Identification and Differentiation of Some Phenothiazine-Type Tranquilizers

By D. K. YUNG and M. PERNAROWSKI

Methods are described for the identification and differentiation of the 13 phenothiazine-type tranquilizers listed in Schedule F of the Canadian Food and Drugs Act. These tranquilizers may be identified by observing the colors formed with certain reagents and by determining the melting points and infrared spectral characteristics of either the picrate or reineckate derivatives.

Most of the substituted phenothiazines possess tranquilizing properties and, consequently, are subject to controls in the market place. The pharmaceutical analyst, particularly if he is employed by an enforcing agency, is often called on to identify substances such as those listed herein. The methods available to the analyst depend mostly on the formation of a characteristic color with a selected reagent.

Feigl (1) described two spot tests for the base material, phenothiazine. Deviller (2) developed methods for the identification of thirteen substituted phenothiazines. Filho and Aichinger (3) showed that phenothiazine, promethazine, diethazine, isothiazine, chlorpromazine, and neozine could be identified by reacting these compounds with a series of reagents. Identification procedures for chlorpromazine hydrochloride, based on color formation, are described in the "British Pharmacopoeia 1958" (4) and the "United States Pharmacopeia XVI" (5). Because of the similarity in chemical structure, many substituted phenothiazines have been included in studies on the identification of antihistamines by color formation (6, 7). Rajeswaran and Kirk (8, 9) reported the identification of 50 tranquilizing and related drugs by means of color reactions and microscopic crystal tests.

Little systematic work has been carried out on the identification of substituted phenothiazines by derivative formation. The preparation of picrate and reineckate derivatives of some of the substituted phenothiazines has been reported (10, 11). Some picrates (12) and reineckates (11) were prepared and used in quantitative procedures involving the colorimetric determination of selected substituted phenothiazines.

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A number of procedures are described in this paper that will aid the analyst in the rapid identification of a restricted group of phenothiazines possessing tranquilizing properties.

EXPERIMENTAL

The following substituted phenothiazines were investigated: acepromazine hydrochloride, chlorpromazine hydrochloride, fluphenazine, levomepromazine maleate, mepazine acetate, perphenazine, prochlorperazine dimaleate, promazine hydrochloride, thiopropazate dihydrochloride, thioridazine hydrochloride, trifluoperazine dihydrochloride, triflupromazine hydrochloride, and trimeprazine tartrate. A Thomas-Hoover capillary melting point apparatus was used to determine the melting points of these compounds. The values so obtained are shown in Table I and are in good agreement with those supplied by the manufacturers or reported in the "Merck Index" (13). The chemical names of these substances may be found in the latter reference.

Reagents for Identification by Color Formation.—(*a*) Reagent I: Add 10 drops of 10% w/w hydrochloric acid to 30 ml. of a 1% aqueous ferric chloride solution; (*b*) Reagent II: 1% aqueous cobalt acetate solution; 10% isopropylamine in acetone; (*c*) Reagent III: 10% aqueous chloramine-T solution; (*d*) Reagent IV: add 1 ml. of hydrochloric acid U.S.P. to 50 ml. of a 0.1% aqueous palladium chloride solution; (*e*) Reagent V: nitric acid; (*f*) Reagent VI: add three ml. of concentrated sulfuric acid to 17 ml. of a 6% solution of uranium nitrate in 95% ethanol. This reagent will not form the desired colors unless it is freshly prepared. (*g*) Reagent VII: 0.5% w/w ammonium vanadate in concentrated sulfuric acid; (*h*) Reagent VIII: Add five drops of 0.1 *N* sulfuric acid to 20 ml. of a 4% aqueous silver nitrate solution.

Identification by Color Formation.—Prepare a 0.15% aqueous solution of the substituted phenothiazine. Heat gently, if necessary, to completely solubilize the phenothiazine. Transfer five drops of this solution to a depression in a white spot plate and add one drop of reagent. Observe the color immediately after mixing. (For the purposes of this paper, one drop is equal to approximately 0.05 ml.)

For reactions involving Reagent II, add one drop of the cobalt acetate solution and then one drop of the 10% isopropylamine in acetone to five drops of the test solution.

For reactions involving silver nitrate, transfer ten drops of the phenothiazine solution to a semimicro

TABLE I.—MELTING POINTS OF THE SALTS, PICRATES, AND REINECKATES OF SOME SUBSTITUTED PHENOTHIAZINES

	M.p., °C. (uncor.)		
	Salt[a]	Picrate	Reineckate
Acepromazine	135.0–136.0	69.0–73.0	154.0–156.0[b]
Chlorpromazine	196.0–196.5[b]	175.5–176.0	207.0–210.5[b]
Fluphenazine	231.0–232.0[b]	234.0–235.0[b]	179.0–181.0[b]
Levomepromazine	188.0–188.5[b]	69.0–79.0[b]	164.5–168.0[b]
Mepazine	70.5–71.5	145.0–146.0[b]	153.0–155.5[b]
Perphenazine	95.5–96.5	239.0[b]	154.5–156.5[b]
Prochlorperazine	219.0–221.0[b]	245.0[b]	156.0–159.5[b]
Promazine	178.5–180.0	145.5–146.0	166.5–168.0[b]
Thiopropazate	222.0–224.0[b]	223.0[b]	149.0–150.5[b]
Thioridazine	162.0–163.0	85.0–95.0	174.5–175.0[b]
Trifluoperazine	169.0–171.0	242.0[b]	183.5–186.0[b]
Triflupromazine	173.0–174.0	152.0–153.0	194.5–195.0[b]
Trimeprazine	162.0–163.0	130.0	168.5–170.0[b]

[a] See first paragraph under *Experimental*. [b] Melt with decomposition.

test tube and add two drops of the reagent. Heat the solution in boiling water for 5 minutes and observe the color.

The colors formed with seven of the eight reagents are reported in Table II. The results obtained for Reagent I are shown in Fig. 1.

Identification by Picrate Formation.—Dissolve 500 mg. of the substituted phenothiazine in 50 ml. of distilled water. If the substance is not completely soluble, stir for 10 minutes and filter. Add slowly, with stirring, 50 ml. of a saturated solution of picric acid in water. Allow the mixture to stand for ten minutes, filter using a sintered-glass filter of medium porosity, and wash the precipitate with a small quantity of distilled water. Recrystallize twice from 95% ethanol and dry the crystals in a desiccator over phosphorous pentoxide.

If recrystallization is not possible, wash the recovered substance thoroughly with distilled water, then with 95% ethanol, and dry as indicated above.

The melting points of the 13 picrate derivatives are listed in Table I.

Purity Criterion for Picrate Derivatives.—Carbon, hydrogen, and nitrogen determinations were carried out on the 13 picrate derivatives. Experimental values agreed satisfactorily with the calculated theoretical values.

The equivalent weights of the picrate derivatives were determined in the following way: (*a*) Method for monopicrates: Dissolve 30 mg., accurately weighed, in 15 ml. of glacial acetic acid and 15 ml. of chloroform. Titrate the solution with 0.02 *N* acetous perchloric acid using four drops of a 0.5% solution of crystal violet in glacial acetic acid as indicator. The color change, from purple to blue, was checked potentiometrically using a Precision-Shell dual titrometer equipped with glass and sleeve-type calomel electrodes. Carry out a blank determination and make any necessary corrections in the calculation. (*b*) Method for dipicrates: Dissolve 50 mg., accurately weighed, in 30 ml. of dimethylformamide. Titrate the solution with 0.1 *N* potassium hydroxide in methanol using four drops of a freshly prepared 0.5% solution of thymol blue in methanol as indicator. The color change, from greenish yellow to green, was checked potentiometrically as indicated above. Protect the contents of the flask from atmospheric conditions. Carry out a blank determination and make any necessary corrections in the calculation.

The equivalent weight is equal to the weight of the sample in grams divided by the product of the volume and normality of the titrant. The equivalent weight of each picrate was determined by using the appropriate method and the experimental and theoretical values compared. All results were found to be satisfactory.

Identification by Reineckate Formation.—Dissolve 150 mg. of the substituted phenothiazine in 50 ml. of 0.1 *N* hydrochloric acid. If the substance is not completely soluble, stir for 10 minutes, and then filter. Add slowly, with stirring, 50 ml. of a saturated solution of ammonium reineckate in water. Refrigerate for 10 minutes, filter through

TABLE II.—COLOR REACTIONS[a] OF SOME SUBSTITUTED PHENOTHIAZINES

	Reagent II	Reagent III	Reagent IV	Reagent V	Reagent VI	Reagent VII	Reagent VIII
Acepromazine	GN.	L.Y.	BR.	O.BR.	Y.O.	BR.	N.R.
Chlorpromazine	L.BL.	GR.	V.	R.	R.	R.	BE.CL.
Fluphenazine	L.BL.	L.Y.	O.	O.BR.	L.O.	Y.BR.	CR.W.
Levomepromazine	L.V.	L.Y.	V.	V.	V.	V.	V.
Meprazine	L.BL.	CR.P.	BL.	O.BR.	O.BR.	BR.	L.PI.
Perphenazine	L.BL.	N.R.	L.O.	PI.	N.R.	PI.	N.R.
Prochlorperazine	L.BL.	L.Y.	O.BR.	R.	R.	R.	P.PI.
Promazine	L.BL.	CR.P.	D.BL.	BR.	O.BR.	BR.	PI.
Thiopropazate	L.BL.	L.Y.	O.BR.	R.	P.R.	R.	BE.CL.
Thioridazine	L.BL.	CR.BL.	R.BR.	BL.	BL.	BL.	BL.CL.
Trifluoperazine	L.BL.	CR.BE.	O.BR.	O.BR.	O.	Y.BR.	CR.W.
Triflupromazine	L.BL.	CR.V.	O.BR.	O.BR.	O.	Y.BR.	CR.W.
Trimeprazine	BL.	L.GR.	BL.	O.BR.	L.PI.	BR.	P.PI.

[a] BE. = beige; BL. = blue; BR. = brown; GN. = green; GR. = gray; O. = orange; P. = purple; PI. = pink; R. = red; V. = violet; W. = white; Y. = yellow; CL. = cloudy; CR. = creamy; D. = dark; L. = light; N.R. = no reaction.

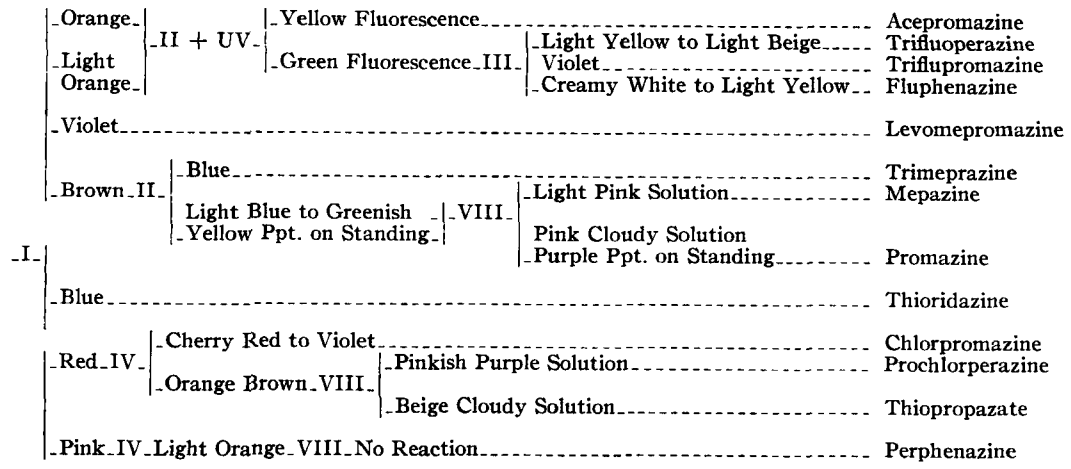

Fig. 1.—Sequence of color tests for differentiation of some substituted phenothiazines. Roman numerals refer to reagents mentioned in text. UV: sample exposed to ultraviolet radiant energy using a Mineralight, model SL, black light lamp.

a sintered-glass filter of medium porosity, wash the precipitate with distilled water, and recrystallize twice from 70% ethanol at 55°. Dry the crystals in a desiccator over phosphorous pentoxide.

The decomposition points of the 13 reineckate derivatives are listed in Table I.

Purity Criterion for Reineckate Derivatives.—The chromium content of each of the reineckates was determined by ashing the sample at 800° for 3 hours. The per cent chromium in the derivatives (calculated from the weight of ash) compared favorably with theoretical values.

Procedure for the Preparation of Infrared Spectrum.—Mix 15 mg. of the picrate or reineckate with 285 mg. of previously dried A.C S. grade potassium bromide in a Wig-L-Bug amalgamator. Transfer the sample to a Beckman rectangular pellet die, evacuate, and subject the mixture for 10 minutes to a pressure of 20,000 lb./sq. in. using a Carver laboratory press. Record the spectrum of the pellet on a Beckman IR 5 infrared spectrophotometer.

The infrared spectra of promazine picrate and trifluoperazine reineckate are shown in Fig. 2. Portions of other spectra are illustrated in Figs. 3 and 4.

DISCUSSION

One of the easiest ways to identify an organic

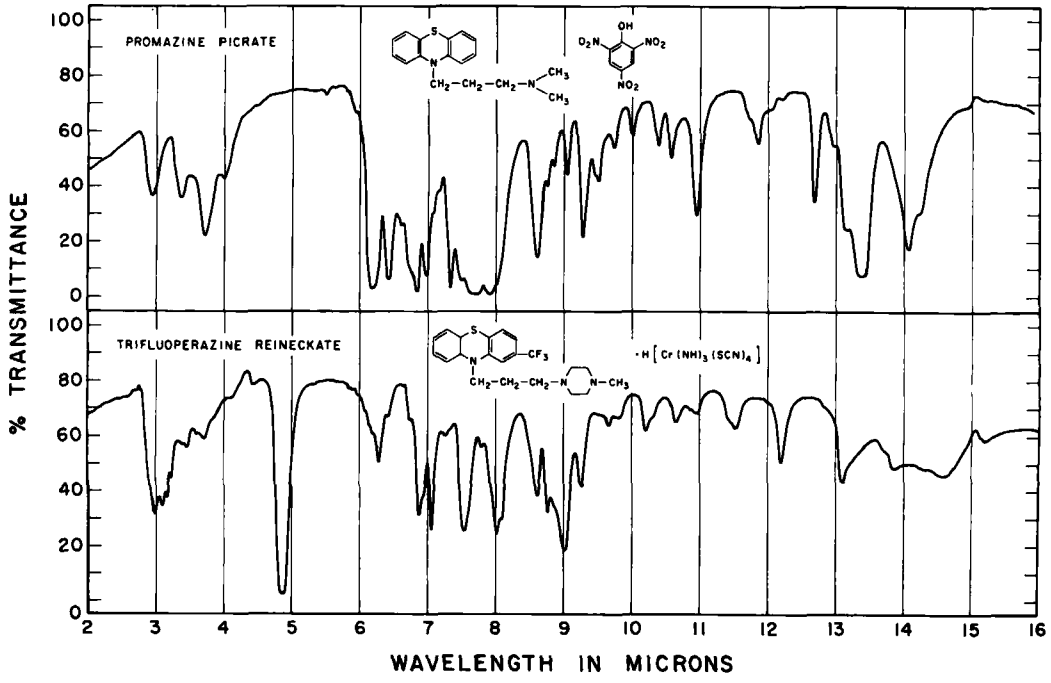

Fig. 2.—Infrared spectra of promazine picrate and trifluoperazine reineckate.

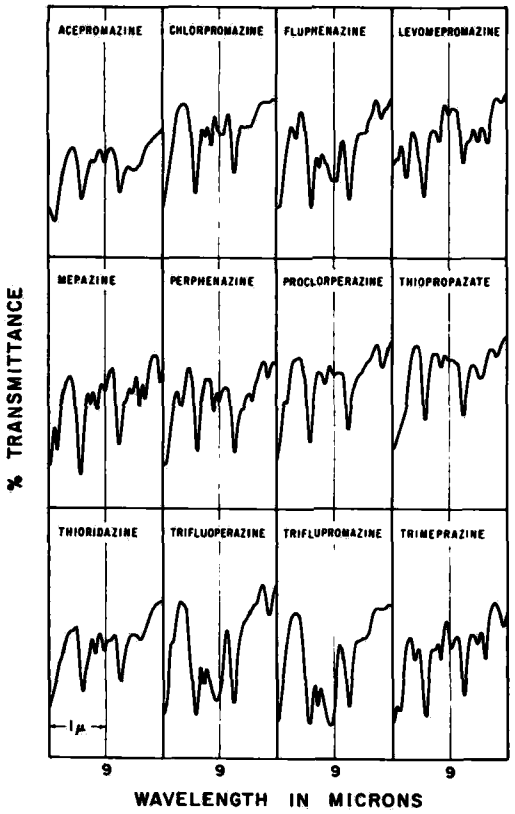

Fig. 3.—Infrared spectra of the picrates of some substituted phenothiazines.

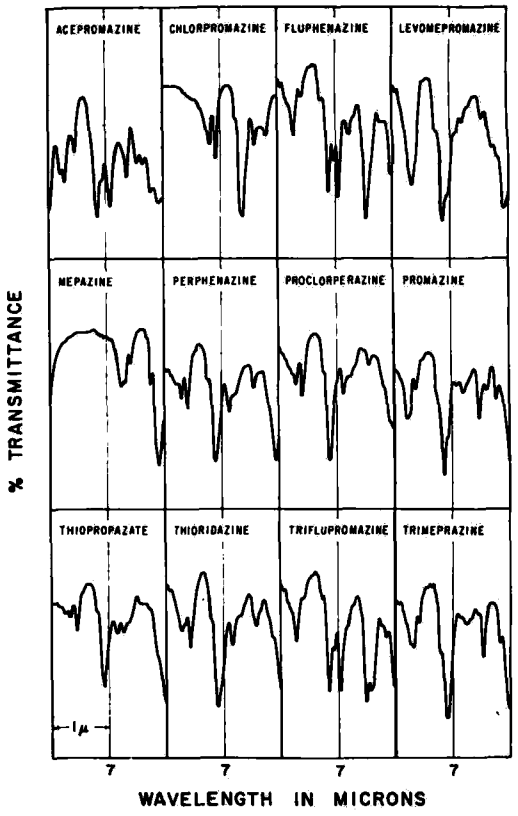

Fig. 4.—Infrared spectra of the reineckates of some substituted phenothiazines.

compound is to carry out a reaction which yields a characteristically colored product. The rationale in most of these tests is that the particular reaction is specific but, unfortunately, such specificity is limited to the conditions outlined by the researcher. Moreover, reaction to color is largely a matter of opinion and, consequently, what is red to one chemist might be orange to another. Tests in sequence are thus better since there is less chance of error in interpretation.

Two sets of data are presented herein. The first set (See Fig. 1) outlines a sequence of color tests that are useful in the identification and differentiation of the substituted phenothiazines listed in this paper. Only some of the reagents listed in the previous section are required for this testing sequence. The second set (See Table II) is a tabulation of the colors formed with seven of the eight reagents and may be useful to the control chemist in setting up a different sequence of tests to cover particular problems involving a more restricted group of phenothiazines.

Tests based on color formation depend upon the amount of material available and on the degree of dilution. In order to standardize conditions, tests were carried out on solutions containing 0.15% of active ingredient. This concentration value was derived from the solubility of the least soluble phenothiazine, prochlorperazine dimaleate. On this basis, reactions were carried out on solutions containing approximately 0.5 to 1.0 mg. of sample. Some of the reagents will detect much smaller quantities of material but some change in color will occur and, consequently, control samples should be run at the same time. A total of approximately 15 mg. of ingredient is generally sufficient to carry out the color tests indicated in Fig. 1.

Levomepromazine gave a violet color with all reagents except chloramine-T. This latter reagent did, however, produce an initial light violet color which then turned to the light yellow color indicated in Table II. Similarly, thioridazine gave a blue color with all reagents except palladium chloride. These two substituted phenothiazines can, therefore, be quickly and fairly accurately characterized on the basis of these particular color tests.

Fluorescent solutions resulted when the substituted phenothiazines were reacted with Reagent II and then exposed to ultraviolet radiant energy. Solutions containing acepromazine yield a bright yellow fluorescence and, on this basis, can be readily differentiated from the other 12 phenothiazines. This technique is of no further value in the identification of any single phenothiazine. It does, however, divide the remaining 12 phenothiazines into two groups, the first yielding a green fluorescence with fluphenazine, trifluoperazine, and triflupromazine and the second giving a basically blue color with the remaining substances. These observations are useful in the differentiation scheme outlined in Fig. 1. It was also observed that aqueous solutions of these substituted phenothiazines fluoresced when exposed to ultraviolet

radiant energy but this did not appear to be useful for identification purposes because the colors formed were not characteristic. Prolonged exposure to ultraviolet radiant energy will result in colored solutions but, again, this does not particularly aid the analyst in the identification of these substances. This color formation is probably due to oxidation reactions caused by the radiant energy.

Color reactions cannot be considered as final criterion in the identification of drugs and, for this reason, picrate and reineckate derivatives of the substituted phenothiazines were prepared. As expected, the monobasic phenothiazines, that is, acepromazine, chlorpromazine, levomepromazine, mepazine, promazine, thioridazine, triflupromazine, and trimeprazine formed monopicrates. The dibasic phenothiazines formed dipicrates. These derivatives can usually be purified by recrystallization from 95% ethanol. Difficulty was experienced, however, in the purification of acepromazine, levomepromazine, perphenazine, prochlorperazine, and thioridazine picrates. These derivatives were washed with water, and then with 95% ethanol, to remove undesired impurities. Levomepromazine picrate is soluble in 95% ethanol and cannot be washed with this solvent. Elementary analyses and equivalent weight determinations indicated that such washings were sufficient for purification purposes.

Dipicrates are insoluble in the glacial acetic acid-chloroform solvent system used in the determination of equivalent weight values. It was, therefore, necessary to develop a secondary technique involving the use of dimethylformamide as solvent and potassium hydroxide in methanol as titrant. The latter technique involves a titration of the picric acid portion of the molecule whereas the acetous perchloric acid method implies a determination of the phenothiazine itself. Both methods gave satisfactory results.

All reineckate derivatives were prepared in acidic medium. It has been reported that high temperatures will decompose some reineckates (14, 15) and, consequently, recrystallizations were carried out at 55°. After two recrystallizations, all derivatives were obtained as the monoreineckates. Chromium determinations on the crude materials indicated that five of the dibasic substituted phenothiazines, namely fluphenazine, perphenazine, prochlorperazine, thiopropazate, and trifluoperazine, first formed direineckates. Trifluoperazine, a dibasic compound, initially formed a monoreineckate. Recrystallization converted the direineckates described above to monoreineckates. Such conversion was also observed by Lee (15) in his study on the use of ammonium reineckate in the identification of nitrogenous organic bases.

Although the melting points of these derivatives are useful in the identification of these substances, overlapping of values does occur and, consequently, creates difficulties but only if such values are viewed alone. The analyst may turn to color formation or to infrared spectroscopy in order to resolve these difficulties. In one case, at least, crystal color aids in differentiating one picrate from another. Mepazine forms a bright yellow picrate, whereas the promazine derivative has a rusty red color.

Infrared spectra are useful in further identifying these derivatives. For the most part, the spectra of these compounds are similar and, consequently, only those of promazine picrate and trifluoperazine reineckate are illustrated over the full 2–16 μ region. It was observed, however, that the spectra of the picrates and reineckates were sufficiently different in the 8–10 and 6–8 μ regions, respectively, to permit identification of the parent substance. Figures 3 and 4 illustrate the significant portions of these spectra.

Certain bands are common to all of the picrates of the substituted phenothiazines. Strong bands occur throughout the 3.00 (OH stretching), 3.30 to 4.00 (CH stretching), 5.90 to 6.26 (C—C stretching), 6.42 (nitro stretching), 6.62 to 7.00 (benzene ring frequency), 7.35 (nitro stretching), and 13.40 to 14.10 (benzene ring frequency) μ regions. It is probable that all of these bands arise from the picric acid portion of the molecule since they are all present in the spectrum of this acid alone. In spite of all of this, special characteristics in the 8–10 μ region makes identification possible. Thus, promazine picrate can be differentiated from fluphenazine picrate by the appearance of bands of weak and medium intensity at 8.85 and 9.50 μ, respectively. Similarly, trimeprazine picrate yields a spectrum which shows two weak bands at 8.10 and 8.40 μ, bands which are not present in the spectrum of promazine picrate.

The spectra of the reineckates of the substituted phenothiazines have certain bands in common. Strong bands occur in the 4.86 and 8.00 to 8.10 (nitrile stretching) μ regions. Broad, intense bands, due to an NH stretching vibration, may be observed in the 2.96 to 3.50 μ region. As in the case of the picrates, differentiation must be based on special features in a restricted portion of the spectra. Even though heavy atoms have a damping effect on the spectrum (16), there is sufficient detail in the 6–8 μ region to permit identification of these substances. For example, fluphenazine reineckate has a band of medium intensity of 6.96 μ whereas trifluoperazine reineckate shows no such absorption. It is difficult to distinguish between perphenazine and prochlorperazine reineckates on the basis of the features in this particular region. However, a weak band does occur at 8.11 μ in the spectrum of perphenazine reineckate. No problems should occur with the evaluation of the other spectra of the derivatives of these substituted phenothiazines.

Several of the substituted phenothiazines deserve special mention. Thus, fluphenazine, trifluoperazine, and trifluopromazine possess a $-CF_3$ group within their molecules. The presence of this particular functional group is indicated in the infrared spectrum by a band at approximately 7.50 μ. Similarly, the presence of the carbonyl groups of acepromazine and thiopropazate may be established on the basis of sharp, intense bands near 5.90 μ. All of these bands occur in the spectra of either the picrate or the reineckate derivatives.

The techniques developed herein were applied to 12 commercial preparations containing various substituted phenothiazines. One of these prepara tions contained fluphenazine in concentrations equal to 0.25 mg. per tablet. This preparation could not be identified by using the techniques described herein because of the difficulties encountered in extracting this quantity of active ingredient

from the tablet mass. No difficulties were encountered with the remainder of the preparations.

CONCLUSION

Several methods are described for the identification of 13 substituted phenothiazines with tranquilizing properties. The methods are rapid and the manipulative techniques are simple.

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Stability Assays of Pharmaceutical Preparations by Quantitative Paper Chromatography II

Quantitation by Spectrophotofluorometry

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A quantitative paper chromatographic method has been developed which is routinely used as a stability assay for estrogenic hormones in castor oil and tablet formulations. The method involves: (*a*) A separation of the estrogenic hormone from interfering degradation products. (*b*) The location of the estrogenic hormone on the chromatogram by the guide strip technique employing a chromogenic agent. (*c*) The elution of the estrogenic hormone from the chromatogram. (*d*) A quantitative spectrophotofluorometric analysis of the eluate. Recoveries in excess of 95% are obtained by simultaneously chromatographing replicates of standard and sample solutions on the same chromatogram. A detailed account of the procedure is presented using as examples the assay of estradiol valerate in castor oil formulations and ethinyl estradiol in tablets.

RECENTLY we have described methods for the quantitative determination of hormone preparations using paper chromatographic separations followed by spectrophotometric analysis (1). In this paper we wish to report on techniques which permit the quantitation of estrogens in ethanol eluates by spectrophotofluorometry. The examples chosen are the quantitative determination of two estradiol derivatives, estradiol valerate in castor oil and ethinyl estradiol in tablets.

The sensitivity of the method permits the chromatography and quantitation of 10 and 20 mcg. quantities. This is important since estrogens, because of their physiological potency, are generally compounded in low concentrations, for example, 0.5% in oil formulations and 0.05% in tablets.

Fluorometric methods may be classified according to whether the fluorescence measured is that of the compound in its native state or is induced by chemical transformations. Most accepted procedures for the quantitation of estrogens fall in this second category. The fluorescence is developed in sulfuric acid and measured in the visible range (2).

The development of the spectrophotofluorometer, capable of activating and measuring fluorescence throughout the visible and ultraviolet regions, has revealed the presence of useful ultraviolet fluorescence in many compounds not previously known to fluoresce in solution.

Duggan, *et al.* (3), in studying the fluorescent properties of a number of compounds of biologi-

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