INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

DEVELOPMENT OF RAPID VISIBLE SPECTROPHOTOMETRIC METHODS FOR THE ESTIMATION OF RANOLAZINE IN FORMULATIONS

Sambasivarao Vattikuti[1]* and Phani. R.S.Ch[2]

[1]SRM College of Pharmacy, Chennai.Tamil Nadu, India.
[2]R.V.Labs, Guntur, Andhra Pradesh, India.

*Corresponding Author: samba.vattikuti@gmail.com

ABSTRACT

Simple, sensitive, accurate, precise and rapid Visible Spectrophotometric methods were developed for the estimation of Ranolazine in pure form and its formulation. For the estimation of Ranolazine, the Ranolazine is subjected to oxidation with 20% Sulphuric acid. The oxidized drug is treated with ferric phenanthroline and potassium permanganate reagents and its absorbances are measured at 510 and 525nm respectively, solvent system employed was methanol. The developed method was used to estimate the total drug content in commercially available oral formulations of Ranolazine and recovery studies were also carried out. Sample recovery in the formulations using the above method was in good agreement with their respective labeled claims, thus suggesting the validity of the method and non-interface of formulation excipients in the estimation. The developed method was found to be stability specific and were validated as per ICH guidelines-2005, USP-2000 and statistical methods.

INTRODUCTION

Ranolazine is chemically known as N-(2,6-dimethylphenyl)-4-[2-hydroxy-3-(2-methoxy phenoxy)propyl]piperazine acetamide, is a Antianginal agent with a piperazine structure. It has a cardioprotective effect against ischemia/ reperfusion injury, without affecting hemo dynamics, both in vitro and in vivo. Its anti-ischemic action is thought to be by modification of metabolism, specifically, activation of pyruvate dehydrogenase and promotion of glucose oxidation with concomitant reduction in fatty acid oxidation. Thus, Ranolazine can enhance utilization of oxygen under conditions of oxygen-supply deficiency. Its Molecular formula $C_{24}H_{33}N_3O_4$ and Molecular weight is 427.537. It is Colour less, amorphous substance, freely soluble in methanol, insoluble in water. It should be kept in well closed container.

The literature review for Ranolazine reveals that methods like LC-MS, LC-MS/MS, LC – APCI –MS and Head space gas chromato-graphic techniques were reported for the determination of Ranolazine in Pharmaceutical formulations and Biological fluids. UV spectroscopic methods have not yet been reported but the work is under progress. The present investigation has been undertaken to develop simple visible spectrophotometric method for the estimation of Ranolazine in pure and its formulations.

REAGENTS PREPARATION

Preparation of Ferric Phenanthroline Reagent:

The reagent was prepared by mixing 0.193 gm of the 1,10-phenanthroline with 2 ml of 1.0 M HCL and 0.16 gm of ferric ammonium sulphate and diluted with distilled water to 100 ml .

Preparation of 1% Potassium Permanganate Solution:

0.1gm of potassium permanganate was accurately weighed dissolved and diluted to 100mL with distilled water.

Preparation of 20% Sulphuric acid:

20 ml of concentrated Sulphuric acid was measured out and made up to 100 ml with distilled water.

Preparation of sample solution (Bulk drug): Ranolazine 100mg was accurately weighed and taken into 100 ml volumetric flask 50 ml methanol and further it was made up to 100 ml with methanol solution. This solution was further serially diluted with methanol solution to get 10, 15, 20, 25, 30 µg/ml solutions.

Preparation of sample solution (Dosage forms):
Twenty tablets were weighed and powdered; the powder equivalent to 100mg of Ranolazine was weighed accurately and taken in 100 ml volumetric flask containing 50 ml of methanol. The contents were shaken well for 30 minutes and made up to the volume 100 ml with methanol. This solution was further diluted with methanol solution to get 10, 15, 20, 25, 30 µg/ml solutions.

PROCEDURE
Method-A
5, 7.5, 10, 12.5, 15 µg/ml solutions were taken separately in 10ml standard flasks, 1ml of 20%v/v Sulphuric acid solution was added to each flask followed by 1ml of 0.1%w/v $KMno_4$ reagent and the solution was allowed to stand for 10 minutes for complete reaction. All the flasks were diluted to 10ml with distilled water and mixed well.
The amount of $KMno_4$ reagent and 20%w/v sulphuric acid solution was optimized by various trails and an amount of 1ml of 0.1%w/v $KMno_4$ reagent and 1ml of 20% sulphuric acid was found to be satisfactory.
This solution was subjected to scanning in visible region. $KMno_4$ exhibits maximum absorbance at about 525nm. The absorbance of the solutions at 525nm was measured and the calibration curves were constructed. Linearity was obeyed in the concentration range of 5-15 µg/ml. The results were tabulated in Table 1, 2 and overlay spectra and linearity plot were shown in Figure 1 and Figure 2 respectively.

Linearity and range
Linearity of Ranolazine by colorimetric method has been done and the corresponding values are tabulated in Table 1.

Table 1: Linearity of Ranolazine – Method A

S. No.	Conc.(µg/ml)	Absorbance
1	5	0.36836
2	7.5	0.50845
3	10	0.62772
4	12.5	0.76265
5	15	0.88133

Fig. 1: Overlay Spectrum of Ranolazine Showing Linearity – Method – A

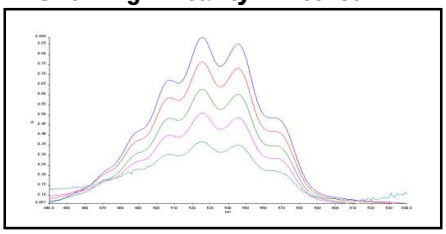


Table 2: Analytical Characteristics of Ranolazine – Method A

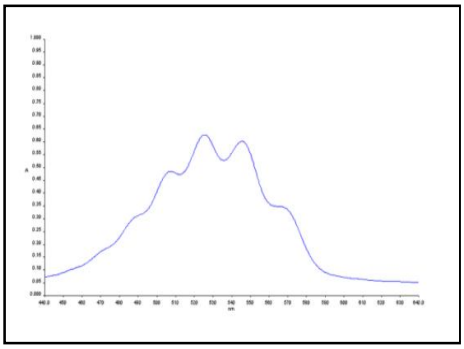
Parameters	Visible Spectrophotometry
Regression equation	y = -0.051x + 1.141
Correlation coefficient	0.9990
Linearity range	5 – 15 µg/ml
Number of data points	5

ASSAY OF RANOLAZINE IN SYNTHETIC MIXTURE (METHOD – A)
The assay was done with six set of samples as described in the procedure for analysis of synthetic mixtures and the results are as follows

Table 3: Assay of Ranolazine – Method A

Set	Weight of placebo	Standard weight	Sample weight	Absorbance of sample	Drug (mg)	% assay
1	0.251	0.0501	0.301	0.6266	49.89	99.78
2	0.252	0.0500	0.302	0.6275	49.82	99.64
3	0.250	0.0500	0.300	0.6263	50.05	100.11
					Average	99.84
					SD	0.20
					%RSD	0.20

Fig. 3: Assay Spectrum of Ranolazine – Method A



SYSTEM PRECISION

System precision was performed by measuring the absorbance of the homogenous solution of standard (10µg/ml) and the results are as follows

Table 4: System Precision of Ranolazine – Method A

S. No.	Standard Absorbance
1	0.62772
2	0.62761
3	0.62784
4	0.62745
5	0.62699
6	0.62619
7	0.62712
8	0.62729
9	0.62698
10	0.62684
Average	0.62720
SD	0.000491
%RSD	0.08

METHOD PRECISION

The method precision was performed by measuring the absorbance of six assay sample solutions prepared simultaneously.

Table 5: Method Precision of Ranolazine Visible Spectrophotometry

Sample	Weight of placebo	Standard weight	Sample weight	Absorbance of sample	Drug present	% assay
1	0.251	0.0501	0.301	0.6266	49.89	99.78
2	0.252	0.0500	0.302	0.6275	49.82	99.64
3	0.252	0.0501	0.302	0.6278	49.82	99.65
4	0.251	0.0501	0.301	0.6270	49.92	99.85
5	0.249	0.0500	0.299	0.6280	50.35	100.71
6	0.250	0.0500	0.300	0.6263	50.05	100.11
Average	0.2500	0.0500	0.300	0.6274	49.98	99.96
SD						0.37
%RSD						0.37

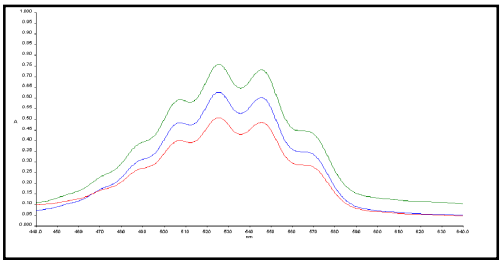
ACCURACY

Accuracy of the method was performed by recovery studies. Accuracy was performed at 80%, 100% and 120% level of target concentration and results are as follows

Table 6: Accuracy of Ranolazine – Visible Spectrophotometry

Accuracy Level	Amount Added (µg/ml)	Amount Present (µg/ml)	Amount Recovered(µg/ml)	% Recovery	Average Recovery
80	5	3	8.06	101.20	100.77 %
100	5	5	9.98	99.52	
120	5	7	12.08	101.60	

Fig. 4: Overlain Spectrum Showing Accuracy for Ranolazine – Method A



Method-B

5, 7.5, 10, 12.5, 15 µg/mL solutions were taken separately in 10ml standard flasks, to each flasks 3ml of Ferric Phenanthroline reagent was added shaken well and were kept in a boiling water bath for 20 minutes. The reaction was quenched by cooling under tap water and each flask was made up to volume with distilled water.

The amount of FPL reagent and the heating time was optimized by various trails and an amount of 3ml of FPL reagent with a heating time of 20 minutes was found to be satisfactory. This solution was subjected to scanning in visible region. Ferric Phenanthroline complex Fe(phen)3]2+ exhibits maximum absorbance at about 510nm. The absorbance of the solutions at 510nm was measured and the calibration curves were constructed. Linearity was obeyed in the concentration range of 5-15 µg/ml. The results were tabulated in Table – 7, 8 and overlay spectra and linearity plot were shown in Figure 5 and Figure 6 respectively.

LINEARITY AND RANGE

Linearity of Ranolazine by visible spectrophotometric method has been done and the corresponding values are tabulated as follows:

Table 7: Linearity of Ranolazine – Method B

S. No.	Theoretical conc.(µg/mL)
1	5
2	7.5
3	10
4	12.5
5	15

Fig. 5: Overlay Spectrum of Ranolazine – Method B

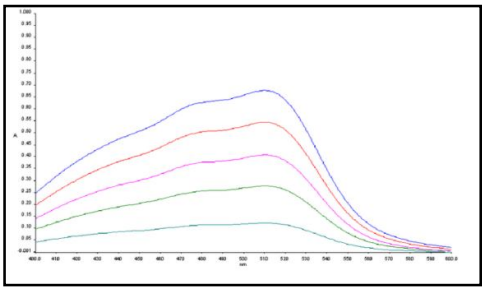


Table 8: Analytical Characteristics of Method B

Parameters	Method A
Regression equation	y = 0.055x - 0.146
Correlation coefficient	0.9992
Linearity range	5 – 15 µg/ml
Number of data points	5

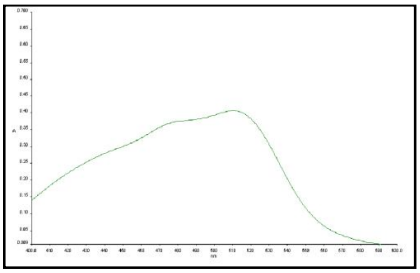
ASSAY OF RANOLAZINE IN SYNTHETIC MIXTURE METHOD B

The assay was done with six set of samples as described in the procedure for analysis of synthetic mixtures under first order derivative spectrophotometric method and the results are as follows

Table 9: Assay of Ranolazine – Method B

Set	Weight of Placebo	Standard Weight	Sample Weight	Absorbance of Sample	Drug Percentage	% Assay
1	0.251	0.0501	0.301	0.4053	49.77	99.55
2	0.252	0.0500	0.302	0.4077	49.91	99.82
3	0.250	0.0500	0.300	0.4087	50.37	100.73
					Average	100.04
					SD	0.51
					%RSD	0.51

Fig. 7: Assay Spectrum of Ranolazine – Method B



SYSTEM PRECISION

System precision was performed by measuring the absorbance of the homogenous solution of standard (3µg/ml) and the results are as follows

Table 10: System Precision of Ranolazine - Method B

S. No.	Standard Absorbance
1	0.2902
2	0.2901
3	0.2899
4	0.2902
5	0.2912
6	0.2898
7	0.2901
8	0.2912
9	0.2910
10	0.2908
Average	0.2905
SD	0.000542
%RSD	0.19
S.NO	**STD ABS**

METHOD PRECISION

The method precision was performed by measuring the absorbance of six assay sample solutions prepared simultaneously

Table 11: Method Precision of Ranolazine – Method B

Sample	Weight of Placebo	Standard Weight	Sample Weight	Absorbance of Sample	Drug Present	% Assay
1	0.251	0.0501	0.301	0.40670	49.72	99.44
2	0.252	0.0500	0.302	0.40712	49.62	99.24
3	0.252	0.0501	0.302	0.40810	49.72	99.45
4	0.251	0.0501	0.301	0.40976	50.09	100.18
5	0.249	0.0500	0.299	0.40610	49.99	99.99
6	0.250	0.0500	0.300	0.40682	49.91	99.83
Average	0.2500	0.0500	0.300	0.40597	49.84	99.69
					SD	0.34
					%RSD	0.34

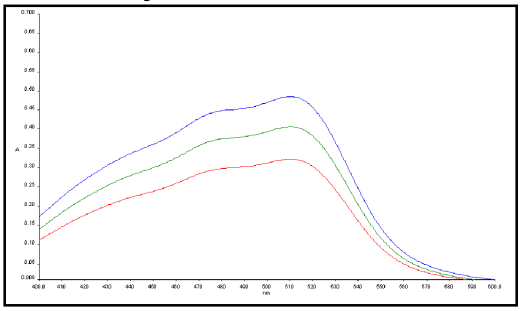
ACCURACY

Accuracy of the method was performed by recovery studies. Accuracy was performed at 80%, 100% and 120% level of target concentration and results are as follows

Table 12: Accuracy of Ranolazine – Method B

Accuracy Level	Amount Added (µg/ml)	Amount Present (µg/ml)	Amount Recovered (µg/ml)	% Recovery	Average Recovery
80	5	3	8.06	100.12	99.83%
100	5	5	10.01	100.30	
120	5	7	11.95	99.07	

Fig. 8: Overlain Spectrum Showing Accuracy for Ranolazine – Method b



CONCLUSION

The developed UV spectrophotometric method for the estimation of Ranolazine was found to be simple and useful with high accuracy, precision, repeatability. Sample recoveries in all formulations using the above method was in good agreement with their respective label claim or theoretical drug content, thus suggesting the validity of the method and non inference of formulation excipients in the estimation. In the selective solvent system, drugs were stable for more than 48 hours, thus suggesting that samples need not be estimated immediately after collection. The developed method was found to be stability specific and were validated as per ICH guidelines (2005) and statistical method.

ACKNOWLEDGEMENTS

We thank to Mr. Seetharaman. R for his assistance in conducting this study.

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