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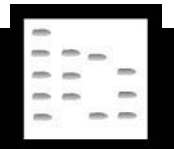
BALSAMS AND RESINS: THIN-LAYER (PLANAR) CHROMATOGRAPHY

See **III / ESSENTIAL OILS / Thin Layer (Planar) Chromatography**

BASES: THIN-LAYER (PLANAR) CHROMATOGRAPHY



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Introduction

According to the Brønsted–Lowry definition (1923) a base is a proton acceptor:

$$\underset{\text{base}}{B} + \underset{\text{proton}}{H^+} = \underset{\text{conjugated acid}}{BH^+}$$

The stronger the base, the larger is its K_b and consequently the smaller is its pK_b and the larger is the pK_a of the conjugated acid.

There are various types of bases, including natural and synthetic compounds, and they occur in a vast array of products, extending from the alkaloids, sulfa drugs (sulfonamides), dyes (azines, indoles), herbicides (simazine, atrazine, promazine), biogenic amines to numerous other groups.

This chapter includes only aliphatic and aromatic amines and their derivatives, heterocyclic bases and miscellaneous compounds (nitrosamines, amides, hydrazines). Thin-layer chromatography is used extensively for the analysis of bases and can achieve separations of complex mixtures comparable to column liquid chromatography.

Aliphatic Amines

The first attempts to separate aliphatic amines were performed on silica gel using chloroform–ammonia (39 : 1), chloroform–methanol–17% ammonia (2 : 2 : 1), butanol–acetic acid–water (4 : 1 : 5) and phenol–water (8 : 3) as eluents. However, highly volatile amines cannot be chromatographed with an ammoniacal solvent. A systematic collection of data on the chromatographic behaviour of a large number of aliphatic amine hydrochlorides, with particular emphasis on eluents, adsorbents and detection reagents, was published by Prandi (see hR_F values in **Table 1**, columns 1, 2 and 3).

Silica gel is particularly useful for the adsorption chromatography of amines that have different polarities but does not resolve the fatty amine series. In particular, the R_F values increase as the aliphatic chain length increases but this increase becomes smaller with increasing chain length.

Reversed-phase partition chromatography on paraffin oil–saturated silica gel is useful for the separation of fatty amines. $R_M[\log(1/R_F)^{-1}]$ values for such amines increase as the length of the aliphatic chain increases and there is a linear relationship between R_M and the number of carbon atoms in the molecule.

Table 1 Retention data (hR_F)* of aliphatic amine hydrochlorides under different experimental conditions[a]

Amine	*Silica gel*[b]		*Po-s Kieselguhr*[c]	*Silica gel + chlorophenol*[d]	*Sil C_{18}-50 + 4 %HDBS*[e]	*AWP*[f]
	Eluent A	*Eluent B*	*Eluent C*	*Eluent D*	*Eluent E*	*Eluent F*
Methylamine	3	6	–	26	92	69
Dimethylamine	4	7	–	31	–	–
Trimethylamine	–	43	–	35	–	–
Ethylamine	7	16	–	46	85	75
Diethylamine	16	32	–	50	–	–
Triethylamine	–	75	–	53	–	–
Ethanolamine	4	10	–	–	–	–
Diethanolamine	5	16	–	–	–	–
Triethanolamine	18	36	–	38	–	–
Ethylethanolamine	11	23	–	–	–	–
Ethyldiethanolamine	30	52	–	–	–	–
2-Ethylhexylethanolamine	65	93	–	–	–	–
Propyldiethanolamine	52	69	–	–	–	–
Propylamine	16	35	–	57	–	–
Di-*n*-propylamine	51	80	–	–	–	–
Isopropylamine	17	36	–	63	–	–
Diisopropylamine	33	66	–	–	–	–
Propanolamine	4	8	–	–	–	–
Triisopropanolamine	52	85	–	–	–	–
Allylamine	–	–	–	60	–	–
Diallylamine	–	–	–	66	–	–
Butylamine	22	48	–	–	55	65
Di-*n*-butylamine	63	95	–	80	–	–
Tri-*n*-butylamine	–	–	–	85	–	–
Isobutylamine	31	58	–	–	62	70
Diisobutylamine	85	99	–	–	–	–
3-Methoxypropylamine	18	43	–	–	–	–
Pentylamine	29	55	–	–	34	60
Isoamylamine	30	56	–	–	45	62
2-Methylbutylamine	36	68	–	–	–	–
Hexylamine	34	65	86	–	24	53
Cyclohexylamine	33	63	–	76	–	–
3-Amino-2,2′-dimethylbutane	51	90	–	–	–	–
2-Amino-3-methylpentane	47	78	–	–	–	–
2-Amino-4-methylpentane	42	73	–	–	–	–
Heptylamine	36	70	82	–	14	elongated spots
Octylamine	37	74	78	–	7	3
2-Ethylhexylamine	54	88	–	–	–	–
Di-2-ethylhexylamine	100	100	–	–	–	–
tert-Octylamine	52	87	–	–	–	–
Nonylamine	39	77	74	–	–	–
Decylamine	40	78	70	–	1	0
Undecylamine	42	79	65	–	–	–
Dodecylamine	44	79	58	–	0	0
Tridecylamine	47	80	50	–	–	–
Tetradecylamine	50	82	43	–	0	0
Pentadecylamine	52	83	38	–	–	–
Hexadecylamine	55	85	30	–	–	–
Heptadecylamine	58	85	24	–	–	–
Stearylamine	60	85	18	–	–	–
1,2-Diaminoethane	2	4	–	22	82	49
1,2-Diaminopropane	3	10	–	–	81	58
1,3-Diaminopropane	–	–	–	–	84	36
1,4-Diaminobutane	–	–	–	–	84	32
1,5-Diaminopentane	–	–	–	–	85	26
1,6-Diaminohexane	–	–	–	–	79	19
1,7-Diaminoheptane	–	–	–	–	72	17
1,8-Diaminooctane	–	–	–	–	56	16
N-(3-Aminopropyl)cyclohexylamine	5	18	–	–	–	–

Table 1 *Continued*

Diethylenetriamine	0	0	–	17	–	–
Spermidine	–	–	–	–	81	10
Spermine	–	–	–	–	72	2
Tetraethylenepentamine	0	0	–	–	–	–

*h$R_f = R_f \times 100$.

[a]Eluents: A and B = chloroform–methanol–17% ammonia in the 82.5 : 15.5 : 2 (A) and 70 : 26 : 4 (B) ratios: C = acetone–17% ammonia (70 : 30 v/v); D = *n*-butanol–acetic acid–water (35 : 5 : 10); E = 1 M acetic acid + 1 M HCl in 30% methanol; F = 2 M NH_4NO_3.

[b]Silica gel G (Merck); detection reagents: 1% ninhydrin solution in ethanol–acetic acid (95 : 5); 1% potassium permanganate + 1% potassium persulfate (1 : 1); 25% iodine methanolic solution; 5% sodium nitroprusside in acetaldehyde–2% sodium carbonate (1 : 1 v/v) solution. Sample volume; 10 μL of a 0.5% water–alchol solution of the amine hydrochloride.

[c]Paraffin oil–saturated Kieselguhr G (Merck) layers were prepared by immersing the plates in a 5% solution of the oil in acetone.

[d]Home-made plates were prepared by spreading a slurry of 50 g of silica gel G (BDH) in 2% *o*-chlorophenol solution (100 mL). The plates were dried for 24 h at 60°C before use. Detection agent: 3 g ammonium thiocyanate and 1 g cobalt chloride in 20 mL of distilled water (blue spots).

[e]The Sil C_{18}-50 impregnated layers (Macherey-Nagel) were prepared by immersing the plates in a 4% dodecylbenzenesulfonic acid (HDBS) solution in 95% ethanol.

[f]Home-made plates were prepared by spreading a slurry of 4 g ammonium tungstophosphate (AWP) and 2 g calcium sulfate hemihydrate in 50 mL of distilled water after stirring 10 min with a magnetic stirrer. Detection agent: 1% ninhydrin solution in a 5 : 1 (v/v) mixutre of pyridine and glacial acetic acid.

Sources: Adapted from Prandi C (1978) Thin-layer chromatography of aliphatic amines. *Journal of Chromatography* 155: 149–157; Srivastava SP, Dua VK and Chauhan LS (1980) Chromatographic behaviour of aliphatic amines on phenol-impregnated thin layers. *Journal of Chromatography* 196: 225–235; Lepri L, Desideri PG, Heinler D and Giannessi S (1982) High-performance thin-layer chromatography of nitrogen compounds on layers of RP-18 and Sil C_{18}-50 untreated or impregnated with dodecylbenzenesulfonic acid and of ammonium tungstophosphate. *Journal of Chromatography* 245: 297–308.

Cellulose and aluminium oxide have also been used as adsorbents. The phenomenon of multiple-spot formation of amines on cellulose thin layers when using neutral or weakly acidic eluents is caused by the presence of carboxyl groups in the cellulose. Partial hydrolysis of the amine hydrochloride, volatilization of the liberated hydrochloric acid and the presence of charged groups in silica gel and alumina layers have also resulted in double-spot formation for specific compounds.

Phosphate and acetate-buffered silica gel and impregnated plates have been used. Hydrogen bond formation between the impregnated plates and aliphatic amines influences their chromatographic behaviour on metal salt-impregnated plates and on phenol-impregnated silica-gel layers.

The hR_F values of some amines, obtained on silica gel impregnated with a 2% solution of *o*-chlorophenol using a butanol–acetic acid–water (35 : 5 : 10) mixture as eluent are reported in Table 1 (column 4). No correlation exists between the pK_a value of the conjugated acid of an amine and its R_M value; it therefore seems that the chromatographic behaviour of such compounds is due to hydrogen bond formation between the amine and silica gel as well as *o*-chlorophenol.

Reversed-phase thin-layer chromatography of several aliphatic mono- and polyamines has been performed on layers of silanized silica gel untreated and impregnated with anionic and cationic surfactants. Ion-exchange and/or partition contribute to the retention of the amines depending on the type of stationary phase, the percentage of surfactant and the apparent pH of the eluent.

Table 1 (column 5) shows the retention data obtained on Sil C_{18}-50 plates impregnated with a 4% dodecylbenzenesulphonic acid solution in 95% ethanol and eluted with 1 M acetic acid + 1 M hydrochloric acid in 30% methanol. On these plates the retention of polyamines is governed chiefly by an ion-exchange mechanism while aliphatic monoamines can be differentiated according to the number of carbon atoms in their side chain.

Aliphatic amines have been studied on layers of weak and strong ion exchangers, Dowex 50-X4 (Na^+ and H^+), sodium carboxymethylcellulose and Rexyn 102 (Na^+), using hydrochloric acid and various buffer and salt solutions as eluents.

The use of ammonium tungstophosphate (AWP) as a layer material is particularly promising since on this exchanger different affinity sequences in comparison with the above-mentioned results are found (see **Table 1**, column 6). The behaviour of polyamines is of interest since it seems to be correlated to the distance between the protonated amino groups involved in the ion-exchange process and, in the case of 1,2-diaminoethane and 1,2-diaminopropane, to the steric hindrance of the methyl group.

Quantitative analysis of the diamine hydrochloride recovered from acid-hydrolysed copolyamides prepared from diamine-diacid has been carried out on silica gel G eluting with phenol–*n*-butanol–formic acid–water (5 : 2 : 1 : 2 v/v) or phenol–formic acid–water (74 : 1 : 25 v/v). Densitometric scanning was performed using a Shimadzu spectrophotometer at 560 nm after spraying the plates with a 0.2% solution of ninhydrin in ethanol and heating at 90°C for 15 min.

Specific procedures have been developed for alkanolamines. The high performance TLC (HPTLC) behaviour of closely related diethanolamines was studied on silica-gel layers eluted with binary solvents (methanol–chloroform, methanol–dichloromethane, methanol–acetone, acetone–chloroform) and on four types of reversed-phase, chemically bonded, silica gel with methanol–water as mobile phase. Alkanolamines, in particular ethanolamines and iso-propanolamines, are used extensively in hydraulic brake fluids and cutting oils as corrosion inhibitors; the derivatives with fatty acids are used as emulsifiers and detergents. Their separation and identification is performed on neutral silica gel using methylene chloride–95%–ethanol–ammonia (0.880) in the proportions 43 : 43 : 15 v/v as eluent. A solution of 0.2% ninhydrin, then alizarin in acetone, is employed to locate the separated alkanolamines. The method has been applied to commercial formulations.

Phenylalkylamines

The structures of alkylamines with an aromatic ring in the side chain are shown in **Figure 1**. The phenylethylamine group comprises a range of natural and synthetic compounds, some of which are used in drug formulations, and includes catecholamines and other biogenic amines which are excreted in the urine. The separation of these compounds by TLC and over-pressured-layer chromatography (OPLC) is very important as shown by the numerous studies dealing with the determination of phenylethylamines in pharmaceutical preparations, with their identification as drugs of abuse and with the determination of catecholamines, their metabolites and their precursors in urine. These studies are carried out on silica gel, cellulose thin layers or using reversed-phase chromato graphy 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 strong and weak ion-exchangers and on layers of AWP, an inorganic synthetic ion exchanger which has been used in the separation of other nitrogen compounds.

Table 2 (column 1) lists the hR_F values of 19 phenylethylamines on reversed-phase OPTI-UPC$_{12}$ plates eluted with 1 M HCl + 3% KCl in water. The presence of potassium chloride in the eluent accounts for the compactness of the spots. Among the diastereoisomers, the differences in the retention allow the separation of norephedrine from norpseudoephedrine.

The Sil C$_{18}$-50 plates impregnated with 4% N-DPC and eluted with 0.5 M Na_2CO_3 in 30% methanol (Table 2, column 2) show considerable differences in the affinity sequence of phenylethylamines with respect to the untreated layers and an improvement in the separation of both the two diastereoisomers of norephedrine and the two isomers of phenylethylamine.

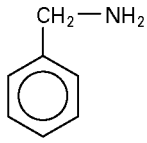
On layers of RP-18 + 4% dodecylbenzenesulfonic acid (HDBS) (**Table 2**, column 3), the retention of the compounds depends on the concentration of hydrochloric acid in the eluent and can be ascribed to the cation-exchange process between the protonated amino group and the surfactant adsorbed on the layer.

A peculiar behaviour is observed on AWP + $CaSO_4 \cdot \frac{1}{2}H_2O$ plates (**Table 2**, column 4) since the steric hindrance of the phenyl group on the carbon atom bound to the amino group allows a complete separation ($\alpha = 1.86$) between the two isomeric phenylethylamines.

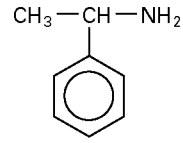
The use of *o*-benzenesulfonamido–*p*-benzoquinone in methanol or acetone has been described as a means to detect and distinguish the 3,4-methylenedioxyamphetamines of the 'Ecstasy' group on silica gel 60 F_{254} plates with ethyl acetate–acetone–methanol–25% ammonia (20 + 20 + 8 + 2) solution as eluent.

Derivatized Amines

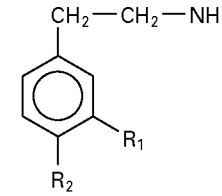
Direct chromatography on thin layers of primary and secondary amines is frequently difficult due to the strong adsorption of the NH_2 or NH groups to the adsorbents employed. Derivatives are often used to overcome these difficulties. A series of reagents has been recommended for the formation of derivatives of primary and secondary amines in order to assist in separating, identifying and determining such compounds on thin layers. The formulae of the most important reagents are shown in **Figure 2**. The chromatographic behaviour of derivatized amines with some common reagents is shown in **Table 3**. Most of these derivatives are intensely coloured (i.e., DADB-, PABS-, DBAB-) or give fluorescent spots (NBD-); in some cases, however, the detection can be considerably improved by exposure of the plates to iodine vapour or by spraying the chromatogram with 0.01 M sulphuric acid (DBAB-amides).



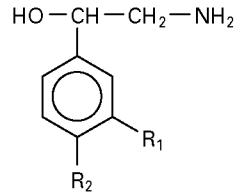
Benzylamine



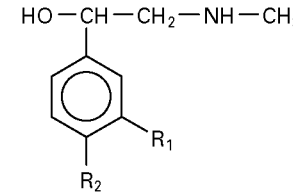
1-Phenylethylamine



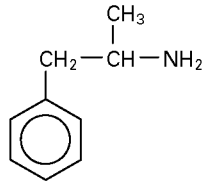
2-Phenylethylamine $R_1=R_2=H$
Tyramine $R_1=H$; $R_2=OH$
Dopamine $R_1=OH$; $R_2=OH$
3-Methoxytyramine $R_1=OCH_3$; $R_2=OH$
3,4-Dimethoxyphenethylamine $R_1=R_2=OCH_3$



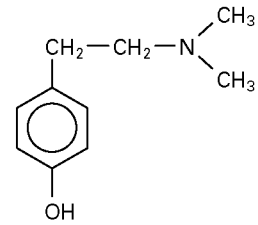
β-Hydroxyphenethylamine $R_1=R_2=H$
Octopamine $R_1=H$; $R_2=OH$
Noradrenaline (norepinephrine) $R_1=R_2=OH$
Normetanephrine $R_1=OCH_3$; $R_2=OH$



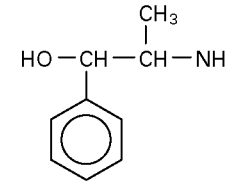
Synephrine $R_1=H$; $R_2=OH$
Adrenaline (epinephrine) $R_1=R_2=OH$
Metanephrine $R_1=OCH_3$; $R_2=OH$



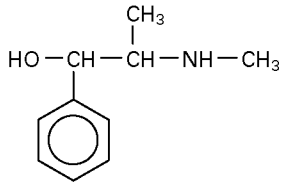
Amphetamine (Benzedrine)



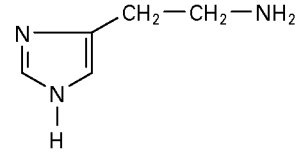
Hordenine



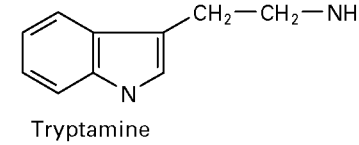
Norephedrine (*erythro*)
Nor-Ψ-ephedrine (*threo*)



Ephedrine (*erythro*)
Ψ-Ephedrine (*threo*)



Histamine



Tryptamine

Figure 1 Structures of phenylalkylamines.

The procedure used for PABS-amides does not allow characterization of the movement of individual compounds by ordinary R_F values, as the chromatography is continued after the solvent front has reached the upper edge of the plate. Therefore, the hR_X values reported in **Table 3** (column 2) represent relative values with respect to the derivative of *n*-butylamine taken as 100.

The dansylated derivatives of ammonia, 1,3-diaminopropane, 1,4-diaminobutane, 1,5-diaminopentane, spermidine, spermine and histamine were separated on silica-gel 60 plates eluted with

Table 2 Retention data (hR_F) of phenylalkylamines under different experimental conditions[a]

Compound	*OPTI-UPC$_{12}$*[b] *Eluent A*	*Sil C$_{18}$-50 + 4% N-DPC*[c] *Eluent B*	*RP-18 + 4% HDBS*[d] *Eluent C*	*AWP*[e] *Eluent D*	*Dowex 50 X4*[f] *(H$^+$) Eluent E*	*Rexyn 102*[g] *(Na$^+$) Eluent F*
Benzylamine	–	–	–	–	28	43
1-Phenylethylamine	28	26	30	42	25	51
2-Phenylethylamine	27	18	24	28	18	38
Tyramine	47	44	66	42	23	31
Dopamine	61	16	75	42	30	21
3-Methoxytyramine	26	46	62	21	–	–
3,4-Dimethoxyphenethylamine	10	34	44	6	–	–
β-Hydroxyphenethylamine	47	38	44	46	–	–
Octopamine	74	70	78	56	41	30
Noradrenaline	84	25	87	57	49	20
Normetanephrine	55	70	77	40	–	–
Synephrine	54	52	74	48	–	–
Adrenaline	68	26	88	–	–	–
Metanephrine	35	52	72	27	–	–
Amphetamine	18	14	24	17	20	43
Norephedrine	28	29	32	41	–	–
Norpseudoephedrine	23	21	32	44	–	–
Ephedrine	16	16	29	27	–	–
Pseudoephedrine	13	15	31	26	–	–
Hordenine	16	13	54	17	–	–
Histamine	–	–	–	–	47	15
Tryptamine	–	–	–	–	4	23

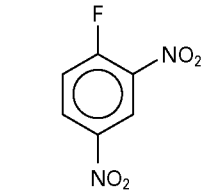
[a]Eluents: A = 1 M HCl + 3% KCl in water; B = 0.5 M Na_2CO_3 in water–methanol (30%); C = 1 M CH_3COOH + 1 M HCl in water–methanol (40%); D = 1 M NH_4NO_3 in water; E = 4 M HCl; F = 0.2 M acetate buffer.
[b]OPTI-UP C_{12} plates (Antec).
[c]The plates were impregnated with 4% *N*-dodecylpyridinium chloride in ethanol.
[d]The plates were impregnated as described in Table 1.
[e]4 g Ammonium tungstophosphate + 2 g calcium sulfate hemihydrate in 50 mL of distilled water.
[f]Home-made Dowex 50-X4 (H^+) layers were prepared by mixing 3 g of the resin (200–400 mesh) with 6 g of microcrystalline cellulose in 40 mL of water.
[g]Home-made Rexyn 102 (Na^+) layers (Fisher) were prepared by mixing 3 g of the resin (200–400 mesh) with 6 g of microcrystalline cellulose in 40 mL of water.
Sources: Adapted from Lepri L, Desideri PG and Coas V (1973) Chromatographic and electrophoretic behaviour of primary mono- and diamines on layers of weak and strong ion exchangers. *Journal of Chromatography* 79: 129–137; Lepri L, Desideri PG and Heimler D (1985) High performance thin-layer chromatography of phenylethylamines and phenolic acids on silanized silica and on ammonium tungstophosphate. *Journal of Chromatography* 347: 303–309.

chloroform–triethylamine (5 : 1 v/v). Putrescine, cadaverine, spermidine and spermine can be quantitatively determined in human urine; higher amounts of the last two amines were found in the urines of cancer patients compared to the values of these substances in normal urine. Dansyl amines can be determined by *in situ* fluorescence on silica gel and, sometimes, on polyamide layers. In favourable cases as little as 10^{-12} moles/spot of a DANS-derivative can be visualized on a normal thin-layer plate.

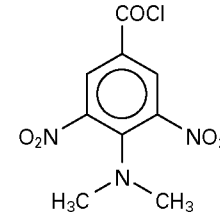
The separation and quantification of dansylated biogenic amines in vegetables have been recently performed on 20 × 20 cm silica-gel HPTLC plates with stepwise gradient elution using the Personal OPLC BS50 overpressured-layer chromatography apparatus (Kovàcs *et al.*, 1998).

Another reagent (NBD-Cl), which itself is not fluorescent but forms fluorescent derivatives with primary and secondary aliphatic amines, seems to have advantages compared to dansyl chloride since it does not produce fluorescent products with phenols, thiols and anilines, or as a consequence of hydrolysis reactions.

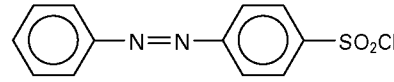
Two-dimensional (2D) TLC allows the separation of nearly every mixture of interest. A comparative study of the chromatographic properties of derivatized biogenic amines with dansyl chloride, dabsyl chloride and 4-chloro-7-nitrobenzoxazole shows that the dansyl chloride should be preferred in terms of both sensitivity and, to a lesser extent, resolution on silica gel by 2D-TLC. Dansylated and dabsylated products were found to have similar TLC characteristics and were adequately resolved by eluting in the



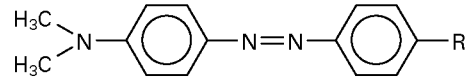
2,4-Dinitrofluorobenzene



4-Dimethylamino-3,5-dinitrobenzoyl chloride (DADB-Cl)

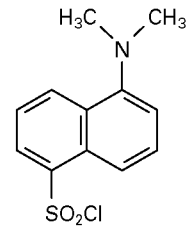


4-(Phenylazo)benzenesulfonyl chloride (PABS-Cl)

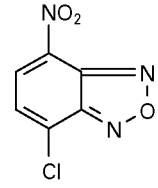


Dabsyl chloride: R=SO_2Cl

p-(*N,N*-Dimethylamino)benzene-*p'*-azobenzoyl chloride; R=COCl



1-Dimethylaminonaphthalene-5-sulfonyl chloride (DANS-Cl)



4-Chloro-7-nitrobenzo-[c]-(1,2,5)-oxadiazole (NBD-Cl)

Figure 2 Structures of the reagents used for derivative formation.

first direction with ethyl acetate–cyclohexane (3 : 2 v/v) and in the second with benzene–triethylamine (5 : 1 v/v). The plates were developed in the dark.

Aromatic Amines

Table 4 shows the hR_F values of several primary aromatic amines under different experimental conditions. Such compounds have been studied on silica-gel G or alumina using organic and aqueous–organic solutions as eluents.

Further studies concern the chromatographic behaviour of aromatic amines on silica gel impregnated with silver compounds as π-complexing metal and manganese, cadmium and zinc salts as complexing agents. On silica gel impregnated with manganese salts, the pK_a values of the conjugated acids of sixteen isomeric methylanilines and chloroanilines

Table 3 Retention data (hR_F or hR_X^* for eluent B) of derivatized amines under different experimental conditions[a]

Amine	*DADB-*[b] *Silica gel + Carbowax Eluent A*	*PABS-*[c] *Alumina Eluent B*	*NBD-*[d] *Silica Eluent C*	*DBAB-*[e] *Silica gel G Eluent D*	*DNP-*[f] *Silica gel HF_{254} Eluent E*
Ammonia	–	–	45	–	3
Methylamine	15	47	57	17	30
Dimethylamine	44	116	–	19	34
Ethylamine	24	71	63	30	59
Diethylamine	58	143	–	36	69
n-Propylamine	33	87	–	36	75
Di-*n*-propylamine	65	150	–	44	–
Isopropylamine	39	92	–	40	74
Diisopropylamine	–	–	–	47	–
Allylamine	–	81	–	39	62
Diallylamine	–	147	–	–	–
n-Butylamine	42	100	71	42	81
Di-*n*-butylamine	68	>150	–	48	–
Isobutylamine	50	106	71	43	82
Diisobutylamine	–	>150	–	48	–
sec-Butylamine	–	104	–	–	–
tert-Butylamine	–	105	–	–	–
n-Amylamine	50	108	–	–	85
Di-*n*-amylamine	–	>150	–	51	–
Isoamylamine	51	113	–	47	86
n-Hexylamine	–	115	–	47	88
Di-*n*-hexylamine	–	–	–	57	–
Cyclopentylamine	–	100	–	–	–
Cyclohexylamine	–	105	–	51	–
Dicyclohexylamine	–	–	–	56	–
Octylamine	–	120	–	–	90
Di-*n*-octylamine	–	–	–	64	–
Decylamine	–	127	–	–	92
Benzylamine	–	87	–	–	67
Dibenzylamine	–	141	–	–	–
1-Phenylethylamine	–	86	–	–	–
2-Phenylethylamine	26	83	–	–	66
Ephedrine	–	57	–	–	–
Amphetamine	–	94	–	–	–
β-Phenylisopropylmethylamine	–	126	–	–	–
Mescaline	–	15	–	–	–
Tryptamine	3	–	55	–	–
Tyramine	8	–	48	–	–
3-Methoxytyramine	–	–	48	–	–
Histamine	–	–	30	–	–
Putrescine	–	–	31	–	–
Cadaverine	–	–	34	–	–
Spermidine	–	–	27	–	–
Spermine	–	–	30	–	–

*$hR_F = R_F \times 100$; $hR_F = R_X$ values $\times 100$ (relative to the hR_X of *n*-butylamine derivative taken equal to 100).

[a]Eluents: A = *n*-hexane–ethyl acetate (7 : 3); B = 25 mL ethyl acetate and 100 mL petroleum ether (62–82° from Shell) saturated with water; C = toluene–acetic acid (4 : 1 v/v); D = cyclohexane–methylethylketone (70 : 30); E = pentane–benzene–triethylanine (45 : 45 : 10).

[b]4-Dimethylamino-3,5-dinitrobenzoyl-; home-made plates were prepared by spreading a mixture of 10 g of Carbowax 400 (Fluka) and 40 g of silica gel G (Merck) in 80 mL of water.

[c]4-(Phenylazo)-benzenesulfonyl-; microchromatoplates (40 × 76 mm) coated with alumina (Fluka, D5).

[d]4-Chloro-7-nitrobenzo-[c]-(1,2,5)-oxadiazole; silica 60 (250 μm) TLC plates (20 × 20 cm) were obtained from Sigma.

[e]*p*-(*N*,*N*-Dimethylamino)benzene-*p*′-azobenzoyl.

[f]2,4-Dinitrophenyl.

Sources: Adapted from Wirotama IPG and Ney KH (1971) Dunnschicht chromatographie von amines als 4-dimethylamino-3,5-dinitrobenzoyl amide. *Journal of Chromatography* 61: 166–168; Jart A and Bigler AJ (1967) Thin-layer chromatographic separation of primary and secondary amines as 4-(phenylazo) benzene sulfonamides. *Journal of Chromatography* 29: 255–258; Price NPG and Gray DO (1993) Mapping of derivatised biogenic amines by two-dimensional thin-layer chromatography. A comparative study. *Journal of Chromatography* 635: 165–170; Churáček J (1970) Einige neue reagenzien zur chromatographischen identifizierung von säuren, alkoholen und amines. *Journal of Chromatography* 48: 241–249; Ilert HI and Hartmann T (1972) Dünnschichtchromatographie der 2,4-dinitrophenyl derivate wasserdampfflüchtiger amine und ihre anwerdung auf die trennung pflanzlicher amine. *Journal of Chromatography* 71: 119–125.

Table 4 Retention data (hR_F) of primary aromatic amines under different experimental conditions[a]

Amine	*Silica gel G*		*Silanized silica + 4% N-DPC*[b]			*AG-1 XA(CH_3COO^-)*[c]		pK_a
	Eluent A	*Eluent B*	*Eluent C*	*Eluent D*	*Eluent E*	*Eluent F*	*Eluent G*	
Aniline	–	–	22	72	92	–	–	4.58
o-Toluidine	42	17	16	61	77	–	–	4.39
m-Toluidine	29	10	14	68	79	–	–	4.69
p-Toluidine	20	5	14	75	81	–	–	5.12
2,4-Dimethylaniline	–	–	7	53	71	–	–	–
2,6-Dimethylaniline	–	–	11	21	50	–	–	–
o-Aminophenol	24	0	–	–	–	46	83	4.72
m-Aminophenol	13	0	–	–	–	30	83	4.17
p-Aminophenol	1	0	–	–	–	70	84	5.49
o-Anisidine	42	15	18	66	78	58	83	4.49
m-Anisidine	30	9	20	53	70	44	83	4.20
p-Anisidine	2	2	26	85	86	74	84	5.29
o-Chloroaniline	75	66	–	–	–	12	40	2.64
m-Chloroaniline	51	40	–	–	–	15	64	3.34
p-Chloroaniline	41	22	6	14	44	18	75	3.98
o-Bromoaniline	78	69	6	13	17	10	27	2.60
m-Bromoaniline	58	44	4	10	28	10	55	3.51
p-Bromoaniline	47	27	5	9	37	12	69	3.91
o-Nitroaniline	55	52	5	7	17	4	8	− 0.29
m-Nitroaniline	44	36	9	10	28	21	31	2.50
p-Nitroaniline	37	29	6	9	23	2	7	1.02
o-Aminobenzoic acid	47	44	–	–	–	–	–	–
m-Aminobenzoic acid	28	12	–	–	–	–	–	–
p-Aminobenzoic acid	37	29	–	–	–	–	–	–
o-Phenylenediamine	0	0	37	81	86	67	83	4.47
m-Phenylenediamine	0	0	49	96	96	71	84	4.88
p-Phenylenediamine	0	0	60	95	97	79	84	6.08
2,4-Dichloroaniline	–	–	6	15	44	–	–	–
2,4-Dinitroaniline	–	–	3	5	15	0	0	− 4.53
2,4-Diaminotoluene	–	–	34	92	94	72	83	–
2,5-Diaminotoluene	–	–	–	–	–	79	84	–
2,6-Diaminotoluene	–	–	50	95	96	73	83	–
3,4-Diaminotoluene	–	–	10	23	34	67	83	–
2,4-Diaminoanisole	–	–	–	–	–	72	83	–
2-Amino-4-nitrophenol	–	–	–	–	–	1	10	–
2-Amino-5-nitrophenol	–	–	–	–	–	1	2	–
4-Amino-2-nitrophenol	–	–	–	–	–	22	65	–
2-Amino-4,6-dinitrophenol	–	–	–	–	–	0	0	–
4-Nitro-*o*-phenylenediamine	–	–	–	–	–	8	16	–
2-Amino-4-chlorophenol	–	–	–	–	–	3	41	–
2-Amino-3,4,6-trichlorophenol	–	–	–	–	–	0	1	–
α-Naphthylamine	–	–	3	9	38	–	–	–
4-Aminodiphenylamine (DPA)	–	–	2	31	53	–	–	–
2-Amino-DPA	–	–	2	11	23	–	–	–
3-Methoxy-4-amino-DPA	–	–	2	29	55	–	–	–
4-Methoxy-4′-amino-DPA	–	–	2	45	56	–	–	–
4,4′-Diamino-DPA	–	–	28	97	97	–	–	–
2,4-Dinitro-4′-amino-DPA	–	–	2	3	26	–	–	–
Benzidine	–	–	6	65	81	–	–	–
o-Tolidine	–	–	2	26	73	–	–	–
o-Dianisidine	–	–	2	7	49	–	–	–

[a]Eluents: A = dibutyl ether ethylacetate–acetic acid (15 : 5 : 1); B = dibutyl ether–acetic acid–*n*-hexane (20 : 1 : 4); C = 0.1 M CH_3COONH_4 + 0.1 M NH_4OH in water–methanol (20%) (pH = 9.20); D and E = 0.1 M and 2 M, respectively, CH_3COOH in water–methanol (20%); F = 0.1 M acetate buffer; G = 1 M acetic acid.

[b]Home-made layers prepared by spreading a mixture of 20 g of silanized silica gel 60HF (Merck) with 4% *N*-dodecylpyridinium chloride in 50 mL of 95% ethanol.

[c]Home-made AG1-X4 (CH_3COO^-) plates prepared by mixing 2 g of the resin (200–400 mesh) and 6 g of microcrystalline cellulose in 40 mL of water.

Sources: Adapted from Gillio-Tos M, Previtera SA and Vimercati A (1964) Separation of some aromatic amines by thin-layer chromatography. *Journal of Chromatography* 13: 571–572; Lepri L, Desideri PG and Heinler D (1979) Soap thin-layer chromatography of sulfonamides and aromatic amines. *Journal of Chromatography* 169: 271–278; Lepri L, Desideri PG and Coas V (1974) Chromatographic and electrophoretic behaviour of primary aromatic amines on anion-exchange thin layers. *Journal of Chromatography* 90: 331–339.

were correlated with their R_M values using benzene–ethyl acetate–acetic acid (2 + 2 + 1 v/v) as mobile phase. Separations via charge-transfer complexes with nitro compounds (picric acid, 2,4,6-trinitrophenyl-*N*-methylnitramine and 2,4-dinitrochlorobenzene) have also been reported.

Reversed-phase planar chromatography has been performed on silanized silica gel untreated or impregnated with cationic and anionic surfactants. 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 and are more strongly retained in the presence of *N*-dodecylpyridinium chloride on the stationary phase.

As the pH of the eluent decreases, a sharp increase in the R_F values is observed on the impregnated layers (see Table 4, columns 3–5). Such behaviour is correlated with the protonation of one or more of the amino groups present in the aromatic amines. On the basis of hR_F values many interesting separations of isomers can be carried out.

Primary aromatic amines can be separated, with difficulty, on polystyrene-based cation exchangers in aqueous–organic solutions and also by elution with concentrated mineral acids owing to the high affinity of such exchangers towards compounds which contain one or more aromatic nuclei. Therefore weak cation exchangers, i.e., carboxymethylcellulose (H^+ or Na^+ form) and alginic acid, or synthetic inorganic exchangers such as ammonium molybdophosphate and tungstophosphate, have been used for separating such compounds. Better results can be achieved using polystyrene-based anion exchangers as shown by the hR_F values obtained on AG-1X4 (CH_3COO^-) plates (see **Table 4**, columns 6 and 7).

As regards the influence of the pH of the eluent, not that the protonated forms of the amines exhibit a lower affinity towards the exchanger than the free base forms.

An equation similar to that suggested for alkaloids can be used for studying quantitatively the influence of eluent pH on the chromatographic characteristics of aromatic amines:

$$(1/R_F) - 1 = (1/R_{F_{alk}} - 1)(K_a/K_a + [H^+])) + (1/R_{F_{ac}} - 1)([H^+]/(K_a + [H^+]))$$

where K_a is the dissociation constant of conjugated acid of the base and $R_{F_{ac}}$ and $R_{F_{alk}}$ are the R_F values of the protonated and the free base form of the amines, respectively.

The detection of aromatic amines has been accomplished with fluorescamine in glacial acetic acid (1 mg mL^{-1}), 5% *p*-dimethylaminobenzaldehyde in a 5 : 1 mixture of ethanol and glacial acetic acid, 0.2% chloranil in chlorobenzene or 9-chloroacridine in 95% ethanol.

Diazotization and coupling can be carried out directly on the layer, i.e., the plates can be exposed to nitrogen dioxide to diazotize the amines and then sprayed with a solution of 0.1 M *β*-naphthol and 0.1 M triethylamine in benzene.

Derivatives of aromatic amines have also been used for separating and identifying these compounds. Therefore, 2,4-dinitrophenyl derivatives and dansyl derivatives have been studied on silica gel with different solvent systems.

Fifty-four aromatic amines used as antioxidants and/or antiozonants for elastomers have been separated on silica gel with a concentrating zone using benzene–ethyl acetate–acetone (100 : 5 : 1 v/v) and benzene–*n*-hexane (50 : 50 v/v) as eluents. The detection reagent is *N*-chloro-2,6-dichloro-*p*-benzoquinone monoimine in buffered alkaline medium.

Heterocyclic Bases

Heterocyclic compounds containing one or more nitrogen atoms have been extensively investigated on thin-layer chromatography as detailed in **Table 5**. Almost all the heterocyclics are chromatographed on polar stationary phases (silica gel, alumina, cellulose, polyamide) and, to a lesser extent, on hydrophobic layers obtained by impregnating polar adsorbents with nonpolar substances or by using silanized silica gel plates. Chemically modified and impregnated layers with cationic and anionic surfactants are also used.

Among the various types of 'simple' nitrogen heterocyclics, the separation of pyridines, indoles, quinolines and pyrimidines is of interest. The indole group of compounds is conventionally divided into the so-called simple derivatives and the indole alkaloids and dyes. A number of simple indole derivatives play important roles in physiological processes. Alkaline and acidic systems are employed on both silica gel and silanized silica for the separation of these compounds (see **Table 6**).

The two-dimensional technique on Sil C_{18}-50 plates can be performed by eluting in the first direction with *n*-hexane–ethyl acetate–acetic acid (72 : 27 : 1 v/v) and in the second direction with 0.1 M ammonia in 40% methanol. This technique allows the separation of 20 indole derivatives; the spots can be identified from their positions on the chromatogram and also from their colours obtained after spraying with 1% *p*-dimethylaminobenzaldehyde solution in concentrated hydrochloride acid–methanol (1 : 1) and heating the plates at 50°C for 20 min.

Table 5 Methods used for the separation of various types of nitrogen heterocyclics

Compound	*Layer*	*Eluent*
Azines	Alumina	Benzene–Chloroform (1 : 1)
Azines	Silica gel with or without a fluorescent indicator (F_{254})	CCl_4–EtOH–Me_2CO (50 + 1 + 2); *n*-PrOH-*n*-hexane in different ratios
Azines	Cellulose MN-300 F_{254}, Aminoplast	CH_3OH–H_2O–CH_3CN (30 + 20 + 5); CH_3OH–aqueous ammonia (30 + 20); CH_3OH–aqueous acetic acid (30 + 20)
Azines	Silica gel F_{254} impregnated with 5% paraffin oil in *n*-hexane	Water–organic solvents
Azines	RP-2, RP-8, RP-18	H_2O–CH_3OH in different proportions
Benzodiazepines	Silica gel and silica gel F_{254}	$CHCl_3$–Me_2O–iPrOH and *n*-hexane–Me_2CO in different ratios; $CHCl_3$–Me_2CO (80 + 20); benzene; BuOH–$CHCl_3$–NH_4OH (50 + 50 + 1); BuOH–C_6H_6–NH_4OH (50 + 50 + 1 and 40 + 10 + 30); $CHCl_3$–MeAc (90 : 10); C_6H_6–iPrOH–25% NH_4OH (85 + 15 + 1)
Benzodiazepines	RP-18, KC18F	Acetate or phosphate buffer + CH_3OH or CH_3CN in different proportions
Benzodiazepines	Silica gel impregnated with oleyl alcohol	NaOH–aqueous buffer solutions (pH 9–9.5) saturated with oleyl alcohol
Benzoquinoxalinone derivatives	Silica gel 60 F_{254}	CH_2Cl_2 + EtAc (17 + 3)
Benzimidazoles and Benztriazoles	Cellulose F_{254}, starch F_{254}	H_2O + CH_3OH or CH_3CN in different proportions
Carbazoles	Silica gel	Benzene; EtAc–MeOH–HCOOH–Pyridine (7.5–7.5–7.5–10); EtOH
Carbazoles	Alumina	Petroleum ether (40–60°C)–$CHCl_3$ (10 : 1); petroleum ether–HAc (10 : 1)
Cyclic amidines	Silica gel F_{254}	CH_3OH + $CHCl_3$ or benzene in different ratios
Imidazolines	Silica gel	Benzene–Me_2CO–25% NH_4OH (4 : 17 : 1)
Imidazoles	Silica gel	$CHCl_3$–Me_2CO–HAc (34 : 4 : 3) EtAc saturated with NH_4OH
Indoles	Silica gel	BuOH–HAc–H_2O (2 : 1 : 1); Me_2O–HAc (100 : 1); phenol–H_2O (4 : 1); iPrOH–25% NH_4OH–H_2O (20 : 1 : 2) BuOH–EtOH–cyclohexylamine (76 : 3 : 6); Me_2CO–$CHCl_3$–HAc–H_2O (8 : 8 : 4 : 1); MeCOEt–$CHCl_3$–conc. NH_4OH (40 : 10 : 1); MeCOEt–CH_3OH–conc. NH_4OH; EtAc–iPrOH–conc. NH_4OH (45 : 35 : 20)
Indoles	Cellulose	BuOH–HAc–H_2O (12 : 3 : 5); benzene–dioxane–H_2O (1 : 1 : 1); benzene–pyridine–H_2O (1 : 1 : 1)
Indoles	Polyamide	$CHCl_3$–EtAc–HAc (7 : 2 : 1); $CHCl_3$–cyclohexane–BuOH–HAc–H_2O (1 : 1 : 1 : 1 : 0.2)
Indoles	Sil C_{18}-50; Sil C_{18}-50 + 4% N-DPC	H_2O–CH_3OH–HAc (59 : 40 : 1); 0.1 M NH_4OH in 40% methanol; *n*-hexane–EtAc–HAc (72 : 27 : 1); 0.1 M NH_4OH + 0.1 M NH_4Cl in 40% methanol; 0.5 M NH_4OH + 0.5 M NH_4Cl in 40% methanol; *n*-hexane EtAc–HAc (67 : 32 : 1)
Indoles	AWP	1 M NH_4NO_3
Indole esters	Silica gel	Octanol–petroleum ether (110–115°C) (1 : 5)
Nitroimidazoles	Silica gel G F_{254} impreganted with a 5% solution of silicone oil in diethyl ether	1 M NH_4OH + 1 M NH_4Cl in water or in various mixtures with methanol
Piperazines	Silica gel	EtAc–CH_3OH (4 : 1); $CHCl_3$–CH_3OH–HAc (14 : 2 : 1)
Piperidines	Alumina	$CHCl_3$ saturated with NH_4OH
Pyrazoles	Silica gel	EtAc (saturated with H_2O); $CHCl_3$–Me_2CO (7 : 3); MeCOEt
Pyrazolones	Silica gel	Cyclohexane–$CHCl_3$–EtOH (4 : 10 : 1); cyclohexane–MeCOEt (4 : 5)
Pyrazolidines	Alumina	
Pyridines	Silica gel	Benzene–MeOH (25 : 1); EtAc–MeOH–HAc (15 : 4 : 1); diethylether–dimethylformamide (99 : 1)
Pyridines	Silica + Ag_2O	Me_2CO–C_6H_6 (2 : 3); MeCOEt–iPrOH (4 : 1); $CHCl_3$–CH_3OH (3 : 2)

Table 5 *Continued*

Pyridine derivatives	Silanized silica gel untreated or impregnated with sodium dodecylsulphate as ion-pairing reagent	Phosphate buffer solution–CH_3OH (1 : 1) with the buffer pH adjusted to the appropriate value (pH 3 to 10)
Pyrimidines	Silica gel	$CHCl_3$–CH_3OH (3 : 1)
Pyrimidines	Dowex 50-X4 (H^+)	0.25–4 M hydrochloric acid
Pyrimidines	Carboxymethylcellulose (Na^+), Dowex 50-X4 (Na^+)	Water; 0.1 and 0.5 M acetate buffer
Pyrroles	Silica gel	*n*-Hexane–$CHCl_3$ (9 : 1); diethylether–2% HAc in *n*-hexane (1 : 1)
Pyrrole acids	Silica gel	$CHCl_3$–96% HAc (1 : 1); benzene–CH_3OH–HAc (45 : 8 : 4)
Quinolines	Silica gel	EtAc–iPrOH–NH_4OH (9 : 6 :4); benzene-EtAc (1 : 1)
Quinolines	Silica gel $60HF_{254}$, aluminium oxide, Florisil	Binary mixtures of *n*-heptane with polar modifiers (iPrOH; dioxane, EtAc, tetrahydrofuran MeCOEt, Me_2CO, iPr_2O, CH_2Cl_2)
Thioindigoid thiazolidinones	Silica gel H	EtOH; CH_3CN; Et_2O; CH_3OH
Triazoles	Alumina	*n*-Hexane–benzene (1 : 1); 1% adipate in xylene–formic acid (49 : 1)

Tryptophan and some of its indole metabolites in urine (5-hydroxytryptophan, tryptamine, serotonin, indolyl-3-acetic acid and 5-hydroxyindolyl-3-acetic acid) have been separated by TLC, stained with van Urk's Salkowski reagent, and determined by scanning densitometry using indolyl-3-butyric acid as internal standard. Sep-Pak C_{18} cartridges are used for extraction of metabolites from urine. The detection limits are 2 $\mu g\,mL^{-1}$ 5-hydroxytryptophan, 1.75 $\mu g\,mL^{-1}$ 5-hydroxyindolyl-3-acetic acid, 1.5 $\mu g\,mL^{-1}$ tryptophan, 0.8 $\mu g\,mL^{-1}$ indolyl-3-acetic acid, 0.9 $\mu g\,mL^{-1}$ indolyl-3-butyric acid, 1.75 $\mu g\,mL^{-1}$ serotonin and 1.25 $\mu g\,mL^{-1}$ tryptamine.

The chromatographic behaviour of several pyridine derivatives, some diazines and their sulfides, dimers and trimers has also been studied on both silica gel and silanized silica (see **Table 7**). The retention of azines and diazines in adsorption TLC on silica gel with *n*-propanol–*n*-hexane eluents can be calculated by the relationship adopted by Kowalska for reversed-phase chromatography:

$$R_F = A + B(X_1)^{1/2} + CX_2$$

where X_1 and X_2 are the volume fractions of alcohol and hydrocarbon, respectively, and A, B, and C the equation constants. In the reversed-phase systems on RP-2, RP-8 and RP-18 plates, R_M values were found to be linearly dependent on methanol concentration (binary methanol–water mixtures) showing the dependence of the retention values on the hydrophobicity and chemical structure of the analysed compounds.

A large number of pyrimidines (nucleobases included) have been studied on plates of silanized silica gel with different characteristics. The layers were also impregnated with anionic surfactants in order to evaluate the role of the ion-exchange process on the chromatographic behaviour of these compounds (**Table 8**). The hR_F sequences of the pyrimidines under the various conditions are related to solvophobic effects, their acid–base characteristics, and the association of the species in solution.

Seven different thin-layer chromatographic systems were investigated to separate 19 pairs of the *E–Z* geometrical isomers of pyrimidine, purine and pyrazole derivatives with potential cytokinin activity. These systems employed silica, silanized silica, silanized silica/Cu(II) cation, chemically bonded RP-8 and RP-18 as stationary phases and a variety of binary mobile phases. The best performance was observed on silica gel with *n*-hexane–ethyl acetate 1 + 9 v/v as mobile phase.

For the location of heterocyclic bases, Dragendorff's reagent is usually employed; other detection agents are tetracyanoethylene and iodine–azide solutions.

Pyrazoles can be visualized with sodium nitroprusside; pyrazolones with 1% mercuric nitrate, iodine–potassium iodide or 10% ferrocyanide–12.5% hydrochloric acid (1 : 1); and imidazoles with iodoplatinate reagent. Indoles can be detected with Ehrlich's, van Urk's and Prochazka's reagents, or *o*-phthalaldehyde–sulfuric acid solution (0.15% w/v). A specific and sensitive fluorescent detection method was proposed for the analysis of nitroimidazoles (titanium (III) chloride followed by spraying with diazotized sulfanilic acid).

Miscellaneous Nitrogen Compounds

Because of the possible presence of nitroso carcinogens in foods, the separation and determination of

Table 6 Retention data (hR_F) of indole derivatives under different experimental conditions[a]

Indole derivative	*Silica gel G*		*Sil C_{18}-50/UV_{254}*		
	Eluent A	*Eluent B*	*Eluent C*	*Eluent D*	*Eluent E*
Indole	84	73	16	13	97
Skatole	87	78	–	–	–
3-Hydroxymethylindole	84	45	–	–	–
Indole-3-aldehyde	81	20	25	18	60
Indole-3-acetaldehyde	86	46	32	22	96
Indole-3-ethanol	–	–	29	23	74
Indole-3-acetone	–	–	24	18	95
Indole-3-acetonitrile	85	46	23	17	95
Indole-2-carboxylic acid	–	–	34	78	63
Indole-3-carboxylic acid	–	–	–	–	–
Indole-5-carboxylic acid	–	–	69	89	75
Indole-3-acetic acid	31	28	62	84	61
Indole-3-propionic acid	38	34	22	77	75
Indole-3-butyric acid	40	38	14	69	81
Indole-3-glyoxylic acid	–	–	61	82	0
Indole-3-lactic acid	–	–	59	80	4
Indole-3-acrylic acid	33	29	15	24	64
5-Hydroxyindole-3-acetic acid	19	4	78	92	18
Indole-3-acetamide	–	–	41	36	34
Indole-3-ethylacetate	–	–	9	7	98
Indole-3-glyoxylamide	–	–	25	20	52
Isatin	75	27	42	40	78
Gramine	77	0	–	–	–
Tryptamine	77	0	47	2	0
Serotonin	65	0	62	8	0
Tryptophan	23	0	64	67	0
5-Hydroxytryptophan	14	0	–	–	–

[a]Eluents: A = methyl acetate–isopropanol–25% ammonium hydroxide (9 : 7 : 4); B = chloroform–96% acetic acid (95 : 5); C = water–methanol–acetic acid (59 : 40 : 1); D = 0.1 M NH_4OH in 40% methanol; E = *n*-hexane–ethyl acetate–acetic acid (72 : 27 : 1).

Source: Adapted from Stahl E and Kaldeuoey H (1951) Trace analysis of physiologically active, simple indole derivatives. *Zeitschrift für Physiologische Chemie* 323: 183–191; Lepri L, Desideri PG and Heinler D (1983) High-performance thin-layer chromatography of indole derivatives on layers of Sil C_{18}-50 untreated or impregnated with N-dodecylpyridinium chloride and on anmonium tungstophosphate. *Journal of Chromatography* 260: 383–389.

N-nitrosamines are of interest. Silica gel, magnesium silicate, cyano- and diol-bonded silica have been used as stationary phases to separate these compounds. Alkyl- and arylnitrosamines can be separated on silica gel with *n*–hexane–diethyl ether–dichloromethane (4 : 3 : 2 v/v) and cyclic nitrosamines with the same solvent mixture in a ratio of 5 : 7 : 10 (v/v).

The chromatographic behaviour of 26 compounds derived from 1,1-diphenylhydrazine was investigated by normal and reversed-phase chromatography. The same techniques were used for the separation of several thiosemicarbazides and 1,2,4-triazoline-3-thiones on silica gel, alumina, and C_{18}-modified silica-gel layers, and nonaqueous and aqueous eluents.

Amides are physiologically active compounds and a knowledge of possible interactions of the amide group and of different substituents under conditions close to those in physiological systems is highly important.

The separation of formanilide and ten para-substituted acetanilides was investigated on starch and cellulose layers, as an alternative to RP-18, using aqueous mobile phases with methanol, acetonitrile, acetone and 1-propanol as modifiers. Because R_M values are related to the partitioning of solute molecules in the given system, they can be regarded as a measure of solute hydrophobicity.

The retention behaviour of three series of aromatic amides was investigated on silica gel with eight binary solvent mixtures (benzene–$CHCl_3$ 20 : 30 v/v; benzene–ethyl acetate 45 : 5 v/v; benzene–acetone 45 : 5 v/v; benzene–dioxane 45 : 5 v/v; heptane–ethyl acetate 40 : 10 v/v; carbon tetrachloride with ethyl acetate, acetone or dioxane in a 45 : 5 v/v ratio). The

Table 7 Retention data (hR_F) of various heterocylic bases under different chromatographic conditions[a]

Compound	*Silica gel G*				*RP*-2	*RP*-8	*RP*-18
	Eluent A	*Eluent B*	*Eluent C*	*Eluent D*	*Eluent E*	*Eluent E*	*Eluent E*
Pyridine	29	54	20	–	70	55	56
2,2′-Bipyridyl	–	–	40	–	72	53	52
2,2′,2″-Tripyridyl	–	–	35	–	66	46	41
2-Methylpyridine	30	54	–	–	–	–	–
3-Methylpyridine	35	55	–	–	–	–	–
4-Methylpyridine (γ-picoline)	27	48	0	26	61	49	46
γ,γ'-Bipicolyl	–	–	24	20	60	49	43
2,4-Dimethylpyridine	28	49	–	–	–	–	–
2,6-Dimethlypyridine	36	59	–	–	–	–	–
2,4,6-Trimethylpyridine	26	51	–	–	–	–	–
2-Ethylpyridine	42	62	–	–	–	–	–
2-*n*-Propylpyridine	47	64	–	–	–	–	–
2-Hydroxypyridine	6	20	–	–	–	–	–
3-Hydroxypyridine	23	53	–	–	–	–	–
4-Hydroxypyridine	0	2	–	–	–	–	–
2-Aminopyridine	27	50	–	–	–	–	–
3-Aminopyridine	18	45	–	–	–	–	–
4-Aminopyridine	5	14	–	–	–	–	–
Pyridine-2-carbinol	18	45	–	–	–	–	–
Pyridine-3-carbinol	13	39	–	–	–	–	–
Pyridine-4-carbinol	4	39	–	–	–	–	–
Pyridine-2-aldehyde	51	67	–	–	–	–	–
Pyridine-3-aldehyde	33	58	–	–	–	–	–
Pyridine-4-aldehyde	36	56	–	–	–	–	–
Pyridine-2-carboxylic acid	2	4	–	–	–	–	–
Pyridine-3-carboxylic acid	6	6	–	–	–	–	–
Pyridine-4-carboxylic acid	5	5	–	–	–	–	–
Pyridine-2,6-dicarboxylic acid	3	5	–	–	–	–	–
2-Acetylpyridine	57	69	–	–	–	–	–
2-Benzoylpyridine	62	71	–	–	–	–	–
2-Fluoropyridine	62	69	–	–	–	–	–
2-Chloropyridine	61	70	–	–	–	–	–
2-Bromopyridine	63	72	–	–	–	–	–
3-Chloropyridine	56	65	–	–	–	–	–
3-Bromopyridine	57	67	–	–	–	–	–
3-Iodopyridine	58	70	–	–	–	–	–
Pyrazine	–	–	10	13	73	54	57
2,2′-Bipyrazyl	–	–	24	40	73	52	51
Quinoline	–	–	33	65	78	58	57
2,2′-Biquinolyl	–	–	70	67	74	53	45
6,6′-biquinolyl sulfide	–	–	5	36	73	36	31
8,8′-Biquinolyl sulfide	–	–	47	78	–	–	–
Quinoxaline	–	–	32	54	82	56	56
2,2′-Biquinoxalyl	–	–	54	83	70	43	32
Thieno[2,3-b; 4,5-b′]biquinoxalyl	–	–	8	–	74	51	39
2-Methylquinoxaline	–	–	20	55	74	55	49
3,3′-Dimethyl-2,2′-biquinoxalyl	–	–	34	59	67	41	28

[a]Eluents: A = ethylacetate; B = acetone; C = chloroform–ethanol–acetone (50 + 1 + 2); D = *n*-propanol–*n*-hexane (45 + 55); E = methanol–water (90 + 10).

Source: Adapted from Petrowitz HJ, Pastuska G and Wagner E (1965) Thin layer chromatography of some pyridines and quinolines. *Chemiker-Zeitung* 89: 7–12; Baranowski I and Swierczek S (1994) A study of the retention of azines and diazines in RPTLC with bonded alkyl stationary phases. *Journal of Planar Chromatography – Modern TLC* 5: 399–405.

anilides used were: *N*-substituted amides of 2,2-dimethylpropanoic acid; *N*-substituted benzamides and α-phenylacetamides, with –F, –Cl, –Br, $-CF_3$, $-CH_3$, $-C_2H_5$, $-OCH_3$, –CN, $-N(CH_3)_2$ or $-N(C_2H_5)_2$ as substituent at the *para* position. Spots were observed under UV light at $\lambda = 254$ nm.

Dialkylaminoethyl dialkylamido fluorophosphates, which exhibit choline esterase-inhibiting effects, were

Table 8 Retention data (hR_F) of pyrimidine derivatives under different experimental conditions[a]

Pyrimidine derivative	*OPTI-UPC$_{12}$*		*Sil C$_{18}$-50 + 4%HDBS*	
	Eluent A	*Eluent B*	*Eluent C*	*Eluent D*
4-Amino-2-hydroxypyrimidine (cytosine)	54	42	7	24
3-Methylcytosine	53	19	8	25
5-Hydroxymethylcytosine	61	52	11	33
5-Methyl-2,4-dihydroxypyrimidine (thymine)	30	29	78	79
1-Methylthymine	13	19	71	73
2,4-Dihydroxypyrimidine (uracil)	55	51	86	86
4,6-Dihydroxypyrimidine	70	75	83	84
4,6-Dihydroxy-2-methylpyrimidine	63	66	72	74
5-Nitrouracil	57	52	84	87
5-Hydroxymethyluracil	63	60	87	90
6-Methyluracil	31	30	80	83
5,6-Dimethyluracil	14	19	67	76
5-Aminouracil	64	64	73	96
6-Aminouracil	54	58	84	85
Uracil-5-carboxylic acid	87	87	84	94
Uracil-6-carboxylic acid (orotic acid)	68	73	90	89
Orotic acid methyl ester	20	29	77	80
Uracil-6-acetic acid	68	71	90	90
6-Chlorouracil	34	56	69	78
5-Chlorouracil	41	47	68	77
5-Bromouracil	36	40	63	73
5-Iodouracil	27	31	57	66
6-Chloromethyluracil	29	34	62	71
5-Trifluoromethyluracil	40	47	60	66
6-Chloro-1,3-dimethyluracil	3	7	44	48
5-Hydroxyuracil (isobarbituric acid)	66	68	91	93
2-Thiouracil	48	56	70	77
4-Phenyl-2-thiouracil	3	17	23	28
5-Methyl-2-thiouracil	28	34	65	70
6-Hydroxy-2-thiouracil (2-thiobarbituric acid)	68	72	83	85

[a]Eluents: A = 3% KCl in water; B = 0.5 M Na_2CO_3 in water; C = 1 M acetic acid in water–methanol (20%); D = 1 M acetic acid + 0.5 M HCl in water–methanol (20%).

Source: Adapted from Lepri L, Coas V, Desideri PG and Zocchi A (1988) Planar chromatography of purines, pyrimidines and nucleosides on untreated and detergent-impregnated silanized silica plates. *Journal of Planar Chromatography – Modern TLC* 1: 317–324.

separated on silica-gel plates developed with methanol–pyridine–formamide, 80 + 15 + 5 (v/v) and detected with ninhydrin.

The lipophilicity of fused-ring nitrogen heterocyclic was determined by reversed-phase TLC on paraffin oil impregnated Silkoplat plates (Labor, MIM) using acetonitrile–aqueous solutions of different pH as eluents.

Lastly, amino-PAHs have been identified in sewage sludge after separation of the DMF extracts on a silicic acid column into three groups: carbazoles, aminoarenes and azaarenes. RP-18 F_{254} (Merck) layers were developed with acetonitrile + water (9 : 1 v/v), observed under UV illumination at $\lambda = 254$ and 365 nm and then sprayed with specific reagents for detection of the amino group. Aminonaphthalene, aminoquinoline and/or aminoisoquinoline, aminofluorene, aminoanthracene and/or aminophenanthrene, aminopyrene, aminophenylnaphthalene and aminochrysene were found in the sludge.

Concluding Remarks

This article demonstrates the current possibilities for the separation of selected organic bases. Most attention has been devoted to the advantages of TLC, retention mechanisms, structure, sample preparation, mobile and stationary phases, usual modes of development and detection procedures, and also two-dimensional techniques.

Quantification of organic bases mainly by *in situ* densitometry has been described. The development of HPTLC and the potential for other innovations such as overpressured thin-layer chromatography (OPTLC) with this group of compounds is of considerable interest.

See also: **II / Chromatography: Thin-Layer (Planar):** Densitometry and Image Analysis; Layers; Modes of Development: Conventional; Modes of Development: Force Flow, Overpressured Layer, Chromatography and Centrifugal; Spray Reagents. **III / Amines: Gas Chromatography. Impregnation Techniques: Thin-Layer (Planar) Chromatography. Pharmaceuticals: Basic Drugs: Liquid Chromatography**.

Further Reading

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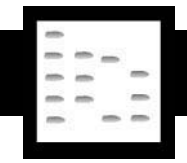
BILE ACIDS



Gas Chromatography

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Introduction

Bile acids, a group of steroidal acids with a carboxyl group in the side chain, are the major end products of cholesterol catabolism, formed in the liver, conjugated with amino acids, glycine and taurine, and secreted into the bile. In most animal species, bile acids contain 24 carbons, with the terminal side chain carbon in the form of a carboxyl group; however, certain reptiles have 27-carbon bile acids as the major biliary bile acids. Primary bile acids are formed via the 5β-saturation of cholesterol double bond by hepatic enzymes, epimerization of 3β-hydroxyl group to α-configuration and further insertion of 7α- and/or 12α-hydroxyl group, shortening of the side chain by three carbons and oxidation of the terminal carbon to a carboxyl group. Structures of some of the bile acids found in animal species are shown in **Figure 1**. Bile acids facilitate the absorption of dietary lipids, including fat-soluble vitamins and cholesterol, via their detergent action. The detergent properties of bile acids result from their unique structure with a nonpolar steroid skeleton and a polar carboxyl group and α-oriented hydroxyl groups, further increased by hepatic conjugation with glycine and taurine (Figure 1). Bile acid conjugates form micelles with phospholipids that solubilize cholesterol in the bile. The primary bile acids, cholic acid and chenodeoxycholic acid, are effectively reabsorbed from ileum during their enterohepatic circulation, but approximately 5% that escape reabsorption seep into the colon, and are subjected to modification to secondary bile acids by intestinal bacteria. These modified bile acids, in particular the 7α-dehydroxylated bile acids, lithocholic acid and deoxycholic acid, are the major faecal bile acids and are also significantly absorbed from the colon and circulate in the enterohepatic circulation, with deoxycholic acid as one of the major plasma and biliary bile acids in humans. Whereas only small amounts of bile acids are excreted into the urine, approximately 500 mg per day is excreted in faeces and forms a major catabolic pathway for the elimination of body cholesterol.

In hepatobiliary and intestinal diseases, the hepatic synthesis and clearance of bile acids and their intestinal absorption are abnormal, which disturbs both cholesterol synthesis and its metabolism, causing increased plasma, urinary and faecal concentrations of bile acids. This results in accumulation of precursors of cholesterol or bile acids and clinical malformations ensue. Early diagnosis of such conditions is often possible from bile acid analysis in bile, serum, urine and faeces. On the other hand, bile acids have therapeutic applications in conditions of abnormal cholesterol biosynthesis and metabolism, and chenodeoxycholic acid, and its 7β-hydroxy epimer, ursodeoxycholic acid, are used for medical treatment of gallstones while ursodeoxycholic acid is also being