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To Develop RP-HPLC Method to Estimate Glimperide in Bulk Dosage Forms

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A B S T R A C T

The present work is aimed to develop a new, simple, fast, rapid, accurate, efficient and reproducible RP-HPLC method for the simultaneous analysis of Glimperide. Literature review reveals that there is no analytical method reported for the analysis of Glimperide by simultaneous estimation by RP-HPLC. Spectrophotometer, HPLC and HPTLC are the reported analytical methods for compounds either individually or in combination with other dosage form. The solutions were chromatographed at a constant flow rate of 1ml/min and Injection volume 20µl, the run time 10min, the linearity range was found to lie from 20µg/ml to 100µg/ml of Glimepride. The correlation coefficient obtained was 0.999 which is in the acceptance limit. The % RSD values of Glimepride are found to be 0.11 and 0.42 indicating less than 2% precision of the method and Intermediate precision for Glimepride found to be 1.09 and 0.45. The percentage recovery varies from 98-102% of Glimperide found to be 99.06% and 99.96. LOD and LOQ were found to be within limit. The results obtained on the validation parameters met ICH and USP requirements. It inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

Keywords: RP-HPLC, HPTLC, Analysis, and Glimperide.

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INTRODUCTION:

Chromatography is a technique which separates components in a mixture due to the differing time taken for each component to travel through a stationary phase when carried through it by a mobile phase. There are many developments which have occurred over years based on the requirements and also technology employed in evaluation of mixtures. High performance liquid chromatography is a very sensitive analytical technique most widely used for quantitative and qualitative analysis of pharmaceuticals. The principle advantage of HPLC compared to classical column chromatography is improved resolution of the separated substance, faster separation times and the increased accuracy, precision and sensitivity [1].

Experimental Methodology

Hplc Method Development:

Mobile Phase Optimization:

Initially the mobile phase tried was methanol: Ortho phosphoric acid buffer and Methanol: phosphate buffer, Acetonitrile: methanol with various combinations of pH as well as varying proportions. Finally, the mobile phase was optimized to Phosphate buffer (pH 3.0), Acetonitrile in proportion 80: 20 v/v respectively [2].

Optimization of Column:

The method was performed with various columns like C18 column Phenomenex column, YMC, and Inertsil ODS column. Inertsil ODS (4.6 x 250mm, 5µm) was found to be

ideal as it gave good peak shape and resolution at 1.0 ml/min flow.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS:

Instrument used and UV detector.	:	Waters HPLC with auto sampler
Temperature	:	Ambient
Column	:	Inertsil ODS(4.6 x 250mm, 5µm)
Buffer	:	3.4g of KH_2PO_4 in 1000 ml of HPLC water Ph was adjusted with OPA up to 3.0.
pH	:	3.0
Mobile phase	:	80% buffer 20% Acetonitrile
Flow rate	:	1 ml per min
Wavelength	:	225 nm
Injection volume	:	20µl
Run time	:	10min.

Preparation of Buffer And Mobile Phase[3]:

Preparation of Phosphate buffer:

3.4g of KH_2PO_4 in 1000 ml of HPLC water Ph was adjusted with OPA up to 3.0.final solution was filtered through 0.44 μm Membrane filter and sonicate it for 10 mins.

Preparation of mobile phase:

Accurately measured 800 ml (80%) of above buffer and 1000 ml of Acetonitrile HPLC (100%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

The Mobile phase was used as the diluent.

Preparation of The Glimepride Af Standard & Sample Solution[4-5]:

Standard Solution Preparation:

Accurately weigh and transfer 20 mg of Glimepride working standard into a 10ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.3ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Sample Solution Preparation:

Table 1: Sample and Standard Details

S. No	Samples
1	Glimepride Tablets 200 mg &12.5 mg
2	Glimepride

Accurately weigh 10 tablets crush in mortor and pestle and transfer equivalent to 20 mg of Glimepride sample into a 10mL clean dry volumetric flask add about 7 mL of Diluent and sonicate it up to 15 mins to dissolve it completely and make volume up to the mark with the same solvent. Then it is Filtered through 0.45 micron Injection filter. (Stock solution)

Further pipette 0.3ml of Glimepride from the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:

Inject 20 µl of the standard, sample into the chromatographic system and measure the areas for Glimepride AF peaks and calculate the %Assay by using the formulae.

SYSTEM SUITABILITY:

Tailing factor for the peaks due to Glimepride in Standard solution should not be more than 2.0

Theoretical plates for the Glimepride peaks in Standard solution should not be less than 2000.

Resolution for the Glimepride peaks in standard solution should not be less than 2.

Calculation: (Glimepride)

$$\%\ Assay = \frac{AT}{AS} * \frac{WS}{DS} * \frac{DT}{WT} * \frac{Average\ weight}{Label\ Claim} * \frac{P}{100} * 100$$

Where:

AT = average area counts of sample preparation.

AS = average area counts of standard preparation.

WS = Weight of working standard taken in mg.

P = Percentage purity of working standard

LC= Label Claim mg/ml.

RESULTS:

System Suitability Results:

1. Tailing factor Obtained from the standard injection is 1.13

2. Theoretical Plates Obtained from the standard injection is 4959.43

3. Resolution Obtained from the standard injection is 5.66

Assay Results: (For GlimeprideAF)

$$\frac{115671}{114706} * \frac{12.5}{10} * \frac{0.6}{10} * \frac{10}{199} * \frac{10}{0.6} * \frac{398}{25} * \frac{99.8}{100} * 100 = 100.64\%$$

METHOD VALIDATION SUMMARY:

PRECISION:

Preparation of stock solution:

Accurately weigh and transfer 20 mg Glimepride working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.3 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:

The standard solution was injected for six times and measured the area for all six. Injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Acceptance Criteria: The % RSD for the area of six standard injections results should not be more than 2% [6].

INTERMEDIATE PRECISION/RUGGEDNESS [7]:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day.

Preparation of stock solution:

Accurately weigh and transfer 20 mg of Glimepride working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.3 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:

The standard solutions prepared in the precision was injected on the other day, for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

The results are summarized for Glimepride

Acceptance Criteria: The % RSD for the area of six standard injections results should not be more than 2%.

SPECIFICITY:

For Specificity Blank and Standard are injected into system there is no any interference of any peak in blank with the retiontime of the analytical peaks.

ACCURACY:

Preparation of Standard stock solution:

Accurately weigh and transfer 20 mg of Glimepride working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.3ml and 0.3 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of Sample solutions:

For preparation of 50% solution (With respect to target Assay concentration):

Accurately weigh and transfer 5mg Glimepride working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.3 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

For preparation of 100% solution (With respect to target Assay concentration):

Accurately weigh and transfer 10 mg of Glimepride working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.3 ml of the above stock solutions into a 10ml volumetric flasks and dilute up to the mark with diluent.

Procedure:

Inject the standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150%solutions.

Calculate the Amount found and Amount added for Glimepride AF and calculate the individual recovery and mean recovery values [8].

Acceptance Criteria: The % Recovery for each level should be between 98.0 to 102.0%

LINEARITY [9]:

Preparation of stock solution:

Accurately weigh and transfer 20 mg of Glimepride AF working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I:

0.1ml of above stock solutions has taken in 10ml of volumetric flask, dilute up to the mark with diluent.

Preparation of Level – II:

0.2ml of above stock solutions has taken in 10ml of volumetric flask, dilute up to the mark with diluent.

Procedure:

Inject each level into the chromatographic system and measure the peak area.

Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

DETECTION LIMIT

LIMIT OF DETECTION:

Preparation of 600µg/ml solution:

Accurately weigh and transfer 20 mg of Glimepride working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.6ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents[10].

Acceptance Criteria:

S/N Ratio value shall be 3 for LOD solution.

LIMIT OF QUANTIFICATION [11]:

Preparation of 600 µg/ml solution:

Accurately weigh and transfer 10 mg of Glimepride working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.3 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank : 65 µV

Signal Obtained from LOQ solution : 192µV

S/N = 659/66 = 9.98

Acceptance Criteria:

S/N Ratio value shall be 10 for LOQ solution.

Procedure for LOD and LOQ:

The LOD and LOQ solutions was prepared injected, for three times and measured the area for all three injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

ROBUSTNESS:

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

A. The flow rate was varied at 0.9 ml/min to 1.1ml/min. Standard solution wasprepared and analysed using the varied flow rates along with method flow rate.

On evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±10%.

System suitability results for:

***** Results for actual flow (1.0ml/min) have been considered from Assay standard.

B. The Organic composition in the Mobile phase was varied from ±10%.

Standard solution was prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition in the method.

On evaluation of the above results, it can be concluded that the variation in 10%.

Organic composition in the mobile phase affected the method significantly. Hence it

Indicates that the method is robust even by change in the Mobile phase ±10

System suitability results:

***** Results for actual Mobile phase composition (80:20)Buffer pH 3: Acetonitrile has been considered from Accuracy standard.

DEGRADATION STUDIES [12]:

The International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. The aim of this work was to perform the stress degradation studies on the Glimepride AF using the proposed method.

Preparation of stock:

Accurately weigh 10 tablets crush in mortor and pestle and transfer equivalent to 20 mg of Glimepride in sample into a 10mL clean dry volumetric flask add about 7 mL of Diluent and sonicate it up to 5 mins to dissolve it completely and make volume up to the mark with the same solvent. Then it is Filtered through 0.44 micron Injection filter. (Stock solution).

Hydrolytic degradation under acidic condition

Pipette 0.6ml of above solution into a 10ml volumetric flask and 3 ml of 0.1N HCl was added. Then, the volumetric flask was kept at 60ºC for 24 hours and then neutralized with 0.1 N NaOH and make up to 10ml with diluent. Filter the solution with 0.44 microns syringe filters and place in vials.

Hydrolytic degradation under alkaline condition

Pipette 0.6ml of above solution into a 10ml volumetric and add 3ml of 0.1N NaOH was added in 10ml of volumetric flask. Then, the volumetric flask was kept at 60ºC for 24 hours and then neutralized with 0.1N HCl and make up to 10ml with diluent. Filter the solution with 0.44 microns syringe filters and place in vials.

Thermal induced degradation

Glimepride sample was taken in petri-dish and kept in Hot air oven at 110^0 C for 3 hours. Then the sample was taken and diluted with diluents and injected into HPLC and analysed.

Oxidative degradation

Pipette 0.6ml above stock solution into a 10ml volumetric flask and 1ml of 12.5% w/v of hydrogen peroxide added in 10 ml of volumetric flask and the volume was made up to the mark with diluent. The volumetric flask was then kept at room temperature for 15 min. Filter the solution with 0.45 microns syringe filters and place in vials.

Photo degradation:

Pipette 0.6 ml above stock solution into a 10ml volumetric flask and expose to sunlight for 24hrs and the volume was made up to the mark with diluent. Filter the solution with 0.45 microns syringe filters and place in vials.

RESULTS AND DISCUSSION

Table 2: Chromatographic Conditions

Chromatographic Conditions	**Trial -1**	**Trial -2**	**Trial -3**	**Trial -4**
Column	Spursil C18 4.6x150mm, 3µm	Spursil C18 4.6x150mm 3µm	Spursil C18 4.5×150mm 3 µm	Spursil C18 4.6×150mm 3µm
Mobile phase ratio	MeOH: H_2O (50:50%v/v)	ACN: 0.1%OPA (70:30%v/v)	Methanol:0.1% OPA (80:20 % v/v)	Methanol:KH_2PO_4 (70:30% v/v)
Detection wavelength	290 nm	290 nm	290 nm	290 nm
Flow rate	1ml/min	1ml/min	1.0ml/min	1.0ml/min
Injection volume	20µl	20µl	20µl	20µl
Run time	10min	10 min	10.0 mins	10 min

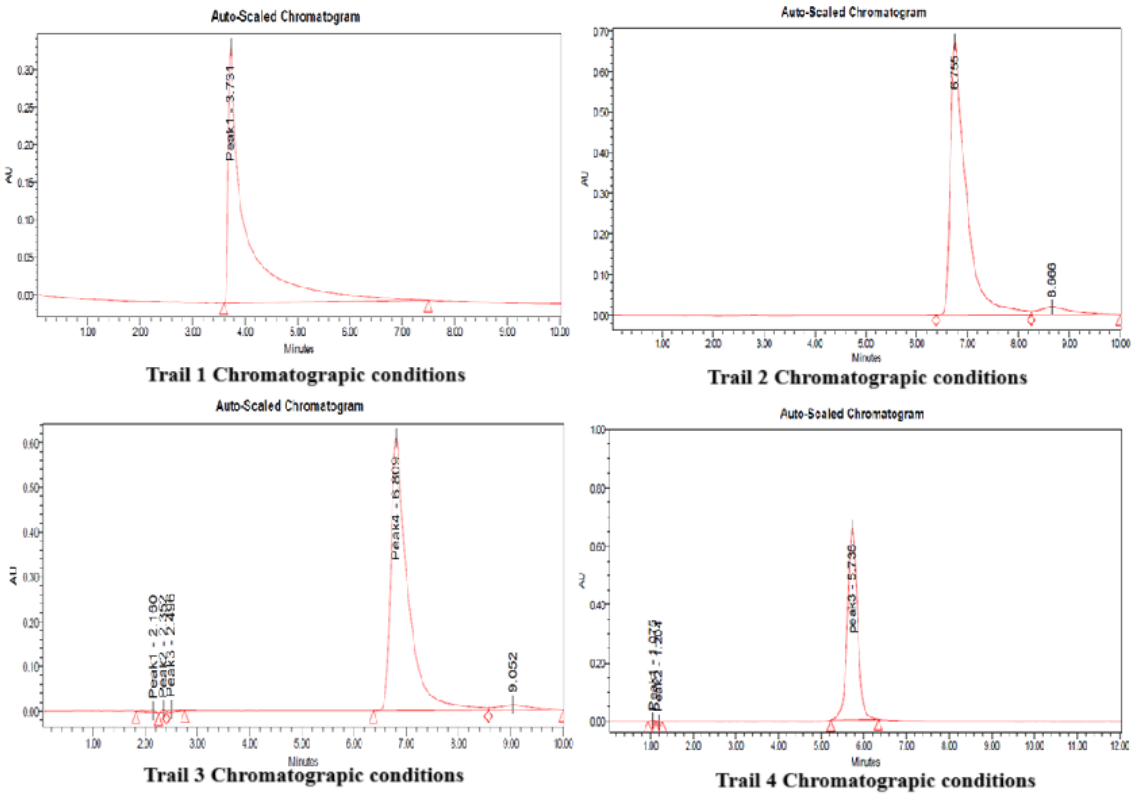

Figure 1: Trail 1 to 4 Chromatograpic conditions

OPTIMIZED CHROMATOGRAPHIC CONDITIONS:

Instrument used : High performance liquid chromatography equipped with Auto Sampler and DAD or UV detector

Temperature : Ambient

Column : Spursil C_{18}-EP,(150×4.6mm, 3µm)

Buffer : Pottasium dihydrogen phosphate buffer(pH-3.0)

Mobile phase : 80% : 20%(KH_2PO_4; ACN)

Flow rate : 1 ml per min

Wavelength : 225 nm

Injection volume : 20 µl

Run time : 10min.

Retention time : 3.584, 4.485

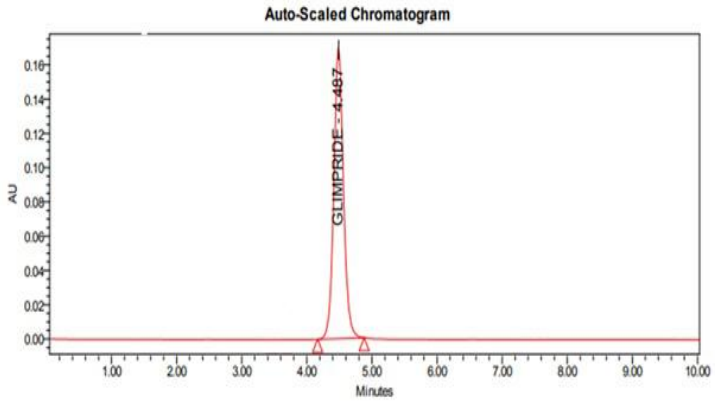

Figure 2: Optimized Chromatographic conditions

Table 3: Chromatographic Conditions for Glimepride

S. No	Peak name	Retention time	Area	Height	USP Tailing	USP plate count
1.	Glimepride	3.584, 4.487	36420, 3834932	43526, 564705	0.98, 0.81	2828, 2835

VALIDATION PARAMETERS

Specificity:

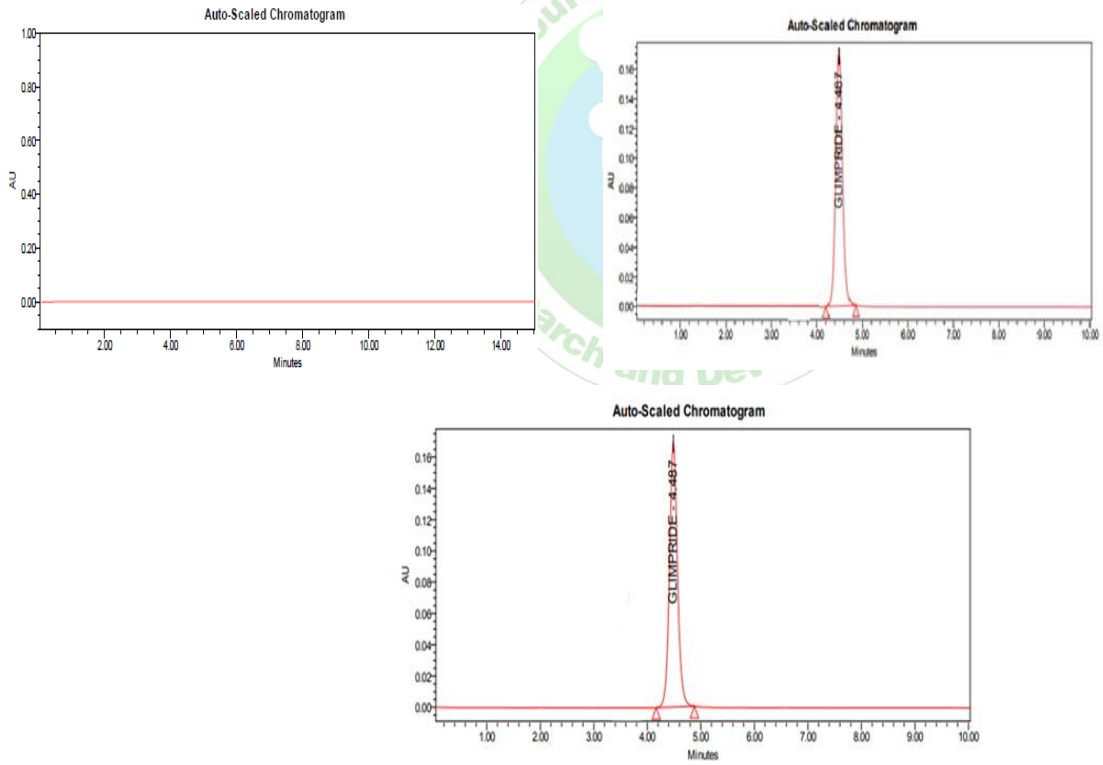

Figure 3: Chromatogram for Blank, Standard and Sample

Table 4: Results of Specificity for Glimepride

S. No	Name	RT (min)	Area (µV sec)	Height (µV)	USP tailing	USP plate count
1.	**Glimepride**(Standard solution)	3.584, 4.487	36425**,** 3834947	564705	0.81	2828
2.	**Glimepride** (Sample solution)	3.584, 4.487	36420, 3834932	564700	1.20	2954

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 more than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

Assay results:

Table 5: Results of Assay for Glimepride

	Label Claim (mg)	**% Assay**
Glimepride	150mg	99.99

Linearity:

The linearity range was found to lie from 20µg/ml to 100µg/ml of Glimepride and chromatograms are shown below.

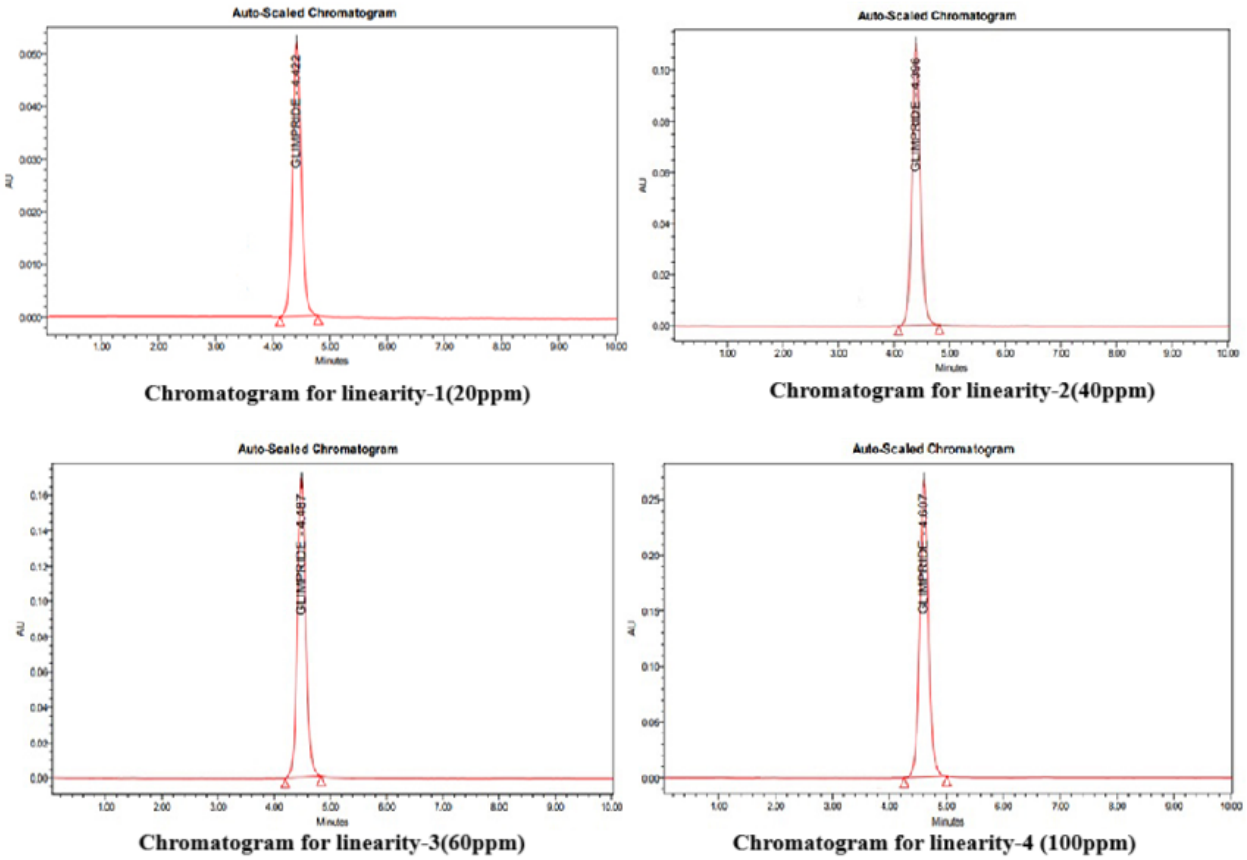

Figure 4: Chromatograms for linearity

Table 6: Area of different concentration of Glimepride

S. No	**Glimepride**		
	Concentration (µg/ml)	**Area**	**Areas**
1	20	12140	1278310
2	40	24280	2456621
3	60	36420	3834932
4	80	47560	5113242
5	100	60700	6391553

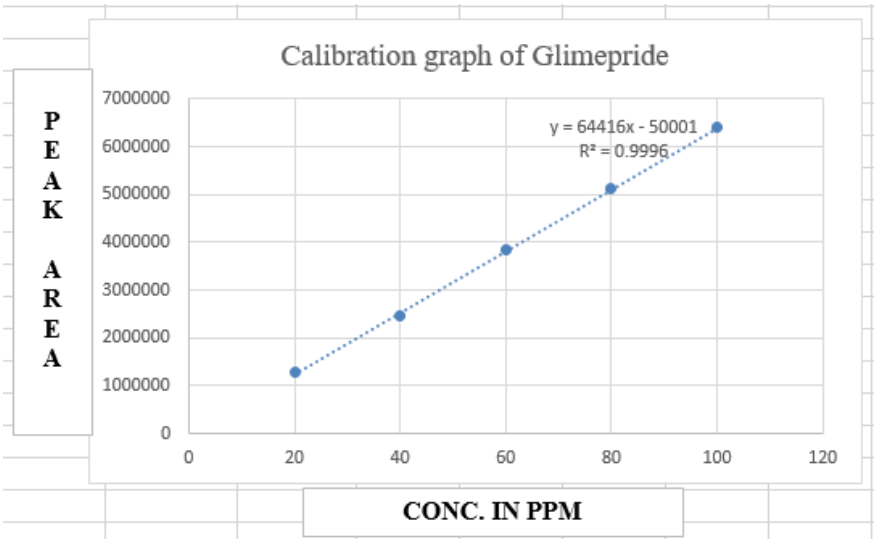

Figure 5: Calibration graph for Glimepride

Table 7: Calibration graph for Glimepride

Parameters	Glimepride	
Slope (m)	602	64416
Intercept (c)	100	50001
Correlation coefficient (R^2)	0.999	0.999

Acceptance criteria:

Correlation coefficient (R^2) should not be less than 0.999

The correlation coefficient obtained was 0.999 which is in the acceptance limit.

Precision:

Precision of the method was carried out for sample solutions as described under experimental work. The corresponding chromatograms and results are shown below.

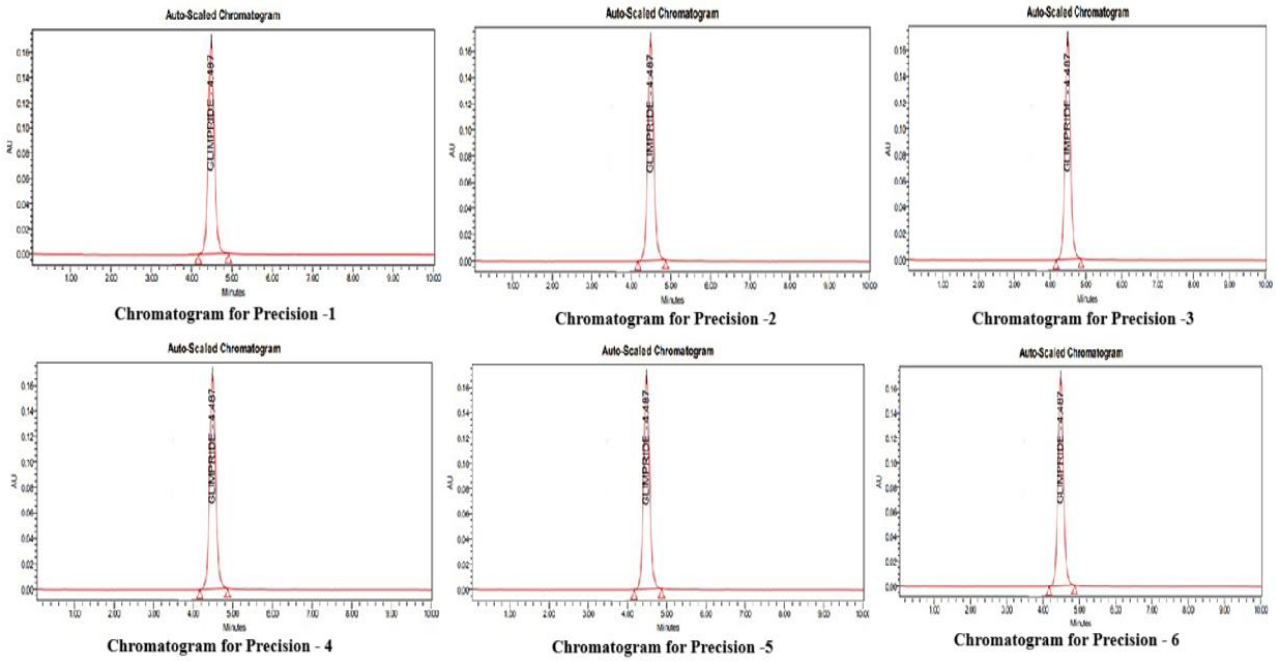

Figure 6: Chromatogram for Precision – 1 to 6

Table 8: Results of Precision for Glimepride

Injection	Area	Areas
Injection-1	36420	3834932
Injection-2	36418	3834932
Injection-3	36520	3833932
Injection-4	36420	3834932
Injection-5	36420	3794932
Injection-6	36420	3834932
Average	36436.33	3828098.6
Standard Deviation	40.9	16253.2
%RSD	0.11	0.42

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 (ruggedness):

There was no significant change in assay content and system suitability parameters at different conditions of ruggedness like day to day and system to system variation.

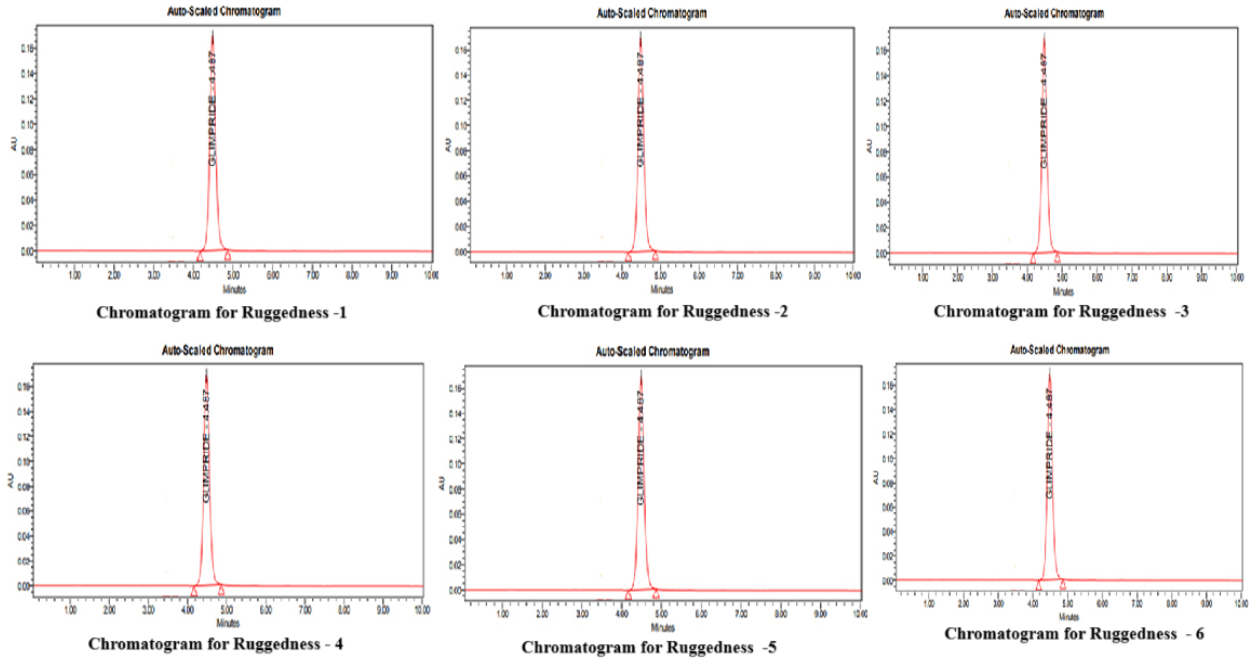

Figure 7: Chromatogram for Ruggedness - 1 to 6

Table 9: Results of Intermediate precision Glimepride

Injection	Area	Area
Injection-1	36420	3834932
Injection-2	36418	3834932
Injection-3	36520	3843932
Injection-4	36420	3834932
Injection-5	36419	3794932
Injection-6	37420	3834932
Average	36602.8	3829765.3
Standard Deviation	402.3	17440.3
% RSD	1.09	0.45

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:

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

Table 10: Accuracy (recovery) data for Glimepride

%Concentration (at specification Level)	Areas*	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	1917466	5	4.90	98.00	99.06%
100%	3834932	10	9.85	99.85	
150%	5752398	15	14.95	99.33	

*Average of three determinations

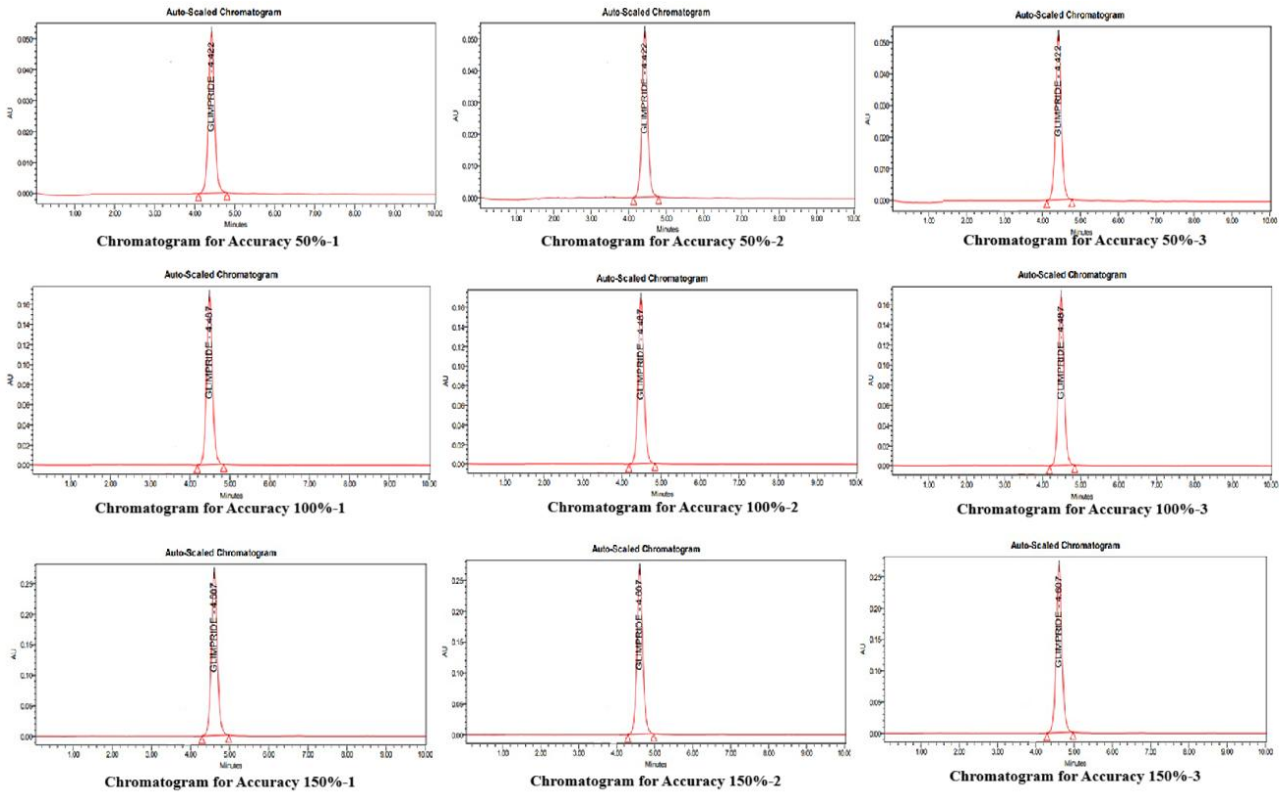

Figure 8: Chromatograms for Accuracy 50%, 100% and 150%-3

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 for Glimepride

The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio.

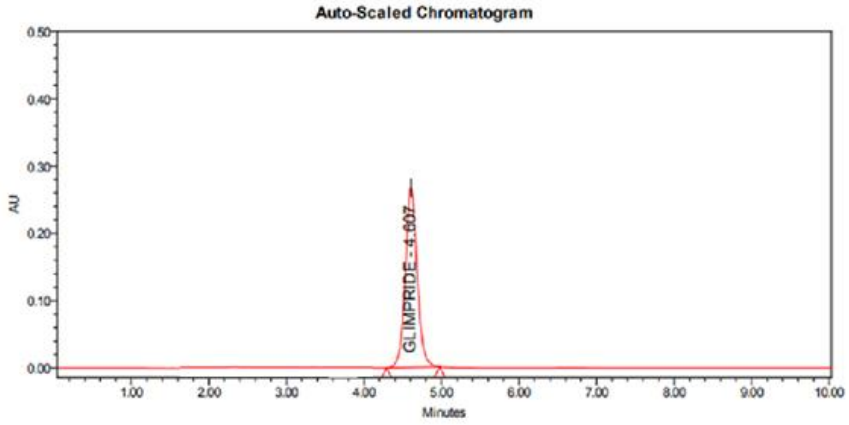

Figure 9: Chromatogram of Glimepride showing LOD

Table 11: Results of LOD

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Glimepride	65	193	2.96

- Signal to noise ratio shall be 3 for LOD solution
- The result obtained is within the limit.

Limit of Quantification for Glimepride

The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio.

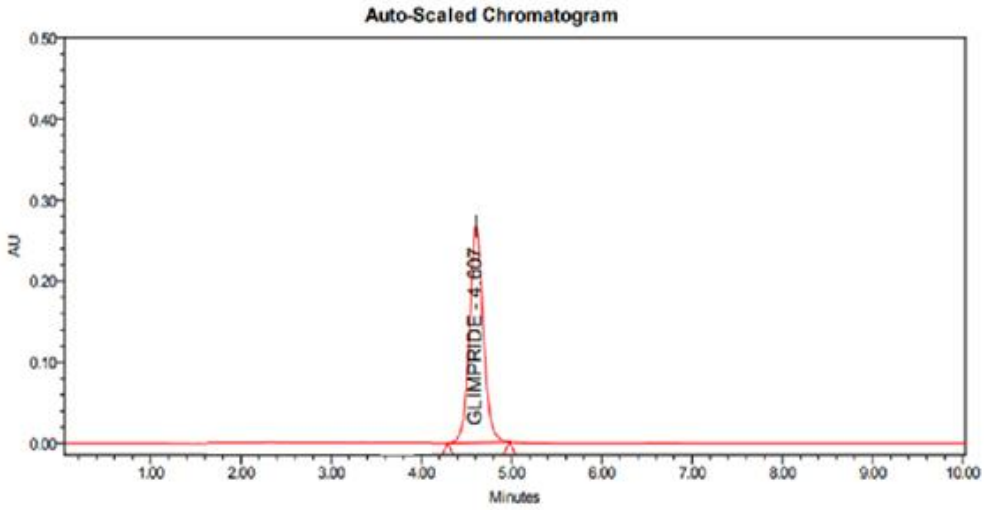

Figure 10: Chromatogram of Glimepride showing LOQ

Table 12: Results of LOQ

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Glimepride	65	645	9.92

- Signal to noise ratio shall be 10 for LOQ solution
- The result obtained is within the limit.

Robustness:

The standard and samples of 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.

a).Variation in flow

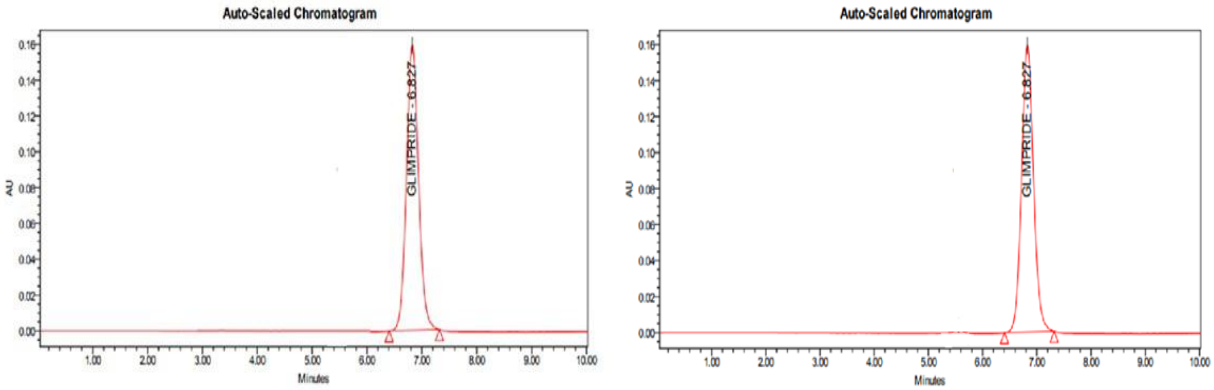

Figure 11: Chromatogram showing less and more flow

b).Variation of organic mobile phase composition in mobile phases:

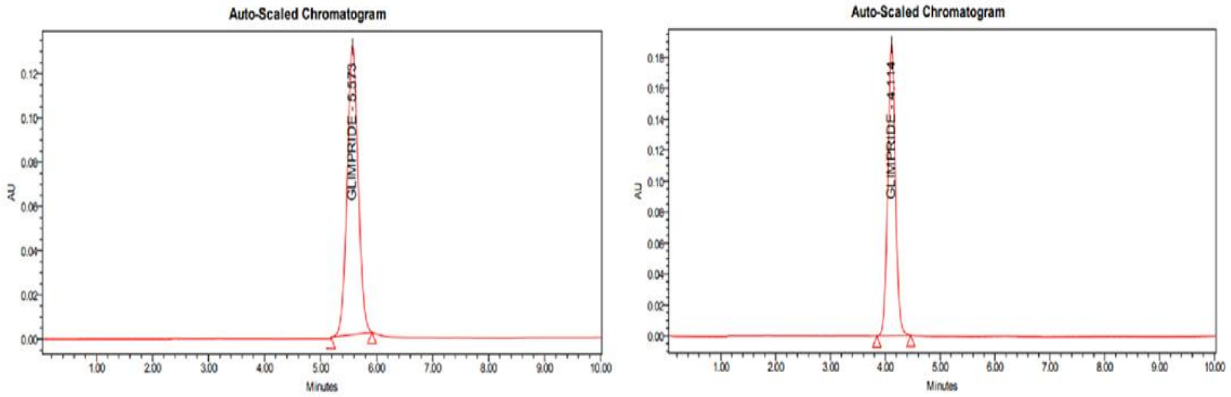

Figure 12: Chromatogram showing less and more organic composition mobile phase

Table 13: Results for variation in flow for Glimepride

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	2428.71	1.11
2	1.0	2828.66	1.19
3	1.2	2808.78	1.32

***** Results for actual flow (1ml/min) have been considered from Assay standard.

Table 14: Results for variation in mobile phase composition for Glimepride

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP
1	10% less	2428.71	1.11
2	*Actual	2828.76	1.75
3	10% more	2808.66	1.19

***** Results for actual Mobile phase composition have been considered from Accuracy standard.

Acceptance criteria:

It was discovered that the USP tailing factor, retention time, USP plate count, and change in mobile phase were all within the acceptable range. Thus, the approach is reliable.

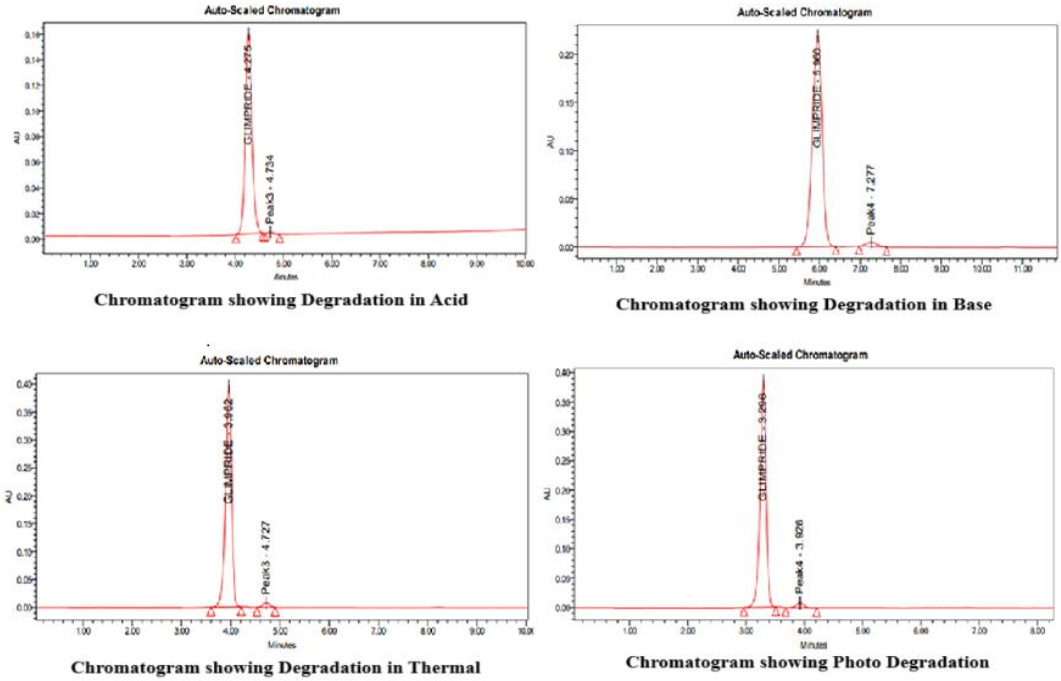

Figure 13: Chromatogram showing Degradations

Table 15: Degradation results for Glimepride

Parameters	Glimepride		
	Area	**Area**	**% Degraded**
Standard	36420	3834932	4.21
Acid	32580	3575770	4.76
Base	22180	3022580	6.14
Peroxide	32475	3422580	4.24
Thermal	36416	3832580	3.39
Photo	31541	3461541	5.63

CONCLUSION:

The results obtained on the validation parameters met ICH and USP requirements. It inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that they have no competing interests.

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