REFERENCES

(1) B. A. Mulley, "Advances in Pharmaceutical Sciences," vol. I, Academic, New York, N. Y., 1964.

(2) P. H. Ellworthy, A. T. Florence, and C. B. Macfarlane, "Solubilization by Surface Active Agents," Chapman & Hall Ltd. London, England, 1968.

(3) K. Kakemi, T. Arita, and J. Muramishi, *Chem. Pharm. Bull.*, **13,** 976(1965).

(4) A. F. Hofman and B. Borgstrom, *Federation Proc.*, **21,** 43(1962).

(5) J. R. Bloor and J. C. Morrison, to be published.

(6) C. T. Rhodes, *Can. J. Pharm. Soc.*, **2,** 16(1967).

(7) J. Harkins and K. Jordan, *J. Amer. Chem. Soc.*, **52,** 1751 (1932).

(8) "Handbook of Chemistry and Physics," 46th ed., Chemical Rubber Co., 1962.

(9) B. A. Brice, M. Halwer, and R. Speiser, *J. Opt. Soc. Amer.*, **40,** 768(1950).

(10) N. A. Lange, "Handbook of Chemistry," 10th ed., McGraw-Hill, New York (1961).

(11) "Non Ionic Surfactants," M. J. Schick, Ed., E. Arnold Ltd., London, England, 1967.

(12) P. Molyneaux, C. T. Rhodes, and J. Swarbrick, *Trans. Faraday Soc.*, **61,** 1043(1965).

(13) K. J. Humphreys and C. T. Rhodes, *J. Pharm. Sci.*, **57,** 79(1968).

(14) P. Debye, *J. Phys. Chem.*, **53,** 1(1949).

(15) K. A. Stacey, "Light Scattering in Physical Chemistry," Butterworths, London, England, 1956.

(16) H. S. Frank and Y. W. Wen, *Discuss. Faraday Soc.*, **24,** 133(1957).

(17) B. A. Wurzshmitt, *Anal. Chem.*, **30,** 118(1950).

(18) H. S. Frank and P. Evans, *J. Chem. Phys.*, **13,** 507(1945).

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DRUG STANDARDS

Trifluoperazine Tablets: Alternative Methods of Analysis

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Abstract □ Procedures utilizing a direct spectrophotometric measurement (such as described in the British Pharmacopoeia) for the analysis of trifluoperazine tablets suffer from several disadvantages. The possibility of excipient interference is not precluded and this factor, coupled with use of a fixed reference absorptivity value, can sometimes lead to erroneous results. Therefore, alternative assay procedures are required to assess accurately the drug content. Two such methods are described. The first is the acid-dye type and involves the partitioning of a trifluoperazine-bromocresol purple complex between an aqueous buffer pH 6 and benzene containing 1% isoamyl alcohol and subsequent measurement of the yellow-colored organic phase at 410 mμ. Acidic and neutral compounds as well as the common excipients do not interfere. The second method employs an alkaline siliceous earth column through which the drug is eluted into a chloroform–methanol–HCl system and the absorbance measured at 259 mμ. The precision and accuracy of the alternative methods, as well as the pharmacopeial procedure are compared using commercial dosage forms and simulated drug–excipient mixtures.

Keyphrases □ Trifluoperazine tablets—analyses □ Acid-dye method—trifluoperazine analysis □ Column chromatography—analysis □ UV spectrophotometry—analysis □ Colorimetric analysis—spectrophotometer

The current BP method for the analysis of trifluoperazine hydrochloride tablets (1) involves dissolution of an aliquot sample of 20 powdered tablets and direct spectrophotometric measurement of the filtrate at 256 mμ using a fixed reference absorptivity value. Although the method is satisfactory in most cases, it suffers from the disadvantage of possible interference from excipients and also from both inter- and intrainstrumental variations. The latter variations may be considerable (2, 3) and introduce unsuspected error into the assay. Therefore, alternative assay procedures which are relatively free from interference and instrumental variation are required on occasion for products with assay results that are suspect by the pharmacopeial method.

While several methods have been reported in the literature as general procedures suitable for the analysis of piperazinyl phenothiazine drugs in pharmaceutical dosage forms and in biological media, there are virtually no data on their direct application to the analysis of trifluoperazine hydrochloride tablets. Blazek and Mares (4) determined drugs of the piperazinyl phenothiazine type gravimetrically by precipitation with silicotungstic acid or by electrochemical titration against the same reagent. Other electrochemical methods include controlled-potential coulometric analysis (5) and polarography (6), the latter being reported as having an accuracy of ±5% when applied to trifluoperazine hydro-

Table I—Analysis of Simulated Drug–Excipient Mixtures

Simulated Mixture No.	% Recovery[a] Direct UV[b]	Acid Dye[c]	Column[d]
1	102.9	101.0	101.6
2	103.8	100.4	101.8
3	104.3	100.8	100.1
4	106.2	100.7	100.8
5	107.2	100.1	100.5
6	109.1	100.3	101.2

[a] Each quoted value in a given row represents the average results of duplicate runs using the same stock solution. Maximum range for duplicates on each sample were: [b] 0.5%, [c] 0.5%, and [d] 0.6%.

chloride formulations. Quantitative paper chromatography involving colorimetric measurement at 610 mμ of the complex formed between the drug and potassium iodoplatinate after elution from the chromatogram was applied to several of the phenothiazine-type drugs by Nadeau and Sobolewski (7). Piperazinyl phenothiazine compounds could be detected in submicrogram quantities by gas chromatography (8) and spectrofluorometry (9), but these techniques have yet to be developed into precise analytical methods.

While the above procedures perhaps could be employed in some instances for the analysis of trifluoperazine hydrochloride tablets, in general they are not sufficiently rapid and accurate or else require the use of equipment which may not be readily available to the analyst.

In the present study, two alternative assay procedures were considered. The first assay procedure is of the acid-dye type and involves the partitioning of a trifluoperazine-bromocresol purple complex between an aqueous buffer of pH 6 and benzene containing 1% isoamyl alcohol, and subsequent measurement of the organic phase at 410 mμ. The second method employs an alkaline-siliceous earth chromatographic column from which the drug is eluted with chloroform (10) and the absorbance measured at 259 mμ. In addition, the use of ultrasonic energy was investigated as a rapid and convenient means to break up the solid dosage form and prepare the sample solution.

In order to measure the accuracy and precision of each technique and to compare against the direct UV procedure (BP method), stock sample solutions were prepared using the ultrasonic disintegration apparatus and aliquots of the filtered solution treated simultaneously by each of the assay procedures.

EXPERIMENTAL

A. Preparation of Sample Solutions—For each product examined, 10 tablets were selected at random and the active ingredient dissolved in a sufficient volume of 1% (v/v) HCl to give a concentration (based on label claim[1]) of approximately 23.6 mg. of salt or the equivalent of 20 mg. of base per 100 ml. (*i.e.*, 10 × 1-mg. tablets in 50 ml, 10 × 2 mg. in 100 ml., 10 × 5 mg. in 250 ml., 10 × 10 mg. in 500 ml., and 10 × 20 mg. in 1000 ml.).

Disintegration of the tablets and dissolution of the drug were accomplished with an ultrasonic probe of 1.27-cm. (0.5-in.) diameter (clamped in a vertical position, tip downward) powered by a 200-w. generator.[2] A basket assembly constructed of stainless steel wire mesh (30 mesh), 5.08 cm. (2 in.) in length and internal diameter slightly larger than the probe, was used to hold each tablet in the area of maximum sonic energy emission. A small rod attached to the basket assembly served as an arm to hold the basket in place during insonation.

In practice, each of the 10 tablets was insonated individually. The first tablet was placed in the wire basket assembly and the basket brought up over the probe so that the tablet came in contact with the tip. A beaker containing the appropriate amount of solvent was raised so that the basket was about half immersed. Ultrasonic energy was applied for 30 sec. at a power setting of 30% maximum with manual tuning for optimum output. This insonation process was repeated until all 10 tablets had been disintegrated. The resulting suspension was stirred briefly and then filtered through a 25-mm. membrane filter of 0.45-μ porosity (with a 1-mm. thick prefilter) using a 5-ml. syringe fitted with a continuous pipeting device. Aliquots of this solution after filtration were subsequently taken for analysis by the chromatographic column procedure, by direct UV measurement, and by the acid-dye technique.

Simulated drug–excipient mixtures were prepared by adding 10 ml. of a solution of trifluoperazine hydrochloride (accurately weighed and made up to a concentration of about 11.8 mg./10 ml. 1% HCl) to 25 ml. of filtered solution prepared as above from insonation of placebos, and adjusting the volume to 50 ml. with 1% HCl (final concentration approximately 23.6 mg. salt/100 ml.). For each successive mixture (Table I), the number of placebos was increased to give about 9% overestimation with the direct UV procedure.

B. Assay by Direct UV Measurement—An aliquot of the filtered sample solution prepared in Part A was diluted twentyfold with 1% HCl to give a concentration (based on label claim) of about 1.18 mg. of salt/100 ml. Dilution was facilitated by the use of an automatic dilutor[3] adjusted for a diluent to sample solution ratio of 19:1. The absorbance of the final solution was measured at 256 mμ against a blank of 1% HCl. The average drug content per tablet was determined by comparison against the average absorptivity value obtained from three separate solutions containing trifluoperazine hydrochloride reference material. For each solution, about 50 mg. of standard (accurately weighed) was made to a volume of 250 ml. in 1% HCl. A twentyfold dilution was made as described with the automatic dilutor.

C. Assay by the Acid-Dye Technique—*Reagents*—(*a*) Buffer solution, pH 6.0 (McIlvaine): 7.37 ml. of 0.1 *M* citric acid plus 12.63 ml. of 0.2 *M* Na_2HPO_4. (*b*) Bromocresol purple (BCP) in buffer solution: bromocresol purple dissolved in buffer solution of pH 6.0 to give a concentration of 37.5 mg./100 ml. This solution was extracted with benzene containing 1% isoamyl alcohol until the extract was virtually colorless. (*c*) Photometric solvent: benzene (A.R.) containing 1% isoamyl alcohol (A.R.) by volume.

Procedure—An aliquot of the filtered sample solution prepared in Part A was diluted tenfold with 1% HCl to give a concentration (based on label claim) of about 118 mcg. of salt per 5 ml. A 5.0-ml. aliquot of this final solution was pipeted into a 42-ml. centrifuge tube along with 5.0 ml. of buffer pH 6, 5.0 ml. of BCP solution, and 10 ml. of photometric solvent (the latter dispensed from an automatic dispenser for convenience). The tube was stoppered and tumbled end-over-end for 5 min. in a device constructed to rotate 15 tubes simultaneously at 100 r.p.m. (alternatively, the stoppered tubes may be shaken vigorously by hand for 1 min.). After mixing, the tube was centrifuged for 2 min.; then the clear supernatant was decanted carefully into a clean dry cell for measurement of absorbance at 410 mμ against a blank prepared in the same manner using 5 ml. of 1% HCl. At the same time, 5.0 ml. of standard reference solution containing approximately 120 mcg. of trifluoperazine hydrochloride (accurately determined from the initial weighing) was carried through the assay procedure. The average drug content per tablet was determined by comparison of the absorbance of the sample solution against that of the standard reference solution.

D. Assay by the Alkaline-Siliceous Earth Column Procedure—*Pretreatment of Diatomaceous Earth*[4]—Acid-washed diatomaceous earth (100 g.) was placed in a 64 × 150-mm. medium-porosity

[1] Tablets were labeled to contain trifluoperazine hydrochloride with the dosage level expressed as the equivalent amount of base.

[2] Blackstone Ultrasonics, model BP-2.
[3] Labindustries, Automatic Dilutor.
[4] Celite 545, Johns-Manville, New York, N. Y.

sintered-glass funnel and 1000-ml. portions of 1:1 hot (boiling) aqueous HCl passed through with the aid of gentle suction. Five hundred milliliters of hot water was then passed through, followed by 1000 ml. of hot 1 *N* NaOH, 500 ml. of chloroform, and 1000 ml. of water. The treated diatomaceous earth was dried for 15 hr. at 110°.

Preparation of Partition Columns—Six grams of the purified diatomaceous earth was mixed well with 2 ml. of 20% NaOH in a small beaker and the damp mass transferred to a 25 × 220-mm. glass column containing a pledget of glass wool at the base to support the diatomaceous earth. The mixture was firmly tamped to produce a column free of air pockets and irregularities.

Procedure—A 4.0-ml. aliquot of the filtered sample solution prepared in Part A was pipeted directly on the surface of the column and allowed to soak in. Water-washed chloroform (90 ml.) was then passed through the column and collected in a 100-ml. volumetric flask containing 8 ml. of methanol and 4 drops of concentrated HCl. The contents of the volumetric flask were made to volume with water-washed chloroform and the absorbance measured at 259 mμ against a blank prepared in the same manner. The average drug content per tablet was determined by comparison against the absorptivity of a reference standard solution prepared in the same manner.

RESULTS AND DISCUSSION

Sample Preparation—Spectrophotometric analysis of drugs in a solid dosage form involves two main steps: the first is to effect quantitative dissolution of the active ingredient from the tablet matrix and the second is to prepare the solution in a suitable form (*i.e.*, clarity and concentration) for final measurement. The techniques used to achieve these goals ultimately govern the overall accuracy and precision of the method.

The current pharmacopeial directive for extracting trifluoperazine hydrochloride from excipient material entails finely grinding 20 weighed tablets and shaking an aliquot of the powder equivalent to about 5 mg. of the drug for 15 min. with 5% HCl. The mixture is then made up to a specified volume with the same solvent, mixed, filtered, and the absorbance measured at 256 mμ. Several factors inherent to this type of sampling sequence tend to diminish the accuracy and precision of the analysis. First are the errors in weighing and those associated with mechanical loss and nonhomogeneous grind. In some cases, there is the added problem of incomplete dissolution of the active drug because of insufficient particle breakup. The use of ultrasonic disintegration as a sampling tool not only precludes the above difficulties but is more rapid and less tedious than the conventional technique. The fact that 1% HCl invariably gave the same assay results as 5% HCl, and because the former acidity was more compatible with the requirements of the acid-dye extraction method and the alkaline-siliceous earth column procedure, militated against the use of the stronger pharmacopeial solvent. Before adopting the ultrasonic technique for routine analytical use, it was necessary to establish its feasibility for the phenothiazine drugs in general and trifluoperazine in particular because there are several well-documented cases in the literature where the ultrasonic field induced chemical changes in the sonified material. For example, the rate of hydrolysis of aspirin was increased when the drug was subjected to ultrasound (11, 12). Skauen (13) has reviewed other instances where ultrasonic waves under certain conditions affected the chemical integrity of medicinal compounds. Because of the lability of most compounds of the phenothiazine family, it was deemed essential to monitor the absence or presence of any destructive action on the trifluoperazine molecule associated with the high-energy emission from the probe. This was done by: (*a*) comparing the thin-layered chromatograms (14, 15) of powdered and sonified tablets; developing systems used were benzene–dioxane–aq. NH_3(60:35:5) giving R_f = 0.69, and *n*-BuOH saturated with 1 *N* NH_3 giving R_f = 0.51; in no instance were any spots found in the sonified material which were not present in the powdered sample; and (*b*) comparing the assay results using the shakeout procedure against those using the ultrasonic-disintegration technique; within experimental error and content uniformity variations, these were in good agreement (usually within 2%). The data, therefore, gave no evidence to suggest any decomposition of any brand of trifluoperazine tablets resulting from exposure to ultrasonic energy under the conditions employed.

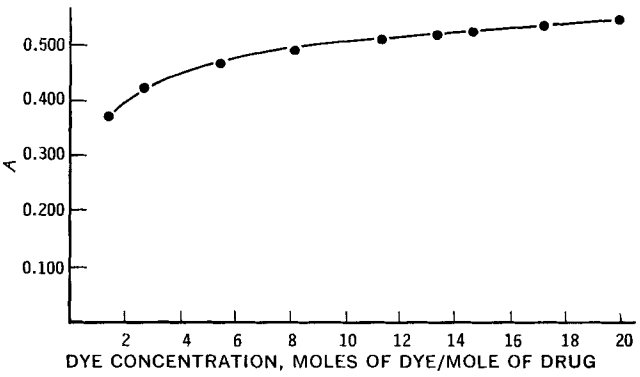

Figure 1—*Effect of dye concentration on absorbance (varying quantity of dye but fixed concentration of drug).*

The official compendium does not specify the type of filtration in the monograph for trifluoperazine tablets. With certain formulations where gravity filtration through Whatman No. 2 paper was employed, the filtrate appeared as a fine opalescent suspension, a situation which could conceivably lead to erroneously high results despite subsequent dilution. While the use of finer porosity paper usually eliminated this difficulty, the filtration step became excessively time consuming and, as a result, the possibility of drug decomposition became an important consideration. Suction filtration even through a thin pad of acid-washed diatomaceous earth was sometimes subject to clogging and was therefore not always satisfactory. Maximum clarity was readily achieved with the use of a membrane filter of micron-range porosity.

Methods—Under the experimental conditions of sample preparation, the effective dye concentration expressed as a molar ratio of dye to drug with the acid-dye technique was approximately 12:1. A plot of this ratio against absorbance is given in Fig. 1. With a fixed concentration of drug, in the case of trifluoperazine hydrochloride, the optimum dye concentration expressed in moles of dye per mole of drug is restricted to a relatively narrow band between 10:1 and 16:1. At lower ratios the color intensity was significantly decreased and was more sensitive to small variations in dye concentration, while higher ratios (*i.e.*, more concentrated dye solution) resulted in the formation of a precipitate. Beer's law was obeyed for drug concentrations in the range of 30 to 150 mcg./5 ml. of sample solution. All solutions which were analyzed by the acid-dye technique in this study fell within this concentration range.

The extent of partition of the drug–dye complex from an aqueous phase of given pH into the organic phase appears to be related to the molecular structure of the particular drug and dye. In the ideal situation, the pH values at which partition of the colored ion pair into the organic phase is a maximum and blank readings a minimum are coincident. This is rarely the case in practice but the pH should usually be kept as high as possible to prevent extraction of the sulfonic acid form of the dye into the organic layer. An optimum

Table II—Analysis of Commercial Dosage Forms

Sample No.	Manufacturer	Dosage Level, mg. Base/Tablet	% of Label Claim[a]		
			Direct UV[b]	Acid Dye[c]	Column[d]
1	A	1	93.9	92.6	90.3
2	B	2	99.7	99.0	100.2
3	B	2	98.6	95.3	97.5
4	C	1	92.6	90.6	91.0
5	C	2	100.7	98.7	100.0
6	D	1	96.9	98.0	95.7
7	D	1	95.1	95.1	93.4
8	E	1	105.7	99.5	100.4
9	E	5	108.8	109.2	106.9

[a] Each quoted value in a given row represents the average results of duplicate runs using the same stock solution. Maximum range for duplicates on each sample were: [b] 0.6%, [c] 0.8%, and [d] 0.9%.

buffer pH of 6.0 was indicated for the trifluoperazine-bromocresol purple system.

In the chromatographic method, the solvent system, methanol (8 ml.) containing concentrated hydrochloric acid (4 drops) and diluted to 100 ml. with water-washed chloroform, is completely transparent down to the cutoff point of 242 mμ. In this solvent system, the absorption maximum for trifluoperazine is at 259 mμ. Nine determinations of the absorptivity of trifluoperazine hydrochloride following partition through the alkaline-siliceous earth column gave an average value of 59.80 with a coefficient of variation of 1%. The commercial diatomaceous earth (see *Footnote 4*), which is often suitable as such for analysis by column chromatography, in this instance gave blank readings which were high and variable (0.072–0.114) and, as a consequence, was not satisfactory for use without further treatment. Washing with hydrochloric acid, then consecutively with aqueous alkali and chloroform to simulate the analytical conditions, led to almost total removal of UV-absorbing impurities and lowered the reference column absorbance values to 0.005 or less. This value, established by several replicate measurements on each treated batch of diatomaceous earth, was quite reproducible and was subtracted as a fixed quantity from the absorbance reading of each solution of the drug eluted from the column.

Results—Studies on the extent of the quantitative interference by excipient substances encountered with the direct spectrophotometric procedure for four brands of trifluoperazine tablets showed that the absorbance contribution of the water-soluble coating materials is generally negligible with the higher dosage forms but is significant at the 1-mg. level where the dilution factor is less. With some of these tablets, the drug content can be overestimated by 2.5%. With placebos of Brand V, for example, an absorbance measurement equivalent to approximately 5% interference was observed. The soluble (in 1% HCl) coating materials of Brands I–IV, and the soluble excipient substances of the placebos of Brand V, however, gave zero absorbance on spectrophotometric analysis by either of the two alternative methods.

To substantiate the validity of these results and to ascertain that complete drug recovery could be effected by the acid dye and the alkaline column procedures, several simulated drug–excipient mixtures were prepared from a stock solution of trifluoperazine hydrochloride and filtered excipient solutions made from sonification of placebos. The data in Table I clearly demonstrate that, as expected with the pharmacopeial method, the presence of excipient material in the simulated solutions resulted in varying levels of interference corresponding to the number of placebos employed, but that no detectable interference and essentially 100% recovery were achieved with all six solutions by the two alternative procedures.

Inspection of the results in Table II for nine commercial formulations shows that, where the extent of interference is about 2.5% or less, the direct spectrophotometric method is of comparable accuracy to that of the two proposed procedures. For most of the lot numbers examined, the assay values are comparable for all three assay procedures, except in the case of sample No. 8 where the degree of overestimation was about 6% by the direct UV method.

The above data underline the fact that with some trifluoperazine tablet formulations, the pharmacopeial method can give erroneously high results, and in these instances where suspicious assay results are encountered, the two alternative procedures described herein should prove useful to assess accurately the drug content.

REFERENCES

(1) "British Pharmacopoeia 1968," General Medical Council, The Pharmaceutical Press, London, England, 1968, p. 1039.

(2) G. Wernimont, *Anal. Chem.*, **39** 554(1967).

(3) M. Winstead, *Amer. J. Med. Technol.*, **32** (1966).

(4) J. Blazek and V. Mares, *Cesk. Farm.*, **15,** 349(1966).

(5) F. H. Merkle and C. A. Discher, *Anal. Chem.*, **36,** 1639 (1964).

(6) N. Chuen and B. E. Riedel, *Can. Pharm. J., Sci. Sect.*, **94,** 51(1961).

(7) G. Nadeau and G. Sobolewski, *J. Chromatogr.*, **2,** 544(1959).

(8) C. McMartin and H. V. Street, *ibid.*, **22,** 274(1966).

(9) J. B. Ragland and V. J. Kinross Wright, *Anal. Chem.*, **36,** 1356(1964).

(10) J. Levine, *J. Pharm. Sci.*, **54,** 485(1965).

(11) E. Mario and R. J. Gerraughty, *ibid.*, **54,** 321(1965).

(12) T. E. Needham, Jr., and R. J. Gerraughty, *ibid.*, **58,** 62(1969).

(13) D. M. Skauen, *ibid.*, **56,** 1373(1967).

(14) J. Cochin and J. W. Daly, *J. Pharmacol. Exp. Ther.*, **139,** 160(1963).

(15) T. J. Mellinger and C. E. Keeler, *J. Pharm. Sci.*, **51,** 1169 (1962).

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