RESEARCH ARTICLE

# Spectrophotometric determination of nateglinide in bulk and tablet dosage forms

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Nateglinide (NTG) is available as tablet dosage form in 60 mg and 120 mg strength. In the present study, two simple, reproducible and efficient UV spectrophotometric methods for the estimation of this drug in bulk and pharmaceutical dosage forms have been developed. In method I, methanol-AR was used as solvent, while in method II, Methanol-AR + 10%V/V 3N NaOH was used as reference solvent. In method I, nateglinide shows $\lambda_{max}$ at 216 nm, which is then shifted to 225.4 nm on increasing the basicity of the reference solvent in method II. The linearity for nateglinide was observed to be statistically in the range of 10-100 µg/ml in method I and 100-1000 µg/ml in method II. Both the methods were validated using ANOVA. The recovery studies confirmed the accuracy of the proposed methods.

***Key words:*** *Absorptivity, nateglinide, $\lambda_{max}$, validation*

## INTRODUCTION

Nateglinide[1-3] chemically [N-(trans-4-isopropyl cyclo hexyl carbonyl)-D-phenylalanine] is a novel, nonsulfonyl urea derivative used for the treatment of type II diabetes mellitus. It is not official in any Pharmacopoeia. Literature survey reveals that micellar electrokinetic chromatography (MEKC)[4] and high performance liquid chromatography (HPLC)[5] methods have been developed for its determination. Besides this, UV spectrophotometric[6] and visible spectrophotometric[7] methods using ethanol as solvent has already been developed for its determination. In the present study, an attempt has been made to develop two different UV spectrophotometric methods for the determination of nateglinide in bulk and marketed formulations using methanol and alkaline methanol as solvents. ANOVA test was applied for comparison of both the methods. The developed methods wee found to be simple, sensitive and reproducible.

## MATERIALS AND METHODS

### Materials

*Instrument*

(1) Elico SL 160 Double beam UV-VIS Spectrophotometer.
   a) Spectral bandwidth of 1.8 nm.
   b) Wavelength accuracy of 0.5 nm.
   c) Matched quartz cells of 10 mm optical path length.
(2) GR 200 Analytical weighing balance (A&D company).
   Minimum sensitivity – 10 mg
   Maximum sensitivity – 210 gm
(3) Sonicator (Enertech Electronics).

*Reagents*

Methanol AR grade was procured from Loba Chemie Pvt. Ltd, New Delhi.

*Drug samples*

Nateglinide was obtained from Glenmark Pharmaceuticals, Mumbai.

*Marketed formulation*

Standard nateglinide tablet; Natilide-60 (60 mg) (Alembic Ltd.), Glinate-60 (60 mg) (Glenmark Pharmaceuticals, Mumbai) were procured from the local market.

### Methods

In method I, 10 mg nateglinide was accurately weighed and dissolved in Methanol AR to obtain a stock solution having a concentration of 100 µg/ml. From this stock solution, working standard solutions of drugs (10-100 µg/ml) were prepared by appropriate dilution.

Working standard solutions were scanned in the entire UV range to determine $\lambda_{max}$. Spectra were overlaid together to obtain $\lambda_{max}$ at 216 nm. Working standard

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solutions with concentrations ranging from 10 to 100 $\mu$g/ml for nateglinide were prepared by using a stock solution with a concentration of 100 $\mu$g/ml. The absorbances of these standard solutions were measured at 216 nm, and a calibration curve was plotted between the concentrations and the absorbances.

For method II, reference solvent was made alkaline (using NaOH) to produce a bathochromic shift ($\lambda_{max}$ shift towards the longer wavelength). Hence, a 10% V/V 3N NaOH solution in Methanol AR was made and used as reference solvent. 100 mg nateglinide was accurately weighed and dissolved in reference solvent to obtain a stock solution with a concentration of 1000 $\mu$g/ml. From this stock solution, working standard solutions of drugs (100-1000 $\mu$g/ml) were prepared by appropriate dilution.

Working standard solutions were scanned in the entire UV range to determine the $\lambda_{max}$. Spectra were overlaid together to obtain $\lambda_{max}$ at 225.4 nm. Standard solutions with concentration ranging from 100 to 1000 $\mu$g/ml for nateglinide were prepared by using a stock solution with a concentration of 100 $\mu$g/ml. The absorbances of these standard solutions were measured at 225.4 nm, and a calibration curve was plotted between the concentrations and the absorbances.

**Preparation of reference solvent and its optimization for method II**

Basic solvents are used for basic drugs for bathochromic effect (Red shift); hence, NaOH was used for nateglinide (a basic drug) to shift $\lambda_{max}$ from 216 nm to 225.4 nm. 10% V/V 3N NaOH solution in methanol was selected as reference solvent because the drug gave optimum absorbance values with better stability with this reference solvent with a given strength and a maximum shift in wavelength maximum. 3N NaOH solution was prepared by dissolving 12 gm of NaOH pellets in distilled water, and the volume was made up to 100 ml using distilled water. This solution was then standardized using potassium hydrogen phthalate. A stock of 250 ml reference solvent was made by mixing 25 ml 3N NaOH solution, and the volume was made up to 250 ml using Methanol AR. Optical parameters and regression characteristics of nateglinide in method I and method II are shown in Table 1.

**Table 1: Optical parameters and regression characteristics of nateglinide**

| Parameter | Method I nateglinide (at 216 nm in methanol AR) | Method II nateglinide (at 225.4 nm in 10% V/V NaOH in methanol AR) |
|---|---|---|
| Linearity range (mg/ml) | 10-100 | 100-1000 |
| Molar absorptivity (l/mole/cm) | $3.4 \times 10^3$ | $0.4 \times 10^3$ |
| Sandell's sensitivity (mg/cm$^2$/0.001 absorbance unit) | $9.2 \times 10^{-2}$ | 0.818 |
| Regression equation (y = a + bc) | 0.0104 | 0.0008 |
| Slope (b) | | |
| Intercept (a) | 0.014 | 0.1321 |
| Correlation coefficient (r) | 0.9995 | 0.9994 |

**Recovery studies and validation of the methods according to ICH guidelines[8,9]**

To study the accuracy, reproducibility and precision of the above proposed methods (method I and method II), recovery studies were carried out by the addition of the standard drug solution to the pre-analyzed samples (Synthetic Mixture and Marketed Formulations) by considering the percentage purity of the added sample bulk drug. The precision of the method was studied by carrying out interday and intraday analysis and is expressed as the percentage coefficient of variation. Specificity was checked by spiking the reference standard by placebo-like magnesium stearate (5 mg), talc (5 mg), starch (1 mg) and lactose (1 mg). The limits of detection and quantitation were studied on the basis of the standard deviation of the absorbances of blank and mean of the absorbances.

The results of recovery studies were found to be satisfactory and are reported in Table 2.

**Estimation of nateglinide in tablet dosage forms**

Twenty tablets (same respective batch number) of two pharmaceutical companies were taken, and IP method was followed to determine the average weight (before and after removing the coating). Average weight of Natilide-60 was 270 mg before removing the coating and 220 mg after removing the coating. Average weight of Glinate-60 was 153.7 mg before removing the coating and 143.2 mg after removing the coating.

These tablets were then finely powdered and triturated well. A quantity of powder (36.67 mg for Natilide-60 and 23.87 mg

**Table 2: Recovery studies of nateglinide in tablet dosage forms**

| Brand | Labeled amount (mg/tab) | | Amount recovered (mg/tab) | | % Recovery[a] ± Standard deviation | |
|---|---|---|---|---|---|---|
| | (Method I) | (Method II) | (Method I) | (Method II) | (Method I) | (Method II) |
| Natilide-60 | 60 | 60 | 59.13 | 58.87 | 98.56 ± 0.481 | 98.113 ± 0.633 |
| Glinate-60 | 60 | 60 | 59.33 | 58.97 | 98.88 ± 0.277 | 98.287 ± 0.349 |

a: average of three readings (Standard added 80,100 and 120%)

for Glinate-60), equivalent to 10 mg of nateglinide (NTG) was transferred to 100 ml volumetric flask, mixed with methanol (50 ml) and sonicated for 15 min. Volume was made up to 100 ml. The solution was filtered through Whatmann filter paper No. 40. The final volume was made up to 100 ml using methanol. This final solution provided 100 $\mu$g/ml of stock solution. From this stock solution, 2 ml was transferred and diluted to mark with methanol in a 10-ml volumetric flask (resultant: 20 $\mu$g/ml).

The absorbances of these solutions were measured at 216 nm using methanol as blank and % recovery was found out, and finally, the amount present in tablet formulations were measured. Results are shown in Table 3.

**Comparative analysis of variance between the results of assay of the above two methods**

Analysis of variance was carried out between method I and method II and within the synthetic mixture (SM), Natilide-60 and Glinate-60. Results are given in Tables 4-6.

**Table 3: Results of estimation of nateglinide in tablet dosage forms**

| Brand | Drug (NTG) | Label claim (mg/tab) | Amount found (mg/tab) | % Recovery |
|---|---|---|---|---|
| Natilide-60 | Method I | 60 | 59.13 | 98.56 |
| | Method II | 60 | 59.14 | 98.57 |
| Glinate-60 | Method I | 60 | 59.33 | 98.88 |
| | Method II | 60 | 58.81 | 98.014 |

a: average of three readings

**Table 4: Percent recovery data**

| | Synthetic mixture | Natilide-60 | Glinate-60 |
|---|---|---|---|
| Method I | 100.37 | 100.592 | 100.042 |
| Method II | 99.4295 | 100.5045 | 98.49 |

**Table 5: Summary**

| Groups | Count | Sum | Average | Variance |
|---|---|---|---|---|
| Method I | 3 | 296.61 | 98.87 | 0.4251 |
| Method II | 3 | 295.03 | 98.34333 | 0.069733 |
| Synthetic mixture (SM) | 2 | 198.24 | 99.12 | 0.4802 |
| Natilide-60 | 2 | 196.73 | 98.365 | 0.13005 |
| Glinate-60 | 2 | 196.67 | 98.335 | 0.00405 |

## RESULTS

**Comparative analysis of variance between the results of assay of above two methods**

Comparative analysis of variance (AVOVA) between the results of assay of two methods show no significant difference (F-cal. < F-crit.), and ANOVA also shows no significant difference within preparations (SM, Natilide-60 and Glinate-60).

## DISCUSSION

In the present work, two UV spectrophotometric methods for estimation of a novel anti-diabetic, Nateglinide (NTG), have been developed. In the first method, Methanol AR was used as solvent, which shows wavelength of maximum absorbance at 216 nm and follows the Beer-Lambert's range between 10 mg/ ml and 100 mg/ml. In the second method, reference solvent consisting of Methanol AR and 10% V/V 3N NaOH was used as solvent, which shows a wavelength of maximum absorbance at 225.4 nm, and follows the Beer-Lambert's range between 100 mg/ml and1000 mg/ml.

The validation parameters according to ICH Q2B guidelines were studied for both the methods. The methods were validated statistically and by recovery studies. The molar absorptivity, and Sandell's sensitivity values show the sensitivity. Linearity obtained for nateglinide was in the concentration ranges of 10-100 mg/ml in method I and 100-1000 mg/ml in method II. The values of coefficient of variance suggest high level of precision of the method. Furthermore, the mean recoveries of nateglinide were found to be 98.718% in method I and 98.20% in method II. The limit of detection and limit of quantitation values for nateglinide were 0.72 and 2.1 mg/ml, respectively, in method I and 1.68 and 5.1mg/ml, respectively, in method II.

It is evident from this study that both the methods are simple, rapid, precise and accurate for the estimation of nateglinide (NTG) in bulk and pharmaceutical formulations. Analysis of variance (ANOVA), employed for both the methods, also show no significant difference; hence, both the methods can be used for routine analysis.

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**Table 6: Analysis of variance**

| Source of variation | SS | df | MS | F-cal. | *P*-value | F-crit. |
|---|---|---|---|---|---|---|
| Between methods | 0.416067 | 1 | 0.416067 | 4.197747 | 0.177016 | 18.51276 |
| Within preparations | 0.791433 | 2 | 0.395717 | 3.992433 | 0.200303 | 19.00003 |
| Error | 0.198233 | 2 | 0.099117 | | | |
| Total | 1.405733 | 5 | | | | |

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