INTERNATIONAL RESEARCH JOURNAL OF PHARMACY

www.irjponline.com ISSN 2230 – 8407

Research Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF GLIPIZIDE AND METFORMIN IN BULK DRUGS AND TABLET DOSAGE FORM

D.Triveni[1], G.V.S Kumar[1]*, S.B. Puranik[1], N.Sateesh Kumar[2], K.A.Sridhar[3]

[1]Department of Pharmaceutical Analysis, East West College of Pharmacy, Bharathnagar, Bangalore, Karnataka, India

[2]Department of Pharmaceutics, East West College of Pharmacy, Bharathnagar, Bangalore, Karnataka, India

[3]Department of Pharmacology, East West College of Pharmacy, Bharathnagar, Bangalore, Karnataka, India

Article Received on: 08/07/12 Revised on: 10/08/12 Approved for publication: 15/09/12

*Email: ewcp.analysis@gmail.com

ABSTRACT

The present work describes development and validation of simple, precise and accurate reversed-phase liquid chromatographic method for simultaneous estimation of glipizide and metformin hydrochloride in both bulk drugs and pharmaceutical dosage forms. The chromatographic separation was achieved on (Enable, symmetry C18, 250mm x 4.6mm, 5µ) analytical column. A mobile phase consisting mixture of potassium dihydrogen phosphate (0.2M, pH 5.8 adjusted with dilute sodium hydroxide) and acetonitrile in ratio (60:40 v/v) at flow rate of 1.0ml/min and UV detector wavelength 258 nm. The retention time of glipizide and metformin Hcl was found to be 7.9 and 2.5 minutes respectively.

The method was successfully validated in accordance to ICH guidelines for accuracy, precision, specificity, linearity, ruggedness and robustness. The linear regression analysis data for calibration plots showed good linear relationship in the concentration range 60-140 µg/mL for both glipizide and metformin hydrochloride.

KEYWORDS: Glipizide, Metformin hydrochloride, RP-HPLC, Validation, Simultaneous estimation.

INTRODUCTION

Glipizide (GPZ) is chemically 1- cyclohexyl -3- [{4–[2-[[(5 – methylpyrazine -2-yl) carboxamido] amino] ethyl] phenyl] sulfaonyl] urea is an oral medium-to-long acting anti-diabetic drug from the sulfonylurea. It is classified as a second generation sulfonylurea, which means that it undergoes enterohepatic circulation. It is an oral diabetes medicine that helps control blood sugar levels. This medication helps pancreas to produce insulin. It is used along diet and exercise to treat type II diabetes. It blocks potassium channel in the beta cells of the islets of langerhans[1].

Metformin HCl (MET) is chemically *N,N*-dimethylimidodicarbonimidic diamide hydrochloride. Metformin improves hyperglycemia primarily through its suppression of hepatic glucose production. It activates AMP-activated protein kinase (AMPK), a liver enzyme that plays an important role in insulin signaling, whole body energy balance, and the metabolism of glucose and fats, activation of AMPK is required for metformin inhibitory effect on the production of glucose by liver cells. Activation of AMPK is required for an increase in the expression of SHP, which in turn inhibits the expression of the hepatic gluconeogenic genes PEPCK and Glc-6-Pase. Metformin increases the amount of cytosolic AMP. Metformin is contraindicated in people with any condition that could increase the risk of lactic acidosis, including kidney disorders, lung disease and liver disease[2].

The literature survey reveals that, GPZ and MET are reported in United State Pharmacopoeia. There have been several publications describing analytical methods for the determination of GPZ[3,4] and MET[5-9] individually or with other drugs as combination. So the aim of our present study was is to develop simple, fast, accurate and specific reversed phase high performance liquid chromatographic method for simultaneous determination of related substances of Glipizide and Metformin Hcl in bulk drugs and tablet formulation. Further validated as per ICH guidelines.

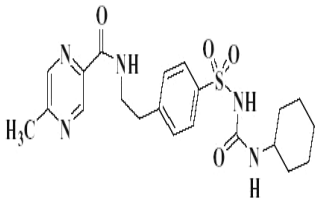


Figure 1: Glipizide

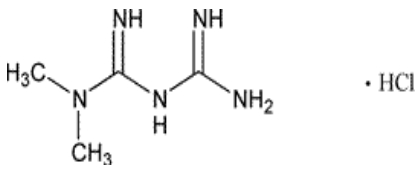


Figure 2: Metformin hydrochloride

EXPERIMENTAL

Chemicals and Reagents

Pure samples of GPZ and MET were provided by Supra Chemicals, Mumbai and Dr. Reddy's, Hyderabad, India. The commercial pharmaceutical preparation Glynase- MF containing 5mg and 500mg of Glipizide and Metformin hydrochloride respectively (manufactured by USV Pvt. Ltd.) were procured from local pharmacy. Acetonitrile HPLC grade, potassium dihydrogen orthophosphate, sodium hydroxide were procured from Thermo fisher scientific India Pvt. Ltd, Mumbai, India. High purity deionised water was obtained from [Millipore, Milli-Q] purification system.

HPLC instrumentation and conditions

HPLC system LC SHIMADZU UFLC-2000 ProminanceLC-20AD Binary Gradient System, Shimadzu Corporation, Japan. The column compartment having temperature control and Photodiode Array/ Ultraviolet (PDA/UV) Detector was employed throughout the analysis. Chromatographic data was acquired using Empower software.

Chromatographic conditions

Enable C-18 (250X4.6mm, 5µm) column was used. Mobile phase consisting of 0.2M KH_2PO_4 buffer (0.2M KH_2PO_4 buffer was prepared by dissolving 27.21g in 1000 ml of

Millipore water or double distilled water and pH adjusted to 5.8 with sodium hydroxide): Acetonitrile,(60:40 v/v). The flow rate was 1.0 ml/min. UV detection was performed at 258 nm at ambient temperature using 20 µL injection volumes.

Standard and sample preparation

Glipizide (10mg) and metformin hydrochloride (10mg) were accurately weighed and transferred to two separate 10ml volumetric flask and dissolved with mobile phase to obtain stock solution of 1000µg/ml each. Standard calibration solutions of glipizide and metformin hydrochloride having concentration in the range of 60-140 µg/mL respectively were prepared by diluting stock solution with mobile phase.

Analysis of Dosage form

Twenty tablets of Glynase-MF (each containing 5mg and 500mg of glipizide and metformin hydrochloride) were made in to fine powder, an amount equivalent to one tablet content was transferred in to 100ml volumetric flask dissolve with mobile phase, filtered through 0.45µ filter and sonicated for 30min. The solution was taken in to a thoroughly cleaned and dried volumetric flask. The sample solution (20µl) was injected to HPLC

Method Validation[10,11]

The optimized chromatographic conditions were validated by evaluating specificity, linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), robustness and system suitability parameters in accordance with the ICH guideline Q2 (R1).

Linearity and Range

Linearity was determined by plotting the standard curve in the concentration range of 60-140µg/mL for both glipizide and metformin hydrochloride **Fig.5**. The linearity of the methods was evaluated by linear regression analysis, using least square method **Table-1**.

Accuracy

This parameter is performed to determine the closeness of test results with that of the true value which is expressed as % recovery. These studies were performed for both glipizide and metformin hydrochloride at three different levels (80%, 100% and 120%), the mixtures were analyzed by the proposed method. The experiment was performed in triplicate and recovery (%), standard deviation (SD) and relative standard deviation RSD (%) of the spiked drugs was calculated. Results are presented in **Table-2**.

Precision

The precision (system, method) of the proposed method was evaluated by carrying out six independent assays of the sample. RSD (%) of six assay values obtained was calculated. The intermediate precision was carried out by analyzing the sample at different days and different analysts and the data is presented in **Table-3**.

Specificity

Specificity of the method was evaluated by injecting the Blank sample, Standard sample separately into HPLC. The subjected drug peaks of GPZ and MET were evaluated with photo diode detector for purity angle **Fig.6**.

Limit of Detection and Limit of Quantification

The limit of detection (LOD) and the limit of quantification (LOQ) for GPZ and MET were determined from standard deviation of the response and the slope. Results are presented in **Table-6.**

$$LOD = \sigma/S \times 3.3;\ LOQ = \sigma/S \times 10$$

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage **Table-4**.

Robustness of the method was investigated under a variety of conditions like change in flow rate by ± 0.2 ml/minute, and change in wavelength by ± 2 nm. The mixed standard solution is injected in five replicates and sample solution of 100% concentration is prepared and injected in triplicate for every condition and % RSD of assay was calculated for each condition.

System suitability

The system suitability parameters with respect to theoretical plates, tailing factor and resolution peak were established. Results are presented in **Table-5.**

Table 1.Linearity data for GPZ and MET

Linearity (n=5)	GPZ	MET
Range	60-140µg/mL	60-140µg/mL
Mean 'r^2' value	0.998	0.998
Regression equation	Y=9072x + 63828	Y= 2549x + 152.1

Table 2.Recovery study of GPZ and MET

Drugs	Levels	Mean recovery(n=3)	±SD(n=3)	% RSD(n=3)
GPZ	L_1	100.9%	0.76	0.76
	L_2	101.7%	0.60	0.59
	L_3	100.3%	0.47	0.44
MET	L_1	100.0%	0.51	0.51
	L_2	99.3%	0.71	0.74
	L_3	102.5%	0.39	0.31

Table 3.Intermediate precision intra-day and inter-day of GPZ and MET

Drugs	Intra-day precision		Inter-day precision	
	% of Label	% RSD(n=3)	% of Label	% RSD(n=3)
GPZ	100.54	0.51	99.94	0.63
MET	99.78	0.64	99.94	1.08

Table 4.Robustness data for change in flow rate and wavelength

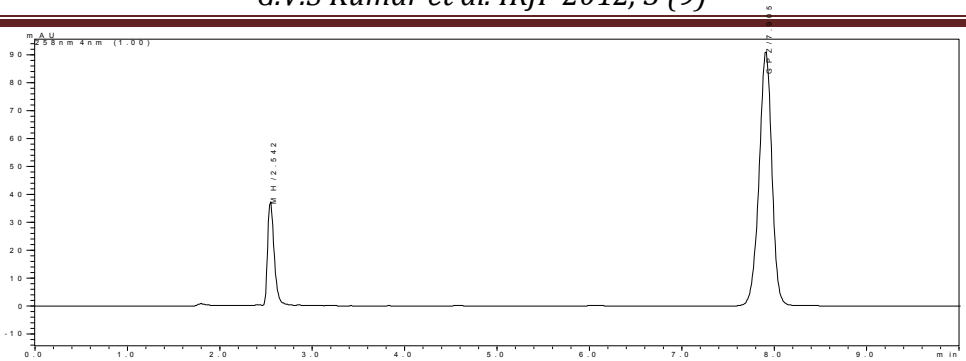
Changing Factor	Level	GPZ (n=3) Mean % assay % RSD	MET (n=3) Mean % assay % RSD
Flow rate	0.8 mL	100.74% (0.97%)	102.08% (1.01%)
	1.2 mL	99.25% (1.17%)	97.74% (1.39%)
Wavelength	256	98.06% (1.54%)	95.57% (1.25%)
	260	100.36% (0.67%)	99.79% (0.83%)

Table 5.System suitability data for GPZ and MET

Drugs	Retention time	Tailing factor	Theoretical plate	Resolution
GPZ	7.92	0.994	14401.391	
MET	2.54	10.41	6494.50	26.45

Table 6. LOD and LOQ data for GPZ and MET

Drugs	LOD	LOQ
GPZ	11.5ng/mL	34.91ng/mL
MET	40.99ng/mL	124.23 ng/mL



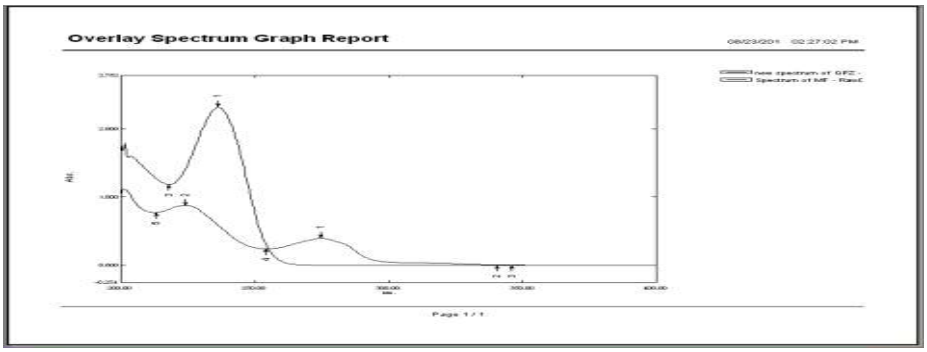


Figure 3.Overlay spectra of GPZ and MET

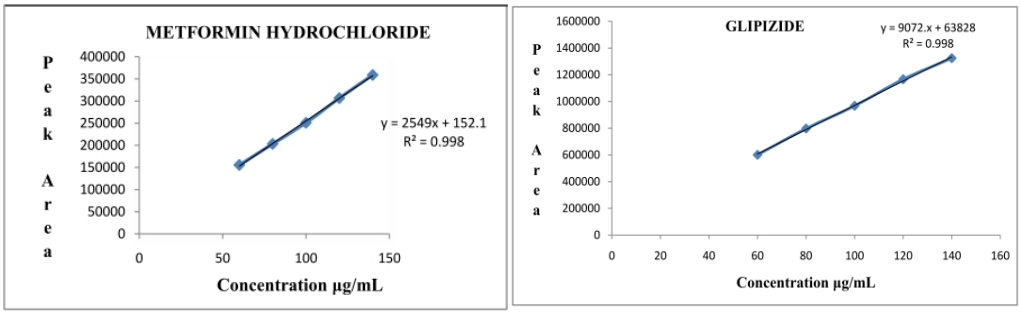


Figure 4.A typical chromatogram of standard solutions of GPZ and MET
Figure 5.Calibration curve of GPZ and MET

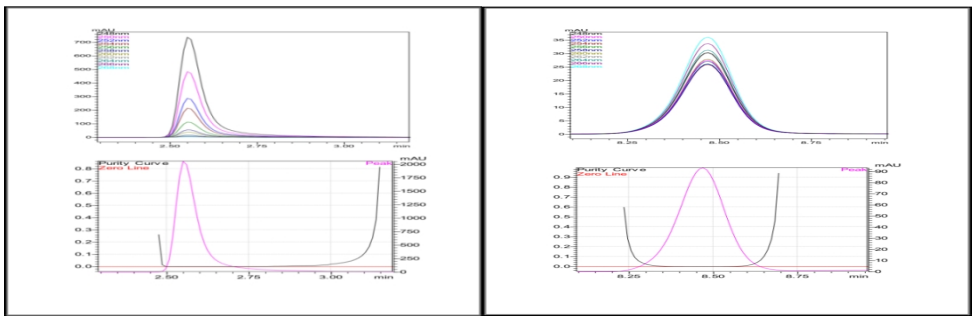


Figure 6.Chromatograms for specificity (GPZ and MET Peak profile and Peak purity)

RESULTS AND DISCUSSION

Method development

A variety of mobile phases were investigated in the development of a method for the analysis of glipizide and metformin hydrochloride in tablet dosage form. The suitability of mobile phase was decided on the basis of selectivity and sensitivity of the assay.

The maximum absorption wavelength of the reference drug solution was found to be 258 nm. This was observed from the UV absorption spectrum **Fig. 3** and was selected as detection wavelength for LC analysis. As the main objective of this chromatographic method was separation of both the drugs.

During the optimization of the method, different ratios of phosphate buffer (PB), water, methanol and acetonitrile were tried as mobile phase to get optimal retention time and other peak parameters. The composition and pH of mobile phase was optimized by several preliminary experimental trials to achieve good peak symmetry and short retention time.

After several trials, using Enable C18 G (250mm x 4.6mm, 5µm) analytical column and the mobile phase consisting 0.2M KH_2PO_4 buffer (pH 5.8) and ACN (60:40% v/v), and the flow rate of 1.0ml/min was considered optimum to achieve adequate retention time and sharp peaks of both the drugs **Fig.4**. System suitability parameters (Tailing factor, HETP, Resolution, Theoretical Plates, Asymmetry) for analyte peaks were evaluated and presented in the **Table-5**.

Method validation

The calibration plot for the method was linear over the concentration range of 60-140µg/mL for both glipizide and metformin hydrochloride. The determination of coefficients

(r^2) was 0.998 and 0.998 for glipizide and metformin hydrochloride, respectively. Values of the method Accuracy was calculated by recovery studies for glipizide and metformin hydrochloride at three levels and found to be 100.3% to 101.7% and 99.3% to 102.5% respectively. For precision and intermediate precision, % RSD of glipizide and metformin hydrochloride were within 2.0% thus confirm good precision of the analytical method development. In Specificity there was no any interference at the retention time of glipizide and metformin hydrochloride in the chromatogram of placebo solution. In peak purity analysis with photo diode detector, purity angle was less than purity threshold for both the analytes. The LOD and LOQ of glipizide and metformin hydrochloride were found to be 11.5ng/mL, 40.99ng/mL and 34.91ng/mL, 124.23ng/mL respectively. Robustness of the method was performed by making deliberate changes in flow rate and wave length and it was by calculating established % RSD values and was within acceptance criteria range of 2.0%.

ACKNOWLEDGEMENT

The author thanks Supra Chemicals, Mumbai and Dr. Reddy's, Hyderabad for providing the gift samples of glipizide and metformin hydrochloride. We thank to the EWCP management for providing the facilities and chemical.

REFERENCES

1. http//www.drug bank.ca/drugs/DB01067 2011May 22; 10:50.
2. http//www.drug bank.ca/drugs/DB00331 2011May 22; 10:55.
3. Swaroop RL, Prashant KP, Ashish AH, Rajesh BN. Development and validation of RP-HPLC method for analysis of glipizide in guinea pig plasma and its application to pharmacokinetic study. Int J Pharm Tech Res 2010; 2(3): 1649-54.
4. Venkata Rayanam, Lakshmana Rao A, Ramana MV. Development and validation of LC Method for estimation of glipizide in pharmaceutical dosage form and serum. Int J Res Pharm Chem 2011; 1(1): 50-4.
5. Venu Gopala Rao J, Swathi K, Chandra Mouli M, Madhavi Latha K, Papa Rao K, Visweswara Rao CH. Quantitative estimation of metformin in diabetic patients serum by RP-HPLC. Int J Res Pharm Chem 2011; 1(1): 73-4.
6. Shweta Havele MS, Dr. Sunil Dhaneshwar. Development and validation of a HPLC method for the determination of metformin hydrochloride, gliclazide and pioglitazone hydrochloride in mulitcomponent formulation. Webmed Central Pharm Sci 2010; 1(10): 1-16.
7. Alexandar S, Rohini Diwedi, Chandrasekar MJN. A RP-HPLC method for simultaneous estimation of metformin and pioglitazone in pharmaceutical formulation. Res J Pharm Bio Chem Sci 2010; 1(4): 858-866.
8. Angshuman Biswas, Arindam Basu. A Novel RP-HPLC Method for Simultaneous Estimation of Metformin Hydrochloride & Glimepiride in Tablet Dosage Form. Int J Pharm Inno 2011; 1(4): 81-7.
9. Ketan P.D, Parthika A.N, Yadvendra K.A. Development and validation spectrophotometric method for simultaneous estimation of gliclazide and metformin hydrochloride in bulk and tablet dosage form by simultaneous equation method. Int J Pharm Sci Res 2011; 2(6): 1559-63.
10. ICH. Note for guidance on validation of analytical methods: Definitions and Terminology Q2A; 1994.
11. ICH. Note for guidance on validation of analytical procedures: Methodology Q2B; 1996.

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