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A new validated stability indicating LC method for simultaneous determination of metoprolol succinate and ramipril in pharmaceutical marketed formulation

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ABSTRACT

A simple and precise stability indicating RP-HPLC method was developed and validated for simultaneous determination of Metoprolol succinate and Ramipril in bulk and Pharmaceutical marketed formulation. Chromatography was carried out on Altima C_{18} (150 x 4.6 mm, 5µ particle size) column in an isocratic mode with mobile phase containing phosphate buffer (adjusted to pH 4.8 with dilute othophosphoric acid, acetonitrile and methanol in the ratio of 35:10:55% v/v/v at a flow rate of 1ml/min. The analyte was monitored using PDA detector at 210 nm. The retention time was found to be 2.203 min and 3.283 min for Metoprolol succinate and Ramipril respectively. The proposed method was found to be having linearity in the concentration range of 5-30 µg/ml for Metoprolol succinate and 0.5-3.0 µg/ml for Ramipril with correlation coefficient value of 0.999 respectively. The mean % recoveries obtained were found to be 99.87-100.24 % for Metoprolol succinate and 99.64-100.08 % for Ramipril respectively. Stress testing which covered acid, base, peroxide, UV light, neutral and thermal degradation was performed on under test to prove the specificity of the method and the degradation was achieved. The developed method has been statistically validated according to ICH guide lines. Thus the proposed method can be successfully applied for the stability indicating simultaneous determination of Metoprolol succinate and Ramipril in bulk and combined tablet dosage form and in routine quality control analysis.

Keywords: Metoprolol succinate, Ramipril, RP-HPLC, Forced degradation, Method validation.

INTRODUCTION

Metoprolol succinate

Chemically (Fig.1), it is {butanedioic acid; 1-[4-(2-methoxyethyl) phenoxy]-3-(propan-2-ylamino) propan-2-ol. It has a molecular formula of $C_{34}H_{56}N_2O_{10}$ and molecular weight of 652.8 g/mol. Metoprolol succinate is an antihypertensive agent (β_1-Adrenergic blocker). Adrenergic beta-antagonists are used for treatment of hypertension, cardiac arrhythmias, angina pectoris, glaucoma, migraine headaches and anxiety. Metoprolol competes with adrenergic neurotransmitters such as catecholamines for binding at β_1-adrenergic receptors in the heart. β_1-receptor blockade results in a decrease in heart rate, cardiac output, and blood pressure.

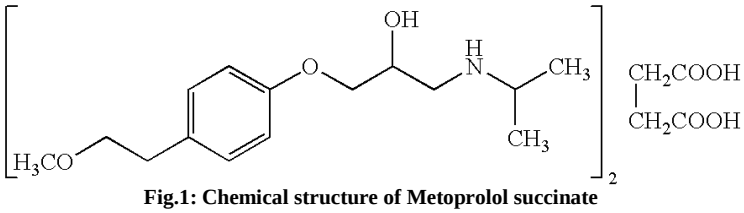

Fig.1: Chemical structure of Metoprolol succinate

Ramipril

Chemically (Fig.2), it is (2S,3aS,6aS)-1-[(2S)-2-[[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino] propanoyl]-3,3a,4,5,6,6a-hexahydro-2H-cyclopenta [b] pyrrole-2-carboxylic acid. It has a molecular formula of $C_{23}H_{32}N_2O_5$ and molecular weight of 416.511 g/mol. Ramipril is an angiotensin converting enzyme inhibitor, used in the treatment of high blood pressure and congestive heart failure. ACE inhibitors inhibit the actions of angiotensin converting enzyme (ACE), thereby lowering the production of angiotensin II and also decreasing the breakdown of bradykinin. The decrease in angiotensin II results in relaxation of arteriole smooth muscle leading to a decrease in total peripheral resistance, reducing blood pressure as the blood is pumped through widened vessels.

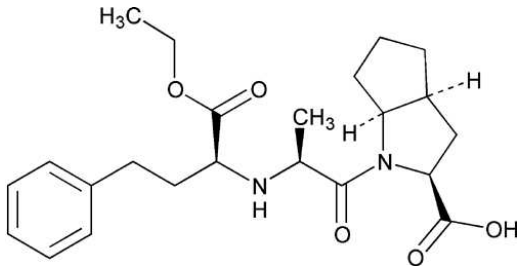

Fig.2: Chemical structure of Ramipril

Literature survey revealed that few analytical methods were reported so far for both drugs in combination or in alone like RP-UPLC [1], RP-HPLC [2-7], LC-MS/MS method in biological fluids [8-10], GC-MS [11] and Spectrophotometric methods [12]. The aim of the present study was to develop a simple, precise, sensitive and selective stability indicating RP-HPLC method with PDA detection for the analysis of Metoprolol succinate and Ramipril in bulk and in combined tablet formulation.

MATERIALS AND METHODS

Chemicals

The Pharmaceutical grade pure samples of Metoprolol succinate and Ramipril were received as gift samples from IPCA Laboratories Ltd., Mumbai. HPLC grade water, methanol and acetonitrile were purchased from E.Merck. Chem.ltd., Mumbai. All the chemicals used were of analytical reagent grade (E.Merck). Fixed dose combination tablet formulation (Starpress R XL 50) containing 50 mg of Metoprolol succinate and 5 mg of Ramipril (Manufactured by Lupin Laboratories Ltd., Mumbai) were procured from local market.

Instrumentation

Quantitative HPLC was performed on Waters 2695 separation module Alliance Isocratic HPLC system and PDA Detector 2996 series equipped with auto injector using empower software 2. An UV-2400PC Series UV/Visible double beam spectrophotometer with 1 cm matched quartz cells was used for all spectral measurements.

Chromatographic conditions

Mobile phase composition	Phosphate buffer (adjusted to pH 4.8 with dilute OPA): acetonitrile: methanol in the ratio of 35:10:55 %v/v/v
Stationary phase	Altima C_{18} column (150 x 4.6mm, particle size 5µ)
Detector wave length	210 nm
Run time	7 min
Flow rate	1.0 ml/min
Injection volume	10µl
Colum temperature	30^0C

Preparation of Phosphate buffer

Accurately weighed quantity of 1.36 gm of Potassium dihydrogen orthophosphate was transferred into a 1000ml volumetric flask. About 900ml of HPLC grade water was added and degassed by subjecting to sonication for 5 min and final volume was made up to the mark with water. Filtered through 0.45µ Millipore nylon filter. Then P^H of the solution was adjusted to 4.8 with dilute orthophosphoric acid solution after adding 1 ml of triethylamine to the solution.

Preparation of Mobile phase

Phosphate buffer, acetonitrile and methanol were taken in the ratio of 35:10:55%v/v/v and mixed and then degassed by subjecting to sonication for 10 min and resultant solution used as mobile phase after filtration using vacuum filtration assembly.

Preparation of diluent
Water and Methanol were taken in the ratio 50:50%v/v, mixed and used as diluent.

Preparation of standard solution
Accurately weighed and transferred each 10 mg of Ramipril and Metoprolol succinate working standards into two 10 ml clean and dry volumetric flasks separately, 3/4thvolume of diluent was added, sonicated to dissolve for 10 minutes and then made up to the final volume with diluent to obtain stock solution of concentration 1000µg/ml. From the above stock solution, 1 ml each was pipette out separately in to a 10 ml volumetric flasks and then volume was made up to mark with diluent to obtain 100µg/ml working standard solution. Then again pipette out 2 ml of Metoprolol succinate and 0.2 ml of Ramipril working standard solutions in to a 10 ml volumetric flask and then volume was made up to mark with diluent to obtain final concentration of 20µg/ml solution of Metoprolol succinate and 2µg/ml solution of Ramipril.

Preparation of Sample solution
20 tablets were accurately weighed and calculated the average weight of each tablet. The tablets were crushed to a fine powder. An amount of powder (435 mg) equivalent to 50mg of Metoprolol succinate and 5 mg of Ramipril were weighed accurately and transferred into a 50ml volumetric flask, 30ml of diluent was added, sonicated for 15 min and volume was made up with diluent. Filtered through 0.45µ Millipore Nylon filter. From the filtered solution, 5ml was pipette out into a 10 ml volumetric flask and then volume was made up to mark with diluent. Then again 0.4 ml of the above solution was further diluted to 10 ml in a 10 ml volumetric flask with diluent to obtain final concentration of 20µg/ml solution of Metoprolol succinate and 2µg/ml solution of Ramipril. Then Injected 10µl of filtered portion of the sample, standard preparation and blank solution into the chromatograph separately. Recorded the responses for the major peaks. Calculated the content of Ramipril and Metoprolol succinate present in each tablet.

Method validation
Analytical validation parameters for this proposed method were determined according to ICH guidelines.

System suitability
System suitability was carried out by injecting five replicate injections of standard solutions of Metoprolol succinate and Ramipril. The system suitability parameters were then evaluated for tailing factor, retention time and theoretical plates of standard chromatograms. % RSD for peak area of five replicate injections of standard solutions (% RSD NMT 2) were within the limits. The results for system suitability studies are presented.

Specificity
The specificity of the method was performed by injecting blank, placebo, standard and sample preparations into the chromatograph. Chromatograms were recorded. Retention times obtained from standard and sample preparations were compared for identification of analytes.

Linearity
The linearity of an analytical method was carried out to check its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. Different concentration levels (25-150%) of linearity solutions were prepared by diluting aliquots (0.1- 0.6 ml) of standard stock solution (500µg/ml for Metoprolol succinate and 50µg/ml for Ramipril) in to each 10 ml volumetric flasks (6 no's) and diluted to final volume with diluent to obtained concentrations in the range of 5-30 µg/ml for Metoprolol succinate and 0.5-3.0 µg/ml for Ramipril respectively to demonstrate linearity for assay and then injected each concentration into the chromatographic system and the chromatograms were recorded. The calibration graphs were plotted between amount of drug concentration (µg/ml) and chromatographic peak areas (mV) of Metoprolol succinate and Ramipril respectively. The linearity of the proposed method was then evaluated by linear regression analysis. The correlation coefficient, slope and intercept were calculated from the graph.

Accuracy
The accuracy of the test method was demonstrated by % recovery across its range by adding a known quantity of the standard to the pre analysed sample. The recovery was carried out at 50%, 100% and 150% concentration levels using standard addition method and at each level, 3 samples were prepared and total of 9 samples were injected separately into the chromatographic system and the contents were then determined from respective chromatograms. From the results obtained we conclude that the method was accurate.

Precision

System precision (Repeatability)

System precision was established by six replicate injections of the working standard solution at 100% concentration level into the chromatographic system. The corresponding peak areas of Metoprolol succinate and Ramipril were measured and % RSD were calculated.

Method precision

The method precision study was performed by injecting six sample preparations of marketed formulations into the chromatographic system. The corresponding peak areas of Metoprolol succinate and Ramipril were measured and % RSD were calculated.

Inter-day precision

Inter-day precision was performed by injecting standard preparations six times into the chromatographic system on different days by maintaining the optimized chromatographic conditions and calculated %RSD of retention time and peak areas for both Metoprolol succinate and Ramipril.

Specificity

Specificity is the ability to assess unequivocally the analytes in the presence of compounds that may be expected to present, such as impurities, degradation products and matrix components. The specificity of the method was assessed by comparing the chromatograms obtained from standard and sample solutions. The retention times of the analytes in standard and the sample solutions were found to be same, so the method was specific and free from interference from excipients present in the tablets.

Limit of Detection (LOD)

Limit of detection is the lowest concentration of the analyte that can be detected by injecting decreasing amount, not necessarily quantity by the method, under the stated experimental conditions. The minimum concentration at which the analyte can be detected is determined from the linearity curve by applying the formula.

LOD = $3.3\ S_a/b$.
S_a is the standard deviation of intercept
b is the slope of calibration curve

Limit of Quantification (LOQ)

Limit of quantification is the lowest concentration of the analyte in a sample that can be estimated quantitatively by injecting decreasing amount of drug with acceptable precision and accuracy under the stated experimental conditions of the method. Limit of quantitation can be obtained from linearity curve by applying the following formula.

LOQ = $10\ S_a/ b$
S_a is the standard deviation of intercept
b is the slope of calibration curve

Robustness

The robustness of the proposed method was determined by analyzing aliquots from homogenous lots by differing physical parameters like mobile organic phase composition, flow rate and column temperature. The standard and sample solutions were injected into the chromatograph at varied conditions of flow rate ± 0.2 ml/min, mobile organic phase ± 10%, mobile phase buffer pH ± 0.2 units and temperature by $\pm$ 5 ^{0}c. The content of the drug was not adversely affected by these changes as evident from the low value of relative standard deviation indicating that the method was robust.

Stability of the solution and Forced degradation studies:

In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 24 hr at room temperature. The results showed that for the solutions, the retention time and peak area of Metoprolol succinate and Ramipril were remained almost similar (%RSD less than 2.0) and no significant degradation within the indicated period, thus indicated that both solutions were stable for at least 24 hr., which was sufficient to complete the whole analytical process. Further forced degradation studies were conducted indicating the stability of the method developed by degrade the sample forcefully under the various stress conditions like acid, base, water, light, heat hydrolysis and peroxide oxidation. The results of the degradation studies are presented.

Acid degradation studies:
To 1 ml of stock solution of Ramipril and Metoprolol, 1ml of 2N Hydrochloric acid solution was added and refluxed for 30 min at 60 ^{0}C and then neutralized the solution with 1 ml of 2N sodium hydroxide solution. The resultant solution was suitably diluted with diluent in a 10 ml volumetric flask to obtain final concentration of 2µg/ml & 20µg/ml solution of Ramipril and Metoprolol respectively. Then 10 µl solutions were injected into the chromatographic system and the chromatograms were recorded to assess the stability of sample**.**

Base degradation studies:
To 1 ml of stock solution of Ramipril and Metoprolol, 1ml of 2N Sodium hydroxide solution was added and refluxed for 30 min at 60 ^{0}C and then neutralized the solution with 1 ml of 2N Hydrochloric acid solution. The resultant solution was suitably diluted with diluent in a 10 ml volumetric flask to obtain final concentration of 2µg/ml & 20µg/ml solution of Ramipril and Metoprolol respectively. Then 10 µl solutions were injected into the chromatographic system and the chromatograms were recorded to assess the stability of sample**.**

Peroxide degradation studies:
To 1 ml of stock solution of Ramipril and Metoprolol, 1 ml of 20% Hydrogen peroxide (H_2O_2) was added and kept for 30 min at 60^{o}c. The resultant solution suitably diluted with diluent in a 10 ml volumetric flask to obtain final concentration of 2µg/ml & 20µg/ml solution of Ramipril and Metoprolol respectively. Then 10 µl solutions were injected into the chromatographic system and the chromatograms were recorded to assess the stability of sample**.**

Thermal degradation studies:
The standard drug solution of Ramipril and Metoprolol was placed in an oven at 105°C for 6 hrs to study dry heat degradation. For HPLC study, the resultant solution was suitably diluted with diluent in a 10 ml volumetric flask to obtain final concentration of 2µg/ml and 20 µg/ml solution of Ramipril and Metoprolol and 10µl solutions were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

Photo stability studies:
It is carried out by exposing 1 ml of stock solution of Ramipril and Metoprolol to UV light, by keeping the beaker in UV Chamber for 7days or 200 Watt hours/m^2 in photo stability chamber. The resultant solution was suitably diluted with diluent in a 10 ml volumetric flask to obtain final concentration of 2µg/ml & 20µg/ml solution of Ramipril and Metoprolol respectively and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral degradation studies:
Stress testing under neutral conditions was studied by refluxing the drug solution on water bath for 6hrs at a temperature of 60^{o}c. For HPLC study, the resultant solution was suitably diluted with diluent in a 10 ml volumetric flask to obtain final concentration of 2µg/ml & 20µg/ml solution of Ramipril and Metoprolol respectively and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

RESULTS AND DISCUSSION

From this study, it was found that a simple, precise, accurate, sensitive and efficient stability indicating RP-HPLC method has been developed and validated for simultaneous estimation of Metoprolol and Ramipril in bulk and pharmaceutical dosage form. Chromatographic separation was carried out using mobile phase composed of phosphate buffer (adjusted to pH 4.8 with dilute OPA): acetonitrile: methanol in the ratio of 35:10:55 %v/v/v on Altima C_{18} (150 x 4.6 mm, 5µ particle size) column at a flow rate 1.0 ml/min using PDA detection at 210 nm. The retention time was found to be 2.203 min and 3.283 min for Metoprolol succinate and Ramipril respectively. System suitability chromatogram as shown in figure 3 and results are shown in table 1. Linearity was evaluated in the concentration range of 5-30 µg/ml for Metoprolol succinate and 0.5-3.0 µg/ml for Ramipril. The calibration curves were described by the equation y = 35395.8x +42.714 and y = 194275x +1564.78 with correlation coefficient of 0.99992 and 0.99971 for Metoprolol succinate and Ramipril respectively as shown in figure 4 and figure 5 respectively. The standard and sample chromatograms in the specifity studies are shown in figure 6 and figure 7. The Limit of detection (LOD) and limit of quantification (LOQ) are shown in figure 8 and figure 9. Accuracy data as shown in table 2. The validation summary parameters and assay results obtained from the marketed formulation is shown in table 3 and table 4. The results of robustness studies are shown in table 5. The %RSD in precision, accuracy and robustness studies were found to be less than 2 %, indicating that the method was precise, accurate and

robust. The stress testing chromatograms for both Metoprolol succinate and Ramipril are shown from figure 10 to figure 15 and results are shown in table 6 and table 7.

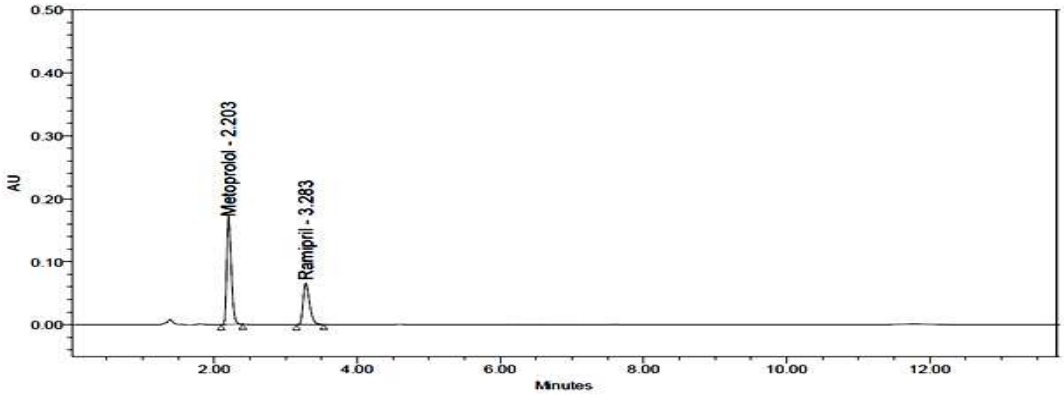

Fig.3: Typical chromatogram of system suitability solution

Table 1: System suitability results

S.No.	System suitability parameters	Metoprolol succinate	Ramipril
1	Tailing factor (T_f)	1.32	1.27
2	Resolution (Rs)	4.32	
3	Retention time (Min)	2.203	3.283
4	Theoretical plates (N)	5469	6515

Table 2: Accuracy data

Sample	Level	Peak area*	Amount added (µg/ml)	Amount recovered (µg/ml)	Mean % Recovery *± SD
Metoprolol succinate	50%	355712	10	10.00	100.07 ±0.67
	100%	719542	20	19.97	99.87±0.48
	150%	1057456	30	30.07	100.24± 0.34
Ramipril	50%	198524	1	1.00	100.08±0.44
	100%	397125	2	1.99	99.64±0.84
	150%	587420	3	2.99	99.91 ±0.62

**Mean of three determinations*

Linearity:

R^2 values were found to be 0.99992 and 0.99971 and regression equation y = 35395.8x +42.714 and y = 194275x +1564.78 for Metoprolol succinate and Ramipril respectively.

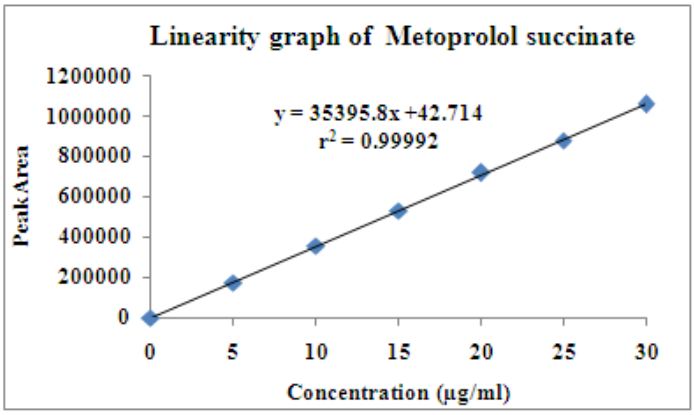

Fig. 4: Linearity Graph of Metoprolol succinate (5-30 µg/ml)

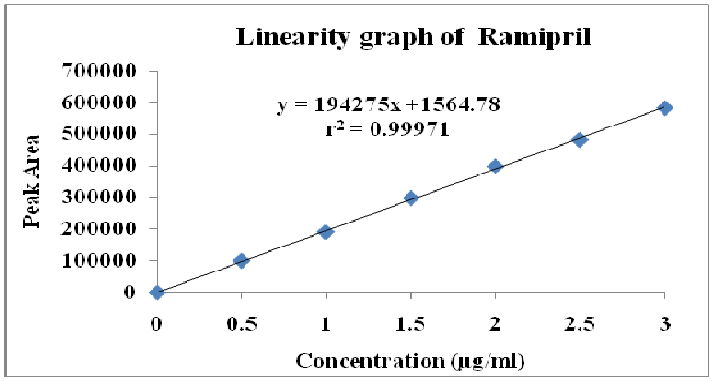

Fig. 5: Linearity Graph of Ramipril (0.5-3.0 µg/ml)

Table 3: Validation Parameters of the proposed RP-HPLC Method

Parameter	Metoprolol succinate	Ramipril
Regression equation	y = 35395.8x +42.714	y = 194275x +1564.78
Correlation coefficient	0.99992	0.99971
LOD (µg/ml)	0.004	0.27
LOQ (µg/ml)	0.01	0.81
System precision (% RSD)	0.12	0.14
Method precision (% RSD)	0.53	0.74
Inter-day precision (% RSD)	0.72	0.46

Table 4: Results of Assay in Marketed Formulation

Brand	Drug	Standard peak area	Sample peak area	Labelled amount (mg/tab)	Amount found (mg/tab)	% Assay	%RSD*
Starpress R XL 50	Metoprolol succinate	763905	765721	50	49.84	99.67%	0.24
	Ramipril	406534	406766	5	4.965	99.33%	0.38

**Mean of three determinations*

Specificity studies:

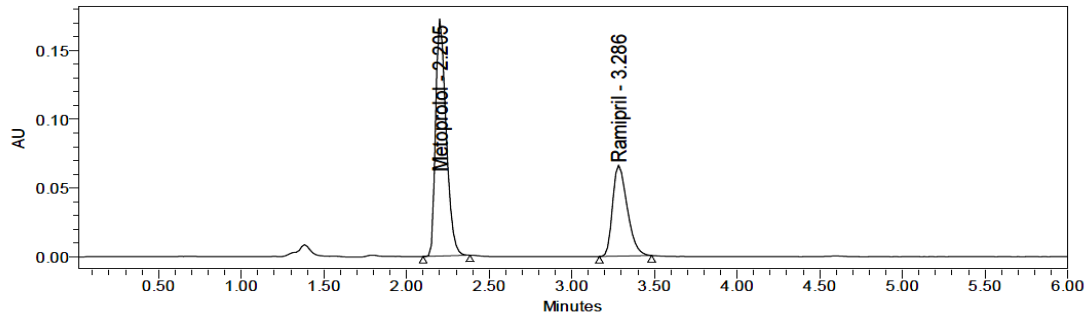

Fig.6: Typical chromatogram of standard

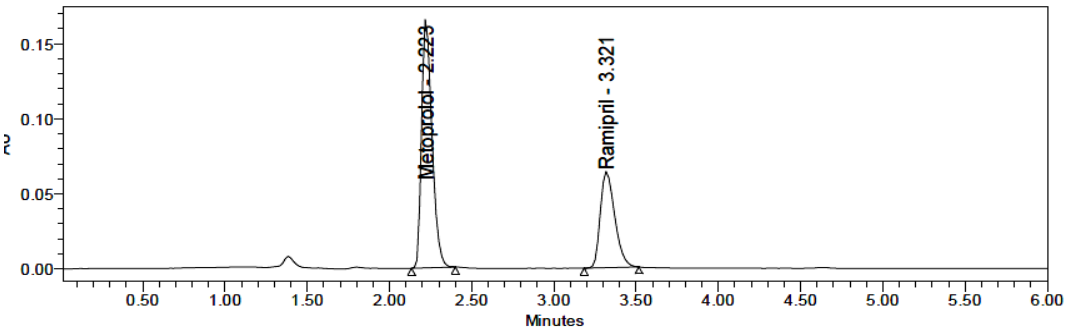

Fig.7: Typical chromatogram of sample

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

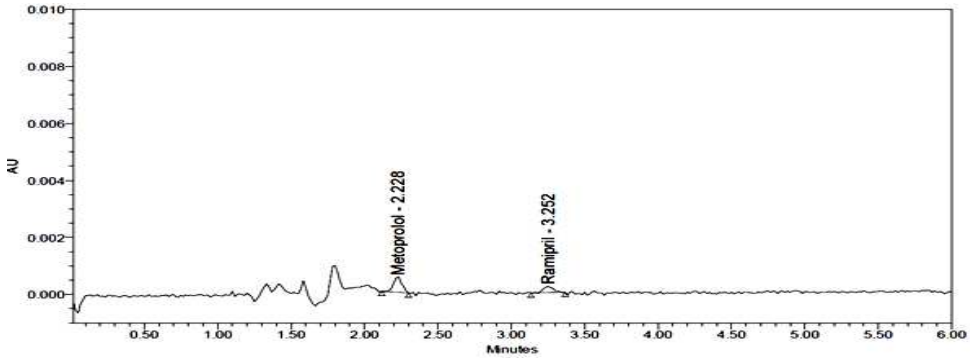

Fig.8: Typical chromatogram of Limit of detection (LOD) solution

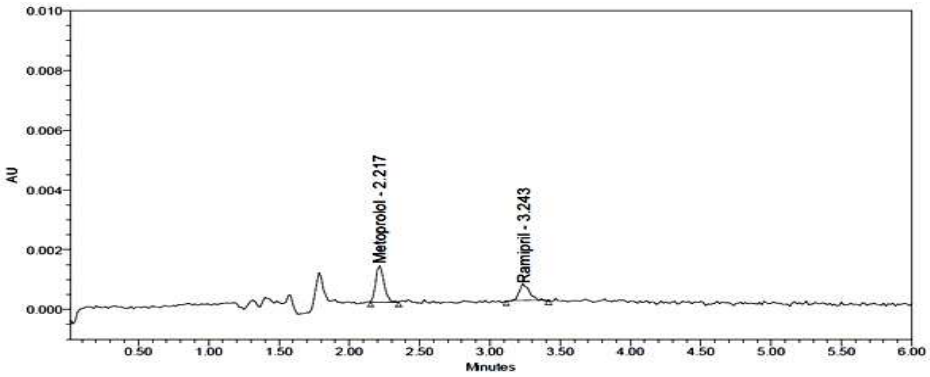

Fig.9: Typical chromatogram of Limit of Quantification (LOQ) solution

Robustness:

The developed method was robust with deliberate changes in variation of mobile organic phase composition, flow rate and temperature for both Metoprolol succinate and Ramipril respectively.

Table 5: Results of Robustness Study

S.No.	Parameter	Change Level	Metoprolol succinate		Ramipril	
			R_t (min)	Tailing factor	R_t (min)	Tailing factor
1.	Flow rate (±0.2 ml/min)	0.8	2.424	1.28	3.548	1.22
		1.2	2.057	1.31	3.104	1.27
2.	Mobile organic phase composition (±10%v/v/v)	33:11:54	2.439	1.22	3.598	1.34
		35:9:56	2.364	1.26	3.388	1.19
3.	Temperature (±5°C)	25 ^{0}C	2.257	1.34	3.291	1.28
		35 ^{0}C	2.209	1.30	3.124	1.24

Forced degradation studies:

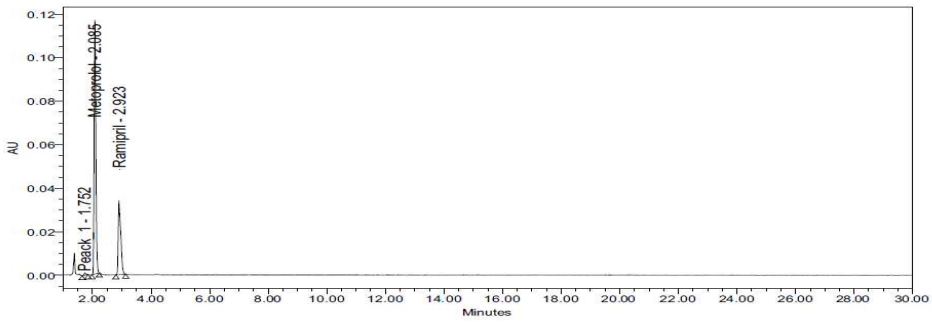

Fig.10: Chromatogram of Acid hydrolysis

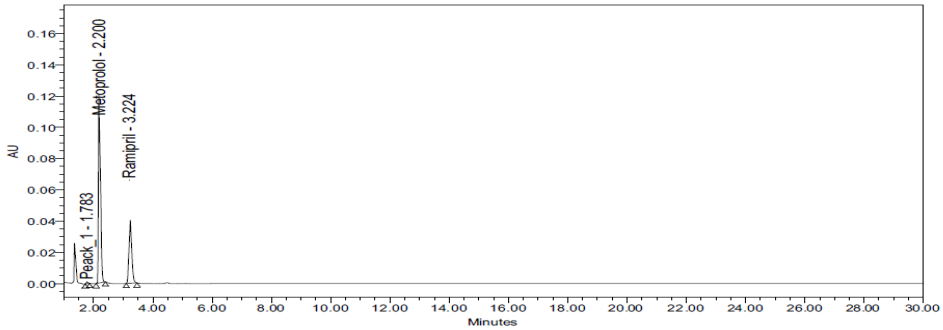

Fig.11: Chromatogram of Base hydrolysis

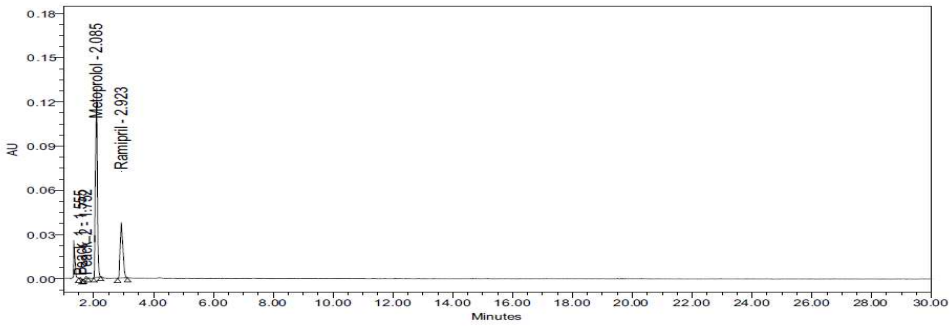

Fig.12: Chromatogram of Oxidation (peroxide) degradation

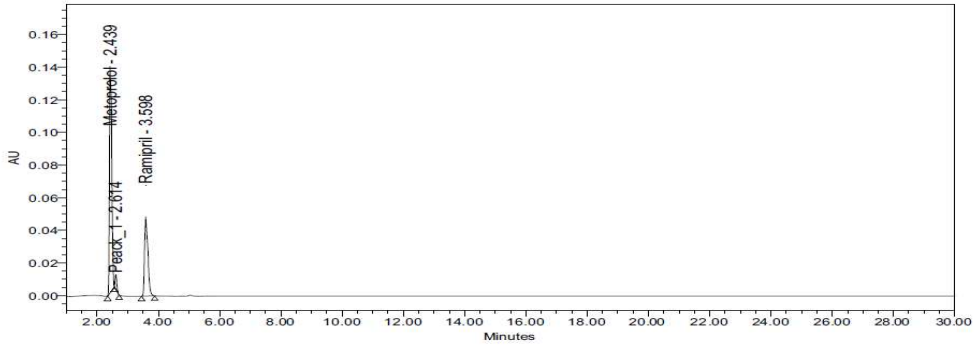

Fig.13: Chromatogram of thermal degradation

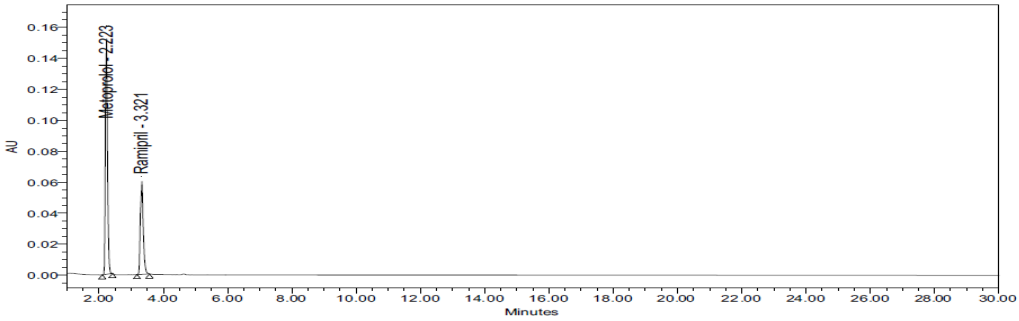

Fig.14: Chromatogram of UV Exposure

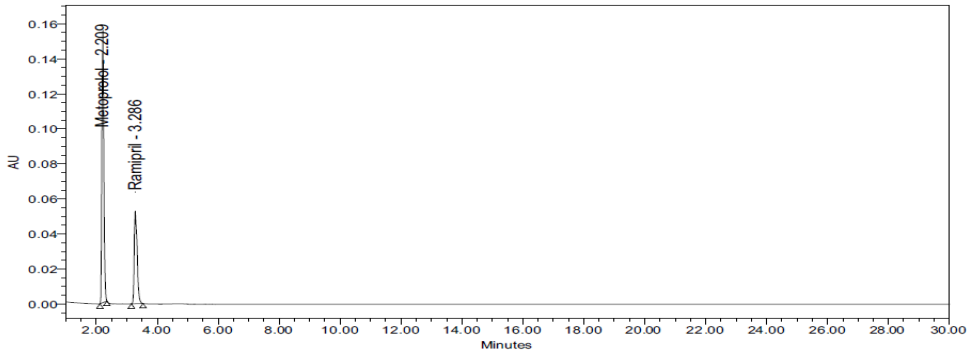

Fig.15: Chromatogram of Neutral degradation

Table 6: Degradation Study of Metoprolol succinate

Stress condition	% Assay	Purity angle	Purity threshold
Acid hydrolysis	92.20	0.125	0.331
Base hydrolysis	93.33	0.237	0.662
Peroxide degradation	94.01	0.247	0.264
Thermal degradation	95.05	0.112	0.308
UV Exposure	98.54	0.250	0.480
Neutral degradation	99.21	0.200	0.358

Table 7: Degradation Study of Ramipril

Stress condition	% Assay	Purity angle	Purity threshold
Acid hydrolysis	92.38	0.265	0.412
Base hydrolysis	93.15	0.124	0.456
Peroxide degradation	94.10	0.211	0.341
Thermal degradation	95.37	0.041	0.340
UV Exposure	98.13	0.256	0.680
Neutral degradation	99.38	0.170	0.487

CONCLUSION

From this study, it is concluded that the proposed Stability indicating RP-HPLC method was found to be simple, accurate, precise, rapid and useful for routine analysis of Metoprolol succinate and Ramipril in bulk & Pharmaceutical dosage form. The statistical parameters and recovery studies were carried out and reported. The obtained results were satisfactory as per ICH guidelines.

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