Assay of Glutethimide Tablets

By EDWARD SMITH

A method is presented for the isolation of glutethimide by partition chromatography and its determination by ultraviolet spectrophotometry. The acid amide, 4-ethyl-4-phenylglutaramic acid, a degradation product of glutethimide, may also be quantitatively isolated and determined. The assay is based on the absorbance of the imide in a chloroform solution at its maximum near 257.5 mμ. The identity of the imide and of the acid amide are confirmed by their ultraviolet characteristics in methanolic potassium hydroxide and by their infrared spectra.

THE N.F. XI assay procedure for glutethimide tablets (1) is a simple extraction of the imide with methanol, followed by measurement of the ultraviolet absorbance of the extract. The method provides neither for the separation of the imide from its degradation product, 4-ethyl-4-phenylglutaramic acid (acid amide), which absorbs strongly at the same wavelength, nor for other methanol soluble components which may be present in the tablets. Assay procedures have been reported which are based on the formation of a colored complex between ferric ion and the hydroxamate resulting from the reaction of the imide with alkaline hydroxylamine (2, 3). This reaction is somewhat specific, in that the imide will form a hydroxamic acid at room temperature while the acid amide will not do so. These procedures and several modifications have been tested in our laboratories and were found unsatisfactory due to the high absorbance readings of the blank, which reduce the precision and accuracy of these methods. Lactose or anions, which complex ferric ions, interfere with these methods (4). A quantitative fluorometric method for glutethimide proposed by Haycock *et al.* (4) does not provide for the separation from the acid amide which interferes.

The quantitative separation of glutethimide from its hydrolytic degradation product by ion exchange resin was recently reported in a study of the stability of the aqueous solutions of glutethimide (5). This procedure required the purification of the reagent grade resin prior to its use and did not provide for the isolation of acid amide present.

In all of the other procedures which have been reported for the determination of glutethimide, the imide is isolated before quantitation. This separation is usually done by extraction with a water immiscible solvent such as chloroform.

Many modifications of the procedure of Goldbaum *et al.* (6) for the determination of glutethimide isolated from biological samples have been published. The original method is based upon measurement of the rate of hydrolysis of glutethimide in alcoholic potassium hydroxide, determined by the ultraviolet absorption at 235 mμ. These procedures, while being suitable for low levels found in biological samples, are not practical for the assay of glutethimide tablets. Szabolcs and Vastagh (7) have determined the imide indirectly by back titrating the excess NaOH after the hydrolysis of the imide. This procedure is subject to error since anything which reacts with the alkali may interfere.

The ultraviolet and infrared absorption characteristics of some therapeutic imides, including glutethimide, have been reported by LaGrange and co-workers (8). They also review the results of the various published methods which they applied to the analysis of these imides. These procedures include the hydroxamic acid method, cobalt amine complex colorimetric procedure, hydrolysis of the imide, and the reaction of the imide with silver ion in nonaqueous media to liberate a proton which is titrated with alcoholic NaOH. The latter was reported by Gautier *et al.* (9).

Goldbaum *et al.* (6) found that glutethimide is

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This procedure has been accepted by the N.F. Committee on Revision for adoption as the official N.F. XII assay method. The chromatographic partition was modified somewhat for simplicity, in that the acid and alkaline segments are combined in a single column. Assay results from the modified method were in agreement with those reported here.

extracted quantitatively by chloroform from aqueous solution at any pH, but above pH 10 the imide tends to decompose. Acidic substances may be removed by washing the $CHCl_3$ with dilute alkali; since glutethimide is an acid too weak to form a salt, it remains in the solvent layer. This property of glutethimide permits the removal of acidic and basic interfering materials from the $CHCl_3$ extracts.

Using partition chromatography, acidic and basic substances can be separated conveniently by trapping ionically in the immobile phases of opposite basicity. Examples of the use of this technique are the N.F. XI determination of aspirin, phenacetin, and caffeine tablets (10) and a variety of pharmaceutical analyses which have been reviewed by Levine (11). With proper immobile phases to retain impurities, the pure imide can be isolated; the acid amide degradation product may also be isolated quantitatively.

METHODS

Assay.—*Preparation of Chromatographic Columns.*—Pack a pledget of fine glass wool in the base of a chromatographic tube (25 × 200-mm. test tube to which is fused a 5-cm. length of 6 or 8-mm. tubing) with the aid of a tamping rod of stainless steel, aluminum, or glass at least 12 in. long and having a disk with a diameter about 1 mm. less than that of the chromatographic tube. To 2.0 Gm. of Celite 545 or a comparable grade of infusorial earth in a 100-ml. beaker, add 2.0 ml. of approximately 4 *N* sulfuric acid and incorporate by kneading thoroughly with a flexible spatula blade until the mixture is fluffy. Transfer to a chromatographic tube, and tamp gently to compress the material to a uniform mass. In like manner, mix intimately 2.0 Gm. of Celite 545 with 2.0 ml. of approximately 1 *N* sodium bicarbonate, transfer to a second chromatographic tube, and gently compress with the tamping rod. Mount the columns so that the effluent from the acidic column flows into the bicarbonate column. Wash the columns with 50 ml. of water-saturated ether, followed by 25 ml. of water-saturated chloroform.

Procedure.—Weigh and finely powder not less than 20 glutethimide tablets. Transfer to a 100-ml. volumetric flask an accurately weighed portion of the powder equivalent to about 250 mg. of glutethimide, dilute to volume with water-saturated chloroform, and mix. Transfer 10 ml. of this solution to the top column and elute with 90 ml. of water-saturated chloroform in two equal portions, collecting the eluate in a 100-ml. volumetric flask. Dilute to volume with water-saturated chloroform and mix. Simultaneously determine the absorbance of this solution and of a solution of N.F. glutethimide reference standard in the same medium at a concentration of about 250 mcg./ml., in 1-cm. quartz cells at the maximum at about 257 mμ with a suitable spectrophotometer, using water-saturated chloroform as the blank.

TABLE I.—RESULTS OF THE ASSAY OF GLUTETHIMIDE TABLETS

Sample	No. Determin.	Av. % Recovery	S.D.	Range
Simulated A	12	100.3	0.66	99.4 to 101.4
Simulated B	12	99.6	1.27	98.3 to 102.1
		Av. % Claim		
Commercial[a]	10	100.3	0.97	98.2 to 101.6
Commercial	10	100.3	0.56	99.3 to 101.3

[a] The chloroform eluate was evaporated, then the residue was dissolved in methanol; all other determinations were made on the chloroform eluate directly.

TABLE II.—ASSAY OF SIMULATED TABLET MIXTURES BY FIVE ANALYSTS

Analyst	% Recovery Sample A	Sample B
I	101.1	99.2
	101.5	99.4
II	100.4	100.8
	101.4	101.2
III	99.8	100.4
	100.6	100.6
IV	99.4	99.3
	100.1	99.7
	100.0	99.0
	100.6	100.6
V	101.0	100.4
	101.5	100.2
	100.6	99.6
	100.3	...

Calculate the quantity, in milligrams, of $C_{13}H_{15}NO_2$ in the portion of the tablets taken by the formula $C(A_u/A_s)$ in which C is the exact concentration of the reference standard solution, in micrograms per milliliter; A_u is the absorbance of the sample solution, and A_s is the absorbance of the reference standard solution.

Identification.—Evaporate to dryness a portion of the eluate obtained in the *Assay*. The infrared absorption spectrum of a potassium bromide dispersion of the residue exhibits absorption maxima at the same wavelengths as those of a similar preparation of N.F. glutethimide reference standard.

DISCUSSION

The accuracy and precision obtained in the recovery of glutethimide are shown in Tables I and II. When known amounts of the acid amide alone and in mixtures of equal and double quantities of glutethimide were chromatographed, recovery of each present was quantitative. The acid amide was recovered from the sodium bicarbonate column by eluting it with 1% acetic acid in water-saturated chloroform. The amount present was determined by ultraviolet absorbance.

To obtain the complete spectrum of glutethimide, the chloroform eluate was evaporated and the residue dissolved in anhydrous methanol. The same quantitative results were obtained as those by the direct reading of the chloroform eluate. The latter procedure eliminated the extra steps and also the danger of decomposing the residue while evaporating the chloroform.

The ultraviolet spectra of glutethimide and of 4-ethyl-4-phenylglutaramic acid in methanol are quite similar with the principal maximum due to the

benzenoid chromophore, appearing at 257 mμ. The spectrum of the acid amide (Fig. 1) is defined more sharply with more pronounced definition of both maxima and minima.

The ultraviolet spectra of the two compounds in alkaline methanol are quite different, thus providing ready means of identifying and differentiating them. The following procedure was used to prove the identity of the separated material. About 1.25 mg. of the dried residue obtained by evaporating the eluate was transferred to a 25-ml. volumetric flask with the aid of 15 ml. of methanol. Two milliliters of 0.5 *N* methanolic KOH was added, and the solution was adjusted to volume with methanol. The ultraviolet spectrum was scanned from 220 to 350 mμ, using methanol as the blank. In alkaline methanol the ultraviolet spectrum of the acid amide is essentially the same as in neutral methanol. With the imide there is a shift in the benzenoid chromophore from sharp peaks to shoulders in the 257 and 263 region and the complete disappearance

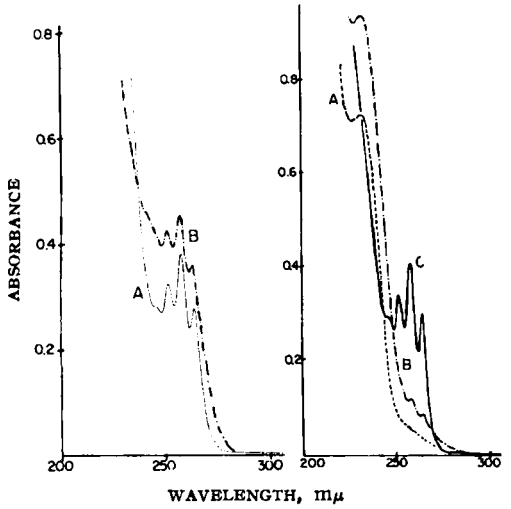

Fig. 1 (left).—Ultraviolet spectra of methanol. Key: *A*, 4-ethyl-4-phenylglutaramic acid, 50 mg./100 ml.; *B*, glutethimide, 25 mg./100 ml. Fig. 2 (right).—Ultraviolet spectra in methanolic potassium hydroxide. Key: *A*, glutarimide, 20 mg./100 ml., N/25 methanolic KOH; *B*, glutethimide, 5 mg./100 ml., N/25 methanolic KOH; *C*, 4-ethyl-4-phenylglutaramic acid, 50 mg./100 ml., N/10 methanolic KOH.

of the maximum at 253 mμ (Fig. 2). For glutethimide, the chromophore most enhanced in alkaline methanol is in the 230 to 235-mμ region. To investigate the assignment of that absorbance, unsubstituted glutarimide was prepared (12) and its spectral properties examined under the same conditions as glutethimide. In alkaline methanol a sharp peak appears in the 230 to 235-mμ region, which changes to a shoulder as the glutarimide is hydrolyzed. β-Alkyl substituted glutarimides have also been shown to exhibit this same spectral property (13, 14). Therefore, this absorbance in the 230 to 235-mμ region is due to the intact glutarimide itself and not due to a shift of the benzenoid chromophore. The absorbance of the glutethimide decreases with time as it is hydrolyzed to the acid

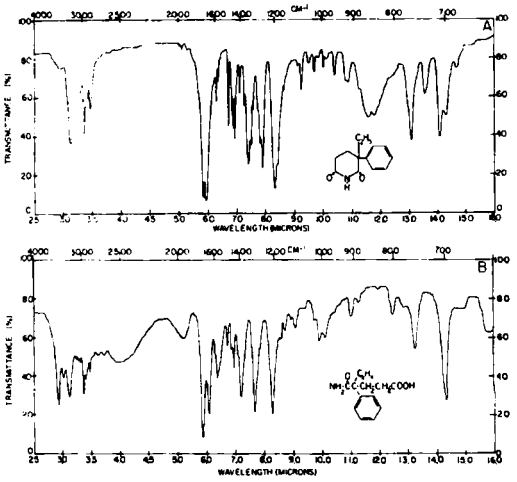

Fig. 3.—Infrared spectra. Key: *A*, glutethimide; *B*, 4-ethyl-4-phenylglutaramic acid.

amide, while the spectrum of the acid amide itself in alkaline methanol remains unchanged for at least 24 hours.

The use of acid washed Celite 545 has often caused problems due to background absorbance of impurities present. The prewashing of the Celite columns removed essentially all the background absorbance. If uncorrected, this background absorbance would cause an error of about 2%.

In the identification test, the infrared spectrum of the residue from evaporation of the eluate in a potassium bromide dispersion was compared to that of a similar preparation of N.F. glutethimide reference standard and was identical.

A similar procedure was used for the identification of any 4-ethyl-4-phenylglutaramic acid which was isolated from the eluate of the bicarbonate column. Its infrared spectrum was compared to that obtained with the acid amide prepared from the hydrolysis of glutethimide reference standard as directed in the N.F. XI identity test B for glutethimide. The infrared spectra are characteristic and suitable for the identification of both glutethimide and the acid amide as illustrated in Fig. 3. The spectra illustrated were obtained with a Perkin-Elmer model 237 spectrophotometer.

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