Simultaneous Spectrophotometric Determination of Glutethimide and Barbiturates

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A simple, rapid, quantitative method for the simultaneous analysis of barbiturates and glutethimide (2-ethyl-2-phenylglutarimide) is described. The procedure involves a single chloroform extraction from a sample buffered at pH 7.4 and requires less than an hour.

The possibility of drug intoxication frequently arises in patients admitted to the hospital in coma. Ingestion of salicylates by children and of barbiturates by adults are the most common causes. Since the introduction of glutethimide (2-ethyl-2-phenylglutarimide) in 1954, there have been increasing reports implicating this drug in drug intoxication. Since the clinical picture of glutethimide intoxication is indistinguishable from that of barbiturates, diagnosis must be confirmed by identifying the drug in blood samples. Well-established ultraviolet spectrophotometric methods are available for identification of these substances (1, 2). Each of these procedures requires about 2 h for completion. Because the laboratory is often asked to look for both barbiturates and glutethimide, it seemed desirable to combine the analytical procedures.

Measurement of glutethimide is based on its characteristic absorption peak at 233 nm in a strongly alkaline solution and its progressive timed hydrolysis. The change in optical density in a given time interval is proportional to its concentration. The method for barbiturates is based upon the observation that these substances have the absorption spectrum of one resonance form at pH 13 and the absorption spectrum of a different resonance form at pH 9.5. The difference in optical density of the solution at pH 13 at 240 and at 260 nm, when the solution at pH 9.5 is used to set the instrument, is directly proportional to the concentration.

Materials and Methods

Reagents

Chloroform. Wash reagent-grade or "spectral grade" chloroform successively with two washes of 1N NaOH and two washes of distilled water to remove impurities. For each liter of chloroform use 100 ml of wash solution.

Phosphate buffer, 0.1M pH 7.40. Dissolve 5.96 g of anhydrous Na2HPO4 and 1.09 g of KH2PO4 in 500 ml of distilled water.

NaOH, 3N and 1N.

NH4Cl. Dissolve 16 g in water and dilute to 100 ml.

Phenobarbital reference solution. Sixty milligrams of phenobarbital (Merck) and 10 ml of 0.5N NaOH are diluted to 100 ml with distilled water. This standard is divided into 2-ml aliquots and stored at −20°C.

Glutethimide reference solution. Dissolve 30 mg of crystalline glutethimide (Ciba) in 100 ml of 95% ethanol. Store at 4°C. The solution is stable for at least one month.

Equipment. The equipment includes: evaporator (Buchi Rotavapor, Fisher, 9-548-150V2), 100-ml evaporating flasks with ground-glass joint (Fisher no. 10-067), standard silica cuvets (Beckman no. 75184), and the Beckman Model DK-2A ratio recording spectrophotometer.

Settings of the instrument are as follows: hydrogen lamp with temperature filament, setting no. 2; photomultiplier tube; resistor, xl; time constant, 0.2; sensitivity, 60; scanning time, 1 (180 nm/min); scale expansion, xl (10 nm/cm); initial wavelength, 320 nm; operator selector, absorbance; absorbance range, −0.3 to +0.7.

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Procedure

Into an appropriately labeled 125-ml separatory funnel (with Teflon stopcock), add 3 ml of serum or plasma to 3 ml of phosphate buffer. Into another separatory funnel, labeled “reference”, add 5.5 ml of phosphate buffer, 0.2 ml of phenobarbital reference solution, and 0.3 ml of glutethimide reference solution. These are equivalent to 4 mg of phenobarbital per 100 ml and 3 mg of glutethimide per 100 ml. To each separatory funnel add 50 ml of chloroform, stopper, and shake vigorously for 1 min. After the layers have separated, filter the (lower) chloroform layer through Whatman no. 42 filter paper, collecting exactly 40 ml of the filtrate in a graduated cylinder. Transfer the chloroform extract to a 100-ml round-bottomed flask and evaporate to dryness on the evaporator, immersing the lower half of the round-bottomed flask in a water bath set at 70°C. This step requires approximately 5 min. Dissolve the residue in 7 ml of water. Solution is facilitated by warming the flask in the water bath and by frequent and vigorous swirling for at least 2 min. A yellow ring of insoluble serum lipids forms at the bottom of the flask. Allow the flask to cool to room temperature. Add 3 ml of dissolved residue to a cuvet containing 0.2 ml of the NH₄Cl solution and 0.4 ml of 1N NaOH and mix. To a duplicate cuvet containing 0.6 ml of 3N NaOH add another 3 ml of dissolved residue and immediately start a timer and mix. This time denotes the onset of hydrolysis of glutethimide, which begins with the addition of NaOH and should, therefore, be uniform for all samples. Place the cuvet containing NH₄Cl and NaOH on the “reference” side and the cuvet containing NaOH on the “sample” side of the spectrophotometer and scan at exactly 1 min from 320 to 220 nm. Scan again at exactly 5 min. If the curve indicates that both barbiturate and glutethimide are present, scan again at 15 min.

The analysis can be performed on 1 ml of serum by taking up the residue in 3 ml of water, distributing 1 ml into each cuvet having a 1.5-ml capacity, and reducing correspondingly the volumes of NaOH and NH₄Cl.

Fig. 1. Differential (pH 13 vs. pH 9.5) absorption spectrum of a 3 mg/100 ml phenobarbital standard

Fig. 2. Differential (pH 13 vs. pH 9.5) absorption spectrum of a 3 mg/100 ml thiopental standard
illustrations

0.00 0.10 0.20 0.30 0.40 0.50 0.60 0.70 0.80 0.90 1.00

mg / 100 ml

Fig. 3. Relationship between absorbance and concentration of several common barbiturates

Results and Discussion

The absorption curve of solutions containing barbiturates (Figure 1) is characteristically biphasic without significant change during the first 15 min of the time period. The curve shifts very slightly downward by the end of 20 min, with the change at 240 nm being somewhat greater than at 260 nm. Thiobarbiturates, such as thiopental, have a different curve (Figure 2), which permits their easy identification. Concentration is directly proportional to the absorbance difference between 240 and 260 nm (253 and 283 nm for thiopental) and is linear up to 20 mg/100 ml. As shown in Figure 3, the absorbance per unit weight of different barbiturates is slightly different. However, for clinical purposes, these differences are not significant, and the unknown values are based on a phenobarbital standard.

Solutions containing glutethimide produce a characteristic absorption peak at 233 nm with progressive decrease of absorbance over 20 min (Figure 4). The concentration is proportional to the change in optical density between any two periods of time. The increment between the 1- and 5-min readings has been chosen for routine calculations because of the large absorbance change per time interval. Other carefully measured time intervals may be chosen if the same interval is used for both standard and unknown. The linear relationship between absorbance and concentration is shown in Figure 5.

When both barbiturates and glutethimide are present, a pattern such as the one in Figure 6 is obtained. Glutethimide is calculated as noted above and barbiturates are calculated from the curve obtained at 20 min.

When specimens are obtained from patients in whom there is a possibility of drug intoxication, it has been our policy to analyze the unknown specimen first. With experience and a good evaporator the curves can be obtained in less than 1 h, permitting one to state whether barbiturates and (or) glutethimide are present in the sample. Based on past experience, one can also indicate the approximate concentration. This information, available in a short period of time, is extremely useful to the attending physician in making a judgment on the nature of his patient's illness as well as the therapeutic regimen to be followed. If one or both of these drugs are present, appropriate standards are run through the procedure and the exact concentrations calculated.

Fig. 4. Differential (pH 13 vs. pH 9.5) absorption spectrum of a 3 mg/100 ml glutethimide standard during 20 min
Buffering the sample at pH 7.4 decreases the solubility of salicylates in chloroform so that concentrations up to 60 mg/100 ml will not interfere with the analysis. Salicylates can be extracted into the chloroform phase by substituting an acetate buffer at pH 5.5. Extration at this pH is satisfactory for forensic screening since barbiturates, glutethimide, and salicylates can be excluded with a single procedure. Unfortunately, salicylates tend to obscure low concentrations of barbiturates, but no interference with glutethimide analysis is noted at this pH. When one analyzes substances such as urine or gastric contents, the pH of the substance must be adjusted to approximately 7.4. If the pH is too alkaline, barbiturates will remain in the aqueous phase.

Turbidity from lipemic sera has been encountered in a method of glutethimide analysis (3) utilizing dichloromethane and subsequent dissolution of the dried extract with ethanol. In none of the sera from the patients examined have we noted turbidity in the final aqueous extracts by our method. Addition of known concentrations of glutethimide and phenobarbital to highly lipemic sera resulted in absorbance curves that were identical to those for the corresponding aqueous standards.

To eliminate glutethimide metabolites, it has been recommended that glutethimide extraction be performed with petroleum ether or that the chloroform extract be washed with NaOH (4). Recognition of the contribution of glutethimide metabolites to the total glutethimide concentration is important, particularly in following the progress of patients with known intoxication.

Since the parent compound accounts for 90% of the total concentration during the first 16 h after ingestion, the unwashed chloroform extract is adequate for initial identification. As a screening procedure for rapid initial recognition and quantitation we have found chloroform to be more satisfactory to use than petroleum ether, primarily because of its lower volatility and specific gravity greater than water.

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