Solvent III: 40 ml isopropanol, 30 ml methyl ethyl ketone and 30 ml 0.880 ammonia solution.

The R_F values of the dyestuffs tested in each of these solvents is given in Table I. Nearly all the compounds showed up as coloured spots, but the intensity of these was increased by spraying the plates with an ethanolic solution of cupric acetate (which forms strongly coloured complexes with most of these dyestuffs).

Solvent I was unsuccessful for azo-dyestuffs soluble in water, since they were virtually insoluble in the non-polar organic solvents. The effectiveness of solvents II and III is shown by the fact that several of the compounds tested contained impurities which separated as very small spots on the plate.

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Received February 24th, 1964

J. Chromatog., 16 (1964) 231–233

Thin-layer chromatography of 4-dimethylaminoazobenzene and some of its metabolites

The potent rat hepatocarcinogen, 4-dimethylaminoazobenzene (DAB), is metabolised by rat liver to a variety of products. 4-Monomethylaminoazobenzene (MAB), 4-aminoazobenzene (AB), 4'-hydroxy-4-dimethylaminoazobenzene (OH-DAB) and possibly 4'-hydroxy-4-monomethylaminoazobenzene (OH-MAB) have been identified amongst those products which retain the azo group[1]. 4'-Hydroxy-4-aminoazobenzene (OH-AB) was not identified. In this work, the dyes were separated by a rather involved procedure by which the non-hydroxylated dyes were chromatographed on an alumina column and the hydroxylated dyes on a column of Hyflo Super Cel.

In the course of a reinvestigation of the metabolism of DAB we developed a simple and rapid method by means of which DAB, MAB, AB and their 4'-hydroxy-derivatives may be separated and identified.

Silica gel G (E. Merck, AG.) was vigorously shaken with 2 parts by weight of distilled water for $1^1/_2$ min and applied by means of the Shandon Unoplan Leveler to glass plates (20 cm × 20 cm) to give a layer 250 μ thick. Brief drying at room temperature was followed by activation at 100°/40 min after which the plates were kept over $CaCl_2$ in a desiccator.

The dyes (1 μg or less) were applied either singly or in admixture in 5 μl of methanol solution along a base-line 3 cm from the lower edge of the plate. The chromatograms were developed with a chloroform (reagent grade washed with water, dried over Na_2SO_4, redistilled and stabilised by addition of methanol to 1%)–methanol mixture (95:5). The solvent front was allowed to ascend 10 cm above

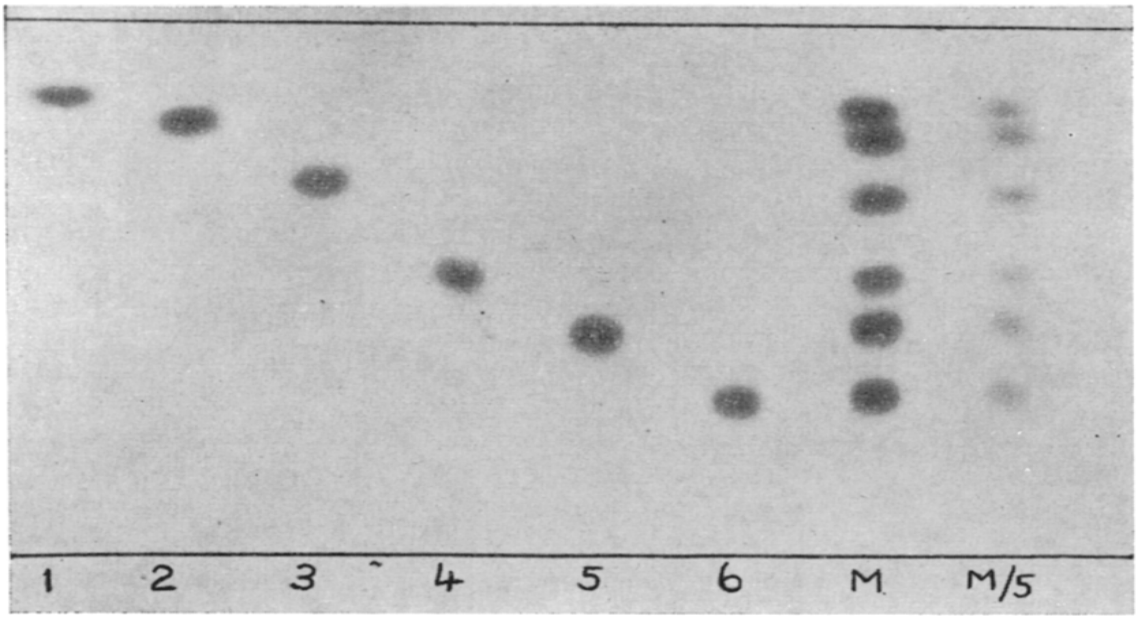

Fig. 1. Thin-layer chromatogram. 1 = DAB; 2 = MAB; 3 = AB; 4 = OH-DAB; 5 = OH-MAB; 6 = OH-AB; 1 μg of each dye. M = mixture consisting of 1 μg of each dye. M/5 = mixture consisting of 0.2 μg of each dye.

the base-line (about 20 min) when the plates were removed from the tank, allowed to dry and then exposed to concentrated HCl fumes for 5 min.

The spots produced were well formed and of characteristic colour and reproducible R_F values (Table I, Fig. 1). The colours of the hydroxy-dyes changed about

TABLE I

R_F VALUES AND COLOURS OF DYES

Dye	*Colours of spots*		R_F *values*
	Immediately	*After 10 min*	
DAB	rose red	rose red	0.85
MAB	orange red	orange red	0.79
AB	orange	orange	0.69
OH-DAB	brown	purple	0.52
OH-MAB	rust-red	purple	0.42
OH-AB	orange	purple red	0.30

10 min after the removal of the plates from the acid vapour; all of the spots faded over night. Mixtures containing all six dyes were readily separated and visualised when each was present in the range 1 μg –0.025 μg.

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Received April 27th, 1964