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METHOD DEVELOPMENT AND VALIDATION FOR DETERMINATION OF RAMIPRIL AND METOPROLOL SUCCINATE BY ZERO ORDER DERIVATIVES IN BULK AND MARKETED FORMULATION

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ABSTRACT

The proposed method involves zero order derivative spectroscopy method. A novel, simple and rapid UV Spectrophotometric determination method for simultaneous estimation of Ramipril (RAM) and Metoprolol Succinate (METO) was successfully developed and validated in bulk and pharmaceutical formulation. In this current work, simultaneous estimation of RAM and METO in methanol was performed by using UV spectroscopy. The zero-order derivative spectroscopy revealed that RAM had a λmax of 205 nm in methanol and METO had λmax of 275 nm respectively. The linearity was found in the range of 3-30 µg/ml for RAM and 20-200 µg/ml for METO. The R^2 value was found to be 0.9994 and slope y=0.0365 for RAM and R^2 = 0.9986 and slope y = 0.0054 for METO.

Keywords *– Ramipril, Metoprolol Succinate, Zero order derivative method.*

1. INTRODUCTION

Analytical methods development, identification, characterization of impurities and method validation play key role in the pharmaceutical's discovery, development, and manufacturing. Derivative spectrophotometry involves the conversion of a normal spectrum to its first, second or higher derivative spectrum. In the context of derivative Spectrophotometry, the normal absorption spectrum is referred to as the fundamental, zeroth order spectrum. The first derivative spectrum[1] of an absorption band is characterized by a maximum, a minimum and a cross-over point at the λmax of the absorption band.

Ramipril (RAM) is a prodrug for the major metabolite ramiprilat formed via ester hydrolysis, which is a highly active inhibitor of angiotensin-converting enzyme (ACE-I)[2]. Atorvastatin (ATOR) calcium belongs to the category of statins, which inhibit 3-hydroxy-3-methylglutaryl-coenzyme A (3-HMG CoA) reductase[3], thus resulting in a decrease in intracellular cholesterol due to increased clearance of low density lipoproteins (LDL) cholesterol in plasma[4]. Although there have been many advances in the management of cardiovascular diseases (CVD) during the last several years, these are still the main cause for morbidity and mortality. The

pathophysiology of CVD reveals that renin angiotensin- aldosterone system (RAAS) and dyslipidemia play an important role in the genesis and progression of CVD risk that is endothelial dysfunction[5]. 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[6].

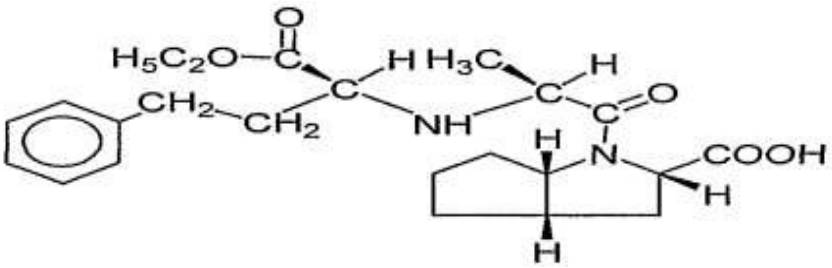

Fig. 1: Structure of Ramipril

Metoprolol succinate is chemically (RS)-1-(Isopropylamino)-3-[4-(2 methoxyethyl)phenoxy]propan-2-ol succinate[7], is a cardio selective β-blocker, used in the treatment of hypertension, angina pectoris, arrhythmia, myocardial infraction and heart failure[8]. It is official in IP[9], BP[10] and USP[11]. 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[12,13].

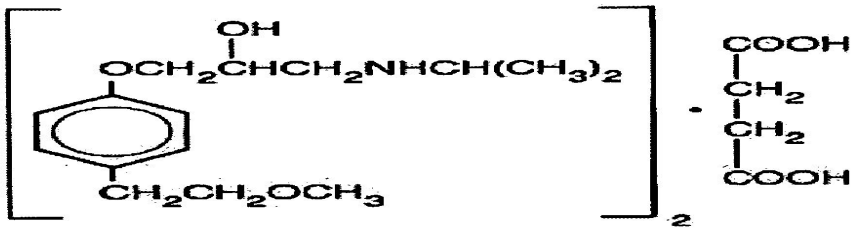

Fig. 2: Structure of Metoprolol Succinate

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.

2.2 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.

2.3 Preparations of solutions

2.3.1 Ramipril standard stock solution: Accurately weighed 100mg of Ramipril was transferred into clean, dry 100 ml 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 (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 100 ml with Methanol to get concentration of 100µg/ml (stock B).

2.3.2 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.

2.3.3 Metoprolol Succinate standard stock solution: Accurately weighed 100mg of Metoprolol Succinate was transferred into clean, dry 100 ml 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.

2.3.4 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 2.0 ml and transferred into different 10 ml 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.

2.3.5 Determination of λmax of Ramipril and construction of calibration curve in methanol (zero order derivative spectra): Wavelength scan was done from 200-400 nm by using 3-30µg/ml solution in double beam UV spectrophotometer Shimadzu UV-1800 series spectrophotometer to get λmax and absorbance of the standard solution were noted down.

2.3.6 Determination of λmax of Metoprolol Succinate and construction of calibration curve in methanol (Zero order derivative spectra): Wavelength scan was done from 200-400 nm by using 20-200µg/ml solution in double beam UV spectrophotometer Shimadzu UV-1800 series spectrophotometer to get λmax and absorbance of the standard solution were noted down.

3. RESULTS AND DISCUSSION

3.1 λ max of Ramipril and calibration curve in methanol (zero order derivative spectra): The λ max of Ramipril was found out at 205nm (Figure 3)and linearity was in the range of 3-30µg/ml and R^2 value was found to be 0.994 , slope Y=0.0365.The absorbance was found at 205nm is mentioned in (figure 4).

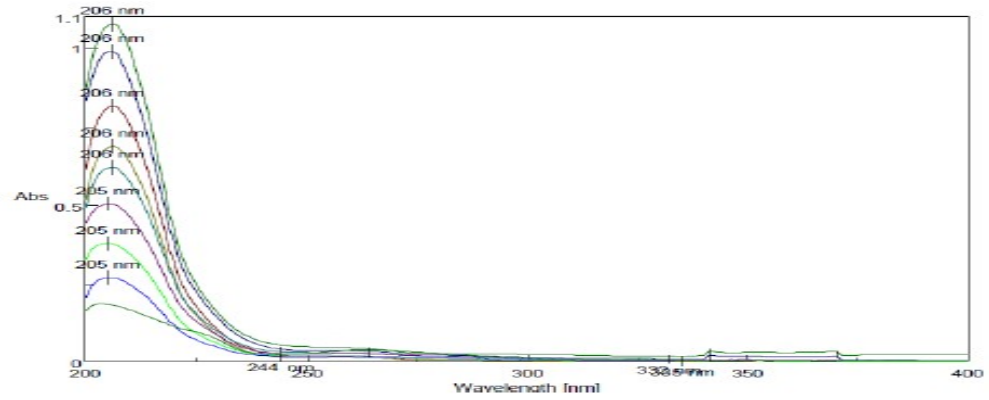

Fig. 3: Overlay of λ max of RAM (3-30µg/ml)

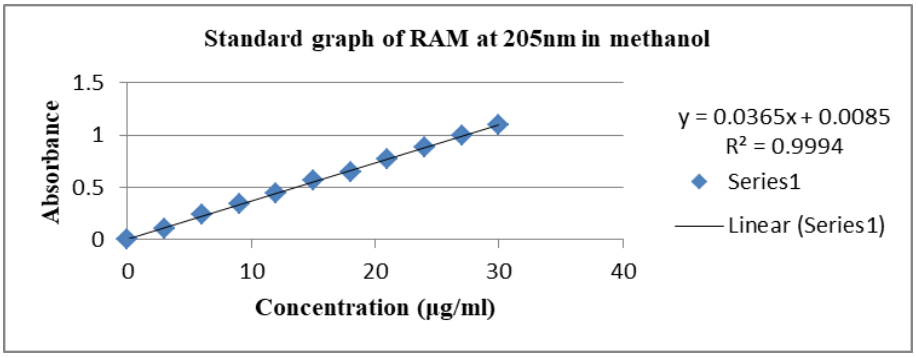

Fig. 4: Standard graph of RAM in methanol at 205nm

3.2 λ max of Metoprolol Succinate and calibration curve in methanol (zero order derivative spectra): The λ max of Metoprolol Succinate was found out at 275nm (Figure 5)and linearity was in the range of 20- 200µg/ml and R^2 value was found to be 0.9986 ,slope Y=0.0054.The absorbance was found at 275nm is mentioned in (figure 6).

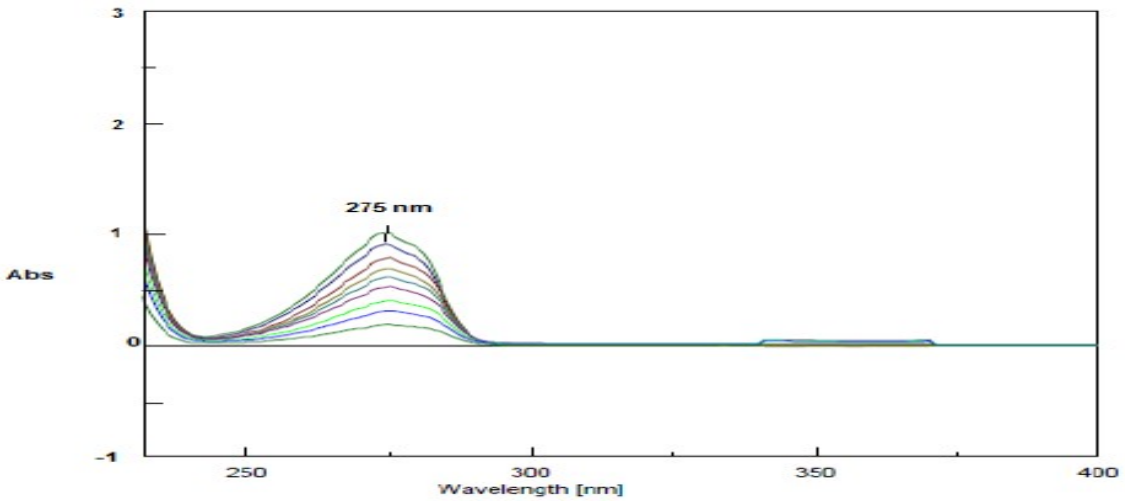

Fig. 5: Overlay of λ max of METO (20-200µg/ml)

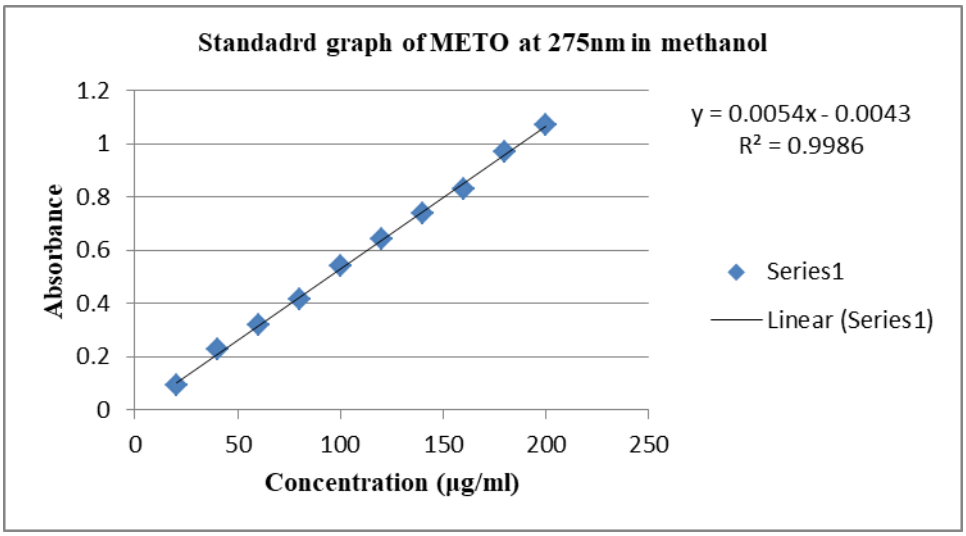

Fig. 6: Standard graph of METO in methanol at 275nm

3.3 Method Validation

The developed analytical method as per the ICH Q2 (R1) guideline it is suitable for the intended purpose with respect to various parameters such as specificity, linearity, range, accuracy, precision, limit of detection, limit of quantification, robustness, system suitability[14, 15].

3.3.1 Sensitivity

Absorbance of standard solutions of Ramipril and Metoprolol Succinate was measured at 205nm and 275nm respectively.

Table 1: Sensitivity Data of Ramipril and Metoprolol Succinate

Conc. µg/ml	Ramipril		Conc. µg/ml	Metoprolol Succinate	
	Absorbance at 205nm	Sensitivity (µg/cm3/Au)		Absorbance at 275nm	Sensitivity (µg/cm3/Au)
3	0.1104	0.0271	**20**	0.0897	0.2229
6	0.2393	0.0250	**40**	0.2284	0.1751
9	0.3467	0.0259	**60**	0.3181	0.1886
12	0.4414	0.0271	**80**	0.4160	0.1923
15	0.5651	0.0212	**100**	0.5414	0.1847
18	0.6510	0.0276	**120**	0.6405	0.1873
21	0.7759	0.0270	**140**	0.7388	0.1894
24	0.8873	0.0270	**160**	0.8320	0.1923
27	0.9987	0.0270	**180**	0.9713	0.1853
30	1.0934	0.0274	**200**	1.0711	0.1867
Mean		**0.02623**	**Mean**		**0.1722**

3.3.2 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 10ml 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 were taken at 205nm and 275nm for zero order derivative and 274nm and 206nm for first order derivative respectively.

Table 2: Linearity Range Data of Ramipril for zero order derivative

Volume of stock solution (ml)	Volume adjusted (ml)	Concentration µg/ml	Absorbance at 205nm	Absorbance at 275nm
0.3	10	3	0.1104	0.0007
0.6	10	6	0.2393	0.0017
0.9	10	9	0.3467	0.0037
1.2	10	12	0.4414	0.0056
1.5	10	15	0.5651	0.0077
1.8	10	18	0.6510	0.0096
2.1	10	21	0.7759	0.0117
2.4	10	24	0.8873	0.0138
2.7	10	27	0.9987	0.0158
3.0	10	30	1.0934	0.0179

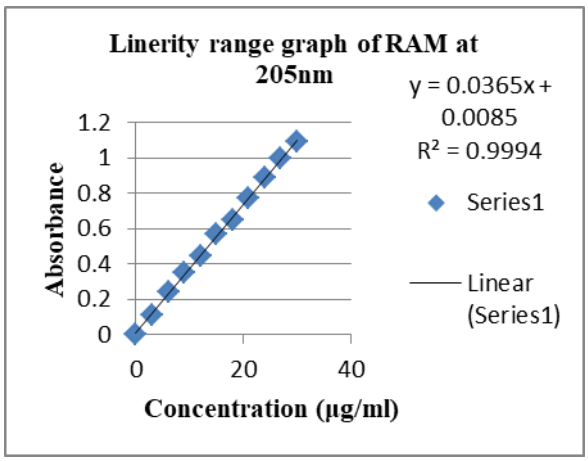

Fig 7: Linearity Range Graph of RAM at 205nm

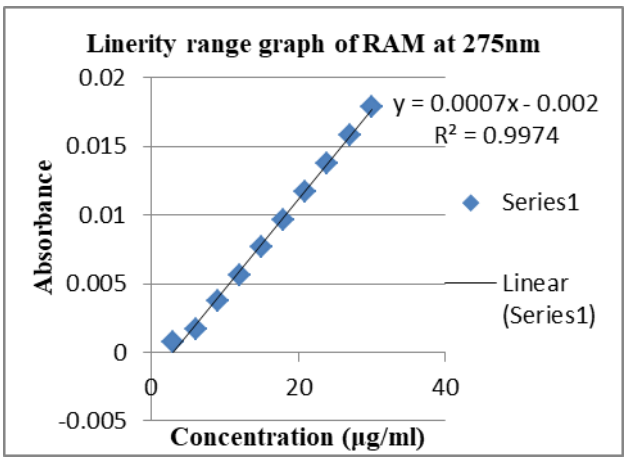

Fig 8: Linearity Range Graph of RAM at 275nm

Table 3: Linearity Range Data of Metoprolol Succinate for zero order derivative

Volume of stock solution (ml)	Volume adjusted (ml)	Concentration µg/ml	Absorbance at 205nm	Absorbance at 275nm
20	10	20	0.8156	0.0897
40	10	40	0.9866	0.2284
60	10	60	1.1576	0.3181
80	10	80	1.3286	0.4160
100	10	100	1.4996	0.5414
120	10	120	1.7335	0.6405
140	10	140	1.9012	0.7388
160	10	160	1.971	0.8320
180	10	180	2.141	0.9713
200	10	200	2.312	1.0711

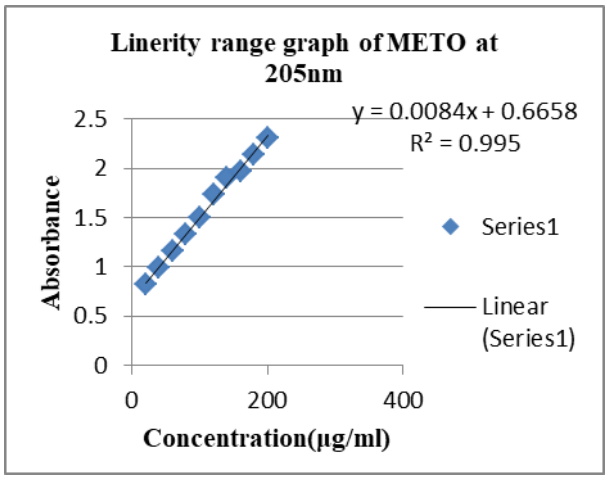

Fig 9: Linearity Range Graph of METO at 205nm

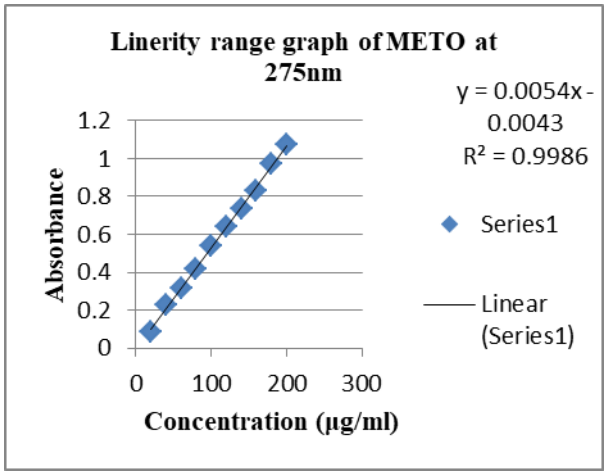

Fig 10: Linearity Range Graph of METO at 275nm

Table 4: Linearity report of Ramipril and Metoprolol Succinate

Parameters	Results observed				Acceptance Criteria
	Ramipril		Metoprolol Succinate		
	205nm	275nm	205nm	275nm	
Linearity range (µg/ml)	3-30µg/ml	3-30µg/ml	20-200µg/ml	20-200µg/ml	-
Regression equation	0.0365x +0.0085	0.0007x -0.002	0.0084x +0.6658	0.0054x -0.0043	-
Correlation coefficient	0.9994	0.9974	0.995	0.9986	0.99
Intercept	0.0365x	0.0007x	0.0084x	0.0054x	-
Slope	0.0085	-0.002	0.6658	-0.0043	-

Table 5: Sample Analysis Data of Ramipril and Metoprolol Succinate

Stock vol.	Concentration obtained(µg/ml)		Amount of drug in tablet		Amount obtained in %	
	RAM	METO	RAM	METO	RAM	METO
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	99.8
3.0	12.55	84.38	2.47	24.95	98.8	99.85
Avg.			**2.46**	**24.96**	**98.4**	**99.85**

3.3.3 Accuracy (Recovery Study)

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. The absorbances of resulting solutions were recorded at wavelengths 205nm and 275nm.

Table 6: Recovery Data of Standard Mixture

Level (%)	Sample conc. (µg/ml)		Total Conc. (µg/ml)		Amount of std. recovered (µg/ml)		% Recovery of Standard	
	RAM	METO	RAM	METO	RAM	METO	RAM	METO
80%	2.97	24.91	4.91	39.79	1.94	14.88	99	99.64
100%	2.94	24.95	5.74	49.73	2.8	24.78	98	99.8
120%	2.92	24.93	6.44	50.63	3.52	25.7	97.33	99.72

3.3.4 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. Precision may be considered at three levels: repeatability, intermediate precision, and reproducibility. The absorbance of six determinations of working solution was recorded at wavelengths 205nm and 275nm. The %RSD was calculated for the absorbance of replicate.

Table 7: System Precision Data of Ramipril and Metoprolol Succinate

Replicates	Absorbance (A1) 205nm	Absorbance (A2) 275nm
1	0.7102	0.0896
2	0.7030	0.0896
3	0.7206	0.0897
4	0.6990	0.0899
5	0.7214	0.0896
6	0.7215	0.0896
Mean	0.7126	0.0896
Standard deviation	0.0089	0.0001
% RSD	1.248	0.1116

Table 8: Method Precision Data of Ramipril and Metoprolol Succinate

Replicates	Concentration in (µg/ml)	
	Ramipril	Metoprolol Succinate
1	2.95	24.91
2	2.98	24.93
3	2.91	24.93
4	2.96	24.98
5	2.98	24.97
6	2.95	24.97
Mean	2.95	24.94
Standard deviation	0.0264	0.03
%RSD	0.8813	0.1202

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

Replicates	Date interval	Concentration in µg/ml	
		Ramipril	Metoprolol Succinate
1	04-10-17, 10am	2.99	24.99
2	04-10-17, 3pm	2.95	24.93
3	05-10-17, 10am	2.93	24.97
4	05-10-17, 3pm	2.97	24.97
5	06-10-17, 10am	2.95	24.96
6	06-10-17, 3pm	2.95	24.92
Mean		2.95	24.95
Standard deviation		0.021	0.027
%RSD		0.711	0.108

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

Replicates	Time interval	Concentration in µg/ml	
		Ramipril	Metoprolol Succinate
1	10:00AM	2.93	24.91
2	11:00AM	2.95	24.93
3	12:00 PM	2.94	24.96
4	1:00 PM	2.99	24.93
5	2:00 PM	2.96	24.92
6	3:00 PM	2.93	24.96
Mean		2.95	24.93
Standard deviation		0.022	0.021
%RSD		0.745	0.084

3.3.5 Limit of Detection (LOD)

Limit of detection was calculated by using formula

DL = 3.3 σ / S

Where, σ – Standard deviation of response (Y intercept)

S – Slope of calibration curve.

Table 11: Report of LOD of Ramipril and Metoprolol Succinate

Drug	LOD (µg/ml)
	Visualization
Ramipril	1.54
Metoprolol Succinate	51.59

3.3.6 Limit of Quantitation (LOQ)

Limit of quantitation was calculated by using formula.

DL = 10 σ / S

Where, σ – Standard deviation of response (Y intercept)

S – Slope of calibration curve.

Table 12: Report of LOQ of Ramipril and Metoprolol Succinate

Drug	LOD (µg/ml)
	Visualization
Ramipril	4.683
Metoprolol Succinate	156.3

3.3.7 Robustness

Robustness was determined by changing the wavelength.

Table 13: Change of Wavelength used for analysis of RAMI and METO

RAMI µg/ml	Wavelength (nm)			METO µg/ml	Wavelength (nm)		
	203	205	207		273	275	277
21	0.8012	0.7011	0.6312	20	0.0898	0.0896	0.0894
21	0.8122	0.7121	0.6302	20	0.0897	0.0897	0.0894
21	0.8140	0.7139	0.6321	20	0.0899	0.0896	0.0895
21	0.8010	0.7009	0.6344	20	0.0897	0.0896	0.0894
21	0.8211	0.7210	0.6332	20	0.0897	0.0898	0.0896
21	0.8130	0.7129	0.6313	20	0.0897	0.0896	0.0894
Mean	0.8104	0.7103	0.6320	Mean	0.0897	0.0896	0.0894
S.D.	0.0111	0.007	0.001	S.D.	0.0001	0.0001	0.0001
%RSD	1.36	0.98	0.15	%RSD	0.1114	0.1116	0.1118

4. CONCLUSION

This method (zero order derivative method) found to be economic, simple, and accurate. Beer-Lambert's Law was obeyed in the concentration range of 3-30µg/ml and 20-200µg/ml for Ramipril and Metoprolol Succinate respectively, with co-efficient of correlation, (R^2) =0.9994 and (R^2) = 0.9986 for RAM and METO, respectively. Straight line equations were obtained from these calibration curves. From the examination of the zero derivative spectra of Ramipril and Metoprolol Succinate, 205nm (λ1) and 275nm (λ2) were selected as working wavelengths for the zero-order derivative spectroscopy. 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.

5. ACKNOWLEDGEMENTS

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