8 RP-HPLC METHOD FOR THE DEVELOPMENT AND VALIDATION OF AMLODIPINE BESYLATE AND METOPROLOL TARTRATE

Amlodipine besylate is a calcium channel blocking agent. Metoprolol tartrate is used for the management of hypertension and long term management of patients with angina pectoris.

8.1 Drug Profile: 136-41

AMLODIPINEBESYLATE: Amlodipine besylate is a calcium channel blocking agent. It inhibits the influx of intracellular and extracellular calcium across the myocardial and vascular smooth muscle cell membranes.

Synonyms: Amlodipine benzene sulfonate, Amlodipine besylate, Amlodipine besylate.

Brand Names: Amlodis, Amlocard, Amvaz, Norvasc.

Molecular Structure:

![Figure 8.1: Structure of Amlodipine besylate(AMD)](image)

Chemical Name: 2-[(2-Aminoethoxy)methyl]-4-(2-chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridine benzenesulfonate.

Molecular Formula: C_{20}H_{25}ClN_{2}O_{5}.C_{6}H_{6}O_{3}S

Molecular Weight: 408.8760 gm/mol

Category: Anti anginals, Anti-hypertensive agents, Vasodilator agents, Calcium channel blockers.

Solubility: It is slightly soluble in water and soluble in methanol.

Physical state: It is a white to pale yellow crystalline powder.

Half-life: 30-50 hours

Absolute bioavailability: 64-90%

Melting point: 178-179°C
Mechanism of Action:

Amlodipine besylate is a calcium channel blocking agent. It inhibits the influx of intracellular and extracellular calcium across the myocardial and vascular smooth muscle cell membranes. The decrease in intracellular calcium inhibits the contractile processes of the myocardial smooth muscle cells causing dilation of the coronary and systemic arteries, increased oxygen delivery to myocardial tissue, decreased total peripheral resistance, and decreased systemic blood pressure.

Therapeutic uses: For the treatment of hypertension, chronic stable angina and confirmed or suspected vasospastic angina. Amlodipine is used with or without other medications to treat high blood pressure. Lowering high blood pressure helps prevent strokes, heart attacks, and kidney problems. Amlodipine belongs to a class of drugs known as calcium channel blockers. It works by relaxing blood vessels so blood can flow more easily. Amlodipine is also used to prevent certain types of chest pain (angina). It may help to increase your ability to exercise and decrease the frequency of angina attacks. It should not be used to treat attacks of chest pain when they occur. Use other medications (such as sublingual nitro-glycerine) to relieve attacks of chest pain as directed by your doctor.

Dosage: Tablet 10mg, 5mg and 2.5mg.

Pharmacodynamics: Amlodipine besylate belongs to the dihydropyridine (DHP) class of calcium channel blockers (CCBs), the most widely used class of CCBs. There are at least five different types of calcium channels in Homo sapiens: L-, N-, P/Q-, R- and T-type. It was widely accepted that DHP CCBs target L-type calcium channels, the major channel in muscle cells that mediate contraction; however, some studies have indicated that Amlodipine besylate also binds to and inhibits N-type calcium channels (see references in Targets section). Similar to other DHP CCBs, Amlodipine besylate binds directly to inactive L-type calcium channels stabilizing their inactive conformation. Since arterial smooth muscle depolarizations are longer in duration than cardiac muscle depolarizations, inactive channels are more prevalent in smooth muscle cells. Alternative splicing of the alpha-1 subunit of the channel gives Amlodipine besylate additional arterial selectivity. At therapeutic sub-toxic concentrations, Amlodipine besylate has little effect on cardiac myocytes and conduction cells.
Pharmacokinetics:

Absorption: Amlodipine besylate is slowly and almost completely absorbed from the gastrointestinal tract. Oral and well absorbed. Peak plasma concentrations are reached 6-12 hour following oral administration. Absorption is not affected by food.

Distribution: Protein Binding: 97.5%

Metabolism: Hepatic

Elimination: Urine

Adverse Reactions:

- Cardiovascular: flushing, palpitations
- CNS: Headache, dizziness, fatigue, somnolence
- Dermatologic: Rashes, pruritis
- Gastrointestinal: nausea, abdominal pain, dyspepsia.

Drug interactions: In patients with severe coronary artery disease, amlodipine besylate can increase the frequency and severity of angina or actually cause a heart attack on rare occasions. This phenomenon usually occurs when first starting amlodipine besylate, or at the time of dosage increase. Excessive lowering of blood pressure during initiation of amlodipine besylate treatment can occur, especially in patients already taking another blood pressure lowering medication. In rare instances, congestive heart failure has been associated with Amlodipine besylate, usually in patients already on a beta blocker.

Contraindications:

- Breast feeding
- Cardiogenic shock
- Unstable angina
- Aortic stenosis: Amlodipine besylate causes vasodilation, which can result in reduced cardiac output in patients with severe aortic stenosis.

Administration: Amlodipine besylate can be taken with or without food. Amlodipine besylate is metabolized mainly by the liver and dosages may need to be lowered in patients with liver dysfunction.

Storage: Store at room temperature of 15° to 30°C.
**METOPROLOL:** It is used for the management of hypertension and long term management of patients with angina pectoris. Metoprolol tartrate is used with or without other medications to treat high blood pressure

**Synonyms:** Metoprolol tartarate, Metoprolol succinate.

**Brand Names:** Tropol-XI, Toprol, Selopral, Seloken, Selo-zok, Betaloc, Beloc, Lopressor.

**Structure:**

![Structure of Metoprolol Tartrate (MTP)](image)

**Chemical Name:** (RS)-1-(Isopropyl amino)-3-[4-(2-methoxyethyl) phenoxy] propan-2-ol

**Molecular Formula:** \((\text{C}_{15}\text{H}_{25}\text{NO}_3)_2\text{C}_4\text{H}_6\text{O}_4\)

**Molecular Weight:** 652.8

**Category:** Antihypertensive agents, Adrenergic beta antagonists, Sympatholytics, Antiarrhythmic agents.

**Solubility:** Soluble in water, methanol and slightly soluble in dichloromethane.

**Physical state:** A white crystalline powder or colourless crystals.

**Half-life:** 3-7 hours

**Absolute bioavailability:** 50%.

**Melting point:** 120°C

**Mechanism of Action:**

Competitively blocks beta-adrenergic receptors in the heart. They lead to decreased heart rate decreasing the work load by the heart. They do not produce coronary vasodilatation but lead to a shift and redistribution of coronary circulation to the ischaemic areas. It decreases the release of renin from the kidney, thus lowering blood pressure. Acts in the Central nervous system to reduce sympathetic outflow and vasoconstrictor tone.
**Therapeutic uses:** It is used for the management of hypertension and long term management of patients with angina pectoris. Metoprolol is used with or without other medications to treat high blood pressure (hypertension). Lowering high blood pressure helps prevent strokes, heart attacks, and kidney problems. This medication is also used to treat chest pain (angina) and to improve survival after a heart attack. Metoprolol belongs to a class of drugs known as beta blockers. It works by blocking the action of certain natural chemicals in your body, such as epinephrine, on the heart and blood vessels. This effect lowers the heart rate, blood pressure, and strain on the heart. This medication may also be used for irregular heartbeats, heart failure, migraine headache prevention, tremors and other conditions as determined by your doctor.

**Dosage:** Available as Injection solution as tartarate 1mg/ml

**Tablets:** 25mg, 50mg, 100mg.

- The dose for treating hypertension is 100-450 mg daily in single or divided doses.
- Angina is treated with 100-400 mg daily in two divided doses.
- Heart attack (acute myocardial infarction) is treated with three 5 mg injections administered 2 minutes apart followed by treatment with 50 mg oral metoprolol every 6 hours for 48 hours. After 48 hours, patients should receive 100 mg orally twice daily for at least 3 months.
- The dose for congestive heart failure is 25 mg/daily initially. Then the dose is increased every 2 weeks to reach a target dose of 200 mg/daily orally.
- Hyperthyroidism is treated with 25 to 30 mg by mouth every 6 hours.

**Pharmacodynamics:** Metoprolol tartrate, a competitive, beta1-selective (cardioselective) adrenergic antagonist, is similar to atenolol in its moderate lipid solubility, lack of intrinsic sympathomimetic activity (ISA) and weak membrane stabilizing activity (MSA).

**Pharmacokinetics:**

**Absorption:** 50%

**Distribution:** Protein binding: 12%

**Metabolism:** Extensively hepatic; significant first pass effect. Metoprolol undergoes a-hydroxylation and O-demethylation as a substrate of the cytochrome liver enzymes CYP2D6 and a small percentage by CYP3A4.
Elimination: Through urine 3-10 % (as unchanged drug)

Side effects: Metoprolol is generally well tolerated. Side effects include abdominal cramps, diarrhoea, constipation, fatigue, insomnia, nausea, depression, dreaming, memory loss, fever, impotence, light-headedness, slow heart rate, low blood pressure, cold extremities, sore throat, and shortness of breath or wheezing. Metoprolol can aggravate breathing difficulties in patients with asthma, chronic bronchitis, or emphysema.

In patients with existing slow heart rates (bradycardias) and heart blocks (defects in the electrical conduction of the heart), metoprolol can cause dangerously slow heart rates, and even shock. Metoprolol reduces the force of heart muscle contraction and can aggravate symptoms of heart failure. In patients with coronary artery disease, abruptly stopping metoprolol can suddenly worsen angina, and occasionally precipitate heart attacks. If it is necessary to discontinue metoprolol, its dosage should be reduced gradually over several weeks.

Initiation of high-dose extended release metoprolol in patients undergoing non-cardiac surgery is associated with bradycardia (slow heart rate), hypotension, stroke, and death. However, long-term therapy with Metoprolol tartrate should not be routinely withdrawn prior to major surgery. Impaired ability of the heart to respond to reflex adrenergic stimuli may increase the risks of general anesthesia and surgery.

Adverse Reactions:

- **CNS:** Drowsiness, insomnia, mental depression
- **Endocrine & metabolic:** Decreased sexual ability
- **Cardiovascular:** Bradycardia, palpitation, edema, congestive heart failure and reduced peripheral circulation.
- **Gastrointestinal tract:** Diarrhoea, nausea, stomach discomfort
- **Respiratory:** Bronchospasm.

Drug interactions: Calcium channel blockers and digoxin (Lanoxin) can lower blood pressure and heart rate to dangerous levels when administered together with metoprolol.

Metoprolol tartrate can mask the early warning symptoms of low blood sugar (hypoglycemia) and should be used with caution in patients receiving treatment for diabetes.
Fluoxetine (Prozac) can increase blood levels of Metoprolol tartrate by reducing breakdown of Metoprolol tartrate, and increase the side effects from Metoprolol tartrate.

**Administration:** Metoprolol should be taken before meals or at bedtime.

**Storage:** Store protected from light.
8.2 LITERATURE REVIEW

Kardile D P et al.,\textsuperscript{107} developed one reverse phase high performance liquid chromatography methods for the simultaneous estimation of Amlodipine besylate and Olmesartan medoximil in tablet dosage form. In reverse phase high performance liquid chromatography analysis is carried out using 0.05M potassium dihydrogen phosphate: ACN (50:50v/v) pH (6.8) as the mobile phase and C18 bonded phase i.e. CAPCEL PACK col No. AKAD 05395 (4.6mmx250mm) with particle size 5μm as stationary phase with detection wavelength of 230.0-260.0nm linearity was obtained in the concentration range of 5 and 20μg/ml for Amlodipine besylate and Olmesartan medoximil respectively.

Permender Rathee et al.,\textsuperscript{108} developed a second derivative UV spectrophotometric method for the simultaneous assay of Amlodipine besylate and Lisinopril in bulk drug and in tablet dosage forms using double distilled water as the solvent. The method is based on simultaneous equation method. The \( \lambda_{\text{max}} \) values for Amlodipine and Lisinopril in the solvent medium were found to be 256.0nm and 216.0nm respectively. The system obeys Beer’s law in the range of 10.0 to 70.0μg/ml and 4.0 to 40.0μg/ml with correlation coefficient of 0.9994 and 0.9996 for Amlodipine and Lisinopril respectively. Repeatability, inter and intraday precision were found to be 0.134, 0.280, 0.349 and 0.205, 0.530, 0.569 respectively.

Priyanka patil R et al.,\textsuperscript{109} developed a spectrophotometric method for simultaneous estimation of Ramipril and Amlodipine. For this simultaneous equation method is used. The method involved measurement of absorbance at two wavelengths, 210.0nm and 238.0nm respectively for Ramipril and Amlodipine. Beer’s law obeyed in the concentration range of 15-35μg/ml and 5-25μg/ml for Ramipril and Amlodipine respectively. The proposed method is recommended for routine analysis since it is rapid, simple, accurate and also sensitive and specific by no heating and no organic solvent extraction.

Basavaiah K et al.,\textsuperscript{110} developed two rapid assay procedures based on visible spectrophotometry and HPLC for the determination of Amlodipine besylate in pharmaceutical formulations. Spectrophotometric method is based on the bromination of Amlodipine with a known excess of bromate-bromide mixture in acid medium followed by the determination of surplus bromine by reacting with metanil yellow and measuring the absorbance at 530.0nm. The HPLC determination was carried out on a reversed phase C\textsubscript{18} column using 0.1% orthophosphoric acid (pH-3.0): acetonitrile
(20:80) at a flow rate of 1.0ml/min with UV detection at 238.0nm. In the spectrophotometric method, it is found to increase linearly with increasing concentration of Amlodipine which is corroborated by the calculated correlation coefficient of 0.9975. The system obeys Beer’s law for 1.25-7.50μg/ml Amlodipine with a molar absorptivity of $2.51 \times 10^4 \text{ mol}^{-1} \text{ cm}^{-1}$ and a sandell’s sensitivity of 16.37ng cm$^{-2}$. The limit of detection and quantitation are calculated to be 0.17 and 0.56μg/ml respectively.

**Rajeswari A. et al.,** developed a reverse phase HPLC method for the simultaneous estimation of Amlodipine besylate and Telmisartan. A Phenominex-luna C18 column (250 x 4.6 mm i.d 5μ) was used with a mobile phase containing a mixture of acetonitrile and phosphate buffer in the ratio of 56:44%v/v pH was adjusted with orthophosphoric acid to 4. The flow rate was 1ml/min and the eluents were monitored at the detector wavelength of 236nm. The retention times of Amlodipine besylate and Telmisartan were found to be 4.32 and 5.32 minutes respectively.

**Shrivastava et al.,** developed and validated an isocratic reversed-phase high-performance liquid chromatographic method (RP-HPLC) for the simultaneous estimation of Amlodipine and Telmisartan in combined dosage form. The chromatographic separation was achieved by using mobile phase acetonitrile and 0.05M sodium dihydrogen phosphate buffer (60:40) adjusted to pH 6.0, a C-18 column, perfectsil target ODS3 (150 mm x 4.6 mm i.d., 5 μm). The mobile phase was pumped at a flow rate of 0.8 ml/min and the eluents were monitored at 254 nm. Retention times were 4.0 min and 8.2 min for Amlodipine and Telmisartan respectively. Linearity for Amlodipine besylate and Telmisartan was established in the range of 5-30 and 10-60 μg/ml, respectively. The recoveries for the two compounds were above 96%.

**A. Kottai Muthu et al.,** developed a method for the simultaneous determination of Amlodipine and Telmisartan in bulk drug and pharmaceutical dosage by RP-HPLC method. Separation was performed on a 5μm Nucleodur® C18 column (250 × 4.6mm ID) with acetonitrile: phosphate buffer at pH 4.5 (60:40v/v) in isocratic elution in less than 9 min with a flow rate of 1.3 ml min$^{-1}$. Good sensitivity for all analytes was observed with UV detection at 238 nm. The method allowed quantification over the 1-11 μg/ml range for Amlodipine and 8-80 μg/ml range for Telmisartan.
S. Rajitha et al.,\textsuperscript{114} developed a reverse phase high performance liquid chromatographic method for simultaneous estimation of Telmisartan and Amlodipine Besylate in tablet dosage form. Symmetry C18 4.6 x 250mm, 5\textmu m particle size was used. The method was carried out in gradient program using mobile phase, 0.02M Potassium dihydrogen orthophosphate: acetonitrile (30:70 v/v) adjusted to pH-5 using dilute ortho phosphoric acid. Flow rate was adjusted to 1.0ml/min and effluents were monitored at 245nm. The retention time obtained for Amlodipine Besylate and Telmisartan was 2.325 and 3.523 min respectively. The calibration curves were linear in the concentration range of 32-96\textmu g/ml for Telmisartan and 4-12\textmu g/ml for Amlodipine.

Agey et al.,\textsuperscript{115} described two methods for simultaneous estimation of Telmisartan and Amlodipine in binary mixture by using UV Spectrophotometry and RP-HPLC. The first method was based on UV-spectrophotometric determination of two drugs which involves absorbance measurement at 297.0 nm (\lambda_{max} of Telmisartan) and 362.0 nm (\lambda_{max} of Amlodipine) in methanol: water (70:30); linearity was obtained in the range of 8-40 \mu g/ml and 1-5 \mu g/ml for Telmisartan and Amlodipine respectively. The second method was based on RP-HPLC and separation of the two drugs was achieved on phenomenex C18 column with acetonitrile and phosphate buffer, pH 2.9 (gradient program). The method was linear with entire range of Telmisartan (16-48 \mu g/ml) and Amlodipine (2-6 \mu g/ml) with coefficient of correlation was above 0.995 for both methods.

Joshi Pryanka et al.,\textsuperscript{116} developed a reverse phase High Performance Liquid Chromatographic method for simultaneous estimation of Metoprolol and Telmisartan in tablet dosage form on RP C-18 Column (Hypersil Gold, 25cm x 4.6mm, 5\mu m) using Acetonitrile and buffer (0.05M KH$_2$PO$_4$, pH 3.0±0.02, 35:65 v/v) as mobile phase at a flow rate of 1.0 ml/min and the detection wavelength was 225nm. The retention time for Metoprolol and Telmisartan was found to be 3.71 and 10.02 min. respectively. Detection response for both Metoprolol and Telmisartan were found to be linear in concentration range of 29.88-69.72mcg/ml and 24.12-56.27mcg/ml respectively in the linearity study, regression equation and coefficient of correlation for Metoprolol and Telmisartan were found to be (y = 33409x + 5518, R$^2$ =0.9999) and (y = 111545x – 5850.4, R$^2$ = 0.9999).
Kevin Vachhani H et al.,\textsuperscript{117} developed and validated a RP-HPLC method for the simultaneous analysis of Metoprolol and Olmesartan medoximil in tablet dosage form. The separation was carried out using a mobile phase consisting of 20mM phosphate buffer and acetonitrile with pH 3.0 adjusted with orthophosphoric acid in the ratio of 60:40%v/v. The column used Thermo C18, (150mmx4.6mm i.d, 5μm) with flow rate of 1ml/min using PDA detection at 223nm. The described method was linear over a concentration range of 0.5-25μg/ml for the assay of Metoprolol and Olmesartan medoximil respectively. The retention times of Metoprolol and Olmesartan was found to be 2.28 and 5.35 min. Results of analysis were validated statistically and by recovery studies. The mean recovery was 99.60± 1.46 and 99.69± 1.31 for Metoprolol and Olmesartan. The limit of detection (LOD) and limit of quantitation (LOQ) for Metoprolol and Olmesartan were found to be 0.137 and 0.416μg/ml and 0.144 and 0.437μg/ml respectively.

Kadem Meral et al.,\textsuperscript{118} developed a spectrofluorometry and HPLC methods for determination of Metoprolol in pure and pharmaceutical dosage forms. The solvent system, wavelength of detection and chromatographic conditions were optimized in order to maximize the sensitivity of both proposed methods. The linearity was established over the concentration range of 50-4000ng/ml for spectrofluorometry and 5.0-300ng/ml for HPLC methods. The intra and inter day relative standard deviation (RSD) was less than 4.14 and 3.86% for spectrofluorometry and HPLC respectively. The limit of quantitation was determined as 30 and 5.0ng/ml for spectrofluorometry and HPLC methods.

Rahman Nafisur et al.,\textsuperscript{119} described a kinetic spectrophotometric method for the determination of Metoprolol Tartarate in commercial dosage forms. The procedure is based on the reaction of the drug with 1-chloro-2, 4-dinitrobenzene (CDNB) in dimethylsulfoxide (DMSO) at 100±1\degree C. The reaction is investigated by measuring the change in absorbance with time at 420nm. Fixed time (AA) and equilibrium methods are chosen for obtaining the calibration curves. Both calibration curves were found to be linear over the concentration range of 5-60μg/ml. The regression analysis of calibration data resulted in the linear regression equations of $\Delta A=1.608\times10^{-4}+3.96\times10^{-3}$ C and $A=7.31\times10^{-4}+1.90\times10^{-2}$C for fixed time and equilibrium methods. The limit of detection (LOD) and limit of quantitation (LOQ) was found to be 1.16 and 0.415μg/ml.
Bilal Yilmaz et al.,\textsuperscript{120} describes a gas chromatography-mass spectrometry method for determination of Metoprolol in human urine after derivatization with N-Methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA). The method employed a one-step extraction of Metoprolol from human urine with a mixture of ethyl acetate and diethyl ether (2:1, v/v) at basic pH using atenolol as internal standard. Calibration curves were linear over the concentration range 50-3000ng/ml. intra and inter day precision values for Metoprolol in human urine were less than 6.0%. The analytical recovery of Metoprolol from human urine averaged 90.88%. The limits of detection and quantification of Metoprolol were 5.0 and 15ng/ml, respectively. Also the developed and validated method was successfully applied to a patient with hypertension who had been given an oral tablet of 100mg Metoprolol.

2 Past Studies on Simultaneous Estimation of Amlodipine and Metoprolol by RP-HPLC

Basavaiah K et al.,\textsuperscript{121} developed and validated a liquid chromatography–tandem mass spectrometry method for quantification of Metoprolol succinate (MPS) and amlodipine besylate (AM) using hydrochlorothiazide (HCTZ) as IS in human plasma. Both the drugs were extracted by simple liquid–liquid extraction with chloroform. The chromatographic separation was performed on a reversed-phase peerless basic C18 column with a mobile phase of methanol–water containing 0.5% formic acid (8:2, v/v). The protonated analyte was quantitated in positive ionization by multiple reactions monitoring with a mass spectrometer. The method was validated over the concentration range of 1–100ng/ml for MPS and 1–15ng/ml AM in human plasma. The MRM transition of $m/z$ 268.10–103.10, $m/z$ 409.10–334.20 and $m/z$ 296.00–205.10 were used to measure MPS, AM and HCTZ (IS), respectively.

Vaijanath G Dongre et al.,\textsuperscript{122} developed a reverse phase HPLC method for the simultaneous determination of Metoprolol succinate (MS) and amlodipine besylate (AB) in tablet dosage form. The chromatographic separation was achieved on Hypersil BDS cyano (250mm× 4.6mm, 5m) column using PDA detector. The mobile phase consisting of buffer (aqueous triethylamine pH 3) and acetonitrile in the ratio of 85:15 (v/v) at a flow rate of 1.0mL/min was used.

CH.M.M.Prasada Rao et al.,\textsuperscript{123} developed a simple rapid and selective HPLC method for quantization of Amlodipine besylate and Metoprolol tartarate from bulk drug and pharmaceutical formulations using a mobile phase consisting mixture of 0.02M Phosphate buffer solution and Acetonitrile as 80:20 at the flow rate of
1ml/min. An inertsil ODS-CV column was used as stationary phase. The retention time of Amlodipine besylate and Metoprolol tartrate were 3.92 and 10.43 respectively. Linearity was observed in the concentration range of 2.5-15µg/ml for Amlodipine besylate and 25-150µg/ml for Metoprolol Tartrate with good linearity response greater than 0.999. Percent recoveries obtained for Amlodipine besylate and Metoprolol Tartrate were 100.3 and 100.48% respectively.

4.3 MATERIALS & METHODS

Instrumentation:
Chromatographic separation was performed using Agilent 1120 compact LC system integrated with a variable wavelength programmable UV detector and a Rheodyne injector equipped with 20µl fixed loop. The separation was performed on a reverse phase C\textsubscript{18} column [Agilent ODS UG 5 column, 250mm x 4.5mm]. Quantitative HPLC was performed on a gradient High Pressure Liquid Chromatography (Shimadzu HPLC class VP series) with two LC-10 AT, VP pumps, variable wavelength programmable UV/Visible detector SPD-10A, VP, CTO-10ASVP column oven (Shimadzu), SCL-10A, VP system controller (Shimadzu) and one column [Phenomenex C18 (250 mm x 4.6 mm) I.D.; particle size 5 mm]. The HPLC system was equipped with the software “class VP series version 5.03 (Shimadzu).

Table 8.1: Chromatographic conditions for MTP & AMD

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stationary phase (column)</td>
<td>C\textsubscript{18} column (Agilent ODS UG column, 250mm x 4.5mm)</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>Acetonitrile, methanol and phosphate buffer, pH 3.0 in ratio 20:50:30, v/v</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1 ml/min</td>
</tr>
<tr>
<td>Column temperature</td>
<td>Ambient</td>
</tr>
<tr>
<td>Volume of injection</td>
<td>20 µL</td>
</tr>
<tr>
<td>Detection wavelength</td>
<td>236 nm</td>
</tr>
<tr>
<td>Retention time</td>
<td>Amlodipine Besylate - 3.3 min</td>
</tr>
<tr>
<td></td>
<td>Metoprolol tartrate - 5.4 min</td>
</tr>
</tbody>
</table>
Preparation of Standard Drug solution for method:

**Amlodipine Besylate:**

25mg of Amlodipine Besylate pure drug was accurately weighed and transferred to a 25ml volumetric flask. The drug was dissolved using mobile phase and the volume was made up with the same solvent to obtain primary stock solution A of conc. 1000µg/ml.

**Metoprolol Tartrate:**

12.5mg of pure sample of Metoprolol Tartrate was weighed accurately and transferred to a 25ml volumetric flask. 10ml of mobile phase was added for the dissolution of the drug and the volume was made up with the same solvent after the dissolution to obtain primary stock solution B of conc. 1000µg/ml.

**Preparation of Working Standard Solutions:**

From the primary stock solutions, 5ml and 12.5ml were pipetted out from Amlodipine Besylate A solution and Metoprolol Tartrate B respectively, transferred to a 50ml volumetric flask and the volume was made up with the mobile phase to obtain final concentrations of 100 and 500µg/ml of Amlodipine Besylate and Metoprolol Tartrate respectively and this solution is working stock solution A.

From this solution, different aliquots 0.5, 1.0, 1.5, 2.0, 2.5ml and 1, 2, 3, 4, 5 were transferred to 10ml volumetric flasks and the volume was made up with the mobile phase to obtain working solutions of concentrations ranging from 5-25µg/ml of Amlodipine Besylate and 50-250µg/ml of Metoprolol Tartrate.

**Preparation of Sample Solution:**

20 tablets of Lopressor 50 were weighed and crushed. Tablet powder equivalent to 5mg of Amlodipine Besylate and 50mg of Metoprolol Tartrate was weighed accurately and transferred to a 50ml volumetric flask. The content was dissolved with 20ml of mobile phase and then sonicated for 15min, the volume was made up to the mark with the mobile phase and filtered with Whatman filter paper no.41. 1ml of this solution was pipetted out and transferred to a 10ml volumetric flask and the volume was made up with the mobile phase to obtain a concentration of 100µg/ml of Amlodipine Besylate and 500µg/ml of Metoprolol Tartrate(working stock solution B).

7ml of the working stock solution B was transferred to a 10ml volumetric flask and the volume was made up with the mobile phase to obtain a conc of 25µg/ml of Amlodipine Besylate and 250µg/ml of Metoprolol Tartrate(working solution 8).
Reagents used:

Acetonitrile (HPLC grade, Merck), Methanol (AR grade, Rankem chemicals Ltd, Merck Specialties Private Limited) Potassium Dihydrogen orthophosphate (AR grade, Merck), and Water (HPLC Grade) were used in the present study.

METHOD DEVELOPMENT:

Purpose:
The purpose of method development is to optimize the chromatographic conditions by conducting various trials that results in a sensitive, precise, accurate and reliable method that enables the simultaneous estimation of Amlodipine Besylate and Metoprolol Tartrate in pharmaceutical formulations.

Procedure:
In developing a HPLC method, a systematic study of effects of various parameters was undertaken by varying one parameter at a time and controlling all other parameters. The following studies are to be conducted for this purpose.

A) Selection of Mode of separation:
The first consideration when developing an HPLC method is to determine the solubility of sample components. As Amlodipine Besylate and Metoprolol Tartrate were soluble in polar solvents, RP-HPLC mode was chosen.

B) Selection of Mobile phase:
In order to get symmetric peaks and optimum resolution between the components, a number of experiments were carried out by varying different parameters like nature and ratios of components of mobile phase by changing one at a time and keeping all other parameters constant. Standard solutions containing Amlodipine Besylate and Metoprolol Tartrate were injected into the column and run using different mobile phases. Different mobile phases of different proportions of the organic phase and buffers of different pH were tried at different flow rates for the better elution and separation of the drugs. Each mobile phase was filtered through 0.45μ membrane filter and sonicated for 15 min before the trials. Standard solutions were injected into the column after obtaining a steady base line to get well resolved and stable peaks for both the drugs. After performing trials with different mobile phases, both the drugs were found to separate well with stable retention times when run with a mobile phase of combination acetonitrile, methanol and phosphate buffer of pH 3.0 in a ratio of 20:50:30, v/v at a flow rate of 1ml/min. so, this mixture was selected as the mobile
phase for the chromatographic method development because of the sharp symmetrical peaks and reproducible retention times obtained.

C) Selection of Stationary phase:
Selection of appropriate stationary phase helps to improve the efficiency of the method. In the present study, in order to get better peak resolution with less tailing factor and more theoretical plates, C\textsubscript{18} column (Agilent ODS UG column, 250mm x 4.5mm) was selected.

Preparation of Optimized Mobile Phase:

Preparation of Phosphate Buffer pH3.0:
Place 50ml of potassium dihydrogen phosphate in 200ml volumetric flask and make up the volume with water to 200ml.

Preparation of Mobile Phase:
The mobile phase was prepared by mixing acetonitrile, methanol and phosphate buffer (pH 3.0) in the ratio 20:50:30 (v/v). The prepared mobile phase was filtered through 0.45\(\mu\)m membrane filter, transferred to the mobile phase reservoir and then sonicated for 15min for the removal of dissolved gases.

Recommended procedure
After systematic and detailed study of the various parameters involved, as described under results and discussion in this chapter, the following procedure was recommended for the determination of MTP & AMD in bulk samples and pharmaceutical formulations.

Method:
Five sets of the drug solutions were prepared in mobile phase containing MTP & AMD at a concentration range of 10-50 \(\mu\)g/ml. The contents of the mobile phase were filtered before use through 0.45\(\mu\)m membrane filter, degassed by sonication. Prior to injection of the drug, the mobile phase was pumped for about 30 min to saturate the column thereby to get the base line corrected, Then 20\(\mu\)L of each of the drug solution were injected for 5 times. Quantity determinations were made by comparisons of the peak area from a standard injection. The amount of MTP & AMD present in the sample was calculated through the standard calibration curve.
8.4 ANALYTICAL METHOD DEVELOPMENT

Standard solutions containing Amlodipine Besylate and Metoprolol Tartrate were injected into the column and run using different mobile phases. Different mobile phases of different proportions of the organic phase and buffers of different pH were tried at different flow rates for the better elution and separation of the drugs. Each mobile phase was filtered through 0.45µ membrane filter and sonicated for 15 min before the trials. Standard solutions were injected into the column after obtaining a steady base line to get well resolved and stable peaks for both the drugs. After performing trials with different mobile phases, both the drugs were found to separate well with stable retention times when run with a mobile phase of combination acetonitrile, methanol and phosphate buffer of pH 3.0 in a ratio of 20:50:30, v/v at a flow rate of 1ml/min. so, this mixture was selected as the mobile phase for the chromatographic method development because of the sharp symmetrical peaks and reproducible retention times obtained. Retention time of Amlodipine Besylate was 3.3 min and Metoprolol Tartrate was 5.4 min. The chromatograms of MTP & AMD blank, placebo and standard were shown in the Figures 8.3, 8.4 & 8.5 respectively.

![Figure 8.3: A typical Chromatogram of Amlodipine Besylate & Metoprolol Tartrate Blank](image)
8.5 METHOD VALIDATION:

8.5.1 Specificity:

**Purpose:** Specificity of a method was determined by testing standard substances against potential interferences. Triplicate injections of 100% test concentration (5µg/ml Amlodipine Besylate +50µg/ml Metoprolol Tartrate) were given to the system for checking the interferences of excipients if any.

**Procedure:**

Common excipients that are usually present in the formulation such as lactose, microcrystalline cellulose and magnesium stearate have been added to the sample solution and injected into the HPLC system by following the test method conditions.
8.5.2 System Suitability:

**Purpose:**
The purpose of this study is to verify that the resolution and reproducibility of the system are adequate for the analysis of MTP & AMD in pharmaceutical dosage forms.

**Procedure:**
Series of six injections from working solution 7 of concentration 25µg/ml of Amlodipine Besylate and 250µg/ml of Metoprolol Tartrate were given for checking system suitability of the proposed method. Retention time (R<sub>t</sub>), number of theoretical plates (N) and tailing factor (T) were reported in Table 8.2 and the chromatogram of was reported in Figure 8.6.

**Table 8.2: Statistical Validation Data of System Suitability of AMD and MTP**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Amlodipine Besylate</th>
<th>Metoprolol Tartrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention time (min)</td>
<td>3.3</td>
<td>5.4</td>
</tr>
<tr>
<td>Theoretical plates (N)</td>
<td>8274</td>
<td>11675</td>
</tr>
<tr>
<td>Tailing factor (T)</td>
<td>0.9</td>
<td>1.1</td>
</tr>
<tr>
<td>Resolution (R&lt;sub&gt;s&lt;/sub&gt;)</td>
<td></td>
<td>2.22</td>
</tr>
</tbody>
</table>

**Figure 8.6: Chromatogram Showing System Suitability of AMD and MTP**

8.5.3 Linearity:

**Purpose:** The purpose of this study is to verify that the detector response is directly proportional to the concentration.

**Procedure:**
Tripplicate injections were given from each working solution and peak areas were recorded. Calibration curves were plotted with peak area against concentration of the drug individually for both the drugs. Regression equations and correlation coefficients
were determined statistically. The results were depicted in Tables 8.3-8.4, calibration curves for Amlodipine Besylate and Metoprolol Tartrate were shown in Figures 8.7-8.8 and chromatograms are shown in Figures 8.9-8.11.

Table 8.3: Calibration Data of AMD

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>Peak area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Injection-1</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.5</td>
<td>1535779</td>
</tr>
<tr>
<td>1</td>
<td>3648639</td>
</tr>
<tr>
<td>1.5</td>
<td>5472081</td>
</tr>
<tr>
<td>2</td>
<td>7184351</td>
</tr>
<tr>
<td>2.5</td>
<td>8865237</td>
</tr>
<tr>
<td>slope</td>
<td>360545</td>
</tr>
<tr>
<td>Intercept</td>
<td>55796</td>
</tr>
<tr>
<td>R²</td>
<td>0.999</td>
</tr>
</tbody>
</table>

Figure 8.7: Standard Calibration Curve of AMD
### Table 8.4: Calibration Data of MTP

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>Peak area</th>
<th>Injection-1</th>
<th>Injection-2</th>
<th>Injection-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>2782524</td>
<td>2784534</td>
<td>2785523</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>5436293</td>
<td>5438292</td>
<td>5439291</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>8353130</td>
<td>8354131</td>
<td>8355130</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>11317337</td>
<td>11327347</td>
<td>11317357</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>14440967</td>
<td>14450968</td>
<td>14470967</td>
<td></td>
</tr>
</tbody>
</table>

slope 575578
Intercept 117447
$R^2$ 0.9992

### Figure 8.8: Standard Calibration Curve of MTP

\[ y = 575578x - 139680 \]
$R^2 = 0.9992$
Figure 8.9: Chromatogram Showing Linearity of Amlodipine Besylate & Metoprolol Tartrate Inj-1

Figure 8.10: Chromatogram Showing Linearity of Amlodipine Besylate & Metoprolol Tartrate Inj-2

Figure 8.11: Chromatogram Showing Linearity of Amlodipine Besylate & Metoprolol Tartrate Inj-3
8.5.4 Precision:

**Purpose:**
The purpose of this study is to establish that the developed RP-HPLC method is precise for the analysis of MTP & AMD in Pharmaceutical formulations. The precision of the method was verified by performing repeatability and intermediate precision studies.

**Repeatability:**

7 ml of working stock solution A was transferred into each of six 10ml volumetric flasks and the volume was made up with the mobile phase.

6 injections from these solutions were given to the chromatographic system on the first day and the peak areas were recorded. %RSD was calculated statistically from the obtained peak areas.

**Intermediate Precision:**

6 injections from the solutions prepared on the first day were given to the system on the third day and peak areas were recorded. %RSD was calculated statistically and reported.

Results of repeatability and intermediate precision were depicted in Tables 8.5 and 8.6 and Figures 8.12 - 8.13.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Peak area</th>
<th>Amlodipine Besylate</th>
<th>Metoprolol Tartrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>9041188</td>
<td>15375691</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>9099771</td>
<td>15620183</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>9001142</td>
<td>15526933</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>9027464</td>
<td>15436114</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>9086372</td>
<td>15323031</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>9090962</td>
<td>15387749</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td>40209.4</td>
<td>109944.4</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>9057817</td>
<td>1544495</td>
</tr>
<tr>
<td>%RSD</td>
<td></td>
<td>0.443</td>
<td>0.711</td>
</tr>
</tbody>
</table>

Table 8.5: Repeatability Data of AMD and MTP
Table 8.6: Intermediate Precision Data of AMD and MTP

<table>
<thead>
<tr>
<th>S. No</th>
<th>Peak area</th>
<th>Amlodipine Besylate</th>
<th>Metoprolol Tartrate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 2</td>
<td>Day 3</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>9051189</td>
<td>9151189</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>9098771</td>
<td>9099771</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>9001242</td>
<td>9011242</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>9026464</td>
<td>9027465</td>
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<tr>
<td>5</td>
<td></td>
<td>9087372</td>
<td>9087372</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>9091962</td>
<td>9092962</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td>39817.8</td>
<td>51287.53</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>9059500</td>
<td>9078334</td>
</tr>
<tr>
<td>%RSD</td>
<td></td>
<td>0.439</td>
<td>0.564</td>
</tr>
</tbody>
</table>

Figure 8.12: Chromatogram Showing Repeatability of AMD and MTP

Figure 8.13: Chromatogram Showing Intermediate Precision of AMD and MTP
8.5.5 Accuracy:

Purpose:
The purpose of this study is to express the extent of closeness of the results obtained by the proposed method to that of true value. Recovery studies were performed by standard addition method for verifying the accuracy of the proposed method. A known quantity of pure drug was added to pre-analysed sample and contents were reanalysed by the proposed method and the percent recovery was reported.

Procedure:

From working stock solution A, different aliquots of 1.7ml, 2.0ml and 2.3ml were transferred to three 10ml volumetric flasks. To these flasks, 1ml of working stock solution B was added to each flask and the volume was made up with the mobile phase. These solutions were labelled as 80, 100 and 120% recovery levels.

Triplicate injections were given from each solution and percent recovery was reported from the peak areas obtained. The result was listed in Table 8.7 and Figures 8.14-8.16.

<table>
<thead>
<tr>
<th>Recovery level (%)</th>
<th>Amlodipine Besylate</th>
<th>Metoprolol Tartrate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amount added (µg/ml)</td>
<td>Amount found (µg/ml)</td>
</tr>
<tr>
<td></td>
<td>Standard</td>
<td>Test</td>
</tr>
<tr>
<td>80%</td>
<td>12</td>
<td>5</td>
</tr>
<tr>
<td>100%</td>
<td>15</td>
<td>5</td>
</tr>
<tr>
<td>120%</td>
<td>18</td>
<td>5</td>
</tr>
<tr>
<td>Mean recovery</td>
<td>99.76-100.95%w/w</td>
<td>99.94-100.03%w/w</td>
</tr>
</tbody>
</table>
Figure 8.14: Chromatogram Showing Accuracy - 80%

Figure 8.15: Chromatogram Showing Accuracy - 100%

Figure 8.16: Chromatogram Showing Accuracy - 120%
8.5.6 Robustness:

Purpose:
The purpose of this study is to determine the capability of the proposed method to remain unaffected by small deliberate variations in method parameters. Robustness of the method was verified by altering the chromatographic conditions like detection wavelength and flow rate.

Procedure:

I) Effect of variation in detection wavelength:
Detection wavelengths were initially changed to 234nm and then to 238nm and a series of six injections of working solution were given at each detection wavelength for reporting the %RSD.

II) Effect of variation of flow rate:
Flow rate was changed to 0.8ml/min and 1.2ml/min and the same procedure was repeated as above and the peak areas were recorded. Peak areas and the %RSD were reported in Tables 8.8 – 8.9 and Figures 8.17-8.18.

Table 8.8: Robustness Data of Change in Detection Wavelength of AMD and MTP

<table>
<thead>
<tr>
<th>S. No</th>
<th>Peak area</th>
<th>234nm</th>
<th>238nm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amlodipine Besylate</td>
<td>Metoprolol Tartrate</td>
<td>Amlodipine Besylate</td>
</tr>
<tr>
<td>1</td>
<td>19833924</td>
<td>15328697</td>
<td>4141649</td>
</tr>
<tr>
<td>2</td>
<td>19933935</td>
<td>15228698</td>
<td>4140648</td>
</tr>
<tr>
<td>3</td>
<td>19823924</td>
<td>15238699</td>
<td>4142649</td>
</tr>
<tr>
<td>4</td>
<td>19843933</td>
<td>15338897</td>
<td>4242657</td>
</tr>
<tr>
<td>5</td>
<td>19943930</td>
<td>15248699</td>
<td>4243649</td>
</tr>
<tr>
<td>6</td>
<td>19944931</td>
<td>15348697</td>
<td>4232657</td>
</tr>
<tr>
<td>SD</td>
<td>59074.9</td>
<td>55532.9</td>
<td>53821.1</td>
</tr>
<tr>
<td>Mean</td>
<td>1988742</td>
<td>1528873</td>
<td>4190651</td>
</tr>
<tr>
<td>%RSD</td>
<td>0.297</td>
<td>0.363</td>
<td>1.28</td>
</tr>
</tbody>
</table>
Table 8.9: Robustness Data for Change in Flow Rate AMD and MTP

<table>
<thead>
<tr>
<th>S. No</th>
<th>Amlodipine Besylate</th>
<th>Metoprolol Tartrate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$R_t$ (min)</td>
<td>Peak area</td>
</tr>
<tr>
<td>1</td>
<td>4.17</td>
<td>11446444</td>
</tr>
<tr>
<td>2</td>
<td>4.17</td>
<td>11456444</td>
</tr>
<tr>
<td>3</td>
<td>4.18</td>
<td>11456445</td>
</tr>
<tr>
<td>4</td>
<td>4.16</td>
<td>11405445</td>
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<tr>
<td>5</td>
<td>4.18</td>
<td>11466445</td>
</tr>
<tr>
<td>6</td>
<td>4.19</td>
<td>11467445</td>
</tr>
<tr>
<td>SD</td>
<td>23044.8</td>
<td>104570</td>
</tr>
<tr>
<td>Mean</td>
<td>11449778</td>
<td>18982987</td>
</tr>
<tr>
<td>%RSD</td>
<td>0.201</td>
<td>0.550</td>
</tr>
</tbody>
</table>

Figure 8.17: Chromatogram for Variation in Flow Rate AMD and MTP

Figure 8.18: Chromatogram Showing Variation in Detection Wavelength AMD and MTP
8.5.7 Limit of Detection (LOD):

The main purpose of this study is to evaluate the sensitivity of the proposed method. Detection limit was determined based on the standard deviation of the response and slope. The detection limit may be expressed as $3.3\sigma/s$, where $\sigma$ is the standard deviation (SD) of the response, $s$ is the slope of the calibration curve.

8.5.8 Limit of Quantitation (LOQ):

A specific calibration curve should be studied using samples, containing an analyte in the range of quantitation limits. The residual SD of a regression line or the SD of y-intercepts of regression lines may be used as SD. LOQ may be expressed as $10\sigma/s$. Results of LOD and LOQ were reported in Table 8.10.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Amlodipine Besylate</th>
<th>Metoprolol Tartrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOD (µg/ml)</td>
<td>0.19</td>
<td>0.20</td>
</tr>
<tr>
<td>LOQ (µg/ml)</td>
<td>0.59</td>
<td>0.61</td>
</tr>
</tbody>
</table>

8.5.9 Assay:

Purpose:

The purpose is to estimate the amount of MTP & AMD present in pharmaceutical formulations.

Procedure:

The content of twenty tablets was accurately weighed. From this tablet powder an amount equivalent to 5 mg of Amlodipine Besylate and 50 mg of Metoprolol Tartrate were taken and the drugs were extracted in 10 ml of mobile phase by sonication for a period of 20 minutes. This solution was filtered through 0.45 µm nylon membrane filter & suitably diluted for analysis and injected into the liquid chromatogram and the chromatograph was recorded (Table 8.11).

<table>
<thead>
<tr>
<th>Drug</th>
<th>Label claim (mg/tab)</th>
<th>Amount recovered (mg)</th>
<th>Amount found in drug (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amlodipine Besylate</td>
<td>5</td>
<td>5.09</td>
<td>101.9</td>
</tr>
<tr>
<td>Metoprolol Tartrate</td>
<td>50</td>
<td>49.9</td>
<td>99.9</td>
</tr>
</tbody>
</table>
8.6 RESULTS AND DISCUSSION:

A RP-HPLC method has been developed and validated by the author for simultaneous estimation of Amlodipine Besylate and Metoprolol Tartrate. The proposed RP-HPLC method were validated as per the International Conference on Harmonisation (ICH) Q2B Guidelines, and was found to be applicable for routine quantitative analysis of Amlodipine Besylate and Metoprolol Tartrate by RP-HPLC using UV detector in pharmaceutical dosage forms. The author has developed this method based on the use of Agilent C18 column and mobile phase composition of Acetonitrile, methanol and phosphate buffer, pH 3.0 in ratio 20:50:30, v/v. The method was validated over a linear concentration range of 5.0–25.0 μg/mL and with a correlation coefficient of \( r^2 \) - 0.999 for Amlodipine Besylate and 0.9992 for Metoprolol Tartrate. The %RSD of the peak response of five replicate injections of standard concentration was found to be below 2, indicating that the proposed method is precise. The percentage recoveries of active pharmaceutical ingredient (API) from dosage forms ranged from 97.0 to 102.0% w/w and 99.02-101.0%ww/w for Amlodipine Besylate and Metoprolol Tartrate, indicating that the proposed method to be accurate. The LOD & LOQ values were found to 0.19µg/ml and 0.20µg/ml, and 0.59µg/ml and 0.61µg/ml respectively for Amlodipine Besylate and Metoprolol Tartrate respectively indicating the sensitivity of the method. Thus the results of analysis of pharmaceutical formulations reveal that the proposed method is suitable for their analysis with virtually no interference of the usual additives presented in pharmaceutical formulations. The proposed method (RP-HPLC) is simple, sensitive and reliable and can be used for the routine determination of Amlodipine Besylate and Metoprolol Tartrate in bulk
samples and pharmaceutical formulations depending upon the need of the specific and arising situation.

8.7 CONCLUSION
A simple, specific, accurate and precise reverse phase high performance liquid chromatography method has been developed which can be used for accurately quantitative estimation of Amlodipine Besylate and Metoprolol Tartrate for routine analysis of individual and combination of drugs. Method was validated as per ICH Q2 (R1) so it can be used by pharmaceutical industries.
SUMMARY:

Development of new analytical methods for the determination of drugs in pharmaceutical dosage forms is more important in pharmacokinetic, toxicological and biological studies. Today pharmaceutical analysis entails much more than the analysis of active pharmaceutical ingredients or the formulated product. The pharmaceutical industry is under increased scrutiny from the government and the public interested groups to consistently deliver to market safe, efficacious products that fulfil unmet medical needs. The pharmaceutical analyst plays a major rule in assuring identity, safety, efficacy, purity and quality of a drug product. The need for pharmaceutical analysis is driven largely by regulatory requirements. The commonly used tests of pharmaceutical analysis generally entail compendia testing method development, setting specifications, and method validation. Analytical testing is one of the more interesting ways for scientists to take part in quality process by providing actual data on the identity, content and purity of the drug products. New methods are now being developed with a great deal of consideration to worldwide harmonization. As a result, new products can be assured to have comparable quality and can be brought to international markets faster.

Pharmaceutical analysis occupies a pivotal role in statutory certification of drugs and their formulations either by the industry or by the regulatory authorities. In industry, the quality assurance and quality control departments play major role in bringing out a safe and effective drug or dosage form. The current good manufacturing practices (cGMP) and the Food Drug Administration (FDA) guidelines insist for adoption of sound methods of analysis with greater sensitivity and reproducibility. Therefore, the complexity of problems encountered in pharmaceutical analysis with the importance of achieving the selectivity, speed, low cost, simplicity, sensitivity, specificity, precision and accuracy in estimation of drugs.

Among the several techniques [HPLC, GC, NMR, MS&spectrophotometry IR] available for the assay of drugs, the author had chosen LC/MS, HPLC.

HPLC is a major tool for the qualitative and quantitative analysis of drugs and pharmaceuticals, chemical and biological materials and also plays very important role in pharmacokinetics studies. HPLC technique has been regarded as the best among various instrumental ones in spite of its heavy cost and maintenance problems.
In the present investigation the author had selected seven potent drugs namely Zolmitriptan (ZMT), Tamsulosin Hydrochloride (TSH), Telmisartan (TSN), Valsartan (VST), Paliperidone (PDN), Amlodipine Besylate (AMB) and Metoprolol tartrate (MTP) for which very few analytical methods were reported and hence there is wide scope for the development of new analytical methods for their quantitative analysis.

Chapter-1 describes brief information on general analytical methodology and also the systematic procedure to be followed for the development of the analytical methods for the selected drugs.

Chapter-2 gives a brief literature survey about the analytical methods available for the estimation of Zolmitriptan. A versatile LC-MS/MS method has been developed for the estimation of Zolmitriptan in pure and in pharmaceutical formulations.

Chapter-3 gives a brief literature survey about the analytical methods available for the estimation of Zolmitriptan. A versatile RP-HPLC method has been developed for the estimation of Zolmitriptan in pure and in pharmaceutical formulations.

Chapters-4 a gives a brief literature survey about the analytical methods available for the estimation of Tamsulosin Hydrochloride. A versatile RP-HPLC method has been developed for the estimation of Tamsulosin Hydrochloride in pure and in pharmaceutical formulations.

Chapter-5 gives a brief literature survey about the analytical methods available for the estimation of Telmisartan. A versatile RP-HPLC method has been developed for the estimation of Telmisartan in pure and in pharmaceutical formulations.

Chapter-6 gives a brief literature survey about the analytical methods available for the estimation of Valsartan. A versatile RP-HPLC method has been developed for the estimation of Valsartan in pure and in pharmaceutical formulations.

Chapter-7 gives a brief literature survey about the analytical methods available for the estimation of Paliperidone. A versatile RP-HPLC method has been developed for the estimation of Paliperidone in pure and in pharmaceutical formulations.

Chapter-8 gives a brief literature survey about the analytical methods available for the simultaneous estimation of Amlodipine Besylate and Metoprolol tartrate. A versatile RP-HPLC method has been developed for the simultaneous estimation of Amlodipine Besylate and Metoprolol tartrate in pure and in pharmaceutical formulations.
CONCLUSION:

Finally it was concluded that, the thesis describes that the proposed methods can be used as alternative methods to reported ones and provides a wide choice for routine determination of the above mentioned drugs.

Thus the purpose of the present investigation was successfully achieved.