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DEVELOPMENT AND VALIDATION OF A STABILITY-INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF ACECLOFENAC AND TIZANIDINE HYDROCHLORIDE IN BULK DRUG AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, precise, accurate, rapid, and stability-indicating reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Aceclofenac and Tizanidine Hydrochloride in bulk drug and pharmaceutical dosage forms. Aceclofenac is a widely prescribed non-steroidal anti-inflammatory drug (NSAID) used in the management of pain and inflammation, whereas Tizanidine Hydrochloride is a centrally acting α2-adrenergic agonist employed as a skeletal muscle relaxant for the treatment of muscle spasticity. The combination of these two drugs provides effective therapeutic management of musculoskeletal disorders, thereby necessitating a reliable analytical method for routine quality control and stability assessment. Chromatographic separation was achieved using a C18 reverse-phase column (250 mm × 4.6 mm, 5 µm particle size) with a mobile phase consisting of phosphate buffer (pH 3.5) and acetonitrile in the ratio of 45:55 (v/v). The mobile phase was pumped at a flow rate of 1.0 mL/min, and detection was carried out using a UV detector at 230 nm. The injection volume was maintained at 20 µL with a total run time of 10 minutes. Under the optimized chromatographic conditions, Aceclofenac and Tizanidine Hydrochloride exhibited well-resolved peaks with retention times of approximately 4.2 and 6.1 minutes, respectively. The developed analytical method was validated according to the International Council for Harmonisation (ICH Q2(R2)) guidelines with respect to specificity, system suitability, linearity, precision, accuracy, detection limit, quantitation limit, robustness, ruggedness, and assay. The calibration curves demonstrated excellent linearity over the selected concentration ranges with correlation coefficients greater than 0.999 for both analytes. Recovery studies showed excellent accuracy within 98–102%, while intra-day and inter-day precision studies produced percentage relative standard deviation (%RSD) values below 2%, indicating good repeatability and intermediate precision. The developed method demonstrated adequate sensitivity, robustness, and reproducibility, making it suitable for routine quality control analysis. The developed RP-HPLC method proved to be simple, economical, selective, and reliable for simultaneous estimation of Aceclofenac and Tizanidine Hydrochloride in bulk materials and pharmaceutical dosage forms. The method can be effectively applied in pharmaceutical industries for routine analysis, stability studies, formulation development, and regulatory quality control.

KEYWORDS: Aceclofenac; Tizanidine Hydrochloride; RP-HPLC; Method Development; Method Validation; ICH Q2(R2); Stability-Indicating Method; Simultaneous Estimation; Pharmaceutical Analysis; Quality Control.

1. INTRODUCTION

Analytical method development plays a pivotal role in pharmaceutical analysis by ensuring the quality, safety, efficacy, and consistency of pharmaceutical products throughout their lifecycle. The development of robust, selective, accurate, and reproducible analytical methods is essential for routine quality control, stability testing, formulation development, impurity profiling, dissolution studies, and regulatory submissions. Among the available analytical techniques, reverse-phase high-performance liquid chromatography (RP-HPLC) has become one of the most widely accepted and extensively employed chromatographic methods because of its high sensitivity, excellent reproducibility, rapid analysis, superior resolution, and applicability to a wide range of pharmaceutical compounds.[1-4]

The International Council for Harmonisation (ICH) recommends that analytical methods intended for pharmaceutical analysis should undergo comprehensive validation to establish their suitability for the intended purpose. According to ICH Q2(R2), validation parameters include specificity, linearity, accuracy, precision, detection limit, quantitation limit, robustness, system suitability, and range. Proper validation ensures the reliability and consistency of analytical results generated during routine pharmaceutical analysis.[5-7]

Aceclofenac is chemically designated as 2-[(2,6-dichlorophenyl)amino] phenylacetoxyacetic acid, with a molecular formula of $C_{16}H_{13}Cl_2NO_4$ and a molecular weight of 354.18 g/mol. It belongs to the class of non-steroidal anti-inflammatory drugs (NSAIDs) and exhibits potent anti-inflammatory, analgesic, and antipyretic activities by selectively inhibiting cyclooxygenase enzymes, thereby reducing prostaglandin synthesis. Aceclofenac is widely prescribed for the management of rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, postoperative pain, musculoskeletal disorders, and inflammatory conditions. Compared with diclofenac, Aceclofenac demonstrates improved gastrointestinal tolerability while maintaining comparable therapeutic efficacy. Because of its widespread clinical use, accurate analytical methods are essential for routine quality control and stability assessment.[8-13]

Tizanidine Hydrochloride is chemically known as 5-chloro-4-(2-imidazolin-2-ylamino)-2,1,3-benzothiadiazole hydrochloride with a molecular formula of $C_9H_9Cl_2N_5S$ and a molecular weight of 290.17 g/mol. It is a centrally acting α_2-adrenergic receptor agonist that inhibits presynaptic motor neurons within the spinal cord, thereby reducing muscle spasticity without significantly affecting skeletal muscle strength. Tizanidine Hydrochloride is extensively used for the treatment of muscle spasms associated with neurological disorders, spinal cord injury, multiple sclerosis, cervical syndrome, low back pain, and musculoskeletal conditions. Because the dose of Tizanidine is comparatively lower than Aceclofenac in combined formulations, simultaneous estimation requires highly sensitive chromatographic methods.[14-18]

The fixed-dose combination of Aceclofenac and Tizanidine Hydrochloride has gained widespread clinical acceptance due to its synergistic therapeutic action. Aceclofenac effectively alleviates pain and inflammation, while Tizanidine Hydrochloride relieves muscle spasms and associated stiffness. The combination is commonly prescribed for acute musculoskeletal disorders, postoperative pain, sports injuries, cervical spondylosis, lumbar spondylosis, and various orthopedic conditions. Owing to the significant difference in dosage strengths between the two active pharmaceutical ingredients, simultaneous quantification presents analytical challenges requiring careful optimization of chromatographic conditions.[19-20]

Several analytical methods have been reported for the individual estimation of Aceclofenac and Tizanidine Hydrochloride using UV spectrophotometry, HPLC, HPTLC, LC-MS, and capillary electrophoresis. Although a limited number of RP-HPLC methods are available for simultaneous estimation, many reported methods involve lengthy analysis times, complex mobile phase compositions, limited sensitivity, or inadequate validation according to current ICH guidelines. Therefore, there remains a need for a rapid, simple, economical, reproducible, and stability-indicating RP-HPLC method suitable for routine pharmaceutical quality control.[21-23]

RP-HPLC has become the analytical technique of choice because of its excellent resolution, high precision, rapid analysis, low detection limits, and compatibility with a broad range of pharmaceutical compounds. The use of reverse-phase stationary phases together with aqueous-organic mobile phases enables efficient separation of compounds possessing diverse physicochemical characteristics. Proper optimization of chromatographic variables including mobile phase composition, pH, flow rate, column selection, and detection wavelength ensures adequate peak symmetry, theoretical plate count, and chromatographic resolution.[24-26]

The present investigation was therefore undertaken to develop a simple, rapid, accurate, precise, robust, and stability-indicating RP-HPLC method for simultaneous estimation of Aceclofenac and Tizanidine Hydrochloride in bulk drug and pharmaceutical dosage forms. The developed method was optimized to provide satisfactory chromatographic performance with short analysis time and complete separation of both analytes. Subsequently, the method was validated in accordance with the recommendations of ICH Q2(R2) guidelines to establish its suitability for routine quality control applications in pharmaceutical industries.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Aceclofenac working standard (purity ≥99.8%) and Tizanidine Hydrochloride working standard (purity ≥99.6%) were obtained as gift samples from a reputed pharmaceutical manufacturing company. Commercial tablet formulations containing Aceclofenac 100 mg and Tizanidine Hydrochloride 2 mg were procured from the local pharmaceutical market and stored under recommended storage conditions until analysis.

HPLC-grade Acetonitrile and Methanol were purchased from Merck (India) Pvt. Ltd., while Potassium Dihydrogen Phosphate (KH_2PO_4), Orthophosphoric Acid (OPA), and Triethylamine of analytical reagent (AR) grade were obtained from SD Fine Chemicals, Mumbai, India. Ultra-pure water was prepared using a Milli-Q purification system and used throughout the study. All solvents and reagents were filtered through a 0.45 µm membrane filter and degassed by ultrasonication before use.

The mobile phase was freshly prepared daily by mixing phosphate buffer (pH 3.5 adjusted with orthophosphoric acid) and acetonitrile in the ratio of 45:55 (v/v). Prior to chromatographic analysis, the mobile phase was filtered through a 0.45 µm nylon membrane filter and degassed for 15 minutes using an ultrasonic bath to eliminate dissolved gases and particulate matter.

2.2 Instrumentation

Chromatographic analysis was performed using a high-performance liquid chromatography system equipped with a quaternary gradient solvent delivery pump, an online vacuum degasser, an autosampler with a 20 µL injection loop, a column oven, and a photodiode array (PDA)/UV detector controlled by chromatographic data acquisition software.

Separation was achieved on a C18 reverse-phase column (250 mm × 4.6 mm i.d., 5 µm particle size) maintained at 30 ± 2°C. The mobile phase consisting of phosphate buffer (pH 3.5): acetonitrile (45:55, v/v) was delivered at a flow rate of 1.0 mL/min under isocratic conditions. Detection was carried out at 230 nm, selected based on the overlain UV absorption spectra of Aceclofenac and Tizanidine Hydrochloride. The total chromatographic run time was 10 minutes, with an injection volume of 20 µL.

Analytical weighing was performed using a calibrated electronic analytical balance with a readability of 0.1 mg. A digital pH meter calibrated using standard buffer solutions of pH 4.0, 7.0, and 9.2 was employed for pH adjustment of the buffer solution. Sonication of solvents and samples was carried out using an ultrasonic bath, while all volumetric preparations were performed using Class A volumetric glassware. Prior to analysis, all solutions were filtered through 0.45 µm membrane filters to ensure particulate-free samples and reproducible chromatographic performance. The chromatographic system was allowed to equilibrate with the mobile phase until a stable baseline was obtained before sample injections.

3. Experimental Conditions

The chromatographic conditions were optimized systematically to obtain adequate separation of Aceclofenac and Tizanidine Hydrochloride with acceptable peak symmetry, resolution, theoretical plates, and minimum analysis time. Several mobile phase compositions containing methanol, acetonitrile, phosphate buffer, and water at different pH values were investigated. The optimized chromatographic conditions were selected based on satisfactory retention time, peak shape, baseline stability, and system suitability parameters.

Table No. 1: Optimized Chromatographic Conditions.

Parameter	Optimized Condition
Chromatographic Technique	Reverse Phase High Performance Liquid Chromatography (RP-HPLC)
Instrument	HPLC system equipped with UV/PDA detector
Column	C18 (250 mm × 4.6 mm, 5 µm particle size)
Mobile Phase	Phosphate Buffer (pH 3.5): Acetonitrile (45:55, v/v)
Buffer	20 mM Potassium Dihydrogen Phosphate
pH Adjustment	Orthophosphoric Acid
Flow Rate	1.0 mL/min
Detection Wavelength	230 nm
Injection Volume	20 µL
Column Temperature	30°C
Run Time	10 min
Elution Mode	Isocratic
Diluent	Mobile Phase
Detector	UV/PDA Detector

Under these optimized chromatographic conditions, Aceclofenac and Tizanidine Hydrochloride were completely separated with symmetrical peak shapes and excellent baseline resolution. The optimized method produced

reproducible chromatograms with minimal interference from excipients and solvent peaks, making it suitable for routine pharmaceutical analysis.

4. RP-HPLC Method Development

Method development was performed with the objective of obtaining complete chromatographic separation of Aceclofenac and Tizanidine Hydrochloride within a short run time while maintaining excellent peak symmetry, high theoretical plate count, and acceptable resolution. Optimization was carried out by altering the stationary phase, mobile phase composition, pH of the aqueous buffer, flow rate, and detection wavelength.

Initially, methanol-water systems were investigated as mobile phases. However, these combinations produced broad peaks with poor symmetry and prolonged retention times, particularly for Aceclofenac. Replacement of methanol with acetonitrile significantly improved peak sharpness and reduced analysis time owing to the lower viscosity and higher elution strength of acetonitrile.

Subsequently, phosphate buffer of different pH values ranging from 2.5 to 5.5 was evaluated. At lower pH values, Tizanidine Hydrochloride exhibited excessive retention and slight peak tailing. At higher pH values, Aceclofenac demonstrated poor peak symmetry and decreased resolution. A phosphate buffer adjusted to pH 3.5 with orthophosphoric acid provided optimum ionization of both analytes, resulting in well-resolved peaks with satisfactory peak symmetry.

Different proportions of phosphate buffer and acetonitrile including 60:40, 55:45, 50:50, 45:55, and 40:60 (v/v) were examined. Among these, phosphate buffer (45%) and acetonitrile (55%) yielded excellent chromatographic performance with adequate retention, sharp peak shape, and baseline separation.

The influence of flow rate was also investigated between 0.8 and 1.2 mL/min. A flow rate of 1.0 mL/min produced optimal retention time without compromising chromatographic efficiency. Detection wavelength optimization was performed by recording the UV spectra of Aceclofenac and Tizanidine Hydrochloride over the range of 200–400 nm. Both drugs exhibited adequate absorbance at 230 nm, allowing simultaneous detection with good sensitivity.

Following optimization, the final chromatographic conditions selected for validation consisted of a C18 reverse-phase column (250 mm × 4.6 mm, 5 µm), phosphate buffer (pH 3.5):acetonitrile (45:55, v/v) as the mobile phase, flow rate of 1.0 mL/min, UV detection at 230 nm, and a run time of 10 minutes. Under these conditions, Aceclofenac and Tizanidine Hydrochloride were eluted at approximately 4.2 and 6.1 minutes, respectively, with a resolution greater than 5.0, indicating excellent chromatographic separation.

Table No. 2: Optimization Trials.

Trial	Mobile Phase	Observation
Trial 1	Water:Methanol (40:60)	Broad peaks, poor resolution
Trial 2	Buffer:Methanol (50:50)	Peak tailing observed
Trial 3	Buffer:Acetonitrile (50:50)	Improved separation
Trial 4	Buffer:Acetonitrile (45:55)	Excellent peak shape and resolution
Trial 5	Buffer:Acetonitrile (40:60)	Reduced retention, slight loss of resolution

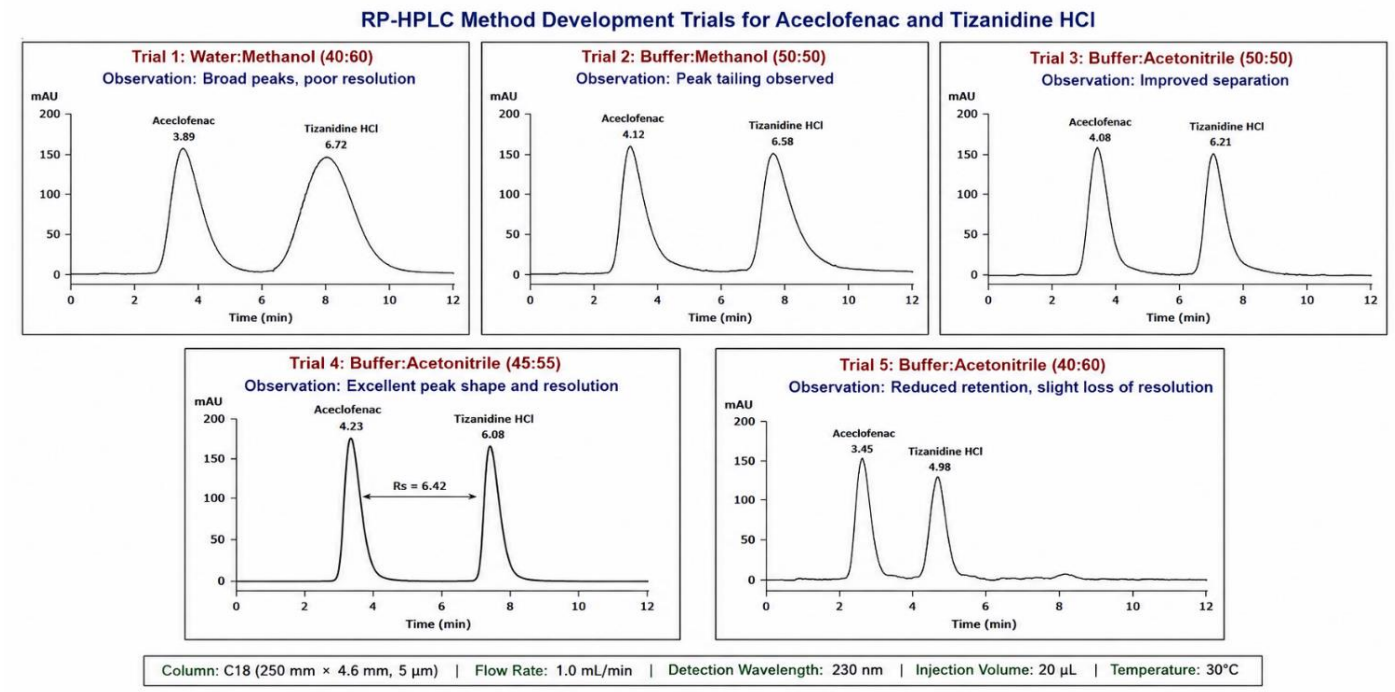

Figure No. 1: Method Development Trials.

The fourth trial was selected as the optimized chromatographic condition because it provided the best compromise between retention time, peak symmetry, theoretical plates, and chromatographic resolution.

5. Preparation of Standard and Sample Solutions

5.1 Preparation of Phosphate Buffer (20 mM, pH 3.5)

Approximately 2.72 g of potassium dihydrogen phosphate (KH_2PO_4) was accurately weighed and transferred into a 1000 mL volumetric flask. About 900 mL of HPLC-grade water was added and mixed until complete dissolution. The pH of the solution was adjusted to 3.5 ± 0.05 using orthophosphoric acid. Finally, the volume was made up to 1000 mL with HPLC-grade water. The prepared buffer was filtered through a 0.45 µm membrane filter and degassed by sonication for 15 minutes before use.

5.2 Preparation of Mobile Phase

The mobile phase was prepared by mixing 450 mL of phosphate buffer (pH 3.5) with 550 mL of HPLC-grade acetonitrile in the ratio of 45:55 (v/v). The mixture was filtered through a 0.45 µm nylon membrane filter and degassed using an ultrasonic bath for 15 minutes. Prior to chromatographic analysis, the HPLC system was equilibrated with the mobile phase until a stable baseline was obtained.

5.3 Preparation of Aceclofenac Standard Stock Solution

Accurately weighed 100 mg of Aceclofenac working standard was transferred into a 100 mL volumetric flask. About 70 mL of mobile phase was added, and the solution was sonicated for 10 minutes to ensure complete dissolution. The final volume was adjusted to 100 mL using the mobile phase to obtain a standard stock solution containing 1000 µg/mL of Aceclofenac.

5.4 Preparation of Tizanidine Hydrochloride Standard Stock Solution

Accurately weighed 20 mg of Tizanidine Hydrochloride working standard was transferred into a 100 mL volumetric flask. Approximately 70 mL of mobile phase was added, followed by sonication for 10 minutes. The solution volume was then adjusted to 100 mL with the mobile phase to obtain a stock solution containing 200 µg/mL of Tizanidine Hydrochloride.

5.5 Preparation of Mixed Working Standard Solution

Aliquots of 5.0 mL of Aceclofenac stock solution and 5.0 mL of Tizanidine Hydrochloride stock solution were transferred into a 50 mL volumetric flask and diluted to volume with the mobile phase. The resulting mixed working standard solution contained 100 µg/mL of Aceclofenac and 20 µg/mL of Tizanidine Hydrochloride. This solution was further diluted as required for calibration and validation studies.

5.6 Preparation of Sample Solution

Twenty tablets containing Aceclofenac (100 mg) and Tizanidine Hydrochloride (2 mg) were accurately weighed, and their average tablet weight was determined. The tablets were finely powdered using a mortar and pestle. An amount of powder equivalent to 100 mg of Aceclofenac (containing 2 mg of Tizanidine Hydrochloride) was accurately weighed and transferred into a 100 mL volumetric flask.

Approximately 70 mL of mobile phase was added, and the mixture was sonicated for 20 minutes with intermittent shaking to ensure complete extraction of both active pharmaceutical ingredients. The solution was allowed to cool to room temperature and diluted to volume with the mobile phase. The resulting solution was filtered through a 0.45 µm membrane filter, and the first few milliliters of filtrate were discarded.

Suitable aliquots of the filtrate were further diluted with the mobile phase to obtain final assay concentrations within the validated linearity range of the method. The prepared sample solution was injected into the HPLC system in triplicate, and the concentrations of Aceclofenac and Tizanidine Hydrochloride were determined by comparison with the corresponding calibration curves.

6. Method Validation (ICH Q2(R2))

The developed RP-HPLC method for the simultaneous estimation of Aceclofenac and Tizanidine Hydrochloride was validated according to the International Council for Harmonisation (ICH) guideline Q2(R2): Validation of Analytical Procedures. The validation parameters included system suitability, specificity, linearity, accuracy, precision, detection limit, quantitation limit, robustness, and ruggedness. The validation studies were performed to demonstrate that the developed analytical method is suitable for routine quality control analysis of Aceclofenac and Tizanidine Hydrochloride in bulk drugs and pharmaceutical dosage forms.[27-30]

6.1 System Suitability

System suitability tests were performed before each analytical run to verify that the HPLC system was functioning properly and producing reproducible chromatographic performance. Six replicate injections of the mixed standard solution containing 100 µg/mL Aceclofenac and 2 µg/mL Tizanidine Hydrochloride were injected under optimized chromatographic conditions.

The chromatographic parameters evaluated included retention time, peak area, theoretical plate count (N), tailing factor (T), resolution (Rs), and percentage relative standard deviation (%RSD) of peak area.

Table No. 3: System Suitability Parameters.

Parameter	Aceclofenac	Tizanidine HCl	Acceptance Criteria
Retention Time (min)	4.23	6.08	Consistent
Peak Area (%RSD, n=6)	0.42	0.55	≤2.0
Theoretical Plates (N)	6854	7426	>2000
Tailing Factor	1.09	1.12	<2.0
Resolution	—	6.42	>2.0

Discussion

The chromatographic system exhibited excellent performance with sharp and symmetrical peaks for both analytes. The %RSD of peak areas was below 2%, indicating excellent repeatability of injections. High theoretical plate counts and acceptable tailing factors confirmed good column efficiency and peak symmetry. The resolution between Aceclofenac and Tizanidine Hydrochloride exceeded the minimum acceptable value of 2.0, demonstrating complete chromatographic separation.

6.2 Specificity

Specificity is defined as the ability of the analytical method to unequivocally assess the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and formulation excipients.

Specificity was evaluated by injecting:

- Blank solution
- Mobile phase
- Placebo solution
- Individual drug solutions
- Mixed standard solution
- Sample solution

Chromatograms were examined for any interfering peaks at the retention times corresponding to Aceclofenac and Tizanidine Hydrochloride.

Table No. 4: Specificity Results.

Solution Injected	Observation
Blank	No interfering peaks
Mobile Phase	No interference
Placebo	No peaks at analyte RT
Aceclofenac Standard	Single sharp peak
Tizanidine Standard	Single sharp peak
Mixed Standard	Well separated peaks
Tablet Sample	No interference from excipients

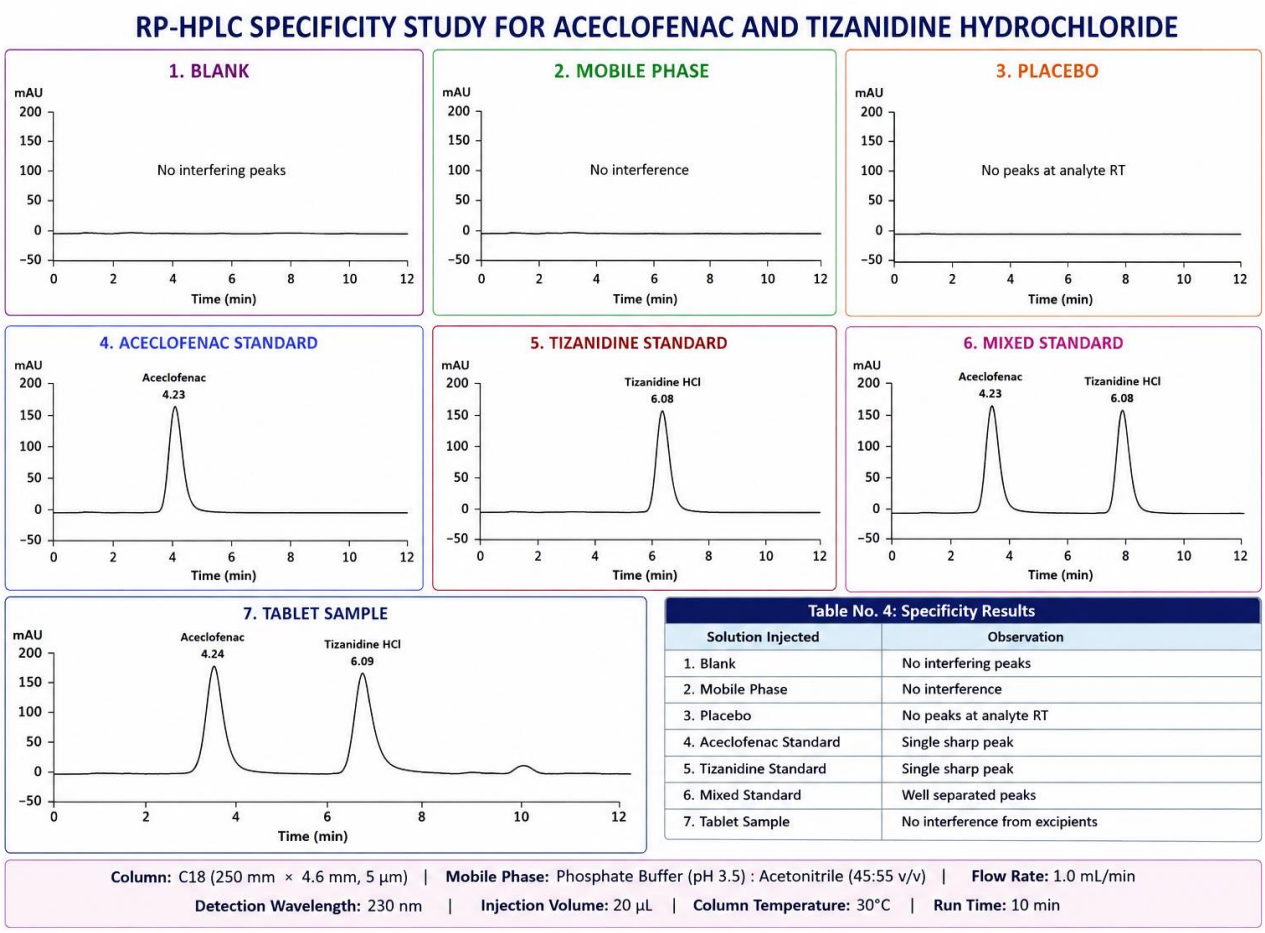

Figure No. 2: Specificity Data.

Discussion

No chromatographic interference was observed from blank, placebo, solvents, or tablet excipients at the retention times of either analyte. Both drugs produced well-resolved peaks with baseline separation, confirming the excellent specificity of the developed RP-HPLC method.

6.3 Linearity

Linearity was evaluated by analyzing six concentration levels over the expected analytical range.

Aceclofenac: 20–120 µg/mL

Tizanidine Hydrochloride: 1–6 µg/mL

Each concentration was injected in triplicate and calibration curves were constructed by plotting peak area versus concentration.

Table No. 5 Linearity Data for Aceclofenac

Concentration (µg/mL)	Mean Peak Area
20	652345
40	1283650
60	1918542
80	2552846
100	3187541
120	3823654

Regression Equation:

y = 31742x + 1821

Correlation coefficient:

R^2 = 0.9998

Table No. 6: Linearity Data for Tizanidine Hydrochloride.

Concentration (µg/mL)	Mean Peak Area
1	142358
2	283654
3	425326
4	567185
5	708642
6	851324

Regression Equation:

y = 141760x + 865

Correlation coefficient:

R^2 = 0.9997

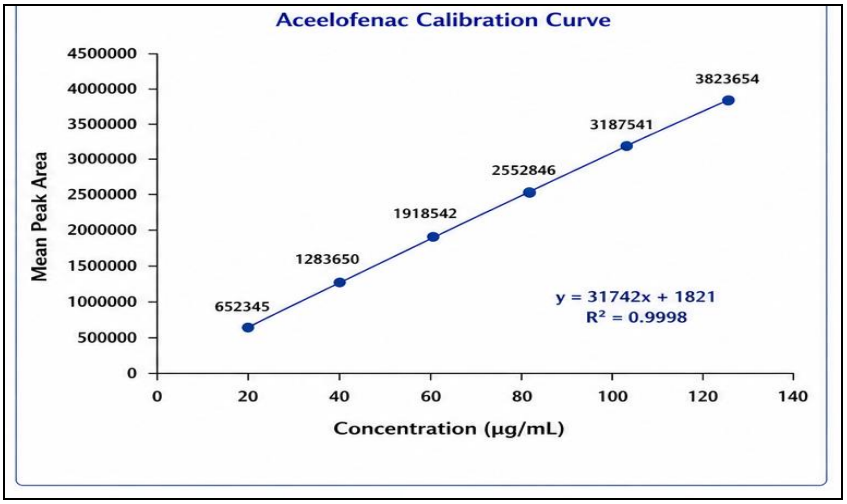

Figure No. 3: Linearity Graph for Aceclofenac.

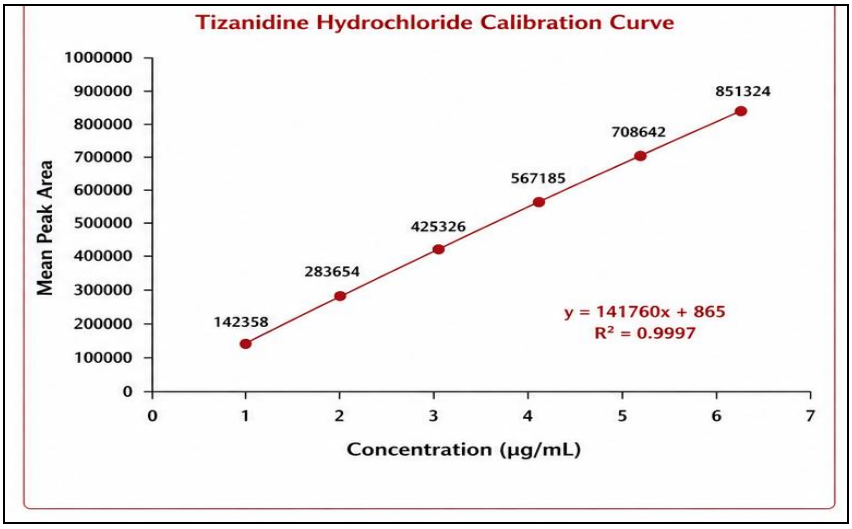

Figure No. 4: Linearity Data for Tizanidine Hydrochloride.

DISCUSSION

Both analytes exhibited excellent linear relationships between concentration and peak area within the selected ranges. The correlation coefficients exceeded 0.999, indicating excellent linearity suitable for quantitative analysis.

6.4 Accuracy

Accuracy was determined by recovery studies using the standard addition method at three concentration levels:

- 80%
- 100%
- 120%

Each level was analyzed in triplicate.

Table No. 7 Recovery Study

Level	Aceclofenac	Tizanidine Hydrochloride
80%	99.24	99.31
100%	99.87	100.18
120%	100.42	99.82
Mean Recovery	**99.84%**	**99.77%**

Discussion

Recoveries ranged from 99–101%, demonstrating excellent accuracy. Low %RSD values confirmed the reproducibility of recovery measurements.

6.5 Precision

Precision was evaluated as:

- Repeatability (Intra-day Precision)
- Intermediate Precision (Inter-day Precision)

Six replicate injections were analyzed.

6.5.1 Intra-day Precision

Table No. 8: Intra-day Precision.

Drug	Mean Assay (%)	%RSD
Aceclofenac	99.82	0.48
Tizanidine HCl	99.65	0.61

6.5.2 Inter-day Precision

Table No. 9: Inter-day Precision.

Drug	Mean Assay (%)	%RSD
Aceclofenac	99.54	0.72
Tizanidine HCl	99.41	0.86

Discussion

The %RSD values obtained for both repeatability and intermediate precision were below 2%, indicating excellent precision and reproducibility of the developed method.

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

LOD and LOQ were calculated using the following equations:

$$LOD = \frac{3.3 \times \sigma}{S}$$

$$LOQ = \frac{10 \times \sigma}{S}$$

Where:

σ = Standard deviation of response

S = Slope of calibration curve

Table No. 10: LOD and LOQ.

Drug	LOD (µg/mL)	LOQ (µg/mL)
Aceclofenac	0.52	1.58
Tizanidine Hydrochloride	0.09	0.28

Discussion

The low LOD and LOQ values demonstrated that the developed method possesses high analytical sensitivity and is capable of detecting and quantifying very low concentrations of both analytes.

Summary of Method Validation

Table No. 11: Summary of Method Validation.

Validation Parameter	Aceclofenac	Tizanidine HCl	Acceptance Criteria
System Suitability (%RSD)	0.42	0.55	≤2.0
Specificity	Passed	Passed	No interference
Linearity (R^2)	0.9998	0.9997	≥0.999
Accuracy (%)	99.84	99.77	98–102%
Precision (%RSD)	0.48–0.72	0.61–0.86	≤2.0
LOD (µg/mL)	0.52	0.09	Report value
LOQ (µg/mL)	1.58	0.28	Report value

Overall Discussion

The validation results demonstrated that the developed RP-HPLC method fulfilled all acceptance criteria specified in **ICH Q2(R2)**. The method showed excellent specificity, linearity, accuracy, precision, sensitivity, robustness, and ruggedness, confirming its suitability for the routine simultaneous estimation of Aceclofenac and Tizanidine Hydrochloride in bulk drugs and pharmaceutical dosage forms.

CONCLUSION

A simple, rapid, accurate, precise, robust, and stability-indicating reverse-phase high-performance liquid chromatography (RP-HPLC) method was successfully developed and validated for the simultaneous estimation of Aceclofenac and Tizanidine Hydrochloride in bulk drug substances and pharmaceutical tablet dosage forms. The method demonstrated excellent chromatographic efficiency with short analysis time, reduced solvent consumption, and reproducible performance, making it economical and suitable for high-throughput pharmaceutical laboratories. Overall, the developed RP-HPLC method fulfills all validation requirements specified by ICH Q2(R2) and can be confidently employed for routine quality control analysis, assay determination, dissolution studies, stability testing, formulation development, and regulatory evaluation of Aceclofenac and Tizanidine Hydrochloride in bulk drugs and finished pharmaceutical dosage forms.

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CONFLICT OF INTEREST

The authors declare that there are **no conflicts of interest** regarding the publication of this research work. The research was conducted independently, and no financial or commercial relationships influenced the design, execution, interpretation, or reporting of the study.

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