World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 6.129

SIMULTANEOUS ESTIMATION OF METFORMIN AND GLIMIPRIDE IN PURE AND TABLET FORM BY RP-HPLC METHOD

Prapulla Putta*

Department of Pharmaceutical Chemistry, Mother Teresa College of Pharmacy N.F.C Nagar, Ghatkesar, and Pin: 501301 Dist: Medchel, Telangana, India.

***Corresponding Author: Prapulla Putta**
Department of Pharmaceutical Chemistry, Mother Teresa College of Pharmacy N.F.C Nagar, Ghatkesar, and Pin: 501301 Dist: Medchel, Telangana, India.

Article Received on 05/11/2019 **Article Revised on 26/11/2019** **Article Accepted on 16/12/2019**

ABSTRACT

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Metformin and Glimepride, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Hypersil C18 (4.6 x 150 mm, 5µm) column using a mixture of Acetonitrile: TEA Buffer pH 4.2 (75:25) as the mobile phase at a flow rate of 1.0 ml/min, the detection was carried out at 259 nm. The retention time of the Metformin and Glimepride was 2.344, 3.282 ±0.02 min respectively. The method produce linear responses in the concentration range of 100-500mg/ml of Metformin and 0.4-2 mg/ml of Glimepride. The method precision for the determination of assay was below 2.0% RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

KEYWORDS: Glimepride, Metformin, RP-HPLC, Validation.

INTRODUCTION

Chromatography is a method of separating a mixture of components into individual components through equilibrium distribution between two phases.[1] Chromatographic methods have taken precedence over the conventional methods of analysis, because of the separation of multiple components during analysis of drug substances. Other than separation of multiple components, the advantage of chromatographic methods is that these possess greater accuracy and sensitivity for even small quantities of degradation products produced. Various chromatographic methods that have been used are thin-layer chromatography (TLC), high performance thin layer chromatography (HPTLC), gas chromatography (GC), High performance liquid chromatography (HPLC) and newer technique like capillary electrophoresis (CE).[2] Metformin is a biguanide antihyperglycemic agent used for treating non-insulin-dependent diabetes mellitus (NIDDM). It improves glycemic control by decreasing hepatic glucose production, decreasing glucose absorption and increasing insulin-mediated glucose uptake. Glimepiride is the first line III generation sulphonyl urea it is a very potent sulphonyl urea with long duration of action. It binds to ATP-sensitive potassium channel receptors on the pancreatic cell surface, reducing potassium conductance and causing depolarization of the membrane.[3] Review of literature for Metformin and Glimepride gave information regarding its physical and chemical properties, various analytical methods that were conducted alone and in combination with other Metformin and Glimepride. Literature survey reveals that certain chromatographic methods were reported for simultaneous estimation of Metformin and Glimepride and single method is available for such estimation by RP-HPLC. In view of the need for a suitable RP-HPLC method for routine analysis of Metformin and Glimepride in formulations, attempts were made to develop simple, precise and accurate analytical method for simultaneous estimation of Metformin and Glimepride and extend it for their determination in formulation.

MATERIALS AND METHODS

Metformin and Glimepride were obtained as a gift sample from Aurobindo Ltd. Acetonitrile served as solvent mixture was also obtained from CDH, New Delhi. All other chemicals/reagents were of analytical grade and were used without further purification.

Preparation of Buffer: **(0.1% OPA)** 1ml of Ortho phosphoric acid solution in a 1000 ml of Volumetric flask add about 100 ml of milli-Q water and final volume make up to 1000 ml with milli-Q water.[4]

Method of validation: The proposed method was validated for various parameters such as linearity and range, accuracy, precision, robustness, ruggedness,

sensitivity and specificity according to ICH Q2 (R1) guideline and USP guidelines.[5]

Method of Linearity and range: The linearity of an analytical procedure is its ability (within a given range) to obtain test result which are directly proportional to the concentration of an analyte in the sample. The range of an analytical procedure is the interval between the upper and lower concentration of an analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity. The linearity of the analytical method was demonstrated over the concentration range investigated by triplicate analysis (n = 3) at a concentration range of 2-20 µg/ml. The absorbance obtained at respective concentration was recorded, and the graph is plotted as concentration (µg/ml) versus absorbance. The linear regression equation and the coefficient correlation were obtained from the UV probe software.[6]

Method of Accuracy: The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness. The accuracy of proposed method was determined on the basis of recovery study. Recovery study was carried out by spiking standard working solution to sample solution (formulation) at three different levels 50%, 100% and 150%. The percentage recovery was calculated as mean ± standard deviation.[7]

Method of Precision: The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the homogeneous sample under the prescribed conditions. The precision of the method was demonstrated by intra-day and inter-day variation studies. In the intra-day precision study, three different solutions of same concentration were prepared and analysed in the same day (morning, noon and evening), whereas in the inter-day precision study, the solutions of same concentration were prepared and analysed, for three consecutive days, and the absorbance were recorded. The result was indicated by calculating percentage RSD.[8]

Method of Robustness: The robustness of an analytical procedure is a measure of its capacity remains unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.[9]

Method of Ruggedness: The ruggedness is a degree of reproducibility of test result under verification of condition like a different analyst, different instruments and different days.[10]

Assay Procedure

Column is equilibrated for 30 min with mobile phase. 20 µl of diluent as blank was injected into the system and recorded the chromatogram for a run time of 30 min. 20 µl of standard preparation-1 was injected into the system and recorded the chromatogram for a run time of 30 min. 20 µl of standard preparation-2 was injected into the system and recorded the chromatogram for a run time of 30 min. Test is valid only when the match factor is in between 0.98 to 1.02. 20 µl of standard preparation-2 into the system was separately injected for four times and recorded each chromatogram for a run time of 30 min. Test is valid only when the five standard preparation-2 injections pass the system suitability.[11]

RESULTS AND DISCUSSIONS

Optimized Chromatogram (Standard)

Mobile phase : Acetonitrile: TEA Buffer pH 4.2 (75:25)
Column : Hypersil C18 (4.6×150mm, 5.0 µm)
Flow rate : 1 ml/min
Wavelength : 259 nm
Column temp : 40ºC
Injection Volume: 10 µl
Run time : 5 minutes

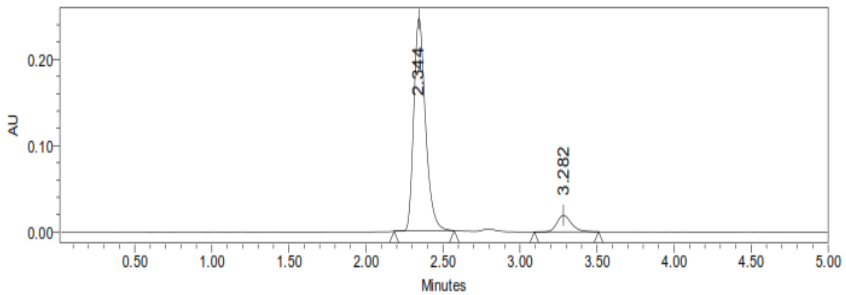

Figure 1: Optimized Chromatogram (Standard).

Table 1: Peak results for optimized.

Sl. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Metformin	2.344	1128848	247861		1.3	4558
2	Glimepride	3.282	14391	19413	6.0	1.2	6031

Observation: From the above chromatogram it was observed that the Metformin and Glimepride peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial.

Column : Hypersil C18 (4.6×150mm, 5.0 µm)
Flow rate : 1 ml/min
Wavelength : 259 nm
Column temp : 40ºC
Injection Volume: 10 µl
Run time : 5 minutes

Optimized Chromatogram (Sample)
Mobile phase: Acetonitrile: TEA Buffer pH 4.2 (75:25)

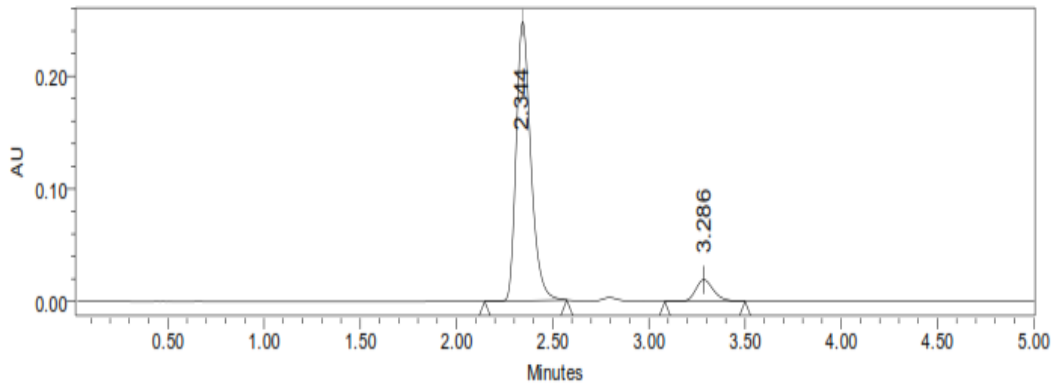

Figure 2: Optimized Chromatogram (Sample).

Table 2: Optimized Chromatogram (Sample).

Sl. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Metformin	2.344	1108849	247851		1.3	4657
2	Glimepride	3.286	14093	19400	6.0	1.2	6075

Acceptance criteria: Resolution between two drugs must be not less than 2

- Theoretical plates must be not less than 2000
- Tailing factor must be not less than 1.2 and not more than 1.3.

Validation
Blank

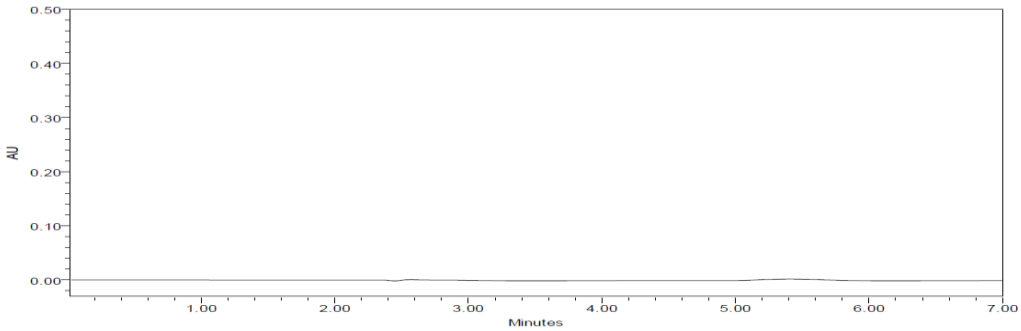

Figure 3: Chromatogram showing blank (mobile phase preparation).

System suitability

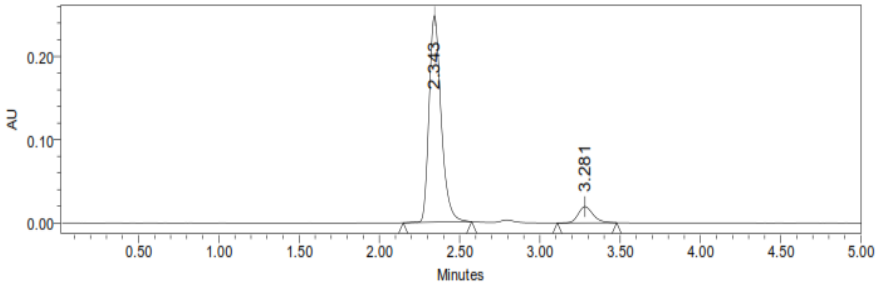

Figure 4: Chromatogram showing injection -1.

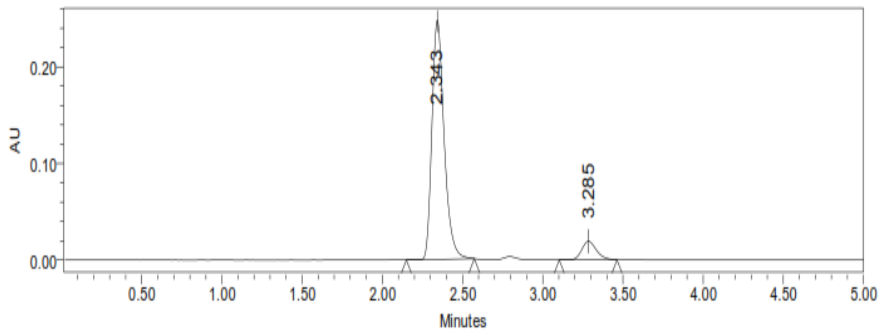

Figure 5: Chromatogram showing injection -2.

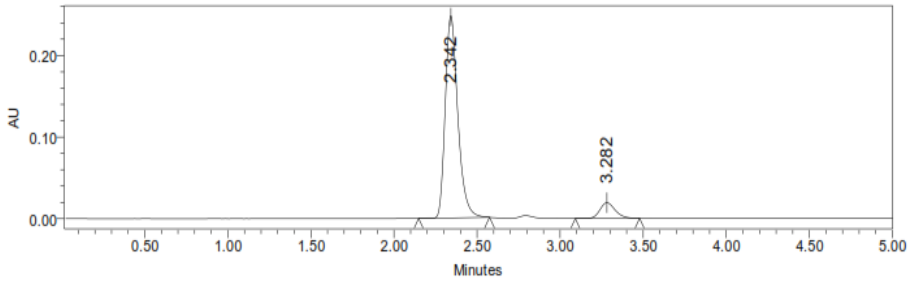

Figure 6: Chromatogram showing injection -3.

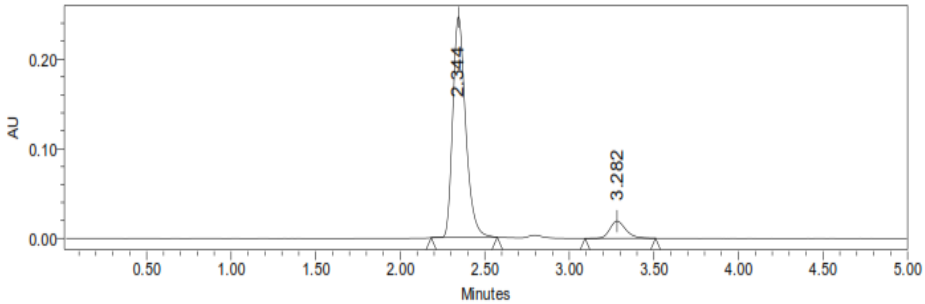

Figure 7: Chromatogram showing injection -4.

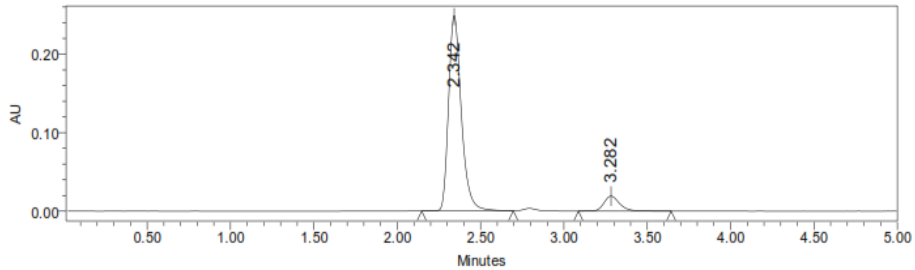

Figure 8: Chromatogram showing injection -5.

Table 3: Results of system suitability for Metformin.

Sl. No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Metformin	2.343	1102519	248455	4506	1.3
2	Metformin	2.343	1102945	249526	4674	1.2
3	Metformin	2.342	1103237	250012	4298	1.2
4	Metformin	2.344	1104076	246695	4032	1.0
5	Metformin	2.342	1109958	248699	4812	1.3
Mean			1104547			
Std. Dev			3077.988			
% RSD			0.27			

Acceptance criteria: % RSD of five different sample solutions should not more than 2.

The % RSD obtained is within the limit, hence the method is suitable.

Table 4: Results of system suitability for Metformin.

Sl. No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Glimepride	3.281	14450	19573	6387.9	1.2
2	Glimepride	3.285	14699	19280	6152.4	1.2
3	Glimepride	3.282	14301	19530	6280.3	1.2
4	Glimepride	3.282	14296	19623	6325.7	1.2
5	Glimepride	3.282	14079	19489	6178.5	1.2
Mean			14365			
Std. Dev			228.8198			
% RSD			1.59			

Acceptance criteria: % RSD for sample should be NMT 2. The % RSD for the standard solution is below 1, which is within the limits hence method is precise.

Specificity

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components.

Analytical method was tested for specificity to measure accurately quantitate Metformin and Glimepride in drug product.

Assay (Standard)

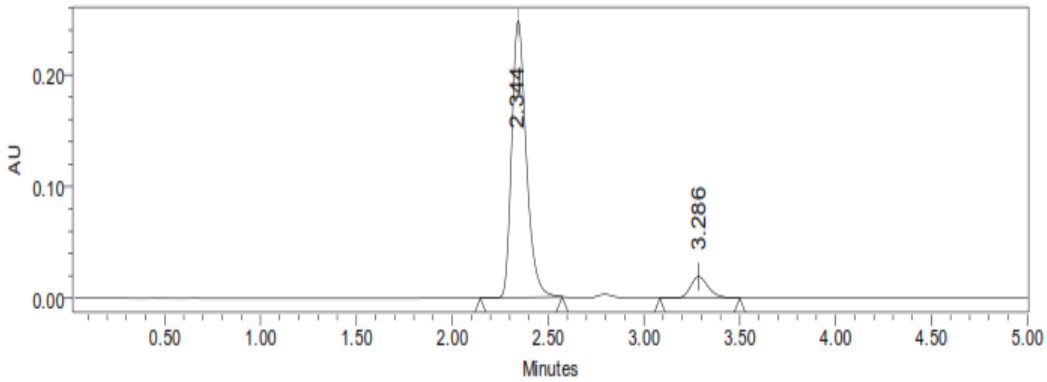

Figure 9: Chromatogram showing assay of standard injection -1.

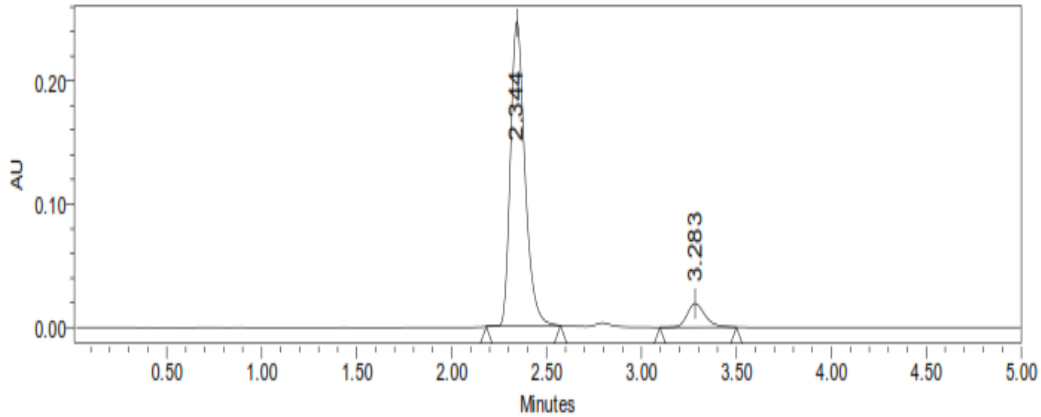

Figure 10: Chromatogram showing assay of standard injection -2.

Table 5: Peak results for assay standard.

Sl. No	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Metformin	2.344	1108495	247282		1.3	4668.9	1
2	Glimepride	3.286	14336	19189	6.0	1.2	6089.7	1

3	Metformin	2.344	1109481	247456		1.3	4677.9	2
4	Glimepride	3.283	14505	19187	6.0	1.2	6098.1	2
5	Metformin	2.344	1117926	247578		1.3	4657.4	3
6	Glimepride	3.283	14903	19210	6.0	1.2	6075.4	3

Assay (Sample)

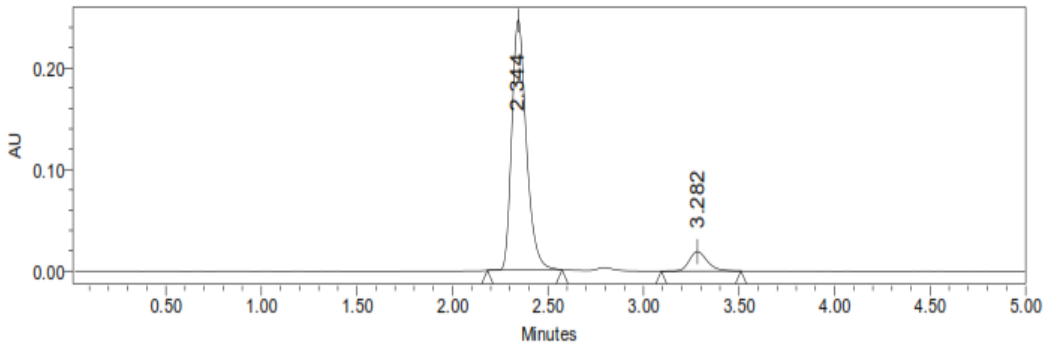

Figure 11: Chromatogram showing assay of sample injection-1.

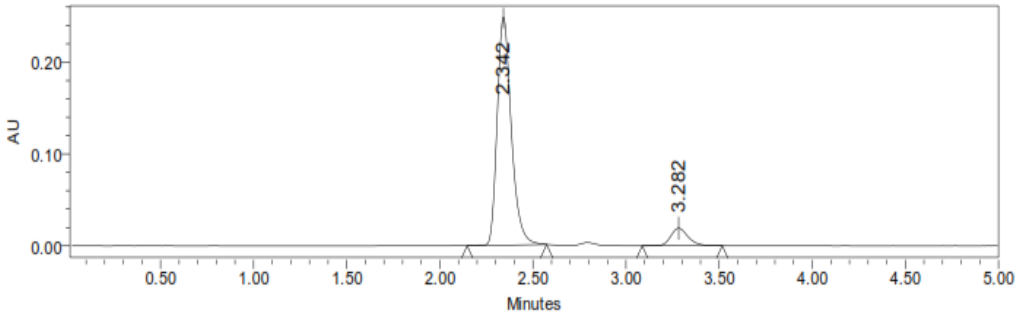

Figure 12: Chromatogram showing assay of sample injection-2.

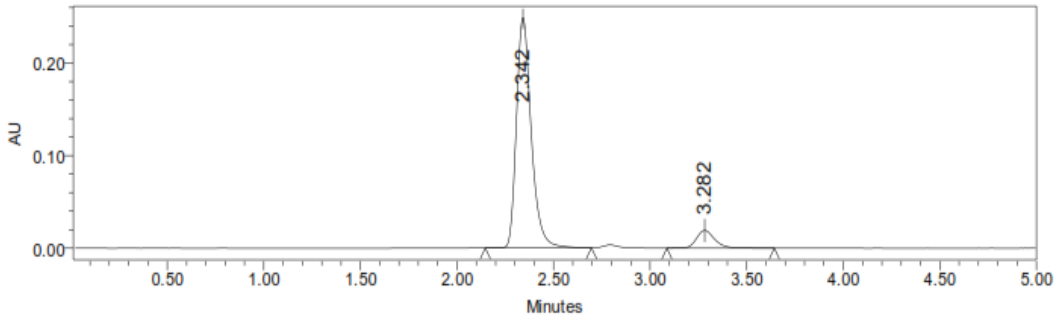

Figure 13: Chromatogram showing assay of sample injection-3.

Table 6: Peak results for Assay sample.

Sl. No	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Metformin	2.344	1107139	246586		1.3	4642.5	1
2	Glimepride	3.282	14452	19117	6.0	1.2	6036.3	1
3	Metformin	2.342	1108903	248422		1.3	4721.5	2
4	Glimepride	3.282	14632	19178	6.0	1.2	6127.3	2
5	Metformin	2.342	1125993	248924		1.3	4701.2	3
6	Glimepride	3.282	14697	19237	6.0	1.3	6090.3	3

$$\text{ASSAY (\%)} = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

= 1114012/1111967×10/300×300/0.0124×99.7/100×0.6219/500×100
= 100.1%

The % purity of Metformin and Glimepride in pharmaceutical dosage form was found to be 100.1 %.

Linearity

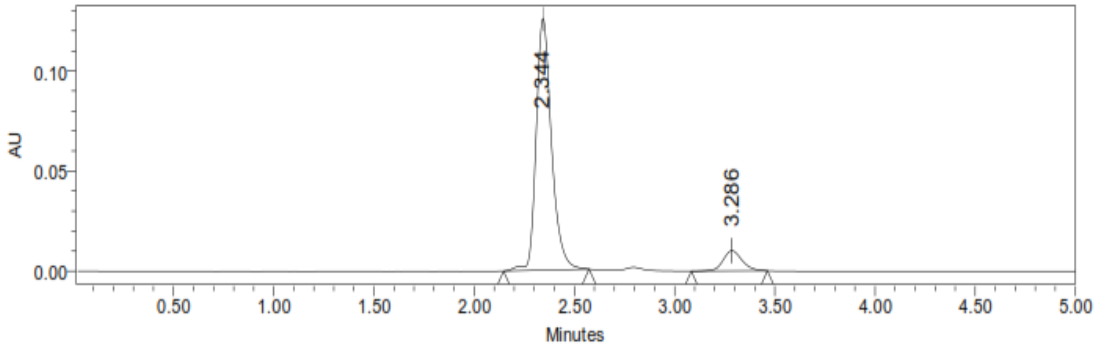

Figure 14: Chromatogram for linearity concentration-100µg/ml of Metformin& 0.4 µg/ml of Glimepride.

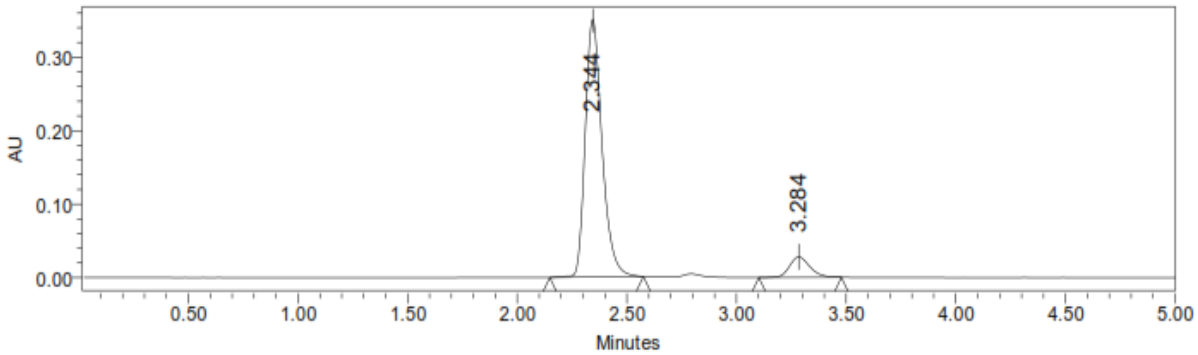

Figure 15: chromatogram for linearity concentration-500µg/ml of Metformin& 2 µg/ml of Glimepride.

Table 7: Chromatographic data for linearity study of Metformin.

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33.3	100	408934
66.6	200	836781
100	300	1203873
133.3	400	1563458
166.6	500	1967084

Table 8: Chromatographic data for linearity study of Glimepride.

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33	0.4	45510
66	0.8	84701
100	1.2	124802
133	1.6	162731
166	2	209732

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

Repeatability

Obtained Five (5) replicates of 100 % accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

Table 9: Results of repeatability for Metformin.

Sl. No	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Metformin	2.345	1102729	248455	4755.2	1.3
2	Metformin	2.344	1102947	249526	4814.8	1.3
3	Metformin	2.343	1103236	250012	4822.2	1.3
4	Metformin	2.344	1103977	246695	4709.2	1.3
5	Metformin	2.345	1109759	248699	4704.8	1.3
Mean			1104530			
Std. Dev			2961.088			
% RSD			0.26			

Acceptance criteria: % RSD for sample should be NMT 2. The % RSD for the standard solution is below 1, which is within the limits hence method is precise.

Table 10: Results of method precession for Glimepride.

Sl. No	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Glimepride	3.287	14149	19573	6387.9	1.2
2	Glimepride	3.287	14066	19280	6152.4	1.2
3	Glimepride	3.288	14271	19530	6280.3	1.2
4	Glimepride	3.285	14291	19623	6325.7	1.2
5	Glimepride	3.288	14056	19489	6178.5	1.2
Mean			14166.6			
Std. Dev			110.7217			
% RSD			0.78			

Acceptance criteria: % RSD for sample should be NMT 2. The % RSD for the standard solution is below 1, which is within the limits hence method is precise.

Intermediate precision

Table 11: Results of Intermediate precision for Metformin.

S. no.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Metformin	2.344	1100148	247140	4703.7	1.3
2	Metformin	2.343	1104520	245696	4645.7	1.3
3	Metformin	2.345	1105937	247870	4707.5	1.3
4	Metformin	2.344	1106476	246764	4639.2	1.3
5	Metformin	2.342	1108271	247280	4642.8	1.3
6	Metformin	2.343	1106582	247166	4631.4	1.3
Mean			1105322			
Std. Dev			2807.405			
% RSD			0.25			

Acceptance criteria: % RSD of five different sample solutions should not more than 2

Table 12: Results of Intermediate precision for Glimepride.

Sl. No	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Glimepride	3.281	14487	19115	6076.6	1.2
2	Glimepride	3.281	14626	19003	6040.0	1.2
3	Glimepride	3.283	14632	19073	6120.1	1.2
4	Glimepride	3.281	14702	19123	6114.0	1.2
5	Glimepride	3.278	14962	19165	6118.5	1.2
6	Glimepride	3.281	14972	19145	6130.3	1.2
Mean			14730.17			
Std. Dev			196.2859			
% RSD			1.33			

Acceptance criteria: % RSD of five different sample solutions should not more than 2. The % RSD obtained is within the limit, hence the method is rugged.

Table 13: Results of Intermediate precision Day 2 for Metformin.

Sl. No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Metformin	2.343	113151	246170	4381.3	1.2
2	Metformin	2.343	113996	245695	4052.5	1.2
3	Metformin	2.342	114390	247869	4140.2	1.2
4	Metformin	2.344	115191	246763	4345.7	1.2
5	Metformin	2.343	114951	247279	4071.1	1.2
6	Metformin	2.344	113161	247165	4657.3	1.2
Mean			114140			
Std. Dev			869.7264			
% RSD			0.76			

Acceptance criteria
% RSD of five different sample solutions should not more than 2.

Table 14: Results of Intermediate precision for Glimepride.

Sl. No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Glimepride	3.281	14041	19573	6381.9	1.0
2	Glimepride	3.285	14093	19280	6052.4	1.1
3	Glimepride	3.282	14198	19530	6140.3	1.1
4	Glimepride	3.286	14032	19623	6345.1	1.1
5	Glimepride	3.283	14098	19489	6071.0	1.2
6	Glimepride	3.287	14100	19573	6657.3	1.0
Mean			14093.67			
Std. Dev			59.19685			
% RSD			0.42			

Acceptance criteria: % RSD of five different sample solutions should not more than 2 The % RSD obtained is within the limit, hence the method is rugged.

Accuracy
Accuracy at different concentrations (50%, 100%, and 150%) was prepared and the % recovery was calculated.

Accuracy 50 %
Table 15: Results of Accuracy for concentration-50 %.

Sl. No	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Metformin	2.344	599156	125163		1.2	4691	1
2	Glimepride	3.284	7888	10063	6.0	1.3	6047	1
3	Metformin	2.343	610507	124410		1.2	4612	2
4	Glimepride	3.282	7800	10066	6.0	1.3	6162	2
5	Metformin	2.343	610315	125429		1.2	4592	3
6	Glimepride	3.284	7811	10018	6.0	1.3	6081	3

Accuracy 100 %:
Table 16: Results of Accuracy for concentration-100 %.

Sl. No	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Metformin	2.344	1189848	246191		1.2	4523	1
2	Glimepride	3.286	14026	19256	6.0	1.3	6234	1
3	Metformin	2.343	1199077	246044		1.2	4512	2
4	Glimepride	3.282	14041	19253	6.0	1.3	6027	2
5	Metformin	2.343	1189849	247851		1.2	4685	3
6	Glimepride	3.283	14003	19400	6.0	1.3	6097	3

Accuracy 150 %
Table 17: Results of Accuracy for concentration-150 %.

S. no.	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Metformin	2.345	1712144	348534		1.2	4685	1
2	Glimepride	3.283	19950	27665	6.0	1.3	6094	1
3	Metformin	2.344	1756259	348167		1.2	4528	2
4	Glimepride	3.282	20992	27646	6.0	1.3	6035	2
5	Metformin	2.343	1855458	348256		1.2	4672	3
6	Glimepride	3.282	19976	27779	6.0	1.3	6098	3

Table 18: Results of the accuracy results for Metformin.

% Concentration	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	606659.3	150	150	100	99.9%
100%	1192925	300	300.3	100.1	
150%	1774609	450	449.3	99.8	

Table 19: Results of the accuracy results for Glimepride.

% Concentration	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	7833	0.6	0.59	98.3	99.1%
100%	14023.3	1.2	1.19	99.1	
150%	20306	1.8	1.8	100	

Acceptance Criteria: The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Limit of Detection

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. **LOD= 3.3 × σ / s**

Where- σ = Standard deviation of the response, S = Slope of the calibration curve

RESULT

Metformin: =3.3 × 20990.9/3904; =17.7µg/ml
Glimepride: =3.3 × 2739.313/10288; =0.8µg/ml

Limit of Quantitation

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

LOQ=10×σ/S

Where- σ = Standard deviation of the response, S = Slope of the calibration curve

Result

Metformin: =10×20990.9/3904; = 53.7µg/ml.
Glimepride: =**10** × 2739.313/10288; = 2.6µg/ml.

Robustness

The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Metformin and Glimepride. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±10%. The standard and samples of Metformin and Glimepride were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

Table 20: Results for Robustness of Metformin.

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	1128848	2.344	4558	1.3
Less Flow rate of 0.9 mL/min	1569971	2.911	7036.3	1.3
More Flow rate of 1.1 mL/min	1114875	2.014	4389	1.4
Less organic phase	1120197	2.361	4508.4	1.4
More organic phase	1107845	2.038	4417	1.4

Acceptance criteria: The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

Table 21: Results for Robustness of Glimepride.

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	14391	3.282	6031	1.2
Less Flow rate of 0.9 mL/min	15550	4.075	7036.3	1.3
More Flow rate of 1.1 mL/min	13951	3.089	6215	1.2
Less organic phase	14406	4.422	6387.7	1.2
More organic phase	14589	3.015	6285	1.2

Acceptance criteria

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Metformin and Glimepride in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Metformin and Glimepride was freely soluble in ethanol, methanol and sparingly soluble in water. Acetonitrile: TEA Buffer pH 4.2 (75:25) was chosen as the mobile phase. The solvent system used in this method was economical. The % RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Metformin and Glimepride in bulk drug and in Pharmaceutical dosage forms.

REFERENCES

1. Sethi PD., Quantitative analysis of Drugs & Pharmaceuticals. CBS publishers and distributors, New Delhi, 3rd edition, 2001 Pg. no 1-120.
2. NR Lad, SI Bhoir, IC Bhoir, M Sundaresan, Concurrent assay of Metformin and Glimepiride in tablets using RP-HPLC with wavelength programming, International Journal of Pharmaceutical Sciences, 2003; 65(6): 650-653.
3. Ramachandra and NVS. Naidu, Validation of RP - HPLC Method for Estimation of Dasatinib In Bulk and Its Pharmaceutical Dosage Forms, International journal of pharmacy and biological sciences, 2014; 61-68: 2230-7605.
4. Kishore konam & Dharmeshwar jadhav, Development and validation of a RP- HPLC method for determination of dronedarone in bulk and pharmaceutical formulation, International Journal of Pharmacy and Biological Sciences, 2014; 4(1): 179-185.
5. International Conference on Harmonization (ICH), Q2A. "Validation of Analytical Procedures: Methodology". Geneva, 1994.
6. Skoog et al., Principles of Instrumental Analysis. Barkhanath publishers, 8th edition, 973-995.
7. Prapulla Putta, Analytical methods Development and Validation of Crizotinib by RP-HPLC Technique, International Journal of Biochemistry and Pharmacology, 2019; 1(1): 1-4.
8. Selvakumar. S, Ravichandran. S, Matsyagiri. L, Development and Validation of analytical method for Simultaneous estimation of Ornidazole and Cefixime trihydrate tablet dosage forms by UV spectroscopy. Asian J. Pharm. Ana, 2016; 6(4): 246-252.
9. Chatwal GR, and Anand SK. Instrumental Methods of Chemical Analysis. 5th Revised and Enlarged ed., Himalaya Publication House, Mumbai, 2002; 2: 630.
10. Matsyagiri. L, Jagadeesh. P, Mounika. B, Srinivas. V, Theja.V, Madhiha Jabeen and Dr. K. Hemamalini, Effect of solvents on Spectrophotometric Estimation of Tinidazole in bulk and dosage forms, World Journal of Pharmaceutical Research, 2018; 7(9): 1742-1754.
11. Jadhav PB, Vivek Shejwal, Development and Validation of an RP-HPLC Method for Crizotinib, International Journal of Pharmacy and Pharmaceutical Research. Human, 2017; 9(2): 100-106.