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Research Article

STATISTICAL CORRELATION AND QUANTIFICATION OF GLICLAZIDE BY SPECTROPHOTOMETRIC METHOD

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ABSTRACT

Objective: A rapid and sensitive Spectrophotometric method was developed for Gliclazide in rat and human plasma.

Methods: The sample was prepared by simple extraction method without derivatization and no use of buffer.Methanol and acetonitrile were used as the solvents in the proposed methods.

Results: Calibration range extended from 0.5mcg/ml to 5 mcg/ml in rat plasma and 2mcg/ml to 20mcg/ml in human plasma with good regression coefficients in both the cases. The Limit of Detection and Limit of Quantification were found out to be 0.51mcg/ml and 1.55mcg/ml in rat plasma and 0.088mcg/ml and 0.266mcg/ml in human plasma. Assay results from the proposed method were found to be 99.92% and 99.91% in rat and human plasma respectively. Stability of the drug in both the plasma was found to be suitable in both refrigerated and ambient conditions.

Conclusion: The current method implied no significance difference as for estimation in rat and human plasma as tested from ANOVA analysis and can be extended pharmacokinetic studies. The proposed method was found prudent to be used in routine QC analysis.

Keywords: Gliclazide, UV-Vis Spectrophotometer, Rat Plasma, Human Plasma, Statistical Correlation

INTRODUCTION

Gliclazide is an oral hypoglycemic (anti-diabetic drug) and is classified as a sulfonylurea. Its classification has been ambiguous, as literature uses it as both a first-generation [1] and second-generation [2] sulfonylurea. Gliclazide was proven to protect human pancreatic beta-cells from hyperglycemia-induced apoptosis.[3] It was also shown to have an antiatherogenic effect (preventing accumulation of fat in arteries) in type 2 diabetes.[4] 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 [5].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 P450s in vitro.[6, 7] But the pharmacokinetics of gliclazide MR is affected mainly by CYP2C19 genetic polymorphism instead of CYP2C9 genetic polymorphism.[8, 9].Literature reveals method development of Gliclazide in human and rat plasma individually but no method has been developed with simultaneous estimation and comparative data for the same[10, 11].

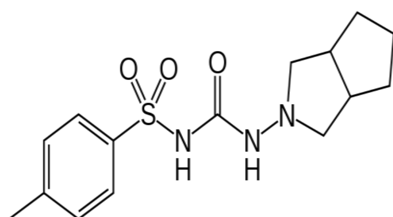


Fig. 1: Structure of Gliclazide

MATERIALS AND METHODS

A double beam UV-Vis Spectrophotometer was used of JASCO.Gliclazide was procured from Panacea Biotech; Baddi.Formulations was available from the local market.Solvents ere procured from Merck, India. 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.

Preparation of stock solution

The stock solution of Gliclazide was prepared in methanol and the volume was made up of with acetonitrile to get a concentration of 100 µg/mL.

Determination of Working Wave Length

In order to determine the wave length of maximum absorption (λmax) drug, different solutions of the drugs in acetonitrile were scanned using spectrophotometer within the wave length region of 200-400 nm against plasma (with acetonitrile) as blank and the wavelength maxima was found at 228nm.

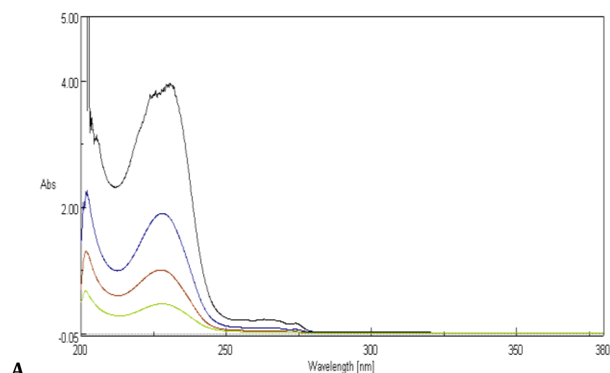
Method Validation

This was carried out by establishing linearity, range, specificity, Limit of Detection, Limit of Quantification, recovery studies and precision studies according to the International Conference on Harmonization Guidelines.

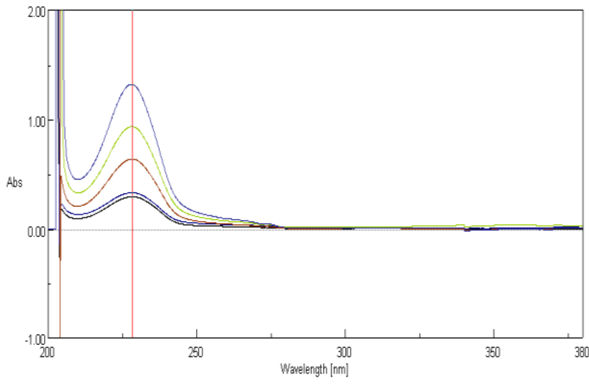
RESULTS AND DISCUSSION

Gliclazide was dissolved with methanol and the volume was made up to the mark with acetonitrile to get a concentration of 100mcg/ml.

Typical overlain spectra for Gliclazide in rat plasma and human plasma are shown in Fig:2

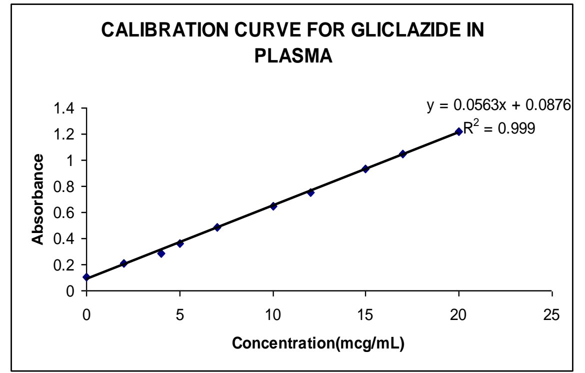


A.



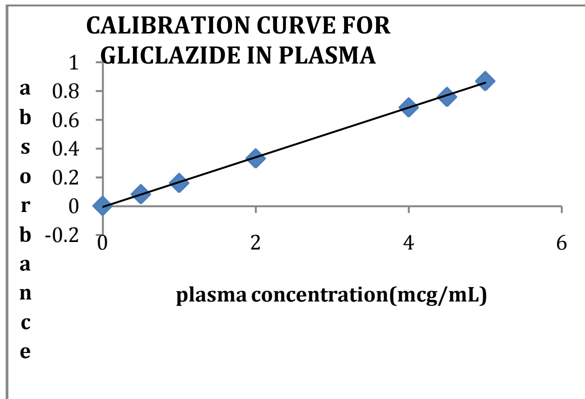
B.

Fig.2: Overlain spectra for Gliclazide in (a) rat plasma (b) human plasma



B.

Fig.3: Calibration Curve for Gliclazide in(a) rat (b)human plasma



A.

Table.1: Analysis in marketed formulations by UV-Vis Spectrophotometry

Label Claim(mg)	Amount Found(mg)		Statistics	
	Rat Plasma	Human Plasma	Rat Plasma	Human Plasma
80	78.16	79.04	Mean=79.39	Mean=79.23
80	79.08	80.16	±S.D=1.113619	±S.D=1.526
80	80	76.32	%RSD=1.402719	%RSD=1.92
80	78.16	79.92		
80	80	80.00		
80	80.91	80.32		

Accuracy and Precision: The within day and between day studies were RSDs were calculated and are given in Table No: 2 and 3.

Table 2: Precision of method in rat plasma and human plasma

Sample	Concentration added(mcg/mL)	Within day			Between Day		
		Mean	±S.D	%RSD	Mean	±S.D	%RSD
Rat Plasma	1.25	100	0.00359	0.2872389	98.00995025	0.004509	2.288959265
	2.5	100	0.003582	0.143263094	99.00744417	0.005	1.253132832
	4.5	99.77778	0.009416	0.209716067	73	0.003	0.413793103
Human Plasma	5.75	100.003	0.028	0.48	99.97	0.019	1.73
	10.75	100.16	0.006	0.06	99.32	0.21	0.98
	14.5	99.53	0.062	0.51	98.15	0.15	1.93

Table 3: Recovery studies of Gliclazide in rat plasma and human plasma

Sample	Quality Control Concentration(µg/mL)	Amount added(µg/mL)	Recovery±S.D
Rat Plasma	1.25	1	99.38±0.0021
		1.25	100.87±0.0015
		1.5	100.62±0.002
	2.5	2	100.30±0.0015
		2.5	99.88±0.0015
		3	100.21±0.0021
	4.5	3.6	96.96±0.002
		4.5	100.21±0.001
		5.4	100.41±0.0021
Human Plasma	5.75	4.6	99.98±0.0007
		5.75	100.20±0.003
		6.9	99.95±0.0002
	10.75	8.6	99.98±0.0003
		10.75	100.04±0.0002
		12.9	100.13±0.014
	14.5	11.6	99.98±0.018
		14.5	99.95±0.002
		17.4	99.04±0.22

Stability of plasma samples

The standard solutions were scanned in the UV range of 200nm to 400nm and 228nm was found as the wavelength maxima. This wavelength was taken for all further measurements. Linearity was determined by plotting the absorbance at its wavelength maxima against the extracted sample concentration from rat and human plasma. Linear regression analysis of the data was found to be 0.999 in both rat and human plasma respectively. The slope and intercept values were very small indicating a high precise method. The Limit of Detection were analyzed from a series of solutions containing a decrease of amounts of Gliclazide in both rat and human extracted plasma and the Relative Standard Deviation computed was not more than 10% and Limit of Quantification did not exceed 20%.

Assay Precision and accuracy were determined by taking three QC samples (1.25, 2.5 and 4.5 mcg/ml in rat plasma and 5.75, 10.75 and 14.5 mcg/ml in human plasma) in six replicates for within day precision and between day precision and the %RSD was found to be less than 2% in all cases.

To determine the stability of Gliclazide in rat and human plasma, 4 sets of quality control concentrations of the spiked calibration standards were divided in to 16 tubes. One was taken as standard (100%).Two of the sets were stored and taken the readings after freeze thaw cycles for 24hours and 1week.The remaining set was stored at -20ºC for 1 week. The results were evaluated comparing these measurements with those of standards and expressed as percentage deviation and the results are summarized in Fig.4: The plasma samples were found to be stable in all the conditions, for only the percentage found was comparatively less in stability at -20ºC.

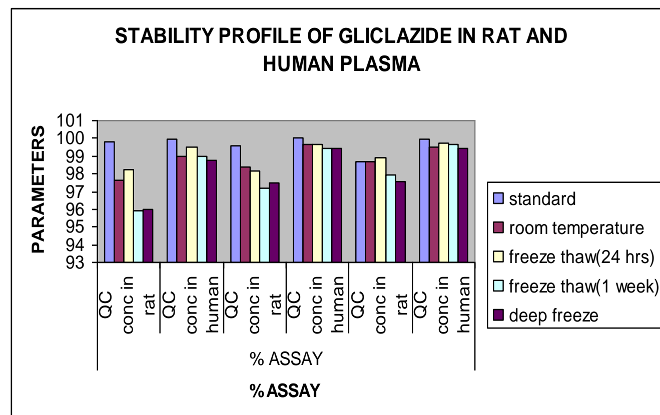


Fig.4: Stability profile of Gliclazide in rat and human plasma

Statistical correlation

To correlate the difference between the two methods for Gliclazide in rat and human plasma, six different samples in the two cases were taken and quantified individually**.** To test difference between the proposed HPLC methods statistical tests were performed for the level of confidence 95% (*P = 0.05*).One way ANOVA was applied to test both method – sample interaction and differences in method precision. In both the cases ***F stat* is less than *F crit*,** signifying the method – sample interaction and the differences between the methods are not significant as shown in Table: 4

Table.4: One way ANOVA data for Gliclazide in rat and human plasma

Anova: Single Factor						
Summary						
Groups	**Count**	**Sum**	**Average**	**Variance**		
RAT PLASMA	6	476.31	79.385	1.23527		
HUMAN PLASMA	6	475.76	79.29333333	2.320426667		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.025208333	1	0.025208333	0.014179125	0.907573	4.964603
Within Groups	17.77848333	10	1.777848333			
Total	17.80369167	11				

CONCLUSION

The present study reveals that method development for Gliclazide in rat plasma and human plasma can be used for routine QC analysis. The method also indicates that there is no significant difference in analysis for the drug in rat plasma and human plasma and the stability of the drug in plasma indicates that it can be considered fair for pharmacokinetic studies.

REFERENCES

1. Ballagi-Pordany, Gyorgy; Koszeghy, Anna; Koltai, Maria-Zsofia; Aranyi, Zoltan; Pogatsa, Gabor (1990). "Divergent cardiac effects of the first and second generation hypoglycemic sulfonylurea compounds". Diabetes Research and Clinical Practice 8 (2): 109–14.
2. S Tatsuhiro; Y Shinya; T Kazuto; K Hidenori; I Eisuke; S Hiroyuki; U Kenji; K Hiroshi et al. (2006). "Gliclazide protects 3T3L1 adipocytes against insulin resistance induced by hydrogen peroxide with restoration of GLUT4 translocation". Metabolism 55 (6): 722–30.
3. D Guerra, S; Grupillo, M; Masini, M; Lupi, R; Bugliani, M; Torri, S; Boggi, U; Del Chiaro, M et al. (2007). "Gliclazide protects human islet beta-cells from apoptosis induced by intermittent high glucose". Diabetes/Metabolism Research and Reviews 23 (3): 234–8.
4. N Katakami; Y Yamasaki.; R H Okano; K Ohtoshi,.; H Kaneto.; M Matsuhisa.; K Kosugi.;M Hori. (2004). "Metformin or gliclazide, rather than glibenclamide, attenuate progression of carotid intima-media thickness in subjects with type 2 diabetes". Diabetologia 47 (11): 1906–13.
5. C. L Lawrence.; P Proks; 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.
6. A Rieutord; I Stupans; GM Shenfield; AS Gross (1995). "Gliclazide hydroxylation by rat liver microsomes". Xenobiotica 25 (12): 1345–54.
7. J.David; C. Benjamin; M. J. Elizabeth; J.Donald; S.Annette;O.John; (2007). "Identification of the human cytochromes P450 catalysing the rate-limiting pathways of gliclazide elimination". British Journal of Clinical Pharmacology 64 (4): 450–7.
8. Y Zhang; D.Si; C Xiaoyan; L Nan; G Yingjie; Z Hui; Z Dafang (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.
9. Xu, H; Williams, K M; Liauw, W S; Murray, M; Day, R O; McLachlan, A J (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.
10. R Talari, J Varshosaz, A Mostafavi,,A Nokhodchi(2011), "Development And Validation Of A novel Rp-Hplc Method For Pharmacokinetic Studies Of Gliclazide In Rat" FARMACIA,59(3):388-395.
11. J F Yang,G L Wei,R Lu,C Liu, B Zheng,P Feng(2005), Bioavailability of Gliclazide Sustained Release Tablet in Healthy Volunteers, Asian Journal of Pharmacodynamics and Pharmacokinetics;6(2)153-160