Table IV—Diuretic Effect after Administration of the Compounds Orally to Rats; Values in Urine as % of Control

Compound No.	Volume	Na^+	K^+	Cl^-
1	125	121	219	277
2	163.5	772.5	118	378.5
4	125.5	182	141.5	296
5	152.5	138	280	287
6	141.5	520.0	162.5	323.0
7	133.5	90	158	266
8	154.5	276.0	164	255
10	155.8	1000	139.3	410
Hydrochloro-thiazide	166	1000	250	538.5

1-phenyl-4-*n*-butylthiosemicarbazide was not depressed.

3-p-Chlorophenyl-6-isopropyl-1,2,4(H)-triazole-5-sulfonamide—A stirred mixture of 3-*p*-chlorophenyl-4-isopropyl-5-mercapto-1,2,4-(H)-triazole (5.0 g.), water (135 ml.), and ferric chloride solution (0.7 ml. of 60%) was stirred and cooled to 0°. Chlorine gas was then passed into the mixture for 1 hr. maintaining the temperature between 0–5°. The reaction mixture was allowed to stand at this temperature for 15 min. more and then filtered. The solid was pressed on filter paper and immediately added to aqueous ammonia (150 ml. of 20%). This solution was left at room temperature for 6 hr. and then filtered. The filtrate was acidified with hydrochloric acid to pH 6. The white solid that separated, on purification by redissolving in alkali and precipitating with acid followed by crystallization from ethanol, gave 3-*p*-chlorophenyl-4-isopropyl-1,2-4(H)-triazole-5-sulfonamide (2.8 g.), m.p. 214–215°.

Anal.—Calcd. for $C_{11}H_{13}ClN_4O_2S$: N, 18.64. Found: 18.69.

Similarly, 3-*o*-chlorophenyl-4-phenyl-1,2,4(H)-triazole-5-sulfonamide was obtained in 32% yield, m.p. 240°.

Anal.—Calcd. for $C_{14}H_{11}ClN_4O_2S$: N, 16.74. Found: N, 16.53.

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DRUG STANDARDS

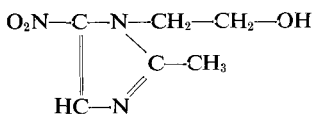
Analysis of Metronidazole

MURRAY M. TUCKERMAN and TATJANA BIĆAN-FIŠTER*

Abstract □ The literature on the identification, assay, and use of metronidazole has been surveyed. Based on published information, private communications, and laboratory experimentation, qualitative tests and quantitative assays have been developed for metronidazole, metronidazole suppositories (vaginal tablets), and metronidazole tablets. Extraction of metronidazole from suppositories and tablets is with hot acetone. Assays are based on titration of metronidazole in acetic anhydride with 0.1 *N* perchloric acid in glacial acetic acid, using malachite green indicator. The visual endpoint coincides with that determined potentiometrically. Supporting data is presented, including UV and IR spectra.

Keyphrases □ Metronidazole dosage forms—analysis □ Colorimetric method—identity □ UV spectrophotometry—identity □ IR spectrophotometry—identity

Metronidazole,[1] $C_6H_9N_3O_3$; mol. wt. 171.15, is 2-methyl - 5 - nitroimidazole - 1 - ethanol or 1 - (2 - hydroxyethyl)-2-methyl-5 nitroimidazole. It was recognized in "Addendum 1964" of the BP 1963 (1) as the drug and in the form of tablets. These two forms and the suppository have been admitted to USP XVIII. The structural formula may be represented as



EXPERIMENTAL

Physical Properties—Metronidazole occurs as white to pale yellow crystals or crystalline powder, stable in air, but darkening on exposure to light. It is sparingly soluble in water, in alcohol, and in chloroform, and is slightly soluble in ether. The melting range is 159–163°.

Identity Tests—*A.*—Heat about 10 mg. in a water bath for 5 min. with 1 ml. of water, 0.25 ml. of hydrochloric acid, and 10 mg. of zinc powder, filter, cool, add 1 ml. of freshly prepared sodium nitrite solution (1 in 100), then remove excess nitrite by addition of 1 ml. of freshly prepared sulfamic acid solution (1 in 100). To 1 ml. of this solution add 1 ml. of betanaphthol T.S.: an intense red color is produced. (This differs from the BP test in that the reaction takes place in an acid medium.)

[1] Flagyl, G. D. Searle & Co., Chicago, Ill.

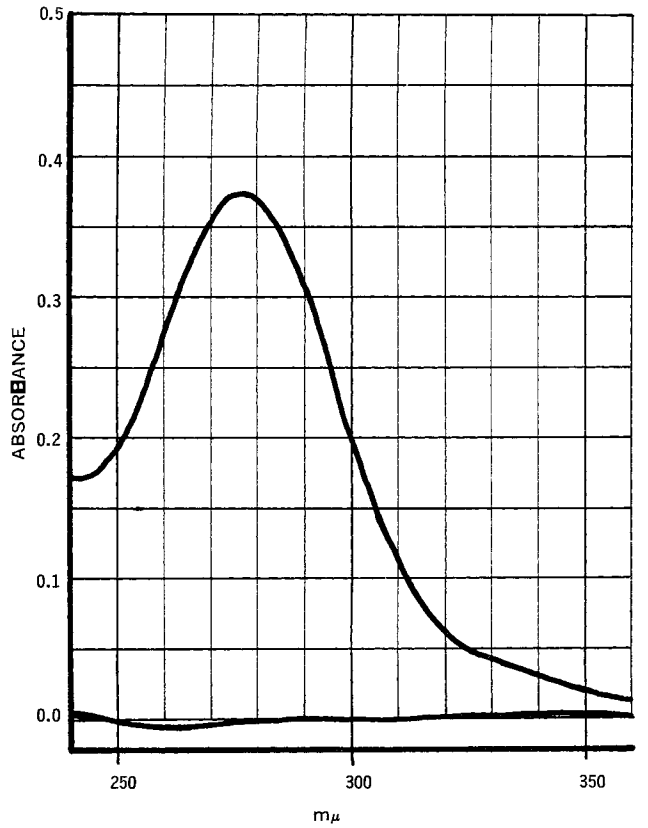


Figure 1—*UV spectrum of 1:10,000 dispersion of metronidazole in 0.1* N *hydrochloric acid.*

B.—Dissolve about 30 mg. in 2 ml. of sodium hydroxide T.S. with the aid of gentle heat: an intense red-violet color is produced that changes to yellow on the addition of excess dilute hydrochloric acid and again turns red-violet on the addition of an excess of sodium hydroxide T.S.

C.—Dissolve about 150 mg. in 10 ml. of 0.1 *N* sulfuric acid and add 10 ml. of trinitrophenol T.S. After standing for about 30 min., wash the precipitate formed with several small portions of cold water, using suction, and dry at 105° for 1 hr. The trinitrophenolate melts between 148 and 152°.

D.—A 1 in 10,000 solution in 0.1 *N* hydrochloric acid exhibits an absorbance maximum about 277 mμ (absorptivity about 3.8). The spectrum is shown in Fig. 1.

E.—The IR spectrum of an approximately 1% dispersion in potassium bromide is shown in Fig. 2.

Method A.—*Assay of Metronidazole*—Dissolve about 150 mg. of metronidazole, accurately weighed, in 20 ml. of acetic anhydride, warming slightly to effect solution. Cool, add two drops of malachite green indicator and titrate with 0.1 *N* perchloric acid in acetic acid to a yellow-green end-point. Perform a blank determination and make any necessary corrections. Each milliliter of 0.1 *N* perchloric acid is equivalent to 17.12 of $C_6H_9N_3O_3$.

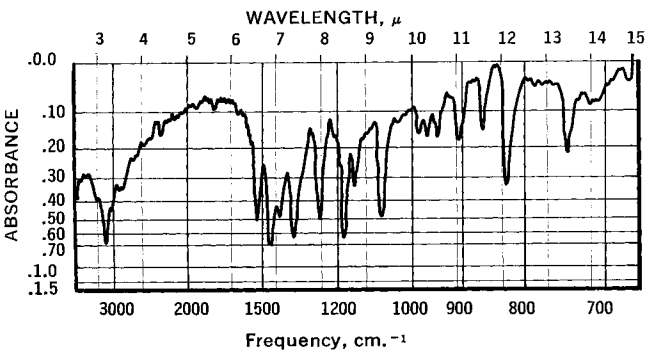


Figure 2—*IR spectrum of approximately 1% dispersion of metronidazole in potassium bromide.*

Malachite green indicator is prepared by dissolving 1 g. of malachite green oxalate in 100 ml. of glacial acetic acid.

Method B.—*Assay of Metronidazole*—Same as Method *A* except that the sample is dissolved in 40 ml. of hot acetone and diluted with 40 ml. of acetic anhydride.

Method C.—*Assay of Metronidazole Tablets and Suppositories*—Weigh and finely powder not less than 20 metronidazole tablets or suppositories. Weigh accurately a portion of the powder equivalent to about 150 mg. of metronidazole and transfer to a coarse-frit sintered-glass funnel. Extract with four 10-ml. portions of hot acetone, mixing the solid material well with each portion, then draining with the aid of gentle suction. To the combined acetone extracts add 40 ml. of acetic anhydride and two drops of malachite green indicator, and titrate with 0.1 *N* perchloric acid in acetic acid to a yellow-green end-point. Perform a blank titration and make any necessary correction. Each milliliter of 0.1 *N* perchloric acid is equivalent to 17.12 mg. of $C_6H_9N_3O_3$.

RESULTS

Assay in triplicate of metronidazole by Method *A* gave values of 100.2 ± 0.2% (range 100.1–100.4%).

Assay in triplicate of metronidazole in acetone-acetic anhydride by Method *B* gave values of 100.2 ± 0.2% (range 100.0–100.4%).

Replicate assay of the same powdered tablet sample by Method *C* gave recoveries of labeled claim of 100.5 ± 0.2% (range 100.2–100.7%).

Assay in triplicate of the same powdered suppository sample by Method *C* gave recoveries of labeled claim of 98.79 ±0.96% (range 98.06–99.88%).

DISCUSSION

The synthesis of metronidazole was reported in 1960 (2) and a claim made for antiprotozoal activity and low toxicity. Antiprotozoal activity, particularly against *Trichomonas vaginalis*, was reported by a number of investigators (3–6) and a dosage regimen established. The oral dose is 250 mg. three times a day for females and twice a day for males, for 10 days. The vaginal dose is 500 mg. daily, combined with 250 mg. twice a day taken orally. Labeling approved by the Food and Drug Administration provides no range from that above.

A widely used method of assay for metronidazole, particularly in biological fluids, is polarography in a variety of media (7–10). The half-wave potential is dependent on the acidity of the medium. Colorimetric methods involving reduction of the nitro group to amine, diazotization, and coupling have been reported (9, 11). This serves as a basis for Identity Test *A*. Spectrophotometric determination has also been used (11). This serves as a basis for Identity Test *D*.

The BP (1) uses nonaqueous titration in acetic acid with *p*-naphtholbenzein as the indicator. The authors found that the indicator change was not sharp and there was some precipitation prior to the end-point. Malachite green did not give a sharp end-point in glacial acetic acid. A sharp end-point in a clear solution containing acetic anhydride which agrees with the result of potentiometric titration was obtained by the suggested method.

The BP assays metronidazole tablets by nonaqueous titration of a suspension of ground tablets in glacial acetic acid. The method suggested in this paper is more conventional for the United States, involving separation of the organic base from possible basic inorganic constituents of the tablet. Hot acetone is used instead of the tetrahydrofuran suggested in a private communication (12). It is necessary that the acetone be hot, as the rate of solubility of metronidazole in cold acetone is low. Time is saved by not evaporating the acetone, but simply adding an approximately equal volume of acetic anhydride and titrating in the mixed solvent system. Extraction on the sintered-glass funnel saves time over the more usual centrifugation.

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TECHNICAL ARTICLES

Shear Cell Measurements of Powders: Proposed Procedures for Elucidating the Mechanistic Behavior of Powder Beds in Shear

E. N. HIESTAND and C. J. WILCOX

Abstract □ A variety of procedures for the use of a shear cell are devised in hopes of elucidating mechanistically the behavior of powders in shear. The processes considered are plastic deformation at regions of true contact and structural changes in the powder bed. Possible structure changes are: (*a*) consolidation or dilation; (*b*) blockage to resist the continuation of motion in the same direction; and (*c*) particle orientation. A series of pulls to just initiate shear is used. These are made either monodirectionally, *i.e.*, all pulls in the same direction, or bidirectionally, *i.e.*, consecutive pull directions reversed. A series of pulls proceeds until a steady state or plateau condition exists. A relative shear force, M, is defined as $M = (\mu_1 - \mu_p)/\mu_p$, where μ_1 and μ_p are the respective friction coefficients using first pull and plateau data. The retention of plateau-condition-shear-strength upon removal of the applied load is obtained by measuring the shear force for the first pull after reducing the load. An index of retention value is defined. Also, an indication of the relative extent of conditioning at plateau condition for various loads is based on a comparison of the shear force observed for one additional pull after increasing the load to an arbitrary constant value.

Keyphrases □ Powders—shear cell measurements □ Shear cell—procedures for use □ IP patterns □ Forces shear—reduced and increased load □ Structural changes—powder bed

In this communication a series of procedures will be proposed, then the experimental use of these will be described. The objective is to develop a mechanistic interpretation of the resistance to shear of powder beds. New parameters and indices will be defined when needed for simplification of the discussion.

The procedures are based on variations of shear cell studies reported previously (1). The primary reference values are the friction data in the form of a plot of the shear force, τ, *versus* the applied load, mg. The simple friction law, Eq. 1, adequately describes these friction data.

$$\tau = \mu(mg + h) \qquad \text{(Eq. 1)}$$

where τ = force required to initiate shear, μ = friction coefficient, mg = load normal to the shear plane, and h = "apparent" cohesion.

As stated in the earlier publication, the observed values of μ and h depend somewhat upon cell design, operational procedure, and the history of the powder bed. Reproducible values are obtained readily when a specific cell and a standard procedure are adopted. The values obtained are useful for making comparisons or detecting changes in the properties of a powder. However, μ and h alone are not adequate to indicate many of the characteristics of a powder bed. Additional meaningful properties must be identified and measured.

Occasional reference will be made to the properties that are associated with good flowability. It is recognized that flowability is not a uniquely defined property. It has meaning only in reference to the conditions under which flow is to occur. Nevertheless, no matter how elusive its precise definition may be, common usage has established a qualitative, intuitive understanding of its meaning. Hereinafter flowability is used in this qualitative connotation.