IAU-ARAK

J. Iran. Chem. Res. 4 (2011) 69-76

Journal of the Iranian Chemical Research

www.iau-jicr.com

UV-Visible spectrophotometry of Repaglinide in bulk and in formulation by using methyl orange as reagents

Smita Sharma [a], Mukesh Chandra Sharma [*,b]

[a] *Department of Chemistry Chodhary Dilip Singh Kanya Mahavidyalya Bhind (M.P) 477001, India*
[b] *School of Pharmacy, Devi Ahilya Vishwavidyalaya, Indore (M.P)-452001, India*

Received 22 October 2010; received in revised form 5 February 2011; accepted 22 April 2011

Abstract

Two sensitive spectrophotometric methods are presented for the assay of Repaglinide in bulk drug and in formulations using methyl orange, as reagents. Oxidant by reacting with a fixed amount of either methyl orange and measuring the absorbance at 485.2 nm (method-I) and measuring the absorbance at 618.9 nm (method-II). Developed method is based on the formation of extractable colored complex of drug with coloring agent Methyl orange dye. A wavelength maximum was found to be 618.9 nm. The concentration range of 15-50 µg ml-1 with linear regression of 0.9995, while the percentage recovery, LOD and LOQ were 99.42-99.08 %, 2.17 µg ml^{-1} and 1.08 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.

Keywords: Repaglinide; Derivative spectrophotometry; Area under curve.

1. Introduction

Stability indicating methods have become an important aspect of any analytical method validation and a part of US FDA requirements [1]. 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 [2-3]. It is official in USP [4] which describes liquid chromatographic method for its quantitation. Literature survey reveals that one HPLC method in human plasma [5], two HPLC [6, 7], one RPTLC [8] and one spectrophtometric method [9] 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 [10, 11]. The purpose of this work was to develop and validate simple,

* Corresponding author.
E-mail address: mukeshcsharma@yahoo.com (M.C. Sharma)

specific, sensitive, accurate, precise, rapid and cost effective HPLC method for the estimation of Repaglinide in bulk and its formulations. Therefore, we have made an attempt to develop a new, simple, accurate stability indicating RP-HPLC method for the determination of Repaglinide in pure and tablet forms.

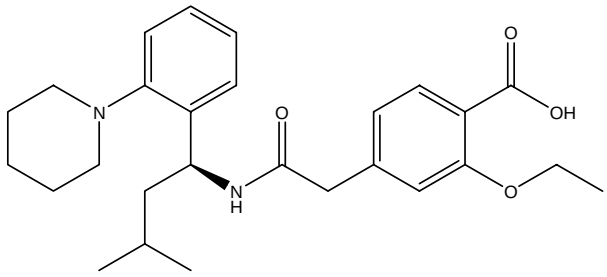

Fig. 1. Chemical Structure of Repaglinide.

2. Experimental

2.1. Material 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), methyl orange (s.d. finechem, Bombay, India).

2.2. Preparation of standard and sample solution

A 200 μg mL^{-1} dye solution was first prepared by dissolving accurately weighed 20.6 mg of dye (S.D. Fine Chem., Mumbai, India, 80 % dye content) in water and diluting to 100 mL in a calibrated flask and filtered using glass wool. It was further diluted to obtain a working concentration of 20 μg mL^{-1}.Standard drug solution (200 μg mL^{-1}) was prepared in double distilled water and was diluted with same, so as to give several dilutions in concentration range 10-50 mg mL^{-1} of drug. To 10 mL of each dilution taken in separating funnel, 10 mL of methyl orange solution was added and shaken gently. Then 5 mL of chloroform was added reaction mixture was shaken gently and allowed to stand so as to separate aqueous and chloroform layer. The chloroform layer was separated out and transferred to 10 mL of volumetric flask. Absorbance of this final extracted chloroform layer was measured at wavelength maxima 618.9 nm against blank. Calibration curve was plotted between concentration of drug and measured absorbance.

2.3. Sample solution

A stock standard solution containing 200 μg mL^{-1} Repaglinide solution was prepared by dissolving accurately weighed 20 mg of pure drug in water and diluting to 100 mL in a calibrated flask with water. The solution was diluted stepwise to get working concentrations of 5 and 20 μg mL^{-1} repaglinide for method I and method II, respectively. Twenty Repaglinide tablets were powdered and an accurately weighed quantity powder equivalent to 20 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 tablet samples were transferred into 100 ml flask and diluted to the mark with absolute ethanol, and the spectrophotometric procedure was followed.

2.4. Preparation of standard stock and binary mixture solutions:

The standard stock solutions of Repaglinide were prepared by dissolving 20 mg of each drug in 10 mL of methyl orange solution and final volume was adjusted with distilled water in 100 mL of volumetric flask. From the above solution 1 mL of solution was taken and diluted to 10 mL with distilled water to get a solution containing 100 μg mL^{-1} of each drug. Working standard solutions were scanned in the entire UV range of 400-200 nm. Both the drug obeyed Beer's law in the concentration range employed for theses methods. For method I and II six mixed standards solutions with concentration of Repaglinide in μg mL^{-1} of 50:15, 45:20, 40:25, 35:30, 20:35, 15:50 were prepared in distilled water by diluting appropriate volumes of the standard stock solutions.

2.5. Method-I: Derivative spectrophotometry method

In this method [12], 20 μg mL^{-1} solution for both the drugs were prepared and scanned in the range of 400 nm to 200 nm. The spectra obtained were derivatized in first order and then recorded, which showed Repaglinide had zero crossing point at 485.2 nm while at the zero crossing point of Repaglinide showed a measurable dA/dλ .Hence wavelengths 485.2 nm selected as analytical wavelengths for estimation of Repaglinide. Calibration curves were plotted for Repaglinide (15-50 μg mL^{-1}) at 485.2 nm as dA/dλ v/s concentration. The concentrations of both the drugs were obtained from the standard calibration curves by interpolation method.

2.6. Method II: Area under curve method (AUC)

AUC method [13] involves the calculation of integrated value of absorbance with respect to wavelength. Area calculation processing item calculates the area of bounded by the curve and horizontal axis. Here horizontal axis represents baseline.

$$(\alpha+\beta)=\int_{\lambda_2}^{\lambda_1} A d\lambda$$

where, α = area of portion bounded by curve data and a straight line connecting the start and end point, β= area of portion bounded by a straight line connecting the start and end point on curve data and horizontal axis, λ_1 and λ_2 are wavelengths representing start and end point of curve region. This method involved calculation of concentration for Repaglinide in the regions of 266-270 nm these regions were selected on the basis of repeated observation that plot area calculation of pure sample drug against the concentration. The UV spectra of Repaglinide with its AUC region.

$$\int_{266}^{270} A d\lambda = K_1 C_1 \text{ .....}\mathbf{Eqn.1} \qquad \int_{299}^{303} A d\lambda = K_2 C_1 \text{ .....}\mathbf{Eqn.2}$$

where C_1 Repaglinide in μg/ml and K_1, K2, were constant having values 0.32161, 0.21543,

$$\int_{266}^{270} A d\lambda = 0.32161 x C_1 + 0.14643 x C_2 \ \mathbf{Eq.3,} \int_{299}^{303} A d\lambda = 0.21543 x C_1 + 0.0154327 x C_2 \ \mathbf{Eq.4}$$

Sample solutions were scanned and area was calculated within the indicated wavelength regions. Concentration of components was calculated using Eqn. 3 and 4.

2.7. Validation of methods

Aliquots of stock transferred into a series of separating funnel then 1 mL of methyl orange reagent and 2 mL phosphate buffer of pH 2.3 was added, then the solutions were allowed to stand for few minutes, followed by accurately measured quantity (10 mL) of methanol and extracted well to give concentration 15-50 μg mL^{-1}, all the solutions were passed through dried sodium sulphate to remove water. Solution was scanned between 400-800 nm which shows λ-max at 618.9 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 15-50 μg mL^{-1}.The effect of methyl orange 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. Higher concentrations caused a distinct decrease in the absorbance. 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 developed methods for simultaneous estimation of Repaglinide were validated as per ICH guidelines. [14]

2.8. Accuracy

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. From that total amount of drug found and percentage recovery was calculated.

2.9. Repeatability

To check the degree of repeatability of the methods, suitable statistical evaluation was carried out. Five samples of the tablet formulations were analyzed for the repeatability study. The standard deviation, coefficient of variance and standard error was calculated.

2.10. Intermediate precision (inter-day and intra-day precision)

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 1.

Table 1
Intraday, Interdays, data of tablet formulation.

Sample	Intra day precision %CV (n=6)	Interday precision %COV		
		Day 1[a]	Day 2[a]	Day 3[a]
I	0.693	0.488	0.329	0.228
II	1.287	1.097	0.873	0.43`1

COV: Coefficient of variance.

2.11. Analysis of combined dosage form

The absorbance of final sample solution was measured against methanol as blank at 417.4 nm. The amount of Repaglinide was computed by adding the absorbance value in simultaneous equation.

2.12. Recovery studies

The method was validated by recovery study were carried out by the addition of different amount of drugs to pre analyze solution (10 μg mL^{-1}). From the stock solution of 100 μg mL^{-1} of each drug 1 mL solution was taken in each of four volumetric flask (10 mL), then 1.2, 0.8, 0.4 mL of mixed standard stock solution (100 μg mL^{-1} of Repaglinide) added in three flasks so that remaining one flask contains no added solution. These solutions were scanned at 381 nm. Percentage recovery was found in the range of 100 % to 105%.

2.13. Robustness and Ruggedness

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 2). 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 2.

Table 2
Robustness and day to day variation of the method.

Parameters Studied	**Recovery (%) ± R.S.D.**
Methyl orange Concentration(%,w/v)	
0.05	101.11 ± 0.63
0.15	99.87 ± 0.27
Wavelength	
485.2 nm	87.25 ± 0.11
618.9 nm	99.97 ± 0.31
Ruggedness(day-to-day variation)	
Day1	101.01 ± 0.14
Day 2	100.12 ± 0.08
Day 3	99.95 ± 0.43

2.14. Analysis of Tablets

A quantity of the finely ground tablet powder equivalent to 20 mg of Repaglinide was accurately weighed into a 100 mL calibrated flask, 60 mL of water was added and shaken for 20 min; the volume was finally diluted to the mark with water, mixed well and filtered using a Whatman No. 41 filter paper. First 10 mL portion of the filtrate was discarded and a suitable aliquot of the subsequent portion (200 μg mL^{-1} Repaglinide) was diluted appropriately to get 5 and 20 μg mL^{-1} concentrations for analysis by method I and method II, respectively.

3. Results and Discussion

In present research work a UV Spectrometric method has been developed for determination of Repaglinide from its tablet formulations. The proposed spectrophotometric methods are indirect and are based on the determination of the residual Methyl orange after allowing the

reaction between Repaglinide and a measured amount of Methyl orange to be complete. The residual Methyl orange was determined by reacting it with a fixed amount. The methods make use of bleaching action of Methyl orange on the dyes, the decolouration being caused by the oxidative destruction of the dyes. Repaglinide when added in increasing concentrations to a fixed concentration of Methyl orange, consumes the latter proportionally and there occurs a concomitant fall in the concentration of Methyl orange.

When a fixed concentration of dye is added to decreasing concentrations of Methyl orange, a concomitant increase in the concentration of dye results. Consequently, a proportional increase in the absorbance at the respective λ max is observed with increasing concentration of Repaglinide. The developed method was based on formation of absolute ethanol extractable complex of drug with methyl orange in double distilled water. For each drug, appropriate dilutions of standard stock solutions were assayed as per the developed methods. For method-I and II, the Beer- Lambert's concentration range was found to be 15-50 µg/ml selected wavelengths and coefficient of correlation were found 0.9975, 0.9996, 0.998 for Repaglinide at 485.2 nm. Wavelength maxima of Repaglinide were found to be at 618.9 nm and linearity was observed in concentration range of 15-50 µg mL^{-1}. 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 3).

Table 3
Accuracy of the proposed method

Sample	Label Claim	Estimated amount (mg/tab)	Spike (%)	Amount of drug added	Amount of drug recovered	Recovery (%)	R.S.D. (%, n=5)
I	2	2.05	50	20	2.11	101.10	0.21
			100	40	1.99	99.97	0.33
			150	60	2.02	100.02	0.15
II	2	1.98	50	20	1.97	99.97	0.87
			100	40	2.08	100.08	0.35
			150	60	2.04	100.03	0.14

A linear correlation was found between absorbance at λ max and concentration of Repaglinide in the ranges given in table 4. Regression analysis of the Beer's law data using the method of least squares was made to evaluate the slope (b), intercept (a) and correlation coefficient (r) for each system and the values are presented in table 4. The optical characteristics such as Beer's law limits and Sandell sensitivity values for both methods are given in Table 4. The limits of detection (LOD) and quantitation (LOQ) calculated according to ICH guidelines are also presented in table 4 and reveal the very high sensitivity of methods. 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:

$A=0.42318\times C-0.2142$ (r=0. 0.9998).

where *A* is the absorbance at 618.9 nm, *C* the concentration of Repaglinide in µg mL^{-1} in the range of 15-50 µg mL^{-1} and *r* is the correlation coefficient. The molar absorptivity (*å*) was found to be: 0.8743×0.0439 lit mol cm^{-1}.

The limit of detection (LOD) and limit of quantitation (LOQ) were determined using the formula: LOD or LOQ = κ S.D.*a*/*b*, where κ = 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 2.17 and 1.08 μg mL^{-1}, respectively.

Table 4
Optical characteristics data of Repaglinide.

Parameters	**Repaglinide**			
	Method I		**Method II**	
Working λ (nm)	485.2 nm		618.9 nm	
Beer's law limit (μg mL^{-1})	5-35	5-35	5-35	0-45
Correlation coefficient *	0.9965	0.9994	0.9998	0.9999
Intercept *	0.0003	0.0014	0.0043	0.0021
Slope *	0.0143	0.0221	0.0651	0.0765
Limit of detection, μg mL^{-1}	2.17		-0.74	
Limit of quantification, μg mL^{-1}	1.08		0.18	

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 99.96-101.05% 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. The results obtained are listed in Table 2. The low relative standard deviation (RSD 0.95; 0.87; 1.01 at three different level (intra-day precision), 1.23, 0.98, 1.45 at three different level (inter-day precision) showed the good precision of the method.

Acknowledgement

The authors are thankful to Head, School of Pharmacy, Devi Ahilya Vishwavidyalaya Indore India to provide facility.

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