

CHROM. 11,713

REVERSED-PHASE AND SOAP THIN-LAYER CHROMATOGRAPHY OF ALIPHATIC MONO- AND POLYAMINES

L. LEPRI, P. G. DESIDERI and D. HEIMLER

Institute of Analytical Chemistry, Via G. Capponi 9, University of Florence, Florence (Italy)

(Received January 10th, 1979)

SUMMARY

The chromatographic behaviour of 25 aliphatic mono- and polyamines has been investigated on layers of silanized silica gel alone and impregnated with anionic and cationic detergents. The influence of the percentage of detergent and of the apparent pH of the eluent on the retention mechanism has been studied. Ion exchange and/or partition contribute to the retention of the amines. Many separations of mono- and polyamine mixtures have also been carried out by two-dimensional technique.

INTRODUCTION

With the recent commercial availability of silanized silica gel, the study of many classes of organic compounds by reversed-phase¹ and soap thin-layer chromatography (TLC)²⁻⁵ has been considerably developed. This paper concerns the behaviour of aliphatic mono- and polyamines on layers of silanized silica gel alone and impregnated with triethanolamine dodecylbenzene sulphonate (DBS) and N-dodecylpyridinium chloride (N-DPC). Such compounds, in addition to being important from a biological standpoint, are suitable for a study of retention mechanisms in the presence and absence of detergents and for a comparison with the results achieved on layers of ion exchangers⁶.

EXPERIMENTAL

The solutions of the test compounds were prepared in 0.01 M hydrochloric acid in a 1:1 (v/v) mixture of water and *n*-propanol. The amount of each amine deposited on the layer was between 1 and 3 μ g. The amines were detected by spraying the wet layers with a solution of 1% ninhydrin in a 5:1 (v/v) mixture of pyridine and glacial acetic acid and then heating the layers at 100° for 5 min.

The layers (thickness 300 μ m) were prepared with a Chemetron automatic apparatus by mixing 20 g of silanized silica gel 60 HF (C₂) (Merck, Darmstadt, G.F.R.) in 50 ml of 95% ethanol with a known concentration of detergent. All of the measurements were carried out at 25°. The migration distance was 11 cm unless otherwise stated.

TABLE I

 R_F VALUES OF PRIMARY MONO- AND DIAMINES IN VARIOUS SYSTEMS

Amine	Silanized silica gel		Silanized silica gel impregnated with DBS					
	A*	B**	1% DBS		2% DBS		4% DBS	
			A*	B**	A*	B**	A*	B**
Methylamine	0.94	0.93	0.77	0.82	0.65	0.78	0.54	0.77
Ethylamine	0.93	0.92	0.73	0.80	0.61	0.76	0.52	0.74
Ethanolamine	0.94	0.94	0.83	0.85	0.71	0.83	0.62	0.82
<i>n</i> -Propylamine	0.89	0.92	0.66	0.79	0.54	0.74	0.49	0.63
<i>n</i> -Butylamine	0.84	0.88	0.51	0.66	0.32	0.58	0.20	0.43
Isobutylamine	0.88	0.92	0.54	0.69	0.38	0.64	0.25	0.50
<i>n</i> -Amylamine	0.72	0.70	0.33	0.44	0.19	0.42	0.10	0.20
Isoamylamine	0.75	0.73	0.34	0.45	0.20	0.43	0.11	0.22
<i>n</i> -Hexylamine	0.59	0.56	0.18	0.37	0.10	0.25	0.04	0.15
<i>n</i> -Heptylamine	0.47	0.44	0.10	0.21	0.04	0.13	0.02	0.07
<i>n</i> -Octylamine	0.33	0.30	0.04	0.12	0.02	0.07	0.01	0.03
<i>n</i> -Decylamine	0.13	0.10	0.01	0.04	0.00	0.03	0.00	0.02
<i>n</i> -Dodecylamine	0.04	0.03	0.00	0.00	0.00	0.00	0.00	0.00
<i>n</i> -Tetradecylamine	0.02	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Benzylamine	0.79	0.73	0.37	0.52	0.23	0.49	0.14	0.32
1,2-Diaminoethane	0.94	0.92	0.52	0.78	0.25	0.74	0.15	0.57
1,2-Diaminopropane	0.94	0.92	0.50	0.77	0.24	0.73	0.15	0.55
1,3-Diaminopropane	0.94	0.92	0.53	0.86	0.28	0.83	0.17	0.63
1,4-Diaminobutane	0.94	0.92	0.50	0.86	0.27	0.83	0.16	0.63
1,5-Diaminopentane	0.94	0.92	0.50	0.84	0.24	0.80	0.11	0.60
1,6-Diaminohexane	0.94	0.90	0.49	0.79	0.22	0.73	0.09	0.52
1,7-Diaminoheptane	0.94	0.90	0.35	0.77	0.18	0.64	0.07	0.37
1,8-Diaminooctane	0.94	0.90	0.22	0.63	0.08	0.48	0.04	0.22
Spermine	0.94	0.92	0.03	0.67	0.01	0.35	0.00	0.06
Spermidine	0.94	0.93	0.15	0.78	0.08	0.54	0.02	0.19

* Eluent A: water-methanol-acetic acid (64:3.30:5.7).

** Eluent B: 0.25 *N* hydrochloric acid in water-methanol (30%)-acetic acid (5.7%).

RESULTS AND DISCUSSION

Aqueous-organic eluents in the presence of acids

In Table I are reported the R_F values of 25 aliphatic mono- and polyamines on layers of silanized silica gel alone and impregnated with increasing amounts of DBS, eluting with aqueous-organic solutions containing the same percentage of methanol (30%) and acetic acid (5.7%) but with different pH values (owing to the presence of hydrochloric acid in one eluent). The DBS concentrations refer to the alcoholic solution in which the silanized silica gel was suspended when the layers were prepared. It should be noted that in the absence of detergent no differences are observed in the chromatographic behaviour of the amines with the two eluents (see results columns 1 and 2 in Table I).

The polyamines run almost with the solvent front ($R_F \geq 0.90$) and the monoamines exhibit different behaviour depending on their molecular weight. The early members of the series (methylamine, ethylamine and ethanolamine) run with the solvent front, but for the others a considerable increase in retention is observed with increasing number of carbon atoms in the side-chain.

From an analytical standpoint, reversed-phase chromatography on silanized silica gel gives rise to good results only for the monoamines (those with lower molecular weight being excluded). It is possible to separate a mixture of eight amines with chain length C_1 (or C_2), C_4 , C_5 , C_6 , C_7 , C_8 , C_{10} and C_{12} , but the C_1 , C_2 and C_3 amines cannot be separated from each other.

On layers impregnated with DBS, the behaviour of the amines is correlated with, in addition to the amount of detergent, also the apparent pH of the eluent (see results columns 3–8 in Table I). On increasing the DBS concentration, the amines are more retained. For most of them, however, a limiting R_F value at high detergent concentrations is not reached, which is different to the results observed with catecholamines². It should also be noted that the presence of DBS does not involve a change in the affinity sequence of those amines which are retained, even on silanized silica gel alone.

The behaviour of the monoamines with low molecular weight is interesting as they exhibit different R_F values even with low percentages of detergent on the layer. In particular, propylamine is more retained than methylamine; ethyl amine and ethanolamine behave differently as the presence of a hydroxyl group in the latter results in the highest R_F value of all the monoamines. The length of the side-chain of monoamines affects their chromatographic behaviour; with diamines, on the other hand, the influence of the side-chain is noticeable only for the members with high molecular weight. It should be noted that the smaller retention of ethanolamine with respect to the monoamines and the weak influence of the aliphatic side-chain with of diamines have already reported for layers of polystyrene-based ion exchangers⁶.

On eluting with hydrochloric acid solution (eluent B), a smaller retention (at a given detergent concentration on the layer) than with eluent A is observed. This effect occurs in general with polyamines, in accordance with their greater charge, and with monoamines only for members with a small number of carbon atoms. Decyl-, dodecyl- and tetradecylamine remain at the starting point, irrespective of the DBS concentration on the layer.

On layers impregnated with N-DPC, the amines are less retained than on layers of silanized silica gel alone or impregnated with DBS. In particular, on layers impregnated with 4% of N-DPC, eluting with eluent A, monoamines with less than six carbon atoms and all the polyamines run with the solvent front. The other amines exhibit R_F values between 0.06 (C_{14}) and 0.90 (C_6).

Aqueous-organic eluents in the absence of acids

Owing to the marked basic characteristics of the aliphatic amines, the lack of acids in the eluent does not result in the disappearance of the positively charged forms. For this reason, on layers of silanized silica gel alone or impregnated with 4% of N-DPC, eluting with water-methanol (7:3), considerable differences in the chromatographic behaviour of the amines in comparison with elution in the presence of 1M acetic acid are not observed.

On layers impregnated with DBS, on the other hand, the absence of acids results in a sharper retention for most amines, confirming the important role played by the functional group of the anionic detergent. The adsorption of amines is so strong that, even eluting with water-methanol (1:1), their R_F values are less than 0.22; all of the polyamines remain at the starting point.

Only at high methanol concentrations in the eluent (70%) is a higher mobility of the amines observed. This effect results in a difference in the chromatographic behaviour of the monoamines of higher molecular weight, whereas with diamines a general levelling of the R_F values is observed (R_F between 0.32 and 0.35).

The increase in R_F values with increasing methanol concentration in the eluent agrees with the results observed with catecholamines², aromatic amines³ and sulphadiazine⁵.

Retention mechanism

On layers of silanized silica gel alone a partition mechanism is operating and the chromatographic behaviour of the amines is not substantially affected either by the apparent pH or by the ionic strength of the eluent (see results columns 1 and 2 in Table I). For homologous series of monoamines, with a partition chromatographic system, the following equation should apply:

$$R_M(B) - R_M(A) = \Delta R_M(\text{CH}_2) \quad (1)$$

where $R_M(B)$ is the R_M value of the amine B, which differs from amino A by a CH_2 group, and $\Delta R_M(\text{CH}_2)$ is the change in R_M on introduction of a CH_2 group and is constant for a homologous series⁷.

Constancy of $\Delta R_M(\text{CH}_2)$, however, occurs only if the R_F values are in the range 0.2–0.8 and if horizontal chromatography is used in order to avoid equilibration difficulties. Furthermore, molecular interactions, which may cause anomalous R_M values, must be absent.

From the data in Table II, it can be seen that constancy of $\Delta R_M(\text{CH}_2)$ substantially occurs on layers of silanized silica gel alone with both eluents. The differences that have been found can probably be ascribed to the equilibration difficulties owing to the ascending chromatography used. Therefore, in these two chromatographic systems the mean value of $\Delta R_M(\text{CH}_2)$ is 0.245.

TABLE II

R_M VALUES OF MONOAMINES ON THIN LAYERS OF SILANIZED SILICA GEL ALONE ELUTING WITH (A) WATER–METHANOL (30%)–ACETIC ACID (5.7%) AND (B) 0.25 *N* HYDROCHLORIC ACID IN WATER–METHANOL (30%)–ACETIC ACID (5.7%)

Amine	$R_{M(A)}$	$\Delta R_{M(A)}(\text{CH}_2)$	$R_{M(B)}$	$\Delta R_{M(B)}(\text{CH}_2)$
<i>n</i> -Amylamine	-0.410	0.252	-0.368	0.263
<i>n</i> -Hexylamine	-0.158	0.210	-0.105	0.210
<i>n</i> -Heptylamine	0.052	0.256	0.105	0.263
<i>n</i> -Octylamine	0.308	0.259	0.368	—
<i>n</i> -Decylamine	0.826	—	—	—

On layers impregnated with DBS, the retention mechanism of the monoamines changes as the percentage of detergent on the layer increases. On layers impregnated with 2% of DBS, the chromatographic system still seems to be controlled by a partition process. The mean value of $\Delta R_M(\text{CH}_2)$ is 0.329 (see Table III).

It should be noted, however, that the scatter of experimental values for this

TABLE III

R_M VALUES OF MONOAMINES ON THIN LAYERS OF SILANIZED SILICA GEL IMPREGNATED WITH 2% DBS SOLUTION ELUTING WITH (A) WATER-METHANOL (30%)-ACETIC ACID (5.7%) AND (B) 0.25 *N* HYDROCHLORIC ACID IN WATER-METHANOL (30%)-ACETIC ACID (5.7%)

Amine	$R_{M(A)}$	$\Delta R_{M(A)}(CH_2)$	$R_{M(B)}$	$\Delta R_{M(B)}(CH_2)$
<i>n</i> -Propylamine	-0.070	0.397	-0.454	0.314
<i>n</i> -Butylamine	0.327	0.303	-0.140	0.280
<i>n</i> -Amylamine	0.630	0.324	0.140	0.337
<i>n</i> -Hexylamine	0.954	—	0.477	0.349
<i>n</i> -Heptylamine	—	—	0.826	—

parameter is wider than that observed on silanized silica gel alone, showing that ion exchange between the protonated amine and the functional group of the detergent is also operating (even if only to a small extent). The increase in the R_F values of the monoamines on increasing the acidity of the eluent (see results columns 5 and 6 of Table I) agrees with such an assumption.

The mean value of $\Delta R_M(CH_2)$ on layers impregnated with 2% of DBS is higher than that found on silanized silica gel alone. This result demonstrates the influence of the detergent on the retention process and gives rise to interesting applications from an analytical standpoint as the separation between two amines that differ by a CH_2 group is sharper on the impregnated layers.

As the percentage of DBS on the layer is increased (to 4%), the retention mechanism seems no longer to be affected mainly by the partition process as the $\Delta R_M(CH_2)$ values for the monoamines (those for which this parameter can be determined, that is, for C_5 and below) change much more than in the two previous instances.

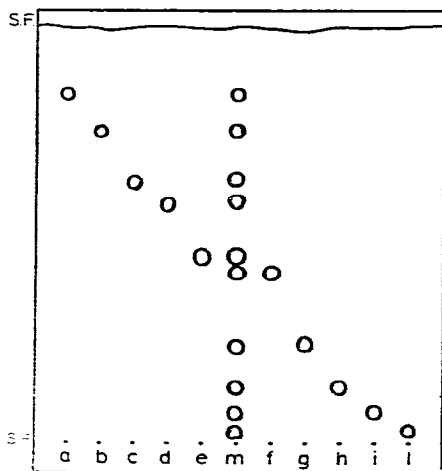


Fig. 1. Thin-layer chromatogram of ten monoamines on silanized silica gel impregnated with 2% DBS solution. Migration distance: 13.5 cm. Eluent: 0.25 *N* hydrochloric acid in water-methanol (30%)-acetic acid (5.7%). (a) Ethanolamine; (b) *n*-propylamine; (c) isobutylamine; (d) *n*-butylamine; (e) benzylamine; (f) *n*-amylamine; (g) *n*-hexylamine; (h) *n*-heptylamine; (i) *n*-octylamine; (l) *n*-decylamine; (m) mixture. S.P. = start point; S.F. = solvent front.

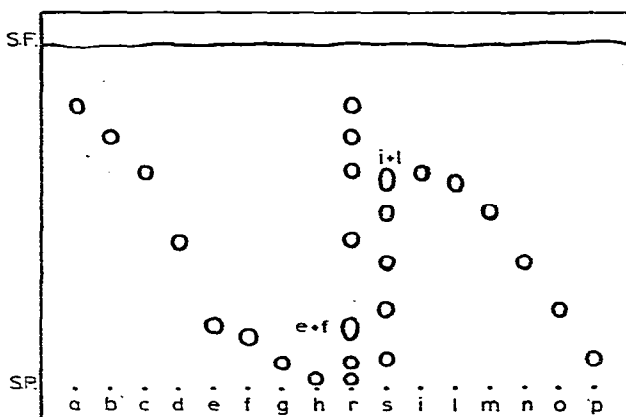


Fig. 2. Thin-layer chromatogram of mono- and polyamines on silanized silica gel impregnated with 4% DBS solution. Eluent: 0.25 *N* hydrochloric acid in water-methanol (30%)-acetic acid (5.7%). (a) Ethanolamine; (b) ethylamine; (c) *n*-propylamine; (d) *n*-butylamine; (e) *n*-amylamine; (f) *n*-hexylamine; (g) *n*-heptylamine; (h) *n*-octylamine; (r) mixture of monoamines; (i) 1,3-diaminopropane; (l) 1,5-diaminopentane; (m) 1,6-diaminohexane; (n) 1,7-diaminoheptane; (o) 1,8-diaminooctane; (p) spermine; (s) mixture of polyamines. S.P. = start point; S.F. = solvent front.

On these layers also the chromatographic behaviour of the diamines does not seem to be controlled by a partition process; $\Delta R_M(\text{CH}_2)$ is not constant. Further, for the same layers, on plotting the R_M values of mono- and diamines as a function of the apparent pH of the eluent, straight lines are generally obtained with slopes of about 0.3 for monoamines and 0.6 for diamines. Such behaviour confirms that in the retention of the amines also the ion-exchange process is operating. This process, with

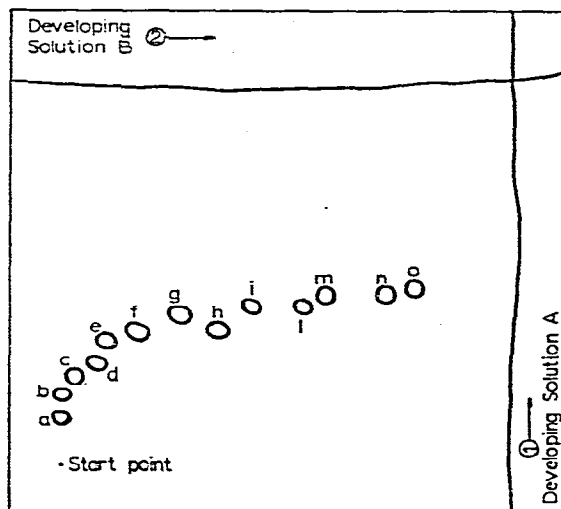


Fig. 3. Two-dimensional separation of 13 aliphatic amines on silanized silica gel impregnated with 4% DBS solution. (1) Development with water-methanol (3:7) (solution A); (2) development with 0.25 *N* hydrochloric acid in water-methanol (30%)-acetic acid (5.7%) (solution B). Migration distance, 12 cm in the first and 13 cm in the second direction. (a) *n*-Tetradecylamine; (b) *n*-dodecylamine; (c) *n*-decylamine; (d) *n*-octylamine; (e) *n*-heptylamine; (f) *n*-hexylamine; (g) *n*-amylamine; (h) benzylamine; (i) *n*-butylamine; (l) isobutylamine; (m) *n*-propylamine; (n) ethylamine; (o) ethanolamine.

polyamines, prevails over the partition mechanism owing to the higher charge of these compounds.

Such an occurrence supports the assumption we made in previous papers^{2,3} that the retention mechanism is controlled by an ion-exchange process in soap TLC only with certain classes of compounds and that even in homologous series its influence may be different for the early members of the series than for those with higher molecular weight.

It is interesting that Terweij-Groen *et al.*⁸, using high-performance liquid chromatography, obtained similar results with aromatic acids on hydrophobic supports covered with a cationic detergent.

Analytical applications

Among the separations that can be effected on the basis of the R_F values obtained with different eluents, we carried out those which cannot be performed on layers of silanized silica gel alone.

Fig. 1 shows the separation of ten monoamines on layers of silanized silica gel impregnated with 2% of DBS, eluting with 0.25 *M* hydrochloric acid in an aqueous-organic mixture containing 30% of methanol and 5.7% of acetic acid.

On increasing the concentration of DBS on the layer (to 4%), the separations of mixtures of mono- and polyamines shown in Figs. 2 and 3 were obtained. The separation of 13 monoamines shown in Fig. 3 was obtained with the two-dimensional technique.

The separation between *n*-butylamine and isobutylamine (see Figs. 1 and 3), which cannot be obtained on layers of silanized silica gel alone, is of interest.

On layers impregnated with a cationic detergent (see Fig. 4), we effected the separations of monoamines between C_6 and C_{14} from each other and from all others which run with the solvent front (polyamines included). With respect to ion-exchange TLC⁶, the resolving power of the layers impregnated with DBS is higher, particularly for the members of the series with higher molecular weights.

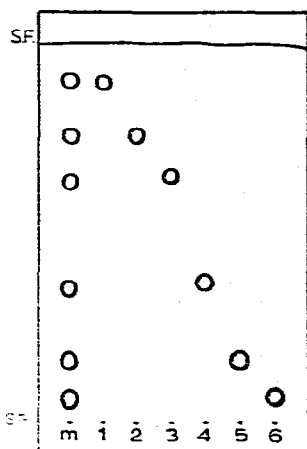


Fig. 4. Thin-layer chromatogram of some monoamines on silanized silica gel impregnated with 4% DPC solution. Migration distance: 12 cm. Eluent: water-methanol-acetic acid (64.3:30:5.7). (1) *n*-Hexylamine; (2) *n*-heptylamine; (3) *n*-octylamine; (4) *n*-decylamine; (5) *n*-dodecylamine; (6) *n*-tetradecylamine; (m) mixture.

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