COMPARISON OF FLUORIMETRIC METHODS OF ASSAY FOR BENZ(c)-ACRIDINE AND BENZO(h)QUINOLINE IN URBAN ATMOSPHERES AND AIR POLLUTION SOURCE EFFLUENTS

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INTRODUCTION

Polynuclear aza heterocyclic compounds have been determined in air pollution source effluents[1] and urban atmospheres[2]. In these determinations the basic fraction obtained by liquid–liquid extraction was separated on the alumina column; the resultant fractions were then analyzed through ultraviolet absorption spectrophotometry. Since this procedure requires several days for analysis of several hundred milligrams of organic airborne particulates, we believe that simpler, more sensitive methods involving less time, and technique and a smaller sample are desirable. An urban airborne particulate sample and a coal tar pitch sample were selected for study. The urban sample was selected because urban air pollution is our main interest; the coal tar pitch sample, because its basic fraction is extremely complicated and should contain a maximal amount of interference.

EXPERIMENTAL*

Equipment

An Aminco-Bowman spectrophotofluorimeter was used with the following settings: sensitivity, 50; slit arrangement No. 2; and phototube RCA type 1P21. An Aminco Fluoro-Microphotometer was used with a mercury vapor lamp; primary filter Aminco No. 4-7112, peaking at 405 mμ; and secondary filter 4-7120, peaking at 490 mμ. The sensitivity was kept at 50 while the meter multiplier readings were held at 0.3. The four holes of the aperture plate were used in the primary filter position.

The absorption spectrophotometric work was performed with a Cary Model 11 Spectrophotometer.

Procedure 1

Benz(c)acridine was analyzed by the following procedure. The sample of urban airborne particulate or air pollution source effluent was extracted with benzene-diethylamine (4:1) in a Soxhlet extractor for 6 h. The solvent was evaporated at

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

room temperature in a vacuum oven. The residue was dissolved in methylene chloride in the proportion of about 5 mg residue to 100 μl of solvent. Then this solution (50 μl per spot) was placed on an alumina thin-layer plate. A standard was run at the same time, e.g. 0.5 μg of benz(c)acridine. The plate was developed with pentane–ether (19:1). Each spot adjacent to the standard was scraped off the plate and extracted with 5 ml of 10 % hydrochloric acid (10 ml concentrated acid diluted to 100 ml with water). Approximately 3 min of shaking was necessary to extract the material. The solution was neutralized with solid sodium carbonate. This mixture was extracted with three 5-ml volumes of methylene chloride. The solution was evaporated and the residue was dissolved in 1 ml of pentane–trifluoroacetic acid (50:1). If necessary, the sensitivity can be increased tenfold by dissolving the residue in 0.1 ml of the acidic pentane. A reading was then taken at F 288/472. The standard and blank were run similarly.

Procedure 2

Benzo(h)quinoline was analyzed similarly, except that two-dimensional thin-layer chromatography was used and readings of the final solution were taken at F 272/420. In analysis for benz(c)acridine, readings were taken at F 288/472. The plate was coated with alumina–cellulose (2:1) and developed with pentane–ether (19:1) followed by dimethylformamide–water (35:65). Standards were run at the same time one-dimensionally.

Terms used in the calculations of procedures 1 and 2 are defined as follows: W_s = weight in nanograms of the standard placed on the thin-layer plate; W_t = weight in milligrams of analyzed organic airborne particulate sample; R_s = product of the meter multiplier and transmittance readings of the acidic pentane solution of the standard spot; R_x = same as R_s but for unknown spot analyzed; and V = volume in cubic millimeters of analyzed air.

The equations used in the analysis of the organic fraction and the air sample for benz(c)acridine and benzo(h)quinoline are:

$$\mu\text{g BcACR/g sample} = \frac{W_s \cdot R_x}{W_t \cdot R_s}$$

and:

$$\text{ng BcACR/m}^3 \text{ air} = \frac{W_s \cdot R_x}{V \cdot R_s}$$

Procedure 3

The separation method was the same as in procedure 2. Then the unknown and standard spots were sprayed with GS Chromatogram-preserving medium (Gallard-Schlesinger Mfg. Corp., Carle Place, New York). Each spot was covered by a 1- by 3-inch strip of Scotch-brand TLC tape (Brinkman, Inc., Great Neck, New York); the coated spot was then peeled from the plate[3] and read at F 288/478. The equations are the same as described for procedure 2, except that values for the areas of the unknown (A_x) and standard (A_s) spot are required.:

$$\mu\text{g BcACR/g sample} = \frac{W_s \cdot R_x \cdot A_x}{W_t \cdot R_s \cdot A_s}$$

TABLE I

COMPARISON OF ANALYTICAL METHODS FOR THE DETERMINATION OF BENZ(c)ACRIDINE AND BENZO(h)QUINOLINE

Procedure	Determination limit (ng/ml)	Total analysis time (h)[a]	Sample	Concentration (μg/g organic sample)			
				Benz(c)acridine	Average	Benzo(h)quinoline	Average
1 TLC —e→ SPF[b]	40	1.5	Air particulates	40, 40, 40, 60, 70, 70, 80, 80, 80	40, 70, 60		
2 2D-TLC —e→ SPF	40	4	Air particulates	40, 40, 50, 50, 60	50, 50	40, 40	40
3 2D-TLC —e→ SPF	4[c]	4	Air particulates	40, 40, 40	70	30, 60, 80	60
4 2D-TLC —e→ F	10	4	Air particulates	40, 40, 40	40	60, 80, 100	80, 100
5 CC —e→ SP	3000	3 days	Air particulates	50, 50	50	25, 35	30
1 TLC —e→ SPF	40	1.5	Coal tar pitch	500, 500, 700, 700, 800, 800, 900	700, 800, 800, 700	700, 1000, 1400	1000

[a] Does not include collection of airborne particulates and 6 h of Soxhlet extraction with benzene–diethylamine (4:1).

[b] —e→ stands for "followed by elution".

[c] Determination limit on the plate.

and:

$$\text{ng BcACR/m}^3 \text{ air} \quad = \quad \frac{W_s \cdot R_x \cdot A_x}{V \cdot R_s \cdot A_s}$$

Procedure 4

Separation and extraction procedures were the same as in procedure 2. The residue obtained after the methylene chloride evaporation was dissolved in 1 ml of nitromethane containing 1 % trifluoroacetic acid. Readings of this solution and a standard were taken on an Aminco Fluoro-Microphotometer with primary filter Aminco No. 4-7112, peaking at 405 mμ, and secondary filter 4-7120, peaking at 490 mμ. Equations were the same as in procedure 2.

Procedure 5

This method of analysis, consisting of the extraction of a basic fraction from the organic particulate fraction followed by column chromatography and absorption spectral analysis of the fractions, has been described[1].

RESULTS

Rapid and sensitive methods are needed to ascertain the presence and concentration of polynuclear aza heterocyclic compounds in air samples. Previous work indicated that the measurement of any one of these compounds should give a quick indication of the presence and approximate concentration of the various aza compounds, some of which are carcinogenic. The advantages and shortcomings of the various procedures were investigated. Some of the data are presented in Table I.

Procedure 1

Benz(c)acridine was readily assayed in urban atmospheric samples with this procedure. Although the one-dimensional thin-layer chromatographic separation of benz(c)acridine was by no means complete, use of the appropriate excitation and emission spectral bands gave the method enough selectivity that the compound could be analyzed in the presence of the many other compounds in the separated spot.

The type of separation and fluorescence spectra obtained in the analysis of benz-(c)acridine is shown in Fig. 1. The fluorescence emission and excitation spectra of the eluted standard and unknown spots show a close resemblance. The absorption spectrum is needed to check the reliability of the excitation spectra. With these spectra characterization and determination are performed simultaneously.

The recovery of benz(c)acridine added to airborne particulates was investigated with this procedure. Three different extraction procedures were tried (Table II). The Soxhlet extraction for 6 h with benzene–Et$_2$NH (4:1) gave the best results. Recovery was 100 $\pm$ 10 %.

This procedure could not be used for urban atmospheric benzo(h)quinoline, since too many fluorescent interferences are present in the separated benzo(h)quinoline spot. The method could be used, however, for the estimation of benzo(h)quinoline in coal tar pitch.
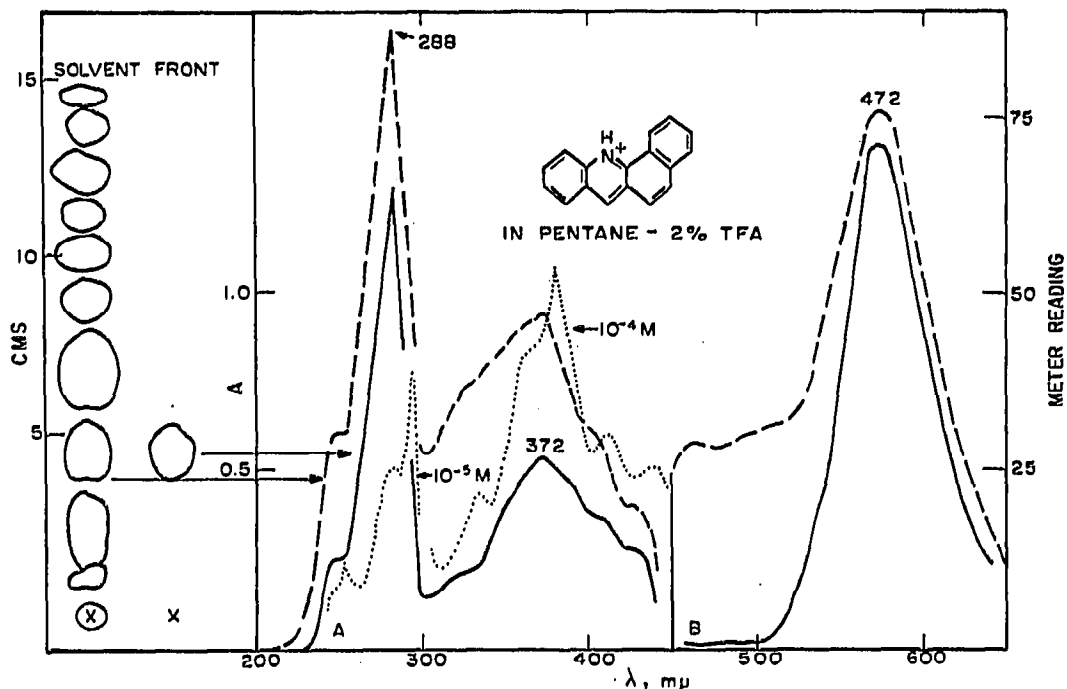
Fig. 1. Separation and fluorimetric and absorption spectra of benz(c)acridine. On left, alumina thin-layer chromatogram of 2.5 mg of the benzene-soluble fraction of urban airborne particulates and 0.1 μg of benz(c)acridine with development by pentane–ether (19:1). On right, fluorescence excitation (A) and emission (B) spectra of unknown spot (- - -) and benz(c)acridine spot (————) F 282/475. Absorption spectra, 10^{-5} and 10^{-4} M (...). All solution in pentane–2 % trifluoroacetic acid.
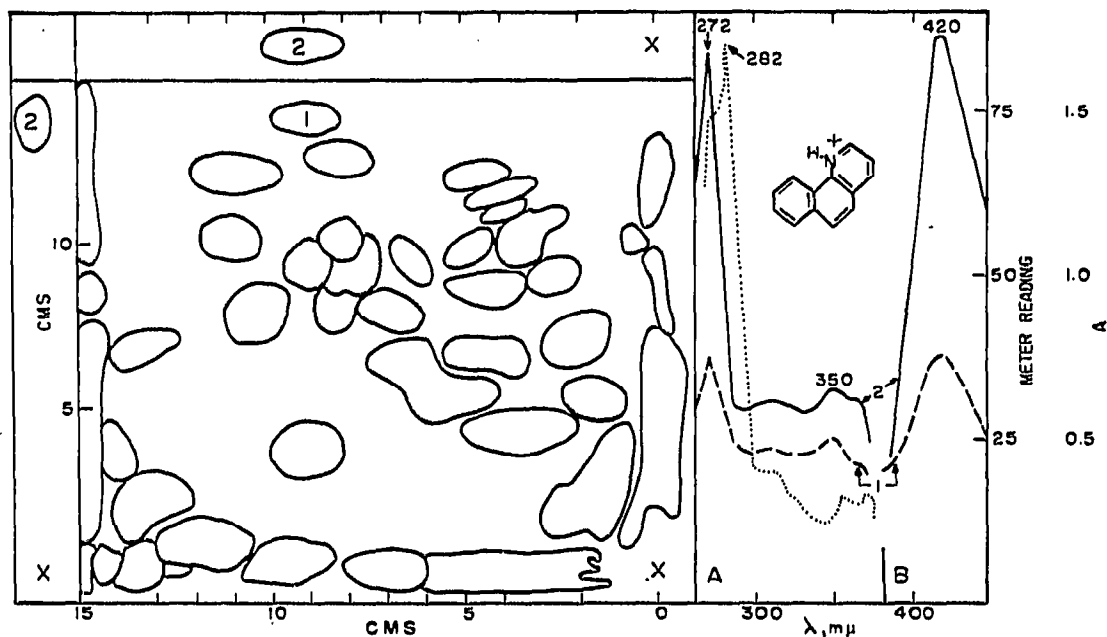

Fig. 2. Separation and fluorimetric and absorption spectra of benzo(h)quinoline. On left, two-dimensional thin-layer chromatogram of 2 mg of the benzene-soluble fraction of alkalinized urban airborne particulates and one-dimensional chromatograms of 1 μg of benzo(h)quinoline. Adsorbent: alumina–cellulose (2:1). Developers: pentane–ether (19:1) followed by dimethylformamide–water (35:65). On right, fluorescence excitation, (A) and emission (B) spectra of spot 1 (- - -) and standard spots 2 (————). F 272/420. Absorption spectrum, 10^{-4} M (...). All solutions in pentane–2 % trifluoroacetic acid.

TABLE II

RECOVERY OF BENZ(c)ACRIDINE FROM AIRBORNE PARTICULATES WITH PROCEDURE I

Extraction process	Results						Average recovery (%)
1 CH$_2$Cl$_2$–Et$_2$NH (4:1); room temperature	44,	44,	50,	50,	63		50
2 Benzene; Soxhlet; 6 h	30,	34,	46,	52,	54,	60	50
3 Benzene–Et$_2$NH (4:1); Soxhlet; 6 h	88,	88,	100,	103,	108,	108	100

Procedure 2

With two-dimensional thin-layer chromatography both benz(c)acridine and benzo(h)quinoline can be determined (Fig. 2). Procedure 2 requires more time for analysis than the first procedure. The whole procedure must be done without delays. If the plates or extracted solutions are left overnight, lower values are obtained.

Procedure 3

This method of two-dimensional thin-layer chromatography on two different adsorbents followed by direct fluorimetric analysis is the most satisfactory for quick characterization. It can be used for the assay of benz(c)acridine and benzo(h)quinoline. It is the most sensitive method of all. With improvements in scanning instrumentation and techniques this method will become much more valuable in characterization and assay of air pollutants. The amount of sample separated ($\sim$ 1 mg) is small enough that well-formed spots are obtained. The method involves more care and technique than all the other methods.

Procedure 4

With the usual solvents neither benz(c)acridine nor benzo(h)quinoline can be determined by filter fluorimetric examination following one- or two-dimensional thin-layer chromatography. A filter fluorimeter can be used in the determination of benz(c)acridine if the sample is first separated two-dimensionally and then eluted, evaporated, and dissolved in acidic nitromethane. Because of the fluorescent interferences present in the separated spot, a solvent must be used that will quench the fluorescence of the impurities and not that of benz(c)acridine. Since the filter fluorimeter is not as selective as the spectrophotofluorimeter, the interference of fluorescence impurities is much more serious. Thus, with the improved separation of two-dimensional thin-layer chromatography and the use of a quenching solvent, benz(c)-acridine could be determined but benzo(h)quinoline could not.

Procedure 5

This method provides by far the most information. A group of aza heterocyclic compounds can be determined and the absorption spectra of a large number of unknown basic compounds can be obtained. The disadvantages of the method are that it requires a large amount of organic particulates ($\sim$ 200 mg), several days for collection of an air sample, and several days for a complete analysis; also, the method has low sensitivity for benz(c)acridine.

RECOMMENDATIONS

Procedure 1 is recommended as the simplest and quickest method for the estimation of benz(c)acridine. If a spectrophotofluorimeter is not available, the filter fluorimetric method of procedure 4 can be used. Either of these methods can be used when the analyst wishes to determine whether an air sample contains polynuclear aza heterocyclic compounds and in what quantity.

Procedure 2 is recommended for the estimation of benzo(h)quinoline. Since benzo(h)quinoline appears to be present in the air and in air pollution samples in approximately the same concentration as benz(c)acridine, this assay can be used as a check on the benz(c)acridine concentration.

With the improvement of scanning equipment, procedure 3 should prove increasingly valuable for the characterization and assay of benz(c)acridine and other fluorescent compounds present in polluted air.

Procedure 5 is recommended to provide the most information about the basic pollutants.

SUMMARY

Five methods for the estimation of benz(c)acridine in urban particulate samples are compared, as are three methods for benzo(h)quinoline. The methods involving thin-layer chromatography followed by some form of fluorimetry are compared with the column chromatographic absorptiometric procedure for aza compounds. Through the quick estimation of benz(c)acridine the analyst should be able to determine the presence and amount of the polynuclear aza heterocyclic compounds in urban atmospheres and other appropriate samples.

REFERENCES

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