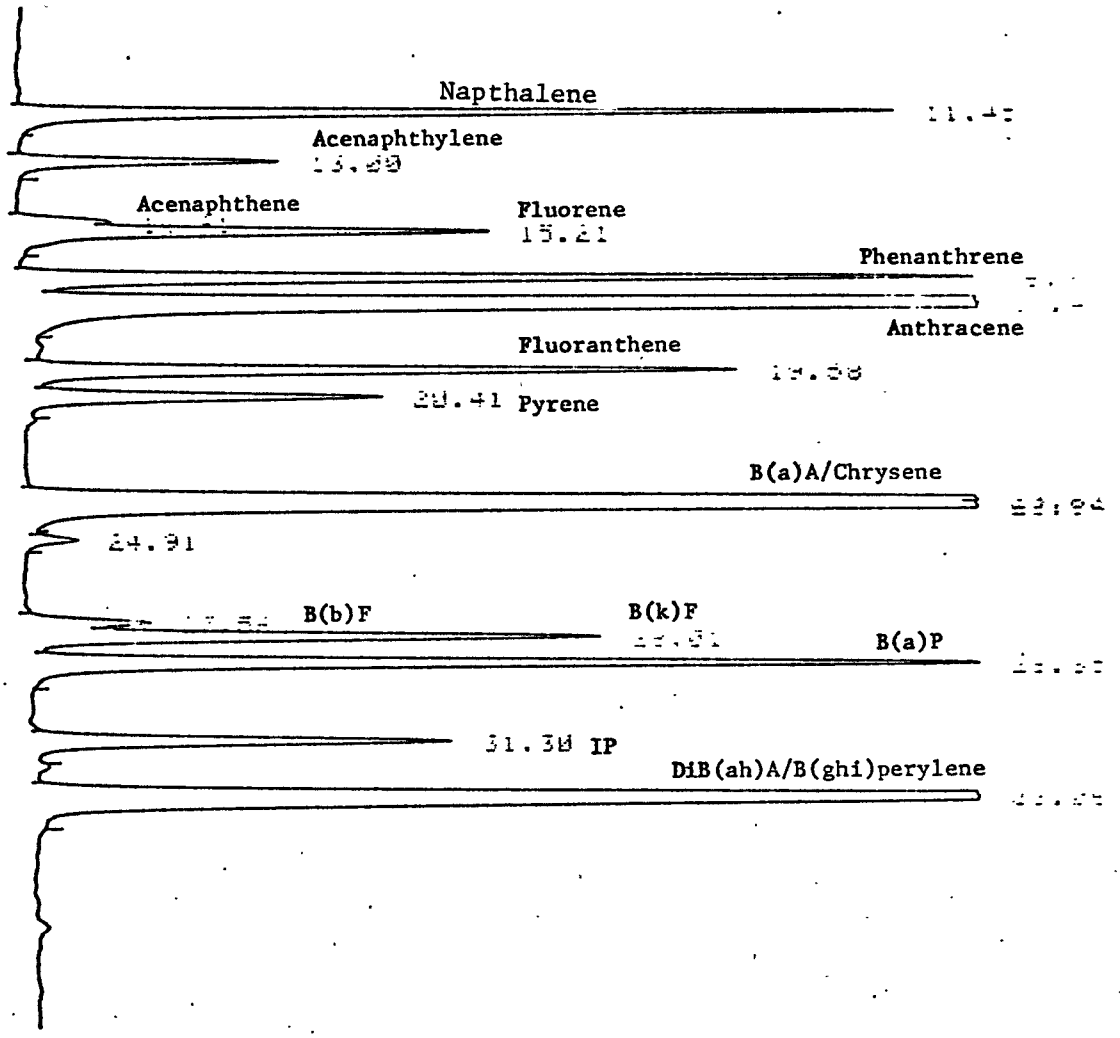


Figure 5. Chromatogram of HPLC separation of 16 PNA standards.

achieved this separation, acetonitrile in water proved to be more selective and gave a flatter base line.

Moderate success on separation of all 16 PAHs was achieved using a gradient elution of 50% acetonitrile to 100% acetonitrile in 50 minutes. An example of this separation is shown in Figure 5. However, the important isomer pair, benz(a)anthracene/chrysene as well as dibenz(a,h)-anthracene/benz(ghi)perylene, are not completely resolved. This latter pair could be resolved using an isocratic step program, but these conditions still left benz(a)anthracene and chrysene unresolved.

Several normal phases for the separation of PAH were investigated. These phases included Lichrosorb Si60, alumina, and Lichrosorb NH_2. Hexane was the mobile phase in all cases. The results of these studies indicated that there were not enough efficiency or selectivity to separate all 16 PAH.

Further investigation of various reverse phase columns indicated that the separation of all 16 PAHs could be achieved using a Perkin-Elmer 10 μ, 0.26 x 25 cm HC-ODS column. Figure 6 shows the separation of all 16 consent decree PAHs using a single stationary phase. The conditions used for this separation were 40% acetonitrile in water isocratic for 5 minutes followed by a gradient elution from 40-100% acetonitrile in 25 minutes at a flow rate of 0.5 ml/min. Fluorescence detection was used with excitation at λex 280 nm and a cut off filter of λem >389 nm. PAH retention times using the Spherisorb and HC-ODS columns are given in Table 3.

Sensitivity

Since all 16 PAH compounds can be resolved using the Perkin-Elmer HC-ODS reverse phase column, it is possible to optimize a fluorescence or UV detector for the best signal to noise ratio for each compound. Fluorescence at λex 280 nm, λem >389 nm gives good sensitivity for most of the larger ring PAH compounds but does not have good sensitivity for the smaller ring compounds like naphthalene and acenaphthylene.

Although these studies were carried out using fluorescence at λex 280 nm, λem >389 nm, samples were spiked at levels approximately 25 times the minimum detection limit (i.e. 25 x the detection limit in ng on column assuming a 1000:1 concentration factor and 5 microliter injection volume), making the choice of wavelength setting of little consequence. However, for increased sensitivity for the smaller ring PAH compounds a UV detector at 254 nm can be placed in series with the fluorescence detector. Sensitivities obtained using these wavelengths are listed in Table 2. Detector response was found to be linear to 25 x these sensitivities in terms of nanograms on column which covered the range in which subsequent samples were spiked.

Details of the recommended analytical procedure for the analysis of PAH in industrial wastewater, based on the results of these studies is

TABLE 2. PAH DETECTION LIMITS

Compound	Capillary GC FID 10:1 split (ng)	UV 254 (ng)	Fluorescence λex 280, λem >389 (ng)
Naphthalene	1.0	2.5	20.0
Acenaphthylene	1.5	5.0	100.0
Acenaphthene	.5	3.0	4.0
Fluorene	2.0	0.5	2.0
Phenanthrene	2.0	0.25	1.2
Anthracene	2.0	0.10	1.5
Fluoranthene	1.0	0.50	0.05
Pyrene	2.0	0.10	0.05
Benz(a)anthracene	2.0	0.20	0.04
Chrysene	4.0	0.20	0.5
Benz(b)fluoranthene	---	1.0	0.04
Benz(k)fluoranthene	6.0	0.30	0.04
Benz(a)pyrene	3.0	0.25	0.04
Dibenz(a,h)anthracene	10.0	1.0	0.08
Benz(ghi)perylene	---	0.75	0.2
Indeno(1,2,3-cd)pyrene	10.0	0.30	0.1

Minimum detectable quantity in nanograms injected at a signal to noise ratio of 10:1

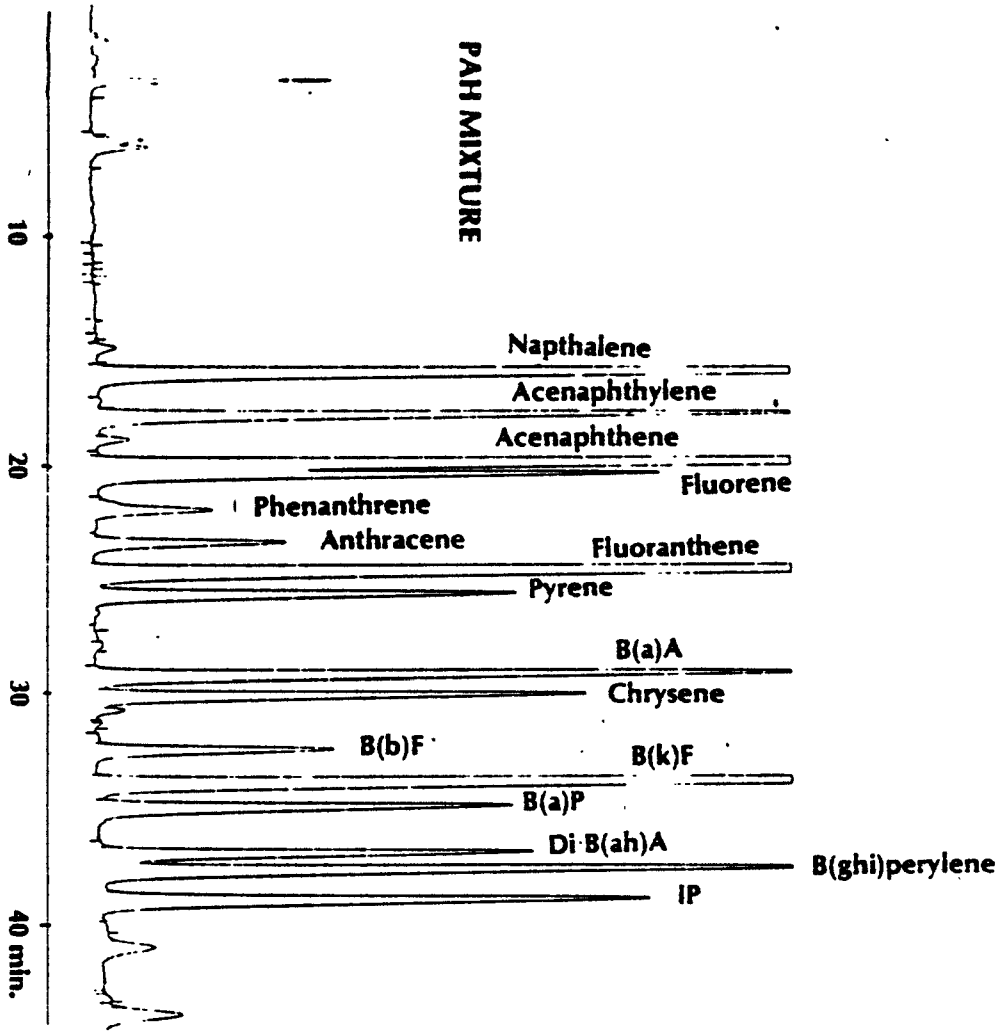


Figure 6. HPLC separation of 16 PAH standards on HC-ODS.

TABLE 3. HPLC RETENTION TIMES FOR PAH ON REVERSE PHASE COLUMNS

Compound	Column: Spherisorb ODS 4.6 x 250 nm Gradient: Acetonitrile/H_2O 50%-100% 50 min Flow: 1 ml/min Retention time (Minutes)	Column: Perkin-Elmer HC-ODS 2.6 x 250 mm Gradient: Acentonitrile/H_2O in 40%-5 min 40%-100%-25 min Flow: 0.5 ml/min Retention time (Minutes)
Naphthalene	11.45	16.17
Acenaphthylene	13.00	18.10
Acenaphthene	14.91	20.14
Fluorene	15.21	20.89
Phenanthrene	16.66	22.32
Anthracene	17.46	23.78
Fluoranthene	19.58	25.00
Pyrene	20.41	25.94
Benz(a)anthracene	23.63	29.26
Chrysene	23.63	30.14
Benz(b)fluoranthene	27.56	32.44
Benz(k)fluoranthene	28.01	33.91
Benz(a)pyrene	28.85	34.95
Dibenz(a,h)anthracene	33.06	37.06
Benz(ghi)perylene	33.06	37.82
Indeno(1,2,3-cd)pyrene	31.30	39.21

given in Appendix A. Excitation, emission, and adsorption spectra for the 16 PAH compounds of interest are shown in Appendix B for use as reference spectra as well as use in evaluation for determining optimum detection wavelengths.

Variance of Detector Response

The variance of the fluorescence detector response was checked to determine if there was any affect on detector response (e.g. dissolved oxygen in the HPLC mobile phases) over a period of time.

Spectral grade acetonitrile and high purity water were passed through a .22 μM Millipore filter prior to boiling each mobile phase for a period of 5 minutes. After allowing them to cool, each mobile phase was poured into its respective container and the HC-ODS column equilibrated by going through one gradient elution with no injection. Following equilibration, a normal separation was performed by injecting a PAH solution. A further PAH standard separation was performed at the end of the day. These standard analyses continued for a period of 3 days which is approximately the duration before the mobile phases need to be replenished. The peak areas for each PAH component were averaged for the six runs and the standard deviation from the average determined. The results which are listed in Table 4 indicate that over a period of 3 days, there is only a slight variance in the detector response ; and since recoveries are calculated using daily calibrations, these variances would not substantially affect analytical results.

Variance in Retention Times

Absolute retention times for individual PAH compounds was found to vary somewhat day to day. Although a microprocessor was used to control the entire gradient and reconditioning of the column prior to injection, these variances nevertheless occurred.

The initial gradient concentration of 40% acetonitrile in water was held for a period of 10 minutes to obtain column equilibrium prior to injection. Holding this concentration for 30 minutes before injection yielded no decrease in the variance. Thus, for shorter total analysis time a 10 minute reconditioning time was used prior to sample injection. prior to sample injection.

The variances which were obtained on the six PAH standard solutions over a period of 3 days are listed in Table 5.

SOLVENT STABILITY STUDIES

Results of the solvent stability studies are shown in Table 6. An ANOVA analysis of this data indicates that acetone and DMSO are virtually equally suitable solvents for the PAHs studied for a time period of 90 days. ANOVA analysis of the data indicated a significant difference favoring acetone over DMSO for fluoranthene however examination

TABLE 4. VARIATION IN FLUORESCENCE DETECTOR RESPONSE

Compound	Relative percent standard deviation λex 280/λem >389 % (n = 6)*
Naphthalene	±3
Acenaphthylene	±3
Acenaphthene	±3
Fluorene	±3
Phenanthrene	±2
Anthracene	±1
Fluoranthene	±2
Pyrene	±2
Benz(a)anthracene	±1
Chrysene	±1
Benz(b)fluoranthene	±1
Benz(k)fluoranthene	±3
Benz(a)pyrene	±7
Dibenz(a,h)anthracene	±2
Benz(ghi)perylene	±5
Indeno(1,2,3-cd)pyrene	±2

* Over a 3 day period.

TABLE 5. VARIANCE OF RETENTION TIME

Compound	Average retention time Time (min)	Standard deviation (n = 6) Time (min)
Naphthalene	16.17	±0.31
Acenaphthylene	18.10	±0.27
Acenaphthene	20.14	±0.50
Fluorene	20.89	±0.24
Phenanthrene	22.32	±0.24
Anthracene	23.78	±0.23
Fluoranthene	25.00	±0.24
Pyrene	25.94	±0.24
Benz(a)anthracene	29.26	±0.23
Chrysene	30.14	±0.22
Benz(b)fluoranthene	32.44	±0.20
Benz(k)fluoranthene	33.91	±0.19
Benz(a)pyrene	34.95	±0.20
Dibenz(a,h)anthracene	37.06	±0.18
Benz(ghi)perylene	37.82	±0.19
Indeno(1,2,3-cd)pyrene	39.21	±0.18

TABLE 6. SOLVENT STABILITY STUDIES

Compound	DMSO					Acetone				
	0 day	30 day	60 day	90 day	Average	0 day	30 day	60 day	90 day	Average
Naphthalene	100±5*	99±5	101±2	95±2	99±4	100±1	96±9	99±2	104±1	100±4
Acenaphthene	98±6	97±7	104±5	100±3	100±5	100±0.6	95±6	98±2	102±3	99±4
Anthracene	100±0.6	97±0.6	99±1	97±0.6	98±2	100±2	98±5	96±2	100±5	98±4
Pyrene	100±0.6	99±2	99±0.6	100±2	100±1	99±2	99±5	100±2	98±3	99±3
Chrysene	100±0	97±0.6	99±0	99±2	99±1	100±4	99±6	100±2	104±0.6	101±4
Indeno(1,2,3-cd)pyrene	101±3	99±2	97±0.6	99±3	99±2	99±2	99±2	97±6	98±5	98±4
Dibenz(a,h)anthracene	101±2	99±4	101±3	100±3	100±2	98±3	95±6	95±9	98±7	97±6
Acenaphthylene	99±2	101±3	98±2	102±2	100±3	100±4	100±4	103±2	104±2	102±3
Fluorene	100±1	101±2	101±2	102±1	101±2	100±7	102±2	104±1	105±3	103±4
Phenanthrene	100±1	100±3	98±2	102±1	100±2	100±3	103±4	102±1	101±3	102±3
Fluoranthene	100±1	98±3	95±2	100±1	98±3	100±3	101±2	100±0.6	101±4	101±2
Benz(a)anthracene	100±0	100±3	96±2	102±0.6	100±3	100±4	103±3	98±5	95±2	99±5
Benz(a)pyrene	100±1	103±5	95±2	103±3	100±4	100±5	89±10	102±2	97±8	97±8
Benz(ghi)perylene	100±4	101±3	95±2	101±3	99±3	100±3	99±2	100±3	99±2	100±2

* Average percent recovery ± standard deviation of triplicate analyses.

of the data shows this to be an artifact due to the high precision of the assay.

EXTRACTION STUDIES

Extraction studies were conducted to evaluate the extraction efficiencies of methylene chloride and 15% methylene chloride in hexane for PAH in water at pH 2, 7, and 10. Results of these analyses are shown in Table 7. ANOVA analysis of the extraction data indicates, as shown in Table 7, that methylene chloride is a significantly better extraction solvent for virtually all of the PAHs. For several of the PAHs, anthracene, pyrene, benz(b)fluoranthene, indeno(1,2,3-cd) pyrene, and dibenz-(a,h) anthracene, pH also had a significant effect, according to the ANOVA analysis. Inspection of the data indicates that in these cases pH 10 gave much lower recoveries than pH 7 or pH 2.

On the basis of these data it is apparent that methylene chloride is the extraction solvent of choice, and that while pH 10 can lead to lower recoveries, pH 7 and pH 2 generally give good recoveries.

PRESERVATION STUDIES

Preservation studies were conducted to evaluate the stability of PAH in water under selected conditions. These conditions include pH 2, 7, and 10; 0 and 2 ppm chloride; and room temperature (RT) and 4°C.

Distilled water which had previously been buffered and/or chlorinated in accordance with the analytical matrix was spiked with PAH standard solution and stored for 7 days. Each storage condition was run in duplicate.

Following the 7 day storage each solution was extracted, concentrated to 1 ml using a Kuderna-Danish evaporator, and analyzed.

The results of these analyses which are corrected for extraction efficiencies are given in Table 8. These data have been presented in alternate format, along with the ANOVA results in Table 9.

The most curious results of these analysis is the zero percent recovery of naphthalene at RT, pH 7, 0 ppm Cl, and acenaphthylene at RT, pH 2, and 2 ppm Cl respectively. These analysis were repeated with fresh stock solution and stored for 7 days under identical conditions. Results of these analysis were identical, i.e., 0 percent recovery for these two compounds. It is possible that chlorine is degrading the acenaphthylene in some way but there seems to be no plausible explanation for the naphthylene results.

TABLE 7. EXTRACTION STUDIES

	15% $MeCl_2$/Hexane			$MeCl_2$			ANOVA Analysis	
	pH 2	pH 7	pH 10	pH 2	pH 7	pH 10	Solvent	pH
Naphthalene	66±8*	66±11	57±9	102±5	100±7	89±8	+	0
Acenaphthene	81±9	76±13	70±9	100±5	99±9	101±8	+	0
Anthracene	87±10	77±12	66±9	100±7	100±7	91±4	+	+
Pyrene	95±16	88±14	75±11	100±3	100±4	84±2	0	+
Chrysene	72±31	79±10	67±8	103±10	100±10	78±1	+	0
Benz(k)fluoranthene	63±25	64±18	59±7	104±14	101±5	63±4	+	+
Indeno(1,2,3-cd)pyrene	96±32	88±17	56±19	106±13	100±4	64±11	0	+
Dibenz(a,h)anthracene	64±20	60±10	41±11	97±11	99±2	59±6	+	+
Acenaphyhylene	91±9	99±14	93±14	81±2	71±2	69±1	+	0
Fluorene	97±8	107±22	110±8	91±0.5	80±2	79±3	+	0
Phenanthrene	106±15	103±25	119±12	90±2	88±3	88±3	+	0
Benz(a)anthracene	97±7	83±14	104±12	90±1	92±6	90±3	+	0
Benz(a)pyrene	72±6	74±16	91±24	86±2	101±7	89±7	+	0
Benz(ghi)perylene	57±13	61±22	67±2	89±2	91±6	88±4	+	0
Fluoranthene	102±11	97±17	129±29	89±1	89±4	91±1	0	0

* Average percent recovery ± standard deviation for triplicate or quadruplicate analyses.

+ Statistically significant differences at the 95% confidence level.

0 No significant difference at the 95% confidence level.

From the data presented in Table 9 it is obvious that added chlorine reduces stability to some extent, although this is highly dependant on the particular compound and the pH level, as will be discussed later. In general stability was lower at RT than at 4°C although for benzo(k)-fluoranthene, indeno(1,2,3-cd)pyrene, and dibenz(a,h)anthracene room temperature gave slightly better stability at RT. The effect of pH on stability varied from compound to compound, although normally stability increased in the order pH2<pH7<pH10. The effect on pH is difficult to interpret due to the fact that pH appears to alter the effect of added chlorine, which has been previously reported [2]. It appears that the effect of added chlorine is greatest at low pH. Three compounds, acenapthalene, benz(a)anthracene, and benz(a)pyrene appear to be extremely unstable in the presence of added chlorine, especially at pH 2.

The data at pH 10 (Table 8) are complicated by the fact that for several of the compounds recoveries greater than 100% were indicated. The reason for the apparently high recoveries are not known, although improper instrument calibration seems the most likely problem.

On the basis of the data presented in Tables 8 and 9 we have concluded that 4°C, pH 7, and removal of chlorine is the optimum protocol for sample storage. Table 8 shows that this condition gives reasonably consistent stabilities (∿80%) for the various compounds. Although pH 10 gives somewhat better average recovery, it appears that this is a result of the artifactually high (>100%) recoveries for a few compounds, and that for the remaining compounds pH 7 and pH 10 give equivalent stabilities. The need for low temperature storage is obvious from the data in Table 8 and is very critical for real world samples where bacterial growth must be prevented. The need for chlorine removal at pH 7 and 4°C is clear for a few of the compounds (e.g. benz(a)pyrene and anthracene) although for the remaining compounds its affect is negligible.

Using pH 7, and 4°C, and removal of chlorine this data indicates that stabilities of ∿80% can be expected for a seven day storage period.

TABLE 8. PRESERVATION STUDIES

	RT 0 Cl	RT 2 Cl	4°C 0 Cl	4°C 2 Cl	RT 0 Cl	RT 2 Cl	4°C 0 Cl	4°C 2 Cl	RT 0 Cl	RT 2 Cl	4°C 0 Cl	4°C 2 Cl
Naphthalene	77±5 *	89±9	81±9	61±5	0	93±7	89±7	97±8	104±8	83±8	111±8	101±8
Acenaphthene	86±5	79±7	90±9	54±5	69±9	66±9	97±9	100±9	102±8	77±8	102±8	98±11
Anthracene	83±7	75±13	85±8	39±7	23±7	22±7	88±7	45±7	100±4	25±4	100±4	38±4
Pyrene	85±12	86±6	91±5	58±3	84±4	79±7	86±6	97±4	111±4	86±3	111±2	93±10
Chrysene	63±12	88±10	79±10	59±10	84±11	53±10	83±10	77±10	109±3	91±1	105±1	95±13
Benz(k)fluoranthene	80±14	91±14	80±14	54±14	69±7	82±5	73±5	65±6	125±4	105±4	121±6	103±4
Indeno(1,2,3-cd)pyrene	77±13	88±13	75±13	46±13	75±16	85±4	78±4	73±4	142±11	117±11	133±11	102±12
Dibenz(a,h)anthracene	73±11	88±11	75±11	61±11	74±9	78±16	70±2	60±8	124±18	88±6	105±6	90±8
Acenaphthylene	74±2	0	75±2	5±3	73±2	17±2	73±2	68±2	78±1	52±1	72±4	84±4
Fluorene	73±2	64±1	80±0.5	69±2	76±2	50±2	68±2	73±3	70±3	48±3	57±6	71±10
Phenanthrene	70±2	59±2	73±5	60±7	72±3	69±4	69±3	72±3	66±3	45±3	57±3	61±3
Fluoranthene	61±5	60±1	67±1	64±2	61±4	45±4	61±4	65±4	64±2	44±2	55±2	59±2
Benz(a)anthracene	61±1	9±1	74±1	10±1	73±12	58±6	65±6	64±6	74±3	50±5	61±3	63±3
Benz(a)pyrene	44±10	26±3	112±5	26±4	84±7	35±7	86±7	46±7	111±7	72±7	89±9	89±7
Benz(ghi)perylene	94±6	63±2	106±9	94±13	86±6	84±6	88±9	88±6	110±5	57±5	88±10	84±8
Average	73	64	78	50	67	61	78	73	95	69	91	82
	pH 2				pH 7				pH 10			

* Average recovery ± standard deviation for duplicate samples.

LC CLEAN-UP STUDIES

The percent recoveries of the representative PAHs from each LC separation scheme are listed in Table 10 below.

TABLE 10. PNA RECOVERY

	Silica				Alumina			
	% Recovery			Total	% Recovery			Total
Elution Volume*	10	20	30		10	20	30	
Naphthalene	<0.5	93.0	<0.5	93	100	<0.5	<0.5	100
Anthracene	0.5	104.5	<0.5	105	86	13.0	<0.5	99
Fluoranthene	0.5	96.5	<0.5	97	78	18.0	<0.5	96
Chrysene	1.0	97.0	<0.5	98	38	53.0	<0.5	91
Dibenz(a,h) anthracene	<0.5	92.0	<0.5	92	0.5	83.0	<0.5	83

* 40 percent methylene chloride in hexane.

From the above data, it is apparent that either LC separation scheme would be suitable as a clean-up procedure. However, silica gel gave somewhat higher recoveries for the larger molecular weight components as well as exhibiting a considerable faster flow rate during the separation procedure. Thus, the use of silica gel was favored over alumina as the stationary phase for these clean-up procedures. A 25 ml elution volume was considered to be optimum, based on this data.

WASTEWATER ANALYSIS

As stated in the Experimental Section, five water samples were selected for analysis using the method outlined. Analysis of the plastic industry wastewater and raw sewage were conducted using the Spherisorb ODS column while subsequent wastewaters were analyzed using the Perkin-Elmer HC-ODS column.

Initial wastewater analysis were conducted by HPLC using a Spherisorb ODS reverse phase column and selective fluorescent wavelength detection based on the work of Das and Thomas (12). Quantitation of benz-(a)anthracene/chrysene using this method involves the use of the ratio of the response at λex 250 and λem >370 to the response at λex 280 and >389.

Analysis of the plastic industry wastewater indicated this source to have a large number of components as well as having a rather high fluorescent background level. A chromatogram illustrating this high background is shown in Figure 7. Triplicate injection of a single sample extract gave good repeatability; however, comparison of different aliquots from the same sample gave erratic peaks which made quantitation

TABLE 9. PRESERVATION STUDIES DATA AND ANOVA ANALYSIS

Compound	Average Stability			ANOVA Analysis	Average Stability		ANOVA Analysis	Average Stability		ANOVA Analysis
	pH 2	pH 7	pH 10	pH	4°C	RT	TCMP	0 ppm	2 ppm	Chlorine
Naphthalene	77	70	100	+	90	61	+	77	87	+
Acenaphthene	77	83	95	+	90	67	+	91	79	+
Anthracene	71	45	57	+	66	55	+	79	41	+
Pyrene	80	87	100	0	89	89	0	94	83	+
Chrysene	72	54	100	0	83	81	0	87	77	+
Benzo(k)fluoranthene	76	72	108	+	83	92	+	91	83	+
Indeno(1,2,3-cd)pyrene	72	78	118	0	85	97	+	97	85	+
Dibenz(ah)anthracene	74	71	96	0	77	87	+	87	78	0
Acenaphthylene	39	58	72	+	64	49	+	74	38	+
Fluorene	72	67	50	+	70	63	0	70	55	0
Phenanthrene	66	71	57	0	65	63	0	68	61	+
Fluoranthene	63	58	55	+	62	55	+	62	56	+
Benz(a)anthracene	39	65	62	+	56	54	0	68	34	+
Benz(a)pyrene	52	63	90	+	75	62	+	88	49	+
Benz(ghi)perylene	89	86	85	0	91	68	+	95	78	+

+ Significantly differenct at 95% confidence level.

0 No significant difference at 95% confidence level.

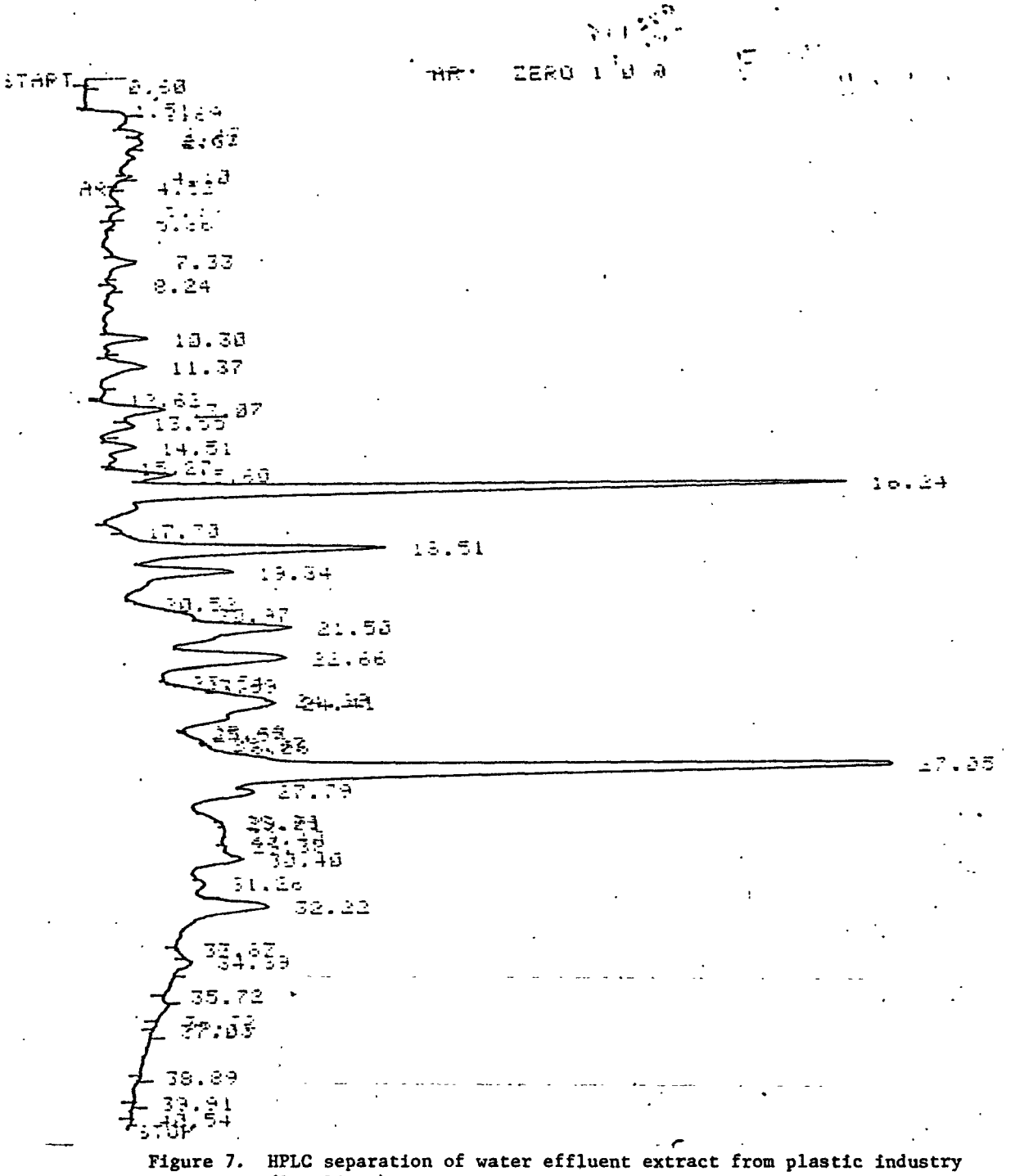


Figure 7. HPLC separation of water effluent extract from plastic industry (1st liter).

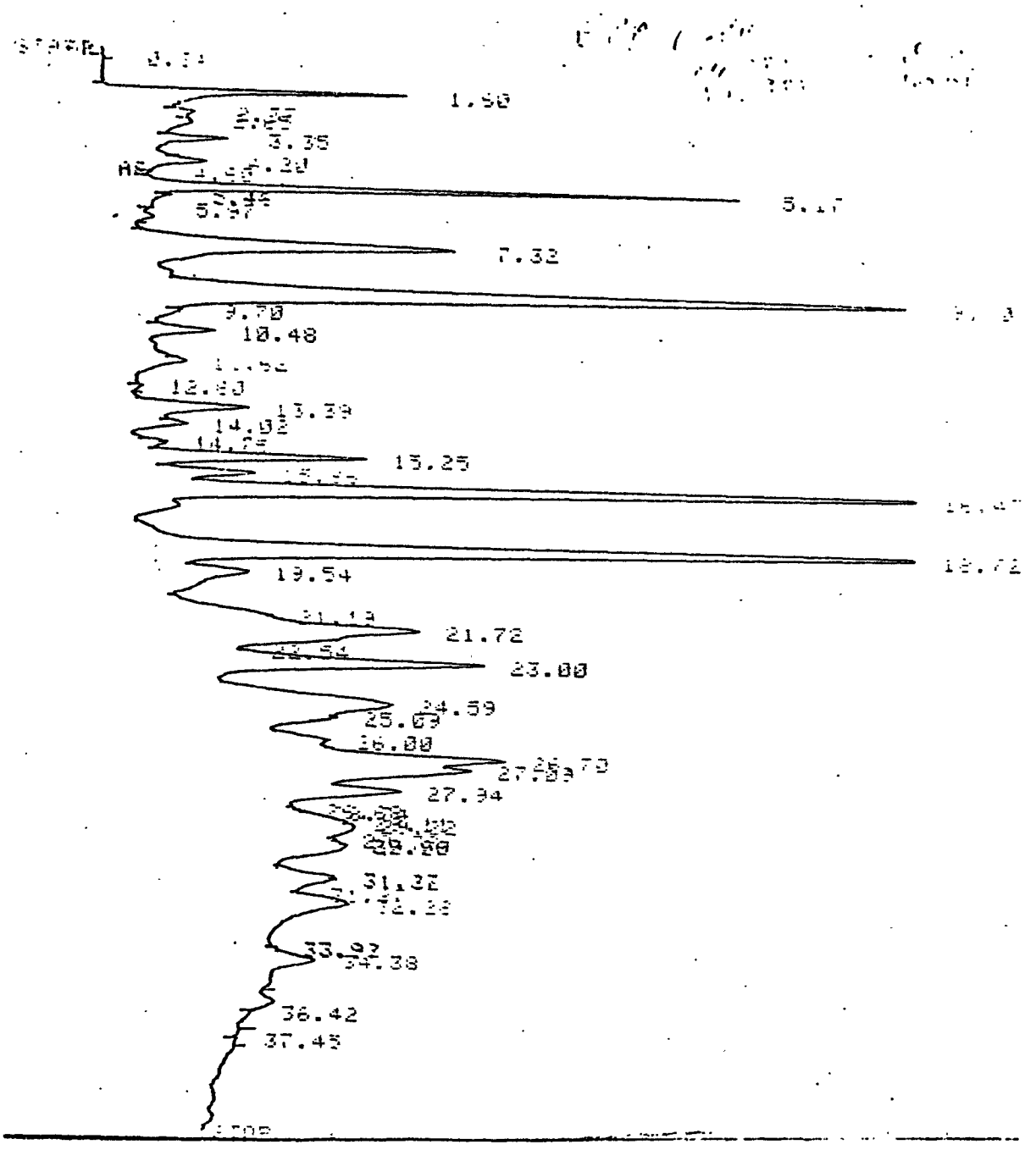


Figure 8. HPLC separation of water effluent extract from plastic industry (2nd liter).

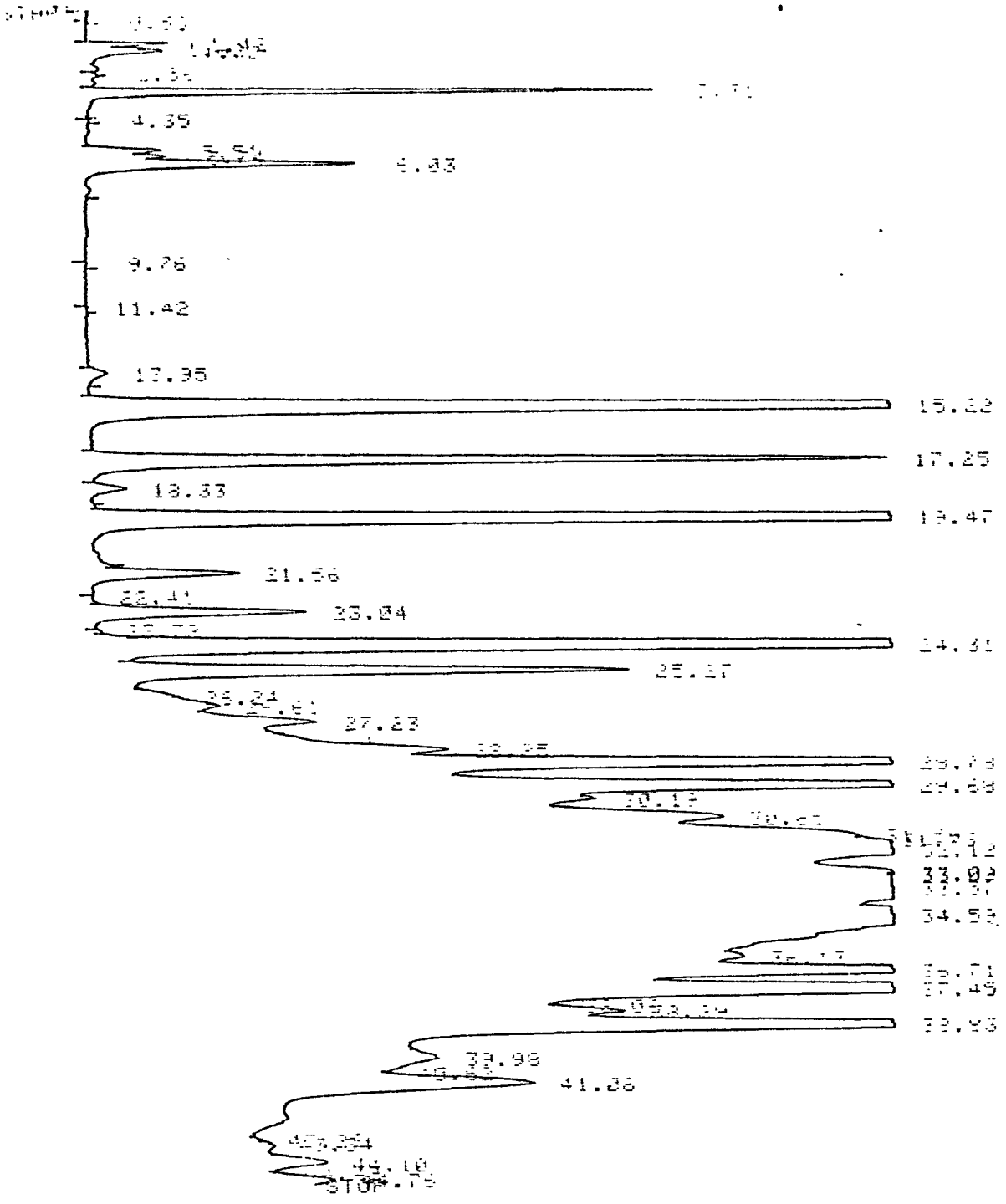


Figure 9. HPLC chromatogram of spiked water effluent extract from plastic industry.

difficult. Retention times between spiked and unspiked samples agreed for some PAH species but the wavelength ratio λex 250 - λem 370/ λex 280 - λem 389 varied widely from the experimentally determined values. Examples of these variations are illustrated by comparison of Figure 7 with that of Figure 8. This variation occurred even among the spiked samples and the high recoveries observed are probably due to this wide variation in background PNA levels.

A possible explanation for these differences is that one aliquot may have contained more particulate material than another. Although the bulk sample bottle was shaken before removing the aliquot for extraction, there may have been differences in particulate content between aliquots. Since PAH is known to be distributed primarily onto the particulate, this is an important aspect in interpretation of the results.

Results of the plastic industry wastewater analysis are listed in Table II.

Subsequent wastewaters were homogenized prior to subdivision into 1 liter aliquots to eliminate these variations in background levels. However, the initial supply of plastic industry wastewater was exhausted and homogenization was not performed on this sample.

Raw municipal sewage from Columbus, Ohio, was obtained as the second wastewater to be studied. Initial extraction studies indicated a fluctuating background similar to that observed with the plastic industry effluent. However, homogenization of this sample produced a more consistent background on subsequent extractions. For real samples an alternate solution would be to collect a 1 liter sample, representative of a particular source, and extract the entire sample.

Analysis indicated the presence of chrysene at 2.4 ppb to be the PAH present in greatest concentration; BaP was present at 0.07 ppb. Results of these analysis are listed in Table 12. Typical chromatograms of spiked and unspiked sewage extract are shown in Figures 10 and 11. The detection limits (DL) for each sample were calculated based on the concentration required for a given compound to yield a peak two times greater than the baseline fluctuation (due to compounds eluting in the vicinicity of the compound of interest) for that sample.

Treated municipal sewage water prior to chlorination was obtained as the third wastewater sample. Again homogenization was performed before aliquots were taken. Sample extracts indicated a lower and more uniform background than had been obtained with the raw sewage. Analysis indicated the presence of fewer PAH compounds than the untreated sewage as well as a lower concentration for chrysene at 0.3 ppb. However, an increase in the concentration of fluoranthene was detected from 0.5 ppb for the untreated sewage to 0.7 ppb for the treated.

Good PAH recoveries from spiked treated sewage were obtained except for naphthalene. These low results are due to the fact that the

TABLE 11. PLASTICS INDUSTRY WASTEWATER

Compound	Spiked	Spiked % recovery		Unspiked	
	Spiked level (ppb)	0 days	7 days	Background ppb	DL ppb
Naphthalene	70	76 ± 14	68 ± 19	<4.0	4.0
Acenaphthylene	450	134 ± 15	117 ± 13	<20	20.0
Acenaphthene	25	192 ± 28	181 ± 37	15.9 ± 5.3	0.8
Fluorene	23	152 ± 27	156 ± 27	0.7 ± 0.4	0.4
Phenanthrene	3.8	183 ± 16	179 ± 33	4.2 ± 2.3	2.1
Anthracene	7.9	181 ± 35	213 ± 48	7.8 ± 3.4	3.0
Fluoranthene	2.2	123 ± 23	182 ± 21	1.0 ± 0.06	0.23
Pyrene	6.9	223 ± 72	168 ± 25	4.8 ± 2.2	0.93
Benz(a)anthracene	0.66	208 ± 45	223 ± 31	0.45 ± 0.21	0.07
Chrysene	6.8	155 ± 26	169 ± 25	1.05 ± 0.30	0.1
Benz(b)fluoranthene	0.24	105 ± 13	135 ± 7	<0.03	0.03
Benz(k)fluoranthene	0.62	221 ± 24	156 ± 38	0.88 ± 0.30	0.03
Benz(a)pyrene	0.30	108 ± 12	122 ± 15	<0.03	0.03
Dibenz(a,h)anthracene	1.7	107 ± 13	115 ± 12	<0.06	0.06
Benz(ghi)perylene	3.4	127 ± 9	126 ± 18	<0.15	0.15
Indeno(1,2,3-cd)pyrene	1.4	133 ± 18	119 ± 13	<0.08	0.08

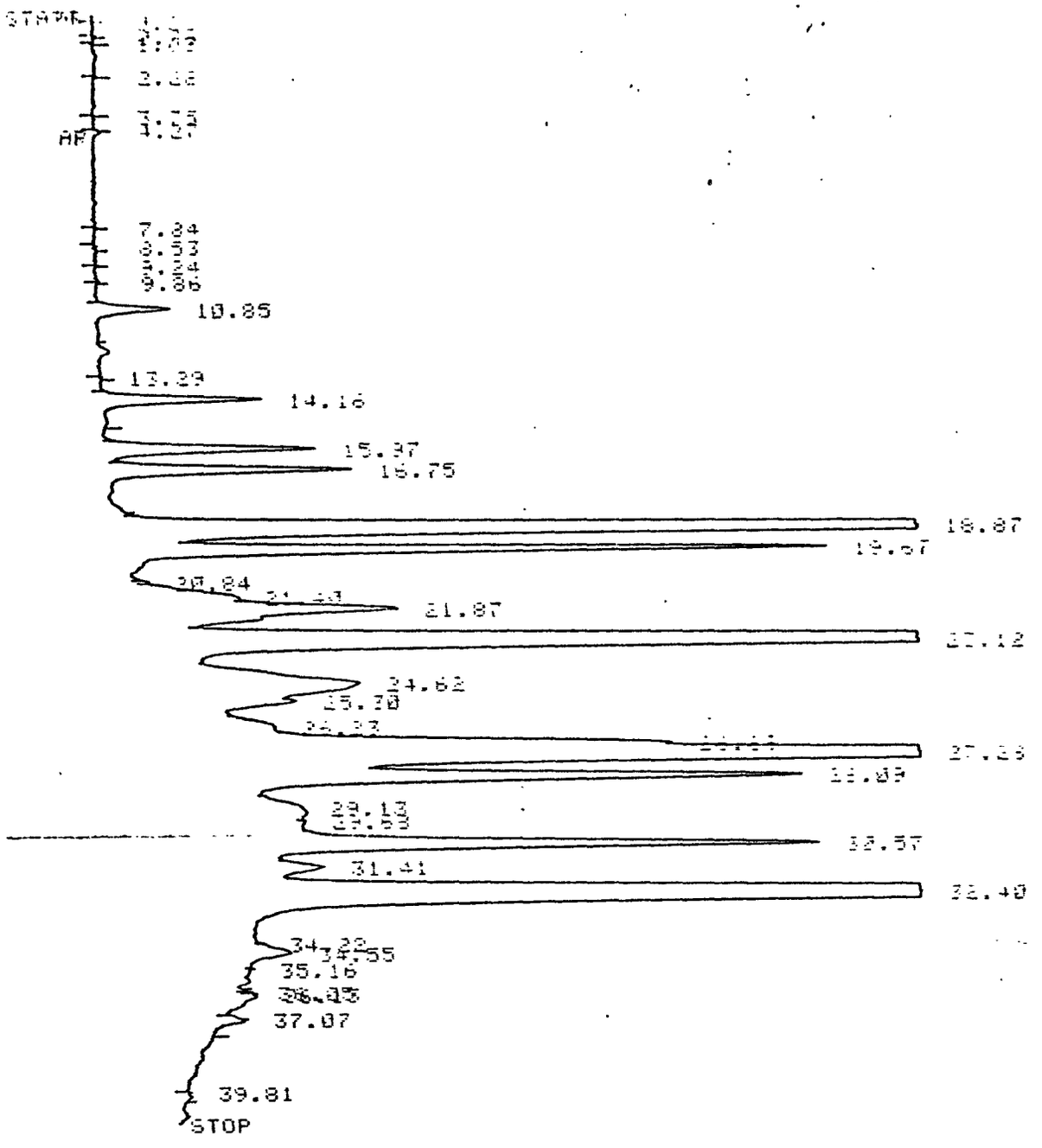


Figure 10. HPLC chromatogram of spiked raw sewage.

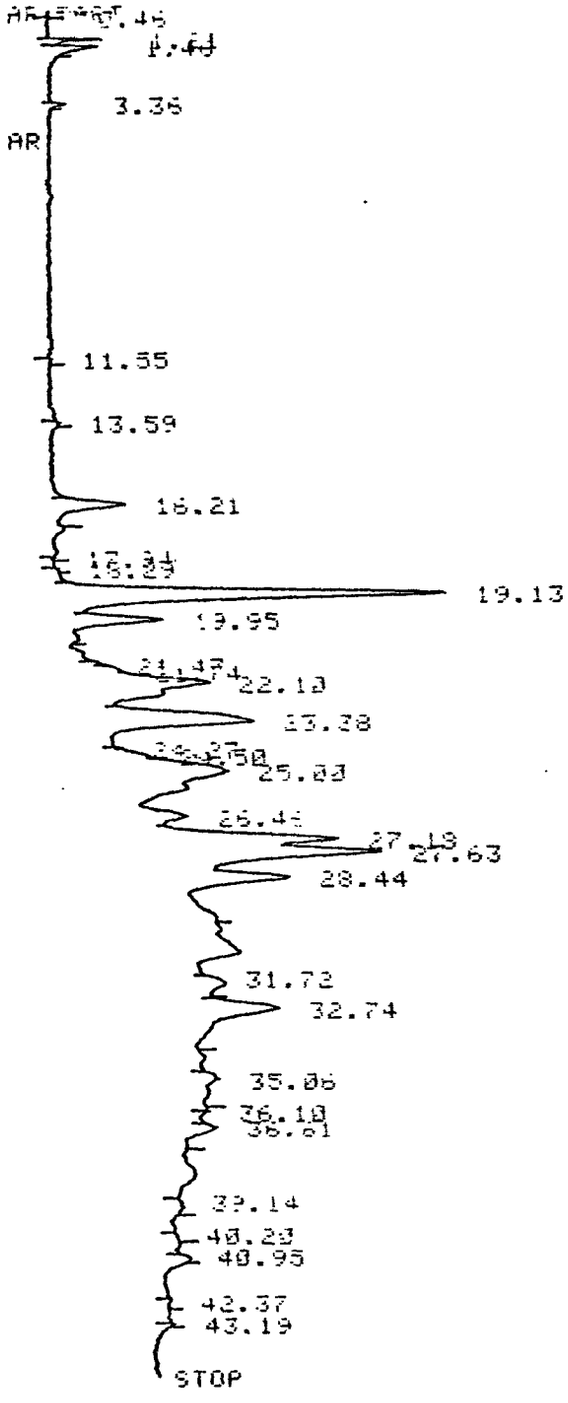


Figure 11. HPLC chromatogram of unspiked raw sewage.

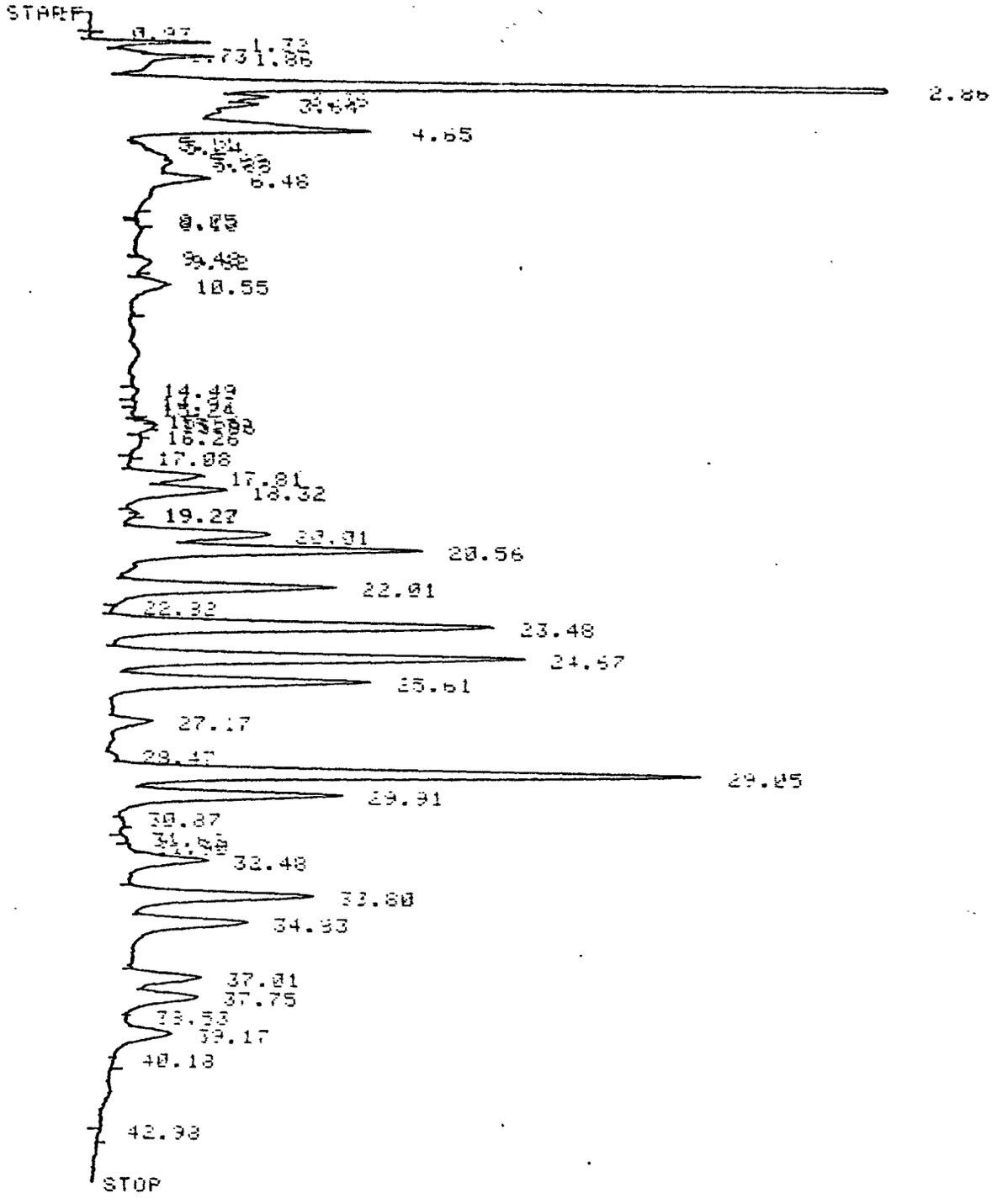


Figure 12. HPLC chromatogram of spiked treated sewage.

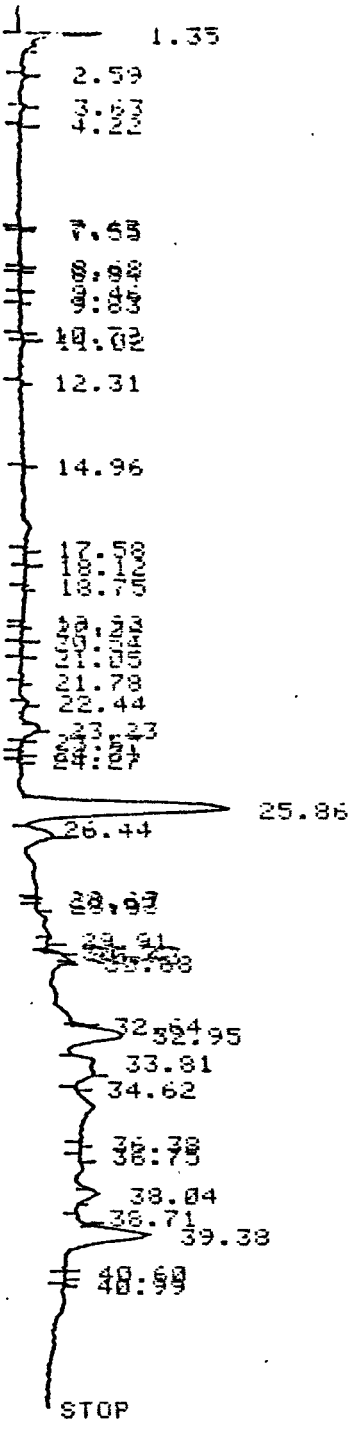


Figure 13. HPLC chromatogram of unspiked treated sewage.

TABLE 12. RAW SEWAGE WASTEWATER

Compound	Spiked	Spiked % recovery		Unspiked	
	Spiked level (ppb)	0 days	7 days	Background ppb	MDL ppb
Naphthalene	70	108 ± 9	113 ± 7	<4	4.0
Acenaphthylene	450	119 ± 5	103 ± 5	<20	20
Acenaphthene	25	93 ± 4	85 ± 5	<0.8	0.8
Fluorene	23	101 ± 7	115 ± 13	<0.4	0.4
Phenanthrene	3.8	115 ± 8	129 ± 12	0.74 ± 0.15	0.3
Anthracene	7.9	114 ± 7	108 ± 7	0.3 ± 0.15	0.3
Fluoranthene	2.2	133 ± 12	134 ± 10	0.47 ± 0.14	0.02
Pyrene	6.9	127 ± 16	113 ± 12	1.0 ± 0.4	0.2
Benz(a)anthracene	0.66	102 ± 7	109 ± 9	0.19 ± 0.08	0.02
Chrysene	6.8	81 ± 3	93 ± 9	2.4 ± 1.05	0.2
Benz(b)fluoranthene	0.24	132 ± 24	133 ± 19	0.10 ± 0.04	0.02
Benz(k)fluoranthene	0.62	112 ± 15	116 ± 14	0.10 ± 0.04	0.02
Benz(a)pyrene	0.30	114 ± 9	90 ± 10	0.07 ± 0.01	0.03
Dibenz(a,h)anthracene	1.7	80 ± 6	89 ± 6	<0.06	0.06
Benz(ghi)perylene	3.4	81 ± 8	81 ± 12	<0.15	0.15
Indeno(1,2,3-cd)pyrene	1.4	98 ± 2	93 ± 7	<0.08	0.08

TABLE 13. TREATED SEWAGE WASTEWATER

Compound	Spiked			Unspiked	
	Spiked level (ppb)	% recovery 0 days	% recovery 7 days	Background ppb	MDL ppb
Naphthalene	20	38 ± 3	24 ± 5	<4	4.0
Acenaphthylene	250	79 ± 8	88 ± 9	<20	20
Acenaphthene	11.6	79 ± 8	86 ± 7	1.8 ± 1.0	1.0
Fluorene	6.1	77 ± 5	75 ± 8	<0.4	0.4
Phenanthrene	5.0	83 ± 6	89 ± 9	<0.3	0.3
Anthracene	11.3	82 ± 6	88 ± 7	<0.3	0.3
Fluoranthene	0.3	127 ± 6	124 ± 7	0.7 ± 0.05	0.05
Pyrene	2.3	88 ± 4	89 ± 5	<0.2	0.2
Benz(a)anthracene	0.64	74 ± 6	71 ± 7	<0.02	0.02
Chrysene	2.0	89 ± 16	92 ± 9	0.3 ± 0.1	0.1
Benz(b)fluoranthene	0.3	88 ± 12	86 ± 9	<0.02	0.02
Benz(k)fluoranthene	0.14	86 ± 4	89 ± 7	<0.02	0.02
Benz(a)pyrene	0.21	101 ± 7	98 ± 8	<0.03	0.03
Dibenz(a,h)anthracene	0.4	86 ± 3	89 ± 5	<0.06	0.06
Benz(ghi)perylene	0.42	86 ± 6	90 ± 5	<0.15	0.15
Indeno(1,2,3-cd)pyrene	0.96	106 ± 6	97 ± 3	<0.08	0.08

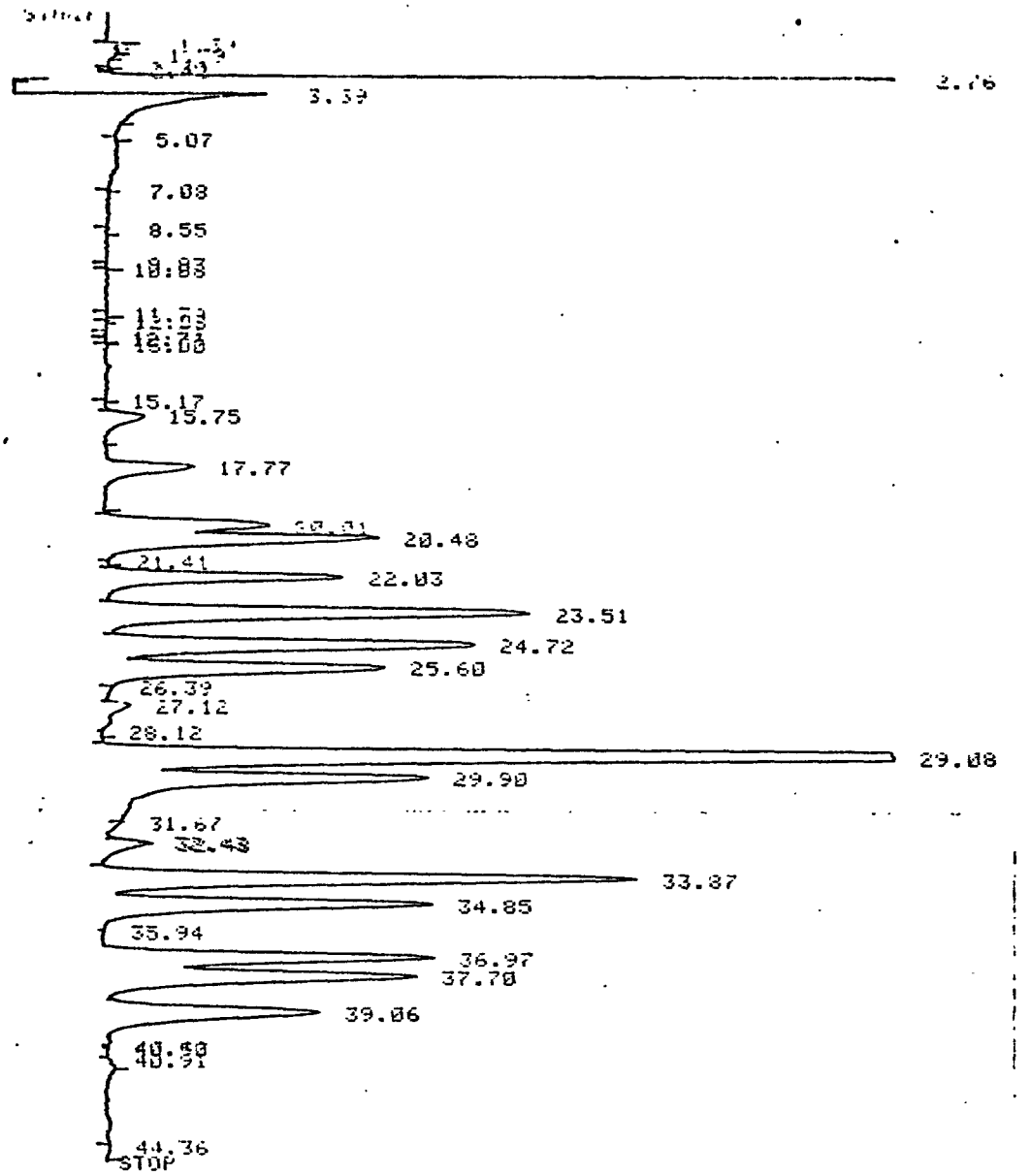


Figure 14. HPLC chromatogram of spiked flyash wash.

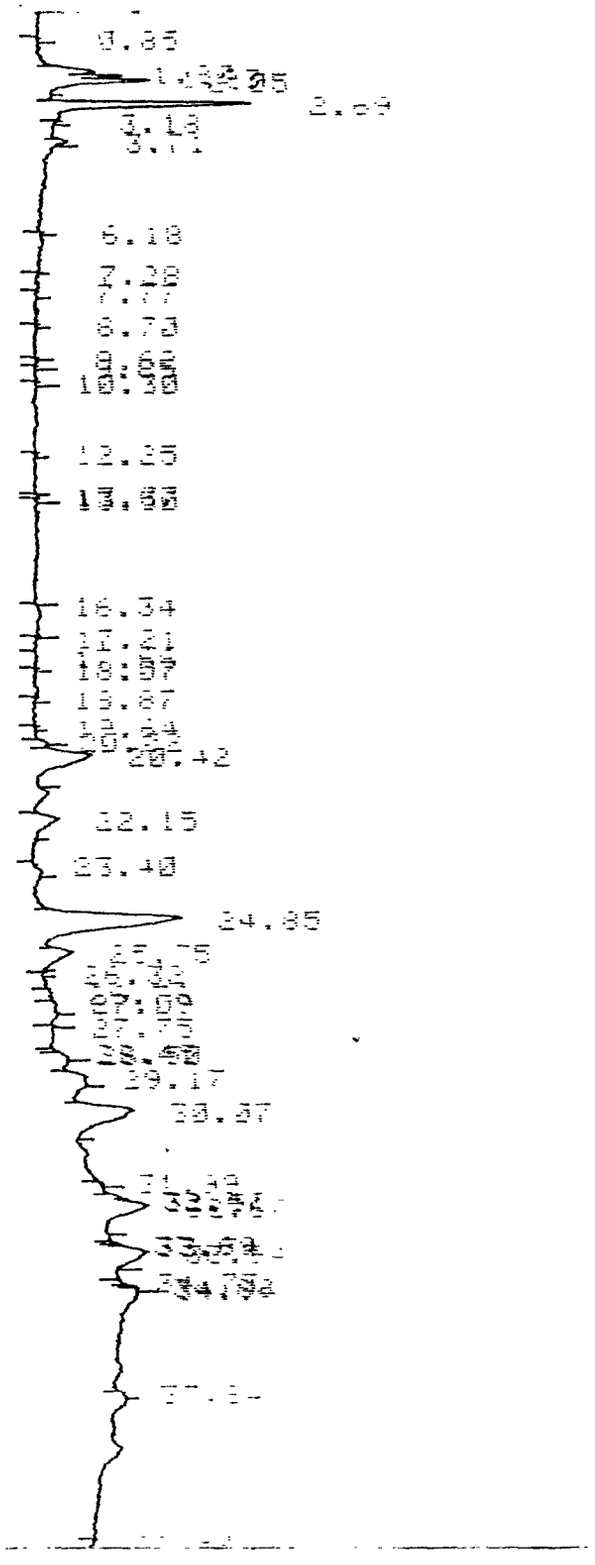


Figure 15. HPLC chromatogram of unspiked flyash wash.

TABLE 14. FLYASH WASH WASTEWATER

Compound	Spiked			Unspiked	
	Spiked level (ppb)	% recovery 0 days	% recovery 7 days	Background ppb	MDL ppb
Naphthalene	40	87 ± 6	85 ± 6	<4	4
Acenaphthylene	250	88 ± 5	85 ± 7	<20	20
Acenaphthene	11.6	91 ± 4	87 ± 5	<0.8	0.8
Fluorene	6.1	86 ± 5	76 ± 7	<0.4	0.4
Phenanthrene	5.0	92 ± 8	94 ± 9	<0.24	0.24
Anthracene	11.3	92 ± 6	87 ± 6	<0.3	0.3
Fluoranthene	0.3	90 ± 6	89 ± 7	0.02 ± 0.01	0.01
Pyrene	2.3	94 ± 5	88 ± 6	<0.1	0.1
Benz(a)anthracene	0.64	91 ± 3	86 ± 10	<0.01	0.01
Chrysene	2.0	92 ± 7	86 ± 9	<0.1	0.1
Benz(b)fluoranthene	0.3	87 ± 7	80 ± 8	<0.01	0.01
Benz(k)fluoranthene	0.14	86 ± 6	86 ± 9	<0.01	0.01
Benz(a)pyrene	0.21	85 ± 5	87 ± 4	<0.01	0.01
Dibenz(a,h)anthracene	0.40	89 ± 7	85 ± 6	<0.02	0.02
Benz(ghi)perylene	0.42	89 ± 5	82 ± 8	<0.05	0.05
Indeno(1,2,3-cd)pyrene	0.96	85 ± 9	83 ± 7	<0.02	0.02

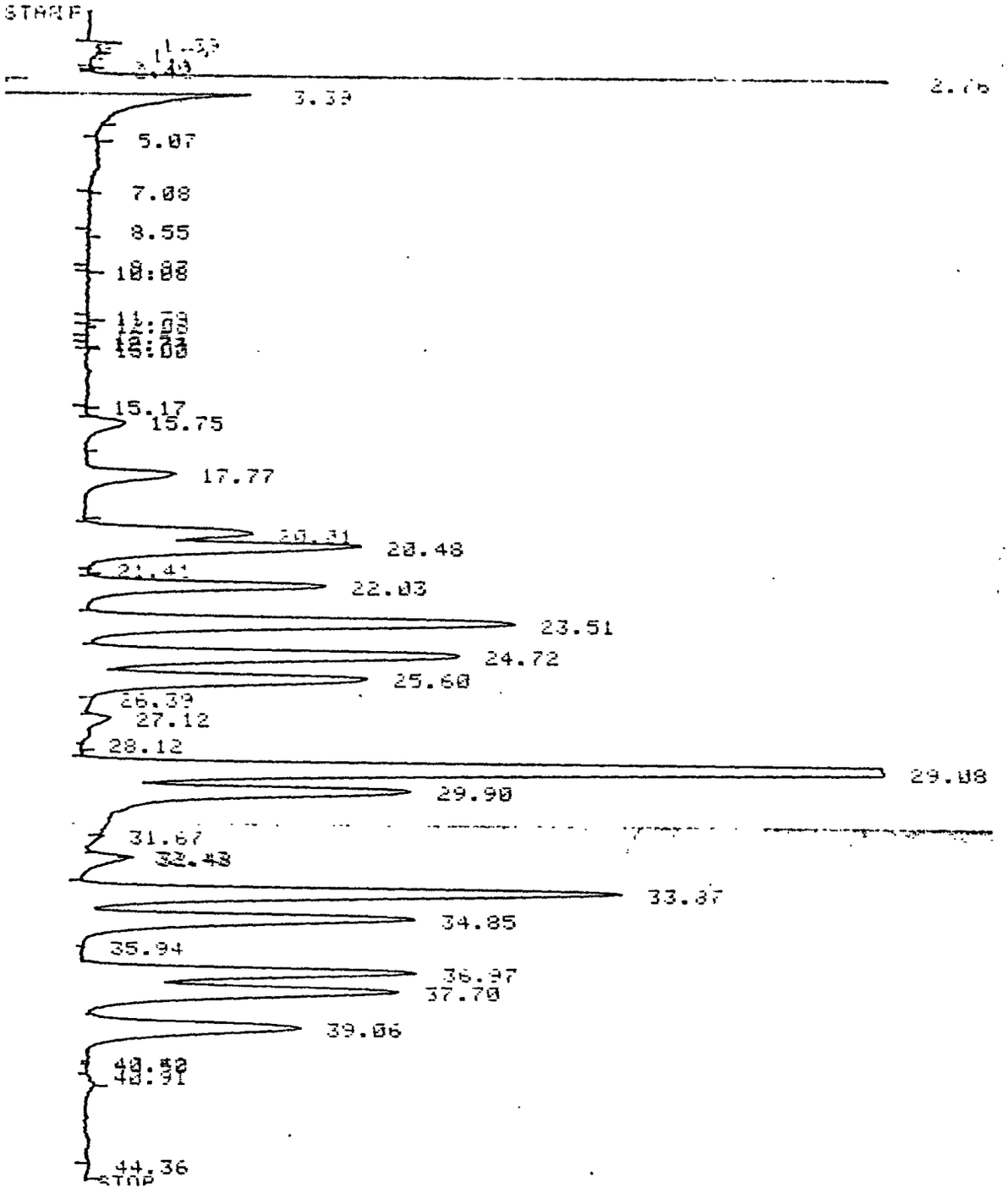


Figure 16. HPLC chromatogram of spiked flyash wash settling pond.

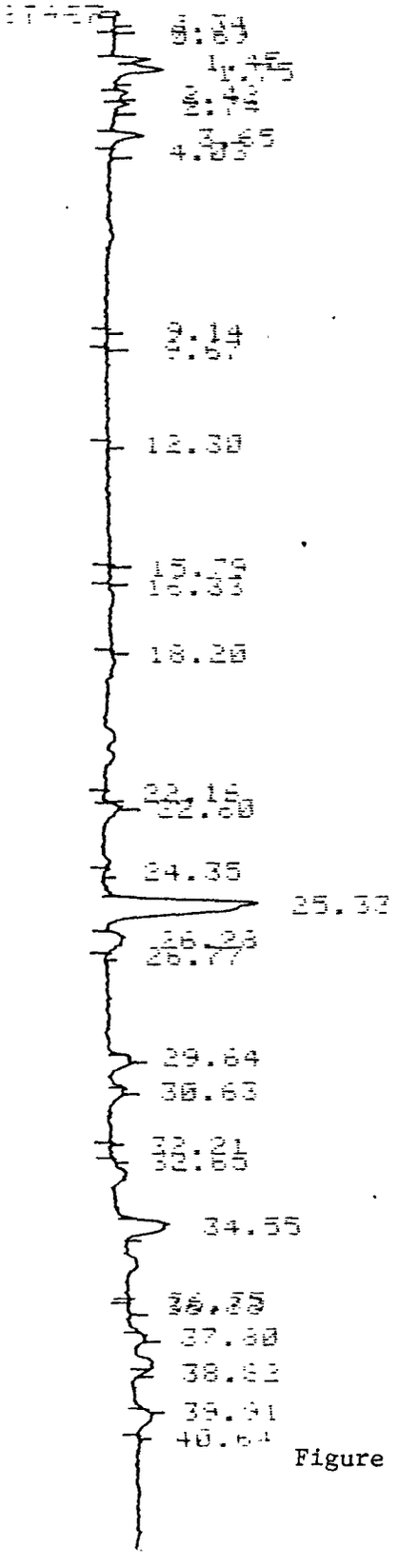


Figure 17. HPLC chromatogram of unspiked flyash wash settling pond.

concentration of naphthalene was close to the detection limit and the integrator did not integrate the sloping shoulders on this small peak. Since recoveries were calculated using peak area, a low result was obtained. Probably peak height would be a more accurate method for quantitation whenever the detection limit of the PAH compound is approached. Since our data was collected using a chromatographic data system it is not possible to calculate recoveries on the basis of peak heights since many of the peaks were offscale on the attenuation setting used. Results of these analysis are listed in Table 13. Typical chromatograms of spiked and unspiked treated sewage are shown in Figures 12 and 13.

Flyash wash water from a coal fired power plant was collected as the fourth wastewater. The flyash which is collected by the electrostatic precipitators is received into large hoppers. These hoppers are emptied every few hours by slurrying the flyash with water which drains into a primary settling pond. The flyash wash samples were collected just at the outfall of the primary settling pond.

No difficulties resulted during the homogenization and subsequent analysis of this wastewater. Fluoranthene was the only PAH apparently present in this sample. As the water contact time with the flyash is relatively short, this is not surprising.

Results of these analyses are given in Table 14. Examples of spiked and unspiked chromatograms are shown in Figures 14 and 15.

An overflow secondary settling pond adjacent to the primary settling pond above was the source of the fifth wastewater. As the sample was collected in late summer, the water in both ponds was relatively low. Thus the primary settling pond water would not have overflowed into the secondary for quite some time. A surface grab sample of this water was obtained at the edge of the pond.

No difficulties resulted during the homogenization and subsequent analysis of this wastewater. Again, fluoranthene was the only PAH apparently present in this sample although at a slightly higher concentration than that found in the primary wash.

Results of these analysis are given in Table 15. Examples of spiked and unspiked chromatograms are shown in Figures 16 and 17.

The recovery data after seven days storage for each of the last four wastewater samples has been combined in Table 16 to give an average recovery for each of the PAHs. The plastics industry sample data has not been included because of the problems encountered with sample homogeneity.

Based on the data obtained for the several wastewater samples it is obvious that the PAHs were stable for seven days using the recommended preservation techniques and that the sample matrix did not seriously

TABLE 15. FLYASH SETTLING POND WASTEWATER

Compound	Spiked			Unspiked	
	Spiked level (ppb)	% recovery 0 days	% recovery 7 days	Background ppb	MDL ppb
Naphthalene	40	87 ± 17	83 ± 5	<4.0	4
Acenaphthylene	250	90 ± 5	89 ± 6	<20	20
Acenaphthene	11.6	96 ± 6	90 ± 5	<0.8	0.8
Fluorene	6.1	97 ± 8	93 ± 7	<0.4	0.4
Phenanthrene	5.0	93 ± 5	88 ± 8	<0.24	0.24
Anthracene	11.3	85 ± 5	86 ± 6	<0.03	0.03
Fluoranthene	0.3	115 ± 12	113 ± 14	0.03 ± 0.01	0.01
Pyrene	2.3	87 ± 6	86 ± 6	<0.1	0.1
Benz(a)anthracene	0.64	93 ± 4	87 ± 6	<0.01	0.01
Chrysene	2.0	86 ± 8	87 ± 5	<0.1	0.1
Benz(b)fluoranthene	0.3	88 ± 6	83 ± 5	<0.01	0.01
Benz(k)fluorantehen	0.14	90 ± 9	88 ± 6	<0.01	0.01
Benz(a)pyrene	0.21	92 ± 9	87 ± 5	<0.01	0.01
Dibenz(a,h)anthracene	0.40	90 ± 8	86 ± 4	<0.02	0.02
Benz(ghi)perylene	0.42	94 ± 6	85 ± 6	<0.05	0.05
Indeno(1,2,3-cd)pyrene	0.96	97 ± 8	89 ± 6	<0.02	0.02

TABLE 16. AVERAGE RECOVERIES FOR PAHs FROM WASTEWATER SAMPLES

Compound	Recovery
Napthalene	76±37*
Acenapthalene	91±8
Acenapthene	87±2
Fluorene	90±18
Phenanthrene	100±19
Anthracene	92±10
Fluoranthene	115±19
Pyrene	94±12
Benz(a)anthracene	88±15
Chrysene	90±4
Benz(b)fluoranthene	95±25
Benz(k)fluoranthene	94±14
Benz(a)pyrene	91±4
Dibenz(a,h)anthracene	87±2
Benz(ghi)perylene	85±4
Indeno(1,2,3-cd)pyrene	91±8

* Average recoveries for four wastewater samples after seven days storage ± the standard deviation of the four measurements.

affect PAH recovery, since the recoveries for distilled water (e.g. preservation studies) and actual wastewater samples are virtually identical. The only serious problem encountered during the wastewater analysis was the problem of sample homogeneity, as discussed previously for the plastics industry sample. It appears that there are two alternative solutions to this problem: 1) thoroughly homogenize the sample before subsampling it or 2) obtain a representative sample from a particular source and analyze the entire sample.

SECTION 7

SUMMARY AND RECOMMENDATIONS

Several important points can be drawn from this analytical methods study for PAH in wastewater. These are:

- Sample homogenization is required in order to obtain a sample of uniform PAH content. Alternatively the entire sample can be extracted.
- For best preservation of PAHs in water, the pH sould be near 7, stored at near freezing temperatures and free chlorine should be removed by addition of a reducing agent.
- Methylene chloride is a suitable extraction solvent for PAHs in water at pH 7 or pH 10.
- All 16 consent decree PAHs can be separated by HPLC on a single stationary phase using a Perkin-Elmer HC-ODS column (2.6 x 250 mm I.D.).
- With proper wavelength selection all consent decree PAHs can be quantitated at sub-ppb levels.
- Although the silica LC clean-up procedure probably removes the majority of the organics in the wastewater extract, a large number of components can still remain in the collected PAH fraction. This was observed in some of the wastewaters. Thus there is a need for unambiguous peak identification. This could be achieved through the use of a stop-flow scanning spectrofluorimeter or by obtaining a UV or fluorescent spectra on collected fractions.
- PAH solutions are stable in DMSO and acetone for at least 90 days.

REFERENCES

1. McGinnes, P.R. and V.L. Snoeyink. Determination of the Fate of Polynuclear Aromatic Hydrocarbons in Natural Water Systems. OWRR A-045-Ill, Illinois Water Resources Center, Urbana, Ill. 55pp., 1974

2. Harrison, R.M., R.A. Wellings. Effect of Water Chlorinations upon Levels of Some Polynuclear Aromatics Hydrocarbons in Water. Env. Sci. Tech., 10(12), 1151-1156, 1976.

3. Pancirov, R.J. and R.A. Brown. Polynuclear Aromatic Hydrocarbons in Marine Tissues. Env. Sci. Tech. 11(10), 989-992, 1977.

4. Bjorseth, Alf. Analysis of PNA's in Air and Water. In Control of Specific Toxic Pollutants, Proceedings, Air Pollution Control Association, Feb. 13 -16, 1979.

5. Haenni, E.O. and J.W. Howard. DMSO A Superior Analytical Extraction Solvent for PNA's and for Some Highly Chlorinated Hydrocarbons. Journal of the AOAC 45(1), 67-72, 1962.

6. Hites, R.A. and K. Biemann. Water Pollution: Organic Compounds in the Charles River, Boston. Science 178, 158-160, 1972.

7. Achieson, M.A., R.M. Harrison, R. Perry, and R.A. Wellings. Factors Affecting the Extraction and Analysis of PNA's in Water. Water Research 10, 207-212, 1976.

8. Baser, D.K. and J. Saxena. Monitoring of PNA's in Water II. Extraction and Recovery of Six Representative Compounds with Polyurethane Foams. Env. Sci. Tech. 12(7), 791-794, 1978.

9. Junk, G.A., C.D. Chriswell, R.C. Chang, L.D. Kissinger, J.J. Richard, J.S. Fritz, and H.J. Svec. Applications of Resins for Extracting Organic Compounds from Water. Anal. Chem. 282, 331-337, 1976.

10. Castex, H., Identification of PNA's by Paper Chromatography and Spectrofluorimetry. Revue De L' Institut Francais Du Petrole 29(2), 155-172, 1974.

11. Soedigo, S., W.W. Angus, and J.W. Flesher. HPLC of PNA's and Some of Their Derivatives. Anal. Biochem. 67, 664-668, 1975.

12. Das, B.S. and G.H. Thomas. Fluorescence Detection in HPLC of PNA's. Anal. Chem. 50(7), 967-973, 1978.

APPENDIX A

POLYNUCLEAR AROMATIC HYDROCARBONS
METHOD 610

1. Scope and Application

1.1 This method covers the determination of certain polynuclear aromatic hydrocarbons (PAH). The following parameters may be determined by this method:

Parameter	STORET No.	Parameter	STORET No.
Acenaphthene	34205	Chrysene	34320
Acenaphthylene	34200	Dibenzo(ah)anthracene	34556
Anthracene	34220	Fluoranthene	34376
Benzo(a)anthracene	34526	Fluorene	34381
Benzo(a)pyrene	34247	Indeno(1,2,3-cd)pyrene	34403
Benzo(b)fluoranthene	34230	Naphthalene	34696
Benzo(ghi)perylene	34521	Phenanthrene	34461
Benzo(k)fluoranthene	34242	Pyrene	34469

1.2 This method is applicable to the determination of these compounds in municipal and industrial discharges. It is designed to be used to meet the monitoring requirements of the National Pollutant Discharge Elimination System (NPDES). As such, it presupposes a high expectation of finding the specific compounds of interest. If the user is attempting to screen samples for any or all of the compounds above, he must develop independent protocols for the verification of identity.

1.3 The sensitivity of this method is usually dependent upon the level of interferences rather than instrumental limitations. The limits of detection listed in Table I represent sensitivities that can be achieved in wastewaters.

1.4 This method is recommended for use only by experienced residue analysts familiar with High Performance Liquid Chromatography (HPLC) or under the close supervision of such qualified persons.

2. Summary of Method

2.1 A 1-liter sample of wastewater is extracted with methylene chloride using separatory funnel techniques. The extract is dried and concentrated to a volume of 10 ml or less. HPLC conditions are described which allow for the accurate measurement of the compounds in the extract.

2.2 If interferences are encountered, the method provides a selected general purpose clean-up procedure to aid the analyst in their elimination.

3. Interferences

3.1 Solvents, reagents, glassware, and other sample processing hardware may yield discrete artifacts and/or elevated baselines causing misinterpretation of gas chromatograms. All of these materials must be demonstrated to be free from interferences under the conditions of the analysis by running method blanks. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be required.

3.2 Interferences coextracted from the samples will vary considerably from source to source, depending upon the diversity of the industrial complex or municipality being sampled. While general clean-up techniques are provided as part of this method, unique samples may require additional, clean-up approaches to achieve the sensitivities stated in Table 1.

3.3 The extent of interferences that may be encountered using liquid chromatographic techniques has not been fully assessed. Although the chromatographic conditions described allow for a unique resolution of the specific PAH compounds covered by this method, other PAH compounds may interfere.

4. Apparatus and Materials

4.1 Sampling equipment, for discrete or composite sampling.

4.1.1 Grab sample bottle - amber glass, liter or quart volume. french or Boston Round design is recommended. The container must be washed and solvent rinsed before use to minimize interferences.

4.1.2 Bottle caps - Threaded to screw on sample bottles. Caps must be lined with Teflon. Foil may be substituted if sample is not corrosive.

4.1.3 Compositing equipment - Automatic or manual compositing system. Must incorporate glass sample containers for the collection of a minimum of 250 ml. Sample containers must be kept refrigerated during sampling. No tygon or rubber tubing or fittings may be used in the system.

4.2 Separatory funnel - 2000 ml, with Teflon stopcock.

4.3 Drying column - A 20 mm ID pyrex chromatographic column with coarse frit.

4.4 Kuderna-Danish (K-D) Apparatus

4.4.1 Concentrator tube - 10 ml, graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked. Ground glass stopper (size 19/22 joint) is used to prevent evaporation of extracts.

4.4.2 Evaporative flask - 500 ml (Kontes K-57001-0500 or equivalent). Attach to concentrator tube with springs. (Kontes K-662750-0012).

4.4.3 Snyder column - three-ball macro (Kontex K503000-0121 or equivalent).

4.4.4 Snyder column - two-ball micro (Kontes K-569001-0219 or equivalent).

4.4.5 Boiling chips - extracted, approximately 10/40 mesh.

4.5 Water bath - Heated, with concentric ring cover, capable of temperature control (±2°C). The bath should be used in a hood.

4.6 HPLC Apparatus:

4.6.1 Gradient pumping system, constant flow.

4.6.2 Reverse phase column, HC-ODS Sil-X, 250 mm x 2.6 mm ID (Perkin Elmer No. 809-0716 or equivalent).

4.6.3 Fluorescence detector, λex 280 nm and λem >389 nm.

4.6.4 UV detector, 254 nm, coupled to fluorescence detector.

4.6.5 Strip chart recorder capatible with detectors, 250 mm (A data system for measuring peak areas is recommended).

4.7 Chromatographic column - 250 mm long x 10 mm ID with coarse fritted disc at bottom and Teflon stopcock.

5. Reagents

5.1 Preservatives:

5.1.1 Sodium hydroxide - (ACS) 10 N in distilled water.

5.1.2 Sulfuric acid - (ACS) Mix equal volumes of conc. H_2SO_4 with distilled water.

5.1.3 Sodium thiosulfate - (ACS) Granular.

5.2 Methylene chloride, Pentane, Cyclohexane, High Purity Water-HPLC quality, distilled in glass.

5.3 Sodium Sulfate - (ACS) Granular, anhydrous (purified by heating at 400°C for 4 hrs.).

5.4 Stock standards - Prepare stock standard solutions at a concentration of 1.00 µg/µl by dissolving 0.100 grams of assayed reference material in high quality acetone or other appropriate solvent and diluting to volume in a 100 ml ground glass stoppered volumetric flask. The stock solution is transferred to ground glass stoppered reagent bottles, stored in a refrigerator, and checked frequently for signs of degradation or evaporation, especially just prior to preparing working standards from them.

5.5 Acetonitrile - Spectral quality.

5.6 Silica gel - 100/120 mesh desiccant (Davison Chemical grade 923 or equivalent). Before use, activate for at least 16 hours at 130°C in a foil covered glass container.

6. Calibration

6.1 Prepare calibration standards that contain the compounds of interest, either singly or mixed together. The standards should be prepared at concentrations covering two or more orders of magnitude that will completely bracket the working range of the chromatographic system. If the sensitivity of the detection system can be calculated from Table I as 100 µg/l in the final extract, for example, prepare standards at 10 µg/l, 50 µg/l, 100 µg/l, 500 µg/l, etc. so that injections of 1-5 µl of each calibration standard will define the linearity of the detector in the working range.

6.2 Assemble the necessary high pressure liquid chromatographic apparatus and establish operating parameters equivalent to those indicated in Table I. By injecting calibration standards, establish the sensitivity limit of the detectors and the linear range of the analytical systems for each compound.

6.3 Before using any clean-up procedure, the analyst must process a series of calibration standards through the system to validate elution patterns and the absence of interferences from the reagents.

7. Quality Control

7.1 Before processing any samples, the analyst should demonstrate through the analysis of a distilled water method blank, that all glassware and reagents are interference-free. Each time a set of samples is extracted or there is a change in reagents, a method blank should be processed as a safeguard against chronic laboratory contamination.

7.2 Standard quality assurance practices should be used with this method. Field replicates should be collected to validate the precision of the sampling technique. Laboratory replicates should be analyzed to validate the precision of the analysis. Fortified samples should be analyzed to validate the accuracy of the analysis. Where doubt exists over the identification of a peak on the chromatogram, confirmatory techniques such as fraction collection and GC-mass spectroscopy should be used.

8. Sample Collection, Preservation, and Handling

8.1 Grab samples must be collected in glass containers. Conventional sampling practices should be followed, except that the bottle must not be prewashed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be free of tygon and other potential sources of contamination.

8.2 The samples must be iced or refrigerated from the time of collection until extraction. Chemical preservatives should not be used in the field unless more than 24 hours will elapse before delivery to the laboratory. If the samples will not be extracted within 48 hours of collection, adjust the sample to a pH range of 6.0-8.0 with sodium hydroxide or sulfuric acid and add 35 mg sodium thiosulfate per part per million of free chlorine per liter.

8.3 All samples must be extracted within 7 days and completely analyzed within 30 days of collection.

9. Sample Extraction

9.1 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a two-liter separatory funnel. Check the pH with wide-range paper and adjust to within the range of 5-9 with sodium hydroxide or sulfuric acid.

9.2 Add 60 ml methylene chloride to the sample bottle and shake 30 seconds to rinse the walls. Transfer the solvent into the separatory funnel, and extract the sample by shaking the funnel for two minutes with periodic venting to release vapor pressure. Allow the organic layer to separate from the water phase for a minimum of ten minutes. If the emulsion interface between layers is more than one-third the size of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, or centrifugation. Collect the methylene chloride extract in a 250-ml Ehrlenmeyer flask.

9.3 Add a second 60-ml volume of methylene chloride to the sample bottle and complete the extraction procedure a second time, combining the extracts in the Erlenmeyer flask.

9.4 Perform a third extraction in the same manner. Pour the combined extract through a drying column containing 3-4 inches of anhydrous sodium sulfate, and collect it in a 500-ml Kuderna-Danish (K-D) flask equipped with a 10 ml concentrator tube. Rinse the Ehrlenmeyer flask and column with 20-30 ml methylene chloride to complete the quantitative transfer.

9.5 Add 1-2 clean boiling chips to the flask and attach a three-ball Synder column. Prewet the Snyder column by adding about 1 ml

methylene chloride to the top. Place the K-D apparatus on a steaming hot (60-65°C) water bath so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed in steam. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15-20 minutes. At the proper rate of distillation the balls of the column will actively chatter but the chambers will not flood. When the apparent volume of liquid reaches 1 ml, remove the K-D apparatus and allow it to drain for at least 10 minutes while cooling. Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1-2 ml of methylene chloride. A 5-ml syringe is recommended for this operation. Stopper the concentration tube and store refrigerated if further processing will not be performed immediately.

9.6 Determine the original sample volume by refilling the sample bottle to the mark and transferring the liquid to a 1000 ml graduated cylinder. Record the sample volume to the nearest 5 ml.

9.7 If the sample requires clean-up before chromatographic analysis, proceed to Section 10. If the sample does not require clean-up, or if the need for clean-up is unknown, analyze an aliquot of the extract according to Section 11.

10. Clean-up and Separation

10.1 Before the silica gel clean-up technique can be utilized, the extract solvent must be exchanged to cyclohexane. Add a 1-10 ml aliquot of sample extract (in methylene chloride) and a boiling chip to a clean K-D concentrator tube. Add 4 ml cyclohexane and attach a micro-Snyder column. Prewet the micro-Snyder column by adding 0.5 ml methylene chloride to the top. Place the micro-K-D apparatus on a boiling (100°C) water bath so that the concentrator tube is partially immersed in the hot water. Adjust the vertical position of the apparatus and the water temperature as required to complete concentration in 5-10 minutes. At the proper rate of distillation the balls of the column will actively chatter but the chambers will not flood. When the apparent volume of the liquid reaches 0.5 ml, remove the K-D apparatus and allow it to drain for at least 10 minutes while cooling. Remove the micro-Snyder column and rinse its lower joint into the concentrator tube with a minimum of cyclohexane. Adjust the extract volume to about 2 ml.

10.2.1 Prepare a slurry of 10g activated silica gel in methylene chloride and place this in a 10 mm ID chrmatography column. Gently tap the column to settle the silica gel and elute the methylene chloride.

10.2.2 Preelute the column with 40 ml pentane. Discard the eluate and just prior to exposure of the sodium sulfate layer to the air, transfer the 2 ml cyclohexane sample extract onto the column, using an additional 2 ml of cyclohexane to complete the transfer.

10.2.3 Just prior to exposure of the sodium sulfate layer to the air, add 25 ml pentane and continue elution of the column. Discard the pentane eluate.

10.2.4 Elute the column with 25 ml of 40% methylene chloride/60% pentane and collect the eluate in a 500 ml K-D flask equipped with a 10 ml concentrator tube. Elution of the column should be at a rate of about 2 ml/min.

10.2.5 Concentrate the collected fraction to less than 10 ml by K-D techniques as in 9.5, using pentane to rinse the walls of the glassware. Proceed with HPLC analysis.

11. High Performance Liquid Chromatography HPLC

11.1 Table I summarizes the recommended HPLC column materials and operating conditions for the instrument. Included in this table are estimated retention times and sensitivities that should be achieved by this method. An example of the separation achieved by this column is shown in Figure 1. Calibrate the system daily with a minimum of three injections of calibration standards.

11.2 To the extract, add 4 ml acetonitrile and a new boiling chip, then attach a micro-Snyder column. Increase the temperature of the hot water bath to 95-100°C. Concentrate the solvent as above. After cooling, remove the micro-Snyder column and rinse its lower joint into the concentrator tube with about 0.2 ml acetonitrile. Adjust the extract volume to 1.0 ml.

11.3 Inject 2-5 µl of the sample extract with a high pressure syringe. Record the volume injected to the nearest 0.05 µl, and the resulting peak size, in area units.

11.4 If the peak area exceeds the linear range of the system, dilute the extract and reanalyze.

11.5 If the peak area measurement is prevented by the presence of interferences, further clean-up is required.

11.6 The UV detector is recommended for the determination of naphthalene and acenaphthylene and the fluorescence detector is recommended for the remaining PAHs.

12. Calculations

12.1 Determine the concentration of individual compounds according to the formula:

$$\text{Concentration, } \mu g/l = \frac{(A)\ (B)\ (V_t)}{(V_i)\ (V_s)}$$

where A = Calibration factor for chromatographic system in nanograms material per area unit.

B = Peak size in injection of sample extract, in area units

V_i = volume of extract injected (µl)

V_t = Volume of total extract (µl)

V_s = Volume of water extracted (ml)

12.2 Report results in micrograms per liter without correction for recovery data. Whenduplicate and spiked samples are analyzed, all data obtained should be reported.

13. Accuracy and Precision

Data is not available at the present time.

BIBLIOGRAPHY

"Determination of Polynuclear Aromatic Hydrocarbons in Industrial and Municipal Wastewaters". Report for EPA Contract 68-03-2624 (In preparation).

TABLE A-1 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY OF PAH's

Compound	Retention time (min)	Detection Limit(ug/l)† UV	Fluorescence
Naphthalene	16.17	2.5	20.0
Acenaphthylene	18.10	5.0	100.0
Acenaphthene	20.14	3.0	4.0
Fluorene	20.89	0.5	2.0
Phenanthrene	22.32	0.25	1.2
Anthracene	23.78	0.10	1.5
Fluoranthene	25.00	0.50	0.05
Pyrene	25.94	0.10	0.05
Benzo(a)anthracene	29.26	0.20	0.04
Chrysene	30.14	0.20	0.5
Benzo(b)fluoranthene	32.44	1.0	0.04
Benzo(k)fluoranthene	33.91	0.30	0.04
Benzo(a)pyrene	34.95	0.25	0.04
Dibenzo(a,h)anthracene	37.06	1.0	0.08
Benzo(ghi)perylene	37.82	0.75	0.2
Indeno(1,2,3-cd)pyrene	39.21	0.30	0.1

HPLC conditions: Reverse phase HC-ODS Sil-X 2.6 x 250 mm Perkin-Elmer column; isocratic elution for 5 min. using 40% acetonitrile/60% water, then linear gradient elution to 100% acetonitrile over 25 minutes; flow rate is 0.5 ml/min.

† Detection limit is calculated from the minimum detectable HPLC response being equal to five times the bacground noise, assuming an equivalent of a 2 ml final volume of the 1 liter sample extract, and assuming an HPLC injection of 2 microliters.

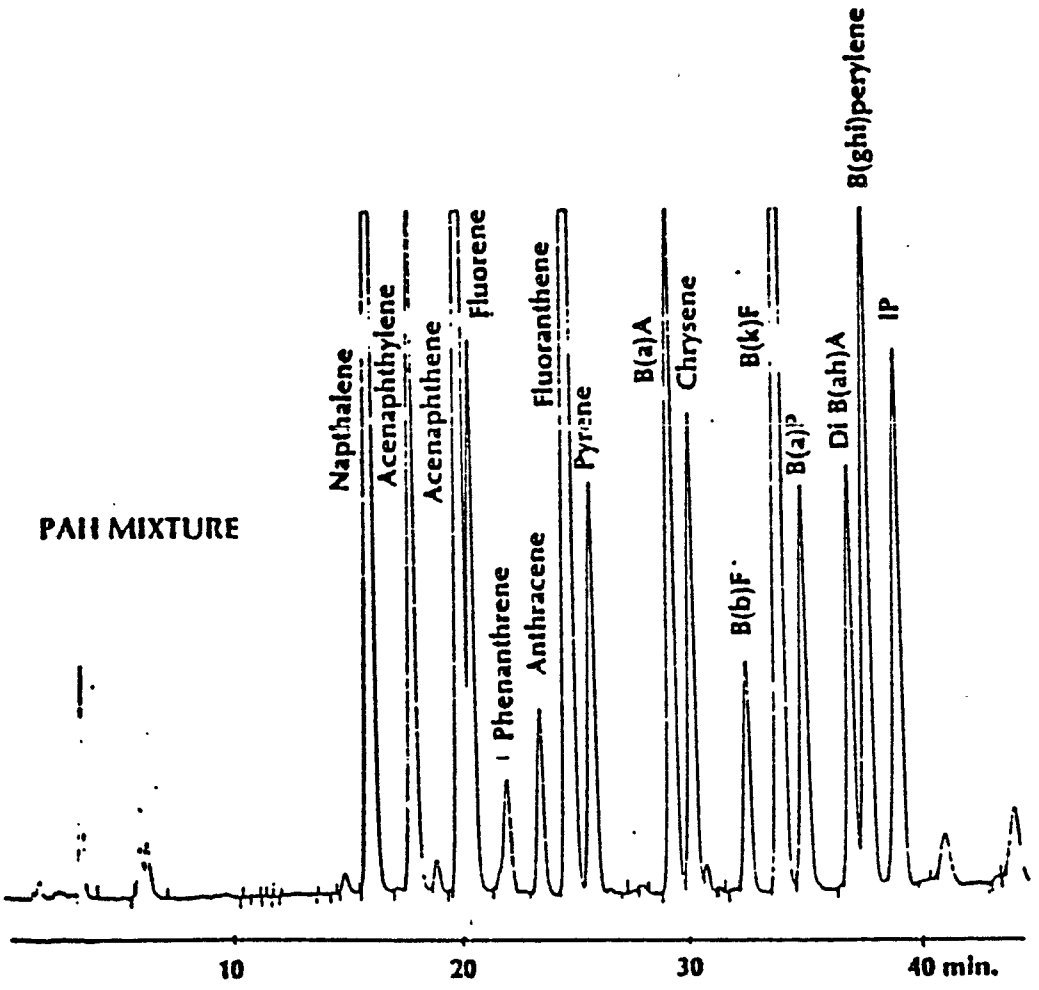
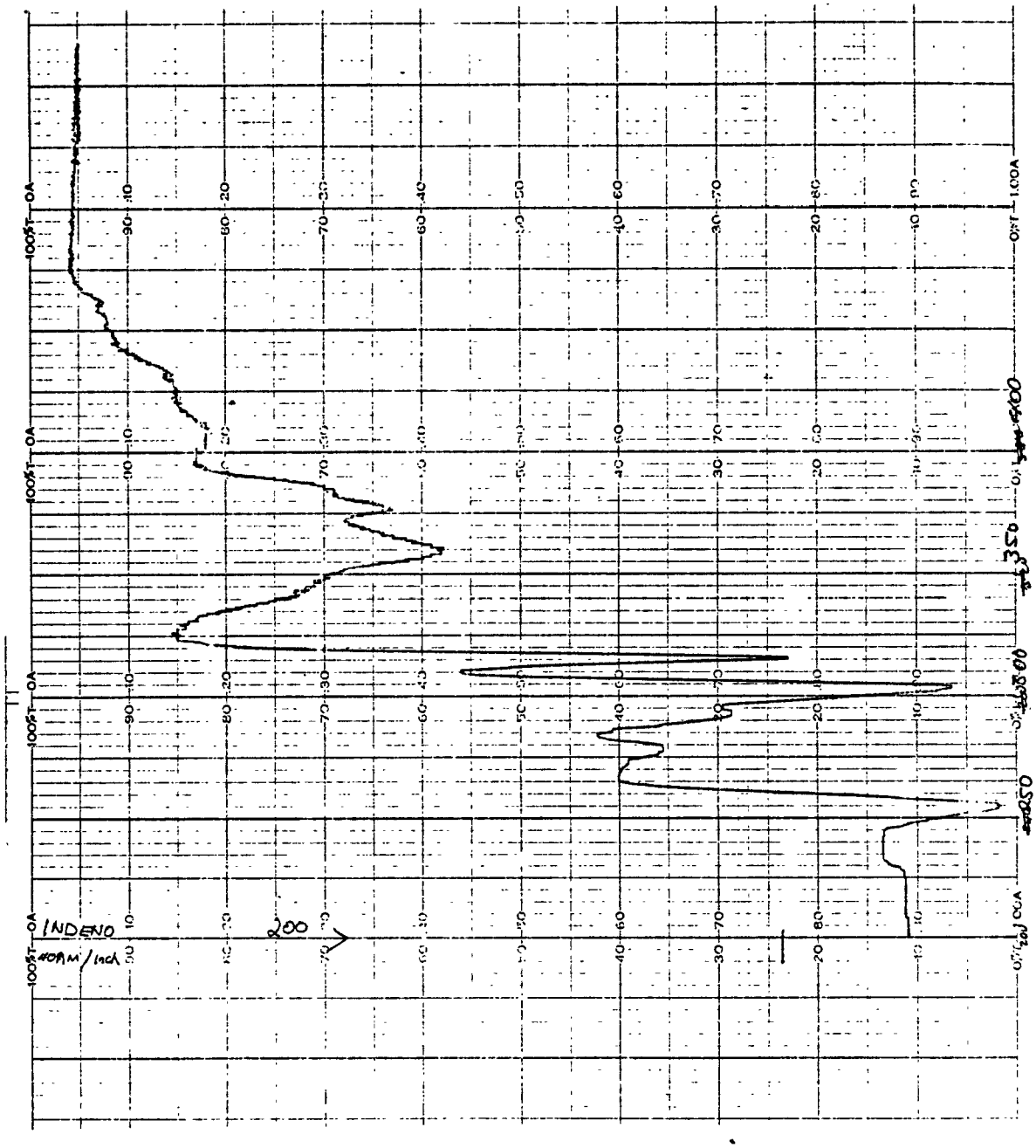


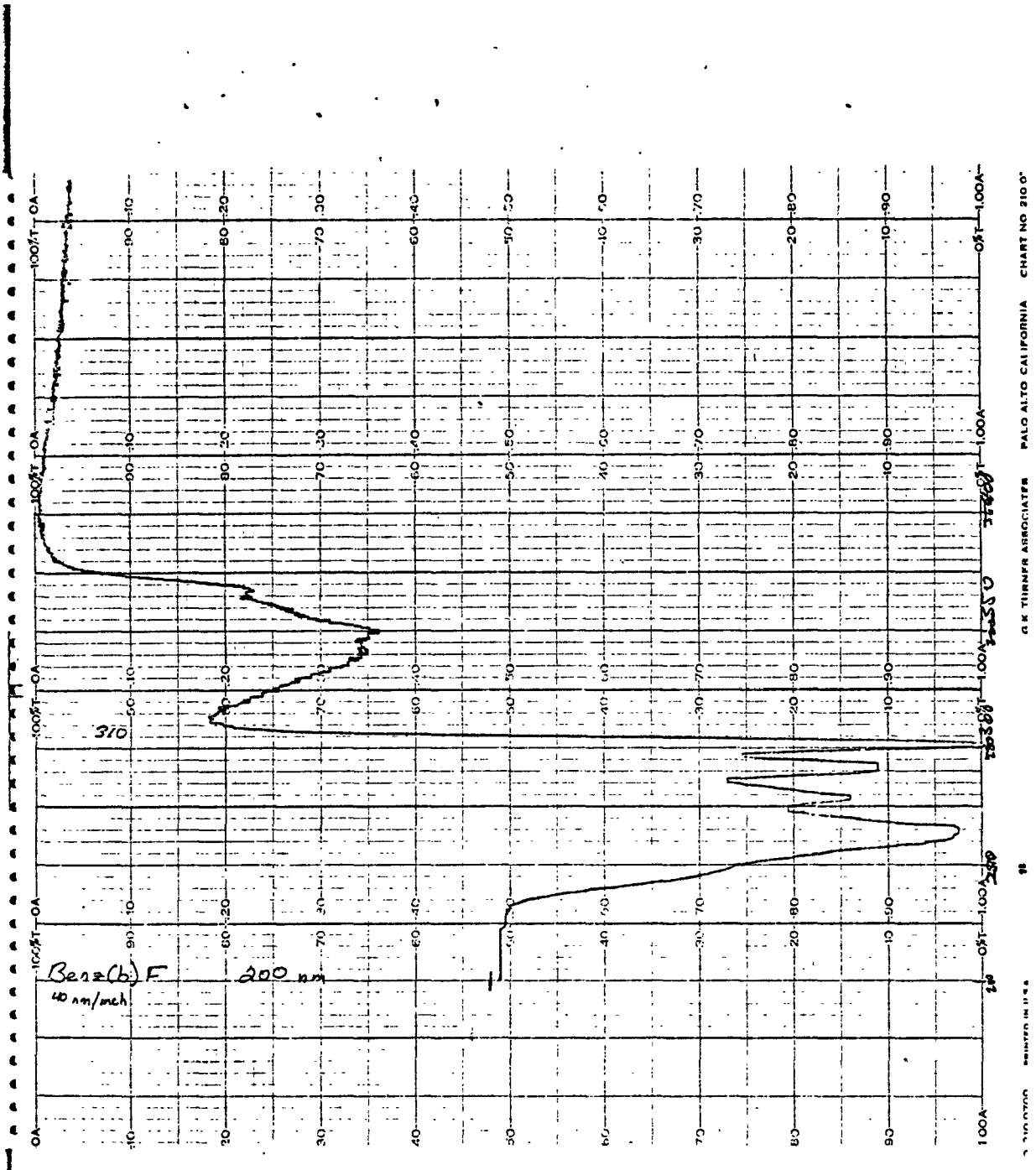
TABLE A-1 HPLC-Fluorescence of PAH's on Column 1.
For Conditions, See Table I.

APPENDIX B

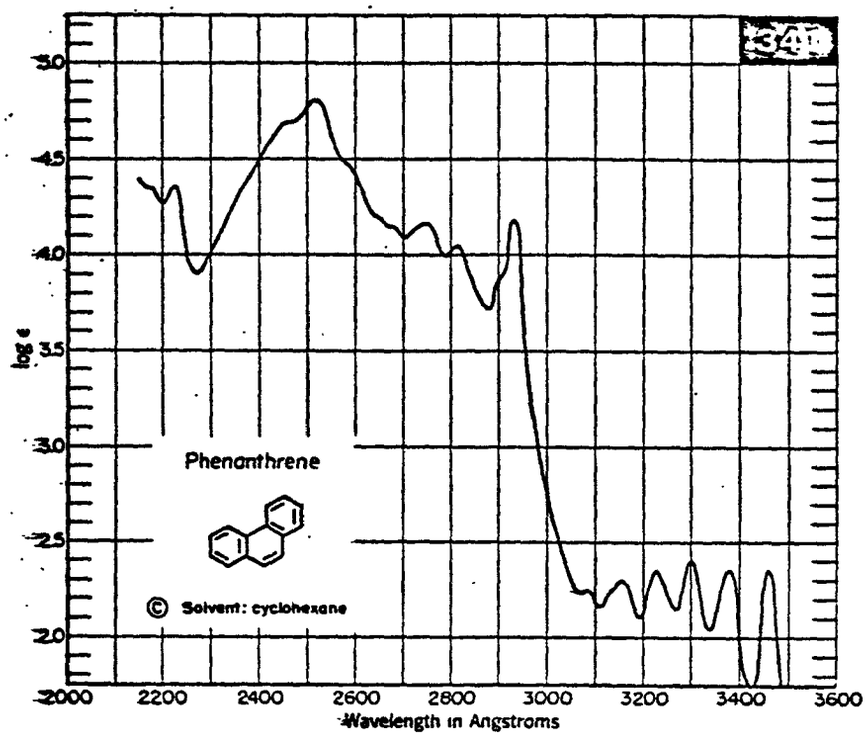
UV and Fluorescence Spectra of Sixteen Selected PNA's

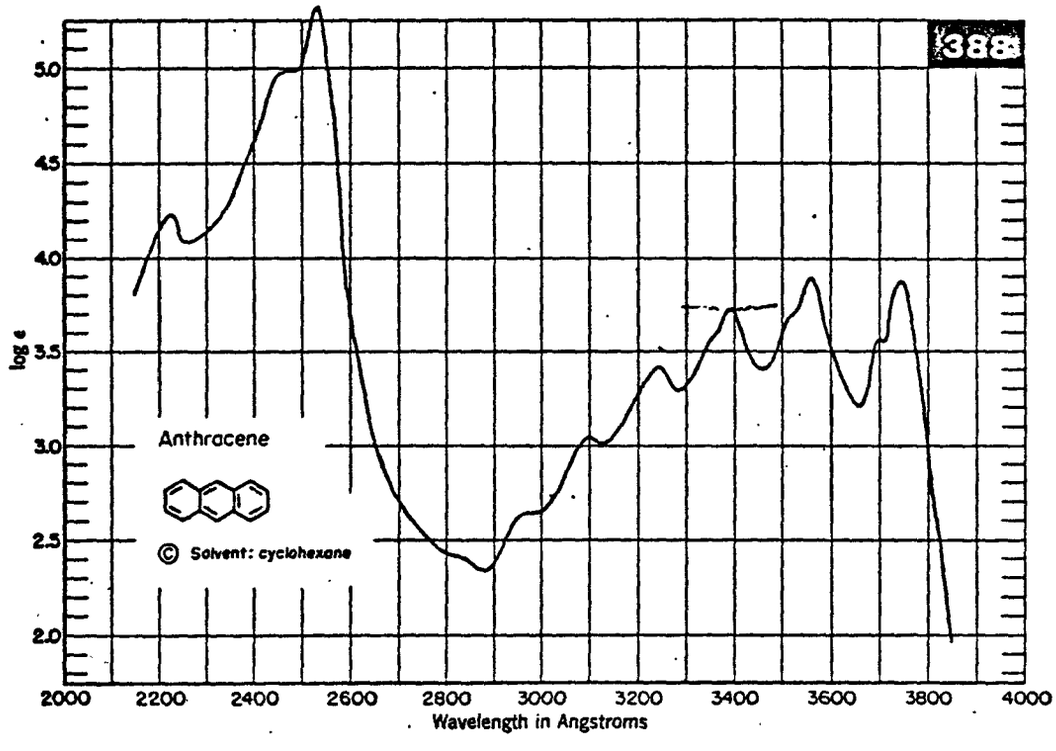


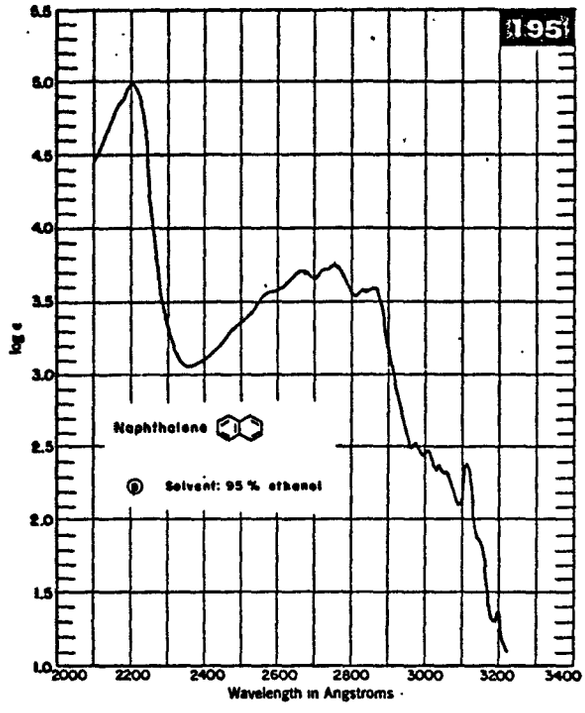
Indeno(1,2,3-cd)pyrene

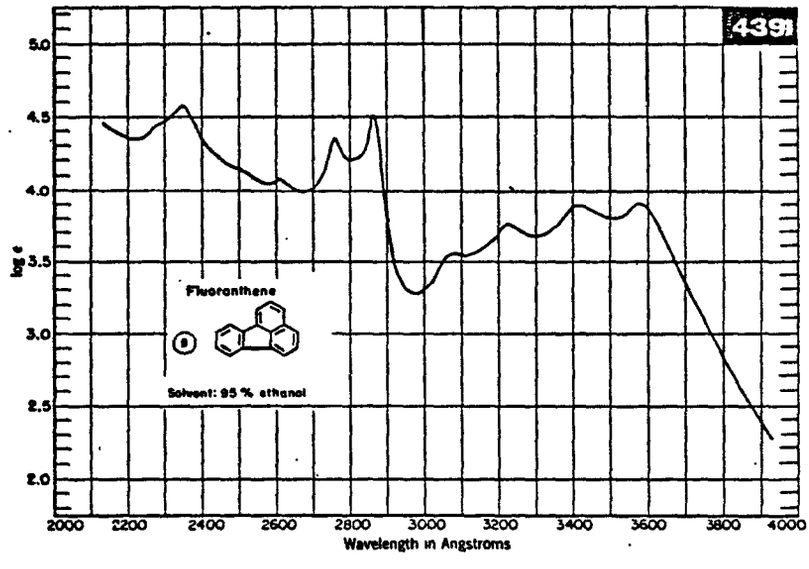


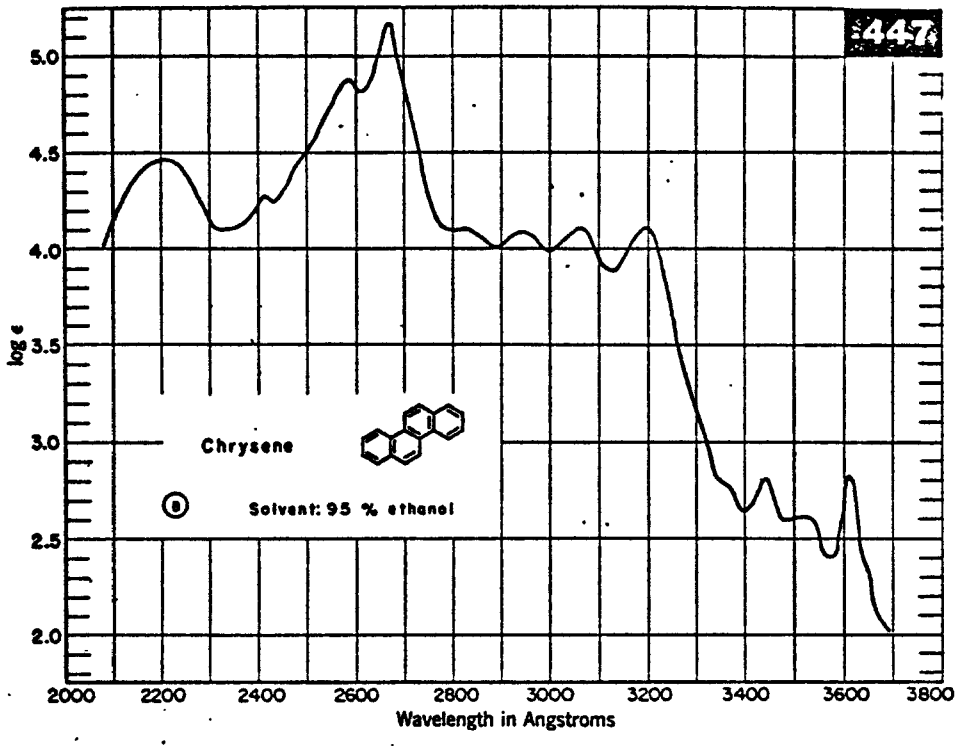
Benz(b)fluoranthene

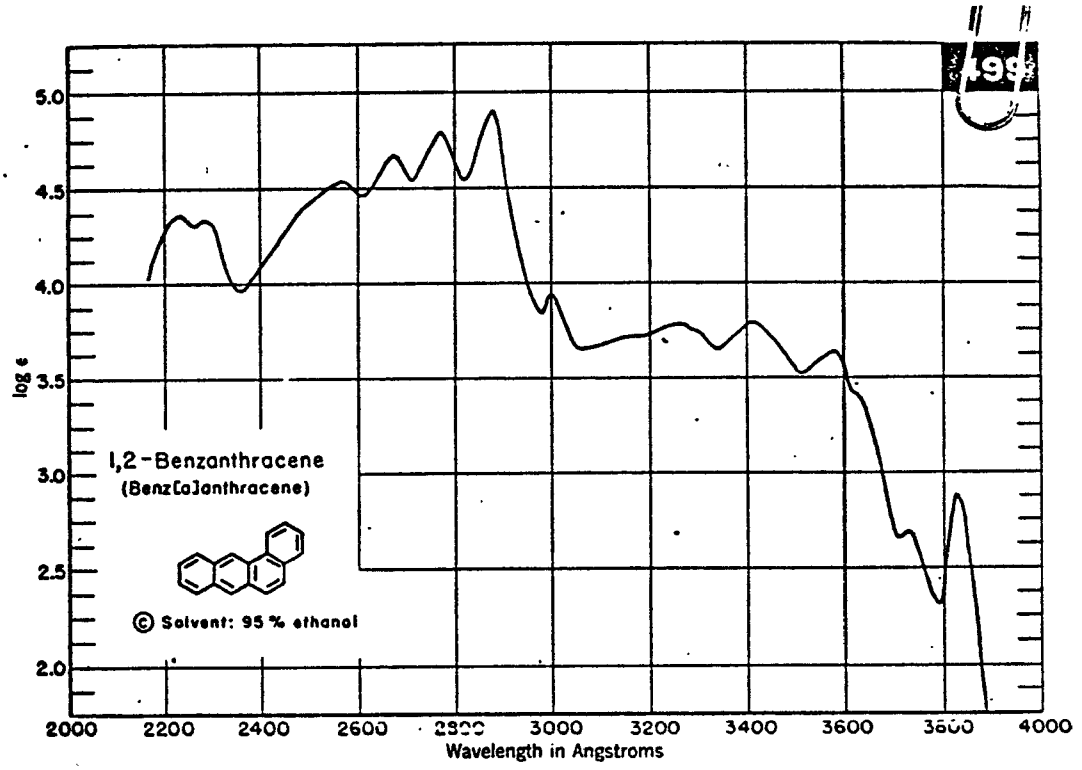

341
log ε
5.0
4.5
4.0
3.5
3.0
2.5
2.0
Phenanthrene
© Solvent: cyclohexane
2000
2200
2400
2600
2800
3000
3200
3400
3600
Wavelength in Angstroms

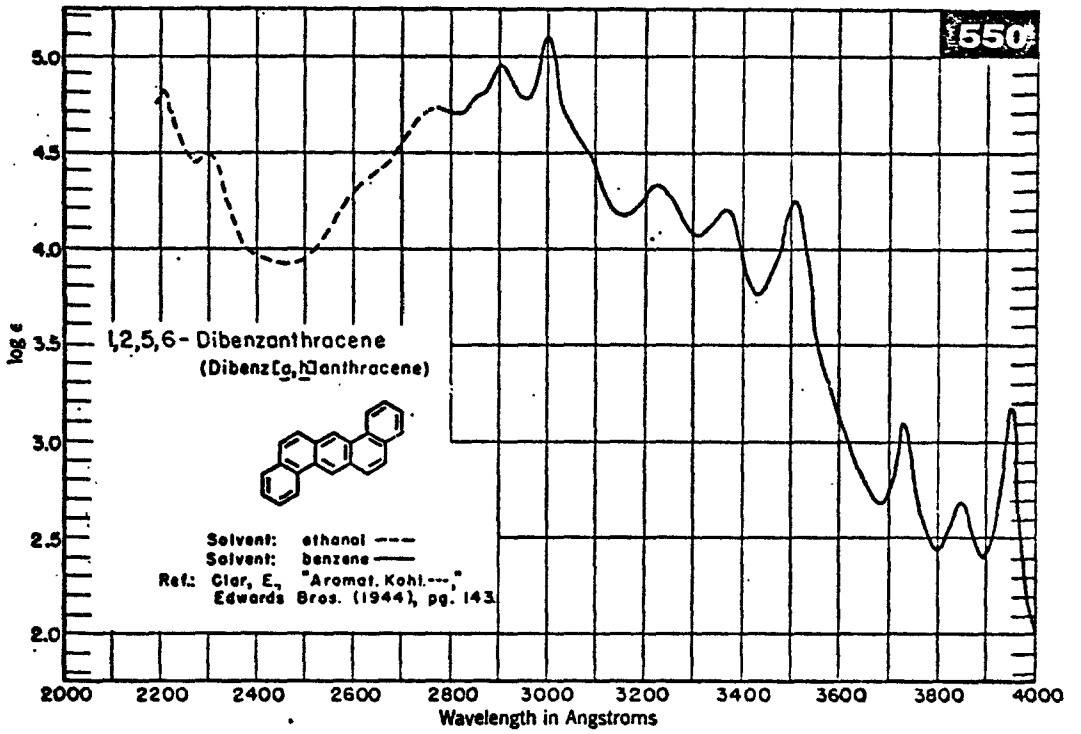

388
log ε
5.0
4.5
4.0
3.5
3.0
2.5
2.0
Anthracene
© Solvent: cyclohexane
2000
2200
2400
2600
2800
3000
3200
3400
3600
3800
4000
Wavelength in Angstroms

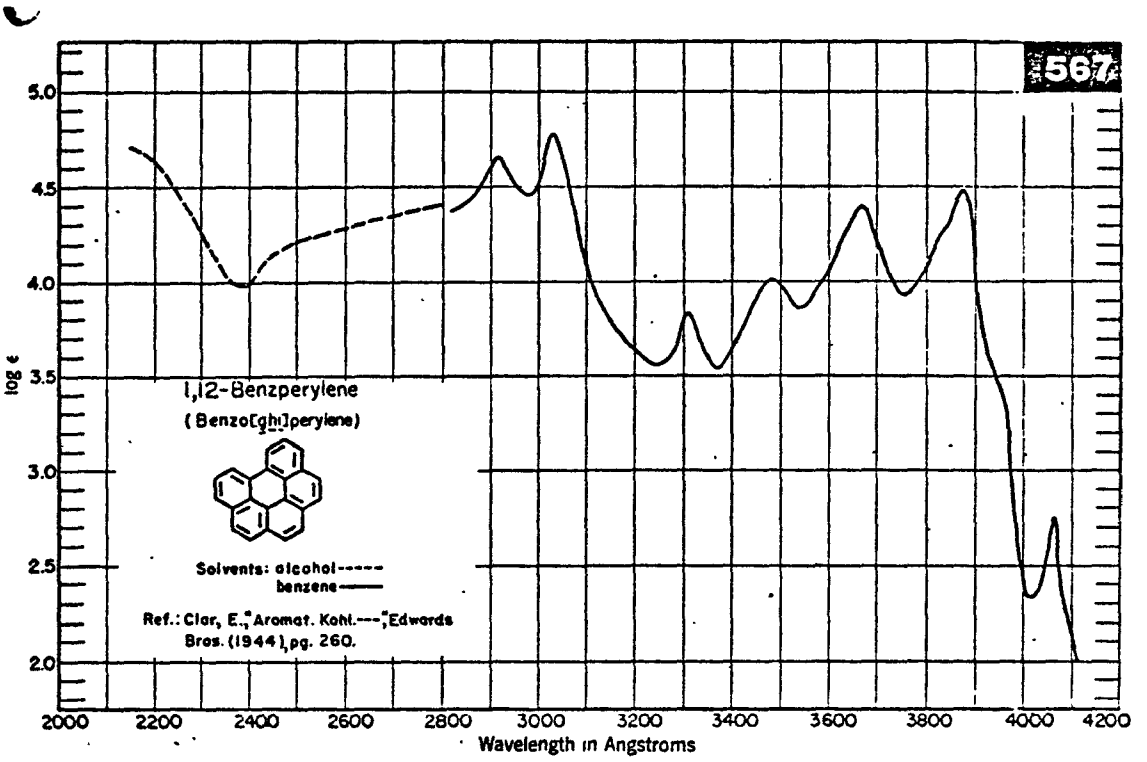

195
log ε
Naphthalene
Solvent: 95 % ethanol
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
2000 2200 2400 2600 2800 3000 3200 3400
Wavelength in Angstroms

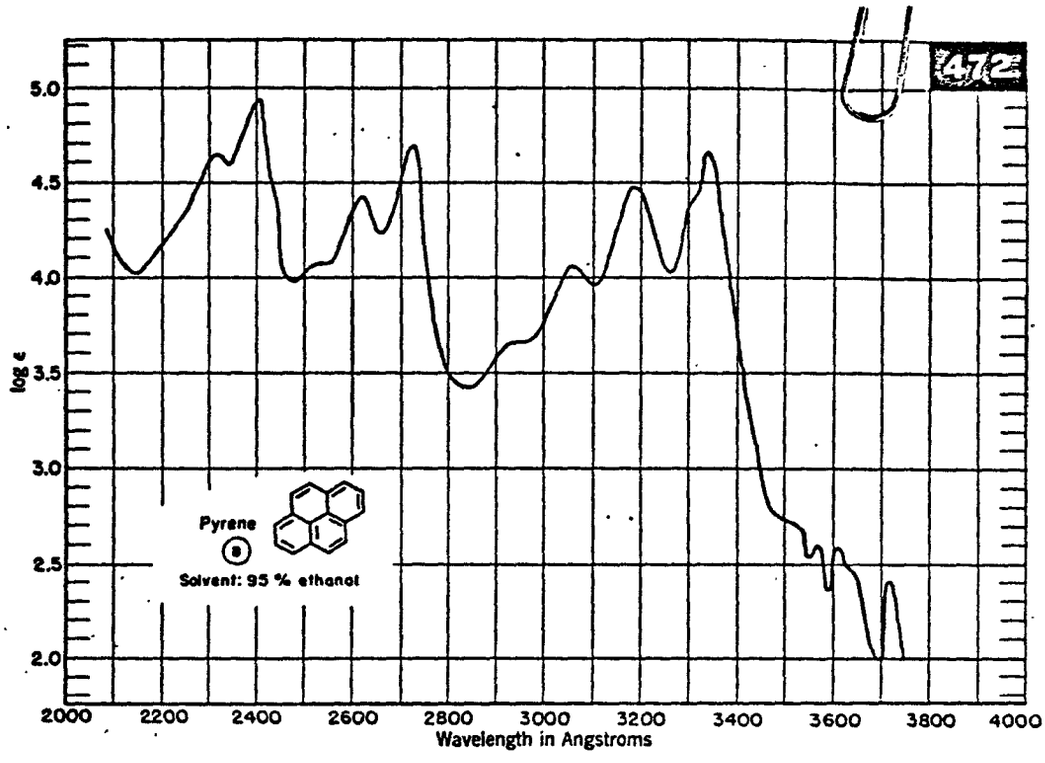

439
log ε
Fluoranthene
Solvent: 95 % ethanol
5.0
4.5
4.0
3.5
3.0
2.5
2.0
2000 2200 2400 2600 2800 3000 3200 3400 3600 3800 4000
Wavelength in Angstroms

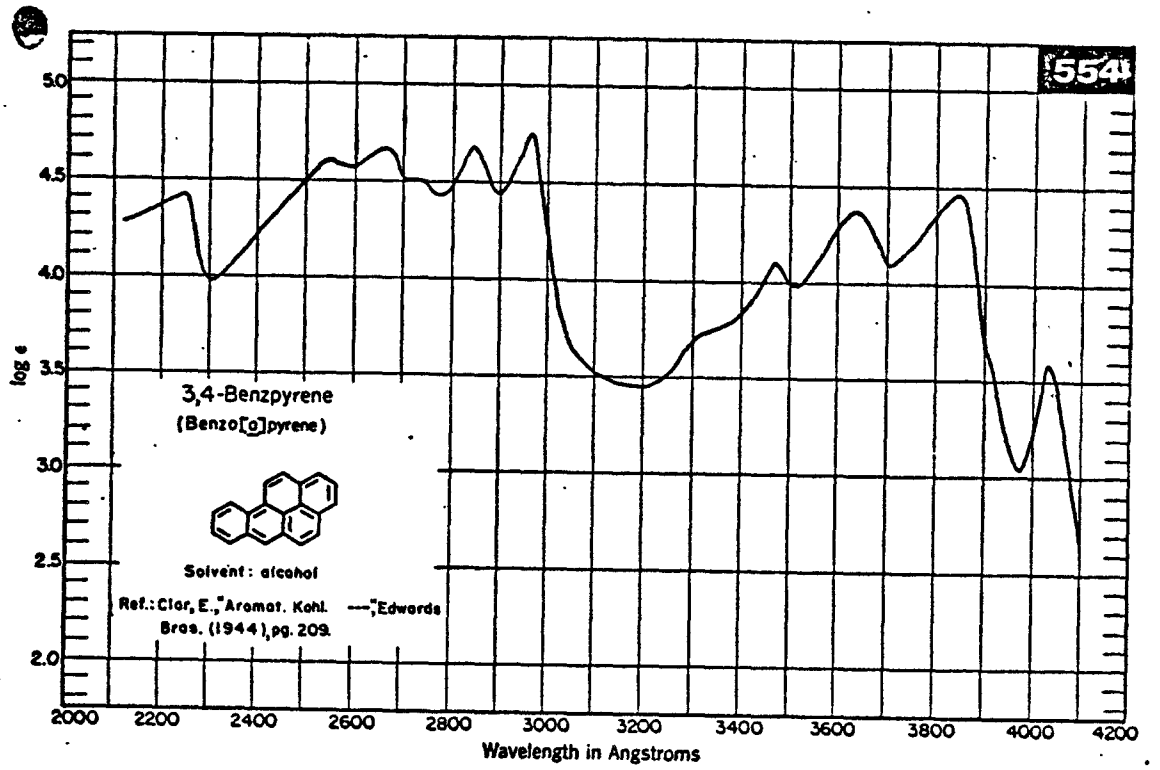

447
5.0
4.5
4.0
3.5
3.0
2.5
2.0
log ε
Chrysene
Solvent: 95 % ethanol
2000
2200
2400
2600
2800
3000
3200
3400
3600
3800
Wavelength in Angstroms

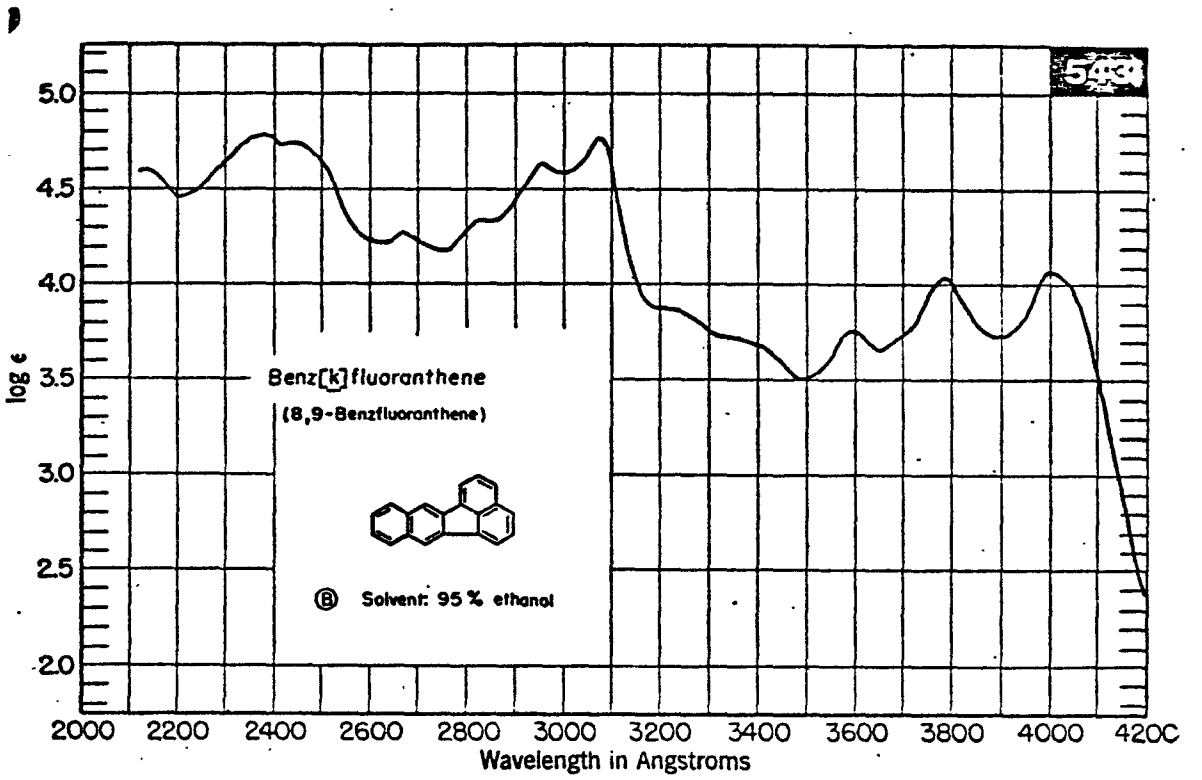

5.0
4.5
4.0
3.5
3.0
2.5
2.0
log ε
1,2-Benzanthracene
(Benz[a]anthracene)
Solvent: 95 % ethanol
2000
2200
2400
2600
3000
3200
3400
3600
4000
Wavelength in Angstroms

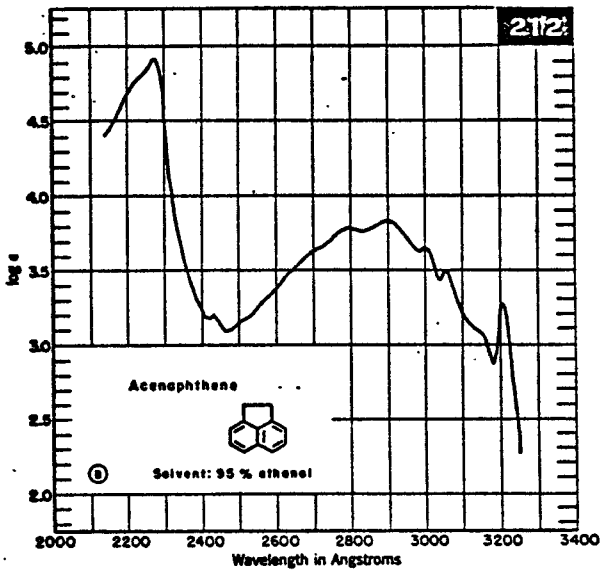

550
log ε
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1,2,5,6- Dibenzanthracene
(Dibenz[a,h]anthracene)
Solvent: ethanol ----
Solvent: benzene ——
Ref: Clar, E., "Aromat. Kohl.---,"
Edwards Bros. (1944), pg. 143.
2000 2200 2400 2600 2800 3000 3200 3400 3600 3800 4000
Wavelength in Angstroms

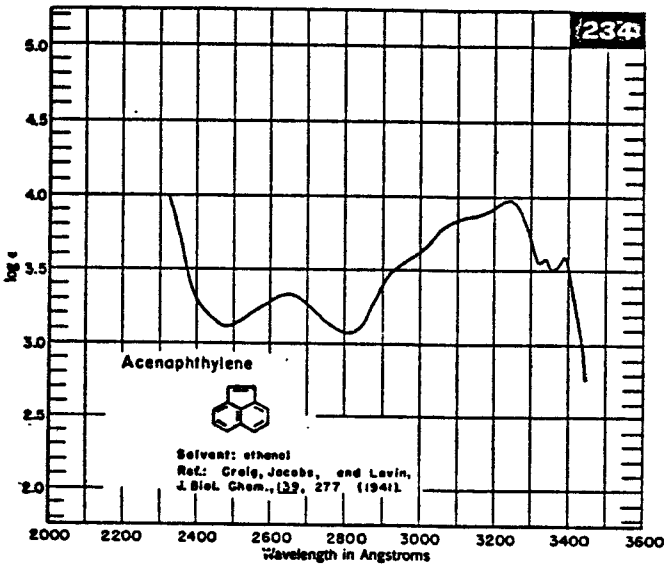

567
log ε
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1,12-Benzperylene
(Benzo[ghi]perylene)
Solvents: alcohol-----
benzene——
Ref.: Clar, E.,"Aromat. Kohl.---",Edwards
Bros. (1944), pg. 260.
2000 2200 2400 2600 2800 3000 3200 3400 3600 3800 4000 4200
Wavelength in Angstroms

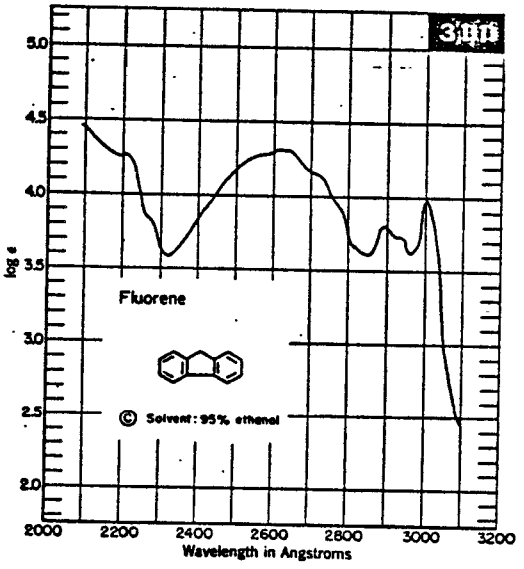

472
5.0
4.5
4.0
log ε 3.5
3.0
2.5
2.0
Pyrene
Solvent: 95 % ethanol
2000
2200
2400
2600
2800
3000
3200
3400
3600
3800
4000
Wavelength in Angstroms

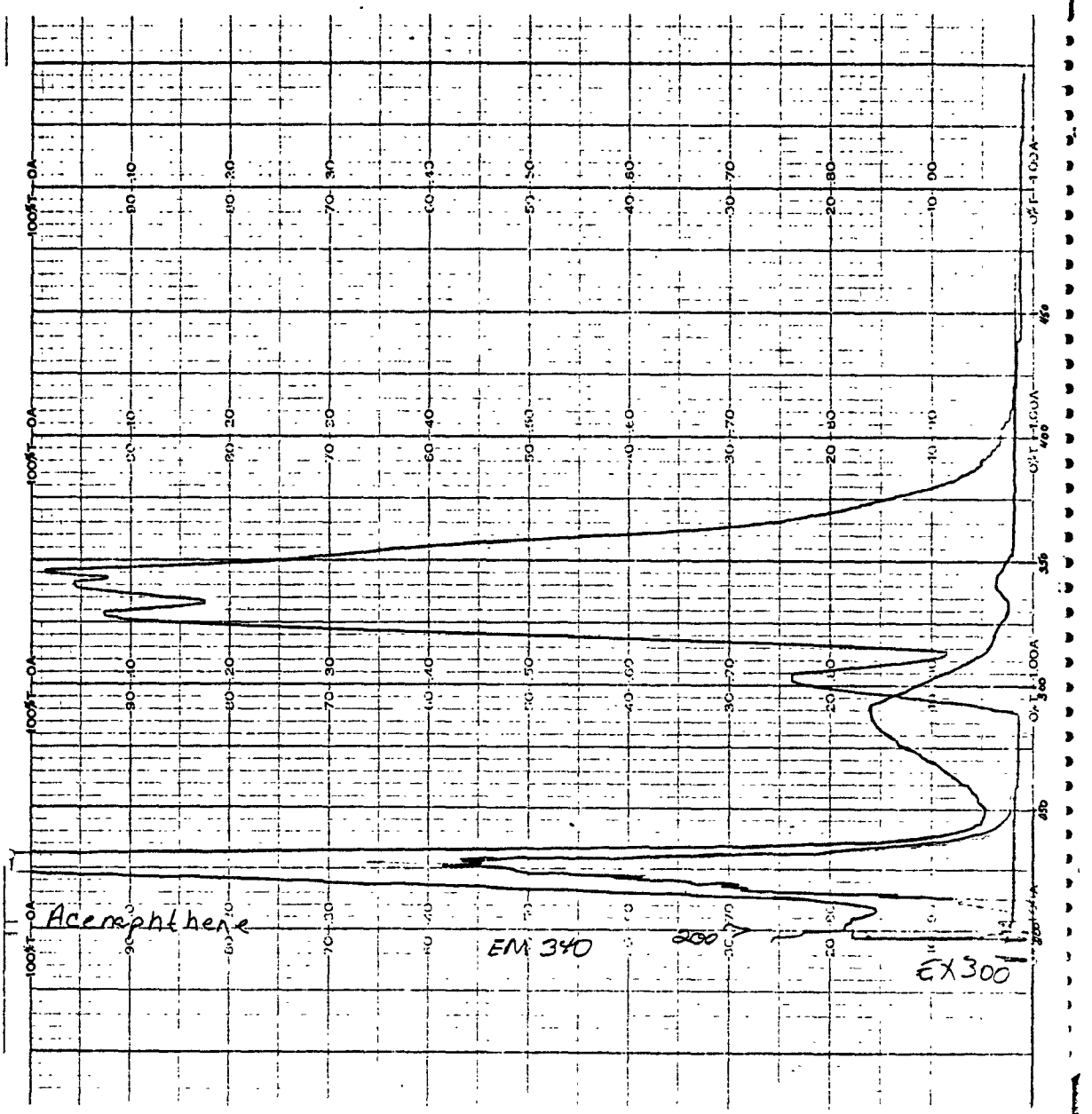

554
3,4-Benzpyrene
(Benzo[a]pyrene)
Solvent: alcohol
Ref.: Clar, E., "Aromat. Kohl. ---"; Edwards Bros. (1944), pg. 209.
log ε
5.0
4.5
4.0
3.5
3.0
2.5
2.0
2000 2200 2400 2600 2800 3000 3200 3400 3600 3800 4000 4200
Wavelength in Angstroms


544
Benz[k]fluoranthene
(8,9-Benzfluoranthene)
Ⓑ Solvent: 95 % ethanol
log ε
5.0
4.5
4.0
3.5
3.0
2.5
2.0
2000 2200 2400 2600 2800 3000 3200 3400 3600 3800 4000 420C
Wavelength in Angstroms

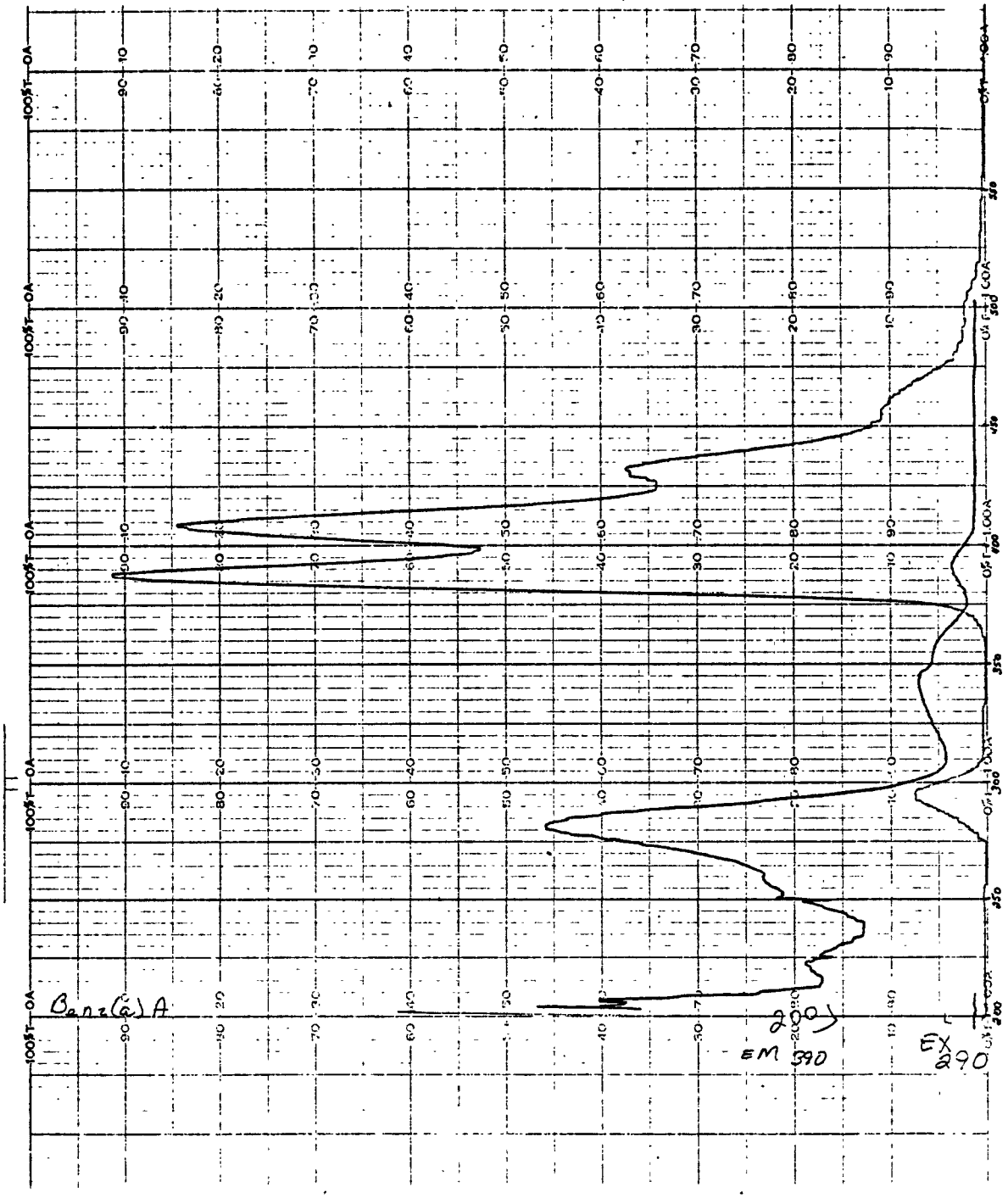

2112
log ε
5.0
4.5
4.0
3.5
3.0
2.5
2.0
Acenaphthene
B
Solvent: 95 % ethanol
2000 2200 2400 2600 2800 3000 3200 3400
Wavelength in Angstroms


2340
log ε
5.0
4.5
4.0
3.5
3.0
2.5
2.0
Acenaphthylene
Solvent: ethanol
Ref.: Craig, Jacobs, and Levin,
J. Biol. Chem., 139, 277 (1941)
2000 2200 2400 2600 2800 3000 3200 3400 3600
Wavelength in Angstroms

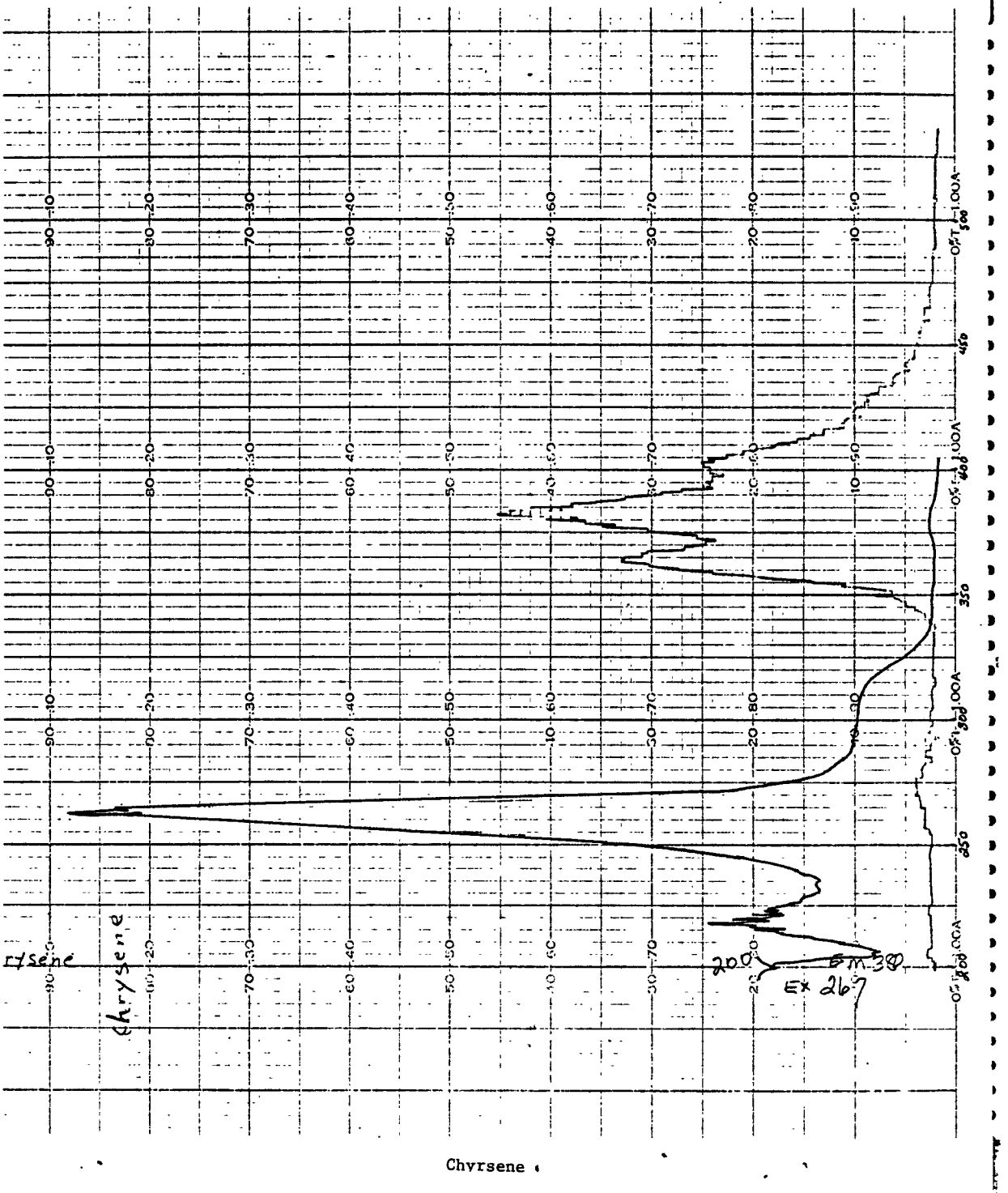

3111
log ε
5.0
4.5
4.0
3.5
3.0
2.5
2.0
Fluorene
C
Solvent: 95% ethanol
2000 2200 2400 2600 2800 3000 3200
Wavelength in Angstroms

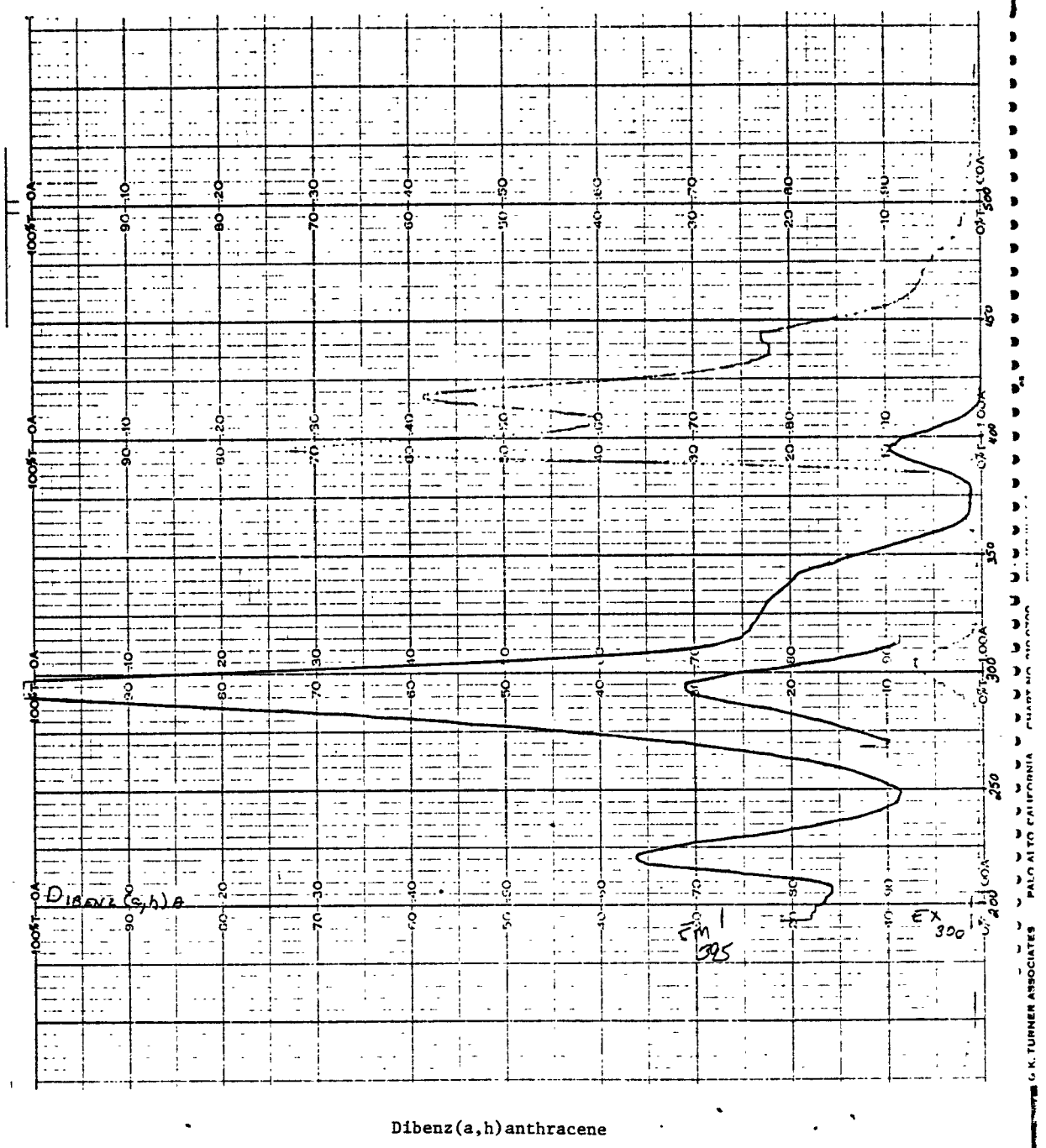


Acenaphthene



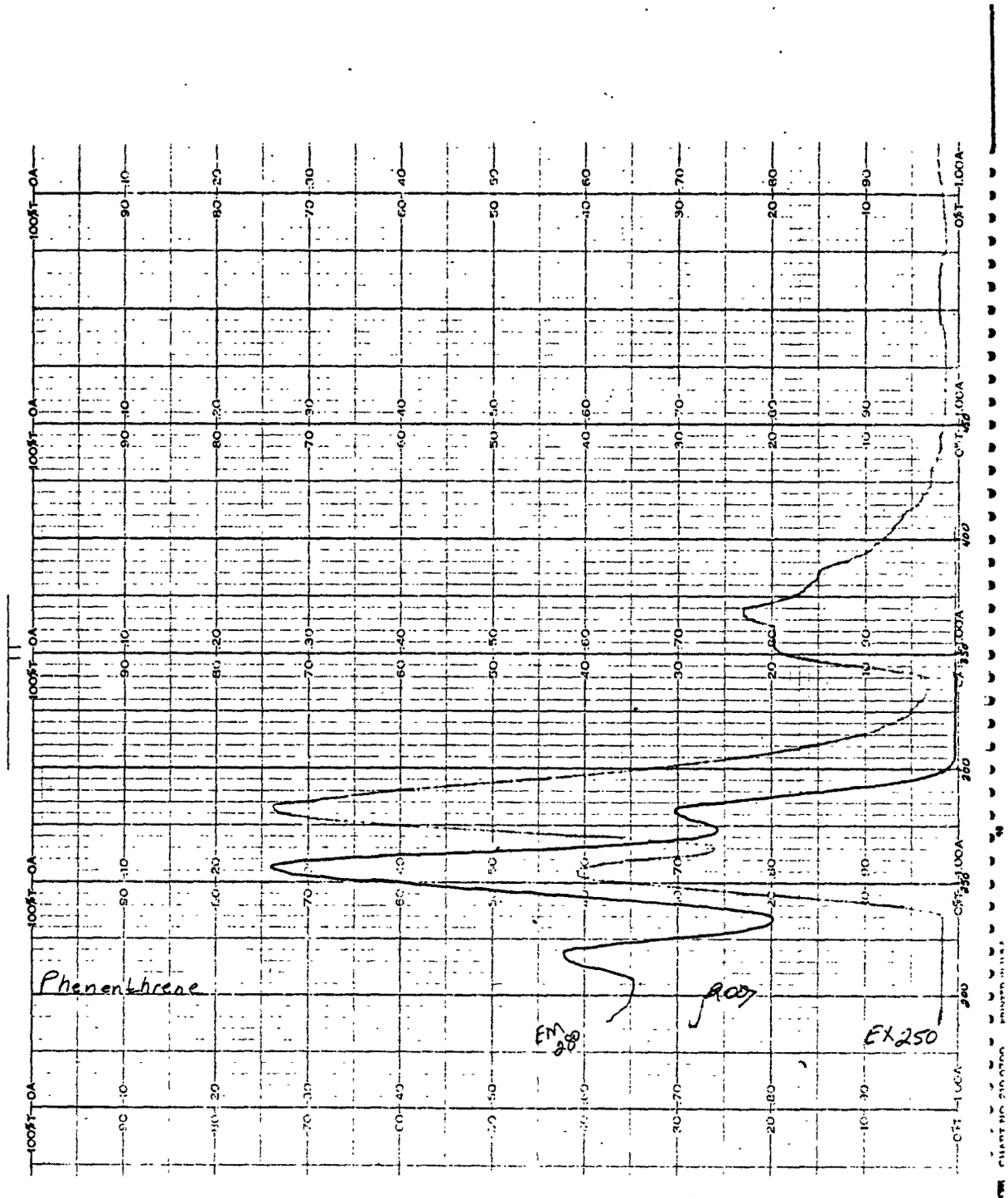
Benz(a)Anthracene


Chrysene
Em 38
Ex 267
Chvrsene

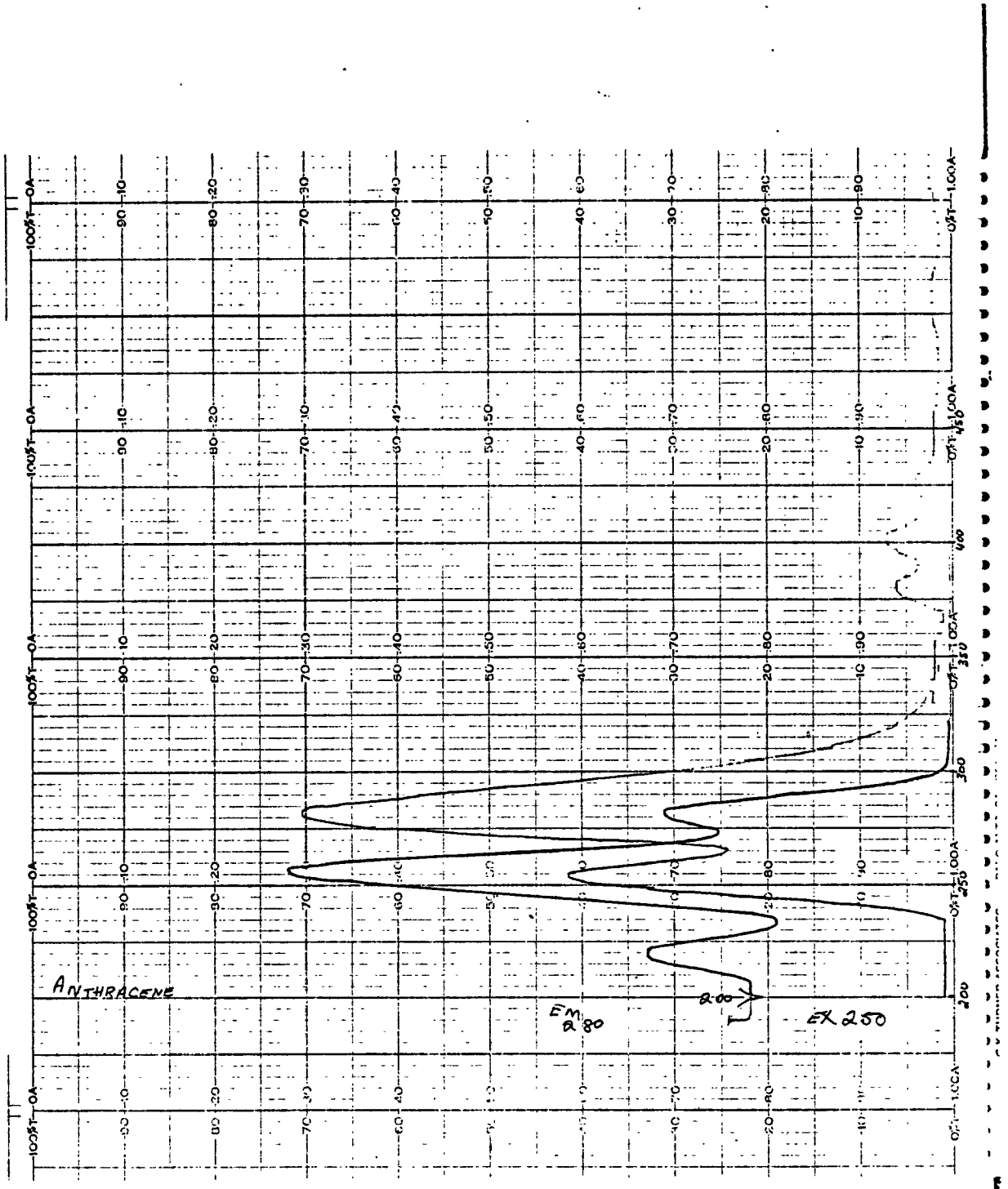


Dibenz(a,h)anthracene

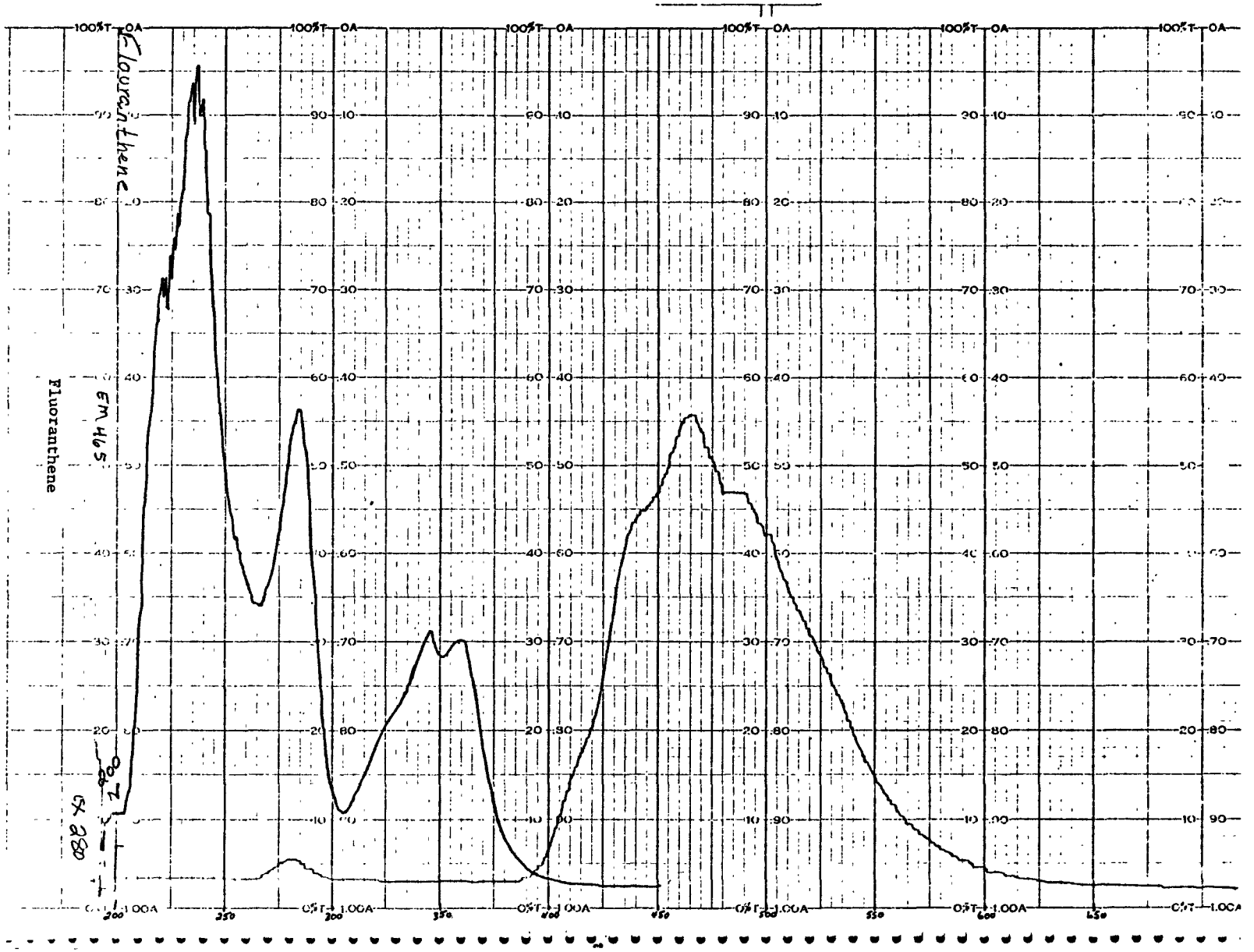
Benzo(g,h,i)perylene

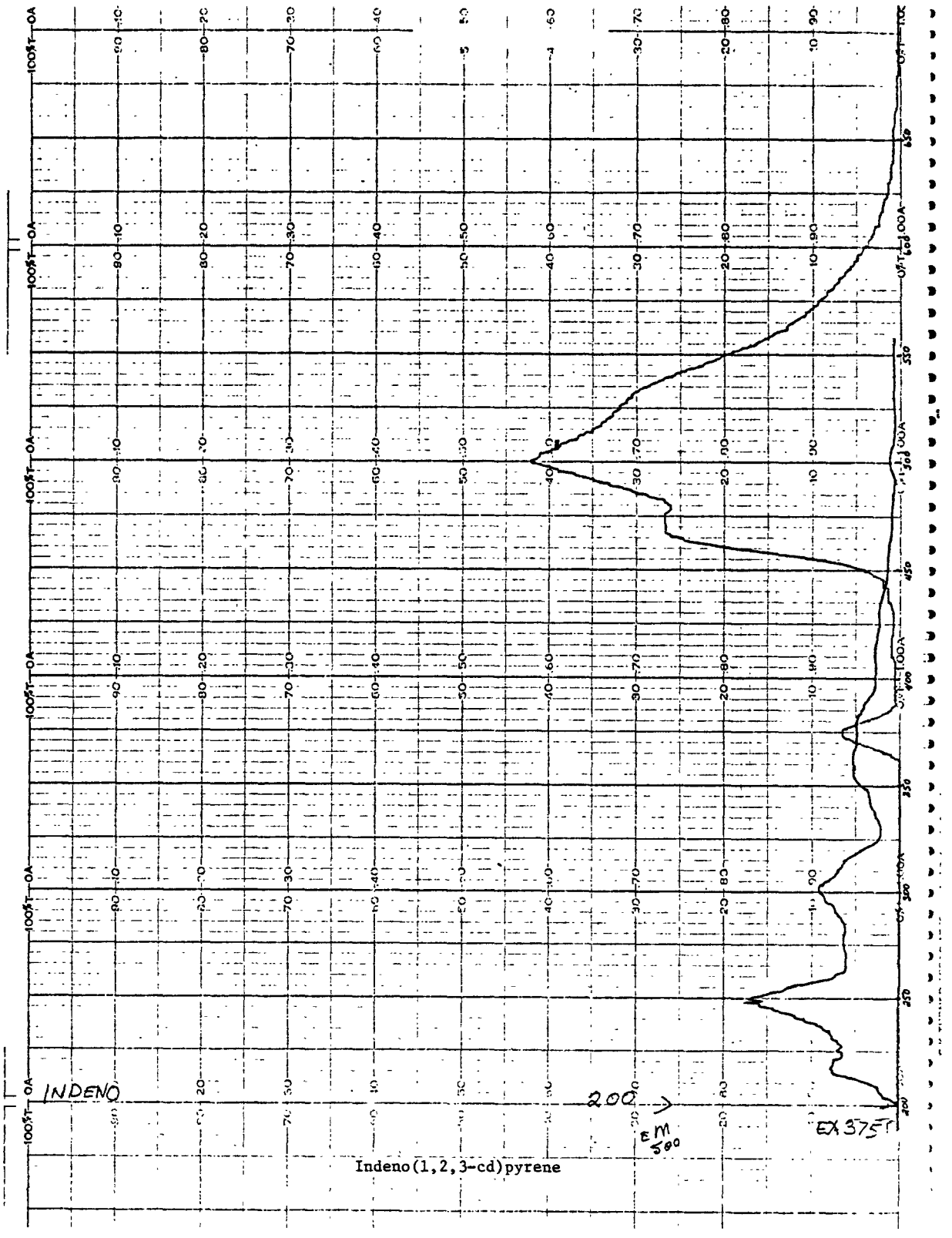


Phenanthrene

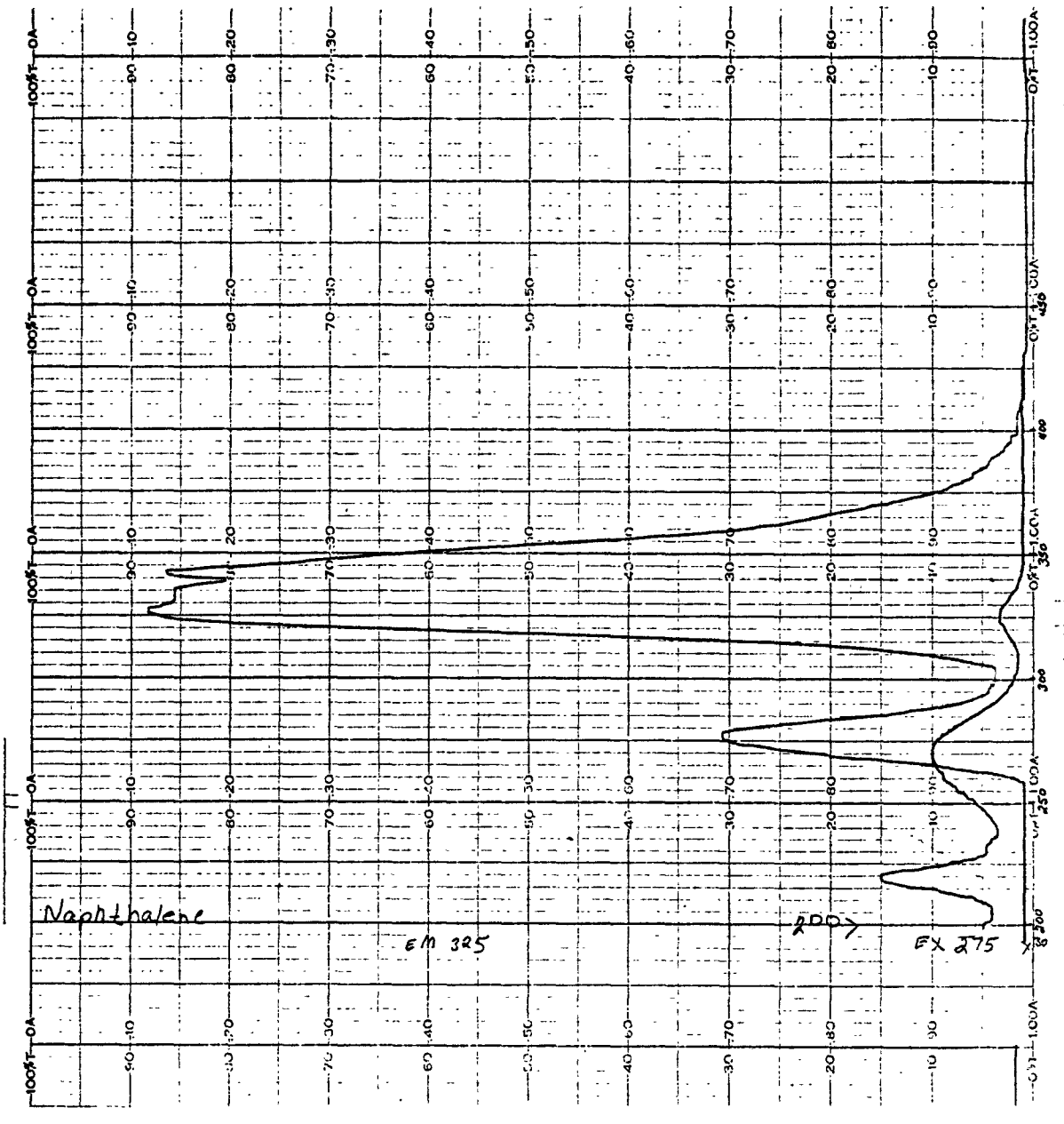


Anthracene

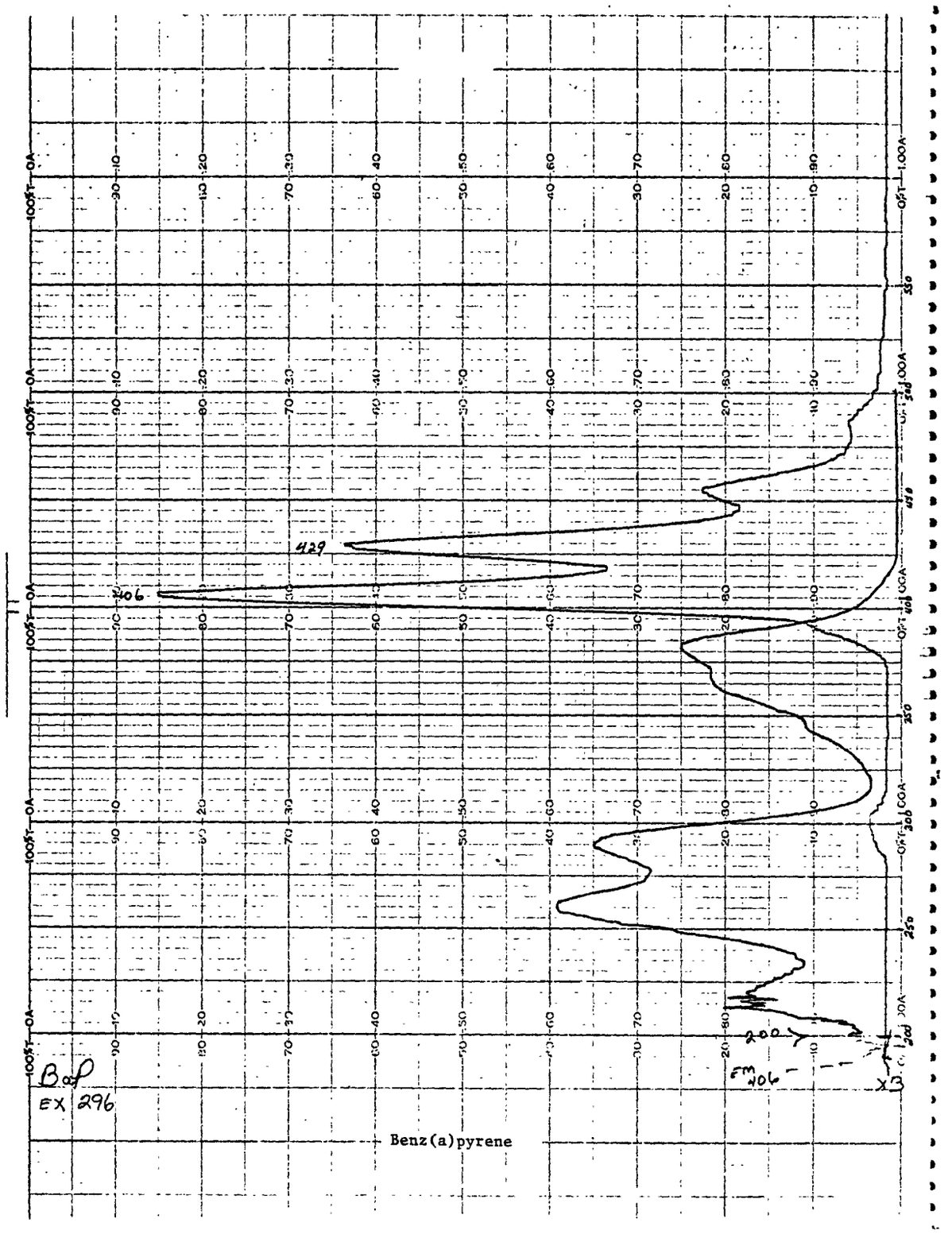

Fluoranthene
EM 465
EX 280

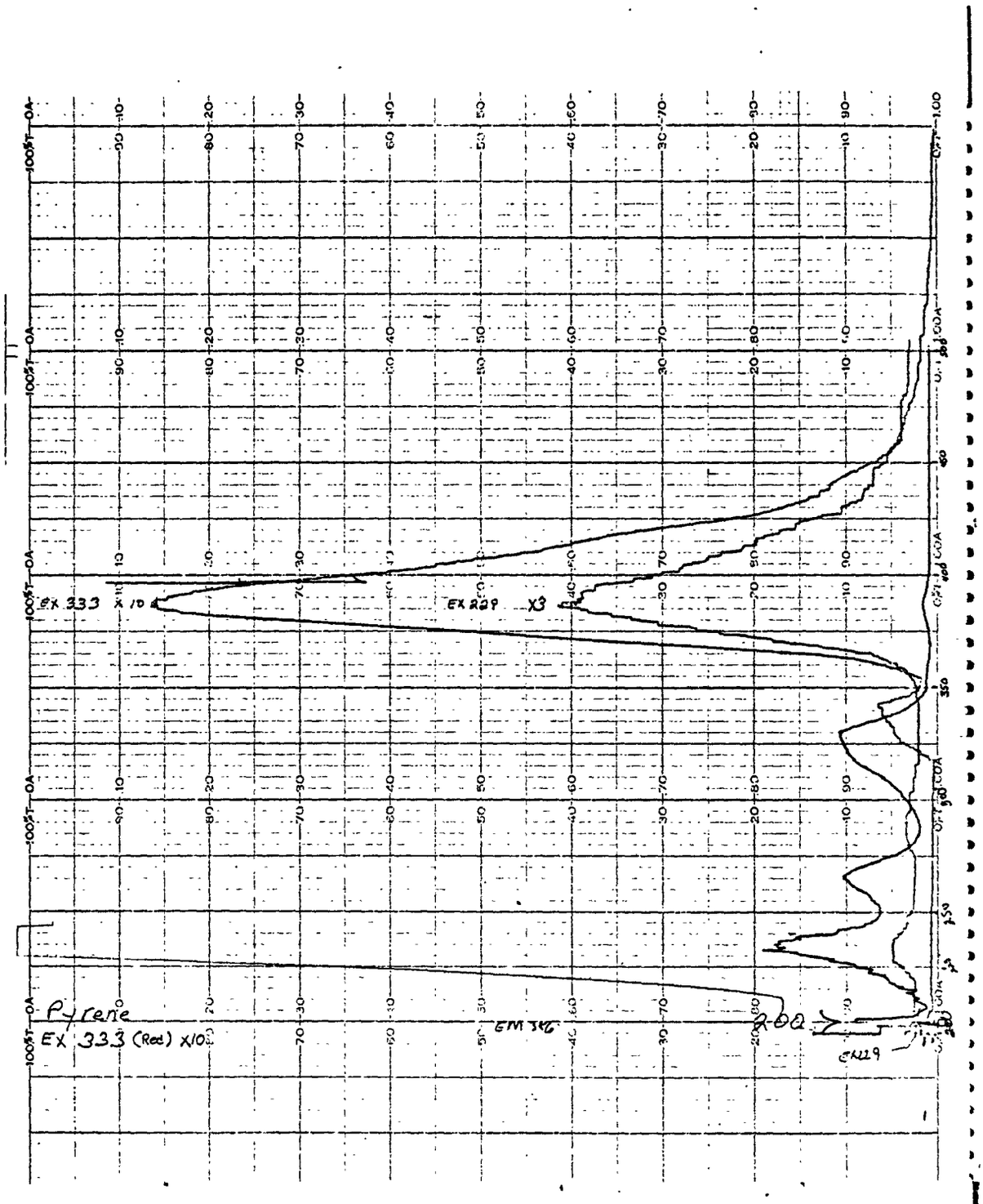


Indeno(1,2,3-cd)pyrene

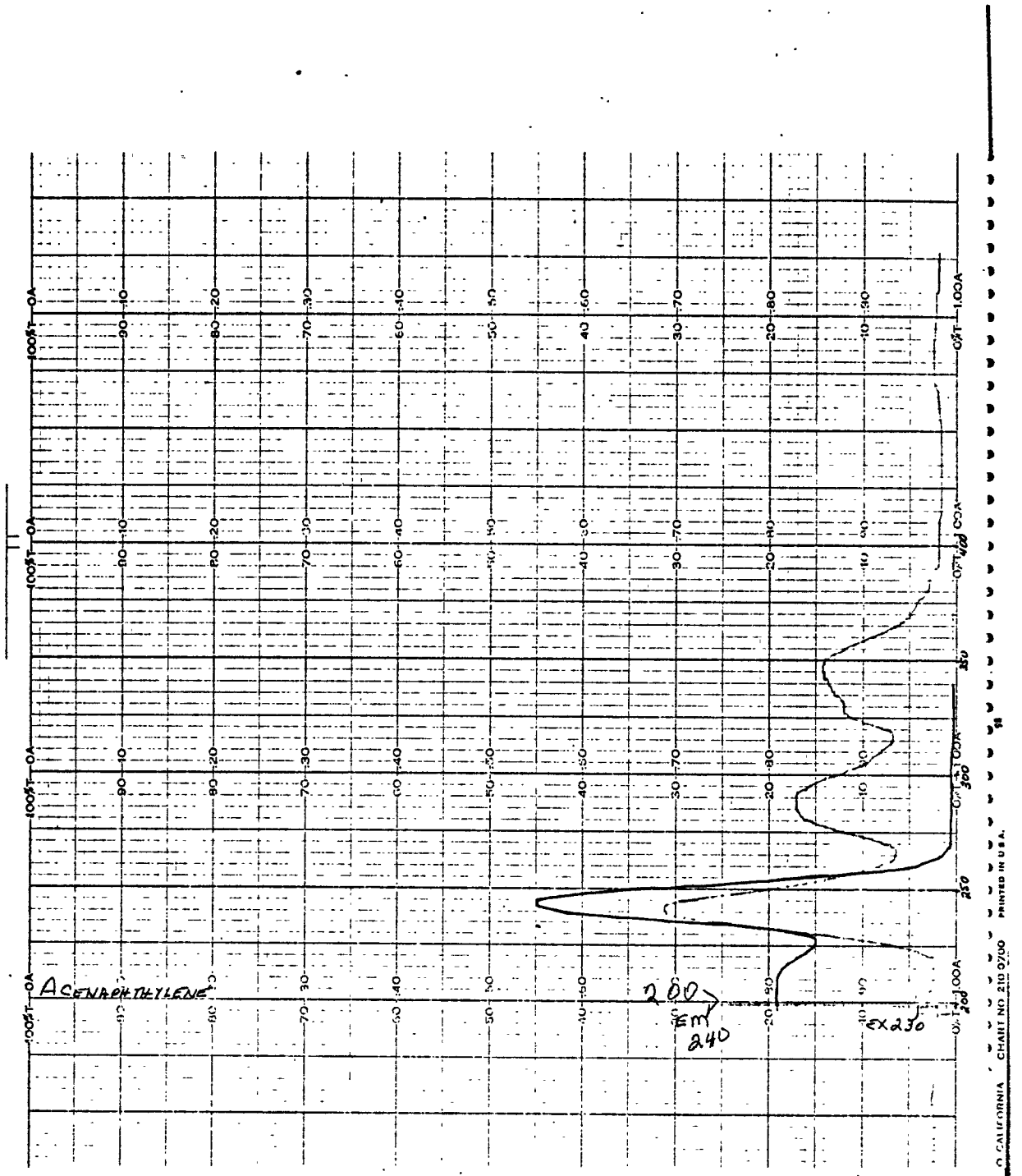


Naphthalene

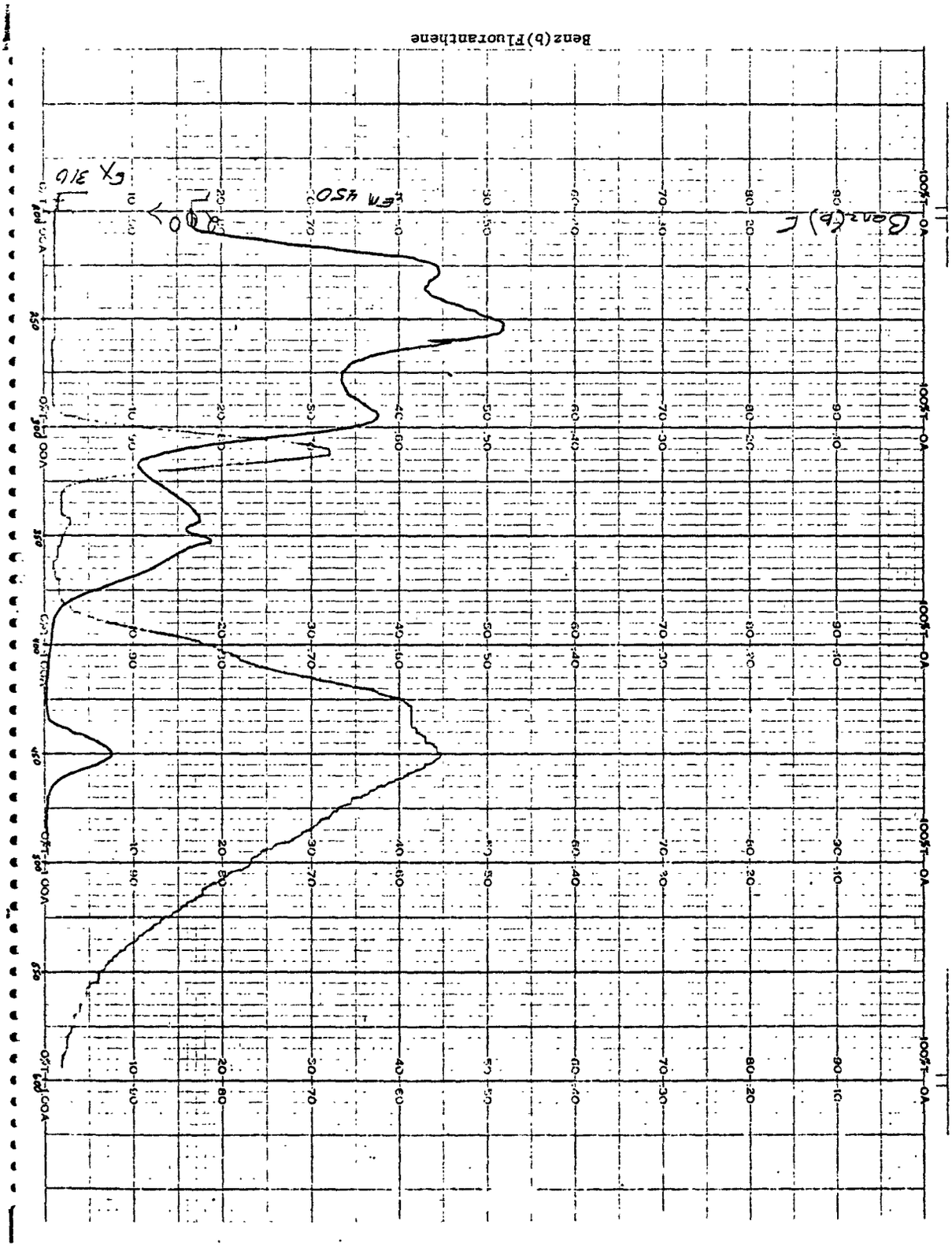

429
406
200
FM 406
BaP
EX 296
Benz(a)pyrene

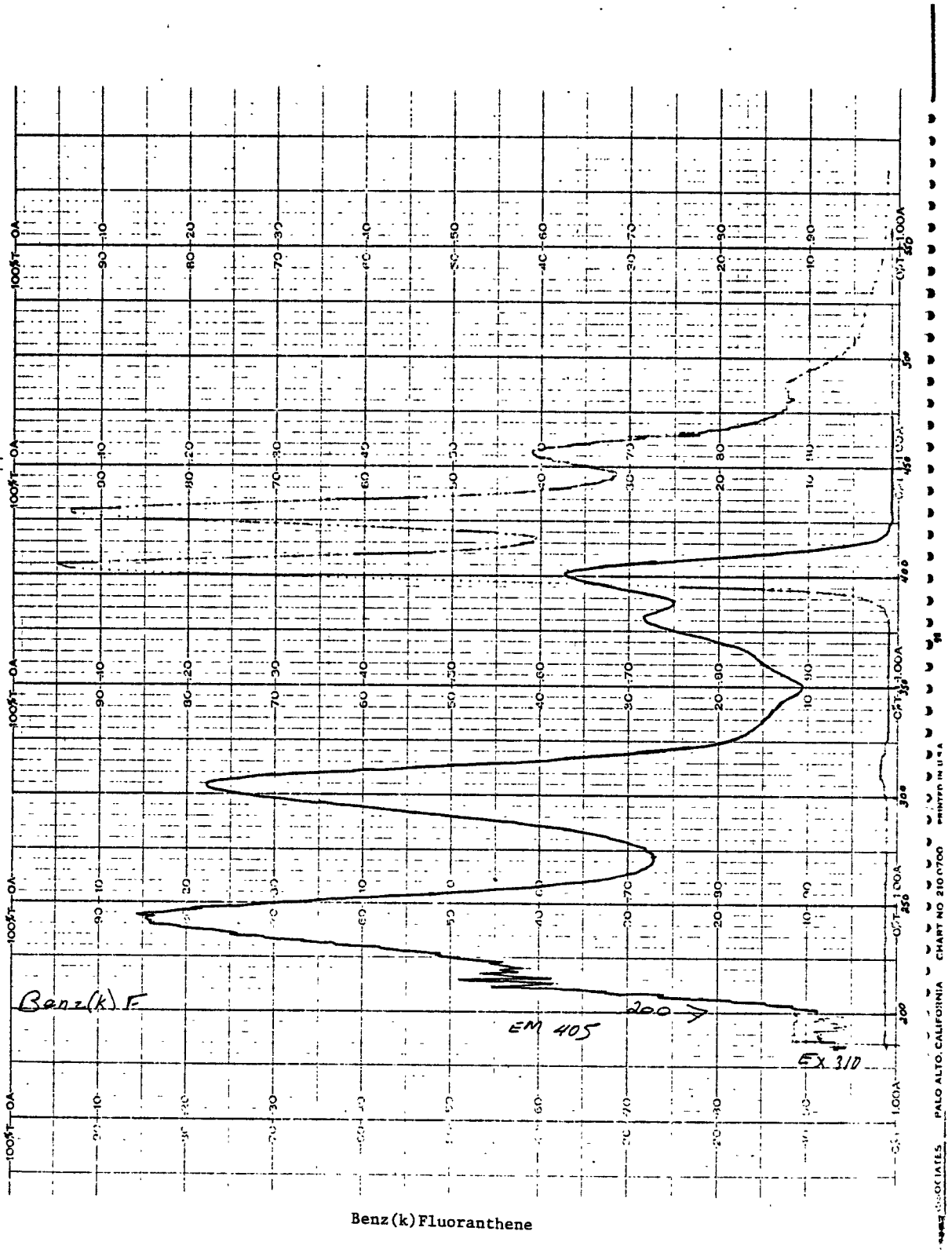


Pyrene



Acenaphthylene


Benz(b)Fluoranthene
Benz(b) F
EX 310
EM 450



Benz(k)Fluoranthene