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Development and Validation of RP-HPLC Method for the Simultaneous Estimation of Ramipril and Metoprolol Tartarate in Combined Dosage Form

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ABSTRACT

A simple, precise and sensitive reverse-phase high performance liquid chromatographic method was developed and validated for the simultaneous estimation of Ramipril and Metoprolol tartarate in pharmaceutical formulations. Chromatographic separation was performed on a High performance liquid chromatography equipped with auto sampler and UV detector. Good sensitivity for all analyte was observed with UV detection at wavelength of 218 nm, Separation was performed on a BDS Hypersil C_{18} (250 X 4.6mm) 5µm, using a mixture of 0.1% Triethylamine buffer pH 3.5 and Acetonitrile in the ratio of (10:90, v/v). The method results in excellent separation with good resolution between the two analytes. The within day variation %RSD values between Ramipril and Metoprolol tartarate were 0.55 and 0.82. The recovery was greater than 98% with %RSD less than 1.00. The method was validated according to ICH guidelines by performing linearity, accuracy, precision, limits of quantitation and selectivity. The results show the method is suitable for its intended use.

Keywords: Ramipril (RAM), Metoprolol tartarate (MTP), HPLC, Simultaneous determination, Relative standard deviation (RSD), Validation.

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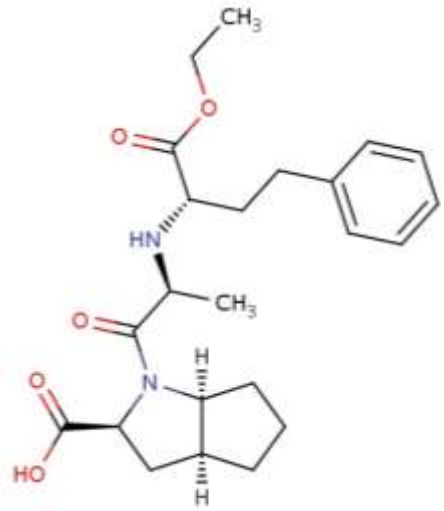
INTRODUCTION

Chemically Ramipril is (2S,3aS,6aS)-1-[(2S)-2-{[(2S)1-ethoxy 1-oxo phenylbutan2yl]amino}propanoyl] octahydrocyclopenta [pyrrole 2 carboxylic acid. (Figure 1) and Metoprolol tartarate is (Figure 2). Ramipril[(1)] is an anti-hypertensive agent. Its mode of action depends on its metabolization into Ramiprilat which works as a competitive ACE inhibitor, the enzyme responsible for the conversion of Angiotension I to Angiotensin II. Thus, it confers blood pressure (BP) lowering effects by antagonizing the effect of Renin Angiotensin Aldosterone System (RAAS). Metoprolol tartarate[2] is a β_1 blocker. Its mechanism of action is based on its competitive binding with adrenergic neurotransmitters such as catecholamines for binding at β_1 adreno receptors in the heart, thereby preventing the release of adrenaline from these receptors and resulting in decrease in the heart rate, cardiac output and blood pressure. The combination of Ramipril and Metoprolol tartarate has been prescribed to the patients who are suffering from myocardial infarction, nephropathy, angina and congestive heart failure. This combination has shown to be safe, effective and well-tolerated treatment for Essential hypertension (which is also called as primary or idiopathic) where this prevents the changes in creatinine kinase isoenzyme system and improves hemodynamic function after myocardial infarction.



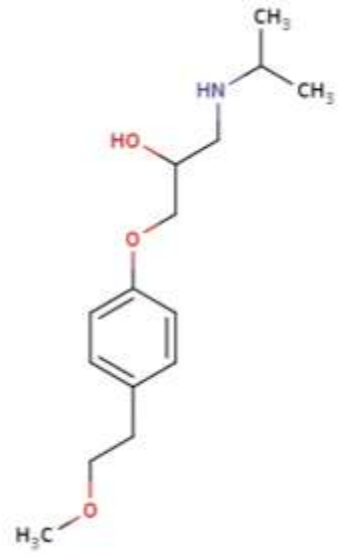


Figure 1: Structure of Ramipril **Figure 2: Structure of Metoprolol tartarate**

The concept of analytical chemistry lies in the precise and accurate measurements. This determination requires highly sophisticated instruments and methods like LC-MS[10-12], GC-MS[13], HPLC[14-21], HPTLC[22-23], UV[24-28] and visible spectrophotometry[29-30] etc. Instrumental methods are sensitive, accurate, precise and desirable for regular determination of drugs in formulations, thereby advantageous than the conventional volumetric methods. On the literature survey, it was

found that both Ramipril and Metoprolol tartarate were estimated independently and in combination with other drugs by several chromatographic, spectrometric and HPTLC methods in pharmaceutical formulations and by LC-MS methods in biological samples. Two analytical methods each of UV and RP-HPLC were found for simultaneous estimation of Ramipril and Metoprolol tartarate in combination.

In view of the need of an analytical method in the quality control laboratories for routine analysis of Ramipril and Metoprolol tartarate in formulations, attempts are being made to develop simple and accurate instrumental methods for simultaneous estimation of Ramipril and Metoprolol tartarate and extend it for their determination in formulation and in laboratory prepared synthetic mixture. The present work describes the development of a simple, precise, accurate and reproducible reverse phase chromatographic method for the simultaneous estimation of RAM and MTP in Pharmaceutical dosage form. The developed method was validated in accordance with ICH Guidelines.

MATERIALS AND METHODS

Instruments:

HPLC apparatus consisting of Shimadzu system, UV detector (set at 218nm), software LC Solutions and a injection volume with a 20µL was used for development and evaluation of this method. Chromatographic separation was performed using a BDS Hypersil C_{18} (250x4.6) 5µ with isocratic elution.

Chemicals and Reagents:

Analytical pure samples of RAM and MTP were provided by BIOLEO labs and WOCKHARDTS Pharmaceuticals as gift samples respectively. Formulation, PROLOMET-R (RAM-5mg+MTP-50mg) manufactured by Sun Pharmaceuticals.LTD New Delhi, India, was purchased from a local pharmacy in Hyderabad.

Preparation of Mobile phase:

0.1 mL of Triethylamine was pipetted and dissolved it in to 100mL of HPLC water. The final pH 3.5 was adjusted using O-phosphoric acid. Finally composition of mobile phase was made to 0.1% Triethylamine pH 3.5: Acetonitrile (10:90). The prepared mobile phase was filter through 0.45µm membrane filter paper.

Preparation of standard (PRIMARY) stock solution:

Initially 10mg of the both the drugs were weighed and diluted to 10ml to get a concentration of 1000µg/mL (Primary stock solution).

Preparation of sample solution:

From this primary stock solution, 1mL is pipetted out to prepare a solution of 100µg/mL (Secondary stock solution), serial dilutions were prepared in 10mL volumetric flasks using mobile phase (10 parts of 0.1% Triethylamine and 90 parts of Acetonitrile adjusted to pH 3.5 with 0.1% orthophosphoric acid) to get concentration ranges of 1-10µg/mL for both the drugs Ramipril and Metoprolol tartarate respectively.

Selection of chromatographic method for separation:

Reverse phase chromatographic technique is selected since both drugs are polar in nature.

Selection of detection wavelength:

Sensitivity of HPLC method that uses ultraviolet (UV) detector depends upon the proper selection of wavelength. An ideal wavelength is the one that gives maximum absorbance and good response for the drug detected at lower concentration also. From the UV spectra obtained for both drugs, 218nm was selected as the wavelength for study.

Selection of Mobile phase:

For developing RP – HPLC method, different mobile phase systems with different ratios were tried, among which 0.1% triethylamine buffer and acetonitrile (pH adjusted to 3.5 using 1% orthophosphoric acid) gave symmetrical peaks with good resolution (Ramipril R_f – 3.2 minutes, Metoprolol tartarate R_f – 5.7 minutes), and hence fixed for further studies. Different mobile phases were tried and their observations are given below in the table 1.

Table 1: Selection of mobile phase

S.No	Mobile phase conditions	Observation
1.	Acetonitrile : Water (90:10 % v/v)	Slight tailing and Less resolution
2.	Acetonitrile : 0.02M Potassium dihydrogen phosphate (90:10 % v/v)	Fronting
3.	Acetonitrile : 0.1% Triethylamine buffer (90:10 %v/v)	Good, symmetrical peaks resolution

Table 2: Statistical data of calibration curve.

Parameter Range	RAM 1-10 µg/mL	MTP 1-10 µg/mL
Slope	30924	34751
Intercept	4352	3762
R^2	0.999	0.998
Wavelength (nm)	218	218
Regression equation (y = mx +c)	y = 30924x + 4352	y = 34751x + 3762
LOD(µg/mL)	0.268	0.629
LOQ(µg/mL)	0.199	0.604

R^2 = correlation coefficient

OPTIMIZATION OF SEPARATION CONDITION

Effect of strength of triethylamine:

Different ionic strengths of triethylamine such as 0.05, 0.1, 0.2% v/v etc., adjusted to pH 3.5 in the ratio of 90:10 (different ionic strengths of acetonitrile: triethylamine) were tried. Among these solutions, acetonitrile: 0.1% triethylamine in the ratio of 90:10 % v/v showed symmetrical peaks where as the other strengths showed peak tailing. Hence 0.1% v/v was selected as the ideal strength of triethylamine.

Effect of ratio of mobile phase:

The mobile phase system consisting of triethylamine and acetonitrile in different ratios such as 70:30, 85:15, 80:20, 90:10 % v/v, adjusted to pH 3.5 using 1% orthophosphoric acid were tried and the chromatograms were recorded at 218 nm at the flow rate of 1 ml/min. The ratio of 90:10 % v/v, gave good separation and symmetrical peaks and hence the ratio of 90:10% v/v was selected for the study.

Effect of pH:

Keeping other conditions constant, chromatograms were recorded with different pH such as 6, 5, 4, 3.5, 3 etc, adjusted using 1% orthophosphoric acid. At the pH of 3.5, the peak shapes of both drugs were good and hence selected for further study.

VALIDATION PARAMETERS:

Linearity and Range:

The linearity of response for both Ramipril and Metoprolol tartarate respectively was determined by preparing standard concentration solutions in the range of 1-10 µg/ml. The Correlation coefficient (r) was found 0.999 for RAM and 0.998 for MTP as shown in Figure 5 and 6 respectively. It indicates that the response was linear over the concentration range. Overlain spectra of RAM and MET (1-10 µg/mL) is shown in figure 3.

Limit of Detection (LOD) and Limit of Quantification (LOQ):

It is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated under the stated experimental conditions. The detection limit (LOD) and quantification limit (LOQ) may be expressed as-

$$LOD = 3.3 \times N/S$$

$$LOQ = 10 \times N/S$$

Where, N is the standard deviation of the peak areas of the drug and S is the slope of the corresponding calibration curve.LOD and LOQ of Ramipril and Metoprolol tartarate were calculated mathematically.

Precision:

The precision of the method was established by carrying out the analysis of the analyte using the proposed developed method on the same day at three time intervals (Intra-day precision) and on three consecutive days (Inter-day precision). The low value of %RSD showed that the methods were precise.

Accuracy:

Accuracy was performed at 50%, 100% and 150% levels by Standard addition method. Each concentration was analyzed 3 times and average recoveries were measured as shown in Table 2. To check the degree of repeatability of the method, suitable statistical evaluation was carried out. The concentrations of two drugs were measured three times on the same day at intervals of 1hr and on three different days for intra and inter day study, respectively. The Standard deviation (SD) and Relative standard deviation (RSD) were calculated.

ANALYSIS OF MARKETED FORMULATION:

Commercial formulation (PROLOMET-R containing 5mg of RAM and 50mg of MTP) was used for analysis. Fixed Chromatographic conditions were applied for the analysis of formulation.

Preparation of Standard Solution:

Stock solution containing concentrations of 100µg/ml of each Ramipril and Metoprolol tartarate was prepared using mobile phase. This solution was suitably diluted to get aliquots of standard solutions containing 1 to 10µg/ml of both Ramipril and Metoprolol tartarate.

Preparation of Sample Solution:

Twenty tablets (PROLOMET-R) are powdered and the average weight was calculated. A quantity equivalent to 20 mg of drug was dissolved in mobile phase and to it 18mg of standard Ramipril was added (standard addition method), such that sample contains 20mg each of Ramipril and Metoprolol tartarate. Finally the volume was made upto the mark to get a working concentration of 6µg/ml each of Ramipril and Metoprolol tartarate.

Recording of Chromatograms:

A steady baseline was recorded with the fixed chromatographic conditions. Standard drug solutions containing 1 to 10µg/ml of Ramipril and Metoprolol tartarate were injected and chromatograms were recorded at 218 nm where both the drugs have shown maximum absorbance values. Retention times of Ramipril and Metoprolol tartarate were found to be 3.2 and 5.7 minutes. This was followed by injection of sample solution obtained from the formulation.

Calibration curves were plotted using peak areas of standard drugs Vs concentration of

corresponding standard solutions. Peak areas of the sample chromatograms were compared and amount of Ramipril and Metoprolol tartarate were calculated and tabulated.

RESULTS AND DISCUSSION

A binary mixture of triethylamine buffer and acetonitrile was selected as the initial mobile phase system for the determination of both drugs. The optimum wavelength for detection was set at 218nm at which much better detector responses for both drug were obtained as seen from the UV spectra (Figure 3).

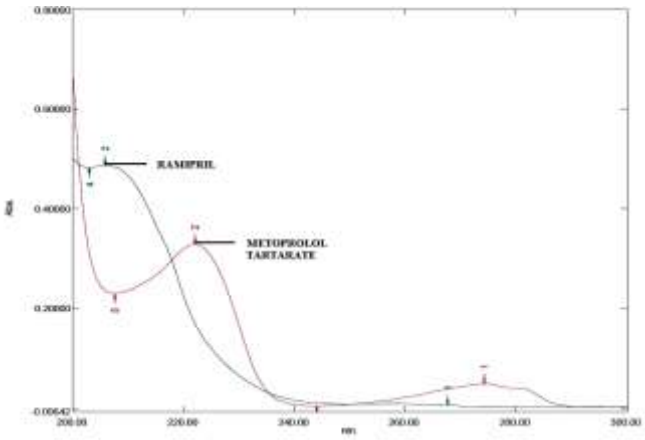


Figure 3: Over lay spectra of RAM and MTP

Firstly, various concentrations of triethylamine buffer were tried. From this, 0.1% triethylamine buffer was found to be ideal for the work. Then different pH's of buffer were tired, out of which triethylamine adjusted to pH 3.5 with orthophosphoric acid gave good peaks. Then the ratio of mobile phase was determined by varying the proportion of triethylamine and Acetonitrile. Finally, the mixture of Acetonitrile and 0.1% triethylamine buffer adjusted to pH 3.5 with orthophosphoric acid (90:10% v/v) was employed for the simultaneous determination of both drugs. The retention times of Ramipril and Metoprolol tartarate were found to be 3.25 and 5.72 minutes respectively as shown in Figure 4.

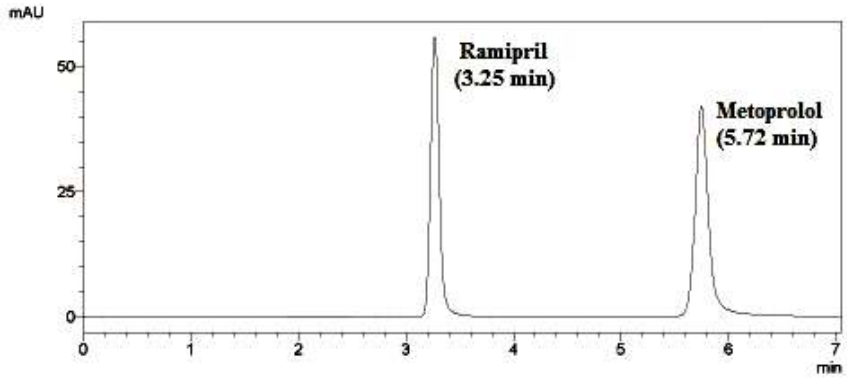


Figure 4: Chromatograms of Ramipril and Metoprolol tartarate at 218nm.

The developed method was validated as per ICH guidelines. Assessed validation parameters include linearity, limit of detection and limit of quantification, selectivity, accuracy and repeatability. The optical regression characteristics and validation parameters are showed in Table 2. Calibration graphs were potted using standard peak areas vs. concentration of standard solutions. The slope, intercept and correlation coefficient values were found to be 30924, 4352, 0.999 for Ramipril and 34751, 3762 and 0.998 for Metoprolol tartarate. Both Ramipril and Metoprolol tartarate was found to be linear in the range of 1 to 10µg/ml. The linear regression graph is given in Figures 5 and 6 of Ramipril and Metoprolol tartarate respectively. The LOD of Ramipril and Metoprolol tartarate were found to be 0.268µg/ml and 0.199µg/ml respectively. The LOQ of Ramipril and Metoprolol tartarate were found to be 0.629µg/ml and 0.631µg/ml respectively. The values of which are shown in Table 2.

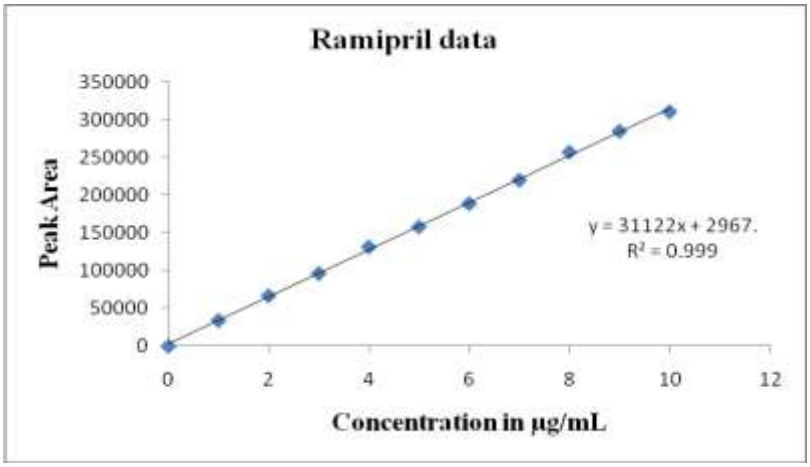


Figure 5: Calibration graph of Ramipril.

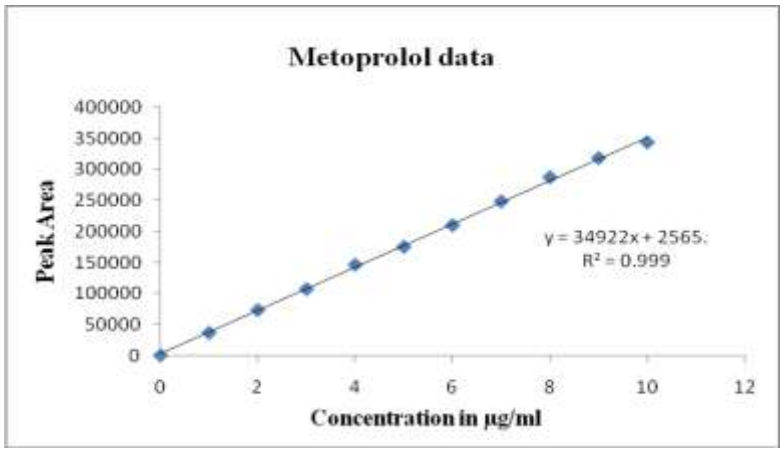


Figure 6: Calibration graph of Metoprolol tartarate

Precision of the developed method was studied under intraday precision, interday precision and repeatability of the injection. Low % RSD values i.e. below 2.0 for both drugs Ramipril and Metoprolol tartarate indicate that the method is precise (Table 3). Recovery studies were

Table 3: Precision values of RAM and MTP

Drug	Concentration (µg/mL)	Intra-day Peak Area	%RSD*	Inter-day Peak Area	%RSD*
Ramipril	4	130004	0.597	130655	0.982
		131457		128767	
		131233		131208	
	5	156154	0.555	156556	0.545
		157789		155207	
		156733		154781	
	6	188432	0.615	189560	0.385
		189562		188316	
		187123		189357	
Metoprolol	4	145332	0.643	144359	0.988
		145177		144074	
		146873		141773	
	5	171715	0.825	172141	1.125
		174315		171795	
		171795		168611	
	6	206678	0.346	205321	0.624
		208025		206634	
		207494		208025	

**= % RSD of 3 observations*

Table 4: Recovery values of RAM and MTP

Drug	Level of Recovery	Amount Present	Amount recovered	% Recovery	%RSD*
Ramipril	50%	3	3.05	101.99%	1.086
			3.03	101%	
			2.99	99.8%	
	100%	6	6.06	101%	0.727
			5.98	99.6%	
			6.04	100.66%	
	150%	9	9.09	101.77%	0.769
			9.02	100.22%	
			9.1	101.11%	
Metoprolol	50%	3	2.99	99.8%	0.633
			3.03	101%	
			3.02	100.77%	
	100%	6	5.83	98.5%	0.349
			5.88	98.00%	
			5.92	98.66%	
	150%	9	8.99	99.8%	0.656
			9.1	101.11%	
			9.03	100.33%	

**= % RSD of 3 observations*

performed. Results indicate that the % recovery values of the pure drugs from the pre-analyzed solutions of formulations were in between 98 - 101%, (Table 4) which indicates that the method

is accurate and reveals that commonly used excipients and additives present in the pharmaceutical formulations did not interfere in the proposed method

Commercial formulation (PROLOMET-R containing 5mg of RAM and 50mg of MTP) was analyzed, amount of drugs present in the formulation was calculated and the results are shown in Table 5 along with %RSD values. Robustness was carried by varying experimental parameters of proposed method. In case of liquid chromatography typical variations are the pH of the mobile phase, the mobile phase composition, and flow rate. No significant change was observed. The values are shown in Table 6. The developed method was found to be robust. System suitability test parameters like Resolution, Retention Time, Theoretical plate and Tailing factor are shown in Table 7. The proposed method was simple, sensitive, and reliable with good precision and accuracy. Hence this validated liquid chromatographic method can be applied for the routine simultaneous analysis of RAM and MTP in bulk and pharmaceutical formulations.

Table 5: Analysis of formulation and its %RSD values

Drug	Label claim(mg)	Amount found (mg)	%Label claim	%RSD*
RAM	5	4.98	99.66%	0.435
		4.96	99.30%	
		4.94	98.8%	
MTP	50	49.57	99.01%	0.390
		49.32	98.65%	
		49.12	98.24%	

*= *%RSD of 3 observations.*

Table 6: Robustness values of RAM and MTP

Factor	Value	Peak Area		%RSD	
		RAM	MTP	RAM	MTP
pH	3.3	188316	205321	0.353	0.532
	3.5	189359	207494		
	3.7	189560	206678		
Mobile Phase	0.1% Triethyl Amine buffer pH 3.5 and Acetonitrile in the ratio of (8:92, v/v)	188889	208025	0.312	0.564
	0.1% Triethyl Amine buffer pH 3.5 and Acetonitrile in the ratio of (10:90, v/v)	188905	206634		
	0.1% Triethyl Amine buffer pH 3.5 and Acetonitrile in the ratio of (12:88, v/v)	188879	205709		
Flow Rate (mL/min)	0.9	156556	172141		
	1.0	155207	171795	0.54	1.12
	1.1	154781	168611		

*= *% RSD of 3 observations*

Table 7: System suitability parameters of RP-HPLC method

Parameter	Ramipril	Metoprolol tartarate
Retention time (min)	3.256	5.720
Theoretical plate	6800	10660
Tailing factor	1.2	1.19
Resolution (min)	2.47	2.47

CONCLUSION

Simultaneous determination of Ramipril and Metoprolol tartarate in their pharmaceutical formulation using HPLC has been successfully achieved. The method is accurate and precise for reliable quality control evaluation of drugs with good accuracy and precision. From these values it is concluded that the new HPLC method is suitable for the simultaneous determination of these two components in their pharmaceutical formulations.

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