CHARACTERIZATION OF TRACE ORGANICS IN SUSPENDED PARTICULATE MATTER BY CHROMATOGRAPHIC TECHNIQUES

by

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GENERAL INTRODUCTION

Evidence for the induction of lung cancer in man via inhalation is extensive. The best evidence is related to occupational exposures, but the general low concentration of certain pollutants in urban atmospheres may be associated with an increased risk of lung cancer. This effect of general air pollution on the induction of cancer is difficult to evaluate because of the overriding effect of cigarette smoking. Epidemiologic evidence linking air pollution to lung cancer has been ambiguous to date, but a modest contribution is possible. However, when one considers the astronomical number of deaths due to lung cancer (over 70,000 cases in the United States each year), the possible contributory factor of air pollution should not be neglected, especially when this type of exposure is involuntary and affects the general population.

Particulate organic matter (POM) is derived mainly from the incomplete combustion of fossil fuels or any organic matter in general. Bioassay studies have shown that POM is carcinogenic in several animal tissues. Present knowledge indicates that POM contains at least two classes of compounds that are known animal carcinogens -- the polycyclic aromatic hydrocarbons (PAH) and the aza-arenes. Our analytical studies logically focus in these two classes of compounds.

Although analytical studies on PAH have been extensive, no satisfactory method was available for the routine analysis

of PAH in POM. Such a method was needed for compiling more comprehensive data on ambient PAH concentrations and to evaluate environmental impacts of "alternate" fuels. The first part of this work is therefore solely devoted to the development of this routine method.

The basic portion of POM which constitutes only a small percentage by weight has been shown to contain tumorigenic agents. Its chemical composition was relatively unexplored, therefore it was believed that the application of modern analytical instrumentation would lead to the identification of many new components. The second part of the thesis characterizes the basic portion through the use of high pressure liquid chromatography and coupled gas chromatography-mass spectrometry.

PART I

HIGH PRESSURE LIQUID CHROMATOGRAPHIC METHOD FOR ROUTINE ANALYSIS OF MAJOR PARENT POLYCYCLIC AROMATIC HYDROCARBONS IN SUSPENDED PARTICULATE MATTER

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAH) are the most studied trace organic compounds in polluted air. The great interest in PAH lies in the fact that several are known carcinogens in experimental animals and some are suspected carcinogens in man (1-2). PAH are formed as trace pollutants through pyro-synthetic routes (3) during incomplete combustions of organic matter under a reducing atmosphere. They are found in particulate organic matter (POM) (4-5), tobacco and marijuana smokes (6-7), gasoline and diesel exhaust (8-10), soots from burning flames (11), coke oven and other industrial effluents (12-13), and barbequed and smoke foods (14). They also occur in various refined and crude petroleum products, including gasoline (15), high-boiling distillates of crude oil (16-17), white petroleum products (18), etc. and constitute major fractions in shale oil (19), coal tar (20), coal liquefaction products (21) and some mineral deposits (22). The presence of PAH in both polluted water (23), drinking water (24), and fish (23) is well publicized. The recent discovery of complex, highly alkylated assemblages of PAH in soils and recent sediments in many depository environments (25-27) has shown their wide natural distribution and has raised interesting questions as to their origin and their possible biological implications in evolution (28).

PAH analysis has been an area of active research in the past ten years. Over one hundred publications appear in the

literature each year. Analytical methods have been well-reviewed (4-5;29-30). Separation methods such as thin-layer chromatography (TLC)(31), paper chromatography (PC)(8), and conventional liquid chromatography (LC)(32), used commonly in the past, are slowly being replaced by modern chromatographic techniques such as gas chromatography (GC) and high pressure liquid chromatography (HPLC)(5).

The presence of carcinogenic PAH in town soot was discovered by Cooper (33) in 1954. Since then, much effort has been expended to characterize organic particulate matter (POM), both in regard to its chemical composition and biological activity. Logically, most of the research has centered around the PAH fraction. Two groups of investigators-Sawicki et al (34-35) and Hoffmann, Wynder and co-workers (4,36-37)-have contributed significantly to our present knowledge about POM. Most of this earlier work employed either TLC or conventional LC for PAH enrichment and uv or fluorescence spectroscopy for identification.

Since detailed chemical composition alone without bioassay data is of little significance in environmental carcinogenesis, the importance fo animal testing to locate the most biologically active fractions can hardly be overemphasized in the study of POM (5).

Several bioassay studies indicate that POM extracts (37-38) and gasoline engine exhaust condensate (39) are carcinogenic in experimental animals. Wynder and Hoffmann (37), using a fractionation scheme similar to the one used in their tobacco

smoke analysis, have shown the neutral aromatic fraction (PAH-containing fraction) to be the most biologically active on mouse skin. More detailed knowledge of the PAH fraction in POM is thus desirable but was difficult at that time because of the limitations of available analytical methods.

Since some PAH have high boiling points (e.g. coronene- b.p. 525 ^{o}C), separation of PAH by GC was generally not very successful before the 60's. Advances in GC, especially the development of high temperature stationary liquid phases in the late 60's, enabled satisfactory separation of PAH by this technique. Separations of PAH in synthetic mixtures and in POM were demonstrated by several workers (12,40-42).

In recent years, the combination of gas chromatography (GC) and mass spectrometry (MS) has seen increasing use as a versatile tool for in-depth analytical studies of environmental problems (43-44). The number of applications of this technique reported exceed 400 each year and are reviewed in periodic publications (45-47).

The direct coupling of a GC to a MS is usually achieved via an interface (separator). These devices increase the sample concentration and allow the GC effluent (at ca. 760 torr) to be introduced into the high vacuum (at ca. 10^{-6} torr) prevailing in the ion source of the MS. GC as an analytical technique is limited by the availability of standard reference materials which must be obtained since GC identifications depend solely on retention times. The use of MS as the GC detector removes this shortcoming by allowing the identidication of

individual components eluted from the GC column in terms of their mass spectrum. Since a formidable amount of information is generated in a GC-MS system, data handling is generally facilitated by a computer. This technique, combining the high separational capability of GC with the molecular definitiveness of MS, enables unparalled speed, sensitivity and completeness of identification of the constituents present in trace amounts in complex mixtures.

In one classic study of POM by Lao et al (48) in 1973, more than 70 PAH were tentatively identified in an air sample by GC-MS analysis. Others quickly followed suit (10,49-52) with equal succes and firmly established the usefulness of GC-MS in this area.

The rapid growth of LC was in some ways even more impressive than that GC. Advances in LC over the past few years has so revolutionized this technique that a new name - "high pressure liquid chromatography" - was required. This area now encompasses a whole new generation of instrumentation and techniques. The speed and efficiency of HPLC to-date even rivals that of GC, but most often the two techniques are complementary (53). The increase in efficiency of HPLC stems mainly from new designs in column packings. Band spreading is reduced by the introduction of superficially porous packings and, more recently, by totally porous microparticulate packings. The columns in HPLC are reusable and since small particle size packing materials are used, the mobile phase is forced through the columns by high pressure pumps. The system also includes new, small dead volume detectors which continously

monitor column effluents and produce "chromatograms". Two types of detectors are most often used - uv absorbance detectors and refractive index detectors. Applications of HPLC are reviewed in periodic publications (47,54).

The explosive growth of HPLC has prompted many chromatographers to apply this technique to PAH analysis. Synthetic mixtures have been separated by HPLC using reversed phases (55-59), adsorption (18,60), partition (61-63), complexation (64-66) and gel permeation (7,67). An excellent example of reversed phase HPLC has been reported by Selkirk et al (68), who separated benzo(a)pyrene and its metabolites formed by rat liver microsomes. To our knowledge, before this work began, no comprehensive quantitative procedure had been published for the analysis of PAH in POM. This was one of the main reasons which inspired our investigation in this area.

Very recently, two papers appeared in the literature concerning the use of selective detectors in HPLC for the analysis of PAH. Fox et al (58) describes a method based on HPLC using a fluorescence detector, which is extremely sensitive and selective to PAH. However, the use of a variable wavelength detector in HPLC reported by Kristulovic et al (59) allows more sensitive and selective monitoring of certain PAH. While we recognize the general usefulness of selective detectors and would have made use of them had they been available during the development of our method, we disagree with the deletion of a prefractionation step in the procedure used by the authors. In our experience, POM are too complex for any single separation method; prefractionation

is essential for PAH analysis. Therefore we strongly discourage the injection of raw particulate matter extract into a chromatograph without prior PAH enrichment, since we believe that quantitative PAH data derived from such procedure are less reliable even when very selective detectors are involved. Also, the expensive microparticulate columns can be easily contaminated by constant injection of dirty samples without prior clean up procedures.

The need for routine monitoring of PAH in POM is apparent for public health reasons. There has been a trend towards lower particulate matter and PAH concentrations in the ambient atmospheres of cities, following the substitution of fuel oil for coal in recent years (69). However, this trend could change quite rapidly because of the supposedly delicate balance between "environment" and "energy". Increased coal consumption would certainly reverse this trend (4). Alternate energy sources such as coal oil and shale oil both contain large quantities of PAH and have been shown to cause skin cancer in workers involved in their production (70-71). Large scale use of these fuel sources should be evaluated in terms of PAH generation before any large scale development is undertaken. A recent change quite significant to environmental health has been the increased consumption of unleaded gasolines which contain higher concentrations of aromatic hydrocarbons resulting in increased quantities of PAH in engine exhaust (4). The catalytic convertor is known to reduce particulate matter and hydrocarbons emission (4), but its long term effectiveness, particularly with aging under normal usage, warrants careful

studies (72). To monitor such trends and to evaluate their environmental impacts requires a simple, rapid and reliable method for PAH analysis.

Several groups have proposed the use of B(a)P as an indicator compound for total PAH (4,37). Such a "quick and dirty" method might thus be implemented to assay B(a)P concentrations and therefore, to obtain a number for total PAH. These methods usually involve a crude chromatographic separation followed by fluorescence measurements. These methods are not satisfactory for PAH analysis in POM, because the relative concentration of B(a)P to the other PAH is a complicated function of combustion temperature (73), emission sources (74) and time of photo-oxidation (4). Thus the supposition that B(a)P is proportional to total PAH entails more assumptions than one can responsibly make and could result in an erroneous picture of the health of our environment. With these facts in mind, a method was developed for rapid routine quantitative assay of major PAH in POM in one chromatogram. HPLC proved to be the ideal tool.

HPLC is clearly superior to conventional LC in terms of resolution, analysis time and analytical precision (53). In the HPLC method described here, a total of approximately 5 hours is needed from the extraction of the air filter to the obtaining of the chromatogram. The liquid chromatographic separation takes 100 min, which can be reduced to 20 min if lower resolution is allowed. A conventional LC separation of comparable resolution takes 2-5 days using a long alumina

column (75).

HPLC offers many advantages over GC in PAH analysis: it operates at lower temperatures (below 80°C); has available a uv detector highly selective for PAH whose molar absorptivities range from 10^4 to 10^5 at 254 nm; fraction collection followed by uv and fluorescence spectrophotometry is simple and convenient; reversed-phase packings are highly selective for separating isomeric PAH such as benzo(a)pyrene and benzo(e)-pyrene which are not normally separated by GC (48) and whose mass spectra are sufficiently similar to make absolute identification with GC-MS difficult.

The ODS Zorbax columns used here have good efficiency, high sample capacity and show long term reproducibility without bleeding. The collected PAH fractions are directly compatible with uv spectophotometry with no prior concentration necessary.

It should be pointed out that some PAH isomers can be separated by GC using capillary (7,49) and long packed (11) columns. Nematic liquid crystals used as a GC stationary phases have been reported by Janini et al (76-77) to show selectivity similar to HPLC for PAH separations. These stationary phases are generally not useful above 260°C which severely limit their applicability in PAH analysis, especially in GC-MS runs which cannot tolerate column bleeding. This major shortcoming, however, might be corrected by chemically bonding these nematic liquid crystals to packing materials (78).

GC-MS will no doubt continue to be the most effective technique for environmental analysis, e.g. for the alkyl and hydro derivatives of PAH. However, the HPLC method developed here does offer an attractive alternative for the routine monitoring of PAH, in terms of cost, accuracy and ease of operation.

EXPERIMENTAL

The experimental procedure is outline in Figure 1.

Materials. Spectro-grade cyclohexane and methanol were obtained from Burdick & Jackson Inc. PAH standards were purchased from Aldrich, K&K, and Eastman Kodak Co. Some PAH were further purified by zone-refining. Pre-coated silica gel-G TLC plates (500 μ thick without fluorescence indicator) were obtained from Analtech Inc.

Sampling. Particulate matter samples were provided by the New York City Department of Air Resources. They were collected at various sampling sites of New York City's Aerometric Network (See Appendix I for details) on glass fiber filters (8"X10" from Mine Safety Appliances Co.) using high volume samplers. The high volume sampler consists of a suction fan operated by a motor and equipped with a filter holder and calibrated air flow gauge. The filter is a rectangular fiber glass sheet of high collection efficiency and low flow resistance. Samplers are exposed inside a louvered box that holds the filter surface, facing upward and horizontal, under a roof that protects the filter from rain, snow, and fallout

particles. Such filtration systems can have high collection efficiency (over 99.9%) for particules of 0.1μ to 10μ in diameter (4). This collection efficiency for small particles is important because incomplete combustion of fossil fuels or organic materials in general is the major source of organic particulate matter in urban areas, and controlled combustions usually produce particles of 0.1-10μ in diameter (4). One study, exploring the distribution of PAH on particulates of different size, has shown that about 60% of the pentacyclic PAH are associated with particle diameter smaller than 1.1μ (79).

The average rate of sampling is 40-50 ft^3/min. The weight of a 24-hr sample ranges from 90 to 200 mg depending on site, season, and weather conditions. Sample S301 was taken from station 3 (South Bronx) and S303 from station 10 (East Manhattan) in November and December of 1974. Both locations are in areas of heavy particulate matter pollution and each sample was a combined extract of 6 filters selected randomly across the two months. S201 was a combination of 18 filters collected during March 1974, randomly selected from different stations.

Extraction. Although cyclohexane, benzene and acetone have all been shown to be nearly 100% efficient in Soxhlet extraction of benzo(a)pyrene from filters (80), cyclohexane appears to be the most suitable since it tends to extract fewer extraneous materials and thus eases the burden on the separation method. Material from two or more filters is required when uv characterization of effluent is employed.

Filters were extracted for six hours using 150 ml of cyclohexane. The extract, a clear yellowish solution, was concentrated to 5 ml in a rotary evaporator.

Sample Prefractionation. Prefractionation is necessary to isolate the PAH from the paraffins, olefins, heterocyclics, etc., also present. The prefractionation procedure of Brocco et al (42) using TLC was employed. A TLC of a typical New York City air particulate extract is shown in Figure 2. Greater than 90% recovery on tested PAH was reported (42) using a similar TLC-GC technique.

Separation and Identification. A Dupont 820 Liquid Chromatograph equipped with fixed wavelength (254 nm) uv detector, refractive index detector and gradient elution accessory was used with a 25-cm long, 2.1mm-i.d. ODS Zorbax column. Carefully prepared synthetic mixture of PAH standards were routinely injected to check column performance and to optimize operating conditions. One to five µl of prefractionated air particulate extract was sufficient for the analysis by the HPLC peak height method. A 30-50 µl sample size was necessary when fractions were to be analyzed by uv. Three-tenth ml fractions, sufficient to fill a uv micro-cell of pathlength 4 mm, were taken. A Cary 14 UV-VIS spectrophotometer and a Perkin Elmer MFP-2A spectrofluorometer were used to obtain spectra. The latter gives spectra uncorrected for source intensity and detector wavelength response variation. Reference spectra were either obtained from PAH standards or from the literature (81-84).

GC-MS Crosschecks. The PAH fraction of air sample S301 and S303 were analyzed by GC-MS, using a Perkin Elmer 800 GC coupled to a Nuclide 12-90-G Mass Spectrometer. Mass spectra of each emerging peak were taken at electron energies of 14 eV and 70 eV. A 12-ft Dexsil-300 GC column was used under conditions similar to those reported by Lao et al (48). Since there is some uncertainty about the split ratio (FID/MS) and about the efficiency of the Biemann-Watson separator, chromatograms were also obtained using the same column in a Perkin Elmer 900 GC for quantitative comparison.

Blank. Extraction of 2 unexposed filters and subsequent analysis using the identical HPLC procedure showed no PAH.

RESULTS AND DISCUSSION

Optimization of Operating Conditions. The ODS Zorbax column, 15% octadecyl silane (ODS) bonded to porous silica microspheres (Zorbax) having a diameter of 5-7 μ, is very similar to the ODS Permaphase (Dupont), 1% ODS bonded to a 37-μ diameter pellicular packing (Zipax)(55).

The optimum operating conditions were chosen empirically by adjusting the amount of MeOH in the mobile phase, temperature and flow rate singly or in combination for best separation in a reasonable time. Our best separation of 2 synthetic mixtures was obtained at 60°C, 65/35 MeOH-H_2O and 0.21 ml/min. as shown in Figures 3 and 4. The calculated number of theoretical plates for the benzo(a)pyrene peak is close to 4000 (similar to the efficiency claimed by the manufacturer).

A gradient of 1% MeOH/min was initiated from 65% to 100% after the benzo(a)pyrene peak eluted to hasten the elution of the more strongly retained compounds. The baseline shift accompanying the use of gradient elution is caused by the changing refractive index of the effluent.

Suspended Particulate Matter Samples. Figures 5 and 6 are liquid chromatograms of 5-μl aliquots of TLC prefractionated air samples (S303, S301 and S201) using the same HPLC conditions as in Figure 3. A gas chromatogram of S303 is shown in Figure 7 for comparison. The envelope under the GC peaks probably represents a conglomeration of minor components also present in the TLC PAH fraction. Samples taken from areas of lower particulate matter pollution show significantly less pronounced envelopes. Only major peaks definitely identified by GC retention time and MS are labelled in Figure 7.

In Figure 5, 18 PAH are identified in air sample S303. Other peaks were only tentatively identified or not identified because of their low concentrations. The major chromatographic peaks group according to the number of aromatic rings. The first pair consists of 3-ring phenanthrene and anthracene; these cannot normally be separated by GC. Similarly, the 4-ring isomers, triphenylene, benz(a)anthracene and chrysene emerge as a single peak in GC (Figure 7). Benz(a)anthracene and chrysene, one of the most difficult pairs to separate, are better separated on ODS Permaphase with 50/50 MeOH-H_2O and 40°C (85). These conditions cannot be used in the ODS Zorbax column because of the slower flow rate and higher stationary phase

loading. GC separates the 5-ring group in 4 peaks: B(j)F, B(b)F and B(k)F, B(a)P and B(e)P. and perylene while in HPLC, B(a)P is well resolved from the rest (Table I for PAH abbreviations).

Liquid chromatograms of most air samples studied show remarkably similar features. All the major PAH are always present but usually vary in relative proportions. This reflects the fact that PAH are formed from combustion processes. e.g., automobiles, space heating, incinerators, etc., which are ubiquitous. According to a study by Jeltes et al (74), the high proportion of pyrene, benzo(ghi)perylene and coronene in S301 is a good indication that the majority of PAH in this sample are derived from automobiles. The reverse is true for S303, indicating domestic fuel burning to be the major particulate matter source.

Figures 8-14 are uv spectra of some of the more important fractions isolated from S303 by HPLC. The solid lines gives the spectrum of the fraction and the dotted lines give the spectrum of the reference PAH at similar concentration. The uv spectrum of the isolated PAH are of significantly better quality than most published spectra of PAH isolated from environmental samples, because of the higher purity of these compounds.

Figures 15-18 show fluorescence and excitation spectra of the B(a)P and other fractions in S303. Collected fractions were first evaporated and samples redissolved in 0.5 ml of cyclohexane for fluorescence measurements.

Quantitation. Table I gives comparision data on the

determination of 20 PAH by 3 different methods on S303, along with average PAH concentrations in US urban areas (34) for comparison.

A brief description of these methods follows. (a) HPLC/UV method. The concentrations of each PAH were calculated from the peak height of the uv spectra of the collected fractions. Molar absorptivities were taken from the literature (82). The baseline method (33) was used in most cases. (b) HPLC peak height method. The height of each PAH peak in the sample was compared with that of the standard of known concentration. (c) GC peak area method. Fluoranthene, which is always present in air samples and which emerges as a well separated peak, was used as an internal reference compound for detector response factor (48). The amount of fluoranthene in the sample was first obtained by comparing peak area with an equal volume injection of standard fluoranthene solution. Since the flame ionization detector response factors of other PAH relative to fluoranthene have been published (48), their quantities in the sample can be calculated from the relative peak areas.

The HPLC/UV method should be the more accurate method since it is subject to less interference. The GC and HPLC results are in reasonably good agreement. The fact that the HPLC/UV always gives the lowest result suggests that some of the chromatographic peaks include more than one compound. Quantitation by HPLC peak heights suffers from insufficient

resolution while the GC peak area method suffers from the nonselective response of the FID.

Future Research in POM. Since the composition of the PAH fraction in POM is already well-known, future analytical research should concentrate on other lesser known fractions which are also biologically active. The neutral oxygenated fraction has been found to induce high incidence of adenomas in mice (38). Due to the non-volatility of most constituents in this fraction, GC-MS is of limited use and its chemical composition remains virtually unknown. The best method for the characterization of this fraction should be offered by HPLC, followed by direct insertion probe mass spectrometry.

The contribution of the tumor promoting activity of the acidic fraction to the overall carcinogenicity of POM (37) should not be neglected. More analytical research, especially when coupled with bioassay data will definitely yield meaningful information on the carcinogenicity of POM.

CONCLUSIONS

In our opinion, any satisfactory routine quantitative method for PAH in POM must fulfill most of the following requirements: accuracy and reliability, giving unambiguous quantitative results for most major PAH, including individual isomeric PAH, and rapidity, enabling the generation of comprehensive data from many locations in a city and allowing pollution control personnel to study PAH emission pattern, long term trends and to evaluate environmental impacts of any new PAH polluting source. The instrument involved should be rugged, relatively easy to operate and maintain, and reasonably inexpensive so as to be available to most local and state pollution agencies.

We believe that the TLC/HPLC method described here is the best method currently available for this purpose. Admittedly, the resolution of our best HPLC column (about 4000 theoretical plates) does not allow detailed analysis of all the PAH isomers and alkyl derivatives of the fraction in POM. HPLC does not now approach capillary GC in terms of resolution and may not in the near future. However, judging from the quality of uv spectra of collected fractions and the good GC-MS crosschecks, our rapid HPLC method does give reliable quantitative information and thus represents the best choice for the routine monitoring of major parent PAH in POM. This method is also adaptable to other environmental sample types such as cigarette smoke condensate, coal tar and petroleum products.

Our data show the general decline of PAH concentrations in New York City. The concentration of B(a)P is only 1.2 µg/1000 m^3 around one of the most polluted sampling stations (station 3). The average B(a)P concentration was 5.7 µg/1000 m^3 in US urban atmospheres in 1963 as reported by Sawicki et al (34). The relative concentration of B(a)P to other PAH is useful to indicate the major contributing particulate source to the sampling site.

SUMMARY

A rapid high pressure liquid chromatographic method is developed and applied to the determination of polycyclic aromatic hydrocarbons in suspended particulate matter samples from New York City's air. Particulate matter collected on filters is Sohxlet extracted using cyclohexane and the extractable matter is prefractionated by thin layer chromatography. The PAH fraction is concentrated and separated by HPLC with an ODS Zorbax column. About 20 PAH are identified in effluent fractions by uv and fluorescence spectrophotometry. Submicrogram quantities of PAH are quantified by the peak height method. The detection limit of benzo(a)pyrene is about 10 ng. Good crosschecks are obtained by gas chromatography-mass spectrometry. Since the analytical procedure takes only 5 hours, this method is most suitable for the routine analysis of PAH in particulate organic matter.

LITERATURE CITED

1. P. Shubik and J.L. Hartwell, "Survey of Compounds Which Have been Tested for Carcinogenic Activity", U.S. Public Health Service Publ. 149, 1951; Suppl. 1, 1957; Suppl. 2, 1969; 1968-69 Vol., 1972; 1961-1967 Vol. Sect. I and Sect. II, 1973; 1970-71 Vol., 1974.

2. J.C. Arcos and M.P. Argus, "Chemical Induction of Cancer", Vol. IIA, Academic Press, New York, 1974.

3. G.M. Badger, "The Chemical Basis of Carcinogenic Activity", Thomas, Springfield, Illinois, 1962, p 16.

4. D. Hoffmann and E.L. Wynder, Organic Particulate Pollutants-Chemical Analysis and Bioassays for Carcinogenicity in "Air Pollution", 3rd Ed. Vol. 2, A.C. Stern, ed., Academic Press, New York, in press.

5. "Particulate Polycyclic Organic Matter", National Academy of Sciences, Washington D.C., 1972.

6. E.L. Wynder and D. Hoffmann, "Tobacco and Tobacco Smoke", Academic Press, New York, 1967.

7. M.L. Lee, M. Novotny and K.D. Bartle, Anal. Chem., 48, 405 (1976).

8. D. Hoffmann and E.L. Wynder, Natl. Cancer Inst. Monogr., 9, 91 (1962).

9. F.W. Karasek, R.J. Smythe and R.J. Laub, J. Chromatogr., 101, 125 (1974).

10. K.W. Boyer and H.A. Laitinen, Environ.Sci. Technol., 9, 457 (1975).

11. B.B. Chakraborty and R. Long, Environ. Sci Technol., 1, 829 (1967).

12. T.D. Searl, F.J. Cassidy, W.H. King and R.A. Brown, Anal. Chem., 42, 954 (1970).

13. E. Sawicki, J.E. Meeker and M.J. Morgan, Int. J. Air Water Pollut., 9, 292 (1965).

14. T. Fazio, R.H. White and J.W. Howard, J. Assoc. Off. Anal. Chem., 56, 68 (1973).

15. C.R. Begeman and J.M. Colucci, Science, 161, 271 (1968).

16. H.J. Coleman, J.E. Dooley, D.E. Hirsch and C.J. Thompson, Anal. Chem., 45, 1727 (1973).

17. J.F. McKay and D.R. Latham, Anal. Chem., 44, 2132 (1972).

18. M. Popl, M. Stejskal and J. Mostecky, Anal. Chem., 47, 1947 (1975).

19. P.C. Uden and S. Siggia, Presented at Centennial American Chemical Society Meeting, (Abstr. Anal. 57), New York, April 7, 1976.

20. K.F. Lang and I. Eigen, Fortschr.Chem. Forsch., 8, 91 (1967).

21. T. Aczel and H.E. Lumpkin, Presented at Centennial American Chemical Society, (Abstr. Anal. 58), New York City, April 7, 1976.

22. M. Blumer, Chem. Geol., in press.

23. G. Gortalum, Eksp. Klin. Onkol., 2, 95 (1975). (Chem. Abstr. - 85; 9926u (1976))

24. J. Borneff, Gas, Wasser, Abwasser, 55, 467 (1975). (Chem. Abstr. - 85; 10272j (1976))

25. W. Giger and M. Blumer, Anal. Chem., 46, 1663 (1974).

26. M. Blumer and W.W. Youngblood, Science, 188, 53 (1974).

27. W.W. Youngblood and M. Blumer, Geochim, Cosmochim. Acta., in press.

28. M. Blumer, Sci. Amer., 234, 34 (1976).

29. O. Hutzinger, S. Safe and M. Zanders, "Polycyclic Aromatic Hydrocarbons", Analab, Inc., Research Notes, 13, 3 (1973).

30. P.K. Mueller and E.L. Kothny, Anal. Chem., 45, 1R (1973).

31. R.C. Pierce and M. Katz, Anal. Chem., 47, 1743 (1975).

32. P.J. Blokzijl and R. Guicherit, TNO-Nieuws, 27, 653 (1972).

33. R.L. Cooper, Analyst, 79, 573 (1954).

34. E. Sawicki, S.P. McPherson, T.W. Stanley, J. Meeker and W.C. Elbert, Int. J. Air Water Pollut., 9, 515 (1965).

35. E. Sawicki, Arch. Environ. Health., 14, 46 (1967).

36. D. Hoffmann and E.L. Wynder, Cancer, 15, 93 (1962).

37. E.L. Wynder and D. Hoffmann, J. Air Pollut. Contr. Assoc., 15, 155 (1965).

38. S. Asahina, J. Andrea, A. Carmel, E. Arnold, Y. Bishop, S. Joshi, D. Coffin and S.S. Epstein, Can. Res., 32, 2263 (1972).

39. D. Hoffmann, E. Theisz and E.L. Wynder, J. Air Pollut. Contr. Assoc., 15, 162 (1965).

40. A. Liberti, G.P. Cartoni and V. Cantuti, J. Chromatogr., 15, 141 (1964).

41. J.R. Wilmshurst, J. Chromatogr., 17, 50 (1964).

42. D. Brocco, V. Cantuti and G.P. Cartoni, J. Chromatogr., 49, 66 (1969).

43. S.R. Heller, J.M. McGuire and W.L. Budde, Environ. Sci. Technol., 9, 211 (1975).

44. A. Alfred, Biomed. Mass Spectrom., 2, 229 (1975).

45. Gas Chromatography-Mass Spectroscopy Abstracts, Science and Technology Agency, London, 1976.

46. S.P. Cram and R.S. Juvet, Anal. Chem., 48, 411R, 1976.

47. Gas and Liquid Chromatographic Abstracts, Applied Science Publishers, Barking, England, 1976.

48. R.C. Lao, R.S. Thomas, H. Oja and L. Dubois, Anal. Chem., 45, 908 (1973).

49. M. Novotny, M. Lee and K.D. Bartle, J. Chromatogr. Sci., 12, 606 (1974).

50. W. Cautreels and K. Van Cauwenberghe, Atmos. Environ., 10, 447 (1976).

51. K. Van Cauwenberghe and W. Cautreels, "Advances in Mass Spectrometry in Biochemistry and Medicine", Vol 1, Spectrum Publications, Inc., 1976, p465.

52. M. Dong, D.C. Locke and E. Ferrand, Anal. Chem., 48,368 (1976).

53. L. Snyder and J.J. Kirkland, in "Introduction to Modern Liquid Chromatography", John Wiley and Sons, New York, 1974, chapter 1.

54. H.F. Walton, Anal. Chem., 48, 52R, 1976.

55. J.A. Schmit, R.A. Henry, R.C. William and J.F. Dieckman, J. Chromatogr. Sci., 9, 645 (1971).

56. C.G. Vaughan, B.B. Wheals and M.J. Whitehouse, J. Chromatogr., 78, 203 (1973).

57. B.B. Wheals, C.G. Vaushan and M.J. Whitehouse, J. Chromatogr., 106, 109 (1975).

58. M.A. Fox and S.W. Staley, Anal. Chem., 48, 992 (1976).

59. A.M. Krstulovic, D.M. Rosie and P.R. Brown, Anal. Chem., 48, 1383 (1976).

60. W. Strubert, Chromatographia, 6, 205 (1973).

61. H.-J. Klimisch, J. Chromatogr., 83 11 (1973).

62. H.-J. Klimisch, Anal. Chem., 45, 11 (1973).

63. R.E. Jentoft and T.H. Gouw, Anal. Chem., 40, 1787 (1968).

64. B.L. Karger, M. Martin, J. Loheac and and G. Guiochon, Anal. Chem., 45, 496 (1973).

65. R. Vililecchia, M. Thiebaud and R.W. Frei, J. Chromatogr. Sci., 10, 411 (1972).

66. C.H. Lochmuller and C.W. Amoss, J. Chromatogr., 108, 85 (1975).

67. M.E. Snook, W.J. Chamberlain, R.F. Severson and O.T. Chortyk, Anal. Chem., 47, 1155 (1975).

68. J.K. Selkirk, R.G. Croy and H.V. Gelboin, Science, 184, 169 (1973).

69. B.T. Commins and L. Hampton, Atmos. Environ., 10, 561 (1976).

70. S.A. Henry, Brit. Med. Bull., 4, 389 (1947).

71. D. Hoffmann, American Health Foundation, Valhalla, private communication.

72. E. Ferrand, New York City Department of Air Resources, New York, private communication.

73. I. Schmeltz, J. Tosk and D. Hoffmann, Anal. Chem., 48, 645 (1976).

74. R. Jeltes, J. Chromatogr. Sci., 12, 599 (1974).

75. G.J. Cleary, J. Chromatogr., 9, 204 (1962).

76. G.M. Janini, K. Johnston and W.L. Zielinski., Anal. Chem., 47, 670 (1975).

77. G.M. Janini, G.M. Muschik and W.L. Zielinski, Anal. Chem., 48, 809 (1976).

78. W.L. Zielinski, Nat. Canc. Inst. Frederick Cancer Research Center, Frederick, Maryland, private communication.

79. A. Albagli, L. Eagan, H. Oja and L. Dubois, Atmos. Environ., 8. 201 (1974).

80. T.W. Stanley, J.W. Meeker and M.J. Morgan, Environ. Sci. Technol, 1, 927 (1967).

81. E.J. Clar, "Polycyclic Hydrocarbons", 2 vols., Academic Press, New York, 1964.

82. "UV Atlas of Organic Compounds", 5 vols., Plenum Press, New York, 1967-68.

83. T.J. Porro, R.E. Anacreon, P.S. Flandreau and I.S. Fagerson, J. Assoc. Off. Anal. Chem., 56, 607 (1973).

84. R.A. Friedel and M. Orchin, "UV Spectra of Organic Compounds", John Wiley and Sons, New York, 1967.

85. M. Dong, D.C. Locke and E. Ferrand, unpublished data.

TABLE I. COMPARISON OF QUANTITATION METHODS ON AIR SAMPLE S303

Compound Names	Abbreviation	Concentration in ug/1000m^3			
		(a) HPLC/UV	(b) HPLC peak height	(c) GC peak area	avg. US urban conc. from (34)
Phenanthrene	Phen	0.36	0.71	0.5	-
Anthracene	A	0.05 } 0.41	0.09 } 0.80	0.1 } 0.6	-
Fluoranthene	F	1.1	1.8	1.9	4
Pyrene	P	1.05	2.7	2.0	1.3-19
Triphenylene	-	0.58	0.95		-
Benz(a)anthracene	B(a)A	1.4 } 3.0 } 3.6	} 3.9*	} 4.3	4
Chrysene	Ch	1.6			1.3-11.6
Benzo(ghi)fluoranthene	B(ghi)F	0.19	0.3	1.0	-
Benzo(c)phenanthrene	B(c)Phen	0.04	-	0.2	-
Benzo(j)fluoranthene	B(j)F	0.3	-	-	-
Benzo(b)fluoranthene	B(b)F	0.8	} 4.0	} 3.2 for	-
Benzo(e)pyrene	B(e)P	1.4		B(a)P & B(e)P	5
Perylene	Per	0.1	0.2	0.2	0.7
Benzo(k)fluoranthene	B(k)F	0.6	0.6	} 3.0 for	0.5-2.0
Benzo(a)pyrene	B(a)P	1.15	1.3	B(b)F & B(k)F	5.7
Benzo(ghi)perylene	B(ghi)Per	0.9	} 2.0*	1.4 } 2.3	8
Indeno(1,2,3-cd)pyrene	-	0.3			-
Anthanthrene	-	0.1	0.18		0.26
Coronene	-	0.4	0.5	} 1.5	2
Dibenzo pyrene	-	-	-		-

*using area

Figure 1. Analytical scheme for determination of PAH in suspended particulate matter.

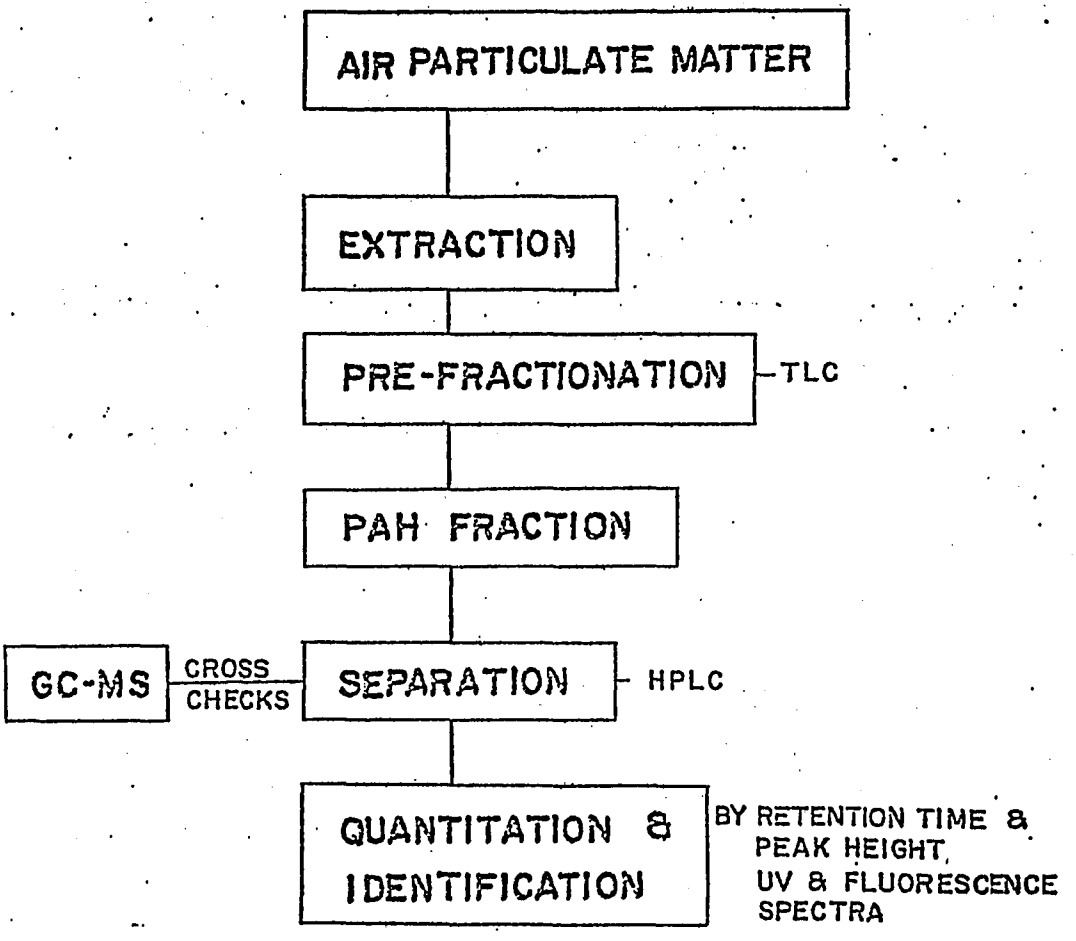

AIR PARTICULATE MATTER
EXTRACTION
PRE-FRACTIONATION
TLC
PAH FRACTION
GC-MS
CROSS CHECKS
SEPARATION
HPLC
QUANTITATION & IDENTIFICATION
BY RETENTION TIME & PEAK HEIGHT, UV & FLUORESCENCE SPECTRA

Figure 2. Typical thin layer chromatogram of the prefractionation of a New York City suspended particulate extract.

TLC SILICA GEL-G 500 microns

CYCLOHEXANE/BENZENE : 1.5 / 1.0

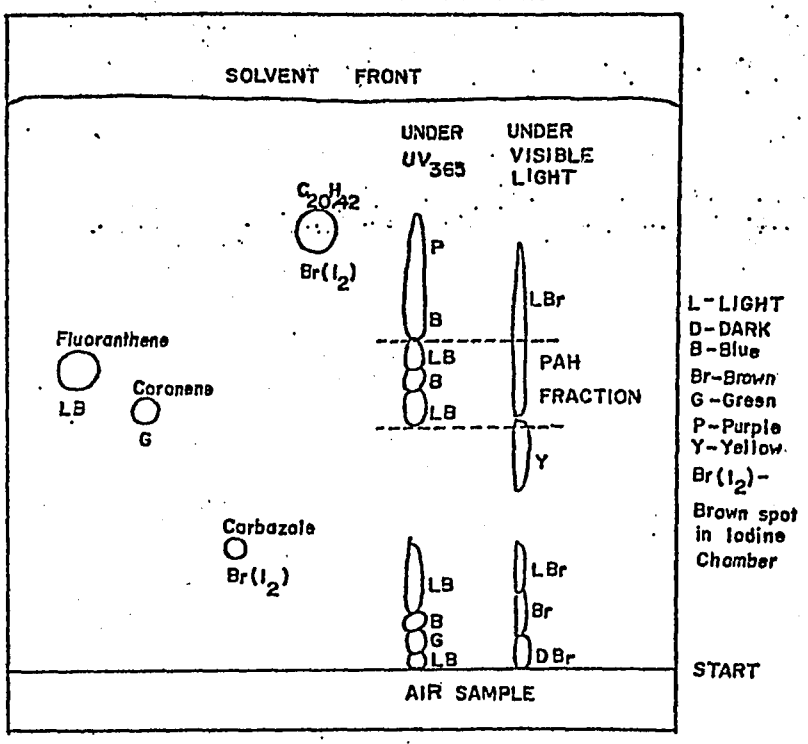


Figure 3. Liquid chromatogram of synthetic PAH mixture # 1.

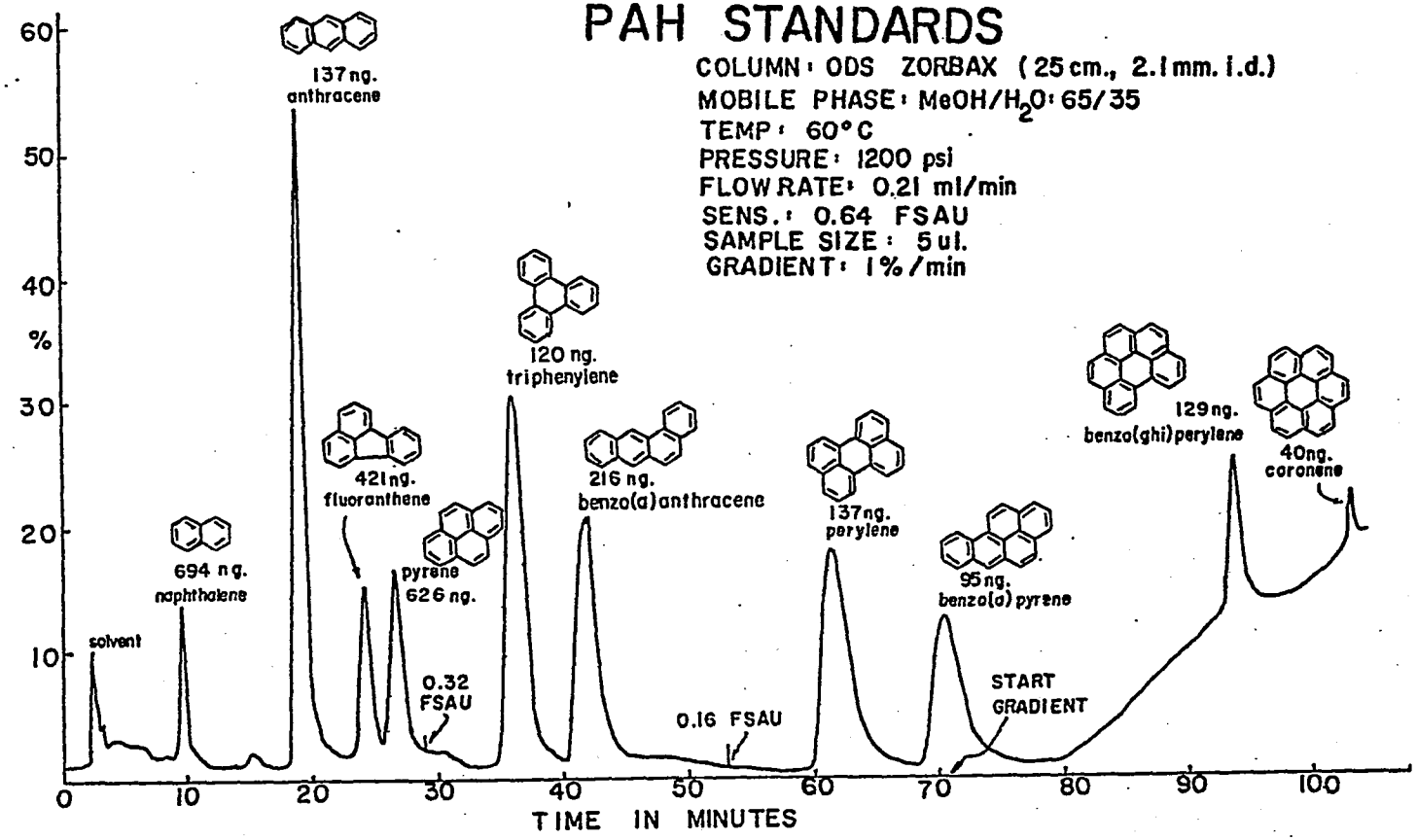

PAH STANDARDS
COLUMN: ODS ZORBAX (25 cm., 2.1 mm. i.d.)
MOBILE PHASE: MeOH/H2O: 65/35
TEMP: 60°C
PRESSURE: 1200 psi
FLOW RATE: 0.21 ml/min
SENS.: 0.64 FSAU
SAMPLE SIZE: 5 ul.
GRADIENT: 1%/min
137 ng. anthracene
120 ng. triphenylene
421 ng. fluoranthene
216 ng. benzo(a)anthracene
694 ng. naphthalene
pyrene 626 ng.
137 ng. perylene
95 ng. benzo(a) pyrene
129 ng. benzo(ghi)perylene
40 ng. coronene
solvent
0.32 FSAU
0.16 FSAU
START GRADIENT
60
50
40
%
30
20
10
0
10
20
30
40
50
60
70
80
90
100
TIME IN MINUTES

Figure 4. Liquid chromatogram of synthetic PAH mixture # 2, chromatographic conditions same as in Figure 3.

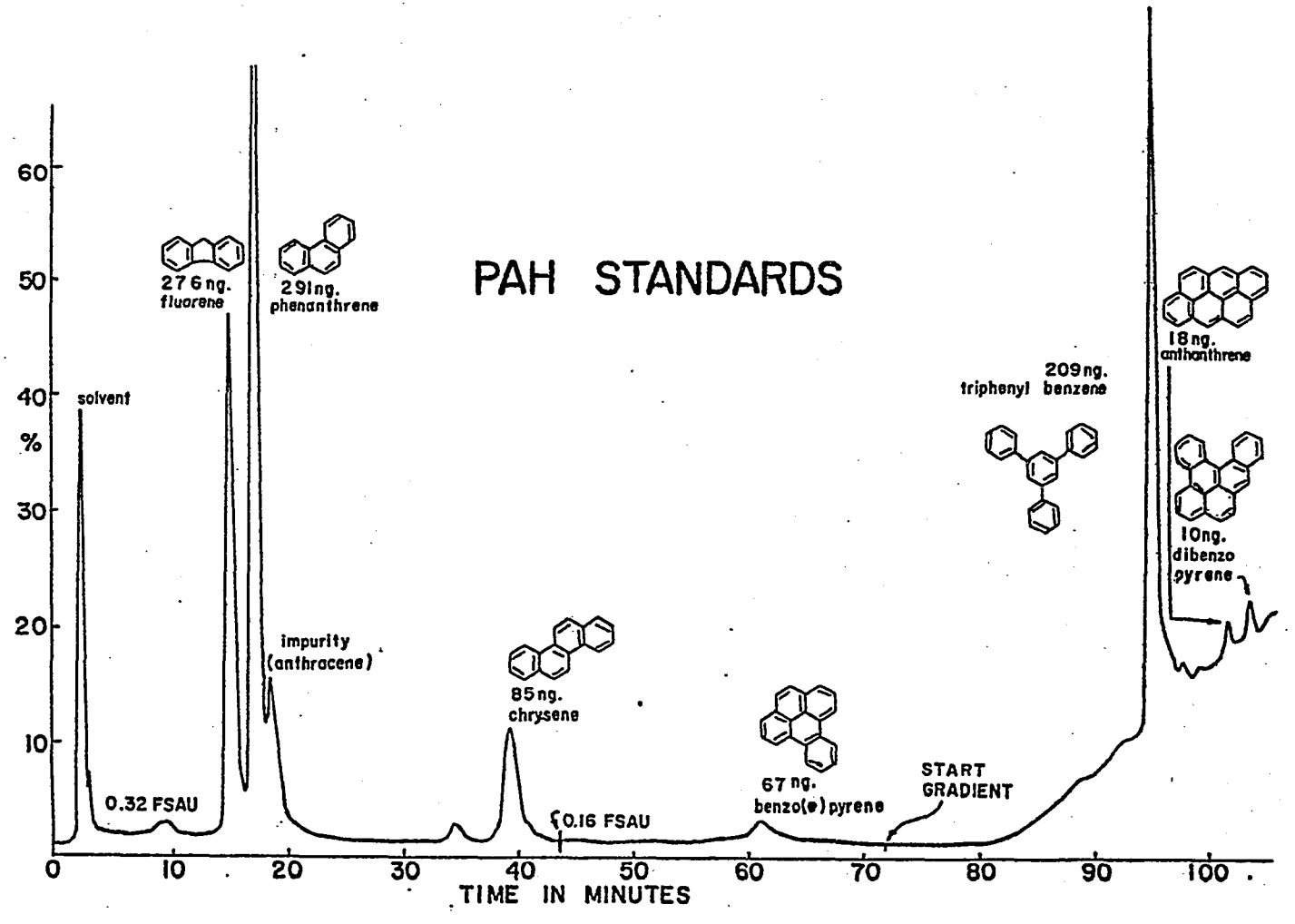

PAH STANDARDS
276 ng.
fluorene
291 ng.
phenanthrene
solvent
impurity
(anthracene)
85 ng.
chrysene
67 ng.
benzo(e)pyrene
209 ng.
triphenyl benzene
18 ng.
anthanthrene
10 ng.
dibenzo
pyrene
0.32 FSAU
0.16 FSAU
START
GRADIENT
%
60
50
40
30
20
10
0
10
20
30
40
50
60
70
80
90
100
TIME IN MINUTES

Figure 5. Liquid chromatogram of Air Sample S303, chromatographic conditions same as in Figure 3.

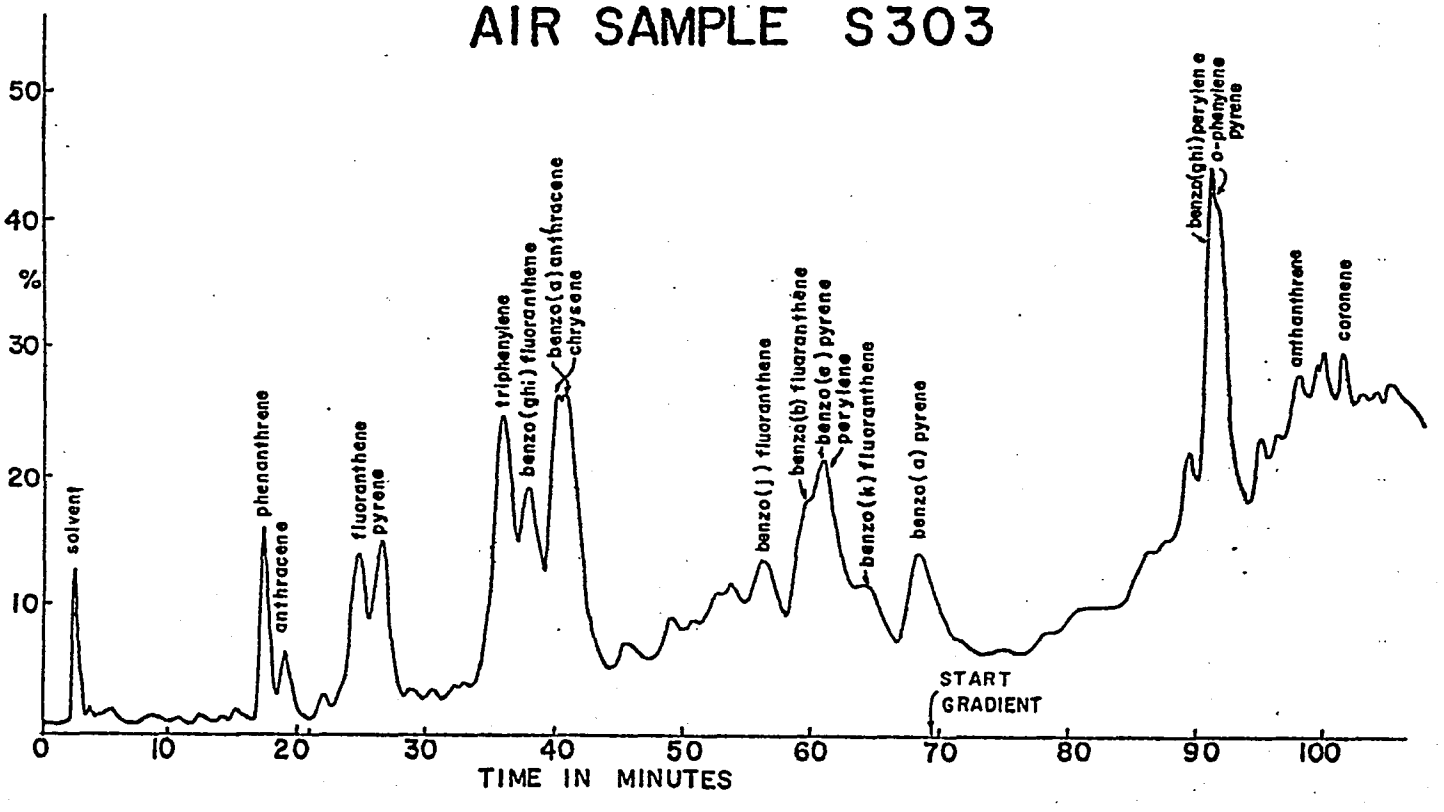

AIR SAMPLE S303
50
40
%
30
20
10
solvent
phenanthrene
anthracene
fluoranthene
pyrene
triphenylene
benzo(ghi) fluoranthene
benzo(a) anthracene
chrysene
benzo(j) fluoranthene
benzo(b) fluoranthene
benzo(e) pyrene
perylene
benzo(k) fluoranthene
benzo(a) pyrene
START
GRADIENT
benzo(ghi) perylene
o-phenylene
pyrene
anthanthrene
coronene
0
10
20
30
40
50
60
70
80
90
100
TIME IN MINUTES

Figure 6. Liquid chromatogram of Air Sample S301 and S201, chromatographic conditions same as in Figure 3.

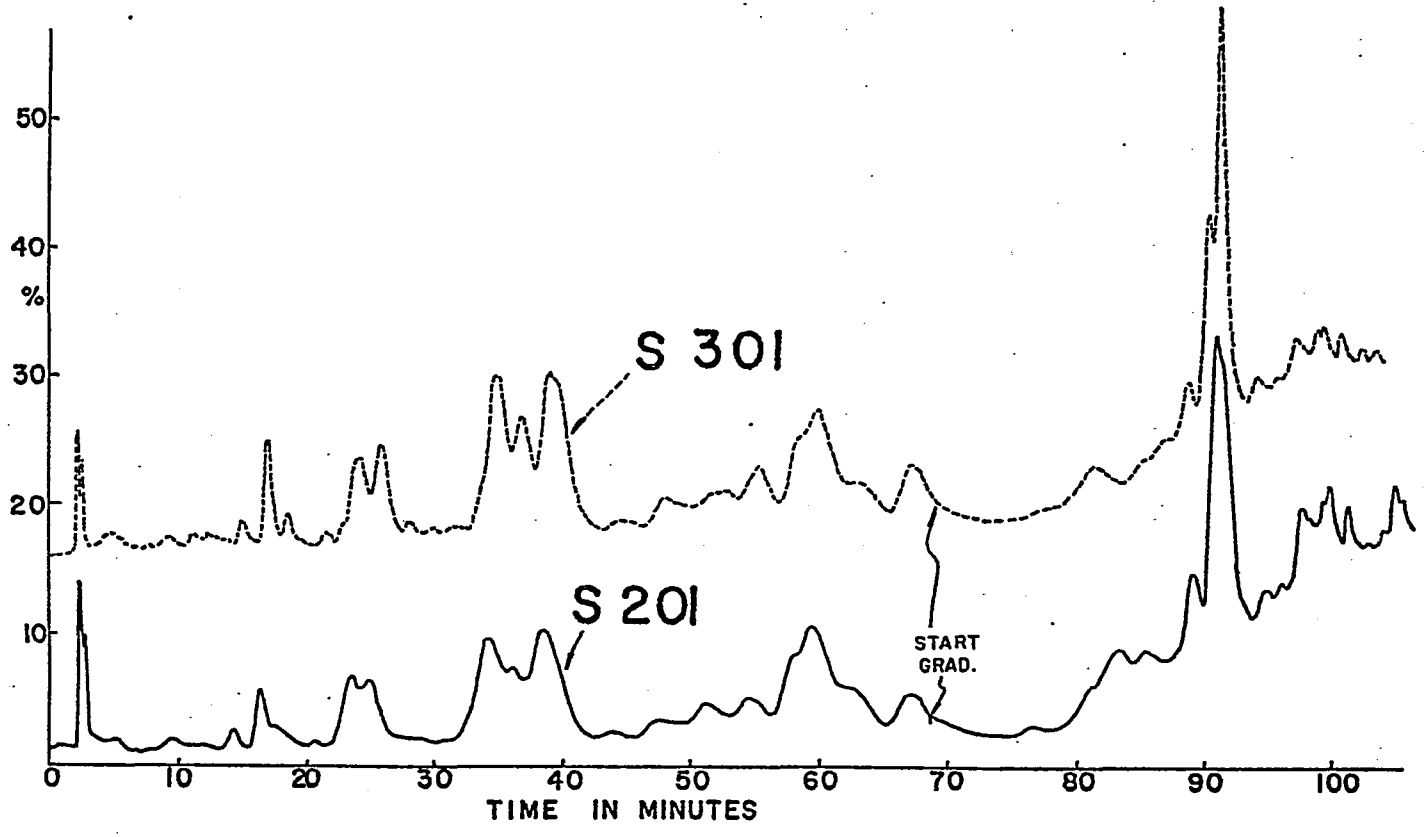

50
40
%
30
20
10
S 301
S 201
START
GRAD.
0
10
20
30
40
50
60
70
80
90
100
TIME IN MINUTES

Figure 7. Gas chromatogram of Air Sample S303. See Table I for PAH abbreviations. (m--methyl, B--benzo)

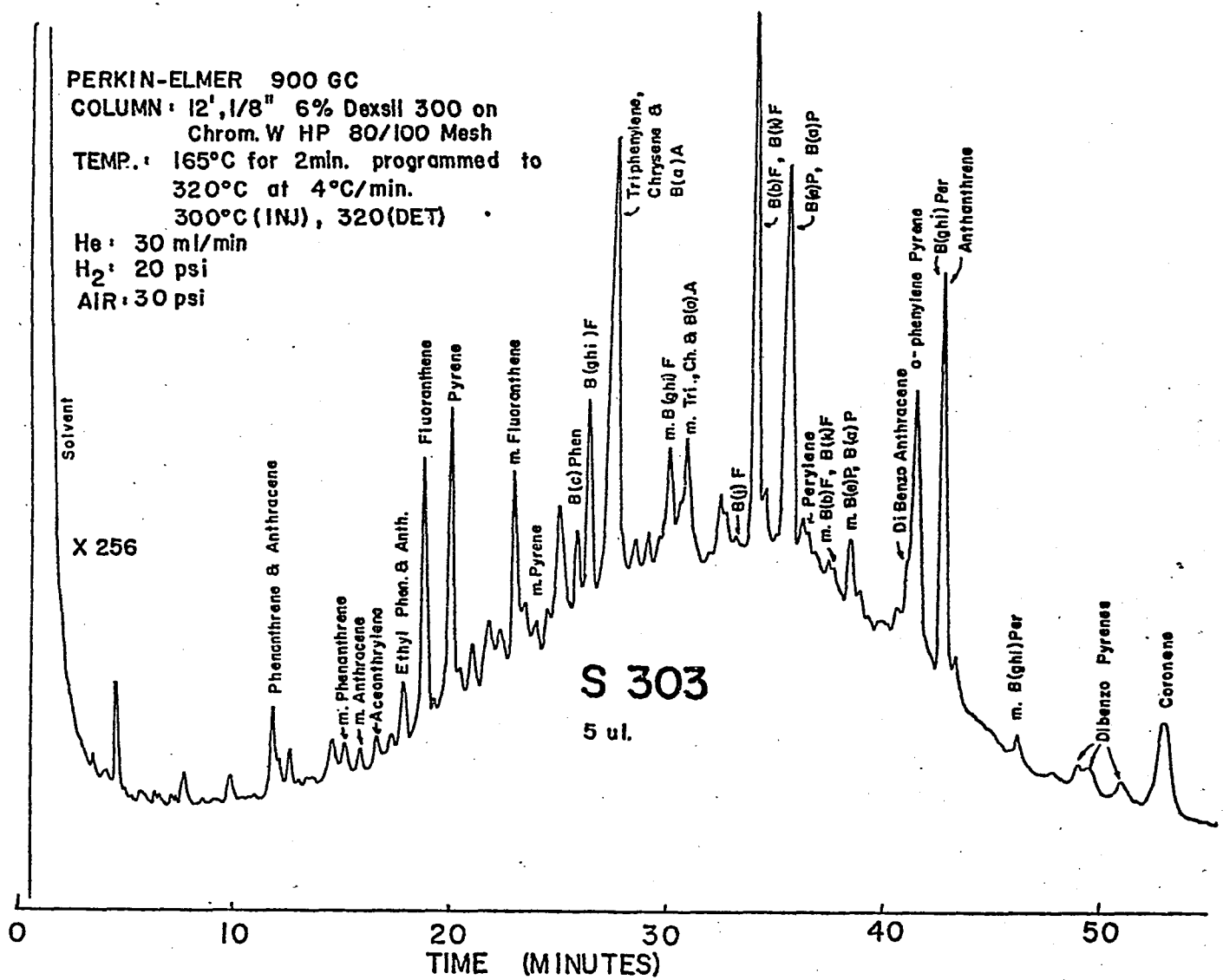

PERKIN-ELMER 900 GC
COLUMN : 12', 1/8" 6% Dexsil 300 on Chrom. W HP 80/100 Mesh
TEMP. : 165°C for 2min. programmed to 320°C at 4°C/min.
300°C (INJ), 320(DET)
He : 30 ml/min
H_2 : 20 psi
AIR : 30 psi
X 256
S 303
5 ul.
Solvent
Phenanthrene & Anthracene
m. Phenanthrene
m. Anthracene
Acenanthrylene
Ethyl Phen. & Anth.
Fluoranthene
Pyrene
m. Fluoranthene
m. Pyrene
B(c) Phen
B(ghi) F
Triphenylene, Chrysene & B(a) A
m. B(ghi) F
m. Tri., Ch. & B(a) A
B(j) F
B(b) F, B(k) F
B(e) P, B(a) P
Perylene
m. B(b) F, B(k) F
m. B(e) P, B(a) P
Di Benzo Anthracene
o-phenylene Pyrene
B(ghi) Per
Anthanthrene
m. B(ghi) Per
Dibenzo Pyrenes
Coronene
0
10
20
30
40
50
TIME (MINUTES)

Figure 8. UV spectrum of fraction 6.

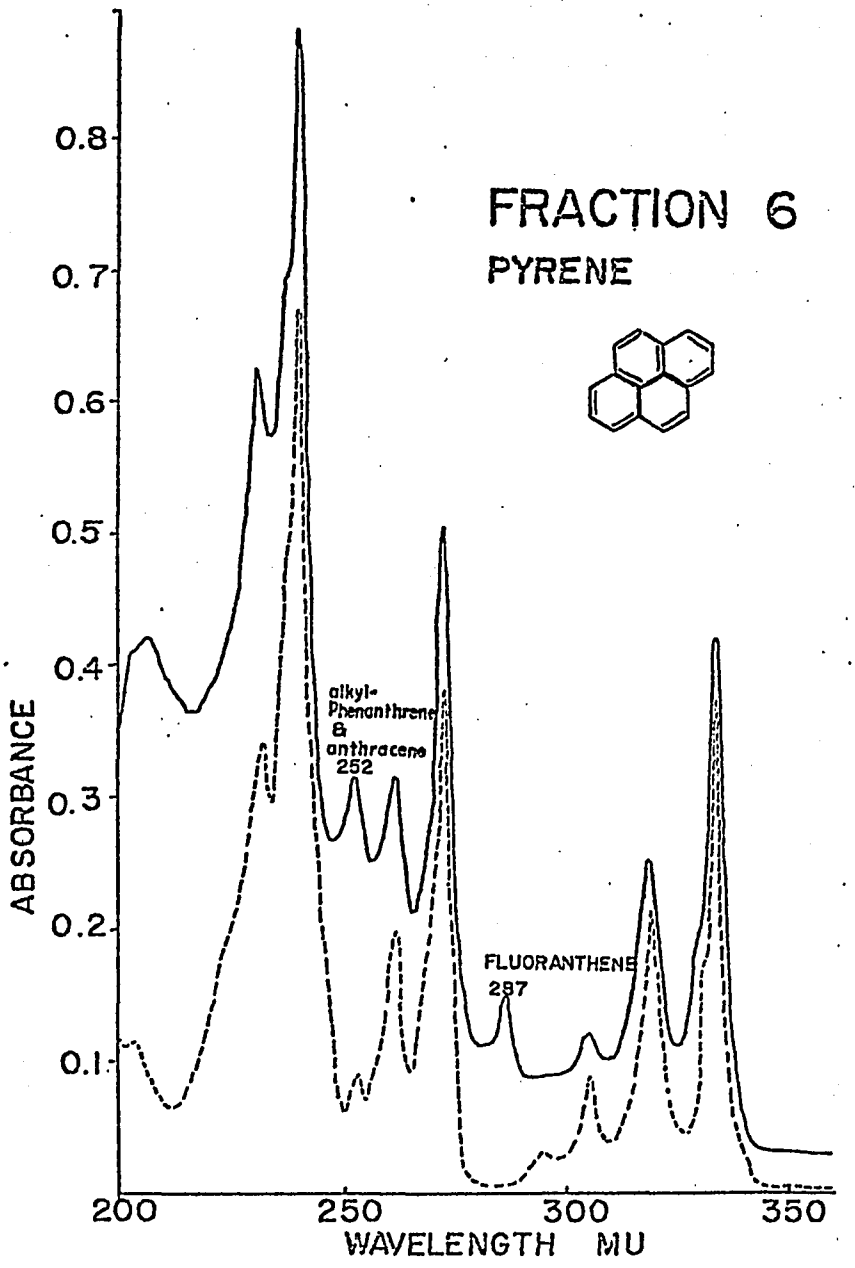

FRACTION 6
PYRENE
ABSORBANCE
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
alkyl-
Phenanthrene
&
anthracene
252
FLUORANTHENE
287
200
250
300
350
WAVELENGTH MU

Figure 9. UV spectrum of fraction 8.

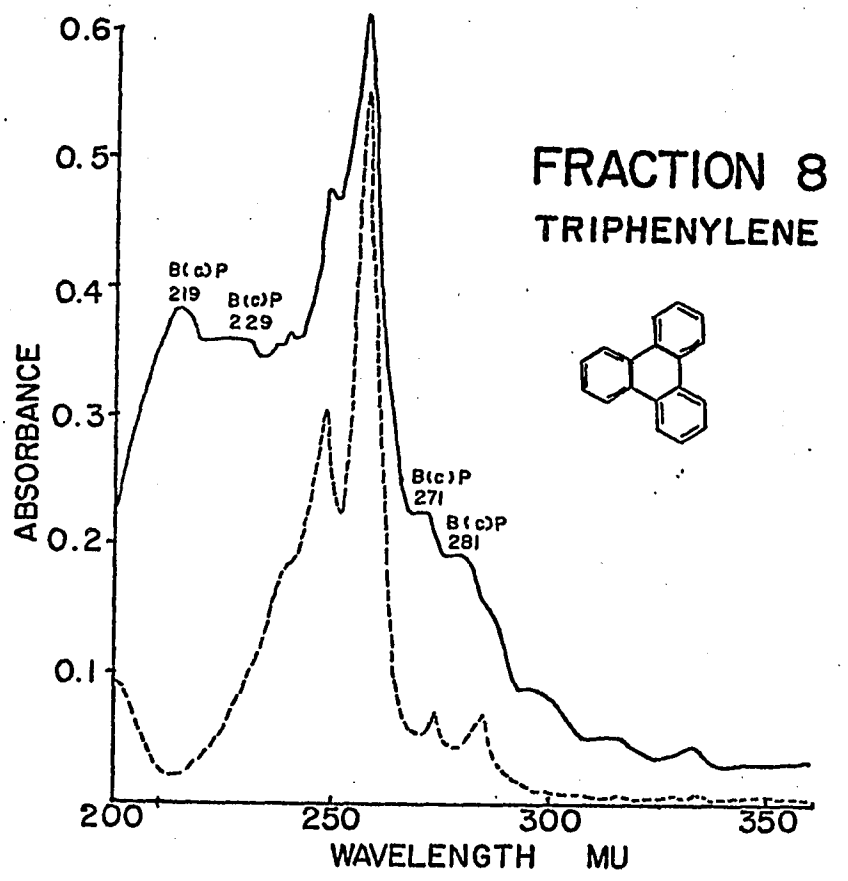

FRACTION 8
TRIPHENYLENE
B(ω)P
219
B(e)P
229
B(e)P
271
B(e)P
281
ABSORBANCE
0.6
0.5
0.4
0.3
0.2
0.1
200
250
300
350
WAVELENGTH MU

Figure 10. UV spectrum of fraction 10.

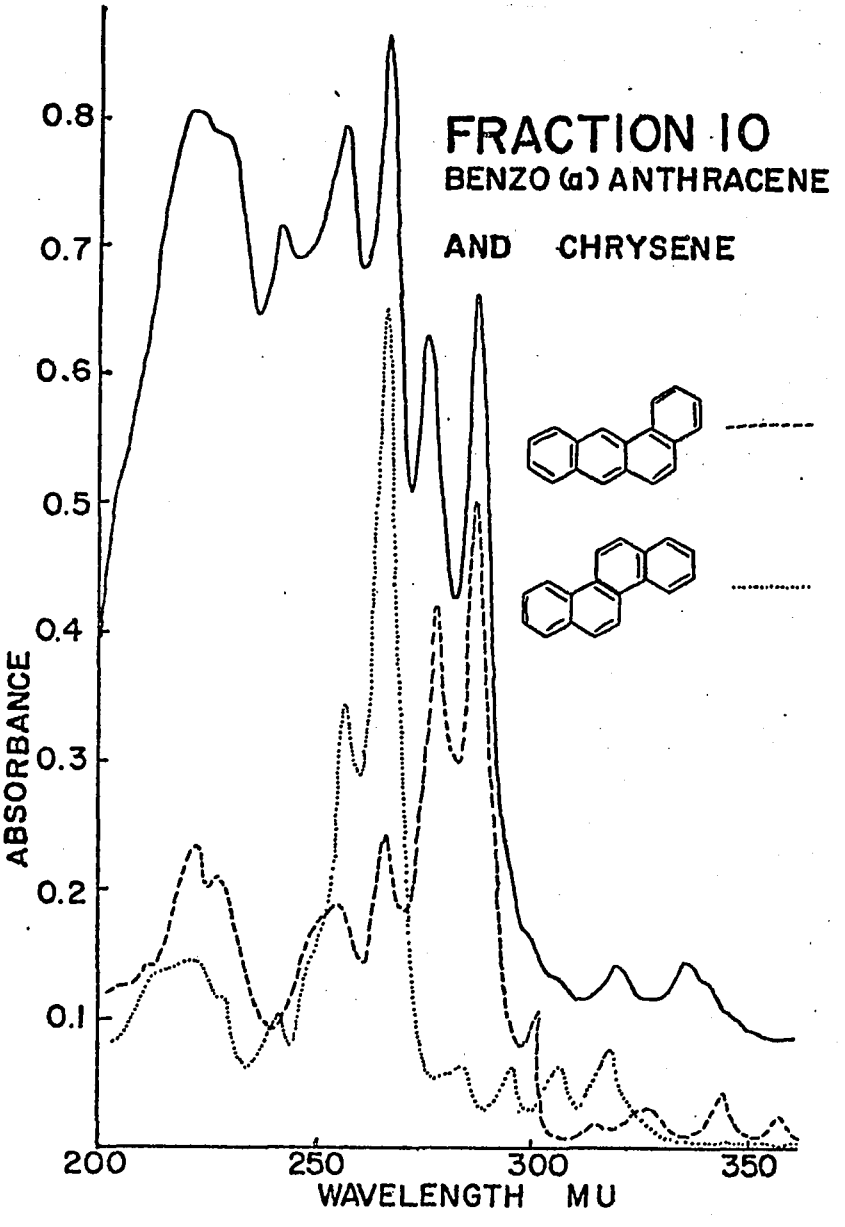

FRACTION 10
BENZO (a) ANTHRACENE
AND CHRYSENE
ABSORBANCE
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
200
250
300
350
WAVELENGTH MU

Figure 11. UV spectrum of fraction 17.

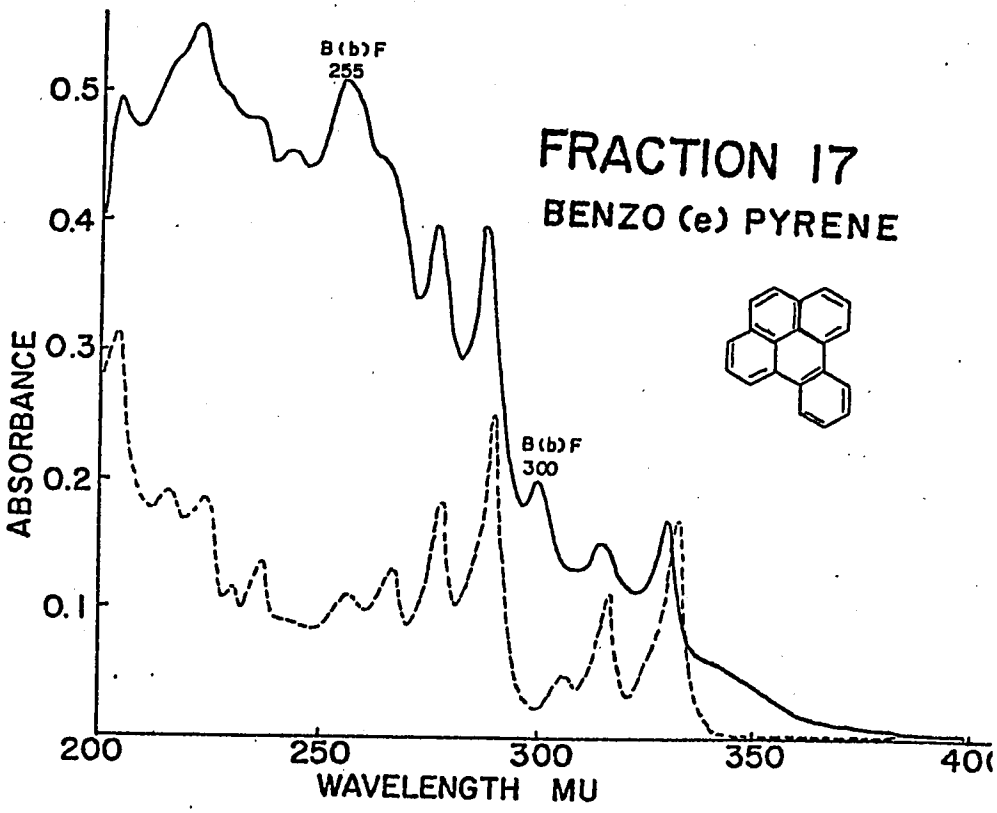

B(b)F
255
FRACTION 17
BENZO (e) PYRENE
B(b)F
300
ABSORBANCE
0.5
0.4
0.3
0.2
0.1
200
250
300
350
WAVELENGTH MU

Figure 12. UV spectrum of fraction 21.

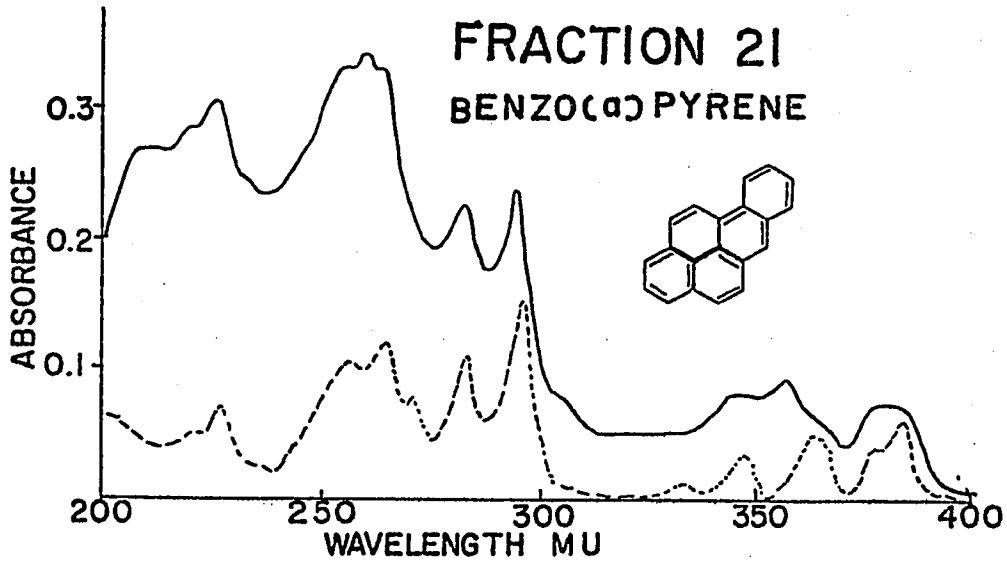

FRACTION 21
BENZO(a) PYRENE
ABSORBANCE
0.3
0.2
0.1
200
250
300
350
400
WAVELENGTH MU

Figure 13. UV spectrum of fraction 31.

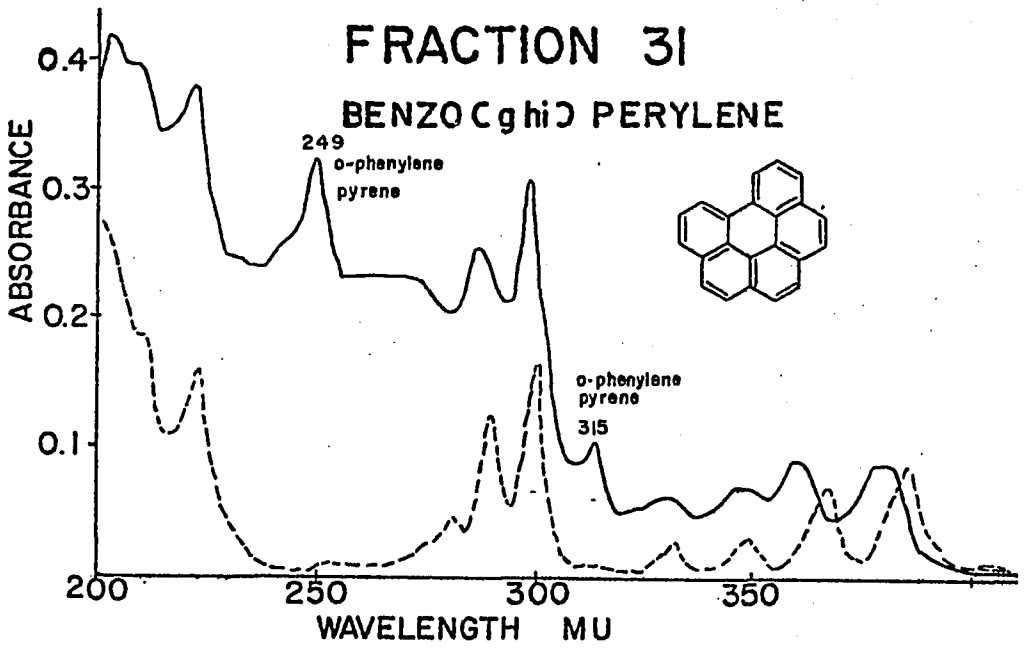

FRACTION 31
BENZO Cghi) PERYLENE
249
o-phenylene
pyrene
o-phenylene
pyrene
315
ABSORBANCE
0.4
0.3
0.2
0.1
200
250
300
350
WAVELENGTH MU

Figure 14. UV spectrum of fraction 41.

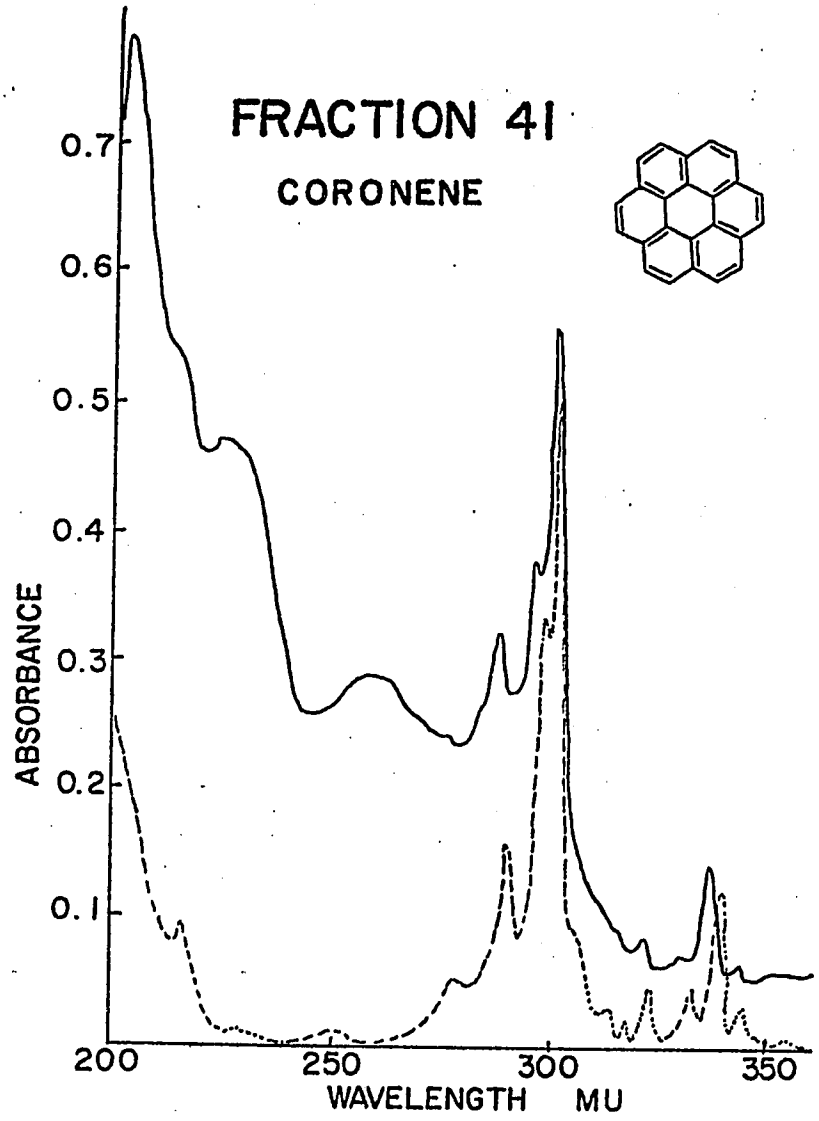

FRACTION 41
CORONENE
0.7
0.6
0.5
0.4
0.3
0.2
0.1
ABSORBANCE
200
250
300
350
WAVELENGTH MU

Figure 15. Fluorescence spectrum of fraction 21, excitation slit width 4 nm, emission slit width 4 nm.

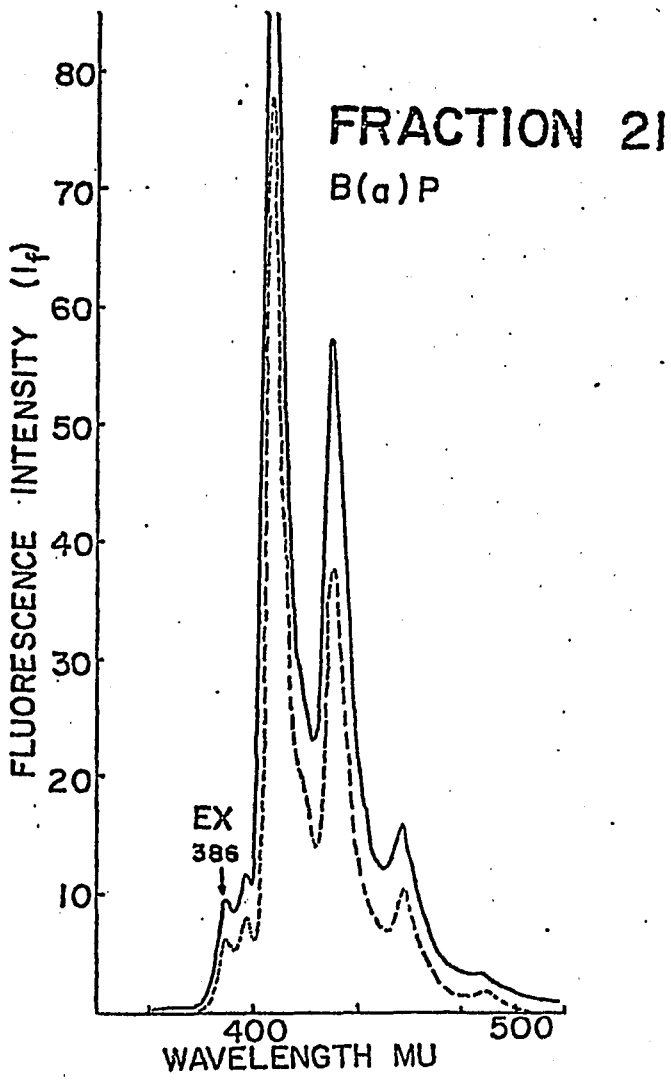

FRACTION 21
B(a)P
FLUORESCENCE INTENSITY (If)
80
70
60
50
40
30
20
10
EX
386
400
500
WAVELENGTH MU

Figure 16. Excitation spectrum of fraction 21, excitation slit width 2 nm, emission slit width 4 nm.

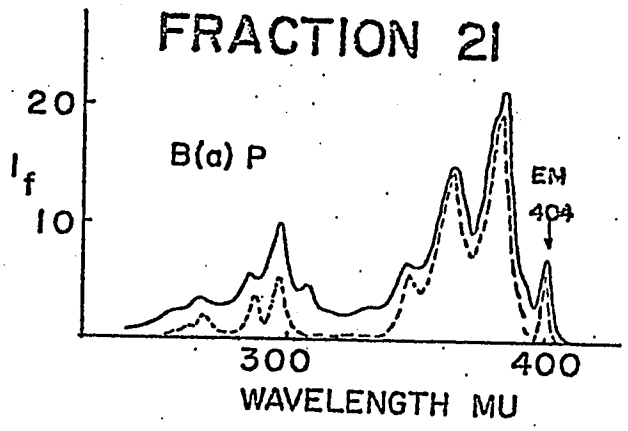

FRACTION 21
20
I_f
10
B(a) P
EM
404
300
400
WAVELENGTH MU

Figure 17. Fluorescence spectrum of fraction 19, excitation slit width 4 nm, emission slit width 4 nm.

FRACTION 19

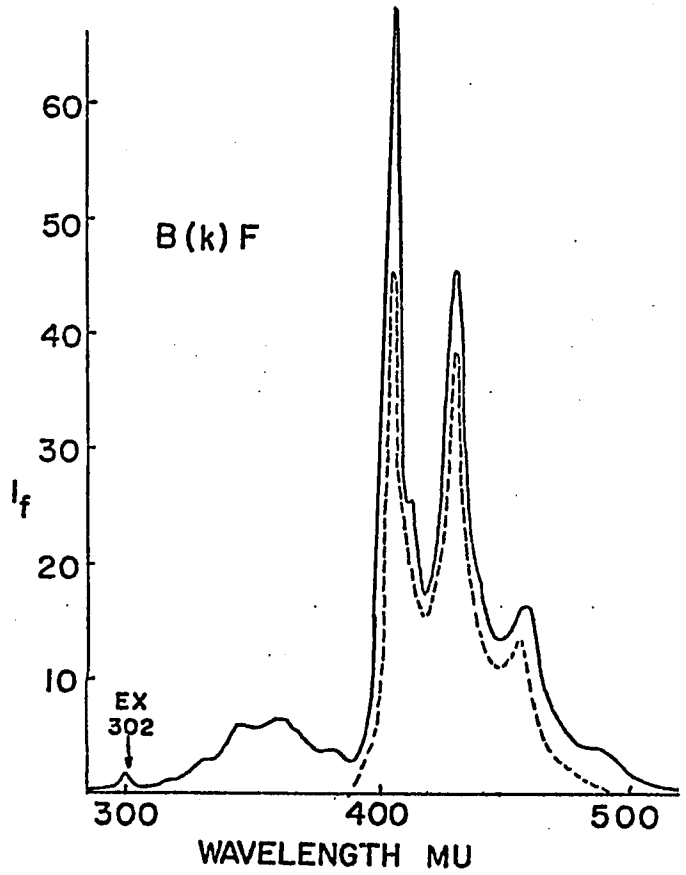
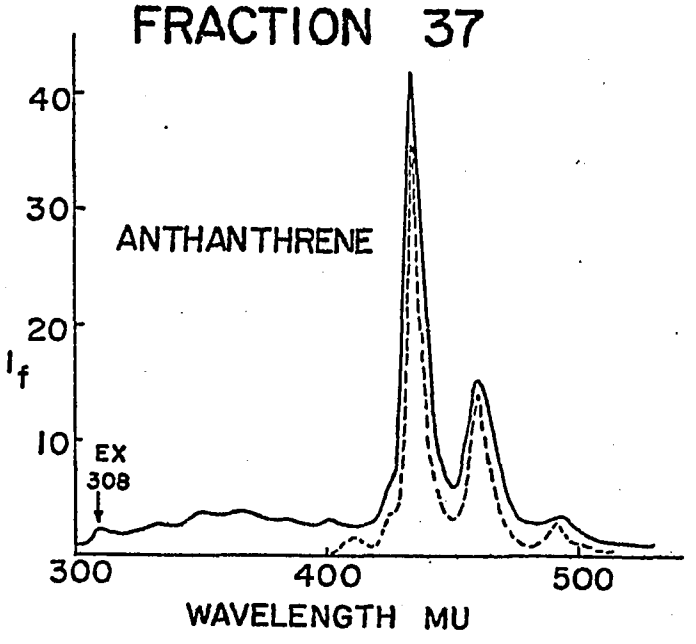


Figure 18. Fluorescence spectrum of fraction 37, excitation slit width 4 nm, emission slit 3 nm.


FRACTION 37
40
30
20
10
I_f
ANTHANTHRENE
EX
308
300
400
500
WAVELENGTH MU

PART II

CHARACTERIZATION OF AZA-ARENES AND OTHER N-BASES IN THE BASIC ORGANIC PORTION OF SUSPENDED PARTICULATE MATTER

INTRODUCTION

Analytical method of polynuclear aza-heterocyclic hydrocarbons (aza-arenes) have received some attention in the past, since a number of these compounds, such as dibenz(a,j)acridine, dibenz(a,h)acridine and some alkyl benzacridines are known animal carcinogens (1-2). Recently, quinoline has been found to induce hepatomas in rats (3). Although bioassays on aza-arenes have been scanty, it is suspected that many of these compounds might have tumorigenic activity (2). Aza-arenes are formed, as trace pollutants, during incomplete combustion of nitrogen-containing substances and are therefore found in urban suspended particulate matter (4-5), tobacco smoke (6-7), automobile exhaust (8), and many pollution source effluents (9).

Separation methods for aza-arenes have been documented. They have been separated by thin-layer chromatography (TLC)(10), paper chromatography (11), electrophoresis (12), and conventional liquid chromatography using adsorption (13) and ion-exchange packings (14). Gas chromatography (GC) has also been successfully applied with a flame ionization detector (15) or with mass spectrometry (MS) as a specific ion detector (4). Various modes of high pressure liquid chromatography (HPLC), including reversed phase (16), adsorption (16), complexation (17), and liquid-liquid partitioning (18) have also been used for the separation of aza-arene standard compounds.

Aza-arenes, with the exception of neutral indole and carbazole homologs,are found in the basic organic fraction of suspended particulate matter. Although this fraction constitutes

a small percentage (0.5-3%) of the organic matter (19), bioassay data have shown the basic fraction to be carcinogenic to infant mice when administered subcutaneously (20). Sawicki and co-workers identified aza-arenes in the basic fraction by spectroscopic and spectrofluorimetric means after chromatographic enrichment (5,9). Nineteen aza-arenes (10 parent ring compounds and 9 alkyl derivatives) were found in a Nashville sample (5). These procedures yield semiquantitative data and were most helpful in the past. More recently, modern analytical instrumentation has been used to recheck earlier results. Alberini et al (15) identified 11 aza-arenes in the basic fraction of suspended particulate matter by their retention times on glass capillary columns. Using a different approach, Brocco et al have employed a TLC-GC method and identified 7 aza-arenes, including quinoline which had not been previously identified (21). The results of the two studies were quite different from those reported by Sawicki et al but since identification were based solely on GC retention times, their data were questionable. Very recently, Cautreels and Cauwenberghe reported a detailed study of the basic fraction by mass fragmentography (4). Fifteen aza-arene compound types were identified in an Antwerp air particulate matter sample. Each type contain many isomers, undistinguished by mass spectrometry.

Frei et al (22) attempted to measure aza-arenes in POM by injecting untreated POM extracts into a silver-impregnated column in their HPLC system. However, no aza-arene peak was identifiable from the chromatogram.

In our approach, we prefractionate the basic fraction by HPLC into 8 subfractions and characterize each subfraction independently by GC-MS and reversed phase HPLC followed by uv and fluorescence spectroscopy of the isolated peak. In view of the complexity of the basic fraction, we believe that this approach offers the most reliable qualitative and quantitative analysis of aza-arenes and other unknown components in the fraction. Since mass spectra of isomeric aza-arenes, e.g. phenanthridine and benzo(f)quinoline, are identical, the use of uv and fluorescence techniques supplements GC-MS and gives unambiguous identification and quantitation of these isomers.

EXPERIMENTAL

A flow chart of the experimental procedure is outlined in Figure 1.

Reagents. Reference compounds were obtained from K&K, Aldrich Chemical Co., and Pfaltz & Bauer. 2-azafluoranthene, 13-azafluoranthene, 4-azapyrene, 11H-indeno(1,2-b)quinoline and four dimethyl quinolines were kindly donated by Dr. Hans Sauerland of the Rutgerswerke AG, Duisburg, West Germany. All solvents used were spectrograde from Fisher Scientific or from Burdick & Jackson.

Sampling. Particulate matter samples were provided by the New York City Department of Air Resources. They were collected at various sites in New York City's Aerometric Network on glass fiber filters (Mine Safety Appliance Co.) using high volume samplers. The average rate of sampling is 40-50 ft^3/min.

The weight of a 24 hr sample ranges from 90-200 mg depending on site, season, and weather conditions. Sample 1 is a composite of 180 samples collected during February to April of 1975, randomly selected from different stations. Sample 2 is a similar composite of 100 samples collected during January to March of 1975.

Extraction. Extraction of organic material from the collection filters was carried out for 8 hours in two large Soxhlet extraction apparati using pre-extracted Whatman cellulose thimbles (123 x 43 mm, Fisher Scientific). Each thimble accomodates five filters. Benzene/methanol (4:1) was used as the extracting solvent. Other workers have used $CHCl_3$ (5), benzene (4,21) or cyclohexane (15). We chose this solvent because it gives the highest amount of extractable organic matter and yields the most biologically active extract (19). We also believe that since aza-arenes are weak bases, and salt formation with inorganic and organic acids on the filter is a possibility, a polar solvent must be used.

Partitioning. The extract was evaporated to near dryness in a rotary evaporator at water bath temperature under 45°C, redissolved in 100 ml of $CHCl_3$ and partition twice with 100 ml water. The aqueous layer containing the water soluble compounds was discarded. The $CHCl_3$ layer was extracted twice with 100 ml of 10% H_2SO_4 and once with 100 ml of 20% H_2SO_4 solution. The use of a strong acid is recommended since aza-arenes are weak bases. A study using a mixture of aza-arene reference compounds has shown that they were quantitatively recovered through such a partitioning scheme and the use of

20% H_2SO_4 solution did not pose any problem of chemical alternation to aza-arenes. Emulsions were often encountered in these partitioning steps, but could be minimized by sonicating in an ultrasonic bath. The acidic layers were combined, cooled in an ice bath, and neutralized by adding saturated NaOH solution until pH 12 was reached. This solution (ca. 300 ml) was then back-extracted 3 times into equal volumes of $CHCl_3$. The combined $CHCl_3$ solution was dried over anhydrous $NaSO_4$, filtered, and concentrated to 0.1 ml. The resulting dark brown solution, the basic fraction of POM, contained aza-arenes and other N-bases.

HPLC Prefractionation. A prefractionation step is necessary because the basic fraction, especially when derived from a benzene/MeOH extraction, is very complex. A high pressure liquid chromatograph model ALC/GPC 202 (Waters Associates) equipped with model M-6000 pumps, model 660 solvent programmer and a 254 nm differential uv detector was used. Lichrosorb SI 60 silanized (30 μ diameter, Brinkman Instruments, Inc.) was dry-packed into a 2.1 mm id x 60 cm stainless steel column for the prefractionation. Using a solvent composed of 0.5% propanol-2 in n-hexane and a flow rate of 3.0 ml/min, most aza-arenes will elute in between 4-25 min. Fifteen minutes after the injection of an aliquot of the basic fraction, the solvent composition was linearly programmed to reach 20% propanol-2 in $CHCl_3$ in 20 min. Eight subfractions (10-50 ml) were collected, concentrated and each redissolved in 100 μl of MeOH.

GC-MS Analysis. Aliquots of these subfractions were injected into a Hewlett Packard model 5982 dual source combined GC-MS system interfaced with a Hewlett Packard model 5933A

computerized data system. Mass spectra were obtained by electron impact at 70 eV. A 10-ft, 1/8" od stainless steel column, packed with 6% Dexsil 300 on Chromosorb W(HP), 80/100 mesh, was used at 60 ml/min flow rate. Temperature programming from 160-300°C at 8°C/min was initiated immediately after injection of each sample. Other gas chromatogram were obtained on a Hewlett Packard 5710A gas chromatograph with dual columns and flame ionization detectors.

HPLC Reversed Phase Analysis. A μ-Bondapak/C_{18} reversed phase column (Waters Associates) was used in the high pressure liquid chromatography described above. A solvent gradient from an initial composition of 20% CH_3CN/H_2O to a final composition of 80% CH_3CN/H_2O at 4%/min and a 3.0 ml/min flow rate was found to give satisfactory separation of most aza-arenes in 20 minutes.

Injections of 1-2% (ca. 1-2 μl) of the subfractions were sufficient for good HPLC chromatograms. However, the use of over 30% (ca. 30 μl) of the subfraction was necessary when effluent fractions were to be analyzed by uv. Fractions (ca. 1 ml) sufficient to fill a uv microcell (4 x 10 mm) were taken. A Cary 14 UV-VIS spectrophotometer and a Perkin Elmer MFP-2A spectrofluorometer were used to obtain spectra. Reference spectra were either obtained from aza-arene standard or from the literature (23).

Extraction of 10 unexposed filters (blanks) and subsequent analysis by the same procedure yielded no aza-arene.

RESULTS AND DISCUSSION

Chromatographic Separation of Aza-arenes. Aza-arenes

were separated using HPLC, GC and TLC chromatographic systems. The pertinent data are listed in Table I. The highlights of these separations are summarized here.

Gas Chromatography. The success of our Dexsil column in separating PAH (24) prompted us to try this system to separate aza-arenes. Excellent separation of aza-arene standard mixture were obtained virtually on the first trial. Although most earlier workers used glass columns for this purpose (15, 21), we experienced no problem with our stainless steel columns, which are rugged and are more convenient to use. Figures 2 and 3 show two gas chromatogram of aza-arene standard mixtures separated under conditions described in the experimental section. A temperature program of 160°C to 300°C at 8°C/min gives adequate separation within a reasonable time. Dibenz-(a,j)acridine elutes at 68 min under these conditions. The final temperature in the program is not determined by the stability of the stationary phase, which can be used up to 400°C, but is dictated by a silicone membrane molecular separator (temperature limit- 300°C) located inside the oven in our GC-MS system. Actually, a temperature program of higher final temperature (e.g. 160°C to 320°C at 8°C/min) gives faster analyses and sharper peaks with little deterioration in resolution.

The order of elution closely follows the boiling points of aza-arenes and is mainly governed by the number of aromatic rings (Table I). Most of the isomeric aza-arenes are well-separated, e.g. quinoline from isoquinoline, benz(a)acridine from benz(c)acridine, dibenz(a,j)acridine from dibenz(a,h)-

acridine, etc, since the difference in their boiling points are substantial. Phenanthridine and benzo(f)quinoline, one of the most difficult isomeric pairs to separate, can be separated by HPLC on an adsorption column (Figure 5).

High Pressure Liquid Chromatography. Both reversed phase and adsorption liquid chromatography have been explored. The separation obtained on reversed phase is more satisfactory and is used for the isolation of individual aza-arenes in the sample. Adsorption chromatography is used for sample prefractionation.

Reversed Phase Chromatography. Figure 4 shows chromatograms of 2 standard synthetic aza-arene mixtures on the μ-Bondapak/C_{18} column. A linear solvent program of 20% to 80% CH_3CN in water at 4%/minute is used. Conditions used in Figure 4 represent a compromise between analysis time and chromatographic efficiency and should not be regarded as optimum conditions for best resolution. The solvent gradient allows samples with a wide range of polarity to be separated while improving peak sharpness, and, consequently, the detection limit. One can easily expand the solvent programming limits to accommodate samples of greater complexity and polarity range.

The structures and retention times of individual aza-arenes are listed in Table I. Similarly to the separation of polycyclic aromatic hydrocarbons (24), the separation of aza-arenes on the C_{18}-reversed phase packing is governed mainly by the number of aromatic rings. The 2-ring quinoline elutes first, followed by compounds having a higher number of rings.

The elution order can presumably be explained by the relative solubilities in the mobile phase as suggested elsewhere (25), although comprehensive solubility data are not currently available in the literature.

Isomeric aza-arenes, e.g. acridine and benzo(f)quinoline (see Figure 4) are often separated by reversed phase chromatography, although they can also be separated by GC. The μ-CN column (Waters Associates) shows essentially the same selectivity towards aza-arenes as the C_{18} bonded phase, but it is less retentive for the same solvent program.

Adsorption Chromatography. The separation of aza-arene standards on the μ-Porasil column (Waters Associates) shown in Figure 5 seems to depend on the steric availability of the aza-nitrogen electron pair, as suggested by a number of workers (10,17). Thus, compounds such as dibenz(a,h)acridine, benzo(h)quinoline and benzo(c)acridine (Figure 5) are weakly retained because their nitrogen electron pairs are sterically well protected. As might be expected, elution order on the μ-Porasil strongly resembles the TLC results reported by Sawicki et at (10). Hexane modified by 1% propanol-2 gives adequate separation of the synthetic mixture in 10 minutes without column activation. Lower percentage of propanol-2 causes excessive tailing of some peaks, e.g. benzo(f)quinoline, and quinoline, presumably as a result of stronger hydrogen bonding to the silanol groups on silica. Other modifiers like CH_3CN and MeOH produce very similar separations. Theoretical plate numbers in excess of 2000 are calculated for most peaks. Selectivity achievable using a Lichrosorb SI 60 silanized

column is similar to that of the μ-Porasil, although aza-arenes are retained to smaller extent. The efficiency of the silanized silica column is also lower because of the larger particle size (30 μ). This results in poorer separation, especially for the compounds eluting later (Fig. 6).

However, since silanized silica gel is a weaker adsorbent than untreated silica, this column is used for the prefractionation of the POM basic portion to prevent any possible irreversible adsorption of the sample.

HPLC Sensitivity Study. Figure 7 shows the separation of a mixture containing 16 ng of each aza-arene using a μ-Bondapak/C_{18} column and an isocratic solvent composed of 60% CH_3CN in H_2O. For good sensitivity, gradient elution is generally not used (26). Figure 7 also shows a similar chromatogram of the same mixture containing 7 ng of each aza-arene separated on a μ-Porasil column, under the conditions described in Figure 5. Except for compounds like quinoline, which has low absorptivity at 254 nm, the detection limit for most aza-arenes is about 1 ng under the above HPLC conditions.

Thin-layer Chromatography. Separation by TLC was also explored. Although relatively poor in resolution and reproducibility, TLC is a useful supplement to HPLC and GC data in sample analysis. Because most aza-arenes are fluorescent, they can be detected selectively under uv light as colored spots on a TLC plate. R_f values are listed in Table I. TLC separation of aza-arene standard mixtures and of the basic POM sample subfractions are shown in Figure 8. Silica gel

TLC plates (250 μ thick with fluorescent indicator, Brinkman Instruments) developed with 10% CH_3CN in $CHCl_3$ were found to give satisfactory separation.

Difficulties in this analysis. The concentrations of aza-arenes in New York City air were found to range from 5-300 ng/1000 m^3 during our sampling period, roughly 10-100 times lower than the concentration of benzo(a)pyrene. The total amount of individual aza-arenes isolated rarely exceeded a few μg even after sampling large volumes of air. The analysis of these trace quantities in a complex environmental matrix presents several difficulties. In the past, these problems were partially circumvented by relying heavily on fluorescence techniques which are very selective methods for identifying aza-arenes. However, we believe that our approach, using 2 different high resolution chromatographic methods followed by a battery of spectroscopic techniques, gives the most detailed and unequivocal characterization of the basic fraction.

Sample contamination is another problem in trace analyses. All glassware was washed in chromic acid, thoroughly rinsed with water, and triply rinsed with spectrograde solvent. Trace contaminants found in TLC and conventional LC packings often gives erroneous result in trace analysis. These packings are therefore avoided completely. A HPLC prefractionation is preferred because the packings are always prewashed. This step gives quantitative recovery and is extremely reproducible as well as convenient. The

entire prefractionation can be completed in one hour, and cut-off points between fractions are easily determined by the uv monitor (Fig. 9).

Sample Size. In our study, composites of over 100 filters were used for detailed analyses. For routine analysis of major aza-arenes, a composite of 10 filters should be quite sufficient.

GC-MS of Subfractions. A computerized data system greatly enhances the power of GC-MS system. It simplifies data handling and allows convenient subtraction of mass spectral background. It enables the use of mass fragmentography or specific ion monitoring (Figures 10 and 11) which has become a powerful tool for routine analysis and sample screening. Also, the use of a computerized mass spectral search system greatly facilitates mass spectral comparisons and, therefore, the identification of unknowns.

It should be indicated that many components of the basic fraction are only tentatively identified or remained unidentified even after detailed searching of reference mass spectra in the literature (27) and in our Hewlett Packard disc reference library. The acute lack of reference mass spectral data, especially in the field of air pollution, is still a major hindrance to a more complete characterization. Fortunately, the aza-arenes as a class give very characteristic fragmentation patterns which are quite easily recognizable. Under electron impact, they yield strong molecular ion peaks and usually M-27 or M-28 peak corresponding to the loss of HCN or H_2CN fragments (28). The mass spectrum of quinoline

shown in Figure 12 is quite representative of other aza-arenes and almost identical to the mass spectrum of isoquinoline. The mass spectrum of the quinoline isolated from Sample 1, Fraction IV, is very similar to that of pure quinoline (Figure 12). Confirmation by mass spectroscopy is important in quantitative analysis by GC since one peak often contains several components. Figures 13-15 are gas chromatograms of Fractions III, IV, and IVA respectively. Most of the labelled peaks were identified by both retention time and by mass spectral data.

Specific Ion Monitoring. Figure 10 shows the simultaneous monitoring of quinoline, isoquinoline and many alkyl quinolines in the sample, and demonstrate the use of mass fragmentography. The traces are normalized with respect to the quinoline peak. The relative concentration of each alkyl quinoline can be estimated from its total integrated area under each chromatographic peak. Figure 10 illustrates isoquinoline eluting after quinoline and shows the surprisingly high concentration of dimethylquinolines in New York City's air (c.f. methylquinolines). The C_3-alkyl quinoline certainly would have escaped detection without the use of this technique. Figure 11 shows similar chromatogram on Fraction IVA. Unlike Figure 10, the chromatograms are normalized with respect to the highest peak in each specific ion trace. Using this type of normalization, the C_3-isoquinolines are quite prominent.

HPLC. Figures 16-18 are reversed phase HPLC chromatograms on subfractions III, IV, and IVA respectively. Most labelled

peaks are identified by retention times, uv and fluorescence spectra of the collected fractions. Compared to the GC traces (Figurs 13-15), HPLC gives cleaner traces since the uv detector is quite sensitive to aza-arenes which have molar absorptivities of 10^4-10^5 at 254 nm. The detection limit for most aza-arenes is 1 ng which compares favorably with that of GC, especially for the 5-ring, high boiling aza-arenes, e.g. dibenzacridines.

UV and Fluorescence Spectra. Figure 19 shows a uv spectrum of isoquinoline isolated from fraction IVA of Sample 1. The dotted line is the spectrum of the pure compound at similar concentration taken from the literature (23). Figures 20 and 21 are similar fluorescence and excitation spectra of isolated benzo(f)quinoline. The spectra are not corrected for source intensity and detector wavelength response variations.

Quantitation. Quantitative data on Samples 1 and 2 were obtained as follows. Two synthetic mixtures of aza-arene reference compounds were carefully prepared. An aliquot of these solutions was injected routinely to generate retention data and to check column reproducibility. The area under each peak was compared to that derived from an aliquot injection of a sample subfraction solution, usually after its identity and purity had been assured by mass spectrometry. Isomers like phenanthridine and benzo(f)quinoline were quantitated from uv spectra of collected fractions separated by HPLC.

Concentrations of Aza-arenes in New York City Suspended Particulate Matter. The concentrations of aza-arenes and other N-bases from the basic fraction are summarized in Table II.

2-ring Aza-arenes. Quinolines escaped identification by Sawicki et al since they possess low molar absorptivity (3.8×10^3 at 308 nm) and do not fluoresce. We have confirmed Cautreels and Cauwenberghe's finding (4) by independently discovering quinoline in our New York City samples, and have extended their observations by demonstrating with quantitative data the complexity of the quinoline (Fraction IV) and isoquinoline (Fraction IVA) fractions. In our two New York City samples, the concentration of isoquinoline exceeds that of quinoline by 3-7 times. The highest concentration of aza-arenes (310 ng/1000m^3 in Sample 1) is associated with a methyl derivative of isoquinoline (Figure 11). The exact structure has not yet been determined because of the lack of a reference compound; however, boiling point data (29) suggests its identity to be a 5- or 8-methylisoquinoline. Its mass spectra is shown in Figure 22. It is possible that there are very specific precursors for this compound.

It should be emphasized that the reported levels of quinolines and isoquinolines represent the minimum concentrations in ambient air because quinoline (b.p. 237°C) and isoquinoline (b.p. 242°C) are probably not quantitatively trapped by the glass fiber filter during sampling. Their retention on glass fiber filter might be correlated to their

hygroscopicity or possibly to their basicity.

3-Rings. Of the five isomers of 3-ring aza-arenes found in our samples (Table II), benzo(f)isoquinoline has the highest concentration (3 times that of acridine in Sample 1) in our samples. This compound has not been previously isolated. It was identified by its chromatographic behavior on silica, mass spectrum (Figure 23), uv (Figure 24) and fluorescence spectra (Figures 25 and 26).

4 and more Rings. Azapyrenes, azafluoranthene and traces of 11H-indeno(1,2-b)quinoline are the only 4-ring aza-arenes found in our samples. No benzacridines or dibenzacridines were found, even though our detection limit for these compounds in the HPLC reversed phase is about 1 ng.

It is interesting to note that we found much higher concentrations of 2-ring aza-arenes and progressively smaller amounts of higher ring compounds in our samples. The reverse observation was reported for the Antwerp sample studied by Cautreels and Cauwenberghe (4), who found relatively higher concentrations of 4- and 5-ring aza-arenes compared to the 2-ring compounds. The fact that European cities generally burn more coal might explain this important difference.

Others. Benzothiazole, a common component in fuel oil was found in Fraction II. It was identified by its GC and HPLC retention times and its mass spectrum (Figure 27). So far, no S-containing organic compound from POM has ever been reported to our knowledge (19). The discovery of more S-containing compounds is expected through the use of a

sulfur selective flame photometric GC detector.

Caffeine, in relatively high concentration, was found in fractions VII and VIII. The ubiquity of coffee roasting plants in New York City and in adjacent New Jersey might explain its presence. This finding is of biological interest since a recent assay has reveal that caffeine can inhibit the carcinogenicity of PAH (30). It was identified by matching the retention time in GC (Figure 28), the retention time in HPLC (Figure 29), uv spectrum (Figure 30) and mass spectrum (Figure 31) to those of pure caffeine. Subsequent analysis of POM from 6 specific stations (Stations 1,9,10,11, 12,31) showed the presence of caffeine in all of those samples. Concentrations of caffeine vary from about 6.0 $\mu g/1000m^3$ at Station 1 (South Bronx), Station 10 (East Manhattan) and Station 11 (Greenpoint, Brooklyn) to less than 2.0 $\mu g/1000m^3$ in the other stations.

Nicotine. Nicotine, derived solely from cigarette smoke, is a common pollutant in indoor air. Its presence in outdoor air has never been reported to our knowledge. During our analyses, nicotine was found to be a major component in Fractions VII and VIII (Figure 28). Its identity was confirmed by GC retention time and mass spectral data (Figure 32). Calculation showed that, if all the nicotine came from ambient air, its concentration would be about 4 $\mu g/1000m^3$ in Sample 1. We questioned these data since nicotine can be introduced during sampling or analysis. Investigation of the smoking habits of the personnel who acted as sample collectors suggested

the possibility of nicotine contamination during the collection of samples. Subsequent analyses of 2 POM samples collected by drivers with smoking habits (Stations 1 and 10) confirmed this hypothesis and showed exceptional high concentrations of nicotine in these samples. Samples from the other stations analyzed had low or no nicotine. We conclude from these findings that most of the nicotine in our samples probably is associated with contamination during collection and is not derived from ambient air. Other tobacco specific components, e.g. myosmine, bipyridyl, etc., were not found because of their much lower concentrations than nicotine in cigarette smoke. Since the determination of ambient outdoor nicotine concentration is desirable, we urge special precaution be exercised to avoid this type of contamination during sampling by any future investigator in this area.

CONCLUSIONS

This analytical study has produced valuable data on the composition of the basic fraction of POM, both in terms of new compounds identified and the relative concentrations of components. The abundance and complexity of the quinoline and isoquinoline fractions, relative to other aza-arenes, should be noted. The recent finding on the carcinogenicity of quinoline is an additional reason for more analytical work on the basic fraction. Nevertheless, because of the minuteness of the observed quantities of aza-arenes and because of

the insufficiency of bioassays on these compounds, we hesitate to assign a biological significance to their presence in the basic fraction of urban pollutants.

Our approach is adaptable to other environmental pollutants such as cigarette smoke, and emission from coal tar and petroleum products processing where aza-arenes are present in significantly higher concentrations. Since aza-arenes, like polynuclear aromatic hydrocarbons, are pyro-synthesized during combustion, it is expected that they are widely distributed in the environment (31), and that they will receive more attention in future environmental research.

SUMMARY

Aza-arenes, formed as trace pollutants by incomplete combustion of N-containing organic matter, are found in the basic fraction of suspended particulate matter from New York City's air. Particulate matter collected on glass fiber filters are Soxhlet-extracted using benzene/methonal (4:1) and the basic fraction derived from the extractable matter is prefractionated by HPLC. The subfractions are further separated by GC-MS and HPLC, followed by spectroscopic identification.

This approach leads to the unambiguous identification of over 20 aza-arenes and other N-bases previously unidentified in ambient samples, and provides insight into the complexity of the basic fraction. Quantitative data also show an unanticipated abundance of quinolines, isoquinoline and their alkyl derivatives in the basic fraction.

LITERATURE CITED

1. P. Shubik and J.L. Hartwell, "Survey of Compounds Which Have Been Tested for Carcinogenic Activity", U.S. Public Health Service Publ. 149, 1951; Suppl. 1, 1957; Suppl. 2, 1969; 1968-1969 Vol., 1972; 1961-1967 Vol. Sect. I and Sect. II, 1973; 1970-1971 Vol., 1974.

2. J.C. Arcos and M.P. Argus, "Chemical Induction of Cancer", Vol. IIA, Academic Press, New York, 1974.

3. K. Hirao, Y. Shinohara, H. Tsuka, S. Fakushima, M. Takahashi and N. Ito, Cancer Res., 36, 329 (1976).

4. W. Cautreels and K. Van Cauwenberghe, Atmos. Environ.,10, 447 (1976).

5. E. Sawicki, S.P. McPherson, T.W. Stanley, J. Meeker and W.C. Elbert, Int. J. Air Water Pollut., 9, 515 (1965).

6. B.L. Van Duuren, J.A. Bilbao and C.A. Joseph, J. Natl. Cancer Inst., 25, 53 (1960).

7. E.L. Wynder and D. Hoffmann, "Tobacco and Tobacco Smoke", Academic Press, New York, 1967.

8. E. Sawicki, J.E. Meeker and M.J. Morgan, Arch. Environ. Health, 11, 773 (1965).

9. E. Sawicki, J.E. Meeker and M.J. Morgan, Int. J. Air Water Pollution, 9, 291 (1965).

10. C.R. Engel and E. Sawicki, J. Chromatogr., 31, 109 (1967).

11. E. Sawicki and J.P. Pfaff, Anal. Chim. Acta., 32,521 (1965).

12. E. Sawicki, M. Guyer and C.R. Engel, J. Chromatogr., 30, 522 (1967).

13. E. Sawicki, T.W. Stanley and W.C. Elbert, J. Chromatogr., 13, 512 (1965).

14. L.R. Snyder, Anal. Chem., 41, 314 (1969).

15. G. Alberini, V. Cantuti and G.P. Cartoni, in "Gas Chromatography". A.B. Littlewood ed., Inst. Petr., London 1967, p.258.

16. M. Dong, D.C. Locke and D. Hoffmann, unpublished works, American Health Foundation, Valhalla, New York, 1976.

17. R. Vivilechia, M. Thiebaud and R.W. Frei, J. Chromatogr. Sci., 10, 441 (1972).

18. S. Ray and R.W. Frei, J. Chromatogr., 71, 45 (1972).

19. D. Hoffmann and E.L. Wynder, in "Air Pollution", 3rd Ed., Vol. 2, A.C. Stern ed., Academic Press, New York, in press.

20. S. Asahina, J. Andrea, A. Carmel, E. Arnold, Y. Bishop, S. Joshi, D. Coffin and S.S. Epstein, Cancer Res., 32, 2263 (1972).

21. D. Brocco, A. Cimmino and M. Possanzini, J. Chromatogr., 84, 371, (1973).

22. R.W. Frei, K. Beall and J. Cassidy, Mikrochim. Acta, 12, 859 (1974).

23. "UV Atlas of Organic Compounds", 5 Vol., Plenum Press, New York, 1967-68.

24. M. Dong, D.C. Locke and E. Ferrand, Anal. Chem. 48, 368 (1976).

25. D.C. Locke, J. Chromatogr. Sci., 12, 433 (1974).

26. L.R. Snyder and J.J. Kirkland in "Introduction to Modern Liquid Chromatography", John Wiley and Sons, New York, 1974, Chapter 1.

27. Q.N. Porter and J. Baldas in "Mass Spectrometry of Heterocyclic Compounds", Wiley-Interscience, New York, 1971.

28. E. Stenhagen, S. Abrahamsson and F.W. McLafferty, "Registry of Mass Spectral Data", John Wiley & Sons, New York, 1974.

29. K.F. Lang and I. Eigen, Forschr. Chem. Forsch., 8, 91 (1967).

30. K. Rothwell, Nature, 252, 69 (1974).

31. M. Blumer and W.W. Youngblood, Science, 188, 53 (1974).

TABLE I. Retention Data of Aza-Arenes

in Several Chromatographic Systems

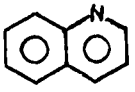
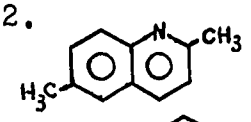
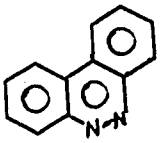
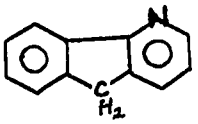
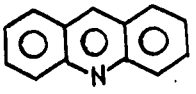
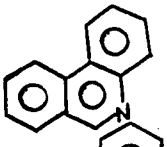
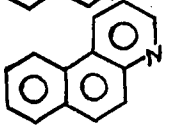
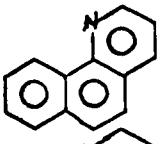
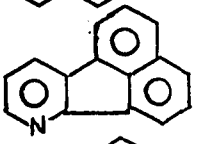
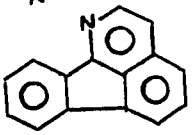
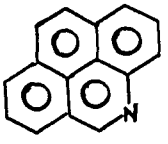
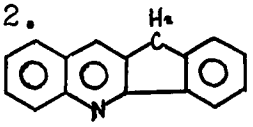
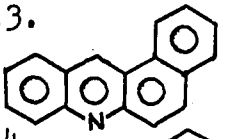
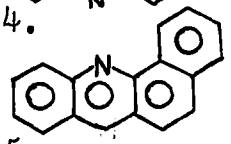
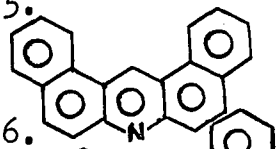
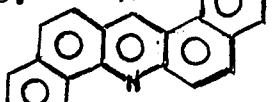
No.	Aza-Arenes	Name	°C bp	(minutes) Retention times I	II	III	R_f IV
1.		Quinoline	237	6.2	4.5	5.2	0.2
2.		2,6-Dimethyl-quinoline	267	10.0	6.3	7.6	-
3.		Benzo(c)cinnoline	-	6.5	4.1	16.8	0.23
4.		4-Azafluorene (5H-Indeno(1,2-b)-pyridine)**	314	8.6	3.7	11.2	0.28
5.		Acridine	343	9.9	3.2	14.0	0.33
6.		Phenanthridine	349	10.0	3.7	14.4	0.28
7.		Benzo(f)quinoline	350	10.3	4.7	14.4	0.23
8.		Benzo(h)quinoline	340	10.5	1.4	13.6	0.48
9.		7-Azafluoranthene (Acenaphtho(1,2-b)-pyridine)	396	11.4	3.7	18.4	0.28
10.		1-Azafluoranthene (Indeno(1,2,3-ij)-isoquinoline	394	11.8	3.2	18.4	0.32
11.		4-Azapyrene (Benzo(lmn)-phenanthridine	407	11.8	3.7	19.2	0.28

TABLE I. (Continued)

No.	Aza-Arenes	Name	°C bp	Retention times (minutes) I	II	III	R_f IV
12.		11H-Indeno(1,2-b) quinoline	410	11.8	1.9	21.0	0.44
13.		Benz(a)acridine	438	13.1	2.9	26.4	0.38
14.		Benz(c)acridine	434	13.8	1.2	25.2	0.58
15.		Dibenz(a,j)-acridine	-	16.3	3.2	68.0	0.38
16.		Dibenz(a.h) acridine	-	17.7	1.2	60.0	0.60

** Nomenclature according to Patterson's Ring Index.

All structures are also drawn according to the Ring Index.

Chromatographic Conditions:

I. HPLC Reversed Phase:(column-μ-Bondapak/C_{18};solvent- 20-80% CH_3CN in H_2O at 2%/min; 3.0 ml/min).

II. HPLC Adsoption: (column- μ-Porasil; solvent- 1.0% propanol -2 in hexane; 4.0 ml/min).

III. GC: (column: Dexsil 300; temperature programmed from 160°C to 300°C at 8°C/min; He flow rate 60 ml/min).

IV. TLC: (Silica gel TLC plate developed by 10% CH_3CN in $CHCl_3$).

TABLE II

CONCENTRATION OF AZA-ARENES IN
NEW YORK CITY SUSPENDED PARTICULATE MATTER

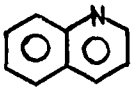
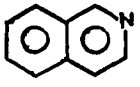
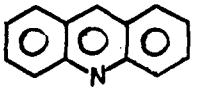
No.	Aza-arenes	Name	Mol. Wt.	Conc. in ng/1000m^3 Sample 1	Sample 2
1.		Quinoline	129	69	22
1a.		Methylquinoline	143	35	33
1b.		Dimethylquinolines	157	48	44
1c.		Ethylquinolines	157	14	22
1d.		3C-quinolines	171	10	ND
2.		Isoquinoline	129	180	140
2a.		5 or 8 methyl-isoquinoline	143	310	170
2b.		Other methyl-isoquinolines	143	76	70
2c.		Dimethyl-isoquinolines	157	62	ND
2d.		Ethyl-isoquinolines	157	160	68
2e.		3C-isoquinolines	171	28	ND
3.		Acridine	179	41	40
3a.		Methylacridines	183	7	ND

TABLE II cont.

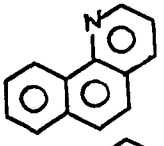
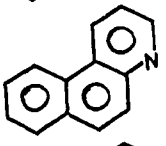
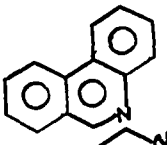
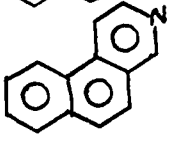
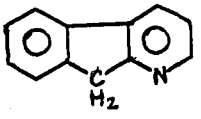
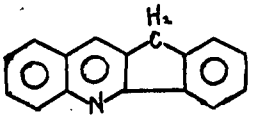
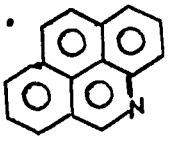
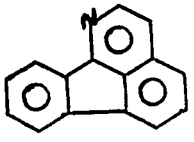
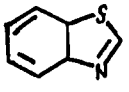
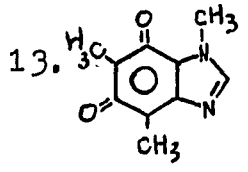
No.	aza-arenes	Name	Mol. Wt.	conc. in ng/1000m³ Sample 1	Sample 2
4.		Benzo(h)quinoline	179	10	13
5.		Benzo(f)quinoline	179	11	10
6.		Phenanthridine	179	22	18
7.		Benzo(f)isoquinoline	179	110	34
8.		4-Azafluorene (5-H-Indeno (1,2-b)-pyridine)*	167	5	5
9.		11H-Indeno (1,2-b) quinoline	217	trace	trace
10.		4-Azapyrenes (Benzo(1mn)phena-thridine	203	21‡	22‡
11.		1-Azafluoranthene (Indeno(1,2,3-ij) isoquinoline)	203	5‡	5‡
12.		Benzothiazole	135	14	20

TABLE II cont.

No.	Aza-arenes	Name	Mol. Wt.	Conc. in ng/1000m^3 Sample 1	Sample 2
13.		Caffeine	194	3400	7000

* Name accordint to Patterson's Ring Index. All structures are also drawn according to the Ring Index.

‡ Includes other isomers.

ND Not Determined

Figure 1. Analytical scheme for the characterization of the basic fraction of suspended particulate matter.

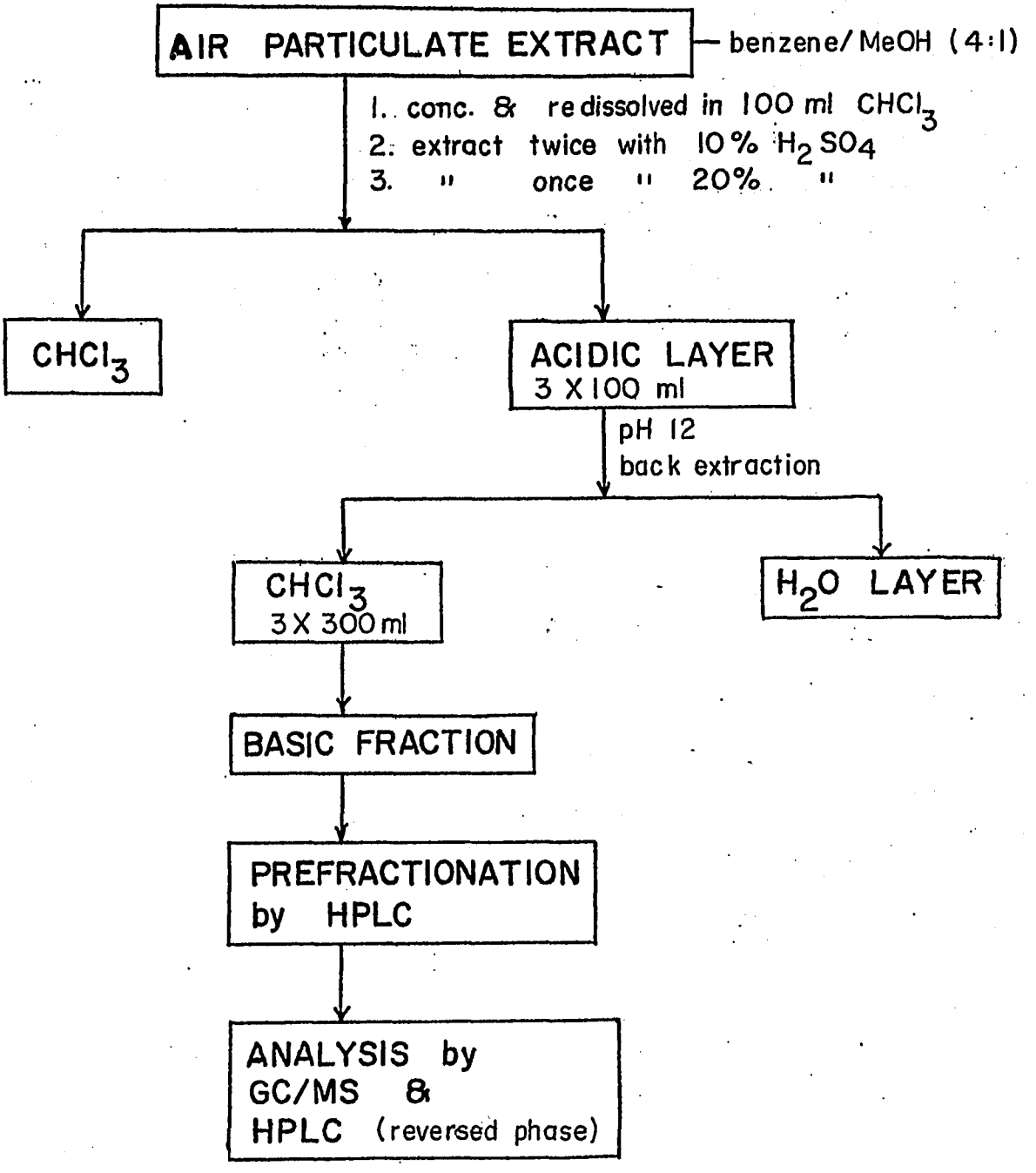

AIR PARTICULATE EXTRACT
benzene/MeOH (4:1)
1. conc. & redissolved in 100 ml $CHCl_3$
2. extract twice with 10% H_2SO_4
3. " once " 20% "
$CHCl_3$
ACIDIC LAYER
3 X 100 ml
pH 12
back extraction
$CHCl_3$
3 X 300 ml
H_2O LAYER
BASIC FRACTION
PREFRACTIONATION
by HPLC
ANALYSIS by
GC/MS &
HPLC (reversed phase)

Figure 2. Gas chromatogram of Standard Aza-Arene mixture # 1. (Chromatographic conditions: 160°C programmed to 300°C at 8°C/min, helium flow rate 30 ml/min, hydrogen pressure 20 psi, air pressure 30 psi. Column: 10 ft, 1/8", 6% Dexsil 300 GC on Chromosorb W (HP), 80/100 mesh, sensitivity: X80. Numbers correspond to compounds listed in Table I)

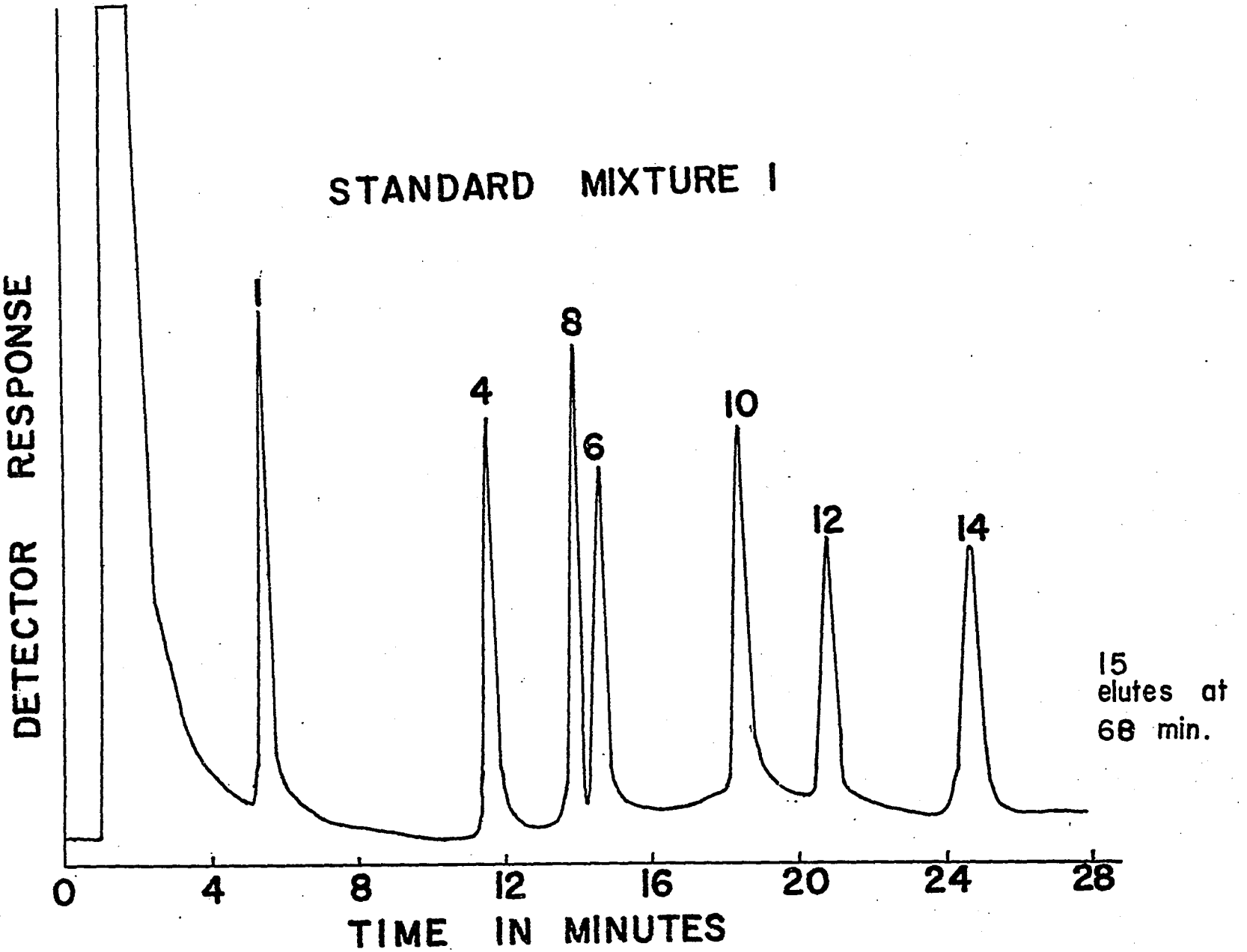
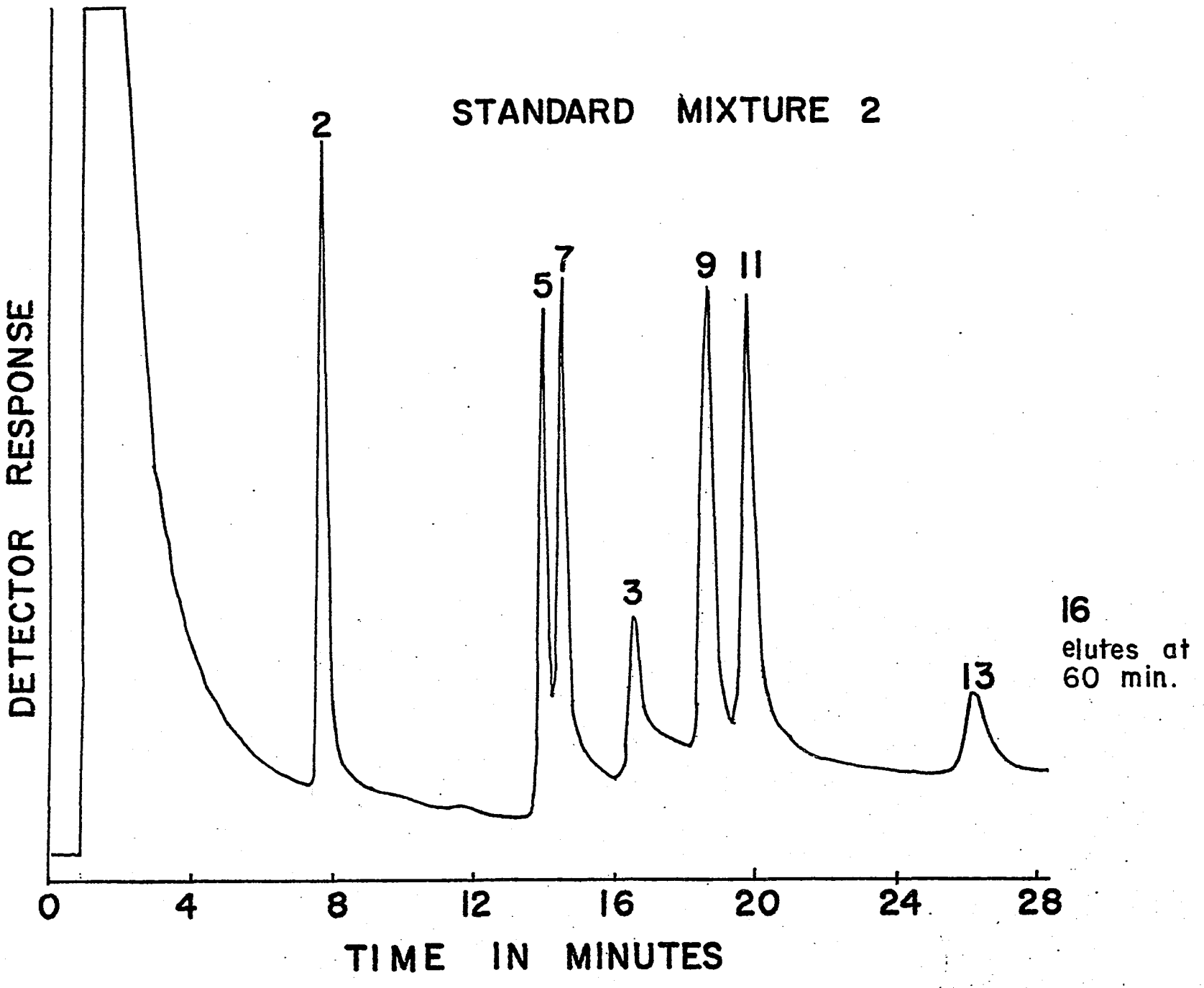

STANDARD MIXTURE I
DETECTOR RESPONSE
1
4
8
6
10
12
14
15 elutes at 68 min.
0
4
8
12
16
20
24
28
TIME IN MINUTES

Figure 3. Gas chromatogram of Standard Aza-Arene mixture # 2. (Chromatographic conditions same as in Figure 2. Numbers corresponds to compounds listed in Table I.)


STANDARD MIXTURE 2
DETECTOR RESPONSE
2
5
7
3
9
11
13
16
elutes at
60 min.
0
4
8
12
16
20
24
28
TIME IN MINUTES

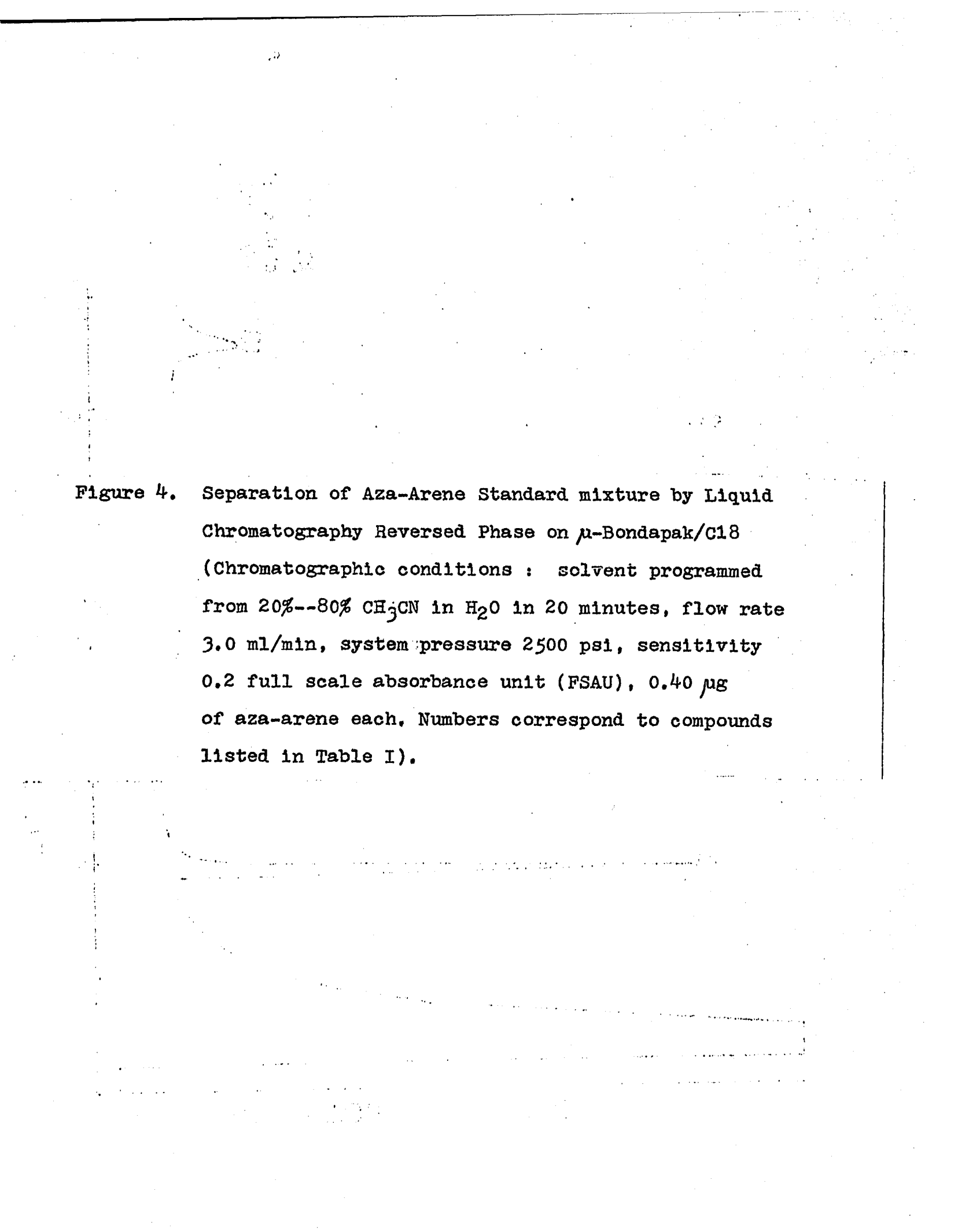


Figure 4. Separation of Aza-Arene Standard mixture by Liquid Chromatography Reversed Phase on μ-Bondapak/C18 (Chromatographic conditions : solvent programmed from 20%--80% CH_3CN in H_2O in 20 minutes, flow rate 3.0 ml/min, system pressure 2500 psi, sensitivity 0.2 full scale absorbance unit (FSAU), 0.40 μg of aza-arene each. Numbers correspond to compounds listed in Table I).

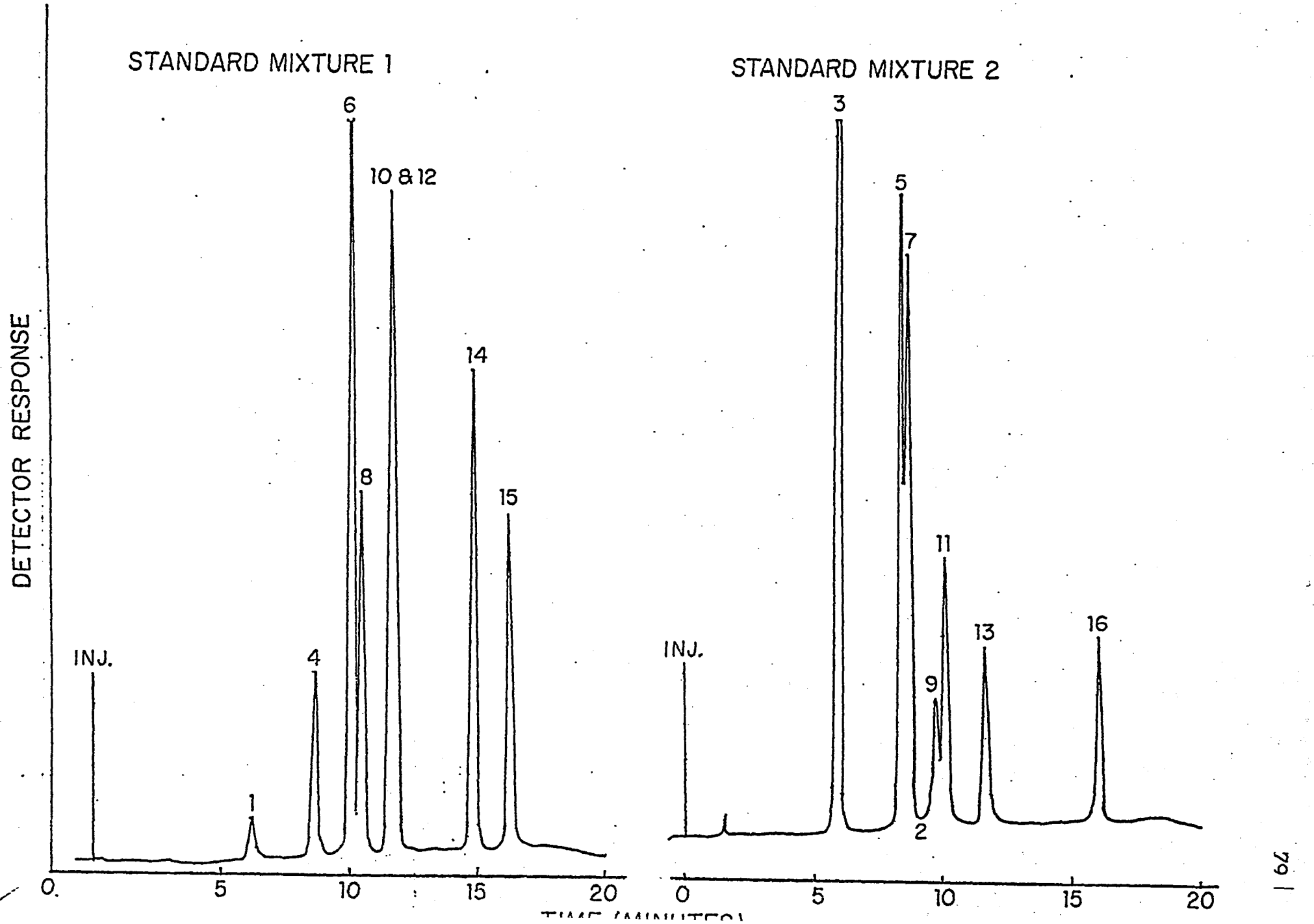

STANDARD MIXTURE 1
STANDARD MIXTURE 2
DETECTOR RESPONSE
INJ.
1
4
6
8
10 & 12
14
15
INJ.
2
3
5
7
9
11
13
16
0.
5
10
15
20
0
5
10
15
20

Figure 5. Separation of Aza-Arene Standard mixtures by Liquid Chromatography Adsorption on μ-Porasil. (Chromatographic conditions: mobile phase 1% propanol-2 in hexane, flow rate 4.0 ml/min, system pressure 1600 psi, sensitivity 0.08 FSAU, 0.16 ug of aza-arene each. Number correspond to compounds listed in Table I.)

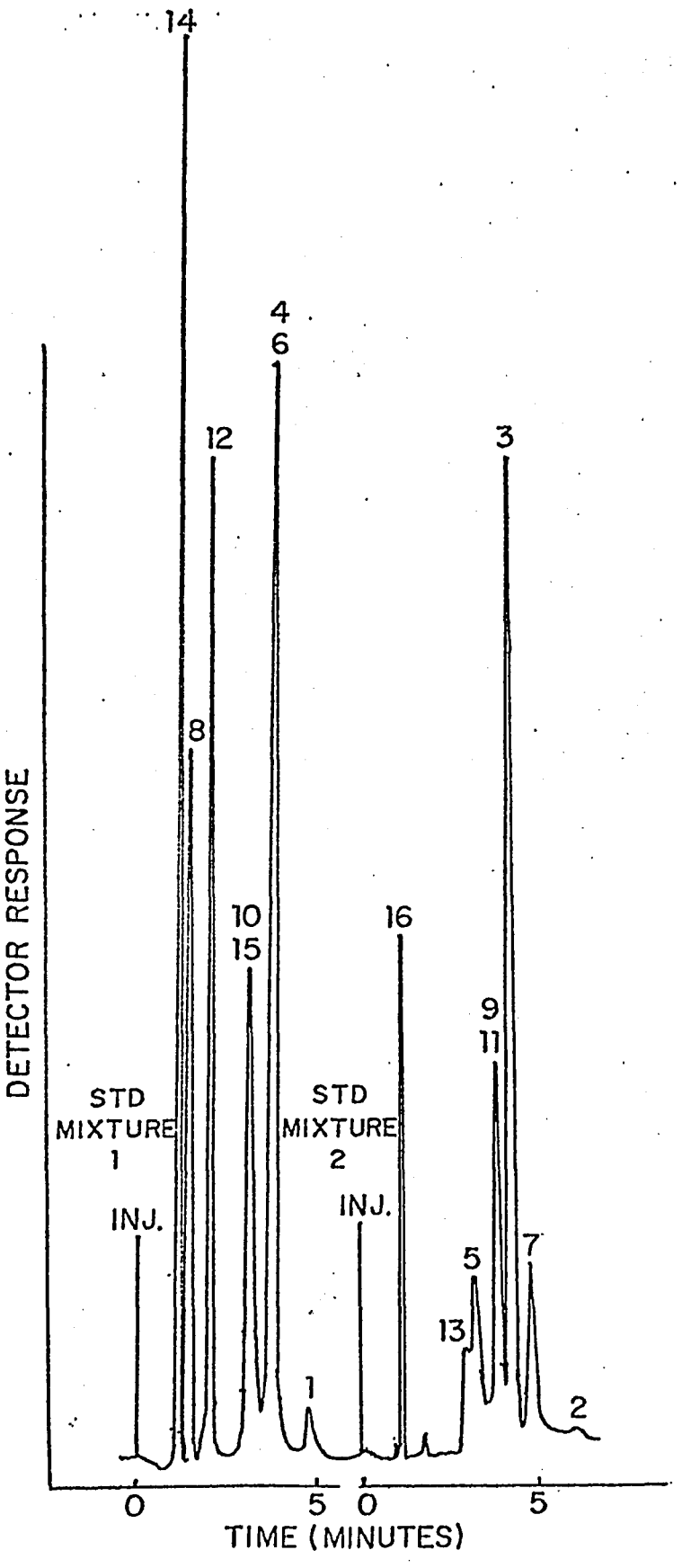

14
4
6
12
3
8
10
15
16
9
11
STD
MIXTURE
1
STD
MIXTURE
2
INJ.
INJ.
5
7
13
1
2
DETECTOR RESPONSE
0
5
0
5
TIME (MINUTES)

Figure 6. Separation of Aza-Arene Standard mixtures by Liquid Chromatography on Lichrosorb SI 60 column. (Chromatographic conditions: mobile phase, 0.5% propanol-2 in hexane, flow rate 3.0 ml/min. Numbers correspond to compounds listed in Table I).

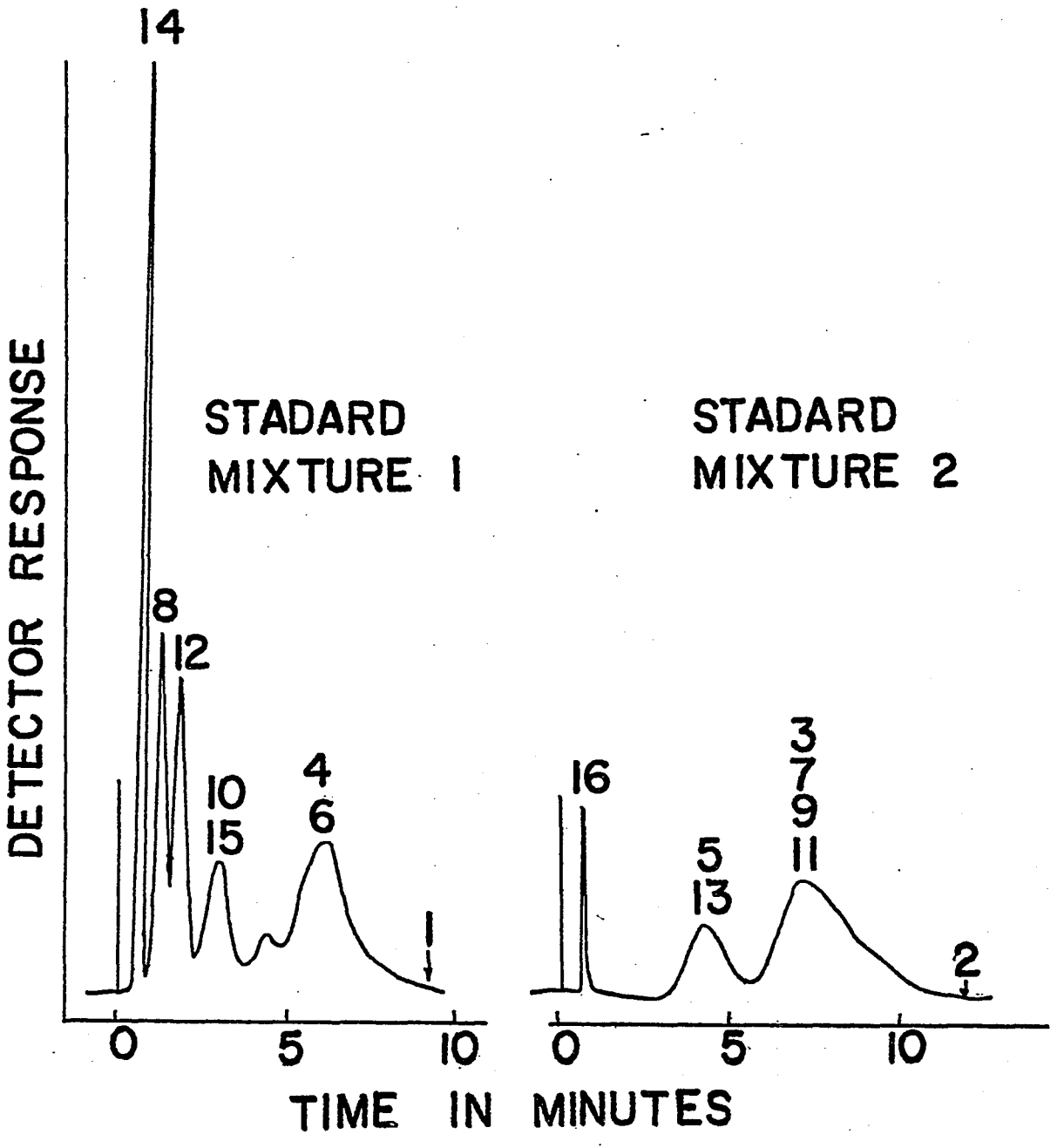
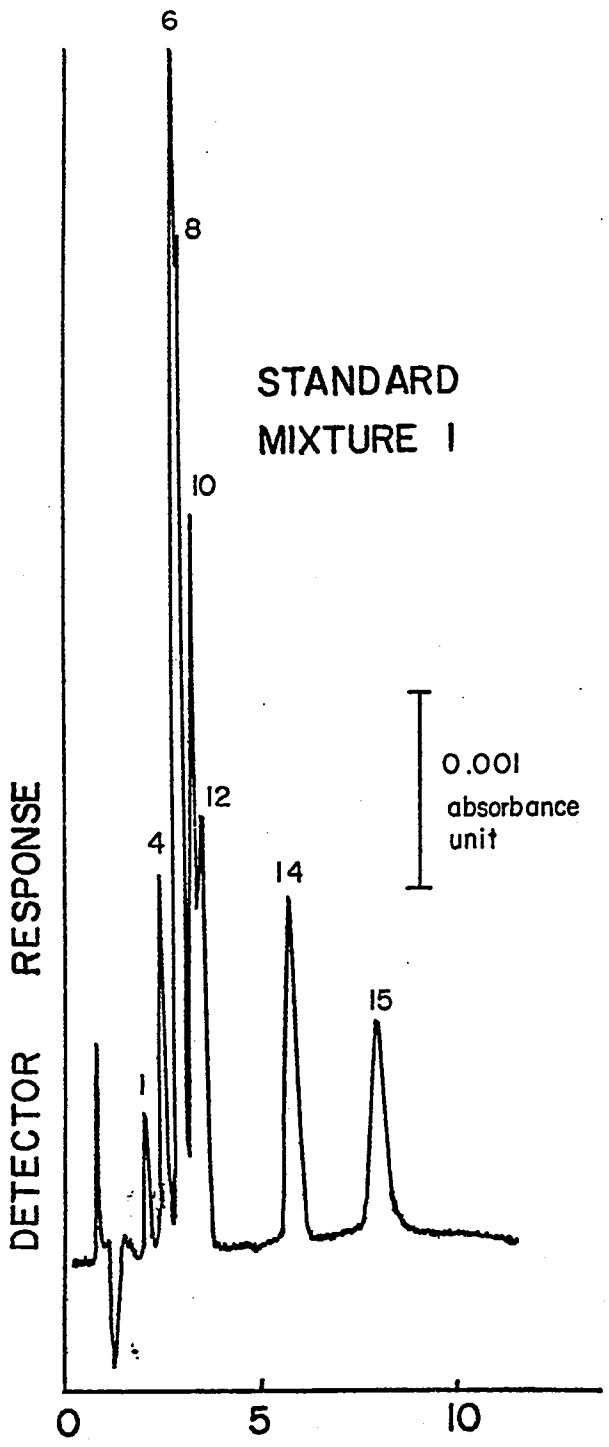

14
STADARD
MIXTURE 1
STADARD
MIXTURE 2
8
12
10
15
4
6
1
16
3
7
9
11
5
13
2
DETECTOR RESPONSE
0
5
10
0
5
10
TIME IN MINUTES

Figure 7. HPLC: Sensitivity study.

Top: Adsorption Chromatography on μ-Porasil. (Chromatographic conditions same as in Figure 5, sample, standard miture #1 containing 7 ng aza-arenes each)

Bottom: Reversed phase Chromatography on μ-Bondapak/C_{18} (Chromatographic conditions: mobile phase, 60% CH_3CN in H_2O, flow rate 3.0 ml/min)

Numbers correspond to compounds listed in Table I.


STANDARD
MIXTURE 1
DETECTOR RESPONSE
0.001
absorbance
unit
6
8
10
12
4
14
15
1
0
5
10

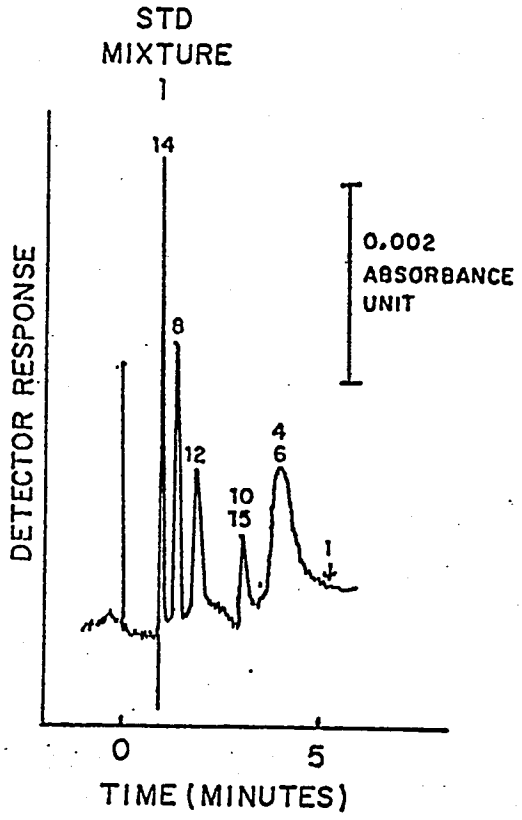

STD
MIXTURE
1
DETECTOR RESPONSE
0.002
ABSORBANCE
UNIT
14
8
12
4
6
10
15
1
0
5
TIME (MINUTES)

Figure 8. Thin Layer Chromatography of Aza-Arene Standard Mixtures and 8 Subfractions of the Basic Fraction of Suspended Particulate Matter.
(Silica Gel-G 250 μ TLC Precoated Plate developed in 10% CH_3CN in $CHCl_3$. The following are colors of the spots when TLC plate is viewed under 365 nm uv light: L, light; P, purple; Y, yellow; B, blue; G, green. Number correspond to compounds listed in Table I.)

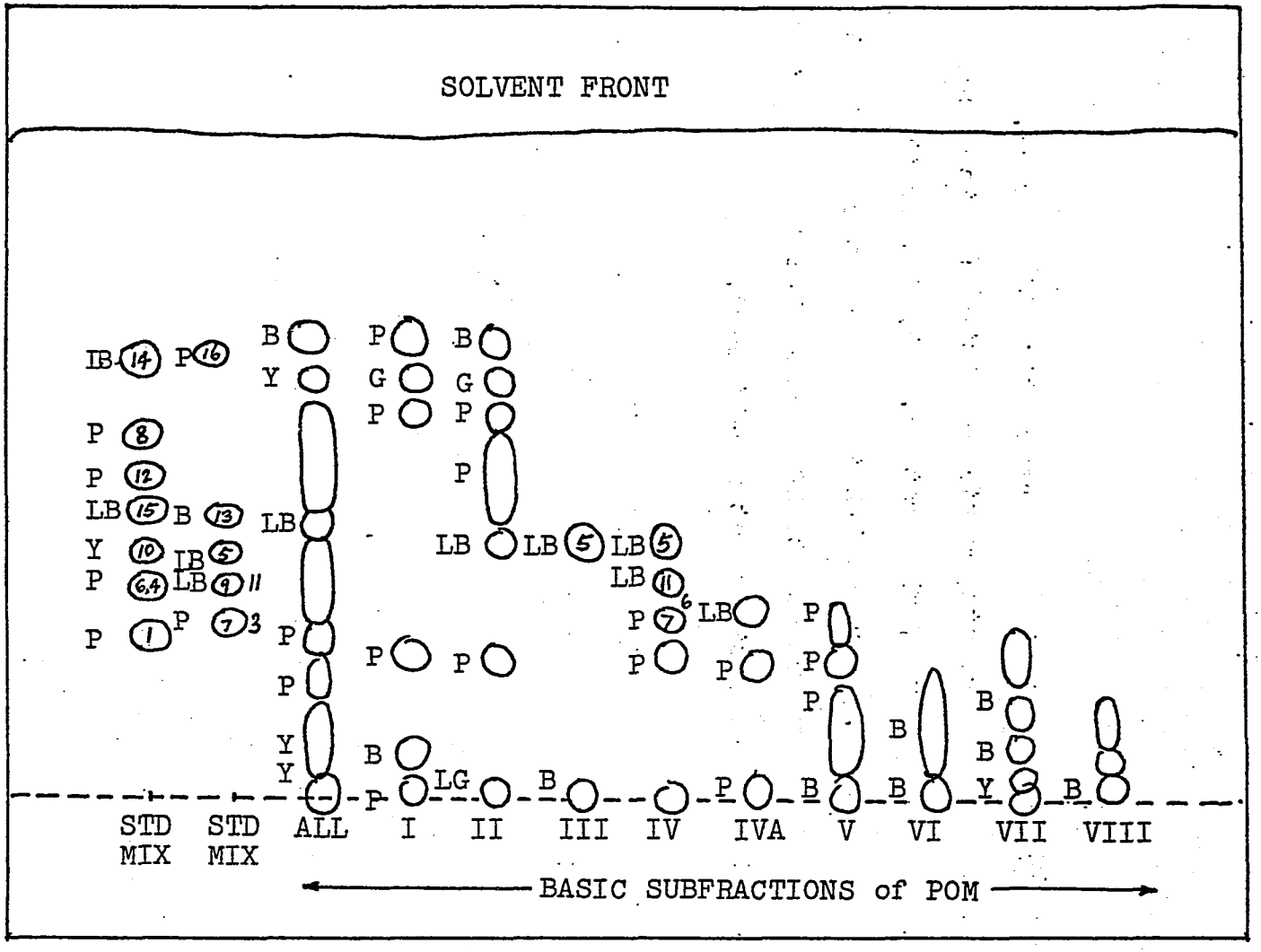

SOLVENT FRONT
STD MIX
STD MIX
ALL
I
II
III
IV
IVA
V
VI
VII
VIII
BASIC SUBFRACTIONS of POM

Figure 9. Typical HPLC Prefractionation Trace. (Chromatographic conditions; Lichrosorb SI 60 column, flow rate 3.0 ml/min, mobile phase 0.5% propanol-2 in hexane after "RUN" solvent programmed to 20% propanol-2 in $CHCl_3$ in 20 min, sensitivity at non-linear).

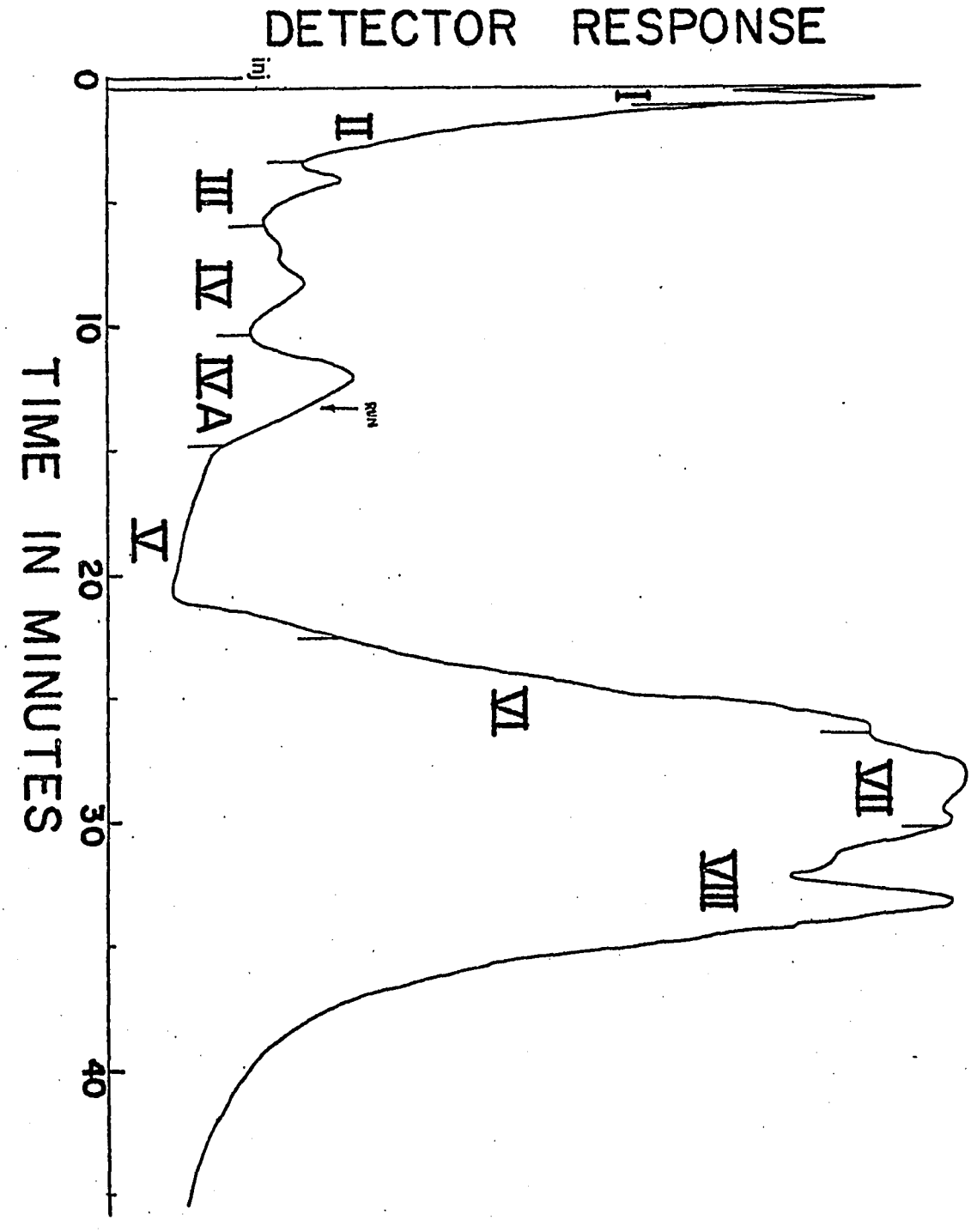

DETECTOR RESPONSE
TIME IN MINUTES
0
10
20
30
40
I
II
III
IV
IVA
V
VI
VII
VIII
RUN

Figure 10. Selected Ion Chromatograms on Fraction IV, the Quinoline Fraction.
(Gas chromatographic conditions the same as Figure 2).

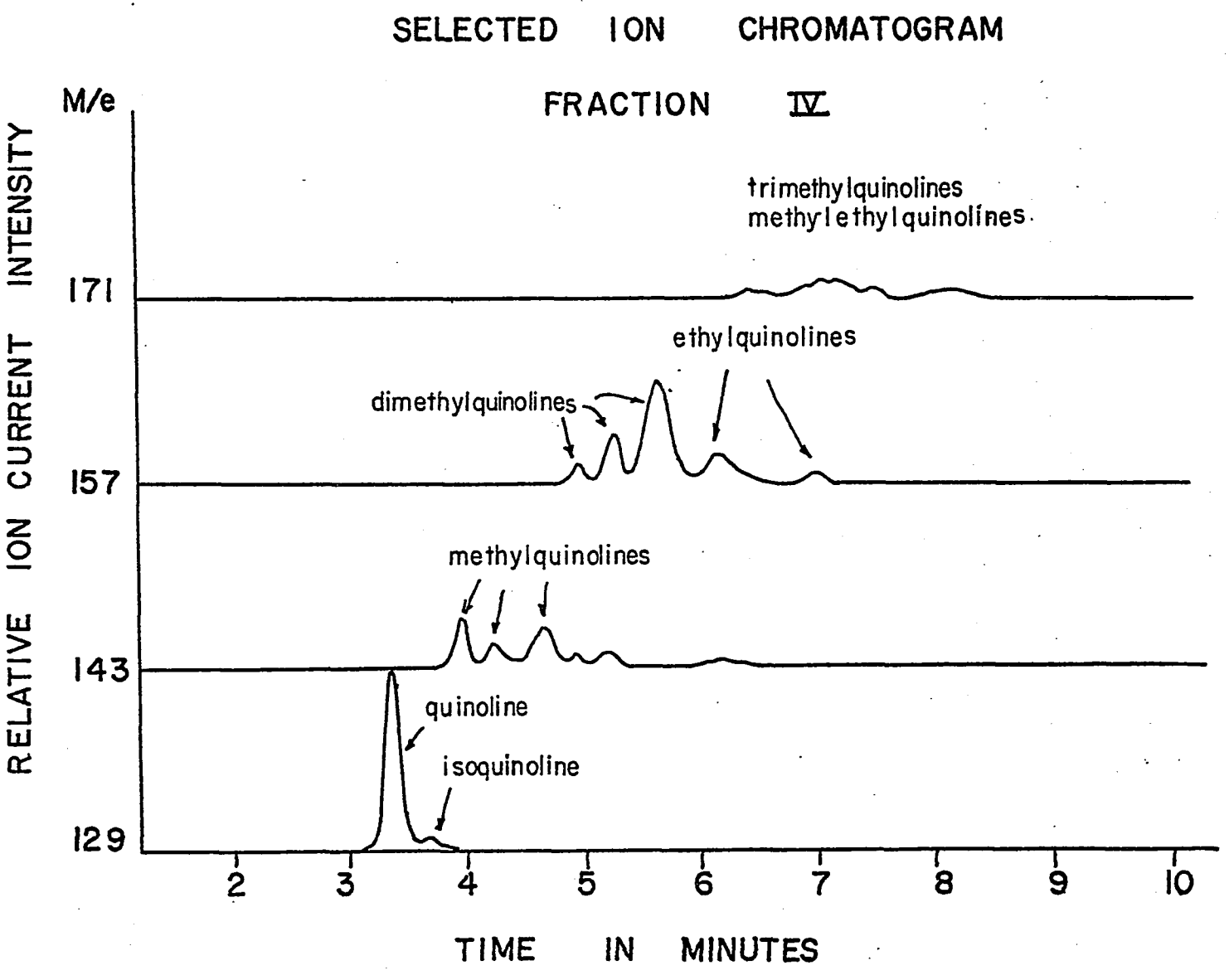

SELECTED ION CHROMATOGRAM
FRACTION IV
M/e
RELATIVE ION CURRENT INTENSITY
171
157
143
129
trimethylquinolines
methylethylquinolines
ethylquinolines
dimethylquinolines
methylquinolines
quinoline
isoquinoline
2
3
4
5
6
7
8
9
10
TIME IN MINUTES

Figure 11. Selected Ion Chromatograms on Fraction IV A, the Isoquinoline Fraction.
(Gas Chromatographic conditions the same as Figure 2).

SELECTED ION CHROMATOGRAM

FRACTION IV A

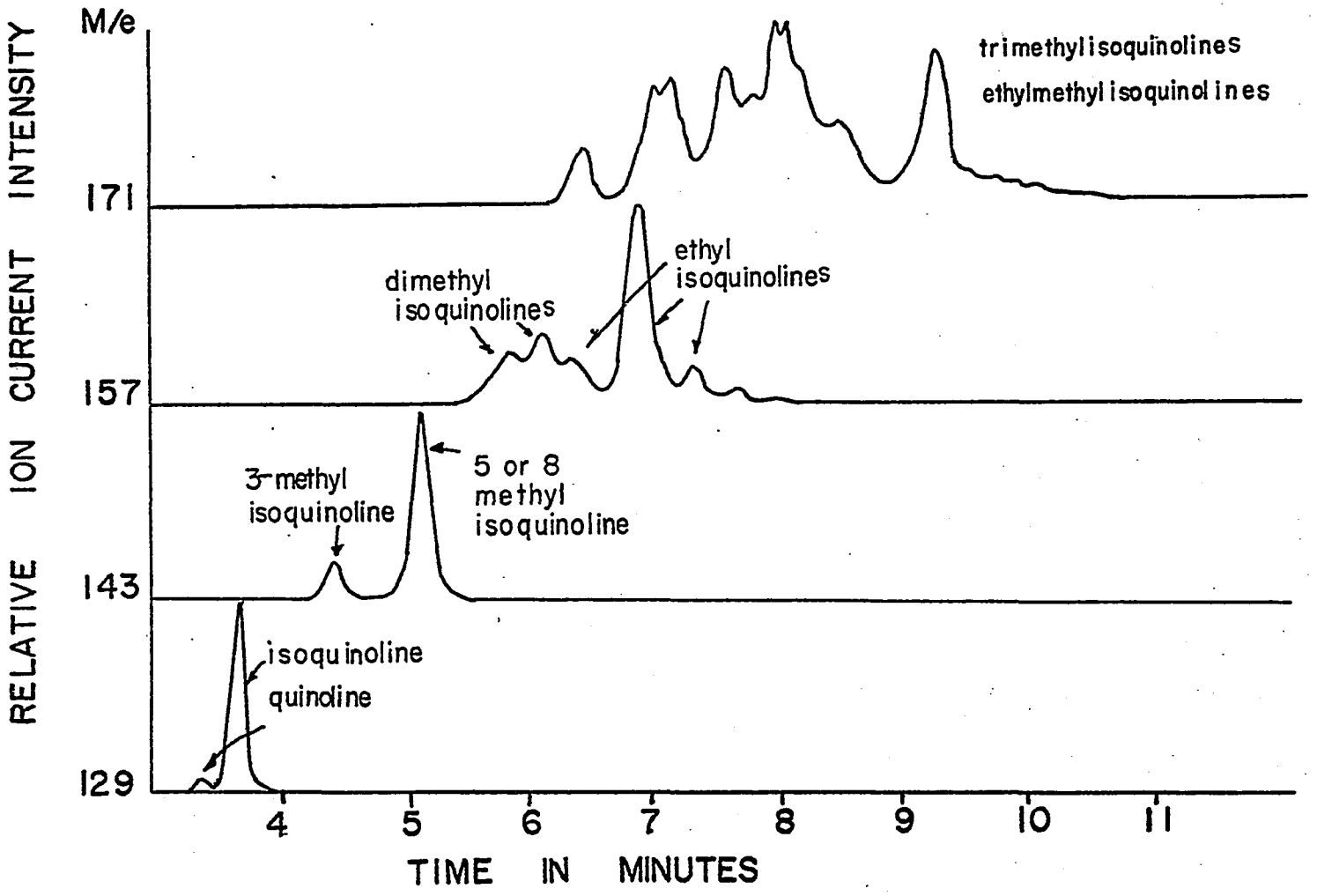
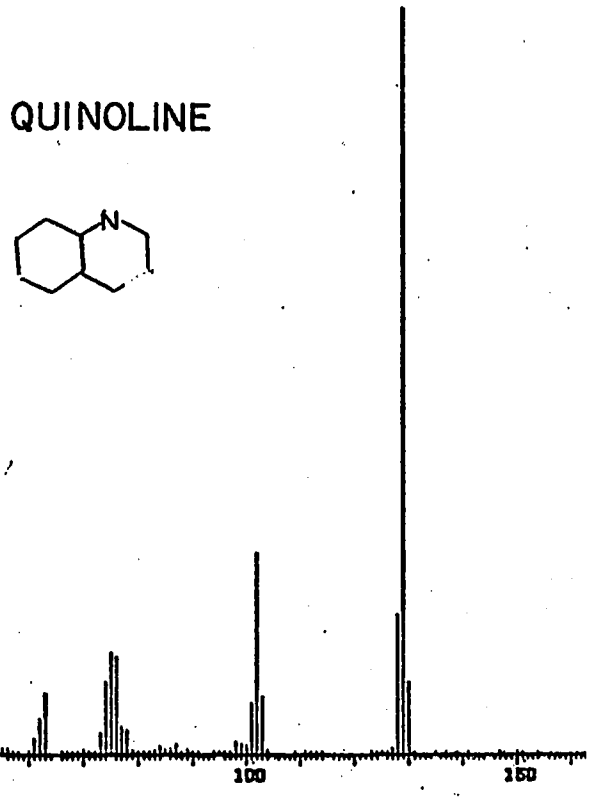


Figure 12. Mass Spectra of pure Quinoline and Quinoline isolated from Sample 1.

QUINOLINE



QUINOLINE (ISOLATED)

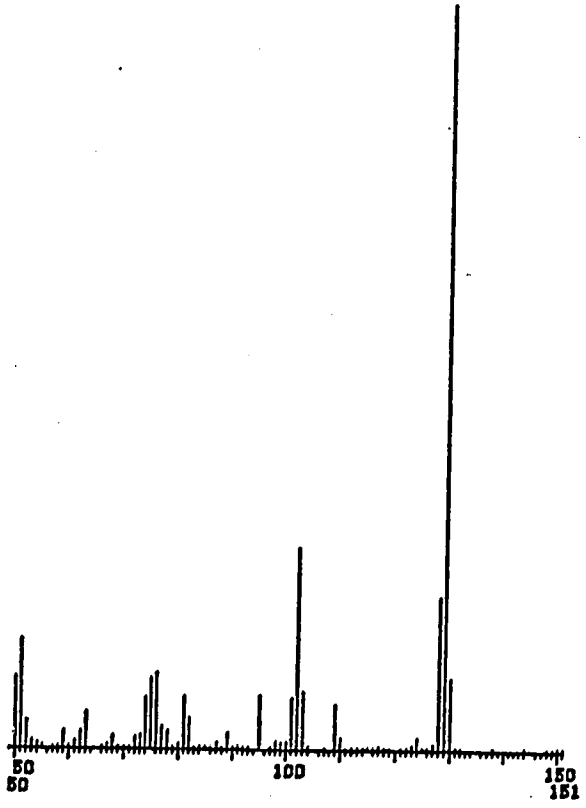


Figure 13. Gas Chromatogram of Fraction III of Sample 1. (Chromatographic conditions same as in Figure 2. Numbers correspond to compounds listed in Table II).

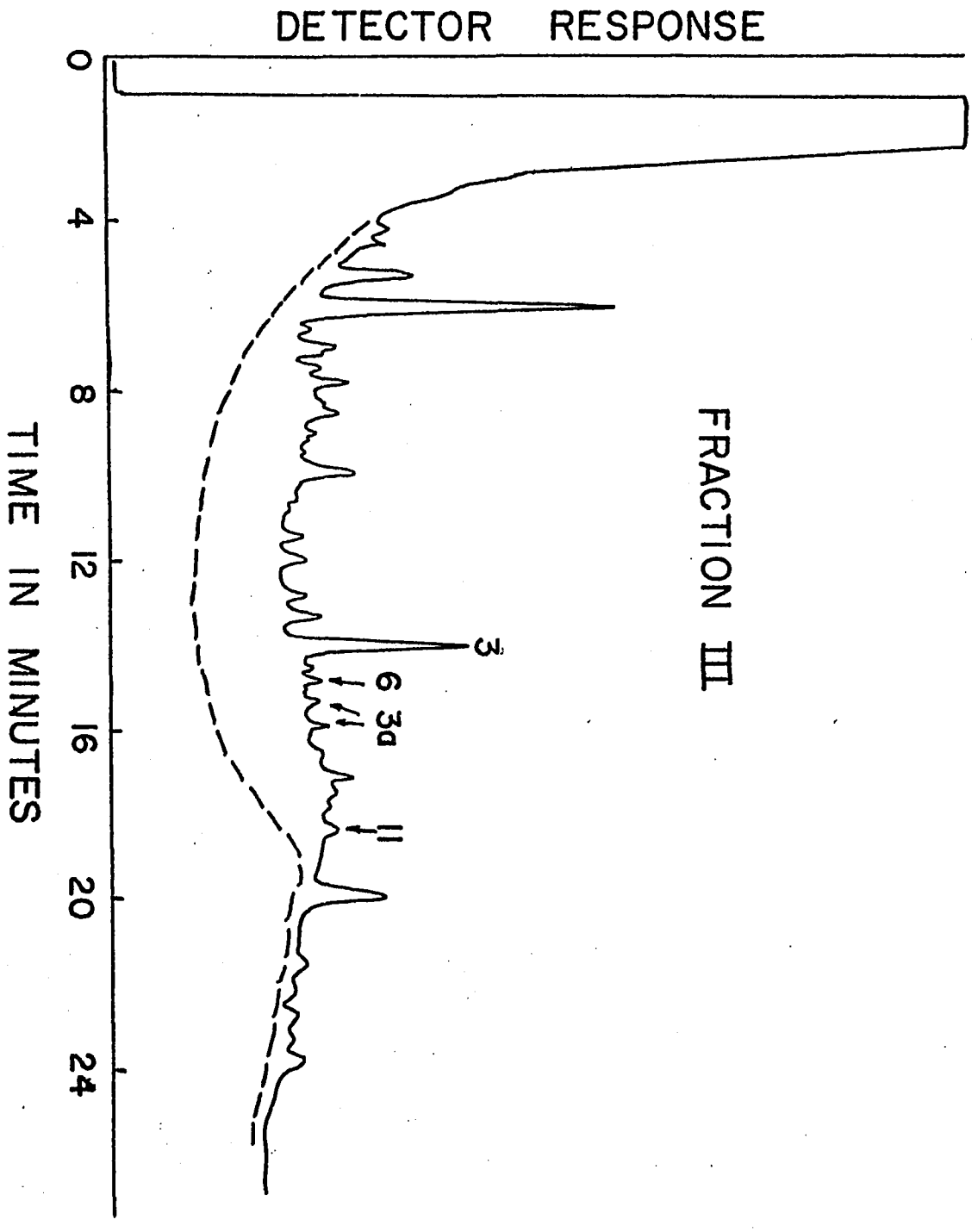

DETECTOR RESPONSE
0
4
8
12
16
20
24
TIME IN MINUTES
FRACTION III
3
6
3a
11

Figure 14. Gas Chromatogram of Fraction IV of Sample 1. (Chromatographic conditions same as in Figure 2. Numbers correspond to compounds listed in Table II).

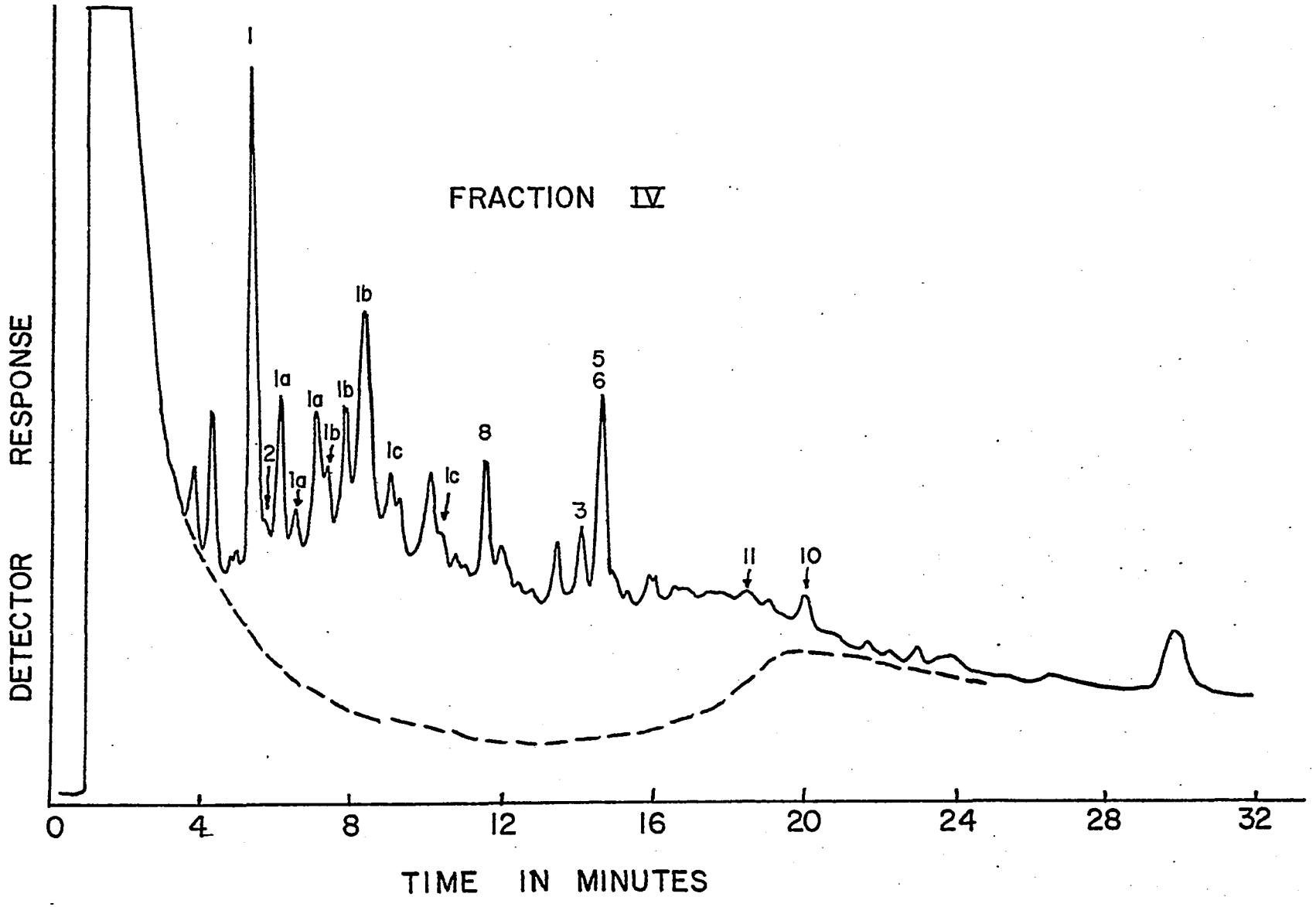

FRACTION IV
DETECTOR RESPONSE
TIME IN MINUTES
0
4
8
12
16
20
24
28
32
1
1a
2
1a
1a
1b
1b
1b
1c
1c
8
3
5
6
11
10

Figure 15. Gas Chromatogram of Fraction IV A of Sample 1. (Chromatographic conditions same as in Figure 2. Numbers correspond to compounds listed in Table II).

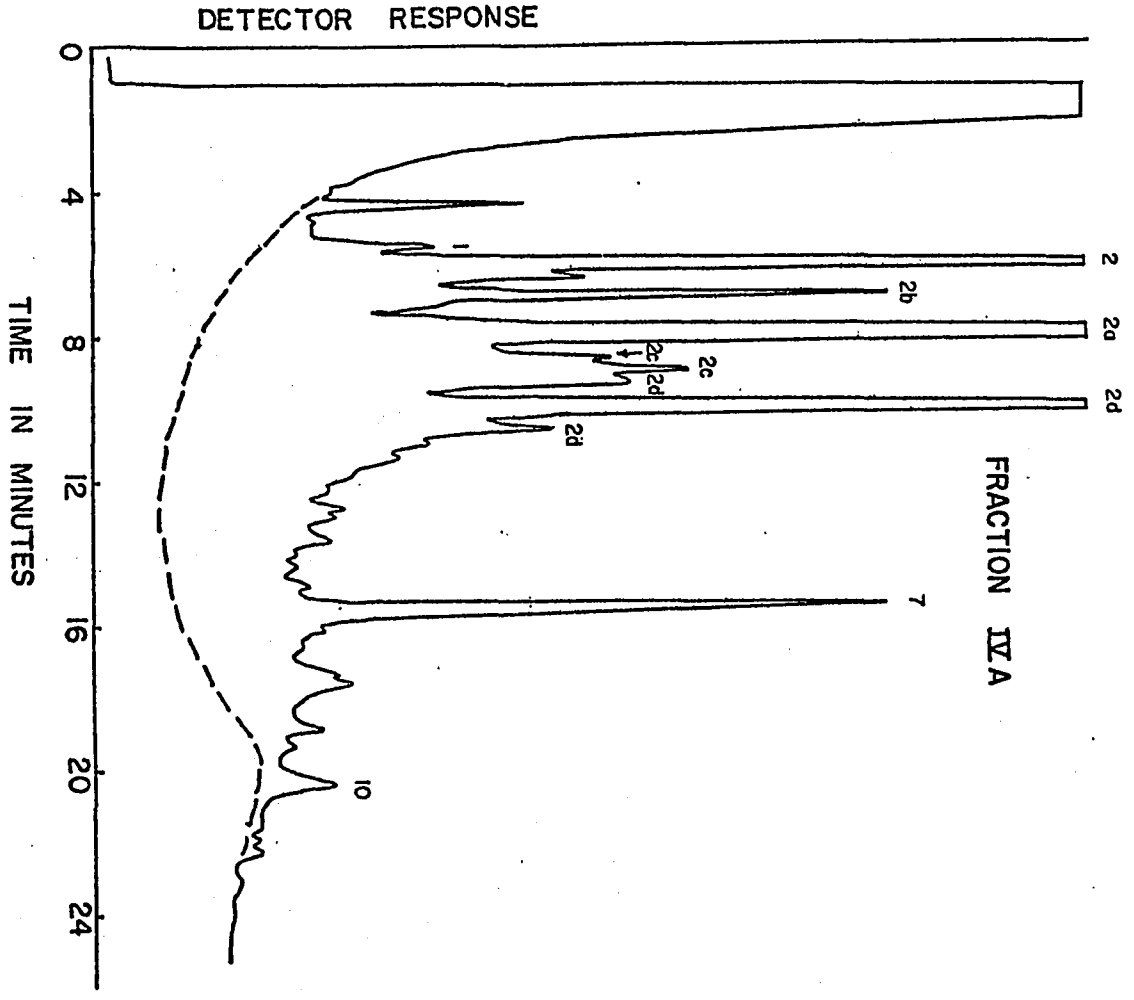

DETECTOR RESPONSE
TIME IN MINUTES
0
4
8
12
16
20
24
2
2a
2b
2c
2d
2e
2f
2d
7
10
FRACTION IX A

Figure 16. Liquid Chromatogram of Fraction III of Sample 1. (Chrmatographic conditions same as in Figure 4. Numbers correspond to compounds listed in Table II).

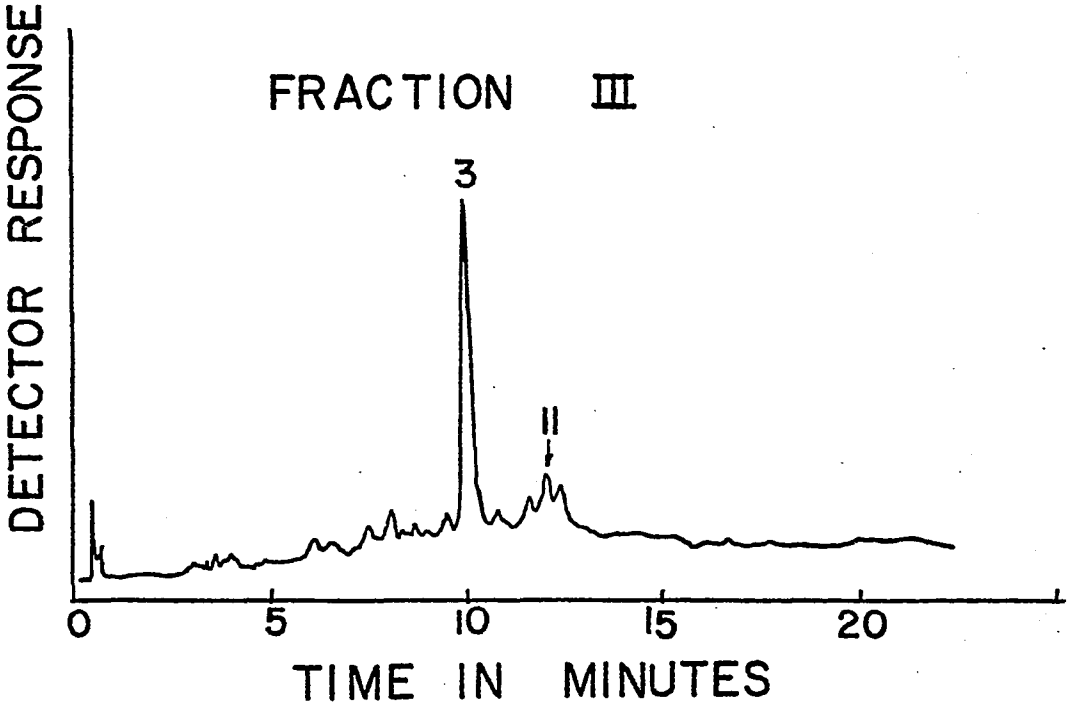
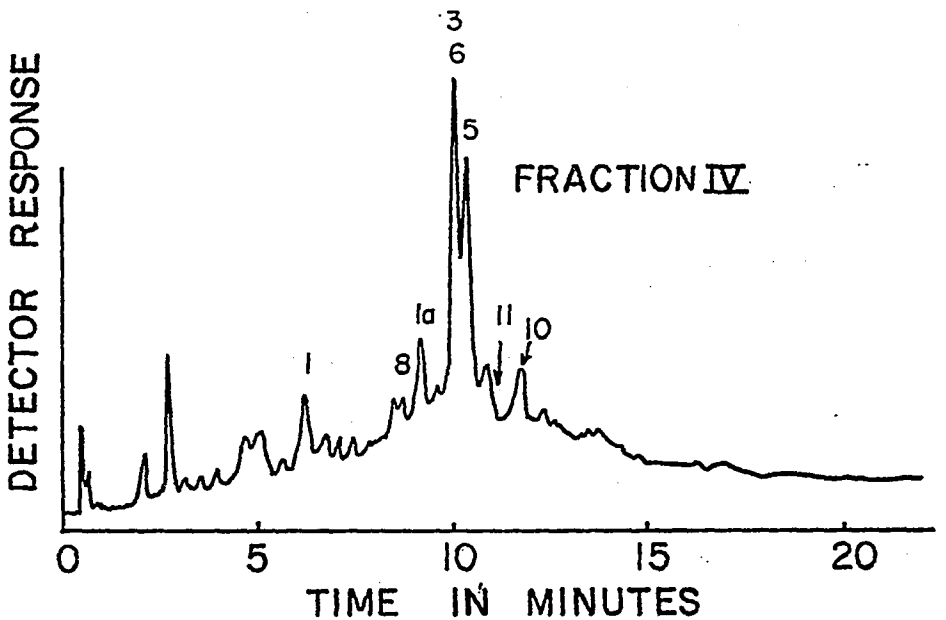

FRACTION III
3
II
DETECTOR RESPONSE
0
5
10
15
20
TIME IN MINUTES

Figure 17. Liquid Chromatogram of Fraction IV of Sample 1. (Chromatographic conditions same as in Figure 4. Numbers correspond to compounds listed in Table II).


DETECTOR RESPONSE
3
6
5
FRACTION IV
1a
11
10
1
8
0
5
10
15
20
TIME IN MINUTES

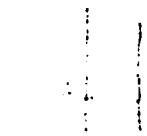


Figure 18. Liquid Chromatogram of Fraction IV A of Sample 1. (Chromatographic conditions same as in Figure 4. Numbers correspond to compounds listed in Table II).

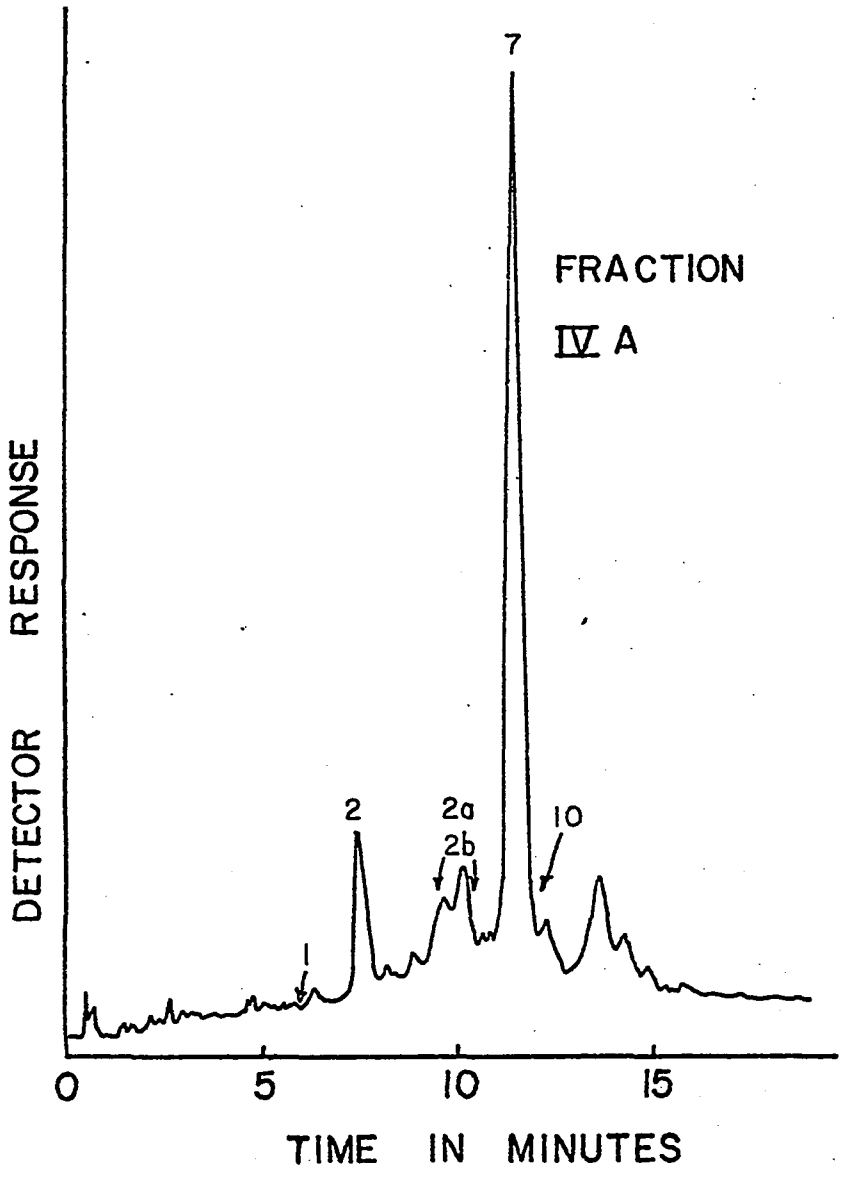

7
FRACTION
IV A
DETECTOR RESPONSE
2
2a
2b
10
1
0
5
10
15
TIME IN MINUTES

Figure 19. UV Spectrum of Isoquinoline isolated in Sample 1.

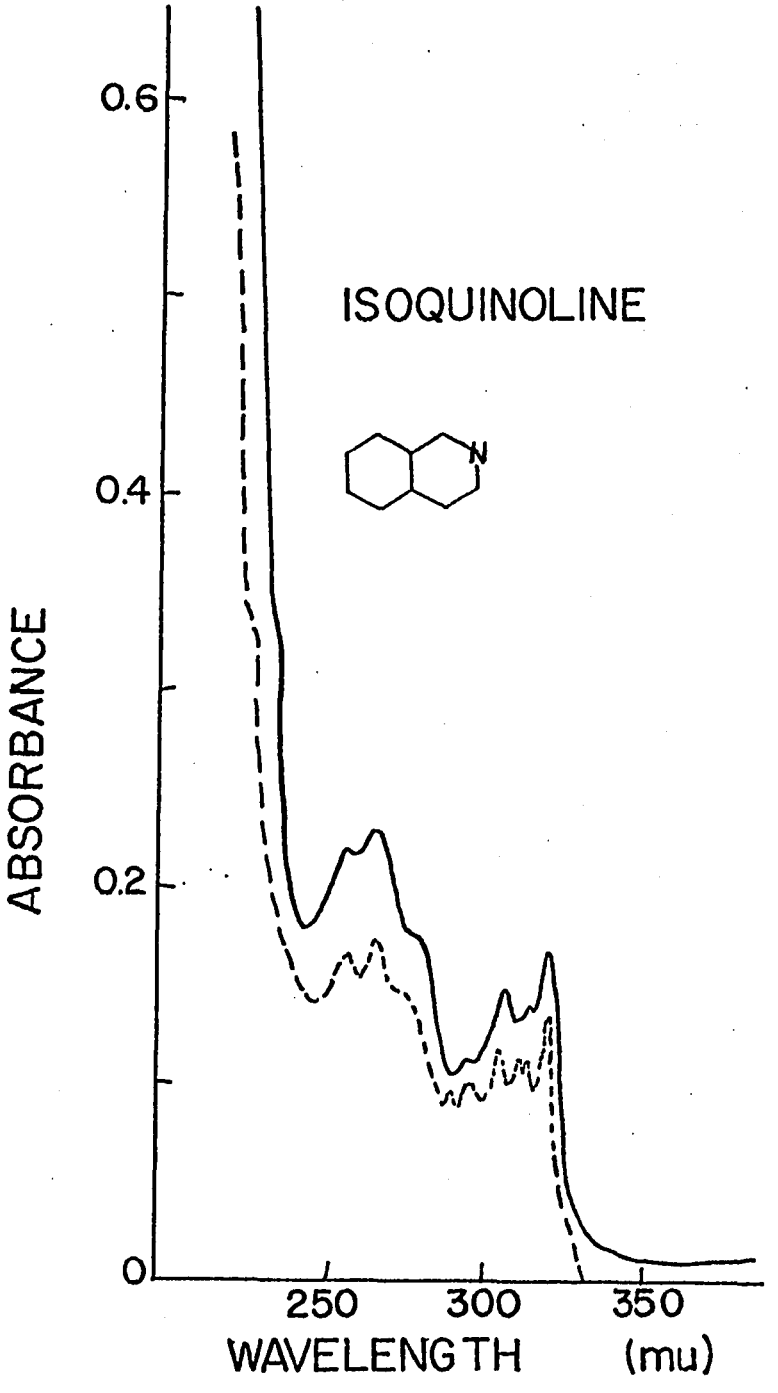

ISOQUINOLINE
N
ABSORBANCE
0.6
0.4
0.2
0
250
300
350
WAVELENGTH (mu)

Figure 20. Fluorescence Spectrum of Benzo(f)quinoline isolated in Sample 1.

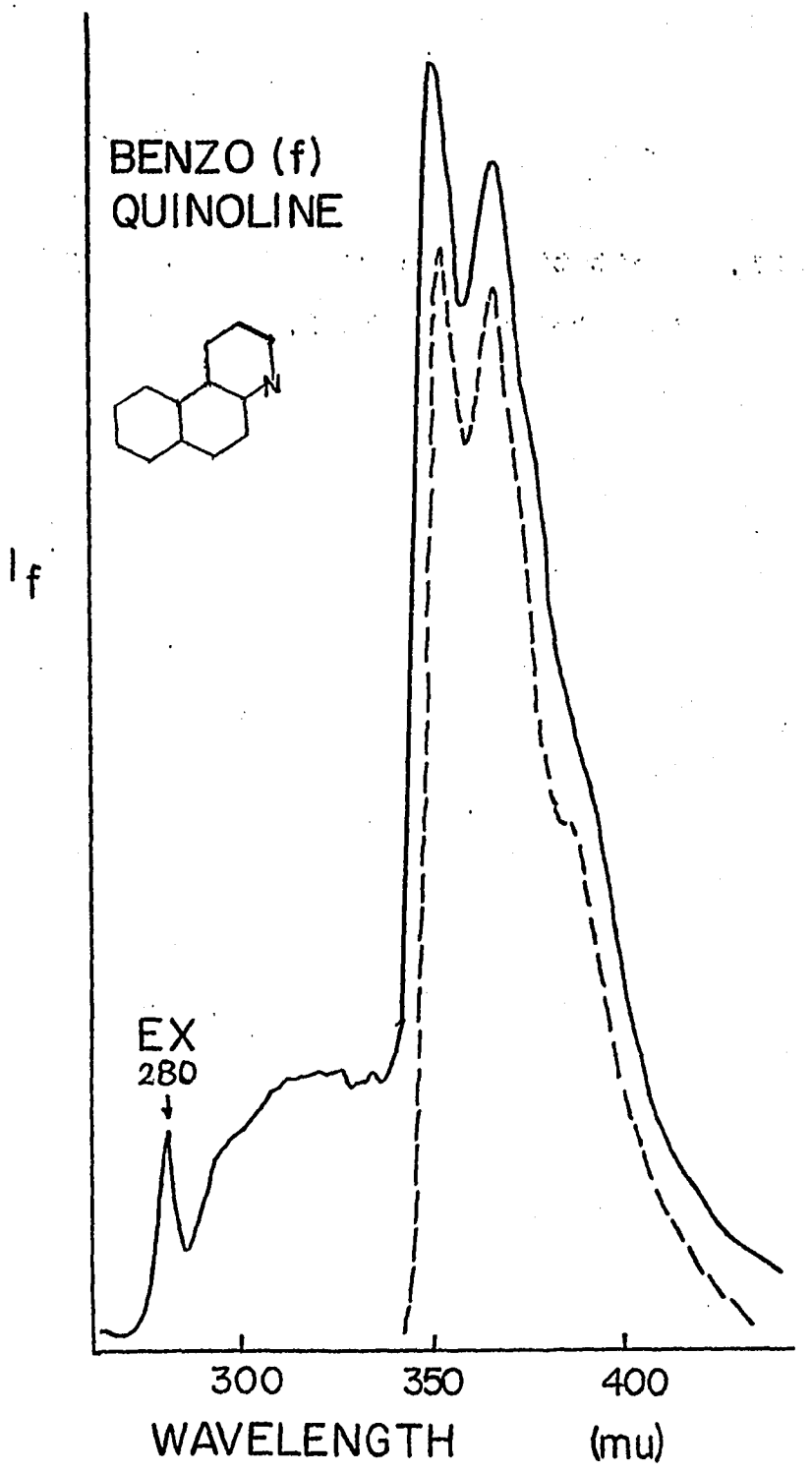

BENZO (f)
QUINOLINE
N
I_f
EX
280
300
350
400
WAVELENGTH
(mu)

Figure 21. Excitation Spectrum of Benzo(f)quinoline isolated in Sample 1.

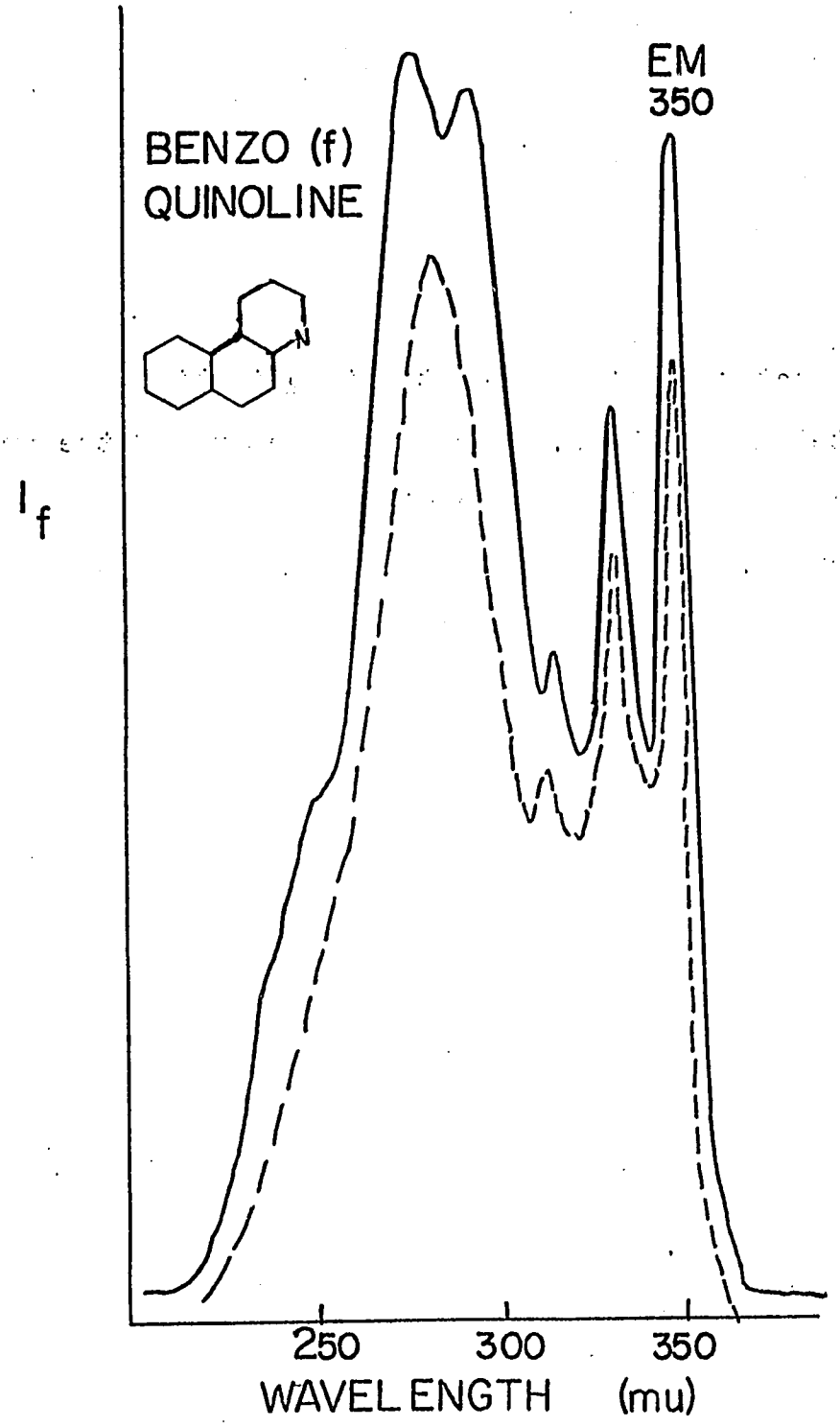

BENZO (f)
QUINOLINE
EM
350
I_f
250
300
350
WAVELENGTH (mu)

Figure 22. Mass Spectrum of 5- or 8-Methylisoquinoline isolated in Sample 1. The Mass spectrum of the pure compound is not available for comparison.

5 OR 8 METHYLISOQUINOLINE (ISOLATED)

Figure 23. Mass Spectrum of Benzo(f)isoquinoline isolated in Sample 1. The mass spectrum of the pure compound is not available for comparison.

BENZO(F)ISOQUINOLINE (ISOLATED)

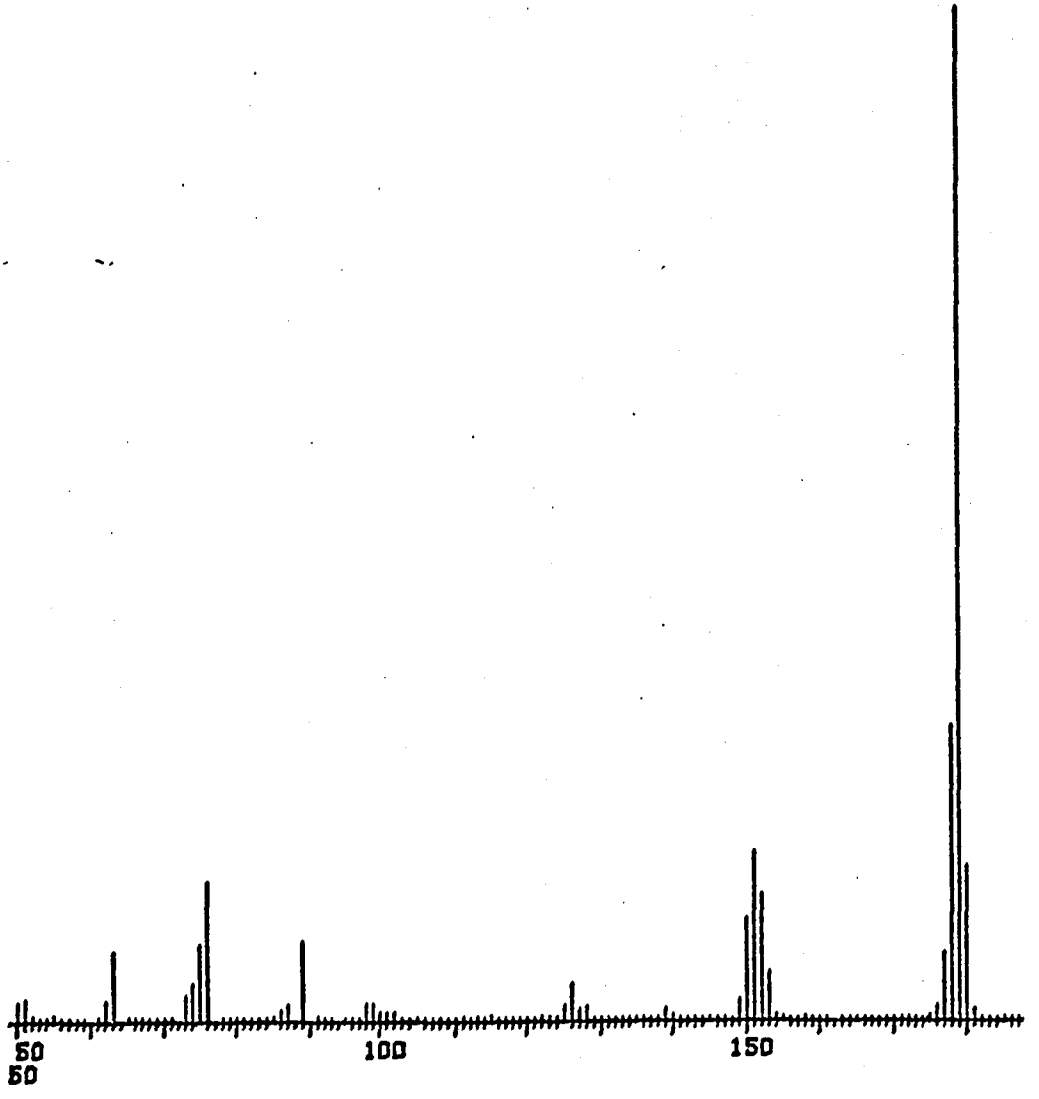


Figure 24. UV Spectrum of Benzo(f)isoquinoline isolated in Sample 1 showing characteristic absorption peaks. Dotted line shows the absorption background caused by Dimethylquinolines also present in the fraction.

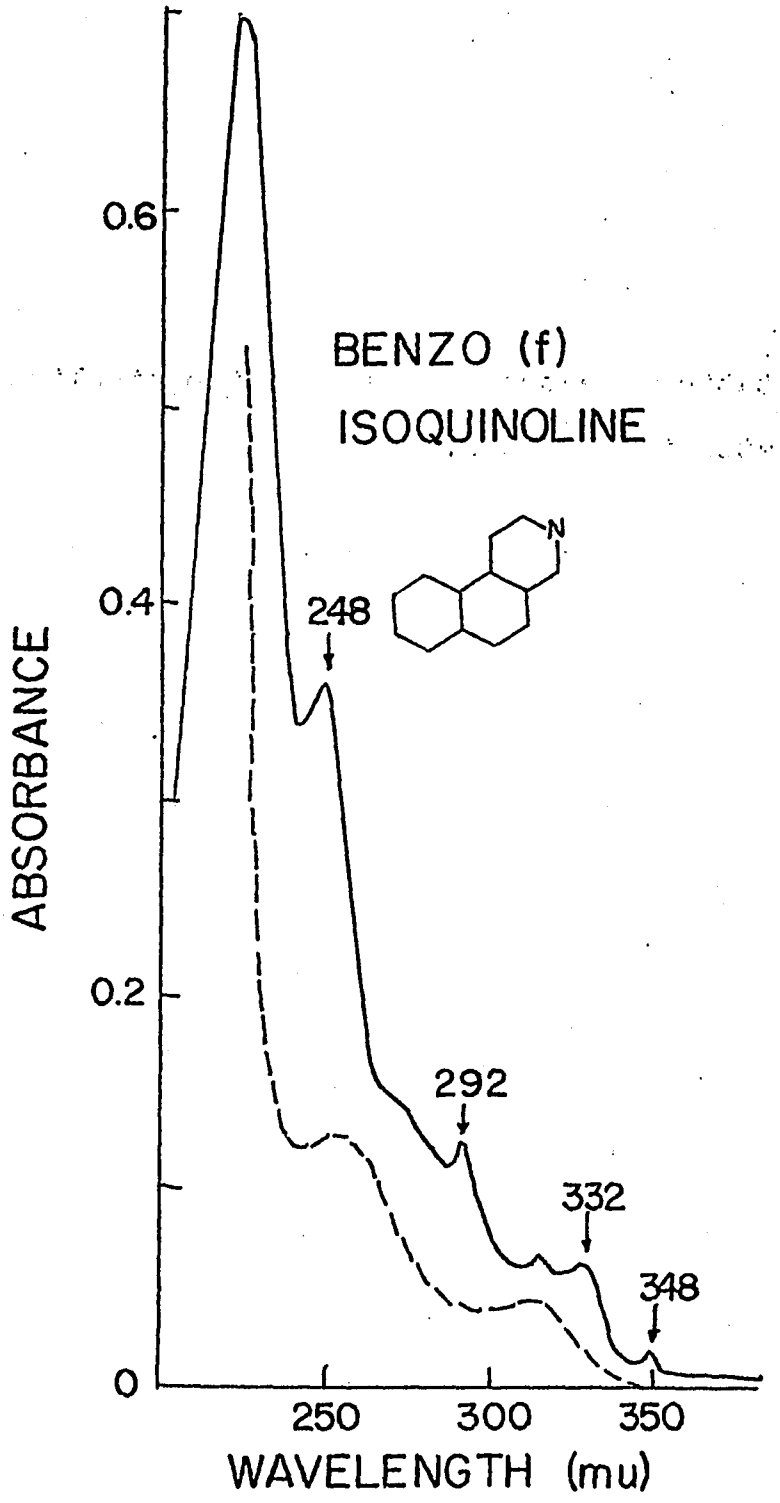

BENZO (f)
ISOQUINOLINE
N
248
292
332
348
0.6
0.4
0.2
0
ABSORBANCE
250
300
350
WAVELENGTH (mu)

Figure 25. Fluorescence Spectrum of Benzo(f)isoquinoline isolated in Sample 1.

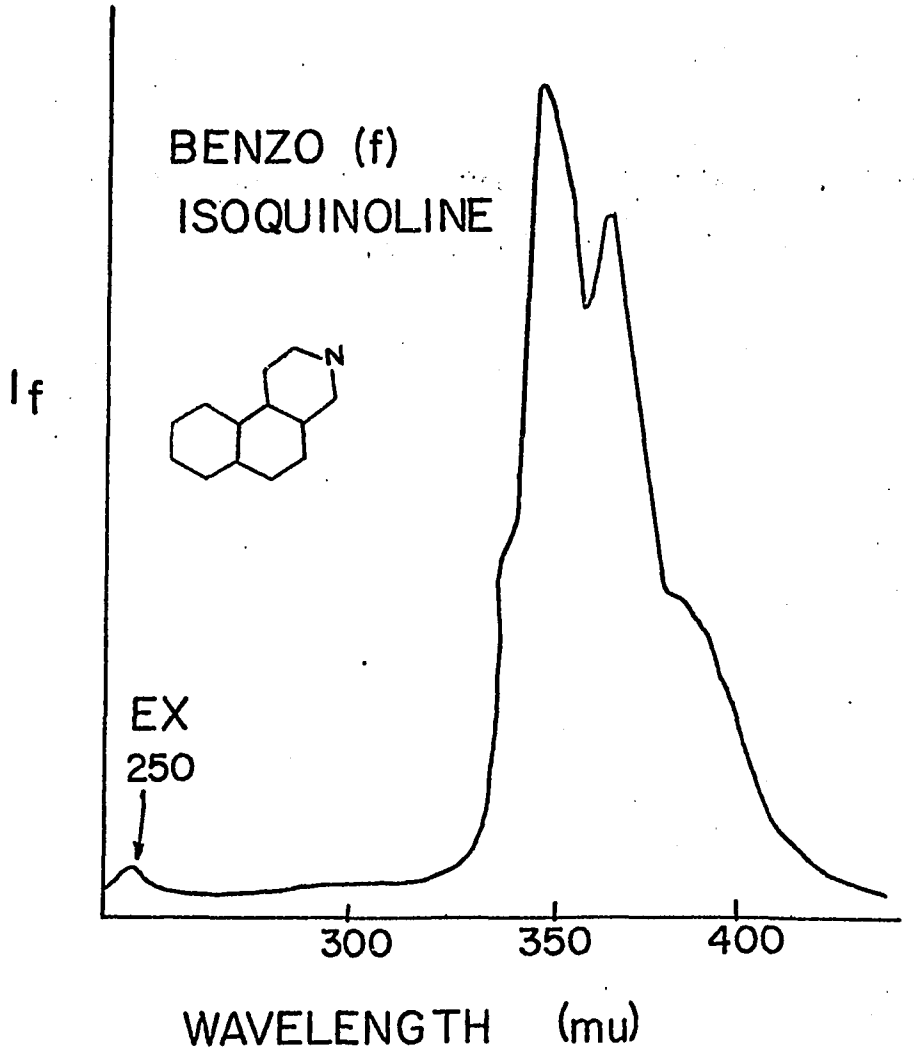

BENZO (f)
ISOQUINOLINE
N
I_f
EX
250
300
350
400
WAVELENGTH (mu)

Figure 26. Excitation Spectrum of Benzo(f)isoquinoline isolated in Sample 1.

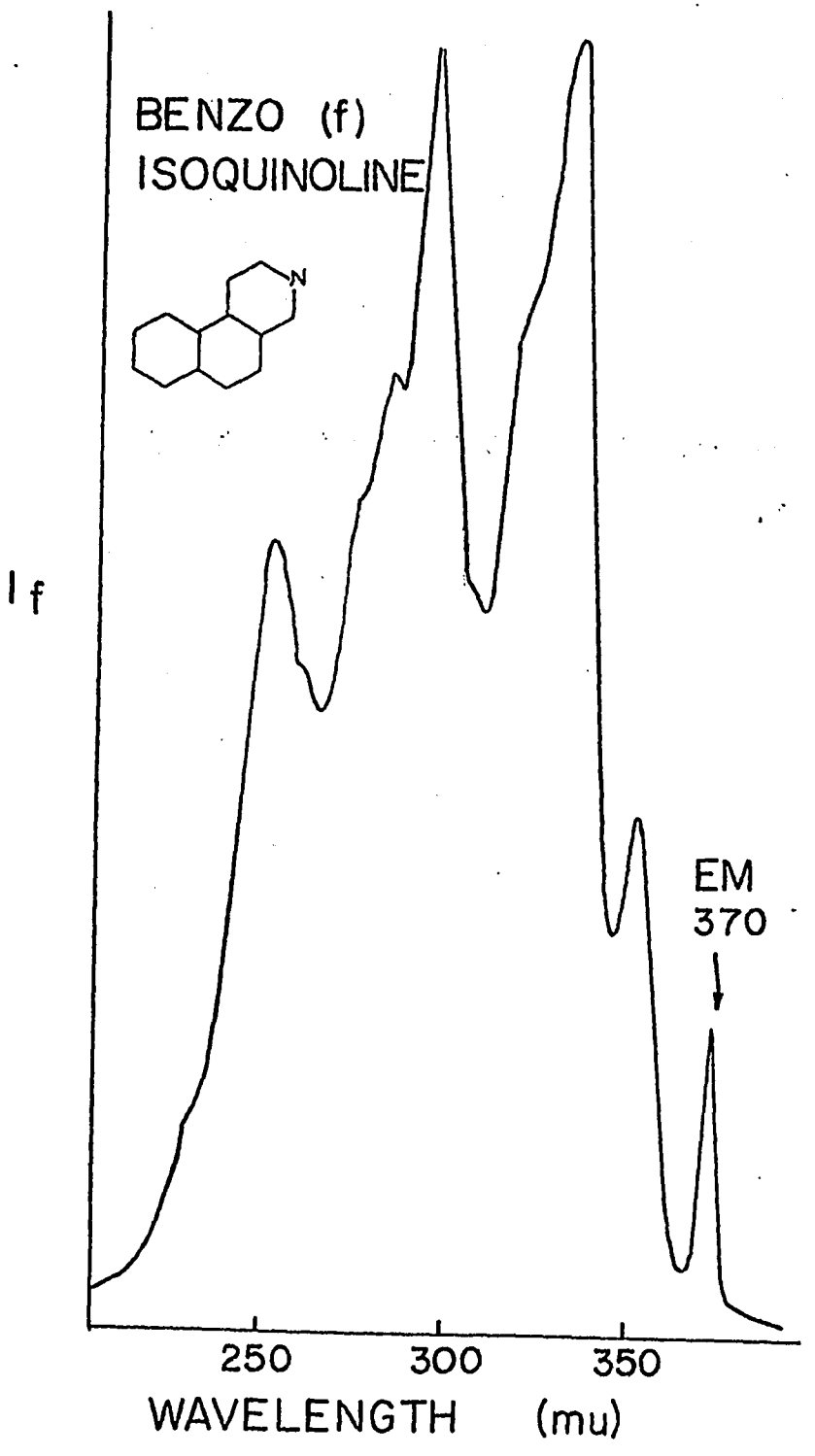

BENZO (f)
ISOQUINOLINE
N
If
EM
370
250
300
350
WAVELENGTH (mu)

Figure 27. Mass Spectrum of Benzothiazole isolated in Sample 1.

BENZOTHIAZOLE (ISOLATED)

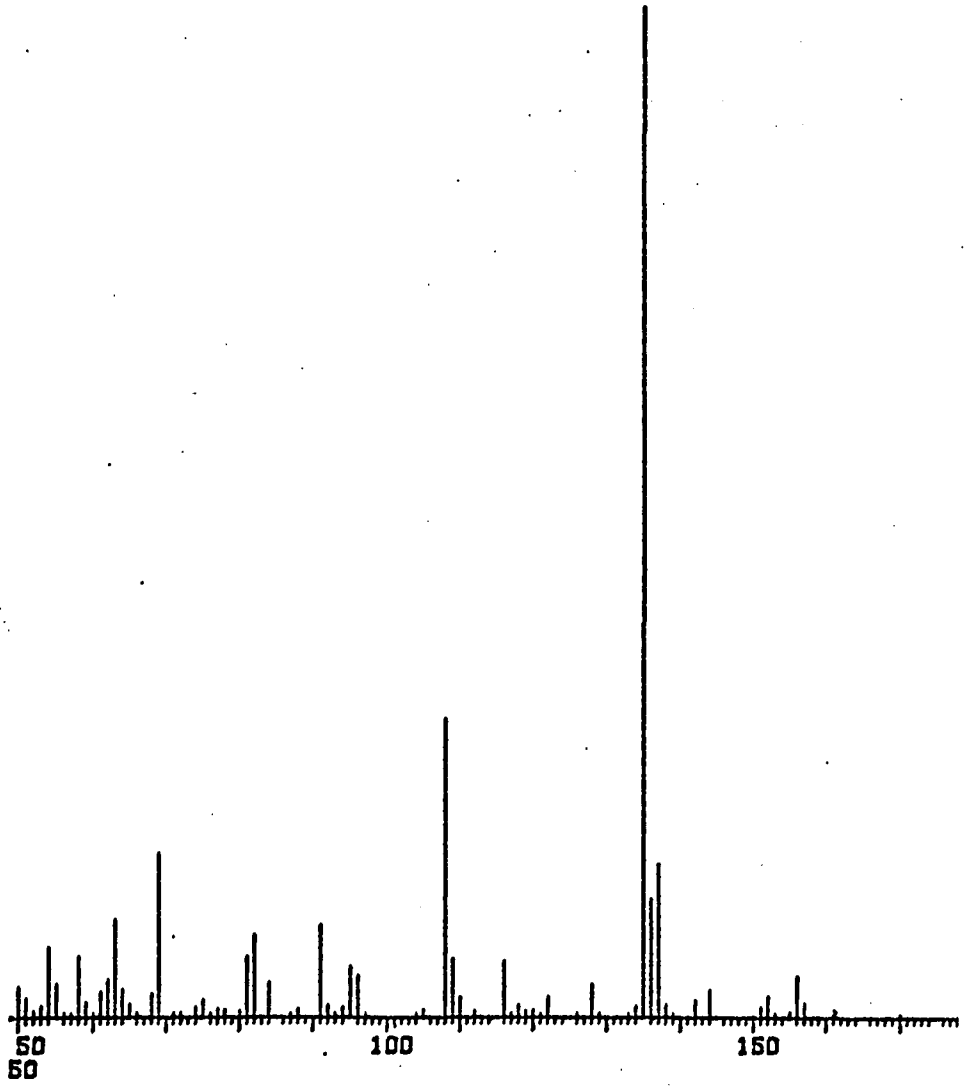


Figure 28. Gas Chromatogram of Fraction VIII of Sample 1.
(Chromatographic conditions same as in Figure 2).

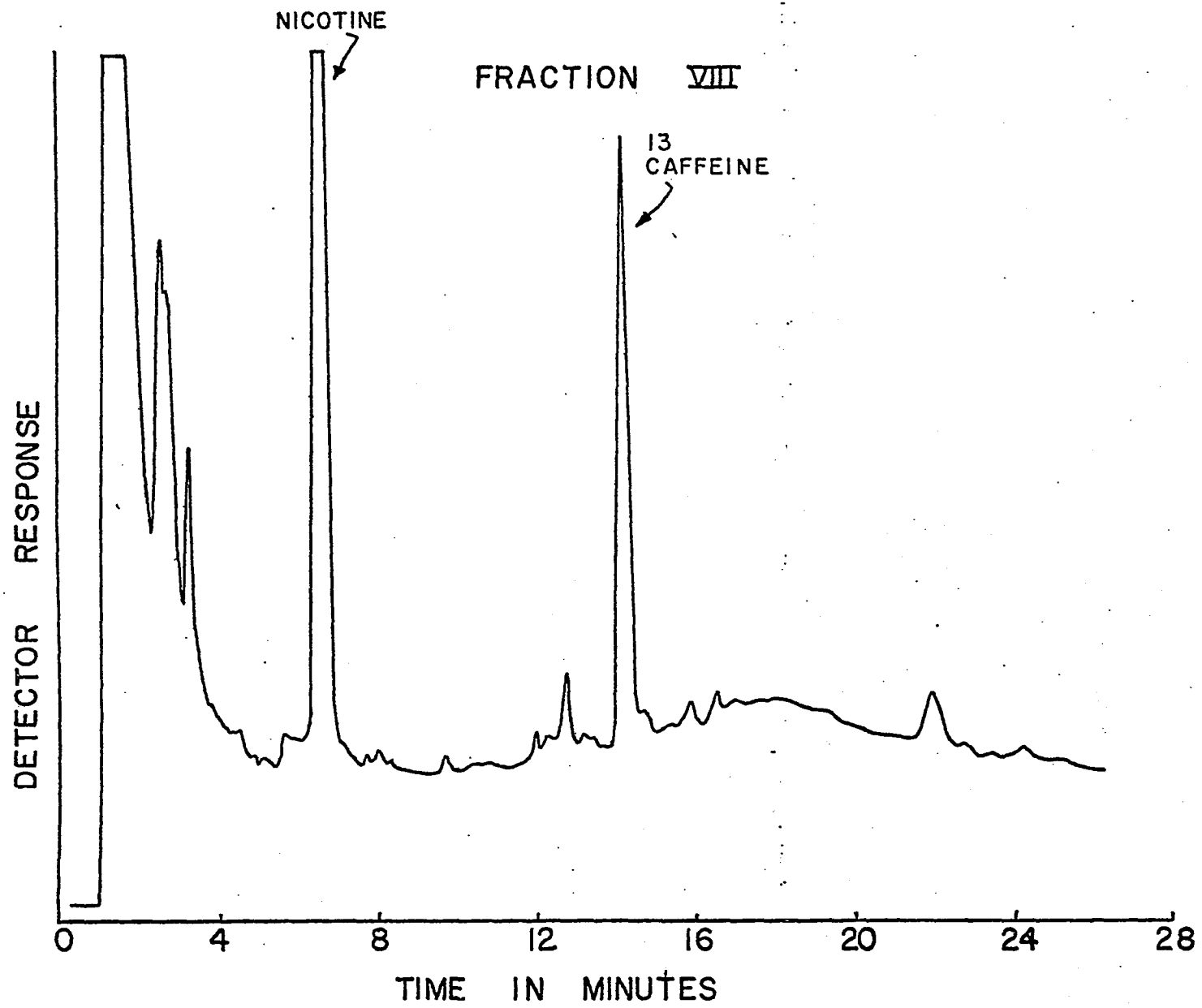

NICOTINE
FRACTION VIII
13
CAFFEINE
DETECTOR RESPONSE
0
4
8
12
16
20
24
28
TIME IN MINUTES

Figure 29. Liquid Chromatogram of Fraction VIII in Sample 1. (Chromatographic conditions same as in Figure 4).

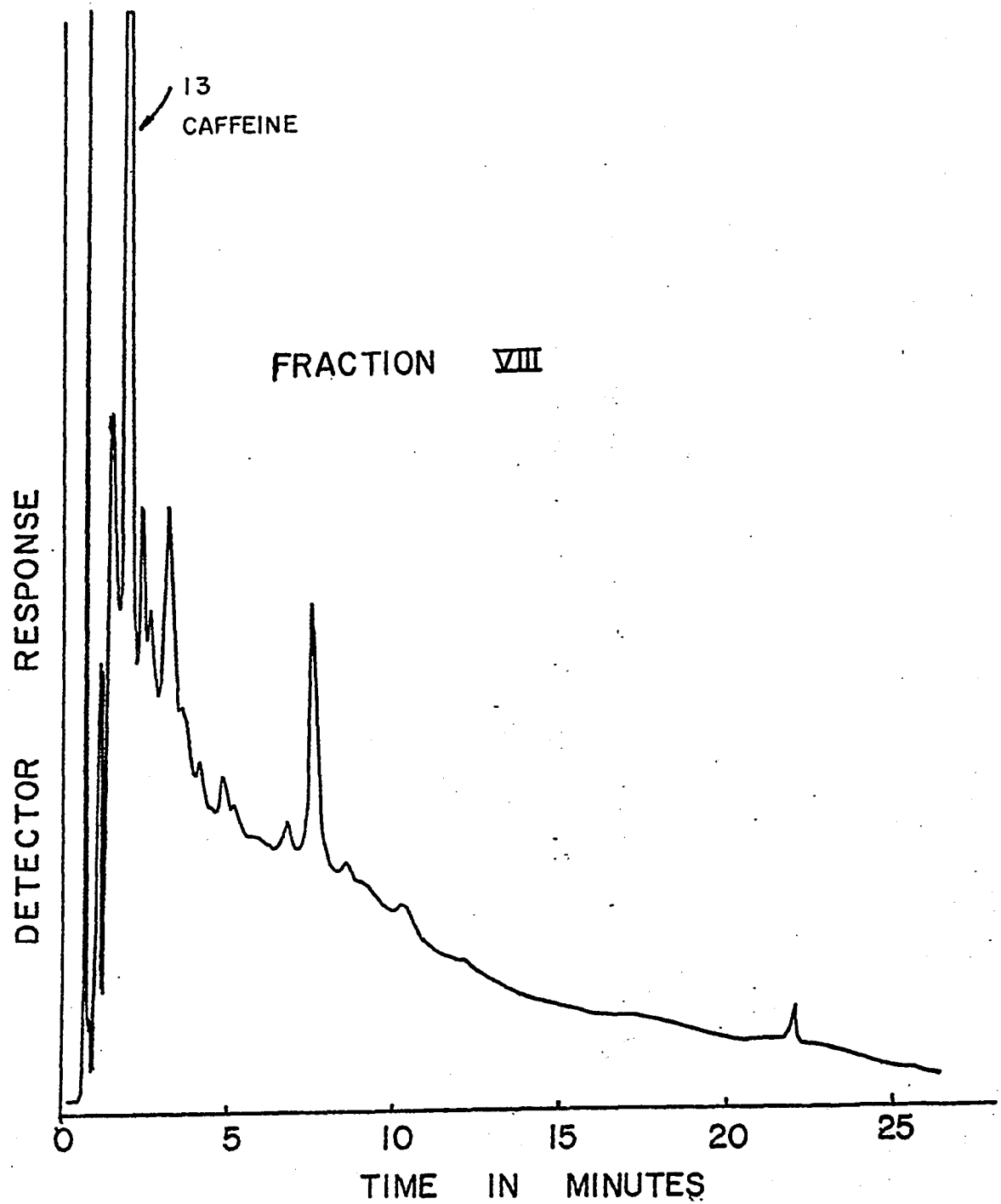

13
CAFFEINE
FRACTION VIII
DETECTOR RESPONSE
0
5
10
15
20
25
TIME IN MINUTES

Figure 30. UV Spectrum of Caffeine isolated in Sample 1.

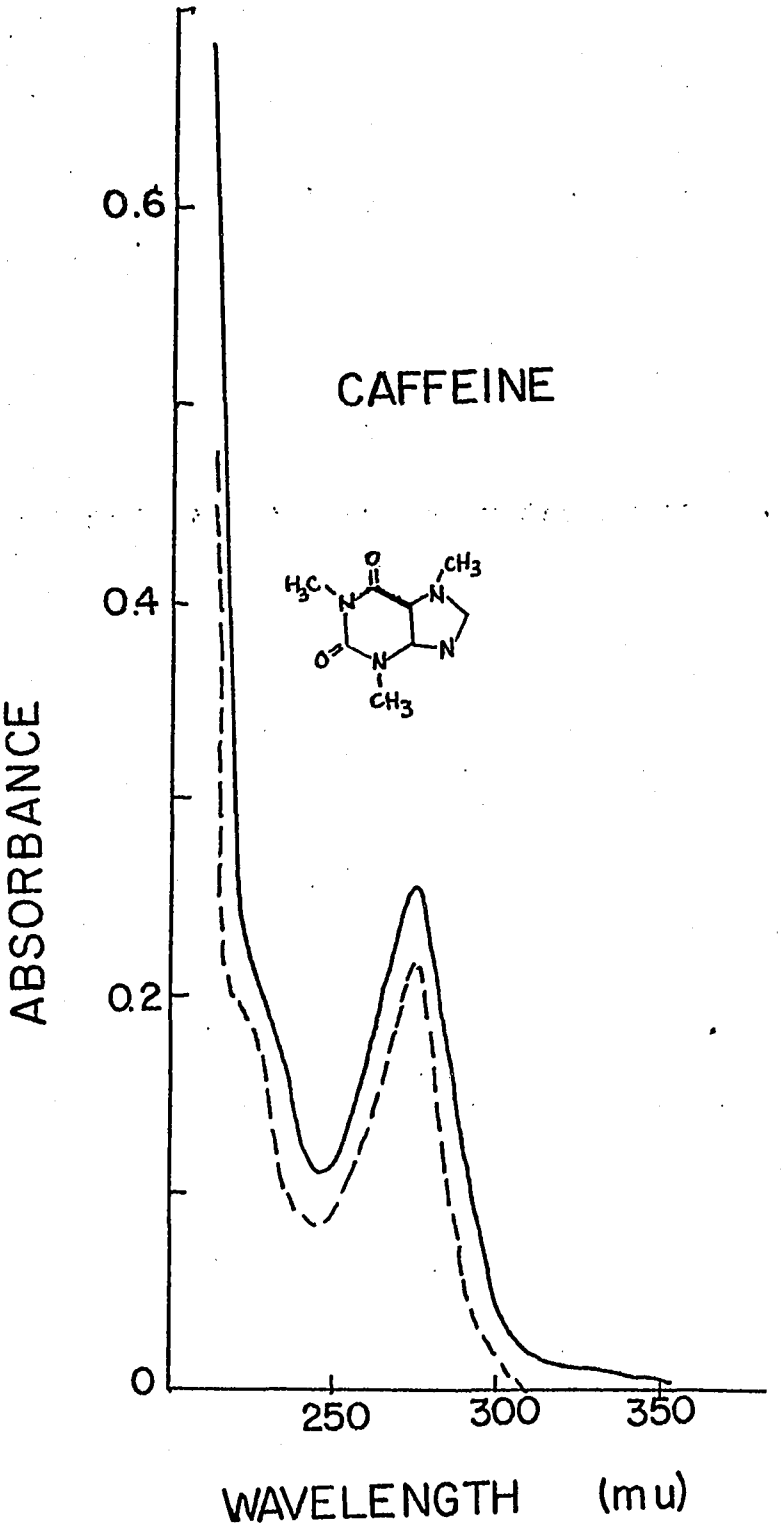

CAFFEINE
ABSORBANCE
0.6
0.4
0.2
0
250
300
350
WAVELENGTH (mu)

Figure 31. Mass Spectrum of Caffeine isolated in Sample 1.

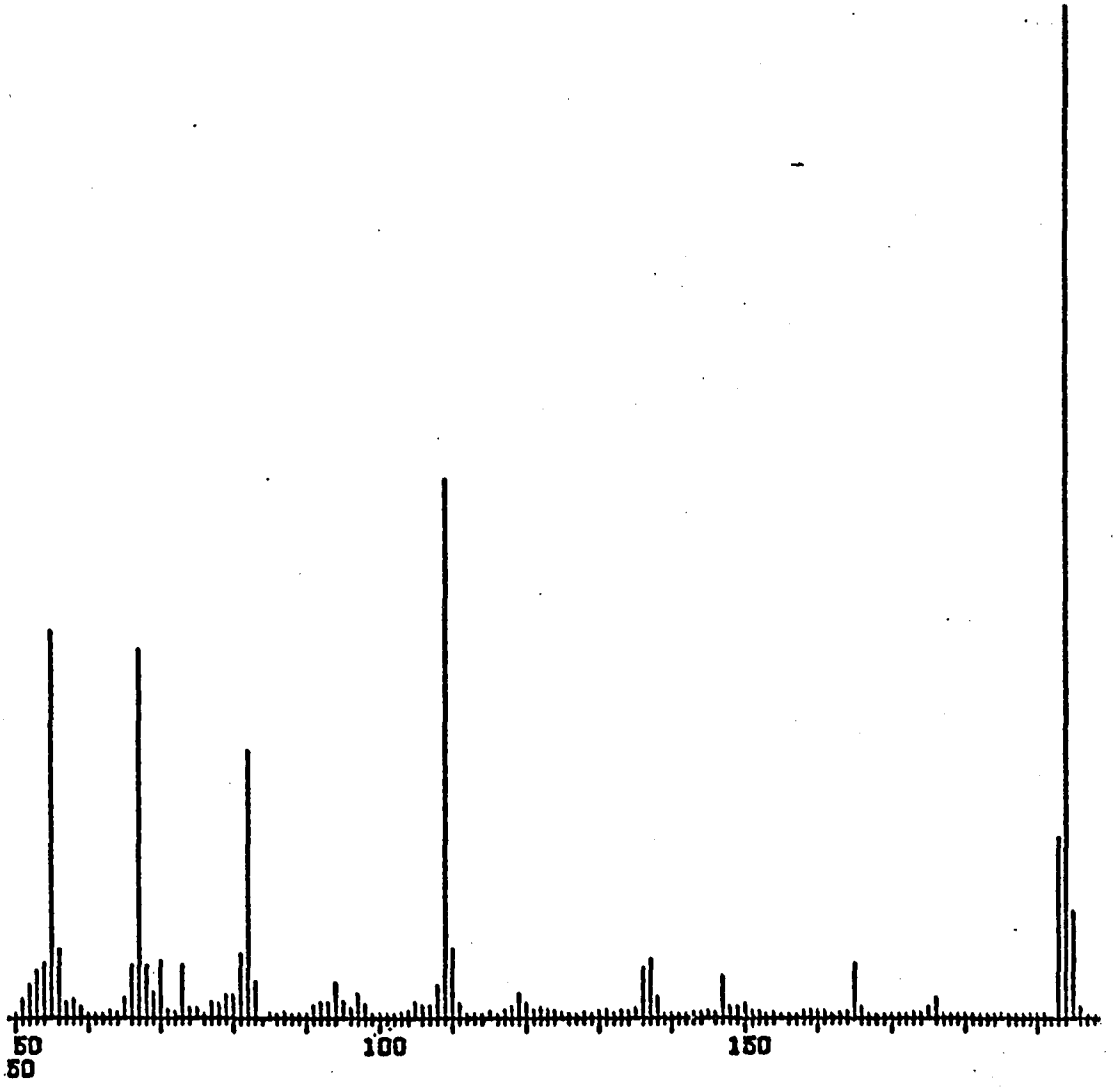
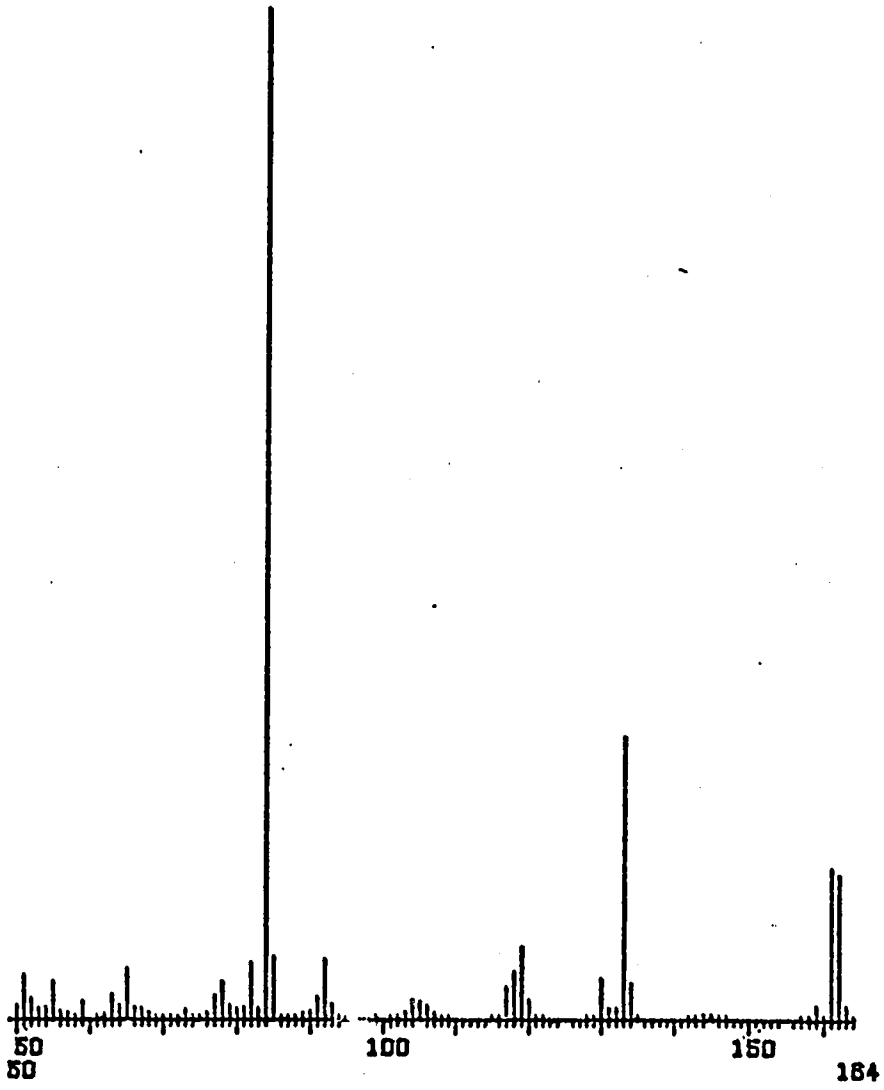

50
100
150
50

Figure 32. Mass Spectrum of Nicotine isolated in Sample 1.


50
50
100
150
164

APPENDIX I

NEW YORK CITY AEROMETRIC NETWORK

The problem of air pollution in New York City is a function of widely distributed sources of contaminants and of meterological conditions which prevail over a large geographical areas. The New York City Aerometric Network, an automated data network operated by the New York City Department of Air Resources, was set up in 1969 for the purpose of effectively monitoring the levels of harmful air pollutants and providing timely, accurate information on air quality. It consists of 28 manual stations and 10 telemetry stations located at 38 strategic sites in the city. Data from these stations are processed in the command station at department headquarters in downtown Manhattan, where research facilities are also available in laboratories for the development of new analytical methods for the measurements and control of air pollution. The locations of these stations are shown in Figure 33.

Equipment packages for the measurement of SO_2, smokeshade, settleable and suspended particulate matter are located at all stations. The telemetry stations have additional equipment for the monitoring of CO_2, air temperature, wind speed and direction.

The functions of this network are:

A. To objectively measure progress toward air quality goals.

B. To indicate where further abatment steps are required.

C. To define ambient air quality in all part of the City.

D. To provide information which would activate an alert warning system.

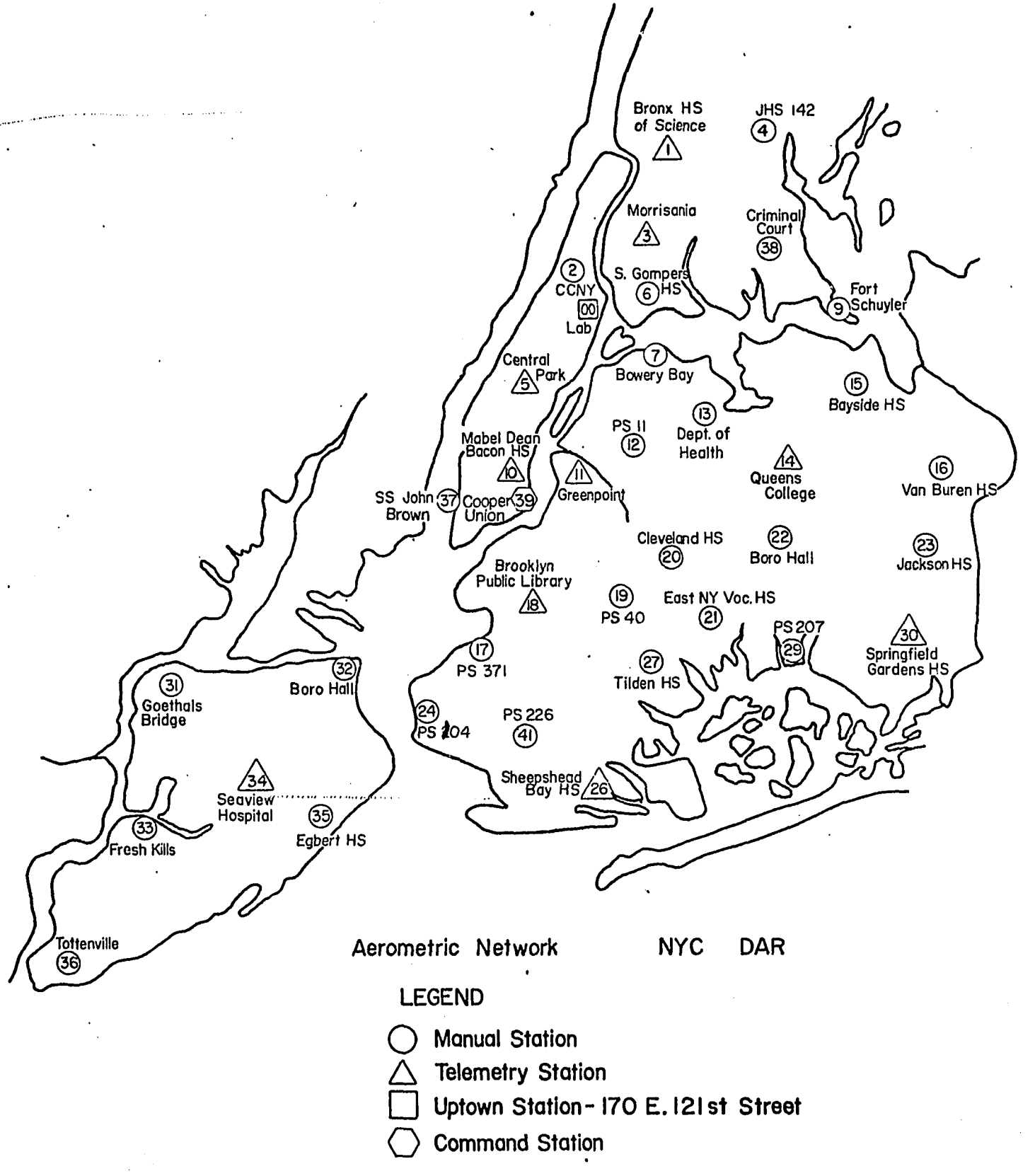
E. To provide daily reports to the public.

F. To establish data bank for epidemiological and other researchers.

G. To provide data for constructing mathematical models.

A brief description concerning the monitoring of particulate matter follows. Settleable particulates are measured in dustfall jars and suspended particulates are collected by high-volume samplers (General Metal Works 200H) on 8" X 10" glass fiber filters (Mine Safety Appliance Co.). The total weight of suspended particulate matter collected daily at each stations are obtained by weighing. Isopleth maps for particulate pollution are plotted from these data on a daily, weekly and monthly basis. 12 metals in suspended particulate matter are routinely analyzed in the laboratory by atomic absorption. For more detailed information on New York City Aerometric Network, consult the City of New York, Environmental Protection Administration, Department of Air Resources, New York, New York 10003.

FIGURE 33


Bronx HS of Science
1
JHS 142
4
Morrisania
3
Criminal Court
38
2
CCNY
00
Lab
S. Gompers HS
6
Fort Schuyler
9
Central Park
5
7
Bowery Bay
15
Bayside HS
13
Dept. of Health
PS 11
12
Mabel Dean Bacon HS
10
11
Greenpoint
14
Queens College
16
Van Buren HS
SS John Brown
37
Cooper Union
39
Cleveland HS
20
22
Boro Hall
23
Jackson HS
Brooklyn Public Library
18
19
PS 40
East NY Voc. HS
21
PS 207
29
30
Springfield Gardens HS
17
PS 371
27
Tilden HS
32
Boro Hall
31
Goethals Bridge
24
PS 104
PS 226
41
Sheepshead Bay HS
26
34
Seaview Hospital
33
Fresh Kills
35
Egbert HS
Tottenville
36
Aerometric Network
NYC DAR
LEGEND
Manual Station
Telemetry Station
Uptown Station - 170 E. 121st Street
Command Station

VITA

Michael W. Dong was born May 5, 1949, in Shanghai, China, the son of Liang-yo and Si-fong Dong. The family moved to Hong Kong in 1951 where he received his primary and secondary schooling. He attended Diocesan Boys' School in Hong Kong and came to the United States in 1968.

In June 1971, he was graduated with a B. S. in Chemistry from Brooklyn College, Brooklyn, New York, where he also did undergraduate research sponsored by the Atomic Energy Commission under Dr. Harmon Finston on various aspects of radiochemistry.

He enrolled at City University of New York Graduate School in September, 1971 and was married to Cynthia Ng of Bayside, New York, in 1972.

A member of the American Chemical Society, he will do postdoctoral research at Naylor Dana Institute for Disease Prevention, American Health Foundation, Valhalla, New York, with Dr. Dietrich Hoffmann and Dr. Irwin Schmeltz in tobacco and cancer research.