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HPLC Estimation of Gliclazide in Formulations and In Pharmacokinetic Studies

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A simple, accurate and sensitive RP-HPLC method was developed and validated for the estimation of gliclazide in bulk drug samples, its formulations and in plasma samples using RP C-18 column with UV detection at 230 nm. The mobile phase consists of water containing 0.1 % w/v sodium phosphate monobasic (pH adjusted to 2.1 using phosphoric acid) and acetonitrile (34:66). The retention time for gliclazide was 5.4 min. The intra- and inter-day coefficient of variation was less than 1.28 % showing high precision of the method. The method was highly accurate with a recovery in the range 99-100 %. The method was found suitable for estimating gliclazide in plasma samples in pharmacokinetic studies. The pharmacokinetic parameters of gliclazide estimated employing the HPLC method developed agreed well with the literature values.

Key Words: HPLC Method, Gliclazide, Pharmacokinetics.

INTRODUCTION

The development of an analytical method for the determination of drugs by HPLC has received considerable attention in recent years because of its importance in quality control of drugs and drug products and in pharmacokinetic studies. Gliclazide, 1-(4 methylbenzenesulphonyl)-3-(3-azabicyclo[3.3.0]octyl)urea, is an oral hypoglycemic drug, belonging to second-generation sulphonylureas, which is used in type II diabetes. Different analytical methods including colorimetry[1], radioimmunassay[2], gas chromatography[3] and HPLC[4-11] have been reported for determination of gliclazide in pharmaceutical dosage forms and in biological fluids. Some reported analytical methods involve time-consuming and laborious extraction steps[6,7], complex derivatization techniques[7], lengthy retention time or large volumes of biological samples[6-8], solid-phase extraction[9] or use of mass spectrometry for detection and identification of the drug[11]. The objective of this study is to develop a simple, rapid and sensitive HPLC method for the analysis of gliclazide in bulk drug samples and its formulations using most commonly employed RP-C_{18} column with UV detection. The method has been applied for the estimation of gliclazide in plasma samples of a pilot *in vivo* study to assess the pharmacokinetics of gliclazide in rabbits.

EXPERIMENTAL

Gliclazide (M/s. Ranbaxy Research Laboratories, Gurgaon). Methanol, HPLC grade (Merck), acetonitrile, HPLC grade (Merck), sodium phosphate monobasic, HPLC grade (Qualigens Fine Chemicals), phosphoric acid, HPLC grade (Qualigens Fine Chemicals) and water, HPLC grade (Qualigens Fine Chemicals) were procured from commercial sources. Glycinorm 40 (M/s. Ipca Laboratories Ltd., Mumbai, B. No. UJ 7006 AK, Mfg. Dt. Dec' 2007) and Glizid 40 (M/s. Panacea Biotech Ltd., Solan (H.P), B. No. 2177504, Mfg. Dt. Dec' 2007) were procured from local market.

The HPLC system (Make: M/s Shimadzu Corporation, Japan) consisted of UV-Visible detector (Shimadzu, Model: SPD-10AVP), C-18 column (Phenomenex, DESC: Gemini 5 µ C18 110A, size: 250 × 4.6 mm, S/No: 288063-23), 2 pumps (Model: LC-10 ATVP) and a microsyringe of capacity 25 µL (Model: Microliter® # 702, Mfd.by: M/s Hamilton) was used.

Chromatographic conditions

Mobile phase: The mobile phase is a mixture of water containing 0.1 % w/v sodium phosphate monobasic (pH adjusted to 2.1 using phosphoric acid) and acetonitrile (34:66). The mobile phase was filtered through 0.45 µ membrane filter before use and was run at a flow rate of 1 mL/min.

Preparation of standard solutions: Gliclazide (50 mg) was dissolved in 50 mL of acetonitrile in a volumetric flask. The above solution was further diluted with diluent (acetonitrile: water, 4:1) to get a concentration of 20 µg/mL (stock solution).

The stock solution of gliclazide was further diluted with the mobile phase to get various concentrations namely 0.2, 0.4, 0.6, 0.8, 1, 2, 4, 6, 8 and 10 µg/mL. Each drug solution was filtered through 0.45 µ membrane filter before use and 20 µL of each were injected into the column.

Detection: The column effluent was monitored at 230 nm.

Validation of the method

Accuracy: The accuracy of HPLC assay method was assessed by adding known amount (20 µg) of drug to drug solution of known concentration (10 µg/mL) and subjecting the samples to the proposed HPLC method. The study was replicated 4 times. The accuracy was expressed in terms of the recovery and calculated by multiplying the ratio of measured drug concentration to the theoretical concentration with 100, so as to give per cent recovery.

Precision: The precision of the HPLC method was determined in terms of intra- and inter-day variation in the peak areas for a set of drug solutions (2 or 4 µg/mL) assayed 4 times on the same day and on 3 different days. The intra- and inter-day variation in the peak areas of drug solutions was calculated in terms of coefficient of variation.

Estimation in formulations: For the estimation of gliclazide in commercial tablet formulations, 10 tablets in each case were weighed and finely powdered.

Accurately weighed tablet powder equivalent to 50 mg of gliclazide was taken into a 25 mL volumetric flask containing 20 mL of acetonitrile and sonicated for 15 min. The volume was made upto 25 mL with acetonitrile and the solution was filtered through 0.45 μ membrane filter. The solution was suitably diluted with mobile phase to produce a concentration of 4 μg/mL and 20 μL of each were injected into the column for assay.

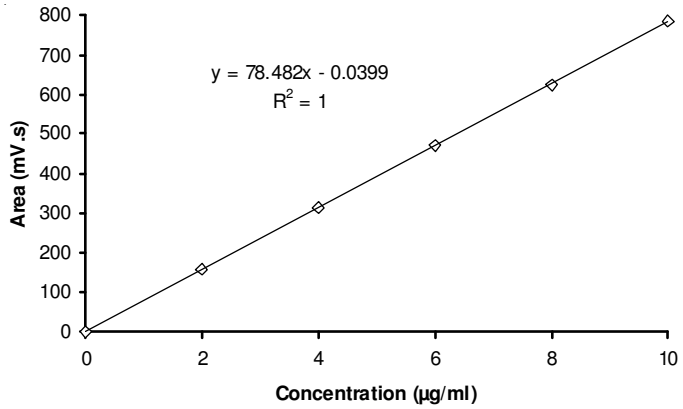

Fig. 1. Calibration curve for the estimation of gliclazide by the HPLC method developed

Estimation of gliclazide in plasma: The HPLC method developed was used for the estimation of gliclazide in plasma samples. For this purpose a calibration curve was constructed by analyzing plasma samples containing different amounts of gliclazide as follows.

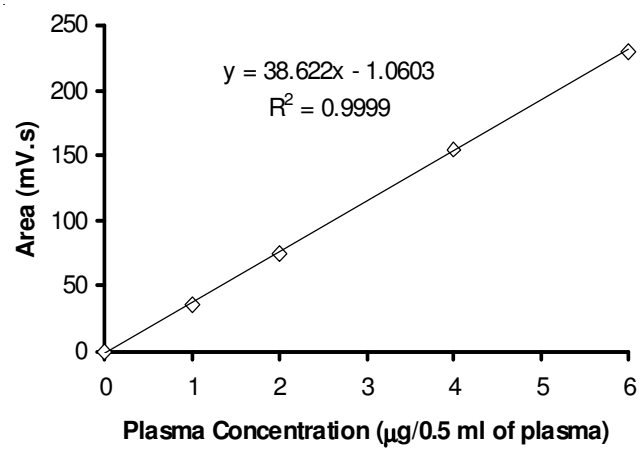

Fig. 2. Calibration curve for the estimation of gliclazide in plasma samples by the HPLC method developed

To a series of tubes containing 0.5 mL of plasma in each, 0.1 mL drug solution containing 1, 2, 4 and 6 µg of gliclazide were added and mixed. To each tube 1 mL of acetonitrile was added, mixed thoroughly and centrifuged at 5000 rpm for 20 min. The organic layer (0.5 mL) was taken into a dry tube and the acetonitrile was evaporated. To the dried residue 0.5 mL of mobile phase [a mixture of water containing 0.1 % w/v sodium phosphate monobasic (pH adjusted to 2.1 using phosphoric acid) and acetonitrile (34:66)] was added and mixed for reconstitution. Subsequently 20 µL were injected into the column for HPLC analysis.

Pharmacokinetic study: The *in vivo* protocols were approved by Institutional Animal Ethics Committee. Healthy rabbits of either sex weighing 1.5-2.5 Kg were fasted over night. Gliclazide was administered at a dose of 10 mg per rabbit (n = 6). After collecting the zero hour blood sample (blank), the product in the study was administered orally with 10 mL of water. Blood samples (3 mL) were collected from marginal ear vein at 0.5, 1, 2, 3, 4, 6, 8, 12, 16, 20 and 24 h after administration. Samples were collected in heparinised tubes and were centrifuged at 10000 rpm for 10 min. The plasma separated was collected into dry tubes and the samples were stored under refrigerated conditions prior to assay. Plasma concentrations of gliclazide were determined by the HPLC method developed as described above. From the time *vs.* plasma concentration data (Fig. 3) various pharmacokinetic parameters such as peak concentration (C_{max}), time at which peak occurred (T_{max}), area under the curve (AUC), elimination rate constant, (K_{el}), biological half-life ($t_{1/2}$), per cent absorbed to various times and absorption rate constant (K_a) were calculated in each case as per known standard methods.

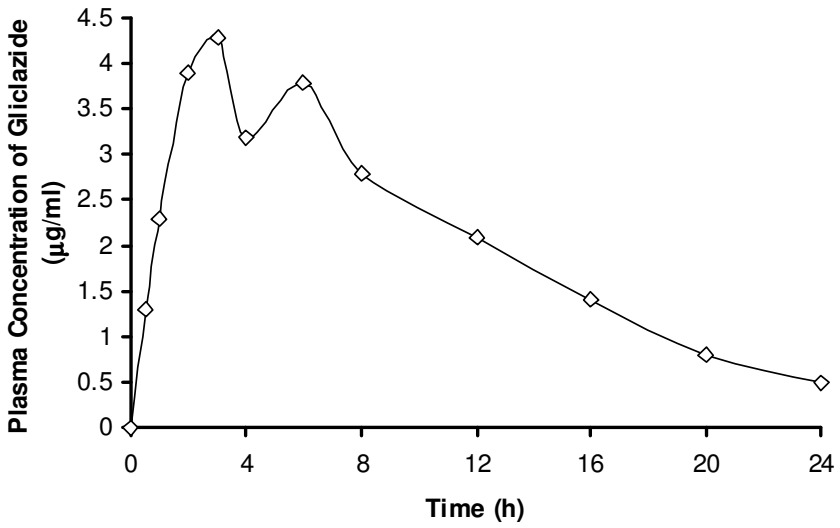

Fig. 3. Time *vs.* Plasma concentration profile following the oral administration of gliclazide in rabbits

RESULTS AND DISCUSSION

The mobile phase consists of water containing 0.1 % w/v sodium phosphate monobasic (pH adjusted to 2.1 using phosphoric acid) and acetonitrile (34:66). The retention time for gliclazide was 5.3 min. The peak areas at different concentrations were reproducible as indicated by low coefficient of variation (1.23 %). When the concentrations of gliclazide and their peak areas were subjected to regression analysis by least squares method, a good linear relationship (r = 0.9999) was observed between the two in the range 0.1-20 μg/mL. The regression of gliclazide concentration (μg/mL) over its peak area was found to be y = 0.7140 + 78.375x where y is peak area and x is concentration of gliclazide (μg/mL). This regression was used to estimate the amount of gliclazide in formulations.

The HPLC method developed was validated for intra- and inter-day variation. When the solutions containing 2 and 4 μg/mL of gliclazide were injected repeatedly on the same day and on other days, the coefficient of variation in amounts estimated was less than 1.28 % (Table-1). The results indicate that the HPLC method is highly reproducible. In the accuracy assessment, the recovery was found to be 99-100 % (Table-2). Thus the method is highly accurate.

TABLE-1
PRECISION OF THE PROPOSED HPLC METHOD

Gliclazide concentration (μg/mL)	Amount of gliclazide (μg/mL) found on	
	Intra-day (% CV) (n=4)	Inter-day (% CV) (n=4)
2	1.997 (0.029)	1.993 (0.28)
4	3.970 (1.280)	3.960 (1.06)

TABLE-2
RECOVERY OF GLICLAZIDE

Amount of drug added (μg)	Mean (± SD) Amount (μg) recovered (n = 4)	Mean (± SD) % of recovery (n = 4)
20	19.95 ± 0.47	99.75 ± 1.68

The HPLC method developed has also been used for assay of two commercial brands of gliclazide. The drug content of the commercial tablets was found to be 97.23-98.70 % of the labeled amount (Table-3). No interfering peaks were found in the chromatograms of tablet assay indicating that excipients used in the tablet formulations tested did not interfere with the estimation of drug by the HPLC method developed.

A good linear relationship was observed between plasma concentration of gliclazide and peak area. The relationship could be expressed by the following linear equation:

$$y = 38.622x - 1.0603$$

TABLE-3
ASSAY OF GLICLAZIDE COMMERCIAL BRANDS

Brand name	Labeled amount (mg)	Estimated amount (mg) (± SD)	Drug content (%)
Glycinorm 40	40	38.89 (0.55)	97.23
Glizid 40	40	39.48 (0.24)	98.70

where x is plasma concentration of gliclazide and y is peak area. This linear regression equation was used for estimating plasma concentrations of gliclazide in the *in vivo* pharmacokinetic study. The precision (RSD) of the method was less than 0.46 %.

The pharmacokinetic parameters estimated following the oral administration of gliclazide are given in Table-4. The elimination rate constant (K_{el}) for gliclazide was found to be 0.1216 h^{-1} and the corresponding half life was found to be 5.7 h following the oral administration of gliclazide. The $t_{1/2}$ value of gliclazide obtained in the present work is in good agreement with the earlier reported[12] value of 6.46 ± 1.05 h. The mean residence time (MRT) was found to be 9.19 h. The absorption rate constant (K_a) was found to be 0.9012 h^{-1}. A C_{max} of 4.3 ± 0.14 µg/mL was observed at 3.0 h after oral administration of gliclazide. A second peak concentration of 3.8 ± 0.12 µg/mL was observed at 6.0 h after administration. The second peak observed is due to the biliary excretion of gliclazide into the G.I tract and subsequent reabsorption. Later the plasma concentrations were decreased rapidly.

TABLE-4
PHARMACOKINETIC PARAMETERS ESTIMATED FOLLOWING
THE ORAL ADMINISTRATION OF GLICLAZIDE

Pharmacokinetic parameter	Estimated value
C_{max} (µg/mL)	4.3
T_{max} (h)	3.0
K_{el} (h^{-1})	0.1216
$t_{1/2}$ (h)	5.7
$(AUC)_o^{24}$ (µg h/mL)	49.575
$(AUC)_o^{\infty}$ (µg h/mL)	53.687
K_a (h^{-1})	0.9012
MRT (h)	9.91

Conclusion

(i) A simple, accurate and sensitive RP-HPLC method was developed and validated for the estimation of gliclazide in bulk drug samples, its formulations and in plasma samples using RP C-18 column with UV detection at 230 nm. (ii) The intra and inter day coefficient of variation was less than 1.28 % showing high precision of the method. The method was highly accurate with a recovery in the range 99-100 %. (iii) The method was found suitable for estimating gliclazide in formulations and also in plasma samples in pharmacokinetic studies. The pharmacokinetic parameters of gliclazide estimated employing the HPLC method developed agreed well with the literature values.

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