Analytical Method Development and Validation of Combined Anti-Hypertensive Agents in Bulk and Dosage Forms Using RP-HPLC According to ICH Guidelines

V Raja lokesh[1], Thirupal[2]
[1,2]*Sun Institute of Pharmaceutical Education and Research*

***Abstract*—A simple, accurate, precise, and robust reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Deserpidine and Methyclothiazide in bulk drug substances and pharmaceutical dosage forms. Chromatographic separation was achieved using an Agilent C18 column (250 × 4.6 mm, 5 µm) with a mobile phase consisting of phosphate buffer (pH 4.0) and acetonitrile in the ratio of 30:70 v/v at a flow rate of 1.0 mL/min. Detection was performed at 254 nm. The retention times of Methyclothiazide and Deserpidine were found to be 2.577 min and 3.503 min respectively. The developed method was validated according to ICH Q2(R1) guidelines for specificity, linearity, accuracy, precision, robustness, LOD, and LOQ. The calibration curves showed excellent linearity in the concentration range of 20–100 µg/mL with correlation coefficients of 0.999 for both analytes. The percentage recoveries ranged from 99–102%, indicating excellent accuracy. Precision studies revealed %RSD values below 2%, confirming method reproducibility. The method demonstrated satisfactory robustness under deliberate variations in chromatographic conditions. The developed RP-HPLC method can be successfully employed for routine quality control analysis of Deserpidine and Methyclothiazide in pharmaceutical formulations.**

***Index Terms*—RP-HPLC, Deserpidine, Methyclothiazide, Method Validation, ICH Guidelines, Pharmaceutical Analysis.**

I. INTRODUCTION

Pharmaceutical analysis plays a crucial role in ensuring the quality, safety, and efficacy of pharmaceutical products. Analytical methods are extensively employed in drug development, process validation, quality control, and regulatory compliance. The development of a reliable analytical method requires optimization of chromatographic parameters to achieve accurate and reproducible quantification of active pharmaceutical ingredients.
High-performance liquid chromatography (HPLC) is one of the most widely used analytical techniques in pharmaceutical industries due to its high sensitivity, specificity, precision, and ability to separate complex mixtures. RP-HPLC has become the preferred chromatographic technique for quantitative estimation of drugs because of its versatility and excellent reproducibility.

Deserpidine is an ester alkaloid antihypertensive drug isolated from Rauwolfia canescens. It acts by inhibiting the ATP/Mg^{2+} pump responsible for neurotransmitter storage in presynaptic vesicles, resulting in depletion of catecholamines and reduction of blood pressure. Methyclothiazide is a thiazide diuretic that inhibits sodium and chloride reabsorption in renal tubules, thereby promoting diuresis and antihypertensive activity. The combination of these drugs is widely used in the management of hypertension.

The simultaneous estimation of Deserpidine and Methyclothiazide requires a selective and validated analytical method capable of accurately quantifying both drugs in the presence of formulation excipients. Therefore, the present study aimed to develop and validate an RP-HPLC method for simultaneous determination of these antihypertensive agents according to ICH Q2(R1) guidelines.

II. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Deserpidine and Methyclothiazide reference standards were obtained from certified pharmaceutical sources. HPLC-grade acetonitrile, methanol, potassium dihydrogen phosphate, orthophosphoric acid, and purified water were used throughout the study. All reagents were of analytical grade.

2.2 Instrumentation

The chromatographic analysis was performed using a Shimadzu HPLC system equipped with LC-20AD pump, SPD-20MA UV detector, autosampler, and LC Solution software. An Agilent C18 column (250 × 4.6 mm, 5 µm) was employed for chromatographic separation.

2.3 Chromatographic Conditions

Parameter	Condition
Column	Agilent C18 (250 × 4.6 mm, 5 µm)
Mobile Phase	Phosphate Buffer (pH 4.0): ACN (30:70 v/v)
Flow Rate	1.0 mL/min
Detection Wavelength	254 nm
Injection Volume	20 µL
Run Time	6 min
Temperature	Ambient
Mode	Isocratic

2.4 Preparation of Standard Solution

Accurately weighed quantities of Deserpidine and Methyclothiazide standards were transferred into volumetric flasks and dissolved in mobile phase to obtain stock solutions. Appropriate dilutions were prepared to obtain working concentrations ranging from 20–100 µg/Ml

2.5 Sample Preparation

Twenty tablets were weighed and powdered. An amount equivalent to the labeled claim was transferred into a volumetric flask, dissolved in diluent, sonicated, filtered, and diluted appropriately to obtain the test concentration.

III. METHOD VALIDATION

The developed RP-HPLC method was validated according to ICH Q2(R1) guidelines for specificity, system suitability, linearity, accuracy, precision, robustness, LOD, and LOQ.

3.1 System Suitability

System suitability testing was performed before analysis to verify chromatographic performance.

Table 1: System Suitability Parameters

Parameter	Methyclothiazide	Deserpidine
Retention Time (min)	2.577	3.503
Theoretical Plates	2936	5824
Tailing Factor	1.2	1.3
Resolution	-	9.4

The obtained values were within acceptable limits, indicating suitability of the chromatographic system.

3.2 Linearity

Linearity was evaluated by analyzing five concentration levels ranging from 20–100 µg/mL.

Table 2: Linearity Data

Concentration (µg/mL)	Methyclothiazide Peak Area	Deserpidine Peak Area
20	214568	175432
40	428695	351284
60	642134	527946
80	856420	702513
100	1069874	878624

Table 3: Regression Analysis

Parameter	Methyclothiazide	Deserpidine
Range (µg/mL)	20–100	20–100
Correlation Coefficient (R^2)	0.999	0.999
Slope	10685	8782
Intercept	1245	987

Excellent linearity was observed over the selected concentration range.

3.3 Accuracy

Accuracy was determined by recovery studies at 50%, 100%, and 150% concentration levels.

Table 4: Accuracy Results

Level	Methyclothiazide Recovery (%)	Deserpidine Recovery (%)
50%	100.2	102.3
100%	101.0	102.8
150%	101.8	102.4
Mean Recovery	101.0	102.5

Recovery values were within the acceptable range of 98–102%, demonstrating the accuracy of the method.

3.4 Precision

Precision was assessed by repeatability and intermediate precision studies.

Table 5: Precision Results

Parameter	Methyclothiazide	Deserpidine
Repeatability (%RSD)	0.5	0.6
Intermediate Precision (%RSD)	0.6	0.7

The low %RSD values indicate excellent precision and reproducibility.

3.5 Limit of Detection and Limit of Quantification

Table 6: Sensitivity Parameters

Parameter	Methyclothiazide	Deserpidine
LOD (µg/mL)	3.02	3.10
LOQ (µg/mL)	10.0	10.1

The method demonstrated adequate sensitivity for routine pharmaceutical analysis.

3.6 Robustness

Robustness was evaluated by deliberately varying chromatographic conditions such as flow rate and mobile phase composition.

Table 7: Robustness Results

Parameter Variation	Methyclothiazide (%RSD)	Deserpidine (%RSD)
Flow Rate ±0.1 mL/min	0.68	0.72
Organic Phase ±2%	0.74	0.81

The method remained unaffected by minor variations, confirming its robustness.

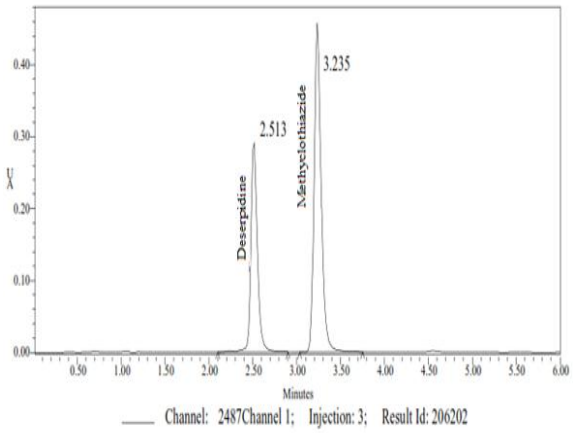

Figure 1: Standard Chromatogram of Deserpidine and Methyclothiazide

IV. RESULTS AND DISCUSSION

A successful RP-HPLC method was developed for simultaneous estimation of Deserpidine and Methyclothiazide. Optimization of chromatographic conditions resulted in well-resolved peaks with retention times of 2.577 min and 3.503 min respectively. The resolution value of 9.4 demonstrated excellent separation between analytes. System suitability parameters such as theoretical plates and tailing factors complied with recommended acceptance criteria.

The method exhibited excellent linearity over the concentration range studied with correlation coefficients greater than 0.999. Recovery studies demonstrated accuracy, while precision studies yielded %RSD values below 2%, confirming repeatability and reproducibility. LOD and LOQ values indicated good sensitivity. Robustness evaluation showed negligible impact on chromatographic performance after deliberate method variations.

The developed analytical procedure offers several advantages including simplicity, short run time, cost-effectiveness, and suitability for routine quality control laboratories. The method is capable of simultaneously quantifying both antihypertensive agents without interference from formulation excipients.

V. CONCLUSION

A validated RP-HPLC method was successfully developed for simultaneous estimation of Deserpidine and Methyclothiazide in bulk and pharmaceutical dosage forms. The method demonstrated excellent specificity, linearity, accuracy, precision, sensitivity, and robustness according to ICH Q2(R1) guidelines. Retention times were observed at 2.577 min and 3.503 min for Methyclothiazide and Deserpidine respectively. The validated method can be reliably employed for routine quality control, stability studies, and pharmaceutical analysis of combined antihypertensive formulations.

VI. FUTURE PERSPECTIVES

Future studies may focus on extending the developed RP-HPLC method to stability-indicating analysis through forced degradation studies. The method can also be adapted to UHPLC platforms for faster analysis and improved sensitivity. Further application in pharmacokinetic and bioequivalence studies may facilitate comprehensive evaluation of Deserpidine and Methyclothiazide formulations.

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