Paper chromatography of some catecholamines and related compounds

McGeer and Clark[1] have recently described the paper chromatography of some catecholamines and related compounds in twenty one solvent systems; in general, four solvents are used for the paper chromatographic separation of catecholamines, *i.e.* water-saturated phenol[2], phenol-0.1 *N* HCl[3], *n*-butanol saturated with hydrochloric acid[4], and *n*-butanol–acetic acid–water (4:1:5)[5]. These solvents require 12–24 h for a satisfactory development of the chromatogram and as a result of the prolonged exposure, oxidation of the catecholamines to the corresponding red aminochromes is usually observed on the paper; the extent to which this oxidation occurs can be reduced by flushing the tank with carbon dioxide, nitrogen or sulphur dioxide[6] prior to development. A further complication in the interpretation of chromatograms of catecholamines may result from the presence of double-spots which are sometimes produced during the chromatography of pure substances[7]. Shepherd and West[8,9] have reported that double-spots are formed when adrenaline and related compounds are chromatographed in the presence of trichloroacetic acid; the author has also observed the presence of double-spots (in addition to that due to the oxidation product) when pure samples of salts (tartrate or hydrochloride) of some of these amines are chromatographed in *n*-butanol–HCl or *n*-butanol–acetic acid–water solvents.

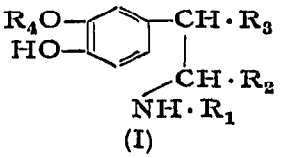
Another investigation[10] in this laboratory required a solvent suitable for the paper chromatography of mixtures containing adrenaline, which required a relatively short development time and gave chromatograms free from any ambiguity due to the artifacts mentioned above. A solvent system found to meet these requirements was methanol (160 ml)–water (40 ml)–quinoline (8 ml), which gave a satisfactory resolution of most of the mixtures examined within 4–5 h; no oxidation of the catecholamines to aminochromes was observed on the chromatograms despite the basic nature of the solvent, presumably due to the relative short development period. The usefulness of the methanol–water–quinoline solvent for the chromatography of catecholamines and some related amines was examined (see Table I). Good separations of the following groups of amines were obtained: (1) noradrenaline, adrenaline and N-isopropylnoradrenaline, (2) adrenaline and its methyl or ethyl ether and (3) metanephrine and normetanephrine. 3-Hydroxytyrosine (dopa) had an R_F value that was sufficiently low to permit its separation from any of the other amines used.

Experimental

The paper chromatographic examination of the amines shown in Table I was carried out in 1 l cylinders at room temperature, using the ascending technique, on Whatman No. 1 paper with methanol (160 ml)–water (40 ml)–quinoline (8 ml) as the developing solvent. A total rise of about 30 cm, taking 4–5 h, was employed. In addition to the catecholamines listed in Table I, the R_F values of two other amines were determined; these were phenylephrine, 1-(3-hydroxyphenyl)-2-methylaminoethanol (R_F = 0.68) and methoxamine, 2-amino-1-(2,5-dimethoxyphenyl)-1-propanol (R_F = 0.74). The catecholamines were detected by spraying the developed chromatograms with 1% potassium ferricyanide followed by exposure to ammonia. Metanephrine and normetanephrine (orange spots) and phenylephrine (yellow spot) were visualized with diazotised sulphanilic acid, and methoxamine (violet spot) with ninhydrin.

TABLE I

PAPER CHROMATOGRAPHY OF SOME PHENOLIC AMINES* IN METHANOL (160 ml)–WATER (40 ml)–QUINOLINE (8 ml)



(I)

Amine (I)	R_1	R_2	R_3	R_4	R_F *value*
Noradrenaline	H	H	OH	H	0.66
Adrenaline	CH_3	H	OH	H	0.72
N-Ethylnoradrenaline	C_2H_5	H	OH	H	0.75
N-Isopropylnoradrenaline	iso-C_3H_7	H	OH	H	0.82
Epinine	CH_3	H	H	H	0.69
Adrenaline methyl ether	CH_3	H	OCH_3	H	0.77
Adrenaline ethyl ether	CH_3	H	OC_2H_5	H	0.84
Noradrenaline methyl ether	H	H	OCH_3	H	0.72
Dopamine	H	H	H	H	0.65
Dopa	H	COOH	H	H	0.42
2-Methyladrenaline	H	CH_3	OH	H	0.77
Metanephrine	CH_3	H	OH	CH_3	0.76
Normetanephrine	H	H	OH	CH_3	0.69

* R_F values for some related amines are given in the experimental section.

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