

DEVELOPMENT AND VALIDATION OF RP HPLC METHOD FOR METFORMIN AND GLICLAZIDE

AMPATI SRINIVAS¹ *, G.SHRAVANI²

^{1,2} Unity College of Pharmacy, Raigir, Bhongir, Yadadhri Bhuvanagiri, Telangana, India 508116

¹Corresponding Author*

Dr.AMPATI SRINIVAS³

³Principal & Professor, Department of Pharmaceutical Analysis, Unity College of Pharmacy

ABSTRACT: A new, simple and validated RP-HPLC method was developed for simultaneous estimation of MetforminHCl (MET) and Gliclazide (GLZ).The separation was achieved by using mobile phase Methanol: 10 mM Phosphate buffer (pH 3) 70:30 % v/v on Kinetics C₁₈ column (250 × 4.6 mm, 5μ). The flow rate was 1 ml/min. The detection was carried out at wavelength 230 nm. The retention time of MET and GLZ was found to be 2.20 min and 4.67 min, respectively. Calibration curves for MET and GLZ were found to be linear in the range of 10-60 μg/ml and 2-12 μg/ml, respectively. The proposed method was validated as per ICH guideline Q2 (R1). The validation parameters studied were linearity, accuracy, precision, specificity and robustness. All validation parameters were found within the acceptable range.

KEYWORDS: Metformin HCl, Gliclazide, RP-HPLC, Analytical Method Validation

INTRODUCTION

Chemically MET is N, N- dimethyl-biguanide¹. It is biguanide class having hypoglycemic activity and used in treatment of diabetes mellitus. Biguanide lowers blood glucose. They increase glucose uptake and utilization in skeletal muscle there by reducing insulin resistance and reduce hepatic glucose production (gluconeogenesis). Additionally MET reduces low density and very low density lipoprotein (LDL and VLDL, respectively).² GLZ belongs to the class of sulfonyl urea. Chemically it is 1-(hexahydrocyclopenta, pyrrol-2(1H-yl))-3-[(4- methyl phenyl) sulfonyl urea].³ The principal action of sulfonyl urea is on stimulating insulin release from β- cells of pancreas by blocking the ATP- sensitive K⁺ channels, resulting in depolarization and Ca²⁺-influx. Also acts by reducing of serum glucagon levels and increasing binding of insulin to target tissues and receptors.⁴ Literature survey revealed several quantitative analytical method for simultaneous

estimation of MET and GLZ in pharmaceutical formulations.⁵⁻⁸ The aim of the present paper was to develop a new and validated RP-HPLC method for the simultaneous estimation of these two antidiabetic drugs; MET and GLZ in tablet formulation

MATERIALS AND METHODS:

Chemicals and Reagents:

Pharmaceutical grade MET and GLZ were kindly supplied as a gift sample from Blue Cross Pharmaceuticals Ltd, Nashik. Methanol and phosphoric acid used in analysis were of HPLC-grade and potassium dihydrogen o-phosphate was of AR-grade, all chemicals and reagents purchased from SD Fine Chemicals, Mumbai, and Maharashtra. A freshly prepared double distilled water used was prepared by All Glass Double Distillation Assembly, purchased from Borosil, Mumbai, Instrumentation and chromatographic conditions: JASCO High Pressure Gradient HPLC system equipped with dual PU 2080 plus

pumps, multichannel UV detector, UV-2077 and injection loop (20 μL capacity), Rheodyne manual loop injector 7725i was used. Data were collected using Borwin Chromatography software (version 1.5). The mobile phase was composed of methanol: 10mM phosphate buffer (pH 3) 70:30 v/v. Isocratic elution was carried out on a Phenomenex Kinetics C18 column (250X4.6 mm, 5 μ) at a flow rate of 1 ml/min. The wavelength was fixed at 230 nm.

Preparation of Standard solution:

Quantity equivalent to 10 mg of MET and GLZ were weighed and transferred to separate 10 ml volumetric flasks and volume was made up to the mark with methanol. The resulting solutions were of 1000 $\mu\text{g/ml}$ of MET and GLZ, respectively.

Calibration Curve Standards:

From above standard stock solution six aliquots were prepared and diluted with mobile phase to get calibration curve standard with concentration of 10, 20, 30, 40, 50, 60 $\mu\text{g/ml}$ for MET and 2, 4, 6, 8, 10, 12 $\mu\text{g/ml}$ for GLZ. These calibration curves were analyzed in triplicates and the mean peak area were plotted on y-axis against respective concentration on x-axis. The intercept, slope and co-efficient of regression were determined.

Estimation of MET and GLZ in Tablets:

For analysis of tablet formulation, tablets containing 500 mg of MET and 80 mg of GLZ were prepared in-house. Twenty tablets were weighed and finely powdered. A tablet power equivalent to 50mg of MET and 8mg of GLZ was transferred to 100 ml volumetric flask and shaken with methanol for 10 min. After filtration the excipients were separated and the volume was made up to the 100 ml with the same solvent. From this stock solution, suitable aliquot was diluted with mobile phase to get concentrations of 50 $\mu\text{g/ml}$ of MET and 8 $\mu\text{g/ml}$ of GLZ and subjected to chromatographic analysis. From the calibration curve standards the amount of MET and GLZ was estimated.

Validation of the Method:

Accuracy and Precision:

Accuracy of the method were determined by analyzing the standard samples at three concentrations level of 80%, 100 % and 120% by standard addition method across the analytical range for MET and GLZ. The precision was established by

three replicates of three levels (80%, 100% and 120%) for the intra-day precision and on three successive days for the intermediate precision. The percent recovery of added concentration and % RSD were taken as measures of accuracy and precision, respectively. Also, the results obtained were subjected to one way ANOVA and the between – day mean square compared to the within – day mean square by F – test.

Specificity:

To evaluate the specificity of the proposed method, blank, placebo, sample, standard solution were injected. Absence of peaks in the chromatographic run at the retention time of the MET and GLZ was taken as indication of specificity.

Robustness:

Robustness of the method was studied by applying 23 full factorial design. The experimental matrix of 8 experiments was prepared with combination of each factor at each level. The independent variables selected were flow rate of mobile phase, concentration of methanol and pH of the buffer. The dependent variable (Response) studied were number of theoretical plates, tailing and retention time of the last eluted peak.

RESULTS AND DISCUSSION:

Various mobile phases were tried to get separation of MET and GLZ. It was observed that a mobile phase with composition of methanol: 10 mM phosphate buffer (pH3) 70: 30 v/v was gave appropriate resolution of MET from GLZ. The optimum wavelength for detection was 230 nm. The retention time of MET and GLZ were observed at 2.20 min and 4.67 min respectively (Fig. 1).

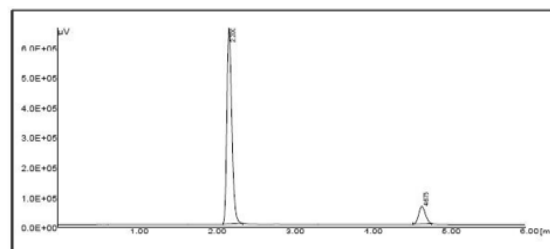


Fig. 1 Representative chromatogram of MET (2.20 min) and GLZ (4.67 min)

In calibration studies, it was found that MET was linear in the range of 10-60 $\mu\text{g/ml}$ and GLZ 2-12 $\mu\text{g/ml}$. The calibration curves with their respective calibration curve equations and regressions are depicted in Fig. 2 and Fig. 3, respectively.

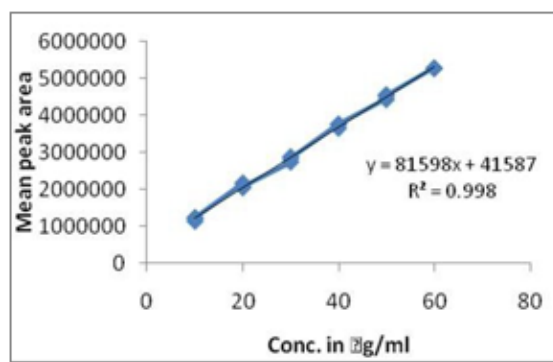


Fig. 2 Calibration curve of MET

When tablets prepared on lab scale were analyzed using the developed method, the results obtained were in good agreement with nominal amount of the drug. The drug content was found to be 99.82 ± 1.94 of the added amount.

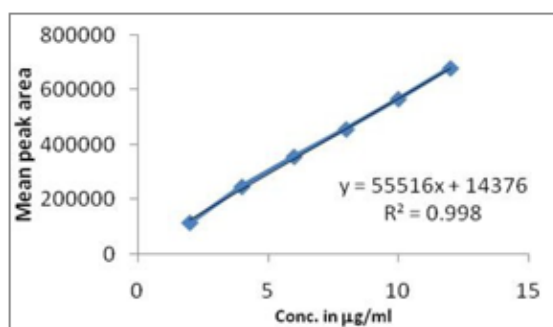


Fig. 3 Calibration curve of GLZ

The results obtained for accuracy and precision are summarized in Table 1 and Table 2, for MET and GLZ, respectively. Mean values of concentration found were close to the concentration added and low values of % RSD indicates the acceptable accuracy and precision of the method. Also, when the results of intra-day and inter-day were subjected to one-way ANOVA and F values were calculated at each QC level, the F values were found to be less than the tabulated F values. This indicated that there was no significant difference between intra-and inter-day variability, suggesting good intermediate precision.

Table 1 Accuracy and Precision Studies of MET

Concentration added (µg/ml)	Concentration Found (µg/ml)			Within Square	Mean	Between Mean Square	F-value
	Day 1	Day 2	Day 3				
25 + 20 = 45 µg/ml (80%)	45.1 45.5 45.2	44.56 44.39 44.98	45.08 44.58 44.53	0.07	0.45	5.86	
Mean	45.27	44.84	44.73				
% RSD	0.46	0.68	0.68				
25 + 25 = 50 µg/ml (100%)	50.5 49.8 49.8	50.1 50.7 50.1	49.9 49.9 50.5	0.13	0.05	0.42	
Mean	50.03	50.30	50.1				
% RSD	0.81	0.69	0.69				
25 + 30 = 55 µg/ml (120%)	54.8 55.8 55.8	55.1 55.8 55.4	56.6 55.7 55.6	0.25	0.26	1.05	
Mean	55.47	55.43	55.97				
% RSD	1.04	0.63	0.98				

Table 2 Accuracy and Precision study of GLZ

Concentration (µg/ml)	added	Concentration Found (µg/ml)			Within Square	Mean	Between Mean Square	F-value
		Day 1	Day 2	Day 3				
4 + 3.2 = 7.2 µg/ml (80%)		7.24 7.24 7.14	7.24 7.18 7.24	7.10 7.18 7.26	0.003	0.001	0.34	
Mean		7.20	7.22	7.18				
% RSD		0.80	0.47	1.11				
4 + 4 = 8 µg/ml (100%)		8.06 8.06 7.90	8.00 8.20 8.00	8.00 7.90 8.00	0.008	0.007	0.90	
Mean		8.00	8.06	7.96				
% RSD		1.05	1.43	0.72				
4 + 4.8 = 8.8 µg/ml (120%)		8.80 8.60 8.65	8.80 8.60 8.63	8.90 8.80 8.81	0.008	0.024	2.89	
Mean		8.68	8.67	8.83				
% RSD		1.19	1.24	0.62				

When blanked tablets were analyzed as per the mentioned chromatographic conditions, no peak was obtained at the retention times of MET and GLZ. During robustness studies, it was observed that there was no significant effect on number of theoretical plates, asymmetry and on retention times of GLZ (last eluted peak) by small but deliberate changes in flow rate, methanol concentration and change of buffer pH. The experimental matrix of 8 experiments for 23 factorial design is depicted in Table 3.

Table 3: 23 factorial design experimental matrix for robustness test

Run No.	Flow rate (ml)	MeOH conc. (%)	Buffer pH(mM)	Theoretical Plate	Asymmetry	R.T. of last eluted peak (i.e. GLZ)
1	0.8	80	3.2	17824	1.0	10.56
2	1.2	80	3.2	17546	1.03	9.07
3	0.8	80	3.2	17002	1.15	5.37
4	1.2	80	3.2	17071	1.10	4.12
5	0.8	60	5.6	14089	1.06	8.14
6	1.2	60	5.6	14050	1.05	7.15
7	0.8	80	5.6	14055	1.03	9.13
8	1.2	80	5.6	14053	1.04	7.15

CONCLUSION:

A new, simple and accurate method was developed for simultaneous estimation of MET and GLZ in tablets. All parameters of validation are within the acceptable range. The developed method is specific as there was no any interfering peak at the retention time of drugs. Hence the developed method was accurate and can be used for routine analysis of MET and GLZ in tablets.

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