PB80-119606

United States
Environmental Protection
Agency

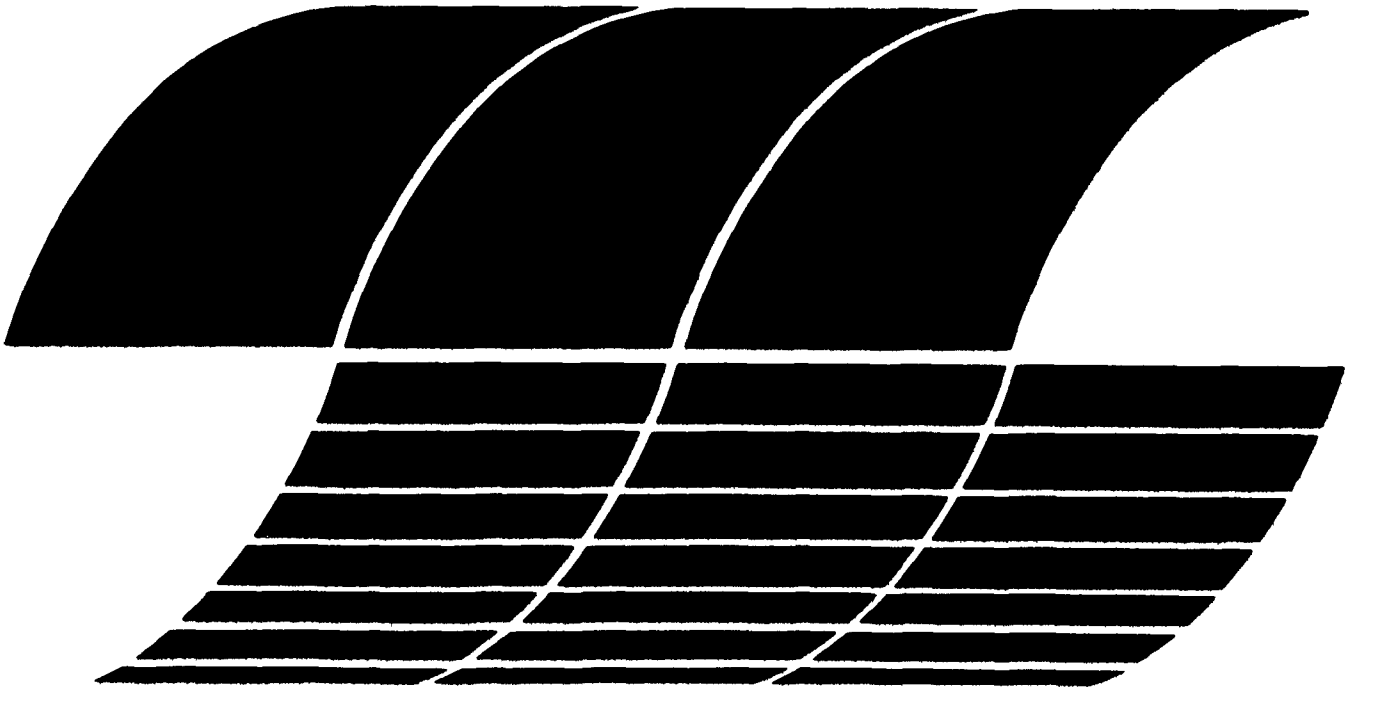
Industrial Environmental Research
Laboratory
Research Triangle Park NC 27711

EPA-600/7-79-191
August 1979

EPA

Measurement of Polycyclic Organic Matter for Environmental Assessment

Interagency Energy/Environment R&D Program Report



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EPA-600/7-79-191

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Measurement of Polycyclic Organic Matter for Environmental Assessment

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Contract No. 68-02-2150
Task No. 10202
Program Element No. INE624

EPA Project Officer: Larry D. Johnson

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Prepared for

U.S. ENVIRONMENTAL PROTECTION AGENCY
Office of Research and Development
Washington, DC 20460

ACKNOWLEDGEMENT

The authors wish to acknowledge the contributions of groups at Battelle Columbus Laboratories and Monsanto Research Corporation for their comments concerning methods of data acquisition on various instruments and analytical procedures for POM analysis.

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SUMMARY

This report discusses methods for the measurement of Polycyclic Organic Matter (POM) for environmental assessment. Two fluorescence methods are described for the estimation of total POM levels in samples. Either of these methods may be used to screen samples for further specific analysis. Three gas chromatography-mass spectrometry (GC/MS) methods are described for the measurement of specific POM compounds. The use of liquid crystal chromatographic phases is recommended for the measurement of a few POMs, i.e., specifically for benzo(a)pyrene. GC/MS methods for a wide range of POMs are discussed for both capillary and packed GC columns. The three methods for specific POM identifications are verified with collected environmental samples of different kinds.

I. INTRODUCTION

There is a great deal of interest in the reliable analysis of polycyclic organic matter (POM). POM has been defined by the National Academy of Sciences[1] as the polycyclic aromatic hydrocarbons (PAH) plus their heterocyclic analogs. POM species are often found in combustion effluents[2] and are widely distributed at varying levels throughout the environment.[3] Since specific POM compounds have been identified as causal agents in the development of cancers, analytical methods for specific POMs are needed to accurately assess potential health hazards. In this document, five areas of effort are covered: 1) pertinent background literature is reviewed to illuminate current activities in the field of POM analysis, 2) several general methods for detection of POM are discussed for use as screening methods, 3) the development of analytical procedures for analyzing specific POM compounds are presented, 4) the verification data for the procedures developed for specific POMs using real samples are presented, and 5) a detailed description of each method is furnished.

A. Historical Perspective

The earliest association of certain cancers with combustion effluents dates to 1776 with the report of high incidence of scrotal cancers in chimney sweeps.[4] By the early 1900's, various workers[5] had succeeded in producing skin tumors in experimental animals by direct application of tar. Work by Dreifus,[6] Kennaway,[7] and Cook,[8] and others established a link between tumor formation and specific POMs (i.e., 1,2,5,6-dibenzanthracene, 1,2-benzopyrene and substituted benzanthracenes). Since these early studies, a great deal of effort has been spent in determining the carcinogenic potential of specific compounds. By 1953, 450 compounds were determined to be carcinogenic of which over 200 were POMs, specifically polycyclic aromatic hydrocarbons, their derivatives and analogs.[9] The carcinogenic activity of POM species changes markedly with relatively minor structural changes in ways that are difficult to predict. Whereas the addition of methyl groups frequently enhances carcinogenicity, addition of a

methyl group at the 5 position of the potent dibenzo (ah or ai) pyrenes reduces their activity and introduction of two methyl groups eliminates the activity completely.[9] The large variation in carcinogenic activity of POMs with slight variations in structure makes the accurate analysis of specific POMs an important factor in environmental monitoring and environmental impact assessment.

Information concerning POMs, their occurrence, health effects, and chemistry is available in many sources. A thorough review of the relationships between POMs and carcinogenesis is found in a document prepared by the Committee on Biologic Effects of Atmospheric Pollutants of the National Academy of Sciences (USA) entitled, "Particulate Polycyclic Organic Matter."[1] This document includes discussion of such topics as the sources of POMs, the physics and chemistry of POMs distributed throughout the environment, testing procedures for carcinogenic, mutagenic and/or teratogenic properties, and clinical studies on exposure to POMs, as well as a set of appendices dealing with the collection and analysis of samples for POMs. This document provides a general overview of the POM problem with respect to health effects.

A series of symposia (International Symposia on the Analysis, Chemistry, and Biology of Polynuclear Aromatic Hydrocarbons) published under the title "Carcinogenesis,"[10,11] offers one method of maintaining current awareness of the status of chemical and biological investigations concerning POMs. The intent of this symposium series, as stated by the editors, is to "provide a valuable forum for discussion and examination of the most recent research findings in the area of analysis, chemistry, and biology of PAHs."[10,11] The types of papers presented at these symposia cover sampling and analysis techniques for POMs, studies of POM formation mechanisms, studies of POM metabolic pathways, and biotesting procedures applicable to the study of POMs.

B. Analytical Methodology

The interest in POMs has given rise to a host of qualitative and quantitative procedures for POM analysis. Of the various procedures, some are intended to give a single quantitative value for a specific

compound, such as for benzo(a)pyrene, while others attempt to give a composite value indicative of the total quantity of POM present in a sample. The main difference between most specific and general analytical techniques is a separation of the species of interest prior to detection.

In the following discussion, the reported methods will be grouped by the general analytical techniques used. Application of each technique to either specific or general analyses will be discussed within that section. For reference, Table 1 contains a list of specific POMs presently of interest in most environmental analyses.

Prior to the detection of POMs, some suitable procedure should be used to isolate POM from interferences and to concentrate the sample. Historically, the techniques of thin layer chromatography and paper chromatography were used to isolate specific POMs for subsequent identification. Current practice utilizes column chromatography to separate POMs from non-POMs (see Appendix A) followed by either high-pressure liquid chromatography or gas chromatography for resolving specific POM isomers from one another. The use of these procedures, in conjunction with POM analysis, is reviewed extensively elsewhere.[12] The emphasis in this report is on the use of gas chromatography (GC) which when combined with mass spectrometry is a powerful tool for the detection and quantification of POMs ranging up to the dibenzopyrenes in molecular weight.

1. Fluorescence Methods

Fluorescence methods were among the very first methods used for the detection of POMs. The work in the early 1930's by J. W. Cook and co-workers[8,13] made extensive use of fluorescence spectroscopy to identify benzo(a)pyrene as a carcinogenic constituent of coal tar. Various workers[14,15,16] have employed fluorescence spectroscopy to follow the metabolic reactions of specific POM compounds during hydroxylation. The analysis of specific POMs has also been undertaken with fluorescence methods. Benz(a)anthracene,[17] benzo(a)pyrene,[18] and indeno(1,2,3-cd)-pyrene[19] are just a few examples of specific POMs which have been analyzed with fluorescence spectroscopy. Fluorescence techniques have been used by Sawicki[20] and others to examine a wide range of POM containing samples.

TABLE 1

Key POMs of Interest in Environmental Samples

	NAS Rating*
Fluorene	-
Anthracene	-
Phenanthrene	-
Fluoranthene	-
Pyrene	-
Benzo(c)phenanthrene	+ + +
Benz(a)anthracene	+
Chrysene	±
Triphenylene	(not rated)
Benzo(b,k or j) fluoranthene	+ +
Benzo(e)pyrene	-
Benzo(a)pyrene	+ + +
Perylene	-
7,12-dimethyl benz(a)anthracene	+ + + +
3-methyl cholanthrene	+ + + +
Indeno(1,2,3-cd)pyrene	+
Benzo(g,h,i) perylene	-
Dibenz(a,h) anthracene	+ + +
Dibenz(a,i or a,j) acridine	+ +
Dibenzo(c,g) carbazole	+ + +
Dibenzo(a,i or a,h) pyrene	+ + +
Coronene	-

*Reference 1.

Fluorescence methods are capable of measuring subnanogram quantities of individual POM species, but tend to be fairly non-selective. The normal spectra obtained from POM and related species tend to be intense and lack resolution in fluid media. To determine levels of specific compounds, thorough separation of the sample constituents is needed to minimize spectral interferences. Efforts to overcome this difficulty have been directed towards low temperature techniques. The fluorescence of various POMs at temperatures of 20°K or less no longer consists of a series of broad, smooth peaks found in room temperature fluid media, but contains many discrete peaks of varying intensity. The unique nature of the spectra obtained with this method is demonstrated by the fact that the six isomeric mono-methylchrysenes can be distinguished from one another by their low temperature fluorescence spectra.[21] However, identification of an unknown species from its spectrum is not an easy task. Compilation of reference spectra and rapid methods of searching reference spectra are not as well developed or as widespread as those often used in infrared or mass spectroscopy. Furthermore, it may be difficult to recognize spectra, even of pure compounds, because of phenomena such as saturation at high concentrations. Also, certain classes of POMs, such as nitrogen containing heterocyclics, exhibit low efficiency towards fluorescence and may not be detected in small quantities.

Work at Oak Ridge National Laboratories[22] has applied synchronous fluorescence and phosphorescence to PAH analysis. With the synchronous technique, the normally broad fluorescence spectra of the PAH species is simplified to a series of distinct and better resolved peaks. The inprovement in the distinguishing features of the different PAH spectra enhances the specificity of these luminescence techniques, and the potential application of these methods to provide more specific information than total POM measurements needs to be further explored.

2. Ultraviolet Absorption Spectroscopy

Ultraviolet absorption spectroscopy (UV) is another analytical detection method which has been widely used. For many years, the preferred approach for POM analysis was to use a combination of liquid chromatography (LC) and thin layer chromatography (TLC) to isolate

specific POMs followed by UV techniques for species identification and quantification.[23] Benz(a)anthracene,[24] benzo(a)pyrene,[25] and indeno (1,2,3-cd) pyrene[26] are typical examples of POMs which may be detected. Compilations of UV data for numerous POMs are found in a number of sources.[27,28] As with fluorescence measurements, the individual spectra for various POMs are unique, although portions of spectra for different compounds may be the same; for example, both chrysene and 4,5-methylene chrysene have similar absorption bands at 361 nm and both benzo(a)pyrene and benzo(ghi)perylene have similar bands at 382 nm. The possibility of spectral overlap requires complete separation of sample components to insure accurate measurement of component levels, as with fluorescence techniques. Also, the overall sensitivity of measurements by UV methods is somewhat lower than for fluorescence methods (one-tenth to one-thousandth that of fluorescence). Hence, the use of UV measurements for POM analysis has declined, being replaced with the more sensitive fluorescence methods and the highly sensitive and specific GC/MS methods.

3. Gas Chromatographic Methods

Gas chromatographic methods have shown the most rapid development for POM analyses in recent years. The first useful application of GC techniques to POM analysis dates to 1965, with methods being found in the Journal of Chromatography[29] and Analytical Chemistry.[30] The instrumentation for a GC separation is relatively inexpensive and samples may be analyzed conveniently with high speed and good reproducibility. Detection of the GC effluent may be by any of a wide range of analytical procedures. Since UV methods have been widely used for POM detection and the UV spectra are specific for different POMs, methods which couple GC for separation and UV for detection have been developed. Other detectors, such as flame ionization detection (FID), electron capture detection (ECD), mass spectrometer (MS) detection or infrared (IR) detection have been coupled with GC separation for sensitive and specific analyses.

Numerous GC column packings have been developed to enhance the separation of specific POMs. Methyl phenyl silicones, such as SE-52, OV-17, or SP2250,and carborane methyl- and methyl phenyl silicones, such as Dexsil 300 or Dexsil 400, have been widely applied to POM separations.

These GC phases will resolve groups of POMs from one another, 3-ring POMs from 4-ring, 4-ring POMs from 5-ring, etc., but the resolution of specific isomers is not possible in every case.

Two developments greatly improved the specificity of GC analyses of POMs. Capillary columns have been known for their inherently high resolution since the work of Golay in 1958.[31] However, extensive use of capillary columns was delayed until the development of efficient injection systems and reproducible coating of the stationary phase on the capillary tube. The high resolution obtainable by capillary GC techniques allows separation of selected isomers unresolved with normal packed columns (see e.g., Lee, et al.[32] for an extensive list of POM relative retention indices). However, capillary columns will only tolerate small sample volumes. Thus, the detector for capillary GC methods must have high sensitivity such as flame ionization detectors (FID), electron capture detector (ECD), or a mass spectrometer (MS). Of these, the last detector system, a mass spectrometer, allows confirmation of species identification and, through the use of computerized data handling procedures, greatly enhances the specificity of the analytical measurement.

The other advance in GC technique which has affected POM analysis is the liquid crystal GC column phase [for example, N,N'-bis(p-phenylbenzylidene) α,α'-bi-p-toluidine (BPhBT)]. In 1975, Janini[33] and coworkers first applied nematic liquid crystals to the separation of polycyclic aromatic hydrocarbons. These columns resolve geometric isomers of different POMs, but the observed elution order of the isomers is frequently different than that observed for the general purpose silicone columns. For example, the normal elution order -- benzo(e)pyrene, benzo(a)pyrene, pyrene -- observed on OV-17 and Dexsil-300 and -400 columns is different from that of a liquid crystal column such as SP-301 (BPhBT) -- benzo(e)pyrene, perylene, and benzo(a)pyrene. To obtain the best separation of POMs, isothermal column operation is required which limits the use of these columns with complex samples. When coupled to a selective detector, e.g., a mass spectrometer, which may be adjusted to minimize the interferences due to other non-POM species in a complex sample, liquid crystal columns are highly useful

for determining quantities of narrow groups of specific POMs.

The power of the combined GC/MS system for POM analyses makes such systems the method of choice for specific POM analyses. Detailed procedures are presented later in this report.

4. Miscellaneous Methods

The intense interest in POM analysis has brought forth a large variety of special techniques for specific analyses. Infrared[34] and Raman spectroscopic techniques, nuclear magnetic resonance[35] methods, polarography,[36] and potentiometric titration[37] are examples of the types of alternative techniques which have been applied to POM analysis. For the most part, these methods tend to be prone to numerous interferences in real samples, and offer no significant advantage over UV, fluorescence or GC methods discussed previously.

In the following chapters, specific analytical techniques are presented for the analysis of POMs. In Chapter 2, two survey methods for POM analysis are presented. These methods are designed to give rapid order of magnitude data on total POM presence in environmental samples. In Chapter 3, three general GC/MS methods for specific POM analysis are presented. The three methods involve current technology and are chosen such that at least one of these methods will be applicable to GC/MS systems which have been or are being produced. In Chapter 4, experimental verification of the three methods described in Chapter 3 is given to provide practical information on the application of these methods to real samples. In Chapter 5, the methods presented in Chapters 2 and 3 are described in further detail for users of these methods.

II. SCREENING METHODS

An initial step in the analysis of samples for POMs may be to estimate the level of POM present. The method used should be rapid, low-cost and simple to perform. Methods which meet these requirements can be used to screen samples to point out those samples which need further specific analysis. If the level of POM present is very low, there may not be sufficient justification to go to the expense of performing specific POM analyses. Two methods are recommended here for use as low-cost screening procedures; both use luminescence spectroscopic techniques.

A. Total POM by Fluorescence

The procedure described in this section is based on the investigations of Sawicki,[38] as adapted for use by Battelle Columbus Laboratories.[39] This procedure makes use of the similarity of the fluorescence spectra for many POMs. In normal fluorescence analyses for POM, specific identifications can only be made for pure POM species. This is due to POMs having overlapping excitation and emission wavelengths. If instead of isolating specific POMs, the sample is fractionated so that one fraction contains only the POMs, then use of a broad excitation source and a broad emission detector will give data representative of the sum of the POMs present.

The first step in determining total POM is to fractionate the sample to isolate the POMs. Use of the Level 1 liquid chromatography procedure (Appendix A) results in the POMs eluting in fractions 2, 3, and 4. These fractions are combined, reduced in volume from 30 mL to a convenient small volume, such as 2 mL, and measured in a spectrofluorimeter set for 350 $\pm$ 5 nm wavelength excitation and 410 $\pm$ 5 nm wavelength emission. After each fluorescence measurement, the sample is diluted and remeasured until the sample is in the linear calibration region. Calibration is conveniently referenced against anthracene to obtain concentration values. Use of this POM measurement procedure yields answers which agree within a factor of three with those found by GC/MS for the same sample.[39] The complete procedure is found in section A of Chapter 5.

B. Sensitized Fluorescence Spot Test

The second method recommended for screening samples for POMs was developed by Arthur D. Little, Inc.[40] and involves the use of sensitized fluorescence. Sensitized fluorescence occurs when two or more fluorescent compounds are present in a solid or crystalline mixture with one being at a much higher concentration than the others and when these compounds are able to have vibrational coupling between their excited singlet energy states (i.e., the compounds have at least one vibrational level frequency in common in the excited state). In such cases, if the mixture is excited and the compounds absorb energy, the fluorescence emission will occur preferentially from the compound which has the lowest vibrational energy level of the excited state. For example, sensitized fluorescence of naphthacene occurs in mixtures of naphthacene and anthracene with the naphthacene level about 10^{-6} that for the anthracene.[41]

The spot test procedure involves drawing three spots on a piece of filter paper, applying sample and/or naphthalene to each spot such that one spot contains naphthalene, a second spot contains the sample of interest, and the third spot contains both naphthalene and the sample, exciting the sample with 254 nm radiation and visually observing the fluorescence. The observed fluorescence of the spots gives an order of magnitude estimate of the total POM level found in a sample. Table 2 shows possible observations and the resulting POM concentration level. For complete details, see reference 40 and section B of Chapter V.

TABLE 2

Fluorescence/Concentration Information for POM Spot Test Procedure* using Naphthalene as Sensitizer

	Observation	POM Concentration
a.	Non-fluorescent when treated with sensitizer	$\leq$ 1 pg/μL
b.	Weakly fluorescent when treated with sensitizer	1-10 pg/μL
c.	Strongly fluorescent when treated with sensitizer but not fluorescent alone	$\geq$ 100 pg/μL
d.	Fluorescent without sensitizer	$\geq$ 10,000 pg/μL

*from Reference 40.

III. SPECIFIC POM DETERMINATION BY GC/MS

The choice of GC/MS as the recommended procedure for specific POM analysis is influenced by several factors. The sensitivity and selectivity of the GC/MS combination is well known. After separation of a complex sample into a large number of chromatographic peaks, the identity of those peaks suspected from their retention time to be POMs can be confirmed from the mass spectra. The use of GC/MS for specific POM determinations is widely accepted and implemented. Due to the instrumental differences between the various GC/MS combinations available and the manner in which different instruments acquire and process sample data, no single combination of instrumental parameters will be directly applicable to all GC/MS systems.

It is not the intent of this document to specify a single instrument for POM analysis by GC/MS to the exclusion of the wide range of other instruments capable of obtaining high quality analyses. For these reasons, the methods outlined in this chapter are somewhat general and intended to be as widely applicable as possible. In the remainder of this chapter, analytical details and procedures for the analysis of POMs are discussed. The general mass spectrometer operating parameters are discussed first,with the discussion of gas chromatography conditions following. The latter discussion is divided into three sections to cover the three major choices for GC separation of POM: packed columns, capillary columns, and liquid crystal phases for GC column packing. The verification data resulting from application of the methods in this section to environmental sample are given in Chapter 4. Detailed descriptions of methods for GC/MS determination of POMs using packed GC columns, capillary GC column and liquid crystal phases in GC columns are given in Chapter 5.

A. Mass Spectrometer Conditions

The exact mass spectrometer conditions chosen for a single analysis depend on many things: the specific manner in which the mass spectrometer acquires and processes data, and instrumental sensitivity required, as well as the reason for the analysis. Although the specific conditions

are highly variable, a number of common conditions are present in any analysis. The discussion in this section focuses on the instrumental options available and their application. The instrumental variables discussed include:

- general instrument set-up
- mode of ionization
- data acquisition/processing
- use of internal standards
- quantitative calibration

The overall instrument operating condition obviously has a great effect on the reproducibility of the data obtained. What is sometimes less obvious, however, is that fully automated GC-MS-DS systems may make it difficult to recognize the overall instrument operating condition. The condition of the ion source affects sample data collected. A source which is dirty shows altered focusing conditions, distorted ion peak profiles, and lower transmission efficiency compared to a clean source. For quadrupole instruments, which are quite sensitive to the transmission of high mass ions, this can lead to loss of sensitivity for high molecular weight POMs. Leaks in the vacuum system will affect instrument performance through ion-molecule reactions and/or depletion of the quantity of the ionizing species (electrons for EI or charged reagent ions for CI). For some automated GC-MS-DS systems, it is difficult to monitor the ion peak profiles to assess the condition of the ion source. For such systems, routine periodic monitoring of the absolute intensities of sets of ions spanning the mass range of interest from a calibration mixture, for example, provides a method of checking instrument performance in lieu of monitoring ion peak profiles directly. A continuous drop in ion intensities (most apparent for higher mass ions) for a constant multiplier voltage, etc., is indicative of a problem in the ion source optics such as a dirty source.

The daily tune of the mass spectrometer is essential in obtaining reproducible data. This is especially critical for quadrupole spectrometers where small changes in lens potentials can drastically change the

ion transmission at higher masses. One may tune, for example, on a bleed peak of the GC column being used (m/z 207, 253, 281, or 315 for many silicone columns) or to a specific compound spectrum such as decafluorotriphenylphosphine specified in the Priority Pollutant Protocol.[41] For GC-MS analysis, tuning the instrument to some component of the GC effluent insures that the spectrometer is correctly focussed for the GC-MS analysis, since for some systems proper focus is dependent upon the location and orientation at which compounds used for tuning and analysis are introduced.

The mode of ionization chosen for POM analysis, either electron impact ionization (EI) or chemical ionization (CI), depends upon the instrument sensitivity as well as sample-related characteristics. Of the two ionization modes, CI tends to be more sensitive for POMs, although the differences are highly instrument dependent. With either mode, the mass spectra for the POMs are simple. For EI, the mass spectrum consists of the molecular ion M^+, the doubly-charged molecular ion M^{2+}, and the loss ions $(M-2)^+$ and $(M-1)^+$. For CI using methane as the reagent gas, the mass spectrum consists of the protonated molecular ion $(M+1)^+$ and the adduct ions at $(M+29)^+$ and $(M+43)^+$ due to the addition of C_2H_5 and C_3H_7. The sensitivity gain with CI is in part due to background noise reduction. Fragment peaks and bleed background tend to be reduced with CI, yielding clean spectra, but identification of other compounds in the sample is somewhat more difficult. For the POMs listed previously in Table 1, either EI or CI may be used for ionization, contingent upon the sensitivity requirements for the analysis. It is recommended that EI ionization be used whenever possible to allow identification of potential interferences by comparison to library reference spectra and, thus, minimize their effect on the analysis.

It is recommended that an internal standard be used in POM analyses to improve the retention time accuracy for qualitative POM identification and provide a quantitative standard. The specific internal standard or standards used for an analysis depends upon the method of data acquisition. d_{10}-anthracene, 9-methyl anthracene, 9-phenyl anthracene, and 9,10-diphenyl anthracene are popular choices as internal standards, since these four

compounds are not normally seen in environmental samples. The choice of using one or more of these compounds for internal standard is up to the analyst. Several cautions should be noted. In acidic media, d_{10}-anthracene may slowly react to replace deuterium with hydrogen. If the internal standard measurement is based on the specific ion of mass 188, artifically high concentration values will be reported unless the exchange is taken into account. Although 9-methyl anthracene is not found in environmental samples, background interferences due to fragment ions at m/z 192 can be substantial with EI ionization, changing the accuracy of the internal standard measurement. The 9,10-diphenyl anthracene also has some problems when used with electron impact ionization. In addition to the molecular ion (m/z 330), 9,10-diphenyl anthracene has a strong fragment ion (about 40% to 50% of the intensity of the parent ion) at m/z 252. Since this compound elutes in the region of the m/z 252 POMs (e.g., benzo(a)pyrene), it represents a potential interference problem. For many POM analyses, 9-phenyl anthracene represents a good choice for use as a single internal standard unless multiple internal standards are required due to unique requirements of the analysis or data acquisition system.

The specific method of data acquisition used for POM analysis depends upon the instrumental capabilities and sensitivity. Data may be acquired with either selected ion monitoring (SIM), selected mass range scanning, or full mass range scanning. In SIM, only a few ions are monitored during a sample run allowing long integration times for each ion. In selected mass range scanning, small portions of the mass range are sampled allowing longer integration times for each mass than in full mass range scanning where each of the masses is sampled. An example of the use of selected mass range scanning is in the measurement of polychlorinated biphenyl where the mass ranges from 254 to 260 and from 288 to 294 are sampled for tri- and tetrachlorobiphenyl.[43] Since SIM and selected mass range scanning are more sensitive than full mass range scanning (due to enhanced signal-to-noise ratio resulting from increased integration time per mass), one of these methods often will be used to enhance the instrumental sensitivity. The disadvantage associated with

using either selected ion monitoring or limited mass range scanning is the limited amount of information acquired. With SIM, there is no way to distinguish between a POM of m/z 252 and, for example, a coeluting silicon bleed peak ion at m/z 252. With limited mass range scanning, this problem is diminished but, with either method, other species which may become of interest would be impossible to assay from the acquired data at a later date. Therefore, we recommend acquisition of full mass scan data for POM analysis whenever possible for detection of a wide range of POMs or selected mass range scanning for detection of a limited number of isomers.

Quantitative data for a specific POM should be determined from calibration curves prepared for that POM from data obtained on the GC-MS system to be used. The calibration curve prepared relates the instrumental response for that POM to its concentration. All work should be based upon response relative to that for an internal standard. For some POMs, reference standards may not be available for preparation of calibration curves and some method, such as the following, would be needed to estimate their response vs. concentration curve. Since the slopes of the response curves for many POMs show a roughly linear decrease with molecular weight, estimation of the response curves for POMs, as needed, can be made by interpolation from the response curve slopes found for POMs of higher and lower molecular weight. The absolute levels of POMs required for use in calibration mixtures will depend on the instrument sensitivity and the samples to be studied. In general, there should be at least four calibration mixtures to yield at least four calibration points for each species, spanning the linear region of the spectrometer with the lowest concentration standard at 2 to 5 times the instrument detection limit for that species.

Since the mass spectra of the individual POMs are dominated by the molecular ion, calibration is conveniently obtained on a relative basis from the area of the POM molecular ion alone vs. that for the internal standard. Variations of this procedure, where several representative ions for each species are summed before obtaining the area, are also

possible but, for whatever procedure is chosen, it must be consistent throughout the analyses.

B. Packed Column GC Procedures

1. General Procedures

Most of the work reported on POM analysis by GC or GC-MS has been carried out on packed columns. Packed columns do not have the high resolution of capillary columns coated with the same phases; however, packed columns have longer lifetimes, will tolerate larger sample sizes, and can give good results for many specific POMs. The methyl silicone, (e.g., OV-101), methyl phenyl silicone (e.g., OV-17), and the carborane methyl- and methyl phenyl silicone (e.g., Dexsil 300 and Dexsil 400) GC phases separate at least some of the specific POM isomers, such as fluoranthene and pyrene, and perylene from benzo(a)pyrene or benzo(e) pyrene. Some non-isomer POM species may not be chromatographically resolved from one another but will be differentiated by molecular weight. To illustrate this point, Figure 1 shows several ion chromatograms for some incompletely resolved POMs obtained from a packed column GC/MS run. Despite the fact that anthracene and phenanthrene or chrysene, triphenylene, and benz(a)anthracene cannot be individually identified, the sum concentration of these non-resolved species is readily determined. For many purposes, the sum concentration value is adequate. Comparison of the sum concentration levels of different samples is one method for assessing the impact on total POM load of different facility-operating conditions.

2. Liquid Crystal GC Procedures

Liquid crystal stationary phases, such as BPhBT (N,N'-bis(p-phenylbenzylidine)α,α'-bi-p-toluidine) allow specific POM identifications to be made on a packed column, in some cases separating isomers which are not resolved even on capillary GC columns. The liquid crystal phases separate geometric isomers of different POMs quite easily. For example, Strand and Andren[44] have compared the separation of benz(a)anthracene and chrysene on Dexsil 300 and two liquid crystal phases. The liquid crystal phases resolve these two isomers which are not resolved on the

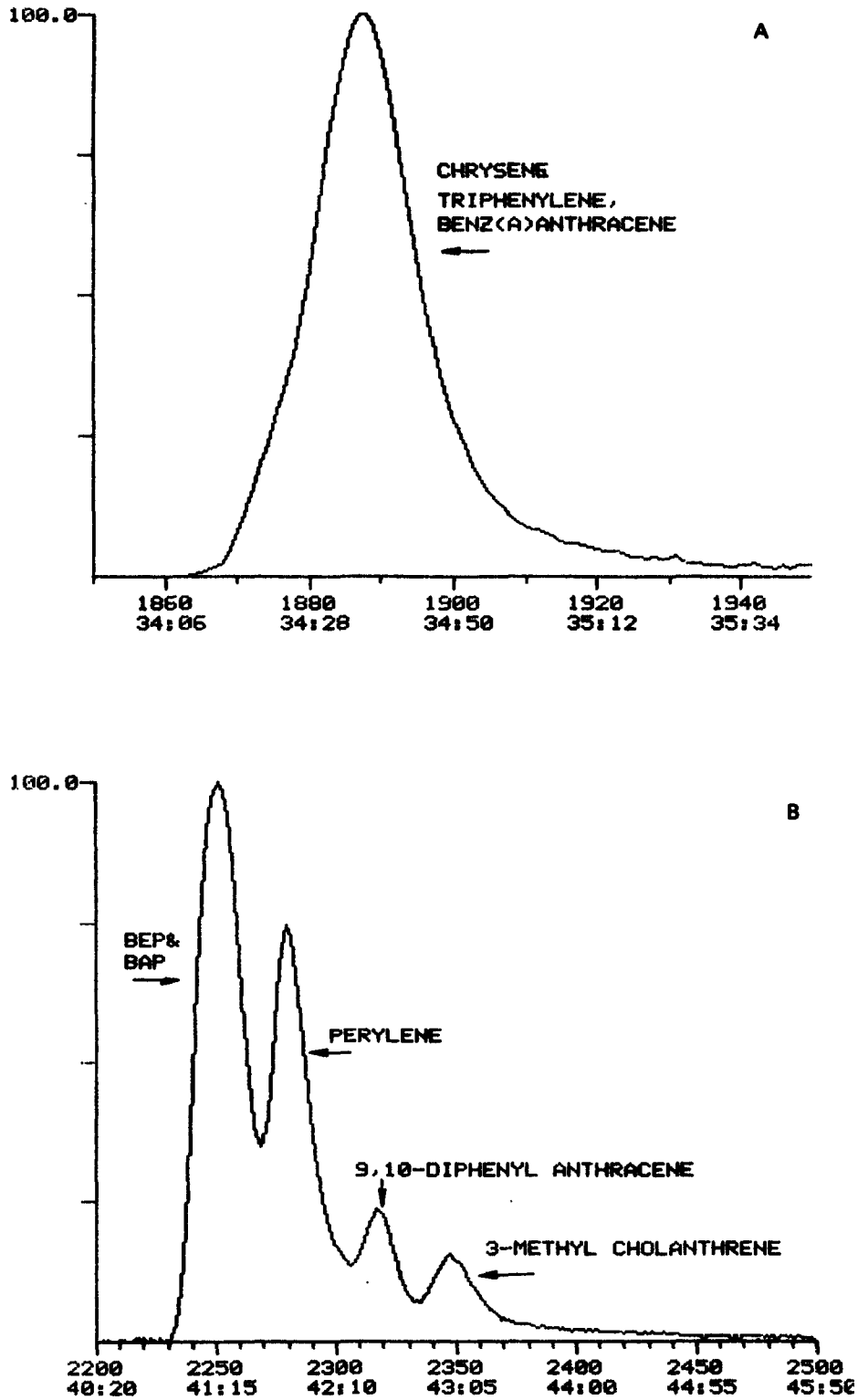

FIGURE 1. Selected Ion Chromatograms for m/z 228 (a) and 252 (b) from Packed Column GC-MS Run Using Dexsil 400 GC Phase.

silicone columns. Another example is the separation of benzo(a)pyrene and benzo(e)pyrene as shown in Figure 2. In part A, the separation is shown for a normal GC phase compared to that found with a liquid crystal GC phase in part B. Unfortunately, the liquid crystal phases are not well suited to temperature programmed use and should be used isothermally slightly above their transition point. When used with temperature programming, two factors become important. First, the column bleed is substantial and, second, at temperatures below the transition point, chromatographic peak shapes are poor and often not resolved from one another. Even under isothermal conditions, the liquid crystal structure continues to undergo phase transition. The effect of this on a benzo(a)-pyrene analysis is to continually alter the benzo(a)pyrene retention time. At the recommended isothermal temperature above the transition point, i.e., 260°C, the retention time continually decreases. Variations in the retention time for benzo(a)pyrene can be from 30 min. or more to 10 min. or less, over a four-hour period. Judicious changes in the temperature can be used to maintain the benzo(a)pyrene retention time within a reasonable range. A quick cooling below the transition point will lengthen the retention time, and a quick heating above the transition point will shorten it. Operation at temperatures close to the transition point extends the limited lifetime of these phases.

An additional point of interest to the analyst is that the elution order is substantially different compared to that from normal GC columns. For most methyl silicone GC phases, the relative retention time for benzo(a)pyrene is smaller than that for perylene, but for liquid crystal phases like SP-301 (BPhBT), the elution order is reversed. Use of liquid crystal GC columns for a few specific POMs, e.g., for benzo(a)pyrene alone, is an attractive procedure but caution must be exercised is using relative retention times from other GC columns to determine POM species elution order.

C. Capillary GC Column Procedures

Of the available chromatographic procedures, capillary GC procedures are best suited to routine determination of a wide range of POM compounds.

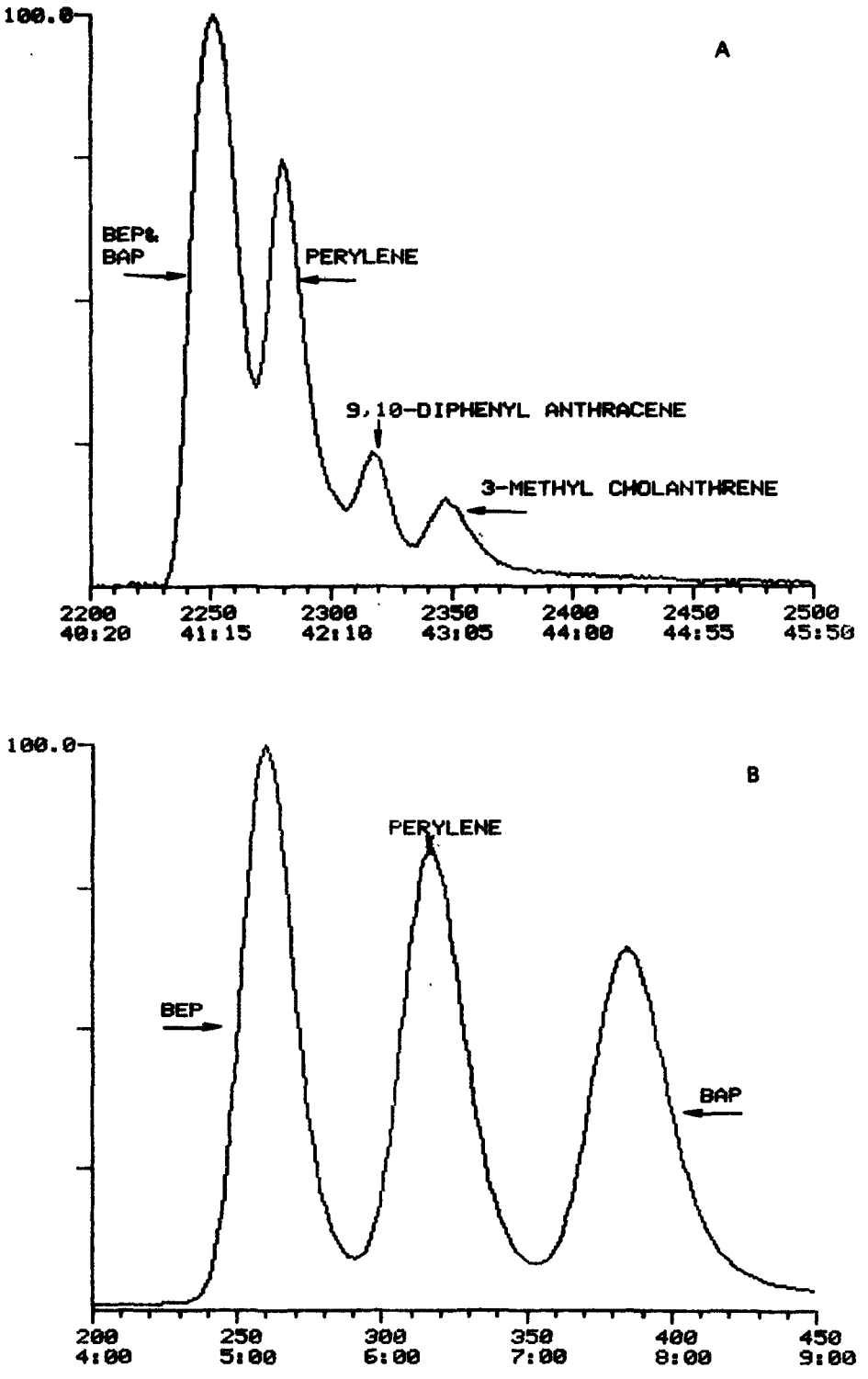

FIGURE 2. Comparison of m/z 252 Selected Ion Chromatograms for Packed Column, Dexsil 400 (a) and Liquid Crystal Column, SP-301 (b).

Of the key POMs listed in Table 1, all but a few are at least partially resolved. Figure 3 shows a mixture of POMs selected from those listed in Table 1 run on an OV-17 capillary using the conditions described in Chapter 4. For this column, phenanthrene and anthracene and chrysene and triphenylene show some separation, which is not the case for packed columns. Figure 4 shows the separation in higher display resolution for these particular pairs of isomers for the same sample displayed in Figure 3. The high resolution of capillary GC columns not only separates POM isomers from one another but also separates potential background interferences from the species of interest. Unlike liquid crystal columns where temperature programming degrades the GC performance, temperature programming may be adjusted to enhance species resolution while simultaneously decreasing analysis time. The prime disadvantage of capillary GC techniques is the small sample size permissible, requiring high sensitivity enhancement procedures discussed in Section A of Chapter 3.

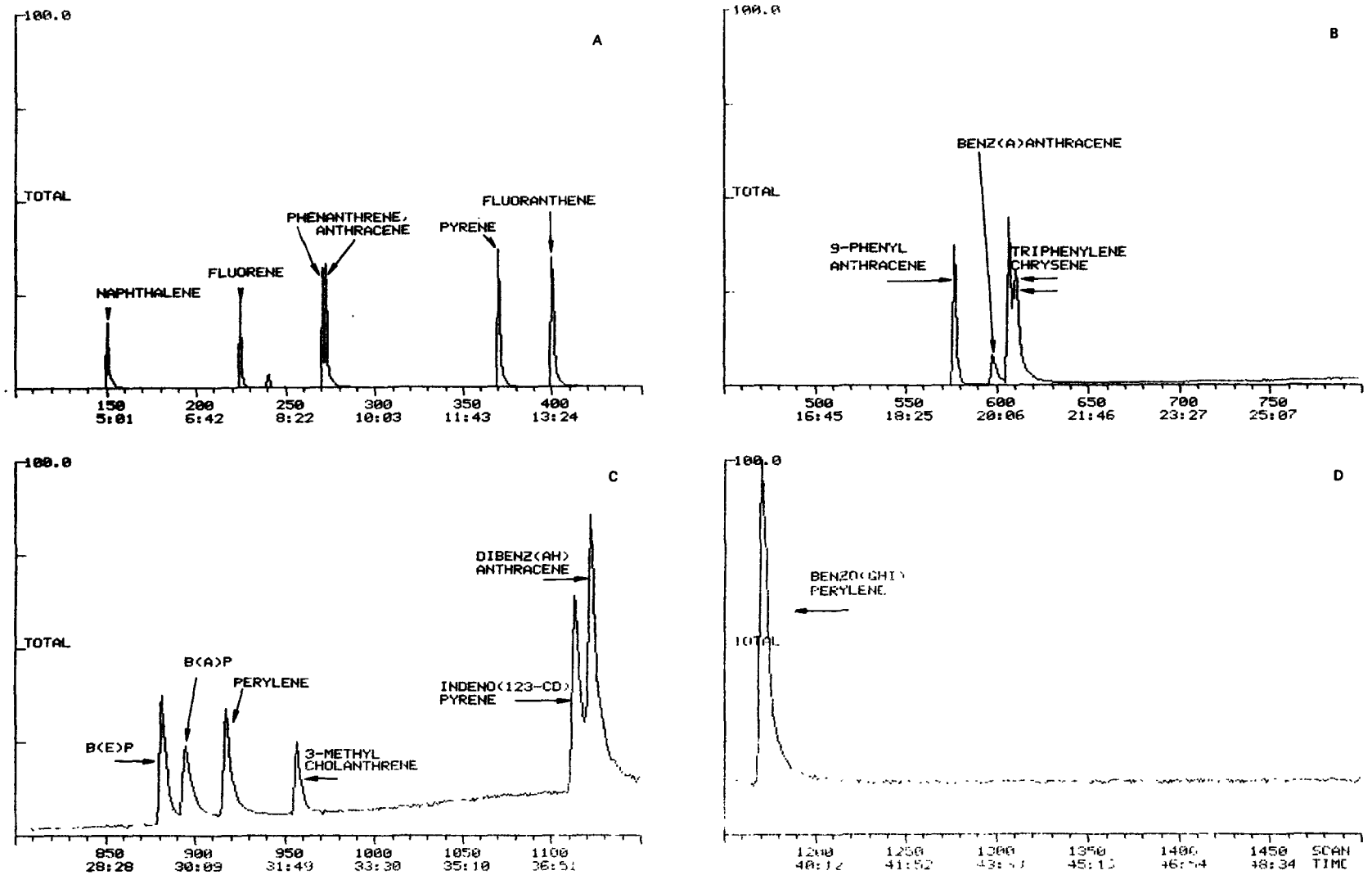

FIGURE 3. Sum Ion Chromatogram for PAH Standard Using OV-17 Capillary GC Column.

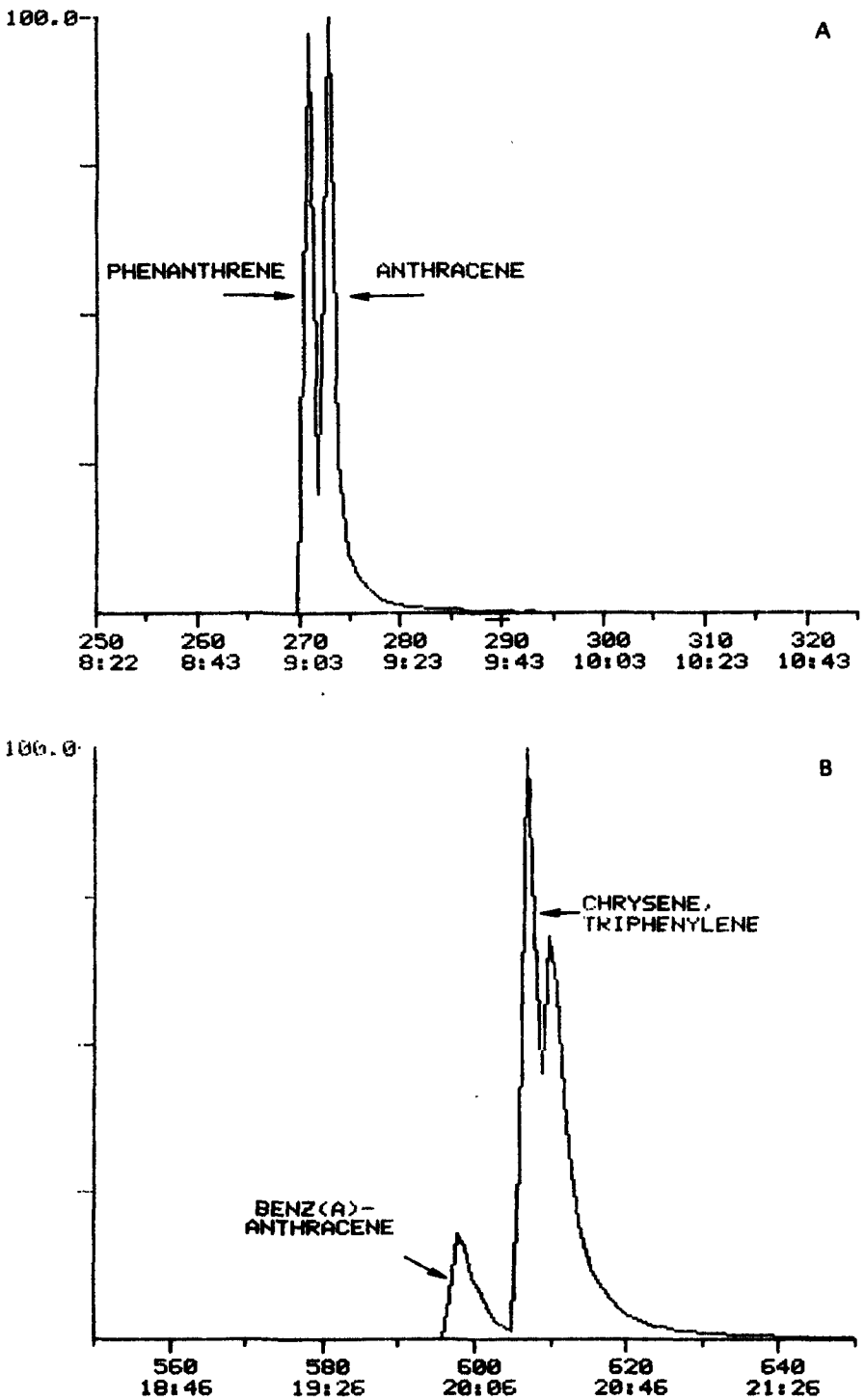

FIGURE 4. Selected Ion Chromatograms for m/z 178 (a) and m/z 228 (b) of Figure 3 Expanded.

IV. VERIFICATION STUDIES

The purpose of this section is to illustrate the use of the general procedures outlined in Chapter 3 on collected environmental samples. The instrument used for all of these studies was a Finnigan Model 4000 GC/MS with either a Finnigan 6110 Data System or an Incos 2300 Data System. Due to the high sensitivity of this instrument in the electron impact mode (limit of detection for Benzo(a)pyrene $\leq$2 ng injected with full mass range scanning for packed GC columns), it was not necessary to use chemical ionization techniques to increase instrument sensitivity or to use selected ion monitoring or limited mass range scanning procedures except as noted.

A. POM Analysis with Packed Column GC/MS

Two types of samples were chosen to demonstrate the capabilities of packed column GC/MS. The first was from a study of the influent to a publicly-owned treatment works, the other was from a study of the emissions from a coke oven quench facility. Specific operating details are included for the analyses described (see i.e., Tables 3,5 etc.).

The first example of samples analyzed by packed column GC/MS techniques consists of methylene chloride extracts from basic solutions of sample influent to a publicly-owned treatment work. The samples of interest were collected, extracted and analyzed under conditions specified in the priority pollutant protocol.[42] Since the conditions specified in the priority pollutant protocol were being followed, the mass spectrometer was tuned to give a mass spectrum for decafluorotriphenyl phosphene with ion intensities within the limits specified. Other GC/MS operating parameters used for these analyses are listed in Table 3. In addition to demonstrating the use of packed column GC/MS procedures for POM analysis, the samples discussed here provide some data on the precision and accuracy which are possible in routine analyses for POM.

The conditions specified in Table 3 were not optimized for POM analyses alone but were selected for a much wider range of species. However, the POM related data, such as calibration data, relative

TABLE 3

GC-MS Conditions for Publicly Owned Treatment Works Sample

GC Conditions

Temperature program	50°C Isothermal for 4 min, linear heating at 8°C/min to 265°C., 265°C isothermal for 30 min.
He flow rate	30 mL/min
Sample Size	2 μL
Internal standard	d_{10}-anthracene

MS Conditions

Mass scanning mode	full mass range scanning
Mass ranges	40-220, 221-425
Integration times	3, 5 msec
EM Voltage	1800 V
Electron energy	50 V
Filament emission	45 ma
Scan rate	3 sec/spectrum
Data system	Finnigan 6110

retention time, etc., obtained under these conditions is similar to those obtained under the conditions used for the analyses of coke oven quench samples reported later. The priority pollutant protocol specifies a small set of POMs to be of interest which are listed in Table 4.

The actual samples were collected over a one-week period. During the analyses of the samples, selected samples were subject to quality assurance (QA) procedures. For each QA sample, the collected wastewater was split into three portions. One portion was extracted in the usual fashion, the remaining two were spiked with reference standards to a level at least five times that of the minimum detection threshold prior to extraction. Two additional samples are prepared to complete the QA sample set. Two high purity water samples are put through the extraction procedure, one as a blank and the other spiked with the same reference standard mixture as was used for the spiked raw wastewater samples.

In the raw wastewater samples, no POMs were found with molecular weights greater than naphthalene (naphthalene is not generally considered to be a problem POM). The spiked raw wastewater and spiked clean water samples provide a means of assessing the precision and accuracy of routine POM measurement. Figures 5 through 7 show typical chromatograms for the three spiked samples associated with a single collected sample. For the POMs listed in Table 4, Table 5 lists the recoveries and standard deviations for seven sets of spiked replicates of the wastewater and spiked clean water including the sample shown in Figures 6-7. The average recoveries for the set of POMs tabulated are 81% and 71% for the clean water and dirty water samples, respectively, with corresponding relative standard deviations of 20% and 30%.

In the steel industry, water from different sources is commonly used to quench the incandescent coke. The water used for quenching may be either clean water, water recycled from other processing procedures, or a mixture of the two. Assessment of the levels of POMs associated with the quench operation was of interest, and samples of the atmospheric emissions under different quenching conditions were collected and analyzed for POM by GC/MS.

The analysis of the coke oven quench emissions for a general list

TABLE 4

POMs Specified in the Priority Pollutant Protocol

	NAS Rating
Naphthalene	-
Acenaphthylene	-
Acenaphthene	-
Fluorene	-
Anthracene/Phenanthrene*	-
Fluoranthene	-
Pyrene	-
Benz(a)anthracene/Chrysene*	+
Benzo(b or k) fluoranthene*	+ +
Benzo(a) pyrene	+ + +
Dibenz(a, h) anthracene	+ + +
Indeno(1,2,3-cd) pyrene**	+
Benzo(g,h,i) perylene	-

*Isomers reported together.

**Not available in this Quality Assurance set.

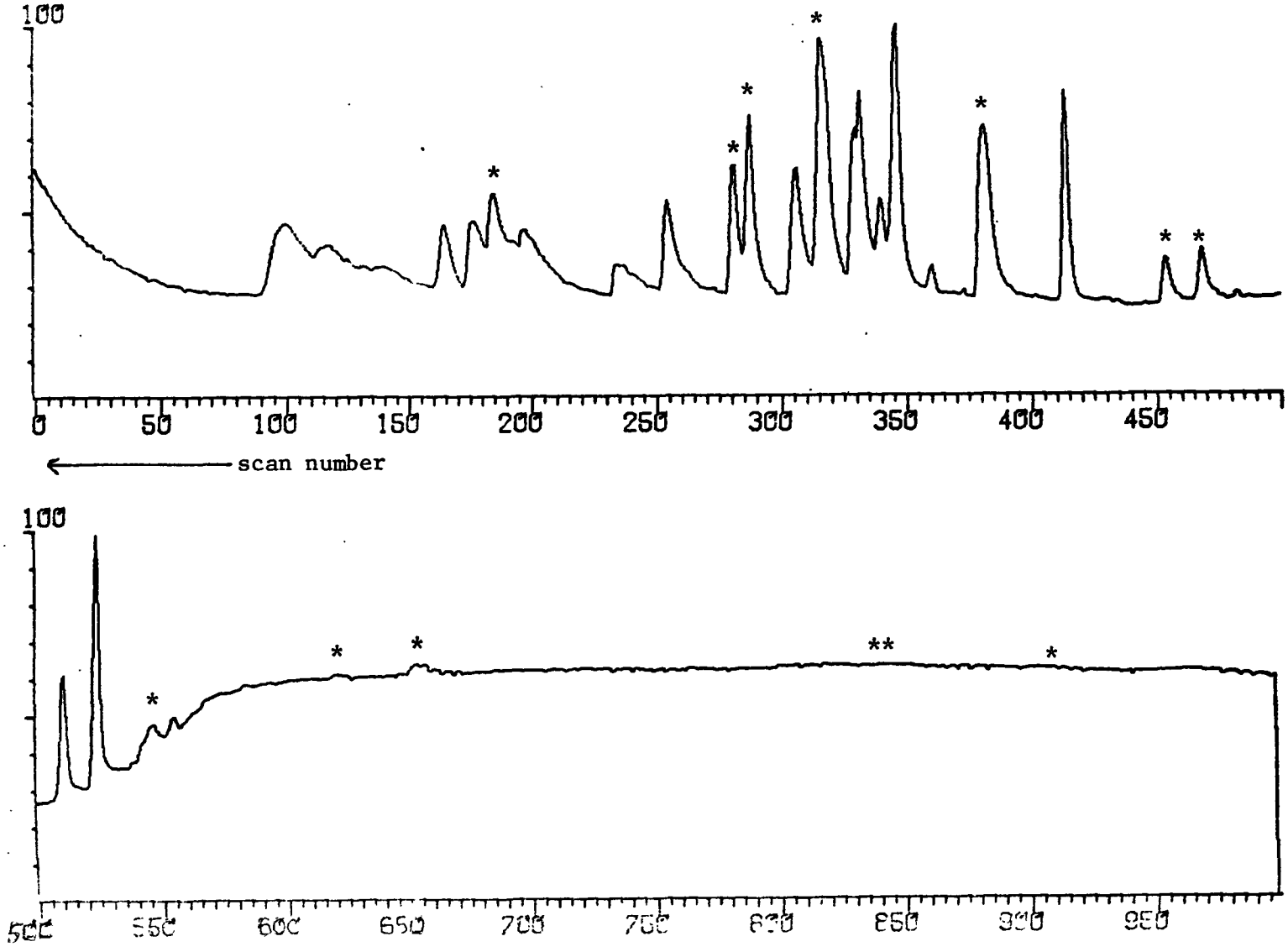

FIGURE 5. Reconstructed Ion Chromatogram for Clean Water Sample Spiked with Priority Pollutants.

* Asterisks denote location of peaks due to various priority pollutant POMs (Table 4).

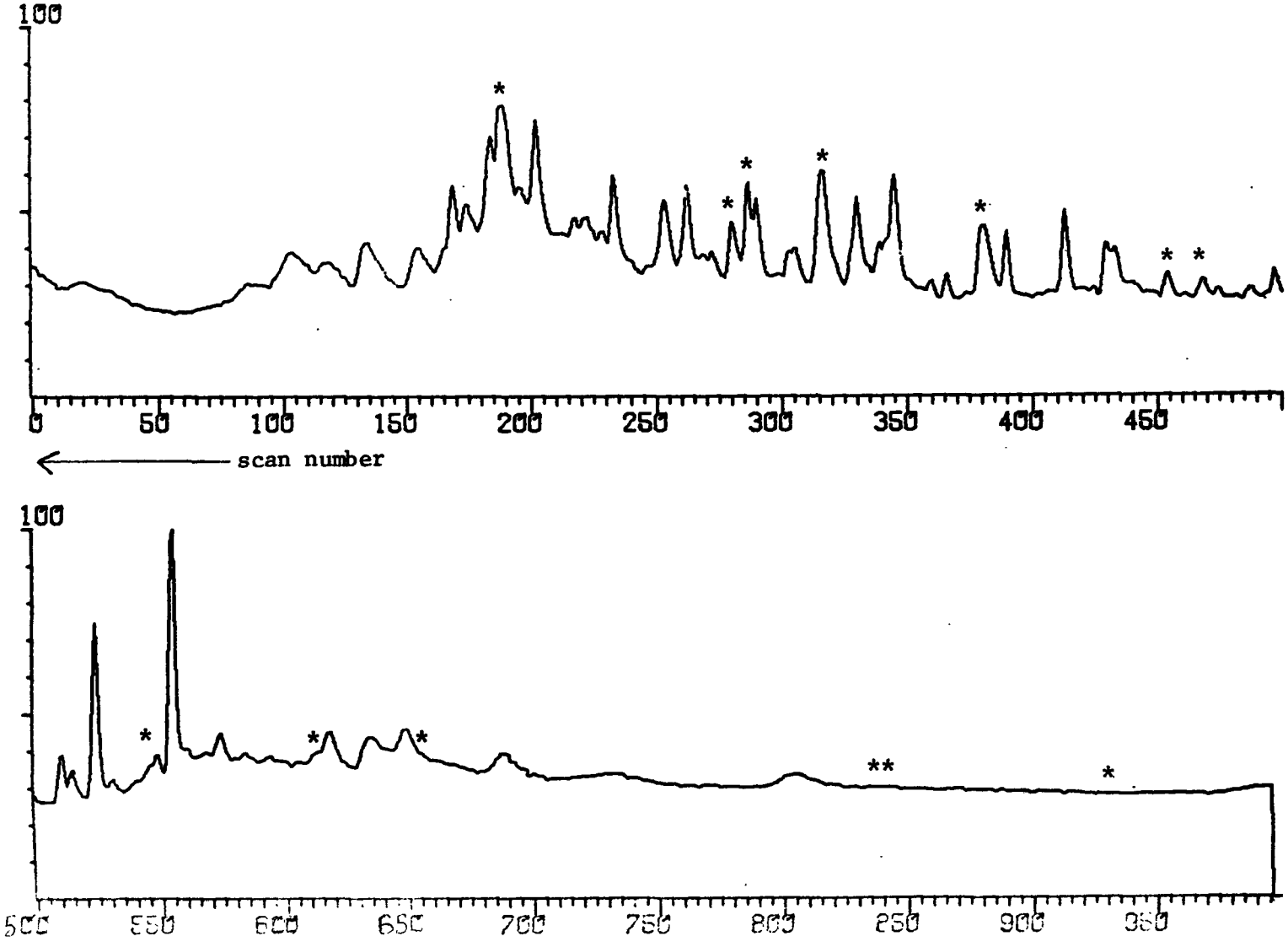

FIGURE 6. Reconstructed Ion Chromatogram for Raw Wastewater Spiked with Priority Pollutants.

* Asterisks denote expected location of peaks due to various priority pollutant POMs (Table 4).

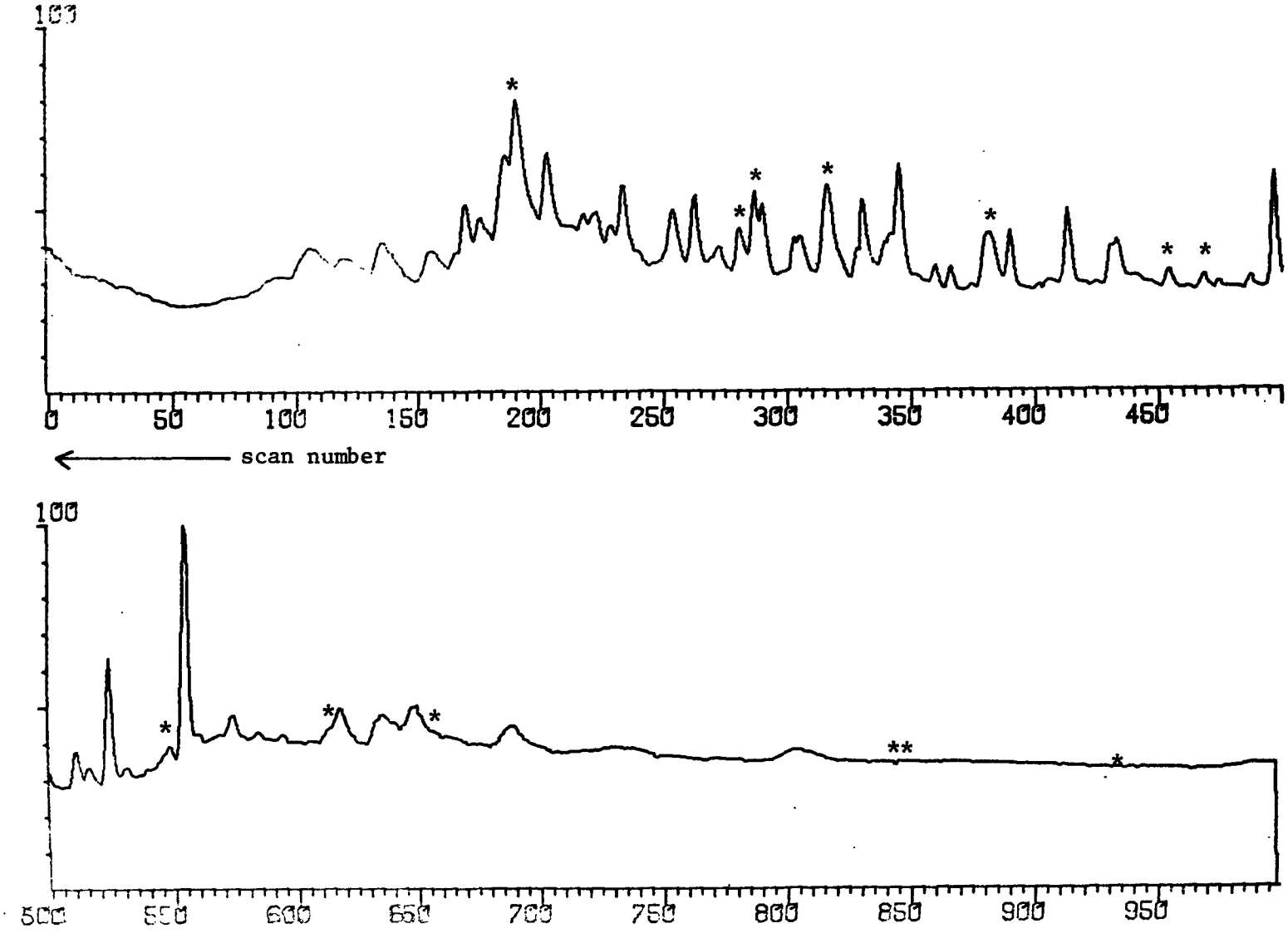

FIGURE 7. Reconstructed Ion Chromatogram for Raw Wastewater Spiked with Priority Pollutants (Replicate).

* Asterisks denote expected location of peaks due to various priority pollutant

TABLE 5

Average Recoveries of POMs from Aqueous Samples

	Clean Water			Waste Water		
Species****	n*	$\bar{P}$**	% Sp***	n*	$\bar{P}$**	% Sp***
Naphthalene	7	78.6	31.6	14	85.5	36.3
Acenaphthylene	7	84.6	19.8	14	80.4	21.4
Acenaphthene	7	89.7	20.5	14	81.6	23.6
Fluorene	7	90.4	15.8	14	73.9	21.2
Anthracene/Phenanthrene	7	106.8	10.1	14	85.6	23.2
Fluoranthene	7	86.3	2.5	14	62.6	21.5
Pyrene	7	86.8	5.1	14	64.3	25.3
Chrysene/Benz(a)-anthracene	7	77.1	14.5	14	69.6	18.8
Benzofluoranthenes	7	57.1	69.3	14	52.0	93.0
Benzo(a) Pyrene	7	67.1	10.5	14	62.8	23.3
Dibenz(a,h)anthracene	7	69.1	24.5	14	64.0	29.0
Benzo(g,h,i) perylene	7	75.4	20.2	14	66.8	23.8
Average for all POMs examined		80.8	20.4		70.8	30.0

*n = Number of samples

**$\bar{P}$ = Average % recovery

***%Sp = Relative standard deviation

**** = POMs listed by increasing elution order

of POMs was performed under the conditions shown in Table 6. Although some POM isomers could not be specifically identified, a wide range of POMs were readily identified. For the purposes of this study, combined values for several isomers were reported together as requested. The POM components were first separated from the other organic constituents in the sample by the Level 1 LC procedure (Appendix A) with the fractions containing the POMs of interest (fractions 2, 3, and 4) being combined and concentrated prior to GC/MS analysis.

The results in Table 7 compare the POM data obtained for samples with clean water or recycled water used for quenching the coke. The samples also contain many other organic species which are not shown in this table. Since full mass range scanning was used during data acquisition, levels for the non-reported species could have been obtained but were not within the scope of the project. The instrumental limit of detection for all of the species reported was ≤1 ng injected.

B. Selected POM Analysis with Liquid Crystal GC Procedure

In Chapter 3, it was recommended that the nematic liquid crystal GC phases such as SP 301 (N,N - bis(p-phenyl-benzylidine)α,α'-bi-p-toluidine or BPhBT) be used for the analysis of a narrow range of POMs. As part of the study of the emissions from a coke oven quench operation, it was requested that the concentration of benzo(a)pyrene be determined exclusive of the other POM isomers with identical molecular weights (i.e., molecular ions of m/z 252) by a second analysis method of high sensitivity. To accomplish this analysis, the samples collected from the coke quench facility were analyzed by liquid crystal GC/MS procedures.

The GC/MS conditions shown in Table 8 were used for the specific analysis of benzo(a)pyrene. 9,10-Diphenyl anthracene was chosen for use as the internal standard for these measurements. 9-Phenyl anthracene, which was used previously as an internal standard in these samples, elutes with the solvent under the GC conditions used, is obscured by the solvent and early eluting POMs, and could not be used as an internal standard for the benzo(a)pyrene measurements. To enhance the sensitivity

TABLE 6

GC-MS Operating Conditions for Coke Oven Quench Sample Analysis

GC CONDITIONS	
Column	Dexsil 400
Temperature Program	Isothermal operation at 170°C for 1 min. Linear operation to 300°C at 15°C/min, isothermal operation at 300°C for 30 min.
Helium Flow rate	30 mL/min
Sample Size	2 - 4 µL
Internal Std	9-phenyl anthracene
MS CONDITIONS	
Mass Scanning Mode	Full mass range scanning
Mass Ranges	70-210, 211-270, 271-350
Integration Times	2, 5, 13 msec
Electron Multiplier Voltage	1800 V
Electron Energy	50 V
Filament Emission	45 ma
Scan Rate	1 sec/spectrum

TABLE 7

Selected POMs from Atmospheric Emissions of Coke Oven Quench Samples Using Clean or Recycled Water*

Species	Concentration $\mu g/m^3$	
	Clean Water Samples	Recycled Water Samples
Naphthalene	0.39	170
Fluorene	0.09	23.4
Carbazole	-	3.6
Anthracene/Phenanthrene	0.80	33
Fluoranthene	0.24	7.6
Pyrene	0.16	8.0
Methyl-Fluoranthene and -Pyrene	0.09	0.96
Chrysene, Benz(a)-anthracene, etc.	-	0.54
Benzofluoranthenes, Benzopyrene	-	0.59

* Concentrations on column range from 0.1 ng up for species detected.

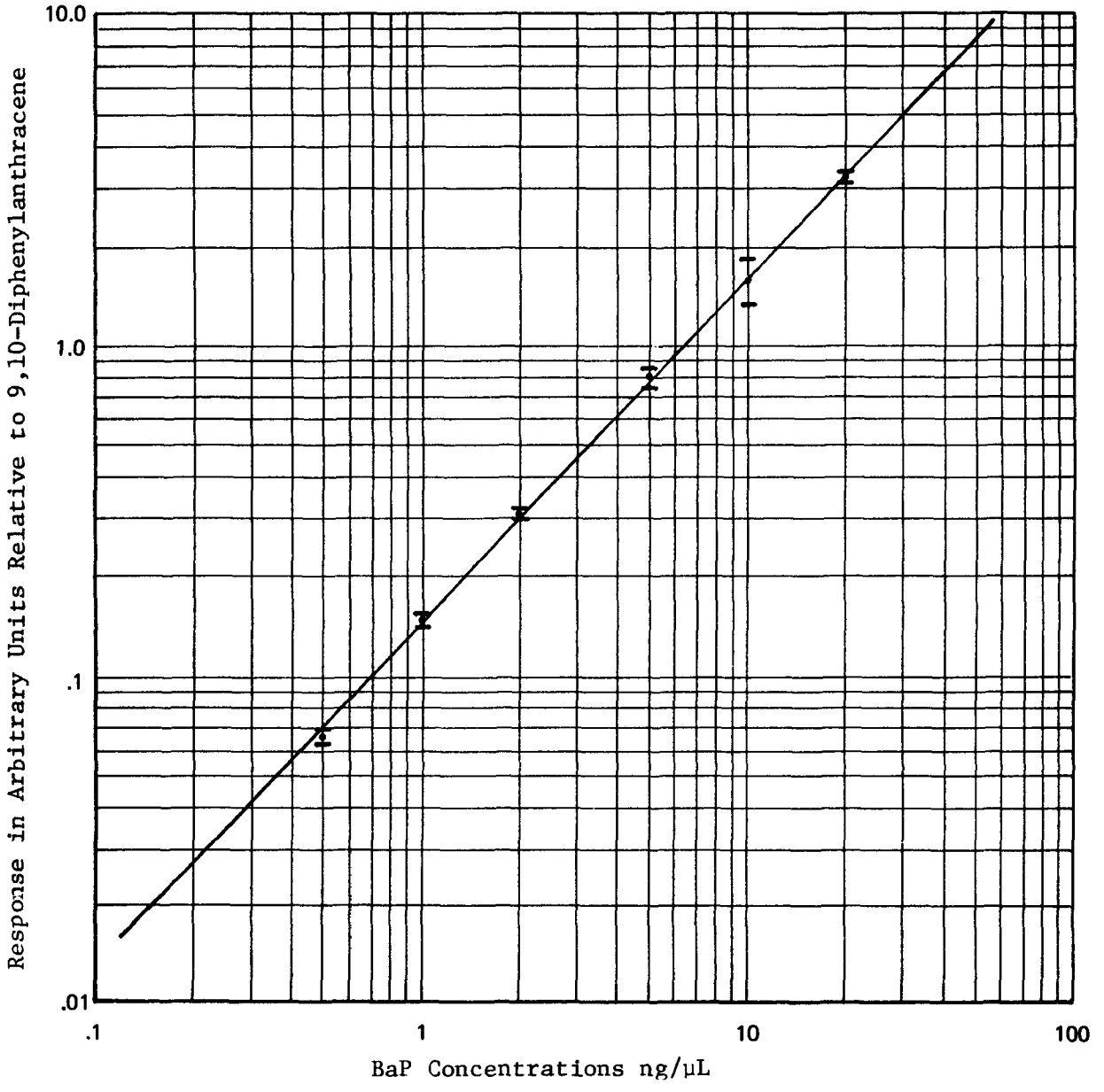

FIGURE 8. Benzo(a)pyrene Calibration Curve Used with Liquid Crystal GC/MS Analysis. Horizontal lines indicate range of responses found for set of five determinations at each concentration, 1 µL injection.

TABLE 9

Comparison of Benzo(a)pyrene Levels from Liquid Crystal to Benzopyrene Levels from Packed Column GC/MS *

Sample Description	Concentration $\mu g/m^3$ Benzo(a)pyrene Liquid Crystal SP-301	Benzopyrene Packed Column**** Dexsil-400
Clean H_2O, green coke**	38	-
Clean H_2O, cured coke**	66	-
Recycled H_2O, cured coke**	98	120
Recycled H_2O, cured coke**	72	89
Recycled H_2O, cured coke***	300	400

* - on column levels of benzo(a)pyrene injected ranged from < 0.1 to 10 ng

** - atmospheric emissions collected

*** - sample of recycled water from settling pond prior to dilution with clean water and use

**** - packed column data for both benzo(e)- and benzo(a)pyrene

minute from a low sulfur fuel with the engine idling. For the sample of interest, the chromosorb trap was extracted with hexane, 9-phenyl anthracene added as the internal standard and the sample run under the conditions listed in Table 10.

Figure 9 shows the chromatogram for this sample with chromatogram peaks labeled as to their identity. A large variety of aliphatic compounds present in these samples were not of interest to the study of jet engine exhaust or the present report. They are not detailed in the following. This sample contained POMs at relatively low levels, as shown in Table 11. The primary interest in showing this example is the resolution of a variety of polar and nonpolar compounds with ease and the detection of various POMs in a different type of sample matrix than was presented previously.

The second sample was collected as part of an occupational exposure study. In this study, a number of roofing pitches and asphalts were heated under laboratory control to simulate the fumes present during a commercial roofing operation. The resultant fumes were collected and concentrated for later use in animal exposure tests. The sample of interest was collected in cyclohexane/acetone from a low burn pitch sample heated to 450^{o}C and was chromatographed with an OV-17 capillary GC column and analyzed using the conditions listed in Table 12.

This sample was rich in POMs as can be seen from Table 13 and Figures 10 and 11. Figure 10 shows the reconstructed gas chromatogram for this sample and the sum ion chromatogram (sum of the individual ion chromatograms) for the specific ions of interest. As can be seen from Figure 10a, the sample of fume emissions is a complex mixture containing many POMs and their alkyl substituents. An estimate of the amount of sample represented by the POMs of interest (found in Table 13) can be made by comparing the area of the two chromatograms in Figures 10 and 11. Using these two areas, the concentrations reported in Table 13 account for about 15% of the total POM in the sample.

The remaining POMs could be quantified at any time using appropriate calibration standards and/or the methods described in Chapter 3 but for the specific program requiring analysis of this sample, only the POMs

TABLE 10

GC-MS Conditions for Jet Engine Exhaust Emissions

Gas Chromatographic Conditions

20 meter glass capillary column coated with OV-101

Grob type - splitless injection

Multilinear temperature program

1) 55° isothermal program for 1.1 min
2) 55°C - 150°C linear program at 25.5°C/min
3) 150°C - 250°C linear program at 4°C/min
4) 260°C isothermal program for 10 min

1 μL sample injections

Mass Spectrometric Conditions

Finnigan Model 4000 mass spectrometer

Mass scanning mode	full mass range scanning
Mass range	100 - 310 amu
Integration	10 ms/amu
Electron multiplier	1800V
Electron energy	70 eV
Filament emission	30 ma
Scan rate	1 sec spectrum

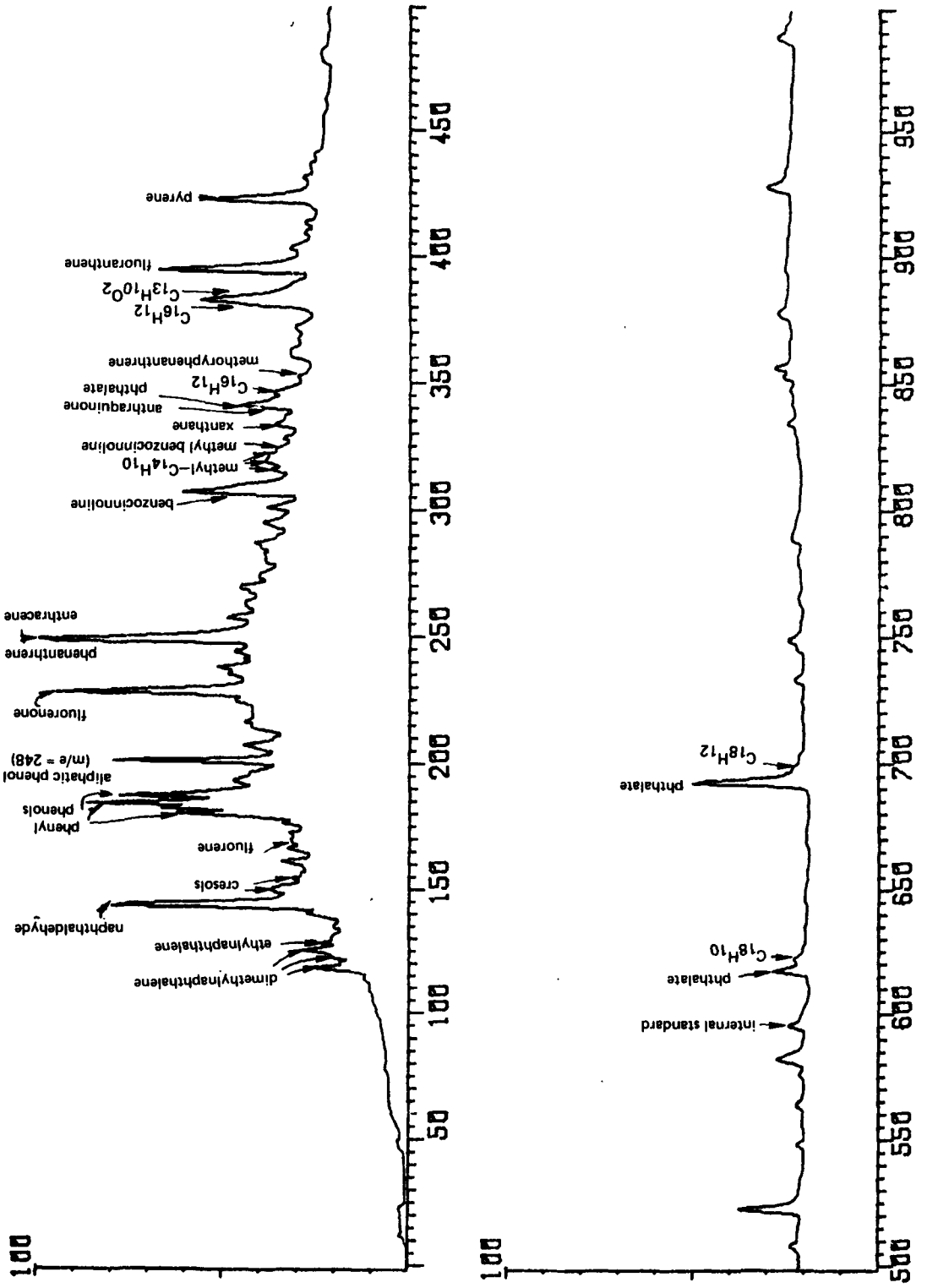

FIGURE 9. Reconstructed Ion Chromatogram for Sample of Jet Engine Exhaust.

TABLE 11

POM Concentrations in Jet Engine Exhaust

Species	Composition	m/z	Run #1***	Run #2***
Fluorene	$C_{13}H_{10}$	166	3.85	7.52
Anthracene Phenanthrene	$C_{14}H_{10}$	178	106.0	223.8
Methyl fluorene	$C_{14}H_{12}$	180	2.57	ND **
Methyl-$C_{14}H_{10}$	$C_{15}H_{12}$	192	27.85	76.19
Fluoranthene	$C_{16}H_{10}$	202	133.6	232.3
Pyrene	$C_{16}H_{10}$	202	46.79	1195.2
Aceanthralyene	$C_{16}H_{12}$	204	13.96	29.10
Benzofluorene	$C_{17}H_{12}$	216	16.19	30.67
Benzophenanthrene Chrysene Naphthacene	$C_{18}H_{12}$	228	48.68	86.66
Benzopyrenes	$C_{20}H_{12}$*	252	37.62	43.19
Perylene	$C_{20}H_{12}$	252	12.11	19.67
Totals			517.0	2038.1

*Sum of signals for both benzo(a)- and benzo(e)pyrene, with benzo(e) pyrene contributing more to the signal than benzo(a)pyrene.

**ND = below instrumental detection limit of 0.010 µg/mL, or 0.001 µg/mL for 10X concentrated samples (Total Sample).

*** Different engine operating conditions.

TABLE 12

GC-MS Conditions for Low Burn Pitch Fumes

GC Conditions

a) Temp. Program 60°C isothermal for 1 min
to 200°C at 20°C/min
to 285°C at 3°C/min
285°C isothermal for 45 min.

b) Split type injection

c) Helium carrier gas @ 2 mL/min through column

d) Sample Size 1 µL

e) Internal Standard 9-phenylanthracene

MS Conditions

a) mass scanning mode full mass range scanning

b) mass range 125 to 310

c) EM voltage 1600V

d) Electron Energy 50V

e) Filament Emission 40 ma

f) Scan Rate 2 sec/spectrum

g) Data System Incos 2300

TABLE 13

Concentrations of Selected POMs in Low Burn Pitch Fume Sample

Species	Analytical m/z	Conc. (µg/mL)*
Naphthalene	128	233
Fluorene	166	278
Anthracene & Phenanthrene	178	>1150**
Fluoranthene	202	>936**
Pyrene	202	>823**
Benzo(c)phenanthrene + Benz(a)anthracene	228	244
Chrysene + Triphenylene	228	267
Benzo(b)fluoranthene	252	57
Benzo(k)fluoranthene	252	75
Benzo(e)pyrene	252	35
Benzo(a)pyrene	252	57
Perylene	252	15
Indeno(1,2,3-cd)pyrene	276	13
Benzo(g,h,i)perylene	276	11
Dibenz(a,h)anthracene	278	2.5

* Concentration prior to dilution of 20X.

** Indicates saturated analytical ion.

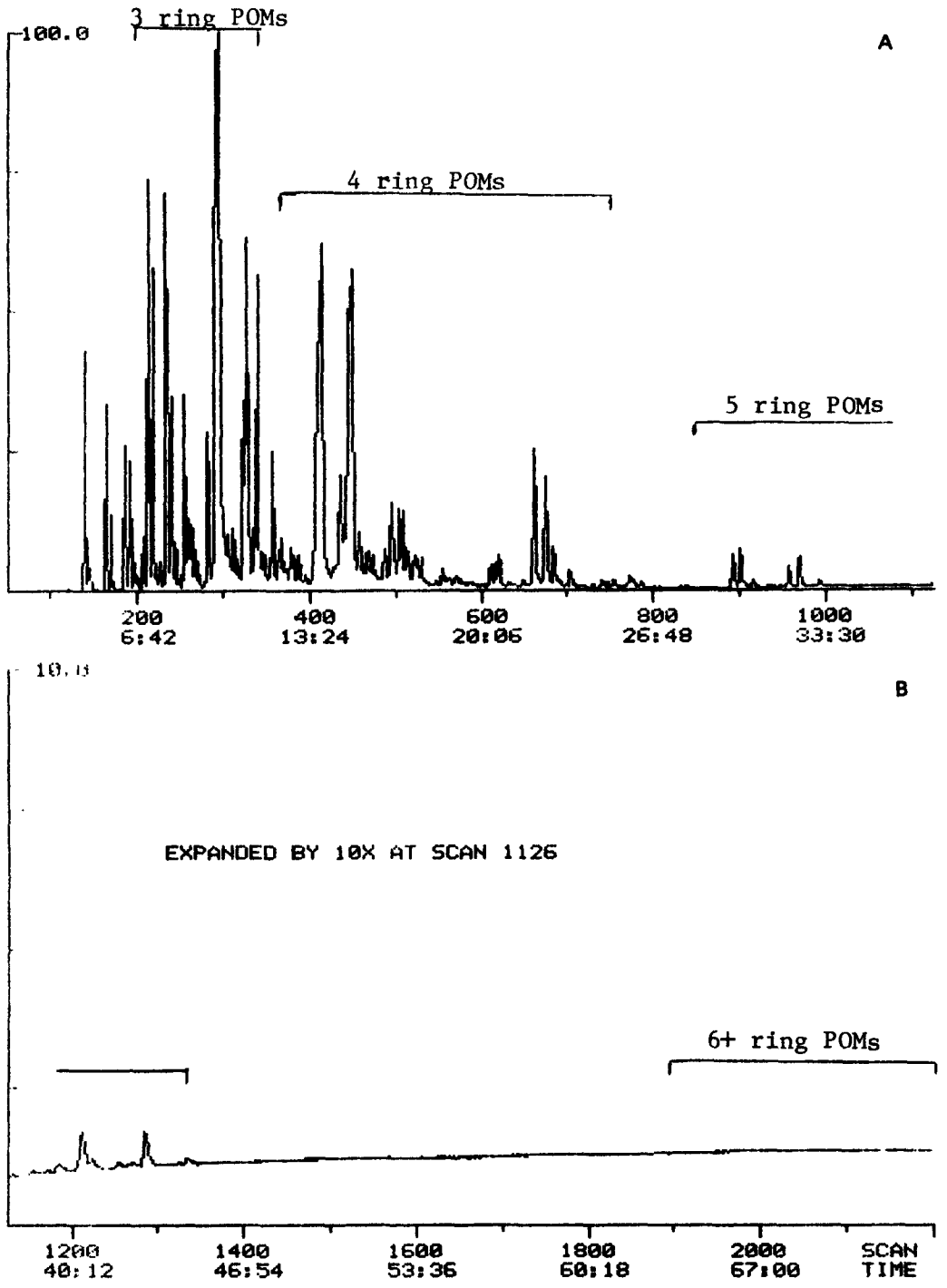

FIGURE 10. Reconstructed Ion Chromatogram for Sample of Fumes from a Low Burn Pitch.

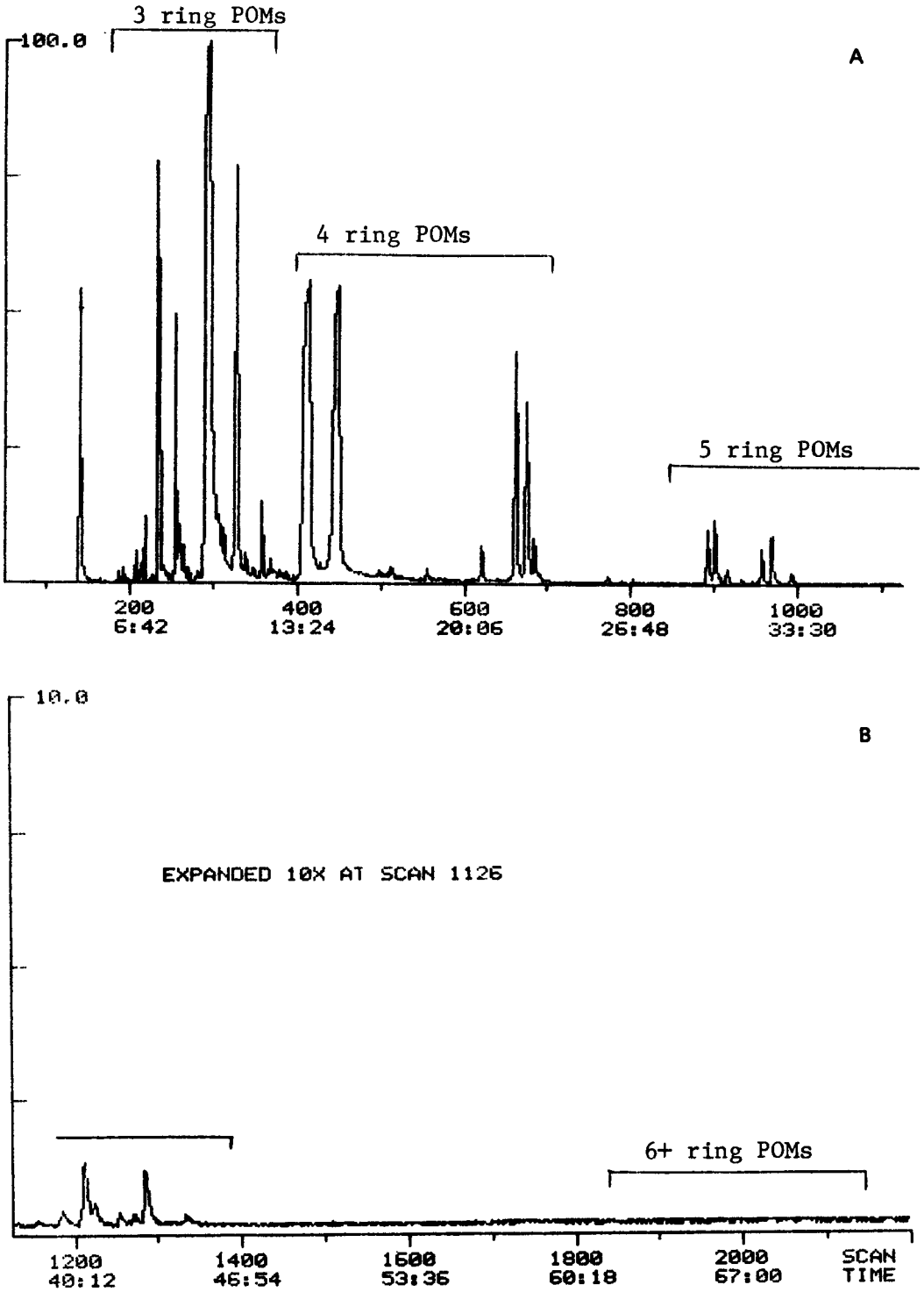

FIGURE 11. Sum Ion Chromatogram for Sample of Fumes from a Low Burn Pitch for Analytical Ions Listed in Table 13.

listed in Table 13 were reported.

To illustrate the resolving power attained under routine operating conditions, Figure 3 (given earlier on p. 22) shows the typical selected ion plots for the molecular ion of some common POMs from a calibration run. Figure 3A shows the separation of equal concentrations of phenanthrene and anthracene under the conditions of Table 12. The valley between the peaks is about 20% of the peak height. Figure 3B shows the partial separation between chrysene and triphenylene which are baseline resolved from benz(a)anthracene. Typically, the former two isomers will not show any separation when analyzed with packed column GC procedures. Figure 3C shows the separation between benzo(e)pyrene, benzo(a)pyrene, and perylene for these conditions. Since this type of separation is typical on glass capillary columns, benzo(a)pyrene may be selectively analyzed with results similar to those obtained using liquid crystal GC procedures.

V. METHODS

This section presents detailed descriptions of the five procedures presented for POM analysis. The first two procedures describe measurement of total POMs, the remaining three describe measurement of specific POM by GC/MS.

A. Total POM Measurement by Solution Fluorescence

1. Abstract

POMs are inherently flourescent materials with fairly broad and intense emission and excitation bands. Since fluorescence emission measurements are linear over a wide range of species concentrations, the fluorescence emission for a sample may be used to estimate total POM for that sample.

2. Interferences

The most prominent interferences on the fluorescence of POMs is due to quenching of the emission by either high POM concentration or by quenching agents such as highly-nitrated aromatics. These will be minimized in dilute solution.

3. Sample Extraction

Sample extractions should be done using distilled-in-glass pentane or methylene chloride. Samples should be concentrated to 1 to 10 mL using a Kuderna-Danish evaporator. If necessary, samples may be further concentrated to .1 mL using a gentle stream of nitrogen.

4. Sample Clean-up

The concentrated extract should be cleaned up using the EPA Level 1 Liquid Chromatography procedures (Appendix A). After clean-up, combine fractions 2, 3, and 4 and reduce in volume to a small, convenient level such as 2 mL using nitrogen.

5. Analysis

Measure the sample for total POM using a spectrofluorimeter with 350 ±5 nm excitation and 410 ±5 nm emission wavelengths. After any

measurement, dilute sample by a factor of 2 to 5 and remeasure until the emission decreases by the same factor; i.e., sample is in linear working region where no significant concentration quenching is occurring. Calibrate the spectrometer emission against standard anthracene solutions ranging in concentration from about 5 to 500 ng/mL of POM as anthracene.

B. Sensitized Fluorescence for Total POM

1. Principle: The fluorescence of a polycyclic aromatic hydrocarbon is greatly enhanced when it is present in trace quantities (10^{-4} to 10^{-6} mole ratio) in a solid aromatic hydrocarbon of lower molecular weight, e.g., anthracene in naphthalene. In the case of isomers, the less linearly conjugated one is a sensitizer for the more linearly conjugated one(s), e.g., phenanthrene for anthracene.

2. Range and Sensitivity: Many PAH can be detected at 10 pg in the presence of 6 to 60 µg of naphthalene. Benzo(a)pyrene has been detected at 1 pg.

3. Interferences: Highly-nitrated aromatic compounds are known to quench fluorescence of PAH. At low levels, however, that effect is probably less likely than the transfer of energy to PAH.

4. Precision and Accuracy: Concentrations of PAH can be estimated within a factor of 10 in the sensitized fluorescent spot test by 1:10 serial dilutions of the sample.

5. Apparatus: Sources are those used during study, and equivalent sources are acceptable.

5.1 Ultraviolet source, 254 nm (Chromatovue Model C5)

5.2 Filter Paper (Whatman #42)

5.3 Pipets (Drummond Microcaps, 1 µL)

6. Reagents:

6.1 Naphthalene (Fisher Scientific Catalog #N-134, "Certified") 60 µg/µL.

6.2 Benzene or methylene chloride (Fisher Scientific, Spectroanalyzed Grade).

7. Procedure: This sensitized fluorescence spot test presupposes that the sample has been obtained in an organic solvent either by direct solution, extraction, or a separation procedure such as liquid chromatography.

7.1 With pencil, mark three circles on filter paper, each approximately 0.25 cm in diameter.

7.2 With the paper supported so that marked spots are not in contact with any other surface, apply 1 μL of sample solution to central portion of each of two marked spots. Allow to air dry, keeping spots from contacting other surfaces.

7.3 Similarly apply 1 μL of naphthalene reagent solution to remaining blank circle and to a spot containing sample.

7.4 Observe spots under 254 nm, viewing either side of substrate. Note whether differences in intensity or color exist between sample-reagent spot and either spot alone, since naphthalene impurities may fluoresce. Any difference indicates sensitized fluorescence. (At 1 ng PAH the fluorescence of the sample spot itself should not be evident.

Since the limits of detection are 1 to 10 pg PAH/spot for sensitized fluorescence and approximately 10 ng/spot for non-sensitized fluorescence, the results of the spot test procedure can be used to make the following estimates of PAH contents in the 1 μL of sample.

a) non-fluorescent when treated with sensitizer : ≤1 pg
b) weakly fluorescent when treated with sensitizer : 1-10 pg
c) strongly fluorescent when treated with sensitizer but not fluorescent alone : ≥100 pg
d) fluorescent without sensitizer : ≥10,000 pg

From such estimates, the decision to proceed with further analyses can be made. In the case of strong sensitized fluorescence, or fluorescence without sensitization, a better estimate of concentration may be made by directly testing dilutions of the sample solution.

8. Calculations: In order to determine the PAH level in the sample solution, the observation should be made on successive 1:10 dilutions until the sensitized fluorescence is no longer observed. Under the conditions for the test--a 1 μL sample volume and 10 pg as the lowest detectable amount of PAH--the concentration of PAH in the solution can be calculated with a factor of 10 as follows:

$$C = 1 \times 10^{(n-6)} \text{ g/L}$$

where n = number of 1:10 dilutions.
The above formula is derived from the more explicit one:

$$C = \frac{10 \times 10^{-12} \text{g}}{1 \times 10^{-6} \text{ L}} \times 10^{n-1}$$

For example, a solution sample that was diluted eight (8) times to reach the point of no recognizable sensitized fluorescence would contain $1 \times 10^{(8-6)} = 100$ g/L.

Since the sample solution used in this test may be an extract, LC fraction, aliquot of another solution, or derived on some other way from an original environmental assessment sample, the appropriate factors must then be applied to compute the PAH content of that original sample.

9. Stability: The naphthalene sensitizer solution, kept in a tightly-stoppered dark brown bottle, has been found to be stable over a one-year period, but should be checked periodically.

C. POM Measurement by Packed Column GC/MS

1. Abstract of Method

The method is designed to measure POMs in environmental samples using packed column GC/MS. Data are acquired to include the molecular ions of all of the POM species of interest. Specific POMs are identified from their retention time relative to an internal standard and confirmed from their mass spectra. Data are reported either for specific POMs or for combinations of specific POMs which are not resolved.

2. Interferences

Interferences in the POM measurements are due to coeluting species

with mass spectra containing the POM molecular ion. Identity confirmation by the mass spectra minimizes the effect of interferences on the POM measurement.

3. Sample Extraction

Sample extractions should be done using distilled-in-glass pentane or methylene chloride. Samples should be concentrated to 1 to 10 mL using a Kuderna-Danish evaporator. If necessary, samples may be further concentrated to 0.1 mL using a gentle stream of nitrogen.

4. Sample Clean-up

The concentrated extract should be cleaned up using the EPA Level 1 Liquid Chromatography procedure (Appendix A). After clean-up, combine fractions 2, 3, and 4 and reduce in volume to a small, convenient level such as 2 mL using nitrogen.

5. Analysis

a. GC Conditions

Use a 2 m x 2 mm I.D. glass column containing any of several phases, i.e., OV-1, OV-101, OV-17, Dexsil 300 or Dexsil 400 at 1% to 3% loading on 80/100 or 100/120 Chromosorb. Temperature program from about 150°C to about 300°C or to the upper temperature limit of the column. An injection of 2-5 µL of sample is made onto the column with the GC gas stream diverted from the mass spectrometer inlet line. After the solvent has eluted, the diverter is closed and data acquisition initiated.

b. MS Conditions

Exact conditions will depend on the spectrometer type and condition and the sensitivity required for the analysis. The spectrometer should collect data for the analytical ions of interest such as the following:

POM	Analytical m/z
Fluorene	166
Anthracene or Phenanthrene	178

POM	Analytical m/z
Fluoranthene or Pyrene	202
Chrysene, Triphenylene, etc.	228
Benzo-pyrenes or -fluoranthenes	252
Indeno pyrene or benzo(ghi)perylene	276
Dibenz-anthracenes, etc.	278
Dibenz pyrenes	302

Full mass scanning over the range of 125-310 is recommended.

6. Qualitative Identification

The relative retention time for the POM of interest, compared to the internal standard present, is used to select the peak from the analytical ion plot containing the POM of interest. The mass spectra of that peak are examined to confirm the identity of the POM.

7. Quantitative Measurement

When the species are confirmed as POMs, individual mass chromatograms for the analytical m/e's are obtained. The peak areas for the POMs of interest and that for the interanl standard are ratioed and compared to a calibration curve for each POM (or POM group). The calibration standards and the samples should have the same amounts of internal standard(s).

D. Measurement of Selected POMs by Liquid Crystal Column GC/MS

1. Abstract of Method

The method is designed to measure a few specific POMs in environmental samples using liquid crystal phases for GC-MS. Data for limited mass regions containing the POM and internal standard analytical ions are acquired. Specific POM isomers are identified from their retention time relative to the internal standard.

2. Interferences

Interferences in the POM measurements are due to coeluting species with mass spectra containing the POM molecular ion. Identity confirmation by the mass spectra minimizes the effect of interferences on the

POM measurement.

3. Sample Extraction

Sample extractions should be done using distilled-in-glass pentane or methylene chloride. Samples should be concentrated to 1 to 10 mL using a Kuderna-Danish evaporator. If necessary, samples may be further concentrated to 0.1 mL using a gentle stream of nitrogen.

4. Sample Cleanup

The concentrated extract should be cleaned up using the EPA Level 1 Liquid Chromatography procedure (Appendix A). After cleanup, combine fractions 2, 3, and 4 and reduce in volume to a small convenient level, such as 2 mL, using nitrogen.

5. Analysis

a. GC Conditions

Use a 2 m x 2 mm ID glass column containing a nematic liquid crystal phase, such as SP-301 coated on 80/100 Supelcoport. Temperature programming has been used but isothermal operation gives best results. For example, for benzo(a)pyrene use isothermal operation at 260°C. Inject a 2-5 μL sample onto the column, and collect data following the elution of the solvent.

b. MS Conditions

Exact conditions will depend on spectrometer type and condition. Either selected ion monitoring or selected mass range scanning should be used to minimize background and enhance the signal-to-noise ratio. The spectrometer should collect data to include the analytical ions of interest. For example, to analyze benzo(a)pyrene with 9,10-diphenyl anthracene as the internal standard, collect data in the following ranges:

Species	Mass Range	Analytical m/z
Benzo(a)pyrene	240 - 260	252
9,10-Diphenyl anthracene	320 - 340	330

6. Qualitative Identification

The relative retention time for the POM of interest, compared to the internal standard present, is used to select the peak from the analytical ion plot containing the POM of interest. The mass spectra of that peak are examined to confirm the identity of the POM.

7. Quantitative Measurement

When the species are confirmed as POMs, individual mass chromatograms for the analytical m/z's are obtained. The peak area for the POMs of interest and that for the internal standard are ratioed and compared to a calibration curve for each POM (or POM group). The calibration standards and the samples should have the same amount of internal standard, such as 9,10-diphenyl anthracene, added to them.

E. POM Measurement by Capillary Column GC-MS

1. Abstract of Method

The method is designed to measure POMs in environmental samples using capillary column GC-MS. Data are acquired to include the molecular ions of all of the POMs of interest. Specific POMs are identified from their retention time relative to an internal standard and confirmed from their mass spectra. Data are reported either for specific POMs or for combinations of specific POMs which are not resolved.

2. Interferences

Interferences in the POM measurements are due to coeluting species with mass spectra containing the POM molecular ion. Identity confirmation by the mass spectra minimizes the effect of interferences on the POM measurement.

3. Sample Extraction

Sample extractions should be done using distilled-in-glass pentane or methylene chloride. Samples should be concentrated to 1 to 10 mL using a Kuderna-Danish evaporator. If necessary, samples may be further concentrated to 0.1 mL using a gentle stream of nitrogen.

4. Sample Cleanup

The concentrated extract should be cleaned up using the EPA Level 1 Liquid Chromatography procedure (Appendix A). After cleanup, combine fractions 2, 3, and 4 and reduce in volume to a small convenient level, such as 2 mL, using nitrogen.

5. Analysis

a. GC Conditions

Use a 20-30 m glass capillary column coated with a silicone phase, e.g., OV-101, OV-17, or SE-52. Depending upon sample concentration, use either split or splitless GC injection of 1 or 2 μL of sample, with splitless on-column injection preferred. Temperature-program the column to separate the various POMs. For example, with a splitless injection of sample in methylene chloride, a typical program might be: 35°C isothermal for 1-4 min., heat at 20°C/min. to 200°C, then heat at 2 or 3°C/min. to just below the upper temperature limit of the column, and hold as necessary to completely elute the sample. Data acquisition should start after the elution of the solvent.

b. MS Conditions

Exact conditions will depend on the spectrometer type and condition and the sensitivity required for the analysis. The spectrometer should collect data for the analytical ions of interest such as the following:

POM	Analytical m/z
Fluorene	166
Anthracene or phenanthrene	178
Fluoranthene or pyrene	202
Chrysene, Triphenylene, etc.	228
Benzo-pyrenes or -fluoranthenes	252
Indeno pyrene or benzo(ghi)perylene	276
Dibenz-anthracenes, etc.	278
Dibenz pyrenes	302

Full mass scanning over the range of 125-310 is recommended.

6. Qualitative Identification

The relative retention time for the POM of interest, compared to the internal standard present, is used to select the peak from the analytical ion plot containing the POM of interest. The mass spectra of that peak are examined to confirm the identity of the POM.

7. Quantitative Measurement

When the species are confirmed as POMs, individual mass chromatograms for the analytical m/e's are obtained. The peak areas for the POMs of interest and that for the internal standard are ratioed and compared to a calibration curve for each POM (or POM group). The calibration standards and the samples should have the same amount of internal standard(s). Use of multiple internal standards, such as d_{10}-anthracene, 9-phenylanthracene, and 9,10-diphenylanthracene, which have boiling points covering the temperature range of the analysis, will minimize errors that might arise due to variation of the split ratio with respect to molecular weight and/or boiling point for the compounds of interest.

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APPENDIX A*

EPA Level 1 - Liquid Chromatographic (LC) Separation

All sample extracts, neat organic liquids, and SASS-train-dried probe/cyclone rinse extracts are subjected to LC separation if sample quantity is adequate. An aliquot of the concentrated extract containing 100 mg of organic matter is preferred for the LC, but smaller quantities down into a lower limit of about 15 mg may be used. The sample components are separated according to polarity on silica gel using a step gradient elution technique. The detailed procedure for the LC separation is given below:

Column: 200 mm x 10.5 mm I.D., glass with Telfon stopcock, waterjacketed with inlet water temperature in the range of 18° to 22°C and sufficient flow to maintain this temperature through to the outlet.

Adsorbent: Davison, Silica Gel, 60-200 mesh, Grade 950 (available from Fisher Scientific Company) is to be used; no other types or grades of silica gel can be substituted. This material should be cleaned prior to use by sequential Soxhlet extractions with methanol, methylene chloride, and pentane. This adsorbent is then activated at 110°C for at least two hours just prior to use and cooled in a desiccator.

Drying Agent: Sodium Sulfate (Anhydrous, Reagent Grade). Clean by sequential Soxhlet extraction for 24 hours each with methanol, methylene chloride, and pentane. Dry for at least two hours at 110°C just prior to use and cool in a desiccator.

9.4.4.1 Procedure for Column Preparation

The chromatographic column, plugged at one end with a small portion

*Reference 46, Section 9.4.4

of glass wool, should be slurry packed with 6.0 g of freshly-activated silica gel in n-pentane. A portion of properly-activated silica gel weighing 6.0 ±0.2 g occupies 9 mL in a 10 mL graduated cylinder. The total height of the silica gel in this packed column is 10 cm. The solvent void volume of the column is 2 to 4 mL. When the column is fully prepared, allow the pentane level in the column to drop to the top of the silica bed so that the sample can be loaded for subsequent chromatographic elution.

After packaging the silica gel column, add 3 g ±0.2 g clean sodium sulfate to the top of the column. Vibrate for 1 min. to compact. The sodium sulfate should occupy 2 mL in a 10 mL graduated cylinder. The sodium sulfate will remove small quantities of water from the organic extract; however, appreciable quantities of water will solidify the sodium sulfate, inhibiting proper flow through the column. Therefore, it is advisable that if enough water is present in the sample to form two layers, it should be removed by another method--pipet or separatory funnel.

9.4.4.2 Evaporation of Sample Extracts with Low Total Chromatographicable Organics (TCO) (≤2 mg original sample)--

For these samples, the aliquot of extract containing 15 mg (minimum) to 100 mg (preferred) of material is added to a small amount of silica gel, the solvent is allowed to evaporate, and the residue plus silica gel is transferred to the LC column with the aid of a microspatula. The container is rinsed as described in Section 9.4.4.5.

9.4.4.3 Solvent Exchange of Sample Extract with High Total Chromatographicable Organics (TCO) (>2 mg original sample)--

An aliquot of methylene chloride extract containing 15 mg (minimum) to 100 mg (preferred) of material is added to 200 mg of silica gel in a graduated receiver. The volume of extract is carefully reduced to 1 mL at ambient temperature under a gentle stream of nitrogen (tapped from a liquid nitrogen cylinder, if possible, to minimize impurities). The solvent evaporates rapidly, so it is important that this operation be done under constant surveillance to insure that the volume is not reduced below 1 mL. It is also necessary to warm the samples slightly, either

by hand or water bath, at $\leq 40^{\circ}C$, to prevent condensation of atmospheric moisture in the sample due to evaporative cooling. One milliliter of cyclopentane is added and mixed by gentle agitation. The volume is reduced to a total of 1 mL as before. A second milliliter of cyclopentane is added, mixed, and the volume is again reduced to 1 mL. The exchange is repeated with a third milliliter of cyclopentane. After the volume has been reduced to 1 mL for this last time, the solvent mixture will be ≤ 5 percent methylene chloride. This is sufficiently low to prevent breakthrough of aromatic sample components into the aliphatic hydrocarbon fraction, LC1.

The cyclopentane and silica gel are transferred to the top of the previously prepared LC column using a Pasteur pipet. The container is rinsed as described in Section 9.4.4.5.

9.4.4.4 Neat Organic Liquids--

A 100 mg sample is weighed into a tared glass weighing funnel and mixed with about 200 mg of silica gel using a microspatula. The sample is then transferred to the top of the column. The container is rinsed as described in Section 9.4.4.5.

When neat organic liquids are fractionated by the liquid chromatography scheme, they have the same theoretical gravimetric detection limitations as other samples separated by this means, 0.1 mg/100 mg or 0.1 percent of the sample applied. Since these aliquots are neat samples and do not have concentration factors as multipliers, the resultant detection limits for minor components are 1 g/kg at best.

9.4.4.5 Chromatographic Separation into Seven Fractions--

Table A-1 shows the sequence for the chromatographic elution. In order to insure adequate resolution and reproducibility, the column elution rate is maintained at 1 mL/min.

The volume of solvents shown in Table A-1 represents the solvent volume added to the column for that fraction. If the volume of solvent collected is less than the volume actually added due to evaporation, restore the fraction volume to the proper level with fresh solvent. In all cases, the solvent level in the column should be at the top of the gel bed, i.e., the sample-containing zone, at the end of the collection

TABLE A-1 LIQUID CHROMATOGRAPHY ELUTION SEQUENCE

Fraction	Solvent Composition	Volume
1	Pentane	25
2	20% Methylene chloride in pentane	10*
3	50% Methylene chloride in pentane	10*
4	Methylene chloride	10*
5	5% Methanol in methylene chloride	10
6	20% Methanol in methylene chloride	10
7	50% Methanol in methylene chloride	10

*POM containing fraction

of any sample fraction. The fractions are retained as solutions for TCO analyses.

After the first fraction is collected, rinse the original sample container or weighing funnel with a few milliliters of Fraction 2 solvent (20 percent methylene chloride/pentane) and carefully transfer this rinsing into the column. Repeat with each successive solvent mixture in turn.

Add each new solvent to the column slowly to minimize disturbing the gel bed and eliminate the trapped air bubbles, particularly in the zone of the sample-containing silica gel.

After each sample is collected, an aliquot (1 to 5 μL) is taken for TCO analysis of each fraction (unless the sample taken for LC had a TCO of ≤2 mg). Also, an aliquot (10 mL for Fraction 1 and 5 mL for Fractions 2-7) is transferred to a tared aluminum micro weighing dish for evaporation and gravimetric analysis. The GRAV data for Fraction 7 must be corrected for a blank contributed by a small quantity of silica gel that dissolves in the highly polar eluent. The blank value is determined by running an LC column in which no sample is added; it is on the order of 0.9 ±0.1 mg in LC7 (10 mL). After TCO and GRAV determinations, the fractions are analyzed by IR and, when the quantity is defficient, by LRMS.

The objective of the LC procedure is to separate the sample into fractions of varying chemical class type to facilitate subsequent analyses. The LC separation procedure is not a high resolution technique and, consequently, there is overlap in class type between many of the fractions. With respect to POM analysis, the three fractions (2, 3, and 4) should be combined and the resulting solution reduced in volume to 2 mL prior to analysis.

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1 REPORT NO	2.	3. RECIPIENT'S ACCESSION NO.
EPA-600/7-79-191		

4. TITLE AND SUBTITLE	5. REPORT DATE
Measurement of Polycyclic Organic Matter for Environmental Assessment	August 1979
	6. PERFORMING ORGANIZATION CODE
7 AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
P. L. Levins, C. E. Rechsteiner Jr., and J. L. Stauffer	
9 PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
Arthur D. Little, Inc. Acorn Park Cambridge, Massachusetts 02140	INE624
	11. CONTRACT/GRANT NO.
	68-02-2150, Task 10202
12 SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
EPA, Office of Research and Development Industrial Environmental Research Laboratory Research Triangle Park, NC 27711	Task Final; 12/76 - 3/79
	14. SPONSORING AGENCY CODE
	EPA/600/13

15. SUPPLEMENTARY NOTES IERL-RTP project officer is Larry D. Johnson, Mail Drop 62, 919/541-2557.

16. ABSTRACT The report discusses methods of measuring polycyclic organic matter (POM) for environmental assessment. It describes two fluorescence methods of estimating total POM levels in samples. Either method may be used to screen samples for further specific analyses. It also describes three gas chromatography/mass spectrometry (GC/MS) methods for measuring specific POM compounds. The use of liquid crystal chromatographic phases is recommended for measuring a few POMs; e.g., specifically for benzo(a)pyrene. It discusses GC/MS methods for a wide range of POMs for both capillary and packed GC columns. The three methods for specific POM identification have been verified with collected environmental samples for different kinds.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Pollution Assessments Measurement Polyclic Compounds Organic Compounds Fluorescence Gas Chromatography Mass Sprectroscopy	Pollution Control Stationary Sources Environmental Assessment Polycyclic Organic Matter Benzo(a)pyrene	13B 14B 07D 07C 20F

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
Release to Public	Unclassified	73
	20. SECURITY CLASS *(This page)*	22. PRICE
	Unclassified	

EPA Form 2220-1 (9-73)