Journal of Chromatography, 75 (1973) 79–86

CHROM. 6336

THIN-LAYER CHROMATOGRAPHY OF PHENOTHIAZINE DERIVATIVES AND ANALOGUES

A. DE LEENHEER

Laboratory of Medical Biochemistry and Clinical Analysis, University Hospitals, 135 De Pintelaan, B-9000 Ghent (Belgium)

(Received August 14th, 1972)

SUMMARY

Two solvent systems for the TLC separation of phenothiazine derivatives and analogues have been investigated. Group differentiation of compounds at the microgram level was achieved by the use of spray reagents with increasing oxidative properties.

INTRODUCTION

Phenothiazines and their analogues are currently used for the treatment of all kinds of psychotic disorders. Numerous workers have described TLC procedures for the identification of phenothiazines[1–12] and benzodiazepines[13–16].

The present paper reports a TLC system developed for the characterization of phenothiazines, azaphenothiazines, thioxanthenes, dibenzazepines and dibenzocycloheptadienes.

EXPERIMENTAL

TLC plates

Amounts of 12.5 g of Cellulose MN300 (Macherey, Nagel and Co.) and of 12.5 g of Silica Gel HF_{254} (Merck) were suspended in 90 ml of water and finely homogenized with the aid of a mixer. Using standard equipment, five 20 × 20 cm glass plates were covered with the slurry to a 250-μm thickness. The plates were dried for 3 h in the air and were ready for use without temperature activation.

Solvent systems

Two eluent combinations were examined: (A) chloroform–acetone–25 % ammonia, 50:50:1 (ml) and (B) chloroform–ethyl acetate–25 % ammonia, 50:50:1 (ml).

Detection

The plates were viewed under UV light of wavelengths 254 and 350 nm. The spray reagents used were concentrated HBr (47–48 %); FPN reagent[17] (5 ml of 5 % $FeCl_3$, 45 ml of 20 % $HClO_4$, 50 ml of 50 % HNO_3); Fe^{3+} reagent[18] (500 mg of $Fe(NO_3)_3$, 80 ml of concentrated H_2SO_4, water to 1000 ml); V^{5+} reagent (500 mg of V_2O_5,

80 ml of concentrated H_2SO_4, water to 1000 ml); Cr^{6+} reagent[19] (200 mg of $K_2Cr_2O_7$, 75 ml of concentrated H_2SO_4, 125 ml of concentrated HNO_3, 50 ml of concentrated $HClO_4$, water to 1000 ml); and iodoplatinate reagent[20] (5 ml of 5% platinum chloride, 45 ml of 10% KI, water to 100 ml).

Solutions

Free bases or salts. Amounts of 10.0 mg were dissolved in 10.0 ml of ethanol.

Sulphoxides. Amounts of 10.0 mg of free bases or salts were transferred to a 10-ml graduated cylinder, dissolved in 1 ml of 15% H_2O_2 and 0.2 ml of acetic acid was added; after a reaction time of 30 min in a water-bath at 60°, the reaction mixture was diluted to 10.0 ml.

Chromatographic procedure

Volumes of 10 μl, representing 10 μg of compound, were applied with a 10-μl micropipette on to 3 cm of the lower edge of a TLC plate in a 2–3 cm line. The plate was placed in a chromatographic tank lined with filter-paper, saturated with the solvent system for at least 30 min, and developed over a 10-cm distance in about half an hour (ambient temperature 18–20°).

RESULTS AND DISCUSSION

Results on chromatographic behaviour in terms of relative R_F values are given in Table I. For phenothiazines, the relative R_F values were calculated with respect to chlorpromazine, whereas for sulphoxide derivatives the corresponding free phenothiazines were used as reference substances. The addition of ammonia to both eluent systems affords identical R_F values for salts and free bases. An

TABLE I

RELATIVE R_F VALUES OF COMPOUNDS EXAMINED WITH TWO ELUENT SYSTEMS

Compounds	*Free bases or salts*				*Sulphoxides*			
	Eluent A[a]		*Eluent B*[b]		*Eluent A*		*Eluent B*	
	Rel. R_F[c]	$(R_F)_{chl.}$[d]	*Rel.* R_F[c]	$(R_F)_{chl.}$[d]	*Rel.* R_F[c]	(R_F)[f]	*Rel.* R_F[c]	(R_F)[f]
1. 1 Aminoalkylphenothiazines								
(a) Dialkylaminoethyl derivatives								
Diethazine·HCl	1.27	(0.62)	1.35	(0.48)	0.64	(0.70)	0.19	(0.43
Dimethoxanate·HCl	0.50	(0.64)	0.45	(0.46)	0.74	(0.73)	0.75	(0.28
Dimetiotazine	0.99	(0.64)	0.95	(0.60)	0.81	(0.75)	0.51	(0.57
Profenamine·HCl	1.42	(0.62)	1.62	(0.48)	0.10	(0.48)	0.16	(0.63
Promethazine·HCl	0.96	(0.62)	0.90	(0.48)	0.65	(0.69)	0.14	(0.14
Propiomazine maleate	0.89	(0.64)	0.93	(0.33)	0.74	(0.77)	0.25	(0.20
Thiazinamium	0.00	(0.64)	0.00	(0.65)	0.00	(0.00)	0.00	(0.00
(b) Dialkylaminopropyl derivatives								
Acepromazine maleate	0.72	(0.66)	0.43	(0.33)	0.31	(0.32)	0.18	(0.27
Alimemazine bitartrate	1.19	(0.66)	1.23	(0.56)	0.45	(0.67)	0.16	(0.50
Aminopromazine fumarate	0.92	(0.66)	0.85	(0.56)	0.00	(0.73)	0.00	(0.27
Chlorproethazine·HCl	1.15	(0.66)	1.11	(0.56)	0.46	(0.52)	0.11	(0.28
Chlorpromazine·HCl	1.00		1.00		0.31	(0.45)	0.09	(0.23
Levomepromazine maleate	1.22	(0.62)	1.24	(0.48)	0.21	(0.58)	0.12	(0.33

TABLE I (*continued*)

Compounds	*Free bases or salts*				*Sulphoxides*			
	Eluent A[a]		*Eluent B*[b]		*Eluent A*		*Eluent B*	
	Rel. R_F[c]	$(R_F)_{chl.}$[d]	*Rel.* R_F[c]	$(R_F)_{chl.}$[d]	*Rel.* R_F[e]	(R_F)[f]	*Rel.* R_F[e]	(R_F)[f]
Methiomeprazine	1.16	(0.64)	1.15	(0.65)	0.08	(0.75)	0.15	(0.72)
Oxomemazine[g]	0.92	(0.64)	0.89	(0.65)				
Promazine·HCl	0.77	(0.66)	0.75	(0.56)	0.40	(0.25)	0.17	(0.12)
Propiopromazine·H_3PO_4	0.87	(0.62)	0.79	(0.49)	0.48	(0.70)	0.20	(0.15)
Triflupromazine·HCl	1.10	(0.66)	1.03	(0.56)	0.42	(0.60)	0.30	(0.30)
I.2 Piperidylalkyl phenothiazines								
Pecazine·HCl·H_2O	1.06	(0.57)	1.07	(0.41)	0.12	(0.40)	0.14	(0.21)
Propericiazine	0.70	(0.64)	0.46	(0.65)	0.50	(0.65)	0.00	(0.17)
Thioridazine	0.93	(0.57)	0.78	(0.42)	0.00	(0.27)	0.21	(0.47)
I.3 Piperazinylalkyl phenothiazines								
Acetophenazine dimaleate	0.31	(0.66)	0.06	(0.33)	0.19	(0.16)	0.00	(0.12)
Dixyrazine	0.66	(0.56)	0.29	(0.46)	0.63	(0.81)	0.26	(0.31)
Fluphenazine·2HCl	0.55	(0.57)	0.24	(0.42)	0.37	(0.78)	0.30	(0.36)
Perazine dimalonate	0.45	(0.56)	0.35	(0.46)	0.14	(0.14)	0.18	(0.33)
Perphenazine	0.50	(0.56)	0.23	(0.46)	0.00	(0.13)	0.00	(0.26)
Prochlorperazine dimaleate	0.62	(0.56)	0.55	(0.46)	0.17	(0.12)	0.28	(0.50)
Thiopropazate·2HCl	1.17	(0.64)	1.09	(0.71)	0.38	(0.60)	0.14	(0.50)
Thioproperazine dimethanesulphonate	0.40	(0.57)	0.18	(0.40)	0.37	(0.46)	0.20	(0.10)
Trifluoperazine·2HCl	0.66	(0.57)	0.50	(0.40)	0.33	(0.33)	0.18	(0.28)
II.1 Azaphenothiazines								
Isothipendyl·HCl	0.90	(0.66)	0.80	(0.33)	0.45	(0.20)	0.33	(0.33)
Prothipendyl·HCl·H_2O	0.71	(0.66)	0.44	(0.38)	0.39	(0.33)	0.24	(0.21)
II.2 Thioxanthenes								
Chlorprothixene·HCl	1.12	(0.68)	1.15	(0.65)				
Methixene·HCl	0.95	(0.64)	0.82	(0.45)				
III.1 Dibenzazepines								
(*a*) *Iminodibenzyl derivatives*								
Desmethylimipramine·HCl	0.32	(0.61)	0.23	(0.56)				
Imipramine·HCl	0.91	(0.71)	0.93	(0.56)				
Trimeprimine maleate	1.20	(0.68)	1.23	(0.65)				
(*b*) *Iminostilbene derivative*								
Opipramol·2HCl	0.49	(0.71)	0.21	(0.56)				
III.2 Dibenzodiazepine								
Dibenzepine·HCl	0.85	(0.68)	0.75	(0.65)				
III.3 Dibenzocycloheptadienes								
Amitriptyline·HCl	1.03	(0.68)	1.06	(0.65)				
Nortriptyline·HCl	0.49	(0.71)	0.45	(0.56)				

[a] Eluent A: chloroform–acetone–25 % ammonia, 50:50:1 (ml).

[b] Eluent B: chloroform–ethyl acetate–25 % ammonia, 50:50:1 (ml).

[c] Rel. $R_F = \frac{R_F \text{ of compound}}{R_F \text{ of chlorpromazine}}$.

[d] $(R_F)_{chl.}$ = R_F of chlorpromazine as measured on a given chromatogram.

[e] Rel. $R_F = \frac{R_F \text{ of sulphoxide}}{R_F \text{ of free base}}$.

[f] (R_F) = R_F of free base as measured on a given chromatogram.

[g] Oxomemazine is different from other free bases or salts in being a sulphone.

TABLE II

DETECTION CHARACTERISTICS[a] OF COMPOUNDS CHROMATOGRAPHED

Compounds	*Free bases or salts*								*Sulphoxides*			
	UV		*HBr*	*FPN*	Fe^{3+}	V^{5+}	Cr^{6+}	*Iodo-platinate*[b]	*UV*		Fe^{3+}	*HBr*
	254 nm	*350 nm*							*254 nm*	*350 nm*		
I.1 Aminoalkylphenothiazines												
(a) Dialkylaminoethyl derivatives												
Diethazine·HCl	+	–	pink	pink	pink	pink	pink	+	+	–	pink	pink–violet
Dimethoxanate·HCl	+	–	–	–	–	–	–	–	+	–	–	–
Dimetiotazine	+	yellow white	pink	pink	pink	pink	pink	+	+	blue	pink	pink
Profenamine·HCl	+	–	pink	pink	pink	pink	pink	+	+	–	–	orange
Promethazine·HCl	+	–	pink	pink	pink	pink	pink	+	+	–	–	pink
Propiomazine maleate	red	red		pink–violet	pink	pink–violet	pink	+	red	green	–	pink
Thiazinamium	+	–	pink	pink	pink	pink	pink	+	+	–	–	pink
(b) Dialkylaminopropyl derivatives												
Acepromazine maleate	red	red	orange	orange	orange	orange	pink	+	red	yellow	pink	pink
Alimemazine bitartrate	+	–	orange	orange	orange	orange	orange	+	+	–	orange	pink
Aminopromazine fumarate	+	–		pink–violet	pink–violet	pink–violet	pink–orange	+	+	–	pink–violet	pink–violet
Chlorproethazine·HCl	+	–	pink	pink	pink	pink	pink	+	+	–	pink	pink
Chlorpromazine·HCl	+	–	pink	pink	pink	pink	pink	+	+	–	pink	pink
Levomepromazine maleate	+	–	blue	blue	blue	blue	blue	+	+	–	–	blue
Methiomeprazine	+	–	blue	blue	blue	blue	blue	+	+	green	pink	blue
Oxomemazine	+	–	–	–	–	–	–	+	+	–	–	–
Promazine·HCl	+	–	orange	orange	orange	orange	orange	+	+	–	–	pink
Propiopromazine·H_3PO_4	red	red		orange	orange	orange	orange	+	purple	green	orange	pink
Triflupromazine·HCl	+	–		yellow–brown	yellow–brown	yellow–brown	yellow–brown	+	+	blue	yellow–brown	yellow–brown
I.2 Piperidylalkyl phenothiazines												
Pecazine·HCl·H_2O	+	–	pink	orange	orange	orange	orange–pink	+	+	–	orange	pink–orange
Propericiazine	pink	yellow		orange	orange	orange	orange	+	yellow	green	pink	pink
Thioridazine	+	–	blue	blue	blue	blue	blue	+	blue	blue	pink	blue–green

I.3 Piperazinylalkyl phenothiazines												
Acetophenazine dimaleate	red	red		orange	orange	orange	pink	+	yellow	green	—	pink
Dixyrazine	+	—	pink	pink	pink	pink	pink	+	—	—	pink	pink
Fluphenazine·HCl	+	—		yellow–brown	yellow–brown	yellow–brown	yellow–brown	+	—	—	orange	pink
Perazine dimalonate	+	—	pink	pink	pink	pink	pink	+	—	—	—	pink
Perphenazine	+	—	pink	pink	pink	pink	pink	+	—	—	—	pink
Prochlorperazine dimaleate	+	—	pink	pink	pink	pink	pink	+	+	—	pink	pink
Thiopropazate·2HCl	+	—	pink–violet	pink–violet	pink–violet	pink–violet	pink–violet	+	+	—	—	pink
Thioproperazine dimethanesulphonate	red	yellow		pink	pink	pink	pink	+	blue	blue	—	pink
Trifluoperazine·2HCl	+	—	yellow–brown	yellow–brown	yellow–brown	yellow–brown	yellow–brown	+	+	blue	pink	pink
II.1 Azaphenothiazines												
Isothipendyl·HCl	+	blue	yellow	orange	yellow	orange	orange	+	+	—	—	yellow
Prothipendyl·HCl	+	blue	yellow	yellow	yellow	yellow–brown	yellow–brown	+	+	blue	yellow	yellow
II.2 Thioxanthenes												
Chlorprothixene·HCl	+	—	orange	orange	orange	orange	orange	+				
Methixene·HCl	+	—	pink	pink	pink	pink	pink	+				
III.1 Dibenzazepines												
(*a*) *Iminodibenzyl derivatives*												
Desmethylimipramine·HCl	+	—	—	—	—	blue	blue	+				
Imipramine·HCl	+	—	—	—	—	blue	blue	+				
Trimeprimine maleate	+	—	—	—	—	blue	blue	+				
(*b*) *Iminostilbene derivative*												
Opipramol·2HCl	red	red	—	—	—	—	yellow	+				
III.2 Dibenzodiazepine												
Dibenzepine·HCl	+	—	—	—	—	pink	yellow	+				
III.3 Dibenzocycloheptadienes												
Amitriptyline·HCl	+	—	—	—	—	—	—	+				
Nortriptyline·HCl	+	—	—	—	—	—	—	+				

[a] UV inspection: + = absorption; — = neither absorption nor fluorescence; colour = fluorescence.
[b] Iodoplatinate reagent: +, colours are only slightly differentiated (blue, violet, brown, blue–brown).

exception to this is thiazinamium, with its inconvertible quaternary ammonium structure. The more polar aminopromazine sulphoxide and perphenazine sulphoxide cannot be chromatographed in either solvent system, and propericiazine sulphoxide and acetophenazine sulphoxide can be developed only in eluent A and thioridazine sulphoxide only in solvent system B. In general, the former seemed to be the most appropriate for the separation of free phenothiazines from their sulphoxides.

Results on the detection of compounds are given in Table II. After spraying a reagent, a hot air stream was applied for a few moments, resulting in more intense stains. The intensity of the colours obtained decreases in the order FPN, Cr^{6+}, V^{5+}, Fe^{3+}. The opposite relationship applies to the stability of the colours. For example, phenothiazines developed with FPN reagent yield very intense colours that exist for only a short time and which may even disappear instantaneously on overspraying; with Fe^{3+} reagent they produce less intense but stable stains.

TABLE III

TLC OF SOME METABOLITES IN ELUENT A

Compounds	R_F	*Rel.* R_F	*Detection*
Levomepromazines			*Fe^{3+} reagent*
Levomepromazine maleate	0.81	1.00	blue
Desmethyllevomepromazine maleate	0.43	0.53	blue
Levomepromazine sulphoxide	0.17	0.21	(blue)[a]
Desmethyllevomepromazine sulphoxide maleate	0.07	0.09	blue
Imipramines			*Cr^{6+} reagent*
Imipramine·HCl	0.65	1.00	blue
Desmethylimipramine·HCl	0.27	0.41	blue
Bidesmethylimipramine·HCl	0.76	1.17	blue
2-Hydroxyimipramine·HCl	0.32	0.50	green, orange edge
2-Hydroxydesmethylimipramine·HCl	0.06	0.09	green, orange edge
Iminodibenzyl	0.88	1.35	green–blue
2-Hydroxyiminodibenzyl·HCl	0.90	1.39	blue
Dibenzepines			*Cr^{6+} reagent*
Dibenzepine·HCl	0.54	1.00	yellow
5-Methyl-10-β-methylaminoethyl-10,11-dihydro-11-oxo-5H-dibenzo-[*b*,*e*] [1,4]-diazepine	0.24	0.44	yellow
5-Methyl-10-β-aminoethyl-10,11-dihydro-11-oxo-5H-dibenzo-[*b*,*e*] [1,4]-diazepine	0.59	1.09	yellow
10-β-Dimethylaminoethyl-10,11-dihydro-11-oxo-5H-dibenzo-[*b*,*e*] [1,4]-diazepine	0.47	0.87	blue
10-β-Methylaminoethyl-10,11-dihydro-11-oxo-5H-dibenzo-[*b*,*e*] [1,4]-diazepine	0.19	0.35	blue
10-β-Aminoethyl-10,11-dihydro-11-oxo-5H-dibenzo-[*b*,*e*] [1,4]-diazepine	0.50	0.92	blue
Amitriptylines			*Iodoplatinate reagent*
Amitriptyline·HCl	0.55	1.00	brown
Amitriptyline-N-oxide	0.00	0.00	brown
Nortriptylines			*Iodoplatinate reagent*
Nortriptyline·HCl	0.25	0.45	brown
Desmethylnortriptyline·HCl	0.90	1.64	brown

[a] Detected by HBr reagent.

Group differentiation following the oxidative series of the reagents is feasible: phenothiazines react better with Fe^{3+} and V^{5+} reagents, phenothiazine sulphoxides with HBr, thioxanthenes with Marquis reagent*, dibenzazepine and dibenzodiazepine derivatives with Cr^{6+} reagent, and dibenzocycloheptadienes only with iodoplatinate reagent. Compounds with additional double or triple bonds in the R_2-substituent, such as propiomazine ($-COC_2H_5$), acepromazine ($-COCH_3$), propiopromazine ($-COC_2H_5$), propericiazine ($-CN$), acetophenazine ($-COCH_3$) and thioproperazine ($-SO_2NMe_2$), or in the tricyclic system, such as opipramol ($-CH=CH-$), can be made visible through their strong fluorescence under UV light. After spraying with Cr^{6+} reagent and inspection under UV light of wavelength 254 nm, chlorprothixene is seen as a pink stain with green–yellow fluorescent edge, while opipramol is detected by its characteristic green–yellow fluorescence.

In general, phenothiazines give yellow, orange, pink or pink–violet colours. Exceptions to this are levopromazine, blue ($R_2 = OCH_3$) and methiomeprazine and thioridazine, blue ($R_2=SCH_3$). Two phenothiazines, dimethoxanate and oxomemazine, cannot be detected with Fe^{3+}, V^{5+} or Cr^{6+} reagent. The first product possesses an esterified carboxyl function located on the ring nitrogen, whereas the latter is characterized by its doubly oxygenated sulphur atom (sulphone).

All experiments were carried out on 10-μg amounts, but detection limits are estimated at the 1–2 μg level. Inspection under short wavelength UV light after spraying with Cr^{6+} reagent lowers the detection limit for chlorprothixene and opipramol to about 0.01 μg. A similar effect occurs for thioxanthenes if Marquis reagent is used as the spray solution combined with viewing under UV light.

In practice, active substances are made visible by applying a definite sequence: UV inspection (254 and 350 nm) for absorption or fluorescence, Fe^{3+} reagent (phenothiazines, azaphenothiazines and thioxanthenes), V^{5+} reagent (as for Fe^{3+} reagent, and also iminodibenzyl derivatives), Cr^{6+} reagent (dibenzazepines and dibenzodiazepines), UV inspection (254 and 350 nm) for typical fluorescence, HBr (sulphoxides) and iodoplatinate reagent (dibenzocycloheptadienes).

It can be seen in Table III that free compounds, metabolites as sulphoxides and N-desmethylated products may simultaneously be separated and detected in eluent A. The described system is now in routine use for the screening of extracts of body fluids in clinical toxicological cases.

REFERENCES

1 T. J. MELLINGER AND C. E. KEELER, *J. Pharm. Sci.*, 51 (1962) 1169.
2 W. PAULUS, W. HOCH AND R. KEYMER, *Arzneim.-Forsch.*, 7 (1963) 609.
3 H. EBERHARDT, O. W. LERBS AND K. J. FREUNDT, *Arzneim.-Forsch.*, 13 (1963) 804.
4 H. EBERHARDT, O. W. LERBS AND K. J. FREUNDT, *Naunyn-Schmiedebergs Arch. Exp. Pathol. Pharmakol.*, 245 (1963) 136.
5 Z. MARGASINSKI, R. DANIELAK, T. POMAZANSKA AND H. RAFALOWSKA, *Acta Pol. Pharm.*, 21 (1964) 5; 21 (1964) 267.
6 A. NOIRFALISE AND M. H. GROSJEAN, *J. Chromatogr.*, 16 (1964) 237.
7 A. NOIRFALISE, *J. Chromatogr.*, 19 (1965) 68.
8 T. CONSTANTINESCU AND S. ENACHE, *Arzneimittelstandardisierung*, 19 (1969) 197.
9 R. L. MITAL AND S. K. JAIN, *J. Chromatogr.*, 47 (1970) 546.
10 C. KORCZAK-ŁABIERKIEWICZ AND G. CIMBURA, *J. Chromatogr.*, 53 (1970) 413.
11 N. KAUL, M. W. CONWAY AND M. L. CLARK, *Nature*, 226 (1970) 372.

* Results not given in Table II; the reagent consists of 2 ml of 37% HCHO dissolved in 100 ml of concentrated H_2SO_4.

12 E. GRUSZ-HARDAY, *Pharmazie*, 26 (1971) 562.
13 E. SCHMID, E. HOPPE, C. MEYTHALER, JR., AND L. ZICHA, *Arzneim.-Forsch.*, 11 (1963) 969.
14 S. LAUFFER, E. SCHMID AND F. WEIST, *Arzneim.-Forsch.*, 19 (1969) 1965.
15 A. VIALA, F. GOUEZO AND C. GOLA, *J. Chromatogr.*, 45 (1969) 94.
16 H. SAWADA AND K. SHINOHARA, *Arch. Toxikol.*, 27 (1970) 71.
17 I. S. FORREST AND F. M. FORREST, *Clin. Chem.*, 6 (1960) 11.
18 H. LEACH AND W. R. C. CRIMMIN, *J. Clin. Pathol.*, 9 (1956) 164.
19 I. S. FORREST AND F. M. FORREST, *Amer. J. Psych.*, 116 (1960) 840.
20 A. STOLMAN, *Progress in Chemical Toxicology*, Vol. 2, Academic Press, New York and London, 1965, p. 357.