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THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION OF PHENOTHIAZINE DERIVATIVE DRUGS

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SUMMARY

Data are presented on the thin-layer chromatographic identification of 20 phenothiazine bases official in the U.S.P. XX. On the basis of four chemically different solvent systems and the color developed with a spray reagent, all phenothiazines could be distinguished from each other. For greater precision, relative R_F values (ratios of the R_F of each phenothiazine to the R_F of chlorpromazine) have been used. The relative efficiencies of various brands of commercially prepared thin-layer plates were evaluated for these solvent systems.

INTRODUCTION

Phenothiazine derivative drugs are a pharmacologically diverse group of substances encompassing therapeutic categories such as antiemetic, antipsychotic, sedative, antipruritic, antidyskinetic, analgesic and antihistaminic. In the U.S.P. XX[1] there are 77 monographs on phenothiazine derivative drugs comprising 20 different phenothiazine bases. The structure of the parent compound and the various substituents are depicted in Table I.

Although the compounds are chemically closely related, there is no uniformity in the analytical methodology for their identification in the monographs. Thin-layer chromatographic (TLC) methods are used for eleven of the substances, while other techniques, such as infrared and ultraviolet spectroscopy, are used for the remaining drugs. In the TLC procedures, two of the mobile solvent systems are identical; the others are diverse.

The British Pharmacopoeia 1980[2] has consolidated its methodology for phenothiazines by including monographs on the identification of phenothiazines and related impurities in phenothiazines in its Appendix. A single solvent system is used for all phenothiazine identifications; three chemically similar mobile solvent systems are used in the test for related impurities in phenothiazines, depending on the particular phenothiazine.

A number of investigators[3–5] have described TLC systems that may be useful in the identification of phenothiazines. Cimbura[6], in a review on methods of analysis for phenothiazines, summarized published work on TLC analysis. TLC systems for

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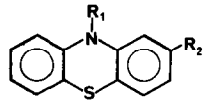
TABLE I
STRUCTURES OF THE PHENOTHIAZINE-TYPE DRUGS

Parent compound:



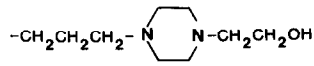
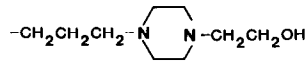
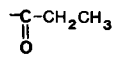
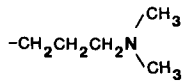
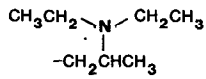
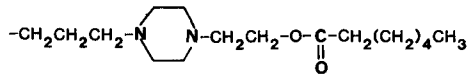
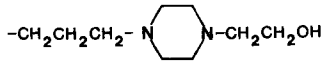
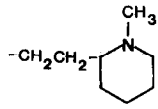
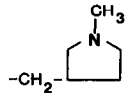
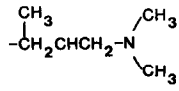
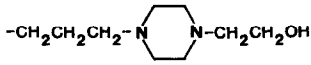
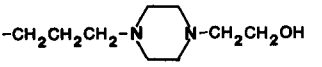
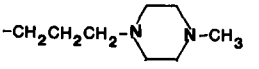
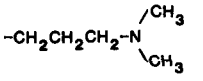
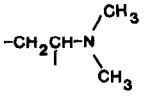
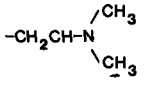
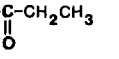
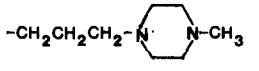
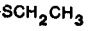
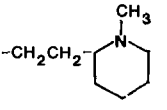
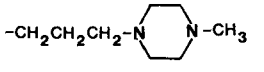
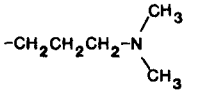
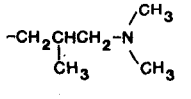
Name	R_1	R_2
Acetophenazine		
Carphenazine		
Chlorpromazine		-Cl
Ethopropazine		-H
Fluphenazine enanthate		$-CF_3$
Fluphenazine		$-CF_3$
Mesoridazine		
Methdilazine		-H
Methotrimeprazine		$-OCH_3$
Perphenazine		-Cl

TABLE I (*continued*)

Name	R_1	R_2
Piperacetazine		
Prochlorperazine		
Promazine		
Promethazine		
Propiomazine		
Thiethylperazine		
Thioridazine		
Trifluoperazine		
Triflupromazine		
Trimeprazine		

separating approximately 20 phenothiazines were described by Cochin and Daly[7]. Kofoes *et al.*[8] described a TLC system applicable to the separation of 40 phenothiazines and their sulfoxides; Korczak-Fabierkiewicz and Cimbura[9] described TLC systems for 19 phenothiazines. Noirfalise[10] investigated 11 phenothiazines and Flinn[11] studied 16 phenothiazines. TLC systems effective in separating phenothiazines and their metabolites were described by Turano *et al.*[12] and Zingales[3,13]. These studies produced data useful for confirmative identification of the individual phenothiazine drugs, but did not provide a coordinated system that would distinguish all of them.

This study was designed to approach the problem of identification in a sequential, systematic manner through the judicious selection of solvent systems and examination of the colors of spots after spraying. It was expected that the appropriate selection of a small number of chemically different solvent systems would provide thin-layer chromatograms for all the official phenothiazine drugs with distinctive R_F values, facilitating the identification of the various phenothiazines. The methodology developed then could be applied to the identification of unknown substances obtained from body tissues of medicated patients, as well as serving for the confirmation of the identity of a suspected phenothiazine.

EXPERIMENTAL

Solvents and chemicals

The following reagents, of ACS reagent grade, were used: chloroform, methanol (Burdick & Jackson Labs., Muskegon, MI, U.S.A.; J. T. Baker, Phillipsburg, NJ, U.S.A.), isopropanol, ethyl acetate (Burdick & Jackson Labs.; Mallinkrodt, St. Louis, MO, U.S.A.), diethyl ether, methylene chloride, butanol-1 (Burdick & Jackson Labs.), ethanol (U.S.Public Health Service Supply Center), ammonia solution and sulfuric acid (J. T. Baker).

Chromatographic plates

The following chromatographic plates (20 × 20 cm), having a 250-μm adsorbent layer and a pre-adsorbent layer, were used: Whatman silica gel LK5; Analtech Uniplates, silica gel GHLF; Whatman LK5F; Baker Si 250 F-PA; Brinkmann Silgur 25 UV_{254} Art. No. 810-023; and EM Merck Cat. No. 11798.

The following plates (20 × 20 cm), having a 250-μm adsorbent layer and no pre-adsorbent layer, were used: Analtech Uniplates, silica gel GF; Analabs Type OF Anasil; Schleicher & Schüll G5100 LS254; and Supelco 5-8167.

Equipment

For development of the plates, glass developing tanks (25 × 30 × 10 cm) were used. Spots were applied with a continuously adjustable precision micropipet. UV detection was accomplished in an Ultra-Violet Products Inc. Chromato-Vue chromatography scanning cabinet.

Compounds chromatographed

The following substances, which are official in the U.S.P. XX[1], were chromatographed: acetophenazine maleate, carphenazine maleate, fluphenazine · HCl,

mesoridazine besylate, methdilazine, methdilazine · HCl, methotrimeprazine, piperacetazine, thiethylperazine malate, trifluoperazine · HCl, triflupromazine · HCl (National Center for Drug Analysis, St. Louis, MO, U.S.A.), chlorpromazine · HCl, ethopropazine · HCl, prochlorperazine maleate, promethazine · HCl (U.S.P. Reference Standard), promazine · HCl, thiethylperazine maleate (N.F. Reference Standard), perphenazine (Schering Corp.), propiomazine (Wyeth Labs.), thioridazine, thioridazine · HCl (Sandoz Pharmaceuticals), fluphenazine enanthate dihydrochloride (E. R. Squibb) and trimeprazine tartrate (Smith, Kline & French Labs.).

In addition, the following extracts of oral dosage forms of the above standards were chromatographed: Tindal, 20 mg acetophenazine maleate; Trilafon, 2 mg perphenazine (Schering); Proketazine, 12.5 mg carphenazine maleate; Sparine, 10 mg promazine · HCl; Phenergan, 12.5 mg promethazine (Wyeth); Thorazine, 10 mg chlorpromazine · HCl; Compazine, 5 mg prochlorperazine maleate; Stelazine, 1 mg trifluoperazine · HCl, Temaril, 2.5 mg trimeprazine tartrate (SKF); Parsidol, 10 mg ethopropazine · HCl (Warner/Chilcott); Prolixin, 1 mg fluophenazine · HCl; Vesprin, 10 mg triflupromazine · HCl (Squibb); Serentil, 10 mg mesoridazine besylate; Torecan, 10 mg thiethylperazine maleate (Boehringer Ingelheim); Tacaryl Chewable, 3.6 mg methdilazine; Tacaryl, 8 mg methdilazine · HCl (Westwood); Quide, 10 mg piperacetazine (Dow Pharmaceuticals); Mellaril, 10 mg thioridazine · HCl (Sandoz).

Procedure

Spotting solutions of *ca.* 2 mg/ml were prepared from the standard phenothiazine materials using 1% ammonia solution in methanol as the solvent.

Oral dosage forms spotting solutions were prepared as follows. Twenty tablets were finely ground and the composite equivalent to *ca.* 8 mg of phenothiazine drug was sonicated and shaken with 4 ml 1% ammonia solution in methanol. The suspension was filtered through Whatman No. 40 paper. A 5-μl volume of standard solution or composite extract was spotted. Development took place in a tank lined with blotting paper saturated with mobile phase. The chromatographic plates were developed for 10 cm from the border of the pre-adsorptive layer and plate support.

The following solvent systems were evaluated on 20 cm × 20 cm plates: (I) diethyl ether–ethyl acetate–ammonia solution (1:1, saturated)[14]; (II) methylene chloride–methanol (50:10)[15]; (III) methanol–butanol-1 (60:40)[7]; and (IV) chloroform–isopropanol–ammonia solution (70:30:1)[16].

The spots were detected by viewing under shortwave ultraviolet light as an initial assessment of the chromatogram. The spots were also revealed by spraying with a solution of 10% (v/v) sulfuric acid in ethanol[7]. Two micrograms of phenothiazine can be detected under these conditions.

RESULTS AND DISCUSSION

Of the 23 phenothiazine drugs official in the U.S.P. XX, four are available as free bases, two as free bases and as salts of these bases, fourteen as salts, one of which is available as a salt of the same base, and two different anions. All are readily soluble in 1% ammonia solution in methanol, and this is the solvent of choice. All drugs are then spotted in the free base form.

In addition to the four solvent systems evaluated, other systems that had been

TABLE II

FREE BASES OF PHENOTHIAZINE-TYPE DRUGS LISTED IN ASCENDING ORDER OF RELATIVE R_F VALUES USING CHLORPROMAZINE AS A REFERENCE STANDARD ACCORDING TO FOUR MOBILE SOLVENT SYSTEMS (R_{CHL})*

*Color***	*I****	R_{CHL}	*II*§	R_{CHL}	*III*§§	R_{CHL}	*IV*§§§	R_{CHL}
Pink	Mesoridazine	0.11	Mesopridazine	0.24	Mesoridazine	0.41	Mesoridazine	0.48
	Perphenazine	0.26	Prochlorperazine	0.87	Chlorpromazine	1.00	Perphenazine	0.73
	Prochlorperazine	0.49	Chlorpromazine	1.00	Perphenazine	1.08	Prochlorperazine	0.85
	Chlorpromazine	1.00	Perphenazine	1.53	Prochlorperazine	1.12	Chlorpromazine	1.00
Blue	Thiethylperazine	0.46	Thioridazine	0.78	Thioridazine	1.04	Thiethylperazine	0.94
	Thioridazine	0.81	Thiethylperazine	0.85	Thiethylperazine	1.22	Thioridazine	1.08
	Methotrimeprazine	1.26	Methotrimeprazine	1.40	Methotrimeprazine	1.31	Methotrimeprazine	1.20
Pink-orange	Promethazine	0.80	Promethazine	1.12	Promethazine	1.24	Promethazine	1.04
Orange	Acetophenazine	0.13	Methdilazine	0.41	Piperacetazine	0.64	Acetophenazine	0.60
	Carphenazine	0.19	Promazine	0.60	Methdilazine	0.66	Carphenazine	0.73
	Fluphenazine	0.30	Trifluoperazine	1.03	Promazine	0.81	Fluphenazine	0.74
	Piperacetazine	0.38	Acetophenazine	1.09	Acetophenazine	0.86	Methdilazine	0.76
	Methdilazine	0.51	Triflupromazine	1.24	Carphenazine	0.97	Piperacetazine	0.76
	Trifluoperazine	0.61	Piperacetazine	1.25	Ethopropazine	1.10	Promazine	0.87
	Promazine	0.74	Trimeprazine	1.33	Fluphenazine	1.13	Trifluoperazine	0.89
	Propiomazine	0.87	Carphenazine	1.36	Trifluoperazine	1.15	Triflupromazine	1.07
	Triflupromazine	1.09	Propiomazine	1.54	Triflupromazine	1.17	Trimeprazine	1.19
	Trimeprazine	1.24	Ethopropazine	1.60	Trimeprazine	1.17	Propiomazine	1.23
	Fluophenazine enanthate	1.44	Fluphenazine	1.77	Propiomazine	1.56	Ethopropazine	1.28
	Ethopropazine	1.54	Fluphenazine enanthate	3.18	Fluphenazine enanthate	2.03	Fluphenazine enanthate	1.43

* Analtech Uniplates silica gel GHLF were used.

** Sprayed with 10% sulfuric acid in ethanol.

*** Solvent system I: diethyl ether–ethyl acetate–ammonia solution (1:1:saturated).

§ Solvent system II: methanol–butanol-1 (60:40).

§§ Solvent system III: methylene chloride–methanol (50:10).

§§§ Solvent system IV: chloroform–isopropanol–ammonia solution (70:30:1).

checked, but were found to be less valuable, were chloroform–cyclohexane–ethanol–acetic acid (50:50:30:10), toluene–methanol–acetic acid (95:2.5:2.5), methylene chloride–methanol (50:10), ethanol–acetic acid–water (50:30:20), chloroform–cyclohexane–methanol (40:40:20), butanol-1–di-*n*-butyl ether–acetic acid (40:80:10), benzene–*p*-dioxane–ammonia solution (60:35:5), acetone and methanol–water–ammonium hydroxide (100:10:3g).

The effect of more unusual solvents, such as dimethyl sulfoxide, tetrahydrofuran, formaldehyde and acetonitrile, in various combinations and proportions was also studied in pilot systems. They were not found to be of particular value in identification, either because of apparent separation of the phases on the silica gel support, or because of too little difference among the R_F values for the various phenothiazines.

The four solvent systems finally selected were sufficiently different from each other chemically to allow the 20 bases to be distinghuished from each other on the basis of R_F values and colors of spots. These four systems were evaluated using different brands of silica gel plates with and without pre-adsorptive bands for spotting.

The data were examined for reproducibility of R_F values using a statistical treatment, and also using relative R_F values (R_X), where R_{CHL} is defined as the ratio of the R_F of a particular phenothiazine divided by the R_F of the reference phenothiazine, chlorpromazine. Several investigators[3,10,18,19] have noted improved reproducibility of data using this ratio instead of R_F alone.

Table II shows the R_{CHL} values for the 20 phenothiazine bases, grouped according to color of spots in ascending order, for the four solvent systems selected. These values are averages for all of the determinations performed. The means were calculated from multiple determinations for the various phenothiazines and were found to be reliable to within ± 10%. There appeared to be no significant difference in R_F values among the plates having a pre-adsorbent layer and those without one, but the spots were less diffuse, better differentiated and less streaky on these plates. Therefore, plates having a pre-adsorbent layer are recommended.

In order to determine statistically the reliability of R_F values with the same solvent system but with different brands of commercially prepared chromatographic plates, a special run of twelve determinations was performed. Nine phenothiazine drugs having a wide range of R_F values in preliminary determinations were spotted on different plates and were developed in solvent system I, which differentiates the most phenothiazines and gives the largest spread of R_F values.

The following plates were used: Baker SI250F-PA (2), Brinkmann (5), EM Merck (1), and Analtech Uniplate silica gel GHLF (4); all had a pre-adsorptive layer. It was found that in general the coefficient of variation for the R_F values is greater than that for the R_{CHL} values. The exceptions involve the series in which all twelve plates of different brands were evaluated, where the greatest variability of both the R_F and R_{CHL} values was exhibited. The discrepancies in the R_{CHL} and R_F values occur exclusively at R_F values less than 0.10, where there may be a substantial error in measurement. The data indicate that the plates of all manufacturers tested were usable for identification.

As an approach to identification, the following procedure is suggested. Chromatograms of the unknown phenothiazine and chlorpromazine are developed with

the four solvent systems and the plates are sprayed with 10% (v/v) sulfuric acid in ethanol. The ratio (R_{CHL}) of the R_F value of the phenothiazine to the R_F value of chlorpromazine is calculated for each solvent. The color of each spot (pink, pink-orange, orange or blue) is noted, and by consulting Table II, the group to which a particular phenothiazine belongs may be established. Then, for each solvent system, the compounds are listed that fall in the $\pm 10\%$ range calculated for the observed spot. The unique compound that occurs in all four systems is the unknown. Further confirmation is obtained by spotting the unknown side-by-side with a standard.

ACKNOWLEDGEMENT

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