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Short communication

A simple and sensitive HPLC method for determination of gliclazide in human serum

Mohammad-Reza Rouini*, Afshin Mohajer, Mohammad-Hosein Tahami

Department of Pharmaceutics, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran 14155-6451, Iran

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Abstract

A simple, rapid and specific method for analysis of gliclazide in serum by a sensitive high-performance liquid chromatographic method is described. Only 100 μl of serum and a little sample work-up is required. A simple procedure of extraction by toluene followed by evaporation to dryness under a gentle stream of air and dissolving the dried residue in mobile was used. The gliclazide peak was separated from endogenous peaks on a C_8 column by a mobile phase of acetonitrile–water (45:55, v/v), pH 3. Gliclazide and internal standard (phenytoin) were eluted at 6.8 and 3.8 min, respectively. The limit of quantitation (LOQ) for gliclazide in serum was 75 ng/ml at 230 nm. The method was linear over the range of 75–10 000 ng/ml with r^2 of 0.999. Mean recovery for gliclazide and internal standard was 84.5 and 87%, respectively.

Keywords: Gliclazide

1. Introduction

Gliclazide, 1-(4 methylbenzenesulphonyl) 3-(3 azabicylco [3.3.0] octyl) urea (I), is an oral hypoglycemic drug, belonging to second-generation sulphonylureas, which is used in type II diabetes, previously known as non-insulin-dependent diabetes mellitus (NIDDM). It has been suggested that due to its short-term acting, gliclazide may be suitable for use in diabetic patients with renal impairment and also in elderly patients whose reduced renal function may increase the risk of hypoglycemia following some sulphonylureas [1].

Different analytical methods including colorimetry [2], radioimmunassay [3], gas chromatography [4] and HPLC [5–12] have been reported for determination of gliclazide in biological fluids. Some reported analytical methods involve time-consuming and laborious extraction steps [7,8], complex derivatization techniques [8], lengthy retention time or large volumes of biologic samples [7–9], solid-phase extraction [10] or use of mass spectrometry for detection and identification of the drug [12]. We developed a simple, rapid and applicable HPLC method, which needs small sample volume and minimal sample work-up. The method is suitable for

*Corresponding author. Tel.: +98-21-611-2325; fax: +98-21-646-1178.
E-mail address: rouini@sina.tums.ac.ir (M.-R. Rouini).

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pharmacokinetic studies in terms of specificity and sensitivity.

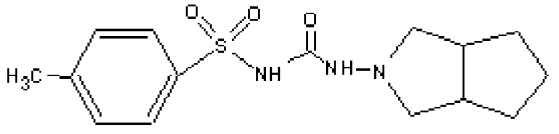
2. Experimental

2.1. Chemicals and reagents

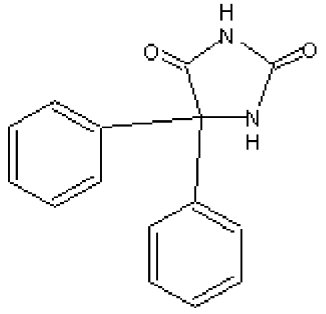
Gliclazide (USP reference standard) and phenytoin working standard (used as internal standard, I.S.) were kindly donated by Dr. Abidi Pharmaceutical (Tehran, Iran) (Fig. 1). HPLC grade acetonitrile and methanol (Merck, Darmstadt, Germany) and double distilled water were used throughout the analysis. All other chemicals and reagents were of analytical grade.

2.2. Apparatus and HPLC conditions

The HPLC system consisted of a model 600 pump, a model 486 UV tunable absorbance detector, a 746 data module and a 600E system controller (all from Waters, Milford, USA).



(I)



(II)

Fig. 1. The structure formula of gliclazide (I) and phenytoin (II).

Separation was performed on a Techsphere C_8 column (3 μm, 150 mm×3.9 mm I.D.; HPLC, UK) at ambient temperature (23–27 °C). The pH of the acetonitrile–water mobile phase (45:55, v/v) was adjusted to 3 with phosphoric acid. Mobile phase was passed through column by a flow rate of 0.9 ml/min and the eluate was monitored at 230 nm. Prepared samples were injected to HPLC column through a 7725i Rheodyne injector (CA, USA) fitted with a 50-μl loop.

2.3. Standards

The stock solution of gliclazide (100 μg/ml) and the I.S. (160 μg/ml) were prepared in acetonitrile and were stable for at least 1 month at 4 °C. The working I.S. solution (12 μg/ml) was prepared in water every day.

2.4. Serum extraction

To 100 μl of serum were added 50 μl of the I.S. working solution and 100 μl of 0.07 *M* phosphate buffer (pH 4.5). After vortex mixing for 10 s, 1 ml of toluene was added and the mixture was shaken vigorously for 1 min. The mixture was then centrifuged for 5 min at 10 000 rpm (8500 *g*) (Eppendorf 5415C, Germany). A 800-μl aliquot of the upper organic layer containing gliclazide and I.S. was transferred to a clean glass tube and evaporated under air stream to dryness at 50 °C. The residue was redissolved in 100 μl of mobile phase and a 50-μl aliquot was injected onto the HPLC column.

2.5. Human study

After overnight fasting, 12 healthy male volunteers, who had given written consent, were administered one 80-mg tablet of gliclazide (formulated by Dr. Abidi Pharmaceutical) or Diamicron (Servier, France) in a double blind cross-over bioequivalency study. The study proposal was approved by the ethical committee of the Ministry of Health. Blood samples (1.5 ml) were taken up to 24 h after drug administration. Blood sugar was also determined every hour up to 12 h.

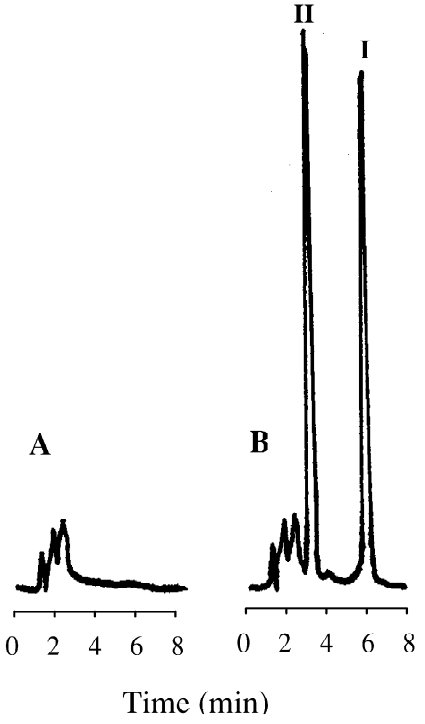


Fig. 2. Chromatograms of (A) blank serum and (B) serum of volunteer 2 h after administration of an 80-mg tablet (I: gliclazide, II: phenytoin; attenuation, 32).

3. Results and discussion

Fig. 2 shows representative chromatograms of extracted serum samples. The retention times of gliclazide and the I.S. were 3.8 and 6.8 min, respectively. No endogenous interference was observed with both gliclazide and the internal standard. The calibration curve was obtained by plotting the peak-height ratios (gliclazide/I.S.) versus concentrations, which was linear over the range of 75–10 000 ng/ml with the regression equation of $y=0.01+0.354x$ and $r^2=0.999$. Estimates of within-day and between-day precisions of the assay were evaluated by analyzing four different known concentrations of gliclazide in serum. The results of six determinations (Table 1) showed a good reproducibility of the proposed method with the mean C.V. of 3.9 and 6.6% for within-day and between-day precision, respectively.

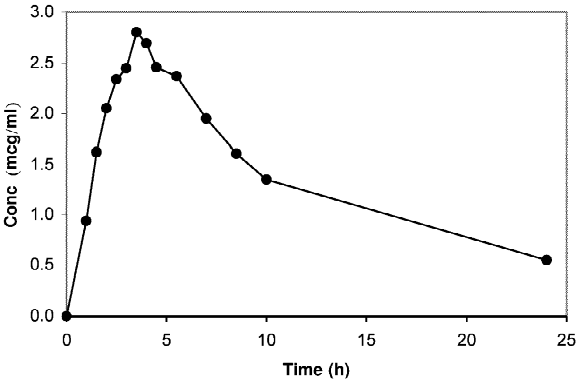


Fig. 3. Serum concentration–time profile of gliclazide after administration of an 80-mg tablet to a healthy volunteer.

The limit of quantitation (LOQ) of gliclazide was 75 ng/ml (signal-to-noise ratio of 5, C.V.<10%) and the minimum detectable level (LOD) was 30 ng/ml (signal-to-noise ratio of 3). The recovery was obtained by comparing the peak height of known serum samples spiked with gliclazide and I.S. to those of their respective aqueous solutions, correcting for volume. The results showed absolute recoveries of 84.5±2.45% and 87.0±4.70% for gliclazide and I.S., respectively. Fig. 3 shows a concentration–time profile of a human volunteer after taking an 80-mg single oral dose of gliclazide.

The solid phase extraction procedure reported by Noguchi et al. [10] for sample preparation is an expensive sample clean-up method. The temperature should be kept at 37 °C during their reported analysis and each run lasts ~12 min. Our method works at

Table 1
Reproducibility and accuracy of the analysis method ($n=6$)

Concentration (μg/ml)	Within-day C.V.	Within-day accuracy	Between-day C.V.	Between-day accuracy
1250.0	3.8	6.7	8.8	9.9
2500.0	3.9	−2.4	5.5	3.5
5000.0	4.7	−1.9	6.3	−4.4
10 000.0	3.3	4.3	5.9	6.1

ambient temperature and uses liquid–liquid extraction, and has a shorter run time. Igaki et al. [8] have developed an HPLC method using fluorescence detection and chemical derivatization, but nevertheless their method is less sensitive than ours (LOQ of 100 vs. 75 ng/ml, respectively). In another study, Charles and Ravenseroft [6] used 3-chlorogliclazide as internal standard, which is not commercially available. The LOQ of their method is ~500 ng/ml, which is far below the therapeutic serum concentrations and sensitivity needed in pharmacokinetic studies.

The methods published by Kimura et al. and Sener et al. [7,9] and Poirier et al. [11] need 0.5 and 0.25 ml of serum samples, respectively, compared to 0.1 ml in our method. Poirier et al. [11] have also used nadoxolol as I.S. which is not easily available. The perchloric acid used in their mobile phase reduces the column life remarkably. We also did not find any difference between the citrate–phosphate buffer used by Poirier et al. compared to phosphate buffer alone in sample preparation. Our method has also the shortest run time in comparison to this and all previously mentioned methods.

The pharmacokinetic parameters obtained from our study using this method of analysis showed a maximum serum concentration of 3 ± 0.58 μg/ml, a t_{max} of 3.5 ± 0.7 h and a $t_{1/2}$ of 9.5 ± 2.4 h. The 24-h serum concentration was ~300 ng/ml after administration of one 80-mg tablet.

In conclusion, our method is a simple, sensitive and specific HPLC method and could easily be used in pharmacokinetic and bioequivalency studies when numerous daily samples have to be assayed relatively quickly; at least 50 samples can be analyzed during an 8-h working day.

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