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Research Article

VALIDATED STABILITY INDICATING HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF AMLODIPINE AND METOPROLOL IN BULK DRUG AND PHARMACEUTICAL FORMULATIONS

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ABSTRACT

A simple stability indicating high performance liquid chromatographic method has been developed for the simultaneous determination of amlodipine besylate in combination with metoprolol succinate using reverse phase C18ace-EPS column (100 × 4.6 mm, 5µm) with UV detection at 237 nm. The mobile phase consisting of acetonitrile and potassium dihydrogen phosphate buffer adjusted to pH 3.0 in a ratio of (50:50, v/v) and at a flow rate of 0.6 mL/min. The method was linear over the concentration range for amlodipine besylate 10-50 µg/mL and for metoprolol succinate 40-200 µg/mL. The recoveries of active pharmaceutical ingredient (API) amlodipine besylate and metoprolol succinate were found to be in the range of 100.4-100.9% and 100.1-102.1% respectively. The method was validated and was successfully employed for the routine quantitative analysis of pharmaceutical formulations containing amlodipine besylate and metoprolol succinate in combined tablet dosage form.

Keywords: Amlodipine, Metoprolol, HPLC, Validation.

INTRODUCTION

Amlodipine besylate (AML) is potent calcium channel blocker used for the treatment of hypertension, congestive heart failure and angina pectoris. It is a dihydropyridine calcium antagonist that inhibits the transmembrane influx of calcium ions into vascular smooth muscle and cardiac muscle. Its chemical name is described as 3-Ethyl-5-methyl (±)-2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-l,4-dihydro-6-methyl-3,5-pyridinedicarboxylate, monobenzenesulphonate[1] (Fig. 1). Metoprolol succinate (MET) is a cardio selective drug used in the treatment of hypertension and various cardiovascular disorders. The action of metoprolol succinate is mediated through the β_1-selective adrenoceptor blockage, thus causing reduction in heart rate and cardiac output. Its chemical name is described as (±)1-(isopropylamino)-3-[p-(2-methoxyethyl) phenoxy]-2-propanol succinate (2:1)[2] (Fig. 2). In the fixed dose combination of amlodipine (calcium channel blocker) and metoprolol (cardioselective beta blocker); both the drugs have two different mechanisms and reduce blood pressure by acting on peripheral vascular resistance, stroke volume and heart rate. Advantages of this combination therapy are it effectively achieves target blood pressure, lower incidence of individual drug's

side-effects, produces synergistic effects, increased patient compliance.

The stability indicating method is defined as validated quantitative analytical method that can detect the change with time in the chemical, physical or microbiological properties of the drug substance and the drug product, that are specific so that the content of active ingredient, degradation can be accurately measured without interference[3]. Stability testing provides information about degradation mechanisms, potential degradation products, possible degradation pathways of the drug as well as interaction between the drug and the excipients in drug product[4].

Literature survey revealed few analytical methods is reported for both the drugs in alone. Very few analytical methods have been reported in combination of amlodipine besylate and metoprolol succinate like, UV[5], HPLC[6-9], HPTLC[10] and LC-MS[11] methods. The aim of the present study was to develop a simple, precise, reliable, sensitive and selective stability indicating HPLC method with UV detection for the analysis of amlodipine and metoprolol in bulk samples and in combined dosage formulation.

EXPERIMENTAL

Chemicals and reagents

The pharmaceutical grade pure samples of amlodipine besylate (99.58%) and metoprolol succinate (99.55%) were received as gift samples from Aurobindo Pharma Ltd., Hyderabad. Amlodipine besylate and metoprolol succinate tablets were purchased from local market. Milli-Q water, HPLC grade acetonitrile and analytical grade potassium dihydrogen phosphate, orthophosphoric acid was obtained from Qualigens Fine Chemicals Ltd., Mumbai.

Apparatus and chromatographic condition

The chromatographic separation was performed on a Waters Alliance HPLC, integrated with Auto Sampler and UV detector. The analytical C18 ace-EPS column (100 × 4.6 mm, 5µm), of make Bischoff Chromatography was used for the separation. The mobile phase consisted of acetonitrile and potassium dihydrogen phosphate (7 grams of KH_2PO_4 into a 1000 mL beaker, dissolved and diluted to 1000 mL with HPLC water). Adjusted the pH to 3.0 with orthophosphoric acid) in a ratio of 50:50(v/v). The mobile phase was prepared freshly, filtered, sonicated before use and delivered at a flow rate of 0.6 mL/min and the detector wavelength was set at 237 nm. The injection volume was 20 µL. The mobile phase was used as diluent.

Preparation of amlodipine and metoprolol standard & sample solution

Standard solution preparation

Accurately weigh and transfer 10 mg of amlodipine and 50 mg of metoprolol working standard into a 100 mL clean dry volumetric flask add about 75 mL of diluent and sonicate to dissolve it completely and make volume up to the mark with diluent. Further pipette 2 mL of amlodipine and metoprolol of the above stock solution into a 10 mL volumetric flask and dilute up to the mark with diluent.

Sample Solution Preparation

Weigh and finely powder not fewer than 20 tablets. Accurately weigh and transfer sample equivalent to 10 mg of amlodipine and 50 mg metoprolol into a 100 mL clean dry volumetric flask add about 75 mL of diluent and sonicate to dissolve it completely and make volume up to the mark with diluent. Further pipette 2 mL of amlodipine and metoprolol of the above stock solution into a 10 mL volumetric flask and dilute up to the mark with diluent.

Procedure

Inject 20 µL of the standard, sample solution into the chromatographic system and measure the peak areas for amlodipine and metoprolol and calculate the % assay value.

RESULTS AND DISCUSSION

All of the analytical validation parameters for this proposed method were determined according to ICH guidelines[12]. Obtained validation parameters are presented in Table1.

Linearity

The linearity for HPLC method was determined at five concentration levels ranging from 10-50 µg/mL for AB and 40-200 µg/mL for MS**.** The calibration curve was constructed by plotting response factor against respective concentration of AML and MET. The plots of peak area Vs respective concentration of AML and MET were found to be linear in the range of 10-50 µg/mL and 40-200 µg/mL with coefficient of correlation (r^2) 0.999 and 0.998 for AML and MET respectively. The linearity of this method was evaluated by linear regression analysis. The slope and intercept calculated for AML and MET were given in Fig.3 and Fig. 4.

Recovery

Three different samples of known concentration ranging from 10-50 µg/mL for

AML and 40-200 µg/mL for MET were prepared and these are analyzed against standard solution**.** The result of recovery analysis of amlodipine besylate and metoprolol succinate was found to be in the range of 100.4-100.9% and 100.1-102.1% respectively. The obtained results are presented in Table 2.

Sensitivity

The limit of detection (LOD) was determined as lowest concentration giving response and limit of quantification (LOQ) was determined as the lowest concentration analyzed with accuracy of the proposed RP-HPLC method. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.89 µg/mL and 2.69 µg/mL for AML and 14.02 µg/mL and 42.48 µg/mL for MET. The LOD and LOQ showed that the method is sensitive for AML and MET.

System suitability test

The specificity of this method was determined by complete separation of AML and MET as shown in Fig. 5 with parameters like retention time, resolution and tailing factor. Here tailing factor for peaks of AML and MET was less than 2% and resolution was satisfactory. The average retention time for AML and MET were found to be 2.868 and 2.075 respectively, for five replicates. The peaks obtained for AML and MET were sharp and have clear baseline separation. Analysis was also performed for active AML and MET, placebo sample (All the ingredients except active AML & MET) both at stressed and unstressed condition. After analysis it was found that there is no interference of peak in the amlodipine and metoprolol region for the stressed, placebo & active sample. Hence the developed method was specific for the analysis of this product**.**

Precision

The method precision study was performed for five sample preparations of marketed formulations. A study was carried out for intermediate precision with the same analyst on the different day for five sample preparations of marketed formulations. Robustness of the method was determined by small deliberate changes in flow rate, mobile phase pH and mobile phase ratio. The content of the drug was not adversely affected by these changes as evident from the low value of relative standard deviation indicating that the method was rugged and robust. The Intra-day and Inter-day precision results are presented in Table 3. The assay results of tablet dosage formulation by the proposed method are presented in Table 4.

Stability

In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 24 hr at room temperature. The results show that for both solutions, the retention time and peak area of AML and MET remained almost similar (% R.S.D. less than 2.0) and no significant degradation within the indicated period, thus indicated that both solutions were stable for at least 24 hr, which was sufficient to complete the whole analytical process. Further forced degradation studies were conducted indicating the stability of the method developed. The results of the degradation studies are presented in Table 5.

Control sample

Weigh and finely powder not fewer than 20 tablets. Accurately weigh and transfer sample equivalent to 10 mg of amlodipine and 50 mg metoprolol into a 100 mL clean dry volumetric flask, add about 75 mL of diluent and sonicate to dissolve it completely and make volume up to the mark with the diluent. Filter the solution through 0.45µm membrane filter. Further pipette 2 mL of the above stock solution into a 10 mL volumetric flask and dilute up to the mark with diluent.

Acid degradation sample

Weigh and finely powder not fewer than 20 tablets. Accurately weigh and transfer sample equivalent to 10 mg of amlodipine and 50 mg metoprolol into a 100 mL clean dry volumetric flask, add about 75 mL of diluent and sonicate to dissolve it for about 30minutes with intermittent shaking at controlled temperature. Then add 5N acid (Hydrochloric acid), refluxed for 60 minutes at 60°C, then cooled to room temperature, neutralize with 5N base (Sodium hydroxide) and make volume up to the mark with diluent and mix. Filter the solution through 0.45 µm membrane filter. Further pipette 2 mL of the above stock solution into a 10 mL volumetric flask and dilute up to the mark with diluent. The typical chromatogram of acid degradation was given in Fig. 6.

Base degradation sample

Weigh and finely powder not fewer than 20 tablets. Accurately weigh and transfer sample equivalent to 10 mg of amlodipine and 50 mg metoprolol into a 100 mL clean dry volumetric flask add about 75 mL of diluent and sonicate to dissolve it for about 30minutes with intermittent shaking at controlled temperature. Then add 10ml of 5N base (Sodium hydroxide), refluxed for 60 minutes at 60°C, then cooled to room temperature, neutralize

with 5N acid (hydrochloric acid) and make volume up to the mark with diluent and mix. Filter the solution through 0.45 µm membrane filter. Further pipette 2 mL of the above stock solution into a 10 mL volumetric flask and dilute up to the mark with diluent. The typical chromatogram of base degradation was given in Fig. 7.

Peroxide degradation sample

Weigh and finely powder not fewer than 20 tablets. Accurately weigh and transfer sample equivalent to 10 mg of amlodipine and 50 mg metoprolol into a 100 mL clean dry volumetric flask add about 75 mL of diluent and sonicate to dissolve it for about 30minutes with intermittent shaking at controlled temperature. Then add 2 mL of 30% Peroxide, refluxed for 60minutes at 60°C, then cooled to room temperature, make volume up to the mark with diluent and mix. Filter the solution through 0.45 µm membrane filter. Further pipette 2 mL of the above stock solution into a 10 mL volumetric flask and dilute up to the mark with diluent. The typical chromatogram of oxidative degradation was given in Fig. 8.

Thermal degradation sample

Weigh and finely powder not fewer than 20 tablets, this powder is exposed to heat at 105°C for about 2 days. Accurately weigh and transfer sample equivalent to 10 mg of amlodipine and 50 mg metoprolol into a 100 mL clean dry volumetric flask. Add about 75 mL of diluent and sonicate to dissolve it for about 30minutes with intermittent shaking at controlled temperature. Then make volume up to the mark with diluent and mix. Filter the solution through 0.45 µm membrane filter. Further pipette 2 mL of the above stock solution into a 10 mL volumetric flask and dilute up to the mark with diluent. The typical chromatogram of thermal degradation was given in Fig. 9.

CONCLUSION

This study presents a simple and validated stability indicating HPLC method for simultaneous estimation of amlodipine besylate and metoprolol succinate in the presence of degradation products. The developed method is specific, accurate, precise and robust. The method was linear response in stated range and is accurate and precise. All the degradation products formed during forced decomposition studies were well separated from the analyte peaks demonstrating that the developed method was specific and stability indicating**.** The method could be applied with success even to the analysis of marketed products of amlodipine and metoprolol combined tablet formulation, as no interference was observed due to excipients or other components present

ACKNOWLEDGEMENTS

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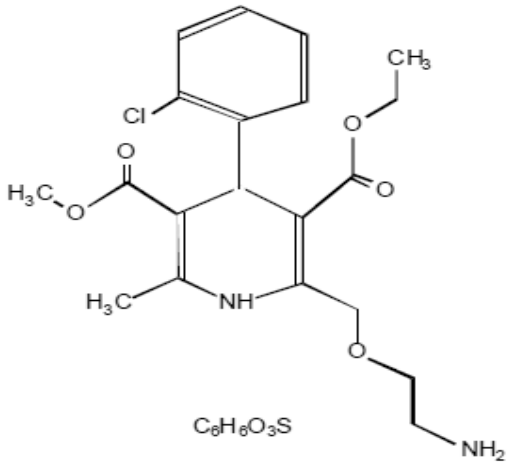

Fig. 1: Chemical structure of amlodipine besylate

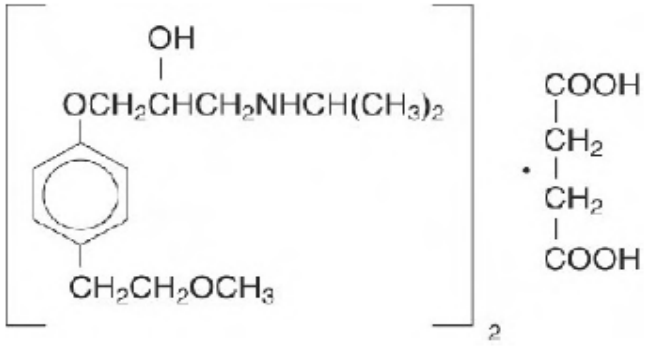

Fig. 2: Chemical structure of metoprolol succinate

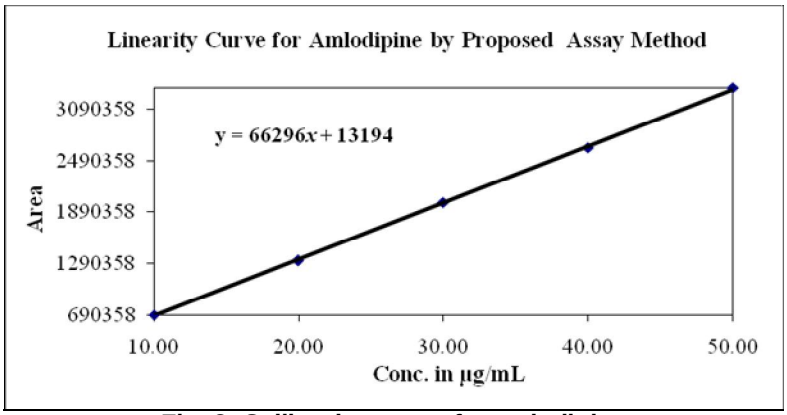

Fig. 3: Calibration curve for amlodipine

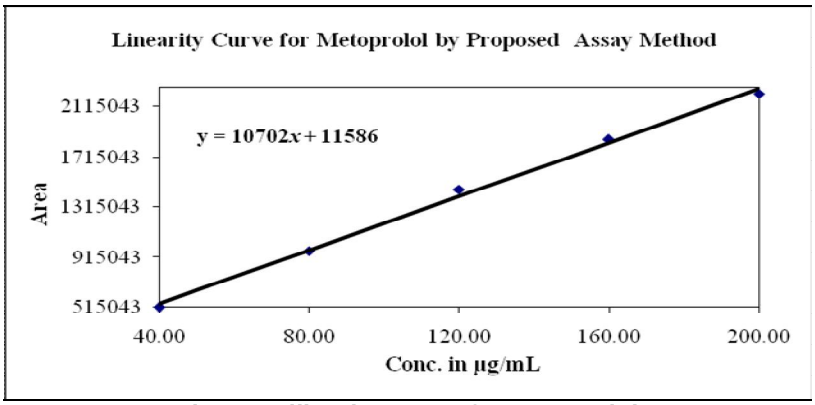

Fig. 4: Calibration curve for metoprolol

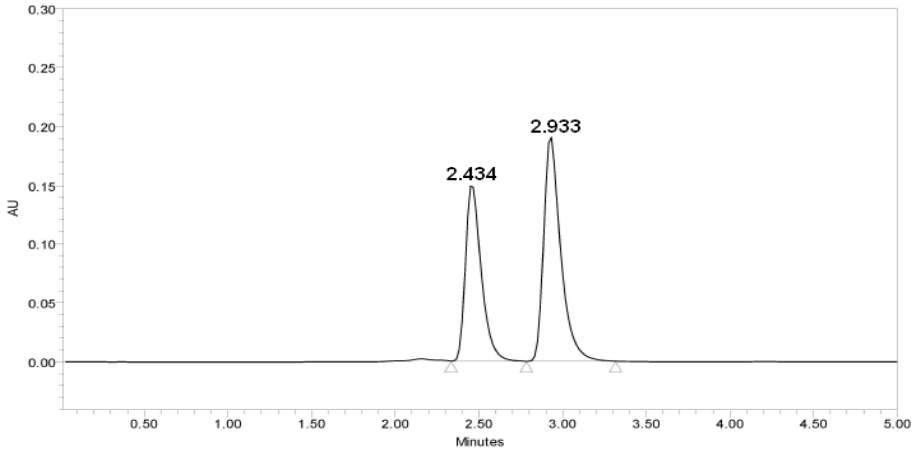

Fig. 5: Typical chromatogram of metoprolol and amlodipine

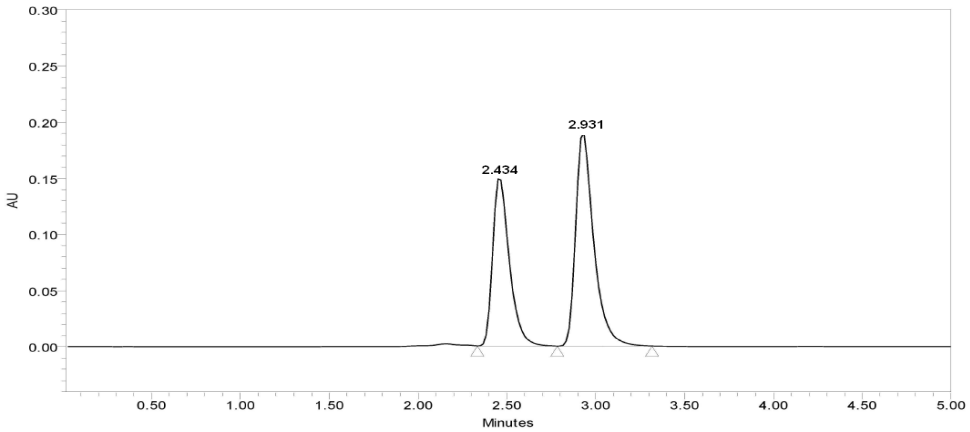

Fig. 6: Acid degradation chromatogram of metoprolol and amlodipine

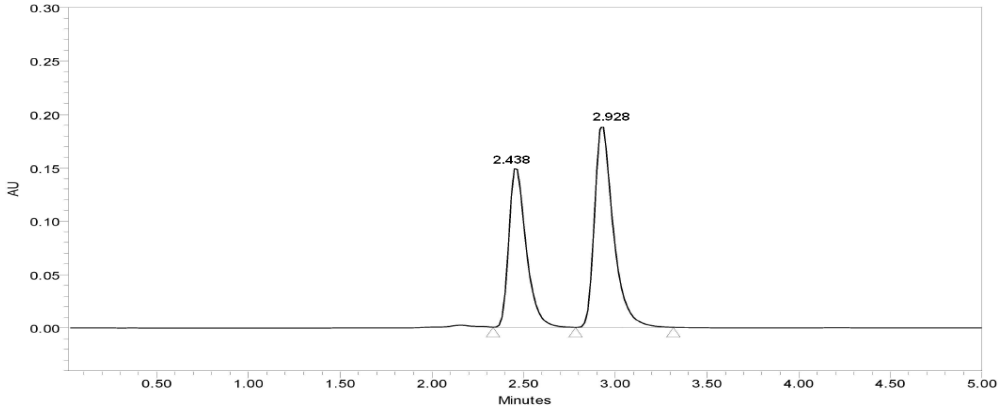

Fig. 7: Base degradation chromatogram of metoprolol and amlodipine

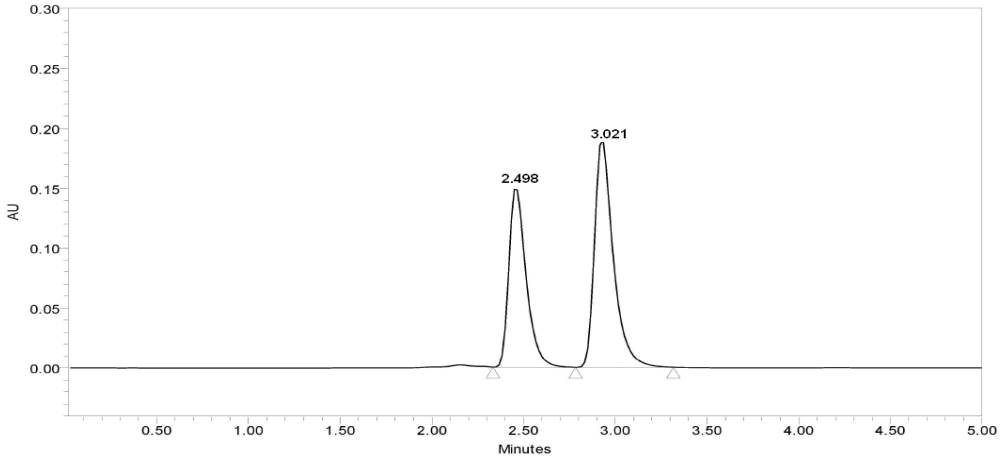

Fig. 8: Peroxide degradation chromatogram of metoprolol and amlodipine

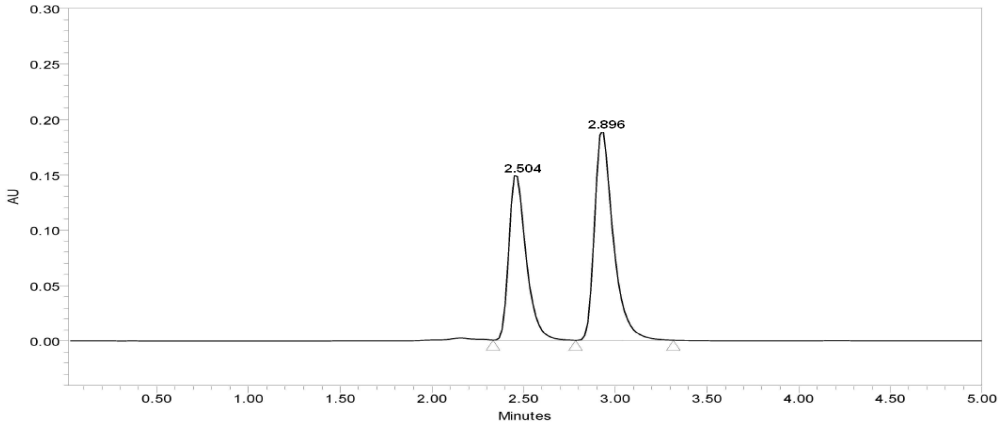

Fig. 9: Thermal degradation chromatogram of metoprolol and amlodipine

Table 1: Analytical validation parameters (System suitability and Linearity)

Parameter	AML	MET
Linearity	10-50 µg/mL	40-200 µg/mL
Slope	66296.0	10702.0
Intercept	13194.0	115866.0
% Y-Intercept	19.9	1082.7
Residual Sum of Squares	17845.0	45466.0
CC(r)	0.9999	0.9983
RSQ(r^2)	0.9998	0.9966
LOD	0.89	14.02
LOQ	2.69	42.48
Theoretical Plates	2537	2537
Tailing Factor	1.4	1.3
Retention Time (min)	2.933	2.434

Table 2: Recovery studies of amlodipine and metoprolol

Recovery data of AML					
Concentration (at specification level)	**Peak Area**	**Amount Added (mg)**	**Amount Found (mg)**	**% Recovery**	**Mean % Recovery**
50%	690254	5.25	5.29	100.8	100.7
100%	1317439	10.09	10.13	100.4	
150%	2006119	15.25	15.38	100.9	
Recovery data of MET					
Concentration (at specification level)	**Peak Area**	**Amount Added (mg)**	**Amount Found (mg)**	**% Recovery**	**Mean % Recovery**
50%	515681	25.85	26.39	102.1	100.8
100%	964991	49.98	50.03	100.1	
150%	1455370	75.25	75.38	100.2	

Table 3: Intra-day and Inter-day precision of amlodipine and metoprolol

Drug	Concentration (µg/mL)	Intra-day precision		Inter-day precision	
		SD	%RSD	SD	%RSD
AML	20	4276.7	0.32	6503.3	0.28
MET	100	2684.4	0.49	2688.7	0.27

Table 4: Assay result of tablet dosage formulation

Drug	Label strength (mg)	Amount found (mg)	% Assay
AML	5	5.01	100.2
MET	25	25.2	100.8

Table 5: Forced degradation studies of amlodipine and metoprolol

Stress Conditions	Degradation Time	Peak Area		% Degradation		% of Active drug present after degradation	
		AML	MET	AML	MET	AML	MET
Control	-	1325526	968640	-	-	-	-
Acid	1 hour	1174374	890166	11.4	8.1	88.6	91.9
Base	1 hour	1144374	878766	13.7	9.3	86.3	90.7
Peroxide	1 hour	1064374	861266	19.7	11.1	80.3	88.9
Thermal	48 hours	1121374	871266	15.4	10.1	84.6	89.9

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