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A validated RP-HPLC method for estimation of Glipizide in it's pure and pharmaceutical dosage form (tablets)

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ABSTRACT

A simple, specific and precise high performance liquid chromatographic method has been developed for estimation of Glipizide in its pure and pharmaceutical dosage forms. A HPLC system consisting of a gradient pump, reverse phase C-18 analytical column, a variable UV-Visible detector set at 225 nm and an integrator was used. The mobile phase consisted of methanol and 0.115%w/v ammonium dihydrogen phosphate buffer and pumped at 1ml/min at ambient temperature. This RP-HPLC method was validated for its linearity, precision and accuracy. The calibration curve was linear in the range of 10 to 70 µg/ml).

Key words: Glipizide, HPLC, Hypurity C18 analytical column, placebo, tablets.

INTRODUCTION

Glipizide[1], 1-cyclohexyl-3- [[p- [2-(5-methylpyrazinecarboxamido) ethyl] phenyl] sulfonyl] urea, is an oral blood-glucose-lowering drug of the sulfonylurea class. The stimulation of insulin secretion by this drug product in response to a meal is undoubtedly of major importance. Literature survey reveals UV-vis Spectrophotometric[2] analytical methods for the estimation of content of the drug from the tablet dosage form. The aim of this work was to develop and validate [3,4,5] a simple, rapid, cost effective and environmental friendly HPLC[6,7] method for the determination of Glipizide in pharmaceutical dosage form in tablets.

MATERIAL AND METHODS

A Thermo separation HPLC, Spectra SERIES chromatograph equipped with P100 pump, UV detector SpectraSERIES, UV100 and an autosampler SpectraSERIES AS100 was used. The column used was Thermo Hypersil – Keystone; Hy Purity, C18 stainless steel column [5µ, 15cm x 4.6mm], operating at room temperature. The elution was carried out isocratically at flow rate of 1ml/min – using methanol: 0.115%w/v of ammonium dihydrogen phosphate in water with pH adjusted to 6.0 with 2M of sodium hydroxide [(45:55) v/v] as mobile phase. The detector was set at a wavelength of 225nm. The responses of peak areas were recorded and integrated using Thermo separation products HPLC data integration software CSW1.

Chemicals and Reagents

Glipizide was provided by the manufacturer M/s Precise ChemiPharma Pvt. Ltd. Placebo / Tablet base for Glipizide tablet formulation was prepared in-house[1]. Ammonium dihydrogen phosphate and sodium hydroxide used were AR grade. Methanol and water used were of HPLC grade purity.

Stationary Phase

Thermo Hypersil – Keystone; Hy Purity C18 stainless steel, 150 mm length, diameter of 4.6mm and 5µ particle size.

Preparation of Mobile Phase

1.15g of Ammonium dihydrogen phosphate was dissolved in 1000 ml of water and adjusted the pH to 6.0 with 2M sodium hydroxide. 550 ml of this solution was mixed with 450 ml of methanol. The solution was filtered through 0.45µ membrane filter paper and degassed before use.

Preparation of standard stock solution

100 mg of Glipizide was taken in a 100 ml volumetric flask and was dissolved in methanol and the volume was made up to the mark to get 1 mg/ml concentration of Glipizide. 10 ml of this solution was diluted to 100 ml with methanol to get standard stock solution of Glipizide (0.1mg/ml)

Working standard solution

25 ml of standard stock solution was diluted to 50 ml with buffer to get a concentration of 50 µg/ml. The solution was mixed thoroughly and clarified through 0.2µ membrane filter and 20µl was injected into the system.

Sample solution

Weighed quantity (90 mg) of placebo (equivalent to 1 tablet base) was transferred into a 100 ml volumetric flask; 10 ml of standard stock solution was added and swirled to mix. The contents were sonicated in 70 ml of methanol for 10 minutes, diluted to volume with methanol and mixed. 25 ml of this solution was taken in a 50 ml volumetric flask and diluted upto the mark with buffer to get a concentration of 50 µg/ml.

System suitability

As per USP - 29 system suitability tests were carried out on freshly prepared working standard solution of Glipizide to check various parameters such as efficiency, retention time and peak tailing which was found to comply with USP requirements. Parameters obtained with 20µl injection volume are shown in Table 3. The instrumental precision as determined by 6 successive injections of the standard preparation gave RSD below 2.0% for RT, asymmetry, peak height and area. Column efficiency was 4567 theoretical plates.

Linearity

Several aliquots of standard stock solution of Glipizide were taken in different 50 ml volumetric flask and diluted upto the mark with buffer such that the final concentration of Glipizide were in the range of 10 to 70 µg/ml. Evaluation of Glipizide were performed with UV detector set at 225 nm. Peak area was recorded for all the peaks and a calibration graph was obtained by plotting peak area versus concentration of Glipizide.

The plot of peak area of each sample against respective concentration of Glipizide was found to be linear in the range of 10 to 70 µg/ml with coefficient of correlation $r^2 = 0.99956$.

Linear regression least square fit data obtained from the measurements are given in the Table I. The respective slope (m), Y-intercept (b) and correlation coefficients (r^2) are all quoted in Table 1.

Assay

20 µl of standard and sample solutions were injected into an injector of liquid chromatograph. From peak area of Glipizide, the amount of the drug sample were computed. The values are given Table 2.

Recovery studies

To study the accuracy and reproducibility and the precision of the proposed method recovery experiments were carried out. Several aliquots of standard stock solution of Glipizide were taken into the placebo (equivalent to 1 tablet base) at 7 different levels. Each level was repeated three times.

RESULTS AND DISCUSSION

As per USP-29 system suitability tests were carried out on freshly prepared working standard solution of Glipizide to check various parameters such as efficiency and peak tailing which was found to comply with the requirements. Parameters obtained with 20µL injection volume are shown in Table 3.

Table 1: Linear Regression (Least Squares Fit) data for calibration curves

Drug	Glipizide
Conc. Range µg/ml	10 - 70
Slope (m)	112.0263
Intercept (b)	-0.612293
r^2	0.99956

Table 2: Results of HPLC Assay Recovery

Amount spiked mg /tablet base	Amount found mg/tablet base
10.03	9.98
	10.00
	10.01
	9.94
	10.02
	9.99
Mean	9.99
SD	0.279
% RSD	0.280

Table 3: System suitability parameter

Parameter	Glipizide	RSD
RT	12.724	0.223
Theoretical plates	4567.833	1.056
Capacity	11.722	0.237
Asymmetry	1.675	0.962
Height	201.860	1.682
Area	5840.516	1.539

Table 4: Recovery study for spiked concentration of Glipizide drug to the tablet base of dosage form

Amount added in mg	Amount recovered in mg*	Percent Recovery
10.03	10.07	100.46
20.06	20.24	100.91
30.09	29.91	99.42
40.12	39.97	99.65
50.15	49.97	99.65
60.18	59.46	98.81
70.21	69.21	98.58

* Each value is average of three determinations

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The limit of detection (LOD) and limit of Quantification (LOQ) for Glipizide were found to be 2 µg/ml and 10 µg/ml.

The optimum mobile phase of methanol: 0.115%w/v of ammonium dihydrogen phosphate buffer [(45:55) v/v)] as mobile phase was selected because it was found to ideally resolve peak of Glipizide (RT-12.724) from other tablet excipients shown in Fig. 1 (chromatogram of test sample).

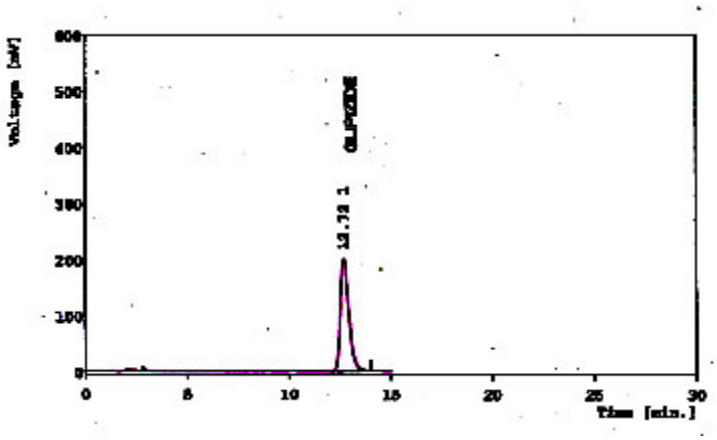

Fig. 1: Typical Chromatogram of the Test Sample

Wavelength was selected by scanning standard drugs over a wide range of wavelength 200 nm to 400nm. The analysis was performed at 225 nm detector setting.

The content of Glipizide per tablet base found by proposed method is shown in Table II. The lower values of RSD of assay indicate the method is precise and accurate. The mean recoveries of Glipizide were in the range of 98.58 to 100.91 %, which shows that there is no interference from excipients (Table 4), which also confirms reproducibility and reliability of the method.

CONCLUSIONS

The proposed method for the determination of Glipizide from tablet formulations is simple rapid and selective. Percent relative deviation (%RSD) was very low, below 2%, which indicates that the method is highly precise Table 3. The accuracy of the method at three different levels ranged from 99.58% to 100.91%, which indicates that the method is highly accurate. The method is free from interferences due to other tablet excipients. Short analysis time (<15 min. RT = 12.7) coupled with simplicity and ease of operation warrants use of the method for analysis of Glipizide from tablet dosage forms. Therefore the method can be useful in routine quality control analysis of these drugs.

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