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Studies of Nitro-PAH on Ambient Air Particulate Matter

by

Yadan Wang Chen

Thesis submitted to the Faculty of the Graduate School
of New Jersey Institute of Technology
in partial fulfillment of the requirements
of the degree of Master of Science
in Chemistry

1989 90

APPROVAL SHEET

Title of thesis: Studies of Nitro-PAH on Ambient Air Particulate Matter

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I specially thank my husband for his patient understanding and help in the last two monthes when I was in a crazy mood working on my thesis.

Contents

ABSTRACT

Title of thesis: Studies of Nitro-PAH on Ambient Air Particulate Matter

Yadan Wang Chen, Master of Science in Chemistry, 1989 90

Thesis directed by: Dr. Arthur Greenberg

A rapid, simple method for routine trace analysis of four isomeric nitropolycyclic aromatic hydrocarbons (nitro-PAH) (1-nitropyrene, 2-nitropyrene, 2-nitrofluoranthene and 3-nitrofluoranthene) in ambient air particulate matter is described. Two types of air particle sample (TSP and IP10) were collected and particle extracts were prefractionated by thin layer chromatography and the appropriate zones of interest analyzed by reversed-phase HPLC with UV and fluorescence detection. The study of Zn/Si post-column reaction chamber and oxygen scrubber for catalytic reduction of target compounds is also described. Six nitro-PAH compounds were identified in TSP and five in IP10 air particle samples. The results of identification and quantification are discussed.

Chapter One

Introduction

Widespread interest in the analytical chemistry of nitrated polycyclic aromatic hydrocarbons (nitro-PAH) is a result of the extraordinary mutagenicity displayed by some members of this compound class. Nitrated pyrenes have been reported to be among the most potent chemical mutagens [1,2]. The first positive identification of a nitro-PAH compound (1-nitropyrene) in an environmental sample was reported by Schuetzle in 1980 [3,4] using a combination of HPLC, GC/MS, and HRMS techniques. In recent years, excellent research from Pitts'[5,6] group has identified 2-nitropyrene and 2-nitrofluoranthene as significant airborne mutagens formed via gas phase reaction of the corresponding PAH with N_2O_5 and/or OH/NO_2 [7,8,9]. The fact that these reactions occur in the gas-phase, explain why levels of nitro-derivatives of pentacyclic and higher PAH are so low, since these latter PAH are nonvolatile. Combined with these findings is the well-known fact that 1-nitropyrene is found at levels 20 times that of benzo(a)pyrene in diesel particulates. Thus, comparison of the levels of 2-nitropyrene, 2-nitrofluoranthene and 1-nitropyrene might provide a measure for the relative contribution of diesel emissions to ambient nitro-PAH. In 1985, Pitts et al. presented a very interesting paper[10, 11, 12] to correct a historical mistake that 3-nitrofluoranthene found in rural

air at winter-time in Denmark was actually 2-nitrofluoranthene.

These findings have caused concern about the possible human health risks resulting from exposure to nitro-PAH. Nitro-PAH adhering to particulate matter can be inhaled into the lungs, and the possibility exists of swallowing sputum containing particulates removed during inhalation [13]. For the above reasons, development of powerful analytical methods is needed to reliably identify ultratrace amounts of individual nitro-PAH in complex sample matrices, and separation of isomeric nitro-PAH is necessary to assess the potential environmental and human health risks associated with environmental samples. Most nitro-PAH in the environment are presumably formed as a result of reaction between PAH and nitrogen oxides and/or nitric acid, all of which are commonly found in combustion effluents[14].

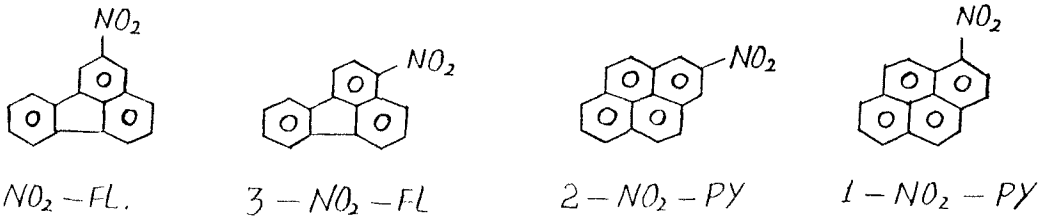
The Necessity of Distinguishing between Isomers

The biological activity of a nitro-PAH is dependent upon its molecular structure[15,16,17]. In order to properly assess the potential risk to the environment and human health, it is necessary to distinguish between isomeric nitro-PAH. The task of isomer differentiation is particularly difficult because of the large number of possible isomeric nitro-PAH. Detection and quantitation of isomeric nitro-PAH in environmental samples have also been hampered by the complexity of the sample matrix, low

concentration levels (ppb to low ppm), and scarcity of analytical standards [18]. As an example of the extreme complexity of nitro-PAH mixture encountered when analyzing combustion emissions, Paputa-Peck et al.[19] detected at least 100 nitro-PAH in a diesel exhaust particulate extract.

Based upon the pioneering work of MacCrehan and May[20], we employed such analytical procedures in our laboratory, involving HPLC separation on a conventional reversed-phase column followed by catalytic reduction using a silica/zinc module. We have investigated several analytical methods for the rapid determination of nitro-PAH in complex mixtures using HPLC. Our study involved (a) Use of a reversed-phase minicolumn in order to allow analysis to be completed in 10 minutes, rather than 50 minutes; (b) Concentration on three isomeric nitro-PAH, which are arguably the most important environmentally; (c) Collection of ambient air particulates at sites heavily impacted by diesel emissions and sites not heavily impacted and analysis of the target nitro-PAH; (d) Comparison of IP10 and TSP samples.

Structures:



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Chapter Two

Identification and Quantitation of Standard Nitro-PAH by HPLC

2.1 Experimental

Solvent and chemicals. The standard nitro-PAH (2-nitrofluoranthene, 3-nitrofluoranthene, 2-nitropyrene and 1-nitropyrene) were commercially obtained from Chemsyn Science Laboratories. The nitro-PAH certified analytical reference standards (SRM 1587) was purchased from the National Institute of Science and Technology. The melting points and concentrations of nitro-PAH standards are given in Table 2-1. All organic solvents for extraction, sample preparation, and chromatography were UV-grade obtained from J. T. Baker and EM Science and these were used as received. Water used in HPLC was charcoal filtered deionized water with pH being adjusted to 5. All solvents were filtered and degassed prior to HPLC use.

Table 2-1 The melting points and concentrations of 4 nitro-PAH standards.

Compound	Melting Point* (°C)	Concentration (μg/ml CH_3CN)
2-nitrofluoranthene	158.0 - 159.5	3.6
3-nitrofluoranthene	------	2.5
2-nitropyrene	201.0 - 202.0	1.7
1-nitropyrene	151.0 - 153.0	3.1

* The melting points were provided by Chemsyn Science Lab.

HPLC System. Two HPLC systems were used in this study. The first was a Waters Associates model 660 gradient liquid chromatograph programmer and two model 6000A solvent delivery systems (pumps) combined with two model 440 UV absorbance detectors and 420AC fluorescence spectrophometer. The columns used were reversed-phase polymeric C18 (Vydac TP 201) column, 25 cm x 4.6 mm and Pecosphere 3x8C C18 minicolumn (Perkin Elmer).

The other HPLC system was a Waters Associates model 990 Photo Diode Array with model 990 integrator. We thank Mr. John Van Antwerp and Dr. Edward Aig for their kindness in instructing us and allowing our use of their Waters Associates Model 990 Photo Diode Array UV detector.

Catalyst. The catalyst used was 7 μm Zn + 37 μm Silica, 1:1 by mass, dry packed in a post-column reaction chamber for catalytic reduction of target compounds. Coarse zinc

powder (100 mesh) from Alfa Products was dry packed in a pre-injector oxygen scrubber column for reaction with dissolved oxygen in the solvent system.

2.2 Qualitative and quantitative analysis of standard nitro-PAH by HPLC

(a) Individual nitro-PAH study. The HPLC retention characteristics of four standard nitro-PAH have been studied using a reverse-phase C18 minicolumn (Perkin Elmer) and the characteristic UV and fluorescence spectral properties were measured. Standard calibration curves for quantitating solutions containing a single nitro-PAH isomer were made for each of four nitro-PAH [Figures 2-1 to 2-4]. The analysis of the nitro-PAH involved their reduction to strongly fluorescing amino-PAH by passing them through a Zn/Si reducer following the UV detector [1,2,3]. The standard nitro-PAH data could be employed for identification of nitro-PAH in real air samples via HPLC.

The instrument standard operating conditions were as follows:

Column: (a) Reversed-phase polymeric C18(Vydac TP201) column 25 cm x 4.6 mm.

(b) Pecosphere 3x8C C18 minicolumn (Perkin Elmer).

Temperature of column: 15°C

Flow rate: 1.0 ml/min

Time range: 0 - 20 minutes

Mobile phase: CH_3CN, Buffer and H_2O

CH_3CN changes from 60% to 90%

Buffer changes from 40% to 10%

Buffer was prepared with 300 ml of potassium acetate solution (0.10 g crystal potassium acetate in 300 ml deionized water) and a couple of drops of glacial acetic acid (99.8%) until the final pH was adjusted to 3 to 5 which was tested by hydrion paper. The buffer was filtered and degassed prior to HPLC use.

After injection of the samples from a 250 μl loop, the column was eluted with buffer-Acetonitrile (3:2) for 20 min. Between 0 and 20 minutes the solvent composition was increased non-linearly to 90% acetonitrile. Figure 2-5 gives the chart of gradient programs (curve #9 on Waters 660 Programmer was selected). The column effluent was monitored by absorbance at 280 nm, 365 nm, and fluorescence[4,5] (excitation: 378 nm and emmission: 460 nm for amino-PAH [2]) after the effluent passed through the zinc reducer. Figure 2-6 gives the order of the apparatus in HPLC system [6].

Nitro-PAH were identified by retention time and UV absorbance ratio at 280 nm to 365 nm and were quantitated by peak area relative to individual nitro-PAH external standards in acetonitrile. HPLC chromatograms of each standard are included in Figures 2-7 to 2-10. Table 2-2 gives the retention times of 4 standard nitro-PAH under standard operating conditions using the minicolumn.

Table 2-2 HPLC retention index of 4 standard nitro-PAH (without the coarse zinc oxygen scrubber).

Compound	RT (min)	UV280/UV365	Fl./UV280
2-nitrofluoranthene	6.75±0.09	5.99±0.18	N/A
3-nitrofluoranthene	6.36±0.09	2.12±0.15	0.12
2-nitropyrene	7.98±0.11	42.0±0.6	0.00
1-nitropyrene	6.22±0.09	1.75±0.06	0.0205

N/A: Data are not available because the analytical fluorescence data for 2-nitrofluoranthene exhibited the largest uncertainty.

(b) Separation. Two standard mixtures of nitro-PAH were prepared to test the resolution of separation. The first contained all 4 nitro-PAH (1-nitropyrene, 2-nitropyrene, 2-nitrofluoranthene and 3-nitrofluoranthene, 1:1:1:1 by volume of their solutions). Separation of the first standard mixture on the column (25 cm x 4.6 mm, Vydac) with Waters 990 Photo Diode Array UV detection is shown in Figures 2-11

and 2-12, and the results are in Tables 2-3(a) and 2-3(b), separation on the minicolumn (3x8C, Perkin Elmer) with Waters 660 UV detection is in Figure 2-13. In Figure 2-13 no clear separation can be observed, three among four, 1-nitro-pyrene, 2-nitrofluoranthene and 3-nitrofluoranthene gave an identical retention time. The second standard mixture (1:1:1 by volume of their solutions) did not have 3-nitrofluoranthene which is assumed not to be present in ambient air particulate matter [4]. Separation on the 3x8C minicolumn (Perkin Elmer) with Waters 660 UV detection is shown in Figure 2-14 and data are in Table 2-4. From Figure 2-14, 1-nitropyrene, 2-nitro-fluoranthene and 2-nitropyrene give a quite clean separation.

Table 2-3(a)

Retention times and UV absorbance areas of four nitro-PAH in a standard mixture on a C18 column (25cm x 4.6mm, Vydac) with Waters 990 Photo Diode Array UV detection at 280 nm and 245 nm.

25.0 ul

Waters 990 Integrator		NPAH.DT3	
Waters			
01-31-1989	10:56:15	Sample name	NPAH S
TANDARD			
Sampling time	28 msec *4	Baseline	
OFF			
Sense	high 4	Resolution	
1.4 nm			
Time range	0 --- 20 min	Interval	
1 sec			
Smoothing	7 points	Slope	.0005
AU/min			
Drift	.002 AU/min	Height	.001
AU			
Width	.02 min	Min. area	.0001
AU*min			
Time double	30 min	Minus peak	
OFF			
Column	mm ID * mm	Packing material	
Mobile phase		Flow rate	
ml/min			
Pressure			

Report File NPAH.DT3

280 nm

No.	Mark	Retention time	Height [AU]	Left time	Right time	Area [AU*min]	Area [%]
1	I	2.66	0.0031	2.51	5.51	0.002752	3.208
2		13.45	0.0499	12.95	13.54	0.012831	14.958
3	V	13.73	0.0367	13.54	13.99	0.012645	14.742
4	V	14.31	0.0723	13.99	15.32	0.024654	28.740
5	I	17.75	0.0811	17.12	19.04	0.032899	38.352

Report File NPAH.DT3

245 nm

No.	Mark	Retention time	Height [AU]	Left time	Right time	Area [AU*min]	Area [%]
1	I	2.66	0.0026	2.51	5.45	0.002192	2.647
2		13.45	0.0972	12.90	13.63	0.030735	37.119
3	V	13.74	0.0470	13.63	13.99	0.012518	15.118
4	V	14.31	0.0668	13.99	15.87	0.023218	28.040
5	I	17.75	0.0348	17.20	19.04	0.014139	17.076

Table 2-3(b)

Retention times and UV absorbance areas of four nitro-PAH in a standard mixture on a C18 column (25cm x 4.6mm, Vydac) with Waters 990 Photo Diode Array UV detection at 365 nm.
25.0 ul

Waters 990 Integrator		NPAH.DT3	
Waters			
01-31-1989	10:56:15	Sample name	NPAH S
TANDARD			
Sampling time	28 msec *4	Baseline	
OFF			
Sense	high 4	Resolution	
Time range	10 --- 20 min	Interval	
1 sec			
Smoothing	7 points	Slope	.0005
AU/min			
Drift	.002 AU/min	Height	.001
AU			
Width	.02 min	Min. area	.0001
AU*min			
Time double	30 min	Minus peak	
OFF			
Column	mm ID * mm	Packing material	
Mobile phase		Flow rate	
ml/min			
Pressure			

365 nm

No.	Mark	Retention time	Height [AU]	Left time	Right time	Area [AU*min]	Area [%]
1		13.43	0.0376	12.98	13.96	0.014320	46.355
2	V	14.31	0.0455	13.96	15.72	0.015685	50.774
3	I	17.75	0.0023	17.36	18.44	0.000887	2.872

Table 2-4 Separation of a standard mixture of 3 nitro-PAH using reversed-phase minicolumn HPLC

Sample: 2-nitrofluoranthene, 2-nitropyrene and 1-nitropyrene

Volumn: 8 μl each

Date : October 19, 1988

280 nm UV

Peak #	Area %	RT (min)	Area
1	23.44	7.72	996553
2	18.18	8.55	772987
3	47.52	9.55	2020554

365 nm UV

Peak #	Area %	RT (min)	Area
1	75.15	7.72	601853
2	17.97	8.55	143957
3	6.31	9.52	50562

Fluorescence

Peak #	Area %	RT (min)	Area
1	100	8.12	27203

Peak #1: 1-nitropyrene.

#2: 2-nitrofluoranthene.

#3: 2-nitropyrene.

(c) Standard nitro-PAH 1587 [8]. Standard Reference Material (SRM) 1587 consists of four vials, each containing approximately 1 ml of a methanol solution of seven nitrated PAH. SRM 1587 sold by National Institute of Science and Technology is Certified and intended primarily for use in calibrating chromatographic instrumentation used for the determination of nitro-PAH. It was also tested using two HPLC systems and the HPLC chromatographic profiles are included in Figures 2-15 to 2-17. The results of separation and quantitation by reversed-phase HPLC with Photodiode Array UV detection are provided in Table 2-5. In addition, certified concentrations of the seven nitro-PAH in standard 1587 are given in Table 2-6. The separation SRM 1587 on a C18 column (Zorbax ODS) using a methanol/water mobile phase with UV detection at 254 nm is given in Figure 2-18. UV spectra for the nitro-PAH were obtained by Photodiode Array UV dectection and are included in Appendix I.

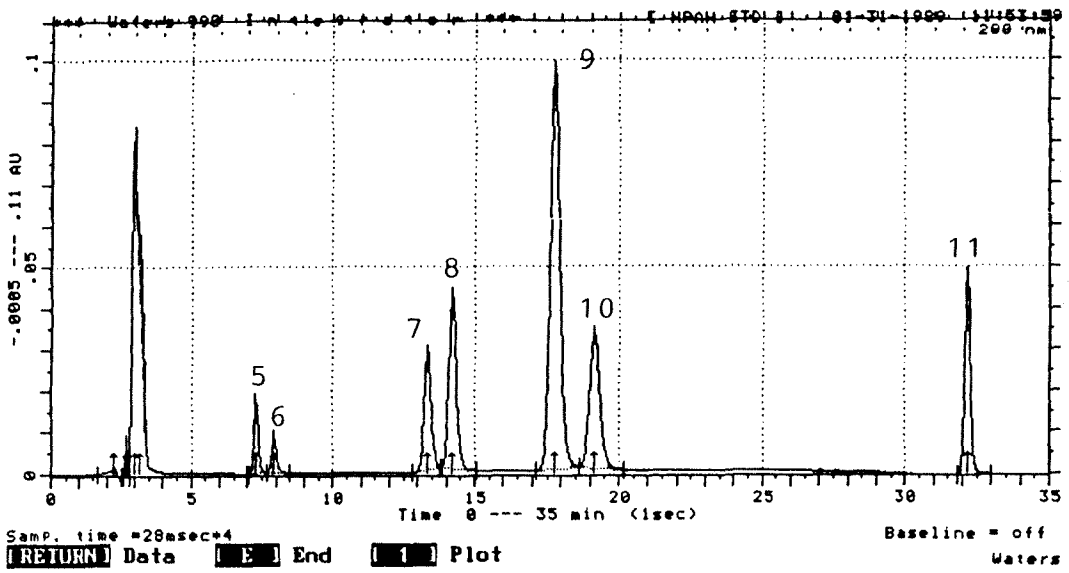

Table 2-5

Retention times and UV absorbance areas of SRM 1587 on a C18 column (25cm x 4.6mm, Vydac) with Photo Diode Array UV detection at 280 nm.

25.0 ul

Waters 990 Integrator Waters		File NBSSTD.DT3			
01-31-1989	11:53:59	Slope	.0005 AU/min		Sample name
Sampling time	28 msec *4	Drift	.002 AU/min		
Sense	high 4	Height	.001	AU	Column
mm ID *	mm				
Resolution	1.4 nm	Width	.02	min	Packing material
Time range	0 --- 15.91 min	Min. area	.0001	AU*min	Mobile phase
Interval	1 sec	Time double	30	min	Flow rate
	ml/min				
Smoothing	7 points	Minus peak		OFF	Pressure
					Baseline
	OFF				

Report File NBSSTD.DT3
280 nm

No.	Retention time	Height [AU]	Left time	Right time	Area [AU*min]	Area [%]	Mark
1	2.27	0.0036	1.69	2.57	0.001481	1.162	I
2	2.72	0.0104	2.56	2.81	0.001206	0.946	
3	2.97	0.0851	2.81	6.95	0.032910	25.814	V
4	3.19	0.0118	3.05	3.39	0.002339	1.835	TA
5	7.29	0.0171	7.02	7.69	0.003198	2.508	I
6	7.90	0.0082	7.65	8.50	0.001732	1.359	I
7	13.34	0.0283	12.80	13.83	0.008371	6.566	I
8	14.20	0.0418	13.80	15.05	0.013276	10.414	I
9	17.77	0.0967	17.14	18.62	0.038424	30.139	I
10	19.17	0.0323	18.62	20.16	0.013504	10.592	I
11	32.17	0.0483	31.84	33.00	0.011047	8.665	I

Table 2-6 Certified concentrations for SRM 1587.

Compound	Concentration	
	(μg/g)*	(μg/ml)*
2-Nitrofluorene	9.67±0.39	7.64±0.31
9-Nitroanthracene	5.01±0.11	3.96±0.09
3-nitrofluoranthene	9.24±0.06	7.30±0.05
1-Nitropyrene	8.95±0.28	7.07±0.22
7-Nitrobenz(a)anthracene	9.27±0.23	7.32±0.18
6-Nitrochrysene	8.13±0.11	6.42±0.09
6-Nitrobenzo(a)pyrene	(6.1)	(4.8)

* μg/g: microgram of nitro-PAH in per gram of the solution.

* μg/ml: microgram of nitro-PAH in per milliliter of the solution. The uncertainties apply only at 23°C. This SRM 1587 may be used between 19 and 27°C, but a concentration change of up to 1% will occur because of the change of the density of methanol with temperature.

2.3 Some experimental problems encountered during quantitation of isomeric nitro-PAH

(a) One practical problem central to any HPLC analysis employing a fluorescence detector is the presence of oxygen in the eluent. While degassing the solvent with filter paper was our deoxygenation procedure, it was quite incomplete. Consequently, the fluorescence responses were reduced by the presence of the oxygen in the solvent[9].

In our first attempt to reduce nitro-PAH, we only used post-column zinc/silica reducer and found that measurable fluorescence was observed only when the catalyst was on line [2]. However, the response of fluorescence of nitro-PAH did not remain constant even if conditions were the same each time. Fluorescence is greatly affected by how much nitro-PAH is reduced to amino-PAH which strongly depends on the freshness of the Zn/Si reducer and pH value of the buffer (no reduction was observed with acetonitrile-water mobile phase [2]). MacCrehan and May [10] used a simple scrubber column containing mossy zinc mounted in-line to conveniently and completely remove oxygen which was interfering with the determination of nitro-PAH. The installation of the oxygen scrubber filled with coarse zinc prior to the injector helps get rid of oxygen dissolved in the solvent and decreases the rate that the zinc/silica reducing column is consumed. As a result, the efficiency of the catalytic reduction was greatly improved. Results of

after-installation of oxygen scrubber are shown in Tables 2-7 to 2-10. The HPLC chromatographic profiles of standard nitro-PAH after-installation of the oxygen-scrubber precolumn are given in Figures 2-19 to 2-22. The comparison of HPLC retention index with and without precolumn is given in Table 2-11 (This part of the work, from Tables 2-7 to 2-12, was done by Ms. Amy Marie Feith, undergraduate student at NJIT.)

Table 2-7 Peak ratios and retention times of 2-nitrofluoranthene after the installation of oxygen-scrubber precolumn.

Injection	Afl/A280	A280/A365	Retention time (min)	
	$*10^{-2}$		UV 280 nm	Fluoresc.
1	14.1	5.79	6.70	6.90
2	13.0	5.63	7.85	8.05
3	14.1	5.11	7.60	7.80
4	12.0	6.49	7.72	7.95
5	11.6	6.12	6.90	7.12
6	14.3	5.86	6.82	7.05
7	13.1	6.12	7.27	7.50
8	13.9	5.89	6.92	7.15
9	13.5	5.89	7.80	8.00
10	12.2	5.59	7.85	8.10
11	14.2	5.88	7.77	8.00
12	12.6	5.75	8.22	8.50
Average:	13.2±0.94	5.75±0.34	7.45±0.5	7.68±0.5

Table 2-8 Peak ratios and retention times of 3-nitrofluoranthene after the installation of oxygen-scrubber precolumn.

Injection	Afl/A280	A280/A365	Retention time (min)	
	$*10^{-2}$		UV 280 nm	Fluoresc.
1	36.1	1.96	7.07	7.20
2	43.5	1.94	7.40	7.50
3	40.2	1.95	7.35	7.47
4	40.0	1.95	7.82	7.95
5	42.8	1.95	6.52	6.65
6	50.7	1.97	7.32	7.42
7	50.2	1.97	7.75	7.85
8	43.0	1.95	7.35	7.45
9	38.8	1.94	6.82	6.92
10	37.7	1.93	6.10	6.22
11	39.0	2.01	7.42	7.55
12	42.0	1.96	7.80	7.92
13	41.2	1.93	7.27	7.40
Average:	41.9±4.15	1.95±0.02	7.23±0.49	7.35±0.48

Table 2-9 Peak ratios and retention times of 2-nitropyrene after the installation of oxygen-scrubber precolumn.

Injection	Afl/A280 $*10^{-2}$	A280/A365	Retention time (min)	
			UV 280 nm	Fluoresc.
1	39.7	40.4	8.55	8.70
2	38.4	41.3	8.67	8.82
3	38.1	42.6	8.55	8.70
4	37.7	42.4	8.55	8.70
5	37.5	41.1	8.62	8.77
6	35.2	41.9	8.47	8.65
7	37.9	40.6	7.86	8.00
8	34.0	42.9	8.27	8.42
9	29.8	41.1	8.47	8.62
10	30.1	41.8	8.40	8.55
11	30.5	41.0	8.45	8.60
12	29.0	41.4	8.25	8.40
13	30.7	41.7	8.58	8.75
Average:	34.5±4.0	41.6±0.7	8.44±0.74	8.59±0.23

Table 2-10 Peak ratios and retention times of 1-nitropyrene after the installation of oxygen-scrubber precolumn.

Injection	Afl/A280 $*10^{-2}$	A280/A365	Retention time (min)	
			UV 280 nm	Fluoresc.
1	19.1	1.67	5.67	5.82
2	20.1	1.68	5.80	5.97
3	21.0	1.67	6.02	6.20
4	19.5	1.69	5.85	6.02
5	19.1	1.70	6.15	6.32
6	17.5	1.69	6.06	6.22
7	15.8	1.68	6.00	6.17
8	12.3	1.71	5.55	5.72
9	9.64	1.69	5.30	5.72
10	17.0	1.63	6.55	6.65
11	12.2	1.64	6.80	6.92
12	11.8	1.65	6.45	6.60
Average:	16.4±3.7	1.68±.02	6.41±.59	6.73±0.65

Table 2-11 The comparison of absorbance and fluorescence ratios of standard nitro-PAH with and without oxygen-scrubber precolumn.

Standard	Afl/A280*10^{-2}		A280/A365	
	with	without	with	without
2-nitrofluoranthene	13.2	N/A	5.75	5.99
3-nitrofluoranthene	41.9	12.0	1.95	2.12
2-nitropyrene	34.5	0.00	41.6	42.0
1-nitropyrene	16.4	2.05	1.68	1.75

N/A: Data are not available because the analytical fluorescence data for 2-nitrofluoranthene exhibited the largest uncertainty.

From Tables 2-7 to 2-10 the lifetime of a certain column was prolonged when the oxygen scrubber was on line. Apparently, the efficiency of the catalytic reduction was significantly improved while basically, the UV profiles of the nitro-PAH were the same and the UV absorbance (A280/A365) was not effected much.

In addition, a limited experiment designed to examine the consistency of three post catalytical reducers was performed, using 1-nitropyrene as the model compound. The results are shown in Table 2-12.

Table 2-12 The consistency of the post column reducer.

Reducer	Afl/A280	
	1st injection	2nd injection
1	1.91	2.01
2	0.964	1.22
3	1.1	1.5

* The volume injected was 20 μl each time.

Table 2-12 shows that the consistency of the post catalytical reducer was not satisfactory in the first two injections and the ratios of Afl. to A280 were slightly higher in the 2nd injection than that of the 1st. This might indicate that the dry-packed catalytical reducer needed a while to become active and stable. Practically, we allowed the buffer with proper pH to pass through the post reducer for a while before the nitro-PAH was injected. The resulting consistency of fluorescence would be helpful in quantitative determination of specific nitro-PAHs.

(b) 3-nitrofluoranthene could not be separated from 2-nitrofluoranthene and co-eluted with the other three nitro-PAH when all 4 isomers were co-injected. This problem was solved satisfactorily when a Photodiode Array UV dectector was applied to reversed-phase HPLC. However, it is not a significant problem since 3-nitrofluoranthene is known not to occur in ambient air.

2.4 Conclusions

(a) We have demonstrated that the standard nitro-PAHs, 2-nitrofluoranthene, 3-nitrofluoranthene, 2-nitropyrene and 1-nitropyrene at μg/ml level concentrations can be rapidly, efficiently and reproducibly determined using reversed-phase C18 column (25 cm x 4.6 mm, Vydac TP201) HPLC with UV and fluorescence detection. Three of them, except 3-nitrofluoranthene, can be cleanly separated by the minicolumn (3x8C Perkin Elmer) under our standard operating conditions. The order of elution is that 1-nitropyrene elutes first, 2-nitrofluoranthene and 3-nitrofluoranthene coelute, and 2-nitropyrene elutes last.

(b) Both the post Zn/Si catalytical reducer and the oxygen scrubber precolumn must be employed in the HPLC system to reduce nitro-PAH to their corresponding amines, enhancing the fluorescence. The efficiency and lifetime of the Zn/Si catalytical reducer are greatly increased by using the oxygen scrubber.

(c) Four target nitro-PAH studied, 2-nitrofluoranthene, 3-nitrofluoranthene, 2-nitropyrene and 1-nitropyrene, can be quantitativbely identified by regular column HPLC with Photodiode Array UV detection.

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[1] W. A. MacCrehan and W. E. May, presented to the Eighth International Symposium on Polynuclear Aromatic Hydrocarbons, Columbus, OH, October, 1983.

[2] S. B. Tejada, R. B. Zweidinger, and J. E. Sigsby, Jr., "Fluorescence Detection and Identification of Nitro Derivatives of Polynuclear Aromatic Hydrocarbons by On-Column Catalytic Reduction to Aromatic Amines", SAE Technical Paper Series 890775 (1982).

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[4] T. L. Gibson, A. I. Ricci, R. L. Williams, "Chemical and Biological Fate: Polynuclear Aromatic Hydrocarbons", M. Cooke, A. J. Dennis, (eds.), Babbelle Press, Columbus, Ohio, 1981, pp 707-719.

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[8] Standard Reference Material 1587, National Bureau of Standards Certificate, June 25, 1985.

[9] C. W. White (ed.), Nitrated Polycyclic Aromatic Hydrocarbons, Huethig, Heidelberg, 1985.

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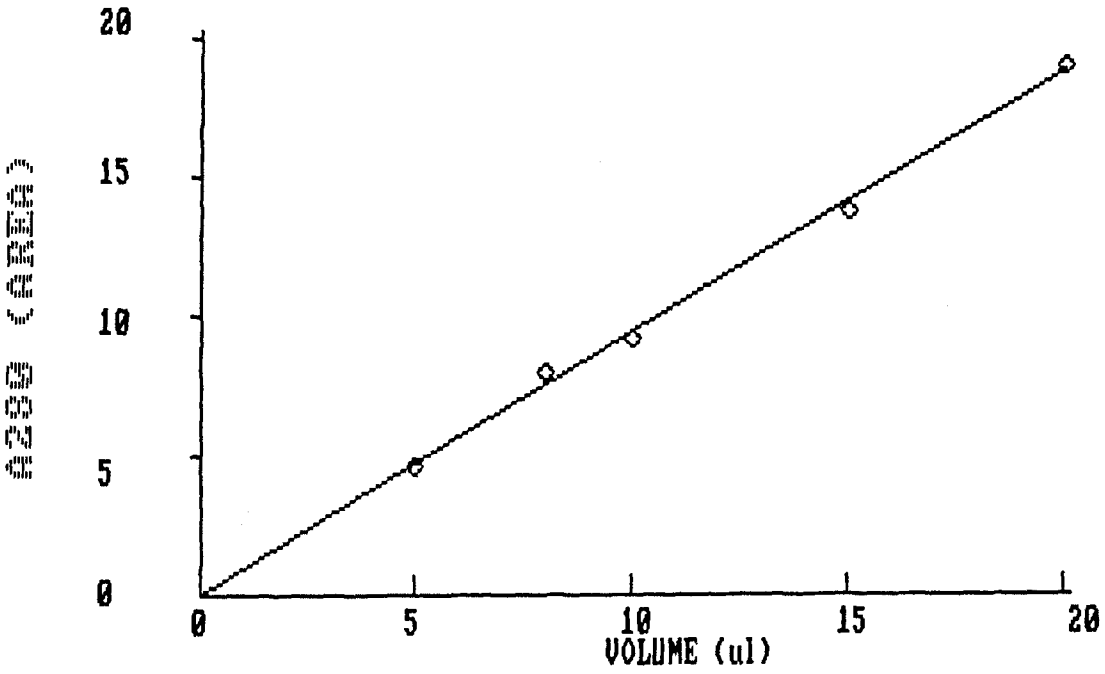


CALIBRATION OF 2-NITROFLUORANTHENE (UV 280 nm)

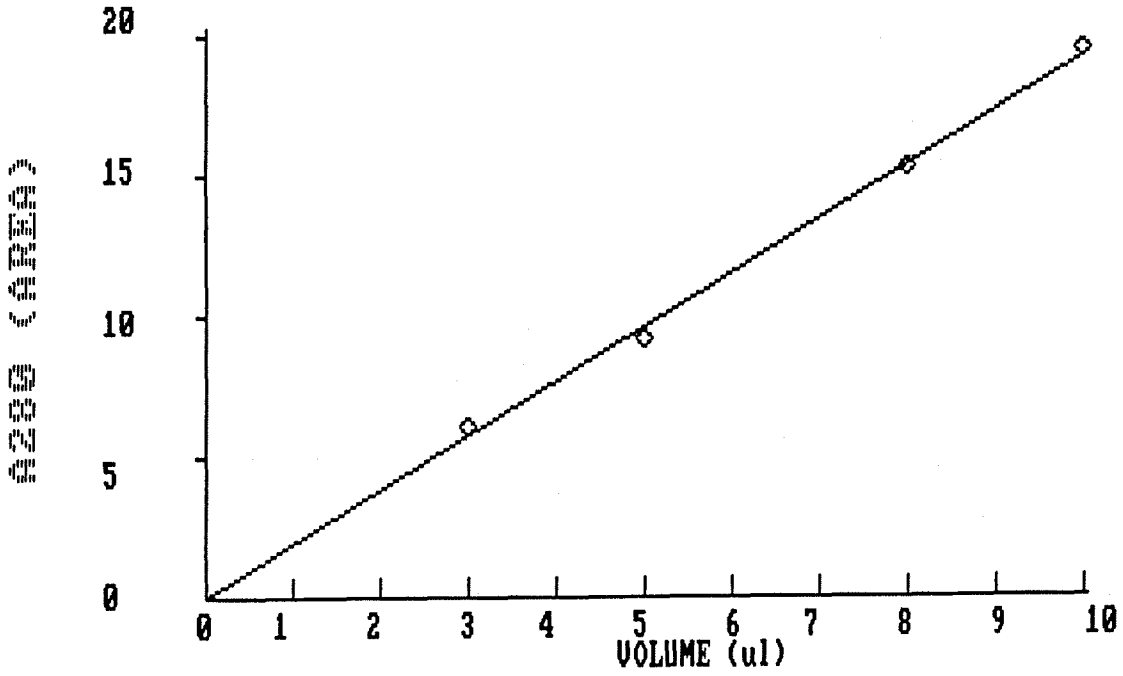
Point	X (Injection Volume)	Y (Absorb. Area)
1	5	4.6
2	8	8.03
3	10	9.24
4	15	13.8
5	20	19.1

Slope = .9441083 +/- 3.298047E-02
Intercept = 2.342987E-03 +/- .391899
Correlation = .9981748 calculated on points 1 to 5

Figure 2-1

Calibration curve of standard 2-nitrofluoranthene at UV 280nm under standard operating conditions.

Column: 3x8C minicolumn (Perkin Elmer).



CALIBRATION OF 3-NITROFLUORANTHENE (UV 280 nm)

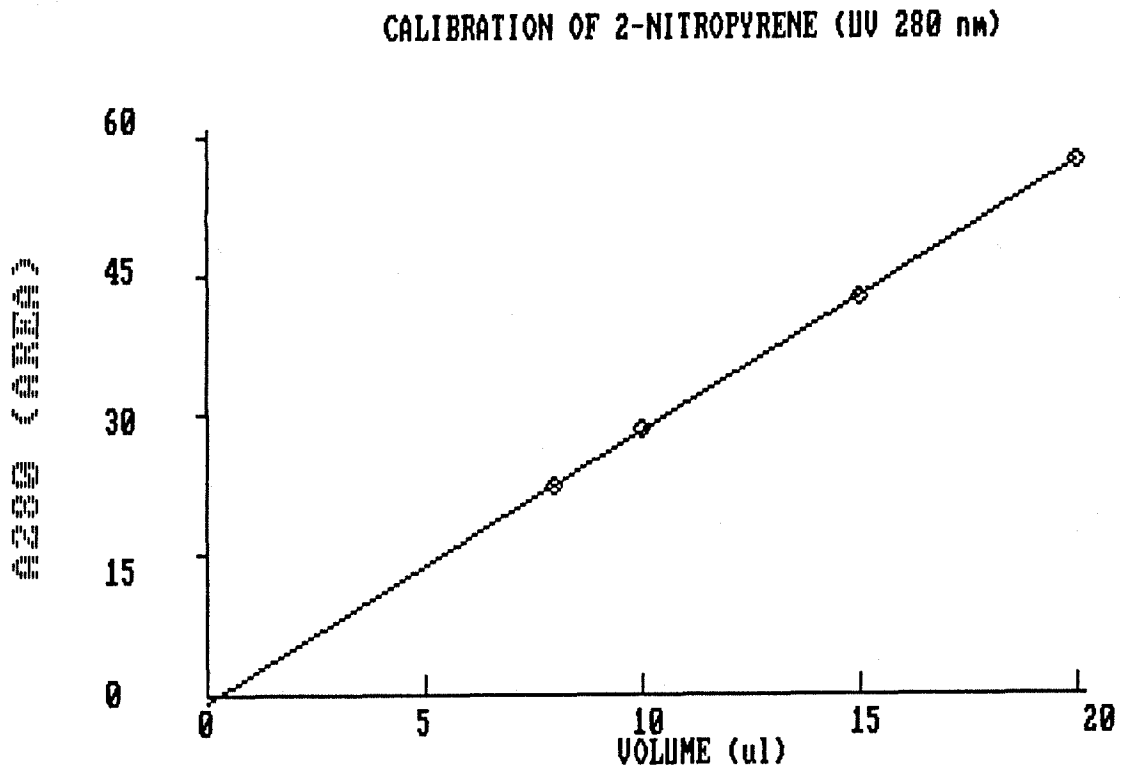
Point	X(Injection Volume)	Y(Absorb. Area)
1	3	6.04
2	5	9.22
3	8	15.3
4	10	19.4

Slope = 1.926897 +/- 6.754732E-02
Intercept = -3.482819E-02 +/- .3637535
Correlation = .9987734 calculated on points 1 to 4

Figure 2-2

Calibration curve of standard 3-nitrofluoranthene at UV 280nm under standard operating conditions.

Column: 3x8C minicolumn (Perkin Elmer).



CALIBRATION OF 2-NITROPYRENE (UV 280 nm)

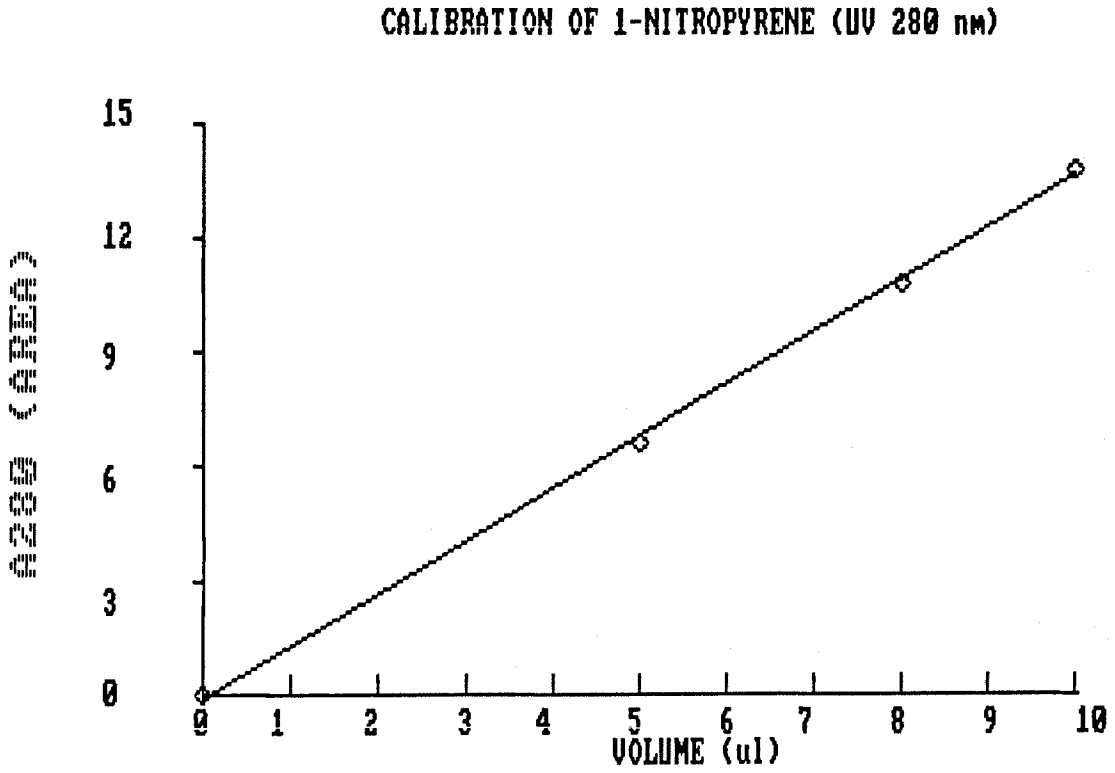
Point	X (Injection Volume)	Y (Absorb. Area)
1	8	22.3
2	10	28.3
3	15	42.6
4	20	57

Slope = 2.884726 +/- 1.257142E-02
Intercept = -.6726227 +/- .1170898
Correlation = .999981 calculated on points 1 to 4

Figure 2-3

Calibration curve of standard 2-nitropyrene at UV 280 nm under standard operating conditions.

Column: 3x8C minicolumn (Perkin Elmer).



CALIBRATION OF 1-NITROPYRENE (UV 280 nm)

Point	X(Injection Volume)	Y (Absorb. Area)
1	5	6.65
2	8	10.8
3	10	13.8

Slope = 1.426315 +/- 3.191668E-02
Intercept = -.5184161 +/- .1135923
Correlation = .9997492 calculated on points 1 to 3

Figure 2-4

Calibration curve of standard 1-nitropyrene at UV 280 nm under standard operating conditions.

Column: 3x8C minicolumn (Perkin Elmer).

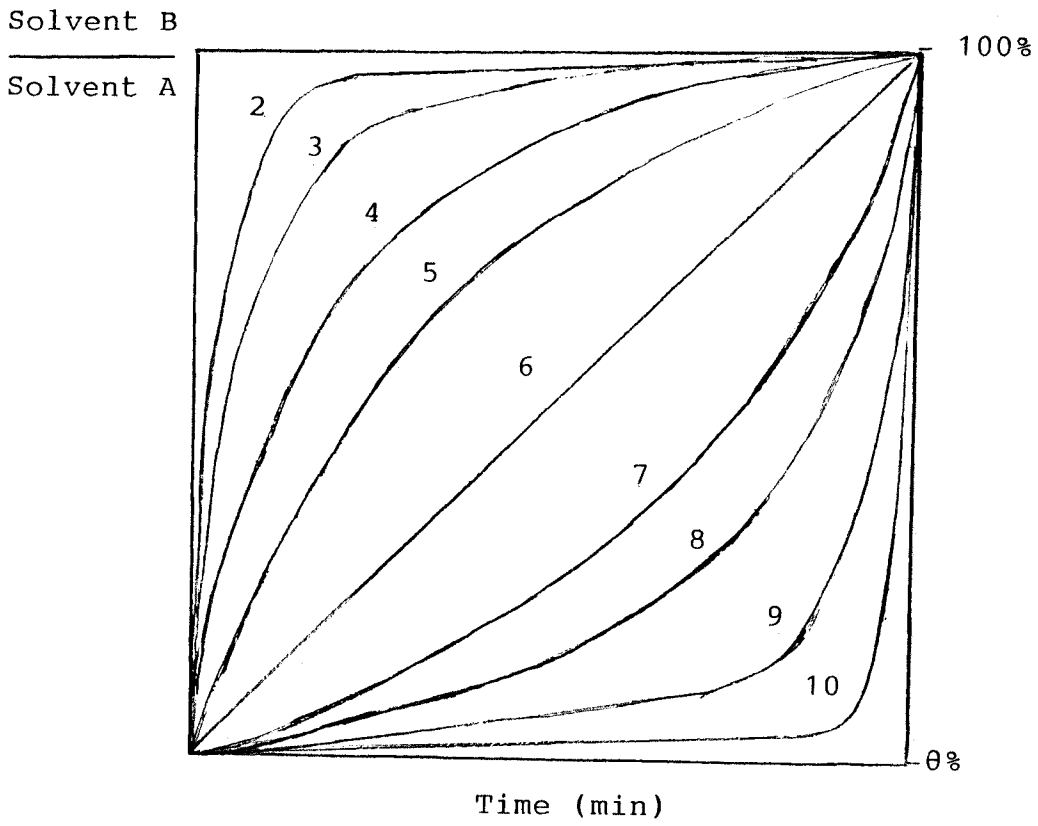


Figure 2-5

The chart of gradient program on Waters 660 Liquid Programmer. (Curve Selection - #9 was used.)

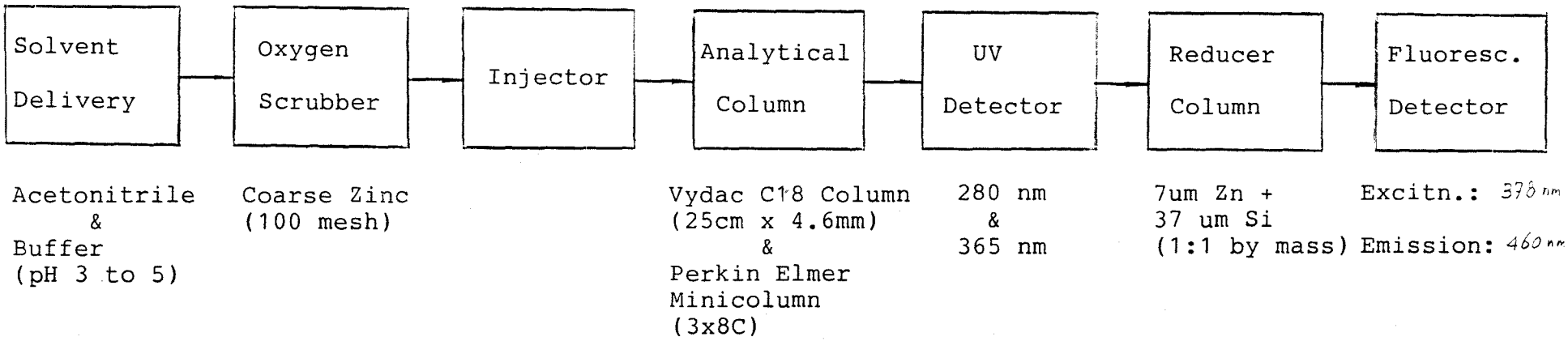


Figure 2-6

HPLC analytical system for nitro-PAH.

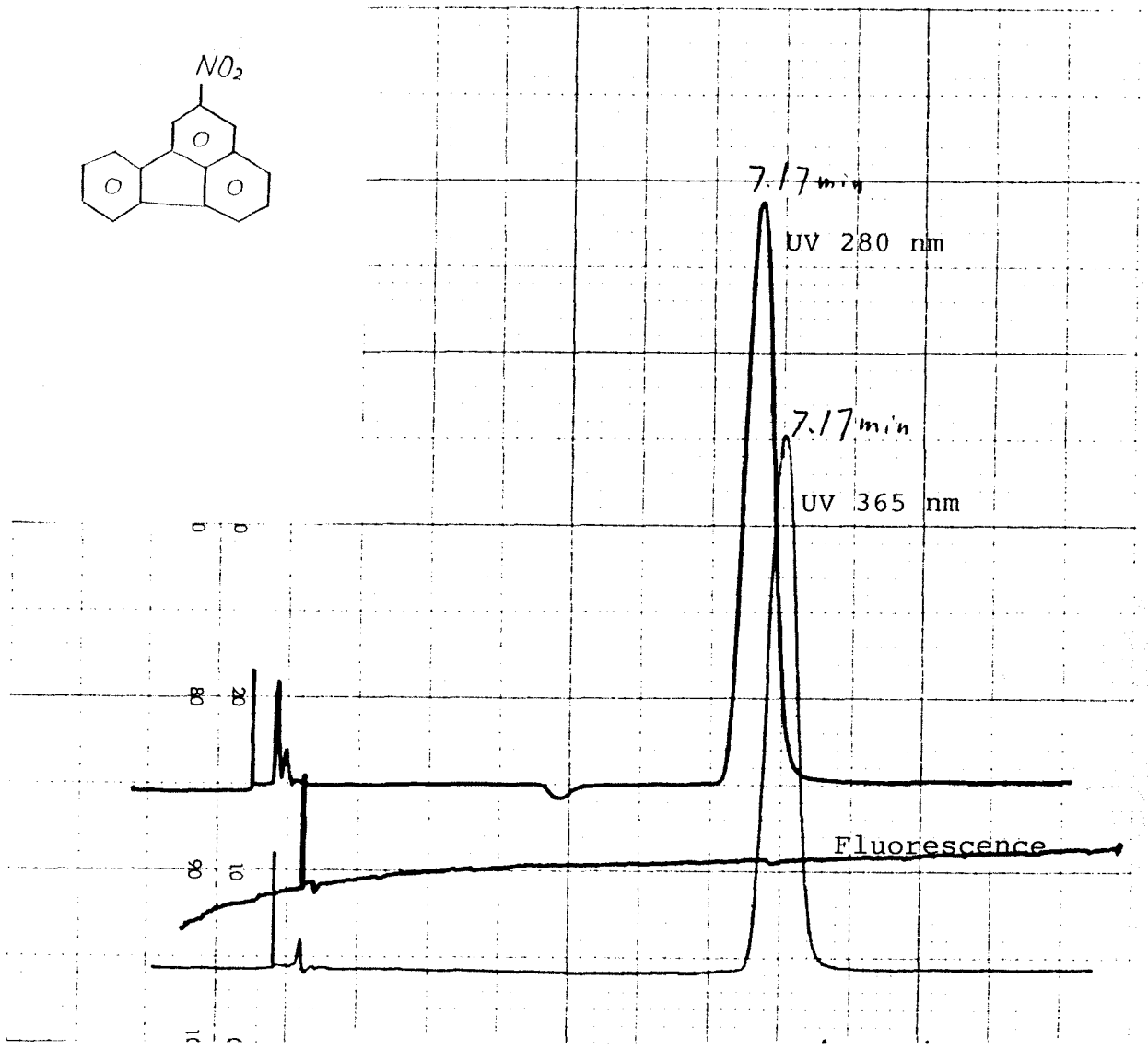


Figure 2-7

HPLC chromatogram of 2-nitrofluoranthene under standard operating conditions (w/o oxygen scrubber).

Concentration:	3.6 μg/ml	Retention Time:	7.17 min
Volume:	20.0 μl	A_{280}/A_{365}:	5.89

Column: 3x8C minicolumn (Perkin Elmer).

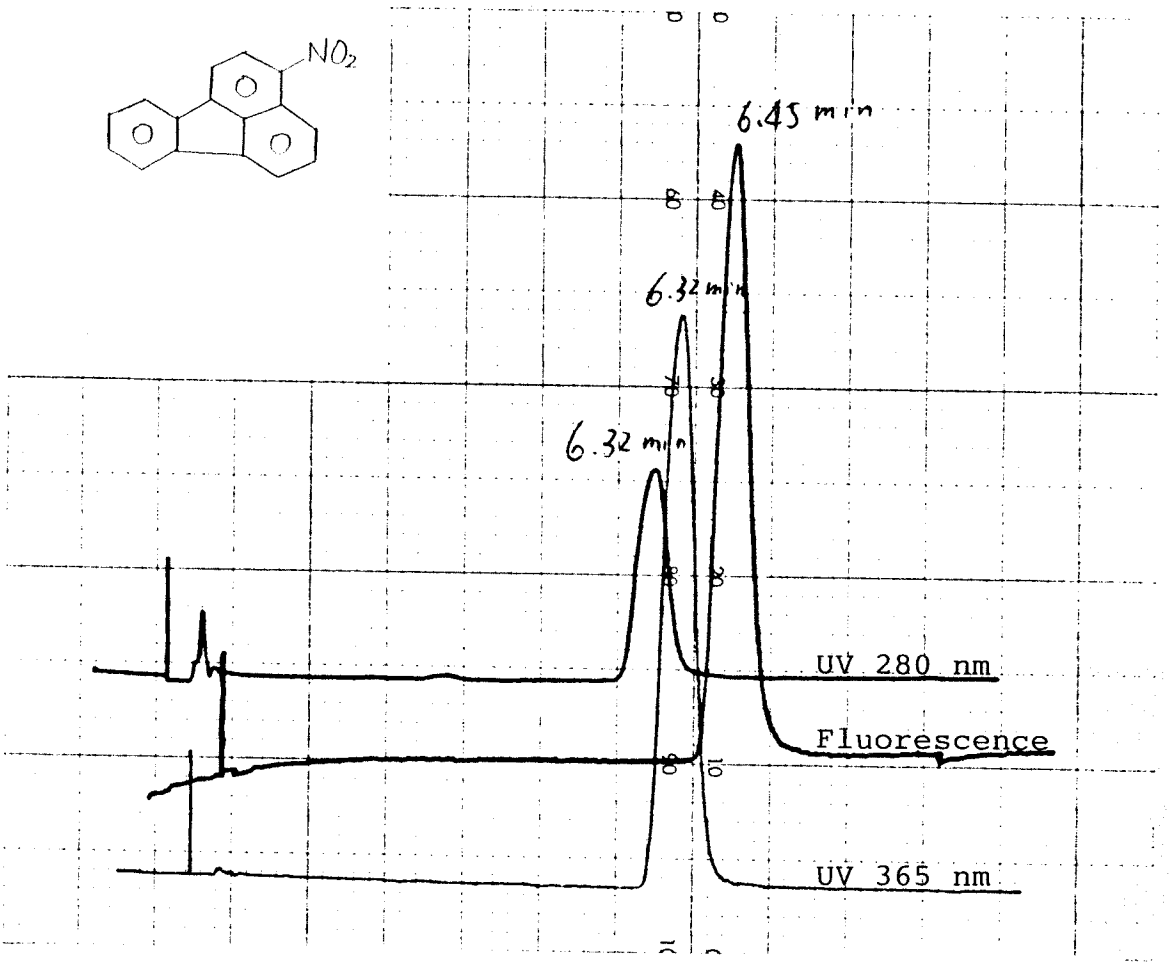


Figure 2-8

HPLC chromatogram of 3-nitrofluoranthene under standard operating conditions (w/o oxygen scrubber).

Concentration:	2.5 μg/ml	Retention Time:	6.45 min
Volume:	3.0 μl	A_{280}/A_{365}:	2.14
		A_{280}/A_{fl}:	7.80

Column: 3x8C minicolumn (Perkin Elmer).

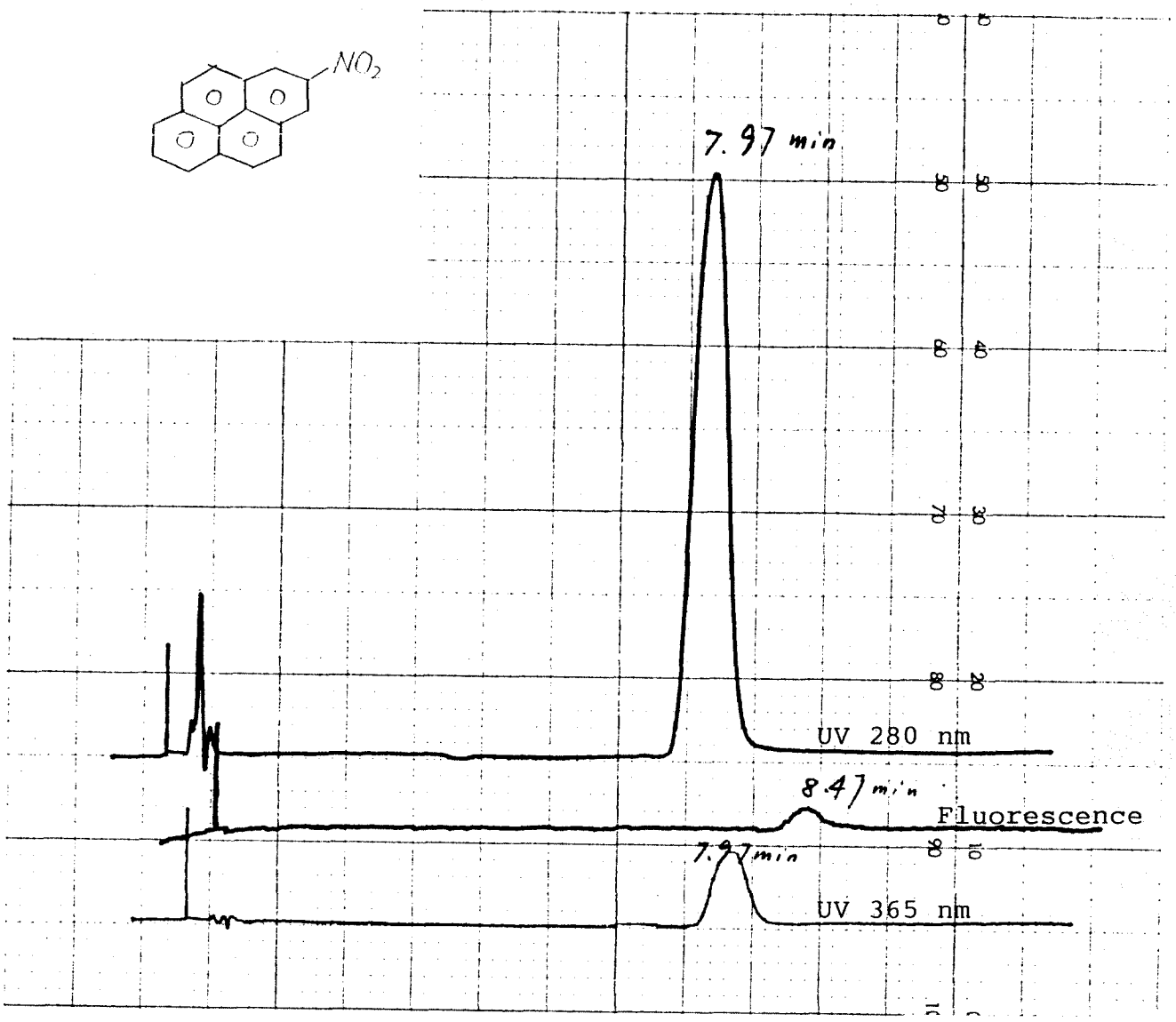


Figure 2-9

HPLC chromatogram of 2-nitropyrene under standard operating conditions (w/o oxygen scrubber).

Concentration:	1.7 μg/ml	Retention Time:	7.97 min
Volume:	8.0 μl	A_{280}/A_{365}:	41.6

Column: 3x8C minicolumn (Perkin Elmer).

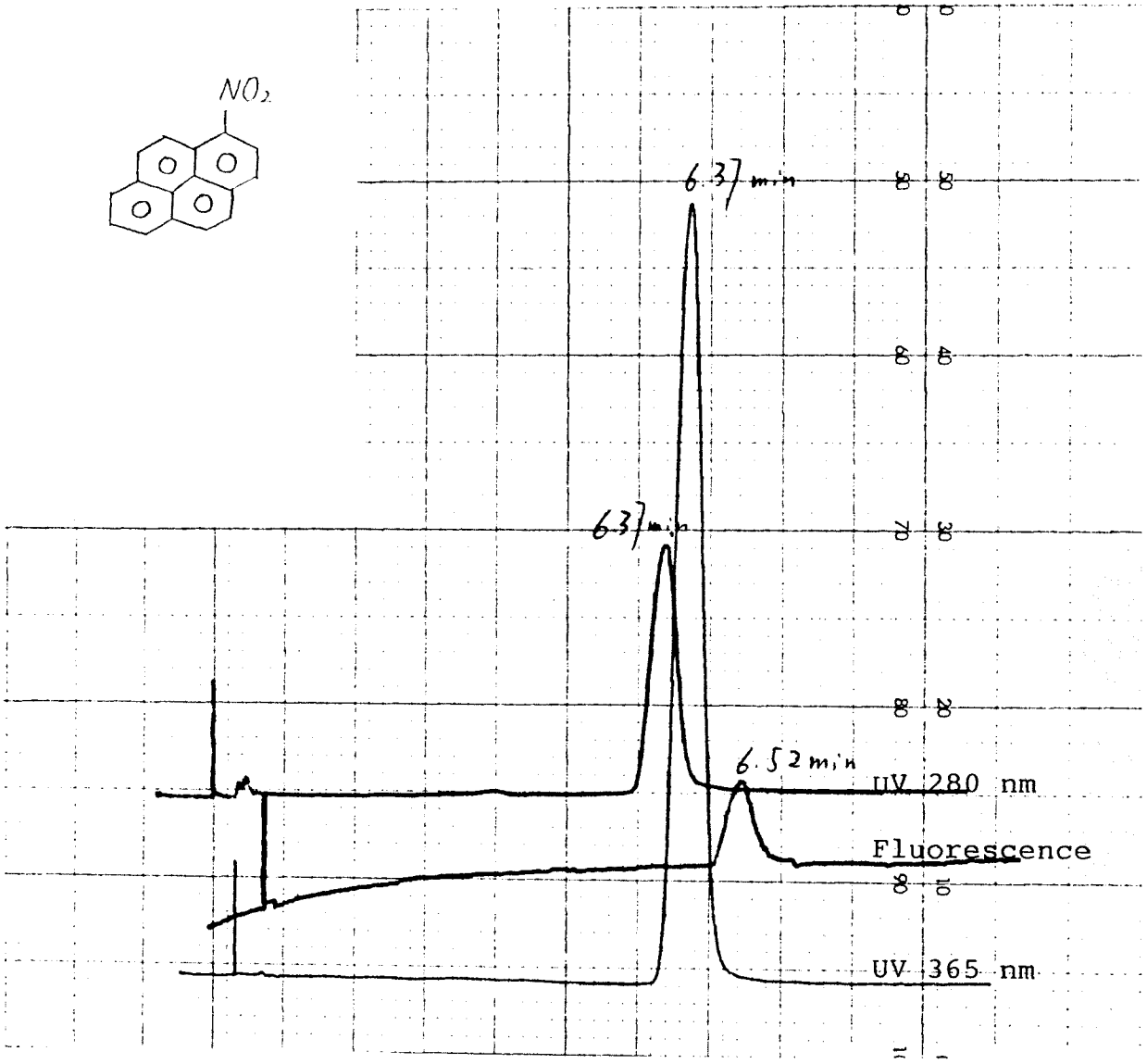


Figure 2-10

HPLC chromatogram of 1-nitropyrene under standard operating conditions (w/o oxygen scrubber).

Concentration:	3.1 μg/ml	Retention Time:	6.52 min
Volume:	5.0 μl	A_{280}/A_{365}:	62.5

Column: 3x8C minicolumn (Perkin Elmer).

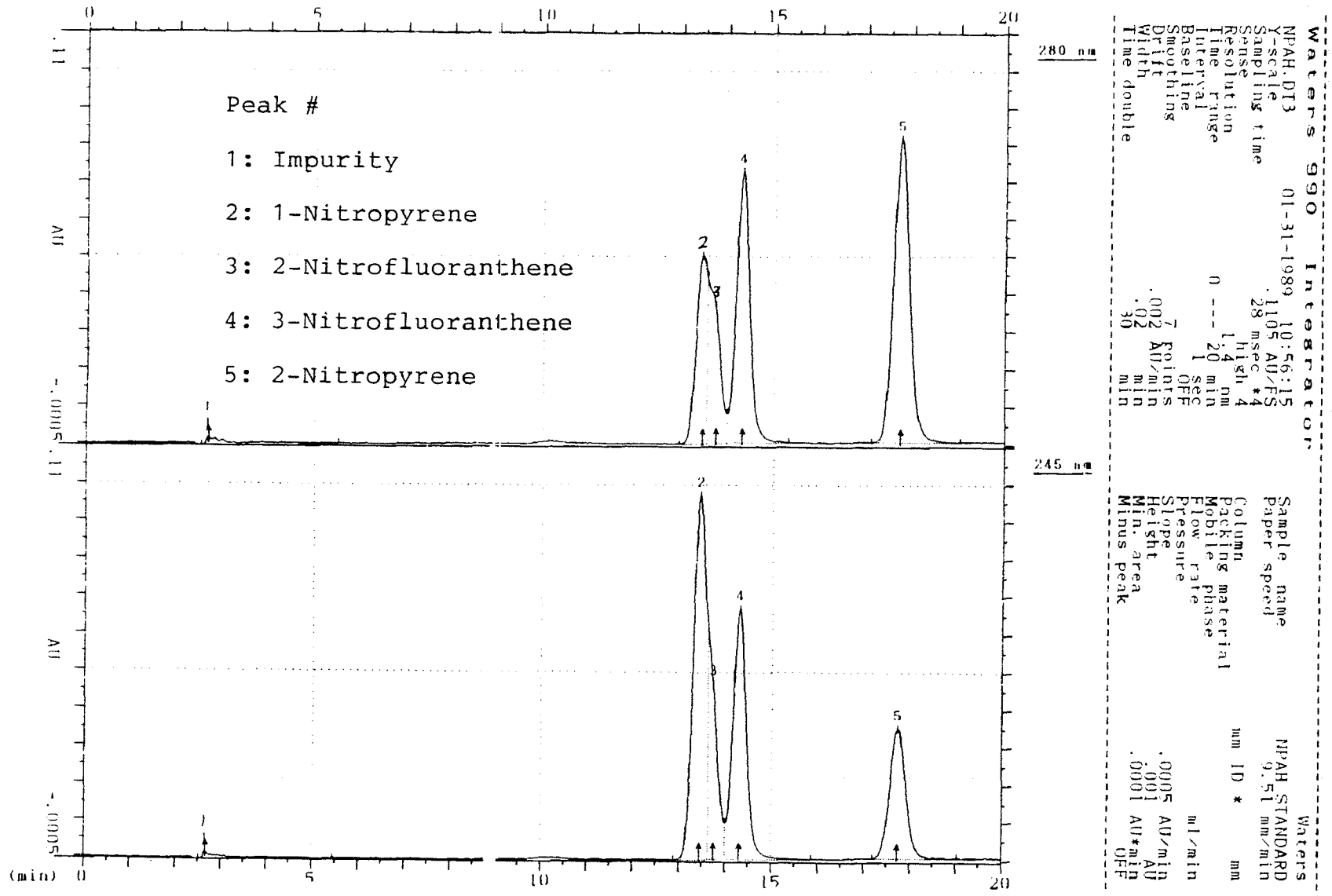


Figure 2-11

Separation of a standard mixture of four nitro-PAH on a C18 column (25cm x 4.6mm, Vydac) with Photo Diode Array UV detection at (a) 280 nm (b) 245 nm.

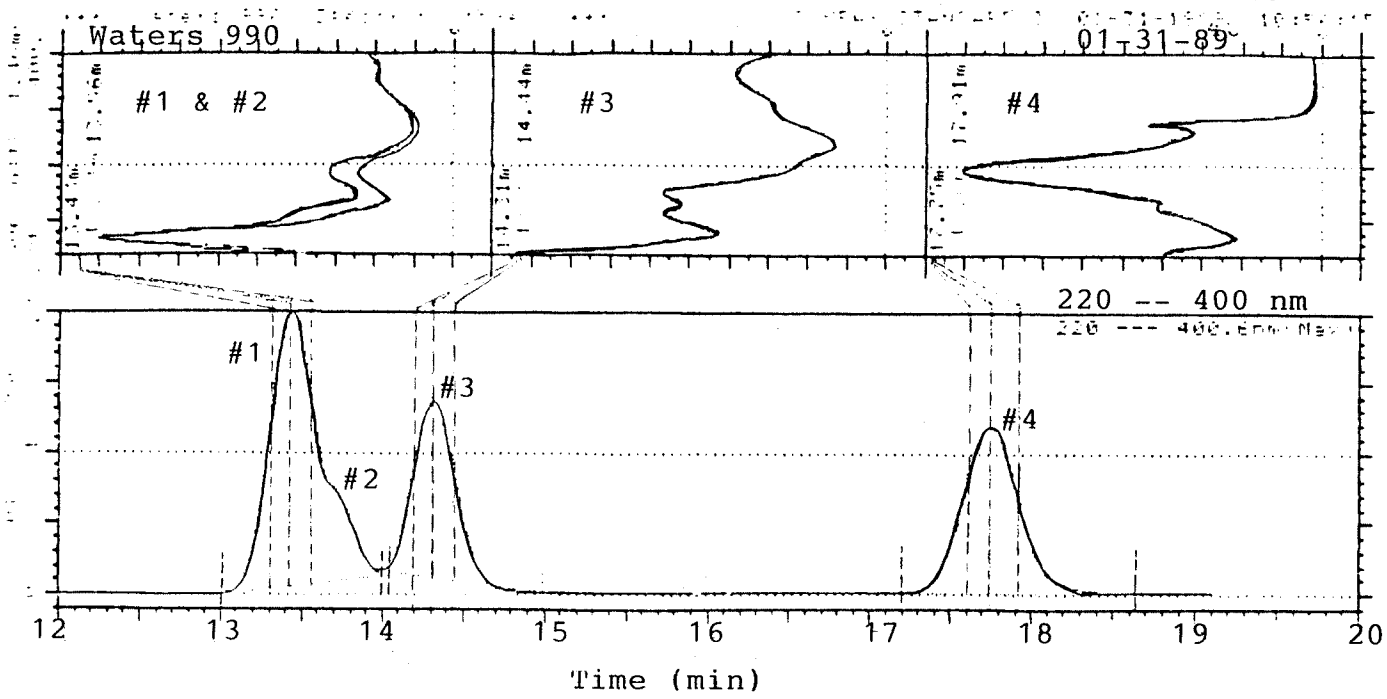
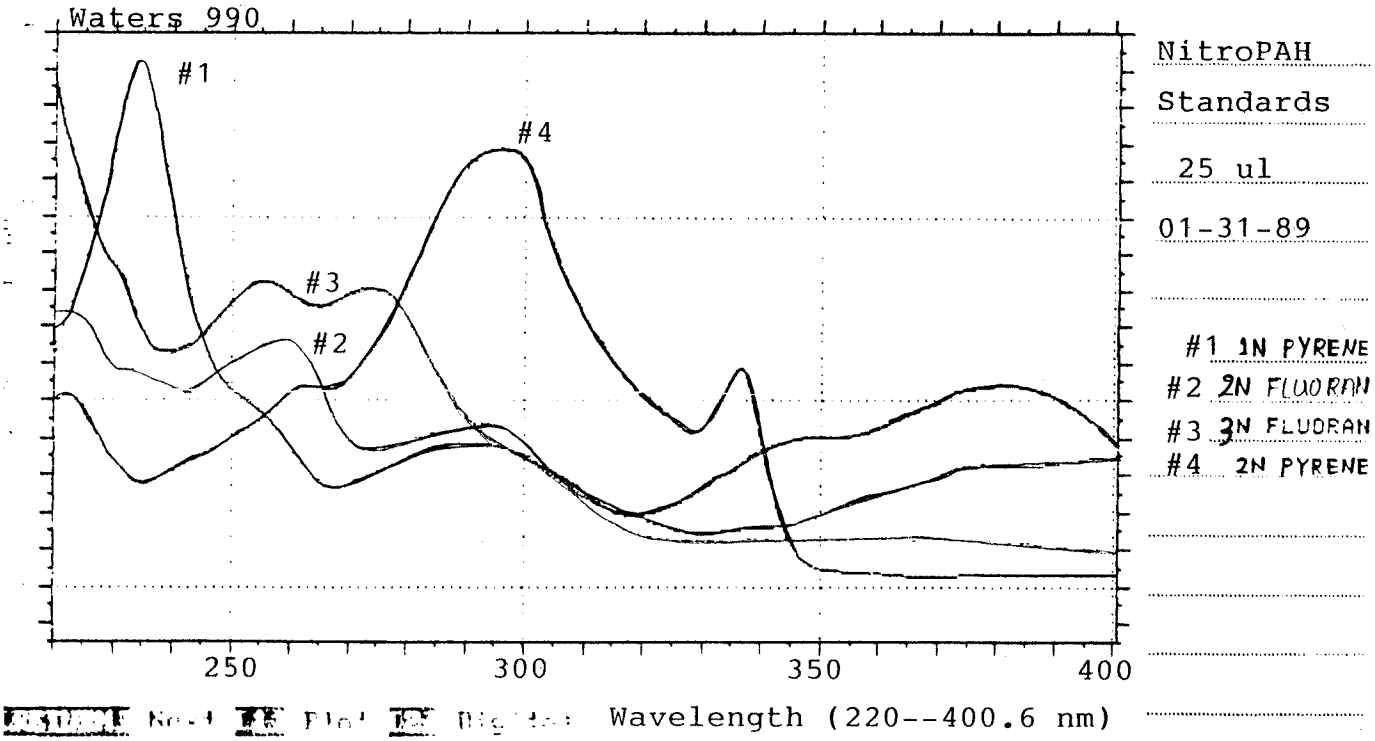


Figure 2-12

UV spectra of four standard nitro-PAH and HPLC chromatogram. Separation and identification on a C18 column (25cm x 4.6mm, Vydac) with Photo Diode Array detection.
(See Figure 2-11 as reference.)



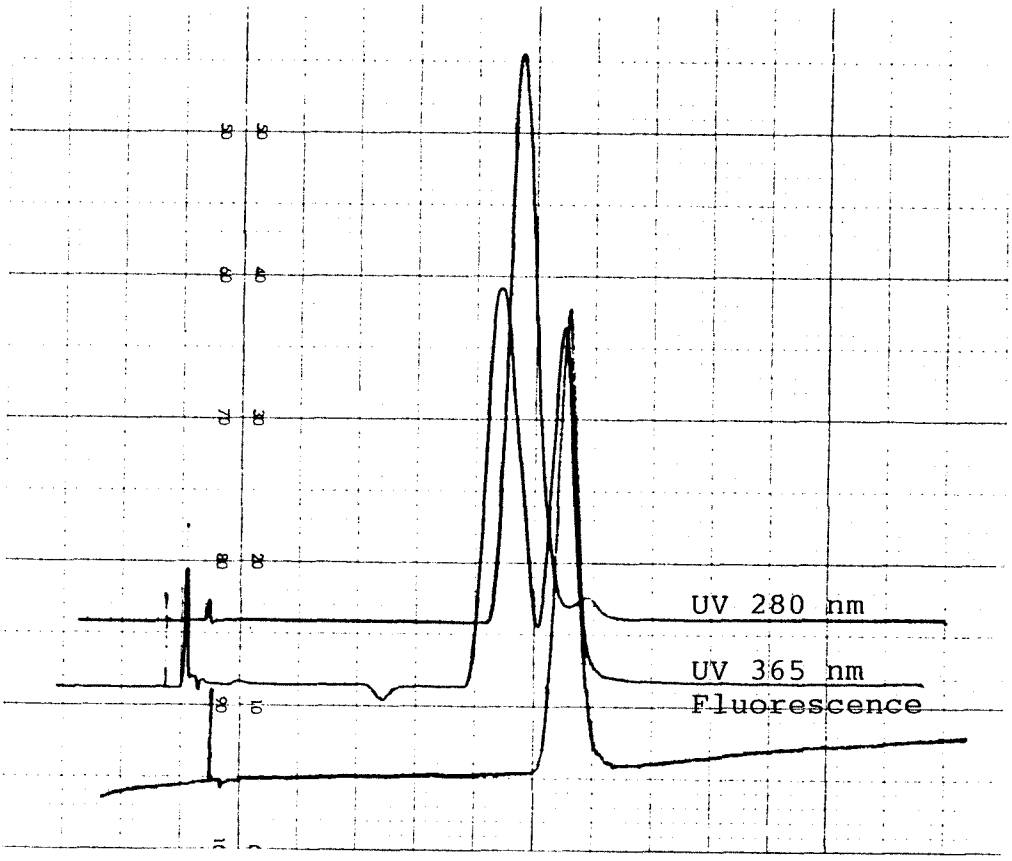


Figure 2-13

Separation of a standard mixture of four nitro-PAH on a 3x8C minicolumn (Perkin Elmer). 5.0 μl each.

No clear separation can be observed.

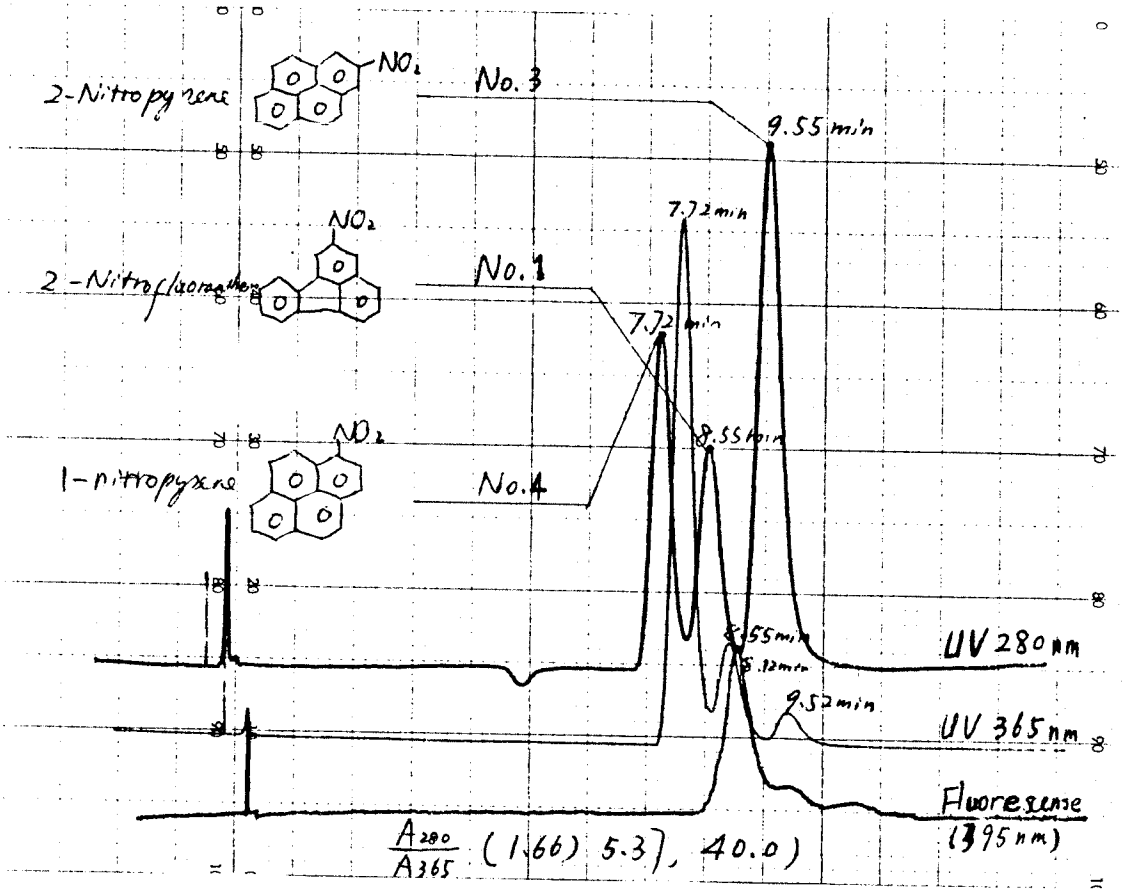


Figure 2-14

Separation of a standard mixture of three nitro-PAH (no 3-nitrofluoranthene) on a 3x8C minicolumn (Perkin Elmer). 8.0 µl each.

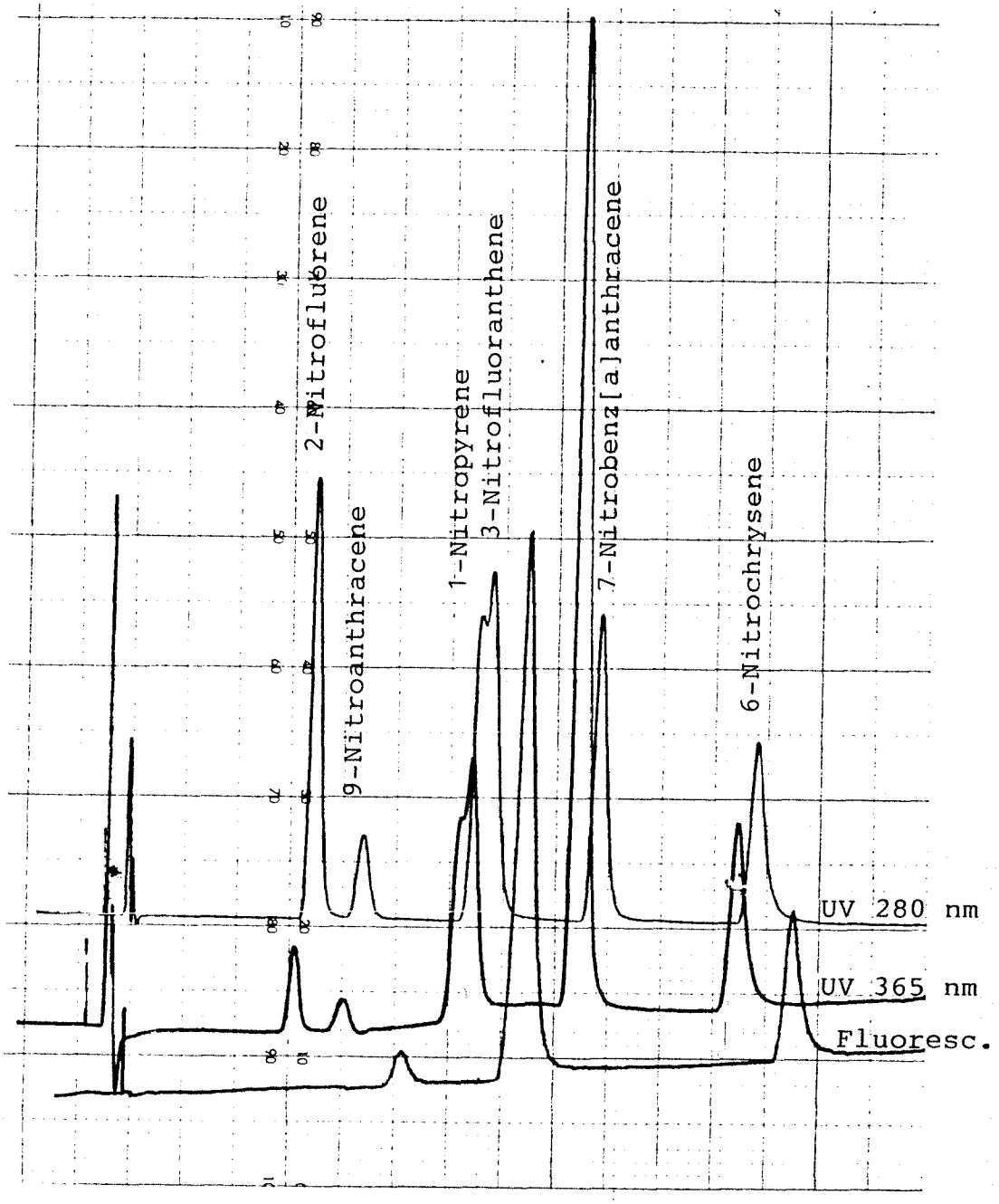


Figure 2-15

Separation of SRM 1587 on a 3x8C minicolumn (Perkin Elmer) under standard operating conditions.

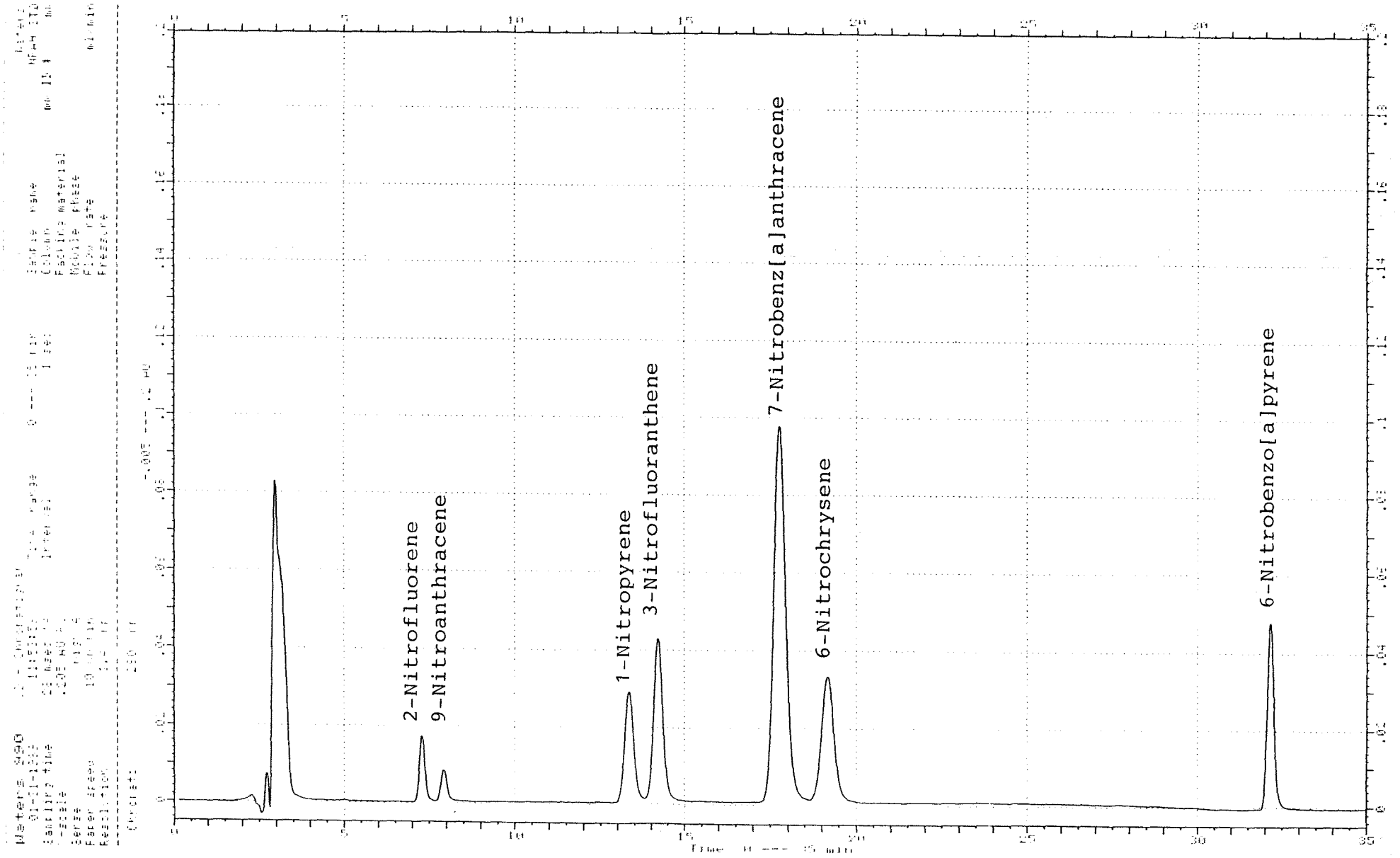


Figure 2-16

Separation of SRM 1587 on a C18 column (25cm x 4.6mm, Vydac) with Photo Diode Array UV detection under standard operating conditions.

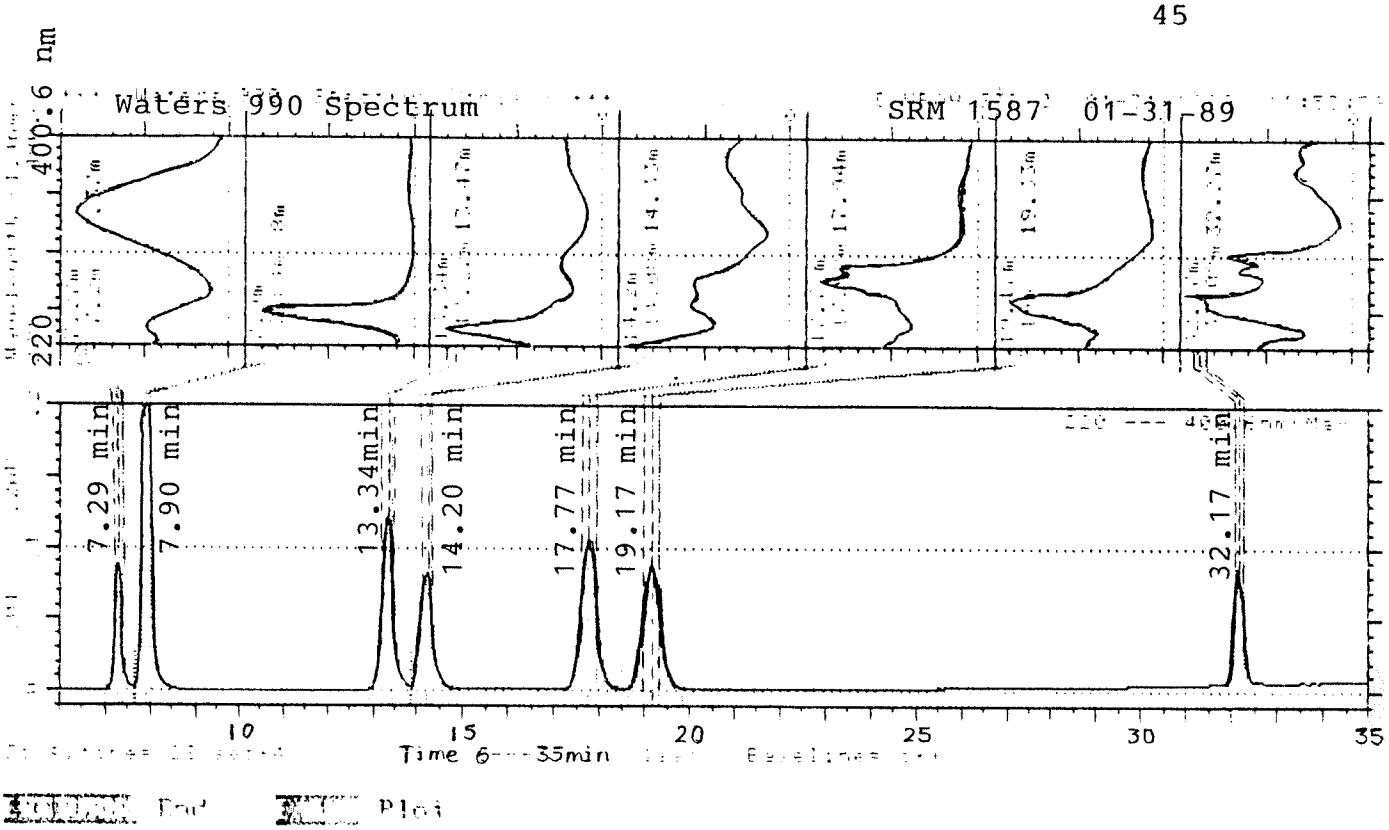


Figure 2-17

HPLC chromatogram(with retention times) and UV spectra of nitro-PAH in SRM 1587.

Separation on a C18 column (25cm x 4.6mm, Vydac) with Photo Diode Array UV detection.

Figure 2. REVERSED-PHASE LC SEPARATION OF SRM 1587 MONO-NITRATED PAH IN METHANOL

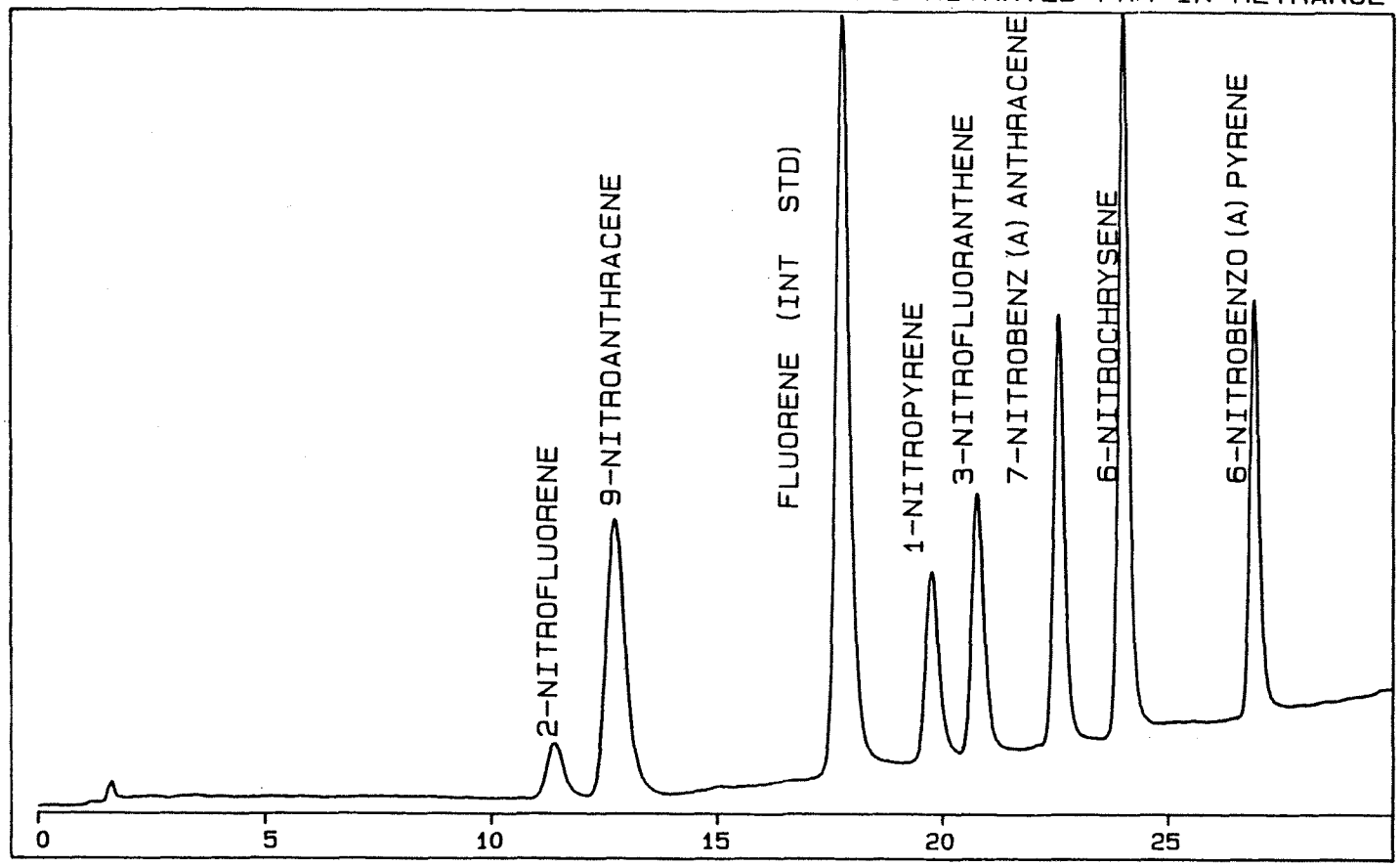


Figure 2-18

Separation of SRM 1587 on a C18 column (Zorbax ODS) with UV detection at 254 nm.

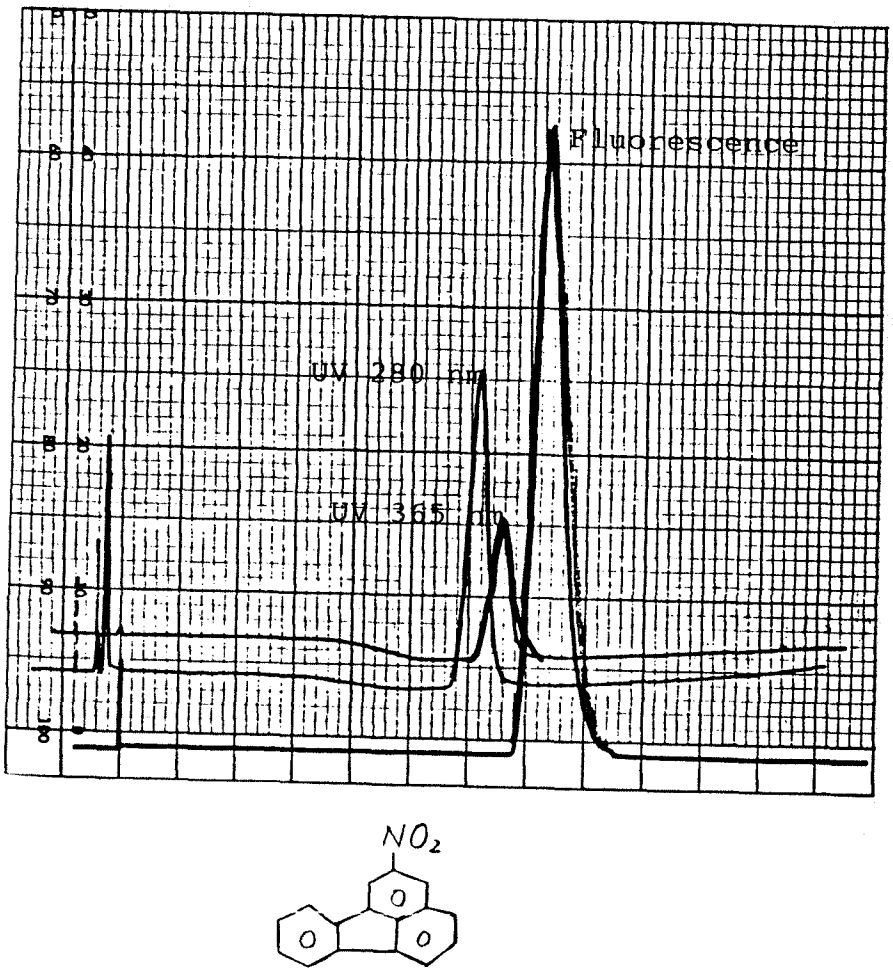


Figure 2-19

HPLC chromatogram of standard 2-nitrofluoranthene with oxygen scrubber on line. (See Figure 2-7 to compare.)

Column: 3x8C minicolumn, Perkin Elmer.

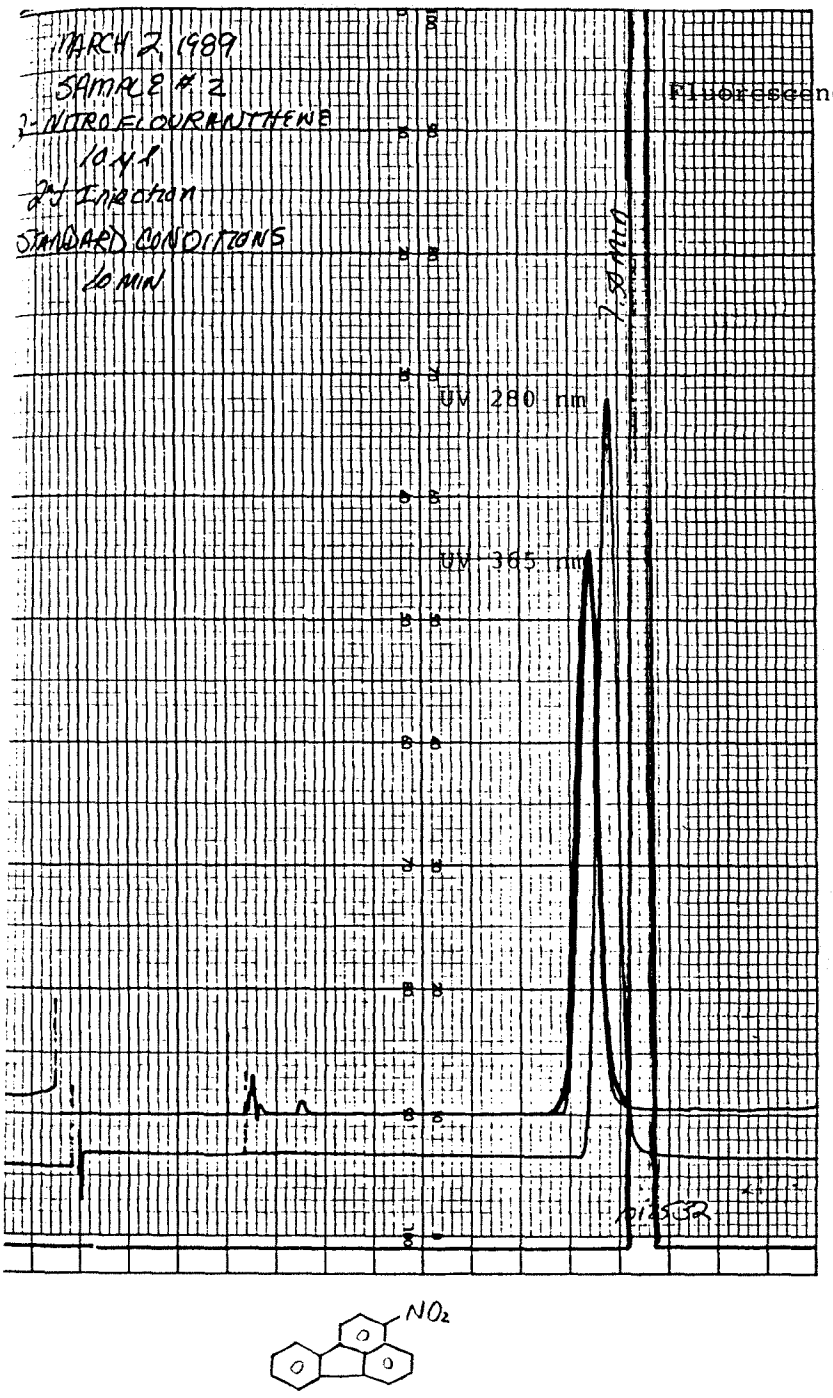


Figure 2-20

HPLC chromatogram of standard 3-nitrofluoranthene with oxygen scrubber on line. (See Figure 2-8 to compare.)

Column: 3x8C minicolumn, Perkin Elmer.

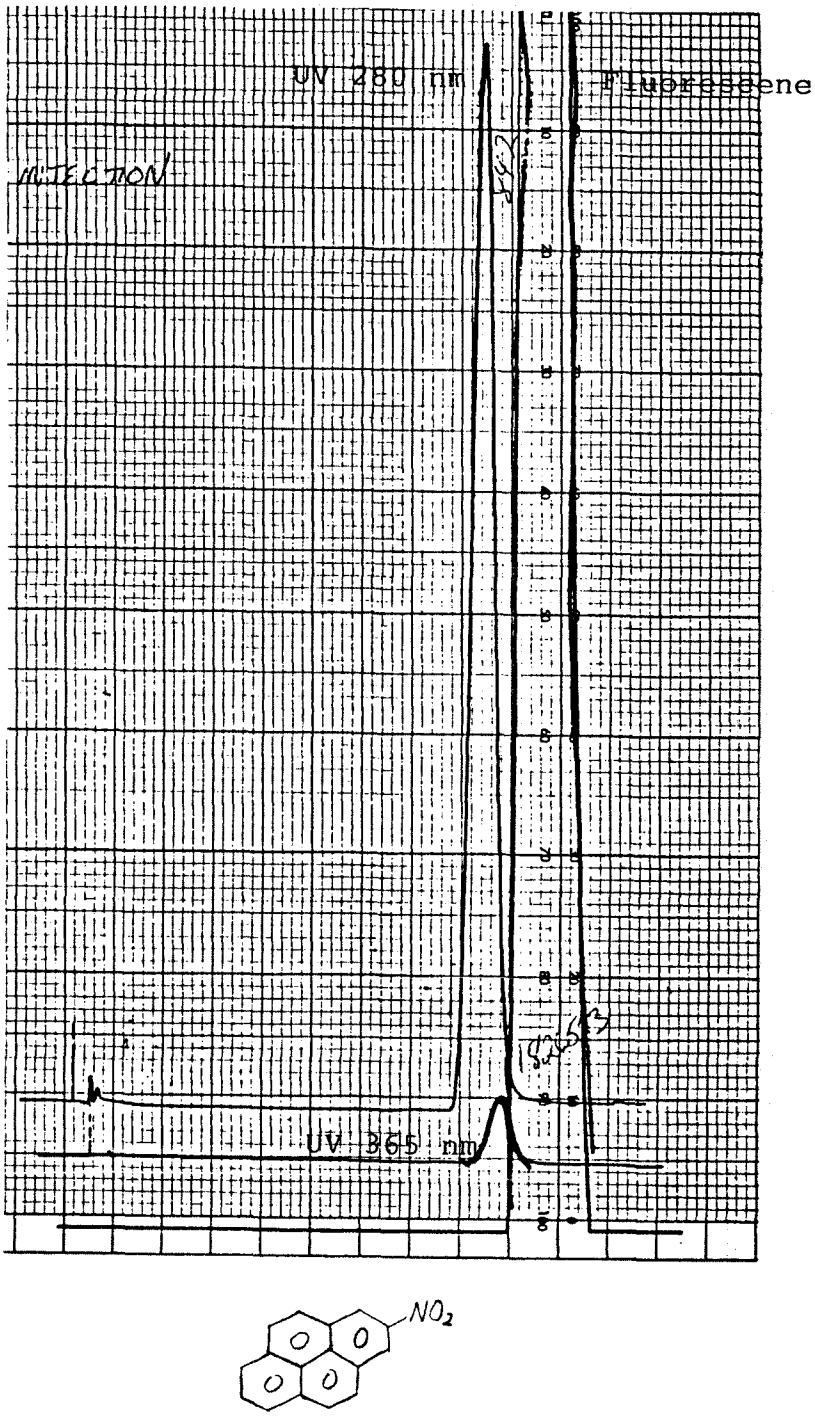


Figure 2-21

HPLC chromatogram of standard 2-nitropyrene with oxygen scrubber on line. (See Figure 2-9 to compare.)

Column: 3x8C minicolumn, Perkin Elmer.

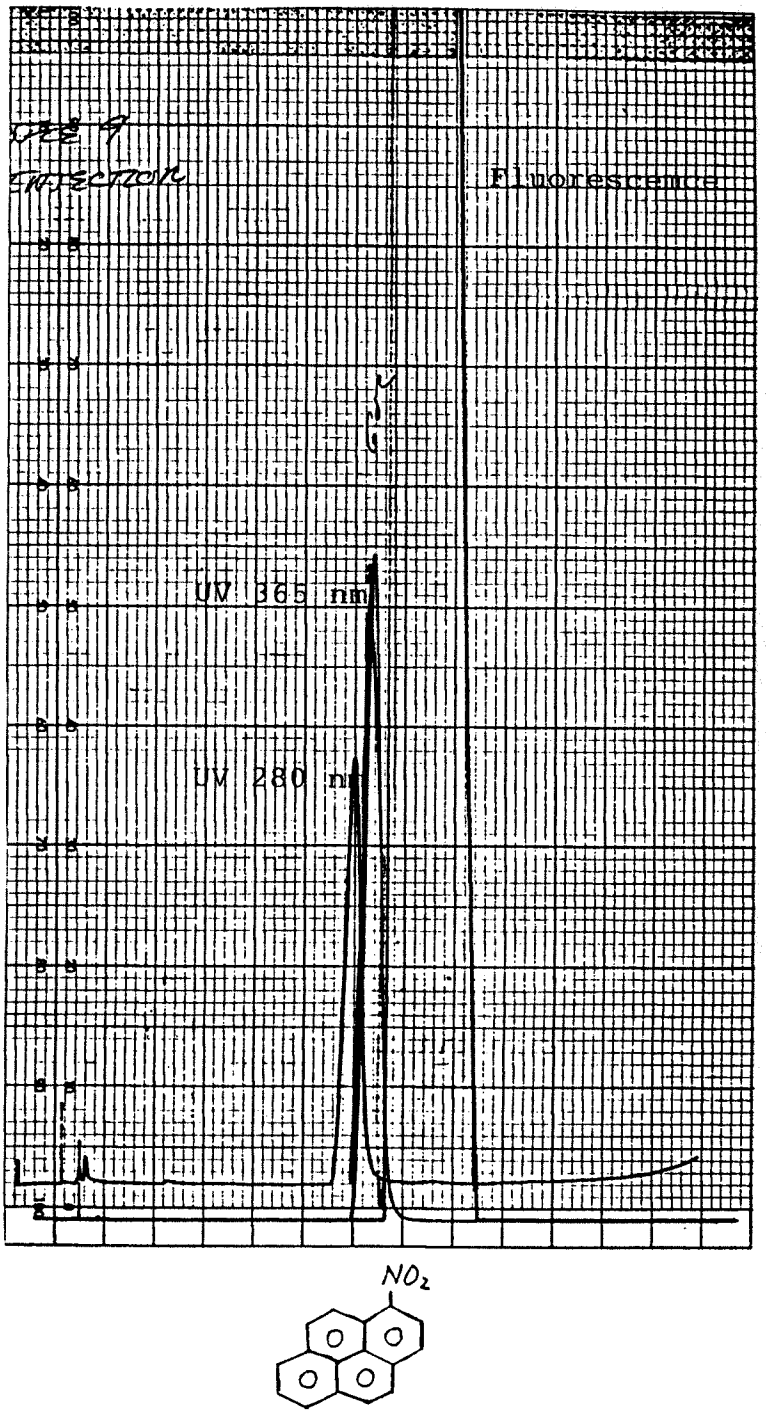


Figure 2-22

HPLC chromatogram of standard 1-nitropyrene with oxygen scrubber on line. (See Figure 2-10 to compare.)

Column: 3x8C minicolumn, Perkin Elmer.

CHAPTER THREE

Analysis of Environmental Samples for Nitro-PAH Using HPLC

3.1 Sample Collection

Airborne particles can be sampled directly by drawing a known flow of air through an appropriate filter. These samples, which are frequently total suspended particulate (TSP), have been collected routinely for many years[1]. However, the TSP samples do not provide a realistic measure for inhalation dosimetry because the lungs do not collect particles like a filter. Instead, inhaled particles are deposited by impaction, sedimentation, or diffusion in different regions of the respiratory system [2,3]. Determining the physical size of particles is important in particulate sampling for inhalation hazard evaluation since the aerodymanic diameter of the particles is the major parameter which governs the particle deposition site in the lungs[4]. Size selective mass sampling of airborne particles would therefore enable classification according to their physical characteristics and is often considered more appropriate in hazard evaluation.

The ambient air particulate samples described in this study were collected by using two different types of co-located samplers for 10 days in Winter, 1988, on the roof of

the Newark Ironbound Boys Club building on Clifford Street in Newark. Samplers used are one IP high volume type which includes two stage fractionators and one hi-vol blower and one TSP sampler. The IP sampler is designed to collect particles less than 10 microns because this size of air particles can be inhaled by humans and are considered lung damaging[4].

The collection period was 12/11-12/12/89 for winter samples.

Before the samples were collected, pre-fired high-volume quartz filters supplied by NJDEP (New Jersey Department of Environmental Protection) were dried in a dessicator for 24 hours and then weighed. The particulate samples collected from Newark were stored overnight in aluminum foil and dried in a dessicator for an additional 24 hours prior to soxhlet extraction. The results of sampling of TSP and IP10 are given in Table 3-1.

Table 3-1 Sampling of TSP and IP10 for 1988 winter

TSP:

Filter #	Weight (1)	Weight (2)	Weight (3)	Time & Pressure
1	4.2838	4.2356	0.0482	48 hrs, 3.8
4	4.3219	4.2700	0.0519	24 hrs, 4.0
6	4.4075	4.2532	0.1543	28 hrs, 3.5
8	4.2814	4.1848	0.0966	24 hrs, 3.8
10	4.2876	4.1809	0.1067	23 hrs, 4.2
11	4.3382	4.2353	0.1029	24 hrs, 3.4
14	4.4245	4.2761	0.1484	24 hrs, 3.5
16	4.4350	4.3421	0.0929	24 hrs, 3.2
18	4.3500	4.2741	0.0759	24 hrs, 3.75
Total: 9			0.8778	16,000 m^3 air

IP10

Filter #	Weight (1)	Weight (2)	Weight (3)	Time & Pressure
2	4.2451	4.2145	0.0306	24 hrs, 3.1
3	4.2902	4.2584	0.0318	24 hrs, 2.6
5	4.3552	4.2727	0.0825	28 hrs, 3.6
7	4.2056	4.1573	0.0483	24 hrs, 3.4
9	4.2741	4.2131	0.0610	22 hrs, 3.3
12	4.2535	4.1941	0.0594	48 hrs, 3.5
13	4.3216	4.2395	0.0821	24 hrs, 3.5
15	4.3074	4.2492	0.0582	24 hrs, 3.5
17	4.3728	4.3271	0.0457	24 hrs, 3.5
Total 9			0.4996	16,000 m^3 air

* Weight (1): weight of sampled filter (gram)

Weight (2): weight of blank filter (gram)

Weight (3): weight of soot (gram)

3.2 Sample Preparation

Particle Extraction. Extracts of environmental particulates contain thousands of individual chemical compounds. The nitro-PAH compounds are minor components and are usually present at low concentrations (below 50 ppm in the particulate extracts)[5] compared to the other chemical species present.

These samples are typically too complex to allow direct HPLC/UV analysis. Positive identification of individual nitro-PAH isomers requires that the particulates be extracted with a suitable organic solvent and prefractionated using chromatography before analysis.

Each filter of TSP and IP10 was first soxhlet extracted in the dark with 250 ml of dichloromethane (DCM), Photrex grade (J.T. Baker) or GC grade (Burdick & Jackson) for 24 hours. Then, each 250 ml extract was concentrated to 5 ml using Kuderna-Danish apparatus, and ultimately all extracts were combined to one extract. During extraction and concentration, every apparatus was wrapped by aluminum foil to protect samples from roomlight to avoid photodecomposition of photosensitive analytes [5]. The extracts were cooled, and blown down by nitrogen to get rid of extra solvent. Final volume was 240 ml for TSP and IP10.

3.3 Separation/Fractionation Procedure

Screening samples for nitro-PAH using TLC method

Thin layer chromatography (TLC) is a practical method for separating nitro-PAH from the large amount of the other chemical species present in particulate extracts without the need for extensive sample cleanup. Thin-layer chromatography was used in early studies of the mutagenicity of diesel and urban air particulate extracts [6,7,8]. Pitts et al. [6] described how nitro-PAH present in the Los Angeles atmosphere were collected, extracted from particulate matter, and chromatographed on a thin-layer plate. The particulate extracts were applied as bands to conventional silica gel TLC plates, and after development, the plate divided into zones. The nitro-PAH zone was indentified by comparison with the retention distance of standards under UV light and then scraped from the plate. Then each TLC band was extracted using DCM and methanol and the components were later detected using HPLC.

The final concentrate extract was injected onto ANASILGF-Precoated plates (250 microns, 20 x 20 cm, Analabs) for TLC with co-injection of nitro-PAH standards and then developed in 100 ml toluene and hexane (1:3) for about 35 minutes. Then the plate was left in a dark hood for 40 minutes to allow the solvent evaporation. Yellow traces could be observed by eye on the dry plate. For

visualization, the dry plate was examined by a UV detector. The nitro-PAH zones showed significant bright fluorescent spots at 9.5 to 10.6 cm when viewed by eye. Standard nitro-PAH (1, 2, 3 and 4) were found in the range 9.5 to 10.6 cm and this zone was thus scraped from the plate.

The silica gel powder containing nitro-PAH was extracted first by 20 ml methanol at 60°C for 20 minutes and then with 20 ml DCM at 40°C for 20 minutes in an ultrasonic bath (Sonic Systems, Inc.). Then DCM and methanol extracts were combined and cooled and blown down by nitrogen in the dark to concentrate. Figure 3-1 gives the flowchart of sample preparation [1,9].

3.4 Identification and Quantitation of Nitro-PAH in Air Samples by HPLC

(a) Analysis by the minicolumn HPLC system with Waters 660 UV detection at 280 nm and 365 nm. The HPLC chromatographic profiles of air samples TSP and IP10 measured by Waters 660 UV detectors are given in Figures 3-2 and 3-3.

Although nitro-PAH were prefractionated by thin layer chromatography from a complex organic matrix before they were applied to HPLC, the presence of significant quantities of PAH was a source of interference. Actually no clear separation could be observed in the range where the target

nitro-PAH are supposed to appear, and as a result, the identification and quantitation became impossible (the retention times and absorbance areas are not shown in this paper). Even the co-injection of standard nitro-PAH (1-nitropyrene and 2-nitropyrene) would not help (Figures 3-4 and 3-5). Thus, a sensitive and selective HPLC detector must be employed.

(b) Analysis by C18 column (25cm x 4.6mm, Vydac) HPLC system with Waters 990 Photo Diode Array UV detection. Presently, the HPLC Photo Diode Array UV detector is one of the most powerful tools to give the necessary precision and accuracy. The UV spectrum of the shoulder, the head and the tail, and even anywhere between, of each peak can be obtained at the same time in one run and identification can be easily done by comparing the UV spectrum obtained with that of interest. Therefore, UV spectra of standard nitro-PAH are required. Consequently, the interference from significant amount of PAH is greatly decreased. If the UV spectrum of the compound is characterized, the accuracy of identification is promised by this method. The assignments should also be aided by applying the retention times of standard nitro-PAH measured under the same conditions when such difficulties occur: (a) Some nitro-PAH isomers have identical UV spectra. The examples are that 9-nitro-anthracene has an identical UV spectrum (Figure 4-3(b)) with 9,10-dinitroanthracene (Figure 4-9) and three hydroxynitro-

PAH isomers have almost identical UV spectra (Figure 4-11 and 4-12). (b) Sometimes the wavelength (or the position) of the main peak moved (usually less than 10 nm) from where that of the standard appears. For example the main peak of 2-nitropyrene in sample IP10 (Figure 4-18) is at 285 nm while it is at 295 nm in the standard UV spectrum (Figure 4-2(b)).

The HPLC chromatographic profiles of particulate matter extracts of TSP and IP10 are provided in Figures 3-6 to 3-7. Matching of the UV spectra with HPLC profiles are shown in Figures 3-8(a) to 3-8(c) for TSP and Figures 3-9(a) and 3-9(b) for IP10. Original data of UV absorbance areas and retention times of air samples with Photo Diode Array UV detection at 280 nm are given in Table 3-2 for TSP and Table 3-3 for IP10.

(c) Results of nitro-PAH in air particulate matter extracts TSP and IP10. The results of identification of TSP and IP10 are give in Tables 3-4 to 3-9. Enlarged HPLC chromatographic profiles with UV spectra in a time range of 12 to 19 minutes where the target nitro-PAH eluted in standard mixtures are given in Figures 3-10 for TSP and 3-11 for IP10. A comparison of PAH distribution in sample TSP and IP10 is made in Table 3-10.

Table 3-2

Retention times and UV absorbance areas of sample TSP on a C18 column (25cm x 4.6mm, Vydac) with Photo Diode Array UV detection at 280 nm.

TSP: 100.0 ul

Report File IPNP.DT3

280 nm

No.	Retention time	Height [AU]	Left time	Right time	Area [AU*min]	Area [%]	Mark
1	2.72	0.0763	1.72	2.82	0.014602	1.851	
2	3.05	0.2141	2.82	4.25	0.123825	15.697	V
3	3.42	0.0195	3.29	3.59	0.002942	0.373	TA
4	4.55	0.0285	4.25	5.05	0.018734	2.375	V
5	5.20	0.0192	5.05	5.84	0.012037	1.526	V
6	5.45	0.0029	5.25	5.69	0.000625	0.079	TA
7	6.57	0.0179	5.84	7.15	0.017455	2.213	V
8	7.47	0.0125	7.15	7.60	0.004801	0.609	V
9	7.74	0.0126	7.60	8.25	0.007012	0.889	V
10	8.52	0.0120	8.25	8.60	0.003651	0.463	V
11	8.82	0.0023	8.67	8.95	0.000367	0.047	TB
12	9.00	0.0190	8.60	9.37	0.009979	1.265	V
13	9.65	0.0065	9.37	9.75	0.002242	0.284	V
14	9.91	0.0062	9.75	9.98	0.001429	0.181	V
15	10.64	0.0091	9.98	11.18	0.006512	0.826	V
16	11.77	0.0035	11.18	12.06	0.001511	0.192	
17	12.80	0.0363	12.06	13.30	0.016455	2.086	V
18	13.83	0.0257	13.25	14.33	0.014133	1.792	
19	14.61	0.0089	14.33	15.11	0.004021	0.510	V
20	15.43	0.0015	15.18	15.68	0.000428	0.054	TB
21	15.73	0.0051	15.10	16.27	0.002999	0.380	I
22	16.89	0.0187	16.26	17.42	0.010316	1.308	
23	17.97	0.0161	17.42	18.77	0.011014	1.396	V
24	18.43	0.0016	18.02	18.67	0.000544	0.069	TA
25	19.23	0.0149	18.96	19.55	0.004324	0.548	TB
26	19.73	0.1041	18.75	21.22	0.082703	10.484	I
27	21.89	0.0210	21.21	23.13	0.013638	1.729	I
28	22.70	0.0010	22.44	23.00	0.000301	0.038	TA
29	24.30	0.0718	23.13	25.54	0.041442	5.254	I
30	26.41	0.0281	25.54	27.45	0.016279	2.064	I
31	28.10	0.0023	27.15	28.50	0.001036	0.131	I
32	29.21	0.0089	28.48	29.61	0.004552	0.577	
33	30.28	0.0181	29.61	30.47	0.009455	1.199	V
34	31.06	0.0535	30.47	31.26	0.024657	3.126	V
35	31.74	0.1716	31.26	32.17	0.072948	9.248	V
36	32.32	0.0403	32.17	32.49	0.012445	1.578	V
37	32.75	0.1413	32.49	33.45	0.050301	6.377	V
38	33.68	0.0014	33.57	33.82	0.000169	0.021	TB
39	33.87	0.0234	33.45	34.38	0.014804	1.877	V
40	34.17	0.0014	34.03	34.33	0.000220	0.028	TA
41	34.70	0.0682	34.38	35.22	0.022812	2.892	V
42	35.55	0.0026	35.12	35.70	0.000384	0.049	TB
43	36.18	0.1015	35.22	36.55	0.042982	5.449	V
44	36.71	0.0132	36.55	37.16	0.003997	0.507	V
45	37.13	0.0086	37.15	37.73	0.002079	0.264	I
46	37.95	0.0076	37.68	38.50	0.002307	0.292	I
47	38.75	0.0041	38.48	39.35	0.001728	0.219	I
48	39.13	0.0013	38.85	39.33	0.000268	0.034	TA
49	40.03	0.0136	39.10	40.38	0.005134	0.651	I
50	40.71	0.0221	40.38	41.36	0.006619	0.839	I
51	42.44	0.1490	41.83	43.39	0.062551	7.930	I
52	43.64	0.0017	43.37	44.39	0.001065	0.135	I

Table 3-3

Retention times and UV absorbance areas of sample IP10 on a C18 column (25cm x 4.6mm, Vydac) with Photo Diode Array UV detection at 280 nm.

IP10: 100 ul

Report File TSPNP.DT3

280 nm

No.	Retention time	Height [AU]	Left time	Right time	Area [AU*min]	Area [%]	Mark
1	2.35	0.0011	1.68	2.40	0.000172	0.015	
2	2.70	0.0436	2.40	2.80	0.008390	0.750	V
3	3.00	0.2718	2.80	4.18	0.146259	13.066	V
4	4.33	0.0344	4.18	4.93	0.020773	1.856	V
5	5.37	0.0230	4.93	5.77	0.016500	1.474	V
6	6.50	0.0019	6.32	6.67	0.000366	0.033	TB
7	6.72	0.0230	5.77	7.18	0.025024	2.236	V
8	7.42	0.0173	7.18	7.53	0.005452	0.487	V
9	7.67	0.0171	7.53	8.15	0.009185	0.821	V
10	8.40	0.0158	8.15	8.50	0.005103	0.456	V
11	8.73	0.0060	8.58	8.91	0.001063	0.095	TB
12	8.96	0.0373	8.50	9.31	0.019736	1.763	V
13	9.54	0.0120	9.31	9.78	0.005251	0.469	V
14	10.03	0.0118	9.78	10.26	0.005453	0.487	V
15	10.57	0.0138	10.26	11.02	0.007829	0.699	V
16	11.80	0.0148	11.02	12.13	0.010970	0.980	V
17	12.72	0.0851	12.13	13.16	0.044145	3.944	V
18	13.71	0.0231	13.16	14.19	0.016146	1.442	V
19	14.56	0.0148	14.19	15.01	0.006927	0.619	V
20	15.34	0.0082	15.09	15.82	0.002516	0.225	TB
21	15.87	0.0276	14.96	16.32	0.017873	1.597	I
22	16.84	0.0321	16.29	17.35	0.016816	1.502	
23	17.83	0.0107	17.35	17.91	0.004103	0.367	V
24	18.25	0.0139	17.91	18.75	0.007174	0.641	V
25	19.28	0.0273	18.70	19.49	0.013503	1.206	
26	19.94	0.0506	19.49	21.19	0.033442	2.988	V
27	21.80	0.0378	21.15	23.10	0.026090	2.331	I
28	22.68	0.0021	22.37	23.00	0.000693	0.062	TA
29	24.23	0.1518	23.11	25.57	0.089587	8.003	I
30	26.37	0.0597	25.59	27.51	0.036789	3.287	I
31	28.20	0.0048	27.53	28.43	0.002746	0.245	
32	28.68	0.0063	28.43	28.86	0.002167	0.194	V
33	29.27	0.0190	28.86	29.71	0.009519	0.850	V
34	30.35	0.0280	29.71	30.57	0.014973	1.338	V
35	31.14	0.0924	30.57	30.62	0.000965	0.086	V
36	31.14	0.0924	30.62	31.35	0.037151	3.319	V
37	31.82	0.2502	31.35	32.28	0.099667	8.904	V
38	32.43	0.0011	32.31	32.56	0.000156	0.014	TB
39	32.86	0.2229	32.28	33.60	0.076444	6.829	V
40	33.83	0.0027	33.71	33.98	0.000349	0.031	TB
41	34.03	0.0397	33.60	34.55	0.021623	1.932	V
42	34.35	0.0015	34.21	34.50	0.000250	0.022	TA
43	34.88	0.1160	34.55	35.50	0.038452	3.435	V
44	35.81	0.0277	35.50	35.88	0.007131	0.637	V
45	36.38	0.1842	35.88	36.79	0.067056	5.990	V
46	36.96	0.0225	36.79	37.46	0.006793	0.607	V
47	37.73	0.0177	37.41	38.03	0.004424	0.395	I
48	38.26	0.0126	37.98	38.76	0.003731	0.333	I
49	39.06	0.0116	38.73	39.76	0.005826	0.520	I
50	39.46	0.0027	39.16	39.69	0.000740	0.066	TA
51	39.96	0.0014	39.81	40.09	0.000211	0.019	TB
52	40.34	0.0175	39.74	40.64	0.007373	0.659	
53	41.07	0.0441	40.64	42.14	0.018532	1.656	V
54	41.91	0.0015	41.66	42.14	0.000391	0.035	TA
55	42.77	0.2033	42.21	43.65	0.087682	7.833	
56	43.82	0.0045	43.65	44.17	0.001311	0.117	V
57	44.32	0.0018	44.17	44.87	0.000397	0.035	I

Table 3-4 Identification of Nitro-PAH in air particulate matter extract IP10 (1988 winter).

Column: Reverse phase C18 polymeric, 25cm x 4.6mm, Vydac.

Compd.	RT (min)		Cite of UV spectrum	
	Std.*	Sample	Std.	Sample
1-Nitropyrene	13.34	UD*	F.4-1(a)	---
2-Nitrofluoranthene	13.73	13.83	F.4-1(b)	F.4-17
3-Nitrofluoranthene	14.20	UD	F.4-2(a)	---
2-Nitropyrene	17.75	17.97	F.4-2(b)	F.4-18
2-Nitrofluorene	7.29	UD	F.4-3(a)	---
9-Nitroanthracene	7.90	8.04	F.4-3(b)	F.4-19
9,10-Dinitroanthracene	N/A*	10.56	F.4-9	F.4-20
7-Nitrobenz[a]anthracene	17.77	UD	F.4-5(a)	---
6-Nitrochrysene	19.17	UD	F.4-5(b)	---
6-Nitrobenz[a]pyrene	32.17	32.32	F.4-6	N/A

* Std.: The standard retention of nitro-PAHs from Tables 2-3 and Table 2-5.

* UD: Undetected or the UV spectrum was not obtained.

* N/A: The standard analysis data are not available.

Table 3-5 Identification of nitro-PAH in air particulate matter extract TSP (1988 winter).

Column: Reverse phase C18 polymeric, 25cm x 4.6mm, Vydac.

Compd.	RT (min)		Cite of UV spectrum	
	Std.*	Sample	Std.	Sample
1-Nitropyrene	13.34	13.46	F.4-1(a)	F.4-14a
2-Nitrofluoranthene	13.73	13.81	F.4-1(b)	F.4-15
3-Nitrofluoranthene	14.20	UD*	F.4-2(a)	---
2-Nitropyrene	17.75	17.77	F.4-2(b)	F.4-14b
2-Nitrofluorene	7.29	UD	F.4-3(a)	---
9-Nitroanthracene	7.90	8.04	F.4-3(b)	F.4-13
9,10-Dinitroanthracene	N/A*	10.57	F.4-9	F.4-16a
7-Nitrobenz[a]anthracene	17.77	UD	F.4-5(a)	---
6-Nitrochrysene	19.17	UD	F.4-5(b)	---
6-Nitrobenz[a]pyrene	32.17	32.43	F.4-6	F.4-16b

* Std.: Retention times of standard nitro-PAH are from Tables 2-3(a) and 2-5.

* UD: Undetected or the UV spectrum was not obtained.

* N/A: No standard 9,10-dinitroanthracene is available.

Table 3-6 UV absorbance at 280 nm of nitro-PAH in SRM 1587 (25 μl) and sample IP10 (100 μl) done by Photo Diode Array HPLC (Vydac column, 25cm x 4.6mm).

Compd.	Standard Absorb.		Sample Absorb.	
	Peak Area	Conc.	Orig.	Estimd.*
1-Nitropyrene	0.00837	7.07	UD*	
2-Nitrofluoranthene	0.0247	3.60	0.0141	0.0106
3-Nitrofluoranthene	0.0133	7.30	UD	
2-Nitropyrene	0.0329	1.70	0.0110	0.0010
9-Nitroanthracene	0.00173	3.96	0.00701	0.0035
6-Nitrobenz[a]pyrene	0.0110	4.8	0.0124	0.0124

* UD: Undetected or the UV spectrum was not obtained.

* Orig: Original data of UV absorbance (peak area) from Table 3-3.

* Estimd: The peak area was estimated when its "Orig." data present a group of coeluted peaks.

Table 3-7 Concentrations of nitro-PAH in sample IP10.

Compd.	Concentration*		
	μg/ml in N-PAH Fraction	μg/g in Particulates	ng/m^3 in Air
1-Nitropyrene	UD	-	-
2-Nitrofluoranthene	0.38	0.89	0.028
3-Nitrofluoranthene	UD	-	-
2-Nitropyrene	0.13	0.030	0.00092
9-Nitroanthracene	2.0	4.6	0.14
6-Nitrobenz[a]pyrene	1.4	3.1	0.097

Total		8.62	

(a) 2-Nitrofluoranthene/2-Nitropyrene: 29.9

(b) The abundance in ambient air particulate matter:

$9\text{-}NO_2\text{-}AN > 6\text{-}NO_2\text{-}B[a]PY > 2\text{-}NO_2\text{-}FL > 2\text{-}NO_2\text{-}PY > 1\text{-}NO_2\text{-}PY$

* (a) Volume of sample IP10 tested: 100 μl

(b) Volume of Nitro-PAH final extract: 1144 μl

(c) Weight of IP10 particulates: 0.4996 grams

(d) Volume of air sampled for IP10: 16,000 m^3

Column: Reverse phase C18 polymeric, 25cm x 4.6mm, Vydac

Table 3-8 UV absorbance at 280 nm of nitro-PAH in SRM 1587 (25 μl) and sample TSP (100 μl) done by Photo Diode Array HPLC (Vydac column, 25cm x 4.6mm).

Compd.	Standard. Peak area	Absorb. Conc.	Sample Absorb. Orig.	Estimd.*
1-Nitropyrene	0.00837	7.07	0.0161	0.00969
2-Nitrofluoranthene	0.0247	3.60	0.0161	0.00805
3-Nitrofluoranthene	0.0133	7.30	UD*	
2-Nitropyrene	0.0329	1.70	0.00410	0.0041
9-Nitroanthracene	0.00173	3.96	0.00919	0.0092
6-Nitrobenz[a]pyrene	0.0110	4.8	0.00165	0.0017

* UD: Undetected or the UV spectrum was not obtained.

* Orig: Original data of UV absorbance (or peak area) from Table 3-2.

* Estimd: The peak area was estimated when its "Orig." data presents a group of coeluted peaks.

Table 3-9 Concentrations of nitro-PAH in sample TSP.

Compd.	Concentration*		
	$\mu g/ml$ in N-PAH Fraction	$\mu g/g$ in Particulates	ng/m^3 in Air
1-Nitropyrene	2.0	2.7	0.15
2-Nitrofluoranthene	0.29	0.38	0.021
3-Nitrofluoranthene	UD*	-	-
2-Nitropyrene	0.053	0.069	0.0038
9-Nitroanthracene	5.3	0.15	0.37
6-Nitrobenz[a]pyrene	0.017	0.022	0.0013
Total		3.32	

(a) 2-Nitrofluoranthene/2-Nitropyrene: 5.56

(b) The Abundance in ambient air particulate matter:

$9\text{-}NO_2\text{-}AN > 1\text{-}NO_2\text{-}PY > 2\text{-}NO_2\text{-}FL > 2\text{-}NO_2\text{-}PY > 6\text{-}NO_2\text{-}B[a]PY$

* (a) Volume of sample TSP tested: 100 μl

(b) Volume of nitro-PAH final extract: 1139 μl

(c) Weight of TSP particulates: 0.8778 grams

(d) Volume of air sampled for TSP: 16,000 m^3

Column: Reverse phase C18 polymeric, 25cm x 4.6mm, Vydac

Table 3-10 Comparison of PAH in sample TSP and IP10.

Compd.	M.W.*	RT (min) TSP/IP10	Peak Area Ratio (TSP/IP)
Phenanthrene	178.23	8.96/8.82	32.3
Fluoranthene	202.08	12.72/12.80	2.68
Pyrene	202.26	14.56/14.66	1.72
Anthracene	178.23	18.25/18.43	13.2
Benzo[ghi]fluoranthene	226.28	21.80/21.89	1.62
Benz[a]anthracene	228.29	24.23/24.30	2.16
Chrysene	228.29	26.37/26.41	2.26
Benzo[j]fluoranthene	252.32	31.14/31.06	0.0587
Benzo[e]pyrene	252.32	31.82/31.74	2.28
Benzo[b]fluoranthene	252.32	32.86/32.75	1.52
Benzo[k]flouranthene	252.32	34.88/34.70	1.69
Benzo[a]pyrene	252.32	36.38/36.18	1.64
Dibenz[a,j]anthracene	278.35	36.96/36.71	1.70
Benzo[ghi]perylene	276.34	42.77/42.44	1.40

The ratio of the particle weight: TSP/IP = 1.757

* M.W.: Source: National Academy of Science, Polycyclic Aromatic Hydrocarbons, NRC Press, Washington, D.C., 1983.

3.5 Discussion:

(1) Waters model 990 Photo Diode Array HPLC is one of the most powerful tools to give satisfactory precision and accuracy for the analysis of nitro-PAH and PAH in ambient air particulate extracts. It overcomes the significant interference of PAH by its uniquly simple method of identifying nitro-PAH. The difficulty that has been long hampering chemists is partly solved.

But the data processing for the results of samples of TSP and IP10 by 990 Photo Diode Array HPLC was not completed because of limited opportunity and limited working time with Waters Associates on this project. Some(not many) valuable UV spectra of the suspected small peaks or the shoulders of other peaks in retention time range of 12 to 19 minutes were not searched for identification and some detailed, more accurate analysis data for quantitative determination were not obtained. Therefore, some nitro-PAH undetected in our samples maybe occur, and an estimated quantitation for the nitro-PAHs has to be made when the UV absorbance area of a group of peaks was given. In this case, an approximate value of peak area for analysis was estimated by carefully checking and comparing the HPLC chromatographic profiles. The concentrations of nitro-PAH given in this paper are approximate and the relative abundance in ambient air might be affected.

(2) We found five nitro-PAH, 2-nitrofluoranthene, 2-nitropyrene, 9-nitroanthracene, 9,10-dinitroanthracene and 6-nitrobenz[a]pyrene in the ambient particle sample IP10 (1988 winter). Their relative abundance in ambient air is

$$9\text{-}NO_2\text{-}AN > 6\text{-}NO_2\text{-}B[a]PY > 2\text{-}NO_2\text{-}FL > 2\text{-}NO_2\text{-}PY$$

1-Nitropyrene and 3-nitrofluoranthene were not detected. The ratio of 2-nitrofluoranthene to 2-nitropyrene is 29.9. The total amount of nitro-PAH in per gram of IP10 particles is 8.62 μg/g.

(3) We found six nitro-PAH, 1-nitropyrene, 2-nitropyrene, 2-nitrofluoranthene, 9-nitroanthracene, 9,10-dinitro-anthracene and 6-nitrobenz[a]pyrene in the ambient particle sample TSP (1988 winter). Their relative abundance in ambient air is

$$9\text{-}NO_2\text{-}AC > 1\text{-}NO_2\text{-}PY > 2\text{-}NO_2\text{-}FL > 2\text{-}NO_2\text{-}PY > 6\text{-}NO_2\text{-}B[a]PY$$

3-Nitrofluoranthene was not detected.
The ratio of 2-nitrofluoranthene to 2-nitropyrene is 5.56. The total amount of nitro-PAH in per gram of TSP particles is 3.32 μg/g.

(4) The results (2) and (3)are somewhat consistant with the following observations which seem to have general validity [10]:

In ambient particulate matter collected in both urban

and rural regions and in the winter as well as summer, 2-nitrofluoranthene is the predominant mononitroarene in ambient particles, followed by 1-nitropyrene and lesser amount of 2-nitropyrene (among the four nitro-PAH which are 2-nitrofluoranthene, 3-nitro-fluoranthene, 2-nitropyrene and 1-nitropyrene); and levels of 3-nitrofluoranthene are very low. Thus, the abundance of nitro-PAH is

$$2\text{-}NO_2\text{-}FL > 1\text{-}NO_2\text{-}PY \geq 2\text{-}NO_2\text{-}PY$$

1-Nitropyrene and 3-nitrofluoranthene were reported to be the major mononitro-PAH present in primary emissions of diesel soot. The abundance of nitro-PAH in diesel soot extracts is

$$1\text{-}NO_2\text{-}PY >> 3\text{-}NO_2\text{-}FL > 8\text{-}NO_2\text{-}FL$$

Table 3-11 shows the concentrations of four nitro-PAH that Pitts.(1987)[10] found in the ambient particulate extract at Riverside, CA.

Table 3-11 Concentrations of the three major nitro-PAH in ambient particulate matter collected over four consecutive time periods at Riverside, CA, 18-19 September 1984.

Date	Collection time (h)	Concentration (ng m^{-3})		
		1-NO_2-PY	2-NO_2-PY	2-NO_2-FL
18/9/84	1200-1800*	0.02	0.003	0.07
18/9/84	1800-2400	0.03	UD*	0.21
19/9/84	2400-0600	0.008	0.01	0.3
19/9/84	0600-1200	0.03	0.02	0.2

* A trace of 3-nitrofluoranthene was detected in the sample.

* UD: = None detected.

Pitts.(1987) also gave a gas-phase daytime reaction mechanism for 2-nitrofluoranthene and 2-nitropyrene in this paper and explained why these two compounds are the major mutagens in ambient particulate extracts when there is relatively abundant fluoranthene in the vapour phase and N_2O_5 is present in ambient atmospheres. The fact that 1-nitropyrene was found in the sample TSP and not in IP10 indicates that most 1-nitropyrene occurs on the surface of large particles which the IP10 sampler does not collect.

The much lower 2-NO_2-FL/2-NO_2-PY in sample TSP than in IP10 might indicate the greatly increased amount of 2-nitropyrene may be formed during sampling by a chemical

pathway totally different from the conventional nitration of mechanism of PAH [11].

The total amount of nitro-PAH detected per gram of IP10 particles (8.62 μg/g) is higher than that per gram of TSP particles (3.32 μg/g). This suggests that most nitro-PAH are formed in the surface of small particles (less than 10 microns) which are inhaled by human beings and should be considered in hazard evaluation.

9-Nitroanthracene, 9,10-dinitroanthracene and 6-nitro-benz[a]pyrene were reported to be detected in ambient particulate extracts [11].

(5) Fourteen PAHs were identified in both TSP and IP10 samples, pyrene and fluoranthene are among them. The order of the elution is quite consistant with the order of molecular weight [12]. Comparison of PAH abundance in sample TSP and IP10 shows that most PAHs except phenanthrene and anthracene have relatively similar distribution in both samples, concerning the TSP/IP10 ratio of particle weight (1.757).

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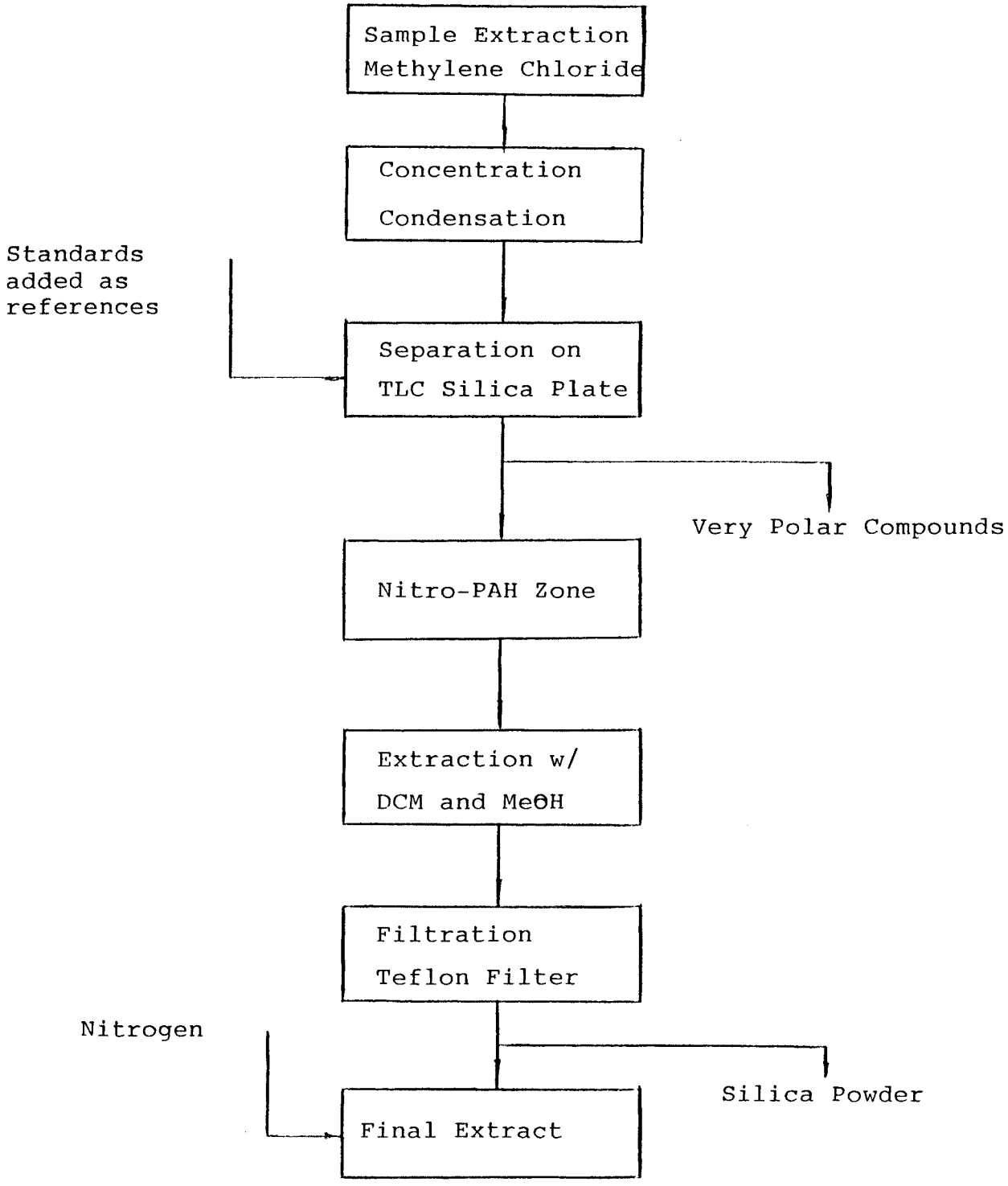


Figure 3-1

The overall separation scheme for air samples TSP and IP10.

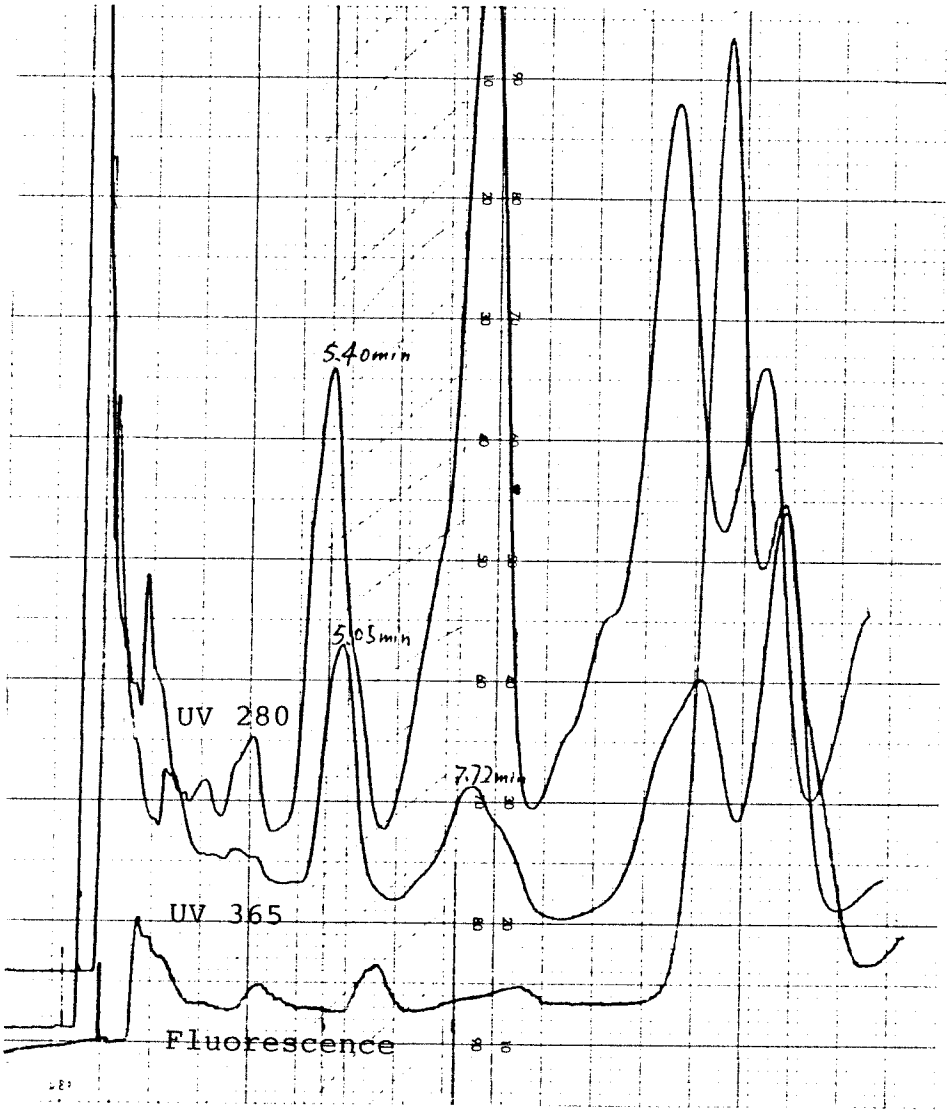


Figure 3-2

HPLC chromatogram of air sample TSP (100 μl) on a 3x8C minicolumn (Perkin Elmer) under standard operating conditions.

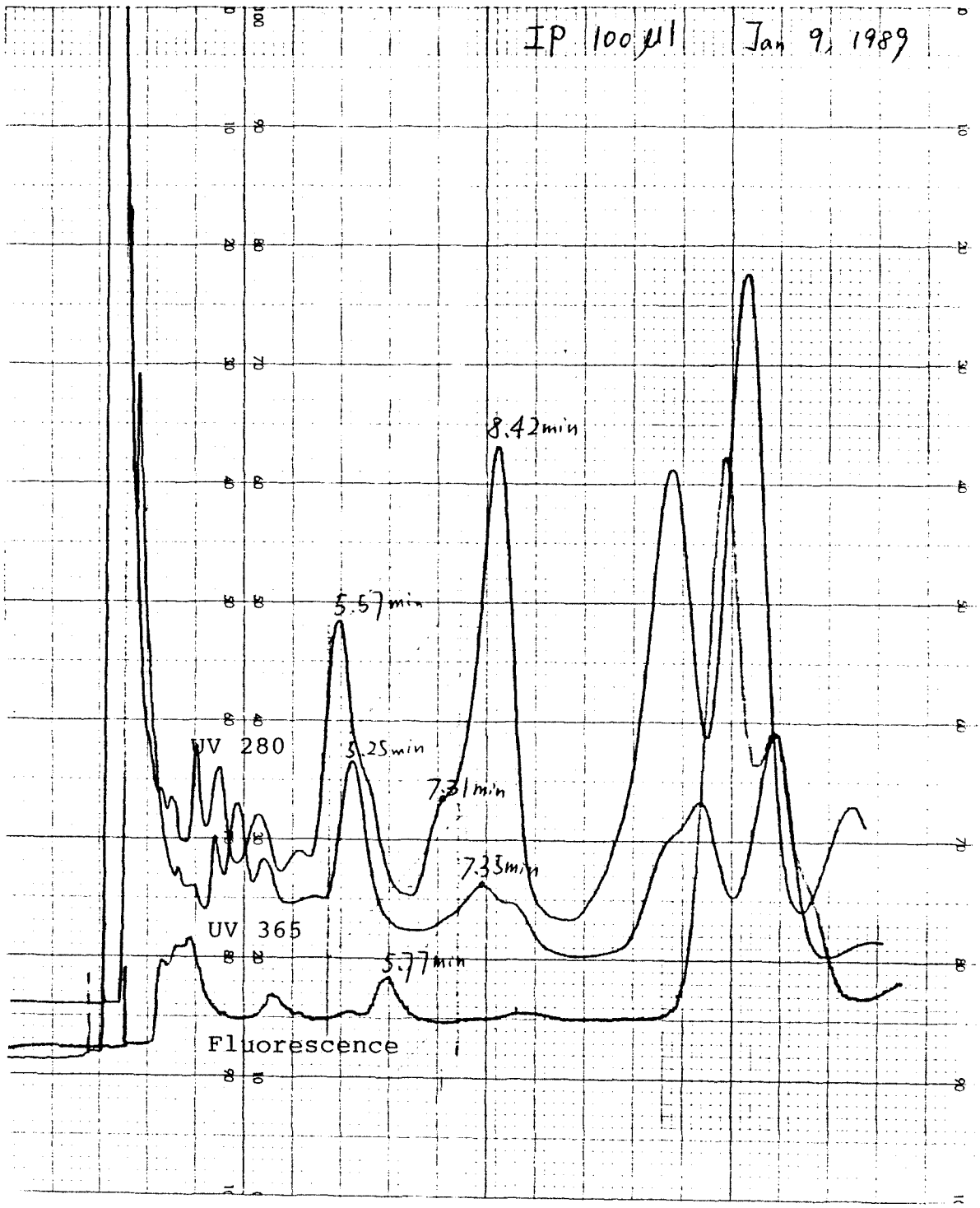


Figure 3-3

HPLC chromatogram of air sample IP10 (100 µl) on a 3x8C minicolumn (Perkin Elmer) under standard operating conditions.

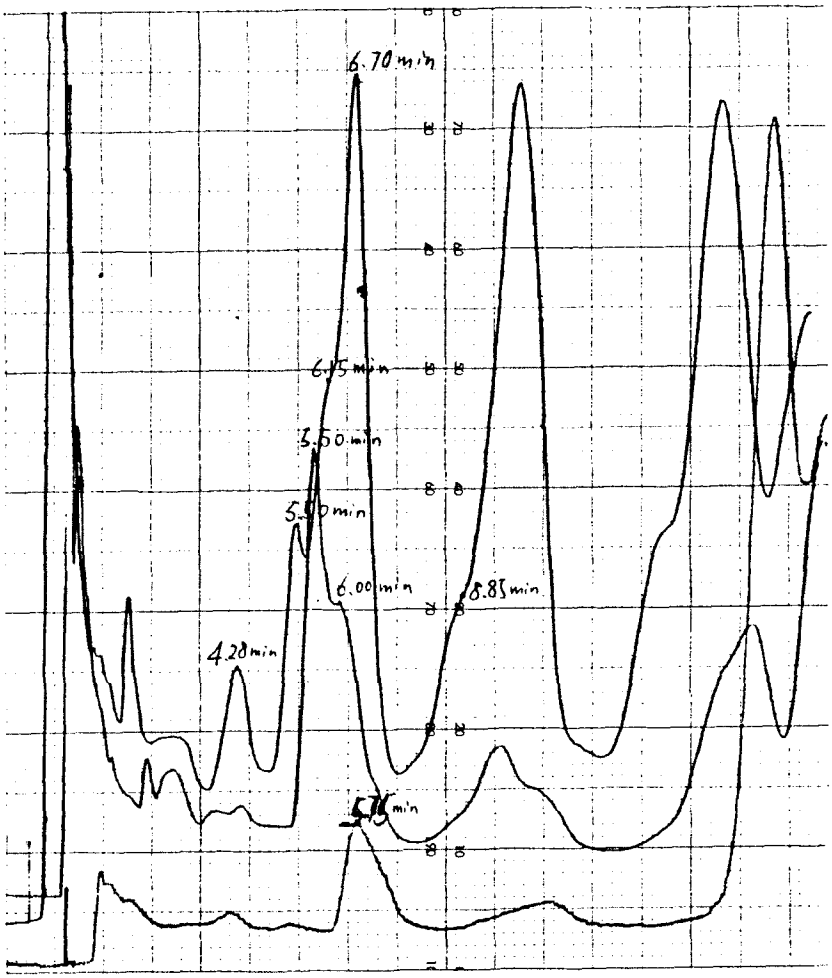


Figure 3-4

HPLC chromatogram of air sample TSP (100 μl) co-injected with 1-nitropyrene and 2-nitropyrene (8 μl each) on a 3x8C minicolumn (Perkin Elmer). (See Figure 3-2 to compare.)

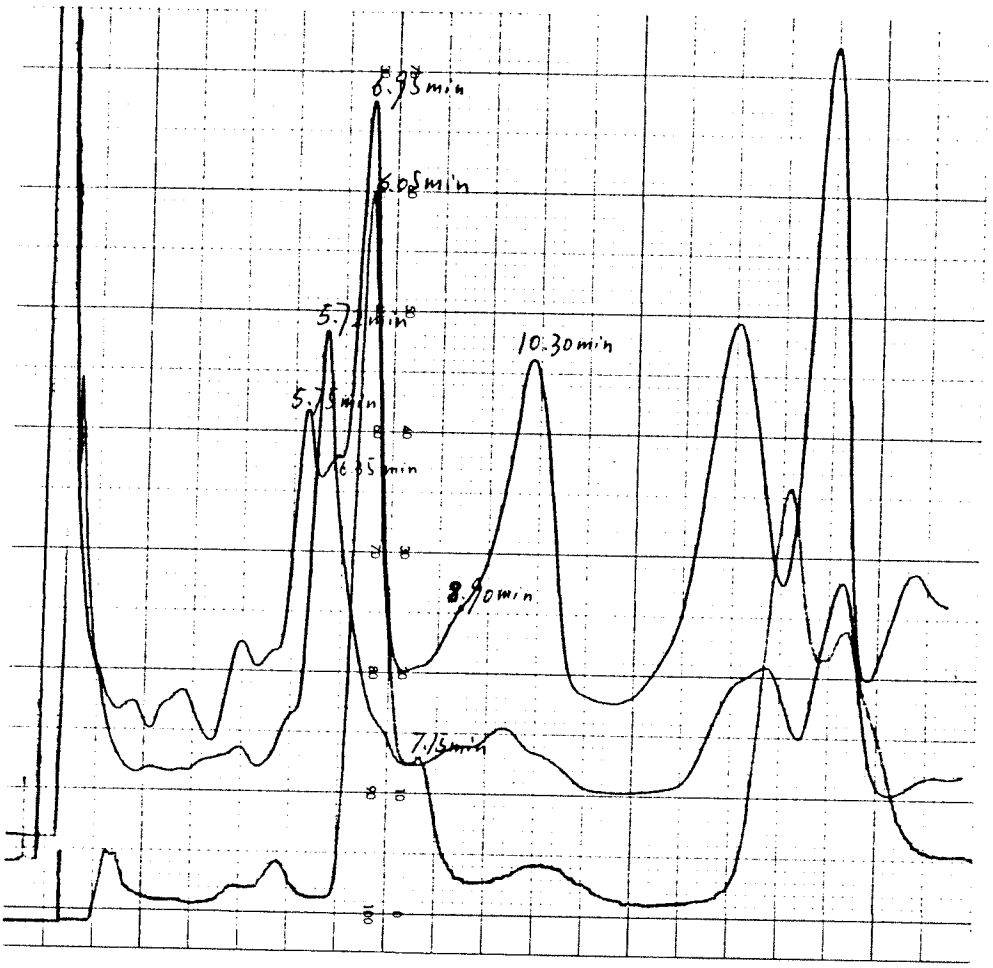


Figure 3-5

HPLC chromatogram of air sample IP10 (100 μl) co-injected with 1-nitropyrene and 2-nitropyrene (8 μl each) on a 3x8C minicolumn (Perkin Elmer). (See Figure 3-3 to compare.)

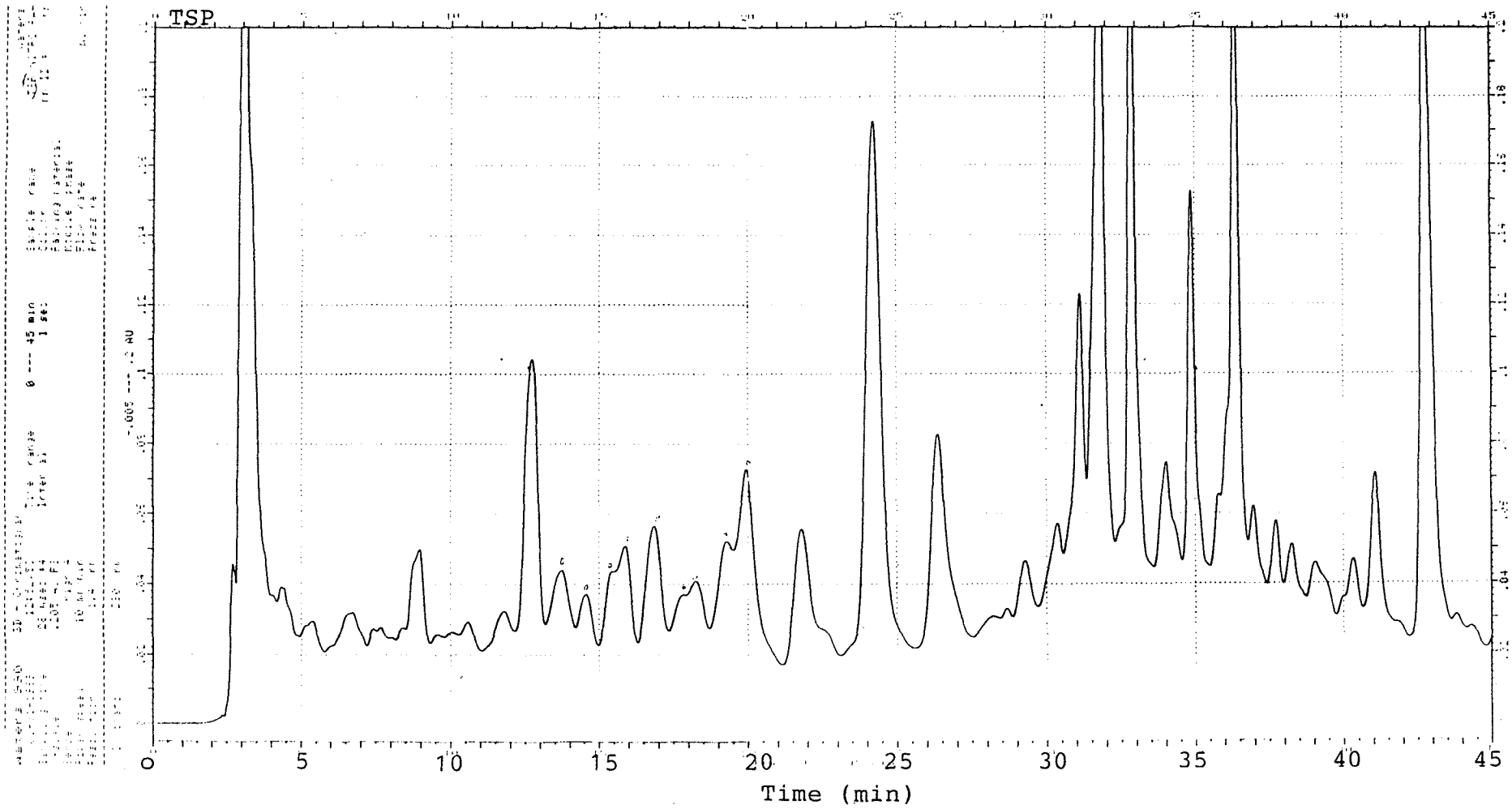


Figure 3-6

HPLC chromatogram of air sample TSP (100 μl) on a C18 column (25cm x 4.6 mm, Vydac) with Photo Diode Array UV detection at 280 nm.

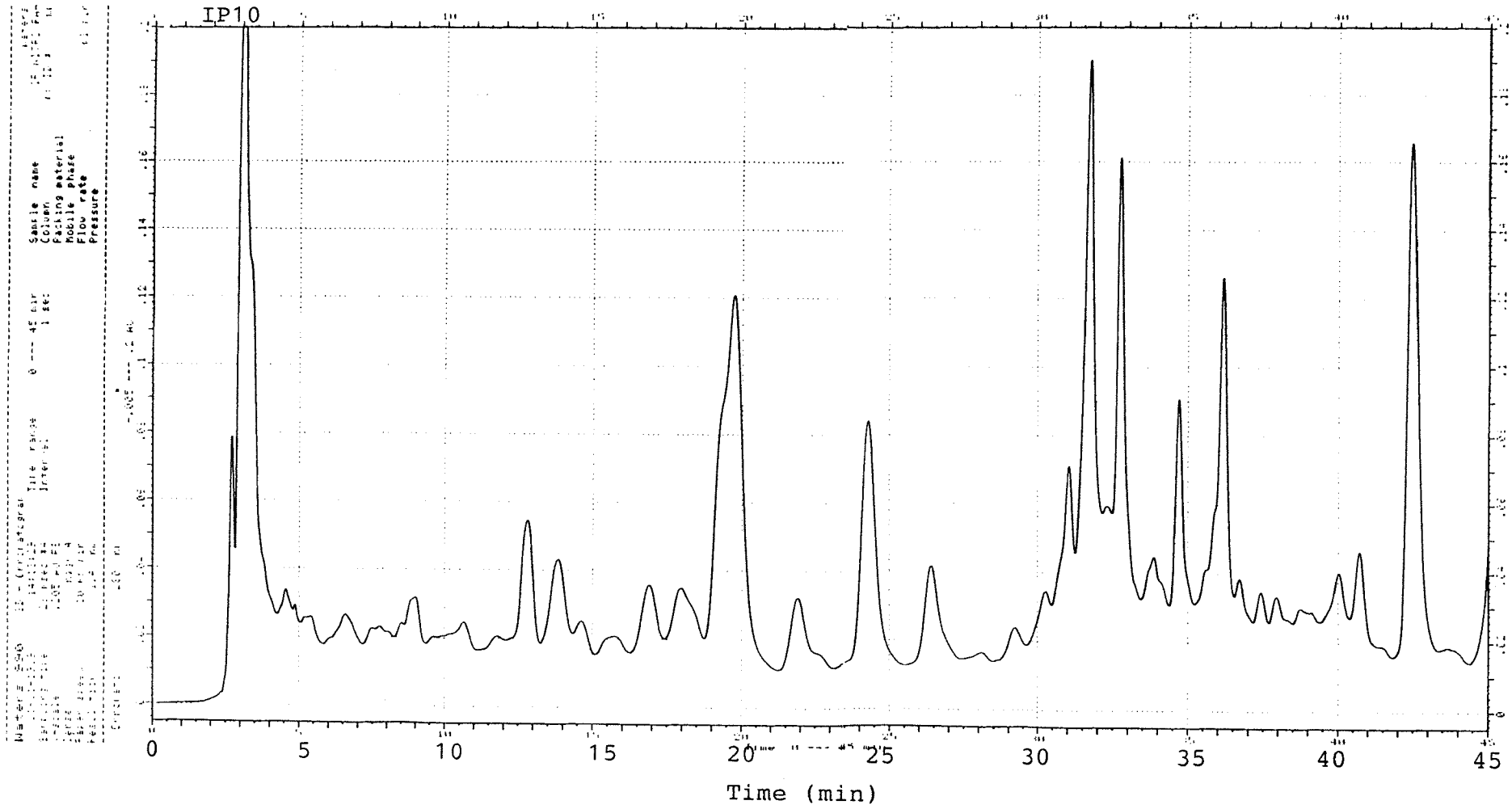


Figure 3-7

HPLC chromatogram of air sample IP10 (100 μl) on a C18 column (25cm x 4.6mm, Vydac) with Photo Diode Array UV detection at 280 nm.

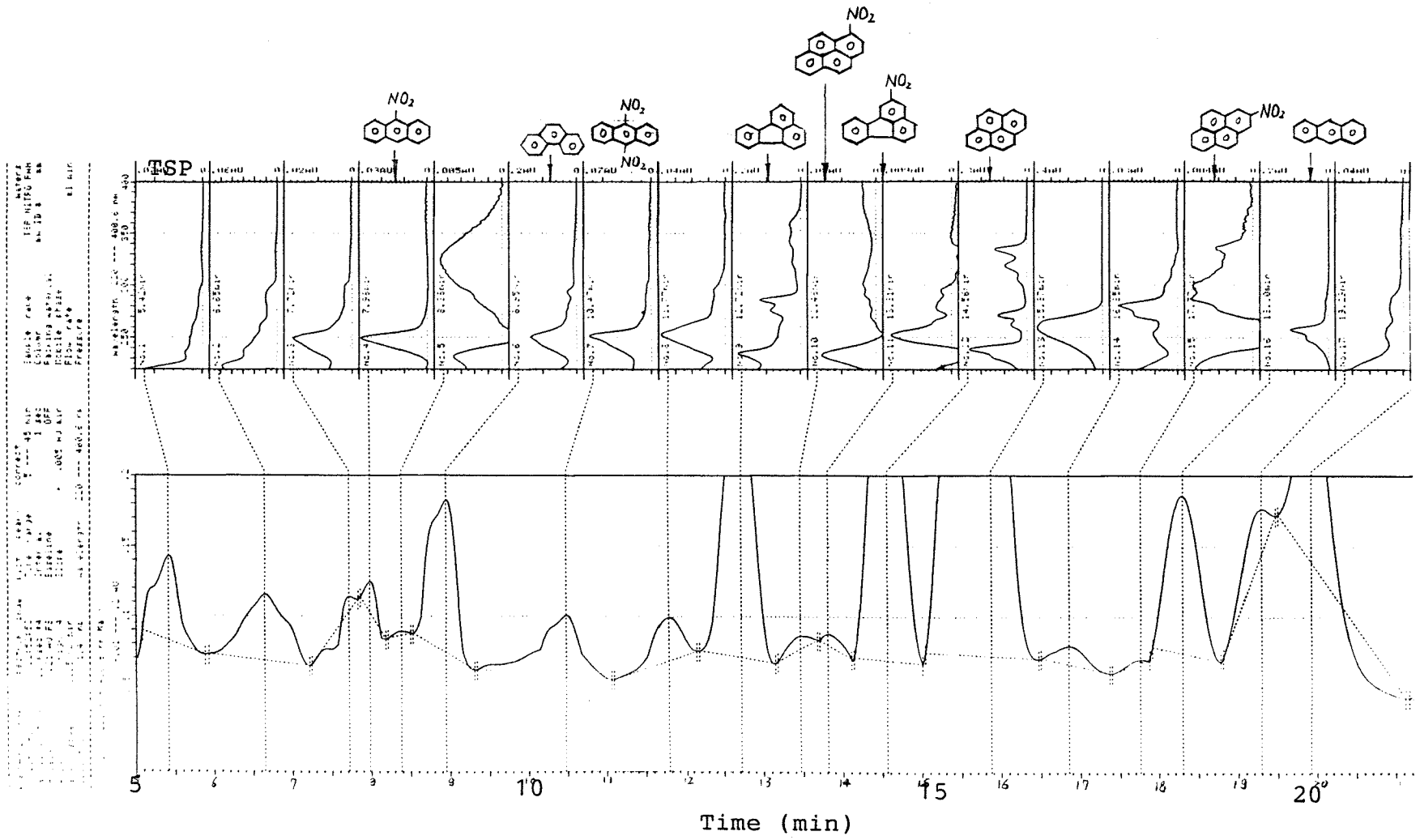


Figure 3-8(a)

Identification of nitro-PAH and PAH in air sample TSP by matching of UV spectra with HPLC chromatogram.

Column: C18 25cm x 4.6mm column, Vydac.

Retention Time: 5 - 21 minutes

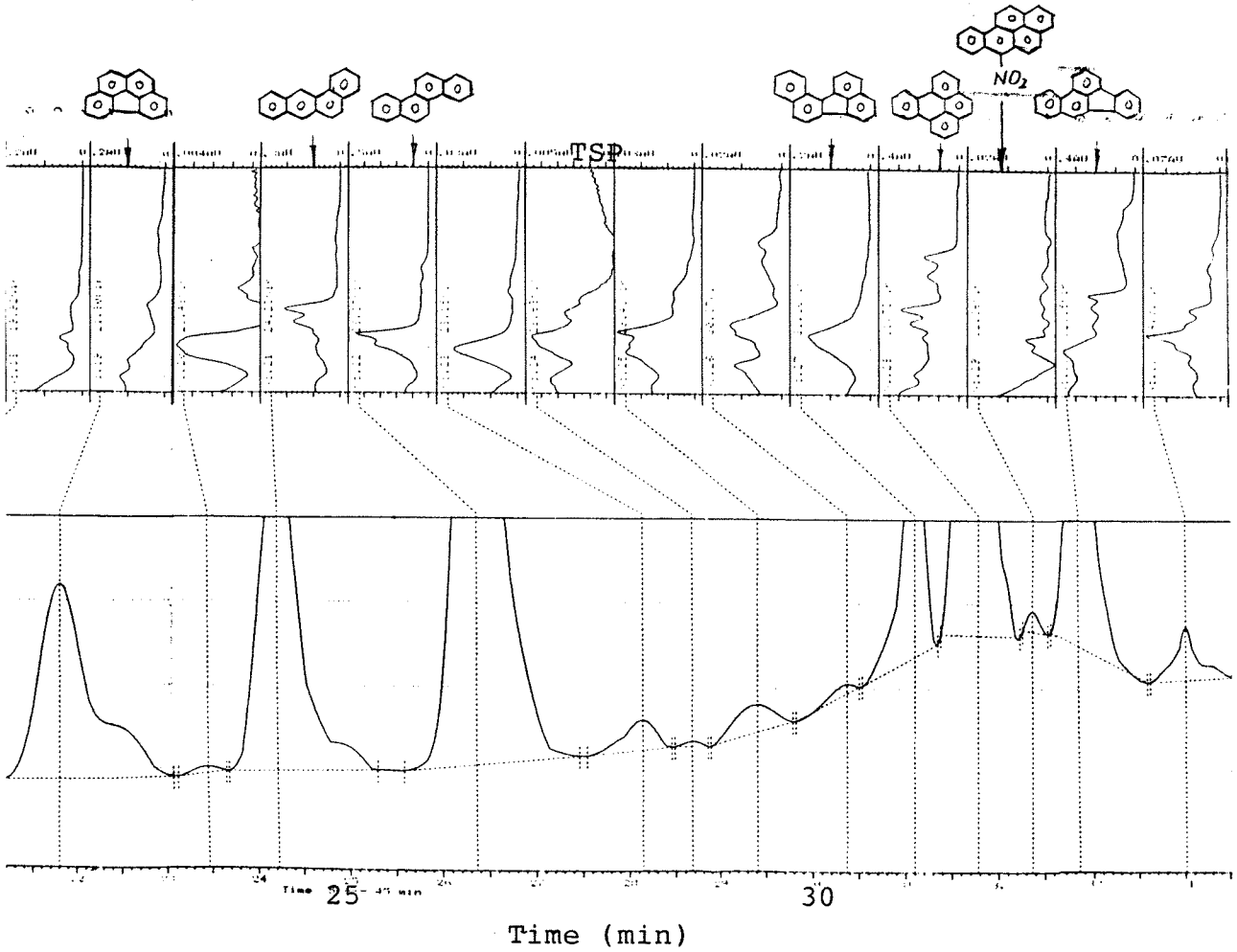


Figure 3-8(b)

Identification of nitro-PAH and PAH in air sample TSP by matching of UV spectra with HPLC chromatogram.

Retention Time: 22 - 34 minutes

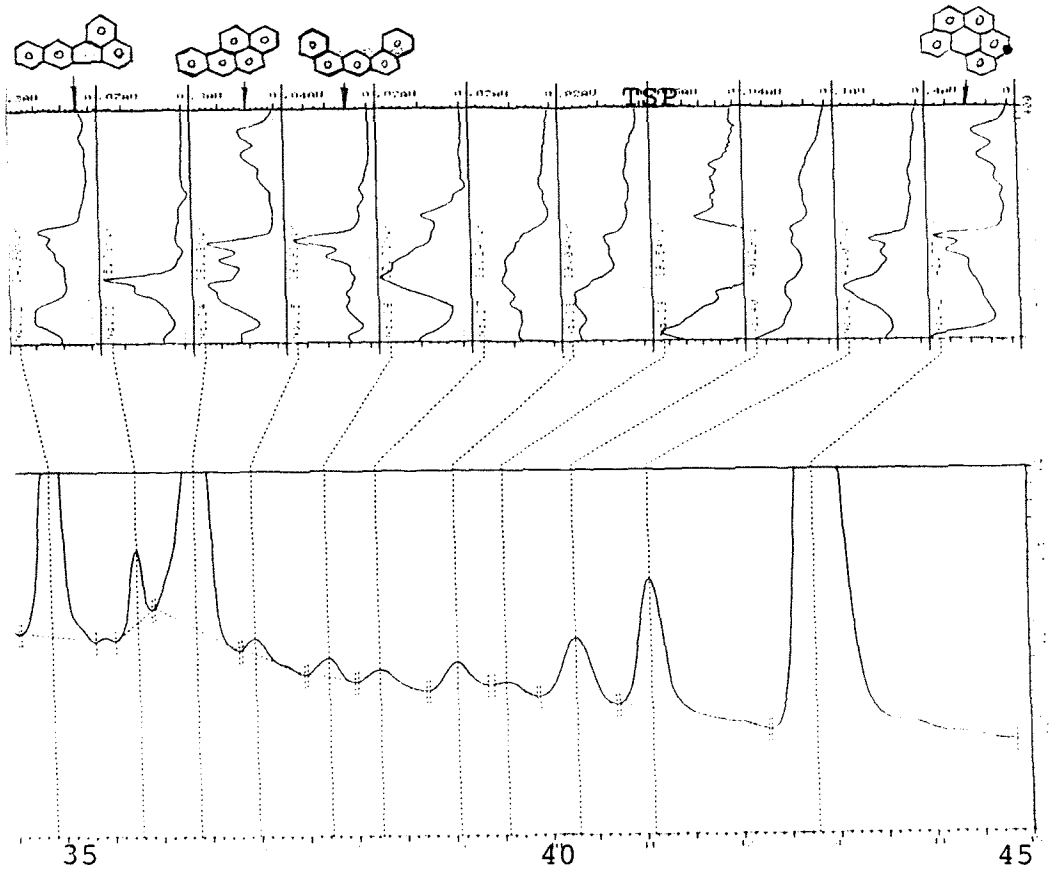


Figure 3-8(c)

Identification of nitro-PAH and PAH in air sample TSP by matching of UV spectra with HPLC chromatogram.

Retention Time: 35 - 45 minutes

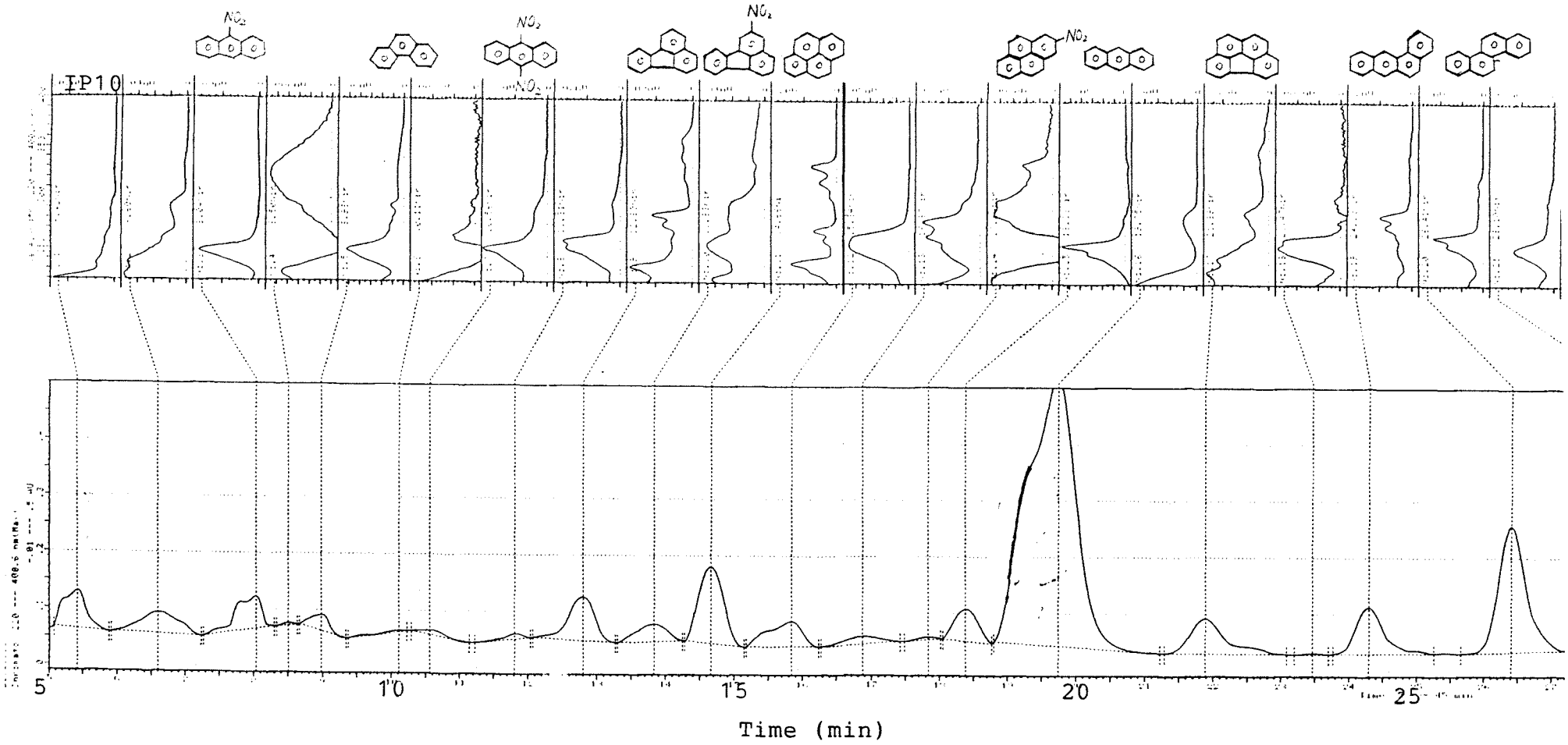


Figure 3-9(a)

Identification of nitro-PAH and PAH in air sample IP10 by matching of UV spectra with HPLC chromatogram.

Column: C18 25cm x 4.6mm column, Vydac

Retention Time: 5 - 27 minutes

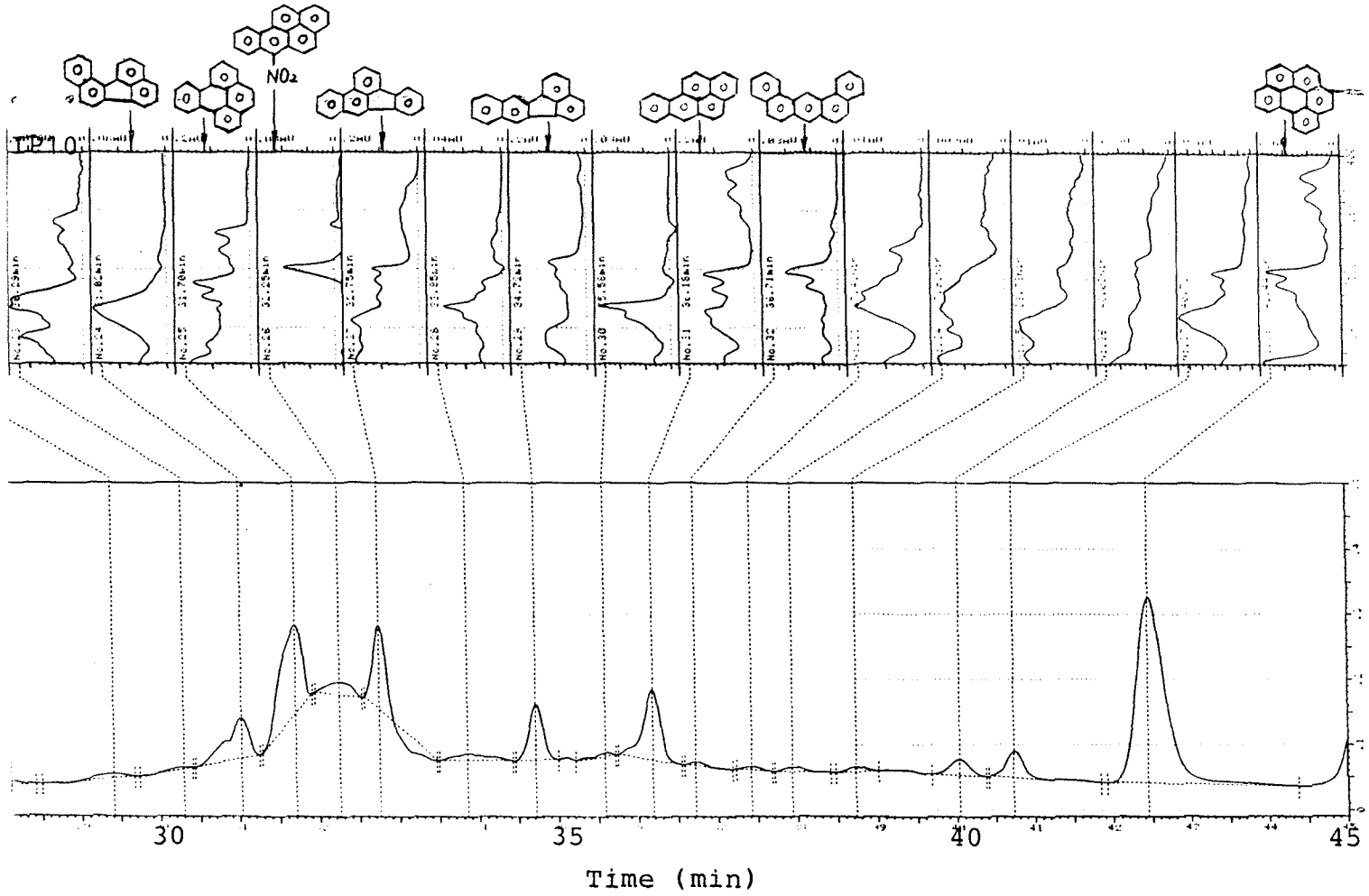


Figure 3-9(b)

Identification of nitro-PAH and PAH in air sample IP10 by matching of UV spectra with HPLC chromatogram.

Retention Time: 28 - 45 minutes

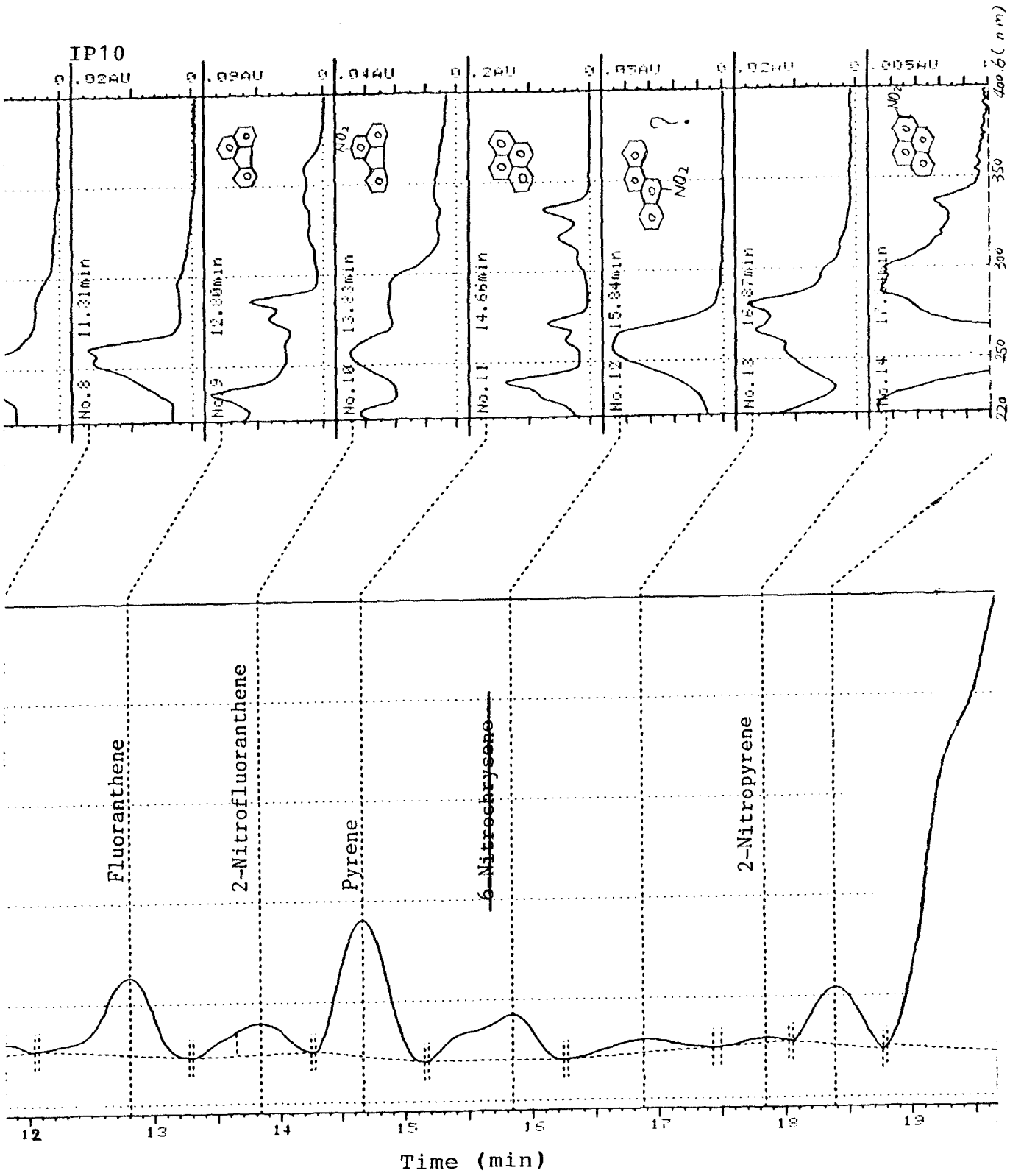


Figure 3-11

A part of enlarged HPLC/UV chromatogram of Figure 3-9 for air sample IP10.

Retention Time: 12 -19 minutes

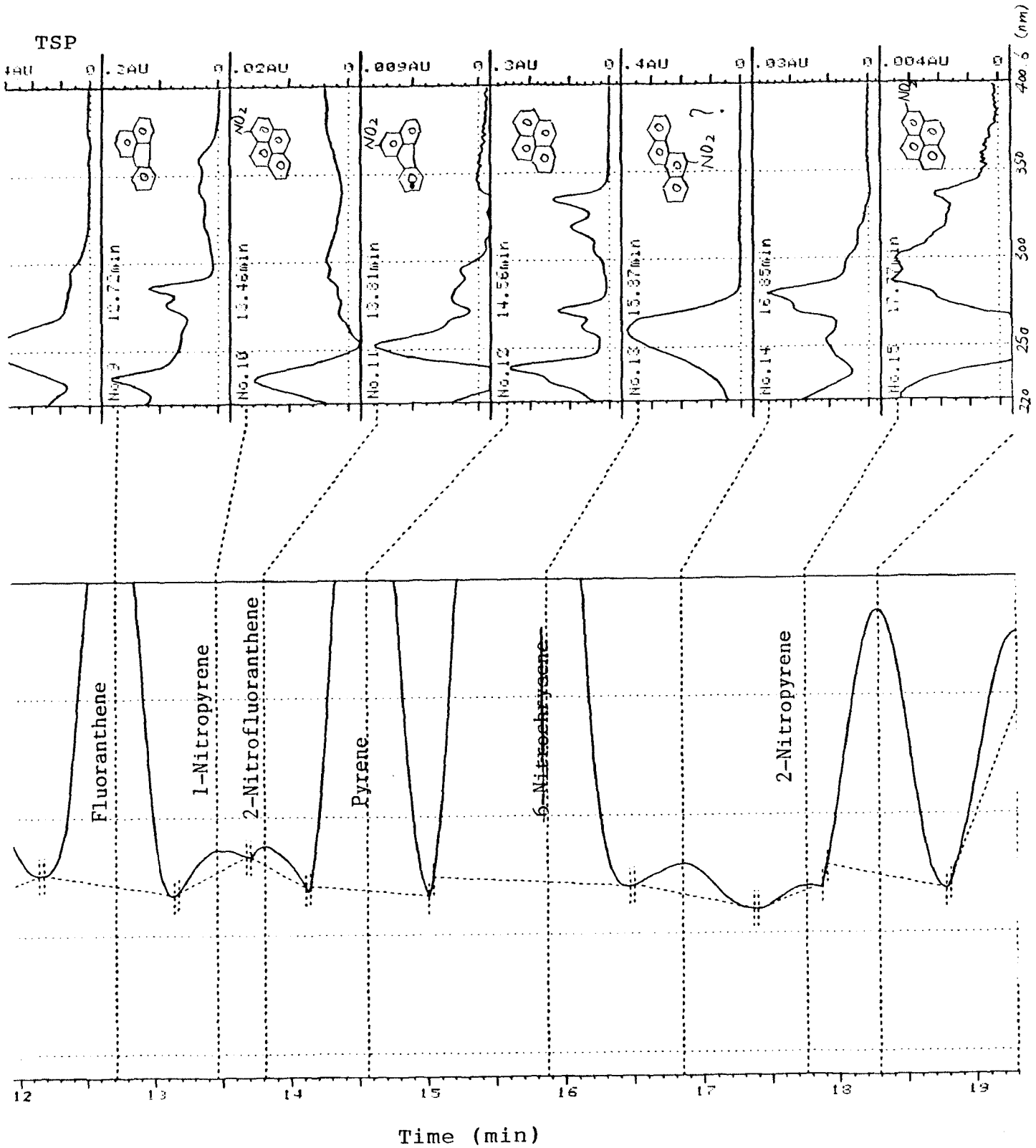


Figure 3-10

A part of enlarged HPLC/UV chromatogram of Figure 3-8 for air sample TSP.

Retention Time: 12 -19 minutes

The Appendix

UV Spectra of Nitro-PAH and PAH

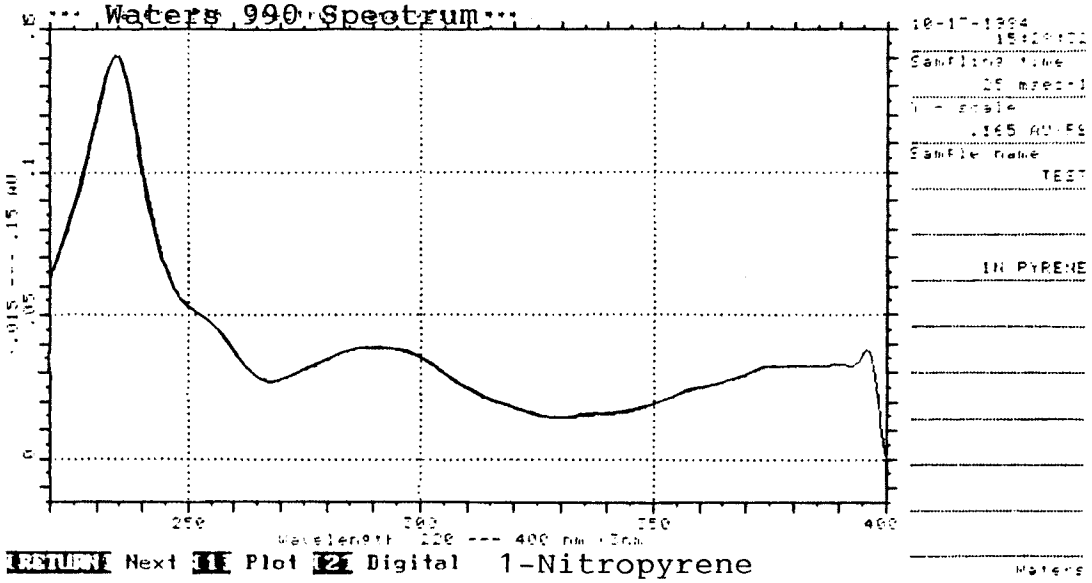


Figure 4-1(a)

UV spectrum of 1-nitropyrene in a standard mixture of four nitro-PAH by Waters 990 Photo Diode Array UV detector.

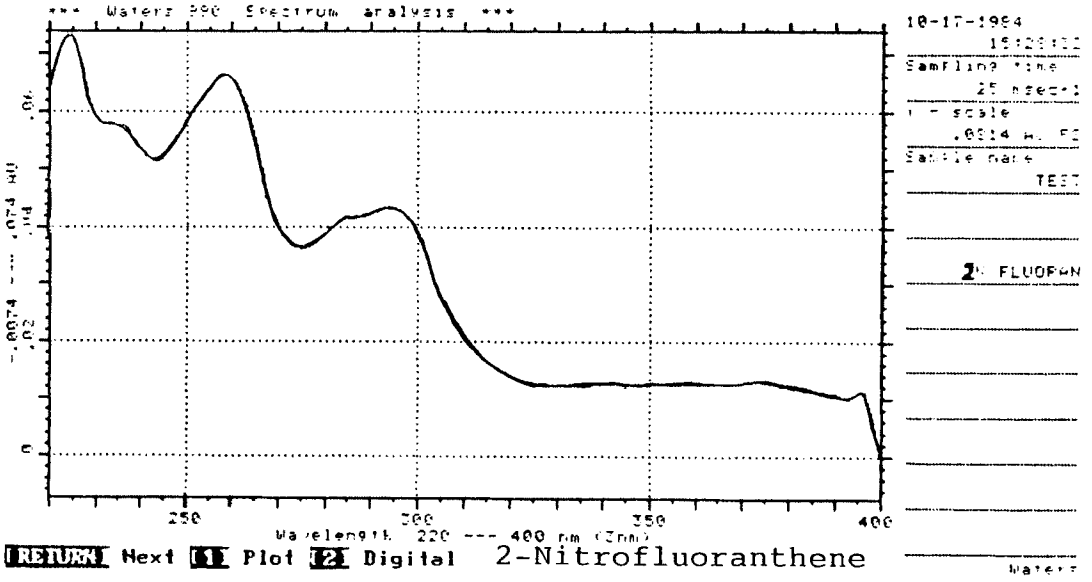


Figure 4-1(b)

UV spectrum of 2-nitrofluoranthene in a standard mixture of four nitro-PAH by Waters 990 Photo Diode Array UV detector.

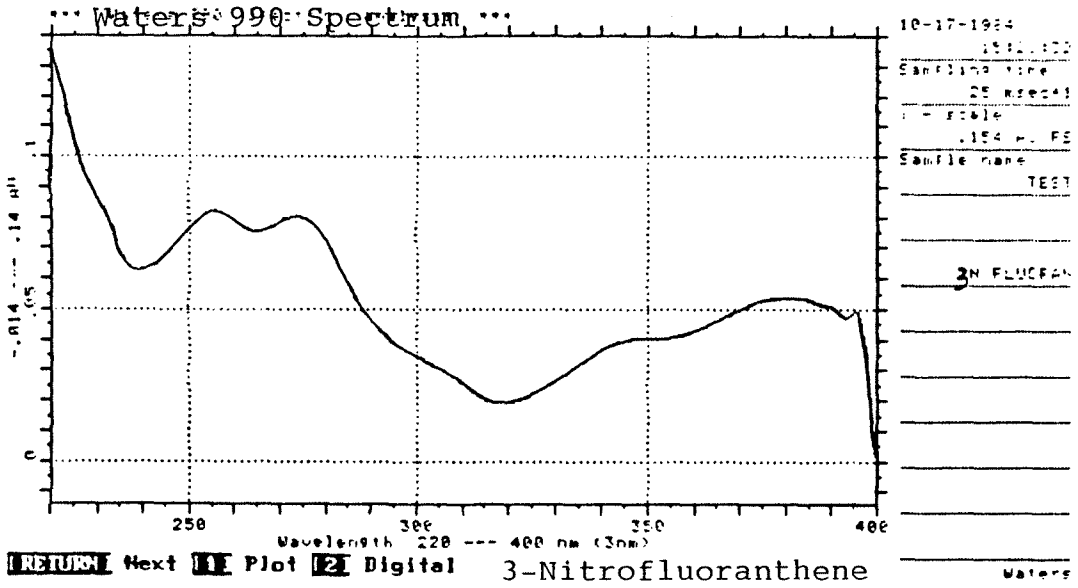


Figure 4-2(a)

UV spectrum of 3-nitrofluoranthene in a standard mixture of four nitro-PAH by Waters 990 Photo Diode Array UV detector.

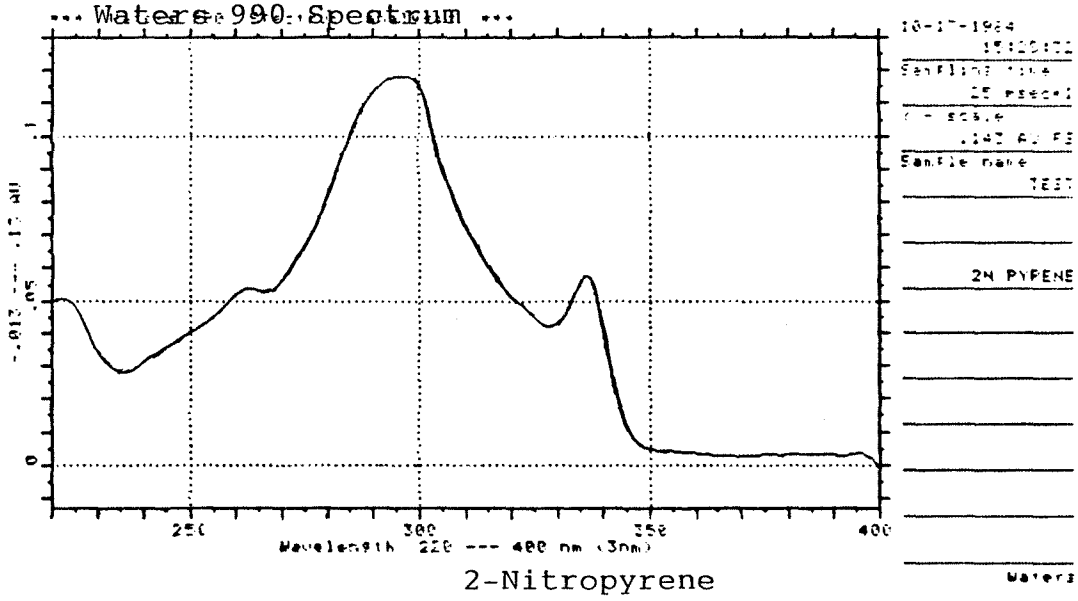


Figure 4-2(b)

UV spectrum of 2-nitropyrene in a standard mixture of four nitro-PAH by Waters 990 Photo Diode Array UV detector.

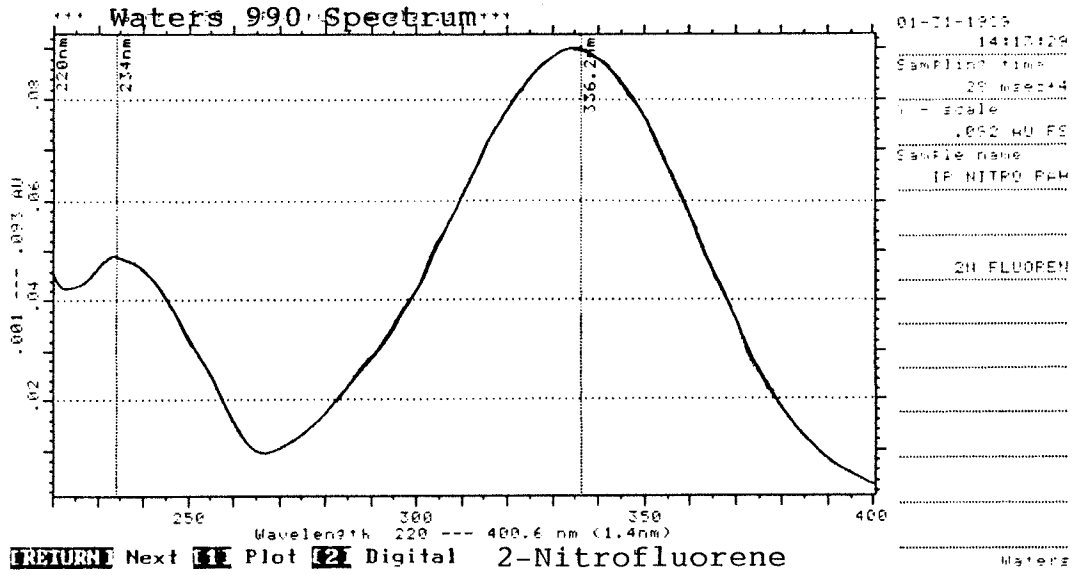


Figure 4-3(a)

UV spectrum of 2-nitrofluorene in SRM 1587 by Waters 990 Photo Diode Array UV detector.

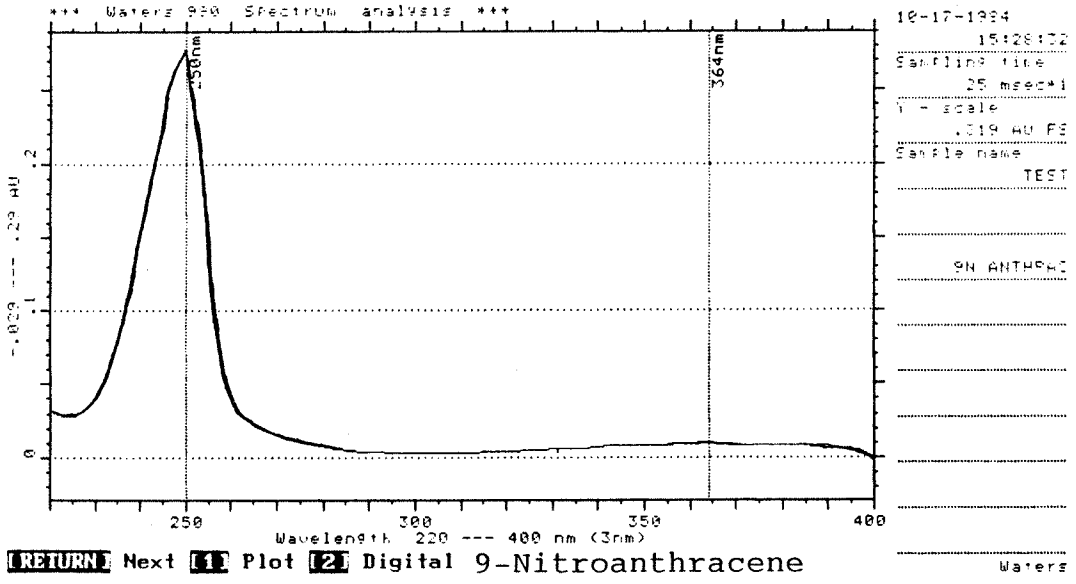


Figure 4-3(b)

UV spectrum of 9-nitroanthracene in SRM 1587 by Waters 990 Photo Diode Array UV detetor.

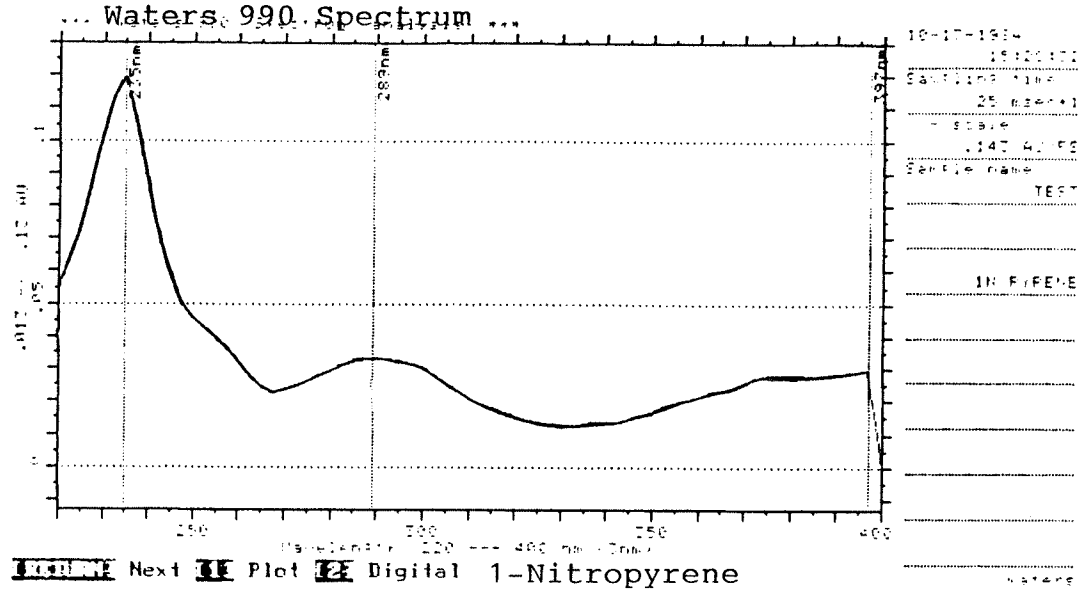


Figure 4-4(a)

UV spectrum of 1-nitropyrene in SRM 1587 by Waters 990 Photo Diode Array UV detectro.

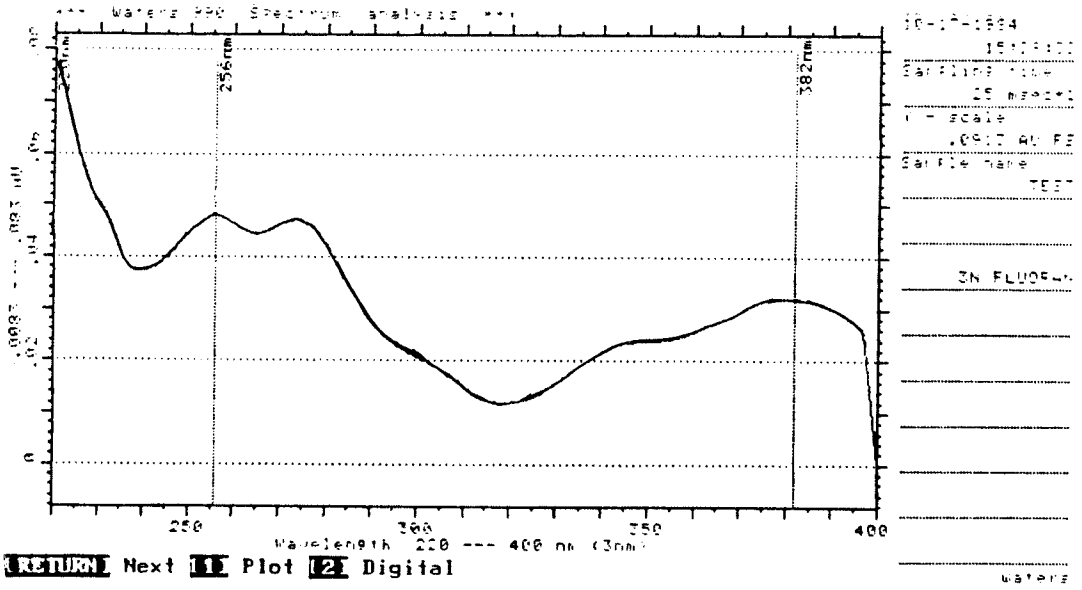


Figure 4-4(b)

UV spectrum of 3-nitrofluoranthene in SRM 1587 by Waters 990 Photo Diode Array UV detector.

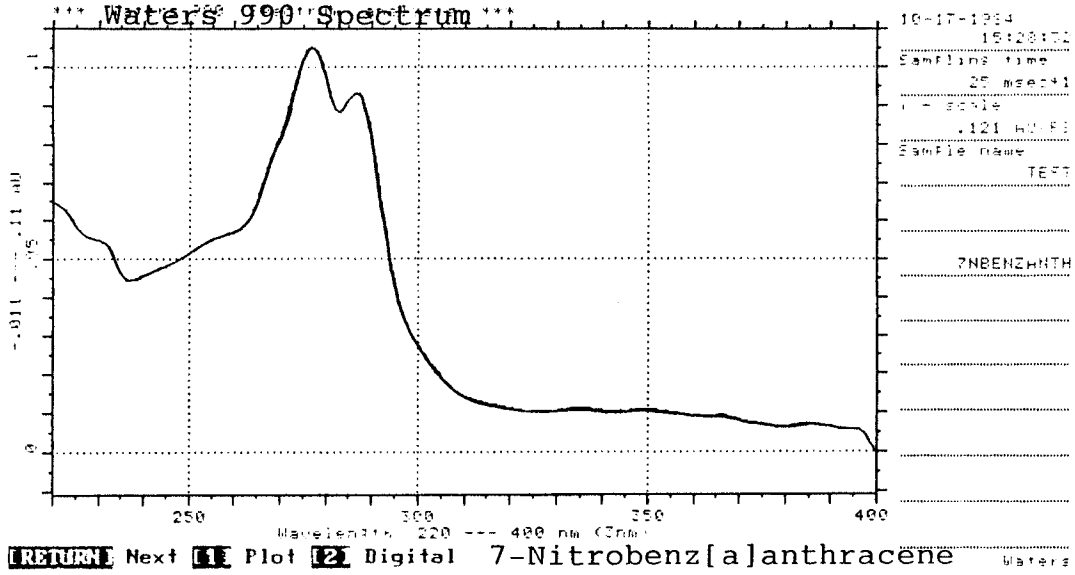


Figure 4-5(a)

UV spectrum of 7-nitrobenz[a]anthracene in SRM 1587 by Waters 990 Photo Diode Array UV detector.

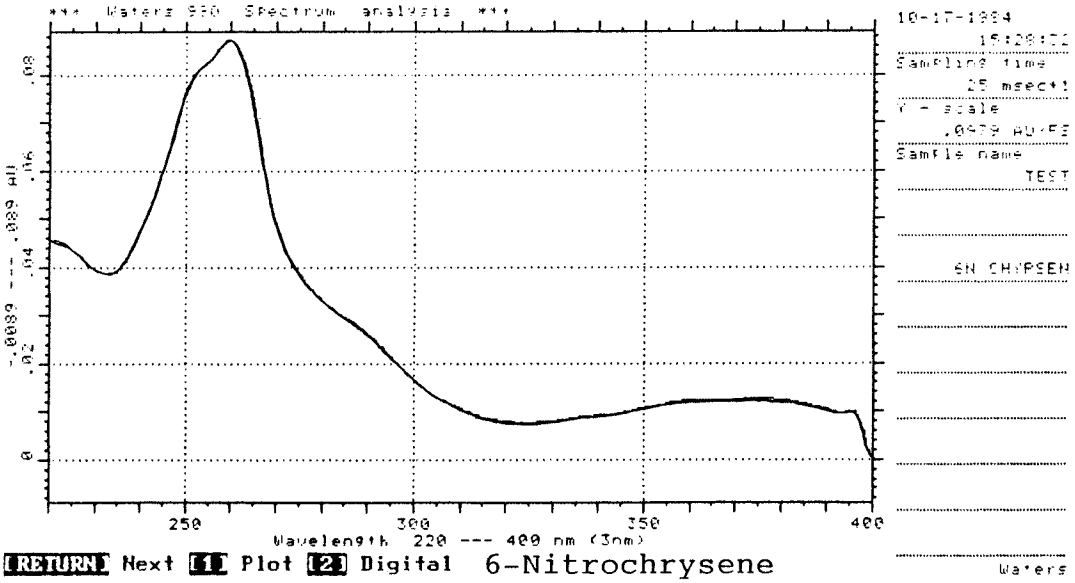


Figure 4-5(b)

UV spectrum of 6-nitrochrysene in SRM 1587 by Waters 990 Photo Diode Array UV detector.

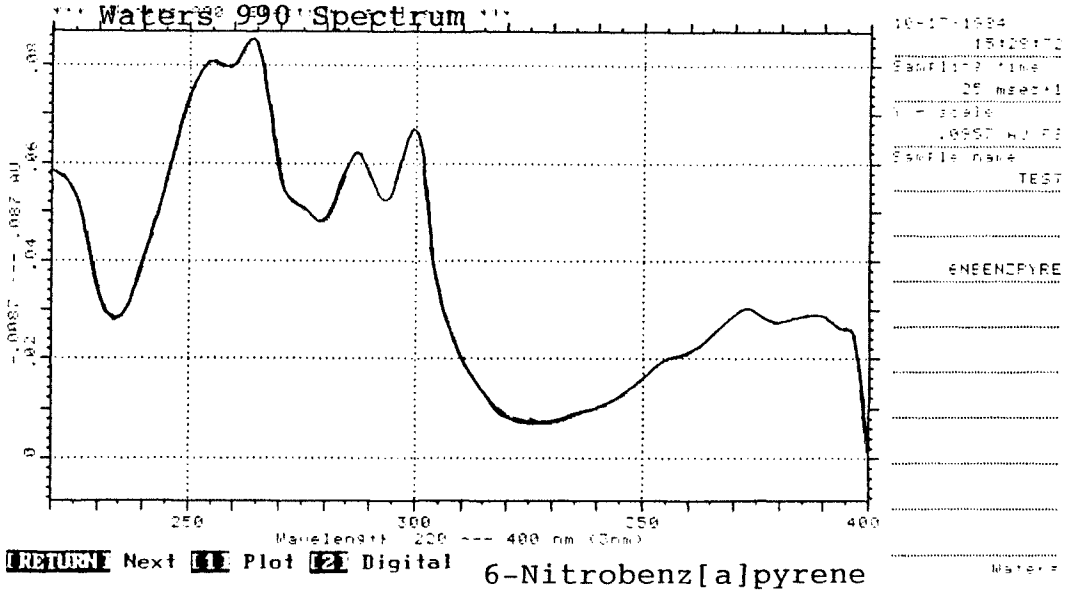
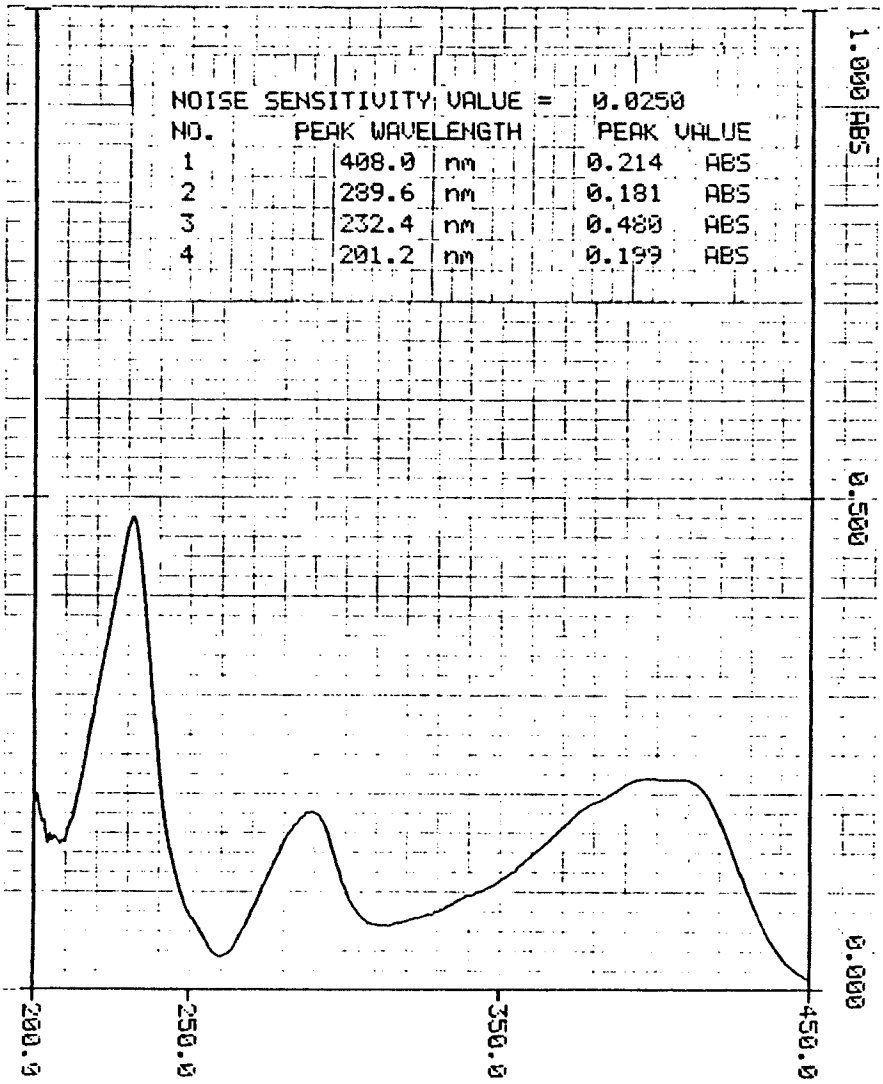


Figure 4-6

UV spectrum of 6-nitrobenz[a]pyrene in SRM 1587 by Wters 990 Photo Diode Array UV detector.



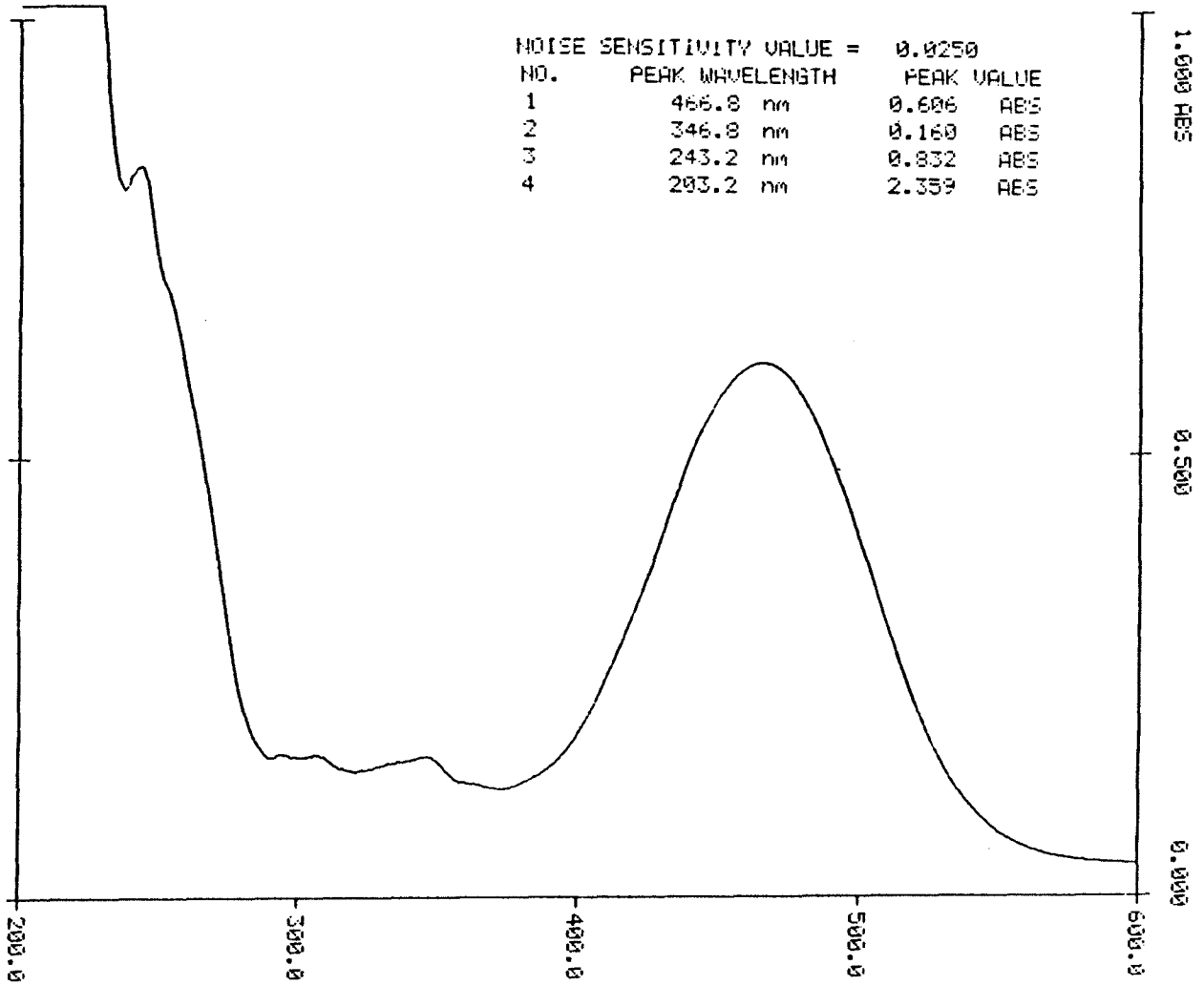
MRI No.: 264 NCI No.: NC0735
Dinitropyrene (mix)
Lot No.: CRC43-15-1
Batch No.: 01

DATE: 01,26,89 OPERATOR: J. Bennett SAMPLE:
CELL: 1cm quartz TEMPERATURE: ambient SOLVENT: 95% ethanol
HUMIDITY: CONC: 0.00443 mg/ml

DATA MODE	:	(2) ABS
BAND WIDTH	:	1.00 nm
TIME CONST	:	0.4 sec
λ SET	:	450.0 ~ 200.0 nm
λ SCALE	:	20 nm/cm
SCAN SPEED	:	100 nm/min
%T ABS SCALE	:	0.000 ~ 1.000 ABS
CYCLE NO	:	1

Figure 4-7

UV spectrum of dinitropyrene (mixture).
Source: Midwest Research Institute.

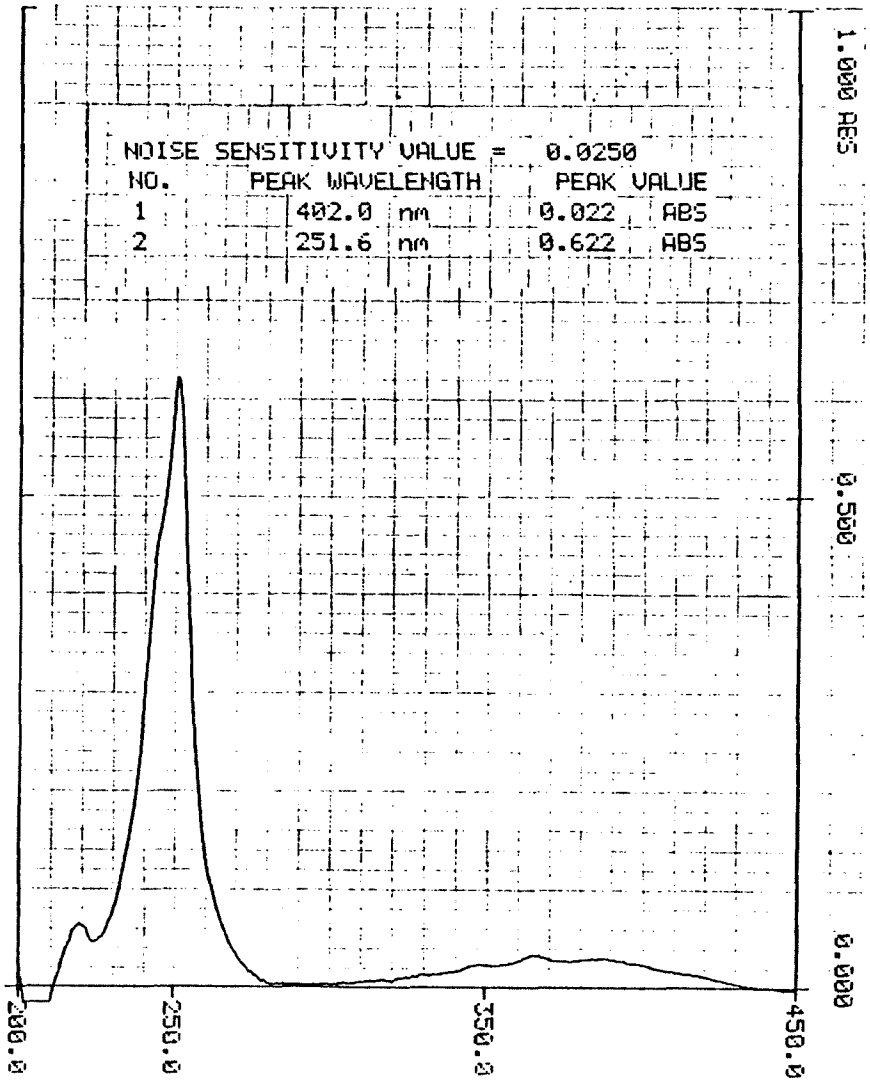


DATE:02.16.89 OPERATOR: A. Schaefer SAMPLE: #232 3-Nitroperylene
CELL: 1cm quartz TEMPERATURE: ambient SOLVENT: 95% Ethanol
HUMIDITY: _____ CONC: 0.009106 mg/ml

DATA MODE	: (2) ABS
BAND WIDTH	: 1.00 nm
TIME CONST	: 0.4 sec
λ SET	: 600.0 ~ 200.0 nm
λ SCALE	: 20 nm/cm
SCAN SPEED	: 100 nm/min
%T ABS SCALE	: 0.000 ~ 1.000 ABS
CYCLE NO	: 1

Figure 4-8

UV spectrum of 3-nitroperylene.
Source: Midwest Research Institute.



MRI No.: 041 NCI No.: NC0738
9,10-Dinitroanthracene
Lot No.: CRC43-15-6
Batch No.: 01

DATE: 01,26,89 OPERATOR: J. Bennett SAMPLE:
CELL: 1cm quartz TEMPERATURE: ambient SOLVENT: 95% ethanol
HUMIDITY: CONC: 0.002980 mg/ml

DATA MODE	: (2) ABS
BAND WIDTH	: 1.00 nm
TIME CONST	: 0.4 sec
λ SET	: 450.0 ~ 200.0 nm
λ SCALE	: 20 nm/cm
SCAN SPEED	: 100 nm/min
%T ABS SCALE	: 0.000 ~ 1.000 ABS
CYCLE NO	: 1

Figure 4-9

UV spectrum of 9,10-dinitroanthracene.
Source: Midwest Research Institute.

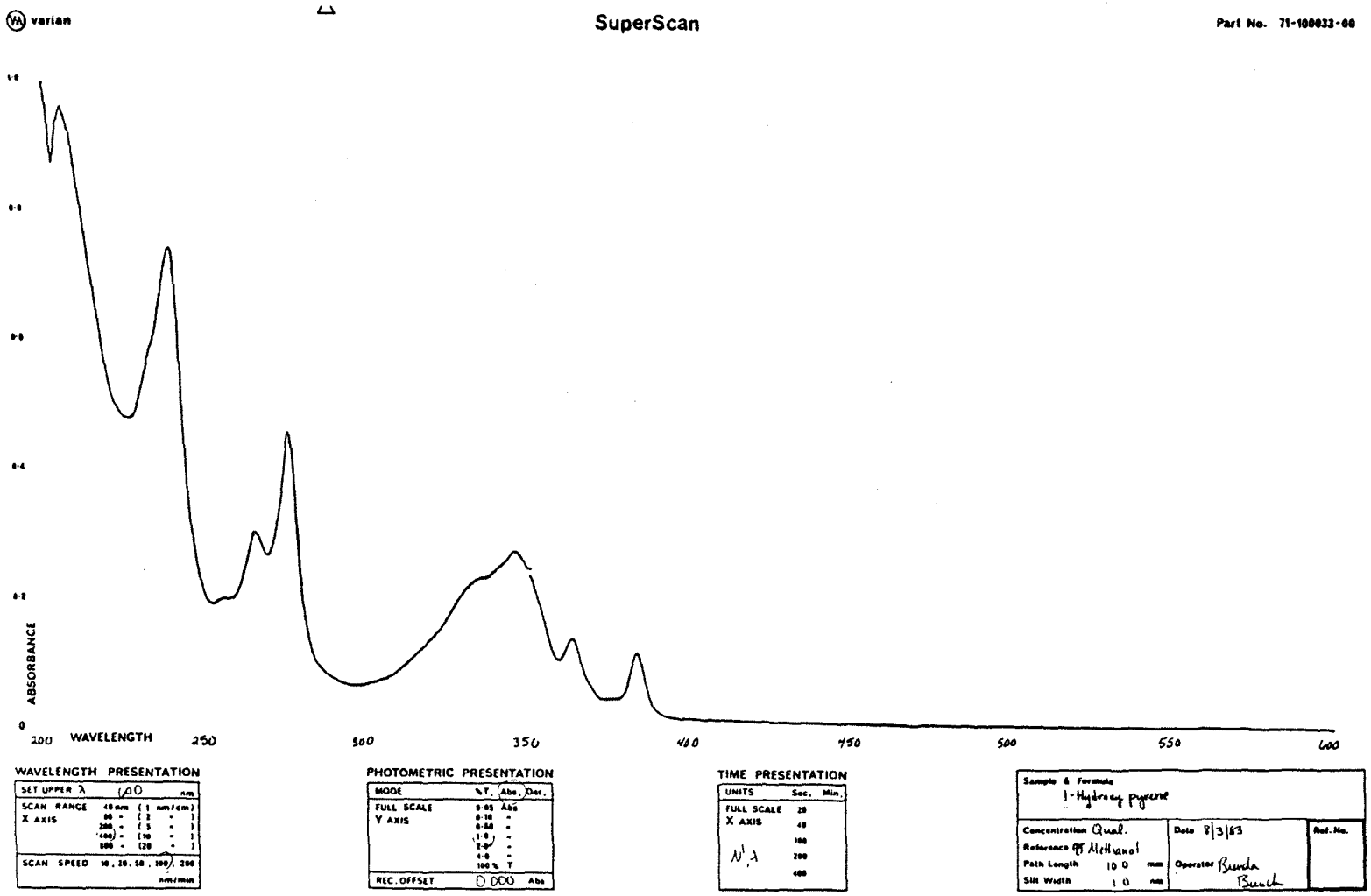
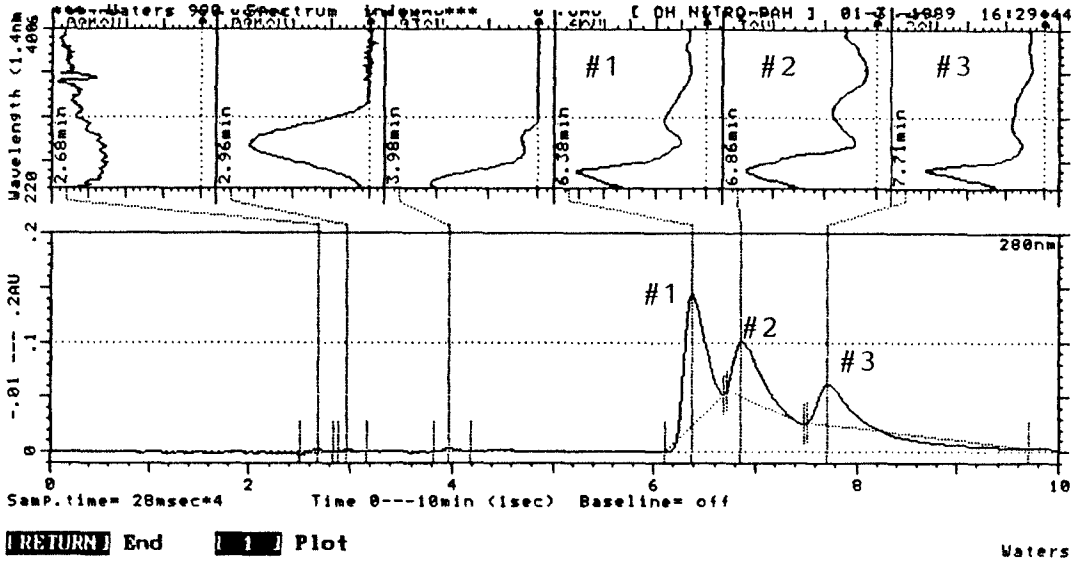


Figure 4-10

UV spectrum of 1-hydroxypyrene.
Source: Midwest Research Institute.



* Compound #1, #2 and #3 are isomers.

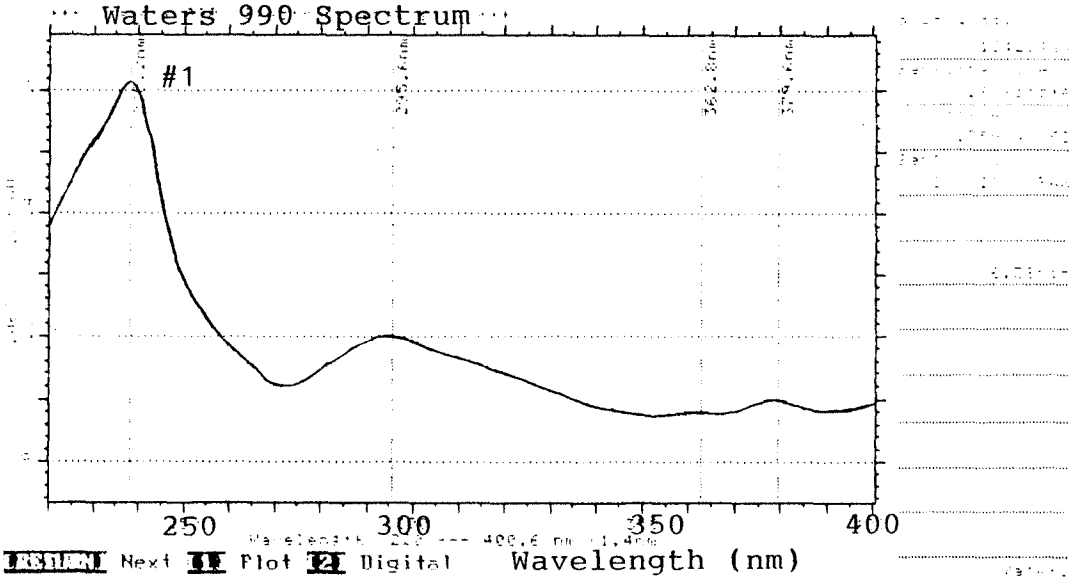


Figure 4-11

UV spectrum of an unknown hydroxy-nitro-PAH (#1) isomer Waters 990 Photo Diode Array UV detector.

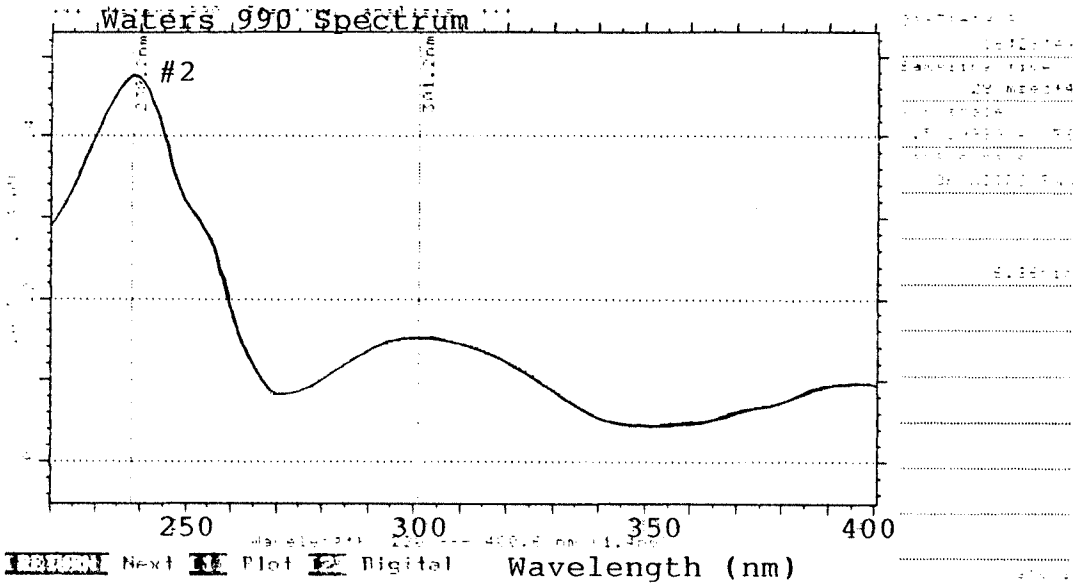


Figure 4-12(a)

UV spectrum of an unknown hydroxy-nitro-PAH (#2) by Waters 990 Photo Diode Array UV detector.

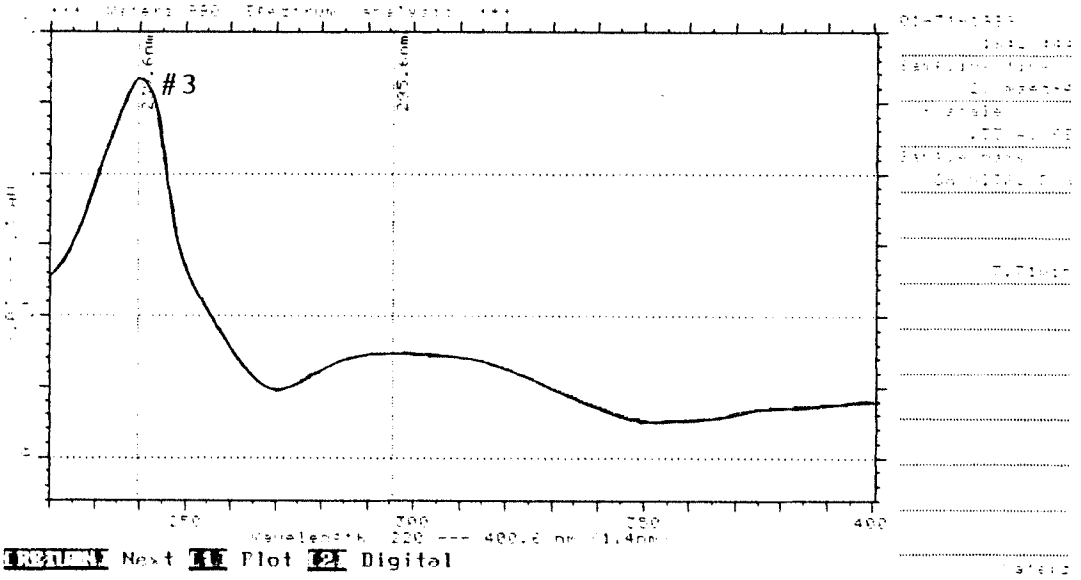


Figure 4-12(b)

UV spectrum of an unknown hydroxy-nitro-PAH (#3) by Waters 990 Photo Diode Array UV detector.

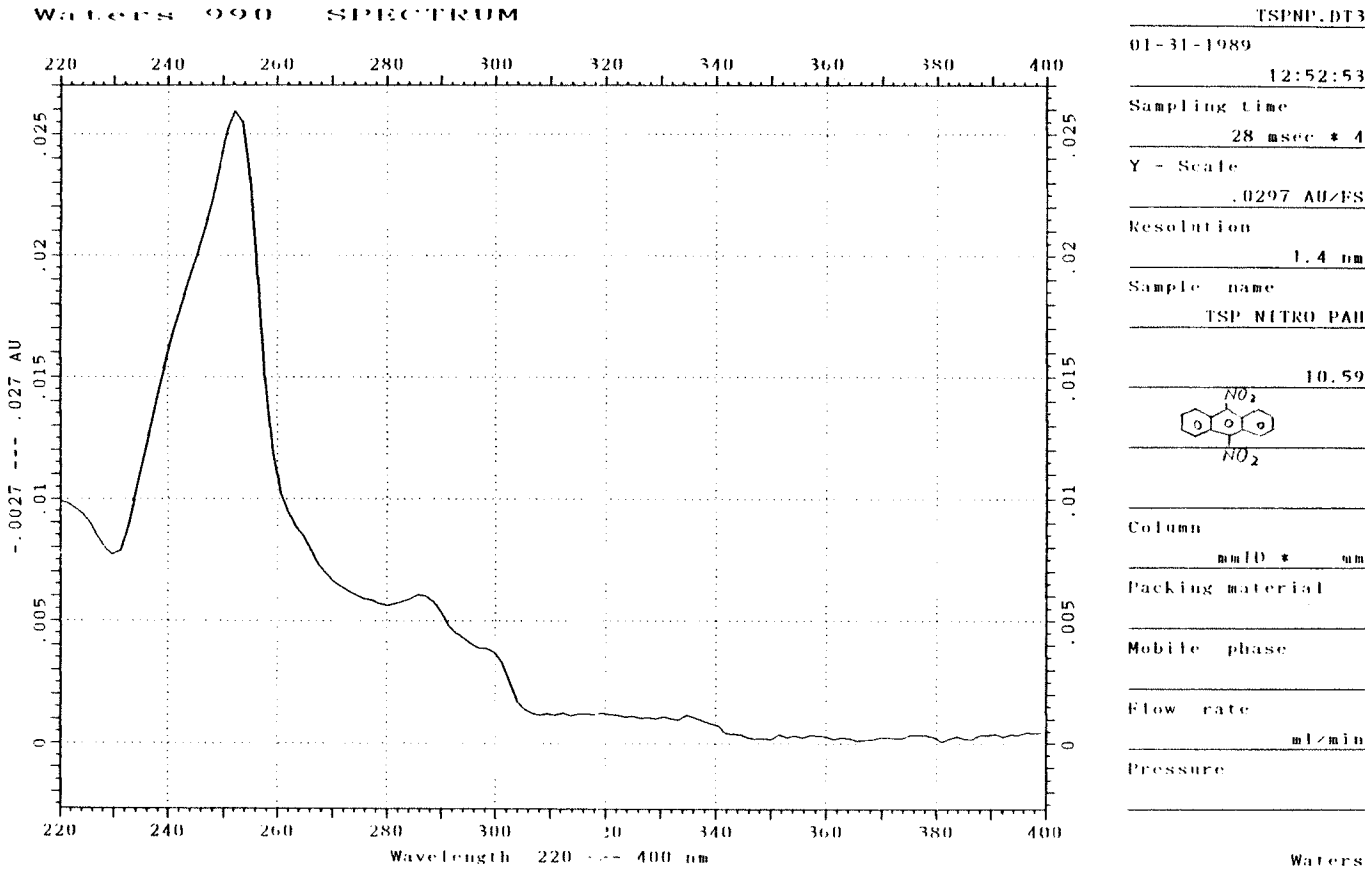


Figure 4-13

UV spectrum of 9,10-dinitroanthracene in air sample TSP by Waters 990 Photo Diode Array UV detector.

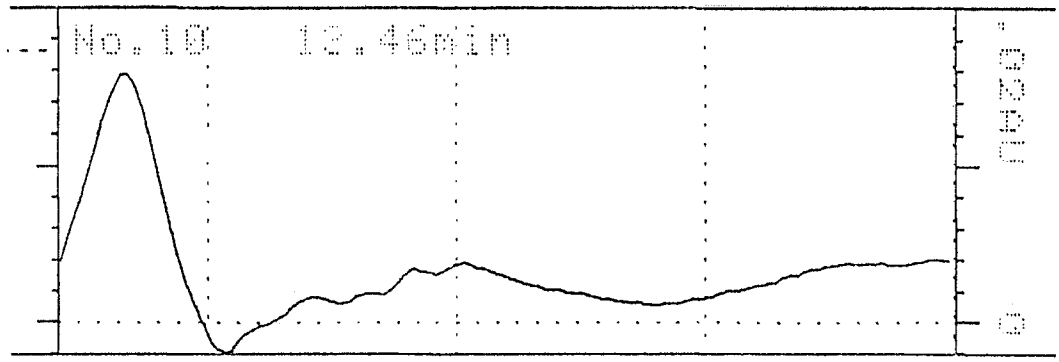


Figure 4-14(a)

UV spectrum of 1-nitropyrene in air sample TSP by Waters 990 Photo Diode Array UV detector.

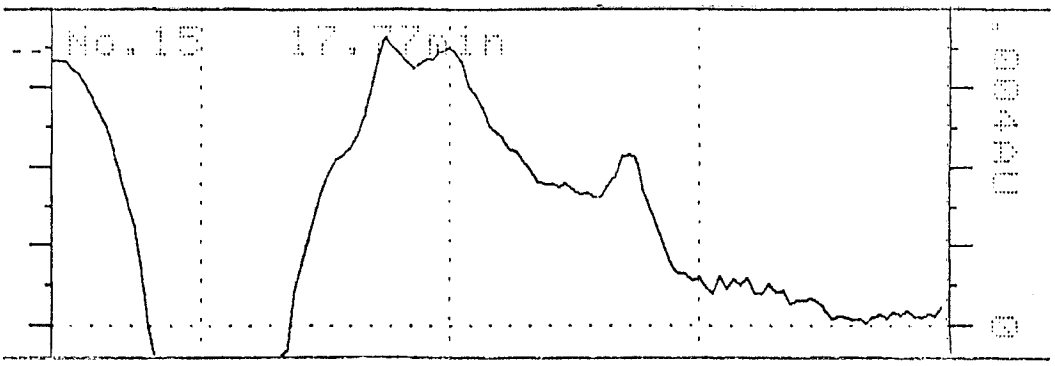


Figure 4-14(b)

UV spectrum of 2-nitropyrene in air sample TSP by Waters 990 Photo Diode Array UV detector.

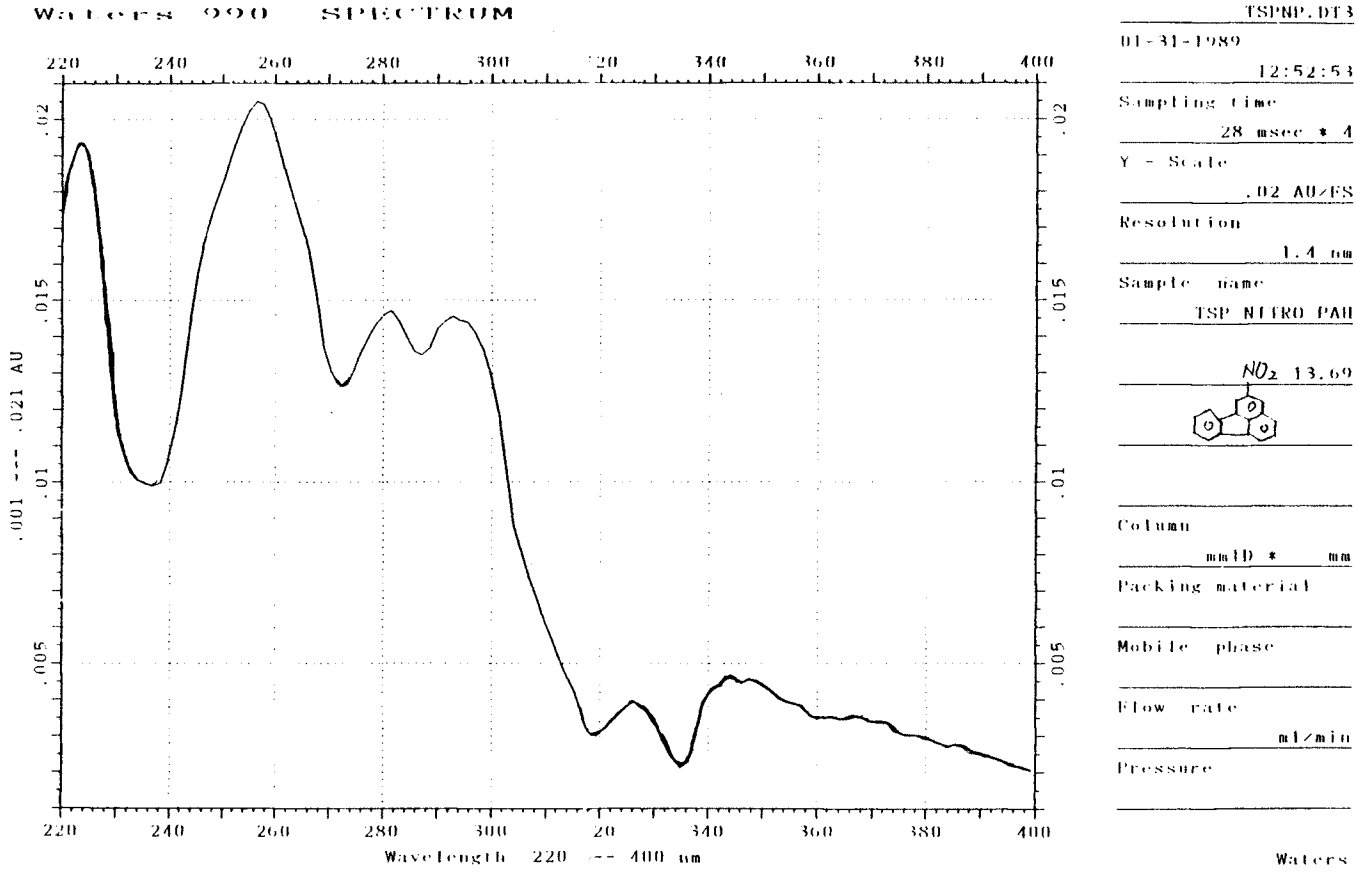


Figure 4-15

UV spectrum of 2-nitrofluoranthene in air sample TSP by Waters 990 Photo Diode Array UV detector.

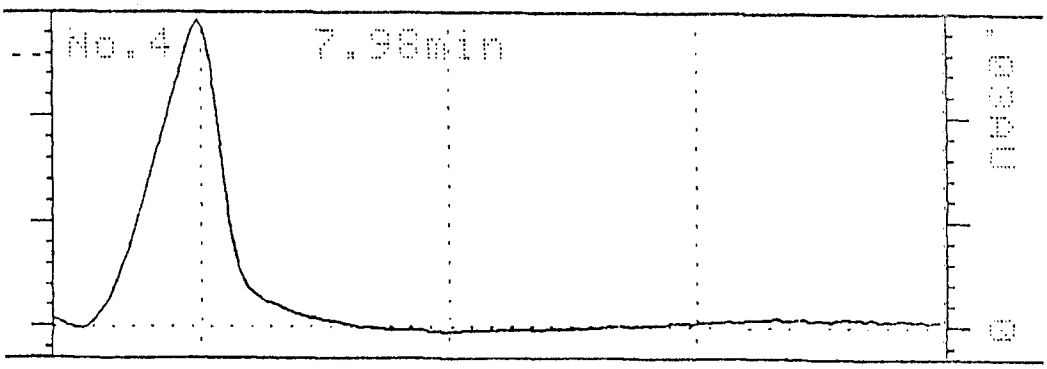


Figure 4-16(a)

UV spectrum of 9-nitroanthracene in air sample TSP by Waters 990 Photo Diode Array UV detector.

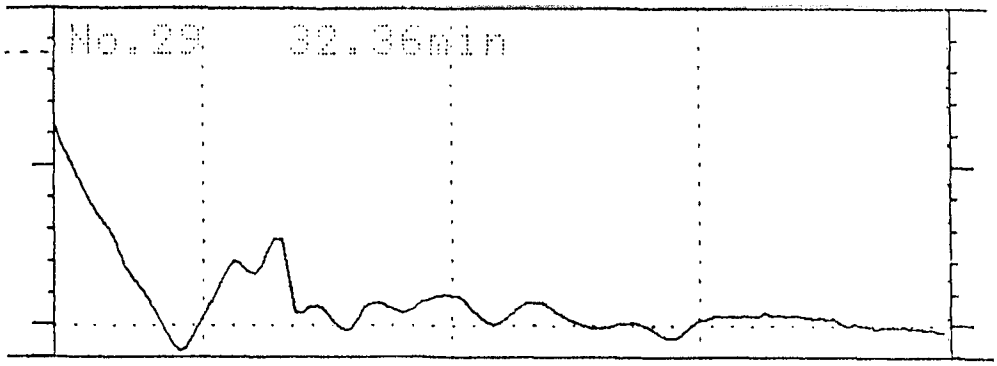


Figure 4-16(b)

UV spectrum of 6-nitrobenz[a]pyrene in air sample TSP by Waters 990 Photo Diode Array UV detector.

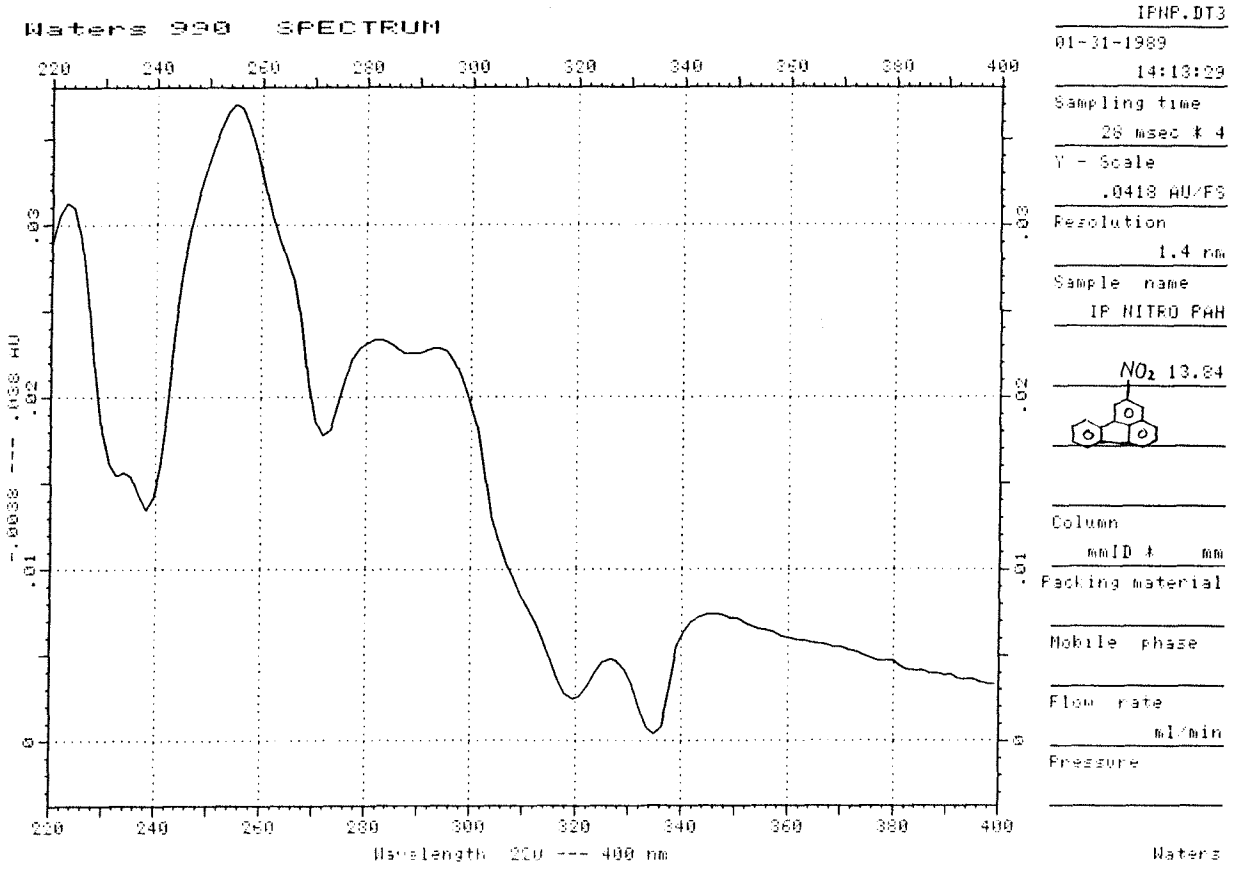


Figure 4-17

UV spectrum of 2-nitrofluoranthene in air sample IP10 by Waters 990 Photo Diode Array UV detector.

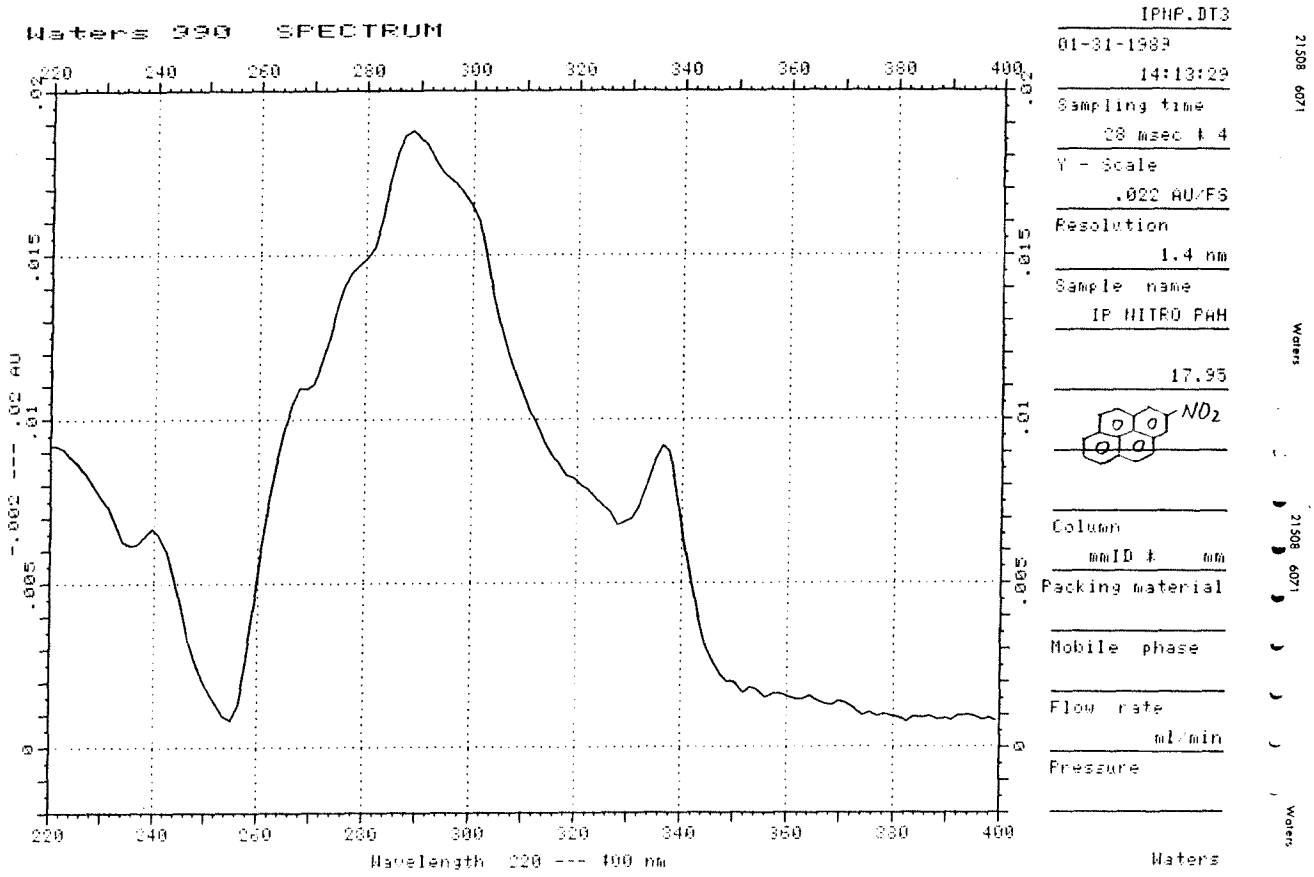


Figure 4-18

UV spectrum of 2-nitropyrene in air sample IP10 by Waters 990 Photo Diode Array UV detector.

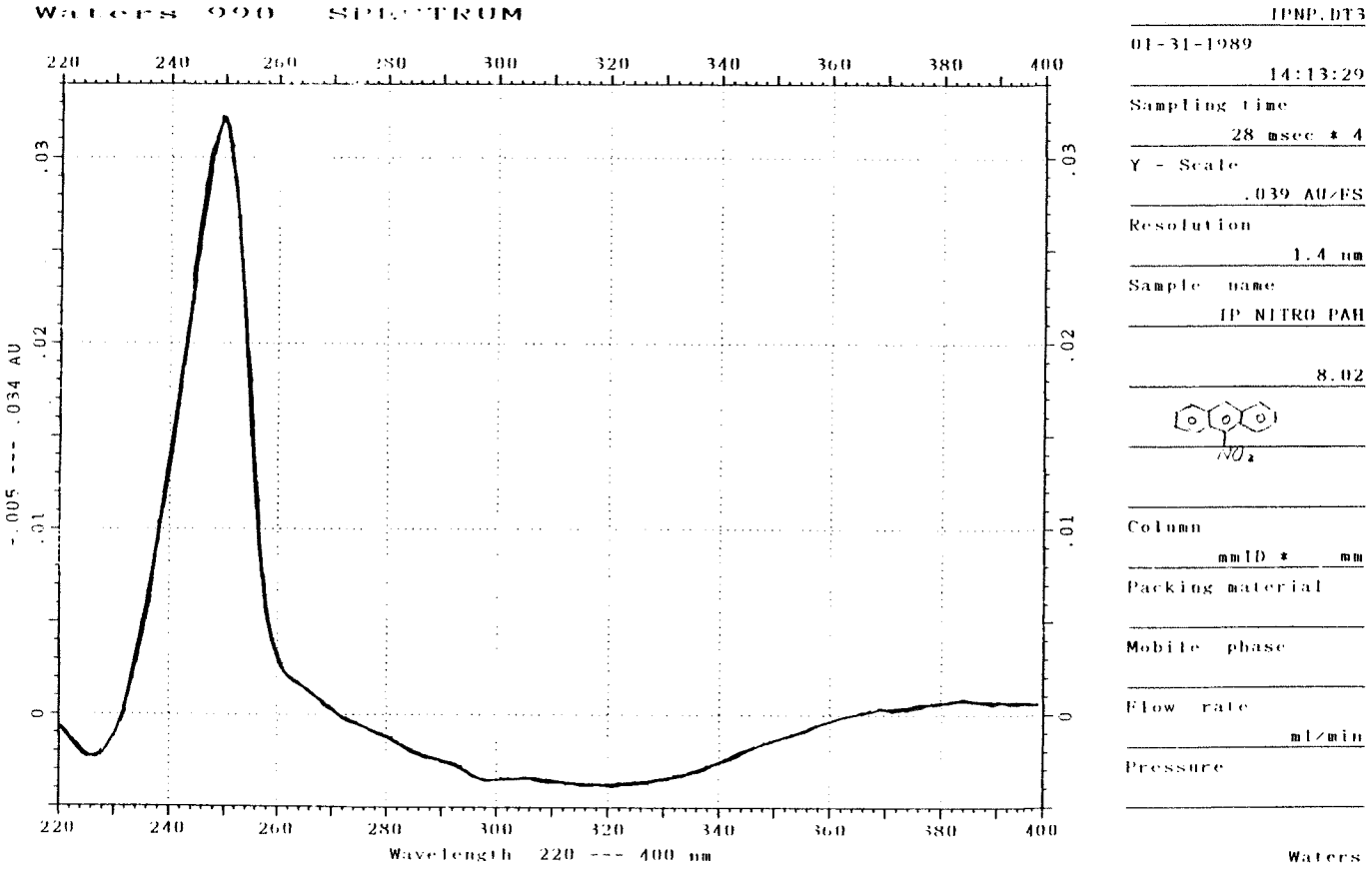


Figure 4-19

UV spectrum of 9-nitroanthracene in air sample IP10 by Waters 990 Photo Diode Array UV detector.

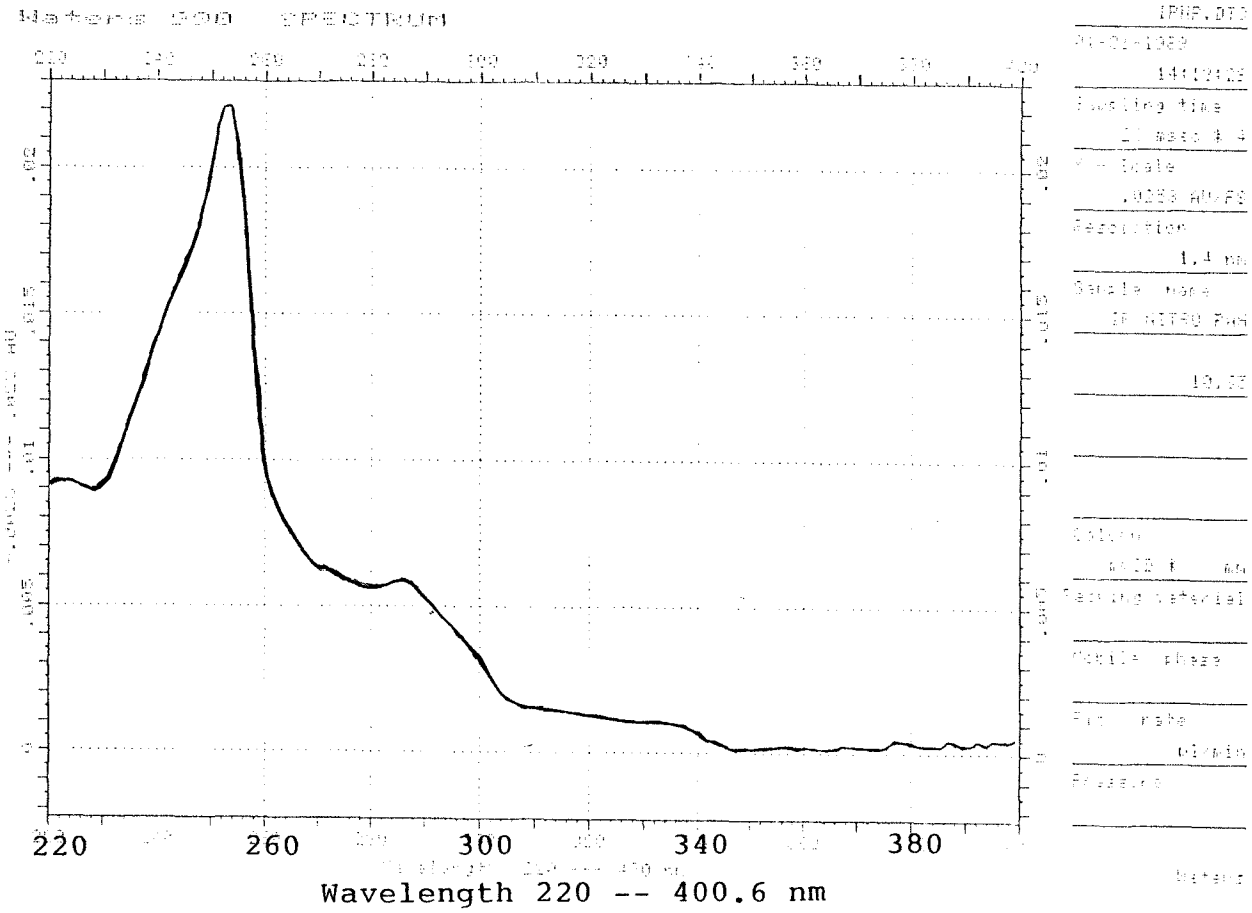


Figure 4-20

UV spectrum of 9,10-dinitroanthracene in air sample IP10 by Waters 990 Photo Diode Array UV detector.

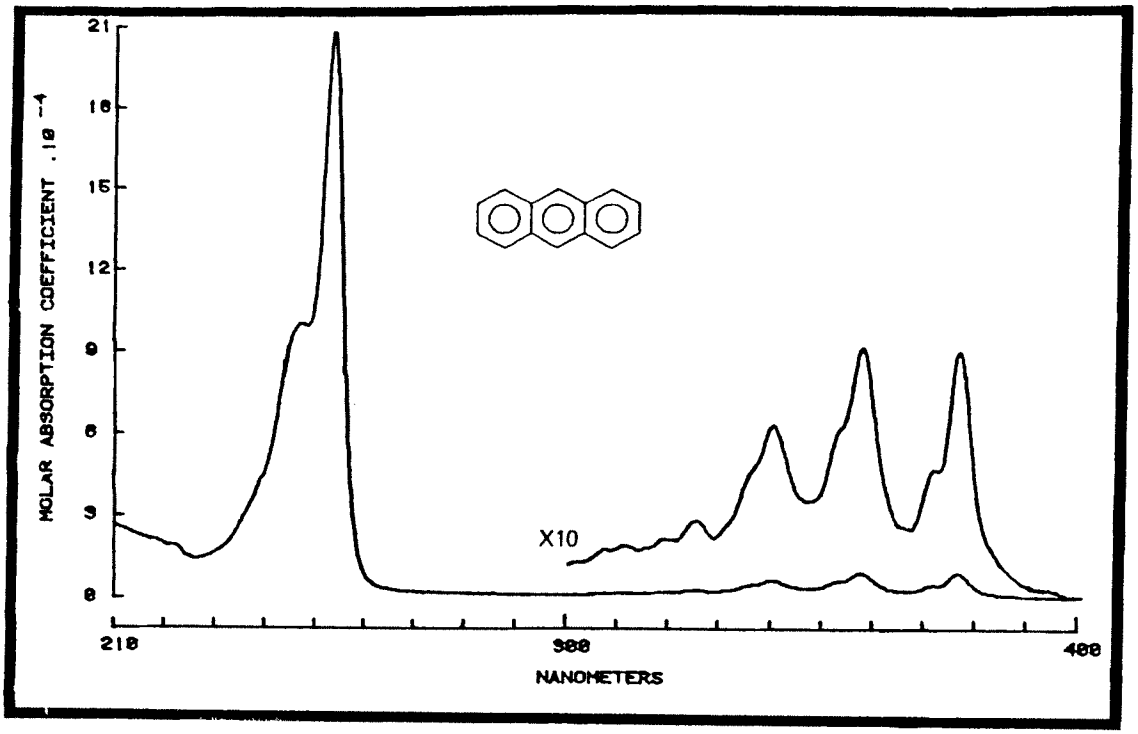


Figure 4-21

UV spectrum of anthracene.

Wavelength (nm)	Molar absorption coefficient ($l\ mol^{-1}\ cm^{-1}\ 10^{-4}$)	Wavelength (nm)	Molar absorption coefficient ($l\ mol^{-1}\ cm^{-1}\ 10^{-4}$)
221	1.90	339	0.63
246	10.0	356	0.91
252	20.8	372	0.465
308	0.180	374	0.89
323	0.291		

Spectrometer	: Perkin-Elmer 555	**Formula**	: $C_{14}H_{10}$
Solvent	: Cyclohexane	**M_r**	: 178.23 u
Concentration	: 0.73 mg l^{-1}	**CA N°**	: 120-12-7
Cell length	: 1.000 cm	**Purity**	: 0.998 g/g
Slit width	: 1 nm	**m.p.**	: 216.4°C

SPECTRUM PREPARED BY JRC PETTEN - DG XII - CEC

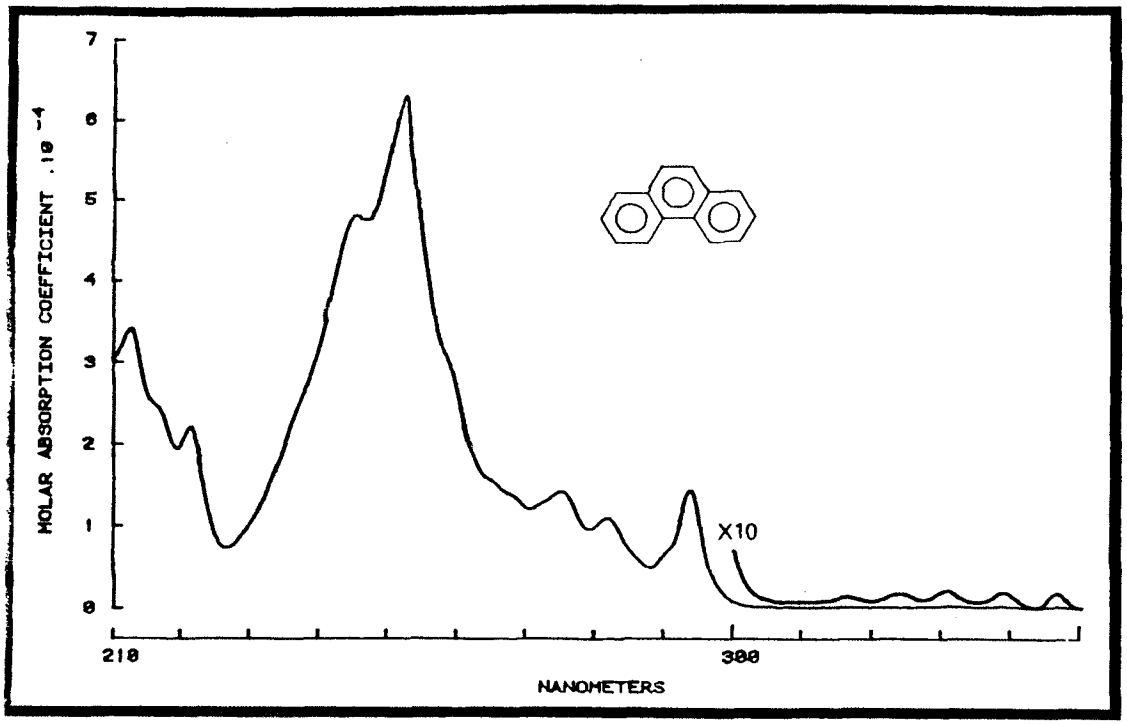


Figure 4-22

UV spectrum of phenanthrene.

Wavelength (nm)	Molar absorption coefficient (l mol^{-1} cm^{-1} 10^{-4})	Wavelength (nm)	Molar absorption coefficient (l mol^{-1} cm^{-1} 10^{-4})
213	3.48	294	1.44
221	2.23	316	0.016
246	4.98	324	0.019
252	6.41	330	0.022
275	1.43	339	0.019
282	1.10	346	0.022

Spectrometer	: Perkin-Elmer 555	**Formula**	: $C_{14}H_{10}$
Solvent	: Cyclohexane	**M$_r$**	: 178.23 u
Concentration	: 5.66 mg l^{-1}	**CA N^0**	: 85-01-8
Cell length	: 1.000 cm	**Purity**	: 0.99 g/g
Slit width	: 1 nm	**m.p.**	: 100.5°C

SPECTRUM PREPARED BY JRC PETTEN - DG XII - CEC

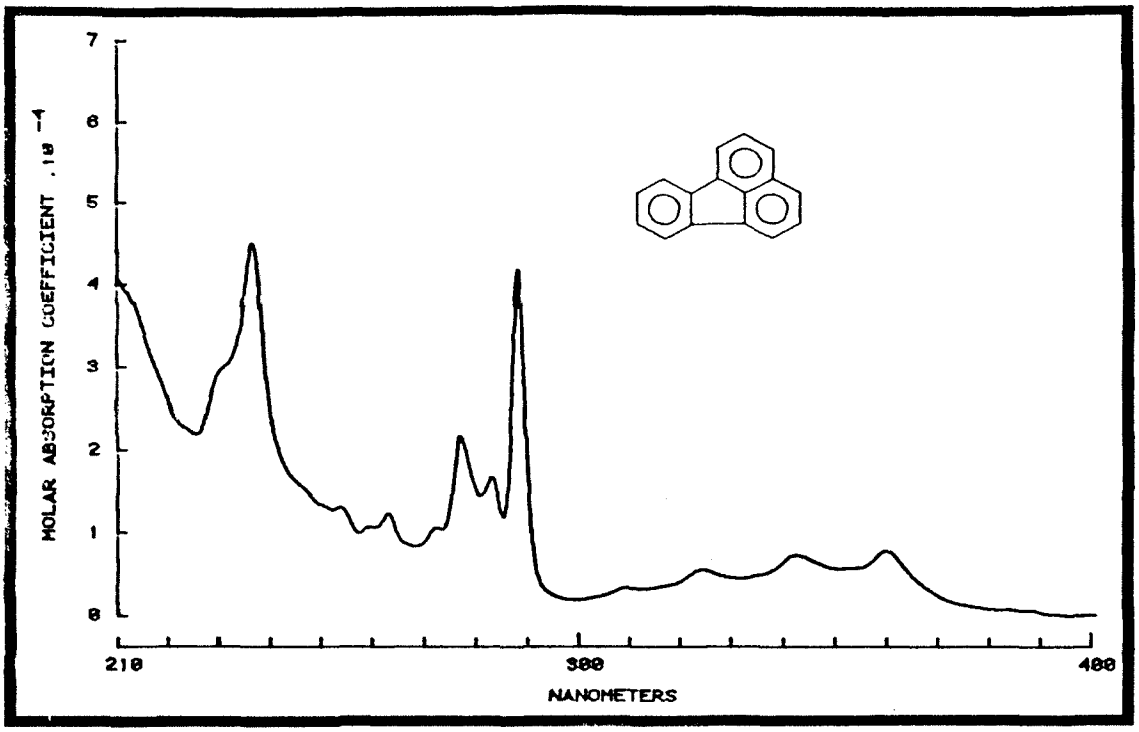


Figure 4-23

UV spectrum of fluoranthene.

Wavelength (nm)	Molar absorption coefficient ($l\ mol^{-1}\ cm^{-1}\ 10^{-4}$)	Wavelength (nm)	Molar absorption coefficient ($l\ mol^{-1}\ cm^{-1}\ 10^{-4}$)
231 (sh)	3.01	283	1.66
237	4.49	288	4.18
254	1.30	309	0.330
260	1.06	324	0.551
263	1.23	342	0.730
272	1.06	359	0.784
277	2.17		

Spectrometer	: Perkin-Elmer 555	**Formula**	: $C_{16}H_{10}$
Solvent	: [illegible]	**M_r**	: 202.08 u
Concentration	: 5.21 mg l^{-1}	**CA N°**	: 206-44-0
Cell length	: 1.000 cm	**Purity**	: 0.996 g/g
Slit width	: 1 nm	**m.p.**	: 108.8°C

SPECTRUM PREPARED BY JRC PETTEN - DG XII - CEC

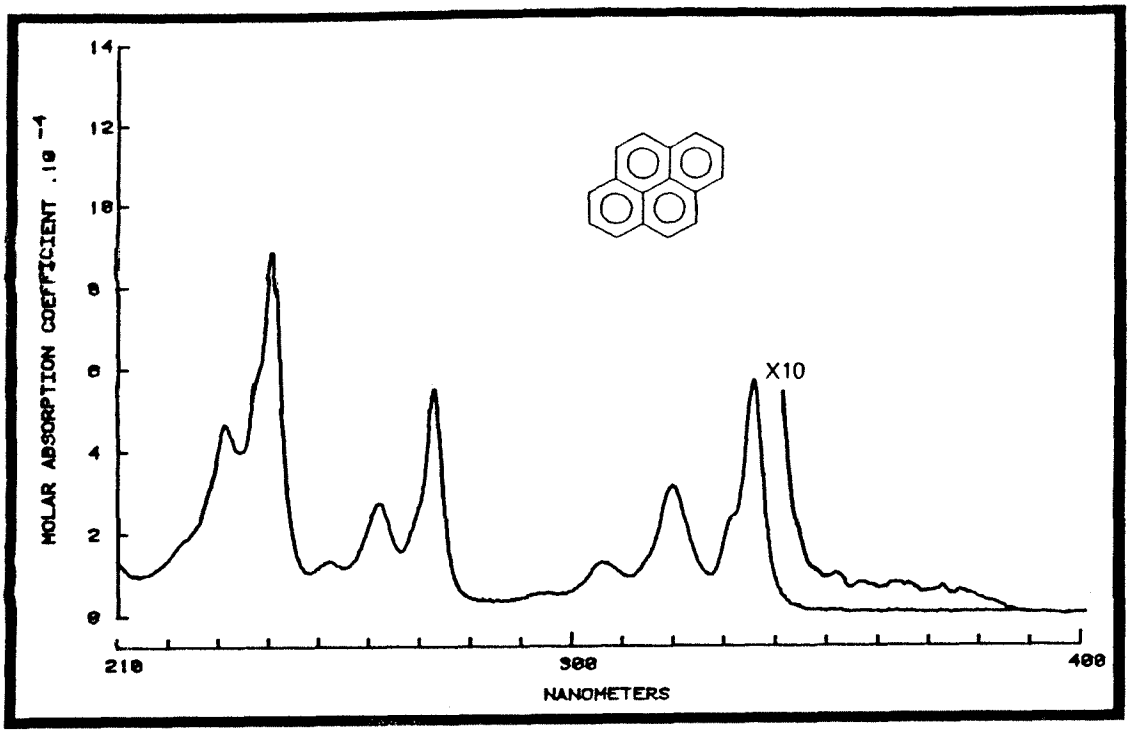


Figure 4-24

UV spectrum of pyrene.

Wavelength (nm)	Molar absorption coefficient ($l\ mol^{-1}\ cm^{-1}\ 10^{-4}$)	Wavelength (nm)	Molar absorption coefficient ($l\ mol^{-1}\ cm^{-1}\ 10^{-4}$)
224 (sh)	1.56	306	1.25
232	4.48	320	3.23
238 (sh)	5.67	332 (sh)	2.49
241.5	8.84	335.5	5.58
253	0.974	351.5	0.0558
263	2.47	357	0.0362
273	5.36	362	0.0343
295	0.439	372	0.0140

Spectrometer	: Perkin-Elmer 555	**Formula**	: $C_{16}H_{10}$
Solvent	: Cyclohexane		
Concentrations	: 0.676 mg l^{-1} (210-300 nm)	**CA N°**	: 129-00-0
	3.38 mg l^{-1} (300-350 nm)	**Purity**	: 0.998 g/g
	33.8 mg l^{-1} (350-380 nm)	**m.p.**	: 150.4°C
Cell length	: 1.000 cm		
Slit width	: 1 nm		

SPECTRUM PREPARED BY JRC PETTEN - DG XII - CEC

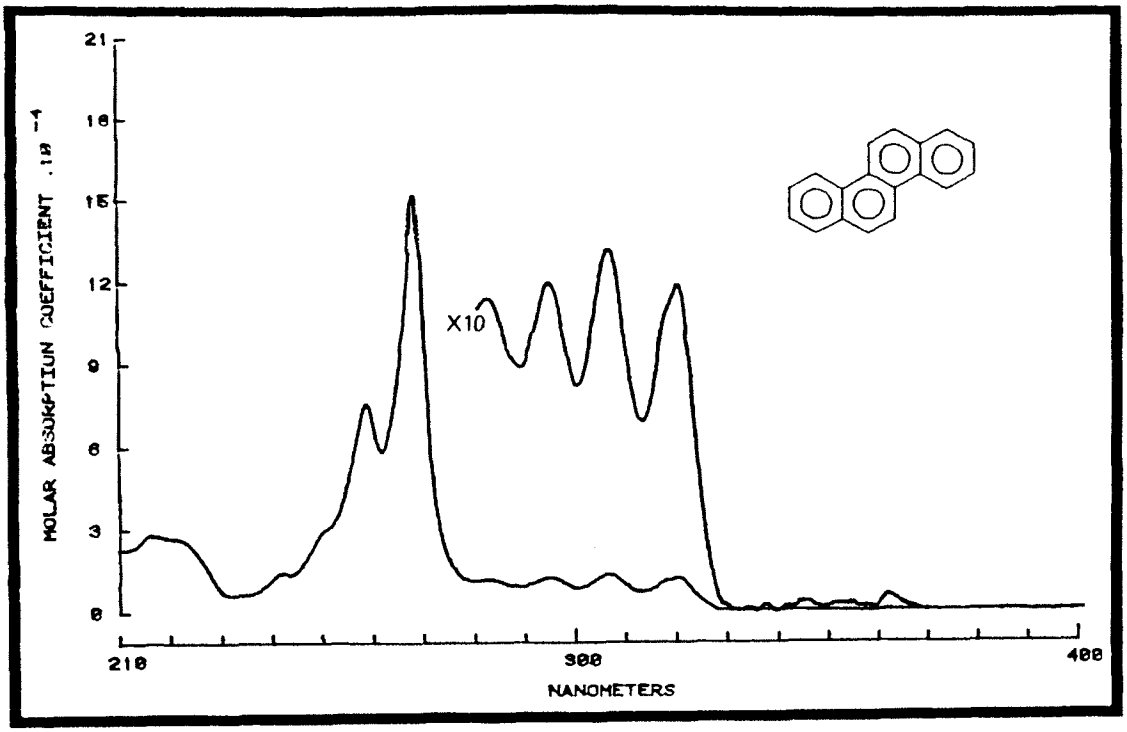


Figure 4-25

UV spectrum of chrysene.

Wavelength (nm)	Molar absorption coefficient ($l\ mol^{-1}\ cm^{-1}\ 10^{-4}$)	Wavelength (nm)	Molar absorption coefficient ($l\ mol^{-1}\ cm^{-1}\ 10^{-4}$)
218	2.90	295	1.20
223	2.85	307	1.32
242	1.83	320.5	1.20
259	7.60	344	0.0389
269	15.2	353	0.0250
283	1.14	361.5	0.0393

Spectrometer	: Perkin-Elmer 555	**Formula**	: $C_{18}H_{12}$
Solvent	: Cyclohexane	**M_r**	: 228.29 u
Concentrations	: 2.91 mg l^{-1} (210-330 nm)	**CA N°**	: 218-01-9
	29.1 mg l^{-1} (330-400 nm)	**Purity**	: 0.998 g/g
Cell length	: 1.000 cm	**m.p.**	: 253.5°C
Slit width	: 1 nm		

SPECTRUM PREPARED BY JRC PETTEN - DG XII - CEC

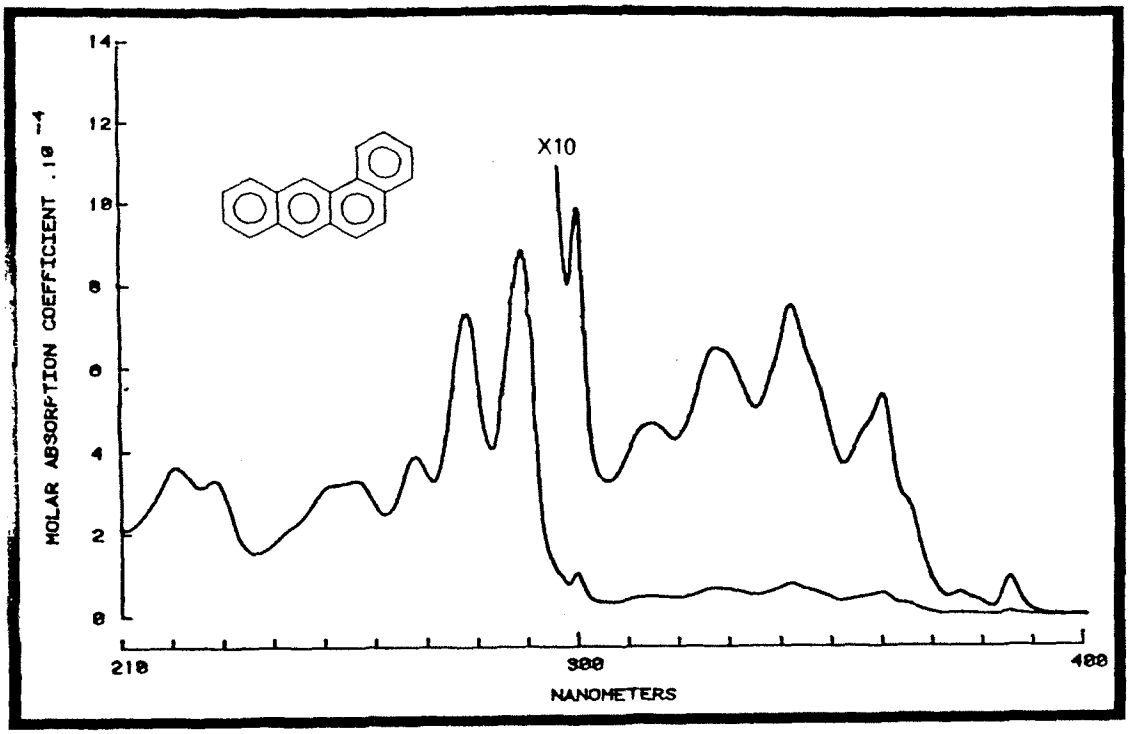


Figure 4-26

UV spectrum of benz[a]anthracene.

Wavelength (nm)	Molar absorption coefficient ($l\ mol^{-1}\ cm^{-1}\ 10^{-4}$)	Wavelength (nm)	Molar absorption coefficient ($l\ mol^{-1}\ cm^{-1}\ 10^{-4}$)
222	3.63	315	0.475
229.5	3.32	327.5	0.655
257	3.32	342	0.764
268.5	3.90	359.5	0.542
278	7.33	375	0.058
289	8.87	385	0.101
300	1.03		

Spectromter	: Perkin-Elmer 555	**Formula**	: $C_{18}H_{12}$
Solvent	: C, -	**M,**	: 228.29 u
Concentration	: 5.17 mg l^{-1}	**CA N°**	: 56-55-3
Cell length	: 1.000 cm	**Purity**	: 0.998 g/g
Slit width	: 1 nm	**m.p.**	: 160.7°C

SPECTRUM PREPARED BY JRC PETTEN - DG XII - CEC