

Method Development and Validation of Nateglinide and Gliclazide by using RP-HPLC

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Abstract

A new methodology was established for co-occurring estimation of Nateglinide and Gliclazide by RP-HPLC methodology. The action conditions were with success developed for the separation of Nateglinide and Gliclazide by mistreatment Xterra C18 5 μ m (4.6*250mm) column, rate was 1ml/min, mobile section magnitude relation was Phosphate buffer (0.05M) pH 4.6: ACN (55:45%v/v) (pH was adjusted with orthophosphoric acid), detection wave length was 255nm. The instrument used was WATERS HPLC automobile Sampler, Separation module 2695, organiser Detector 996, Empower-software version-2. The analytical methodology was valid consistent with ICH tips (ICH, Q2 (R1)). The dimensionality study for Nateglinide and Gliclazide was found in concentration vary of 1 μ g-5 μ g and 100 μ g-500 μ g and correlation (r²) was found to be zero.999 and 0.999, this does not imply recovery was found to be 100% and one hundred. 5%, %RSD for repeatability was 0.7 and 0.4, nothing R SD for intermediate preciseness was zero.18 and 0.39 severally.

Keywords

Nateglinide, Gliclazide HPLC, new method development, validation.

1. INTRODUCTION

Nateglinide It is an oral antihyperglycemic agent used for the treatment of NIDDM It belongs to the Meglitinide class of short-acting insulin secretagogues It lowers blood glucose by stimulating the release of insulin from the pancreas, by closing

ATP dependent potassium channels in the membrane of the β cells This depolarizes the β cells and causes voltage gated calcium channels to open The resulting calcium influx induces fusion of insulin containing vesicles with the cell membrane and insulin.²

Structure of drug

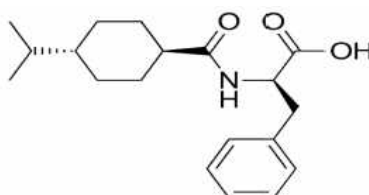


Fig 1: Structure of Nateglinide

GLICLAZIDE

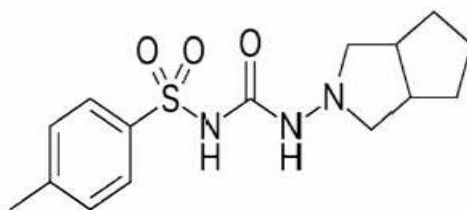


Fig 2: Structure of GLICLAZIDE

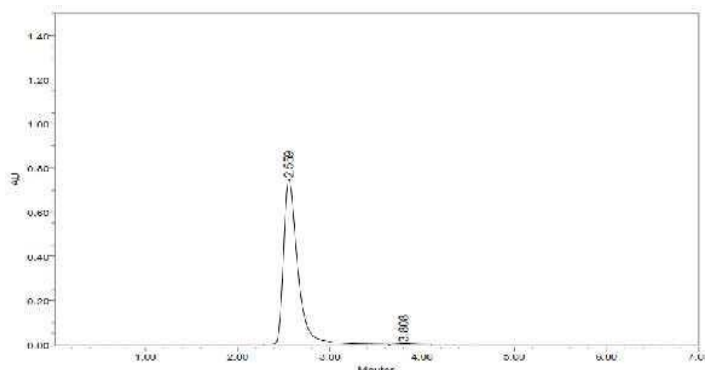


Fig 3: Chromatogram of nateglinide and gliclazide

VALIDATION RESULTS

1. Accuracy:

The accuracy study was performed for five hundredth, 100% and a hundred and fifty you look

after Nateglinide and Gliclazide. every level was injected in triplicate into natural process system. The world of every level was used for calculation of twenty-two recoveries.

Chromatograms square measure shown in Figs. 4-6 and results square measure tabulated in Tables

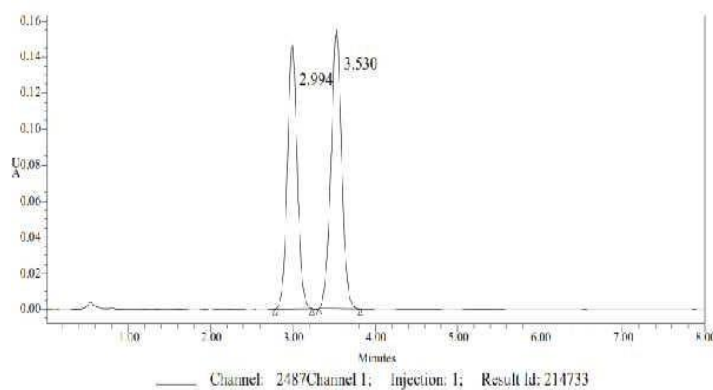


Fig 4: Chromatogram showing accuracy 50% injection 1

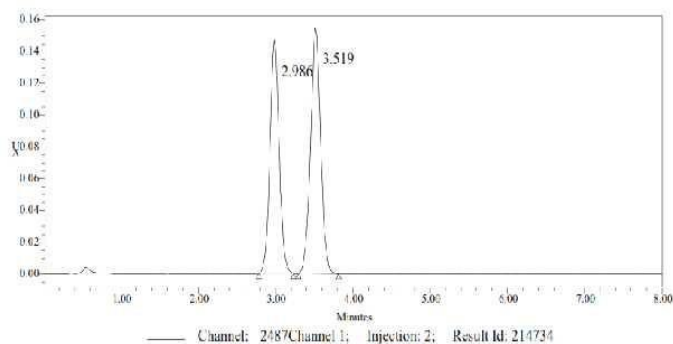
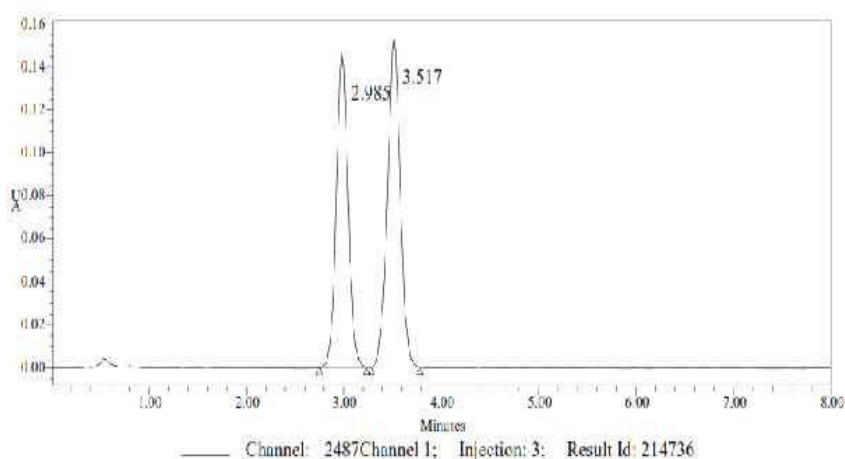
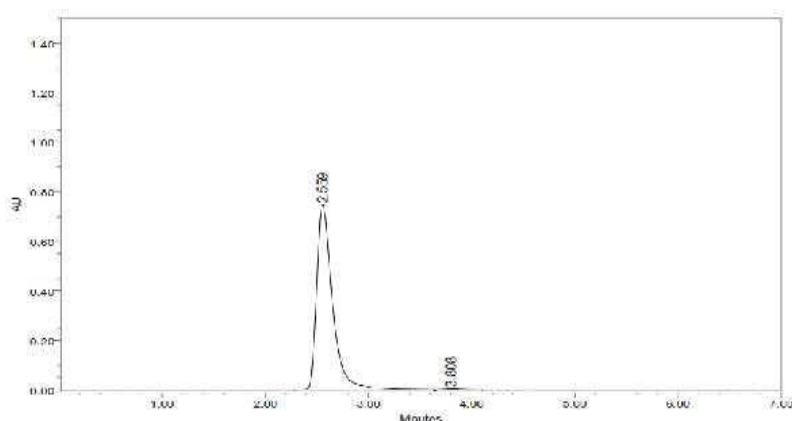


Fig 5: Chromatogram showing accuracy 50% injection 2



Chromatogram showing accuracy 50% injection-3



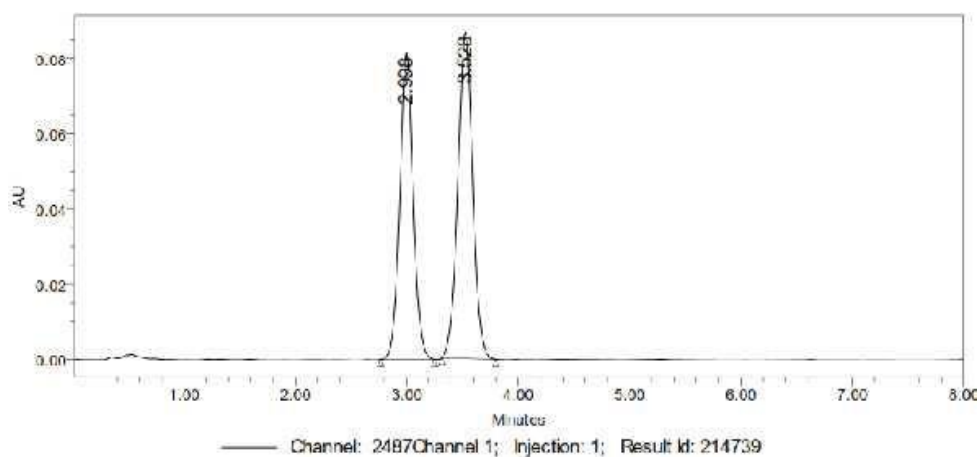
Name: Nateglinide

	Name	RT	Area
1	Nateglinide	2.994	544164
2	Nateglinide	2.986	542589
3	Nateglinide	2.985	547381
Mean			544711
Std. Dev.			2442.4
% RSD			0.44

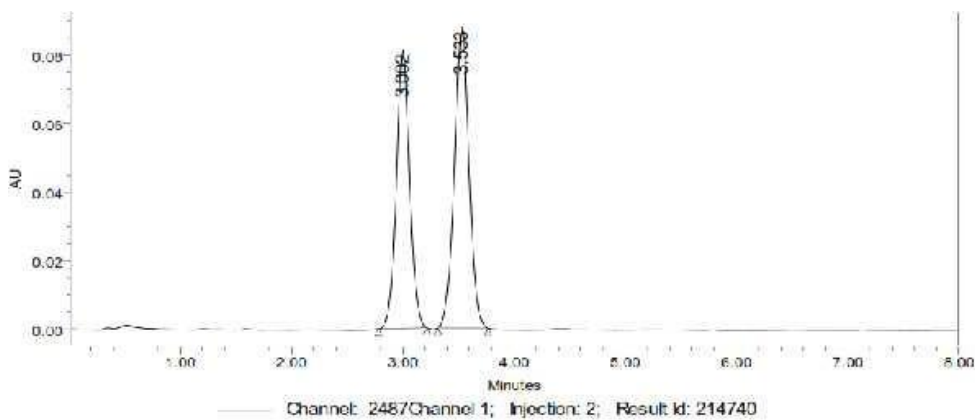
Name: Gliclazide

	Name	RT	Area
1	Gliclazide	3.530	641412
2	Gliclazide	3.519	644644
3	Gliclazide	3.517	648238
Mean			644765
Std. Dev.			3414.8
% RSD			0.52

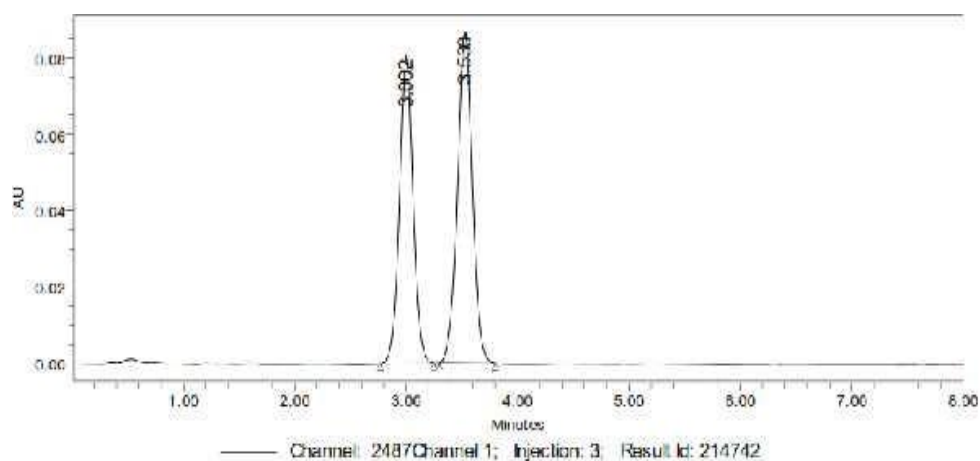
Table 7.8 Accuracy 50%



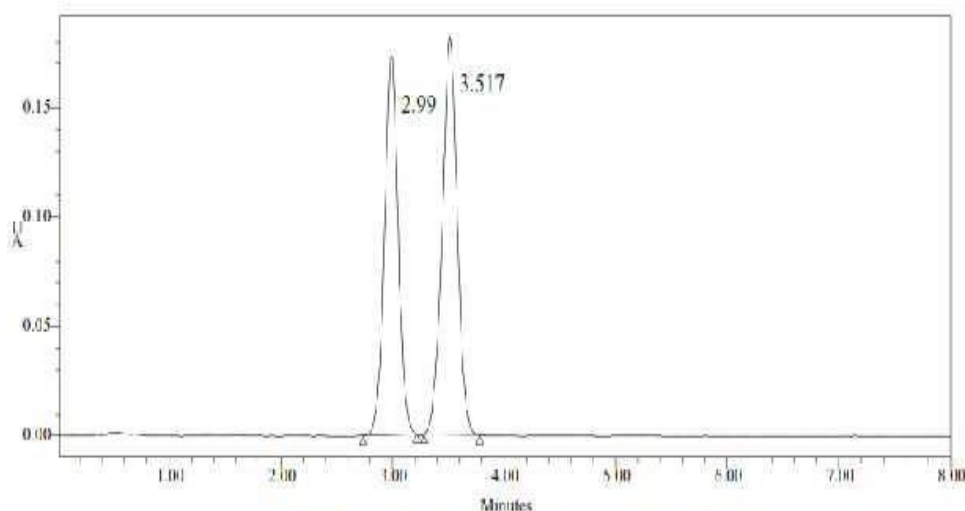
Chromatogram showing accuracy 100% injection – 1



Chromatogram showing accuracy 100% injection - 2



Chromatogram showing accuracy 100% injection - 3



Channel: 2487Channel I; Injection: 1; Result Id: 214749

2	Nateglinide	3.533	803075
3	Nateglinide	3.530	809247
Mean			803722
Std. Dev.			5232.4
% RSD			0.65

Name : Gliclazide

	Name	RT	Area
1	Gliclazide	2.998	676367
2	Gliclazide	3.002	673158
3	Gliclazide	3.002	678282
Mean			675935
Std. Dev.			2588.9
% RSD			0.38

Fig 7.19 Chromatogram showing accuracy150% injection-1

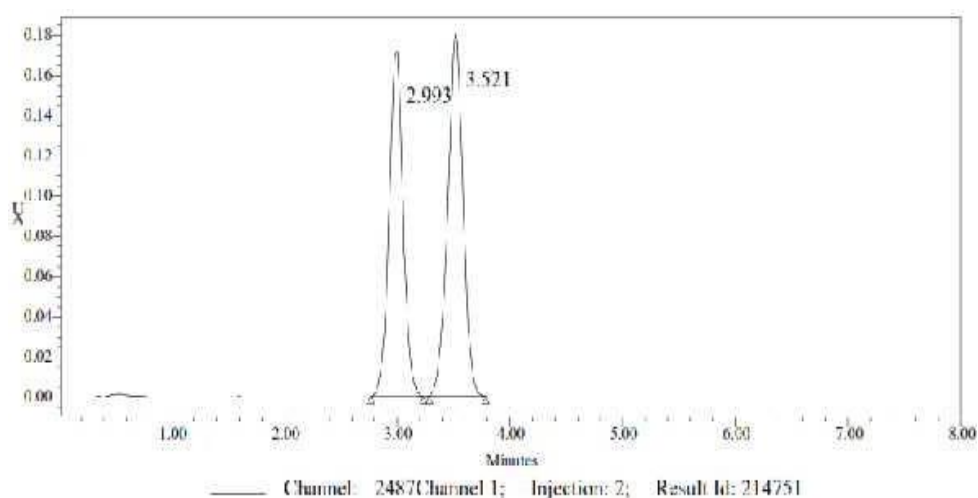


Fig 7.20 Chromatogram showing accuracy 150% injection-2

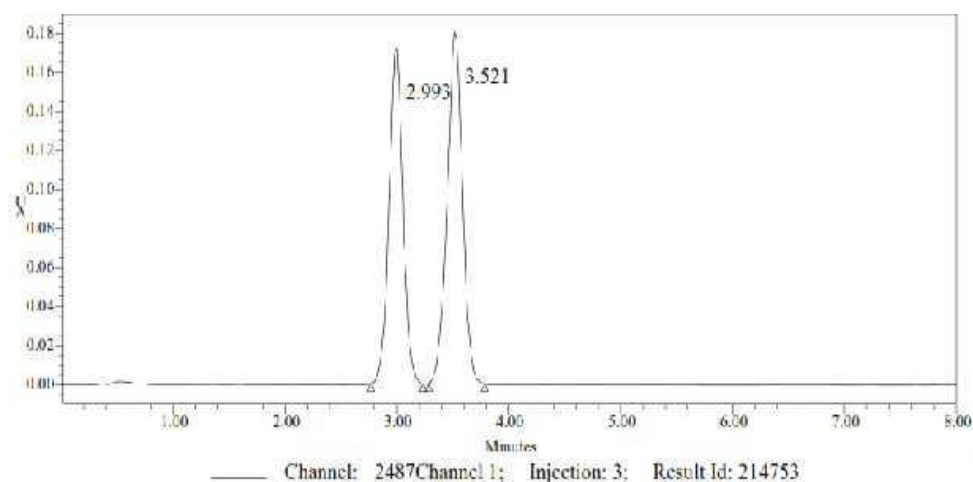


Fig 7.21 Chromatogram showing accuracy 150% injection-3

Name: Nateglinide

	Name	RT	Area
1	Nateglinide	3.517	960574
2	Nateglinide	3.521	964089
3	Nateglinide	3.521	964089
Mean			962917
% RSD			0.2

Name: Glizalazide

	Name	RT	Area
1	Glizalazide	2.991	813332
2	Glizalazide	2.993	812480
3	Glizalazide	2.993	812480
Mean			812764
% RSD			0.06

Table 7.10 Accuracy 150%

Table 7.11 Accuracy results of Gliclazide

%Concentration (at specification Level)	Area	Amount added (mg)	Amount found (mg)	%Recovery	Mean Recovery
50%	5447115		5.10	101.8%	
100%	67593510		9.99	99.9%	
150%	81276415		14.9	99.1%	100.5%

Acceptance Criteria:

The % Recovery for each level should be between 98.0 to 102.0%.

The accuracy results for Nateglinide
Table 7.12 Accuracy results of Nateglinide

%Concentration (at specification level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	6447655		5.0	101.3%	
100%	80372210		9.94	99.4%	100.0%
150%	96291715		14.8	99.2%	

Accepta Criteria:

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

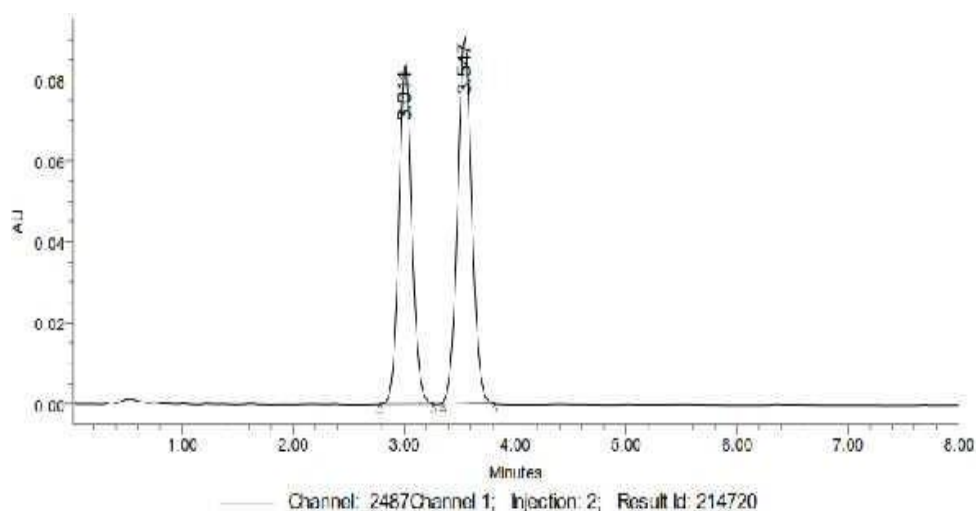
2. Precision

- I. Repeatability
- II. Intermediate precision(Ruggedness)

Repeatability

The exactness study was performed for 5 injections of Nateglinide and Gliclazide. every commonplace

injection was injected in to chromatographical system. the realm of every commonplace injection was used for calculation of twenty-two RSD.

Fig 7.22 Chromatogram of Standard Inj-1


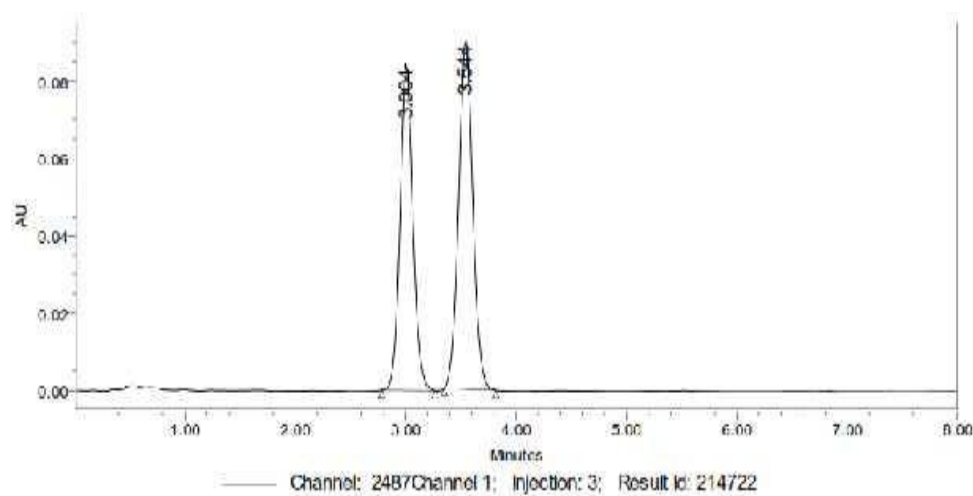
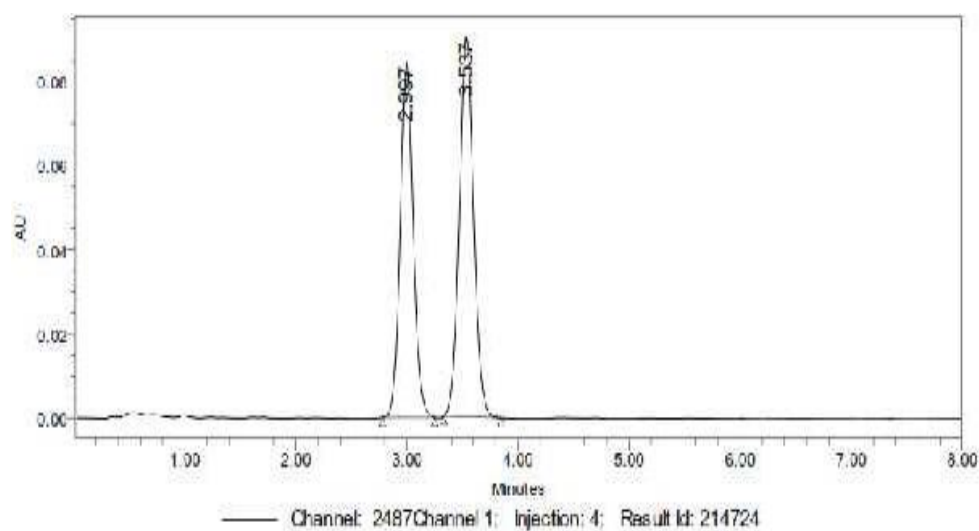
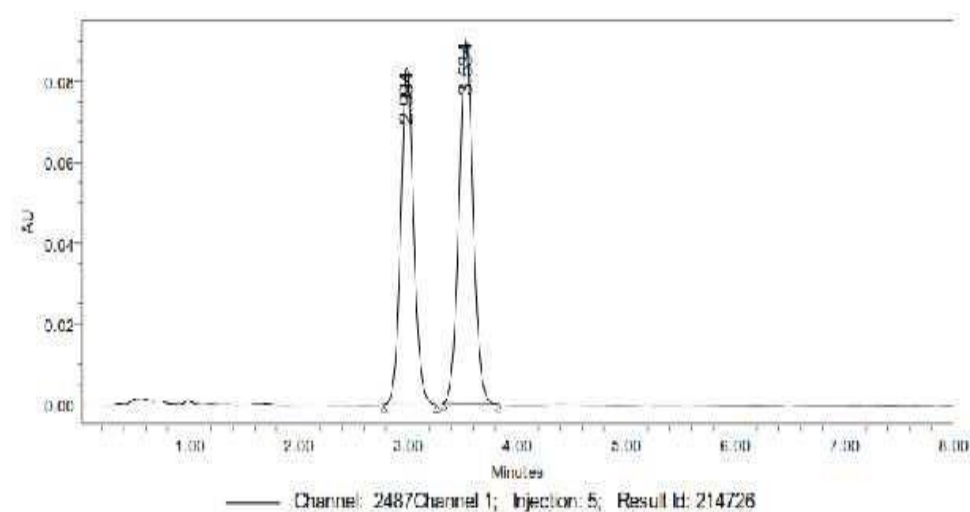
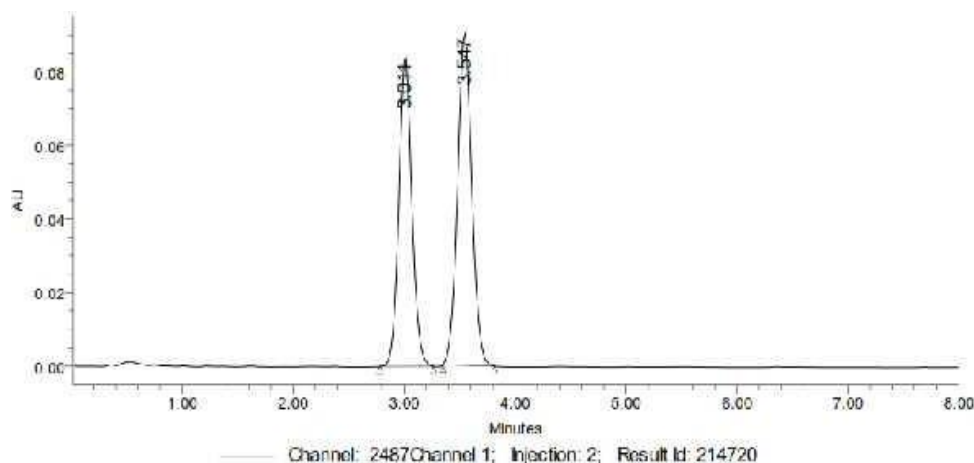
Chromatogram of Standard Inj-2

Chromatogram of Standard Inj-3

Fig 7.25 Chromatogram of Standard Inj-4


Fig 7.26 Chromatogram of Standard Inj-5



Name : Nateglinide

	Name	RT	Area
1	Nateglinide	3.019	691143
2	Nateglinide	3.011	685431
3	Nateglinide	3.004	683543
4	Nateglinide	2.997	683564
5	Nateglinide	2.994	683532
Mean			685443
Std. Dev.			3289.7
% RSD			0.48

Name : Gliclazide

	Name	RT	Area
1	Gliclazide	3.557	819305
2	Gliclazide	3.547	807157
3	Gliclazide	3.544	804070
4	Gliclazide	3.537	808474
5	Gliclazide	3.534	804505
Mean			808702
Std. Dev.			6203.7
% RSD			0.77

Table 7.13 Repeatability results of Gliclazide and Nateglinide

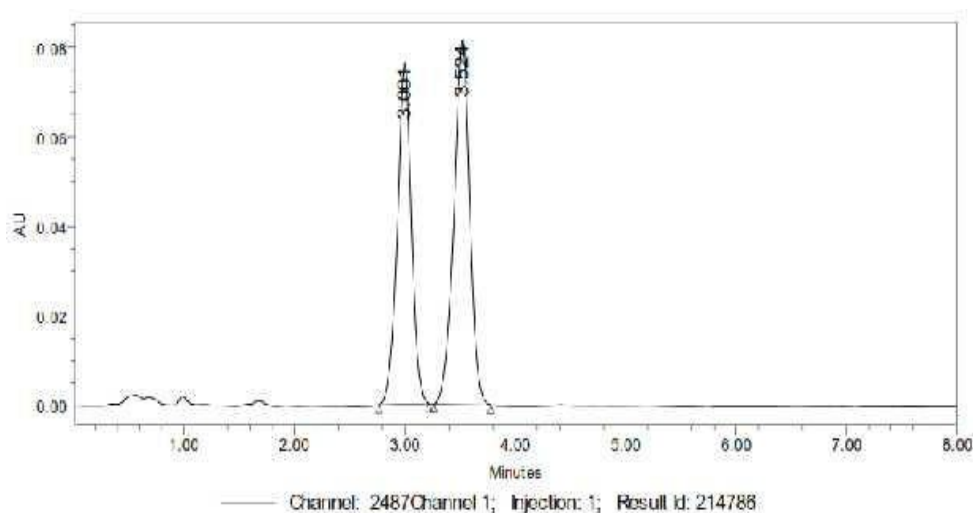
Acceptance Criteria:

The half RSD for the realm of 5 customary injections results mustn't be over 2%

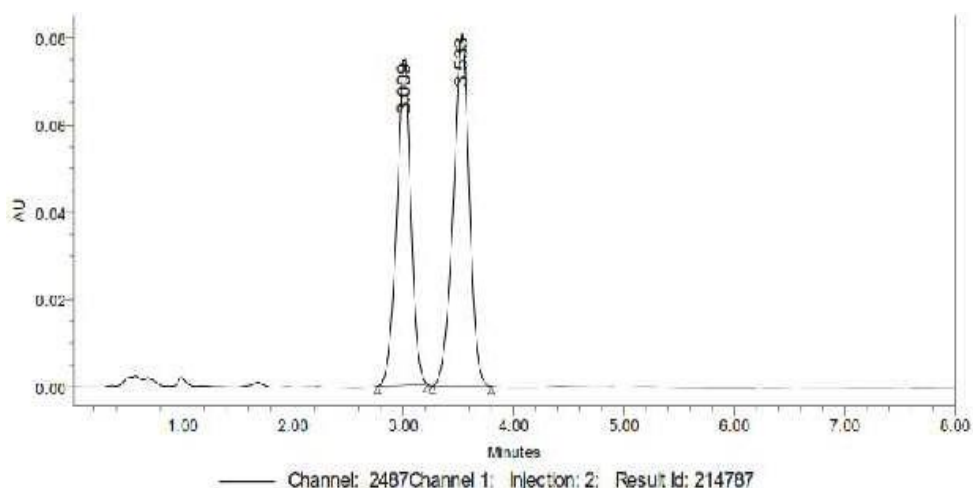
The Method exactitude study was performed for the %RSD of Nateglinide and Gliclazide was found to be zero.7 and 0.4 (NMT2).

Intermediate precision/Ruggedness

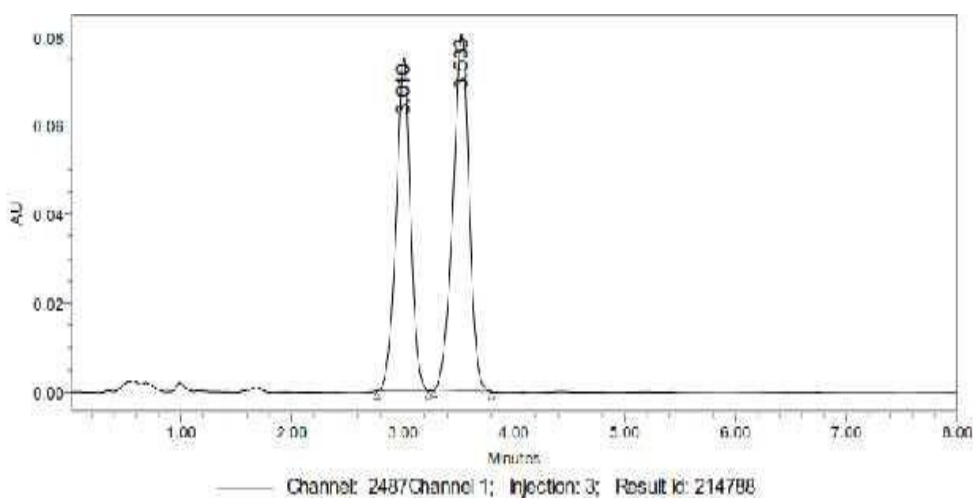
The intermediate exactness study was performed for 5 injections of Nateglinide and Gliclazide. every normal injection was injected into activity system. the world of every normal injection was used for calculation of nadaRSD.



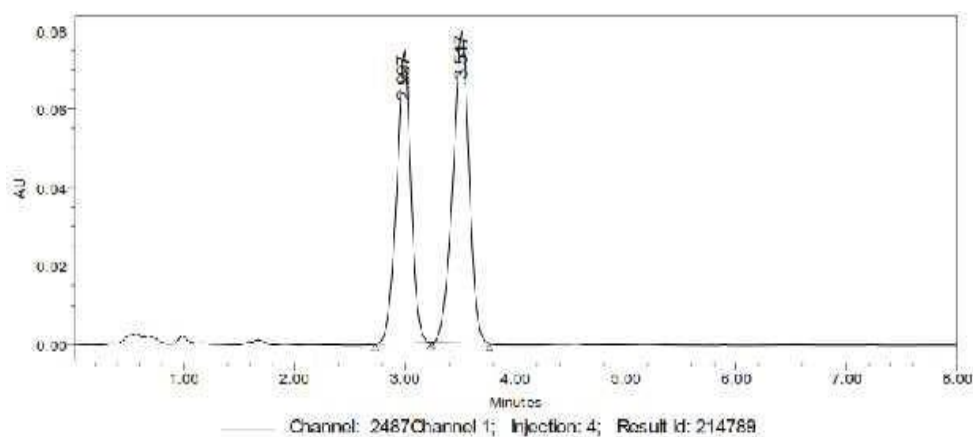
Chromatogram of Standard Inj-1(ID Precision)



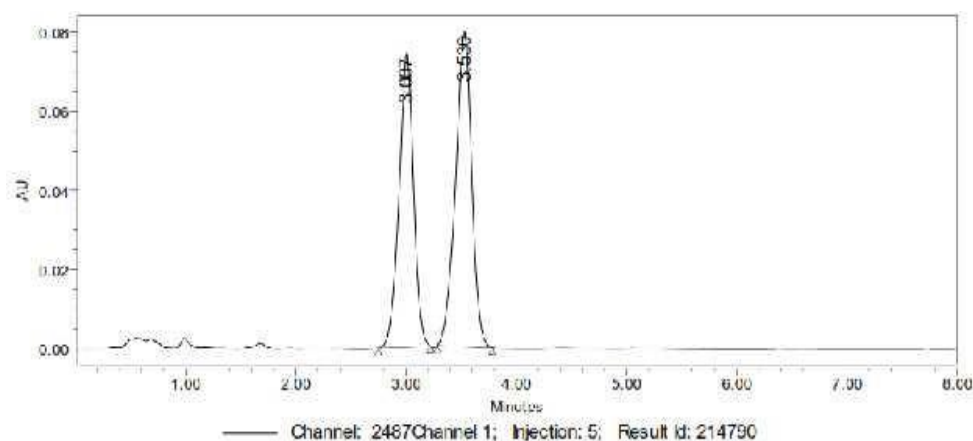
Chromatogram of Standard Inj-2(ID Precision)



Chromatogram of Standard Inj-3(ID Precision)



Chromatogram of Standard Inj-4(ID Precision)



Chromatogram of Standard Inj-5(ID Precision)

NAME: Nateglinide

	Name	RT	Area
1	Nateglinide	3.524	813507
2	Nateglinide	3.533	817673
3	Nateglinide	3.533	815189
4	Nateglinide	3.517	815816
5	Nateglinide	3.530	815356
Mean			815508
Std.Dev.			1492.7
% RSD			0.18

NAME: Gliclazide

	Name	RT	Area
1	Gliclazide	3.001	673725
2	Gliclazide	3.009	672535
3	Gliclazide	3.010	676216
4	Gliclazide	2.997	679037
5	Gliclazide	3.007	677101
Mean			675723
Std.Dev.			2611.5
% RSD			0.39

The results are summarized Gliclazide Ruggedness results of Gliclazide Acceptance

Criteria:

The % RSD for the realm of 5 commonplace injections results mustn't be quite a pair of the intermediate preciseness was performed for %RSD of Nateglinide and Gliclazide was found to be 0.18 and 0.39 severally (NMT 2).

3. Specificity:

The system quality for specificity was meted out to work out whether or not there's any interference of any impurities in retention time of analytical peak. The study was performed by injecting blank. The chromatograms are shown in Fig.4.37, 4.38.

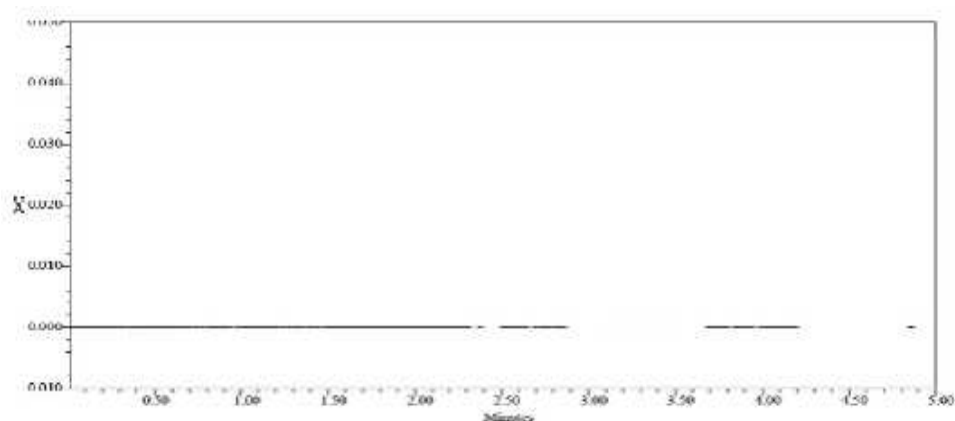


Fig. 7.32 Chromatogram of blankInjection

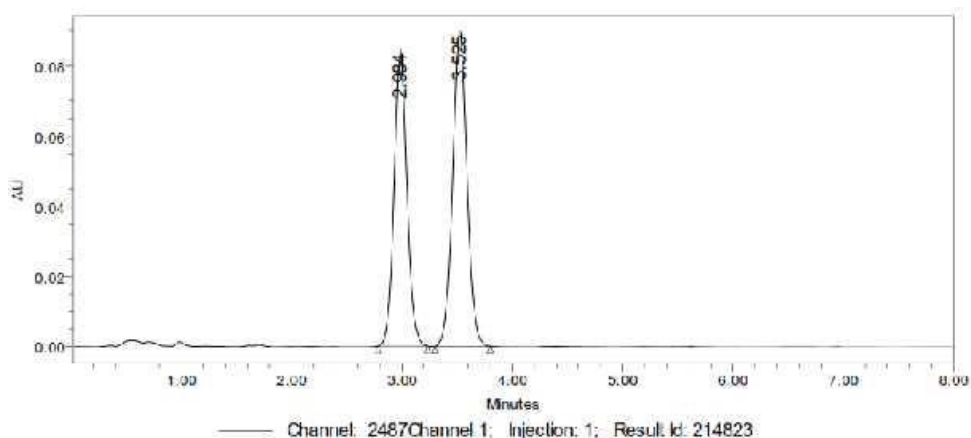


Fig. 7.33 Chromatogram of StandardInjection

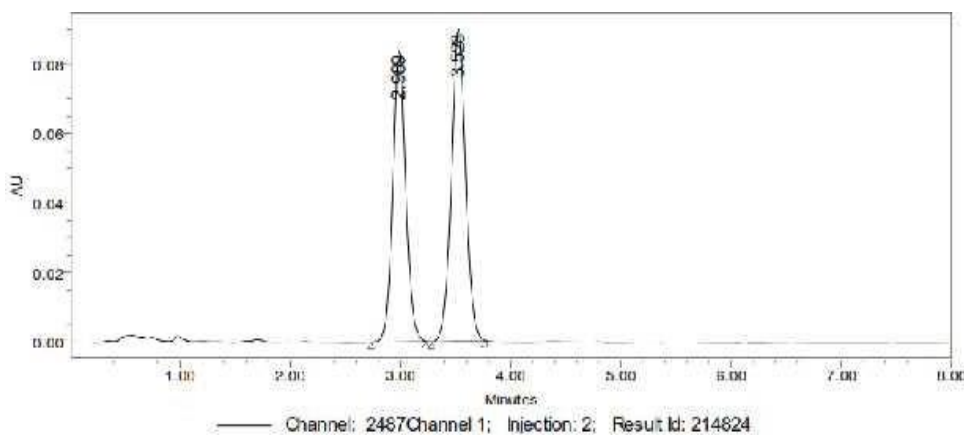


Fig. 7.34 Chromatogram of Standard Injection

Name: Nateglinide

	Name	RT	Area	USP Plate Count	USP Tailing
1	Nateglinide	2.984	681469	3115.4	1.1
2	Nateglinide	2.989	683696	3209.7	1.1
Mean			682582	3162.5	1.1
Std. Dev.			1575.2		
% RSD			0.23		

Name: Glucalazide

	Name	RT	Area	USP Plate Count	USP Tailing	USP Resolution
1	Glucalazide	3.525	810802	3527.8	1.0	2.4
2	Glucalazide	3.528	808790	3566.2	1.0	2.3
Mean			809796	3547.0	1.0	
Std. Dev.			1422.2			
% RSD			0.18			

Table 7.15 Details of Standard Inj

Specificity

The specificity takes a look at was performed for Nateglinide and Glucalazide. it absolutely was found that there was no interference of impurities in retention time of analytical peak.

supported the quality deviation of y- intercepts of regression lines.

Formula:

$$LOD = 3.3 \times \frac{\sigma}{S}$$

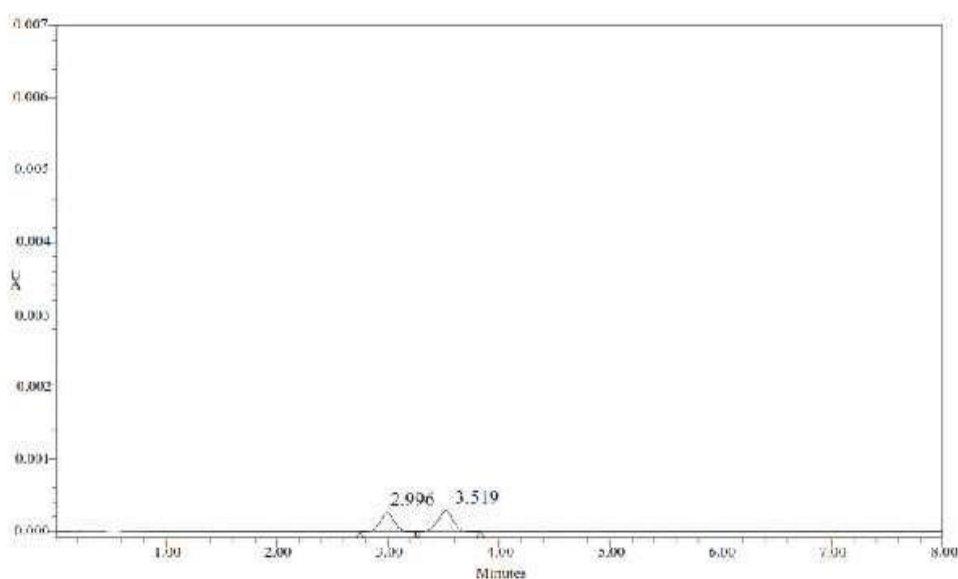
4. Detection of limit:

LOD's are often calculated supported the quality deviation of the response (SD) and therefore the slope of the activity curve (S) at levels approximating the LOD in keeping with the formula. the quality deviation of the response is often determined

Where

σ - Standard deviation (SD)

S – Slope



Chromatogram of LOD

5. Quantitation Limit: LOQ's are often calculated supported the quality deviation of the response (SD) and therefore the slope of the activity curve (S) at levels approximating the LOQ in keeping with the

formula. the quality deviation of the response is often determined supported the quality deviation of y-intercepts of regression lines.

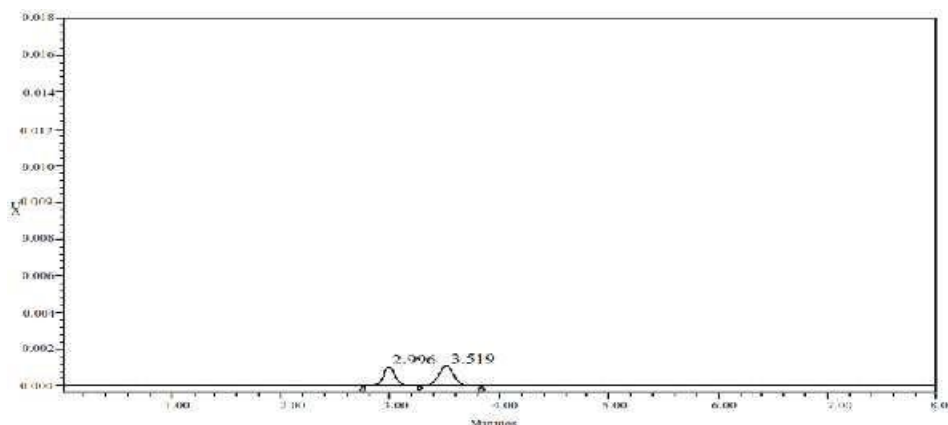


Fig. 7.36 Chromatogram of LOQ

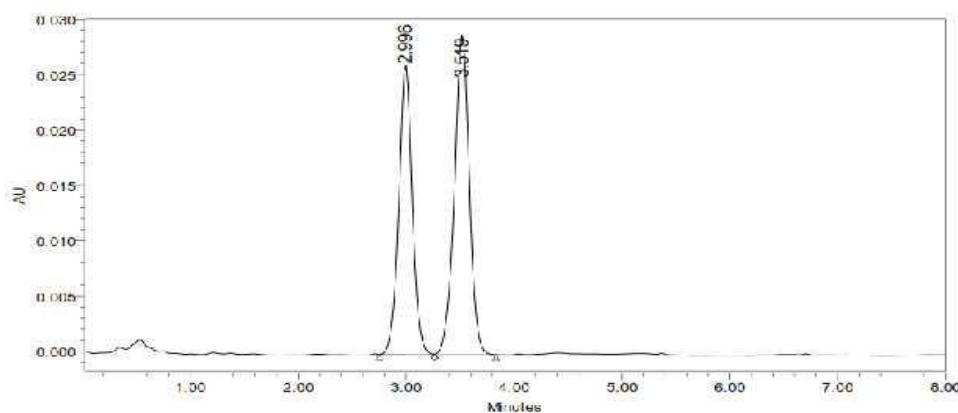
Acceptance criteria:

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

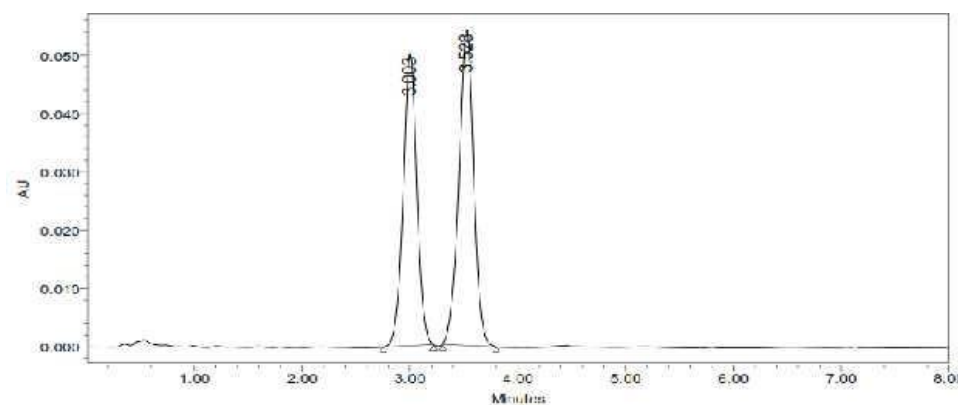
The LOQ was performed for Nateglinide and Gliclazide was found to be 9.87 and 10 respectively.

The dimensionality study was performed for the concentration of 100ppm to 500ppm and 1ppm to 5ppm level. every level was injected into action system. the realm of every level was used for calculation of coefficient of correlation.

Linearity:



Chromatogram for Inj Level-I



Chromatogram for Inj Level-II

Linearity results of Nateglinide

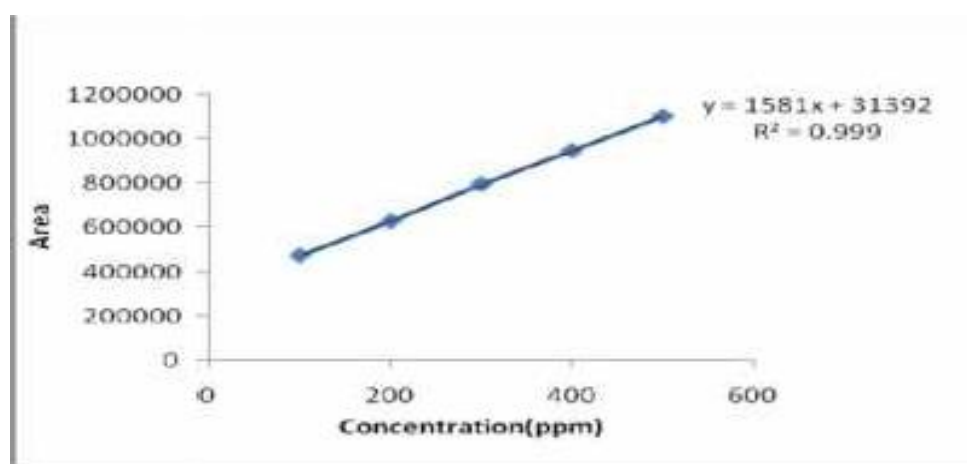
S.No	Linearity	Concentration	Area
1	I	1ppm	277182
2	II	2ppm	521695
3	III	3ppm	808274
4	IV	4ppm	1033875
5	V	5ppm	1285804
Correlation Coefficient			0.999

Acceptance Criteria:

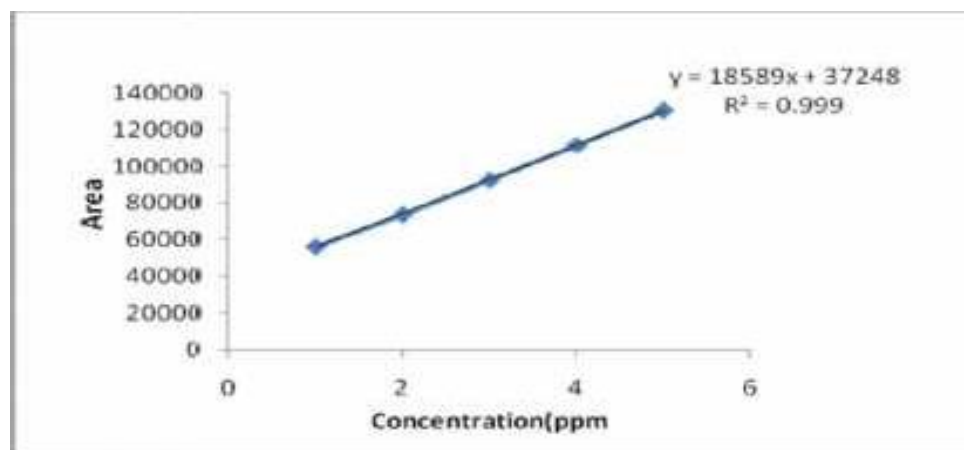
Correlation coefficient should be not less than 0.999.

Plotting of calibration graphs:

The resultant areas of linearity peaks are plotted against Concentration



Calibration curve of Gliclazide



Calibration curve of Nateglinide

6. Range

The dimensionality study was performed for concentration vary of 1 μ g - 5 μ g and 100 μ g-500 μ g of Nateglinide and Gliclazide and also the coefficient of correlation was found to be 0.999 and 0.999. (NLT 0.999).

7. Robustness

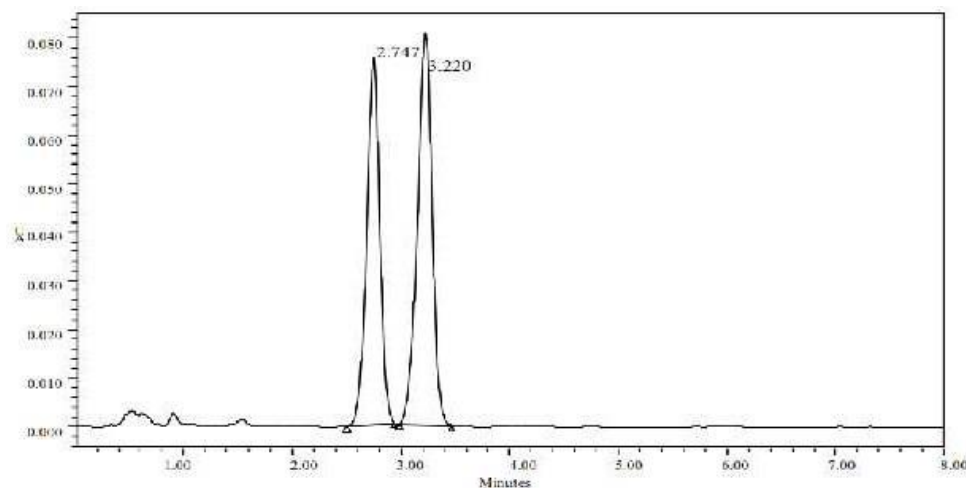
As a part of the robustness, deliberate modification within the rate of flow, Mobile part composition, Temperature Variation was created to gauge the impact on the tac

A) Flow Rate:

The robustness was performed for the rate variations from zero.8 ml/min to 2ml/min. normal answer three hundred μ g/ml of Gliclazide & 3 μ g/ml of Nateglinide was ready and analysed victimisation the

various Mobile part composition beside the particular mobile part composition within the methodology.

Fig 7.44 Chromatogram for Robustness more flow



	Name	Retention Time (min)	Area (μV*sec)	Height (μV)	USP Plate Count	USP Tailing	USP Resolution
1	Nategli	2.747	623847	75117	2503.3	0.9	
2	Gliclaz	3.220	756748	80446	2685.4	0.9	1.9

Table 7.19 Details of Robustness more flow

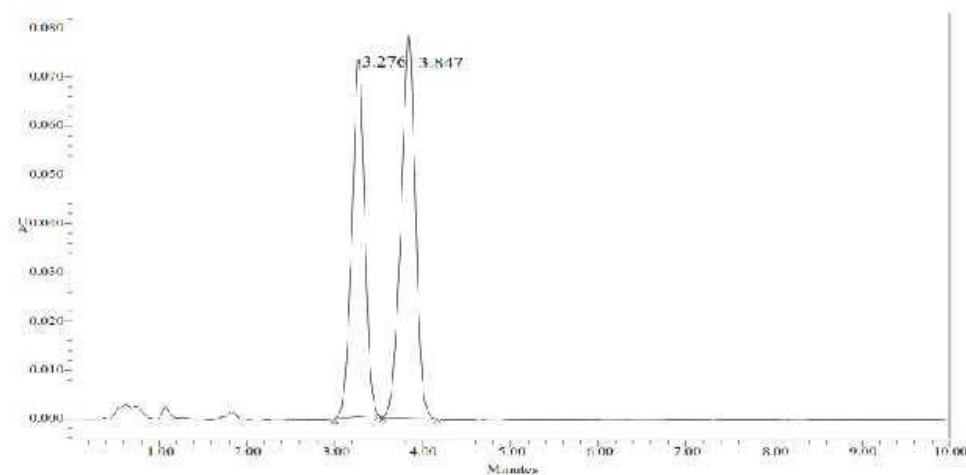


Fig. 7.45 Chromatogram for Robustness less flow

	Name	Retention Time (min)	Area (μV*sec)	Height (μV)	USP Plate Count	USP Tailing	USP Resolution
1	Nategli	3.276	740721	73704	2690.4	0.9	
2	Glicla	3.847	903225	78896	2716.2	0.9	1.9

Table 7.20 Details of Robustness less flow

The results square measure summarized on analysis of the on top of results, it is often ended that the variation in rate of flow affected the strategy considerably. therefore, it indicates that the strategy is strong even by amendment within the rate of flow ± 0.2 ml/min.

System suitability results for Gliclazide:
Table 7.21 System suitability results for Gliclazide (Flow rate)

S.No	Flow Rate(ml/min)	System suitability results	
		USP Plate count	USP Tailing
1	0.8	2690	0.9
2	1.0	3115	1.1
3	1.2	2503	0.9

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

System suitability results for Nateglinide (Flow rate)

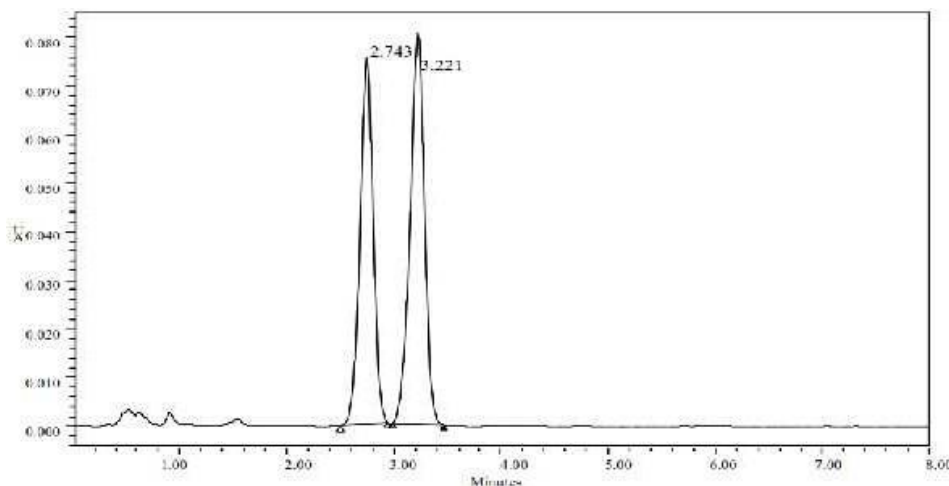
S.No.	Flow Rate(ml/min)	System suitability results	
		USP Plate count	USP Tailing
1	0.8	2716	0.9
2	1.0	3527	1.0
3	1.2	2685	0.9

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

B) Mobile Phase:

The Organic composition within the Mobile part was varied from seventieth to hr. customary answer three hundred μ g/ml of Gliclazide & 3 μ g/ml of

Nateglinide was ready and analyzed exploitation the numerous Mobile part composition at the side of the particular mobile part composition within the technique.


Chromatogram for Robustness more organic

	Name	Retention Time (min)	Area (μ V*sec)	Height (μ V)	USP Plate Count	USP Tailing	USP Resolution
1	Nategli	2.743	623812	75134	2707.1	1.1	
2	Glicla	3.221	756795	80412	3001.8	1.0	1.9

Details of Robustness more org.

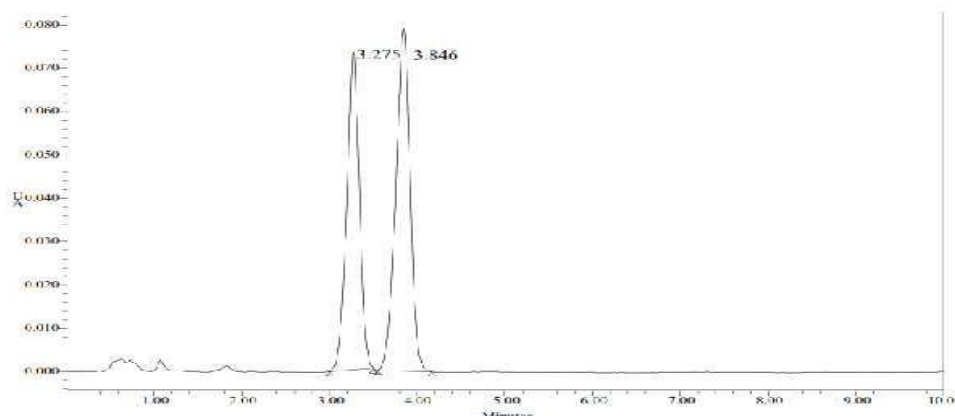


Fig 7.47 Chromatogram for Robustness less organic

	Name	Retention Time (min)	Area (μV*sec)	Height (μV)	USP Plate Count	USP Tailing	USP Resolution
1	Nategli	3.275	740841	73795	2818.9	1.1	
2	Glicla	3.846	903365	78845	3107.7	1.0	1.9

Table 7.24 Details of Robustness les organic

The results area unit summarized. On analysis of the higher than results, it is terminated that the variation in 100 percent Organic composition within the

mobile section affected the tactic considerably. therefore, it indicates that the tactic is strong even by amendment within the Mobile section±10

System suitability results for Gliclazide:

Table 7.25 System suitability results for Gliclazide (Mobile phase)

S.No.	Change in Organic Composition in the Mobile Phase	System suitability results	
		USP Plate count	USP Tailing
1	10% Less	2818	1.1
2	Actual	3125	1.1
3	10% More	2707	1.1

Results for actual Mobile phase composition (55:45Buffer: ACN) have been considered from Accuracy standard

CONCLUSION

A validated stability indicating isocratic RP-HPLC method has been developed for the assay of nateglinide in pure and in tablet dosage form.

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- Patil Sudarshan S. et al Development and Validation of Analytical Method for Simultaneous Estimation of Gliclazide and Nateglinide HCl in Bulk and Tablets Using UV – Visible Spectroscopy.
- Shammi Goyal et al Development and Validation of RP-HPLC Method for Estimation of Glipizide in Bulk Drug and Pharmaceutical Formulation
- Arayne MS et al Development and Validation of RP-HPLC Method for The Analysis of Nateglinide.
- Shammi Goyal et al Development and Validation of RP-HPLC Method for Estimation of Glipizide in Bulk Drug and Pharmaceutical Formulation
- Arayne MS et al Development and Validation of RP-HPLC Method for The Analysis of Nateglinide.