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Research Article

VALIDATED RP-HPLC METHOD FOR THE ESTIMATION OF NATEGLINIDE IN FORMULATION

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ABSTRACT

A simple, selective, linear, precise and accurate RP-HPLC method was developed and validated for rapid assay of Nateglinide in tablet dosage form. Isocratic elution at a flow rate of 1.0ml/min was employed on a symmetry C18 (250x4.6mm, 5µm in particle size) at ambient temperature. The mobile phase consisted of methanol: acetonitrile: 0.05M KH_2PO_4 20:55:25 (V/V/V). The UV detection wavelength was 215nm and 20µl sample was injected. The retention time for Nateglinide was 6.040 min. The percentage RSD for precision and accuracy of the method was found to be less than 2%. The method was validated as per the ICH guidelines. The method was successfully applied for routine analysis of Nateglinide in tablet dosage form.

Keywords: Nateglinide, RP-HPLC, UV detection, recovery, precise.

INTRODUCTION

Nateglinide Molecular formula **$C_{19}H_{27}NO$**. Molecular weight 317.423 g/mol. IUPAC Name 3-phenyl-2-[(4-propan-2ylcyclohexane carbonyl) amino] propanoic acid. Nateglinide lowers blood glucose by stimulating the release of insulin from the pancreas. It achieves this by closing ATP-dependentpotassium 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 secretion occurs[1-5].

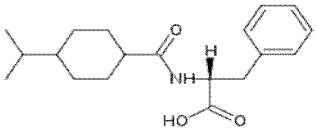

Fig.1: Chemical Structure of Nateglinide

EXPERIMENTAL

Chemicals and reagents

HPLC grade actonitrile and methanol were purchased from Merck Specalities Pvt. Ltd.

Instrumentation and analytical conditions

The analysis of drug was carried out on a PEAK HPLC system equipped with a reverse phase C18 column (250x4.6mm, 5µm in particle size), a LC-P7000 isocratic pump, a 20µl injection loop and a LC-UV7000 absorbance detector and running on PEAK Chromatographic Software version 1.06. Isocratic elution with methanol: acetonitrile: 0.05M KH_2PO_4 20:55:25 (V/V) (P^H-3.5) was used at a flow rate of 1.0ml/min. The mobile phase was prepared freshly and degassed by sonicating for 5 min before use.

Stock and Working standard solutions

Accurately weigh and transfer 10mg of Nateglinide working standard into a 10ml volumetric flask add diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45μm nylon filter paper and finally 5$\mu g/ml$ were prepared. The calibration curve was plotted with the five concentrations of the 1$\mu g/ml$ - 5$\mu g/ml$ working standard solutions. Calibration solutions were prepared daily and analyzed immediately after preparation.

Assay of Nateglinide tablets

Weigh 20 Nateglinide (Glinate-120mg) tablets and calculate the average weight. Accurately weigh and transfer the sample equivalent to 10mg of Nateglinide in to a 10ml volumetric flask. Add diluent and sonicate to dissolve it completely and make volume up to the mark with diluents. Mix well and filter through 0.45um filter. Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to mark with diluents and finally 4$\mu g/ml$ were prepared. Mix well and filter through 0.45um filter. An aliquot of this solution was injected into HPLC system. Peak area of Nateglinide was measured for the determination. The results are furnished in Table 3.

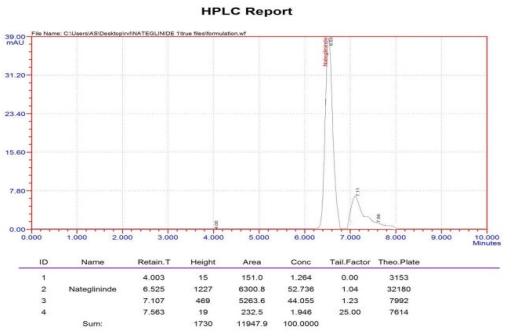

Fig 2: Typical chromatogram of Nateglinide Formulation

Validation procedure

The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The method was validated for linearity, precision (repeatability and intermediate precision), accuracy, specificity, stability and system suitability. Standard plots were constructed with five concentrations in the range of 1$\mu g/ml$ to 5$\mu g/ml$ prepared in triplicates to test linearity. The peak area of Nateglinide was plotted against the concentration to obtain the calibration graph. The linearity was evaluated by linear regression analysis that was calculated by the least square regression method. The precision of the assay was studied with respect to both repeatability and intermediate precision. Repeatability was calculated from six replicate injections of freshly prepared Nateglinide test solution in the same equipment at a concentration value of 100% (4$\mu g/ml$) of the intended test concentration value on the same day. The experiment was repeated by assaying freshly prepared solution at the same concentration additionally on two consecutive days to determine intermediate precision. Peak area of the Nateglinide was determined and precision was reported as %RSD.

Method accuracy was tested (% recovery and %RSD of individual measurements) by analyzing sample of Nateglinide at three different levels in pure solutions using three preparations for each level. The results were expressed as the percentage of Nateglinide recovered in the samples. Sample solution short term stability was tested at ambient temperature (20±10^{0}C) for three days. In order to confirm the stability of both standard solutions at 100% level and tablet sample solutions, both solutions protected from light were re-injected after 24 and 48 hours at ambient temperature and compared with freshly prepared solutions.

RESULT AND DISCUSSION

Optimization of the chromatographic conditions

Proper selection of the stationary phase depends up on the nature of the sample, molecular weight and solubility. The drug Nateglinide in non-polar. Non-polar compounds preferably analyzed by reverse phase columns. Among C8 and C18, C18 column was selected. Non-polar compound is very attractive with reverse phase columns. So the elution of the compound from the column was influenced by polar mobile phase. Mixture of methanol, acetonitrile and 0.05M KH_2PO_4 was selected as mobile phase and the effect of composition of mobile phase on the retention time of Nateglinide was thoroughly investigated. The concentration of the water, methanol, acetonitrile and 0.05M KH_2PO_4 were optimized to give symmetric peak with short run time (Fig.3).

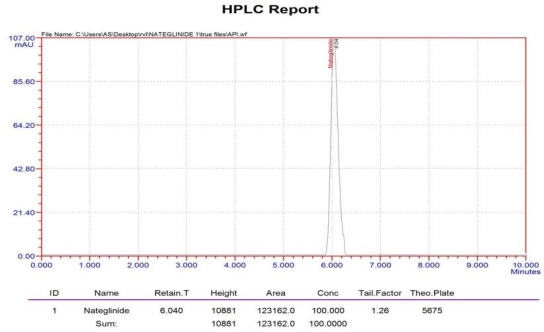

Fig 3: Typical chromatogram of Nateglinide

Validation of method

Linearity

Five points graphs was constructed covering a concentration range 1-5$\mu g/ml$ (Three independent determinations were performed at each concentration). Linear relationships between the peak area signal of Nateglinide the corresponding drug concentraton was observed. The standard deviation of the slope and intercept were low. The statistical analysis of calibration is shown in Table 1.

Precision

The validated method was applied for the assay of commercial tablets containing Nateglinide. Sample was analyzed for five times after extracting the drug as mentioned in assay sample preparation of the experimental section. The results presented good agreement with the labeled content. Low values of standard deviation denoted very good repeatability of the measurement. Thus it was showing that the equipment used for the study was correctly and hence the developed analytical method is highly repetitive. For the intermediate precision a study carried out by the same analyst working on the same day on two consecutive days indicated a RSD of 1.195. This indicates good method precision.

Stability

The stability of Nateglinide in standard and sample solutions containing determined by storing the solutions at ambient temperature (20 ± 10^0C). The solutions were checked in triplicate after three successive days of storage and the data were compared with freshly prepared samples. In each case, it could be noticed that solutions were stable for 48 hrs, as during this time the results did not decrease below 98%. This denotes that Nateglinide is stable and standard and sample solutions for at least 2 days at ambient temperature.

System suitability

The system suitability parameter like capacity factor, asymmetry factor, tailing factor and number of theoretical plates were also calculated. It was observed that all the values are within the limits (Table.3). The statistical evaluation of the proposed method was revealed its good linearity, reproducibility and its validation for different parameters and let us to the conclusion that it could be used for the rapid and reliable determination of Nateglinide in tablet formulation. The results are furnished in Table 3.

Table 1: Linearity of Nateglinide

S. No.	Linearity level	concentration	Area
1	I	1µg/ml	43495.9
2	II	2µg/ml	81507.0
3	III	3µg/ml	121621.7
4	IV	4µg/ml	164464.6
5	V	5µg/ml	206090.5

Table 2: Recovery studies of Nateglinide

% Concentration	% Recovery	Mean Recovery
50%	99.15%	
100%	99.33%	99.28%
150%	99.38%	

Table 3: System stability parameters

Parameters	Values
λ max (nm)	215
Beer's law limit (µg/ml)	1-5
Correlation coefficient	0.999
Retention time	6.040
Theoretical plates	5675.44
Tailing factor	1.26
Limit of detection (ng/ml)	15
Limit of quantification (ng/ml)	50
Slope	40814.68
Intercept	991.9
accuracy	99.97%
R.S.D.	1.195
% of Nateglinide in formulation	19.21%

Table 4: Assay

Formulation	Label claim (mg)	% Amount found
Glinate	120mg	19.21%

Table 5: Chromatographic conditions

Mobile phase	Methanol:Acetonitrile:0.05M KH_2PO_4 (20:55:25)
PH	5.5
UV detection	215nm
Analytical column	C18
Flow rate	1.0ml/min
Temperature	ambient
Injection volume	20µl
Runtime	10min
Retention time	6.040 min

CONCLUSION

A validated RP-HPLC method has been developed for the determination of Nateglinide in tablet dosage form. The proposed method is simple, rapid, accurate, precise and specific. Its chromatographic run time of 10 min allows the analysis of a large number of samples in short period of time. Therefore, it is suitable for the routine analysis of Nateglinide in pharmaceutical dosage form.

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