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Research Article

Development of Stability Indicating RP-HPLC Method for Tizanidine Hydrochloride in Bulk Drug and Pharmaceutical Dosage Form

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Abstract

The quantitative analysis of tizanidine hydrochloride in both pharmaceutical dosage form and bulk medication has been developed and validated using an easy, affordable, quick, and unique isocratic HPLC approach. Tizanidine hydrochloride was separated isocratically using Waters symmetry C18 ODS as the stationary phase (250×4.6 mm, 5µm particle size), a flow rate of 1.0 ml/min, and a UV detector to track the eluate at 230 nm. The drug and its degradation products could be separated using the mobile phase, which was made up of acetonitrile: phosphate buffer (20:80 v/v) and pH-adjusted to 3.0 by ortho-phosphoric acid. Linearity, accuracy (recovery), precision, specificity, and robustness of the approach were all validated. For the range of 4-80 µg/ml, the linearity of the technique was satisfactory (correlation coefficient 0.999). Between 100.4 to 101.3% of the tizanidine hydrochloride was recovered from the medicinal dosage form. In order to analyze the samples, tizanidine hydrochloride was put under stress conditions, including hydrolysis (acid, base), oxidation, photolysis, and heat degradation. The tizanidine hydrochloride forced degradation study demonstrated that it decomposed under minimal conditions. Under the other stress scenarios examined, the medication remained steady. It was discovered that tizanidine hydrochloride was significantly more stable in its solid state than in its solution condition. The breakdown products were clearly distinguishable from the primary peak. The validation method may be useful for routine analysis of tizanidine hydrochloride as bulk drug, in respective dosage forms, for dissolution studies, and as a stability indicating assay method in pharmaceutical laboratories and industries because the forced degradation study proves the method's stability indicating power.

Keywords: RP-HPLC, Tizanidine hydrochloride, Forced degradation, Method validated

INTRODUCTION

5-Chloro-N-(4,5-dihydro-1H-imidazol-2-yl)-2,1,3-benzothiadiazol-4-amine hydrochloride is tizanidine hydrochloride (TIZ, Figure 1). It is an alpha2 adrenergic agonist and a muscle relaxant[1]. It is used to treat medical conditions such multiple sclerosis, spastic diplegia, back pain, and several other ailments to the spine or central nervous system that result in muscular spasms[2,3], cramping, and tightness. When dried, TIZ has a C9H8ClN5S.HCl content that is between 99.0% and 101.0%. It is a white to light yellow-white crystalline powder when it is present. It is essentially insoluble in acetic anhydride and acetic acid, but somewhat soluble in water and ethanol. The equivalent of 2 mg of the base administered as a single dosage is the typical first daily dose for the therapy of spasticity. TIZ is used in doses equating to 2 to 4 mg of the base three times per day in order to relieve painful muscle spasm. In terms of clinical practice, NSAIDs are the most often prescribed medications by doctors for inflammatory disorders[4]. An essential step in the process of developing a medicinal product is stability testing.

The goal of stability testing is to demonstrate how the quality of a drug ingredient or drug product changes over time under various environmental circumstances, including as temperature, humidity, and light. This information is used to determine storage conditions, retest intervals, and shelf lives[5,6]. The assay of the active ingredient and the degradation products produced during stability studies are the two key components of stability studies that are crucial for determining shelf lives. The International Conference on Harmonization (ICH) [7] recommends using a stability indicating method for the assay of a drug product in a stability test sample. The goal of this work was to create an analytical LC approach that was quick, accurate, and could be used to analyze a dose form of TIZ and determine its stability. The United States Pharmacopoeia (USP) suggests using the HPLC technique to identify tizanidine (I) in tablets and raw

materials[8]. Other techniques for determining tizanidine hydrochloride have been published in the literature, including spectrophotometry[9–16], voltammetry[17–19], GC[20–21], TLC[22–24], and HPLC[16, 22, 25–28]. None of the available methodologies permits analysis of the tizanidine hydrochloride in pharmaceutical dosage forms in the presence of their breakdown products. This article presents the creation and validation of a speedy, affordable, precise, and accurate stability-indicating isocratic reversed phase HPLC technique for the measurement of tizanidine hydrochloride in the presence of its degradation products in compliance with ICH recommendations 12. This study primarily focuses on the forced degradation of tizanidine hydrochloride under stressful conditions like acidic and basic hydrolysis, oxidation, heat, and light, as well as the validation of a method for precisely quantifying tizanidine hydrochloride in bulk medication and solid dosage form.

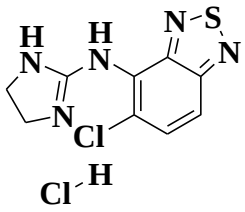


Figure 1: Molecular structure of tizanidine hydrochloride

EXPERIMENTAL

Chemicals and reagents

Endoc pharma labs limited Rajkot (Gujarat, India) generously provided tizanidine hydrochloride bulk medicine (quality 98.9%), and Sirdalud 2/4mg tizanidine tablets were obtained from the neighborhood market. The following chemicals were purchased from Merck Fine Chemicals Mumbai, India: acetonitrile (HPLC grade), orthophosphoric acid, sodium hydroxide (NaOH), hydrochloric acid (HCl), and hydrogen peroxide (H2O2). Throughout the experiment, water of the double HPLC grade was used. The other compounds were analytical- or HPLC-grade substances. Working standard was dissolved in mobile phase and diluted with the same solvent to create standard stock solution (1 mg/ml) of TIZ. This stock solution was diluted with diluent to create standard calibration solutions (4-80µg/ml) for the evaluation of linearity. **Chromatography**

With a 2695 separation module, 2996 PDA detector, performance plus 4 channel in line degasser, auto injector, quaternary high pressure gradient pump, 100µl sample loop, 200µl syringe, and column heater, the Waters alliance HPLC system is geared for high-performance separation. Using Empower login software, all of the data from the Waters Alliance HPLC system was collected and processed. Using a mobile phase of acetonitrile: phosphate buffer (20:80 v/v) and ortho-phosphoric acid to adjust pH to 3.0, the chromatographic analysis was carried out. Before use, these were degassed by sonication and filtered through a 0.45-inch membrane filter. During analysis at room temperature, the mobile phase was pumped isocratically at a flow rate of 1.0 ml/min. The injection volume was 20µl, the run time was 10 min, and the eluent was detected at 230 nm on a C18 ODS with Waters symmetry (250 × 4.6 mm, 5µm particle size).

Analysis of dosage forms

Twenty tablets (2-4mg each) were weighed, and the mean weight of the tablets was calculated. Two milligrams of TIZ were diluted in 50 milliliters of diluent, sonicated for 30 minutes, and then filtered through whatman paper no. 41. The filtrate was properly diluted to achieve a concentration of 20, 40, and 60µg/ml before being tested.

Forced degradation study

To study the impact of acid, 10 mg of TIZ that had been precisely weighed was dissolved in 9 ml of mobile phase, and the volume was then made up to 10 ml with 1N HCl to get a concentration of 1000µg/ml (i.e., the concentration of HCl in solution was 0.1 N). This solution was then kept on a water bath at 80°C for 60 minutes. To make the 40µg/ml solution, aliquots of the aforesaid solution were neutralized with 1N NaOH and diluted with diluents. A chromatogram was created when the sample solution was examined. To explore the impact of the alkali, 10 mg of TIZ that had been precisely weighed was dissolved in 9 ml of mobile phase and then volume was increased to 10 ml with 1N sodium hydroxide to create a solution of 1000µg/ml (i.e. conc. of NaOH in solution was 0.1N). The aforementioned mixture was heated to 80°C in a water bath for 60 minutes. To make the 40µg/ml solution, aliquots of the aforesaid solution were neutralized with 1N HCl and diluted with diluents. The sample solution's chromatogram was recorded after analysis. To investigate the impact of oxidizing environments, precisely weighed 10 mg of TIZ were dissolved in 9 ml of mobile phase, and then volume was made up to 10 ml with 30.0% hydrogen peroxide to achieve a solution of 1000µg/ml (i.e., the concentration of hydrogen peroxide in the solution was 3%) and kept on a water bath at 80°C for 60 min. To create a 40µ g/ml solution, aliquots of the aforesaid solution were neutralized with 1N HCl and diluted with diluents. A chromatogram was recorded after the sample solution was examined. For three days, 1.0 gram of precisely weighed TIZ was maintained in an oven set at 80 °C to study the effects of temperature. Every 24 hours, a sample containing 10 mg of the medication was removed and diluted in accordance with protocol. A chromatogram was recorded after the preparation of aliquots of a 40µg/ml concentration. In order to investigate the impact of UV radiation, precisely weighted about 1.0 gram TIZ was exposed to short- and long-wavelength UV radiation (222 and 366 nm, respectively) for 48 hours. Samples containing 10 mg of the drug were taken out and diluted as directed after every 24 hours. A chromatogram was recorded after the preparation of aliquots of a 40µg/ml concentration.

Method validation

According to ICH criteria, the method was validated for linearity, specificity, limits of detection (LOD), limits of quantification (LOQ), system adaptability, accuracy, precision, robustness, and stability. Peak purity was obtained using a photodiode-array detector to evaluate specificity. Test solutions of TIZ were made at six concentrations ranging from 4-80µg/ml to verify linearity. The calibration graphs were created by graphing peak area against concentration after each solution was injected in triplicate. On three consecutive days, linearity was examined over the same concentration range. Also, the calibration plot's Y-intercept and slope's RSD (%) were calculated.

The limits of detection (LOD) and quantification (LOQ) for TIZ were determined, as the levels for which signal-to-noise ratios were 3:1 and 10:1, respectively, by injecting a series of dilute solutions of known concentration. Precision was achieved by measuring the drug concentration in the injection six times to estimate RSD (%). Two analysts evaluated intermediate (inter-day) precision on separate days in the same laboratory. By measuring recovery after adding known dosages of the medicine (80, 100, and 120% of the label claim of 1gm TIZ per injection) to the placebo, the accuracy of the procedure was investigated. At each recovery level, three samples were collected, and the results were computed using the calibration

plot. By purposefully changing the experimental circumstances and analyzing the impact on the resolution of TIZ from the main product produced by deterioration under standard conditions, the robustness of the approach was evaluated. Instead of using acetonitrile: phosphate buffer (20:80 v/v) and adjusting the pH to 3.0 with ortho-phosphoric acid, acetonitrile: phosphate buffer (25:75 v/v) and adjusting the flow rate to 1.0 ml/min, 0.8 ml/min, and 1.2 ml/min were used as the mobile phase and flow rates, respectively. All other conditions were maintained constant during these testing at their ideal levels. Analysis after 24, 48, and 72 hours, comparison of the results with those from freshly prepared standard solutions, and calculation of RSD were used to test the stability of TIZ and sample solutions (at ambient temperature).

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

To achieve resolution between TIZ and its degradation products was the main goal of creating this stability-indicating HPLC technique. Waters Alliance HPLC system with 2695 separation module, 2996 PDA detector, and C18 column was used for the planned work to achieve this. An attempt was made to quantify TIZ using acetonitrile: phosphate buffer (20:80 v/v) and ortho-phosphoric acid to adjust the pH to 3.0 as the mobile phase with acceptable system suitability parameters (RT, tailing factor, number of theoretical plates, and HETP) at 230 nm as the detection wavelength. With a correlation coefficient of r^2 = 0.9998 and the equation AUC = 41.716Conc-2.250, linearity was shown to exist between 4-80µg/ml. 25°C was the column's temperature. Retention periods were around 5.417 ±0.5 min for the primary peak and less than 10 min for the degradation products, while the tailing factor for TIZ was < 2. (Figure 2). High productivity and low cost of analysis per sample were produced as a result of the reduced overall runs time.

Forced degradation study

While determining the stability-indicating characteristics of analytical procedures, Bakshi et al.[30] recommended a target degradation of 20–80% because even intermediate degradation products shouldn't interfere with any stage of drug analysis. Even after prolonged exposure to acid, base, and oxidizing agents, the parameters employed for forced degradation could not be altered to achieve deterioration in this range under any other circumstances. Peak purity test findings indicated that the TIZ peak was homogeneous under all the stress conditions examined. The mass balance of TIZ in stress samples was nearly 100%, and furthermore, the method's capacity to indicate stability was confirmed by the assay of unaffected TIZ in the tablets. Table 1 provides a summary of the findings from studies on forced deterioration. Data on the chromatographic peak purity were taken from the spectrum analysis report; a peak with a peak purity of greater than 99 is considered to be homogeneous. The degradation experiments' peak purity for TIZ was in the range 99.9-100.0, suggesting homogenous peaks and proving the method's specificity. Figure 3 displays, respectively, chromatograms from the solutions obtained following deterioration under acidic, basic, oxidizing, and photolytic conditions. There were no peaks that co-eluted with the TIZ peak, indicating that the approach allowed for a more precise study of TIZ and the presence of its breakdown products.

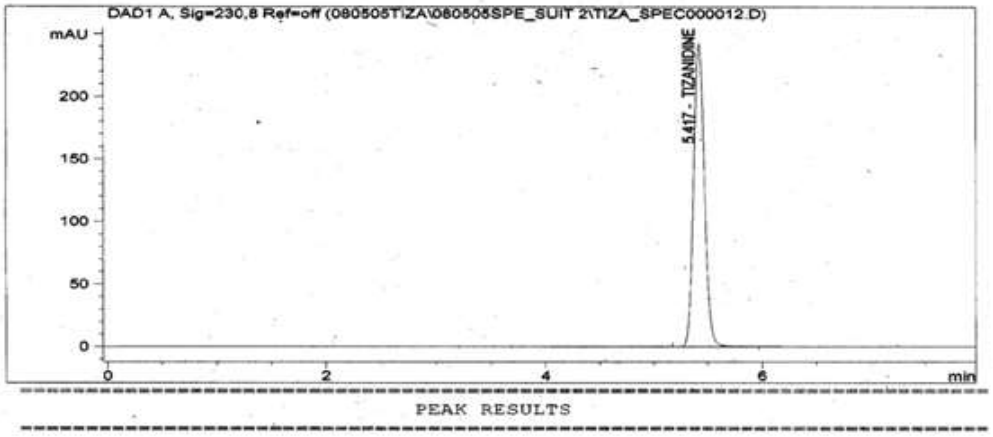
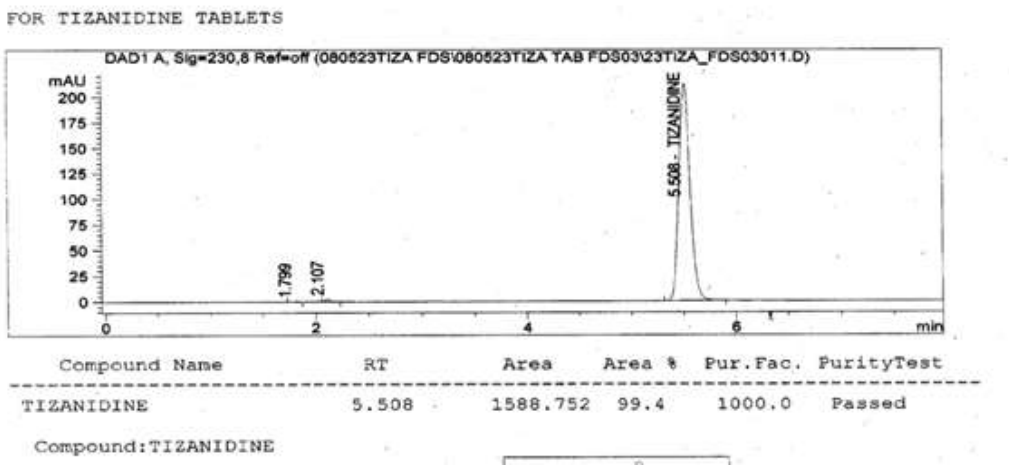
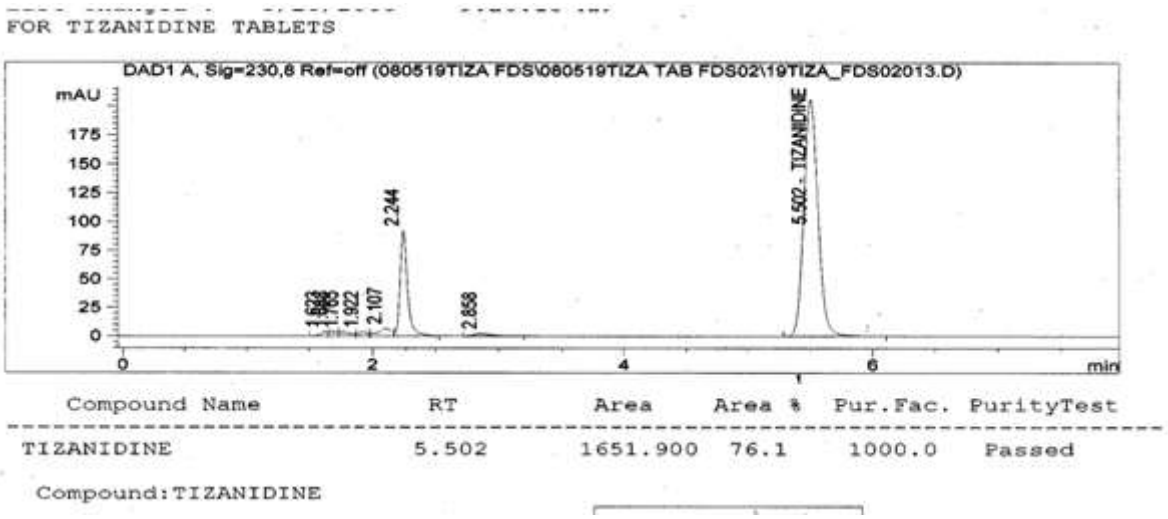


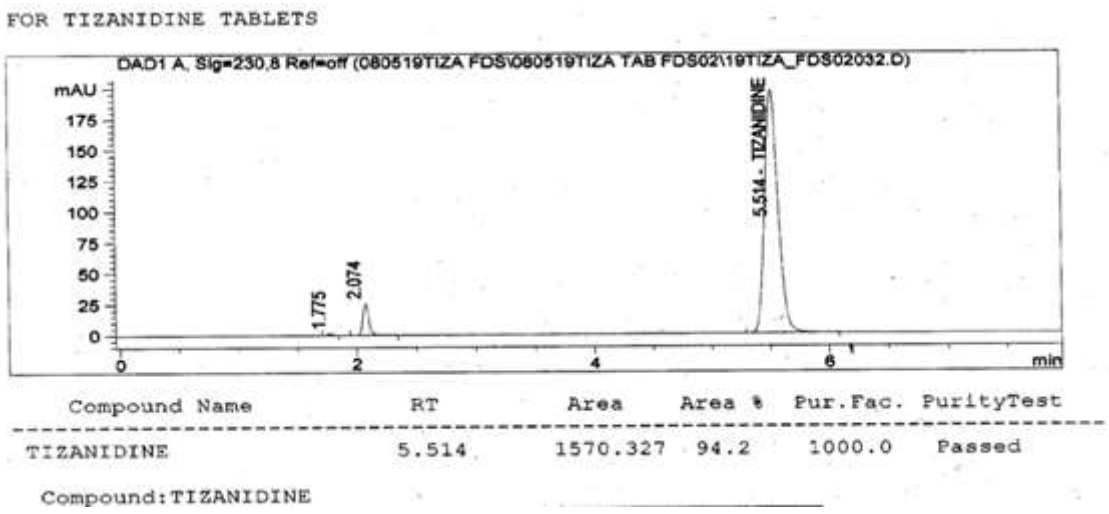
Figure 2: Standard chromatogram of tizanidine hydrochloride



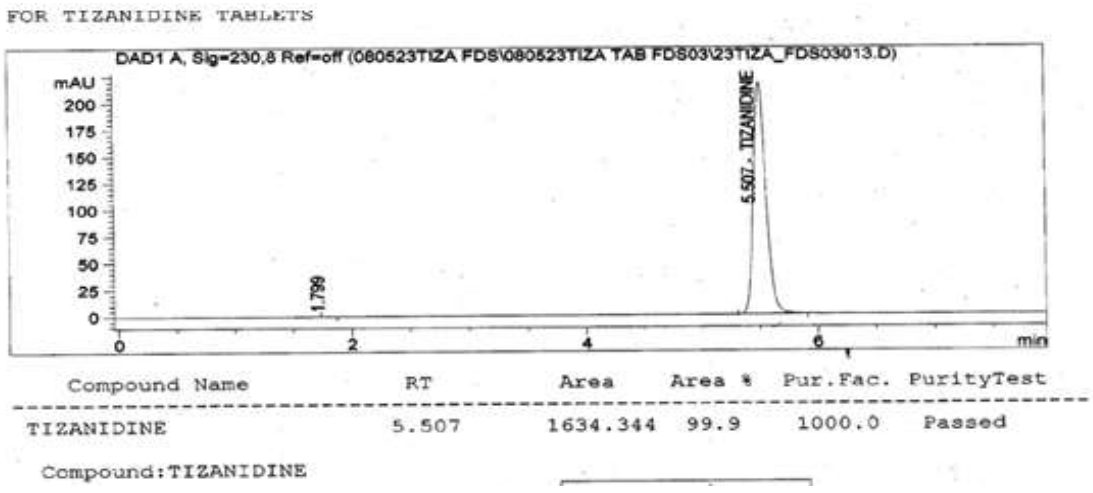
(A)



(B)



(C)



(D)

Figure 3: Typical chromatogram obtained after degradation of tizanidine hydrochloride under (A) acidic conditions, (B) basic conditions, (C) oxidizing conditions, (D) photolytic condition

Method validation

Peak purity for the drug substance and drug degradation products was >99.9% at 230 nm, demonstrating the purity of the analyte peaks and excluding the interference of formulation excipients and degradation products. For a 20µl injection volume, the LOD and LOQ for TIZ were 0.05 and 0.22µg/ml, respectively. Table 2 contains a list of the regression analysis findings together with information on system suitability. The RSD of TIZ peak area was less than 2% when precision was assessed using a six-fold examination of drug injection, demonstrating the method's dependability.

Table 3 contains the outcomes of the precision evaluation. Table 4 lists the results of the recovery determination, whereas Table 5 displays the outcomes of the robustness test.

Table 1: Results from analysis of samples from the forced degradation study, showing percentage degradation andpeak purity of tizanidine hydrochloride

Stress condition and duration	% degradation	Peak purity*
Acid degradation 0.1N HCl–80°C /60 min	4.8	99.928
Base degradation 0.1N NaOH–80°C/60 min	56.56	99.983
Oxidizing degradation (3% H2O2)-60 min	9.3	99.912
Thermal degradation- 80°C /48 hrs	0	99.971
UV light/222nm/48 h	0	100.23
UV light/366nm/48 h	0	101.34

*Peak purity values in the range of 99-100 indicate a homogeneous speak

Table 2: Results from regression analysis and system suitability data

Parameters	Tizanidine hydrochloride
Retention time*	5.417± 0.5 min
Tailing factor*	1.10
Theoretical plate*	13965
Linear range (µg/ml)	4-80
Limits of detection (µg/ml)	0.05
Limits of quantification (µg/ml)	0.22
Linear equation	41.716Conc-2.250
Slope	41.716
Intercept	-2.250
Correlation coefficient	0.9998

*Mean of six readings

Table 3: Result of precision of test method of tizanidine hydrochloride

Std. conc. (µg/ml)	Repeatability	Intermediate precision	
		Day to day	Analyst to analyst
4	100.2	99.80	99.26
20	102.7	103.9	100.19
40	99.60	102.3	99.80
60	101.00	100.5	99.02
80	100.19	100.9	100.12
Mean	100.738	101.48	99.678
S.D.	1.205	1.632	0.519
% R.S.D.	1.196	1.608	0.520

*Mean of fifteen determinations (3 replicates at 5 concentration level)

Table 4: Recovery of tizanidine hydrochloride

Level of addition	Std. drug sol. added (µg/ml)	% mean* recovered
50	20	100.4
100	40	101.0
150	60	101.3

Table 5: Results from robustness testing

Change in Parameters	Values	Component	RT (min)	T. Plates	Tailing Factor	% Assay	SD	% RSD
Normal conditions	As per specified method	Tizanidine	5.420	13974	1.11	98.3	1.1314	1.15
Flow Rate (±0.2ml)	0.8	Tizanidine	6.777	15657	1.13	98.1		
	1.2	Tizanidine	4.523	12531	1.09	98.0		
Column Change	Inertsil ODS 3V, 250 x 4.6 mm, 5µ	Tizanidine	5.686	15091	1.11	98.3		
pH change (±0.2)	2.8	Tizanidine	5.531	14602	1.11	99.5		
	3.2	Tizanidine	5.642	14681	1.11	99.0		
Composition of Mobile Phase (Buffer : ACN)	78:22	Tizanidine	4.761	13469	1.11	99.7		
	82:18	Tizanidine	6.810	15220	1.11	99.6		
Column Temp. (50^0C±5)	45°C	Tizanidine	5.835	14574	1.12	96.8		
	55°C	Tizanidine	5.463	14818	1.12	96.4		

CONCLUSION

The technique created for the quantitative analysis of tizanidine hydrochloride is quick, exact, precise, and selective. Peak purity tests under all stress situations revealed that the drug peak was pure, proving that the process is stable. In other words, it can be said that the method created can be used to successfully quantify the drug even when its degradation product and excipients are present. All of the characteristics studied yielded satisfactory findings, and the methodology was fully validated. It is possible to evaluate the stability of tizanidine hydrochloride in the bulk medication using the stability-indicating method. In pharmaceutical laboratories and businesses, the approach is easily applied for routine analysis of tizanidine hydrochloride as a bulk drug, in the appropriate dosage forms, for dissolving investigations, and as a stability-indicating test method.

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