REVIEW ARTICLE JPAR 2022

Journal of Pharmaceutical Advanced Research
(An International Multidisciplinary Peer Review Open Access monthly Journal)
Available online at: www.jparonline.com

RP-HPLC analytical method for simultaneous estimation of percentage assay of Glimepiride and Metformin HCl in combined dosage forms

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Received: 24.04.2022 Revised: 10.06.2022 Accepted: 20.06.2022 Published: 30.06.2022

ABSTRACT: Background: Diabetes mellitus is considered a public health problem. The initial line of treatment involves improving the lifestyle and changes in the diet. When the changes do not bring about any marked impact on the condition, it becomes inevitable to take medication. Metformin inhibits hepatic gluconeogenesis. Glimepiride was the potent first-generation sulfonylurea derivative which till date is widely used in the treatment of non-insulin-dependent type-II Diabetes mellitus. **Aim:** To develop a simple, rapid, precise, and reliable reverse phase HPLC method for the separation and estimation of two drugs viz. glimepiride and metformin in bulk drugs and their pharmaceutical dosage forms. **Method:** The method was developed using Inertsil ODS-3V 250 × 4.6 mm, 5 µm using a mobile phase consisting of acetonitrile, methanol, and buffer consisting of 5mM Pentane Sodium salt and 20 mM potassium phosphate at pH (adjusted 3 with phosphoric acid) with gradient flow rate and detection at 230 nm. Both Metformin and Glimepiride were well resolved with a retention time of 6 and 25 min for Metformin and Glimepiride respectively. **Results:** The method was validated according to the ICH guidelines and values of accuracy, precision, and other statistical analysis were found to be in good accordance with the specified acceptance criteria. **Conclusion:** The validated method was successfully applied for the estimation of Metformin and Glimepiride in a combined dosage form for routine analysis.

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Keywords: Glimepiride, Metformin hydrochloride, ICH guidelines, RP-HPLC, Validation.

INTRODUCTION:

In the current scenario, the most commonly attacking disease to a common man has been found to be diabetes. Recent studies indicate that the prevalence of type-2 diabetes is rapidly increasing in society. Type-2 diabetes is a progressive disorder with a consistent and steady increasing glycosylated hemoglobin (HbA_1C) over time associated with an enhanced risk of micro and macrovascular complications and a substantial reduction in life expectancy [1]. There are three major pathophysiologic abnormalities associated with type-2

diabetes: (i) impaired insulin secretion, (ii) excessive hepatic glucose output, and (iii) insulin resistance in skeletal muscles, liver, and adipose tissue. These defects have been treated by the use of oral insulin secretagogues (sulphonyl ureas/ glinides) or insulin, biguanides, and thiazolidinediones [2-4].

Glimepiride is a medium-to-long-acting sulphonyl urea antidiabetic drug. It is chemically 1-[[p-[2-(3-Ethyl-4-methyl-2-oxo-3-pyrroline-1 carboxamido) ethyl] phenyl] sulfonyl]-3-(trans-4-methyl cyclohexyl) urea. The primary mechanism of action of glimepiride in lowering blood glucose appears to be dependent on stimulating the release of insulin from functioning pancreatic beta cells. Metformin hydrochloride is also an antidiabetic drug in the biguanide class and it is chemically 1,1-dimethyl biguanide monohydrochloride [5,6]. It decreases hepatic glucose production, decreases intestinal absorption of glucose, and improves insulin sensitivity by increasing peripheral glucose uptake and utilization. The combination of Glimepiride and metformin sustained release complements each other and provides better glycemic control in the management of Type-2 diabetes and probably in the prevention of its associated macrovascular and microvascular complications [7-9].

The chemical structures of the drugs are shown in Fig 1 and 2. Keeping the medical importance in mind, the group of drugs used for treating diabetes, namely, glimepiride and metformin have been selected for the method development and validation [10,11].

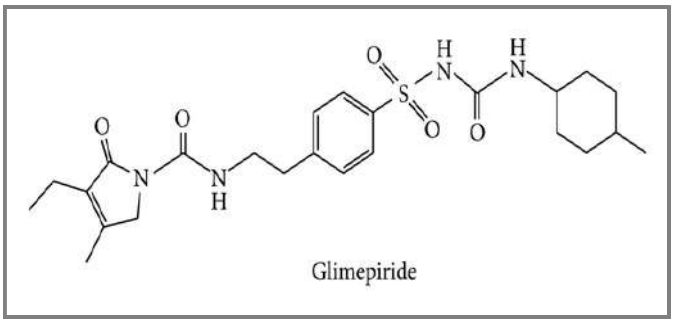

Fig 1. Chemical structure of Glimepiride.

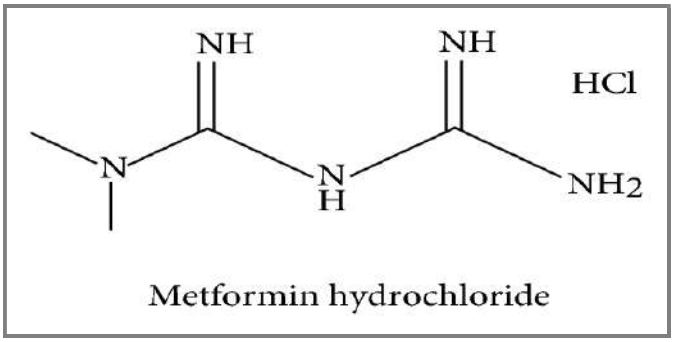

Fig 2. Chemical Structure of Metformin HCl.

For individual estimation of each drug, several methods are available in the literature. There are limited methods where even multiple drug moieties are estimated at a time. Very limited work has been done for the simultaneous estimation of glimepiride and metformin [12].

For contributing to such a novel cause, through this article, we have tried our best to develop a fast, stability-indicating, and user-friendly methodology for the simultaneous estimation of Glimepiride and metformin, using reverse phase-HPLC in the combined dosage forms [13].

MATERIALS AND METHODS:

Chemicals and reagents:

Glimepiride and metformin hydrochloride standards were used available in Oman Pharmaceutical Products L.L.C. Combination drug tablets were taken from the commercial batch manufactured at Oman Pharmaceutical Products L.L.C. HPLC-grade acetonitrile, methanol, and orthophosphoric acid were obtained from Merck, Darmstadt, Germany. All other chemical reagents were of analytical grade.

Table 1. Gradient program.

Time (min)	Flow rate (ml/min)	% Mobile Phase A	% Mobile Phase B	% Mobile Phase C
0.0	1.0	90	8	2
5.0	1.0	90	8	2
15.0	1.8	45	5	50
30.0	1.8	45	5	50
30.1	1.0	90	8	2
35.0	1.0	90	8	2

Preparation of mobile phase:

A buffer solution containing 5 mM Pentane Sulfonic acid, Sodium salt, and 20 mM potassium phosphate was prepared. About 0.87 g pentane sodium salt and 2.74 g potassium phosphate were weighed and transferred into a beaker containing 1000 ml of Milli-Q grade water and mixed. The pH was adjusted to 3.0 with phosphoric acid. The prepared mobile phase was considered as a mobile phase A, methanol as mobile phase B, and acetonitrile as a mobile phase C. The diluent was buffer: methanol: acetonitrile in the proportion of 90: 08: 2 (Table 1).

Glimepiride standard stock solution:

The solutions of the strength of 12.5 mg/ml metformin and 0.5 mg/ml glimepiride in methanol were prepared.

Accurately 5 mg of glimepiride working standard was weighed and transferred into a 250 ml clean and dry volumetric flask, and about 200 ml of methanol was added and sonicated to dissolve. Finally, the volume was made up to the mark with methanol and mixed it well.

Preparation of standard solutions:

A solution containing 0.25 mg/ml of metformin and 0.001 mg/ml glimepiride was prepared in the diluent. Accurately 25 mg of metformin HCl working standard was weighed and transferred into a 100 ml clean and dry volumetric flask. About 5 ml of glimepiride standard stock solution was added into the same flask containing metformin HCl standard. Next, 10 ml of methanol was added and sonicated to dissolve. Finally, the solution was diluted up to the mark with diluent and mixed well.

Preparation of placebo solution:

Accurately, the placebo powder equivalent to 5 Tablets (Equivalent weight of 800 mg) was weighed and transferred into a 100 ml volumetric flask. About 20 ml of water was added and sonicated for 15 min with intermittent swirling. Then, about 50 ml of acetonitrile was added and sonicated for 10 min with intermittent swirling. Finally, the solution was diluted to volume with methanol and mixed well. Next, the solution was centrifuged at 10000 RPM for 10 min and the supernatant solution was used. Further, 2 ml of the supernatant solution was transferred into a clean and dry 200 ml volumetric flask and diluted up to the mark with diluent.

Preparation of sample solution:

About 5 tablets were weighed and transferred into a 100 ml volumetric flask. About 20 ml of water was added and sonicated for 15 min with intermittent swirling. Then, about 50 ml of acetonitrile was added and sonicated for 10 min with intermittent swirling. Finally, the solution was diluted to volume with methanol and mixed well. Next, the solution was centrifuged at 10000 RPM for 10 min and the supernatant solution was used. Further, 2 ml of the supernatant solution was transferred into a clean and dry 200 ml volumetric flask and diluted up to the mark with diluent.

Evaluation of system suitability:

The chromatographic conditions are given in Table 2. The column and system were equilibrated at the initial composition for 30 min. The diluent as blank was injected into the liquid chromatographic system and the chromatogram was recorded. The standard solution was injected five times into the liquid chromatographic system and the chromatograms were recorded. The percentage RSD for metformin and glimepiride peak areas of five injections from STD should be not more than 2.0.

Table 2. Chromatographic conditions.

Column:	Inertsil ODS-3V 250 × 4.6mm,5µ (Cat # 5020-01802)
Injection volume:	10µl
Wavelength:	230 nm
Column temp:	35 °C
Elution:	Gradient
Run time:	35 min
Retention time:	Metformin: About 6 min Glimepiride: About 25 min

The sample solution was injected into the liquid chromatography and the chromatogram was recorded. The retention time of metformin and glimepiride was about 6.0 and 25.0 min respectively. The percentage of the metformin (POM) which is expressed as a percentage label claim, was calculated by using the following equation 1.

$$POM=\frac{A_{T1}}{A_{S1}}\times\frac{W_{S1}}{20}\times\frac{1}{50}\times\frac{100}{5}\times\frac{200}{2}\times\frac{P_1}{100}\times\frac{100}{L_1} \quad ....(1)$$

Where A_{T1} is the area of metformin peak in sample preparation, A_{S1} is the average area of metformin peak in standard preparation, W_{S1} is the weight of metformin working standard taken in mg, P_1 is the purity of metformin working standard (on as is basis), and L_1 is label claim of metformin in mg.

The percentage of the glimepiride (POG) which is expressed as a percentage label claim, was calculated by using the following equation 2.

$$POG=\frac{A_{T1}}{A_{S1}}\times\frac{W_{S1}}{20}\times\frac{1}{50}\times\frac{100}{5}\times\frac{200}{2}\times\frac{P_1}{100}\times\frac{100}{L_1} \quad ...(2)$$

Where A_{T1} is the area of glimepiride peak in sample preparation, A_{S1} is the average area of glimepiride peak in standard preparation, W_{S1} is the weight of glimepiride working standard taken in mg, P_1 is the purity of glimepiride working standard (on as is basis), and L_1 is label claim of glimepiride in mg.

RESULTS AND DISCUSSION:

The developed method for the determination of the percentage assay of metformin and glimepiride was validated by using the following parameters.

System suitability:

System suitability follows the procedure described in the methodology and establishes the system suitability before starting the analysis. The standard solution is as mentioned in Table 3.

Table 3. System suitability – Standard.

Injection #	Metformin HCl	Glimepiride
	Area	Area
1	9817291	17193
2	9771257	17073
3	9703982	17130
4	9756142	17346
5	9677244	16888
Mean	9745183	17126
SD	55481.409	167.599
% RSD	0.6	1.0

Specificity:

There were no interfering peaks in the retention times of the metformin and glimepiride in the presence of excipients. Further, to demonstrate the specificity of the method, the sample had been subjected to acid, base, oxidation, thermal, and photolytic degradation. This was evaluated by comparing the purity angle with the purity threshold as referred in Fig 3 to Fig 10 for the chromatograms and Table 4 to 7 for the peak purity analysis data.

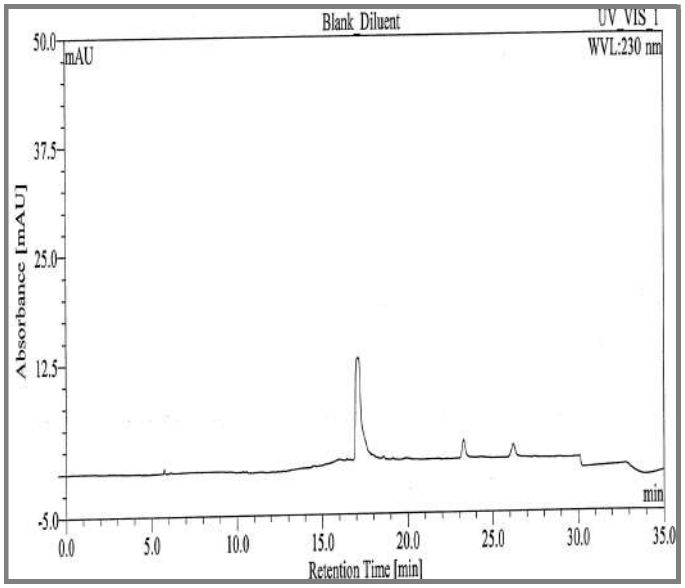

Fig 3. Reference chromatogram of the blank.

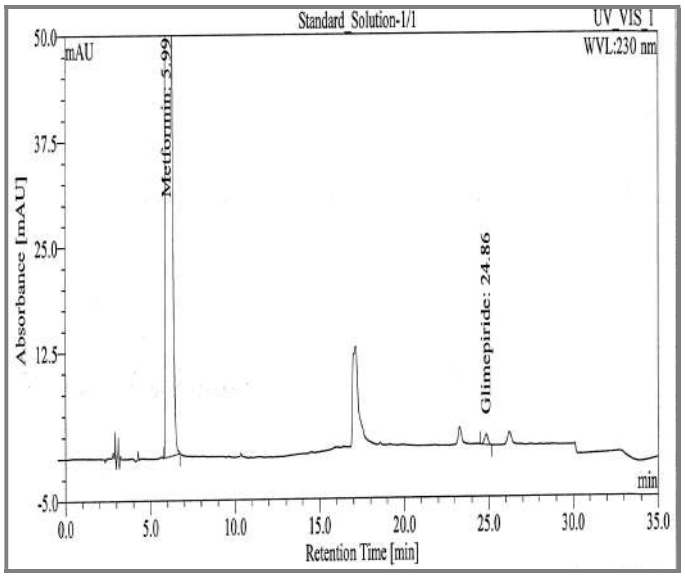

Fig 4. Reference chromatogram of standard solution.

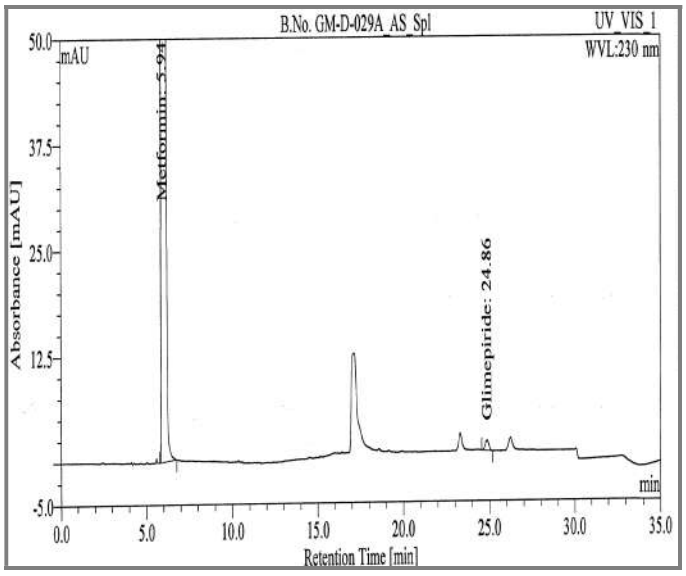

Fig 5. Reference chromatogram of sample solution.

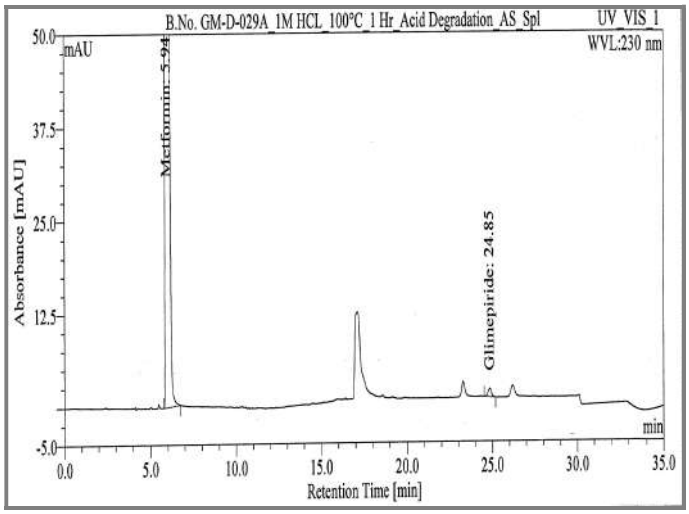

Fig 6. Reference chromatogram of acid degradation.

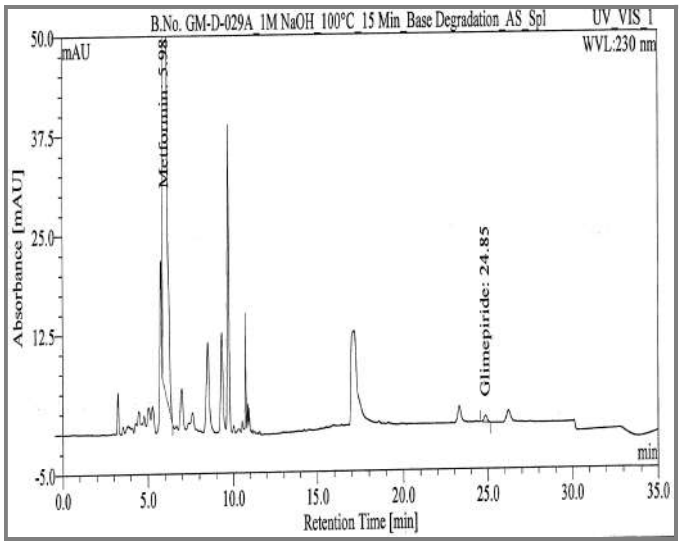

Fig 7. Reference chromatogram of base degradation.

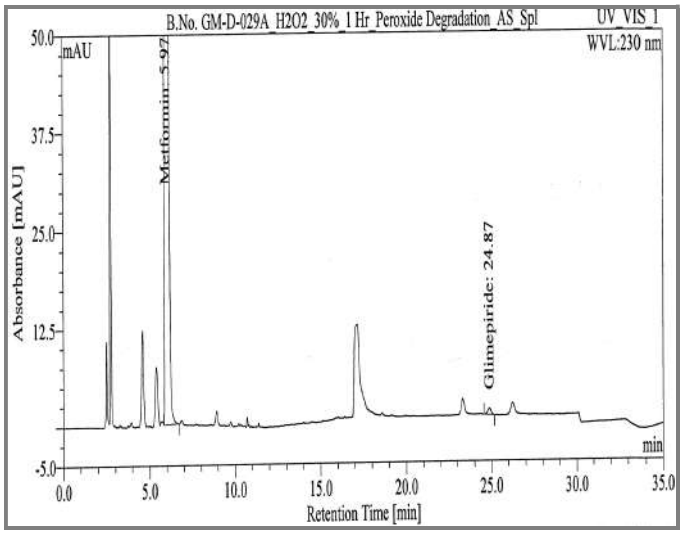

Fig 8. Reference chromatogram of peroxide degradation.

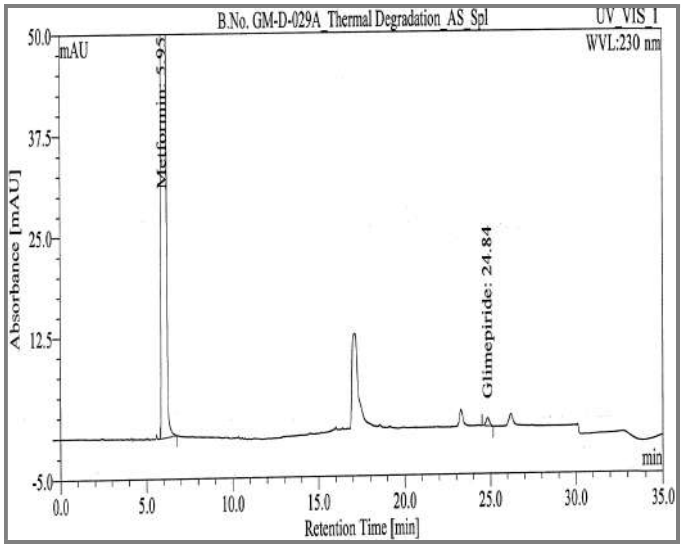

Fig 9. Reference chromatogram of Thermal degradation.

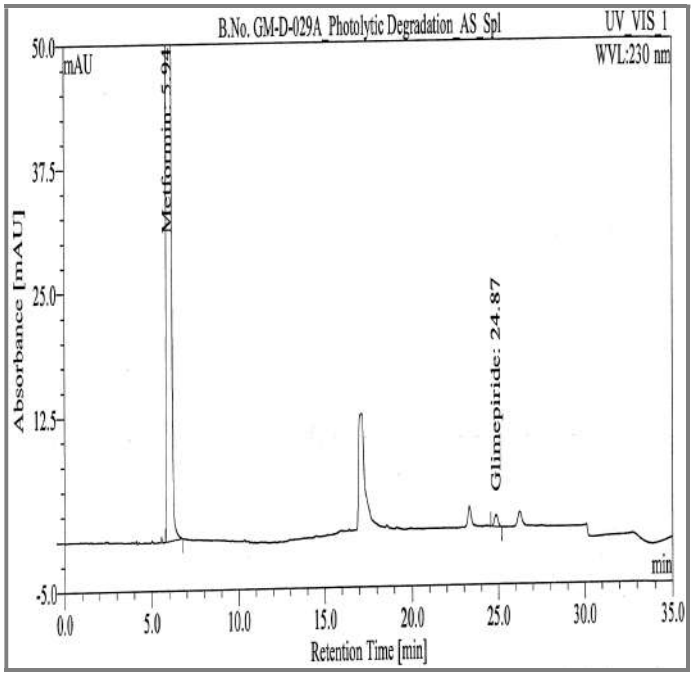

Fig 10. Reference chromatogram of Photolytic degradation.

Table 4. The percentage Interference study.

Obser-vation	Placebo preparation	Blank preparation	Individual Impurity
% Interference	No Interference	No Interference	No Interference

Table 5. Peak purity analysis.

Name of moiety	Component	Peak purity
Metformin Standard	Metformin	1.0000
Glimepiride Standard	Glimepiride	0.9993
Metformin HCl 500 mg and Glimepiride 2 mg Film-Coated Tablets (Un spiked sample)	Metformin	1.0000
	Glimepiride	0.9991
Metformin HCl 500 mg and Glimepiride 2 mg Film-Coated Tablets (Spiked sample)	Metformin	1.0000
	Glimepiride	0.9947

Precision:

The sample was prepared in six replicates as per methodology and the chromatograms were recorded. The percentage assay was calculated for each preparation. The percentage RSD was deduced for percentage assay from six replicate preparations. The data obtained had been presented in Table 8.

Table 6. Forced degradation study for Metformin.

Sample Name	% Assay	% Degradation	% Peak purity
As such sample (Unstressed)	108.4	-	1.0000
Acid Degradation 1 N HCl_1 h_100°C	107.3	1.01	1.0000
Alkaline Degradation 1 M NaOH_1 h_100°C	20.0	81.55	1.0000
Alkaline Degradation 1 M NAOH_15 min_100°C	57.3	47.14	1.0000
Peroxide degradation 30 % H_2O_2_1h_100°C	78.2	27.86	1.0000
TD_105°C_1 day	106.3	1.94	1.0000
Photolytic Degradation UV_1.2M Lux h	108.3	0.1	1.0000

Table 7. Forced degradation for Glimepiride.

Sample Name	% Assay	% Degradation	% Peak purity
As such sample (Unstressed)	100.0	-	0.9999
Acid degradation 1 N HCl_1 h_100°C	95.0	5.0	0.9994
Alkali degradation 1 M NAOH_1 h_100°C	50.0	50.0	0.9979
Alkali degradation 1 M NAOH_15min_100°C	70.0	30.0	0.9987
Peroxide degradation 30 % H_2O_2_1h_100°C	65.0	35.0	0.9984
Thermal degradation thermal_105°C_1 day	85.0	15.0	0.9989
Photolytic degradation UV_1.2M Lux h	100.0	0.0	0.9993

Ruggedness:

Intermediate precision was demonstrated by injecting six sample preparations on different days by a different analyst and using a different HPLC system. The mean, standard deviation, and relative standard deviation were calculated. Also mean, standard deviation, and percentage relative standard deviation were reported for all the sample solutions of repeatability and intermediate precision. The data obtained have been presented in Tables 9 and 10.

Table 8. Method precision study.

No of Injection	Metformin		Glimepiride	
	Area	%	Area	%
1	9329133	99.7	16861	100.2
2	9414956	100.6	17136	101.9
3	9405179	100.5	16937	100.7
4	9339300	99.8	16711	99.4
5	9421331	100.7	17046	101.3
6	9296069	99.4	16888	100.4
Mean	-	100.1	-	100.7
SD	-	0.549	-	0.873
% RSD	-	0.5	-	0.9

Table 9. Method intermediate precision study.

No of Injection	Metformin		Glimepiride	
	Area	% Assay	Area	%
1	928932	97.2	17369	102.0
2	931639	97.4	17137	100.6
3	931233	97.4	17500	102.8
4	947148	99.1	17020	100.0
5	926871	96.9	17136	100.6
6	928535	97.1	16762	98.4
Mean	-	97.5	-	100.7
SD	-	0.799	-	1.542
% RSD	-	0.8	-	1.5

Linearity and range:

The standard solutions containing metformin and glimepiride were prepared. Linearity was determined by duplicate injections of five different concentrations (50, 80, 100, 120, and 150 % of the target concentration). The average peak areas were plotted against concentrations. Then linearity was evaluated using the calibration curve to calculate the coefficient of correlation, slope, and intercept. In general, a value of correlation coefficient (r^2) > 0.99 was considered the evidence of an acceptable fit for the data to the regression line. The data obtained had been presented in Tables 11 to 14 and Fig 11 and 12.

Table 10. Precision v/s. intermediate precision study comparison.

No of Injection	Metformin		Glimepiride	
	Precision	Intermediate Precisio	Precision	Intermediate Precision
1	99.7	97.2	100.2	102.0
2	100.6	97.4	101.9	100.6
3	100.5	97.4	100.7	102.8
4	99.8	99.1	99.4	100.0
5	100.7	96.9	101.3	100.6
6	99.4	97.1	100.4	98.4
Mean	100.1	97.5	100.7	100.7
SD	0.549	0.799	0.873	1.542
% RSD	0.5	0.8	0.9	1.5
Overall Mean	98.8		100.7	
Overall SD	1.507		1.196	
Overall % RSD	1.5		1.2	

SD – Standard deviation.

Table 11. Linearity of metformin.

Level No.	Concentration (µg/ml)	Mean area
50 %	124.84	4781813
80 %	199.75	7580066
100 %	249.69	9449904
120 %	299.63	11294648
150 %	374.53	14329717
Slope		38094.8059
Intercept		-24612.4103
CC		0.9998
R^2		1.000

Table 12. Linearity of Glimepiride.

Level No.		Concentration (µg/ml)	Mean area
1	50 %	0.55	9086
2	80 %	0.87	14750
3	100 %	1.09	18122
4	120 %	1.31	21337
5	150 %	1.64	28484
Slope			17371.3125
Intercept			-638.4931
CC			0.9971
R^2			0.994

Table 13. Range at a lower (50 %) and higher (150 %) concentration for Metformin.

Inj. No.	Linearity Level-1	Linearity Level-5
1	4795014	14203836
2	4780002	14203670
3	4787588	14214351
4	4790775	14270376
5	4791316	14201821
6	4791893	14217102
Mean	4789431	14218526
SD	5194.104	26166.524
RSD (%)	0.1	0.2

Table 14. Range at a lower (50 %) and higher (150 %) concentration for Glimepiride.

Inj. No.	Linearity Level-1	Linearity Level-5
1	9457	24939
2	9082	24795
3	9345	24659
4	9440	24602
5	9440	24173
6	9365	24354
Mean	9355	24587
SD	141.126	282.228
RSD (%)	1.5	1.2

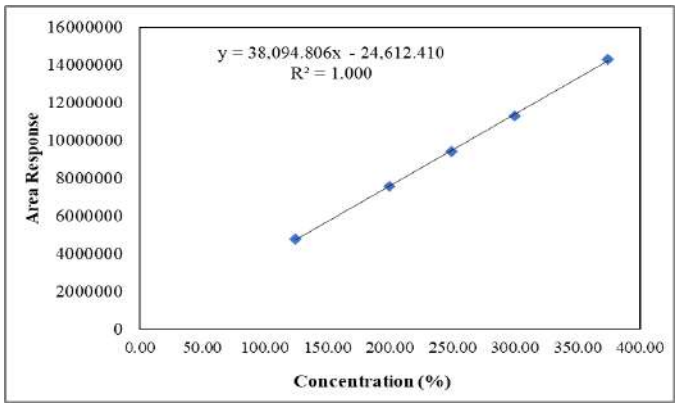

Fig 11. Linearity graph for metformin.

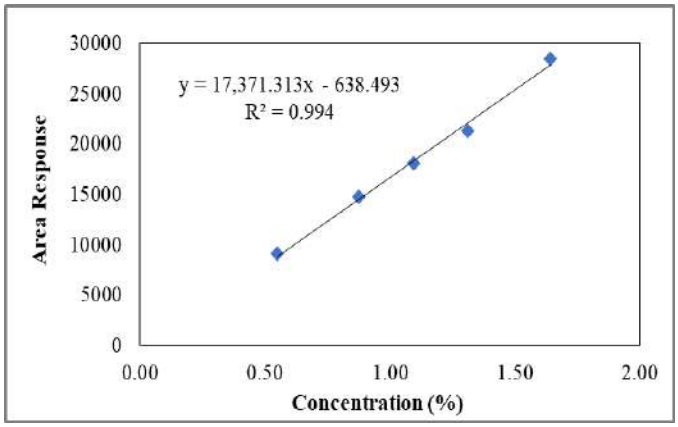

Fig 12. Linearity graph for glimepiride.

Table 15. Accuracy of Metformin.

Sl. No.	Level	Sample Area	Amount recovered (µg/ml)	Amount added (µg/ml)	% Recovery	Mean % Recovery	
1	50%-1	4644587	124.1153	124.6669	99.6	Mean	99.4
2	50%-2	4636128	123.8893	124.6729	99.4	SD	0.200
3	50%-3	4628780	123.6929	124.6310	99.2	% RSD	0.2
4	100%-1	9402846	251.2683	249.3148	100.8	Mean	100.8
5	100%-2	9404908	251.3234	249.3397	100.8	SD	0.000
6	100%-3	9406814	251.3743	249.3198	100.8	% RSD	0.0
7	150%-1	13910227	371.7171	373.9468	99.4	Mean	99.3
8	150%-2	13864421	370.4931	373.9338	99.1	SD	0.153
9	150%-3	13889120	371.1531	373.9428	99.3	% RSD	0.2

SD – Standard deviation.

Table 16. Accuracy of Glimepiride.

Sl. No.	Level	Sample Area	Amount recovered (µg/mL)	Amount added (µg/mL)	% Recovery	Mean % Recovery	
1	50%-1	8336	0.4955	0.4930	100.5	Mean	100.2
2	50%-2	8242	0.4899	0.4887	100.2	SD	0.252
3	50%-3	8113	0.4822	0.4821	100.0	%RSD	0.3
4	100%-1	17579	1.0449	1.0244	102.0	Mean	101.2
5	100%-2	17800	1.0580	1.0442	101.3	SD	0.907
6	100%-3	17883	1.0630	1.0609	100.2	%RSD	0.9
7	150%-1	25181	1.4967	1.5033	99.6	Mean	100.9
8	150%-2	26116	1.5523	1.5310	101.4	SD	1.136
9	150%-3	26520	1.5763	1.5506	101.7	%RSD	1.1

SD – Standard deviation.

Accuracy:

An accuracy study was conducted by spiking metformin and glimepiride in triplicate at three different levels (50, 100, and 150 %) of the specification level in the placebo. The samples were analyzed as per methodology and percentage recovery at each level was calculated. The data obtained had been presented in Tables 15 and 16.

CONCLUSION:

This intended study can be concluded as: the proposed method is economical, simple, ultra-fast, sensitive, and reliable and is found to be accurate, precise, specific, stability-indicating, and rugged. All these parameters considered for verification meet the predefined acceptance criteria. So, the method is proposed for the quantitative estimation percentage assay of metformin and glimepiride in metformin HCl and glimepiride film-coated tablets 500/2 mg for intended applications.

ACKNOWLEDGEMENT:

The authors wish to thank the management of Oman Pharmaceutical Products Co. LLC, for providing the library and laboratory facility to carry out this analytical method validation for Glimepiride and Metformin HCl combined dosage form formulation.

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Conflict of Interest: None
Source of Funding: Nil
Paper Citation: Das P*, Prajapati M, Maity A. RP-HPLC analytical method for simultaneous estimation of percentage assay of Glimepiride and Metformin HCL in combined dosage forms. J Pharm Adv Res, 2022; 5(6): 1569-1577.