Anal.—Calcd. for $C_{18}H_{24}Cl_2N_4S_2$: C, 50.11; H, 5.61; N, 12.91; S, 14.86. Found: C, 50.43; H, 5.86; N, 12.72; S, 15.21.

(Carboxamidinium)methyl Disulfide Sulfate—A solution of α-(carboxamidino)methanethiolsulfuric acid (1) (10.0 Gm., 0.059 mole) in 6 *N* hydrochloric acid (250 ml.) was heated at 96° for 17 hr. The reaction mixture was evaporated *in vacuo* and the yellow gummy residue recrystallized from 50% aqueous alcohol (4.5 Gm., 55%), m.p. 225–227° dec.; infrared,† 1700 cm.$^{-1}$ (ν C=N$^+$); NMR (D_2O), δ 3.82 (s, CH_2S–S–).

Anal.—Calcd. for $C_4H_{12}N_4O_4S_3$: C, 17.38; H, 4.38; N, 20.27; S, 34.81. Found: C, 17.62; H, 4.50; N, 20.00; S, 35.01.

(N-Benzylcarboxamidino)methanesulfonic Acid—(*a*) To an aqueous solution of *N*-benzyl-α-chloroacetamidinium chloride (1) (5 Gm., 0.019 mole) in 30 ml. was added an aqueous sodium sulfite solution (2.42 Gm. 0.019 mole in 22 ml.) and the mixture heated at the reflux for 0.5 hr. The crystals (1.2 Gm., 37%) which separated were recrystallized from methanol–ether to give the product (0.20 Gm., 47%), m.p. 245–246°; infrared, 1690 (ν C=N$^+$), 1645 cm.$^{-1}$ (δ C=NH_2^+); NMR (D_2O), δ 4.10 (s, CH_2S), 4.60 (*s*, CH_2N), 7.45 (s, C_6H_5).

Anal.—Calcd. for $C_9H_{12}N_2O_3S$: C, 47.35; H, 5.30; N, 12.27; S, 14.05. Found: C, 47.25; H, 5.52; N, 12.27; S, 13.90.

(*b*) A solution of (*N*-benzylcarboxamidinium)-methyl disulfide dichloride (0.40 Gm.), 30% hydrogen peroxide solution (5 ml.), and 6 *N* hydrochloric acid (10 ml.) were heated at 96° (17 hr.). Solvents were removed *in vacuo* and the residue crystallized and found to be identical to that described in *a*.

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Keyphrases

α-mercaptoacetamidinium chlorides—synthesis
S-alkyl phosphorothioates—intermediate products
IR spectrophotometry—structure
NMR spectrometry

Drug Standards

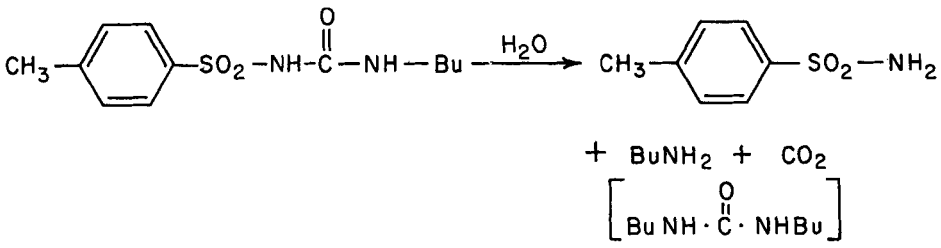
Selective Determination of Tolbutamide in Pharmaceutical Dosage Forms by Reaction with Ninhydrin

By K. K. KAISTHA and W. N. FRENCH

A colorimetric method is described for the quantitative determination of tolbutamide in pharmaceutical dosage forms. The procedure is based on the interaction of *n*-butylamine, liberated *in situ* from tolbutamide, and ninhydrin to form a blue complex which can be quantitated spectrophotometrically at 585 mμ. No color is produced by *p*-toluenesulfonamide or dibutylurea, two possible decomposition products of tolbutamide. Urea and its acyl derivatives, carbamates, amidines, guanidine, and common tablet excipients do not undergo the reaction. A modified procedure is described for the removal of *n*-butylamine, a major hydrolytic product of tolbutamide, from samples of the drug that have undergone decomposition. Results of the application of the assay procedure to a stability study of tolbutamide tablets are presented.

ALTHOUGH ULTRAVIOLET SPECTROPHOTOMETRIC analysis, as described in the USP XVII (1), is commonly used for tolbutamide preparations, this method is not satisfactory if ultraviolet-absorbing degradation products such as *p*-toluenesulfonamide or impurities such as *p*-toluenesulfonylurea are present in the sample. Similarly, a procedure involving total nitrogen determination, as described in the BP 1963 (2),

Received May 19, 1967, from the Research Laboratories, Food and Drug Directorate, Department of National Health and Welfare, Ottawa, Ontario, Canada.
Accepted for publication October 26, 1967.
Presented to the Drug Standards, Analysis and Control Section, APhA Academy of Pharmaceutical Sciences, Las Vegas meeting, April 1967.
The authors thank Dr. Leo Levi for his interest and encouragement.



Scheme I

is unsatisfactory if nitrogen-containing contaminants are present. Spingler and Kaiser (3) determined tolbutamide in serum by recording the absorption at 228 mμ, while Spingler (4) reported a colorimetric procedure based on the treatment of an amyl acetate extract of serum with 1-fluoro-2,4-dinitrobenzene followed by measurement of the absorbance at 380 mμ. The latter technique was adopted by Wiseman and co-workers for the estimation of a number of sulfamylureas and their metabolites in plasma and urine (5).

It was observed that a blue color was formed when an ethanolic solution of tolbutamide was heated with ninhydrin. The color was not formed with *p*-toluenesulfonamide or dibutylurea and ninhydrin, but was generated with *n*-butylamine. On the basis of this reaction, a method wherein the *n*-butylamine is generated *in situ* from tolbutamide and subsequently reacts with ninhydrin is presented.

The main hydrolytic decomposition of tolbutamide occurs as shown in Scheme I. Therefore, this method may be applied to decomposed formulations for the selective determination of tolbutamide by prior removal of any *n*-butylamine present.

EXPERIMENTAL

Reagents—Ninhydrin solutions: (*a*) 0.3% ninhydrin (reagent grade) in 80% ethanol. (*b*) 0.3% ninhydrin (reagent grade) in absolute ethanol.

Standard Reference Solution—Tolbutamide USP reference standard (200 mg.) in absolute ethanol (200 ml.).

Assay Procedure for Tolbutamide in the Absence of *n*-Butylamine—Procedure I

Procedure—Twenty tablets were weighed and finely powdered. An accurately weighed aliquot containing about 200 mg. of tolbutamide was quantitatively transferred to a 200-ml volumetric flask with the aid of absolute ethanol. The mixture was made to volume with absolute ethanol, shaken for 10 min., and filtered. A 25-ml. aliquot of the filtrate was heated under reflux in a 100-ml. flask fitted with a water-cooled condenser by immersing the flask up to the level of its contents in a stirred paraffin oil bath maintained at 96–100°. A small boiling chip was added to promote continuous and uniform boiling. After 90 min., the solution was cooled, transferred quantitatively into a 50-ml. volumetric flask, and made to volume with absolute ethanol.

A 2-ml. aliquot of the latter solution was pipeted into a 50-ml. flask and diluted with 3 ml. of the 80% ethanolic ninhydrin solution and 35 ml. of absolute ethanol. The solution was heated under reflux for 4 hr. as described above, cooled, then transferred quantitatively to a 50-ml. volumetric flask, and made to volume with absolute ethanol. The absorbance of the sample solution and that of tolbutamide standard carried simultaneously through the assay procedure were measured at 585 mμ against absolute ethanol.[1]

Calculations are as follows:

$$\text{mg. tolbutamide per tablet} = \frac{A_U}{A_S} \times \frac{W_S \times W_O}{W_U}$$

where:

A_U = absorbance of sample solution
A_S = absorbance of standard reference solution
W_S = weight of standard tolbutamide (mg.)
W_U = weight of sample (mg.)
W_O = average weight per tablet (mg.)

Procedure for Partially Degraded Tolbutamide Formulations—Procedure II

Total Unchanged Tolbutamide (Part A)—An aliquot of sample originally containing the equivalent of 200 mg. of tolbutamide was placed in a 200-ml. volumetric flask and chloroform added to volume. The mixture was shaken for 10 min., filtered, and a 25-ml. aliquot of filtrate transferred to a 60-ml. separator. The chloroform layer was extracted with 20 ml. of 0.1 *N* hydrochloric acid, and the aqueous extract in turn washed with 4 × 10-ml. portions of chloroform. The combined chloroform extracts were taken to dryness by means of a rotary film evaporator and the residue dissolved in 25 ml. of absolute ethanol. The color was developed as described in the assay procedure for tolbutamide (*Procedure I*) beginning at "A 25-ml. aliquot. . .was heated under reflux. . . ."

n-Butylamine (Part B)—The aqueous extract obtained as described under Part A was washed with 5 ml. of benzene (discarded), made alkaline with 5 ml. of 30% NaOH, and extracted successively with 9 ml. and three 5-ml. portions of benzene. The combined benzene extracts were diluted to 25 ml. with benzene in a volumetric flask, mixed, and passed through a pledget of glass wool.

A 5-ml. aliquot of the filtrate was transferred to a 50-ml. flask, 3 ml. of ethanolic ninhydrin solution

[1] This blank gives the same absorbance as a true "reagent blank."

(0.3% in absolute ethanol), and 25 ml. of absolute ethanol were added, and the color developed by heating under reflux for 3.5 hr. The reaction solution was cooled, diluted with absolute ethanol to 50 ml. in a volumetric flask, and its absorbance determined at 585 mμ. A standard solution of *n*-butylamine in benzene (0.1 mg./ml. ≡ 1.37×10^{-3} mmole/ml.) was analyzed concurrently for comparison purposes. The content of *n*-butylamine in the sample was calculated from the respective absorbance values.

Free Tolbutamide (Part C)—A 25-ml. aliquot of the chloroform filtrate obtained as described in Part A was extracted with three 15-ml. portions of water. The combined aqueous extracts were then extracted once with 15 ml. of chloroform, and the combined chloroform solutions evaporated to dryness by means of a rotary film evaporator. The residue was dissolved in 25 ml. of absolute ethanol, and the color developed as described under the assay procedure for tolbutamide (Procedure I) starting at "A 25-ml. aliquot ... was heated under reflux ..." The amount of free tolbutamide was calculated from the absorbance values of standard and sample.

Tolbutamide—n-Butylamine Salt (Part D)—The combined aqueous extracts obtained as described in Part C were acidified with 10 ml. of *N* HCl, and extracted with three 15-ml. portions of chloroform. The combined chloroform extracts were evaporated to dryness by means of a rotary film evaporator, and the residue dissolved in 25 ml. of absolute ethanol. The color was developed as described under the assay procedure for tolbutamide (Procedure I) starting at the words "A 25-ml. aliquot ... was heated under reflux ..." Ten-milliliter aliquots of this refluxed solution which had been diluted to 50 ml. with absolute ethanol were used for the color development instead of 2 ml. The amount of tolbutamide forming a salt with *n*-butylamine was calculated from the absorbance values of standard and sample.

p-Toluenesulfonamide (Part E)—A 25-ml. aliquot of the chloroform filtrate isolated as described in Part A was assayed according to the USP XVII procedure for tolbutamide tablets, and the apparent amount of tolbutamide calculated. The weight of *p*-toluenesulfonamide was calculated by subtracting the weight of tolbutamide per tablet as determined by the colorimetric method (Part A) from the apparent weight of tolbutamide per tablet as determined by the USP assay procedure.

Thin-Layer Chromatography—Silica Gel GF and layer thickness of 250 μ were used throughout. For the separation and detection of *p*-toluenesulfonamide and/or *n*-butylamine in tolbutamide preparations, a solvent system of benzene–acetone–methanol–acetic acid (70:20:5:5) (6) was used. R_f values were 0.60, 0.05, and 0.70, respectively. Examination under short-wave ultraviolet light enabled the detection of the aromatic compounds. Spraying with 0.5% ethanolic ninhydrin solution followed by heating at 80–100° for 10–15 min. caused the formation of orange to pink spots for *n*-butylamine and tolbutamide (limiting sensitivities of 2 and 10 mcg., respectively). *p*-Toluenesulfonamide was visualized with a spray of 3% $KMnO_4$ in H_2SO_4 (limiting sensitivity of 6 mcg.). Alternatively, chlorine treatment followed by benzidine reagent (7) gave blue spots for *p*-toluenesulfonamide (limiting sensitivity of 5 mcg.). Semiquantitative thin-layer chromatographic estimation was accomplished by comparing spot areas of unknown and standard.

RESULTS AND DISCUSSION

The blue color formed during the assay of tolbutamide arises from the interaction of *n*-butylamine and ninhydrin. Although *n*-butylamine is formed readily from tolbutamide in boiling ethanol, the ethanolysis proceeds only partially when no reagent such as ninhydrin is present to remove *n*-butylamine from the equilibrium. Thus, when ethanolic tolbutamide solutions (1 Gm./100 ml.) were refluxed for 1 or 40 hr., the reaction was found to have proceeded to the extent of about 10% only, as indicated by semiquantitative thin-layer chromatography for the amount of released *n*-butylamine. Further evidence that *n*-butylamine was the species interacting with ninhydrin was furnished by isolation of *n*-butylamine as its hydrochloride salt from a refluxed ethanolic solution of tolbutamide, by its absence in the solution after complete color development with ninhydrin (as determined by thin-layer chromatography), and by the fact that similar absorbance curves (Fig. 1) were obtained for the final solution when either tolbutamide or *n*-butylamine was used in the assay procedure. The absorption maximum near 585 mμ was independent of the ninhydrin concentration in the reaction solution while the maximum near 410 mμ was affected by ninhydrin concentration.

Although it was observed that the colorimetric reaction went to completion on heating tolbutamide in absolute ethanol in the presence of ninhydrin (as indicated by the absence of tolbutamide by thin-layer chromatography), the color formed was not sufficiently reproducible for quantitative analysis. Only by refluxing a solution of tolbutamide in ethanol for 90 min. followed by dilution and subsequent reaction of an aliquot with ninhydrin were accuracy and reproducibility achieved.

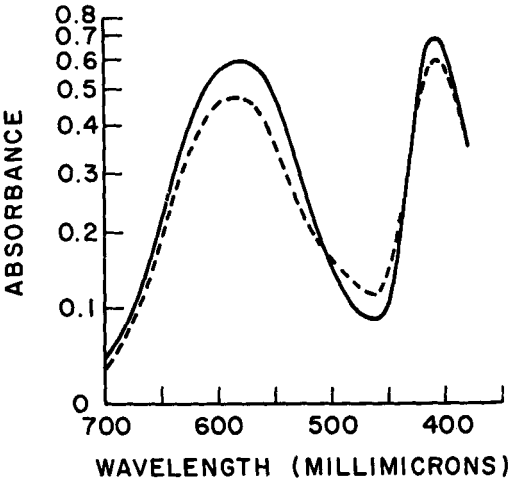


Fig. 1—Absorption spectra of final solutions. Key: ——, tolbutamide and ninhydrin after 4 hr. (concentration equivalent to 7.39×10^{-5} mmoles of tolbutamide per ml.); —, n-butylamine and ninhydrin after 3.5 hr. (concentration equivalent to 5.48×10^{-5} mmoles of n-butylamine per ml.).

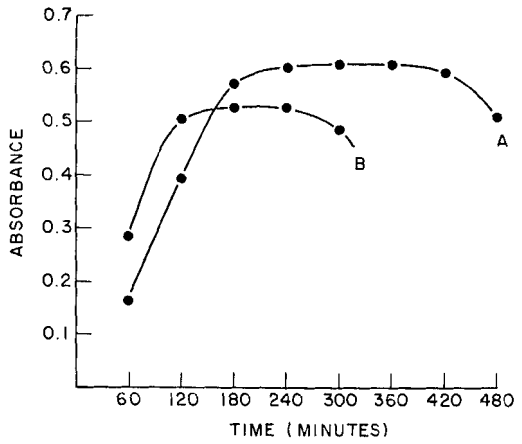


Fig. 2—Effect of time on color formation. Curve A, *tolbutamide concentration equivalent to 7.39 × 10^{-5} mmoles/ml. after dilution to final volume; Curve* B, *n-butylamine concentration equivalent to 6.08 × 10^{-5} mmoles/ml. after dilution to final volume.*

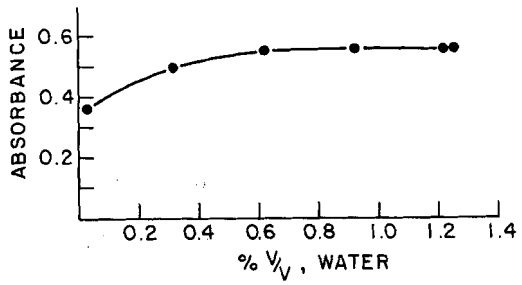


Fig. 3—Effect of water content on color formation after 4 hr. refluxing of the reaction solution (concentration equivalent to 20 mcg. of tolbutamide per ml. after dilution to final volume).

Variables examined to obtain optimum color development and reproducibility were: (*a*) Ninhydrin concentration—Satisfactory color development occurred using a molar ratio of 13.5:1 of ninhydrin to tolbutamide, although molar ratios as high as 22.8:1 did not adversely affect the reaction. On the other hand, a molar ratio of 4.6:1 was insufficient for maximum color development. (*b*) Reaction time—A minimum reflux time of 90 min. for tolbutamide in ethanol prior to color development was found to be an essential requirement to obtain reproducible results. Longer reflux times affected neither color intensity nor rate of color formation. Maximum color development in the ninhydrin reaction occurred within 4–6 hr. for tolbutamide and 3–4 hr. for *n*-butylamine alone (Fig. 2). (*c*) Water content of solvent—Variations in color intensity occurred with the use of "absolute" ethanol. The presence of 0.6 to 1.2% water (v/v) in the final reaction solution resulted in more reproducible results as well as maximum color development (Fig. 3). A higher water content did not adversely affect the amount of color formed, but did cause more nonreproducibility than a water content of 0.6–1.2%. It is essential, however, to use absolute ethanol in the initial reflux stage to achieve reproducible results. (*d*) Temperature of reaction—Reproducible color development occurred only when the reaction solution was maintained under continuous but gentle solvent reflux. This was achieved by immersing the flasks containing the reaction solutions in a stirred paraffin oil bath kept at 96–100°.

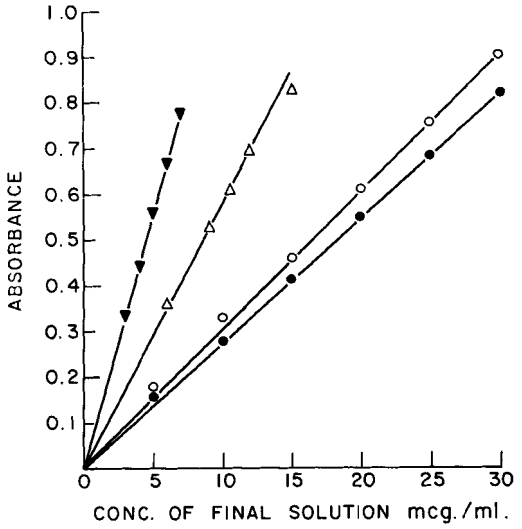


Fig. 4—Relationship between concentration and absorbance. Key: ●, *reaction solution of tolbutamide at 585 mμ;* ○, *at 410 mμ;* △, *reaction solution of* n-*butylamine in absolute ethanol;* ▼, *reaction solution of* n-*butylamine using benzene (5 ml.) with absolute ethanol (28 ml.).*

As shown in Fig. 4, a linear relationship exists between concentration and absorbance for both tolbutamide and *n*-butylamine throughout the region most practical for absorbance measurement. A final reaction solution containing the equivalent of 20 mcg./ml. of tolbutamide or 5.4 mcg./ml. of *n*-butylamine (7.38 × 10^{-5} mmole/ml.) gave an absorbance value of about 0.58. The color intensity of *n*-butylamine in ethanol–benzene was less than that obtained in ethanol alone. In all cases, the color developed was stable for at least 1 hr. with the absorbance decreasing 1–2% after 3 hr.

Results obtained by applying assay Procedure I to six products containing tolbutamide are shown in Table I. Comparison of the experimental data with

Table I—Analysis of Tolbutamide Tablets

Product	Ninhydrin Method: % of Label Claim, Av.	S.D.	No. of Detns.	USP Method: % of Label Claim, Av.	S.D.	No. of Detns.
A	100.9	1.34	10[a]	100.6	1.80	5
B	102.9	0.90	10[a]	103.3	0.70	4
C	103.0	0.13	4	103.5	...	2
D	101.4	0.65	4	101.8	...	2
E	100.4	0.57	4	100.3	...	2
F	99.1	0.32	4	100.9	...	2

[a] Performed on three separate aliquots of 20 tablets (4, 3, and 3 determinations on each aliquot, respectively).

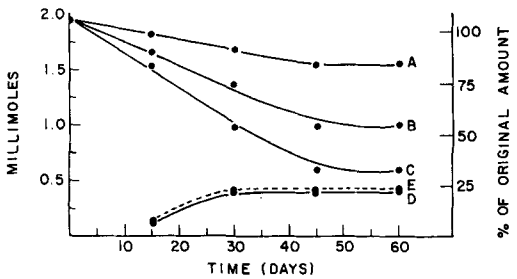


Fig. 5—Decomposition of tolbutamide tablets at 70° and 75% humidity. A, assay values by USP XVII method; B, total tolbutamide; C, free tolbutamide; D, tolbutamide (combined with n-butylamine); E, n-butylamine (combined with tolbutamide).

dilute hydrochloric acid, and subsequent color development of the remaining tolbutamide. The amount of *n*-butylamine in the aqueous phase then could be determined by extraction (after making alkaline with NaOH) with benzene, followed by ninhydrin color development. The *n*-butylamine generated during the hydrolytic decomposition of tolbutamide in the powdered tablets formed a salt with unchanged tolbutamide, and this salt could be readily extracted into water from a chloroform extract. Analysis of these aqueous solutions gave curves D and E of Fig. 5 while the chloroform-soluble tolbutamide, which had not formed a salt with *n*-butylamine, yielded curve C.

Curve A shows the assay values obtained by the USP XVII procedure. Although *p*-toluenesulfonamide was carried through this assay procedure, a

TABLE II—AMOUNT OF *p*-TOLUENESULFONAMIDE IN DECOMPOSED TABLETS

Decomposition Period, Days[a]	Assay Values for Tolbutamide[b] (%) USP Method	Ninhydrin Method	% Error[c] in USP Method	*p*-Toluenesulfonamide[d] (%) Photometric[e]	TLC
0	103.29	102.88	0.39	...	...
15	97.93	90.13	8.65	7.80	8
30	90.94	73.98	22.92	16.96	14
45	84.57	53.04	59.44	31.53	30
60	84.35	53.05	59.0	31.30	30

[a] 70° and 75% relative humidity. [b] Calculated relative to the label claim. [c] % Error = $\frac{\%\ \text{USP} - \%\ \text{Ninhydrin}}{\%\ \text{Ninhydrin}} \times 100$. [d] % by weight relative to the labelled amount of tolbutamide. [e] As determined by the difference between the USP assay values and the ninhydrin procedure assay values.

those obtained by the USP XVII method shows a good correlation between the two, with comparable precision for products A and B.

Although the direct colorimetric procedure (Procedure I) is applicable to dosage forms of tolbutamide containing degradation products such as *p*-toluenesulfonamide or impurities such as *p*-toluenesulfonylurea, it is not applicable to those containing *n*-butylamine. Therefore, for stability studies, or for the analysis of decomposed samples, the procedure was modified to allow for the separation of this interfering amine prior to color development. For decomposed products, the presence of *n*-butylamine may be readily determined by thin-layer chromatography (sensitivity of 2 mcg.) and the appropriate procedure followed.

The specificity of the modified procedure (Procedure II) was demonstrated by its application to tolbutamide tablets which had been subjected to hydrolytic conditions. Weighed aliquots of powdered tablets were stored in individual open vials in a chamber maintained at a temperature of 70° and a relative humidity of about 75% (by means of a saturated sodium chloride solution), and analyzed at various time intervals by assay Procedure II. Results are shown in Fig. 5. As determined by thin-layer chromatography, the decomposition products were *p*-toluenesulfonamide and *n*-butylamine. Dibutylurea, which has been reported as a decomposition product (8), was not detected in the present study.

Since only *n*-butylamine and tolbutamide were found to form colored products with ninhydrin under the reaction conditions, the amount of total unchanged tolbutamide (curve B of Fig. 5) could be readily determined by removal of *n*-butylamine from the chloroform extract of the tablet powder into

small decrease in assay values with length of hydrolytic exposure was observed because this compound's molecular extinction coefficient is lower than that of tolbutamide. Of interest, however, is the fact that both compounds have the same absorptivity value. Therefore, the difference in assay values obtained by the USP procedure and the ninhydrin method is equivalent to the weight of *p*-toluenesulfonamide present in the sample. Since the determination involves a difference between two relatively large numbers, each of which may have significant inherent errors, assay values must be treated with caution if the content of *p*-toluenesulfonamide in a tolbutamide sample is relatively low.

A comparison of the results obtained for tolbutamide by the USP procedure and the ninhydrin method is shown in Table II along with the percent error in results obtained by the USP method. The amount of *p*-toluenesulfonamide, deduced from the difference between these assay values, is also depicted along with the amounts ascertained by thin-layer chromatography as a check on the overall procedure.

Under the conditions described, the ninhydrin method was found to be specific for tolbutamide and did not give a color with many related compounds such as carbamates, urea, barbiturates, amidines, guanidine, and excipients commonly used in tablet formulations.

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Keyphrases

Tolbutamide dosage forms—analysis
Degradation products—tolbutamide
TLC—separation
Colorimetric analysis
Ninhydrin, *n*-butylamine—color formation

Determination of Microgram Quantities of Belladonna Alkaloids in Neomycin-Kaolin-Pectin Suspension

By ALFRED BRACEY and GEORGE SELZER

A method combining liquid–liquid extraction and column chromatography has been developed to determine microgram quantities of belladonna alkaloids in kaolin-pectin suspensions. The alkaloids are first separated from the suspension by several ethanol-ether extractions. Interfering substances, such as methylparaben and propylparaben, are eliminated by extracting the alkaloids with chloroform from a basic solution. The alkaloids are further purified by column chromatography. The chloroform extract is passed through an acid siliceous earth column, which retains the alkaloids. The column is made basic with ammonium hydroxide, and the alkaloids are eluted with chloroform. The isolated alkaloids are nitrated, then reduced to the aromatic amine, and finally diazotized and coupled with the Bratton-Marshall reagent for color formation. Recoveries of atropine sulfate added to blank kaolin-pectin suspensions ranged from 92.9 to 99.4 percent.

THE BELLADONNA ALKALOIDS, atropine sulfate, hyoscyamine sulfate, and scopolamine hydrobromide, are often formulated with kaolin and pectin in aqueous suspensions for treatment of intestinal disorders. These preparations may also contain neomycin. The suspensions combine the adsorbent and detoxifying effects of kaolin (which functions in the stomach and small intestines) and pectin (which functions in the large intestines) with the antispasmodic effect of the belladonna alkaloids to help control hypermotility and hypersecretion in the gastrointestinal tract.

Methods for assaying belladonna alkaloids in multicomponent formulations include Koch, Levine, and Zenker's method (1) for belladonna alkaloids in combination with phenobarbital and Woodson's procedure (2) for determining atropine sulfate and hyoscyamine in the presence of methylene blue. However, no procedure has been reported that involves the assay of microgram quantities of belladonna alkaloids in suspension with strong adsorbents such as kaolin.

This paper presents a method of extracting the alkaloids from kaolin–pectin suspensions (3) and determining their total concentration, using the color development procedure of Koch, Levine, and Zenker (1).

Since atropine sulfate and hyoscyamine sulfate are optical isomers, and are not differentiated by this assay procedure, they are considered analytically equivalent. The third alkaloid component of the preparation is scopolamine hydrobromide, which constitutes only 5% of the total alkaloid dose. This compound has been demonstrated to be equivalent to atropine sulfate in the assay procedure except for a small difference in the colorimetric absorptivity, which is made insignificant by its low concentration. Therefore, the belladonna alkaloids are expressed as the equivalent of atropine sulfate.

ASSAY PROCEDURE

Reagents—The following reagents are used: absolute ethanol; ethyl ether; fuming nitric acid (90%); concentrated hydrochloric acid; ammonium

Received August 4, 1967, from the Division of Antibiotics and Insulin Certification, Bureau of Science, Food and Drug Administration, U. S. Department of Health, Education, and Welfare, Washington, DC 20204
Accepted for publication October 10, 1967.