RESEARCH ARTICLE

Liquid Chromatography-Tandem Mass Spectrometry Method for the Estimation of Gliclazide in Human Plasma: Application to Bioequivalence Study

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ABSTRACT

Gliclazide was estimated in human plasma using a very sensitive and fast liquid chromatography method coupled to mass spectroscopy (LC-MS), where glipizide was used as an internal standard (IS). Samples were prepared using the liquid-liquid extraction method and analyzed by employing a C18 column with a mobile system composed of methanol (90):water (10):formic acid (0.1) (v/v/v). Gliclazide was chromatographed and analyzed by MS detector equipped with electro-spray ionization (ESI) method under positive ion mode with multiple reactions monitoring (MRM) for recording the transition for gliclazide (m/z parent ion 324.1 and daughter ion 127.2) and IS (m/z for parent ion 446.2 and daughter ion 321.1). In this linear method (20-9125 ng/mL), the minimum quantifiable value was 20.1 ng/mL. The recovery of the extraction process for gliclazide was 100.5% and for glipizide (internal standard) was 102.5 % from a biological matrix. The validation of the method was done as per USFDA regulatory guidelines so that this method can be used to precisely evaluate pharmacokinetic parameters in the regulated bioequivalence studies.

Keywords: Analysis, Bioequivalence, Gliclazide, Mass spectrometry, Pharmacokinetics, Validation, Stability.

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INTRODUCTION

The past three decades have witnessed type 2 diabetes mellitus and its complexities reaching the global levels, especially in developing countries, accounting for about 75% of the patient population. Sulfonylurea derivatives have been successfully used to treat T2DM, the major advantage being their long-term safety and economic benefits.[1,2] Gliclazide, a second-generation sulfonylurea, shows the antidiabetic effect by activating islet cells of the pancreas engaged in secreting insulin via inhibition of ATP-dependent K^+ channels in the β cells of the pancreas and through enhancement of peripheral tissues response to insulin.[3] Gliclazide exhibits effectiveness similar to glimepiride and glibenclamide and has shown an effective lowering of the glycated hemoglobin level (HbA1c) compared to glipizide.[2] The usual recommended amount of drug is 4. 0 mg–8.0 mg per day, which can be slowly raised to 320 mg per day, if necessary. A modified-release (MR) tablet dosage form with a starting dose of 30 mg once daily is also available, which can be maximized to 120 mg daily if required. It is rapidly taken up by GIT and is exclusively attached to plasma proteins. The drug has a duration of action of 12–24 h and a half-life of about 10–12 h.[4]

Various chromatographic procedures, including gas chromatography, HPLC semi-micro HPLC, and LC/MS, must be used and validated to quantify gliclazide in biological samples.[5-12] The greater retention time,[3-6] lesser sensitivity[7] longer analytical time and usage of large volumes of plasma[8] and expensive solid-phase extraction procedures[9] are the major limitations of the existing chromatographic methods for gliclazide estimation.[10-12] This necessitates changes in methods for fast detection of gliclazide in bio-specimens, specially for bioequivalence studies.

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This investigation attempts to develop and validate a fast and accurate analytical method for estimating gliclazide, employing LC-MS with a triple quadrupole mass analyzer in human plasma. The method described in the present research work has the advantages of smaller quantity with a short analysis time compared to the existing techniques. The present process must be used to analyze pharmacokinetic parameters upon a single oral dose of 80 mg gliclazide to twenty-six healthy subjects (no. of volunteers = 26). A pharmacokinetic comparison between different gliclazide 80 mg tablets was performed to establish bioequivalence of the two products of Gliclazide® (Generic version) and Diamicron® (Servier Laboratories Ltd., United Kingdom).

MATERIALS AND METHODS

Chemicals and Reagents

Standard (for reference) for gliclazide (99.27% purity) with glipizide (99.69% assay) was purchased from Vivan Life Sciences, Mumbai, India. Plasma samples (human) with anticoagulant, tripotassium ethylene diamine tetra acetate (K3EDTA), were obtained as a sample from Mediplas Laboratories, Hyderabad, India. Components of human plasma samples were separated chromatographically to check for interferences before use. The batches of plasma that were found with no traces of significant interferences were pooled to prepare calibration standards and quality control (QC) samples for evaluation during method validation. All the plasma matrices were stored at -20 ± 10ºC. HPLC-grade methanol and water were used during the analysis.

Liquid Chromatographic-Mass Spectrometric Parameters

The separation using chromatography was obtained using the HPLC system from Shimadzu supported using Ascentis Express C18 column (100 x 4.6 mm, 2.7 µm) kept at a temperature of 40 ± 2ºC using Shimadzu Column Oven CTO-20A. The mobile phase consisted of Methanol: Water: Formic Acid (90:10:0.1 (v/v/v) at a flow rate of 1.0 ml/minute (with splitter). The run time was 2.0 minutes.

The triple quadrupole mass spectrometer (MS) (API 3000™) (AB Sciex) coupled with an electro-spray ionization source was run in the positive mode of ion, and measurements were carried out with multiple reactions monitoring (MRM). The following instrument conditions were set after optimization, nebulizer (NEB) gas was set at 12.00 psi, collision activated dissociation (CAD) gas at 6.00 psi, Curtain Gas (CUR) at 12.00 psi, Ion Spray Voltage (ISV) was 3000.00 V, Source Temperature was 500^0 C and Entrance Potential (EP) 10.00 V. Parameters depending on a compound like, Collision Energy (CE) was set at 27.00 V and 21.00 V for Gliclazide and IS, respectively. Focusing potential (FP), De-Clustering Potential (DP), and Cell Exit Potential (CXP) were kept at 350.00 and 310.00, 56.00 and 46.00, 12.00 and 10.00, respectively for Gliclazide along with Internal Standard. The multiple reaction monitoring pair was m/z 324.1/127.2 for gliclazide and for internal standard m/z 446.2/321.1, and Dwell time was set at 200.00 ms.

Calibration Standards and Quality Control Samples

Stock solutions of gliclazide and glipizide (IS) (2 mg/mL) were prepared using methanol as the diluent. Gliclazide and IS final concentrations were adjusted to account for potency and the actual amount weighed. Working solutions in the concentration range of 20–9125 ng/mL were made by diluting the stock solution using methanol as the diluent. The prepared stock solutions were diluted with methanol: water (50:50 v/v). The internal standard working solution of 25000 ng/mL was prepared. Before use, all samples were stored at 2-8°C and brought to room temperature before use. Standards for preparing calibration curves for gliclazide were prepared by mixing 20 µL of working standard solutions of various concentrations with 1000 µL of blank plasma to obtain calibration standards of concentration 20.1, 40.3, 309.8, 2065.0, 4130.1, 5817.0, 7756.0, and 9124.8 ng/mL.

The control standards were separately prepared by a similar method at the concentrations of 20.2, 57.6, 3789.5, and 7579.0 ng/mL as control samples at the limit of quantitation (LoQ), low-quality control (LQC), middle-quality control (MQC) and high-quality control (HQC). These plasma standards were required to be taken out from respective storage areas for the preparation of samples for each batch of analysis, including unknown samples.

Preparation of Sample

Blank samples, standards for the calibration curve, and control samples were removed from the sample storage equipment and thawed at room temperature. Models and standards used in the batch analysis were vortexed to mix the contents uniformly. 200 µL specimen was sampled into a vial, and 50 µL of IS dilution (25000 ng/mL) and 200 µL of 0.1% solution of formic acid were added to it and vortexed. Further, extraction solvent (3 mL) (diethyl ether: dichloromethane, 7:3) was then added and vortexed. Such tubes were spun in a refrigerated centrifuge at 4500 rpm for 5 minutes at 5ºC. A 1 mL of the supernatant from the vial was transferred into the glass tubes and dried on N_2 evaporator at about 50ºCand 20 psi until it was dry completely. Then samples were reconstituted using 1 mL of reconstitution solution (methanol: water, 9:1) and vortexed. The solution was then transferred to clean autosampler vials. 10 µL was injected into LC-MS/MS for testing. Low light conditions were used for testing.

Procedure for Development and Validation of Protocol

Ten lots of plasma, including one lipaemia and hemolysis plasma with tripotassium ethylene diamine tetra acetate, were tested to ascertain interference from the endogenous parts with determinations of gliclazide and IS. The plasma batch that interfered least with the determinations of gliclazide and IS was used to prepare and evaluate by chromatography six specimens at the LOQ level.

To evaluate the sensitivity of the method, the extracted placebo samples were compared with the extracted LoQ specimens along with the internal standard, and the percentage

interference was calculated by the peak response area of blank samples at the RT of Gliclazide and IS compared to the average response area of LOQ at a retention time of Gliclazide and IS respectively.

A ratio of the peak area covered by gliclazide to the peak area of IS was used for the quantification of gliclazide in plasma samples. The standard plot was prepared with an area under peak ratios on Y-axis, the respective standard gliclazide concentration was used on X-axis, and the standard plot was based on the equation, $Y = A + BX$ with weighted $(1/x^2)$ least squares linear regression.

For reproducibility assessment, three sets of precision plus accuracy samples were used for the analysis. The accuracy of the determination was calculated as the ratio of the back-calculated value of the QC samples to the respective nominal values and expressed as a percentage (% nominal).

The percentage recovery of extracted analyte and the IS were checked at three QC levels, LQC, MQC, and HQC. The procedure involved measuring the mean area of peak response for the analyte and IS in spiked QC compared to the mean area of peak response of analyte and IS for an aqueous QC sample of the same concentration.

The effect of diluting the biological sample on sample concentration was evaluated by analyzing six control samples at the level of 16333.8 ng/mL, which was almost twice the maximum quantifiable limit, and then diluting it 2 times and 4 times respectively with blank plasma and analysing using freshly spiked calibration curve standard samples processed at the same time.

Matrix effect was observed by analyzing duplicate specimens at LQC & HQC samples spiked in six different blank plasma lots with six replica samples of aqueous injections at an equivalent level and comparing the area peak response of the matrix-based samples against the aqueous samples of the same class.

The ruggedness of the bioanalytical technique was tested by analyzing six replicas each of control samples of LLOQ, LQC, MQC, and HQC and analyzing them against the set of calibration standards with another same make chromatographic column by another analyst following the same procedures.

The stability of stock solutions and the reference mix containing Gliclazide and IS was assessed at the respective storage conditions of RT and temperature of 2 to 8ºC for the desired period of storage. The stability was calculated by observing the peak area response observed in the stability samples with the fresh sample dilutions at the same concentration of Gliclazide and IS, respectively.

Benchtop extraction stability (samples were kept idle at the bench top after every critical step during the extraction for a period of 2 hours), and long-term stability in human plasma (samples stored for 18 days in a cold room at -20–10°C) were determined for gliclazide. The stability samples were assessed against freshly spiked and prepared calibration curve standards along with four sets of freshly spiked and prepared QC samples (comparison samples) at concentration levels of LQC and HQC.

Pharmacokinetic Study

Healthy people participated in an open-label, randomized, two-treatment, two-sequence, two-period, single-dose, crossover bioequivalence research of Gliclazide 80 mg tablets and Diamicron® 80mg tablets (containing 80 mg gliclazide) (Servier labs ltd., United Kingdom). The Sentinel Independent Ethics Committee set the pharmacokinetic parameters. The subjects signed the informed consent in written format according to the principles of the Declaration of Helsinki (1964) revised version of 1996 (Somerset West). Blood samples (5 mL) were collected at intervals of 1 to 72 hours from the forearm vein, before and post administration of gliclazide, and stored in K3EDTA heparin-containing tubes. Human Plasma samples were removed by centrifugation at 3200 rcf for up to 15 minutes with storage at −20°C till the end of the analysis. The validated method determined the plasma level of Gliclazide and Glipizide (internal standard) in the blood samples. The ADME parameters were determined via concentration-time data using Phoenix WinNonlin software (version 8.3).

Under fasting conditions, healthy people participated in a randomized, double-blind, two-treatment, two-sequence, single-dose, crossover bioequivalence research comparing Gliclazide 80 mg tablets with Diamicron® 80mg tablet (containing 80 mg gliclazide) (Servier labs ltd., United Kingdom). Statistical analyses were done on plasma Gliclazide with SAS version for Windows, Version 9.2 or above (SAS Institute Inc., USA), or the WinNonlin version 5.0.1 or above. A mixed effects ANOVA model with a type III sum of squares was used to examine the log-transformed pharmacokinetic parameters (Cmax, $AUC_{0\to t}$, and $AUC_{0\to\infty}$). The analysis of the ln-transformed data will yield intrasubject variability, which reflects residual variability after accounting for differences within subjects, periods, sequence, and formulations, and inter-subject variability, which reflects residual variability after accounting for differences between subjects, periods, sequence, and formulations.[13] For two products to be bioequivalent, the 90% confidence interval for the ratio of the average of pharmacokinetic parameters C_{max} $AUC_{0\to t}$ and $AUC_{0\to\infty}$ for test and reference product (geometric least square means) should be between 80% and 125% for the log-transformed data

Method Development

Different detection techniques are currently being used. However, hyphenated technique-liquid chromatography (LC) with mass spectrometry (MS) has proven superiority over other separation and detection techniques for conducting biological and analytical determinations with improved detection reliability, more selectiveness, and specificity[14-15]. Gliclazide and glipizide (IS) could be detected in positive ionization mode using ESI with increased selectiveness obtained by tandem mass spectrometric testing using multiple reactions monitoring (MRM) functions at m/z 324.1/127.2 for gliclazide and 446.2/321.1 for IS. The minimal level of formic acid in the mobile solution markedly promoted the analytes ionization, thus significantly improving the MS response of gliclazide with increased sensitivity[3]. Using the described chromatographic

conditions, a good peak symmetry was observed in both the analyte and IS. The mobile phase composition of Methanol: Water: Formic Acid [90:10:0.1 (v/v/v)] was optimized to get symmetrical peak shapes, reduced time of sample run, and diminished background noise and matrix effect. The method was employed for the pharmacokinetic evaluation of the gliclazide tablet dosage form.

Method Validation

Selectivity and Specificity

To test the selectivity of the method, ten lots of plasma (including one lipemic and haemolysed plasma with K3EDTA) were tested by this set extraction and chromatographic procedure. The Peak area response observed was compared to the same for the aqueous sample prepared for gliclazide and internal standard at a quantification limit (LOQ) concentration level. Sample chromatograms of reference mix solution, a blank sample of plasma, and blank plasma with IS and QC samples (LOQQC, LQC, MQC, and HQC) are presented in Figure 1 (A-G) No remarkable interferences were observed at the detection window of analyte or IS. The retention time was 1.00 and 0.95 min for Gliclazide and IS, respectively.

Linearity and Sensitivity

Linearity and sensitivity were checked by measuring regression of least square analysis using a weighting factor of 1/concentration^2 using eight points calibration curve. The results were found to be linear for the calibration curve range of 20.1-9124.8 ng/mL for gliclazide with a coefficient of regression (r) value of 0.9965 and coefficient of determination (r^2) value of 0.9930 (Figure 2). This method provided a quantitation limit (LOQQC) of 20.1 ng/mL. At LOQQC, the concentration for gliclazide, calculated with the analyte peak area to that of IS, was found to be 7.2 and 98.6%, respectively.

Accuracy and Precision

Three batches were tested to assess these parameters, and the result is presented in Table 1. The correctness of the assay can be expressed as the ratio from calculated mean values to their respective average range, shown in percent. The accuracy was expressed as the %CV for the concentration range of QC samples.

The inter-day accuracy was evaluated using accuracy with precision batches analyzed on two different days from

Table 1: Intra-day and inter-day precision and accuracy of gliclazide in human plasma (n = 12).

Sample ID	*Nominal Concentration (ng/mL)*	*Observed Concentration (ng/mL)*	*%Accuracy*	*%CV*
Intra-day				
LOQQC	20.2	19.64 ± 1.43	97.2	7.3
LQC	57.6	62.28 ± 4.28	108.1	6.9
MQC	3789.5	3915.9 ± 143.54	103.3	3.7
HQC	7579.0	7229.14 ± 194.52	95.4	2.7
Inter-day				
LOQQC	20.2	20.56 ± 1.13	101.8	5.5
LQC	57.6	61.06 ± 4.87	106.0	8.0
MQC	3789.5	3975.27 ± 125.39	104.9	3.2
HQC	7579.0	7474.98 ± 318.30	98.6	4.3

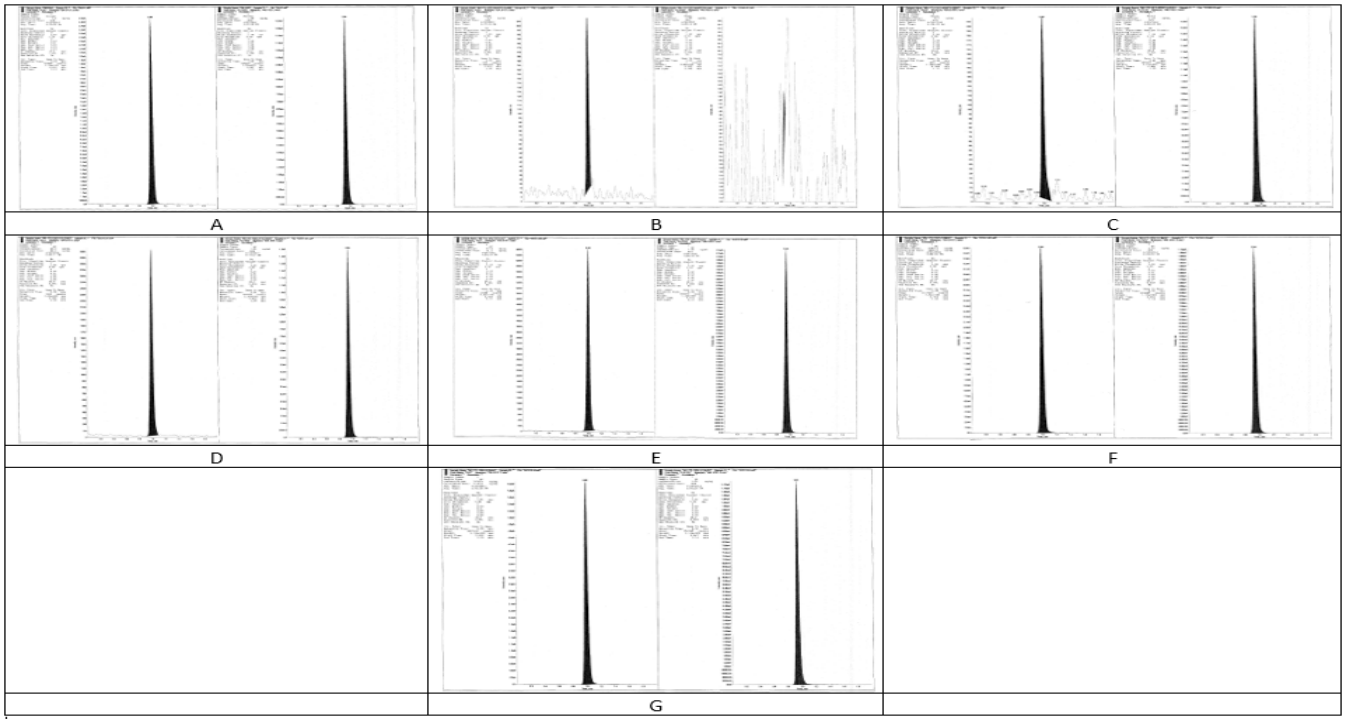

Figure 1: Representative chromatograms of reference mix solution (A), blank plasma (B), blank plasma with IS (C), and quality control (QC) samples; LOQQC (D), LQC (E), MQC (F), and HQC (G)

98.6–106.0% with 3.2–8.0%, respectively. Then intra-day accuracy and precision were evaluated using two precision and accuracy batches analyzed on the same day ranging from 95.4 to 108.1% and 2.7 to 7.3%, respectively, for gliclazide. The results were found as acceptable values. This indicated that an analytical technique was precise and hence acceptable for in-vivo applications.16

Extended Precision and Accuracy

Extended precision was assessed for a complete batch comprising about 118 samples which included standard calibration as well as control standards. The overall outcome is provided in Table 2. Accuracy was 95.5-103.0%, and obtained precision was 2.4–7.6% for gliclazide.

Recovery

The percent recovery of Gliclazide at LQC, MQC, and HQC QC specimens was 96.6, 103.0, and 102.0%. A %CV for recovery of an inter-quality control sample of gliclazide was 3.4%. The mean percent recovery for gliclazide was 100.5%. The percentage recovery of IS at MQC was found to be 102.5%.

Dilution Integrity, Matrix Effect, and Ruggedness

The dilution integrity was checked at diluting to the sample at 1:4 times and 1:2 times, and the percentage accuracy was found to be 109.1 and 97.6%, and precision was calculated to be 7.6% and 2.9% at 4 times and 2 times dilution level, respectively.

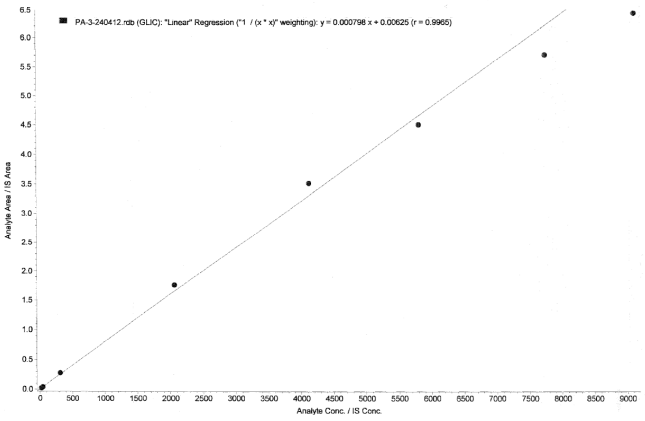

Figure 2: Calibration curve of gliclazide

Due to the presence of matrix in biological samples, the matrix effect suppresses or enhances the ionization of analytes. Matrix factor was calculated as the peak area ratio of Analyte to IS in the presence of matrix to peak area ratio of Analyte to IS in a sample with no matrix. The mean matrix factor for LQC and HQC samples was found to be 1.05 and 0.95, respectively, with the percent CV calculated to be 4.6% and 5.0%, respectively, at LQC and HQC.

The QC samples (6 sets) and standard calibration (one precision and accuracy batch) were analyzed using another column of the same type to determine the robustness of the extraction method along with the chromatographic method. Table 3 summarises the findings. Inter batches precision was 1.3–7.0%, with within batch accuracy ranging from 98.6 to 107.1%. The developed method met the acceptance criteria of linearity, precision, and accurate data of the QC samples, based on the results obtained.

Stability in Aqueous Samples

The stability of gliclazide in an aqueous solution was tested at Room temperature, and storage condition was maintained at temperature (2-8°C) for stock solutions. The samples for stability assessment were kept for the intended duration at applicable storage conditions. The %stability of the sample was checked by measuring the mean area response of samples with respect to the average area response of fresh samples prepared at the same concentration level.

The reference mix sample was found to be stable for 28 hours when stored at Room temperature. The percent stability of the reference mix for Gliclazide with Glipizide was 99.4%. The stock solution stability was 96.8 and 99.6% throughout 26.52 and 26.42 hours for gliclazide and IS, respectively. Stability of the gliclazide solution and Internal Standard was performed after a storage period of 19 days at refrigerated temperature (2 to 8°C)**,** and the percentage stability of the stock solution for gliclazide was 99.4% and for IS was 100.8%, respectively.

Stability in Human Plasma

All the stabilities were evaluated by comparing the mean peak area response of QC samples analyzed against a fresh prepared standard calibration curve. The comparative stability studies, including Freeze-Thaw, Bench-Top, Bench-Top Extraction,

Table 2: Extended Precision and Accuracy analysis of gliclazide

Sample ID	*Nominal Concentration (ng/mL)*	*Observed Concentration (ng/mL)*	*%Accuracy*	*%Precision*
LOQ	57.6	58.61 ± 4.43	101.8	7.6
MQC	3789.5	3901.73 ± 93.81	103.0	2.4
HQC	7579.0	7236.85 ± 207.88	95.5	2.9

Table 3: Ruggedness data calculated for QC samples (n = 6) using a different column (same type) by another analyst

Sample ID	*Nominal Concentration (ng/mL)*	*Observed Concentration (ng/mL)*	*%Accuracy*	*%Precision*
LOQQC	20.2	19.92 ± 0.86	98.6	4.3
LoQ	57.6	61.72 ± 4.31	107.1	7.0
MQC	3789.5	3968.60 ± 208.14	104.7	5.2
HQC	7579.0	7531.18 ± 94.67	99.4	1.3

In-Injector, and Long-Term studies of gliclazide in human plasma using K3EDTA as an anticoagulant ranged from 99.4 to 103.2%, 95.0 to 105.3%, 95.4 to 102.4%, 96.3 to 106.4 and 101.9 to 106.7%, respectively (Table 4).

The current study describes a reliable and accurate analytical technique for the routine testing of gliclazide in biological specimens to assess its pharmacokinetic profile. The technique is suitable for regular use owing to its simple extraction procedure and short analysis time.

Application in Healthy Volunteers

Gliclazide concentrations in plasma samples, collected at various time intervals upon single oral dose of 80 mg (test formulation) and Diamicron® 80 mg tablets (containing 80 mg gliclazide) (reference formulation) to (no. of subjects = 26) healthy volunteers, were detected using this technique. Samples of blood were taken at predetermined Time intervals up to 72 hours. Figure 3 explains the mean plasma concentration-time profile of gliclazide. The ADME values are summarized in Table 5. The least-square mean (LSM) in geometric values with 90% CI toward pharmacokinetic parameters of gliclazide are presented in Table 6. It was observed that the ratio and 90%

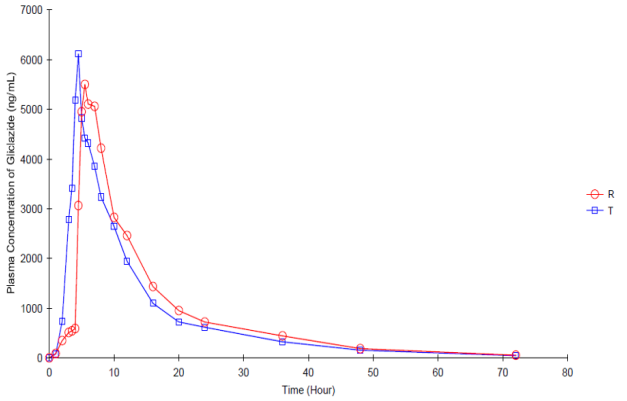

Figure 3: Plasma-concentration time profile of gliclazide after single oral administration of 80 mg gliclazide as test and reference formulation

Table 4: Stability of gliclazide and IS under different storage conditions

	Observed concentration (ng/mL)			
	LQC (57.6 ng/mL)		*HQC (7579.0 ng/mL)*	
Conditions	*Stability samples*	*Comparison samples*	*Stability samples*	*Comparison samples*
Freeze-thaw stability (after 3 cycles)	60.35 ± 5.52	58.60 ± 8.05	7505.55 ± 245.64	7569.30 ± 91.96
% Accuracy: 99.4 - 103.2				
Bench-top stability (at ambient conditions for 6.52 hours)	65.63 ± 3.66	62.33 ± 6.09	7218.20 ± 129.72	7602.08 ± 426.62
% Accuracy: 95.0 - 105.3				
Bench-top extraction stability (at bench top after every critical step during the extraction for a period of 2 h)	57.33 ± 0.74	59.90 ± 0.92	7660.50 ± 143.63	7458.38 ± 254.73
% Accuracy: 95.4–102.4				
In-injector stability (for 94.47 hours)	65.60 ± 3.64	61.75 ± 0.33	7347.33 ± 429.97	7648.15 ± 207.26
% Accuracy: 96.3–106.4				
Long-term stability of gliclazide in human plasma using K3EDTA as an anticoagulant (stored at -20 ± 10°C for 18 days)	56.48 ± 2.76	52.73 ± 2.89	7496.23 ± 371.23	7332.10 ± 203.13
% Accuracy: 101.9 – 106.7				

Table 5: Pharmacokinetic (PK) parameters of gliclazide after peroral administration of Gliclazide 80 mg tablets (Test formulation) and Diamicron® tablet (containing 80 mg Gliclazide) (Reference formulation).

Pk parameters	*Reference formulation*	*Test formulation*
T_{max} (h)	5.712 ± 1.0207	5.039 ± 1.2088
C_{max} (ng/mL)	5273.59 ± 891.025	5571.53 ± 1144.433
$AUC_{0\text{-last}}$ (h*ng/mL)	85515.1074 ± 32544.08458	86116.0947 ± 36192.02773
$AUC_{0\text{-}\infty}$ (h*ng/mL)	91116.8904 ± 39035.10386	92861.0452 ± 44237.26701
Vz_F_obs (mL)	24354.01 ± 1867.11	29114.03 ± 3140.60
Cl_F_obs (mL/hr)	1156.42 ± 167.82	1179.60 ± 215.87

Table 6: Summary statistics of Gliclazide test and reference formulation

		Geometric LSM		*T/R ratio*		*ISCV*	*Power*
Parameter	*Units*	*Ref.*	*Test*	*(%)*	*90% CI*	*(%)*	*(%)*
$Ln(C_{max})$	ng/mL	5273.59	5571.53	104.88	99.82%-110.19%	10.4	0.97
$Ln(AUC_{0\text{-last}})$	h*ng/mL	85515.1074	86116.0947	99.40	96.14% - 102.77%	7.0	0.99
$Ln(AUC_{0\text{-}\infty})$	h*ng/mL	91116.8904	92861.0452	100.10	96.55% - 103.78%	7.6	0.99

CI of the C_{max}, $AUC_{0\text{-last}}$, and $AUC_{0\text{-}\infty}$ geometric LSM of the test and reference formulations were within the acceptable bio-equivalence range of 80 to 125 %.[17-18] The ISCV (%) was observed to be 10.4, 7.0, and 7.6 for C_{max}, $AUC_{0\text{-last}}$, and $AUC_{0\text{-}\infty}$, respectively. ANOVA on the log values for the pharmacokinetic parameters for the difference between all the effects, namely treatment, sequence, and period, were found to be statistically insignificant ($p>0.05$), which indicated no significant differences between the test and the reference formulation.

CONCLUSION

The developed analytical method was found to be accurate, specific, and precise for detecting gliclazide in the concentration range of 20-9125 ng/mL using Glipizide as IS in human plasma. The lower sample run time can enhance sample throughput and is crucial for a large batch of samples typically in bioequivalence studies. The validated technique was successfully used for the assessment of pharmacokinetic data following an oral dose of a reference and test formulation for the bioequivalence studies in humans. The two gliclazide products were found to be bioequivalent.

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