

Results and discussion

R_F values obtained with five solvent systems are given in Table I. Separation is achieved by these solvent systems and the detection limit of each compound is approximately 2 μg . It has been found that small and sharp spots are obtained with the addition of salts. The time required to ascend 10 cm from origin is 3–4 h. Separation is based on the formation of hydrogen bonds between the polyamide and the sample and adsorption or partition between the silica gel and the sample. The method described gives good resolution and reproducibility. The layer did not crack or peel and can be stored easily. Both sides of the glass are independent of each other and chromatography can be performed simultaneously on both sides.

Department of Pharmacy, Taipei Medical College,
Taipei, Taiwan (Republic of China)

HUNG-CHEH CHIANG

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Received December 10th, 1968

J. Chromatog., 40 (1969) 189–190

CHROM. 3888

The paper chromatography of tar polycyclic hydrocarbons

A correction of previous results

In 1957 a paper chromatographic solvent system was described which, without any previous column or other pretreatment, separated that fraction of the neutral portion of naturally occurring high-temperature tar mixtures which contains the carcinogenic polycyclic hydrocarbons¹. Then a new kind of the semi-quantitative spot evaluation *in situ* was added, *viz.* the horizontal diameter measurement calibration².

But some hydrocarbons were identified incorrectly^{1–3} due to the lack both of some authentic compounds and of reliable data concerning their fluorescence. In addition, the R_F values are higher when employing 45 × 45 cm Whatman No. 4 paper impregnated with a 10% petroleum ether–paraffin oil solution; this paper is thinner than the 46 × 57 cm sheets and therefore the separation is better. A characteristic paper chromatographic pattern was obtained and a revised identification of the hydrocarbons was made (Fig. 1). Previously a benzene–coal tar solution of suitable concentration had been applied. The spots were outlined over a U.V. transilluminator. Thus the highest analytical sensitivity was achieved. The R_F values may vary from run to run, but the sequence of the hydrocarbons is constant. The solution was applied

J. Chromatog., 40 (1969) 190–191

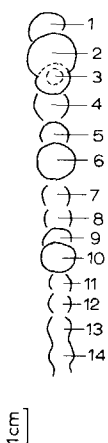
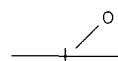


Fig. 1. R_F values, fluorescence colour, and identification of the strongly fluorescing hydrocarbons of the coal tar neutral portion fraction containing carcinogenic polycyclic hydrocarbons. (1) 0.46, violet, anthracene (the R_F value of authentic phenanthrene is 0.01 R_F unit lower; fluorescence violet, but it sublimes readily). (2) 0.44, emerald green, fluoranthene. (3) 0.42, green with violet ring, pyrene. (4) 0.39, blue-violet, chrysene. (5) 0.37, violet with touch of yellow, 1,2-benzanthracene. (6) 0.34, blue-green, 3,4-benzofluoranthene. (7) 0.32, blue, perylene with 1,2-benzopyrene. (8) 0.30, violet, 3,4-benzopyrene. (9) 0.28, violet with touch of yellow, dibenzanthracenes (1,2,5,6 and 1,2,3,4). (10) 0.26, ripe lemon yellow, 1,2,3,4-dibenzopyrene. (11) 0.24, violet, 1,12-benzoperylene. (12) 0.22, blue, anthanthrene. (13) 0.20, yellow with a touch of blue, not yet identified. (14) 0.18, yellow with a touch of blue, possibly coronene (this is unconfirmed because the standard hydrocarbon was not of a sufficient purity). O = origin.



with Kirby's automatic micropipette, 0.75 μ l in capacity, with the whole volume always being applied at once to the origin (1.5 cm from the edge), the depth of the ascending mobile phase (*viz.* methanol saturated with paraffin oil) being 1.0 cm. A glass cylinder, 20 cm in diameter and 40 cm in height, with ground glass stopper, was employed as a chromatographic tank. The developing time was 5 h at room temperature.

Research Institute of Industrial Hygiene and Occupational Medicine, ERNEST MALÝ
Dukelská 20, Bratislava (Czechoslovakia)

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Received November 25th, 1968