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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF METOPROLOL SUCCINATE AND RAMIPRIL IN BULK AND MARKETED FORMULATION

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ABSTRACT

The main aim of this research work is to estimate and validate the Metoprolol Succinate and Ramipril in bulk and marketed formulation. Method development was carried out by autosampler HPLC Shimadzu 2030C 3D plus was used with stationary phase YMC column (150 × 4.6 mm, 3 µm) with ambient temperature. The mobile phase-A consisting of 0.1 ml orthophosphoric acid in 100 ml water and mobile phase-B consisting of Acetonitrile in gradient mode was pumped into the column at a flow rate of 1.0 mL/min. The injection volume was 20 µL with photo diode array detector at 225 nm. Validation was done according to ICH Q2 (R1) guidelines. Linearity for Metoprolol Succinate was 50-250µg/mL, and Ramipril was 10-50µg/mL with the correlation coefficient [R^2] 0.9998and 0.9994. The percentage recoveries were found to be within limits of acceptance criteria between the ranges of 98-102%. Precision results were found to be within limits and method was found to be robust with %RSD limit of NMT 2.0. The method was validated statistically and was applied successfully for estimation of Metoprolol Succinate and Ramipril. All the parameters like theoretical plates, resolution, tailing factor and % RSD was within the acceptance limits. Hence the proposed method can be successfully applied to routine analysis.

Keywords: Metoprolol Succinate, Ramipril, RP-HPLC, Validation

INTRODUCTION

Metoprolol Succinate **(Figure 1)**, it is {butanedioic acid; 1-[4-(2-methoxyethyl) phenoxy]-3-(propan-2-ylamino) propan-2-ol. Ithas a molecular formula of $C_{35}H_{56}N_2O_{10}$and molecular weight of 652.8 g/mol. Metoprolol succinate is an antihypertensive agent (β1-Adrenergic blocker). Adrenergic beta-antagonists are used for treatment of hypertension,cardiac arrhythmias, angina pectoris, glaucoma, migraine headaches and anxiety. Metoprolol competes with a drenergic neurotransmitters such as catecholamines for binding at β1-adrenergic receptors inthe heart. β1-receptorblockade results in a decrease in heart rate, cardiac output, and blood pressure **[1]**.

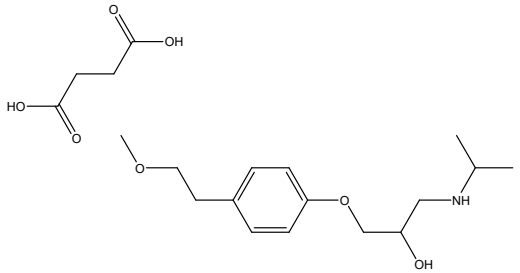

Figure 1: Structure of Metoprolol Succinate

Ramipril **(Figure 2)**, it is 4-[2-(1-ethoxycarbonyl-3-phenyl-propyl) aminopropanoyl]-4-azabicycloctane-3-carboxylic acid is a long-actingangiotensin converting enzyme (ACE) inhibitor and it is a prodrug, which is hydrolysed after absorption to form the active metabolite ramiprilate which has a long elimination half-life, permitting once daily administration. Ramipril may be effective in indications such as displayed beneficial effects in patients with moderate to serve congestive heart failure **[2]**.

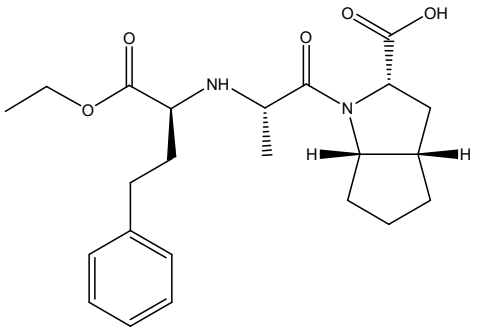

Figure 2: Structure of Ramipril

MATERIALS AND METHODS

The pharmaceutical grade samples of Metoprolol Succinate and Ramipril were procured from Sigma Aldrich. Orthophosphoric acid, Acetonitrile and HPLC grade water of analytical grade were obtained from national scientific products. The tablet formulation containing 25mg of Metoprolol Succinate and 2.5mg of Ramipril were procured from local market.

Chromatographic conditions:

A HPLC equipped with PDA detector was used. The chromatographic analysis was performed on column of YMC (150 x 4.6mm)3µm. Mobile phase-A consisting of Orthophosphoric acid and mobile phase-B is Acetonitrile was used in gradient mode, with detection of 225nm. An injection volume of 20µL was used, keeping flow rate of 1mL/min.

Preparation of Orthophosphoric acid:

Accurately 0.1mL oforthophosphoric acid was pipette out in 100mL volumetric flask

and the volume was made up to 100mL with HPLC water and it was filtered through 0.45µ membrane filter and sonicated for 10minutes.

Preparation of standard stock solution:

About 10mg of Metoprolol Succinate and Ramipril were weighed and transferred into a separate 10mL volumetric flasks and dissolved in acetonitrile and water (50:50v/v) and made up to the volume to obtain 1000µg/mL.

Preparation of sample solution:

About 10mgof tablet powder was accurately weighed which is calculated based on average weight of 20 tablets and dissolved in acetonitrile and water (50:50v/v) in 10mL volumetric flask. From the above stock solution 0.1mL was pipette out in 10mL volumetric flask and the solution was made up to the volume with acetonitrile and water (50:50v/v).

Method Development:

Optimized chromatographic conditions:

Column: YMC C_8 (150×4.6mm) 3µm

Wavelength: 225nm

Flow rate: 1.0 mL/min

Injection volume: 20µL

Run time: 11.01min

Table 1: Gradient Mode of Mobile Phase

Time (min)	MP-A (0.1% OPA)	MP-B (ACN)
0.01	75	25
3.00	75	25
5.00	50	50
7.10	25	75
9.00	75	25
11.00	75	25
11.01	Stop	

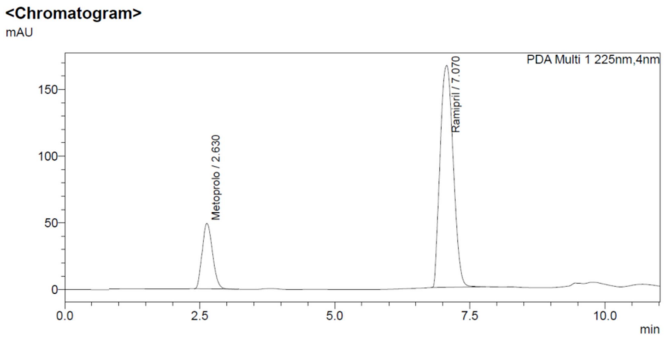

Figure 3: Standard Chromatogram

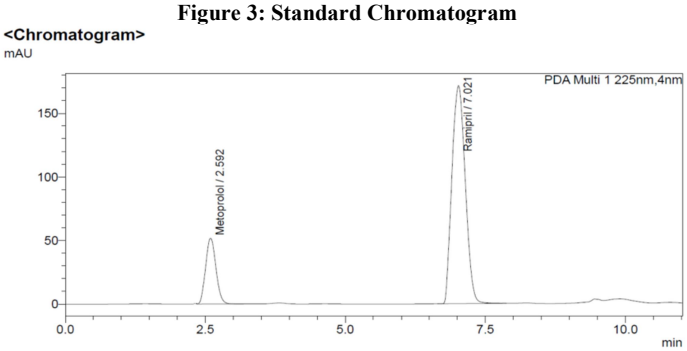

Figure 4: Sample Chromatogram

Method Validation:

The proposed method was validated according to the ICH guidelines which include system suitability, specificity, linearity, accuracy, precision, Limit of Detection (LOD), Limit of Quantification (LOQ) and robustness. Under the validation study, the following parameters were studied.

RESULTS

System Suitability:

HPLC system was optimized as per the chromatographic conditions. 20 µLof standard solutions of drugs were injected in six times into the chromatographic system. To ascertain the system suitability for the proposed method, the parameters such as retention time, number of theoretical plates, resolution, tailing factor and % RSD were calculated and compared with standard specification of system **(Table 2)**.

Specificity:

The specificity of method was determined by comparing the chromatograms of blank, standard and sample.

Linearity:

Linearity of the method was analyzed by preparing calibration curves using different concentrations of the standard solutions. Linearity was established from 50-250µg/mL for Metoprolol Succinate and 10-50µg/mL for Ramipril **(Figure 5-6, Table 2)**.

Accuracy:

The accuracy was performed by preparing known amount of samples at 50%, 100% and 150% levels. They were injected in a triplicate at each level **(Table 4)**.

Precision:

System and Method precision was carried out for Metoprolol Succinate and Ramipril by giving six injections **(Table 5)**.

Limit of Detection (LOD) and Limit of Quantification (LOQ):

The LOD of Metoprolol Succinate and Ramipril was found to be 5µg/mL and 1µg/mL and LOQ for Metoprolol Succinate and Ramipril was found to be 15µg/mL and 3µg/mL which indicates the sensitivity of the method.

Robustness:

The standard solutions of Metoprolol Succinate and Ramipril were injected by changing the chromatographic conditions like flow rate of the mobile phaseand wavelength **(Table 6)**.

Assay:

The % Purity of Metoprolol Succinate and Ramipril was found to be 100.07% and 100.70% respectively.

Table 2: Data of System Suitability

Metoprolol Succinate			Ramipril	
Injection No	Retention Time (min)	Peak Area	Retention Time (min)	Peak Area
1	2.630	653811	7.070	2823553
2	2.648	658428	7.083	2821908
3	2.75	651354	7.009	2862771
4	2.585	659494	7.024	2847090
5	2.592	662339	7.021	2848972
6	2.569	650238	7.023	2853414
Mean655944			2842951	
Standard Deviation4854.72			16581.8	
%RSD0.74			0.58	
Tailing factor		1.202	1.143	
Theoretical plates		5213	4512	

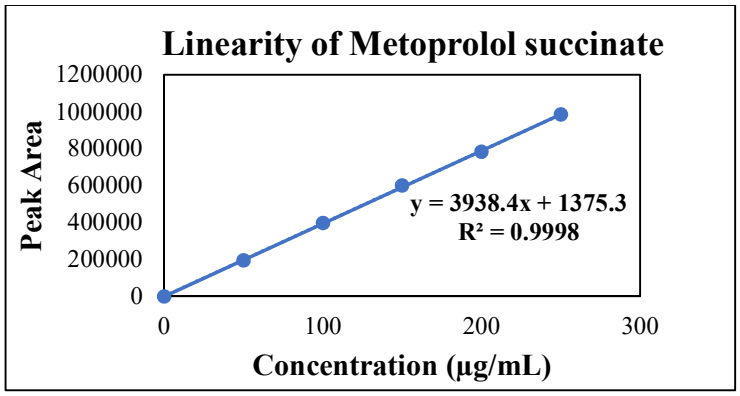

Figure 5: Calibration Curve of Metoprolol Succinate

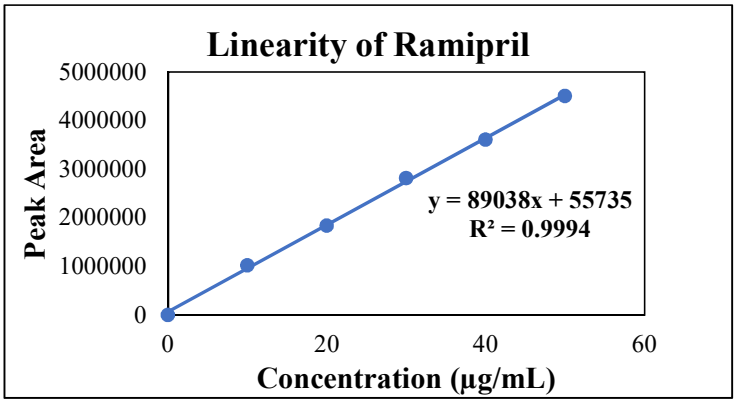

Figure 6: Calibration Curve of Ramipril

Table 3: Data of Linearity

Metoprolol Succinate		Ramipril	
Concentration (µg/mL)	Peak area	Concentration (µg/mL)	Peak area
50	195088	10	1019757
100	397871	20	1841386
150	600913	30	2816518
200	783227	40	3607326
250	984944	50	4505067
R^2= 0.9998		R^2= 0.9994	

Table 4: Data of Accuracy

Drug	% Level	Standard peak area	Sample peak area	% Recovery	% Average Recovery	% Overall Mean recovery
Metoprolol Succinate	50%	28422951	1442951	100.46	100.43	100.56%
		2842951	1425951	100.59		
		2842951	1412951	100.24		
	100%	2842951	2869415	100.55	100.19	
		2842591	2819168	99.81		
		2842591	2870991	100.22		
	150%	2842591	4308080	101.30	101.04	
		2842591	4298591	100.98		
		2842591	4306891	100.91		
Ramipril	50%	655944	330128	101.31	100.66	100.78%
		655944	329845	100.33		
		655944	328954	100.34		
	100%	655944	658745	101.05	100.67	
		655944	658745	99.90		
		655944	656897	101.06		
	150%	655944	996589	101.03	101.02	
		655944	992584	100.90		
		655944	991458	101.14		

Table 5: Data of System Precision and Method Precision

Injection No	System Precision		Method Precision	
	Metoprolol Succinate	Ramipril	Metoprolol Succinate	Ramipril
1	651411	2823553	650241	2823953
2	658418	2821908	657428	2824908
3	659354	2862771	651054	2862571
4	657944	2847090	653029	2847090
5	650149	2848972	656839	2848572
6	658958	2843514	659958	2853314
Mean	656039	2841301	654758.2	2843401
SD	4120.896	15806.93	3888.748	15660.91
%RSD	0.63	0.56	0.59	0.55

Table 6: Data of Robustness

S. No	Parameters	Metoprolol Succinate			Ramipril		
		R_t (min)	Peak area	%RSD	R_t (min)	Peak area	%RSD
1.	Change in flow rate- 0.8mL/min	3.08	601287	0.39	7. 749	2826513	0.75
		3.10	604592	0.36	7.752	2856590	0.78
2.	Change in flow rate- 1.2mL/min	2.105	606987	0.35	6.361	2828427	0.70
		2.108	603690	0.35	6.364	2856548	0.72
3.	Change in wavelength 220nm	2.575	5892856	0.27	7.009	2216548	0.48
		2.579	5870134	0.26	7.011	2231680	0.51
4.	Change in wavelength 230nm	2.585	6255530	0.25	7.024	2953142	0.56
		2.588	6279401	0.25	7.028	2976830	0.58

CONCLUSION

A simple, sensitivity, precise and specificreverse phase high performance liquid chromatography (RP-HPLC) method was developed and validated for estimation of Metoprolol Succinate and Ramipril in tablet dosage form. The separation was performed on YMC (150mm x 4.6mm, 3µm) chromatographic column with the mobile phase-A was Orthophosphoric acid buffer and mobile phase-B was Acetonitrile with the flowrate of 1.0mL/min and detection wavelength at 225nm. The developed method was validated according

to ICH guidelines. The linearityrange was 50-250µg/mL for Metoprolol Succinate and 10-50µg/mL for Ramipril with a correlation coefficient of 0.9998 and 0.9994 respectively. The percentage recoveries were found to be within limits of acceptance criteria between the ranges of 98-102%. Precision results were found to be within limits and method was found to be robust with %RSD limit of NMT 2.0. The method was validated statistically and was applied successfully for estimation of Metoprolol Succinate and Ramipril.

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