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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF METOPROLOL SUCCINATE AND RAMIPRIL IN TABLET DOSAGE FORM BY RP-HPLC

VIPUL PATEL[1], DR. SAURABH PANDYA[2]

1. L B Rao institute of pharmaceutical education and research, Khambhat- 388620, Anand, Gujarat, India.
2. Shree Swaminarayan Sanskar Pharmacy College, Zundal, Gujarat 382421.

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Abstract: A specific, accurate and precise RP-HPLC method has been developed and validated for simultaneous estimation of Metoprolol Succinate and Ramipril in Tablet dosage form. The separation was achieved by using Phenomenex C_{18} (250mm X 4.6mm), 5µm column and Acetonitrile: 1.4% Sodium Perchlorate Monohydrate (42:58, v/v) pH-2.7 set with Orthophosphoric acid as mobile phase with flow rate 1.0 mL/min and detection was carried out at 215 nm. The retention time of Metoprolol Succinate and Ramipril were found to be 4.217 min and 10.472 min respectively. The method was validated as per ICH guidelines. Over the range from 19-171 µg/mL for Metoprolol Succinate and 2-18 µg/mL for Ramipril with co-relation coefficients of 0.9982 and 0.9993, respectively. The developed method was simple, specific, and economic and can be used in routine analysis for simultaneous estimation of Metoprolol Succinate and Ramipril Tablet dosage form.

Keywords: Metoprolol Succinate, Ramipril, RP-HPLC method and Tablet dosage form.

Corresponding Author: MR. VIPUL PATEL

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INTRODUCTION

Metoprolol Succinate is Beta-adrenoceptor antagonist. It is chemically known as 1-[4-(2-methoxyethyl)phenoxy]-3-(propan-2-ylamino)propan-2-ol butanedioate (2:1) (Fig 1). It reduces the oxygen requirements of the heart by blocking catecholamine-induced increases in heart rate, in velocity and extent of myocardial contraction, and in blood pressure, thus making it useful in the long-term management of angina pectoris.

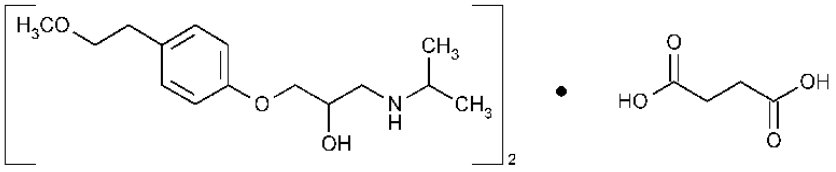

Figure 1: Structure of Metoprolol succinate

Ramipril is an Angiotensin Converting Enzyme Inhibitor. It is chemically known as (2S, 3aS, 6aS)-1-[(2S)-2-{[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino}propanoyl]-octahydrocyclopenta[b] pyrrole-2-carboxylic acid (Fig 2). It inhibits the actions of Angiotensin Converting Enzyme (ACE), thereby lowering the production of angiotensin II. 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 vessel. [1-7]

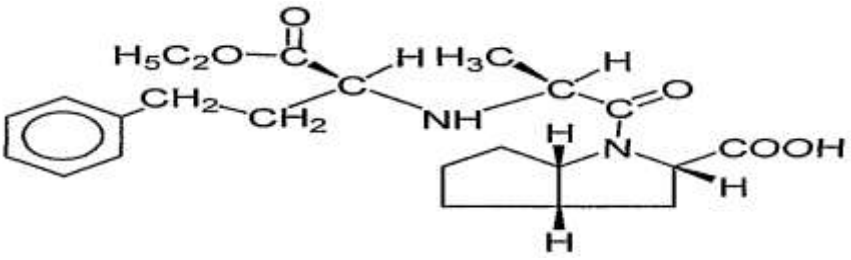

Figure 2: Structure of Ramipril

A combination of ACE inhibitors and beta blockers helps for the improvement in cardiac function after myocardial infarction.

A literature survey revealed that number of analytical methods are available for analysis of Metoprolol Succinate and Ramipril individually in pharmaceutical formulations as well as in combination with other drugs but no any single method is reported for the determination of combination of Metoprolol Succinate and Ramipril in tablet dosage form by RP-HPLC method [8-15]. Therefore, the rationale of the reported research was to develop accurate and precise

analytical method for simultaneous estimation of Metoprolol Succinate and Ramipril in their combined dosage form.

MATERIAL AND METHODS

Chemicals and reagents:

Metoprolol succinate (MES) and Ramipril (RAM) were procured as gratis sample from Sun Pharmaceutical Pvt. Ltd., (Baroda, India). Sodium perchlorate monohydrate (E. Merck, Mumbai, India), Ortho phosphoric acid (Spectrochem), Methanol, Acetonitrile (E. Merck, Mumbai, India) were used for the preparation of mobile phase. Tablet formulation (Embeta-R 2.5 mg, Intas Pharmaceuticals Ltd.,) containing labelled amount of 23.75 mg of Metoprolol succinate and 2.5 mg of Ramipril were purchased from local market.

Chromatographic condition:

A reversed phase Phenomenex C_{18} (250mm X 4.6mm), 5µm column equilibrated with mobile phase comprising of Acetonitrile: 1.4% Sodium Perchlorate Monohydrate (42:58, v/v) pH-2.7 set with Orthophosphoric acid was used. Mobile phase flow rate was maintained at 1 mL/min and effluent was monitored at 215 nm. A 20 µL of sample was injected, and the total run time was 15 min. All the chromatographic separations were carried out at controlled room temperature (25 ± 2 ^{o}C).

Preparation of mobile phase and stock solution:

420 mL of Acetonitrile and 580 mL of 1.4 % w/v solution of Sodium Perchlorate Monohydrate were taken and pH 2.7 was adjusted with Orthophosphoric acid. It was mixed properly. Then mobile phase was filtered through 0.45 µm filter paper with vacuum filtration assembly. Mobile Phase was transferred to mobile phase bottle and sonicated for 15 min prior to use. Acetonitrile and water in the ratio of 40:60 was used as diluent.

Accurately weighed quantity of 95 mg of MES and 10 mg of RAM were transferred into 100 mL volumetric flask, dissolved and diluted up to mark with diluent, it gives a stock solution having strength of 950 µg/mL of MES and 100 µg/mL of RAM. Aliquots from the stock solutions were appropriately diluted with diluent to obtain working standards of 95 µg/mL of MES and 10 µg/mL of RAM.

Calibration curves for MES and RAM:

Appropriate aliquots of working standard solution were taken in different 10 mL volumetric flasks. The volume was made up to the mark with diluent to obtain final concentrations of 19, 57, 95, 133, 171µg/mL of MES and 2, 6, 10, 14, 18 µg/mL of RAM. 20 µL of each solution was

injected and chromatograms were recorded. Calibration curves were constructed by plotting peak area versus concentrations of both drugs and regression equations were computed.

Analysis of Marketed Formulation:

Twenty tablets were weighed accurately and finely powdered. Tablet powder equivalent to 95 mg of MES and 10 mg of RAM was taken in 100 mL volumetric flask, 50 mL of diluent was added to the above flask, sonicated it for 15 minutes and volume was made up to the mark with diluent**.** The sample solution was filtered through 0.2 µm Nylon membrane filter paper with the use of syringe filter. First few mL was discarded.

Appropriate volume of the filtrate was transferred to a 10 mL volumetric flask and the volume was made up to the mark with diluent to obtain a solution containing 95 µg/mL of MES and 10 µg/mL of RAM. The solution was sonicated for 5 min. It was injected as per the above chromatographic conditions and peak area was recorded. The quantifications were carried out by keeping these values to the linear equation of calibration curve.

Method validation:

The method was validated according to the ICH guidelines with respect to linearity, accuracy, specificity, precision, detection limit, quantitation limit and robustness. [16]

Linearity:

The linearity was obtained at 5 concentration levels of MES and RAM standard solution. The solutions were injected into liquid chromatographic system using above chromatographic condition. The linearity was evaluated by least square regression method.

Accuracy:

The accuracy of the method was determined by calculating recoveries of MES and RAM by method of standard additions. Known amounts of standard samples of MES and RAM at 80%, 100% and 120% levels were added to the preanalysed tablet sample (57 µg/mL MES and 6 µg/mL RAM).and the amount of MES and RAM were estimated by measuring the peak area and by fitting these values to the straight-line equation of calibration curve.

Specificity:

The specificity study was carried out to check the interference from the excipients used in the formulation by injecting blank, standard and sample solution prepared as per method and injected into HPLC system, The chromatogram showed peaks for the drug without any interfering peak.

Precision:

The precision of the method was checked by repeatedly injecting (n = 6) standard solution of 95 µg/mL MES and 10 µg/mL RAM under the same chromatographic condition and peak area was measured. The results are reported in terms of % relative standard deviation. The intra-day and inter-day precision study of MES and RAM were carried out by estimating the corresponding responses 3 times on the same day and on 3 different days (first, second and third day) for 3 different concentrations of MES (57, 95 and 133 µg/mL) and of RAM (6, 10 and 14 µg/mL). The results are reported in terms of % relative standard deviation (RSD).

Limit of detection and quantification:

The detection limit is defined as the lowest concentration of an analyte that can reliably be differentiated from background levels. Limit of quantification of an individual analytical procedure is the lowest amount of analyte that can be quantitatively determined with suitable precision and accuracy. LOD and LOQ were calculated using following equation as per ICH guidelines. LOD = 3.3 ×σ /S and LOQ = 10 ×σ /S, where σ is the standard deviation of y-intercepts of regression lines and S is the slope of the calibration curve.

Robustness

Robustness of the method was studied by deliberately changing the experimental conditions like flow rate, wavelength and percentage of organic phase. The system suitability parameters were calculated.

RESULTS AND DISCUSSION

Optimization of mobile phase was carried out by taking different proportions of aqueous and organic phases to obtain rapid, simple assay method for simultaneous determination of Metoprolol succinate and Ramipril with appropriate run time, asymmetric factor and theoretical plates. Mobile phase consisting of Acetonitrile: 1.4% Sodium Perchlorate Monohydrate (42:58, v/v) pH-2.7 set with Orthophosphoric acid was found to be satisfactory which gave symmetric and sharp peak at 1 mL/min. For quantitative analytical purpose wavelength was set at 215 nm. Under the chosen experimental conditions, the liquid chromatogram of MES and RAM showed well separated peaks of the drugs around retention time of 4.217 min and 10.472 min, respectively.

Linearity:

The calibration curve for MES and RAM were obtained by plotting the peak area versus concentration. It was found to be linear in the range of 19-171 µg/mL for MES and 2-18 µg/mL

for RAM. Peak area and concentrations were subjected to least square regression analysis to calculate calibration equation and correlation coefficient (Fig 3). The data of the calibration curve are shown in Table 1. The correlation coefficient (r^2) was found to be 0.9982 and 0.9993 for MES and RAM, respectively.

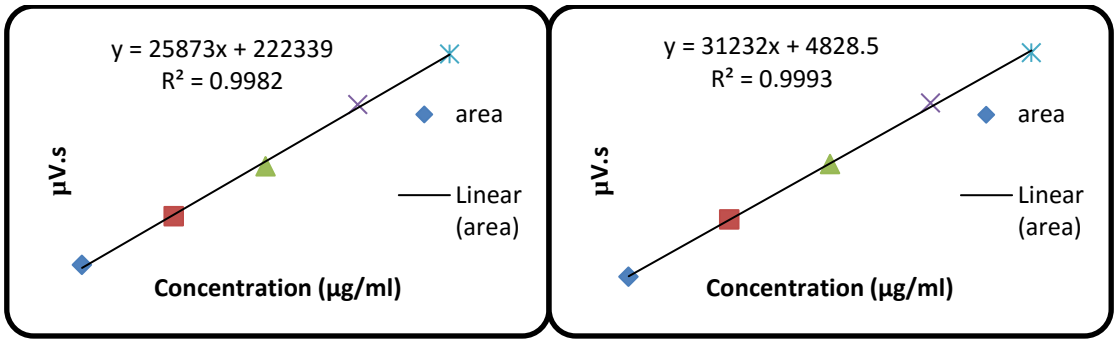

Figure 3a: Calibration curve of MES **Figure 3b: Calibration curve of RAM**

Accuracy:

The accuracy of the method was determined by calculating recoveries of MES and RAM by method of standard addition. The recoveries found to be 98.73 - 100.44% for MES and of 99.07 – 101.67% for RAM (Table 2). The high values indicate that the method is accurate.

Specificity:

The chromatogram (Fig 4) showed that there was no interference from excipients at the retention tome of MES and RAM.

Precision:

Instrument precision was determined by performing injection repeatability test and the % RSD was found to be 0.86% for MES and 1.39% for RAM. The % RSD for intra-day was found to be 0.24 – 0.53 % for MES and 0.23 – 1.01 % for RAM. The % RSD for inter-day precision was found to be 0.66 – 1.41 % for MES and 0.64 – 1.17 % for RAM (Table 2). The low %RSD values indicate that the method is precise.

Limit of detection and limit of quantification:

The detection limit and quantitation limit was found to be 0.84 µg/mL and 2.55 µg/mL for MES and 0.28 µg/mL and 0.85 µg/mL for RAM respectively (Table 2). The above data shows that a nanogram quantity of the drug can be accurately and precisely determined.

Robustness:

The method was found to be robust, as small but deliberate changes in the method parameters have no detrimental effect on the method performance as shown in table 4. The low value of relative standard deviation was indicating that the method was robust.

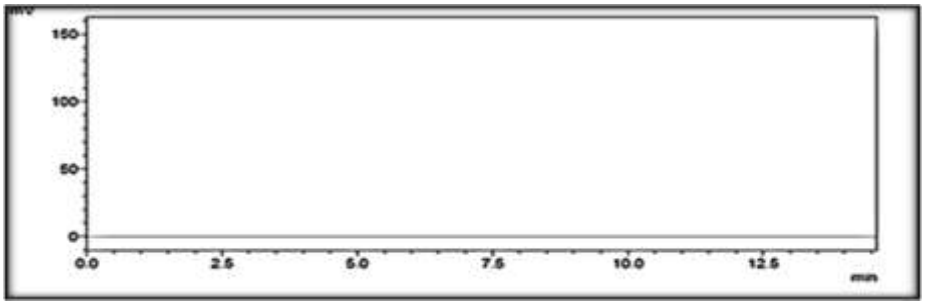

Figure 4a: Chromatogram of blank

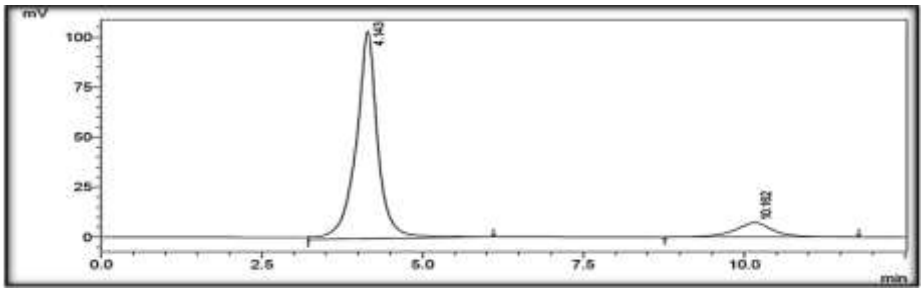

Figure 4b: Chromatogram of standard solution

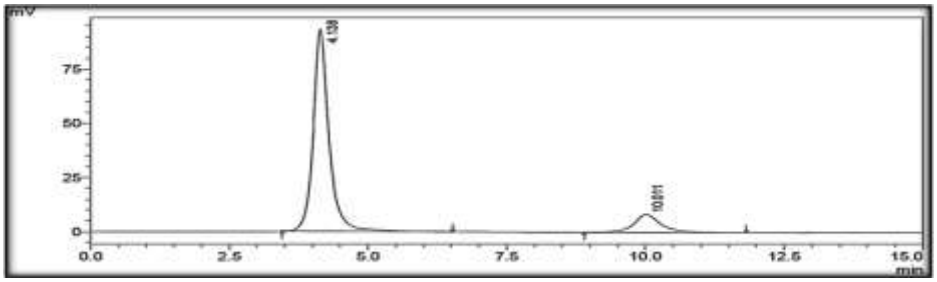

Figure 4c: Chromatogram of sample solution

Analysis of marketed formulation:

The proposed liquid chromatographic method was applied to the determination of MES and RAM in Tablet formulation. The % recovery for MES and RAM was found to 99.16 ± 1.24 and 99.60 ± 2.88, (mean value ± standard deviation) respectively of three determinations which was comparable with the corresponding labeled amounts.

CONCLUSION

A rapid, simple, reproducible and specific RP-HPLC method was developed for simultaneous estimation of Metoprolol succinate and Ramipril in tablet dosage form. The developed method was validated as per ICH guideline for linearity, accuracy, specificity, precision and robustness and was found to be linear, accurate, precise and repeatable. The developed method could be successfully used to analyze the drugs in marketed formulation and also the routine analysis of Metoprolol Succinate and Ramipril.

ACKNOWLEDGEMENT:

The authors are grateful to Sun Pharmaceuticals Ltd., Baroda for providing gift sample of Metoprolol Succinate and Ramipril.

Table 1: **Regression analysis of the calibration curve for the assay of Metoprolol succinate (MES) and Ramipril (RAM)**

Parameters	MES	RAM
Linearity range (µg/mL)	19 – 171	2-18
Slope	60033.4	8045.64
Standard deviation of intercept	15319.56	682.4588
Correlation coefficient	0.9982	0.9993

Table 2: Summary of validation parameters

Parameters	MES	RAM
Detection limit (µg/mL)	0.84	0.28
Quantitation limit (µg/mL)	2.55	0.85
Accuracy (%)	98.73-100.44 %	99.07-101.67 %
Precision (RSD[a], %)		
Intra-day precision (n=3)	0.24-0.53 %	0.23-1.01 %
Inter-day precision (n=3)	0.66-1.41 %	0.64-1.17 %
Instrument precision (RSD[a] , %)	0.86 %	1.39 %

[a]RSD indicates relative standard deviation

Table 3: System suitability test parameters for the proposed method

System suitability parameters	MES	RAM
Retention time (min)	4.371±0.036	10.344±0.065
Theoretical plates/ meter	2265.3±67.94	2952.2±128.22
Tailing factor	0.904±0.047	0.880±0.067

Table 4: Data derived from robustness for proposed method

Sr no	Parameter	%RSD	
		MES	RAM
1	Flow rate (± 0.2 mL/min)	0.84-1.35	0.81-1.58
2	Wavelength (± 2 nm)	0.81-1.51	0.88-0.97
3	Mobile phase (± 5% of organic phase)	0.57-1.56	0.58-1.64

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