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NEW METHOD DEVELOPMENT AND VALIDATION FOR DETERMINATION OF RAMIPRIL AND METOPROLOL SUCCINATE IN BULK AND MARKETED FORMULATION

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ABSTRACT

Two simple, accurate, precise, reproducible and an economical spectrophotometric method were developed for the simultaneous estimation of Ramipril (RAM) and Metoprolol Succinate (METO) in pharmaceutical bulk and synthetic mixture. The first method was developed on the basis of Q-absorbance ratio method for analysis of both the drugs. Wavelengths selected for analysis in Q-absorbance ratio method were 205nm (λmax of Ramipril) and 243nm (iso-absorptive wavelength) in methanol. Ramipril and Metoprolol Succinate were found to be linear over the range of 3-30µg/ml and 20-200µg/ml respectively. It could be concluded from the results obtained in the present investigation that the method for the simultaneous estimation of Ramipril and Metoprolol Succinate in tablet dosage form are simple, rapid, accurate, precise and economical and can be used, successfully in the quality control of pharmaceutical formulations and other routine laboratory analysis.

Keywords *– Ramipril, Metoprolol succinate, absorption ratio method, linearity, λmax.*

1. INTRODUCTION

Ultraviolet and visible spectrophotometry is one of the most frequently employed analytical tools in the pharmaceutical industry. Spectrophotometry is mainly concerned with the following regions of spectrum: ultraviolet, visible and infrared [1]. Ultraviolet and visible absorption spectrophotometry involves the measurement of the absorption of monochromatic radiation by solutions of chemical substances, in the range of 185nm to 380nm, and 380nm to 780nm of the spectrum, respectively [2]. The amount of absorption depends on the wavelength of radiation and the structure of the compound. The absorption of radiation is an electron transition phenomenon; UV is sometimes called electronic spectroscopy [3].

The new drugs introduced into the market are increasing every year in number. These drugs may be totally new or partial structural modification in existing molecules. Hence, analytical method development may be made for new products or existing products or modified products. Methods are developed for new products when no official methods are available. Alternate methods for existing (Non-Pharmacopoeial) products are developed to reduce the cost and time for better precision and ruggedness. Trial runs are conducted, method is optimized and validated. When alternate method proposed is intended to replace the existing procedure comparative laboratory data including merit/demerits are made available.

Ramipril {(2S, 3aS, 6aS) 1[(S)1(ethoxycarbonyl)-3-phenylpropyl]amino] propanoyl] octahydrocyclopental[b] pyrrole -2-carboxylic acid} is a prodrug belonging to the angiotensin-converting enzyme (ACE) inhibitor class of medications[4]. 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). Ramipril may be used in the treatment of hypertension, congestive heart failure, nephropathy, and to reduce the rate of death, myocardial infarction and stroke in individuals at high risk of cardiovascular events [5]. Molecular Formula C23H32N2O5, Molecular weight 416.5, freely soluble in methanol, sparingly soluble in water, bioavailability 60-87%, half -life 2-3 hours, a white or almost white crystalline powder [6].

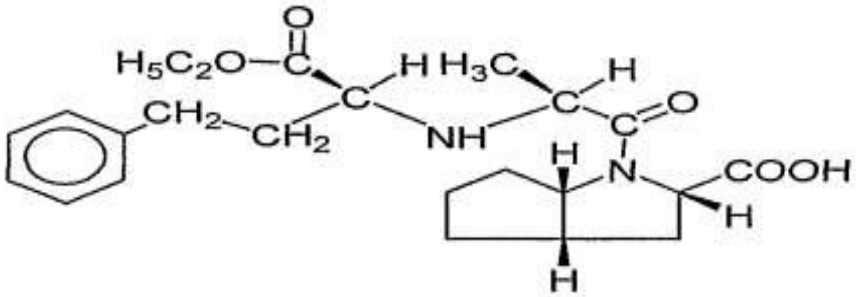

Fig. 1: Structure of Ramipril

Metoprolol Succinate[7] {butanedioic acid;1-[4-(2-methoxyethyl)phenoxy]-3-(propan-2-ylamino)propan-2-ol} is Beta-adrenoceptor antagonist. 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[5]. Molecular formula C34H56N2O10, molecular weight 652.8, it is freely soluble in water, soluble in Methanol, sparingly soluble in alcohol and slightly soluble in isopropyl alcohol, bioavailability 50% for single dose and 70 % for repeated administration, half-life 3-7 hours, a white, crystalline powder or colorless crystals [8,9].

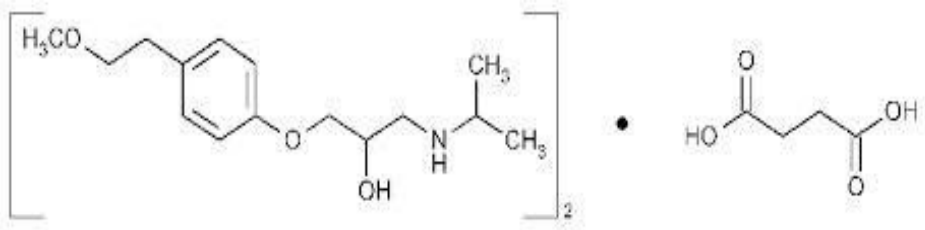

Fig. 2: Structure of Metoprolol Succinate

2. MATERIALS AND METHODS

2.1 Material

The Ramipril and Metoprolol Succinate were purchased from Yarrow Chem Products Mumbai (INDIA). All other chemicals and reagents used were of analytical grade.

2.1.1 Apparatus and conditions

A double beam Shimadzu UV-1800 series spectrophotometer was used. Absorption and overlain spectra of both test and standard solutions were recorded over the wavelength range of 200-400nm using 1cm quartz cell at fast scanned speed and fixed slit width of 1.0nm. All weighing of ingredients was done on digital weighing balance.

2.1.2 Preparation of standard stock solution

Stock solution (1000µg/ml) of Ramipril and Metoprolol Succinate were prepared by accurately weighing 100mg of the drug in minimum quantity of Methanol and finally, diluted with Methanol, to make the volume up to 100ml. A series of standard drug solution in concentration range of 3-30µg/ml for Ramipril and 20-200µg/ml for Metoprolol Succinate respectively were prepared by diluting appropriate volumes of standard stock solutions. The scanning for solution of Ramipril and Metoprolol Succinate acid were carried out in the range of 200-400nm against Methanol as a blank for obtaining the individual absorption spectra (as shown in figure 3 & 4) as well as overlain spectra (as shown in figure 5) that were used in the analysis.

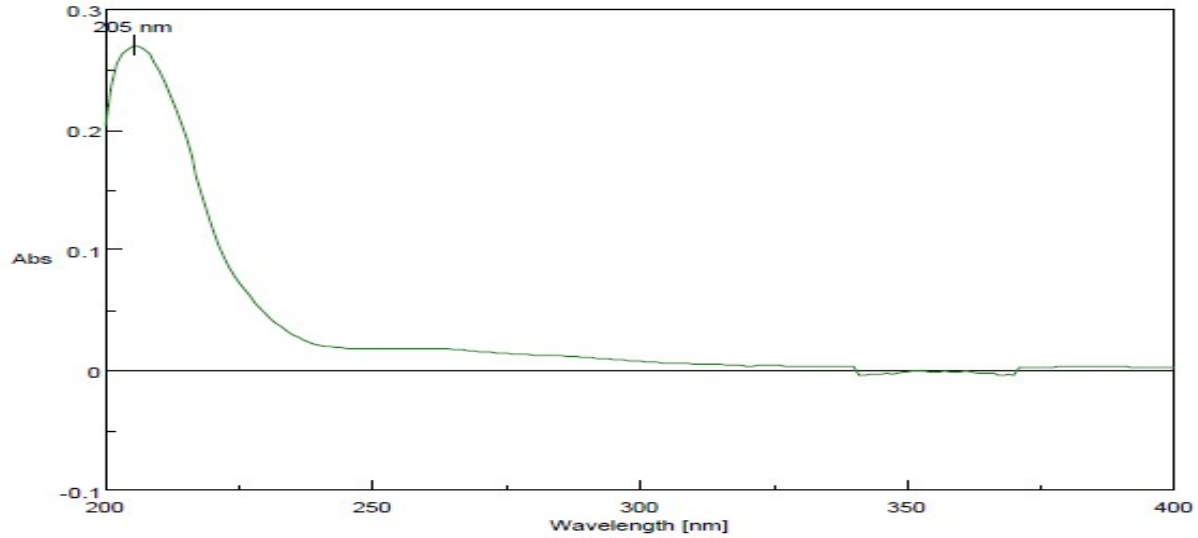

Fig. 3: Absorption spectra of Ramipril

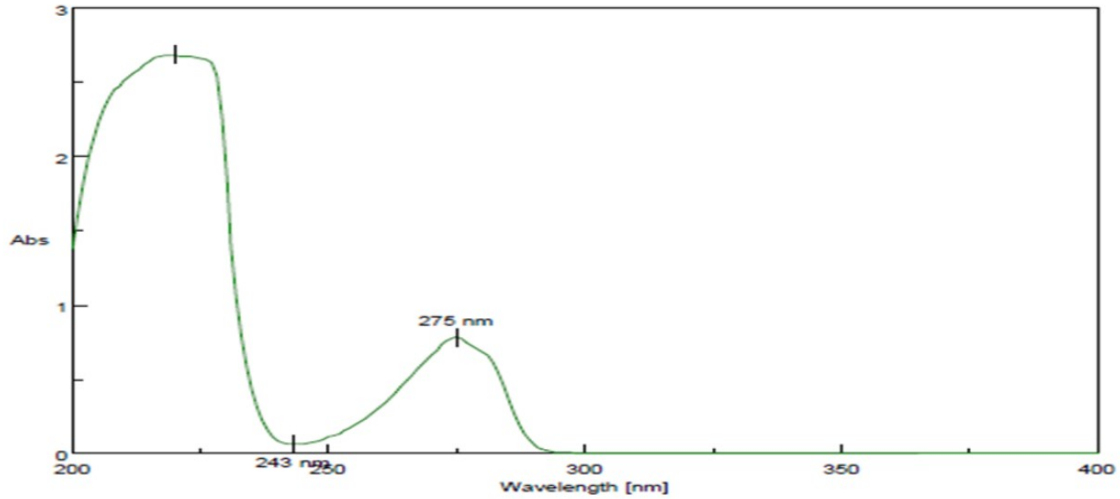

Fig. 4: Absorption spectra of Metoprolol Succinate

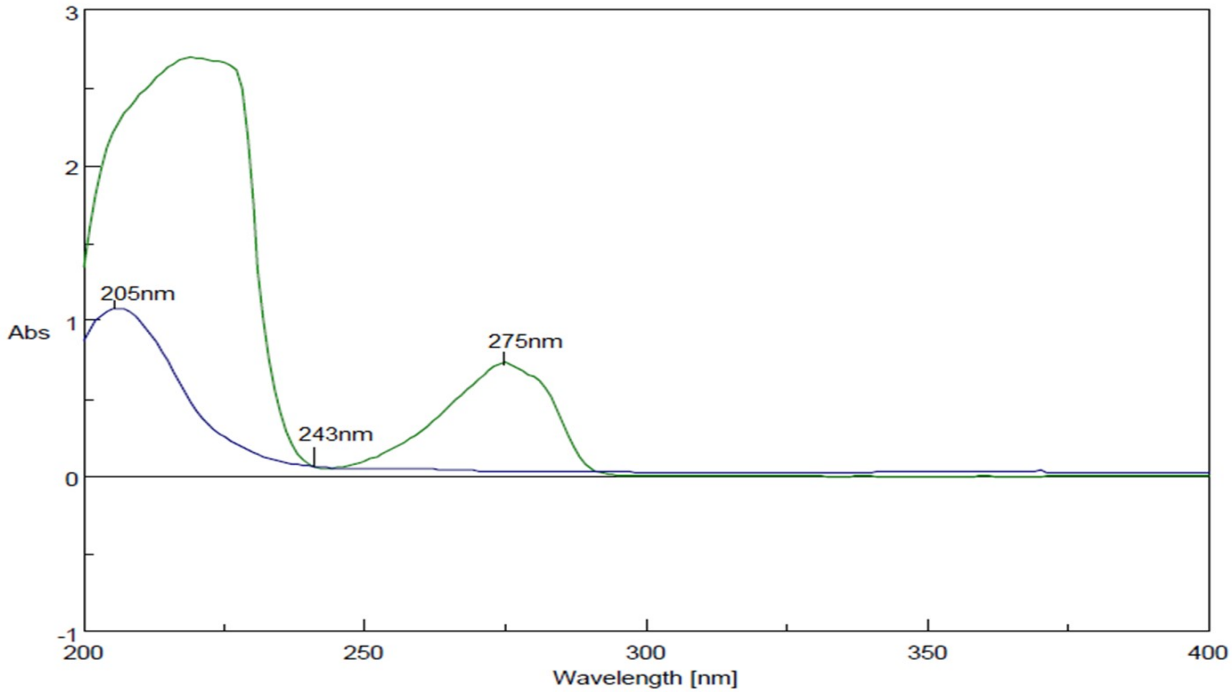

Fig. 5: Overlain spectra of Ramipril and Metoprolol Succinate

The maximum absorption (λmax) of Ramipril was found at 205nm and iso-absorptive point at 243nm. Absorption and absorptivity for a series of standard solutions were recorded at selected wavelengths.

2.2 Methodology

Absorption ratio method uses the ratio of absorptions of two selected wavelength, one of which is iso-absorptive point and other being the λmax of one of the two components. From the overlain spectra of two drugs (as shown in figure 5), it shows that Ramipril and Metoprolol Succinate having iso-absorptive point at 243nm. The second wavelength used is 205nm, which is the λmax of Ramipril. Working standard solutions having concentration 3-30µg/ml for Ramipril and 20-200µg/ml was prepared in Methanol and the absorbance at 243nm (iso-absorptive point) and 205nm (λmax of Ramipril) were measured and absorptivity coefficient were calculated using calibrations curve. The concentration of two drugs in the mixture can be calculating by using the equation,

$$Cx = \{(QM-Qy)/(Qx-Qy)\} * (A1/ax1)$$

$$Cy = \{(QM-Qx)/(Qy-Qx)\} * (A1/ay1)$$

where, A1 and A2 are the absorbance of mixture at 243nm and 205nm; ax1 and ay1 are absorptivities of Ramipril and Metoprolol Succinate at 243nm; ax2 and ay2 are absorptivities of Ramipril and Metoprolol Succinate at 205nm;

$$QM = A2/A1,\ Qx = ax2/ax1,\ Qy = ay2/ay1.$$

2.3 Validation of proposed method

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: sensitivity, linearity and range, accuracy, precision, system precision and method precision, intraday precision and interday precision [10] .

2.3.1 Sensitivity

Ramipril standard stock solution: Accurately weighed 100mg of Ramipril was transferred into clean, dry 100ml volumetric flask and dissolved with sufficient volume of Methanol. The volume was made up to 100ml with Methanol to get concentration of 1000µg/ml. Working Standard stock solution.

Working Standard stock solution: Aliquot's from stock solution was transferred into separate 10ml volumetric flasks and volume made up to 10ml with Methanol to obtain the concentrations 3, 6, 9, 12, 15, 18, 21, 24, 27 and 30µg/ml respectively.

Metoprolol Succinate standard stock solution: Accurately weighed 100mg of Metoprolol Succinate was transferred into clean, dry 100ml volumetric flask and dissolved with sufficient volume of Methanol. The volume was made up to 100 ml with Methanol to get concentration of 1000µg/ml.

Working Standard stock solution: Aliquot's from stock solution was transferred into separate 10ml volumetric flasks and volume made up to 10ml with Methanol to obtain the concentrations 20, 40, 60, 80, 100, 120, 140, 160,180 and 200µg/ml respectively.

Determination: Absorbance of working standard solutions of Metoprolol Succinate and Ramipril was taken at 205nm and 243nm (iso absorptive point).

2.3.2 Linearity and Range

Ramipril standard stock solution: Accurately weighed 100mg of Ramipril was transferred into clean, dry 100ml volumetric flask and dissolved with sufficient volume of Methanol. The volume was made up to 100ml with Methanol to get concentration of 1000µg/ml (stock A). Further 10ml was withdrawn from stock A in 100ml volumetric flask and dissolved with sufficient volume of Methanol. The volume was made up to 100ml with Methanol to get concentration of 100µg/ml (stock B).

Working standard solution: Aliquots from standard solution were withdrawn in the volumes of 0.3, 0.6, 0.9, 1.2, 1.5, 1.8, 2.1, 2.4, 2.7 and 3.0ml and transferred into different 10 ml volumetric flasks. The volumes were made up with Methanol to get concentrations ranging from 3, 6, 9, 12, 15, 18, 21, 24, 27 and 30µg/ml respectively.

Metoprolol Succinate standard stock solution: Accurately weighed 100mg of Metoprolol Succinate was transferred into clean, dry 100ml volumetric flask and dissolved with sufficient volume of Methanol. The volume was made up to 100ml with Methanol to get concentration of 1000µg/ml.

Working standard solution: Aliquots from standard solution were withdrawn in the volumes of 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8 and 2.0ml and transferred into different 10ml volumetric flasks. The volumes were made up with Methanol to get concentrations ranging from 20, 40, 60, 80, 100, 120, 140, 160,180 and 200µg/ml respectively.

Determination: Five replicates per concentration were studied. Absorbance of working standard solutions of Metoprolol Succinate and Ramipril was taken at 205nm and 243nm. Graph of concentration (on X- axis) Vs mean response (on Y- axis) was plotted for both the drugs separately. The regression equation, Y- intercept and correlation coefficient were calculated.

2.3.3 Accuracy

Preparation of standard stock solution: Accurately weighed 2.5mg of Ramipril and 25mg Metoprolol Succinate transferred in to a clean, dry 100ml volumetric flask and dissolved in sufficient volume of Methanol. The volume made up to 100ml with Methanol to get the concentration of 25µg/ml of Ramipril and 250µg/ml of Metoprolol Succinate

Preparation of sample stock solution: Twenty tablets containing 2.5mg of Ramipril and 25mg of Metoprolol Succinate were weighed and finely powered. Accurately weighed 250mg of powder which is equivalent to 2.5mg of Ramipril and 25mg of Metoprolol Succinate was transferred into a clean, dry 100ml volumetric flask. To this Methanol was added and sonicated for 15min. The resulting suspension was then filtered through whatmann filter. The volume of filtrate was made up to 100ml with Methanol to get the concentration 25µg/ml of Ramipril and 250µg/ml of Metoprolol Succinate

Preparation of standard and sample mixture

Level I (80%): Volume of 1ml sample stock solution and 0.8ml standard stock solution was transferred into 10ml volumetric flask and volume made up with Methanol.

Level II (100%): Volume of 1ml sample stock solution and 1ml standard stock solution was transferred into 10ml volumetric flask and volume made up with Methanol.

Level III (120%): Volume of 1ml sample stock solution and 1.2ml standard stock solution was transferred into 10ml volumetric flask and volume made up with Methanol.

Determination: The absorbances of resulting solutions were recorded at wavelengths 205nm and 243nm. Concentrations of Ramipril and Metoprolol Succinate in each solution were calculated from Absorbance Ratio equation.

2.3.4 Precision

Preparation of standard mixture solution: Accurately weighed 2.5mg of Ramipril and 25mg Metoprolol Succinate transferred in to a clean, dry 100ml volumetric flask and dissolved in sufficient volume of Methanol. The volume made up to 100ml with Methanol to get the concentration of 25µg/ml of Ramipril and 250µg/ml of Metoprolol Succinate.

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

Determination

System Precision: The absorbance of six determinations of working solution was recorded at wavelengths 205nm and 243nm. The % RSD was calculated for the absorbance of replicates.

Method Precision: The absorbance of six determinations of working solution was taken at wavelengths 205nm and 243nm. Concentration of Ramipril and Metoprolol Succinate in each replicate was calculated from simultaneous equation. The % RSD was calculated from the concentrations of Ramipril and Metoprolol Succinate.

Inter-day Precision: The absorbance of six determinations of working solution was taken at wavelengths 205nm and 243nm on different days. Concentration of Ramipril and Metoprolol Succinate in each replicate was calculated from simultaneous equation. The % RSD was calculated from the concentrations of Ramipril and Metoprolol Succinate in six determinations of working solution. The standard deviation and relative standard deviation were calculated.

Intra-day Precision: The absorbance of six determinations of working solution was taken at wavelengths 205nm and 243nm on different intervals in the same day. Concentration of Ramipril and Metoprolol Succinate in each replicate was calculated from simultaneous equation. The % RSD was calculated from the concentrations of Ramipril and Metoprolol Succinate in six determinations of working solution. The standard deviation and relative standard deviation were calculated.

3. RESULTS AND DISCUSSION

31 Sensitivity

Absorbance of standard solutions of Ramipril and Metoprolol Succinate was measured at 205nm and 243nm. Sandell's sensitivity (Π) for both the drugs was calculated from formula, at both the wavelengths.

Sandell' s sensitivity = (concentration of drug in µg/100ml)/absorbance×0.001

Table 1: Sensitivity Data of Ramipril

Concentration (µg/ml)	Ramipril	
	Absorbance at 243nm	Sensitivity (µg/cm3/Au)
3	0.086	0.348
6	0.095	0.0631
9	0.159	0.0566
12	0.251	0.0478
15	0.332	0.0451
18	0.421	0.0427
21	0.488	0.0430
24	0.534	0.0449
27	0.608	0.0444
30	0.698	0.0429
	Mean	0.04653

Table 2: Sensitivity Data of Metoprolol Succinate

Concentration (µg/ml)	Metoprolol Succinate	
	Absorbance at 243nm	Sensitivity (µg/cm3/Au)
20	0.212	0.0943
40	0.423	0.0945
60	0.632	0.0949
80	0.739	0.1082
100	1.012	0.0988
120	1.257	0.0954
140	1.428	0.0980
160	1.614	0.0991
180	1.803	0.0998
200	2.135	0.0936
	Mean	0.09766

3.2 Linearity and Range

The linearity in response for Ramipril and Metoprolol Succinate was observed in the concentration range of 3-30µg/ml and 20-200µg/ml respectively for both the drugs, with percentage curve fittings found to be well within the limits of acceptance criteria.

Table 3: Linearity Range Data of Ramipril

Volume of stock solution(ml)	Volume adjusted(ml)	Concentration µg/ml	Absorbance at 205nm	Absorbance at 243nm
0.3	10	3	0.1531	0.0004
0.6	10	6	0.2393	0.0024
0.9	10	9	0.3467	0.0047
1.2	10	12	0.4414	0.0067
1.5	10	15	0.5651	0.0087
1.8	10	18	0.6510	0.0114
2.1	10	21	0.7759	0.0136
2.4	10	24	0.8873	0.0156
2.7	10	27	0.9987	0.0175
3.0	10	30	1.0934	0.0196

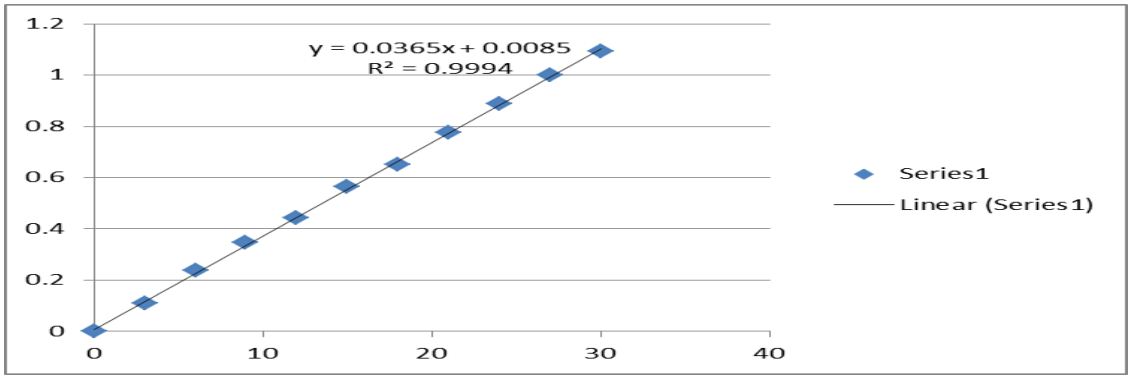

Fig. 6: Linearity range graph of Ramipril at 205nm

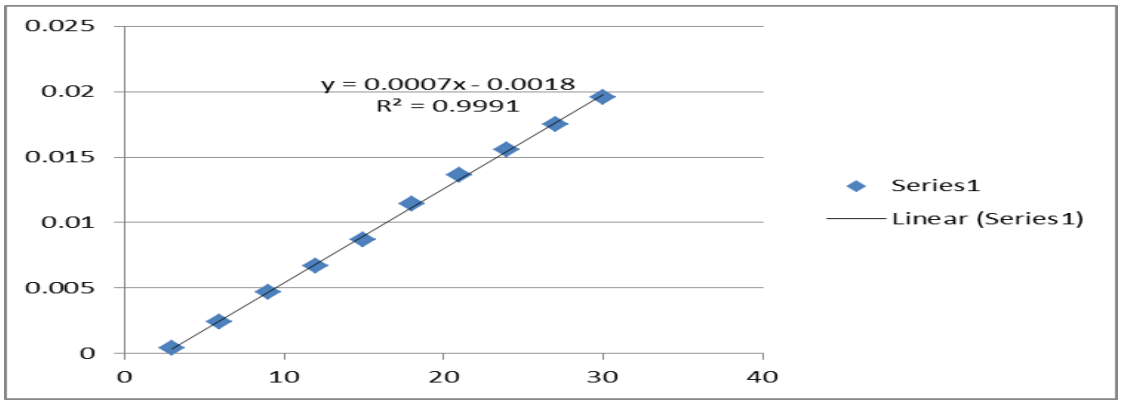

Fig. 7: Linearity range graph of Ramipril at 243nm

Table 4: Linearity Range Data of Metoprolol Succinate

Volume of stock solution(ml)	Volume adjusted(ml)	Concentration µg/ml	Absorbance at 205nm	Absorbance at 243nm
0.2	10	20	0.8156	0.0014
0.4	10	40	0.9866	0.0095
0.6	10	60	1.1576	0.0159
0.8	10	80	1.3286	0.0223
1.0	10	100	1.4996	0.0381
1.2	10	120	1.7335	0.0368
1.4	10	140	1.9012	0.0463
1.6	10	160	1.9717	0.0515
1.8	10	180	2.141	0.0597
2.0	10	200	2.312	0.0643

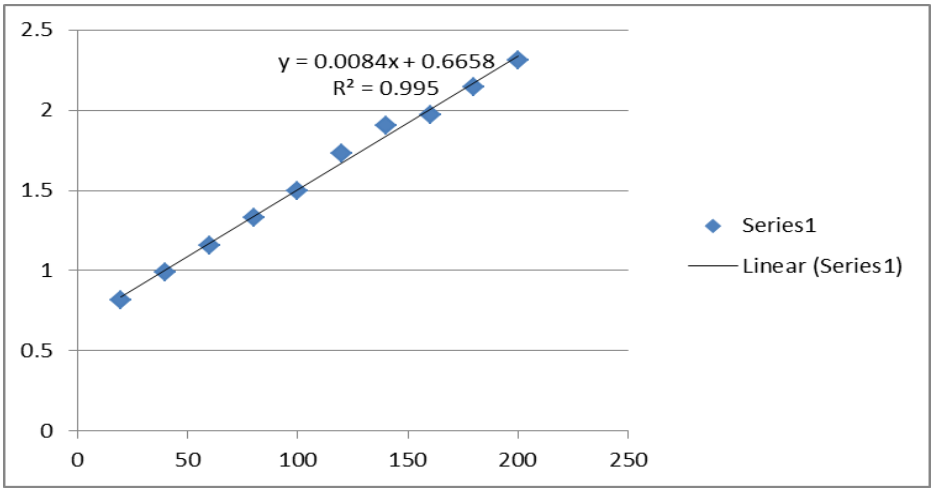

Fig. 8: Linearity range graph of Metoprolol Succinate at 205nm

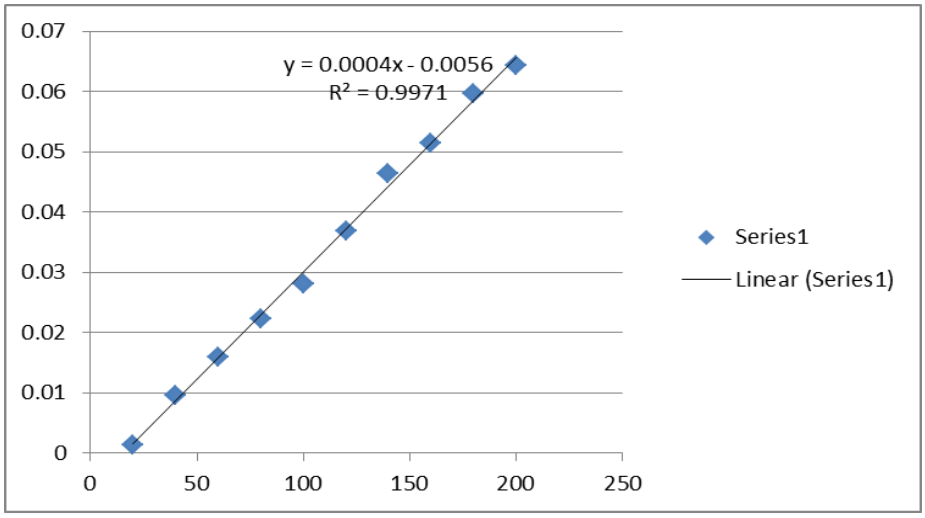

Fig. 9: Linearity range graph of Metoprolol Succinate at 243nm

Table 5: Linearity report of Ramipril and Metoprolol Succinate

Parameters	Results observed				Acceptance criteria
	Ramipril		Metoprolol Succinate		
	205nm	243nm	205nm	243nm	
Linearity range (µg/ml)	3-30 µg/ml	3-30 µg/ml	20-200 µg/ml	20-200 µg/ml	-
Regression equation	0.0365x + 0.0085	0.0007x - 0.0018	0.0084x + 0.6658	0.0004x - 0.0056	-
Correlation coefficient	0.9994	0.9991	0.995	0.9971	0.99
Intercept	0.0365x	0.0007x	0.0084x	0.0004x	-
Slope	0.0085	- 0.0018	0.6658	- 0.0056	-

Table 6: The content of Ramipril and Metoprolol Succinate found in tablets (Assay of marketed formulation)

Stock volume	Concentration obtained(µg/ml)		Amount of drug in tablet (mg)		Amount obtained in %	
	Ramipril	Metoprolol succinate	Ramipril	Metoprolol succinate	Ramipril	Metoprolol succinate
1.0	2.47	24.97	2.47	24.97	98.8	99.88
1.5	4.96	39.81	2.47	24.97	98.8	99.88
2.0	7.42	54.45	2.45	24.97	98	99.88
2.5	9.93	69.43	2.44	24.96	97.6	99.84
3.0	12.55	84.38	2.47	24.95	98.8	99.8
Average			2.46	24.96	98.4	99.85

3.3 Accuracy

The absorbances of resulting solutions were recorded at wavelengths of 243nm and 205nm. Concentrations of Ramipril and Metoprolol Succinate in each solution were calculated from absorbance ratio equation.

Table 7: Recovery Data of Standard Mixture

Level (%)	Sample concentration (µg/ml)		Total Concentration (µg/ml)		Amount of standard recovered(µg/ml)		% Recovery of standard	
	RAM	METO	RAM	METO	RAM	METO	RAM	METO
80%	2.98	24.93	4.91	39.79	1.93	14.86	96.5	99.06
100%	2.95	24.97	5.74	49.73	2.79	24.76	93	99.04
120%	2.80	24.94	6.44	50.63	3.64	25.69	91	99.80

3.4 Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

3.5 System Precision

The absorbance of six determinations of working solution was recorded at wavelengths 205nm and 243nm. The % RSD was calculated for the absorbance of replicates.

3.6 Method Precision

The absorbance of six determinations of working solution was taken at wavelengths 205nm and 243nm. Concentration of Ramipril and Metoprolol Succinate in each replicate was calculated from simultaneous equation. The % RSD was calculated from the concentrations of Ramipril and Metoprolol Succinate.

3.7 Inter-day Precision

The absorbance of six determinations of working solution was taken at wavelengths 205nm and 243nm on different days. Concentration of Ramipril and Metoprolol Succinate in each replicate was calculated from simultaneous equation. The % RSD was calculated from the concentrations of Ramipril and Metoprolol Succinate in six determinations of working solution. The standard deviation and relative standard deviation were calculated.

3.8 Intra-day Precision

The absorbance of six determinations of working solution was taken at wavelengths 205nm and 243nm on different intervals in the same day. Concentration of Ramipril and Metoprolol Succinate in each replicate was calculated from simultaneous equation. The % RSD was calculated from the concentrations of Ramipril and Metoprolol Succinate in six determinations of working solution. The standard deviation and relative standard deviation were calculated.

Table 8: System Precision Data of Ramipril and Metoprolol Succinate

Replicates	Absorbance A1 205nm	Absorbance A2 243nm
1	0.7104	0.2032
2	0.7032	0.2041
3	0.7204	0.2134
4	0.6989	0.2188
5	0.7212	0.2013
6	0.7212	0.2179
Mean	0.7123	0.2097
Standard Deviation	0.0016	0.0015
%RSD	0.2246	0.7153

Table 9: Method Precision Data of Ramipril and Metoprolol Succinate

Replicates	Concentration (µg/ml)	
	Ramipril	Metoprolol Succinate
1	2.98	24.97
2	2.98	24.93
3	2.95	24.93
4	2.92	24.98
5	2.96	24.97
6	2.98	24.97
Mean	2.96	24.95
Standard Deviation	0.0489	0.0583
%RSD	1.6520	0.2336

Table 10: Inter-day Precision Data of Ramipril and Metoprolol Succinate

Replicates	Date interval	Concentration (µg/ml)	
		Ramipril	Metoprolol Succinate
1	4/10/2017	2.95	24.97
2	4/10/2017	2.94	24.97
3	4/10/2017	2.93	24.94
4	4/10/2017	2.97	24.93
5	4/10/2017	2.95	24.92
6	4/10/2017	2.95	24.96
Mean		2.94	24.94
Standard Deviation		0.0178	0.0583
%RSD		0.605	0.2337

Table 11: Intra-day Precision Data of Ramipril and Metoprolol Succinate

Replicates	Time interval	Concentration (µg/ml)	
		Ramipril	Metoprolol Succinate
1	10:00AM	2.94	24.97
2	11:00AM	2.93	24.93
3	12:00AM	2.95	24.96
4	1:00PM	2.96	24.93
5	2:00PM	2.94	24.92
6	3:00PM	2.93	24.96
Mean		2.94	24.94
Standard Deviation		0.0325	0.02
%RSD		1.1054	0.081

4. CONCLUSION

The proposed absorption ratio method was found to be simple, sensitive and accurate method for determination of Ramipril and Metoprolol Succinate in the tablet dosage form. Ramipril and Metoprolol Succinate have been estimated at 205nm and 243nm in methanol. Ramipril and Metoprolol Succinate obey Beer-Lamberts law in concentration range of 3-30µg/ml and 20-200µg/ml respectively. The method was validated as per ICH and USP guidelines. In this method the solvent used will be easily available and was also economic for estimation of tablet dosage form. This method was accurate, simple, rapid, precise, reliable, sensitive, reproducible and can be used for further quantitative analysis of Ramipril and Metoprolol Succinate.

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