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Research Article

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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR NATEGLINIDE IN FORMULATION AND BULK DRUG

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In the present work, simple and sensitive RP-HPLC method has been developed for the quantitative estimation of Nateglinide in bulk and in Pharmaceutical formulations.Isocratic elution at a flow rate of1.0ml/min was employed on a waters X- Bridge C18 column 5µm 4.6x250mm at ambienttemperature. The mobile phase consisted of acetonitrile: 0.1% formic acid (pH 3.1) 60:40 (V/V/). In the range of 2-64 µg/ml, the linearity of Nateglinide shows a correlation co-efficient of 0.999. The UV detection wavelength was 215nm and 20µl sample was injected. The retention time for Nateglinide was 6.503 min. The percentage RSD for precision and accuracy of the method was found to be less than 2%.In the pharmaceutical formulations,(LOQ) of 1.247µg/ml and (LOD) of 0.156µg/ ml. 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[1](fig.1) is an oral anti-diabetic agent used in the management of Type 2 diabetes mellitus [also known as non-insulin dependent diabetes mellitus (NIDDM) or adult-onset diabetes]. (-)-N-[(trans-4- isopropylcyclohexane) carbonyl]-D-phenylalanine (Fig.1.) 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. NTG is an amino acid derivative that induces an early insulin response to meals decreasing postprandial blood glucose levels. NTG is included in the official drafts of United States Pharmacopoeia (United States Pharmacopoeia 2011) and European Pharmacopoeia (EDQM 2011) which recommend HPLC method for its assay.

In the literature, several HPLC methods, (Madhira VNS Ramprasad et al. April-2014, is available for estimation of nateglinide in human plasma)[10],and for pharmaceutical formulations (N.V. Krishna Reddy et al. 2011; Aylin Hacioğlu et al. 2015;CijoMadathil Xavier et al.(2012;J. Sachin; et al. 2012;ShaliniAsthana et al. 2010 Jan-Mar; G. RaveendraBabu et al. 2013)[3-9], are available.

According to literature survey, there are quite a few publications on HPLC method development, and The primary objective of this study was to develop and validate an RP-HPLC method that could separate drug in the bulk and formulated forms from its potential related substances and to establish an in-depth understanding of the method and build in the quality during the method development to ensure optimum method performance over the lifetime of the product

METHODS

Materials and Reagents

Nateglinide (99.8% purity) was received as gift sample from Glenmark Pharmaceuticals Ltd, Mumbai, Maharashtra, India. HPLC grade Acetonitrile, Formic acid and Analytical grade Sodium Hydroxide (Merck, Mumbai, India). Pharmaceutical formulation GLINATE tablet (Glenmark Pharmaceuticals Ltd, Mumbai, Maharashtra, India)(label claim 60 mg Nateglinide) was used in HPLC analysis. HPLC grade water obtained in-house by using Direct-Q water purification system (Millipore, Milford, USA) was used in HPLC study.

Chromatographic conditions and equipment:

The Agilent 1120 Compact LC HPLC system consisting of gradient pump (LC-10AT vp pump) (4MPa or 40barr), rheodyne injector, UV variable wavelength detector, Standard cell and agilent syringe was used. The separations were achieved on a waters X- Bridge C18 column 5µm 4.6x250mm with UV detection at 215nm. Analytical weighing balance (Shimadzu AUX 220) was used for weighing. Double beam UV Visible spectrophotometer (SHIMADZU-UV 1700) was used for wavelength detection. The EZ Chrome Elite software-single channel was used for acquisition, evaluation and storage of chromatographic data. Mobile phase used was

(60:40) acetonitrile and formic acid buffer (pH 3.1)

Fig 1: Structure of Nateglinide

Instrumental parameters

After several trials with the different combination and ratio of solvents, the mobile phase Acetonitrile: Formic acid buffer (60:40 v/v, pH 3.01±0.1) was selected, because it was found that it ideal with retention time (R_t) 6.503 min and the same is shown in Fig.4. Wavelength was selected by scanning the standard drug over a wide range of wavelength 200 nm to 400 nm. The component show reasonably good response at 215 nm (fig 2).

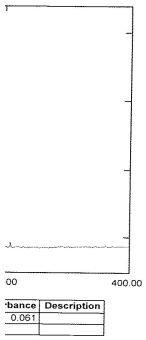

Fig. 2 UV Spectrum of Nateglinide at 215nm

Preparation of Stock Solution

Accurately weighed 100 mg of Nateglinide in 100 ml volumetric flask and dissolved in methanol and the volume were made up to the mark with the same solvent. From the above 10 mL solution was pipette out into separate 100 ml volumetric flask and volume was made up to the mark with the same solvent. This gave the concentration of 100 µg mL^{-1} of Nateglinide, from this six dilutions of Nateglinide were prepared in between 2-64 µg mL^{-1} with methanol and used in HPLC estimation.

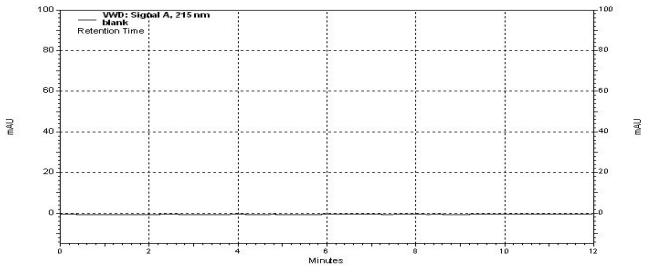

Fig.3Blank chromatogram

Procedure for Preparation of Calibration Curve

Calibrationstudy was carried out individually at six different concentration levels. All stock and working solutions were sonicated for 10 min then filtered through the nylon membrane filter (0.45µ) prior to use. Triplicate of 20µL injections were made for each concentration and chromatographed under specified condition at ambient temperature (24^0C).

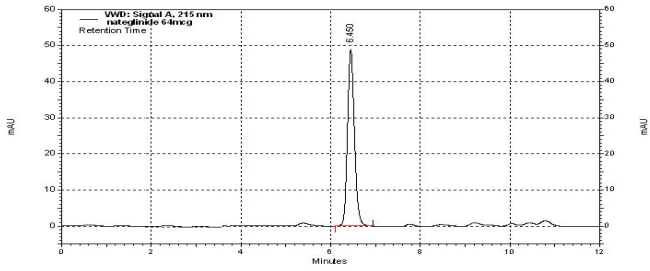

Fig. 4 Chromatogram of Nateglinide with optimized method

S.NO	Concentration(µg/ml)	Peak Area
1.	2	442697
2	4	563135
3.	8	1034348
4.	16	2142972
5.	32	4316278
6.	64	8743069

Table.1. Linearity data for Nateglinide

Preparation of Tablet Extracts and Assay Procedure

Twenty tablets each containing 60 mg of Nateglinide wereweighedandpowdered for further study.Thepowderequivalentto 50mg of Nateglinide was accuratelyweighedandtransferredto50ml volumetricflask containing 25ml of methanolandsonicatedfor10min.

Theabovesolutionwascarefully filtered through Whatmannfilterpaper(No.41) and the residue was washed with 3 portions of 5 ml of solvent. The volumewasmade up to themark withmethanol. From this solution, required dilutions for HPLC method were prepared by using methanol as a solvent.

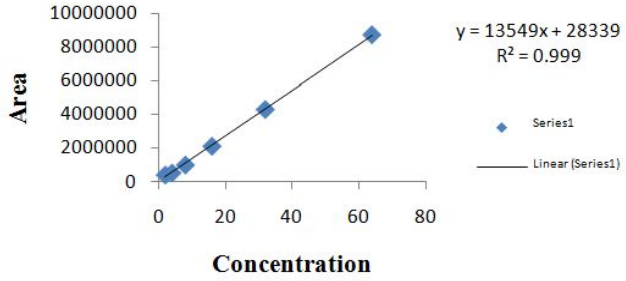

Fig.5.Linearity curve for Nateglinide at 215nm

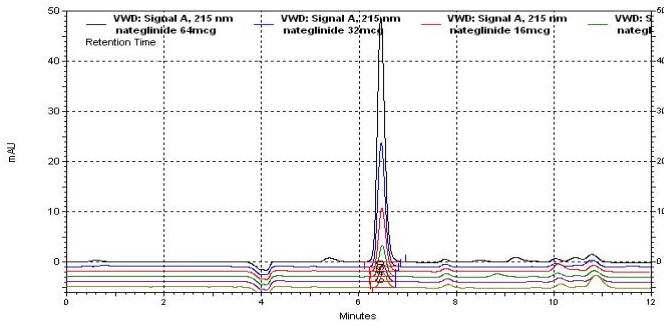

FIG.6 Overlay chromatograms of nateglinide of linearity

Procedure for Method Validation

Specificity: blank interference

A study to establish the interference of blank was conducted. Mobile phase was injected as per the test method. Chromatogram of blank (fig-3) should not show any peak at the retention time of analyte peak.

Linearity:

Linearity of the proposed HPLC method for determination of Nateglinide was evaluated by analyzing a series of different concentrations of standard drug. In this study six concentrations were chosen ranging between 2-64µg mL^{-1} for Nateglinide fig.6. Each concentration was injected three times and obtained information on variation in the peak area response of pure analyte was plotted against corresponding concentrations and result was shown in Table 1.

Precision	Conc(µg/ml)	Area	
Nateglinide		Morning	Afternoon
	Injection 1	2150302	2156789
	Injection 2	2178432	2168430
	Injection 3	2156734	2173403
	Injection 4	2148679	2148673
	Injection 5	2153489	2157894
	Injection 6	2170734	2160542
	Average	2159728	2160955
	STDEV	12081.31	8815.574
	RSD %	0.559	0.407

The linearity of the calibration graph was validated by the high value of correlation coefficient, slope and the intercept value was shown in Fig.5. The optimized method parameters are given in the Table1.

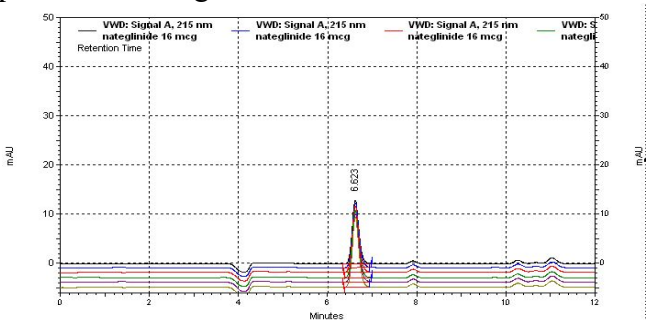

Fig. 7. Overlay chromatograms of intermediate precision-Morning

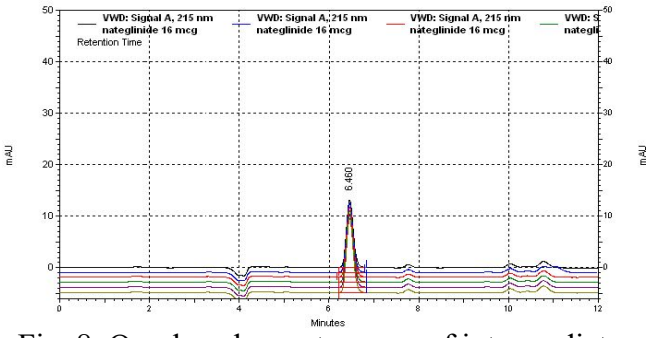

Fig. 8. Overlay chromatograms of intermediate precision-afternoon

Precision:

Precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample.It was demonstrated by

repeatability and intermediate precision measurements of peak area and peak symmetry parameters of HPLC method for the title ingredient. The repeatability(fig.7and 8) (within-day) and intermediate precision(fig.9and 10) (for 2 days) were carried out at one concentration levels and six replicates for compound. The obtained results were within and between the acceptable range. The precision expressed as % RSD is given in Table 2 and 3.

Accuracy:

Accuracy of an analytical method is the closeness of test results obtained by that method to the true

Precision	Concentration (µg/ml)	Area	
Nateglinide		Day 1	Day 2
	Injection 1	2156780	2150506
	Injection 2	2167423	2160405
	Injection 3	2150568	2150567
	Injection 4	2150543	2170532
	Injection 5	2170453	2150593
	Injection 6	2160563	2150854
	Average	2159388	2155576
	STDEV	8380.98	8305.706
	RSD %	0.388	0.385

Table .3 Precision (interday) study results of prepared sample

value. The accuracy of an analytical method should be established across its linearity range. Accuracy was performed in three different levels, each level in triplicate for Capecitabine using standards at 80%, 100% and 120% (Fig 11,12 and 13).Each sample was analysed in triplicate for each level. From the results, % recovery is calculated and shown in Table 4 and 5.

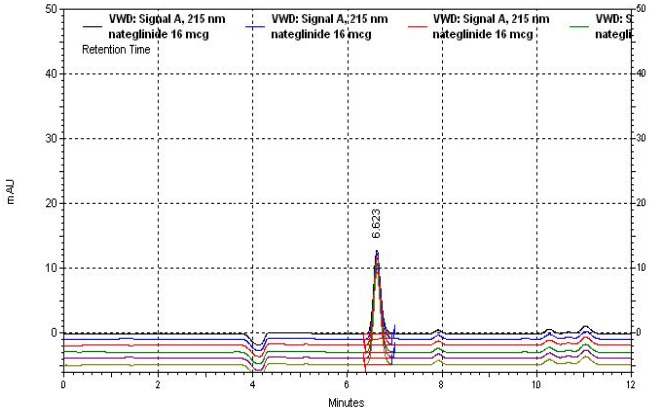

Fig. 9. Overlay chromatograms of intermediate precision day-1

Limit of Detection (LOD) and Limit of Quantitation (LOQ):

It is calculated according to ICH recommendations where the approach is based on the signal-to-noise ratio. Chromatogram signals obtained with known low concentrations of analytes were compared with the signals of blank samples. A signal-to-noise ratio 3:1 and 10:1 was considered for calculating LOD and LOQ respectively. The values of LOD and LOQ were given in Table 6.

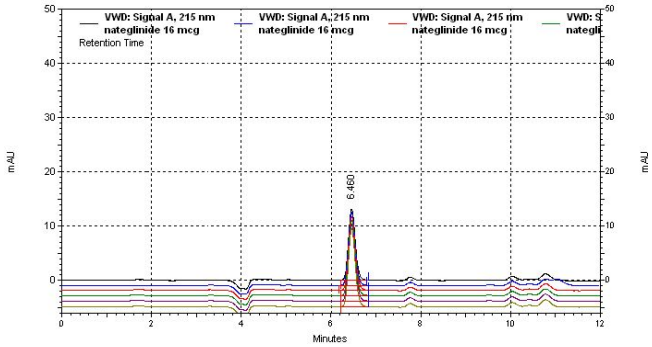

Fig. 10. Overlay chromatograms of intermediate precision-day-2

RESULT AND DISCUSSION

The objective of the proposed work was to develop a method for the determination of Nateglinide to validate the methods according to USP and ICH guidelines and applying the same for its estimation in laboratory prepared mixtures. HPLC method developed was found to be rapid, simple, precise, accurate and economic for routine estimation of Nateglinide in laboratory prepared mixtures.

In HPLC method, HPLC conditions were optimized to obtain, an adequate separation of eluted compound. Initially, various mobile phase compositions were tried, to separate title ingredient. Mobile phase and flow rate selection was based on peak parameters (height, tailing, theoretical plates, capacity or symmetry factor), run time, and resolution. The system with acetonitrile: 0.1% formic acid buffer (pH 3.1)

(60:40 v/v) with 1ml.min^{-1} flow rate is quite robust.

S.No.	Level in %	Area Response	Mean % recovery
		Nateglinide	Nateglinide
1	80	2156702	101
2		2165403	
3		2172031	
1	100	2156340	100.56
2		2150403	
3		2160345	
1	120	2150323	100.62
2		2155302	
3		2164220	

Table.4. Mean % recovery of Nateglinide

The optimum wavelength for detection was 215 nm at which better detector response for the title drug was obtained. The retention time for nateglinide was found to be 6.503 min respectively. The calibration was linear in concentration range of 2-64 µg mL^{-1} withregression 0.9999, intercept 28339 and slope 13549 for Nateglinide. The low values of % R.S.D indicate the method is precise and accurate. The mean recoveries were found in the range of 101 – 100.62 %.

Sample to sample precision and accuracy were evaluated using three samples of five different

S.no	Labelled in mg	Amount added in mg	Amount found in mg*	%Recovery
	Nateglinide	Nateglinide	Nateglinide	Nateglinide
1	60	48	60.6	101
2	60	60	60.33	100.56
3	60	72	60.37	100.62

Table.5. Assay results of Nateglinide
*Average of three determinations

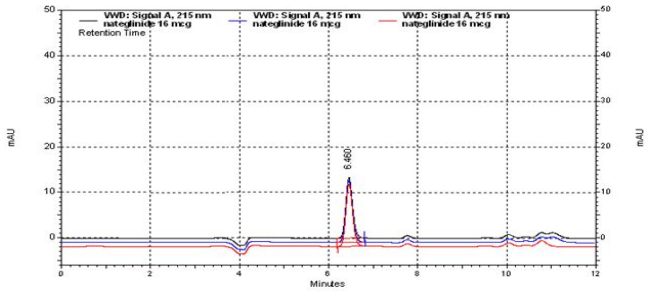

Fig.11 . Overlay chromatograms of 80% recovery

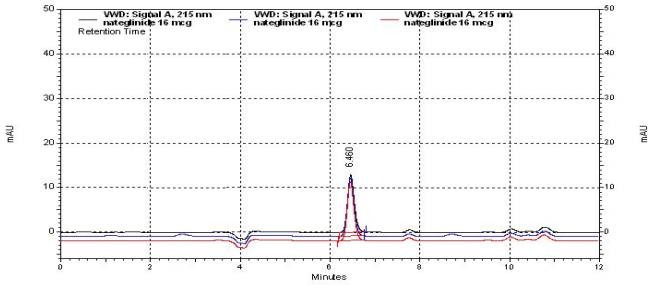

Fig. 12. Overlay chromatograms of 100% recovery

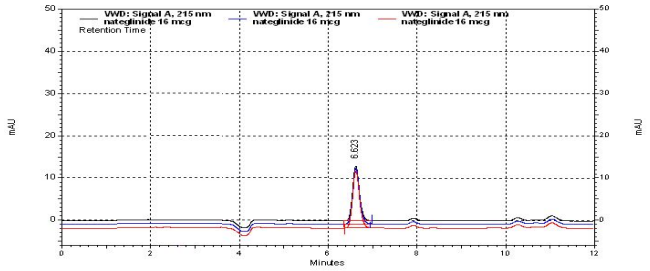

Fig. 13. Overlay chromatograms of 120% recovery

concentrations, which were prepared and analyzed on same day. Day to day variability was assessed using three concentrations analyzed on

Parameters	Nateglinide
LOD(µg/ml)	0.156
LOQ(µg/ml)	1.247

Table .6. Result of LOD and LOQ

three different days over a period of three days. These results show the accuracy and reproducibility of the assay. The % R.S.D. reported was found to be less than 2 %.The proposed method was validated in accordance

with ICH parameters and the applied for analysis of the same in laboratory prepared mixtures.

The proposed methods are accurate, simple, rapid and selective for the estimation of Nateglinide in laboratory prepared mixtures. Hence, these methods can be conveniently adopted for the routine analysis of Nateglinide in quality control laboratories.

Limit of Detection (LOD) and Limit of Quantitation (LOQ):

It is calculated according to ICH recommendations where the approach is based on the signal-to-noise ratio. Chromatogram signals obtained with known low concentrations of analytes were compared with the signals of blank samples. A signal-to-noise ratio 3:1 and 10:1 was considered for calculating LOD and LOQ respectively. The values of LOD and LOQ were given in Table 5.7

SUMMERY AND CONCLUSION

On the basis of the experiments, we can conclude that the RP-HPLC & method developed for the Estimation of Nateglinide can be used for routine analysis Q.C. Samples. Nateglinide was determined by reverse phase HPLC using Formic acid (pH 3.1): Acetonitrile (60:40v/v) as mobile phase, and Waters X Bridge C 18Column, 5µ 250×4.6mm as a stationary phase. Detection was carried out using UV detector at 215 nm. After development of the method, it was validated for system suitability, specificity and linearity, limit of detection and limit of quantification, precision, and accuracy.

Validation Parameters	Nateglinide
Mobile phase	Acetonitrile: 0.1% Formic acid (60:40v/v)
Flow rate	1.0ml/min
Detection Wavelength	215
Rt	6.503
Run Time	12min
Theoretical Plates	8456
LOD	0.156µg/ml
LOQ	1.247µg/ml
Linearity	2-64 µg/ml
Precision	% RSD < 2

Summary of the present study (RP-HPLC)

- The system suitability was found to be within the limits. The limit was Not more than RSD <2. The retention time of Nateglinide is 6.503 mins. The data regarding the system suitability is shown in table 5.1.
- The Specificity of Nateglinide is shown in Chromatogram there was no interference. In this method it means no impurity was interfered and also reveals that commonly used excipients and additives present in the pharmaceutical formulations were not interfering in the proposed methods.
- The precision was found to be within the limits. The limit were not more than RSD <2. This indicates that the method is precise. The data regarding the precision are shown in table 2 and 3.
- From the linearity table 1, it was found that, the drug obeys Beer's Law. For HPLC the

calibration plot of Nateglinide was observed as linear in the range 2-64µg/ml and the correlation coefficients were found to be 0.999 respectively.

- From the results shown in the accuracy table 4 and 5, it was found that Recovery value of pure drug from the solution were between 101 – 100.62 %.This indicates that the method is accurate.

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