INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
Available online http://www.irjponline.com
Research Article

SENSITIVE AND SELECTIVE ANALYTICAL METHOD FOR THE QUANTIFICATION OF GLIPIZIDE IN HUMAN PLASMA

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Article Received on: 09/11/10 Revised on: 29/12/10 Approved for publication: 09/12/10

ABSTRACT
A sensitive and selective high performance liquid chromatographic method for determination of Glipizide in Human plasma has been developed. The plasma samples were acid buffered, and samples were extracted with benzene, and organic layer was evaporated to dryness. The residue was reconstituted in mobile phase 0.05M Potassium Dihydrogen Orthophosphate: Methanol [15: 85 %v/v, pH 7.0 ± 0.05, pH adjusted with 1% Triethylamine] and an aliquot of 20 µL was chromatographed on C_{18} Intersil [4.6(id) x 250 mm] column. Quantification was achieved by monitoring the ultraviolet absorbance at 225nm. The retention time of Glipizide was found to be 3.21 min and the response was linear (10-2000 ng cm^{-3}) and detection limit was 5ng cm^{-3} in plasma. The developed method was validated in terms of accuracy, precision, linearity, limit of detection and limit of quantitation and can be used for the estimation of these drugs in pharmaceutical dosage forms.

KEY WORDS: RP-HPLC, Glipizide, Human plasma, potassium dihydrogen orthophosphate.

INTRODUCTION
Glipizide is chemically N-[2-[4 (cyclohexylcarbamoylsulfamoyl) phenyl] ethyl]-5-methyl-pyrazine-2-carboxamide. Glipizide is an effective oral hypoglycemic agent, widely used in the treatment of non-insulin-dependent diabetes mellitus (Type II diabetes)[1,2]. As a second- generation sulphonylurea, glipizide is given in low doses. In connection with pharmacokinetic and metabolic investigation of glipizide, a sensitive and selective analytical method for determination of plasma levels of the drug is required.
A few bioassays for analysis of glipizide in plasma or serum have been reported[3-8]. The determination of glipizide in plasma has been performed by radioimmunoassay technique[3]. However, the selectivity of these methods has not been verified. Gas Chromatography (GC) has also been used for determination of glipizide in plasma[4]. However, GC requires a time consuming derivatization step to give volatile and thermally stable derivatives. One of the GC techniques[4] also lack selectivity, since structurally similar sulphonylurea (e.g. Glibenclamide) may form identical derivatives. Wahlin-Boll and Melander[5] described a sensitive high performance liquid chromatographic (HPLC) technique for the measurement of glipizide concentrations in human serum. The method is limited by a relatively long elution time (25- 30 min) for each sample. Hakan Emilsson also described high performance liquid chromatographic (HPLC) technique for determination of glipizide in human plasma and urine[6], the method is again limited by long elution time (10.5 min). Glipizide in combination with other drugs has been determined by several techniques including Liquid Chromatography- Tendem Mass Spectrometry[7,8].
A good method for monitoring plasma levels is needed for defining accurate doses of glipizide and for analysis of samples in connection with hypoglycemic crises and incidents with other kinds of severe side effects. In order to find a sensitive, selective, simple and more rapid method for determination of intact

glipizide in human plasma samples, the HPLC assay described in this paper was developed which provide a good choice to study the pharmacokinetics and pharmacodynamics of Glipizide in vivo. Plasma samples with glipizide were analyzed to demonstrate the utility of the method.

MATERIALS AND METHODS

Acetonitrile, HPLC grade; water, HPLC grade; orthophosphoric acid, AR grade; methanol, HPLC grade; Triethylamine, HPLC grade and potassium dihydrogen orthophosphate, GR grade were procured from Loba Chemicals, Mumbai, India. Reference standards of drugs were procured from Cipla Pvt. Ltd, Mumbai, India

Chromatographic separation was performed on a Jasco PU 1580 intelligent pump, variable wavelength UV/VIS detector (Jasco UV 1575), precision loop injector (Rheodyne 20µl) Borwin Software (version 1.21.60). Column C18 Intersil (250 X 4.6 i.d., particle size 10µm) was used for the separation.

Preparation of mobile phase and standard solutions

The mobile phase used was a mixture of 0.05M Potassium Dihydrogen orthophosphate : Methanol [15: 85 %v/v, pH 7.0 ± 0.05, pH adjusted with 1% Triethylamine]. It was filtered through Whatman filter paper No. 42. Standard stock solutions of glipizide 1000 µg mL^{-1} were prepared in methanol. Working solutions of appropriate concentrations were made by dilution of the stock solution with mobile phase. The standard calibration curve were prepared by adding known amount of Glipizide to blank plasma, and contained 10- 2000 ng mL^{-1} glipizide. The mobile phase was delivered at a flow rate of 1 mL/min with detection at 225 nm. The injection volume was 20 µL; Analysis was performed at room temperature.

Preparation of sample solutions

Twenty tablets (GLYNASE®, USV Limited, Mumbai, India.) containing 5 mg of Glipizide was taken, average weight was determined. Weight equivalent to 5 mg of Glipizide was taken in 100 ml volumetric flask and 40 mL of methanol was added and sonicated for 30 min, finally volume was made up to the mark with methanol. The extracts were filtered through Whatman filter paper No. 42 and required dilutions were made with mobile phase to get the final concentration containing 500 ng mL^{-1} of Glipizide.

Extraction procedure from plasma

Into a series of 15 cm^3 stopperd tubes 1 cm^3 of blank plasma was taken. To this 1cm^3 of 10, 20, 50,100, 200, 500, 1000 and 2000 ng/cm^3 standard glipizide solution were added. To each of the test tubes 3ml of 0.05 M HCL were added, resulting in a pH of 3, and vortex for one minute. The mixture was extracted with 3 x 3ml of benzene and tubes were shaken gently for 15 minutes and centrifuge at 3250 rpm for 5 min. The organic layer was transferred to a conical tube for evaporation to dryness under the stream of air (a well ventilated fume chamber was used because of benzene vapors). The residue was reconstituted mobile phase by vortexing. An aliquot of 20 µl were injected into chromatograph.

The same treatment was given to 1 cm^3 of blank plasma without any drug solution. All these solutions were injected into the chromatographic system under the set experimental conditions and chromatograms were recorded. The peak area was calculated at each level.

A graph of the concentration of glipizide in ug cm^{-3} against the peak area was plotted. Calibration curves were constructed on successive days to assess the repeatability of the method.

ASSAY METHOD

With the optimized chromatographic conditions, a steady baseline was recorded, the mixed standard solutions without and with plasma were injected and the chromatogram was recorded. The retention time of Glipizide without and with plasma were found to be 3.21 ± 0.07 and 3.21 ± 0.05 min, respectively. This procedure was repeated for the sample solution obtained from the formulation. The response factor (peak area) of the standard solution and sample solution was recorded. The analyte concentration of the drugs was calculated and presented in Table 1.

RESULT

The method described enables to the quantification of Glipizide in human plasma. The proposed HPLC conditions ensure sufficient resolution and the precise quantification of the compound. Results from statistical analysis of the experimental results were indicative of satisfactory precision and reproducibility.

Thus, the HPLC assay described in this paper with minor modification can be suitable for monitoring glipizide in clinical therapy and in pharmacokinetic /or metabolic studies, and may also be suitable during hypoglycemic crises. The assay is sufficiently selective, sensitive, rapid and simple to allow accurate and precise measurements of plasma level of glipizide under therapeutic conditions.

DISCUSSION

Glipizide is a weak acid with pka 5.94 and relatively lipophilic compound as undissociated free acid (pH ≤ 4.5). In accordance with these properties, the pH of 3.0 – 3.5 seemed to be appropriate for the extraction from plasma samples. Different organic solvents used for extraction of Glipizide from human plasma, amongst them, only benzene was found to be suitable for the extraction of Glipizide from human plasma. The average extraction recovery of glipizide was 93-94 %, when benzene and acidified plasma (pH 3.0) were used.

Estimation of Glipizide without and with plasma in solid dosage forms by RP-HPLC method was carried out using optimized chromatographic conditions. The standard and sample solutions were prepared. The chromatograms were recorded. The typical chromatogram of sample solution without and with plasma is given in Fig 1 and Fig 3. The peak area of standard and sample solutions without and with plasma was calculated. Table 1 shows the result of analysis of pharmaceutical dosage form. The results of analysis shows that the amount of drugs was in good agreement with the label claim of the formulation.

To study the accuracy and precision of the proposed method from human plasma recovery experiments were carried out by standard addition method. Known amounts of standard glipizide were added to blank plasma and analyzed using the proposed method. The recovery experiments were performed three times. The recovery levels were 80%, 100%, 120%. The linearity of the method was determined at five concentration levels ranging from 80-120%. The results obtained for the recovery experiment are given in Table 2. The low values of SD and COV % obtained indicate that the method is highly precise. The absence of any peak at retention time of drug indicates the specificity of the method.

According to USP, 80% to 120% of test concentration was taken and dilution was done appropriately. The calibration curve was linear in the range of 10-2000 ng mL^{-1} of glipizide. The equation of the best fit line was Y= 69.766X (R^2 = 0.9992) for Glipizide without plasma and Y = 64.185X (R^2 = 0.9990) for Glipizide with plasma. The results show that an excellent correlation exists between response factor and concentration of drugs within the concentration range indicated above.

The column efficiency and peak asymmetry were calculated for the standard solutions Table 3. The values obtained demonstrated the suitability of the system for the analysis of this drug without and with plasma.

ACKNOWLEDGEMENT

Authors are grateful to Aristo Pharmaceutical, Raisen (M.P), India. for gift sample. Above all, the authors would like to offer their gratitude to the authorities of Institute of Pharmaceutical Education and Research, Wardha for providing all the facilities.

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Table 1: Result of Tablet assay

Glipizide (without plasma)			Glipizide (with plasma)		
Amount claimed mg/tablet	Amount found mg/tablet	% found*	Amount claimed mg/tablet	Amount found mg/tablet	% found*
5	5.2	99.98	5.5	5.4	92.64
	5.05	100.08		5.3	92.35
	5.1	99.00		5.3	92.06
	4.8	99.92		5.4	92.41
	5.01	99.79		5.4	92.18
Mean	5.032	99.75	Mean	5.36	92.33
S. D.	0.1478	0.4344	S. D.	0.05477	0.2236
C. O. V.	0.0018	0.43	C. O. V.	0.0044	0.241

*Mean of five determinations

S.D- Standard Deviation, C.O.V- Coefficient of Variation

Table 2: Recovery study data of Tablet formulation

Glipizide (without plasma)			Glipizide (with plasma)		
Amount added (mg)	Amount found (mg)	% Recovery*	Amount added (mg)	Amount found (mg)	% Recovery*
0.0004	0.0009	100.4	0.0004	0.0009	93.63
0.0005	0.0010	100.08	0.0005	0.0010	94.31
0.0006	0.0011	99.89	0.0006	0.0011	94.43

*mean of three estimations

Table 3: System suitability parameters

Parameter	Glipizide (without plasma)	Glipizide (with plasma)
Retention Time	3.21 ± 0.07	3.21 ± 0.05
Asymmetry	1.098	1.09
No. of Therotical Plates	2418.43	2415.31
Calibration Range ng mL^{-1}	10-2000 ng mL^{-1}	10-2000 ng mL^{-1}
Limit of detection ng mL^{-1}	5 ng mL^{-1}	5 ng mL^{-1}
Limit of quantitation ng mL^{-1}	15 ng mL^{-1}	15 ng mL^{-1}

*mean of five estimations

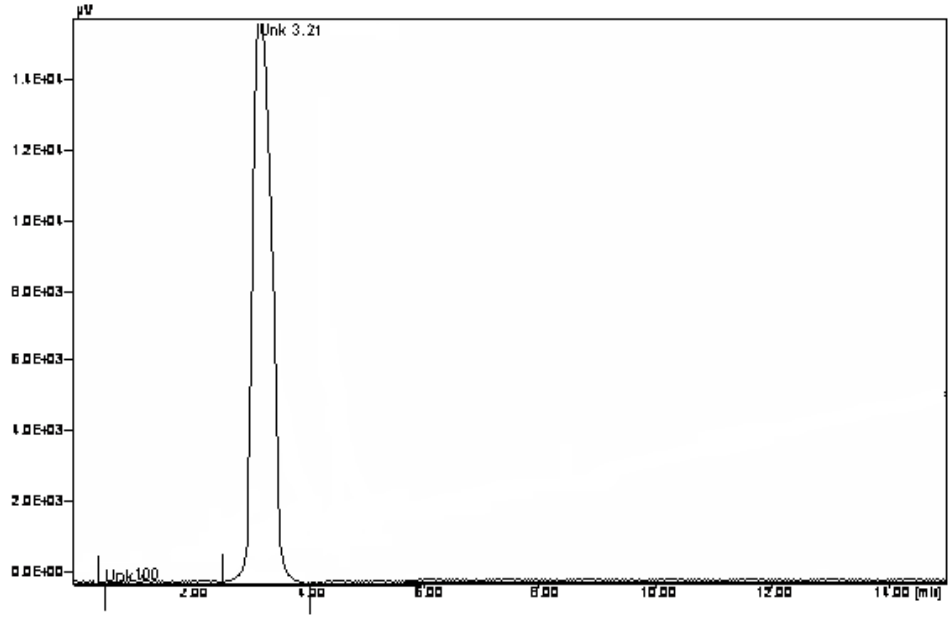

Fig. No. I: Typical Chromatograms of Glipizide (Rt 3.21 ± 0.07) without plasma

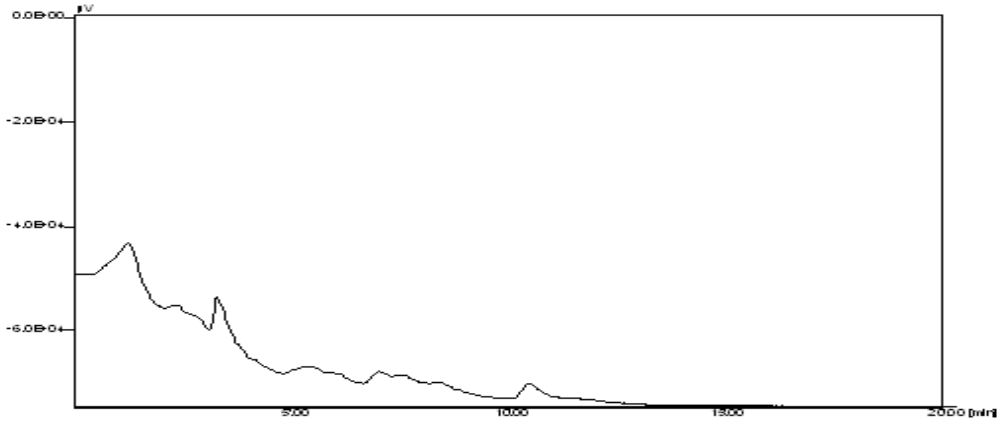

Fig. No. II: Typical Chromatograms of Blank plasma

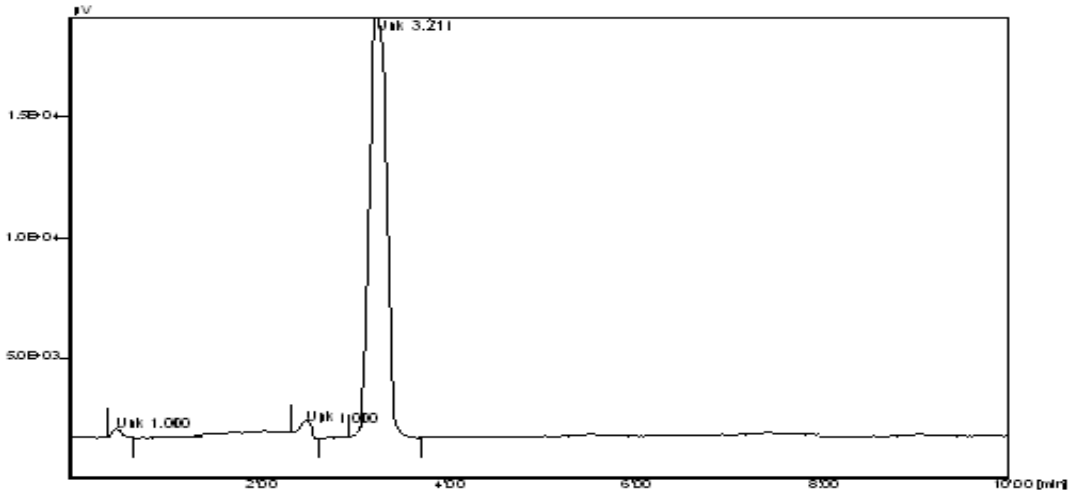

Fig. No. III: Typical Chromatograms of Glipizide (Rt 3.21 ± 0.05) with plasma

Source of support: Nil, Conflict of interest: None Declared