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DEVELOPMENT AND VALIDATION OF A LIQUID CHROMATOGRAPHIC METHOD FOR ESTIMATION OF GLIMEPIRIDE IN TABLET DOSAGE FORM

Vaishali V. Karkhanis*[1] and Anandkumari D. Captain [2]

A.R. College of Pharmacy [1], Vallabh Vidhyanagar - 388120, Dist: Anand, Gujarat, India
G.H. Patel Institute of Pharmacy [2], Vallabh Vidhyanagar - 388120, Dist: Anand, Gujarat, India

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Correspondence to Author:

Vaishali V. Karkhanis

A.R. College of Pharmacy [1], Vallabh Vidhyanagar - 388120, Dist: Anand, Gujarat, India

E-mail: vesli@rediffmail.com

ABSTRACT: An accurate, sensitive and precise RP-HPLC method has been developed and validated for the estimation of Glimepiride (GLM) from bulk drug and Pharmaceutical Dosage form. The separation was achieved by Hypersil C18 column (250mm X 4.6mm, 5µm) in isocratic mode, with mobile phase comprises of Acetonitrile : Buffer in proportion of 60:40v/v, buffer was 0.02M Potassium Di-hydrogen Phosphate (pH 4.5 adjusted with Ortho Phosphoric Acid). The flow rate of mobile phase was 1.0ml/min and employing UV detection with 232nm wavelengths. The retention time of GLM was 5.420 min.The calibration curve was found to be linear within the concentration range of 50µg/ml to 150µg/ml. The regression data for calibration curve shows good linear relationship with r^2 = 0.9965. The method was validated in accordance with the requirements of ICH guidelines. Moreover, the proposed analytical method was applied to monitor the formulation commercially available.

INTRODUCTION: Glimepiride is widely used in the treatment of non-insulin dependent Type II diabetes mel-litus[1]. It acts by stimulating insulin secretions from the beta cells of pancreas and is also known to increase peripheral insulin sensitivity thereby decreasing insulin resistance. It can be used in combination with metformin, thiazolidinediones, alpha-glucosidase inhibitors and in-sulin[2]. After oral administration, it is completely absorbed from the gastrointestinal tract. Peak plasma concentration is reached 2 - 3 hrs after dosing. Its bioavailability changes a little with food and glimepiride (99.5%) are bound to proteins.

QUICK RESPONSE CODE

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Glimepiride is completely metabolised in liver. The structure of glimepiride is shown in **Figure 1**.

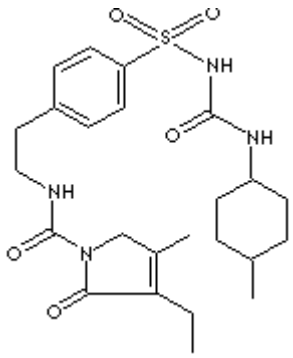

FIGURE 1: GLIMEPIRIDE

Several different methods have been reported for qualitative and quantitative analysis of glimepiride; these include micellar electrokinetic capillary chromatography (MECC) with diode-array detection (DAD) or ultraviolet (UV) detection, high performance liquid chromatography (HPLC) with DAD and UV detection and derivative UV Spectro-photometric detection [3, 4], liquid chromatography-

electrospray ionization mass spectrometry (LC-ESI/MS) [5-8], an HPLC method for the quantification of glimepiride in single dose formulation [9-11], in human plasma [12-13], the quantification of cis-isomer of glimepiride by normal phase chromatography[14].A simple accurate and precise HPLC method has been developed for estimation of glimepiride in single dosageform.

MATERIALS & METHODS:

Material: The HPLC system consisted of following components: Shimadzu- Model LC20AT. Rhenodyne valve with 20µl fixed loop, isocratic system pump, Chromatographic analysis was performed on Hypersil BDS C18 column 250×4.6 mm, 5µm particle size. Analytically pure glimepiride was procured as gift samples from Hetero Labs Ltd, Andhra Pradesh, India. All other chemicals and reagents used were analytical grade and purchased from Merck Chemicals, India. Tablets were procured from the local market.

Methods:

a. **Preparation of standard stock solution and solutions for calibration curve:** Stock solutions of Glimepiride were prepared by dissolving 100 mg of Glimepiride in 100 ml of volumetric flask with diluent. Aliquot of 5.0ml of the standard stock solution of glimepiride was transferred into 50 ml volumetric flask and from that appropriate aliquots were taken to give concentration range of 50-150µg/ml for calibration curve.

b. **Chromatographic conditions:** Chromatographic estimation was performed using a Hypersil BDS C18 column (250mm×4.6mm i.d.), mobile phase consisting of Acetonitrile: Buffer in proportion of 60:40v/v, buffer was 0.02M Potassium Di-hydrogen phosphate (pH 4.5 adjusted with Ortho Phosphoric Acid 5%). Detection was done at wavelength of 232nm. The sample was injected using a 20µl fixed loop, flow rate 1ml/min and the total run time was 8 minutes.

c. **Validation:** The method was validated as per the ICH guideline.

i. **Regression analysis**: Regression of analytical method is expressed in terms of correlation co-efficient of the regression analysis. Accuracy- For determination of Accuracy, recovery study was carried out. That was performed by standard addition method at three different levels (80%, 100%, and 120%), to the pre-analyzed samples and the subsequent solutions were re-analyzed. At each level, three determinations were performed.

ii. **Precision**: The precision of analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogeneous samples. Intraday precision- Intraday variance for the Glimepiride was done at the interval of 3 hrs. Interday precision- Interday variance for the Glimepiride was done at the interval of one day.

iii. **Limit of Detection (LOD):** LOD was found out based on the standard deviation of the response and the slope method. Limit Of Quantification (LOQ) - LOQ was foundout based on the standard deviation of the response and the slope method. Specificity- Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present.

d. **Determination of Glimepiride in Tablet Dosage Form:** Twenty tablets were weighed, finely powdered, and an accurately weighed sample of powdered tablets equivalent to 10mg of Glimepiride was treated with mobile phase in a 100mL volumetric flask using ultra sonicator. This solution was filtered through 0.45 µm filter paper. Suitable aliquot of the filtered solution was added to a volumetric flask and make up to volume with mobile phase to get appropriate concentration in range.

RESULTS AND DISCUSSION: Several mobile phase compositions were tried to resolve the peak of GLM. The mobile phase containing Acetonitrile: Buffer in proportion of 60:40v/v, buffer was 0.02M Potassium Di-hydrogen (pH 4.5 adjusted with Ortho Phosphoric Acid) was found ideal to resolve the peak of GLM. Retention time of GLM was 5.420 min (**Figure 2**).

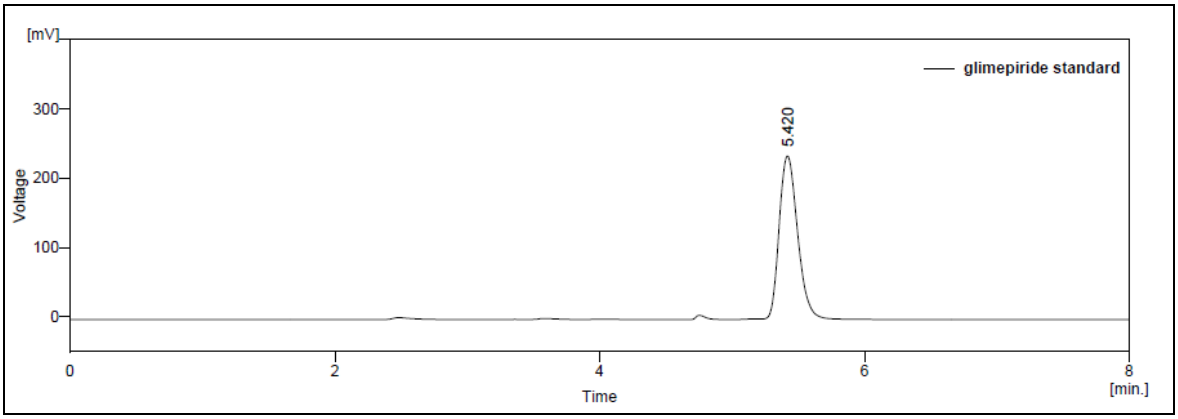

FIGURE 2: CHROMATOGRAM OF GLM STANDARD

Linear regression data showed a good liner relationship over a concentration range of 50-150µg/ml for GLM. The correlation coefficient (r^2) was 0.9965 (**Figure 3**).

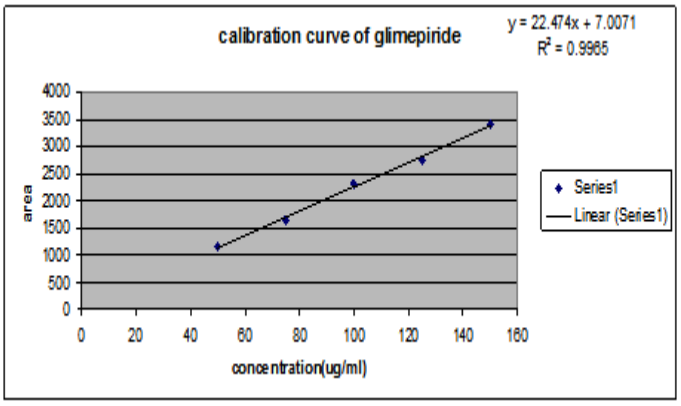

FIGURE 3: CALIBRATION CURVE OF GLM

The system suitability parameters are shown in **Table 1.**

TABLE 1: SYSTEM SUITABILITY PARAMETERS

System suitability Parameters	
Retention times (RT)	5.420
Theoretical plates (N)	6982
Tailing factor (AS)	1.4
%RSD(n=5)	0.2

The accuracy of the method was evaluated by carrying out recovery studies, were performed by standard addition method at three different levels I, II and III (80%, 100%, and 120%), to the pre-analyzed samples and the subsequent solutions were re-analyzed. At each level, three determinations were performed (**Table 2**).

TABLE 2: RECOVERY STUDY DATA FOR GLM

Level	Area	Amount Recovered (µg/ml)	%recovery	%RSD
80	1818.176667	80.58	100.22	0.673
100	2256.281	100.08	99.59	1.056
120	2714.528333	120.47	99.72	1.054

The limit of detection and limit of quantification were found to be 1.82ug/ml and 5.52ug/ml respectively. The intra-day and inter day precision was determined by analyzing standard solution of 50, 100 and 150 µg/mL and the results are reported in terms of relative standard deviation (**Table 3**).

TABLE 3: PRECISION DATA

	intraday			interday		
concentration	**50(µg/ml)**	**100(µg/ml)**	**150(µg/ml)**	**50(µg/ml)**	**100(µg/ml)**	**150(µg/ml)**
Mean	1136.306	2302.746667	3442.83	1132.375	2270.558	3360.371
S.D	13.64	29.01	35.20	13.93	26.79	43.02
% RSD	1.20	1.26	1.02	1.23	1.18	1.28

The assay result was repeated for three times which was found to be 99.76-98.11 % of labelled claim (**Table 4**).

TABLE 4: ASSAY RESULT

Formulation	Mg/tablet	%assay
Tablet	2	99.76

CONCLUSIONS: A method of quantitative determination of Glimepiride using HPLC has been developed. The validation results have demonstrated that this method is accurate, precise and linear. The method can also be applied for drug content in pharmaceutical preparations.

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REFERENCES:

1. http://www.drugbank.ca/drugs/DB00222.
2. S. N. Davis: The Role of Glimepiride in the Effective Management of Type 2 Diabetes. Journal of Diabetes and its Complications 2004; 18: 367-376.
3. Altinoz, S. and Tekeli, D.: Analysis of glimepiride by using derivative UV spectrophotometric method. Journal of Pharmaceutical and Biomedical Analysis 2001; 24:507-515.
4. Anju G, Singhvi I.: Simultaneous spectrophotometric estimation of rosiglitazone maleate and glimepiride in tablet dosage forms. Indian Journal of Pharmaceutical Sciences 2007; 69(6):780-783.
5. I. I. Salem, J. Idrees and J. I. Al Tamimi: Determination of Glimepiride in Human Plasma by Liquid Chromatography-Electrospray Ionization Tandem Mass Spectrometry. Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences 2004; 799(1): 103-109.
6. H. Kim, K. Y. Chang, H. J. Lee and S. B. Han: Determination of Glimepiride in Human Plasma by Liquid Chromatography-Electrospray Ionization Tandem Mass Spectrometry," Bulletin of the Korean Chemical Society 2004; 25(1): 109-114.
7. H. Kim, K. Y. Chang, C. H. Park, M. S. Jang, J. A. Lee, H. J. Lee and K. R. Lee: Determination of Glimepiride in Human Plasma by LC-MS-MS and Comparison of Sample Preparation Methods for Glimepiride. Chromatographia 2004; 60(1-2): 93-98.
8. N. Yuzuak, T. Ozden, S. Eren and S. Ozilhan: Determination of Glimepiride in Human Plasma by LC-MS-MS. Chromatographia 2007; 66(1):165-168.
9. Wanjari, D.B, Gaikwad N.J.: Reversed phase HPLC method for determination of glimepiride in tablet dosage form. Indian Journal of Pharmaceutical Sciences 2005; 67:253-255.
10. S. P. Pawar, G. A. Meshram and M. U. Phadke: Simultaneous LC Estimation of Glimepiride and Metformin in Glimepiride Immediate Release and Metformin Sustained Release Tablets. Chromatographia 2008; 68(11-12): 1063-1066.
11. I. U. Khan, F. Aslam, M. Ashfaq and M. N. Asghar: Determination of Glimepiride in Pharmaceutical Formulations Using High Performance Liquid Chromatography and First-Derivative Spectrophotometric Methods. Journal of Analytical Chemistry 2009; 64(2):171-175.
12. K. H. Lehr and P. Damm: Simultaneous Determination of the Sulphonylurea Glimepiride and Its Metabolites in Human Serum and Urine by High-Performance Liquid Chromatography after Pre-Column Derivatization. Journal of Chromatography-Biomedical Applications 1990; 526(1): 497-505.
13. Y. K. Song, J. E. Maeng, H. R. Hwang, J. S. Park, B. C. Kim, J. K. Kim and C. K. Kim: Determination of Glimepiride in Human Plasma Using Semi Microbore High Performance Liquid Chromatography with Column switching. Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences 2004; 810(1):143-149.
14. D. B. Pathare, A. S. Jadhav and M. S. Shingare: RP-LC Determination of the Cis-Isomer of Glimepiride in a Bulk Drug Substance. Chromatographia, 2007; 66(7-8): 639-641.

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