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Short Communication

Determination of gliclazide in serum by high-performance liquid chromatography using solid-phase extraction

Hideto Noguchi, Naomi Tomita and Shunsuke Naruto

Exploratory Research Laboratories, Dainippon Pharmaceutical Co., Ltd., Enoki-cho 33-94, Suita, Osaka 564 (Japan)

Shigeyuki Nakano

Department of Clinical Pharmacology and Therapeutics, Oita Medical University, Hasama-machi, Oita 879-55 (Japan)

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ABSTRACT

A simple and sensitive high-performance liquid chromatographic method for a routine assay of gliclazide in serum is described. Serum samples spiked with glibenclamide (internal standard) were applied to Bond Elut C_{18} cartridges. After washing with phosphate buffer (pH 7.5) and water, the cartridge was eluted with 60% methanol. The eluate was evaporated to dryness. The residue was dissolved in methanol and injected onto an octadecyl silica column (5 μm, 150 mm × 4.6 mm I.D.). The mobile phase was 0.04 *M* potassium dihydrogenphosphate (pH 4.6)–acetonitrile–isopropyl alcohol (5:4:1, v/v). Ultraviolet detection at 227 nm was used. The minimum detectable level of gliclazide was 20 ng/ml.

INTRODUCTION

Gliclazide, 1-(4-methylbenzensulphonyl)-3-(3-azabicyclo[3,3,0]octyl)urea, is a potential oral hypoglycemic drug which is useful for the treatment of non-insulin-dependent diabetes mellitus. Gas chromatography [1,2] and high-performance liquid chromatography (HPLC) [3–6] have been used for the determination of gliclazide in biological fluids. However. these assay methods involve laborious solvent extraction procedures [1–6] or derivatization steps [1,2,6]. Charles and Ravenscroft [7] reported a simple procedure using acetonitrile as deproteinization and extraction solvent, but the minimum detectable level of gliclazide in plasma was 0.5 μg/ml. In a search for a more simple and precise assay method, at a concentration of gliclazide less than 0.1 μg/ml of serum, we examined the use of solid-phase extraction. Although Iegushi *et al.* [8] used solid-phase extraction, they did not describe any details of the detection limit and recovery of the drug. Kanatsuna *et al.* [9] reported that the maximum plasma level of gliclazide was in the range 2.1–3.5 μg/ml after single oral administration of 40 mg of gliclazide.

Correspondence to: H. Noguchi, Exploratory Research Laboratories, Dainippon Pharmaceutical Co., Ltd., Enoki-cho 33-94, Suita, Osaka 564, Japan.

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This paper deals with a simple and sensitive method for the routine determination of gliclazide in human serum by the combination of solid-phase extraction and HPLC with UV detection.

EXPERIMENTAL

Chemicals and reagents

Gliclazide (Lot No. 7C405/1) was supplied by Servier Labs. (Neuilly, France). Glibenclamide, the internal standards (I.S.), was extracted and purified from Euglucon tablets (Yamanouchi Pharm., Tokyo, Japan). Blank human serum Type I-A was obtained from Sigma (St. Louis, MO, USA). Bond Elut C_{18} cartridges were purchased from Uniflex (Tokyo, Japan). The cartridges were conditioned by washing them succesfully with 3 ml of methanol, 3 ml of distilled water and 1 ml of 0.2 *M* sodium phosphate (pH 7.5). HPLC-grade acetonitrile, isopropyl alcohol and distilled water were purchased from Wako (Osaka, Japan). Other chemicals were of analytical grade.

Apparatus

The HPLC system consisted of a Model LC-6A (Shimadzu, Kyoto, Japan) and a SPD-6A UV detector (Shimadzu). The analytical column was a STR ODS-M (5 μm, 150 × 4.6 mm I.D.) (Shimadzu).

HPLC conditions

The mobile phase was 0.04 *M* potassium dihydrogenphosphate (pH 4.6)–acetonitrile–isopropyl alcohol (5:4:1, v/v). The column temperature was 35°C, and the flow-rate was 0.9 ml/min. The eluate was monitored at 227 nm with 0.02 a.u.f.s. sensitivity.

Standards

The stock solution of gliclazide (2 mg per 20 ml) in methanol–water (1:1, v/v) (50% methanol) and of the I.S. (0.05 mg/ml) in 50% methanol were stable for three months at 4°C. The working standard solutions of gliclazide in the concentration range 0.5–50 μg/ml were made by dilution of the stock solution with 50% methanol.

Sample preparation

A 20-μl volume of the stock solution of the I.S. was added to 0.5 ml of serum sample. After vortex-mixing for 1 min, the mixture was applied to the pretreated Bond Elut C_{18} cartridge. The cartridge was successively washed with 2 ml of 0.2 *M* sodium phosphate (pH 7.5) and 3 ml of distilled water, and then eluted with 1 ml of 60% methanol. The eluate was evaporated to dryness under reduced pressure at 60°C. The residue was dissolved in 200 μl of methanol. After centrifugation (1500 *g*, 2 min), a 10-μl aliquot of the supernatant was injected into the column.

RESULTS AND DISCUSSION

Gliclazide and the I.S. were retained on the pretreated Bond Elut C_{18} cartridge. Because various serum endogenous components gave interfering peaks in the chromatogram, several washing and elution conditions were examined to improve the recovery of gliclazide and the I.S. The recoveries were determined by comparison of the HPLC peak areas of the spiked serum samples with those of each 50% methanolic solution. Despite successive washing with 2 ml of phosphate buffer (pH 7.5) and 3 ml of water, neither gliclazide nor the I.S. could be liberated from the cartridge. The recovery of the I.S. increased with increasing concentration of methanol in water (30 to 80% methanol). Gliclazide was completely eluted with 40% methanol. Elution with 70% methanol afforded several interfering peaks in the chromatograms, thus elution with 1 ml of 60% methanol was selected as the best compromise. The recoveries of gliclazide and the I.S. from the extraction procedure were 101.3 and 99.9%, respectively.

Fig. 1 shows typical chromatograms of the extracts from serum samples. The calibration curve that was obtained by plotting the peak-area ratios (gliclazide/I.S.) *versus* concentrations (μg per 0.5 ml of serum) was linear over the range 0.1–10 μg/ml, with a correlation coefficient of 0.999.

The intra-day precision was evaluated with serum samples spiked with known concentration of gliclazide. The coefficients of variation (C.V.) af-

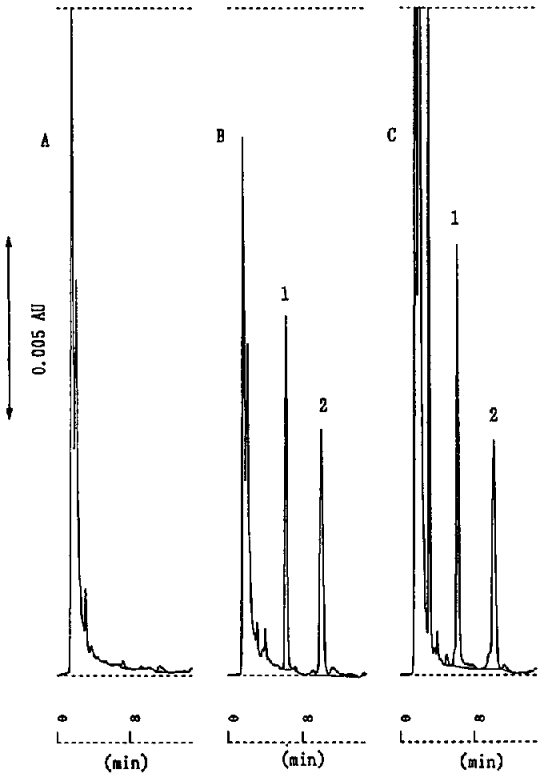

Fig. 1. Chromatograms of extracts: (A) blank human serum; (B) human serum spiked with 2.26 μg/ml gliclazide and 1 μg/ml glibenclamide; (C) serum from a volunteer 3 h after administration of 40 mg of gliclazide. Peaks: 1 = gliclazide; 2 = glibenclamide (I.S.).

ter five determinations at three different concentrations are shown in Table I. The inter-day C.V. was 2.2% (assay of 4 μg/ml, $n = 10$). These data show a very good reproducibility of the proposed method. The limit of determination of gliclazide was 0.1 μg/ml and the limit of detection was 20 ng/ml (signal-to-noise ratio 5:1).

Gliclazide levels in serum obtained from patients receiving gliclazide have been determined by the proposed assay method to give precise and sensitive results. For instance, we determined the plasma gliclazide level after a single oral administration of a tablet containing 40 mg of gliclazide to nine male healthy volunteers under several conditions in a cross-over manner. The detailed results of these clinical trials will be reported elsewhere. It was found that the method was applicable to rat plasma samples with a minor modification using a rat blank plasma. In addition, it has been shown that theophylline, pentobarbital, acetohexamide, carbamazepine, phenytoin, phenobarbital and primidone do not co-chromatograph with either gliclazide or the I.S.

TABLE

INTRA-DAY PRECISION FOR MEASUREMENT OF GLICLAZIDE ADDED TO HUMAN SERUM

Concentration added (μg/ml)	Concentration found (mean ± S.D., $n = 5$) (μg/ml)	C.V. (%)
0.10	0.136 ± 0.004	2.9
4.07	4.12 ± 0.018	0.4
10.17	10.24 ± 0.265	2.6

The method is thus simple, sensitive and readily adaptable to routine determination of therapeutic concentrations of gliclazide in serum and plasma.

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