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METHOD DEVELOPMENT FOR ANALYSIS OF GLICLAZIDE IN HUMAN PLASMA BY USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Since 1970, dissolution requirements have been included in tablets and capsules monographs, in general, in response to concerns for bioavailability of equal significance is the recognition of the immense value of dissolution testing as a tool for quality control. Thus, equivalence in dissolution behaviour was sought in the light of both bioavailability and quality control considerations (Coppack *et al.,* 1990). Nonetheless, dissolution profiles are often considered by the industry to ascertain the release rates of drug from tablet formulations as a quality assurance tool. However, in terms of sensitivity, precision and specificity, high-performance liquid chromatographic (HPLC) method may offer additional advantages (Charles & Ravenscroft, 1984 and Nawaz, 2001).

INTRODUCTION

Selective and sensitivie analytical method for quantitative determination of drugs and their metabolites are essential for successful evaluation of clinical pharmacology, pharmacokinetics (PK), bioavailability (BA) and bioequivalence (BE) studies (Raghow & Meyer, 1981).

Gliclazide is known to possess low aqueous solubility (Nawaz, 2001). Large inter- and intra-individual responses following administration of sulphonylurea (glibenclamide etc.) reparations have also been reported (El-Sayed *et al.,* 1989, Marchetti & Navalesi, 1989 and Charles & Ravenscroft 1984). Such variations are undesirable and may expose susceptible patients to the danger of hypoglycaemia or other associated hazards when a patient's therapy is changed from one preparation to another.

MATERIALS AND METHODS

Potassium di hydrogen phosphate, guaranteed reagent tested according to International Standard Organization, Merck specification Acetonitrile were obtained from Merck and Reference Standard (RS) was obtained through the courtesy of Nabi-Qasim Pharmaceutical Industries. All stock solutions were prepared by dissolving RS in methanol followed by mobile phase/plasma.

Chromatographic System:

The chromatographic system used in this study consisted of a LC-5A, 6A system (Shimadzu-Japan) attached by UV. μ bonda pack C_{18} 300 mm x 3.9 mm internal diameter were used protected by Octa Decyl Silane guard column (pre column). A degassed and filtered mixture of Potassium Dihydrogen Phosphate (0.685 g in 1000 ml water) buffer pH 2.5 and Acetonitrile 30: 70 (v/v) was used as mobile phase. The flow rate was maintained at 1 ml/min. Detection was performed at 229 nm and separation was carried out at ambient temperature.

Preparation of Stock Solution in Mobile Phase:

Standard solution of Gliclazide prepared in acetonitrile was diluted in described mobile phase according to the study design.

Preparation of Stock Solution in Plasma:

Standard solution of Gliclazide prepared in acetonitrile was also diluted in described fresh plasma according to the study design.

A calibrated Microsyring, Juster and glassware (Pyrex Japan) were used for this purpose.

Extraction of Gliclazide from Plasma:

Samples of serum were mixed with equal quantity of acetonitrile in eppendorff tube followed by vortex mixing for 01 minute and centrifuged for 05 minutes at 3000 rpm which resulted in deproteinization. The supernatant layer was transferred to another eppendorff tube. Sufficient quantity of ammonium sulphate was added followed by vortex for 1 minute and centrifuged for 05 minutes at 3000 rpm resulting in reasonably clean extract that allowed quantitative determination of the subject. On the other hand, acetonitrile layer gave unhindered quantitation of Gliclazide from plasma constituents. A 20-microliter sample was injected in the chromatographic system for estimation of Gliclazide.

Retention Time of Drug:

Retention time observed was 8.35 min, 15.8 min at 1 ml/min and 0.5 ml/min respectively and variation in retention time was not significant at different injections.

Assay Procedures and Validation:

Stock solutions of 10 mg/10 ml of gliclazide RS in methanol and further dilution were prepared in mobile phase (acetonitrile: phosphate buffer pH 2.9; 30: 70% v/v), separately. Standard solutions of varying concentrations of gliclazide (0.625, 1.25, 2.5, 5.0 and 10 ug/ml) were prepared. The range of dilutions from 5 ug/ml was selected keeping in view the previously reported maximum concentration of Gliclazide in plasma. A mobile phase composing of KH_2PO_4 buffer (0.685 gram Potassium Di Hydrogen Phosphate per liter pH was adjusted with phosphoric acid up to 2.5) and acetonitrile (30: 70) and it was delivered isocratically at 1 ml/min. The retention times for the gliclazide peak were 8.1 minutes. To obtain the optimum retention time of the gliclazide peak, the composition of the acetontirile in the mobile phase was adjusted up to 70% v/v. While the mean retention time for the gliclazide peaks was 8.3 minutes with relative standard deviation (RSD) values are insignificant (n=50). This showed that the precision of this method was acceptable.

For the assessment of intra-day (Table 2) precision, complete set of gliclazide standards in mobile phase was injected (n=24) at 4 different times over 12-hour period, namely in the early morning 9.00 am, noon at 1 pm, mid-evening at 4 pm and in the night at 8 pm. This was reported over 3 days to measure inter-day (Table 3) precision (n=18).

To further validate the accuracy of the assay technique regarding recovery from plasma five (5) different known concentration (Table 4) of the gliclazide standard in mobile phase and plasma were analyzed. Further validation of method was checked by recovery of additional amounts of Glicalizde added.

A part from measuring the retention times of the analyte peaks, calibration curves of peak area ratios (PAR) of plasma standard (plasma sample) of glicalizde versus the standard solution gliclazide concentrations were also constructed.

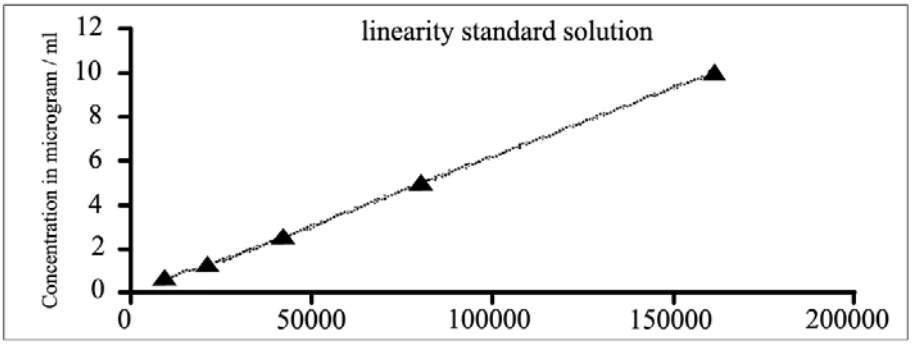

Fig. 1: Showing the method sensitivity and the linearity between standard solution and peak area.

Method Linearity:

The calibration curve for gliclazide was linear in the concentration range 0.625 to 10 ug/ml (R^2=0.9996) as shown in Fig.1 and Table 1, using peak area of gliclazide standard solution in mobile phase.

Table 1
Showing the reproducibility and accuracy of the method

Injection	10 ug	5 ug	2.5 ug	1.25 ug	625 ng
1st	160914	80302	42274	21080	9532
2nd	162472	80174	42106	21001	9012
3rd	161710	80222	41998	21030	9471
4th	162320	80247	42470	22043	9322
5th	162013	80319	42521	20990	9340
6th	161047	81101	42191	21513	9612
7th	160023	79850	42950	21402	9678
8th	162604	81316	42444	21322	9546
9th	161419	79560	42843	21702	9436
10th	161317	80444	42154	21073	9410
Mean	161584	80353.5	42395.1	21315.6	9435.9
± SD	805.75	520.81	314.13	353.68	187.01
RSD	0.50	0.65	0.74	1.66	1.98

Table 2
Showing the intra day variation

Conc.	9.00 am	1.00 pm	4.00 pm	8.00 pm	Mean	± SD	RSD
5 ug	80302	80319	79850	79560	80007.75	369.15	0.46
	80174	81101	81316	80444	80758.75	538.10	0.67
Mean	80238	80710	80583	80002	80383.25	453.63	0.56
2.5 ug	21080	21030	21402	21702	21303.50	312.65	1.47
	21001	22043	21322	21073	21359.75	475.81	2.23
Mean	21040.5	21536.5	21362.0	21387.5	21331.6	394.2	1.8
0.625 ug	9532	9471	9340	9436	9444.75	80.31	0.85
	9012	9322	9612	9410	9339.00	249.52	2.67
Mean	9272.0	9396.5	9476.0	9423.0	9391.8	164.2	1.7

Table 3
Showing the inter day variability

Conc.	1st day	2nd day	3rd day
5 ug	80302	78143	77250
	80174	78347	78210
Mean	80238	78245	77730
2.5 ug	21080	20249	20031
	21001	21013	20541
Mean	21040.5	20631	20286
0.625 ug	9532	9034	8994
	9012	9122	8541
Mean	9272.00	9078	8767.5

Table 4
Showing the determination of gliclazide from standard solution prepared in mobile phase and standard prepared in plasma and recovery of additional amount of gliclazide

Standard solution	Area (MP)	Area (Ps)	% Re-covery	Amount Added	Area (MP)	% Re-covery	Area (Ps)	% Re-covery
10 ug/ml	160914	163455	101.58	2.5 ug/ml	202371	99.59	211519	103.38
5 ug/ml	80302	79502	99.00	1.25 ug/ml	102472	101.07	101317	101.78
2.5 ug/ml	42274	41137	97.31	2.5 ug/ml	83175	98.37	81054	98.5
1.25 ug/ml	21080	20041	95.07	2.5 ug/ml	62982	99.41	61982	101.3
0.625 g/ml	9532	8871	93.07	5 ug/ml	88473	98.48	84431	95.53

MP = Mobile phase, Ps = Plasma

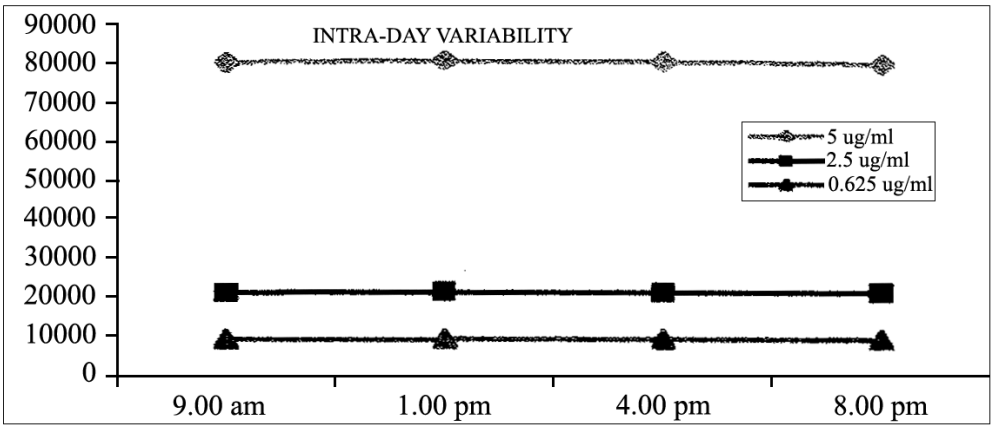

Fig. 2: Showing the intra day variability

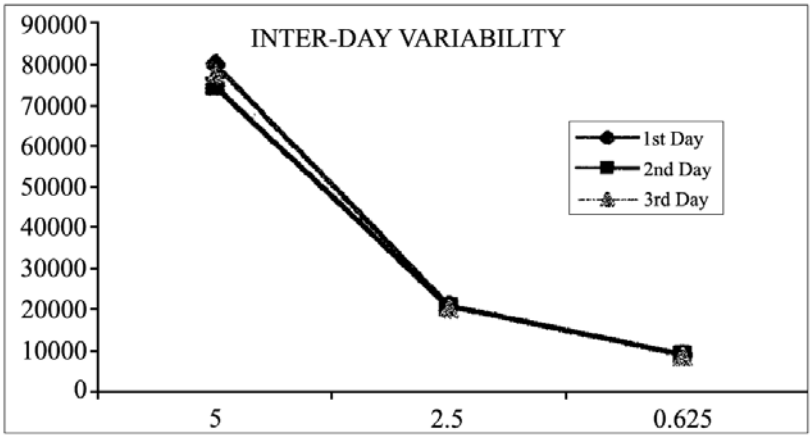

Fig. 3: Showing the inter day variability.

RESULT AND DISCUSSION

Both solutions prepared in mobile phase and plasma were analyzed by described chromatographic system and in each case i.e. Mobile phase and plasma solutions, a linear relationship between peak area and concentration was observed as shown in figure 2 of chromatogram. The recovery data clearly shows the accuracy of the method and the reproducibility data shows the precision and validity of the method. During a exercise the change of pH and acetonitrile concentration were found to vary with retention time. Sensitivity (limit of quantification and detection) and specificity of the method is achieved by using different absorbance at UV. During the initial stages of selection of a suitable mobile phase to separate gliclazide by reverse phase HPLC, it was established that the drug did not elute from the C_{18} column when a buffer free mobile phase was used. The differences between the concentration values of standard prepared in mobile phase and the values of standard solution prepared in plasma were not significant are reflected in Table 4. This further validated the accuracy of the assay method.

CONCLUSION

A rapid HPLC method for the detection and quantification of gliclazide from *in vivo* studies had been successfully developed, with optimum retention time. The method is able to determine gliclazide concentrations as low as 0.625 ug/ml with a relative standard deviation ranging between 0.5% and 1.98% as shown in Table 1. Apart from the greater precision and sensitivity attained using this HPLC method, the specificity offered is undoubtedly another advantage compared to the UV Spectrophotometric and other methods/techniques of analysis. The outcome justifies the use of HPLC and the validation data convincingly demonstrates the adequate performance of the proposed method of analysis that may help in therapeutic drug monitoring, *in vivo* studies as well as analysis of pharmaceutical formulation containing gliclazide.

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