

# Thin Layer Chromatographic Separation and Spectrophotometric Determination of Benzo[*a*]Pyrene in Organic Extracts of Airborne Particulates

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IN RECENT YEARS much attention has been given to the analysis of benzo[*a*]pyrene (BaP) and other biologically active polynuclear hydrocarbons found in organic extracts of urban airborne particulates. The analytical procedure generally employed is a very lengthy process involving a 24-hour collection of particulates, a 6-hour Soxhlet extraction, column chromatographic separation, and investigation of many fractions by ultraviolet-visible absorption. This method (1), with various modifications, has been applied to the investigation of contaminated atmospheres, and considerable information has been published (2-5). Because the method requires above-average technical skill and 50- to 100-mg samples of organic extracts, it has not been widely accepted for routine analysis.

With recent advances in thin layer chromatographic techniques, a paper has been published describing the separation and the spectrophotometric and spectrophotofluorimetric determination of benzo[*a*]pyrene in organic extracts of airborne particulates (6). The procedures described for the determination of benzo[*a*]pyrene in air particulates offer advantages of speed, simplicity, and sensitivity not previously available. Recently, however, routine application of these procedures has disclosed certain objectionable disadvantages in the suggested separation procedures, elution solvents, sample sizes, interferences, recoveries, and reproducibilities. In this paper we report several important changes in this method that make it more applicable to the routine thin layer separation and spectrophotometric determination of benzo[*a*]pyrene present in organic extracts of airborne particulates. The modified procedures are compared with other methods, and data obtained by technicians of different technical capabilities are reported.

## EXPERIMENTAL

**Reagents and Apparatus.** Pentane and dichloromethane were redistilled, and fractions boiling at 35° and 40° C, respectively, were collected and used without drying. Other solvents were used as received from the supplier.

Benzo[*a*]pyrene, used as a standard, was recrystallized three times from spectrograde *n*-hexane and washed with several small volumes of redistilled pentane. The recrystallized standard still contained about 0.07% perylene and a

trace of anthanthrene. Additional crystallization did not help.

A Cary Model 15 recording spectrophotometer and a Beckman Model DU were used for all ultraviolet measurements. One-milliliter cells of 1-cm path length were also used.

Fluorescence measurements were made on an Aminco-Bowman spectrophotometer with the following settings: sensitivity, 50; slit arrangement, No. 2; and phototube, RCA type 1P21.

All thin layer chromatographic equipment and adsorbents were purchased from Brinkmann Instruments, Inc., Great Neck, N. Y.

Developed chromatograms were observed in a Chromatovue cabinet equipped with a 3600-Å light source (Ultra-Violet Products, Inc., San Gabriel, Calif.).

**Preparation and Storage of Plates.** Directions for preparing layers of aluminum oxide, 250-μ thick, were obtained from the supplier. After preparation of the 20- by 20-cm glass plates, proper activation was achieved by heating at 100° C for 30 minutes. Activated plates were stored in a vacuum desiccator adjusted to 45% relative humidity with aqueous sulfuric acid.

**Development Procedure.** One hundred milliliters of redistilled pentane was poured into a development chamber (12 × 3.875 × 10.875 inches) and allowed to equilibrate for 1 hour. By use of a labeling template (Brinkmann Instruments, Inc.), a 0.5- to 5-mg aliquot of a dichloromethane solution of organic residue of air particulates was spotted (proportionated among 5 to 10 spots) 1.5 cm from the bottom of a thin layer plate. A 2-μg aliquot of a dichloromethane solution of pure benzo[*a*]pyrene (proportioned between two spots) was carefully added to the same plate. Precautions were followed to ensure integrity of each spot, keep areas small, and avoid exposure to direct light. The plates were then placed in the development chamber and the solvent was allowed to travel 15 cm from the origin. The developed chromatogram was observed under a 3600-Å light source, and the fluorescent area of the pure standard and a corresponding area of the sample were quickly scored with a sharp stylus. Exposure to ultraviolet radiation was kept to a minimum. (Extended exposure to ultraviolet radiation resulted in visible darkening of the benzo[*a*]pyrene area of the sample chromatogram, and lower results were usually obtained.) The adsorbent containing the benzo[*a*]pyrene fraction and the pure standard were transferred to test tubes for subsequent elution and analysis.

**Elution and Analysis.** Ten milliliters of anhydrous ether were added to the test tubes containing sample and standards, and the slurries were mixed mechanically for 3 minutes. The mixtures were then quantitatively transferred to fine-porosity sintered-glass funnels positioned in 50-ml filtering flasks attached to a vacuum line and maintained at 50° ± 2° C in a water bath. Washing of the adsorbents was continued, at a rate approximately equal to the rate of evaporation, until 100 ml of ether had been used. When

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