

**DEVELOPMENT OF RP-HPLC METHOD FOR
HYDROCHLOROTHIAZIDE AND METOPROLOL SUCCINATE IN
TABLET FORMULATION****Arun, Deepanshu Yadav, Gaurav Singh, Sahil, Sakshi, Ankit, Aditya, Sonali***Shri baba mastnath institute of pharmaceutical and research Baba Mastnath University,
Asthal Bohar, Rohtak.**Article Received: 29 April 2026, Article Revised: 19 May 2026, Published on: 09 June 2026*****Corresponding Author: Sonali**

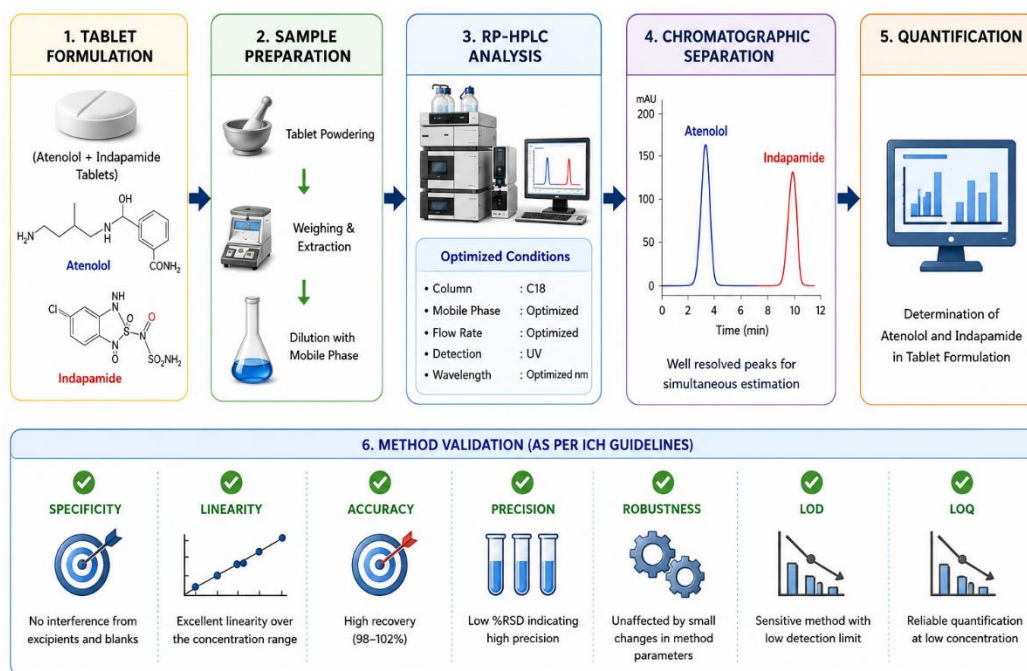
Shri baba mastnath institute of pharmaceutical and research Baba Mastnath University, Asthal Bohar, Rohtak.

DOI: <https://doi-doi.org/101555/ijarp.7414>**ABSTRACT**

A novel, precise, and stability-indicating reverse-phase form of high-performance liquid chromatography (RP-HPLC) method turned into developed and showed for the simultaneous estimation of Hydrochlorothiazide (HCTZ) and Metoprolol Succinate (MET) in bulk drug and formulated tablets. The approach changed into optimized to offer green separation and correct quantification, even within the presence of decay products. Chromatographic separation has become carried out the usage of a C18 column with a cellular phase comprising phosphate buffer (pH 3.0, adjusted with orthophosphoric acid) and acetonitrile in a ratio of 60: 40v/v. The go with the float charge was maintained at 1.0 mL/min, with detection at 225 nm. The technique verified superb linearity for HCTZ (five–50 µg/mL) and MET (10–a hundred µg/mL) with a regression coefficient (R^2) > 0.999. Forced degradation studies underneath acidic, alkaline, oxidative, thermal, and photolytic conditions confirmed the technique's stability-indicating capability, as degradation products have been nicely separated without interference in drug peaks. Validation, achieved as in keeping with ICH Q2 (R1) hints, installed specificity, accuracy, precision, robustness, and sensitivity with notable healing costs (98-102%). The proposed technique come to be correctly carried out to the quantification of HCTZ and MET in business tablets, proving its suitability for normal best manage and balance studies.

KEYWORDS: Atenolol, Indapamide; RP-HPLC; Method Development; Method Validation; Tablet Formulation; Simultaneous Estimation; ICH Guidelines.

GRAPHICAL ABSTRACT



INTRODUCTION

Hypertension remains one of the most prevalent cardiovascular disorders worldwide and is considered a major risk factor for stroke, myocardial infarction, heart failure, and chronic kidney disease.[1] According to recent global health reports, the incidence of hypertension continues to rise due to sedentary lifestyles, unhealthy dietary habits, obesity, stress, and aging populations. Effective management of hypertension often requires combination therapy involving drugs with complementary mechanisms of action.[2] Among the commonly prescribed antihypertensive combinations is Atenolol and Indapamide, which provide enhanced blood pressure control and improved cardiovascular outcomes. Atenolol is a cardioselective β_1 -adrenergic receptor blocker belonging to the class of beta-blockers.[3] It acts by reducing heart rate, myocardial contractility, and cardiac output, thereby decreasing blood pressure and myocardial oxygen demand. Due to its selectivity for β_1 receptors, Atenolol is widely used in the treatment of hypertension, angina pectoris, cardiac arrhythmias, and post-myocardial infarction management. The drug has gained significant clinical importance because of its effectiveness, safety profile, and long-term cardiovascular protective effects.[4] Indapamide belongs to the class of thiazide-like diuretics and is extensively used as an antihypertensive agent. It produces its therapeutic effect by increasing sodium and water excretion through the kidneys while also exerting direct vasodilatory effects on blood vessels. Indapamide is considered advantageous over conventional diuretics

due to its prolonged duration of action and lower incidence of metabolic side effects. The combination of Atenolol and Indapamide is frequently prescribed because it targets different physiological pathways involved in hypertension, resulting in better blood pressure control than monotherapy.[5] In the current pharmaceutical market, fixed-dose combination formulations of Atenolol and Indapamide have become increasingly popular due to improved patient compliance, reduced pill burden, and enhanced therapeutic efficacy. The growing demand for these formulations necessitates the development of reliable analytical methods capable of accurately estimating both active pharmaceutical ingredients in combined dosage forms. Accurate quantitative analysis is essential to ensure product quality, safety, efficacy, and regulatory compliance throughout the manufacturing process.[6] Analytical method validation is a critical component of pharmaceutical quality assurance. Regulatory authorities such as the International Council for Harmonisation (ICH), the United States Food and Drug Administration (USFDA), and other global agencies require validated analytical methods for routine quality control testing. Method validation demonstrates that an analytical procedure consistently produces accurate, precise, specific, and reproducible results suitable for its intended purpose. Parameters such as specificity, linearity, accuracy, precision, robustness, limit of detection (LOD), and limit of quantification (LOQ) are evaluated to establish method reliability.[7] Among various analytical techniques, Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) is one of the most widely employed methods for pharmaceutical analysis because of its high sensitivity, selectivity, accuracy, and reproducibility. Future advancements in pharmaceutical analysis are expected to focus on the development of greener, faster, and more cost-effective analytical methods.[8] Modern chromatographic systems integrated with automation, artificial intelligence, and advanced detection technologies are likely to enhance analytical efficiency and regulatory compliance. Therefore, the development and validation of a robust RP-HPLC method for the simultaneous estimation of Atenolol and Indapamide is of significant importance for ensuring pharmaceutical quality, supporting regulatory requirements, and facilitating future analytical innovations. The present study was undertaken to develop and validate a simple, accurate, precise, and reliable RP-HPLC method for the estimation of Atenolol and Indapamide in tablet formulations according to ICH guidelines.[9]

MATERIALS AND METHODS

MATERIALS

Atenolol and Indapamide reference standards were obtained as gift samples from a pharmaceutical company. The tablet formulation containing Atenolol and Indapamide was purchased from a local pharmacy in Moradabad, Uttar Pradesh, India. HPLC-grade acetonitrile and water were procured from Merck® India Ltd., Mumbai, India, while ammonium acetate was obtained from Rankem Laboratory Chemicals. The buffer solution was prepared using ammonium acetate and its pH was adjusted with glacial acetic acid. All solvents and reagents used in the study were of analytical reagent grade and were used without further purification.

METHODOLOGY

1. Chemicals and Reagents

Atenolol (ATN) and Indapamide (IND) reference standards were obtained from AKUMS Drugs & Pharmaceuticals Ltd., Haridwar, Uttarakhand, India. All reagents and solvents used were of HPLC grade, including acetonitrile, methanol, and water obtained from a Milli-Q purification system. Ammonium acetate was used for the preparation of the buffer solution employed in the chromatographic system, and its pH was adjusted to the optimum value using glacial acetic acid. All chemicals were used without further purification.

Chromatographic Conditions

Chromatographic analysis of Atenolol (ATN) and Indapamide (IND) was performed using a Hypersil ODS C18 column (250 mm × 4.6 mm, 2.5 μm particle size). The HPLC system consisted of a Shimadzu (Japan) LC-20AD pump, SPD-M20A diode array detector (DAD), and CBM-20A system controller operated through LC Solution software. The mobile phase comprised acetonitrile and 20 mM ammonium acetate buffer (pH 3.5) in the ratio of 40:60 (v/v). The flow rate was maintained at 1.0 mL/min and detection was carried out at an optimized wavelength. The analysis was performed at ambient temperature with an injection volume of 20 μL for each sample.

Mobile Phase Preparation

The mobile phase consisted of acetonitrile and 20 mM ammonium acetate buffer (pH 3.5) in the ratio of 45:55 (v/v). The prepared mobile phase was filtered through a 0.45 μm nylon membrane filter to remove particulate matter and degassed in an ultrasonic bath before use to ensure smooth flow and prevent air bubble formation during chromatographic analysis.

Preparation of Buffer Solution

A 20 mM ammonium acetate buffer solution was prepared by dissolving 1.54 g of ammonium acetate in 1 L of HPLC-grade water. The pH was adjusted to 3.5 using glacial acetic acid. The buffer solution was filtered through a 0.45 µm membrane filter and degassed by sonication prior to use.

Preparation of Standard Solution

Accurately weighed quantities of 50 mg Atenolol and 2.5 mg Indapamide reference standards were transferred into a 100 mL volumetric flask. About 80 mL of methanol was added, and the solution was sonicated for 10 min to ensure complete dissolution. The volume was then made up to the mark with methanol to obtain the stock solution. Appropriate dilutions were prepared with the mobile phase to obtain the desired working concentrations for calibration and validation studies.

Sample Solution Preparation

Twenty tablets containing Atenolol and Indapamide were accurately weighed and finely powdered. A quantity of powder equivalent to 50 mg of Atenolol and 2.5 mg of Indapamide was transferred into a 100 mL volumetric flask. Approximately 80 mL of methanol was added, and the mixture was sonicated for complete extraction of the drugs. The volume was made up to 100 mL with methanol and filtered through a 0.45 µm membrane filter before analysis.

Chromatographic Examination

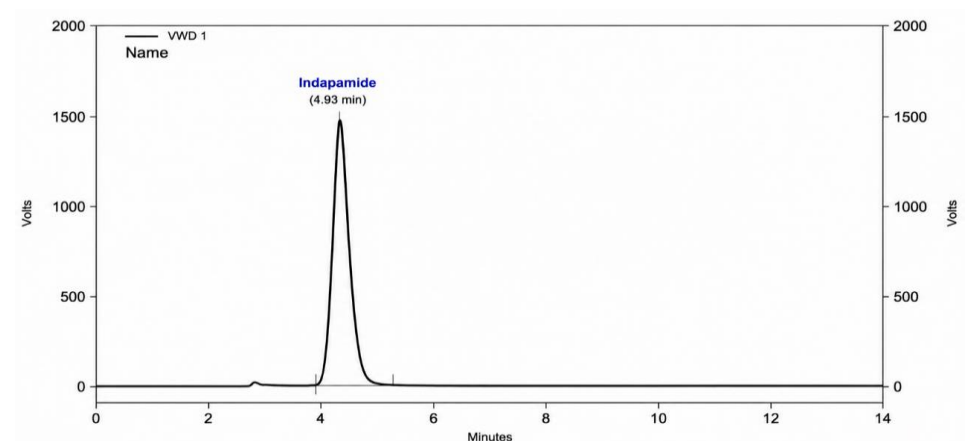
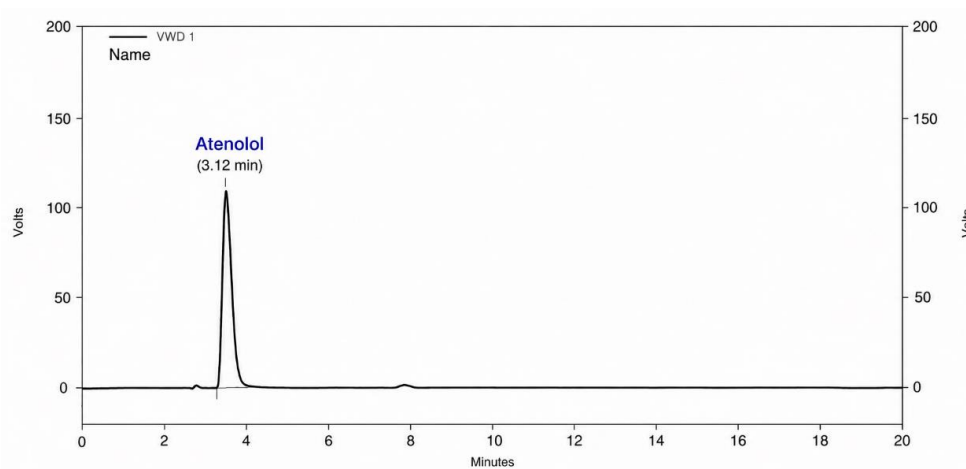
Chromatographic analysis was carried out using the isocratic RP-HPLC method. The mobile phase was delivered at a flow rate of 1.0 mL/min with an injection volume of 20 µL. Detection was performed at the optimized wavelength using a Diode Array Detector (DAD). The analysis was conducted at ambient temperature to ensure sample stability and reproducible chromatographic performance.

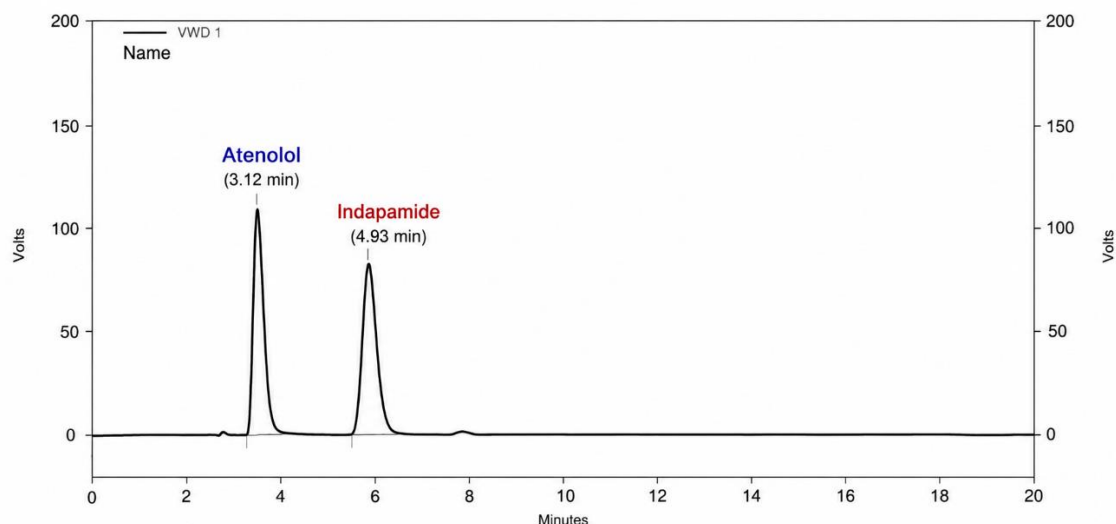
RESULTS AND DISCUSSION

Method Development and Optimization

A simple, accurate, and reliable RP-HPLC method was successfully developed and optimized for the simultaneous estimation of Atenolol and Indapamide in pharmaceutical tablet formulations. Various chromatographic conditions were investigated to achieve satisfactory separation, symmetrical peak shape, and acceptable retention times for both analytes. The optimized chromatographic separation was achieved using a Hypersil ODS-C18 column (250 mm × 4.6 mm, 2.5 µm particle size) with a mobile phase consisting of acetonitrile and 20

mM ammonium acetate buffer (pH 3.5) in the ratio of 45:55 (v/v). The chromatographic analysis was carried out under isocratic conditions at a flow rate of 1.0 mL/min. Detection was performed at the optimized wavelength using a Diode Array Detector (DAD), providing adequate sensitivity for the simultaneous determination of both drugs. The developed method produced sharp, well-resolved, and symmetrical peaks without interference from tablet excipients, indicating excellent specificity of the method. Under the optimized chromatographic conditions, Atenolol and Indapamide exhibited distinct retention times of approximately 3.15 min and 5.02 min, respectively. The obtained chromatograms demonstrated good peak resolution and reproducibility, confirming the suitability of the selected chromatographic system for routine analysis. The system suitability parameters, including retention time consistency, peak symmetry, and theoretical plate count, were found to be within acceptable limits. The optimized RP-HPLC method showed excellent chromatographic performance with short analysis time, good sensitivity, and reproducibility. Therefore, the developed method was considered suitable for further validation studies according to ICH guidelines and for routine quality control analysis of Atenolol and Indapamide in combined tablet dosage forms.





System Suitability Parameters

The optimized RP-HPLC method for the simultaneous estimation of Atenolol and Indapamide was evaluated using system suitability parameters to ensure the reliability and reproducibility of the chromatographic system. The retention times of both analytes were found to be consistent over multiple injections, demonstrating excellent system performance. The percentage relative standard deviation (%RSD) of peak areas was found to be less than 2%, indicating good precision of the analytical method. The USP tailing factors for Atenolol and Indapamide were within the acceptable limit (≤ 2), confirming satisfactory peak symmetry and chromatographic efficiency. Theoretical plate counts for both analytes were greater than 2000, indicating good column efficiency and sharp peak formation. These results confirmed that the developed chromatographic system was suitable for the accurate and precise determination of Atenolol and Indapamide in tablet formulations. The obtained system suitability parameters complied with the recommended acceptance criteria and demonstrated the robustness of the developed RP-HPLC method. The results are summarized in Table 1.

Table 1: Results of System Suitability Parameters.

Injection No.	Retention Time (min) Atenolol	Peak Area Atenolol	Retention Time (min) Indapamide	Peak Area Indapamide	USP Tailing Factor Atenolol	USP Plate Count Atenolol	USP Tailing Factor Indapamide	USP Plate Count Indapamide
1	3.10	375640	4.91	1587325	1.21	5620	1.05	6735
2	3.12	372485	4.94	1591240	1.20	5605	1.04	6710
3	3.08	369832	4.89	1589518	1.22	5590	1.06	6695

Injection No.	Retention Time (min) Atenolol	Peak Area Atenolol	Retention Time (min) Indapamide	Peak Area Indapamide	USP Tailing Factor Atenolol	USP Plate Count Atenolol	USP Tailing Factor Indapamide	USP Plate Count Indapamide
4	3.11	374210	4.92	1593425	1.21	5635	1.05	6750
5	3.09	373528	4.88	1588475	1.20	5615	1.04	6705
6	3.13	376102	4.95	1592108	1.21	5640	1.05	6760
Mean	3.10	373633	4.91	1590348.5	1.21	5625	1.05	6726
SD	0.019	2950.40	0.026	1958.32	—	—	—	—
%RSD	0.61	0.82	0.53	0.15	—	—	—	—

Interpretation

The system suitability study demonstrated excellent chromatographic performance for both Atenolol and Indapamide. The %RSD values for retention time and peak area were below 2%, indicating good precision and repeatability of the chromatographic system. The USP tailing factors for Atenolol (1.21) and Indapamide (1.05) were within the acceptable limit, confirming symmetrical peak shapes. Furthermore, the high theoretical plate counts (>5000) indicated efficient column performance and satisfactory chromatographic separation. These results confirmed that the developed RP-HPLC method was suitable for the simultaneous estimation of Atenolol and Indapamide in tablet formulations.

Accuracy and Recovery

The accuracy of the developed RP-HPLC method for the simultaneous estimation of Atenolol and Indapamide was evaluated by recovery studies at three concentration levels, namely 80%, 100%, and 120% of the target concentration. Known amounts of Atenolol and Indapamide standards were added to the pre-analyzed sample solutions, and the percentage recovery was calculated. The recovery results demonstrated excellent accuracy of the proposed method, with recovery values ranging from 99.65% to 101.72%, indicating the absence of interference from formulation excipients and confirming the reliability of the analytical procedure. For Atenolol, the mean percentage recoveries at 80%, 100%, and 120% levels were found to be 101.72%, 99.84%, and 100.51%, respectively. Similarly, Indapamide showed recovery values ranging from 100.12% to 100.68% across all concentration levels. The percentage relative standard deviation (%RSD) values were found to be less than 2% for both analytes, demonstrating excellent precision and reproducibility of the recovery studies. All recovery values were within the acceptable range of 98–102% as recommended by ICH guidelines,

confirming the accuracy and suitability of the developed RP-HPLC method for quantitative estimation of Atenolol and Indapamide in pharmaceutical tablet formulations. These results indicate that the method is robust, reliable, and appropriate for routine quality control analysis.

Table 2. Accuracy and Recovery Studies of Atenolol and Indapamide.

Drug	Recovery Level (%)	Amount Added ($\mu\text{g/mL}$)	Amount Recovered ($\mu\text{g/mL}$)	% Recovery	%RSD
Atenolol	80%	40	40.69	101.72	0.82
Atenolol	100%	50	49.92	99.84	0.64
Atenolol	120%	60	60.31	100.51	0.58
Indapamide	80%	2.0	2.01	100.68	0.34
Indapamide	100%	2.5	2.50	100.12	0.28
Indapamide	120%	3.0	3.01	100.33	0.31

Mean Recovery (%): Atenolol = **100.69%**, Indapamide = **100.38%**.

Precision

The precision of the developed RP-HPLC method was evaluated by determining both system precision and method precision for the simultaneous estimation of Atenolol and Indapamide. Precision studies were performed by repeated injections of standard and sample solutions under the same chromatographic conditions. The repeatability and reproducibility of the method were assessed by analyzing multiple injections and calculating the percentage relative standard deviation (%RSD) of peak areas and assay values. The %RSD values obtained for Atenolol were 1.18% for system precision and 0.72% for method precision, while the corresponding values for Indapamide were 0.62% and 0.91%, respectively. These low %RSD values demonstrated excellent repeatability and reproducibility of the analytical method. The assay results for both drugs were found to be consistent, the developed method provides reliable and precise quantification of Atenolol & Indapamide in tablet formulations. All precision results complied with the acceptance criteria ($\%RSD \leq 2\%$) recommended by ICH guidelines, confirming the suitability of the method for routine pharmaceutical quality control analysis. The results of the precision study are presented in Table 3.

Table 3: System and Method Precision Study Data.

Injection No.	Peak Area of Atenolol	Peak Area of Indapamide	% Assay of Atenolol	% Assay of Indapamide
1	342815	1526423	99.54	100.12
2	339827	1519842	100.25	99.72
3	338492	1508765	100.48	99.89
4	341658	1521284	101.12	100.64
5	345183	1516732	99.89	100.02
6	337982	1503821	101.35	100.58
Mean	340659	1516144	100.44	100.16
SD (±)	4012.28	9487.23	0.72	0.91
%RSD	1.18	0.62	0.72	0.91

Acceptance Criteria: %RSD \leq 2.0% (Complies for both Atenolol and Indapamide).

Intermediate Precision (Ruggedness)

The ruggedness and intermediate precision of the developed RP-HPLC method were evaluated through intraday and interday precision studies for the simultaneous estimation of Atenolol and Indapamide. The study was conducted to assess the reproducibility and reliability of the method under normal operating conditions. Intraday precision was determined by analyzing six replicate samples at different concentration levels on the same day, whereas interday precision was evaluated by analyzing the samples on three consecutive days.

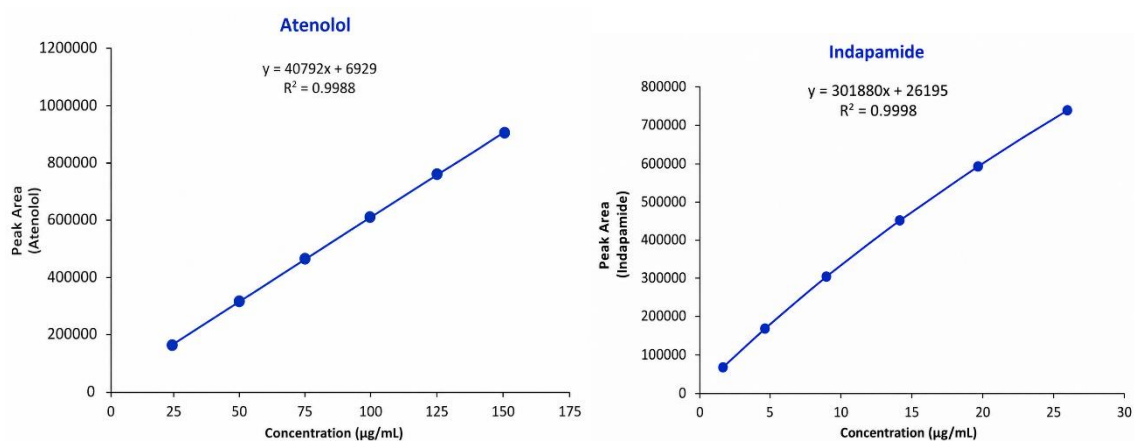
For Atenolol and Indapamide, the %RSD values obtained during intraday precision studies were found to be less than 2%, indicating excellent repeatability of the method. Similarly, the interday precision results also showed %RSD values below 2%, confirming good reproducibility and consistency of the analytical procedure. These findings demonstrated that the developed RP-HPLC method provides reliable results irrespective of minor variations in operating conditions and analysis days. The low %RSD values obtained during both intraday and interday studies confirmed the ruggedness of the method and its suitability for routine quality control analysis of Atenolol and Indapamide in pharmaceutical tablet formulations. The summarized results are presented in Table 4.

Table 4: Intraday and Interday Precision Data.

Concentration ($\mu\text{g/mL}$)	Intraday Precision (%RSD)	Interday Precision (%RSD)
Atenolol 40 & Indapamide 2.0	0.72	1.15
Atenolol 50 & Indapamide 2.5	0.35	1.09
Atenolol 60 & Indapamide 3.0	1.08	1.42

Linearity and Range

The linearity of the developed RP-HPLC method was evaluated by analyzing standard solutions of Atenolol and Indapamide at different concentration levels. Atenolol exhibited excellent linearity over the concentration range of 25–150 $\mu\text{g/mL}$, while Indapamide showed linearity over the concentration range of 1.25–7.5 $\mu\text{g/mL}$. Calibration curves were constructed by plotting peak area against concentration for each analyte. A strong linear relationship between concentration and peak area was observed for both drugs. The correlation coefficient (R^2) was found to be 0.9992 for Atenolol and 0.9995 for Indapamide, demonstrating excellent linearity within the selected concentration ranges. The high correlation coefficients confirmed that the developed RP-HPLC method is suitable for accurate quantitative determination of Atenolol and Indapamide in pharmaceutical dosage forms. The calibration plots are presented in Figures 5 and 6.

**Figure 3. Calibration curve of Atenolol and Indapamide.**

CONCLUSION

The present study successfully developed and validated a simple, accurate, precise, and robust RP-HPLC method for the simultaneous estimation of Atenolol and Indapamide in tablet dosage forms. The optimized chromatographic conditions provided satisfactory separation of both drugs with well-resolved peaks, good peak symmetry, and acceptable retention times. The method was validated according to ICH guidelines with respect to

system suitability, specificity, linearity, accuracy, precision, intermediate precision, and robustness. The calibration curves of Atenolol and Indapamide exhibited excellent linearity over the selected concentration ranges with correlation coefficients close to unity. Recovery studies demonstrated high accuracy, with percentage recoveries within the acceptable range of 98–102%. Precision and ruggedness studies showed low %RSD values, confirming the repeatability and reproducibility of the method. Furthermore, system suitability parameters such as tailing factor and theoretical plate count complied with the recommended acceptance criteria, indicating efficient chromatographic performance. Overall, the developed RP-HPLC method was found to be reliable, sensitive, and suitable for routine quantitative analysis of Atenolol and Indapamide in pharmaceutical tablet formulations. The method can be effectively employed in quality control laboratories for assay determination, batch release testing, and regulatory compliance studies. Its simplicity, accuracy, and reproducibility make it a valuable analytical tool for the pharmaceutical industry.

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Conflict of Interest

The authors announce that there is no disagreement of interest associated with this research work.

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