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Development and Validation of Stress Induced Stability Indicating UV-Spectroscopic Method for Nateglinide Bulk and Pharmaceutical Formulations

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ABSTRACT

The present study describes a simple, accurate, precise and cost effective UV-VIS Spectrophotometric method for the estimation of Nateglinide, an anti-diabetic drug, in bulk and pharmaceutical dosage form. The solvent used was 0.1 N HCl+ 0.5 SLS solution and the λ max or the absorption maxima of the drug was found to be 212 nm. A linear response was observed in the range of 10- 60µg/ml with a regression coefficient of 0.999. The method was then validated for different parameters such as Linearity, Accuracy, Precision, Specificity, Robustness, Ruggedness, Limit of Detection and Limit of Quantification (LOQ). This method can be used for the determination of Nateglinide in quality control of formulation without interference of the excipients. Nateglinide was subjected to stress degradation under different conditions recommended by ICH such as acid, alkali, photolytic, dry heat and oxidative. The samples so generated were used for degradation studies using the developed method.

Keywords: Nateglinide, validation parameters, stress degradation study

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INTRODUCTION

The Nateglinide (NTG) is the anti-diabetic drug used in diabetes mellitus. Nateglinide is an amino-acid derivative that lowers blood glucose levels by stimulating insulin secretion from the pancreas.[1] This action is dependent upon functioning beta-cells in the pancreatic islets. Nateglinide interacts with the ATP-sensitive potassium (K^+ATP) channel on pancreatic beta-cells. The subsequent depolarization of the beta cell opens the calcium channel, producing calcium influx and insulin secretion. The extent of insulin release is glucose dependent and diminishes at low glucose levels. The tablets have to be taken 10-20 minutes before meal.[2] However, no UV-VIS spectrophotometric method was proposed for the estimation of Nateglinide in bulk and pharmaceutical dosage forms. The literature survey also indicates that no stability indicating spectrophotometric method was proposed for Nateglinide. The aim of this work is to develop and validate an analytical method by using UV-VIS spectrophotometry for the estimation of Nateglinide in bulk and pharmaceutical dosage forms and also perform stress degradation studies on the drug as per ICH Guidelines using the developed method.[3, 4]

The present investigation reports the development and validation of a UV spectrophotometric method for quantification of NTG in tablet and study of its degradation profile. Stress testing of NTG was carried out according to International Conference on Harmonization (ICH) guidelines entitled 'stability testing of new drug substances and products' and investigates the degradation studies in thermal degradation, acid hydrolysis, alkali hydrolysis and oxidation. The proposed method was demonstrated to be simple, selective and cost-effective compared to many reported methods.[5, 6]

MATERIALS AND METHODS:

The instrument used for the study was an UV-VIS double beam spectrophotometer (Model U-2900, Hitachi) with 1cm matched pair quartz cells. The solvent used was 0.01N HCl + 0.5% SLS and was of AR grade, purchased from SD Fine Chemicals Limited, India.

Solubility Test:

Solubility test for the drug Nateglinide was performed by using various solvents. The solvents include Water, 0.01N HCl + 0.5% SLS, Ethanol, 0.1 N Hydrochloric Acid (HCl), 0.1 N Sodium Hydroxide (NaOH). However, 0.01N HCl + 0.5% SLS was chosen as a solvent for developing the method.

Preparation of stock solution:

10 mg of Nateglinide was accurately weighed and transferred to 100 ml volumetric flask. To this

sufficient quantity of 0.01 N HCl containing 0.5% SLS solution was added to dissolve the drug and then volume was made upto 100 ml so as to obtain a stock solution of 100 µg/ml.

Determination of λ max:

Preparation of Working Standard Solution:

From the above stock solution, 3ml was pippetted into a 10ml volumetric flask and the volume was made up to the mark with 0.01N HCl + 0.5% SLS to prepare a concentration of 30µg/ml. Then the sample was scanned in UV-VIS Spectrophotometer in the range 400-200nm using 0.01N HCl + 0.5% SLS as a blank and the wavelength corresponding to maximum absorbance (λ max) was found to be 212nm. Shown in Figure. 1.

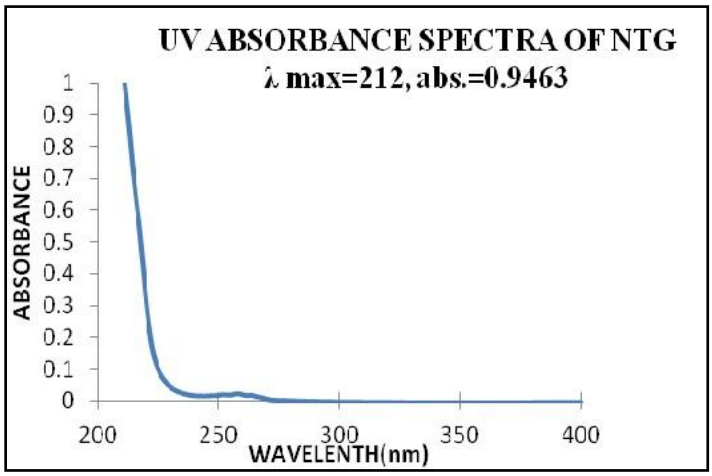

Figure. 1 UV **Spectra of Nateglinide**

Construction of calibration curve of Nateglinide:

Appropriate dilutions from the stock solution were made with 0.01 N HCl containing 0.5% SLS solution in concentration range of 10-60 µg/ml. The absorbances of the resulting drug solutions were recorded spectrophotometrically against 0.1 0N HCl containing 0.5% SLS solution as blank. The curve was drawn absorbance verses concentration which is shown in Figure. 2

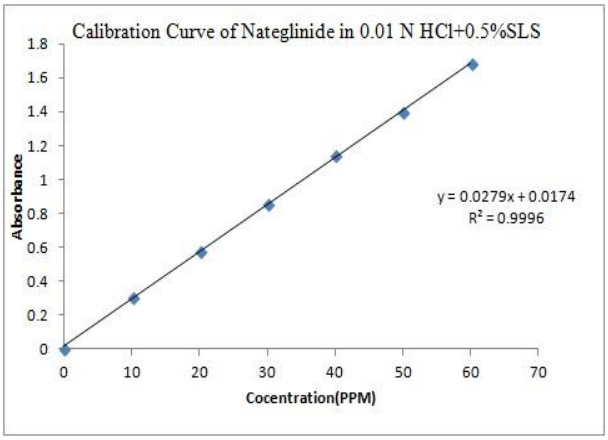

Figure. 2 Calibration curve for Nateglinide

Assay of Nateglinide (NATELIDE-60mg):

A quantity of powder equivalent to 10mg of Nateglinide was taken in a 100 ml volumetric flask and it was dissolved and diluted upto the mark with 0.1 N HCl+ 0.5 % SLS solution. The resultant solution was ultrasonicated for 5 minutes. The solution was then filtered using Whatmann filter paper No.40. From the filtrate, appropriate dilutions were made in methanol to obtain the desired concentration (30µg/ml). This solution was then analysed in UV and the result was indicated by % recovery given in table 11.

METHOD VALIDATION:

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 for different parameters like Linearity, Accuracy, Precision, Specificity, Robustness, Ruggedness, Limit of Detection (LOD) and Limit of Quantification (LOQ).[4, 5, 6, 7]

Linearity:

Various aliquots were prepared form the stock solution (100µg/ml) ranging from 10-60µg/ml. The samples were scanned in UV-VIS Spectrophotometer using 0.01N HCl + 0.5% SLS as blank. It was found that the selected drug shows linearity between the 10-60µg/ml reported in table 1 and Figure. 2.

Accuracy:

The accuracy of the method was determined by preparing solutions of different concentrations that is 80%, 100% and 120% in which the amount of marketed formulation(NATELIDE-60mg) was kept constant (10mg) and the amount of pure drug was varied that is 8mg, 10mg and 12mg for 80%, 100% and 120% respectively. The solutions were prepared in triplicates and the accuracy was indicated by % recovery shown in table 2.

Precision:

Precision of the method was demonstrated by intraday and interday variation studies. In intraday variation study, 8 different solutions of same concentration that is 30µg/ml were prepared and analysed repeatability and three times in a day i.e. morning, afternoon and evening and the absorbances were noted. The result was indicated by % RSD. (Table 4) In the interday variation study, solutions of same concentration 30µg/ml were prepared and analysed for three consecutive days and the absorbances were noted. The result was indicated by % RSD which is reported in table 3, 4, 5.

Specificity:

10 mg of Nateglinide was spiked with 50% (5mg), 100% (10mg), and 150% (15mg) of excipient mix (Magnesium Stearate) and the sample was analysed for % recovery of Nateglinide shown in table 6.

Robustness:

Robustness of the method was determined by carrying out the analysis at two different wavelengths. The respective absorbances were noted and the result was indicated by % RSD shown in table 7.

Ruggedness:

Ruggedness of the method was determined by carrying out the analysis by two different analysts and the respective absorbances were noted. The result was indicated by % RSD reported in table 8.

Limit of Detection (LOD):

The limit of detection (LOD) was determined by preparing solutions of different concentrations ranging from 0.1-1µg/ml. The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected but not necessarily quantitated as an exact value shown in table 9.

Limit of Quantification:

The LOQ is the concentration that can be quantitated reliably with a specified level of accuracy and precision. Sensitivity and regression parameters were reported in table 10.

DEGRADATION STUDIES:

The International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. The aim of this work was to perform the stress degradation studies on the Nateglinide using the method developed. [8,9,10]

1. **Stress degradation by hydrolysis under acidic condition:**

To 2 ml of stock solution(100µg/ml) of Nateglinide, 2 ml of 3 N HCl was added in 10 ml of volumetric flask and the volume was made up to the mark with 0.01N HCl + 0.5% SLS. Then, the volumetric flask was kept at normal condition for 90 minutes. After 60 min. time interval, dilution was the appropriate concentration of (20µg/ml). This solution was taken in cuvette. And take absorbance again blank. After 90 minutes, again 1ml of the solution was pippetted out from the flask and the above procedure was repeated.

2. **Stress degradation by hydrolysis under alkaline condition:**

To 2 ml of stock solution(100µg/ml) of Nateglinide, 2 ml of 1 N NaOH l was added in 10 ml of

volumetric flask and the volume was made up to the mark with 0.01N HCl + 0.5% SLS. Then, the volumetric flask was kept at normal condition for 90 minutes. After 60 min. time interval, dilution was the appropriate concentration of (20µg/ml). This solution was taken in cuvette. And take absorbance again blank. After 90 minutes, again 1ml of the solution was pippetted out from the flask and the above procedure was repeated.

3. Stress degradation under oxidative condition:

To 2 ml of stock solution(100µg/ml) of Nateglinide, 2 ml of 30% was added in 10 ml of volumetric flask and the volume was made up to the mark with 0.01N HCl + 0.5% SLS. Then, the volumetric flask was kept at normal condition for 30 minutes. After 15 min. time interval, dilution was the appropriate concentration of (20µg/ml). This solution was taken in cuvette. And take absorbance again blank. After 30 minutes, again 1ml of the solution was pippetted out from the flask and the above procedure was repeated.

4. Stress degradation under photolytic condition:

Take 50mg of Nateglinide in a Petri dish place it in for sunlight for 8 Hrs. after 4Hrs. quantity of powder equivalent to 10mg of Nateglinide was taken in a 100 ml volumetric flask and it was dissolved and diluted upto the mark with 0.01N HCl + 0.5% SLS. The resultant solution was ultrasonicated for 5 minutes. From this 2 ml of stock solution was removed prepared dilution of the appropriate concentration of (20µg/ml). This solution was taken in cuvette. And take absorbance again blank. After 8Hrs. again take 10 mg of powder and repeat procedure.

5. Stress degradation under Dry heat condition:

Take 50mg of Nateglinide in a Petri dish place it in for hot air oven for 8 Hrs. after 4 Hrs. A quantity of powder equivalent to 10mg of Nateglinide was taken in a 100ml volumetric flask and it was dissolved and diluted upto the mark with 0.01N HCl + 0.5% SLS. The resultant solution was ultrasonicated for 5 minutes. From this 2 ml of stock solution was removed prepared dilution of the appropriate concentration of (20µg/ml). This solution was taken in cuvette. And take absorbance again blank. After 8 Hrs. again take 10 mg of powder and repeat procedure. Results of Stress Degradation Studies were reported in table 11.

RESULTS AND DISCUSSION:

The calibration curve was constructed absorbance verses concentration in ppm shows linearity of drug was obtain in range between 10-60 ppm of Nateglinide. And of regression coefficient was found to be 0.9996 and assay indicated by % recovery 99.12%. Accuracy of the method was determined through the recovery studies of the drugs. Recovery of the drugs was well within the

acceptance limit. Precision of the method was determined by analyzing the drug formulation by repeatability, interday and intraday. Percent RSD of the analyte was found to be within the limit of 1.45%, thus the developed method was found to provide high degree of precision and repeatability.

Ruggedness was determined by performing the assay with same condition on different days, by different analysts and different day and time. The test results were found within limit 99.88 – 100.2%. The results were found to be reproducible, in spite of variations in conditions which could be normally expected from analysts to analysts. Robustness was determined by carrying out the assay during change Wavelength. Percent RSD was found to be within the limit NMT 3.63%. The values of RSD obtained with the change in wavelength ratio makes it possible to carry out the method for Nateglinide with a small variation in wavelength. The Limit of Detection (LOD) was found to be 0.8 µg/mL, Limit of quantification (LOQ) was found to be 0.82 µg/mL. System suitability was determined by performing the assay with the same sample repeatedly.

Table 1 Linearity table of Nateglinide in working standard

Concentrations(µg/ml)	**Absorbance**
0	0
10	0.308
20	0.580
30	0.854
40	1.145
50	1.402
60	1.682
Regression Equation	y=0.0279x+0.017
Slope	0.027
Intersect	0.017

Table 2 Accuracy readings of Nateglinide

Sr. No.	**Concentration**	**Absorbance**	**Result**
1.	80%	0.298	**Mean** = 0.295
		0.295	**S. D.** = 0.001
		0.294	**% R.S.D.** = 0.704
2.	100%	0.308	**Mean** = 0.306
		0.305	**S. D.** = 0.0009
		0.307	**% R.S.D.** = 0.498
3.	120%	0.323	**Mean** = 0.327
		0.330	**S. D.** = 0.0027
		0.328	**% R.S.D.** = 1.110

Table 3 Precision results showing Repeatability of Nateglinide:

Concentrations(µg/ml)	Absorbance	Statistical Analysis
30	0.875	
30	0.878	**Mean**= 0.8831
30	0.907	
30	0.873	**SD**= 0.01278
30	0.880	
30	0.897	**%RSD**= 1.45%
30	0.885	
30	0.870	

Table 4 Intra-assay precision:

Concentrations(µg/ml)	Absorbance		
30	0.875	0.868	0.872
30	0.878	0.879	0.878
30	0.907	0.880	0.871
30	0.873	0.870	0.869
30	0.880	0.875	0.862
30	0.897	0.874	0.875
30	0.885	0.871	0.880
30	0.870	0.868	0.882
Mean	0.883	0.882	0.873
SD	0.012	0.013	0.006
%RSD	1.45%	1.54%	0.75%

Table 5 Inter-Day assay precision:

Concentrations(µg/ml)	Absorbance		
	Day-1	Day-2	Day-3
30	0.879	0.861	0.878
30	0.871	0.879	0.875
30	0.885	0.871	0.880
30	0.887	0.865	0.866
30	0.881	0.869	0.881
30	0.879	0.872	0.881
30	0.895	0.868	0.879
30	0.881	0.873	0.875
Mean	0.881	0.869	0.876
SD	0.0050	0.0054	0.007
%RSD	0.57%	0.62%	0.57%

Table 6 Test for Specificity showing no effect of excipient

Excipient Conc.(%)	Nateglinide(added) (mg)	Nateglinide(recovered) (mg)	Mean NTG recovered	SD	%RSD
50	10	9.7			
100	10	10.01	9.87	0.15	1.59
150	10	9.9			

Table 7 Robustness of method for Nateglinide

Concentrations (µg/ml)	Wavelength (nm)	Absorbance	Statistical Analysis
30	212	0.895	**Mean**=0.876
		0.856	**SD**=0.019
		0.878	**%RSD**=2.23%
30	222	0.182	**Mean**=0.187
		0.195	**SD**=0.0068
		0.185	**%RSD**=3.63%

Table 8 Ruggedness of method for Nateglinide

Parameter	Set I		Set II	
System	Hitachi-U2900		Hitachi-U2900	
Analyst	885		1002	
Sample	30ppm		30ppm	
Absorbance	0.856	Mean=0.858	0.853	Mean=0.856
	0.858	SD=0.002	0.857	SD=0.002
	0.861	%RSD=0.29%	0.859	%RSD=0.35%
Assay	100.2		99.88	

Table 9 Limit of Detection (LOD):

Concentrations(µg/ml)	Absorbance
0.8	0.002
1.0	0.008

Table 10 Sensitivity and regression parameters

Parameter	Proposed method
Wavelength (λ max, nm)	212
Linear range, (µg/mL)	10– 60
Limit of detection (LOD),(µg/mL)	0.8
Limit of quantification (LOQ), (µg/mL)	0.82
Regression equation, y	y =0.0279x+0.017
Intercept (a)	0.017
Slope (b)	0.027
Regression coefficient (r)	0.9996
Assay indicated by % recovery	99.12%

Table 11 Summary of Result of Stress Degradation Studies

Condition	Time	% Degradation
Hydrolytic degradation of 3 N HCl	60 min	90.63
	90 min	96.47
Hydrolytic degradation of 1 N NaOH	60 min	93.50
	90 min	95.81
Photolytic degradation	4 Hrs	10.65
	8 Hrs	12.43
Dry Heat degradation	4 Hrs.	14.74
	8 Hrs.	15.67
Oxidative degradation	15 min	16.09
	30 min.	18.99

CONCLUSION:

This is conclude that all the above factors proposed method is accurate, precise, simple, sensitive, robust and cost effective and can be applied successfully for the estimation of Nateglinide in bulk and pharmaceutical formulation. The proposed method is also useful for determination of stress induced stability study of Nateglinide in sample of bulk and pharmaceutical dosage forms.

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