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Development and Validation of a High-Performance Liquid Chromatography Method for the Analysis of Amlodipine and Telmisartan in a Fixed-Dose Drug Combination

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Abstract: *A sensitive and reliable Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation [1] of Amlodipine (AML) and Telmisartan (TEL) in a fixed-dose combination tablet. The chromatographic separation was achieved on a C18 column (4.6 x 250 mm) using an isocratic mobile phase consisting of 0.2% Ortho Phosphoric Acid in water and Acetonitrile in the ratio of 70:30 (v/v) at a flow rate of 1.0 ml/min. UV detection was performed at 258 nm. The method was validated according to USP guidelines for system suitability, precision, accuracy, linearity, robustness, and specificity. System suitability parameters were within acceptable limits. The method demonstrated good precision with %RSD values for system and method precision below 2.0%. Accuracy was confirmed by recovery studies, yielding mean recoveries between 98-102% for both analytes. Linearity was established with a correlation coefficient (R^2) of 0.998 for both AML and TEL over the concentration range of 50-150%. The method was found to be robust to small deliberate changes in chromatographic conditions and specific as no interference was observed from the placebo. The developed and validated RP-HPLC method is simple, rapid, and suitable for routine quality control analysis of Amlodipine and Telmisartan in pharmaceutical formulations.*

Keywords: RP-HPLC, Method Development, Method Validation, Simultaneous Estimation, Amlodipine, Telmisartan, Fixed-Dose Combination, Pharmaceutical Analysis

I. INTRODUCTION

Analytical chemistry, a cornerstone of the chemical sciences, provides the essential methodologies for elucidating the chemical composition of matter. Its scope encompasses the identification of constituent elements and compounds (qualitative analysis) and the determination of their respective quantities (quantitative analysis) within a given sample. The applications of analytical chemistry are pervasive, underpinning quality control in industries, environmental monitoring, medical diagnostics, and forensic science.

The history of analytical chemistry is intertwined with the evolution of chemistry itself. Early advancements laid the groundwork for instrumental analysis, exemplified by the development of flame emissive spectrometry in the mid-19th century (Bunsen and Kirchhoff, 1860), which facilitated the discovery of new elements. The 20th century witnessed a significant paradigm shift, with analytical chemistry becoming an indispensable tool across diverse scientific disciplines.

Method development in analytical chemistry is a critical yet often intricate process, demanding a blend of expertise, innovation, logical reasoning, and meticulous experimentation. While modern software and automation have aided this

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endeavor, achieving optimal method performance, particularly in terms of resolution, frequently necessitates a systematic process of preliminary investigations and subsequent fine-tuning.

Prior to embarking on the development of a new analytical method, a comprehensive literature review is of paramount importance. This thorough search should encompass existing methodologies for the analytes of interest or structurally related compounds, including compendial monographs (USP, EP), scientific literature, manufacturer information, and online databases. While a directly applicable method may not always be available, such a review often provides a valuable foundation or relevant references for the development process.

The necessity for new analytical methods arises in several scenarios: the analysis of novel chemical entities, situations where existing methods exhibit limitations in reliability, sensitivity, or cost-effectiveness, the advent of new instrumentation offering superior performance, and the requirement for alternative methods for regulatory compliance.

The selection of an appropriate analytical method is a crucial decision for the analyst, requiring careful consideration of various factors. These include the nature of the analysis (elemental or molecular, routine or specialized), the complexity and potential challenges posed by the sample matrix (e.g., reactivity, interferences), the concentration range of the analytes, the required accuracy, the availability of instrumentation, time constraints, and the anticipated number of analyses.

The chosen analytical method should ideally possess characteristics such as simplicity, high specificity, productivity, cost-effectiveness, and accuracy and precision commensurate with the analytical requirements. Furthermore, critical components should ideally have readily available and reliable sources, and the method should undergo thorough optimization before validation to ensure its performance characteristics.

Analytical methods can be broadly classified into classical and instrumental techniques. Classical methods rely on gravimetric and titrimetric measurements, often involving observable chemical reactions. While these methods can be accurate and do not require highly specialized equipment, they often suffer from limitations in sensitivity, specificity, and are typically more time-consuming. Instrumental methods, on the other hand, measure physical properties of the analytes using sophisticated instruments, offering advantages such as high sensitivity, speed, and the ability to analyze complex matrices. These methods encompass a wide range of techniques, including spectroscopic and chromatographic methods.

Spectrophotometric methods, based on the Beer-Lambert Law, are widely employed for quantitative analysis due to their simplicity, speed, and reasonable accuracy. These methods measure the absorption or emission of electromagnetic radiation by the analyte. Chromatographic techniques, such as High-Performance Liquid Chromatography (HPLC), separate mixtures based on the differential distribution of analytes between a mobile and a stationary phase. Following separation, a detector quantifies the eluted components.

High-Performance Liquid Chromatography (HPLC) is a modern and versatile chromatographic technique that utilizes high pressure to pump a mobile phase through a column packed with a stationary phase, achieving efficient separation of analytes. HPLC finds extensive applications in the pharmaceutical industry for the analysis of drug substances and drug products.

Fixed-dose combinations (FDCs) containing multiple active pharmaceutical ingredients are increasingly common to improve patient compliance and therapeutic outcomes. This research focuses on the development and validation of a robust analytical method for the simultaneous determination of Amlodipine (AML) and Telmisartan (TEL), two widely used antihypertensive drugs, in a fixed-dose combination tablet formulation using RP-HPLC. The developed method aims to provide a reliable and efficient means for quality control analysis of this important pharmaceutical product.

II. METHOD DEVELOPMENT IN HPLC

The principle of HPLC separation relies on the differential partitioning of analytes between a mobile phase (liquid) and a stationary phase (solid or liquid coated on a solid support) as the mobile phase carries the sample through the column.

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The efficiency of separation is influenced by various factors, including the nature of the stationary phase, the composition and flow rate of the mobile phase, the column temperature, and the detection wavelength.

Developing an effective HPLC method involves a systematic optimization process. Initial scouting runs are performed using different combinations of stationary phases and mobile phase compositions to achieve adequate separation of the analytes of interest. Parameters such as solvent strength, pH of the aqueous component of the mobile phase, and the use of modifiers are evaluated to improve peak resolution, symmetry, and retention times. This often involves a degree of trial-and-error, guided by the physicochemical properties of the analytes.

Reversed-Phase HPLC (RP-HPLC) is a widely used mode in pharmaceutical analysis, particularly suitable for separating non-polar to moderately polar compounds. It employs a non-polar stationary phase (typically a C8 or C18 bonded silica) and a polar mobile phase (a mixture of water or an aqueous buffer with organic modifiers like methanol or acetonitrile). The separation mechanism is primarily based on hydrophobic interactions; more non-polar compounds interact more strongly with the stationary phase and elute later. Given the lipophilic nature of both Amlodipine and Telmisartan, RP-HPLC with a C18 column was deemed appropriate for this analysis.

The selection of the mobile phase (0.2% Ortho Phosphoric Acid in water and Acetonitrile 70:30 v/v) was based on its ability to provide satisfactory separation of AML and TEL with acceptable retention times and peak shapes, as observed during the method optimization phase (refer to "Procedure for Method Optimization" in the experimental section). Ortho Phosphoric Acid was used in the aqueous phase to control the pH and improve peak symmetry. Acetonitrile, as the organic modifier, adjusts the eluting strength of the mobile phase.

The detection wavelength of 258 nm was chosen as a common wavelength where both Amlodipine and Telmisartan exhibit significant UV absorbance, allowing for their simultaneous detection and quantification (refer to "Determination of Common Wavelength"). The optimized chromatographic parameters achieved a baseline separation of Telmisartan and Amlodipine with retention times of approximately 5.1 minutes and 11.96 minutes, respectively, indicating the successful development of the HPLC method for their simultaneous estimation

III. EXPERIMENTAL WORK

This section details the materials, instruments, and procedures employed in the development and validation of the HPLC method for the simultaneous estimation of amlodipine (AML) and telmisartan (TEL) in a fixed-dose drug combination tablet formulation.

3.1 Materials and Instruments:

3.1.1 Materials:

3.1.1.1. Pure Drugs:

Drug	Supplied by	Quantity	Purity (Assay)
Telmisartan	Arrow Chem Mumbai.	10 g	99.9 % w/w
Amlodipine	Arrow Chem Mumbai	10 g	99.5% w/w

Table No. 1: Details of API

The active pharmaceutical ingredients (APIs), Telmisartan and Amlodipine Besilate, were obtained from Arrow Chem, Mumbai, India. Their details are provided in Table No. 1

3.1.1.2. Marketed Preparation:

The fixed-dose combination tablet, Telma-AM® Tab, manufactured by Glenmark Pharmaceuticals Ltd., containing 5 mg of Amlodipine and 40 mg of Telmisartan, was procured from the local market (Table No. 2).

Brand Name	**Mfd by**	**Content**	**Quantity**
Telma-AM® Tab	Glenmark Pharmaceuticals Ltd	Amlodipine	5 mg
		Telmisartan	40mg

Table No. 2: Details of Marketed Preparation

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3.1.1.3. Reagents and Chemicals:

All reagents and chemicals used were of AR grade and HPLC grade:

- Methanol (HPLC grade)
- Acetonitrile (HPLC grade)
- Disodium hydrogenphosphate (AR grade)
- Distilled Water (HPLC grade)
- Triethylamine (HPLC grade)
- Ortho Phosphoric Acid (HPLC grade)

3.1.1.2. Instruments: The instruments used in this study are listed in Table No. 3.

Sr. No	Instruments	Make	Model
1	UV-Visible Spectrophotometer	Shimadzu	UV-1900
2	HPLC	Waters 600	996 PDA Detector
3	pH Meter	Hanna	-
4	Balance	Citizen	CY 104 (Micro Analytical Balance)
5	Ultra sonicator	-	1.5 L 50

Table No. 3: Instruments Used

3.1.2. Study of Functional Group by Using Infra-Red Spectroscopy:

The functional groups of the pure drug substances were confirmed by IR spectroscopy using the Diffused Attenuated Reflectance (DAR) mode with dried KBr. 3 mg of each API (Amlodipine Besilate and Telmisartan) was mixed with 300 mg of dried KBr, triturated, and the IR spectrum was recorded.

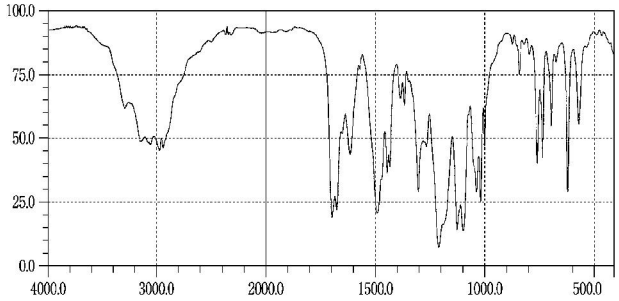

Fig. No. 1: IR Spectra of Amlodipine Besilate

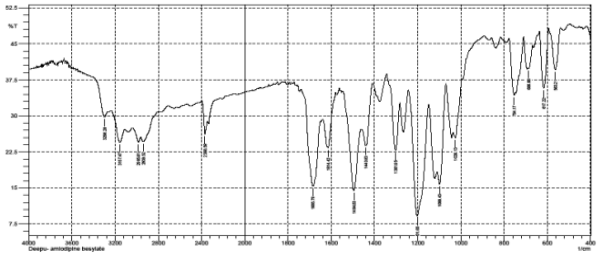

Fig. No. 2: Reference IR Spectra of Amlodipine Besilate

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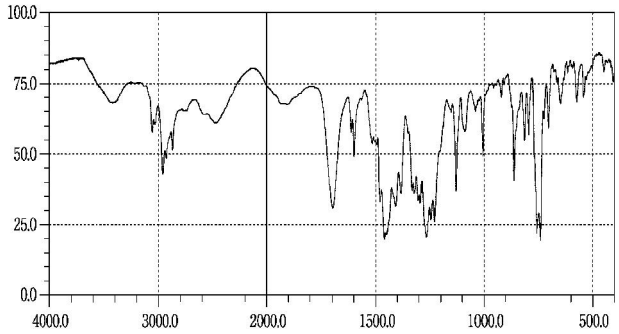

Fig. No. 3: IR Spectra of Telmisartan

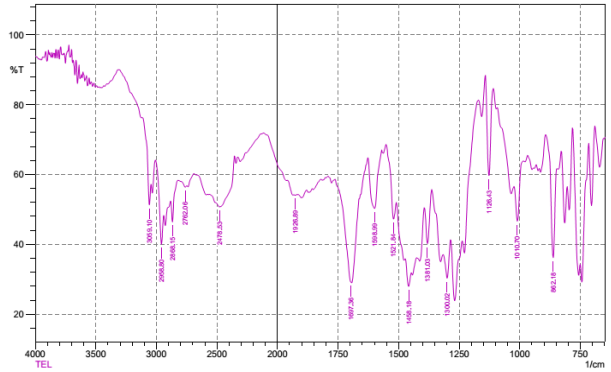

Fig. No.4: Reference IR Spectra of Telmisartan

Conclusion: The IR spectra of the test drugs matched the reference IR spectra, confirming the identity of the functional groups.

Determination of Common Wavelength:

Standard stock solutions of Amlodipine Besilate (50 mg/ml in methanol) and Telmisartan (400 mg/ml in methanol) were prepared. These stock solutions were further diluted to 10 mg/ml of each drug. The UV absorbance spectra of these diluted solutions were recorded in the range of 400-200 nm using a 1 cm cell against a methanol blank.

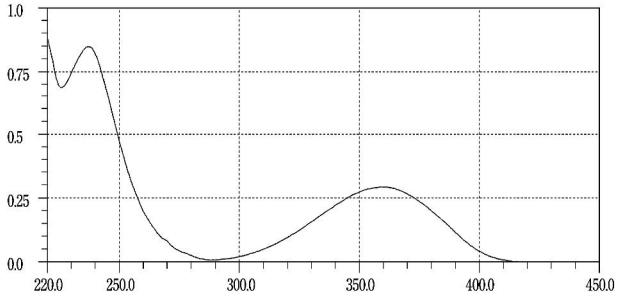

Fig. No. 5: Wavelength Maxima for AML

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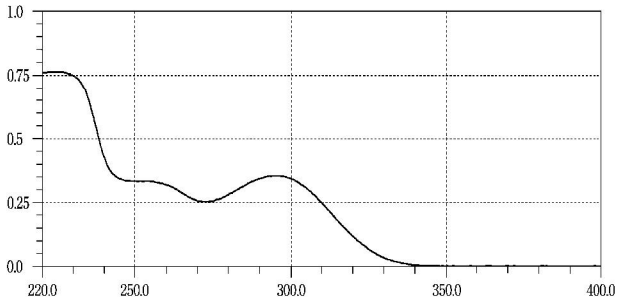

Fig. No. 6: Wavelength Maxima for TEL

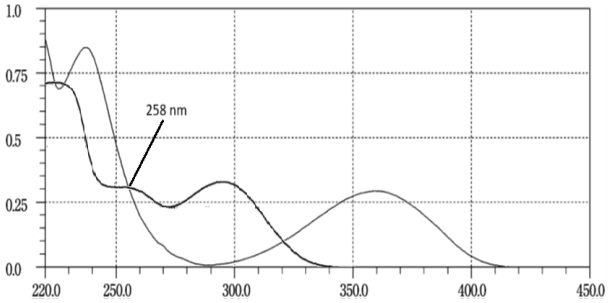

Fig No. 7: Overlain spectra of AML and TEL showing 258 nm as common wavelength

A common wavelength of 258 nm was selected for HPLC analysis as both drugs exhibited significant absorbance at this wavelength.

3.2. DEVELOPMENT OF HPLC METHODS FOR ESTIMATION OF AMLODIPINE AND TELMISARTAN

3.2.1. Method Development Strategy:

3.2.1.1. Selection of Common Solvent (Diluent):

HPLC grade methanol was chosen as the common solvent for preparing stock solutions and studying the spectral characteristics of the drugs. Further dilutions were made using the mobile phase. This selection was based on the good solubility of both drugs in methanol.

3.2.1.2. Preparation of Standard Stock Solution:

Accurately weighed 5 mg of AML and 40 mg of TEL were dissolved in 100 ml of methanol to prepare a standard stock solution.

3.2.1.3. Preparation of Diluent:

HPLC grade methanol was used as the diluent for preparing working standard and sample solutions.

3.2.1.4. Procedure for Method Optimization:

The mobile phase was allowed to equilibrate with the stationary phase until a stable baseline was obtained. Standard solutions containing a mixture of AML and TEL were injected under different chromatographic conditions (various solvent combinations) to achieve good separation and stable peaks. All solutions were filtered through a 0.15 µm membrane filter. Several mobile phase compositions were evaluated to obtain acceptable separation based on peak resolution, symmetry, and retention time, considering the solubility and stability of the analytes. The final optimized chromatographic conditions were kept constant throughout the method.

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3.2.1.5.Optimized Chromatographic Parameters:

Sr.No	Peak area		Retention Time		Symmetry		No .of theoretical Plates	
		AML	TEL	AML	TEL	AML	TEL	AML
1	308350	61060.2	5.19	12	0.622	0.756	8149.1	4138.7
2	308850	61629.2	5.25	11.8	0.623	0.754	8100.2	4042.8
3	309750	61111.0	5.20	11.9	0.622	0.771	8143.3	4190.0
4	306820	61012.4	5.16	12.1	0.626	0.756	8194.1	4167.3
5	308150	61120.2	5.21	11.9	0.627	0.778	8181.5	4100.2
Mean	308384	61386.6	5.202	11.94	0.624	0.763	8153.64	4127.8
S.D	2272.6	349.8	0.05	0.11	0.002	0.010	36.73	58.17
%R.S.D.	0.66	0.90	0.65	0.95	0.37	1.41	0.45	1.40

Column: C18 (Symmetry C18 Column), 4.6 x 250 mm
Flow Rate: 1.0 ml/min
Wavelength: 258 nm
Injection Volume: 20 µl
Column Oven Temperature: Ambient (25°C)
Run Time: 15 minutes
Mobile Phase: 0.2% Ortho Phosphoric Acid (OPA) in water and Acetonitrile (ACN) in the ratio of 70:30 (v/v).
Preparation of 0.2% OPA: 2 ml of ortho phosphoric acid was diluted to 1000 ml with HPLC water.

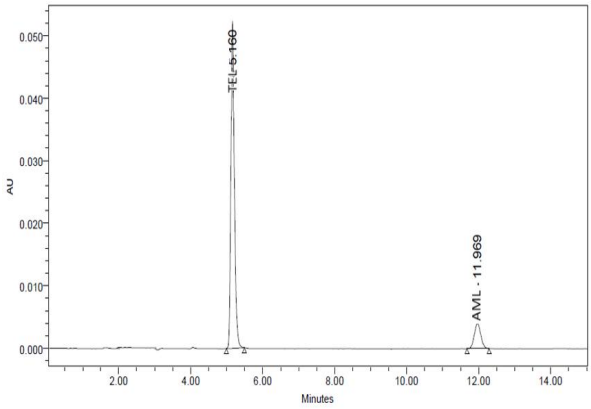

Fig. No. 8: Separation of the two drugs in selected mobile phase Showing R.T. of 5.1 min for TEL and 11.96 for AML

Under these optimized conditions, Telmisartan eluted at approximately 5.1 minutes, and Amlodipine eluted at approximately 11.96 minutes, with good separation between the peaks.

3.2.2 System Suitability Studies: System suitability tests were performed as per pharmacopoeial requirements to verify the resolution and reproducibility of the chromatographic system for the intended analysis. Five replicate injections of the standard solution containing a mixture of AML and TEL were made under the optimized chromatographic

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conditions. System suitability parameters such as peak area, retention time, symmetry (tailing factor), and the number of theoretical plates were recorded and evaluated.

Procedure: The filtered mobile phase was allowed to equilibrate, and five replicate injections of the standard drug solution were made. The system suitability parameters were recorded (Table No. 4).

Sr.No	Peak area		Retention Time		Symmetry		No .of theoretical Plates	
		AML	TEL	AML	TEL	AML	TEL	AML
1	308350	61060.2	5.19	12	0.622	0.756	8149.1	4138.7
2	308850	61629.2	5.25	11.8	0.623	0.754	8100.2	4042.8
3	309750	61111.0	5.20	11.9	0.622	0.771	8143.3	4190.0
4	306820	61012.4	5.16	12.1	0.626	0.756	8194.1	4167.3
5	308150	61120.2	5.21	11.9	0.627	0.778	8181.5	4100.2
Mean	308384	61386.6	5.202	11.94	0.624	0.763	8153.64	4127.8
S.D	2272.6	349.8	0.05	0.11	0.002	0.010	36.73	58.17
%R.S.D.	0.66	0.90	0.65	0.95	0.37	1.41	0.45	1.40

Table No. 4: Result of System Suitability Test

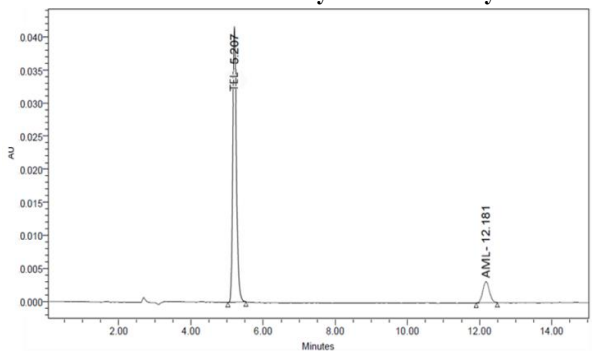

Fig. No 9 : Separation of the Two drugs in selected mobile phase Showing R.T. for TEL at 5.207 min AML at 12.181 min respectively

The system suitability parameters were within the acceptable limits (%RSD for peak area and retention time NMT 2.0, tailing factor NMT 2.0, and theoretical plates NLT 2000), indicating that the chromatographic system was suitable for the analysis.

3.3 Application of Proposed Method for Estimation of TEL and AML in Tablet Formulation:

a) Preparation of Standard Solutions:

Telmisartan Standard Stock Solution: 40 mg of TEL dissolved in 100 ml methanol (400 µg/ml).

Telmisartan Standard Working Solution: 1 ml of stock solution diluted to 10 ml with methanol (40 µg/ml).

Amlodipine Standard Stock Solution: 5 mg of AML dissolved in 100 ml methanol (50 µg/ml).

Amlodipine Standard Working Solution: 1 ml of stock solution diluted to 10 ml with methanol (5 µg/ml).

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Mix Standard Working Solution: 1 ml each of TEL (40 µg/ml) and AML (5 µg/ml) working standard solutions were mixed and diluted to 10 ml with methanol (Resultant concentrations: TEL 40 µg/ml, AML 5 µg/ml). **Note:** There seems to be a discrepancy in the provided text regarding the final concentrations in the mix standard. Assuming 1 ml of each was diluted to 10 ml, the concentrations should be TEL 4 µg/ml and AML 0.5 µg/ml. However, the formula in the next section suggests the concentrations used were TEL 50 µg/ml and AML 5 µg/ml. We will proceed with the concentrations implied by the formula.

b) Sample Solution Preparation:

Twenty tablets were weighed, and the average weight was determined (280 mg). The tablets were powdered, and a quantity equivalent to 50 mg of TEL and 5 mg of AML was transferred to a 100 ml volumetric flask. 50 ml of methanol was added, sonicated for 10 minutes, and the volume was made up to the mark with methanol. This stock solution was filtered through a 0.45 µm membrane filter. 1 ml of this stock solution was further diluted to 10 ml with methanol and filtered through a 0.45 µm membrane filter before HPLC analysis.

Procedure: Equal volumes (20 µl) of standard and sample solutions were injected separately after equilibration of the stationary phase. Chromatograms were recorded, and the peak areas were measured. The content of TEL and AML in the tablets was calculated by comparing the sample peak areas with those of the standard using the following formula:

Amount of drug in tablet (mg)=AsAt×DsWs×WtDt×Average weight×Purity of standard

Where: At = Area count for sample solution. As = Area count for standard solution. Ds = Dilution factor for standard. Dt = Dilution factor for sample. Ws = Weight of standard (mg). Wt = Weight of sample (mg).

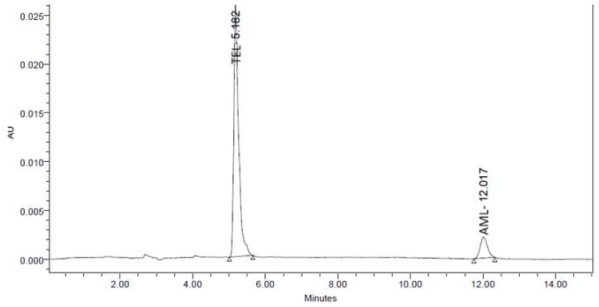

Fig. No.10 : Chromatogram obtained by tablet formulation of TEL and AML showing retention time 5.18 min and 12.01 min respectively

Table No. 5: Results and statistical data for estimation of TEL and AML in marketed formulation

Sr.No.	TEL		AML	
	Assay (mg)	Assay % of LC	Assay (mg)	Assay % of LC
1	49.9	99.8	4.95	99.0
2	49.8	99.6	4.90	98.0
3	49.9	99.8	4.90	98.0
Average	49.86	99.73	4.92	98.33
SD	0.05	0.11	0.02	0.58
% RSD	0.11	0.11	0.58	0.58

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The assay results for both Telmisartan and Amlodipine in the marketed formulation were within the acceptable limits of 90-110% of the label claim, with low %RSD values, indicating the accuracy and precision of the developed method for pharmaceutical analysis.

3.4 Validation Parameters:

The developed HPLC method was validated for the following parameters:

Accuracy

Precision (System Precision and Method Precision)

Ruggedness (Intermediate Precision)

Robustness

Linearity and Range

Specificity (Placebo Interference Study)

IV. VALIDATION

The developed RP-HPLC method for the simultaneous estimation of Telmisartan (TEL) and Amlodipine (AML) was validated according to USP guidelines for the following parameters: Precision, Linearity & Range, Accuracy, Robustness, and Specificity.

4.1. Precision:

Precision was evaluated at two levels: System Precision and Method Precision (Repeatability).

System Precision:

System precision was assessed by injecting the standard solution containing both TEL and AML in three replicates. The results for system suitability parameters are presented in Table No. 6.

Sr. No.	Parameter	Observations		Limits
		TEL	AML	
1	The % RSD of peak area response for three replicate injections of standard	0.67	0.7	NMT 2.0
2	Theoretical plates	7197.53	8057.53	NLT 2000
3	Tailing factor	1.10	1.19	NMT 2.0

Table No. 6: Data showing System Precision

The %RSD for peak area response was well within the acceptance limit of NMT 2.0 for both TEL and AML. The number of theoretical plates exceeded the minimum requirement of NLT 2000, and the tailing factor was within the limit of NMT 2.0, indicating acceptable system precision.

Method Precision (Repeatability):

Method precision was evaluated by performing replicate estimations of the tablet formulation using the proposed method. The assay results for three sample preparations are shown in Table No. 7

Sr.no.	TEL		AML	
	Assay (mg)	Assay % of LC	Assay (mg)	Assay % of LC
1	50.00	100.0	4.98	99.60
2	50.01	100.02	5.00	100.0
3	49.95	99.99	4.98	99.60
Average	49.99	100.0	4.99	99.74
SD	0.03	0.015	0.01	0.23

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% RSD	0.06	0.015	0.23	0.23

Table No. 7: Method Precision Studies Set

The %RSD values for the assay of TEL and AML were less than 2.0, indicating good repeatability of the method.

Intermediate Precision (Ruggedness):

Intermediate precision was assessed by analyzing six sample solutions prepared as per the test method using different HPLC systems, columns, and analysts on different days. The data for intermediate precision is not provided in this section. However, the text mentions that the study was performed. For a complete research paper, the data obtained for intermediate precision (assay values and %RSD) should be included in a table similar to Table No. 7. The acceptance criterion for %RSD in intermediate precision is also typically NMT 2.0.

4.2. Linearity & Range:

Linearity was established by analyzing standard solutions at five concentration levels ranging from 50% to 150% of the target concentration for both TEL and AML. A graph of concentration versus mean peak area was plotted, and the correlation coefficient (R^2) was determined. The R^2 values were found to be 0.998 for TEL and 0.998 for AML, indicating excellent correlation between concentration and peak area within the tested range. This demonstrates the linearity of the method.

4.3. Accuracy

Accuracy was evaluated by performing recovery studies using the standard addition method. Known amounts of pure TEL and AML standards were added to the placebo, and the recovery of the added analytes was determined. The results of the accuracy studies are presented in Table No. 8

	TEL			**AML**		
	Levels			**Levels**		
	80%	**100%**	**120%**	**80%**	**100%**	**120%**
Amt added (µg/ml)	**40**	**50**	**60**	**4**	**5**	**6**
	40	**50**	**60**	**4**	**5**	**6**
	40	**50**	**60**	**4**	**5**	**6**
Amt taken (µg/ml)	**40**	**50**	**60**	**4**	**5**	**6**
	40	**50**	**60**	**4**	**5**	**6**
	40	**50**	**60**	**4**	**5**	**6**
Amt recovered (µg/ml)	**40.0**	**49.9**	**59.9**	**3.95**	**4.98**	**5.98**
	39.8	**50.0**	**59.8**	**4.0**	**4.98**	**5.97**
	39.6	**49.8**	**60.0**	**3.95**	**5.00**	**5.98**
% Recovery	**100.0**	**99.8**	**99.83**	**98.75**	**99.6**	**99.66**
	99.5	**100.0**	**99.66**	**100.00**	**99.6**	**99.5**
	99.9	**99.6**	**100.00**	**98.75**	**100.0**	**99.66**
Mean % recovery	**99.80**	**99.80**	**99.83**	**99.16**	**99.73**	**99.60**
% RSD	**0.26**	**0.20**	**0.17**	**0.72**	**0.23**	**0.09**

Table No. 8: Result of Accuracy Studies

The mean percentage recoveries for TEL and AML at all spiking levels were within the acceptable range of 98-102%, and the %RSD values were NMT 2.0, indicating the accuracy of the developed method.

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4.4. Robustness

The robustness of the method was evaluated by making deliberate small changes in critical chromatographic parameters:

1. Effect of variation in flow rate: ± 10% (0.9 ml/min and 1.1 ml/min).

Sr. No.	System Suitability parameter		Observations for flow rate			Limits
			Unchanged	0.9 ml	1.1 ml	
1	The % RSD of peak area response for five replicate injections	TEL	1.057	0.95	0.86	NMT 2.0
		AML	0.155	0.20	0.05	
2	Theoretical plates	TEL	8197.53	8138.7	8157.9	NLT 2000
		AML	4157.53	4165.7	4128.9	
3	Tailing factor	TEL	1.38	1.81	1.30	NMT 2.0
		AML	1.26	0.910	1.10	
4	Retention Time (Min)	TEL	5.3	5.4	12.1	
		AML	12.1	5.1	11.9	

Table No. 9: Effect of variation in flow rate

2. Effect of organic phase composition: ± 10% Acetonitrile in the mobile phase.

Sr. No.	System Suitability parameter		Observations for flow rate			Limits
			Unchanged	- 10%	+10%	
1	The % RSD of peak area response for five replicate injections	TEL	1.017	0.655	0.046	NMT 2.0
		AML	0.173	0.021	0.030	
2	Theoretical plates	TEL	8197.53	7996	8347.6	NLT 2000
		AML	4157.53	4060	4386.6	
3	Tailing factor	TEL	1.28	1.166	1.08	NMT 2.0
		AML	1.06	1.062	0.88	
4	Retention Time (Min)	TEL	5.2	5.2	5.1	
		AML	11.2	12.3	11.9	

Table No. 10: Effect of organic phase composition

3. Effect of wavelength: ± 5 nm (253 nm and 263 nm). **Note:** The previous data mentioned ± 2 units, this section states ± 5 units. Assuming ± 2 units as per the experimental section.The text states that all system suitability parameters remained within the acceptable limits under these deliberate changes, indicating that the method is robust.

Sr. No.	System Suitability parameter		Observations for wavelength			Limits
			Unchanged	256nm	260nm	
1	The % RSD of peak area response for five replicate injections	TEL	1.017	0.3638	0.141	NMT 2.0
		AML	0.173	0.065	0.016	

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2	Theoretical plates	TEL	7197.53	6186.2	5693.5	NLT 2000
		AML	8057.53	7987.9	6678.3	
3	Tailing factor	TEL	1.28	1.08	1.95	NMT 2.0
		AML	1.06	1.00	0.94	
4	Retention Time (Min)	TEL	5.1	5.3	5.1	
		AML	11.8	12.2	11.1	

Table No. 10: Effect of wavelength

4.5. Specificity:

Specificity was assessed by injecting blank and placebo solutions prepared according to the test method. The chromatograms were examined for any peaks eluting at the retention times of TEL and AML. The text indicates that no interference was found at the retention times of TEL and AML from the blank or placebo, demonstrating the specificity of the method. Figure No. 11 showing the chromatograms of the placebo interference study would be included here in a full research paper.

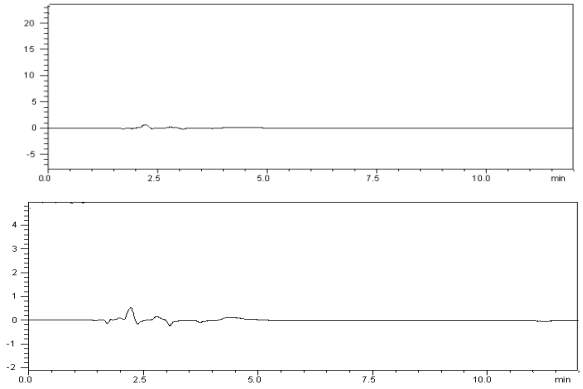

Fig. No.11. Chromatograms of Placebo Interference Study of sample matrix and placebo preparation.

V. SUMMARY AND CONCLUSION

5.1. SUMMARY

A sensitive and simple RP-HPLC method was developed for the simultaneous estimation of Telmisartan (TEL) and Amlodipine (AML) in a fixed-dose combination tablet formulation. The chromatographic separation was achieved using a C18 column and a mobile phase composed of water (0.2% OPA) and acetonitrile (70:30 v/v) under isocratic conditions with a flow rate of 1.0 ml/min and UV detection at 258 nm. The total run time was 15 minutes.

The developed method was validated according to USP guidelines and met the acceptance criteria for all validation parameters:

- **Linearity and Range**: Excellent linearity was observed for both TEL and AML in the concentration range of 50-150% of the target concentration, with a correlation coefficient (R^2) of 0.998 for both analytes.
- **Accuracy:** Recovery studies using the standard
- **Precision**: The method showed good system precision (%RSD for peak area < 2.0) and method precision (%RSD for assay < 2.0).addition method demonstrated good accuracy, with mean recoveries within the range of 98-102% and %RSD < 2.0 for both TEL and AML.
- **Robustness:** The method proved to be robust, as small deliberate changes in flow rate, organic phase composition, and wavelength did not significantly affect the system suitability parameters.

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- **Specificity**: The method was specific, with no interference observed from the placebo at the retention times of TEL and AML.

5.2. CONCLUSION

The developed and validated RP-HPLC method is accurate, precise, specific, linear, and robust for the simultaneous estimation of Telmisartan and Amlodipine in their combined tablet dosage form. The method is simple and rapid, making it suitable for routine quality control analysis in pharmaceutical laboratories. The successful validation of the method ensures its reliability and applicability for the intended purpose.

REFERENCES

[1]. Khopkar S. M. Basic concepts of analytical chemistry, New Age International*Ltd. Publishers, New Delhi (1998); 2:178-179.
[2]. Settle F. Handbook of Instrumental techniques for analytical chemistry, Prentice Hall PTR, NJ (1997); 17(19): 56-57.
[3]. Skoog D. A. Holler F. J, Crouch S. R. Principle of Instrumental Analysis, Thomson Publications, India (2007); 6: 1-3, 145-147, 180.
[4]. Mendham J, Denney R. C, Barnes J. D, Thomas M. Vogel's Textbook of Quantitative Analysis, Pearson Education, Singapore (2003); 8-9.
[5]. Sharma B. K. Instrumental Methods of Chemical Analysis, Goel Publication, Meerut (1983); 25, 3, 6.
[6]. Christian G. D. Analytical Chemistry, John Wiley and Sons (2003); 5: 35-42, 131-132.
[7]. Beckett A. H, Stenlake J. B. Practical Pharmaceutical chemistry, CBS Publisher and Distributor, New Delhi (1997); 2:1-85.
[8]. Christianah M. A, Pui-Kai L. Analytical Profile of Drug Substances. Edi. By Klaus Florey, 124-141.
[9]. Dong M. W. Modern HPLC for Practicing Scientist. John Wiley and sons, (2006).
[10]. Willard H. H, Merritt L. L, Dean J. A, Settle F. A. Instrumental Methods of Analysis. Seventh ed., CBS Publishers and Distributors, New Delhi, (2001).
[11]. Synder L. R, Kirkaland J. T, Glajch J. L. Practical HPLC Method Development. Second ed, John Wiley and Sons Publication, Inc, New York, (1997).
[12]. Kasture A. V, Mahadik K. R, Wadodkar S. G, More H. N. Pharmaceutical Analysis, Nirali Prakashan, (1999); 2: 6-7, 28-30, 49, 64, 67.
[13]. Scott R. P. W. Technique and Practice of chromatography. Marcel Dekker, New York, (2003); 70:1-12.
[14]. Brown P. R. Advances in Chromatography. Marcel Dekker, New York, (2001); 41.
[15]. Sethi P D. HPLC-Quantitative analysis of pharmaceutical formulations. CBS publishers and distributors, New Delhi, (2001); 1: 1-5, 58-67, 116-120.
[16]. Pattan S, Jamdar S. RP-HPLC Method for Simultaneous Estimation Of Paracetamol and Etoricoxib from bulk and Tablet, Journal of Chemical and Pharmaceutical Research(2009);1(1): 329-335.
[17]. Malesuik MD, Cardoso SG, Bajerski L, Lanzanova FA. Determination of amlodipine in pharmaceutical dosage forms by liquid chromatography and ultraviolet spectrophotometry. J AOAC Int. 2006 Mar-Apr;89(2):359-64.
[18]. Gowramma B, Rajan S. A Validated RP-HPLC Method for simultaneous estimation of Paracetamol and Diclofenac pottasium in pharmaceutical formulation, International journal of chemtech research(jan-mar 2010);2(1):676-680.
[19]. Reddy P, Battu. The Simultaneous RP-HPLC determination of Nimesulide and Paracetamol in tablet, International journal of Pharmtech Research (july- sept2009);1(3):514-516.

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[20]. Gopinath R, Rajan S, Meyyanathan S. N, Krishnaveni N, Suresh B. (RP- HPLC) method was developed for Paracetamol, Aceclofenac and Etoricoxibin pharmaceutical dosage forms, Indian Journal of pharmaceutical sciences, 2007; 69 (1): 137-140