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# DETERMINATION OF GLICLAZIDE IN HUMAN SERUM BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY USING AN ANION-EXCHANGE RESIN

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## SUMMARY

A method for the routine clinical examination of serum gliclazide by high-performance liquid chromatography (HPLC) on a column packed with a macroporous anion-exchange resin, Diaion CDR-10, was developed. The elution was performed with acetonitrile—methyl alcohol—1.2 *M* ammonium perchlorate (4:3:7, v/v/v) at a flow-rate of 0.4 ml/min. The retention time of gliclazide was 15 min. It seems that the retention mechanism of gliclazide under the HPLC conditions described is not only ion-exchange mode but reversed-phase mode between the anion-exchange resin and the mobile phase. The detection limit of gliclazide was 0.2 μg/ml in plasma. The coefficient of variation for the within-day assay was 5.0% (0.2 μg/ml, $n=8$). The decay curve of serum gliclazide in diabetic patients was determined.

## INTRODUCTION

Gliclazide, N-(4-methylphenylsulfonyl)-N'-(3-azabicyclo[3,3,0]octyl)urea, is one of a series of sulfonylureas recently evaluated as a potential oral hypoglycemic drug. A simple efficient method for the determination of serum gliclazide was required for clinical monitoring of the serum drug levels in diabetic patients. This method had to be quick and highly sensitive. Conventional methods for the determination of serum sulfonylureas, such as tolbutamide, chlorpropamide and acetohexamide, have been based on colorimetry [1], gas—liquid chromatography (GLC) [2—8] or high-performance liquid

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chromatography (HPLC) [9]. Highly sensitive methods for the determination of gliclazide in human serum by GLC with electron-capture detection (ECD) [10] and by reversed-phase HPLC [11] have been developed; however, these methods present the following problems: the gas—liquid chromatographic method uses extraction and derivatization steps which are time-consuming, and the reversed-phase packing (chemical bond silica) used in the HPLC method tends to deactivate through routine assays of many samples.

In the present work, a more suitable method for the routine clinical examination of serum gliclazide by HPLC with the use of a macroporous anion-exchange resin, which is chemically stable, was developed.

## EXPERIMENTAL

### *Reagents*

A pure reference sample of gliclazide was obtained from Dainippon Pharmaceutical (Osaka, Japan). Methyl benzoate, methyl alcohol, acetic acid, sodium acetate, chloroform, acetonitrile and ammonium perchlorate (analytical reagent grade) were obtained from Wako Pure Chemicals (Osaka, Japan). All other chemicals were of analytical reagent grade. All aqueous solutions containing reagents were passed through a filter (Millipore type CS, 0.22 μm, Millipore, Bedford, MA, U.S.A.) before use.

### *Apparatus*

A high-performance liquid chromatograph, Model Tri Rotar, equipped with a variable-wavelength spectrometric detector, Model UVIDEC—100 II (Japan Spectroscopic, Tokyo, Japan) was used. The packed column (250 mm × 4.6 mm I.D.) of Diaion CDR-10, a macroporous anion-exchange resin particle (size 7 μm) was obtained from Mitsubishi Chemicals (Tokyo, Japan). The following conditions were used: column temperature, ambient (25—27°C); detection wavelength, 227 nm; mobile phase flow-rate, 0.4 ml/min; sample volume injected, 10 μl; mobile phase, acetonitrile—methyl alcohol—1.2 *M* ammonium perchlorate (4:3:7, v/v/v).

### *Procedure for extraction of gliclazide from serum*

A mixture of 250 μl of human serum and 1 ml of 0.5 *M* phosphate buffer (pH 7.0) was shaken vigorously for 15 min with 4 ml of chloroform. The mixture was centrifuged for 15 min at 1400 *g*, then 3 ml of the chloroform layer withdrawn, mixed with 2 ml of 1 *N* sodium hydroxide, and shaken for 10 min. After centrifugation of the mixture for 5 min, 1.5 ml of the aqueous layer was added to a mixture of 0.5 ml of 3 *N* hydrochloric acid, 2.5 ml each of 0.5 *M* phosphate buffer (pH 7.0) and chloroform, followed by shaking for 10 min. After centrifugation for 5 min, 2 ml of the chloroform layer was evaporated to dryness under a stream of nitrogen at 50°C. The residue was redissolved in 50 μl of methyl alcohol containing the internal standard (IS), methyl benzoate, 0.4 μg/50 μl, and a 10-μl aliquot was injected onto the HPLC column. The extractability of gliclazide from serum using the present procedure was maintained at over 96% down to pH 7.0. The methyl alcohol solution containing the extracted drug was stored at 2—4°C, and assayed within 3 days.

*Preparation of calibration curve*

Serum was obtained from the blood of a normal male. De-ionized water and serum found to have no drugs detectable by the present method were used to make up a solution ranging in concentration from 0.3 to 10 μg/ml of gliclazide. The calibration curve was constructed from peak height ratios of gliclazide to IS. De-ionized water and serum blanks were analyzed with each set of standard solutions. Serum samples from gliclazide-administered diabetic patients were extracted with chloroform and chromatographed in the same manner.

## RESULTS AND DISCUSSION

A highly sensitive method for the determination of serum gliclazide was required because of the small drug dosage, resulting in low serum levels. At the same time, separation of the drug from several endogenous serum components could also be an important problem when using HPLC. Therefore, the procedure for the extraction of serum gliclazide was studied.

Conventionally, the extraction of sulfonylureas with chloroform from serum has been performed by using acetate buffer (pH 3.95, Walpole's buffer). Since the $pK_a$ values of gliclazide are 5.98 and 2.21, the possibility of extraction with chloroform at around pH 7 was considered. The extractability of gliclazide from serum with chloroform at different pH values in the range 1.0 to 10.0 was examined. As shown in Fig. 1, gliclazide almost quantitatively passed into

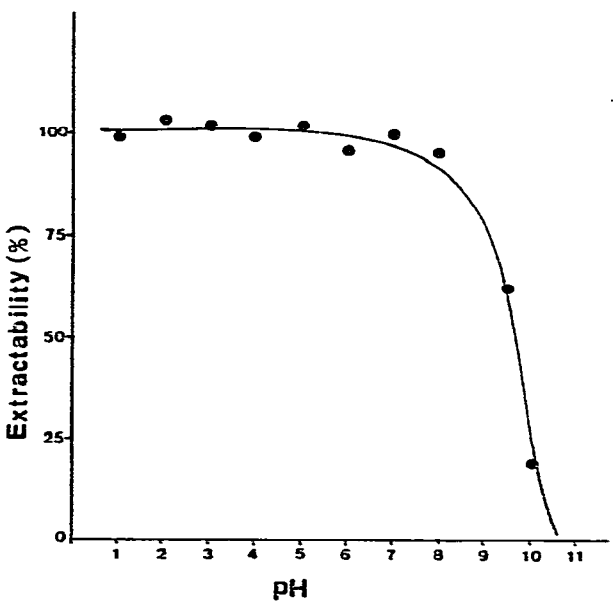


Fig. 1. Effect of pH on extractability of serum gliclazide. The same serum used for the preparation of the calibration curve was also used for the examination of drug extractability. The serum contained 5 μg/ml of the drug and extraction was carried out as described in Experimental. The extractability was calculated from the peak height ratio of gliclazide to IS (methyl benzoate). Each point represents the mean value of three separate experiments.

the chloroform layer from the mixture of serum and phosphate buffer below pH 7.0, while it was extracted with only 20% yield at pH 10.0. The present extraction procedure including a back-extraction may be tedious; however, a highly sensitive analysis is required to minimize some of the endogenous serum components or other drugs in the extracted sample.

Fig. 2 shows two HPLC chromatograms of endogenous serum components

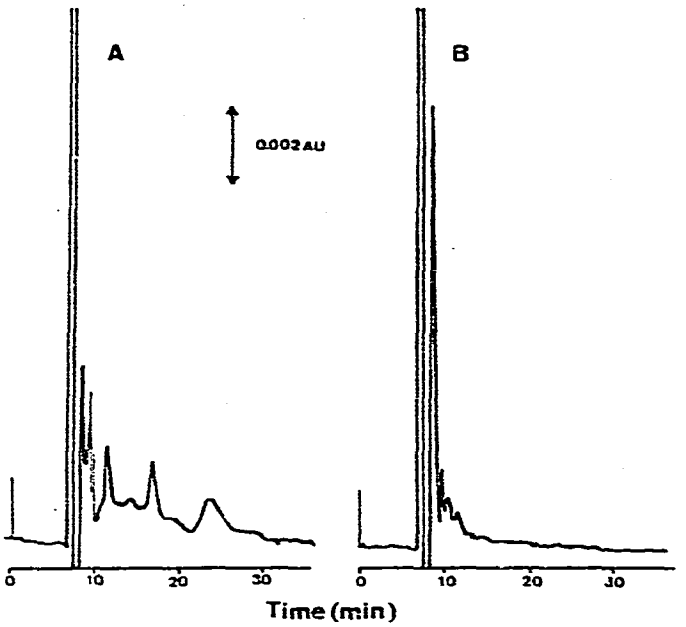


Fig. 2. Chromatograms of serum extracts: (A) extracted at pH 3.95 and (B) extracted at pH 7.0.

back-extracted with chloroform from serum at pH 7.0 and 3.95. As shown in Fig. 2A, six serum component peaks were detected at retention times in the range 7—24 min. In the case of extraction at pH 7.0, only two serum component peaks were detected at retention times in the range 7—12 min (Fig. 2B). Thus, the extraction using phosphate buffer at pH 7.0 seems to be more advantageous than when using acetate buffer (pH 3.95) for the determination of serum gliclazide by HPLC. Fig. 3 shows a typical chromatogram of gliclazide and IS (methyl benzoate) in serum. The gliclazide and IS peaks were detected at retention times of 15 and 19 min, respectively, whereas the serum component peaks were detected earlier (within 12 min).

Recently, Diaion CDR-10, a macroporous anion-exchange column was developed for the HPLC analysis of nucleotides, carbohydrates and fatty acids. It is known that the macroporous resins have a relatively higher cross-linkage, larger surface area, and larger porosity than the microporous resins [12]. Usually, 0.1—6.0 *M* acetate buffer (pH 4.0) is recommended to be used in the mobile phase when using this resin. But an attempt to use this mobile phase failed because of gliclazide is hard to dissolve. Furthermore, the use of an alkaline buffer of high ionic strength did not give good separation. In order to overcome

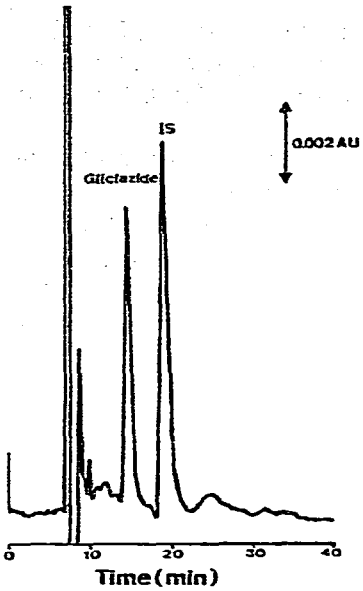


Fig. 3. Chromatogram of control human serum plus gliclazide and IS (methyl benzoate).

these difficulties, it was found that the addition of acetonitrile and methyl alcohol to 1.2 *M* ammonium perchlorate in the mobile phase gave sharper peaks and better separation. It seems that the retention mechanism of a weak acidic and hydrophobic compound (gliclazide) under the HPLC conditions described is not only ion-exchange mode but also reversed-phase mode between the styrene—divinylbenzene skeleton of the anion-exchange resin and the mobile phase. Therefore, this column was tried for the determination of serum gliclazide.

The calibration curve was linear (correlation coefficient, $r = 0.99$) over a range of 0.3—10 μg/ml of gliclazide, and the detection limit of gliclazide was estimated to be 0.2 μg/ml, using an injection volume of 10 μl. The sensitivity of the spectrophotometer was kept at 0.02 a.u.f.s. throughout.

Within-run precision and recoveries were determined for various sera concentrations of gliclazide (Table I). The coefficients of variation (C.V.) for the

TABLE I

WITHIN-DAY PRECISION OF GLICLAZIDE ASSAY

| Added (μg/ml) | Found (mean ± S.D., μg/ml) | C.V. (%) | Recovery (mean ± S.D., %) | *n* |
|---|---|---|---|---|
| 0.2 | 0.2 ± 0.01 | 5.0 | 100 ± 5.0 | 8 |
| 0.4 | 0.4 ± 0.02 | 5.0 | 100 ± 5.0 | 8 |
| 2.0 | 2.1 ± 0.10 | 4.8 | 109 ± 1.7 | 6 |
| 5.0 | 5.0 ± 0.14 | 2.8 | 101 ± 1.7 | 6 |
| 8.0 | 7.9 ± 0.28 | 3.5 | 96 ± 3.5 | 5 |

pooled sera containing 0.2, 0.4, 2, 5 and 8 μg/ml gliclazide were 5.0, 5.0, 4.8, 2.8 and 3.5% ($n$ = 5—8), respectively. The recovery of the drug ranged from 96 to 101%. The coefficient variation for the day-to-day assay was 2.7% from the results measured nine times during three weeks.

To show the applicability of the proposed method, typical decay curves of serum gliclazide in a diabetic patient given 80 mg of the drug orally were determined by the present method and by GLC with electron-capture detection [10] (Fig. 4). The data show that the two methods gave the same results. The serum level of gliclazide rapidly reached a maximum (ca. 7 μg/ml) about 2 h after administration of the drug; it subsequently declined to the pre-administration level within 48 h.

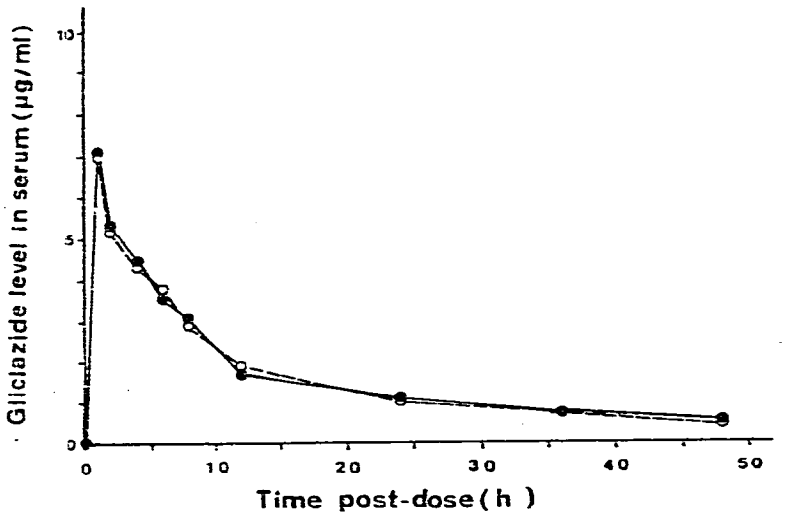


Fig. 4. Decay curves of serum gliclazide levels after oral administration of 80 mg to a diabetic patient. Each point represents the mean value of duplicate determinations. The same sample was determined by the present HPLC method (•) and by GLC with electron-capture detection [10] (○).

The therapeutic oral dose of gliclazide, which is a more potent hypoglycemic than other sulfonylureas, was not more than 1/5 of that of tolbutamide. However, the detection limits of the conventional methods for assay of serum sulfonylureas by colorimetry and by pyrolysis—GLC are 10 and 5 μg/ml [13], respectively. These methods are not satisfactory for the determination of serum gliclazide. On the other hand, the detection limit of GLC with electron-capture detection is 0.1 μg/ml of gliclazide [10] which would seem to be useful for the clinical examination of serum gliclazide, but this method usually requires tedious procedures, such as acylation of the extracted sample, and gives lower reproducibility (C.V. = 6.4%, $n$ = 11) [10]. Neither co-administered drugs (e.g. vitamins or antibiotics) nor possible metabolites were detected in the present analytical system. Probably, they were removed through the extraction procedure. Recently, a high-potent hypoglycemic drug, glipizide was determined in blood with high sensitivity (detection limit 10 ng/ml) by HPLC using a μBondapak $C_{18}$ column [14].

## CONCLUSION

The present method for the determination of serum gliclazide by HPLC with use of an anion-exchange resin is comparable to GLC with electron-capture detection in sensitivity (0.2 μg/ml), specificity and speed (shorter retention time). Diaion CDR-10 resin is mechanically stable when operated at high pressures, and is easy to handle. The present method is preferred for routine clinical examination because of its simplicity and high reproducibility.

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