A 1:1 mole ratio complex also was formed between 9-ethyladenine and barbital in the solid state (11), but a continuous chain of molecules was formed in this case. The formation of strongly bonded dimers or chains of molecules would increase the glass transition temperature of the barbiturate–citric acid glass, and evidence of a 1:1 molecular reaction is found in the presence of the glass transition temperature maximum, which occurs at approximately 50 mole % [51.8% (w/w) pentobarbital, 52.9% (w/w) hexobarbital, and 54.4% (w/w) heptabarbital, respectively]. At this concentration, each barbiturate molecule is complexed with one citric acid molecule. Therefore, the interaction and the deviation of the glass transition temperature are greater.

X-ray diffraction could not be used to investigate complex formation because the glass phase was amorphous. The devitrified glasses were simple eutectic mixtures, which suggested that crystallization involved the breakage of citric acid–barbiturate bonds to produce crystals of the pure components. Therefore, the X-ray diffraction patterns of the devitrified systems could not help to elucidate the structures of the complexes.

The increase in the glass transition temperature in these three dispersions was not great and did not exceed the glass transition temperature of the pure barbiturate. It may be possible to increase the glass transition temperature further by selecting other glass-forming compounds as carriers or formulating three-component glass systems. By selection of suitable barbiturates, glassy dispersions can be prepared for further studies to test the feasibility of the glass phase as a solid dispersion system. Work is continuing on this aspect.

Conclusions—Many barbiturates will form glasses when fused; the viscosity and glass transition temperature of the glass vary with the molecular configuration of the barbiturate molecule. The position of the glass transition temperature within the barbiturate series can be rationalized in terms of the intermolecular bonding in the glass and the restriction of molecular movement imposed by substituents in the C-5 position. Substituting a methyl group for hydrogen on the N-1 position reduces intermolecular hydrogen bonding and results in a relatively low glass transition temperature.

When fused with citric acid, heptabarbital, hexobarbital, and pentobarbital form binary glasses. The glass transition temperatures of these systems do not show a linear increase with composition but attain a maximum at approximately 50% (w/w) of the barbiturate. This deviation is consistent with the formation of a 1:1 mole ratio complex between the two components; it is postulated that there is a greater number of hydrogen bonds in the complex, probably of greater strength, than in glasses of the individual components.

REFERENCES

(1) W. L. Chiou and S. Riegelman, *J. Pharm. Sci.*, **58**, 1505 (1969).
(2) M. P. Summers and R. P. Enever, *ibid.*, **65**, 1613 (1976).
(3) *Ibid.*, **66**, 825 (1977).
(4) R. F. Boyer, *Rubber Chem. Technol.*, **36**, 1303 (1963).
(5) L. D. Pye, in "Introduction to Glass Science," L. D. Pye, H. J. Stevens, and W. C. La Course, Eds., Plenum, New York, N.Y., 1972.
(6) W. A. Lee and G. J. Knight, *Br. Polym. J.*, **2**, 73 (1970).
(7) C. Hansch and S. M. Anderson, *J. Med. Chem.*, **10**, 745 (1967).
(8) Y. Kyogoku, R. C. Lord, and A. Rich, *Nature*, **218**, 69 (1968).
(9) M. Gordon and J. S. Taylor, *J. Appl. Chem.*, **2**, 493 (1952).
(10) K. H. Illers, *Kolloid-Z.*, **190**, 16 (1963).
(11) D. Voet, *J. Am. Chem. Soc.*, **94**, 8213 (1972).

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Improved Spectrophotometric Determination of Antazoline

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Abstract □ A simple, precise, and accurate spectrophotometric determination of antazoline salts was developed by improving the ceric sulfate procedure. Replacement of water with acetic acid for the preparation of all assay solutions permitted reproducible measurements of the chromogen that absorbed at 505 nm. An appreciable increase in color stability was attained by the controlled addition of perchloric acid to the ceric reagent prior to interaction with antazoline at room temperature. Evidence is provided to account for the oxidation of antazoline at the expense of a complex ceric species. Other 2-imidazolines or phenylephrine did not interfere with the investigated color reaction. In addition to the high value of the chromogen molar absorptivity, ideal adherence of color absorption to Beer's law permitted accurate and reproducible estimation of antazoline over the 1–10-μg range. The procedure was applied to the analysis of different antazoline dosage forms.

Keyphrases □ Antazoline—spectrophotometric analysis in prepared solutions and dosage forms □ Spectrophotometry—analysis, antazoline in prepared solutions and dosage forms □ Antihistaminics—antazoline, spectrophotometric analysis in prepared solutions and dosage forms

The frequent formulation of antazoline[1], 2-[(*N*-phenyl)benzylaminomethyl]-2-imidazoline, with other pharmaceutical amines and 2-imidazoline congeners motivated the development of a selective determination of this antihistamine.

BACKGROUND

Previous antazoline analyses primarily were based on spectrophotometry (1–8). Of the diverse chromogenic reagents adopted, only the sodium nitrite (5–7) and ceric sulfate procedures showed pronounced selectivity. The well-documented and popular ammonium reineckate (1, 2), sodium nitroprusside (3), and dipicrylamine (4) methods are not specific; other pharmaceutical amines and 2-imidazoline derivatives, likely to be present along with antazoline, interfere (9). Moreover, the nitroprusside method, while apparently specific for the intact imidazoline ring, is affected by the experimental buffer concentration and species, the age of reagents, and the intrinsic color of the sample (10).

The utility of the nitrite method is handicapped by interference of phenylephrine with color production (6), and the accuracy of the ceric sulfate procedure is questionable. Discrepancies in recovery studies attained by this latter method reveal considerable nonreproducibility, especially when compared to the nitrite procedure (11). In addition, no quantitative data could be supplied when the ceric reagent was not properly cooled prior to interaction with antazoline or when much water was present. These findings attest to the thermolabile nature of the antazoline–ceric chromogen as well as to its marked sensitivity to pH variations.

Such shortcomings in the available methods for the estimation of an-

[1] Antistine.

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Table I—Effect of Perchloric Acid Addition to Antazoline Reaction Medium on Color Development (c 5 μg/ml)

Milliliters Added per 10 ml of Assay Solution			
0.05% Ceric Reagent	Perchloric Acid	Absorbance	Stability, min
0.5	1.0	0.400	60
0.5	2.0	0.360	70
0.5	3.0	0.310	80
1.0	1.0	0.395	70
1.0	2.0	0.410	90
1.0	3.0	0.405	90
2.0	1.0	0.385	70
2.0	2.0	0.390	80
2.0	3.0	0.378	80

tazoline prompted development of a more accurate and precise method. The antazoline–ceric color reaction and its application to the analysis of pure antazoline salts and some of their pharmaceutical formulations were investigated.

EXPERIMENTAL

Instrumentation—A double-beam UV–visible spectrophotometer[2] with 1-cm glass cells and an analytical balance[3] were used.

Materials—Pharmaceutical grade hydrochloride and methanesulfonate salts of antazoline were utilized as the working standards. The following commercially available formulations were analyzed: antazoline tablets[4], antazoline injection[5], and antazoline–naphazoline solution[6]. Other chemicals were analytically pure.

Reagents—*Ceric Sulfate Solution*—By gentle warming, 50 mg of the dried salt was dissolved in 50 ml of 98% (w/w) sulfuric acid. This solution was then cooled and diluted stepwise and quantitatively to 100 ml with formate-free acetic acid. The clear and bright-yellow solution obtained was quite stable at room temperature.

Standard Antazoline Solution—An accurately weighed amount of the appropriate, well-dried antazoline salt was dissolved in acetic acid, and the solution was diluted quantitatively and stepwise with the same solvent to obtain a final concentration of 50 μg of the working standard/ml of the solution.

Assay Samples—*Tablets*—A single powdered tablet, or its equivalent (150 mg) from a composite of 20 tablets, was placed in a 100-ml volumetric flask. Then ~25 ml of acetic acid was added, and the flask was allowed to stand for 30 min with occasional shaking. The solution was diluted to volume with acetic acid, mixed well, and filtered through a dry filter into a dry flask, the first portions of the filtrate being rejected. An aliquot of this sample was properly diluted with acetic acid to obtain ~50 μg of the claimed antazoline salt content/ml of the assay solution.

Injections and Solutions—For these dosage forms, 1.0 ml of the injection, or the measured content of a single-dose container, was pipetted into a suitable volumetric flask and diluted with acetic acid to obtain ~50 μg of the claimed antazoline salt content/ml of the assay solution.

Procedure—Ceric sulfate reagent (1.0 ml) was transferred into a 10-ml volumetric flask, 2.0 ml of 70% (w/w) perchloric acid was added, and the solution was mixed well. Then 1.0 ml of the standard or assay solution of antazoline was added, and the solution was diluted to volume with acetic acid and mixed well. The solution was transferred directly into a 1-cm glass cell, and the absorbance of the color was determined at 505 nm *versus* a blank prepared from 1.0 ml of acetic acid and treated similarly.

RESULTS AND DISCUSSION

Antazoline–Cerium(IV) Interaction—The original application of ceric sulfate as a color reagent for the identification of medicinal imidazoline derivatives revealed its high sensitivity for the detection of antazoline (12). However, this test could not be quantified because of the instability of the red color formed in 15% aqueous sulfuric acid at room temperature. Enhancing chromogen stability for not more than 30 min by raising the acid concentration to 50%, added with essential cooling before interaction, could not bring about a fully accurate estimation of antazoline; the recovery was ~94–99% (11).

Table II—Effect of Temperature Rise on Antazoline–Ceric Interaction (c 5 μg/ml)

Temperature[a]	Absorbance[b]
25°	0.408
40°	0.383
75°	0.255
95°	0.125

[a] Of a thermostated water bath, as the reaction temperature. [b] At 505 nm after 10-min interaction time.

Water was utilized for the preparation of antazoline and ceric sulfate solutions (11). The chromogen formed is much less stable on addition of water to the reaction mixture (11, 12); therefore, the antazoline–ceric sulfate reaction was performed in 50% sulfuric acid, and the chromogen formed was extracted by organic solvents. However, this procedure was handicapped by the insolubility of the color in chloroform, ether, or benzene.

Further investigation of the behavior of the chromogen in different nonaqueous solvents revealed its complete instability in absolute ethanol, methanol, dimethylformamide, dimethyl sulfoxide, dioxane, acetonitrile, and formic acid. However, marked stability was noticed with acetic acid as the reaction solvent, an observation that recommended its use for the preparation of all solutions. The utility of acetic acid for the ceric sulfate oxidation of organic compounds is well documented (13).

Preliminary interaction at room temperature of antazoline hydrochloride with a 0.05% (w/v) ceric sulfate solution in a 50% (v/v) sulfuric–acetic acid mixture resulted in the instantaneous production of a purple-red color with a maximum absorption at 505 nm. Maximum color intensity was manifested directly after mixing and measurement, with a steady rate of light absorption over 45 min. Repetition of this interaction using lower concentrations of sulfuric acid brought about a measurable decrease in the stability of the chromogen, with a negligible drop of its maximum absorption (Fig. 1).

These data reflect the significance of protonation for the stabilization of the chromogen formed. Further investigation of the effect of higher sulfuric acid concentrations was hindered by the syrupy consistency of the solutions during preparation of the corresponding mixtures in acetic acid. However, this problem was overcome by addition of a measured volume of 70% (w/w) perchloric acid to the ceric reagent, prepared in a 50% (v/v) sulfuric–acetic acid mixture, prior to interaction with the antazoline solution. In contrast to sulfuric acid, perchloric acid offered no

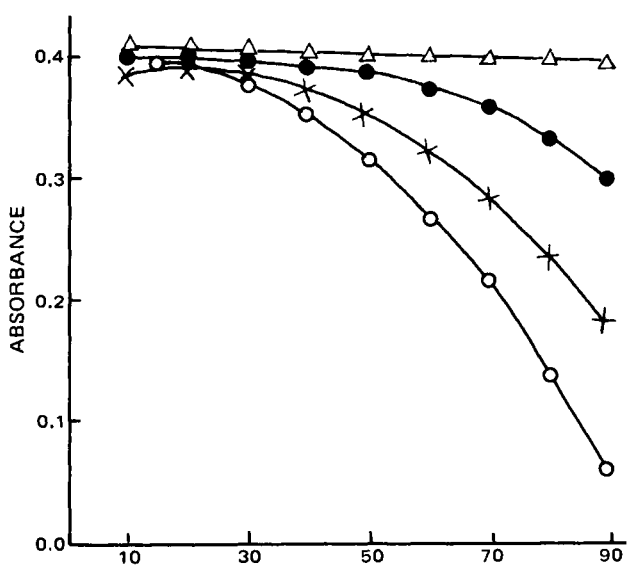

Figure 1—*Absorbance–stability behavior of antazoline chromogen at different sulfuric acid concentrations. Key:* ●, *50% sulfuric acid in glacial acetic acid;* ×, *30% sulfuric acid in glacial acetic acid;* ○, *15% sulfuric acid in glacial acetic acid; and* △, *same as* ● *after addition of 2 ml of perchloric acid.*

[2] MOM, Budapest, Hungary.
[3] WA-33, Warsaw, Poland.
[4] Antistine (Ciba-Geigy, West Germany) contains 100 mg of antazoline hydrochloride/tablet.
[5] Histazine (CID, Egypt) contains 100 mg of antazoline methanesulfonate/2-ml ampul.
[6] Antistine-Privine (Ciba-Geigy) contains 5 mg of antazoline sulfate and 0.25 mg of naphazoline nitrate/1 ml of solution.

Table III—Replicate Analysis of Antazoline Hydrochloride Working Solutions (c 5 μg/ml)

Replication	Absorbance at 505 nm
1	0.410
2	0.408
3	0.408
4	0.409
5	0.410
6	0.412
7	0.407
Average	0.409
SD	$\pm 1.676 \times 10^{-3}$

difficulties with respect to consistency or exothermic interactions. As revealed from the data given in Table I, 90 min of maximum stability was demonstrated by the color developed when 1.0 ml of the ceric reagent was mixed with 2.0 ml of 70% (w/w) perchloric acid and the mixture formed was further interacted with 1.0 ml of the working antazoline hydrochloride solution.

Under optimum conditions for color production, interaction of antazoline with ceric sulfate was seriously affected by a temperature rise (Table II), thus confirming the thermolability of the chromogen as initially postulated. Discrepancies in antazoline estimation, as achieved by the "aqueous" ceric sulfate method, can be attributed to some inaccuracies in controlling the local exothermic effect brought about by water–acid mixing.

Investigation of the effect of the mode of addition on the sensitivity of color production indicated that the antazoline solution should be added to the ceric reagent. Slightly lowered light absorption values were observed with the reverse order.

Reaction Interpretation—Substitution of phenylephrine or other 2-imidazoline derivatives for antazoline in the given interaction failed to induce color formation. These derivatives included 2-methyl-2-imidazoline, naphazoline[7], oxymetazoline[8], tolazoline[9], xylometazoline[10], and tetrahydrozoline[11] hydrochloride salts. With phentolamine[12], a transient blue color appeared for a few moments and faded into pale yellow However, with different aniline derivatives, including *N*-ethylaniline, *N,N*-dimethylaniline, and *o*-toluidine, an instantaneous red-brown color was observed.

These data and the fact that different aniline derivatives are frequently utilized as redox indicators in cerimetric titrations (14) suggested that the antazoline–cerium(IV) color interaction could be explained in terms of the oxidation of the antazoline aniline moiety. That the imidazoline ring is not vulnerable to such an effect is in accord with the stability of this heterocycle toward oxidation (15). This stability is further enhanced in the antazoline salts by their resonance-stabilized guanidinium function. The appreciable stabilization of the antazoline chromogen in the presence of strong acids is also in agreement with the properties of other *N*-disubstituted aniline chromogens (16). Establishment of the exact nature of the investigated color product was not attempted because of its insolubility in different organic solvents, sensitivity to pH variations, thermolability, and instability in aqueous solutions.

Addition of excess perchloric acid to the ceric sulfate reagent prior to the interaction with antazoline lowered reaction sensitivity (Table I). Such lowering was not observed when this excess was added after the interaction of antazoline with the reagent alone. This paradox in the effect of perchloric acid attests to the role of an anionic complex cerium species (17), mostly as $Ce(SO_4)_3^{2-}$, for an effective oxidation of antazoline. The formation of this complex in the presence of higher perchloric acid concentrations is doubtful (18). In support of this view, no color response could be evidenced up to a sensitivity limit of ~100 μg of antazoline/ml when a 0.05% solution of ceric perchlorate in a 50% (v/v) perchloric–acetic acid mixture was reacted under the optimum conditions mentioned.

Quantitative Analysis—For the investigated antazoline–cerium(IV) interaction, the intensity of color absorption was a function of the amount of antazoline interacted. A linear regression analysis of the Beer's plot of antazoline hydrochloride revealed an excellent correlation ($r = 0.9996$) over a concentration range of 1.0–10 μg/ml, with a slope value of 0.082 and an apparent molar absorptivity of 2.47×10^4. For antazoline methanesulfonate, these values were r, 0.9995; concentration range, 2.0–12 μg/ml; slope, 0.066; and apparent molar absorptivity, 2.05×10^4. These results permitted the development of the investigated color reaction into a sensitive spectrophotometric analysis of antazoline salts.

[7] Privine.
[8] Nasivin.
[9] Priscol.
[10] Otrivine.
[11] Tyzine.
[12] Regitine.

Table IV—Recovery Analysis of Antazoline Hydrochloride Working Solutions

	Antazoline Hydrochloride			
Sample	Weight, mg	Level of Analysis, μg/ml	Found[a], mg	Recovery, %
1	25	4	24.9	99.6
2	25	8	25.1	100.4
3	50	3	49.9	99.8
4	50	6	50.8	101.6
5	100	2	99.5	99.5
6	100	10	99.3	99.3
Mean recovery, %				100.03
Relative *SD*				$\pm 8.545 \times 10^{-3}$

[a] Average of four determinations.

Table V—Analysis of Antazoline Commercial Formulations

	Antazoline Salt Content, mg/unit			
Preparation[a]	Label Claim	Found[b]	Added	Recovered[b]
Tablets	100	101.4	50	151.2
Injections[c]	50	49.3	10	59.4
Solution[d]	5.0	5.1	100	105.5

[a] See *Experimental* for details. [b] Average of three determinations. [c] Antazoline methanesulfonate per milliliter. [d] Calculated as antazoline hydrochloride and multiplied by 1.0415.

Replicate analysis of these salts by the improved method (Table III) proved to be very precise, with a relative standard deviation of 4.098×10^{-3}. Recovery studies of working solutions of antazoline hydrochloride, analyzed at different concentrations (Table IV), afforded a mean percent recovery of 100.03 ± 0.85, in comparison to 102.6 ± 0.4 attained by the sodium nitrite method (5–7). No discrepancies in the recovery data of antazoline assayed by the presented method were observed, in contrast to the former ceric sulfate procedure (8, 11). Application of the presented color reaction to the analysis of different, commercially available antazoline dosage forms (Table V) proved satisfactory.

The presented improved ceric sulfate colorimetric determination of antazoline salts offers several advantages over the previously reported method in terms of accuracy, precision, and convenience and surpasses the sodium nitrite procedure on account of its high selectivity and stability of the ceric reagent. These considerations recommend the method for the automated analysis of antazoline formulations.

REFERENCES

(1) F. J. Bandelin, E. D. Slifer, and R. E. Pankratz, *J. Am. Pharm. Assoc., Sci. Ed.*, **39**, 277 (1950).

(2) K. I. Lee, *J. Pharm. Pharmacol.*, **12**, 666 (1960).

(3) S. C. Slack and W. J. Mader, *J. Am. Pharm. Assoc., Sci. Ed.*, **46**, 742 (1957).

(4) E. Selles and A. M. Rodriguez, *Galenica Acta*, **9**, 33 (1956).

(5) S. A. Ibrahim and A. F. Youssef, *Indian J. Pharm. Sci.*, **36**, 73 (1974).

(6) N. M. Sanghavi and H. S. Chardramohan, *ibid.*, **36**, 137 (1974).

(7) M. M. Amer, M. S. Tawakkol, and S. A. Ismaiel, *Analyst*, **99**, 487 (1974).

(8) M. M. Amer, M. S. Tawakkol, and S. A. Ismaiel, *Aust. J. Pharm. Sci.*, **3**, 58 (1974).

(9) P. F. G. Boon and W. Sudds, *J. Pharm. Pharmacol.*, **19**, 88S (1967).

(10) J. A. Mollica, G. R. Padmanabhan, and R. Strusz, *Anal. Chem.*, **45**, 1859 (1973).

(11) S. A. Ismaiel, Master dissertation, Cairo University, Cairo, Egypt, 1974, p. 119.

(12) A. Pitrowska, *Farm. Pol.*, **24**, 71B (1968).

(13) S. V. Gorbachev and Y. I. Vabel, *Zh. Fiz. Khim.*, **28**, 1782 (1954).

(14) L. Chatten, "Pharmaceutical Analysis," vol. I, Dekker, New York, N.Y., 1966, p. 163.

(15) K. Hoffmann, in "The Chemistry of Heterocyclic Compounds,"

vol. 6, A. Weissberger, Ed., Wiley–Interscience, New York, N.Y., 1953, pp. 1–420.
(16) H. S. Tan, G. A. Bruemmer, and D. Shelton, *J. Pharm. Sci.*, **66**, 1037 (1977).
(17) T. J. Hardwick and E. Robertson, *Can. J. Chem.*, **29**, 828 (1951).
(18) E. L. King and M. L. Pandow, *J. Am. Chem. Soc.*, **74**, 1966 (1952).

Effect of Formulation and Process Variables on Bioequivalency of Nitrofurantoin I: Preliminary Studies

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Received December 1, 1977, from the *Department of Industrial Pharmacy, Massachusetts College of Pharmacy, Boston, MA 02115.* Accepted for publication March 22, 1978.

Abstract □ Fifty-two combinations of nitrofurantoin were developed to assess the effect of dosage form type, particle size, diluent, and process on *in vitro* availability. With the official procedure and conditions, dissolution rates fell in a 66-fold range. Statistical analysis of the dissolution rates indicated no significant differences as a result of particle size, processing method, or compression force. The diluent choice and dosage form type significantly influenced the dissolution rate. Based on *in vitro* screening, six formulations presenting a broad range of dissolution rates were selected for further study relating to human bioavailability and bioequivalence.

Keyphrases □ Nitrofurantoin—*in vitro* dissolution rate, effect of formulation and process variables □ Dissolution rate, *in vitro*—nitrofurantoin, effect of formulation and process variables □ Antibacterials, urinary—nitrofurantoin, *in vitro* dissolution rate, effect of formulation and process variables

Nitrofurantoin, an antibacterial agent used in urinary tract infections, was included by the Food and Drug Administration on its list of drugs requiring bioavailability/bioequivalence testing (1). Because of its physicochemical properties, dissolution testing might be used as a means for eliciting inferences concerning its bioavailability. Formulation and manufacturing processes may affect its dissolution and ultimate bioavailability (2), and dissolution is particle size dependent (3–5).

No useful correlation was observed between the extent of urinary excretion and either the disintegration or dissolution characteristics of nitrofurantoin tablets (6, 7). However, the third supplement of USP XIX (8) requires that not less than 25% of the labeled amount of nitrofurantoin must dissolve in 60 min. Since the bioavailability characteristics of commercially produced nitrofurantoin tablets were examined in previous investigations without regard to specific formulation and process variability information, a study was undertaken utilizing predetermined variables to assess their effect on bioequivalence.

EXPERIMENTAL

Particle-Size Reduction and Analysis—To determine the effect of particle size, crystalline nitrofurantoin USP[1] was used both in its commercially available form and in micronized form prepared by processing the commercial material through a fluid energy jet mill[2]. A differential pressure of 30% was used between the inlet and opposing jets.

The original and micronized crystals were then suspended in peanut oil, and the particle size was determined by microscopy (9).

Formulations and Processing—Fifty-two formulations were prepared, incorporating the following variables:

1. Three types of dosage forms (chewable tablets, swallow tablets, and hard gelatin capsules).
2. Two particle sizes of nitrofurantoin crystals.
3. Two processes of incorporation (wet granulation and direct blending–compression).
4. Two diluents [compressible sugar[3] and mannitol[4]–lactose[5] (2:1)].
5. Three levels of hardness or compression force for the tablet formulations.

The formulations are summarized in Tables I–III.

For wet granulation, all ingredients except the lubricant were blended in a cuboidal blender[6] for 10 min at 35 rpm. Granulation then was accomplished in a planetary mixer[7] at 120 rpm for 10 min, using sufficient distilled water to produce the proper consistency. This mixture was granulated through a 12-mesh screen, dried overnight at 48°, and sized through a 16-mesh screen. The granulation was blended with the lubricant in the cuboidal blender for 10 min at 35 rpm. Compression was accomplished on an instrumented rotary tablet press[8] at three levels of force.

For direct compression, all ingredients were blended in the cuboidal blender for 10 min at 35 rpm and compressed as described.

For the hard gelatin capsules, the blends were prepared as for direct compression. The blend was filled into hard gelatin capsules[9] (size 0) using a hand-operated capsule-filling machine[10].

The tablets were physically evaluated on the basis of weight variation, hardness variation, friability, and disintegration. The capsules were physically evaluated on the basis of weight variation only. All dosage forms were evaluated chemically on the basis of composite average assay and content uniformity using a slight modification of the method originally proposed by Conklin and Hollifield (10). An accurate sample equivalent to approximately 100 mg of nitrofurantoin was mixed with approximately 10 ml of dimethylformamide, filtered, quantitatively transferred with adequate rinsing, and diluted to 50 ml with dimethylformamide. A 1.0-ml aliquot of this solution was diluted to 50.0 ml with 10% dimethylformamide. A 1.0-ml aliquot of this dilution was acidified with 2.0 ml of 0.2 *M* HCl and extracted with 5.0 ml of nitromethane. Then 3.0 ml of the nitromethane layer was combined with 0.5 ml of 0.04 *M*

[1] Lot 12060, Berry and Withington Co., Cambridge, Mass.

[2] Gem-T research model, Trost Air Mill Department, Newton, Pa.
[3] Nu-Tab, lot DB917M, Specialty Products by SuCrest, Pennsauken, N.J.
[4] Granular, lot 1219, ICI America, Wilmington, Del.
[5] Anhydrous, lot 4NM10, Sheffield Chemical Co., Union, N.J.
[6] Model KB-15, Erweka-G.m.b.H., Frankfurt, West Germany.
[7] Model N-50, Hobart Manufacturing Co., Troy, Ohio.
[8] Model B-2, Stokes Division, Pennwalt Corp., Warminster, Pa.
[9] Elanco Products Co., Indianapolis, Ind.
[10] Model SGR-O Capsulator, Spielman and Co., Clifton, N.J.

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