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Note

High-performance thin-layer chromatography of phenylethylamines and phenolic acids on silanized silica and on ammonium tungstophosphate

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The phenylethylamine group includes natural and synthetic compounds which are used in drug formulations, catecholamines and other biogenic amines which are excreted in the urine together with phenolic acids and glycols. The separation of these compounds by thin-layer chromatography (TLC) is very important, as shown by the numerous papers dealing with the determination of phenylethylamines in pharmaceutical preparations[1], with their identification as drugs of abuse[2,3] and with the determination of catecholamines, their metabolites and precursors in urine[4–7]. These studies were carried out on silica gel or cellulose thin layers, taking only one group of compounds and/or a restricted number of them into account.

Using reversed-phase TLC, good results were obtained in the separation of some primary phenylethylamines[8] of the three most important catecholamines[9] and of some phenolic acids[10,11].

We therefore considered it useful to study a large number of phenylethylamines and phenolic acids and glycols on different ready-for-use plates of silanized silica gel untreated and impregnated with anionic and cationic detergents.

The chromatographic behaviour of these compounds has also been studied on layers of ammonium tungstophosphate (AWP), an inorganic synthetic ion exchanger, which was already been used in the separation of primary aromatic amines[12] and other nitrogen compounds[13].

EXPERIMENTAL

Standard solutions of amines and phenolic acids and glycols (1–2 mg/ml) were prepared by dissolving the compounds in water–methanol (1:1), except 3,4-dihydroxymandelic acid, which was dissolved in water.

The amount deposited on the layer was between 0.2 and 0.5 μg for silanized silica gel and between 0.5 and 1 μg for ammonium tungstophosphate.

The amines were rendered visible by spraying the layers with 1% ninhydrin solution in pyridine–acetic acid (5:1) and heating the plates for 5 min at 100°C. Hordenine, phenolic acids and glycols were detected by the Boute[14] reaction, exposing the layers successively to nitrogen dioxide and ammonia vapours.

The silanized silica gel layers (Sil C_{18}-50, Macherey, Nagel & Co., Düren,

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TABLE I

R_F VALUES OF PHENYLETHYLAMINES, PHENOLIC ACIDS AND GLYCOLS ON DIFFERENT PLATES WITH THE FOLLOWING ELUENTS (a) 1 *M* CH_3COOH + 3% KCl IN WATER; (b) 1 *M* HCl + 3% KCl IN WATER; (c) 1 *M* CH_3COOH IN WATER–METHANOL (20%); (d) 1 *M* CH_3COONa IN WATER–METHANOL (30%); (e) 0.5 *M* Na_2CO_3 IN WATER–METHANOL (30%); (f) 1 *M* CH_3COOH + 1 *M* HCl IN WATER–METHANOL (40%); AND (g) 1 *M* NH_4NO_3 IN WATER

No.	*Compound**	*OPTI-UP* C_{12}		*Sil* C_{18}*-50*	*Sil* C_{18}*-50 + 4% N-DPC*		*RP-18 + 4% HDBS*	*AWP + CaSO₄ (4:2)*
		a	*b*	*c*	*d*	*e*	*f*	*g*
1	1-Phenylethylamine	0.29	0.28	0.54	0.65	0.26	0.30	0.42
2	2-Phenylethylamine	0.29	0.27	0.51	0.56	0.18	0.24	0.28
3	Tyramine	0.54	0.47	0.68	0.70	0.44	0.66	0.42
4	Dopamine	0.66	0.61	0.74	0.68	0.16	0.75	0.42
5	3-Methoxytyramine	0.44	0.26	0.66	0.72	0.46	0.62	0.21
6	Nor-adrenaline	0.84	0.84	0.83	0.67	0.25	0.87	0.57
7	Adrenaline	0.70	0.68	0.77	0.67	0.26	0.88	n.d.**
8	Amphetamine	0.26	0.18	0.48	0.55	0.14	0.24	0.17
9	β-Hydroxyphenethylamine	0.50	0.47	0.62	0.62	0.38	0.44	0.46
10	3,4-Dimethoxyphenetylthylamine	0.14	0.10	0.50	0.67	0.34	0.44	0.06
11	Nor-metanephrine	0.69	0.55	0.79	0.78	0.70	0.77	0.40
12	Octopamine	0.78	0.74	0.79	0.76	0.70	0.78	0.56
13	Nor-ephedrine	0.39	0.28	0.55	0.56	0.29	0.32	0.41
14	Nor-ψ-ephedrine	0.34	0.23	0.54	0.56	0.21	0.32	0.44
15	Ephedrine	0.26	0.16	0.53	0.55	0.16	0.29	0.27
16	ψ-Ephedrine	0.22	0.13	0.52	0.55	0.15	0.31	0.26
17	Synephrine	0.63	0.54	0.72	0.75	0.52	0.74	0.48
18	Metanephrine	0.53	0.35	0.69	0.75	0.52	0.72	0.27
19	Hordenine	0.29	0.16	0.60	0.52	0.13	0.54	0.17
20	3,4-Dihydroxy-MA	0.89	0.73	0.95	0.56	0.62	0.90	0.95
21	*p*-Hydroxy-MA	0.82	0.57	0.90	0.56	0.62	0.87	0.94
22	*m*-Hydroxy-MA	0.75	0.46	0.83	0.46	0.53	0.87	0.92
23	4-Hydroxy-3-methoxy-MA	0.64	0.39	0.88	0.59	0.64	0.85	0.83
24	Homogentisic acid	0.64	0.37	0.70	0.30	0.47	0.87	0.90
25	Homovanillic acid	0.19	0.07	0.41	0.44	0.52	0.75	0.69
26	3,4-Dihydroxy-PG	0.74	0.61	0.77	0.59	0.66	0.89	0.91
27	4-Hydroxy-3-methoxy-PG	0.45	0.28	0.65	0.61	0.65	0.85	0.74
28	*o*-Hydroxy-PA	0.33	0.19	0.35	0.11	0.23	0.52	0.72
29	*m*-Hydroxy-PA	0.34	0.19	0.43	0.27	0.44	0.65	0.72
30	*p*-Hydroxy-PA	0.33	0.19	0.53	0.36	0.55	0.77	0.74

* MA = mandelic acid; PG = phenylglycol; PA = phenylacetic acid.

** n.d. = not determined.

F.R.G.; OPTI-UP C_{12}, Antec, Bennwil, Switzerland; RP-18, E. Merck, Darmstadt, F.R.G.) were impregnated with N-dodecylpyridinium chloride (N-DPC) or with dodecylbenzene sulphonic acid (HDBS) according to a previous work[15] and the AWP and its layers were prepared as described previously[12,16].

The migration distance was 6 cm for the ready-for-use plates and 10 cm with the inorganic exchanger, unless stated otherwise. The chromatographic measurements were carried out at 25°C.

RESULTS AND DISCUSSION

Untreated layers of silanized silica gel

The behaviour of the amines and phenolic acids and glycols was studied on layers of OPTI-UP C_{12}, Sil C_{18}-50 and RP-18. The RP-18 plates are ill-suited, as eluents containing at least 60% of methanol must be used and under such elution conditions most compounds are only very slightly retained.

Table I lists the chromatographic characteristics of nineteen phenylethylamines and eleven phenolic acids and glycols on OPTI-UP C_{12} and Sil C_{18}-50 plates eluted with aqueous solutions at different pH values (columns 1 and 2) and with a 20% methanol content (column 3), respectively.

The presence of potassium chloride in the aqueous eluents accounts for the compactness of the spots. Eluent (a) allows the separation of most amines, with the exception of the two phenylethylamine isomers.

The presence in the molecule of one or more hydroxy groups decreases the retention of the compounds considerably, while an opposite behaviour occurs with replacement of a phenolic group with a methoxy group, *i.e.*, dopamine (R_F = 0.66)/3-methoxytyramine (R_F = 0.44)/3,4-dimethoxyphenetylamine (R_F = 0.14); 3,4-dihydroxymandelic acid (R_F = 0.89)/4-hydroxy-3-methoxymandelic acid (R_F = 0.64); 3,4-dihydroxyphenylglycol (R_F = 0.74)/4-hydroxy-3-methoxyphenylglycol (R_F = 0.45).

Secondary amines are more strongly retained than primary amines. Among the diastereomers, the differences in the retention allow the separation of nor-ephedrine from nor-ψ-ephedrine.

Differing behaviour depends on the position of the phenolic group, which causes different retentions of the two isomers of hydroxymandelic acid but does not affect the behaviour of the three isomers of hydroxyphenylacetic acid.

On the basis of the R_F values obtained with eluent (a), many interesting separations can be achieved; we separated compounds 2, 3, 4, 7, 10 and 5, 9, 11, 12, the four mandelic acid derivatives and homovanillic acid from vanylmandelic acid (these two compounds are generally excreted in human urine) and also from all the other phenolic acids and glycols.

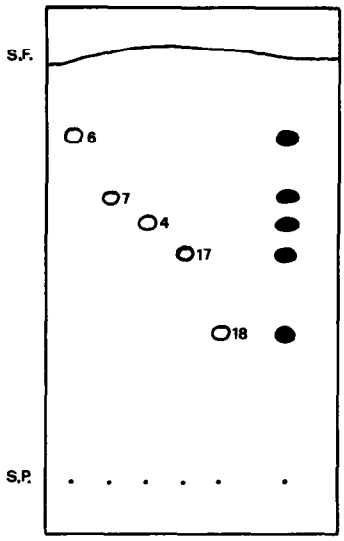
On eluting with hydrochloric acid solution (eluent b), the behaviour of the compounds is connected, in addition to the sharp acidity increase, with the lack of an organic solvent, such as acetic acid, in the eluent. The strongest retention observed for most compounds can be ascribed more to the absence of acetic acid than to the acidity increase and/or to the higher ionic strength of the eluent.

In fact both amines, which are in an ionized form in the two eluents, and the un-ionized compounds, such as the two glycols, are more strongly retained with

eluent (b). Furthermore, elution with 2 *M* hydrochloric acid + 3% potassium chloride in water does not lead to substantial R_F differences for most compounds. With eluent (b), some separations that cannot be carried out with eluent (a) can be effected, such as that between 2-phenylethylamine and amphetamine and that among the three most important catecholamines. The separation of the three catecholamines and of two other biogenic amines on OPTI-UP C_{12} layers is shown in Fig. 1.

Eluting with alkaline solutions (*i.e.*, 1 *M* sodium acetate in water) the amines are more retained, while the phenolic acids migrate more and in a different sequence. The strongest retention of the amines must be ascribed to their smaller degree of protonation, as shown also from the further decrease in their R_F values on eluting with more alkaline solutions (*i.e.*, 0.5 *M* sodium carbonate in water). Fig. 2 shows the separation on OPTI-UP C_{12} plates of the most important phenolic acids and glycols that are excreted in urine.

On Sil C_{18}-50 plates (see column 3 in Table I) the following differences can be seen with respect to the behaviour on OPTI-UP C_{12} layers: (1) a smaller retention of the compounds, which can be attributed both to the higher hydrophobicity of the layer and to the presence of methanol in the eluent; (2) a levelling of the R_F values of the phenylethylamines as the chromatographic behaviour of the ephedrine diastereomers shows; and (3) a good separation among the three isomers of hydroxyphenylacetic acid.



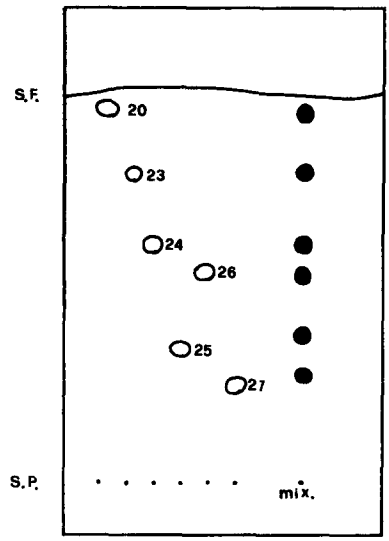


Fig. 1. Thin-layer chromatogram of biogenic amines on OPTI-UP C_{12} plates. Migration distance, 8 cm. Eluent: 1 *M* HCl + 3% KCl in water. Spots: 6, nor-adrenaline; 7, adrenaline; 4, dopamine; 17, synephrine; 18, metanephrine.

Fig. 2. Thin-layer chromatogram of urinary phenolic acids and glycols on OPTI-UP C_{12} plates. Migration distance, 7.5 cm. Eluent: 1 *M* CH_3COONa in water. Spots: 20, 3,4-dihydroxy-MA; 23, 4-hydroxy-3-methoxy-MA; 24, homogentisic acid; 25, homovanillic acid; 26, 3,4-dihydroxy-PG; 27, 4-hydroxy-3-methoxy-PG.

Sil C_{18}-50 and RP-18 plates impregnated with 4% N-DPC or 4% HDBS

We impregnated Sil C_{18}-50 and RP-18 plates only, as on OPTI-UP C_{12} plates the detergents are very weakly adsorbed, and migrate during elution with aqueous or aqueous–organic solutions, giving rise to very irregular solvent fronts.

Impregnation of the Sil C_{18}-50 plates with 4% N-DPC solution allows the retention of both acids and amines to be increased on eluting with alkaline solutions.

Table I (columns 4 and 5) gives the data obtained on eluting with 1 *M* sodium acetate (eluent d) and 0.5 *M* sodium carbonate (eluent e) in water–methanol (30%). With respect to the untreated layers, considerable differences are observed both in the retention and in the affinity sequence of the classes of compounds examined. This indicates that the detergent adsorbed on the layer not only gives rise to anion-exchange reactions but also modifies the hydrophobic characteristics of Sil C_{18}-50.

Under these elution conditions, a good separation between the two phenylethylamine isomers and an improvement, with respect to the untreated layers, in the separation of the three hydroxyphenylacetic acids are achieved.

Elution with 0.5 *M* sodium carbonate in water–methanol (30%) gives a further increase in the retention of amines with respect to eluent (d) owing to the stronger adsorption of the unprotonated species.

With the acids, the smaller retention can be attributed to the higher ionic strength of the eluent. With this eluent, the separation of nor-metanephrine and octopamine from all the other amines and that between the two diastereomers of nor-ephedrine should be noted.

The layers of RP-18 were impregnated with a 4% HDBS solution in order to increase the retention power and the selectivity of the stationary phase. On impregnated layers, the percentage of organic solvent may be decreased to 40%. In Table I (column 6) are reported the R_F values obtained on eluting with a mixture of 1 *M* acetic acid + 1 *M* hydrochloric acid in water–methanol (6:4) (eluent f). With respect to the trend observed on the two above-mentioned supports, on RP-18 + 4% HDBS a greater influence of the number and position of the hydroxy groups on the chromatographic behaviour of phenylethylamines can be noted (*cf.*, R_F values of compounds 2, 3, 4 and 6 in Table I). With acid compounds, the differences among the

TABLE II

R_M VALUES OF PHENYLETHYLAMINES ON THIN-LAYERS OF OPTI-UP C_{12} AND SIL C_{18}-50, ELUTING WITH (a) 1 *M* ACETIC ACID + 3% KCl IN WATER, (b) 1 *M* HYDROCHLORIC ACID + 3% KCl IN WATER AND (c) 1 *M* ACETIC ACID IN WATER–METHANOL (20%)

Amine	*OPTI-UP C_{12}*				*Sil C_{18}-50*	
	$R_{M(a)}$	$\Delta R_{M(a)}(OH)$	$R_{M(b)}$	$\Delta R_{M(b)}(OH)$	$R_{M(c)}$	$\Delta R_{M(c)}(OH)$
Nor-adrenaline	−0.720	0.432	−0.720	0.526	−0.689	0.235
Dopamine	−0.288		−0.194		−0.454	
Nor-metanephrine	−0.347	0.452	−0.087	0.541	−0.575	0.287
3-Methoxytyramine	0.105		0.454		−0.288	
Octopamine	−0.550	0.480	−0.454	0.506	−0.575	0.248
Tyramine	−0.070		0.052		−0.327	

R_F values are less marked than on the other two layers, even though the separation of the three isomers of hydroxyphenylacetic acid is still possible.

The retention of phenylethylamines depends on the concentration of hydrochloric acid in the eluent and can be ascribed to the cation-exchange process between the protonated amino group and the detergent adsorbed on the layer. In fact, if the R_M values of the amines are plotted as a function of the logarithm of the hydrochloric acid activity in the eluent, straight lines are obtained for most amines; the slopes are between 0.7 and 0.9 and are, therefore, in accordance with the theoretical values[8]. Among the different separations that can be achieved on this layer, of interest is that between the two phenylethylamine isomers, which is not feasible on home-made layers of silanized silica gel (C_2) impregnated with 4% DBS[8].

Retention mechanism

On layers of silanized silica gel the retention of the compounds is probably controlled by a liquid–liquid partition mechanism. We wanted to see whether, for the phenylethylamine derivatives also, the introduction into the molecule of a functional group, such as a hydroxy or N-methyl group, might give rise to a specific contribution to the retention on untreated OPTI-UP C_{12} and Sil C_{18}-50 plates.

Table II gives the ΔR_M(OH) values, calculated on the basis of the chromatographic data in Table I. The ΔR_M(OH) values are substantially constant for the three pairs of phenylethylamines. The mean value of ΔR_M(OH) is 0.525 for eluent (b), 0.455 for eluent (a) and 0.256 for eluent (c). Such a decrease further demonstrates the R_F levelling caused by an increase in the percentage of organic solvent in the eluent.

Table III gives the ΔR_M(N-CH_3) values calculated on OPTI-UP C_{12} plate with eluents (a) and (b) from Table I. The data on Sil C_{18}-50 plates are not reported because, owing to the presence of 20% of organic solvent in the eluent, the differences between the R_M values are too small. The ΔR_M(N-CH_3) valuews refer to six pairs of

TABLE III

R_M VALUES OF PHENYLETHYLAMINES ON THIN LAYERS OF OPTI-UP C_{12}, ELUTING WITH (a) 1 *M* CH_3COOH + 3% KCl IN WATER AND (b) 1 *M* HCl + 3% KCl IN WATER

Amine	$R_{M(a)}$	$\Delta R_{M(a)}$(N-CH_3)	$R_{M(b)}$	$\Delta R_{M(b)}$(N-CH_3)
Nor-adrenaline	−0.720	0.352	−0.720	0.393
Adrenaline	−0.368		−0.327	
Nor-metanephrine	−0.347	0.295	−0.087	0.356
Metanephrine	−0.052		0.269	
Octopamine	−0.550	0.319	−0.454	0.384
Synephrine	−0.231		−0.070	
Nor-ephedrine	0.194	0.260	0.410	0.310
Ephedrine	0.454		0.720	
Nor-ψ-ephedrine	0.288	0.262	0.525	0.301
ψ-Ephedrine	0.550		0.826	
Tyramine	−0.070	0.459*	0.052	0.668*
Hordenine	0.389		0.720	

* This value is relative to the dimethylamino group and is double that for N-CH_3.

amines and are consistent among them. The mean values of $\Delta R_M(\text{N-CH}_3)$ are 0.344 (eluent b) and 0.278 (eluent a).

Ammonium tungstophosphate plates

On layers of this exchanger, eluting with aqueous solutions of 1 *M* ammonium niotrate (column 7 in Table I), a peculiar behaviour is observed with amines. Such behaviour, as has already been pointed out in previous papers[12,13], depends on the structural characteristics of the compounds. In fact, the steric hindrance of the phenyl group on the carbon atom bound to the amino group allows a good separation between 1- and 2-phenylethylamine. The N-methyl groups increase the affinity of the compound towards the exchanger; the following sequence of R_F values is observed: primary amine > secondary amine > tertiary amine. The replacement of a phenolic group by a methoxy group also results in an increase in retention; this increase is more marked than on the other supports.

The acid compounds, as was predictable, are retained less than on silanized silica gel; some separations, however, can be effected, such as that concerning compounds 20, 23 and 25 in Table I.

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