"DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC AND HPTLC METHODS FOR SIMULTANEOUS ESTIMATION OF AMLODIPINE BESYLATE AND TELMISARTAN IN THEIR COMBINED DOSAGE FORM"

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in

Pharmaceutical Analysis

BY

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DECLARATION

I declare that the thesis entitled "DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC AND HPTLC METHODS FOR SIMULTANEOUS ESTIMATION OF AMLODIPINE BESYLATE AND TELMISARTAN IN THEIR COMBINED DOSAGE FORM" has been prepared by me under the guidance of Dr. Hardik G. Bhatt, Assistant professor, Department of Pharmaceutical Analysis, Nirma University. No part of this thesis has formed the basis for the award of any degree or fellowship previously.

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CERTIFICATE

This is to certify that Mr. YOGESH B. KOLADIYA has prepared his thesis entitled "DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC AND HPTLC METHODS FOR SIMULTANEOUS ESTIMATION OF AMLODIPINE BESYLATE AND TELMISARTAN IN THEIR COMBINED DOSAGE FORM", in partial fulfillment for the award of M. Pharm. degree of the Nirma University, under my guidance. He has carried out the work at the Department of Pharmaceutical Analysis, Nirma University.

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Chapter 1
INTRODUCTION

1. Introduction

Drugs are an inevitable part of our life. The quality of the drug is an essential feature as it directly affects the life of the consumer. The quality of any product or material can be judged by analyzing it, only. Science provides truth through its investigative process called analysis. Analytical chemistry is the science of obtaining, processing and communicating information about the composition and structure of matter. The number of the drugs introduced in the market is increasing every day.

These drugs are either new entity or the structural modification of the existing drugs. Very often there is time lag from the date of introduction of a drug in to market to the date of its inclusion in pharmacopoeia. Under these conditions, standard and analytical process for these drugs may not be available in pharmacopoeia. It becomes necessary to develop new analytical methods for such drugs.

The reasons for the development of newer analytical methods of drugs are:

- The drug / drug combination may not be official in any pharmacopoeia.
- Proper analytical procedure of drug may not be available in literature due to patent regulations.
- Analytical methods may not be available in the form of formulations.
- Analytical methods for the quantification in the biological fluids may not be available.
- Existing analytical method may require expensive reagent or solvent.
- Analytical method for the drug which is indicative of stability may not be available.

The aim of the analytical studies is to obtain quantitative and qualitative information about the compounds of interest (analyte) in a sample. Pharmaceutical formulations are formulated with more than one drug, typically referred to as combination products. These are intended to meet desired patient need by combining their therapeutic effects of two or more drugs in one product. These combination products can present challenges to the analytical chemists responsible for the development and validation of the analytical method for their analysis.

Testing a pharmaceutical product involves chemical, instrumental and sometimes microbiological analysis. Simultaneous estimation of drugs in combination can be carried out by using spectrophotometric and spectrofluorimetric methods and some chromatographic techniques like HPLC, HPTLC, SFC, LC/MS etc.

1.1) Introduction to Hypertension [1-3]

Hypertension is the term used to describe high blood pressure. Blood pressure readings are measured in millimeters of mercury (mmHg) and usually given as two numbers. For example, 120 over 80 (written as 120/80 mmHg).

- The top number is your systolic pressure, the pressure created when your heart beats. It is considered high if it is consistently over 140.
- The bottom number is your diastolic pressure, the pressure inside blood vessels when the heart is at rest. It is considered high if it is consistently over 90.

Either or both of these numbers may be too high.

Pre-hypertension is when your systolic blood pressure is between 120 and 139 or your diastolic blood pressure is between 80 and 89 on multiple readings. If you have pre-hypertension, you are more likely to develop high blood pressure.

Causes

Blood pressure measurements are the result of the force of the blood produced by the heart and the size and condition of the arteries.

Many factors can affect blood pressure, including:

- How much water and salt you have in your body
- The condition of your kidneys, nervous system, or blood vessels
- The levels of different body hormones

High blood pressure can affect all types of people. High blood pressure is more common in African Americans than Caucasians. Smoking, obesity, and diabetes are all risk factors for hypertension.

Most of the time, no cause is identified. This is called essential hypertension.

High blood pressure that results from a specific condition, habit, or medication is called secondary hypertension. Too much salt in your diet can lead to high blood pressure. Secondary hypertension may also be due to:

- Adrenal gland tumor
- Alcohol abuse
- Anxiety and stress
- Arteriosclerosis
- Birth control pills
- Coarctation of the aorta
- Cocaine use
- Cushing syndrome
- Diabetes
- Kidney disease, including:
    - Glomerulonephritis (inflammation of kidneys)
    - Kidney failure
    - Renal artery stenosis
    - Renal vascular obstruction or narrowing
- Medications
    - Appetite suppressants
    - Certain cold medications
    - Corticosteroids
    - Migraine medications
- Hemolytic-uremic syndrome
- Obesity
- Pain

- Pheochromocytoma
- Pregnancy (called gestational hypertension)
- Primary hyperaldosteronism
- Renal artery stenosis
- Retroperitoneal fibrosis
- Wilms' tumor

Symptoms

Most of the time, there are no symptoms. Symptoms that may occur include:

- Chest pain
- Confusion
- Ear noise or buzzing
- Irregular heartbeat
- Tiredness
- Vision changes.

Exams and Tests

Your health care provider will perform a physical exam and check your blood pressure. If the measurement is high, your doctor may think you have high blood pressure. The measurements need to be repeated over time, so that the diagnosis can be confirmed.

Other tests may be done to look for blood in the urine or heart failure.

These tests may include:

- Chem-20
- Echocardiogram
- Urinalysis
- Ultrasound of the kidneys

Treatment

The goal of treatment is to reduce blood pressure so that you have a lower risk of complications.

There are many different medicines that can be used to treat high blood pressure, including:

- Alpha blockers
- Angiotensin-converting enzyme (ACE) inhibitors
- Angiotensin receptor blockers (ARBs)
- Beta-blockers
- Calcium channel blockers
- Central alpha agonists
- Diuretics
- Renin inhibitors, including Aliskiren
- Vasodilators

Possible Complications

- Aortic dissection
- Blood vessel damage (arteriosclerosis)
- Brain damage
- Congestive heart failure
- Kidney damage
- Kidney failure
- Heart attack
- Hypertensive heart disease
- Stroke
- Vision loss

Prevention

Adults over 18 should have their blood pressure checked routinely.

Lifestyle changes may help control your blood pressure:

- Lose weight if you are overweight. Excess weight adds to strain on the heart. In some cases, weight loss may be the only treatment needed.
- Exercise regularly. If possible, exercise for 30 minutes on most days.
- Eat a diet rich in fruits, vegetables, and low-fat dairy products while reducing total and saturated fat intake (the DASH diet is one way of achieving this kind of dietary plan).
- Avoid smoking.
- If you have diabetes, keep your blood sugar under control.
- Do not consume more than 1 or 2 alcoholic drinks per day.
- Try to manage your stress.

Chapter 2
DRUG PROFILES & LITERATURE REVIEW

2) Drug profiles and Literature review

2.1) RATIONAL FOR COMBINATION:

Amlodipine, dihydropyridine calcium channel blocker (CCB), and Telmisartan, a beta blocker are established antihypertensive agents. A fixed dose tablet formulation of the Amlodipine and telmisartan is widely used combination in India for the treatment of morning hypertension in patients.

- The combination of telmisartan, an angiotensin receptor blocker (ARB), and amlodipine, a CCB, brings together their synergistic and complementary mode of actions to achieve substantial and sustained 24-hour blood pressure lowering.

- The combination of an ARB with a CCB has been shown to reduce the incidence of peripheral oedema, a common side effect of CCBs, such as amlodipine.

- Unlike many anti-hypertensive treatments, telmisartan and amlodipine:

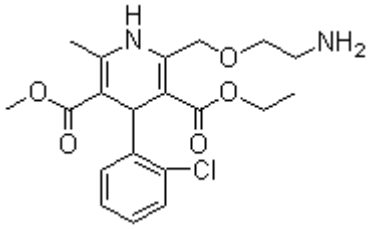
    - ✓ both have long plasma half-lives and have demonstrated 24-hour effective BP reductions.
    - ✓ have proven evidence bases in CV outcomes demonstrating cardio-protective benefits.
    - ✓ have a well established safety and tolerability profile.
    - ✓ have once daily dosing to aid patient compliance.

- The telmisartan and amlodipine combination offers an effective and well tolerated option for BP control, particularly for difficult-to-treat patients at risk of CV events and those not controlled by amlodipine alone.

2.2) DRUG PROFILES:

A) Drug Profile for Amlodipine:

1. Drug class: Antihypertensive
2. Category: Second generation dihydropyridamol Calcium channel blocker
3. CAS number[4]: 88150-42-9
4. Official status: Drug is official in USP, IP, BP and EP.
5. Chemical name[4]: 3-Ethyl-5-methyl-2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylate.
6. Molecular formula[4]: $C_{20}H_{25}ClN_2O_5$
7. Molecular weight[4]: 408.879 g/mol
8. Structural formula[4]:



9. Physicochemical properties:
    a) Description and Solubility[6]: It is a white powder. It is slightly soluble in water and isopropanol; freely soluble in methanol; and sparingly soluble in dehydrated alcohol.
    b) Melting point: 199 - 201°C
    c) Optical rotation[7]: Between -0.10° & +0.10° at 20°C (solution 10mg/ml in methanol).
    d) Dissociation constant (Pk_a)[8]: 8.6
    e) Partition coefficient (log p)[8]: (o/w) 3.0

10. Storage condition[7]: Preserve in tight container, protected from light, store at room temperature.
11. Pharmacological action and clinical pharmacology:
    a) Mechanism of action[5]: Amlodipine had a strong blocking action on both the L-type and N-type Ca^{2+} channels expressed in the oocyte. The potency of the amlodipine block on the N-type Ca^{2+} channel was comparable to that on the L-type Ca^{2+} channel.

    The blocking action of amlodipine on the N-type Ca^{2+} channel was dependent on holding potential and extracellular pH, as has been observed with amlodipine block on the L-type Ca^{2+} channel. A depolarized holding potential and high pH enhanced the blocking action of amlodipine,

    Amlodipine is a peripheral arteriolar vasodilator; thus it reduces after load.

    b) Dosage and dosage form[9]:
        - For hypertension, angina pectoris, stable angina and prinzmental's angina:

          Adult: initially 5mg once daily increased to 10 mg once daily orally.

          Elderly: 2.5mg daily orally.

    c) Pharmacokinetics[5]: After oral administration of therapeutic doses of the drug, absorption produces peak plasma concentrations between 6 and 12 hours. Absolute bioavailability has been estimated to be between 64 and 90%. The bioavailability of the drug is not altered by the presence of food.

    The pharmacokinetics of amlodipine is not significantly influenced by renal impairment. Patients with renal failure may therefore receive the usual initial dose.

Elderly patients and patients with hepatic insufficiency have decreased clearance of amlodipine with a resulting increase in AUC of approximately 40-60%, and a lower initial dose may be required. A similar increase in AUC was observed in patients with moderate to severe heart failure.

d) Metabolism[5]:

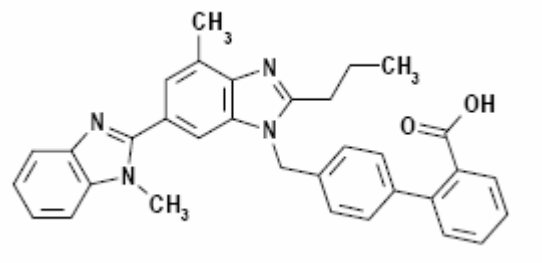
Amlodipine Undergoes minimal presystemic metabolism. It is extensively (about 90%) converted to inactive metabolites via hepatic metabolism with 10% of the parent compound and 60% of the metabolites excreted in the urine. *Ex vivo* studies have shown that approximately 93% of the circulating drug is bound to plasma proteins in hypertensive patients. Elimination from the plasma is biphasic with a terminal elimination half-life of about 30-50 hours. Steady-state plasma levels of amlodipine are reached after 7 to 8 days of consecutive daily dosing.

e) Side effects:[4]

- Very often: peripheral edema (feet and ankles)
- Often: dizziness, palpitations, muscle, stomach or headache, dyspepsia, nausea
- Sometimes: blood disorders, development of breasts in men (gynecomastia), impotence, depression, insomnia, tachycardia
- Rarely: erratic behavior, hepatitis, jaundice, hyperglycemia, tremor, Stevens-Johnson syndrome

B) Drug Profile for Telmisartan:

1. Drug class: Antihypertensive
2. Category: Angiotensin II receptor antagonist
3. CAS number[10]: 144701-48-4
4. Chemical name[10]: 2-(4-{[4-methyl-6-(1-methyl-1*H*-1,3-benzodiazol-2-yl)-2-propyl-1*H*-1,3-benzodiazol-1-yl]methyl}phenyl)benzoic acid
5. Molecular formula[10]: $C_{33}H_{30}N_4O_2$
6. Molecular weight[10]: 514.617 g/mol
7. Structural formula[10]:



8. Physicochemical properties[12]:
    a. Description and Solubility: It is white to off-white, odorless crystalline powder.
    b. It is freely soluble in methanol. It is practically insoluble in water or an aqueous solution in the pH range of 3 to 9, and sparingly soluble in a strong acid, with the exception of hydrochloric acid in which it is insoluble. Telmisartan is soluble in a strong base.
    c. Melting point[13]: 261 – 264°C

9. Dose: The usually effective dose is 40 mg once daily. Some patients may already benefit at a daily dose of 20 mg. In cases where the target blood pressure is not achieved, telmisartan dose can be increased to a maximum of 80 mg once daily.
10. Storage condition[13]: Store at 25°C (77°F); excursions permitted to 15°-30°C (59°-86°F).

11. Mechanism of action[17]: Telmisartan is an Angiotensin Receptor Blocker (ARB) that shows high affinity for the angiotensin II type 1 (AT_1) receptors, has a long duration of action, and has the longest half-life of any ARB. In addition to blocking the Renin-Angiotensin System (RAS), telmisartan acts as a selective modulator of Peroxisome proliferator-activated receptor gamma (PPAR-γ), a central regulator of insulin and glucose metabolism. It is believed that it has dual mode of action may provide protective benefits against the vascular and renal damage caused by diabetes and cardiovascular disease (CVD). Telmisartan has binding affinity 3000 times with AT-2 receptor than AT-1 receptor.

12. Pharmacokinetics[15]: Following oral administration, peak concentrations (C_{max}) of telmisartan are reached in 0.5-1 hour after dosing. Food slightly reduces the bioavailability of telmisartan, with a reduction in the area under the plasma concentration-time curve (AUC) of about 6% with the 40 mg tablet and about 20% after a 160 mg dose. The absolute bioavailability of telmisartan is dose dependent. At 40 and 160 mg the bioavailability was 42% and 58%, respectively. The pharmacokinetics of orally administered telmisartan is nonlinear over the dose range 20-160 mg, with greater than proportional increases of plasma concentrations (C_{max} and AUC) with increasing doses.
    a. **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).
    b. **Protein binding:** ≥99.5%

c. **Biotransformation:**

Minimally metabolized by conjugation to form a pharmacologically inactive acylglucuronide; the glucuronide of the parent compound is the only metabolite that has been identified in human plasma and urine. The cytochrome P450 isoenzymes are not involved in the metabolism of telmisartan.

d. **Half life:** 24 hours

13. Side effects:[10] most common telmisartan side effects include:

- Upper respiratory infection, such as the common cold or flu -- up to 7 percent of people
- Back pain -- up to 3 percent of people
- Diarrhea -- up to 3 percent of people
- Inflammation of the sinuses (sinusitis) -- up to 3 percent

Some of these possible rare side effects of telmisartan include, but are not limited to:

- Insomnia
- Impotence
- Constipation
- Dry mouth
- Gout
- Diabetes
- Depression
- Middle ear infection (otitis media)
- Asthma

- Vision problems
- Serious breakdown of muscle (known medically as rhabdomyolysis).

14. Interactions[15]**:**

Drug -drug.

- ✓ Diuretics, such as torsemide, furosemide , hydrochlorothiazide, and others
- ✓ Potassium supplements
- ✓ Potassium-sparing diuretics, such as spironolactone , triamterene, and amiloride
- ✓ Digoxin
- ✓ Warfarin.

Table 2.1: Marketed formulations for combination of Amlodipine besylate (5 mg) and Telmisartan (40 mg)**.**

Formulation	Brand Name	Name of Manufacturer
Tablet	TELMA-AM	Glenmark Pharmaceuticals Ltd.
Tablet	TELSAR-A	Glenmark Generics Ltd.
Tablet	TELSARTAN-AM	Dr Reddy's Laboratories Ltd.

2.3) LITERATURE REVIEW

- **LITERATURE REVIEW FOR INDIVIDUAL DRUGS:**

A) Literature Review for Amlodipine:

Numerous analytical methods had been developed for the estimation of amlodipine besylate in pharmaceutical formulation, human plasma and serum in an attempt to optimize treatment outcomes. These techniques vary with regards to method of detection and separation such as UV-spectrophotometer, HPLC, HPTLC, GC etc. and in combination such as HPLC-UV, LC-MS, GC-MS etc. to prepare plasma sample for analysis, clinical samples are subjected to chemical procedures including solid phase extraction and liquid- liquid extraction. These methods are differing in sensitivity, selectivity, accuracy and precision. These variables are dependent upon the laboratory personnel, the analytical detection method and the sample preparation method utilized.

Table 2.2: UV - Vis Spectrophotometric method for estimation of Amlodipine Besylate

Sr. no.	Matrix	Solvent system	Wavelength of determination	Quantitative analysis
1[18]	Tablets & Capsules	Methanol	238 nm	**Linearity and range:** 10-35 µg /ml
2[19]	Tablets	Methanol	360 nm	**Linearity and range:** 5-30 µg/ml
3[20]	Tablets	Methanol	361 nm	**Linearity and range:** 5-30

				µg/ml
4[21]	Tablets	Methanol	363nm	**Linearity and range:** 0-20 µg/ml
			For simultaneous equations method at 245 nm and 363 nm	**Linearity and range:** 0-20 µg/ml

Table 2.3: Colorimetric method for estimation of Amlodipine Besylate

Sr. no.	Matrix	Solvent system	Wavelength of determination	Quantitative analysis
1[22]	Tablets	Formation of colored chloroform extractable ion pair complexes with Bromocresol green (BCG), Bromophenol blue (BPB) and Methylene blue(MB) in acidic medium.	409.0 nm (BCG). 409.0 nm (BPB) and 668.2 nm (MB)	**Linearity and range:**0-80 µg/ml
2[23]	Tablets	Formation of colored chloroform extractable complex of drug with rhodizonic acid	450nm	**Linearity and range:** 0.1 – 1.5 mg/ml
3[24]	Tablets	Oxidation of the drug with Fe(III)	500nm	**Linearity and range:** 2-10µg/ml **Molar absorptivity:** 2.9×10^4 L mol^{-1} cm^{-1}
		Estimation of Fe(II) produced after chelation	515nm	**Linearity and range:** 4–

		with either 1,10-phenanthroline or 2,2′-bipyridyl		14 μg /ml **Molar absorptivity:** 2.7×10^4 L mol^{-1} cm^{-1}
		With ammonium heptamolybdate tetrahydrate, which resulted in the formation of molybdenum blue	825nm	**Linearity and range:** 15-59 μg/ml **Molar absorptivity:** 1.8×10^4 L mol^{-1} cm^{-1}
4[25]	Bulk drug and Pharmaceutical formulations	Charge transfer complexation reaction: with 2,3-dichloro 5,6-dicyano 1,4-benzoquinone (DDQ) to give colored product	580nm	**Linearity and range:** 1–125 μg/ml
		Interaction of drug with ascorbic acid in *N*,*N*-dimethyl formamide to give purple red colored product	530nm	**Linearity and range:** 10–140 μg/ml
5[26]	Bulk drug and Pharmaceutical formulations	Reduction of iron(III) by the studied drugs in acid medium and subsequent interaction of iron(II) with ferricyanide to form Prussian blue colored product	760nm	**Linearity and range:** 5–15 μg /ml **Molar absorptivity:** 1.76×10^4 L mol^{-1} cm^{-1}
6[27]	Bulk drug and Pharmaceutical formulations	Interaction of the primary amino group of the drug with ninhydrin in *N*,*N*′-dimethylformamide (DMF) medium	595nm	**Linearity and range:** 10–60 μg /ml **Molar absorptivity:** 6.52×10^3 L mol^{-1} cm^{-1}

		producing a colored complex		

Table 2.4: Spectrofluorimetric method for estimation of Amlodipine Besylate

Sr. no.	Matrix	solvent system	Excitation and Emission wavelength	Quantitative measurement
1[28]	Tablets	Reaction of drug with ninhydrin and phenyl acetaldehyde in buffered medium (pH 7.0) resulting in formation of a green fluorescent product	λ_{ex} = 375 nm λ_{em} =480 nm	**Linearity and range:** 0.35–1.8 µg/ml **LOD:** 0.09 µg/ml
		Reaction of AML with 7-chloro-4-nitro-2,1,3-benzoxadiazole (NBD-Cl) in a buffered medium (pH 8.6) resulting in formation of a highly fluorescent product.	λ_{ex} = 480 nm λ_{em} =535 nm	**Linearity and range:** 0.55–3.0 µg ml **LOD:** 0.16 µg/ml

Table 2.5: HPLC method for estimation of Amlodipine Besylate

Sr. no.	Matrix	Column and solvent system	Wavelength of measurement	Quantitative measurement
1[29]	Raw material and	RP_{18} column Mobile phase: phosphate buffer	238nm	---

	tablets	(pH 3): methanol :acetronitrile (50:35:15) Flow rate: 1 ml/min		
2[30]	Tablets & Capsules	RP_{18} column Mobile phase: 0.1% (v/v) ortho-phosphoric acid (pH 3.0) – acetonitrile (60:40, v/v) Flow rate: 1.0 ml/min	238 nm	**Linearity and range:** 10-30 µg/ml
3[31]	Tablets	Mobile phase: acetonitrile and 0.03M phosphate buffer pH 2.9 (55:45, v/v).	362 nm	**Linearity and range:** 0.1-20 µg/ml
4[32]	Tablets	C18-column Mobile phase: 25 mM ammonium acetate adjusted to pH 5.0 and methanol (65: 35, v/v) Flow rate: 0.8 ml/min	230 nm	**Retention time:** 3.91 min **Linearity and range:** 8-33 µg/ml
5[33]	Tablets	phenomenex Luna C-18 (250 x 4.6 mm, 5 µm) Mobile phase: methanol: acetonitrile: 50 mM KH_2PO_4 (20:50:30, v/v/v; pH 3.5) Flow rate: 1.0 ml/min	240nm	**Retention time:** 3.2min **Linearity and range:** 5-100 µg/ml
6[34]	Bulk powder	Inertsil ODS 3V (150 mm × 4.6 mm, 5 µm) Mobile phase: mixture of 1% triethyl amine, pH adjusted to 3.0 with orthophosphoric acid :	220nm	-----

		acetonitrile (65:35 v/v) Flow rate: 1.0 ml/min		
7[35]	Tablets	Hypersil BDS cyano (250 mm × 4.6 mm, 5 µm) Mobile phase: buffer (aqueous triethylamine pH 3) : acetonitrile (85:15 v/v) Flow rate: 1.0 ml/min	------	-----
8[36]	Tablets	Perfectsil® Target ODS-3 (250 × 4.6 mm, 5 µm) Mobile phase: acetonitrile: 0.025 M NaH_2PO_4 buffer (pH 4.5) (55:45, v/v) Flow rate: 1.0 ml/min	237nm	**Linearity and range:** 1–20 µg/ml **LOQ:** 1 µg/ml **LOD:** 0.35 µg/ml
9[37]	Tablets	Zorbax SB C18 (250 mm × 4.6 mm, 5 µm) Mobile phase: phosphate buffer (PH 7.0) : acetonitrile (65:35 v/v) Flow rate: 1.0 ml/min	240nm	**Linearity and range:** 6–14 µg/ml
10[38]	Bulk powders	JASCO-metaphase ODS (250 mm × 4.0 mm, 5 µm) Mobile phase: acetonitrile : 0.01 *M* KH_2PO_4, with pH 3.5 adjustments done with H_3PO_4 Flow rate: 1.5 ml/min	250nm	**Retention time:** 2.57 min **Linearity and range:** 25–3200 ng/ml **LOD:** 15 ng/ml

Table 2.6: LC Fluorescence method for estimation of Amlodipine Besylate

Sr.no.	Matrix	Column and solvent system	Excitation and Emission wavelength	Quantitative measurement
1[39]	Human plasma	precolumn derivatization with 4-chloro-7-nitrobenzofurazan (NBD-Cl) C-18 column Mobile phase: sodium phosphate buffer (pH 2.5) containing 1 ml/l triethylamine and methanol Flow rate: 2.8 ml/min	λ_{ex} = 480 nm λ_{em} =535 nm	**Linearity and range:** 0.25–16 ng/ml **LOD:** 0.25 ng/ml
2[40]	Plasma	precolumn derivatization with 4-chloro-7-nitrobenzofurazan (NBD-Cl) C-18 column Mobile phase: sodium phosphate buffer (pH 2.5) containing 1 ml/l triethylamine and methanol	λ_{ex} = 480 nm λ_{em} =535 nm	**Linearity and range:** 0.25–18 ng/ml

		Flow rate: 2.8 ml/min		

Table 2.7: LC-MS method for estimation of Amlodipine Besylate

Sr. no.	Matrix	Column and solvent system	Detection	Quantitative measurement
1[41]	Human plasma	C18 column Mobile phase: methanol–water (8:2, v/v) containing 0.5% formic acid	*m/z* 409.10–334.20	**Linearity and range:** 1–15 ng/ml
2[42]	Human plasma	Chiral AGP column containing α_1-acid glycoprotein as chiral selector Mobile phase: 10-mM acetate buffer (pH 4.5) - 1-propanol (99:1, v/v).	m/z 409 to 238	**LOQ:** 0.1ng/ml

Table 2.8: HPTLC method for estimation of Amlodipine Besylate

Sr. no.	Matrix	TLC plate and solvent system	Detection	Quantitative measurement
1[43]	Pharmaceutical dosage	Precoated TLC silica gel F254 (10 x 10 cm) mobile phase: toluene,	-----	-----

	forms	acetone, ethanol, ammonia (56.0 : 34.5 : 6.0 : 3.5, v/v/v/v)		
2[44]	Tablets	Precoated silica gel 60F254. mobile phase: methylene chloride: methanol: ammonia solution (25% NH_3) (8.8:1.3:0.1; v/v/v)	230nm	**Retention factor (R_f) :** 0.75 **Linearity and range:** 10–500 µg /ml

Table 2.9: Adsorptive square-wave anodic stripping voltammetry for estimation of Amlodipine Besylate

Sr.no.	Matrix	Electrode	Detection	Quantitative measurement
1[45]	Tablets and plasma	glassy carbon electrode	0.510 V (versus Ag/AgCl)	**Linearity and range:** 4.0×10^{-8} to 2.0×10^{-6} M **LOD:** 1.4×10^{-8} M

B) Literature Review for Telmisartan:

Numerous analytical methods had been developed for the estimation of telmisartan in pharmaceutical formulation, human plasma, serum and other matrices, using different analytical techniques. These methods are utilized for various purposes like quality control of pharmaceutical dosage forms, stability studies, study of pharmacokinetics of analyte and its metabolites.

Table 2.10: UV – Vis Spectrophotometric method for estimation of telmisartan

Sr. no.	Matrix	Solvent system	Wavelength of determination	Quantitative analysis
1[46]	Bulk drug and Pharmaceutical formulations	Methanol	Telmisartan exists in two different forms in acidic and basic mediums that differ in their UV spectra. Difference spectrum, obtained by keeping telmisartan in 0.01 N NaOH in reference cell and telmisartan in 0.01 N HNO_3 in sample cell, showed two characteristic peaks at 295 nm and 327 nm with positive and negative absorbance respectively. Difference of absorbance between these two maxima was calculated to find out the amplitude, which was plotted against concentration.	**Linearity and range:**2-12 µg/ml
2[47]	Tablets	Methanol	296 nm	**Linearity and range:** 4-28

				µg/ml
3[48]	Tablets	Methanol	328 nm	**Linearity and range:** 5-30 µg/ml

Table 2.11: Colorimetry method for estimation of Telmisartan

Sr. no.	Matrix	Solvent system	Wavelength of determination	Quantitative analysis
1[49]	Pharmaceutical formulations, Urine & Plasma	Formation of colored complex of Telmisartan and Congo red in pH 2.5 HCL-NaAc Buffer solution	593 nm	-----
2[50]	Tablets	Formation of colored chloroform extractable ion pair complexes with Bromocresol green (BCG)	440 nm	-----

Table 2.12: Fluorimetric method for estimation of Telmisartan

Sr. no.	Matrix	solvent system	Excitation and Emission wavelength	Quantitative measurement
1[51]	Bulk drug, Pharmaceutical formulations & Plasma	Reaction of Telmisartan with 1 M NaOH solution, which shows strong native fluorescence	λ_{ex} = 230 nm λ_{em} =365 nm	-----

Table 2.13: LC-MS method for estimation of Telmisartan

Sr. no.	Matrix	Column and solvent system	Quantitative measurement
1[52]	Human plasma	Zorbax-C_{18} column Mobile phase: methanol–10 mM ammonium acetate (85:15, v/v) adjusted to pH 4.5 after mixing with formic acid	**Linearity and range:** 0.5–600.0 ng/ml

Table 2.14: HPLC method for estimation of Telmisartan

Sr. no.	Matrix	Column and Solvent system	Wavelength of determination	Quantitative analysis
1[53]	Tablets	Hypersil C18 BDS (25×0.46 cm) Mobile phase: acetonitrile : Methanol (60:40 v/v) Flow rate: 1.2 ml/min	245nm	**Retention time:** 1.92 min **Linearity and range:** 4-12 µg/ml
2[54]	Tablets	Hypersil C18 BDS (25×0.46 cm) Mobile phase: acetonitrile : 0.05M KH_2PO_4 pH 3.0 (60:40, v/v) Flow rate: 1.0 ml/min	271nm	**Retention time:** 5.19 min **Linearity and range:** 4.1-20.48µg/ml
3[55]	Tablets	Genesis C18(25×0.46 cm, I.D.,10 µm) Mobile phase: 0.01M KH_2PO_4 pH 3.4 : Methanol : acetonitrile (15:15:70 v/v/v) Flow rate: 1.0 ml/min	210nm	**Retention time:** 4.98 min **Linearity and range:** 28-52 µg/ml **LOQ:** 1.5 µg/ml **LOD:** 0.5 µg/ml

Table 2.15: HPLC- Fluorimetric method for estimation of Telmisartan

Sr. no.	Matrix	Column and solvent system	Excitation and Emission wavelength	Quantitative measurement
1[56]	Urine	Novapak C18 column (3.9×150 mm, 4 µm). Mobile phase: acetonitrile–phosphate buffer (pH 6.0, 5 mM) (45:55, v/v	λ_{ex} = 305 nm λ_{em} =365 nm	**Linearity and range:** 1-1000 µg/l
2[57]	Urine	Chromolith RP-18e monolithic (250 x 4.6mm,10mm)	λ_{ex} = 259 nm λ_{em} =399 nm	**Linearity and range:** 1-2500 ng/ml

Table 2.16: LC method for estimation of Telmisartan

Sr. no.	Matrix	Column and solvent system	Detector	Quantitative measurement
1[58]	Tablets	C-18 column Mobile phase: Buffer (pH 3.0): acetonitrile (55:45 v/v). Buffer	-----	**Linearity and range:** 20-400 µg/ml

		contains 0.1M sodium perchlorate monohydrate in double distilled water. Flow rate: 2.8 ml/min		

Table 2.17: HPTLC method for estimation of Telmisartan

Sr. no.	Matrix	TLC plate and Solvent system	Wavelength of determination	Quantitative analysis
1[59]	Tablets	Precoated silica gel 60F254 Mobile phase: chloroform : methanol: toluene) (2:5:5; v/v/v)	272nm	**Retention factor:** 0.33 **Linearity and range:** 250–500 ng /spot **LOQ:** 190 ng/spot **LOD:** 75 ng/spot
2[60]	Tablets	Precoated silica gel 60F254. M phase: butanol : ammonia 25% (8:2v/v)	295 nm	-----

2.4 LITERATURE REVIEW FOR SIMULTANEOUS ESTIMATION OF AMLODIPINE BESYLATE AND TELMISARTAN

Table 2.18: HPLC method for simultaneous estimation of Amlodipine Besylate and Telmisartan

Sr. no.	Matrix	Column and Solvent system	Wavelength of determination	Quantitative analysis
1[61]	Tablet	Merck C18 (250 x 4.0 mm, 5 µm) Mobile phase: acetonitrile: Phosphate buffer pH 4.5 (60:40 v/v) Flow rate: 1.3 ml/min	238nm	**Retention time:** Less than 9 min **Linearity and range:** 1-11 µg/ml for amlodipine 8-80 µg/ml for telmisartan

Chapter 3
AIM OF PRESENT WORK

3. AIM OF PRESENT WORK:

3.1 Aim of Present Work:

Hypertension is a highly prevalent disease in country like India. Numbers of drugs and their products are available in the Indian market for the treatment and maintenance of it. The drug products are available either as single or Multicomponent dosage forms, where Multicomponent dosage forms are more effective than single component dosage form. Among these dosage forms amlodipine and telmisartan found to be most widely used combination.

Amlodipine is a Ca^{++} channel blocker where telmisartan is Angiotensin receptor blocker, and combination of these drugs will have synergistic effect. As this combination is taken by number of patients its quality and performance must be in the acceptance limit. To check the quality and performance reliable and accurate assay method must be there. The development of assay procedures for such dosage forms poses considerable challenges to the analytical chemist, owing to the complexity of these dosage forms as it contains multiple drug entities.

Extensive literature survey reveals that not any spectrophotometric and HPTLC has been reported for simultaneous determination of Amlodipine besylate and Telmisartan. So this combination was selected for development of spectrophotometric and HPTLC method for their estimation.

- ✓ Prime objective of work was to develop a specific, simple, rapid, sensitive and reproducible spectrophotometric and HPTLC method for quantitative estimation of amlodipine and telmisartan in their combined dosage form.

3.2 Objective of Present Work:

- ➢ To develop and validate spectrophotometric method for simultaneous estimation of amlodipine besylate and telmisartan.
- ➢ To develop and validate HPTLC method for simultaneous estimation of amlodipine besylate and telmisartan.

Chapter 4
METHODS & VALIDATION INTRODUCTION

4.1. SPECTROPHOTOMETRIC METHOD FOR ANALYSIS OF DRUG COMPONENTS

THEORY OF SPECTROPHOTOMETRY

Wavelength and Energy:

Absorption and emission of radiant energy by molecules and atoms is the basis for optical spectroscopy. By interpretation of these data both qualitative and quantitative information can be obtained. Qualitatively, the positions of the absorption and emission lines or bands, which occur in the electromagnetic spectrum, indicate the presence of a specific substance. Quantitatively, the intensities of the some absorption and emission lines or bands for the unknown and standards are measured. The concentrations of the unknown is then determined from these data [62, 63].

The absorption and the emission of energy in the electro-magnetic spectrum occur in discrete packets of photons. The relation between the energy of a photon and the frequency appropriate for the description of its propagation is

$E = hv$

Where E = Energy in ergs

v = Represents frequency in cycles per second

h = Plank's constant (6.6256×10^{-27} erg-sec)

The data obtained from a spectroscopic measurement are in the form of a plot of radiant absorbed or emitted as a function of position in the electromagnetic spectrum. This is known as a spectrum and the position of absorption or emission is measured in units of energy, wavelength or frequency[64].

Beer-Lambert's law:

UV and visible absorption bands are due to electronic transitions in the region of 200 nm to 780 nm. In case of organic molecules, the electronic transitions could be ascribed to a s, p or n electron transition from the ground state to an excited state (s*, p* or n*).There are four types of absorption bands that occur due to the electronic transition of a molecule[65,66]:

R - Bands: n ® p*, in compounds with C=O or NO_2 group

k - Bands: p ® p*, in conjugated systems.

b - Bands (Benzenoid bands): Due to aromatic and heteroaromatic systems

E - Bands (ethylenic bands): In aromatic systems.

When light (monochromatic or heterogeneous) falls upon a homogeneous medium, a portion of the incident light is reflected, a portion is absorbed within the medium and the remainder is transmitted. If the intensity of the incident light is expressed by I, that of the absorbed light by I_a, that of the transmitted light by I_t, and that of the reflected light by I_r, then:

$$I = I_a + I_t + I_r \quad \text{..... (1)}$$

Credit for investigating the change of absorption of light with the thickness of the medium is frequently given to Lambert; Beer later applied similar experiments to solutions of different concentrations and published his results. The two separate laws governing absorption are usually known as Lambert's law and Beer's law. In the form they are referred to as the Beer-Lambert law. Mathematically, the radiation-concentration and radiation-path-length relation can be expressed by[67]

$$\frac{dI}{dc} = -k_1 I \text{ and } \frac{dI}{dt} = -k_2 I \quad \text{..... (2)}$$

The more familiar equation used in spectrometry

log (I/I) = Î cl .....(3)

Where, I = intensity of the incident energy

I = intensity of the emergent energy

c = concentration

l = thickness of the absorber (in cm and

Î = molar absorbtivity for concentration in moles/L

, which is encountered less frequently in the literature, represents a concentration of 1% w/v and 1 cm cell thickness and is used primarily in the investigation of those substances of unknown or undetermined molecular weight. A typical UV absorption spectrum, shown in fig. 1, is the result of plotting wavelength v/s absorbtivity, $\hat{I}_{max}$ is denoted by l_{max}.

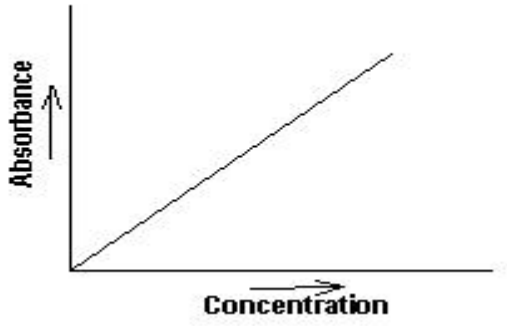


Fig. 4.1: A representative Beer-Lambert law plot

- **PRINCIPLE OF QUANTITATIVE SPECTROPHOTOMETRIC ASSAY OF MEDICINAL SUBSTANCES[68]:**

The assay of an absorbing substance may be quickly carried out by preparing a solution in a transparent solvent and measuring its absorbance at a suitable wavelength. The concentration of

the absorbing substance calculated from the measured absorbance using one of three principal procedures.

Use of a standard absorbtivity value:

This procedure is adopted by official compendia, e.g. British Pharmacopoeia, for substances such as methyl testosterone that has reasonably broad absorption variation of instrumental parameters e.g. slit width, scan speed.

Use of a calibration graph:

In this procedure the absorbances of a number (typically 4-6) of standard solutions of the reference substance at concentrations encompassing the sample concentrations are measured and a calibration graph is constructed. The concentration of the analyte in the sample solution is read from the graph as the concentration corresponding to the absorbance of the solution.

- **DIFFERENT SPECTROPHOTOMETRIC SIMULTANEOUS ESTIMATION METHODS FOR MULTICOMPONENT SAMPLES[69]**

The spectrophotometric assay of drugs rarely involves the measurement of absorbance of sample containing only one absorbing component. The pharmaceutical analyst frequently encounters the situation where the concentration of one or more substances is required in samples known to contain other absorbing substances, which potentially interfere in the assay.

The basis of all the spectrophotometric techniques for multi-component samples is the property that all wavelengths:

(a)The absorbance of a solution is the sum of absorbances of the individual components; or

(b)The measured absorbance is the difference between the total absorbance of the solution in the sample cell and that of the solution in the reference (blank) cell.

In multi-component formulations the concentration of the absorbing substance is calculated from the measured absorbance using one of the following procedures:

(a)Assay as a single-component sample: The concentration of a component in a sample which contains other absorbing substances may be determined by a simple spectrophotometric

measurement of absorbance, provided that the other components have a sufficient small absorbance at the wavelength of measurement.

(b) Simultaneous equation method: If a sample contains two absorbing drugs (X and Y) each of which absorbs at the l_{max} of the other, it may be possible to determine both drugs by the technique of simultaneous equations (Vierodt's method).

$$C_x = \frac{A_2\, a_{y1} - A_2\, a_{y2}}{A_{x2}\, a_{y1} - a_{x1}\, a_{y2}}$$

$$C_y = \frac{A_1\, a_{x2} - A_2\, a_{x1}}{A_{x2}\, a_{y1} - a_{x1}\, a_{y2}}$$

Where:

a) The absorptivity of X at λ_1 and λ_2, a_{x1} and a_{x2} respectively.

b) The absorptivity of Y at λ_1 and λ_2, a_{y1} and a_{y2} respectively.

c) The absorbances of the diluted sample at λ_1 and λ_2, A_1 and A_2 respectively.

Criteria for obtaining maximum precision, based upon absorbance ratios that place limits on the relative concentrations of the components of the mixture.

The criteria are that the ratios

$$\frac{A_1 / A_2}{a_{x2} / a_{x1}} \quad \text{and} \quad \frac{a_{y2} / a_{y1}}{A_2 / A_1}$$

should lie outside the range 0.1- 2.0 for the precise determination of Y and X respectively. These criteria are satisfied only when the λ max of the two components are reasonably dissimilar. An additional criterion is that the two components do not interact chemically.

To reduce the random errors during measurements, sometimes instead of carrying out analysis of two components at two wavelengths, it is carried out at 3 or 4 wavelengths. The equations will no longer have a unique solution but the best solution can be finding out by the least square criterion.

(c) Absorbance ratio method[70]: The absorbance ratio method is a modification of the simultaneous equations procedure. It depends on the property that, for a substance, which obeys Beer's law at all wavelengths. Q-analysis is based on the relationship between absorbance ratio value of a binary mixture and relative concentrations of such a mixture. The ratio of two absorbance determined on the same solution at two different wavelengths is constant. This constant was termed as "Hufner's Quotient' or Q-value which is independent of concentration and solution thickness e.g. Two different dilutions of the same substances give the same absorbance ratio A_1/ A_2. In the USP this ratio is referred to as a Q value. In the quantitative assay of two components in admixture by the absorbance ratio method, absorbance are measured at two wavelengths, one being the λ max of one of the components (λ_2) and the other being a wavelength of equal absorptivity of the two components (λ_1), an iso-absorptive point.

$$Cx = \quad Qm - Qy / \ Qx - Qy \ . \quad A_1/ a_{x1}$$

Equation gives the concentration of X in terms of absorbance ratios, the absorbance of the mixture and the absorptivity of the compounds at the iso-absorptive wavelengths. Accurate dilutions of the sample solution and of the standard solutions of X and Y are necessary for the accurate measurement of A_1 and A_2 respectively.

(d) Geometric correction method: A number of mathematical correction procedures have been developed which reduce or eliminate the background irrelevant absorption that may be present in samples of biological origin. The simplest of this procedure is the three point geometric procedure, which may be applied if the irrelevant absorption is linear at the three wavelengths selected. If the wavelengths λ_1, λ_2 and λ_3 are selected to that the background absorbances B_1, B_2 and B_3 are linear, then the corrected absorbance D of the drug may be calculated from the three absorbances A_1, A_2 and $A_3$of the sample solution at λ_1, λ_2 and λ_3 respectively as follows

Let $_vD$ and $_wD$ be the absorbance of the drug alone in the sample solution at λ_1 and λ_3 respectively, i.e. v and w are the absorbance ratios vD/D and wD/D respectively.

$B_1 = A_1 - vD$, $B_2 = A_2 - D$ and $B_3 = A_3 - wD$

Let y and z be the wavelengths intervals ($\lambda_2 - \lambda_1$) and ($\lambda_3 - \lambda_2$) respectively

$D = y(A_2 - A_3) + z(A_2 - A_1) / y(1-w) + z(1-v)$

This is a general equation which may be applied in any situation where A_1, A_2 and A_3 of the sample, the wavelength intervals y and z and the absorbance ratio v and w are known.

(e) Orthogonal polynomial method[71]: The technique of orthogonal polynomials is another mathematical correction procedure, which involves more complex calculations than the three-point correction procedure. The basis of the method is that an absorption spectrum may be represented in terms of orthogonal functions as follows

$A(\lambda) = p\,P(\lambda) + p_1 P_1(\lambda) + p_2 P_2(\lambda) \ldots\ldots p_n P_n(\lambda)$

Where A denotes the absorbance at wavelength λ belonging to a set of n+1 equally spaced wavelengths at which the orthogonal polynomials, $P(\lambda)$, $P_1(\lambda)$, $P_2(\lambda)$ ….. $P_n(\lambda)$ are each defined.

The accuracy of the orthogonal functions procedure depends on the correct choice of the polynomial order and the set of the wavelengths. Usually, quadratic or cubic polynomials are selected depending on the shape of the absorption spectra of the drug and the irrelevant absorption. The set of the wavelengths is defined by the number of wavelengths, the interval and the mean wavelength of the set (λ m). Approximately linear irrelevant absorption is normally eliminated using six to eight wavelengths, although many more up to 20, wavelengths may be required if the irrelevant absorption contains high-frequency components. The wavelengths interval and λ m are best obtained from a convulated absorption curve. This is a plot of the absorptivity coefficient for a specified order of polynomial, a specified number of wavelengths and a specified wavelengths interval against the λ m of the set of wavelengths. The optimum set of wavelengths corresponds with a maximum or minimum in the convoluted curve of the analyte

and with a coefficient of zero in the convoluted curve of the irrelevant absorption. In favorable circumstances the concentration of an absorbing drug in admixture with another may be calculated if the correct choice of polynomial parameters is made, thereby eliminating the contribution of the drug from the polynomial of the mixture.

(f)Difference spectrophotometry[72-76]: Difference spectroscopy provides a sensitive method for detecting small changes in the environment of a chromophore or it can be used to demonstrate ionization of a chromophore leading to identification and quantitation of various components in a mixture. The selectivity and accuracy of spectrophotometric analysis of samples containing absorbing interferents may be markedly improved by the technique of difference spectrophotometry. The essential feature of a difference spectrophotometric assay is that the measured value is the difference absorbance (Δ A) between two equimolar solutions of the analyte in different forms which exhibit different spectral characteristics.

The criteria for applying difference spectrophotometry to the assay of a substance in the presence of other absorbing substances are that:

A) Reproducible changes may be induced in the spectrum of the analyte by the addition of one or more reagents.

B) The absorbance of the interfering substances is not altered by the reagents.

The simplest and most commonly employed technique for altering the spectral properties of the analyte properties of the analyte is the adjustment of the pH by means of aqueous solutions of acid, alkali or buffers. The ultraviolet-visible absorption spectra of many substances containing ionisable functional groups e.g. phenols, aromatic carboxylic acids and amines, are dependent on the state of ionization of the functional groups and consequently on the pH of the solution.

If the individual absorbances, A_{alk} and A_{acid} are proportional to the concentration of the analyte and path length, the Δ A also obeys the Beer-Lambert law and a modified equation may be derived

$$\Delta A = \Delta abc$$

Where Δ a is the difference absorptivity of the substance at the wavelength of measurement.

If one or more other absorbing substances is present in the sample which at the analytical absorbance A_x in the alkaline and acidic solutions, its interference in the spectrophotometric measurement is eliminated

$$\Delta A = (A_{alk} + A_x) - (A_{acid} + A_x)$$

The selectivity of the Δ A procedure depends on the correct choice of the pH values to induce the spectral change of the analyte without altering the absorbance of the interfering components of the sample. The use of 0.1M sodium hydroxide and 0.1M hydrochloric acid to induce the Δ A of the analyte is convenient and satisfactory when the irrelevant absorption arises from pH-insenstive substances. Unwanted absorption from pH-sensitive components of the sample may also be eliminated if the pK_a values of the analyte and interferents differ by more than 4.

(g) Derivative spectrophotometry: Direct spectrophotometric determination of multicomponent formulation is often complicated by interference from formulation matrix and spectral overlapping; such interferences can be treated in many ways like solving two simultaneous equations, using absorbance ratios at certain wavelengths, but still may give erroneous results[77]. Other approaches include PH induced differential least squares[78] and orthogonal function methods[79]. Also the compensation technique can be used to detect and eliminate unwanted or irrelevant absorption. Derivative spectrophotometry is a useful means of resolving two overlapping spectra and eliminating matrix interferences or interferences due to an indistinct shoulder on side of an absorption band[80]. Derivative spectrophotometry involves the conversion of a normal spectrum to its first, second or higher derivative spectrum. In the context of derivative spectrophotometry, the normal absorption spectrum is referred to as the fundamental, zeroth order or D spectrum. The absorbance of a sample is differentiated with respect to wavelength λ to generate first, second or higher order derivative

$[A] = f(\lambda)$: zero order

$[dA/d\lambda] = f(\lambda)$: first order

$[d^2A/d\lambda^2] = f(\lambda)$: second order

The first derivative spectrum of an absorption band is characterized by a maximum, a minimum, and a cross-over point at the λ max of the absorption band. The second derivative spectrum is characterized by two satellite maxima and an inverted band of which the minimum corresponds to the λ max of the fundamental band.

The spectral transformation confer two principal advantages on derivative spectrophotometry, firstly an even order spectrum is of narrower spectral bandwidth than its fundamental spectrum, secondly derivative spectrophotometry discriminates in favor of substances of narrow spectral bandwidth substances. This is because the derivative amplitude.

The enhanced resolution and bandwidth discrimination increases with increasing derivative order. However it is also found that the concomitant increase in electronic noise inherent in the generation of the higher order spectra, and the consequent reduction of the signal to noise ratio, place serious practical limitations on the higher order spectra.

The important features of derivative technique include enhanced information content, discrimination against back ground noise and greater selectivity in quantitative analysis. It can be used for detection and determination of impurities in drugs, chemicals and also in food additives and industrial wastes[81].

4.2 HPTLC METHOD FOR ANALYSIS OF DRUG COMPONENTS

4.2.1 Introduction to HPTLC

HPTLC added a new dimension to chromatography as it was demonstrated that precision could be improved ten-fold, analysis time could be reduced by a similar factor, less mobile phase was required, and the development distances on the layers could be reduced. The technique could now be made fully instrumental to give accuracy comparable with HPLC[82].

For multi-component samples fractions of interest from an HPLC separation can be collected and subsequent re-chromatography of these on HPTLC can give a ‘‘fine tuned’’ separation of the components of the fractions[83-85]. HPTLC has been successfully hyphenated with high performance liquid chromatography (HPLC), mass spectroscopy (MS), Fourier transform infra-red (FTIR), and Raman spectroscopy, to give far more detailed analytical data on separated compounds[86]. Even the UV/visible diode array technique has been utilized in HPTLC to determine peak purity or the presence of unresolved analytes. HPTLC uses the same type of silica gel 60 layers with a thickness of 0.20-0.25 mm. However the particle size is much smaller, typically ranging from 4-8 μm, with optimum 5-6 μm[87].

Table 4.1 Comparison of HPTLC and TLC

PARAMETERS	HPTLC	TLC
Particle size	5-6 μm	10-12 μm
Pore diameter	60 Å	60-100 Å
Layer thickness	0.20-0.25 mm	0.20-0.25 mm
Spot size recommended	~1 mm	2-5 mm
Spot loading	50-200 nl	1-5 μl

Band size recommended	5-10 mm	10-15 mm
Band loading	1-4 µl	5-10 µl
Sensitivity limit	Upper pg	Ng
Normal development time	2-30 minutes	15-120 minutes

❖ **Mechanism of HPTLC separation**

- Adsorption
- Partition
- Ion-exchange

4.2.2 Steps involved in HPTLC [87]

- Selection of chromatographic layer
- Sample and standard preparation
- Layer pre-washing and pre-conditioning
- Application of sample and standard
- Chromatographic development
- Detection of spots
- Scanning and Documentation of chromatic plate

❖ **Sample Application**

The solvent used to apply the sample to the TLC plate can have a decisive influence on the spot size. The least polar single solvent or mixture of solvents in which the analyte are completely soluble or completely extracted from the sample matrix can be used.

Although the spot can be easily applied manually using a glass capillary, the application of sample as a band usually require more dexterity and is more accurately accomplished with semi- or fully automated equipment. The Linomat 5 offers semi-automatic sample application for qualitative and quantitative analyses as well as for preparative separations

Table 4.2 Advantages of Band Application over Spot Application

Advantage of Band over Spot Application	Application of Spot over Band Application
Better resolution of analytes-near origin	Can require less automation

More even distribution of sample	Can be very inexpensive
Less error on choice of scanner slit width	Application usually less time consuming
Greater accuracy – lower % standard deviation	
More flexibility in sample loading	

❖ **Solvent Selection**

Selectivity of separation is greatly influenced by the choice of solvent or solvent mixture.

- Trial and error
- Experience and Literature
- 3 - 4 component mobile phase should be avoided
- Multi component mobile phase once used not recommended for further use
- Twin trough chambers are used only 10 -15 ml of mobile phase is required
- Components of mobile phase should be mixed introduced into the twin - trough chamber

❖ **Development chambers**

There are a variety of different types of chambers, each designed with particular features to control to the greater or lesser extent the parameters of chromatogram development reproducibility.

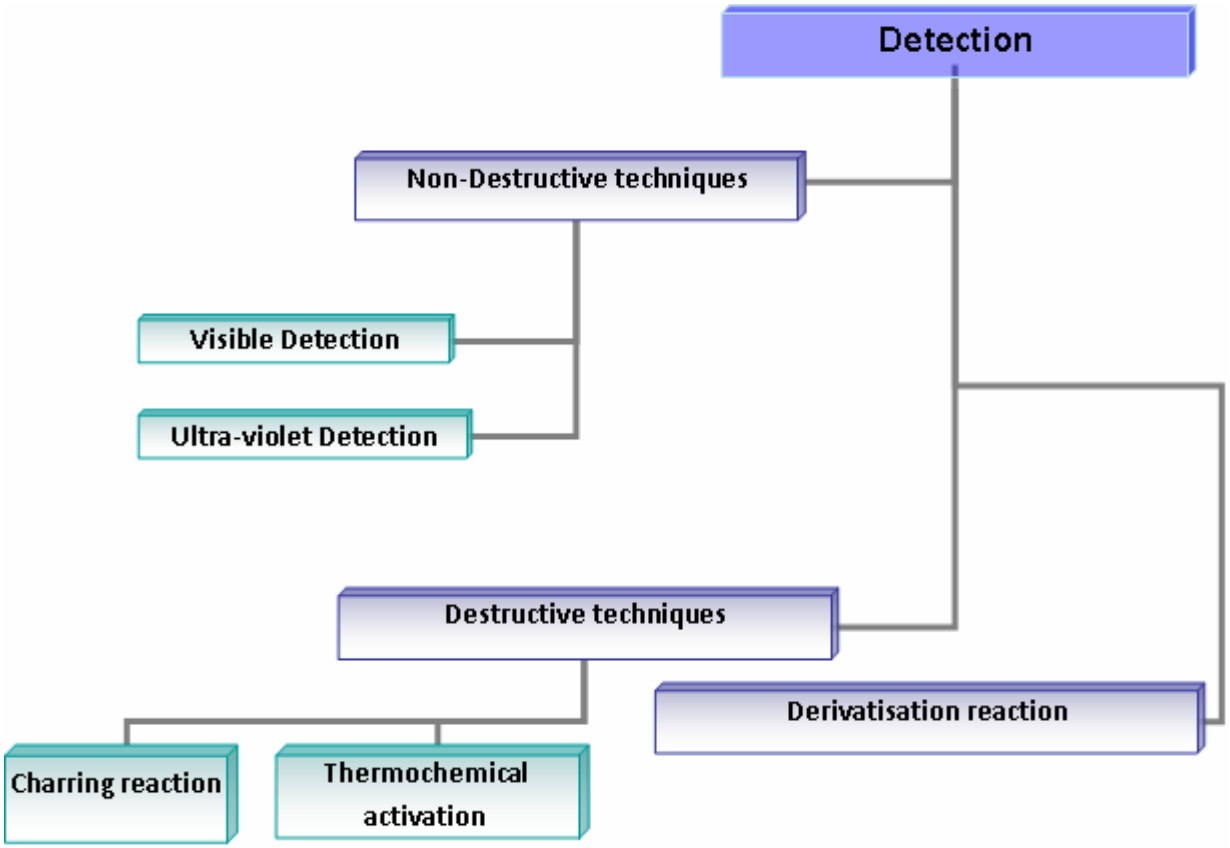
As solvent vapour saturation, sorbent vapour adsorbed, solvent vapour "demixing" and solvent front and edge effects on the chromatographic layer can have a bearing on separation achieved, it is important to eliminate unwanted effects and to utilize those features that will improve resolution.

The types of HPTLC chambers are

- Nu-chamber
- Ns-chamber
- Twin-through chamber
- Su-chamber
- Ss- chamber

- Horizontal chamber
- Automatic development chamber (ADC)
- Vario chambers

❖ **Detection**



❖ **Quantification**

1) Densitometry

Densitometry is a means of measuring the concentration of the chromatographic zones on the developed HPTLC layer without damaging the separated substance.

There are three possible scanning modes, single beam, single wavelength, double beam using a beam splitter and dual wavelength, double beam combined into a single beam. The single beam format is most popular, as the beam of electromagnetic radiation hits the chromatographic layer, some passes into and through the layer whilst the remainder is reflected back from the surface. Reflectance occurs due to the opaqueness of the layer. This reflected radiation is measured by the photomultiplier unit or photoelectric cell in the instrument.

The spectrodensitometric scanner scan separate tracks and wavelength produces vast amount data. These data includes peak heights and areas, and position of zones (start, middle and end) for every resolved component on every chromatographic track on the HPTLC plate. A baseline adjustment is applied so that all peaks can be accurately integrated ready for possible quantification.

2) Video imaging and Densitometry

The developed chromatogram is illuminated from above with visible, 254 nm (UV) or 366 nm (UV) light, depending on the radiation required to visualize the analytes. Illumination from below the plate can often improve the brightness of the image. With the plate suitably lit, an image acquisition device, usually CCD (charged coupled device) camera with zoom attachment is positioned vertically above. The CCD camera transmits a digital signal to a computer and video printer.

4.3 VALIDATION OF ANALYTICAL METHODS

Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice.

Validation is an act of proving that any procedure, process, equipment, material, activity or system performs as expected under given set of conditions and also give the required accuracy, precision, sensitivity, ruggedness etc. When extended to an analytical procedure, depending upon the application, it means that a method works reproducibly, when carried out by some different persons, in same or different laboratories, using different reagents, different equipments, etc.

Advantage of analytical method validation

The biggest advantage of method validation is that it builds a degree of confidence, not only for the developer but also to the user. Although the validation exercise may appear costly and time consuming, it results are inexpensive and eliminates frustrating repetitions, leads to better time management in the end. Minor change in the conditions such as reagents supplier or grade, analytical setup are unavoidable due to obvious reasons but the method validation absorbs the shock of such conditions and pays for more than invested on the process.

The objective of the analytical procedure should be clearly understood since this will govern the validation characteristics that are evaluated. Typical validation characteristics that may be considered are listed below:

1. Accuracy
2. Precision

3. Linearity
4. Range
5. Limit of Detection (LOD) and Limit of Quantification (LOQ)
6. Specificity
7. Selectivity
8. Robustness

4.3.1 Accuracy

The accuracy of an analytical method is the extent to which test results generated by the method and the true value agree. As per ICH guideline, accuracy is defined as "the closeness of agreement between the value that is adopted, either as a conventional, true or accepted reference value, and the value found". Accuracy is a measure of exactness of the analytical method.

Accuracy can be measured by several methods:

The true value can be obtained from an established reference method. In this approach assumes that the uncertainty of the reference method is known.

Accuracy can be assessed by analyzing a sample with known concentration, for example, a certified reference material, and comparing measured value with the true value as supplied with the material.

Recovery is found from the following formula [88]

$$\% \text{ Recovery} = \frac{N(\sum xy) - (\sum x) * (\sum y)}{N(\sum x^2) - (\sum y^2)}$$

Where N = Number of observations

x = Amount of standard drug added

y = Amount of drug added

or

% Recovery = ($C_{fortified} - C_{unfortified}$) x 100 / $C_{std\ added}$ (QAAC)

Where, $C_{fortified}$ is concentration of drug from matrix with standard addition of drug

$C_{unfortified}$ is concentration of drug without addition

$C_{std\ added}$ is standard added drug in solution of drug

4.3.2 Precision

The precision of a method is the extent to which the individual test results of multiple injections of a series of standards agree. As per ICH guideline, precision of a method is "express the closeness of agreement (degree of scatter) between a series of successive measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions"[89, 90].

The measured standard deviation can be subdivided into 3 categories:

- Repeatability
- Intermediate precision
- Reproducibility

Repeatability gives the degree of precision obtained when the method is replicated in the same laboratory within short intervals and in the same conditions. Reproducibility represents precision obtained under variations in conditions of assays such as different analyst, equipment and reagents, laboratory and days.

The precision of an analytical method is usually expressed as the standard deviation (SD) or relative standard deviation (RSD).

The standard deviation is calculated from the following formula

$$SD = \sqrt{\frac{\sum (X_i - X)^2}{N - 1}}$$

Where, Xi = Individual measurement in the set

X = Arithmetic mean of the set

N = Number of replicates taken in the set

$$RSD = \frac{SD}{X}$$

%RSD or coefficient of variance (CV) is expressed as

$$\%\ RSD = CV = \frac{SD}{X} * 100$$

4.3.3 Linearity

The linearity of an analytical method is its ability to elicit test results that are directly proportional to the concentration of analytes in samples within a given range or proportional by means of well-defined mathematical transformations. Linearity may be demonstrated directly on the test substance (by dilution of a standard stock solution) and/or by using separate weighings of synthetic mixtures of the test product components, using the proposed procedure[91].

The relationship between the sample concentration and its signal is first order type. This line, known as the calibration line, is expressed by an estimated first order equation.

y = mx + c

Where, y = measured signal

x = concentration of sample

c = intercept

m = slope of line

4.3.4 Range

The range of an analytical method is the interval between the upper and lower levels (including these levels) that have been demonstrated to be determined with precision, accuracy and

linearity. The range is normally expressed in the same units as the test results (e.g., percentage, parts per million) obtained by the analytical method[91].

4.3.5 Specificity and Selectivity

Specificity of an analytical method is the ability to measure accurately and specifically the analyte in presence of components that may be expected to be present in the sample matrix[91].

Specificity is expressed as degree of bias of test results obtained by analysis of sample containing added impurities, degradation product, related chemical compounds or placebo ingredients when compared with test results from samples without added samples. Bias may be expressed as difference in assay results between two group samples. Thus specificity is a measure of the degree of interference in the analysis of complex sample mixture.

Selectivity is ability of analytical method to differentiate various substances in the sample[91]. One basic difference in selectivity and specificity is that, selectivity is restricted to qualitative detection of the components from the sample matrix whereas specificity means quantitative measurement of one or more analytes. Selectivity generally applies to a separative method whereas specificity is applicable to a non-separative method. The titration methods are good examples of specificity and chromatographic methods are both selective and specific.

The selectivity is an essential requirement for all types of methods used in identification i.e. spectroscopic, chromatographic or chemical. On the other hand, the selectivity is an essential for non chromatographic assay methods and for stability indicating assays whether chromatographic or non chromatographic[92].

Procedure for establishment of selectivity of a method

1. Analyze sample and reference materials by the candidate and other independent methods.
2. Assess the ability of the methods to confirm free identity analyte and their ability to measure the analyte in solution from the interference present
3. Choose the most appropriate method
4. Analyze sample containing various suspected interference in the presence of the analyte of present
5. Examine the effect of interferences and whether further development is required.

4.3.6 Limit of detection (LOD) and Limit of Quantification (LOQ):

Limit of detection (LOD)

It is the lowest amount of analyte in sample that can be detected but not necessarily quantitated under the stated experimental conditions. It can be determined by three methods:

(I) Based on visual evaluation: It is determined by the analysis of samples with known concentrations of analyte and establishing the minimum level at which the analyte can be reliably detected.

(II) Based on signal to noise ratio: Determination of the signal to noise ratio is performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and establishing the minimum concentration at which the analyte can be reliably detected. A signal to noise ratio of 2:1 or 3:1 is generally considered acceptable for estimating the detection limit.

(III) Based on standard deviation of the response and the slope: The detection limits may be expressed as:

$$DL = 3.3 \sigma / S$$

Where, σ = the standard deviation of the response

S = the slope of calibration curve

From the linearity curves of both the drugs the standard deviation of the intercept was calculated and the value obtained was substituted in the above equation to get limit of detection for both the drugs respectively.

Limit of Quantification

It is the lowest concentration of analyte in the sample that can be determined with the acceptable precision and accuracy condition. . It can be determined by three methods:

(I) Based on visual evaluation: It is determined by the analysis of samples with known concentrations of analyte and establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision

(II) Based on signal to noise: Determination of the signal to noise ratio is performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and establishing the minimum concentration at which the analyte can be reliably quantified. A signal to noise ratio of 10:1 is generally considered acceptable for estimating the quantitation limit.

(III) Based on standard deviation of the response and the slope: The quantitation limits may be expressed as:

$$DL = 10\,\sigma / S$$

Where, σ = the standard deviation of the response

S = the slope of calibration curve

From the linearity curves of both the drugs the standard deviation of the intercept was calculated and the value obtained was substituted in the above equation to get limit of quantification for both the drugs respectively.

Chapter 5

EXPERIMENTAL WORK

5.) Experimental work

5.1 IDENTIFICATION OF DRUGS:

Drug samples were received from pharmaceutical companies; these samples were subjected to identification. Identification of these drugs was carried out by following methods:

5.1.1 Determination of Melting Point:

Melting point of both the drug samples were determined by capillary method and obtained results as under:

Table 5.1 Melting point of drugs

Drugs	Standard	Obtained
Amlodipine besylate	199- 201°C	200- 201°C
Telmisartan	261 – 264°C	260 – 262°C

5.1.2 Determination of UV-Spectrum:

5.1.2.1 UV-spectrum of Amlodipine Besylate:

UV-spectrum of Amlodipine besylate was obtained at 10 µg/ml concentration in methanol. Amlodipine besylate was found to show absorption maxima at 360 nm and 237 nm (Figure 5.1)

5.1.2.2 UV-spectrum of Telmisartan:

UV-spectrum of Telmisartan was obtained at 10 µg/ml concentration in methanol. Telmisartan was found to show absorption maxima at 296 nm (Figure 5.2).

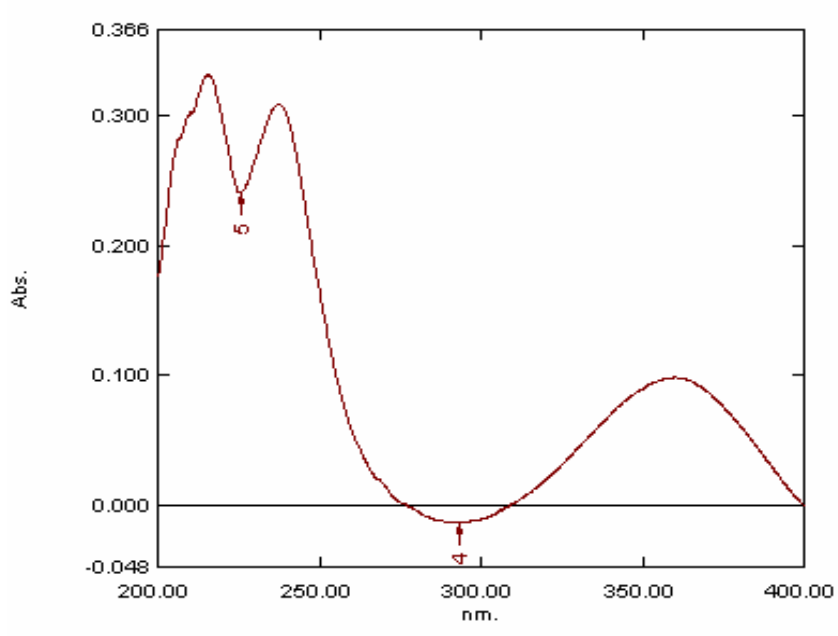


Figure 5.1: UV-Spectrum of Amlodipine Besylate (10 µg/ml) in methanol

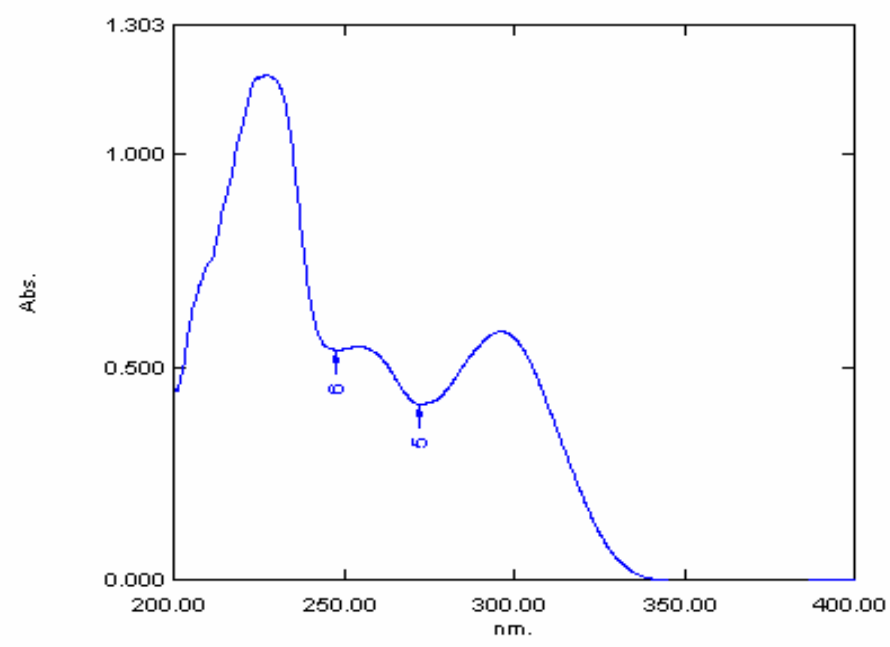


Figure 5.2: UV-Spectrum of Telmisartan (10 µg/ml) in methanol

Table 5.2 Comparison of Wavelength maxima of drugs

Drugs	Standard	Obtained
Amlodipine besylate	361 nm and 237 nm	360 nm and 238 nm
Telmisartan	296 nm	296 nm

5.1.3 Determination of Infrared (IR) Spectra:

5.1.3.1 Infrared spectrum of Amlodipine besylate:

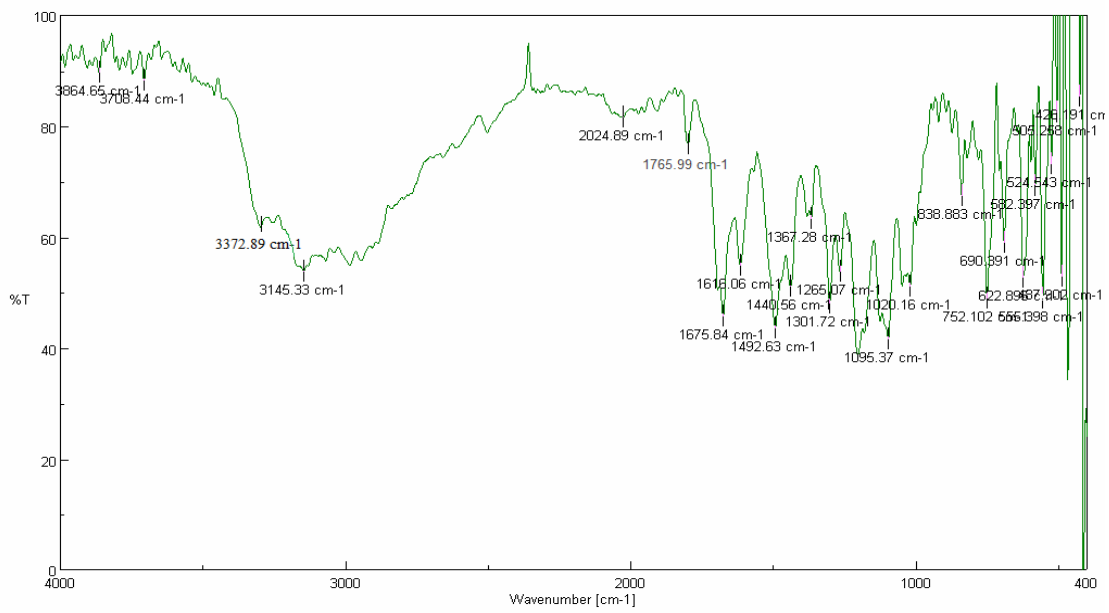


Figure 5.3: IR-spectrum of amlodipine besylate

Table 5.3 Specification of Infrared spectrum of Amlodipine besylate

Wavenumber (cm^{-1})	Specification
3372.89	$-NH_2$ stretching
3145.33	N-H (pyridine)
2950.55	C-H bending
1675.84	C=O Ester
1265.07	C-O Ester
1367.27	$S(=O)_2$

5.1.3.2 Infrared spectrum of Telmisartan:

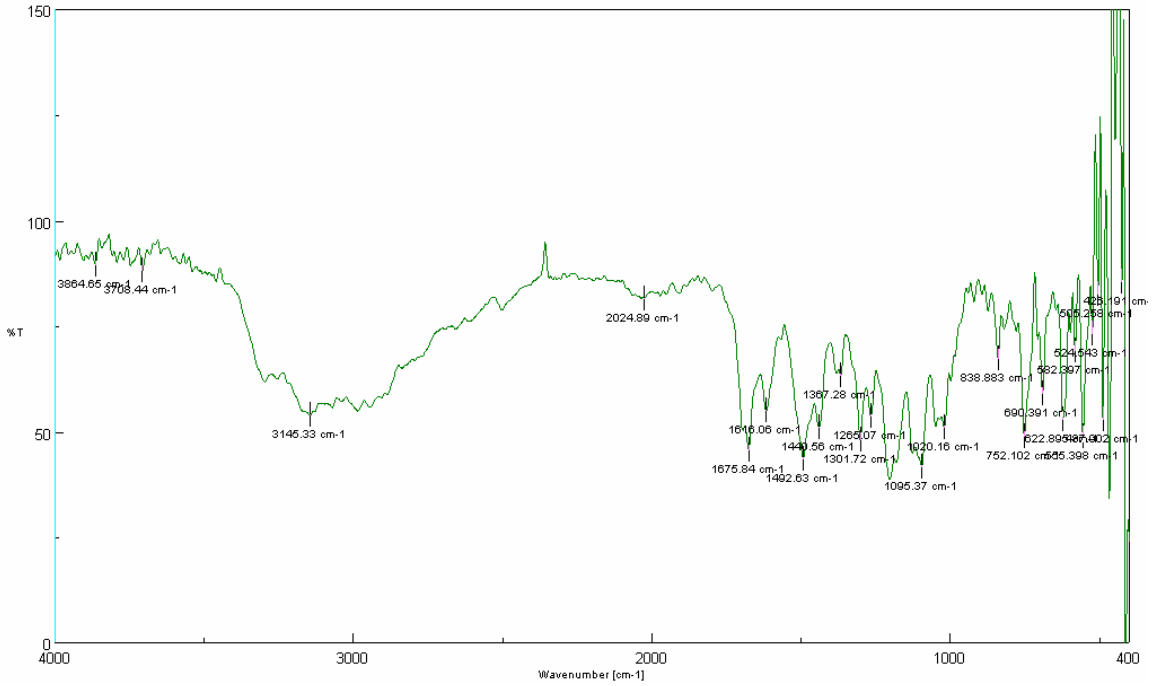


Figure 5.4: IR-spectrum of Telmisartan

Table 5.4 Specification of Infrared spectrum of Telmisartan

Wavenumber (cm^{-1})	Specification
2958.27	C-H stretching
1700.91	-C=O stretching
1457.92	C-H Bending
1270.86, 1128.15	C-N stretching

5.2 Development and Validation of Spectrophotometric method for simultaneous estimation of Amlodipine besylate and Telmisartan in tablet dosage form:

Abstract

A simple, accurate, economical and reproducible spectrophotometric method for simultaneous estimation of two-component drug mixture of amlodipine besylate and telmisartan in combined tablet dosage form has been developed. Developed method is based on direct estimation of amlodipine besylate at 360.0 nm, as at this wavelength telmisartan has zero absorbance and hence does not interfere. For estimation of telmisartan developed method is based on first derivative spectroscopy using 289.0 nm as zero crossing point for estimation of telmisartan. Linearity was observed in the concentration range of 5-35 µg/ml for AMLO and 10-70 µg/ml for TELMI. LOD was found to be 0.50 and 0.63 µg/ml for amlodipine besylate and telmisartan, respectively. The method was validated statistically and recovery study was performed to confirm the accuracy of the method.

5.2.1 INSTRUMENTATION:

5.2.1.1 UV-Visible Double-Beam spectrophotometer:

Matched quartz cell (1cm)

Model: UV-2450 Pc series

Manufacturer: Shimadzu Inc. Japan.

Wavelength range: 190 nm to 1100 nm

Baseline Flatness: ±0.001 abs

Resolution: 0.1 nm

5.2.1.2 Analytical Balance:

Model: KEROY

Manufacturer: Keroy (balance) Pvt. Ltd. Varanasi, India.

Maximum capacity: 100gm.

5.2.1.3 Sonicator:

Model: TRANS-O-SONIC; D-compect.

Capacity: 2 Lit.

5.2.2 MATERIALS & REAGENTS:

- Amlodipine Besylate (obtained as gift samples from Torrent Research Center, Bhat.)
- Telmisartan (obtained as gift samples from Torrent Research Center, Bhat.)
- Methanol (AR Grade) – S.D. Fine Chemicals Ltd., Bombay, INDIA
- Tablet dosage form: Telma-AM, Telsar-A, Telsartan-AM

5.2.3 METHOD:

5.2.3.1 Selection of solvent:

Selection of solvent depends on solubility of both drugs. Amlodipine and Telmisartan are soluble in methanol. So, it is used as solvent for preparation of standard and sample solutions.

5.2.3.2 Selection of wavelength:

From the overlain spectra of AMLO and TELMI in methanol solution [Figure -5.5], it was observed that TELMI has zero absorbance at 360.0 nm, where AMLO has substantial absorbance so AMLO was estimated directly at this wavelength. For estimation of TELMI first order derivative spectroscopy method was used. From first order derivative spectra of AMLO and TELMI in methanol [Figure -5.6] zero crossing point 289.0 nm was selected for estimation of TELMI (Scaling factor = 10).

5.2.3.3 Preparation of Standard Stock Solution:

- Amlodipine besylate standard stock solution (1000µg/ml): weighed accurately 100mg of pure amlodipine besylate powder and quantitatively transferred to 100 ml volumetric

flask. Added 70ml of methanol to solubilize the powder and make up the volume to 100ml with the same.

- Telmisartan standard stock solution (1000µg/ml): weighed accurately 20mg of pure Telmisartan powder and quantitatively transferred to 100 ml volumetric flask. Added 70ml of methanol to solubilize the powder and make up the volume to 100ml with the same.

5.2.3.4 Preparation of working Standard Solution:

1. Amlodipine besylate working standard solution (100µg/ml): Pipette out 10 ml of standard stock solution and transfer it to 100ml volumetric flask. Make up the volume with methanol up to the mark.

2. Telmisartan working standard solution (100µg/ml): Pipette out 10 ml of standard stock solution and transfer it to 100ml volumetric flask. Make up the volume with methanol up to the mark.

5.2.3.5 Preparation of Binary Mixture of Amlodipine Besylate and Telmisartan for Linearity and Range:

Appropriate volumes of aliquots from working standard solution of amlodipine besylate and telmisartan were transferred to 10 ml volumetric flask. Volumes were adjusted to mark with methanol to obtain different concentration of amlodipine besylate and telmisartan in the mixtures.

5.2.3.6 Method Validation:

1. Preparation of Linearity Range

Different standard solutions of drugs were prepared in the range of 5-35 µg/ml for AMLO and 10-70 µg/ml for TELMI as per section 5.2.3.5. The standard solutions were scanned in UV-visible spectrophotometer at 200-400 nm and the absorbance was recorded for both drugs. Linearity curve of concentration v/s absorbance was plotted for both the drugs.

2. Precision

- **Intraday and Interday precision**

Intraday and interday precision were measured in terms of %RSD. For intraday precision, the experiment was repeated three times in a day using three different concentrations (10, 15, 20 µg/ml for amlodipine besylate and 20, 30, 40 µg/ml for telmisartan). For interday precision, the experiment was repeated on three different days using three different concentrations.

- **Reproducibility**

To study the reproducibility, six determinations at 100% test concentrations (i.e. 20 µg/ml of amlodipine and 40 µg/ml of telmisartan) were carried out. Reproducibility of the method was measured in terms of %RSD.

3. LOD and LOQ

From the linearity curve equation, the standard deviation (SD) of the intercepts (response) was calculated. Then LOD and LOQ were measured by using mathematical expressions given in section 4.3.7.

4. Accuracy

It was determined by calculating the recovery of Amlodipine and Telmisartan by Standard addition method. To a fixed amount of Amlodipine (10 µg/ml) and Telmisartan (20 µg/ml), increasing amount of Amlodipine and Telmisartan was added at three levels(80%, 100% and 120%) of calibration curve and the amount of both drugs were calculated at each level.

5.2.3.7 Sample Preparation for Assay:

For the assay, 10 tablets were weighed and powdered. Weighed tablet powder equivalent to 40 mg of telmisartan and 5mg of Amlodipine besylate was transferred quantitatively to 50 ml of volumetric flask. Add 30ml of methanol and sonicated for 15 minutes. Volume was made up to the mark with methanol. Aliquots of 0.8 ml were transferred to 10 ml volumetric flasks and volume was made up to the mark with methanol. The solution was filtered and amplitudes of the solution were measured at particular wavelengths for both the drugs.

5.2.4 RESULTS AND DISCUSSION:

The mixture solution was prepared in methanol and scanned in range of 200-400 nm and spectrum was recorded. Then spectrum was converted in to 1st order derivative spectrum.

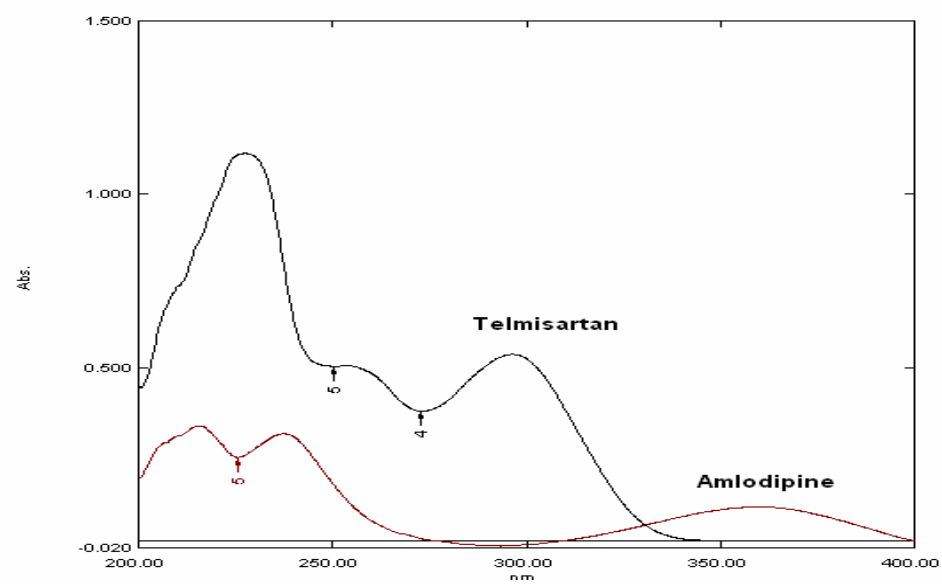


Figure 5.5: Overlain spectra of Amlodipine besylate and Telmisartan

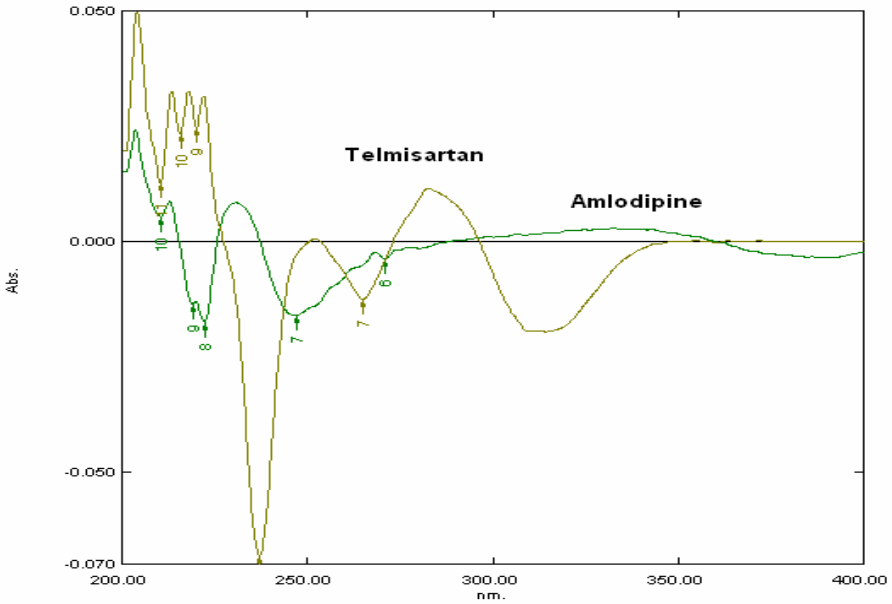


Figure 5.6: Overlain (first order derivative) spectra of Amlodipine besylate and Telmisartan

Validation Parameters

5.2.4.1 Linearity

Linearity range of Amlodipine and Telmisartan were found to be 5-35 µg/ml and 10-70 µg/ml with correlation co-efficient 0.999 and 0.998 respectively.

Table 5.5 Calibration data of Amlodipine

Sr. No.	Concentration (µg/ml)	Absorbance
		Mean(n=3) ± SD
1	5	0.047 ± 0.004
2	10	0.100 ± 0.005
3	15	0.155 ± 0.002
4	20	0.200 ± 0.002
5	25	0.260 ± 0.004
6	30	0.303 ± 0.003
7	35	0.359 ± 0.003

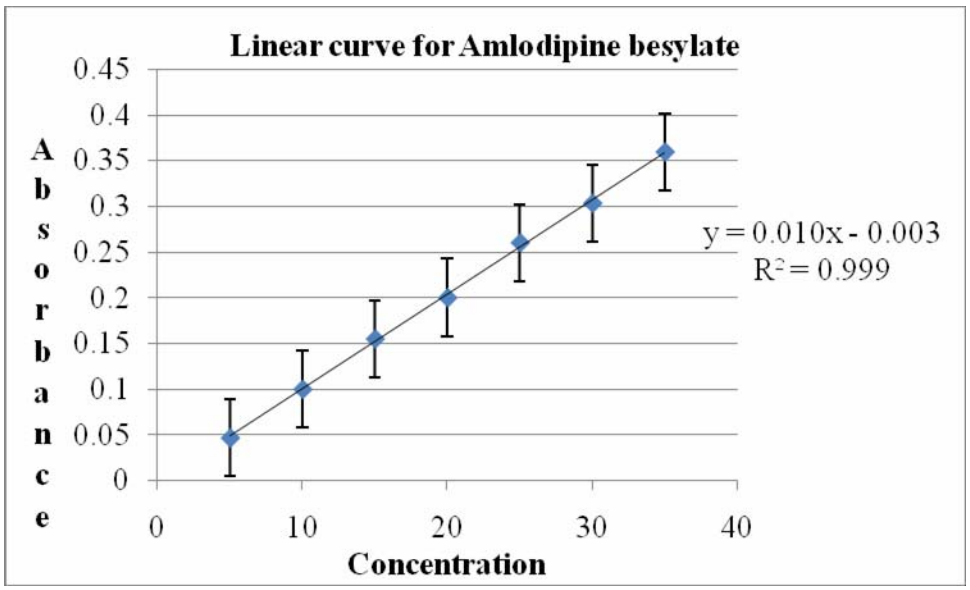


Figure 5.7: Linearity curve for Amlodipine besylate

Table 5.6 Calibration data of Telmisartan

Sr. No.	Concentration (µg/ml)	Absorbance
		Mean ± SD (n=3)
1	10	0.085± 0.005
2	20	0.172 ± 0.004
3	30	0.251 ± 0.002
4	40	0.342 ± 0.003
5	50	0.424 ± 0.002
6	60	0.501 ± 0.003
7	70	0.566 ± 0.005

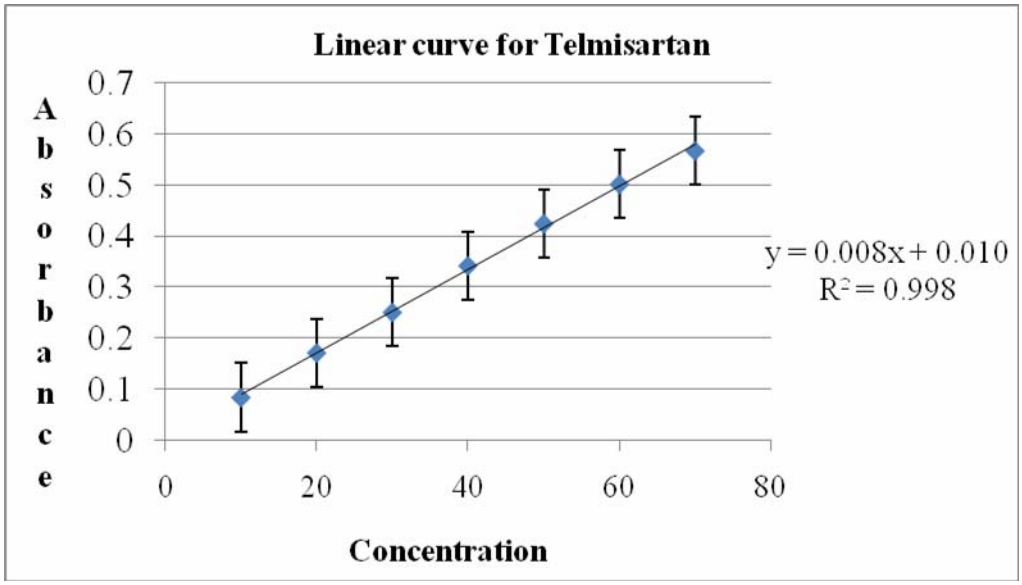


Figure 5.8: Linearity curve for telmisartan

5.2.4.2 Precision

Precision 5.2.4.2.1 Repeatability

Table 5.7 Repeatability data of Amlodipine by UV-Visible Spectophotometry

Time	Absorbance
1st	0.203
2nd	0.200
3rd	0.206
4th	0.201
5th	0.209
6th	0.204
Mean ± SD	0.203 ± 0.0033
%RSD	1.62

Table 5.8 Repeatability data of Telmisartan by UV-Visible Spectophotometry

Time	Absorbance
1st	0.342
2nd	0.347
3rd	0.339
4th	0.350
5th	0.341
6th	0.347
Mean ± SD	0.344 ± 0.0042
%RSD	1.24

5.2.4.2.2 Intraday and Interday Precision:

Intraday precision for both the drugs was done by analyzing three different concentrations (µg/ml) within linearity range, three times in a day (3*3 determinations).

Interday precision for both the drugs was done by analyzing three different concentrations (µg/ml) within linearity range, on three consecutive days.

Table 5.9 Intraday precision data of Amlodipine besylate by UV-Visible Spectophotometry

Sr. No.	Concentration (µg/ml)	Absorbance	
		Mean ± SD (n=3)	%RSD
1	10	0.101 ± 0.004	1.98
2	15	0.154 ± 0.003	1.62
3	20	0.203 ± 0.005	0.75

Table 5.10Interday precision data of Amlodipine besylate by UV-Visible Spectophotometry

Sr. No.	Concentration (µg/ml)	Absorbance	
		Mean ± SD (n=3)	%RSD
1	10	0.100 ± 0.003	1.52
2	15	0.151 ± 0.006	1.37
3	20	0.206 ± 0.004	0.74

Table 5.11 Intraday precision data of Telmisartan by UV-Visible Spectophotometry

Sr. No.	Concentration (µg/ml)	Absorbance	
		Mean ± SD (n=3)	%RSD
1	20	0.171 ± 0.004	0.88
2	30	0.254 ± 0.003	0,78
3	40	0.341 ± 0.007	1.21

Table 5.12 Interday precision data of Telmisartan by UV-Visible Spectophotometry

Sr. No.	Concentration (µg/ml)	Absorbance	
		Mean ± SD (n=3)	%RSD
1	20	0.176 ± 0.005	1.13
2	30	0.250 ± 0.003	1.21
3	40	0.348 ± 0.004	1.14

5.2.4.2.3 Accuracy

Accuracy of the measurement of Amlodipine besylate and Telmisartan was determined by standard addition method. Standard addition was done at three levels, 80%, 100% and 120% of a concentration in the linearity range.

Table 5.13 Accuracy data of Amlodipine besylate by UV-Visible Spectophotometry

Initial conc. (µg/ml) (A)	Quantity of std. Added (µg/ml) (B)	Total Amount (A + B)	Accuracy	
			Absorbance Mean ± S.D (n=3)	%Recovery Mean ± S.D (n=3)
10	8	18	0.183 ± 0.004	99.92 ± 0.82
10	10	20	0.203 ± 0.006	99.48 ± 1.01
10	12	22	0.225 ± 0.002	99.41± 0.67

Table 5.14 Accuracy data of Telmisartan by UV-Visible Spectophotometry

Initial conc. (µg/ml) (A)	Quantity of std. Added (µg/ml) (B)	Total Amount (A + B)	Accuracy	
			Absorbance Mean ± S.D (n=3)	%Recovery Mean ± S.D (n=3)
20	16	36	0.297 ± 0.003	99.65 ± 1.25
20	20	40	0.335 ± 0.006	101.66 ± 1.40
20	24	44	0.360 ± 0.004	99.43 ± 1.23

5.2.4.2.4 Limit of detection

The minimum detectable concentration of Amlodipine besylate was found to be **0.50 µg/ml**

The minimum detectable concentration of Telmisartan was found to be **0.63 µg/ml**

5.2.4.2.5 Limit of quantification

The lowest quantifiable concentration of Amlodipine besylate was found to be **1.52 µg/ml**

The lowest quantifiable concentration of Telmisartan was found to be **1.90 µg/ml**

5.2.4.2.6 Summary of Validation parameters:

Table 5.15 Summary of Validation parameters with UV detection

Sr. No	Parameters	Amlodipine besylate	Telmisartan
1	Linearity range (µg/ml)	5-35	10-70
2	Regression equation	y = 0.010x -0.003	y = 0.008x +0.001
3	Correlation coefficient (r^2)	0.999	0.998
4	Precision		
	Intraday % RSD (n = 3)	1.45	0.95
	Interday % RSD (n = 3)	1.21	1.16
	Repeatability of measurements		
	% RSD	1.62	1.24
5	Limit of detection (µg/ml)	0.50	0.63
6	Limit of quantification (µg/ml)	1.52	1.90

5.2.4.2.7 Estimation of Amlodipine besylate and Telmisartan in marketed Tablet:

The developed method was used to estimate Amlodipine besylate and Telmisartan in the tablet dosage form. Two different brands of tablet formulations were procured from the market for analysis by the proposed method. The percentage of Amlodipine and Telmisartan was found from the calibration curve of the standard drug respectively.

Table 5.16 Estimation of Amlodipine besylate and Telmisartan in tablet by UV detection

Drug	Brand Names	Labeled Claim (mg/tablet)	Amount found(mg/tablet) (n=3)	Assay result % Recovery ± S.D (n=3)
Amlodipine besylate	Telma-AM	5	5.04 ± 0.07	100.8 ± 0.44
	Telsar-A	5	4.87 ± 0.06	97.40 ± 1.20
	Telsartan-AM	5	4.95 ± 0.06	99.0 ± 1.31
Telmisartan	Telma-AM	40	39.33 ± 0.46	98.32 ± 1.17
	Telsar-A	40	39.69 ± 0.22	99.22 ± 0.55
	Telsartan-AM	40	39.79 ± 0.37	99.47 ±0.92

5.3 Development and Validation of HPTLC method for simultaneous estimation of Amlodipine besylate and Telmisartan in tablet dosage forms.

5.3.1 Abstract

A High Performance Thin Layer Chromatography method was developed for simultaneous estimation of Amlodipine besylate and Telmisartan in their pharmaceutical dosage forms. The separation was achieved by TLC silica gel 60 F_{254} plates and chloroform: methanol: toluene (5:3.5:1.5, v/v/v) as eluent. Detection was carried out at 238 nm. The retention factor of Amlodipine besylate and Telmisartan were found to be 0.23 and 0.75 respectively. The method has been validated for linearity, accuracy, precision. Linearity for Amlodipine besylate and Telmisartan were found in the range of 300-900 ng/spot and 600-1800 ng/spot respectively. The developed method was found to be precise, selective and rapid for estimation of Amlodipine besylate and Telmisartan in their pharmaceutical dosage forms.

5.3.2 Materials and Methods

5.3.2.1 Apparatus and Instruments:

- Pre-coated silica gel aluminum Plate 60F–254 (20 × 20 cm with 250 µm thickness) (E. Merck)
- Camag – 100 µl Applicator syringe (Hamilton, Bonaduz, Schweiz)
- Camag Applicator-Linomat V
- Camag – Twin trough chamber (10 × 10) with stainless steel Lid
- Camag TLC scanner3
- UV cabinet with dual wavelength UV lamp (254 nm and 366 nm)
- Balance Model: KEROY®, Keroy (Balance) Pvt. Ltd.
- Ultra Sonicator, Trans-o-sonic, India
- Amber colored volumetric flask – 100 ml, 50 ml, and 10 ml.

5.3.2.2 Reagents and Materials:

- Amlodipine besylate standard & Telmisartan standard (gifted by Torrent Research Center, Bhat.)
- Methanol, Baroda chemicals Pvt. Ltd., Baroda
- Chloroform, AR grade, S.D. fine chemicals, Mumbai
- Toluene, AR grade, S.D. fine chemicals, Mumbai
- Tablets containing Amlodipine besylate and telmisartan.

5.3.2.3 Chromatographic conditions:

- **Stationary phase:** Pre-coated silica gel aluminum Plate 60F–254 pre-washed with Methanol then dried for 20 minute at 60°C.
- **Mobile phase:** Chloroform: Methanol : Toluene (5 : 3.5 : 1.5, v/v/v)
- **Chamber saturation:** 30 min
- **Band width:** 4 mm
- **Distance run:** 80 mm
- **Run time:** 20 minutes
- **Scanning Wave length:** 238 nm
- **Slit dimension:** 4 x 0.45 mm
- **Evaluation mode:** Absorbance

5.3.2.4 Preparation of Mobile Phase

A mixture of 5 ml of chloroform, 3.5 ml of methanol and 1.5 ml of toluene was mixed properly and it was used as a mobile phase.

5.3.2.5 Preparation of standard stock solution of Amlodipine besylate:

Standard amlodipine besylate (25 mg) was accurately weighed and transferred to 25 ml volumetric flask. It was dissolved properly and diluted up to mark with methanol to obtain final concentration of 1000 µg/ml. Suitable aliquot of this solution was transferred in a 50 ml volumetric flask and diluted up to mark with methanol to obtain final concentration of 50 µg/ml. This solution was used as working standard solution.

5.3.2.6 Preparation of standard stock solution of Telmisartan:

Standard telmisartan (25 mg) was accurately weighed and transferred to 25 ml volumetric flask. It was dissolved properly and diluted up to mark with methanol to obtain final concentration of 1000 µg/ml. Suitable aliquot of this solution was transferred in a 50 ml volumetric flask and diluted up to mark with methanol to obtain final concentration of 100 µg/ml. This solution was used as working standard solution.

5.3.2.7 HPTLC Analysis

1) Activation of Silica gel plate

Analysis was performed on 10 cm × 10 cm TLC silica gel 60 F_{254} plates. Layers were cleaned by predevelopment to the top with methanol and dried in a hot air oven at 60ºC for 20 minutes.

2) Spotting

Standard zones were applied to the layer as bands by means of a Camag (Wilmington, NC, USA) Linomat V semi-automated spray-on applicator equipped with a 100-µl syringe and operated with the settings band length 4 mm, application rate 15 nl/s, distance between bands 4 mm, distance from the plate side edge 100 mm, and distance from the bottom of the plate 80 mm.

3) Development

Spotted plates were developed in saturated HPTLC twin-through chamber up to 80 mm solvent front. Developing time was 20 minutes. HPTLC twin-trough chamber is presaturated with mobile phase for 30 minutes with help of filter papers. After development, plates were removed immediately and dried in oven at 60ºC for 5 minutes.

4) Scanning of Plates

The developed plates were scanned with the help Camag TLC Scanner III with a deuterium and tungsten source, slit dimension was 4 × 0.45 mm, and a scanning rate of 10 mm s^{-1} using WINCATS-3 software.

5.3.2.8 Method validation

1) Preparation of Linearity Curve

From the standard stock solution of both drugs aliquots of 6,8,10,12,14,16 and 18µl were spotted on the TLC plate under nitrogen stream using Linomat V to obtain final concentration range of 300-900 ng/spot for amlodipine besylate and 600-1800 ng/spot for telmisartan. Plates were developed and peak area of the spots was measured at 238 nm in the absorbance mode with Camag TLC scanner III. Calibration curve of peak area v/s concentration was plotted for the drugs.

2) Precision

- **Intraday and interday precision**

  Intraday and interday precision were measured in terms of %RSD. The experiment was repeated three times in a day using three different concentration (500, 600, 700 ng/spot for amlodipine besylate and 1000, 1200, 1400 ng/ml for telmisartan) for intraday precision and three different concentrations on three different days for interday precision.

- **Reproducibility**

  To study the reproducibility, six determinations at 100% test concentrations (i.e. 500 ng/spot of amlodipine besylate and 1000 ng/spot of telmisartan) were carried out. Reproducibility of the method was measured in terms of %RSD.

3) LOD and LOQ

From the linearity curve equation, the standard deviation (SD) of the intercepts (response) was calculated. Then LOD and LOQ were measured by using mathematical expressions given in section 4.3.7.

4) Accuracy

It was determined by calculating the recovery of Amlodipine and Telmisartan by Standard addition method. To a fixed amount of Amlodipine (300 ng/spot) and Telmisartan (600 ng/spot), increasing amount of Amlodipine and Telmisartan was added at three levels(80%, 100% and 120%) of calibration curve and the amount of both drugs were calculated at each level.

5) Specificity

- Specificity is a measure of the degree of interference in the analysis of complex sample mixture.

5.2.3.9 Quantification of Amlodipine besylate and Telmisartan in Tablet:

- **Preparation of test stock solution:**

Total 20 tablets were weighed accurately and powdered. An amount equivalent to one tablet (containing 5 mg of Amlodipine besylate and 40 mg of Telmisartan) was taken and dissolved in 15 ml methanol in 50 ml volumetric flask. The solution was sonicated for 15 minutes and then diluted with methanol to the mark. The solution was filtered by using whatmann filter No.41. Now, 5 ml of the above solution was taken diluted up to 10 ml to get the final concentration 50 µg/ml Amlodipine besylate and 400 µg/ml of Telmisartan.

- **Analysis of prepared samples:**

10µl of this solution was spotted over the plate and analyzed for the content of Amlodipine besylate and 3 µl of this solution was spotted over the plate and analyzed for the content Telmisartan.

5.3.3 RESULTS AND DISCUSSION:

5.3.3.1 Selection and optimization of solvent and mobile phase:

- Selection and optimization of a proper mobile phase is a challenging task in HPTLC method development. Several factors affects the selection of mobile phase such as polarity of the drugs, desired R_f values, practical problems such as diffusion of spots, tailing, proper peak shape after scanning.

  Table 5.17 Observation and remarks of Mobile phase optimization

Sr. No.	Trials	Observation	Remarks
1	Chloroform : methanol (8 : 2, v/v) Run length = 60mm	High R_f value, run along with solvent front	Not satisfactory
2	Chloroform : methanol (7 : 3, v/v) Run length = 70mm	Very high R_f values, diffused spots	Not satisfactory
3	Chloroform : methanol : toluene (5 : 4 : 1, v/v) Run length = 70mm	Diffused spots	Not satisfactory
4	Chloroform : methanol : toluene (5 : 3.5 : 1.5, v/v/v) Run length = 80mm	Good resolution, compact spots.	Satisfactory

- The solvent methanol was used for preparation of solution of both drugs because both drugs are freely soluble in methanol, so it was selected as a solvent for preparation amlodipine besylate and telmisartan solutions for the experimental work.
- The mobile phase Chloroform: methanol: toluene (5: 3.5:1.5) was found to be appropriate, as both the drugs were separated with good R_f values. Moreover, densitometric evaluation was done which bypassed the use of spraying reagents which made the analysis simple and rapid.

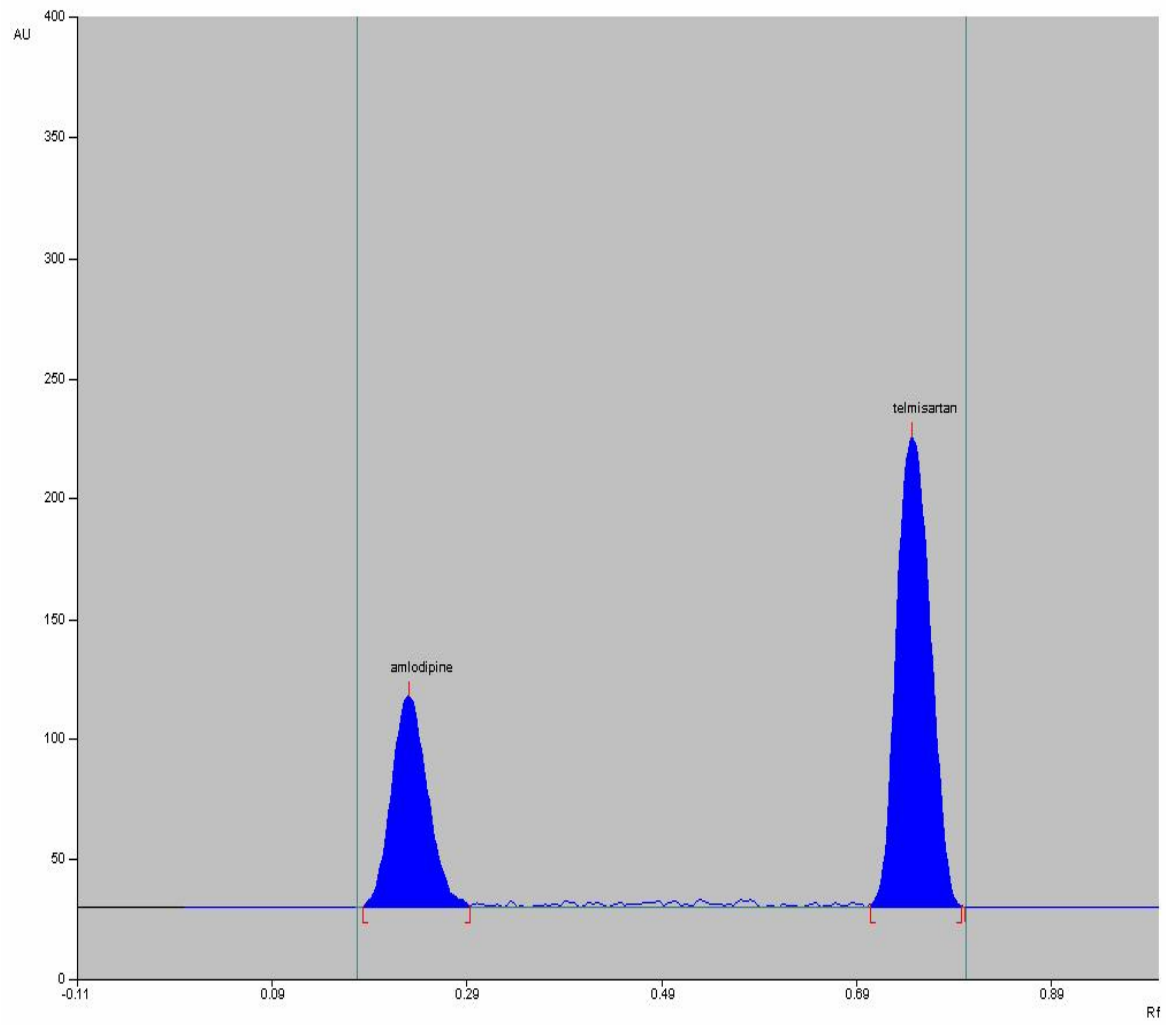


Figure 5.9: HPTLC chromatogram of amlodipine besylate (R_f = 0.23) and telmisartan (R_f = 0.75) standard mixture.

5.3.3.2 Validation Parameters

1. Linearity

Linearity range were found to be 300-900 ng/spot for Amlodipine besylate and 600-1800 ng/spot for Telmisartan with correlation co-efficient 0.993 and 0.984 respectively.

Table 5.18 Calibration data of Amlodipine besylate by HPTLC with UV detection

Sr. No.	Concentration (ng/spot)	Peak area
		Mean ± SD (n=3)
1	300	1687.8 ± 59.87
2	400	2034.17 ± 118.07
3	500	2527.07 ±53.85
4	600	2837.45 ±110.69
5	700	3096.61 ± 71.84
6	800	3507.98 ± 89.02
7	900	4024.38 ± 85.75

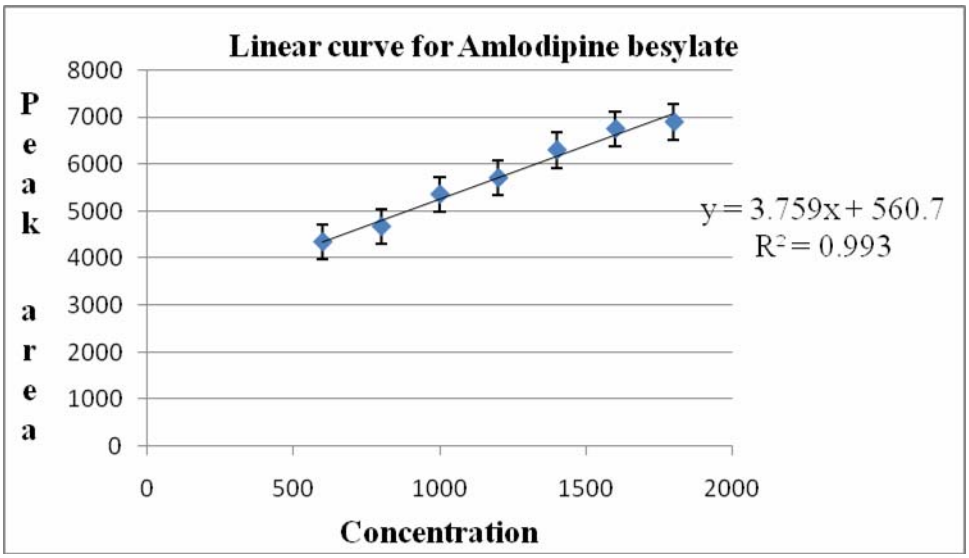


Figure 5.10 Linearity curve for Amlodipine besylate

Table 5.19 Calibration data of Telmisartan by HPTLC with UV detection

Sr. No.	Concentration (ng/spot)	Peak area
		Mean ± SD (n=3)
1	600	4344.30 ± 70.54
2	800	4670.02 ± 42.26
3	1000	5356.80 ± 60.67
4	1200	5708.17 ±32.97
5	1400	6302.14 ± 55.36
6	1600	6752.25 ± 59.34
7	1800	6898.06 ± 39.96

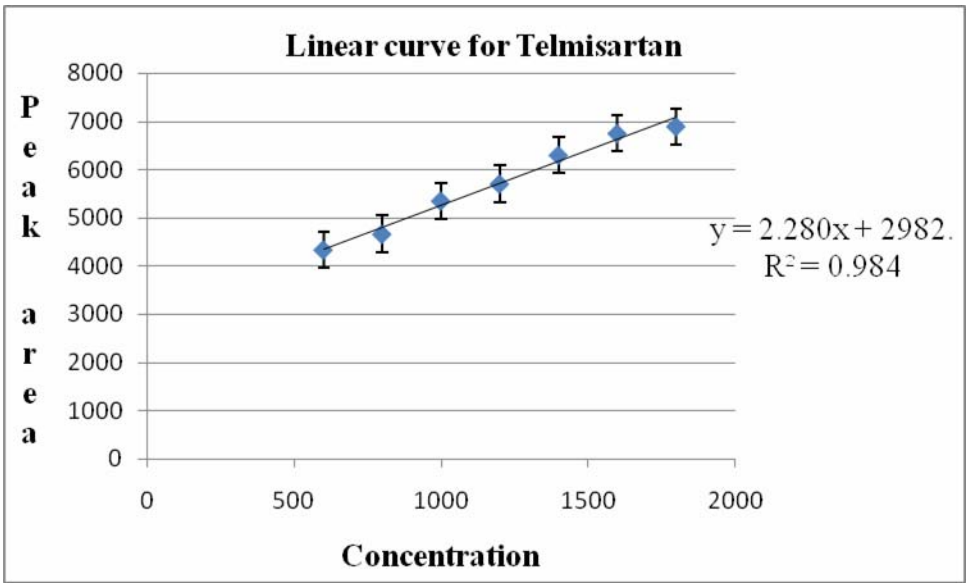


Figure 5.11 Linearity curve for telmisartan

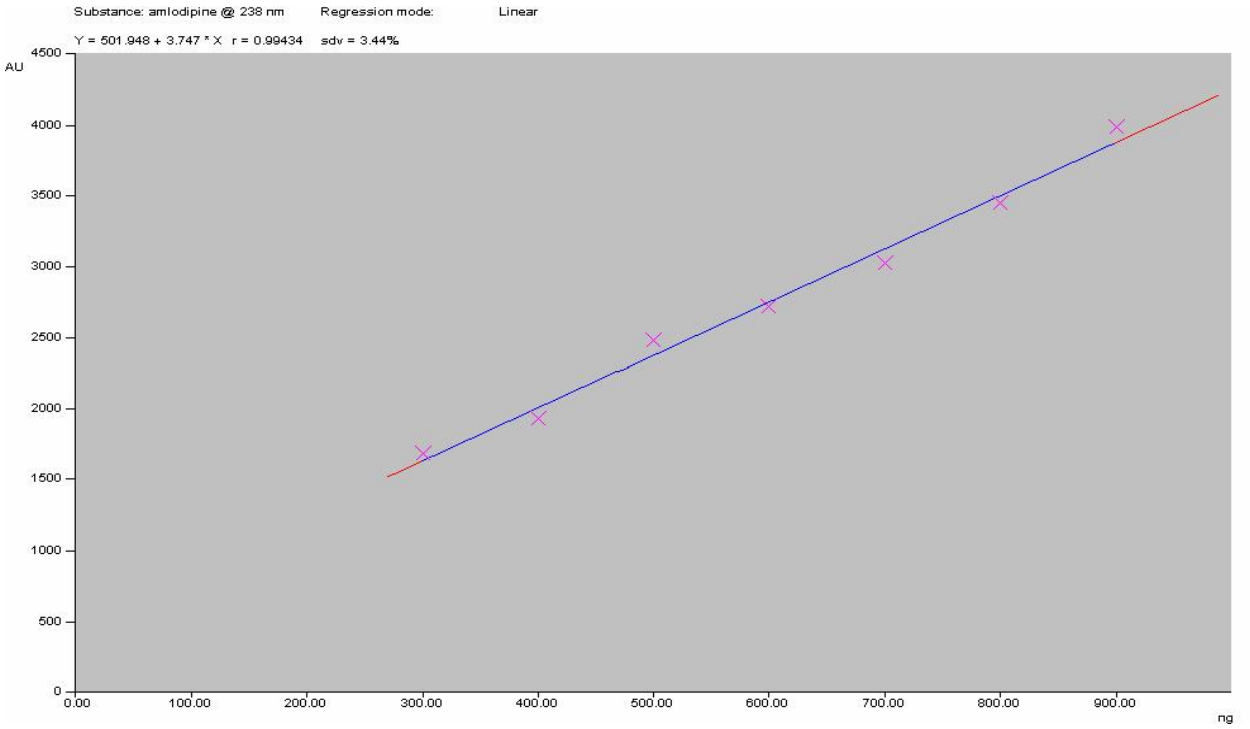


Figure 5.12 Linearity curve for Amlodipine besylate from winCATS software

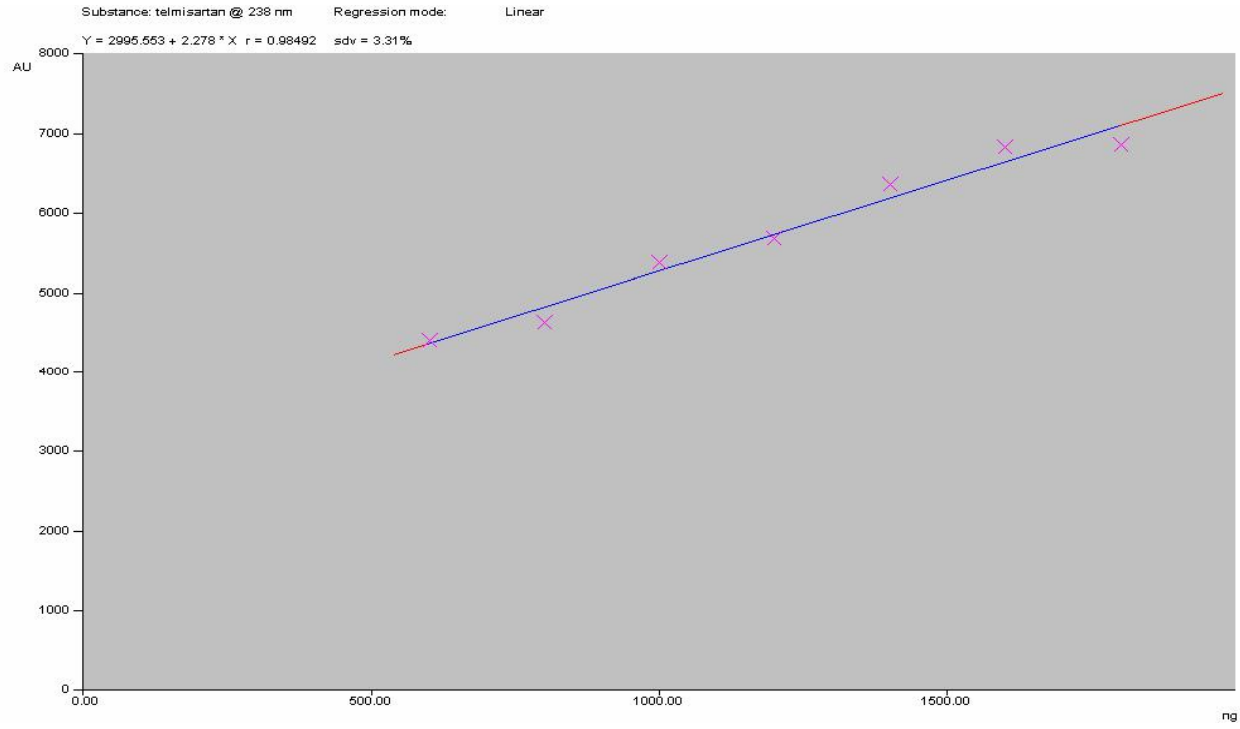


Figure 5.13 Linearity curve for telmisartan from winCATS software

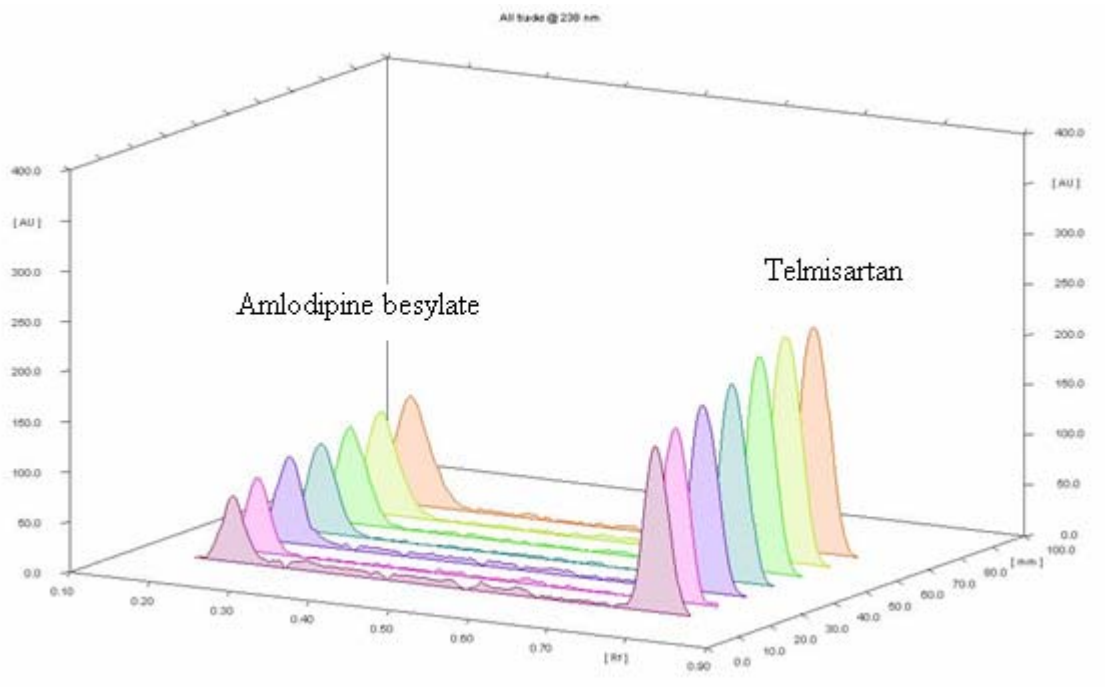


Figure 5.14 HPTLC chromatogram (3D view) for linearity of amlodipine and Telmisartan

2. Precision

2.1 Repeatability

Table 5.20 Repeatability data of Amlodipine besylate by HPTLC

Time	Peak Area
1st	2484.02
2nd	2509.75
3rd	2547.98
4th	2478.93
5th	2563.41
6th	2487.32
Mean ± SD	2511.90 ± 35.85
%RSD	1.42

Table 5.21 Repeatability data of Telmisartan by HPTLC

Time	Peak Area
1st	5384.8
2nd	5398.43
3rd	5287.19
4th	5432.25
5th	5267.94
6th	5359.29
Mean ± SD	5354.98± 64.71
%RSD	1.20

2.2 Intraday and Interday Precision:

Table 5.22 Intraday precision data of Amlodipine besylate by HPTLC with UV detection

Sr. No.	Concentration (ng/spot)	Peak Area	
		Mean ± SD (n=3)	%RSD
1	500	2487.30 ± 21.23	0.85
2	600	2776.34 ± 40.56	1.46
3	700	3174.08 ± 36.26	1.11

Table 5.23 Interday precision data of Amlodipine besylate by HPTLC with UV detection

Sr. No.	Concentration (ng/spot)	Peak Area	
		Mean ± SD (n=3)	%RSD
1	500	2461.37 ± 25.14	1.02
2	600	2715.72 ± 32.36	1.19
3	700	3092.87 ± 44.15	1.42

Table 5.24 Intraday precision data of Telmisartan by HPTLC with UV detection

Sr. No.	Concentration (ng/spot)	Peak Area	
		Mean ± SD (n=3)	%RSD
1	1000	5292.21 ± 38.15	0.72
2	1200	5732.72 ± 52.54	0.91
3	1400	6179.18 ± 67.38	1.09

Table 5.25 Interday precision data of Telmisartan by HPTLC with UV detection

Sr. No.	Concentration (ng/spot)	Peak Area	
		Mean ± SD (n=3)	%RSD
1	1000	5371.95 ± 34.72	0.64
2	1200	5701.81 ± 33.85	0.58
3	1400	6302.14 ± 55.36	0.87

3. Accuracy

Accuracy of the measurement of Amlodipine besylate and Telmisartan was determined by standard addition method. Standard addition was done at three levels, 80%, 100% and 120% of a concentration in the linearity range.

Table 5.26 Accuracy data of Amlodipine besylate by HPTLC with UV detection

Initial conc. (ng/spot) (A)	Quantity of std. Added (ng/spot) (B)	Total Amount (ng/spot) (A + B)	Accuracy	
			Peak area Mean ± S.D (n=3)	%Recovery Mean ± S.D (n=3)
300	240	540	2574.64 ± 26.5	99.21 ± 1.30
300	300	600	2811.02 ±22.88	99.77 ± 1.01
300	360	660	3004.32 ±24.24	98.49± 0.97

Table 5.27 Accuracy data of Telmisartan by HPTLC with UV detection

Initial conc. (ng/spot) (A)	Quantity of std. Added (ng/spot) (B)	Total Amount (ng/spot) (A + B)	Accuracy	
			Peak area Mean ± S.D (n=3)	%Recovery Mean ± S.D (n=3)
600	480	1080	5470.89±43.99	101.07 ± 1.78
600	600	1200	4100.35±21.61	99.75 ± 0.78
600	720	1320	5986.44±28.69	99.82± 0.95

4. Limit of detection (LOD) & Limit of quantification (LOQ)

The LOD and LOQ of Amlodipine besylate was found to be **30.79 ng/spot** and **93.32 ng/spot** respectively.

The LOD and LOQ of Telmisartan was found to be **23.39 ng/spot** and **70.58 ng/spot** respectively.

5. Specificity

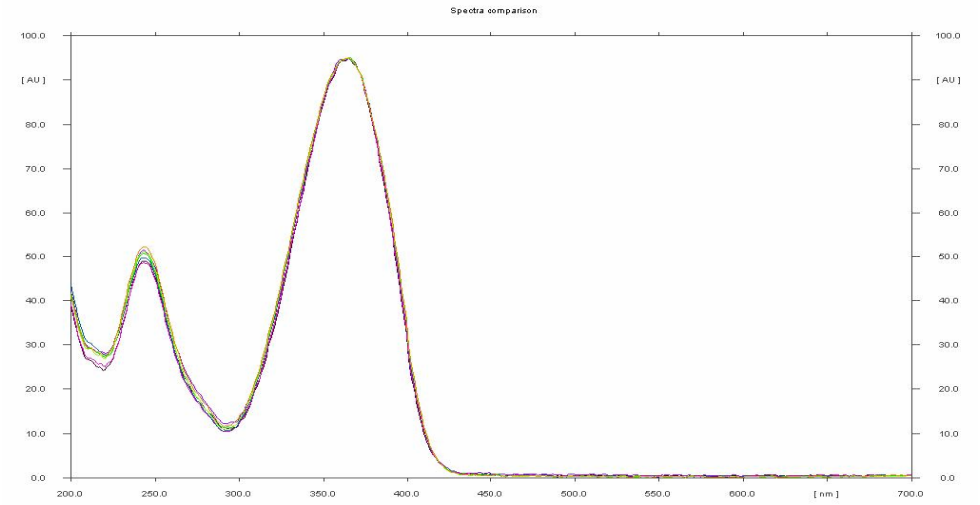


Figure 5.15 Purity spectrum for Amlodipine besylate from winCATS software

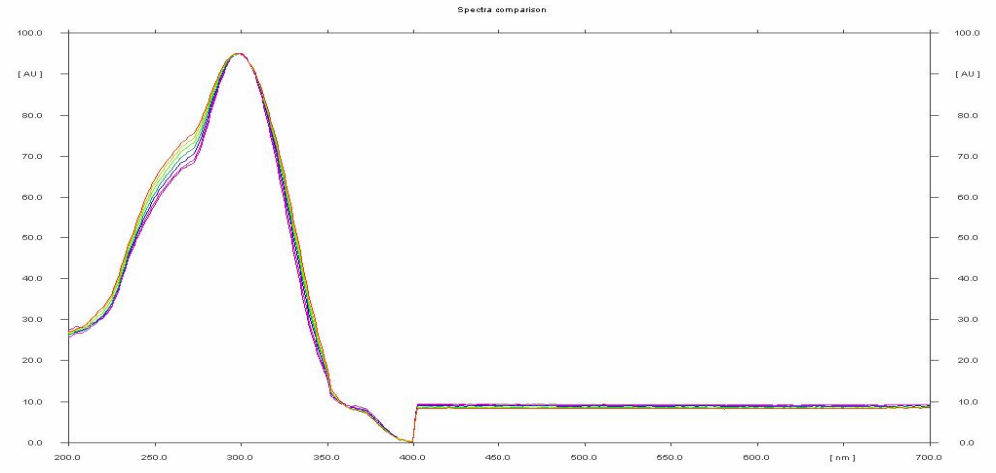


Figure 5.16 Purity spectrums for Telmisartan from winCATS software

6. Summary of Validation parameters:

Table 5.28 Summary of Validation parameters by HPTLC with UV detection

Sr. No	Parameters	Amlodipine besylate	Telmisartan
1	Linearity range (ng/spot)	300-900	600-1800
2	Regression equation	y = 3.759x + 560.7	y = 2.280x + 2982
3	Correlation coefficient (r^2)	0.993	0.984
4	Precision Intraday % RSD (n = 3) Interday % RSD (n = 3) Repeatability of measuremen % RSD	 1.15 1.21 1.54	 0.9 0.7 1.21
5	Limit of detection (ng/spot)	30.79	23.39
6	Limit of quantification (ng/spot)	93.32	70.58
7	Specificity	Specific	Specific

5.3.3.3 Estimation of Amlodipine besylate and Telmisartan in marketed Tablet:

The developed method was used to estimate Amlodipine besylate and Telmisartan in the tablet dosage form. Three different brands of tablet formulations were procured from the market for analysis by the proposed method. The percentage of Amlodipine besylate and Telmisartan was found from the calibration curve of the standard drug respectively.

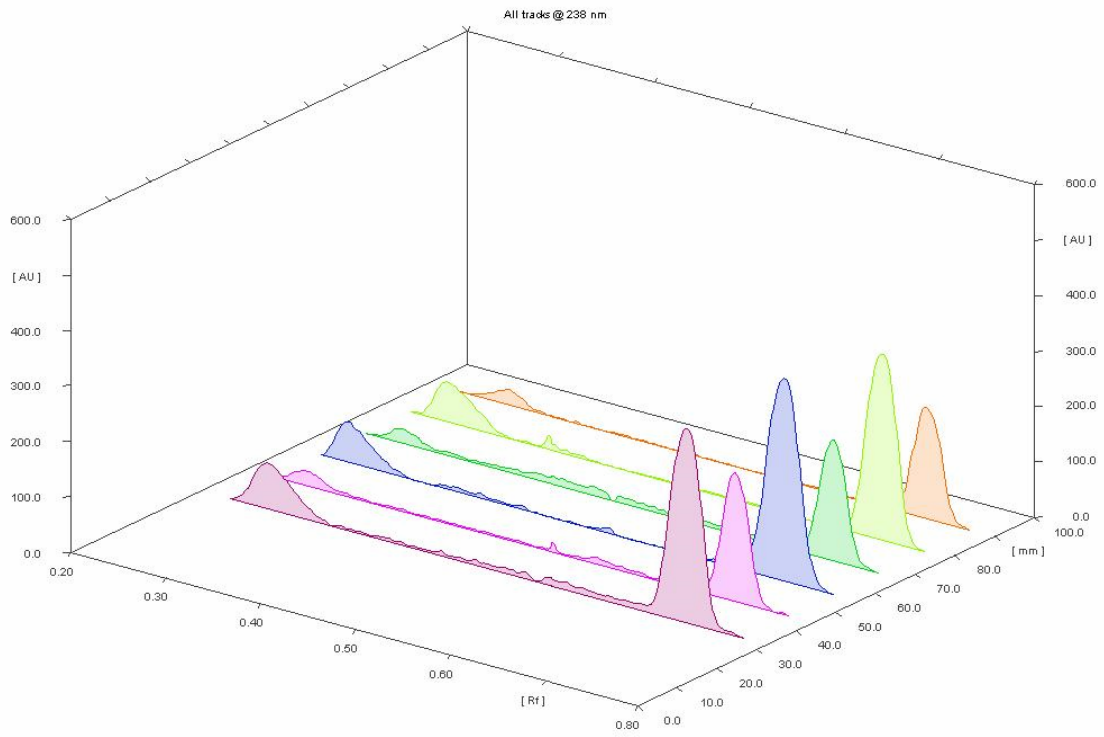


Figure 5.17 HPTLC chromatogram of assay of tablet samples (500 ng/spot for amlodipine besylate and 1000 for telmisartan)

Track 1: Telsartan-AM= Amlodipine

Track 2: Telsartan-AM= Telmisartan

Track 3: Telma-AM= Amlodipine

Track 4: Telma-AM= Telmisartan

Track 5: Telsar-A= Amlodipine

Track 6: Telsar-A= Telmisartan

Table 5.16 Estimation of Amlodipine besylate and Telmisartan in tablet by HPTLC

Drug	Brand names	Labeled Claim (mg/tablet)	Amount found(mg/tablet) (n=3)	Assay result % Recovery ± S.D (n=3)
Amlodipine besylate	Telma-AM	5	4.94 ± 0.08	98.82 ± 1.63
	Telsar-A	5	4.91 ± 0.06	98.20 ± 1.24
	Telsartan-AM	5	5.01 ± 0.11	100.21 ± 2.30
Telmisartan	Telma-AM	40	39.50 ± 0.29	98.75 ± 0.73
	Telsar-A	40	39.47 ± 0.55	98.67 ± 1.39
	Telsartan-AM	40	39.63 ± 0.61	99.07 ±1.52

5.4 Comparison of UV-Spectophotometry and HPTLC methods for estimation of Amlodipine and Telmisartan in Pharmaceutical dosage form

- Comparison of developed methods was performed by applying Student-t-test (paired two samples for means) for all 3 dosage forms.

Table 5.29 Comparison of U.V and HPTLC methods for assay results.

Brand name	Drugs	%assay results	
		U.V	**HPTLC**
TELMA-AM	Amlodipine besylate	99.6	97
		102.4	99.2
		100.4	100.2
	Telmisartan	99.62	98.8
		97.35	99.4
		98.00	97.95
TELSAR	Amlodipine besylate	96.2	96.2
		98.6	99.2
		97.4	98.6
	Telmisartan	99.42	98.37
		99.65	100.2
		98.6	97.45
TELSAR-AM	Amlodipine besylate	97.6	101
		99.2	97.6
		100.2	102
	Telmisartan	98.65	99.62
		99.3	97.35
		100.47	100.25

Table 5.30 Results of Student t-test

T-TEST: PAIRED TWO SAMPLE FOR MEANS					
Brand Name	**Parameter**	**Amlodipine besylate**		**Telmisartan**	
		UV	**HPTLC**	**UV**	**HPTLC**
TELMA-AM	Mean	100.8	98.8	98.32	98.75
	Variance	2.08	2.68	1.37	0.54
	Observations	3	3	3	3
	Pearson Correlation	0.474		-0.103	
	Df	2		2	
	T Stat (t_{cal})	2.182		-0.508	
	P (T≤t) one-tail	0.008		0.330	
	t Critical one tail	2.919		2.919	
	P (T≤t) two-tail	0.160		0.661	
	t Critical two tail (t_{crit})	4.302		4.302	
TELSAR-A	Mean	97.4	98.2	99.22	99.15
	Variance	1.44	1.56	0.30	0.46
	Observations	3	3	3	3
	Pearson Correlation	0.960		-0.465	
	Df	2		2	
	T Stat (t_{cal})	-4		0.122	
	P (T≤t) one-tail	0.028		0.456	
	t Critical one tail	2.919		2.919	
	P (T≤t) two-tail	0.057		0.913	
	t Critical two tail (t_{crit})	4.302		4.302	

TELSARTAN -AM	Mean	99	100.2	99.47	99.07
	Variance	1.32	5.32	0.85	2.32
	Observations	3	3	3	3
	Pearson Correlation	0.085		0.362	
	Df	2		2	
	T Stat (t_{cal})	-0.813		0.471	
	P (T≤t) one-tail	0.250		0.341	
	t Critical one tail	2.919		2.919	
	P (T≤t) two-tail	0.501		0.683	
	t Critical two tail (t_{crit})	4.302		4.302	

5.5 Conclusion

The proposed UV and HPTLC methods were fast, accurate and precise for determination of Amlodipine besylate and Telmisartan in combined dosage forms. High recoveries show that the method is free from the interference from excipients used in the commercial pharmaceutical preparations. Hence, it can be successfully applied for routine estimation for Amlodipine besylate and Telmisartan in quality control laboratories. The results of validation parameters are satisfactory level indicates the accuracy of proposed method for estimation of Amlodipine besylate and Telmisartan. Assay results of tablets obtained by both methods were compared using student t-test. Here tcal < tcrit for all 3 dosage forms .Hence, it was concluded that both the methods do not differ significantly and can be successfully applied for the analysis of Amlodipine besylate and Telmisartan in pharmaceutical dosage forms.

Chapter 6
SUMMARY

6 SUMMARY

- UV-Spectophotometry and HPTLC method were developed for the simultaneous determination of Amlodipine besylate and Telmisartan in their combine dosage form. The developed methods were validated in terms of linearity, precision, accuracy, repeatability, limit of detection and limit of quantitation.
- UV-Spectophotometry method showed linearity in the range of 5-35 µg/ml for amlodipine besylate and 10-70 µg/ml for Telmisartan with coefficient of 0.999 and 0.998 for Amlodipine besylate and Telmisartan, respectively. The method was found accurate, precise, specific, selective and repeatable. The minimum detectable concentration of Amlodipine besylate and Telmisartan was found to be 0.50 µg/ml and 0.63 µg/ml respectively. The lowest quantifiable concentration of Amlodipine besylate and Telmisartan was found to be 1.52 µg/ml and 1.90 µg/ml respectively.
- For, HPTLC method the developed method consisted of Chloroform: Methanol: Toluene (5: 3.5:1.5, v/v/v) as mobile phase. Saturation time was kept 30 minutes with run length of 80 mm. the drugs were separated at the R_f value of 0.23 for amlodipine besylate and 0.75 for telmisartan.
- The drugs showed linearity in the range of 300-900 ng/spot for amlodipine besylate and 600-1800 ng/spot for telmisartan with correlation coefficient of 0.993 and 0.984 respectively. The method was found accurate, precise, specific, selective and repeatable. The minimum detectable concentration of Amlodipine besylate and Telmisartan was found to be 30.79 ng/spot and 23.39 ng/spot respectively. The lowest quantifiable concentration of Amlodipine besylate and Telmisartan was found to be 93.32 ng/spot and 70.58 ng/spot respectively.
- The developed methods were compared by student-t-test for the assay of tablets containing Amlodipine besylate and Telmisartan, of three different brands.
- Here, tcal < tcrit for all 3 dosage forms. Hence, it was concluded that both the methods do not differ significantly and can be successfully applied for the analysis of Amlodipine besylate and Telmisartan in pharmaceutical dosage forms.

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