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METHOD DEVELOPMENT, VALIDATION AND FORCED DEGRADATIONSTUDIES OF CILNIDIPINE AND TELMISARTAN BY QBD APPROACH USING RP-HPLC METHOD

Payal P. Jaju [1], Dr. Vinayak M.Gaware [2*], Mr. Rahul D. Khaire [3], Dr. Vivekanand A. Kashid [4]

1. PG Scholar, PRES's College of Pharmacy (For Women), Chincholi, Tal. Sinnar, Dist. Nashik.
2. Associate Professor, PRES's College of Pharmacy (For Women), Chincholi, Tal. Sinnar, Dist. Nashik.
3. Assistant Professor, PRES's College of Pharmacy (For Women), Chincholi, Tal. Sinnar, Dist. Nashik.
4. Principal, MABD Institute of Pharmaceutical Education and Research, Baghulgaon, Tal. Yeola, Dist. Nashik.

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ABSTRACT

Several spectrophotometric and HPLC methods have been reported for the determination of Telmisartan and Cilnidipine in drugs and pharmaceutical dosage forms. The present study focuses on optimizing, developing, and validating a sensitive and specific reversed-phase high-performance liquid chromatography (RP-HPLC) method for the quantification of Cilnidipine and Telmisartan in bulk and tablet formulation. Design of Experiments (DoE) was employed to optimize the RP-HPLC method. A forced degradation study was conducted under various stress conditions, including acidic, basic, oxidative (H_2O_2), photolytic, and thermal degradation.

In the developed RP-HPLC method, a mobile phase consisting of Methanol and Water (70:30 %v/v) was utilized at a flow rate of 1.0 ml/min on an HPLC system equipped with a UV detector and HPLC Workstation software. The chromatographic separation was achieved using a Cosmosil C18 column (250 mm x 4.6 mm ID, 5 µm particle size), with detection carried out at 232 nm. The retention times were found to be 4.623 minutes for Telmisartan and 10.379 minutes for Cilnidipine. The robustness values were less than one, indicating method stability.

The method's performance was validated in terms of solution stability, specificity, linearity, accuracy, precision (repeatability and intermediate precision), limit of detection (LOD), limit of quantification (LOQ), and robustness. The sensitivity, accuracy, precision, specificity, and robustness of the developed RP-HPLC method were found to be in accordance with regulatory guidelines.

Keywords: RP-HPLC, Cilnidipine, Telmisartan, Analytical Method, Validation, QBD.

INTRODUCTION

Hypertension is a prevalent and significant health concern globally, often requiring a combination of antihypertensive agents for effective management. Telmisartan, an angiotensin II receptor antagonist, and Cilnidipine, a calcium channel blocker, are commonly co-administered to achieve synergistic effects in reducing blood pressure. The effective quantification of these drugs in bulk and tablet formulations is crucial for ensuring their quality, efficacy, and safety. Several analytical methods, including spectrophotometric and high-performance liquid chromatography (HPLC) techniques, have been reported for the determination of Telmisartan and Cilnidipine individually and in combination. However, there remains a need for a more robust, sensitive, and specific method that can address the complexities associated with their simultaneous analysis. [1]

Reversed-phase high-performance liquid chromatography (RP-HPLC) is a widely accepted analytical technique due to its high resolution, sensitivity, and precision. Despite numerous reports on RP-HPLC methods for these drugs, there is a continuous demand for improved methodologies that can provide accurate results under various conditions, including stress testing for stability studies. Forced degradation studies are essential to understand the intrinsic stability of pharmaceutical compounds and to develop stability-indicating methods. These studies involve subjecting the drugs to stress conditions such as acidic, basic, oxidative, photolytic, and thermal environments to assess their degradation behaviour. [2]

The Quality by Design (QbD) approach has gained prominence in analytical method development, offering a systematic framework for understanding and controlling variability in analytical processes. By employing QbD principles, method development becomes more efficient, ensuring consistent quality and regulatory compliance. The application of Design of Experiments (DoE) within the QbD framework allows for the optimization of method parameters, leading to a robust and reliable analytical procedure. [3]

In this study, we aim to develop and validate a sensitive and specific RP-HPLC method for the simultaneous quantification of Telmisartan and Cilnidipine in bulk and tablet formulations. The method will be optimized using the QbD approach, and its robustness will be evaluated through forced degradation studies under various stress conditions. This comprehensive approach will ensure the method's suitability for routine quality control and stability testing of these antihypertensive agents, ultimately contributing to better therapeutic outcomes and patient safety. [4]

The significance of this study lies in its potential to provide a validated, regulatory-compliant analytical method that can be widely adopted in pharmaceutical quality assurance. By addressing the

limitations of existing methods and incorporating advanced analytical techniques, this research aims to enhance the reliability and efficiency of Telmisartan and Cilnidipine analysis, supporting the ongoing efforts to improve hypertension management. [5]

MATERIALS AND METHODS

Identification of Drug:

Organoleptic Properties:

The color and odour of Cilnidipine and Telmisartan samples were examined.

Melting Point Determination: The melting points were determined using a melting point apparatus.

Solubility Analysis: Cilnidipine and Telmisartan were tested for solubility in various solvents and found soluble in ethanol, methanol, and isopropyl alcohol but poorly soluble in water. [6]

Selection of Analytical Wavelength:

Solvent Selection: Methanol was selected to dissolve both drugs.

Wavelength Selection: UV spectra of 25 µg/ml solutions in methanol were observed, and 232 nm was selected for detection. [7]

Chromatographic Conditions:

RP-HPLC was chosen for its simplicity and suitability. Methanol: Water (70:30 %v/v) was used as the mobile phase at a flow rate of 1.0 ml/min. The detection wavelength was set at 232 nm using a Cosmosil C18 column (250 mm x 4.6 mm, 5 µm). [8]

Preparation of Standard Stock Solutions:

Twenty tablets were crushed, and an average weight of 43.65 mg was dissolved in 100 mL methanol to obtain a 1000 ppm solution. Further dilutions were prepared for analysis: 30 ppm Cilnidipine and 120 ppm Telmisartan solutions. [9]

Optimization of Mobile Phase Strength:

Various mobile phases were tested, and a methanol-water gradient program (70:30 %v/v) was selected for optimal resolution and peak symmetry. The mobile phase was filtered through a 0.45 µm membrane filter and degassed by sonication for 20 minutes. [10]

HPLC Method Optimization:

Trial Conditions:

- Trial 1: Cilnidipine (Methanol:Water 60:40, 0.8 ml/min, 232 nm)
- Trial 2: Telmisartan (Methanol:Water 80:20, 0.8 ml/min, 234 nm)
- Trial 3: Combination (Methanol:Water 70:30, 1.0 ml/min, 232 nm)
- Trial 4: Cilnidipine Optimized (Methanol:Water 70:30, 1.0 ml/min, 232 nm)

- Trial 5: Telmisartan Optimized (Methanol:Water 70:30, 1.0 ml/min, 232 nm)
- Trial 6: Combination Optimized (Methanol:Water 70:30, 1.0 ml/min, 232 nm)

Optimized Chromatographic Conditions:

Parameter	Condition
Mobile phase	Methanol: Water (70:30)
Column	Cosmosil C18 (250 mm x 4.6 mm, 5 μm)
Injection volume	20 μL
Pressure	10-11 MPa
Flow rate	1.0 ml/min
Detection wavelength	232 nm
Retention time	Cilnidipine (10.379 min), Telmisartan (4.623 min)
Tailing Factor	Cilnidipine (1.237), Telmisartan (1.168)

Table No 1: Optimized Chromatographic Conditions

Validation of Developed RP-HPLC Method

Linearity Procedure:

Linearity was assessed by preparing solutions of Cilnidipine and Telmisartan in the range of 10%-50% and 40%-200% of working concentration, respectively. Calibration curves were plotted using five concentrations and each level was injected in triplicate. [11]

Acceptance Criteria:

- Correlation Coefficient: NLT 0.98
- Intercept and Slope: Reported

Precision Study Procedure:

Intraday and Interday Precision:

Test solutions were analyzed three times a day for intraday precision and on two different days for interday precision. Results were reported as %RSD. [12]

Acceptance Criteria:

- %RSD: NMT 2.0

LOD and LOQ:

Detection and Quantitation Limits:

LOD and LOQ were determined based on the standard deviation of the y-intercept and slope from the linearity curves using the formulas: 13]

- LOD = 3.3 σ / S

- LOQ = 10 σ / S

Accuracy (% Recovery):

Samples were prepared covering 50% to 150% of the nominal concentration. Three preparations at each level were analyzed, and recoveries were calculated. [14-17]

Robustness:

The method's robustness was evaluated by making deliberate changes to the pH and detection wavelength. System suitability parameters were assessed for variations. [18-21]

Ruggedness:

Ruggedness was assessed by analyzing test solutions under different conditions (system, analyst, and atmospheric changes). Solutions were injected at 1.0 mL/min by two different analysts.[22]

Assay of Marketed Formulation:

Marketed Sample (Telista CL):

Contains Cilnidipine 10 mg and Telmisartan 40 mg. Tablets were weighed, crushed, and a 1000 ppm solution was prepared for analysis. Dilutions of 30 ppm Cilnidipine and 120 ppm Telmisartan were made. [23]

System Suitability: System suitability was verified using five replicate injections of standard drug solution. [24]

Acceptance Criteria:

- Resolution: >1.75
- Theoretical Plates: >2000
- Tailing Factor: <2

Forced Degradation Study

Acid-Induced Degradation: Drug content in 1N HCl at 60°C for 30 min, neutralized, filtered, and analyzed.

Base-Induced Degradation: Drug content in 1N NaOH for 30 min, neutralized, filtered, and analyzed.

Peroxide Degradation: Drug content in 30% v/v H2O2 at 80°C for 1 hour, filtered, and analyzed.

Photolytic Degradation:

Drug content exposed to UV light for 24 hours.

Thermal Degradation: Solid drug exposed to dry heat at 80°C. [25-27]

RP-HPLC Method Development by QbD Approach

Quality Target Product Profile (QTPP): Identified retention time, theoretical plates, and peak asymmetry.

Critical Quality Attributes (CQAs): Mobile phase composition and pH were controlled to maintain QTPP parameters. [28]

QTPP:

- Identified retention time, theoretical plates, and peak asymmetry.[29]

CQAs:

- Controlled mobile phase composition and pH to maintain QTPP parameters.[30]

Factorial Design:

- Central composite design applied to optimize mobile phase composition and pH.
- A 2-factor, 3-level design using Design Expert® (Version 11.0, Stat-Ease Inc., M M) to explore interactions and quadratic effects.[31]

Evaluation of Experimental Results:

- Assessed retention time, theoretical plates, and peak asymmetry.
- Optimized conditions using CCD approach, ensuring method robustness and reproducibility.[32]

Risk Assessment:

- Applied QbD principles from ICH Q8 and ICH Q9 guidelines.
- Evaluated method robustness and ruggedness under various conditions.[33]

Control Strategy:

- Implemented to ensure consistent performance and quality.
- Analytical control strategy set for sample preparation, measurement, and replicate control.[34]

Continual Improvement:

- Monitoring and maintaining quality consistency through periodic instrument maintenance and software updates.[35,36]

RESULT AND DISCUSSION

The results and discussion presented provide a comprehensive analysis of the identification, solubility, stability, analytical method development, validation, and performance characteristics for the simultaneous estimation of Cilnidipine and Telmisartan using RP-HPLC.

Identification of Drugs

Organoleptic Properties

The organoleptic properties of the drugs were evaluated, as detailed in Table 1. Cilnidipine appears as a light green powder, while Telmisartan is a white to slightly yellowish solid, and both are odourless.

Sr. No.	Organoleptic Property	Cilnidipine	Telmisartan
1	**Color**	Light green	White to slightly yellowish solid.
2	**Odor**	Odorless	Odorless

Table No. 2: Organoleptic properties of drugs

Melting Point

The melting points for the drugs were determined as shown in Table 2. Cilnidipine exhibited a melting point in the range of 105-110°C, and Telmisartan exhibited a melting point in the range of 261-263°C.

Sr. No.	Name of Drug	Melting Point in ^{0}C
1	**Cilnidipine**	105-110 ^{0}C
2	**Telmisartan**	261-263 ^{0}C

Table No. 3: Melting point study

Solubility Study

The solubility of both drugs in various solvents was assessed and presented in Table 3. Cilnidipine was found to be insoluble in water, freely soluble in methanol, and sparingly soluble in aqueous buffer. Telmisartan was practically insoluble in water, soluble in methanol, and sparingly soluble in aqueous buffer.

Sr. No	Solvent	Cilnidipine	Telmisartan
1	Water	Insoluble	Practically Insoluble
2	Methanol	Freely Soluble	Soluble
3	Aqueous Buffer	Sparingly soluble	Sparingly soluble

Table No. 4: Solubility Study

Stability Study

Stability studies indicated no degradation of the samples in methanol and water, confirming the stability of both drugs under the experimental conditions.

Selection of Analytical Wavelength

The wavelength of maximum absorbance (λmax) was determined using a UV spectrum of methanol as a blank and an overlay UV spectrum of Cilnidipine and Telmisartan (Fig. 1). The chosen analytical wavelength was 232 nm.

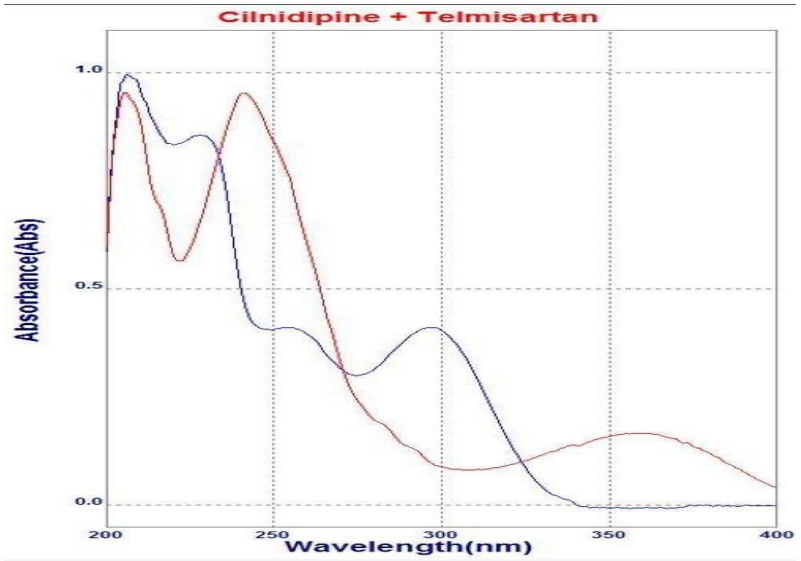

Figure No. 1 Overlay UV spectrum of Cilnidipine and Telmisartan

Development of Simultaneous RP-HPLC Method

The RP-HPLC method was developed for the simultaneous estimation of Cilnidipine and Telmisartan, with the mobile phase comprising Methanol: Water (70:30) and detection at 232 nm. The chromatographic separation was achieved with a Cosmosil C18 column. The optimized chromatographic conditions and results from the trials are summarized in the following:

- Retention Times: Telmisartan (4.623 min) and Cilnidipine (10.379 min)
- Tailing Factors: Telmisartan (1.168) and Cilnidipine (1.237)
- Theoretical Plates: Telmisartan (7565.394) and Cilnidipine (8360.216)

Linearity

The linearity of the method was established within a concentration range, and the regression analysis for both drugs showed high correlation coefficients ($r^2 > 0.9995$). The calibration curves (Figs. 2 and 4) and linearity data (Table 5) demonstrate the method's accuracy and reliability.

Sr. No.	Cilnidipine		Telmisartan	
	Concentration (ppm)	Area	Concentration (ppm)	Area
1	10	247157	40	613205
2	20	497139	80	1098001
3	30	744442	120	1648316
4	40	986201	160	2196168
5	50	1212951	200	2743234

Table No 5: - Linearity Data for Cilnidipine and Telmisartan by RP-HPLC method

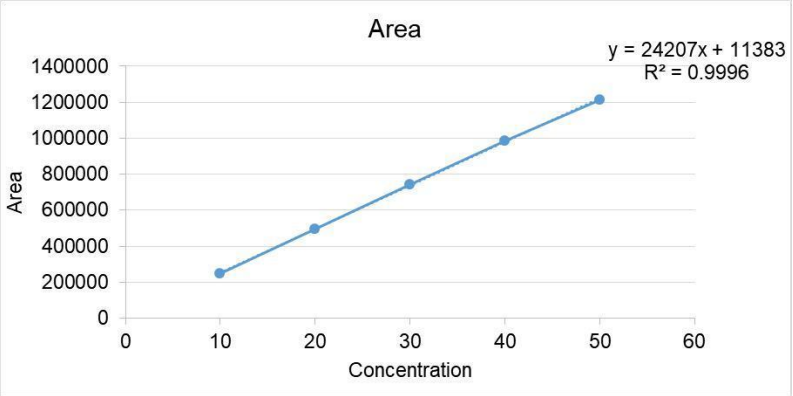

Figure No. 2 Calibration curve of Cilnidipine

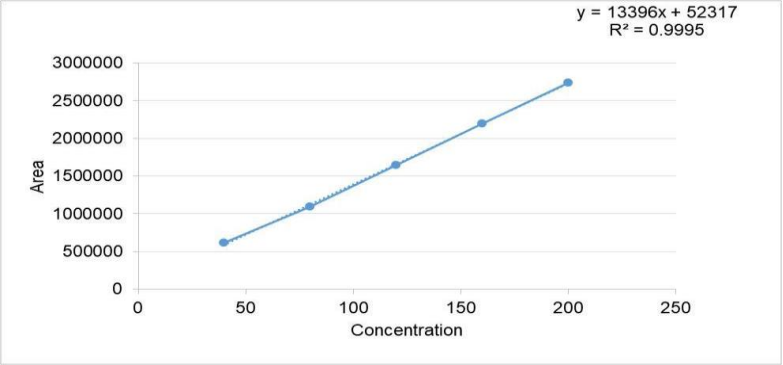

Figure No. 3 Calibration curve of Telmisartan

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Accuracy

The accuracy of the method was confirmed by recovery studies, with results close to 100% recovery for both drugs, indicating minimal deviation from true values (Tables 5 and 6).

Cilnidipine			Standard Deviation		Accuracy	Precision
Sr. No.	Conc.	Area	Mean	SD	%SD	%RSD
1	10 10 10	247157 245224 246603	246328	995.4099658	0.404099	
2	30 30 30	744442 745107 744870	744806.3333	337.0405515	0.045252	0.203817796
3	50 50 50	1212951 1219289 1209966	1214068.667	4760.931246	0.392147	

Table No.6: Data for recovery study of Cilnidipine by RP-HPLC

Telmisartan			Standard Deviation		Accuracy	Precision
Sr. No.	Conc.	Area	Mean	SD	%SD	%RSD
1	40 40 40	613205 616248 613206	614219.6667	1756.588265	0.285987	
2	120 120 120	1648316 1663341 1657175	1656277.333	7552.616125	0.455999	0.241999882
3	200 200 200	2743234 2717881 2759381	2740165.333	20919.48939	0.763439	

Table No.7: Data for recovery study of Telmisartan by RP-HPLC

Precision

Precision was evaluated in terms of inter-day and intra-day variability. Both Cilnidipine and Telmisartan exhibited low %RSD values, indicating high precision in the analytical method (Tables 8 and 9).

Interday			Standard Deviation		Accuracy	Precision
Sr. No.	Conc.	Area	Mean	SD	%SD	%RSD
1	30 30 30	744442 745107 744870	744806.3333	337.0405515	0.0452521	0.33113455
2	30 30 30	747190 740682 747423	745098.3333	3826.430756	0.5135471	

Table No 8:- Result for Cilnidipine of Intra- day and Inter- Day and intraday Precision oftest sample assay.

Intraday			Standard Deviation		Accuracy	Precision
Sr. No.	Conc.	Area	Mean	SD	%SD	%RSD
1	30 30 30	744442 745107 744870	744806.3333	337.0405515	0.0452521	0.20032424
2	30 30 30	751514 748334 746672	748840	2460.339001	0.3285534	

Table No 9:- Result for Telmisartan of Intra- day and Inter- Day and intraday Precision oftest sample assay.

Robustness

Robustness testing showed that small deliberate changes in pH and wavelength did not significantly affect the %RSD values for either drug, confirming the method's robustness as per ICH guidelines (Table 10).

Sr. No	Parameter	Conc.	Telmisartan			
			Area	Mean	SD	%RSD
1	Change in P^H	80	1098001	1092084.3	6244.435	0.5717906
2		80	1085557			
3		80	1092695			
1	Change in Wavelength (nm)	80	1098001	1093845.3	3641.379	0.3328971
2		80	1092322			
3		80	1091213			
Sr. No	Parameter	Conc.	Cilnidipine			
			Area	Mean	SD	%RSD
1	Change in P^H	20	497139	495931	2791.45	0.5628706
2		20	492739			
3		20	497915			
1	Change in Wavelength (nm)	20	497139	498184	947.7663	0.1902442
2		20	498988			
3		20	498425			

Table No.10: Data for Robustness study of Telmisartan and Cilnidipine

Ruggedness

Ruggedness studies were performed by different analysts, and the %RSD values remained within acceptable limits, further validating the method's reliability (Table 11).

Sr. No.	Cilnidipine		Telmisartan	
	Concentration (ppm)	Area	Concentration (ppm)	Area
1	10	232998	40	611840
2	20	486588	80	1099153
3	30	742736	120	1637477
4	40	961630	160	2149219
5	50	1208545	200	2701397

Table No.11: Data for Ruggedness Study of Cilnidipine and Telmisartan

Assay of Marketed Formulation

The % assay of the marketed formulation (Telista CL) was found to be within the acceptable range of 99-100% for both Cilnidipine and Telmisartan (Tables 12, 13).

Sr. NO.	Conc.	Area of Standard	Area of Sample	% Assay
1	**30ppm**	744442	738923	99.2586394

Table No. 12: % Assay of Cilnidipine

Sr. NO.	Conc.	Area of Standard	Area of Sample	% Assay
1	**120ppm**	1648316	1636235	99.2670701

Table No. 13: % Assay of Telmisartan

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ were calculated for both drugs, with LOD values of 0.04594671 µg/mL for Cilnidipine and 0.432719767 µg/mL for Telmisartan, and LOQ values of 0.13923245 µg/mL for Cilnidipine and 1.31127202 µg/mL for Telmisartan (Table 14).

Sr. No.	Drug	SD	Slope	LOD	LOQ
1	Telmisartan	1756.58	13396	0.432719767	1.31127202
2	Cilnidipine	337.04	24207	0.04594671	0.13923245

Table No14: Result Summary of LOD and LOQ

Discussion: The developed RP-HPLC method for the simultaneous estimation of Cilnidipine and Telmisartan is validated with respect to linearity, accuracy, precision, robustness, ruggedness, and specificity. The method is suitable for routine analysis of these drugs in bulk and dosage forms.

Forced Degradation Studies

Forced degradation studies were conducted to assess the stability and specificity of Cilnidipine and Telmisartan under different stress conditions, including acid, base, oxidative, photolytic, and thermal environments.

Telmisartan:

- **Acid Degradation**: The percentage degradation was found to be 13.16% with a significant decrease in peak area (from 2743234 to 2382211).
- **Base Degradation**: Telmisartan showed a higher degradation of 15.46%, indicating susceptibility under basic conditions.
- **Oxidative Degradation**: The degradation was minimal at 3.52%, suggesting resistance to oxidative stress.
- **Photolytic Degradation**: Telmisartan showed negligible degradation (0.17%) under photolytic conditions, indicating high photostability.
- **Thermal Degradation**: The degradation was also minimal at 1.08%, suggesting thermal stability.

Cilnidipine:

- **Acid Degradation**: Cilnidipine exhibited 12.06% degradation, with a decrease in peak area from 1212951 to 1066721.
- **Base Degradation**: A higher degradation of 15.92% was observed, indicating sensitivity to basic conditions.
- **Oxidative Degradation**: Degradation was minimal at 3.20%, showing oxidative stability.
- **Photolytic Degradation**: Cilnidipine was highly photostable with only 0.83% degradation.

- **Thermal Degradation**: The degradation was minimal at 1.81%, indicating stability under thermal stress.

Chromatographic Performance:

- For both Telmisartan and Cilnidipine, the Retention Time (RT), Theoretical Plate Count, and Tailing Factor were within acceptable ranges across all stress conditions. The resolution between the peaks was consistently high, indicating good separation and method specificity.

RP-HPLC Method Development and Optimization:

The RP-HPLC method was developed and optimized using a Central Composite Design (CCD) approach. The method parameters, including mobile phase composition, flow rate, and wavelength, were systematically varied to achieve optimal separation and quantification of Telmisartan and Cilnidipine. The final optimized conditions consisted of a mobile phase composition of 70% acetonitrile, a flow rate of 0.9 mL/min, and detection at 234 nm. Under these conditions, Telmisartan had a retention time of 5.88 minutes, and Cilnidipine had a retention time of 11.98 minutes.

ANOVA Analysis:

ANOVA results for the RP-HPLC method confirmed the significance of the model with an F-value of 8.15 for Cilnidipine's asymmetry factor and 7.88 for its area, indicating that the model reliably predicts the outcomes. Significant factors influencing the Cilnidipine peak area included mobile phase composition (A) with a p-value of 0.0040 and flow rate (B) with a p-value of 0.0015. The interaction between mobile phase composition and wavelength (AC) was also significant, with a p-value of 0.0394 for asymmetry. The model fit was strong, with an R^2 value of 0.91 for asymmetry, demonstrating that the developed method is robust and reliable.

The optimization ensured that the method produced sharp, well-resolved peaks with theoretical plate counts exceeding 6,500 for Telmisartan and 9,000 for Cilnidipine, confirming excellent column efficiency. Peak asymmetry was within the acceptable range, with values close to 1.0 for both compounds, indicating symmetrical peak shapes essential for accurate quantification.

Discussion: The forced degradation and method development studies successfully established a robust RP-HPLC method for the simultaneous estimation of Telmisartan and Cilnidipine. The method was validated for stability-indicating capacity, with significant degradation observed under specific stress conditions. The optimized chromatographic conditions provided accurate, precise, and reproducible results, making this method suitable for routine analysis in quality control laboratories.

The statistical analysis of the RP-HPLC method for the quantification of Cilnidipine and Telmisartan is summarized below with specific results and interpretations.

Response: TEL AF

- Model Significance: The Model F-value of 9.95 indicates that the model is significant with a very low probability (0.11%) that this F-value could occur due to noise.
- Significant Factor: Factor A (Composition) is significant with a p-value of 0.000273287.
- Model Fit: R^2: 0.6966, indicating that approximately 69.66% of the variability in the response is explained by the model.
- Adjusted R^2: 0.6266, which accounts for the number of predictors in the model.
- Predicted R^2: 0.3654, which is not as close to the Adjusted R^2, suggesting potential issues such as a large block effect.
- Adequate Precision: 9.765, showing that the model has a good signal-to-noise ratio.
- Regression Equation (Coded Factors)
- TEL AF = 0.9948 - 0.05625 **A + 0.020125 B - 0.017625 C**

Response: TEL Area

- Model Significance: The Model F-value of 9.80 indicates that the model is significant with a very low probability (0.12%) that this F-value could occur due to noise.
- Significant Factor: Factor B (Flowrate) is significant with a p-value of 0.000120818.
- Model Fit: R^2: 0.6934, indicating that approximately 69.34% of the variability in the response is explained by the model.
- Adjusted R^2: 0.6227, which accounts for the number of predictors in the model.
- Predicted R^2: 0.3735, which is not as close to the Adjusted R^2, indicating possible issues such as a large block effect.
- Adequate Precision: 8.480, indicating a good signal-to-noise ratio.
- Regression Equation (Coded Factors):
- TEL Area = 692182.5882 - 3996.625 **A - 51929.625 B - 2301.75 C**

Response: TEL RT

- Model Significance: The Model F-value of 1771.65 indicates that the model is highly significant.
- Significant Factors: Factors A (Composition), B (Flowrate), AB interaction, and A^2 are significant.

- Model Fit: R^2: 0.9996, indicating that 99.96% of the variability in the response is explained by the model.
- Adjusted R^2: 0.9990, showing excellent fit.
- Predicted R^2: 0.9930, which is in reasonable agreement with the Adjusted R^2.
- Adequate Precision: 117.449, indicating an excellent signal-to-noise ratio.
- Regression Equation (Coded Factors):
- TEL RT = 5.885 - 7.697375 **A - 0.98075 B + 0.107625 C + 0.47575 AB + 5.08775 A^2**

Response: TEL TP

- Model Significance: The Model F-value of 29.00 implies the model is significant.
- Significant Factors: Factors A (Composition), B (Flowrate), A^2, and B^2 are significant
- Model Fit: R^2: 0.9739, indicating that 97.39% of the variability in the response is explained by the model.
- Adjusted R^2: 0.9403, suggesting a very good fit.
- Predicted R^2: 0.5821, which is not as close to the Adjusted R^2, indicating possible issues with model or data.
- Adequate Precision: 15.902, indicating a strong signal-to-noise ratio.
- Regression Equation (Coded Factors):
- TEL TP = 5967.511 + 32601722 **A + 2494071 B + 1063232 C + 193463.6 AB + 514218.1 AC + 1096062 BC + 27991246 A^2 + 3677120 * B^2 + 74470.2 C^2**

ANOVA for Quadratic Model of Resolution

The quadratic model for the resolution response was found to be significant with a Model F-value of 70.50 ($p = 0.0000049$), indicating a strong predictive capability. Significant model terms included Composition (A) and its squared term (A^2), with p-values of 1.64E-07 and 1.76E-06, respectively. Flowrate (B), Wavelength (C), and their interactions were not significant ($p > 0.1$).

The standard deviation of the model was 0.886995, with a mean response of 11.69929, yielding a coefficient of variation (CV %) of 7.58%, which is acceptable. The adjusted R^2 (0.9751) and predicted R^2 (0.8254) were in close agreement, showing that the model is well-fitted with minimal overfitting. The adequate precision ratio was 20.709, indicating an adequate signal for navigating the design space.

Coefficients in Coded Factors

The final equation in coded factors for resolution was derived, showing that an increase in the composition (A) significantly increases the resolution, with a coefficient of 6.435375. The negative

coefficient for A^2 (-6.27488) suggests a quadratic relationship where resolution decreases after a certain level of composition. The low VIF values (all around 1) indicate minimal multicollinearity among the factors.

Design Summary and Responses

The design utilized a Box-Behnken quadratic model with three factors: Composition (%), Flowrate (ml/min), and Wavelength (nm). The study included 17 randomized runs. The observed responses showed substantial variability, with resolution ranging from 1.408 to 16.502 units. The mean resolution was 11.69929, with a standard deviation of 5.616231. This wide range and high ratio (11.72) indicate a significant effect of the experimental conditions on resolution.

Prediction and Validation

The predicted mean resolution for the optimal composition (70%), flow rate (1 ml/min), and wavelength (232 nm) was 15.50163 units, with a confidence interval (CI) ranging from 13.6852 to 17.3180 units. The model's PRESS value of 88.11707 and other fit statistics, such as the -2 Log Likelihood (29.0826), BIC (57.41473), and AICc (85.74927), further confirmed the model's robustness.

Discussion: The study demonstrated that the composition had the most significant impact on resolution, both linearly and quadratically. The model's high precision and accurate predictions suggest it is reliable for optimizing resolution in similar settings. This model can effectively guide future experiments to achieve desired resolution outcomes within the design space.

Design Summary:

- **File Version** 13.0.5.0
- **Study Type** -Response and Subtype- Randomized
- **Design Type-** Box-Behnken
- **Run** -17
- **Design Model-** Quadratic
- **Blocks-** No
- **Build Time**-24

Factor	Name	Units	Type	Subtype	Minimum	Maximum	Coded	Values	Mean
A	Composition	%	Numeric	Continuous	60	80	False	1.000=80	70
B	Flowrate	ml/ min	Numeric	Continuous	0.8	1	False	1.000=1	0.9
C	Wavelength	nm	Numeric	Continuous	232	236	False	1.000=236	234

Response	Name	Units	Obs	Minimum	Maximum	Mean	Std. Dev.	Ratio
R1	TEL RT	min	17	2.831	19.758	8.150176	6.083825	6.979159
R2	CIL RT	min	17	6.404	23.099	12.93294	5.042292	3.606964
R3	TEL Area	AU	17	609636	767533	692182.6	44269	1.259002
R4	CIL Area	AU	17	175800	351657	229644.5	38561.49	2.000324
R5	Resolution	Units	17	1.408	16.502	11.69929	5.616231	11.72017
R6	TEL TP	Unit	17	2126.7	8773.59	5967.511	2099.058	4.125448
R7	CIL TP	Units	17	8365.489	10530.66	9563.957	597.1696	1.258821
R8	TEL AF	Units	17	0.91	1.093	0.994765	0.052771	1.201099
R9	CIL AF	Units	17	1.11	1.3	1.204765	0.063827	1.171171

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CONCLUSION

In conclusion, the developed RP-HPLC method for the simultaneous estimation of Cilnidipine and Telmisartan in bulk and pharmaceutical dosage forms has proven to be simple, accurate, precise, and robust, adhering to ICH guidelines. The method demonstrated excellent linearity with correlation coefficients of 0.9996 for Cilnidipine and 0.9995 for Telmisartan. The retention times were 4.623 min for Cilnidipine and 10.379 min for Telmisartan, with adequate sensitivity as indicated by low LOD and LOQ values. Additionally, stress degradation studies confirmed the method's specificity, effectively separating degradation products from the active drugs. This validated method is suitable for routine analysis and quality control of Cilnidipine and Telmisartan in both bulk and tablet dosage forms.

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