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METHOD DEVELOPMENT, VALIDATION AND STABILITY INDICATING ASSAY ON GLIMEPIRIDE IN TABLET DOSAGE FORM BY RP-UFLC

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Keywords:

Glimepiride, RP-UFLC, Acetonitrile, Validation, ICH Guidelines, Degradation

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ABSTRACT: The study aimed to develop a specific, exact, selective, precise and accurate Reversed-Phase Stability Indicating Ultra-fast Liquid Chromatography (RP-UFLC) strategy is created and validated for the determination of glimepiride in the tablet dosage form. The method showed an adequate separation for glimepiride from their degradation products. The optimum separation was achieved by using 250 mm × 4.6 mm C18 column (5 µm) with a mixture of potassium dihydrogen orthophosphate adjusted to pH 4.6 using (orthophosphoric acid) and acetonitrile in the ratio of 75:25 at a flow rate of 1 ml/min. The detection was carried out at 236 nm, and the retention time was found to be 5.9 min. Linearity was observed (correlation coefficient r^2 0.9989). % R.S.D was found to be less than 2%. Accuracy of the method found to be in the range of 98.0 to 102.00 (% w/w). The above method was validated concerning system suitability, linearity, precision, the limit of detection (LOD) and limit of quantification (LOQ), accuracy (recovery) and robustness according to ICH guidelines. The linearity of the above methods was found to be 2-10 µg/ml for glimepiride and force degradation were carried out. Hence these methods can be used for routine analysis in quality control laboratories.

INTRODUCTION: Glimepiride, sold under the trade name amaryl among others, is medium-to-long-acting sulfonylurea anti-diabetic medication. It is taken by mouth. It is sometimes classified as either the first third-generation sulfonylurea or as second-generation. In 2016 it was the 61st most prescribed medication in the United States with more than 12 million prescriptions. Glimepiride is indicated to treat type 2 diabetes mellitus; its mode of action is to increase insulin secretion by the pancreas.

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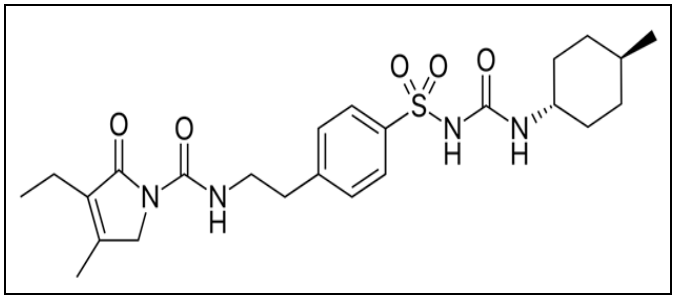
However, it requires adequate insulin synthesis as a prerequisite to treating appropriately. It is not used for type 1 diabetes because in type 1 diabetes the pancreas is not able to produce insulin. Like all sulfonylureas, glimepiride acts as an insulin secretagogues. It lowers blood sugar by stimulating the release of insulin by pancreatic beta cells and by inducing the increased activity of intracellular insulin receptors. Not all secondary sulfonylureas have the same risk of hypoglycemia. Glibenclamide (glyburide) is associated with an incidence of hypoglycemia of up to 20-30%, compared to as low as 2% to 4% with glimepiride. Glibenclamide also interferes with the normal homeostatic suppression of insulin secretion in reaction to hypoglycemia, whereas glimepiride does not. Also, glibenclamide diminishes glucagon secretion in reaction to hypoglycemia, whereas glimepiride does not react to hypoglycemia [1, 2].



MATERIALS AND METHODS:

Preparation of Mobile Phase: To a 1000mL volumetric flask, add 1.36 gm of potassium dihydrogen orthophosphate was taken and diluted with millipore water and made up to the mark, and the pH was adjusted to 4.6 using orthophosphoric acid. The solution was then filtered through a 0.45µ membrane filter.

Preparation of Diluent: The diluent is a mixture of 100 parts of acetonitrile.

Preparation of Standard Stock Solution for Glimepiride: 100 mg of glimepiride was taken into 100 mL volumetric flask. To this add 50 mL of diluent and sonicate to dissolve and the volume was made up to the mark with diluent (1000 µg/ml). Pipette 1ml of the above solution into 10 mL volumetric flask and make up the volume using diluent (100 µg/ml).

Preparation of Solutions for Linearity: The solutions for linearity were prepared from the stock solution by diluting with diluent. The concentration ranging from 2, 4, 6, 8, 10 µg/mL were prepared for Glimepiride**.** Pipette 0.2, 0.4, 0.6, 0.8, 1.0 mL in 10 mL volumetric flasks and make up the volume using diluent to get the above concentrations.

Chromatographic Conditions:

TABLE 1: CHROMATOGRAPHIC CONDITION

S. no.	Chromatographic Conditions	
1	Column	Phenomenex Kinetex C18 (250 × 4.6 mm)
2	Flow Rate	1.0mL/min
3	Wavelength	236nm
4	Detector	PDA detector
5	Injection volume	10µl
6	Mobile phase	Potassium Di-hydrogen orthophosphate and Acetonitrile(75:25)
7	pH	4.6 with Orthophosphoric acid
8	Run time	10 min

Method Validation: [3-10]

Linearity and Range: The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of an analyte in samples within a given range.

Procedure: 2-10 ug/ml for glimepiride was prepared. The regression line obtained was linear. From the data obtained, co-relation coefficient, slope, and Y-intercept were calculated. Ideally co-relation coefficient should be not less than 0.99 and statistical Y-intercept should be not more than 2.0.

Accuracy: Accuracy is performed in three different levels for glimepiride using standard addition method at 50%, 100%, and 150%. A known quantity of sample was spiked to the standards. Samples are analyzed in triplicate for each level. From the results, % recovery was calculated. % recovery at each spike level shall be not less than 98.0% and not more than 102.0%, % RSD for the duplicate observations shall be not more than 2.0. Overall % RSD for the % Recovery shall be not more than 2.0.

% Recovery = (Amount of drug recovered) / (Amount of drug added) × 100

Procedure:

Accuracy at 50%: A known amount of standard drug solution of glimepiride (25 µg/mL) from the stock solution was added to the sample solution of a determined concentration (50 µg/mL) the solutions were taken in a 10 mL volumetric flask and the volume was made up with diluent and filtered through 0.2 µ syringe filter. The solutions were injected, analyzed and the recovery was calculated.

Accuracy at 100%: A known amount of standard drug solution of Glimepiride (50 µg/mL) from the stock solution was added to the sample solution of a determined concentration (50 µg/mL). The solutions were taken in a 10 mL volumetric flask and the volume was made up with diluent and filtered through 0.2 µ syringe filter. The solutions were injected, analyzed and the recovery was calculated.

Accuracy at 150%: A known amount of standard drug solution of glimepiride (75 µg/mL) from the

stock solution were added to the sample solution of a determined concentration (50 µg/mL) the solutions were taken in a 10 mL volumetric flask and the volume was made up with diluent and filtered through 0.2 µ syringe filter. The solutions were injected, analyzed and the recovery was calculated.

Precision:

System Precision: The system precision is checked by injecting 6 sample injections and checking the reproducibility in the retention time and area. The % RSD calculated must be less than 2%.

Method Precision:

Intraday Precision: The intraday precision is checked by using standard glimepiride samples to ensure that the analytical system is precise. The retention time and area of three determinations was measured and RSD was calculated. % RSD of the assay value for three determinations shall not be more than 2.0%.

Interday Precision: The interday precision is checked by using the same standard glimepiride samples analyzed for intraday precision on an alternate day to ensure that the analytical system is precise. The retention time and area of three determinations was measured and RSD was calculated. % RSD of the assay value for three determinations shall not be more than 2.0%.

Limit of Detection (LOD) and Limit of Quantification (LOQ): LOD and LOQ were calculated using the mathematical equations.

$$LOD = 3.3\ \sigma/S$$

Where, σ = the standard deviation of the response; S = the slope of the calibration curve

$$LOQ = 10\ \sigma/S$$

Where, σ = the standard deviation of the response; S = the slope of the calibration curve

Robustness: The robustness of an analytical method 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.

Procedure: Robustness was done by changing the flow rate (± 0.1 mL), and the mobile phase ratio (± 2) and pH (± 0.1). All the system suitability parameters must meet as per the method. The flow rate of the mobile phase was increased to 1.1 mL/min and decreased to 0.9 mL/min from 1.0 mL/min. The final variation was done by changing the mobile phase (acetonitrile: potassium dihydrogen orthophosphate) ratio to 75:25 and 60:40 from 70:30. The pH was changed to 4.5 and 4.7 from 4.6.

System Suitability: Five replicate injections of standard solutions were injected, and the chromatograms were recorded. The system is suitable for analysis if

- The theoretical plates in five replicate injections should be not less than in 2000.
- USP tailing factor for glimepiride peaks should be not more than 2.0.
- The % relative standard deviation for five replicate injections should not be more than 2%.

Procedure: The standard solution (10 µg/mL) was prepared and injected into the UFLC system six times. The tailing factor and theoretical plate count were recorded.

Method Validation:

System Suitability: System suitability tests are used to verify the reproducibility of the chromatographic system. To ascertain its effectiveness, system suitability tests were carried out on freshly prepared stock solutions.

TABLE 2: SYSTEM SUITABILITY RESULTS FOR GLIMEPIRIDE

S. no.	System suitability parameters	Glimepiride	Acceptance criteria
1	% RSD for six replicate injections of analyte peak in standard solution	1.2511	<2
2	Tailing factor for analyte peak in standard solution	0.84	<2
3	USP plate count for analyte peak in standard solution	5428	>2000

Data Interpretation: It was observed from the data tabulated above that the method complies with system suitability parameters. Hence, it was concluded that the system suitability parameter met the requirement of method validation.

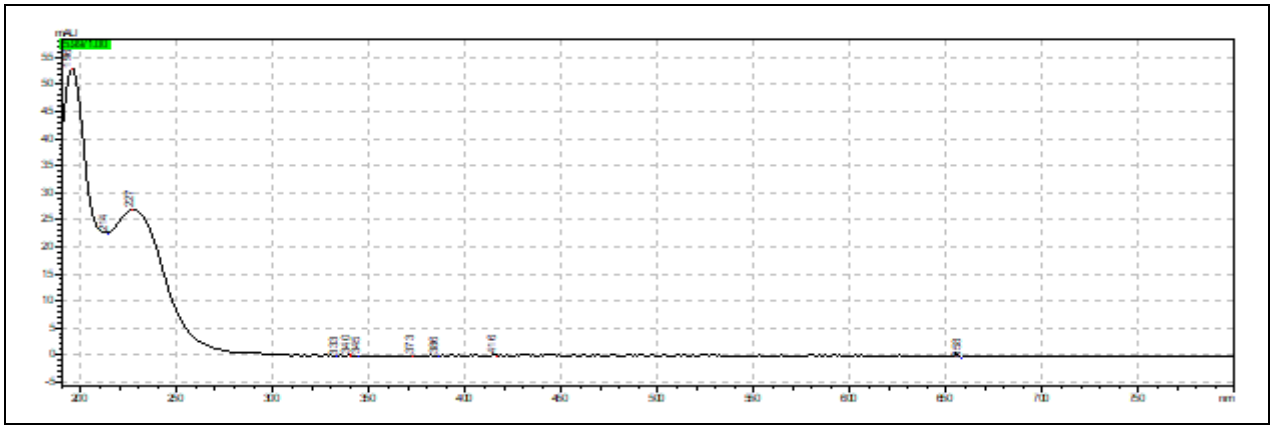


FIG. 1: SPECTRUM VIEW

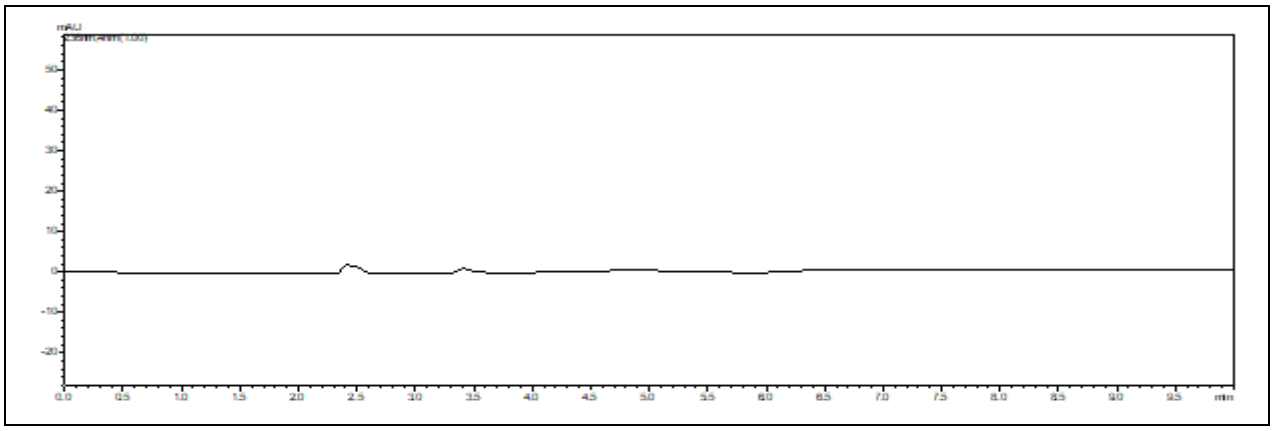


FIG. 2: CHROMATOGRAM OF BLANK

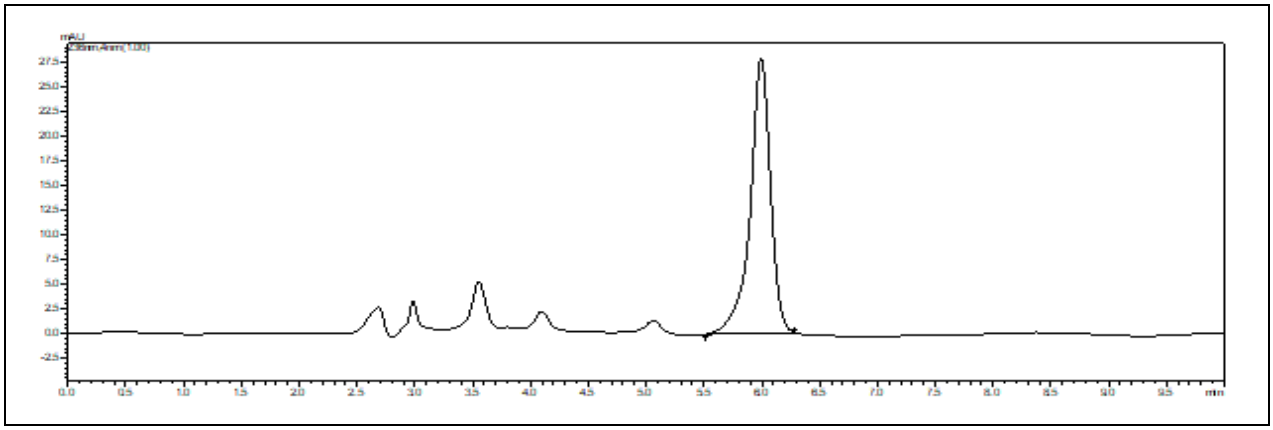


FIG. 3: CHROMATOGRAM OF STANDARD

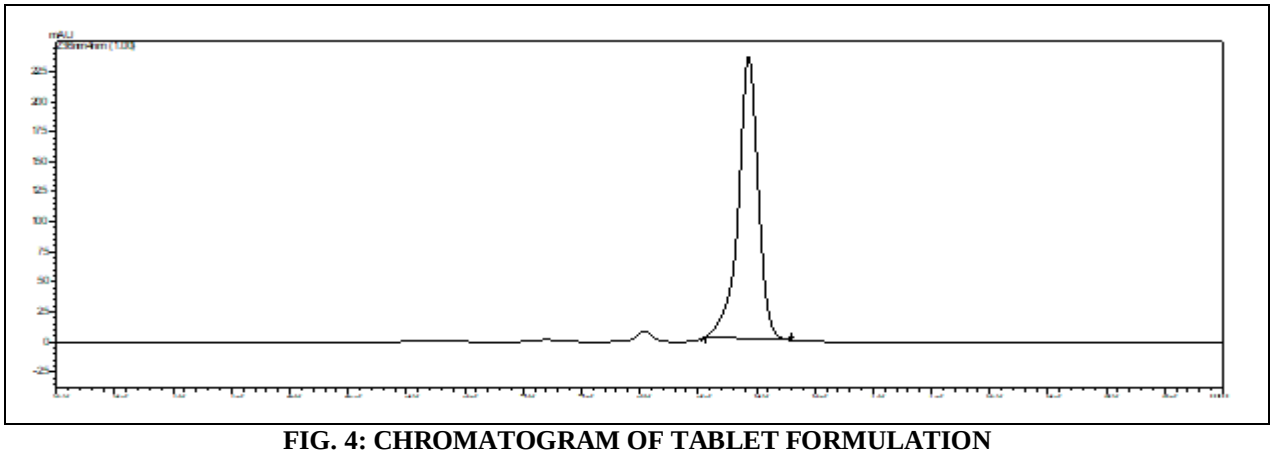


FIG. 4: CHROMATOGRAM OF TABLET FORMULATION

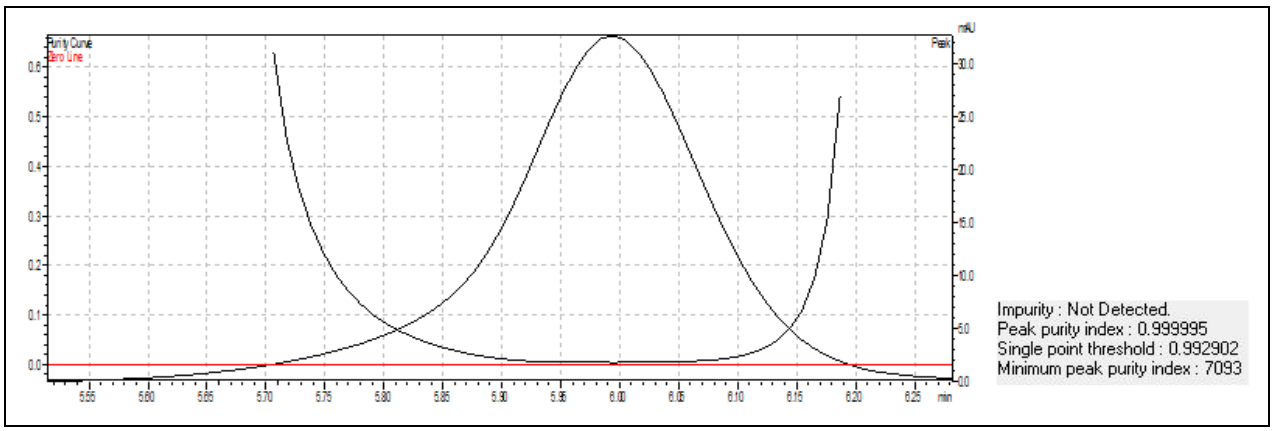


FIG. 5: PEAK PURITY

Linearity and Range:

Glimepiride: The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of an analyte in samples within a given range of 2-10 ug/ml as reported in **Table 3**.

TABLE 3: LINEARITY

S. no.	Concentration	Peak area of Glimepiride
1	2	129261
2	4	176176
3	6	236622
4	8	287503
5	10	340174
Slope		26658
Intercept		74001
Co-efficient of correlation		0.9989
Acceptance criteria		Coefficient of Correlation shall be not less than 0.999

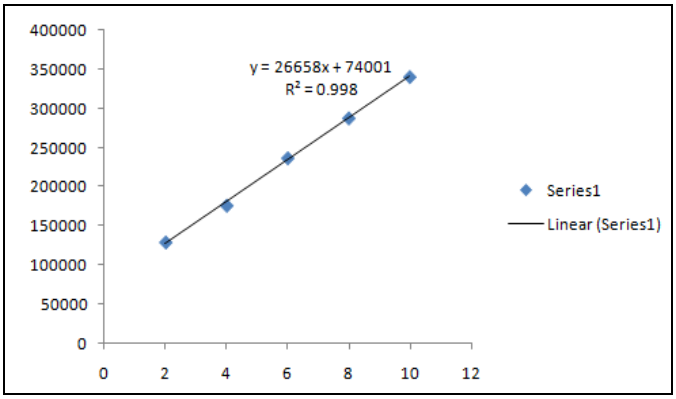


FIG. 6: CALIBRATION CURVE FOR GLIMEPIRIDE

Data Interpretation: The method for glimepiride was found to be linear in the concentration range of 2 µg/ml to 10 µg/ml and the correlation coefficient obtained is 0.9693.

Precision: The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of a homogeneous sample.

System Precision: The system precision was carried out to ensure that the analytical system was working properly. The system precision is performed by 6 sample injections and checking the reproducibility in the peak area.

Data Interpretation: It is observed from the data tabulated above, that the area responses are consistent as evidenced by the values of relative standard deviation. Hence, it can be concluded that the system precision parameter meets the requirement of method validation.

Method Precision:

Intraday Precision:

TABLE 4: METHOD PRECISION FOR GLIMEPIRIDE

S. no.	2 µg	6 µg	10 µg
1	129261	236622	340174
2	128456	236652	342178
3	130641	238234	344161
4	131201	235896	348174
5	132001	241621	350110
6	133366	243612	352177
Avg.	130821	238772.8333	346162.3
St. dev	1636.716734	2864.149578	4311.757
% RSD	1.251111621	1.199529083	1.245588

TABLE 5: SYSTEM PRECISION FOR GLIMEPIRIDE

S. no.	2 µg	6 µg	10 µg
1	149228	316539	360872
2	149480	321135	361845
3	150208	310590	370814
4	151188	311529	371881
5	153267	312589	372870
6	154020	321054	374578
Avg.	151231.8333	315572.6667	368810
St. dev	1828.031766	4320.471451	5396.35
% RSD	1.208761228	1.369089249	1.463179

The precision express reliability of the method, where it defines the extent for the individual test results can agree to repeated test result on the same operating conditions at a short time period. Repeatability of the method is accepted.

Interday Precision: Repeatability method procedure was repeated on the next day. The method passed the test, as both retention time (<1%) and response peak areas (<2%), % RSD obtained were in the limits.

Data Interpretation: From the above **Table 4** and **5** results, it was concluded that the method is precise.

Limit of Detection and Limit of Quantitation: Limit of detection is the lowest amount of analyte in a sample that can be detected, but not necessarily quantitated, under the stated experimental conditions.

Limit of Quantitation is the lowest amount of analyte in a sample that can be quantitated with acceptable accuracy and precision, under the stated experimental conditions.

Limit of Detection (LOD) and Limit of Quantitation (LOQ) was calculated based on the residual standard deviation of response and slope.

TABLE 6: RESULTS OF LOD & LOQ OF GLIMEPIRIDE

Parameter	Glimepiride
LOD (µg/ml) 3.3*sd/slope	2.95917628
LOQ (µg/ml) 10*sd/slope	8.95917628

Accuracy: The accuracy of an analytical method is the closeness of test results obtained by that method to the true value (Standard value).

% Recovery = Amount of drug × 100 / Amount of drug added

TABLE 7: RECOVERY RESULTS FOR GLIMEPIRIDE

Level of recovery	Amount of formulation	Amount of Pure drug	Total amount of drug	Peak area	Difference	% recovery	Mean
50	40	20	60	319101	119259	67.6931024	
	40	20	60	329103	199842	113.433158	98.60386
	40	20	60	331309	202048	114.685315	
100	40	40	80	312002	135826	77.0967669	
	40	40	80	369001	192825	109.450209	98.75484
	40	40	80	369472	193296	109.717555	
150	40	60	100	379642	143020	81.1801835	
	40	60	100	405021	168399	95.5856643	95.32816
	40	60	100	429039	192417	109.218622	

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 8: RESULTS FOR ROBUSTNESS OF GLIMEPIRIDE

WL	C	A	WL	C	A
	6	0.115		6	0.116
523	6	0.113	525	6	0.114
	6	0.115		6	0.115
	Average	0.114333333		Average	0.115
	St. dev	0.001154701		ST DEV	0.001
	%RSD	1.009942162		%RSD	0.86956522

Ruggedness:

TABLE 9: RESULTS FOR RUGGEDNESS OF GLIMEPIRIDE

C	T1	T2	Mean	SD	%RSD
By changing the analyst					
0	0	0	0	0	0
2	0.04	0.039	0.0395	0.000707107	1.79014375
4	0.073	0.074	0.0735	0.000707107	0.962050042
6	0.116	0.114	0.115	0.001414214	1.229750924
8	0.183	0.178	0.1805	0.003535534	1.958744546
10	0.204	0.209	0.2065	0.003535534	1.712122957
By changing the instrument					
0	0	0	0	0	0
2	0.04	0.041	0.0405	0.000707107	1.74594267

4	0.073	0.074	0.0735	0.000707107	0.962050042
6	0.115	0.112	0.1135	0.00212132	1.869004708
8	0.17	0.176	0.798	0.004242641	0.531659234
10	0.203	0.205	0.204	0.001414214	0.693241942

Assay of Marketed Formulation:

Sample Preparation: In case of marketed formulations, twenty tablets were taken, weighed, finely powdered and an accurate amount equivalent to 100 mg of drug powder was transferred into a 100 ml volumetric flask. The stock solution was further diluted with diluents and it was filtered through 0.45µ nylon filter to obtain a concentration of 100 µg/ml of glimepiride and then the resultant solution is analyzed.

Forced Degradation Studies: The stress studies were performed Glimepiride drug at 50 µg/ml concentration. Here the bulk drug is exposed to acidic stress by addition of 1.0 ml of 0.1M HCl to drug solution and counteracted with 1.0 ml of 0.1M NaOH, at 0 min, 30 min, 1 h, 2 h, 4 h, 8 h, 6 h, and 32 h respectively. Similarly, the basic stress studies were performed by adding 1.0 ml of 0.1 M NaOH and neutralized with 1ml of 0.1M HCl. Oxidation studies were achieved on the bulk drug by addition of 1.0 mL of 3% H_2O_2. Thermal studies were performed by heating the sample at 60 ºC and UV studies were also carried out by sample at UV-Lamp 45 ºC respectively. Entire samples were placed in a different volumetric flask (10 mL) and dissolved in HPLC grade acetonitrile. Final drug concentration for the assay was made up of Acetonitrile and injected in the chromatographic system. For all these stability studies, the development of degradable item was affirmed by contrasting and the chromatogram of the arrangement kept under ordinary unstressed conditions. Every stressed sample was analyzed by improved RP-UFLC method. The degradation of data for glimepiride was shown below [11-16].

Acid Stress: For 2 ml sample add 2 ml 0.1N HCl keep aside for 5 min and then add 2 ml of 0.1N NaOH, then inject this sample for 36 h as intervals 30 min, 1 h, 1.30 min respectively.

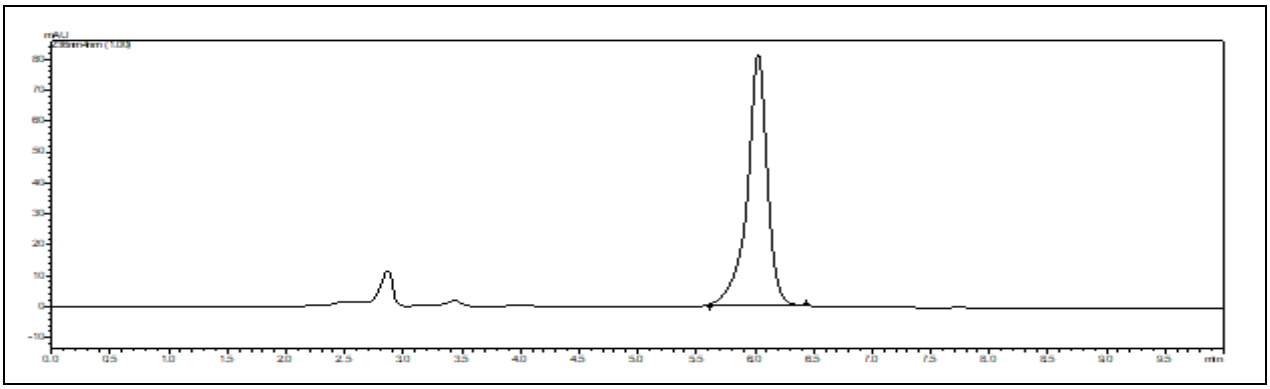


FIG. 7: CHROMATOGRAM FOR ACID STRESS

Base Stress: For 2 ml sample add 2 ml of 0.1N NaOH keep aside for 5 min and then add 2 ml of 0.1N HCL, and inject the sample.

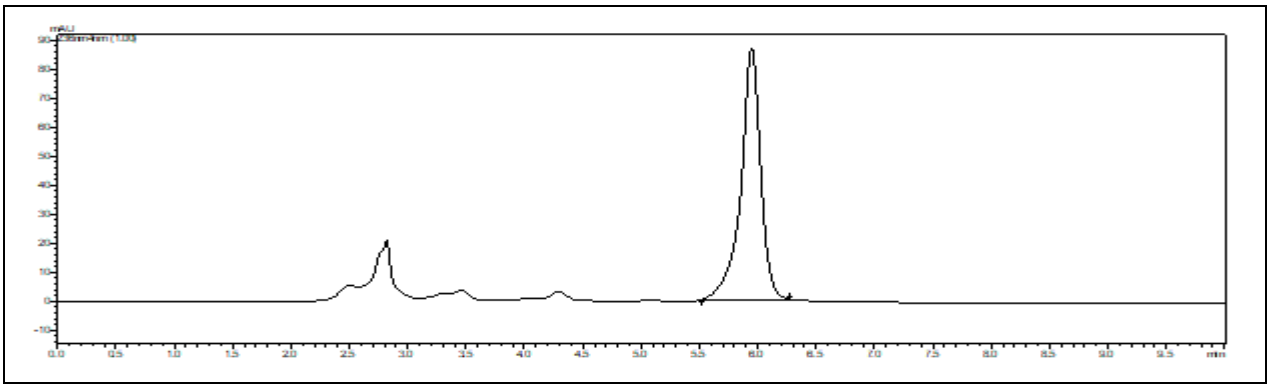


FIG. 8: CHROMATOGRAM FOR BASE STRESS

Peroxide Stress: For 2 ml sample add 1 ml of 3% peroxide solution and inject this sample.

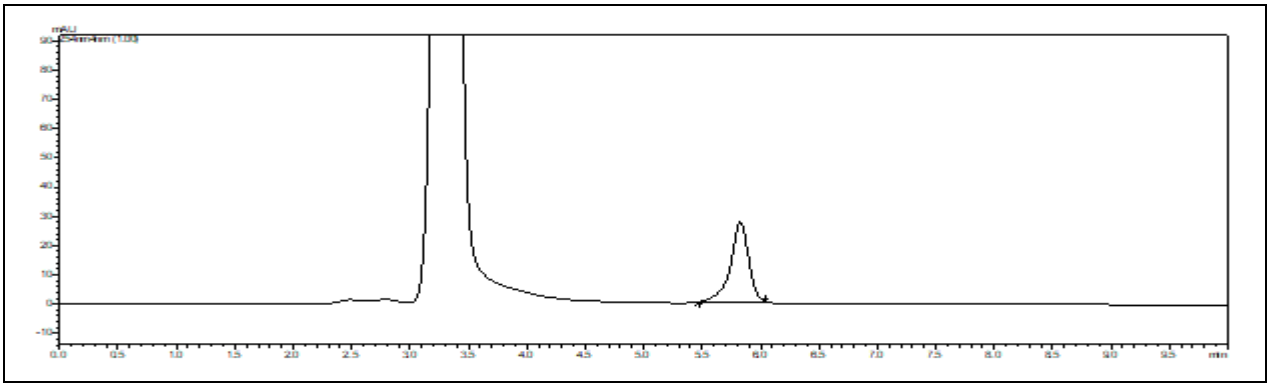


FIG. 9: CHROMATOGRAM FOR PEROXIDE STRESS

Heat Stress: Take 2 ml sample and heat for 1 h at 8 ºC and inject the sample.

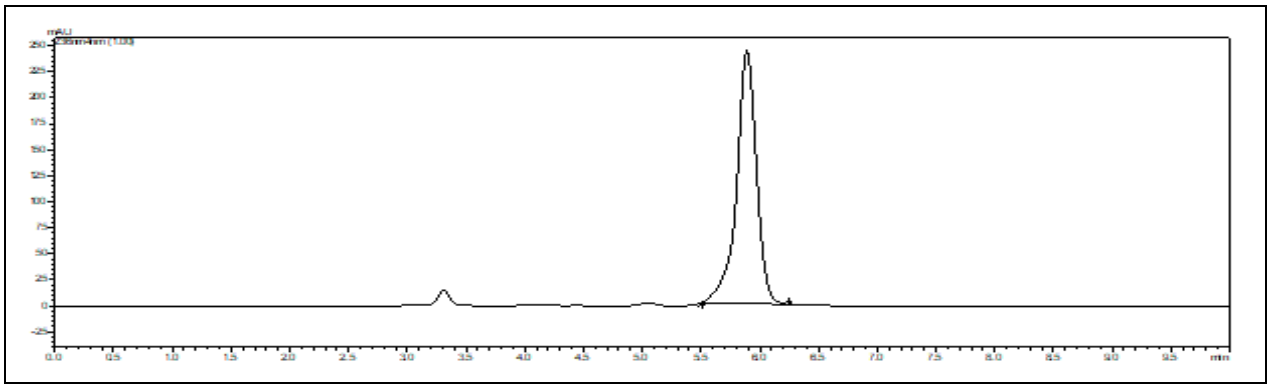


FIG. 10: CHROMATOGRAM FOR HEAT STRESS

Photolytic Stress: Take 2 ml sample and place in a UV chamber for 1 h UV- Lamp 45 ºC respectively and then inject the sample.

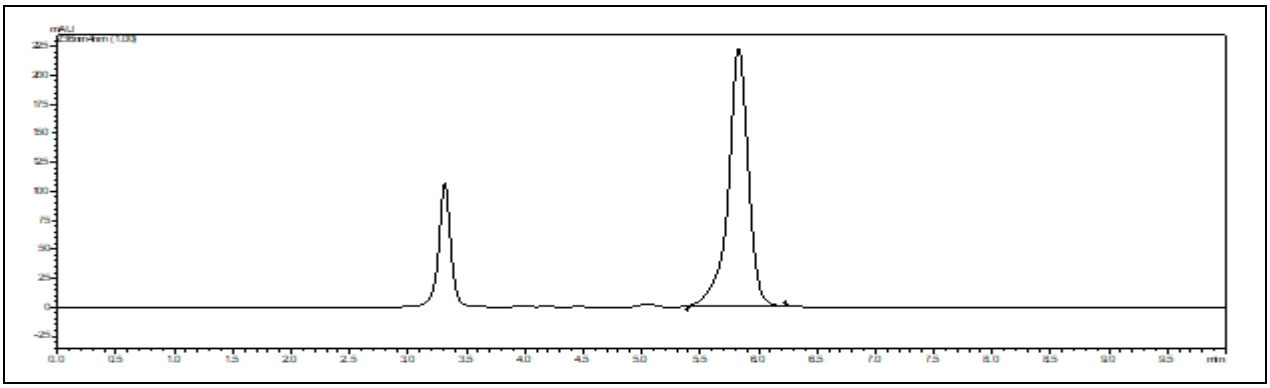


FIG. 11: CHROMATOGRAM FOR PHOTOLYTIC STRESS

CONCLUSION: A novel, simple, rapid and cost-effective RP-UFLC method was successfully developed for method development, validation and stability-indicating assay on glimepiride in tablet dosage form by RP-UFLC form the proposed method was optimized and validated for the various experimental parameters. Influence of pH of the mobile phase, mobile phase ratio, and flow rate on the analysis of glimepiride was evaluated. All the analytes were well resolved and separated in less than 10 min. The developed method can be conveniently used by quality control outfits to determine the contents of glimepiride in routine and stability samples. This method could be used for the analysis of the drugs in pharmaceutical preparations and routine laboratory analysis. Overall, the proposed method provides high throughput for determination of glimepiride with

excellent accuracy, precision, selectivity, and reproducibility.

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CONFLICT OF INTEREST: None

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