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METHOD DEVELOPMENT AND VALIDATIONFOR ESTIMATION OF NATEGLINIDE IN TABLET DOSAGE FORM BY REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

An effortless, hasty, unambiguous, robust, exactas well as precise isocratic reverse phase high performance liquid chromatographic technique has been urbanizedin addition to validated for the assessment of Nateglinide in tablet dosage form. The chromatographic severance was accomplished on Phenomenex kinetex C_{18}(250mm×4.6mm i.d, 5µm) column by means of a mobile phase mixture containing methanol: buffer of pH 6.8: ACN in the proportion of 47:23:30 respectively at a flow rate of 1ml/min with injection volume of 20µl and recognition wavelength of 216 nm at ambient temperature. The retention time was established to be 4.823mins by way of a run time of 7mins. The linearity was obtained in the range of 20 to 300µg/ml with correlation coefficient of 0.9996. The mean entitlement recuperation at every level was established to be within the limits of 98% and 105%.The optimized method was used to assay the pharmaceutical dosage form and assay value was found to be 96.94%. The anticipated technique was validated as per ICH guidelines as well as applied for the investigation of Nateglinide in tablet dosage form.

Keywords: Nateglinide, Assay, RP-HPLC, Method development, Validation

INTRODUCTION:

Nateglinide is an amino acid derivative that induces an early insulin response to meals decreasing postprandial blood glucose levels. It is belonging to the meglitinide

class of short-acting insulin secretagogues, which act by binding to β cells of the pancreas to stimulate insulin release. Nateglinide 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. Nateglinide is an amino acid derivative that 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 **[1-3]**.

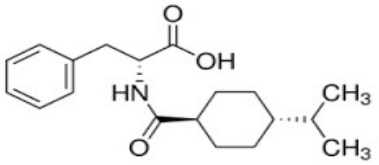

Figure 1: Chemical structure of Nateglinide

MATERIALS AND METHODS:

Equipment: Chromatographic separation was conceded on WATERS HPLC system which is outfitted with the 515 dual head reciprocating pump & a 2489 UV Visible detector. The software used is Empower-2 software and Phenomenex kinetex C_{18} (250mm×4.6mm i.d, 5µm) column is used for the investigation.

Chemicals and reagents: Nateglinide drug was gifted by Aurobindo Pharmaceuticals, Hyderabad, Telangana, India. Acetonitrile, methanol, HPLC grade water and mono sodium hydrogen orthophosphate and di sodium hydrogen ortho phosphate were procured from local manufacturers.

Preparation of buffer: 0.1gm of mono sodium hydrogen orthophosphate and 0.1gm of di sodium hydrogen ortho phosphate was precisely gauged and moved in to a 500ml volumetric jar, broken up by count HPLC water weakened stamp with water. Mix 51 ml of mono sodium hydrogen orthophosphate with 49 ml of di sodium hydrogen orthophosphate and adjust the pH to 6.8 with orthophosphoric acid.

Preparation of mobile phase: Methanol, Mono and disodium Hydrogen orthophosphate buffer of pH 6.8 and acetonitrile were blended in the proportion of 47:23:30 %V/V and the portable stage was then sifted through 0.45µm layer channel and sonicated for 5min in ultrasonicator shower and moved in to dissolvable repository staying away from air pockets.

Preparation of standard solution: 25mg of Nateglinide (Bulk) was weighed precisely and moved in to 25ml volumetric flagon. Required amount of methanol was added to break up the medication. At that point, volume was made up check with methanol. This arrangement was set apart

as standard stock arrangement (1000µg/ml). 1ml of standard stock arrangement was pipette out in to 10ml volumetric cup and volume was made sufficient with diluent. This was marked as 'working standard solution' (100µg/ml).

Preparation of the test solution: All three tablets are accurately ground and crushed to form fine powder. The amount of powder is estimated at 25mg of Nateglinide, and it is taken into a 25ml cup containing 25ml of methanol. The flagon was then sonicated for 5 min and then fused with methanol. This leads to a stock arrangement of Nateglinide, which contains a group of 1000µg/ml. The arrangement of the stock was then isolated with Whatman channel paper and washed with methanol, which was shed by 0.45µm layer channel. Pipette 1ml of solution into a 10ml volumetric jar and is further diluted to volume with methanol. The setting was separated as a test routine (100µg/ml).

Selection of detection wavelength: The effective standard reserved solution was organized according to the route given above and the wavelength was decided by scanning the standard solution among 200 to 400nm. The scanned outcome revealed that maximum absorbance was experimental at 287nm. For this reason, this wavelength was selected for the investigation and the spectrum obtained was shown in **Figure 2**.

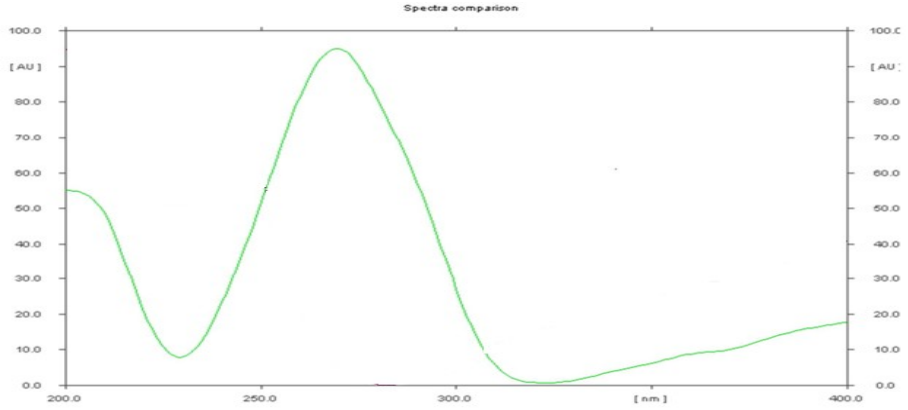

Figure 2: UV spectrum of Nateglinide

METHOD DEVELOPMENT [4-6]

Optimized Chromatographic conditions:

Column:Phenomenex kinetex C_{18} (250mm×4.6mm i.d, 5µm) column

Mobile phase:Methanol: Mono and disodium Hydrogen orthophosphate buffer of pH 6.8: acetonitrile (47:23:30 %V/V)

Flow rate: 1ml/min

Injection volume: 20µl

Detection wavelength: 287nm

Mode of elution: Isocratic

Column temperature: Ambient

VALIDATION OF THE METHOD [7-10]

System suitability test: Solution for system suitability test was all set by moving 1ml of standard stock arrangement (1000µg/ml) into 10ml volumetric flagon, weakening to check with diluent and sonicated. This preparation was injected six times into the HPLC system for assessing parameters like number of hypothetical plates (N), peak area and tailing factor. The results were shown in **Table 1** and the overlain chromatogram for system suitability was shown in **Figure 3**.

Linearity: Working standard solution was prepared according to the procedure and after filtering and sonicating the solution for 5mins further dilutions were made to get different concentration levels ranging from 20 to 300µg/ml. Every solution was injected into HPLC system as well as linearity was appraised. The calibration curve was designed taking concentration on X-axis along with peak area on Y-axis. The linearity plot was shown in figure 4and overlain chromatogram for linearity was shown in **Figure 5**.

Range: Working standard arrangements were set up by moving 0.2 and 3.0 ml of standard stock arrangement in to 10ml volumetric jars independently and volume was made up to mark with diluent. At that point, the working standard arrangements were sonicated and injected 6 times into the HPLC system and the upper and lower levels of analyte were found to be 300 and 20µg/ml respectively. The overlain chromatograms for lowest and highest concentrations of range were shown in **Figures 6 and 7** respectively and the range data was shown in **Table 2**.

Precision:

System Precision: This was performed by infusing six repeat injections of the standard system in 60µg/ml focus under the same test conditions. The mean worth, standard deviation and %RSD were resolved for every one of the six recreates infusions and the results are shown in **Table 3** and overlain chromatogram for system precision was shown in **Figure 8**.

Method precision: Method precision was establishedby infusing six relaxation infusions of the standard system fixation under the same test conditions. The relative standard deviation obtained was shown in **Table 4** and overlain chromatogram for system precision was shown in **Figure 9**.

Intermediate Precision: It communicates exactness inside research facility varieties. It incorporates full examination on various days. This was done by injecting six replicate injections of test regime at 100µg / ml. Interday precision results are shown in table 5 and overlain chromatograms were shown in **Figure 10 and 11**.

Specificity: An investigation to build up specificity was led by injecting a volume of

20µl of diluents at optimized conditions in to the HPLC system and chromatogram was recorded as shown in **Figure 12**.

Accuracy: Accuracy studies were accomplished at three inimitable levels (50%, 100%, and 150%) by expansion standard medication to pre-break down example arrangement each in triplicate. Mean rate accuracy esteems at three distinct convergences of medication were determined and the results are shown in **Table 6**.

Robustness: Six replicate injections were given and the impacts of varieties were seen in the separately recorded chromatograms and the %RSD of the peak areas were determined for the medication at every one of the accompanying conditions.

- At diverse Flow rate (±0.1ml/min)
- At various grouping of versatile stage proportion (±2ml)
- At distinctive wavelength(±3nm)

The results were shown in **Tables 7, 8 and 9**.

Table 1: System suitability parameters

Injection	Peak area	Retention time (Rt)	Plate count	Tailing factor
1	421628	4.8	7071	1.03
2	422368	4.8	7118	1.03
3	421529	4.8	7069	1.01
4	430428	4.8	7001	1.02
5	428632	4.8	7091	1.01
6	424706	4.8	7026	1.02
Mean	424881	4.8	7062.6	1.02
SD	3821.07	0.004	42.71	0.006
%RSD	0.89	0.08	0.60	0.6

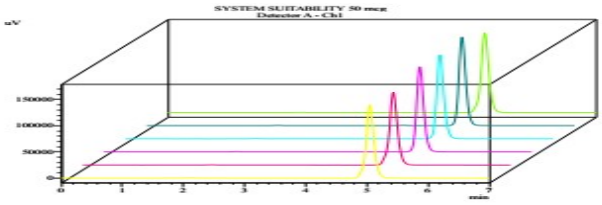

Figure 3: Overlain chromatogram for System Suitability

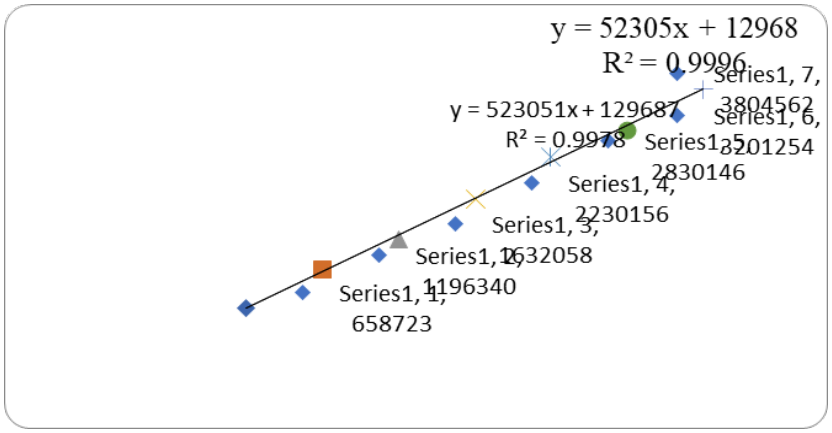

Figure 4: Linearity plot of Nateglinide

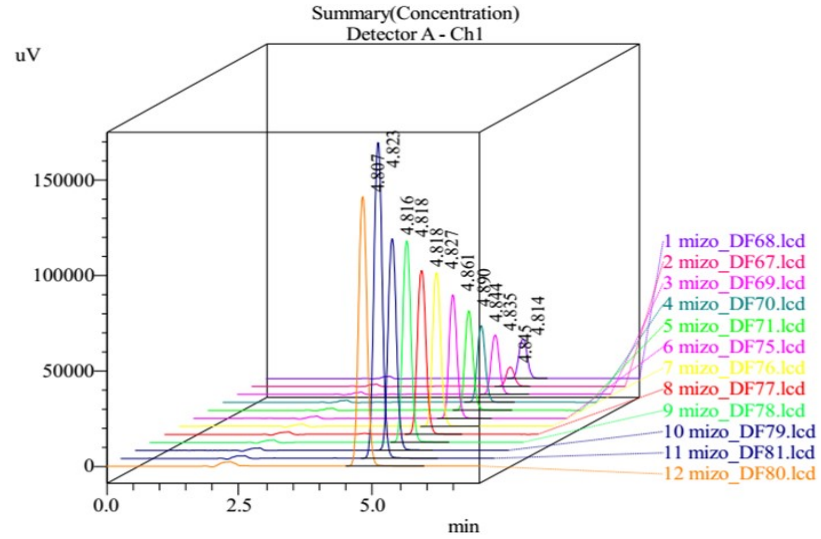

Figure 5: Overlain chromatogram for linearity

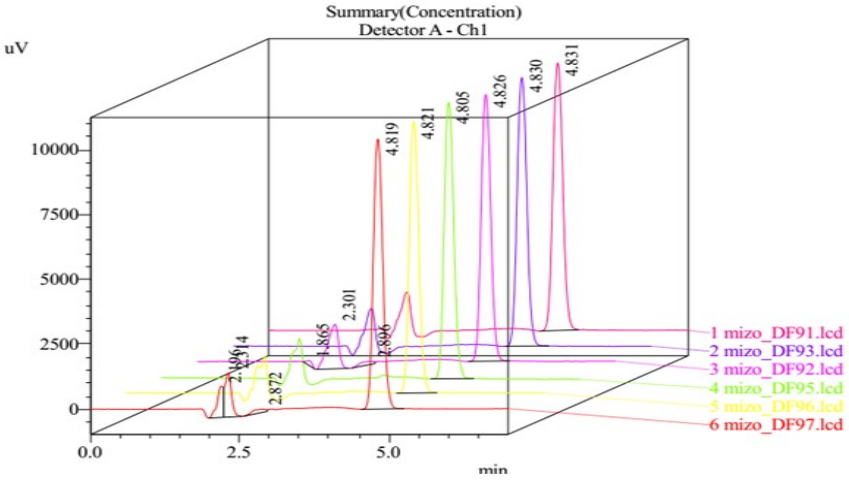

Figure 6: Overlain Chromatogram for lowest concentration of Range

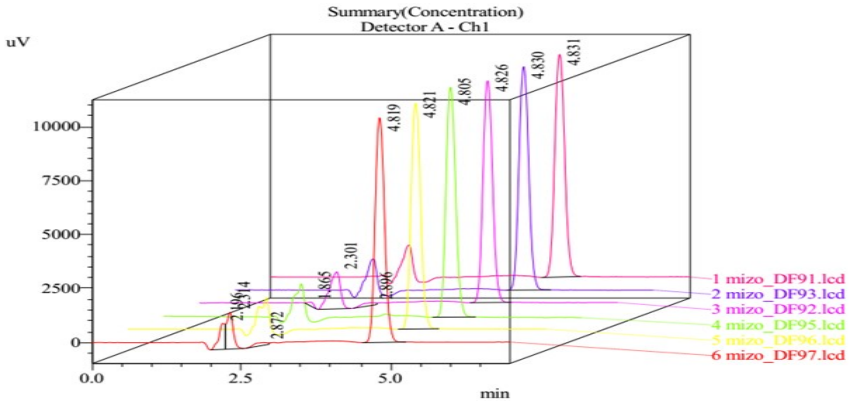

Figure 7: Overlain Chromatogram for highest concentration of Range

Table 2: Range data for Nateglinide

S. No.	Nateglinide	
	Peak area lowest concentration (20µg/ml)	Peak area highest concentration (300µg/ml)
1	118399	1769071
2	114775	1837505
3	117458	1835914
4	119767	1807302
5	116981	1797621
6	114569	1815512
Mean	1169915	1810488
S.D	2033.891	25662.11
% RSD	1.73	1.41

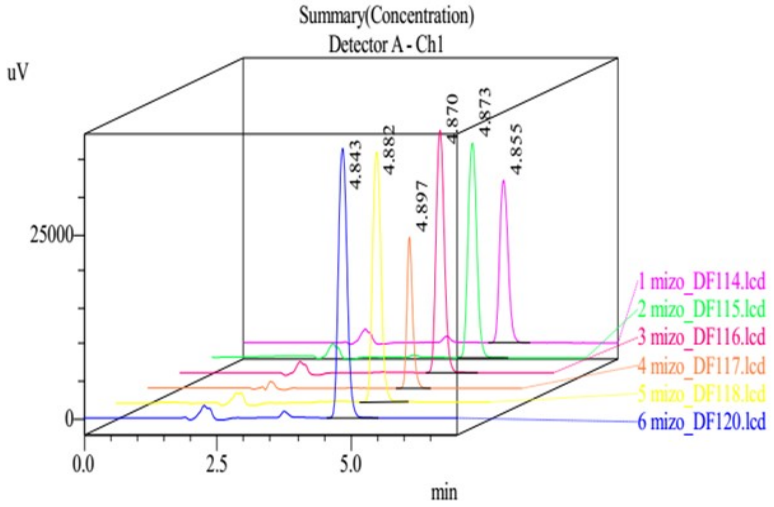

Figure 8: Overlain chromatogram for system precision

Table 3: Results of System Precision

Injections	Peak area
1	2292371
2	2267211
3	2286432
4	2317321
5	2323173
6	2319594
Mean	2301017
S.D	22504.1
% RSD	0.97

Table 4: Results of Method Precision

Injections	Peak area
1	242401
2	332447
3	352967
4	156645
5	377027
6	416042
Mean	354147
S.D	223134.41
% RSD	1.04

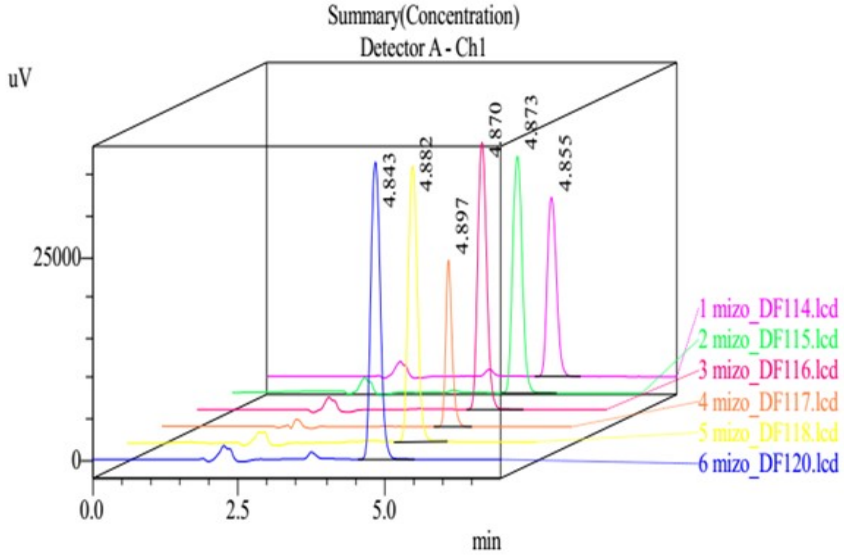

Figure 9: Overlain chromatogram for Method precision

Table 5: Interday precision results

S. No.	Day I	Day II
Injections	Peak area	Peak area
1	975506	965982
2	971984	963463
3	974353	965666
4	975486	966217
5	980225	952363
6	974403	973699
Mean S.D % RSD	975326.2 2721.243 1.23	964565 6920.728 0.79

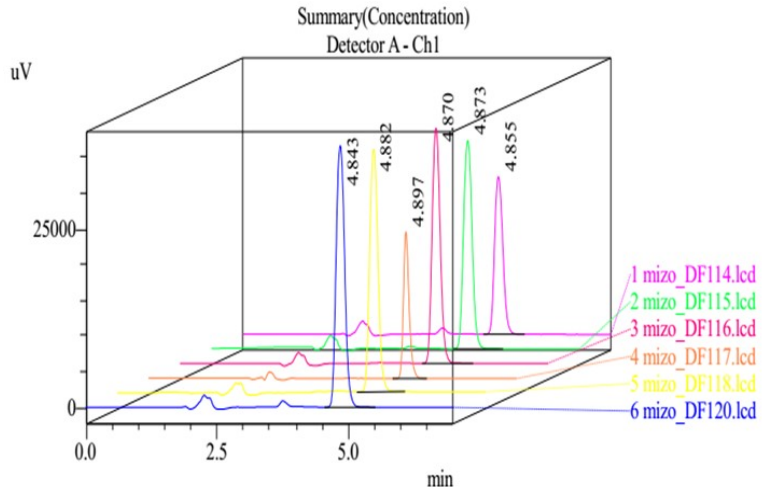

Figure 10: Overlain Chromatogram for Inter day precision (Day-1)

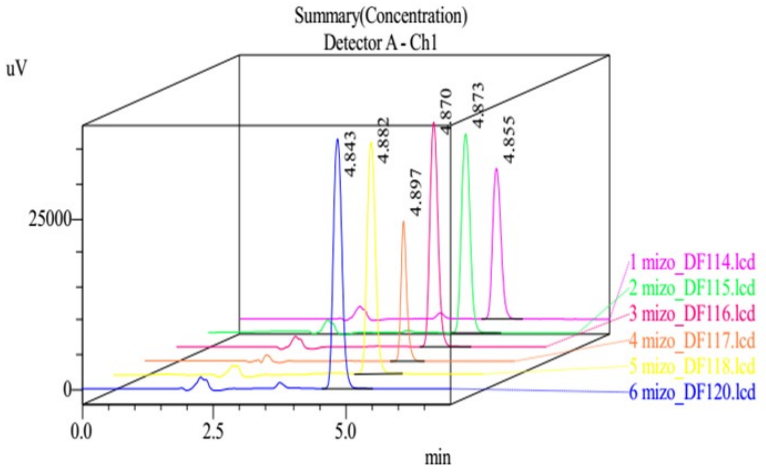

Figure 11: Overlain Chromatogram for Inter-day precision (Day-2)

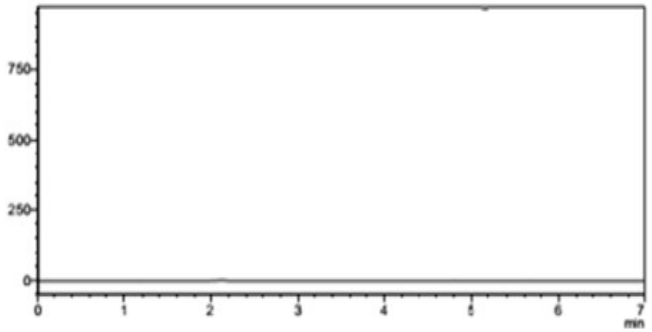

Figure 12: Chromatogram of blank

Table 6: Accuracy Study data

Spikedlevel	Standard		TestPeak area	SpikedPeak area	%Recovery
	Conc. (µg/ml)	Peak area			
50%	50	341713	725006	1058792.3	98.7
		329370		1048421.1	
		330276		1037341.2	
100%	100	644076		2637400	98
		636958		2625221	
		631360		2613434	
150%	150	873887		1616883	101
		896944		1513684	
		904800		1512048	

Table 7: Robustness Study (Change in flow rate) data

Flow rate	Parameters	Replicate injections						Statistical analysis		
		1	2	3	4	5	6	Mean	S.D	%RSD
0.9ml/min	Peak area	4951485	4859216	4832101	4803638	4883961	4986101	4886084	70311	1.43
	Rt	4.921	4.953	4.936	4.918	4.920	4.904	4.9253	0.0169	0.34
1ml/min	Peak area	5293216	5325926	5382185	5265832	5307295	5323756	5301574	56255.3	1.45
	RT	5.052	5.045	5.056	5.074	5.065	5.073	5.056	0.0188	0.375
1.1ml/min	Peak area	5504321	5607885	5637361	5762585	5672628	5581324	5627684	87260.03	1.55
	Rt	5.129	5.132	5.162	5.106	5.187	5.142	5.148	0.028	0.54

Table 8: Robustness Study (Change in mobile phase ratio) data

Mobile phase	Parameters	Replicate injections						Statistical analysis		
		1	2	3	4	5	6	Mean	S.D	%RSD
A:B:68:32	Peak area	1932960	1957501	1925482	1915264	1962354	1952983	1941091	19181.3	0.98
	Rt	5.125	5.105	5.131	5.102	5.101	5.142	5.117	0.017	0.33
A:B:70:30	Peak area	1892932	1865956	1853672	1893427	1852837	1861627	1850075	1855.9	0.99
	Rt	5.01	5.02	5.04	5.01	5.03	5.01	5.02	0.012	0.25
A:B:72:28	Peak area	1785321	1765238	1742658	1768523	1795875	1785625	1773873	19135.3	1.078
	Rt	4.92	4.91	4.93	4.97	4.92	4.95	4.93	0.022	0.45

Table 9: Robustness Study (Change in detection wavelength) data

Wave length	Parameters	Replicate injections						Statistical analysis		
		1	2	3	4	5	6	Mean	S.D	%RSD
285nm	Peak area	5602732	5529012	5677220	5557438	5573709	5661005	5600186	58709.17	1.04
	Rt	3.87	3.88	3.87	3.87	3.87	3.87	3.871	0.0040	0.10
287nm	Peak area	4182927	4290124	4250194	4241044	4231380	4310519	4251031	45148.2	1.06
	Rt	3.83	3.83	3.83	3.83	3.82	3.82	3.826	0.0051	0.13
289nm	Peak area	2605905	2574553	2645246	2583799	2559668	2601012	2595031	29905.22	1.15
	Rt	3.87	3.87	3.86	3.86	3.86	3.86	3.86	0.0051	0.13

Assay of formulation: In view of the establishment of chromatographic conditions, the device was balanced to obtain a permanent gauge. At that time, clear standard solutions and equal amounts of the test solutions (prepared as per procedures given above) were independently distributed three times into the HPLC system and chromatograms were recorded as shown in **Figures 13 and 14**. The maximum area response of the analyzed peak was estimated and shown in **Table 10**. The amount recovered in mg/tablet was calculated by using the following formulae:

$$\text{Amount} = \frac{\text{Concentration obtained X Dilution factor X Average weight}}{\text{Sample taken for analysis}}$$

$$\text{Amount} = \frac{0.296 \text{ X } 83.33 \text{ X } 143}{362} = 9.694\text{mg}$$

$$\%\ \text{Assay} - \frac{\text{Amount obtained}}{\text{Label claim}} \text{X100}$$

$$\%\ \text{Assay} = \frac{9.69}{10} \text{X100} = 96.94\%$$

Table 10: Results of Assay Data

Parameter	Mizolastine
Mean peak area	698531
Weight taken(mg)	362
Label claim (mg)	10
Average weight (mg)	143
Amount recovered	9.694mg
%Assay	96.94%

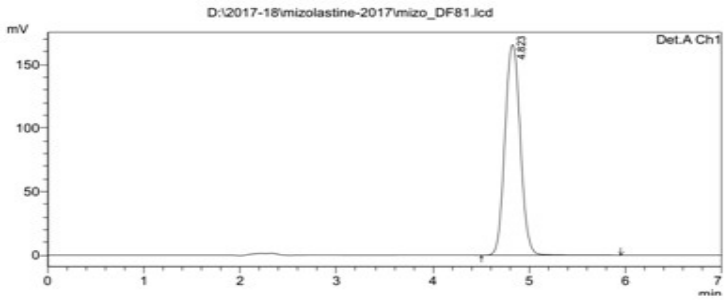

Figure 13: Chromatogram of standard

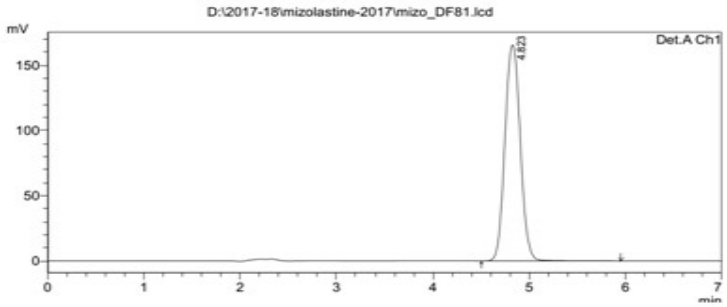

Figure 14: Chromatogram of test formulation

RESULTS AND DISCUSSION

At first the solubility tests were directed on the standard of Nateglinide. In view of the consequences of the solubility test, a blend of methanol, buffer of pH 6.8 and acetonitrile in a proportion of 47:23:30 was chosen as mobile phase.

Ideal consequences have been accomplished by means of a Phenomenex kinetex C_{18} (250mm×4.6mm i.d, 5µm) column at flow rate of 1ml/min at a recognition wavelength of 287nm. The peak of Nateglinide was eluted at retention time of 4.8min and run time of 7minswith great goals, top size and balance. From system suitability results, it ensures that both methodology and instrumentation are within the expectations. All the system suitability parameters were satisfied; hence the method passed the system suitability test.

The optimized method was used to assay for tablets. Assay value was found to be 96.94%. Not showing any interference due to excipients and diluents. The method is found suitable for the determination of Nateglinide in making tablets. The method is subject to verification as per ICH guidelines. Linearity was set up over the focus scope of 20-300µg/ml. The regression equation was given as Y= 52305X+ 12968. Correlation coefficient was seen as 0.9996.These results show that there was an outstanding correlation among peak area as well as concentration of drug in the focused range.

The robustness studies were carried out by changing the flow rate, mobile phase composition and wavelength. The% RSD at 0.9ml/min was seen as 1.43 and 1.1ml/min at 1.55. At wavelength of 285nm, the %RSD was observed as 1.04 and at 289nm it was 1.15. In the portable stage of methanol and phosphate buffer in the relation of 68:32, the %RSD was found to be 0.98 and in the 72:28 ratio methanol and buffer, it was found to be 1.07% individually. %RSD of the peak areas was seen to be below 2% even after changing the mobile phase composition, detection wavelength and stream rate and finally it indicates that the method was robust.

Chromatogram of blank solution did not show any peaks at the retention time of Nateglinide. Hence the method was found to be more specific as there was no blank interference.

CONCLUSION

HPLC technique creates enormous measure of value information, which fills in as exceptionally ground-breaking and helpful expository apparatus. A basic, exact, precise, powerful technique was produced for the normal investigation of the Nateglinide by performing different preliminaries. The example recovery from the plan was in great concurrence with the name guarantee, which recommended non-obstruction of detailing excipients and diluents in the estimation. The technique was effectively approved as far as linearity, exactness, precision and robustness according to ICH rules. The strategy gives a straight reaction over a wide scope of focuses. Subsequently it tends to be presumed that the proposed technique was a decent approach for acquiring solid outcomes and saw as reasonable for the normal investigation and quality control of Nateglinide in bulk and tablet dosage form.

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CONFLICT OF INTEREST: The authors declare that they have no conflict of interests.

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