A rapid simple gas liquid chromatographic technic has been developed for the identification and quantitation of glutethimide in biologic fluids. The method requires no evaporation of extracting fluid and will estimate glutethimide levels in the range of 1 mg./100 ml. or higher.

With the enormous development in numbers and types of ataraxic drugs and nonbarbiturate sedatives, the clinical laboratory has been literally bombarded with requests for special toxicologic analyses. One of the most popular of the nonbarbiturate sedatives is glutethimide (Doriden, Ciba) or \(\alpha\)-ethyl-\(\alpha\)-phenylglutarimide. This agent has sedative-hypnotic properties and the usual dose is 250–500 mg. taken at bedtime. McBay and Algieri have reported death occurring after the ingestion of 10 gm. (1).

Glutethimide is slowly metabolized and, thus, elevated blood concentrations can be lowered by hemodialysis. It is, therefore, important from a clinical standpoint to be able to identify and quantitate this drug in the blood. In 1956, Sheppard described a colorimetric method dependent upon the formation from glutethimide of the iron salt of hydroxamic acid (2). We have used this technic for qualitative identification of glutethimide. It cannot be used to assay toxic drug levels, however, because of the low sensitivity of the reaction. In 1960, Goldbaum et al. (3) described a spectrophotometric procedure for the determination of glutethimide in body fluids. These investigators measured the decrease in ultraviolet absorbance of glutethimide in alkaline solution at 235 m\(\mu\). There have been several modifications of this technic including a recent paper of Dauphinais and McComb (4). The major objections to the spectrophotometric procedures are (1) the relatively long period necessary for the completion of the assay and (2) the many
manipulations required by the method. In 1963, Kazyak and Knoblock described the application of gas chromatography to analytic toxicology (5). Glutethimide was one of many compounds that was investigated. They obtained their best results on a 1% SE 30 column with column temperature of 165° using an argon beta detector. The aqueous fluids were extracted with chloroform and evaporated to dryness. After reconstitution, the residue was injected into the gas chromatograph. Recently, Korzun et al. described another gas chromatographic technic for the estimation of glutethimide using methylene chloride as the extracting fluid (6). The separation was accomplished with borosilicate columns packed with 2% carbowax on the Gas Chrom Q apparatus. Like the method of Kazyak and Knoblock, this method involves evaporation and reconstitution.

We have developed a simple 1-step microprocedure which permits rapid determination of glutethimide without evaporation and reconstitution of the organic solvent.

Materials and Method

Apparatus and Reagents

Instrument

The gas chromatograph instrument used for this study was obtained from the Instrument Division of the Warner-Chilcott Laboratories (Model 1660). It consists of an oven, a proportioning temperature controller, a recorder with electrometer amplifier and a multiple flow meter. The oven is equipped with a hydrogen-flame ionization detector and has fittings for 2 GLC columns. Routinely, separation of toxicologic agents is done on a 3 ft. × 1/8 in. capillary stainless steel column packed with 5% DC-200 on acid-washed Chromosorb W, 60–80 mesh. The second column, a 6 ft. × 1/8 in. capillary glass column packed with 3% SE 30, on acid-washed Chromosorb W, DMCS, 60–80 mesh was used to compare the separating properties of SE 30 and DC-200.

The temperature in the column and in the detector cell is 190°; in the injection port, it is 400°. The carrier gas is nitrogen with a 40 ml/min. flow rate. The attenuation of the electrometer is 1/100 of maximum sensitivity.

Reagents

Chloroform, reagent grade

Standards Pure glutethimide powder obtained from Ciba Corporation was prepared as a stock standard of 50 mg./100 ml. of water (0.5 μg./μl.). This standard was stable for 2 weeks in the refrigerator. Working standards were prepared fresh daily from the stock to have 1, 2, and 5 mg. Doriden/100 ml. These working standards were not
stable. Sodium salts and acids of various barbiturates were also prepared in a similar fashion.

**Procedure**

1. Place 1.0 ml. of serum and 1.0 ml. of each of the working standards in glass-stoppered 3.0-ml. narrow tapered centrifuge tubes.
2. Add 0.4 ml. of chloroform to each tube. Insert the stopper and mix the tubes for 15 sec. on a Vortex mixer.
3. Centrifuge the tubes at 2500 rpm for 5 min. Two layers separated by a protein button are usually found in the serum sample; the lower layer is chloroform and contains the glutethimide.
4. Insert a 10-μl syringe (either Hamilton or York) into the organic lower layer and withdraw 2 μl. of solvent; inject this into the inlet port of the column.
5. The amount of glutethimide in the serum sample can be determined in 2 ways. A plot of the peak-area curve (obtained by multiplying the peak height in millimeters by the width in millimeters at 1/2 peak height) of the standards can be drawn and the reading of the unknown obtained directly from the curve, since the same injection volume (2.0 μl.) are used for the sample and each of the standards (Fig. 1).

Since there is a straight line relationship between peak area and glutethimide concentration up to 5 mg./100 ml., the following alternate formula can be also used for quantitation.

\[
\frac{\text{Peak area of unkn. in serum}}{\text{Peak area of std.}} \times \text{amt. of std., mg./100 ml.} = \text{amt. of unkn., mg./100 ml.}
\]

Values higher than 5 mg./100 ml. can be assayed by diluting the serum sample or by using a lower injection volume.
Results and Discussion

Under conditions cited in the method, the retention time for glutethimide was 5.6 min. with an 80–90% recorder deflection at a concentration of 5.0 mg./100 ml. At an injection temperature of 300°, the recorder response was only 50–60% of full scale deflection, and the retention time was 6.5 min. Further changes in these parameters were seen at lower injection temperatures. Similar results were found when the column temperature was varied. Electrometer response was markedly decreased at 150°, while at 220° the retention time was less than 3 min. The temperature parameters chosen for this procedure provided optimal recorder response with a relatively short retention time and still achieved good separation when other drugs were present in the sample. This is demonstrated in Fig. 2A, which shows the separation of a mixture of barbiturates and glutethimide. All samples were injected at a 3 mg./100 ml. concentration. The peak height represents the electrometer sensitivity and indicates that glutethimide has the maximal response under the conditions cited. Separation of the same compounds under the identical conditions on the SE 30 borosilicate column is shown in Fig. 2B. Although the electrometer response is superior for some compounds, it is quite obvious that the separation on the DC 200 column is better.

The recovery of glutethimide from a serum sample is shown in Table 1, indicating that at high concentrations of the drug (5 mg./100 ml.) 0.4 ml. of chloroform is an efficient volume of extracting fluid, al-
though at therapeutic levels (0.5–1.0 mg./100 ml.) only 75% of the drug is recovered. A study was also performed to determine the effect of the presence of various drugs in the serum sample on the recovery of glutethimide. Several barbiturates, as well as meprobamate and meperidine, were added to blood samples containing varying amounts of glutethimide. The results of the study are shown in Table 2. It is apparent that the presence of other drugs does not appreciably alter glutethimide recovery.

A recorder tracing of a patient who had had an original glutethimide level of 3.1 mg./100 ml. is shown in Fig. 3A. This patient was a young child who had inadvertently taken the drug; 48 hr. later, her serum level was down to 0.8 mg./100 ml. (Fig. 3B), and she was awake and well. In a recent semicomatose patient, a low level of glutethimide (less than 1.0 mg./100 ml.) was found in his blood. The reason for the patient’s condition was the presence in his blood of 2.5 mg./100 ml. of nembutal (sodium pentobarbital) which was clearly shown on the recorder chart (Fig. 4).

Table 1. Recovery of Glutethimide from Human Serum as Determined by Gas-Liquid Chromatograph

<table>
<thead>
<tr>
<th>Glutethimide Added (mg./100 ml.)</th>
<th>Glutethimide Found (mg./100 ml.)</th>
<th>Recovered (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHLOROFORM (0.2 ml.)*</td>
<td></td>
<td></td>
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<tr>
<td>0.50</td>
<td>0.37</td>
<td>74.5</td>
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<tr>
<td>1.00</td>
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<td>84.0</td>
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<tr>
<td>2.00</td>
<td>1.75</td>
<td>86.0</td>
</tr>
<tr>
<td>5.00</td>
<td>2.62</td>
<td>83.5</td>
</tr>
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<td>CHLOROFORM (0.4 ml.)*</td>
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<td></td>
</tr>
<tr>
<td>0.50</td>
<td>0.39</td>
<td>78.6</td>
</tr>
<tr>
<td>1.00</td>
<td>0.91</td>
<td>91.0</td>
</tr>
<tr>
<td>2.00</td>
<td>1.90</td>
<td>94.5</td>
</tr>
<tr>
<td>5.00</td>
<td>5.00</td>
<td>100.0</td>
</tr>
</tbody>
</table>

* Extracting fluid.

Table 2. Recovery of Glutethimide from Human Serum Containing Various Drugs*

<table>
<thead>
<tr>
<th>Glutethimide Added (mg./100 ml.)</th>
<th>Glutethimide Found (mg./100 ml.)</th>
<th>Recovered (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>0.40</td>
<td>79.0</td>
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<tr>
<td>1.0</td>
<td>0.89</td>
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<td>2.0</td>
<td>1.85</td>
<td>87.0</td>
</tr>
<tr>
<td>5.0</td>
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</table>

* Using 0.4 ml. chloroform as the extracting fluid.
We have investigated several dozen samples of serum, plasma, and gastric washings with this technic and have been able to demonstrate the presence of glutethimide in a number of unexpected instances.

**Fig. 3A.** GLC separation showing a 3.1 mg./100 ml. level of glutethimide in serum. **B.** GLC separation showing a 0.8 mg./100 ml. level of glutethimide in serum.

**Fig. 4.** GLC separation showing presence of sodium pentobarbital as well as a low level of glutethimide in the same serum sample.

**References**