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ANALYSIS OF NITRATED POLYCYCLIC HYDROCARBONS, PAH-QUINONES AND RELATED COMPOUNDS IN AMBIENT AIR

by
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Thesis submitted to the Faculty of the Graduate School of
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ABSTRACT

Title of Thesis: Analysis of Nitrated Polycyclic Hydrocarbons, PAH-quinones and Related Compounds in Ambient Air

Yalan Wang, Master of Science, 1987

Thesis Directed by: Dr. Arthur Greenberg, Professor of Department of Chemical Engineering and Chemistry

Studies were conducted to characterized airborne particulate extractable organic matter (EOM) with respect to chemical composition and biological activity. It has been well established that the nitrated polycyclic hydrocarbons (nitro-PAH) are environmental contaminants. To identify nitro-PAH in ambient air sample, a technique was developed involving introduction of a reducer column into an reverse-phase high performance liquid chromatography (HPLC) system. Strong fluorescence signals were achieved from nitro-PAH and detected together with u.v. signals for the qualitative and quantitative analysis of nitro-PAH. 2-Nitrotriptycene was synthesized according to published procedures and used as an internal standard. By use of the above technique, 9-nitroanthracene, 1-nitropyrene, 7-nitrobenz(a)anthracene, 6-nitrochrysene and 6-nitrobenzo(a)pyrene were detected in NBS diesel particulate sample.

PAH-quinones may be formed from the appropriate PAH's by their photochemical oxidation in the atmosphere. In this

study, an analytical method for the identification of PAH-quinones in airborne particulates was developed. The same HPLC system was employed as that for nitro-PAH. 3,6-BaP-dione and 7,8-BaP-dione were identified in both CYC extract and DCM extract of an ambient air sample by using the analytical method developed.

Since hydroxynitropyrenes have been identified as making a significant contribution to the mutagenicity of airborne particulate matter, this prompted us to investigate one such compound, 1-nitropyren-2-ol. This compound was synthesized according to published procedures. 1-Nitropyren-2-ol was detected in one of the ambient air samples and in an NBS diesel particulate sample by using our reverse-phase HPLC system monitoring at 546 nm.

To provide more data for estimating the photochemical products of 1-nitropyrene, the filters coated with 1-nitropyrene were exposed either to sunlight or to u.v. light. The breakdown products were analyzed using HPLC. No 1-nitropyren-2-ol was found in the filters exposed in both ways. Further work is needed to identify the breakdown products.

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CHAPTER I : Introduction

1-1. Background

The organic fractions extracted from airborne particles have been shownto exhibitmutagenic activityby in-vitro bioassays reported by several investigators [15, 17, 75, 99, 105]. In the New York City area, all of the analyzed particulate samples have been mutagenic and they have exhibited a seasonal variation with the highest activity occurring in the winter [16]. Tokiwa et al.[103] reported that ambient aerosols collected from an industrial area were more mutagenic than those collected from residential areas. In general, the mutagenic activities of airborne particles were mostly direct-acting and were very similar to those found in combustion-related aerosols [48] and diesel exhaust particles [12, 39, 73, 93]. The search for major mutagens associated with airborne particulate matter has evolved from investigations of polycyclic aromatic hydrocarbons(PAH) through studies of nitro-PAH, PAH-quinones and other oxygenated derivatives to analysis of various mixed functionality compounds such as nitrohydroxy-PAH [69, 90]. It has been shown that the PAH class itself only accounts for a minor part of the mutagenicity in ambient airborne particulate matter [2]. Therefore, interest in nitro-PAH, PAH-quinones and the oxygenated derivatives of PAHs has grown in recent years.

Nitro-PAHs have been a source of increasing health-

related concern due to their direct-acting mutagenic response in salmonella test [54, 65, 85], positive mutagenic responses in mammalian cells [14], and carcinogenic activity in animal experiment [69]. Schuetzle et al.[89] have estimated that approximately 30% of the direct-acting mutagenicity of the solvent-extractable portion of diesel engine exhaust is due to the presence of one compound, 1-nitropyrene. Nitro-PAH are found in diesel exhaust gases and particulates [79, 88, 109], in carbon black [84] and in other environmentally significant material.

In addition to nitro-PAHs, many oxygenated PAH may be associated with particulate matter in ambient air. For instance, model experiments on chemical and, particularly, photochemical PAH stability suggest that several of the PAH that are emitted in sizable amounts from various natural and anthropogenic sources are degraded in the atmosphere by sunlight or by interactions with other reactive airborne species [3, 24, 25, 46, 47, 49, 50, 76, 78, 98]. To some extent, relatively stable oxidation products such as quinones are generated by these reactions. Furthermore, analytical investigations of various source emissions have shown that oxygenated PAH may readily be formed by incomplete combustion of organic materials [1, 22, 35, 72, 89, 91, 96]. Pierce and Katz [74] have reported the identification of 9,10-anthraquinone, benzo(a)pyrene-6,12-quinone, benzo(a)pyrene-1,6-quinone, benzo(a)pyrene-

3,6-quinone, and dibenzo(b,def)chryscene-7,14-quinone in the samples of suspended particulate matter collected in Toronto, Ont. In the course of other investigations on airborne particulates, anthracene-10,9-dione, and were also identified dibenzo[cd,jk]pyrene-6,12-dione [11]. However, very little information has been amassed on the PAH-quinones.

Strongly mutagenic and carcinogenic 1-nitropyrene has been found in environmental samples, for example, diesel exhaust and airborne particulate matter [66, 68, 89, 106, 107]. Therefore, the fate of 1-nitropyrene in the environment is of interest since it is fairly unstable and reactive toward heat or light. It is very important to know what products occur through thermolysis or photolysis and whether the reaction products are mutagenic (or carcinogenic). Recently,the thermolysis product of 1-nitropyrene was tentatively identified as 1-aminopyrene [97] and the photolysis product was determined to be 1-nitro-2-hydroxypyrene (1-nitro-2-pyrenol) by A.Yasuhara [108]. The hydroxynitropyrenes (HNP) have been identified as making a significant contribution to the mutagenicity of airborne particulate matter [90]. Among the HNP are 3-nitro-1-hydroxypyrene, 6-nitro-1-hydroxypyrene and 8-nitro-1-hydroxypyrene [28, 29, 69, 71, 91]. Aside from being responsible for a significant part of the mutagenic activity of airborne particulates, these compounds may be useful markers of the age of air particulate, their concentrations

possibly increasing relative to more unstable pollutants as the particulate matter ages [28, 29]. Another source of interest in these compounds is their natural occurrence as metabolites of 1-nitropyrene [5].

1-2. Nitro-PAHs

1-2-1. Sources and Formation

The strong, directly-acting bacterial mutagenicity of collected respirable ambient particulate matter is attributed in part to mono- and dinitroarenes [41,82, 102]. A major source of these nitroarenes in particulate matter appears to be their direct emission in combustion-generated fine particles such as auto and diesel exhaust particulate [79, 90, 107] and soot from wood-burning fireplaces [27].

Additionally, on the basis of studies in simulated atmospheres containing NO_2 and traces of HNO_3, it has been suggested that some nitro-PAH in ambient particulate matter may be formed by heterogeneous reactions of adsorbed PAH with these gaseous nitrogenous co-pollutants during the transport through the atmosphere, collection on filters, or both [76, 77]. Subsequently, this suggestion has been supported by other laboratory experiments [42, 83, 101], including several with PAH adsorbed on environmentally relevant substrates such as fly ash [38], soot [10] and ambient particulate matter [8]. However, Grosjean et al [33] have expressed a dissenting opinion on this issue. J.N.

Pitts also reported that in the gas-phase reactions in air of N_2O_5 with ppm levels of naphthalene, significant yields of 1-nitronaphthalene and 2-nitronaphthalene (18% and 7.5% respectively) were obtained [80]. J.N. Pitts and others [38, 42, 76] have suggested that these reactions are particularly important because the nitro-PAH in environmental samples may be sampling artifacts formed by nitration of PAH on sample collection media. J.N. Pitts and coworkers [76] have shown that PAH deposited on filters are converted to nitro-PAH when NO_2 is passed over the filter. Lane and Katz [50] have shown that PAH are reactive to oxidants under sampling conditions. More recently, Schuetzle [91] has investigated the artifact formation of nitro-PAH on sample collection media during dilution-tube sampling of vehicle emissions and concluded that "chemical conversion of PAH to nitro-PAH during dilution-tube sampling of particles on Teflon filters and gases on XAD-2 resin is a minor problem (representing 10-20%, on the average , of 1-nitropyrene found in extracts) at short sampling time (23 min), at low sampling temperature (42 C), and in diluted exhaust containing 3 ppm NO_2. Nevertheless, under some sampling conditions, formation of nitro-PAH artifacts can be a serious problem. It should be noted that the overall amount of nitro-PAH formed during sample collection is potentially different for each source sampled and for each different sampling condition. Short collection times and low sampling temperatures minimize artifact formation.

1-2-2. Mutagenicity and Carcinogenicity

The discovery of the potent bacterial mutagenicity of some nitro-PAH coupled with the recognition of the almost ubiquitous distribution of these chemicals in the environment has generated a great deal of interest in their properties [84, 86]. The mutagenicity of nitro-PAH was found to be optimal in Salmonella typhimurium TA98, a plasmid-containing strain which detects frameshift mutations. H.S. Rosenkranz and R. Mermelstein [87] have summarized the mutagenicity of nitro-PAHs as shown in Table 1.

Table 1. Mutagenicity of nitro-PAH for Salmonella typhimurium TA98 (See Ref. 87).

Chemical	Mutants per nmol -S9	Mutants per nmol +S9	References
1-Nitronaphthalene	0.05		[60]
2-Nitronaphthalene	0.2		[60]
2-Methyl-1-nitronaphthalene	0		[19]
1-Methyl-2-nitronaphthalene	0.2		[19]
3-Methyl-2-nitronaphthalene	1.0		[19]
1,3-Dinitronaphthalene	0.9		[60]
1,5-Dinitronaphthalene	3.3		[60]
1,8-Dinitronaphthalene	0		[60]
2,4-Dinitro-1-naphthol	0.2		[60]
2,3,5-Trinitronaphthalene	32		[95]
1,3,6,8-Tetranitronaphthalene	0.2		[60]
3-Nitro-1,8-naphthalic anhydride	2.6		[60]
5-Nitroacenaphthene	4.6		[62, 63]
2-Nitrofluorene	14		[61]
3-Nitro-9-fluorene	383		[73]
2,7-Dinitrofluorene	471		[61]
2,7-Dinitro-9-fluorene	1,459		[61]
2,4,7-Trinitro-9-fluorene	2,125		[61]
2,4,5,7-Tetranitro-9-fluorene	860		[61]
2-Nitroanthracene	892		[92]
9-Nitroanthracene	0.5		[31]
2-Nitrophenanthrene	<0.5		[31]
1-Nitrofluoranthene	544		[31]
3-Nitrofluoranthene	5,439		[31]
7-Nitrofluoranthene	74		[31]
8-Nitrofluoranthene	11,125		[31]
1-Nitropyrene	453		[65]
1-Nitrosopyrene	2,130		[64]
2-Nitropyrene	2,225		[31]
8-Hydroxy-1-nitropyrene	31	19	
6-Hydroxy-1-nitropyrene	22	87	
3-Hydroxy-1-nitropyrene	103	36	
2-Hydroxy-1-nitropyrene	0	22	[56]
1,3-Dinitropyrene	144,760		[65]
1,6-Dinitropyrene	183,570		[65]
1,8-Dinitropyrene	254,000		[65]
1-Amino-8-nitropyrene	266		[9]
1,3,6-Trinitropyrene	40,700		[65]
1,3,6,8-Tetranitropyrene	15,590		[65]
2-Nitrochrysene	<0.6	27	[31]
5-Nitrochrysene	<0.6	2.7	[100]
6-Nitrochrysene	269		[31]
7-Nitrobenz[a]anthracene	0.3	1.4	[31]

Table 1, continued

Chemical	Mutants per nmol -S9	+S9	References
6-Nitrobenzo[a]pyrene	0	466	[79,34]
1-Nitrobenzo[a]pyrene	1,567		[79,34]
3-Nitrobenzo[a]pyrene	1,070		[79,34]
1-Nitrobenzo[e]pyrene	39		[31]
3-Nitrobenzo[e]pyrene	890		[31]
3-Nitroperylene	<30	1,784	[31]
4-Nitrobenzo[g,h,i]perylene	<0.6	1,925	[31]
7-Nitrobenzo[g,h,i]perylene	<0.3	0.6	[31]
5,8-Dinitrobenzo[g,h,i]-perylene	21,500	7,200	[104]
5,10-Dinitrobenzo[g,h,i]-perylene	4,000	368	[104]
1-Nitrotriphenylene	0		[55]
2-Nitrotriphenylene	12,300		[55]
1-Nitrocoronene	0	340	[104]
1-Nitrocarbazole	0	0	[51]
2-Nitrocarbazole	10.3		[51]
3-Nitrocarbazole	0.2	0.8	[51]
4-Nitrocarbazole	0.01	0.06	[51]
5-Nitroquinoline	0.1		[43]
6-Nitroquinoline	0.05		[43]
8-Nitroquinoline	0		[43]

Some of the important features regarding their mutagenicity were summarized by F.A. Beland et al [5] as follows:

1. Nitro-PAHs generally exhibit their highest mutagenicity in strain TA98 (a frameshift detector) in the absence of an S9 activating system. This direct mutagenic activity contrasts with the response observed with PAHs and amino-PAHs which normally require S9 to induce mutations.

2. Some of the nitro-PAHs (e.g., 1,3-, 1,6-, and 1,8-dinitropyrene are among the most mutagenic compounds ever tested in the S. typhimurium reversion assay.

3. Nitro-PAHs which show greater activity in the

presence of S9 (e.g., 6-nitro-Bap) may have a fundamentally different reactive intermediate than the direct-acting nitro-PAHs.

4. There can be dramatic variations in mutagenic potential between nitro-PAH isomers. For instance, 6-nitro-Bap is not a direct-acting mutagen whereas 1- and 3-nitro-Bap are potent direct-acting mutagens.

5. Dinitro-PAHs appear to be more mutagenic than their mononitrated analogues.

Except for 1-nitronaphthalene, the other nitro-PAHs tested are all found to possess some carcinogenicity [87] (Table 2). Perhaps the most important finding is that regarding 1-nitropyrene's ability to cause tumors at the site of injection as well as metastases at a distant organ [36]. Concerning the carcinogenic activity,the ranking of the carcinogenic potency of dinitropyrenes given subcutaneously to rats is of the same order as that of benzo[a]pyrene and 3-methylcholanthrene. Because the nitro-PAH are much more mutagenic to bacteria than either benzo[a]pyrene or 3-methylcholanthrene, this indicates that the mutagenic potency is not necessarily translated into carcinogenic potentials. If this should be true for other organs, species, and modes of administration, it would indeed be a reassuring finding.

Table 2. List of nitro-PAHs tested for their carcinogenicity [87].

Chemical	Chemical
5-Nitroacenaphthene	1-Nitronaphthalene
2-Nitronaphthalene	2-Nitrofluorene
1-Nitropyrene	1,3-Dinitropyrene
1,6-Dinitropyrene	1,8-Dinitropyrene
3-Nitrofluoranthene	6-Nitrofluoranthene
6-Nitrochrysene	6-Nitrobenzo[a]pyrene
3-Nitroperylene	

1-3. PAH-quinones

1-3-1. Sources and Formation

PAH-quinones may be formed from the appropriate PAHs by their photochemical oxidation in the atmosphere [24, 58]. There is evidence that PAHs are degraded in the atmosphere by photooxidation and by reaction with atmospheric oxidants [67]. The most likely reactions of these hydrocarbons produce oxygenated compounds. Some oxygenated compounds exist in the atmosphere, and the oxygenated fractions of several air extracts appear to be carcinogenic [20, 21]. Several investigations [7, 59] have indicated that various PAHs are metabolized in mammalian cells with some of the end products being PAH-quinones.

Schuetzle et al. [73, 88] found that the most mutagenic fractions of diesel particulate extracts contain primarily oxygenated PAH derivatives including hydroxy-PAH, ketones, and quinones, etc. The observed ease of oxy-PAH formation from the oxidation of PAH also raised the question regarding

the origin of these species, i.e., whether they are produced as "native" products during the engine combustion process, in the tail pipe, or instead, formed as a result of chemical conversion to produce artifacts during the sampling or analysis procedures. Lee [52] showed that the oxidation of PAH is greatly enhanced by the direct deposition of the PAH on the quartz or glass fiber as done in the experiments by Pitts and Lane. Lee also found that PAH deposited on Teflon filter or adsorbed in particulates are much more stable toward chemical conversion. These effects are demonstrated by the recovery of BaP during various conditions of ambient air sampling as shown in Figure 1. D. Schuetzle has found that PAH react with molecular oxygen to form quinones and ketones and other oxygenated derivatives. It is shown by the following equations:

$$PAH + O_2 \text{ --- } PAH\text{-ketones} + PAH\text{-quinones}$$
$$PAH\text{-dicarboxylic acids} + PAH\text{-aldehydes}$$

$$PAH + HNO_3 \text{ --- } PAH\text{-}NO_2 + PAH\text{-}(NO_2)_2 + H_2O$$

$$PAH\text{-}(NO_2)_2 \text{ --- } PAH\text{-quinones}$$

Very little is known about the formation of oxy-PAH in the engine and tail pipe vehicle exhaust. Schuetzle [91] suggested that more studies concerning their chemistry and reaction kinetics under this condition is needed.

1-3-2. Mutagenicity

Recent investigations on diesel exhaust have shown that the so-called moderately polar fraction, which normally

contains the oxygenated PAH, exhibit most of the direct-acting Ames mutagenicity [91]. In contrast to most of the mutagenic parent-PAH, these substances require no activation by mammalian enzymes to cause mutagenic responses. It is easy to imagine that similar compounds may also occur in association with general airborne particulate matter. Thus, it would be of special interest to determine their concentration in the ambient air samples.

1-4. Nitrohydroxypyrenes

1-4-1. The Formation and the Mutagenicity of Nitrohydroxypyrenes

Compared to the studies of PAH and nitro-PAH, little information about nitrohydroxypyrenes (or nitrohydroxy-PAH in general) is available. Recent research on nitrohydroxypyrenes seem to suggest that nitrohydroxypyrene are formed in the atmosphere from PAH or nitro-PAH under ultraviolet irradiation [28]. Gibson [28] reported that hydroxynitropyrene increased from 4 ug/g in fresh diesel particulate matter to 10 ug/g in the aerosol exposed for 8 hours to exhaust gases and u.v. irradiation. He also found that the concentration of hydroxynitropyrenes were higher in rural samples and especially those from the remote site compared to suburban and urban samples. This is strong evidence that these compounds are formed in atmospheric reactions as they are in smog-chamber experiments.

In another study on hydroxynitropyrene, Yasuhara and

Fuwa synthesized 1-nitro-2-hydroxypyrene (1-nitropyren-2-ol) from 1-nitropyrene just by exposing 1-nitropyrene solution under U.V. light for 5 hours [108a]. This also supports the suggestion that this groups of compounds are formed in the atmospheric reaction because the U.V. irradiation is available there. However, their tentative and admittedly incomplete study of airborne particulates turned up no evidence for the presence of this compound in air [108b].

The mutagenicity of 1-nitropyrene-2-ol has been tested by Lofroth et al [56].The result indicated that it is not directly mutagenic but its activated mutagenicity is comparable to that of benzo(a)pyrene. In contrast, the other HNP tested are direct mutagens, and the seemingly anomalous biological activity of 1-nitropyrene-2-ol has been attributed to the presence of intramolecular hydrogen bonding.

The goals of this work were: (1) to develop an analytical technique for the determination of nitro-PAHs in ambient air samples; (2) to develop an analytical method for the separation and identification of PAH-quinones in ambient air samples; (3) to set procedures for the analysis of 1-nitro-2-hydroxypyrene in ambient air samples.

CHAPTER II: Experimental Section

2-1. Materials

All Solvents employed for extraction and chromatography are HPLC grade. 9-Nitroanthracene and 1-nitropyrene were obtained from Aldrich Chemical company and used without any further purification. Other nitro-PAHs as well as the seven benzo[a]pyrene (BaP) diones and one BeP dione were purchased from the National Cancer Institute Chemical Repository(Midwest Research Institute synthesized the compounds). 2-Nitrotriptycene was synthesized and used as an internal standard for nitro-PAH analysis and the standard 1-nitropyren-2-ol, used for the identification of this substance in ambient air samples, was synthesized as described later in this chapter. 3-Nitropyren-1-ol, 6-nitropyren-1-ol and 8-nitropyren-1-ol were obtained from Dr. Louise Ball, University of North Carolina. the sample of 2-nitrofluoranthene was obtained from Drs. Janet Sweetman and Barbara Zielinska, University of California at Riverside.

2-2. Sample Collection and Fractionation

Twenty-four hour samples were collected during Fall (October-December), 1984 at the ATEOS Newark site [53] using an Anderson high volume sampler (AD <10 micron) and prefired quartz filters. These were soxhlet extracted for 20-24 hours each using the sequence cyclohexane (CYC), dichloromethane (DCM), and acetone (ACE). Each extract was streaked onto a separate silica gel GF plate (Analabs,

Inc.). The cyclohexane extract was developed using 1:1 hexane/toluene and five fractions (#5, least polar; #1, most polar) were scraped from the plate and the adsorbent washed with hot CYC and hot methanol sequentially and then the two washings were combined. The DCM and ACE plates were developed using DCM and five DCM extract fractions (#5, least polar; #1, most polar) and six ACE extract fractions (#5, least polar; #0, most polar) were obtained by scraping the adsorbent from the plate and washing with hot DCM and hot methanol sequentially and the combining the washings. Figures 2, 3, and 4 show the positions of each fraction on the TLC plate respectively. For Ames assays, the 15 extracts of thesample were composited and then fractionatedby TLC. Subsequent (1985) TLC developments were done with somewhat different conditions and different fractions were scraped.

2-3. Bioassay of Fractions

The solutions of each fractions were clarified and 100 ul and 50 ul aliquots taken for EOM (extractable organic matter) determination using a Cahn 26 automatic electrobalance [53]. Quarterly composites of whole extracts and fractions were assayed using TA98.

Masses and mutagenic activities of the 16 fractions obtained from the three extracts of a quarterly composite (October-December, 1984) are shown in Figure 5. Most of the mass is concentrated in the least polar fraction (cyclohexane #5) and the most polar fraction (acetone #0).

However, the greatest mutagenic activity , both on the basis of rev/mass of EOM and rev/m^3 is found in the most polar fraction (#1) of the DCM extract. It is apparent that the only fraction to manifest a major increase in mutagenicity upon metabolic activation is cyclohexane fraction #4 which has at least 90% of the PAH [30]. The acetone extract has 6-10% of its total mass due to NO_3^- and another 1% due to NH_4^+ with a negligible contribution from SO_4^{--}. These ions are concentrated in the acetone #0 fraction. It is likely that other inorganic substances also contribute to #0 which dominates the ACE EOM.

The use of greater amounts of EOM devoted to Ames assays in another quarterly composites (January- May, 1985) which represent winter and spring sampling dates permitted the detection of mutagenicity in fractions which had previously shown marginal sensitivity. All fractions of the CYC extract showed the mutagenicity without S9 with the response distributed fairly evenly throughout the plate except in the lighter most nonpolar fraction 6 which contains alkanes. Addition of S9 lowered the mutagenicity of the unfractionated extract, but increased the response slightly for fraction 0 and 1, significantly for fraction 2 which contains ketones and quinones, fraction which 3-4 contains nitro-PAH and fraction 5 which contains PAH (Figure 6). The DCM extract contained the most potent EOM and showed direct mutagenicity both in the unfractionated extract and in the individual subfractions. "Hot" fractions were

fraction 0 and 1, which corresponded to fraction 1 of the Fall, 1984 sample. Fraction 4, however, although representing approximately 1/3 of the mass found in either fraction 0 or 1, had a very high response, 15 times greater in rev/ug than the unfractionated extract (Figure 7). Since this fraction represents the less polar portion of the DCM extract, it may contain dinitro-PAH. However, the small mass of fraction 4 is reason for hesitancy in quantitative interpretation of this result.

The ACE extract was directly mutagenic in the unfractionated composite as well as in all fractions tested. The "hottest" fraction was fraction 2. Fraction1 which contains most of the ACE mass was 1/5 as mutagenic per ug mass as the lighter fraction 2 (Figure 8). A significant portion of the mass in fraction 1 may be due to inorganic salts.

2-4. Analysis of Nitro-PAHs

2-4-1. High Performance Liquid Chromatography System

A Waters gradient HPLC system was used consisting of two Model 6000 A pumps, a Model U6K injector, a Model 660 solvent programmer, a Model 440 absorbance detector operating at 280 nm and 365 nm, and a Model 420 fluorescene detector operating at 395 nm (excitation) and 460 nm (emission). The column used was a Vydac 201 TP54 column. The solvent flow was 1 mL/min and a linear gradient of 60% aq CH_3CN to 100% CH_3CN in 35 mins was used.

For the reduction of nitro-PAH to amino-PAH, a reducing system was introduced into the above HPLC system by employing essentially the same technique as that described by MacCrehan and May [57] except that modifications have been made on the zinc/silica reducer column. The flowchart of the whole system and the structure of the reducer column are shown in Fig.9 and Fig.10a, 10b, 10c and 10d respectively.

When nitro-PAH which do not fluoresce or weakly fluoresce pass through the reducer, they are reduced to amino-PAH which strongly fluoresce. Thus, the fluorescence signal can be taken as a measure to identify nitro-PAH.

The reducer column was made as follows:

(1). Preparation of packing material

Packing material is a mixture of zinc powder (7.1 micron, from Alpha Products) and silica (37 micron, from Whatman, Inc.). The ratio of Si to Zn is 1:1 by mass.

Si and Zn powder (in 1:1 ratio) were poured dry into a small vial. The material was mixed thoroughly to produce a well mixed packing material.

(2). Packing Zn reducer postcolumn cartridges

Carefully remove top frits from precolumn inserts for Water Associates Guard-Pak precolumn Module, P/N 080040. Empty out packing material and wash with methanol. Dry pack the empty column with the well mixed Zn-Si mixture, and tap against table gently until the level of the previous packing is reached. Replace frits carefully applying

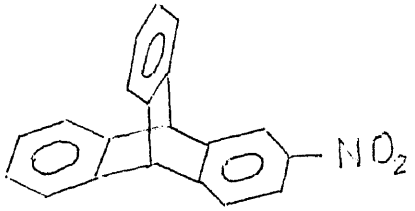
sufficient pressure to firmly pack the column. Make sure that they sit flat on the packing material. Before placing cartridge in module, wet down thoroughly with methanol and remove any excess packing with a chemwipe.

(3). Using post-column reducer module

Insert the packed reducer cartridge into Guard-Pak Precolumn Module. Plumb module to column switching valve (Valco) using 1/16" stainless steel tubing. This permits HPLC reverse-phase separation with and without postcolumn reduction. The reducer cartridges allow at least 40 hours of use before loss of resolution or efficiency of reduction. Efficiency of chemical reduction appears to be stable thorough the usable period as evidence by the Beer's Law calibration plots.

2-4-2. Synthesis of Internal Standard for Nitro-PAH Analysis

We are currently using 2-nitrotriptycene (1) as an internal standard for nitro-PAH analysis. As a nearly spherical molecule, it elutes much earlier than its



(1)

companion nitro-PAH. Nitrotriptycene was synthesized and purified according to a published procedure [44]: To a solution of 5 g of triptycene in 150 ml of acetic anhydride at 29°C was added a solution of 3.0 ml of concentrated

nitric acid in 5.0 ml of glacial acetic acid, the temperature being maintained at 27-29°C. After 3 hr the reaction mixture was added to 400 ml of water. The precipitate which formed upon standing was removed by filtration, suspended in 25 ml of methylene chloride, chromatographed on a Florisil column (2 cm x 30 cm; 60-100 mesh) packed in petroleum ether (bp 35-60°C), and eluted with 1:9 methylene chloride-petroleum ether. The first fraction contained triptycene and 2-nitrotriptycene; the second fraction contained pure 2-nitrotriptycene as shown by checking by TLC (thin layer chromatography) and HPLC. 2-Nitrotriptycene has a 280 nm/365 nm ratio of 10.6. The 365 nm band disappears upon reduction.

2-4-3. Results and Discussion

For nitro-PAH analysis, fraction 3 of cyclohexane extract was used. After washing with hot CYC and hot methanol sequentially , the combined washings were clarified and then blow down under high purity nitrogen.

Figure 11 gives a list of nitro-PAH standards used and their structures.

Use of nitro-PAH standards indicated that all of the mononitro-PAH are found in the cyclohexane extract fraction #3. This was the most mutagenic of the cyclohexane fractions both with and without activation. Some dinitro-PAH may be also found in this fraction. Fig.12a and Fig.12b show HPLC chromatograms of NBS SRM 1587 without and with reduction.

Quantitative conversion to highly fluorescent amino-PAH are achieved. Fig.13a and Fig.13b show corresponding chromatograms (without reduction and with reduction) for cyclohexane fraction #3 of NBS SRM 1650 diesel particulate.

The mutagenicities of selected nitro-PAH tested in this study by Dr. T. Atherholt (Institute for Medical Research) are shown in Table 3.

Table 3. Mutagenicities of Selected Nitro-PAH Tested in This Study (by Dr. T. Atherholt) in Revertants per nmol Using TA98.

	Mutants per nmol		
	-S9	+S9	TA98 NR
1-Nitropyrene	247	ND	20
2-Nitrofluoranthene	227	ND	40
3-Nitrofluoranthene	3310	ND	720
1-Nitropyren-2-ol	ND	19	16*
3-Nitroperylene	53	315	321*
6-Nitrobenzo(a)pyrene	ND	68	71*
9-Nitroanthracene	0.2	ND	0.02
2-Nitrofluorene	19	ND	4
9,10-Dinitroanthracene	0.5	.....	0.1
2-Nitrotriptycene	ND	ND	
2,6-Dinitrotriptycene + 2,7-Dinitrotriptycene	ND	ND	
2-Methyl-1-Nitro-anthraquinone	0.5	0.8	
6-Nitrochrysene	3.3	41	25*

* This is TA98 NR (+S9); others are TA98 NR (-S9).

Table 4 shows the data of nitro-PAH for 41 ambient air samples (from Winter, 1985 to Winter 1986) obtained by Faye Darack et al using the technique developed.

Table 4.

(pg/M^3)

Date	1NPY	2NFA	7NBA	9NA	2NFL	6N
01/31/85	190	420	62	NA		730
02/12/85	230	320	65	72		34
04/13	40	20	73	44		15
04/19	54	54	49	35		230
04/25	25	100	4.4	12		
05/07	45	45		160		39
05/13	130	160	51	110		120
05/19	300	150	70	78		41
05/25						6400
06/06	4600	2400	520	340		5100
06/12	11	11				
06/18	120	120	250			
06/24			9	20		
07/12	300	250		15		
07/24	2000	2000	9	43		66
07/30	320	2700	14	7.2		
08/11				330		
08/17	2200	1400	11			77
08/23	230	230	16			86
08/29	300	240	6			220
09/04	30	30		6.1		81
09/10	250	300	7.3			
09/16				7.7		
09/22			50			21
09/28	10	350	110			21
10/10	150	150		34		
10/16	600	600	82			
10/22			4.7	43	41.99	54
11/15					155.23	
11/21			8.1		597.39	610
11/27	30	2200	34	39		320
12/03	180	180		19		
12/09				3.7		
12/15	0	0		4.4	3316.20	
12/21			4.8			
01/02/86	140	140	125	4.7		530
01/08/86	300	300	4	11	76.79	
01/14			13	15	425.68	65
01/20	90	850	15	68	2150.70	
02/01	1000	1000	180	11		240
02/19	250	250	1.6	45	1263.27	13

Note: 1NPY: 1-Nitropyrene; 2NFA: 2-Nitrofluoranthene;

7NBA: 7-Nitrobenz(a)anthracene;

9NA: 9-Nitroanthracene;

2NFL: 2-Nitrofluorene;

6N: 6-Nitrobenzo(a)pyrene.

2-5. Analysis of PAH-quinones

2-5-1. High Performance Liquid Chromatography System

The same HPLC system was employed for reduction and analysis of PAH-quinones as that for nitro-PAH. Strong fluorescence signals were achieved from the reduction of PAH-quinones in the system. The linear gradient of 65% of aq. CH_3CN to 100% of CH_3CN in 35 mins was used.

2-5-2. Results and Discussion

Fraction #2 of the cyclohexane extract and fraction # 4 of the DCM extract were used for the identification of PAH-quinones. Figure 14 gives the names and structures of PAH-quinone standards employed in this study. Fig.15a shows an HPLC chromatogram without reducer column for 7 BaP-diones and 1 BeP-dione standards. Fig.15b shows the result of reduction in producing dihydroquinones that strongly fluoresce under the conditions employed. The results of the fraction #3 of cyclohexane extract and the fraction #4 of DCM extract of an ambient air sample (both with reducer and without reducer) are shown in Fig.16a, 16b and 17a, 17b respectively.

BaP-3,6-dione and BaP-7,8-dione were identified in both cyclohexane extract and DCM extract of the ambient air

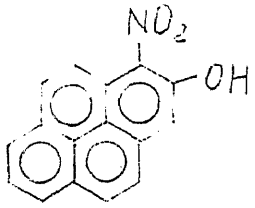
sample (from Newark, 09/27/84). About the same amount of these two compounds were present in the cyclohexane extract as that in DCM extract. More data are needed to find out the percentage of the distribution of PAH-quinones in these two extracts.

The identification of PAH-quinones from the ambient air samples is important because the oxidation of PAH to PAH-quinones may be a very significant disappearance pathway of PAHs.

2-6. Identification of One of The Nitrohydroxypyrene: 1-Nitro-2-hydroxypyrene

2-6-1. Synthesis of 1-nitro-2-hydroxypyrene

A published method was used to synthesize standard 1-nitro-2-hydroxypyrene [108]: 0.4 g 1-nitropyrene was dissolved in acetonitrile (600 ml) and the solution was irradiated with u.v. lamp for 5 hours with stirring. Reaction products were checked with thin-layer silica gel chromatography , where the solvent used was carbon tetrachloride. The spot with Rf value of 0.35 is 1-nitropyrene; the spot with Rf value of 0.53 is 1-nitro-2-hydroxypyrene. The 1-nitro-2-hydroxypyrene (2) was separated from the reaction mixture by column chromatography using silica gel (40-140 mesh), where elution solvent was carbon tetrachloride. After recrystallization from benzene, red needle-shaped crystals were obtained.



2

2-6-2. High Performance Liquid Chromatography System

The same HPLC system was employed as that for nitro-PAH except that no any reduction was used for the identification of 1-nitropyren-2-ol in the ambient air sample. Analysis was performed employing a linear solvent program of 65% - 100% aq. acetonitrile in 35 mins and monitoring using 365 nm, 546 nm and fluorescence (395 nm excit.; >460 nm emiss.) and subsequently using 546 nm alone. The peak for 1-nitropyren-2-ol was identified by its retention time and a 365 nm/546 nm ratio identical with those of the standard. Coinjection was also performed. No fluorescence peak was observed.

2-6-3. Results and Discussions

The substance 1-nitropyren-2-ol has been found to have following features:

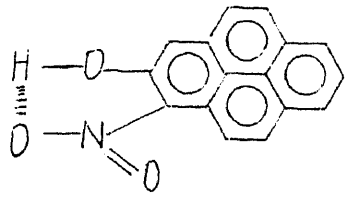
(1). indirect-acting mutagenicity;

(2). relative nonpolar (under DCM development it migratestoward the top of a silica gel plate);

(3). violet color;

These features suggest that intramolecular hydrogen

bonding (2a) is present and that if it is found in ambient air it should be found in cyclohexane fraction #3 or DCM #5.



2a

The cyclohexane extract was developed by the TLC method and the part of the plate corresponding to the migration of the standard solution of 1-nitropyren-2-ol scraped and the absorbent washed with cyclohexane followed by methanol with combination of the washings and concentration under nitrogen. Fig.18a shows the HPLC chromatogram, using detection at 546 nm, of the cyclohexane extract for a Newark, New Jersey August 28, 1984 high-volume 24-hour sample. Fig.18b shows the same sample spiked with a standard solution of 1-nitropyren-2-ol. Figure 18c is the chromatogram of 1-nitropyren-2-ol standard. Placement of a zinc/silica reduction column just prior to the detector caused the disappearance of the 546 nm absorption peak and the appearance of a very strong fluorescence peak consistent with the presence of nitro-PAH. The level found for the 8/28/84 sample was 1.5 ng/m^3. The concentration was found to be highly variable but comparable to benzo(a)pyrene levels and much higher than ambient concentrations of 1-

nitropyrene. The reported finding of this compound is at variance with the highly tentative results of Yasuhara and Morita [108b].

An urban dust sample (NBS Standard Reference Material #1649) and diesel particulate matter samples (NBS Standard Reference Material #1650) were also analyzed for 1-nitropyren-2-ol in their CYC extracts (Fig. 19). Figure 19 shows the TLC plate portion scraped for this compound. Figure 20 shows the chromatogram of the urban dust sample. No 1-nitropyren-2-ol was found. Figure 21a shows the chromatogram of the diesel particulate matter sample and Figure 21b shows the chromatogram of the diesel particulate matter sample spiked with 1-nitropyren-2-ol standard solution. The level of 1-nitropyren-2-ol found in the diesel particulate matter sample was, at most, 2.3 ug of 1-nitropyren-2-ol in 1 gram of diesel particulate matter.

1-Nitropyrene adsorbed to glass or teflon-coated filters or to particulate matter is stable when stored in the dark at ambient temperature [6]. It does not decomposed significantly when exposed in the dark to ambient air [32, 33]. When coated on glass plate and exposed outdoors in sunlight, about 72% remained after one month; the products did not include 3-nitropyren-1-ol, 6-nitropyren-1-ol, or 8-nitropyren-1-ol [6]. 1-Nitropyrene has also been reported to have a half-life of 1.2 days in dimethyl sulfoxide solution and 6 days when coated onto silica and exposed to light. Here, one of the decomposition products was reported

to be 8-nitropyren-1-ol [37]. None of these researchers appeared to look for 1-nitropyren-2-ol, whose nonpolar properties would place it in a different fraction from the other HNP. The known photochemical reactivity of 1-nitropyrene and the manner of synthesis of 1-nitropyren-2-ol suggest that this molecule may be present in the atmosphere. Our results are somewhat ambiguous. The observation of this compound in airborne particulates collected by high volume sampler (Figure 18a) seems to conflict with its absence in NBS SRM 1649 urban dust (Figure 20). Since the latter is collected in a baghouse, it could be the 1-nitropyren-2-ol is an artifact of high volume sampling. We note, however, its absence in the tentative results of Yasuhara and Morita [108b] although they did not include details of collection or separation of airborne particulates and extracts. Clearly, more work will have to be done in this area.

2-7. Environmental Transformation of 1-Nitropyrene on Quartz Filters

2-7-1. Background

The photochemistry of nitroaromatic compounds in solution has been the subject of many investigations [13, 18, 40, 108]. Dopp [18] details photochemical reactions of nitroaromatic compounds, which include hydrogen abstraction, photoreduction of the nitro group and nucleophilic aromatic photosubstitution. Chapman and coworkers [13] report photoisomerization of 9-

nitroanthracene to give the nitrite ester. Subsequent reactions produce anthraquinone by photo-oxidation, bianthrone by photodimerization and a nitrosoketone. Yasuhara and Fuwa [108a] reported the formation of 1-nitropyren-2-ol by irradiation of 1-nitropyrene with u.v. light. In the studies described above, compounds were dissolved in organic solvents, so results may not be applicable to the types of reactions that may be expected to take place in the atmosphere, where compounds exist as solids, often adsorbed to particles.

The following tests were done to provide more data for estimating the photochemical products of 1-nitropyrene.

2-7-2. Methods of Tests

1-Nitropyrene solution (approximate 0.1 mg/ml) was made by dissolving 1-nitropyrene in methanol. Previously exposed filters (12 cm x 10 cm, sampled as described in sec. 2-1) were used for the tests. In each test, two filters were needed. First, filter 1 was cut into two equal pieces and 1 ml of the above solution was applied to each piece respectively by spotting. Care was taken to apply the solution evenly. One of the spotted piece was wrapped in aluminum foil and kept at room temperature in the dark. The other piece was placed outside to exposed to the sunlight and all other environmental conditions for 14 days.

Secondly, filter 2 was cut and 1-nitropyrene solution applied in the same way as in filter 1. One of the spotted

piece was wrapped in aluminum foil and kept at room temperature in the dark. The other piece was exposed to u.v. light for 5 hours.

Filter 1	Piece 1	Kept at room temperature in the dark.	
	Piece 2	Exposed to the sunlight and all other environmental conditions.	
Filter 2	Piece 1	Kept at room temperature in the dark.	
	Piece 2	Exposed to u.v. light.	

After the exposure, the filters were extracted by cyclohexane and dichloromethane sequentially and the resulting extracts were separated on TLC plates respectively in the same way as described in sec. 2-1.

The fractions which could have 1-nitropyren-2-ol (as determined by standards) were scraped from the TLC plates (Figure 19) and the adsorbent washed with hot methanol by ultrasonic bath. These fractions were then analyzed using HPLC.

2-7-3. High Performance Liquid Chromatography System

The same HPLC system was employed as that for 1-nitropyren-2-ol with a solvent program of 65% - 100% aq. acetonitrile in 35 mins and monitoring wavelengths at 365nm and 546nm.

2-7-4. Results and Discussions

Breakdown to compounds of higher polarity than 1-

nitropyrene occurred after 1-nitropyrene was exposed to sunlight and air for 14 days or u.v. light alone for 5 hours.

Significant decomposition (about 70%) of 1-nitropyrene was found in sunlight and air, but no evidence indicated that 1-nitropyren-2-ol was one of the breakdown products (Figure 22a and 22b). Since our TLC test (moble phase: 50% Toluene,50% Hexane) shows that 3-nitropyren-1-ol, 6-nitropyren-1-ol and 8-nitropyren-1-ol do not migrate, these three compounds were not in the fraction scraped as shown in Fig.19. Therefore, these three compounds were not breakdown products found in this fraction. It is possible that the products are some of the PAH-quinones or PAH-ketones because the retention times of those compounds are quite similar at the same conditions (Figure 23a and 23b).

No significant decrease in 1-nitropyrene concentration was found after the exposure to u.v. light for 5 hours, but there were some more polar compounds produced (Figure 24a and 24b). Still, there was no evidence of 1-nitro-2-pyrenol being produced. It seems that the breakdown products are similar to those found in sunlight.

In a similar test, J.M. Benson et al [16] investigated the photochemical/chemical transformation of solid 1-nitropyrene on glass surfaces in natural light. They reported that the breakdown of 1-nitropyrene to 1-nitro-2-pyrenol did not occur and the breakdown appeared to have resulted in loss of the nitro group with formation of

hydroxypyrene, possibly pyrene dione and dihydroxypyrene. They also found that the mutagenic activities of breakdown products were significantly less than that of 1-nitropyrene, probably because of the loss of the nitro group.

P.C. Howard et al [37] in a recent test exposed several nitroarenes and their parent PAHs to light > 310 nm, at 0.6 Watt/cm^2 either dissolved in DMSO (dimethyl sulfoxide) or coated onto glass or silica. Their results showed that 1-nitropyrene had half-lifes of 1.2 and 6 days when dissolved in DMSO or coated onto silica and exposed to light. The photodecomposition products were found to co-migrate on HPLC with phenolic and quinone derivatives of the parent PAHs, and with 1,8-dinitropyrene one of the principal photodecomposition products was found to 1-nitropyren-8-ol. they indicated that these compounds could arise not only from oxidation of a nitro-PAH, but could also result from photodecomposition of dinitro-PAH.

Results of our studies seem to support the idea that mutagenic 1-nitropyrene is broken down in sunlight and air to several compounds that may be less mutagenic than 1-nitropyrene and possibly less hazardous to man. Further work is needed to identify those breakdown products.

CHAPTER III : Conclusions

To identify nitro-PAH in ambient air sample, a technique was developed involving introduction of a reducer column into an reverse-phase HPLC system. Strong fluorescence signals were achieved from nitro-PAH and detected together with u.v. signals for the analysis of nitro-PAH. 2-Nitrotriptycene was synthesized and used as an internal standard. By use of the above technique, 9-nitroanthracene, 1-nitropyrene, 7-nitrobenz(a)anthracene, 6-nitrochrysene and 6-nitrobenzo(a)pyrene were detected in NBS diesel particulate sample and the data for 41 ambient air samples were reported.

In this study, an analytical method for the identification of PAH-quinones in airborne particulates was also developed. 3,6-BaP-dione and 7,8-BaP-dione were identified in both CYC extract and DCM extract of an ambient air sample by using the analytical method developed.

Since the hydroxynitropyrenes have been identified as making a significant contribution to the mutagenicity of airborne particulate matter, this prompted us to investigate one such compound, 1-nitropyren-2-ol. This compound was synthesized according to the published procedures. 1-Nitropyren-2-ol was detected in one of the ambient air sample and in an NBS diesel particulate sample by useing our reverse-phase HPLC system monitoring at 546 nm.

To provide more data for estimating the photochemical

products of 1-nitropyrene, the filters coated with 1-nitropyrene were exposed either to sunlight or to u.v. light. The breakdown products were analyzed using HPLC. No 1-nitropyren-2-ol was found in the filters exposed in both ways. Further work is needed to identify the breakdown products.

APPENDIX A: FIGURES

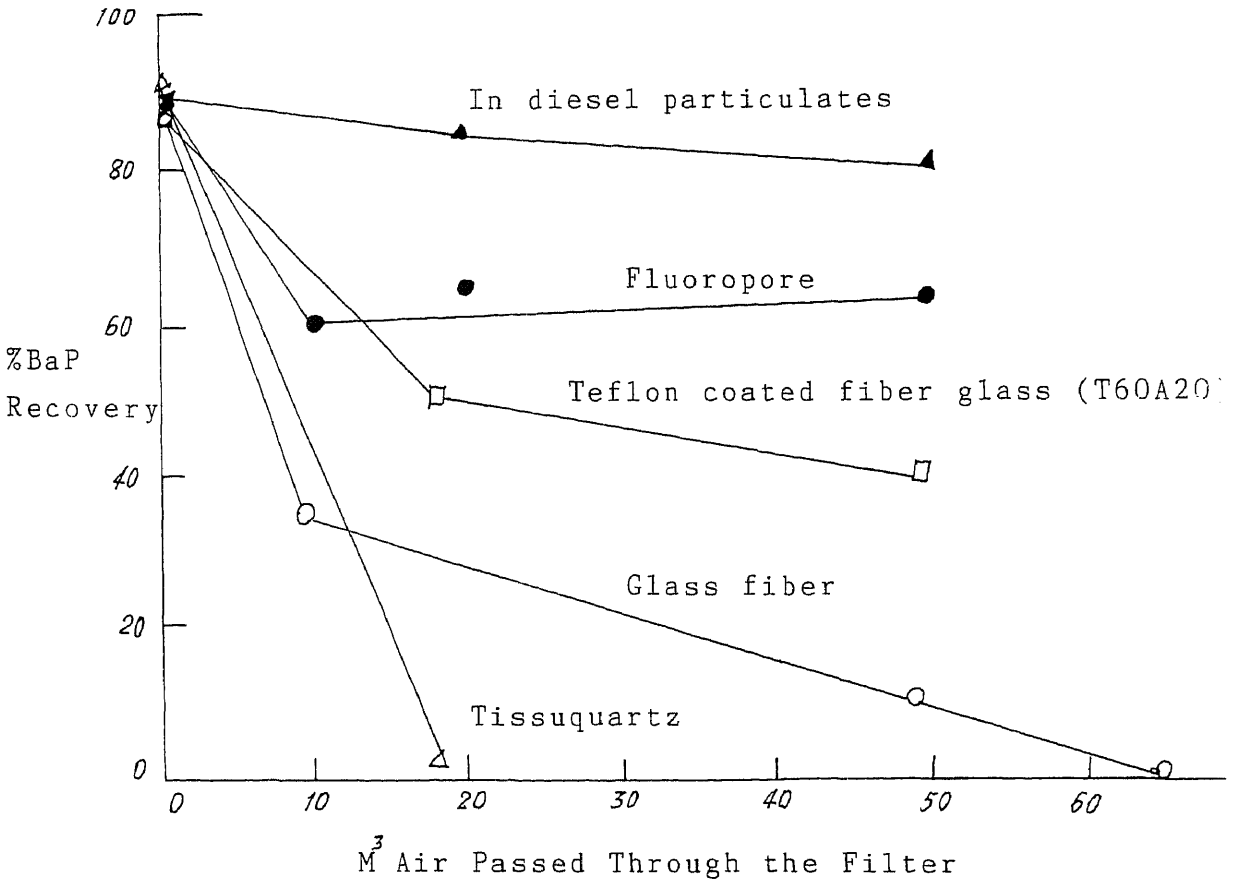


Fig.1. Recovery of BaP during various conditions of ambie air sampling for 27 ppm of BaP in diesel particula on a T60A20 filter and for 500 ug of BaP spiked on various filter media (Ref.52).

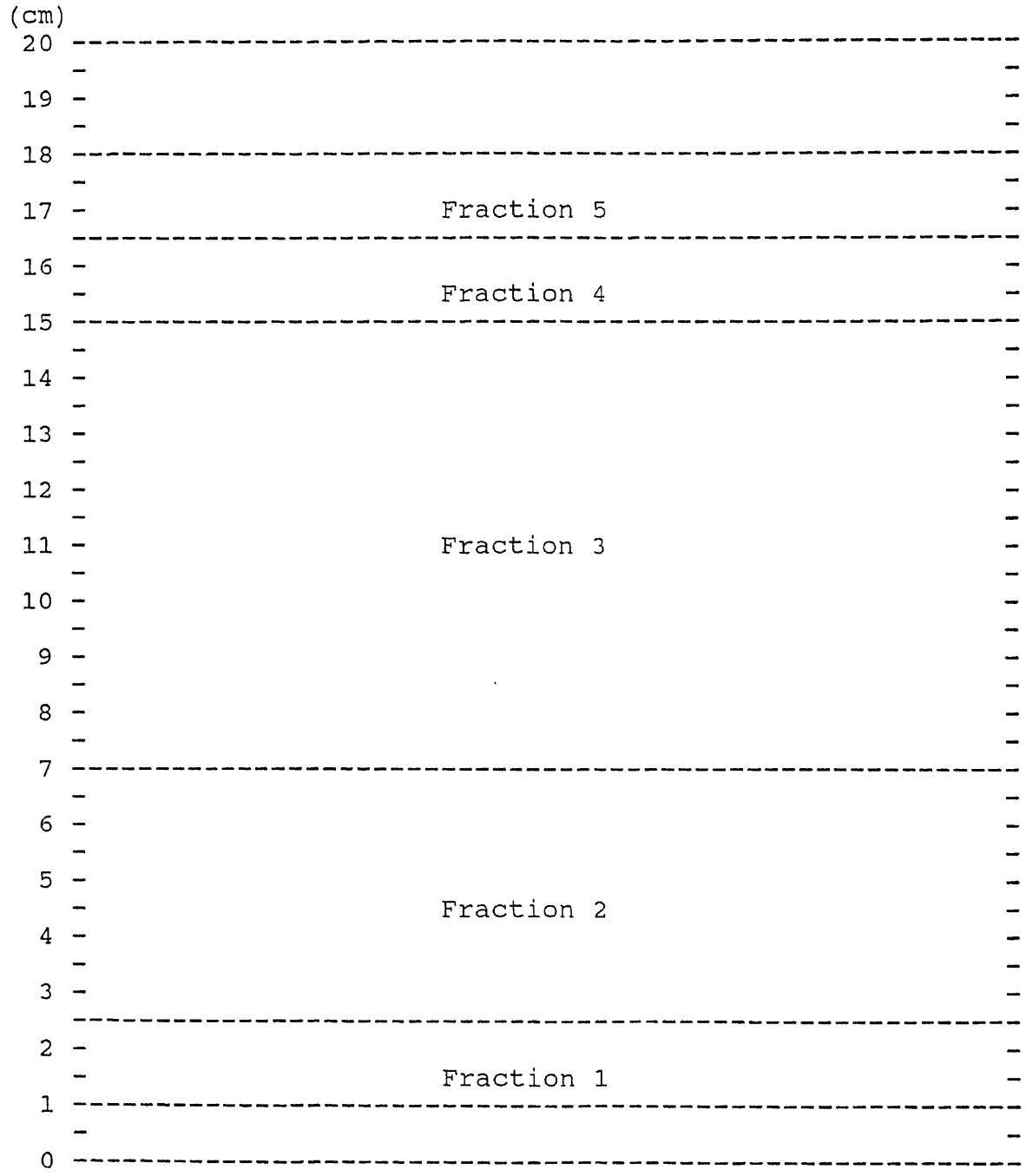


Fig.2. The positions of each fraction of CYC extract on TLC plate (Composite sample taken during Fall,1984).

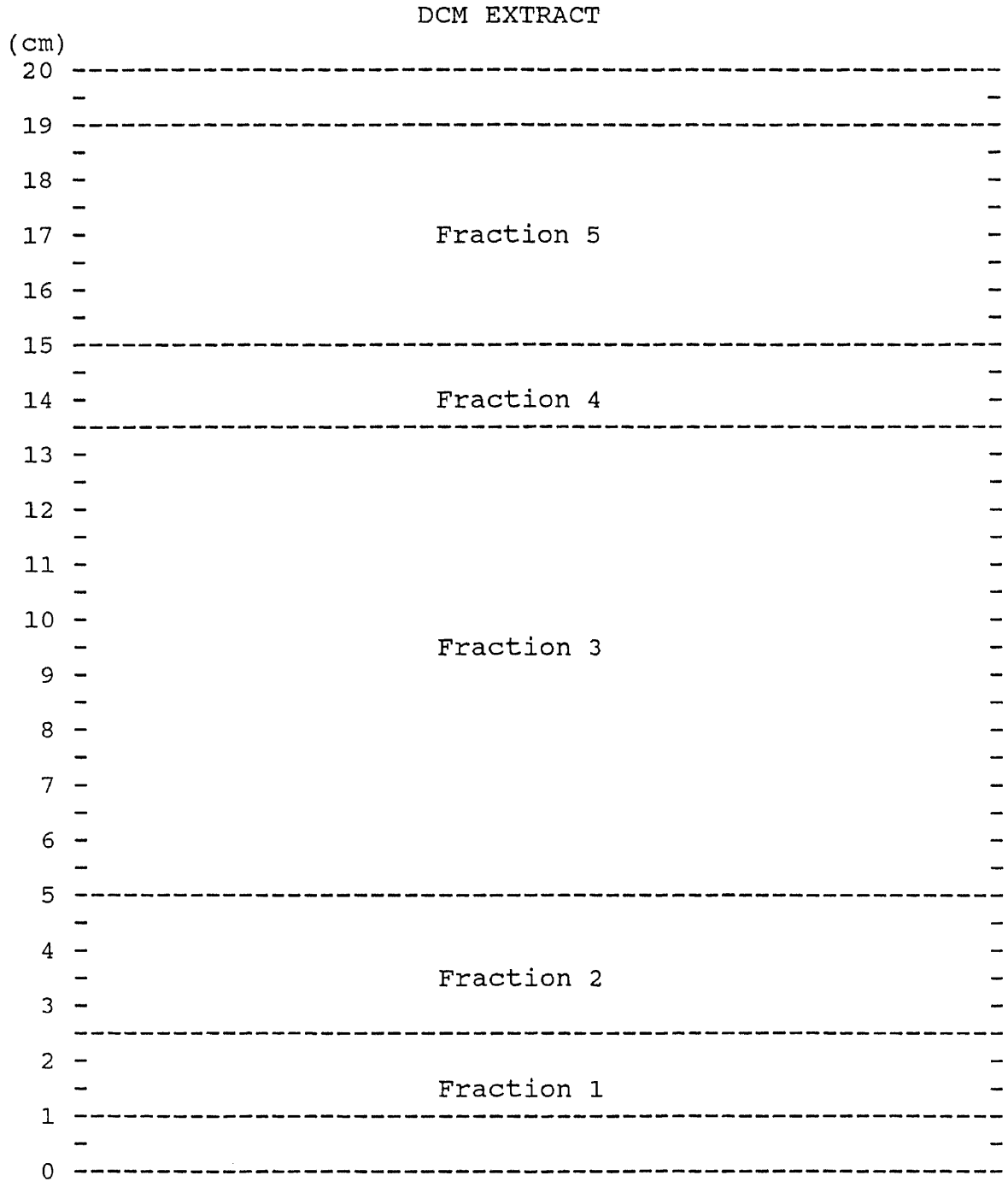


Fig.3. The positions of each fraction of DCM extract on TLC plate (Composite sample taken during Fall,1984).

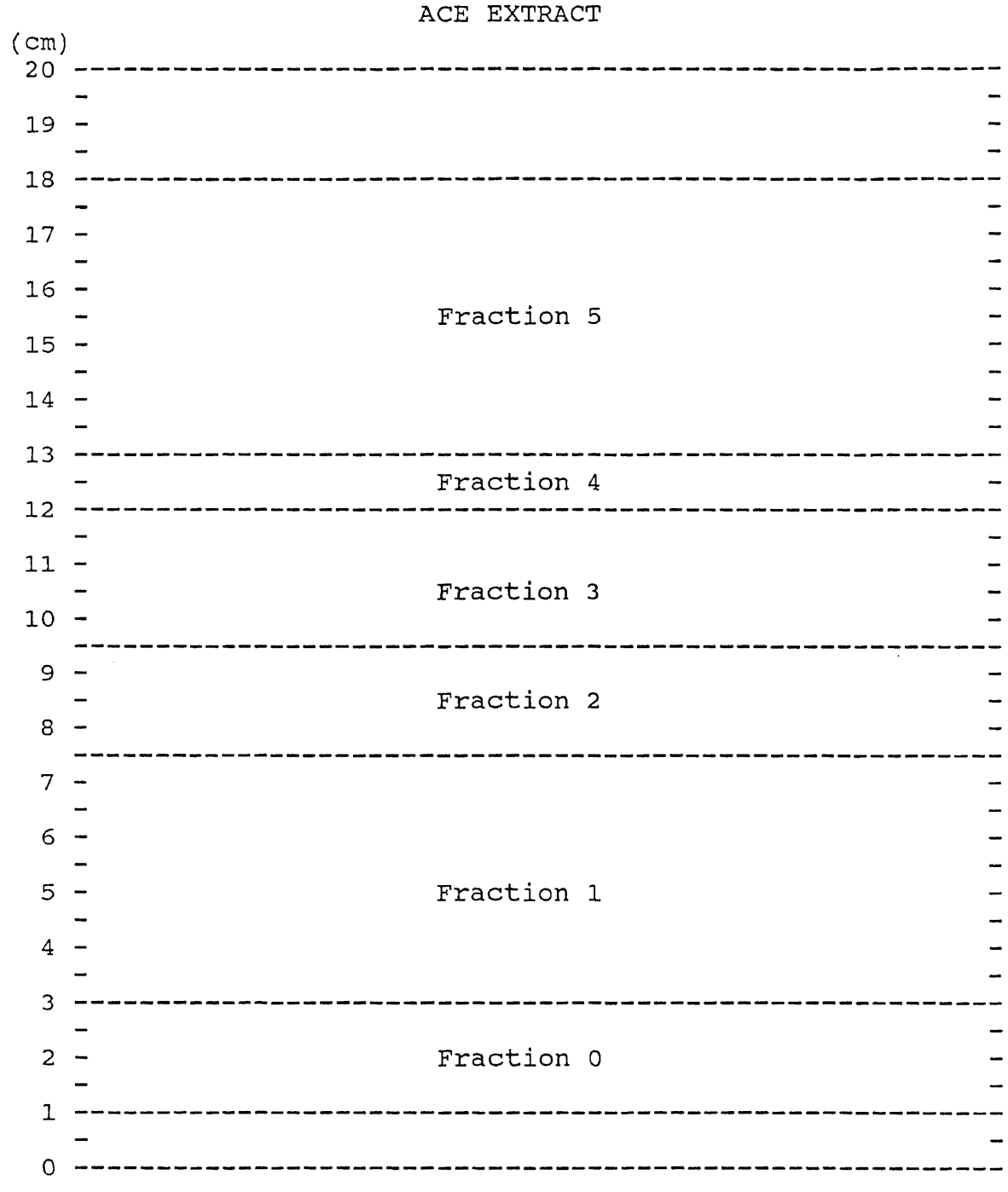


Fig.4. The positions of each fraction of ACE extract on TLC plate (Composite sample taken during Fall,1984).

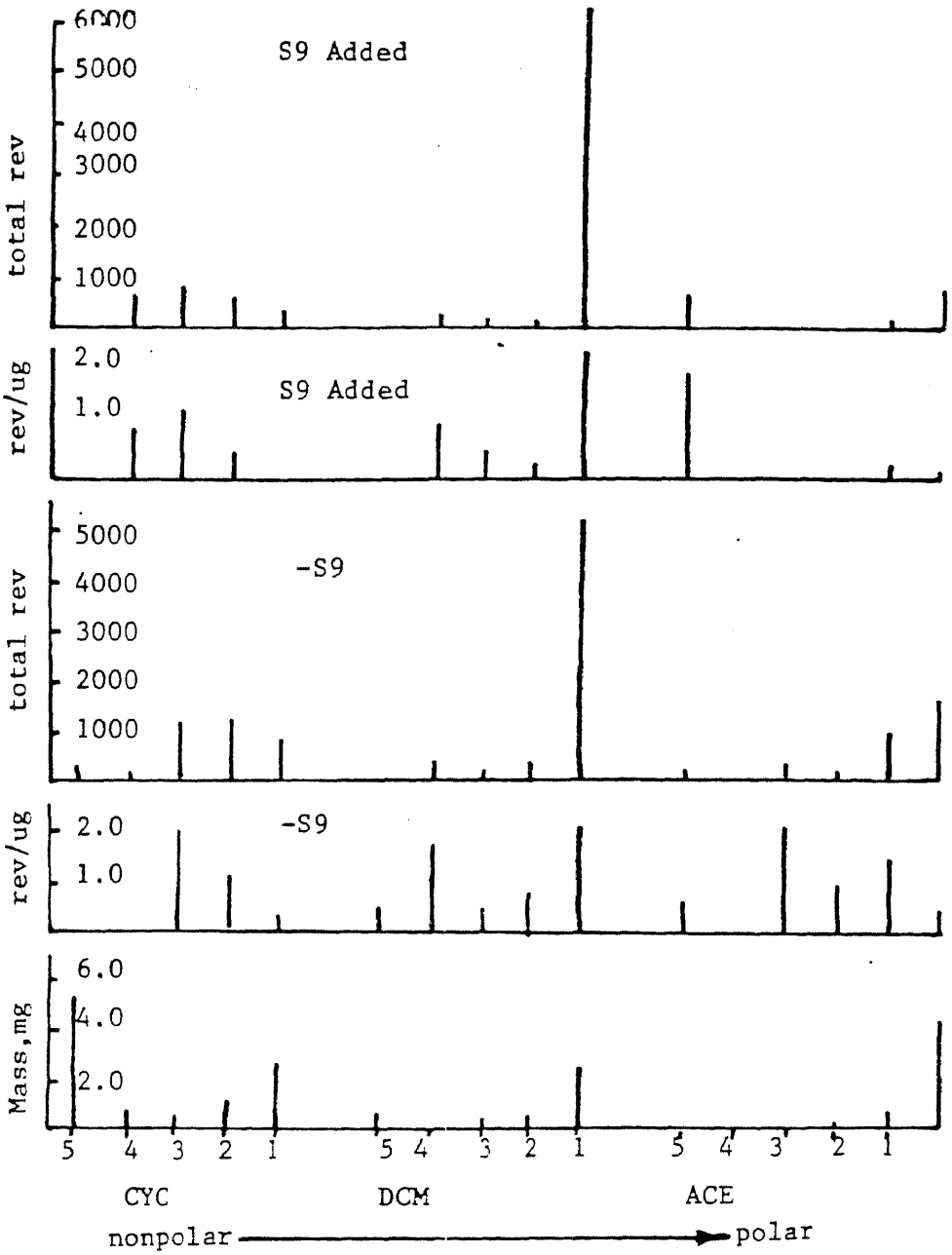


Fig.5. TA98 assays on 16 fractions from ambient airborne particulate (October-December, 1984).

Sample	±S9	Doses	Rev/ug ± 95% C.I.	r^2
Unfractionated	-S9	5	0.69±0.13	.94
	+S9	5	0.32±0.06	.95
Fraction 0	-S9	4	0.64±0.12	.95
	+S9	5	0.81±0.20	.90
Fraction 1	-S9	5	0.43±0.13	.86
	+S9	5	0.62±0.16	.90
Fraction 2	-S9	5	0.59±0.15	.89
	+S9	5	1.10±0.17	.86
Fraction 3-4	-S9	5	0.71±0.13	.94
	+S9	5	1.32±0.25	.94
Fraction 5	-S9	5	0.62±0.15	.90
	+S9	5	1.16±0.24	.93
Fraction 6	-S9	5	0.19±0.04	.93
	+S9	5	0.30±0.06	.94

Fig.6. TA98 assays on 7 fractions from CYC extract composite (January-May, 1985).

Sample	±S9	Dose	Rev/ug±95% C.I.
Unfractionated	-S9	6	2.56±0.23
	+S9	6	1.81±0.09
Fraction 0	-S9	5	4.05±0.33
	+S9	5	2.81±0.17
Fraction 1	-S9	4	9.13±1.86
	+S9	5	4.07±0.44
Fraction 2	-S9	5	0.78±0.17
	+S9	4	0.44±0.33
Fraction 3	-S9	5	0.59±0.09
	+S9	5	0.66±0.08
Fraction 4	-S9	3	32.93±9.28
	+S9	4	13.22±1.80
Fraction 5	-S9	5	2.79±0.39
	+S9	5	1.17±0.22

Fig.7. TA98 assays on 7 fractions from DCM extract composite (January-June, 1985).

Sample	±S9	Doses	Rev/ug±95% C.I.	r
Unfractionated	-S9	5	1.18±0.14	.
	+S9	6	0.53±0.05	.
Fraction 1	-S9	6	0.69±0.06	.
	+S9	6	0.47±0.04	.
Fraction 2	-S9	4	3.18±0.37	.
	+S9	5	1.96±0.21	.
Fraction 3-4	-S9	5	0.39±0.13	.
	+S9	5	negative	
Fraction 5	-S9	4	0.27±0.20	.
	+S9	5	negative	
Fraction 6-7	-S9	5	0.20±0.11	.
	+S9	6	0.20±0.05	.
Fraction 8	-S9	5	0.32±0.10	.
	+S9	4	0.38±0.27	.

Fig.8. TA98 assays on 7 fractions from ACE extract composit (January-May, 1985).

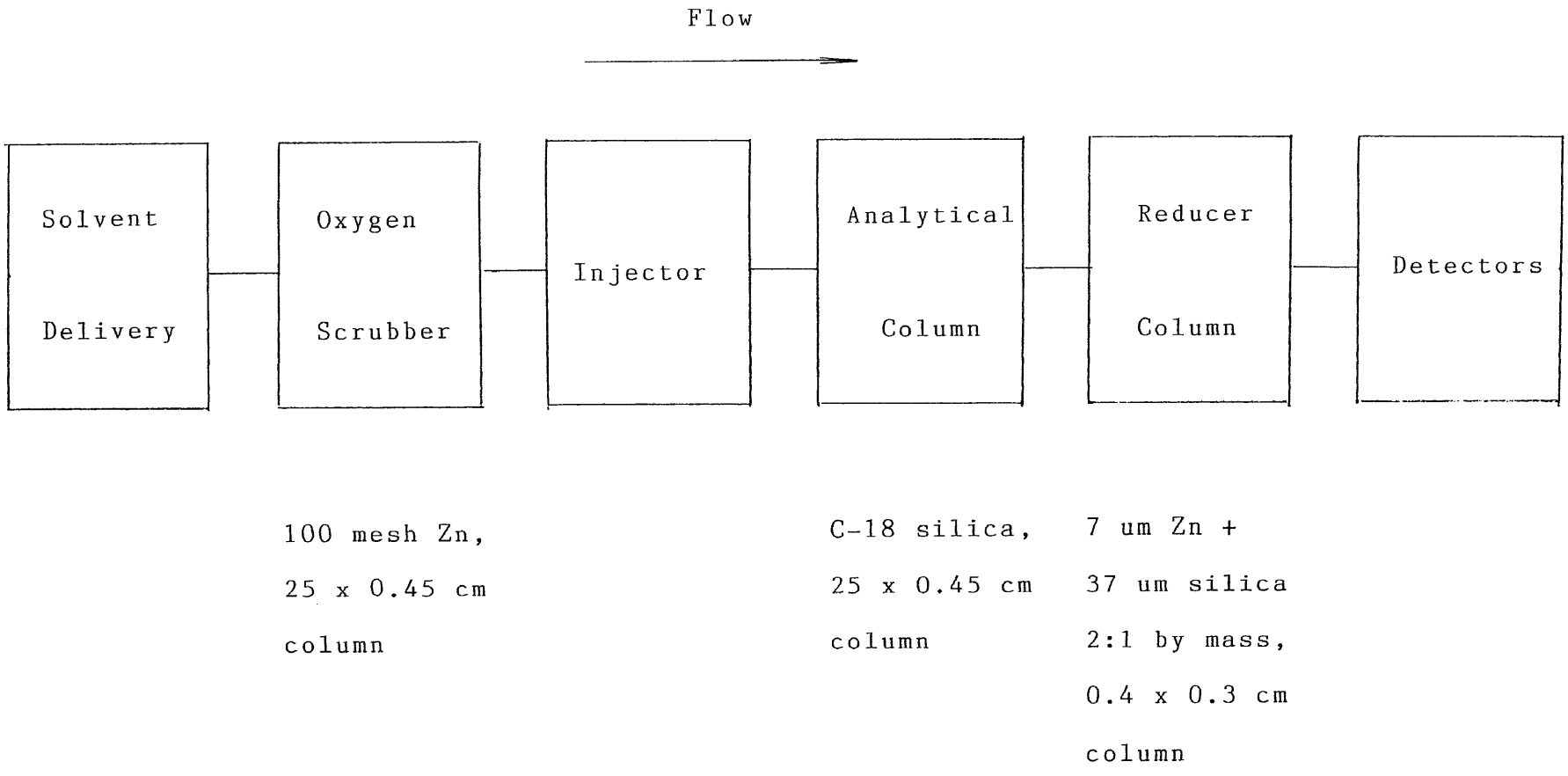


Fig.9. The flowchart of the HPLC system for nitro-PAH analysis.

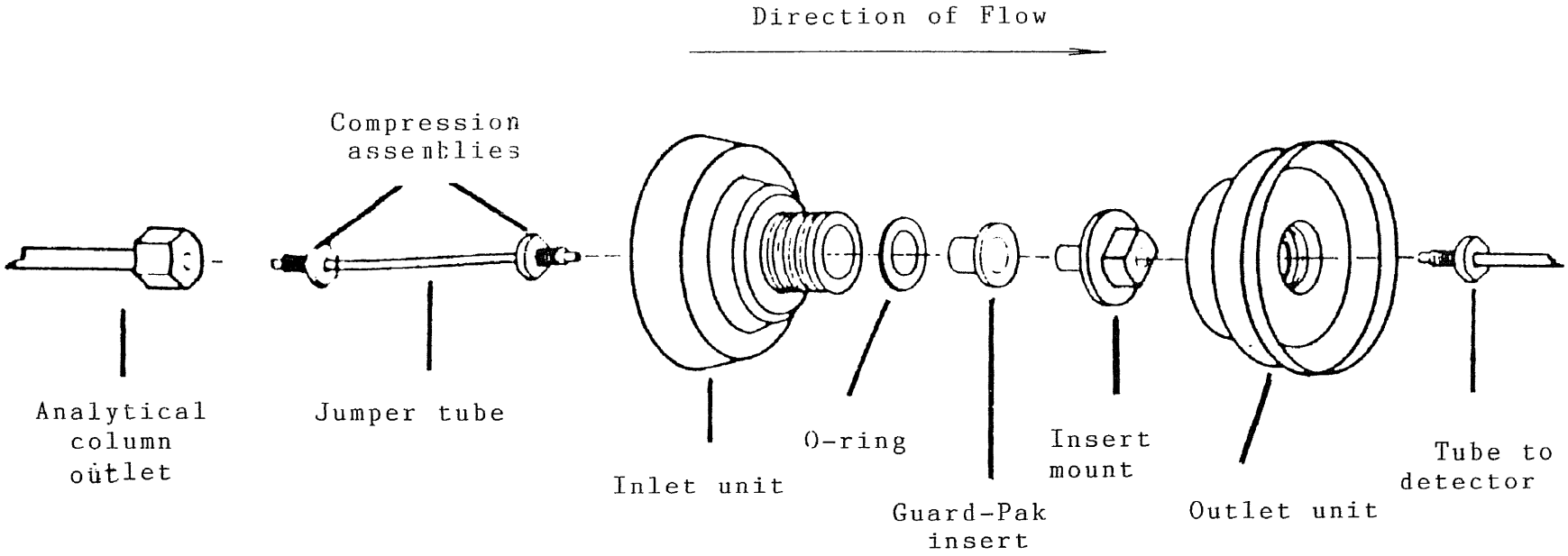


Fig.10a. The structure of the reducer column.

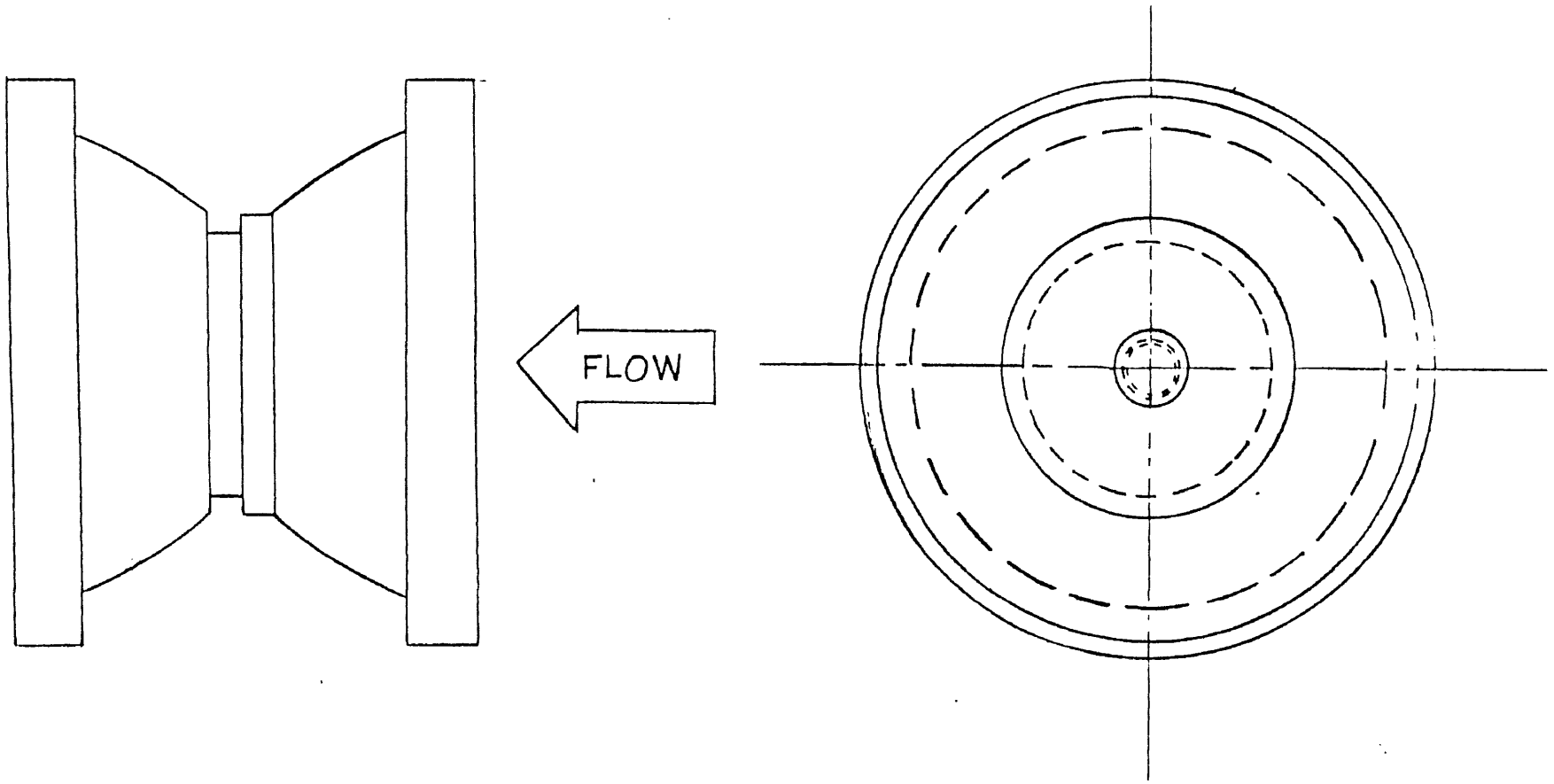
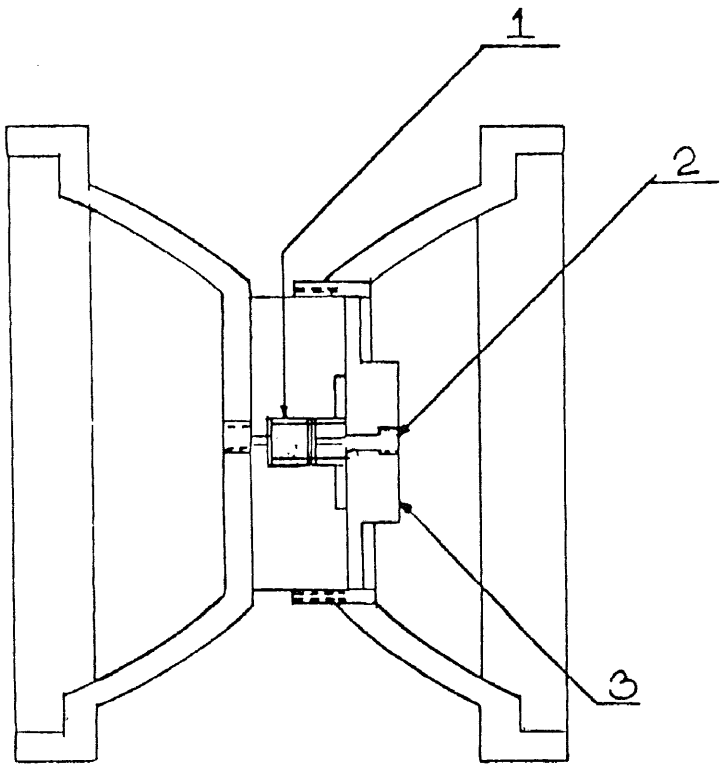


Fig. 10b. The structure of Guard-Pak precolumn module P/N 080040.

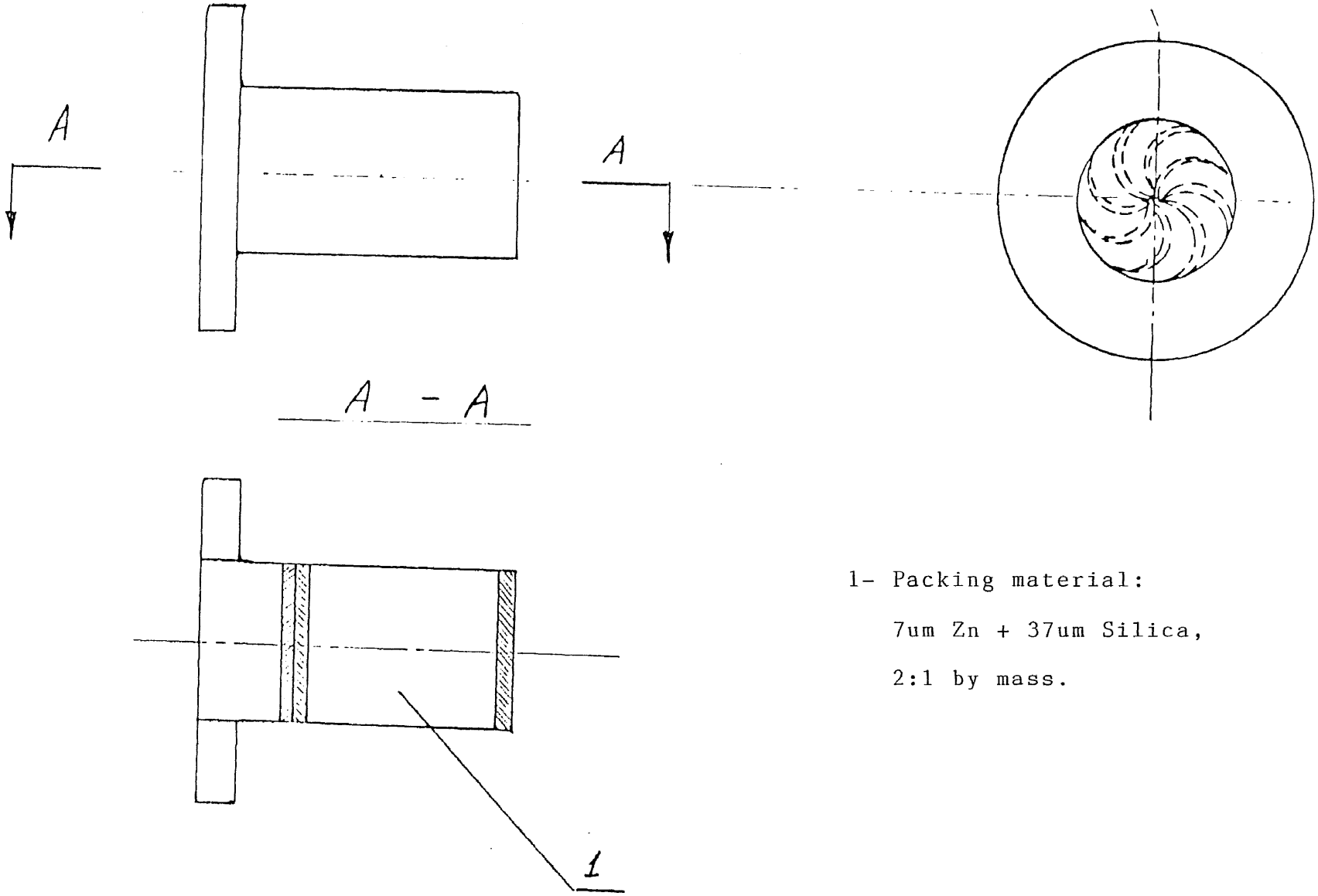


1- Modified Guard-Pak

2- Waters 1/16" Tubing Fitting

3- Steel Compression Fitting

Fig. 10c. The structure of the reducer column.

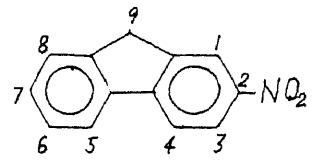


1- Packing material:

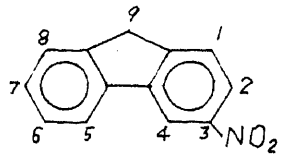
7um Zn + 37um Silica,

2:1 by mass.

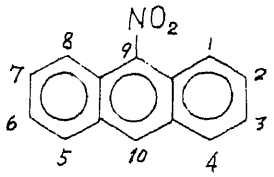
Fig. 10d. The structure of Guard-Pak insert.



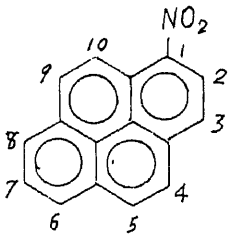
2-nitrofluorene



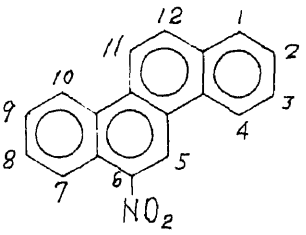
3-nitrofluorene



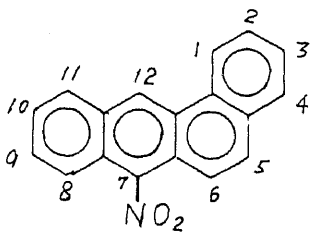
9-nitroanthracene



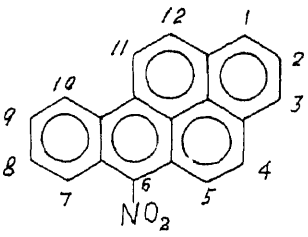
1-nitropyrene



6-nitrochrysene



7-nitrobenz(a)anthracene



6-nitrobenzo(a)pyrene

Fig.11. The names and structures of nitro-PAH standards used in this work.

Vydac 201 TP54

60% aq CH_3CN to 100% CH_3CN

35 min linear gradient

1 : 2-Nitrofluorene
2 : 9-Nitroanthracene
3 : 3-Nitrofluor.
4 : 1-Nitropyrene
5 : 7-NitroB(a)A
6 : 6-Nitrochrysene
7 : 6-NitroB(a)P

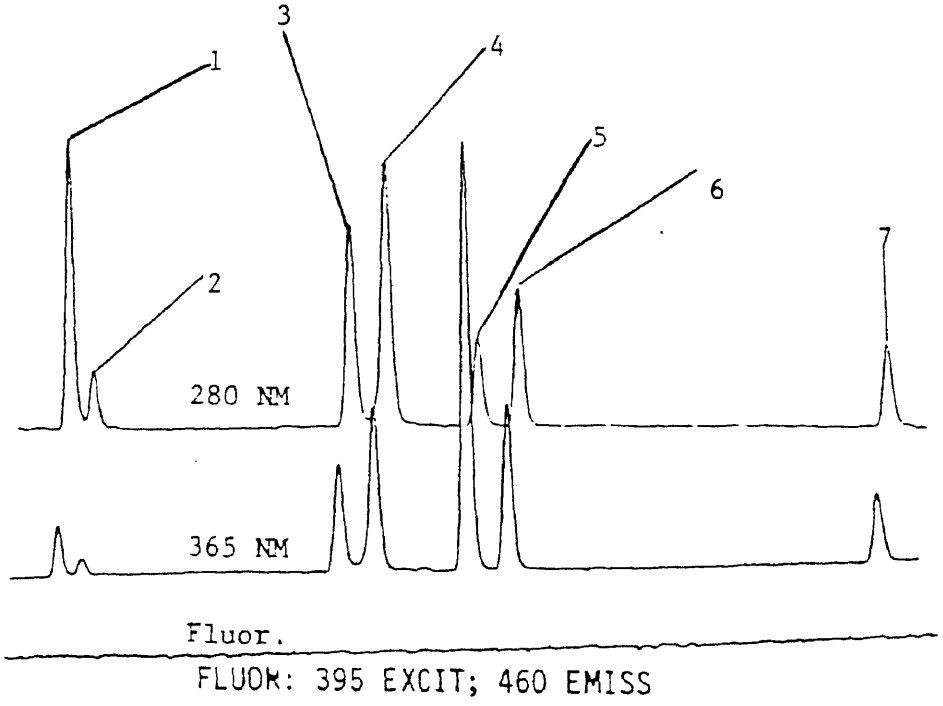


Fig. 12a. The HPLC chromatogram of NBS SRM #1587 without reduction.

1 : 2-Nitrofluorene
2 : 9-Nitroanthracene
3 : 3-Nitrofluorene
4 : 1-Nitropyrene
5 : 7-Nitro B(a)A
6 : 6-Nitrochrysene
7 : 6-Nitro B(a)P

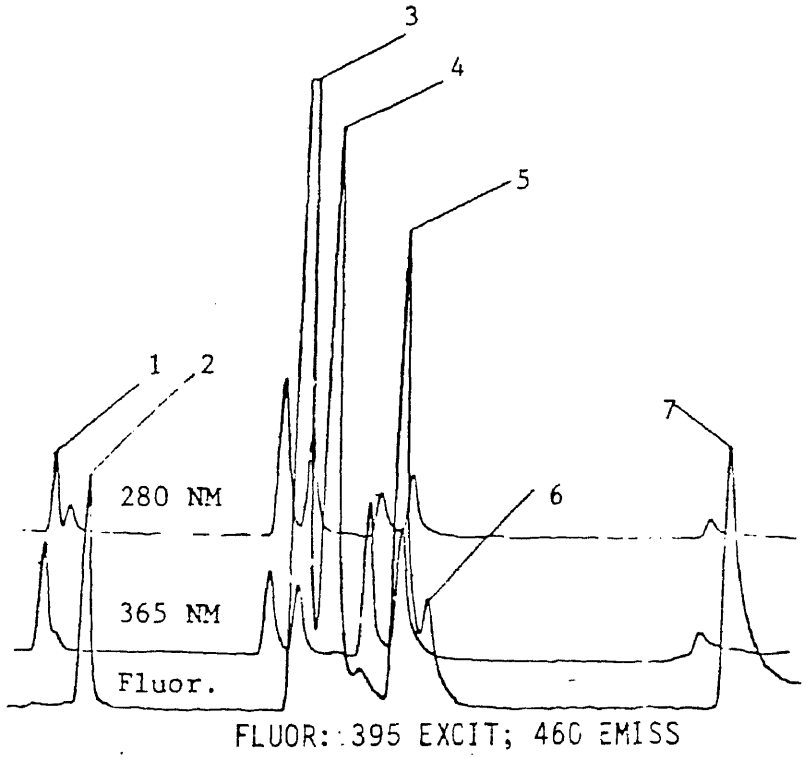


Fig. 12b. The HPLC chromatogram of NBS SRM #1587 with reduction.

1 : 9-Nitroanthracene
2 : 1-Nitropyrene
3 : 7-Nitro B(a)A
4 : 6-Nitrochrysene
5 : 6-NitroB(a)P

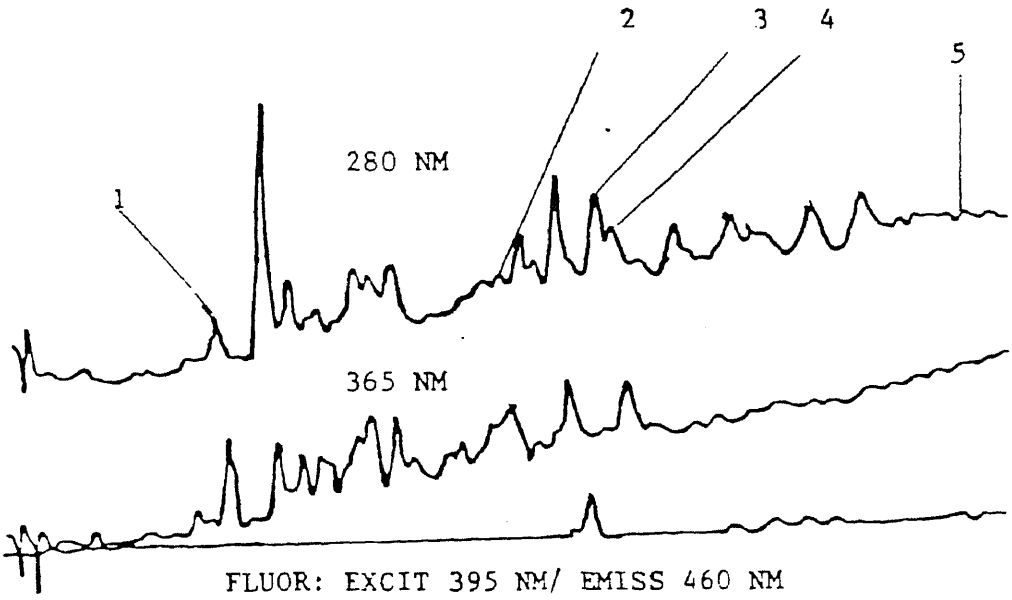
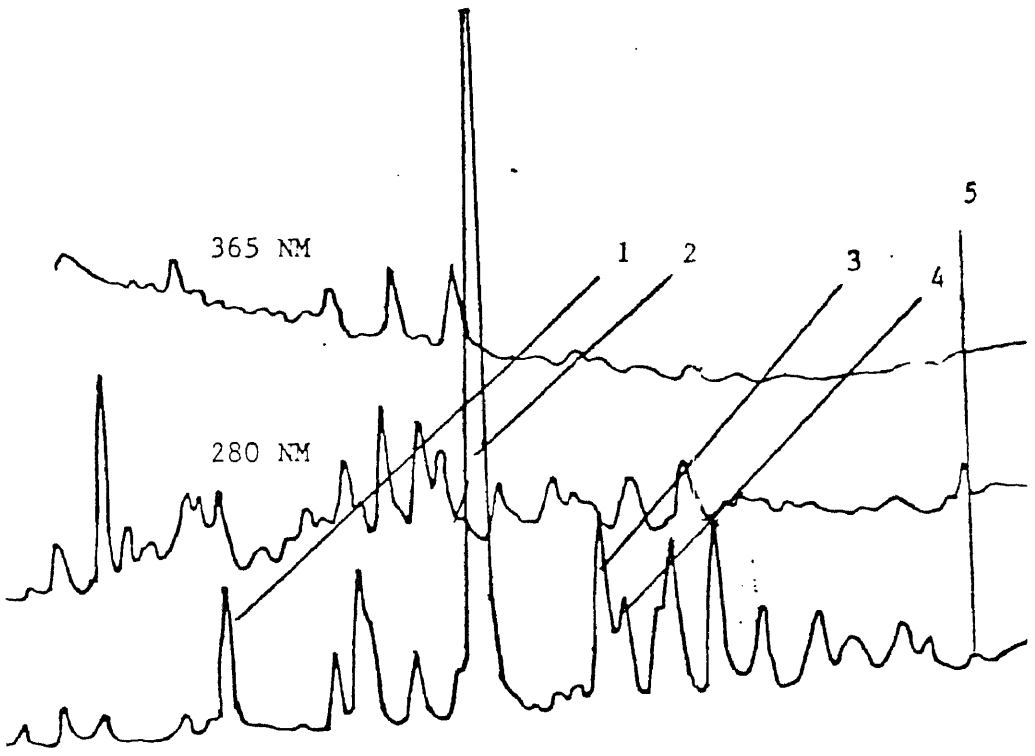


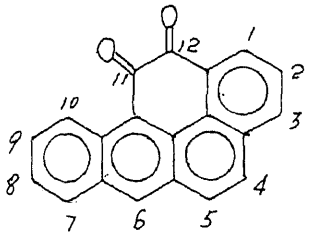
Fig. 13a. The HPLC chromatogram for CYC fraction #3 of NBS SRM #1650 diesel particulate without reduction.

1 : 9-Nitroant
2 : 1-Nitropyrene
3 : 7-Nitro B(a)A
4 : 6-Nitrochrysene
5 : 6-Nitro B(a)P

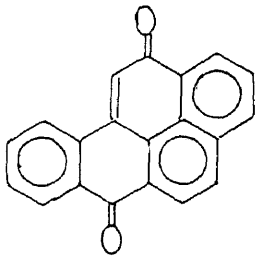


FLUOR : EXCIT 395 NM/ EMISS 460 NM

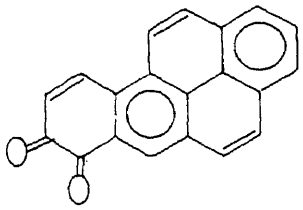
Fig. 13b. The HPLC chromatogram for CYC fraction #3 of NBS SRM #1650 diesel particulate with reduction.



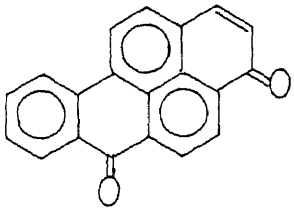
11,12-BaP-dione



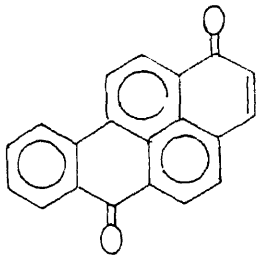
6,12-BaP-dione



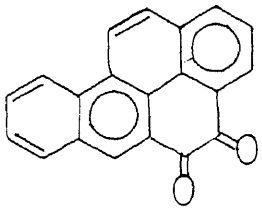
7,8-BaP-dione



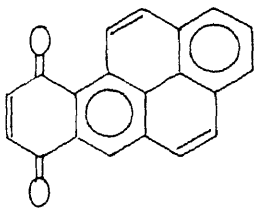
3,6-BaP-dione



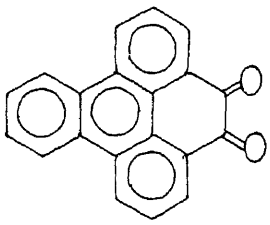
1,6-BaP-dione



4,5-BaP-dione



7,10-BaP-dione



4,5-BeP-dione

Fig.14. The names and structures of PAH-quinone standards employed in this

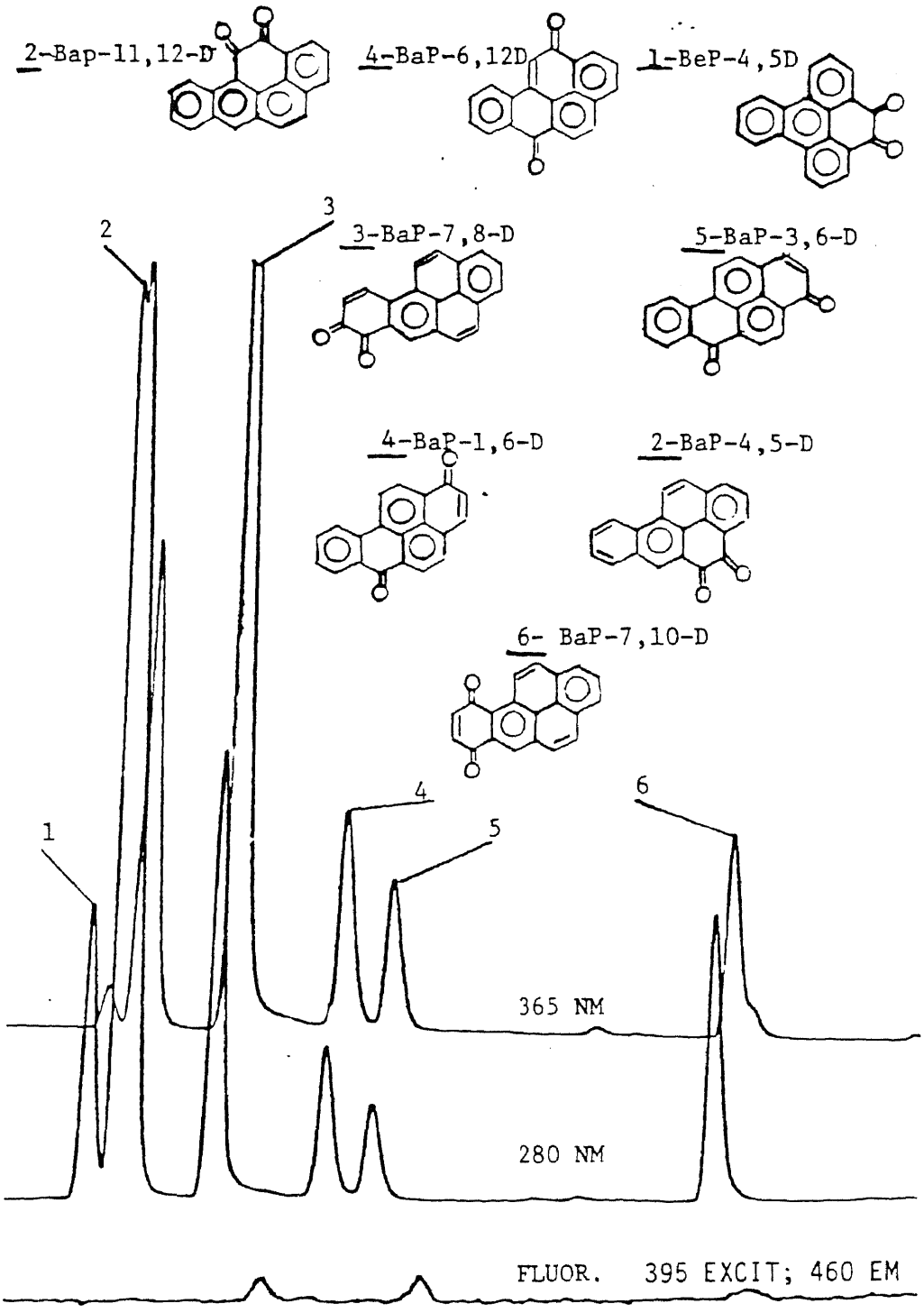


Fig. 15a. The HPLC chromatogram for 7 BaP-diones and 1 BeP-dione standards without reduction.

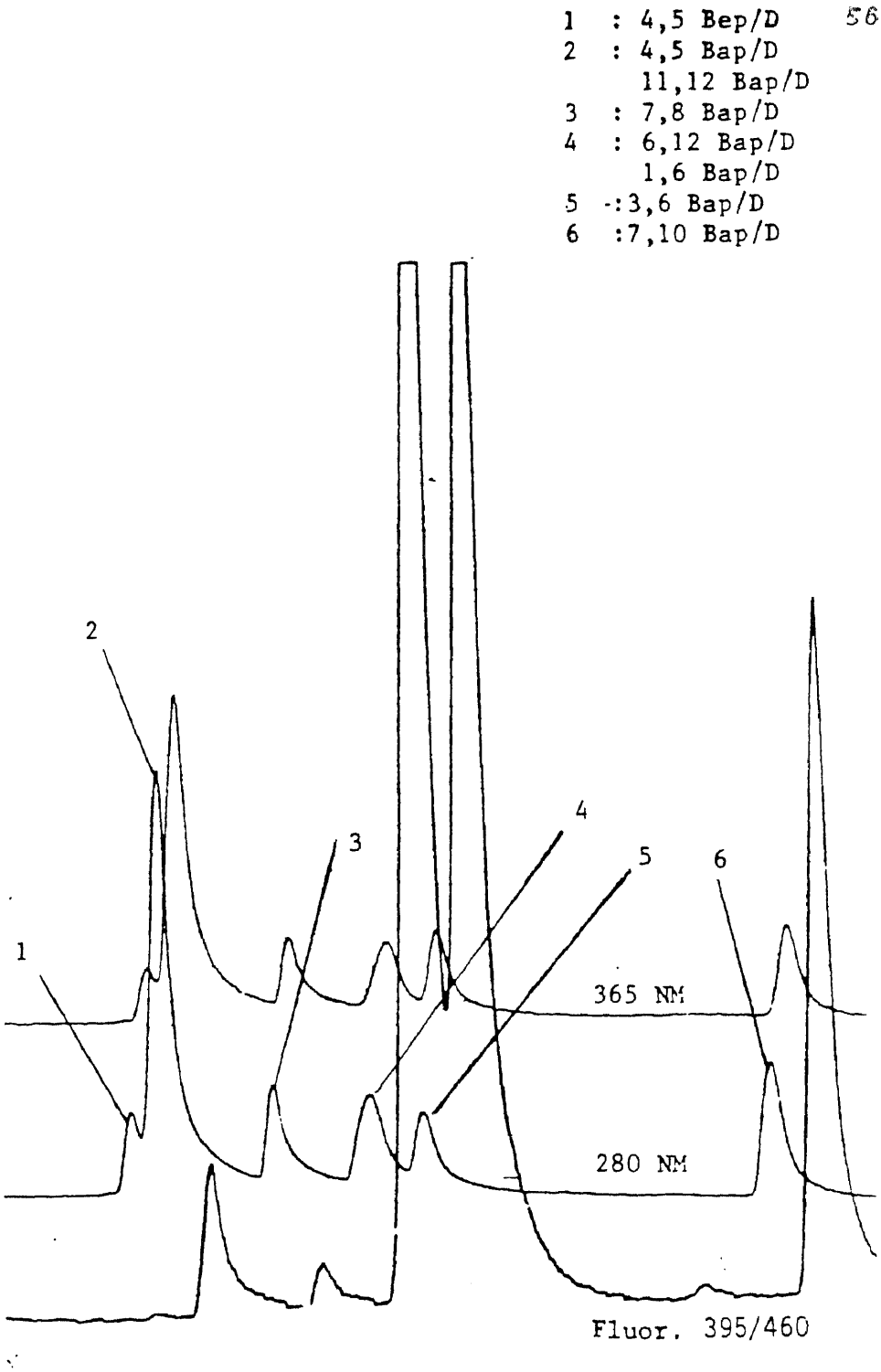


Fig. 15b. The HPLC chromatogram for 7 BaP-diones and 1 BeP-dione standards with reduction.

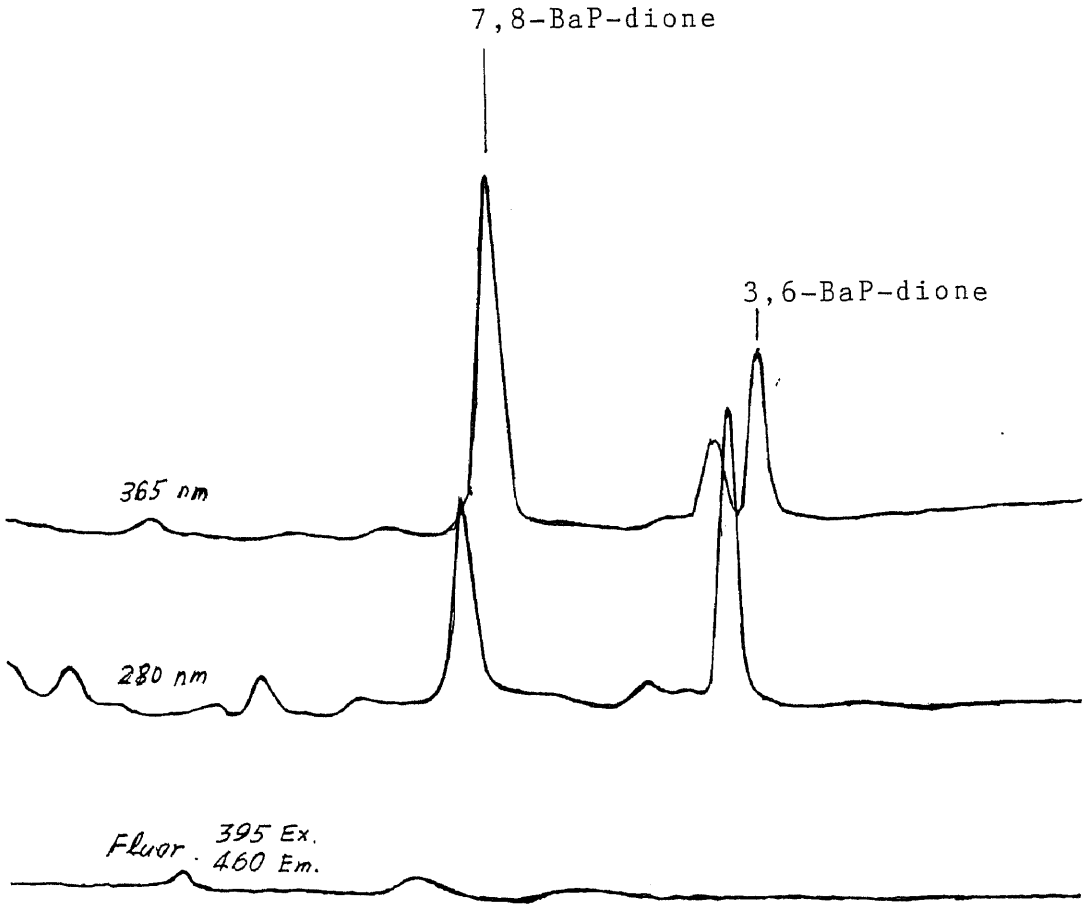


Fig.16a. The HPLC chromatogram for fraction #3 of CYC extract of an ambient air sample (Newark, 09/27/84) without reduction.

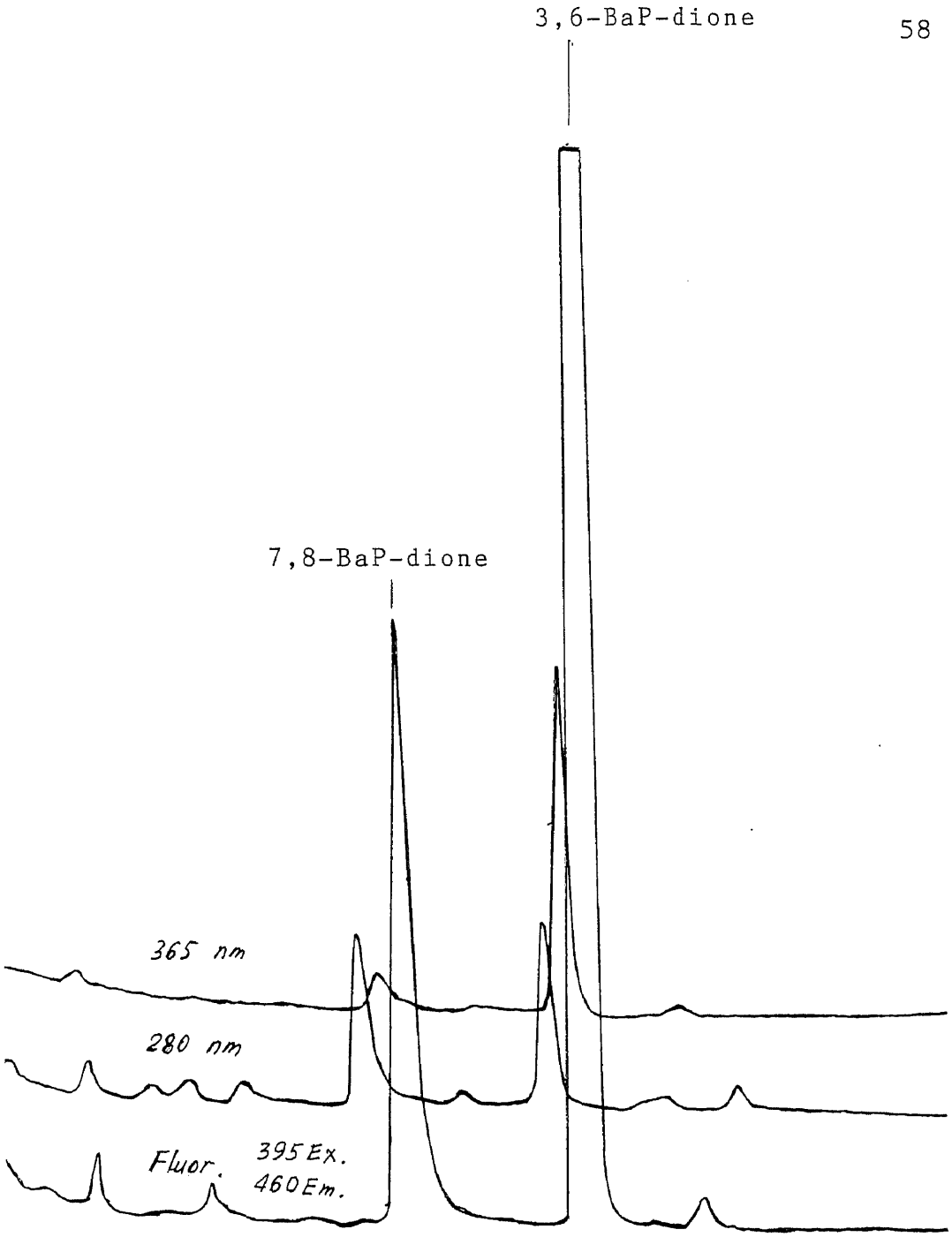


Fig.16b. The HPLC chromatogram for fraction #3 of CYC extract of an ambient air sample (Newark, 09/27/84) with reduction.

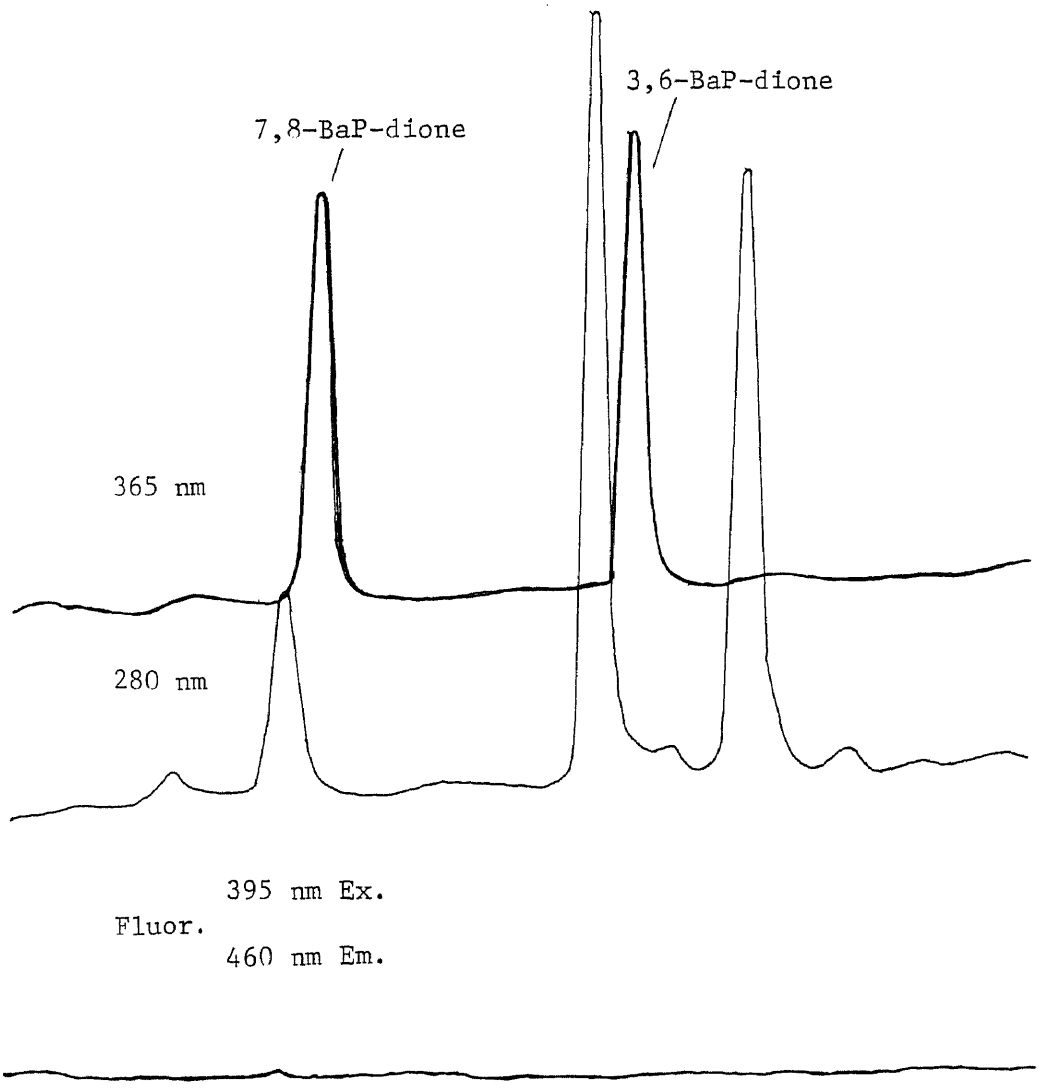


Fig.17a. The HPLC chromatogram for fraction #4 of DCM extract of an ambient air sample (Newark, 09/27/84) without reductio

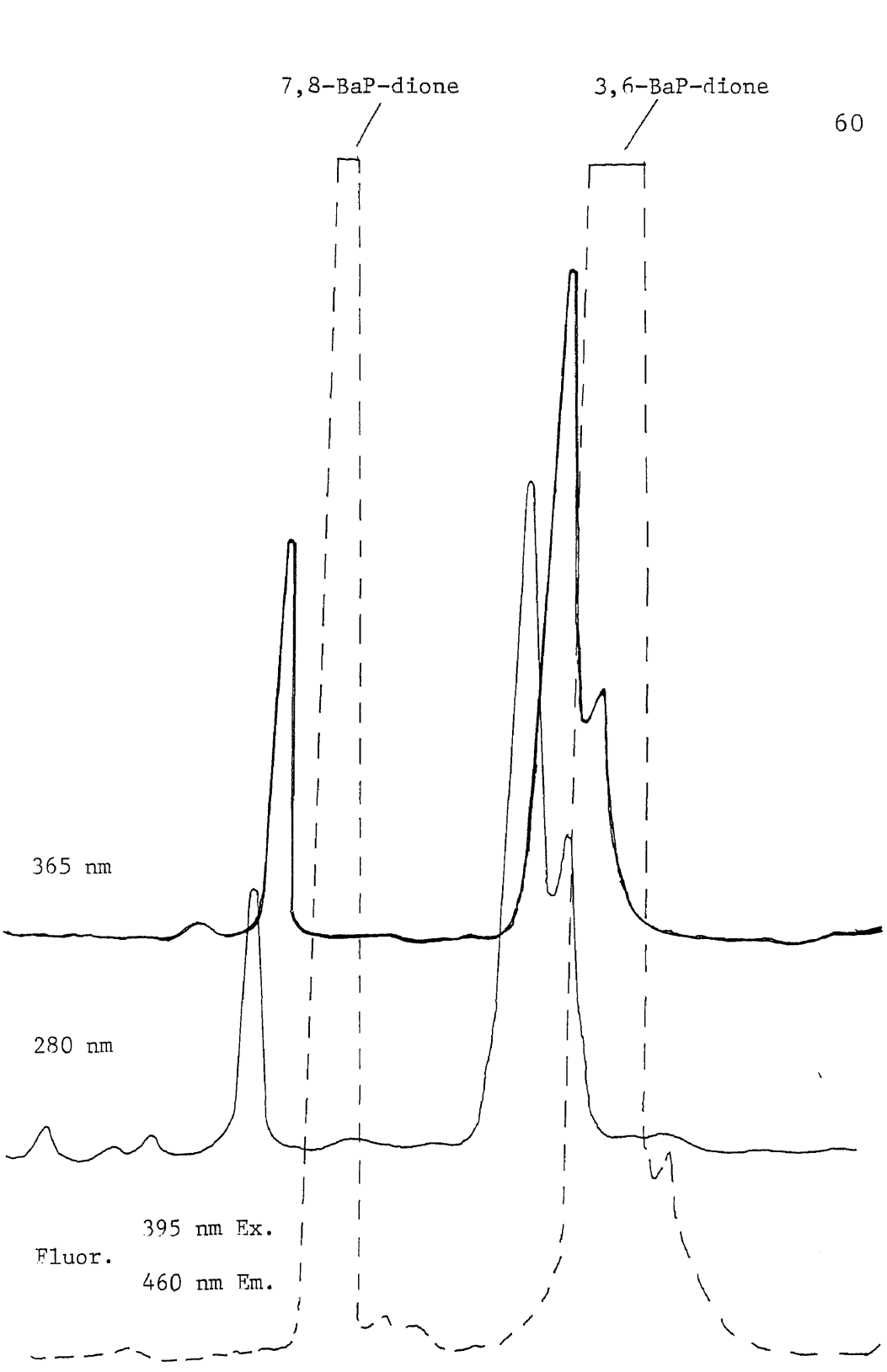


Fig.17b. The HPLC chromatogram for fraction #4 of DCM extract of an ambient air sample (Newark, 09/27/84) with reduction

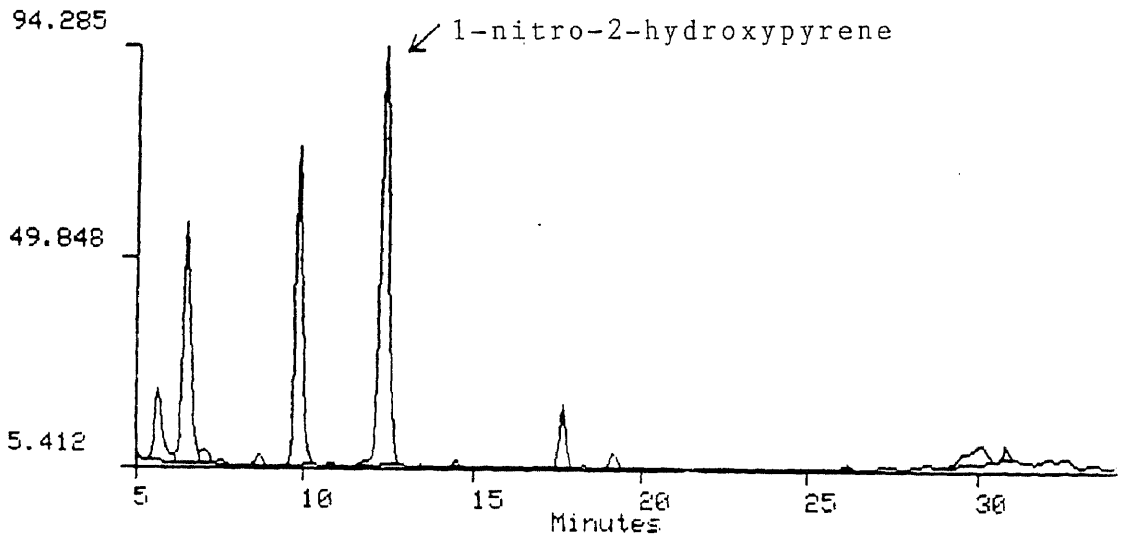


Fig. 18a. The HPLC chromatogram of the CYC extract an ambient air sample (Newark, 08/28/84). Peaks are monitored at 546 nm.

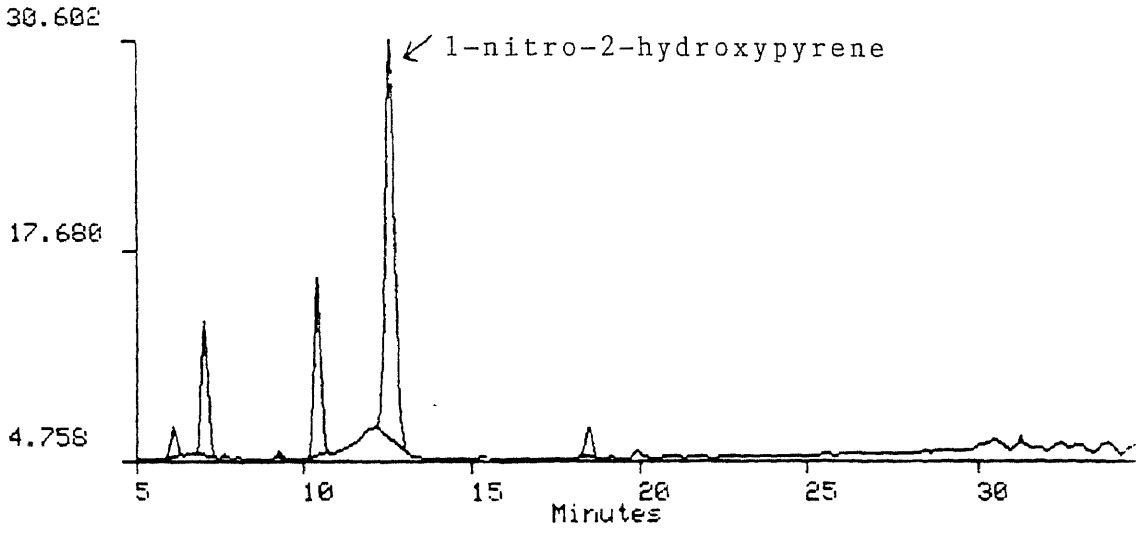


Fig. 18b. The HPLC chromatogram of the CYC extract for an ambient air sample (Newark, 08/28/8 spiked with 1-nitropyren-2-ol standard. Peaks are monitored at 546 nm.

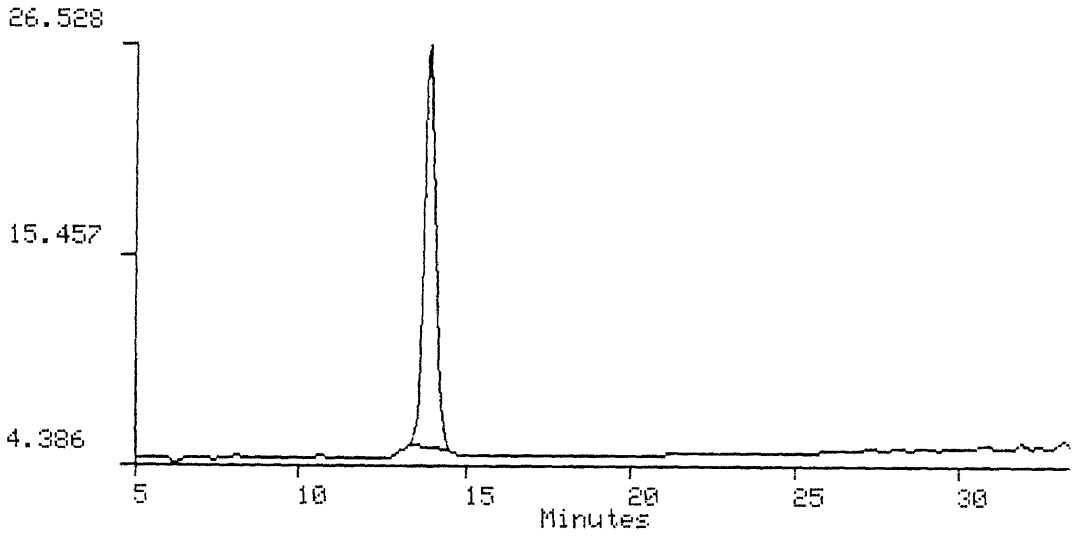
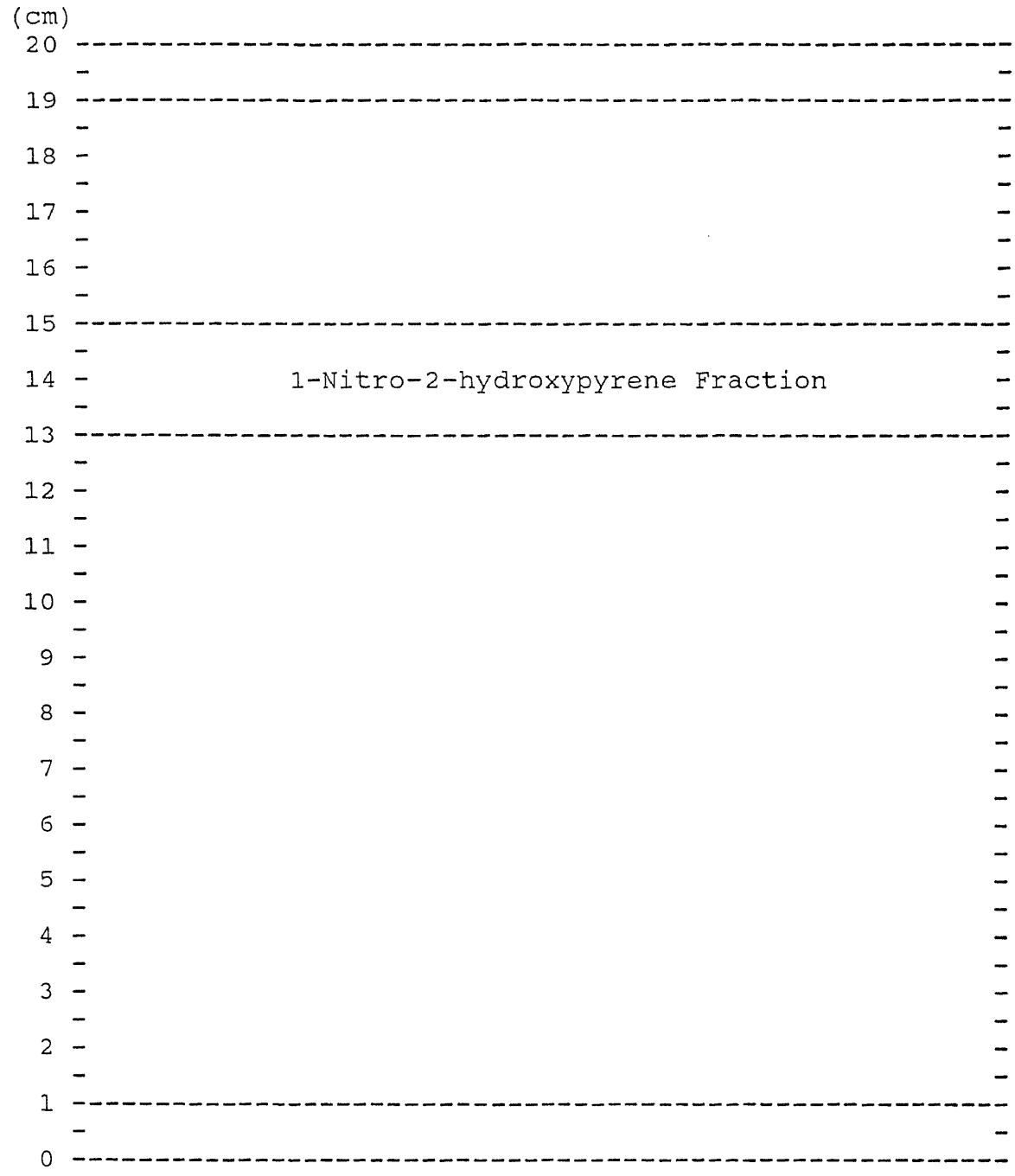


Fig.18c. The HPLC chromatogram of 1-nitropyren-2-ol standard (monitoring at 546 nm).



Mobil Phase: Toluene 50%; n-Hexane 50%.

Fig.19. The position of 1-nitropyren-2-ol on TLC plate.

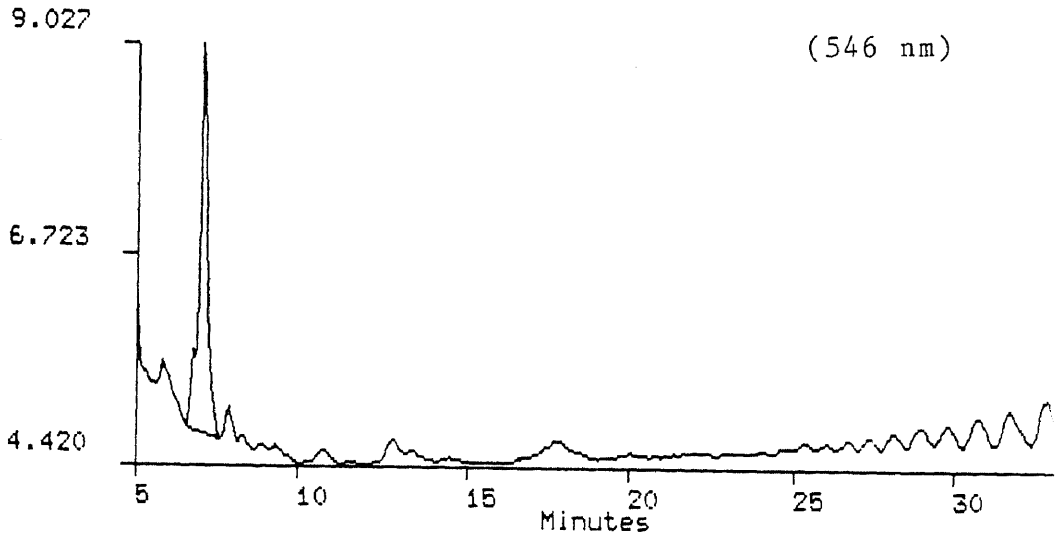


Fig.20. The HPLC chromatogram of NBS SRM #1649 (urban dust sample).

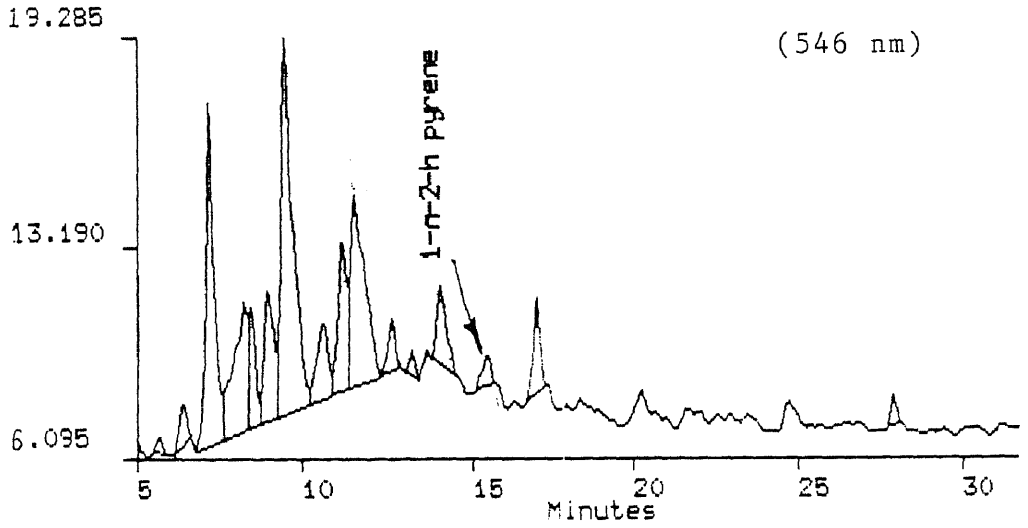


Fig.21a. The HPLC chromatogram of NBS SRM #1650 (diesel particulate sample).

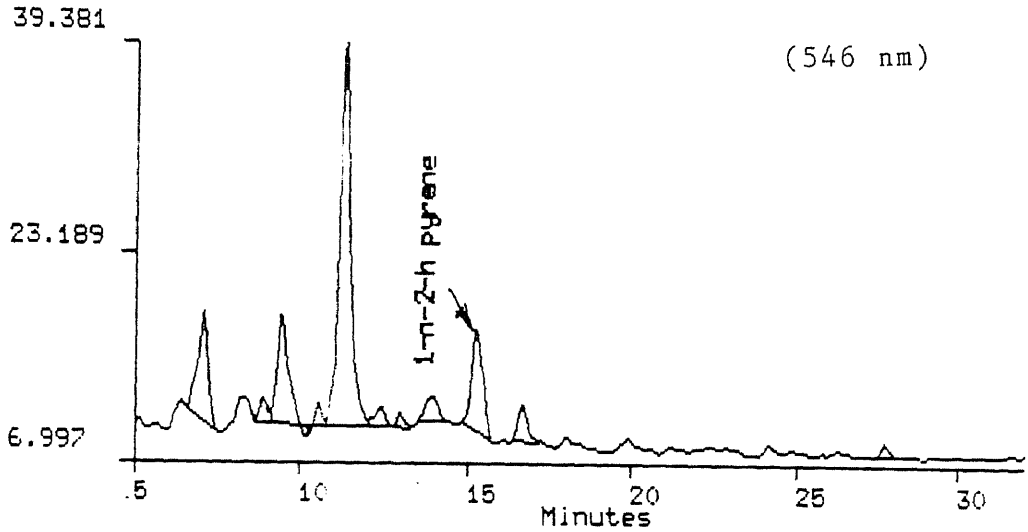


Fig.21b. The HPLC chromatogram of NBS SRM #1650 (diesel particulate sample) spiked with 1-nitropyren-2-ol standard.

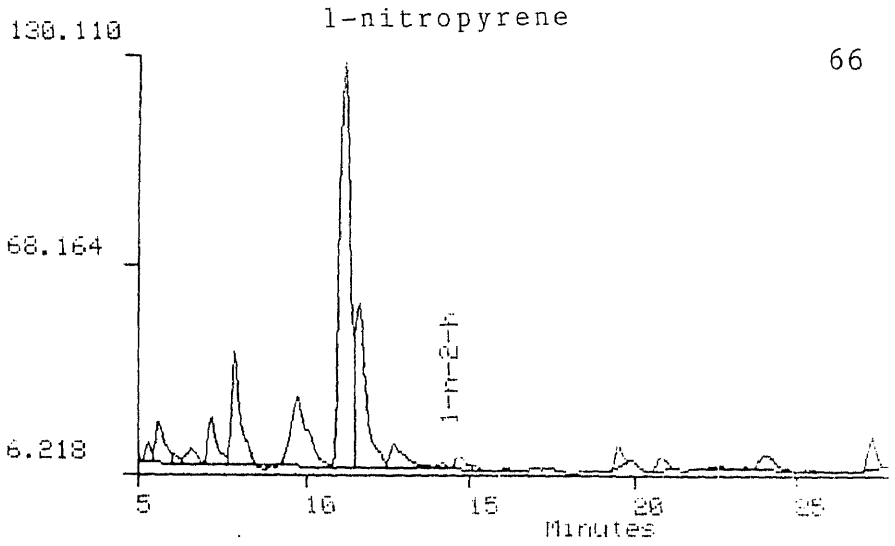


Fig. 22a. The HPLC chromatogram for the extract of the filter spotted with 1-nitropyrene without exposure to sunlight (kept in dark).

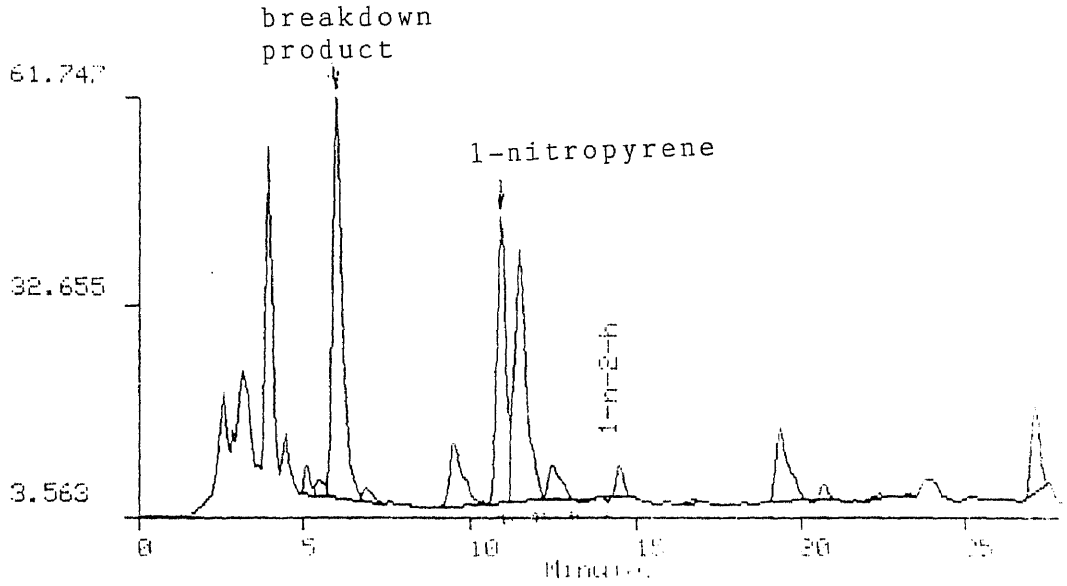
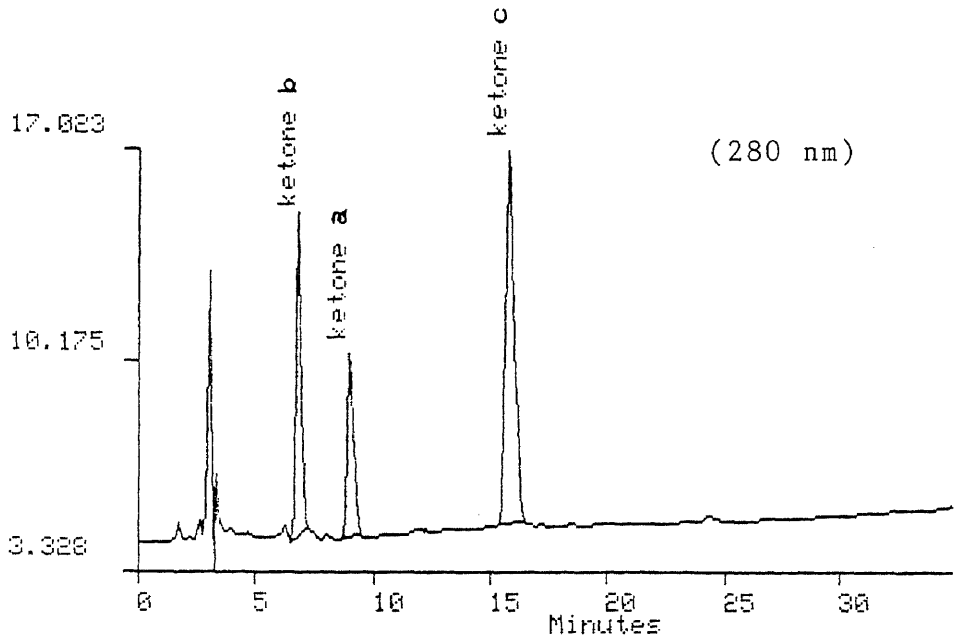


Fig.22b. The HPLC chromatogram for the extract of the filter spotted with 1-nitropyrene with exposure to sunlight.

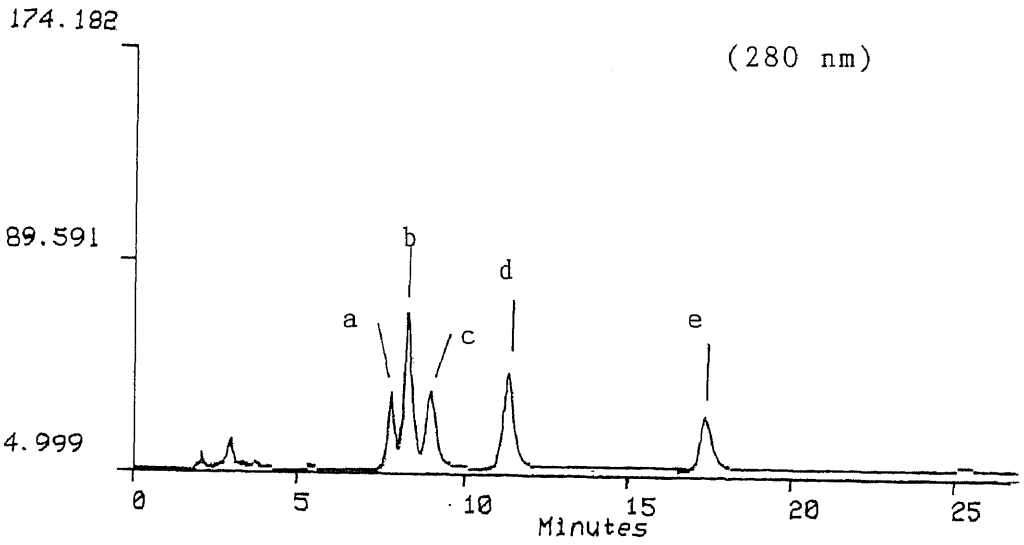


Ketone a: 1-methyl-4H-cyclopenta(def)phenanthrene-4-one.

Ketone b: 4H-cyclopenta(def)phenanthrene-4-one.

Ketone c: 4H-cyclopenta(def)chrysene-4-one.

Fig.23a. The HPLC chromatogram of PAH-ketone standards.



Peak a: 4,5-BeP-dione.

Peak b: 4,5-BaP-dione and 11,12-BaP-dione.

Peak c: 7,8-BaP-dione.

Peak d: 1,6-BaP-dione and 3,6-BaP-dione.

Peak e: 7,10-BaP-dione.

Fig.23b. The HPLC chromatogram of PAH-quinone standards.

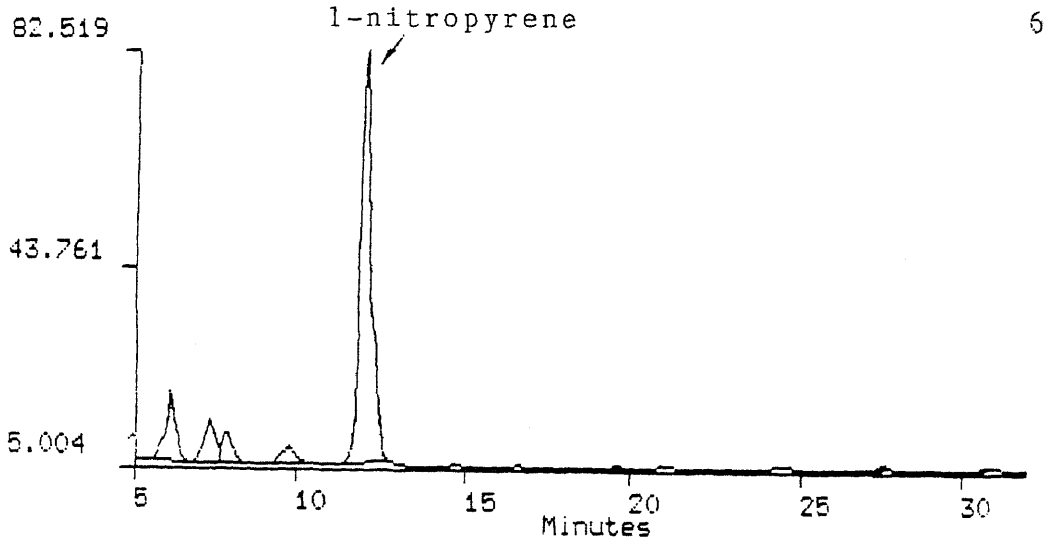


Fig.24a. The HPLC chromatogram for the extract of the filter spotted with 1-nitropyrene without exposure to u.v. light(kept in dark).

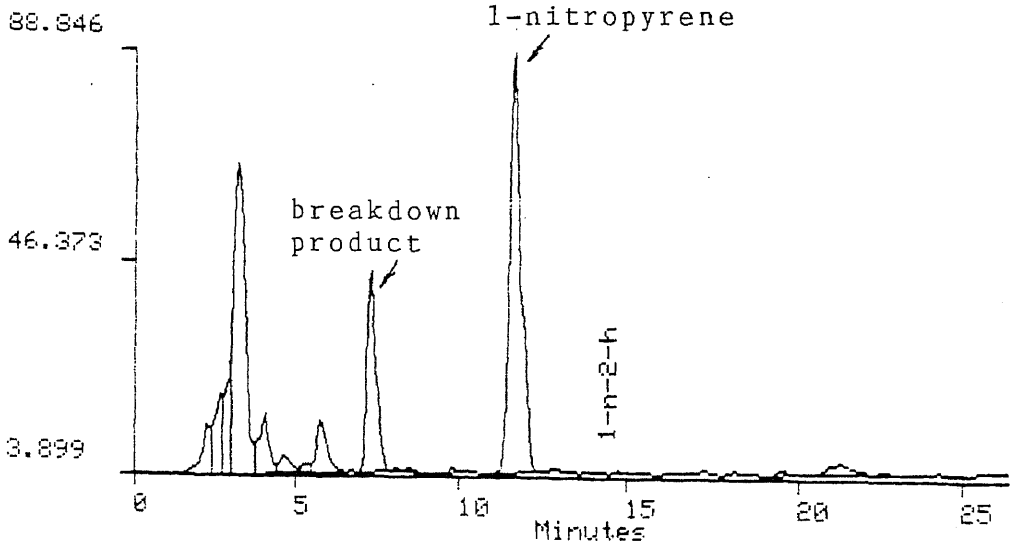


Fig.24b. The HPLC chromatogram for the extract of the filter spotted with 1-nitropyrene with exposure to u.v. light.

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