TLC Differentiation of Butyrophenone and Diphenylbutylpiperidine Compounds from Phenothiazine Derivatives

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Abstract □ A procedure is described for TLC detection and differentiation of the butyrophenone–diphenylbutylpiperidine group and phenothiazine derivatives at the microgram level. A two-dimensional TLC method to separate butyrophenone and diphenylbutylpiperidine compounds is reported. A variety of possible detection reagents were examined. The solvent systems and spray reagents described should be useful for the identification of these drugs in various dosage forms.

Keyphrases □ Butyrophenones—analysis, TLC, differentiation from phenothiazine derivatives □ Diphenylbutylpiperidines—analysis, TLC, differentiation from phenothiazine derivatives □ TLC—analysis, butyrophenones, diphenylbutylpiperidines, phenothiazine derivatives

Butyrophenones, diphenylbutylpiperidines, and the pharmacologically analogous phenothiazines are currently used for the treatment of various psychotic disorders. A rapid detection and identification method is needed for screening pharmaceutical formulations for these drugs.

Numerous investigators (1–13) described various solvent systems for chromatography of psychotropic drugs, mainly phenothiazine derivatives. However, none of these systems completely separates the two groups of neuroleptic compounds. In the proposed analytical system, group differentiation depends on R_f values and on color reactions to spray reagents. Identification of the butyrophenone or diphenylbutylpiperidine compounds can be accomplished with the described two-dimensional TLC method.

Table I—Guide R_f Values for Phenothiazine Derivatives (Eluent A)

Compound	R_f[a]	RR_f[b]
Acepromazine	0.23	0.72
Acetophenazine	0.20	0.62
Alimemazine (trimeprazine)	0.35	1.09
Chlorpromazine	0.32	1
Dixyrazine	0.31	0.97
Fluphenazine	0.33	1.03
Levomepromazine (methotrimeprazine)	0.36	1.12
Perazine	0.18	0.56
Perphenazine	0.31	0.97
Piperacethazine	0.18	0.56
Prochlorperazine	0.29	0.90
Promazine	0.23	0.72
Promethazine	0.37	1.16
Propericiazine (pericyazine)	0.30	0.94
Prothipendyl	0.17	0.53
Thiopropazate	0.71	2.22
Thioproperazine	0.23	0.72
Thioridazine	0.32	1
Triethylperazine	0.30	0.94
Trifluoperazine	0.33	1.03
Trifluopromazine	0.36	1.12

[a] Mean of six values. [b] Relative R_f value with chlorpromazine as standard.

EXPERIMENTAL

Adsorbent—Precoated TLC silica gel 60 F 254[1] plates were used.

Eluent Systems—Solvents were analytical grade and were used as received from commercial sources[1]. Eluent A consisted of ethyl acetate–chloroform–methanol–0.1 *M* sodium acetate buffer (pH 4.7) (54:23:18:5). Eluent B consisted of chloroform–ethanol (90:10).

Detection—The following spray reagents were used:

Reagent A—To 250 mg of vanadium pentoxide, 40 ml of concentrated sulfuric acid was added. The mixture was diluted with distilled water to 500 ml (12).

Reagent B—To 5 ml of 5% aqueous iron(III) chloride, 45 ml of 20% perchloric acid and 50 ml of 50% nitric acid were added (12, 13).

Reagent C—Five grams of *p*-dimethylaminobenzaldehyde was dissolved in 100 ml of 18 *N* H_2SO_4 (13).

Reagent D—A 40% (v/v) aqueous sulfuric acid solution was used. After spraying, the TLC plate was heated at 110° for 5–10 min (14).

Reagent E—To 0.1% ethanolic bromcresol purple solution were added a few drops of a 10% ammonia solution until the color changed (15, 16).

Reagent F—Forty milligrams of bromcresol green was dissolved in 100 ml of ethanol, and 0.1 *N* NaOH was added until a blue color appeared (15, 16).

Reagent G—Aconitic acid, 250 mg, was dissolved in 40 ml of acetic anhydride by heating; the mixture was diluted with toluene to 100 ml. After spraying, the TLC plate was heated at 100° for 30 min (17, 18).

Drug Solutions—Ten milligrams of the pure drug reference standards[2] were dissolved in 10 ml of chloroform–methanol (7:3). The compounds investigated are summarized in Tables I and II.

Procedure—*Rapid Group Detection*—Volumes of 10 μl, corresponding to 10 μg of a compound, were applied to a precoated silica gel plate with a syringe[3]. The chromatoplate was developed over 15 cm using Eluent A in a tank[4] without filter paper lining. Use of a saturated paper-lined development tank resulted in a poor separation because of the lower R_f values of all compounds and because many of the phenothiazine derivatives did not migrate from the application point.

The plate was air dried at room temperature and viewed under shortwave UV light[5]. The spots were outlined with a pencil. Finally, the plate was sprayed with one detection reagent for group identification.

Identification of Butyrophenones and Diphenylbutylpiperidines—When the rapid detection method showed the presence of a neuroleptic compound of the butyrophenone–diphenylbutylpiperidine group, identification of this compound could be confirmed using two-dimensional TLC. With a 10-μl syringe, the butyrophenone–diphenylbutylpiperidine mixture was applied to the three corners of a TLC plate prepared as shown in Fig. 1.

After development over 15 cm using Eluent A in a tank without paper lining, the TLC plate was removed and air dried at room temperature. The plate was then developed for 15 cm at right angles to the first run in a second tank containing Eluent B and saturated by lining the walls with filter paper moistened with the mobile phase. The chromatogram

[1] Merck, Darmstadt, West Germany.

[2] Pure drug samples were supplied by Janssen Pharmaceutica (all butyrophenones and diphenylbutylpiperidines), Farmitalia, Searle, Schering, Rhône-Poulenc, Smith Kline & French, Sandoz, Clin-Byla, Pitman-Moore, Promonta, and Homburg and Union Chimique Belge (phenothiazine derivatives).

[3] Hamilton, Bonaduz, Switzerland.

[4] Desaga, Heidelberg, West Germany.

[5] Chromato-vue cabinet CC-20, Ultra-Violet Products, San Gabriel, Calif.

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Table II—Guide R_f Values for Butyrophenone and Diphenylbutylpiperidine Compounds

Compound	Eluent A			Eluent B	
	R_f[a]	RR_f[b]	RR_f[c]	R_f[a]	RR_f[c]
Butyrophenones					
Benperidol	0.55	1.72	1	0.29	1
Droperidol	0.63	1.97	1.14	0.37	1.27
Fluanisone	0.76	2.37	1.38	0.62	2.14
Haloperidol	0.44	1.37	0.80	0.16	0.55
Pipamperone	0.22	0.69	0.40	0.06	0.21
Trifluperidol	0.47	1.47	0.85	0.16	0.55
Diphenylbutylpiperidines					
Fluspirilene	0.67	2.09	1.22	0.43	1.48
Penfluridol	0.77	2.40	1.40	0.37	1.27
Pimozide	0.75	2.34	1.36	0.52	1.79

[a] Mean of four values. [b] Relative R_f value with chlorpromazine as standard. [c] Relative R_f value with benperidol as standard.

was air dried at room temperature and evaluated by quenching the 254-nm-induced fluorescence.

RESULTS

Guide R_f values for the examined compounds are summarized in Tables I and II.

The described one-dimensional TLC method with Eluent A allows almost a complete differentiation of the phenothiazine group and the butyrophenone–diphenylbutylpiperidine group. With this solvent system, nearly all compounds of the latter group were located between R_f 0.44 and 0.76. Pipamperone (R_f 0.22) was the only compound that did not migrate into this region. Fluanisone, penfluridol, and pimozide were not separated with this eluent. The phenothiazine thiopropazate (R_f 0.71) interfered with these three compounds. All other phenothiazines migrated into the R_f 0.18–0.37 region where pipamperone interference was possible.

Combination of R_f values and the color reactions can facilitate the identification of the compounds. All compounds investigated quenched the 254-nm-induced fluorescence. Group differentiation was possible with different reagents (Table III).

The phenothiazine group reacted positively with Reagents A–D, whereas no color reaction was seen for the butyrophenones and diphenylbutylpiperidines. Reagents C and D gave more intense colors than did Reagents A and B. All compounds examined reacted with Reagents E and F to give lightly bluish-colored spots. Reagent G reacted with butyrophenones and diphenylbutylpiperidines, but spot intensities were so light that identification was very difficult.

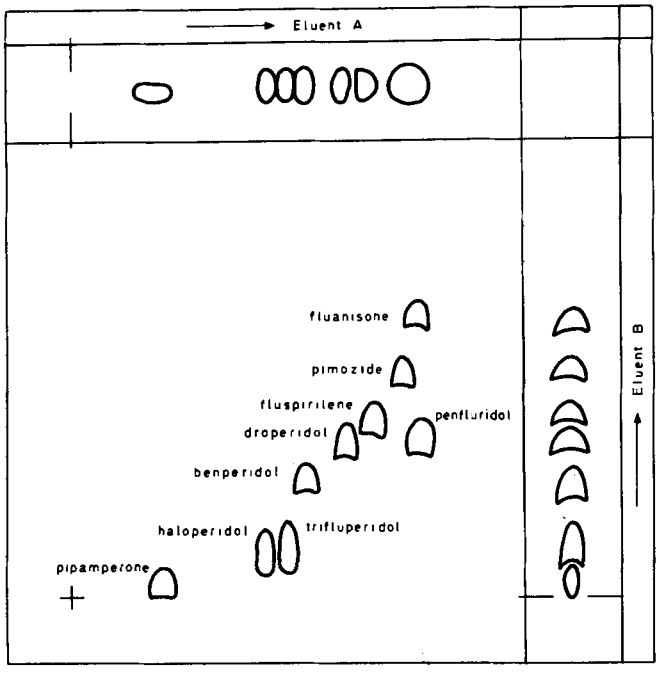

Figure 1—*Type chromatogram of the two-dimensional identification test for butyrophenones and diphenylbutylpiperidines.*

Table III—Color Reaction to Various Spray Reagents

Compound	UV, 254 nm	Detection Reagent[a]						
		A	B	C	D	E	F	G
Phenothiazines								
Acepromazine	+	RO	R	RO	RB	BL	BL	–
Acetophenazine	+	R	R	R	R	BL	BL	–
Alimemazine	+	RO	RO	RO	RB	BL	BL	–
Chlorpromazine	+	R	R	R	R	BL	BL	–
Dixyrazine	+	RO	R	R	R	BL	BL	–
Fluphenazine	+	O	O	O	O	BL	BL	–
Levomepromazine	+	P	P	BL	BL	BL	BL	–
Perazine	+	RO	R	R	R	BL	BL	–
Perphenazine	+	R	R	R	R	BL	BL	–
Piperacethazine	+	R	R	R	R	BL	BL	–
Prochlorperazine	+	R	R	R	R	BL	BL	–
Promazine	+	RO	RO	RO	RB	BL	BL	–
Promethazine	+	BO	B	B	RB	BL	BL	–
Propericiazine	+	RO	R	R	RO	BL	BL	–
Prothipendyl	+	O	Y	Y	B	BL	BL	–
Thiopropazate	+	WR	WR	R	R	BL	BL	–
Thioproperazine	+	R	R	R	R	BL	BL	–
Thioridazine	+	P	P	BL	BL	BL	BL	–
Triethylperazine	+	P	P	BL	BL	BL	BL	–
Trifluoperazine	+	O	O	O	O	BL	BL	–
Trifluopromazine	+	O	O	O	O	BL	BL	–
Butyrophenones								
Benperidol	+	–	–	–	–	BL	BL	W+
Droperidol	+	–	–	–	–	BL	BL	W+
Fluanisone	+	–	–	–	–	BL	BL	W+
Haloperidol	+	–	–	–	–	BL	BL	W+
Pipamperone	+	–	–	–	–	BL	BL	W+
Trifluperidol	+	–	–	–	–	BL	BL	W+
Diphenylbutylpiperidines								
Fluspirilene	+	–	–	–	–	BL	BL	W+
Penfluridol	+	–	–	–	–	BL	BL	W+
Pimozide	+	–	–	–	–	BL	BL	W+

[a] B = brown, BL = blue, O = orange, P = purple, R = red, Y = yellow, W = weak, + = detectable, and – = not detectable.

The color of a compound after TLC and spraying is generally dependent on the concentration of the substance in the sample, on the mode of reagent application, and on the amount of reagent used for detection. In this TLC procedure, identification depends on R_f values (or relative R_f values with, for example, chlorpromazine as a standard) and on color. Neither is an absolute.

Identity of the butyrophenone or diphenylbutylpiperidine compound can be accomplished by the described two-dimensional TLC method. Guide R_f values of the compounds for the two solvent systems are summarized in Table II. A two-dimensional chromatogram is shown in Fig. 1. Because of the great similarity in chemical structure of the butyrophenones and diphenylbutylpiperidines, it is very difficult to find a specific reagent for each compound of this group. Therefore, unspecific detection such as shortwave UV light was used.

Iodine vapors or the Munier–Macheboeuf modification of Dragendorff's reagent can be used also. The use of unspecific detecting agents, however, makes identification dependent on R_f values, which are influenced by numerous parameters. Comparison with reference samples would be absolutely necessary.

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High-Pressure Liquid Chromatographic Analysis of Antipyrine in Small Plasma Samples

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Received August 28, 1978, from the *College of Pharmacy and Allied Health Professions, Northeastern University, Boston, MA 02115.* Accepted for publication January 31, 1979.

Abstract □ A high-pressure liquid chromatographic (HPLC) method was developed for the assay of antipyrine in small (0.1-ml) plasma samples using aminopyrine as the internal standard and a reversed-phase microparticulate column. The assay sensitivity (1 μg/ml) permits development of a plasma level–time curve using a single rat. The mean (± *SE*) plasma elimination half-life in rats was 1.28 ± 0.14 hr. A comparison of the spectrophotometric method with the HPLC method yielded a correlation coefficient of 0.98. The HPLC assay for antipyrine is rapid and precise and can be used for hepatic drug metabolism study in a single animal.

Keyphrases □ Antipyrine—analysis, high-pressure liquid chromatography, plasma, rats □ High-pressure liquid chromatography—analysis, antipyrine, plasma, rats □ Analgesics—antipyrine, high-pressure liquid chromatographic assay, plasma, rats

The plasma antipyrine elimination rate is commonly used to study hepatic drug metabolism *in vivo* (1–5). Antipyrine is metabolized almost completely by the liver enzymes (6), and protein binding of the drug is minimal (7).

BACKGROUND

The spectrophotometric assay of Brodie *et al.* (8) generally is used for measuring plasma antipyrine levels. The spectrophotometric method is tedious and may yield high blank values. Furthermore, the method requires 1–2 ml of plasma, which precludes obtaining multiple samples from a small animal such as the rat.

GLC analyses of antipyrine also have been reported (3, 9). Although these methods appear to be more precise than the spectrophotometric method, they still require a 1.0-ml plasma sample and drug extraction. Another assay for plasma antipyrine using ^{14}C-label drug (4) is very sensitive but requires expensive equipment and involves the hazards of radioactivity.

High-pressure liquid chromatography (HPLC) has been applied to the separation and quantitation of many drugs. A sensitive HPLC procedure for antipyrine using a silica gel column was reported (10). This procedure requires 0.5 ml of plasma for drug extraction. A rapid, sensitive method was needed that utilizes smaller blood samples, 0.1–0.2 ml, so that an antipyrine pharmacokinetic profile can be obtained in a single laboratory animal such as the rat. Recently, HPLC procedures utilizing reversed-phase chromatography were developed for drug analysis in biological tissues (11). The advantage of reversed-phase HPLC is that minimal sample cleanup is required prior to chromatography; therefore, smaller tissue samples may be used.

This report describes a rapid HPLC procedure which is more precise than the spectrophotometric method and utilizes small plasma sample volumes.

EXPERIMENTAL

Materials—Antipyrine NF and aminopyrine NF were used as standards[1]. All other reagents, analytical grade or better, were purchased from commercial sources.

Animals—Adult, male, Sprague–Dawley strain rats[2], 200–350 g, were used.

Plasma Assay and Animal Dosing—For standard curves, heparinized blood samples were removed from the abdominal aorta of nonmedicated ether-anesthetized rats, placed into centrifuge tubes, and centrifuged to obtain the plasma fraction. Plasma aliquots (0.1 ml) were pipetted into 1.5-ml microtest tubes[3] and spiked with 10 μl of concentrated antipyrine solutions to yield final drug concentrations corresponding to 0, 1.5, 3, 4.5, 6, 12, 25, and 50 μg/ml of plasma.

To each microtest tube was added 0.05 ml of 20% (w/v) $ZnSO_4 \cdot 7H_2O$ in 50% (v/v) methanol–water containing aminopyrine, 100 μg/ml, as the internal standard. The mixture was mixed on a vortex mixer. Then 0.05 ml of saturated barium hydroxide solution was added to the mixture. The final mixture was vortexed and centrifuged for 10 min. A 10-μl sample of the supernate was removed and subjected to HPLC.

Rats were given 100 mg of antipyrine/kg ip (injection volume of 0.5 ml/200 g). Blood samples of approximately 250–300 μl were removed from the tail vein at 0, 2, 3, 4, 5, and 6 hr. The samples were placed into heparinized microcentrifuge tubes and centrifuged to obtain the plasma fraction. Plasma aliquots, 0.1 ml, were processed along with the spiked plasma standards described.

HPLC—Samples were chromatographed on a high-pressure liquid chromatograph[4] equipped with a universal liquid chromatographic injector, a UV (254-nm) absorbance detector, and a strip-chart recorder[5]. The deproteinated plasma samples (10 μl) were chromatographed at room temperature on a microparticulate[6] reversed-phase HPLC column, 4 mm × 30 cm, with an eluting mobile phase of methanol–water (50% v/v). The mobile phase flow rate was adjusted to 1 ml/min with an inlet pressure of ~1750 psig. The chart speed was 0.25 cm/min.

A standard curve was obtained by comparing the peak height ratio of antipyrine to aminopyrine and the spiked plasma antipyrine concentration. Unknown plasma sample concentrations were calculated by comparing the peak height ratios of the samples to the processed standards.

Correlation with Spectrophotometric Assay—Ten plasma samples obtained from nonmedicated rats were spiked with antipyrine at con-

[1] Merck & Co., Rahway, N.J.
[2] Charles River CD.
[3] Eppendorf, Brinkmann Instrument, Westbury, N.Y.
[4] Model ALC/GPC 204, Waters Associates, Milford, Mass.
[5] Fisher Recordall, series 500, Fisher Scientific Co., Pittsburgh, Pa.
[6] μBondapak C_{18}, Waters Associates, Milford, Mass.

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