Journal of Chronotherapy and Drug Delivery

ORIGINAL RESEARCH PAPER

Development and Validation of RP-HPLC Method for Estimation of Metoprolol and Ranolazine in Bulk and in Formulation

Poornima K[1]*, Channabasavaraj KP[2]

[1] Department of Pharmaceutical Chemistry, East West College of Pharmacy, Bengaluru 560 018 (India)
[2] Department of Pharmaceutical Chemistry, Government College of Pharmacy, Bengaluru 560 027(India)

* For correspondence
Email: poori.harshit@gmail.com

Key words
Metaprolol, Ranolazine, RP-HPLC, Simultaneous estimation

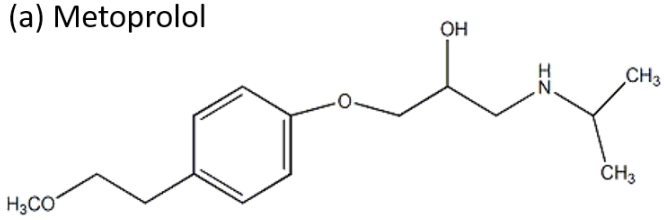
Abstract
A simple, sensitive, precise and rapid reverse phase high performance liquid chromatography (RP-HPLC) method was developed and validated for simultaneous estimation of Metoprolol (MET) and Ranolazine (RAN) in bulk drug and tablet dosage forms. The separation was achieved by using Gemini-NX 10 micron (250 mm X 4.6 mm) column with a mobile phase consisting of buffer (0.05 M potassium dihydrogen ortho phosphate) and acetonitrile in ratio 70:30 % v/v at a flow rate of 1.0 mL/min. Analysis was performed at ambient temperature with detection at 272 nm. The retention times of MET and RAN were found to be 3.4 and 6.9 min and the calibration curves were linear (r^2=0.999) over a concentration range from 10-50 µg/mL for MET and RAN respectively. The Limit of detection (LOD) of MET and RAN was observed to be 0.001 µg/mL and 0.1 µg/mL respectively, the Limit of quantitation (LOQ) of MET and RAN was observed to be 1 µg/mL for both. The developed method was validated for parameters like system suitability, specificity, linearity, accuracy, precision, ruggedness and robustness as per ICH guidelines and the results were found to be within the limits. So it can be used for the routine quality control of MET and RAN in bulk sample and tablet dosage forms.

J Chronother Drug Deliv | Vol 6| Issue 2| 2015: 41-48

INTRODUCTION

Metoprolol, chemically is a {2-hydroxy-3-[4-(2-methoxyethyl)phenoxy]propyl}(propan 2- yl)amine (Fig 1a). It is a cardio selective β1-adrenergic blocking agent, used for angina pectoris, acute myocardial infarction, heart failure and mild to moderate hypertension. It is also used for supraventricular and tachyarrhythmias and prophylaxis for migraine headaches. At low doses, Metoprolol selectively block cardiac β1-adrenergic receptors with little activity against β2-adrenergic receptors of the lung and vascular smooth muscle. Receptor selectivity decreases with higher doses.[1] Ranolazine is an anti-anginal drug (Fig 1b). It can be used in combination with beta blockers, nitrates, calcium channel blockers, antiplatelet therapy, lipid-lowering therapy, ACE inhibitors, and angiotensin receptor blockers.[2] Metoprolol along with the Ranolazine is given for chronic angina treatment.[3]

(a) Metoprolol



(b) Ranolazine

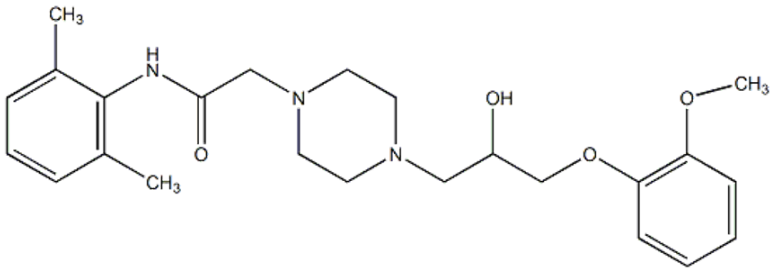


Fig 1. Structures of selected drugs

Literature survey revealed that various methods such as RP-HPLC,[4,5] rapid spectrofluorometry,[6] Ultraviolet Spectroscopy[7] and Derivative Spectroscopy[8] for Metoprolol and RP-HPLC,[9-11] Visible Spectroscopy,[12] LC-MS[13] for the estimation of Ranolazine were reported individually and in combination with other drugs. But no method was developed and validated to estimate them simultaneously.

Hence, in the present study, an attempt has been made to estimate the Metoprolol and Ranolazine simultaneously. It can also be applied for routine analysis of either one or both of this content in bulk and formulations using RP-HPLC technique.

MATERIALS AND METHODS

Chemicals and Reagents

Metoprolol and Ranolazine standards were obtained as gift sample from Micro Labs, Bengaluru, along with Certificate of Assurance (COA). HPLC grade solvents, acetonitrile and methanol were purchased from Merck, India. HPLC grade water is collected from Milli-Q3 water purifier system. Class A apparatus were used throughout the experiment. Formulations are purchased from local markets of Bengaluru for recovery studies and specificity determination.

Preparation of Working Standard Solutions of Metoprolol and Ranolazine

Standard Preparation

Accurately 10 mg of Metoprolol and 10 mg of Ranolazine were weighed into a clean and dry 10 mL volumetric flask separately, dissolved with sufficient volume of diluent. The final volumes were made up to 10 mL with diluent to get the concentration of 1000 µg/mL for Metoprolol and Ranolazine,

respectively (Stock I). From the stock I solution, 1 mL was pipetted out into 10 mL volumetric flasks separately to get concentration of 100 µg/mL for Metoprolol and Ranolazine, respectively (stock A) .

Sample Preparation

Twenty tablets that contain Metoprolol (50 mg) and Ranolazine (500 mg) were weighed and crushed to a fine, homogenous powder. Weigh accurately 0.0873 g of tablet triturate that contains 5 mg of Metoprolol and 50 mg of Ranolazine were weighed into clean and dry 10 mL volumetric flask, dissolved with sufficient volume of diluent. The final volume was made up to 100 mL with diluent to get the concentration of 500 µg/mL of Metoprolol and 5000 µg/mL for Ranolazine (stock II). From the stock II solution, 1 mL was pipetted out into 10mL volumetric flasks separately to get concentration of 50 µg/mL of Metoprolol and 500 µg/mL for Ranolazine (Stock B).

Instrumentation and Chromatographic System

HPLC System: Shimadzu, 20 AT model attached with pump, degasser, auto sampler, Ultra-violet detector.
Mobile Phase: Potassium dihydrogen ortho phosphate buffer (0.05M) : acetonitrile (70:30% v/v)
Chromatographic Column: Gemini-NX 10µ, C-18 (250×4.6 mm)
Flow Rate: 1.0 mL/min.
Acquisition Time: 10 min.
Detection Wavelength: 272 nm
Injection Volume: 20 µL
HPLC Method was evolved on trial and error basis and mobile phase potassium dihydrogen orthophosphate buffer (0.05M): acetonitrile (70:30% v/v) found to give a good resolution.

Method Validation

The method was validated as per ICH guidelines. The method was validated in terms of linearity, specificity, accuracy, precision, Limit of Detection (LOD) and Limit of Quantification (LOQ).

Linearity

In the present study, linearity was established using 5 final concentrations viz. 10, 20, 30, 40, 50 µg/mL which was prepared by pipetting 1.0 mL to 5.0 mL from stock II and diluted to get final concentration. Peak area and retention time were recorded and linearity was derived by plotting the standard calibration curve for Metoprolol and Ranolazine.

Accuracy

Accuracy of the method was determined by recovery experiments using the standard addition method.

Recovery

The percent recovery of added standard to the known samples was calculated using formula:
Percentage Recovery= {(C1-C2)/ C3} ×100
Where, C1 is the total concentration of analyte found, C2 is the concentration of analyte present in formulation, and C3 is the concentration of standard added to formulation. The solutions were injected in triplicate and percent recovery was calculated.

Precision

Precision (system precision) was determined by interday precision and intraday precision. Successive six injections of fixed concentration were taken for calculating intraday precision for both standard and sample. Similarly results were recorded after 24 hours at ambient temperature which shows the stability of solutions and interday precision. Relative Standard Deviation (RSD) was calculated for both six injections and found within 2%.

Repeatability (Method Precision)

Repeatability was established by six replicate injections and measurements of peak area for Metoprolol and Ranolazine. Relative Standard Deviation (RSD) was calculated for six injections (six replicates) and found within 2%.

Specificity

The specificity of the method was evaluated by assessing whether excipients present interfered with the analysis. Metoprolol and Ranolazine were used mutually as internal standard in varied concentrations.

Limit of Detection and Limit of Quantification

Visualization method was used for determination of LOD and LOQ of Metoprolol and Ranolazine respectively. As per this method, lowest dilutions of standard drugs Metoprolol and Ranolazine were prepared successfully, injected to obtain chromatogram and the response was recorded.

System Suitability

This is to ensure that analytical system is working properly and gives accurate and precise results.

Robustness

It is a measure of capacity of method to remain unaffected by small but deliberate variation of the operating conditions. This was tested by studying the changes in the pH of mobile phase by 0.5, also by varying amount of buffer by 10%.

Assay

The proposed method was used to estimate the total drug content in commercially available pharmaceutical dosage form. The assay of drug was done in a sample tablet and compared with the standard drugs. Percentage drug recovery was calculated.

RESULTS AND DISCUSSION

Literature search revealed that no analytical methods have been reported for the simultaneous estimation of Metoprolol and Ranolazine in bulk and tablet dosage form till date. Hence, the newer, simpler, rapid and reliable RP-HPLC method was established for the simultaneous estimation of these two drugs either alone and/ or in combination in formulation. The RP-HPLC method was developed with mobile phase consisting of buffer (0.05 M potassium dihydrogen orthophosphate) and acetonitrile in ratio 70:30 % v/v. The mobile phase was delivered at a flow rate of 1.0 mL/min on Gemini-NX 10µ (250 mm X 4.6 mm) column as stationary phase. Analysis was performed at ambient temperature with detection at 272 nm gave a satisfactory chromatogram of Metoprolol and Ranolazine.

The retention time for Metoprolol and Ranolazine was found to be 3.4 and 6.9 min respectively. For determination of linearity, peak area and retention time were recorded and linearity was derived by plotting the standard calibration curve for Metoprolol and Ranolazine (Fig 2). The linearity and regression coefficient value is 0.999 for both Metoprolol and Ranolazine and thus the response is linear for the concentration range of 10-50 µg/mL for both.

The results of accuracy determination are given in Table 1 and those of precision and repeatability studies are given in Table 2. The results were within the acceptable limits, showing that the developed method was accurate and precise.

Fig 3 shows the results are specificity study; and Table 3 shows the results of system suitability study. The results suggest that the developed method was suitable and specific.

The LOD was determined by visualization method were found to be 0.001 and 0.1 µg/mL for Metoprolol and Ranolazine, respectively. The LOQ was determined by visualization method were found to be 1 µg/mL for both Metoprolol and Ranolazine. The results are given in Table 4.

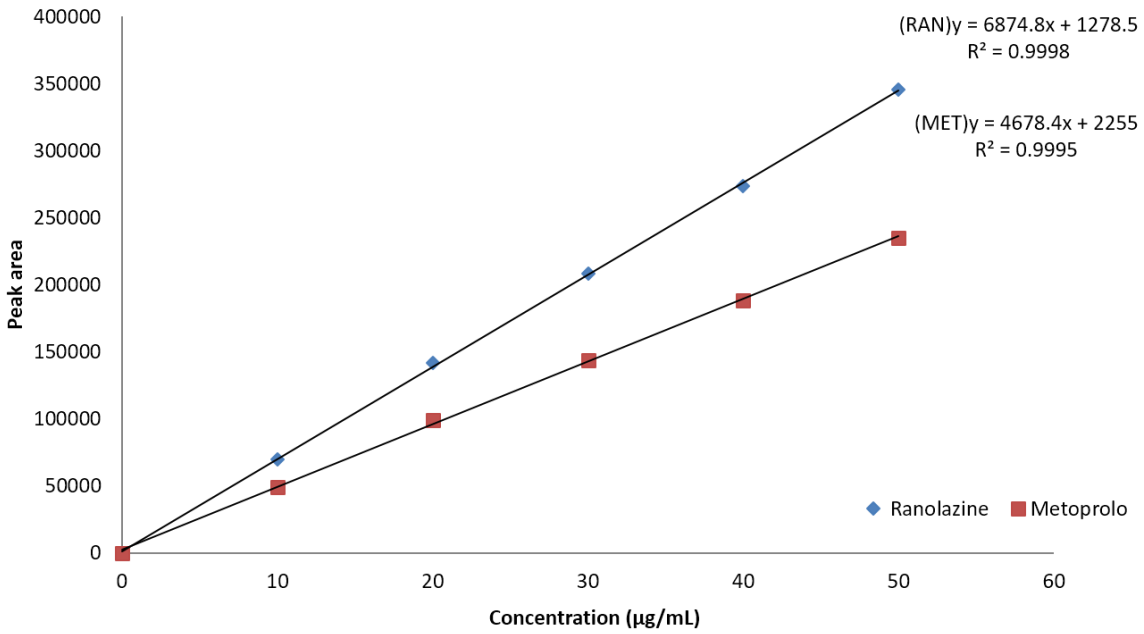


Fig 2. Linearity study of metoprolol and ranolazine using the developed HPLC method

Table 1. Results of accuracy of metoprolol and ranolazine using the developed HPLC method

Levels (%)	Metoprolol		Levels (%)	Ranolazine		Conc. of MET (µg/mL)	Conc. of RAN (µg/mL)	Amount of standard recovered (µg/ml)		Percentage Found	
	Std. soln (µg/mL)	Sample mix. soln (µg/mL)		Std. soln (µg/mL)	Sample mix. soln (µg/mL)			MET	RAN	MET	RAN
90	4	5	**75**	25	50	9	75	8.85	78.55	98.33	104.7
	4	5		25	50	9	75	8.85	75	98.33	100
	4	5		25	50	9	75	8.85	78.4	98.33	104.5
100	5	5	**90**	40	50	10	90	9.55	88.72	95.5	98.57
	5	5		40	50	10	90	9.63	88.76	96.3	98.63
	5	5		40	50	10	90	9.54	88.57	95.4	98.41
200	10	5	**100**	50	50	15	100	14.64	105.65	97.63	105.65
	10	5		50	50	15	100	14.74	105.68	98.26	105.68
	10	5		50	50	15	100	14.44	105.20	96.32	105.2

Table 2. Results of precision parameters of simultaneous estimation of metoprolol and ranolazine

Precision Parameters	Metoprolol % RSD*	Ranolazine % RSD*	Acceptance Criteria
System Precision	0.654	0.170	< 2.0%
Method Precision	0.244	0.165	< 2.0%
Intraday Precision	0.356	0.356	< 2.0%
Interday Precision	1.06	0.44	< 2.0%

* RSD: Relative Standard Deviation

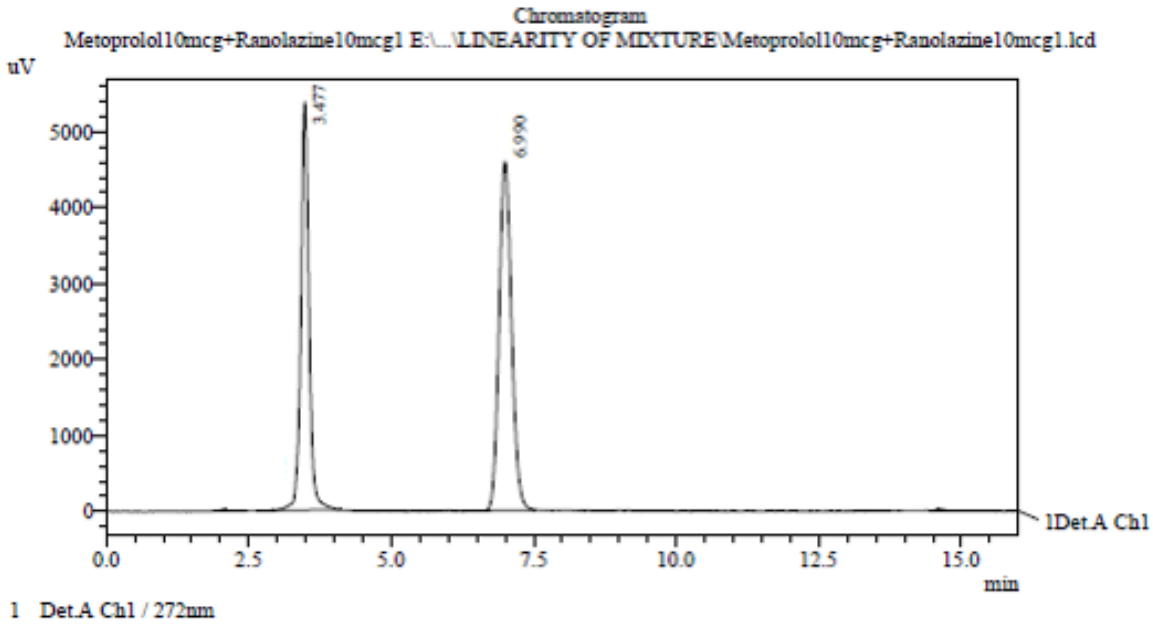


Fig 3. Chromatogram of Metoprolol and Ranolazine

Table 3. System suitability parameters for RP-HPLC estimation of Metoprolol and Ranolazine

System Suitability Factor	Metoprolol	Ranolazine	Acceptance Criteria
Theoretical plates	21221	31266	>2000
HETP (mm)*	47.136	31.720	-
Tailing factor	1.06	1.16	< 2
Resolution	10.7		> 2

HETP: Height equivalent to theoretical plate

Table 4. Results of limit of detection (LOD) and limit of quantification (LOQ)

Parameter	Metoprolol		Ranolazine	
	Peak Area*	Conc. (µg/mL)	Peak Area*	Conc. (µg/mL)
LOD	2195	0.001	1125	0.1
LOQ	6806	1	8847	1

*n=6

Results of robustness study are shown in Table 5 and the assay results are shown in Table 6. The results show that the developed method was robust and gives accurate results, as confirmed by the assay.

Table 5. Results of robustness study (n = 6)

Change in	% Assay of Metoprolol*	% Assay of Ranolazine*
Flow rate (mL)		
0.8	101.9	100.2
1.0	100.2	100.6
1.2	96.5	91.78
Mobile phase ratio v/v		
65:35	101.8	97.66
70:30	100.5	100.1
75:35	93.26	94.99

Table 6. Results of assay

Replicates	Metoprolol Peak Area*		Ranolazine Peak Area*	
	Standard	Sample	Standard	Sample
Average peak area	21302	21040	344354.1	362113
Amount of drug recovered (mg)	49.38		527.5	
% Assay	98.76		105.5	

* n = 6

CONCLUSION

A simple Rapid and reliable RP-HPLC method has been established for the simultaneous estimation of Metoprolol and Ranolazine either alone and/ or in combination in formulation. The method has several advantages like rapid, simple sample preparation, no need of any special reagents, high sensitivity etc. It is suitable for analysis of these drugs in binary formulation in a single isocratic run. This makes the method suitable for routine analysis of the combination product in quality control laboratories.

ACKNOWLEDGEMENT

The authors would like to thank the Principal, GCP, HOD of Pharmaceutical chemistry department GCP, Chief Scientific Officer and Scientific Officer Drug testing Laboratory, Bengaluru, for providing laboratory facilities to carry out this work and Micro labs for providing gift sample of Metoprolol and Ranolazine.

DECLARATION OF INTEREST

It is hereby declared that this paper does not have any conflict of interest.

REFERENCES

1. Metoprolol compound summary. [Online]. Available from URL:http://en.wikipedia.org/wiki/Metoprolol. 21.12.14; 2:00. URL:http://www.drugbank.ca /drugs/DB00264.
2. Ranolazine compound summary. [Online]. Available from URL:http://en.wikipedia.org/wiki/Ranolazine. 21.12.14; 2:00. URL:http://www.drugbank.ca/drugs/DB00243.
3. Matassini MV, Guerra F, Scappini L, Urbinati A, Capucci A. New anthyarrhythmic drugs for atrial fibrillation. Future Cardiol. 2015 Nov; 11(6): 705-717.
4. Bhargavi DK, et al. RP-HPLC method for estimation of Metoprolol in bulk drug. Int J Sci Innov Discov. 2011; 1(2): 151-157.

5. Aqil M, Ali A, Ahad A, Sultana Y, Najmi AK and Saha N. A validated HPLC method for estimation of Metoprolol in human plasma. Acta Chromatographica. 2007; 19: 130-140.
6. Bilal Y, Kadem M, Ali A, Yavuz O. Determination of Metoprolol in pure and pharmaceutical dosage forms by spectrofluorometry and high performance liquid chromatography. Chem Ind Chem Quart. 2011; 17(1): 25-31.
7. Jadhav AS, Tarkase KN, Deshpande AP. Quantitative determination of Metoprolol succinate in bulk and tablet dosage form through comparative study of UV and derivative spectroscopy. Der Pharmacia Lettre. 2012; 4(3): 763-767.
8. Yilmaz B. Determination of Metoprolol in pharmaceutical preparations by zero, first, second and third order derivative spectrophotometric method. Int J Pharma Bio Sci. 2010; 1(1): 1-15.
9. Boovizhikannan T, Palanirajan VK. Estimation of Ranolazine using reversed phase high performance liquid chromatography technique Am J Pharm Tech Res. 2012; 2(6): 348-353
10. Tapas KL, Dibya RP, Subrata S. Validated stability indicating reversed phase high performance liquid chromatographic method of Ranolazine dihydrochloride and characterization of its degradation products Int J Pharm Pharmaceut Sci. 2013; 5(1): 61-66.
11. Phani KV, Sunandamma Y. New RP-HPLC method development and validation for analysis of Ranolzine in tablet dosage form. J Pharm Res. 2011; 4(10): 368-370.
12. Sambasivarao V, Phani RS. Development of a rapid visible Spectrophotometric method for the estimation of Ranolazine in formulations. Int J Res Pharm Chem. 2011; 1(1): 66-72.
13. Tripti S, Swapan MK, Sudam CSI, Dannana SG. Stability-indicating LC method for the determination of Ranolazine hydrochloride in the bulk drug and in pharmaceutical dosage form. Int J Pharm Pharmaceut Sci. 2011; 3(4): 327-332.
14. Limei Z, Hao L, Yao J, Riyang P, Pengfei L, Jingkai G. Determination of Ranolazine in human plasma by liquid chromatographic– tandem mass spectrometric assay. Int J Chromatographic Sci. 2008; 46: 697-700.
15. ICH Guidance. Validation of analytical methods - definition and terminology. Q2A. Geneva: International Conference on Harmonization. Nov 2005.
16. ICH Guidance. Validation of analytical procedures - methodology. Q2B. Geneva: International Conference on Harmonization. Nov 2005.

Received: June 23, 2015; Revised: July 15, 2015; Accepted: July 25, 2015