Research Article

QUANTITATIVE ESTIMATION BY METHOD DEVELOPMENT AND VALIDATION OF NATEGLINIDE IN BULK AND ITS MARKETED FORMULATION BY RP-HPLC METHOD

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ABSTRACT

A simple, rapid, specific and accurate reverse phase high performance liquid chromatographic method has been developed for the validated of Nateglinide in bulk as well as in marketed pharmaceutical dosage form. This separation was performed on a Symmetry ODS C18 (4.6×250mm, 5µm) column with Methanol: Phosphate Buffer (55:45) V/V as mobile phase at a flow rate of 1.0 ml/ min with UV detection at 225 nm; the constant column temperature was Ambient. The run time under these chromatographic conditions was less than 8 min. The retention time of Nateglinide was found to be 2.252. The calibration plot was linear over the concentration range of 6–12 µg/ ml with limits of detection and quantification values of 1.2 and 3.6 µg/ mL respectively. The mean % assay of marketed formulation was found to be 99.86%, and % recovery was observed in the range of 98-102%. Relative standard deviation for the precision study was found <2%.The developed method is simple, precise, specific, accurate and rapid, making it suitable for estimation of Nateglinide in bulk and marketed pharmaceutical dosage form dosage form.

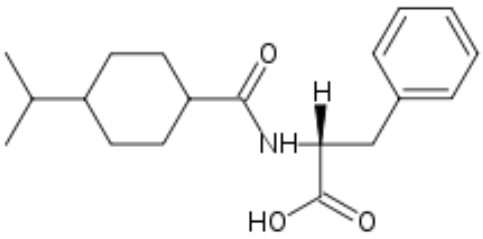
Keywords: Nateglinide, RP-HPLC, Phosphate buffer, Methanol, Starlix tablets, Sonicator.

INTRODUCTION

Pharmaceutical analysis comprises of the procedures necessary to determine the identity, strength, quality and purity of substances of therapeutic importance. It also deals not only with medicaments (drugs and their formulations) but also with their precursors i.e. with the raw material on which degree of purity and quality of medicament depends. The quality of the drug is determined after establishing its authenticity by testing its purity and the quality of pure substance in the drug and its formulations.

HPLC is also called as high pressure liquid chromatography since high pressure is used to increase the flow rate and efficient separation by forcing the mobile phase through at much higher pressure. The HPLC is the method of choice in the field of analytical chemistry, since this method is specific, robust, linear, precise and accurate and the limit of detection is low and also it offers the following advantages.

1. Improved resolution of separated substances
2. column packing with very small (3,5 and 10 µm) particles
3. Faster separation times (minutes)
4. Sensitivity
5. Reproducibility
6. continuous flow detectors capable of handling small flow rates
7. Easy sample recovery, handling and maintenance.



Structure of Nateglinide

Nateglinide is an oral anti-hyperglycemic 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 is freely soluble in methanol, ethanol, and chloroform, soluble in ether, sparingly soluble in acetonitrile and practically insoluble in water.

Mechanism of Action

Nateglinide activity is dependent on the presence functioning β cells and glucose. It potentiates the effect of extracellular glucose on ATP-sensitive potassium channel and has little effect on insulin levels between meals and overnight. Nateglinide is more effective at reducing postprandial blood glucose levels than fasting blood glucose levels and requires a longer duration of therapy. Nateglinide appears to be selective for pancreatic β cells and does not appear to affect skeletal or cardiac muscle or thyroid tissue.

Side Effects

An overdose may result in an exaggerated glucose-lowering effect with the development of hypoglycemic symptoms.

Marketed Formulation

Literature review showed that the methods were not reported by using phosphate buffer and methanol in suitable ratio for Nateglinide. Hence, the current method focused on the development of accurate, simple, precise analytical method and was validated according to ICH guidelines.

EXPERIMENT

Instruments used: RP-HPLC, UV-Visible Spectrophotometer, Ultra Sonicator, pH meter and weighing balanc**e.**

Chemicals used: Nateglinide pure sample (startech Labs, Hyderabad.), Methanol, Phosphate buffer, Starlix tablets available from local market.

Method:

Preparation of stock solution:

Accurately weigh and transfer 10 mg of Nateglinide working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol. Further pipette 0.1ml of the above Nateglinide stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Optimization of the method:

The standard solution is scanned for wavelength in UV-Visible Spectrophotometer from 200-400nm and the wavelength is found to be 225nm.

The method was developed by varying different conditions and parameters like columns like phenomenox column, Symmetry ODS column, Devilosil ODS column etc., and mobile phases like acetonitrile, methanol, water and phosphate buffer etc.,

The optimized method is observed in the following conditions:

Mobile phase ratio	: Methanol: Phosphate Buffer (55:45) V/V
Column	: Symmetry ODS C18 (4.6×250mm, 5µm)
Column temperature	: Ambient
Wavelength	: 225nm
Flow rate	: 1ml/min
Injection volume	: 10µl
Run time	: 8min

This method is considered as optimized because the tailing factor is less than 2 and plate count is more than 2000.

Validation:

The present method is validated for the following parameters like Accuracy, Precision, Linearity, Range, Robustness, LOD, LOQ and Ruggedness etc., as per the ICH guidelines.

Validation Parameters:

Assay:

Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Nateglinide working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.1ml of the above Nateglinide stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Preparation of Sample Solution:

Weight 10 mg equivalent weight of Nateglinide sample into a 10mL clean dry volumetric flask and add about 7mL of diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 0.1ml of Nateglinide above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure:

Inject the three replicate injections of standard and sample solutions and calculate the assay.

Linearity:

The solutions were prepared in the concentration of 6ppm to 14ppm concentrations. 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.

$$\textbf{Slope} = y = mx + c$$

Precision:

The precision was calculated for Intermediate precision and repeatability. The solutions were prepared and injected 3 times and observed the RSD and standard deviation.

Accuracy:

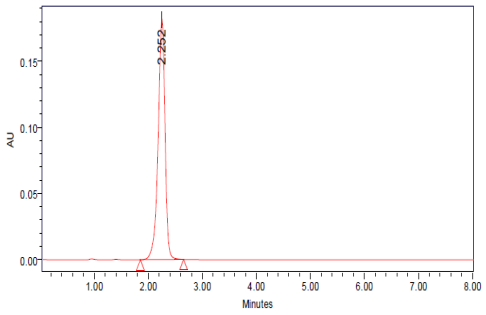
The different concentrations like 50%, 100% and 150% spiked concentrations are prepared and injected. The chromatograms are recorded and the peak responses are measured. Calculate the Amount found and Amount added for Nateglinide and calculate the individual recovery and mean recovery values.

Robustness:

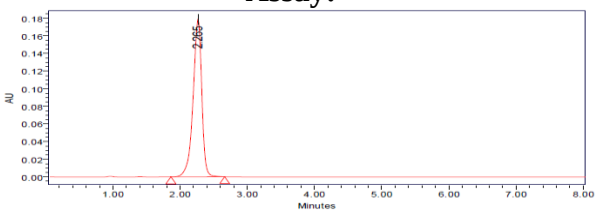
The analysis was performed in different conditions to find the variability of test results like change in flow rate and mobile phase and run time.

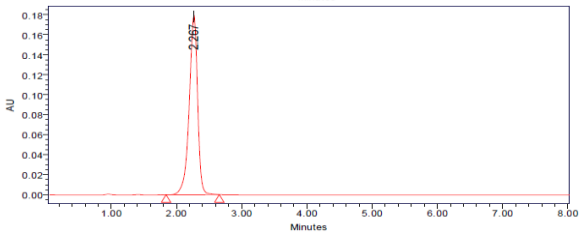
Results:
Optimized chromatogram

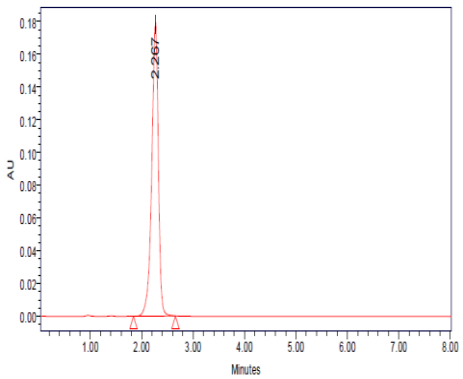
S. No.	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Nateglinide	2.252	1658242	185421	1.24	6569



Assay:



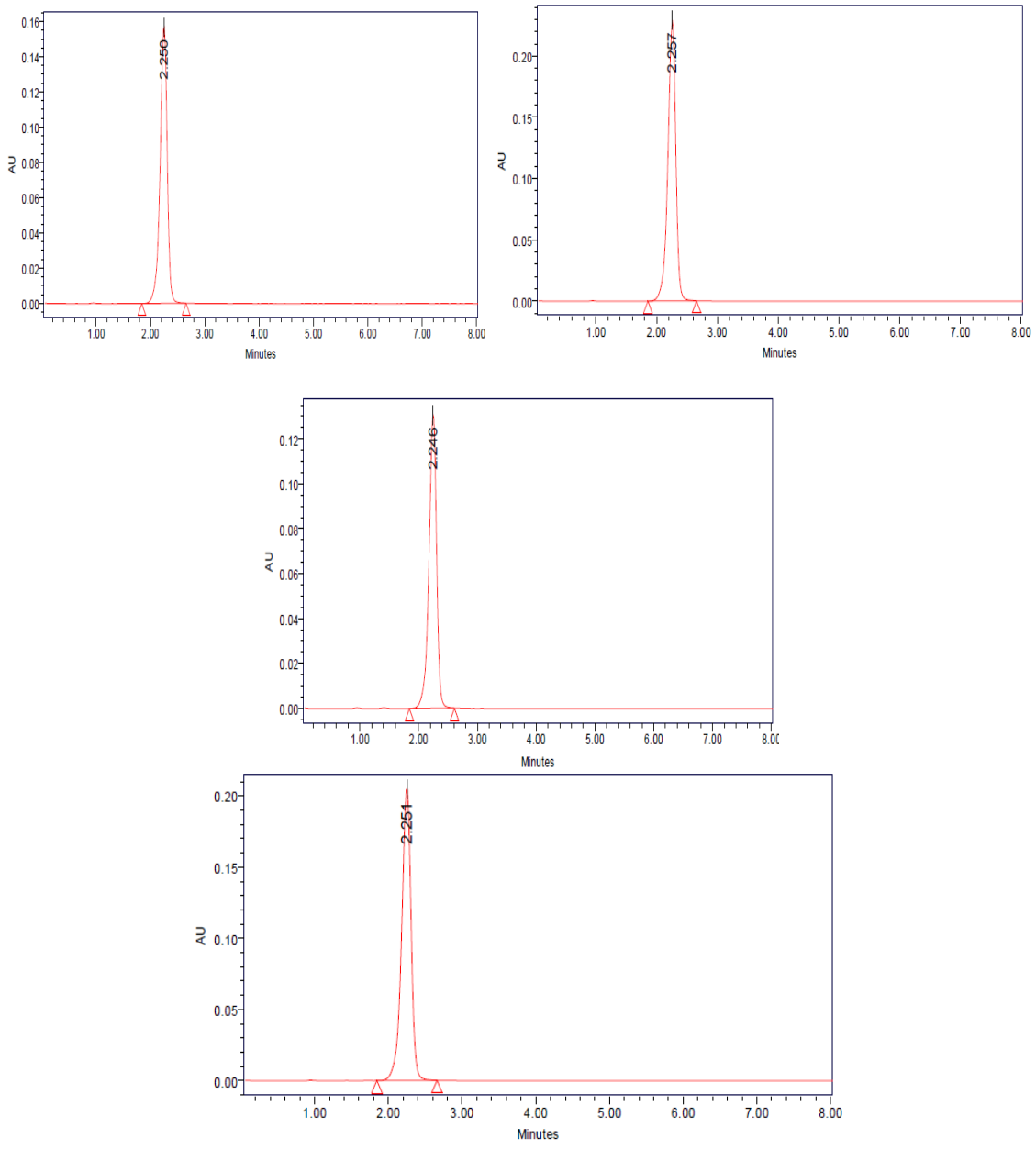


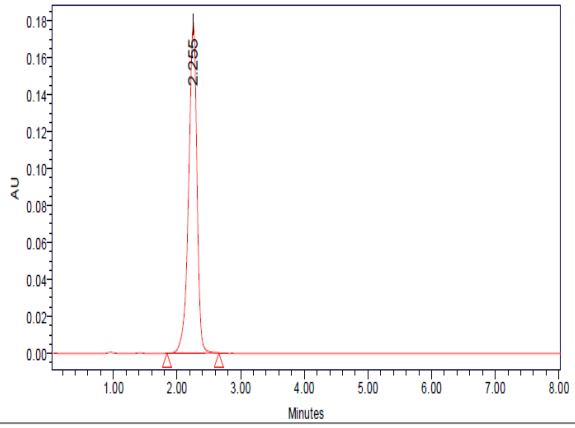


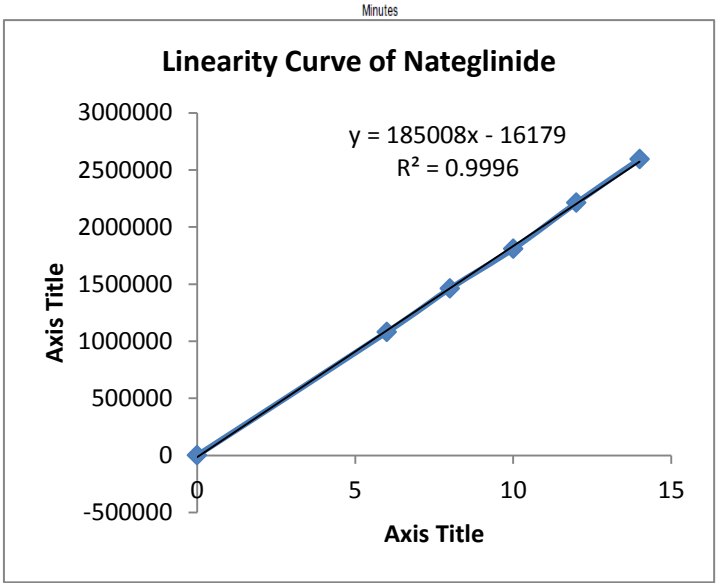
S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Nateglinide	2.265	1658254	185468	1.24	6391	1
2	Nateglinide	2.267	1658475	184524	1.23	6549	2
3	Nateglinide	2.267	1658471	186598	1.25	6682	3

The % purity of Nateglinide in pharmaceutical dosage form was found to be 99.86%.

Linearity:







Repeatability:

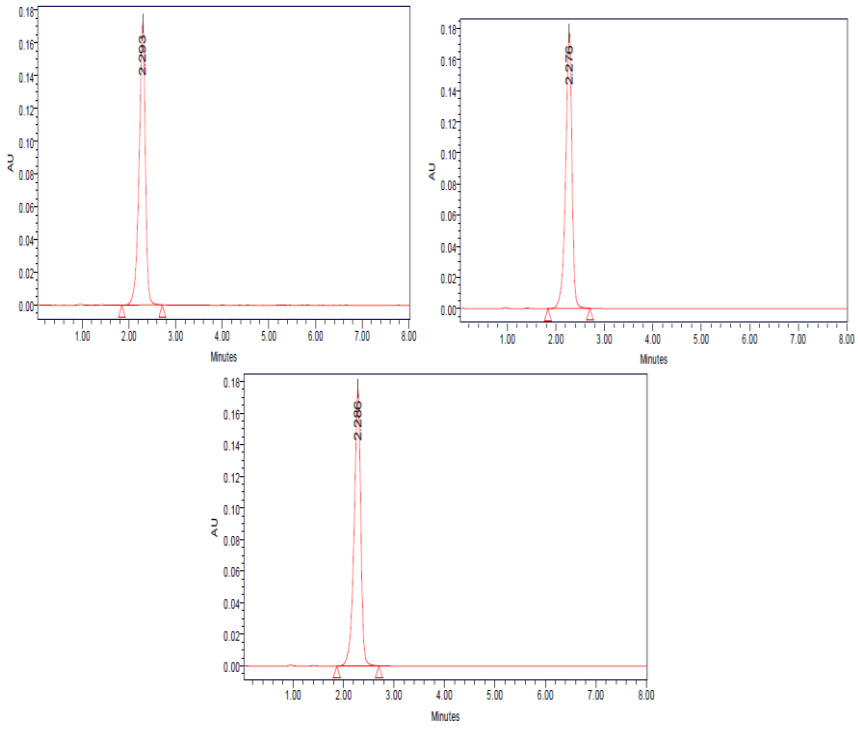
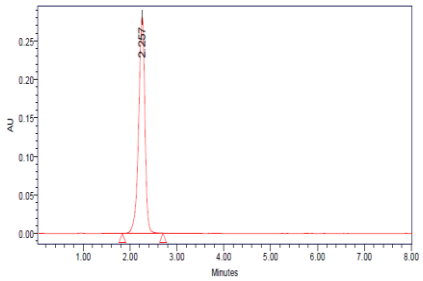


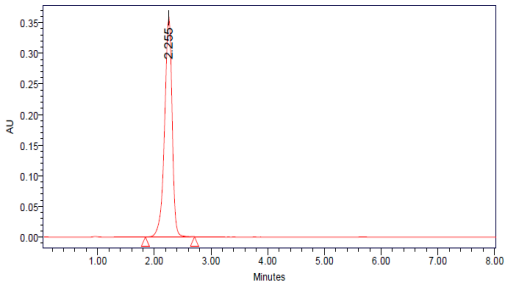
Table showing repeatability results:

	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Nateglinide	2.293	1658954	186958	1.26	6785
2	Nateglinide	2.276	1658745	187548	1.27	6854
3	Nateglinide	2.286	1659865	189854	1.26	6852
Mean			1657118			
Std.dev			2913.592			
%RSD			0.175823			

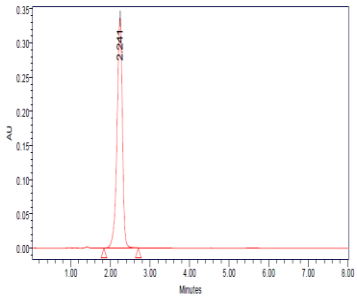
Accuracy:



50% concentration



100% concentration



150% concentration

Table showing Accuracy results:

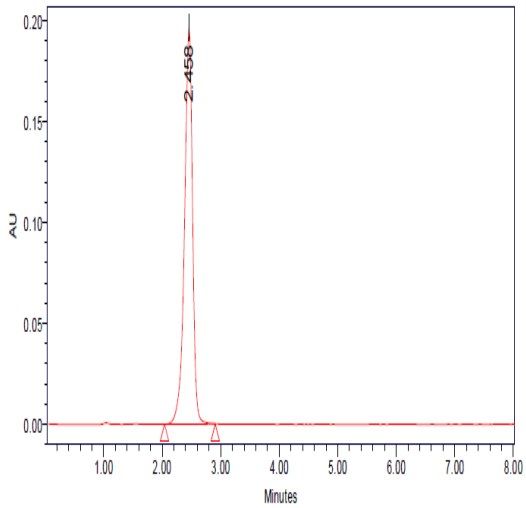
%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	109068.3	5	5.021	100.420%	100.72%
100%	202187	10	10.054	100.540%	
150%	297032.3	15	15.181	101.206%	

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.

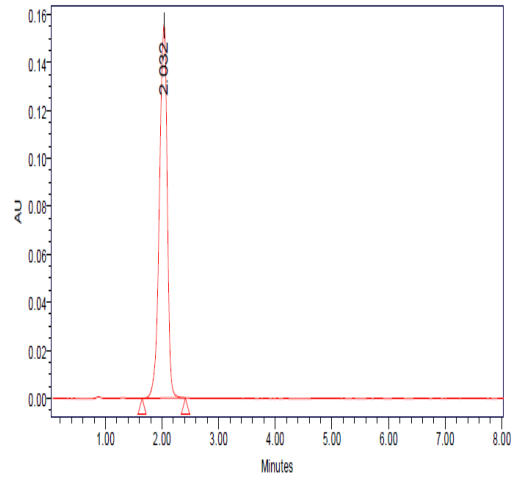
Limit of detection: It was calculated from calibration curve values and it was found to be 1.2µg/ml

Limit of quantification: It was calculated from calibration curve values and it was found to be 3.6µg/ml.

Robustness:



Flow rate 0.8 ml/min



Flow rate 1.2 ml/min

Robustness values:

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 ml/min	1658242	2.312	6569	1.24
Less Flow rate of 0.9 ml/min	1854215	2.458	6865	1.35
More Flow rate of 1.1 ml/min	1758468	2.032	6254	1.32

Acceptance criteria:
The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000

CONCLUSION:
In the present study/work, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Nateglinide in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps.
Nateglinide was freely soluble in methanol, ethanol, and chloroform, soluble in ether, sparingly soluble in acetonitrile and practically insoluble in water. Methanol was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the

method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Nateglinide in bulk drug and in Pharmaceutical dosage forms.

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