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Development and Validation of RP-HPLC Method for Estimation of Glipizide in Bulk Drug and Pharmaceutical Formulation

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Abstract: A rapid, simple and sensitive RP-HPLC method was developed and validated for the determination of Glipizide in bulk drug and pharmaceutical formulation. Optimum separation was achieved by using 150 mm × 4.6 mm C18 column (3 µm) with a mixture of acetonitrile (ACN): water in the ratio of 60:40 at a flow rate of 1 ml/min. The detection was carried out at 276 nm and retention time was found to be 2.4 min. Linearity was observed (correlation coefficient r2 0.9995) in the concentration range of 0.5–18 µg/ml. The limit of detection (LOD) and limit of quantitation (LOQ) were found to be 0.3 and 0.5 µg/ml, respectively. % R.S.D was found to be less than 2 %. Accuracy of the method found to be in the range of 99.60 to 101.20 (% w/w).
Key Words: Glipizide (GPZ), HPLC, Acetonitrile, Validation.

INTRODUCTION

Glipizide (structure given in figure 1) is N-(4-[N-(cyclohexylcarbamoyl)sulfamoyl]phenethyl)-5-methylpyrazine-2-carboxamide widely used as anti-diabetic drug (second generation sulfonylurea) producing action by blocking potassium channels in the beta cells of the islets of Langerhans. By partially blocking the potassium channels, it will increase the time the cell spends in the calcium release stage of cell signaling leading to an increase in calcium. The increase in calcium will initiate more insulin release from each beta cell. Methods for the determination of GPZ in pharmaceutical formulations and biological materials which have been reported previously included high performance liquid chromatography (HPLC), capillary electrophoresis, and spectrophotometric determination[1-3].

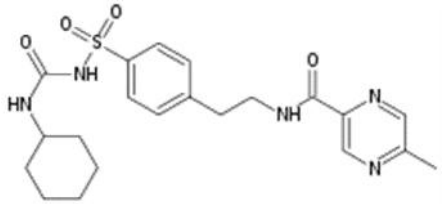

Figure: 1 Structure of GPZ

The main purpose of the present study was to establish a relatively simple, single - step, sensitive, validated and inexpensive RP-HPLC method for the determination of GPZ in pure form and in pharmaceutical dosage form, since most of the previous methods have been found to be relatively complicated and expensive. The developed methods were relatively more sensitive and the limit of detection (LOD) and limit of quantitation (LOQ) values for proposed methods were lower than the Reported method in the literature[4-7].

MATERIALS AND METHODS

Materials

GPZ was procured as a gift sample from concern pharmaceuticals (Ludhiana). HPLC grade acetonitrile, water were purchased from Rankem Ltd. (New Delhi, India). All other chemicals and solvents were of analytical reagent grade. All solutions were prepared in HPLC grade water[6,7].

HPLC Instrument and Software

The analysis was performed on HPLC system of WATERS (Milford, USA) composed of 515 solvent delivery system equipped with Rheodyne injection valve with a 20 µl loop, PDA detector set at wavelength range 190-800 nm. Separation was performed on a WATERS C18 column 150 mm × 4.6 mm (3µm). Chromatographic data were recorded and processed using EMPOWER-2 software[6-8].

Chromatographic conditions

Mobile phase consist of ACN-water in the ratio of 60:40 at a flow rate of 1 ml/min and UV detection was performed at 276 nm. The mobile phase was degassed by an ultrasonic bath before each use. The column was equilibrated for at least 30 min with the mobile phase flowing through the system. Each solution was injected in triplicate.

Preparation of stock and standard solutions

Stock solution of GPZ (1 mg/ml) was prepared in mobile phase and stored at 2–8 ˚C until used. Aliquots were diluted stepwise with the mobile phase to obtain 10 µg/ml. This solution was used for the optimization of the proposed method[1].

Optimization of chromatographic conditions

The effects of different chromatographic conditions on the instrument response create a situation where one has to compromise between different experimental variables in order to achieve the best chromatographic separation. Chromatographic separations are significantly affected by the mobile phase conditions, such as the type and composition of the organic modifiers and therefore before selecting the conditions for the optimization, a number of preliminary trials were conducted with different combinations of different organic solvents and buffers at various pH, compositions, and flow rate to check the retention time, shape, resolution, and other chromatographic parameters. From those experiments the mobile phase combination of ACN and water was found to be most suitable[6,7].

In order to achieve an optimum separation, following conditions were studied: (i) Mobile phase composition varied at 60:40, 70:30, 85:15, 90:10, 55:45 and 50:50 and flow rate kept constant at 7.4 and 1.0 ml/min, respectively. (iii) Flow rate was varied (0.8, 1.0, and 1.2 ml/min) with mobile phase composition 60:40. Moreover, the effects of different level of all these factors were systematically addressed on system suitability parameters such as resolution, theoretical plates, retention time, capacity factor, separation factor, asymmetry, and HETP etc.

After applying the different chromatographic parameters during the method development, the recommended mobile phase consisted of ACN: water of 60:40 % v/v/v. The best resolution and sensitivity of the method was obtained at 276 nm and mobile phase flow rate of 1 ml/min. typical chromatogram at the optimized condition give sharp and symmetric peak with retention time of 2.4 min[9,11].

Validation of the proposed method

After chromatographic method development and optimization it was validated. The validation of an analytical method verifies that the characteristics of the method satisfy the requirements of the application domain. The proposed method was validated according to ICH guidelines for linearity, precision, sensitivity, and recovery. For linearity studies, working standard solutions equivalent to 0.5 to 18 µg/ml of GPZ were prepared with the mobile phase. Calibration graph was prepared by plotting the mean peak area versus concentration of GPZ.

Precision & Accuracy

According to ICH, precision is the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions and may be considered at three levels: repeatability, intermediate precision and reproducibility. The intra-day and inter-day variations of the method were determined using five replicate injections of four concentrations and analysed on the same day and three different days.

The accuracy of an analytical method expresses the closeness between the theoretical value and experimental value. To ensure the reliability and accuracy of the method, the recovery studies were carried out by spiking method. Accuracy was evaluated by injecting the GPZ about five times, at three different concentrations equivalent to 80, 100, and 120 % of the active ingredient, by adding a known amount of GPZ standard to a sample of known concentration and calculating the recovery of GPZ for each concentration.

Detection and quantification limits

The limits of detection and quantification were calculated by the method based on standard deviation (σ) and slope (S) of the calibration plot using the formula LOD = $3.3\sigma/S$ and LOQ = $10\sigma/S$.

Specificity
The specificity test of proposed method demonstrated that excipients from tablets do not interfere in the drug peak. Furthermore, well shaped peaks indicate the specificity of the method.

Assay
Twenty tablets were weighed and powdered equivalent to 10 mg of GPZ was accurately weighed and diluted up to 10 ml. Working dilution was prepared using same diluents and used for analysis.

RESULTS AND DISCUSSION

Method development
Chromatographic conditions were optimized for the proposed method. A solution of GPZ was injected five times and was monitored by UV detection at 276 nm. A mobile phase consisting of ACN: water in the ratio 60:40 (% v/v/v) was selected after several preliminary experiments. At a flow rate of 1 ml/min the retention time was 2.4 min as shown in Figure 2. Under the described experimental conditions, the peak was found to be symmetric. System suitability parameters for the peak are as shown in the Table 1.

Validation Parameter
Method was validated by calculating different parameters which are shown in Table 2 along with their results.

Linearity
Linearity was found to be in the range of 0.5-18 µg/ml (Correlation coefficient r2=0.9995). The overlay of peaks is as shown in Figure 3.

Precision
The intra-day and inter-day variations of the method were determined using three replicate injections of four concentrations and analysed on the same day and three different days. The result revealed the precision with % RSD (1.329 % and 1.188 %) respectively for intra-day and inter-day. Results of intra-day and inter-day precision are as shown in Table 3.

Accuracy (% Recovery)
To examine the accuracy of the method, recovery studies were carried out by standard addition method. Accuracy was found to be 100.08±2.42. The recovery results are as shown in Table 4.

Assay
Assay for the pharmaceutical formulation was established with present chromatographic conditions developed and it was found to be accurate and reliable. Figure 4 shows the selective & specific estimation of GPZ in presence of excipients of pharmaceutical formulation and % purity was found to be 98.3 % w/w. There was no interference found due to excipient. The obtained results were as shown in Table 5.

CONCLUSION

The data obtained indicated that proposed method is simple accurate, precise and less time consuming and can be useful for routine determination of GPZ in bulk drug and pharmaceutical formulation.

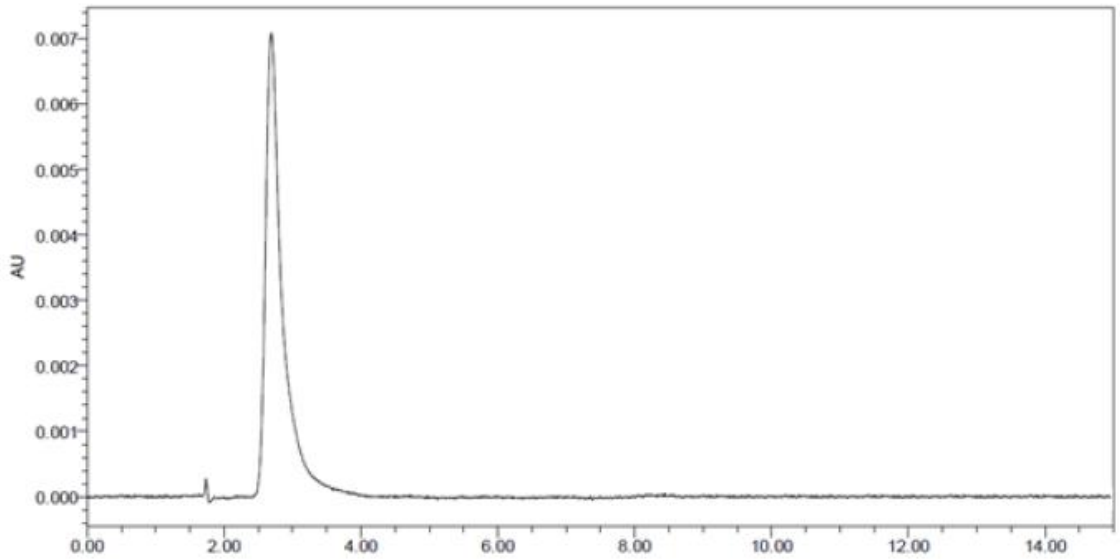

Figure: 2 Separation results of GPZ under Optimized Conditions

Table: 1 The system suitability parameters for the peak

System suitability parameters	Results	Acceptable limits
Asymmetry Factor	1.3	<1.5
Tailing Factor	1.5	,2
Plate No.	>2000	>2000
HETP	0.011cm	

Table: 2 Validation parameters

Parameters	Results
Linearity range	0.5-18 µg/ml
Regression equation	Y= 9544x-778.2
Slope[a]	9544
Intercept[b]	778.2
Correlation Coefficient (r^2)	0.9995
Limit of detection	0.3µg/ml
Limit of quantification	0.5µg/ml

Regression equation Y=aX-b, a: slope, b: intercept

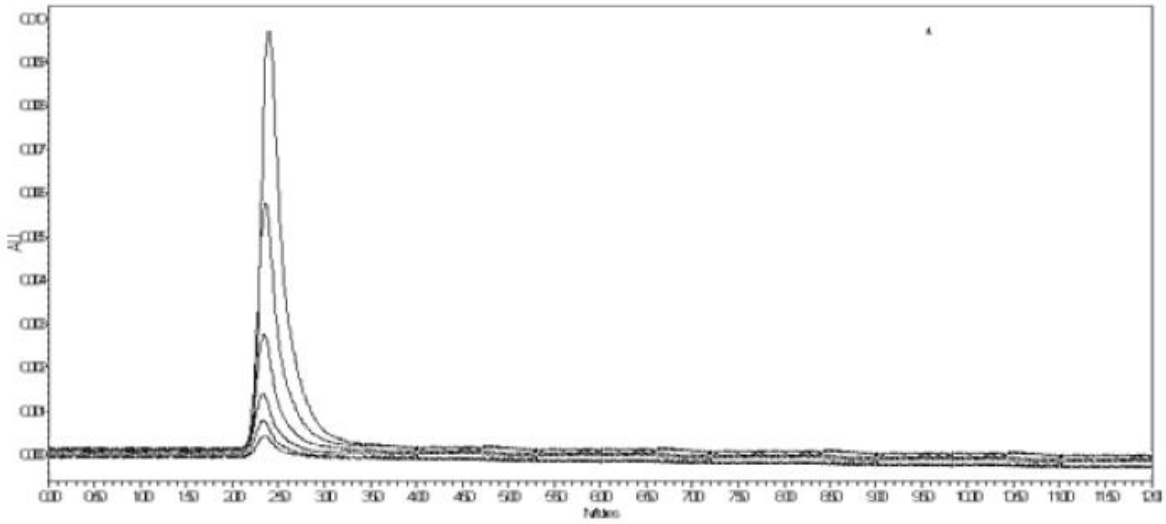

Figure: 3 Overlay of peaks in range of 0.5-16 µg/ml

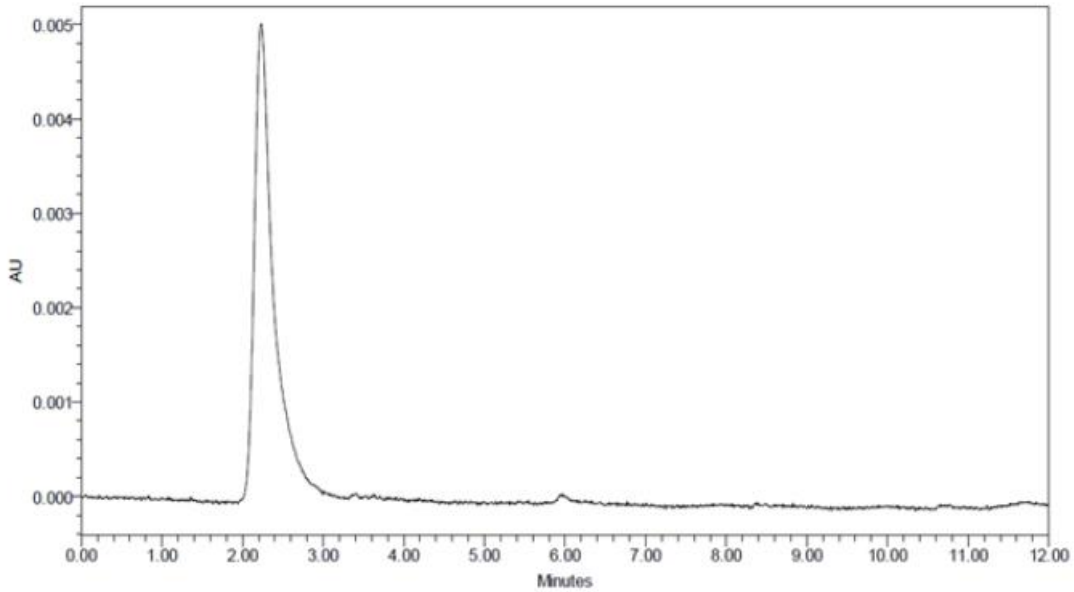

Figure: 4 Separation result of pharmaceutical formulation

Table: 3 The **intra-day and inter-day Precision**

Concentration (µg/ml)	Observed Concentration* (µg/ml)			
	Intra-day	% RSD	Inter-day	% RSD
1	1.02	1.535	0.94	1.23
2	1.96	1.87	1.94	1.67
4	3.97	0.9205	4.01	1.04
8	7.98	0.9860	7.87	0.812

*mean of six values (n=6)

Table: 4 Recovery results of the method

Sample Amount (µg/ml)	Amount added (µg/ml)	Amount recovered* (µg/ml)	% Recovery (w/w)
2	1.6	1.58	98.75
2	2	1.98	99
2	2.4	2.46	102.5

*mean of six values (n=6)

Table: 5 The Results of assay of pharmaceutical formulation

Sample amount(µg/ml)	8
Amount estimated(µg/ml)	7.8
Mean* ± SD	8±0.414
% Purity	98.38%

*mean of six values (n=6)

REFERENCES

1. Poole P. Pantoprazole, Am. Health Syst. Pharm., 2001;58 (11): 999-1008.
2. Hitt E. Uses of metformin may extend beyond patients with type 2 diabetes. Drugs 1995;63:1879-94.
3. Adel Aly M, Mazen Qato K, Mahrous Ahmad O,"Enhancement of the dissolution rate and bioavailability of glipizide through cyclodextrin inclusion complex", Drug Del Ind Pharm., 2002;32: 578-584.
4. Mutalik, S., Udupa, N. Transdermal delivery of glibenclamide and glipizide: in vitro permeation studies through mouse skin. Pharmazie, 2002;12:838–841.
5. Vasudevan M, Ravi J, Ravishankar S, Suresh B. Ion-pair liquid chromatography technique for the estimation of metformin in its multi-component dosage forms. J Pharm Biomed Anal. 2001;25:77-84
6. Cheng CL, Chou CH. Determination of metformin in human plasma by high-performance liquid chromatography with spectrophotometric detection. J Chromatogr B Biomed Sci Appl. 2001;762:51-58.
7. Emilsson, H. High performance liquid chromatographic determination of glipizide in human plasma and urine. Journal of Chromatography.1987;421:319–326.
8. Yuen KH, Peh KK. Simple high performance liquid chromatographic method for the determination of metformin in human plasma. J Chromatogr B Biomed Sci Appl 1998;710:243
9. Klepser TB, Kelly MW. Metformin hydrochloride: an antihyperglycemic agent. Am J Health System Pharm 1997;54:893-903.
10. Arayne MS, Sultana N, Zuberi MH. Development and validation of RP-HPLC method for analysis of metformin. Pak J Pharm Sci 2006;19:231-235.
11. Verma KR, Garg S. "Development and Evaluation of Osmotically Controlled Oral Drug
12. Delivery System of Glipizide", Eur J Pharm Biopharm., 2004; 57: 513-525.
13. Verma, K.R, Kaushal M.A, Garg S. "Development and Evaluation of Extended Release Formulations of Iso-sorbide MonoNitrate based on Osmotic Technology", Int J Pharm., 2003; 263: 9-24.
14. Mansour AM, Sorour OM. High-performance liquid chromatographic determination of pantoprazole in tablet dosage form, Chromato graphia, 53;478-479:2001.
15. Tanaka M, Yamazaki H. Direct determination of pantoprazole enantiomers in human serum by reversedphase high-performance liquid chromatography using a cellulose-based chiral stationary phase and columnswitching system as

a sample cleanup procedure, Anal. Chem.1996;68 (9);1513-1516.
16. Ekpe A, Jacobsen T. Effect of various salts on the stability of lansoprazole, omeprazole, and pantoprazole as determined by high-performance liquid chromatography, Drug Dev. Ind. Pharm. 1999;25 (9):1057-1065.
17. Tivesten A, Folestad S, Schonbacher V, Svensson K. Nonaqueous capillary electrophoresis for the analysis of labile pharmaceutical compounds, Chromatographia. 1999; 49 (1): 7-11.
18. Daniela E, Rolf PH, Reinhard K. Chiral resolution of pantoprazole sodium and related sulfoxides by complex formation with bovine serum albumin in capillary electrophoresis, J. Chromatogr. A. 1997;759:185-192.
19. Abdel-Aziz MW, Omayma A, Azza AG, Hoda M, Marwa SM. Spectrophotometric determination of omeprazole, lansoprazole and pantoprazole in pharmaceutical formulations, J. Pharm. Biomed. Anal. 2002;30:1133-1142.
20. Bruni AT, Leite VB, Ferreira MM. Conformational analysis: a new approach by means of chemometrics, J. Comput. Chem. 2002;23 (2): 222-236.

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