Spectrophotometric Methods for the Determination of Nateglinide in Tablets

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Two simple and sensitive spectrophotometric methods for the determination of nateglinide in bulk samples and tablets are described. Method A (λmax 520 nm) is based on the reaction of drug with brucine and sodium metaperiodate under acidic conditions forming light pink coloured brucipuinone derivative while method B (λmax 455 nm) is based on the reaction of the drug with sodium 1,2–napthoquinone–4-sulphonate forming reddish orange chromogen. The concentration measurements are reproducible with a relative standard deviation of 1%.

Nateglinide (NTG) is N-(trans-4-isopropyl cyclohexane carbonyl)-D-phenylalanine. It is a new drug and finds place in Martindale-extra pharmacopoeia[1] and Merck index[2]. It is a non–sulfonylurea insulin secretagogue[3]. Literature survey reveals the presence of two reverse phase HPLC[4,5] methods for its estimation. As there is no report on visible spectrophotometry, the need for fast, low cost and selective methods are obvious especially for routine quality control analysis of pharmaceutical products containing NTG. Hence in this paper, the authors report two simple, sensitive and reproducible spectrophotometric methods for the determination of NTG in pure form as well as some tablets. In method A, brucine under acidic conditions oxidizes to quinine, which in turn undergoes nucleophilic attack on the coupler (NTG) to give coloured 1-mono substituted monoquinone derivative (λmax 520 nm). In the method B, aliphatic primary amine of the drug reacts with NQS to give reddish orange coloured complex having absorption maximum at 455 nm.

An Elico SL 171 spectrophotometer with 1 cm matched quartz cells was used in the present study. Aqueous solutions of brucine (0.2%w/v), sodium metaperiodate ($NaIO_4$, 0.2%w/v) and sodium 1,2-napthoquinone-4-sulphonate (NQS, 0.3% w/v) were prepared. H_2SO_4 (2 M) was prepared by dissolving 14 ml of concentrated sulphuric acid in 100 ml of distilled water.

A standard solution containing 1 mg/ml of NTG was prepared in methanol by dissolving 100 mg of pure NTG in 100 ml of methanol. From this solution, working standard solutions were prepared by dilution with methanol, 200 µg/ml for both method A and method B. The sample drug solution was prepared by taking tablet powder equivalent to 50 mg of NTG in a 50 ml volumetric flask, shaken thoroughly with 25 ml of methanol and subsequently diluted to 50 ml with methanol. It was filtered if necessary to obtain clear solution and further diluted as in standard solution preparation.

Method A comprises of transferring into a series of 10 ml volumetric flasks, aliquots of NTG (1.0-5.0 ml, 200 µg/ml) were added followed by 3.0 ml of brucine solution, 1.5 ml of $NaIO_4$ solution and 2.0 ml of 2.0 M H_2SO_4 were added to each tube and the volume was made up to 9.0 ml with distilled water, shaken well and placed in boiling water bath for 15 min. The reaction mixture was cooled and made up to 10 ml with methanol. The absorbance of the coloured solution was measured at 520 nm during the next 30 min

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against a reagent blank prepared simultaneously.

In method B, aliquots of standard drug solution (1.0-6.0 ml, 200 µg/ml) were transferred into a series of 10 ml volumetric flasks. Then 1 ml of NQS reagent was added and heated on a boiling water bath for 15 min, cooled and made up to volume with methanol. The absorbance of the reddish orange coloured chromogen formed is measured at the wavelength at the wavelength of maximum absorption 455 nm against the reagent blank. Different concentrations of the sample drug preparations were taken in 10 ml volumemetric flasks and above procedure is subsequently followed. The amount of drug (NTG) in each method was computed from the appropriate calibration curve.

TABLE 1: OPTICAL CHARACTERISTICS AND PRECISION OF THE PROPOSED METHODS

Parameter	Method A	Method B
λmax (nm)	520	455
Beer's law Limit (µg/ml.)	20-100	20-120
Molar absorptivity (1/mol.cm)	$3.108X10^3$	$3.94X10^5$
Sandell's sensitivity (mg cm^{-2}per 0.001 absorbance unit)	$4.37X10^{-2}$	0.049
Regression equation (y=a+bC)*		
Slope (b)	$9.197X10^{-3}$	$3.7X10^{-3}$
Intercept (a)	$2.66X10^{-4}$	$4.0X10^{-4}$
Correlation coefficient (r)	0.9999	0.9998
Relative standard deviation (%)**	0.475	0.270
% Range of error (confidence limits)**		
0.05 level	0.498	0.289
0.01 level	0.782	0.444
% Error in bulk samples***	-0.543	-0.135

*Y=a+bC, where C is concentration of analyte and Y is absorbance unit, **average of six determinations, ***average of three determinations.

Optimum operating conditions used in the procedures were established adopting variation of one variable at a time (OVAT) method. The optical characteristics of the methods are presented in Table 1. The precision and accuracy of the methods was tested by measuring six replicate samples of the drug in Beer's law limits. Commercial formulations containing NTG were successfully analyzed by the proposed methods. The results are presented in Table 2 None of the usual excipients employed in the formulation of dosage forms interfere in the analysis of NTG by the proposed methods. As an additional check of accuracy, recovery experiments were performed by standard addition method. When tablets containing NTG were analyzed, the results obtained by the proposed methods were in good agreement with the labeled amounts. The recovery with the methods was found to be 99-101%. The proposed methods are simple, convenient, accurate, sensitive and reproducible. They can be employed for routine analysis of NTG in bulk drug and formulations.

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TABLE 2: ASSAY OF NTG IN PHARMACEUTICAL FORMULATIONS

Drug*	Label Claim mg/tablet	Amount found by Proposed Methods		% Recovery by proposed Methods **	
		Method A	Method B	Method A	Method B
Tablet 1	60	59.76	59.51	99.9 ± 0.16	99.8 ± 0.33
Tablet 2	120	119.76	119.27	99.9 ± 0.16	99.71 ± 0.41

*Glinate, 60 and 120 mg, Glenmark pharmaceuticals; **Recovery of 10 mg added to the pre-analyzed pharmaceutical dosage forms (average of 3 determinations).

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Synthesis and Biological Activities of Some 1,3,4-Oxadiazoles, Thiadiazoles, Triazoles and Related Compounds Possessing Benzofuran moiety

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The condensation of 5-chloro-3-methyl-2-benzofuran carbohydrazide (4) with phenylisothiocynate gave 5-chloro-3-methylbenzofuran-2-carbo-N-phenylthiosemicarbazide (5). The cyclisation of 5 under different reaction conditions furnished 1,3,4-oxadiazole, 1,3,4-thiadiazole, 1,2,4-triazole, thiadiazolidinone and thiopyrimidone. Their chemical structures have been assigned by IR, ^{1}HNMR, Mass and elemental analyses. All the compounds synthesized were evaluated for antibacterial and antifungal activity*.

There is considerable interest in the chemotherapeutic activity of heterocycles such as oxadiazoles, thiadiazoles, triazoles, thiadiazolidinones and pyrimidines[1-4]. Recently we have reported that biheterocycles containing benzofuran and pyrazoline ring system possessed significant antimicrobial activity[5]. In continuation of our research on synthesis of pharmacologically active benzofuran derivatives, we now report the synthesis of oxadiazoles, thiadiazoles, triazoles, thiadiazolidinones and pyrimidones coupled with benzofuran moiety and the biological activity exhibited by them.

5-Chloro-3-methyl-2-benzofuran carbohydrazide (4), an intermediate in the synthesis of title compounds was synthesized from ethyl 5-chloro-3-methyl-2- benzofuran carboxylate (3). 5-Chloro-2-hydroxy acetophenone (1) on reaction with ethyl chloroacetate in anhydrous acetone in presence of anhydrous potassium carbonate gave 5-chloro-2-ethoxycarbomethoxy acetophenone (2), the ester (2) underwent Thorpe-Zeigler cyclisation in anhydrous dimethyl formamide in presence of anhydrous potassium carbonate to afford ethyl 5-chloro-3-methyl-2-benzofuran carboxylate (3). The compound (3) on reaction with hydrazine hydrate in ethanol gave 5-chloro-3-methyl-2-benzofuran carbohydrazide (4).

The carbohydrazide (4) was subjected to condensation with phenyl isothiocynate in ethanol to produce 5-chloro-3-methylbenzofuran-2-carbo-N-phenylthiosemicarbazide (5). Thiosemicarbohydrazide (5) underwent cyclisation in boiling ethanol with iodine and potassium iodide which resulted in the formation of 5-anilino-2-(5-chloro-3-methyl benzofuran-2-yl)-1,3,4-oxadiazole(6).

Cyclisation of 5 in concentrated sulphuric acid at low temperature furnished the formation of 1,3,4-thiadiazole (7). Thiosemicarbohydrazide (5) on refluxing with aqueous sodium hydroxide offered 5-(5-chloro-3-methylbenzofuran-2-yl)-4-phenyl-2,3-dihydro(3H)1,2,4-triazol-3-thione (8). The treatment of compound 5 with chloroacetic acid in presence of sodium acetate in ethanol produced thiazolidinone

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