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DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC AREA UNDER CURVE METHOD FOR QUANTITATIVE ESTIMATION OF RANOLAZINE IN API AND TABLET FORMULATION

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ABSTRACT

The aim of present work was to develop an accurate, precise, reproducible and economical UV spectrophotometric Area Under Curve method for estimation of Ranolazine. This Area Under Curve method of UV spectrum between 261 to 281 nm was validated as per ICH guideline Q2 (R1). The method has followed linearity in the range of 75-200 µg/ml. The value of correlation coefficient was 0.998. Satisfactory values of Percent relative standard deviation for the intra-day and inter-day precision studies indicated that method is precise. Results of the recovery studies (99.42% to 99.97%) showed accuracy of the method. LOD and LOQ were calculated as 10.77 µg/ml and 32.63 µg/ml, respectively. The developed method can be used for routine estimation of Ranolazine in bulk and tablet formulation.

KEYWORDS: Area Under Curve, Ranolazine, UV spectrophotometry, Quantitative estimation.

INTRODUCTION

Ranolazine is an Antianginal drug and chemically it is a Piperazine derivative. Structurally it is N-(2,6- dimethylphenyl)-2-[4-[2-hydroxy-3-(2-Methoxyphenoxy)propyl]piperazin-1yl] acetamide.[1] Ranolazine is believed to have its effects via altering the trans-cellular late sodium current. It is by altering the intracellular sodium level that Ranolazine affects the sodium-dependent calcium channels during myocardial ischemia. Thus, Ranolazine indirectly prevents the calcium overload that causes cardiac ischemia. Ranolazine is indicated for the treatment of chronic angina. Ranolazine may be used with beta blockers, nitrates, calcium

channel blockers, antiplatelet therapy, lipid-lowering therapy, ACE inhibitors, and angiotensin receptor blockers. Literature survey revealed that some methods have been developed for the determination by spectrophotometry[2,3,4] and HPLC[5,6], however no method was found for the Ranolazine by Area Under Curve UV spectrophotometry using water as a solvent. Earlier reported methods were Performed by using methanol which is a expensive solvent. The purpose of this work was to develop a simple, accurate, precise, reproducible and economical UV spectrophotometric Area Under Curve method for estimation of Ranolazine.

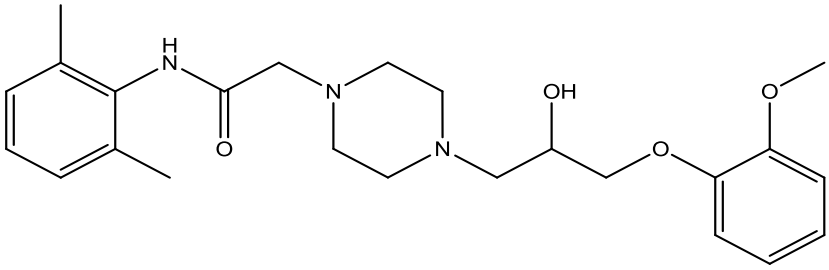

Figure 1: Chemical Structure of Ranolazine

MATERIALS AND METHODS

INSTRUMENTATION AND APPARATUS

Shimadzu UV 1800 with UV Prob Software, was employed for this work. Single pan electronic balance (Shimadzu, ATY 224) was used for weighing purpose. Sonication of the solutions was carried out using an Ultrasonicator (Spectra lab UCB 40, India). Calibrated volumetric glasswares (Borosil®) were used in this study.

MATERIALS

Active pharmaceutical ingredient (API) of Ranolazine was gifted by M. J. Biopharm Pvt. Ltd. Navi Mumbai, India**.** Commercially available tablets RANCAD® containing 500 mg of Ranolazine was procured from local pharmacy. Methanol (AR) was purchased from Merck India Ltd., Mumbai, India

METHOD DEVELOPMENT

Preparation of standard solution

The standard stock solution of Ranolazine was prepared by transferring, accurately weighed, 100 mg of API to100 ml of volumetric flask. The drug was dissolved with sonication in 10 ml of methanol and volume was made up to 100 ml by using distilled water. The standard stock

solution (1000 µg/ml) was further diluted with distilled water to obtain the concentration of 100 µg/ml.

Selection of wavelength range

The standard solution of 100 µg/ml was scanned between 400 nm to 200 nm in UV spectrophotometer against distilled water as blank after baseline correction. Wavelength range was selected around wavelength maxima (271nm). Different working standards were prepared in the concentration from 75-200 µg/ml. Various wavelength range were tried and final range between 261-281 nm was selected on the basis of linear relationship between area and corresponding concentration (Figure- 2).

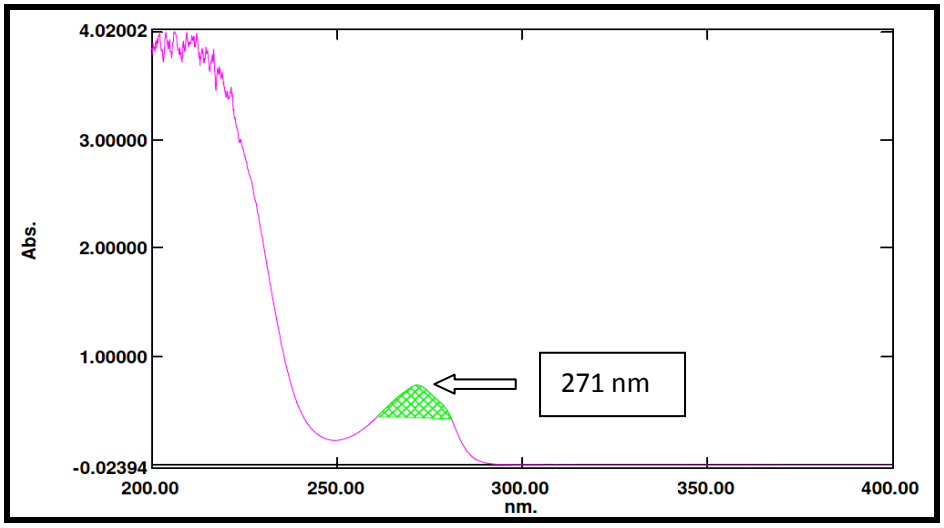

Figure 2: Area Under Curve graph of 100 µg/ml Ranolazine solution

Area under curve (Area calculation)

This method involves calculation of integrated value of absorbance with respect to wavelength in indicated range. Area calculation processing item calculates the area bounded by the curve and horizontal axis. Here horizontal axis represents baseline.

$$\text{Area calculation } (\alpha+\beta) = \int_{\lambda 2}^{\lambda 1} A d\lambda$$

Whereas, α is area of portion bounded by curve data and a straight line connecting the start and end point, β is area of portion bounded by a straight line connecting the start and end point on curve data and horizontal axis, λ1 and λ2 are wavelengths representing start and end point of curve region. In this study area was integrated between wavelength ranges from 261 to 281 nm.

Preparation of calibration curve

Solutions for calibration curve were prepared from standard stock solution of 1000 µg/ml, by further dilution with distilled water to obtain the concentrations of 75, 100, 125, 150, 175, and 200 µg/ml, respectively. These solutions were scanned from 400 to 200 nm and Area Under Curve (AUC) was integrated in the range of 261-281 nm. The calibration curve was plotted between Area under curve against concentration (Figure- 3).

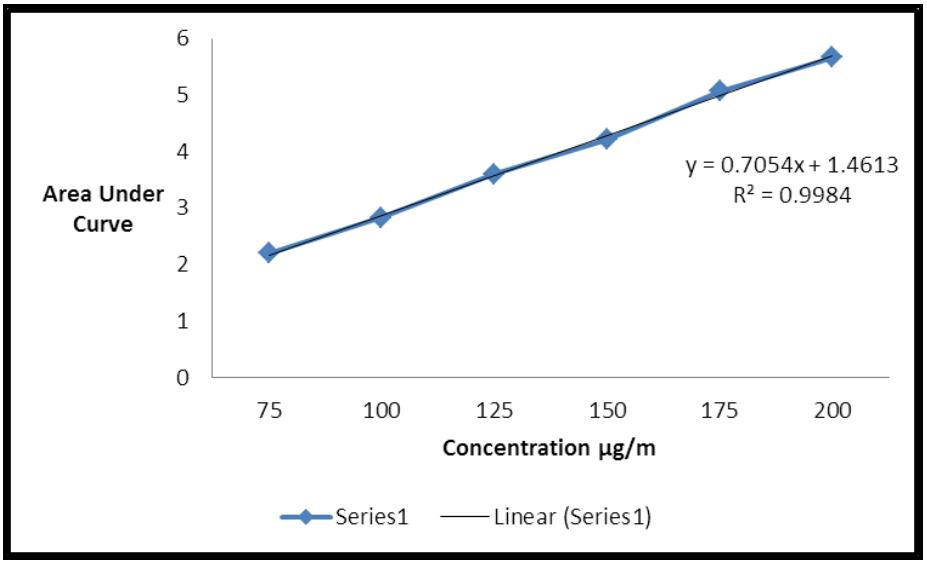

Figure 3: Calibration curve of Ranolazine

METHOD VALIDATION

The objective of validation of an analytical procedure is to demonstrate whether the procedure is suitable for its intended purpose. The proposed method was validated for various parameters such as Linearity & Range, Precision, Accuracy, Limit of detection (LOD) and Limit of Quantitation (LOQ) according to ICH Q2 (R1) guideline.[7]

Linearity and Range

The linearity was determined by using working standard solutions between 75-200 µg/ml. The spectrums of these solutions were recorded and area under curve was integrated in wavelength range 261-281 nm. Calibration curve of Area under curve *v/s* concentration was plotted after suitable calculation and simple linear regression was performed (Figure 3). Regression equation and correlation coefficient were obtained. The range of solution has been decided according to statistical parameters of generated equation.

Table1 1. Linearity and range of Ranolazine.

Concentration µg/ml	Area Under Curve
75	2.195
100	2.8345
125	3.5998
150	4.2143
175	5.067
200	5.6702

Method Precision

Repeatability

The precision of the method was checked by repeatedly analyzing (n= 6) standard solutions of Ranolazine (25µg/ml). Area under curve of each of these solutions was measured in the range of 261-281 nm. Relative standard deviation (% RSD) was calculated.

Reproducibility

The intra-day and inter-day precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different dilutions (125, 150 and 175 µg/ml) of Ranolazine. The results were reported in terms of percentage relative standard deviation (%RSD). The results are tabulated in Table 2.

Table 2. Intraday and Interday precision study of Ranolazine

Drug	Concentration µg/ml	% RSD*	
		Intra-day	Inter-day
Ranolazine	125	0.39	0.69
	150	0.089	0.085
	175	0.17	0.5

*n=3

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Five sets of known concentrations of 75-200 µg/ml were prepared. Calibration curves were plotted for each set. LOD= 3.3 * SD/S and LOQ= 10 * SD/S were calculated.

Where, SD is standard deviation of y-intercept of the calibration curves and S is mean slope of five calibration curves.

Accuracy

The accuracy for the analytical procedure was determined at 80%, 100% and 120% levels of tablet solution. Area under curve was measured in the range of 261-281 nm and results were

expressed in terms of % recoveries. Three determinations were performed at each level and % RSD was calculated. The results are tabulated in Table 3.

Table 3. Recovery studies for AUC method

Accuracy level	Total amount taken µg/ml	Mean amount recovered µg/ml	Mean % Recovery ± S.D.	Mean % RSD
80%	120	119.583	99.65 ± 0.17	0.17
100%	150	149.95	99.97 ± 0.21	0.21
120%	180	178.97	99.42 ± 0.20	0.20

***n=3**

ASSAY OF TABLET FORMULATION

Twenty tablets were weighed and average weight was calculated. These tablets were crushed and powdered in a glass mortar. The tablet powder equivalent to 100 mg of Ranolazine was accurately weighed, and transferred to a 100 ml volumetric flask containing 10 ml of methanol and then diluted up to mark with distilled water. The solution was filtered with Whatmann filter paper No. 41 and the first few ml of filtrate was discarded. This solution was further diluted to obtain 100 µg/ml solution with same solvent and subjected for UV analysis. This procedure was repeated in triplicate.

RESULTS AND DISCUSSION

An attempt was made to develop a simple and specific AUC spectrophotometric method for the determination of Ranolazine in tablet dosage form. The generated regression equation was $\int_{281}^{261} Ad\lambda$ = 0.0282x + 0.0506 (R^2= 0.998), where, $\int_{281}^{261} Ad\lambda$ is area under curve between 261 to 281 nm, 'x' is the concentration and R is correlation coefficient. The R^2 value as 0.998 indicates that developed method was linear. The proposed method was found to be precise as % R.S.D values for intraday as well as for interday precision were satisfactory. The drug at each of the 80%, 100% and 120% levels showed good recoveries. Hence, it can be said that this method was accurate. The LOD and LOQ were calculated as 10.77 µg/ml and 32.63 µg/ml, respectively. The result of the analysis of tablet formulation by the developed method was consistent with the label claim, highly reproducible and reliable. The method can be used for the routine analysis of the Ranolazine in tablet dosage form. The validation parameters are summarized in Table 4.

Table 4. Summary of Validation parameters.

Parameter		Results
Linearity range		75 – 200 µg/ml
Regression Equation(y= mx + c)		y = 0.0282x + 0.0506
Slope (m) ± SD* (n=5)		0.02978 ± 0.00027
Intercept (c) ± SD* (n=5)		0.1767 ± 0.0972
Correlation Coefficient (R^2)		R^2 = 0.998
Precision Studies (%RSD)	Intra-day	0.21
	Inter-day	0.42
LOD (µg/ml)		10.77
LOQ (µg/ml)		32.63
Accuracy Studies (n=3)	Mean % Recoveries ± S.D.	
	80%	99.65 ± 0.17
	100%	99.97 ± 0.21
	120%	99.42 ± 0.20

CONCLUSION

The proposed spectrophotometric method was found to be simple, sensitive, accurate and precise for determination of Ranolazine in API and Tablet formulation. The method utilizes easily available and cheap solvent for analysis hence the method was also economic for estimation of Ranolazine in API and tablet formulation. The common excipients and other additives are usually present in the tablet formulation do not interfere in the analysis of Ranolazine in method, hence it can be conveniently adopted for routine analysis of the drugs in tablet formulation.

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