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Simultaneous Spectrophotometric Determination of Repaglinide in Pharmaceutical Dosage form Using *Indigo carmine*

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Abstract: The present study describes a simple, accurate, precise and cost effective UV-VIS Spectrophotometric method for the estimation of extractable colored complex of drug with coloring agent Indigo Carmine. A wavelength maximum was found to be 562 nm. The concentration range of 10-30 μg ml^{-1} with linear regression of 0.9997, while the percentage recovery, LOD and LOQ were 100.03-101.01 %, 0.15 μg ml^{-1} and 1.19 μg ml^{-1}, respectively. The result of analysis have been validated statistically and also by recovery studies. From the percentage recovery and specificity studies it was concluded that there was no interference of common additives during the estimation. This proves the suitability of this method for the routine quality control analysis of the Repaglinide in formulation.

Key words: Repaglinide · Spectrophotometric · Indigo Carmine

INTRODUCTION

Repaglinide is a meglitinide antidiabetic used in the management of type 2 diabetes mellitus, chemically *S*(+)2-ethoxy-4(2((3-methyl-1-(2-(1-piperidinyl) phenyl)-butyl) amino)-2-oxoethyl) benzoic acid [1-2]. It is official in USP [3] which describes liquid chromatographic method for its quantitation. Literature survey reveals that one HPLC method in human plasma [4], two HPLC [5-6], one RPTLC [7] and Spectrophotometric method [8] in pharmaceutical dosage form. In recent times, there is an increase tendency towards the development of stability-indicating assay, using the approach of stress testing as mentioned in the ICH guidelines (Q1A). It also recommends carrying out of stress testing on the drug substance to establish its inherent stability characteristics and to support the suitability of the proposed analytical procedure. The stress testing encompasses the influence of temperature, humidity, light, oxidizing agent as well as susceptibility over a wide range of pH values [9, 10]. Author of the article and his research team has developed a UV Method development in different pharmaceutical dosage form [11-25] using Indigo Carmine [26].The purpose of this work was to develop and validate simple, specific, sensitive, accurate, precise, rapid and cost effective UV method with help Indigo Carmine dye for the estimation of Repaglinide in formulations.

MATERIALS AND METHODS

UV Visible spectrophotometer (Shimadzu Model 1601) was employed with spectral bandwidth of 1 nm and wavelength accuracy of 0.3 nm (with automatic wavelength correction with a pair of 1 cm matched quartz cells). Single component tablet formulations of repaglinide (2 mg) (formulation A Eurepa, manufactured by Torrent Pharma. Ltd., Ahmedabad) were purchased from local market. All chemicals and reagents used were of AR/HPLC grade, Chloroform, ammonia (SD'S) and methanol (A.R., Ranbaxy Ltd., New Delhi) were used for mobile phase preparation and as solvent. All chemicals used in this study were analytical grade and used without further purification. Chloroform (s.d. finechem, Bombay, India), Indigo Carmine.

Solubility Test: Solubility test for the drug repaglinide was performed by using various solvents. The solvents include chloroform, Methanol and Ethanol. However, Distilled water was chosen as a solvent for developing the method.

Determination of λ_{max}

Standard and Sample Solution Preparation: 1 mg ml^{-1} stock solution of repaglinide was prepared by dissolving 100 mg of repaglinide in appropriate volume of double

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distilled water and made up to 100 ml in volumetric flask and used as stock solution.

Twenty repaglinide tablets were powdered and an accurately weighed quantity powder equivalent to 100 mg of repaglinide from each brands were dissolved in methanol. The excipients were separated by filtration using Whatman filter paper (No.41) and the filter paper washed three times with distilled water for effective liberation of drug from the core. Filtrate and washings of the tablets samples were transferred into 100 ml flask and diluted to the mark with absolute ethanol and the spectrophotometric procedure was followed.

Reagent Preparation: 0.1 gm of Indigo Carmine was weighed and transferred into a 100 ml standard flask and the volume was made up to the mark to get the required concentration (0.5%w/v).

Calibration Curve: Standard drug solution (100µg/ml) was prepared in double distilled water and was diluted with same, so as to give several dilutions in concentration range 10-30 µg ml^{-1} of drug. To 10 ml of each dilution taken in separating funnel, 10 ml of Indigo Carmine solution was added and shaken gently. Then 5 ml of ether was added reaction mixture was shaken gently and allowed to stand so as to separate aqueous ether layer. The ether layer was separated out and transferred to 10 ml of volumetric flask. Reaction mixture was extracted further with 50 ml fresh ether. The ether layer was separated out and transferred to 100 ml of volumetric flask. Absorbance of this final extracted chloroform layer was measured at wavelength maxima 562 nm against blank. Calibration curve was plotted between concentration of drug and measured absorbance and combined it with previously extracted chloroform layer containing complex. Absorbance of this final extracted ether layer was measured at wavelength maxima 562 nm against blank. Calibration curve was plotted between concentration of drug and measured absorbance.

Method Development: Aliquots of stock transferred into a series of separating funnel then 1 ml of Indigo Carmine reagent and 2 ml phosphate buffer of pH 4.1 was added, then the solutions were allowed to stand for few minutes, followed by accurately measured quantity (10ml) of methanol and extracted well to give concentration 10-30 µg ml^{-1}, all the solutions were passed through dried sodium sulphate to remove water. Solution scanned between 400-800nm which shows λ_{max} at 642 nm. The above λ_{max} was used for its analysis of repaglinide in formulation. Formed ion pair complex was obeying Beer's law in the range of 10-30 µg ml^{-1}. The developed methods for simultaneous estimation of repaglinide were validated as per ICH guidelines [27]. The effect of Indigo Carmine concentration on the reaction was checked out at room temperature and away from direct sunlight. The reaction of repaglinide was dependent on the concentration of dye used. A concentration of 0.1% (w/v) was selected as the optimum reagent concentration. The absorbance of the solution was measured after 10 minutes after adding reagent and up to 3 hrs, the reaction was slow and the formed colour was stable up to 3 hrs. The method was validated by recovery study were carried out by the addition of different amount of drugs to pre analyze solution (10µg/ml). From the stock solution of 100µg/ml of each drug 1ml solution was taken in each of four volumetric flask (10ml), then 1.2, 0.8, 0.4 ml of mixed standard stock solution (100µg/ml of repaglinide) added in three flasks so that remaining one flask contains no added solution. These solutions were scanned at 514 nm. Percentage recovery was found in the range of 100 % to 105%. Robustness and Ruggedness of the method were also studied by altering wavelength of estimation and changing the dye's concentration which were also within the acceptable limit with respect to % RSD (Table. 3). In case of ruggedness difference in the estimation was studied by means of analyzing the samples in two different days by following same procedure and the results were summarized in Table 3. To check the accuracy of the developed methods and to study the interference of formulation additives, analytical recovery experiments was carried out by standard addition method [28]. From that total amount of drug found and percentage recovery was calculated. To check the degree of repeatability of the methods, suitable statistical evaluation was carried out. Five samples of the tablet formulations

Table 1: Accuracy of the proposed method

Sample	Label Claim	Estimated amount (mg/tab)	Spike Level (%)	Amount of Drug Added	Amount of Drug recovered	%Recovery	RSD(%n=6)
1	2	2.11	50	20	2.02	101.02	0.83
			100	40	2.00	100.00	1.08
			150	60	1.99	99.99	0.65

Table 2: Intraday, Interdays, data of tablet formulation

		Interday precision %COV		
sample	Intra day precision %COV (n=6)	Day 1[a]	Day 2[a]	Day 3[a]
I	0.987	0.863	0.743	0.659

COV: Coefficient of variance

Table 3: Robustness and day to day variation of the method

Parameters Studied	Recovery(%±RSD
Indigo CarmineConcentration(%,w/v)	
10.00	100.03±0.21
30.00	101.08±0.98
Wave length	
496 nm	65.32 ±0.32
583 nm	87.93 ±0.28
623 nm	100.02±0.03
Ruggedness(day-to-day variation)	
Day1	100.03±0.17
Day 2	99.98 ±0.76
Day 3	99.76 ±0.8

were analyzed for the repeatability study. The standard deviation, coefficient of variance and standard error was calculated. The intra and inter-day precision was calculated by assay of the sample solution on the same day and on different days at different time intervals, respectively. The results are presented in Table 2.

Analysis of Combined Dosage Form: The absorbance of final sample solution was measured against methanol as blank at 642 nm. The amount of repaglinide was computed by adding the absorbance value in simultaneous equation.

RESULTS AND DISCUSSION

In present research work a UV Spectrometric method has been developed for determination of Repaglinide from its tablet formulations. The developed method was based on formation of absolute ethanol extractable complex of drug with Indigo Carmine in double distilled water. Wavelength maxima of Repaglinide was found to be at 438.6 nm and linearity was observed in concentration range of 10-30 μg/ml. Percentage label claim estimated for tablet formulation was found to be in the range of 99.42-99.08 % and respective values of standard deviation were found in the range of 0.3821-0.6520 for two different batches of tablet formulations of Repaglinide (Table 1). To fix the linearity a calibration curve was constructed by plotting the absorbance as a function of the corresponding concentrations. The regression equation for the results was:

$$A=6.36123\ *-1.5432\ (r = 0.\ 0.9997)$$

Where A is the absorbance at 642 nm, C the concentration of Repaglinide in $\mu g ml^{-1}$ in the range of 10-30 $\mu g\ ml^{-1}$ and r is the correlation coefficient. The molar absorptivity ($\hat{a}$) was found to be 3.8531* 0.9654 lit mol cm^{-1}.

The limit of detection (LOD) and limit of quantitation (LOQ) were determined (ICH 2002) using the formula: LOD or LOQ = ? S.D.a/b, where ? = 3 for LOD and 10 for LOQ, S.D.a is the standard deviation of the intercept and b is the slope. The LOD and LOQ were 0.15 $\mu g\ ml^{-1}$ and 1.19 $\mu g\ ml^{-1}$, respectively. The detection and quantitation limits determined were 0.74 and 0.18 mg ml^{-1}, respectively. These low values indicated the high sensitivity of the purposed method. Recovery studies were carried out by adding a known quantity of pure drug to a pre-analyzed formulations and the proposed method was followed. From the amount of drug found, percentage recovery was calculated. The results of analysis and recovery studies are given in Table.1. The accuracy expresses the agreement between the accepted value and the true value. The mean percentage recovery was found to be LOD and LOQ were 100.03-101.01 %, for tablets (Table 1). This value proves the good accuracy of the purposed method.

Intra-day precision was calculated from results obtained from fivefold replicate analysis of samples at three different concentrations on the same day. Inter-day precision was calculated from results from the same samples analyzed on five consecutive days. This method utilizes the active analogue principle that lies at the spectroscopic method [11-26].

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