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Rapid Methods of Analysis for Trace Quantities of Polynuclear Aromatic Hydrocarbons and Phenols in Automobile Exhaust, Gasoline, and Crankcase Oil

CRC-APRAC Project CAPE-12-68

R. A. Brown, T. D. Searl, W. H. King, Jr.,
W. A. Dietz and J. M. Kelliher
Esso Research and Engineering Company
Linden, New Jersey

CRC-APRAC PROJECT CAPE-12-68

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Panel

F. P. Hochgesang, Project Leader (Mobil Research & Development Corp., Paulsboro, N. J.)

C. R. Begeman (General Motors Research, Warren, Michigan)

K. Habibi (E. I. duPont de Nemours & Co., Penns Grove, N. J.)

L. W. Mixon (American Oil Research & Development, Whiting, Indiana)

E. Sawicki (Environmental Protection Agency/Air Pollution Control Office, Research Triangle Park, North Carolina)

CRC-APRAC Headquarters

A. E. Zengel, Project Manager

T. C. Belian, Assistant Project Manager

Table of Contents

	Page
Objective	1
Summary	1
Introduction	3
Method for Benz(a)Anthracene, Benzo(a)Pyrene and Other Polynuclear Aromatic Hydrocarbons in Automobile Exhaust, Gasoline and Crankcase Oil	4
Preparation of Samples with Carbon-14 Labeled BaA and BaP	4
Caustic Extraction	4
Preparation of a PNA Concentrate by Adsorption Chromatography	6
Gas Chromatography and Trapping of Peaks	6
Ultraviolet Absorption Spectrophotometry	8
Radioactivity Measurements	11
Calculation of PNA's in GC Peaks	11
Test for Accuracy	14
Calculation to a Sample Basis	15
Application of the Method	16
Measurement of Total Polynuclear Aromatic Hydrocarbons By Low Voltage Mass Spectrometery	19
Procedure for Mass Spectrometer Analysis	21
Calibration	21
Calculation	25
Application of the Method	26
Method for Phenols in Automobile Exhaust Gas	28
Description of Method	28
Preparation of Sample for Gas Chromatograph	28
Gas Chromatographic Separation of Phenols	29
Application of the Method	30
Appendix A - Method for Benz(a)anthracene, Benzo(a)pyrene, and Other Polynuclear Aromatic Hydrocarbons in Automobile Exhaust, Gasoline and Crankcase Oil	34
Appendix B - Measurement of Total Polynuclear Aromatic Hydrocarbons by Low Voltage Mass Spectrometry	45
Appendix C - Method for Measurement of Phenols in Aqueous Condensate of Automobile Exhaust	49

Final Report

Rapid Methods of Analysis for Trace Ouantities of Polynuclear Aromatic Hydrocarbons and Phenols in Automobile Exhaust, Gasoline, and Crankcase Oil

OBJECTIVE

Objectives of CAPE-12-68 project were:

- Develop and put to practice a rapid method to measure benz-(a)anthracene and benzo(a)pyrene in tar recovered from automobile exhaust, in gasoline, and in crankcase oil.
- Extend this method to additional polynuclear aromatic (PNA) hydrocarbons.
- Develop a method to measure total PNA's in auto exhaust tar.
- Develop a method to measure individual phenols in auto exhaust tar.

SUMMARY

A. A rapid method was developed to measure benz(a)anthracene (BaA) and benzo(a)pyrene (BaP) in auto exhaust tar, gasoline, and crankcase oil. Nine other polynuclear aromatic hydrocarbons were also included in the measurement. These included: pyrene, chrysene, triphenylene, methyl BaA, dimethyl BaA, benzo(e)pyrene (BeP), methyl BaP, methyl BeP, and benzo(g,h,i)perylene. Each of the 11 compounds occurred in concentrations of 10-2000 ppm and were measured as 0.1 micrograms and higher. The method can also be extended to other PNA hydrocarbons, such as benzofluoranthenes, dibenzanthracenes, anthanthrene, and coronene.

A cyclohexane solution of the sample was extracted with aqueous caustic, then eluted through a column of partially deactivated alumina and all fractions containing PNA's were combined. A portion of the PNA concentrate was fractionated by gas chromatography and individual chromatographic peaks trapped. Concentrations of individual PNA's in the trapped fractions were measured by UV absorption spectrophotometry. Analytical losses were accounted for by isotope dilution.

Twenty technician-hours were required to measure the 11 individual PNA's.

The precision (1σ) for nine of the compounds was in the range 4-12 relative percent. The preceision for benzo(g,h,i)perylene was 18 relative percenter, and methyl BaP 45 relative percent. Absolute accuracy for BaA and BaP was within 10 percent.

B. Total PNA's were measured by low voltage mass spectrometry, using the same concentrates that were prepared for injection into the gas chromatograph in the GC/UV procedure. PNA's are defined by class and molecular weight. Quantitation is achieved by using tripheylbenzene as an internal standard. Compound classes range from tricyclic to heptacyclic aromatics. Of the total four and higher ring PNA hydrocarbons in one sample, BaA represented only 0.95% and BaP only 0.4%.

C. A solvent extraction-gas chromatographic method was developed for the quantitative determination of individual phenols in the aqueous condensate of auto exhaust. An internal standard, chlorophenol, was added and neutral compounds were removed by extraction of the alkaline sample. After acidification, phenols were removed by extraction with ethyl ether. After removing organic acids from this extract, evaporation to a residue is carried out. An aliquot of the residue was then analyzed by capillary column gas chromatography. In two samples of exhaust tar, phenol was 65 and 100 ppm, individual cresols ranged from 7 to 15 ppm, and xylenols were less than 1 ppm.

These methods were developed principally for the CAPE-6-68 Project.

INTRODUCTION

The initial and most important objective in the CAPE-12-68 Project was to develop a rapid method to measure BaA and BaP in tar recovered from automobile exhaust, in gasoline, and in crankcase oil. In selecting an approach, we were in position to learn from fifteen years of experience in the assay of petroleum fractions for PNA's. Our choice of technique was based on a recent method which was developed at Esso Research and Engineering to measure several PNA compounds in coke oven effluents (1). Based on combined use of gas chromatography and ultraviolet absorption spectrophotometry, the new method (GC/UV) appeared to be more advantageous compared with the other most promising approach in which thin layer chromatography would provide the final fraction(s) for each PNA measurement. This approach was in agreement with a conclusion reached earlier in 1967 by McKee and McMahon (2). They surveyed existing methods for analyzing PNA and concluded that "gas chromatography appears to show the greatest promise of providing a rapid yet reliable and accurate method to determine a number of PNA compounds."

The coke oven effluent method was extensively modified in achieving a satisfactory procedure for the CAPE-12-68 project.

Another objective of CAPE-12-68 was to provide a means of measuring all of the PNA's in a sample. Some of the promising approaches included: ultraviolet absorption spectrophotometry, summation of GC peaks, and low voltage mass spectrometry. The mass spectrometric capability offered the best potential for comprehensiveness and specificity. The value of low voltage ionization measurements was first demonstrated by Field and Hastings (3). They showed that olefins and aromatic hydrocarbons can be selectively measured in the presence of other compounds. This capability arises from the fact that ionization potentials of olefins and aromatics are one or two volts lower than those of aliphatic and cycloparaffins. By using a low ionization potential, selective ionization occurs. This principle has been extensively used in the petroleum industry to analyze complex hydrocarbon mixtures.

In achieving another objective of CAPE-12, a method was developed to measure individual phenols at the ppm level. This development was based primarily on the use of an extraction procedure evaluated by Hoffman and Wynder (4) to prepare a sample for analysis by capillary gas chromatography (5, 6).

(1) T. D. Searl, F. J. Cassidy, W. H. King, Jr., R. A. Brown, Anal. Chem. 42, 954 (1970).
(2) H. P. McKee, W. A. McMahon, Technical Report No. 1, Project No. 21-2139, Committee for Air and Water Conservation, American Petroleum Institute.
(3) F. H. Field, S. H. Hastings, Anal. Chem. 28, 1248 (1956).
(4) Dietrich Hoffman, Ernest L. Wynder, Beiträge zur Tobakforschung 3, 101 (1961).
(5) Jan Hrivnak, J. Chromatog. Sci. 8, 602 (1970).
(6) D. S. Payn, Chemistry & Industry, 1090 (August 20, 1960).

METHOD FOR BENZ(a)ANTHRACENE, BENZO(a)PYRENE AND OTHER POLYNUCLEAR AROMATIC HYDROCARBONS IN AUTOMOBILE EXHAUST, GASOLINE AND CRANKCASE OIL

The method used carbon-14 labeled BaA and BaP as internal standards, starting from the first step in the analysis. Some polar compounds were removed by caustic and then a preliminary separation of PNA's was made by liquid-solid adsorption column chromatography. Specific compounds were next measured by a combined gas chromatography/ultraviolet absorption spectrophotometry procedure. In the following sections, details of the method are discussed, as well as its applicability to tar from automobile exhaust, gasoline, and crankcase oil.

The method was developed and tested during 1969-70. From mid-1970 on, it was in routine use in CAPE-6-68. Figure 1 shows the method of analysis for PNA's in automobile exhaust tar, and complete details of the method are given in Appendix A.

Preparation of Samples With Carbon-14 Labeled BaA and BaP

In this work, the starting sample of exhaust tar was in solution as several gallons of cyclohexane/acetone solvent containing the tar (7). It was necessary to distill off solvent to a residual volume of one liter. Prior to starting the distillation, ^{14}C BaA and ^{14}C BaP were added to the sample.

For gasoline, a starting sample of 50-1000 ml was spiked with ^{14}C BaA and ^{14}C BaP and evaporated on a steam bath over nitrogen. As evaporation proceeded the sample was twice replenished with cyclohexane and finally reduced to 25 ml. The sample was then ready for charging to the alumina column.

In the case of used crankcase oil, carbon-14 standards were added to a weighed amount of sample ($\sim$500 mg), and the sample charged to the deactivated alumina column.

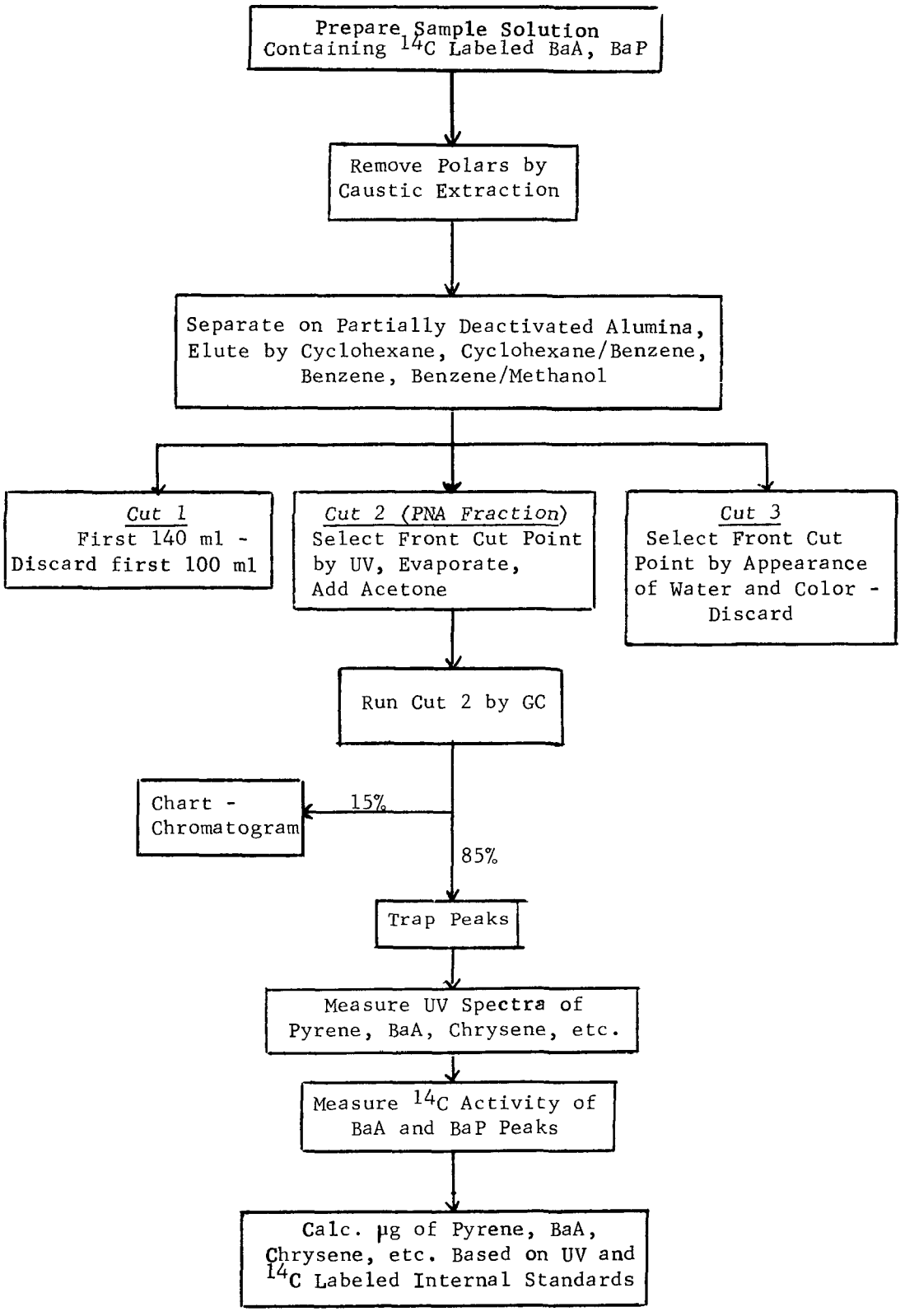
Caustic Extraction

Caustic extraction was needed only for tar samples. 500 ml of the one-liter sample was extracted three times with 0.5 N aqueous sodium hydroxide and then washed successively with 0.1 N HCl and water. This extraction step reduced the background of the gas chromatogram. 250 ml of the caustic treated sample (one-quarter of the original sample) was charged to the alumina column. This volume usually contained 100-400 mg of tar.

(7) G. P. Gross, "Gasoline Composition and Vehicle Exhaust Gas Polynuclear Aromatic Content," U.S. Clearinghouse Federal Science Technology Information, PB Rep. Issue No. 200266 (1971) 124 pp.

Figure 1

Outline of GC/UV Method for CAPE-12-68



Preparation of a PNA Concentrate
By Adsorption Chromatography

A PNA concentrate was prepared using the materials and conditions listed in Table I.

Table I

Conditions For Stepwise Elution
Of PNA's From Auto Exhaust Tar

Column Dimensions: 50 cm length and 1.1 cm i.d.

Alumina: Woelm neutral alumina dried at 160°C and then deactivated with 2 wt.% water.

Elution Schedule: 100 ml of cyclohexane
100 ml of cyclohexane/benzene (4:1)
100 ml of benzene
100 ml of benzene/methanol (1:1)

The first 100 ml of cyclohexane eluted was discarded. Then, 10 ml fractions were collected and inspected by UV for the appearance of pyrenes (absorption band at 340 nm). The first fraction containing pyrene and all subsequent fractions to the appearance of a two liquid phase boundary were combined as the PNA concentrate. The appearance of pyrene has varied from the second to the seventh 10 ml fraction of the cyclohexane/benzene elution step. Selection of the first fraction containing pyrene is illustrated in Figure 2. In this example, cut B2-3 was the initial fraction of the PNA concentrate.

The alumina column fraction containing the PNA's will normally consist of ~160 ml of a cyclohexane/benzene solution. To prepare a sample for injection into the gas chromatograph, the solution is evaporated to ~40 µl.

- Evaporate solvent, under a small jet of nitrogen, using a 150 ml beaker on a steam bath. Add portions of the solution and reduce to 2 ml.
- Transfer the 2 ml residue to a one-dram vial and continue the evaporation on a steam table to constant volume. Add acetone to bring residual volume up to ~40 µl.

Gas Chromatography and Trapping of Peaks

The operating conditions for the gas chromatographic separation are shown in Table II.

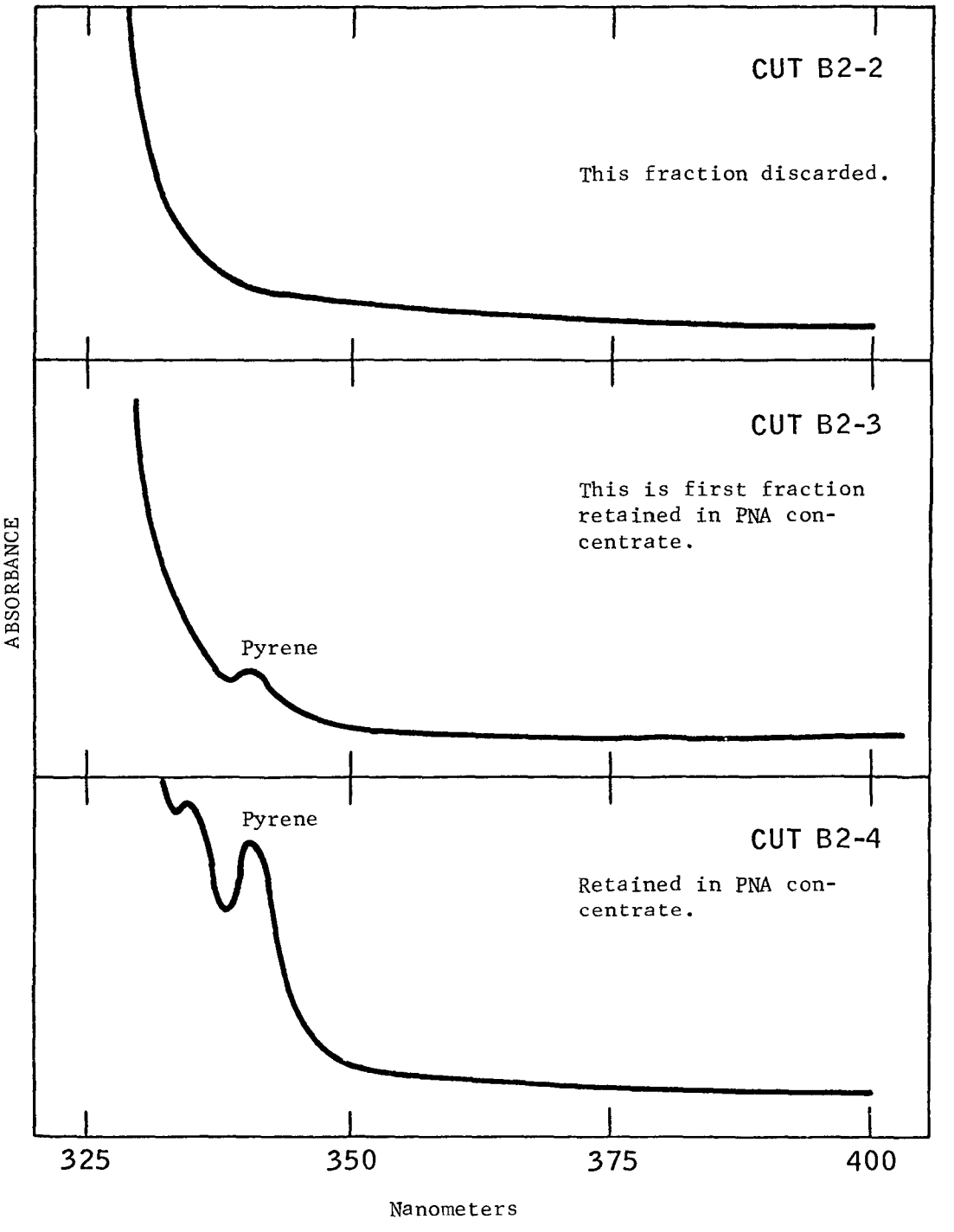
Table II

Operating Conditions for GC Separation of PNA Fractions

Chromatograph - Perkin-Elmer Model 900.
Gas Flow - Helium at 30 ml/min.
Column - 300 cm-0.22 cm i.d., packed with 2% SE 30 (GC grade) on Chromosorb "G" 80/100 mesh (acid washed and DMCS treated).
Injection Port - 305°C
Detector, F.I.D. - 345°C
Program - 175° to 300°C at 4°/min., then hold

Figure 2

SELECTION BY UV OF FRONT CUT PT.
OF PNA FRACTION FROM Al_2O_3 COLUMN



The recovery of individual PNA's, as influenced by operating conditions, indicated that the temperature at the sample injection port was important. This is shown by the recovery data in Table III.

Table III

Recovery vs. Sample Injector Temperature

Temp. programmed to 300°C
Trap line at 345°C

Sample Injector (°C)	No. Runs	Mean % Recovery Pyrene	BaA	BaP	B(g,h,i)P
260	2	87	82	84	58
275	6	87	81	81	73
305	4	91	83	84	77
345	3	85	75	70	62
400	1	79	71	63	59

Optimum recovery occurred at 305°C injection port temperature. Even for these conditions, the recovery of B(g,h,i)P is ~10 relative % lower than BaA and BaP. For this reason, a correction of 1.10 is applied to the measurement of B(g,h,i)P.

Figure 3 shows the gas chromatograph trapping assembly. To trap a fraction the stainless steel tube was slid into position as shown.

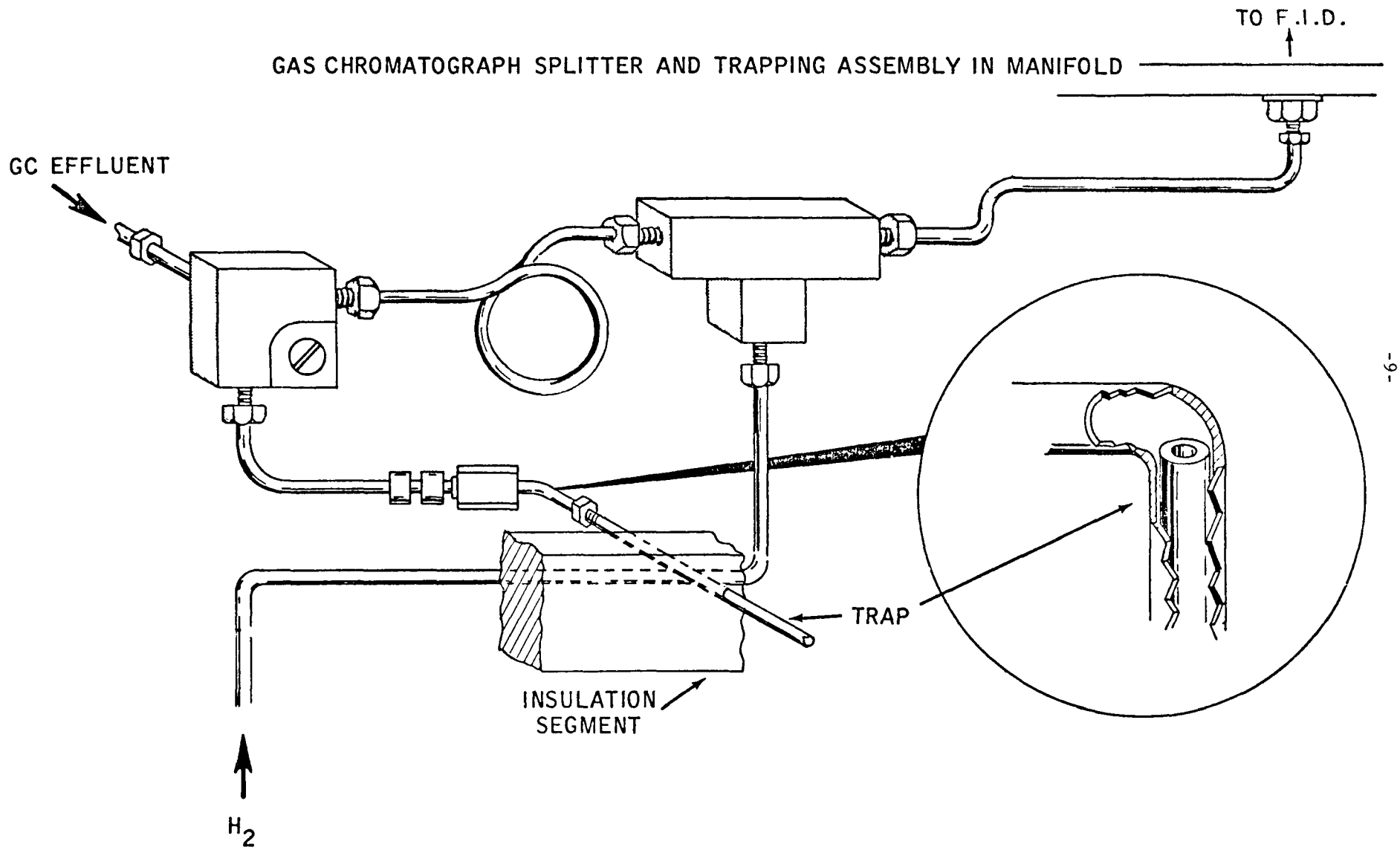
As the sample was run on the chromatograph, selected peaks were trapped and the trap content rinsed out with cyclohexane for UV measurement. Selection of peaks coincided with the retention times listed near the top of Table IV. In some instances retention times were virtually identical for two or three different PNA's. For example, BaA, chrysene and triphenylene elute together and are taken into one trap. BaP and BeP also elute together. Methyl BaP and methyl BeP elute together but in three different peaks; each peak is trapped separately. A typical chromatogram is illustrated in Figure 4. All of the PNA's currently measured plus others are shown.

Ultraviolet Absorption Spectrophotometry

Absorptivity coefficients were measured for those PNA's of interest which could be obtained. These included: pyrene, BaA, chrysene, triphenylene, some methyl and dimethylbenz(a)anthracenes, BaP, BeP, and benzo(g,h,i)perylene. These calibrations were corrected for the observed purity of each compound (purity was established by GC and MS measurements). Coefficients for methyl BaP and methyl BeP were estimated as being 0.815 of the parent compounds.

To prepare the trapped fraction for UV spectroscopy, the trap containing the GC fraction was rinsed with cyclohexane and made up to 3.8 ml. UV absorbance was measured with 1 cm path cell. For low absorbing solutions a 5-cm cell was used.

Figure 3


TO F.I.D.
GAS CHROMATOGRAPH SPLITTER AND TRAPPING ASSEMBLY IN MANIFOLD
GC EFFLUENT
TRAP
INSULATION SEGMENT
H_2

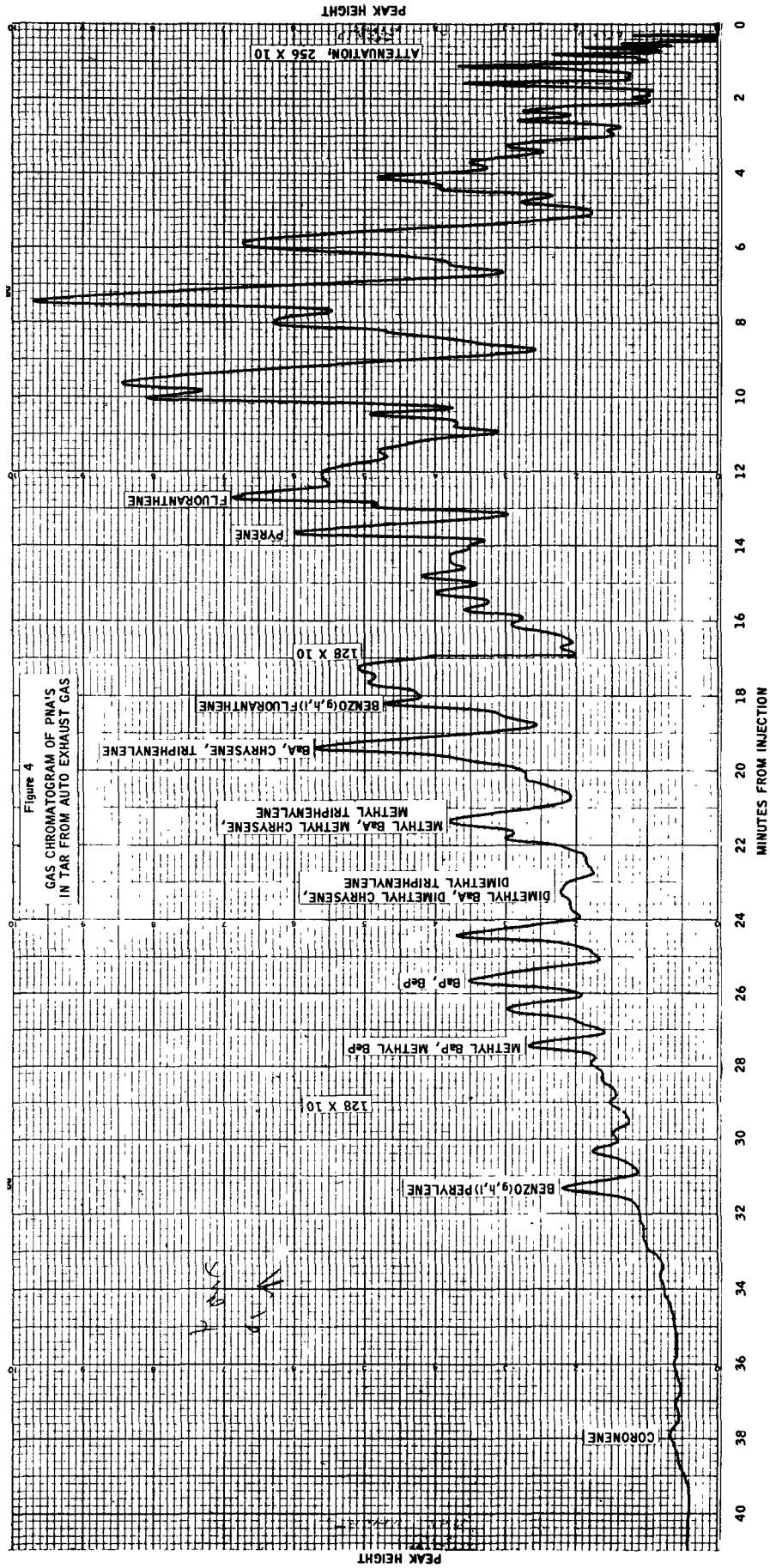


Figure 4
GAS CHROMATOGRAM OF PNA'S IN TAR FROM AUTO EXHAUST GAS

Radioactivity Measurements

The most accurate and finally used radioactive count was obtained in a long count using a Packard Tri-Carb Counter. Counting was conducted for fifty minutes or 50 K, whichever came first. Background and counting efficiency was measured on each sample. On occasion, a waiting period of one week was encountered in getting this count. To avoid this delay in reporting an analysis, a tentative analysis was based on a short count. This short count was obtained for one ml of cyclohexane solution using an Intertechnique SL 20. This count was always in close agreement with the long count.

Calculation of PNA's in GC Peaks

Concentrations of the individual PNA's were calculated from UV spectra of GC peaks based on absorptivity coefficients listed in Table IV. Slant base line measurements were used and the table lists the anchor points and the analytical peaks for each compound.

BaA, Chrysene, Triphenylene - Fig. 5 is an UV absorption spectrum of the GC peak containing BaA, chrysene and triphenylene. To calculate the concentration of each compound, a set of three simultaneous equations is solved. The array of calibration coefficients from Table IV is shown below.

Table V

Calibration Matrix for Calculation of BaA, Chrysene, and Triphenylene

	BaA	Chrysene	Triphenylene	
(1)	0.332	0	0	A289
(2)	0.039	0.464	0	A269
(3)	0.015	0.107	0.480	A259

A289, A269, A259 = UV absorbance of GC peak in a 1 cm cell.

The equations can be solved directly by substitution.

$$\text{BaA} = \frac{\text{A289}}{0.332}$$

$$\text{Chrysene} = \frac{\text{A269} - \text{BaA} \times 0.039}{0.464}$$

$$\text{Triphenylene} = \frac{\text{A259} - \text{BaA} \times .015 - \text{Chrysene} \times 0.107}{0.480}$$

TABLE IV

GC AND UV CALIBRATION DATA FOR SOME PNA HYDROCARBONS

		Pyrene	BaA	Chrysene	Triphenylene	Methyl BaA	Dimethyl/ethyl BaA	BaP	BeP	Methyl BaP	Methyl BeP	Benzo-(g,h,i) perylene
GC Retention Times (min)		13.6	19.4	19.6	19.4	21.4	23.3	25.8	25.7	-----27.5----- -----28.0----- -----29.0-----		31.4
Wavelength nm												
Peak	Base Line											
		Absorptivity (ml/μg cm)										
336	327-343	0.240										
289	283-295		0.332									
269	263-277		0.039	0.464								
259	252-263		0.015	0.107	0.480							
293[a]	285-300					0.27						
293[a]	285-300						0.27					
383	373-390							0.092				
333	325-338								0.135			
384[b]	373-390									0.075		
335	325-340										0.110	
382	370-390											0.074

[a] Occurs in range, 290-293 nm

[b] Occurs in range, 383-385 nm

Radioactivity Measurements

The most accurate and finally used radioactive count was obtained in a long count using a Packard Tri-Carb Counter. Counting was conducted for fifty minutes or 50 K, whichever came first. Background and counting efficiency was measured on each sample. On occasion, a waiting period of one week was encountered in getting this count. To avoid this delay in reporting an analysis, a tentative analysis was based on a short count. This short count was obtained for one ml of cyclohexane solution using an Intertechnique SL 20. This count was always in close agreement with the long count.

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Concentrations of the individual PNA's were calculated from UV spectra of GC peaks based on absorptivity coefficients listed in Table IV. Slant base line measurements were used and the table lists the anchor points and the analytical peaks for each compound.

BaA, Chrysene, Triphenylene - Fig. 5 is an UV absorption spectrum of the GC peak containing BaA, chrysene and triphenylene. To calculate the concentration of each compound, a set of three simultaneous equations is solved. The array of calibration coefficients from Table IV is shown below.

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Calibration Matrix for Calculation of BaA, Chrysene, and Triphenylene

	BaA	Chrysene	Triphenylene	
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$$\text{Chrysene} = \frac{\text{A269} - \text{BaA} \times 0.039}{0.464}$$

$$\text{Triphenylene} = \frac{\text{A259} - \text{BaA} \times .015 - \text{Chrysene} \times 0.107}{0.480}$$

TABLE IV

GC AND UV CALIBRATION DATA FOR SOME PNA HYDROCARBONS

	Pyrene	BaA	Chrysene	Triphenylene	Methyl BaA	Dimethyl/ethyl BaA	BaP	BeP	Methyl BaP	Methyl BeP	Benzo-(g,h,i) perylene
GC Retention Times (min)	13.6	19.4	19.6	19.4	21.4	23.3	25.8	25.7	-----27.5----- -----28.0----- -----29.0-----		31.4

Wavelength nm Peak	Base Line	Absorptivity (ml/μg cm)										
336	327-343	0.240										
289	283-295		0.332									
269	263-277		0.039	0.464								
259	252-263		0.015	0.107	0.480							
293[a]	285-300					0.27						
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383	373-390							0.092				
333	325-338								0.135			
384[b]	373-390									0.075		
335	325-340										0.110	
382	370-390											0.074

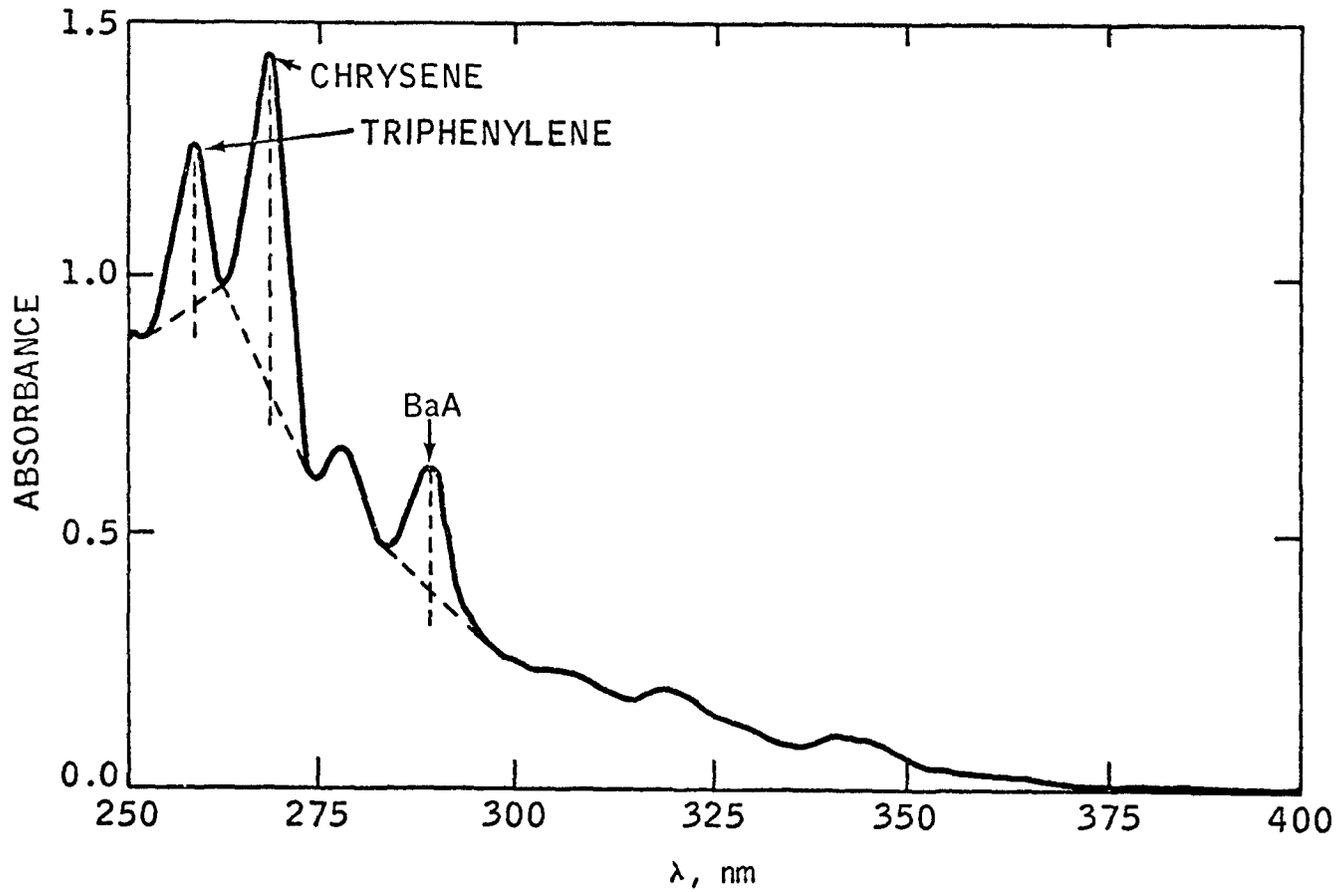
[a] Occurs in range, 290-293 nm

[b] Occurs in range, 383-385 nm

Figure 5

UV SPECTRUM OF GC
FRACTION CONTAINING BaA,
CHRYSENE AND TRIPHENYLENE

- 1 cm cell -



Test for Accuracy

The accuracy of the UV analysis of these three component mixtures was found to be satisfactory as indicated by the results in Table VI for some known blends.

Table VI

UV Analyses of Known Blends Containing Benz(a)anthracene, Chrysene and Triphenylene

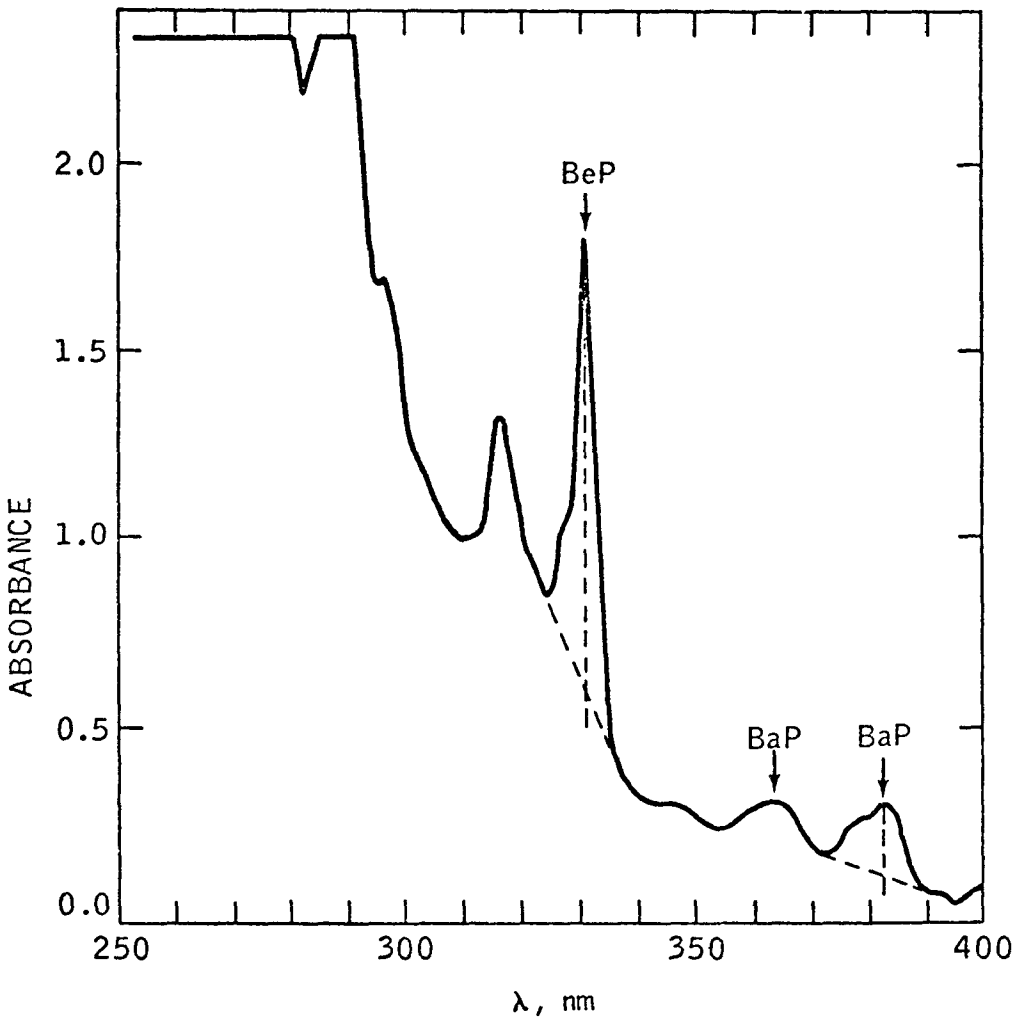
	Known	Found	Known	Found	Known	Found
	μg/ml					
BaA	0.4	0.5	1.5	1.5	0.5	0.5
Chrysene	1.2	1.0	1.5	1.3	1.5	1.4
Triphenylene	2.4	2.3	1.5	1.5	1.0	1.0

BaP, BeP - Spectrum of a GC peak containing BaP and BeP is shown in Figure 6. Base line constructions are shown for these compounds.

Figure 6

UV Spectrum of GC Fraction Containing BaP and BeP

- 5 cm cell -



Methyl BaP, Methyl BeP - Of the other PNA's measured, the calculation of pyrene, methyl BaA, and dimethyl BaA is straightforward. Benzo(g,h,i)perylene is corrected upward by 1.10 as previously noted (p. 8). Methyl substituted BaA and BeP isomers require special handling as they occur in three separate GC peaks. In practice, each of the three peaks is separately trapped and measured by UV. Summed values are then used to calculate the quantity of each methyl isomer. A more efficient approach would be to blend the solutions of the three fractions prior to the UV measurement. This is not done because there are variations in the wave length position of the maximum absorption and the UV absorption of the blend would therefore be non-additive. For methyl BaP the band position was observed to vary from 384 to 386 nm, and for methyl BeP it was 334 to 337 nm (see Table VII).

Composition information was obtained for one CAPE-6 sample in which the GC peaks were examined by UV and mass spectrometer. Results are summarized in Table VII. Peak 20 contained BaP and BeP.

Table VII

Identification of GC Fractions Containing Alkyl Substituted BaP and BeP

Peak No.	BaP Series nm	BeP Series nm	Mol. Wt.	Identification
20	383	332	252	BaP, BeP
22	384	334	266 - 100%	Methyl BaP, Methyl BeP
23	384 to 388[a]	334,337	266 - 58%	Methyl BaP, Methyl BeP
			280 - 42%	(Dimethyl/Ethyl BaP, (Dimethyl/Ethyl BeP
24	385	335	266 15%	Same as Fraction No. 23 above
			280 - 85%	

a = Broad peak.

Peak 22 showed only 266 as a molecular weight (MW) ion which indicates the presence of only methyl BaP and methyl BeP. In peak 23, however, only 58% of MW 266 was found and there was 42% of MW 280. MW 280 is probably due to dimethyl and/or ethyl BeP and BeP. Peak 24 is primarily MW 280. An estimate of the overall distribution of the methyl and dimethyl/ethyl species in the three peaks was:

methyl (BaP + BeP) = 0.8
dimethyl/ethyl (BaP + BeP) = 0.2

Calculation to a Sample Basis

Based on the measurements of the previous steps, the micrograms of each PNA in the sample were calculated from the aliquot factor (activity ratio), the PNA concentrations as measured for each GC peak, and a correction for the weights of added ^{14}C BaA and ^{14}C BaP.

Details are in Appendix A.

Application of the Method

Time Requirement - During the past two years, this method has been routinely used to analyze all of the sample types for which it was intended plus others. Experience with the method indicates that a technician can be trained in all of the steps during a 1-2 week period. Trained technicians can analyze two or three samples in parallel and at this level of proficiency, each sample requires 20 man hours of effort.

Expansion of the Method - In addition to the eleven PNA's currently being measured, the gas chromatographic separation provides numerous other well-defin ed peaks that can be trapped and analyzed. Based upon our experience other 4+ ring PNA's can be added, including: fluoranthene, benzo(b)fluoranthene, benzo-(j)fluoranthene, benzo(k)fluoranthene, perylene, anthanthrene, and coronene.

Analysis of Tar - A principal test of the method was to compare results on 11 samples with measurements by an independent procedure. In the latter method, which also employed carbon-14 labeled BaA and BaP as internal standards, a preliminary separation was carried out in a deactivated alumina column. A composite of these fractions was next separated on a thin layer chromatographic (TLC) plate containing 20% acetylated cellulose. A fraction from the plate, rich in BaA (or BaP) was then examined by UV and counted for radioactivity to obtain a quantitative measurement.

Comparison between the two methods is shown graphically in Figure 7. Shown are the ratios of GC to TLC. Measurements of BaP by GC/UV are consistently higher, particularly for small quantities. We have no explanation of this, although only small differences in absolute amounts are involved. An opposite relationship exists for BaA in that the measurement by TLC is higher than by GC/UV. High values by TLC can be attributed to the presence of methyl BaA in TLC fractions (8).

Precision of the method is indicated by several analyses of a tar sample as shown in Table VIII.

Table VIII

Precision[a] of GC/UV Method as Applied to a Tar

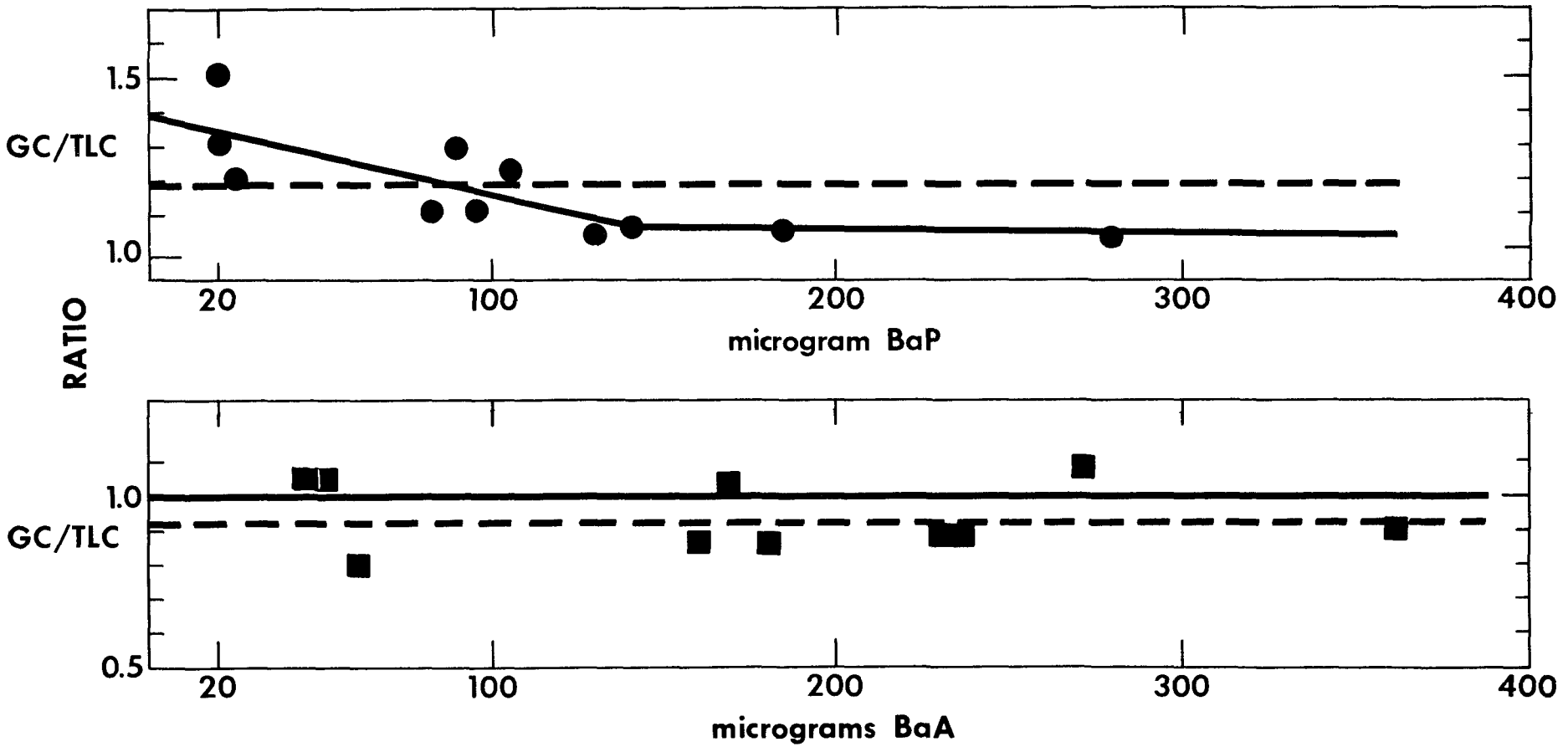
Compound	Amount (ug)	σ (ug)	σ (%)
Pyrene	676	25.4	3.8
BaA	117	5.7	4.9
Chrysene	111	13.1	11.8
Triphenylene	25	1.4	5.6
Methyl BaA	58	4.0	6.9
Dimethyl/ethyl BaA	14	1.1	7.9
BaP	15	1.4	10.0
BeP	153	8.5	5.6
Benzo(g,h,i)perylene	137	24.9	18.2
Methyl BaP	5	2.2	45.2
Methyl BeP	63	6.2	9.8

a = six analyses from March-October, 1971.

(8) R. A. Brown, J. M. Kelliher, Amer. Petrol. Inst. Proc., Div. Ref. 51, 349 (1971).

Figure 7

COMPARISON OF TAR ANALYSES



Analysis of Gasoline - Repetitive analysis of a gasoline was obtained at a time when only 2-5 compounds were being measured. These results are in Table IX.

Table IX

Analysis of a Gasoline

Compound	No. of Measurements	µg per Gallon Amount	µg per Gallon σ	σ (%)
Pyrene	4	5865	618	11
BaA	5	878	107	12
Chrysene	4	2458	360	15
BaP	5	511	38	7
BeP	4	519	25	5

Analysis of Crankcase Oil - A sample of unused multi-grade oil was analyzed. Two grams of oil were eluted through a deactivated alumina column as described in the procedure. Both the "PNA" fraction and the more polar fraction which elutes afterwards were inspected by GC/UV. BaP and BaA were below the detectable limit. Three known blends of BaA and BaP in this oil were then analyzed with accuracy as shown in Table X.

Table X

Measurement of BaA and BaP
As Added to Unused Multi-Grade Oil

Sample	BaA		BaP	
	- - - micrograms in 250 ml - - -			
	Added	Found	Added	Found
A	12	12	11	11
B	23	23	17	19
C	48	47	44	43

A 250 mg sample of crankcase oil, estimated to have been used for 3,000 miles, was found to contain 6 µg BaA and 7 µg BaP, equivalent to 24 and 28 ppm, respectively. These concentrations are comparable to those reported by Begeman and Colucci (9).

(9) Charles R. Begeman, Joseph M. Colucci, SAE Trans., 79, 1782 (1970).

MEASUREMENT OF TOTAL POLYNUCLEAR AROMATIC HYDROCARBONS BY LOW VOLTAGE MASS SPECTROMETRY

Low voltage mass spectrometry appears to be an ideal tool for measuring PNA's in a mixture. It is common practice in the petroleum industry to use this technique for olefins and aromatics. Lumpkin and Aczel (10) observed aromatics in gas oil and numerous others have reported on its use. The low voltage technique can be used with either high or low resolution spectrometers. High resolution offers the advantage of unequivocal measurement by empirical formula. A shortcoming of this approach is that is requires expensive, complex equipment. For general utility, a low resolution (1/600) mass spectrometric method is attractive.

A partial spectrum (Figure 8) shows the nature of a measurement for a polynuclear aromatic mixture. Monoisotopic peaks are shown for the mass number ranges 188-190 and 220-228. Individual peaks are identified by molecular weight.

Compound(s) in a given series start at the lowest number consistent with the molecular weight of the parent compound(s) and occur at intervals of fourteen mass units in ascending value. Successive substitution of methyl, dimethyl or ethyl, and higher alkyls account for the regular increase of fourteen mass units. Phenanthrenes occur as the series 178, 192, 206, etc., and pyrenes and fluoranthenes at 202, 216, 230, etc. Based on this behavior, a spectrum provides additional insight into identifications. For example, the spectrum in Figure 8 shows an intense peak at 202 and a weak 14 units lower, which identifies the 202 as the parent peak of pyrene and/or fluoranthene. Another series, benzo-(g,h,i)fluoranthenes (mol wt 226), starts at 226, and the much lower peak at 212 indicates that the first member of this series does indeed start at MW 226. Another series starts at MW 228 (benzanthracenes, etc.) although a much smaller peak at 214 indicates a small amount of another class of aromatics.

Cyclopentaphenanthrenes are handled as if their lowest molecular weight is 190 and this is consistent with a small 176 peak that is observed but which is not shown in Figure 8.

The sample to be analyzed by mass spectrometer is the aromatic fraction from the chromatographic separation in the alumina column. Oxygenated compounds are present in the original sample, and even though they largely are separated into a polar fraction, there is a likelihood that some would also occur in the aromatic fraction. There was concern that such oxy's might be present so as to subsequently be measured along with PNA's.

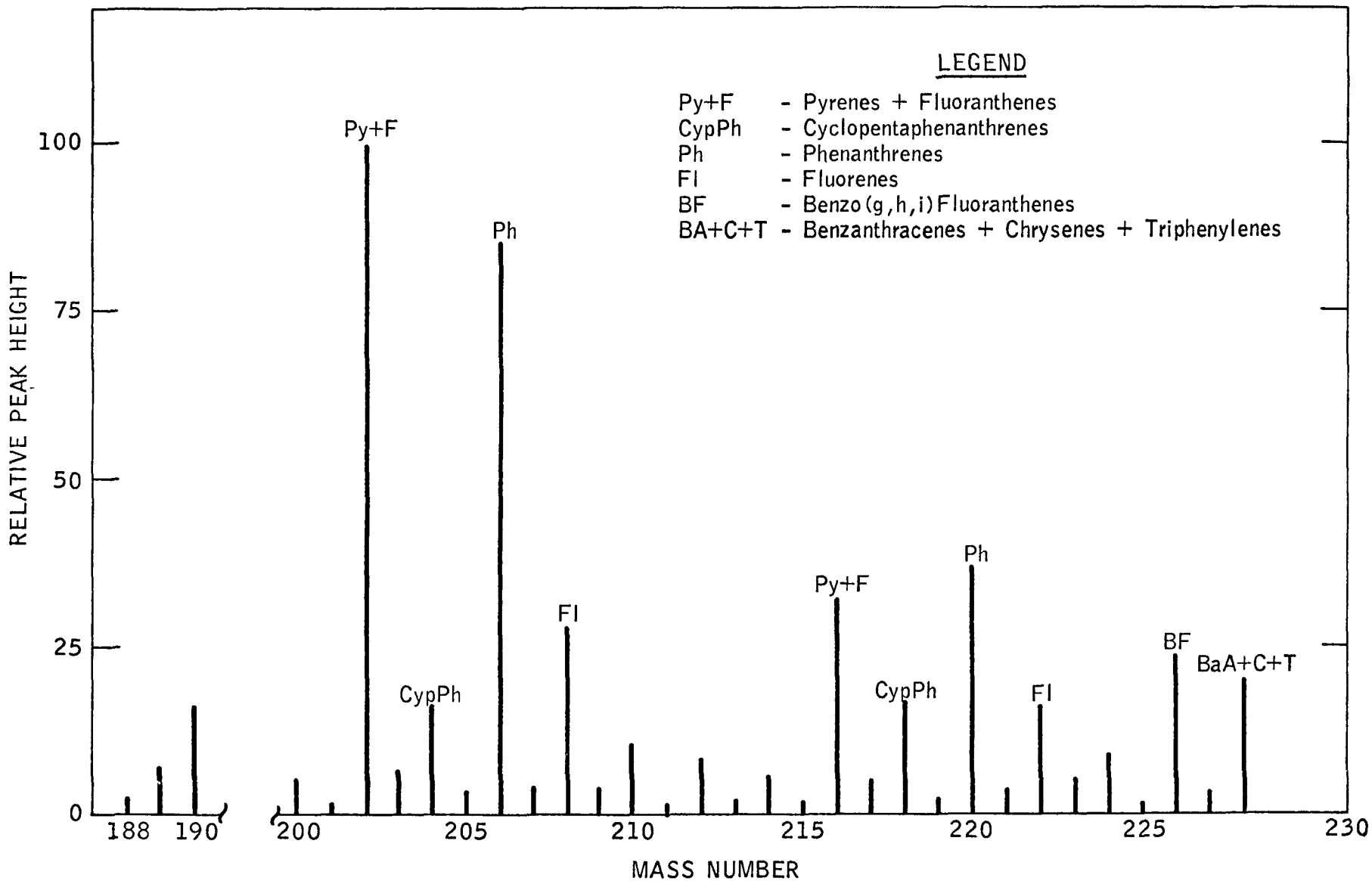
In order to evaluate this source of error, a typical PNA concentrate from a CAPE-6 run was analyzed by high resolution mass spectrometry[a]. The analysis showed that oxygenates were absent in the spectral region of interest for this method.

(10) H. E. Lumpkin, Thomas Aczel, Anal. Chem. 36, 181 (1964).

a = Analysis was done by H. E. Lumpkin, Esso Research and Engineering Company, Baytown, Texas.

Figure 8

PARTIAL LOW VOLTAGE SPECTRUM OF PNA FRACTION



Procedure for Mass Spectrometer Analysis

A sample to be analyzed is spiked with a known quantity (40 micrograms) of triphenylbenzene as the internal standard. The sample is then charged to the alumina column and handled the same way as for preparing the PNA concentrate for GC. Three μl of this sample is charged to the mass spectrometer.

A Model 21-102 mass spectrometer manufactured by Consolidated Electrodynamics Corporation was used. This instrument is equipped with an all-glass sample inlet system that is maintained at a temperature of 316°C. It was important to demonstrate that typical compounds could be quantitatively handled by the spectrometer. This implies the ability to quantitatively introduce a sample to the inlet system, measure its spectrum, and pump out without decomposition occurring.

Investigation of important variables had been done previously in some other work. This previous work showed that hydrocarbons were stable but that benzanthrone, an oxygenated aromatic, does decompose at certain conditions. Even though we were not concerned with this type of compound, we used operating conditions for its stability. This involved operating at a lowered ion source temperature. In all of our work the ion source was operated at a n-hexadecane cracking pattern of $\frac{m/e\ 127}{m/e\ 226} \sim 0.8$. This was obtained by running at a relatively low temperature in the ion source. This is in contrast to that associated with a cracking pattern of 1.40 obtained at higher temperatures.

At these conditions, some partially hydrogenated compounds behaved normally (9,10-dihydrophenanthrene, dihydropyrene); others were found to partially decompose, as also reported by Shultz (11). Decomposition occurred for 9,10-dihydroanthracene and 3-methylcholanthrene. In agreement with Shultz, we found that decomposition of these compounds was greatly reduced at sample bottle temperature of 260°C, as compared with 316°C. The lower temperature was not put to practice, however, because an actual sample of tar analyzed at 316°C and 260°C gave similar compositional results. Vulnerable compounds were thereby shown to be absent or present at an insignificant level.

Calibration

Low voltage measurements are based on the intensities observed for molecular or parent ions. For quantitative work it is necessary to calibrate the mass spectrometer to establish peak intensities per unit weight, mol or volume. We chose to measure peak intensity per microgram (sensitivity) of each compound. Triphenylbenzene was used as an internal standard with each calibration blend. In the calibration step, therefore, the sensitivity of individual compounds was measured on an absolute basis, and also in comparison with triphenylbenzene. Sensitivities were observed by examining blends of 4-5 compounds, each at a known concentration of approximately one milligram per milliliter of solution.

(11) J. L. Shultz, Spectroscopy Letters, 1 (8 & 9), 345 (1968).

Compounds for each blend were selected to be of different molecular weights so that each compound could be separately observed. Toluene was the solvent used for 3-5 ring compounds, but 6+ ring compounds required pyridine solvent.

Samples of 1-3 microliters are introduced with the sintered disk at 40°C. This temperature is then raised to 316°C prior to running.

More than thirty calibration blends were run in duplicate. Results for a typical blend are tabulated in Table XI.

Table XI

Calibration of a Typical Blend (# LV-21) of PNA's

Compound	Concentration (mg/ml)	Mol. Wt.	Purity[a] (%)	Sensitivity (PkHt/μg)
2-Methylphenanthrene	2.03	192	100	111, 123
2,3-Dihydro-1H-cyclo-penta [1] phenanthrene	2.08	218	84	86, 94
BaP	2.00	252	100	62, 67
Dibenz (a, h) anthracene	2.08	278	100	54, 58
Triphenylbenzene	1.96	206	100	42, 46

a = Purity was measured by GC and MS.

Sensitivities were measured for fifty-three compounds as tabulated in Table XII. Each sensitivity value represents the average of duplicate measurements. Replicate measurements are based on a given compound being present in different blends. 2-methyl pyrene, for example, was measured in three different blends.

Based on sensitivities from Table XII, average sensitivities were estimated for groups of compounds having identical empirical formulae, viz., phenanthrenes and anthracenes of the formula, C_nH_{2n-18}. Parent compound sensitivities were first calculated and then estimates were made for the alkyl-substituted isomers. When available, knowledge of composition was employed to obtain weighted averages. For example, in auto exhaust and in gasoline, phenanthrenes occur in greater abundances than anthracene. Thus, the average for this group was weighted in the direction of phenanthrenes. Values as used from Table XII are:

Phenanthrene = 138
Anthracene = 126

Mean = 132

Instead of the mean of 132, a value of 134 was selected.

Table XII

Low Voltage Sensitivities of 3-7 Ring PNA Hydrocarbons

Compound	Mol Wt	Sensitivity (mm/μg)
Phenanthrenes, Anthracenes		
Phenanthrene	178	138
1-Methyl Phenanthrene	192	106
2-Methyl Phenanthrene	192	133
9,10-Dimethyl Phenanthrene	206	115
Anthracene	178	126, 127
2-Methyl Anthracene	192	78, 95
9-Methyl Anthracene	192	136
2,6-Dimethyl Anthracene	206	128, 131
2,7-Dimethyl Anthracene	206	113
Fluorenes, Others		
Fluorene	166	84
2-Methyl Fluorene	180	121
9-Methyl Fluorene	180	97
9,10-Dihydrophenanthrene	180	93
9,10-Dihydroanthracene	180	69
Pyrenes, Fluoranthenes, Benzofluorenes		
Pyrene	202	91, 101
1-Methyl Pyrene	216	97
2-Methyl Pyrene	216	85, 79, 74
4-Methyl Pyrene	216	106
1,9-Dimethyl Pyrene	230	95
Fluoranthene	202	80
5H-Benzo(a)fluorene	216	75
11H-Benzo(b)fluorene	216	58
Cyclopentaphenanthrenes, Other		
Dihydropyrene	204	90
2,3-Dihydro-1H-cyclopenta[l]phenanthrene	218	102
Benzo(g,h,i)fluoranthenes, Cholanthrenes		
Benzo(g,h,i)fluoranthene	226	61
Cholanthrene	254	--
3-Methyl Cholanthrene	268	63
4-Methyl Cholanthrene	268	52

(continued)

Table XII (continued)

Compound	Mol Wt	Sensitivity (mm/μg)
Benzanthracenes, Chrysenes, Triphenylenes		
BaA	228	93
7-Methyl BaA	242	73
8-Methyl BaA	242	76
12-Methyl BaA	242	90
7,12-Dimethyl BaA	256	71, 75
Chrysene	228	99
Triphenylene	228	68
Benzo(c)phenanthrene	228	79
Benzopyrenes, Benzofluoranthenes		
Benzo(a)pyrene	252	81
Benzo(e)pyrene	252	76
Perylene	252	67
Benzo(b)fluoranthene	252	62
Benzo(k)fluoranthene	252	--
Benzoperylenes, Anthanthrenes		
Benzo(g,h,i)perylene	276	63
Anthanthrene	276	26
Dibenzanthracenes		
Dibenz(a,h)anthracene	278	63, 62
Dibenz(a,e)anthracene	278	57
3,4,5,6-Dibenzophenthrene	278	58
Picene	278	51
3,4-Benztetraphene	278	61
Pentacene	278	-a-
Coronenes		
Coronene	300	48
Dibenzopyrenes		
Dibenzo(a,e)pyrene	302	42
Dibenzo(a,h)pyrene	302	38
Dibenzo(a,i)pyrene	302	36
2,3,4,5-Dibenzopyrene	302	40
1-Methyl-2,3,7,8-dibenzopyrene	316	-a-
5-Methyl-3,4,8,7-dibenzopyrene	316	-a-

- - - - - - - - - - - - -

-a- - No or slight vaporization in GC and MS.

Sensitivities for alkyl-substituted compounds were estimated from the sensitivity behavior of methyl and dimethyl isomers of anthracene, phenanthrene, pyrene, and benz(a)anthracene. This is summarized below in terms of relative values (Table XIII).

Table XIII

Relative Low Voltage Sensitivities

	Phenanthrenes Anthracenes	Pyrenes	Benz(a)Anthracenes	Mean
Parent	1.00	1.00	1.00	1.00
Methyl Subst.	0.93	0.98	0.90	0.94
Dimethyl	0.91	0.98	0.79	0.90

This trend of diminishing sensitivity with increased substitution on the ring is common to simpler aromatics. Ratios of 1.00, 0.94, 0.90, 0.88, 0.85, and 0.82 were used to represent compounds ranging, successively, from the parent compound to its alkyl substituted form of up to 5 carbon atoms. Applied to the pyrene, fluoranthene series this refers to compounds of molecular weights: 202, 216, 230, 244, 258. Both pyrene and fluoranthene have a molecular weight of 202. A methyl-substituted pyrene has a molecular weight of 216, a dimethyl or ethyl pyrene is 230, etc.

Average sensitivity data for the method are shown in Appendix B.

In the calculation of the mass spectrum, monoisotopic peaks are first obtained and then, based on the sensitivities supplied in the method, microgram quantities are calculated. Quantities on an original sample basis are obtained by proportioning the amount of triphenylbenzene found in the mass spectrometer to the forty micrograms initially added.

Calculation

Peaks are read from a chart or selected from a digitizer/computer printout. All peaks in the m/e range, 178-330, are recorded.

- Calculate monoisotopic peaks for all even m/e numbers.
- Calculate the triphenylbenzene peak at m/e 306 by correcting for sample background as follows:

m/e	Peak Height
292	117.0
306	266.1
320	70.1

$$\text{Triphenylbenzene peak (M/e 306)} = 266.1 - \left[(117.0 + 70.0)/2\right] = 172.6.$$

- Divide monoisotopic peaks by appropriate sensitivity to obtain micrograms of each mol wt species in the sample.
- Based on the internal standard, triphenylbenzene, convert all microgram quantities to an original sample basis.

Calculation of a partial spectrum is outlined in Table XIV.

Table XIV

Partial Calculation of a Sample

Type of Compound	Mol Wt (m/e)	Peak Height	Sensitivity	Micrograms (Peak ÷ Sensitivity)	Micrograms in Sample
Pyrenes, Fluoranthenes	202	4150	88	47.1	540
	216	2262	86	26.3	300
	230	1066	80	13.4	153
	244	596	78	7.6	87
	258	353	75	4.7	54

This illustrated calculation covers the group, pyrenes and fluoranthenes, for MW's from 202 to 258. Micrograms of each MW species are shown in column 5; for example, 4150 ÷ 88 = 47.1 micrograms. The quantity of internal standard (triphenylbenzene) is calculated.

172.6 (peak height) ÷ 50 (sensitivity) = 3.5

Since 40 micrograms of triphynylbenzene was originally added to the sample, the micrograms by MW (column 5) can be converted to a total sample basis by multiplying each quantity by 40 ÷ 3.5, or 11.4.

Application of the Method

Two samples, X and Y, were analyzed as shown in Table XV. Including the tricyclics, eleven groups of compounds are reported and several of these include different molecular structures. For example, one group includes benzanthracenes, chrysenes, and tripheylenes. If we count molecular structure variations, the number is eighteen. This includes 3- to 7-ring structures.

In addition to unsubstituted PNA's, many alky-substituted PNA's are present in auto tar. The parent compounds represent the principal isomer in each group, although the sum total of substituted compounds generally exceeds that of the parent compound itself.

In the GC/UV analysis of sample Y, BaA was found to be 174 μg, and BaP was 73 μg. Considering that this sample contained 18,294 μg of 4+ ring PNA hydrocarbons, BaA represented only 0.95% and BaP was only 0.4% of the total PNA content.

Table XV

Total PNA's in Tar from Auto Exhaust
For Samples X and Y

Phenanthrenes			Fluorenes, Dihydrophenanthrenes			Pyrenes, Fluoranthenes, Etc.			Cyclopenta-Phenanthrenes			Benzo(g,h,i)-fluoranthenes		
Mol Wt	X	Y	Mol Wt	X	Y	Mol Wt	X	Y	Mol Wt	X	Y	Mol Wt	X	Y
Micrograms														
178	3,620	5,060	180	2,800	6,665	202	1,630	2,725	204	590	1,040	226	645	640
192	5,075	12,990	194	2,580	5,265	216	1,095	1,810	218	940	1,520	240	310	305
206	2,325	5,340	208	1,255	2,660	230	860	1,035	232	780	1,020	254	270	245
220	1,350	2,345	222	770	1,230	244	480	555	246	475	570	268	220	240
234	575	750	236	410	490	258	310	300	260	265	270	282	125	195
248	90	300	250	210	225	272	130	110	274	135	165			
													1,620	1,645
	13,035	26,785		8,025	16,535		4,505	6,535		3,185	4,585			

Benzanthracenes, Chrysenes, Triphenylenes			Benzopyrenes Benzofluoranthenes			Benzoperylenes Anthanthrenes			Dibenzanthracenes			Coronenes			Dibenzopyrenes		
Mol Wt	X	Y	Mol Wt	X	Y	Mol Wt	X	Y	Mol Wt	X	Y	Mol Wt	X	Y	Mol Wt	X	Y
Micrograms																	
228	550	585	252	845	920	276	660	595	278	195	160	300	265	125	302	180	125
242	460	540	266	475	520	290	215	175	292	165	135	314	85	80	316	100	100
256	280	330	280	245	260	304	110	110	306	100	100	328	65	70	330	70	80
270	135	155	294	125	125	318	75	85	328	60	70						
284	75	85											415	275		350	305
				1,690	1,825		1,060	965		520	465						
	1,500	1,695															

METHOD FOR PHENOLS IN AUTOMOBILE EXHAUST GAS

The internal standard method described in this report was developed as the last part of the CAPE-12-68 project. Due to lack of funds, the procedure was not thoroughly tested. Limited data indicate that it adequately handles an aqueous sample. Since most of the phenols in automobile testing occur in the water condensate, the method satisfies most of the needs in this kind of work. Furthermore, the method can be extended directly to measure phenols in hydrocabon samples.

For an aqueous condensate sample of 25 ml, sensitivity of the method is 1 μg (40 ppb). Since CAPE-6 tests generally gave 5 liters of the condensate, it is possible to measure much lower quantities of a phenol by using a larger starting sample.

Description of Method

A known amount of an internal standard, o-chlorophenol, is added to the original sample. Neutral compounds are removed by extraction of the alkaline sample. The extract is acidified, saturated with sodium sulfate, and the phenols are extracted by five successive portions of ethyl ether. Organic acids are then removed by a sodium bicarbonate extraction. The ether is distilled in a special concentrator leaving a small residue which is injected into a capillary column gas chromatograph. The recorded peaks are identified by retention time. Peak areas are measured and multiplied by the appropriate factors to convert the area to weight.

Preparation of Sample for Gas Chromatograph

To prepare a sample for the gas chromatograph, the phenols are separated from the aqueous sample and then purified by a classical solvent extraction technique. A similar separation procedure was evaluated by Hoffman and Wynder (4) in their study on tobacco smoke. In one step, these workers separate the phenols from impurities by steam distillation. This causes a five-fold increase in volume and was found to be unnecessary in the present work.

After adding 0.8 mgs of o-chlorophenol, the internal standard, to 25 ml of the aqueous condensate and making it alkaline, the neutral compounds are removed by ethyl ether extraction. The aqueous phase is then acidified, saturated with sodium sulfate and extracted five times with ether to isolate the phenols. Any organic acids in the aqueous condensate are extracted along with the phenols. They are removed from the combined ether solution by extracting with a small volume of saturated sodium bicarbonate. Finally, dissolved water is removed by anhydrous sodium sulfate. Ether is distilled off in a "concentrator" and a phenol-rich residue gathers in a depression of the glass still pot. Using a medicine dropper the residue is transferred to a small vial and 10 drops of methanol added. This volume is reduced to 0.2 ml. An aliquot of this sample is injected to the gas chromatograph for the phenols measurement.

Gas Chromatographic Separation of Phenols

As listed in Table XVI, a number of GC columns were evaluated prior to selecting a capillary column described by Hrivnak (5). Retention times and response factors of twenty-three phenols were obtained and are presented in Table XVI.

Table XVI

Analytical Data on Phenols

Source[a]	B.P.-°C	Compound	Minutes Past Injection	Flame Relative Sensitivity[b]
E	80	Benzene	2.76	1.12
E	176	o-Chlorophenol	7.40	0.45
P	182	Phenol	9.5	0.61
P	192	o-Cresol	12.2	0.66
P	203	m-Cresol	15.7	0.60
P	207	o-Ethyl phenol	18.6	0.63
A	218	m-Ethyl phenol	25.8	0.61
P	219	p-Ethyl phenol	24.8	0.60
P	218	2,3-Xylenol	24.2	0.62
P	212	2,4-Xylenol	19.8	0.60
P	242	2,5-Xylenol	20.5	0.68
P	212	2,6-Xylenol	13.0	0.68
P	219	3,5-Xylenol	28.0	0.69
P	225	3,4-Xylenol	29.5	0.61
N		m-Isopropylphenol	35.5	0.64
A	220	m-n-Propylphenol	29.1	0.70
N	229	p-Isopropylphenol	42.2	0.56
P	236	2,3,5-Trimethylphenol	41.6	0.60
A	220	2,4,6- "	22.0	0.62
A	249	3,4,5- "	63.0	--
A	247	2,3,5,6-Tetramethylphenol	51.6	0.47
K	242	Indol-4	56.1	0.66
CS	255	Indol-5	70.5	0.59
CS		3-tert. Butylphenol	52.5	--

(a) E Eastman Kodak Co., Rochester, N.Y.
A Aldrich Chemical Co., Inc., Milwaukee, Wisconsin
N Newton Maine Res. Chem. Co., England
CS Chemical Samples Co., Columbus, Ohio
K K&K Chemical Co., New York, N.Y.
P Polyscience Corporation, Evanston, Illinois

(b) For flame ionization detector, divide area by relative sensitivity to get corrected area. Normalize corrected areas to get weight percent.

In brief, relative sensitivities were measured by preparing weighed blends of selected compounds with a known internal standard (one of the C_8 aromatics). In many cases, the relative sensitivity values are the average of twelve determinations, involving four or more blends. The values are estimated to be accurate within ± 3 relative %. These calibration data are also reported elsewhere (12).

Table XVII

GC Columns for Separation of Phenols

Column Substrate	Type	Support	Column Length, ft.	Diameter	Temp. -°C
Didecylphthalate + 10% H_3PO_4	Capillary	Chromo G	150	0.01", i.d.	120
2% Didecylphthalate + 0.2% H_3PO_4	Packed	Chromo T	6	0.125" o.d.	125
2% Didecylphthalate + 2% Bentone 34	"	"	"	"	"
10 wt.% FFAP (free fatty acid phase)	"	"	10	"	225
Chromosorb 102	"	Chromo 102	4	"	150
Chromosorb 104	"	Chromo 104	"	"	200

Application of the Method

The method was first evaluated with a synthetic blend containing nine phenols. Results are presented in Table XVIII and the chromatogram is reproduced in Figure 9.

Table XVIII

Analysis of Phenol Synthetic

Retention Time	Phenol	ppm Known	ppm Found
8.0	Chlorophenol	Int. Std.	--
10.2	Phenol	32.5	32.0
13.1	o-Cresol	11.2	12.9
14.0	2,6-Xylenol	10.8	10.8
15.8	p-Cresol	12.4	13.6
16.4	m-Cresol	12.4	13.3
20.8	2,4-Xylenol	10.8	9.2
21.3	2,5-Xylenol	11.2	11.2
25.8	p-Ethyl Phenol	10.8	11.6
20.8	3,4-Xylenol	10.8	12.0

(12) W. A. Dietz, J. Chromatog. Sci. 10, 423 (1972)

A water condensate from a CAPE-6 test was analyzed as shown in Table XIX. Figure 10 is the chromatogram of the sample. The sum of the individual phenols is 141 ppm compared with 125 ppm as found by the routine method for measuring total phenols.

Table XIX

Phenol Content of Aqueous Condensate, Commercial Fuel

Phenol	Amount, ppm
Phenol	101.0
o-Cresol	9.0
P-Cresol	15.0
m-Cresol	12.0
2,6 Xylenol	0.8
2,4 Xylenol	0.8
2,5 Xylenol	0.4
3,5 Xylenol	0.5
3,4 Xylenol	0.5
o-Ethyl Phenol	0.7
Total By GC	141
Total by UV	125

FIGURE 9

NINE COMPONENT BLEND TAKEN THROUGH EXTRACTION PROCEDURE

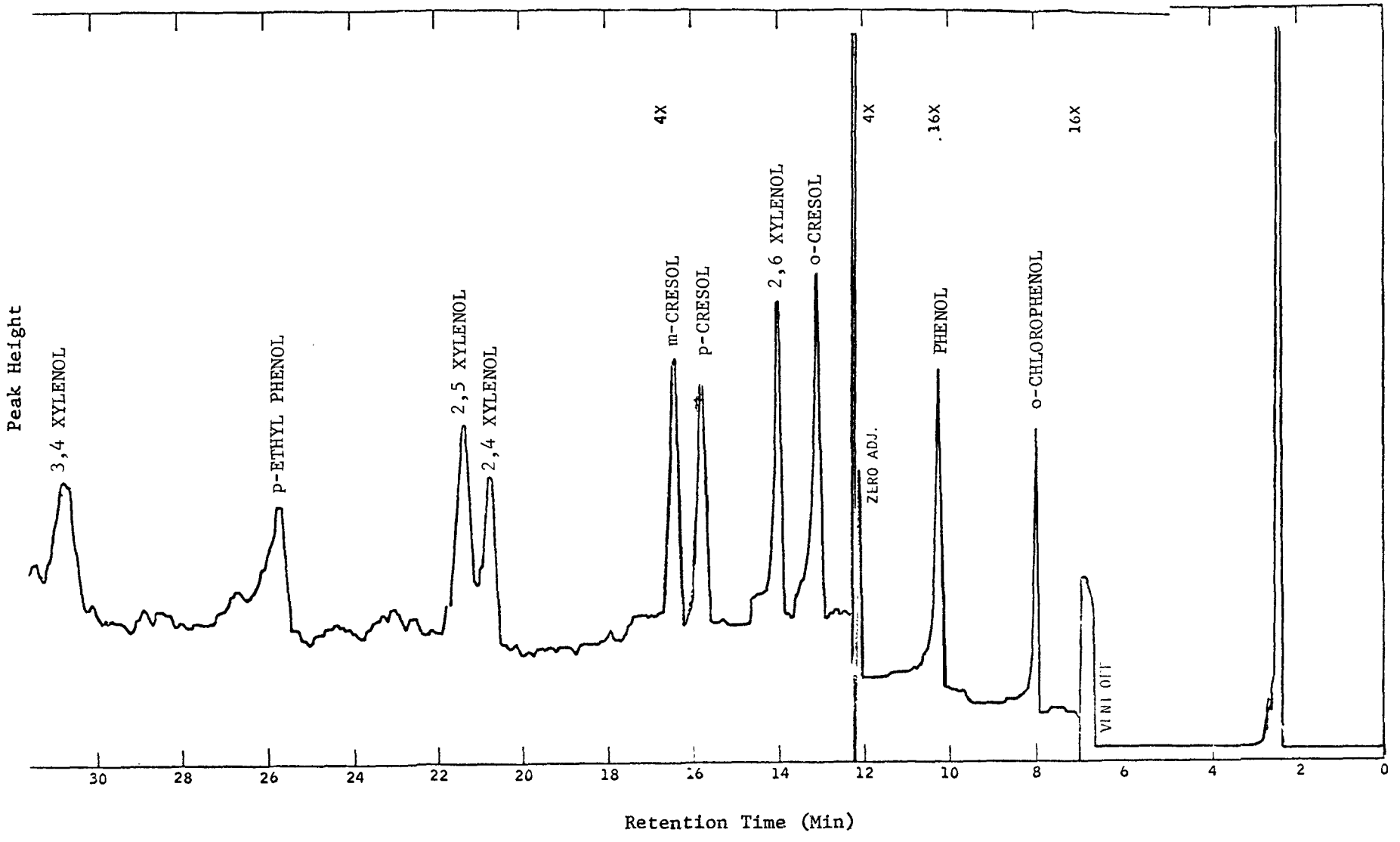
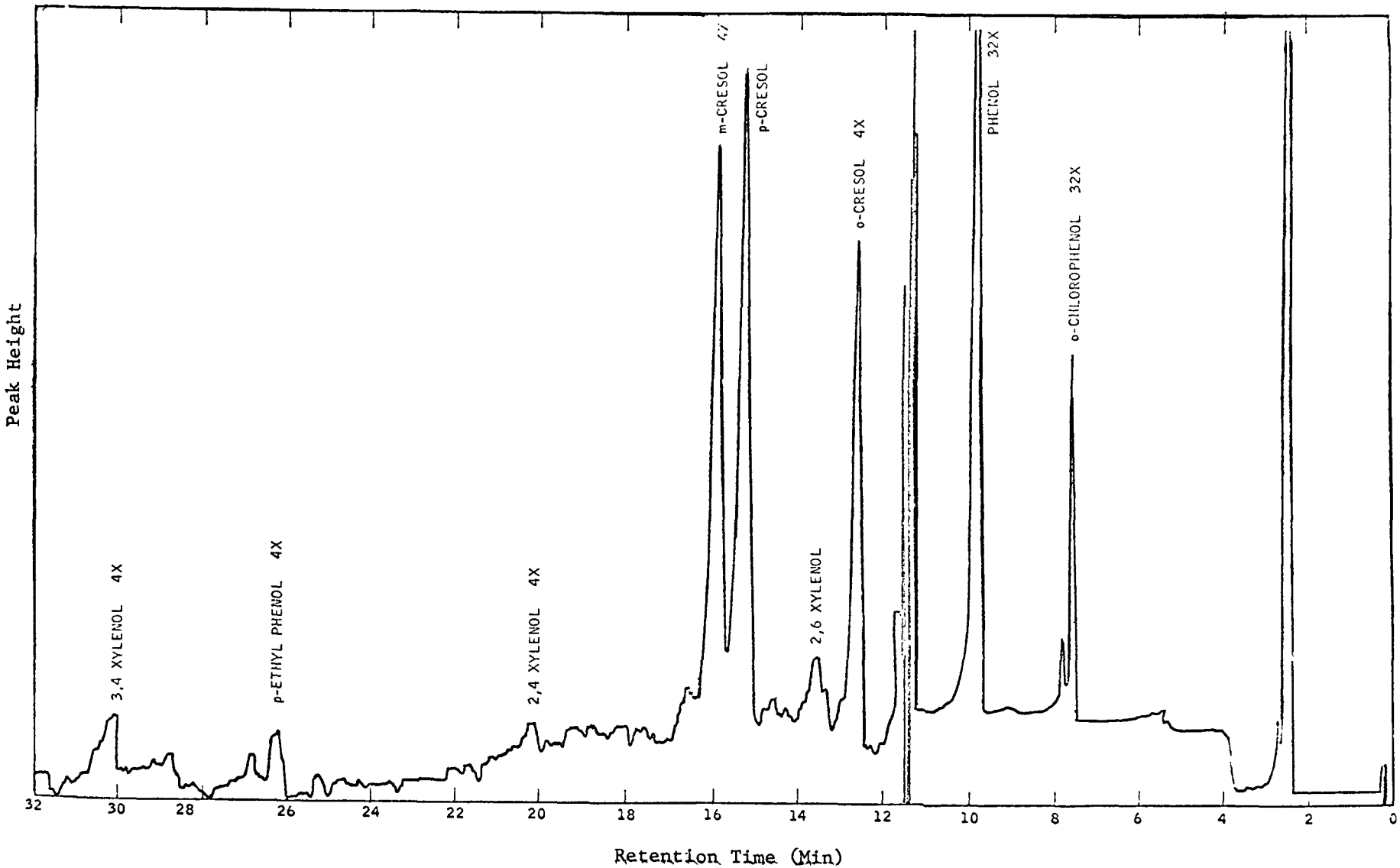


FIGURE 10

CHROMATOGRAM FROM AQUEOUS CONDENSATE



APPENDIX A

Method for Benz(a)anthracene, Benzo(a)pyrene, And Other Polynuclear Aromatic Hydrocarbons in Automobile Exhaust, Gasoline and Crankcase Oil

Introduction

This method was developed to determine polynuclear aromatic hydrocarbons in gasoline, crankcase oil, and tar from auto exhaust.

Scope[a]

The method covers polynuclear aromatics (PNA) ranging from pyrene to benzo(g,h,i)perylene. As described herein, eleven PNA's are measured, including: pyrene, benz(a)anthracene, chrysene, triphenylene, methylbenz-(a)anthracene, dimethyl and/or ethylbenz(a)anthracene, benzo(a)pyrene benzo(e)pyrene, methylbenzo(a)pyrenes, methylbenzo(e)pyrenes and benzo(g,h,i)-perylene. Additional PNA's can be included. The procedure was successfully demonstrated for gasoline, exhaust tars and crankcase oils.

Summary

A sample to be analyzed is spiked with known quantities of carbon-14 labeled benzo(a)anthracene (BaA) and benzo(a)pyrene (BaP). Some polar compounds are removed by a caustic treat[b] and then a PNA hydrocarbon concentrate is obtained by solvent elution off a column of partially deactivated alumina. The solvents are cyclohexane, cyclohexane-benzene, benzene, and benzene-methanol. The fraction containing the PNA's is reduced to a small volume by evaporation on a steam bath. An aliquot of this sample is injected into a gas chromatograph and fractions are collected for measurement by UV and, in the case of BaA and BaP peaks, also for carbon-14 activity. These activities, compared with known concentrations originally added, give factors by which to relate the concentrations of each PNA to its total weight in the sample.

Apparatus & Isotope Dilution

1. The Gas Chromatograph is equipped with a flame ionization detector, a linear temperature programmer and a flow controller. The injection port consisted of an aluminum block with a stainless steel tube which slides into the block. A quartz sleeve fits inside of the stainless steel tube. The chromatograph is modified by the addition of a by-pass and trap so that 15% of the column effluent will go to the detector and 85% to the trap. This arrangement is shown in Figure 3 (p.9). A Perkin-Elmer 900 chromatograph was used in the development of this method, but equivalent instruments should perform satisfactorily.

a = At the time of this report, work is in progress to also include fluoranthene, benzo(b)fluoranthene, benzo(j)fluoranthene, benzo(k)fluoranthene, perylene, and coronene.

b = The caustic treat is done on tar samples only.

2. Recorder, 0-1 millivolt, 2 min./inch chart speed.

3. Trapping Tubes, 20 or more stainless steel tubes, 18 cm long by 0.32 cm diameter.

4. Column, 300 cm of .22 cm I.D. stainless steel tubing packed with 2% SE-30 (GC Grade) on Chromosorb G (acid washed and DMCS treated), 80/100 mesh.

5. Syringe, 10 microliter.

6. Spectrophotometer, spectral range 225-400 nanometers, with spectral slit width of 2 nm or less. Under instrument operating conditions for these absorbance measurements, the spectrophotometer shall also meet the following performance requirements: absorbance repeatability, ± 0.01 at 0.4 absorbance; absorbance accuracy, ± 0.05 at 0.4 absorbance; wavelength repeatability, ± 0.2 nm; wavelength accuracy, ± 1.0 nm.

7. Spectrophotometric Cells, fused quartz cells having optical path lengths of 1.000± 0.005 cm and 5 cm cells. The 5-cm cells are microcells and contain about 3 ml of solution.

8. Graduate Glass, 5 ml.

9. Vials, one- and three-dram with cap.

10. Steam Bath equipped with nitrogen outlet for purging vials.

11. Medicine Dropper, with tip drawn out.

12. Special Low UV Emission Room Lights, Westinghouse W-40-gold fluorescent lamps.

13. Chromatographic Column, two feet long 0.5 inch OD and 0.43 inch ID with 300 ml receiving bulb on top and stopcock with Teflon barrel on the bottom.

14. Radiation Counters used are Intertechnique SL-20 and Packard Tri-Carb.

15. Counting Vials, 20 ml with cap.

Reagent and Materials

Reagent grade chemicals must be used throughout. Benzo(a)pyrene was repurified but other polynuclear compounds were used as purchased. Compounds of comparable purity from other suppliers may be employed.

1. Cyclohexane. As a large amount of cyclohexane is used, it must be of highest purity. To check purity, evaporate 180 ml down to 5 ml. Run a UV scan on this residue in a one-cm cell from 280-400 nm. The absorbance should

not exceed 0.01 units. To purify, percolate through activated silica gel, Grade 12, in a glass column, 90 cm long and 5-8 cm diameter. "Distilled in Glass Solvents" are generally of suitable purity.

2. Toluene.

3. Acetone.

4. Sodium hydroxide.

5. Hydrochloric Acid.

6. Benzene B&J.

7. Methanol B&J.

8. Benz(a)anthracene $C_{18}H_{12}$, MW 228, Eastman 4672.

9. Benzo(a)pyrene $C_{20}H_{12}$, MW 252, Eastman 4951.

10. Pyrene $C_{16}H_{10}$, MW 202, Eastman 3627.

11. Benzo(g, h, i)perylene $C_{22}H_{12}$, MW 276, Columbia Organic.

12. Test Blend.

CAUTION

Exercise care when handling these compounds to avoid inhaling them or getting them on the skin. Wash hands thoroughly after handling.

Weigh exactly 25.0 mgs of each of the four compounds listed as Items 8 through 11. Place in a 25-ml volumetric flask, add 20 ml of toluene and swirl until compounds are in solution. Then make up to volume. This will contain 1 microgram of each compound per microliter of solution. Pour into a small, narrow neck brown bottle and keep in a cool, dark place, preferably a refrigerator.

13. Organic Counting Solution, 8 gm of BBOT in a liter of toluene.

14. ^{14}C benz(a)anthracene, ^{14}C benzo(a)pyrene, Mallinkrodt Chemical Works.

Method

Precautionary Note: Because of the sensitivity of the test, the possibility of errors arising from contamination is great. It is of the greatest importance that all glassware be scrupulously cleaned to remove all organic matter such as oil, grease, detergent residues, etc. Examine all glassware, including stoppers and stopcocks, under ultraviolet light to detect any residual fluorescent contamination. As a precautionary measure it is recommended practice to rinse all glassware with purified isooctane immediately before use. No grease is to be used on stopcocks or joints. Great care to avoid contamination of samples in handling and to assure absence of any extraneous material arising from inadequate packaging or storing is essential. Because some of the polynuclear hydrocarbons sought in this test are very susceptible to photooxidation, the entire procedure is to be carried out under subdued light. Avoid use of fluorescent lamps. Special yellow lights are available. Tygon tubing must absolutely be avoided to eliminate di-isooctyl phthalate (DOP) contamination which interferes in both the GC and UV.

Radioactive Materials must be handled and disposed of by accepted methods. Glassware should be rinsed with chloroform-acetone solvent and washed in dichromate-sulfuric acid cleaning solution.

A. Sample Preparation

1. Distillation of Tar Samples

Before commencing the distillation prepare the radioactive BaA and BaP spikes. Add 50 λ of BaA to a 25 ml. volumetric flask and 50 λ of BaP to a second. Make up to volume with cyclohexane and remove duplicate 100 λ aliquots from each for counting. Pour the contents of the flasks into the still and rinse out well with cyclohexane and acetone. Conduct the distillation as described elsewhere (1A).

2. Gasoline

The volume of starting sample is varied according to the level of PNA's. PNA-rich gasolines can be analyzed by using 50 ml and spiking with 50 λ each of ^{14}C labeled BaA and BaP. For gasolines of low PNA content, 1000 ml of sample is spiked with 10 λ each of the BaA and BaP internal standards.

3. Used Crankcase Oil

A weighed amount of sample (~ 500 mg) is made up to 25 ml of cyclohexane which has been previously spiked with 10 λ each of carbon-14 labeled BaA and BaP.

(1A) G. P. Gross, "Gasoline Composition and Vehicle Exhaust Gas Polynuclear Aromatic Content," U.S. Clearinghouse Federal Science Technology Information, PB Rep. Issue No. 200266 (1971) 124 pp.

B. Caustic Extraction (Tar Samples)

1. The volume of still bottoms will be about one liter. Take exactly one-half of this and place in a one-liter separatory funnel.

2. Add 50 ml of 0.5 N aqueous sodium hydroxide solution, shake one minute, and remove lower aqueous phase.

3. Repeat the extractions with 30 and 20 ml portions of sodium hydroxide.

4. Wash with 50 ml of water, then 50 ml of 1/10 N HCl.

5. Finally, wash with 25 ml of water.

6. Evaporate cyclohexane to about 150 ml and filter, if necessary.

7. Evaporate filtrate to below 50 ml, place in a 50 ml volumetric flask and make up to volume with cyclohexane.

8. Take 25 ml for placing on the alumina chromatographic column.

C. Column Chromatographic Separation

1. Seventy-five grams of Woelm Neutral alumina is dried for one hour at 150°C. The alumina is transferred to a bottle and allowed to reach room temperature. 1.5 ml of water are added dropwise with shaking. The bottle is capped and placed on a paint shaker for 15 minutes to obtain a uniform mixture.

2. A glass wool plug is placed in the end of the chromatographic column and the alumina is poured in along with gentle tapping to pack the alumina. The column is filled to within one inch of the top and another glass wool plug is placed on top.

3. Ten ml of cyclohexane are placed on top of the column and allowed to run into the column under nitrogen pressure of 2 pounds. The sample is then poured into the 200 ml bulb on top and allowed to run into the column. 90 ml cyclohexane is added to the bulb.

4. When the cyclohexane has run into the column below the top glass wool plug, 100 ml of cyclohexane benzene (4:1) are added and run into the column. The first 100 ml of cyclohexane is collected and set aside. The following solutions are collected in 3-dram vials and capped. Each should contain 10 ml of solution.

5. After the cyclohexane/benzene (4:1) is below the top of the glass wool plug, 100 ml of benzene are added to the column. Collection of 10 ml fractions is continued.

Method

Precautionary Note: Because of the sensitivity of the test, the possibility of errors arising from contamination is great. It is of the greatest importance that all glassware be scrupulously cleaned to remove all organic matter such as oil, grease, detergent residues, etc. Examine all glassware, including stoppers and stopcocks, under ultraviolet light to detect any residual fluorescent contamination. As a precautionary measure it is recommended practice to rinse all glassware with purified isooctane immediately before use. No grease is to be used on stopcocks or joints. Great care to avoid contamination of samples in handling and to assure absence of any extraneous material arising from inadequate packaging or storing is essential. Because some of the polynuclear hydrocarbons sought in this test are very susceptible to photooxidation, the entire procedure is to be carried out under subdued light. Avoid use of fluorescent lamps. Special yellow lights are available. Tygon tubing must absolutely be avoided to eliminate di-isooctyl phthalate (DOP) contamination which interferes in both the GC and UV.

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2. Add 50 ml of 0.5 N aqueous sodium hydroxide solution, shake one minute, and remove lower aqueous phase.

3. Repeat the extractions with 30 and 20 ml portions of sodium hydroxide.

4. Wash with 50 ml of water, then 50 ml of 1/10 N HCl.

5. Finally, wash with 25 ml of water.

6. Evaporate cyclohexane to about 150 ml and filter, if necessary.

7. Evaporate filtrate to below 50 ml, place in a 50 ml volumetric flask and make up to volume with cyclohexane.

8. Take 25 ml for placing on the alumina chromatographic column.

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2. A glass wool plug is placed in the end of the chromatographic column and the alumina is poured in along with gentle tapping to pack the alumina. The column is filled to within one inch of the top and another glass wool plug is placed on top.

3. Ten ml of cyclohexane are placed on top of the column and allowed to run into the column under nitrogen pressure of 2 pounds. The sample is then poured into the 200 ml bulb on top and allowed to run into the column. 90 ml cyclohexane is added to the bulb.

4. When the cyclohexane has run into the column below the top glass wool plug, 100 ml of cyclohexane benzene (4:1) are added and run into the column. The first 100 ml of cyclohexane is collected and set aside. The following solutions are collected in 3-dram vials and capped. Each should contain 10 ml of solution.

5. After the cyclohexane/benzene (4:1) is below the top of the glass wool plug, 100 ml of benzene are added to the column. Collection of 10 ml fractions is continued.

6. After all the benzene has passed the top glass wool plug, 100 ml of benzene/methanol (1:1) is added to the column. The benzene/methanol front can usually be followed by the movement of an orange colored band. The front can also be detected by a warm front moving down the column which is apparent to the touch or it can be followed by a UV lamp. When the front reaches the end of the column, a two-phase system becomes visible in the collecting vial. At this point, the remaining solution is collected in one large bottle and allowed to run off the column (cut 3). The column should not be stopped or allowed to run dry between solvent additions.

7. The solutions in the individual three-dram vials are scanned in order of elution on a UV spectrophotometer. The peak at 340 nm is used as a guide in determining the start of elution of tetracyclic PNA compounds. The solution whose spectrum shows a 340 peak is the start and all remaining solutions are combined as the PNA fraction (cut #2).

D. Preparation of Concentrated PNA Solution

1. Transfer the contents of the first 4 vials of cut #2 to a 150 ml beaker and rinse each vial twice with 1 to 2 ml of cyclohexane and combine with the contents of the beaker.

2. Place the 150 ml beaker on the steam bath under a small jet of nitrogen.

3. As evaporation progresses, add the contents of the remaining vials with similar rinsing to the beaker. Do not evaporate to less than two mls and in no case let the contents go to dryness.

4. Transfer the concentrated solution to a one-dram vial. Wash beaker with cyclohexane and add to vial.

5. Place the vial in a 30 ml beaker for support. The beaker is placed on a steam bath under a gentle jet of nitrogen.

6. Rinse the 150 ml beaker with 3 or 4 small portions of cyclohexane (~1 ml) and combine with the contents of the one-dram vial as evaporation proceeds.

7. If, as is preferable, it is unnecessary to obtain the weight of the PNA residue, evaporate the solution down to about 50 µl (0.05 ml). Add about 0.5 ml of acetone and again evaporate to 50 µl.

8. If the weight of the residue must be obtained, exercise great care as the solution approaches dryness. Keep the vial under constant observation and the instant all the solvent has been removed, cool and weigh the vial.

9. Return the vial to the beaker under nitrogen for an additional minute, cool and reweigh. Repeat this procedure until a constant weight is reached. For the GC analysis add 10 to 20 microliters of acetone to lower the viscosity of the sample. Cap and save.

E. Gas Chromatographic Analysis

Parameters

The following parameters are employed in developing the gas chromatographic separation with the Perkin-Elmer 900. Other instruments might use different conditions.

Carrier Gas - Helium
Flow Rate - 30 ml./min.
Hydrogen and Air - at manufacturers recommendation, or optimum rates.
Injection Port - 300°C.
Detector - 340°C.
Program - 175°C to 300°C at 4° per minute. The temperature is held at 300° until all peaks are eluted, but in any case, for 20 minutes.

Column Conditioning

1. The Supelco Co. states that only minimum conditioning is needed on the column specified in (4) of apparatus. Connect the column to the inlet of the GC but not with the detector.

2. Pass helium through at 30 ml. per minute program at one degree per minute from 150°C to 275°C. Keep at 275 for 30 minutes. Cool and connect to the detector.

3. If SE 30 as supplied by the manufacturer is used in place of GC grade, connect as in (1). Purge with helium for 10 minutes and then put a cap on the exit end, keeping a helium pressure on the column.

4. Raise the oven to 350°C and bake for 3 hours.

5. Cool to room temperature, remove cap, pass helium through at 30 ml. per minute and continue conditioning column as shown below without attaching to the detector.

Temp., °C.	150	200	250	275
Time, Min.	30	30	30	60

6. Cool to room temperature and attach to detector. The column is now ready for use.

Performance Test

This test is used to measure retention times and demonstrate suitable recovery of individual PNA's. Recoveries of ≥ 83% are required except for benzo(g,h,i)perylene. For this compound, a recovery of ≥ 75% is satisfactory. With the column conditioned and the parameters set as described in 1, prepare to inject a sample.

7. Flush the 10 µl syringe twice with the test blend.

8. The third time fill the syringe and hold vertically point up. Stick the tip in a rubber septum and depress the plunger to compress air bubbles which rise to the top.

9. Now advance the plunger to the desired amount, for instance 2 µl; the rubber is removed, and the plunger retracted until the air-liquid meniscus enters the glass bore.

10. Note the volume from meniscus to plunger.

11. Insert the syringe up to the hilt in the GC injection port, depress the plunger. Start the recorder.

12. Remove the syringe from the port and retract the plunger.

13. Note the volume remaining (generally about 0.2 µl) and subtract from the previously noted volume to determine the net volume injected.

14. Have stainless steel trapping tubes and one-dram vials available. The vials should be supported in small holes drilled in a board. A plastic cup filled with ice surrounds the stainless steel insert.

15. As a peak approaches, insert a tube. Remove as the trace returns to the baseline. Mark the peak No. 1 and place the tube in a one-dram vial labeled 1.

16. Continue taking cuts, putting each tube in a separate vial labeled 2, 3, etc.

17. Typical retention times obtained in setting up this method are shown in Table IA.

Table IA

OBSERVED RETENTION TIMES [a]

Compound	Retention Time, Min.
Pyrene	13.6
Triphenylene	19.4
Benz(a)anthracene	19.4
Chrysene	19.6
Methylbenz(a)anthracenes	21.4
Dimethyl/ethylbenz(a)anthracenes	23.3
Benzo(e)pyrene	25.7
Benzo(a)pyrene	25.8
Methylbenzo(e)pyrenes[b]	) 27.5, 28.0, 29.0
Methylbenzo(a)pyrenes[b]	)
Benzo(g,h,i)perylene	31.4

a = Conditions given on page 40.
b = Measured in three different peaks.

18. When the run is completed take the first tube and place in a 5-ml graduate. With an elongated medicine dropper add enough cyclohexane [a] through the top of the tube to fill the spectrophotometric cell to be used. In developing this method, 3.8 ml was a convenient volume to employ, but all cuts must be made up to the same volume.

19. Pour the solution into the original vial, cap and protect from light. Rinse out the graduate with cyclohexane and use for the next tube. Save the fractions for UV, and calculation of recoveries.

20. When the run is completed, measure and record the retention time in minutes from injection point for each compound including the internal standard.

Analysis of Samples

21. From the vial containing the PNA concentrate, take 5-10 µl of sample and inject into the GC unit. Trap the peaks at the retention times obtained on the test blend and also trap any other peak that might be of interest. Trapped fractions must be made up to the same volume employed for the test blend.

a - At the time of this report, some preliminary data indicated that benzo(g,h,i)-perylene might not be completely dissolved by cyclohexane, resulting in low recovery. A preferred step would be to use acetone. In this case, the UV measurement might be done with the cyclohexane/acetone mixed solvent or it might be necessary to replace the acetone with cyclohexane by careful evaporation.

F. Ultraviolet Spectrophotometric Analysis and Scintillation Counting

1. Prepare solutions of known concentrations of each PNA in cyclohexane, e.g., 2.4 μg/ml.

2. With cyclohexane in the reference cell, obtain the spectrum of each solution from 400 to 225 nm.

3. From the spectra obtain the absorbances of the various compounds at the wavelength shown in Table IV (p. 12). Calculate absorptivity from the known concentrations. Typical calibration data are also shown in the table.

4. Rinse the cell thoroughly with cyclohexane between spectra and remove the last traces of solvent by vacuum or a gentle jet of air.

5. Obtain the UV spectrum of each selected fraction as directed in step E-21. Measure the absorbance at the specified wavelength and reference point as determined by the base line having anchor points as listed in Table IV.

6. One ml of each fraction containing BaA and BaP is placed in a counting vial, along with 9 ml of toluene and 10 ml of counting solution. Counts per minute are measured using a scintillation counter.

G. Calculation to a Sample Basis

1. To calculate the micrograms of the individual PNA's in the sample, the aliquot factor (activity ratio) must be determined based on radioactivity per ml of the cyclohexane solutions of GC fractions containing ^{14}C BaA and BaP, respectively.

Equation 1. $$M = \frac{ON}{(K-L)}$$

M = activity ratio
Q = activity in DPM added to sample
K = average CPM in a measured volume (ml) of the solution containing the GC fraction
L = background in CPM
N = counting efficiency CPM per DPM

CPM is counts per minute
DPM is disintegrations per minute.

The concentration of each component is determined from the UV absorbance.

Equation 2. $$D = \frac{A}{BC}$$

D = concentration of each PNA (μg/ml)
A = absorbance of each PNA
B = absorptivity of each PNA (ml/μg cm)
C = cell length (cm)

The weight of added radio tracer is determined.

Equation 3. $$X = \frac{Q}{S}$$

X = weight of radio tracer added, e.g., 1 μg of ^{14}C BaP.
S = specific activity (DPM/μg)
Q = activity in DPM added to sample.

Calculate weight of PNA in the sample.

Equation 4. $$T = MVD - X$$

T = weight of PNA in the sample.
V = volume in ml of the solution containing the GC fraction taken for counting.

The subtraction of X (Equation 4) is done only for BaA and BaP. The weight of benzo (g,h,i) perylene is corrected upward by 1.10 (p. 8).

Where more than one internal standard is used, all components eluting in the same peak with a standard are calculated based on that standard's activity ratio. All other components are calculated using the smallest activity ratio since this represents the maximum recovery of standard.

APPENDIX B

Measurement of Total Polynuclear Aromatic Hydrocarbons by Low Voltage Mass Spectrometry

Introduction

This method was developed to measure total polynuclear aromatic hydrocarbons in gasoline, crankcase oil, and tar from auto exhaust.

Scope

The method covers eleven groups of polynuclear aromatics (PNA) ranging from 3-ring to 7-rings and including alkyl substituted compounds. Compound classes include: phenanthrenes; fluorenes and dihydrophenanthrenes; pyrenes and fluoranthenes; cyclopentaphenanthrenes; benzo(g,h,i)fluoranthenes and cholanthrenes; benzanthracenes, chrysenes and triphenylenes; benzopyrenes and benzofluoranthenes; benzoperylenes and anthanthrenes; dibenzanthracenes; coronenes; and dibenzopyrenes. This procedure was demonstrated for auto exhaust tar. It should be applicable also to gasoline and crankcase oil.

Summary

A sample to be analyzed is spiked with a known quantity (40 micrograms) of triphenylbenzene. PNA hydrocarbons are removed from the total sample by separation on a column of partially deactivated alumina. The solvents used are cyclohexane, cyclohexane/benzene, benzene and benzene/methanol. The fraction containing the PNA's is reduced to about 30 μl by evaporation on a steam bath. An aliquot of this sample is injected into the mass spectrometer for a low voltage measurement of its spectrum. Quantitative measurement of PNA groups are calculated by applying sensitivity calibrations to monoisotopic peaks. Sample quantity is based on the internal standard, triphenylbenzene.

Apparatus

1. The mass spectrometer is equipped with an all glass sample inlet system that is heated to 316°C. Samples are introduced through a glass frit sealed with gallium. Resolution of one part in six hundred is maintained over a mass range of 12-600. A Consolidated Electrodynamics Corp. Model 21-102 was used but any comparable instrument is suitable.

2. An Infotronics Digital Readout System, Model CRS-160 is used for printout of the spectrum. Peaks can also be read manually or acquired by on line computer.

3. Micropipette, 3 μl.

Materials

1. 1,3,5-Triphenylbenzene (recrystallized) Pract. MW 306.41 Aldrich Chemical T8200-7.

2. Selected PNA hydrocarbons.

Method

1. Settings for the Mass Spectrometer

With the mass spectrometer in a high voltage (70 volts) mode of operation, adjust the ion source temperature so as to give a 127/226 peak ratio of 0.8 for n-hexadecane. At high voltage, the metastable suppressor dial reads 89, its maximum setting. The first step in changing to low voltage is to set this dial at 40, thereby lowering the ionizing voltage to approximately 15 volts. The inner repeller dial is set at its maximum position, which is 97, and the outer repeller turned to 60. The low voltage switch is moved from the 'N' to the 'L' position, and the metastable suppressor dial is adjusted to give a meter value of 11 for the ionization voltage. The ionizing current is maintained at 20 microamperes, with no change in the inner and outer focus slits.

2. Sample Introduction

A 3 μl volume is charged to the sample inlet with the sintered disk at approximately 40°C. The disk temperature is then raised to 316°C (600°F).

3. Scan of Sample

The sample is scanned from m/e 150 to m/e 400.

4. Calculation

Peaks are read from a chart or selected from a digitizer/computer printout. All peaks in the m/e range, 178-330, are recorded. Steps to be followed from this point include:

a. Calculate monoisotopic peaks for all even m/e numbers.

b. Calculate the triphenylbenzene peak at m/e 306 by correcting for sample background as illustrated:

m/e	Peak Height
292	117.0
306	266.1
320	70.0

$$\text{Triphenylbenzene Peak (m/e 306)} = 266.1 - \left(\frac{117.0 + 70.0}{2}\right) = 172.6$$

c. Divide monoisotopic peaks by appropriate sensitivity from Table 1B to obtain micrograms of each mol wt species as contained in the mass spectrometric sample.

d. Based on the internal standard, triphenylbenzene, convert all microgram quantities to a total sample basis.

Table IB

Sensitivities of Polynuclear Aromatic Hydrocarbons for Low Voltage Mass Spectrometer

Phenanthrenes, Anthracenes		Fluorenes, Dihydro-phenanthrenes		Pyrenes, Fluoranthenes		Cyclopenta-phenanthrenes		Benzo(g,h,i) fluoranthenes, Cholanthrenes		Benz-anthracenes, Chrysenes, Triphenylenes	
Mol Wt	Sens.[a]	Mol Wt	Sens.	Mol Wt	Sens.	Mol Wt	Sens.	Mol Wt	Sens.	Mol Wt	Sens.
178	134	180	102	202	88	204	96	226	61	228	92
192	125	194	97	216	86	218	92	240	60	242	87
206	122	208	93	230	80	232	87	254	58	256	83
220	118	222	90	244	78	246	84	268	57	270	81
234	114	236	87	258	75	260	81	282	56	284	79
248	110	250	84	272	73	274	78				

Benzopyrenes, Benzo fluoranthenes		Benzo-perylenes, Anthanthrenes		Dibenz-anthracenes		Coronenes		Dibenzo-pyrenes	
Mol Wt	Sens.	Mol Wt	Sens.	Mol Wt	Sens.	Mol Wt	Sens.	Mol Wt	Sens.
252	71	276	55	278	58	300	48	302	39
266	68	290	52	292	55	314	45	316	37
280	63	304	50	306	52	328	43	330	35
294	62	318	48	320	51				
	60								

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a - sensitivity = peak height (mm) per microgram.

Mass Spectrometer Conditions:

CEC Model 21-102
Ionizing Voltage = 11 volts (nominal)
Ionizing Current = 20 microamps
Sample Bottle = 316°C
$^{n}C_{16}$ peaks 127/276 = ~0.8

APPENDIX C

Method for Measurement of Phenols in Aqueous Condensate of Automobile Exhaust

Introduction

This method was devised specifically for the determination of phenols in the aqueous condensate obtained from automobile exhaust. Individual phenols are measured, including phenol, cresols, xylenols, ethyl phenols, and a few others. The method is applicable to water samples or extracts from other sources.

Summary

A known amount of an internal standard, o-chlorophenol, is added to the sample. Neutral compounds are removed by extraction of the alkaline sample with cyclohexane. The extract is acidified, saturated with sodium sulfate, and the phenols are extracted by five successive portions of ethyl ether. Organic acids are then separated by a sodium bicarbonate extraction. The ether is distilled in a special concentrator leaving a small residue which is injected into a capillary column gas chromatograph. The recorded peak areas are identified by the retention time, measured and multiplied by the appropriate factors which relate the area to weight.

Apparatus

1. The gas chromatograph is equipped with a hydrogen flame ionization detector, a sample splitter and an oven capable of isothermal temperature control to at least 120 $\pm$ 0.2°C. A Perkin-Elmer 900 chromatograph was used in the development of this method, but equivalent instruments should perform satisfactorily.

2. Recorder, 0-1 millivolt, 1 second full scale deflection, 2 inches/minute chart speed.

3. Column, 150' by 0.020" I.D. stainless steel capillary tubing coated with a mixture of didecyl phthalate and 10 percent phosphoric acid.

4. Syringe, 10 μl.

5. Vials, 1 dram.

6. Concentrator, Cat.# K-283500, Kontes Glass Co., Vineland, New Jersey, or equivalent apparatus for evaporation.

Reagents

1. Ethyl Ether, anhydrous.

2. Sulfuric Acid, pour 100 ml of water in a beaker and cool the beaker. Slowly and carefully add 100 ml of concentrated sulfuric acid, H_2SO_4.

3. Sodium Sulfate, anhydrous.

4. Sodium Hydroxide, 50% solution.

5. Sodium Hydroxide, 0.5N.

6. Sodium Bicarbonate, saturated solution.

7. O-Chlorophenol Solution A. Weigh approximately 400 mgs to the nearest 0.1 mg. Dissolve in methanol and make up to 100 ml in a volumetric flask.

8. O-Chlorophenol Internal Standard. Pipette 10.0 ml of Solution A into a 100-ml volumetric flask and dilute to volume with water.

9. Standard Phenol Solution. To prepare a standard for checking retention times, resolution and factors for quantitative analysis, weigh out from 100 to 300 milligrams of each compound and make up to volume in a 10-ml volumetric flask. Benzene, chlorophenol, and phenol should be included in the standard, along with some phenols which will cover the b.p. range of interest. Table XVII contains data on a number of phenols. It should not be necessary to calibrate with each phenol. Data in Table XVII, for example, should be applicable as shown. Retention times, on the other hand, can be established by cross comparison and interpolation.

Calibration of the Gas Chromatograph

The following operating conditions were employed with the Perkin-Elmer 900. Other instruments might use different parameters.

Sample Splitter - No. 3 (400-1)
Carrier Gas - 4 ml/min
Injection Temp. - 250°C
Detector - 250°C
Column - 125°C

1. Set the instrument controls as described above.

2. Inject 2 µl of toluene and allow helium to flow for 30 minutes to see if column is clean and base line straight.

3. Inject about 0.5 µl of the synthetic phenol standard (Reagent 9). Determine the retention time and relative sensitivity of each compound. By comparing retention times with those shown in Table XVII and by interpolation, the retention time of each compound can be determined. Relative sensitivities of Table XVII can be used directly although this should be confirmed by satisfactory agreement between the on-hand measurement and each corresponding value in Table XVII. Retention times decrease as the column is used so the standard should be checked frequently.

Method

A. Extraction of Phenol and Separation of Neutral and Acidic Components

1. Pipette 25.0 ml of the aqueous sample into a 60-ml separatory funnel and then add by pipette 2.0 ml of the internal standard.

2. Neutralize with 50% sodium hydroxide and add 1 ml in excess.

3. Add 15 ml of ethyl ether (ether) and shake for one minute. Draw off the aqueous phase into a second separatory funnel.

4. Add 10 ml of ether to the aqueous phase and shake for one minute. Draw off the aqueous phase into a 50-ml beaker and save for Step 6.

5. Combine this ether with the first ether extract and add 5 ml of 0.5N sodium hydroxide solution. Shake for one minute, draw off the aqueous phase into the 50-ml beaker and discard the ether.

6. Acidify the contents of the 50 ml beaker with (1:1) sulfuric acid. Add 5 g of anhydrous sodium sulfate to the beaker, stir until the crystals are dissolved, and pour into a 60-ml separatory funnel.

7. Add 25 ml ethyl ether to the beaker, then transfer combined ether and water phase to a separatory funnel.

8. Shake gently and vent. Repeat the gentle shaking and venting several times and then shake vigorously for 2 minutes. Drain the aqueous phase into a second 60-ml separatory funnel, and pour the ether phase into a 125-ml separatory funnel.

9. With aqueous phase, repeat steps 3 and 4 four times using 20, 15, 15, 10 ml of ether, combining all of the ether washings in the 125-ml separatory funnel.

10. Add five ml of saturated sodium bicarbonate solution to the combined ether extracts and shake for one minute.

11. Draw off the bicarbonate solution into a 60-ml separatory funnel, extract the ether with another 5 ml of bicarbonate and add the bicarbonate solution to the first.

12. Extract the bicarbonate solutions successively with two 5-ml portions of ether, and add the ether to the main ether fraction.

13. Add 4 g of anhydrous sodium sulfate to the combined ether phase and shake for two minutes to remove dissolved water.

B. Evaporation of Ether

14. Assemble the condenser and receiver of the evaporator, and decant the ether into the special still pot. Connect and distill off the ether at a gentle but steady rate.

15. When about 1 ml remains in the pot, and the distilling rate has slowed down considerably, remove the flask from the heat and cool immediately with a damp cloth.

16. With an elongated medicine dropper, transfer the ether from the still to a one-dram vial. Rinse the still with 10 drops of ether and add the rinsings to the vial. Repeat with 10 drops of methanol.

17. Place the vial on a steam bath or other controlled heat source and carefully evaporate off the ether. A gentle stream of nitrogen may be applied to the vial, but keep the vial warm so that moisture from the air does not condense on the sides. Remove from the heat when about 0.2 ml remains.

18. Cap the vial and place in a cool location until the GC analysis can be performed.

C. Gas Chromatographic Analysis

19. Set the instrument controls as described under Calibration.

20. Inject 2 μl of the residue and run at 125°C for at least 70 minutes.

21. Measure the retention times and the area of each peak by a suitable method.

Calculations

1. Determine the phenols present by comparing the retention times of the sample with those of the standard or from the table.

2. Calculate the amount of each phenol present by the following equation:

$$A = B \times \frac{C}{E} \times \frac{F}{D} = \frac{BC}{E} \times \frac{0.45}{D} = \frac{0.45\ BC}{ED}$$

where: A = weight of respective component,
B = weight of internal standard,
C = measured area of respective component,
D = relative sensitivity of component,
E = measured area of internal standard,
F = relative sensitivity for Int. Std. (O-chlorophenol = 0.45).