The Separation and Determination of Polycyclic Aromatic Hydrocarbons by Thin Layer Chromatography

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This study presents a method for the separation and the quantitative determination of polycyclic aromatic hydrocarbons by thin-layer adsorption chromatography.

Anthracene, pyrene, 1, 2-benzanthracene, 3, 4-benzpyrene, perylene and 1, 12-benzperylene were used as samples. They were all proved to be chromatographically pure. As the adsorbent layer, silica gel Woelm for thin layer chromatography (M. Woelm · Eschege, Germany) was used in a thickness of about 300 μ. The chromatoplate was activated by heating at 100°C for 15 min. in an electric furnace and then by heating it at 60°C and 0.1 mmHg for 30 min. in a vacuum desiccator. The activated plate was transferred quickly onto a hot metal plate of about 60°C, and 3 μl. of a benzene solution of the test sample were spotted on it. The ascending development was carried out in a chromatographic chamber using as the developing solvent *n*-hexane, *o*-dichlorobenzene and pyridine (10 : 1 : 0.5). The humidity in the chromatographic chamber was about 25%. The developing time was about 60 min., and the height of development was about 12 cm. Spots of the separated hydrocarbons on the plate were detected by fluorescence under a 365 mμ ultraviolet light.

The minimum values of aromatic hydrocarbons detectable by this method were within the range of 0.13 μg. to 0.0017 μg., as Table I shows. As these values obtained by acetylated paper chromatography have been reported to be 2 μg. to 5 μg.,[1] this method is very sensitive in detecting these compounds.

TABLE I. R_f VALUES AND MINIMUM DETECTABLE VALUES OF POLYCYCLIC AROMATIC HYDROCARBONS BY THIN LAYER CHROMATOGRAPHY

Substance	R_f value[1]	Minimum detectable value, μg.
Anthracene	0.51	0.03
Pyrene	0.41	0.13
1,2-Benzanthracene	0.32	0.010
3,4-Benzpyrene	0.23	0.007
Perylene	0.19	0.0017
1,12-Benzperylene	0.15	0.002

1) To obtain these R_f values, samples were spotted in 5 min. on a hot metal plate (ca. 60°C) in a room of 49% relative humidity at 29°C and developed in a chromatographic chamber of 27% relative humidity at 29°C for 60 min.

It must be noticed that the R_f values were highly affected by the humidity in the chromatographic chamber as well as by that in the environmental air in which samples were spotted. An increase in the humidity gave larger R_f values, resulting in an unsatisfactory separation of the aromatic hydrocarbons.

In this study, a linear relationship was found between the area of spot and the logarithm of the amount of aromatic hydrocarbon (Fig. 1). On the basis of this relationship, aromatic hydrocarbons were determined with an accuracy of ±5% if such experimental conditions as activation and humidity were fixed. In this connection, it is recommended that the amount of an unknown sample is calculated using a calibration curve which is drawn from three or more spots of known amounts of pure hydrocarbon on the plate on which the unknown sample is spotted.

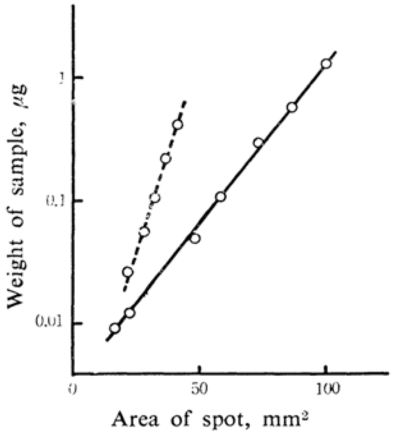

Fig. 1. Relationship between area of spot and logarithm of weight for 3,4-benzpyrene (——) and for 1,2-benzanthracene (----) on the thin layer chromatoplate.

This method might offer a useful tool for the microdetermination of carcinogenic hydrocarbons such as 3, 4-benzpyrene in air pollutants, exhaust gases or the smoke of cigarettes.

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[1] Th. Wieland and W. Kracht, *Angew. Chem.*, **69**, 172 (1957).