

**REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC
METHOD FOR THE ANALYSIS OF GLIPIZIDE IN
PHARMACEUTICAL DOSAGE FORMS**

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ABSTRACT

A rapid and sensitive reverse phase high performance liquid chromatographic methods depicted for the qualitative and quantitative assay of glipizide in pharmaceutical dosage forms. Glipizide was chromatographed on reverse phase C₁₈ column with mobile phase consisting of 0.05 M Potassium Dihydrogen Orthophosphate: Methanol [15: 85 %v/v, pH 7.0 ± 0.05, adjusted with 1% Triethylamine]. The mobile phase was pumped at a flow rate 1 mL/min. Quantification was achieved by monitoring the ultraviolet absorbance at 225 nm. The average retention time for Glipizide was found to be 3.21 ± 0.07. With this method, linearity was observed in the range of 10 – 2000 ng/ml. The LOD and LOQ were found to be 5 ng/ml and 15 ng/ml respectively. The method was applicable for the analysis of drug in tablet formulation. The results of analysis were validated statistically.

KEY WORDS: HPLC, UV, Glipizide, Methanol, Triethylamine, Potassium Dihydrogen Phosphate

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INTRODUCTION

Glipizide ¹⁻² {*N*-[2-[4 (cyclohexylcarbamoylsulfamoyl) phenyl] ethyl methyl-pyrazine-2-carboxamide]} is sulfonylurea oral hypoglycemic drugs used in Type II Diabetes Mellitus. It produces its action by blocking potassium channels in the beta cells of the islets of langerhans. A few bioassays for analysis of glipizide in plasma or serum have been reported ³. 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- Tandem Mass Spectrometry ⁷⁻⁸.

The HPLC methods using the most commonly available columns and detectors like UV are preferred. The present study describes the determination of glipizide in pharmaceutical dosage forms by using RP-C₁₈ column with UV detectors. Owing to the widespread use of HPLC in routine analysis, it is important that well validated HPLC methods are to be developed for estimating glipizide. The aim of this study is development of simple, precise, rapid, accurate and economical reverse phase HPLC method for the estimation of glipizide in different pharmaceutical dosage forms.

MATERIALS AND METHODS

Instrument

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

Materials

All the chemicals and solvents used during project work were of analytical grade. Standard gift samples of Glipizide were procured from Aristo Pharmaceutical, Pvt, Ltd. Raisen (M.P), India.

Preparation of Mobile Phase

The mobile phase used was a mixture of 0.05M Potassium Dihydrogen orthophosphate: Methanol [15: 85 %v/v, pH 7.0 \pm 0.05, pH adjusted with 1% Triethylamine]. It was filtered through Whatman filter paper No. 42. The elution was carried out isocratically at the flow rate of 1.0 mL/min. Detection was carried out at 225 nm.

Preparation of Standard Solution

Standard stock solutions of glipizide 1 mg 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 was prepared contained 10-2000 ng mL⁻¹ glipizide. Twenty micro liters of each solution was injected into the HPLC system to obtain the chromatogram. From these chromatograms, the areas under the peaks of the drug were noted. The regression of the drug concentration over these ratios was computed. The method follows the regression equation $y = 69.766x$ with coefficient of correlation $R^2 = 0.9992$. The recovery studies were carried out by adding known amount of glipizide to preanalysed samples and then analyzing them by the proposed HPLC method.

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 and mixed well. Each of these solutions (20 μ L) was then injected 5 times into the column. The mean peak area of the drug of 5 such determination was calculated and the drug content in the tablets was quantified using the regression equation obtained for pure sample.

Assay Method

With the optimized chromatographic conditions, a steady baseline was recorded, the mixed standard solutions were injected and the chromatogram was recorded. The retention time of Glipizide was found to be 3.21 ± 0.07 min. 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 quantified using the regression equation obtained for the pure sample.

To study the accuracy, reproducibility and precision of the above proposed methods, recovery studies were carried out by addition of standard drug to pre-analyzed sample.

RESULTS

Results of recovery studies were found to be satisfactory and reported in **Table 1**. The low coefficient of variation and recovery close to 100% indicating the reproducibility and accuracy of the assay of glipizide in tablets.

DISCUSSION

The drug content in the tablets was quantified using the proposed analytical method. The mean amount of glipizide in tablet dosage form is shown in Table 1. The absence of additional peaks in the chromatogram indicates non-interference of the common excipients used in the tablets. The tablet were found to contain 99.77- 99.88% of the labeled amount of the drug.

CONCLUSION

With HPLC assay method, the response factor (peak area) of the standard solution and sample solution was recorded. The analyte concentration of the drugs was quantified using the regression equation obtained for the pure sample. Each of the samples was injected 5 times and almost same retention times were obtained in all cases. When glipizide solutions analyzed by proposed method for finding out inter- and intra-day variations, a low coefficient of variation was observed (0.0709). This shows that the present HPLC method is highly precise. A recovery of 99.96 % of glipizide from the preanalysed samples shows that the present method is highly accurate.

It can be concluded that the proposed HPLC method is sensitive and reproducible for the analysis of glipizide in pharmaceutical dosage forms within a short analysis time.

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Table 1: Recovery Study Data of Tablet Formulation

Drug	Assay results		Amount added µg/mL	Amount recovered µg/mL (n=5)	Recovery (%)	Average recovery (%)
	Actual concentration (mg)	Concentration found (%) (n=5)				
Glipizide	5.0	99.86	0.4	0.398	100.01	99.96
		99.77	0.5	0.499	99.98	
		99.88	0.6	0.597	99.89	

*Mean of three determinations

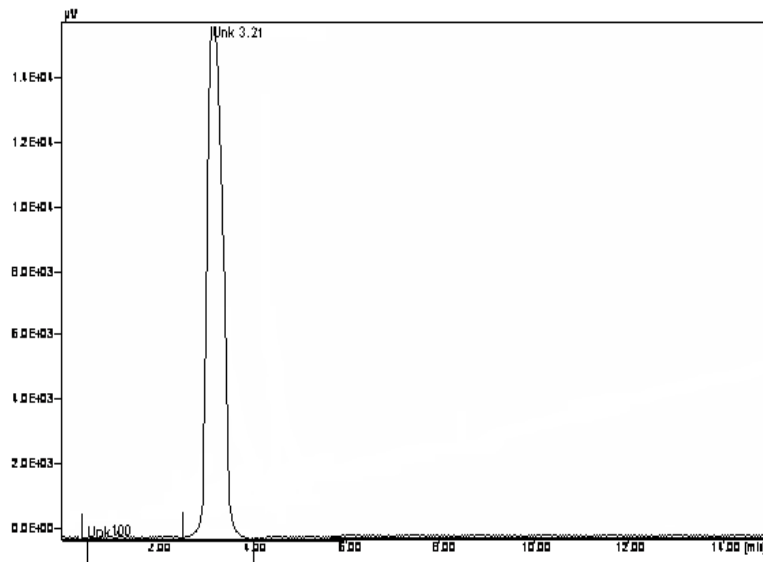


Figure 1: Typical chromatograms of Glipizide (Rt 3.21 ± 0.07)

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