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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF RAMIPRIL HCL AND METOPROLAL TARTRATE IN PURE AND IN COMBINATIONS BY UV SPECTROPHOTOMETRY AND RP-HPLC

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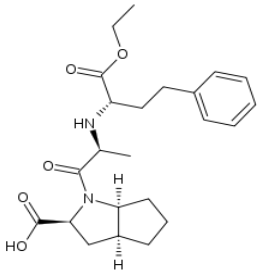
ABSTRACT

Simple, rapid and accurate UV Spectroscopic (Simultaneous Equation method and First order derivative method) and an isocratic RP – HPLC methods showed excellent sensitivity, reproducibility, accuracy, and repeatability. In simultaneous UV method, the overlaid spectra of mixture of Ramipril HCl and Metoprolol Tartrate were recorded. From the spectra, 206nm for Ramipril HCl and 222nm for Metoprolol Tartrate was selected as wavelength to construct simultaneous equation. The percentage label claim present in tablet formulation was found to be 101% and 103% for Ramipril HCl and Metoprolol Tartrate respectively. In second method, the same spectrums were derivatised and 205 nm selected for detection of Ramipril HCl where Metoprolol Tartrate shows zero crossing and also 210 nm selected for detection of Metoprolol Tartrate where Ramipril HCl shows zero crossing. The percentage label claim present in formulation was found to be 98.5% and 101% for Ramipril HCl and Metoprolol Tartrate respectively. In RP-HPLC method, mobile phase used is acetonitrile: methanol: acetate buffer pH 5.0 (30:50:20 V/V) with flow rate of 0.9 ml per min, the retention time of Metoprolol Tartrate and were found to be 2.84 and, 3.55respectively. The percentage purity was found to be 99.76% and 99.82% for Metoprolol Tartrate and Ramipril HCl, respectively. The low % RSD values for recovery indicated that the method was found to be accurate.

Keywords: Analytical Method Development and Validation, Ramipril, Metoprolal, UV Spectrophotometry, RP-HPLC.

INTRODUCTION

Ramipril HCl, the active metabolite, competes with angiotensin I for binding at the angiotensin-converting enzyme, blocking the conversion of angiotensin I to angiotensin II. As angiotensin II is a vasoconstrictor and a negative-feedback mediator for renin activity, lower concentrations result in a decrease in blood pressure and an increase in plasma renin. Ramipril HCl may also act on kininase II, an enzyme identical to ACE that degrades the vasodilator bradykinin [1-3].



RAMIPRIL HCL

Molecular Formula: $C_{23}H_{32}N_2O_5$

Chemical Name: [2s,3as,6as]-1-[(2s)-2-{[(1s)-1-(ethoxycarbonyl)-3-phenyl propyl] amino}-1-oxopropyl]-octahydrocyclopenta(b) pyrrole-2-carboxylic acid

Molecular Weight: 416.5 g / mol

METOPROLOL TARTRATE SUCCINATE

Like betaxolol and atenolol, Metoprolol Tartrate competes with adrenergic neurotransmitters such as catecholamines for binding at beta(1)-adrenergic receptors in the heart and vascular smooth muscle. Beta(1)-receptor blockade results in a decrease in heart rate, cardiac output, and blood pressure.

Molecular Formula: $C_{15}H_{25}NO_3$

Chemical Name: 1-[4-(2-methoxyethyl)phenoxy]-3-(Propan-2- ylamino) propan-2-ol

Molecular Weight: 267.36 g / mol

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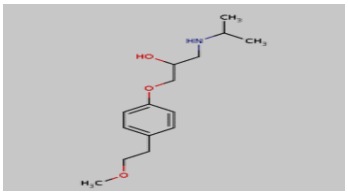


METOPROLOL TARTRATE SUCCINATE

MATERIALS AND METHODS

Methods employed for UV-spectroscopy [7-9]

In the present work an attempt was made to develop and validate simple, precise and accurate method for the estimation of Ramipril HCl and Metoprolol Tartrate in pure and in combined tablet dosage form by UV-spectrophotometry and RP-HPLC method.

UV-Spectrophotometry

A. Simultaneous equation method
B. Derivative spectrophotometric method

Simultaneous Equation method

The linearity was carried out individually for both the drugs adequate dilution were made from stock solution to get concentration ranging from 1-5 mcg / ml in Ramipril HCl and Metoprolol Tartrate concentration ranging from 10-50 mcg / ml. Absorbance of these solutions were measured at 206 and 222 nm.

Derivative method

Adequate dilution were made from stock solution to get concentration ranging from 1-5 mcg / ml in Ramipril HCl and Metoprolol Tartrate concentration ranging from 10-50 mcg / ml. These solutions were scanned and derivatised to produce first derivative spectra ($\Delta\lambda$=1). Derivatised values were measured at 205 nm and 222 nm.

Selection of Solvent

The solubility and stability for both Ramipril HCl and Metoprolol Tartrate were evaluated. The absorbance of both drugs were higher and exhibited distinct λmax in methanol followed by distilled water in the final dilution, since it was decided to prepare drug solutions in methanol followed by distilled water.

Preparation of Standard Stock Solution

Pure raw materials Ramipril HCl and Metoprolol Tartrate 100 mg were accurately weighed and dissolved separately in methanol, followed by distilled water to produce 100 mcg / ml solutions.

Selection of Wavelength

For the selection of wavelength for estimation of Ramipril HCl and Metoprolol Tartrate, a suitable standard solution to contain a 10 mcg/ml of Ramipril Hcl and Metoprolol Tartrate were prepared individually and scanned in the entire range from 200-400 nm, an overlain spectra was made. From the spectra the λmax of Ramipril HCl was found to be 206 nm and λmax for Metoprolol Tartrate was 222 nm. Hence, the λmax of two drugs was selected for the simultaneous equation method. For Derivative Spectroscopic method, the zero order spectrum was derivatised to first order spectrum in that 222 nm was selected for the estimation of Ramipril HCl, which is zero crossing for Metoprolol Tartrate and 205 nm was selected for the estimation of Metoprolol Tartrate which is zero crossing for Ramipril HCl [4-6].

Quantification in Formulation [10-12]

Twenty tablets were weighed and average weight was found out and it was finely powdered. Powdered tablet equivalent to 25 mg of Metoprolol Tartrate was transferred to a 25 ml standard flask and the content of the flask was dissolved in methanol by sonication for 15 minutes and then made up to the required volume. This solution was then filtered through whatmann filter paper (no.41).The solution was diluted to get a concentration of 30 mcg/ml using distilled water. Absorbance of the diluted sample solutions were measured for the methods as described in above said wavelengths.

VALIDATION

Precision

Precision of the method was demonstrated by repeatability studies. Repeatability studies were done by consequently analyzing the sample solution for six times. Intraday and inter day precision were established by repeating the determination on the same day and on three consecutive days.

Recovery Studies

In order to ensure the accuracy of the proposed methods recovery studies were carried out. To the pre-analyzed sample solution, a definite concentration was added and then its recovery was studied. 1ml of pre-analyzed formulation was taken in three separate 10 ml volumetric flasks, with these, known concentration of pure drug samples (Ramipril Hcl and SMetoprolol Tartrate) at 60%, 80% and100% levels were added. The absorbances of resulting solutions were measured at their corresponding wavelengths and the percentage recovery was then calculated.

Limit of Detection and Limit of Quantification

Preparation of calibration curve from the serial dilution of standard was repeated for six times. The limit of detection and limit of quantification was calculated by using the average value of slope and standard deviation.

Loss on drying = 3σ / s

Correlation co-efficient = 10σ / s

RP-HPLC Method

Chromatographic method depends up on the nature of the sample, molecular weight solubility etc., the drug selected for the present study is polar compound, can be

separated either by normal phase or reverse phase chromatography. Reverse phase chromatographic technique was selected for initial separations from the knowledge of properties of compound, C_{18} column was chosen as stationary phase and various mixtures of phosphate buffer (with different pH), acetate buffer, methanol and acetonitrile were considered as mobile phase.

Selection of Mobile Phase and λmax

Different mixtures of mobile phase with different ratios were selected and their chromatograms were recorded. From this acetate buffer (pH -5) Acetonitrile: Methanol was selected as mobile phase, since these two drugs were eluted with sharp peak and with better resolution. Hence this mobile phase was used to optimize the chromatographic conditions.

Optimized Chromatographic Conditions

The following parameters were used for RP-HPLC analysis of Metoprolol Tartrate and Ramipril HCl,
Mode of operation: Isocratic
Stationary phase: C_{18} column (150mm × 4.6 mm I, d., 5m)
Mobile phase: Acetate buffer (pH-5): Methanol: acetonitrile
Ratio: 20:50:30
Detection wavelength: 210 nm
Flow rate: 1 ml / min
Temperature: Ambient
Sample volume: 20 µl
Operating pressure: 150 kgf

Preparation of the Standard Stock Solution

Weighed accurately 25 mg of Ramipril HCl and Metoprolol Tartrate, transferred into a 25 ml standard volumetric flask separately and dissolved with minimum quantity of methanol and the volume was made up to the mark with methanol. From the above solutions 1 ml were transferred to a 10 ml flask and diluted with water to get the concentration of 100 µg / ml.

Linearity and Calibration

From the standard solution, pipetted 0.5-2.5 ml into a series of five 10ml flask and made up to the mark with mobile phase to obtain the concentration range from 5-25 mcg/ml for Metoprolol Tartrate and 0.5-2.5 mcg/ml for Ramipril HCl solution were injected and chromatogram was recorded. The calibration curve was plotted between concentration and peak area.

Quantification of Ramipril HCl and Metoprolol Tartrate

Twenty tablets containing 2.5 mg of Ramipril HCl and 25 mg Metoprolol Tartrate were accurately weighed and powdered tablets equivalent to 25 mg of Metoprolol Tartrate was transferred to a 25 ml volumetric flask and dissolved in methanol and sonicated for 15 minutes. The final concentration was 1000 mcg / ml. The above solution, was filtered through whatmann filter paper and the clear solution was collected, 1 ml was pipetted into a 10 ml volumetric flask and made up to the mark with mobile phase to produce 10 mcg/ml solutions. The peak area measurements were done by injecting sample (20 µl) six times and the amount of Ramipril HCl and Metoprolol Tartrate were calculated from their respective calibration curve.

Recovery Studies

To ensure the reliability of the method, recovery studies were carried out by mixing a known quantity of standard drug solution with the pre-analyzed sample formulation and the content were mixed and made to the volume with mobile phase and re-analyzed by the proposed method, the percentage recovery was calculated.

Limit of Detection and Limit of Quantification

Preparation of calibration curve from the serial dilution of standard was repeated for six times. The limit of detection and limit of quantification were calculated by using the average value of slope and standard deviation of response (intercept).

System Suitability Studies

The system suitability studies were carried out as specified in I.P. the parameter like column efficiency, tailing factor, asymmetric factor, theoretical plate number and were calculated.

RESULTS AND DISCUSSION

UV-Spectroscopic Studies

The solubility of Ramipril HCl and Metoprolol Tartrate were determined in a variety of solvents using schefter and higuchi method. 10 mg samples were taken in test tube and checked their solubility with variety of solvents as per IP and the profiles.

From the solubility studies, methanol followed by distilled water was chosen as solvent for UV- visible spectroscopic studies in bulk and in formulations. Based upon its easy availability, cost factor and the stability conditions methanol and water was selected as solvent. Two simple, sensitive and precise UV methods namely, Simultaneous equations method, and Derivative spectroscopic methods were selected for the determination of Ramipril HCl and Metoprolol Tartrate in pharmaceutical formulations.

The drugs were dissolved in methanol and followed by water to produce µg / ml. Scanned in the range of 400-200nm and it shows constant λmax at 222 nm for Metoprolol Tartrate and 206 nm for Ramipril HCl and overlain spectra was made. This is shown in Fig. 1, 2 and 3 respectively Stability of absorbance at their λmax was also checked.

The linearity of Ramipril HCl and Metoprolol Tartrate was constructed in the range of 1-5 and 10-50 mcg/ml and their calibration curves were shown in the Fig. 4 and 5 respectively. The optical characteristics such as Beer's law limit (1-5 and 10-50 mcg/ml), molar extinction co-efficient, sandell's sensitivity, correlation co-efficient,

Figure 1. UV-Spectrum of Metoprolol Tartrate

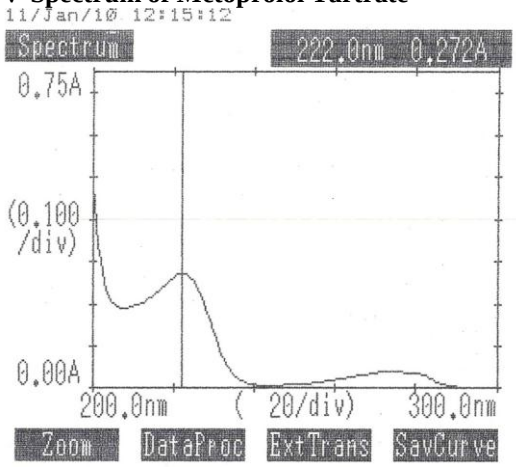


Figure 2. UV-Spectrum of Ramipril HCl

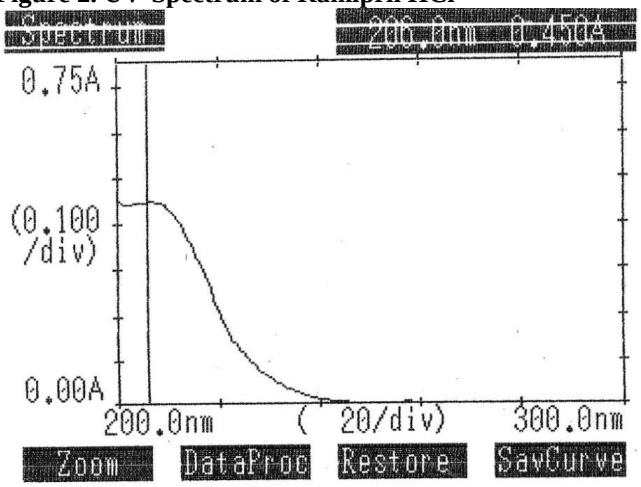


Figure 3. Overlain Spectrum of Metoprol and Ramipril HCl

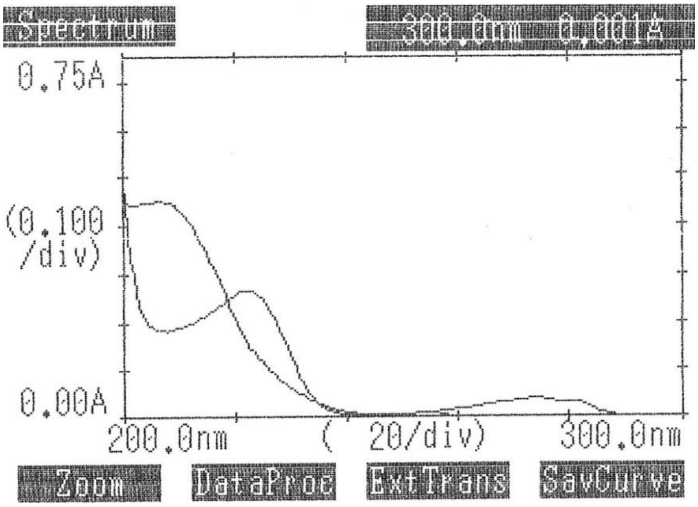


Figure 4. Calibration Curve for Metoprolol Tartrate at 206 nm

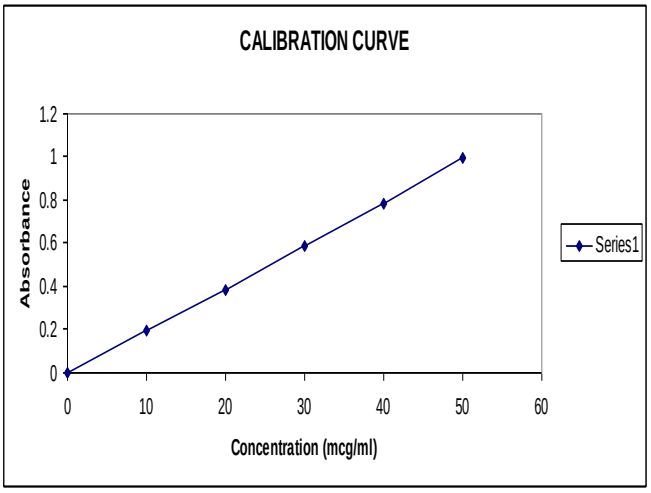


Figure 5. Calibration Curve for Metoprolol Tartrate at 222 nm

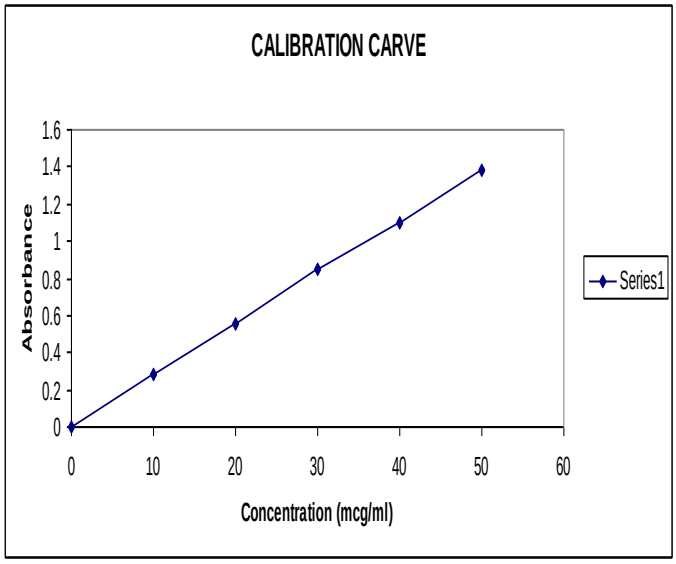


Figure 6. Calibration Curve for Ramipril HCl At 206 nm

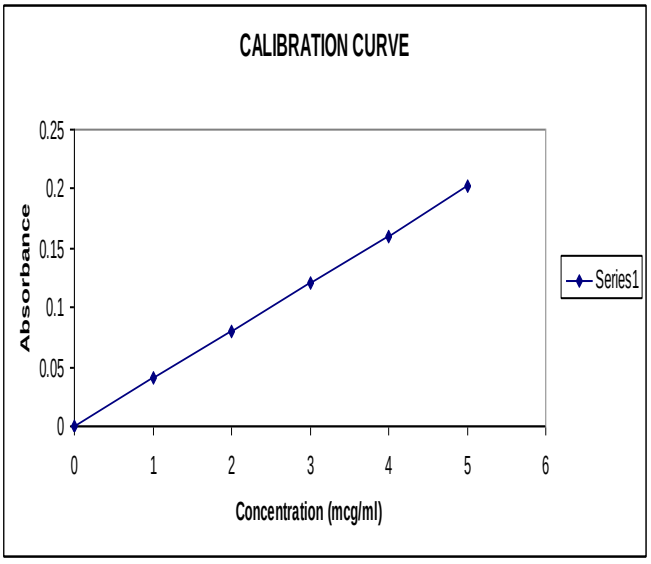


Figure 7. Calibration Curve for Ramipril HCl At 222 nm

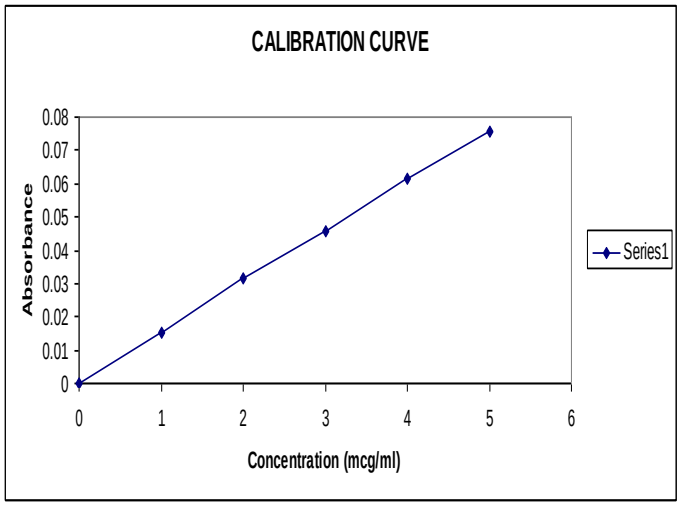


Figure 8. Derivative Spectrum of Metoprol Tartrate

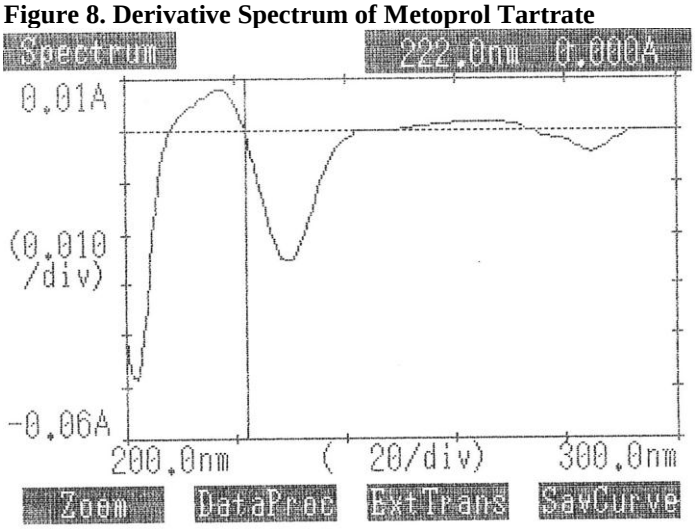


Figure 9. Derivative Spectrum of Ramipril HCl

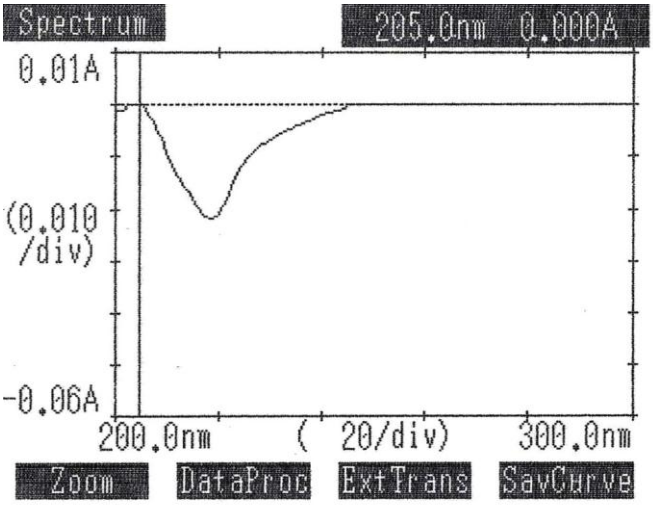


Figure 10. Overlain Derivative Spectrum of Metoprolol Tartrate And Ramipril HCl

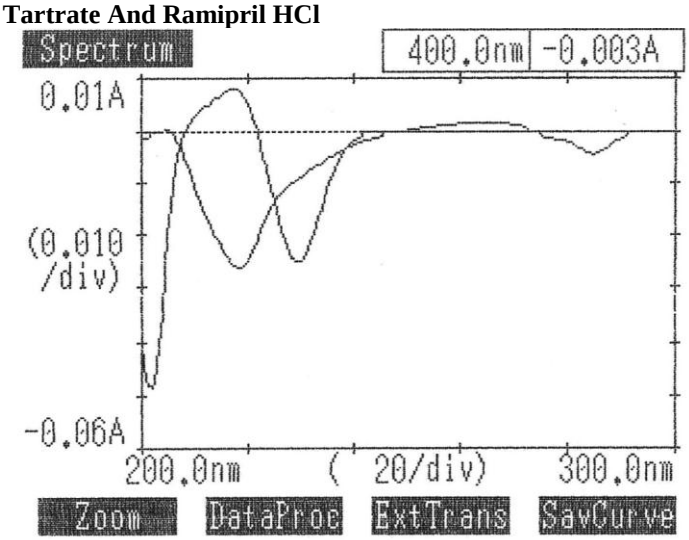


Figure 11. Calibration Curve For Metoprolol Tartrate at 205 Nm By Derivative Spectroscopy

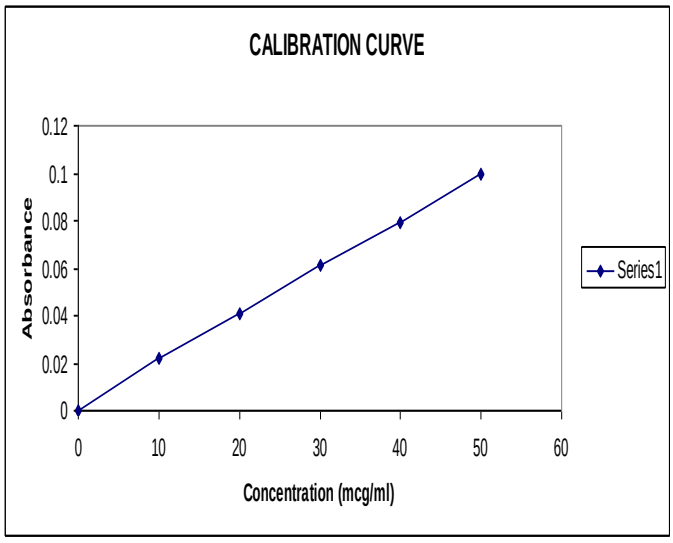


Figure 12. Calibration Curve for Ramipril HCl at 222 nm by Derivative Spectroscopy

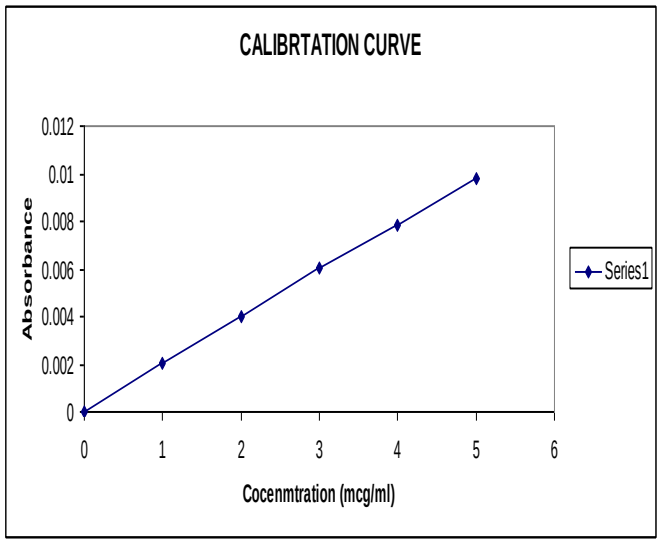


Figure 13. Calibration Curve for Metoprolol Tartrate By RP-HPLC

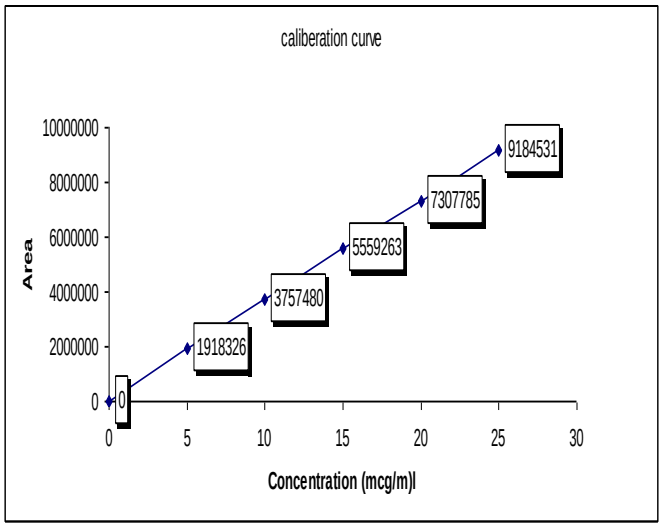


Figure 14. Calibration Curve For Ramipril HCl RP-HPLC

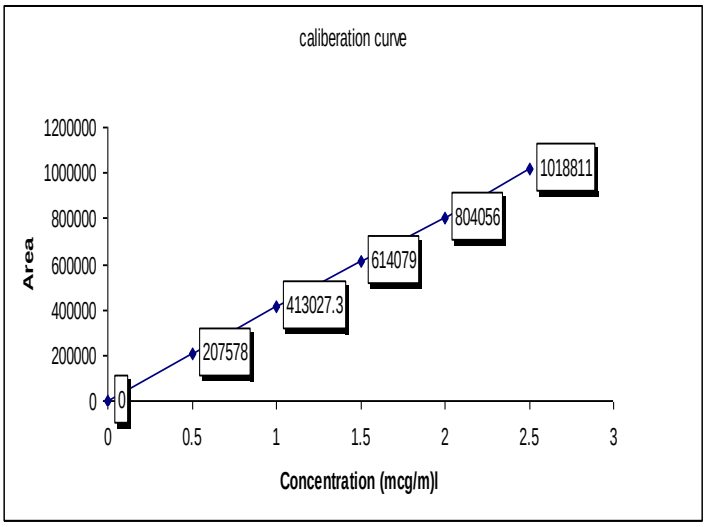
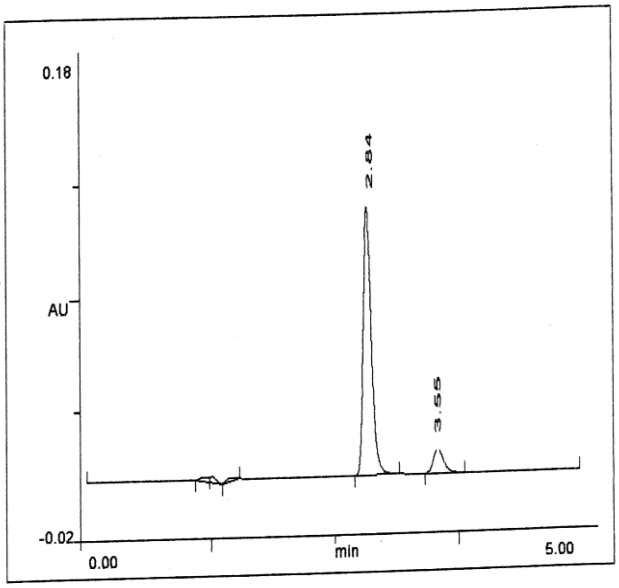


Figure 15. Quantification of Metoprol and Ramipril HCl in Formulation

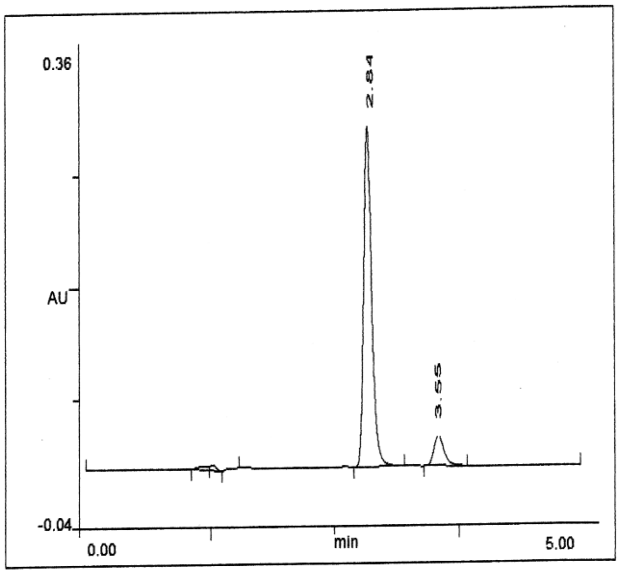
Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	65	5	4	5.0



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	2.84	26353	3698325	91.9440	90.0617	BB	0.094
2	3.55	2309	402594	8.0560	9.9383	BB	0.119
		3e+04	4050919				

Figure 16. Linearity Chromatogram of Metoprolol and Ramipril HCl (25 mcg/ml)

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	65	5	4	5.0



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	2.84	66797	9060931	91.8867	89.8669	BB	0.093
2	3.55	5898	1021681	8.1133	10.1331	BB	0.118
		7e+04	10082612				

Table 1. Assay of Commercial Formulation by UV- Spectroscopy (Simultaneous Equation Method)

Formulation	Drug	S No	Amount Labeled (mg/tab)	Amount Estimated (mg/tab)	% Amount found	S.D (+/-)	R.S.D	S.E
PROLOMET-R	MET	1	10	9.836	98.36	0.977	0.978	0.398
		2		9.993	99.36			
		3		9.774	97.74			
		4		10.59	105.9			
		5		10.11	101.1			
		6		9.893	98.93			
	RAM	1	50	50.88	101.3	0.863	0.86	0.378
		2		49.13	98.81			
		3		50.47	104.7			
		4		49.8	99.87			
		5		49.54	99.46			
		6		48.81	98.12			
SELORAM™	MET	1	20	20.89	104.4	1.147	1.154	0.811
		2		19.14	95.73			
		3		20.58	102.9			
		4		19.47	97.35			
		5		19.36	96.87			
		6		19.83	99.15			
	RAM	1	50	50.14	100.2	0.86	0.960	0.55
		2		50.48	100.9			
		3		48.91	97.82			
		4		49.45	98.91			
		5		49.81	99.63			
		6		49.31	98.63			

MET: Metoprolol Tartrate; RAM: Ramipril HCl

Table 2. Assay of Commercial Formulation by UV-Spectroscopy (Derivative Spectroscopy Method)

Formulation	Drug	S No	Amount Labeled (mg/tab)	Amount Estimated (mg/tab)	% Amount found	S.D (+/-)	R.S.D	S.E
PROLOMET-R	MET	1	10	9.89	98.93	1.7	1.07	0.43
		2		10.16	101.6			
		3		9.62	96.2			
		4		10.43	104.3			
		5		9.83	98.33			
		6		10.18	101.8			
	RAM	1	50	50.36	100.3	0.46	0.46	0.18
		2		50.41	100.4			
		3		49.81	98.12			
		4		48.21	98.72			
		5		48.44	98.74			
		6		51.21	102.7			
SELORAM™	MET	1	20	20.81	104.5	2.02	2.01	0.43
		2		19.38	96.90			
		3		20.34	101.7			
		4		19.26	96.3			
		5		20.16	100.8			
		6		19.98	99.93			
	RAM	1	50	51.12	102.2	1.42	1.43	0.82
		2		48.41	96.82			
		3		49.21	98.42			
		4		49.91	99.82			
		5		50.63	101.2			
		6		50.41	100.8			

MET: Metoprolol Tartrate; RAM: Ramipril HCl

Table 3. Precision (Interday)

Formulation	Drug	S No	Amount Labeled (mg/tab)	Amount Estimated (mg/tab)	% Amount found	S.D (+/-)	R.S.D	S.E
PROLOMET-R	MET	1	10	9.833	98.33	1.33	1.03	0.76
		2		10.13	101.3			
		3		10.38	103.8			
	RAM	1	50	50.89	100.8	0.98	0.99	0.56
		2		48.25	98.25			
		3		49.88	98.42			
SELORAM™	MET	1	20	19.69	98.45	0.11	0.13	0.06
		2		20.31	101.5			
		3		19.17	95.85			
	RAM	1	50	49.69	99.38	0.24	0.24	0.12
		2		51.55	103.1			
		3		47.24	94.62			

MET: Metoprolol Tartrate; RAM: Ramipril HCl

Table 4. Precision (Intraday)

Formulation	Drug	S No	Amount Labeled (mg/tab)	Amount Estimated (mg/tab)	% Amount found	S.D (+/-)	R.S.D	S.E
PROLOMET-R	MET	1	10	9.711	97.11	0.67	0.64	0.31
		2		9.96	99.65			
		3		10.03	100.3			
	RAM	1	50	50.89	100.8	0.88	0.83	0.34
		2		48.25	98.25			
		3		49.88	98.96			
SELORAM™	MET	1	20	19.69	98.54	1.74	1.43	0.58
		2		20.38	101.9			
		3		20.83	104.2			
	RAM	1	50	49.81	99.62	0.20	0.21	0.19
		2		49.25	98.5			
		3		50.91	101.8			

MET: Metoprolol Tartrate; RAM: Ramipril HCl

Table 5. Recovery Studies –Simultaneous Equation Method

Formulation	Drug	S No	Amount Present (µg/ml)	Amount Added (µg/ml)	Amount Found (µg/ml)	Amount Recovered (µg/ml)	% Recovery
PROLOMET-R	MET	1	0.8	4	4.78	3.98	99.54
		2	0.8	6	6.91	6.10	101.6
		3	0.8	8	8.85	8.05	100.6
	RAM	1	4	4	7.94	3.94	98.52
		2	4	6	10.5	6.05	100.8
		3	4	8	12.23	8.23	102.9
SELORAM™	MET	1	1.6	4	5.73	4.13	103.2
		2	1.6	6	7.58	5.98	99.68
		3	1.6	8	9.59	7.99	99.87
	RAM	1	4	4	8.41	4.11	101.2
		2	4	6	9.87	5.87	97.83
		3	4	8	12.1	8.19	102.3

MET: Metoprolol Tartrate; RAM: Ramipril HCl

Table 6. Recovery Studies – Derivative Spectroscopy Method

Formulation	Drug	S No	Amount Present (µg/ml)	Amount Added (µg/ml)	Amount Found (µg/ml)	Amount Recovered (µg/ml)	% Recovery
PROLOMET-R	MET	1	0.8	4	4.85	0.85	101.6
		2	0.8	6	6.91	0.91	103.7
		3	0.8	8	8.91	0.91	106.2
	RAM	1	4	4	7.89	3.89	97.25
		2	4	6	10.22	4.22	105.5
		3	4	8	12.41	4.41	110.2
SELORAM™	MET	1	1.6	4	5.54	1.54	96.25
		2	1.6	6	7.71	1.71	106.8
		3	1.6	8	9.90	1.78	102.7
	RAM	1	4	4	8.21	4.21	105.25
		2	4	6	9.94	3.94	98.5
		3	4	8	11.89	3.89	97.25

MET: Metoprolol Tartrate; RAM: Ramipril HCl

Table 7. Optical Characteristics of Metoprolol Tartrate and Ramipril HCl BY RP-HPLC Method

Parameters	Metoprolol Tartrate	Ramipril HCl
λ max	210 nm	210 nm
Beer's law limit(µg/ml)	5-25	0.5-2.5
Correlation Co- efficient	0.9998	0.9999
Slope (m)	272159.04	187484.83
Intercept (c)	30617.3	-5990.75
LOD (µg/ml)	0.1919119	0.62941843
LOQ (µg/ml)	0.581549668	1.9073285
Standard Error	329160.45	209542.88

Table 8. Assay of Commercial Formulation (SELORAM™) by RP-HPLC Method

Drug	Sample No.	labeled Amount (mg/tab)	Amount Found (mg/tab)	Percentage Obtained	Average %	S.D (+/-)	% R.S.D
MET	1	10	10.44	104.4	100.45	0.54	0.51
	2	10	10.26	102.6			
	3	10	10.48	104.8			
	4	10	9.932	99.32			
	5	10	9.442	94.48			
	6	10	9.72	97.2			
RAM	1	50	50.32	100.64	98.96	0.76	0.71
	2	50	49.50	99.01			
	3	50	50.56	101.12			
	4	50	47.86	95.72			
	5	50	45.84	94.68			
	6	50	48.3	96.6			

MET: Metoprolol Tartrate; RAM: Ramipril HCl

Table 9. Recovery Studies of Metoprolol Tartrate and Ramipril HCl by RP-HPLC Method

Drug	% level	Amount Present (µg/ml)	Amount Added (µg/ml)	Amount Estimated (µg/ml)	Amount Recovered	% Recovery	S.D (+/-)	% R.S.D
MET	150	2.5	5	7.876	2.876	105.01	1.93	1.88
	200	2.5	7.5	11.34	6.34	113.4		
	250	2.5	10	11.19	6.19	89.54		
RAM	100	12.5	2.5	14.94	2.45	99.96	0.43	0.43
	120	12.5	5	17.69	5.19	101.18		
	130	12.5	7.5	18.98	7.48	99.90		

Table 10. System Suitability

Parameters	Metoprolol Tartrate	Ramipril HCl
Tailing factor	1.30	1.22
Asymmetrical factor	1.44	1.33
Capacity factor	0.82	4.37
Theoretical plate per unit length	36.26	53.54
Resolution	18.02	

slope and intercept were calculated. The Limit of detection and the Limit of quantification were determined from the linearity studies which was done 6 times and calculated by using slope and standard deviation of response (intercept).

The formulations PROLOMET-R, SELORAM™ were selected for analysis. The amount present were determined by calculating the average of six replicate analysis and its percentage purity was found to be in the range of 97-103 % by the two methods. It is shown in Table 1and 2 respectively.

To evaluate the accuracy of the method, recovery studies were carried out, known amount of pure drug was added to the previously analyzed solution containing formulation and the mixture was re-analyzed by the proposed methods, and their recoveries were calculated. The percentage recovery of Ramipril HCl and Metoprolol Tartrate in the formulations PROLOMET-R and SELORAM™ were found to be in the range of 97-103%. These values are shown in Table 5 and 6.

Precision of the method was studied by making repeated analysis of the same sample and it was carried out three times in a day and for three days. The % RSD and standard deviation for inter-day and intraday analysis was found to be less than 2 indicate the methods is precise, which are shown in Table 3 and 4.

RP-HPLC Method

An involvement was made in this project to device, a simple, accurate, less expensive and sensitive RP-HPLC method of estimation of Ramipril HCl and Metoprolol Tartrate I solid dosage form. Since the drug is polar reverse phase high performance liquid chromatography was selected.

Acetonitrile was preferred because of its lower viscosity and high UV transparency; Methanol was selected due to its inexpensiveness. Acetonitrile: Methanol: Water in the ratio 40: 20: 40 was selected. This give tailing and broaden peaks, hence choice of buffers were incorporated. An attempt was made in phosphate buffer of pH 4 instead of water in different ratio, this also gave broaden peaks. Hence attempt was made in different pH and different ratio of acetate buffer: Methanol: Acetonitrile in the ratio 10: 60: 30, this also gave merged and broad peak, and hence acetate buffer pH 5: Methanol: Acetonitrile, in the ratio 20: 60: 20 this also gave sharper peaks.

The detection wavelength was measured by scanning the solution of Ramipril HCl and Metoprolol Tartrate in mobile phase. In UV-spectrophotometry, over lining spectra and select the wavelength of maximum absorption was selected as 220nm. With the optimized chromatographic conditions, stock solutions of Ramipril HCl and Metoprolol Tartrate were prepared in mobile phase and prepared the mixture in the concentration range 0.5-2.5µg / ml of Ramipril HCl and 5-25µg / ml of Metoprolol Tartrate. 20µl of each solution was injected and records the chromatogram at 220nm.

The chromatogram as Fig. 15 and 16, the calculation curve was plotted using concentration against peak area. The procedure was repeated for three times. The correlation coefficient was found to be above 0.999 for all the drugs. The calibration graph of Ramipril HCl and Metoprolol Tartrate are shown in Fig. 13 and 14 respectively. The optical characteristics of Ramipril HCl and Metoprolol Tartrate shown in table 7. The tablet dosage form Prolomet-R was selected for the analysis. The ostensible concentration 1µg / ml of Ramipril HCl and 10µg / ml of Metoprolol Tartrate in the mobile phase were prepared. 20µl of each solution was injected and chromatograms were recorded. The percentage purity was found to be 99.82% Ramipril HCl and 99.76% of Metoprolol Tartrate respectively.

The precision of the method was confirmed by repeatability of formulation for six times and the chromatograms are shown in fig. 15. The percentage RSD was found to be 1.43 respectively. The data is shown in table 9.

The accuracy of the method was performed by recovery studies to the pre analysed formulation, a known quantity of Ramipril HCl and Metoprolol Tartrate raw material solutions were added at different levels, injected the solutions. The percentage recovery was found to in the range between 101% Ramipril HCl and 100% Metoprolol Tartrate. The percentage RSD was found to be 0.64 and 0.83 respectively. The low percentage of RSD values for recovery indicated that the method was found to be accurate. The high percentage recovery revealed that no interference was produced due to the excipients used in formulation. Therefore developed method was found to be accurate.

All the above parameters with the ease of operations ensure that the projected methods could be applied for the routine analysis of Ramipril HCl and Metoprolol Tartrate n pure form and in tablet dosage form.

SUMMARY AND CONCLUSION

Ramipril HCl is a long-acting angiotensin-converting enzyme inhibitor. It is a prodrug that is transformed in the liver to its active metabolite Ramipril

HCl. Metoprolol Tartrate is a selective adrenergic beta-1-blocking agent with no stimulatory action. It's binding to plasma albumin is weaker than alprenolol and it may be useful in angina pectoris, hypertension, or cardiac arrhythmias.

The proposed analytical methods are simple, economical, rapid, sensitive, reproducible and accurate for the estimation of Ramipril HCl and Metoprolol Tartrate. The methods adopted for studies were

1. UV- spectroscopic method for the estimation of Ramipril HCl and Metoprolol Tartrate in combined dosage form by

- Simultaneous equations method
- Derivative spectroscopic method

2. RP-HPLC method.

The drug samples containing Ramipril HCl and Metoprolol Tartrate in combined dosage forms were analyzed by UV-spectroscopic method using methanol followed by distilled water as a solvent and the contents of drug determined in each formulations (PROLOMET-R and SELORAM™) were found to be satisfactory.

Simultaneously, RP-HPLC method has been developed for the estimation of both drugs in bulk and in formulation. The proposed method gives reliable assay results with short analysis time, using mobile phase Acetate buffer (pH-5): Methanol: Acetonitrile in the ratio of 20:50:30. The contents of drug present in the formulation were found to be satisfactory and system suitability parameters are in desired limit.

All the above methods do not suffer from any interference due to common excipients. It indicates that methods were accurate. Therefore the proposed methods could be successfully applied to estimate commercial pharmaceutical products containing Ramipril HCl and Metoprolol Tartrate.

Thus the above studies findings would be helpful to the analytical chemists to apply the analytical methods for the routine analysis of the analyte in pharmaceutical dosage forms after their approval from FDA.

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