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Research Article

DEVELOPMENT AND VALIDATION OF REPAGLINIDE BY REVERSED PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY(RP-HPLC) IN TABLET DOSAGE FORMS

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Abstract

Repaglinide is an oral antihyperglycemic agent used for the treatment of non-insulin-dependent diabetes mellitus (NIDDM). A new RP-HPLC method was developed for the estimation of Repaglinide which is simple and less time consuming using an economical column. The analysis was resolved by using a mobile phase (Potassium di hydrogen phosphate: Methanol (80:20)) at a flow rate of 1 ml per minute using ACE- C18 (250 x 4.0, 5 microns) on an isocratic HPLC system. There was no interference observed from other impurities and Repaglinide has been eluted with good peak shape, response within 12 minutes and retention time at 3.016 minutes. The detection was made at 285 nm.The assay of the sample has been carried out using this new method and it was found to be 99.7%. The developed method has been validated for different parameters like Specificity, Precision, Linearity, Accuracy, Robustness, Limit of Detection (LOD) and Limit of Quantitation(LOQ) are studied as reported in the International Conference on Harmonization (ICH) guidelines. All the results obtained were within the acceptance limits indicating that the developed method is simple, specific accurate and economical. The method may now be recommended for routine and quality control analysis of Repaglinide.

Key Words: *Repaglinide, Reversed phase high performance liquid chromatography, limit of detection and limit of quantitation.*

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INTRODUCTION

Repaglinide is an oral antihyperglycemic agent used for the treatment of non-insulin-dependent diabetes mellitus (NIDDM). It belongs to the meglitinide class of short-acting insulin secretagogues, which act by binding to β cells of the pancreas to stimulate insulin release[1]. Repaglinide induces an early insulin response to meals decreasing postprandial blood glucose levels. It should only be taken with meals and meal-time doses should be skipped with any skipped meal. Approximately one month of therapy is required before a decrease in fasting blood glucose is seen. Meglitnides may have a neutral effect on weight or cause a slight increase in weight. The average weight gain caused by meglitinides appears to be lower than that caused by sulfonylureas and insulin and appears to occur only in those oral antidiabetic agents[2].

Chemicals and Reagents used for the Study

1. Repaglinide working standard (WS)
2. Repaglinide Sample
3. Methanol HPLC gradient water
4. High performance liquid chromatography **(Waters e2695)**
5. Electronic Balance **(sartorius)**
6. Ultra Sonicator **(Fast-Clean)**
7. Thermal Oven **(newtech lab equipments)**
8. Column (ACE- C_{18}: 250 x 4.0 *5 µ)
9. Potassium hydrogen phosphate

Instrumentation used for the Study

High Performance liquid chromatography Agilent Technologies 1200 series with auto sampler and Waters e2695 High Performance liquid chromatography with auto sampler, Liquid chromatogram equipped with maximum pressure 400 bar, Auto sampler, UV-Detector (Standard cell) and data handling system (chemstation and LC solutions software).

Apparatus

Analytical balance, volumetric flasks, Pipettes, P^H meter, Ultra Sonicator, Filtration unit and 0.45 µ membrane filters.

Preparation of Standard solution

Accurately weigh and transfer about 560.75 mg of Repaglinide Working standard in 100 mL volumetric flask. Add about 20 mL of mobile phase and sonicate to dissolve it completely and make up volume up to the mark with mobile phase. Dilute 10 mL of this solution to 10 mL in volumetric flask with the mobile phase (0.1 mg/mL).

Preparation of Test solution

20 tablets (Repaglinide) were weighed accurately to obtain the average tablet weight; the tablets were then crushed and triturated in a mortar until a fine powder was obtained. An amount of the powder equivalent to 560.75 mg of Repaglinide was weighed accurately and transferred to be examined in 50 ml volumetric flask and make up the volume to the mark with diluents using HPLC grade water and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

RESULTS AND DISCUSSION

1. Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. (Fig No: 2). Results are reported in Table 1.

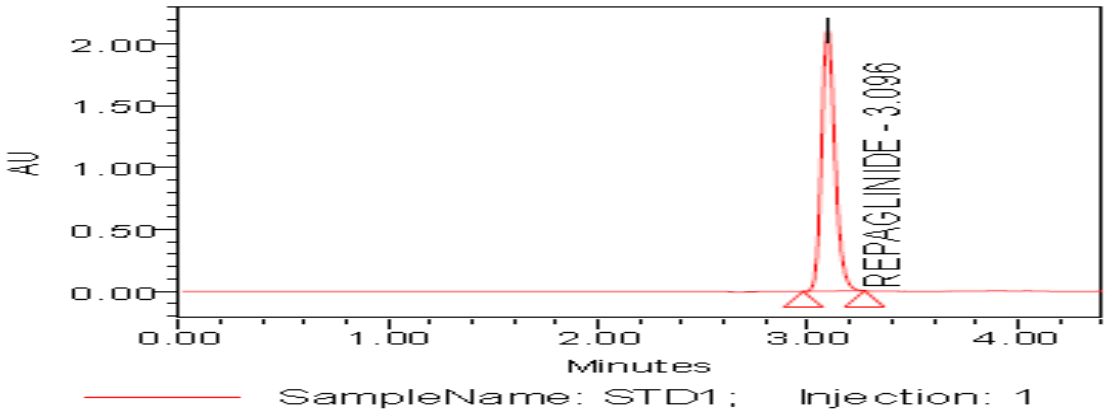
System suitability

System suitability of test method has been demonstrated by injecting system suitability solution and standard solution as per described procedure (Fig No: 1).

Acceptance criteria: % RSD of assay of six preparations is NMT 2.00

Table No.1: Specificity Evaluation Comparing Retention Time

Repaglinide	RT	Area
Inj-1	3.091	9518004
Inj-2	3.89	9519557
Inj-3	3.88	9518338
Inj-4	3.92	9513639
Inj-5	3.93	951886
Inj-6	3.93	9518448
AVG	-	9518448
STDEV	-	0.02
%RSD	-	0.02



Component Summary Table

Name: REPAGLINIDE

	SampleName	Inj	Name	RT	Area	USP Resolution	USP Tailing	USP Plate Count	s/n
1	STD1	1	REPAGLINIDE	3.096	9388370		1.186	11538	22.48
Mean					9388370				
% RSD					0.2				

Fig No.1: Specificity – System Suitability

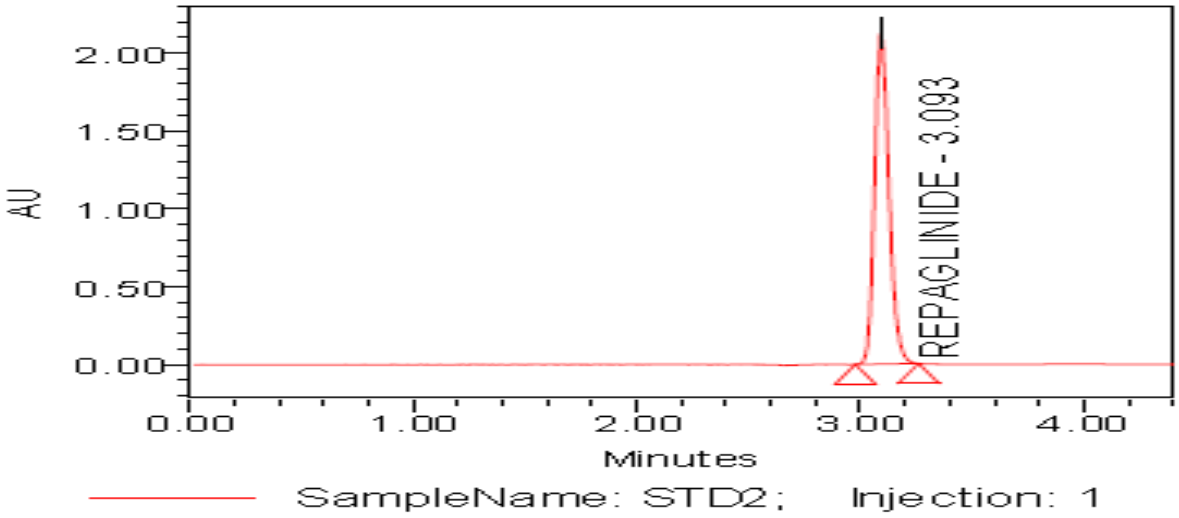


Fig No.2: Specificity 1

2. Presision

To experiment the repeatability of Standard solution obtained by this method. The precision has been demonstrated by preparing six standard solutions as per the test method. The precision of method has been evaluated by computing the %RSD of the Area count (Fig No: 3). Results are reported in Table 2.

Acceptance criteria: %RSD of assay of six preparations is NMT 2.00

Table No.2: Precision

System Precision	RT	Area	% Assay
Precision -1	3.091	9518004	99
Precision -2	3.089	9519557	99
Precision -3	3.088	9518338	99
Precision -4	3.092	9513639	99
Precision -5	3.093	9516836	99
Precision -6	3.092	9518866	99
AVG	-	9518448	99
STDEV	-	99.1	0.02
%RSD	-	0.02	0.02
Assay Range (98.0% to 102.0%)	-	-	99.1

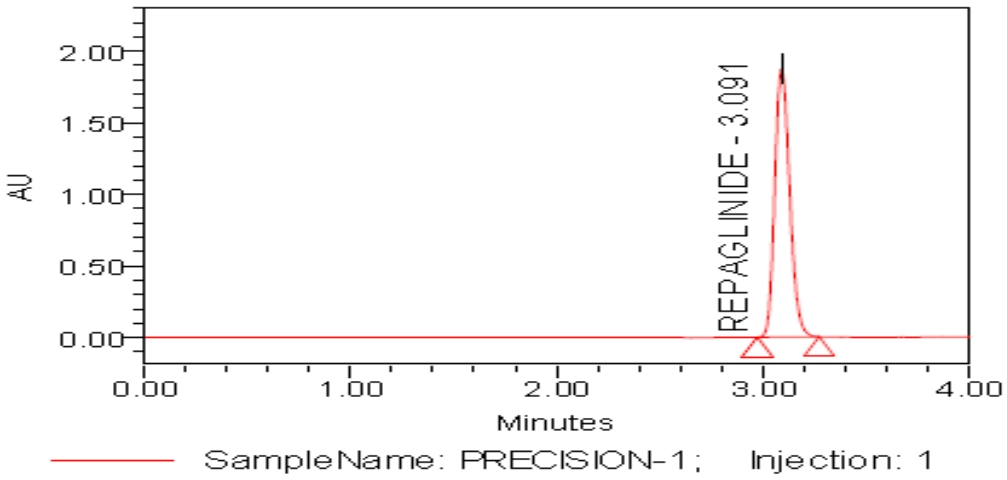


Fig No.3: Precision

3. Linearity

Demonstrates the linearity of analyte over the range of 50% to 150% of standard concentration.

Preparation of linearity stock solution

Accurately weigh and transfer about 560.75 mg of Repaglinide working standard in 250 mL volumetric flask, add about 250 mL of mobile phase and sonicate to dissolve it completely and make the volume up to the mark with mobile phase.

Inject these solutions into the HPLC system and record the area of analyte peak. Plot a graph of concentration (X-axis) *vs.* analyte peak (Y-axis). Evaluate the correlation co-efficient between concentration and peak area. (Fig No: 4 - 5). Results are reported in Table 3.

Acceptance criteria

The correlation coefficient is NLT 0.99

Table No: 3 Linearity

CONC%	Area	ug/ml	% Assay
50	4752281	2.5	99
75	7134135	3.75	99
100	9513233	5.00	99
125	11894473	6.25	99
150	14292279	7.5	99

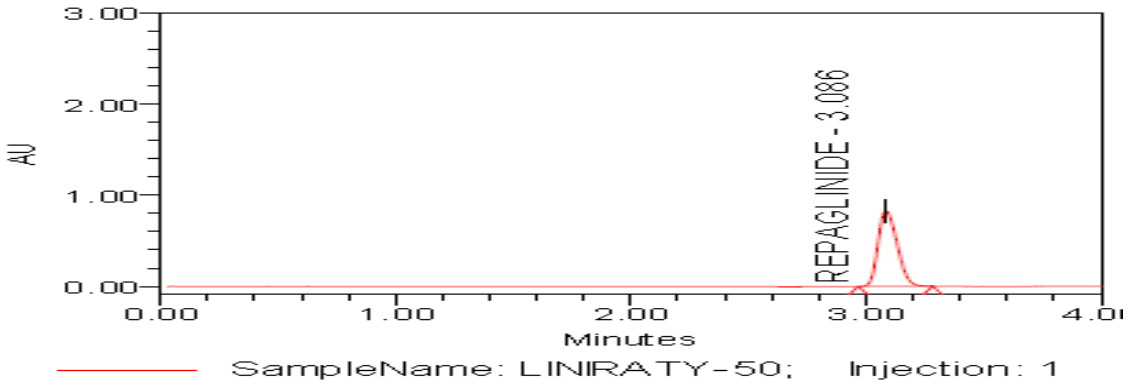


Fig No.4: Linearity 1

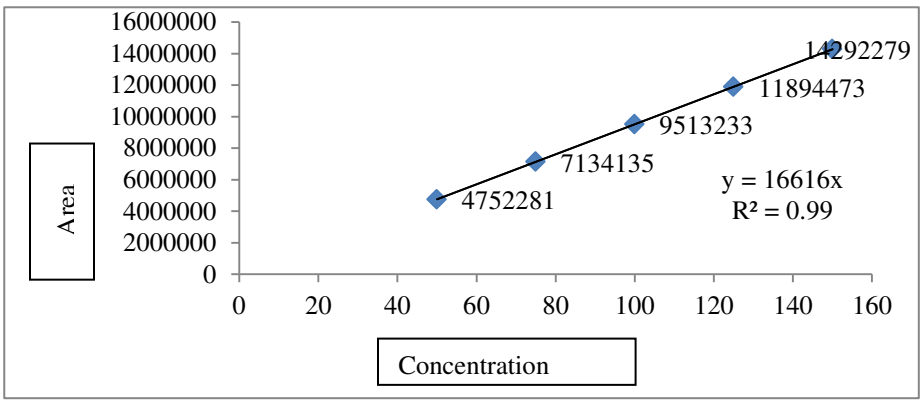


Fig No.5: Linearity graph

4. Accuracy

Accuracy of the test method has been demonstrated by preparing accuracy samples in 50 mL volumetric flask at the level of 50%, 100%, and 150% of samples concentration. The accuracy samples will be prepared in triplicate in each level.

Solution preparations:

Accuracy 50%

Accurately weigh and transfer about 560.75 mg of Repaglinide test sample in three separate 100 mL volumetric flask. Add about 20 mL of mobile phase and sonicate to dissolve completely and make up the volume up to the mark with mobile phase. Transfer 1 mL into a 10 mL volumetric flask using clean pipette and dilute to 10 mL with mobile phase (0.10 mg/mL) (Fig No: 6).

Accuracy 100%

Accurately weigh and transfer about 560.75 mg of Repaglinide test sample in three separate 100 mL volumetric flask. Add about 20 mL of mobile phase and sonicate to dissolve completely and make up the volume up to the mark with mobile phase. Transfer 1

mL into a 10mL volumetric flask using clean pipette and dilute to 10 mL with mobile phase (0.20 mg/mL) (Fig No: 7)

Accuracy 150%

Accurately weigh and transfer about 560.75 mg of Repaglinide test sample in three separate 100 mL volumetric flask. Add about 20 mL of mobile phase and sonicate to dissolve completely and make up the volume up to the mark with mobile phase. Transfer 1 mL into a 10mL volumetric flask using clean pipette and dilute to 10 mL with mobile phase (0.30 mg/mL) (Fig No: 8).

Results are reported in Table 4.

Acceptance criteria

The Recovery for all levels should be between 98%-102%.

Table No. 4 Accuracy

REPAGLINIDE						
Spiked Level	Sample Weight	Sample Area	µg/ml added	µg/ml found	% Recovery	% Mean
50%	280.40	4753601	2.470	2.47	100	100
50%	280.40	4752206	2.470	2.47	100	
50%	280.40	4755261	2.470	2.48	100	
50%	280.40	4759925	2.470	2.48	100	
50%	280.40	4750716	2.470	2.47	100	
50%	280.40	4756806	2.470	2.48	100	
100%	560.75	9510725	4.940	4.95	100	100
100%	560.75	9515979	4.940	4.95	100	
100%	560.75	9513054	4.940	4.95	100	
150%	841.15	14299240	7.410	7.44	100	100
150%	841.15	14295684	7.410	7.44	100	
150%	841.15	14291621	7.410	7.44	100	
150%	841.15	14236213	7.410	7.41	100	
150%	841.15	14286047	7.410	7.44	100	
150%	841.15	14278837	7.410	7.43	100	

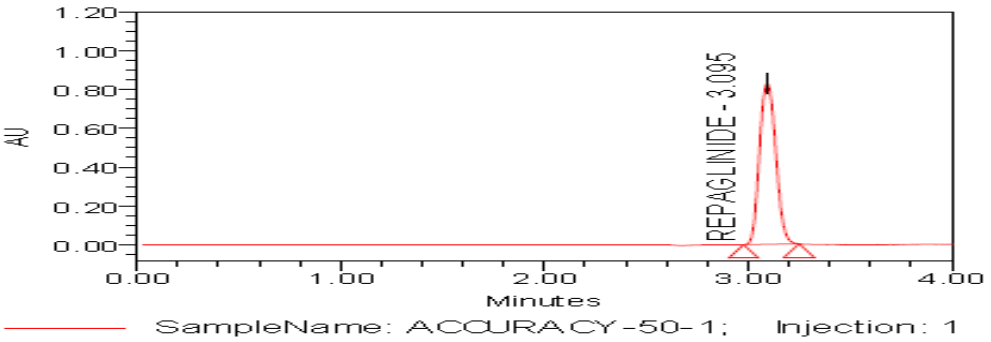


Fig No.6: Accuracy 50% 1

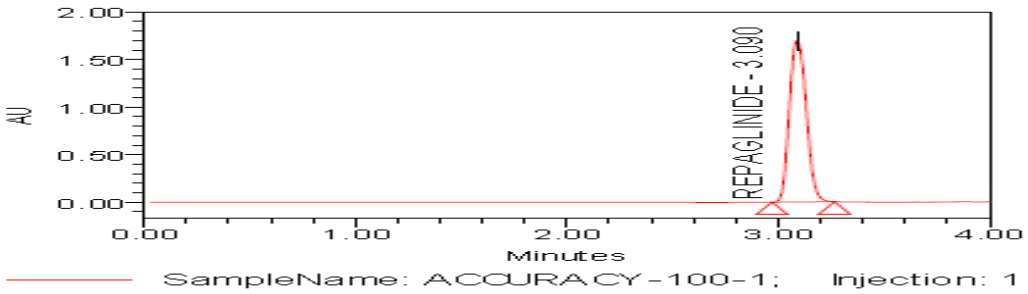


Fig No.7: Accuracy 100% 1

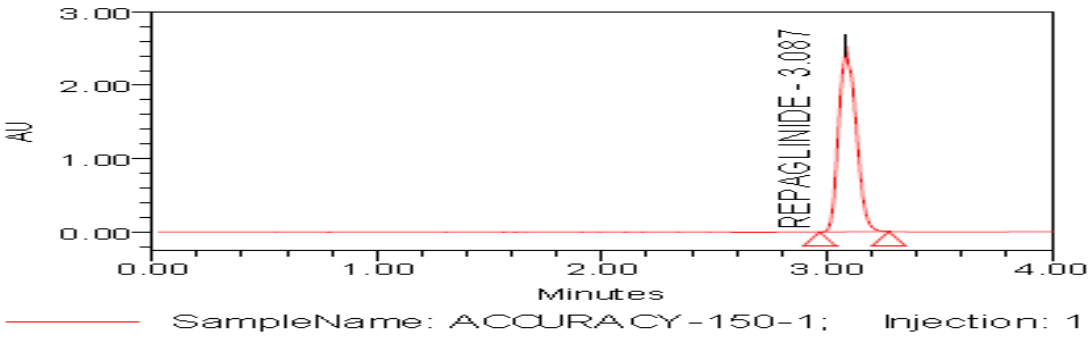


Fig No.8: Accuracy 150% - 1

5. LOD and LOQ

The Limit of Detection (LOD) and Limit of Quantitation (LOQ) of REPAGLINIDE by the proposed methods were determined using calibration standards. LOD and LOQ were calculated as 3.3 s/S and 10 s/S, respectively, where S is the slope of the calibration curve and s is the standard deviation of y-intercept of regression equation. The results of the same are shown in Table5, 6. The low values indicated the good sensitivity of the method proposed (Fig No: 9 - 10).

Table No. 5: LOD and LOQ

Analyte	LOD	LOD	LOQ	LOQ
	Concentration (mg/ml)	S/N ratio	Concentration (mg/ml)	S/N ratio
Repaglinide	2.5	0.667	6.25	2.224

Table No. 6: LOD and LOQ

Component Summary Table
Name: REPAGLINIDE

	SampleName	Inj	Name	RT	Area
1	LOD	1	REPAGLINIDE	3.086	163280
2	LOQ	1	REPAGLINIDE	3.089	424376
Mean	22.48				
Std. Dev.	0.6				
% RSD	0.99				

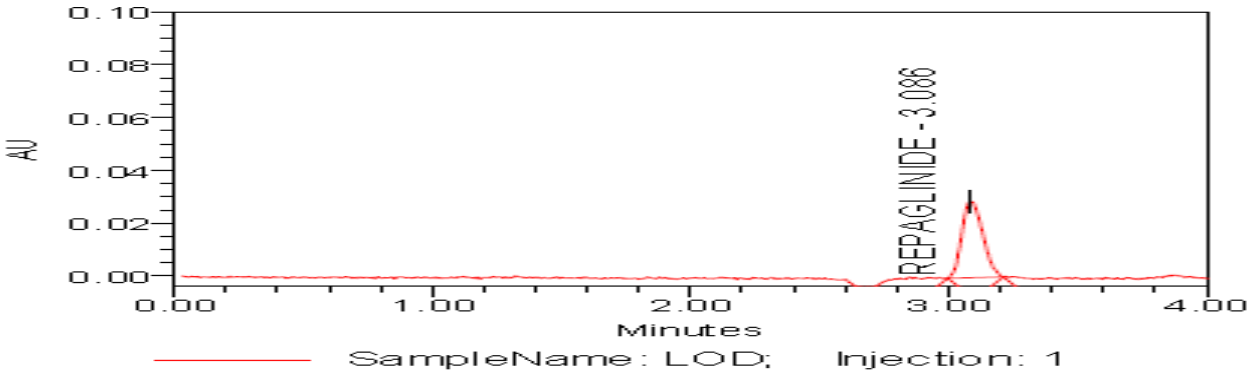


Fig No.9: LOD 1

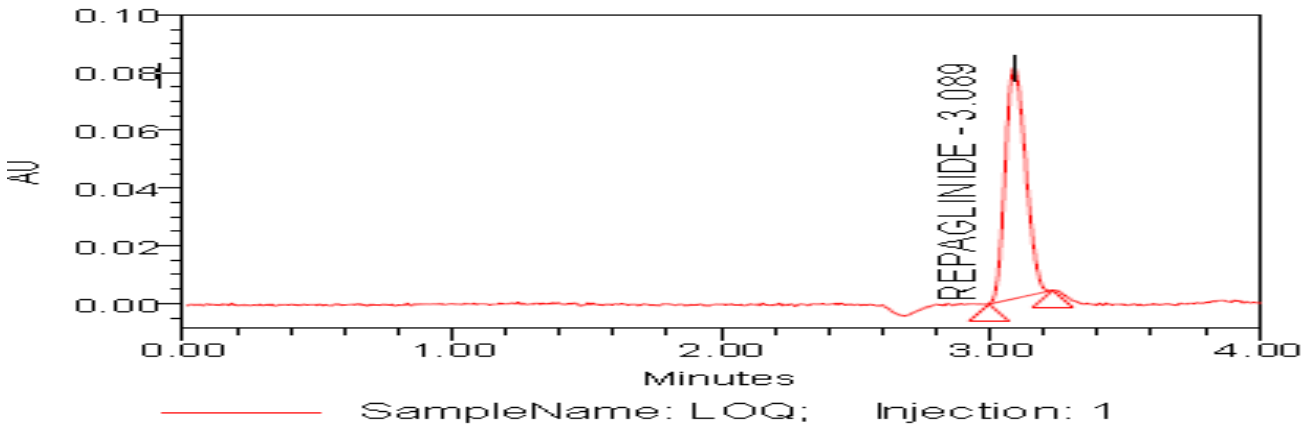


Fig No.10: LOQ 1

6. Robustness:

To establish the robustness of test method and to demonstrate its reliability for minor changes in chromatographic conditions (Fig No: 11). Robustness of test method will be demonstrated by carrying out system suitability under normal condition and each of the altered conditions. Results are reported in Table 7, 8.

Table No. 7: Robustness

S.No	Sample Name	RT	Area	USP Tailing	USP Plate count	s/n
1	TEMP1	3.9	1194	1.229	7735	1272.78
2	TEMP2	3.1	9486	1.198	8767	1970.77
3	FLOW1	3.1	9440	1.201	8549	1888.12
4	FLOW2	3.1	9454	1.23	8606	1735.58
MEAN		112.5				
%RSD		0.02				

Table No. 8: Robustness

S.NO	Parameter	Normal condition	Altered condition-1	Altered condition-2
1.	Flow rate	0.8 mL/min	0.6 mL/min	1 mL/min
2.	Temperature	20 ^{0}C	25^{0}C	30^{0}C

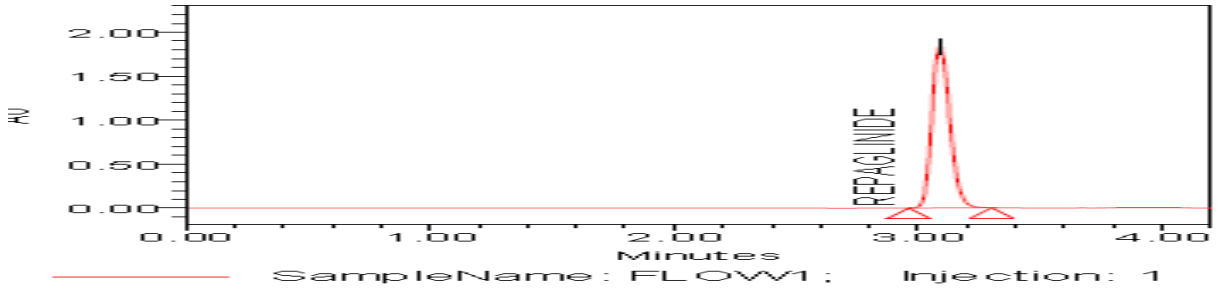


Fig No.11: Robustness 1

Acceptance criteria

Following system suitability parameters to be evaluated should comply with all altered conditions.

%RSD for area response of Repaglinide peak obtained with altered conditions NMT 2.00

no. of theoretical plates for repaglinide peak in altered conditions: NLT 3000

CONCLUSION

The proposed method is suitable for the estimation of Repaglinide which is simple, less time consuming using an economical column. The developed method has been validated for different parameters like Specificity, Method Precision, System Precision and Intermediate precision, Intermediate precision Analyst-II, Linearity, Accuracy and Robustness. All the results obtained were within the acceptance limits indicating that the developed method is simple, specific accurate and economical. This method may now be recommended for routine and quality control analysis of Repaglinide.

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