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Research Article

STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF METFORMIN AND GLIPIZIDE

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ABSTRACT

A novel, precise and accurate stability indicating RP-HPLC method was developed and validated for the simultaneous estimation of Metformin and Glipizide in combined pharmaceutical dosage form. Chromatographic separation was achieved on Microsorb-MV C18 column (250 × 4.6 mm, 5 µm) with UV detection at 257 nm. The mobile phase consists of acetate buffer (pH 4.0) and acetonitrile in the ratio of 60:40 v/v and at a flow rate of 1.0 mL/min. The method was linear over the concentration range of 60-140 µg/mL for Metformin and 10-50 µg/mL for Glipizide. The retention times for Metformin and Glipizide were found to be 2.434 min and 5.710 min respectively. The mean percentage recoveries of Metformin and Glipizide were found to be 100.42% and 100.39% respectively. The method was validated and was successfully employed for the routine quantitative analysis of pharmaceutical formulations containing Metformin and Glipizide in combined pharmaceutical formulation.

INTRODUCTION

Metformin (Fig. 1) is biguanide anti hyperglycemic agent used for treating non-insulin-dependent diabetes mellitus[1]. Chemically it is 1,1-dimethylbiguanide. Metformin decreases hepatic glucose production, decreases intestinal absorption of glucose and improves insulin sensitivity by increasing peripheral glucose uptake and utilization[2,3].

Glipizide (Fig. 2) is a second-generation sulfonyl-urea, is used to lower blood glucose in patients with diabetes mellitus type II[4]. Chemically it is N-[2-(4-[(cyclohexylcarbamoyl) amino] sulfonyl} phenyl) ethyl]-5-methylpyrazine-2-carboxamide. Glipizide bind to ATP-sensitive potassium-channel receptors on the pancreatic cell surface, reducing potassium conductance and causing depolarization of the membrane. Depolarization stimulates calcium ion influx through voltage-sensitive calcium channels, raising intracellular concentrations of calcium ions, which induces the secretion, or exocytosis, of insulin[5,6].

Literature survey revealed that few HPLC methods[7-11] were reported for simultaneous estimation of Metformin and Glipizide in combined pharmaceutical dosage form. But no stability indicating HPLC method was reported. Hence the objective of this method is to develop and validate a simple, rapid, precise and accurate stability indicating RP-HPLC method in accordance with ICH guidelines[12,13] for the simultaneous estimation of Metformin and Glipizide in combined pharmaceutical dosage form.

MATERIALS AND METHODS

Materials

Metformin and Glipizide pure drugs were obtained from Yarrow Chemicals, Mumbai, India. Combination of Metformin and Glipizide tablets (Glynase-MF Tablets) were obtained from local pharmacy store. Acetonitrile, glacial acetic acid, triethylamine and distilled water were obtained from Rankem Chemicals Ltd., Mumbai, India.

Instrumentation

The analysis of drugs was carried out on Agilent 1260 infinity binary pump HPLC system on Microsorb-MV C18 column (250 × 4.6 mm, 5 µm). The instrument is equipped with auto injector with

20 µL sample loop. A 20 µL Hamilton syringe was used for injecting the samples. Data was analyzed by using Open Lab software. A double-beam Schimadzu UV-1800 UV-Visible spectrophotometer was used for measuring absorbance for Metformin and Glipizide solutions. Degassing of the mobile phase was done by using an ultrasonic bath sonicator. A Shimadzu balance was used for weighing the materials.

Mobile phase

A mobile phase consisting of mixture of acetate buffer (pH 4.0) and acetonitrile in the ratio of 60:40 v/v was prepared.

Preparation of standard stock and working solutions

Standard stock solutions of Metformin and Glipizide were prepared by dissolving 50 mg of Metformin and Glipizide each dissolved in sufficient mobile phase. After that filtered the solution using 0.45 micron filter paper and sonicated for 5 min and dilute to 50 mL with mobile phase. 1 mL from the resulting solution was transferred to 100 mL volumetric flask and diluted with mobile phase to obtain 100 µg/mL. Further dilutions of Metformin and Glipizide were made from stock solution using mobile phase.

Preparation of sample stock and working solutions

20 tablets (each tablet contains 500 mg of Metformin and 5 mg of Glipizide) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of Metformin and Glipizide were prepared by dissolving weight equivalent to 500 mg of Metformin and 5 mg of Glipizide and dissolved in sufficient mobile phase. After that filtered the solution using 0.45 µ syringe filter and sonicated for 5 min and dilute to 100 mL with mobile phase. Further dilutions are prepared by adding 1 mL of stock solution to 10 mL of mobile phase.

METHOD DEVELOPMENT

Various trails were performed by using different mobile phases and based on peak parameters the chromatographic conditions (Table 1) were optimized and optimize chromatogram was shown in Fig. 3.

METHOD VALIDATION

System suitability parameters

The system suitability parameters were determined by preparing standard solutions of Metformin (10 µg/mL) and Glipizide (10 µg/mL) and the solutions were injected six times and the parameters like USP plate count, peak tailing and resolution were determined. All the system suitability parameters were within the range and satisfactory as per ICH guidelines.

Specificity

Specificity is the parameter used to check the interference in the optimized method. We should not find interfering peaks in blank, placebo, standard and sample at retention times of these drugs in this method. So this method was said to be specific.

Linearity

Five linear concentrations of Metformin (60-140 µg/mL) and Glipizide (10-50 µg/mL) are prepared and injected. The results were furnished in Table 2 and calibration curves were shown in Fig. 4 & 5.

Precision

Precision of method was studied by performing intra-day and inter-day precision. Intra-day precision (Table 3) and inter-day precision (Table 4) was studied by injecting the 6 replicates of standard solution in a single day and six days. Calculate the %RSD and it should not be more than 2.0.

Accuracy

The accuracy of the method was established by calculating percentage recovery of Metformin and Glipizide by the method of addition. Known amount of Metformin and Glipizide at 80%, 100% and 120% was added to a prequantified sample solution. The recovery studies (Table 5 & 6) were carried out in the tablet in triplicate each in the presence of placebo. The mean percentage recovery at each level was not less than 98% and not more than 102%.

Sensitivity

Limit of detection (LOD) was calculated by standard deviation method. Limit of quantitation (LOQ) was calculated by standard deviation method.

Degradation studies

Acid degradation studies

To 1mL of stock solution of Metformin and Glipizide, 1 mL of 2N hydrochloric acid was added and refluxed for 30 mins at 60^0C. The resultant solution was diluted to obtain 140 µg/mL & 50 µg/mL solution and 20 µL solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali egradation studies

To 1 mL of stock solution of Metformin and Glipizide, 1 mL of 2N sodium hydroxide was added and refluxed for 30 mins at 60^0C. The resultant solution was diluted to obtain 500 µg/mL & 50 µg/mL solution and 20 µL were injected into the

system and the chromatograms were recorded to assess the stability of sample.

Oxidative degradation studies

To 1 mL of stock solution of Metformin and Glipizide, 1 mL of 20% hydrogen peroxide was added separately. The solutions were kept for 30 mins at 60^{0}C. For HPLC study the resultant solution was diluted to obtain 500 µg/mL & 50 µg/mL solution and 20 µL were injected into the system and the chromatograms were recorded to assess the stability of sample.

Thermal degradation studies

The standard drug solution was placed in oven at 105°C for 6 hrs to study thermal degradation. For HPLC study the resultant solution was diluted to 500 µg/mL & 50 µg/mL solution and 20 µL were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Neutral degradation studies

Stress testing under neutral conditions was studied by refluxing the drug in water for 6 hours at a temperature of 60ºC. For HPLC study the resultant solution was diluted to 500 µg/mL & 50 µg/mL solution and 20 µL were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Degradation studies results of Metformin and Glipizide were tabulated in Table 7 & 8.

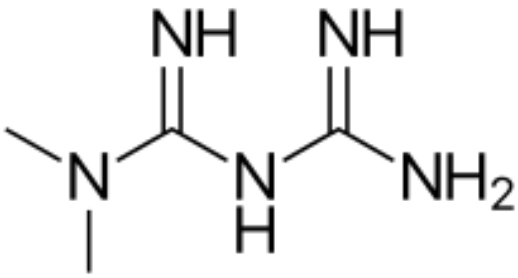

Fig. 1: Structure of Metformin

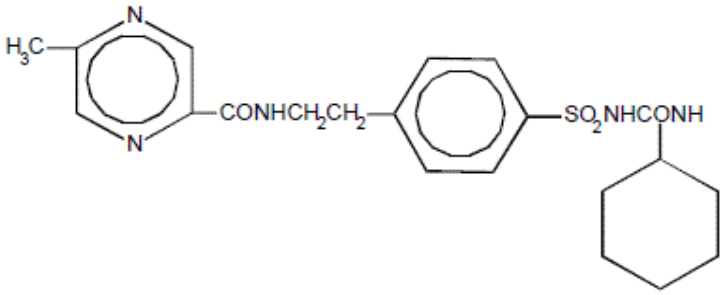

Fig. 2: Structure of Glipizide

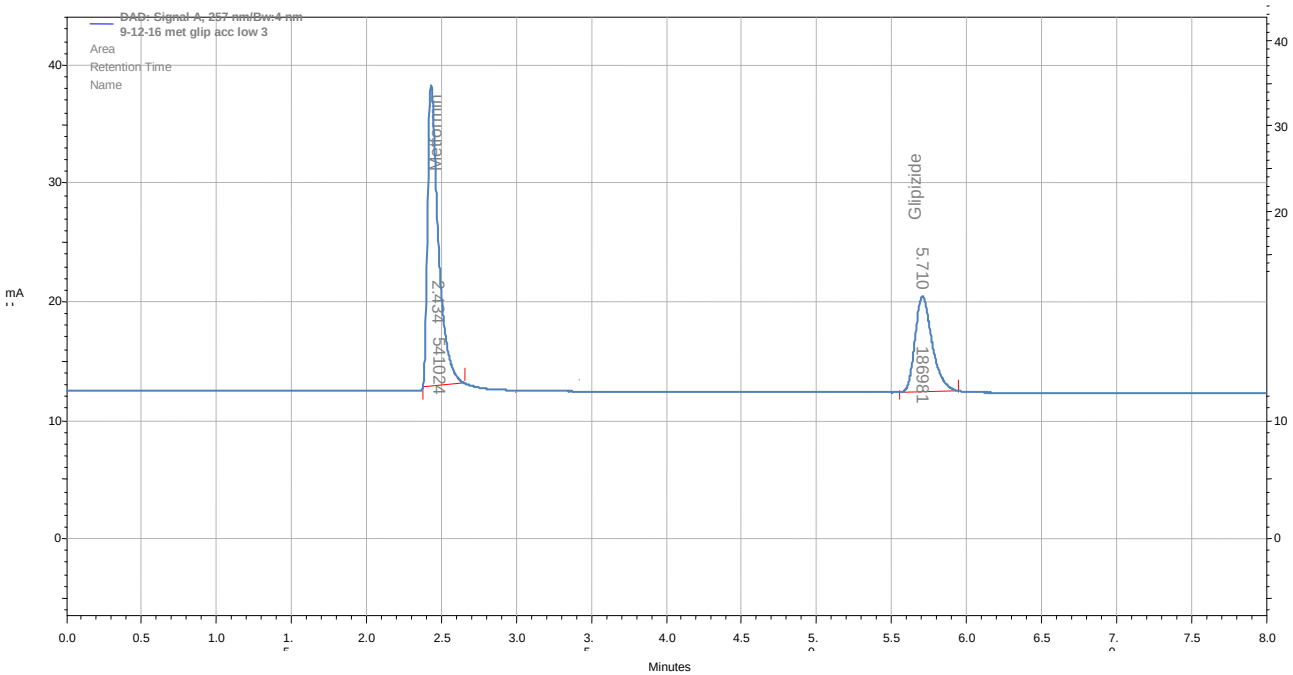

Fig. 3: Optimized chromatogram of Metformin and Glipizide

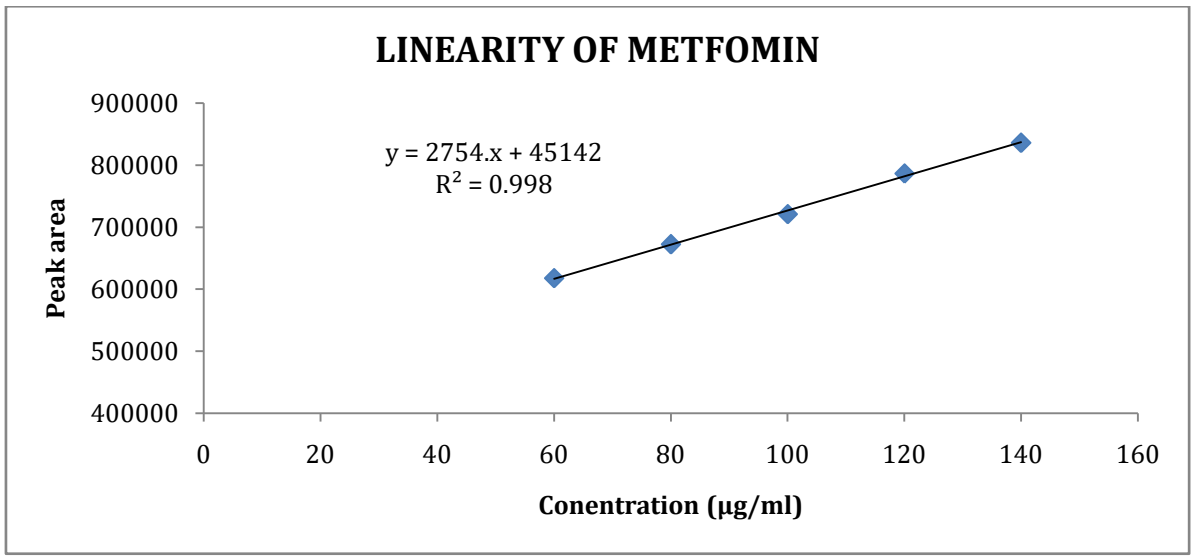

Fig. 4: Calibration curve of Metformin

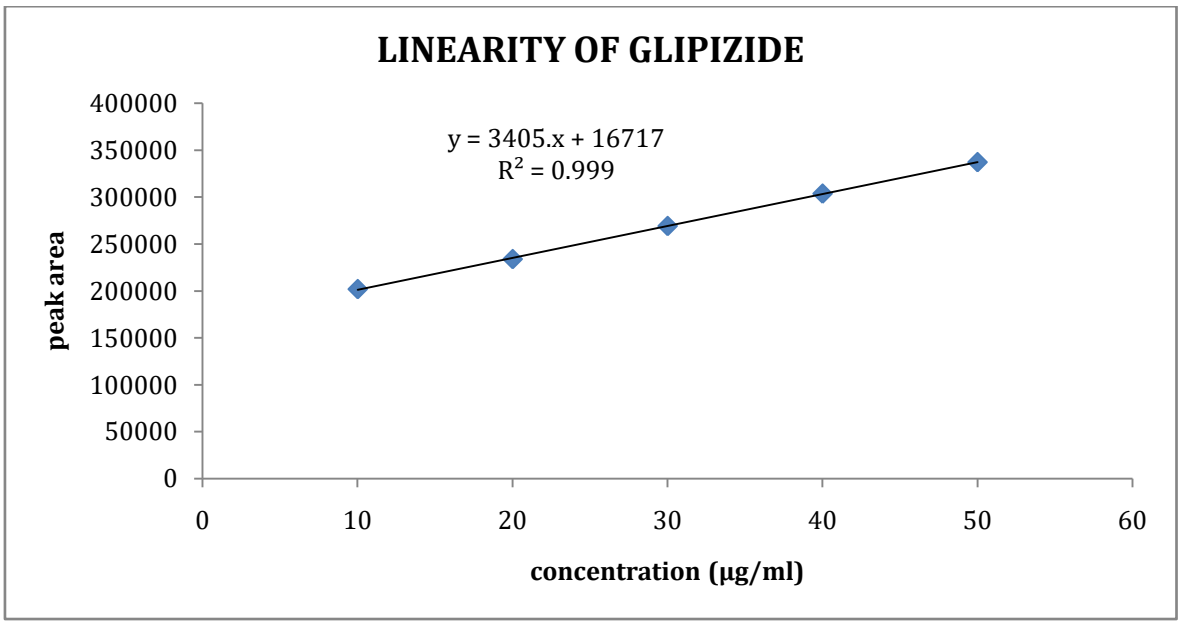

Fig. 5: Calibration curve of Glipizide

Table 1: Optimized chromatographic conditions

Mobile phase	Acetate buffer (pH 4.0):acetonitrile, 60:40 v/v
Flow rate	1 mL/min
Column	Microsorb-MV C18 (250 x 4.6 mm, 5 µm)
Detector wave length	257 nm
Column temperature	25°C
Injection volume	20 µL
Run time	8 min
Diluent	Mobile phase
Retention time	Metformin: 2.434 min; Glipizide: 5.710 min
Theoretical plates	Metformin: 10205; Glipizde: 11067

Table 2: Linearity results for Metformin and Glipizide

S. No.	Concentration of Metformin (µg/mL)	Peak area	Concentration of Glipizide (µg/mL)	Peak area
1	60	617813	10	202048
2	80	672456	20	234061
3	100	721269	30	269332
4	120	786922	40	303857
5	140	836059	50	337429

Table 3: Intra-day precision results for Metformin and Glipizide

S. No.	Metformin Peak area	Glipizide Peak area
1	715899	219871
2	716892	219396
3	719354	218521
4	720101	220945
5	714879	219887
6	717681	219680
Mean	717467.7	219266.7
Std. Dev.	2001.575	1368.826
%RSD	0.27	0.62

Table 4: Inter-day precision results for Metformin and Glipizide

S. No.	Metformin Peak area	Glipizide Peak area
1	719354	218521
2	719575	220538
3	714879	218938
4	720046	220456
5	719678	219871
6	715899	219936
Mean	718238.5	219710
Std. Dev.	2241.843	815.9414
%RSD	0.31	0.37

Table 5: Accuracy results of Metformin

Recovery level	Amount taken (µg/mL)	Area	Average area	Amount recovered (µg/mL)	% Recovery	Mean % Recovery
80%	80	539581	541146	80	100.87	100.42
	80	542834				
	80	541024				
100%	100	596575	586936	100	100.22	
	100	564879				
	100	599354				
120%	120	651605	654468	120	100.17	
	120	657164				
	120	654636				

Table 6: Accuracy results of Glipizide

Recovery level	Amount taken (µg/mL)	Area	Average area	Amount recovered (µg/mL)	%Recovery	Mean% Recovery
80%	80	186679	187023	20	99.58	100.39
	80	187409				
	80	186981				
100%	100	201598	201314	30	100.80	
	100	200949				
	100	201395				
120%	120	217865	218786	40	99.50	
	120	219340				
	120	219153				

Table 7: Degradation data of Metformin

S. No.	Degradation condition	Peak area	% Assay	Amount degraded %
1	Acid	1383287	95.00	5.00
2	Alkali	1087654	98.91	1.09
3	Oxidative	1035678	97.16	2.84
4	Thermal	1399673	96.12	3.88
5	Neutral	1414765	97.85	2.15

Table 8: Degradation data of Glipizide

S. No.	Degradation condition	Peak area	% Assay	Amount degraded %
1	Acid	283176	95.58	4.42
2	Alkali	263176	96.12	3.88
3	Oxidative	250591	95.98	4.02
4	Thermal	321386	98.58	1.42
5	Neutral	324896	97.22	2.78

RESULTS & DISCUSSION

A stability indicating RP-HPLC method was developed and validated for the simultaneous estimation of Metformin and Glipizide by using mobile phase consisting of acetate buffer (pH 4.0) and acetonitrile in the ratio of 60:40 v/v. The retention times for Metformin and Glipizide were found to be 2.434 min and 5.710 min respectively. The proposed method was validated as per ICH guidelines. The theoretical plates for Metformin and Glipizide were found to be 10205 and 11067 respectively, which indicates the efficient performance of the column. Linearity range was found to be 60-140 µg/mL for Metformin and 10-50 µg/mL for Glipizide. The %RSD values for intra-day precision values of Metformin and Glipizide were found to be 0.27 and 0.62 respectively. The %RSD values for inter-day precision values of Metformin and Glipizide were found to be 0.31 and 0.37 respectively and hence the proposed method is precise. The mean percentage recoveries of Metformin and Glipizide were found to be 100.42% and 100.39% respectively and the method is found to be accurate. LOD for Metformin and Glipizide were found to be 0.287 µg/mL and 0.065 µg/mL respectively. LOQ for Metformin and Glipizide were found to be 0.870 µg/mL and 0.196 µg/mL respectively. Degradation studies were carried out in acid, alkali, oxidative, thermal and neutral stressed conditions. The results revealed that both the drugs are stable in described conditions. Thus it is evident that the described method can be adopted for routine estimation of Metformin and Glipizide in combined pharmaceutical dosage form.

CONCLUSION

The present method was proposed for the simultaneous estimation of Metformin and Glipizide by using RP-HPLC in tablet dosage form is found to be simple, rapid, accurate and precise. Retention times were decreased and that run time was

decreased, so the method developed was simple and economical that can be applied in regular quality control tests in pharmaceutical industries.

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