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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF METOPROLOL AND AMLODIPINE IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A new, simple, precise, accurate and reproducible RP-HPLC method for Simultaneous estimation of Metoprolol and Amlodipine in bulk and pharmaceutical formulations. Separation of Metoprolol and Amlodipine was successfully achieved on a Agilent zorbax XDB C18 (150 mm×4.6mm,5µ)or equivalenting an isocratic mode utilizing Potassium dihydrogen phosphate (pH-4.8): Methanol (40:60) v/v at a flow rate of 1.0mL/min and eluate was monitored at 235nm,with are Retention time of 1.694 and 3.234 minutes for Metoprolol and Amlodipine. The developed method was validated and the response was found to be linear in the drug concentration range of 50µg/mL to 150 µg/mL for Metoprolol and 50µg/Ml to 150µg/mL for Amlodipine. The values of the slope and the correlation coefficient were found to be y=26615x+ 43142 and 0.999 for Metoprolol and y=5560x-1243 and 0.999 for Amlodipine respectively. The LOD and LOQ for Metoprolol were found to be 1.36,4.12 respectively. The LOD and LOQ for Amlodipine were found to be 1.0, 3.0 respectively. This method was found to be good percentage recovery for Metoprolol and Amlodipine were found to be 100.00 and 100.31 respectively indicates that the proposed method is highly accurate. The specificity of the method shows good correlation between retention times of standard with the sample so, the method specifically determines the analyte in the sample without interference from excipients of pharmaceutical dosage forms. The method was extensively validated according to ICH guidelines for Linearity, Range, Accuracy, Precision, Specificity and Robustness.

KEYWORDS: Metoprolol, Amlodipine, RP-HPLC, Simultaneous estimation.

INTRODUCTION

Metoprolol is a selective beta-1 blocker commonly employed as the succinate and tartrate derivatives depending if the formulation is designed to be of immediate release or extended release.[1] The possibility of the generation of these formulations comes from the lower systemic bioavailability of the succinate derivative.[2] To this date, it is one of the preferred beta-blockers in general clinical guidelines and it is widely prescribed in the Netherlands, New Zealand, and the US.3 Metoprolol was developed since 1969 by US Pharmaceutical Holdings I and FDA approved in 1978. Metoprolol is a beta-1-adrenergic receptor inhibitor specific to cardiac cells with negligible effect on beta-2 receptors. This inhibition decreases cardiac output by producing negative chronotropic and inotropic effects without presenting activity towards membrane stabilization nor intrinsic sympathomimetics.[3] IUPAC Name is 1-[4-(2-methoxyethyl) phenoxy]-3-[(propan-2-yl) amino] propan-2-ol. Molecular formula is $C_{15}H_{25}NO_3$. Molecular weight is 267.3.

Amlodipine is a popular antihypertensive drug belonging to the group of drugs called dihydropyridine calcium channel blockers. Due to their selectivity for the peripheral blood vessels, dihydropyridine calcium channel blockers are associated with a lower incidence of myocardial depression and cardiac conduction abnormalities than other calcium channel blockers.[4] Amlodipine is commonly used in the treatment of high blood pressure and angina. Amlodipine has antioxidant properties and an ability to enhance the production of nitric oxide (NO), an important vasodilator that decreases blood pressure.[5] The option for single daily dosing of amlodipine is an attractive feature of this drug. IUPAC Name is 3-ethyl 5-methyl 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine -3,5 dicarboxylate; benzenesulfonic acid. Molecular formula is C26H31ClN2O8S. Molecular weight is 567 g/mol. It is slightly soluble in water and sparingly soluble in ethanol.

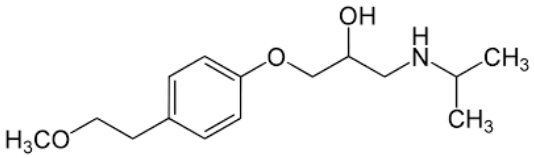

Figure 1: Structure of Metoprolol.

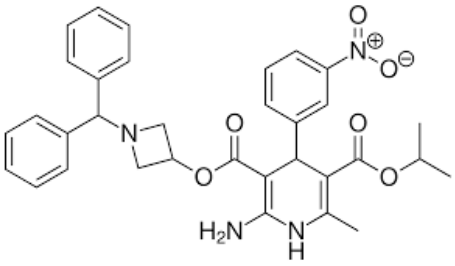

Figure 2: Structure of Amlodipine.

Literature survey shows that a number of methods have been reported for estimation of Metoprolol And Amlodipine individually or in combination with other drugs.[6-11] However, there is only few HPLC methods are reported for the simultaneous estimation of these drugs in combined dosage forms. I got better results than already published one. The aim of the present study was A New Rp-Hplc Method for Simultaneous Estimation of Metoprolol and Amlodipine in Its Bulk and pharmaceutical Dosage Form.

MATERIALS AND METHODS

Chemicals and Reagents: Metoprolol and Amlodipine were Purchased from Hetero drugs. NaH_2PO_4 was analytical grade supplied by Finerchem limited, Orthophosphoric acid (Merck), and Water and Methanol for HPLC (Lichrosolv (Merck).

Equipment and Chromatographic Conditions: The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, UV detector and Empower 2 software. Analysis was carried out at 235 nm with column Agilent Zorbax XDB C18 (150mm×4.6mm,5µ), dimensions at 25^0C temperature. The optimized mobile phase consists of Potassium dihydrogen phosphate: Methanol (40:60). Flow rate was maintained at 1 ml/min.

Preparation of solutions

PREPARATION OF MOBILE PHASE

Transfer 1.36086gof Potassium dihydrogen phosphate into 1000ml of beaker and adjust pH 4.80 with orthophosphoric acid (OPA).

Transfer the above solution 400ml and 600ml of methanol is used as mobile phase. They are mixed and sonicated for 20min.

Preparation of the metoprolol and amlodipine standard and sample solution

Preparation of standard solution

Accurately weigh and transfer 50 mg of Metoprolol and 50 mg of Amlodipine into 50 ml of volumetric flask and add 10ml of water and sonicate 10min (or) shake 5min and make with water.

Transfers 5ml of the above solution into 25ml volumetric flask, make up the volume with water**.**

Preparation Of Sample Stock Solution

Commercially available six tablets ware weighed and powdered the powdered equivalent to the 585.58 mg of Metoprolol and Amlodipine of active ingredients were transfer into a 50 ml of volumetric flask and add 10ml of methanol and sonicate for 20min (or) shake 10min and makeup with water.

Transfers above solution 5ml into 25ml of the volumetric flask dilute the volume with water. And the solution was filtered through 0.45µm filter before injecting into HPLC system.

RESULTS AND DISCUSSION

METHOD

The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness and robustness as per ICH guidelines.

System suitability parameters: To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 1.0 ml/min to equilibrate the column at ambient temperature. Chromatographic separation was achieved by injecting a volume of 10 µL of standard into AgilentZorbax XDB C18(150mm×4.6mm,5µ), the mobile phase of composition Potassium dihydrogen phosphate: Methanol (40:60) was allowed to flow through the column at a flow rate of 1.0 ml per minute. Retention time, tailing factor and USP theoretical plate count of the developed method are shown in table 1.

Table 1: System suitability parameters.

parameter	Metoprolol	Simethicone	Acceptancecriteria
Retention time	1.694	3.234	_
Theoretical plates	4508	5387	>2500
Tailing factor	1.69	1.55	<2.00
% RSD	0.02	0.03	<2.00

Assay of pharmaceutical formulation: The proposed validated method was successfully applied to determine Metoprolol and Amlodipine in their pharmaceutical dosage form. The result obtained for was comparable with the corresponding labeled amounts and they were shown in Table-2.

Table 2: Assay results for Metoprolol and Amlodipine.

	Label Claim (mg)	% Assay
Metoprolol	50	100.25
Amlodipine	50	98.99

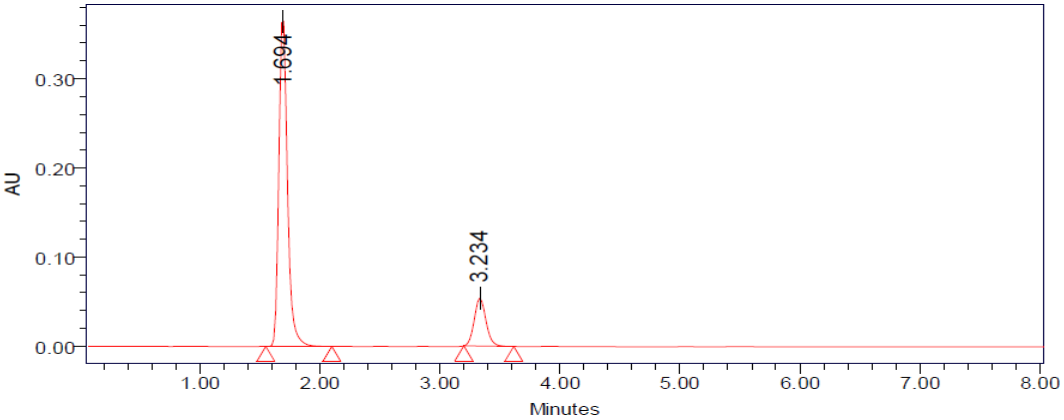

Figure 3: Standard chromatogram.

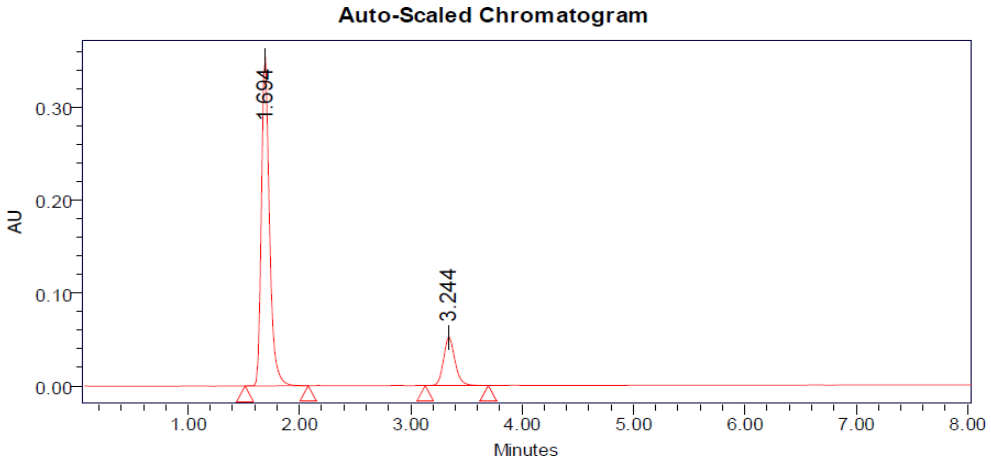

Figure 4: Sample chromatogram.

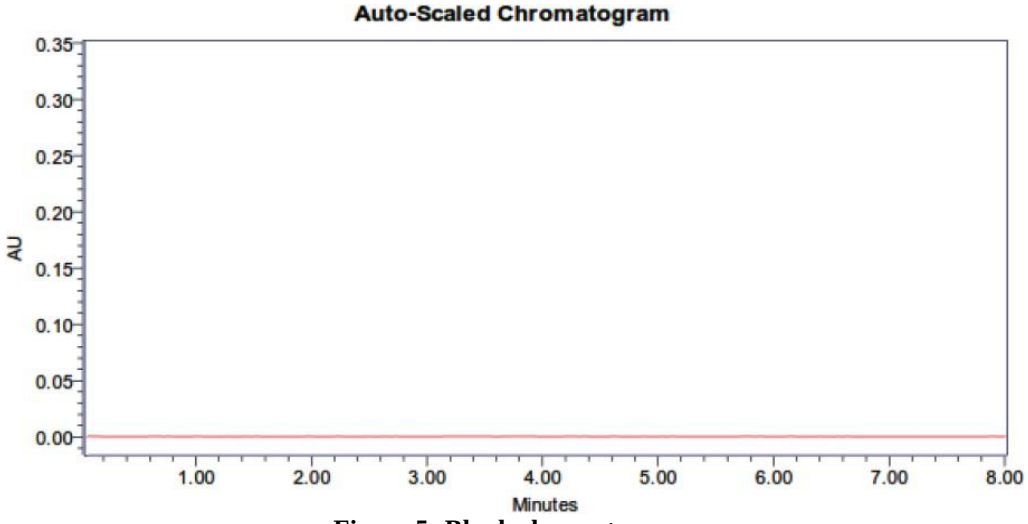

Figure 5: Blank chromatogram.

Validation of Analytical method

Linearity: The linearity study was performed for the concentration of 50 ppm to 150 ppm and 50 ppm to 150 ppm level. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient. Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. The results are shown in table 3,4.

Table 3: Linearity results of Metoprolol.

S. no	Concentration (µg/ml)	Rt	Area
1.	50	1.689	1254871
2.	75	1.691	1895454
3.	100	1.692	2653415
4.	125	1.689	3258474
5.	150	1.688	3986547

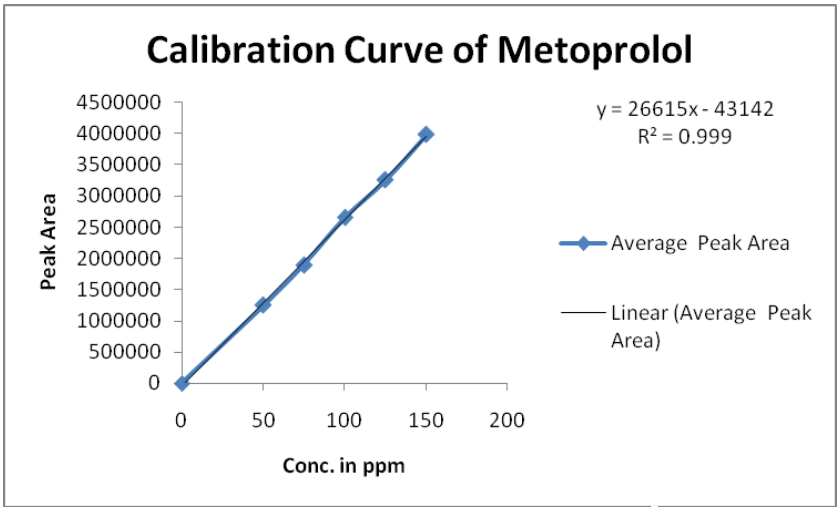

Figure 6: Linearity graph for Metoprolol.

Table 4: Linearity results of Amlodipine.

S. no	Concentration (µg/ml)	Rt	Area
1.	50	3.203	269658
2.	75	3.299	418753
3.	100	3.294	559858
4.	125	3.290	695847
5.	150	3.288	828654

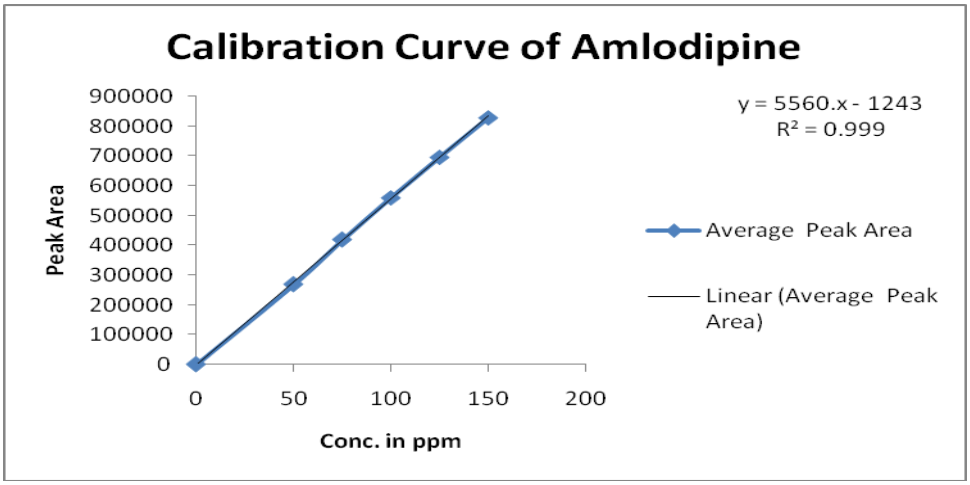

Figure 6: Linearity graph for Amlodipine.

Accuracy studies: The accuracy was determined by help of recovery study. The recovery method carried out at three level 50%, 100%, 150% and 50%, 100%, 150% Inject the standard solutions into chromatographic system. Calculate the Amount found and Amount added for Metoprolol and Amlodipine and calculate the individual recovery and mean recovery values. The results are shown in table 5,6.

Table 5: Showing accuracy results for Metoprolol.

S. no	Accuracy Level	Sample name	µg/ml added	µg/ml found	% Recovery	% Mean
1	50%	1	50	49.761	99.522	99.99
		2	50	50.053	100.106	
		3	50	50.171	100.342	
2	100%	1	100	99.581	99.581	100.073
		2	100	100.446	100.446	
		3	100	100.194	100.194	
3	150%	1	150	149.885	99.923	99.956
		2	150	149.757	99.838	
		3	150	150.164	100.109	

Table 6: Showing accuracy results for Amlodipine

S. no	Accuracy Level	Sample name	µg/ml added	µg/ml found	% Recovery	% Mean
1	50%	1	50	50.358	100.716	100.66
		2	50	50.518	101.036	
		3	50	50.114	100.228	
2	100%	1	100	100.454	100.454	100.25
		2	100	100.822	100.822	
		3	100	99.475	99.475	
3	150%	1	150	150.379	100.252	100.03
		2	150	149.462	99.641	
		3	150	150.297	100.198	

Precision Studies: precision was calculated from Coefficient of variance for six replicate injections of the standard. The standard solution was injected for six times and measured the area for all six Injections in HPLC. The %RSD for the area of six replicate injections was found. The results are shown in table 7.

Table 7: Precision results for Metoprolol and Amlodipine.

S. no	Intraday precision for Metoprolol			Intraday precision for Amlodipine		
	Peakarea	Mean peakarea	%RSD	Peakarea	Mean peakarea	%RSD
1	2653415			567898		
2	2654514			568887		
3	2685475	2667028	0.556	569275	568727	0.137
4	2658426			569858		
5	2664858			568586		
6	2685479			567858		

Robustness: As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method. The flow rate was varied at 0.8 ml/min to 1.2 ml/min. The results are shown in table 8,9.

Table 8: Robustness results for Metoprolol

Parameter	Rt	Theoretical plates	Tailing factor
Decreased flow rate (0.8ml/min)	1.868	4052	1.60
Increased flow rate (1.2ml/min)	1.544	4941	1.49
Decreased temperature (20^0c)	1.731	4475	1.61
Increased temperature (30^0c)	1.675	4581	1.61

Table 9: Robustness results for Amlodipine

Parameter	Rt	Theoretical plates	Tailing factor
Decreased flow rate (0.8ml/min)	3.621	5230	1.45
Increased flow rate(1.2ml/min)	2.998	5828	1.41
Decreased temperature (20^0c)	6.242	5484	1.50
Increased temperature (30^0c)	2.302	5494	1.50

LOD and LOQ: The sensitivity of RP-HPLC was determined from LOD and LOQ. Which were calculated from the calibration curve using the following equations as per ICH guidelines. The results are shown in table 10.

LOD = 3.3σ/S and

LOQ = 10 σ/S, where

σ= Standard deviation of y intercept of regression line,

S = Slope of the calibration curve

Table 10: LOD, LOQ of Metoprolol and Amlodipine.

Drug	LOD	LOQ
Metoprolol	1.36	1.0
Amlodipine	4.12	3.0

CONCLUSION

The validated HPLC method developed for the quantitative quality control determination of Metoprolol and Amlodipine in combination was evaluated for system suitability, specificity, sensitivity, linearity, range, accuracy (recovery), precision (repeatability and intermediate precision), and robustness. All the validation results were within the allowed specifications of ICH guidelines. The developed method has proven to be rapid, accurate, and stability-indicating for the simultaneous determination of combined Metoprolol and Amlodipine in pharmaceutical dosage form in the presence of excipients and the degradation products. As a result, the proposed HPLC method could be adopted for the quantitative quality control and routine analysis of the pharmaceutical dosage form.

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