Spectrophotometric Determination of Nateglinide

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Two spectrophotometer methods have been developed for the determination of nateglinide in pure and its pharmaceutical dosage from. In UV spectrophotometric method, nateglinide showed absorption maxima at 210 nm in ethanol (95 %), where as in visible spectrophotometric method it imparted a purple color with Ninhydrin reagent showing absorption maxima at 570 nm. The methods obeyed beer's law in the concentration range of 20–100 μg/ml and 200–1000 μg/ml, respectively. The methods are statistically evaluated for accuracy and precision.

Nateglinide (NG) is chemically [N-(trans-4-Isopropylcyclohexylcarbonyl)–D-phenylalanine]. It is a novel, non sulfonyl urea derivative, used for the treatment of type-II diabetes mellitus. It is not official in any Pharmacopoeia. Literature survey revealed complicated HPLC methods for its determination in plasma and urine[1-2]. No simple spectrophotometric method is reported so far in the literature. Hence we describe a UV and a visible spectrophotometric method for the determination of NA in bulk and its formulation, in the present communication.

A Systronics model No. 118 single beam UV/Vis spectrophotometer was used for absorbance measurements. One hundred milligrams of pure NA obtained from M/S Alembic Pharmaceuticals was accurately weighed and dissolved in 100 ml of ethanol.

In method A, aliquots of standard stock solution corresponding 20-100 μg/ml were taken in a series of 10 ml volumetric flask and diluted up to the mark with ethanol (95 %). The absorbance measurements of these solutions were carried out against ethanol as blank at 210 nm. This data in this range was further considered for statistical validation and regression analysis. A graph of concentration of NG in μg/ml on abscissa Vs absorbance values on the ordinate was plotted. Regression analysis of the calibration data was carried out to determine the relationship between the absorbance and concentration.

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About 50 mg of NG was accurately weighed and transferred into a 50 ml volumetric flask. It was dissolved and diluted up to the mark with ethanol (95 %). Six millilitres of this solution was then transferred to a 100 ml volumetric flask and diluted up to the mark with ethanol (95 %). Absorbance of this solution was measured at 210 nm. Using the calibration curve, amount of NG present was calculated. The average of five determinations was taken to determine the content of NG in the bulk drug.

Two solid dosage forms, Glinate (M/s Healtheon) and Natilide (M/s Alembic) were used for analyzing the content of NG. The average weight of each tablet was calculated by weighing 10 tablets and the weighed tablets were powdered finely in a glass mortar. Powder equivalent to 100 mg of NG was accurately weighed and transferred into 50 ml volumetric flask. The contents were dissolved and diluted up to the mark with ethanol (95 %) and filtered through a Whatman No 1 filter paper. Six millilitres of this solution was transferred in to a 100 ml volumetric flask and diluted up to the mark with ethanol (95 %). Absorbance was measured at 210 nm, for standard and sample solutions. The average of five observations was taken to determine the content of NG in the formulation. The amount of NG present in Glinate (120 mg) and Natilide (120 mg) was found to be 119.7 and 120 8 mg, respectively.

Freshly prepared Ninhydrin solution (1 mg/ml in acetonitrile) was used for colorimetric analysis, in method B. From the stock solution, volumes equivalent to 200, 400, 600, 800 and 1000 μg /ml were transferred into 10 ml volumetric flasks.

Two milliliters of 0.1% w/v Ninhydrin reagent was added and mixed well. Volumetric flasks were placed in a hot water bath maintained at 80° for 20 min, to form a purple colored chromogen. Volumetric flasks were then taken out and allowed to cool. Then the contents were diluted up to the mark with acetonitrile and shaken. Absorbance of all these solutions was measured at 570 nm using a reagent blank.

Tablet powder equivalent to 100 mg was accurately weighed and dissolved in a 100 ml volumetric flask with ethanol (95%). After filtration, 8 ml of this solution was transferred into a 100 ml volumetric flask and it was treated as before for color development. Absorbance was measured at 570 nm against reagent blank. The amount of NG per tablet was calculated by comparing the absorbance values of standard and sample solutions. An average of five observations was taken to determine the content of NG in the bulk drug and its formulation. The amount of NG present in Glinate (120 mg) and Natilide (120 mg) was found to be 121 and 119 mg, respectively.

To study the accuracy, reproducibility, and precision and to check whether any positive or negative interference observed, a recovery study was performed. The recovery studies were conducted by addition of different amount of pure drug with known concentration to a preanalyzed sample solution. The recovery of added sample was studied at four different levels 0.0, 5.0, 10.0, and 20.0 mg. Each level was repeated 5 times. From the amount of drug found, the percentage recovery was calculated by using the formula, % recovery=$N(\Sigma XY)-(\Sigma X)(\Sigma Y)/N(\Sigma X^2)-(\Sigma X)^2 \times 100$.

Optimum conditions were established by varying one parameter at a time and keeping the others constant by observing the effect produced on the absorbance of the colored species. Various parameters involved in the color development that include, concentrations of the various reagents (2 ml of 0.1 % w/v Ninhydrin reagent) and time involved for maximum color development (20 min) were optimized. A linear correlation was obtained between absorbance and concentration over a range of 20-100 μg/ml (r=0.9999) for method A and 200-1000 μg/ml (r=0.9999) for method B thereby obeying Beer's law in those ranges. The regression equation was found to be Y=0.0024+0.0025 X for method A and Y=0.0015+0.0016 X for method B, where X is the concentration of NG in μg/ml and Y is the absorbance of respective absorption maxima. Molar absorptivity (1/mole.cm) value was found to be 79854.78 for method A and 50662.84 for method B, respectively. The percent RSD was found to be 0.01 for both the methods. Recovery for both the methods was found to be 99.5-100%. Recovery experiments indicated the absence of interference from the commonly encountered pharmaceutical additives and excipients. The low values of standard deviation (Glinate-0.01, Natilide-0.06) and coefficient of variance (Glinate-0.01, Natilide-0.05) indicates the proposed methods can be employed for the routine determination of NG in bulk sample and solid dosage forms.

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