RP-HPLC method for simultaneous estimation of amlodipine and metoprolol in tablet formulation

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A reverse phase high performance liquid chromatography (RP-HPLC) method for the simultaneous estimation of amlodipine and metoprolol in marketed formulation is developed. The determination was carried out on a Kromasil C18 (250 x 4.6 mm, 5 μm) column using a mobile phase of 0.02 M phosphate buffer solution: acetonitrile (70:30v/v, pH 3.0). The flow rate was 1.0ml/min with detection at 221 nm. The retention time for amlodipine was 2.57 min and for metoprolol 4.49 min. Amlodipine and metoprolol showed a linear response in the concentration range of 10-110 μg/ml. The correlation co-efficient ('*r*' value) for amlodipine and metoprolol was 0.9991 and 0.9992, respectively. The results of analysis have been validated statistically and by recovery studies. The percentage recoveries obtained for amlodipine and metoprolol ranges from 100.04 to 100.57%.

Key words: *Amlodipine, metoprolol, RP-HPLC*

INTRODUCTION

Amlodipine (AMD) is chemically a 2-[(2-Aminoethoxy) methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridine dicarboxylic acid- 3-ethyl 5-methyl ester and it belongs to the class of calcium channel blocker.[1,2] Several spectroscopic,[3,4] RP-HPLC,[5,6] HPTLC,[7] LC-MS/MS[8] and LC-MS[9] have been reported for the estimation of amlodipine individually and in combination with other drugs. Metoprolol (MET) is beta blocker, which is official in IP,[10] chemically it is 1-[4-(2-methoxyethyl) phenoxy]-3-[(1-methylethyl)amino]-2-propanol.[2,3] Literature reveals UV spectrosctroscopy,[11] HPLC,[12-15] chemometric-assisted spectrophotometric and HPLC method,[16,17] GC-MS,[18] liquid chromatography tandem mass spectrometry methods[19] have been reported for the estimation of metoprolol. But no method is developed so far for the combination of AMD and MET. A successful attempt is made to estimate the two drugs simultaneously. Therefore it was thought worthwhile to develop an accurate and rapid RP-HPLC method for simultaneous estimation of AMD and MET from tablet formulations.

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EXPERIMENTAL

Instrumentation
A Gradient HPLC (Merck Hitachi) with L-7100 double reciprocating pump, L-7400 UV detector, and RP-C18 column (5 μm particle size) was used. The RP-HPLC system was equipped with winchrom software for data processing.

Chemicals and reagents
The solvents used were of HPLC/AR grade standard samples of AMD and MET are obtained as gift samples from Glenmark Pharmaceuticals Limited, Nashik and Cipla Ltd., Kurkumbh, respectively. Marketed formulation Supermet*AM (Nicholas Piramal) was procured from the local market.

Chromatographic conditions
Method was developed using a Kromasil C18 (250 x 4.6 mm, 5 μm) column. Mobile phase used was 0.02 M phosphate buffer solution : acetonitrile (70:30 v/v, pH 3.0). Flow rate employed was 1.0 ml/min. Detection was carried out at 221 nm.

Standard stock solution
About 20 mg of each of reference standard of AMD and MET was weighed accurately and transferred to two separate 100 ml volumetric flask. Both drugs were dissolved in 50 ml of mobile phase with shaking and

volume was made upto the mark with mobile phase to get 200 µg/ml of standard stock solution of each drug. These stock solutions were filtered through 0.2 µm Nylon 6, 6(N_{66}) membrane filter paper.

Calibration curves

For each drug, appropriate aliquots were pipetted out from each standard stock solution into a series of 10 ml volumetric flasks. The volume was made upto mark with mobile phase to get set of solutions having concentration range 10, 20, 30, 40, 50, 60, 70, 80, 90,100 and 110 µg/ml for each drug. Triplicate dilutions of each concentration of each drug were prepared separately. From these triplicate solutions, 20 µl injections of each concentration of each drug were injected into the RP-HPLC system separately and chromatographed under the conditions as described above. Evaluation of both drugs was performed with UV detector at 221nm. Peak areas were recorded for all the peaks and peak areas were plotted against the concentrations to obtain the standard calibration curves.

Analysis of the marketed formulations

Twenty tablets were weighed and crushed to fine powder. The tablet powder equivalent to 5 mg of amlodipine and 47.5 mg of metoprolol was transferred to a 100 ml volumetric flask and dissolved in mobile phase and the content was kept in ultrasonicator for 30 min. Finally, the volume was made up to the mark with mobile phase. The solution was filtered through 0.2 µm Nylon 6, 6(N_{66}) membrane filter paper. This solution was further diluted with mobile phase and standard stock solution of AMD was added to obtain mixed sample solution containing 47.5 mg amlodipine and 47.5 mg metoprolol. A 20 µl of sample solution was injected into sample injector for six times under chromatographic condition as described above. Area of each peak was measured at 221 nm. The amount of each drug present in the sample ($n=6$) was determined from peak area of AMD and MET present in the pure mixture and percent label claim and standard deviation (SD) was calculated. The results are given in Table 1. Typical chromatogram of AMD and MET present in tablet formulation is given in Figure 1.

Validation of HPLC method - The proposed RP-HPLC method was validated as per ICH guidelines.

Specificity

The specificity of the RP-HPLC method was determined by comparison of the chromatogram of mixed standards and sample solutions. The parameters like retention time (t_R), resolution (R_S) and tailing factor (T_f) were calculated. Good
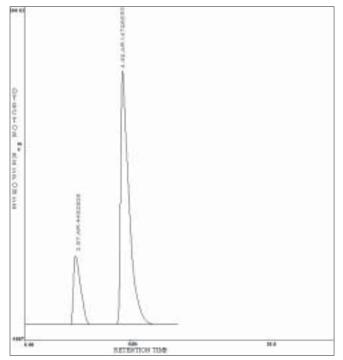

Figure 1: Typical chromatogram of AMD (RT=2.57 min) and MET (RT=4.49 min) in tablet formulation

correlation was found between the results of mixed standards and sample solutions.

Precision

Precision study was performed to find out intra-day and inter-day variations. The %relative standard deviation (RSD) for intra-day precision was 0.138% for AMD and 0.129% for MET and for inter-day precision was 0.590% for AMD and 0.414% for MET, respectively which is less than 2% indicating high degree of precision.

Accuracy (Recovery studies)

Recovery studies were performed by standard addition method at three levels i.e., 80%, 100% and 120%. Known amounts of standard AMD and MET were added to pre-analyzed samples and they were subjected to proposed HPLC method. Results of recovery studies are shown in Table 1.

Limit of detection (LOD) and limit of quantitation (LOQ)

The LOD and LOQ were separately determined based on the calibration curves. The standard deviation of the y-intercepts and slope of the regression lines were used. Results of LOD and LOQ are given in Table 2.

Table 1: Result of marketed formulation analysis

Marketed formulation	Drug	Label claim (mg/tablet)	Estimated % of label claim ± SD*	%Recovery ± S.D*		
				80	100	120
Supermet*AM	AMD	5	100.51±0.342	100.13±0.853	100.57±0.514	100.51±0.405
(Nicholas Piramal)	MET	47.5	100.19±0.689	100.54±0.536	100.05±0.614	100.04±0.127

*Average of six determinations

Table 2: System suitability parameters

Parameter	AMD	MET
Tailing factor	1.74	1.25
Resolution (Rs)	1.60	
Separation factor	1.56	
Capacity factor	2.01	3.15
Limit of detection (μg/ml)	0.029	0.025
Limit of quantitation (μg/ml)	0.090	0.075

Robustness

The robustness study was done by making small changes in the optimized method parameters like ±0.1 change in pH, ±1% change in mobile phase ratio and column temperature. There was no significant impact on the retention time and tailing factor.

Ruggedness

The ruggedness study was done by the two analysts. The %RSD for analyst-I was 0.1088% for AMD and 0.1078% for MET and for analyst-II was 0.3208% for AMD and 0.7329% for MET, respectively.

RESULTS AND DISCUSSION

The present work describes RP-HPLC method for estimation of AMD and MET in tablets. Both the drugs were resolved on Kromasil C18 (250 x 4.6 mm, 5 μm) column using 0.02 M phosphate buffer solution: acetonitrile (70:30v/v, pH 3.0) as mobile phase with a flow rate of 1.0 ml/min, UV detection was performed at 221 nm. Linearity response was found in the concentration range of 10-110 μg/ml for both the drugs. The correlation co-efficient ('*r*' value) for AMD and MET was 0.9991 and 0.9992, respectively. The %RSD for the tablet analysis and recovery studies was less than 2% indicating high degree of accuracy. The %RSD of AMD and MET for intra-day precision and inter-day precision was less than 2% indicating high degree of precision. The results of the robustness study also indicated that the method is robust and is unaffected by small variations in the chromatographic conditions. The results of ruggedness study was found to be satisfactory. Hence, it can be concluded that the developed RP-HPLC method is accurate, precise, and selective and can be employed successfully for the estimation of AMD and MET in both bulk and multicomponent formulation.

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