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Research Article

SIMULTANEOUS DETERMINATION AND VALIDATION OF AMOLODIPINE AND METAPROLOL IN PHARMACEUTICAL DOSAGE FORMS BY REVERSE PHASE HPLC METHOD

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ABSTRACT

A rapid, specific and accurate isocratic HPLC method was developed and validated for the assay of Amolodpine besylate and Metaprolol in pharmaceutical dosage forms. The assay involved an isocratic – elution of Amolodpine besylate and Metaprolol in ODS- C18 column using mobile phase composition consists of 70:30(v/v) of Methanol and 0.1M potassium di hydrogen ortho phosphate with 0.1% W/V 1-octane sulfonic acid with pH adjusted to 3.0.The wavelength of detection is 210 nm.The method showed good linearity in the range of 5.0-50.0 µg/mL for Amolodipine and 50 to 500 µg/mL for Metaprolol. The runtime of the method is 10 mins. The proposed method can be used for routine quality control samples in industry in bulk and in finished dosage forms. In present study, a rapid specific precise and validated HPLC method for the quantitative estimation of anolodpine and metaprolol in pharmaceutical dosage forms has been reported. The developed method can be applied to directly and easily to the analysis of the pharmaceutical tablet preparations. The percentage recoveries were near 100% for given methods. The method was completely validated and proven to be rugged. The excipients did not interfere in the analysis. The results showed that this method can be used for rapid determination of amolodipine and metaprolol in pharmaceutical tablet with precision, accuracy and specificity.

Keywords: Amolodipine and metaprolol HCL, Assay, Reverse phase, HPLC.

INTRODUCTION

Amlodipine[1, 2](AB), chemically, 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-1, 4- dihydro- 6-methyl-3, 5-pyridinedicarboxylic acid3-ethyl, 5-methyl ester, is an anti-hypertensive and an antianginal agent in the form of the besylate salt, amlodipine besylate. Amlodipine[3] exists in "left- and right-handed" chiral forms (more accurately called the R-(+)**Fig-1A** and the S-(-)- enantiomers) **Fig-1B**. Only the (S)- form of this molecule is active against hypertension. When a mixture of (R) and (S)-forms (called a racemic mixture) is used, patients may experience adverse side-effects such as headache, dizziness, abdominal pain, flushing, peripheral oedema etc. Obviously, in cases where only one of the enantiomers is active, it is preferable to use only the chirally pure form. (-) Amlodipine is a widely prescribed calcium channel blocking (CCB) antihypertensive agent. However, amlodipine is a racemate with an equal proportion of two enantiomers "S" and "R", thus patients receiving amlodipine are in fact taking two different drugs.S-Amlodipine and R-Amlodipine which do not have the same level of antagonistic effect on the calcium channel receptor.The S-enantiomer of amlodipine is active and the R-enantiomer is inactive in terms of calcium channel blocking activity. S(-)Amlodipine has 1000 fold stronger calcium channel blocking activity than R- amlodipine.S(-)Amlodipine is therefore responsible for all of the CCB-mediated pharmacodynamic action of amlodipine including its anti-anginal activity without the concomitant liability of adverse effects associated with the racemic mixture of amlodipine. As S-Amlodipine is more active than the R-enantiomer, the faster release of S-enantiomer from a dosage form is very essential to treat the emergency conditions like hypertension and angina.Like other calcium channel blockers, amlodipine acts by relaxing the smooth muscle in the arterial wall, decreasing peripheral resistance and hence reducing blood pressure; in angina it increases blood flow to the heart muscle. Amlodipine besylate is a white crystalline powder with a molecular weight of 567.1. It is slightly soluble in water and sparingly soluble in ethanol. Amlodipine Besylate Tablets USP are formulated as white to off-white tablets equivalent to 2.5 mg, 5 mg and 10 mg of Amlodipine for oral administration. In addition to the active ingredient, Amlodipine besylate, each tablet contains the following inactive ingredients: dibasic calcium phosphate anhydrous, magnesium stearate, microcrystalline cellulose and sodium starch glycolate. The usual initial antihypertensive oral dose of Amlodipine besylate tablet is 5 mg once daily with a maximum dose of 10 mg once daily. Bioavailability is 64% to 90%. About 93% is protein bound..Metoprolol Succinate **Fig-2**(RS)-1- (isopropylamino)-3-[4-(2-methoxyethyl)phenoxy] propan-2-ol, is a cardio selective drug used in the treatment of hypertension and various cardiovascular disorders. The action of Metoprolol succinate[4] is mediated through the beta1-selectiveadrenoceptorblockage, thus causing reduction in heart rate and cardiac output. It is a beta1-selective drug which belongs to the chemical class of beta blockers and is(±)1-(isopropylamino)-3-[p-(2-methoxyethyl)phenoxy]-2-propanolsuccinate (2:1)(salt) with molecular formula of $(C_{15}H_{25}NO_3)_2$. $C_4H_6O_4$ and molecular weight of.652.81. At low doses, metoprolol selectively blocks cardiac β-1-adrenergic receptors with little activity against β2-adrenergic receptors of the lungs and vascular smooth muscle. Receptor selectivity decreases with higher doses. Unlike propranolol and pindolol, metoprolol does not exhibit membrane-stabilizing or intrinsic sympathomimetic activity. Membrane-stabilizing effects are only observed at doses much higher than those needed for β-adrenergic blocking activity. Metoprolol possesses a single chiral centre and is administered as a racemic mixture.

The Adsorbtion of Metaprolol is rapid and complete. Absorbed readily and completely for the GI tract (oral); peak plasma concentrations after 1.5-2 hours.Distributed widely, crosses the placenta and enters breast milk. Protein-binding: 12%.Its water solubility is 16.9mg/ml.The melting point of Metaprolol in its tartrate form is about 120^{0}C.Metaprolol exist in salts forms of tatrate and succinate. For healthy adults, the starting dose is 25 to 100 mg daily in single or divided doses and the maximum dose is 400 to 450 mg/day Metoprolol tartrate was developed by Novartis and received approval in the United States August 7, 1978.Generic metoprolol succinate was developed by Sandoz and received approval in the United States on July 31, 2006. It is marketed under the brand name Lopressor by Novartis, and Toprol-XL (in the USA); Selokeen (in the Netherlands); as Minax by Alphapharm (in Australia), Metrol by Arrow Pharmaceuticals (in Australia), as Betaloc by AstraZeneca, as Neobloc by Unipharm (in Israel) and as Corvitol by Berlin-Chemie AG (in Germany). In India, this drug is available under the brand names of Metolar and Starpress. A number of generic products are available as well. The active substance metoprolol is employed either as metoprolol succinate or metoprolol tartrate (whereas 100 mg metoprolol tartrate corresponds to 95 mg metoprolol succinate), respectively as prolonged-release or conventional-release formulation. Both Amodlopine besylate and Metaprolol is used as combination for treatment of Hypertension, they are many generic version supplied by various companies in various dosage forms such as AMTAS-M (INTAS) containing 25 mg and 5 mg of Metaprolol and

Amodlopdine respectively, CORVADIL-M(UNICHEM) containing Metaprolol and Amolodpine 25 mg and 2.5 mg respectively, SITELOL-AM(SANOFI-AVENTIS) Containing 25 mg and 5mg of Metaprolol and Amolodipine respectively. METSTAMLO(DRL) capsules Contaning 25 mg and 5mg of Metaprolol and Amolodpine respectively.METALOL-AM of 25and 50 Tablets (CIPLA) containing relatively corresponding amounts of both metaprolol and Amolodpine respectively. From literature survey separate methods have been developed for amolodipine and metaprolol. Several methods have been reported for the quantitative determination of amolodipine in bulk, and pharmaceutical and biological samples. These methods include UV-Visible spectrophotometric and UV-derivative [5-7], HPTLC[8-12], HPLC-UV-detector[12-19], HPLC-Ameperometric detection[20-21], HPLC with Mass-spectrophotometer detector[22], Gas-chromotography-chromatography-Mass-spectrophtometry[23]. Similarly individual methods have been developed for metaprolol which include UV-Visible spectrophotometric and UV-derivative[24], HPLC-UV-detector[25-34], HPLC-UV-Floroscence detector[35], HPLC with solid-phase extraction[36], Super-critical fluid liquid chromatography[37], Hyphenated techniques such as GC-MS[38].

However from literature surevey it has been observed very few and scanty methods have been developed for determination of Amolodpine for both (R) and (S)-enatiomeric[39-40] forms and simultaneous determination of amolodpine and metaprolol by HPLC[41-42]Besides many of the above said methods could not separate amolodpine in its R and S forms.Hence the author has attempted to develop a method for simultaneous determination of metoprolol and Amolodipine. By HPLC [43-44] and UV [45].

MATERIALS AND METHODS

Chemicals and reagents

Amolodipine (99.92%) working standard was gift samples from corpuscle research solutions and Metaprolol (99.89% pure, was procured from corpuscle research solutions. acetontrile (HPLC grade) was obtained from Qualigens fine chemicals. Milli-Q water was purchased from Ranbaxy fine chemicals limited (RFCL). 1-Octane-sulfonic-Acid was purchased from Merck. All chemicals used were of HPLC grade.

Instrumentation

The HPLC system consisted of a Shimadzu Class VP Binary pump LC-10Atvp, SIL-10Dvp Auto sampler, CTO-10Avp column temperature Oven, PDA-UV Detector. All the components of the system are controlled using SCL-10Avp System Controller. Data acquisitions was done using LC-solution software. The mobile phase consists of 70:30 (v/v) of Methanol and 0.1M potassium dihydrogen orthophosphate with 0.2% w/v 1-octane sulfphonic acid as modifier, with pH adjusted to 3.0 with otho phosphoric acid. operated on isocratic mode. Analysis was carried out at 210 nm. The chromatographic separation of Amodlodipine and metaprolol was carried out using Waters spherisorb, 250X 4.6 mm X 5µm, ODS-2 Column. The flow rate is 1.0 ml/min. The injection volume is 20µL.The run time of the method is 10 mins. Diluents consists of mobile phase.

Preparation of solutions

Drug stock solution and internal standard

Two different Stock solution of Amolodipine working standard and Metaprolol was prepared by dissolving accurately weighed 10mg of drug in 10 ml of methanol , so that final concentration is 1mg/1ml.The prepared stock solution is stored in 4^0C protected from light. Suitable dilutions of both the drugs were prepared by using diluent solution.

Calibration standards and quality control samples

An eight point linear calibration curve standards were prepared using diluents solutions in the concentration range of 5.0 to 50.20µg/ml, and 50 to 500 µg/ml for amolodipine and Metaprolol respectively. Calibration standards were prepared at the concentration of 5.05, 10.09, 15.14, 20.19, 31.55, 44.17, 50.47 µg/ml for amolodipine and 50, 100, 150, 200, 300, 400, and 500 µg/ml of metaprolol from first standard stock solution of the linear calibration standard auto sampler for analysis. Three quality control samples were at the concentrations of 12.62, 25.24 and 37.86 µg/ml for amolodipine and 126.88, 253.77 and 375.77 µg/ml of metaprolol were prepared for analysis representing low, medium and high concentration respectively.

Sample preparation

Commercially available tablets of are taken from two different brands and tested for assay. Twenty tablets of each brand are taken and crushed to powder. A powder equivalent to 1mg/ml and 1mg/ml of amolodpine and metaprolol is taken and transferred into a stoppered conical flask to which 25ml of methanol is added. The contents are transferred into a stoppered flask and shaken for 20 mins to extract the drug. Contents are carefully transferred into a centrifuge tube and centrifuged for 4000 rpm for 20 mins. The supernatant liquid is taken and diluted with diluents, to obtain approximately final concentration of 10 µg/ml and 100 µg/ml. This sample is analyzed in triplicate. The accuracy and concentration is determined using regression equation.

Method Validation

System Suitability

System Suitability is an integral part of the LC method. They are used to verify that resolution. reproducibility are adequate. This is based on the concept that equipment, electronics, analytical operations, analytical operator, and sample constitute an integral system that can be evaluated as such. The system suitability was assessed by six replicate analysis of the drug at a concentration of 25 µg/ml and 250 µg/ml concentrations of amolodipine and metaprolol respectively.The acceptance criterion is ± 1% for the per cent coefficient of the variation for the peak area and retention times for the both drug and internal standard. The parameters which we have evaluated are USP Theoretical plates, USP tailing factor and USP Resolution. The following formulas has been used for the calculation of

USP resolution: $Rs = 2(T_1-T_2)/W_1-W_2$

USP Theoretical plates = $5.54\ [T_R/W_R]^2$

USP Tailing = $W_{0.5}$ / 2d

Detection and Quantization Limits (sensitivity)

Limits of detection (LOD) **Fig-3** and quantization (LOQ), **Fig-4** were estimated from both linearity calibration curve method and signal to noise ratio method. The detection limit was defined as the lowest concentration level resulting in a peak area of three times the baseline noise. The quantization limit was defined as the lowest concentration level that provided a peak area with signal to noise ratio higher than 10, with precision (%CV) and accuracy with (±) 10%

Linearity (Calibration Curve)

The calibration curve were constructed with eight concentration ranging from 2.01 to 50.20 µg/ml. The peak area ratio of the drug to the internal standard was evaluated by linearity graph. The linearity was evaluated by linear regression analysis, which was calculated by least square method. It is depicted in **Fig-5(a), Fig-5(b) and Fig-5(c)** respectively.

Accuracy and Precision

Accuracy of assay method was determined for both intra-day and inter-day variations using triplicate analysis of the QC samples. Precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day). Repeatability refers to the use of the analytical procedure within the laboratory over the shorter period of the time that was evaluated by assaying the QC samples during the same day. Intermediate precision was assessed by comparing the assays on different days (3 days).

Specificity

Specificity of the method was determined by injecting 3 samples

1) Chromatogram of Blank.(**Fig-6**).

2) Chromatogram of "R" and "S"-Amolodipine besylate.**Fig-7**).

3) Chromotogram of Metaprolol.(**Fig-8**).

A less than 20% interference of the peak area at the retention time of the drug in the blank sample and zero blank samples are taken as acceptance criteria for the analyte. The interference of the internal standard the peak area at the retention time of the internal standard must the less than 5% in the blank sample. Specificity is also observed in the degradation study of the drug. None of the degraded products must interfere with the quantification of the drug.

Stability

The stability of the drug is determined by using QC samples for the short term stability by keeping at room temperature upto 12 hours and then analyzing them. Further, auto-sampler stability for upto 24 hrs was also established.

RESULTS AND DISCUSSIONS

Method Development and Validation

The HPLC procedure was optimized with a view to develop a stability indicating assay method. Different permutations and combinations, at different pH values ranging from pH 3.0 to pH 11.0 using various columns like Hypersil-BDS-C18, Symmetry C18, YMC-PACK C18YMC-PACK PRO, have been tried with different buffer salts such ammonium acetate, potassium-di-hydrogen orthophosphate, di-potassium hydrogen orthophosphate, in combination with acetonitrile, methanol and tetrahydrofuran. However good resolution, less tailing and high theoretical plates are obtained with Waters spherisorb column C18 250 X 4.6 mm, 5 µm The mobile phase consists of 70:30 (v/v) of Methanol and 0.1M potassium dihydrogen orthophosphate with 0.2% w/v 1-octane sulfphonic acid as modifier, with pH adjusted to 3.0 with otho phosphoric acid. operated on isocratic mode. Analysis was carried out at 210 nm. The chromatographic separation of Amodlodipine and metaprolol was carried out using Waters sphrosorb, 250X 4.6X 5µm, ODS-2 Column. The flow rate is 1.0 ml/min.The injection volume is 20µL.The run time of the method is 10 mins. Diluents consists of mobile phase. The column temperature is maintained at 250 C. At the reported flow rate peak shape was excellent, however increasing or decreasing the flow rate increased the tailing factor and resulting in poor peak shape and also resolution between the drug and internal standard also decreased. Hence 1.0 ml/min was optimized flow rate decreasing the consumption of the mobile phase, which in turn proves to be cost effective for long term routine quality control analysis. There was no interference in the drug and internal standard, from the blank. The peak shape and symmetry were found to be good when the mobile phase composition of 70:30 v/v was used, with better resolution between the (R)- and (S)-Amolidipine and metaprolol drugs.

Method Validation

System Suitability

The % RSD of the peak area and the retention time for both drug and internal standard are within the acceptable the range **Table 1(a).**The efficiency of the column was expressed as the number of USP theoretical plates for the six replicate injections was around 4946 ± 85 and the USP tailing factor was 1.41 ± 0.01 for (R)-amolodipine, 6615 ± 22 and 1.57±0.01 and the USP resolution between the (R)- and (S)-amolodipine is 17.28 ± 0.08. From **Table 1(b)** the efficiency is determined by USP theroretical plates which is around 6498 ± 53, the USP tailing is around 1.85 ± 0.03, and USP resolution between metaprolol and (S)-amolodipine is 8.64 ± 0.02.

Detection and quantization limits (sensitivity)

(**Fig-3)** and (**Fig-4)** represents the six replicate injections of the limit of detection and limit of quantification. The method is found to be sensitive which can be determined from the data obtained from the (**Table 2)** and (**Table 3)**.The LOD Concentration is = 0.25µg/ml, and 2.5 µg/ml for amolodipine and metaprolol respectively. The LOQ concentration is = 0.50 µg/ml and 5.0 µg/ml for amolodipine and metaprolol respectively.

Linearity

The calibration curve constructed was evaluated by its correlation coefficient. The peak area ratio of the drugs, (S)-amolodipine, (R)-amolodipine and metaprolol was linear, and the range, is 5.0 and 50.00µg/ml, for amolodipine and 50 to 500µg/ml, for metaprolol.The linearity was determined in three sets, the correlation coefficient (R^2) was consistently greater then 0.990 (**Table 4(a), Table 4(b), Table 4(c)**). From the data in **Table 4(a), Table 4(b), Table 4(c)** regression equation, limit of quantification and limit of detection was determined from the calibration curve method.

Regression equation:

(1) y = 33472x – 41210, for (R)-amolodipine.

(2) y = 78656x – 11305, for (S)-amolodpine.

(3) y = 24802x + 23877, for metaprolol.

Accuracy and Precision

Accuracy and precision calculated for the QC samples during the intra- and inter –day run are given the (**Table 5(a), Table 5(b), Table 5(c))**.The intra-day (day-1) and inter-day accuracy ranged from 99.15 to 102.18. The results obtained from intermediate precision (inter-day) also indicated a good method precision.All the data were within the acceptance criteria.

Specificity

Specificity was determined from Blank (**Fig-6**) representative chromatograms of (S)- amolodipine, (R)-amolodipine (**Fig-7**) and representative chromatogram of metaprolol (**Fig-8**).

Stability

Stability studies were done for short term stability upto 12 hrs, auto sampler stability upto 24hrs at three different concentrations of low QC, medium QC, High QC levels conditions and the mobile phase is stable upto 72 hrs.These data can be interpreted from **Table 6(a) and Table 6(b)**, for amolodpine and metaprolol respectively.

Robustness study

Robustness is the measure of method capacity to remain unaffected by deliberate small changes in the chromatographic conditions. The experimental conditions were deliberately altered to test evaluate the robustness of the method. The impact of flow-rate(1.0±0.1), column temperature (15^0C 25^0C, 30^0C)changes and effect of mobile-phase composition(±10%) was evaluated on the important system suitability factors such as retention time, theoretical plates, tailing factor, and resolution were studied. The experimental results were presented in the (**Table 7(a) and Table 7(b) Table 7(c)).**

Application of the method to dosage forms

The HPLC method developed is sensitive and specific for the quantitative determination of Metaprolol and Amolodipine besylate. Also the method is validated for different parameters, hence has been applied for the estimation of drug in pharmaceutical dosage forms. Metaprolol and Amolodipine besylate tablets of 25mg, 5mg strength from two different manufacturers were evaluated for the amount of Metaprolol and Amolodipine besylate tablets. The amount of Metaprolol in tablet 1 is 97.06 ± 0.56 and tablet 2 is 99.24 ± 0.32 and amolodpine besylate in tablet 1 is 98.79 ± 0.86 and tablet 2 is 99.59 ± 0.90 (**Table 8**).None of the tablets ingredients interfere with the analytic peak.

CONCLUSION

A rapid sensitive and specific method for the determination of amolodipine and metaprolol in the pharmaceutical; formulations has been developed using external and simultaneous determination method. The method gave accurate and precise results in the concentration range of 5.0-50.00µg/ml, for amolodipine and 50.00

to 500µg/ml for metaprolol respectively. 70:30(v/v) of Methanol and 0.1M potassium di hydrogen ortho phosphate with 0.1% W/V 1-octane sulfonic acid with pH adjusted to 3.0.The wavelength of detection is 210 nm. The flow rate of 1.0 ml/min. The retention times of (R) –amolodpine, (S)-amolodpine and metaprolol are 2.11 ± 0.01, 8.51 ± 0.03 and 5.52 ± 0.01 respectively.

Abbreviations

Rs **=** USP Resolution.

T_1, T_1 = retention time of the Peak 1, Peak 2.

W1, W2 = peak width 1 and 2 at the base line.

T_R = retention time along the baseline from the point of injection to the perpendicular dropped from the maximum of the peak corresponding to the analyte.

W_R = width of the peak at half the height.

$W_{0.5}$ = width of the peak at 1/20 of peak height.

d = distance between perpendicular dropped from peak maximum to the leading edge of the peak at 1/20 of peak height.

Table 1(a): System Suitability Study

	R-Amolodipine			S-Amolodipine			Resolution
	R.T	T.P	Tailing	R.T	T.P	Tailing	
Inj-01	2.08	4830	1.4	8.56	6594	1.56	17.4
Inj-02	2.12	4932	1.43	8.54	6601	1.57	17.29
Inj-03	2.12	5067	1.41	8.5	6606	1.58	17.29
Inj-04	2.12	4927	1.41	8.49	6608	1.59	17.20
Inj-05	2.12	5022	1.4	8.49	6632	1.57	17.26
Inj-06	2.12	4899	1.41	8.48	6652	1.58	17.21
Mean	2.11	4946.17	1.41	8.51	6615.5	1.575	17.28
S.D	0.02	85.60	0.011	0.032	22.02	0.010	0.072
RSD	0.77	1.73	0.78	0.38	0.33	0.67	0.42

Table 1(b): System Suitability Study

	Metaprolol			Resolution
	R.T	T.P	Tailing	
Inj-01	5.52	6394	1.8	8.68
Inj-02	5.54	6528	1.88	8.64
Inj-03	5.52	6495	1.86	8.62
Inj-04	5.51	6518	1.89	8.61
Inj-05	5.51	6513	1.85	8.64
Inj-06	5.51	6545	1.84	8.63
Mean	5.52	6498.83	1.85	8.64
S.D	0.012	53.95	0.03	0.02
RSD	0.21	0.83	1.73	0.28

Table 2: Limit of detection

Injection. No	R-Amolodipine	S-Amolodipine	Metaprolol
01	26942	18065	57142
02	27190	14264	51879
03	27697	16840	53392
04	27456	17786	54345
05	27543	17498	54564
06	27987	17543	54634
Mean	27469.17	16999.33	54326
S.D	369.19	1400.44	1728.69
RSD	1.34	8.24	3.18

Table 3: Limit of Quantification

Injection. No	R-Amolodipine	S-Amolodpine	Metaprolol
01	111323	37127	111471
02	111228	37625	111424
03	111706	37706	112872
04	111675	37435	111675
05	111543	37453	111876
06	111198	37564	111956
Mean	111445.5	37485	111879
S.D	225.21	203.16	530.49
RSD	0.20	0.54	0.47

Table 4(a): Results and regression analysis of linearity data of R-Amolodipine

Mean ± S.D(n=3)	
Slope	33472 **±** 0.06
Intercept	-41210± 0.08
Correlation coefficient(R^2)	0.999 ± 0.0003

Each mean value is a result of triplicate analysis (n=3)

Table 4(b): Results and regression analysis of linearity data of S-Amolodipine

Mean ± S.D(n=3)	
Slope	38656 ± 0.06
Intercept	-11305± 0.08
Correlation coefficient(R^2)	0.998 ± 0.0003

Each mean value is a result of triplicate analysis (n=3)

Table 4(c): Results and regression analysis of linearity data of Metaprolol

Mean ± S.D(n=3)	
Slope	24802 ± 0.06
Intercept	23807 ± 0.08
Correlation coefficient(R^2)	0.994 ± 0.0003

Each mean value is a result of triplicate analysis (n=3)

Table 5(a): Intra-day and Inter-day precision and accuracy of HPLC assay of (R)-amolodipine

	Nominal concentration		
	12.62 µg/ml	**25.24 µg/ml**	**37.86 µg/ml**
Day=1			
Mean (n=3)	384538.3	812357	1226008
S.D	459.5197	1624.629	1818.633
R.S.D	0.119499	0.19999	0.148338
Recovery(%)	100.7887	101.0338	99.99751
Day=2			
Mean(n=3)	386164	814087	1225262
S.D	1025.655	4566.34	3938.109
R.S.D	0.265601	0.560915	0.32141
Recovery(%)	101.4066	101.2386	99.15198
Day-3			
Mean (n=3)	386003.7	809787	1223920
S.D	1051.966	5770.014	3368.271
R.S.D	0.272527	0.287129	0.275204
Recovery(%)	101.1356	100.7296	99.83269

Each mean value is a result of triplicate analysis (n=3)

Table 5(b): Intra-day and Inter-day precision and accuracy of HPLC assay of (S)-amolodipine

	Nominal concentration		
	12.62 µg/ml	**25.24 µg/ml**	**37.86 µg/ml**
Day=1			
Mean (n=3)	996594.7	2009734	3004266
S.D	2984.33	5669.38	1377.12
R.S.D	0.30	0.28	0.05
Recovery(%)	99.54	100.80	100.26
Day=2			
Mean(n=3)	996155.3	2009734	3007316
S.D	2852.65	5669.38	7239.91
R.S.D	0.29	0.28	0.24
Recovery(%)	101.49	101.61	101.61
Day-3			
Mean (n=3)	995972	2009557	3008385
S.D	1051.97	5770.01	9247.74
R.S.D	0.27	0.29	0.31
Recovery(%)	101.14	100.73	101.40

Each mean value is a result of triplicate analysis (n=3)

Table 5(c): Intra-day and Inter-day precision and accuracy of HPLC assay of Metaprolol

	Nominal concentration		
	126.88 µg/ml	**253.77 µg/ml**	**375.77 µg/ml**
Day=1			
Mean (n=3)	3238524	6303843	9418967
S.D	37659.81	2433.858	23158.41
R.S.D	1.16	0.04	0.25
Recovery(%)	100.15	99.78	100.81
Day=2			
Mean(n=3)	3238473	6303730	9417750
S.D	37236.79	1836.932	11810.22
R.S.D	1.15	0.03	0.13
Recovery(%)	102.15	99.78	100.79
Day-3			
Mean (n=3)	3239393	6303307	9406182
S.D	36155.95	2040.434	2305.39
R.S.D	1.12	0.03	0.02
Recovery(%)	102.18	99.77	100.67

Each mean value is a result of triplicate analysis (n=3)

Table 6(a): Short-term, long term and auto-sampler stability of (R)-amolodpine

	Nominal concentration		
	12.62 µg/ml	**25.24 µg/ml**	**37.86 µg/ml**
Short term stability (12 hrs)			
Mean (n=3)	374210	802441	1217409
S.D	2417.469	5712.005	5183.077
R.S.D	0.643164	0.706325	0.423809
Recovery(%)	98.34366	100.6002	99.75814
Auto sampler stability(24 hrs)			
Mean(n=3)	372098	800987	1206075
S.D	2236.3	6786.191	9450.504
R.S.D	0.596689	0.840859	0.775426
Recovery(%)	96.01184	100.4062	99.42474

Table 6(b): Short-term, long term and auto-sampler stability of (S)-amolodpine

	Nominal concentration		
	12.62 µg/ml	**25.24 µg/ml**	**37.86 µg/ml**
Short term stability (12 hrs)			
Mean (n=3)	993456	2006477	3001202
S.D	2984.333	2593.831	3243.113
R.S.D	0.299453	0.129446	0.107998
Recovery(%)	101.2212	101.6373	101.1616
Auto sampler stability(24 hrs)			
Mean(n=3)	989856	2000134	3000089
S.D	4427.479	1573.54	3005.572
R.S.D	0.444901	0.078623	0.100112
Recovery(%)	100.8585	101.3178	101.1242

Table 6(c): Short-term, long term and auto-sampler stability of Metaprolol

	Nominal concentration		
	126.88 µg/ml	**253.77 µg/ml**	**375.77 µg/ml**
Short term stability (12 hrs)			
Mean (n=3)	3165439	6302098	9405057
S.D	19234.74	3321.786	23109.7
R.S.D	0.61	0.05	0.25
Recovery(%)	99.83	99.75	100.66
Auto sampler stability(24 hrs)			
Mean(n=3)	3160970	6300089	9390100
S.D	18335.36	4930.179	12430.46
R.S.D	0.58	0.08	0.13
Recovery(%)	99.69	99.72	100.50

Table 7(a): Effect of Various parameters in assessment of method for (R)-Amolodipine

Parameters	**Variation**	**Observed values**			
		R.T	**T.P**	**Tailing**	**Resolution**
Flow rate	0.9ml/min	2.38	4946	1.44	18.16
	1.0ml/min	2.11	4947	1.41	17.28
Column temperature	150C	2.11	4804	1.4	17.64
	250C	2.11	4947	1.41	17.28
	300C	2.11	4869	1.39	17.63
Mobile phase	100% organic	2.11	4947	1.41	17.28
	110% organic	1.83	3992	1.6	13.6
Analyst-Analyst Variation					
Analyst-1		2.11	4947	1.41	17.28
Analyst-2		2.12	4778	1.47	17.66
Analyst-3		2.11	4786	1.48	17.64
Analyst-4		2.07	4766	1.44	17.71

Table 7(b): Effect of Various parameters in assessment of method for (S)-Amolodipine

Parameters	Variation	Observed values			
		R.T	T.P	Tailing	Resolution
Flow rate	0.9ml/min	6.33	6615	1.94	17.64
	1.0ml/min	5.52	6616	1.85	17.28
Column temperature	150C	5.68	6421	1.85	17.63
	250C	5.52	6616	1.85	17.28
	300C	5.68	6424	1.86	13.6
Mobile phase	100% organic	5.52	6616	1.85	
	110% organic	4.7	6544	2.27	17.28
Analyst-Analyst Variation					
Analyst-1		5.52	6616	1.85	17.64
Analyst-2		5.69	6535	1.84	17.77
Analyst-3		5.68	6475	1.84	17.64
Analyst-4		5.89	6539	1.86	17.28

Table 7(c): Effect of Various parameters in assessment of method for Metaprolol

Parameters	Variation	Observed values			
		R.T	T.P	Tailing	Resolution
Flow rate	0.9ml/min	9.98	6920	1.65	9.26
	1.0ml/min	8.51	6615	1.58	8.64
Column temperature	150C	8.94	6589	1.54	9.03
	250C	8.51	6615	1.58	8.64
	300C	8.94	6620	1.54	9.02
Mobile phase	100% organic	8.51	6615	1.58	8.64
	110% organic	7.84	6850	1.69	7.6
Analyst-Analyst Variation					
Analyst-1		8.51	6615	1.58	8.64
Analyst-2		8.93	6562	1.61	8.97
Analyst-3		8.93	6547	1.59	8.98
Analyst-4		8.64	6341	1.56	9

Table 8: Results of amolodipine and metaprolol in marketed product

Marketed formulation	Drug	% Amount obtained	% RSD
Brand-1	Amolodipine -5 mg	98.79± 0.86	0.30
Brand-2	Amolodipine -5 mg	99.59 ±0.90	0.32
Brand-1	Metaprolol-25 mg	99.06 ± 0.56	0.56
Brand-2	Metaprolol-25 mg	99.24 ±0.32	0.48

Each value is a result of triplicate analysis.

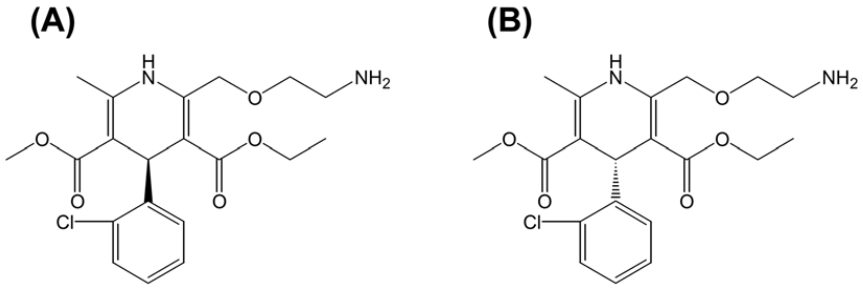

Fig. 1: Chemical structures of amlodipine (A) R- and (B) S-isomers.

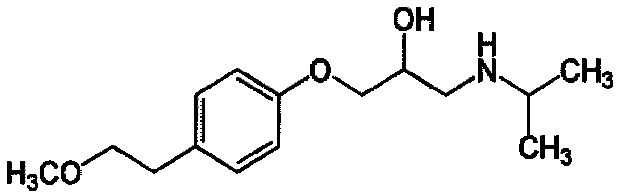

Fig. 2: Struture of Metaprolol

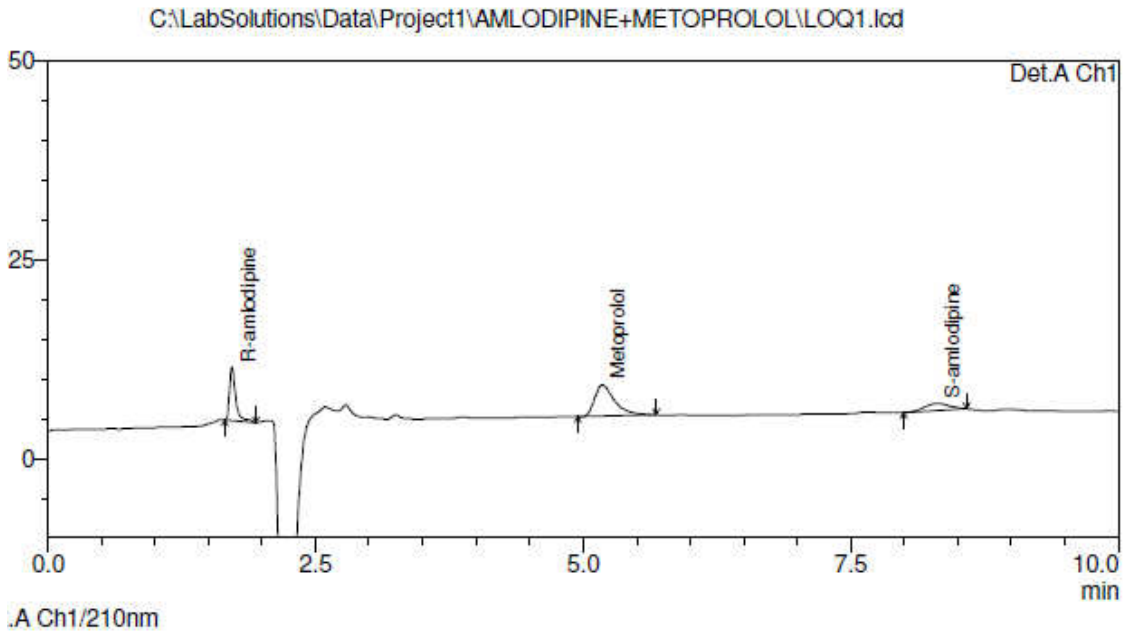

Fig. 3: LOD Chromatogram of (R)-and (S)-Amolodpine and Metaprolol

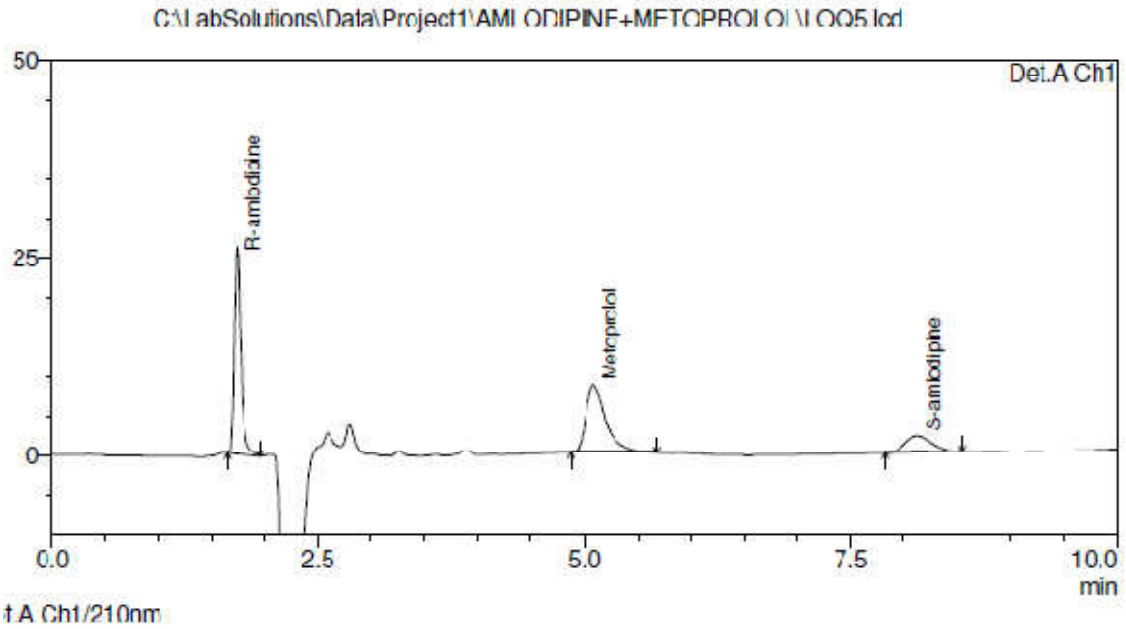

Fig. 4: LOD Chromatogram of (R)-and (S)-Amolodpine and Metaprolol

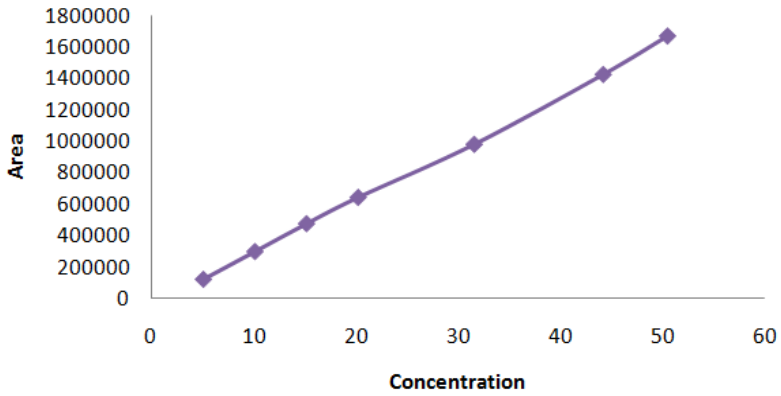

Fig. 5(a): Linear calibration curve of (R)-Amolodipine besylate

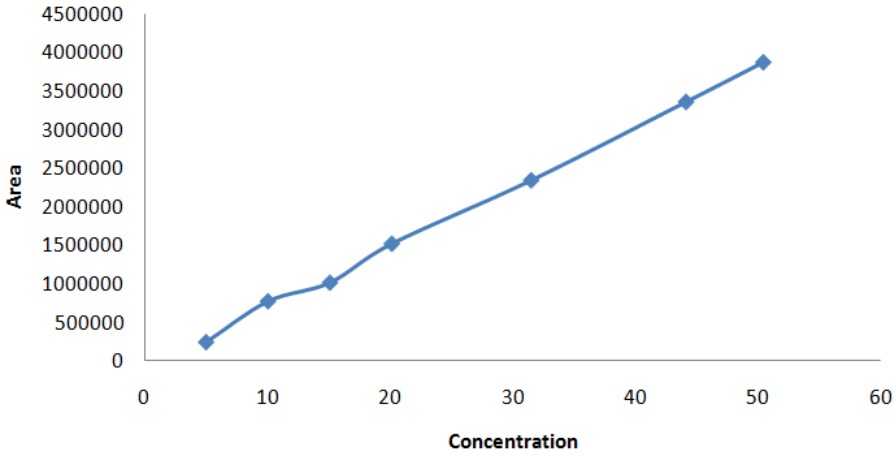

Fig. 5(b): Linear calibration curve of (S)-Amolodipine besylate

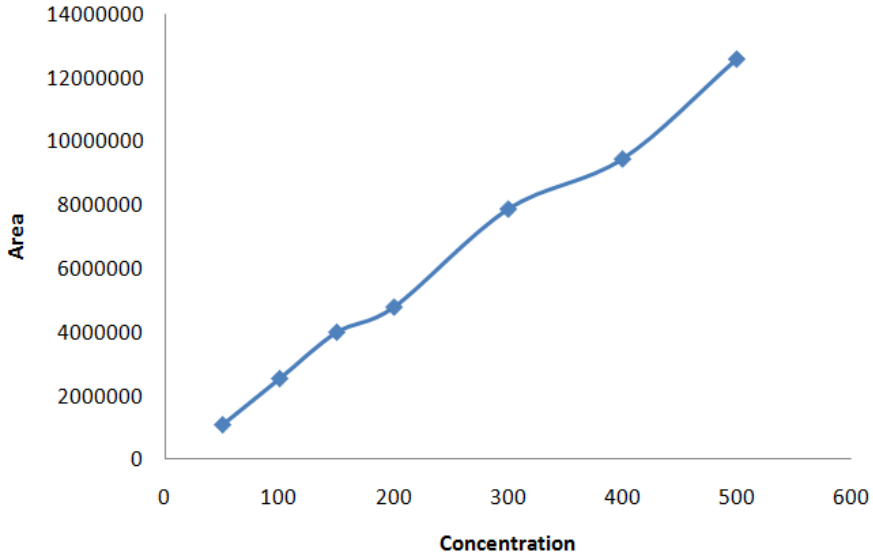

Fig. 5(c): Linear calibration curve of Metaprolol succinate

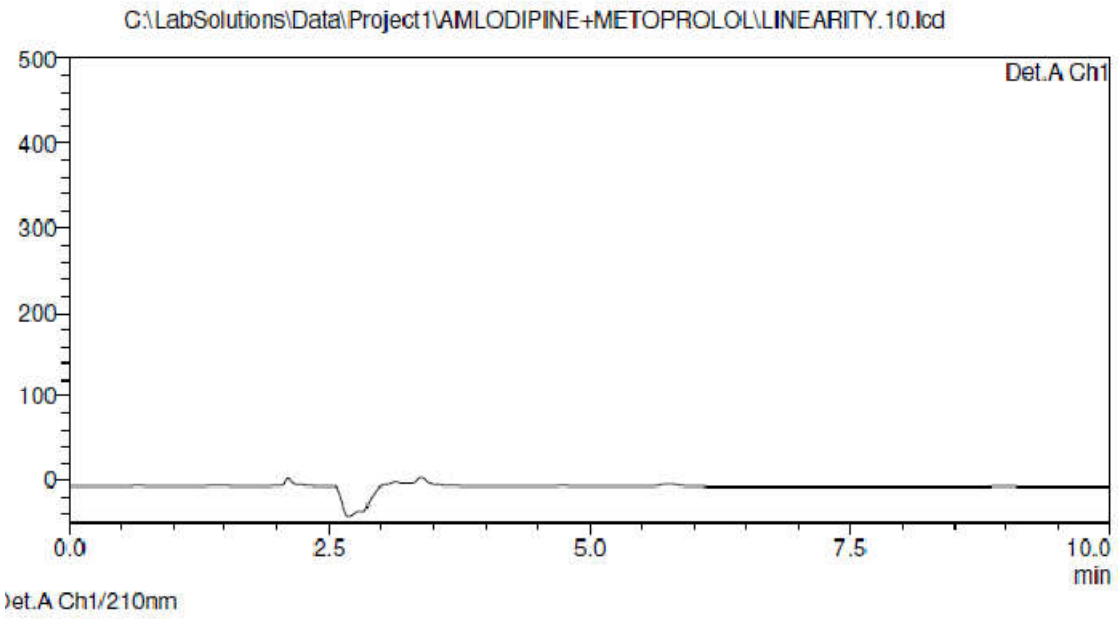

Fig. 6: Blank Chromatogram

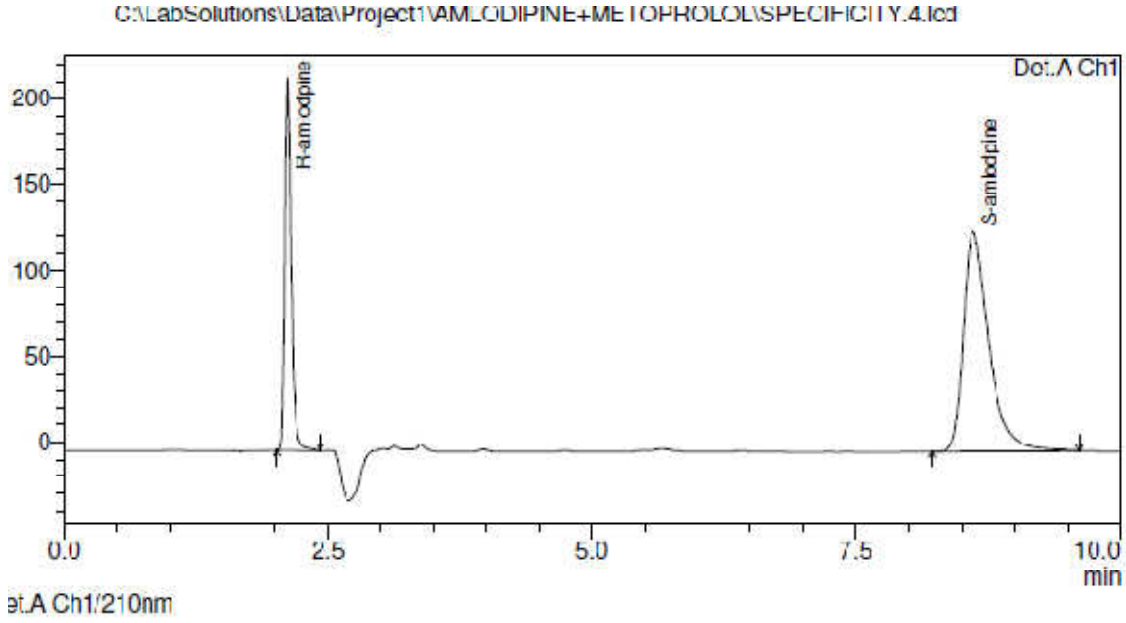

Fig. 7: (R)- and (S)-Amolodipine Chromatogram

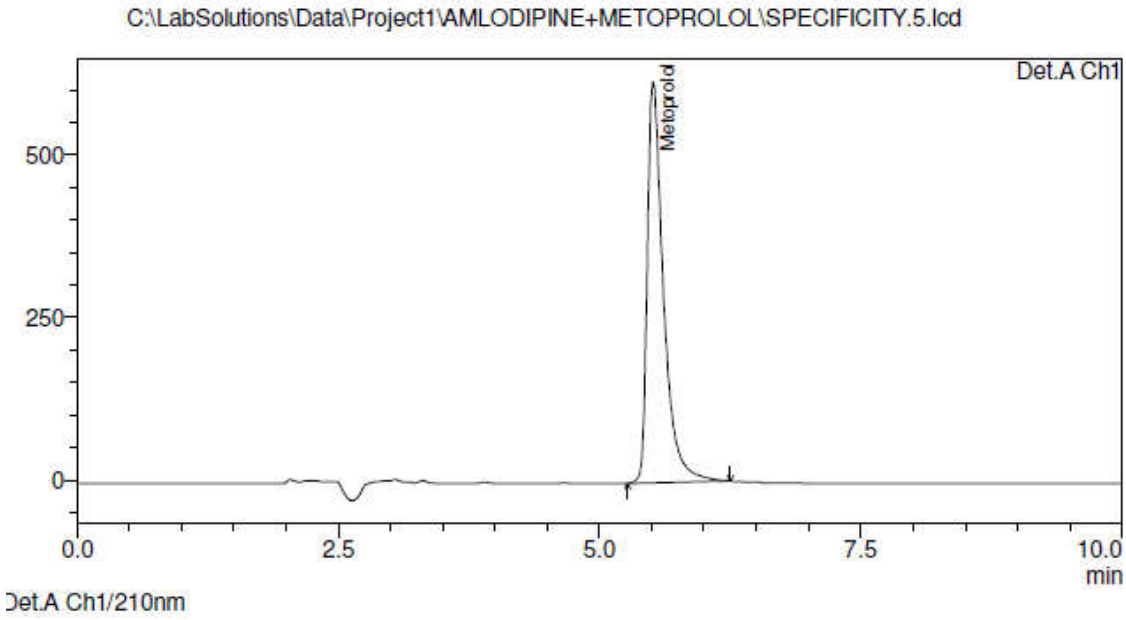

Fig. 8: Metaprolol Chromatogram

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