International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.6, No.2, pp 692-700, April-June 2014

RP-HPLC determination of Gliclazide in Rat Plasma and its Application in Pharmacokinetic Studies

[1]*Lopamudra Adhikari, [2]U.S. Mishra, [2]P.N. Murthy

[1]School of Pharmaceutical Sciences;S'O'A University;Kalinga Nagar; Ghatikia; Bhubaneswar-751003; Odisha; India

[2]Royal College of Pharmacy and Health Sciences; Andhapasara Road; Berhampur; Odisha; India

*Corres.author: lopamudraadhikari@soauniversity.ac.in

Abstract: A very simple liquid chromatographic method was developed for Gliclazide for its quantification and was established by validating the method in rat plasma. The method was further extended for its pharmacokinetic studies as an application of the proposed method. As per the results obtained all the values were found to be within the limit as per ICH guidelines.Significantly, the proposed method proceeds without any use of buffer which apparently settles on with the advantages over other methods. An isocratic mobile phase of methanol, acetonitrile and water was involved with a detection wavelength set at 228nm.Male Albino rats were selected fulfilling all there prerequisites and the extraction procedure pursues simple solvent extraction steps. The stability of Gliclazide was evaluated with three studies, short term stability, long term stability and, freeze thaw stability and the results obtained were reasonable.C_{max} was observed at 2426.76±90.74 ng/ml with a T_{max} of 7.33±1.16 hours. The method was further extended for determination of other pharmacokinetic parameters with the present method and was found prudent to be used in routine QC analysis.
Keywords: Bioanalytical method;Gliclazide;Validation;Pharmacokinetic studies.

Introduction

Gliclazide is a second generation sulphonylurea that is widely used in the treatment of patients with type 2 diabetes because it has similar efficacy to other sulphonylureas but a lower risk of hypoglycaemia.[1-2]. Gliclazide selectively binds to sulfonylurea receptors (SUR-1) on the surface of the pancreatic beta-cells. It was shown to provide cardiovascular protection as it does not bind to sulfonylurea receptors (SUR-2A) in the heart.[3]Gliclazide undergoes extensive metabolism to several inactive metabolites in humans, mainly methylhydroxygliclazide and carboxygliclazide. CYP2C9 is involved in the formation of hydroxygliclazde in human liver microsomes and in a panel of recombinant human P450sin vitro.[4-5] But the pharmacokinetics of gliclazide MR are affected mainly by CYP2C19 genetic polymorphism instead of CYP2C9 genetic polymorphism.[6-7]Existing literature reports about method development of Gliclazide in its bulk and pharmaceutical dosage form in single and multicomponent dosage forms.[8] but single dosage form can also be effective and as per existing data it has got 94 percent protein binding factor. The present study involves development of a simple bioanalytical method for Gliclazide in its tablet dosage form.

Materials and Methods

Gliclazide pure drug was procured from Actavis pharmaceuticals LTD, Indrad, Dist. Mehsana (Gujarat). Formulations were available from the local market.Slovents were procured from Merck, India.

Other instruments used in the method development and validation include vortex mixer Remi, India), sonicator (Model SONICA® 2200 MH, Soltec, Italy), refrigerated centrifuge (Model C-24 BL, Remi, India) and deep freezer (Model BFS-345-S, Celfrost Innovations Pvt. Ltd., India). Membrane filters of 0.22 μm (Millipore, USA) were used for filtration of aqueous phase used in the mobile phase system**.**

Male Albino rats were procured from the Department of Pharmacology, SPS; SOA University with prior permission from IAEC.Human plasma was collected from SUM Hospital, Kalinga Nagar; Bhubaneswar.

Chromatographic Conditions:

An endcapped C18 reverse phase (RP) column Xterra column with a PDA detector from JASCO was used in the present investigation. The isocratic mobile phase consisted of methanol:acetonitrile:water(65:20:15). The HPLC system was stabilized for 1 h at 1 mL min^{-1} flow rate, through baseline monitoring prior to actual analysis. Gliclazide was monitored at wavelength of 228nm. An injection volume of 10 μL was optimized for final method.

Selection of Mobile phase:

In the process of analytical method development for Gliclazide, mobile phase composition and flow rate were optimized by trying different aqueous phase and non-aqueous phase combinations at different flow rates. Mobile phase composition and flow rate were finally selected based on the criteria of peak properties (retention time and asymmetric factor), sensitivity (height and area), ease of preparation and applicability of the method for in vivo studies in rats.

Collection and Separation of Blood Plasma

Blood was collected from retro-orbital plexus of Albino rats weighing between 120 to 200g. Prior permission was obtained for all experiments involving animals from the Institutional Animal Ethics Committee. Clear supernatant plasma was separated from blood after the centrifugation at 10000 rpm, 4° C, for 10 min. Samples were kept at −20 °C till further analysis.

Calibration Curve:

Aliquots of standard stock solutions of drugs were taken in 10 ml volumetric flask and diluted up to the mark with methanol in such a way that the final concentration of drugs were in the range of 250-5000 ng/ml and analytical quality control samples for studying the absolute recovery of plasma quality control samples (275,1500,4500 ng/ml) were prepared by making appropriate dilutions in methanol.

Blank sample was prepared by spiking 10 μL of methanol (drug diluent) in 90 μL of blank plasma. All solutions were stored at 4 °C until further use.

Extraction Technique:

The blood sample was centrifuged at 10000pm for 10 minutes. The supernatant liquid was separated out and twice the volume of acetonitrile was added to the sample and centrifuged at 10000 rpm for 5 minutes.

Required drug at required proportion was added and 10ml of acetonitrile was added and centrifuged. It was then filtered through Whattmann filter paper.The solution was then filtered through membrane filter and taken for further analysis in HPLC.

Method Validation

Linearity:

Plasma calibration standards were prepared and analyzed in five independent runs. Daily standard curves were constructed using the observed peak area to that of nominal concentration. Unknown concentrations were

computed from the linear regression equation of the peak area against the concentration. Calibration curve was constructed from a blank sample (plasma sample processed without drug) and eight non-zero concentrations ranging from 250-5000 ng/ml.

Accuracy:

For determining the accuracy of the proposed method, different quality control (QC) levels of Gliclazide in plasma (LQC = 275 ng/ml, MQC = 1500 ng/ml and HQC = 4500 ng/ml) were prepared independently and analyzed (n = 6).

Precision:

Repeatability was determined by analyzing all three QC levels of drug concentrations. Inter-day and intra-day variation and analyst variations were studied to determine intermediate precision of the proposed method. Three QC levels of drug concentrations in triplicates were prepared twice in a day and studied for intra-day variation (n = 6). The same protocol was followed for three different days to study inter-day variation (n = 18). The percent relative standard deviation (%RSD) was calculated from the predicted concentrations obtained by regression equation.

Recovery Study:

Recovery of the drug was determined by comparing the area obtained from plasma (extracted) samples with analytical standard (unextracted) samples. For the recovery experiment, plasma extracted samples were prepared by spiking Gliclazide at three different concentration levels (LQC, MQC and HQC) in triplicate.

Stabilty Studies

The stability of Gliclazide was evaluated with three studies,short term stability,long term stability and,freeze thaw stability.Plasma blank samples were spiked with the selected drugs individually at a concentration of 275ng/mL(LQC),1500ng/mL (MQC)and 4500 ng/mL(HQC).Each concentration was carried out for 6 times.Plasma extraction of drugs was processed in the same way as described previously.

Pharmacokinetic Study

Gliclazide formulation (for oral administration) was prepared by triturating the formulation and making a suspension with 0.2ml of Tween-80 and 5 ml water just before the commencement of study. Formulation was administered orally to the male Albino rats (n=6) weighing between 120 to200g.The animals were housed in a temperature controlled environment prior the experiment Blood samples were drawn from retro-orbital plexus of Albino rats at 0.3,0.5,0.7,0.8,0.9,1.0,1.5,1.8,2.0,2.5, 5.0,10.0, 20.0,24.0,28.0,30.0 and 36.0 hours post dose in microfuge tube pretreated with sodium citrate solution (3.8% w/v). All samples were processed according to the procedure described earlier and analyzed using the validated HPLC method.

Various pharmacokinetic parameters were calculated from measured Gliclazide plasma concentrations verses time profiles after oral administration using non-compartmental model.

From the data of plasma concentration at each sample time, the maximum plasma concentration (Cmax, ìg/ ml); and the corresponding time for the maximum plasma concentration (t_{max}, h) were directly determined from each individual animal.

Table.1: Optimized chromatographic conditions for RP-HPLC method for Gliclazide in rat plasma

Parameters	**Conditions**
Stationary phase(column)	XTERRA RP 18 5µm
Mobile Phase	Methanol:Acetronitrile:Water(60:20:15)
Flow rate (ml/min)	01
Run time (minutes)	10
Column temperature(^{0}C)	Ambient
Volume of injection loop (µl)	10
Detection wave length (nm)	228
Drug R_t (min)	3.15
Linearity (ng/ml)	250-5000

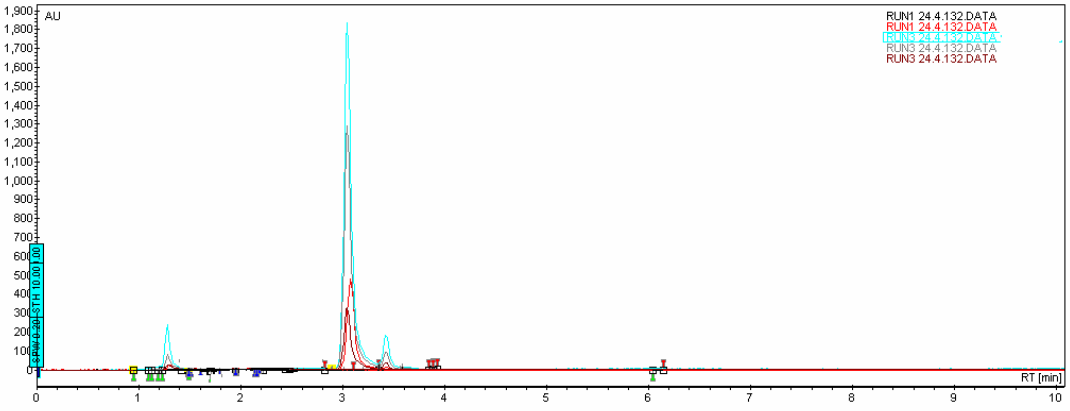


Fig.1: Overlain chromatograms for Gliclazide in rat plasma

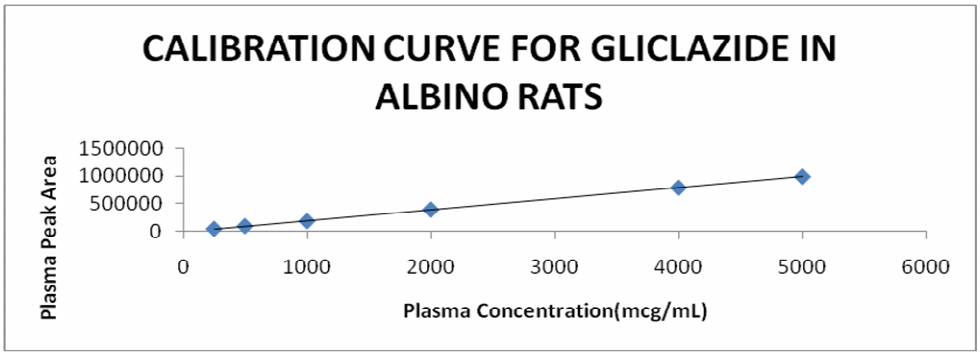


Fig.2: Calibration Curve for Gliclazide in rat plasma

Results and Discussion

Method Development

The isocratic mobile phase consisted of methanol: acetonitrile: water (65:20:15) at a flow rate of 1 mL min^{-1} was selected as optimal condition for the developed method. With optimized mobile phase condition, retention time of Gliclazide was found to be 3.15 ± 0.21 min with an asymmetric factor of 1.01 ± 0.10.

Construction of calibration Curve

An overlain chromatogram has been depicted in Figure 1 and a graph was plotted between plasma peak area and plasma concentration (Figure:2) which gives a regression of 0.999 and slope and intercept values of 199.3 and -2834 respectively.

Method Validation

Validation of the bioanalytical method was through and the individual statistical results were found to be within the limit as certain from Table 2,3 and 4.

Stability Studies for Gliclazide in rat plasma by RP-HPLC method

Stability studies as a part of validation was done in three different phases like short term stability, long term stability and freeze thaw stability and the corresponding concentrations were calculated which did not show much variations including the elution time of Gliclazide in plasma as depicted in Figure 3,4 and 5.

Table.2: Accuracy data of RP-HPLC method for Gliclazide in rat plasma

Q C Conc. (ng/mL)	%Amount added	Accuracy*	%Recovery*	Statistics*
275	**80**	-0.24	99.76	**Mean=99.8 ±S.D=0.12 %RSD=0.13**
	100	-0.23	99.76	
	120	-0.13	99.86	
1500	**80**	-0.01	99.99	**Mean=99.83 ±S.D=0.15 %RSD=0.17**
	100	-0.18	99.81	
	120	-0.30	99.69	
4500	**80**	-0.09	99.91	**Mean=99.94 ±S.D=0.03 %RSD=0.05**
	100	-0.04	99.96	
	120	-0.05	99.94	

*Mean of six readings

Table.3: Results and statistical data of Intra-day study and Inter-day study of RP-HPLC method for Gliclazide in rat plasma

Sl.No.	Quality Control Concentration (ng/mL)	*Statistical Analysis	
		Intraday	**Interday**
		Mean±S.D	
1	275	99.16±1.06	98.85±1.16
2	1500		
3	4500		

*Mean of six readings

Table.4: Limit of detection & limit of quantitation of RP-HPLC method for Gliclazide in rat plasma

Sl.No	Parameters	S.D	Slope(b)	Formula	Calculation (µg/ml)
1	**LOD**	1.16	199.3	$3.3\left(S.\frac{D}{b}\right)$	0.019
2	**LOQ**	1.16	199.3	$10\left(S.\frac{D}{b}\right)$	0.058

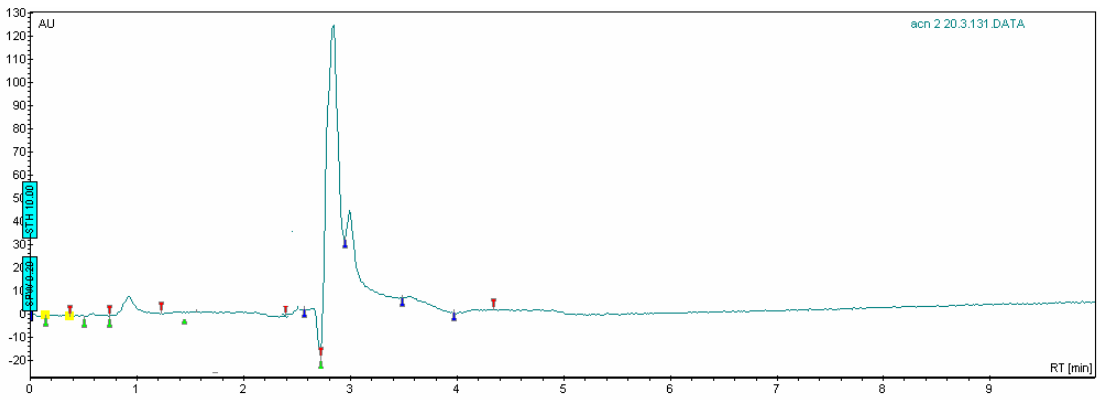


Fig.3: Representative chromatogram for Gliclazide for short term stability at 1500 ng/mL

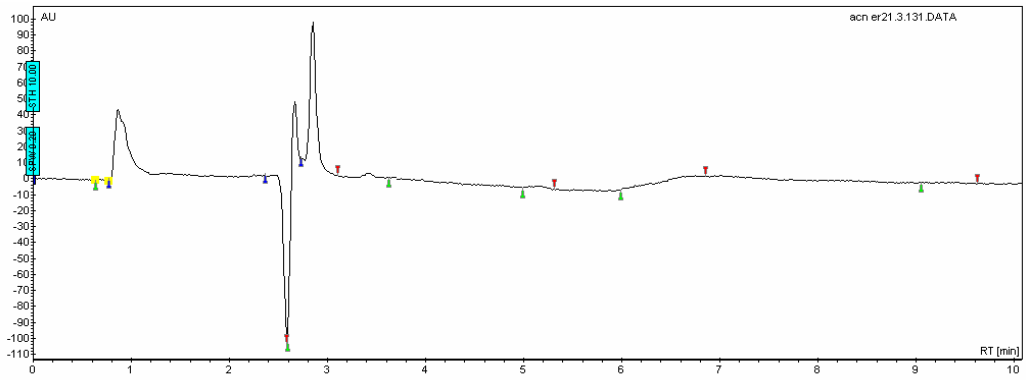
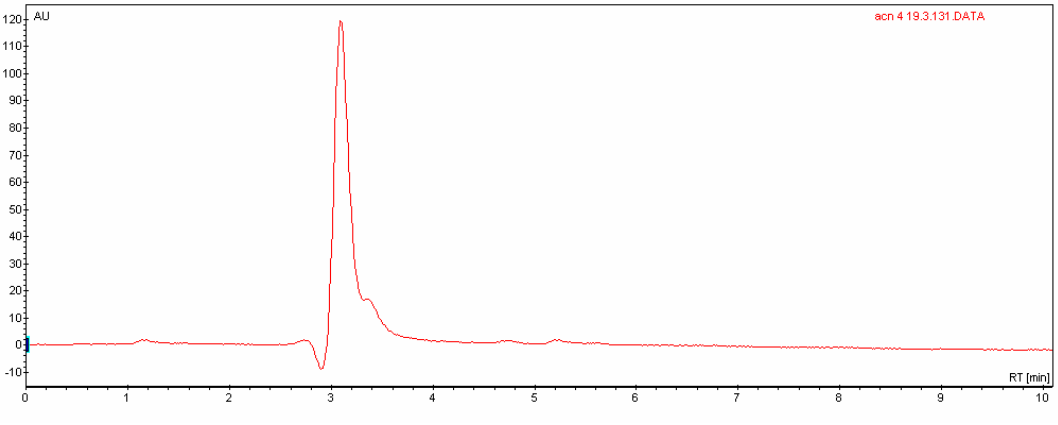
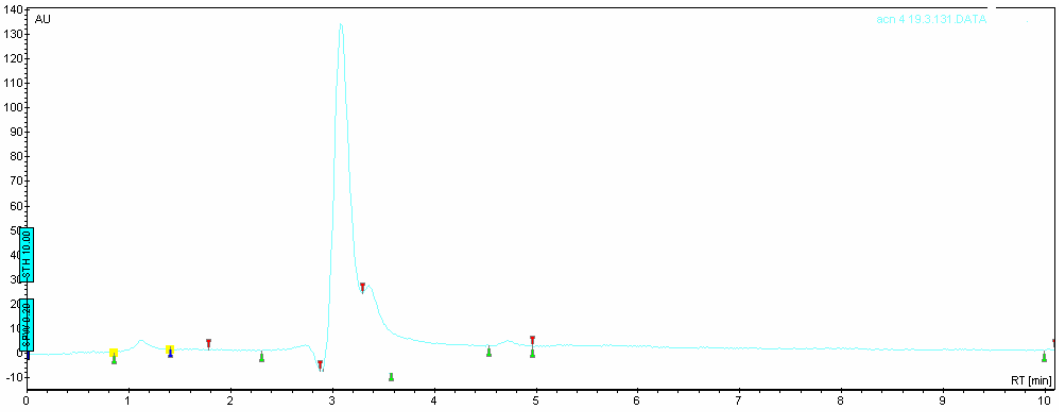


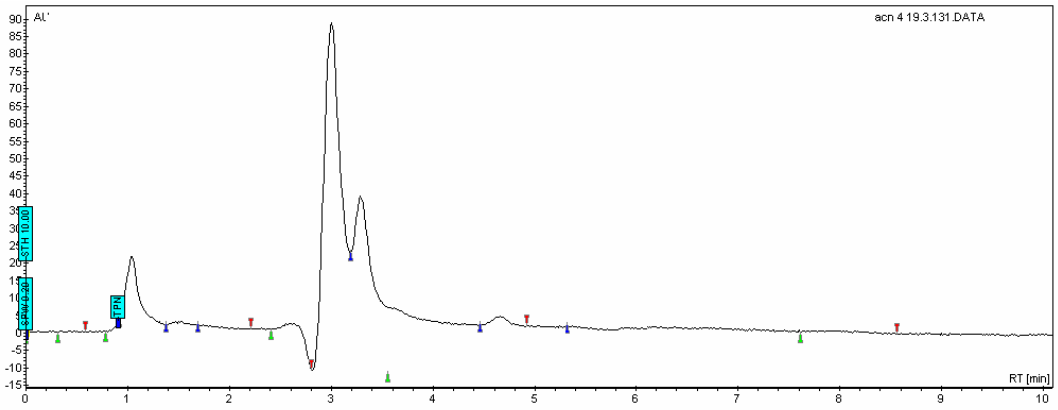
Fig.4: A typical chromatogram showing the effect of long term stability on Gliclazide in plasma



(a)



(b)



(c)

Fig.5 (a) ;(b) ;(c): Chromatograms showing effect of Freeze thaw stability on Gliclazide in rat plasma at three QC concentrations

Table.5: Analysis from Zero Moment Curve for Gliclazide in rat plasma

C_{max}(ng/mL)	T_{max}(hours)	$AUC_{(0-t^*)}$(nghr/mL)	$AUC_{(t^*-\alpha)}$ (nghr/mL)	$AUC_{(0-\alpha)}$ (nghr/mL)	$K_{elimination}$ ($hour^{-1}$)
2426.76±90.74	7.33±1.16	34630±22.87	30150±51.22	64790±271.57	0.095±0.02

Table.6: Clinical Implications by Gliclazide in rat plasma

$AUMC_{(0-t^*)}$ (ng hr/mL)	$AUMC_{(t^*-\alpha)}$ (ng hr/mL)	$AUMC_{(0-\alpha)}$ (nghr/mL)	Mean Residence Time (hr)	Half life(hrs)	Cl_{total} ($litrehour^{-1}$)	Vd(liter)
$7.8x10^{-6}$±250.09	$2.5x10^{-5}$±123.65	10.22×10^{-4}±11.09	1.90±0.12	7.29±2.87	0.07±0.002	0.074±0.02

Pharmacokinetic Study

The developed and validated HPLC method was applied to determine the pharmacokinetic parameters following oral administration of Gliclazide formulation in rats. The mean plasma concentration versus time profile of Gliclazide obtained following the oral administration is given in Figure 9. Sample injected ,extracted from rat plasma was found with no change in the retention time with different time intervals.Model chromatograms are shown in figure 7 and 8.

The pharmacokinetic parameters obtained from the study using non-compartmental and compartmental analysis:

Area Under the Curve (AUC): The peak plasma concentration and time to C_{max} were determined from the observed plasma concentration –time table and other pharmacokinetic parameters were computed.

The first moment is calculated as concentration times time (Cp • t). The AUMC is the area under the concentration times time versus time curve.

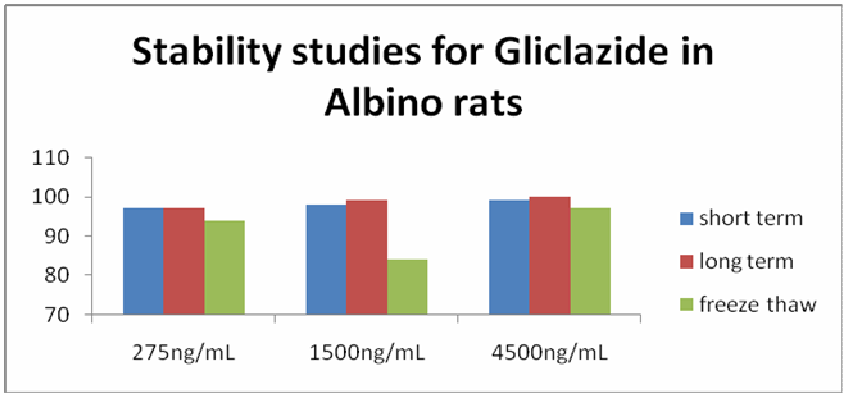


Fig.6: Graphical representation for stability studies in Gliclazide

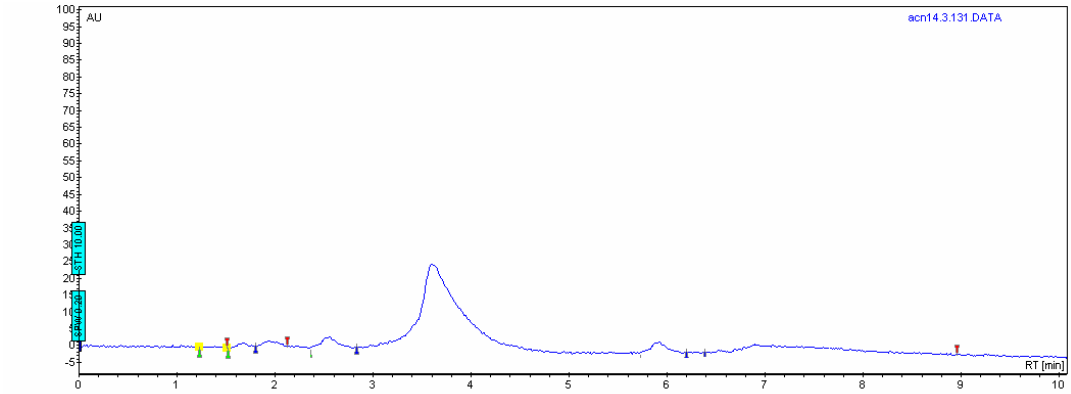


Fig.7: Chromatogram for Gliclazide at 0.5 hour of drug administration in rat plasma

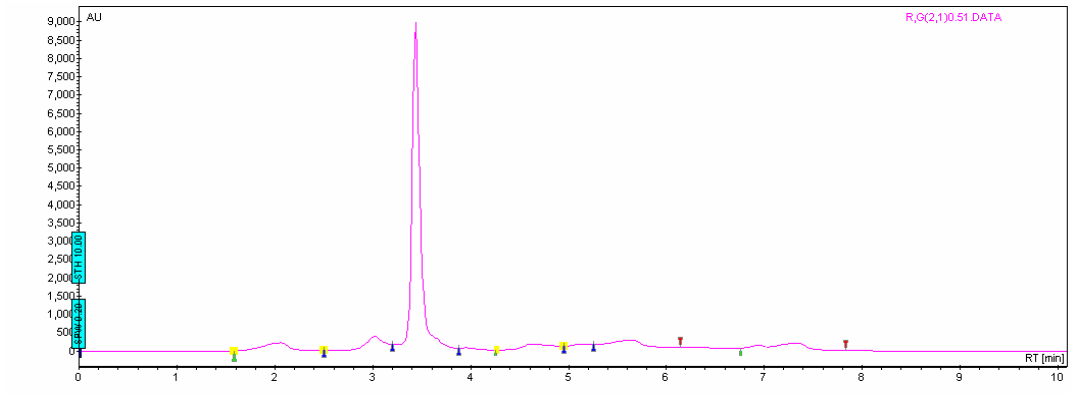


Fig.8: Chromatogram for Gliclazide at 2 hour of drug administration in rat plasma

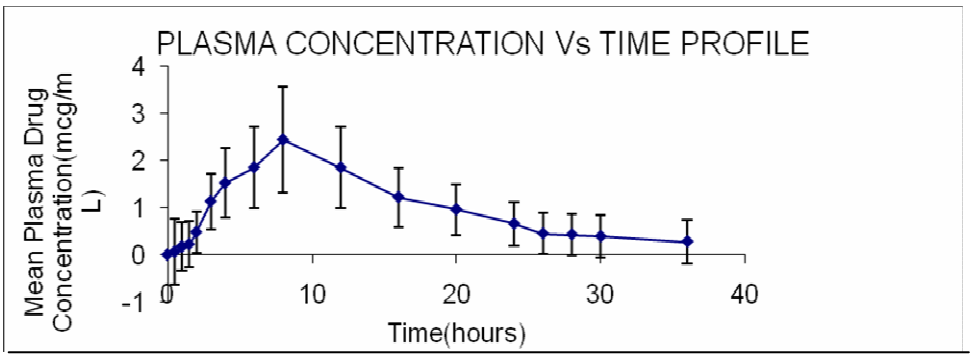


Fig.9:Area Under Zero Moment Curve for Gliclazide

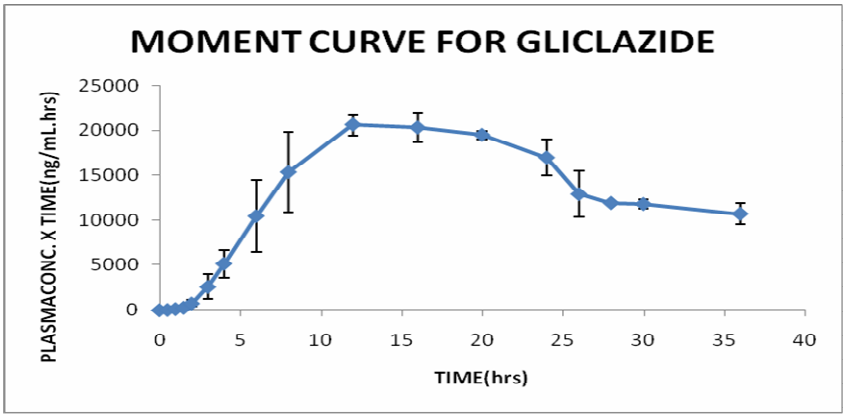


Fig.10: Area Under First Moment Curve for Gliclazide in rat plasma

Conclusion

From the present study it was concluded that Gliclazide can be quantified in rat plasma using simple organic solvents and the extraction procedure can be trouble-free and less time consuming with the same.The present work affirms a simple,economical and less time consuming bioanalytical method ,extended to its estimation of pharmacokinetic parameters.

References

1. A.J .Garber, 2000. Using dose–response characteristics of therapeutic agents for treatment decisions in type 2 diabetes. Diabetes Obes. Metab .2,139-147.
2. Lindblad, U. and Melander, A. 2000. Sulphonylurea dose response relationships: relation to clinical practice. Diabetes Obes. Metab .2, 25-31.
3. C. L.Lawrenc-e; P.Proksn; G. C .Rodrigo; P. Jones; Y.Hayabuchi; N. B. Standen; F. M. Ashcroft, (2001). "Gliclazide produces high-affinity block of K ATP channels in mouse isolated pancreatic beta cells but not rat heart or arterial smooth muscle cells". Diabetologia 44 (8): 1019–25.
4. A Rieutord; I Stupans; GM Shenfield; AS Gross, (1995). "Gliclazide hydroxylation by rat liver microsomes". Xenobiotica 25 (12): 1345–54.
5. Elliot, J. David; Lewis, C. Benjamin; Gillam, M. J. Elizabeth; Birkett, J. Donald; Gross, S.Annette; Miners, O. John; Miners, JO (2007). "Identification of the human cytochromes P450 catalysing the rate-limiting pathways of gliclazide elimination". British Journal of Clinical Pharmacology 64 (4): 450–7.
6. Y Zhang,; Si, Dayong; X Chen; N Lin; Y Guo; H Zhou; D Zhong, (2007). "Influence of CYP2C9 and CYP2C19 genetic polymorphisms on pharmacokinetics of gliclazide MR in Chinese subjects". British Journal of Clinical Pharmacology 64 (1): 67–74.
7. H Xu; K M Williams; W S Liauw; M Murray; R O Day; A J McLachlan, (2009). "Effects of St John's wort and CYP2C9 genotype on the pharmacokinetics and pharmacodynamics of gliclazide". British Journal of Pharmacology 153 (7): 1579–86.
8. P Venkatesh, T Harisudhan, H Choudhury, R Mullangi , NR Srinivas.(2006).” Simultaneous estimation of six anti-diabetic drugs--glibenclamide, gliclazide, glipizide, pioglitazone, repaglinide and rosiglitazone: development of a novel HPLC method for use in the analysis of pharmaceutical formulations and its application to human plasma assay”. Biomed Chromatogr. Oct; 20(10):1043-8.

*****