SPECTROPHOTOMETRIC METHODS FOR ESTIMATION OF NATEGLINIDE IN BULK DRUG AND ITS DOSAGE FORM

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INTRODUCTION
Nateglinide1-3 chemically [N-[(trans-4-isopropyl cyclo hexyl carbonyl)]-D-phenylalanine] is a novel, nonsulfonyl urea derivative used for the treatment of type II diabetes mellitus4-6. It is used in novel drug delivery system7 and In-vitro studies of drugs8. It is not official in any Pharmacopoeia. Literature survey reveals that micellar electrokinetic chromatography (MEKC)9, Spectrophotometric methods10-15, HPLC16-17, Liquid Chromatography18, HPTLC19-20. In the present study, an attempt has been made to develop three different UV spectrophotometric methods for the determination of nateglinide in bulk and marketed formulations using phosphate buffer. ANOVA test was applied for comparison of both the methods. The developed methods were found to be simple, sensitive and reproducible.

MATERIALS AND METHODS

Instrumentation
The present work was carried out on ElicoSL164 UV-visible spectrophotometer having double beam detector configuration. The absorption spectra of reference and test solution were carried out in a 1 cm quartz cell over the range of 200-800 nm.

Chemicals
All chemicals of analytical grade used as it is.

Preparation of standard solution
A stock solution of 1 mg/ml was prepared in phosphate buffer pH is 6.8. This solution is diluted with phosphate buffer pH is 6.8 to obtain required concentrations.

Preparation of sample solutions
20 tablets were weighed and powdered to 100mg of Nateglinide was weighted and transferred to
the 100ml volumetric flask. To it 50 ml of phosphate buffer was added and shake until the drug is dissolved. The solution was filtered and made up to 100ml with phosphate buffer. This solution was suitably diluted to obtain the required concentration. The same in other methods with respective solvent.

**PROCEDURE**

Aliquots of working standard solution of Nateglinide 1-6ml (100μg/ml) were transferred into a series of 10ml volumetric flask. The volumetric flasks are made up to the volume with the same solvent phosphate ph is 6.8 (method-I), (method-II), (method-III). Then the absorbance of the samples are measured spectrophotometrically at 239nm for method-I using phosphate buffer (ph-6.8) at 248nm for method-II using phosphate buffer and at 415nm for method-III using phosphate buffer against a reagent blank.

**VALIDATION**

Validation of the developed method was done according to ICH guidelines. Linearity

The linearity of the method is its ability to elicit test results that are directly proportional to the concentration of the analyte in samples. The calibration curve was taken in the range of 2-16 μg/mL, 2-12 at the respective λmax for method-I, method-II, and 2-16 μg/ml for method-III. The correlation coefficient of the linearity were found for three methods and reported in table No.1

**Precision and Accuracy**

The precision of an analytical method is determined by assaying a sufficient number of aliquots of a homogeneous sample to be able to calculate statistically valid estimate of % Relative Standard Deviation (%RSD). Intermediate precision was done to express within laboratory variation, on different days. Five replicates of 10 μg/ml concentration of the working standard mixture and sample solution were analyzed %RSD was found to be less than 2%. Accuracy were determined for three methods and results were reported in table no.2.

**Specificity**

Results of tablet solution showed that there is no interference of the excipients when compare with the working standard solution. Thus, the method was said to be specific.

**RESULT AND DISCUSSIONS**

The optimum conditions for methods I, II and III have been established by varying the parameters one at a time and keeping the other parameters fixed and observing the effect of products on the absorbance of the sample and colored species. Beer’s law limits, molar absorbivity, Sandal’s sensitivity, % range of error and % relative standard deviation are summarized in Table I. The regression analysis using the method of least squares was made for the slope (b), intercept (a) and correlation coefficient (r) obtained from different concentrations are given in Table I. The results showed that these methods have reasonable precision. To evaluate the validity and reproducibility of the methods, known amounts of pure drug were added to the previously analyzed pharmaceutical dosage forms and the mixtures were analyzed by the proposed methods. The percentage recoveries are given in Table 2. The interference studies veiled that the common excipients and other additives that are usually present in the injection dosage forms did not interfere at their regularly added levels.
Fig. 3: Determination of Nateglinide by first order derivative spectrophotometric method

Fig. 4: Determination of Nateglinide by second order derivative spectrophotometry method

Fig. 5: Calibration curve for method I

Fig. 6: Calibration curve for method II
Table 1: Optical regression characteristics, precision and accuracy of the proposed methods

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method I</th>
<th>Method II</th>
<th>Method III</th>
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<tbody>
<tr>
<td>λmax (nm)</td>
<td>239</td>
<td>248</td>
<td>217</td>
</tr>
<tr>
<td>Beer's law limits (μg.ml⁻¹)</td>
<td>2.16</td>
<td>2.12</td>
<td>2.16</td>
</tr>
<tr>
<td>Molar absorbivity (lit. mole⁻¹.cm⁻¹)</td>
<td>0.0492 x 10⁴</td>
<td>0.0054 x 10⁴</td>
<td>22.22 x 10²</td>
</tr>
<tr>
<td>Sandell's sensitivity (μg.cm⁻²/0.001 abs.unit)</td>
<td>0.020</td>
<td>0.018</td>
<td>0.014</td>
</tr>
<tr>
<td>Regression equation (Y=a+bx)</td>
<td>Y=0.048C+0.009</td>
<td>Y=0.053C+0.012</td>
<td>Y=0.068C+0.019</td>
</tr>
<tr>
<td>slope (b)</td>
<td>0.048</td>
<td>0.053</td>
<td>0.068</td>
</tr>
<tr>
<td>Intercept (a)</td>
<td>0.009</td>
<td>0.012</td>
<td>0.019</td>
</tr>
<tr>
<td>Correlation Co-efficient (r)</td>
<td>0.9999</td>
<td>0.998</td>
<td>0.999</td>
</tr>
<tr>
<td>RSD</td>
<td>0.648</td>
<td>0.313</td>
<td>0.713</td>
</tr>
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Y = a + bx where x is the concentration of Nateglinide μg/ml and Y is the absorbance at the respective λ max.

Table 2: Assay of Nateglinide in Pharmaceutical formulation

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Labeled amount in mg</th>
<th>Amount found by proposed Method-M₁</th>
<th>Amount found by proposed Method-M₂</th>
<th>Amount found by proposed Method-M₃</th>
<th>%Recovery* proposed by Method-M₁</th>
<th>%Recovery* proposed by Method-M₂</th>
<th>%Recovery* proposed by methods -M₃</th>
<th>%Recovery* proposed by methods -M₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tablet-1</td>
<td>59.904</td>
<td>59.67</td>
<td>60.18</td>
<td>99.84</td>
<td>99.45</td>
<td>100.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

R. Reference was UV method developed in the laboratory.*Recovery amount is the average of six determinations.

CONCLUSIONS
The proposed spectrophotometric methods were accurate, precise and reliable for the measurement of SIM in dosage form. The developed spectrophotometric method was validated for estimation of SIM using linearity, range, accuracy and precision. The RSD for all parameters was found to be less than one, which indicates the validity of method and assay results obtained by this method are in fair agreement. The developed method can be used for routine quantitative estimation of SIM in pharmaceutical preparation.

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REFERENCES
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