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Quantitative Determination of Butaperazine by TLC

A. J. KAPADIA, M. A. BARBER, and A. E. MARTIN

Abstract ☐ A method for the separation and determination of butaperazine in the presence of its degradation products is described. A sample is streaked onto a thin layer of silica gel G under a stream of nitrogen. The chromatogram is developed with isopropyl alcohol–ammonia (1 N) (4:1). The separated butaperazine is removed from the silica gel by elution with methanol and is determined quantitatively by UV spectroscopy. Details of the elution technique are described. Using the proposed method, quantitative recoveries are obtained from tablets and syrups.

Keyphrases ☐ Butaperazine in dosage forms—analysis ☐ TLC—separation ☐ UV spectrophotometry—analysis

The use of psychotropic drugs for the treatment of patients with emotional or mental disorders has led to widespread use of phenothiazine derivatives. One such derivative is butaperazine,[1] 2-(n-butyryl)-10-[3-(4-methyl-1-piperazinyl)-propyl]-phenothiazine.

The literature provides ample indication that several types of decomposition take place in these compounds. One type involves oxidation at the sulfur atom, leading to sulfoxide and eventually to sulfone (1). The quinonoid-type oxidation products of phenothiazine have been described (2–4). Huang and Sands (5, 6) studied the effect of UV irradiation on chlorpromazine solution under aerobic and anaerobic conditions. They found that under the former condition, oxidation prevails and the sulfoxide and N-oxide are formed; however, under the latter condition, the polymerization processes predominate.

The degree of deterioration and the type and amount of decomposition product pose difficult problems for the analyst. Consequently, for the purpose of establishing stability, it is necessary to devise a relatively simple but versatile separation, one that would be applicable to the quantitative determination of the phenothiazine derivative in experimental formulations.

Recently, Blazek (7) reviewed the procedures available for quantitative determination of phenothiazine derivatives. Included among the methods for these compounds are colorimetric (8), titrimetric (9), UV absorption spectrophotometric, and chromatographic procedures. One might choose any one of these except

for the following considerations. The first two procedures are not selective for the undegraded compound. The UV method (10, 11) is an accurate and convenient means of assaying formulations containing phenothiazine derivatives, but it is unsuitable in badly degraded formulations because of the presence of other UV absorbing species. A paper chromatography technique (12, 13) was not selected because of degradation and tailing occurring during analysis (14, 15). Gas chromatographic procedures have been used for phenothiazines, but the present authors observed that butaperazine, because of its high boiling point and low thermal stability, was too low in volatility to be eluted quantitatively without extensive thermal decomposition from the several columns that were tried. Thus, they eliminated GLC from further consideration.

Since its introduction by Stahl (16), TLC has assumed a position of analytical importance for both the separation and analysis of complex inorganic, organic, and biological mixtures. Several papers have been published which describe quantitative thin-layer techniques (16–19). These methods can be classified as direct or indirect.

In the direct method, the developed chromatogram is quantitatively evaluated by measuring spot size or area or by densitometry. Thus, measurement is accomplished without removing the sought-for substance from the support. An indirect method implies removal of the separated substance from the plate. This may be followed by elution of the sample from the adsorbent and analysis of the eluant, usually by spectrophotometry or colorimetry. Indirect methods have the advantage in that spectra of the samples are readily obtained as part of the analysis. These may provide important additional information concerning identity and purity (20). Spencer and Beggs (21) have pointed out certain precautions which must be taken if an indirect method is to give precise, accurate results.

The authors report an application of the indirect method, utilizing TLC for the physical separation of butaperazine from its degradation products and, subsequently, quantitative determination of butaperazine using UV spectroscopy. Possible sources of error in the method are examined. The initial steps in the oxidative decomposition of butaperazine are shown.

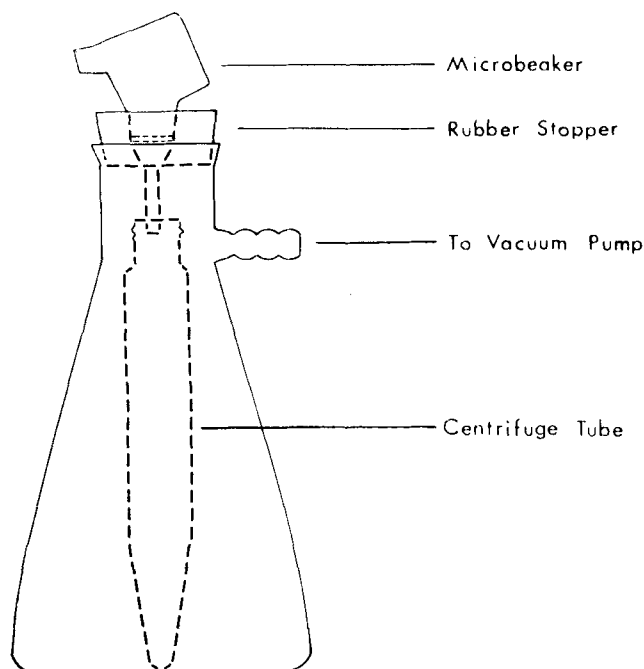[1] Marketed as Repoise by A. H. Robins Co., Richmond, VA 23220

Figure 1—*Elution assembly for quantitative recovery of sample.*

EXPERIMENTAL

Apparatus—The following were used: Hamilton syringe, fixed needle, blunt tip, 100 and 250 μl.; microbeaker, Pyrex, filter type with fine-porosity fritted disk; glass plates (20 × 20 cm.); and Shandon Chromatank (Cat. No. SAB-2843) lined with solvent-saturated filter paper.

Chemicals—All chemicals and reagents used were analytical reagent grade. The chemical purity of standard butaperazine was checked by melting point and TLC. Solubility analysis gave further indication of the purity of the standard.

Phosphate Buffer Solution, pH 5.3—Sodium dihydrogen phosphate monohydrate ($NaH_2PO_4 \cdot H_2O$), 38.0 g., and 2.0 g. disodium hydrogen phosphate (Na_2HPO_4) were dissolved and diluted to 1 l. with water.

Preparation of Thin-Layer Plates—A slurry of silica gel G was prepared from 30 g. of Merck silica gel G, grain size 10–40 μ, and 60 ml. water. This was applied to glass plates with an appropriate spreader set at 250 μ. The plates were air dried for 15 min. and then activated at 105° and stored over anhydrous silica gel. Subsequent work has shown that the air-dried plates without activation yield the same type of separation.

Developing Solvent—The developing system consisted of 4 parts isopropyl alcohol and 1 part 1 *N* ammonia. The system was prepared just prior to use and used only once.

Preparation of the Standard—The standard solution of butaperazine phosphate or maleate was prepared in phosphate buffer solution (pH 5.3) such that it contained a concentration of the salt equivalent to 1.91 mg./ml. of the base. The solution was thoroughly mixed, and 10-ml. aliquots were transferred to a separator. The aqueous solution was extracted, using successively one 10-ml. and three 5-ml. portions of chloroform. The standard solution was prepared just prior to use.

Application of Drug and Development of Chromatogram—Using a precision syringe, a predetermined amount of chloroform extract was applied to a plate. The organic extract was transferred as a narrow streak 4 cm. long. An appropriate streak was achieved by applying successive 10 -μl. portions of the solution, evaporating each portion with a slow stream of nitrogen. This application was repeated until a total of about 0.1 mg. of butaperazine base was applied on the same streak. One hundred microliters of the standard solution previously described was applied as a single streak.

Another streak using the sample solution was applied in the same manner on the other side of the plate. The exact quantity applied depended, of course, on the expected sample concentration.

The prepared plates (two or more) were placed into separate chromatographic chambers. Each chamber was equilibrated for 2 hr.

Table I—Precision of the Whole Procedure

Amount Butaperazine Taken, mcg.	Absorbance at 278 mμ after TLC (Uncorrected)	Absorptivity
76.58	0.392	51.2
76.58	0.398	52.0
77.09	0.396	51.4
96.37	0.484	50.2
96.37	0.489	50.7
96.37	0.495	51.4
96.37	0.498	51.7
76.58	0.391	51.1
76.58	0.393	51.3
76.58	0.404	52.8
$\bar{x}$		51.3
SD		0.56

and contained 150 ml. developing solvent. The chromatogram was allowed to develop in the dark and terminated when the solvent front ascended to a height of 15–16 cm. The developed plate was dried in a vacuum desiccator and was subsequently examined briefly under an UV lamp (254 mμ) to locate the separated butaperazine. The butaperazine appeared as a bright-orange to pink fluorescent streak at R_f approximately 0.5. This area was carefully marked, taking care not to overlap any other fluorescent areas.

Quantitative Determination of Butaperazine—Quantitative determination was accomplished by carefully removing the portion of silica gel adsorbent containing the butaperazine. The powder was removed by scraping onto nonabsorbent paper and was then transferred to a microbeaker with the aid of a small funnel. Methanol (3 ml.) was added and, after mixing gently, the mixture was allowed to stand for 15 min. The methanolic extract was collected by filtration, using the filtration assembly shown in Fig. 1. Elution of butaperazine was continued, using two additional 3-ml. portions of methanol. The combined extracts were finally diluted to 10 ml. with
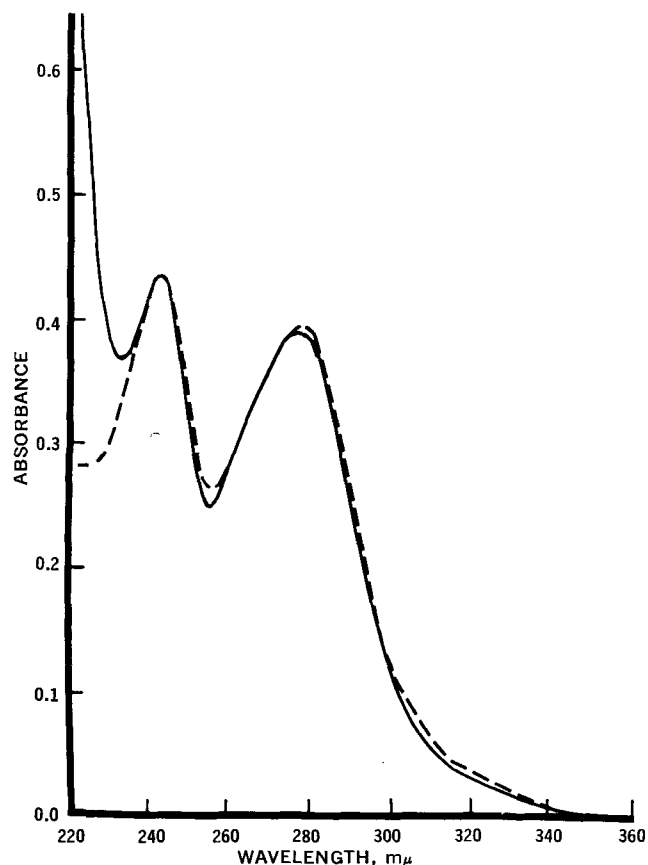

Figure 2—*UV absorption spectra of butaperazine in methanol. Key:* ——, *before TLC; and* - - -, *after TLC.*

Table II—Total Recovery: Butaperazine following TLC

Absorbance before TLC, 278 mμ	Absorbance after TLC,[a] 278 mμ	Recovery, %
0.396	0.384	97.0
0.394	0.390	99.0
0.391	0.388	99.2
0.495	0.476	96.2
0.495	0.481	97.2
0.495	0.487	98.4
0.497	0.490	98.6
0.391	0.383	98.0
0.395	0.385	97.5
0.389	0.395	101.8
$\bar{x}$		98.3

[a] Corrected for analytical blank.

methanol and mixed. The UV spectrum of the methanolic solution was then recorded in 1.0-cm. cells, using methanol as the reference.

Assay of Experimental Preparations—Each formulation was treated individually because of differences in the concentration of butaperazine. The general procedure for film-coated tablets involved reducing several weighed tablets in a mortar to a fine powder and passing the grind through a No. 50 sieve. A weighed portion of the sieved grind was then transferred to a separator using phosphate buffer solution. After mixing thoroughly, the mixture was extracted completely with chloroform. Each portion of the chloroform extract was transferred through a pledget of chloroform-washed cotton into a flask. During the transfer, the withdrawal of any insoluble matter was avoided. The chloroform extract was either concentrated or diluted to volume, depending upon the concentration of butaperazine. It was then chromatogrammed and assayed. Usually the standard and sample were run concurrently; therefore, absorbance values of the butaperazine eluates were not corrected for adsorbent blank.

RESULTS AND DISCUSSION

To compare UV spectra, a standard butaperazine maleate solution was prepared and extracted with chloroform as described. One aliquot was diluted directly with methanol. An equal aliquot was chromatographed, the butaperazine was eluted, and the solution was finally diluted to volume with methanol. As seen in Fig. 2, absorbance spectra before and after TLC were essentially identical above 240 mμ. Below this wavelength, the spectrum of an unchromatographed sample exhibits a higher absorbance. This observed spectral difference reflects the presence of chloroform in the unchromatographed sample. Although 242 mμ could not be utilized as an analytical wavelength because of interferences, use of the absorbance maximum at 278 mμ provided a direct measure of intact butaperazine. At this wavelength, there is little interference in the analytical blank or from formulation excipients. The position of maximum absorbance and absorbancy coefficient did not change significantly above 240 mμ during chromatography. This observation indicates that the responsible chromophore is not modified during chromatography.

Two-dimensional TLC, using the same solvent system for development in both directions, confirmed the stability of butaperazine during chromatography. No evidence of decomposition or alteration of the butaperazine spot was noted.

The spectral absorbance of the silica gel was obtained by collecting portions of the surface layer in a region near the butaperazine

Table III—Recovery of Butaperazine in the Presence of Its Oxidation Products

Butaperazine	mg. Taken — Butaperazine Sulfoxide	Butaperazine Sulfone	Intact Butaperazine mg. Found	Recovery, %
19.15	1.7	1.7	18.58	97.0
19.87	3.4	3.4	19.35	97.4
19.87	5.1	5.1	19.35	97.4

Table IV—Degradation of Butaperazine with 30% Hydrogen Peroxide

Interval after Addition of H₂O₂, min.	Absorbance at 278 mμ 30% H₂O₂ Added, ml. 0.3	0.6
0	0.496	0.475
30	0.459	0.432
60	0.441	0.395
120	0.389	0.290

spot and processing them in exactly the same manner as the sample. Absorbance of the filtrate was found to be 0.008 at 278 mμ, using a 1.0-cm. pathlength. This value is on the order of a few percent of the absorbance for the chromatographed samples.

Treatment of the eluant to remove most of the finely divided, insoluble particles of the adsorbent is an important step in improving the precision of the method. The 0.008 figure cited, which was repeatedly obtained, agreed closely with the published value of Spencer and Beggs (21).

A standard curve was prepared from aqueous solutions of butaperazine carried through the procedure described. At 278 mμ, a linear relationship exists between the absorbance and the amount of butaperazine in the range 45–123 mcg.

The precision of the method was established by measuring the absorbance at 278 mμ and calculating the absorptivity for known samples. Table I summarizes the precision data obtained for the whole procedure (including sample preparation, extraction, TLC, and elution). The experimentally determined mean absorptivity of a set of 10 assay runs on known samples was 51.3. The standard deviation (single measurement) was 0.56.

Experiments were conducted to determine the total recovery of butaperazine after TLC and elution (Table II). The average recovery (10 trials) was 98.3% (range 96–102%).

These high recoveries may be attributed partly to the method of elution chosen. Thus, butaperazine on the silica gel was exposed repeatedly to fresh solvent. Minimizing the volume of eluting solvent and filtering concurrently decreased losses from filtration and transfer.

To assess application of the method to degraded samples, mixtures of butaperazine, butaperazine sulfoxide, and sulfone were prepared so as to simulate various degrees of oxidation. These mixtures were assayed for intact butaperazine (Table III). As expected, recoveries were uniformly high, averaging better than 97%.

The assay method was further tested for its applicability in the presence of oxidative degradation products of butaperazine. Buffered aqueous solutions of butaperazine maleate were treated with 0.3 ml. and 0.6 ml. hydrogen peroxide (30%) for 30, 60, and 120 min. After each interval, the solutions were extracted with chloroform. The amount of butaperazine remaining was determined, using the procedures described (Table IV). In summary, hydrogen peroxide treatment causes a loss of butaperazine, which increases with time of exposure. Table III reveals that, as the amount of peroxide is increased, decomposition of butaperazine increases. Examination of TLC plates confirmed that the butaperazine spot became smaller and fainter with time and/or increasing peroxide concentration. The other change noted was in the sulfoxide spot, which increased in size and intensity with time. Thus, the sulfoxide appeared to be the major product for this oxidative degradation.

Table V—Assay of Butaperazine in Experimental Formulations: Application of TLC Method

Formulation	Storage Conditions	Labeled Amount of Butaperazine	Label Claim Found, %
Tablets	12 months at RT	51.3 mg./tablet	97.2
	12 months at 37°		99.4
	12 months at 50°		97.8
Syrup	Initial	5 mg./ml.	98.2
	12 months at RT		98.3
Injectables	3 months at RT	5 mg./ml.	96.8
	3 months at 37°		98.5
	3 months at 50°		99.0

Unbuffered aqueous solutions of butaperazine phosphate were placed in a commercial light-stability testing unit. After 7 and 15 days of exposure, the solutions were assayed. As expected, the butaperazine content had decreased by 16 and 22%, respectively.

Aqueous solutions of butaperazine buffered at pH 5.3 were sealed in glass ampuls and autoclaved at 115° for 30, 60, and 120 min. The ampuls were cooled, opened, and assayed for butaperazine. An initial 10% loss was noted, which did not increase with time. Atmospheric oxygen initially present in the vial and test solution was suspected. When consumed, no further oxidation occurred. This suspicion was confirmed by autoclaving ampuls in which oxygen was replaced with nitrogen. As predicted, these solutions did not show a significant loss of butaperazine.

Tablets, syrups, and injectables were assayed by the method described. These results are shown in Table V. The excipients, coloring agents, diluents, and fillers employed in the various formulations did not interfere.

It is evident that the formulations tested exhibited no significant decomposition. Storage at elevated temperatures (37 and 50°) for extended periods did not change this overall picture.

Several experimental factors were found to be critical in obtaining precise, accurate results. The area of the sample streak increases during development; therefore, care must be taken to keep the initial application narrow and uniform. Evaporation of the organic solvent must be accomplished quickly with a nonoxidizing drying agent. This precaution minimizes the size of the sample streak and prevents oxidative decomposition.

Use of the solvent system for more than one separation is not permissible, because decreased separation of butaperazine and decomposition products occurs and the R_f values change unpredictably.

Investigation was begun early to elucidate the major decomposition products of butaperazine and to compare them to those of other phenothiazines.

Chloroform solutions of butaperazine, its sulfoxide, and sulfone[2] were compared by TLC to the chloroform extract of degraded aqueous samples of butaperazine. These results showed that the sulfoxide and sulfone migrate slower than butaperazine in an isopropanol-NH_3 system. In addition, all were well resolved from butaperazine. In a degraded sample, one of the bands corresponded to the position of the sulfoxide streak. This band was eluted with methanol. The UV spectrum of the eluate was identical to that of the synthetic sulfoxide. The band corresponding to the synthetic sulfone could not be isolated by the techniques employed.

When a developed TLC plate was exposed for a few days to normal atmosphere, it was noted that the fluorescence of the butaperazine band changed with time. The resulting fluorescence was similar to that of a degraded sample.

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[2] The identity of these synthesized compounds was established by CHN analysis and by UV, IR, and NMR spectroscopy.

TLC of grossly degraded samples often revealed the presence of products other than those described. Oxidative decomposition apparently is not the only degradation pathway, although, undoubtedly, the predominant one. Degradation apparently proceeds as follows: [butaperazine]...[sulfoxide]...[sulfone]...[other oxides].

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