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Development and validation of an HPLC method for the analysis of nateglinide in pharmaceutical dosage forms

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ABSTRACT

A simple reverse phase HPLC method was developed for the determination of nateglinide present in pharmaceutical dosage forms. A Hypersil ODS C18, 4.6mm×μ250 mm, 5μm column with mobile phase acetonitrile: phosphate buffer pH-5.5,70:30%v/v was used. The flow rate was 1.0 ml/min and effluent was monitored at 210 nm. Ezetemibe is used as internal standard. The retention times were 4.47min and 3.308min for nateglinide and ezetimibe respectively. The linearity range was found to be 0.5-200μg/ml for nateglinide. The proposed method was also validated.

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KEYWORDS

Nateglinide;
Anti-diabetic;
Validation;
HPLC.

INTRODUCTION

Nateglinide, chemically (-)-N-[(trans-4-isopropyl cyclohexane)carbonyl]-D-phenylalanine[1], is an oral antidiabetic agent used in the management of type 2 diabetes mellitus. It is structurally unrelated to the oral sulfonylurea insulin secretagogues. It is freely soluble in methanol, ethanol, and chloroform, soluble in ether, sparingly soluble in acetonitrile and octanol, and practically insoluble in water. Nateglinide is an 'insulotrophic' agent, i.e., it lowers blood glucose by stimulating insulin production from the pancreas[2]. A search on literature reveals that a few HPLC methods have been reported for nateglinide in biological fluids[3-4] and in bulk dosage forms[5-6], and also a few spectrophotometric[7-9] methods were reported. The aim of present study is to develop a simpler, rapid cost-effective RP-HPLC method for the determination of teglinide in bulk and in pharmaceutical dosage forms.

MATERIALS AND METHODS

Instrumentation

Quantitative HPLC was performed on a binary gradient HPLC with Shimadzu LC10AT and LC10AT VP series HPLC pumps, with a 20μl injection of sample loop (manual), and SPD 10A VP UV-visible detector. The output signal was monitored and integrated using Shimadzu CLASS-VP Version 6.12 SP1 software. Hypersil ODS C_{18} (46 mm×25 cm, 5mcm) column was used for the separation. The pH of the solution was adjusted by using digital pH meter, model DI 707 (Digisun electronics, Hyderabad, India).

Standards and chemicals

Nateglinide and ezetimibe used as an internal standard (IS) were gifts obtained from Aurobindo pharma (Hyderabad, India) and Ranbaxy Laboratories Limited (Mohali, India). Natelid tablets (Sun pharma.) and

Glinate tablets (Torrent pharma) containing 120 mg and 120 mg of nateglinide, were purchased from local market. Purified water was prepared using a Millipore Milli-Q (Bedford, M.A., USA) water purification system. Methanol of HPLC grade was purchased from Burdick and Jackson (Muskagon, MI, USA), ammonium acetate of A.R. grade and formic acid of A.R. grade were purchased from local suppliers.

6.80 gms of Potassium dihydrogen orthophaphate was dissolved in 1000 mL of water and mixed the contents well. Then adjust the pH 5.5 with dilute phosphoric acid. The contents are filtered through 0.45µ membrane filter. Acetonitrile and phosphate buffer (pH-5.5) were properly mixed in the ratio of 70:30% v/v and pH was adjusted using orthophosphoric acid.

Preparation of standard drug solutions

Stock solution of nateglinide was prepared by dissolving 25 mg of nateglinide in 25 mL of volumetric flask containing 20 mL acetonitrile. The solution was sonicated for about 20 min and then made up to volume with mobile phase. Daily working standard solutions of nateglinide was prepared by suitable dilution of the stock solution with appropriate mobile phase. Similarly stock solution of internal standard was prepared by dissolving 25mg of ezetimibe in 25 mL of acetonitrile.

Chromatographic conditions

The mobile phase used in this study was a mixture of acetonitrile:phosphate buffer pH-5.5, 70:30%v/v. The stationary phase is a Hypersil ODS C18, 4.6mm×250 mm, 5µm column. The contents of the mobile phase were filtered before use through a 0.45µ membrane and degassed for 15 min. The mobile phase was pumped from the solvent reservoir to the column at a flow rate of 1ml/min. The column temperature was maintained at 23±1^{0}C. The eluents were monitored at 210nm using UV-detecctor. The identification of the separated nateglinide and ezetimibe were confirmed by running the chromatograms of the individual compounds under identical conditions.

Calibration of standards

Calibration standards were prepared by spiking working standard solutions into methanol containing in 5 mL volumetric flasks to yield concentrations of 0.5, 1, 2, 5, 7.5, 10, 15, 20, 50, 100, 150, and 200µg /mL. To the above solutions 20µg/mL of ezetimibe (internal standard) was added and the final volume was made up to the mark. The linearity data were shown in TABLE 1. Calibration curve was plotted between peak area ratio of drug vs internal standard against concentration of the drug.

TABLE 1: Linearity TABLE for nateglinide

Concentration (µg/mL)	Peak area ratio's	Statistical analysis
0.5	0.0669	
1	0.1335	
2	0.267	
5	0.665	Slope:0.1319
10	1.332	Intercept:0.0529
20	2.675	
40	5.3	Correlation
60	8.025	Coefficient:0.9999
80	10.7	
100	13.35	
200	26.69	
300	39.38	

TABLE 2: Amount of nateglinide in tablet dosage forms by proposed HPLC method

Formulation	Labeled amount (mg)	Mean ±s.d (amount mg recovered)n = 3	Mean ±s.d (% of recovery)
Glinate	120	118.25 ± 1.3185	98.51±1.1152
Natelide	120	120.23 ± 0.0808	100.19±0.0672

Recovery of nateglinide in tablets

Twenty tablets were weighed, finely powdered and an accurately weighed sample of powdered tablets equivalent to 25 mg of nateglinide was extracted with acetonitrile in a 25mL volumetric flask using ultra sonicator. This solution was filtered through Whatmann No 1 filter paper. The solution obtained was diluted with the mobile phase so as to obtain a concentration in the range of linearity previously determined. An aliquot of the internal standard was added to the sample solution prior to the dilution. All determinations were carried out in six replicates. The represented data were shown in TABLE 2.

RESULTS

The developed method was used based upon its ability to detect and quantitate nateglinide with the use of standard high-performance liquid chromatographic

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system equipped with UV-Visible detector.

Method validation[10,11]

1. Specificity and selectivity

The HPLC chromatogram recorded for the drug-matrix (mixture of the drug-excipients) showed almost no peaks within a retention time range of 10 min. as shown in figure 1. The figure shows that nateglinide is clearly separated from its internal standard. The retension time, asymmetric factor and peak area ratio of the marketed formulations were not affected with excipients present in formulation. Thus, the HPLC method presented in this study is selective and specific for nateglinide.

2. Linearity

The standard curve was obtained in the concentration range of 0.5-200g/mL. The linearity of these methods was evaluated by linear regression analysis, which was calculated by least square method. The mean ± standard deviation (SD) for the slope, intercept and correlation coefficient of standard curves (n=6) were calculated.

3. Limit of detection (L.O.D) and limit of quantification (L.O.Q)

Limit of detection was found to be 0.523µg/mL (signal to noise ratio is 3) and limit of quantification was found to be 1.743µg/mL (signal to noise ratio is 10).

Precision and accuracy

The precision of the method was demonstrated by inter day and intra day variation studies. In the intra day studies, six repeated injections of standard solutions were made and the response factor of drug peaks and percentage coefficient of variance (C.V) were calculated and presented in TABLE 3. In the inter day variation studies, six repeated injections of standard and sample solutions were made for three consecutive days and response factor of drug peaks and percentage C.V were calculated and presented in TABLE 3. From the data obtained, the developed RP-HPLC method was found to be precise. The accuracy of the method was determined by recovery experiments. The recovery studies were carried out six times and the percentage recovery and standard deviation of the percentage recovery were calculated and presented in TABLE 4. From the data obtained, added recoveries of standard drugs were found to be accurate.

TABLE 3: Precision studies

S.no	Concentration Taken (µg/ml)	Intra-day Measured concentration (µg/ml) Mean ± S.D	% C.V	Inter-day Measured Concentration (µg/ml) Mean ± S.D	% C.V
1.	5	4.97±0.02	0.47	5.09±0.03	0.68
2.	10	9.97±0.06	0.65	10.09±0.08	0.87
3.	25	25.06±0.16	0.67	25.98±0.24	0.96

TABLE 4: Accuracy studies

Sl. no.	Amount of standard drug added to preanalysed formulation	Amount of pure drug recovered Mean ±S.D (µg) (n=6)	% of recovery Mean ±S.D
1	120	119.56±0.34	99.63±0.28
2	150	150.45±0.54	100.30±0.36
3	180	179.54±0.65	99.74±0.36

System suitability

For system suitability, six replicates of standard sample were injected and studied the parameters like plate number (N), tailing factor (k), resolution (R) and relative retention time (α), Height Equivalent Theoritical Plate (HETP), capacity factor (k^1), plates per meter and peak symmetry of samples. The represented data

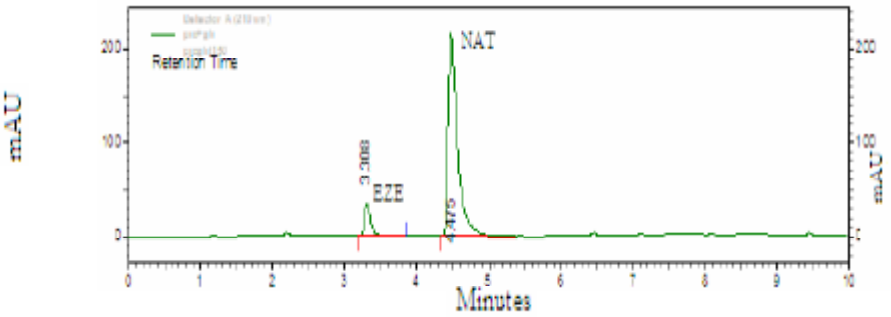

Figure 1: A typical chromatogram of nateglinide in formulation

TABLE 5 : System suitability parameters of nateglinide

Sl.no.	Parameter	Value
1	Resolution	2.27
2	Capacity factor	2.13
3	Theoritical plates	2759
4	Tailing factor	0.914
5	HETP	0.091×10^{-5}
6	Asymmetry	1.02

was shown in TABLE 5.

Robustness

The percent recoveries of nateglinide was good under most conditions and didn't show any significant change when the critical parameters were modified. The tailing factor for nateglinide was always less than 2.0 and the components were well separated under all the changes carried out. Considering the modifications in the system suitability parameters and the specificity of the method, as well as carrying the experiment at room temperature may conclude that the method conditions were robust.

DISCUSSION

The chromatographic method was optimized by changing various parameters, such as pH of the mobile phase, organic modifier and buffer used in the mobile phase. Retention of nateglinide has more dependence on pH of the mobile phase when compared to ezetimibe. The separation of peaks was also dependent on pH of the buffer and the percentage of methanol.

Under the presently prescribed conditions, the recovery studies of nateglinide were found to be from 99.63 to 100.30% respectively. This method is very useful for determination of nateglinide in pharmaceutical dosage forms, clinical studies and pharmacokinetic studies. The observation of C.V less than 2.0 for both intra- and inter-day measurements indicates high degree of precision. In the present method, a hypersil ODS C18 column has been used and the buffer pH in the mobile is 5.5, which is within the limits (pH 2-8) specified by the manufacturers. In the present method, we have established a linearity range of 0.5-200µg /mL; this linearity range covers all the strengths of nateglinide. Hence this method can be applied for quantifying the low levels of nateglinide in pharmaceutical dosage forms and other pharmacokinetic studies.

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