Development and Validation of RP-HPLC Method for Simultaneous Determination of Metoprolol Succinate, Telmisartan, and Amlodipine Besylate in Synthetic Mixture

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Abstract:

A novel, simple, rapid, precise and highly efficient, isocratic liquid chromatography (RP-HPLC) method has been developed and validated for simultaneous estimation of amlodipine, metoprolol and telmisartan. Separation of all three compounds was achieved using Cosmosil C18 (250x4.6), 5µ column set at 25 °C temperature. Mobile phase consisted of a mixture of 20mMol KH2PO4: Acetonitrile:Methanol (30:30:40)

%v/v flowing at a flow rate of 1.5 mL/min. Detection was carried out by UV detector set at 234 nm. Injection volume was kept at 5 µL. The retention times were found to be 3.913 min, 2.107, 8.859 minutes for amlodipine, metoprolol and telmisartan respectively. The calibration curve demonstrated linearity over the range of 25-75 µg/mL, 250-750 µg/mL, 200-600 µg/mL for amlodipine, metoprolol and telmisartan respectively with correlation coefficients of 0.999 for all three drugs. The recoveries were found to be in the ranges of 100.1-100.4%, 100.1-100.8%, 99.2-100.0% for amlodipine, metoprolol and telmisartan respectively. The method was found to be precise and robust with very less %RSD values. The method can be employed for routine quality control analysis.

Keywords: Amlodipine, Metoprolol, telmisartan, RP-HPLC, validation, ICH

INTRODUCTION:

1) **Hypertension: [1, 2, 3]**

Hypertension, defined as persistent systolic blood pressure (SBP) at least 130 mm Hg or diastolic BP (DBP) at least 80 mm Hg. Systolic Blood Pressure represents the pressure in the arteries when the heart beats whereas Diastolic Blood Pressure represents the pressure in the arteries when your heart is at rest between beats.

Hypertension is a component of the metabolic syndrome and a stand-alone risk factor for cardiovascular and cerebrovascular illnesses. It has been determined to be the third most prevalent cause of disability globally. A survey by the World Health Organization found that non-communicable diseases account for over two thirds of all fatalities in India. Of these, 27% are attributable to cardiovascular disease, which affects 45% of adults in the 40–69 age range. **[4, 5]**

World Health Organization/health topics/hypertension] According to the 2019–2020 National Family Health Survey (NFHS-5), the prevalence of hypertension was found to be 24% in men and 21% in women, up from 19% and 17%, respectively, in the 2015–16 survey cycle.**[6]**

The signs of hypertension can be difficult to recognize and can take years to manifest. In actuality, a lot of people don't even realize they have high blood pressure until their readings become potentially fatal. This illness has been called the "silent killer" because damage to the body can happen over many years without a person noticing. When symptoms do appear, they may include ringing in the ears,

headaches, nosebleeds, irregular heartbeats, and abnormalities in eyesight. In addition to being a significant risk factor for heart disease and stroke, severe hypertension can cause exhaustion, nausea, confusion, chest pain, and kidney damage.

1.1.1 Types of hypertension

There are two main types of hypertension:

Primary Hypertension: Also known as essential hypertension, the most common type when the cause is unidentifiable. It often develops gradually over time and is associated with lifestyle factors and genetics.

Secondary Hypertension: This type is caused by any underlying medical illness or side effects of medications. It tends to have a sudden onset and can be related to issues like kidney disease, hormonal imbalances, or certain medications.

1.1.1 Monitoring and diagnosis:

The procedure of monitoring blood pressure is simple and rapid. Blood pressure cuffs are easily available at local pharmacy store or can be checked at the registered medical practitioner.

Table 1: Blood Pressure Ranges and their meaning

BLOOD PRESSURE CATEGORY	SYSTOLIC mm Hg (upper number)	and/or	DIASTOLIC mm Hg (lower number)
NORMAL	LESS THAN 120	and	LESS THAN 80
ELEVATED	120 – 129	and	LESS THAN 80
HIGH BLOOD PRESSURE (HYPERTENSION) STAGE 1	130 – 139	or	80 – 89
HIGH BLOOD PRESSURE (HYPERTENSION) STAGE 2	140 OR HIGHER	or	90 OR HIGHER
HYPERTENSIVE CRISIS (consult your doctor immediately)	HIGHER THAN 180	and/or	HIGHER THAN 120

Complications of hypertension

Hypertension, if left uncontrolled, can lead to several serious complications. Some of the common complications associated with hypertension are depicted in figure 1.1.

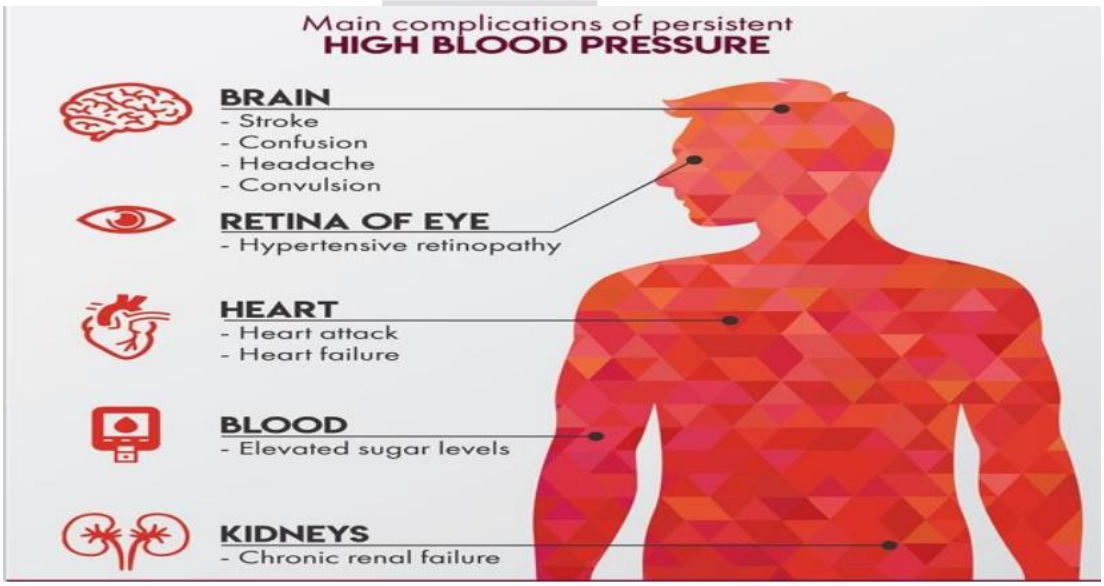


Figure 1: Complications of hypertension

1.1.1 Treatment:

The first line of treatment for hypertension is changing one's lifestyle, which includes cutting back on or giving up alcohol/smoking, exercising, maintaining a healthy diet that emphasizes potassium and low sodium intake, and losing weight.

Individual lifestyle factors each have a partially additive influence on blood pressure reduction, which increases the effectiveness of pharmaceutical therapy. BP level and the existence of high atherosclerotic CVD risk should be taken into consideration when deciding whether to start antihypertensive medication.

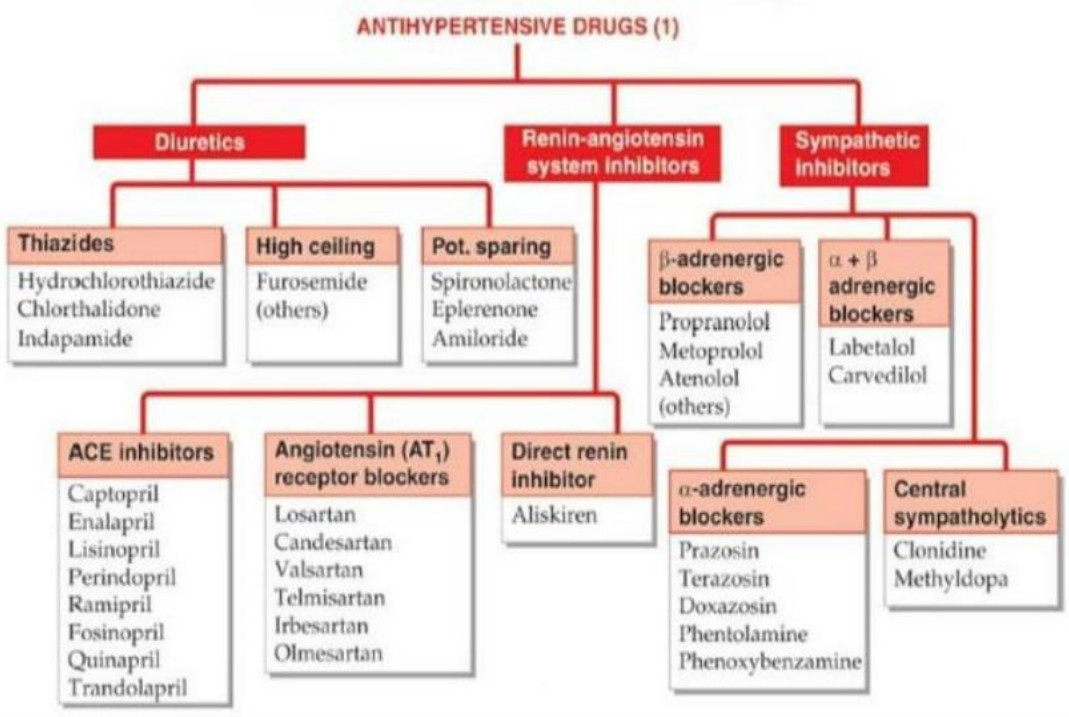


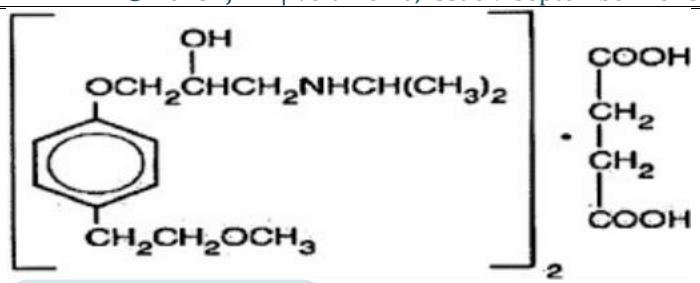
Figure 2:Classification of anti-hypertensive drugs

DRUG PROFILE[24-26]

1.1 Metoprolol succinate 22, 23

Table 2: Drug profile of Metoprolol Succinate

Chemical profile:	
Name	Metoprolol succinate
Molecular Formula	C15H25NO3
IUPAC Name	1-[4-(2-methoxyethyl)phenoxy]-3-[(propan-2- yl)amino]propan-2-ol
Molecular Weight	267.3639 gram/mol
Description	A white, crystalline powder or colourless crystal**[28]**

Chemical Structure	
Solubility	(freely soluble) water, (soluble) methanol, (slightly soluble) ethanol, (very slightly soluble) acetate**[30]**
Melting Point	120-123°C
Log P	1.8
PKa	(strongest acidic)- 14.09 (strongest basic)- 9.67
Therapeutic Category	Anti-hypertensive and anti-anginal

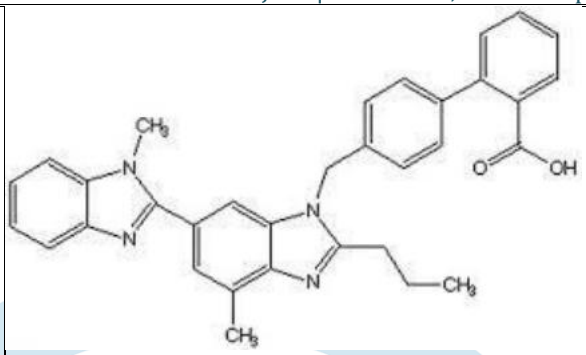
Side Effects	Chest pain or discomfort, dilated neck veins, extreme fatigue, irregular breathing or heartbeat, swelling of the face, fingers, feet, or lower legs, trouble breathing, or weight gain.
Mechanism of Action	Metoprolol is a beta-1-adrenergic receptor inhibitor specific to cardiac cells with negligible effect on beta-2 receptors. This inhibition decreases cardiac output by producing negative chronotropic and inotropic effects without presenting activity towards neither membrane stabilization nor intrinsic sympathomimetics.
Indications	Metoprolol is indicated for the treatment of angina, heart failure, myocardial infarction, atrial fibrillation, atrial flutter and hypertension.

Pharmacokinetic Data:	
Absorption	When administered orally, it is almost completely absorbed in the gastrointestinal tract. The maximum serum concentration is achieved 20 min after intravenous administration and 1-2 hours after oral administration. The bioavailability of metoprolol is of 100% when administered intravenously and when administered orally it presents about 40% for the succinate derivative.
Protein Binding	Metoprolol is not highly bound to plasma proteins and only about 11% of the administered dose is found bound. It is mainly bound to serum albumin.
Excretion	Metoprolol is mainly excreted via the kidneys. From the eliminated dose, less than 5% is recovered unchanged.
Half-life	3-7 hours

1.1 Telmisartan: [24, 25]

Table 3: Drug profile of Telmisartan

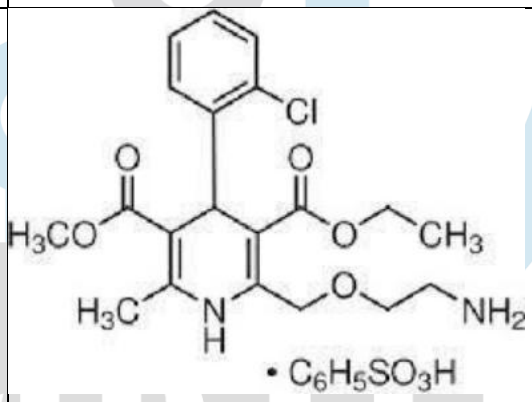
Chemical profile:	
Name	Telmisartan
Molecular Formula	C33H30N4O2
IUPAC Name	4'-{[4-methyl-6-(1-methyl-1H-1,3-benzodiazol-2- yl)-2-propyl-1H-1,3-benzodiazol-1-yl]methyl}- [1,1'-biphenyl]-2-carboxylic acid
Molecular Weight	514.6169 gram/mol
Description	A white to off-white crystalline powder **[32]**

Chemical Structure	
Solubility	(practically insoluble) water, (slightly soluble) methanol, (sparingly soluble) methylene chloride**[33]**
Melting Point	261-263 °C
Log P	6.66
pKa	(strongest acidic)- 3.62 (strongest basic)- 5.86
Therapeutic Category	Anti hypertensive

Side Effects	Changes in vision, dizziness, light-headedness, fainting, fast heartbeat, large hives.
Mechanism of Action	Telmisartan interferes with the binding of angiotensin II to the angiotensin II AT1-receptor by binding reversibly and selectively to the receptors in vascular smooth muscle and the adrenal gland. As angiotensin II is a vasoconstrictor, which also stimulates the synthesis and release of aldosterone, blockage of its effects results in decreases in systemic vascular resistance.
Indications	Used alone or in combination with other classes of antihypertensive for the treatment of hypertension. Also used in the treatment of diabetic nephropathy in hypertensive patients with type 2 diabetes mellitus, as well as the treatment of congestive heart failure (only in patients who cannot tolerate ACE inhibitors).
Pharmacokinetic Data:	
Absorption	Absolute bioavailability depends on dosage. Food slightly decreases the bioavailability (a decrease of about 6% is seen when the 40-mg dose is administered with food).
Protein Binding	Telmisartan is highly bound to plasma proteins (>99.5%), mainly albumin and alpha 1-acid glycoprotein. Binding is not dose-dependent.
Excretion	Following either intravenous or oral administration of 14C-labeled Telmisartan, most of the administered dose (>97%) was eliminated unchanged in feces via biliary excretion; only minute amounts were found in the urine (0.91% and 0.49% of total radioactivity, respectively).
Half-life	24 ours

1.1 Amlodipine besylate: [26, 27]

Table 4: Drug profile of Amlodipine besylate

Chemical profile:	
Name	Amlodipine besylate
Molecular Formula	C20H25ClN2O5
IUPAC Name	3-ethyl 5-methyl 2-[(2-aminoethoxy)methyl]-4- (2-chlorophenyl)-6-methyl-1,4-dihydropyridine- 3,5-dicarboxylate
Molecular Weight	408.876 gram/mol
Description	White or almost white powder **[38]**
Chemical Structure	
Solubility	(Slightly soluble) water and 2-propanol (freely soluble) methanol, (sparingly soluble) anhydrous ethanol **[38]**
Melting Point	178-179°C
Log P	2.22
pKa	(Strongest acidic)- 19.12 (Strongest basic)- 9.45
Therapeutic Category	Anti hypertensive

Side Effects	Treat high blood pressure and coronary artery disease. Swelling, fatigue, palpitations, and flushing.

Mechanism of Action	Mechanism of action on blood pressure Amlodipine is considered a peripheral arterial vasodilator that exerts its action directly on vascular smooth muscle to lead to a reduction in peripheral vascular resistance, causing a decrease in blood pressure. Amlodipine is a dihydropyridine calcium antagonist (calcium ion antagonist or slow-channel blocker) that inhibits the influx of calcium ions into both vascular smooth muscle and cardiac muscle. Experimental studies imply that amlodipine binds to both dihydropyridine and nondihydropyridine binding sites, located on cell membranes. The contraction of cardiac muscle and vascular smooth muscle are dependent on the movement of extracellular calcium ions into these cells by specific ion channels.
Indications	Amlodipine may be used alone or in combination with other antihypertensive and antianginal agents for the treatment of the following conditions Label: Hypertension Coronary artery disease Chronic stable angina Vasospastic angina (Prinzmetal's or Variant angina) Angiographically documented coronary artery disease in patients without heart failure or an ejection fraction < 40%
Pharmacokinetic Data:	
Absorption	Amlodipine absorbed slowly and almost completely from the gastrointestinal tract. Peak plasma concentrations are achieved 6-12 hours after oral
	administration. The estimated bioavailability of Amlodipine is 64-90%. Steady-state plasma Amlodipine levels are achieved after 7-8 days of consecutive daily dosing. Absorption is not affected by food
Protein Binding	About 98%

Excretion	Amlodipine is 10% excreted as unchanged drug in the urine. Amlodipine can be initiated at normal doses in patients diagnosed with renal failure.
Half-life	30-50 hours

EXPERIMENTAL WORK

7.1 List of Instrumentation

Table 5: Instrumentation List

Sr. No.	Name
1.	Shimadzu LC 2010 CHT HPLC
2.	Agilent Cary 630 FTIR Spectrometer
3.	Shimadzu UV-1800 spectrophotometer
4.	pH Analyser
5.	Sartorius Electronic Balance
6.	Ultra Sonicator
7.	Eppendorf Micropipette
8.	YMC pack pro C18 column (100 × 4.6mm, 5µ)

7.2 List of samples, chemicals and solvents

Table 6: Solvents and Chemicals

Sr. No.	Name	Manufacturer
1.	Metoprolol Succinate	USP Standards/Torrent Pharmaceuticals
2.	Telmisartan	USP Standards/Torrent Pharmaceuticals

3.	Amlodipine besylate	USP Standards/Torrent Pharmaceuticals
4.	Povidone, Sorbitol, Magnesium Stearate	S. D. Fine
5.	Microcrystalline Cellulose	Merck
6.	Potassium di hydrogen phosphate	Merck
7.	Milli - Q/HPLC water	Merck
8.	Acetonitrile (HPLC grade)	Rankem
9.	Methanol (HPLC grade)	Rankem

7.1 Preliminary studies

Preliminary studies were conducted to assess the correctness of the received APIs and to assess the feasibility of developing a rapid and accurate method for quantifying metoprolol, telmisartan and amlodipine in combined dosage forms.

7.1.1 Melting point determination

Melting points of AMLO, METO and TELMI were determined using melting point apparatus. Melting point results are tabulated in table-18.

Table 7: Melting point results

Sr. No.	Drug Name	Melting point result (°C)	Reference (°C)
1.	Amlodipine besylate	177-178	178-179
2	Metoprolol succinate	120-121	120-123
3.	Telmisartan	261-263	261-263

Conclusion: Melting point when compared with reference range, they were found to be matching. So received APIs maybe correct.

7.1.2 Fourier transmission infrared spectroscopy

FT-IR spectra were scanned in the range of 400-4000 cm^{-1} using Agilent Cary 600 FT-IR, using ATR crystal which eliminates the need for KBr disc sampling. FT-IR spectra comparison was done with reference spectra available in pharmacopoeia. Also from the structure, functional groups stretching and bending were identified.

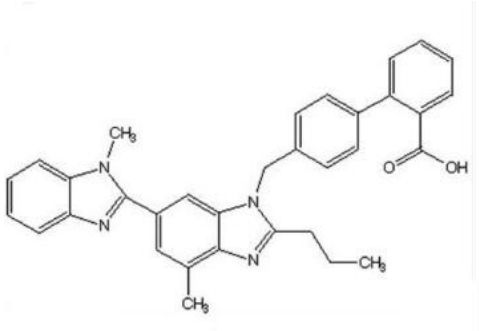


Figure 7 Structure of Telmisartan

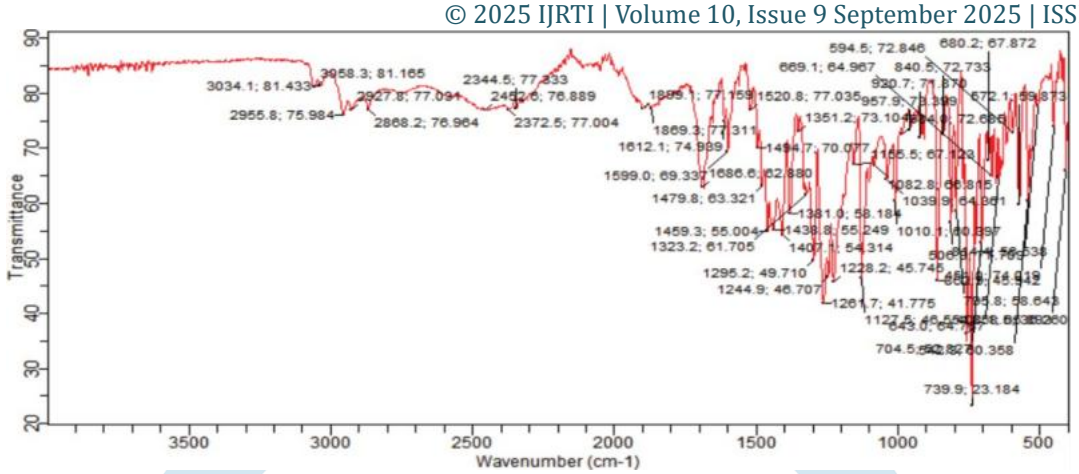


Figure 8 Sample FT-IR spectrum of Telmisartan

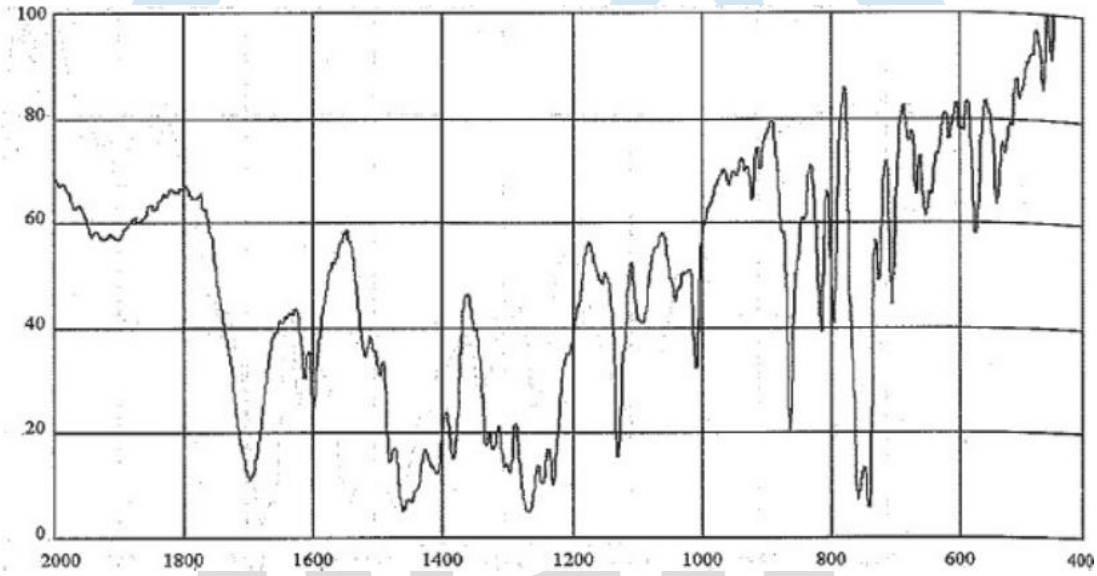


Figure 9 Reference IR spectrum of telmisartan74

Table 8: Structural interpretation of telmisartan

Functional Group	**Measured Frequency (cm-1)**	**Reference Frequency (cm-1)**
O-H Stretch	3034.1	3400 – 3000
C=O Stretch	1686.6	1680 – 1630
C-H Aliphatic Stretch	2868.2, 2927.8, 2955.8	3000 – 2850
C-H bending	1459.3	1470-1450
C-N Stretch	1261.7	1350 – 1000

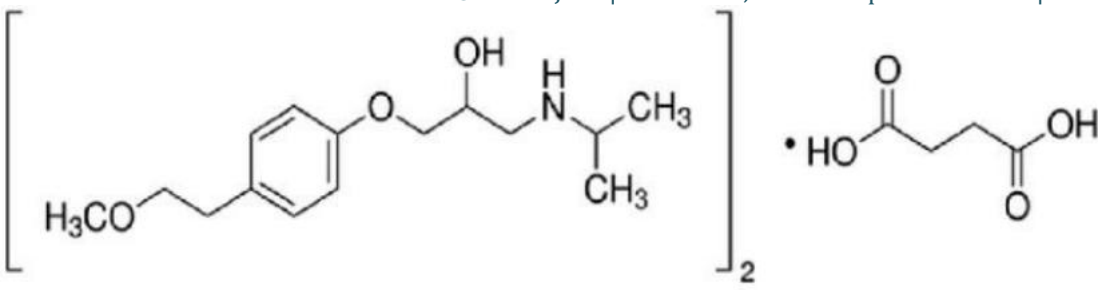


Figure 10 Structure of metoprolol succinate

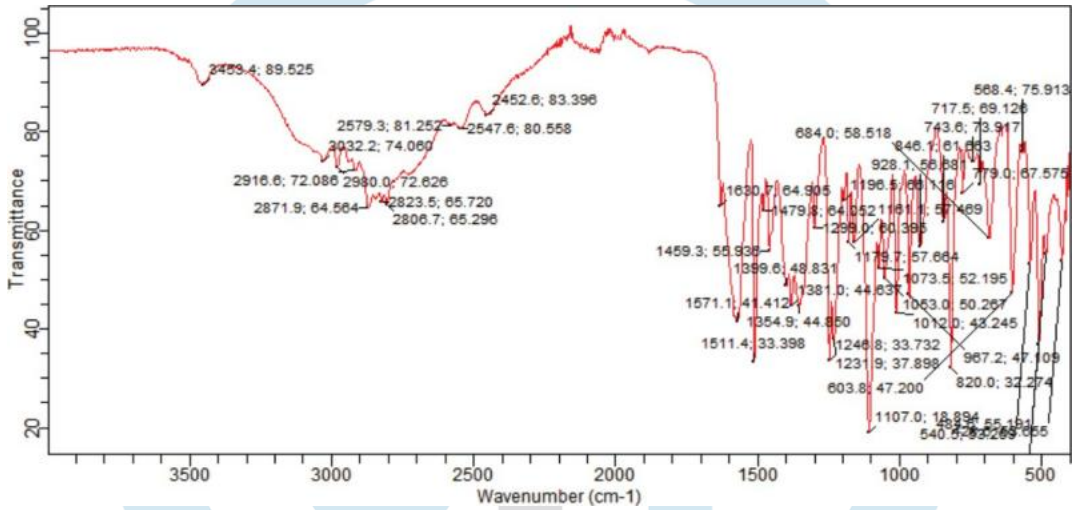


Figure 11 Sample FT-IR spectrum of Metoprolol succinate

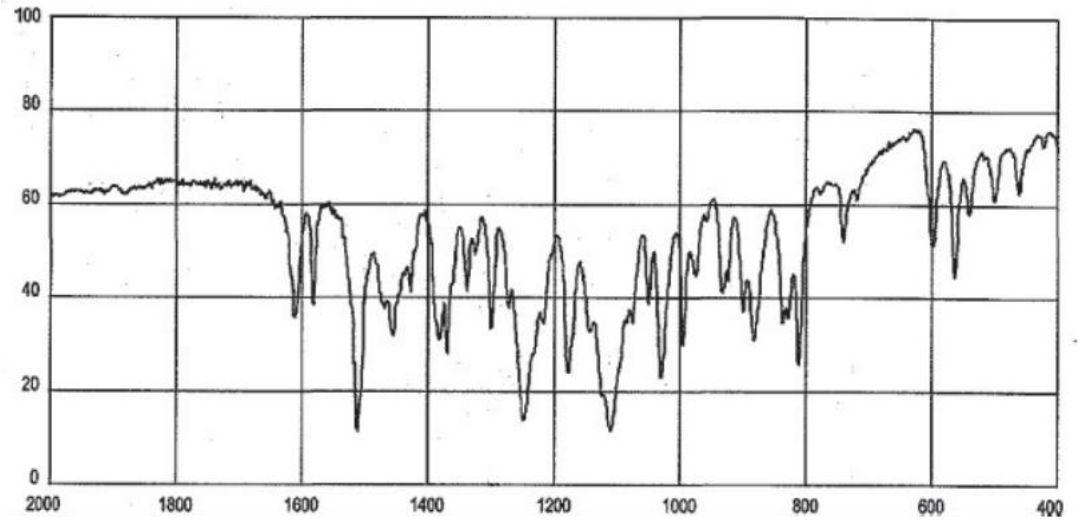


Figure 12 Reference IR spectrum of Metoprolol succinate 75

Table 9: Structural interpretation of metoprolol succinate

Functional Group	Measured Frequency (cm-1)	Reference Frequency (cm-1)
N-H Stretch	3453.4	3500-3200
O-H Stretch	3032.2	3300-3000

C=O Stretch	1630.7	1750-1600
C-H stretch of aliphatic group	2803.7 2823.5, 2871.9	3000 – 2850
CH3-O-Ar	1107.0	1300-1000

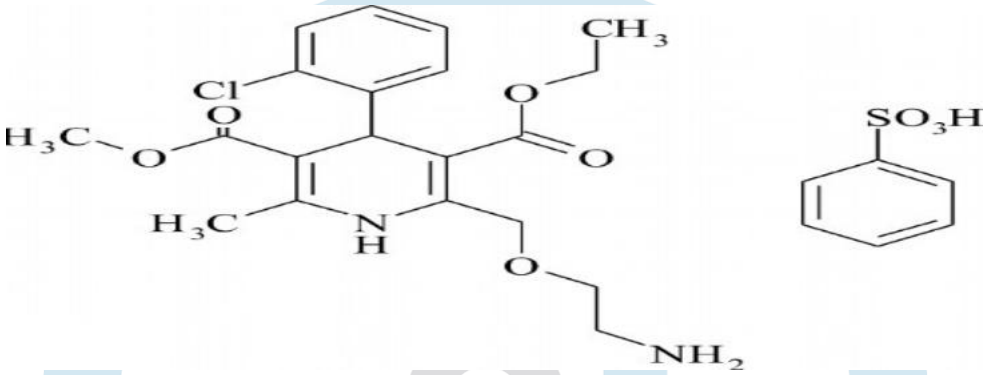


Figure 13 Structure of amlodipine besylate

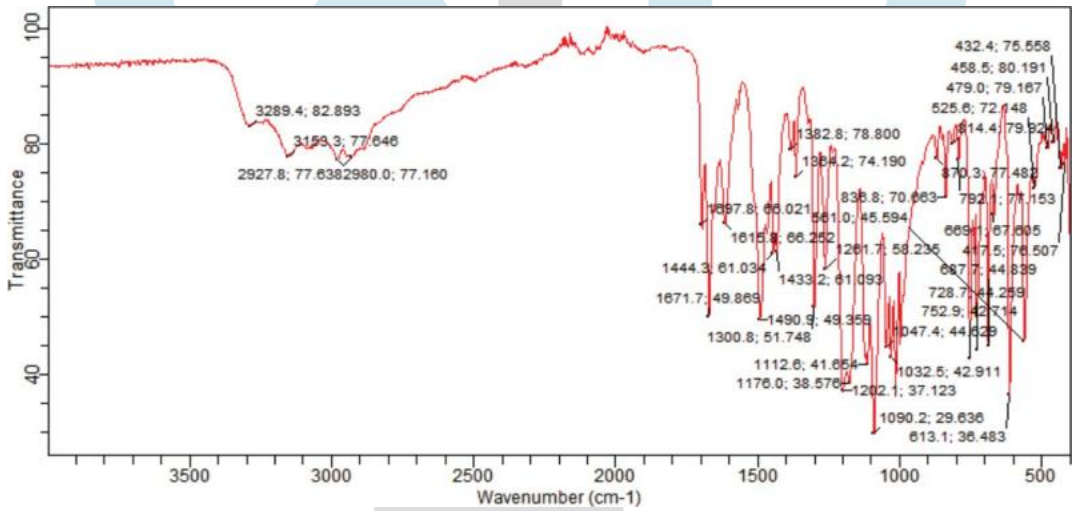


Figure 14 Sample FT-IR spectrum of Amlodipine Besylate

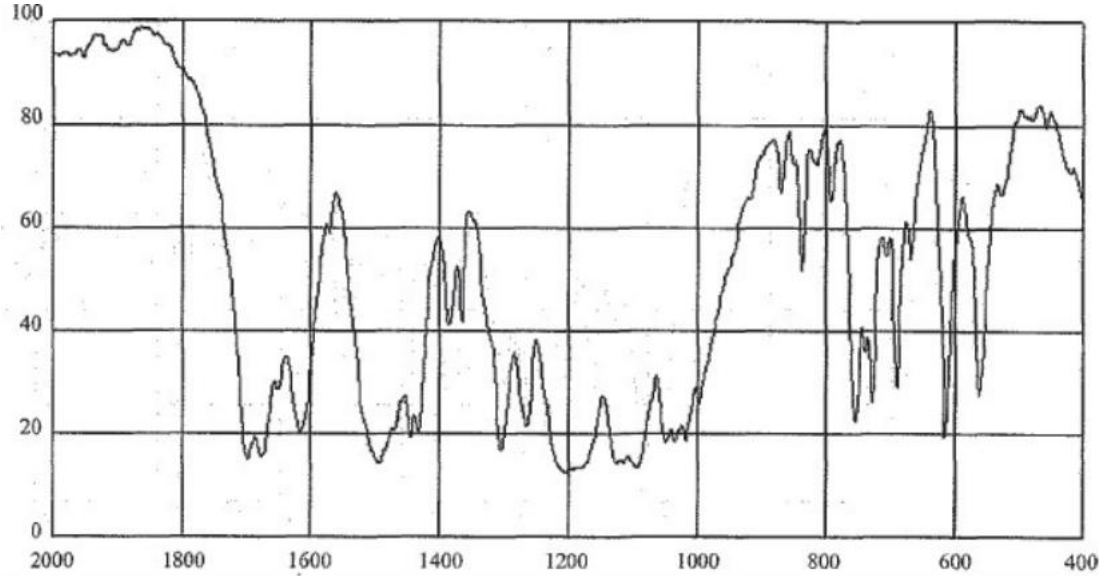


Figure 15 Reference IR of Amlodipine besylate76

Table 10: Structural interpretation of AMLO besylate

Functional Group	Measured Frequency (cm-1)	Reference Frequency (cm-1)
O-H Stretch	3289.4	3300-3000
>C=O Stretch	1671.7	1680 – 1630
C-H Aliphatic Stretch	2927.8, 2928.0	3000-2840
N-H	1615.3	1650-1580
S=O Stretch	1090.2	1160-1120
C-Cl Stretch	613.1	785 – 540

Conclusion: The FT-IR spectrum results and structural elucidation are in conformance of the amlodipine besylate, metoprolol succinate, and telmisartan structure and are matching with reference spectrum available in Indian pharmacopoeia.

7.1.1 Solubility study

Accurately weighed 10 mg of APIs were taken in three different clean test tubes and labelled them. Then chosen solvent was incrementally added by 1 mL with thorough

stirring until saturation is achieved. Then the volume of added solvent was recorded and determined the solubility in approximate mg/mL. Solubility study results are tabulated in table 22.

Table 11 Solubility testing results of telmisartan, metoprolol succinate and amlodipine besylate

Solvent	Telmisartan	Metoprolol Succinate	Amlodipine Besylate

Water	Practically insoluble in water	Freely soluble	Slightly soluble
Methanol	Slightly soluble	Freely soluble	Freely soluble
Acetonitrile	Slightly soluble	Soluble	Soluble
0.1 N NaOH	Freely soluble	Freely soluble	Freely soluble
0.1 N HCL	Insoluble	Freely soluble	Freely soluble

Conclusion: TELMI is insoluble in water, so water cannot be taken as solvent. It is freely soluble in 0.1 NaOH, therefore for making stock, we will use 0.1 N NaoH to dissolve the compound. Then methanol can be used along with buffer as a diluent to make volume. METO and AMLO are freely soluble, so methanol can be taken as a solvent.

7.1.1 UV-VIS spectroscopy

As all three compounds are freely soluble in 0.1 N NaOH, 10 µm/mL solutions of each were prepared, then put into UV cuvette and scanned in 200-400 nm. Spectrum noted and overlain on each other.

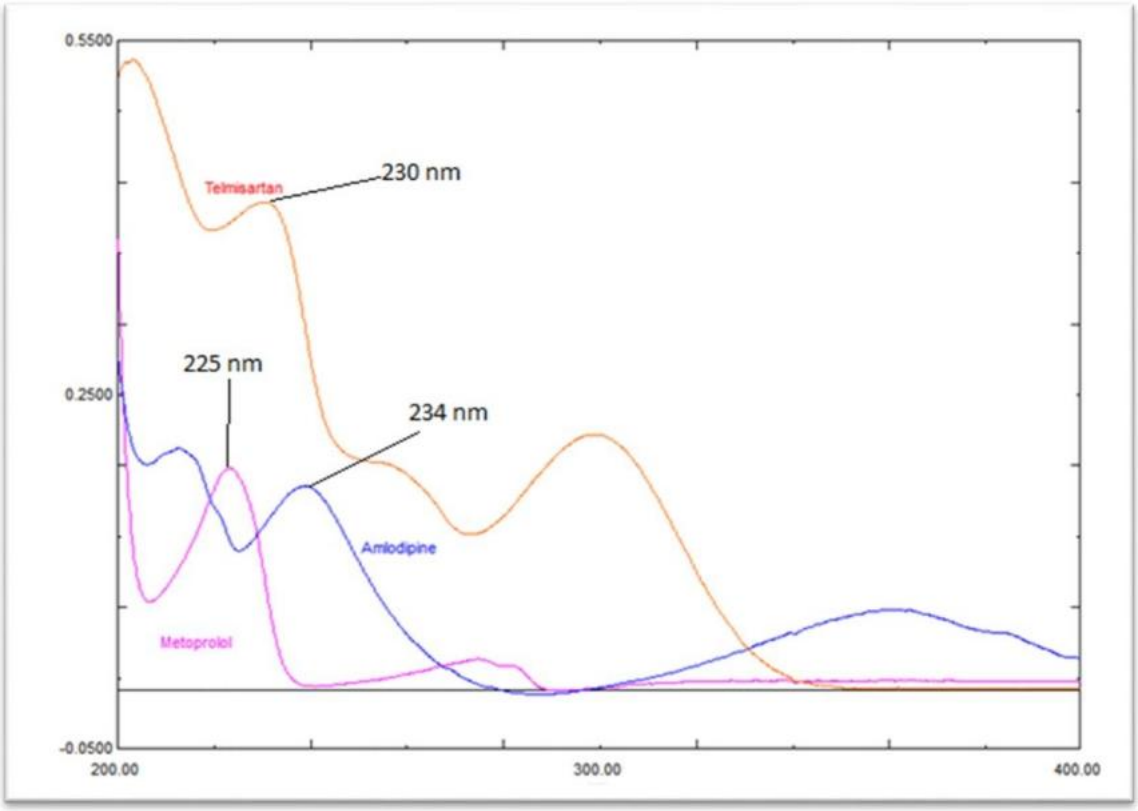


Figure 16 Overlay UV of TELMI, METO and AMLO

Conclusion: There is no single isosbestic point for all three. Absorbance maxima of METO, AMLO and TELMI are 225, 234 and 230 nm. Therefore any wavelength can be chosen which gives optimized results.

7.1 Solution preparation

7.1.1 Synthetic Mixture77-78

Telmisartan 40.0 mg

Amlodipine besylate 7.0 mg (eq. to amlodipine 5 mg) Metoprolol succinate 47.5 mg (eq.tometoprolol tartrate 50 mg) Sodium hydroxide 6.0 mg

Povidone 24.0

Sorbitol 337.0

Magnesium stearate 8.00

Microcrystalline

Cellulose 100.00

Total Weight: 569.5 mg

7.1.1 Metoprolol Stock (5000 µm/mL)

METO stock was prepared by taking 95 mg of metoprolol succinate (equivalent to 100 mg metoprolol tartrate) into 20 mL flask. 5 mL methanol was added and flask was sonicated 10 minutes. Volume made with Methanol.

7.1.2 Amlodipine Stock (500 µm/mL)

AMLO stock was prepared by taking 14 mg of amlodipine besylate (equivalent to 10 mg amlodipine) into 20 mL flask. 5 mL methanol was added and flask was sonicated 10 minutes. Volume made with Methanol.

7.1.3 Telmisartan Stock (4000 µm/mL)

TELMI stock was prepared by taking 80 mg of telmisartan workings standard into 20 mL flask. 1 mL 0.1 N NaOH was added and flask was sonicated 10 minutes. Volume made with Methanol.

7.1.4 Standard solution (500 µm/mL METO, 400 µm/Ml TEL, 50 µm/mL AMLO)

Working mix standard solution was prepared by diluting 1 mL aliquot of each stock standard to 10 mL with the diluent. Filtered through a 0.45 µm membrane filter before analysis.

7.1.5 Sample solution for assay (500 µm/mL METO, 400 µm/mL TEL, 50 µm/mL AMLO)

569.5 mg of synthetic mixture powder was taken into 100 mL volumetric flask. To this 10 mL 0.1 N NaOH was added. Dissolved by sonication and then diluted with diluent to make 100 mL. Filtered through a 0.45 µm membrane filter before analysis.

7.2 Method development:

Selection and optimization of suitable stationary phasewas done from different column length, i.e. 15 cm and 25 cm, different particle sizes, i.e. 3.5 µ and 5 µ, different particle types, i.e. C8 and C18, out of which C18 column with 250 mm length and 5 µ particle size gave optimum responses.

- Selection and optimization of suitable mobile phase begin with water:methanol or water:acetonitrile, then taken trials with buffer in combination with organic solvents. Then the combination with higher efficiency and better resolution was selected, which is Phosphate buffer:ACN:methanol 30:30:40%v/v. We preferred isocratic elution mode.

Optimization of all the chromatographic parameterswas done and analytical method was finalized. Summary of optimization of chromatography parameter is given below:

Rational:Dual wavelength used for analysis and organic ratio modified to shorten the run time

MP:20mMol KH2PO4(pH:5): Acetonitrile:Methanol (30:30:40)

Column:Cosmosil C18 (250x4.6), 5µ

Flow:1.5 ml/min

Wavelength:225 nm, 234 nm(dual wavelength mode) Inj volume: 5µL

Column temp: 25°C

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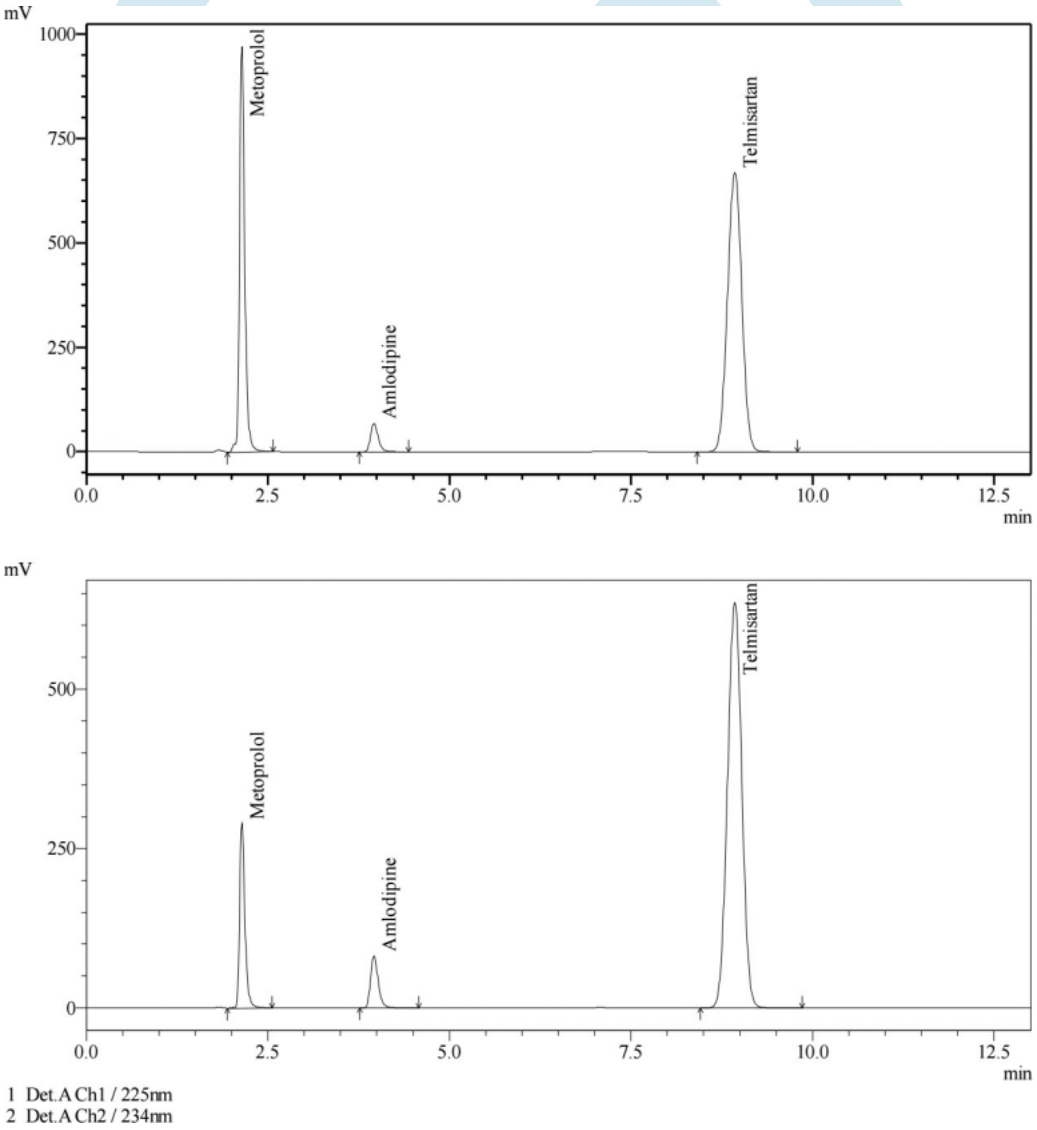

PeakTable

Detector A Ch1 225nm

Peak#	Ret. Time	Name	Area	Area %	Resolution	T.Factor	T. Plate
1	2.137	Metoprolol	4809137	33.16	0.000	1.42	3795
2	3.956	Amlodipine	502419	3.46	10.677	1.33	6142
3	8.924	Telmisartan	9190827	63.37	17.390	1.03	9332
Total			14502383	100.00			

PeakTable

Detector A Ch2 234nm

Peak#	Ret. Time	Name	Area	Area %	Resolution	T.Factor	T. Plate
1	2.138	Metoprolol	1471404	13.60	0.000	1.47	3431
2	3.956	Amlodipine	604056	5.58	10.452	1.33	6140
3	8.924	Telmisartan	8746608	80.82	17.379	1.03	9315
Total			10822068	100.00			

Figure 22 Chromatogram of trial final

Conclusion: Dual wavelength used for analysis because amlodipine concentration is low in standard solution composition and smaller peak responses maybe difficult to evaluate during 50% linearity and recovery level testing. So another wavelength tried was 234 nm which is a maxima of amlodipine besylate. Organic ratio and flow rate modified to shorten the run time, Buffer:ACN:Methanol (33:30:40), Flow rate 1.5 mL/min. At 234 nm, response of AMLO increased to 20%. Metoprolol and telmisartan peak optimum responses. Trial finalized.

Table 23 RP-HPLC method for estimation of METO, AMLO and TELMI

Parameters	**Specifications**
Mode of Elution	**Isocratic**
Injection Volume	**5 µL**
Column	**Cosmosil C18 (250x4.6), 5µ**
Mobile phase	**20mMol KH2PO4: Acetonitrile:Methanol (30:30:40) %v/v**
Column Temperature	**25°C**
Flow rate	**1.5 ml/min**
Wavelength	**234 nm**
Run time	**13 minutes**

Analytical method validation confirms the reliability, precision, and consistency of analytical outcomes, which ensures suitability for the intended use. It is also a mandatory requirement by the regulatory bodies and industry norms. Analytical method validation was performed as per the ICHQ2(R1) guidelines. Various validation characteristics performed in laboratory setting are as follows:

7.1 Validation summary

Table 38 AMLO, METO, TELMI method validation summary

Parameters	AMLO	METO	TELMI
System Suitability			

Theoretical Plates (N)	6563	4002	9339
Asymmetry	1.35	1.65	1.02
Retention Tim	3.913	2.107	8.859
Precision			
Repeatability (%RSD)	0.08	1.71	0.05
Intraday (% RSD)	1.13	1.13	1.13
Interday (% RSD)	0.06-0.50	0.15-0.88	0.21-0.67
Robustness	Robust		
Linearity			
Range (µg/ml)	25-75	250-750	200-600
Correlation co-efficient (R^2)	0.999	0.999	0.999
Accuracy (%)	100.1-100.4%	100.1-100.8	99.2-100.0
Assay	98.6	99.6	99.1

7.1 Conclusion

A novel, simple, accurate and efficient RP-HPLC method was developed for simultaneous analysis of amlodipine, metoprolol and telmisartan. The key advantage of the method is its cost-effectiveness, short analysis time, consistency and precision. Method is well reproducibility as indicated by low RSD values on intra-day and inter- day studies. Method demonstrated excellent recoveries, hence accurate for the analysis.

Considering all the parameters mentioned above, it seems quite useful for analysis of bulk and in synthetic mixture. The method can be explored for analysis of marketed formulation when they are available in the market.

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