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Visible Spectrophotometric Determination of Nateglinide in Pharmaceutical Formulations

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A sensitive visible spectrophotometric method for the analysis of nateglinide either in pure form or pharmaceutical formulations has been developed. The drug was extracted from formulations with chloroform from an alkaline medium and reacted with citric acid-acetic anhydride reagent to produce a bluish-violet colour having absorption maximum at 580 nm. Beer's law is obeyed in the concentration range 2–28 μg/mL. The method is quantitatively evaluated and found to be precise and accurate.

Key Words: Citric acid-acetic anhydride reagent, Nateglinide, Visible spectrophotometry.

INTRODUCTION

Nateglinide (NTG) is an amino acid derivative of D-phenylalanine and is a non-sulfonylurea insulin secretagogue[1]. Chemically it is N-(trans-4-isopropyl cyclohexane carbonyl)-D-phenylalanine. Nateglinide belongs to the meglitinide class of oral hypoglycemic agents. It causes insulin secretion *via* inhibition of the ATP-sensitive potassium channels in the pancreatic beta cells[2]. Literature survey reveals the presence of only one method of estimation of the drug using RP-HPLC method[3]. Hence, a new and simple visible spectrophotometric method has been developed for routine analysis of nateglinide in pharmaceutical formulations.

EXPERIMENTAL

An Elico SL 171 mini-spectrophotometer with 1 cm matched quartz cell was used for all spectral measurements. A Systronics model 361-pH-meter was used for pH measurements.

All the chemicals used were of analytical grade and all the solutions were prepared with distilled water. Freshly prepared solutions were always used. .Citric acid-acetic anhydride (C-A) was prepared by dissolving 1.2 g citric acid monohydrate (E. Merck) in 5 mL anhydrous methanol and diluting up to 100 mL with acetic anhydride (E. Merck).

Preparation of Standard Drug Solution

Nateglinide (25 mg) was treated with 20 mL of 0.1 N NaOH solution and

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transferred into a 125 mL separator. The free base of the drug was extracted with 4 successive 25 mL portions of chloroform. The total chloroform extract was filtered through a pledget of cotton carrying 2 g anhydrous sodium sulphate and made up to 250 mL with chloroform for getting the working standard solution (100 μg/mL of NTG). Tablet powder equivalent to 25 mg of the drug was taken and the sample solution was prepared in the same manner as mentioned under standard drug solution preparation.

Analysis of Pure Samples and Pharmaceutical Formulations

Aliquots of standard and sample solutions (50–300 μg of NTG) were taken in a series of 25 mL graduated tubes and gently evaporated on a boiling water bath to dryness. To this, 1.0 mL C-A reagent was added and the tubes were immersed in a boiling (95 ± 2°C) water bath for 30 min. The tubes were cooled to room temperature and made up to the mark with acetic anhydride. The absorbances of the coloured solutions were measured after 15 min at 580 nm against reagent blank. The amount of the drug was computed from the calibration graph.

RESULTS AND DISCUSSION

The optimum conditions for the development of the method were established by varying the parameters one at a time and keeping the others fixed, and observing the effect produced on the absorbance of the coloured species. The optimum experimental conditions were incorporated in the recommended procedures for colour development. Beer's law was found to be valid over the concentration ranges given in Table-1 at appropriate λ_{max} values.

Analytical Data

The Beer's law limits, molar absorptivity, Sandell's sensitivity, detection limits, regression equation and correlation coefficients obtained are given in Table-1. The per cent S.D. and the per cent range of error for each method are given in Table-1.

TABLE-1
OPTICAL CHARACTERISTICS OF THE PROPOSED METHOD

Parameters		Method
λ_{max} (nm)		580
Beer's law limits (μg/mL)		2–28
Molar absorptivity (L mol^{-1} cm^{-1})		3.416×10^4
Sandell's sensitivity (mg cm^{-2} per 0.001 absorbamce unit)		1.50×10^{-2}
Regression equation (y = a + BC)* Slope (b)		5.40×10^{-2}
Intercept (a)		6.50×10^{-5}
Correlation coefficient (r)		0.9999
Relative standard deviation (%)†		0.3340
%Range of error (confidence limits)†:	0.05 level	0.3070
	0.01 level	0.6030
% Error in bulk samples‡		0.1300

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

Interference studies

The effect of excipients and other additives present in the formulations of NTG in the determination under optimum conditions were investigated. Commercial formulations (tablets) containing NTG were successfully analysed by the proposed method. The interfering excipients should be removed by selectively extracting the NTG initially with chloroform. Accuracy and recovery experiments were performed by adding a fixed amount of the drug to the preanalysed formulations. These results are summarized in Table-2.

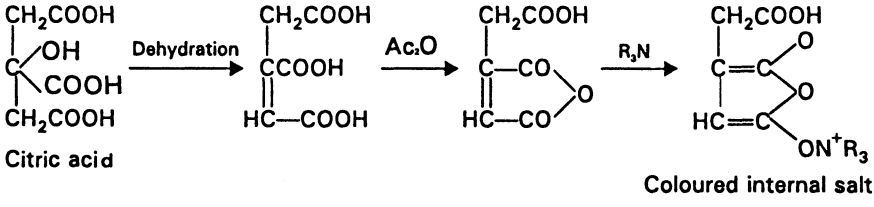
TABLE-2
ASSAY OF NATEGLINIDE IN PHARMACEUTICAL FORMULATIONS

Drug*	Lable claim (mg/tablet)	Amount found by proposed method†	% Recovery by proposed method‡
Tablet 1	60	59.83 ± 0.15	99.83
Tablet 2	60	59.63 ± 0.48	99.63
Tablet 3	120	119.77 ± 0.28	99.89

†Drug from different pharmaceutical companies.
†Average ± standard deviation of 6 determinations.
‡Recovery of 10 mg added to the pre-analyzed pharmaceutical dosage forms (average of 3 determinations).

Chemistry of Coloured Species

When basic tertiary amines are heated with a solution of citric acid (or *cis*-aconitic anhydride[4]) in acetic anhydride, a red to violet colour develops. The colour reaction was reported to be selective for tertiary amines[5]. In preparing citric acid-acetic anhydride reagent, citric acid is heated with Ac_2O.[6] The *trans*-configuration of aconitic acid[7] initially forms through dehydration besides *cis*-aconitic anhydride and subsequently yields α,γ-anhydro aconitic acid[8]. α,γ-Anhydride develops violet colour in the presence of tertiary amine which allows the colorimetric determination of class of compounds. The coloured compound formed appears to be internal salt resulting from the tertiary amine and aconitic anhydride **(Scheme-1).**



Scheme-1

Conclusions

The proposed method is simple, sensitive and useful for the routine determination of nateglinide in pure samples and pharmaceutical formulations. Moreover, no spectrophotometric methods have been reported for the drug and hence this

method can be used for analysis, where HPLC is not available. The method is selective for compounds containing the tertiary amino group in their molecules.

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