Paper Chromatography of Primary Aromatic Amines

BIRGER EKMAN

Laboratory of Clinical Chemistry and Department of Physiological Chemistry, University of Lund, Sweden

The method of Consden, Gordon and Martin [1] for the analysis of amino acids by paper chromatography has been applied for the separation, identification and quantitative determination of various other substances, for instance sugars (Partridge [2], Flood, Hirst and Jones [3]), organic acids (Lugg and Overell [4]), adrenaline and associated compounds (James [5]), flavin nucleotides (Crammer [6]).

We have used this method for the analysis of primary aromatic amines. The procedure and the apparatus have been in principle the same as that of Consden *et al.*[1]. For the development of the spots we have diazotized the amines with sodium nitrite in acid solution. By coupling the resulting diazonium salts with ethyl-α-naphtylamine a characteristic red-violet colour is developed. Mixtures of benzene, amyl and methyl alcohol saturated with water have been found most suitable as solvents with regard to the differentiation of various amines and have the advantage of a short running time (4—6 hours at room temperature).

Procedure. Spots comprising the samples and containing 10—20 γ of the amines no. 1—13 and 100—200 γ of no. 14—17 in Table 1. are placed in the usual way on the paper. According to Edman [7] we have used the quick-filtering paper no. OB (50 × 50 cm), supplied by J. H. Munktells Pappersfabriksaktiebolag, Grycksbo, Sweden. After running the paper, it is dried at about 80° C till most of the organic solvents have disappeared. The paper is then sprayed with a 0.2 % sodium nitrite solution in 0.1 *N* HCl. After drying for some minutes at 50—80°, the paper is sprayed with a solution of 0.2 % ethyl-α-naphtylaminehydrochloride in conc. ethyl alcohol. The spots generally turn coloured immediately and are not influenced by heating. It is, however, easier to observe spots only faintly coloured, if the paper is dry.

Results. The R_F values for 17 primary aromatic amines run in various combinations of benzene, amyl and methyl alcohol, with or without the addition of hydrochloric acid or ammonia, are given in Table 1. Mixtures of amines are easily separated.

The compounds were as a rule recrystallized from ethanol. The compounds derived from tutocaine, trasentin, amidone and nupercaine were purified by dissolving in ethanol, resp. acetone and precipitating with ether.

The quaternary compounds are white, beautifully crystalline compounds, slightly soluble in alcohol and almost insoluble in ether and benzene. The bromides are easily soluble in water, the iodides, however, are slightly soluble also in water.

The methoiodide of trasentin is unstable when moist: It rapidly turns brown and deliquesces. When pure and dry it is quite stable.

The melting points and analyses of the quaternary derivatives are presented in Table 1.

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Received July 9, 1948.

Table 1. R_F values for 17 primary aromatic amines run in various solvents.

Solvents:					
% methyl alcohol	40	35	35	35	30.8
» amyl alcohol	20	17.5	17.5	17.5	15.2
» benzene	20	35	35	35	46
» water	20	12.5	12.5 (2 *N* HCl)	12.5 (4%NH_3)	8
R_F values for:					
1. sulphatiazole	0.86	0.78	0.53	0.64	0.64
2. sulphanilamide	0.78	0.73	0.45	0.69	0.53
3. sulphapyridine	0.87	0.82	0.53	0.73	0.72
4. diaminodiphenylsulphone	0.89	0.84	0.80	0.84	0.79
5. *p*-aminobenzoic acid	0.88	0.56	0.79	0.41	0.71
6. *o*- » »	0.90	0.61	0.82	0.53	0.84
7. *m*- » »	0.81	0.42	0.78	0.45	0.76
8. *p*-aminosalicylic »	0.79	0.44	0.79	0.46	0.71
9. sulphanilic »	0.74	0.73	0.50	0.50	0.35
10. α-naphtylamine	0.92	0.95	0.92	0.95	0.98
11. β »	0.92	0.93	0.89	0.95	0.93
12. *m*-phenylendiamine	0.62	0.75	0.35	0.71	0.50
13. *p*- »	0.25	0.75	0.28	0.70	0.46
14. *m*-toluidine	0.89	0.85	0.87	0.86	0.83
15. *o*- »	0.88	0.88	0.86	0.89	0.87
16. aniline	0.88	—	0.71	0.91	0.84
17. *p*-aminophenol	0.51	0.82	0.33	0.74	0.46

So we have, for instance, observed that on standing in alcoholic solution *p*-aminophenol with an R_F value of 0.31 (in benzene, amyl and methyl alcohol and 2 *N* HCl) gives oxidation products with R_F values of 0.26, 0.41, 0.63, and 0.73. A mixture of sulphanilamide, sulphatiasole, sulphapyridine and *p*-aminobenzoic acid could be differentiated in four spots distinctly separated after running the paper first with 46 % benzene, 15.2 % amyl alcohol, 30.8 % methyl alcohol and 8 % water and then at cross angles with 20 % benzene, 20 % amyl alcohol, 40 % methyl alcohol and 20 % *N* HCl.

Other organic solvents, for instance phenol, acetone and ethyl acetate have less favourable dissolving properties and the spots are drawn out. Combinations with pyridine have no advantages and run very slowly.

Discussion. The above method was intended for the analysis of aromatic amines present in urines from patients with tumors in the digestive tract or in the urine bladder (Ekman and Strömbeck [8]). Further it will be used in studies of the metabolism of cancerogenic substances which yield aromatic amines in the body, for instance azotoluene and acetaminofluorene. The method may also be of value in investigations of the antagonism between *p*-aminobenzoic acid and sulphapreparations in bacterial metabolism.

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Received August 27, 1948.