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RAPID FLUORIMETRIC METHODS BY INSTANT THIN-LAYER CHROMATOGRAPHY FOR THE ASSAY OF PHENALEN-1-ONE AND 7H-BENZ(*de*)-ANTHRACEN-7-ONE IN POLLUTED URBAN ATMOSPHERES

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SUMMARY

Through the use of glass-fiber paper impregnated with silica gel (called instant thin-layer chromatography, or ITLC), the rapid determination of phenalen-1-one (PO) and 7H-benz(*de*)anthracen-7-one (BO) is facilitated. One method is introduced for the specific determination of PO, and one method for the determination of both PO and BO at the same time. Both methods are superior to previous methods in terms of speed, selectivity, simplicity, and sensitivity. In addition, they give reasonable accuracy and precision.

Samples from urban atmospheres and air pollution source effluents can be analyzed rapidly for PO and BO by these methods. Analysis of one sample of organic extracts would take less than 30 minutes; analysis of a dozen samples would take less than 2 h. For those laboratories without instrumentation the amount of PO and BO can be estimated by eye after separation; eight analyses for either compound can be accomplished in about 20 minutes.

INTRODUCTION

The first indication of the presence of phenalen-1-one (PO) and the carcinogenic 7H-benz(*de*)anthracen-7-one (BO) in polluted air was obtained by SAWICKI AND JOHNSON¹. At that time, the use of trifluoroacetic acid fumes (TFA) for locating basic compounds on a thin-layer chromatogram was introduced. The chromatograms from urban air samples separated on aluminum oxide with pentane-ether (19:1, v/v) as the developer, were found to contain, after TFA fuming, a brilliant emerald green spot and a moderately brilliant pink-to-orange fluorescent spot. Because these fluorescent colors faded rather rapidly but could be brought back by retreatment with TFA fumes, it was believed that these two compounds were weakly basic, and probably aromatic carbonyl compounds.

Examination of the column and thin-layer chromatographic separation and the fluorescence characteristics of a large number of polynuclear ring carbonyl compounds²

disclosed that two of the compounds, PO and BO, showed separation and fluorescence color properties similar to those of the two unknown spots.

Through the use of thin-layer chromatography and direct spectrophotofluorimetric examination of thin-layer plates³, the emerald green and orange TFA-treated spots were identified as PO and BO, respectively, and their amounts in airborne particulate samples were estimated.

Based on the previous work a group of eight methods involving TLC separation, filter fluorometry, quenchofluorimetry, and spectrophotofluorimetry were developed and compared⁴.

In the work described in this paper, use was made of fast separation on glass-fiber paper impregnated with silica gel (ITLC) to develop methods for the determination of phenalen-1-one and 7H-benz(*de*)anthracen-7-one that are faster, simpler and as accurate as the previously described methods.

REAGENTS AND APPARATUS*

Phenalen-1-one (perinaphthenone) and 7H-benz(*de*)anthracen-7-one (benzanthrone) were obtained from the Aldrich Chemical Company, Milwaukee, Wisc., and crystallized to a constant melting point. The remainder of the reagents were obtained from commercial sources and crystallized or distilled until they were chromatographically pure.

Chromatography was performed on glass-fiber paper impregnated with silica gel. This type of separation is called instant thin-layer chromatography (ITLC) by the Gelman Instrument Company, Ann Arbor, Mich. The chromatograms were examined for fluorescence colors in a Chromato-Vue cabinet (Kensington Scientific Corp., 1717 Fifth Street, Berkeley 10, Calif.) under a 3660 Å light source.

An Aminco-Bowman spectrophotofluorimeter was used in determining fluorescence spectra in solution, with the following settings: sensitivity 50, slit arrangement No. 2, and phototube RCA type 1P21. In the assay of PO, Corning filter No. CS3-72 (Corning Glass Works, Corning, N.Y.) was used to exclude wavelengths below 435 m μ . Where indicated in the procedure, Aminco filter No. 4-7164 (American Instrument Company, Silver Springs, Md.) was used in the assay of BO to exclude wavelengths below 535 m μ .

Procedure for assay of phenalen-1-one

The separation of extracts of urban airborne particulates and the subsequent location of PO in the samples were performed with pentane-trifluoroacetic acid (50:1, v/v) as the developer; 20 minutes were required for the solvent front to travel 15 cm. After development, the emerald green spot was located under ultraviolet light and intensified with the use of TFA fumes. The spot was then cut out and extracted with three 1-ml volumes of boiling acetone; the resulting extract was evaporated to dryness in a boiling water bath. The residue was dissolved in 0.5 ml of concentrated sulphuric acid, and a reading was then obtained at an emission wavelength of 498 m μ with the spectrophotofluorimeter set at an excitation wavelength of 400 m μ . Filter No. CS3-72 was used in the instrument to exclude wavelengths below 435 m μ . In this

* Mention of commercial products does not constitute endorsement by the Public Health Service.

fashion much of the background fluorescence that interfered with the analysis of air samples was removed. The standard PO spot and a spot used as the blank, of the same size and R_F value as the unknown and standard spots, were cut out and treated in the same manner as the unknown.

Investigation of various temperatures and volumes of acetone in the extraction procedure proved that extraction with three successive 1-ml volumes of boiling acetone was sufficient for quantitative extraction.

Calculation of the amount of PO in the unknown sample was based on the linear relation of fluorescence intensity *versus* concentration. Conformance with Beer's law was observed from 20 to at least 1000 ng.

Procedure for assay of phenalen-1-one and 7H-benz(de)anthracen-7-one

The location of both PO and BO in the extracts of urban airborne particulates was accomplished with pentane-methylene chloride (3:1, v/v) as the developer. About 18 min were required for the solvent front to travel 15 cm. A separation time of 6 min was possible when the solvent front was allowed to travel 10 cm. After development, the paper was examined under ultraviolet light, and the PO and BO spots, opposite the appropriate standards, were located with TFA fumes. The extraction technique and estimation of PO in the sample were done as described in the preceding section. Estimation of BO was made at an excitation wavelength of 370 m μ with the spectrofluorimeter set at an emission wavelength of 560 m μ . Calculation of the amount of BO in the unknown sample was based on the linear relation between the fluorescence intensity of BO and its concentration. Conformance with Beer's law was observed from 10 to at least 1000 ng. For this calculation, the amount of transmittance between the wavelength maximum of 370 m μ and the wavelength minimum of 390 m μ was read. A Beer's law relationship existed if the readings were made with the instrument set at maximum sensitivity of 50 or at a sensitivity of 25.

An alternative reading procedure was necessary for one sample that was difficult to analyze by the method described because of the unusually low amounts of BO present. With Aminco filter No. 4-7164, the reading was taken at emission wavelength 560 m μ with the instrument set at an excitation wavelength of 360 m μ .

Estimation of PO and BO by eye

The unknown sample and spots containing PO and BO in 0.025, 0.05, 0.1, 0.2, and 0.4 μ g amounts were separated by the pentane-methylene chloride procedure. The chromatogram was placed under ultraviolet light. All spots were sprayed with trifluoroacetic acid fumes and the amount of PO and BO in the unknown sample was estimated immediately by eye through comparison of the unknown and standard spots. The PO method could also be used.

RESULTS AND DISCUSSION

The method for the determination of PO with pentane-trifluoroacetic acid (50:1, v/v) as the developer has the advantage that the analysis can be performed in much less time than by previous literature methods, with the result that more samples can be analyzed in the same amount of time. In addition, the method is simple, sensitive, and selective, and the aza heterocyclic compounds do not interfere because they

remain at the origin. Fig. 1 shows the emerald green spot found in the separation of 1 mg of coal-tar-pitch and 1 mg of a benzene-soluble fraction of urban airborne particulate, which gives the same fluorescence color and R_F value as the PO standard. Both the BO and 9-xanthenone standards traveled to the solvent front. The mixture of aza heterocyclic compounds that remained at the origin includes 1 μ g each of benzo(*f*)quinoline, benzo(*h*)quinoline, benz(*a*)acridine, and benz(*c*)acridine.

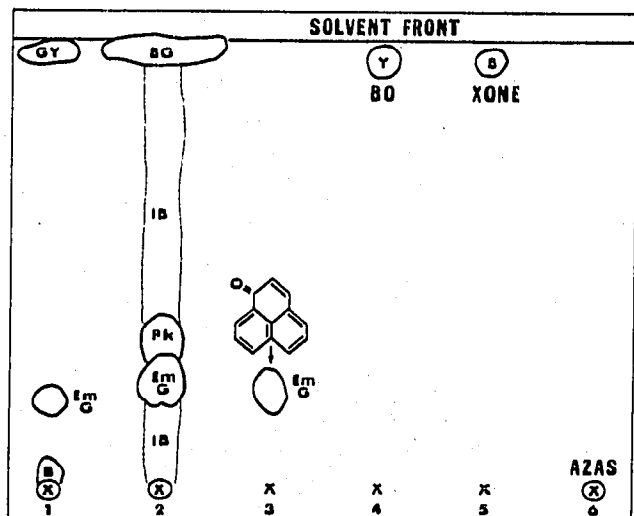


Fig. 1. Thin-layer chromatogram on silica gel glass-fiber paper with pentane-trifluoroacetic acid (50:1, v/v) as the developer. The fluorescence colors of the compounds shown appeared after the chromatogram had been sprayed with trifluoroacetic acid fumes. Color abbreviations are as follows: B = blue, Em G = emerald green, GY = green yellow, O = orange, BG = blue green, Pk = pink, Y = yellow, l = light. Amounts of the compounds separated are as follows: (1) 1 mg of coal-tar-pitch, (2) 1 mg of a benzene-soluble fraction of urban airborne particulates, (3) 1 μ g of phenalen-1-one, (4) 1 μ g of 7H-benz(*de*)anthracen-7-one, (5) 1 μ g of 9-xanthenone, and (6) a mixture containing 1 μ g each of benzo(*f*)quinoline, benzo(*h*)quinoline, benz(*a*)acridine, and benz(*c*)acridine.

The method for the determination of BO and PO with pentane-methylene chloride (3:1, v/v) as the developer offers the advantage that a large number of samples can be assayed for these two compounds in a relatively short time because separation time is reduced to 18 min or less, depending on the distance the solvent front is allowed to travel. The results calculated for PO by this procedure are analogous to those obtained by the specific procedure for PO.

Since PO and BO can be determined at the 10 to 20 ng level both methods provide a high order of sensitivity. Compared to all previous procedures, both methods are superior in simplicity and speed. The methods show high selectivity, since we know of no interferences in the determination of PO and BO by these methods. Although the natural mixtures contain many thousands of compounds, the PO and BO spots are so easily obtained and stand out so vividly on the chromatograms that the procedure should prove useful for teaching purposes.

Because of the vividness of the spots, PO and BO can be estimated by eye with the help of standards. The spots must be fumed with trifluoroacetic acid and read immediately. Although the emerald green color of the PO spot fades gradually, it is still vivid after several hours. PO must be more basic than BO, since the orange color

of the BO cation fades more quickly. Since this fading or loss of trifluoroacetic acid is much more rapid on paper chromatograms or on alumina or silica gel thin-layer plates, the silica gel glass-fiber paper must hold trifluoroacetic acid much more tenaciously. This phenomenon should prove of value in future research.

Recoveries of PO and BO from an extract of urban airborne particulates collected indoors were studied. Under the conditions of the analysis the material gave an indication of less than 10 μg of both PO and BO per gram of particulate. The two chemicals were added to this particulate and a Soxhlet extraction for 6 h was performed with benzene in one flask and methylene chloride in another flask. These extracts served as the "test" extracts. At the same time, a 6-h Soxhlet extraction of the particulate without the added BO and PO was carried out, and these extracts represented "blanks" in the analyses. After analysis in triplicate for PO and BO by the respective methods, the results were as follows: PO was recovered from the benzene extract in a 101 % yield and from the methylene chloride extract in a 104 % yield; BO was recovered from the benzene extract in a 91 % yield and from the methylene chloride extract in a 106 % yield. Because of the small amount of BO and the presence of unknown interfering substances in this particular sample, the final step in the assay of the extract for BO was modified slightly. Instead of obtaining a reading at the excitation wavelength of 370 $\text{m}\mu$ with the spectrophotofluorimeter set at an emission wavelength of 560 $\text{m}\mu$, the reading was taken at the emission wavelength of 560 $\text{m}\mu$ with the instrument set at an excitation wavelength of 360 $\text{m}\mu$. An Aminco filter No. 4-7164 was also used; this filter, which excluded light of wavelengths less than 535 $\text{m}\mu$, proved to be the best one for maximum sensitivity and minimum interference from other compounds. The modification appeared to work well in this analysis and could be used in place of the original technique when the amount of BO in the sample is low.

A study involving the efficiency of extraction of the PO and BO spots from the silica gel glass-fiber paper was also conducted. Results obtained for BO were slightly higher when chloroform was used to extract the paper than when acetone was used; the results for PO, however, were the same with chloroform and acetone. In all cases the efficiency of extraction from the paper for both PO and BO was close to quantitative, regardless of which solvent was used.

APPLICATION

The separation of a 1 mg sample of a benzene-soluble fraction of urban airborne particulates from Greenville, S.C., and the subsequent identification of BO and PO in the sample are shown in Fig. 2. The fluorescence spectra in Fig. 2 are those of the extract from the standard BO spot and the extract from the unknown spot opposite the BO standard.

The assay results for PO and BO in the benzene-soluble fraction of urban airborne particulates are shown in Table I.

For the estimation of PO in the samples, the specific method for PO was used, with pentane-trifluoroacetic acid (50:1, v/v) as the developer. Results obtained with and without a filter in the instrument were compared. The average value clearly shows that the use of the filter reduced the percent deviation considerably. In addition, the value thus obtained for PO was comparable to that found by an earlier method³.

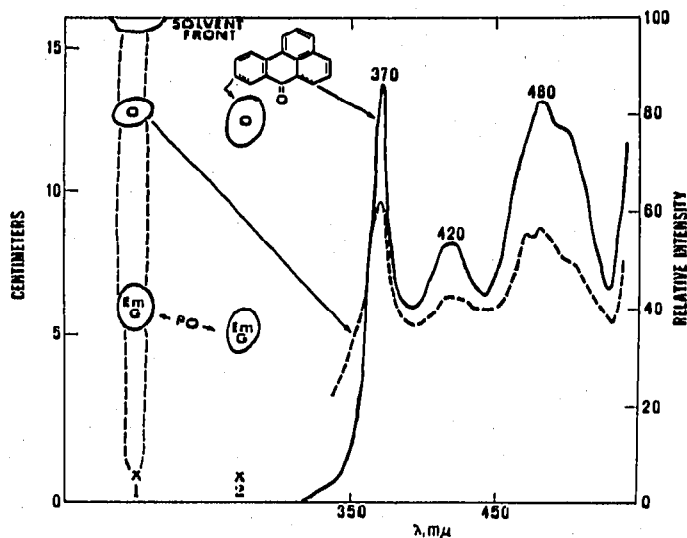


Fig. 2. On the left side appears the separation on silica gel glass-fiber paper of (1) 1 mg of a benzene-soluble fraction of urban airborne particulate from Greenville, S.C., and (2) a mixture containing 0.8 μ g of phenalen-1-one and 0.8 μ g 7H-benz(*de*)anthracen-7-one, with pentane-methylene chloride (3:1, v/v) as the developer. The fluorescence colors shown appeared after TFA fuming. For color abbreviations refer to Fig. 1. On the right side are shown the excitation spectra at emission λ 560 and meter multiplier (MM) 0.01 of (—) the extract from the BO standard spot in 0.5 ml concentrated sulfuric acid and (---) the extract from the unknown spot opposite the BO standard spot in 0.5 ml concentrated sulfuric acid.

TABLE I

ASSAY FOR PHENALEN-1-ONE AND 7H-BENZ(*de*)ANTHRACEN-7-ONE IN THE BENZENE-SOLUBLE FRACTION OF URBAN AIRBORNE PARTICULATES^a

Sample size (mg)	PO method ^b (mg/g benzene-soluble fraction)	Sample size (mg)	PO method ^c (mg/g benzene-soluble fraction)	Sample size (mg)	BO method (mg/g benzene-soluble fraction)
1.0	0.33	0.6	0.25	1.0	0.29
1.0	0.27	0.6	0.27	1.0	0.30
1.0	0.24	0.6	0.24	1.0	0.35
1.0	0.24	0.6	0.25	1.0	0.33
0.8	0.28	0.6	0.29	1.0	0.27
0.6	0.20	0.6	0.29	1.0	0.53
0.4	0.23	0.6	0.27	1.0	0.46
0.04	0.33	0.6	0.24	1.0	0.35
		0.4	0.26		
Average ^d	0.26 \pm 0.047		0.26 \pm 0.019		0.36 \pm 0.12

^a Corrected values based on recovery data.

^b Determination without filter.

^c Determination with filter No. CS3-72.

^d Average value of 0.2 mg PO/g was obtained by previous method^a.

Investigation of various filters for use in the instrument for the assay of PO showed that Filter CS3-72 was superior to the other filters. A 0.6 mg sample of a benzene-soluble fraction of urban airborne particulate from New Rochelle, N.Y., was separated and analyzed for PO in the prescribed manner. Fig. 3 shows the spectra of

the extract from the unknown spot opposite the PO standard, run without a filter and with various filters placed in the instrument. This investigation indicated that filter No. CS3-72 should be used in the assay for PO to give maximum sensitivity and minimum interference from other fluorescing compounds in the mixture present in the spot.

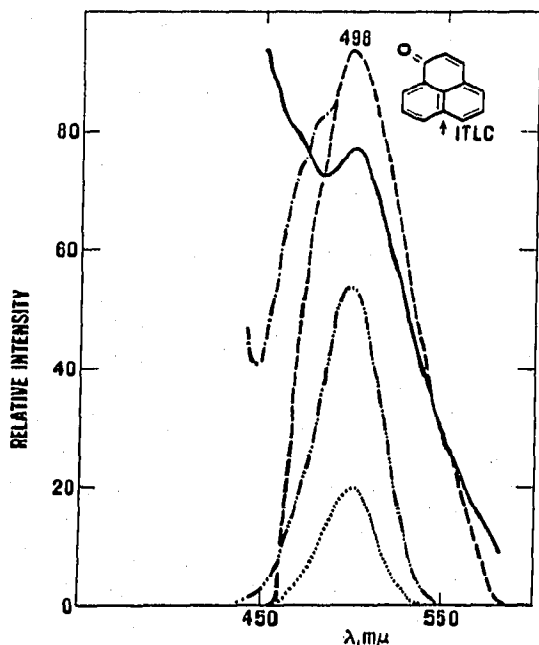


Fig. 3. Effect of various filters on the emission spectrum of the extract of an unknown spot opposite the phenalen-1-one spot from the separation of 0.6 mg of a benzene-soluble fraction of urban airborne particulate from New Rochelle, N. Y., with pentane-trifluoroacetic acid (50:1, v/v) as the developer. The readings were taken at an emission λ of 498 $m\mu$ with the excitation λ set at 400 $m\mu$. The extract was in 0.5 ml of concentrated sulfuric acid. (—) No filter, MM = 0.01. (---) Corning Glass filter No. CS3-72, MM = 0.01; cuts off light below 435 $m\mu$. (-·-·) Corning Glass filter No. CS3-70, MM = 0.01; cuts off light below 490 $m\mu$. (-·-·-·) Filter No. 65A, MM = 0.01, Aminco Catalogue No. 4-7160; peaks at 495 $m\mu$. (·····) Filter No. 75, MM = 0.01, Aminco Catalogue No. 4-7120; peaks at 490 $m\mu$.

Table I also gives the average value of BO found in 1 g of the benzene-soluble fraction of urban airborne particulates. The assay was performed according to the described procedure, with pentane-methylene chloride (3:1, v/v) as the developer.

All the values listed for PO and BO in the tables represent corrected values based on recovery data, the results of which will be discussed in a later section.

The results of the assay for BO and PO by their specific methods, in samples from the urban atmospheres of various cities are shown in Tables II and III. An appropriate amount (~ 1 mg) of the urban sample to be analyzed was spotted on a silica gel glass-fiber paper, and the analysis for either BO or PO was performed according to the described methods.

The values for BO and PO listed for the various cities represent a general means of comparison. The values do not represent the specific amounts present in each city, for this would involve the analysis of numerous samples collected in many parts of each city and at different times of the year. If this were done, an overall average value of each city could be calculated, and the values for the cities specifically compared.

TABLE II

ASSAY^a FOR 7H-BENZ(*de*)ANTHRACEN-7-ONE ON ITLC

Location ^b	mg BO/g sample ^c	Average value
Harrisburg, Pa.	0.13, 0.20, 0.10	0.14
Greenville, S.C.	0.40, 0.43, 0.48	0.44
Ashville, N.C.	0.46, 0.45, 0.50	0.47
Mt. Vernon, N.Y.	0.22, 0.22, 0.22	0.22
Lynn, Mass.	0.20, 0.25, 0.28	0.24
New Rochelle, N.Y.	0.13, 0.14, 0.16	0.14
Memphis, Tenn.	0.20, 0.23, 0.25	0.23
Ft. Wayne, Ind.	0.23, 0.31, 0.29	0.28
Wilkes-Barre, Pa.	0.13, 0.14, 0.14	0.14
Glen Cove, N.Y.	0.14, 0.13, 0.13	0.13
Saginaw, Mich.	0.25, 0.34, 0.32	0.31

^a Developer: pentane-methylene chloride (3:1, v/v); adsorbent: glass-fiber paper impregnated with silica gel; time for solvent front to travel 15 cm = 18 min.

^b Sample size was 1 mg for each determination.

^c Corrected value based on recovery data.

TABLE III

ASSAY^a FOR PHENALEN-1-ONE ON ITLC

Location ^b	mg PO/g sample	Average value
Memphis, Tenn.	0.22, 0.17, 0.17	0.19
Saginaw, Mich.	0.33, 0.30, 0.33	0.32
Harrisburg, Pa.	0.22, 0.21, 0.22	0.22
Glen Cove, N.Y.	0.12, 0.09, 0.16	0.12
Wilkes-Barre, Pa.	0.13, 0.12, 0.14	0.13
Ft. Wayne, Ind.	0.43, 0.41, 0.43	0.42
New Rochelle, N.Y.	0.24, 0.25, 0.22	0.24
Mt. Vernon, N.Y.	0.12, 0.11, 0.12	0.12
Lynn, Mass.	0.23, 0.25, 0.23	0.24
Ashville, N.C.	0.52, 0.52, 0.51	0.52
Greenville, S.C.	0.38, 0.36, 0.40	0.38

^a Developer: pentane-TFA (50:1, v/v); adsorbent: glass-fiber paper impregnated with silica gel; time for solvent front to travel 15 cm = 20 min; filter No. CS3-72 used in SPF.

^b Sample size in all determinations was 0.6 mg except for Ashville, N.C. and Greenville, S.C., which were 0.4 mg samples.

Nevertheless, the values listed in these tables do give a general idea as to the differences in amounts of BO and PO found in the cities.

Fig. 4 shows the emission spectrum of the extract of the unknown emerald green spot opposite the PO standard spot obtained in the separation of 0.4 mg of a benzene-soluble fraction of urban airborne particulate from Ashville, N.C. The spectrum for the unknown is the average of triplicate determinations made on this sample. The emission spectrum of the PO standard is shown, as well as that of the blank, which was cut out at the same R_F value as that of the standard and unknown. All the spots were extracted in the same way.

In an attempt to ascertain the presence of BO and PO in samples from air pollution sources, an extract of an effluent from a coffee-roasting industrial plant was

separated. The results indicated the presence of two well-separated spots of BO and PO, which gave the same R_F values as their respective BO and PO standards and the same fluorescent colors with TFA fumes. This preliminary work indicates the presence of these two compounds in various types of polluted atmospheres. The procedures should be used in the future investigation of various samples of polluted atmospheres for BO and PO.

For those laboratories without instrumentation, the amounts of PO and BO can be estimated by eye after separation. This means that eight complete analyses can

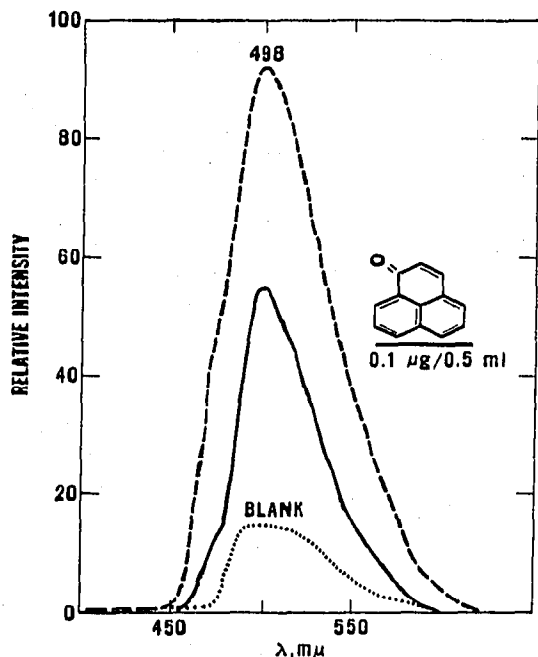


Fig. 4. Emission spectra of extracts from silica gel glass-fiber paper developed with pentane-trifluoroacetic acid (50:1, v/v) and read at F 400/498. All extracts were read in 0.5 ml concentrated sulfuric acid. (---) Unknown spot opposite phenalen-1-one standard in the separation of 0.4 mg of a benzene-soluble fraction of urban airborne particulate from Ashville, N.C. Average of three determinations, $MM \cdot T = 0.81 \pm 0.01$. (—) 0.1 μ g of phenalen-1-one. (·····) Blank, scan area cut out at same R_F value as standard and unknown. All spots were extracted in the same manner.

TABLE IV

ESTIMATION OF PHENALEN-1-ONE AND 7H-BENZ(*de*)ANTHRACEN-7-ONE BY EYE

Sample site	PO^a (mg/g benzene-soluble fraction)		BO^b (mg/g benzene-soluble fraction)	
	SPF	Eye	SPF	Eye
Saginaw, Mich.	0.29	0.30	0.28	0.25
Harrisburg, Pa.	0.20	0.15	0.13	0.20
Ft. Wayne, Ind.	0.39	0.30	0.25	0.15
New Rochelle, N.Y.	0.22	0.15	0.13	0.10
Lynn, Mass.	0.22	0.20	0.22	0.20

^a Sample size for analysis: 0.6 mg of benzene-soluble fraction.

^b Sample size for analysis: 1.0 mg of benzene-soluble fraction.

be done for either compound in about 20 min; the only equipment required is glass-fiber paper impregnated with silica gel, a developing tank, an ultraviolet light, pentane, trifluoroacetic acid, methylene chloride, phenalen-1-one, 7H-benz(*de*)anthracen-7-one, and reasonably good eyesight. The results obtained by this estimation are compared with those given by the spectrophotofluorimetric method in Table IV.

CONCLUSIONS

With the described separation methods and the use of glass-fiber paper impregnated with silica gel, the determination of phenalen-1-one and the carcinogen, 7H-benz(*de*)anthracen-7-one, can be performed in a fraction of the time necessary for previous methods. In addition, these methods are simple, selective, and sensitive. Preliminary investigation shows that urban atmospheres and air pollution source effluents can be analyzed for the presence of PO and BO by these methods. For those laboratories without fluorescence instrumentation PO and BO can be readily estimated by eye under ultraviolet light after separation of the air sample and appropriate standards on silica gel glass-fiber paper.

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