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UV-Spectrophotometric Determination of Nateglinide in Bulk and Pharmaceutical Dosage Form Using Hydrotropic Solubilization Technique

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ABSTRACT

Nateglinide is practically insoluble in water, sparingly soluble in strong acid; soluble in strong bases. It is an anti-diabetic, effective in treatment of diabetes. Solubility of Nateglinide is increased by using 0.1M Piperazine as a hydrotropic agent. Nateglinide showed the maximum absorbance at 220nm. At this wave length, hydrotropic agent and other tablet excipients did not show any significant interference in the spectrophotometric assay. The developed methods were found to be linear in the range of 5-30 μ g/ml with correlation coefficient r^2 of 0.9966. The mean percent label claim of tablets of Nateglinide in formulation estimated by the proposed method was found to be 98.05%, 98.07%, 99.15%. The developed methods were validated according to ICH guidelines and values of accuracy, precision and other statistical parameters were found to be in good accordance with the prescribed values. As hydrotropic agent was used in the proposed methods, these methods were eco-friendly and it can be used in routine quantitative analysis of drug in bulk and dosage form in industries.

Keywords: Nateglinide, AUC, Hydrotropic Solubilization Technique, First order Derivative Spectroscopy.

INTRODUCTION

Hydrotropic solution be a proper choice to preclude the use of organic solvents so that, a simple, accurate, novel, safe and precise method could be developed for estimation of poorly water soluble drug like Nateglinide (fig No.1). [1] The term hydrotropic agent was first introduced by Newberg (1916), to designate anionic organic salts which, at high concentrations, considerably increase the aqueous solubility of poorly soluble solutes. The hydrotropic agents are defined as non-micelle-forming substances, either liquids or

solids, organic or inorganic, capable of solubilizing insoluble compounds. [2-4] Hydrotropic agents consist generally of two essential parts, an anionic group and a hydrophobic aromatic ring or ring system. The anionic group is obviously involved in bringing about high aqueous solubility, which is a prerequisite for a hydrotropic substance. On other hand, planarity of the hydrophobic part has been emphasized as an important factor in the mechanism of hydrotropic solubilization. Hydrotropic agents commonly used include sodium salicylate, nicotinamide, urea, trisodium citrate, sodium ascorbate, piperazine, caffeine, potassium citrate etc. Hydrotropic agents have been observed to enhance the solubility of various substances in water.

Nateglinide chemically is 3- phenyl-2-[(4-propan-2-ylcyclo hexane carbonyl) amino] propanoic acid. Nateglinide is practically insoluble in water, sparingly

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soluble in strong acid; soluble in strong bases. It is an anti-diabetic, effective in treatment of diabetes. Literature survey revealed that there are many methods like Visible [5], HPTLC [6], UV spectrophotometric [7-8], RP-HPLC [9], Bioanalytical [10], Simultaneous determination by using Micellar Liquid Chromatography methods for determination of Nateglinide using organic solvents. [11] Drawbacks of organic solvents include higher cost, toxicity, pollution and error in analysis due to volatility. Therefore, hydrotropic solution may be a suitable alternative to exclude the use of organic solvents. Therefore it was thought useful to utilize the hydrotropic agents to extract the Nateglinide from its commercially available tablet dosage form to carry out UV spectrophotometric estimation.

The objective of the present investigation is to develop simple, precise, accurate and eco-friendly UV-spectrophotometric estimation (AUC and first order derivative methods) for determination of Nateglinide in bulk and in the tablet dosage form using piperazine as a hydrotropic solubilizing agent. The developed methods were validated as per the ICH guidelines.

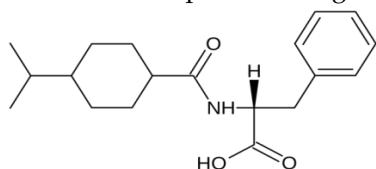


Fig. 1: Chemical structure of Nateglinide

MATERIALS AND METHOD

Apparatus

UV Visible spectrophotometer (Shimadzu Model 1800) was employed with spectral band width of 1nm attach with computer loaded with Shimadzu UV PC software (UV Probe) version 2.31 and using a pair of 10mm matched quartz cells.

Chemicals and Reagents

Piperazine (A. R. Grade; Qualigens) and distilled water used for the study.

Drug samples

Nateglinide (99.3%) was obtained as gift sample from Hetero Labs., Hyderabad, India. Pharmaceutical tablet formulation of Starlix purchased from local market.

Method development

Selection of solvent

0.1M piperazine solution was selected as a solvent for developing spectral characteristics of a drug. The selection was made after assessing the solubility in different hydrotropic solvents like sodium acetate, sodium benzoate, urea, sodium chloride, citric acid.

Preparation of Reagent solution

0.1M piperazine solution was prepared by 8.61 g of piperazine pure chemical was weighed and dissolved in 10 ml distilled water and volume was made up to the mark with distilled water in 100 ml volumetric flask.

Preparation of standard stock solution

Working standard Nateglinide 10 mg was weighed accurately and transferred to a 10 ml volumetric flask

and dissolved in 1 ml of 1M piperazine solution. The flask was shaken and volume was made up to the mark with distilled water to give a solution of 1000µg/ml. It was further diluted with distilled water to get a concentration of 100µg/ml. From this solution a series of aliquots were prepared for further method development.

Method A: Absorption Maxima Method

For the selection of analytical wavelength, 10µg/ml solution of Nateglinide was prepared by appropriate dilution of standard stock solution and scanned in the spectrum mode from 200nm to 400nm. From the spectrum λ_{max} of Nateglinide, 220nm was selected for the analysis. The calibration curve was prepared in the concentration range of 5-30µg/ml at 220nm. The calibration curve (Fig. 2) for Nateglinide was plotted in the concentration v/s absorbance and regression equation was calculated.

Method B: Area under Curve method

For the selection of analytical wavelength, 10µg/ml solution of Nateglinide was prepared by appropriate dilution of standard stock solution and scanned in the spectrum mode from 200nm to 400nm. Area under Curve (AUC) method involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelengths 215nm-225nm. Area calculation processing item calculates the area bound by the curve and the horizontal axis. The horizontal axis is selected by entering the wavelength range over which the area has to be calculated. The wavelength range is selected on basis of repeated observations so as to get the linearity between area under curve and concentration. From this regression equation was calculated (Fig. 3).

Method C: First order derivative spectroscopy

It involves the conversion of a normal spectrum to its zero, first, second or higher derivative spectrum. In derivative spectrophotometry, spectra are obtained by plotting the first or a higher order derivative of absorbance with respect to wavelength as a function of wavelength. Often, these plots reveal spectral detail that is lost in an ordinary spectrum. In addition, concentration measurements of an analyte in the presence of interference or of two or more analytes in a mixture can sometimes be made more easily or more accurately using derivative methods. In this method, 10µg/ml solution of Nateglinide was prepared by appropriate dilution of standard stock solution and scanned in the spectrum mode from 215nm to 225nm. The absorption spectra thus obtained were derivatized from zero to second order. First order derivative spectra of drug showed a sharp peak at 228nm, which was selected for its quantization. The calibration curve for Nateglinide was plotted in the concentration range of 5-30µg/ml at 220nm. The concentration of the drug present in the test solution was determined against the calibration curve in quantization mode (Fig. 4).

Estimation of Nateglinide in Tablet Formulation

For the estimation of Nateglinide in the commercial formulations, Starlix tablets each containing 60 mg of Nateglinide were weighed and average weight was calculated. The tablets were crushed and powdered in glass mortar. For the analysis of drug, quantity of powder equivalent to 10 mg of Nateglinide was transferred to 10 ml volumetric flask and dissolved in sufficient quantity of 0.1M by measuring amplitude difference at $\lambda_{\max}$ 228 nm. Results of tablet analysis are shown in Table 1. The assay procedure was repeated six times (n=6) Piperazine solution. It was filtered with whatman filter paper no. 41 and then the volume made up to the mark with water to obtain a stock solution of 1000 μ g/ml of Nateglinide. Further dilutions of the stock solution were made in distilled water to get required concentration. In method A, the concentration of Nateglinide was determined by measuring absorbance of sample solutions at 220nm($\lambda_{\max}$ of Nateglinide). In method B, the concentration of Nateglinide was determined by measuring absorbance of sample solutions in wavelength range of 215-225nm. In method C, first order derivative spectroscopy the concentration of Nateglinide was determined.

Validation of proposed methods

Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics. The method was validated as per ICH guide lines for different parameters like linearity, accuracy, precision.

Linearity

The linearity of the proposed UV spectroscopic methods were evaluated by analyzing different concentrations of standard solutions of Nateglinide and by plotting absorbance of analyte against concentrations of the analyte. Beer's law was obeyed for all four methods in the concentration range of 5 – 30 μ g/ml. A good linear relationship ($R^2=0.99$) was observed between the concentrations of Nateglinide and the corresponding absorbance. The regression analysis was made for slope, intercept and correlation coefficient values.

Accuracy

Accuracy is express as degree of closeness of experimental value to the true value. To study the accuracy of the proposed method and to check the interferences from excipients used in the dosage forms, recovery experiments were carried out by standard addition method. This parameter is evaluated by percent recovery studies at concentration levels of 50, 100 and 150% which includes addition of known amounts of Nateglinide working standard to a prequantified sample solution. Each of the dilution was observed six times. The samples were reanalyzed by proposed methods. The amount of Nateglinide was estimated by applying obtained values to regression equation. The percentage recovery of the drug was calculated. The results were shown in the Table 2.

Precision

Precision is the level of repeatability of results as reported between samples analyzed on the same day (Intra-day) and samples run on three different days (Inter-day). To check the intra-day and inter-day variation of the methods, solutions containing 5, 10 and 15 μ g/mL concentrations of Nateglinide were subjected to the proposed spectrophotometric methods of analysis and the recoveries obtained were noted. The precision of the proposed method *i.e.* the intra and inter-day variations in the absorbance of the drug solutions was calculated in terms of %RSD. Statistical evaluation revealed that relative standard deviation of drugs at different concentration levels for three times was less than 2.0 (Intra-day – 0.76, inter-day – 0.69). The values were shown in Table. 3.

RESULTS AND DISCUSSION

For quantitative estimation of Nateglinide in bulk and tablet dosage form three validated methods was proposed for method A the absorbance maxima was found to be at 220 for method B area under the curve in the range of 215-225nm and for method C $\lambda_{\max}$ at 228 nm was selected for first order derivative spectra were selected for the analysis. The % assay by the three methods was found to be in the range 98.05-99.15 for Nateglinide. No interference was observed from the pharmaceutical excipients. The % recovery obtained for absorption maxima, first order derivative spectroscopy and area under the curve was found to be 98.05%, 98.07%, 99.15% respectively. Hence, the proposed methods were validated in terms of linearity, precision and accuracy. The present work provides an accurate and sensitive method for the analysis of Nateglinide in Bulk and Tablet formulation.

The three spectrophotometric methods were developed and validated as per ICH guidelines. The standard deviation and % RSD calculated for the proposed methods are within limits, indicating high degree of precision of the methods. The results of the recovery studies performed indicate the methods to be accurate.

Table 1: Result of marketed formulation analysis

Proposed methods	Label claim (mg)	Test concentration μ g/ml	Mean amount found (n=6)
A	60 mg	10	98.05
B	60 mg	10	98.07
C	60 mg	10	99.15

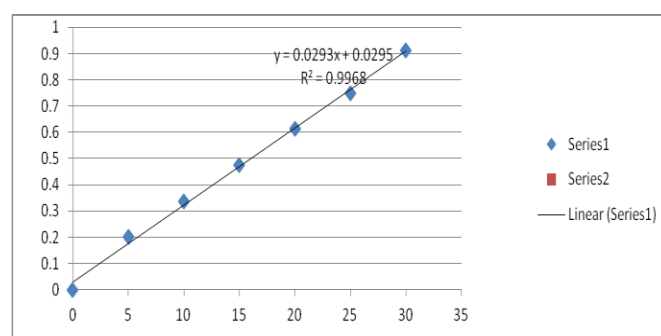


Fig. 2: Calibration curve of Nateglinide

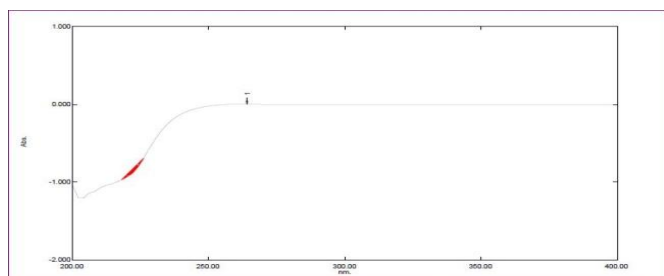


Fig. 3: UV-Spectrum indicating AUC

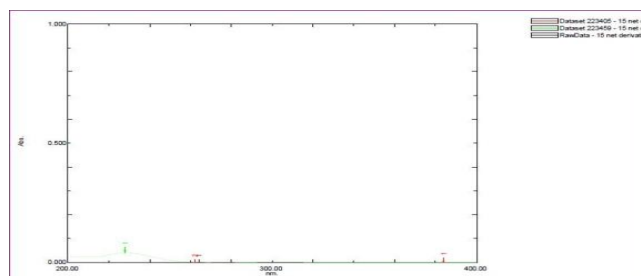


Fig. 4: First order derivative spectrum

Table 2: Recovery studies of Nateglinide

Conc. taken ($\mu\text{g/ml}$)	Recovery level (%)	Amount added ($\mu\text{g/ml}$)	Amount found ($\mu\text{g/ml}$) [n =6]			% Recovery		
			A	B	C	A	B	C
10	50	5	14.34	14.48	14.45	95.60	96.53	96.33
10	100	10	19.96	19.77	19.84	99.3	98.85	99.20
10	150	15	24.55	24.69	24.65	99.2	98.76	98.60

Table 3: Intraday and inter day precision studies of Nateglinide

Concentration taken ($\mu\text{g/ml}$)	Intra-day precision		Inter-day precision	
	Mean $\pm$ SD; (n=3)	%RSD	Mean $\pm$ SD; (n=3)	%RSD
5	4.89 $\pm$ 0.173	0.93	4.59 $\pm$ 0.28	0.98
10	9.89 $\pm$ 0.753	0.86	9.78 $\pm$ 0.980	0.92
15	14.86 $\pm$ 1.38	1.50	14.75 $\pm$ 1.53	1.78

Table 4: summary of optical characteristics and validation parameters

S. No.	Parameter	Method-A	Method-B	Method-C
1	λ_{max} (nm)	220nm	215-225nm	228nm
2	Beer's range ($\mu\text{g/ml}$)	5-30	5-30	5-30
3	Correlation coefficient (r^2)	0.9847 $\pm$ 0.0015	0.9945 $\pm$ 0.00176	0.999 $\pm$ 0.00176
4	Regression equation	Y=0.0243x+0.1192	Y=0.1593x+1.7538	Y=0.001x+0.000533
5	% Recovery	98.51	99.57	98.94

Hence, it can be concluded that the developed spectrophotometric methods are accurate, precise and can be employed successfully for the estimation of Nateglinide in bulk and formulation. The proposed methods were found to be simple, economical, eco-friendly, rapid, precise and accurate for the determination of Nateglinide in tablet dosage form. There is a good scope for other poorly water soluble drugs which may be tried to get solubilized in 0.1M piperazine solution (as hydrotropic agent) to carry out their spectrophotometric analysis excluding the use of costlier and unsafe organic solvents. Thus, it can be easily and conveniently adopted for routine quality control analysis.

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