Research Article

Development and Validation of New Analytical Methods for Simultaneous Estimation of Ramipril and Metoprolol succinate by HPLC method in Combined Tablet Dosage Form

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ABSTRACT

In the Present simple and sensitive HPLC method have been developed for the quantitative estimation of Metoprolol Succinate and Ramipril in combination using bulk and pharmaceutical dosage forms. Normal Phase HPLC method in which the determination of Metoprolol Succinate and Ramipril was carried on a normal phase C 18 column using mobile phase consisting of Acetonitrile and Methanol in the ratio 70:30. The mobile phase was pumped at a rate of 1ml/min and detection was carried out at 216.5 nm. The Linearity was found to be in range of 5-50µg/ml and 0.5-5µg/ml with regression coefficient (r^2=0.999 and r^2=0.998) for Metoprolol Succinate and Ramipril respectively. The peaks obtained were sharp having baseline separation with a retention time of 3.78 and 1.65min for Metoprolol Succinate and Ramipril respectively. The method was validated statistically.

Keywords: Metoprolol Succinate, Ramipril, Normal phase HPLC Method.

INTRODUCTION

The development of the pharmaceuticals brought a revolution in human health. These pharmaceuticals would serve their intent only if they are free from impurities and are administered in an appropriate amount. Analytical methods are required to define the quality of products and to retain their qualification in world markets. The drug substance and drug product composition during all phases of pharmaceutical development are characterised by analytical methods. Early phase methods must support changes in synthetic routes and dosage form and elucidate the structures and levels of impurities. In later phases, goals change to the development of rapid and robust methods for release and stability evaluation.Therefore, the need to develop new analytical methods for assurance of quality, safety and efficacy of drugs and pharmaceuticals is quite important because of their use not only as health care products but also life saving substances.[1]

.High performance liquid chromatography (HPLC) is the fastest growing analytical technique for the analysis of drugs. Its simplicity, high specificity, and wide range of sensitivity make it ideal for the analysis of many drugs in both dosage forms andbiological fluids[6].The technique is based on the same modes of separation as classical column chromatography, i.e. adsorption, partition, ion exchange and gel permeation,but it differs from column chromatography in that the mobile phase is pumped through the packed column under high pressure[2]. The HPLC system consists of: [3] solvent reservoir system, pump, sample inlet system, column, detector, and recording unit.

Method validation is the process of demonstrating that analytical procedures are suitable for their intended use and that they support the identity, quality, purity and potency of the drug substances and drug products. The various validation parameters are[4] Specificity, linearity, range, accuracy, precision, limit of detection, limit of quantification, robustness, system suitability should be carried out.

Metoprolol Succinate[5]: Metoprolol succinate, is a $beta_1$ selective (cardioselective) adrenoreceptor blocking agent, for oral administration,available as extended-release tablets.The tablets comprise a multiple unit system containing metoprolol succinate in a multitude of controlled release pellets.

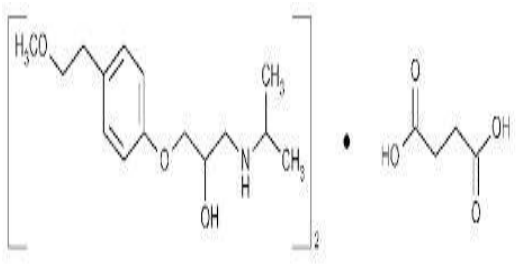

Fig. 1: Chemical Structure of Metoprolol Succinate

Brief Introduction[6]: IUPAC name: (±)1+ (isopropylamino)-3-[p-(2-methoxyethyl)phenoxy]-2propanol succinate, Empirical formula: $(C_{15}H_{25}NO_3)_2$ $C_4H_6O_4$, Molecular weight: 652.81, Bioavailability: 40-50%, Metabolism: 90%, Solubility: Soluble in water, freely soluble in ethanol, Soluble in Methanol, Halflife: 5-7 hours. The mechanism of the antihypertensive effects of beta-blocking agents has not been elucidated. However, several possible mechanisms have been proposed :(1) competitive antagonism of catecholamine's at peripheral (especially cardiac) adrenergic neuron sites, leading to decreased cardiac output; (2) A central effect leading to reduced sympathetic outflow to the periphery (3) suppression of renin activity.

Ramipril[7]:

Ramipril is a prodrug belonging to the angiotensin-converting enzyme (ACE) inhibitor class of medications. It is metabolized to ramiprilat in the liver and, to a lesser extent, kidneys. Ramiprilat is a potent, competitive inhibitor of ACE, the enzyme responsible for the conversion of angiotensin I (ATI) to angiotensin II (ATII). ATII regulates blood pressure and is a key component of the renin-angiotensin-aldosterone system (RAAS).

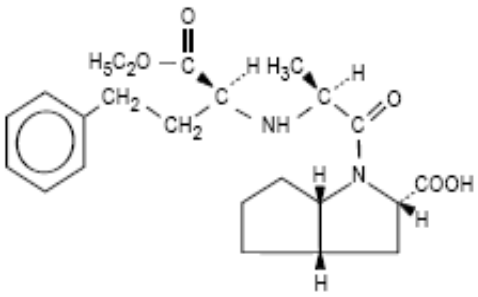

Fig. 2: Chemical Structure of Ramipril

Brief Introduction[8, 9]: IUPAC name: (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[d]pyrrole-2-carboxylic acid, Molecular weight: 416.511, Halflife: 2 to 4 hours. Ramipril causes an increase in plasma renin activity likely due to a loss of feedback inhibition mediated by ATII on the release of renin and/or stimulation of reflex mechanisms via baroreceptors.

MATERIALS AND METHODS

Reagents and chemicals used

- ✓ Acetonitrile**,** HPLC grade
- ✓ Methanol, HPLC grade
- ✓ Metoprolol Succinate
- ✓ Ramipril

Table 1: Chromatographic conditions

Instrument	High Performance Liquid Chromatography LC JASCO
Column	C18 Column
Injector	Manual injector
Injection Volume	20µl

Preparation of standard stock solution: Accurately weighed 50 mg of Metoprolol Succinate and 5mg of Ramipril was transferred into a clean, dry 10 ml volumetric flask and dissolved with sufficient volume of mobile phase respectively. The volume was made up to 100 ml with mobile phase to get concentration of 500 µg/ml for Metoprolol Succinate and made upto 100ml for Ramipril to get of 50 µg/ml.

Assay determination of Metoprolol Succinate and Ramipril

Preparation of sample stock solution

Twenty tablets containing 50 mg of Metoprolol Succinate and 5mg Ramipril were weighed and finely powered. Acccurately weighed 125.1mg of powder which is equivalent to 50mg of Metoprolol Succinate and 5mg of Ramipril was transferred into a clean, dry 100 ml volumetric flask. The powder was first dissolved in sufficient volume of mobile phase by sonication. The resulting suspension was then filtered through whatmann filter.

The volume of filtrate was made up to 100 ml with mobile phase to get the concentration 500 µg/ml and 50 µg/ml.

Working sample solutions

From the above stock 1.0, 1.5, and 2.0ml of the stock solution was transferred into three different 10 ml volumetric flasks and volume made up to 10 ml with mobile phase.

Determination

Volume of 20 µl of each working standard solutions were injected with flow rate of 1

ml/min. The chromatograms were recorded. With substitution of peak area in the linearity equation y = 1811.x+2264 for Metoprolol Succinate, y = 1274.x- 144.6 for Ramipril the concentration of drug in sample solution was determined and the amount present in marketed dosage form reported.

Validation of HPLC method for the Assay of Metoprolol Succinate and Ramipril

Validation of an analytical method is process to establish that the performance characteristics of the developed method meet the requirements of the intended analytical application. Typical analytical parameters used in assay validation according to ICH guidelines are: Specificity, linearity, range, accuracy, precision, limit of detection, limit of quantification, robustness, system suitability.

1. Specificity

The specificity was determined by injecting the placebo into the chromatograph. The placebo here represents the possible excipients of the formulation. The placebo sample solution was prepared with commonly used excipients for tablet formulation like microcrystalline cellulose; cellulose acetate phthalate and starch. Accurately weighed 40mg microcrystalline cellulose, 40mg Hydroxypropyl Methyl cellulose and 10 mg starch were mixed with mobile phase in 100 ml volumetric flask. The resulting suspension was filtered through whatmann filter paper. Working placebo sample solution was prepared by diluting 0.5 ml of filtrate up to 10 ml with mobile phaseand the chromatogram was recorded.

2. Linearity and Range

Accurately weighed each 50 mg of Metoprolol Succinate and 5mg Ramipril were transferred into a clean and dry 100 ml volumetric flask and dissolved with sufficient volume of mobile phase. The volume was made up to 100ml with mobile phase to get concentration of 500 µg/ml and 50 µg/ml of Metoprolol Succinate and Ramipril respectively.

Working standard solution and Determination

Aliquots from standard solution were withdrawn in the volumes of 0.5 ml to 5 ml and transferred into different 10 ml volumetric flasks. The volumes were made up with the mobile phase to get concentrations ranging from 5–50 µg/ml of Metoprolol Succinate and 0.5-5µg/ml of Ramipril. Volume of 20µl of each working standard solutions were injected with flow rate of 1 ml/min. The chromatograms were recorded.

3. Accuracy

Preparation of standard stock solution

Accurately weighed each 50 mg of Metoprolol Succinate and Ramipril were transferred into a clean and dry 100 ml volumetric flask and dissolved with sufficient volume of mobile phase. The volume was made up to 100ml with mobile phase to get concentration of 500 µg/ml and 50 µg/ml of Metoprolol Succinate and Ramipril respectively.

Preparation of sample stock solution

Twenty tablets containing 50 mg of Metoprolol Succinate and 5mg of Ramipril were weighed and finely powered. Accurately weighed 125.1mg of powder which is equivalent to 50mg of Metoprolol Succinate and 5mg of Ramipril, was transferred into a clean, dry 100 ml volumetric flask. The powder was first dissolved in sufficient volume of mobile phase by sonication. The resulting suspension was then filtered through whatmann filter. The volume of filtrate was made up to 100 ml with mobile phase to get the concentration 500 µg/ml and 50 µg/ml.

Preparation of standard and sample mixture

Level I (80%)

Volume of 1 ml sample stock solution and 0.8ml standard stock solution was transferred into 10 ml volumetric flask and volume made up with mobile phase.

Level II (100%)

Volume of 1 ml sample stock solution and 1 ml standard stock solution was transferred into 10 ml volumetric flask and volume made up with mobile phase.

Level III (120%)

Volume of 1 ml sample stock solution and 1.2 ml standard stock solution was transferred into 10 ml volumetric flask and volume made up with mobile phase.

Determination

Solutions of each level I, II and III of 20 µwere injected into the chromatograph. The percentage recovery of standard was calculated from the peak area.

4. Precision

Preparation of standard mixture solution

Accurately weighed 50 mg of Metoprolol Succinate and 5 mg of Ramipril was transferred into clean, dry 100 ml volumetric flask and dissolved in sufficient volume of mobile phase. The volume was made up to 100 ml with mobile phase to get the

concentration of 500 µg/ml of Metoprolol Succinate and 50 µg/ml of Ramipril.

Working solution
Standard mixture solution of volume 1.0 ml was transferred in to 10 ml volumetric flask and volume adjusted with mobile phase to get the concentration 50 µg/ml of Metoprolol Succinate and 5 µg/ml of Ramipril.

Repeatability
Repeatability expresses the precision under the same operating conditions over a short interval of time. Replicate injections of one concentration were injected into the chromatograph under the same operating conditions over a short interval of time to determine repeatability. The standard deviation and relative standard deviation was determined.

A. Method Precision
Successive six injections of 20 µl of working standard solution (Mixture of 50 µg/ml Metoprolol Succinate and 5 µg/ml of Ramipril) were injected and chromatograms recorded. The % relative standard deviation was calculated for the concentration of drug in replicates.

B. System Precision
Successive six injections of 20 µl of working standard solution (Mixture of 50 µg/ml Metoprolol Succinate and 5 µg/ml of Ramipril) were injected and chromatograms recorded. The % relative standard deviation was calculated for the peak area of replicates.

Inter-day Precision
Successive six injections of 20 µl of working standard solutions (Mixture of 50 µg/ml Metoprolol Succinate and 5µg/ml of Ramipril) were injected on different days and chromatograms were recorded. The % relative standard deviation was calculated for concentration of drug in replicates. The standard deviation and relative standard deviation were calculated from the statistical formula.

Intra-day Precision
Successive six injections of 20 µl of working standard solution (Mixture of 50 µg/ml Metoprolol Succinate and 5µg/ml of Ramipril) were injected at different intervals in the same day and chromatograms were recorded. The % relative standard deviation was calculated for concentration of drug in replicates.

5. Limit of Detection (LOD)
LOD calculated by using the values of slopes and intercepts of the calibration curves for both the drugs.

Preparation of standard stock solution
Accurately weighed 50 mg of Metoprolol Succinate and 5mg of Ramipril was transferred into a clean, dry 10 ml volumetric flask and dissolved with sufficient volume of mobile phase respectively. The volume was made up to 100 ml with mobile phase to get concentration of 500 µg/ml for Metoprolol Succinate and made upto 100ml for Ramipril to get of 50 µg/ml.

Working standard stock solution
volumes of 0.9 to 0.1 ml were transferred into different 100 ml volumetric flasks. The volumes were made up with the mobile phase to get the concentration of 0.09 to0.01 µg/ml for Metoprolol succinate.
Volumes of 0.9 to 0.1 ml were transferred into different 100 ml volumetric flasks. The volumes were made up with the mobile phase to get the concentration of 0.045 to 0.005 µg/ml for Ramipril.

6. Robustness
Robustness was performed by changing the chromatographic conditions. The flow rate of the mobile phase was changed and composition of the buffer in mobile phase was changed.

7. Ruggedness
Ruggedness of the method was performed by two different analysts using same experimental and environmental conditions.

RESULTS AND DISCUSSION

1. Specificity
This parameter was performed to assess and ensure that the impurities, degraded products and diluents do not affect the samples analyzed. As no peaks were found at retention time of 3.78 min and 1.65 min, the proposed method was specific for detection of Metoprolol Succinate and Ramipril.

2. Linearity and Range
The linearity in response for Metoprolol Succinate and Ramipril was observed in the concentration range of 5 to 50 µg/ml and 0.5-5 µg/ml respectively for both the drugs, with percentage curve fittings found to be well within the limits of acceptance criteria.

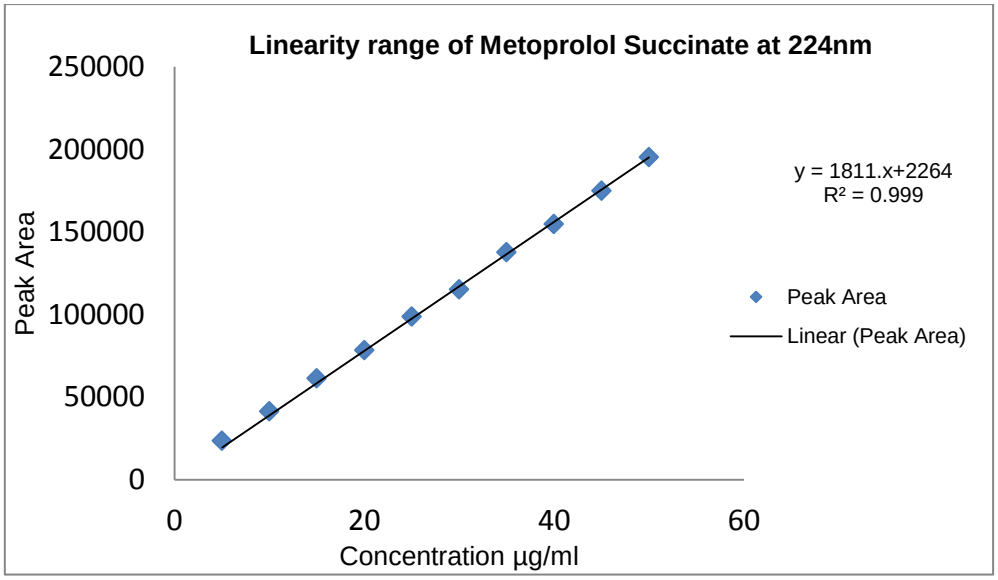

Fig. 3: Linearity Range Graph of Metoprolol Succinate

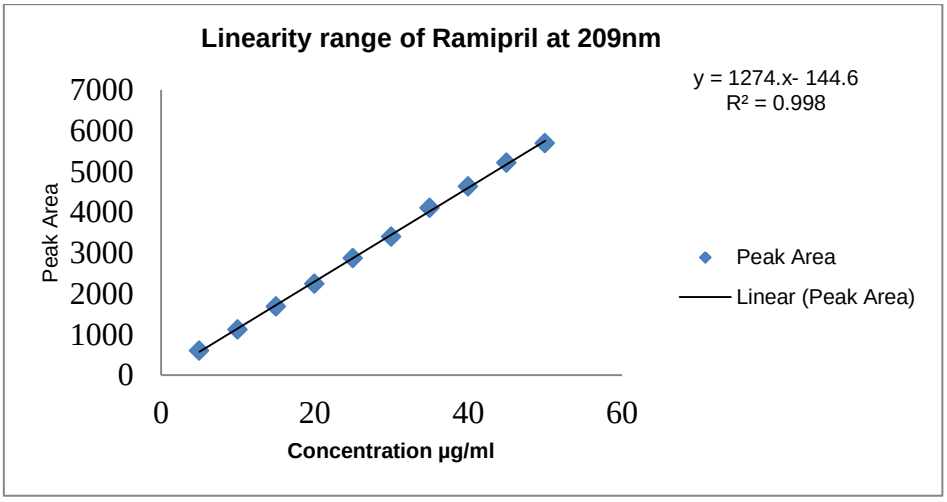

Fig 4: Linearity Range Graph of Ramipril

Table 2: Linearity Data of Metoprolol Succinate and Ramipril

Parameters	Metoprolol Succinate	Ramipril	Acceptance Criteria
Linearity Range	5-50µg/ml	0.5-5 µg/ml	-
Regression Equation	y = 1811.x+2264	y = 1274.x- 144.6	-
Correlation Coeficient	0.999	0.998	0.99
Percentage curve Fitting	99.9%	99.8%	99%
Intercept	2264	-144.6	-
Slope	1811	1274	-

Assay of marketed formulation

The content of Metoprolol Succinate and Ramipril found in the tablets by the proposed method are shown in Table.

Accuracy: The method was found to be accurate as the % recovery ranged from 97.19 to 98.99% for Metoprolol Succinate and 94.98 to 97.87 % for Ramipril which was well within the acceptance range of 90 to 110%.

Precision

Method precision: The % RSD for method precision was found to be 0.078 and 0.274 for Metoprolol Succinate and Ramipril respectively. As the results were within the acceptance limits i.e. < 2.0%, so the method provides good precision.

System precision: The % RSD for system precision was found to be 0.0125and 0.1301for Metoprolol Succinate and Ramipril respectively. As the results were within the acceptance limits i.e. < 2.0%, so the system provides good precision.

Inter-day and Intra-day Precision: The % RSD for method precision was found to be 0.011 and 0.59 for intraday precision; 0.031 and 0.589 for inter day precision of Metoprolol Succinate and Ramipril respectively. As the results were within the acceptance limits so both method as well as the system provides good precision.

Chromatograms for Assay of Metoprolo Succinate and Ramipril

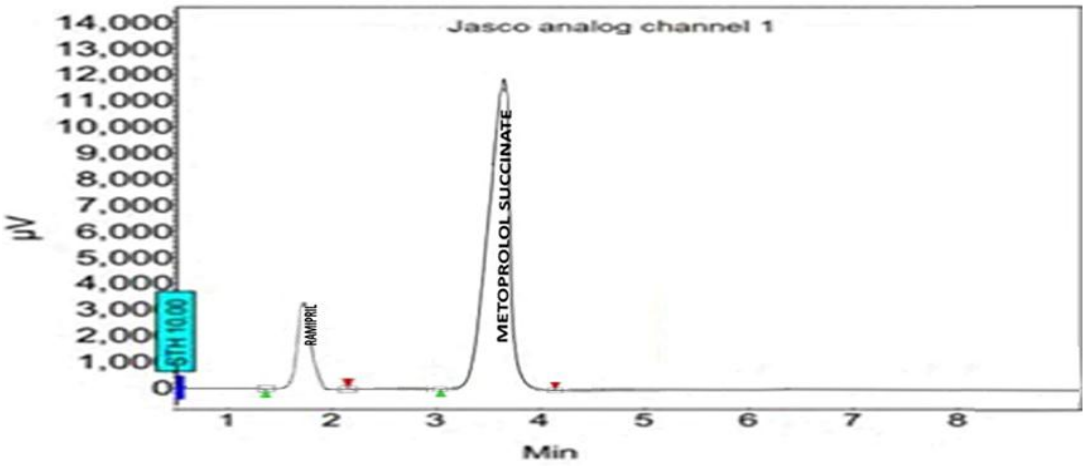

Fig. 5: Chromatogram for Metoprolol Succinate (50µg/ml) and Ramipril (5µg/ml)

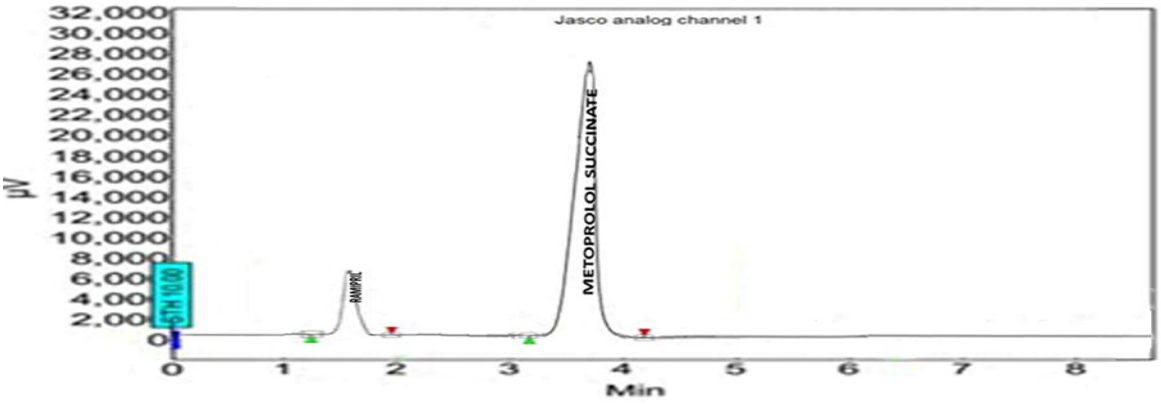

Fig. 6: Chromatogram for Metoprolol Succinate (75µg/ml) and Ramipril (7.5µg/ml)

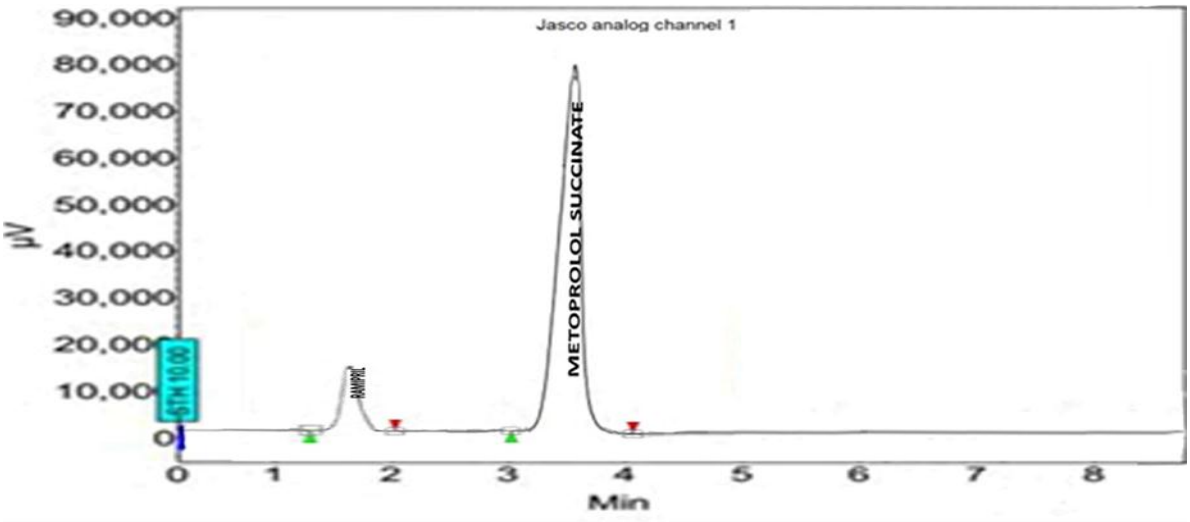

Fig. 7: Chromatogram for Metoprolol Succinate (100µg/ml) and Ramipril (10µg/ml)

Table 3: Assay Report of Sample Metoprolol Succinate and Ramipril

Volume of stock Solution (ml)	Metoprolol Succinate			Ramipril		
	Peak area	Concentration (µg/ml)	Amount per tablet (mg)	Peak area	Concentration (µg/ml)	Amount per tablet (mg)
1.0	90360	49.89	49.89	6300	4.94	4.94
1.5	135612	74.88	49.92	9542	7.48	4.98
2.0	180945	99.91	49.95	12693	9.96	4.98
Average			49.92	Average		4.96

Table 4: Recovery Report of Metoprolol Succinate

Level	Standard Conc. (µg/ml)	Sample Conc. (µg/ml)	Peak area	Total Conc. (µg/ml)	Amount of Standard Recovered	%Recovery
80%	40	49.92	162000	89.453	39.533	98.83
100%	50	49.92	180045	99.417	49.497	98.99
120%	60	49.92	196014	108.23	58.315	97.19

Table 5: Recovery Report of Ramipril

Level	Standard Conc. (µg/ml)	Sample Conc. (µg/ml)	Peak area	Total Conc. (µg/ml)	Amount of Standard Recovered	%Recovery
80%	4	4.96	11307	8.875	3.91	97.87
100%	5	4.96	12435	9.76	4.8	96.01
120%	6	4.96	13580	10.65	5.699	94.98

Table 6: Method Precision Data of Metoprolol Succinate and Ramipril

Replicates	Metoprolol Succinate		Ramipril	
	Peak area	Concentration µg/ml	Peak area	Concentration µg/ml
1	90366	49.89	6312	4.95
2	90432	49.93	6332	4.97
3	90227	49.82	6283	4.93
4	90314	49.86	6219	4.88
5	90262	49.84	6351	4.98
6	90407	49.92	6298	4.94
Mean	**90334.66**	**49.87**	**6299.16**	**4.94**
Standard deviation	**72.757**	**0.0385**	**16.93615**	**0.01354**
%RSD	**0.080**	**0.078**	**0.268**	**0.274**

Table 7: System precision data for Metoprolol Succinate and Ramipril

Replicates	Metoprolol Succinate	Ramipril
	Peak area	Peak area
1	90362	6299
2	90324	6310
3	90336	6286
4	90349	6212
5	90354	6356
6	90297	6318
Mean	**90337**	**6296.83**
Standard deviation	**11.33578**	**8.192985**
%RSD	**0.0125**	**0.1301**

Table 8: Intra-day Precision Data of Metoprolol Succinate and Ramipril

Replicates	Time interval	Metoprolol Succinate		Ramipril	
		Peak area	Concentration (µg/ml)	Peak area	Concentration (µg/ml)
1	10.45 am	90328	49.87	6325	4.96
2	11.45 am	90279	49.85	6212	4.87
3	12.45 pm	90308	49.86	6218	4.88
4	1.45 pm	90367	49.89	6246	4.90
5	2.45 pm	90432	49.93	6232	4.89
6	3.45 pm	90474	49.95	6331	4.96
Mean		**90364.66**	**49.89**	**6260**	**4.91**
Standard deviation		**10.169**	**0.005774**	**38.589**	**0.029**
%RSD		**0.0112**	**0.0115**	**0.616**	**0.59**

Table 9: Inter-day Precision Data of Metoprolol Succinate and Ramipril

Replicates	Time interval	Metoprolol Succinate		Ramipril	
		Peak area	Concentration (µg/ml)	Peak area	Concentration (µg/ml)
1	30/01/2017, 10.30 am	90391	49.91	6299	4.94
2	30/01/2017, 3.30 pm	90488	49.96	6324	4.96
3	31/01/2017, 10.30 am	90423	49.92	6228	4.88
4	31/01/2017, 3.30 pm	90467	49.95	6316	4.95
5	01/02/2017, 10.30 am	90409	49.92	6332	4.97
6	01/02/2017, 3.30 pm	90470	49.95	6276	4.92
Mean		**90441.33**	**49.93**	**6295.83**	**4.94**
Standard deviation		**28.80**	**0.0156**	**34.844**	**0.029155**
%RSD		**0.031**	**0.0312**	**0.553**	**0.589**

Table 10: Report of Precision for Metoprolol Succinate and Ramipril

Precision Parameters	Metoprolol Succinate % RSD	Ramipril % RSD	Acceptance criteria % RSD
Method Precision	0.078	0.274	2%
System Precision	0.012	0.130	2%
Intra- day Precision	0.011	0.590	2%
Inter day Precision	0.031	0.589	2%

LIMIT OF DETECTION: The Limit of detection was calculated for Metoprolol Succinate and Ramipril by visualization method. It was found from the chromatogram that the concentration of 0.01µg/ml Metoprolol Succinate and 0.015µg/ml Ramipril, peaks or response were observed but no area was observed. LOD for Metoprolol Succinate and Ramipril were found to be 0.01µg/ml and 0.015µg/ml.

Table 11: LOD Data of Metoprolol Succinate

Volume of stock solution (ml)	Volume made up to(ml)	Concentration (µg/ml)	Peak area
0.3	100	0.03	4250
0.2	100	0.02	1904
0.1	100	0.01	-

Table 12: LOD Data of Ramipril

Volume of stock solution (ml)	Volume made up to(ml)	Concentration (µg/ml)	Peak area
0.3	100	0.015	-
0.4	100	0.020	864
0.5	100	0.025	1423

Robustness: In all varied conditions, the RSD of contents of Metoprolol Succinate and Ramipril were found to be well within the acceptable limit of 2%. The tailing factor for both the peaks was found to be <1.5.

DISCUSSION

A combination of Metoprolol Succinate and Ramipril is mainly used as Antihypertensive drugs. However, no method is so far reported for simultaneous estimation of these drugs in combined dosage form by Normal Phase-HPLC method.

- The LOD was determined by visualization method and found to be at the concentration of 0.01µg/ml and 0.015µg/ml for Metoprolol succinate and Ramipril respectively.
- The linearity was determined by injecting replicates of working standard solution and found to be in the concentration range of 5-50µg/ml for both Metoprolol Succinate and 0.5-5µg/ml of Ramipril. The regression equation for linearity was found to be Y = 1811.x+2264 for Metoprolol Succinate and Y = 1274.x-144.6 for Ramipril. The linearity graph for both the drugs was satisfactory as observed from the correlation coefficient values which were 99.9% and 99.8% for Metoprolol Succinate and Ramipril respectively. The slope of the linearity graph was found to be 1811 and 1274 for Metoprolol Succinate and Ramipril. The intercept was found to be 2264 and -144.6 for Metoprolol Succinate and Ramipril respectively.
- The precision of method and system was determined by replicate injections of standard solution. In method precision the % RSD of the assay was found to be 0.078% and 0.274% for Metoprolol Succinate and Ramipril. In system precision the % RSD of the peak area was found to be 0.012% and 0.13% for Metoprolol Succinate and Ramipril respectively. For intra-day precision the % RSD of the assay was found to be 0.011% and 0.59% for Metoprolol Succinate and Ramipril. For inter-day precision the % RSD of the assay was found to be 0.031%and 0.589% for Metoprolol Succinate and Ramipril. As all the values of % RSD for precision study obtained was within the acceptance criteria of less than 2%, the proposed method was found to be providing good degree of precision.
- The specificity of the method was determined. The method was specific for estimation of Metoprolol Succinate and Ramipril as no peak was detected in the retention time of 15 min with placebo.
- The accuracy was determined through recovery study of the drug by spiking the standard drug of Metoprolol Succinate and Ramipril at three different levels of 80 %, 100 % and 120 % with previously analyzed samples of known fixed concentration.
- The percentage recovery was found to be 97.19% to 98.99% for Metoprolol Succinate and 94.98% to 97.87% for Ramipril. The percentage recovery was in total agreement with acceptance criteria of 90 %-110 %.
- A HPLC method was developed with mobile phase system Acetonitrile: Methanol in the ratio of 70:30 with flow rate of1 ml/ min on C18 column (250 x 4.6 mm, 5µm particle size). The retention time of Metoprolol Succinate and Ramipril was observed at 3.78 min and 1.65 min respectively.

CONCLUSION

For HPLC, the chromatograph used was LC Jasco with manual injector 20µl and the column C18, 250 x 4.6 mm, 5µm. The mobile

phase comprised of Acetonitrile:Methanol in the ratio of 70:30 v/v and flow rate of 1 ml/min, with detection at 224 nm, produced peaks of Metoprolol Succinate and Ramipril in the chromatogram which were well resolved with retention time of 3.78 min and 1.65 min respectively. The HPLC method was validated for various parameters like accuracy, precision, and specificity as per ICH guidelines.

The proposed methods were applied for determination of Metoprolol Succinate and Ramipril in marketed formulations. The proposed method was found to be satisfactory and could be used for the routine analysis of Metoprolol Succinate and Ramipril in their marketed tablet dosage formulations.

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