ejpmr, 2016,3(3), 265-270

SJIF Impact Factor 3.628

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 3294-3211

EJPMR

A VALIDATED STABILITY INDICATING RP-HPLC METHOD FOR ESTIMATION OF NATIGLINIDE IN BULK AND PHARMACEUTICAL DOSAGE FORMS

Challa Sudheer[1*], B. Tirumaleswara Rao[2], S. Madhavi[3] and C. Rambabu[*]

[1,2]Department of Chemistry, Vikas Degree College (P.G Courses), Vissannapeta, Krishna Dist,-521215, (A.P.) India.
[3]Department of Pharmacy, Vikas Pharmacy College, Vissannapeta, Krishna Dist-521215, (AP), india.
[*]Department of Chemistry. Acharya Nagarjuna University, Nagarjunanagar, Guntur, (AP) India.

***Author for Correspondence: Dr. Challa Sudheer**
Department of Chemistry, Vikas Degree College (P.G Courses), Vissannapeta, Krishna Dist,-521215, (A.P.) India.

Article Received on 04/01/2016 **Article Revised on 24/01/2016** **Article Accepted on 14/02/2016**

ABSTRACT

A new simple, accurate, stability indicating RP-HPLC method has been developed and validated for assay of Nateglinide in pure and dosage forms. Chromatographic analysis of Nateglinide in the present assay was carried out isocratically with a mobile phase consisting of 0.02M ammonium dihydrogen phosphate buffer (pH-4.0) and acetonitrile in the ratio of 40:60v/v. The flow rate was 1.0ml/min. The effluent was monitored spectrophotometrically at a wavelength of 226nm at ambient temperature with a retention time of 4.189 min respectively. The proposed RP-HPLC method was validated according to ICH guidelines with respect to specificity, precision, linearity and accuracy. The drug substance (Nateglinide) was subjected to stress conditions of hydrolysis, photolysis degradation. The degradation of Nateglinide was observed under acid, base and photolyticstress conditions and was found to be stable in other stress conditions studied.the oxidative stress studies revealed that natiglinide(Rt=4.189 min) was not fully degraded and its degradation products were eluted separatly at different retention times respectively. The percentage RSD for precision and accuracy of the proposed method for Nateglinide was found to be less than 2%. The proposed RP-HPLC method was successfully applied for routine analysis of Nateglinide in tablet dosage form.

KEYWORDS: Nateglinide, RP-HPLC, Validation.

INTRODUCTION

Nateglinide[1,2], chemically (-)-N-[(trans-4isopropylcyclohexane)carbonyl]-D is an oral antidiabetic agent used in the treatment of Type 2 diabetes mellitus which is also known as non-insulin dependent diabetes mellitus (NIDDM) or adult-onset diabetes.

In the present paper a sensitive, accurate, precise, validated stability indicating RP-HPLC method for the assay of Nateglinide in pure and in tablet dosage forms in accordance with ICH guidelines have been described.

MATERIALS AND METHODS

a) INSTRUMENTATION

The development and validation of the present RP-HPLC method was performed on a isocratic HPLC system (Waters Alliance 2695 separations module) equipped with 600e controller pump, 776 auto sampler and 2487 dual variable wavelength UV detector. The output signal was recorded with Empower software on Dell computer. The analytical column used was a stainless steel ODS, C_{18} RP-Column (4.6mmx250mm) purchased from Waters Corporation (Bedford, MA, USA) protected by a guard column of the same material.

b) CHEMICALS AND MATERIALS

The pharmaceutical grade pure sample of nateglinide was procured from Hetero Drugs Ltd. Acetonitrile solvent of analytical grade was obtained from E Merck Ltd, Mumbai, India. Ammonium dihydrogen phosphate and Orthophosphoric acid of AR grade was procured from Qualigens Fine chemicals, Mumbai, India. The HPLC grade water was obtained from a Milli-Q RO water purification system.

c) PREPARTION OF BUFFER SOLUTION

2.3gms of ammonium dihydrogen phosphate was weighed and dissolved in 1000ml HPLC grade water and then adjusted to pH 4.0 with Orthophosphoric acid. This buffer solution is filtered and degassed prior to the assay.

d) PREPARATION OF MOBILE PHASE

The mobile phase used in the present assay is prepared by dissolving 0.02M ammonium dihydrogen phosphate buffer(pH-3.5)and Acetonitrile in the ratio of 40:60 v/v.

This Mobile phase is filtered and degassed prior to the assay.

e) PREPARATION OF DILUENT
Mobile phase is used as diluent in the present assay.

f) STANDARD STOCK SOLUTION
An accurately weighted sample of 10mg of Nateglinide was dissolved in 100ml of diluent to give standard stock solution of 100µg/ml. A series of working standard solutions (2.0µg/ml - 10µg/ml) were obtained by diluting this stock solution with diluent. All the volumetric flasks containing working standard solutions of nateglinide were wrapped with aluminium foil and stored in the dark.

g) PREPARATION OF TABLETS CONTAINING THE DRUG
The proposed RP-HPLC method has been validated for the assay of nateglinide in tablet as per guidelines of ICH. Ten tablets are procured and powdered. An accurately weighed portion of powder equivalent to 25mg of nateglinide was dissolved in 25ml of diluent and filtered through 0.45µm membrane filter. From this filtrate, 0.1ml was pipetted in to 10ml graduated test tube and made up to volume with the mobile phase. 20µL of this sample was injected into the column and the drug content in the tablet was quantified using the regression equation.

RESULTS AND DISCUSSION
i) METHOD DEVELOPMENT
Several systematic trials were performed by the author in developing an accurate stability indicating RP-HPLC method for the analysis of nateglinide in pure and pharmaceutical dosage forms that include the following.

a) Selection of Wavelength
The sensitivity of the any HPLC method depends upon the proper selection of wavelength and therefore the author had chosen the selection of the appropriate wavelength is the first step in the development of the present chromatographic assay. Spectroscopic analysis of nateglinide showed maximum UV absorbance (λ_{max}) at 226nm and therefore, in the present study the chromatographic assay was performed at 226 nm using a U.V detector as nateglinide exhibited good response at this λ_{max}.

b) Selection of Mobile Phase
Based on the literature and p^{Ka} values of nateglinide an appropriate mobile phase was selected containing a mixture of 0.02M ammonium dihydrogen phosphate buffer(pH-4.0) and acetonitrile in different ratios was studied. Experimentally it was observed that peak splitting occurred with above said same mobile phase in the ratios of 40:50 and 50:60. But with the same mobile phase in the ratio of 40:60 resulted in well resolved peaks for nateglinde and the same mobile phase is used for carrying out further stability studies.

c) Effect of Flow Rate on Separation
After having fixed the ratio of the mobile phase, the mobile phase was pumped into the system at different flow rates such as 0.8ml/min,1.0ml/min and 1.2ml/min for the better separation of the above said drug and was found that flow rate of 1.0ml/min gave ideal peak resolution with an retention time of 4.189 min respectively.

d) Chromatographic conditions
Chromatographic analysis of repaglinide in the present assay was carried out isocratically with a mobile phase consisting of 0.02M ammonium dihydrogen phosphate buffer(pH-4.0) and acetonitrile in the ratio of 40:60v/v. The flow rate was 1.0ml/min. The effluent was monitored spectrophotometrically at a wavelength of 226nm at ambient temperature. The optimized chromatographic conditions carried out in the present proposed method for natelinide assay is represented in **Table.1.1.**

On this finalized chromatographic conditions, nateglinde was well separated and eluted with good peak symmetry and higher theoretical plates. The typical chromatogram of sample solution of nateglinide is shown in **Fig.1.2**.

ii) METHOD VALIDATION
After developing the assay method for nateglinide various validation studies were carried out in accordance to ICH guidelines which include system suitability studies, specificity, stability studies (Forced degradation studies), linearity, sensitivity, precision and accuracy studies respectively.

a) SYSTEM SUITABILITY STUDIES
The system suitability studies were carried out as specified in ICH guidelines. These parameters include column efficiency, resolution and percentage coefficient of variation. In the present study column efficiency (N) resolution (Rs.), capacity factor and peak asymmetry factor were calculated and are present in **Table.1.2**.

b) SPECIFICITY
The specificity test of the proposed RP-HPLC method showed almost no peaks at the retention time of nateglinide for the blank and placebo demonstrating that the excipients present in the tablets did not interfered with the drug peak revealing the specificity of the proposed method.

c) FORCED DEGRADATION STUDIES
In the present study forced degradation studies was performed for the selected drug (nateglinide) by subjecting to acidic, alkaline, oxidizing, thermal and photolytic conditions. For this studies powdered tablets, equivalent to 50mg of nateglinide was transferred into a 250ml round bottomed flask and were subjected to the

given below degradation conditions. After subjecting to different degradation treatments, the stress content solutions were allowed to equilibrate to room temperature and diluted with the diluent and was injected into the column and the respective chromatograms were recorded and evaluated (**Table.1.3)**.

i. Acidic condition

In acidic degradation repaglinide was heated under reflux with 1.0N HCl at 80°C for 1hr and the mixture was neutralized with 1 N NaOH solution. In acidic degradation, it was found that nateglinide was not degraded.

ii. Alkaline condition

In alkaline degradation nateglinide was treated with 0.1 N NaOH at room temperature for 100mins and the mixture was neutralized with 0.1 N HCl solution. No degradation was found in alkali condition.

iii. Oxidative condition

In oxidative degradation repaglinide was heated under reflux with 3% hydrogen peroxide at 80°C for 1 hrs. In oxidative degradation, it was found that the drug was not fully and its degradation products eluted separately at different retention times respectively.

v. Photolytic condition

Photolytic degradation was performed by exposing the drug content in sunlight for 72hrs. Iwas observed thatnateglinide was not degraded in photolytic condition.

d) LINEARITY

The linearity of the present RP-HPLC method was determined at five concentration levels ranging from 2.0-10.0µg/ml for repaglinide. The calibration curve was constructed by plotting response factor against concentration of nateglinide. The slope and intercept value of calibration curve (**Fig.1.3**) for nateglinide were **Y = 188414X + 385650(R^2= 0.9999)** [where **Y** represents the ratio of peak area ratio of analyte and **X** represents analyte concentration]. These results revealed that significant correlation exists between response factor and concentration of nateglinide within the concentration range indicated on Y-axis (**Table.1.4)**.

e) LIMITS OF DETECTION(LOD) AND QUANTIFICATION (LOQ)

In the present study the limit of detection and quantitation were calculated by the method based on the standard deviation (σ) and the slope (S) of the calibration plot, using the formulae LOD = 3.3σ/S and LOQ = 10σ/S.

The LOD and LOQ values were found to be 0.0082µg/ml and 0.46µg/ml revealing that the developed method exhibited good sensitivity for nateglinide assay respectively.

e) PRECISION

In the present assay, precision studies of the developed RP-HPLC method were established by evaluating method precision and intermediate precision study.

In the present study method precision of the present RP-HPLC method was determined by performing method precision on another day under same experimental condition. Assay of all six replicate sample preparations was determined and mean % assay value, standard deviation and % relative standard deviation and 95% confidence intervals was calculated and are presented in **Table.1.5.** From the data obtained in **Table.1.5**, the developed RP-HPLC method for nateglinide was found to be precise.

f). ACCURACY [RECOVERY STUDIES]

Accuracy of the proposed method was carried out with a known quantity of the pure drug was added to the placebo sample at the levels of 50%, 100% and 150% of the test concentration. The results of this study were determined from the respective chromatograms and are reported in **Table.1.6** respectively. The mean recoveries revealed that there is no interference from excipients. These results showed that the amounts of drug (nateglinide) were in good agreement with the label claim of the formulation.

g) RUGGEDNESS AND ROBUSTNESS

The ruggedness of the present RP-HPLC method was determined by carrying out the experiment by different analysts using different columns of similar types. The percentage of assay of different preparations assay values with two different analysts and columns were 99.5%, 98.7% respectively.

Robustness of the method was determined by small deliberate changes in flow rate and temperature. The robustness limit for flow rate variation and temperature variation were well within the limit, indicating that the proposed method is robust under given set of defined experimental conditions **Table.1.7**.

h) APPLICATION OF THE DEVELOPED METHOD

The present proposed RP-HPLC method was used for the assay of nateglinide in tablet dosage forms and the values of proposed RP-HPLC method was compared with a previously published HPLC method[10] using statistical approach and found that no significant differences in using both methods **Table. 1.8**.

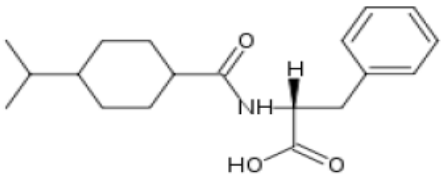

Fig. 1.1. Structure of nateglinide.

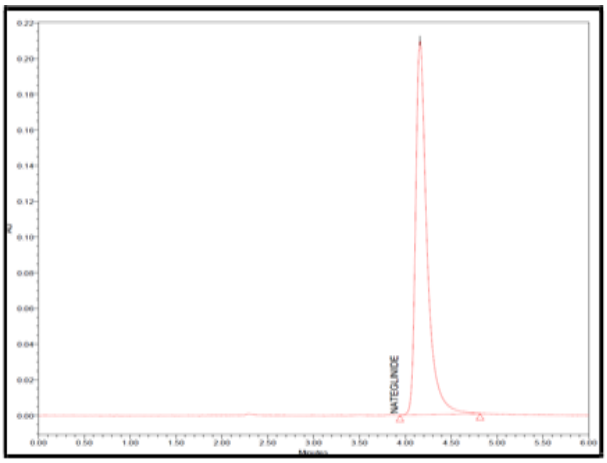

Fig. 1.2. Chromatogram of nateglinde.

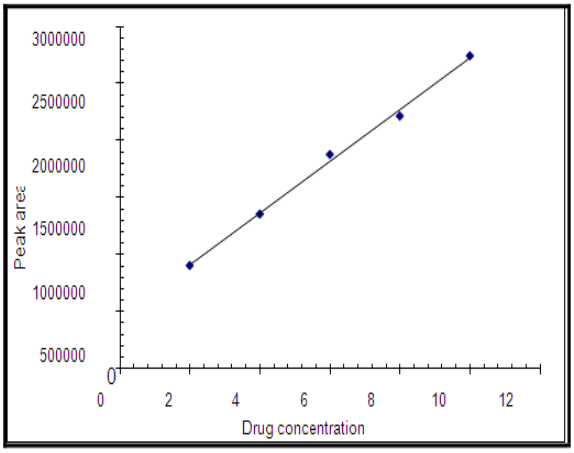

Fig 1.3 Calibration curve of nateglinide.

Table. 1.1: Optimized chromatographic conditions.

CHROMATOGRAPHIC PARAMETERS	PEAK HPLC
Elution	Isocratic
Mobile phase	0.02M ammonium dihydrogen phosphate buffer(pH-4.0): Acetonitrile in the ratio of 40:60 v/v
API Concentration range	2.0 - 10 µg/ml
Column	ODS C-18 RP (4.6 mm i.d x 250 mm)
Flow rate	1.0 min/ ml
Detection	UV at 226nm
Injection volume	20 micro liters
Temperature	Ambient
Retention time	4.189 minutes
Run time	10 minutes
Area	1877424mAU
pH	4.0
Theoretical plates	3999
Pressure	20-25 Mpa
Tailing factor	1.6

Table. 1.2: System suitability parameters.

PARAMETERS	NATEGLINIDE
Retention time	4.189
USP Plate count	3999
USP Tailing	1.6
Linearity Range (µg /ml)	2.0-10
Limit Of Detection (LOD) (µg /ml)	0.746
Limit Of Quantitation (LOQ) (µg/ml)	2.330

Table. 1.03: Results of forced degradation parameters.

S.No	Sample Weight	Sample Area-1	% Assay	% Assay	%DEG
ACID	644	1941205	106	12500	7
BACE	644	1964105	107	12500	8
PEROXIDE	644	1851063	101	12500	2
LIGHT	644	1988207	108	12500	9
Avarage Assay:		1604440.5	87	12500	-12
STD		787676.1	42.87	0.00	
%RSD		49.1	49.09	0.00	

Table. 1.4: Results of linear regression data.

CONCENTRATION (µg.mL)	AREA (mAU)
2	763865
4	1138858
6	1518042
8	1884950
10	2474960
Regression equation; Intercept (a)	38560
Slope (b)	188414
Correlation coefficient	0.9999
Standard deviation on intercept (S_a)	517.824
Standard deviation on slope (S_b)	879.61
Standard error on estimation (S_e)	5677.02
LOD	.0082
LOQ	.0466

Table. 1.5: Results of precision data of nateglinide.

DRUG NAME	REPAGLINIDE
TYPE OF PRECISION	**METHOD PRECISION**
PARAMETERS STUDIED	**PEAK AREA**
Set-1	2457727
Set-2	24588488
Set-3	2445094
Set-4	12469610
Set-5	2418823
Set-6	2417194
***Average**	2444489.3
***Standard deviation**	21938.1
***%RSD**	0.9

All the values are the averages of six determinations.

Table-1.6: Recovery studies of the proposed hplc methods.

NATEGLINIDE						
Spiked Level	**Sample Weight**	**Sample Area**	**µg/ml added**	**µg/ml found**	**% Recovery**	**% Mean**
50%	351.00	1014005	258.999	264.92	102	101
50%	352.00	1006221	259.737	262.88	101	
50%	352.00	1007307	259.737	263.17	101	
50%	356.00	1011339	262.688	264.22	101	
50%	359.00	1010366	264.902	263.97	100	
50%	354.00	1008771	261.212	263.55	101	
100%	644.0	1828637	475.200	477.75	101	101
100%	642.0	1840814	473.724	480.93	102	
100%	641.0	1823906	472.986	476.51	101	
150%	962.00	2727154	709.848	712.49	100	100
150%	961.00	2729858	709.111	713.20	101	
150%	969.00	2738546	715.014	715.47	100	
150%	964.00	2724130	711.324	711.70	100	
150%	968.00	2726179	714.276	712.24	100	
150%	969.00	2731132	715.014	713.53	100	

All the values are the averages of three determinations.

Table. 1.07: Results of robustness study.

ROBUST CONDITIONS		NATEGLINIDE	
		RT	PEAK AREA
FLOW RATE	0.8 ml/min	4.147	1832716
	1.2 ml/min	4.133	1828191
TEMPERATURE	40^{O}C	4.260	1829190
	45^{O}C	4.147	1821475

Table. 1.08: Results of analysis of nateglinide formulations.

PHARMACEUTICAL FORMULATION	AMOUNT OF REPAGLINIDE		% RECOVERY
	LABELED	*FOUND	
BENDIT	60 mg	59.89	99.89 %

All the values are the averages of three determinations.

CONCLUSIONS

A validated stability indicating isocratic RP-HPLC method has been developed for the assay of nateglinide in pure and in tablet dosage form. The proposed method is simple, rapid, accurate, precise and specific. Its chromatographic run time of 4.189 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.

REFERENCES

1. Z.X. Yang, Q. Wang, J.Z. Liuxi, HPLC method for the determination of contents and dissolution of nateglinide capsules by HPLC, *Strait Pharmaceutical Journal*, 2002; 14: 22-24.
2. B. Steffen, S. Elke, S. Julia, K. Claudia, M. Jurgen, R. Ivar, Rapid and simple method for the analysis of nateglinide in human plasma using HPLC analysis with UV detection, *Journal of Pharmaceutical and Biomedical Analysis*, 2003; 31: 551-555.
3. L.P. Gu, Tablet of nateglinide and its L-isomer in the HPLC determination of L-Isomer in nateglinide and its tablets by HPLC, *Chinese Journal of Pharmaceuticals*, 2004; 35: 486-487.
4. E.S. Michael, Ultra performance liquid chromatography (UPLC), An introduction, *Separation Science Redefined*, 2005; 8-14.
5. Z. Mao, Y.Q. Liang, Y.Z. Qin, Column-witching RP-HPLC determination of plasma concentrations of nateglinide, *West China Journal of Pharmaceutical Sciences*, 2006; 21: 456-458.
6. D.B. Pathare, A.S. Jadhav, M.S. Shingare, A validated stability indicating LC method for nateglinide, *Drug Development and Industrial Pharmacy*, 2007; 33: 551–557.
7. M. Srinivas, K. Srisailam, M. Chandra, M.E. Madhusudan, K.E.S. Gerhard, Development and validation of a stereoselective HPLC method for the determination of the in vitro transport of nateglinide enantiomers in rat intestine, *Journal of Separation Science*, 2007; 30: 1875-1880.
8. X.H.He, Z.Y. Yan, C.G. Xiaoyan, Determination of nateglinide and its related substances by HPLC, *Chinese Journal of Hospital Pharmacy*, 2005; 25: 48-50.
9. Y. Jia, G. Wang, S. Sun, RP-HPLC mobile phase addition method of chiral separation and determination of nateglinide enantiomer, *Chinese Journal of Pharmaceuticals*, 2007; 38: 440-441.
10. M.S. Jolly, G.S. MayuR, B.S. Vijay, C. Rajashree, Nateglinide quantification in rabbit plasma by HPLC: Optimization and application to pharmacokinetic study, *Journal of Pharmaceutical and Biomedical Analysis*, 2007; 44: 196-204.
11. B.H.Li, B.J. Tian, L.Y. Kaic, Simultaneous chiral separation of geometry isomers and enantiomers of Nateglinide by capillary electrophoresis with mixture of CD derivations as chiral selector, *Analytical Letters*, 2008; 41: 3177-3186.
12. K.S. Lakshmi, T. Rajesh, S. Shrinivas, Liquid chromatographic method for the determination of metformin and nateglinide in pharmaceutical formulations, *Asian Journal of Chemistry*, 2010; 22: 5231-5236.
13. N.V. Krishna Reddy, R.S. Phani, R. Rameshraju, Validated RP-HPLC method for the estimation of Nateglinide in formulation, *International Journal of Research in Pharmacy and Chemistry*, 2011; 1(1): 46-49.
14. Cijo Madathil Xavier, Kanakapura Basavaiah, Pavagada Jaganathamurthi Ramesh, Kanakapura Basavaiah Vinay And Hosakere Doddarevanna Revanasiddappa, RP-UPLC method development and validation for the determination of nNateglinide in bulk drug and pharmaceutical formulations, *Malaysian Journal of Pharmaceutical Sciences*, 2012; 10(1): 23-44.
15. Cijo Madathil Xavier, Kanakapura Basavaiah, Pavagada Jaganathamurthi Ramesh, Kanakapura Basavaiah Vinay And Hosakere Doddarevanna Revanasiddappa, RP-UPLC method development and validation for the determination of nNateglinide in bulk drug and pharmaceutical formulations, *Malaysian Journal of Pharmaceutical Sciences*, 10(1): 23-44, 2.