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Note

Reversed two-dimensional technique for multiple separations of benzo[*a*]-pyrene from atmospheric aerosol samples

MICHAEL McGUIRK and SYLVIA J. MAINWARING*

Department of Industrial Science, University of Melbourne, Parkville, Victoria (Australia)

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There has been increasing concern in recent years over the potential cancer producing properties of polluted city air, particularly relating to levels of polynuclear aromatic hydrocarbons (PAHs) associated with atmospheric aerosols. Benzo[*a*]pyrene (B*a*P), because of its carcinogenic activity and its universal occurrence, is frequently used as an indicator of the potential health risk of the atmosphere. The present reported method arose out of such a study of B*a*P concentrations in the city of Melbourne, the results of which are published elsewhere[1].

Interferences in the ultraviolet and fluorescent spectra[2] between compounds within the PAH group necessitates, in most cases[3], their separation from each other as well as from other organic compounds present in atmospheric particulate extracts. Such separations can be satisfactorarily accomplished using thin-layer chromatography (TLC) and this has become the most frequently employed method to date.

The present paper outlines a novel two-dimensional TLC technique which greatly increases the speed and efficiency of treating multiple samples by processing up to eight per plate.

EXPERIMENTAL

Samples were collected on the roof of the Industrial Science building at the University of Melbourne, Melbourne, Australia. A "Staplex" high-volume sampler was used in a housing of recommended design[4]. Sampling periods were generally 24 h at flow-rates of approximately 80 m^3/h. The glass fibre paper (Vokes Australia VFM149) used as a collecting medium was pre-conditioned by pyrolysis at 400° for ½ h to remove organic binders. Papers were weighed before and after sample collection without any special equilibration procedures[5].

Filters were Soxhlet extracted within 4 h of collection, using 60 ml of analytical-grade benzene for a total of 100 cycles (approximately 4 h). The benzene extract was evaporated down to 3 ml and then made up to exactly 5 ml with benzene. An alternative method involving evaporation to dryness and dissolution in chloroform–ether (1:1) was discontinued because the chloroform–ether mixture caused significant retention of pure B*a*P at the point of application on the TLC plates used.

* To whom correspondence should be addressed.

Materials and procedures for plate preparation have been described elsewhere[1]. TLC separations of the extracted air samples were carried out as follows: (a) on aluminium oxide H (Merck, Darmstadt, G.F.R.), developed with *n*-pentane–diethyl ether (19:1), followed by a separation on cellulose acetate 20% (Machery, Nagel & Co., Düren, G.F.R.)[6]; and (b) on mixed aluminium oxide–cellulose acetate plates[7].

Preliminary experiments on aluminium oxide H at various levels of activation gave consistently good separations of the benzene-soluble fraction on plates activated at 100° for 30 min and equilibrated at room temperature for a further 20 min. The PAH band was eluted with diethyl ether after removal from the plate. A second separation of this band was carried out on aluminium oxide prior to separation trials on cellulose acetate.

Ethanol–methylene dichloride–water (20:10:1) and ethanol–toluene–water (17:4:4) solvent systems both gave good separations of the PAHs on the cellulose acetate plates although the first system separated B*a*P more completely.

Two-dimensional separations of the benzene-soluble fraction were carried out on mixed cellulose acetate–aluminium oxide plates (1:3) using *n*-pentane–diethyl ether (19:1) in the first and ethanol–methylene dichloride–water (20:10:1) in the second direction at 90° to the first. The position of B*a*P on these two-dimensional plates indicated that a reversed two-dimensional development might successfully separate this component as well as offer certain other advantages. The procedure used in this case was identical with that already described above except that the development in the second direction was run at 180° to the first.

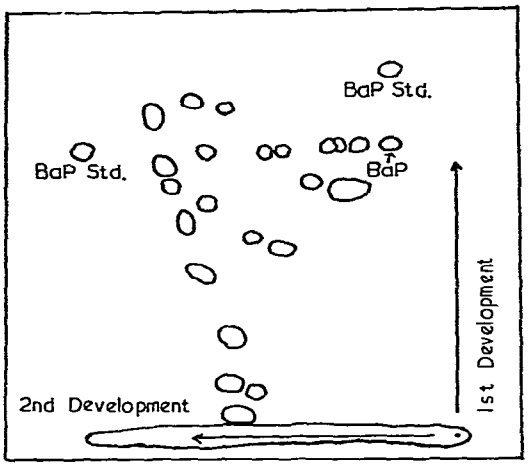
Identification and quantitative analysis were carried out using a Hitachi Model 204 spectrofluorometer equipped with an R212 photomultiplier tube and a Seiwa type 9820070 xenon arc lamp. The instrument was operated in the direct mode at a scanning speed of 60 nm/min and with fixed slit widths of 5 nm.

Fluorescent spectrofluorimeter standards, and internal standards were dilutions in cyclohexane of B*a*P supplied by Eastman-Kodak Co., Liverpool, Great Britain.

DISCUSSION

The most complete separation of the PAHs in the Melbourne aerosol sample was obtained from two successive separations of the benzene soluble fraction on aluminium oxide followed by a two-dimensional separation on cellulose acetate using ethanol–methylene dichloride–water (20:10:1) in the first direction and ethanol–toluene–water (17:4:4) in the second. Not only is this a time-consuming procedure but the number of extraction steps involved leads to low overall recoveries. When B*a*P is being taken as representative of the whole PAH group this long and repetitive procedure becomes unnecessary.

Satisfactory separations of B*a*P can be readily obtained by a conventional two-dimensional separation of the benzene-soluble fraction on mixed aluminium oxide–cellulose acetate plates, thereby reducing the number of thin-layer chromatograms per sample to one (Fig. 1). Internal standards can be used if only one compound is of interest by separate application of the standard prior to each development. Although this procedure is faster it still limits the number of samples per chromatogram to one.



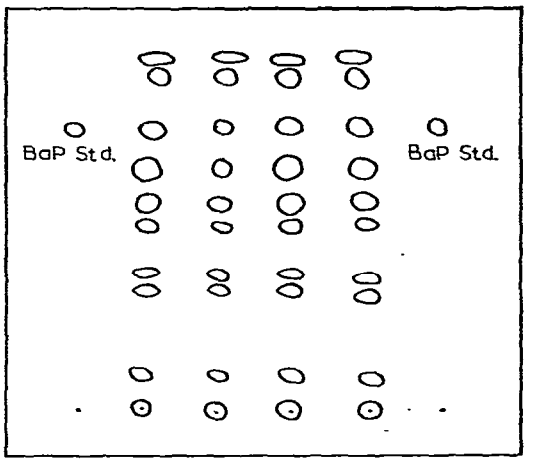
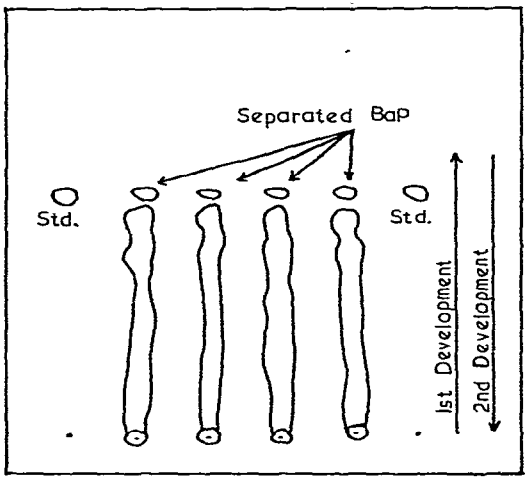


Fig. 1. Two-dimensional chromatogram of an extracted air sample on mixed aluminium oxide–cellulose acetate.

Fig. 2. Chromatogram of an extracted air sample on a mixed plate after development with *n*-pentane–diethyl ether.

Close inspection of the separation obtained in Fig. 1 showed that after the first development the B*a*P spot lay close to the top of the plate whereas after the second development the B*a*P spot had moved a small but significantly shorter distance than the spots above it. This suggested the use of the reversed separation through 180° which would have the advantage of allowing up to eight air samples to be developed on a single plate.

The results of this reverse procedure are shown in Figs. 2 and 3. Fig. 2 shows



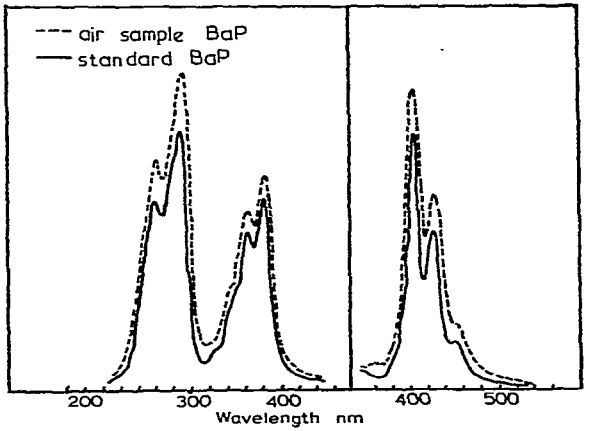


Fig. 3. Chromatogram of the sample shown in Fig. 2 after development in the reversed direction.

Fig. 4. Fluorescence excitation and emission spectra of B*a*P from a benzene-soluble fraction sample separated by the reversed two-dimensional method.

the separation in the first direction with the PAH band of compounds containing B*a*P lying towards the top of the plate. Fig. 3 is the same plate after development through 180° showing the B*a*P spots clearly removed the other fluorescing compounds at the top of the plate.

Spectra of the B*a*P spot from an air sample and a standard run on the same plate are compared in Fig. 4. The coincidence of the spectra is good with no variation in emission peak height ratios found on varying the excitation wavelength, further confirming the excellence of the separation. The degree of activation of the aluminium oxide is not critical for this method, good reversed separations being obtained on plates of very low activity.

There are two factors which justify the use of B*a*P as representative of the whole PAH group. Firstly there is as yet no established relationship between the carcinogenic hazard of the atmosphere and the concentrations of PAHs[8] and secondly the concentration ratios of individuals in the group tend to be constant in any one location. Monitoring B*a*P, the most carcinogenic of the PAHs in the atmosphere, would seem, in the light of present knowledge, to be a sufficient indicator and the method described here a useful advance on existing TLC techniques.

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