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THE ASSAY OF GLUTETHIMIDE IN PLASMA

PHILLIDA GRIEVESON AND J. S. GORDON
Research Division, CIBA Laboratories Ltd., Horsham, Sussex (Great Britain)
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SUMMARY

A gas–liquid chromatographic method for the estimation of glutethimide in plasma is described. The method allows the estimation of concentrations of the drug as low as 0.3 μg/ml. Results are presented showing plasma levels of the drug up to twenty-four hours after a therapeutic dose.

INTRODUCTION

Glutethimide (α-ethyl-α-phenyl glutarimide)* is a widely used non-barbiturate hypnotic. A sensitive method for the assay of this drug in plasma was required in order to compare plasma levels in normal subjects with those achieved in subjects in various clinical states after a therapeutic dose of the drug, and to assess the efficacy of different methods of treating glutethimide poisoning.

Various methods have been published for the determination of glutethimide in plasma. Some depend on the determination of the drug by UV spectrophotometry[1–3], others on the use of GLC[4–6]. None of these methods was found to be adequate for the assay of glutethimide in plasma from subjects who had received a normal dose of glutethimide (250–500 mg). It has been shown with radioactive glutethimide that the concentration of the drug in plasma may be less than 1 μg/ml 5 h after a dose of 250 mg[7]. Of the available methods those in which determination is by UV spectrophotometry suffer from the disadvantage that the non-specific absorbance due to normal plasma constituents may be high in comparison with the absorbance due to the drug. This problem has not been overcome by any of the different extraction procedures used. Of the GLC methods available that of WINSTEN AND BRODY[6] was found capable of determining glutethimide in plasma only at concentrations higher than 10 μg/ml. The method of FINKLE[4] was capable of determining accurately levels of more than 3 μg/ml. The method of KORZUN *et al.*[5] was found to be unsuitable owing to the use of an excessively high column temperature which caused bleeding of the liquid phase from the column with consequent reduction of the sensitivity. In addition, the use of dimethylformamide as a solvent for the glutethimide caused progressive deterioration of the column owing to reaction between the solvent and the liquid phase, a polyethylene glycol, Carbowax 20 M.

* Doriden®.

The method described in this article is suitable for the assay of glutethimide in plasma down to levels of 0.3 μg/ml and has been used to assay blood samples taken from volunteers up to 24 h after oral administration of 500 mg of glutethimide.

MATERIALS AND METHODS

Chromatography

A Pye Series 104 gas chromatograph fitted with a dual flame ionisation detector head was used in this work, so that it could be used as a dual column–dual detector instrument. Thus two separate determinations could be carried out simultaneously.

Glass columns (9 ft. × 1/4 in. O.D.) were used. They were inactivated before use by rinsing with dichlorodimethylsilane, followed by methanol. They were air dried.

Chromosorb G* (100–120 mesh) was used as the support. It was prepared by washing for one hour with several volumes of concentrated HCl. The acid was removed by repeated washing with distilled water. The fine particles were removed by decanting after the last wash. The material was dried at 110°. It was then inactivated by refluxing for 12 h in twice its volume of a mixture of 10% trimethylsilane and 10% diethylamine in hexane. The material was dried by suction, washed with *n*-propanol, followed by petroleum ether (60–80°) and then dried at 110°.

The liquid phase used was Carbowax 20M*. The required volume of a 0.5% solution of Carbowax 20M in chloroform (1 ml/g Chromosorb G) was added to the support material and the chloroform was removed under vacuum at 70° using a rotary evaporator. The coated support, when completely dry, was packed into the columns under nitrogen at a pressure of 14 p.s.i., with vibration. Columns were preconditioned by heating at 210° for 48 h with a slow flow of nitrogen through the column. Operating conditions were as follows: column oven 200°; detector oven 260°; carrier gas nitrogen, flow rate 100 ml/min; hydrogen flow rate 80 ml/min; air flow rate 800 ml/min. All samples were dissolved in tetrahydrofuran. 1 μl samples were injected into the column. Glutethimide had a retention time of 12 min under these conditions.

Extraction procedure

Duplicate 1 or 2 ml plasma samples were extracted by shaking for 10 min with 10 ml redistilled dichloromethane. After centrifugation at 3000 r.p.m. for 5 min the aqueous phase was removed and the organic phase containing the drug washed with 4 ml 1 *N* NaOH, followed by 4 ml 1 *N* HCl and then 4 ml distilled water. After each wash tubes were centrifuged in order to ensure adequate separation of the phases, before the aqueous phase was removed. A measured aliquot of the organic phase (usually between 5 and 7 ml) was then transferred to a 10 ml conical Quickfit testtube and the dichloromethane removed on a rotary evaporator, at 30°. The tubes were then sealed with a rubber disc and aluminium cap of the type used in sealing vials. The residue was then redissolved in 100 μl of tetrahydrofuran which was introduced into the tube through the seal with a (50 μl) Hamilton syringe. When the solvent had been added tubes were stored at 0°. These precautions were necessary in order to prevent concentration of the sample through the evaporation of the solvent.

* Applied Science Laboratories, Inc.

Standards, for the calibration of the instrument, were prepared by drying a suitable volume of a solution of glutethimide in ethanol on to a conical testtube and were then treated in the same way as the samples. The detector response was calibrated each day.

It was found that the concentration of glutethimide in each sample could be accurately estimated by the measurement of the height of the peak above the extrapolated baseline, provided that not more than 1 μl samples were injected. With constant injection volume peak width is constant and triangulation of the peak is therefore unnecessary.

In order to test the recovery of glutethimide from plasma known volumes of the standard solution of glutethimide in ethanol were dried on to testtubes. The residue was then dissolved in 1 or 2 ml of plasma from normal volunteers who had not taken glutethimide for at least two weeks.

In order to assess the usefulness of the method in assaying plasma levels of glutethimide after a therapeutic dose of the drug, six normal volunteers swallowed two tablets (500 mg) approximately 1 h after a light breakfast. Blood samples were taken by venepuncture at 1/2, 1, 2, 4, 6, 8, 12 and 24 h after the dose had been taken. The heparinised blood was centrifuged within 15 min and the plasma was frozen and stored at $-20°$ until the assay was carried out.

RESULTS AND DISCUSSION

The reproducibility of the response of the detector to injections of 1 μl samples of glutethimide at two different concentrations (10 and 100 ng/μl) was determined. The standard deviation at both concentrations was found to be 3.3%. The response of the detector to glutethimide was shown to be linear over the range 2.5 ng–1 μg. The smallest quantity that can be accurately measured is 2.5 ng.

The recovery of glutethimide from plasma is between 90 and 100%. Details are shown in Table I. Lower concentrations than 0.3 μg/ml glutethimide in plasma cannot be estimated because of interference by normal plasma constituents.

Using this method it was found possible to assay glutethimide in blood samples

TABLE I

RECOVERY OF GLUTETHIMIDE FROM PLASMA

Concentration of glutethimide in plasma (μg/ml)	*Plasma volume extracted (ml)*	*Apparent concentration (μg/ml)*	*% recovery*
0.5	1	0.45	90
0.5	1	0.5	100
1	1	0.95	95
1	1	0.90	90
2.5	1	2.5	100
2.5	1	2.3	92
5	1	4.5	90
5	1	4.7	94
10	1	9.5	95
10	1	9.7	97

taken from subjects who had taken the drug between 30 min and 24 h beforehand. Results are shown in Table II. Fig. 1 shows a typical tracing from chromatography of (a) a normal, drug-free plasma extract and (b) an extract of plasma containing glutethimide.

Analysis of the results of duplicate assays of plasma samples indicate that the overall error in the method is 8%.

TABLE II

PLASMA GLUTETHIMIDE LEVELS (μg/ml) AFTER INGESTION OF 500 mg OF GLUTETHIMIDE IN TABLET FORM BY SIX SUBJECTS

The results of duplicate assays on each sample are shown.

Subject	*Time (h)*							
	1/2	*1*	*2*	*4*	*6*	*8*	*12*	*24*
SWMH	1.4	3.2	3.6	2.6	1.6	1.5	1.1	0.5
	1.4	3.7	3.6	2.5	1.8	—	1.0	0.5
CMcM	1.2	4.4	7.9	4.4	3.8	3.1	2.0	0.4
	1.4	4.9	6.2	4.1	3.8	3.3	2.0	0.4
AMW	0.9	3.5	2.6	2.2	1.7	1.4	0.9	0.7
	1.2	4.3	3.6	2.2	—	1.4	1.4	0.9
PMG	4.0	6.5	4.8	2.4	1.7	1.5	1.1	0.4
	3.7	4.5	5.2	2.6	1.6	1.4	1.0	0.4
RKR	0.8	3.2	2.9	2.1	1.5	1.3	1.1	0.6
	0.9	3.5	3.1	—	—	1.4	1.2	0.7
GK	<0.3	0.7	1.3	1.6	2.8	2.4	2.1	0.8
	<0.3	0.8	1.4	1.6	2.9	2.4	2.1	0.8

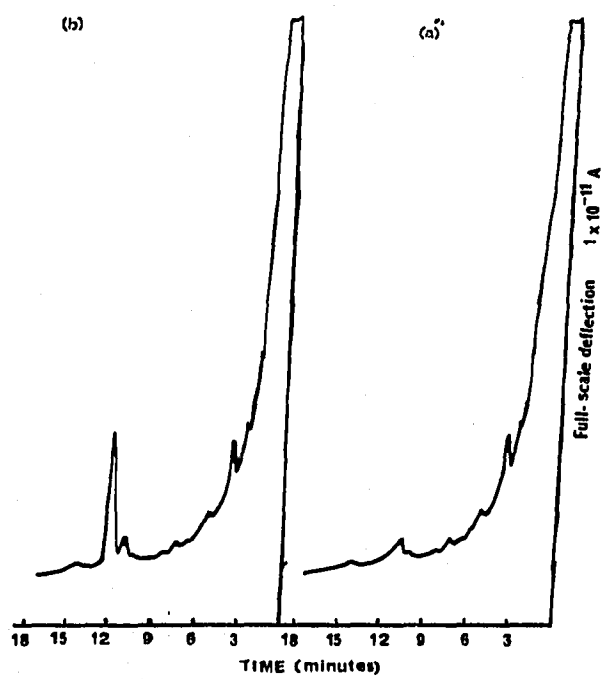

Fig. 1. Tracing obtained by chromatography of (a) extract from normal plasma and (b) extract from plasma containing glutethimide (1 μg glutethimide per ml plasma).

It can be seen from Table II that plasma levels vary considerably between subjects. Peak plasma levels were attained more rapidly in some subjects than in others, the time of the maximum concentration ranging from 1 to 6 h. The peak levels varied from 2.9 to 7.1 μg/ml. This variation could not be accounted for by differences in body weight of the subjects. At 24 h after the dose plasma levels ranged from 0.4 to 0.8 μg/ml. The sensitivity of the method was adequate for the assay of all samples except one. In the subject from whom this sample was taken the drug appeared to be absorbed very slowly and in the 30 min sample less than 0.3 μg/ml of glutethimide could be detected.

The results suggest that the rate and extent of absorption of glutethimide from tablets is variable. Irregularities in the plasma decay curves indicate that absorption continues throughout the first twelve hours and that the concentration of the drug in the plasma during this period is dependent both on the rate of absorption from the gut and on the rate of its elimination from the plasma by metabolism and excretion.

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