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GAS CHROMATOGRAPHIC DETERMINATION OF THE HYPOGLYCAEMIC AGENT GLICLAZIDE IN PLASMA

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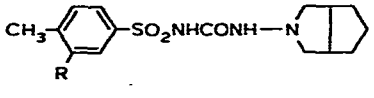
SUMMARY

A gas chromatographic method has been developed that permits the accurate and specific determination of the hypoglycaemic agent gliclazide in plasma. Gliclazide is extracted with chloroform and, after clean-up, derivatized with diazomethane followed by heptafluorobutyric anhydride to form N-methyl-N'-heptafluorobutyrylgliclazide, which is assayed on a gas chromatograph equipped with a flame ionization detector, an electron-capture detector or a nitrogen—phosphorus sensitive detector.

Accurate determinations are possible with flame ionization detection over a concentration range of 1—15 μg/ml of gliclazide in plasma with a relative standard deviation of 5.2%. The minimum detectable concentration with electron-capture detection is 0.02 μg per sample. Plasma levels of gliclazide in dogs following single oral administration (40 mg per dog) have also been determined.

INTRODUCTION

Gliclazide {1-(4-methylbenzenesulphonyl)-3-(3-azabicyclo[3.3.0]octyl)urea} (Fig. 1) [1, 2] is a new oral hypoglycaemic drug of the sulphonylurea type. In order to study the pharmacokinetics of gliclazide, a sensitive and specific assay method for the unchanged drug is necessary.



R = H Gliclazide
R = Cl Internal standard

Fig. 1. Structural formulae of gliclazide and internal standard.

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Sulphonylurea drugs have been most frequently determined by gas chromatography [3—8] after methylation. These methods utilize the thermal fragmentation of the N-methyl derivative to N-methylsulphonamide in the injection port, and therefore the unchanged drug cannot be determined in the presence of some metabolites or other sulphonylurea drugs such as tolbutamide and tolazamide. These disadvantages were eliminated after the thermally stable N-methyl-N'-perfluoroacyl derivative was introduced [9, 10], and the specific determination of the unchanged drug is possible.

In this paper we describe a gas chromatographic method for the determination of unchanged gliclazide in plasma based on methylation with diazomethane followed by acylation with heptafluorobutyric anhydride (Fig. 2).

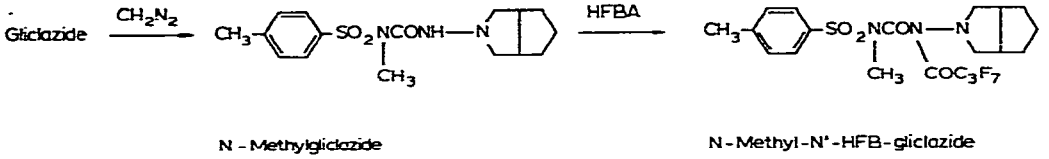

Fig. 2. Derivatization of gliclazide with diazomethane and heptafluorobutyric anhydride.

EXPERIMENTAL

Chemicals and reagents

Gliclazide was a gift from Lab. Servier (Suresnes, France). 3-Chlorogliclazide (m.p. 161—162°C), used as an internal standard, was synthesized in our laboratory [11] (Fig. 1). Heptafluorobutyric anhydride (HFBA) was obtained from Tokyo Kasei Kogyo Co. (Tokyo, Japan). An ethereal diazomethane solution was prepared as described previously [12]. All other chemicals were of analytical-reagent grade.

Gas chromatography

Flame ionization and electron-capture detection. A JEOL Model JGC-20K gas chromatograph equipped with a flame ionization detector (FID) and a 10-mCi ^{63}Ni electron-capture detector (ECD) was used. A silanized glass column (100 cm × 2 mm I.D.) was packed with 3% XE-60 on Chromosorb W AW DMCS (80 -100 mesh). Nitrogen was used as the carrier gas at a flow-rate of 30 ml/min. The column temperature was 220°C and the injector and detector temperatures were 280°C.

Nitrogen—phosphorus sensitive detection. A Hewlett-Packard Model 5840A gas chromatograph equipped with a nitrogen—phosphorus sensitive detector (N—P FID) was used. A silanized glass column (120 cm × 2 mm I.D.) was packed with 2% OV-101 on Chromosorb W AW DMCS (100—120 mesh). Helium was used as the carrier gas at a flow-rate of 30 ml/min. The column, injector and detector temperatures were 220, 280 and 300°C, respectively.

Preparation of N-methylgliclazide

Diazomethane solution was added to a solution of gliclazide (2 g) in methanol and the mixture was allowed to stand at room temperature for 1 h,

then evaporated to dryness in vacuo. The residue dissolved in chloroform was applied to a silica gel column (20 × 2 cm I.D.), eluted with chloroform and evaporated to dryness in vacuo. The residue was recrystallized from diethyl ether—light petroleum to give 1.1 g of N-methylgliclazide (53% yield, colourless needles, m.p. 133—134.5°C). Calculated for $C_{16}H_{23}N_3O_3S$: C, 56.95; H, 6.87; N, 12.45; S, 9.50%. Found: C, 56.92; H, 7.04; N, 12.19; S, 9.36%.

Preparation of N-methyl-N'-heptafluorobutyryl gliclazide

HFBA (2 ml) and pyridine (0.5 ml) were added to a solution of N-methylgliclazide (1 g) in ethyl acetate and the mixture was heated at 65°C for 30 min. The reaction mixture was shaken with 0.5 *M* sodium carbonate solution followed by water. The organic layer was dried over anhydrous sodium sulphate and evaporated in vacuo, leaving a colourless oil. The oil was distilled to give 1.17 g of N-methyl-N'-heptafluorobutyrylgliclazide (74% yield, b.p. 110—113°C at 30 mmHg). Calculated for $C_{20}H_{22}F_7N_3O_4S$: C, 45.24; H, 4.16; F, 24.93; N, 7.88; S, 6.01%. Found: C, 45.24; H 4.40; F, 24.67; N, 7.67; S, 6.18%.

Assay procedure for gliclazide in plasma

The assay procedure with the FID was as follows. To 1 ml of plasma sample were added 2 ml of 0.25 *M* acetate buffer (pH 3.95) and 8 ml of chloroform containing 15 μg of internal standard in a glass-stoppered 15-ml centrifuge tube, and the tube was shaken for 15 min. The organic layer (6 ml) was shaken with 4 ml of 1 *N* sodium hydroxide solution for 10 min. The aqueous layer (3 ml) was transferred into a glass-stoppered 15-ml centrifuge tube containing 1 ml of 3 *N* hydrochloric acid and 5 ml of 0.25 *M* acetate buffer (pH 3.95), and then shaken with 5 ml of chloroform for 10 min. The organic layer (4 ml) was transferred into another centrifuge tube and evaporated to dryness under a gentle stream of nitrogen.

The residue was dissolved in 0.1 ml of methanol, 0.5 ml of diazomethane solution was added and the solution was allowed to stand at room temperature for 10 min. The reaction mixture was evaporated to dryness under a gentle stream of nitrogen. The residue was dissolved in a mixture of 45 μl of ethyl acetate, 5 μl of pyridine and 50 μl of HFBA, and the solution was heated at 65°C for 10 min. The reaction mixture was shaken with 4 ml of *n*-hexane for 30 sec, 4 ml of 5% ammonia solution were added and the mixture was again shaken for 5 min. After centrifugation, the organic layer (3 ml) was evaporated to dryness. The residue was dissolved in 30 μl of ethyl acetate and a 5-μl aliquot of the solution was injected into the gas chromatograph.

When the ECD or N—P FID was used, the assay procedure was as described above except that the amount of the internal standard was reduced to 2 μg in each instance.

Calibration graph

Samples (1 ml) of the control plasma containing 1—15 μg of gliclazide were treated as described under *Assay procedure for gliclazide in plasma*. Peak-area ratios of gliclazide to the internal standard were measured with the FID and plotted against the amount of gliclazide present.

Animal experiment

Gliclazide was administered orally at a dose of 40 mg to male beagle dogs (9—12 kg) fasted for 16 h and about 5 ml of blood were drawn by venipuncture 1, 2, 4, 6, 8, 10, 24 and 48 h after dosing. Blood samples were centrifuged and plasma samples were kept frozen until taken for analysis.

RESULTS AND DISCUSSION

Derivatization of gliclazide

Sulphonylurea drugs have usually been methylated with diazomethane [3, 4] or dimethyl sulphate [5—7] for determination by gas chromatography. The reaction of gliclazide with these reagents was evaluated.

Treatment of gliclazide with excess of diazomethane gave two products, N-methylgliclazide and N,N′-dimethylgliclazide (Fig. 3). The former was a major product and the yield was 74.8 ± 1.2% ($n = 6$). N,N′-Dimethylgliclazide (m.p. 144—145°C) was considered to be an inner salt of the quaternary amine [12], as revealed by nuclear magnetic resonance spectrometry and elemental analysis, and did not volatilize under the usual gas chromatographic conditions. Dimethyl sulphate also gave two products, but the quaternary amine was the major product and the yield of N-methylgliclazide was less than 5%. Similarly, extractive alkylation [8] with methyl iodide afforded largely the quaternary amine derivative. Therefore, diazomethane was used for the methylation of gliclazide.

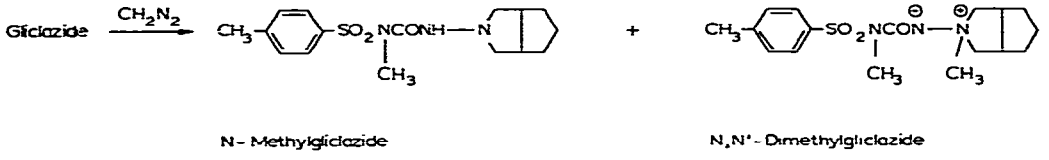

Fig. 3. Reaction of gliclazide with diazomethane.

The reaction of N-methylgliclazide with HFBA proceeded to completion at 65°C within 5 min, and no decomposition products were found after 120 min. The reaction was not affected by the temperature used in the range 50—80°C.

The mass spectrum of N-methyl-N′-HFB-gliclazide obtained by gas chromatography—mass spectrometry showed prominent ions at *m/z* 476 ($[M-57]^+$, 2%), 321 ($[M-212]^+$, 100%), 155 ($[M-378]^+$, 64%) and 91 ($[M-442]^+$, 19%), suggesting that no thermal fragmentation had occurred.

The purification step after derivatization, viz., addition of *n*-hexane followed by washing with 5% ammonia solution, was necessary owing to the large solvent peaks of pyridine and HFBA. However, treatment with these reagents in the reverse order resulted in decomposition of the HFB derivative.

N-Methyl-N′-trifluoroacetyl(TFA)gliclazide (m.p. 93—93.5°C) was also synthesized. Both TFA and HFB derivatives had excellent gas chromatographic properties and gave sharp and symmetrical peaks, but were less stable to the usual washing with alkaline solution to remove acyl reagents. The TFA derivative was hydrolysed to N-methyl-*p*-toluenesulphonamide, whereas the

HFB derivative was stable when dissolved in *n*-hexane followed by washing with 5% ammonia solution. Therefore, the HFB derivative was used in this work.

Specificity

Gliclazide is known to be metabolized in animals and man with major metabolic conversions of the tolyl and azabicyclooctyl groups [13]. The pyrolysis gas chromatographic method commonly used for the determination of sulphonylurea drugs would be simpler and more rapid than our method. However, two hydroxyazabicyclooctyl metabolites have already been isolated from urine of rats receiving 10 mg/kg of gliclazide and therefore these metabolites, if present in plasma, would also be determined as the unchanged drug. In contrast, in the present method these metabolites gave individual peaks (t_R 7.4 and 9.5 min) that were well separated from gliclazide (t_R 6.0 min).

Sensitivity

The minimum detectable concentrations of gliclazide in plasma using the FID, ECD and N—P FID methods are shown in Table I. The sensitivity increased in the order FID < N—P FID < ECD, and the ECD and N—P FID methods were ten times or more sensitive than the FID method.

As the mean plasma level of gliclazide in humans receiving a single 80-mg dose of the drug was about 0.8 μg/ml 24 h after dosing [13], the FID method required a sample volume of 1 ml for study of the pharmacokinetics of gliclazide. In contrast, the sample volume could be decreased to 0.1 ml when using the ECD and N—P FID methods.

TABLE I

SENSITIVITY TOWARDS N-METHYL-N'-HFB-GLICLAZIDE USING THE FID, ECD AND N—P FID

Detector	Minimum detectable concentration (mole/sec)*	Sample volume (ml)
FID	$9.4 \cdot 10^{-14}$	1
ECD	$3.1 \cdot 10^{-16}$	0.1
N—P FID	$1.0 \cdot 10^{-15}$	0.1

*The amount that gives a signal three times the background noise level.

Calibration graph

The calibration graph obtained with 1—15 μg of gliclazide in 1 ml of plasma using the FID was rectilinear and passed through the origin. The precision of the method was 5.2% (relative standard deviation), and the recovery of gliclazide was 85.1 ± 2.7% (n = 8) of the theoretical value.

Fig. 4 shows typical chromatograms for plasma samples obtained using the FID, ECD and N—P FID.

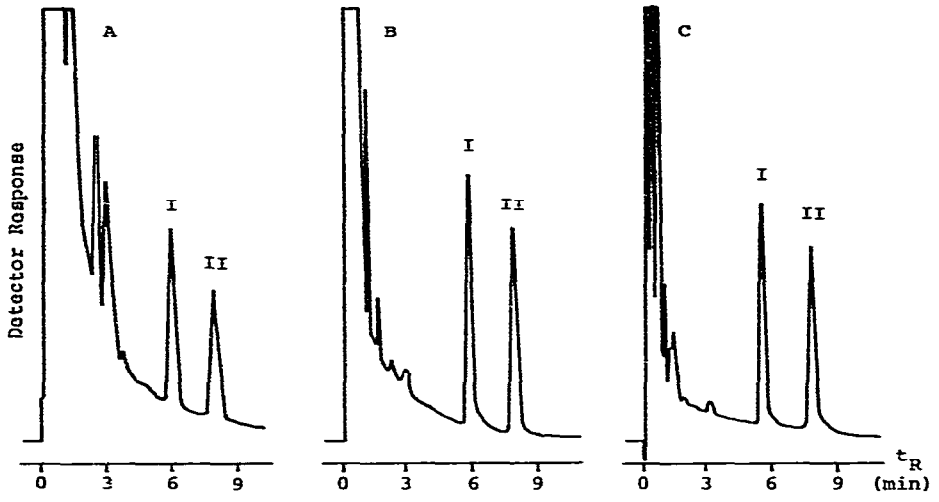

Fig. 4. Typical chromatograms of gliclazide in plasma obtained using (A) FID, (B) ECD and (C) N—P FID. A: Gliclazide (I, 10 μg) and internal standard (II, 15 μg) were added to 1 ml of plasma. B and C: I (1 μg) and II (2 μg) were added to 0.1 ml of plasma.

Determination of gliclazide in dog plasma

Plasma levels of gliclazide following single oral administrations of 40 mg (ca. 4 mg/kg) are shown in Fig. 5. The drug levels were maximal 4 h after dosing with a mean peak level of 7.9 μg/ml, followed by a first-order decrease with a half-life of 10 h. These results are consistent with previous findings [13].

The method described here should be sufficiently sensitive and specific for the determination of unchanged gliclazide in plasma and would therefore

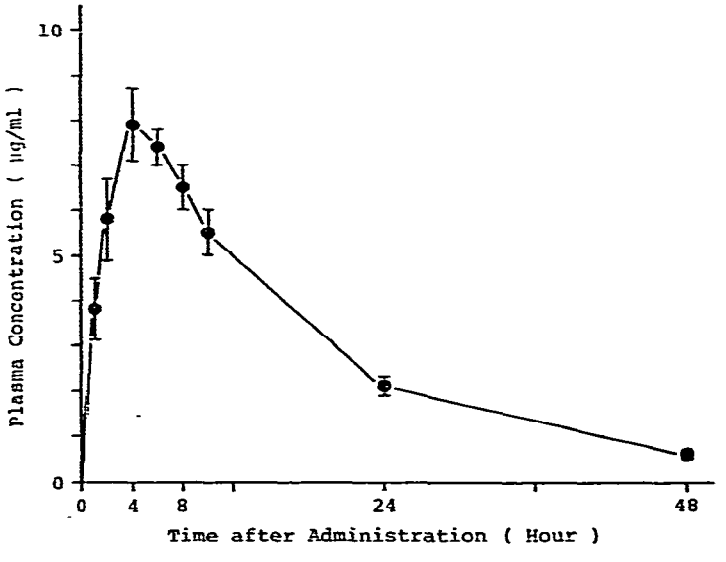

Fig. 5. Plasma levels of gliclazide in dogs following single oral administration of 40 mg of gliclazide. The points are mean values ± standard errors from six dogs.

permit pharmacokinetic studies of gliclazide in both man and experimental animals, as the peak plasma levels of gliclazide were 2—5 μg/ml in human subjects receiving a clinical dosage of 80 mg [13].

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