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### A validated RP-HPLC method of Metoprolol Succinate and Amlodipine Succinate from bulk drugs

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#### Abstract

*A simple, rapid and precise method is developed for the quantitative simultaneous determination of Metoprolol succinate and Amlodipine Besylate in a combined pharmaceutical-dosage form. The method is based on High Performance Liquid Chromatography (HPLC) on a reversed-phase column, Inertsil ODS – CV C<sub>18</sub> (150 X 4.6 mm), using a mobile phase of Sodium acetate buffer (the pH was adjusted to 3.0 ± 0.05 with Ortho Phosphoric acid), acetonitrile and methanol (80:20 v/v). The buffer used in the mobile phase contains Sodium acetate in double-distilled water. The chromatographic conditions are flow rate of 1ml/min, column temperature at 30°C and detector wavelength of 215nm. Both the drugs were well resolved on the stationary phase and the retention times were around 10.3 minute for Metoprolol succinate and 3.8 minute for amlodipine. The method was validated and shown to be linear for Metoprolol succinate and amlodipine. The correlation coefficients for Metoprolol succinate and amlodipine are 0.999963 and 0.999979, respectively. The relative standard deviations for six replicate measurements in two sets of each drug in the tablets is always less than 2% and mean % error of active recovery not more than ± 1.5%. The method was validated for precision and accuracy. The proposed method was successfully applied to the pharmaceutical dosage forms containing the above-mentioned drug combination without any interference by the excipients.*

**Key Words:** Reverse phase liquid chromatography, Metoprolol succinate, Amlodipine Succinate, HPLC, specificity, validation

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## INTRODUCTION

Metoprolol succinate [1] (MS) is a cardio selective drug used in the treatment of hypertension and various cardiovascular disorders. It is a beta1-selective drug which belongs to the chemical class of beta blockers and is ( $\pm$ ) 1-(isopropyl amino)-3-[p-(2-methoxyethyl) phenoxy]-2-propanol succinate (2:1) (salt). Amlodipine besylate (AM), 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1, 4-ihdropyridine, is a benzene sulfonate (besylate) salt of amlodipine, which is a dihydropyridine calcium channel blocker. AM is a calcium antagonist that inhibits the transmembrane influx of calcium ions into vascular smooth muscles and cardiac muscles, which in turn affects their contractile process and results in reduced blood pressure. It is used in the treatment of hypertension and angina. Literature survey revealed that various methods have been reported for estimation of MS in Tandem Spectroscopy bulk drugs[2] plasma[3-7] serum[8-10] pharmaceutical dosage forms [11,12] ,HPTLC[13] and Urine[14,15]. The focus of the present study was to develop and validate a economic, rapid reversed-phase high-performance liquid chromatographic method for the quality control of Metoprolol succinate and amlodipine besylate in pharmaceutical preparations with lower solvent consumption along with the short analytical run time leads to an environmentally friendly chromatographic procedure that allows the analysis of a large number of samples in a short period of time. The proposed method is applicable as well as for routine analysis and content uniformity test of MS and AM in tablets and complies well with the validation requirements in the pharmaceutical industry.

## MATERIALS AND METHODS

### Materials and reagents

Acetonitrile and methanol were of HPLC grade and were purchased from E. Merck, Darmstadt, Germany. Sodium acetate, Ortho Phosphoric acid and other reagents were of analytical-reagent grade and purchased from E.Merck, Darmstadt, Germany. The HPLC grade water was obtained by double distillation and purification through Milli-Q water purification system. Water was deionised and double distilled. Camlostin Plus tablets were kind gift from Square Pharmaceuticals Ltd Bangladesh. Each tablet was labeled to contain 50 mg Metoprolol succinate and 5 mg amlodipine. The excipients present in the tablets are: microcrystalline cellulose, maize starch, sodium starch glycolate, pigments blend-24843 (pink), colloidal silicon dioxide, magnesium stearate and purified talc.

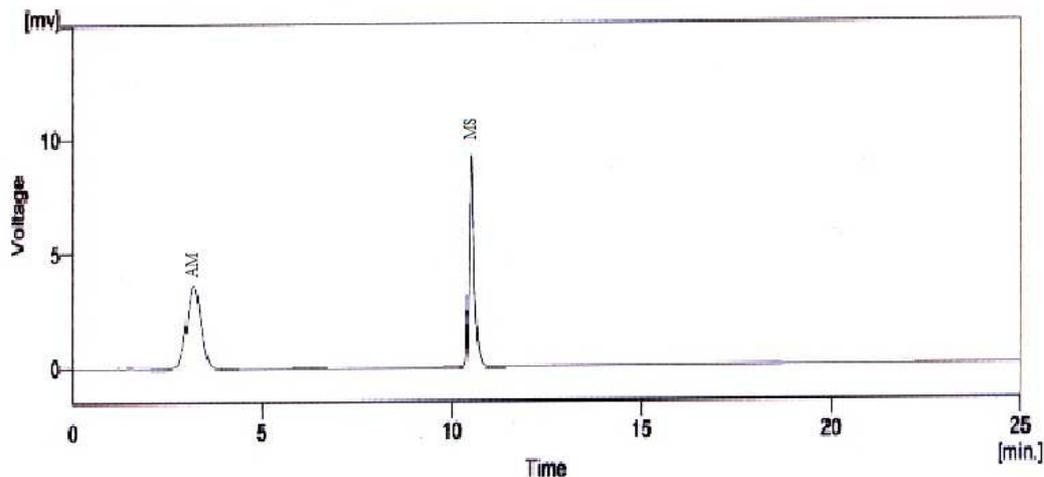
### Preparation of mobile phase and Stock Solution:

#### *Mobile Phase:*

7.8gm Sodium dihydrogen phosphate ( $\text{NaH}_2\text{PO}_4$ ) dissolved in water made up the volume to 1000ml with water. Then pH was adjusted with Ortho Phosphoric acid  $3.0 \pm 0.5^{\circ}\text{C}$ . 800ml of above buffer mixed with 200ml of acetonitrile and degassed by filtering through 0.45membrane. 70mg of amlodipine besylate dissolved mobile phase 50ml volumetric flask volume made with mobile phase. Then 5 ml 100ml volumetric flask volume made with mobile phase and brought out 50mcg/ml of amlodipine besylate. 47.5mg of metoprolol Succinate dissolved mobile phase 100ml volumetric flask volume made with mobile phase and brought out 500mcg/ml of Metoprolol succinate.

**Working Standard Solution of Amlodipine besylate and Metoprolol succinate.**

5.0ml of amlodipine besylate standard stock solution and 5.0ml of Metoprolol succinate standard stock solution mixed 25ml volumetric flask and sonicated volume made up with mobile phase to get the concentration of 10mcg/ml of amlodipine besylate and 100mcg/ml of metoprolol Succinate. The Retention time of Amlodipine besylate and Metoprolol Succinate 3.8 and 10.3 respectively in figure no 1.

**Figure 1. Representative chromatogram of Amlodipine besylate and Metoprolol Succinate.****Chromatographic Condition**

A reverse phase C-18 column, equilibrated with mobile phase buffer: acetonitrile (80:20%v/v) was used. The active principle was eluted isocratically and the mobile phase flow rate was maintained at 1.0 ml/min. The effluents were monitored at 215 nm with the detector. The sample was injected using a 20  $\mu$ l fixed loop, and the total run time was 17.5 min.

**Linearity and Range**

Appropriate aliquots were pipetted out from standard stock solution into series of 10 ml volumetric flasks. The volume was made up to the mark with mobile phase to get solutions having concentration range at least 6 different concentrations in the range between 2.5-15 $\mu$ g/ml for Amlodipine Besylate and 25-150 $\mu$ g/ml for Metoprolol Succinate. Each concentration was analysed in triplicate. Evaluation of drugs was performed at 215 nm.

**Validation of HPLC method:****Specificity:**

The specificity of the RP-HPLC method was determined by comparison of the chromatogram of standard and sample solution. The parameters like retention time (tR), resolution (Rs) and tailing factor (Tf) were calculated.

**Table: 1. System suitability Parameters**

Parameters	Values found in Amlodipine Besylate	Values found in Metoprolol succinate
Resolution	0.0000	15.623
Tailing factor	1.439	1.690
Number of Theoretical plates	3751.417	5165.927
RSD	0.16948	0.06854

**Precision**

Precision study was performed to find out intra-day and inter-day variation. It was carried out by estimating the corresponding responses 3 times on the same day and on 3 different day (first, second and fifth day) for 3 different concentration of MS (1, 15, 30 µg/ml) and the results are reported in terms of relative standard deviation (RSD). The repeatability studies were carried out by estimating response of 3 different concentration of MS (1, 15, 30 µg/ml) in triplicate. Precision of procedure was calculated by within day and between day variations.

**Accuracy (Recovery studies):**

Recovery studies were performed by standard addition method at three levels i.e. 80%, 100% and 120%. A known amount of standard Amlodipine besylate and Metoprolol succinate were added to preanalyzed sample and was subjected to proposed HPLC method. Accuracy of method was measured as percentage of deviation between added and measured concentrations (recovery study). The results are shown in table no 2.

**Table: 2. Recovery study.**

Drug	Tablet amount (µg/ml)	Amount added (µg/ml)	Amount recovered (µg/ml)	Recovery (%)
Amlodipine besylate	80	57.4	58.2	100.03
	100	70.1	71.3	99.67
	120	83.2	83.6	99.84
Metoprolol succinate	80	38.6	39.1	100.48
	100	48.9	50.2	99.28
	120	58.9	59.3	99.74

**Limit of Detection and Quantification:**

The limit of detection (LOD) and limit of Quantification (LOQ) was estimated from the standard calibration curve. The residual standard deviation of regression line or standard deviation of y intercepts of regression lines used to calculate LOD and LOQ. Here,  $LOD=3.3 * D/S$  and  $LOQ=10 * D/S$ . Where, D is the standard deviation of y intercept of regression line and S is the slope of calibration curves.

**Analysis of tablets:**

A total number of 20 tablets accurately weighed and powdered in a mortar. Quantities of the powdered tablets equivalent to 5 mg of AM and 50 mg of MS were accurately weighed and transferred in a 50 mL volumetric flask. Weighed powder was dissolved in 30 mL of acetonitrile and vortexed for 15 minutes. Then the volume made up to 50 mL with acetonitrile, mixed thoroughly and kept under mechanical shaking for 15 minutes. Solution obtained was filtered through whatman no. 42 filter paper and diluted with the same solvent to get the concentration within linearity and used for the measurement of derivative spectra. The assay content of Amlodipine besylate and Metoprolol succinate was found to be 99.7% and 101.6%.

**Stability:**

In order to demonstrate the stability of the standard solution of MS during analysis, the solution was stored over a period of 24 hr at room temperature and then analyzed.

**Robustness:**

Robustness of the method was studied by changing the composition of organic phase by  $\pm 5\%$  and pH by 0.2, and also by observing the stability of the drug for 24 hr at ambient temperature in mobile phase.

**RESULTS AND DISCUSSION:**

The development of the RP-HPLC method for the determination of drugs has received considerable attention in recent years because of its importance in routine quality control analysis. Different analytical columns with various stationary phases were tested. Good separation was achieved using a RP Inertsil ODS – CV C<sub>18</sub> (150 X 4.6 mm column and was finally used for analysis. A RPHPLC method was proposed as a suitable method for the estimation of MS and AB in bulk drug. The chromatographic conditions were adjusted in order to provide a good performance of the assay. The method involved a mobile phase consisting of Sodium Phosphate buffer and acetonitrile (80:20%v/v) accomplished at 215nm. The retention time of Amlodipine Besylate and Metoprolol Succinate about was 4mins and 10mins respectively at a flow-rate of 1 ml/min. The total run time for an assay was approximately 17 min. The mobile phase was chosen after several trials with other solvent combinations. Mobile phase selection was based on peak parameters (symmetry, tailing), run time, ease of preparation and cost. Figures 2 (A, B, C) show a representative chromatogram using the proposed method. As shown in these figures, MS was eluted forming symmetrical peak and well separated from the solvent front. Observed retention time (5.1 min) allowed a rapid determination of the drug (Table 1).

**Linearity:**

The linearity range of 2.5-15µg/ml for Amlodipine Besylate and 25-150µg/ml for Metoprolol Succinate was found to obey linearity with the correlation coefficient of 0.999949 for Amlodipine Besylate and 0.998808 for Metoprolol Succinate. In the linearity assay, the coefficient of variation (C.V) of the response factor was 4.82. The linearity of the calibration graph and conformity of RPHPLC value to Beer's Law were proven by the high correlation coefficients (r) for the regression equations.

**Accuracy and precision:**

Repeatability is given as inter- and intra-day precision and accuracy evaluated by analyzing three different concentrations of MS and AM. The precision of the RP-HPLC method was demonstrated by the relative standard derivation (RSD %) of lower than 0.62% for intra-day and 1.26% for inter-day.

**Sensitivity:**

The LOQ is defined as the lowest concentration on the calibration curve at which both accuracy and precision should be within 20%. LOQ value of the RP-HPLC method was determined as 0.75 µg/ml. The LOD for MS determination was approximately 0.25 µg/ml.

**Stability:**

The results for stability studies revealed that for the solutions, retention time and peak area of MS and AM remained almost unchanged.

**CONCLUSION**

Proposed study describes new and simple RP-HPLC method for the estimation of MS and AM in bulk drugs. The method was validated and found to be simple, sensitive, accurate and precise. Therefore the proposed method can be used for quantification of MS and AM in bulk drugs as well as for routine analysis in quality control.

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