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SOAP THIN-LAYER CHROMATOGRAPHY OF SULPHONAMIDES AND AROMATIC AMINES*

LUCIANO LEPRI, PIER GIORGIO DESIDERI and DANIELA HEIMLER

Institute of Analytical Chemistry of the University of Florence, Florence (Italy)

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SUMMARY

Using soap thin-layer chromatography, we investigated the behaviour of sulphonamides and many primary aromatic amines. The influence on the chromatographic behaviour of the kind of detergent, the organic solvent percentage in the eluent in the presence and absence of acids, and the apparent pH of the eluent has been widely investigated. Many separations that cannot be effected on ion exchangers have been carried out.

INTRODUCTION

Soap thin-layer chromatography (TLC) has been employed with good results in the separation of catecholamines[1], primary aromatic amines[2] and food dyes[3]. This paper describes the application of this technique to the separation of several sulphonamides (which have already been studied on anion and cation exchange thin layers[4]) on layers of silanized silica gel impregnated with triethanolamine dodecylbenzensulphonate (DBS) and N-dodecylpyridinium chloride (N-DPC).

In order to obtain a complete picture of the chromatographic behaviour of the primary aromatic amines, we also studied their behaviour, other than on layers impregnated with DBS[2], even in the presence of N-DPC.

EXPERIMENTAL

The solutions of the test compounds were prepared in acetone (sulphonamides) or in 0.1 *M* hydrochloric acid in a 1:1 mixture of water and *n*-propanol (aromatic amines). The amount of compound deposited on the layer was between 1 and 4 μg. The compounds were detected with a solution of 5% N,N-dimethyl-*p*-aminobenzaldehyde in a 5:1 mixture of ethanol and glacial acetic acid.

The layers (thickness 300 μm) were prepared with a Chemetron automatic apparatus by mixing 20 g of silanized silica gel 60 HF (Merck, Darmstadt, G.F.R.) in 50 ml of 95% ethanol solution with a known concentration of detergent. All the

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measurements were carried out at 25°. The migration distance was 11 cm unless otherwise stated.

RESULTS AND DISCUSSION

Sulphonamides

   Aqueous-organic eluents in the presence of acids. Table I lists the R_F values of 13 sulphonamides and of sulphanilic acid on layers of silanized silica gel alone and impregnated with 4% DBS and N-DPC eluting with an aqueous–organic solution of 1 M acetic acid in 30% methanol (apparent pH 2.75). On layers of silanized silica gel alone (column 1) the sulphonamides are generally retained while sulphanilic acid runs with the solvent front. The affinity of the sulphonamides towards the stationary phase can be compared with that found in the case of the primary aromatic amines under the same elution conditions[2]. In the presence of DBS the sulphonamides exhibit R_F values that do not greatly differ from those on silica gel alone. In fact, in the presence of 4% DBS (see column 2), the mean decrease of the R_F values is about 0.08 unit and the affinities sequence is different only in the case of sulphaguanidine and sulphaisomidine. This decrease is noticeably smaller than that observed in the case of primary aromatic amines[2].

TABLE I

R_F VALUES OF SULPHONAMIDES ON THIN LAYERS OF SILANIZED SILICA GEL ALONE (a) AND IMPREGNATED WITH 4% DBS (b) OR 4% N-DPC (c) SOLUTION

Sulphonamide	1 M CH₃COOH in water–methanol (30%)			Water–methanol (7:3)			Water–methanol (4:1) (b)	pK$_{a2}$
	(a)	*(b)*	*(c)*	*(a)*	*(b)*	*(c)*		
Sulphathiazole	0.52	0.45	0.41	0.35	0.47	0.18	0.37	7.12
Sulphaguanidine	0.79	0.67	0.57	0.58	0.61	0.45	0.55	0.5
Sulphamerazine	0.43	0.40	0.39	0.33	0.74	0.23	0.67	7.06
Sulphadiazine	0.51	0.45	0.42	0.44	0.84	0.27	0.83	6.48
Sulphamethazine	0.42	0.35	0.36	0.27	0.30	0.24	0.23	7.37
Sulphanilamide	0.82	0.74	0.58	0.75	0.65	0.47	0.60	10.43
Sulphanilic acid	0.94	0.88	0.29	0.96	0.92	0.07	0.88	–
Sulphabenzamide	0.31	0.20	0.15	0.15	0.67	0.90	0.02	4.57
Sulphacetamide	0.66	0.59	0.46	0.79	0.73	0.25	0.62	5.38
Sulphapyridine	0.50	0.41	0.36	0.34	0.33	0.23	0.22	1.0
Sulphisomidine	0.64	0.34	0.59	0.44	0.73	0.35	0.65	–
Sulphisoxazole	0.36	0.33	0.18	0.65	0.86	0.05	0.84	–
Sulphallantoin	0.76	0.74	0.54	0.77	0.67	0.46	0.62	–
Sulphaquinoxaline	0.18	0.15	0.11	0.24	0.80	0.04	0.74	–

   The influence of substituent groups on the chromatographic behaviour of the sulphonamides can be deduced from columns 1 and 2, referring to sulphanilamide. The replacement of the amidic hydrogen by different groups involves (sulphaguanidine alone excepted) a sharp increase of the affinity towards the stationary phase.
   As the eluent acidity is increased by addition of hydrochloric acid, a sharp

increase of the R_F values on both layers is observed; the difference between the R_F values on the two layers, however, remains practically constant (*i.e.* 0.09 unit with 0.5 M hydrochloric acid). On the basis of such behaviour we can exclude the possibility that the small influence of DBS on the retention of the sulphonamides is due to protonation of the primary amino group ($pK_{a1} \approx 2$) and therefore that a cation exchange mechanism is operating as in the case of primary aromatic amines[2]. On layers impregnated with N-DPC (column 3) the sulphonamides are more retained than on silica gel alone or impregnated with DBS, with the exception of sulphisomidine which is more retained in the presence of DBS than of N-DPC. The strong retention of sulphanilic acid can be ascribed to an anion exchange process with the detergent. The small influence of the anion exchange process in the case of sulphaguanidine and sulphapyridine, notwithstanding their negative charge, can be ascribed to the presence of a second strongly basic azotate group which is in the protonated form at the pH of the eluent. In the case of the other sulphonamides a partition mechanism is operating, as on silica gel alone. The stronger retention in the presence of DBS and overall of N-DPC can be therefore ascribed to the non-ionic interactions of the sulphonamides with the detergent. Since most sulphonamides are in the neutral or in the cationic form at the pH of the eluent, the formation of ion pairs on N-DPC layers must be excluded.

As the percentage of methanol in the eluent is increased, at a constant acetic acid concentration (1 M), a sharp increase and a levelling of the R_F values of the sulphonamides is observed both on layers of silica gel alone and on those impregnated with cationic and anionic detergents. The strongest resolution is achieved at low methanol percentages, analogous to what observed for aromatic amines[2] and catecholamines[1].

Aqueous–organic eluents in the absence of acids. On elution with water–methanol mixtures (see columns 4, 5 and 6 of Table I), the sulphonamides behave differently than in the presence of acetic acid. A strong retention of all the sulphonamides is observed on layers impregnated with N-DPC; on silica gel alone or impregnated with DBS many compounds exhibit a smaller affinity towards the stationary phase. The different behaviour on the three layers can be explained by considering that in the water–methanol mixtures the primary amino group of the sulphonamides ($pK_{a1} \approx 2$) is in the free base form and many compounds are in the anionic form (see pK_{a2} values of Table I).

For these reasons the sulphonamides are more retained on layers impregnated with N-DPC, since the disappearance of the positive charge of the molecule and the possible formation of an anionic species involve a higher affinity of these compounds towards the stationary phase. Also on layers of silica gel alone the stronger retention of most sulphonamides with respect to elution with acid solutions must be ascribed to the disappearance of the positive charge on the primary amino group; the higher mobility of the remaining sulphonamides must be ascribed to the anionic form of these compounds. The behaviour of sulphabenzamide and sulphacetamide supports such an assumption; these compounds, in fact, are in the anionic form and exhibit R_F values higher than those obtained in the presence of acetic acid. Such behaviour is amplified by the presence of DBS on the layer. In this case, in fact, even those sulphonamides that, on the basis of their pK_{a2} values, are only partly in the anionic form, exhibit higher R_F values than those obtained eluting with acid eluents.

Similar behaviour has been observed for the same compounds on layers of polystyrene-based strong cation exchangers eluting with aqueous–organic mixtures[4].

As the percentage of methanol in the eluent is decreased (*i.e.* 20%), the layers of silanized silica gel cannot be used because the eluent rises very slowly and irregularly. On layers impregnated with 4% detergent, on the other hand, elution can also be effected with eluents containing small percentages (5–10%) of organic solvent. In Table I (column 7) are listed the R_F values of the sulphonamides eluting with water–methanol (4:1). Under such elution conditions the resolution power of the layer is noticeably increased, as shown by the improvement of the separation of sulphamerazine, sulphadiazine and sulphamethazine (Fig. 1), which cannot be effected on layers of anion and cation exchangers[4].
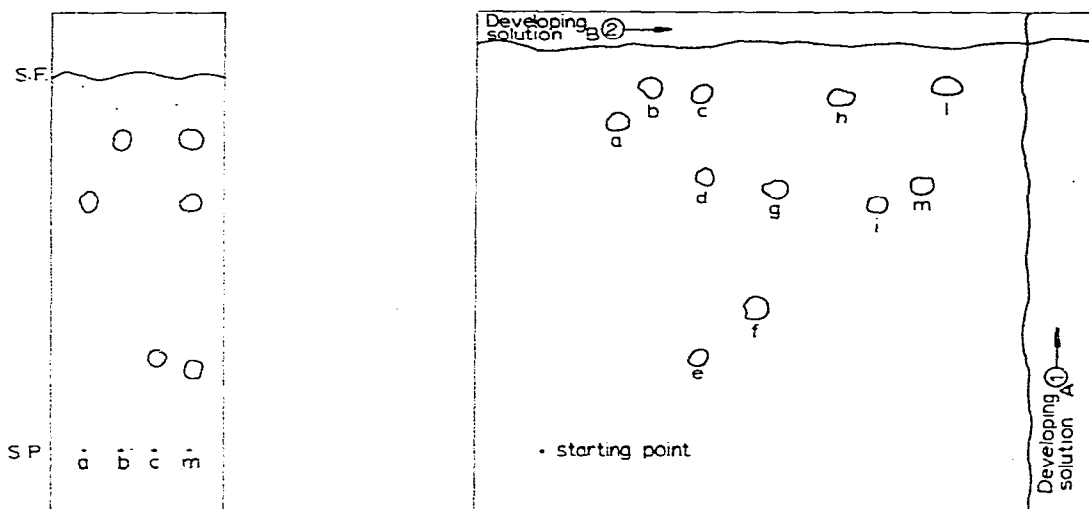

Fig. 1. Thin-layer chromatogram of some sulphonamides on silanized silica gel impregnated with 4% DBS solution. Migration distance, 12 cm; eluent, water–methanol (4:1). (a) Sulphamerazine; (b) sulphadiazine; (c) sulphamethazine; (m) mixture. S.P. = Starting point; S.F. = solvent front.

Fig. 2. Two-dimensional separation of 10 sulphonamides and of sulphanilic acid on silanized silica gel impregnated with 4% DBS solution. (1) Development with water–methanol (4:1) (solution A); (2) development with water–methanol–acetic acid (64.3:30:5.7) (solution B). Migration distance, 13 cm in the first and 14 cm in the second direction. (a) Sulphaquinoxaline; (b) sulphabenzamide; (c) sulphisoxazole; (d) sulphamerazine; (e) sulphamethazine; (f) sulphathiazole; (g) sulphacetamide; (h) sulphadiazine; (i) sulphaguanidine; (l) sulphanilic acid; (m) sulphallantoin.

With the two-dimensional techniques, 10 sulphonamides and sulphanilic acid can be separated from each other (see Fig. 2) eluting in the first direction with water–methanol (4:1) and in the second with water–methanol–acetic acid (64.3:30:5.7).

The replacement of methanol with acetone involves a general increase of the R_F values without improvement from an analytical standpoint, since for many compounds a levelling of the R_F values is observed. With more polar solvents, such as N,N-dimethylformamide, these layers cannot be employed since the eluent does not rise.

Aromatic amines

Table II lists the R_F values of 35 primary aromatic amines on layers of silanized silica gel alone (column 1) and impregnated with 4% N-DPC (column 2) eluting with 0.1 M ammonium acetate and 0.1 M ammonia in 30% methanol (apparent pH 9.25). The aromatic amines, which are in the free base form at the pH of the eluent, exhibit a high affinity towards the silanized silica gel. In the presence of detergent they are

TABLE II

R_F VALUES OF PRIMARY AROMATIC AMINES ON THIN LAYERS OF SILANIZED SILICA GEL ALONE AND IMPREGNATED WITH 4% N-DPC

Eluents: 1 = 0.1 M CH$_3$COONH$_4$ + 0.1 M NH$_3$ in water–methanol (30%); 2 = 0.1 M CH$_3$COONH$_4$ + 0.1 M NH$_3$ in water–methanol (20%) (pH 9.20); 3 = 0.1 M CH$_3$COOH + 0.1 M NaCl in water–methanol (20%) (pH 3.25); 4 = 0.1 M HCl + 0.1 M CH$_3$COOH in water–methanol (20%) (pH 1.40); 5, 6 and 7 = 0.1 M, 1 M and 2 M, respectively, CH$_3$COOH in water–methanol (20%).

Amine	Silica gel, 1	Silica gel impregnated with 4% N-DPC						
		1	2	3	4	5	6	7
Aniline	0.54	0.26	0.22	0.71	0.75	0.72	0.73	0.92
m-Toluidine	0.36	0.19	0.14	e.s.	0.67	0.68	0.74	0.79
o-Toluidine	0.38	0.19	0.16	e.s.	0.57	0.61	0.71	0.77
p-Toluidine	0.35	0.18	0.14	0.68	0.71	0.75	0.75	0.81
2,4-Dimethylaniline	0.24	0.10	0.07	e.s.	0.53	0.53	0.67	0.71
2,6-Dimethylaniline	0.29	0.14	0.11	0.15	0.29	0.21	0.42	0.50
m-Nitroaniline	0.35	0.11	0.09	0.09	0.30	0.10	0.16	0.28
o-Nitroaniline	0.24	0.09	0.05	0.05	0.05	0.07	0.10	0.17
p-Nitroaniline	0.34	0.10	0.06	0.06	0.10	0.09	0.14	.23
2,4-Dinitroaniline	0.23	0.05	0.03	0.03	0.04	0.05	0.09	0.15
m-Bromoaniline	0.25	0.07	0.04	0.06	0.30	0.10	0.16	0.28
p-Bromoaniline	0.25	0.08	0.06	0.08	0.30	0.13	0.14	0.17
p-Bromoaniline	0.23	0.07	0.05	0.06	0.30	0.09	0.24	0.37
p-Chloroaniline	0.28	0.09	0.06	0.10	0.29	0.14	0.35	0.44
2,4-Dichloroaniline	0.15	0.09	0.06	0.08	0.08	0.15	0.34	0.44
m-Anisidine	0.40	0.24	0.20	e.s.	0.56	0.53	0.66	0.70
o-Anisidine	0.34	0.21	0.18	0.59	0.67	0.66	0.72	0.78
p-Anisidine	0.42	0.31	0.26	0.80	0.83	0.85	0.85	0.86
m-Phenylenediamine	0.73	0.52	0.49	0.94	0.96	0.96	0.96	0.96
o-Phenylenediamine	0.67	0.41	0.37	0.78	0.80	0.81	0.85	0.86
p-Phenylenediamine	0.75	0.67	0.60	0.94	0.98	0.95	0.97	0.97
2,6-Diaminotoluene	0.67	0.53	0.50	0.91	0.90	0.95	0.95	0.96
2,4-Diaminotoluene	0.65	0.40	0.34	0.87	0.87	0.92	0.92	0.94
3,4-Diaminotoluene	0.46	0.14	0.10	0.11	0.29	0.23	0.23	0.34
α-Naphtylamine	0.21	0.07	0.03	0.06	0.30	0.09	0.24	0.38
4-Amino-DPA	0.15	0.05	0.02	0.17	0.29	0.31	0.48	0.53
2-Amino-DPA	0.10	0.03	0.02	0.02	0.06	0.11	0.11	0.23
3-Methoxy-4-amino-DPA	0.09	0.04	0.02	0.14	0.29	0.29	0.47	0.55
3-Methoxy-4'-amino-DPA	0.14	0.05	0.02	0.22	0.29	0.45	0.50	0.56
4,4'-Diamino-DPA	0.37	0.28	0.28	0.93	0.96	0.97	0.97	0.97
2,4-Dinitro-4'-amino-DPA	0.07	0.02	0.02	0.02	0.26	0.03	0.13	0.26
Benzidine	0.23	0.08	0.06	e.s.	0.65	0.65	0.76	0.81
o-Tolidine	0.12	0.04	0.02	0.10	0.29	0.26	0.64	0.73
o-Dianisidine	0.07	0.02	0.02	e.s.	0.29	0.07	0.31	0.49

DPA = Diphenylamine; e.s. = elongated spot.

more strongly retained. Such behaviour can be ascribed to their interactions with the side-chain of the cationic detergent.

The presence of the detergent involves also a higher resolving power of the layer towards those compounds with two amino groups in the molecule, such as phenylenediamines and diaminotoluenes. These compounds, which cannot be separated from each other on silanized silica gel, exhibit well differentiated R_F values on layers impregnated with N-DPC.

The influence of the substituent groups on the chromatographic behaviour of the amines can be seen from the data referring to aniline in Table II. The presence of $-CH_3$, $-NO_2$, $-Cl$ and $-Br$ groups involves an increase of the retention by the two layers. The opposite behaviour is observed if an $-NH_2$ group is present in the ring. When two substituent groups of opposite behaviour are present, the chromatographic behaviour cannot be predicted, because it depends on the mutual position of the three groups in the molecule (*i.e.* diaminotoluenes). The presence of a second aromatic ring results in sharp decrease of the R_F value.

Influence of methanol in the eluent. On changing the percentage of methanol in the eluent, the behaviour of aromatic amines is similar to that of sulphonamides (see Table II, columns 2 and 3). Comparison of these data indicates that the decrease of the methanol percentage involves a lowering of the R_F values and in some cases an increase of the resolving power of the layer.

Influence of the pH of the eluent. The R_F values of the amines on layers impregnated with 4% N-DPC eluting with solutions at apparent pH in the range 9.2 and 1.4, constant ionic strength (0.1) and 20% methanol are reported in Table II (columns 3, 4 and 5). In the pH range 6.95–3.25 many compounds (*i.e.* benzidine and its derivatives, toluidines, dimethylanilines and anisidines) give rise to elongated spots. Elution with 0.1 M acetic acid and 0.1 M hydrochloric acid (column 5) results in a double front, the first with $R_F = 0.30$. The data in column 5 show that many amines run with the first solvent front.

As regards the affinity of the amines towards the stationary phase, a sharp decrease is observed for most compounds as the pH is decreased. Such affinity decrease is correlated with the protonation of one or more amino groups in the molecule. The amines with more marked acidic characteristics are either not at all (*o*-nitroaniline, 2,4-dinitroaniline, 2,4-dichloroaniline) or only weakly (*p*-nitroaniline, chloro- and bromoanilines) affected by the pH decrease.

Fig. 3 shows some typical R_F/pH trends. In the curves (a), (b) and (c) the sharp R_F change occurs in the pH range 9.20–6.95. On the basis of the pK_{a1} values of these amines (between 4.47 and 6.08), such compounds should generally be in the free base form at both these pH values. In order to explain the results we carried out pH measurements on the layer as described in a previous paper[5], when we found that the pH of the layer is markedly lower than that of the eluent at pH 6.95 and that it decreases changing from the starting point to the solvent front.

Change of the acetic acid concentration. Columns 6, 7 and 8 in Table II list the R_F values of the amines on changing the acetic acid concentration in the eluent at constant methanol concentration (20%). The data in column 6, which refer to a 0.1 M acetic acid concentration (apparent pH 3.25), can be compared with those of column 4, which refer to an eluent with the same pH value, the same acetic acid concentration but a different ionic strength. The R_F values are lower in the presence of 0.1 M NaCl.
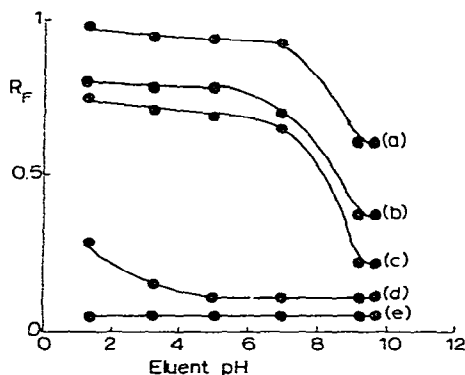
Fig. 3. R_F values versus apparent pH of the eluent for some aromatic amines on thin layers of silanized silica gel impregnated with 4% N-DPC solution. Eluents, 0.1 M hydrochloric acid in water–methanol (20%)–acetic acid (0.57%) (pH 1.4); 0.1 M sodium chloride in water–methanol (20%)–acetic acid (0.57%) (pH 3.25); 0.1 M sodium acetate in water–methanol (20%)–acetic acid (0.57%) (pH 5.00); 0.1 M ammonium acetate in water–methanol (20%) (pH 6.95); 0.1 M ammonium acetate and 0.1 M ammonia in water–methanol (20%) (pH 9.20); 0.1 M ammonium acetate and 0.5 M ammonia in water–methanol (20%) (pH 9.60). (a) p-Phenylendiamine; (b) o-phenylendiamine; (c) aniline; (d) 2,6-dimethylaniline; (e) o-nitroaniline.

Furthermore, without NaCl, compact spots are obtained even for those amines that give rise to elongated spots.

As the acetic acid concentration is increased, an increase of the R_F values is observed for all the amines, including those not protonated in the pH range 3.25–2.45. Such an increase may therefore be ascribed not only to the pH decrease of the eluent but also to the increase in the percentage of organic solvent (acetic acid plus methanol).
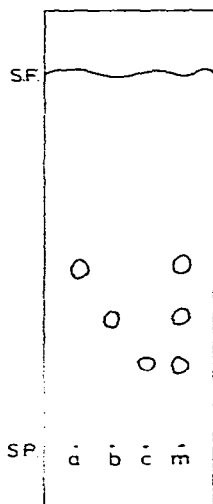

Fig. 4. Thin-layer chromatogram of some aromatic amines on silanized silica gel impregnated with 4% N-DPC solution. Migration distances, 12 cm; eluents, 0.1 M ammonium acetate and 0.1 M ammonia in water–methanol (20%). (a) 2,6-Diaminotoluene; (b) 2,4-diaminotoluene; (c) 3,4-diaminotoluene; (m) mixture of diaminotoluenes.

Analytical applications

On the basis of the R_F values and of the compactness of the spots many interesting separations can be effected. Fig. 4 shows the separation of the three isomers of diaminotoluene, which cannot be achieved on polystyrene or cellulose-based anion exchangers. Figs. 5 and 6 show some separations we carried out. The separation of the three isomers of toluidine and that of 4-aminodiphenylamine and 4-amino-4'-methoxydiphenylamine can be carried out on other supports only with great difficulty.
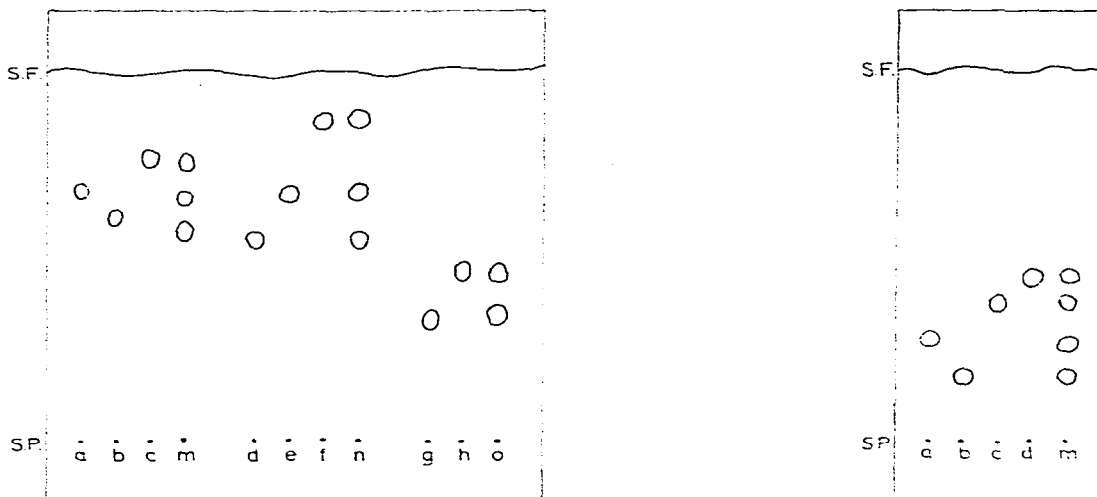

Fig. 5. Thin-layer chromatogram of some aromatic amines on silanized silica gel impregnated with 4% N-DPC solution. Migration distances, 12 cm; eluents, water–methanol–acetic acid (79.43:20:0.57) (a) *o*-Toluidine; (b) *m*-toluidine; (c) *p*-toluidine; (m) mixture of the toluidines; (d) *o*-anisidine; (e) *m*-anisidine; (f) *p*-anisidine; (n) mixture of the anisidines; (g) 4-aminodiphenylamine; (h) 4-amino-4'-methoxy-diphenylamine; (o) mixture of aminodiphenylamines.

Fig. 6. Thin-layer chromatogram of some aromatic amines on silanized silica gel impregnated with 4% N-DPC solution. Migration distances, 12 cm; eluents, water–methanol–acetic acid (68.6:20:11.4). (a) *m*-Bromoaniline; (b) *o*-bromoaniline; (c) *p*-bromoaniline; (d) *p*-chloroaniline; (m) mixture of chloro- and bromoanilines.

The separation of the three bromoanilines and *p*-chloroaniline has also been achieved on AG 1-X4 layers[6]. The R_F sequence on these two layers is the same, which indicates a close similarity in the behaviour of the amines on layers impregnated with N-DPC and on polystyrene-based anion exchangers.

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