# Determination of Glutethimide, Aminoglutethimide, and Bemegride by Nonaqueous Titration

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**A simple nonaqueous titration procedure is presented for the determination of glutethimide, aminoglutethimide, and bemegride and their dosage forms. The solvent is dimethylformamide, and the titrant is sodium methoxide in benzene–methanol. By the proposed procedure it is possible to titrate differentially glutethimide and its degradation product, 4-ethyl-4-phenylglutaramic acid. Dosage forms are analyzed without preliminary extraction of the active constituent.**

GLUTETHIMIDE (2-ethyl-2-phenylglutarimide), aminoglutethimide [2-(*p*-aminophenyl)-2-ethylglutarimide], and bemegride (3-ethyl-3-methylglutarimide) are useful pharmacological agents having in common a substituted glutarimide structure. They are poorly soluble in water and possess the weakly acidic imide grouping. Glutethimide is a widely used sedative and hypnotic. Aminoglutethimide is an anticonvulsant, while bemegride is a central nervous system stimulant and of value in the treatment of barbiturate coma.

Glutethimide is official in N.F. XII (1) as the pure compound and as a tablet dosage form. The assay involves ultraviolet spectrophotometry, and in the case of the tablets, partition chromatography is employed to remove 4-ethyl-4-phenylglutaramic acid, the degradation product of glutethimide which absorbs at the same wavelength. This procedure is based on the method reported by Smith (2). In his paper Smith reviews critically the literature on the assay of glutethimide. Glutethimide is official in the B.P. (3) as the pure compound and as the tablet. The assay involves hydrolysis of the compound with standard alcoholic KOH.

Bemegride is official in U.S.P. XVII (4) and the B.P. (3) as the pure compound and as an injection. The pure compound is assayed by the Kjeldahl nitrogen method according to the U.S.P. and B.P. The injection, according to the U.S.P., is assayed spectrophotometrically, whereas the B.P. employs the Kjeldahl determination.

Aminoglutethimide, while not official, is commercially available as a tablet. A commercial producer of the tablet assays the product for quality control by forming the hydroxamic acid derivative with alkaline hydroxylamine followed by complexation with ferric ion to produce a purple coloration. The absorptivity is measured at 530 mμ. It is based on a procedure proposed by Sheppard *et al.* (5).

The application of nonaqueous titrimetry to the determination of these compounds has been limited. Haycock *et al.* (6) refer to a visual nonaqueous titration procedure for glutethimide in which pyridine or ethylenediamine is the solvent and sodium methoxide is the titrant. A similar method was described by Ellert *et al.* (7). Ethylenediamine as the solvent yielded satisfactory results, but pyridine and dimethylformamide were found to be unsuitable. Dosage forms were not analyzed.

In the present paper a simple nonaqueous titration procedure is described which is applicable to the three compounds and their dosage forms. Titration may be effected visually or potentiometrically using a Fisher titrimeter equipped with a calomel and platinum electrode system. The solvent is dimethylformamide, and the titrant sodium methoxide in benzene–methanol. By the proposed procedure it is possible to differentially titrate glutethimide and its degradation product, 4-ethyl-4-phenylglutaramic acid. Dosage forms are analyzed without preliminary extraction of the active constituent.

## EXPERIMENTAL

**General Assay Procedure.**—About 200 mg. of reference standard glutethimide, accurately weighed, was dissolved in 40 ml. of dimethylformamide contained in a 150-ml. beaker. The solution, magnetically stirred, was titrated potentiometrically with 0.1 *N* sodium methoxide in benzene–methanol, prepared and standardized as described earlier (8). Potentiometric measurements were made with a Fisher titrimeter, model 35, equipped with a sleeve-type calomel and platinum electrode system. The end point in the titration was determined from the inflection in the curve obtained by plotting volume of titrant (ml.) *versus* millivolt (mv.) readings. Titration was also effected visually by adding 3 drops of azo violet indicator solution (saturated solution in benzene) to the titration beaker. The proper indicator color change was noted by using indicator in conjunction with a potentiometric titration. The color change corresponding to the graphic end point was from a purple to a blue and in most cases was

Received July 21, 1965, from the Department of Pharmacy, University of Illinois at the Medical Center, Chicago.
Accepted for publication September 7, 1965.
The authors thank Ciba Pharmaceutical Co. and Abbott Laboratories for supplying the reference standards and drug dosage forms used in this investigation.

readily detectable with the addition of 1 drop of titrant at the end point.

**Analysis of Glutethimide Tablets.**—The general assay procedure described above was applied to commercially available glutethimide tablets. Twenty tablets were weighed and reduced to a fine powder. An accurately weighed portion of the powder mass equivalent to 1 meq. of glutethimide was added to a 150-ml. beaker containing 40 ml. of dimethylformamide. The mixture was stirred magnetically for several minutes and then titrated potentiometrically with 0.1 *N* sodium methoxide in benzene–methanol.

**Analysis of Aminoglutethimide and Bemegride Dosage Forms.**—Aminoglutethimide reference standard and bemegride reference standard were analyzed as described for glutethimide. Commercially available aminoglutethimide tablets were assayed in the same manner as glutethimide tablets. A commercially available bemegride injection containing in each ml. 5 mg. of bemegride, 9 mg. of benzyl alcohol as a preservative, and sodium chloride for isotonicity was analyzed by pipetting exactly 20 ml. of the injection into a 150-ml. beaker. The solution was evaporated to dryness by heating on a Glas-Col mantle at a temperature of 70–80°. This required about 2 hr. The residue was dissolved in 40 ml. of dimethylformamide, and the solution, magnetically stirred, was titrated with 0.1 *N* sodium methoxide in benzene–methanol.

**Analysis of Degraded Glutethimide.**—Since it has been demonstrated that the hydrolytic degradation product of glutethimide is 4-ethyl-4-phenylglutaramic acid (9), a differentiating titration procedure was developed for the determination of glutethimide in the presence of its degradation product.

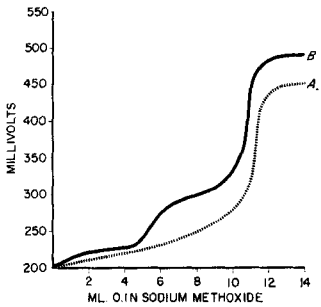


Fig. 1.—Key: *A*, titration curve for glutethimide, reference standard or tablet dosage form; *B*, titration curve for glutethimide and 4-ethyl-4-phenylglutaramic acid mixture.

4-Ethyl-4-phenylglutaramic acid was prepared according to the method described by Wesolowski (10). Synthetic mixtures of glutethimide and 4-ethyl-4-phenylglutaramic acid were prepared and were titrated potentiometrically as described for pure glutethimide. The titration curve exhibited two inflections; the first for 4-ethyl-4-phenylglutaramic acid and the second for glutethimide.

For comparative purposes, a number of the dosage forms were assayed by the official procedure or by the method utilized by the manufacturer of the commercial product.

## RESULTS AND DISCUSSION

The weakly acidic nature and the poor water solubility of substituted glutarimides make nonaqueous titrimetry an ideal procedure for analyzing glutethimide and related drugs. Titration is readily effected in dimethylformamide using sodium methoxide in benzene–methanol as solvent. In most instances visual titration is possible with azo violet as the indicator. For potentiometric measurements a calomel and platinum electrode system was found most suitable. The proposed assay procedure is applicable to dosage forms and does not require preliminary extraction of the active constituent.

A typical titration curve for glutethimide in an aliquot of powdered tablets is shown in Fig. 1, curve *A*. Assay results for the determination of glutethimide content in the powder and tablet dosage forms are indicated in Table I. In the case of the 500-mg. tablet, comparison was made with the N.F. XI assay procedure (11) which is an ultraviolet spectrophotometric method. Smith (2) criticized the N.F. XI assay procedure for the tablets because provision was not made for the separation of the degradation product, 4-ethyl-4-phenylglutaramic acid, which absorbs strongly at the same wavelength as glutethimide. Smith recommended a partition chromatographic procedure for the removal of acidic impurities from the sample taken for analysis. Celite 545 or purified siliceous earth which was treated with sodium bicarbonate was used for trapping the 4-ethyl-4-phenylglutaramic acid. Intact glutethimide appeared in the eluate.

Wesolowski (10), in studying the kinetics of the degradation of glutethimide, devised an ion exchange method for the separation of 4-ethyl-4-phenylglutaramic acid from glutethimide. The strongly basic anion exchange resin, Dowex 2-4X, was employed to remove the degradation product. The glutethimide being weakly acidic did not exchange with the resin.

Table I.—Analysis of Glutethimide, Aminoglutethimide, and Bemegride in Dosage Forms

| Compd. | Dosage Form | Labeled Amt.,[a] mg. | Recovery, % | Assay Method |
|---|---|---|---|---|
| Glutethimide | Powder | ... | 101.89 ± 0.73[b] | Proposed |
| | Tablets | 250 | 97.00 ± 0.00 | Proposed |
| | | 500 | 101.10 ± 0.99 | Proposed |
| | | 500 | 100.32 ± 1.14 | N.F. XI |
| Aminoglutethimide | Powder | ... | 98.84 ± 1.11 | Proposed |
| | Tablets | 250 | 98.71 ± 0.50 | Proposed |
| | | 250 | 98.65 ± 1.28 | Hydroxamic acid[c] |
| Bemegride | Powder | ... | 98.59 ± 1.07 | Proposed |
| | Injection | 50 | 97.29 ± 0.35 | Proposed |
| | | 50 | 97.75 ± 0.07 | U.S.P. XVII |

[a] Per unit dosage. [b] Standard deviation based on at least 4 determinations. [c] See *Reference 5*.

TABLE II.—ANALYSIS OF MIXTURES OF GLUTETHIMIDE AND 4-ETHYL-4-PHENYLGLUTARAMIC ACID BY DIFFERENTIAL TITRATION

| Glutethimide | | 4-Ethyl-4-phenylglutaramic Acid | |
|---|---|---|---|
| Weighed Amt., mg. | Recovery, % | Weighed Amt., mg. | Recovery, % |
| 147.5 | 99.72 | 141.5 | 103.67 |
| 103.4 | 102.80 | 149.3 | 98.19 |
| 147.4 | 100.75 | 130.6 | 101.45 |
| 163.7 | 99.42 | 45.75 | 101.68 |
| 170.0 | 100.65 | 52.15 | 97.34 |
| 160.2 | 100.75 | 52.55 | 99.26 |

The proposed assay permits the differential titration of glutethimide and its breakdown product. Synthetic mixtures of glutethimide and 4-ethyl-4-phenylglutaramic acid in varying ratios of the two components were analyzed by the proposed method. A typical differential titration curve is shown in Fig. 1, curve *B*. The data for the analysis of the synthetic mixtures are recorded in Table II. The significant difference in pKa values (glutethimide, 9.2; 4-ethyl-4-phenylglutaramic acid, 4.6) accounts for the two inflections in the titration curve. The data in Table II indicate that excellent resolution of the mixtures was achieved.

The proposed nonaqueous titration was applied to aminoglutethimide powder and tablets and bemegride powder and injection. The data are shown in Table I.

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(11) "The National Formulary," 11th ed., J. B. Lippincott Co., Philadelphia, Pa., 1960, p. 153.

# *Notes*

## Solubility of Pentaerythritol Tetranitrate-1,2-$^{14}$C in Water and Saline

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**The solubility of clinically useful pentaerythritol tetranitrate has been determined in water and saline utilizing the radioactive labeled compound. The solubility agrees with one of the two previously reported values. The synthetic work has been included.**

Two vastly differing values for the solubility of pentaerythritol tetranitrate (PETN) have been reported. Desvergnes (1) found the solubility to be 100 mcg./ml., while Leslie (2) reported a value of 1.5 mcg./ml. When PETN labeled with $^{14}$C was prepared for an extensive biological evaluation, it became possible to re-evaluate this solubility problem and resolve this discrepancy. The older methods for the determination of the solubility of compounds having a limited solubility suffer from the inherent error of weighing a small amount of solid, or in this case, of an analytical method designed to detect inorganic nitrate ions and not intact PETN. If hydrolysis of the PETN had occurred, the results would be high due to the ready solubility of the nitrate ion.

### DISCUSSION

Essentially, the method was to equilibrate 5 mg. of PETN-1,2-$^{14}$C with 5 or 15 ml. of water or saline, filter to remove colloidal material,[1] then count[2] an aliquot in a scintillation solution composed of 0.7% PPO, 0.03% dimethyl POPOP, and 100 Gm. of naphthalene adjusted to 1 L. with freshly distilled 1,4-dioxane. The counting efficiency was 69%, and each sample was corrected for quenching by the addition of benzoic acid-$^{14}$C as an internal standard.

It was found necessary to use a 0.22-$\mu$ filter (Millipore)[3] to remove the colloidal material completely. However, successive passes through similar filters removed material from solution. At these low concentrations, this phenomena is not unexpected (3–5). These results are shown in Table I.

Received July 12, 1965, from the Department of Chemical Research, Warner-Lambert Research Institute, Morris Plains, N. J.
Accepted for publication August 4, 1965.
The author thanks Mrs. N. Eltvedt for technical assistance and the staff of the Research Center, Hercules Powder Co.

[1] This was brought to the author's attention by Mr. M. Geller, Applied Analytical Research Department, Warner-Lambert Research Institute, Morris Plains, N. J.
[2] Tri-Carb scintillation spectrometer, model 314 X, Packard Instrument Co.
[3] Millipore Filter Corp., Bedford, Mass.