Research Article

Development and validation of the quantitative estimation of Nateglinide in bulk and its marketed formulation by RP-HPLC method

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Abstract

A simple, rapid, explicit, and extremely expensive fluid chromatographic technique, Nateglinide mass and claimed drug structure have been established. Methanol: Phosphate support (55:45) v/v as cell area at a float cost of 1.0 ml min1; the temperature of the reliable section was ambient. This section was divided using a balance of ODS C18 (four. 6250 mm, five meters). The run time under these chromatographic conditions was less than an hour. Finally, 2.52 were chosen as the maintenance interval for Nateglinide. In the symmetry plot over the visual range of 6–14ng, there were two cutoff values for identification and measurement: 1.2 and 3.6 ng per milliliter, respectively. While the percent mending was not quite resolved in the region of 98-102 percent, the recommended percent test of promoted extra compounds came out to be 99.86 percent. The relative stranded deviation of the accuracy study was found to be 2%. The novel method is excellent, simple, quick, precise, and suitable for evaluating Nateglinide in large quantities while maintaining the drug's quantity strategy.

Keywords: Nateglinide, RP-HPLC, accuracy, precision, marketed formulation

Introduction

Nateglinide, a derivative of amino acid and an oral hypoglycemic agent, functions by stimulating the secretion of insulin from the pancreas and is employed in the treatment of type 2 diabetes. The IUPAC name of Nateglinide is 3-phenyl-2-[(4-propan-2-ylcyclohexanecarbonyl) amino] propanoic acid. The chemical formula is $C_{19}H_{27}NO_3$ and molecular weight is 317.42 g/mol. The mechanism of action of Nateglinide is as a Potassium Channel Antagonist. It is a white powder. It is soluble in methanol, chloroform, ethanol, acetonitrile, and octanol, but insoluble in water. The structural formula of Nateglinide was shown in figure 1.

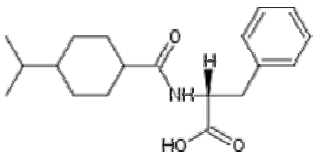

Figure 1: structure of Nateglinide

Materials and methods

Instrument and Chemical resources

HPLC Waters alliance 2695 separation module Empower-2 software, 996 PDA detectors, pH meter lab India, Digital balance Sartorius and Digital ultra sonicater, Nateglinide (Bulk & tablet), Acetonitrile and Methanol (Merck)

Method development

Preparation of standard stock solution

Weigh accurately about 10mg of Nateglinide and transfer into 10ml volumetric flask to this add 7ml of methanol and sonicate it finally make up the volume with methanol(1000ppm). Pipette out 1ml of the above solution and make up to 10 ml with methanol (100ppm).

Preparation of sample stock solution

Weigh accurately equivalent to 10mg of Nateglinide and transfer into 10ml volumetric flask to this add 7ml of

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methanol and sonicate it finally make up the volume with methanol (1000ppm) The stock solution is degassed for 10min and filter with 0.45µm nylon filter (Stock solution). Pipette out 1ml of the above solution and make up to 10 ml with methanol (100ppm).

Preparation of mobile phase

Measure accurately about 600ml of Acetonitrile and 400ml water were mixed and degassed in Ultra Sonicater for 10 min and then filtered through 0.2µ filter under vacuum filtration.

Chromatographic condition

HPLC system (Waters HPLC with auto Sampler and PDA detector) column ODS C18 (250 x 4.6mm; 5µm) HPLC grade, acetonitrile, methanol and phosphate buffer were used for the preparing the mobile phase. A freshly prepared, methanol: phosphate buffer (pH -3.5) (55:45) v/v) was used as the mobile phase. The solvent was filtered through a 0.45µ membrane filter and sonicated before use. The flow rate of the mobile phase was maintained at 1.0 mL/min, Column temperature was maintained at room temperature and the detection of the drug was carried out at 225 nm.

Validation

Linearity

Five duplicate assessments were carried out over a specific time frame to determine the linearity range of 10, 20, 30, 40, and 50 µg/ml. Calibration standards were meticulously prepared for the linearity studies. The calibration curve was then constructed using the drug's concentration and the corresponding peak areas. To assess the precision, the % RSD was calculated (Pravalika and Lavanya, 2023; Bhadru et al., 2023; Patel et al., 2023; Tanpure et al., 2023; Swapna et al., 2018).

Accuracy

In order to evaluate the precision of the developed technique, a study was conducted to recover Ulipristal Acetate. The accuracy of the method was determined by employing the conventional addition method to calculate the recoveries of Nateglinide. A pre-quantified sample solution (10µg/ml) was combined with a known quantity of Nateglinide standard solutions (80%, 100%, and 120%). By utilizing a calibration curve, the quantity of Nateglinide present was determined (Boggula et al., 2023; Reddy et al., 2023; Pravalika and Lavanya, 2023; Bhadru et al., 2023).

Precision

The precision of the analytical method was assessed by conducting multiple samplings of a homogeneous sample. To demonstrate the reproducibility of the method, measurements of peak area and peak symmetry parameters were taken for both repeatability and intermediate precision. The intermediate precision was tested using single concentration levels over a period of two days, while repeatability was tested within a day in triplicates. Six injections were carried out, and the results were expressed as a percentage of RSD within and between trial days (Srinidhi et al., 2016; Tanpure et al., 2023; Bhadru et al., 2023).

Limit of detection (LOD) and Limit of quantification (LOQ)

Calculation of LOD and LOQ, as per ICH guidelines, relies on the signal-to-noise ratio. A signal to noise ratio of 3:1 and 10:1 was considered when determining LOD and LOQ (Pravalika and Lavanya, 2023; Bhadru et al., 2023; Patel et al., 2023; Tanpure et al., 2023; Swapna et al., 2018).

Robustness

Intentional alterations to the protocol, such as adjusting the rate of flow and the wavelength of detection, were implemented to assess the reliability of the technique (Pravalika and Lavanya, 2023; Bhadru et al., 2023; Patel et al., 2023; Tanpure et al., 2023).

Results and discussion

The aim of this study was to develop a simple, accurate and precise HPLC method for the analysis of Nateglinide in bulk and tablet dosage forms using mobile phase and commonly employed Symmetry C18 column with UV detector at 285 nm. Chromatograms obtained under optimal conditions underwent a system suitability assessment to confirm various criteria, such as theoretical, resolution, asymmetry, and tailing factor. The typical chromatogram of Nateglinide was shown in Figure 2.

Optimized chromatographic conditions

Column: Symmetry ODS C18 (250 x 4.6mm; 5µm)

Mobile phase ratio : Phosphate buffer: Methanol (45:55% v/v)

Detection wavelength	: 225 nm
Flow rate	: 1.0 ml/min
Injection volume	: 10µl
Column temperature	: Ambient
Run time	: 8 min
Retention time	: 2.252 min

Linearity

In order to check the linearity for the developed method, solutions of five different concentrations ranging from 60µg/mL - 140µg/mL were prepared. The chromatograms were recorded, and the peak areas were given in table 2.

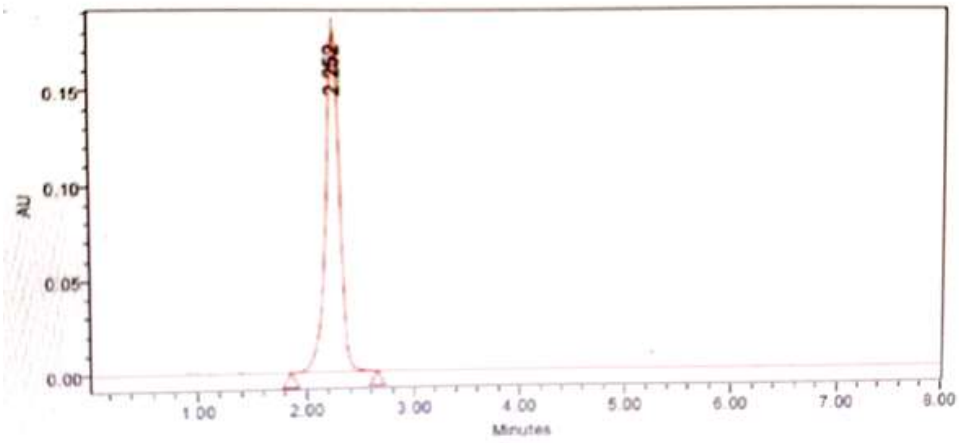

Figure 2: Chromatogram of Nateglinide in Optimized Condition

Table 1: Summary of method optimization

Column Used	Mobile Phase	Retention time(min)	Peak Area	Plate Count	Tailing Factor	Flow Rate
Symmetry ODS C_{18} RP Column, 250 mm x 4.6 mm, 5µm	Phosphate buffer: Methanol	8.0	1658242	6569	1.24	1.0ml/min

Linearity graph was shown in figure 3.

Accuracy: Recovery studies were used to determine the method's accuracy, and the % recovery was calculated. Nateglinide recovery rates were reported to be in the range of 99.91 percent. Nateglinide concentration was determined using the suggested Liquid Chromatographic technique. For Nateglinide, the results were comparable to the labeled amounts was shown in table 3.

Precision

Repeatability

The peak areas and retention periods acquired by real determination of six replicates of a given quantity of medication were used to determine the precision of each approach individually. Nateglinide is a type of Nateglinide that (API). The % relative standard deviation for Nateglinide was calculated and is shown in table 4.

Intermediate Precision

The Intermediate Precision consists of two methods:

Intra and Inter Day: In Intra Day process, the 80%, 100% and 120% concentration are injected at different intervals of time in same and different days.

LOD and LOQ

The slope of line and variance acquired from accuracy studies were used to evaluate the limit of detection (LOD) and the limit of quantization (LOQ) parameters.

The limit of detection LOD = 3.3(SD/S)

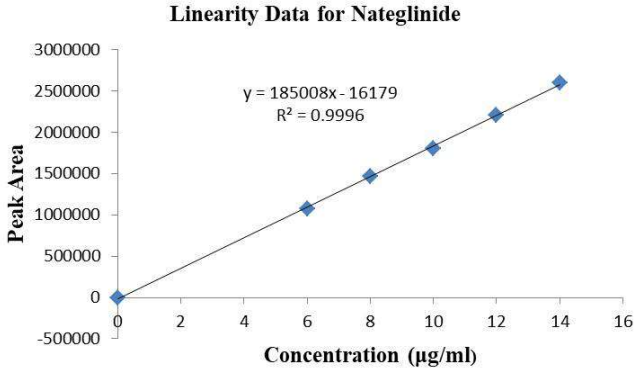

Figure 3: Calibration Curve of Nateglinide

Table 2: Linearity Data for Nateglinide

Concentration (µg/ml)	Area	Statistical data of Nateglinide	
6	1078475	**Y= mx +c**	
8	1461129	Slope	18500
10	1808358	Intercept	16179
12		Correlation	0.999
	2211573	coefficient(r^2)	
14	2593778		

Table 3: Shown accuracy observation of Nateglinide

Accuracy	Amount taken(mg)	Amount Added(mg)	Amount Recovered	Peak Area	% Recovery	Mean recovery
80%	100	80	80.698	603517	100.997	
	100	80	80.773	604598	100.841	100.57
	100	80	80.656	605213	100.945	
100%	100	100	99.833	746471	99.933	
	100	100	100.083	745574	100.083	100.22
	100	100	100.565	747652	100.365	
120%	100	120	120.390	894415	100.241	
	100	120	120.301	896762	100.167	100.25
	100	120	120.242	895541	100.368	

Table 4: Repeatability data for Nateglinide

S. No.	Injection	Peak Area
1	Injection 1	743826
2	Injection 2	745277
3	Injection 3	742506
4	Injection 4	747576
5	Injection 5	746715
6	Injection 6	741278
7	**Average**	**744529.6667**
8	**SD**	**2440.4116**
9	**% RSD**	**0.32777**

Table 6: Data of System Suitability Parameter

S. No	Parameters	Limit	Results
1	Retention time	Rt>2	4.783
2	Asymmetry	T≤2	1.35
3	Theoretical plates	N>2000	2865
4	Tailing factor	T<2	1.37

Table 5: Results of intra-assay & inter-assay

Concentration of (API) (µg/ml)	Observed concentration of Nateglinide (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Mean(n=6)	%RSD	Mean(n=6)	%RSD
80	80.38	0.56	80.45	0.56
100	100.17	0.71	100.50	0.77
120	120.89	0.89	120.91	0.85

The limit of quantization LOQ= 10(SD/S)

Where as,

SD =Standard deviation

S =Slope

The lowest concentration level at which the analyte can be reliably detected (LOD) and quantified (LOQ) was found to be 0.07 g/ml and 0.21 g/ml, respectively.

System Suitability

Many analytical processes include system suitability testing as part of the process. The tests are founded on the idea that the equipment, electronics, analytical activities, and samples to be studied are all part of a larger system that may be evaluated. The parameters for the system suitability test were established.

Method Robustness: Minute changes in chromatographic conditions such as flow rate 1.0 ml (0.1 ml/min), Wavelength of detection 284 (2nm), and organic phase content in mobile phase (5%) were studied to determine the method's robustness, and the results of (percent RSD 2%) were in shown in table 7.

Estimation of Nateglinide in Pharmaceutical Dosage Form

To determine the average weight of 20 tablets crush with mortar and pestle. A quantity of powder equivalent to 25 mg

Table 7: Result of method Robustness test

Changes in parameters	%RSD
Flow(1.1ml/min)	0.45
Flow(0.9ml/min)	0.38
More organic	0.76
Less organic	0.65
Wavelength of detection(286nm)	0.98
More organic	0.93

Table 8: Recovery Data for estimation of Nateglinide

Brand Name	Labeled amount(mg)	Mean(±SD)	Assay%
Glinate	60mg	60.10 (±0.468)	100.34

of powder was transfer into 25 ml volumetric flask and sonicated for 15 minutes, and the volume was made up to 25 ml with the mobile phase. Then, 10 mL of the aforementioned solution was diluted to 100 mL. The results were recorded in table 8.

Conclusion

For the analysis of Nateglinide API, a sensitive and selective RP-HPLC technique has been designed and validated. Additionally, the suggested RP-HPLC method possesses high sensitivity, precision, and repeatability. The results suggest that the developed approach is yet another suitable assay, purity, and analysis method for Nateglinide in various formulations.

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